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Zastosowanie nowatorskich technologii preparatyki próbek do analiz antydopingowych oraz terapeutycznego monitorowania leków w złożonych matrycach biologicznych

Rozprawa na stopień doktora nauk farmaceutycznych

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1. Sobczak, Ł.; Goryński, K. Evaluation of swabs from 15 commercially available oral fluid sample collection devices for the analysis of commonly abused substances: Doping agents and drugs of abuse. *Analyst* **2020**, *145*, 7279–7288. DOI: 10.1039/d0an01379j

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2. Sobczak, Ł.; Kołodziej, D.; Goryński, K. Modifying current thin-film microextraction (TFME) solutions for analyzing prohibited substances: Evaluating new coatings using liquid chromatography. *J. Pharm. Anal.* **2022**, *12*, 470–480. DOI: 10.1016/j.jpha.2021.12.007

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3. Sobczak, Ł.; Kołodziej, D.; Goryński, K. Benefits of Innovative and Fully Water-Compatible Stationary Phases of Thin-Film Microextraction (TFME) Blades. *Molecules* **2021**, *26*, 4413. DOI: 10.3390/molecules26154413

Punktacja: IF: 4,927; ministerialny wykaz czasopism punktowanych: 140

4. Kołodziej, D.; Sobczak, Ł.; Goryński, K. Polyamide Noncoated Device for Adsorption-Based Microextraction and Novel 3D Printed Thin-Film Microextraction Supports. *Anal. Chem.* **2022**, *94*, 2764–2771. DOI: 10.1021/acs.analchem.1c03672

Punktacja: IF: 8,008; ministerialny wykaz czasopism punktowanych: 140

5. Kołodziej, D.; Sobczak, Ł.; Goryński, K. Innovative, Simple, and Green: A Sample Preparation Method Based on 3D Printed Polymers. *Talanta* **2023**, *257*, 124380. DOI: 10.1016/j.talanta.2023.124380

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Keywords:

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Wykaz skrótów:

AAS – steryd anaboliczny (ang. *anabolic-androgenic steroid*)

ADHD – zespół nadpobudliwości psychoruchowej z deficytem uwagi (ang. *attention deficit hyperactivity disorder*)

C_8 – ugrupowanie oktylowe (ang. *octyl group*)

C_{18} – ugrupowanie oktadecylowe (ang. *octadecyl group*)

CN – ugrupowanie 3-cyjanopropylowe (ang. *3-cyanopropyl group*)

DVB – diwinylobenzen (ang. *divinylbenzene*)

ESI – jonizacja przez elektrorozpylanie (ang. *electrospray ionization*)

HPLC – wysokosprawna chromatografia cieczowa (ang. *high-performance liquid chromatography*)

LLE – ekstrakcja ciecz-ciecz (ang. *liquid-liquid extraction*)

MKOl – Międzynarodowy Komitet Olimpijski (ang. *International Olympic Committee*)

MS – spektrometria mas (ang. *mass spectrometry*)

NPS – nowa substancja psychoaktywna (ang. *new psychoactive substance*)

OUN – ośrodkowy układ nerwowy (ang. *central nervous system*)

PANDA – (mikroekstrakcja typu) PANDA (ang. *polyamide noncoated device for adsorption-based (microextraction)*)

PDMS – polidimetylosiloksan (ang. *polydimethylsiloxane*)

PED – substancja poprawiająca wydolność (ang. *performance enhancing drug*)

POChP – przewlekła obturacyjna choroba płuc (ang. *chronic obstructive pulmonary disease*)

POLADA – Polska Agencja Antydopingowa (ang. *Polish Anti-Doping Agency*)

SARM – selektywny modulator receptora androgenowego (ang. *selective androgen receptor modulator*)

SERM – selektywny modulator receptora estrogenowego (ang. *selective estrogen receptor modulator*)

SPE – ekstrakcja do fazy stałej (ang. *solid-phase extraction*)

SPME – mikroekstrakcja do fazy stałej (ang. *solid-phase microextraction*)

TDM – terapia monitorowana stężeniem leku (ang. *therapeutic drug monitoring*)

THC – Δ^9-tetrahydrokannabinol (ang. Δ^9-*tetrahydrocannabinol*)

TFME – mikroekstrakcja do cienkiej powłoki (ang. *thin-film microextraction*)

TMS – ugrupowanie trimetylosililowe (ang. *trimethylsilyl group*)

TUE – zezwolenie na stosowanie leku (ang. *therapeutic use exemption*)

UHPLC – ultra wysokosprawna chromatografia cieczowa (ang. *ultra high-performance liquid chromatography*)

WADA – Światowa Agencja Antydopingowa (ang. *World Anti-Doping Agency*)

1. Wprowadzenie

Wiodące metody instrumentalne chemii analitycznej stosowane obecnie w badaniach laboratoryjnych już teraz zapewniają znakomitą jakość wyników, a jednocześnie bezustannie są one udoskonalane. Uściślając pojęcie jakości wyników, mowa tutaj o podstawowych charakterystykach metody analitycznej takich jak m.in.: **selektywność** (względnie również specyficzność) zapewniająca pewność co do prawdziwości otrzymanego wyniku; **liniowość** oznaczającą, w ściśle określonym zakresie wartości, brak przekłamania spowodowanego wielkością analizowanego parametru; **czułość** określającą najmniejszą możliwą od wykrycia (jakościowo), jak również i do oznaczenia (ilościowo) wartość parametru; **precyzję** stanowiącą o powtarzalności uzyskiwanych wyników; **dokładność** będącą miarą zbieżności uzyskiwanych wyników z ich rzeczywistą wartością; a także inne, niewymienione tutaj, lecz niezaprzeczalnie istotne cechy [1,2]. Zanim przedstawione zostaną przykłady konkretnych metod analitycznych, warto już teraz zasygnalizować konieczność kompleksowego spojrzenia na zagadnienie jakim jest ostatecznie stosowany protokół analityczny będący w praktyce połączeniem kilku różnych technik, odmiennych i wyspecjalizowanych w swojej funkcji. Dopiero czerpanie z komplementarnych korzyści każdej z rozpatrywanych metod zapewnia w rezultacie uzyskanie znakomitej jakości wyników.

Za wiodące metody instrumentalne powszechnie uważane są m.in. zastosowane w prezentowanych w przedłożonej rozprawie doktorskiej badaniach: wysokosprawna chromatografia cieczowa (HPLC, ang. *high-performance liquid chromatography*); tzw. miękkie metody jonizacji (ang. *soft ionization*); oraz tandemowa spektrometria mas (MS, ang. *mass spectrometry*). Co niezwykle znamienne, żadna z wymienionych metod nie należy do najnowszych osiągnięć nauki.

Technika rozdzielania chromatograficznego, która umożliwia wyodrębnienie poszczególnych składników mieszaniny w celu ułatwienia ich identyfikacji oraz precyzyjniejszego pomiaru, znana jest już ponad sto lat. Za pioniera chromatografii kolumnowej uważany jest pracujący na początku XX wieku w Warszawie (Uniwersytet Warszawski oraz Politechnika Warszawska) Michaił Cwiet (ros. Михаил Цвет), stosujący kolumny własnej produkcji do rozdzielania i identyfikacji barwników roślinnych [3]. Udoskonalony wariant kolumnowej chromatografii cieczowej – HPLC – do użytku wprowadzony został we wczesnych latach sześćdziesiątych XX wieku [4].

Pierwsze doniesienia naukowe o zastosowaniu HPLC ukazały się kilka lat później na łamach wysoce prestiżowych czasopism *Nature* [5] oraz *Analytical Chemistry* [6].

Rozdzielenie składników próbki jest jednak zaledwie początkiem całego procesu analizy instrumentalnej. Aby wykryć i oznaczyć składniki próbki, zarówno takiej poddanej uprzedniej preparatyce, jak i nie, niezbędne jest zastosowanie metody detekcji. Wykorzystana w prezentowanej rozprawie doktorskiej metoda detekcji za pomocą spektrometrii mas wymaga wcześniejszego obdarzenia ładunkiem elektrycznym (jonizacji) wszystkich analizowanych składników próbki. W tym celu w prezentowanych badaniach użyta została metoda jonizacji przez elektrorozpylanie (ESI, ang. *electrospray ionization*), która należy do tzw. miękkich metod jonizacji. Jest to grupa metod pozwalających na nadanie cząsteczce ładunku elektrycznego bez degradacji (fragmentacji) jej struktury, a tym samym umożliwia określenie masy całej cząsteczki. Wprowadzona w 1968 roku [7] metoda ESI, obecnie wykorzystuje w praktyce późniejsze osiągnięcia Johna Fenna. Prowadzone przez niego od lat osiemdziesiątych XX wieku badania zostały w 2002 roku wyróżnione Nagrodą Nobla w dziedzinie chemii [8], w szczególności za przełomowe wdrożenie ESI jako metody jonizacji sprzężonej ze spektrometrią mas [9].

Z kolei najstarszą spośród omawianych metryką, swoimi początkami sięgającą aż XIX wieku, może pochwalić się metoda spektrometrii mas [10]. Pierwszy detektor typu kwadrupol (ang. *quadrupole* – jego nazwa nawiązuje do czterech prętów generujących pole elektryczne filtrujące cząsteczki obdarzone ładunkiem elektrycznym) stworzony został w 1953 roku [10]. Osiągnięcie to stworzyło podwaliny dla konstrukcji analizatorów mas typu pułapka jonowa (ang. *ion trap*). Badania te zostały w 1989 roku wyróżnione Nagrodą Nobla, tym razem w dziedzinie fizyki, dla Wolfganga Paula – twórcy pierwszego kwadrupola [11]. Współczesne kwadrupole w praktyce często posiadają jednak nie cztery, a osiem prętów zapewniających korzystniejszą charakterystykę pracy. Następnym krokiem na ścieżce rozwoju spektrometrii mas było stworzenie urządzeń wykorzystujących kilka połączonych ze sobą analizatorów mas. Przykładem tandemowego spektrometru mas jest wykorzystywany w badaniach prezentowanych w rozprawie doktorskiej detektor typu potrójny kwadrupol (ang. *triple quadrupole*). Potrójny kwadrupol (a tym samym technika tandemowej spektrometrii mas – MS/MS) po raz pierwszy zastosowany został w 1978 roku [12]. Dzisiaj jego konfundująca nazwa jest już tylko zaszłością historyczną, gdyż stosowane obecnie potrójne kwadrupole posiadają jedynie dwa analizatory mas typu kwadrupol – a trzeci

(środkowy) zastąpiony w nich został specjalną komorą zderzeń (ang. *collision cell*) umożliwiającą fragmentację wybranej cząsteczki w celu poznania jej struktury oraz zwiększenia selektywności uzyskiwanych pomiarów (ze względu na występowanie specyficznych par jon molekularny – jon fragmentaryczny).

Omówione powyżej przeszło sto lat rozwoju wybranych metod analitycznych (Ryc. 1) przekłada się bezpośrednio na osiąganą obecnie znakomitą jakość wyników, m.in. uzyskiwanych dzięki stosowanemu w prezentowanych w rozprawie doktorskiej badaniach protokołowi analitycznemu (Ryc. 2).

Ryc. 1. Linia czasu wybranych osiągnięć w dziedzinie rozwoju metod analitycznych.

Jednakże ta doskonała jakość wyników obarczona jest jednocześnie dużą wrażliwością opisywanej aparatury na wszelkie zanieczyszczenia. Wśród przyczyn upatrywać można m.in. wykorzystania w urządzeniach tego typu licznych elementów podatnych na występowanie zatorów. Przykłady stanowią: ciasno upakowane kolumny chromatograficzne o małej wielkości cząsteczek wypełnienia – szczególnie cząsteczek poniżej 2 μm średnicy stosowanych w standardzie ultra wysokosprawnej chromatografii cieczowej (UHPLC, ang. *ultra high-performance liquid chromatography)* – w zamian charakteryzujących się bardzo wysoką zdolnością rozdzielczą; wąskie rurki i połączenia zestawu (U)HPLC operujące w warunkach wysokiego ciśnienia atmosferycznego (nawet rzędu tysiąca atmosfer dla metod UHPLC), które zapewniają warunki pracy niezbędne dla osiągnięcia kompatybilności ze wspomnianymi wcześniej ciasno upakowanymi kolumnami; a także wąskie kapilary źródła jonów oraz analizatora mas – zapewniające dużą dokładność pomiaru. Ponadto wysoce podatne na zabrudzenie nielotnymi solami oraz makrocząsteczkami (np. peptydy i białka) jest samo źródło jonów typu ESI, co może skutkować koniecznością żmudnego czyszczenia. Oznacza to zarazem przestój w pracy całego instrumentu analitycznego.

Rozwiązaniem tego problemu jest stosowanie wyłącznie kosztownych odczynników o klasie czystości *LC-MS*. Odczynniki takie są pozbawione cząsteczek wielkości powyżej 0,2 µm, a także charakteryzują się one bardzo niskim stężeniem wszelkich elektrolitów – a zatem jonów, które mogłyby za sprawą konkurowania o ładunek elektryczny osłabiać jonizację badanych substancji. Jednakże przede wszystkim, skutecznym remedium jest wdrożenie metody preparatyki próbek. Jest to jednocześnie tzw. „wąskie gardło" całego protokołu analitycznego, ponieważ sam proces przygotowania próbki przekłada nawet na 1/3 wypadkowo uzyskanego błędu pomiarowego [13,14]. Wśród wiodących metod przygotowania próbek znajdują się będące newralgiczną częścią tej rozprawy doktorskiej metody mikroekstrakcyjne (rozdziały 8 i 9) [15]. Pierwsza spośród tych metod, technika mikroekstrakcji do fazy stałej (SPME, ang. *solid-phase microextraction*) została wprowadzona i spopularyzowana przez pochodzącego z Gdańska profesora Janusza Pawliszyna [16]. Dalsze informacje na temat metod mikroekstrakcyjnych znajdują się w rozdziale 7.

Ryc. 2. Schematyczne przedstawienie kolejnych etapów protokołu analitycznego oraz metod stosowanych w przedstawionej rozprawie doktorskiej.

Doskonałym polem do praktycznego zastosowania, a zarazem do dalszego rozwoju wszystkich wymienionych metod są badania antydopingowe, badania kierowców i wykonywane w miejscu pracy, analizy kryminalistyczne i toksykologiczne, a także terapia monitorowana stężeniem leku (TDM, ang. *therapeutic drug monitoring*) [17-22]. W szczególności dotyczy to kontroli używania nowoopracowanych substancji niezatwierdzonych do stosowania u ludzi (ang. *designer drugs*) oraz środków odurzających takich jak narkotyki (w tym m.in. opioidowe leki przeciwbólowe), substancje psychotropowe (w tym m.in. leki nasenne, przeciwlękowe i uspokajające), a także tzw. dopalacze (NPSs, ang. *new psychoactive substances*). Wspólną cechą większości z wymienionych specyfików jest ich duża siła działania, co przekłada się bezpośrednio na stosowanie ich w małych dawkach (np. rzędu nanogramów), a tym samym wypadkowo na niskie stężenia analitów (np. rzędu pg/mL (= ng/L) [23]) obecne w płynach ustrojowych wykorzystywanych w badaniach. Oczywiste staje się wówczas, że analiza takich substancji wymaga zastosowania bardzo czułej metodyki. Dodatkowo wyjątkową charakterystyką wymienionych badań jest konieczność dokładnego i precyzyjnego określenia stężenia substancji, ze względu na występowanie ściśle określonych dla niej stężeń progowych. Wartości te są newralgiczne biorąc pod uwagę podejmowane na ich podstawie decyzje (np. o dyskwalifikacji i zawieszeniu sportowca, o wydaniu wyroku, czy o zwolnieniu dyscyplinarnym pracownika). Niezbędne jest, aby stosowana metodologia zapewniała absolutną pewność i powtarzalność wyników, a tym samym nie mogła zostać zakwestionowana jako dowód w postępowaniu. Temat specyfiki badań związanych z wykrywaniem i oznaczaniem substancji zakazanych (dopingu farmakologicznego i środków odurzających) został dogłębniej opisany w rozdziałach 3 i 4.

Na koniec wprowadzenia zasygnalizowana zostanie problematyka złożoności materiału biologicznego stosowanego do badań laboratoryjnych w celu wykrywania wspomnianych substancji. Najczęściej jest on płynem ustrojowym (krew, mocz lub ślina) z obecnymi substancjami drobnocząsteczkowymi (w tym leki) oraz niepożądanymi podczas badań makromolekułami (takimi jak białka i peptydy, a czasami nawet całe komórki). Zagadnienie materiałów biologicznych stosowanych do badań oraz metod pobierania próbek zostanie omówione w rozdziałach 5 i 6.

2. Cel pracy

Głównym celem prowadzonych badań było udoskonalenie metod przygotowania próbek, ze szczególnym uwzględnieniem zastosowania metod mikroekstrakcyjnych do późniejszego oznaczania substancji zakazanych (dopingu farmakologicznego i środków odurzających) w próbkach śliny przy wykorzystaniu aparatury HPLC-ESI-MS/MS. Dodatkowe cele stanowiły wykorzystanie śliny jako tzw. alternatywnego materiału do badań (względem zazwyczaj stosowanych materiałów takich jak krew i mocz), a także opracowanie zupełnie nowej metody przygotowania próbek.

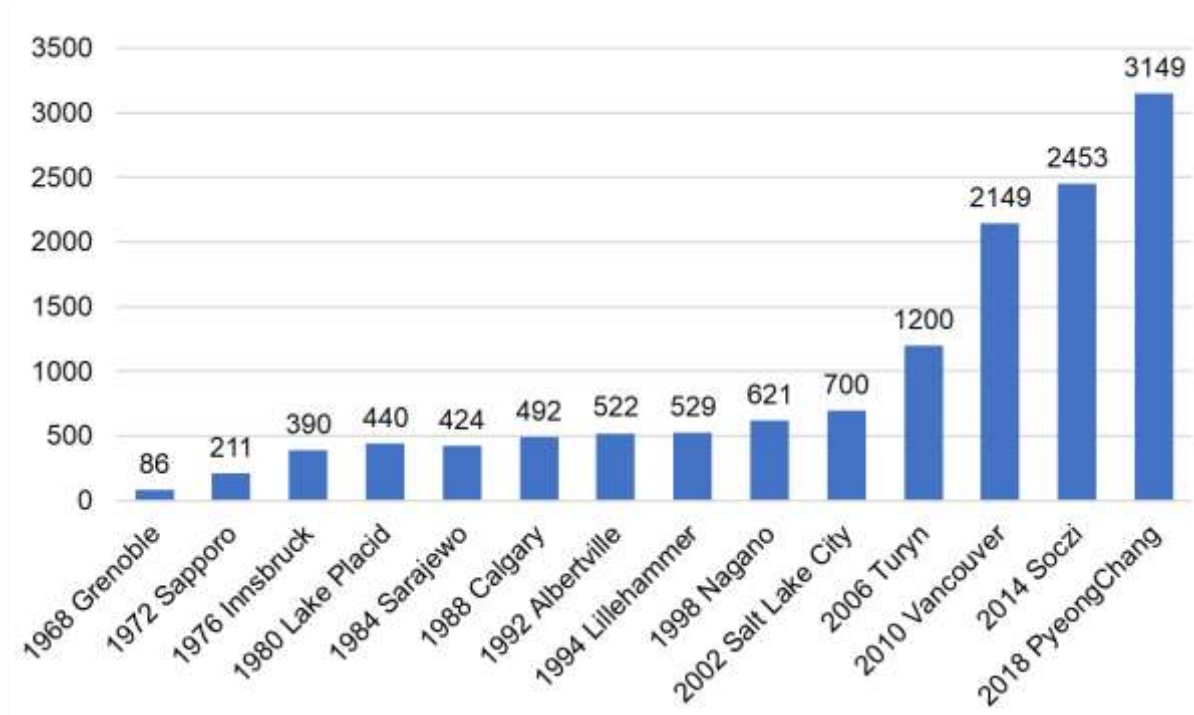
Na potrzeby realizacji przedstawionych celów kompleksowo ocenione zostały dostępne komercyjnie urządzenia służące do pobierania próbek śliny. Dodatkowo wdrożone oraz poddane dogłębnej analizie zostały nowe rodzaje pokryć urządzeń do mikroekstrakcji do cienkiej powłoki (TFME, ang. *thin-film microextraction*). Ponadto z wykorzystaniem najnowszych osiągnięć technologii druku 3D przygotowane zostały innowacyjne urządzenia ekstrakcyjne.

3. Krótka historia badań antydopingowych

Nie sposób dzisiaj stwierdzić co jest zjawiskiem wcześniejszym – sportowe współzawodnictwo czy zażywanie substancji poprawiających wydolność organizmu (PEDs, ang. *performance enhancing drugs*). Natomiast z całą pewnością wiadomo, że już w starożytności zażywano liczne substancje uważane obecnie za doping farmakologiczny, takie jak: sterydy anaboliczne (AASs, ang. *anabolic-androgenic steroids*) [24]; stymulanty ośrodkowego układu nerwowego (OUN, ang. *central nervous system*) [25], np. efedryna [26] i kokaina [27]; kannabinoidy [28-29]; oraz środki przeciwbólowe, np. opium zawierające morfinę [29-30]. Za doskonały przykład posłużyć może Milon z Krotonu – zapaśnik i sześciokrotny mistrz igrzysk olimpijskich rozgrywanych w VI wieku p.n.e. [24]. Jego dieta składała się głównie z mięsa wołowego, którego spożywał aż kilka kilogramów dziennie, w szczególności zaś obfitowała ona w bycze jądra (bogate w endogenne AAS – głównie w testosteron) [24]. Nie należy jednak przeceniać podaży AAS pochodzącej z tego typu diety. Bycze jądra zawierają około 4 μg testosteronu w 100 g tkanki [31], co przy biodostępności testosteronu po podaniu doustnym wynoszącej około 3,5% [32] przekłada się zaledwie

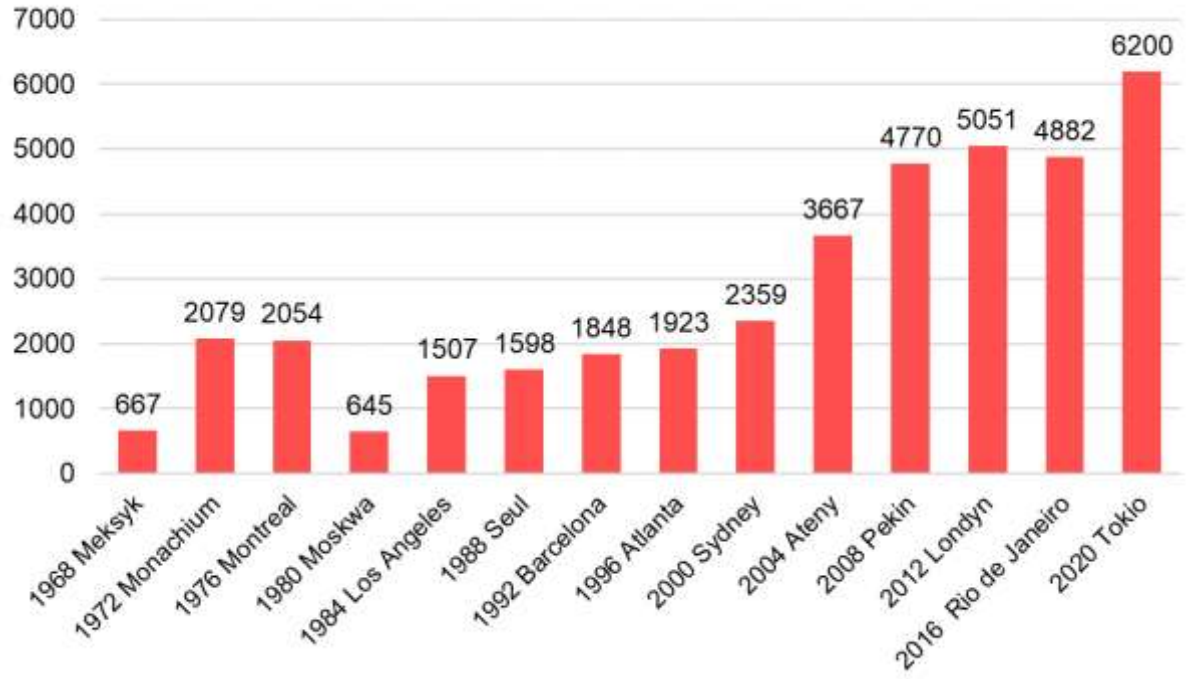
na około 0,14 µg/100 g. Terapeutycznie stosowane dawki testosteronu wynoszą zazwyczaj 158–237 mg, ponadto podawane są 2 razy dziennie [33]. Prosta kalkulacja wskazuje na konieczność spożycia niemal 8 ton byczych jąder, aby osiągnąć dawkę stosowaną terapeutycznie.

Wiadomo również, że już od czasów antycznych doping uważano za poważny problem. Za podawanie środków pobudzających koniom wyścigowym, winnego skazywano nawet na karę śmieci [34]. Jednak aż do XX wieku, nie istniały metody badań laboratoryjnych, które pozwoliłyby wykryć oszustwo bez złapania kogoś na tzw. gorącym uczynku. Pionierem badań antydopingowych (właśnie w wyścigach konnych) był warszawski farmaceuta Alfons Bukowski [35]. W 1910 roku opracował on metodę wykrywania alkaloidów (m.in. heroina, kofeina oraz kokaina) w końskiej ślinie. Swoją metodę stosował z powodzeniem na międzynarodowych wyścigach organizowanych w Budapeszcie, Moskwie, Petersburgu, Warszawie oraz Wiedniu [35-36]. Pomimo wdrożenia w 1928 roku pierwszych przepisów zabraniających stosowania dopingu u sportowców [37-38], na pierwsze badania próbek pochodzących od ludzi trzeba było poczekać jeszcze kolejne 40 lat – do Zimowych Igrzysk Olimpijskich w Grenoble w 1968 roku [38]. Przebadano wówczas zaledwie 86 próbek (Ryc. 3), z których wszystkie okazały się być negatywne [39].



Ryc. 3. Liczba próbek poddanych testom antydopingowym podczas kolejnych Zimowych Igrzysk Olimpijskich. Dane na podstawie informacji prasowej MKOl [39].

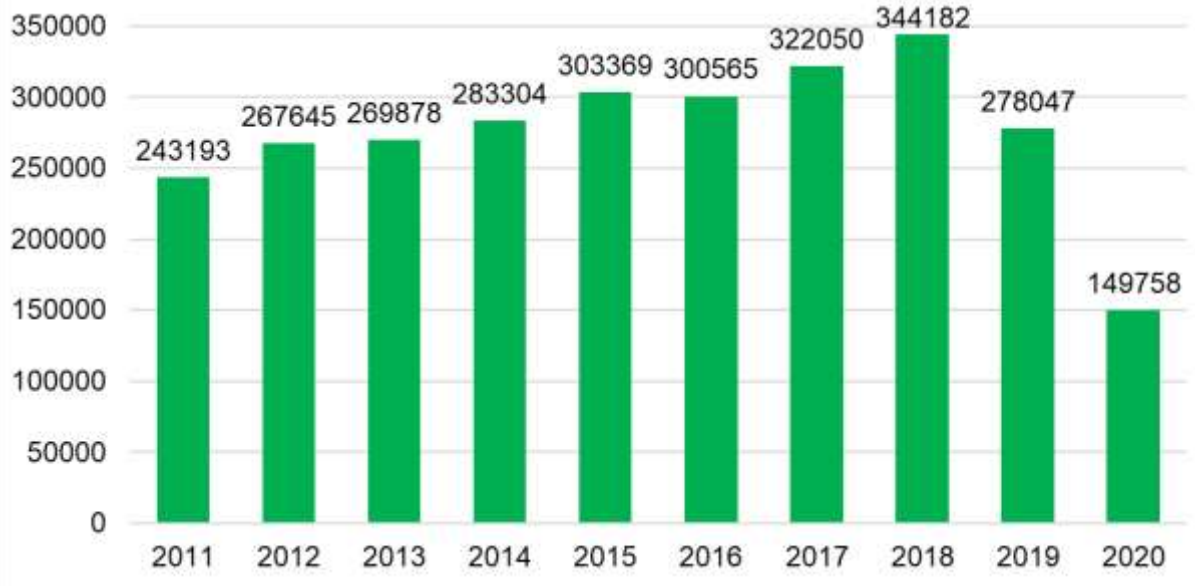
Dla porównania podczas rozgrywanych w 2021 roku Letnich Igrzysk Olimpijskich w Tokio przebadano już 6200 próbek (Ryc. 4) [40].



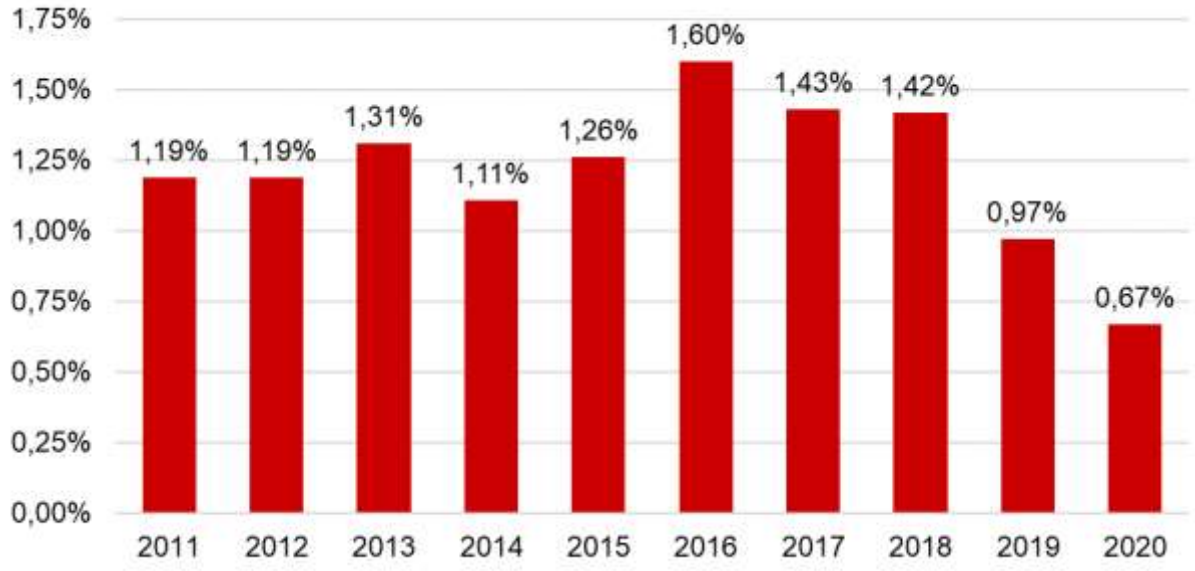
Ryc. 4. Liczba próbek poddanych testom antydopingowym podczas kolejnych Letnich Igrzysk Olimpijskich. Dane na podstawie informacji prasowej MKOl [39] oraz danych International Testing Agency [40] w przypadku Igrzysk w Tokio 2020 (ze względu na pandemię COVID-19 rozegranych w 2021 roku).

Poczynając od 1968 roku badano wyłącznie próbki moczu, a nadzór nad testowaniem sportowców sprawowała Komisja Medyczna Międzynarodowego Komitetu Olimpijskiego (MKOl, ang. *International Olympic Committee*) [36-37]. Krew jako materiał do badań antydopingowych, po raz pierwszy wykorzystano podczas Zimowych Igrzysk Olimpijskich w Lillehammer w 1994 roku. Pięć lat później, w 1999 roku, utworzono Światową Agencję Antydopingową (WADA, ang. *World Anti-Doping Agency*) – wyspecjalizowaną organizację zrzeszającą krajowe instytucje zajmujące się zwalczaniem dopingu, w tym m.in. Polską Agencję Antydopingową (POLADA, ang. *Polish Anti-Doping Agency*)). W 2004 roku WADA przejęła od Komisji Medycznej MKOl nadzór nad walką z międzynarodowym procederem dopingu [36].

W ostatnich latach, w skali całego świata WADA przeprowadza nawet 344 tys. testów antydopingowych rocznie (Ryc. 5), z czego zazwyczaj ponad 1% z nich dostarcza wyników pozytywnych – czyli wskazuje na obecność niedozwolonych substancji w organizmie (Ryc. 6) [41-50].



Ryc. 5. Łączna liczba próbek poddanych testom antydopingowym w ciągu danego roku. Dane na podstawie raportów WADA [41-50]. Na wykresie począwszy od 2019 roku widać wyraźny spadek ilości wykonywanych testów antydopingowych spowodowany pandemią COVID-19 [49-50].



Ryc. 6. Odsetek wyników pozytywnych dla próbek poddanych testom antydopingowym w ciągu danego roku. Dane na podstawie raportów WADA [41-50].

Obecnie jednak, w dalszym ciągu do badań pobierane są wyłącznie próbki moczu oraz (zdecydowanie rzadziej [41-50]) krwi. Analiza materiałów takich jak pot, włosy, czy stosowana w prezentowanych badaniach ślina pozostaje wyłącznie domeną laboratoriów zajmujących się rozwojem metod analitycznych.

4. Ogólna charakterystyka substancji stosowanych jako doping farmakologiczny oraz środki odurzające

4.1. Substancje dopingujące

WADA rokrocznie publikuje listę substancji oraz metod, których stosowanie jest u sportowców niedozwolone [51]. Współczesne wydania listy wyszczególniają 11 kategorii substancji zakazanych, spośród których część jest zabroniona przez cały czas (kategorie S0-S5), natomiast inne wyłącznie w trakcie trwania zawodów sportowych (S6-S9). Poszczególne kategorie substancji dopingujących według wykazu WADA [52] zostały przedstawione na Ryc. 7. Istnieje również specjalna kategoria substancji zakazanych wyłącznie w wybranych dyscyplinach (P1 – β-blokery). Do wspomnianych dyscyplin należą m.in.: golf, łucznictwo, skoki narciarskie, sporty motorowe, czy strzelectwo. Są to zatem dyscypliny kładące szczególny nacisk na precyzję wykonywanych ruchów, tym samym takie w których stosowanie β-blokerów zmniejszających drżenie mięśniowe byłoby szczególnie korzystne.

Ponadto u sportowców niedozwolone jest także wykonywanie pewnych zabiegów, które według wykazu WADA podzielone zostały na 3 kategorie niedozwolonych metod (M1-M3). Wśród wspomnianych metod znajdują się m.in.: transfuzje krwi (wykonywane w celu eliminacji niedozwolonych substancji z organizmu), wlewy dożylne powyżej 100 ml na 12 godzin (powodujące obniżanie stężenia analitów w próbce krwi oraz przyspieszanie ich eliminacji z moczem (lub ich metabolitów)), a także podmiana i manipulowanie (np. rozcieńczanie) próbkami pobranymi do badań [52].

Kategoria WADA	Przykłady substancji wykrywanych w testach antydopingowych [41-50]	Przykłady korzyści z niedozwolonego stosowania przez sportowców [36,53]
S0 **Substancje niezatwierdzone do stosowania u ludzi**	• nowo uzyskane substancje chemiczne • leki znajdujące się w trakcie badań klinicznych • leki weterynaryjne	• trudne do określenia
S1 **Środki anaboliczne**	• sterydy anaboliczne (testosteron, nandrolon) • klenbuterol (nielegalnie stosowany również do zwiększania masy zwierząt hodowlanych) • eksperymentalne leki z grupy SARM (ostaryna, ligandrol)	• rozrost tkanki mięśniowej (wzrost siły)
S2 **Hormony peptydowe, czynniki wzrostu i substancje o zbliżonym działaniu**	• erytropoetyna (EPO) • somatostatyna (GH) • analogi greliny (ibutamoren) • insuliny	• zwiększenie wydolności tlenowej organizmu • rozrost tkanki mięśniowej i wzrost ukrwienia mięśni • spalanie tkanki tłuszczowej
S3 **β_2-mimetyki**	• leki przeciwastmatyczne i na POChP (terbutalina, wilanterol) • higenamina (składnik suplementów diety stosowanych na odchudzanie)	• zwiększenie wydolności oddechowej • rozrost tkanki mięśniowej • spalanie tkanki tłuszczowej
S4 **Modulatory hormonalne i metaboliczne**	• leki przeciwnowotworowe z grupy SERM (tamoksyfen) oraz inhibitorów aromatazy (anastrozol, letrozol) • leki przeciwniedokrwienne (meldonium) • leki na bezpłodność (klomifen)	• zwiększenie wydzielania testosteronu (SERM) • łagodzenie skutków ubocznych stosowania środków anabolicznych (inhibitory aromatazy)
S5 **Leki moczopędne i środki maskujące**	• leki na nadciśnienie (furosemid, hydrochlorotiazyd) • leki na dnę moczanową (probenecyd) • leki na nietrzymanie moczu z grupy analogów wazopresyny (desmopresyna)	• przyspieszenie wydalania innych środków dopingujących z moczem • zwiększenie objętości osocza (obniżenie stężenia innych środków)
S6 **Substancje pobudzające OUN**	• leki na ADHD (metylofenidat) • substancje odurzające (kokaina, amfetamina) • leki na nieżyt nosa (pseudoefedryna)	• wzrost motywacji do wysiłku, spadek wrażliwości na zmęczenie • wzrost koncentracji • spadek masy ciała
S7 **Narkotyki**	• opioidowe leki przeciwbólowe (fentanyl, morfina, oksykodon) • substancje odurzające (heroina)	• działanie przeciwbólowe (znoszenie wpływu urazów)
S8 **Kannabinoidy**	• produkty pochodzące z konopi zawierające psychoaktywną substancję THC (marihuana, haszysz) • syntetyczne analogi kanabinoidów (niektóre dopalacze)	• zmniejszenie podatności na stres
S9 **Glukokortykosteroidy**	• leki na stany zapalne skóry (triamcynolon) • leki przeciwzapalne (prednizolon, betametazon)	• działanie przeciwzapalne (znoszenie wpływu urazów)
P1 **β-blokery**	• leki na nadciśnienie (propranolol, bisoprolol)	• zmniejszenie drżenia mięśni • działanie uspokajające

Ryc. 7. Kategorie substancji dopingujących według najnowszego wykazu WADA [52] wraz z przykładami substancji wykrywanych u sportowców [41-50] oraz potencjalnymi korzyściami wynikającymi z ich stosowania [36,53].
Wprowadzone skróty: ADHD – zespół nadpobudliwości psychoruchowej z deficytem uwagi (ang. *attention deficit hyperactivity disorder*); POChP – przewlekła obturacyjna choroba płuc płuc (ang. *chronic obstructive pulmonary disease*); SARM – selektywny modulator receptora androgenowego (ang. *selective androgen receptor modulator*); SERM – selektywny modulator receptora estrogenowego (ang. *selective estrogen receptor modulator*); THC – Δ^9-tetrahydrokannabinol (ang. *Δ^9-tetrahydrocannabinol*)

Wiele spośród substancji zakazanych w sporcie jest powszechnie stosowanych jako leki [53], w tym również jako leki dostępne bez recepty w aptekach. Dlatego, przepisy antydopingowe dopuszczają zażywanie wybranych leków przez sportowców na zasadzie uzyskania specjalnego zezwolenia (TUE, ang. *therapeutic use exemption*). Warunkiem otrzymania TUE jest m.in. orzeczenie lekarskie wydane na podstawie badania stwierdzającego występowanie konkretnej dolegliwości oraz brak alternatywnych leków na tę dolegliwość (nieuwzględnionych w wykazie substancji dopingujących). Jednak przede wszystkim, o TUE należy ubiegać się przed rozpoczęciem stosowania danego leku [54-55]. W wyjątkowych i udokumentowanych sytuacjach (takich jak stany nagłe, np. interwencja na oddziale ratunkowym) możliwe jest wsteczne (retroaktywne) przyznanie TUE [36]. W przypadku wątpliwości sportowcy mają także możliwość konsultacji z instytucjami zajmującymi się zwalczaniem dopingu. Przykładem jest m.in. POLADA udostępniająca specjalną wyszukiwarkę online: *Baza Leków Zabronionych* [56].

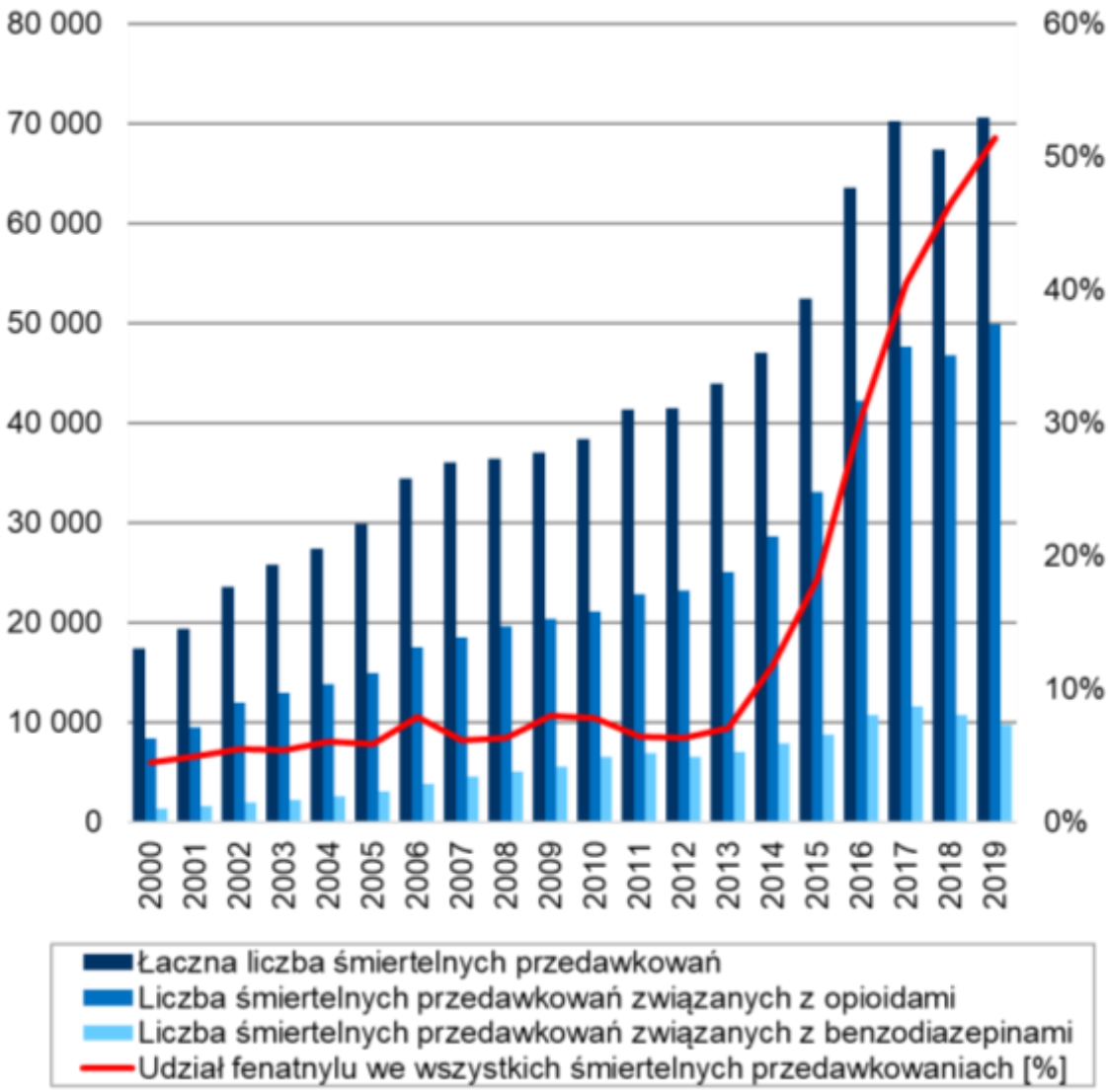
Do najczęściej stosowanych substancji dopingujących należą przede wszystkim sterydy anaboliczne (np. stanozolol, dehydrochlorometylotestosteron i drostanolon), substancje pobudzające OUN (metylofenidat, kokaina, amfetamina) oraz diuretyki (furosemid, hydrochlorotiazyd, kanrenon).

4.2. Środki odurzające

W świetle przepisów polskiego prawa środki odurzające (zwyczajowo określane mianem narkotyków, a od niedawna również i dopalaczy) określa Rozporządzenie Ministra Zdrowia z dnia 17 sierpnia 2018 wraz z późniejszymi zmianami [57]. Rozporządzenie dzieli wymienione środki na substancje psychotropowe (grupy I-P, II-P, III-P i IV-P), narkotyki (grupy I-N, II-N, III-N i IV-N) oraz nowe substancje psychoaktywne (grupy I-NPS, II-NPS, III-NPS, IV-NPS i V-NPS).

Poza substancjami stosowanymi wyłącznie w celach pozamedycznych, ministerialny wykaz zawiera także liczne lekarstwa. Wśród tych najczęściej stosowanych znajdują m.in. opioidowe leki przeciwbólowe (np. fentanyl, morfina, czy oksykodon) oraz działające poprzez receptory benzodiazepinowe leki nasenne, przeciwdrgawkowe, przeciwlękowe i uspokajające (np. alprazolam, klonazepam i zolpidem). Według przepisów WADA, wiele spośród środków odurzających należy również do wykazu substancji dopingujących, w szczególności zaś do kategorii S6 (substancje pobudzające OUN), S7 (narkotyki) oraz S8 (kannabinoidy) [52].

Konieczność ścisłego nadzoru nad stosowaniem substancji odurzających doskonale obrazują doroczne statystyki opisujące przypadki zgonów spowodowanych przedawkowaniem leków (Ryc. 8).



Ryc. 8. Roczna liczba zgonów spowodowanych przedawkowaniem leków w USA [58].

Na przykładzie Stanów Zjednoczonych Ameryki, na przestrzeni 20 lat, widoczny jest znaczący wzrost liczby śmiertelnych przedawkowań. Dane wskazują na 70630 takich przypadków w 2019 roku, co stanowi ponad czterokrotny wzrost względem 17415 przypadków zarejestrowanych w roku 2000. Największy udział we wspomnianym zjawisku mają opioidy oraz pochodne benzodiazepiny (odpowiednio 70,6% i 13,7% wszystkich przypadków w 2019 roku) [58].

Szczególnie niepokojący jest zauważalny w ciągu ostatniej dekady (Ryc. 8) gwałtowny wzrost udziału syntetycznych opioidów, głównie fentanylu i jego pochodnych (od 7,1% w 2013 roku do 51,5% w 2019 roku [58]). Zjawisko to określane mianem *epidemii opioidowej* stanowi niezwykle poważny problem społeczny [59-60], ale także nowe wyzwanie dla laboratoriów analitycznych [61].

5. Rodzaje materiałów biologicznych stosowanych do badań laboratoryjnych ze szczególnym uwzględnieniem materiałów wykorzystywanych do wykrywania niedozwolonych substancji

Jak zostało uprzednio wspomniane (rozdział 3), jedynymi stosowanymi obecnie materiałami do badań antydopingowych sankcjonowanych przez WADA są krew oraz mocz [62]. Są to jednocześnie materiały biologiczne najczęściej wykorzystywane do rutynowych oznaczeń laboratoryjnych [63], takich jak m.in. badanie poziomu hormonów, mikro- i makroelementów oraz oznaczania rozmaitych biomarkerów. Z tego względu oba płyny ustrojowe zwykło się określać mianem *klasycznych* (bądź też *konwencjonalnych*) materiałów do badań [64-65]. Jednakże ani krew, ani mocz nie są idealnymi materiałami analitycznymi.

W przypadku krwi, większość analizowanych substancji drobnocząsteczkowych (leków) obecnych jest w postaci związanej z białkami (głównie z albuminami i z α_1-glikoproteinami [66-67]), co wymaga wdrożenia specjalnych metod rozbicia takich połączeń w celu ukazania całkowitego stężenia oznaczanej substancji. Przykładem metody rozbijania połączeń lek-białko jest denaturacja białek z wykorzystaniem rozpuszczalników organicznych, występująca w trakcie przeprowadzania najpopularniejszej techniki ekstrakcji analitów z próbek – ekstrakcji ciecz-ciecz (LLE, ang. *liquid-liquid extraction*) [63]. Szczególnym przypadkiem są natomiast sytuacje, w których badaczowi zależy wyłącznie na określeniu stężenia frakcji wolnej leku, która to jest postacią aktywną farmakologicznie. Ma to miejsce np. w przypadku monitorowania farmakoterapii oraz medycyny spersonalizowanej [68]. Wówczas z powodzeniem zastosowanie znajdują m.in. metody mikroekstrakcyjne [68-69].

W przypadku próbek moczu, badane substancje występują najczęściej w postaci metabolitów. Mogą to być zarówno metabolity I fazy (zmienione chemicznie [70-71]), jak również i metabolity II fazy (sprzężone z innymi cząsteczkami lub grupami funkcyjnymi ułatwiającymi ich wydalanie z organizmu poprzez zmniejszenie hydrofobowości, a tym samym zwiększenie rozpuszczalności w wodzie będącej głównym składnikiem moczu [72]). Z obecnością analitów w postaci sprzężonych metabolitów II fazy wiąże się drobna niedogodność w postaci konieczności dodawania

enzymów rozdzielających takie połączenia, w celu określenia całkowitego stężenia substancji w moczu. Przykładem jest WADA wymagająca dodawania β-glukuronidazy do każdej próbki moczu analizowanej w aspekcie wzajemnych proporcji endogennych sterydów anabolicznych (tzw. profil sterydowy) [73]. Alternatywą jest sumowanie stężeń niezmetabolizowanego związku i wszystkich jego metabolitów [74], co wymaga dodatkowych czynności i może przyczyniać się do powiększenia niepewności pomiaru. Znacznie istotniejsza jest jednak konieczność znajomości metabolizmu danego związku chemicznego – warunek często niemożliwy do spełnienia w przypadku nowo powstałych substancji dopingujących oraz środków odurzających.

Przytoczona charakterystyka krwi i moczu jako materiału do oznaczania substancji zakazanych oraz wynikające z niej niedogodności, a także dodatkowe problemy dotyczące pobierania próbek wymienionych materiałów (opisane w rozdziale 6), są niewątpliwie jedną z głównych motywacji do wdrożenia tzw. *alternatywnych* materiałów do badań [75]. Do grupy tej zaliczane są m.in. pot, ślina, włosy oraz wydychane powietrze [65,75]. W dziedzinie kryminalistyki sporym uznaniem cieszy się analiza próbek włosów i paznokci, których główną zaletą jest kumulacja analitów umożliwiająca określenie historii stosowania badanej substancji na przestrzeni tygodni, a nawet lat [76-78]. Istotnymi problemami, są jednak niskie stężenie analitów, a przede wszystkim brak możliwości dokładnego określenia czasu spożycia substancji oraz brak bezpośredniej korelacji między stężeniem substancji a zastosowaną dawką [77]. Szczególnie dwie ostatnie cechy praktycznie wykluczają potencjalne zastosowanie włosów i paznokci w badaniach antydopingowych, gdzie stosowanie wielu substancji zabronione jest wyłącznie w trakcie trwania zawodów (klasy S6-S9), a w przypadku innych niedozwolone jest wyłącznie powyżej określonej dawki [52].

Obiecującym materiałem do badań antydopingowych jest natomiast ślina [79]. Jest ona zarazem materiałem powszechnie stosowanym do kontrolowania trzeźwości kierowców oraz w miejscu pracy [80]. Ogólnodostępne są również domowe narkotesty wykorzystujące próbki śliny do wykrywania najpopularniejszych środków odurzających. U źródeł popularności śliny leży jej charakterystyka. Jest ona wydzielana do jamy ustnej jako bezpośredni przesącz osocza, w związku z czym zawiera praktycznie wszystkie obecne w nim substancje drobnocząsteczkowe, a także peptydy i białka. Istnieje kilka różnych mechanizmów, za pomocą których dochodzi do przenikania leków do śliny. Najważniejszym z nich jest dyfuzja prosta (transport pasywny). Istotne są również ultrafiltracja przez pory (dotycząca głównie małych

(<300 Da) i polarnych struktur) oraz transport aktywny z wykorzystaniem przenośników w postaci białek błonowych (dotyczący większych (>1000 Da) struktur takich jak peptydy i białka) [81]. Wśród zalet stosowania śliny do wykrywania substancji zakazanych wymienić można obecność analitów w postaci niezmetabolizowanej, a także ich wyższe stężenia (względem moczu i osocza [82]). Ostatnia cecha wynika wprost ze specyfiki zjawiska dyfuzji biernej, któremu podlegają cząsteczki niezjonizowane, zgodnie z gradientem stężeń. Substancje zasadowe obecne w osoczu (większość leków), po przeniknięciu do śliny ulegają w niej jonizacji na skutek znalezienia się w kwasowym środowisku (fizjologiczne pH osocza znajduje się zakresie 7,38-7,42 [83], natomiast pH śliny wynosi zazwyczaj 6,7 [84]). Po tym jak ulegną jonizacji, cząsteczki nie biorą już udziału w ustalaniu różnicy stężeń formy niezjonizowanej substancji (warunkującego dyfuzję bierną). W związku z czym, do śliny przenikają kolejne cząsteczki leku ulegające w niej jonizacji, a cały proces powtarza się [79]. Wypadkowe stężenie analitu jest zatem wyższe w ślinie. Natomiast okres występowania analitów w ślinie jest zazwyczaj nieznacznie krótszy niż ma to miejsce w przypadku osocza i moczu [82]. Zdecydowanie szybciej trafiają one jednak z osocza do śliny niż do moczu. Najważniejszym ograniczeniem w stosowaniu śliny do kontroli antydopingowej wydaje się jednak brak egzogennego markera pozwalającego na określenie stężenia próbki (a tym samym np. weryfikacji czy nie została ona rozcieńczona w celu ukrycia faktu stosowania dopingu). Przykładowo w tym celu, dla próbek moczu WADA zakłada oznaczanie stężenia kreatyniny [73].

Wspólną cechą dla większości najpopularniejszych matryc biologicznych do badań laboratoryjnych, poza ich złożonością, jest także podatność niektórych z obecnych w nich analitów na czynniki zewnętrzne. Do najważniejszych z nich należą temperatura przechowywania próbki, ekspozycja na światło czy kontakt z oksydantami (powietrze) [85]. Istotna jest również wrażliwość substancji badanych na rozkład enzymatyczny [85], działalność drobnoustrojów oraz utratę analitów w wyniku kontaktu z powierzchnią urządzeń do pobierania próbek [86], co szczegółowo omówione zostanie w rozdziale 6.

6. Metody pobierania materiałów biologicznych do wykrywania niedozwolonych substancji ze szczególnym uwzględnieniem próbek śliny

Wśród konwencjonalnych materiałów biologicznych do badań antydopingowych najczęściej stosowany jest mocz [41-50]. Próbki moczu pobiera się do specjalnych polimerowych pojemników z nakrętką. Jednak sam proces wiąże się przede wszystkim z ryzykiem podmiany próbki [79]. Jest to szczególnie istotne w przypadku badań antydopingowych, ale także w przypadku narkotestów wykonywanych w miejscu pracy. W związku ze wspomnianym ryzykiem, WADA wymaga obecności specjalnej osoby nadzorującej oddawanie próbki moczu podczas kontroli [62]. Uzyskane w ten sposób ograniczenie sposobności do podmiany próbki, wiąże się jednocześnie z naruszeniem prywatności i dyskomfortem dla badanych sportowców – również tych uczciwych. W przypadku kontroli wykonywanej po wysiłku fizycznym (np. po treningu, czy po zawodach sportowych), problem może stanowić również pobranie stosownej objętości próbki do badań – na skutek występowania stanu odwodnienia organizmu.

Próbki krwi pobierane są przy wykorzystaniu specjalnych probówek próżniowych. Wytworzona w ten sposób różnica ciśnień zapewnia pasywny przepływ krwi do probówki [87]. Zazwyczaj do celów analizy substancji drobnocząsteczkowych stosuje się polimerowe probówki z czerwoną nakrętką – zawierające aktywator krzepnięcia (w postaci naniesionej na ścianki cienkiej warstwy zmikronizowanych cząsteczek krzemionki), za to pozbawione antykoagulantów, konserwantów oraz żelu oddzielającego elementy morfotyczne krwi od osocza [87]. Z technicznego punktu widzenia do badań wykorzystywana jest zatem najczęściej surowica, czyli krew pozbawiona skrzepu powstałego na skutek aktywacji fibrynogenu (zawierającego w sobie również erytrocyty) [88]. Samo pobieranie próbek krwi jest natomiast zabiegiem inwazyjnym – wiążącym się z naruszeniem ciągłości tkankowej osoby badanej [79]. Dosyć rozpowszechnionym zjawiskiem jest także fobiczny lęk przed igłami (łac. *trypanophobia*). Szacuje się, że dolegliwość ta dotyczyć może nawet 10% całego społeczeństwa [89].

Wymienione powyżej czynniki oraz te dotyczące samej natury materiałów stosowanych do badań (omówione uprzednio na łamach rozdziału 5), skłaniają do poszukiwania mniej uciążliwych rodzajów próbek biologicznych. Jak również zostało

wspomniane, takim właśnie obiecującym materiałem do badań jest ślina. Ponadto, oddawanie próbek śliny jest preferowane przez same osoby poddawane badaniom (względem oddawania próbek moczu lub krwi) [90-91]. W najprostszym wariancie, ślina może być pobrana do polimerowej probówki poprzez pasywne sączenie (plucie). Szeroko rozpowszechnione są jednak specjalne rozwiązania do pobierania śliny, składające się najczęściej z elementów absorpcyjnych (wacików) oraz probówek.

Jednak w trakcie wcześniejszych eksperymentów (przeprowadzonych podczas realizacji pracy magisterskiej autora) zauważono istotne straty niektórych substancji badanych po pobraniu próbek z wykorzystaniem bawełnianych wacików. W celu określenia optymalnej metody pobierania próbek śliny oraz dokładniejszego poznania zjawiska utraty analitów podczas pobierania próbek, w trakcie realizacji rozprawy doktorskiej dokonano kompleksowej analizy wszystkich dostępnych komercyjnie (w momencie przeprowadzania eksperymentu) urządzeń zawierających waciki chłonne. Przebadano również zwyczajne polimerowe probówki laboratoryjne o różnych pojemnościach. Wyniki tej analizy zostały opublikowane w poniższej publikacji:

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Evaluation of swabs from 15 commercially available oral fluid sample collection devices for the analysis of commonly abused substances: doping agents and drugs of abuse†

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Oral fluid testing is steadily building its position as a valuable complement or alternative to plasma and urine analyses in everyday laboratory practice. However, the great significance of the sample collection process in the attainment of representative results is not always paralleled by the attention given to its informed selection. Few evaluations of commercially available sample collection devices have been published until now, and the current work intends to fill this gap by presenting an evaluation of swabs from 15 different devices for the analysis of 49 popular drugs. Swabs, derived from sample collection devices, were used to collect a drug-fortified mixture. Then, swab-retrieved samples were subjected to instrumental analysis with the high-performance liquid chromatography coupled with tandem mass spectrometry method. Results within the 80–120% range were considered to have no significant impact on analyte concentration (thus satisfactory) and were observed in 44.1% of all results. Out of the 15 evaluated swabs, 7 provided results in the aforementioned range for more than half of the substances under study. The possibility of matrix effects originating from swab materials was also investigated. The selection of an appropriate oral fluid sample collection method plays a critical role in the success of the analytical procedure, a fact that is well-illustrated by the tremendous differences between analyte concentrations observed in this research. Perhaps, the tedious labour of improving sample preparation and analysis methods already in-use could be spared if only greater emphasis were to be put on the improvement and better selection of suitable solutions for oral fluid collection.

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Introduction

Oral fluid is a direct filtrate of blood, well resembling the small molecule content of its native plasma. Drug transfer from plasma and its subsequent oral fluid concentration depend on multiple factors. Some of the key determinants of this process include the chemistry of the drug (molecular weight, acidity, and lipophilicity), its circulating form (electric charge, extent of plasma protein binding), and ratio of pH values between both matrices. Small (<500 Da), basic, lipophilic, neutral, unbound, and unmetabolized drugs are generally expected to be present in oral fluid.[1–4] Basic substances are especially biased towards higher oral fluid concentrations due to the so-called ion-trapping phenomenon. Such basic drugs are present in plasma (pH = 7.35–7.45) as neutral molecules, but ionize after diffusion to more acidic oral fluid (pH = 6.2–7.4), subsequently becoming no longer involved in the equilibrium-driven process of passive diffusion, and thus remaining trapped in oral fluid at increasing total concentrations.[2]

Oral fluid is an already well-established bioanalytical specimen alternative or complementary to plasma or urine in the fields of forensics and therapeutic drug monitoring (TDM), as well as for examination procedures related to driving under the influence of drugs and workplace testing.[5–9] The use of oral fluid in doping control is also an increasing trend.[3,10,11]

The provision and collection of oral fluid specimens is convenient, non-invasive, and preferred over blood or urine donation according to the results of several survey-based studies.[12–14] Oral fluid samples can be collected by passive drool (or simply spitting) into plastic tubes/vials, *via* rinsing with a special extraction solution (as in the saliva collection system device from Greiner Bio-One International), or collected with absorptive materials. The use of commercially available devices made of absorptive materials is more popular

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with donors and their supervisors, being praised as more hygienic and easier to handle.[6] However, passive drool remains unparalleled in terms of sample quality due to it carrying the lowest number of factors negatively affecting the collected oral fluid and therefore providing the truest results among the described collection methods.

However, despite possible drawbacks such as analyte loss due to unspecific binding to collection swabs, swab handle and transport tube materials (this issue is especially important for Δ^9-tetrahydrocannabinol (THC) analysis[15–17]), or contaminants originating from collection device elements, the use of absorptive devices is currently predominant in oral fluid sample collection. Rarely are such commercial products validated for specific analyte collection (usually an endogenous hormone – *e.g.* Salivette® Cortisol from Sarstedt), and reports on device performance for exogenous substance collection are scarce and limited only to the most popular drugs.[18,19] Such a situation hinders the development of oral fluid testing in general. To address this uncertainty, several evaluations of commercial products have been already published in scientific literature, but these are unfortunately limited only to a single or few devices at a time.[17,20–26] Research by Langel *et al.*[27] (comparing 9 different devices) stands out as perhaps the most comprehensive work to date. Regrettably, more than a decade after its publication in 2008, only 5 out of 9 tested devices still remain available on the market, decreasing its usefulness for contemporary researchers. Therefore, the herein presented evaluation of swabs from 15 various devices for the analysis of 49 commonly abused substances could be of substantial aid in the selection of an appropriate collection device for a given drug or class of drugs and may help avoid numerous difficulties at the very first step of the analytical process – sample collection. This may play a significant role in the successful analysis of potent drugs present in biological matrices only at trace amounts.

Experimental section

Substances analysed

A total of 49 commonly abused substances (drugs of abuse and doping agents) were selected for a comparison of recoveries from swabs based on the latest report of The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA),[28] and laboratory testing figures of The World Anti-Doping Agency (WADA).[29] Among the chosen compounds, 21 are considered recreational drugs and 43 are substances banned in sports (from all classes listed in WADA's Prohibited List[30]) – see Table A1 in ESI A† for a detailed list. The selected substances include 6 most abused drugs in Europe: cannabis (as THC and its long-lasting metabolite 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH)), cocaine, heroin, amphetamine, methamphetamine, and ecstasy (3,4-methylenedioxymethamphetamine, MDMA).[28] Also, 13 of the 15 most frequently detected doping agents were analysed: clenbuterol (with 320 reported offences in 2018 according to the latest annual report by the WADA), stanozolol (235), furosemide (172), nandrolone (150), THC-COOH (141), methandienone (131), hydrochlorothiazide (127), meldonium (111), amphetamine (95), and methylphenidate (91), as well as boldenone, cocaine, and terbutaline (87 offences each).[29]

Sample collection devices

A total of 15 different swabs from oral fluid collection devices from 10 different manufacturers were compared during experiments (see Fig. 1). Their characteristics based on available product information (instructions and leaflets) are summarized in Table 1. It should be noted that this work evaluated only the swabs, and as such it may be less relevant in the case of 5 devices, where the processed sample should be placed into an extraction fluid (buffer and/or preservative solution), according to the recommended protocol, if the device is used as recommended. These particular devices are the Oasis Diagnostics® Accu-SAL™, OraSure Technologies Intercept® i2™, Thermo Scientific™ Oral-Eze™, StatSure™ Saliva Sampler™, and Immunalysis™ Quantisal®.

HPLC-MS/MS analysis

All samples were analysed on a Shimadzu LCMS-8060 triple quadrupole system. Samples (8 μL injection volume) were separated on an Agilent InfinityLab Poroshell 120 EC-C18 column

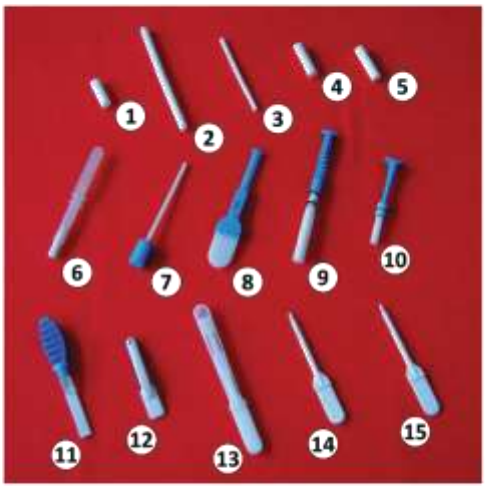

Fig. 1 Oral fluid collection devices. Devices presented: 1 – Salimetrics® SalivaBio Oral Swab; 2 – Porex® Saliva Collection Swab (*type I*); 3 – Porex® Saliva Collection Swab (*type II*); 4 – Sarstedt Salivette®; 5 – Sarstedt Salivette® Cortisol; 6 – Dräger DCD™ 5000; 7 – Malvern Medical Developments Oracol; 8 – Oasis Diagnostics® Versi-SAL®; 9 – Oasis Diagnostics® Super-SAL™; 10 – Oasis Diagnostics® Micro-SAL™; 11 – Oasis Diagnostics® Accu-SAL™; 12 – OraSure Technologies Intercept® i2™; 13 – Thermo Scientific™ Oral-Eze™; 14 – StatSure™ Saliva Sampler™; 15 – Immunalysis™ Quantisal®.

Table 1 Characteristics of tested oral fluid collection devices

Design	Collection device	Manufacturer	Specification		Collection time	Collected volume	Volume adequacy indicator	Recommended sample extraction	Recommended sample handling
			Absorptive material(s)	Collection method					
Swab	SalivaBio Oral Swab (SOS)	Salimetrics®	Synthetic fibre	Keep under tongue	1–2 min (max 5 min)	Up to 2 mL (0.2–1 mL recovered)	No	Centrifugation (1500*g*, 15 min)	Storage tube
	Saliva Collection Swab (*type I*) (8 × 125 min)	Porex® Filtration Group	Synthetic fibre (LDPE/LDPP)	N/A	N/A	N/A	No	N/A	N/A
	Saliva Collection Swab (*type II*) (4.5 × 90 min)	Porex® Filtration Group	Synthetic fibre (LDPE/LDPP)	N/A	N/A	N/A	No	N/A	N/A
	Salivette®	Sarstedt	Cotton fibre	Chewing	1 min	N/A	No	Centrifugation (1000*g*, 2 min, 20 °C)	Storage tube
	Salivette® Cortisol	Sarstedt	Synthetic fibre (PP/LDPE)	Chewing	1 min	0.8–1.4 mL (recovered)	No	Centrifugation (1000*g*, 2 min, 20 °C)	Storage tube
Swab on handle	DCDP™ 5000	Dräger Safety	N/A	Keep between gums and cheek	Up to 4 min	0.4 mL	Yes	N/A	Storage tube
	Oracol	Malvern Medical Developments	Synthetic sponge	Rub gums	1 min	Up to 1 mL	No	Centrifugation	Storage tube
	Versi-SAL®	Oasis Diagnostics® Corporation	Non-cellulosic and non-cotton material	Keep in oral fluid pooled under tongue	1–3 min (max 15 min)	1.2–1.4 mL (0.7–1 mL)	Yes	Dedicated extractor	2 mL Eppendorf tube
	Super-SAL™	Oasis Diagnostics® Corporation	Non-cotton material	Keep in oral fluid pooled under tongue	1–5 min (max 15 min)	1 mL (0.75–1.2 mL)	Yes	Dedicated extractor	2 mL Eppendorf tube
	Micro-SAL™ for children	Oasis Diagnostics® Corporation	N/A	Keep in oral fluid pooled under tongue	N/A	0.5 mL	No	Dedicated extractor	2 mL Eppendorf tube
	Accu-SAL™	Oasis Diagnostics® Corporation	N/A	Keep in oral fluid pooled under tongue	1–3 min (max 10 min)	1 mL (0.5–1.1 mL)	Yes	Centrifugation	Storage tube with extraction buffer
	Intercept® 12™	OraSure Technologies	Cotton fibre	Keep under tongue	3–4 min (max 15 min)	1 mL	Yes	Centrifugation (3000 rpm or 600–800*g*, 5 min)	Storage tube with preservative solution
	Oral-Eze™	Thermo Scientific™	Cotton fibre	Keep between gums and cheek	3–5 min (max 10 min)	1 mL	Yes	Dedicated extractor	Storage tube with extraction buffer and preservative (sodium azide)
	Saliva Sampler™	StatSure™ Diagnostic Systems	Cellulose fibre	Keep in oral fluid pooled under tongue	2–15 min (max 15 min)	1 mL	Yes	N/A	Storage tube with extraction buffer and preservative (sodium azide)
	Quantisal®	Immunalysis™ Corporation	Cellulose fibre	Keep under tongue	2–10 min	1 mL (0.907–1.079 mL)	Yes	N/A	Storage tube with extraction buffer and preservative (non-azide)

LDPE = low-density polyethylene; LDPP = low-density polypropylene; PP = polypropylene.

(3 × 100 mm, 2.7 μm) with a matching Agilent InfinityLab Poroshell 120 EC-C18 guard column (3 × 5 mm, 2.7 μm), using gradient elution in reversed-phase mode. Column oven temperature was set at 25.0 °C. Phase A consisted of LC-MS grade water (LiChrosolv®, Merck) with 0.1% formic acid (Optima™ LC/MS, Fisher Chemical), and phase B consisted of LC-MS grade acetonitrile (CHROMASOLV™, Honeywell) with 0.1% formic acid (Optima™ LC/MS, Fisher Chemical). Gradient starting conditions were 10% phase B (maintained for 0.5 min), followed by a linear increase of phase B concentration to 100% (for 25.5 min), hold at 100% phase B (for 3 min) and column re-equilibration with 10% phase B (6 min). Total running time was 35 min per sample. The total flow rate was 300 μL min^{-1}. Investigated analytes and their respective monitored *m/z* ratios are listed in Table A2 in ESI A.†

Experimental design

In order to obtain as reliable results as possible, LC-MS grade water was used to mimic oral fluid in a preparation of testing solution spiked with the analytes. By doing so, widely known factors that would have negative impact on data quality, if oral fluid was to be tested instead of ultrapure water, were omitted. For example, irreproducibility of oral fluid could be an outcome of circadian secretion rhythms of endogenous hormones, separation of oral fluid emulsion (formation of layers with different densities and protein contents[21]), bacterial and enzymatic activity,[21] and sample contamination with the residue of food or mouth hygiene products among other reasons. However, such design of the experiment raises concerns over the relevance of its results in accordance with the actual oral fluid testing. To address these concerns and reassure that the results are reliable, a follow-up experiment involving oral fluid samples was performed to compare recovery values recorded for both matrices, the results of which are presented and discussed in this work.

As a means to enable substance recovery comparisons between various devices under evaluation, unified proceedings were always applied, which sometimes necessitated alterations of the protocols recommended by manufacturers of said devices. Also, only the absorptive parts (swabs) of the devices were used for sampling, without attached handles or dedicated extractors that accompany certain devices for collection from living donors.

Drug recovery

Drug recoveries from oral fluid collection device swabs were tested by inserting swabs into a spiked mixture with the above specified analytes at individual concentrations of 10 ng mL^{-1}. The first step was carried out in 15 mL Falcon type plastic tubes. Swabs were fully immersed in the mixture for 60 min to allow the analytes to bind to the absorption materials. Next, each swab was removed with single-use nitrile gloves and squeezed with a disposable needle-less plastic syringe (5 or 10 mL capacity, depending on swab size) into a 2 mL Eppendorf plastic tube. However due to the stiff structure of Dräger DCD™ 5000 swabs it was not possible to squeeze them with a syringe barrel, and instead centrifugation in special tubes with pre-cut holes in their bottom was incorporated. Centrifuged samples were then transferred to the 2 mL Eppendorf tubes. All of the 2 mL tubes were centrifuged (1500*g*, 3 min, 4 °C) to force sedimentation of particulate residues originating from the swab (the formation of which was already reported in the literature[23]). The supernatant was then transferred into silanized 2 mL HPLC vials with PTFE-bonded/silanized caps. Each swab type was tested in quadruplicate. Drug recoveries were calculated as mean values relative to the mean values (n = 4) of the reference sample (portion of the drug-spiked mixture, measured twice before and twice after the samples of each swab type).

Non-specific binding of drugs to laboratory plastics

While prepared samples were kept in silanized vials in order to reduce drug losses related to non-specific binding, the experimental proceedings required the use of multiple laboratory plastics (see the *Drug recovery* paragraph). Given this, non-specific binding of the analytes to 15 mL Falcon tubes, 5 or 10 mL syringes, and 2 mL Eppendorf tubes was assessed by incubating the drug mixture inside plastics for 60 min, and then measuring such samples against reference samples. Each experiment was repeated 4 times.

Matrix effect assessment

In order to test the hypothesis that some of the tested swabs from oral fluid collection devices could contaminate samples, which could in turn affect MS/MS detection, an additional experiment was performed. Drug-free water was collected with each swab and subsequently recovered from swabs using the same protocol described in the *Drug recovery* paragraph. Samples of matrices retrieved from the sample collection device swabs, as well as the neat matrix, were then spiked with the drug mixture (each substance present in the concentration of 10 ng mL^{-1}) and subjected to instrumental analysis. Results were calculated as signals measured from the spiked matrix collected with swabs relative to signals from the spiked neat matrix. Each type of swab was tested in triplicate.

Eligibility of testing recovery from swabs with ultrapure water

Oral fluid (negative for all the analytes) provided by two volunteers was spiked with 43 drugs at a concentration of 10 ng mL^{-1} each. Informed consent was obtained from the participants prior to sampling, and all experiments were performed in compliance with applicable policies and guidelines (permission to conduct experiments with oral fluid was issued by the Bioethics Committee of Collegium Medicum in Bydgoszcz at Nicolaus Copernicus University in Toruń, permission number KB 651/2018). Six drugs used for previous experiments were excluded from this test, due to the insufficient recovery of the extraction method (hydrochlorothiazide and psilocybin), or intense matrix effect causing the previously tested recovery values to exceed 150% (bisoprolol, meldonium, methylphenidate, and phencyclidine).

Drug-fortified oral fluid samples were collected with Sarstedt Salivette® swabs and handled with the same protocol as described in the *Drug recovery* paragraph. Prior to instrumental analysis, the analytes were extracted from oral fluid recovered from the swabs with the use of C18 SPME LC fiber probes (Supelco®). The following 6-step extraction protocol was used: I – preconditioning #1 (1.5 mL methanol/water (90/10, v/v), 60 min, 850 rpm agitation); II – preconditioning #2 (1.5 mL methanol/water (10/90, v/v), 60 min, 850 rpm agitation); III – rinse #1 (1.5 mL of water, 2 s, no agitation); IV – extraction (700 μL of oral fluid, 60 min, 850 rpm agitation); V – rinse #2 (1.5 mL of water, 2 s, no agitation); VI – desorption (150 μL acetonitrile/water/formic acid (80/19.9/0.1, v/v), 60 min, 850 rpm agitation). The injection volume was 1.2 μL (4 × 0.3 μL) in *POISe* injection. Swabs were tested in triplicate, and the recovery values were compared with the ones recorded after the use of ultrapure water as the matrix.

Results and discussion

The detection method was linear in the anticipated concentration range of 1–10 ng mL^{-1}. Coefficient of determination values from 7-point calibration curves for all substances exceeded $R^2 = 0.99$, both without and with 1/a or $1/a^2$ weighting applied.

The stability of the tested drugs during the 60 min sampling period at room temperature and during the period of time in which samples were placed in a refrigerated autosampler (maintained at 4 °C) until LC-MS/MS analysis should not influence the results, as all reference samples were stored under exactly the same conditions throughout the entire process.

Drug recovery

No significant drug losses (recovery values in the 80–120% range) for the majority of the tested substances were observed for 7 out of the 15 evaluated swabs (full results are presented in Table B2 in ESI B†). The greatest number of recovery values in such a range (29 out of 49 drugs) was achieved with 3 different swabs: Porex® Saliva Collection Swab (*type I*), Porex® Saliva Collection Swab (*type II*), and Dräger DCD™ 5000. The majority of results were also satisfactory for the Salimetrics® SalivaBio Oral Swab (28/49), StatSure™ Saliva Sampler™ (27/49), Immunalysis™ Quantisal® and Oasis Diagnostics® Accu-SAL™ (both 25/49). Significant analyte loss (recoveries below 80%) for more than half of the tested drugs was observed with 4 swabs. The lowest numbers of undesired results (below 80%) were observed with Dräger DCD™ 5000 (only 8 out of 49) and Porex® Saliva Collection Swab (*type II*) (9/49).

In terms of the drugs being analysed, anastrozole, betamethasone, nandrolone, and prednisone proved to be the least affected by the collection process, with recoveries in the 80–120% range for 14 out of the 15 tested swabs. On the other hand, compounds such as LSD, methadone, nebivolol, stanozolol, and THC could not be recovered by any of the tested swabs in the 80–120% range. The highest measured values of these drugs were achieved with OraSure Technologies Intercept® i2™ for LSD (69.9%), Oasis Diagnostics® Accu-SAL™ for methadone (77.7%), Oasis Diagnostics® Versi-SAL® for nebivolol (59.9%) and stanozolol (77.6%), and StatSure™ Saliva Sampler™ for THC (17.3%).

Non-specific binding of drugs to laboratory plastics

The majority of the tested substances were not affected by non-specific binding to laboratory plastics utilized in this research (detailed results for this experiment are presented in Table B1 in ESI B†). Cases of recoveries below 80% of reference sample included phencyclidine, canrenone, and stanozolol loss on the 15 mL Falcon tubes (76.1%, 62.1%, and 61.8% recoveries, respectively). With THC-COOH, substance loss was observed on 3 of the 4 tested plastics (22.4% recovery from 15 mL Falcon tubes, 40.3% from 5 mL syringes, and 47.7% from 10 mL syringes). The greatest decline in concentration was measured for THC (20.5% recovery from 15 mL Falcon tubes, 23.9% from 5 mL syringes, 24.5% from 10 mL syringes, and 41.3% from 2 mL Eppendorf tubes).

All of the affected substances have hydrophobic structures, with log *P* values of 2.7 for canrenone, 3.6 for phencyclidine, 4.5 for stanozolol, 6.3 for THC-COOH, and 7.0 for THC (all data according to the PubChem database[12]). Non-specific binding of non-polar analytes to the surfaces of various polymers has been previously reported in the literature (most notably for THC).[20,27–33]

Matrix effect

While matrix effects are mostly dependent on the selected LC-MS conditions, differences in observed matrix effects between samples collected with different sample collection swabs, but measured by the same analytical method, indicate that the presence of undesirable impurities may have played a role in the generation of matrix effects, resulting in signal enhancement or suppression in MS analysis.

The majority of the drug recovery results described in the *Drug recovery* paragraph (393 out of 735 (53.5%)) does not seem to be significantly biased, as measured values of corresponding matrix effects were within the 80–120% range (see Table B3 in ESI B†).

Among the tested substances, alprazolam and anastrozole were the least prone to matrix effects, as results obtained with all evaluated swabs fell within the aforementioned range. For betamethasone, nandrolone, and prednisone, only 1 adverse result was recorded. In contrast, results for phencyclidine were only unbiased with Dräger DCD™ 5000 (97.8%). Only 2 out of the 15 tested swabs yielded matrix effect values within the desired range (80–120%) for each of the following compounds: clenbuterol (Porex® Saliva Collection Swab (*type II*) – 86.5% and Sarstedt Salivette® Cortisol – 89.5%), cocaine (Sarstedt Salivette® – 106.9% and Oasis Diagnostics® Micro-SAL™ – 96.2%), methadone (Malvern Medical Developments Oracol – 110.8% and Oasis Diagnostics® Micro-SAL™ – 117.4%), and

metoprolol (Porex® Saliva Collection Swab (*type II*) – 92.9% and Sarstedt Salivette® Cortisol – 99.0%).

A total of 160 out of 735 results were below 80% – indicating signal suppression. Results below 80% were measured with 8 swabs for fenoterol, meldonium, propranolol, and zolpidem, and with as much as 9 swabs for LSD. No cases of signal suppression were present with the Oasis Diagnostics® Accu-SAL™. Only 1 such case occurred with the OraSure Technologies Intercept® i2™, Immunalysis™ Quantisal® and StatSure™ Saliva Sampler™.

On the other hand, 182 obtained results were over 120% – suggesting signal enhancement. Elevated matrix effect values were registered with 10 of the 15 tested swabs for methadone and methylphenidate, 11 swabs for buprenorphine, 12 swabs for atenolol, and 13 swabs for phencyclidine. Only 2 such cases occurred with the Oasis Diagnostics® Micro-SAL™ and Malvern Medical Developments Oracol.

Nevertheless, the majority of results were within 80–120% for 8 out of 15 swabs. The greatest number of unbiased results was obtained with the Dräger DCD™ 5000 (for 33 out of 49 analysed drugs). The remaining 7 swabs are Porex® Saliva Collection Swab (*type II*) (32/49), Porex® Saliva Collection Swab (*type I*) (31/49), Salimetrics® SalivaBio Oral Swab and Thermo Scientific™ Oral-Eze™ (both 30/49), Sarstedt Salivette® Cortisol (29/49), Immunalysis™ Quantisal® (27/49), and StatSure™ Saliva Sampler™ (26/49).

Assessment of matrix effect influence on the results

Establishment of matrix effect values from sample collection swabs enables an assessment of corrected recovery values for each substance. For this purpose, following the methodology previously proposed by Matuszewski *et al.*,[14] the equation corrected drug recovery [%] = (drug recovery [%]/matrix effect [%]) × 100% was used to compute corrected recovery values.

While such approach results in only putative results, it is justified by a nearly 2-fold decline in the number of suspiciously high recovery values – above 120% (from 127 to 76 cases) and a 6-fold decline in especially peculiar cases of more than doubled recovery values (from 30 to just 5 cases). Among the 735 computed values, 5 cases contributed to less than 0.7% frequency within all corrected results. Corrected recovery values for each drug and each sample collection swab are presented in Table B4 in ESI B.†

With respect to drug recovery results, after applying correction for matrix effects, 13 out of 15 swabs (instead of 7/15) provided results within the 80–120% range for more than half of the evaluated substances, although the greatest numbers of such results (36/49) were still achieved with the Porex® Saliva Collection Swab (*type II*) and Dräger DCD™ 5000 (previously, 29/49 before correction).

It is also worth mentioning that matrix effects exceeding 200% after collection of samples with swab-based devices for some of the substances analysed in this research (*e.g.* buprenorphine, LSD, methadone, THC, and zolpidem) have already been reported in the literature, with matrix effect values as high as 3358% for THC (before IS correction).[35]

Further discussion

Recovery results from this research are mostly in agreement (within ±20% of given value) with values previously reported in scientific literature. In total, 64 eligible results for 4 various swabs were found in 10 unique reports. All the corresponding results are listed in Table 2. It should be emphasized that a direct comparison is only possible for Malvern Medical Developments Oracol and Sarstedt Salivette®, while for the Immunalysis™ Quantisal® and StatSure™ Saliva Sampler™ the substance recovery values could have been altered by this work not using dedicated extraction buffers. It is likely an explanation for poorer recovery of some analytes (like THC), and for weaker correlation between the results (especially for the StatSure™ Saliva Sampler™).

For the Malvern Medical Developments Oracol, the attained results are in compliance for 4 out of 6 common analytes after applying correction for matrix effects (and also for 4 out of 6 before that correction).[27] The Pearson's correlation coefficient value is $r = 0.66$ ($r = 0.73$ before correction), $n = 5$.

For the Immunalysis™ Quantisal®, results are compliant for 8 out of 9 substances (4/9 before correction).[17,20,21,26,27,36] The correlation coefficient value is $r = 0.59$ ($r = 0.43$ before correction), $n = 20$.

With the StatSure™ Saliva Sampler™, 4 out 6 drugs have similar recovery values (3/6 before correction).[26,27] The Pearson's coefficient value is $r = 0.22$ ($r = -0.19$ before correction), $n = 6$.

The Sarstedt Salivette®, as the most thoroughly studied device, enabled a comparison of results for 12 drugs. Results were in agreement for 9 of them (6/12 before correction).[2,12,27,37,38] The correlation coefficient is $r = 0.56$ ($r = 0.54$ before correction), $n = 21$.

Influence of non-specific binding on the results

Although non-specific binding of some especially non-polar analytes to surfaces of polymers used in this research was noted and described in the *Non-specific binding of drugs to laboratory plastics* paragraph, the issue affected only a marginal fraction of results and all of the 15 evaluated swabs were equally burdened by this phenomenon. Therefore, the recovery results were not corrected for its influence. However, this could partially explain lesser recovery values than previously reported for non-polar substances (particularly THC and THC-COOH). For example, if the THC recoveries would be corrected for the influence of the non-specific binding using an established value of 79.5% substance loss on the 15 mL Falcon tubes (as the analytes had the longest exposure time (1 h) with this particular type of plastic), the recovery values would increase from the 0.4–17.3% range (with a mean value of 6.9% and a median of 7.4%) to the 1.8–84.4% range (with a mean value of 33.8% and a median of 36.3%). Nonetheless, the differences between the swabs would still remain exactly proportional to the ones prior to such correction.

Eligibility of testing recovery from swabs with ultrapure water

The outcome of the follow-up experiment seems to reassure the relevance of results achieved with ultrapure water in

Table 2 Comparison of substance recovery values [%] from oral fluid collection devices established in this research with corresponding data previously reported in scientific literature

Collection device	Substance	Substance recovery	Recovery corrected for matrix effect	Previously reported recovery	Source
Malvern Medical Developments Oracol	Alprazolam	22.3	24.3	19.2	27
	Amphetamine	65.4	73.3	69.1	27
	Cocaine	66.2	94.2	35.1	27
	MDMA	31.5	54.1	52.0	27
	Morphine	83.7	103.0	81.5	27
	THC	0.4	0.3	<12.5	27
Immunalysis™ Quantisal®	Alprazolam	91.3	96.9	111.0	27
	Amphetamine	98.1	90.6	98	26
				94.2–96.9	22
				89.7	27
				≥80	36
	Cocaine	230.7	130.3	97	26
				91.2–95.7	22
				81.7	27
				≥80	36
	Ketamine	101.3	98.8	≥80	36
	MDMA	135.5	91.2	82.3	27
				<80	36
	Methamphetamine	135.3	96.5	93.1–103.8	22
				100	26
				≥80	36
	Morphine	112.5	90.6	91.9–98.7	22
				98	26
				82.7	27
				≥80	36
	THC	9.9	6.7	94	26
				81.3–91.4	22
				80	20
				75–85	17
				48.7–67.5	36
				55.8	27
	THC-COOH	69.2	109.8	93	26
StatSure™ Saliva Sampler™	Alprazolam	93.7	93.0	91.1	27
	Amphetamine	97.2	85.5	88.7	27
	Cocaine	232.3	135.5	85.6	27
	MDMA	133.0	85.8	86.3	27
	Morphine	103.8	88.4	88.5	27
	THC	17.3	6.7	85.4	27
				>73	17
Sarstedt Salivette®	Alprazolam	85.3	91.3	27.3	27
	Amphetamine	71.8	94.4	85.63–86.07	12
				54–57	2
				51.8	27
	Buprenorphine	58.0	44.9	20.2–21.7	37
	Cocaine	108.1	101.1	91–92	2
				90.84–91.56	12
				81.7–91.4	38
				>73.3	37
				33.3	27
	Heroin	100.0	87.4	79.2–85.2	38
				>73.3	37
	MDMA	71.9	80.3	26.5	27
	Methadone	69.6	51.0	47.4–50.8	37
	Methamphetamine	110.3	97.8	87.97–88.81	12
				55–59	2
	Morphine	99.4	72.4	78.46–81.63	12
				73.9–78.3	38
				>73.3	37
				38–46	2
				35.2	27
	Phencyclidine	195.6	99.4	76.34–81.71	12
	THC	5.7	7.1	<12.5	27
	THC-COOH	7.3	8.7	12.17–47.41	12
				12–47	2

accordance with testing oral fluid samples. Recovery values measured using both matrices are similar for tested swabs, and there is a strong positive correlation between both datasets (Pearson's correlation coefficient value is $r = 0.73$, $n = 43$). Less than a quarter of compared pairs (oral fluid *vs.* water) differ by more than 20%. On average, recoveries were 5.8% greater from the oral fluid, than from water (median of 7.0%), and that small difference was likely caused by the use of an extraction method, which was necessitated for testing biological matrices. Impact of the sample preparation process on the final result is commonly acknowledged.[39] And as was aptly concluded by majors: *Sample processing typically accounts for at least one-third of the error generated*.[40] RSD values are also higher with oral fluid samples (average of 5.1%, and median of 3.4%). Increased variation of the results was expected for biological matrices, as this was the main rationale behind using ultrapure water for the main evaluation of the swabs. Full results of this comparison are presented in Table B5 in ESI B.†

Conclusions

The choice of an adequate oral fluid sample collection method is critical to the attainment of representative results, as certain methods can enormously influence analyte concentrations. Differences in analyte recovery values as great as 100 fold between samples collected with different collection swab types were observed in this research. Yet, this issue seems to be massively underrecognized and underappreciated. Many researchers tend to prioritize improving existing sample preparation and analysis methods, while many difficulties could be easily avoided by focusing attention on obtaining samples of superior quality – with emphasis placed on the sample collection step. This paper aims to initiate further discussion on this subject. Furthermore, the disclosed findings could be of substantial aid in the selection of an appropriate oral fluid sample collection method for HPLC-MS/MS analysis of 49 widespread drugs of abuse and doping agents.

Conflicts of interest

There are no conflicts to declare.

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Electronic Supplementary Material (ESI) for Analyst.

List of contents:

Tab. A1 Detailed list and classification of analysed substances.

substance	drug of abuse	therapeutic drug	doping agent (WADA class)*	analytical standard supplier
(+\-)-amphetamine	✓	✓	S6 stimulants	LGC
(+\-)-methamphetamine	✓	×	S6 stimulants	LGC
(+\-)-3,4-methylenedioxymethamphetamine (MDMA)	✓	×	S6 stimulants	LGC
methylhexanamine (4-methylhexan-2-amine, DMAA)	×	×	S6 stimulants	Sigma
cocaine	✓	✓	S6 stimulants	LGC
methylphenidate	✓	✓	S6 stimulants	LGC
nikethamide (N,N-diethylnicotinamide)	×	✓	S6 stimulants	Aldrich
strychnine	×	×	S6 stimulants	Sigma
(-)-Δ^9-tetrahydrocannabinol (THC)	✓	✓	S8 cannabinoids	LGC
(-)-11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH)	×	×	S8 cannabinoids	LGC
morphine	✓	✓	S7 narcotics	LGC
heroin (diacetylmorphine)	✓	✓	S7 narcotics	LGC
hydrocodone	✓	✓	×	Cerillant®
oxycodone	✓	✓	S7 narcotics	LGC
(+\-)-methadone	✓	✓	S7 narcotics	Cerillant®
buprenorphine	✓	✓	S7 narcotics	Cerillant®
fentanyl	✓	✓	S7 narcotics	LGC
ketamine	✓	✓	×	LGC
phencyclidine (PCP)	✓	×	S0 non-approved substances	LGC
lysergic acid diethylamide (LSD)	✓	×	S0 non-approved substances	LGC
psilocybin	✓	×	S0 non-approved substances	Cerillant®
alprazolam	✓	✓	×	LGC
clonazepam	✓	✓	×	Cerillant®
flunitrazepam	✓	✓	×	LGC
zolpidem	✓	✓	×	LGC
boldenone (Δ^1-testosterone / 1-dehydrotestosterone)	×	✓	S1 anabolic agents	VETRANAL™ (Sigma-Aldrich)
methandienone (methandrostenolone)	×	✓	S1 anabolic agents	Cerillant®
nandrolone (19-nortestosterone)	×	✓	S1 anabolic agents	LGC
stanozolol	×	✓	S1 anabolic agents	LGC
clenbuterol	×	✓	S1 anabolic agents	LGC
ibutamoren (MK-677)	×	×	S2 peptide hormones, growth factors, related substances and mimetics	Cayman Chemicals
fenoterol	×	✓	S3 beta-2 agonists	LGC
salbutamol (albuterol)	×	✓	S3 beta-2 agonists	LGC
terbutaline	×	✓	S3 beta-2 agonists	LGC
anastrozole	×	✓	S4 hormone and metabolic modulators	LGC
meldonium	×	✓	S4 hormone and metabolic modulators	EDQM
canrenone	×	✓	S5 diuretics and masking agents	Sigma
chlorothiazide	×	✓	S5 diuretics and masking agents	Sigma-Aldrich
hydrochlorothiazide	×	✓	S5 diuretics and masking agents	LGC
furosemide	×	✓	S5 diuretics and masking agents	Sigma
betamethasone	×	✓	S9 glucocorticoids	LGC
prednisolone	×	✓	S9 glucocorticoids	Sigma
prednisone	×	✓	S9 glucocorticoids	Sigma
atenolol	×	✓	P1 beta-blockers	LGC
bisoprolol	×	✓	P1 beta-blockers	LGC
carteolol	×	✓	P1 beta-blockers	USP
metoprolol	×	✓	P1 beta-blockers	LGC
nebivolol	×	✓	P1 beta-blockers	Sigma
propranolol	×	✓	P1 beta-blockers	LGC

*according to 2020 Prohibited List. (https://www.wada-ama.org/en/resources/science-medicine/prohibited-list-documents, WADA 2019)

Tab. A2 List of selected MS/MS parameters for the analytes. Substances arranged by increasing retention time.

substance	precursor ion [m/z]	product ions [m/z]			retention time [min]
		quantifier	qualifier(s)		
meldonium	147.00	58.10	59.15	-	1.499
psilocybin	284.90	58.10	205.20	240.05	2.433
morphine	286.10	152.10	201.15	165.10	2.642
salbutamol	240.00	148.10	222.25	166.20	2.913
terbutaline	226.00	152.10	107.05	125.10	3.015
atenolol	267.00	145.10	190.20	74.15	3.089
fenoterol	304.00	107.15	135.15	286.10	4.281
nikethamide	178.90	108.05	80.10	72.10	4.453
carteolol	293.00	237.15	202.15	74.10	4.548
amphetamine	135.90	91.10	119.15	65.10	4.625
oxycodone	316.10	298.20	241.20	256.10	4.629
hydrocodone	300.00	199.15	171.10	128.15	4.928
methamphetamine	150.00	91.10	65.10	119.20	5.001
chlorothiazide	294.00*	214.05	179.10	215.10	5.012
methylhexanamine	116.20	57.10	41.10	-	5.115
MDMA	194.00	163.10	105.10	133.10	5.185
strychnine	334.90	184.10	156.20	129.15	5.330
hydrochlorothiazide	296.00*	269.00	205.10	77.95	5.421
ketamine	237.90	125.05	220.10	207.15	5.665
metoprolol	267.90	116.15	74.15	72.10	6.407
clenbuterol	277.10	203.05	132.10	168.15	6.531
methylphenidate	234.00	84.15	56.10	91.10	6.650
heroin	370.10	165.15	268.20	211.10	6.866
cocaine	303.90	182.20	82.10	105.10	7.348
zolpidem	307.90	235.15	236.20	263.20	7.383
LSD	323.90	223.20	208.15	207.10	7.688
bisoprolol	326.20	116.20	74.05	72.10	7.869
phencyclidine	244.00	91.05	86.10	159.20	8.607
propranolol	260.10	116.20	183.10	155.20	8.775
fentanyl	337.00	188.20	105.15	103.10	9.114
prednisolone	361.00	147.15	325.20	307.15	9.277
prednisone	359.00	341.35	267.30	-	9.409
buprenorphine	468.10	55.15	396.25	414.25	9.672
ibutamoren	529.00	267.10	91.15	263.15	10.382
betamethasone	393.10	373.25	355.15	279.10	10.798
furosemide	329.05*	284.85	205.10	126.10	11.043
nebivolol	406.10	151.10	123.10	103.10	11.098
methadone	310.00	265.15	105.10	77.05	11.322
alprazolam	308.90	281.15	205.15	274.10	11.393
anastrozole	294.00	225.20	210.20	115.05	11.412
stanozolol	329.10	81.15	95.15	121.10	11.496
boldenone	287.00	121.20	135.20	77.15	11.942
clonazepam	316.00	270.10	214.05	207.15	11.970
nandrolone	275.10	109.10	239.10	257.30	12.320
methandienone	301.20	121.05	149.25	77.00	12.582
flunitrazepam	313.90	268.15	239.10	183.10	12.750
canrenone	341.00	107.15	187.25	235.15	15.029
THC-COOH	345.00	327.10	299.25	193.30	19.874
THC	315.00	193.10	123.10	259.15	24.788

*negative ionization mode

List of contents:

Tab. B1 Drug recoveries [%] from laboratory plastics. Relative standard deviations (RSDs) [%] given in brackets, drugs arranged by increasing retention time, n=4.

substance	15 mL Falcon tube	5 mL syringe	10 mL syringe	2 mL Eppendorf tube
meldonium	92.4 (3.8)	99.6 (0.7)	100.3 (1.0)	100.6 (1.3)
psilocybin	98.5 (1.1)	99.2 (0.6)	100.8 (0.8)	99.1 (1.2)
morphine	110.8 (3.2)	101.7 (2.9)	105.6 (2.5)	100.5 (2.3)
salbutamol	103.2 (0.7)	100.3 (1.1)	101.5 (1.2)	99.7 (1.4)
terbutaline	105.3 (1.5)	101.2 (1.5)	100.5 (1.2)	100.3 (0.7)
atenolol	97.3 (8.6)	97.4 (7.6)	102.5 (3.8)	110.0 (6.7)
fenoterol	108.8 (1.7)	99.4 (1.4)	97.6 (0.8)	99.3 (0.8)
nikethamide	103.0 (1.0)	99.6 (1.7)	99.8 (0.9)	100.7 (0.9)
carteolol	96.3 (9.2)	98.1 (9.1)	104.2 (3.5)	110.3 (7.5)
oxycodone	101.3 (11.4)	97.9 (9.0)	102.3 (2.4)	117.3 (9.7)
amphetamine	101.9 (3.5)	101.1 (4.5)	104.2 (3.8)	103.0 (5.0)
chlorothiazide	99.5 (0.8)	100.2 (0.7)	102.1 (0.9)	98.6 (0.9)
hydrocodone	114.5 (16.7)	101.5 (12.8)	106.3 (6.5)	118.7 (13.1)
methamphetamine	96.5 (12.2)	101.6 (9.9)	105.7 (7.3)	113.1 (11.6)
methylhexanamine	101.6 (5.8)	101.9 (5.7)	104.1 (4.3)	104.1 (7.1)
MDMA	94.3 (11.7)	101.2 (10.7)	103.4 (7.0)	114.9 (12.9)
hydrochlorothiazide	100.7 (3.1)	100.8 (4.3)	103.4 (3.7)	99.7 (1.8)
strychnine	111.6 (22.4)	100.9 (13.2)	104.8 (7.4)	122.7 (18.3)
ketamine	105.6 (2.7)	100.3 (2.4)	103.3 (0.8)	100.4 (2.6)
metoprolol	96.4 (10.4)	97.3 (9.1)	103.0 (4.7)	111.4 (10.6)
clenbuterol	97.2 (7.5)	98.0 (6.3)	104.1 (2.6)	107.1 (7.3)
methylphenidate	173.3 (4.7)	97.4 (5.0)	105.6 (4.6)	100.6 (4.9)
heroin	127.2 (2.5)	99.9 (3.5)	108.9 (2.0)	102.8 (2.1)
zolpidem	104.0 (2.0)	100.7 (2.2)	102.9 (1.5)	101.2 (1.9)
cocaine	117.0 (21.0)	99.7 (16.6)	103.9 (8.6)	121.7 (19.3)
LSD	103.2 (1.4)	99.6 (1.1)	101.0 (1.0)	100.2 (1.2)
bisoprolol	89.2 (15.9)	94.5 (11.0)	100.5 (2.7)	115.9 (13.7)
phencyclidine	76.1 (20.2)	91.8 (10.7)	92.6 (3.5)	123.9 (23.4)
propranolol	102.8 (5.2)	100.1 (6.0)	103.1 (3.6)	105.3 (6.0)
prednisolone	98.7 (0.9)	97.7 (3.1)	97.9 (1.3)	98.7 (3.2)
fentanyl	105.5 (20.2)	101.3 (15.8)	108.4 (5.8)	121.6 (14.8)
prednisone	96.5 (1.0)	101.1 (4.3)	99.0 (1.1)	101.5 (2.5)
buprenorphine	106.4 (4.5)	99.2 (5.0)	105.6 (3.5)	110.6 (4.5)
ibutamoren	115.4 (0.7)	104.0 (5.0)	109.1 (3.9)	107.2 (4.4)
betamethasone	89.2 (2.3)	95.7 (3.8)	101.0 (3.0)	98.7 (4.7)
furosemide	94.9 (0.5)	97.8 (1.3)	101.4 (2.2)	98.7 (1.8)
nebivolol	118.0 (2.1)	99.1 (4.1)	104.4 (4.0)	103.3 (7.0)
alprazolam	98.3 (1.1)	100.5 (1.2)	100.5 (0.7)	101.7 (0.9)
anastrozole	100.9 (1.0)	100.9 (1.4)	101.0 (0.7)	100.9 (1.9)
stanozolol	61.8 (0.6)	94.9 (1.9)	96.7 (2.8)	99.8 (3.8)
methadone	94.8 (12.1)	96.4 (10.2)	101.3 (3.1)	114.2 (17.0)
boldenone	92.3 (0.8)	98.9 (1.4)	99.4 (0.8)	100.4 (1.5)
clonazepam	99.0 (0.6)	99.1 (1.2)	100.7 (0.7)	100.8 (1.2)
nandrolone	92.2 (1.9)	98.2 (1.8)	98.8 (2.1)	99.3 (1.3)
methandienone	90.8 (0.5)	99.3 (2.1)	99.3 (1.2)	100.3 (1.5)
flunitrazepam	98.0 (1.3)	99.7 (1.7)	100.6 (1.0)	99.7 (0.8)
canrenone	62.1 (2.6)	97.8 (2.0)	98.9 (1.3)	100.5 (2.7)
THC-COOH	22.4 (6.4)	40.3 (8.7)	47.7 (15.3)	87.7 (3.8)
THC	20.5 (3.5)	23.9 (6.1)	24.5 (11.9)	41.3 (11.0)

Tab. B2 Drug recoveries [%] from oral fluid sample collection device swabs. Relative standard deviation (RSD) [%] given in brackets, n=4.

substance	SalivaBio Oral Swab	Saliva Collection Swab (type I)	Saliva Collection Swab (type II)	Salivette®	Salivette® Cortisol	DCD™ 5000	Oracol	Versi•SAL®	Super•SAL™	Micro•SAL™	Accu•SAL™	Intercept® i2®	Oral-Eze®	Saliva Sampler™	Quantisal™
meldonium	17.5 (6.7)	25.6 (14.6)	38.7 (15.3)	86.0 (5.2)	54.2 (2.7)	87.8 (21.5)	46.3 (15.9)	38.1 (9.4)	71.1 (9.1)	102.5 (6.5)	76.7 (9.2)	23.3 (14.7)	105.0 (5.9)	105.7 (3.7)	104.2 (5.8)
psilocybin	101.0 (2.2)	101.9 (1.1)	96.6 (1.2)	333.3 (4.6)	99.5 (3.1)	93.1 (5.4)	98.3 (1.9)	96.6 (2.3)	97.5 (0.8)	99.5 (3.3)	302.1 (7.5)	484.0 (13.0)	206.2 (10.2)	114.6 (3.3)	157.8 (7.2)
morphine	98.1 (2.6)	102.7 (2.1)	116.6 (1.5)	99.4 (4.7)	64.0 (19.7)	118.0 (1.2)	83.7 (1.6)	107.0 (4.4)	115.1 (1.8)	114.6 (4.2)	109.4 (2.5)	176.9 (2.2)	130.8 (3.9)	103.8 (2.8)	112.5 (1.0)
salbutamol	95.5 (4.9)	97.7 (4.5)	106.6 (1.5)	81.2 (2.8)	40.0 (23.5)	107.8 (0.7)	69.7 (0.8)	96.6 (6.3)	101.5 (2.9)	85.4 (6.4)	97.8 (0.9)	116.0 (0.6)	95.2 (1.0)	96.5 (2.9)	96.5 (2.2)
terbutaline	90.5 (5.1)	94.3 (4.7)	104.8 (1.7)	55.0 (1.9)	36.7 (30.6)	109.4 (0.4)	64.0 (9.8)	92.8 (7.4)	102.0 (4.2)	67.5 (10.2)	86.7 (1.2)	112.5 (0.8)	80.8 (0.7)	89.7 (3.4)	93.6 (2.6)
atenolol	134.6 (7.7)	134.8 (5.6)	146.6 (1.2)	119.4 (3.6)	87.8 (16.3)	147.2 (0.8)	95.1 (4.0)	110.0 (6.4)	138.4 (3.3)	127.5 (6.1)	119.9 (1.0)	156.3 (1.0)	127.0 (2.2)	120.1 (3.0)	131.2 (2.1)
fenoterol	41.8 (4.0)	58.0 (7.8)	83.6 (6.2)	35.0 (5.9)	17.6 (60.2)	84.9 (28.3)	16.5 (9.6)	47.6 (4.8)	26.4 (3.2)	36.4 (9.7)	54.6 (10.4)	78.3 (17.5)	23.3 (3.0)	50.5 (13.8)	56.9 (20.2)
nikethamide	91.1 (2.5)	87.8 (4.9)	94.7 (1.8)	69.3 (1.8)	84.5 (3.1)	97.7 (2.3)	48.7 (1.7)	66.2 (2.7)	75.5 (2.9)	65.8 (5.8)	99.2 (1.6)	88.5 (2.3)	76.3 (1.3)	101.8 (1.2)	100.6 (2.1)
carteolol	103.0 (6.7)	94.3 (10.6)	121.6 (4.6)	60.1 (4.3)	34.1 (40.9)	135.9 (2.5)	66.4 (2.1)	61.2 (6.2)	59.6 (5.5)	65.0 (7.6)	99.9 (1.4)	138.5 (0.9)	87.2 (1.0)	100.4 (5.7)	100.7 (3.7)
oxycodone	121.9 (1.6)	113.0 (4.5)	147.0 (3.6)	79.2 (2.8)	69.8 (32.6)	168.8 (3.0)	96.9 (3.1)	84.4 (5.9)	46.5 (2.9)	79.7 (8.4)	160.3 (2.2)	178.8 (3.2)	139.8 (2.7)	168.0 (4.1)	169.6 (2.4)
amphetamine	90.2 (6.0)	94.7 (6.2)	108.6 (4.9)	71.8 (5.7)	15.9 (49.1)	99.0 (5.4)	65.4 (3.3)	79.6 (7.1)	47.2 (3.4)	72.2 (11.0)	105.3 (2.2)	104.0 (3.3)	91.2 (1.7)	97.2 (3.1)	98.1 (2.8)
chlorothiazide	119.6 (2.5)	121.6 (3.0)	117.5 (1.8)	120.8 (2.7)	129.3 (5.7)	105.5 (0.8)	48.8 (12.5)	119.0 (2.3)	110.2 (2.3)	146.8 (4.7)	103.2 (0.7)	77.8 (10.2)	95.2 (2.5)	107.5 (1.6)	109.4 (1.1)
hydrocodone	118.3 (5.6)	112.2 (4.0)	150.5 (3.2)	90.6 (2.8)	57.1 (37.2)	185.2 (1.8)	74.4 (4.0)	96.9 (5.6)	64.7 (3.5)	111.8 (8.7)	174.5 (1.7)	198.6 (4.8)	165.7 (1.5)	190.0 (2.6)	212.3 (2.0)
methamphetamine	138.2 (4.0)	137.1 (4.3)	151.8 (2.5)	110.3 (3.5)	23.3 (51.2)	137.3 (4.3)	52.0 (4.3)	114.6 (5.0)	99.1 (1.6)	111.1 (4.5)	141.7 (3.4)	147.5 (2.9)	123.7 (2.2)	129.2 (3.6)	135.3 (3.2)
methylhexaneamine	102.3 (1.4)	107.1 (3.8)	117.7 (3.6)	94.0 (3.8)	16.3 (53.1)	103.1 (7.1)	63.6 (2.9)	86.5 (4.1)	75.3 (1.4)	90.0 (10.7)	117.9 (4.4)	123.6 (2.7)	105.6 (3.4)	109.7 (3.5)	111.8 (3.3)
MDMA	89.9 (2.6)	86.9 (6.2)	117.6 (5.4)	71.9 (5.6)	24.1 (51.1)	143.8 (3.8)	31.5 (6.4)	59.5 (4.8)	33.5 (5.4)	65.3 (12.1)	141.1 (3.3)	150.4 (3.9)	127.6 (1.9)	133.0 (3.4)	135.5 (3.0)
hydrochlorothiazide	94.2 (4.8)	97.0 (4.5)	100.9 (1.5)	89.2 (2.0)	105.2 (3.5)	91.7 (6.5)	12.7 (25.4)	89.5 (4.6)	95.9 (4.1)	99.5 (7.4)	89.7 (5.0)	73.6 (6.7)	78.4 (4.4)	93.0 (6.2)	93.9 (4.3)
strychnine	168.2 (11.2)	163.4 (7.6)	204.7 (4.0)	65.3 (8.0)	23.6 (63.7)	202.5 (6.4)	34.8 (11.9)	56.6 (6.3)	129.3 (4.0)	108.7 (17.1)	185.2 (4.5)	275.5 (1.8)	191.3 (4.3)	210.6 (5.2)	218.2 (3.2)
ketamine	60.9 (2.7)	63.6 (5.9)	78.7 (4.4)	53.3 (4.0)	21.9 (46.1)	91.9 (4.1)	53.4 (3.9)	42.5 (4.0)	26.8 (5.6)	55.7 (11.0)	96.9 (3.0)	102.8 (3.1)	90.4 (2.1)	95.6 (3.6)	101.3 (0.8)
metoprolol	58.6 (3.5)	63.9 (8.2)	82.6 (7.6)	66.9 (5.4)	22.8 (54.1)	121.9 (2.1)	52.9 (1.1)	44.0 (2.2)	46.7 (3.7)	66.4 (14.5)	126.3 (3.5)	139.1 (2.0)	120.2 (2.2)	119.9 (2.7)	121.6 (2.8)
clenbuterol	53.0 (3.2)	58.6 (5.7)	77.3 (7.3)	55.6 (6.4)	12.5 (60.0)	109.3 (3.4)	14.6 (12.7)	42.3 (3.5)	52.9 (3.7)	56.9 (16.9)	102.6 (2.7)	127.7 (0.8)	97.1 (2.2)	96.5 (3.4)	96.1 (1.9)
methylphenidate	112.4 (3.2)	132.8 (8.1)	151.2 (7.5)	168.8 (5.6)	22.8 (48.0)	145.8 (3.9)	103.7 (5.8)	78.8 (11.8)	172.5 (4.1)	185.8 (16.1)	256.6 (6.1)	344.4 (3.8)	215.1 (8.3)	212.4 (15.3)	204.0 (9.8)
heroin	87.5 (2.5)	97.0 (3.0)	108.7 (2.7)	100.0 (3.2)	35.3 (52.1)	115.8 (0.9)	77.0 (4.5)	87.8 (4.2)	116.2 (1.9)	115.2 (6.4)	125.7 (3.4)	156.8 (2.4)	121.6 (1.9)	122.0 (5.9)	128.2 (2.6)
zolpidem	32.6 (3.1)	37.3 (6.1)	48.8 (6.8)	30.7 (9.1)	15.5 (62.2)	91.3 (1.7)	14.4 (18.8)	28.8 (3.0)	38.9 (2.2)	46.1 (16.0)	75.8 (3.6)	79.0 (2.4)	73.4 (3.1)	78.2 (4.0)	82.4 (1.5)
cocaine	76.4 (6.0)	83.4 (7.6)	108.6 (10.7)	108.1 (9.3)	33.3 (47.6)	192.1 (4.4)	66.2 (6.6)	61.0 (3.4)	91.9 (4.7)	118.6 (17.1)	248.1 (4.6)	332.7 (3.3)	229.4 (2.7)	232.3 (7.9)	230.7 (3.9)
LSD	16.5 (8.8)	20.5 (11.4)	34.6 (13.8)	19.3 (18.4)	2.8 (61.7)	64.6 (4.5)	4.6 (23.8)	17.5 (2.2)	19.8 (6.3)	23.7 (32.1)	37.1 (2.3)	69.9 (4.5)	42.7 (10.8)	46.8 (8.9)	62.6 (7.2)
bisoprolol	102.2 (2.2)	107.3 (2.2)	127.4 (4.4)	158.7 (2.9)	27.9 (59.2)	157.8 (2.3)	108.4 (2.5)	107.2 (1.8)	108.5 (2.6)	121.1 (5.2)	160.7 (5.6)	186.9 (1.8)	157.0 (2.4)	148.4 (2.5)	153.8 (1.4)
phencyclidine	223.3 (6.8)	228.2 (4.5)	254.8 (6.9)	195.6 (7.5)	18.1 (56.6)	155.0 (11.8)	16.8 (19.3)	225.6 (4.1)	228.8 (2.4)	117.4 (18.6)	238.4 (12.3)	300.1 (4.8)	225.6 (7.1)	213.7 (5.9)	222.4 (3.7)
propranolol	45.1 (12.9)	55.8 (12.7)	82.0 (6.3)	48.9 (11.1)	2.8 (64.3)	51.5 (10.3)	3.4 (21.2)	60.0 (2.8)	70.5 (10.6)	21.1 (24.4)	59.7 (9.5)	87.6 (5.0)	54.0 (6.3)	57.6 (8.7)	47.6 (6.6)
prednisolone	89.7 (4.0)	93.7 (2.1)	92.1 (1.7)	84.4 (4.4)	128.7 (6.2)	97.5 (2.5)	44.1 (7.4)	88.2 (2.0)	91.0 (3.0)	76.5 (1.8)	94.7 (0.7)	91.9 (5.8)	98.1 (2.0)	97.5 (2.9)	97.6 (1.9)
fentanyl	102.9 (5.4)	113.9 (6.2)	139.8 (3.5)	76.1 (6.7)	8.1 (63.7)	88.6 (10.2)	6.5 (26.2)	129.8 (3.6)	148.8 (4.5)	34.4 (27.1)	109.8 (11.5)	161.8 (4.3)	114.6 (6.4)	105.4 (9.4)	112.6 (2.6)
prednisone	91.8 (2.9)	95.5 (2.4)	96.0 (2.9)	95.4 (1.1)	143.1 (4.7)	102.6 (0.9)	100.8 (5.1)	90.7 (1.9)	92.8 (1.4)	92.7 (1.6)	97.1 (2.7)	95.5 (2.8)	97.7 (1.3)	98.1 (1.3)	99.2 (1.2)
buprenorphine	89.5 (7.0)	105.0 (6.9)	109.7 (5.4)	58.0 (6.7)	5.0 (67.7)	40.1 (9.2)	7.5 (25.6)	117.7 (4.8)	108.1 (4.9)	61.6 (16.2)	74.3 (13.1)	128.2 (10.0)	80.0 (8.1)	70.2 (10.7)	64.3 (6.5)
ibutamoren	71.6 (14.1)	85.0 (9.3)	110.0 (3.5)	56.2 (9.8)	3.4 (64.8)	56.9 (14.4)	9.5 (23.0)	95.2 (6.0)	83.6 (11.0)	40.1 (18.2)	40.7 (3.9)	76.2 (14.3)	46.1 (18.1)	43.8 (16.9)	42.7 (10.1)
betamethasone	98.4 (1.4)	100.6 (4.5)	103.3 (2.2)	88.1 (3.4)	85.1 (11.5)	95.9 (1.7)	22.9 (8.8)	94.2 (1.4)	95.6 (1.7)	89.0 (3.4)	92.6 (2.4)	94.6 (3.0)	93.5 (1.1)	93.0 (1.0)	88.4 (2.4)
furosemide	80.6 (2.0)	83.3 (3.6)	88.2 (3.8)	69.2 (4.5)	77.9 (7.1)	98.4 (1.8)	2.2 (46.5)	74.2 (3.1)	83.1 (1.6)	76.4 (5.6)	100.5 (0.9)	90.4 (2.4)	100.9 (1.1)	109.6 (2.4)	102.6 (1.7)
nebivolol	36.8 (6.0)	39.0 (17.1)	48.5 (30.6)	18.9 (10.1)	3.3 (63.7)	26.0 (26.2)	0.5 (42.0)	59.9 (19.0)	44.6 (16.5)	7.1 (19.1)	24.0 (21.0)	27.2 (26.4)	24.4 (18.9)	33.8 (24.8)	21.2 (24.5)
alprazolam	87.2 (3.7)	90.1 (3.3)	91.5 (0.9)	85.3 (4.6)	50.1 (16.4)	88.9 (2.7)	22.3 (9.3)	83.5 (2.2)	85.1 (3.1)	74.5 (4.4)	94.1 (4.0)	93.1 (0.8)	89.6 (1.8)	93.7 (0.5)	91.3 (2.0)
anastrozole	97.3 (1.7)	100.7 (1.1)	99.5 (1.1)	96.8 (7.0)	98.2 (3.2)	100.4 (2.2)	19.0 (14.9)	99.8 (2.2)	97.2 (1.3)	93.3 (0.6)	101.8 (1.9)	100.5 (1.3)	100.3 (2.1)	102.0 (0.4)	100.2 (1.3)
stanozolol	60.0 (8.9)	64.8 (3.4)	77.4 (4.0)	13.9 (9.0)	7.5 (80.0)	66.6 (6.9)	1.2 (12.4)	77.6 (6.7)	52.8 (7.0)	24.8 (18.9)	22.8 (3.3)	39.5 (10.2)	29.0 (15.2)	37.0 (5.9)	28.9 (8.6)
methadone	147.1 (10.2)	161.4 (6.7)	163.1 (11.8)	69.6 (4.2)	3.5 (78.0)	40.6 (21.0)	10.2 (23.9)	157.9 (7.8)	135.4 (5.0)	32.2 (38.9)	77.7 (19.0)	122.3 (9.5)	76.1 (10.9)	69.8 (15.9)	69.3 (2.2)
boldenone	84.4 (1.3)	86.7 (2.4)	90.1 (1.5)	75.6 (5.5)	72.0 (11.8)	91.4 (2.5)	7.8 (14.7)	79.8 (1.2)	84.9 (2.6)	74.1 (4.5)	90.8 (2.8)	92.8 (1.2)	90.3 (2.3)	90.7 (0.8)	87.8 (0.8)
clonazepam	90.0 (1.2)	93.5 (1.0)	95.5 (2.0)	71.7 (4.0)	72.6 (8.5)	93.3 (2.6)	3.4 (20.9)	89.8 (0.8)	90.0 (1.5)	78.5 (2.2)	93.7 (2.6)	93.9 (1.7)	92.0 (2.1)	94.3 (1.0)	91.9 (1.2)
nandrolone	101.0 (4.5)	103.9 (2.1)	100.3 (2.8)	84.5 (3.2)	87.5 (7.4)	87.7 (4.9)	11.1 (18.8)	104.7 (2.3)	96.1 (1.7)	91.7 (1.4)	82.0 (3.3)	108.1 (3.9)	83.2 (0.9)	87.5 (1.0)	85.5 (0.6)
methandienone	88.4 (3.0)	89.4 (2.2)	91.7 (1.7)	77.3 (5.1)	76.6 (9.2)	88.0 (2.8)	10.7 (16.7)	72.4 (2.6)	85.6 (2.5)	73.9 (4.5)	85.1 (2.9)	89.5 (3.0)	88.7 (1.3)	88.9 (0.5)	86.7 (1.4)
flunitrazepam	88.0 (3.2)	98.6 (5.0)	104.9 (1.3)	77.1 (3.7)	83.1 (7.1)	94.2 (2.6)	4.1 (19.4)	69.2 (3.3)	92.8 (2.2)	73.4 (2.6)	93.5 (0.4)	93.6 (2.5)	98.0 (1.4)	96.9 (0.8)	95.3 (0.7)
canrenone	61.1 (2.6)	71.2 (5.6)	86.0 (1.3)	70.4 (3.2)	43.0 (20.2)	84.8 (3.7)	1.4 (25.1)	67.4 (3.8)	60.2 (3.4)	56.4 (3.4)	83.4 (2.5)	81.5 (2.9)	77.3 (2.8)	81.3 (2.5)	76.0 (3.2)
THC-COOH	23.6 (14.0)	27.9 (28.2)	67.3 (33.3)	7.3 (6.8)	10.7 (75.1)	80.4 (13.4)	0.4 (37.0)	28.0 (21.0)	22.4 (9.8)	25.3 (40.0)	57.7 (3.7)	59.6 (6.9)	55.1 (8.1)	64.2 (9.0)	69.2 (5.4)
THC	3.6 (9.6)	4.7 (37.4)	5.2 (57.3)	5.7 (33.8)	0.8 (37.4)	9.2 (23.0)	0.4 (29.2)	12.3 (25.4)	7.6 (12.0)	1.5 (88.3)	8.9 (42.9)	7.4 (65.9)	9.3 (32.9)	17.3 (21.0)	9.9 (35.8)

Tab. B3 Matrix effects presented as percentage of signals measured from spiked matrix from oral fluid sample collection device swabs relative to signals from spiked neat matrix. Relative standard deviation (RSD) [%] given in brackets, n=3.

substance	SalivaBio Oral Swab	Saliva Collection Swab (type I)	Saliva Collection Swab (type II)	Salivette®	Salivette® Cortisol	DCD™ 5000	Oracol	Versi•SAL™	Super•SAL™	Micro•SAL™	Accu•SAL™	Intercept® i2™	Oral-Eze™	Saliva Sampler™	Quantisal™
meldonium	27.1 (10.3)	31.0 (10.0)	46.7 (7.9)	62.7 (9.8)	57.7 (4.7)	92.3 (14.5)	60.4 (24.0)	31.8 (15.8)	81.7 (10.0)	98.8 (12.8)	86.0 (22.9)	8.3 (26.0)	114.0 (9.9)	111.8 (1.1)	105.2 (12.6)
psilocybin	108.3 (4.1)	103.9 (3.0)	113.3 (2.5)	389.0 (4.2)	116.2 (7.7)	113.1 (3.4)	99.1 (6.0)	92.9 (3.1)	97.9 (5.6)	103.9 (9.8)	366.7 (2.4)	649.4 (9.0)	282.1 (14.2)	122.2 (2.3)	185.1 (8.0)
morphine	105.5 (6.0)	100.2 (2.7)	112.0 (2.7)	137.3 (2.9)	116.9 (6.2)	114.5 (3.1)	81.3 (14.9)	89.1 (8.3)	98.2 (1.1)	90.4 (3.6)	138.6 (9.0)	171.4 (13.5)	145.7 (11.2)	117.5 (4.5)	124.2 (13.5)
salbutamol	109.9 (3.7)	99.3 (2.4)	107.9 (2.6)	100.3 (4.3)	87.0 (3.2)	108.4 (0.8)	81.8 (7.7)	85.5 (6.4)	96.6 (4.6)	76.4 (8.5)	113.3 (7.8)	126.6 (3.3)	104.2 (12.2)	107.0 (4.6)	105.4 (10.6)
terbutaline	110.6 (10.2)	103.5 (6.9)	111.0 (3.9)	95.3 (3.2)	97.1 (4.1)	117.0 (1.3)	73.5 (18.7)	83.7 (7.0)	103.2 (6.4)	70.1 (6.2)	115.5 (9.4)	135.2 (3.4)	100.1 (9.2)	108.7 (5.1)	110.4 (12.6)
atenolol	125.2 (2.9)	121.1 (5.1)	135.9 (2.8)	148.6 (4.4)	125.6 (3.6)	137.4 (3.8)	93.6 (12.1)	96.7 (11.7)	120.2 (3.4)	109.2 (9.1)	143.8 (5.4)	161.8 (6.3)	134.6 (13.8)	135.0 (1.8)	134.6 (8.3)
fenoterol	65.6 (14.6)	83.3 (8.0)	98.5 (4.3)	86.2 (9.2)	106.2 (6.5)	109.6 (2.6)	65.9 (1.1)	52.2 (16.4)	34.5 (2.9)	37.4 (14.6)	92.9 (2.7)	112.5 (9.1)	42.1 (7.6)	74.6 (18.9)	94.7 (8.9)
nikethamide	97.5 (4.2)	93.5 (2.3)	109.3 (1.5)	66.7 (7.2)	92.0 (4.6)	113.9 (2.6)	64.1 (4.8)	66.2 (6.1)	69.3 (5.8)	64.3 (5.3)	107.8 (9.2)	93.8 (2.7)	61.6 (11.8)	99.9 (2.5)	95.2 (13.0)
carteolol	104.8 (2.7)	100.4 (5.8)	121.9 (6.3)	86.7 (8.2)	111.0 (7.9)	133.7 (5.4)	85.7 (19.5)	57.3 (10.3)	53.6 (4.8)	58.7 (5.9)	141.9 (4.8)	142.6 (3.8)	101.0 (13.1)	133.7 (0.8)	127.6 (8.7)
oxycodone	117.1 (8.9)	114.5 (7.9)	149.4 (5.3)	108.9 (12.3)	152.5 (10.2)	167.0 (3.3)	91.6 (21.8)	72.4 (13.7)	47.4 (6.6)	67.7 (2.1)	185.4 (6.5)	180.0 (2.7)	113.6 (11.3)	185.2 (7.0)	165.7 (11.6)
amphetamine	96.3 (2.8)	90.8 (3.5)	95.4 (5.8)	76.1 (6.6)	85.5 (9.8)	99.7 (4.5)	89.2 (4.8)	66.6 (5.5)	47.5 (5.3)	67.1 (6.8)	121.3 (7.3)	109.2 (2.8)	109.0 (15.2)	113.7 (2.4)	108.3 (9.6)
chlorothiazide	110.4 (3.1)	107.9 (2.0)	119.7 (3.4)	105.5 (7.3)	126.7 (6.4)	116.1 (8.0)	137.3 (6.5)	82.5 (6.6)	85.7 (4.5)	119.7 (17.5)	110.8 (8.9)	87.9 (7.6)	99.0 (5.4)	101.2 (3.2)	98.0 (11.2)
hydrocodone	102.0 (6.9)	100.7 (15.9)	152.2 (5.3)	117.7 (9.0)	134.5 (14.2)	173.1 (3.0)	54.4 (39.5)	76.4 (8.8)	54.8 (9.7)	80.6 (11.7)	186.3 (5.8)	181.5 (2.3)	159.5 (11.2)	170.6 (1.7)	171.1 (11.4)
methamphetamine	115.4 (3.5)	111.5 (7.7)	111.5 (7.1)	112.9 (5.6)	107.2 (3.8)	111.2 (10.7)	75.2 (16.6)	82.6 (7.0)	66.5 (4.7)	90.6 (7.0)	157.6 (6.6)	153.5 (2.5)	140.7 (12.5)	145.2 (0.8)	140.2 (10.6)
methylhexanamine	99.9 (6.0)	94.7 (2.6)	96.3 (4.6)	94.8 (5.0)	90.2 (5.6)	95.9 (10.8)	77.1 (8.4)	67.7 (7.1)	59.2 (2.8)	79.9 (7.6)	134.5 (7.5)	125.5 (2.1)	118.9 (12.4)	121.1 (0.5)	117.8 (12.3)
MDMA	89.3 (6.1)	86.5 (8.1)	106.5 (6.9)	89.6 (9.2)	107.4 (4.2)	123.4 (10.7)	58.2 (17.3)	50.6 (10.5)	33.2 (4.1)	63.9 (3.5)	167.3 (4.2)	162.6 (3.2)	152.3 (11.1)	154.9 (0.8)	148.7 (8.5)
hydrochlorothiazide	94.3 (7.1)	88.9 (7.4)	109.3 (5.5)	73.4 (9.7)	104.9 (3.3)	116.2 (6.2)	71.3 (7.6)	69.1 (4.4)	69.8 (5.9)	88.8 (14.3)	108.2 (10.7)	87.1 (3.9)	95.7 (12.9)	101.9 (3.7)	92.6 (12.2)
strychnine	108.3 (10.6)	105.3 (22.7)	159.9 (7.0)	90.8 (14.8)	109.2 (12.6)	165.0 (1.9)	45.7 (44.0)	35.1 (14.7)	50.7 (15.8)	62.7 (12.8)	222.4 (7.5)	220.7 (1.1)	199.5 (16.5)	205.0 (4.0)	197.0 (5.8)
ketamine	73.0 (2.7)	69.4 (0.5)	93.3 (4.7)	62.5 (8.0)	96.8 (2.9)	109.7 (3.4)	70.8 (13.9)	38.8 (9.8)	31.7 (1.4)	56.0 (5.8)	116.5 (8.6)	107.8 (3.8)	101.9 (10.6)	106.0 (3.8)	102.6 (13.0)
metoprolol	61.0 (2.2)	59.6 (5.5)	92.9 (12.6)	71.7 (8.0)	99.0 (5.5)	125.7 (2.4)	70.9 (15.2)	36.7 (10.7)	48.0 (1.6)	61.3 (1.6)	149.8 (6.5)	146.8 (2.7)	133.1 (15.4)	138.3 (2.8)	132.1 (7.9)
clenbuterol	57.1 (4.2)	57.4 (4.6)	86.5 (7.8)	69.6 (8.3)	89.5 (3.1)	123.5 (6.0)	69.1 (12.5)	35.0 (12.1)	54.7 (2.6)	65.5 (4.0)	137.6 (4.8)	137.0 (3.4)	125.3 (9.4)	128.3 (2.9)	123.0 (13.4)
methylphenidate	138.1 (45.1)	122.1 (26.3)	85.1 (21.2)	176.6 (28.4)	149.0 (43.2)	107.6 (20.7)	114.9 (11.1)	53.2 (19.4)	87.3 (11.5)	126.9 (30.2)	209.9 (41.7)	242.2 (28.5)	150.2 (11.5)	140.6 (16.4)	153.7 (26.3)
heroin	84.3 (7.3)	80.7 (4.9)	99.2 (4.3)	114.3 (7.7)	148.9 (2.4)	119.2 (8.3)	84.6 (16.9)	54.7 (11.3)	88.6 (4.5)	91.7 (10.1)	140.7 (13.0)	138.8 (8.1)	119.2 (8.3)	123.1 (1.7)	118.4 (14.9)
zolpidem	42.2 (3.7)	41.6 (4.2)	65.5 (8.8)	50.6 (13.2)	88.4 (2.6)	109.1 (1.9)	64.1 (14.2)	28.7 (5.6)	48.3 (2.6)	67.5 (9.9)	115.0 (9.9)	109.3 (1.7)	100.9 (13.2)	103.1 (3.8)	100.2 (13.8)
cocaine	59.3 (19.9)	58.3 (7.0)	77.0 (25.4)	106.9 (16.8)	121.2 (3.3)	129.3 (15.0)	70.3 (30.7)	39.7 (4.6)	61.8 (12.8)	96.2 (8.7)	208.8 (12.1)	226.6 (4.6)	178.0 (16.8)	171.5 (11.9)	177.1 (6.3)
LSD	28.3 (1.9)	28.8 (8.9)	57.3 (13.4)	50.5 (6.6)	64.7 (3.6)	109.1 (2.7)	67.5 (15.2)	19.7 (4.1)	33.6 (3.1)	48.3 (4.6)	111.7 (6.7)	107.6 (1.4)	100.6 (11.6)	104.3 (2.9)	99.7 (11.5)
bisoprolol	78.0 (2.4)	78.9 (12.0)	112.8 (8.1)	144.1 (4.2)	133.6 (11.5)	147.1 (9.7)	95.7 (22.0)	77.5 (6.2)	95.4 (4.1)	102.0 (1.6)	180.7 (4.0)	180.7 (4.5)	167.4 (13.5)	168.0 (2.6)	162.0 (7.6)
phencyclidine	145.0 (8.5)	151.5 (22.4)	150.5 (21.1)	196.6 (13.3)	144.6 (2.9)	97.8 (18.7)	74.1 (36.4)	168.0 (1.8)	174.5 (11.8)	174.9 (10.8)	299.3 (4.1)	315.8 (7.4)	278.0 (18.6)	276.4 (3.5)	266.3 (1.7)
propranolol	65.2 (4.6)	63.3 (3.9)	41.4 (6.3)	85.7 (12.0)	44.1 (32.5)	47.8 (18.5)	42.8 (12.6)	61.0 (7.1)	88.5 (1.1)	54.8 (3.5)	124.4 (8.5)	130.9 (2.9)	111.4 (10.5)	114.4 (4.9)	112.1 (6.5)
prednisolone	92.2 (16.3)	89.1 (6.7)	104.9 (3.0)	76.3 (8.9)	167.1 (13.4)	108.8 (2.4)	77.8 (2.2)	82.4 (2.1)	91.9 (4.8)	72.9 (4.9)	107.5 (6.2)	97.7 (4.6)	95.2 (9.7)	100.5 (2.6)	94.7 (14.8)
fentanyl	96.4 (7.5)	97.0 (12.4)	125.8 (6.4)	101.2 (15.6)	108.9 (4.6)	104.6 (3.7)	62.5 (38.5)	104.7 (3.7)	128.1 (3.2)	77.9 (8.6)	167.9 (7.5)	168.6 (0.8)	153.0 (9.4)	153.3 (3.8)	148.9 (11.4)
prednisone	105.4 (2.3)	96.8 (3.6)	110.2 (1.6)	87.5 (7.1)	175.7 (6.9)	110.2 (4.0)	91.8 (7.3)	86.0 (4.6)	94.2 (4.7)	84.2 (5.7)	109.8 (7.3)	99.9 (5.1)	96.7 (9.8)	101.3 (3.4)	95.7 (13.1)
buprenorphine	181.7 (3.9)	171.3 (7.5)	110.2 (8.8)	129.0 (12.7)	159.4 (2.2)	53.3 (26.0)	116.4 (22.5)	162.7 (5.5)	179.6 (1.6)	113.2 (4.2)	190.7 (7.4)	200.9 (8.4)	157.5 (4.1)	187.6 (8.2)	180.0 (4.8)
ibutamoren	136.1 (16.0)	127.8 (9.2)	119.5 (17.3)	143.2 (9.4)	137.6 (26.0)	115.5 (9.2)	99.2 (11.0)	114.7 (6.4)	118.0 (3.7)	109.9 (20.2)	123.7 (22.8)	141.8 (10.6)	118.0 (4.7)	128.3 (14.5)	123.8 (13.3)
betamethasone	106.6 (5.8)	104.0 (6.2)	114.9 (2.1)	87.4 (5.0)	144.8 (6.3)	105.9 (6.3)	82.7 (4.6)	91.2 (4.6)	97.7 (8.8)	85.4 (4.4)	110.1 (6.6)	106.0 (2.0)	97.5 (12.2)	99.0 (4.5)	95.4 (13.0)
furosemide	90.8 (3.6)	88.4 (2.4)	100.5 (3.8)	68.9 (9.8)	85.7 (2.7)	111.7 (3.2)	81.9 (5.7)	73.4 (4.1)	83.9 (4.4)	76.6 (10.3)	109.1 (8.4)	99.9 (4.4)	96.0 (10.2)	100.0 (1.2)	90.8 (18.4)
nebivolol	143.7 (24.5)	127.1 (14.3)	14.0 (33.9)	95.1 (13.7)	111.4 (38.5)	21.9 (7.3)	25.1 (17.4)	125.4 (8.1)	120.5 (9.5)	50.5 (9.0)	121.9 (31.3)	112.9 (15.0)	72.7 (9.3)	133.0 (21.7)	120.4 (12.3)
alprazolam	103.1 (4.2)	96.7 (2.6)	113.4 (0.5)	93.4 (11.6)	108.0 (14.0)	106.7 (2.2)	91.6 (4.6)	83.9 (4.3)	92.6 (3.9)	83.9 (6.1)	111.0 (8.0)	106.4 (3.4)	97.4 (10.8)	100.7 (2.9)	94.2 (16.3)
anastrozole	107.9 (4.2)	103.3 (2.1)	115.0 (2.0)	90.7 (12.8)	117.7 (6.9)	114.7 (3.3)	96.5 (3.9)	91.2 (3.3)	97.5 (5.1)	94.6 (10.1)	111.7 (8.5)	104.1 (3.5)	99.3 (7.3)	101.9 (3.0)	96.7 (16.2)
stanozolol	111.5 (7.3)	101.0 (1.8)	78.9 (29.8)	59.0 (15.5)	88.1 (14.7)	61.3 (10.0)	71.5 (5.9)	92.7 (2.6)	98.9 (2.9)	76.2 (18.7)	107.3 (10.5)	101.4 (7.8)	96.9 (5.3)	102.5 (10.3)	86.5 (25.3)
methadone	161.2 (5.7)	147.3 (15.7)	22.3 (36.3)	136.3 (14.2)	77.1 (29.1)	3.7 (45.4)	110.8 (29.0)	147.4 (4.2)	136.4 (5.0)	117.4 (14.1)	177.5 (15.9)	220.4 (9.5)	151.0 (6.9)	163.6 (6.2)	168.8 (6.3)
boldenone	96.5 (1.7)	93.0 (2.5)	106.4 (1.0)	72.1 (11.2)	99.9 (6.7)	106.6 (3.1)	55.3 (4.7)	72.6 (5.7)	91.2 (4.2)	71.3 (4.1)	109.3 (7.3)	105.5 (4.6)	96.9 (11.1)	101.3 (3.0)	94.4 (14.7)
clonazepam	102.2 (3.4)	97.1 (2.6)	107.6 (1.9)	76.1 (10.3)	99.7 (3.5)	110.4 (3.0)	73.6 (8.2)	81.3 (3.9)	92.9 (4.3)	82.6 (2.8)	109.9 (7.1)	106.4 (2.8)	98.0 (9.0)	101.9 (2.8)	96.4 (13.7)
nandrolone	114.0 (2.5)	109.9 (2.2)	117.7 (2.9)	75.4 (9.9)	115.8 (2.4)	115.3 (3.0)	88.8 (6.1)	85.1 (3.2)	104.0 (6.8)	91.7 (8.1)	112.2 (7.7)	96.3 (4.5)	100.2 (6.0)	103.0 (3.9)	98.8 (17.6)
methandienone	95.1 (2.2)	92.9 (1.9)	110.0 (3.4)	77.7 (11.6)	114.7 (4.7)	108.3 (3.1)	88.8 (4.2)	55.9 (8.8)	94.0 (3.3)	78.0 (3.2)	110.1 (7.8)	105.2 (5.2)	97.8 (8.1)	104.3 (5.7)	95.7 (15.6)
flunitrazepam	99.8 (5.9)	100.7 (2.3)	124.5 (5.2)	76.0 (10.0)	111.3 (3.4)	110.0 (2.1)	74.0 (4.6)	55.5 (8.8)	105.6 (4.9)	79.0 (3.3)	110.8 (8.9)	104.9 (3.5)	98.0 (11.1)	103.5 (4.5)	97.5 (14.4)
canrenone	66.1 (7.8)	65.9 (3.8)	90.5 (7.0)	84.4 (7.5)	70.9 (2.2)	94.9 (5.1)	86.8 (1.5)	59.2 (13.2)	56.2 (5.0)	81.4 (10.9)	111.4 (10.2)	105.3 (4.6)	102.0 (11.6)	102.1 (3.4)	91.6 (21.3)
THC-COOH	111.9 (6.2)	109.0 (5.9)	109.3 (0.6)	83.5 (14.9)	106.5 (6.0)	90.2 (3.7)	95.3 (5.1)	46.4 (20.0)	92.9 (2.5)	83.1 (27.5)	104.0 (12.6)	95.4 (16.8)	99.1 (17.0)	102.8 (2.9)	63.0 (59.3)
THC	232.1 (32.2)	202.2 (21.6)	11.0 (63.2)	80.1 (15.8)	181.8 (51.7)	43.4 (24.1)	125.7 (37.4)	194.8 (21.7)	239.0 (13.0)	58.8 (28.7)	228.7 (39.7)	88.4 (16.8)	94.2 (24.1)	257.3 (22.1)	149.4 (54.1)

Tab. B4 Drug recoveries [%] from oral fluid sample collection device swabs (data taken from Tab. B1) and presumptive drug recovery correction values [%] given in brackets – calculations based on matrix effects for each substance and device type (see Tab. B2).

substance	SalivaBio Oral Swab	Saliva Collection Swab (type I)	Saliva Collection Swab (type II)	Salivette®	Salivette® Cortisol	DCD™ 5000	Oracol	Versi•SAL™	Super•SAL™	Micro•SAL™	Accu•SAL™	Intercept® i2™	Oral-Eze®	Saliva Sampler®	Quantisal®
meldonium	17.5 (+47.1)	25.6 (+57.1)	88.7 (+44.2)	86.0 (+51.3)	54.2 (+39.7)	87.8 (+7.3)	46.3 (+30.3)	38.1 (+81.8)	71.1 (+15.9)	102.5 (+1.2)	76.7 (+12.5)	23.3 (+257.2)	105.0 (-12.9)	105.7 (-11.1)	104.2 (-5.2)
psilocybin	101.0 (-7.8)	101.9 (-3.8)	96.6 (-11.3)	333.3 (-247.6)	59.5 (-13.9)	93.1 (-10.8)	98.3 (+0.9)	98.6 (+7.4)	97.5 (+2.1)	99.5 (-3.7)	102.1 (-219.7)	484.0 (-409.5)	206.2 (-133.1)	114.6 (-20.8)	157.8 (-72.5)
morphine	98.1 (-5.2)	102.7 (-0.2)	116.6 (-12.5)	99.4 (-27.0)	64.0 (-9.3)	118.0 (-14.9)	83.7 (+19.2)	107.0 (+13.1)	115.3 (+2.1)	114.6 (+12.2)	109.4 (-30.5)	176.9 (-73.6)	130.8 (-41.0)	103.8 (-15.5)	112.5 (-21.9)
salbutamol	95.5 (-8.6)	97.7 (+0.7)	106.6 (-7.8)	81.2 (-0.1)	48.0 (+6.0)	107.8 (-8.4)	69.7 (+15.5)	96.6 (+16.4)	101.5 (+3.6)	85.4 (+26.4)	97.8 (-11.5)	116.0 (-24.8)	95.2 (-3.9)	96.5 (-6.3)	96.5 (-4.9)
terbutaline	90.5 (-8.7)	94.3 (-3.2)	104.8 (-10.4)	55.0 (+2.7)	36.7 (+1.1)	109.4 (-15.9)	64.0 (+23.1)	92.8 (+18.1)	102.0 (-3.1)	67.5 (+28.5)	86.7 (-11.6)	112.5 (-29.5)	80.8 (-0.1)	89.7 (-7.1)	93.6 (-8.8)
atenolol	134.6 (-27.1)	134.8 (-23.5)	146.6 (-38.8)	119.4 (-89.0)	87.8 (-17.9)	147.2 (-40.0)	95.1 (+6.5)	110.0 (+3.8)	138.4 (-23.3)	127.5 (-10.8)	119.9 (-96.5)	156.3 (-59.7)	127.0 (-32.6)	120.1 (-31.1)	131.2 (-33.7)
fenoterol	41.8 (+22.0)	58.0 (+11.7)	83.6 (+1.3)	35.0 (+17.9)	17.6 (-1.0)	84.9 (-7.5)	16.5 (+8.5)	47.6 (+43.6)	26.6 (+50.1)	36.4 (+63.0)	54.6 (+4.2)	78.3 (-8.7)	23.3 (+32.1)	50.5 (+17.2)	56.9 (+3.2)
nikethamide	91.1 (+2.4)	87.8 (+6.1)	94.7 (-8.0)	69.3 (+34.6)	84.5 (+7.4)	97.7 (-12.0)	48.7 (+27.3)	66.2 (+33.8)	75.5 (+33.5)	65.8 (+36.8)	99.2 (-7.2)	88.5 (+5.5)	76.3 (+47.6)	101.8 (+0.1)	100.6 (+5.0)
carteolol	103.0 (-4.7)	94.3 (-0.4)	121.6 (-21.8)	60.1 (+9.2)	34.1 (-3.4)	135.9 (-94.2)	66.4 (+13.1)	61.2 (+45.6)	59.6 (+51.7)	65.0 (+45.7)	99.9 (-29.5)	138.5 (-41.4)	87.2 (-0.8)	100.4 (-25.3)	100.7 (-21.8)
oxycodone	121.9 (-17.8)	113.0 (-14.3)	147.0 (-48.6)	79.2 (-6.5)	69.8 (-24.0)	168.8 (-67.7)	96.9 (+8.9)	84.4 (+32.2)	46.5 (+51.5)	79.7 (+38.0)	160.3 (-73.9)	178.8 (-79.5)	133.8 (-16.7)	168.0 (-77.3)	168.6 (-67.2)
amphetamine	90.2 (+3.5)	94.7 (+9.6)	108.6 (+5.2)	71.8 (+22.5)	15.9 (+2.7)	93.8 (+0.9)	65.4 (+7.9)	79.6 (+89.9)	47.2 (+52.3)	72.2 (+55.4)	105.3 (-18.5)	104.0 (-8.7)	51.2 (-7.5)	97.2 (-11.7)	98.1 (-7.5)
chlorothiazide	119.6 (-11.2)	121.6 (-8.9)	117.5 (-19.4)	120.8 (-6.3)	129.3 (-27.3)	105.5 (-14.6)	48.8 (-13.3)	119.0 (+25.2)	110.2 (+18.4)	146.8 (-24.1)	103.2 (-10.1)	77.8 (+10.7)	95.2 (+1.0)	107.5 (-1.3)	109.4 (+2.2)
hydrocodone	118.3 (-2.3)	112.2 (-0.8)	150.5 (-51.6)	90.6 (-13.7)	57.1 (-14.6)	185.2 (-78.2)	74.4 (+62.5)	96.9 (+29.9)	64.7 (+53.4)	111.8 (+26.9)	174.5 (-80.9)	198.6 (-89.2)	165.7 (-61.8)	190.0 (-78.6)	212.3 (-88.2)
methamphetamine	138.2 (-18.4)	137.1 (-14.2)	151.8 (-15.7)	110.3 (-12.6)	25.9 (-1.6)	137.3 (-19.8)	52.0 (+17.1)	134.6 (+24.1)	99.1 (+49.9)	111.1 (+11.5)	141.7 (-51.8)	147.5 (-51.4)	123.7 (-35.8)	129.2 (-40.2)	135.3 (-38.8)
methylhexanamine	102.3 (+0.1)	107.1 (+6.0)	117.7 (+6.6)	94.0 (+5.1)	16.3 (+1.8)	100.1 (+4.4)	63.6 (+18.9)	86.5 (+41.3)	75.3 (+51.9)	98.0 (+22.6)	117.9 (-30.3)	123.6 (-25.1)	105.6 (-16.8)	109.7 (-19.1)	111.8 (-16.9)
MDMA	89.9 (+10.8)	86.9 (+13.5)	117.6 (-7.1)	71.9 (+8.4)	24.1 (-1.7)	143.8 (-27.3)	31.5 (+22.6)	59.5 (+58.1)	33.5 (+67.5)	65.3 (+36.9)	141.1 (-56.7)	150.4 (-57.9)	127.6 (-43.8)	133.0 (-47.1)	135.5 (-44.4)
hydrochlorothiazide	94.2 (+5.7)	97.0 (+12.1)	100.9 (-8.6)	89.2 (+32.3)	105.2 (-4.5)	91.7 (-12.8)	12.7 (+5.1)	89.5 (+40.1)	95.9 (+41.4)	99.5 (+12.6)	89.7 (-6.8)	73.6 (+10.9)	78.4 (+8.6)	93.0 (-1.7)	93.9 (+7.5)
strychnine	168.2 (-12.9)	163.4 (-8.2)	204.7 (-76.7)	65.3 (+6.6)	23.6 (-2.0)	202.5 (-79.8)	34.8 (+41.4)	56.6 (+104.8)	129.3 (+125.8)	108.7 (+64.7)	185.2 (-101.5)	275.5 (-150.6)	151.3 (-92.5)	218.6 (-107.9)	218.2 (-107.4)
ketamine	60.9 (+22.5)	65.6 (+28.0)	78.7 (+5.7)	53.3 (+52.0)	21.9 (+0.7)	91.9 (-8.1)	53.4 (+22.0)	42.5 (+67.1)	26.8 (+57.8)	55.7 (+43.8)	96.9 (-13.7)	102.8 (-7.4)	90.4 (-1.7)	95.6 (-5.4)	101.5 (-2.5)
metoprolol	58.6 (+37.5)	63.9 (+43.2)	82.6 (+6.4)	66.9 (+26.4)	22.8 (+0.2)	121.9 (-24.9)	52.9 (+21.7)	44.0 (+75.8)	46.7 (+50.6)	66.4 (+41.9)	126.3 (-40.0)	139.1 (-44.3)	120.2 (-29.9)	119.9 (-33.2)	121.6 (-29.6)
clenbuterol	53.0 (+39.8)	58.6 (+43.5)	77.3 (+12.1)	55.6 (+24.3)	12.5 (+1.5)	109.3 (-20.8)	14.6 (+6.5)	42.3 (+78.7)	52.9 (+43.9)	56.9 (+29.5)	102.6 (-28.0)	127.7 (-34.5)	97.1 (-19.6)	96.5 (-21.3)	96.1 (-18.0)
methylphenidate	112.4 (-31.0)	132.8 (-24.0)	151.2 (+26.5)	168.8 (-73.2)	22.8 (-7.5)	145.8 (-10.3)	109.7 (-13.5)	78.8 (+69.3)	172.5 (+25.1)	185.8 (+89.4)	256.6 (-190.8)	344.4 (-202.2)	215.1 (-71.9)	212.4 (-61.4)	204.0 (-71.3)
heroin	87.5 (+16.3)	97.0 (+23.2)	108.7 (+0.9)	100.0 (-12.5)	35.3 (-11.6)	115.8 (-18.6)	77.0 (+14.0)	87.8 (+72.8)	116.2 (+15.0)	115.2 (+10.5)	125.7 (-36.4)	156.8 (-43.8)	121.6 (-19.5)	122.0 (-22.9)	128.2 (-19.9)
zolpidem	32.6 (+44.6)	37.3 (+52.3)	48.8 (+25.7)	30.7 (+30.0)	15.5 (+2.0)	91.3 (-7.6)	14.4 (+8.1)	28.8 (+71.6)	38.9 (+41.6)	46.1 (+22.2)	75.8 (-9.9)	79.0 (-6.8)	73.4 (-0.7)	78.2 (-2.3)	82.4 (-0.2)
cocaine	76.4 (+52.4)	83.4 (+59.6)	189.6 (+32.4)	108.1 (-7.0)	33.3 (-5.8)	192.1 (-43.5)	66.2 (+27.9)	61.0 (+92.7)	91.9 (+56.9)	118.6 (+4.6)	248.1 (-129.3)	332.7 (-185.8)	229.4 (-100.5)	232.3 (-96.9)	230.7 (-100.4)
LSD	16.5 (+41.7)	20.5 (+50.7)	34.6 (+25.8)	19.3 (+18.9)	2.8 (+1.5)	64.6 (-5.4)	4.6 (+2.2)	17.5 (+71.0)	19.8 (+39.2)	23.7 (+25.4)	37.1 (-3.9)	68.9 (-4.9)	42.7 (-0.3)	46.8 (-1.9)	62.6 (+0.2)
bisoprolol	102.2 (+28.9)	107.3 (+28.8)	127.4 (-14.5)	158.7 (-48.6)	27.9 (-7.0)	157.8 (-50.6)	108.4 (+4.8)	107.2 (+31.1)	108.5 (+5.2)	121.1 (-2.3)	160.7 (-71.8)	186.9 (-83.4)	157.0 (-63.2)	148.4 (-60.1)	153.8 (-58.9)
phencyclidine	223.3 (-68.3)	228.2 (-77.5)	254.8 (-85.5)	195.6 (-96.1)	18.1 (-5.6)	155.0 (+3.5)	16.8 (+5.9)	225.6 (-91.3)	228.8 (-97.7)	117.4 (-50.3)	238.4 (-158.7)	300.1 (-205.1)	225.6 (-144.5)	213.7 (-136.4)	222.4 (-138.9)
propranolol	45.1 (+24.1)	55.8 (+32.3)	82.0 (+116.0)	48.9 (+8.2)	2.8 (+3.5)	51.5 (+56.2)	3.4 (+4.5)	60.0 (+38.3)	70.5 (+9.1)	21.1 (+17.4)	59.7 (-11.7)	87.6 (-20.7)	54.0 (-5.5)	57.6 (-7.3)	47.6 (-5.1)
prednisolone	89.7 (+7.6)	93.7 (+11.5)	92.1 (-4.3)	84.4 (+26.2)	128.7 (-51.7)	97.5 (-7.9)	44.1 (+12.6)	88.2 (+18.8)	91.0 (+8.0)	76.5 (+28.5)	94.7 (-6.6)	91.9 (+2.2)	98.1 (+4.9)	97.5 (-0.5)	97.6 (+5.5)
fentanyl	102.9 (+3.8)	113.9 (+3.5)	139.8 (-28.7)	76.1 (-0.9)	8.1 (-0.7)	88.6 (-3.9)	6.5 (+3.9)	129.8 (-5.8)	148.8 (-32.6)	34.6 (+9.7)	109.8 (-44.4)	161.8 (-65.9)	114.6 (-39.7)	105.4 (-36.6)	112.6 (-37.0)
prednisone	91.8 (-4.7)	95.5 (+3.2)	96.0 (-8.9)	95.4 (+13.6)	143.1 (-61.6)	102.6 (-9.5)	100.8 (+9.0)	90.7 (+14.8)	92.8 (+5.7)	92.7 (+17.4)	97.1 (-8.6)	95.5 (+0.1)	97.7 (+3.4)	98.1 (-1.2)	99.2 (+4.5)
buprenorphine	89.5 (-40.3)	105.0 (-45.7)	109.7 (-10.2)	58.0 (-19.0)	5.0 (-1.9)	40.1 (+95.1)	7.5 (-1.1)	117.7 (-45.3)	108.1 (-47.9)	61.6 (-7.2)	74.3 (-35.3)	128.2 (-64.4)	80.0 (-29.2)	78.2 (-32.8)	64.9 (-28.6)
ibutamoren	71.6 (-19.0)	85.0 (-18.5)	110.0 (-18.0)	56.2 (-17.0)	3.4 (-0.9)	56.9 (-7.6)	9.5 (+0.1)	95.2 (-12.2)	83.6 (-12.7)	40.1 (-3.6)	40.7 (-7.8)	76.2 (-22.5)	46.1 (-7.0)	43.8 (-9.7)	42.7 (-8.2)
betamethasone	98.4 (-6.1)	100.6 (-3.9)	103.3 (-13.4)	88.1 (+12.7)	85.1 (-26.3)	95.9 (-5.3)	22.9 (+4.8)	94.2 (+9.0)	95.6 (+2.2)	89.0 (+15.2)	92.6 (-8.5)	94.6 (-5.4)	93.5 (+2.4)	93.0 (+1.0)	88.4 (+4.3)
furosemide	80.6 (+8.2)	83.3 (+11.0)	88.2 (-0.5)	69.2 (+31.3)	77.9 (+13.0)	98.4 (-10.3)	2.2 (+0.5)	74.2 (+26.9)	83.1 (+15.9)	76.4 (+23.3)	100.5 (-8.4)	90.4 (+0.1)	100.9 (+4.3)	103.6 (±0.0)	102.6 (+10.4)
nebivolol	36.8 (-11.2)	39.0 (-8.3)	48.5 (+297.8)	18.9 (+1.8)	3.3 (-0.3)	26.0 (+92.7)	0.5 (+1.6)	59.9 (-12.1)	44.6 (-7.6)	7.1 (+7.0)	24.0 (-4.3)	27.2 (-3.1)	24.4 (+9.2)	33.8 (-8.4)	21.2 (-3.6)
alprazolam	87.2 (-2.6)	90.1 (+3.0)	91.5 (-10.8)	85.3 (+6.8)	50.1 (-3.7)	88.9 (-5.6)	22.3 (+2.1)	83.5 (+16.0)	85.1 (+6.8)	74.5 (+14.3)	94.1 (-9.3)	93.1 (-5.6)	89.6 (+2.4)	93.7 (-0.7)	91.3 (+5.6)
anastrozole	97.3 (-7.2)	100.7 (-3.2)	99.5 (-13.0)	96.8 (+9.8)	98.2 (-14.7)	100.4 (-12.9)	19.0 (+0.7)	99.8 (+9.6)	87.2 (+2.4)	93.3 (+5.3)	101.8 (-10.7)	100.5 (-3.8)	100.3 (+0.7)	102.0 (-1.9)	100.2 (-3.5)
stanozolol	60.0 (-6.2)	64.8 (-0.6)	77.4 (+20.7)	13.9 (+9.8)	7.5 (+1.0)	66.6 (+41.9)	1.2 (+0.5)	77.5 (+6.1)	52.8 (+0.6)	24.8 (+7.8)	22.8 (-1.6)	39.5 (-0.6)	29.0 (+0.9)	37.0 (-0.9)	28.9 (+4.5)
methadone	147.1 (-55.9)	161.4 (-51.8)	163.1 (+566.8)	69.6 (-18.5)	3.5 (+1.0)	40.6 (+1048.3)	10.2 (-1.0)	157.9 (-90.8)	135.4 (-36.1)	32.2 (-4.8)	77.7 (-33.8)	122.3 (-66.8)	76.1 (-25.7)	69.8 (-27.2)	69.3 (-28.5)
boldenone	84.4 (+3.0)	86.7 (+6.5)	90.1 (-5.4)	75.6 (+29.3)	72.0 (+0.1)	91.4 (-5.6)	7.8 (+6.3)	79.8 (+30.1)	84.9 (+8.2)	74.1 (+29.9)	90.8 (-7.7)	92.8 (-4.9)	90.3 (+2.9)	90.7 (-1.1)	87.8 (+5.2)
clonazepam	90.0 (-1.5)	93.5 (+2.8)	95.5 (-6.8)	71.7 (+22.5)	72.6 (+0.2)	93.3 (-8.8)	3.4 (+1.2)	89.8 (+20.7)	90.0 (+6.5)	78.5 (+18.5)	93.7 (-8.5)	93.9 (-5.7)	92.0 (+1.9)	94.3 (-1.8)	91.9 (+3.5)
oxandrolone	101.0 (-12.4)	103.9 (-9.4)	100.3 (-15.1)	84.5 (+27.6)	87.5 (-12.0)	87.7 (-11.6)	11.1 (+1.4)	104.7 (+18.3)	96.1 (-3.7)	91.7 (+8.3)	82.0 (-8.9)	108.1 (+4.1)	83.2 (-8.1)	87.5 (-2.6)	85.5 (+1.1)
methandienone	88.4 (+4.6)	89.4 (+6.9)	91.7 (-8.3)	77.3 (+22.2)	76.6 (-9.8)	88.0 (-6.7)	10.7 (+1.4)	72.4 (+57.0)	85.6 (+5.4)	73.9 (+20.8)	85.1 (-7.8)	89.5 (-4.4)	88.7 (+2.0)	88.9 (-3.6)	86.7 (+3.9)
flunitrazepam	88.0 (+0.2)	98.6 (-0.7)	104.9 (-20.6)	77.1 (+24.4)	85.1 (-8.4)	94.2 (-8.6)	4.1 (+1.4)	69.2 (+55.6)	92.8 (-4.9)	73.4 (-19.5)	93.5 (-9.1)	99.6 (-4.4)	98.0 (+2.0)	96.9 (-3.3)	95.3 (+2.5)
canrenone	61.1 (+31.4)	71.2 (+36.8)	86.0 (+9.0)	70.4 (+13.0)	43.0 (+17.7)	84.8 (+4.6)	1.4 (+0.2)	67.4 (+46.4)	60.2 (+46.9)	56.4 (+12.9)	83.4 (-8.5)	81.5 (-4.1)	77.3 (-1.5)	81.3 (-1.7)	76.0 (+7.0)
THC-COOH	23.6 (-2.5)	27.9 (-2.3)	67.3 (-5.7)	7.3 (+1.4)	10.7 (-0.7)	80.4 (+8.7)	0.4 (±0.0)	28.0 (+32.3)	22.4 (+1.7)	25.3 (+5.1)	57.7 (-2.2)	59.6 (+2.5)	55.1 (+0.5)	64.2 (-1.8)	68.2 (+40.6)
THC	3.6 (-2.0)	4.7 (-2.4)	5.2 (+42.0)	5.7 (+1.4)	0.8 (-0.4)	9.2 (+12.0)	0.4 (-0.1)	12.3 (-6.0)	7.6 (-4.4)	1.5 (+1.0)	8.9 (-5.0)	7.4 (+1.0)	9.3 (+0.6)	17.3 (-10.6)	9.9 (-3.3)

Tab. B5 Drug recoveries [%] from Sarstedt Salivette® swabs – comparison of results recorded from spiked oral fluid (n=3) and spiked ultrapure water (n=4). Relative standard deviations (RSDs) [%] given in brackets.

substance	spiked oral fluid	spiked ultrapure water
morphine	89.2 (7.4)	99.4 (4.7)
salbutamol	87.1 (23.9)	81.2 (2.8)
terbutaline	85.2 (12.1)	55.0 (1.9)
atenolol	84.2 (23.7)	119.4 (3.6)
fenoterol	65.1 (11.2)	35.0 (5.9)
nikethamide	85.6 (12.1)	69.3 (1.8)
carteolol	66.3 (6.3)	60.1 (4.3)
oxycodone	63.7 (4.6)	79.2 (2.8)
amphetamine	78.8 (4.6)	71.8 (5.7)
chlorothiazide	108.1 (30.7)	120.8 (2.7)
hydrocodone	66.9 (7.5)	90.6 (2.8)
methamphetamine	74.1 (6.1)	110.3 (3.5)
methylhexanamine	91.7 (14.7)	94.0 (3.8)
MDMA	70.5 (2.6)	71.9 (5.6)
strychnine	63.2 (13.0)	65.3 (8.0)
ketamine	73.7 (9.6)	53.3 (4.0)
metoprolol	68.5 (4.1)	66.9 (5.4)
clenbuterol	69.3 (6.9)	55.6 (6.4)
heroin	77.1 (9.0)	100.0 (3.2)
zolpidem	66.8 (6.6)	30.7 (9.1)
cocaine	72.1 (5.4)	108.1 (9.3)
LSD	54.3 (10.5)	19.3 (18.4)
propranolol	61.9 (7.9)	48.9 (11.1)
prednisolone	94.7 (5.3)	84.4 (4.4)
fentanyl	87.4 (8.8)	76.1 (6.7)
prednisone	94.5 (5.6)	95.4 (1.1)
buprenorphine	84.4 (7.8)	58.0 (6.7)
ibutamoren	82.2 (13.0)	56.2 (9.8)
betamethasone	80.4 (21.7)	88.1 (3.4)
furosemide	74.4 (21.6)	69.2 (4.5)
nebivolol	14.3 (29.6)	18.9 (10.1)
alprazolam	85.7 (4.0)	85.3 (4.6)
anastrozole	91.5 (12.0)	96.8 (7.0)
stanozolol	25.7 (25.3)	13.9 (9.0)
methadone	88.8 (8.9)	69.6 (4.2)
boldenone	90.3 (7.9)	75.6 (5.5)
clonazepam	82.4 (7.4)	71.7 (4.0)
nandrolone	84.7 (10.3)	84.5 (3.2)
methandienone	95.8 (6.8)	77.3 (5.1)
flunitrazepam	87.0 (6.6)	77.1 (3.7)
canrenone	88.2 (8.7)	70.4 (3.2)
THC-COOH	64.6 (13.4)	7.3 (6.8)
THC	16.4 (18.7)	5.7 (33.8)

7. Metody oczyszczania próbek materiałów biologicznych ze szczególnym uwzględnieniem metod mikroekstrakcyjnych

Najprostszą, a zarazem najczęściej stosowaną, metodą przygotowania próbek jest ich rozcieńczenie [63]. Jednak ze względu na to, że nie dochodzi wówczas do oczyszczenia próbki, nie jest to metoda zapewniająca najwyższą jakość wyników, zwłaszcza w przypadku badania złożonych materiałów biologicznych posiadających liczne składniki utrudniające analizę. Dla prowadzenia analiz z wykorzystaniem aparatury HPLC-ESI-MS/MS szczególnie niekorzystne są makrocząsteczki (np. białka i tłuszcze), a także sole (szczególnie nielotne, takie jak fosforany [92]) i elektrolity obecne we wszystkich płynach ustrojowych [93]. Poza prozaiczną niedogodnością w postaci konieczności częstszego czyszczenia zabrudzonych instrumentów analitycznych, niepożądane elementy materiałów biologicznych stosowanych do badań pogarszają także samą jakość uzyskiwanych wyników poprzez obniżanie lub podwyższanie sygnału rejestrowanego przez detektor. Uzyskiwane wówczas wyniki stają się niedokładne (to znaczy odbiegają od wartości rzeczywistej), a zjawisko to znane jest jako tzw. *efekt matrycy* (ang. *matrix effect*) [94]. Główną przyczyną występowania efektu matrycy jest obecność w płynach ustrojowych fosfolipidów (w tym produktów ich fragmentacji takich jak nielotne reszty fosforanowe) [95], a także licznych jonów konkurujących z analitami o elektrony podczas procesu jonizacji [96].

Aby przezwyciężyć opisane problemy stosowane są metody ekstrakcyjne służące oddzieleniu analitu od zawartych w próbce zanieczyszczeń. Wśród trzech najbardziej rozpowszechnionych metod ekstrakcyjnych znajdują się ekstrakcja ciecz-ciecz (LLE), ekstrakcja do fazy stałej (SPE, ang. *solid-phase extraction*), oraz mikroekstrakcja do fazy stałej (SPME) [63]. Biorąc pod uwagę stopień oczyszczenia próbki, SPE zapewnia lepsze rezultaty od LLE, natomiast najlepsze rezultaty można uzyskać z wykorzystaniem metod mikroekstrakcyjnych takich jak SPME [97-98]. Wynika to z unikatowego mechanizmu działania SPME, w ramach którego urządzenie ekstrakcyjne umieszczane jest w próbce (lub nad próbką w przypadku analizy związków lotnych), następnie po związaniu analitów jest zabierane z próbki. Wyekstrahowane związki są wówczas poddawane desorpcji. Podczas procesu nie dochodzi do ekstrakcji makromolekuł takich jak peptydy i białka [99-101], a ekstrakcja

szczególnie niepożądanych fosfolipidów jest marginalna [98]. W przypadku faz ekstrakcyjnych wiążących analizowane substancje za pomocą mechanizmu adsorpcji (większość pokryć stosowanych w SPME, zawierających m.in. grupy funkcyjne: diwinylobenzenową (DVB, ang. *divinylbenzene*); polidimetylosiloksanową (PDMS, ang. *polydimethylsiloxane*); oraz oktadecylową (C_{18}) [102]) nie dochodzi również do ekstrakcji jonów metali, a zatem obecne w próbkach biologicznych elektrolity ulegają oddzieleniu od analitów. Wobec eliminacji najistotniejszych czynników generujących efekt matrycy [94-95], dla próbek oczyszczonych za pomocą SPME uzyskiwane są bardzo dokładne wyniki. Ewentualne utrzymujące się wzmocnienie lub osłabienie sygnału rejestrowanego przez detektor po zastosowaniu SPME można tłumaczyć wpływem innych substancji drobnocząsteczkowych, jest to jednak problem możliwy do rozwiązania z wykorzystaniem metod chromatograficznych. Głównym mankamentem metody SPME, w stosunku do LLE i SPE, jest niecałkowity odzysk analitów z próbki. Wynika on z niewyczerpującego mechanizmu ekstrakcji, która w przypadku SPME zachodzi poprzez dążenie do osiągnięcia stanu równowagi stężeń (analitu) pomiędzy fazami ekstrakcyjną (urządzenie SPME) a ekstrahowaną (próbka) [103]. Ten problem jest z kolei możliwy do rozwiązania przy odpowiedniej optymalizacji metody [98], czyli dobraniu odpowiednich do danej aplikacji parametrów SPME takich jak: rodzaj fazy ekstrakcyjnej, metoda desorpcji oraz warunki samego procesu desorpcji (m.in. czas trwania, temperatura procesu, a także intensywność mieszania próbki) [103].

Przytoczona powyżej charakterystyka podstawowych metod oczyszczania materiałów biologicznych wskazuje na zalety skłaniające do zastosowania metody mikroekstrakcyjnej – TFME (będąca modyfikacją SPME) – w badaniach prezentowanych w rozprawie doktorskiej. TFME (w porównaniu z SPME) zapewnia kilka korzyści szczególnie pożądanych przy analizie substancji niedozwolonych, m.in. większą powierzchnię fazy ekstrakcyjnej zapewniającą większy odzysk analitów, a zatem wyższą czułość metody [104], a także kompatybilność z wysokoprzepustowym standardem 96-dołkowych płytek (zapewniających możliwość przygotowywania jednocześnie 96 lub nawet większej ilości próbek) [105-110].

8. Nowe rozwiązania w mikroekstrakcji (część 1): Zastosowanie nowych rodzajów pokryć urządzeń TFME

Badania antydopingowe, podobnie jak oznaczanie środków odurzających, wymagają jednoczesnego monitorowania próbek w aspekcie obecności niezwykle dużej ilości substancji. Potencjalny cel stanowią tysiące różnorodnych związków chemicznych [51]. Wymusza to konieczność stosowania licznych, komplementarnych względem siebie metod analitycznych do wykonywania wyspecjalizowanych oznaczeń [36]. Wpływa to także na potrzebę wdrażania kompromisowych rozwiązań w trakcie procesu optymalizacji konkretnych metod. Wśród decyzji takich znajduje się m.in. wybór metody ekstrakcji (w tym konkretnego rodzaju faz ekstrakcyjnych) zapewniających ekstrakcję jak najszerszego spektrum analitów.

Jak zostało zauważone (również przez innych autorów), jednym z głównych ograniczeń wpływających na możliwość zastosowania metod mikroekstrakcyjnych w codziennej praktyce laboratoryjnej jest mała różnorodność dostępnych komercyjnie rodzajów pokryć (faz ekstrakcyjnych) takich urządzeń [111-112]. W ramach badań przedstawionych w rozprawie doktorskiej dokonano kompleksowego porównania 12 zróżnicowanych rodzajów pokryć, w większości nie stosowanych wcześniej w opracowanych protokołach mikroekstrakcyjnych. Analizowane pokrycia różnią się między sobą przede wszystkim typem oddziaływań międzycząsteczkowych zapewniających wiązanie analitów do urządzeń ekstrakcyjnych.

Kolejnym krokiem było porównanie właściwości cząsteczek stosowanych do wykonywania pokryć. Do tego badania wykorzystano wyłącznie jeden, najpopularniejszy rodzaj cząsteczek (z przyłączonymi grupami C_{18}), za to o różnych rozmiarach porównywanych cząsteczek. Zweryfikowano również wpływ grup pomocniczych, zapewniających większą kompatybilność faz ekstrakcyjnych z wodnym środowiskiem oczyszczanych próbek biologicznych.

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Original article

Modifying current thin-film microextraction (TFME) solutions for analyzing prohibited substances: Evaluating new coatings using liquid chromatography

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ABSTRACT

For identifying and quantifying prohibited substances, solid-phase microextraction (SPME) continues to arouse interest as a sample preparation method. However, the practical implementation of this method in routine laboratory testing is currently hindered by the limited number of coatings compatible with the ubiquitous high-performance liquid chromatography (HPLC) systems. Only octadecyl (C_{18}) and polydimethylsiloxane/divinylbenzene ligands are currently marketed for this purpose. To address this situation, the present study evaluated 12 HPLC-compatible coatings, including several chemistries not currently used in this application. The stationary phases of SPME devices in the geometry of thin film-coated blades were prepared by applying silica particles bonded with various functional ligands (C_{18}, octyl, phenyl-hexyl, 3-cyanopropyl, benzenesulfonic acid, and selected combinations of these), as well as unbonded silica, to a metal support. Most of these chemistries have not been previously used as microextraction coatings. The 48 most commonly misused substances were selected to assess the extraction efficacy of each coating, and eight desorption solvent compositions were used to optimize the desorption conditions. All samples were analyzed using an HPLC system coupled with triple quadrupole tandem mass spectrometry. This evaluation enables selection of the best-performing coatings for quantifying prohibited substances and investigates the relationship between extraction efficacy and the physicochemical characteristics of the analytes. Ultimately, using the most suitable coatings is essential for trace-level analysis of chemically diverse prohibited substances.

1. Introduction

Solid-phase microextraction (SPME) is an established and highly regarded sample preparation technique that has been successfully used for various applications [1], including the determination of prohibited substances such as drugs of abuse and doping agents [2,3]. However, for more than 30 years after its introduction in 1990 [4], the full potential of this method has yet been realized. In particular, the limited selection of commercially available coating chemistries limits the practical implementation of SPME in conjunction with liquid chromatography [5,6]. Currently, only octadecyl (C_{18}) and polydimethylsiloxane/divinylbenzene (PDMS/DVB) ligands are marketed as SPME stationary phases compatible with the ubiquitous high-performance liquid chromatography (HPLC) systems.

Thin-film microextraction (TFME) was proposed as an alternative format of SPME [7]; compared to SPME fibers, TFME increases the volume of the extracting phase and may improve both recovery and sensitivity for trace level analysis [8]. At the same time, the higher area-to-volume ratio means the extraction time was not extended. The first TFME devices were prepared using PDMS as the stationary phase [7]. However, PDMS is prone to swelling and phase-bleeding when introduced to some LC solvents [9]. In addition, PDMS was found to be unsuitable for the extraction of certain

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drugs (including some benzodiazepines) because of the slow kinetics of the process. Accordingly, C_{16}-amide or C_{18}-bonded silica particles were proposed as better SPME coatings for this application [10].

To date, only a handful of TFME coatings have been used for the extraction of prohibited substances. The published applications predominantly used C_{18} as the stationary phase [11–15]. However, other chemistries more suitable for extraction from aqueous biological samples were also investigated. Examples include mixed-mode C_{18} (C_{18} and benzenesulfonic acid), polar end-capped C_{18}, polar enhanced polystyrene (PS)-DVB, hydrophilic-lipophilic balance (HLB), and phenylboronic acid (PBA) [16]. Several studies that compared these chemistries have produced diverse results. C_{18} and HLB coatings were evaluated for the extraction of doping agents (β-blockers and β_2-agonists) from plasma and urine. The results of this study are favorable for the HLB coating [17]. PBA and PS-DVB were used for the extraction of several drugs (including benzodiazepines) from the plasma. A greater efficacy for benzodiazepines was achieved with PS-DVB [18]. Another study evaluated C_{18}, HLB, and PS-DVB coatings for the extraction of prohibited substances from plasma. The HLB coating performed the best in terms of the greatest extraction efficacy, and no significant carry-over was observed [19]. A more comprehensive study examined four coatings (C_{18}, mixed-mode C_{18}, PBA, and PS-DVB) for the extraction of 110 doping agents from urine. The authors concluded that the C_{18} coating performed best in terms of the highest efficacy and lowest carry-over effect [20]. The most recent study compared as many as eight different coatings (graphene, graphene oxide, multiwalled carbon nanotubes (MWCNTs), carboxylated MWCNTs, C_{18}, HLB, PBA, and PS-DVB) for the extraction of various analytes, including some prohibited substances. Once again, the C_{18} coating was found to be superior for the extraction of nonpolar substances [21].

The present study focused on assessing those alternative stationary phases that are popular in LC applications, but have mostly not been evaluated as microextraction coatings. The experiment was conducted in two consecutive parts. Initially, the six homogenous coatings tested were C_{18}, octyl (C_8), phenylhexyl (Phe-Hex), 3-cyanopropyl (CN), benzenesulfonic acid (SCX), and unbonded silica (SIL). The C_{18} coatings served as a reference because of their popularity as microextraction coatings [11–17,19–21]. Based on the results acquired, mixed compositions were selected for the second part. The principle for selection of the mixed compositions was to combine different extraction mechanisms to achieve optimal extraction efficacy and the broadest possible analyte coverage. As a result, 12 LC-compatible coatings were evaluated for the extraction of 48 prohibited substances. The introduction of novel stationary phases was combined with the important advancements of the last few years; these include device biocompatibility achieved by incorporating biologically inert polyacrylonitrile (PAN) to immobilize the stationary phases, and high-throughput TFME blades, which improved processing time to under 2 min per sample in this study. Additionally, microextraction methods are known to allow the implementation of green analytical chemistry principles, including reduction of the required sample volume and consumption of organic solvents; microextraction also enables simultaneous sample collection, extraction, and analyte preconcentration [1,22,23]. In doping control and forensic applications, there are particular advantages to convenient tailored chemistry that can efficiently extract the structurally diverse analytes present at trace levels; these fields will therefore greatly benefit from microextraction methods. This study is an important step toward fulfilling this goal.

2. Materials and methods

2.1. Chemicals

2.1.1. Coating particles

In this study, five types of silica particles bonded with functional groups, as well as SIL, were evaluated as the stationary phases of TFME devices (Fig. 1).

Additionally, six coating compositions were created by combining nonpolar C_8 particles with polar SIL or less-nonpolar CN-type particles, which were characterized by different extraction mechanisms. For each mixed composition, three different proportions, namely, 3:1, 1:1, and 1:3 *(m/m)*, were tested, increasing the number of coatings in this comparison to 12 different types.

All particles were supplied by Phenomenex (Phenomenex Inc.; Torrance, CA, USA) and had very similar parameters to the silica (such as particle size and pore diameter), which enabled unbiased and credible comparison of the bonded functional groups. See Table 1 for further details.

2.1.2. Analytes

Forty-eight prohibited substances, either drugs of abuse and/or doping agents, were used to compare the coatings. The selection of analytes was based on the worldwide popularity of their misuse [24–26]. The drugs of abuse were cannabinoids, central-nervous-system stimulants, opioids, hallucinogens, and sedatives. In total, 21 analytes were in this category. Substances prohibited in sports according to the World Anti-Doping Agency [27] were selected based on the prevalence of doping offences involving their use in recent years [26]. Of the 42 selected doping agents, 27 were unique compounds and 15 were in common with the 21 drugs of abuse. A complete list of the analytes and their suppliers is presented in Table S1.

The test mixture for extraction was prepared by spiking analytical standards into LC-MS grade water to achieve the 50 μg/L concentration of each analyte. Following an approach that has previously been used successfully [28], water was used to mimic biofluids composed mainly of this solvent (such as plasma, oral fluid, or urine). This approach minimizes factors that could bias the results, in particular, the affinity of drug-protein binding, enzymatic activity, microbial activity, sample density, and sample ionic strength, all of which may differ between individual biological samples and may, in turn, affect the veracity of the results. The impact of blood hematocrit levels on the SPME method, for example, has been reported in the literature [29].

2.1.3. Other chemicals

The following chemical reagents were used in this work: acetonitrile (LC-MS grade; Chromasolv, Honeywell International Inc., Charlotte, NC, USA), ammonium hydroxide (LC-MS grade; Fluka, Honeywell International Inc., Charlotte, NC, USA), *N,N*-dimethylformamide (DMF; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), formic acid (LC-MS grade; Optima, Fisher Chemical, Thermo Fisher Scientific Inc., Waltham, MA, USA), 2-propanol (LC-MS grade; Chromasolv, Honeywell International Inc., Charlotte, NC, USA), methanol (LC-MS grade; Chromasolv, Honeywell International Inc., Charlotte, NC, USA), PAN (Aldrich, Merck KGaA, Darmstadt, Germany), and water (LC-MS grade; LiChrosolv, Merck KGaA, Darmstadt, Germany).

2.2. Preparation of TFME blades

The precut metal blade supports were purchased from PAS Technology (PAS Technology Deutschland GmbH, Magdala, Germany), each with 12 pins in a row. Prior to spraying, the blades

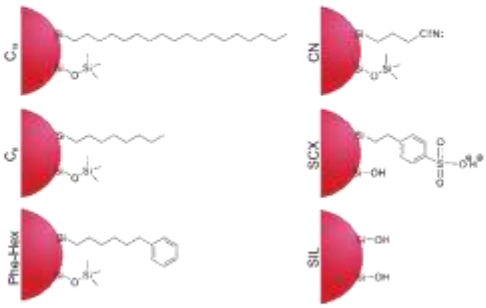

Fig. 1. Chemical structures of ligands bonded with silica particles. C_{18}: octadecyl; C_8: octyl; Phe-Hex: phenyl-hexyl; CN: 3-cyanopropyl; SCX: benzenesulfonic acid; SIL: unbonded silica.

were prepared by ultrasound-assisted etching in concentrated hydrochloric acid (Fluka, Honeywell International Inc., Charlotte, NC, USA) for 1 h. The process produced a black layer of anhydrous iron (III) chloride, which was carefully scrubbed to expose the prepared metal surface.

The tip of each pin (1 cm) was spray-coated with one of the preparations described in Section 2.1.1, following a protocol slightly modified from that of Mirnaghi et al. [12]. A coating slurry was prepared by dispersing the particles in DMF solution of PAN. The proportions used were constant for each type of coating, i.e., PAN:DMF:particles (1.000:18.380:2.375, *m/m/m*). The blades were covered in 10 thin-film layers; after application, each layer was cured immediately in a 110 °C oven for 3 min (180 °C for 2 min for the C_{18} particles).

2.3. Extraction method

All extractions were performed in 2 mL 96-well Deep-well Plates (Nunc, Thermo Fisher Scientific Inc., Waltham, MA, USA) and with a 96-well plate-compatible benchtop SH10 Heater-Shaker (Ingenieurbüro CAT, M. Zipperer GmbH; Ballrechten-Dottingen, Germany).

The extraction protocol comprised six steps: 1) first preconditioning (1 mL of methanol:water (50:50, *V/V*), 2 h, 850 min^{-1} agitation); 2) second preconditioning (1 mL of methanol:water (50:50, *V/V*), 0.5 h, 850 min^{-1} agitation); 3) first rinse (1 mL of water, 5 s, no agitation); 4) extraction (1 mL of test mixture, 2.5 h, 850 min^{-1} agitation); 5) second rinse (1 mL of water, 5 s, no agitation); and 6) desorption (1 mL of desorption solvent, 2 h, 850 min^{-1} agitation).

Eight desorption solvent (DS) compositions were used to optimize the desorption: DS1 = acetonitrile/water/formic acid (80:19.9:0.1, *V/V/V*); DS2 = acetonitrile:methanol:water:formic acid (40:40:19.9:0.1, *V/V/V/V*); DS3 = methanol:water:formic acid (80:19.9:0.1, *V/V/V*); DS4 = acetonitrile:2-propanol:methanol:water:formic acid (30:25:25:19.9:0.1, *V/V/V/V/V*); DS5 = acetonitrile:water:ammonium hydroxide (80:19.9:0.1, *V/V/V*); DS6 = acetonitrile:methanol:water:ammonium hydroxide (40:40:19.9:0.1, *V/V/V/V*); DS7 = methanol:water:ammonium hydroxide (80:19.9:0.1, *V/V/V*); and DS8 = acetonitrile:2-propanol:methanol:water:ammonium hydroxide (30:25:25:19.9:0.1, *V/V/V/V/V*). Each coating-desorption solvent combination was tested in triplicate.

2.4. HPLC-MS/MS method

All samples were analyzed with a Shimadzu LCMS-8060 triple quadrupole (Shimadzu Corporation, Kyoto, Japan) system fitted with an Agilent InfinityLab Poroshell 120 EC-C_{18} analytical column (3 × 100 mm, 2.7 μm) and guard column (3 × 5 mm, 2.7 μm) (Agilent; Santa Clara, CA, USA). Separations were run in gradient elution mode, with the column temperature fixed at 25.0 °C, and a 300 μL/min total flow rate of both mobile phases. Phase A comprised water with 0.1% formic acid, and phase B comprised acetonitrile with 0.1% formic acid. The gradient plot of phase B concentrations was as follows: 10% for 0.5 min, linearly increased to 100% (25.5 min), an isocratic hold at 100% (3 min), then 10% for column re-equilibration (6 min), totaling 35 min per sample. The resultant chromatograms of the analyzed substances under optimum conditions are presented in Fig. 2.

The high organic content (80%) of the desorption solvents used in this study was significantly higher than the starting composition of the conventional gradient elution reversed-phase HPLC method (mostly aqueous at the time of sample injection). As such, it can decrease retention of polar analytes. To address this issue, a preliminary test was performed to determine the maximal injection volume of the 50 μg/L analyte mixture in each variant of the desorption solvent used. As a result, an injection volume of 0.3 μL was established as an upper limit compatible with our HPLC

Table 1
Characteristics of the particles used for preparation of the stationary phases.

Particle	Particle type	Bonded ligand	End-capping	Total carbon load (%)	Surface coverage (μmole/m)	Silica particle parameters			Recommended applications	Main interaction mechanisms
						Particle size (μm)	Pore diameter (Å)	Surface area (m^2/g)		
C_{18}	Luna C_{18}	Octadecyl	Yes	16.38	3.01	8.37	104	381	Very hydrophobic compounds	Hydrophobic
C_8	Luna C_8	Octyl	Yes	12.60	3.95	8.57	103	399	Hydrophobic compounds	Hydrophobic
Phe-Hex	Luna PREP Phe-Hex	Phenyl with hexyl linker	Yes	15.09	2.67	9.94	104	384	Aromatic compounds and non-polar compounds	π-π (aromatic), hydrophobic, and dipole-dipole
CN	Luna CN	3-Cyanopropyl	Yes	7[a]	N/A	8.48	105	374	Polar compounds and −COOH, −CO, $-NH_2$, −NHR, or $-NR_2$ containing compounds	π-π, dipole-dipole, and hydrophobic
SCX	Luna SCX	Benzenesulfonic acid with ethyl linker	No	0.61	0.53	8.48	105	374	Positively charged compounds and amine and polyamine containing compounds	Ion-exchange, π-π (aromatic), and hydrophobic
SIL	Luna silica	None (unbonded silica)	No	–	–	8.37	104	381	Polar compounds	Hydrogen-bonding and ion-exchange

[a] Theoretical value (no experimental data available). –: no data; C_{18}: octadecyl; C_8: octyl; Phe-Hex: phenyl-hexyl; CN: 3-cyanopropyl; SCX: benzenesulfonic acid; SIL: unbonded silica.

method. The retention time of the analytes and ions monitored in tandem MS detection is listed in Table S1.

The extracts for the batch-to-batch reproducibility study were analyzed using a Shimadzu LCMS-8045 triple quadrupole system (Shimadzu Corporation; Kyoto, Japan). To compensate for the lower sensitivity of the instrument (in comparison with the Shimadzu LCMS-8060), the 0.4 mL aliquots of the extracts were evaporated dry using a CentriVap refrigerated concentrator with a −50 °C cold trap (Labconco; Kansas City, MO, USA) and reconstituted in 50 μL of LC-MS grade water. The injection volume for the reconstituted extracts was 1.6 μL, and all other HPLC-MS/MS parameters were the same as those used previously for the main study.

2.5. MS/MS data processing

The results of the experiments were presented as ratios created by stacking the mean signal measured for the sample ($n = 3$) against the mean measurement of the reference sample ($n = 4$). The reference sample comprised a portion of the same test mixture used for all extractions and was stored under identical conditions for the duration of the experiment. The reference sample was measured in quadruplicate with each batch of samples extracted with a certain type of coating.

Such a ratio would be synonymous with the extraction yield, if not for the open-bed configuration of the 96-well plates that enabled evaporation of the solvents during desorption at room temperature.

To mitigate the impact of the desorption solvent composition on the analyte ionization efficacy in the electrospray ion source, each ratio was additionally stacked against the signal measured from the analyte mixture spiked into the corresponding desorption solvent composition. Potential autosampler carry-over effects induced by the sample solvent composition change throughout the analysis (by elution of the analyte residue from the injection system resulting from introducing a stronger solvent) were also addressed and corrected.

2.6. Statistical analysis

To assess normality, the dataset was subjected to a log-transformation. Normal distribution was verified using the Kolmogorov-Smirnov test, Lilliefors test, and Shapiro-Wilk test.

One sample Student's *t*-test was used to determine which coating and desorption solvent were associated with the highest extraction efficacy relative to the reference established by the mean value recorded for the entire dataset. The null hypotheses were accepted for the following coating-desorption solvent combinations: C_8 (DS2–DS8), C_{18} (DS1, DS3–DS8), Phe-Hex (DS6), C_8 + CN (3:1) (DS1, DS5–DS8), C_8 + CN (1:1) (DS1–DS8), C_8 + CN (1:3) (DS1, DS5–DS8), and C_8 + SIL (3:1) (DS7). The acceptance of null hypotheses for these combinations signified that the recorded number of quantified analytes exceeded the reference point. This corresponds well with the results shown in Fig. 3. However, statistical analysis revealed that although the highest number of quantified analytes was obtained for the C_8 and C_{18} coatings, the most universal type of coating was C_8 + CN (1:1), as only the results obtained for the C_8 + CN (1:1) coating allowed the acceptance of the null hypothesis for every tested type of desorption solvent.

The dataset was analyzed using IBM SPSS Statistics for Macintosh, version 26.0. (IBM Corp., Armonk, NY, USA) and Statistica version 13 (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and discussion

3.1. Comparison of coatings

The large number of results generated in this research necessitated statistical analysis instead of a direct comparison; complete

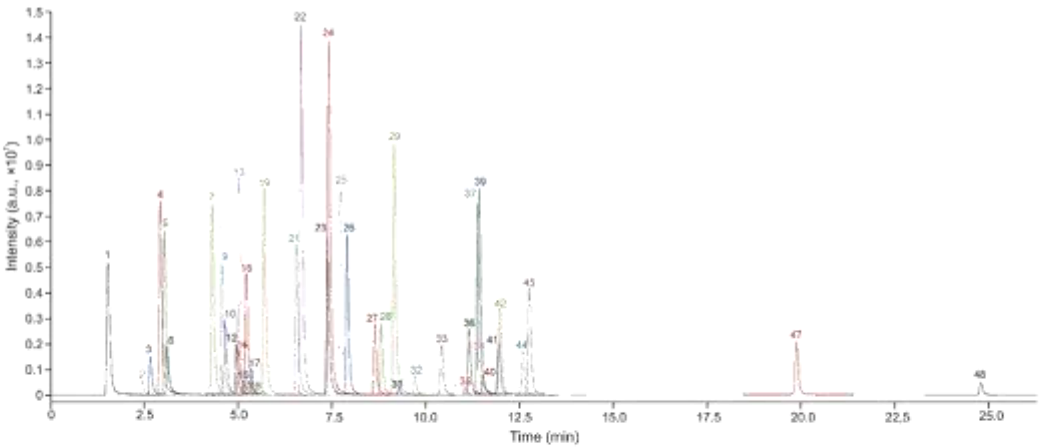

Fig. 2. Chromatogram of analyzed substances under optimum conditions. Order of the peaks: 1: meldonium; 2: psilocybin; 3: morphine; 4: salbutamol; 5: terbutaline; 6: atenolol; 7: fenoterol; 8: nikethamide; 9: carteolol; 10: amphetamine; 11: oxycodone; 12: hydrocodone; 13: methamphetamine; 14: chlorothiazide; 15: methylhexanamine; 16: 3,4-methylenedioxymethamphetamine; 17: strychnine; 18: hydrochlorothiazide; 19: ketamine; 20: metoprolol; 21: clenbuterol; 22: methylphenidate; 23: cocaine; 24: zolpidem; 25: lysergic acid diethylamide; 26: bisoprolol; 27: phencyclidine; 28: propranolol; 29: fentanyl; 30: prednisolone; 31: prednisone; 32: buprenorphine; 33: ibutamoren; 34: betamethasone; 35: furosemide; 36: nebivolol; 37: methadone; 38: alprazolam; 39: anastrozole; 40: stanozolol; 41: boldenone; 42: clonazepam; 43: nandrolone; 44: methandienone; 45: flunitrazepam; 46: canrenone; 47: 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol; 48: Δ^9-tetrahydrocannabinol.

tables with 4608 ratios and relative standard deviation (RSD) values are available in Tables S2 and S3. Subsequently, all results were divided into 8 segments corresponding to the 8 desorption solvent compositions tested. A direct comparison within such segments should not bias the outcome, as only identical solvents were characterized with identical solvent evaporation intensities during the desorption step, and with the same impact on the electrospray ionization efficacy. For each segment, the numbers of results (from 48 possible, the number of analytes tested) in the 2nd quartile (Q_2, upper half), 3rd quartile (Q_3, upper quarter), and the 90th percentile (P_{90}, in this case a single highest result) were assessed for each of the 96 compared coating-desorption solvent combinations, as shown in Table 2.

To further highlight the differences between the evaluated coatings, the results for all 8 segments were summarized within a single coating type. Fig. 3 shows a graphical representation of this approach.

The results showed that the C_8 and C_{18} coatings were superior in terms of the number of quantified analytes in the 2nd quartile, with 323 and 313 results, respectively. The highest count of 323 results in Q_2 for C_8 was 8.5 times higher than that of the overall worst-performing C_8 + SIL-type coatings. However, mixed coatings comprising C_8 + CN performed much better in all three proportions tested. With 285 results in Q_2, the coating comprising C_8 – CN (1:1) generated 7.5 times as many such results as did the C_8 + SIL (1:1) coating, with only 38 results.

Moreover, the coating comprising C_8 + CN (1:1) generated the greatest number of quantified analytes in both the 3rd quartile and 90th percentile. With 197 results in Q_3, this particular type of coating outperformed the C_8 type by 9 results (188 results) and the C_{18} type by 60 results (137). With 110 results in P_{90}, the C_8 + CN (1:1) coating surpassed the C_8 type by 23 results (87) and the C_{18} type by 69 results (41), which constituted more than a 2.5-fold difference.

The three best-performing coatings (C_8, C_{18}, and C_8 + CN (1:1)), in terms of efficacy, were similarly well repeatable and had similarly low coefficients of variation. For the C_8 coating, the median RSD was in the range of 1.6%–4.9%, depending on the analyte and desorption solvent used. For the C_{18} coating, the median RSD was in the range of 1.7%–7.1%, while for the C_8 – CN (1:1)-type coating, the median RSD was in the range of 1.5%–4.8%.

Certain analytical challenges were encountered in this study. In 23 out of the 96 tested coating-desorption solvent combinations, psilocybin was not extracted in a quantifiable amount for the analytical method used; no coating type was found to be sufficiently effective to enable its quantification after the attempted desorption to DS1. Furosemide, one of the analytes ionized in negative mode, could not be quantified after extraction with polar or less nonpolar coatings (CN, SCX, and SIL types) with desorption to any of the 8 tested desorption solvent compositions. This was probably due to the generally lower extraction efficacy of these coatings exacerbated by the presence of formic acid in HPLC mobile phases, which hindered the electrospray ionization in the negative mode. Hydrochlorothiazide, another analyte ionized in negative mode, could not be quantified after extraction with SCX coating and desorption to DS1 (also containing formic acid as an additive).

3.2. Extraction with C_8 + CN (1:1) coating

The coating comprising C_8 + CN (1:1) particles provided the greatest number of quantified analytes in both Q_3 and P_{90} and excelled in terms of the number of quantified analytes in Q_2 and in repeatability based on low RSD values. Therefore, of the 12 tested coatings, C_8 – CN (1:1) was the best composition for the extraction of the 48 commonly abused substances that were investigated in this study.

The C_8 particles provided hydrophobic-type interactions between the extracted analyte and extraction phase ligands. The CN particles provided π-π and dipole-dipole type interactions [30,31] as well as hydrophobic interactions. According to the hydrophobic-subtraction model [32–38], CN particles were characterized by a hydrophobicity parameter (H) nearly half as low as that of C_8: 0.45 vs. 0.88 [39], where both H values are relative to the typical C_{18}-bonded type-B silica particles [32]. C_8 particles are recommended for the extraction of hydrophobic compounds, while CN particles are best suited for the extraction of polar and certain functional group-containing (–COOH, –CO, $–NH_2$, –NHR, and $–NR_2$) compounds. With the mixed C_8 + CN (1:1) coating,

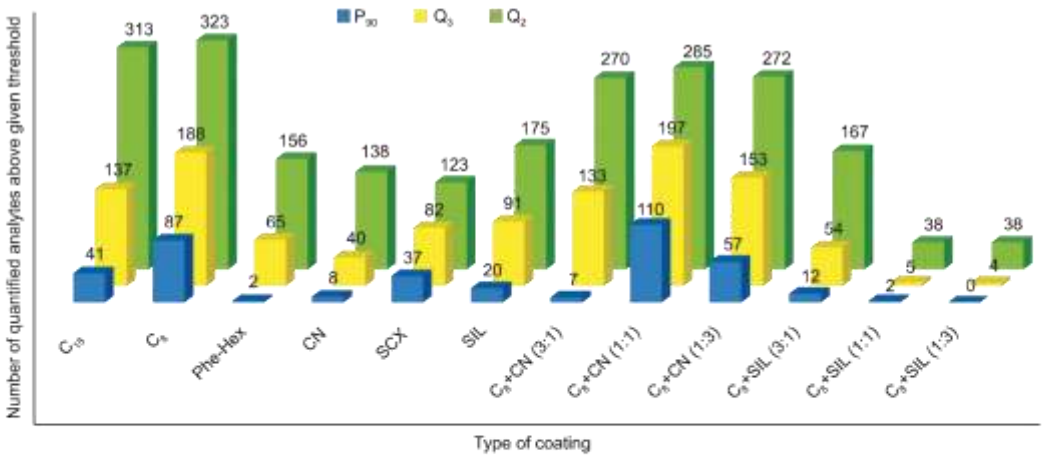

Fig. 3. Summarized number of quantified analytes in the 2nd quartile (Q_2), 3rd quartile (Q_3), and 90th percentile (P_{90}) for each coating type.

Table 2
Number of quantified analytes in the 2nd quartile (Q_2), 3rd quartile (Q_3), and 90th percentile (P_{90}) for each coating-desorption solvent combination.

Desorption solvent		Coating											
		C_{18}	C_8	Phe-Hex	CN	SCX	SIL	C_8 + CN (3:1)	C_8 + CN (1:1)	C_8 + CN (1:3)	C_8 + SIL (3:1)	C_8 + SIL (1:1)	C_8 + SIL (1:3)
DS1	Q_2	33	33	10	7	5	11	42	45	42	34	11	9
	Q_3	4	8	3	1	2	1	34	40	31	15	1	1
	P_{90}	2	1	0	0	0	1	3	28	7	4	1	0
DS2	Q_2	39	40	20	13	1	24	39	41	42	18	6	5
	Q_3	10	24	5	5	0	11	22	31	26	8	1	1
	P_{90}	3	11	0	1	0	3	0	24	5	1	0	0
DS3	Q_2	46	42	36	20	2	19	37	34	29	16	4	3
	Q_3	25	31	20	5	0	10	14	23	8	5	2	1
	P_{90}	7	13	1	2	0	3	1	15	5	1	0	0
DS4	Q_2	44	44	19	19	3	27	29	32	38	22	4	7
	Q_3	27	26	8	7	0	21	8	21	16	9	0	1
	P_{90}	9	7	0	2	0	11	0	13	5	1	0	0
DS5	Q_2	41	35	13	18	28	24	37	35	31	18	5	3
	Q_3	16	13	6	5	23	14	16	27	21	2	1	0
	P_{90}	9	0	0	0	17	0	0	16	4	1	1	0
DS6	Q_2	40	42	22	22	28	24	30	36	28	15	0	1
	Q_3	20	29	7	4	18	11	14	20	19	2	0	0
	P_{90}	5	16	1	1	6	1	1	2	14	1	0	0
DS7	Q_2	25	42	11	15	27	19	42	36	36	24	6	5
	Q_3	7	19	2	6	15	8	24	29	26	8	0	0
	P_{90}	2	10	0	1	5	0	2	9	17	2	0	0
DS8	Q_2	45	45	25	24	29	27	14	26	26	20	2	5
	Q_3	28	38	14	7	24	15	1	6	6	5	0	0
	P_{90}	4	29	0	1	9	1	0	3	0	1	0	0

Compositions of desorption solvents: DS1 = acetonitrile/water/formic acid (80:19.9:0.1, *V/V/V*); DS2 = acetonitrile:methanol:water:formic acid (40:40:19.9:0.1, *V/V/V/V*); DS3 = methanol:water:formic acid (80:19.9:0.1, *V/V/V*); DS4 = acetonitrile:2-propanol:methanol:water:formic acid (30:25:25:19.9\0.1, *V/V/V/V/V*); DS5 = acetonitrile:water:ammonium hydroxide (80:19.9:0.1, *V/V/V*); DS6 = acetonitrile:methanol:water:ammonium hydroxide (40:40:19.9:0.1, *V/V/V/V*); DS7 = methanol:water:ammonium hydroxide (80:19.9:0.1, *V/V/V*); DS8 = acetonitrile:2-propanol:methanol:water:ammonium hydroxide (30:25:25:19.9:0.1, *V/V/V/V/V*).

moderate (Pearson's coefficients (r) in the range of 0.300–0.500) to strong (r > 0.500) two-way significant correlations could be observed between the analyte pK_a value and its extraction efficacy, and between the analyte logP value and its extraction efficacy.

With logP values calculated by ChemAxon software [40], correlations (r) were in the range of 0.421–0.536 (n = 45, P < 0.004; median = 0.500, mean = 0.490) for the C_8 + CN (1:1) coating. While the correlations were higher for the hydrophobic C_8 coating with values in the range of 0.627–0.790 (n = 45, P < 0.001; median = 0.755, mean = 0.746), they were not observed (not significant for five out of eight desorption solvents) for the CN coating on its own, with r values in the range of 0.243–0.381 (n = 45, P < 0.108; median = 0.280, mean = 0.300).

The correlations between pK_a (strongest acidic) values [40] of the analytes and their extraction efficacy for the C_8 + CN (1:1) coating were strong, in the range of 0.661–0.738 (n = 33, P < 0.001; median = 0.700, mean = 0.698), while they were weaker for the CN (r = 0.523–0.631 (n = 33, P < 0.002; median = 0.557, mean = 0.573)) and C_8 (r = 0.442–0.542 (n = 33, P < 0.001; median = 0.481, mean = 0.483)) coatings on their own. Therefore, analytes with higher pK_a (strongest acidic) values were preferred by these coatings, especially by the 3-cyanopropyl group-containing C_8 + CN (1:1) and CN types. With higher pK_a (strongest acidic) values, these analytes were present either as cationic or neutral species during the extraction. To some degree, the increased interaction of the positively charged species with the 3-cyanopropyl ligands could be explained by the occurrence of the cation–π interactions [41], as the 3-cyanopropyl ligands are known to exert π interactions [31].

All correlations discussed in this section are summarized in Table 3.

3.3. Desorption from C_8 + CN (1:1) coating

Several factors affected the desorption from coatings prepared with two distinct types of particles. For C_8 ligands, the steric repulsion of the analytes was greater for methanol-based solvents than for acetonitrile-based solvents [42,43]. This phenomenon was clearly visible in the results of this study, with desorption being more effective in solvents containing 80% methanol than in those containing 80% acetonitrile (Table 2) with both formic acid (DS3 vs. DS1) and ammonium hydroxide (DS7 vs. DS5) as additives. Moreover, an acetonitrile-rich environment enabled analyte bonding by the stationary phase via both the adsorption mechanism (interaction of the analyte-solvent complex with the ligand) and the partition mechanism (analyte-ligand direct interaction), while methanol only enabled bonding by the partition mechanism [44]. In theory, this could further enhance desorption from C_8 particles to methanol-based solvents.

For CN particles, acetonitrile present in the desorption solvent should suppress π-π and dipole-dipole interactions between the ligands and analytes [31], enhancing desorption from this particular coating in comparison with methanol. However, the results of this study do not provide evidence for the significance of this phenomenon.

A comparison of the desorption efficacy of the mixed C_8 + CN (1:1) coating for each desorption solvent composition tested is shown in Fig. 4.

The results indicate that DS1 performed best in terms of the number of quantified analytes in Q_3 and P_{90}, but it was not possible to quantify psilocybin after desorption to this composition. Therefore, DS7 was the second best in terms of the number of quantified analytes in Q_3 and P_{90}, the best composition in terms of the number of quantified analytes in Q_2, and the best for versatility enabling quantification of every tested analyte.

In general, acidic or basic additives seemed to shift the preference between acetonitrile and methanol as the optimal solvents for desorption from this type of coating. With formic acid as an additive, the acetonitrile-based DS1 was superior to the methanol-based DS3. With ammonium hydroxide as an additive, the methanol-based DS7 was more effective than acetonitrile-based DS5, and a mixture of acetonitrile and methanol in even proportions (DS6) was between the two in terms of the number of quantified analytes in Q_2, Q_3, and P_{90}.

3.4. Batch-to-batch reproducibility of C_8 + CN (1:1) coatings

The inter-batch reproducibility of the C_8 + CN (1:1) coatings was assessed based on the extraction of the testing mixture with five individually prepared batches of TFME blades. Four samples were extracted for each batch and the RSD values were calculated. The evaluated coatings provided reproducible extraction efficacies with a median RSD value of 7.5% (n = 47, mean = 9.9%). Detailed results are presented in Table S4.

3.5. Impact of hydrophobicity

C_{18}, C_8, and Phe–Hex were the three most hydrophobic coatings in this study. According to the hydrophobic-subtraction model [32], the hydrophobicity parameter H is the greatest contributor to analyte retention [34,37]. Based on this model, the hydrophobicity of the column packing materials used for the preparation of TFME coatings is 1.00 for C_{18}, 0.88 for C_8, and 0.78 for Phe-Hex [39].

The three most nonpolar coatings also showed strong two-way significant correlations between the extraction efficacies and logP values of the analytes. The correlations were calculated with four datasets of logP values computed by the ACD/Labs [45], ALOGPS [40], ChemAxon [40], and XLogP 3.0 [46] programs.

The correlation coefficients (r) for the C_{18} coating with ACD/Labs data were in the range of 0.480–0.665 (n = 47, P < 0.001; median = 0.626, mean = 0.606), depending on the desorption solvent composition (calculated for all eight variants). The correlations computed with the ALOGPS data were in the range of 0.508–0.657 (n = 45, P < 0.001; median = 0.623, mean = 0.608), those with the XLogP data were in the range of 0.527–0.659 (n = 48, P < 0.001; median = 0.619, mean = 0.612), and the highest values were observed with data from ChemAxon in the range of 0.602–0.737 (n = 45, P < 0.001; median = 0.703, mean = 0.693).

Stronger correlations were observed for the C_8 coating, with Pearson's coefficients with ACD/Labs, ALOGPS, XLogP, and ChemAxon data in the range of 0.482–0.700 (n = 47, P < 0.001; median = 0.661, mean = 0.644), 0.540–0.713 (n = 45, P < 0.001; median = 0.680, mean = 0.667), 0.574–0.737 (n = 48, P < 0.001; median = 0.696, mean = 0.687), and 0.627–0.790 (n = 45, P < 0.001; median = 0.755, mean = 0.746), respectively. This outcome, a stronger analyte-hydrophilicity/extraction-efficacy correlation for the less-hydrophobic of the two compared alkyl ligands, might be somewhat explained by the S^* parameter, defined in the hydrophobic-subtraction model as "steric resistance to insertion of bulky solute molecules into the stationary phase" [33]. According to this model, the longer alkyl chain of the C_{18} ligand is less accessible to the analytes than the shorter C_8 chain. Moreover, C_8 particles had approximately 25% higher surface coverage than C_{18} particles (3.95 vs. 3.01 μmol/m) based on the certificates of analyses.

For the Phe-Hex coating, similar but slightly lower correlation coefficient values were observed than those for C_{18}. The data from ACD/Labs correlations produced an r value of 0.371–0.553 (n = 47, P < 0.011; median = 0.496, mean = 0.487), while data from ALOGPS gave r = 0.467–0.608 (n = 45, P < 0.002; median = 0.562,

Table 3
Correlations between the selected physicochemical properties of the analytes (pK_a (strongest acidic) and logP values) and their extraction efficacies with C_8 + CN (1:1), C_8, and CN coatings. Pearson's r values are presented, with P values given in brackets.

Coating	Desorption solvent	logP (n = 45)	pK_a (strongest acidic) (n = 33)
C_8 + CN (1:1)	DS1	0.468** (0.001)	0.708** (0.000)
	DS2	0.497** (0.001)	0.706** (0.000)
	DS3	0.521** (0.000)	0.694** (0.000)
	DS4	0.529** (0.000)	0.691** (0.000)
	DS5	0.444** (0.002)	0.661** (0.000)
	DS6	0.421** (0.004)	0.668** (0.000)
	DS7	0.503** (0.000)	0.718** (0.000)
	DS8	0.536** (0.000)	0.738** (0.000)
C_8	DS1	0.627** (0.000)	0.452** (0.008)
	DS2	0.751** (0.000)	0.451** (0.008)
	DS3	0.777** (0.000)	0.442* (0.010)
	DS4	0.790** (0.000)	0.476** (0.006)
	DS5	0.750** (0.000)	0.542** (0.001)
	DS6	0.754** (0.000)	0.513** (0.002)
	DS7	0.756** (0.000)	0.506** (0.003)
	DS8	0.759** (0.000)	0.485** (0.004)
CN	DS1	0.256 (0.090)	0.617** (0.000)
	DS2	0.360* (0.015)	0.631** (0.000)
	DS3	0.381** (0.010)	0.599** (0.000)
	DS4	0.332* (0.026)	0.560** (0.001)
	DS5	0.286 (0.057)	0.549** (0.001)
	DS6	0.273 (0.069)	0.553** (0.001)
	DS7	0.267 (0.077)	0.553** (0.001)
	DS8	0.243 (0.107)	0.523** (0.002)

Two-way significant correlation, *P < 0.05, **P < 0.01.

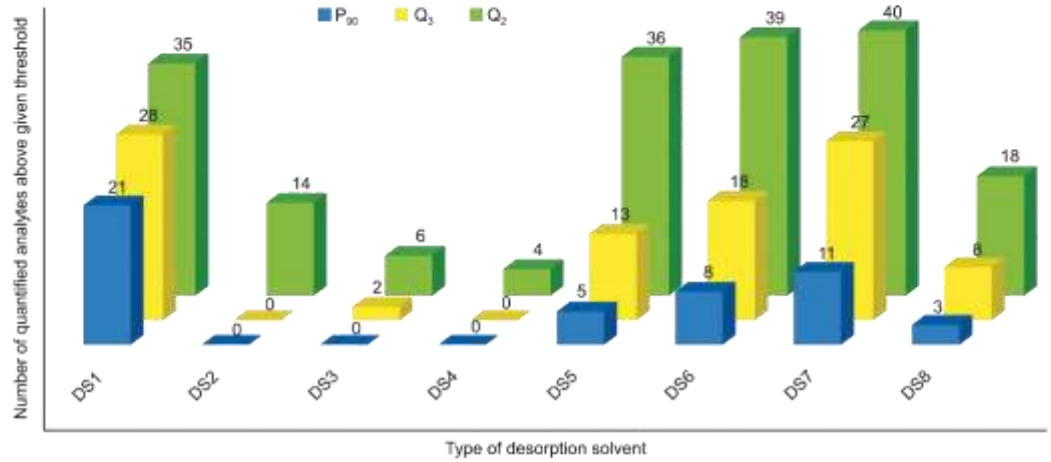

Fig. 4. Number of quantified analytes in the Q_2, Q_3, and P_{90} for the C_8 + CN (1:1) coating and every desorption solvent tested. Compositions of desorption solvents: DS1 – acetonitrile:water:formic acid (80:19.9:0.1, *V/V/V*); DS2 – acetonitrile:methanol:water:formic acid (40:40:19.9:0.1, *V/V/V/V*); DS3 – methanol:water:formic acid (80:19.9:0.1, *V/V/V*); DS4 – acetonitrile:2-propanol:methanol:water:formic acid (30:25:25:19.9:0.1, *V/V/V/V/V*); DS5 – acetonitrile:water:ammonium hydroxide (80:19.9:0.1, *V/V/V*); DS6 – acetonitrile:methanol:water:ammonium hydroxide (40:40:19.9:0.1, *V/V/V/V*); DS7 – methanol:water:ammonium hydroxide (80:19.9:0.1, *V/V/V*); DS8 – acetonitrile:2-propanol:methanol:water:ammonium hydroxide (30:25:25:19.9:0.1, *V/V/V/V/V*).

mean = 0.556), data from XLogP gave $r = 0.527–0.647$ ($n = 48$, $P < 0.001$; median = 0.590, mean = 0.593), and data from ChemAxon gave $r = 0.603–0.733$ ($n = 44$, $P < 0.001$; median = 0.688, mean = 0.683). The Phe-Hex ligand, although comprising 12 carbon atoms, had characteristics different from those of the C_{12} alkyl chain because of the presence of a phenyl group providing an additional π-π interaction mechanism between the ligand and the analyte. Phe-Hex ligands also had lower accessibility for analytes [38], which further complicates the direct comparison of the observed correlations with those for the C_8 and C_{18} alkyl ligands.

Table 4
Correlations between hydrophobicity of the analytes (determined by logP values) and their extraction efficacies with C_{18}, C_8, and Phe-Hex coatings. Pearson's r values are presented, with P values given in brackets.

Coating	Desorption solvent	logP dataset			
		XLogP3.0 ($n = 48$)**	ALOGPS ($n = 45$)**	ChemAxon ($n = 45$)**	ACD/Labs ($n = 47$)
C_{18}	DS1	0.527 (0.000)	0.508 (0.000)	0.602 (0.000)	0.480** (0.001)
	DS2	0.602 (0.000)	0.584 (0.000)	0.679 (0.000)	0.576** (0.000)
	DS3	0.639 (0.000)	0.630 (0.000)	0.724 (0.000)	0.629** (0.000)
	DS4	0.659 (0.000)	0.657 (0.000)	0.737 (0.000)	0.665** (0.000)
	DS5	0.615 (0.000)	0.617 (0.000)	0.699 (0.000)	0.622** (0.000)
	DS6	0.601 (0.000)	0.604 (0.000)	0.686 (0.000)	0.605** (0.000)
	DS7	0.623 (0.000)	0.628 (0.000)	0.706 (0.000)	0.630** (0.000)
	DS8	0.629 (0.000)	0.634 (0.000)	0.707 (0.000)	0.639** (0.000)
C_8	DS1	0.574 (0.000)	0.540 (0.000)	0.627 (0.000)	0.482** (0.001)
	DS2	0.708 (0.000)	0.677 (0.000)	0.751 (0.000)	0.656** (0.000)
	DS3	0.722 (0.000)	0.696 (0.000)	0.777 (0.000)	0.673** (0.000)
	DS4	0.737 (0.000)	0.713 (0.000)	0.790 (0.000)	0.700** (0.000)
	DS5	0.676 (0.000)	0.665 (0.000)	0.750 (0.000)	0.646** (0.000)
	DS6	0.691 (0.000)	0.679 (0.000)	0.754 (0.000)	0.660** (0.000)
	DS7	0.694 (0.000)	0.681 (0.000)	0.756 (0.000)	0.661** (0.000)
	DS8	0.697 (0.000)	0.687 (0.000)	0.759 (0.000)	0.674** (0.000)
Phe-Hex	DS1	0.527 (0.000)	0.467 (0.001)	0.603 (0.000)	0.371* (0.010)
	DS2	0.615 (0.000)	0.568 (0.000)	0.701 (0.000)	0.504** (0.000)
	DS3	0.591 (0.000)	0.533 (0.000)	0.667 (0.000)	0.458** (0.001)
	DS4	0.647 (0.000)	0.608 (0.000)	0.733 (0.000)	0.553** (0.000)
	DS5	0.588 (0.000)	0.570 (0.000)	0.693 (0.000)	0.501** (0.000)
	DS6	0.579 (0.000)	0.552 (0.000)	0.677 (0.000)	0.481** (0.001)
	DS7	0.587 (0.000)	0.556 (0.000)	0.682 (0.000)	0.490** (0.000)
	DS8	0.612 (0.000)	0.592 (0.000)	0.711 (0.000)	0.538** (0.000)

Two-way significant correlation, *$P < 0.05$, **$P < 0.01$.

All correlations discussed in this Section are summarized in Table 4.

Finally, it is worth considering that the C_{18}, C_8, Phe-Hex, and all mixed-coating compositions containing C_8 particles could have suffered to some degree from the stationary phase collapse phenomenon during the extraction step performed in 100% aqueous conditions, with which they were not compatible. This issue may explain the deviations between the experimental and theoretically anticipated extraction efficacies for these coatings, as previously described by Sobczak et al. [16].

3.6. Impact of ionic interactions

The acidity of the analyte determines the optimal pH value for the extraction and desorption steps of the microextraction method. Ionic interactions are present between the analyte and the ligands of the stationary phase, as well as between the desorption solvent and the extracted analyte. The pH value is especially important for coatings with extraction driven by ion-exchange mechanisms, such as strong cation exchange (SCX). The intensity of the ionic interactions is determined by whether the particles of the stationary phase are end-capped or not, with interactions more prominent for non-end-capped particles because of the free silanol groups present on their surface. In this evaluation, two coatings were prepared exclusively with non-end-capped particles: SCX and SIL. Ionic interactions, to some degree, also influence all other silica-based stationary phases, including those relying on hydrophobic interaction mechanisms, such as C_{18} [34–36] or C_8. An explanation for this is the incomplete substitution of residual silanols during the end-capping process resulting from the steric impedances [47]. Ionic interactions are especially high at a pH value of 7.0, owing to the ionization of both acidic silanols and basic solutes such as amines [48]. Usually, silanols interact only with cations, not with anions or uncharged molecules [49]. However, the interaction of ion-paired anions is still possible [50].

The SCX coating exhibits a strong contribution of silanols [51], most likely because of its low bonding density with benzenesulfonic acid ligands (only 0.53 μmol/m). The optimal sample pH value for this coating should be 2 units below the pK_a value of the analyte during the extraction, while the pH value of the desorption solvent should be 2 units above that value during the desorption step. The median pK_a (strongest basic) value of the tested analytes was 8.69 ($n = 44$) [40]; therefore, basic desorption solvent compositions with 0.1% ammonium hydroxide (DS5–DS8) provided much more effective desorption from the SCX coating than the corresponding (same solvent content) acidic compositions with 0.1% formic acid (DS1–DS4). In addition, the amine modifier (NH_4OH) may compete for ion-exchange sites with the analytes [52], aiding the desorption process. There was a strong correlation between the pK_a value of the analyte and the efficacy of its extraction with this coating, suggesting an increased extraction of more basic drugs. The correlation coefficients were in the range of 0.746–0.873 ($n = 44$, $P < 0.001$; median = 0.863, mean = 0.843).

Another stationary phase where ionic interactions provided the main extraction mechanism was the SIL, which acted through hydrogen bonds created by hydroxyl groups (−OH) and ionic bonds created by ionized silanols ($-O^{\ominus}$) [52]. Similar to the SCX coating, there was also a strong correlation between the pK_a value of the analyte and the efficacy of its extraction with silica-based coating. In this case, the correlation coefficients were in the range of 0.861–0.876 ($n = 44$, $P < 0.001$; median = 0.870, mean = 0.869). Therefore, with the SCX and SIL coatings, basic drugs are strongly preferred over acidic drugs. In this study, the extraction efficacies of the most basic drugs (buprenorphine (pK_a 12.54), phencyclidine (pK_a 10.56), and methamphetamine (pK_a 10.21) were, on average, over 14 times higher than the corresponding extraction efficacies for the most acidic drugs (THC (pK_a −4.90), THC-COOH (pK_a −4.90), and canrenone (pK_a −4.80) [40]. This could be explained by the high reactivity of silanols with basic compounds [53]. All correlations discussed in this Section are summarized in Table 5.

Table 5
Correlations between pK_a (strongest basic) of the analytes and their extraction efficacies with SCX and SIL coatings. Pearson's r values are presented, with P values given in brackets.

Coating	Desorption solvent	pK_a (strongest basic) ($n = 44$)**
SCX	DS1	0.859 (0.000)
	DS2	0.807 (0.000)
	DS3	0.746 (0.000)
	DS4	0.850 (0.000)
	DS5	0.873 (0.000)
	DS6	0.867 (0.000)
	DS7	0.869 (0.000)
	DS8	0.871 (0.000)
SIL	DS1	0.869 (0.000)
	DS2	0.876 (0.000)
	DS3	0.870 (0.000)
	DS4	0.875 (0.000)
	DS5	0.870 (0.000)
	DS6	0.866 (0.000)
	DS7	0.866 (0.000)
	DS8	0.861 (0.000)

** Two-way significant correlation ($P < 0.01$).

4. Conclusions

Currently, commercially available SPME devices prepared with C_{18} or PDMS/DVB coatings do not fulfil all of the specific demands created by the analysis of prohibited substances. A broader selection of readily available stationary phases is desirable for thoroughly utilizing the advantages of microextraction methods in everyday analytical practice. This work takes a significant step toward fulfilling this goal, proposing a novel mixed coating comprising $C_8 + CN$ (1:1) particles as the most suitable type for the extraction of 48 representative prohibited substances. In comparison with the commonly used C_{18} particles, which provide extraction exclusively by hydrophobic-type interactions, this mixed composition provides additional π-π and dipole-dipole type interactions to enhance the extraction efficacy and analyte coverage of the microextraction devices. In addition, it was determined that for the extraction of a diverse panel of analytes, the established C_{18} coatings were outperformed by the less hydrophobic C_8 coatings. In terms of repeatability, all of these coatings provided very good results, with the C_8 – CN (1:1) coating having the lowest RSD values. Therefore, this new mixed coating provides an opportunity to improve the performance of future microextraction devices.

CRediT author statement

Łukasz Sobczak: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing - Original draft preparation, Reviewing and Editing; **Dominika Kołodziej:** Data curation, Formal analysis, Methodology, Writing - Original draft preparation, Reviewing and Editing; **Krzysztof Goryński:** Conceptualization, Funding acquisition, Methodology, Investigation, Project administration, Resources, Supervision, Writing - Reviewing and Editing.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2021.12.007.

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Table S1. Analyzed substances.

analyte	manufacturer	retention time [min]	precursor ion [m/z]	product ions [m/z]		
metildienum	EDQM (European Directorate for the Quality of Medicines, Strasbourg, France)	1.499	147.00	58.10	59.15	-
psilocybin	Cerilliant® (Merck KGaA, Darmstadt, Germany)	2.433	284.90	58.10	205.20	240.05
morphine	LGC Standards (LGC Group, Teddington, UK)	2.642	286.10	152.10	201.15	165.10
salbutamol	LGC Standards (LGC Group, Teddington, UK)	2.913	240.00	148.10	222.25	166.20
terbutaline	LGC Standards (LGC Group, Teddington, UK)	3.015	226.00	152.10	107.05	125.10
atenolol	LGC Standards (LGC Group, Teddington, UK)	3.089	267.00	145.10	190.20	74.15
fenoterol	LGC Standards (LGC Group, Teddington, UK)	4.281	304.00	107.15	135.15	286.10
nilorthamide	Aldrich® (Merck KgaA, Darmstadt, Germany)	4.453	178.90	108.05	80.10	72.10
carteolol	USP (United States Pharmacopeia, Rockville, MD, USA)	4.548	293.00	237.15	202.15	74.10
amphetamine	LGC Standards (LGC Group, Teddington, UK)	4.625	135.90	91.10	119.15	65.10
oxycodone	LGC Standards (LGC Group, Teddington, UK)	4.629	316.10	298.20	241.20	256.10
hydrocodone	Cerilliant® (Merck KGaA, Darmstadt, Germany)	4.928	300.00	199.15	171.10	128.15
methamphetamine	LGC Standards (LGC Group, Teddington, UK)	5.001	150.00	91.10	65.10	119.20
chlorothiazide	Sigma-Aldrich® (Merck KgaA, Darmstadt, Germany)	5.012	294.00	214.05	179.10	215.10
methylhexanamine	Sigma® (Merck KgaA, Darmstadt, Germany)	5.115	116.20	57.10	41.10	-
MDMA (3,4-methylenedioxymethamphetamine)	LGC Standards (LGC Group, Teddington, UK)	5.185	194.00	163.10	105.10	133.10
strychnine	Sigma® (Merck KgaA, Darmstadt, Germany)	5.330	334.90	184.10	156.20	129.15
hydrochlorothiazide	LGC Standards (LGC Group, Teddington, UK)	5.421	296.00	269.00	205.10	77.05
ketamine	LGC Standards (LGC Group, Teddington, UK)	5.665	237.90	125.05	220.10	207.15
metoprolol	LGC Standards (LGC Group, Teddington, UK)	6.407	267.90	116.15	74.15	72.10
clenbuterol	LGC Standards (LGC Group, Teddington, UK)	6.531	277.10	203.05	132.10	168.15
methylphenidate	LGC Standards (LGC Group, Teddington, UK)	6.650	234.00	84.15	56.10	91.10
cocaine	LGC Standards (LGC Group, Teddington, UK)	7.348	303.90	182.20	82.10	105.10
zolpidem	LGC Standards (LGC Group, Teddington, UK)	7.383	307.90	235.15	236.20	263.20
LSD (lysergic acid diethylamide)	LGC Standards (LGC Group, Teddington, UK)	7.688	323.90	223.20	208.15	207.10
bisoprolol	LGC Standards (LGC Group, Teddington, UK)	7.869	326.20	116.20	74.05	72.10
phencyclidine	LGC Standards (LGC Group, Teddington, UK)	8.607	244.00	91.05	86.10	159.20
propranolol	LGC Standards (LGC Group, Teddington, UK)	8.775	260.10	116.20	183.10	155.20
fentanyl	LGC Standards (LGC Group, Teddington, UK)	9.134	337.00	188.20	105.15	103.10
prednisolone	Sigma® (Merck KgaA, Darmstadt, Germany)	9.277	361.00	147.15	325.20	307.15
prednisone	Sigma® (Merck KgaA, Darmstadt, Germany)	9.409	359.00	341.35	267.10	-
buprenorphine	Cerilliant® (Merck KGaA, Darmstadt, Germany)	9.672	468.10	55.15	396.25	414.25
ibutamoren	Cayman Chemical (Cayman Chemical, Ann Arbor, MI, USA)	10.382	529.00	267.10	91.15	263.15
betamethasone	LGC Standards (LGC Group, Teddington, UK)	10.766	393.10	373.25	355.15	279.10
furosemide	Sigma® (Merck KgaA, Darmstadt, Germany)	11.043	329.05	284.85	205.10	126.10
nebivolol	Sigma® (Merck KgaA, Darmstadt, Germany)	11.096	406.10	151.10	121.10	103.10
methadone	Cerilliant® (Merck KGaA, Darmstadt, Germany)	11.322	310.00	265.15	105.10	77.05
alprazolam	LGC Standards (LGC Group, Teddington, UK)	11.393	308.90	281.15	205.15	274.10
enrastronle	LGC Standards (LGC Group, Teddington, UK)	11.412	294.00	225.20	210.20	115.05
stanozolol	LGC Standards (LGC Group, Teddington, UK)	11.496	329.10	81.15	95.15	121.10
boldenone	VETRANAL™ (Merck KgaA, Darmstadt, Germany)	11.942	287.00	121.20	135.20	77.15
clonazepam	Cerilliant® (Merck KGaA, Darmstadt, Germany)	11.970	316.00	270.10	234.05	207.15
nandrolone	LGC Standards (LGC Group, Teddington, UK)	12.320	275.10	109.10	239.10	257.30
methandienone	Cerilliant® (Merck KGaA, Darmstadt, Germany)	12.582	301.20	121.05	149.25	77.00
flunitrazepam	LGC Standards (LGC Group, Teddington, UK)	12.750	313.90	268.15	239.10	183.10
cannenone	Sigma® (Merck KgaA, Darmstadt, Germany)	15.029	341.00	107.15	187.25	235.15
THC-COOH (11-nor-9-carboxy-Δ9-tetrahydrocannabinol)*	LGC Standards (LGC Group, Teddington, UK)	19.874	345.00	327.10	299.25	193.30
THC-OH (11-Hydroxy-Δ9-tetrahydrocannabinol)**	Cerilliant® (Merck KGaA, Darmstadt, Germany)	20.089	331.05	313.25	55.2	193.15
THC (Δ9-tetrahydrocannabinol)*	LGC Standards (LGC Group, Teddington, UK)	24.788	315.00	193.10	123.10	259.15

* used for the main study only
** used for the batch-to-batch reproducibility study only

Table S2. Extraction ratios, n=3.

coating/DS type	meldonium	psilocybin	morphine	salbutamol	terbutaline	atenolol	fenoterol	nikethamide	carteolol	oxycodone	amphetamine	chlorothiazide	hydrocodone	methamphetamine	methylhexedrone	MDMA	hydrochlorothiazide	strychnine
C18 DS1	0,01	-	0,64	0,77	0,73	0,85	0,88	0,43	0,97	0,95	0,98	0,08	1,01	0,99	1,03	1,01	0,14	0,86
C18 DS2	0,01	0,02	0,71	0,76	0,75	0,84	0,87	0,45	1,03	0,97	0,92	0,08	1,03	0,91	0,90	0,93	0,14	0,98
C18 DS3	0,01	0,03	0,64	0,73	0,71	0,84	1,07	0,46	1,11	0,96	0,88	0,09	1,01	0,92	0,86	0,93	0,13	0,94
C18 DS4	0,01	0,02	0,59	0,65	0,64	0,80	1,04	0,44	1,17	0,99	0,87	0,09	1,14	0,94	0,87	0,95	0,15	1,14
C18 DS5	0,01	-	0,72	0,73	0,67	0,91	1,05	0,45	1,34	1,09	1,02	0,09	1,13	1,03	0,93	1,04	0,14	1,29
C18 DS6	0,01	0,03	0,79	0,81	0,75	0,95	1,20	0,46	1,48	1,16	1,09	0,12	1,44	1,11	1,06	1,20	0,15	1,38
C18 DS7	0,01	0,03	0,74	0,70	0,65	0,84	1,02	0,45	1,30	1,00	0,97	0,12	1,28	1,05	0,99	1,04	0,12	1,21
C18 DS8	0,01	0,01	0,75	0,76	0,70	0,92	1,21	0,47	1,46	1,26	1,03	0,14	1,44	1,09	1,09	1,18	0,17	1,40
C8 DS1	0,01	-	0,61	0,78	0,74	0,88	0,83	0,47	0,91	0,92	1,09	0,07	0,97	1,09	1,09	1,06	0,07	0,81
C8 DS2	0,01	0,03	0,66	0,77	0,74	0,88	0,96	0,46	1,05	0,98	0,97	0,07	1,08	1,00	0,97	1,00	0,08	0,99
C8 DS3	0,01	0,04	0,61	0,77	0,74	0,88	1,04	0,51	1,07	0,95	0,96	0,08	0,95	1,00	0,98	0,99	0,16	0,89
C8 DS4	0,01	0,02	0,62	0,70	0,69	0,85	1,01	0,47	1,14	0,98	0,97	0,07	1,13	1,04	0,96	1,05	0,16	1,08
C8 DS5	0,01	-	0,63	0,66	0,63	0,83	0,98	0,40	1,24	1,04	1,05	0,07	1,28	1,08	0,96	1,13	0,11	1,21
C8 DS6	0,01	0,04	0,73	0,78	0,76	0,95	1,16	0,47	1,41	1,22	1,22	0,07	1,45	1,26	1,15	1,32	0,18	1,39
C8 DS7	0,01	0,03	0,67	0,74	0,72	0,92	1,08	0,46	1,31	1,14	1,16	0,08	1,35	1,24	1,17	1,22	0,13	1,29
C8 DS8	0,01	0,02	0,76	0,78	0,74	0,97	1,21	0,51	1,47	1,36	1,24	0,10	1,51	1,29	1,22	1,34	0,18	1,47
Pho-Hex DS1	0,01	-	0,52	0,50	0,50	0,58	0,65	0,27	0,72	0,73	0,80	0,11	0,80	0,82	0,78	0,83	0,13	0,73
Pho-Hex DS2	0,01	0,02	0,52	0,45	0,48	0,56	0,75	0,26	0,85	0,84	0,73	0,10	0,92	0,79	0,72	0,85	0,14	0,91
Pho-Hex DS3	0,01	0,02	0,59	0,59	0,63	0,72	0,92	0,32	1,00	0,89	0,87	0,13	0,88	0,93	0,86	0,94	0,20	0,85
Pho-Hex DS4	0,01	0,02	0,51	0,45	0,48	0,57	0,79	0,27	0,92	0,85	0,75	0,10	0,94	0,82	0,73	0,87	0,18	1,01
Pho-Hex DS5	0,01	-	0,52	0,46	0,42	0,57	0,69	0,26	0,96	0,87	0,72	0,10	0,99	0,78	0,67	0,88	0,16	1,07
Pho-Hex DS6	0,01	0,03	0,46	0,53	0,48	0,66	0,79	0,31	1,05	0,95	0,83	0,11	1,08	0,91	0,77	1,02	0,19	1,15
Pho-Hex DS7	0,01	0,03	0,52	0,45	0,44	0,58	0,76	0,29	0,99	0,88	0,78	0,11	1,00	0,87	0,76	0,94	0,15	1,09
Pho-Hex DS8	0,01	0,03	0,40	0,41	0,41	0,52	0,73	0,28	0,94	0,95	0,71	0,11	0,97	0,78	0,67	0,83	0,13	1,10
CN DS1	0,01	-	0,75	0,71	0,76	0,77	0,78	0,08	0,76	0,77	0,93	0,10	0,79	0,94	0,91	0,85	0,16	0,70
CN DS2	0,01	0,03	0,78	0,85	0,77	0,79	0,93	0,09	0,94	0,92	0,87	0,12	0,93	0,91	0,80	0,93	0,21	0,90
CN DS3	0,01	0,02	0,71	0,64	0,77	0,79	0,87	0,08	0,98	0,88	0,81	0,11	0,86	0,90	0,83	0,91	0,19	0,91
CN DS4	0,01	0,04	0,82	0,68	0,82	0,84	1,02	0,08	1,03	0,95	0,88	0,11	0,96	0,97	0,86	0,99	0,18	1,02
CN DS5	0,01	-	0,96	0,85	0,98	0,96	1,07	0,08	1,15	1,13	0,93	0,10	1,11	1,01	0,90	1,04	0,17	1,13
CN DS6	0,02	0,02	0,68	1,08	1,10	1,15	1,20	0,10	1,28	1,22	1,07	0,13	1,21	1,16	1,06	1,17	0,17	1,26
CN DS7	0,01	0,01	0,67	0,98	0,99	1,05	1,07	0,10	1,15	1,12	1,02	0,12	1,08	1,09	1,04	1,08	0,19	1,14
CN DS8	0,01	0,02	0,74	1,02	1,11	1,11	1,24	0,10	1,27	1,45	1,09	0,13	1,24	1,18	1,02	1,22	0,20	1,24
SCX DS1	0,04	-	0,83	0,85	0,85	0,87	0,82	0,21	0,65	0,85	0,94	0,01	0,63	0,85	0,98	0,78	-	0,43
SCX DS2	0,02	-	0,35	0,56	0,59	0,45	0,67	0,18	0,48	0,35	0,51	0,02	0,29	0,41	0,55	0,40	0,09	0,23
SCX DS3	0,01	0,01	0,27	0,62	0,64	0,48	0,71	0,18	0,50	0,26	0,45	0,03	0,17	0,39	0,56	0,35	0,14	0,15
SCX DS4	0,03	-	0,47	0,75	0,78	0,63	0,92	0,18	0,71	0,46	0,59	0,04	0,38	0,58	0,69	0,56	0,09	0,35
SCX DS5	0,07	-	1,12	1,21	1,22	1,24	1,30	0,23	1,34	1,34	1,13	0,04	1,34	1,19	1,13	1,18	0,10	1,25
SCX DS6	0,07	0,01	0,96	1,30	1,26	1,33	1,37	0,24	1,39	1,32	1,16	0,04	1,34	1,21	1,10	1,20	0,13	1,32
SCX DS7	0,06	0,01	0,88	1,18	1,13	1,21	1,23	0,20	1,29	1,21	1,07	0,03	1,17	1,12	1,04	1,10	0,12	1,19
SCX DS8	0,06	0,01	1,05	1,31	1,28	1,33	1,40	0,20	1,45	1,53	1,18	0,03	1,39	1,24	1,17	1,24	0,03	1,35
SIL DS1	0,06	-	0,82	0,83	0,81	0,84	0,72	0,16	0,75	0,79	0,95	0,04	0,81	0,94	0,95	0,85	0,07	0,69
SIL DS2	0,05	0,02	0,87	0,82	0,85	0,91	0,83	0,16	0,98	0,96	0,92	0,04	0,99	0,96	0,93	0,95	0,07	0,96
SIL DS3	0,04	0,02	0,75	0,77	0,81	0,89	0,85	0,16	1,01	0,87	0,86	0,04	0,88	0,95	0,90	0,93	0,06	0,96
SIL DS4	0,06	0,02	0,93	0,91	0,95	1,01	0,88	0,16	1,13	1,08	0,97	0,04	1,06	1,06	1,02	1,06	0,05	1,11
SIL DS5	0,04	-	1,07	0,97	0,98	1,05	1,01	0,18	1,22	1,19	1,03	0,04	1,18	1,09	1,00	1,10	0,07	1,19
SIL DS6	0,06	0,01	0,70	1,11	1,05	1,19	1,11	0,17	1,30	1,23	1,11	0,03	1,25	1,19	1,06	1,20	0,03	1,27
SIL DS7	0,04	0,01	0,65	0,97	0,94	1,05	1,01	0,17	1,18	1,10	1,06	0,03	1,03	1,08	1,05	1,10	0,06	1,11
SIL DS8	0,05	0,01	0,77	1,09	1,08	1,17	1,11	0,17	1,32	1,53	1,17	0,04	1,27	1,19	1,08	1,22	0,06	1,30
C8+CN (1:1) DS1	0,01	-	0,72	0,70	0,71	0,79	1,07	0,45	1,11	1,04	0,95	0,09	1,18	1,00	0,98	1,11	0,18	1,22
C8+CN (1:1) DS2	0,01	0,03	0,68	0,65	0,67	0,74	0,96	0,44	0,95	0,94	0,87	0,09	1,06	0,87	0,84	0,93	0,14	1,07
C8+CN (1:1) DS3	0,01	0,04	0,62	0,61	0,63	0,68	0,96	0,43	0,92	0,89	0,77	0,09	0,96	0,84	0,83	0,86	0,17	1,02
C8+CN (1:1) DS4	0,01	0,02	0,64	0,58	0,61	0,86	0,96	0,41	0,92	0,94	0,82	0,08	1,04	0,85	0,87	0,92	0,10	1,04
C8+CN (1:1) DS5	0,01	0,04	0,92	0,64	0,69	0,80	1,20	0,45	1,05	1,14	0,87	0,09	1,30	0,82	0,73	0,90	0,15	1,14
C8+CN (1:1) DS6	0,01	0,04	1,07	0,75	0,82	0,91	1,20	0,50	1,08	1,17	0,98	0,11	1,31	0,94	0,90	1,02	0,16	1,39
C8+CN (1:1) DS7	0,01	0,05	0,98	0,79	0,84	0,89	1,19	0,55	1,11	1,20	1,02	0,11	1,35	1,01	0,98	1,05	0,18	1,42
C8+CN (1:1) DS8	0,01	0,03	0,63	0,68	0,73	0,80	1,02	0,46	1,04	1,14	0,86	0,09	1,23	0,92	0,84	0,98	0,16	1,33
C8+CN (1:3) DS1	0,01	-	1,03	0,90	0,97	0,96	1,26	0,42	1,08	1,15	1,05	0,11	1,41	1,06	1,05	1,17	0,15	1,48
C8+CN (1:3) DS2	0,01	0,02	0,98	0,87	0,75	0,75	1,12	0,38	0,91	1,02	0,94	0,10	1,22	0,91	0,90	0,96	0,12	1,27
C8+CN (1:3) DS3	0,01	0,02	0,84	0,57	0,63	0,63	1,06	0,34	0,86	0,92	0,79	0,07	1,10	0,80	0,84	0,82	0,13	1,23
C8+CN (1:3) DS4	0,01	0,01	0,79	0,53	0,61	0,61	1,05	0,33	0,86	0,93	0,83	0,08	1,14	0,82	0,85	0,90	0,13	1,24
C8+CN (1:3) DS5	0,01	0,04	1,24	0,66	0,78	0,80	1,30	0,41	0,94	1,23	0,88	0,11	1,50	0,78	0,76	0,84	0,15	1,61
C8+CN (1:3) DS6	0,01	0,03	1,40	0,81	0,95	0,94	1,34	0,45	1,03	1,25	1,02	0,13	1,50	0,93	0,94	0,98	0,18	1,65
C8+CN (1:3) DS7	0,02	0,04	1,17	0,77	0,88	0,85	1,25	0,48	1,03	1,20	1,00	0,13	1,47	0,96	0,95	1,01	0,17	1,64
C8+CN (1:3) DS8	0,01	0,05	0,71	0,61	0,71	0,71	1,03	0,42	0,93	1,12	0,84	0,12	1,31	0,85	0,78	0,91	0,20	1,53
C8+CN (1:3) DS1	0,02	-	0,98	0,93	0,96	0,96	0,99	0,36	0,98	1,03	1,11	0,15	1,09	1,11	1,15	1,08	0,19	1,12
C8+CN (1:3) DS2	0,02	0,02	0,96	0,84	0,90	0,89	1,02	0,34	0,97	1,00	1,00	0,15	1,04	0,97	0,98	0,96	0,18	1,11
C8+CN (1:3) DS3	0,02	0,02	0,88	0,82	0,84	0,83	0,96	0,33	0,92	0,92	0,90	0,15	0,95	0,89	0,98	0,83	0,19	1,00
C8+CN (1:3) DS4	0,02	0,02	0,95	0,82	0,89	0,89	1,09	0,33	1,00	1,01	1,01	0,14	1,06	0,98	1,05	0,99	0,17	1,12
C8+CN (1:3) DS5	0,02	0,04	1,13	0,58	0,69	0,76	1,19	0,33	0,94	1,15	0,88	0,14	1,25	0,76	0,69	0,78	0,18	1,41
C8+CN (1:3) DS6	0,02	0,03	1,48	0,68	0,79	0,88	1,27	0,35	1,03	1,19	1,17	0,11	1,42	0,96	0,96	0,97	0,13	1,49
C8+CN (1:3) DS7	0,02	0,03	1,16	0,68	0,77	0,86	1,27	0,35	1,07	1,18	1,18	0,12	1,40	1,01	1,02	1,04	0,15	1,48
C8+CN (1:3) DS8	0,01	0,02	0,88	0,65	0,76	0,81	1,09	0,33	0,98	1,22	0,95	0,13	1,18	0,93	0,86	0,94	0,18	1,43
C8+SIL (1:1) DS1	0,04	-	0,83	1,02	0,98	0,91	0,86	0,57	0,89	0,79	0,92	0,10	0,73	0,67	0,96	0,82	0,15	0,70
C8+SIL (1:1) DS2	0,03	-	0,69	0,82	0,80	0,75	0,79	0,52	0,74	0,84	0,74	0,10	0,57	0,66	0,73	0,64	0,14	0,56
C8+SIL (1:1) DS3	0,02	0,03	0,59	0,82	0,78	0,72	0,79	0,53	0,72	0,53	0,69	0,10	0,47	0,65	0,75	0,60	0,14	0,45
C8+SIL (1:1) DS4	0,03	0,02	0,71	0,84	0,83	0,76	0,85	0,55	0,77	0,66	0,75	0,11	0,59	0,68	0,83	0,69	0,14	0,56
C8+SIL (1:1) DS5	0,03	0,03	0,86	0,77	0,82	0,78	1,00	0,53	0,87	0,93	0,83	0,10	0,87	0,73	0,71	0,73	0,12	0,86
C8+SIL (1:1) DS6	0,02	0,03	0,81	0,80	0,82	0,85	0,95	0,52	0,91	0,88	0,87	0,10	0,86	0,82	0,81	0,80	0,12	0,88
C8+SIL (1:1) DS7	0,03	0,03	0,71	0,92	0,93	0,91	1,03	0,61	0,97	0,94	0,94	0,11	0,92	0,90	0,90	0,88	0,16	0,92
C8+SIL (1:1) DS8	0,02	0,03	0,76	0,86	0,88	0,85	0,96	0,55	0,90	1,04	0,84	0,11	0,86	0,84	0,79	0,82	0,15	0,92
C8+SIL (1:3) DS1	0,03	-	0,46	0,56	0,52	0,64	0,55	0,36	0,71	0,65	0,76	0,06	0,64	0,81	0,84	0,74	0,09	0,65
C8+SIL (1:3) DS2	0,02	0,02	0,44	0,49	0,47	0,56	0,58	0,31	0,76	0,66	0,70	0,06	0,64	0,73	0,74	0,76	0,09	0,69
C8+SIL (1:3) DS3	0,02	0,03	0,37	0,46	0,44	0,56	0,57	0,30	0,76	0,54	0,63	0,06	0,53	0,68	0,71	0,72	0,08	0,61
C8+SIL (1:3) DS4	0,02	0,02	0,42	0,49	0,47	0,58	0,61	0,31	0,85	0,66	0,69	0,06	0,64	0,76	0,79	0,83	0,09	0,73
C8+SIL (1:3) DS5	0,02	0,04	0,58	0,53	0,55	0,64	0,76	0,32	0,87	0,83	0,72	0,07	0,77	0,71	0,65	0,75	0,10	0,87
C8+SIL (1:3) DS6	0,01	0,02	0,55	0,62	0,64	0,75	0,77	0,28	0,94	0,79	0,84	0,06	0,74	0,84	0,80	0,86	0,07	0,85
C8+SIL (1:3) DS7	0,02	0,02	0,39	0,63	0,65	0,76	0,79	0,30	0,99	0,76	0,86	0,06	0,75	0,88	0,87	0,95	0,09	0,82
C8+SIL (1:3) DS8	0,01	0,03	0,46	0,52	0,53	0,63	0,70	0,29	0,87	0,91	0,75	0,06	0,70	0,78	0,75	0,84	0,08	0,86
C8+SIL (1:3) DS1	0,04	-	0,69	0,75	0,72	0,77	0,66	0,29	0,76	0,78	0,86	0,06	0,77	0,89	0,96	0,84	0,09	0,74
C8+SIL (1:3) DS2	0,04	-	0,70	0,89	0,69	0,73	0,74	0,25	0,85	0,83	0,83	0,05	0,81	0,82	0,84	0,85	0,08	0,80
C8+SIL (1:3) DS3	0,03	0,02	0,58	0,63	0,62	0,71	0,72	0,24	0,86	0,86	0,74	0,05	0,68	0,78	0,80	0,80	0,07	0,70
C8+SIL (1:3) DS4	0,03	-	0,68	0,66	0,67	0,76	0,78	0,27	0,93	0,82	0,80	0,06	0,80	0,85	0,91	0,91	0,06	0,86
C8+SIL (1:3) DS5	0,03	0,03	0,69	0,52	0,56	0,62	0,74	0,26	0,80	0,93	0,71	0,06	0,89	0,66	0,62	0,72	0,07	0,91
C8+SIL (1:3) DS6	0,02	0,02	0,59	0,57	0,58	0,70	0,72	0,24	0,90	0,83	0,74	0,05	0,85	0,78	0,72	0,83	0,06	0,89
C8+SIL (1:3) DS7	0,03	0,03	0,44	0,59	0,60	0,76	0,77	0,28	0,95	0,82	0,80	0,06	0,85	0,82	0,83	0,91	0,07	0,88
C8+SIL (1:3) DS8	0,02	0,03	0,59	0,56	0,58	0,69	0,72	0,25	0,87	1,05	0,76	0,06	0,83	0,78	0,74	0,82	0,08	0,95

Abbreviations used for coatings: octadecyl (C18); octyl (C8); phenyl-hexyl (Pho-Hex); 3-cyanopropyl (CN); benzenesulfonic acid (SCX); and unbonded silica (SiL)
Compositions of desorption solvents: DS1 = acetonitrile/water/formic acid (80/19.9/0.1, V/V); DS2 = acetonitrile/methanol/water/formic acid (40/40/19.9/0.1, V/V); DS3 = methanol/water/formic acid (80/19.9/0.1, V/V)

ketamine	metoprolo	clenbutero	methylphe	zolpidem	cocaine	LSD	bisoprolol	phencyclid	propranolo	prednisolo	fentanyl	prednisone	buprenorp	ibutamore	betametha	furosemid	nebivolol	alprazolam
1,00	1,01	1,03	2,03	0,82	1,35	0,76	1,02	0,98	0,86	1,12	0,90	1,20	0,86	0,77	1,10	0,35	0,74	1,25
0,94	0,98	1,02	1,91	0,97	1,43	0,94	1,03	1,01	0,95	0,99	1,06	1,10	1,06	1,07	1,10	0,34	0,98	1,14
1,02	1,08	1,11	2,02	1,04	1,47	0,99	1,16	1,15	1,06	1,15	1,16	1,21	1,14	1,18	1,17	0,38	1,11	1,23
1,01	1,13	1,15	2,12	1,14	1,62	1,11	1,20	1,19	1,13	1,06	1,25	1,17	1,22	1,26	1,18	0,37	1,17	1,20
1,14	1,24	1,29	2,34	1,20	1,82	1,22	1,33	1,22	1,26	1,06	1,32	1,25	1,28	1,33	1,08	0,40	1,27	1,19
1,22	1,32	1,36	2,42	1,29	1,89	1,37	1,36	1,30	1,41	1,10	1,38	1,21	1,31	1,52	1,17	0,49	1,33	1,14
1,10	1,14	1,22	2,16	1,17	1,70	1,22	1,24	1,20	1,20	1,02	1,29	0,98	1,19	1,33	1,08	0,40	1,24	1,02
1,26	1,33	1,37	2,49	1,32	1,98	1,41	1,42	1,34	1,36	1,07	1,46	1,23	1,43	1,56	1,26	0,40	1,40	1,19
1,04	1,02	1,01	1,59	0,79	1,10	0,70	1,00	0,92	0,77	1,12	0,84	1,16	0,79	0,67	1,11	0,40	0,64	1,25
1,01	1,06	1,07	1,52	0,99	1,22	0,93	1,08	1,02	0,94	1,07	1,06	1,14	1,08	1,07	1,16	0,43	0,97	1,21
1,05	1,10	1,12	1,55	1,03	1,16	0,90	1,18	1,10	0,99	1,10	1,11	1,17	1,16	1,15	1,19	0,46	1,05	1,23
1,04	1,10	1,16	1,62	1,11	1,32	1,05	1,19	1,16	1,08	1,04	1,21	1,18	1,21	1,21	1,18	0,38	1,13	1,21
1,14	1,21	1,24	1,77	1,16	1,52	1,17	1,29	1,22	1,19	1,06	1,26	1,20	1,26	1,26	1,10	0,39	1,23	1,16
1,30	1,34	1,41	1,98	1,34	1,70	1,38	1,41	1,37	1,39	1,22	1,41	1,31	1,36	1,42	1,27	0,52	1,34	1,28
1,22	1,23	1,33	1,84	1,24	1,59	1,28	1,33	1,29	1,25	1,17	1,35	1,16	1,29	1,31	1,23	0,45	1,29	1,18
1,36	1,40	1,45	2,08	1,36	1,78	1,43	1,47	1,42	1,40	1,15	1,48	1,34	1,49	1,56	1,37	0,54	1,44	1,32
0,84	0,85	0,84	0,89	0,79	0,88	0,72	0,91	0,86	0,76	0,98	0,82	1,09	0,83	0,73	1,03	0,37	0,66	1,14
0,86	0,92	0,93	0,89	0,97	0,96	0,96	1,03	1,03	0,95	0,96	1,06	1,05	1,07	1,05	1,03	0,33	0,97	1,13
0,98	1,09	1,07	0,96	1,05	0,94	1,00	1,17	1,11	1,08	1,08	1,13	1,12	1,13	1,13	1,15	0,34	1,06	1,23
0,88	1,00	1,00	0,91	1,09	1,02	1,07	1,14	1,15	1,10	0,93	1,21	1,05	1,17	1,17	1,07	0,38	1,13	1,15
0,91	1,03	1,05	0,94	1,13	1,13	1,13	1,20	1,17	1,20	0,95	1,27	1,15	1,26	1,27	1,02	0,37	1,18	1,14
1,01	1,09	1,15	1,03	1,22	1,21	1,21	1,25	1,23	1,27	1,07	1,31	1,16	1,26	1,27	1,22	0,45	1,20	1,20
0,96	1,07	1,10	0,98	1,14	1,18	1,14	1,18	1,16	1,18	1,02	1,26	0,96	1,18	1,15	1,10	0,41	1,15	1,12
0,92	1,02	1,07	0,95	1,24	1,16	1,24	1,25	1,22	1,24	1,02	1,34	1,14	1,34	1,34	1,14	0,45	1,28	1,23
0,89	0,86	0,84	0,83	0,82	0,83	0,74	0,82	0,78	0,69	0,25	0,75	0,31	0,74	0,62	0,43	-	0,62	0,61
0,94	0,94	0,97	0,81	1,00	0,88	1,00	0,97	1,01	0,93	0,26	1,03	0,34	1,02	0,98	0,46	-	0,93	0,70
0,92	0,99	1,03	0,81	1,02	0,84	1,01	1,03	1,06	1,06	0,25	1,08	0,32	1,04	1,00	0,41	-	1,00	0,70
0,98	1,04	1,07	0,86	1,06	0,92	1,10	1,03	1,11	1,07	0,25	1,12	0,30	1,06	1,03	0,39	-	1,01	0,58
1,11	1,14	1,15	0,94	1,17	1,06	1,20	1,12	1,15	1,14	0,27	1,22	0,34	1,16	1,11	0,43	-	1,08	0,57
1,21	1,20	1,26	1,00	1,27	1,15	1,29	1,22	1,24	1,23	0,30	1,28	0,36	1,21	1,18	0,51	-	1,13	0,65
1,15	1,15	1,16	0,92	1,16	1,11	1,19	1,14	1,14	1,17	0,28	1,21	0,31	1,12	1,11	0,40	-	1,08	0,60
1,23	1,25	1,31	1,03	1,30	1,17	1,36	1,24	1,26	1,30	0,28	1,33	0,33	1,25	1,28	0,44	-	1,23	0,59
0,78	0,82	0,83	1,06	0,61	0,83	0,54	0,83	0,74	0,64	0,08	0,69	0,11	0,72	0,59	0,11	-	0,60	0,48
0,44	0,53	0,54	0,55	0,41	0,40	0,35	0,61	0,46	0,45	0,07	0,48	0,08	0,59	0,58	0,08	-	0,54	0,42
0,45	0,56	0,57	0,53	0,35	0,28	0,26	0,65	0,44	0,43	0,06	0,43	0,10	0,49	0,48	0,06	-	0,49	0,31
0,62	0,73	0,76	0,75	0,61	0,53	0,57	0,83	0,68	0,67	0,04	0,73	0,06	0,78	0,79	0,05	-	0,73	0,31
1,30	1,29	1,32	1,53	1,24	1,43	1,30	1,32	1,31	1,24	0,05	1,34	0,06	1,26	1,29	0,05	-	1,17	0,29
1,30	1,27	1,35	1,52	1,29	1,48	1,37	1,32	1,30	1,30	0,05	1,37	0,09	1,27	1,20	0,07	-	1,19	0,35
1,22	1,20	1,25	1,41	1,21	1,43	1,25	1,25	1,19	1,21	0,04	1,30	0,05	1,20	1,14	0,04	-	1,14	0,33
1,38	1,34	1,41	1,61	1,35	1,54	1,44	1,40	1,36	1,36	0,04	1,45	0,06	1,36	1,31	0,05	-	1,29	0,31
0,90	0,85	0,82	0,93	0,80	0,90	0,70	0,82	0,77	0,68	0,05	0,74	0,06	0,71	0,58	0,05	-	0,61	0,30
0,98	0,97	1,00	0,95	1,00	0,99	0,99	1,02	1,06	0,96	0,03	1,07	0,07	1,02	0,96	0,05	-	0,95	0,35
0,95	1,02	1,02	0,92	1,02	0,93	0,98	1,09	1,08	1,09	0,03	1,10	0,04	1,01	0,96	0,04	-	0,98	0,34
1,07	1,14	1,11	1,03	1,13	1,06	1,10	1,16	1,20	1,15	0,04	1,22	0,05	1,13	1,10	0,04	-	1,09	0,32
1,19	1,21	1,23	1,11	1,21	1,22	1,22	1,22	1,26	1,25	0,04	1,29	0,06	1,18	1,14	0,04	-	1,12	0,27
1,26	1,23	1,29	1,15	1,31	1,31	1,31	1,30	1,31	1,32	0,03	1,35	0,04	1,26	1,26	0,05	-	1,21	0,30
1,17	1,18	1,21	1,05	1,22	1,25	1,21	1,21	1,21	1,25	0,03	1,28	0,03	1,16	1,13	0,03	-	1,15	0,29
1,31	1,31	1,37	1,21	1,40	1,35	1,40	1,35	1,37	1,39	0,03	1,44	0,04	1,32	1,36	0,06	-	1,30	0,31
1,09	1,18	1,15	1,34	1,08	1,27	1,00	1,32	1,29	1,16	1,46	1,21	1,33	1,32	1,09	1,21	0,40	1,29	1,51
0,97	1,04	1,01	1,17	1,03	1,12	0,98	1,12	1,15	1,00	1,22	1,06	1,22	1,13	1,04	1,13	0,37	1,12	1,28
0,96	0,97	0,99	1,14	1,04	1,07	0,94	1,11	1,16	0,98	1,22	1,04	1,18	1,14	0,99	1,25	0,34	1,14	1,25
0,97	1,00	1,00	1,16	1,05	1,13	1,02	1,14	1,16	1,04	1,27	1,09	1,17	1,22	1,03	1,23	0,33	1,25	1,30
1,16	1,12	1,05	1,34	1,19	1,35	1,21	1,23	1,08	1,10	1,37	1,27	1,35	1,43	1,06	1,36	0,33	1,45	1,43
1,14	1,15	1,11	1,34	1,21	1,36	1,23	1,23	1,14	1,14	1,38	1,25	1,26	1,39	1,07	1,40	0,37	1,44	1,43
1,24	1,19	1,15	1,41	1,29	1,41	1,30	1,27	1,30	1,20	1,34	1,36	1,30	1,33	1,09	1,28	0,44	1,33	1,33
1,17	1,09	1,06	1,35	1,22	1,37	1,27	1,17	1,23	1,14	1,12	1,28	1,10	1,22	1,23	1,02	0,36	1,18	1,06
1,15	1,16	1,14	1,87	1,11	1,62	1,04	1,34	1,51	1,23	1,60	1,31	1,34	1,52	1,19	1,29	0,41	1,58	1,70
0,99	1,00	0,98	1,57	1,09	1,41	1,02	1,17	1,32	1,05	1,45	1,12	1,26	1,35	1,06	1,17	0,32	1,44	1,52
0,97	0,93	0,93	1,51	1,07	1,34	0,97	1,16	1,31	1,00	1,43	1,11	1,32	1,33	1,02	1,31	0,32	1,44	1,52
0,98	0,96	0,94	1,52	1,09	1,40	1,05	1,17	1,31	1,07	1,40	1,16	1,24	1,42	1,06	1,38	0,27	1,52	1,49
1,21	1,02	0,96	1,78	1,22	1,69	1,23	1,18	1,16	1,08	1,47	1,34	1,39	1,59	1,05	1,42	0,29	1,60	1,53
1,19	1,06	1,04	1,78	1,24	1,68	1,24	1,21	1,25	1,13	1,49	1,31	1,27	1,50	1,05	1,41	0,34	1,55	1,49
1,23	1,14	1,08	1,84	1,31	1,71	1,31	1,23	1,42	1,17	1,40	1,42	1,31	1,47	1,03	1,31	0,40	1,45	1,41
1,16	1,02	0,98	1,74	1,24	1,68	1,28	1,13	1,34	1,11	1,23	1,34	1,16	1,32	1,11	1,00	0,42	1,25	1,14
1,06	1,08	1,04	0,89	0,99	1,03	0,86	1,18	1,18	0,94	1,31	1,06	1,28	1,11	0,82	1,33	0,26	0,97	1,40
1,00	1,01	0,99	0,79	1,03	0,94	0,96	1,13	1,15	0,97	1,22	1,08	1,12	1,18	0,89	1,11	0,23	1,13	1,25
0,97	0,95	0,94	0,74	0,99	0,86	0,88	1,11	1,14	0,92	1,13	1,04	1,11	1,13	0,96	1,08	0,22	1,11	1,22
1,00	1,03	1,02	0,79	1,06	0,96	1,02	1,17	1,18	1,05	1,15	1,15	1,05	1,22	1,04	1,25	0,23	1,25	1,23
1,14	1,01	0,96	0,88	1,24	1,14	1,22	1,21	1,01	1,04	1,33	1,35	1,28	1,51	1,10	1,39	0,24	1,57	1,48
1,11	1,09	1,06	0,88	1,20	1,16	1,17	1,33	1,15	1,17	2,11	1,26	1,73	1,55	1,09	1,94	0,25	1,77	2,32
1,17	1,15	1,10	0,93	1,27	1,24	1,26	1,39	1,30	1,23	1,84	1,37	1,52	1,83	1,09	1,75	0,29	1,80	1,99
1,15	1,04	1,03	0,89	1,23	1,19	1,26	1,22	1,21	1,14	1,20	1,35	1,16	1,36	1,23	1,00	0,23	1,30	1,19
0,84	0,92	0,88	0,75	0,85	0,67	0,71	0,97	0,80	0,87	1,34	0,78	1,37	0,84	0,80	1,27	0,39	0,86	1,35
0,69	0,77	0,73	0,58	0,74	0,51	0,60	0,82	0,65	0,70	1,21	0,63	1,18	0,72	0,73	1,01	0,33	0,73	1,17
0,70	0,75	0,73	0,56	0,69	0,46	0,52	0,81	0,68	0,67	1,19	0,59	1,16	0,69	0,66	1,04	0,31	0,69	1,15
0,74	0,81	0,78	0,62	0,78	0,56	0,67	0,87	0,73	0,79	1,22	0,70	1,15	0,79	0,78	1,19	0,28	0,83	1,20
1,03	0,93	0,90	0,85	1,06	0,86	0,98	0,97	0,81	0,96	1,28	0,99	1,25	1,04	0,91	1,28	0,34	1,12	1,30
1,00	0,94	0,94	0,85	1,07	0,87	1,01	1,02	0,86	1,03	1,24	0,96	1,21	0,99	0,92	1,19	0,39	1,13	1,29
1,11	1,04	1,03	0,96	1,17	0,99	1,10	1,09	0,98	1,08	1,25	1,09	1,31	1,08	0,90	1,27	0,45	1,15	1,36
1,06	0,94	0,96	0,91	1,10	0,91	1,06	1,01	0,94	1,09	1,18	1,02	1,18	1,01	1,09	1,07	0,38	1,11	1,15
0,78	0,90	0,79	0,67	0,79	0,72	0,62	0,94	0,88	0,69	1,10	0,72	1,30	0,73	0,53	1,24	0,44	0,60	1,18
0,77	0,88	0,81	0,59	0,89	0,68	0,80	1,01	0,96	0,83	1,01	0,85	1,02	0,87	0,78	0,99	0,41	0,82	1,09
0,75	0,91	0,84	0,56	0,90	0,64	0,80	1,01	0,96	0,88	0,88	0,88	0,95	0,86	0,80	0,91	0,43	0,79	1,01
0,80	1,01	0,91	0,63	0,97	0,73	0,91	1,06	1,03	0,95	0,93	0,94	0,98	0,89	0,85	1,11	0,36	0,86	1,02
0,96	1,00	0,95	0,71	1,09	0,83	1,01	1,07	0,99	0,98	0,96	1,06	1,05	0,88	0,79	1,10	0,33	0,92	1,06
0,93	1,01	0,98	0,70	1,05	0,83	1,00	1,09	1,01	1,05	0,92	0,99	1,05	0,91	0,91	1,01	0,27	0,93	1,02
0,98	1,13	1,05	0,76	1,17	0,93	1,09	1,19	1,13	1,12	1,01	1,12	1,11	1,04	0,89	1,09	0,38	1,02	1,17
0,93	1,02	0,98	0,72	1,11	0,85	1,06	1,14	1,09	1,06	1,03	1,07	1,07	0,99	0,99	1,00	0,36	0,99	1,06
0,88	0,89	0,83	0,88	0,83	0,85	0,67	0,93	0,89	0,72	0,94	0,79	1,22	0,79	0,58	1,17	0,18	0,63	1,10
0,88	0,91	0,87	0,78	0,92	0,80	0,83	0,97	0,96	0,84	0,87	0,92	0,97	0,93	0,83	0,95	0,14	0,85	0,99
0,84	0,89	0,87	0,74	0,92	0,74	0,82	1,00	0,95	0,91	0,85	0,93	0,98	0,93	0,87	0,87	0,16	0,88	1,01
0,89	1,00	0,94	0,82	1,00	0,85	0,94	1,04	1,01	0,99	0,91	0,99	1,00	0,97	0,93	1,12	0,15	0,95	1,02
1,01	0,89	0,91	0,86	1,11	0,94	1,04	0,99	0,94	0,95	0,88	1,12	1,00	1,04	0,84	1,09	0,24	0,99	1,05
0,93	0,92	0,91	0,83	1,06	0,92	1,02	1,02	0,95	1,04	0,88	1,04	1,00	0,98	0,87	1,00	0,25	0,99	1,00
0,99	1,05	1,01	0,91	1,17	1,03	1,10	1,14	1,06	1,11	0,94	1,17	1,09	1,12	0,80	1,17	0,31	1,09	1,14
0,98	0,95	0,97	0,89	1,12	0,99	1,11	1,10	1,04	1,06	0,98	1,15	1,06	1,09	0,95	1,08	0,31	1,09	1,09

; DS4 = acetonitrile/-propanol/methanol/water/formic acid (30/25/25/19.9/0.1, V/V); DS5 = acetonitrile/water/ammonium hydroxide (80/19.9/0.1, V/V); DS6 = acetonitrile/methanol/water/ammonium hydroxid

anastrozole	stanozolol	methadone	boldenone	clonazepam	nandrolone	methandie	flunitrazepam	renone	THC-COOH	THC
1,15	0,84	0,80	1,09	1,03	1,04	1,09	1,07	0,89	1,12	1,93
1,10	1,04	0,96	1,09	1,05	1,13	1,09	1,05	1,07	0,97	1,95
1,23	1,12	1,07	1,21	1,17	1,27	1,21	1,19	1,20	1,10	2,22
1,23	1,16	1,14	1,18	1,10	1,25	1,22	1,16	1,16	1,15	2,41
1,23	1,16	1,24	1,20	1,13	1,22	1,20	1,15	1,21	1,13	2,50
1,30	1,20	1,31	1,23	1,13	1,21	1,22	1,14	1,23	1,18	2,46
1,17	1,08	1,19	1,06	1,01	1,08	1,07	1,02	1,13	1,10	2,34
1,33	1,22	1,37	1,21	1,17	1,21	1,27	1,21	1,29	1,27	2,78
1,18	0,84	0,70	1,10	1,06	1,08	1,12	1,11	0,90	1,24	1,91
1,18	1,08	0,92	1,17	1,14	1,21	1,18	1,15	1,18	1,23	2,30
1,27	1,08	0,98	1,25	1,22	1,30	1,26	1,23	1,26	1,30	2,33
1,25	1,18	1,05	1,19	1,16	1,28	1,23	1,19	1,19	1,30	2,34
1,19	1,06	1,17	1,16	1,11	1,17	1,15	1,13	1,17	1,10	2,17
1,26	1,20	1,33	1,30	1,23	1,32	1,29	1,26	1,32	1,33	2,56
1,27	1,15	1,24	1,19	1,16	1,26	1,19	1,19	1,24	1,24	2,45
1,39	1,26	1,41	1,29	1,29	1,29	1,34	1,31	1,34	1,43	2,75
1,04	0,78	0,76	1,00	0,94	0,96	1,04	1,00	0,91	0,90	0,53
1,04	1,02	1,06	1,08	0,99	1,08	1,06	1,02	1,11	0,93	0,55
1,16	1,17	1,09	1,15	1,11	1,14	1,18	1,13	1,21	1,03	0,58
1,10	1,13	1,17	1,12	1,03	1,13	1,13	1,07	1,16	1,02	0,62
1,13	1,07	1,25	1,14	1,05	1,13	1,13	1,09	1,20	0,81	0,58
1,20	1,14	1,29	1,20	1,12	1,18	1,23	1,20	1,26	0,91	0,58
1,13	1,10	1,18	1,11	1,06	1,11	1,14	1,09	1,19	0,85	0,54
1,19	1,19	1,32	1,22	1,15	1,22	1,27	1,20	1,29	0,95	0,58
0,62	0,58	0,73	0,53	0,49	0,52	0,60	0,54	0,70	0,23	0,17
0,69	0,83	1,08	0,62	0,55	0,58	0,65	0,59	0,84	0,22	0,20
0,67	0,80	1,12	0,57	0,55	0,55	0,64	0,57	0,86	0,23	0,21
0,63	0,86	1,14	0,56	0,49	0,51	0,63	0,53	0,86	0,24	0,19
0,66	0,83	1,23	0,57	0,50	0,59	0,63	0,57	0,86	0,20	0,20
0,72	0,89	1,32	0,63	0,56	0,61	0,71	0,64	0,99	0,22	0,22
0,67	0,82	1,17	0,58	0,54	0,55	0,66	0,57	0,87	0,19	0,18
0,67	0,87	1,32	0,60	0,54	0,58	0,67	0,59	0,91	0,19	0,18
0,19	0,39	0,70	0,17	0,19	0,15	0,18	0,21	0,26	0,03	0,04
0,18	0,45	0,54	0,18	0,18	0,15	0,17	0,20	0,29	0,03	0,08
0,19	0,42	0,49	0,16	0,16	0,15	0,17	0,18	0,29	0,05	0,08
0,16	0,45	0,73	0,13	0,13	0,12	0,14	0,14	0,24	0,03	0,07
0,17	0,51	1,35	0,14	0,15	0,14	0,15	0,16	0,26	0,04	0,07
0,23	0,53	1,32	0,15	0,16	0,15	0,17	0,18	0,31	0,04	0,08
0,16	0,47	1,21	0,12	0,14	0,13	0,14	0,15	0,26	0,04	0,07
0,17	0,47	1,38	0,13	0,13	0,13	0,14	0,15	0,25	0,03	0,08
0,13	0,28	0,71	0,09	0,11	0,09	0,10	0,13	0,17	0,06	0,04
0,15	0,33	1,10	0,10	0,12	0,11	0,10	0,14	0,20	0,10	0,07
0,15	0,36	1,09	0,09	0,11	0,09	0,09	0,13	0,20	0,11	0,08
0,15	0,33	1,23	0,10	0,12	0,10	0,10	0,14	0,20	0,08	0,07
0,14	0,32	1,32	0,10	0,11	0,11	0,10	0,14	0,19	0,07	0,04
0,12	0,26	1,38	0,09	0,11	0,10	0,09	0,14	0,18	0,05	0,04
0,12	0,25	1,22	0,09	0,10	0,09	0,09	0,13	0,17	0,05	0,04
0,13	0,33	1,40	0,09	0,12	0,09	0,10	0,14	0,19	0,06	0,05
1,10	0,97	1,12	1,43	1,31	1,25	1,45	1,47	1,16	0,54	0,23
1,14	1,05	0,98	1,20	1,17	1,23	1,25	1,27	1,11	0,49	0,33
1,12	1,03	0,97	1,27	1,18	1,19	1,24	1,29	1,13	0,53	0,31
1,11	0,99	1,03	1,24	1,18	1,14	1,27	1,28	1,14	0,50	0,32
1,19	1,11	0,90	1,38	1,29	1,26	1,35	1,40	1,22	0,57	0,36
1,19	1,15	0,98	1,35	1,31	1,26	1,38	1,40	1,24	0,61	0,38
1,28	1,24	1,12	1,36	1,24	1,35	1,31	1,33	1,26	0,64	0,38
1,18	1,01	1,13	1,11	1,02	1,12	1,16	1,05	1,09	0,58	0,35
1,31	1,21	1,20	1,54	1,42	1,32	1,55	1,60	1,24	0,57	0,43
1,15	1,23	1,03	1,37	1,32	1,26	1,39	1,48	1,20	0,53	0,47
1,14	1,26	1,03	1,47	1,31	1,26	1,37	1,48	1,21	0,51	0,45
1,13	1,16	1,09	1,39	1,26	1,19	1,35	1,43	1,19	0,51	0,45
1,23	1,28	0,90	1,46	1,34	1,29	1,41	1,48	1,26	0,61	0,50
1,19	1,30	1,00	1,41	1,35	1,23	1,41	1,46	1,25	0,61	0,49
1,26	1,27	1,15	1,41	1,24	1,31	1,35	1,39	1,26	0,69	0,54
1,18	1,03	1,14	1,14	1,05	1,14	1,19	1,10	1,11	0,64	0,51
1,18	0,93	0,96	1,25	1,19	1,13	1,31	1,29	1,01	0,50	0,21
1,04	1,03	1,02	1,17	1,10	1,05	1,20	1,18	1,07	0,44	0,23
1,04	1,09	0,99	1,15	1,09	1,08	1,18	1,16	1,06	0,47	0,24
1,05	1,07	1,08	1,16	1,09	1,08	1,20	1,16	1,05	0,46	0,24
1,14	1,18	0,90	1,40	1,29	1,24	1,38	1,40	1,22	0,52	0,27
1,26	1,53	1,06	1,83	1,82	1,42	1,76	2,09	1,39	0,54	0,27
1,28	1,48	1,20	1,70	1,63	1,46	1,63	1,83	1,39	0,58	0,28
1,14	1,15	1,15	1,19	1,07	1,10	1,21	1,14	1,16	0,58	0,26
1,26	1,08	0,73	1,30	1,27	1,22	1,33	1,35	1,15	0,66	0,29
1,10	0,92	0,96	1,15	1,16	1,11	1,17	1,20	1,08	0,54	0,22
1,10	0,93	0,95	1,17	1,14	1,14	1,16	1,20	1,07	0,55	0,21
1,12	1,02	0,67	1,20	1,16	1,14	1,19	1,20	1,07	0,59	0,24
1,18	1,08	0,71	1,26	1,21	1,21	1,23	1,28	1,14	0,62	0,26
1,15	1,01	0,80	1,19	1,21	1,14	1,23	1,26	1,13	0,61	0,23
1,27	1,13	0,91	1,31	1,29	1,24	1,31	1,35	1,24	0,69	0,26
1,17	1,04	0,92	1,13	1,12	1,06	1,18	1,15	1,10	0,67	0,28
1,13	0,91	0,74	1,14	1,02	1,06	1,14	1,10	0,96	0,66	0,18
1,02	0,78	0,94	1,07	0,97	1,02	1,07	1,03	1,11	0,65	0,22
1,00	0,71	0,97	0,99	0,92	1,02	1,02	0,97	1,05	0,56	0,20
1,03	0,73	1,06	1,02	0,95	1,08	1,05	0,99	1,06	0,55	0,20
1,06	0,83	0,94	1,06	0,96	1,07	1,05	1,03	1,09	0,62	0,21
1,00	0,86	1,01	0,96	0,93	1,02	1,01	0,97	1,06	0,58	0,22
1,15	0,96	1,17	1,13	1,02	1,20	1,14	1,10	1,15	0,56	0,22
1,07	0,87	1,13	1,08	0,96	1,06	1,10	1,03	1,09	0,51	0,21
1,07	0,65	0,77	1,08	0,91	1,05	1,10	1,02	0,91	0,59	0,18
0,94	0,74	0,96	1,02	0,86	0,96	1,01	0,94	1,06	0,54	0,23
0,93	0,84	0,98	0,99	0,84	1,03	1,01	0,94	1,06	0,55	0,22
0,99	0,85	1,06	1,04	0,88	1,06	1,06	0,96	1,05	0,58	0,23
1,00	0,82	0,95	1,06	0,89	1,06	1,04	0,98	1,11	0,63	0,24
0,96	0,75	1,00	0,99	0,83	1,01	1,02	0,95	1,06	0,60	0,24
1,09	0,84	1,14	1,13	0,95	1,20	1,14	1,07	1,18	0,67	0,26
1,03	0,89	1,10	1,10	0,93	1,07	1,11	1,03	1,14	0,64	0,24

c (40/40/19.9/0.1, v/v); DS7 = methanol/water/ammonium hydroxide (80/19.9/0.1, v/v); and DS8 = acetonitrile/1-propanol/methanol/water/ammonium hydroxide (30/25/25/19.9/0.1, v/v).

Table S3. RSD values, n=3.

coating/DS type	meldoniur	psilocybin	morphine	salbutamo	terbutalin	atenolol	fenoterol	nikethami	carteolol	oxycodon	amphetan	chlorothia	hydrocod	methamp	methylhex	MDMA	hydrochlo	strychnine
C18 DS1	7,6%		11,2%	11,5%	11,8%	6,1%	6,5%	4,3%	6,7%	7,9%	6,3%	4,1%	6,0%	7,6%	5,7%	8,0%	18,6%	7,3%
C18 DS2	23,7%	7,5%	9,2%	4,4%	5,5%	3,1%	4,0%	5,3%	6,7%	4,6%	2,9%	11,9%	5,8%	3,9%	1,4%	5,9%	17,5%	3,7%
C18 DS3	9,9%	37,7%	5,3%	4,1%	4,6%	2,1%	0,8%	6,5%	1,5%	0,8%	3,4%	1,7%	2,7%	1,3%	1,1%	0,4%	37,7%	3,2%
C18 DS4	6,3%	50,5%	11,5%	9,4%	10,5%	8,0%	6,1%	7,5%	5,1%	5,5%	6,6%	12,5%	4,9%	7,6%	8,4%	7,3%	24,2%	6,5%
C18 DS5	20,5%		11,2%	8,1%	10,8%	7,3%	6,3%	2,9%	4,5%	4,4%	6,9%	8,5%	2,6%	4,6%	4,3%	4,3%	7,8%	2,5%
C18 DS6	29,3%	12,8%	16,5%	15,2%	14,3%	9,9%	5,4%	11,7%	3,7%	5,9%	11,0%	18,1%	4,7%	7,1%	9,2%	5,6%	13,3%	3,3%
C18 DS7	7,0%	14,6%	2,0%	2,8%	5,3%	2,0%	2,1%	1,3%	1,3%	1,8%	2,3%	7,7%	2,5%	0,3%	2,5%	1,7%	16,4%	2,4%
C18 DS8	16,0%	22,0%	7,3%	7,5%	6,7%	6,6%	2,0%	2,9%	2,6%	3,2%	2,9%	10,0%	2,9%	1,2%	3,8%	2,0%	53,7%	1,4%
C8 DS1	8,9%		7,2%	4,0%	5,7%	4,1%	3,1%	4,5%	2,7%	3,1%	4,9%	15,4%	3,4%	4,1%	4,1%	4,5%	29,7%	6,0%
C8 DS2	6,9%	16,6%	3,8%	3,5%	3,8%	3,5%	3,1%	2,8%	1,1%	1,9%	4,0%	5,5%	2,7%	2,9%	4,0%	2,8%	31,7%	2,9%
C8 DS3	17,3%	12,4%	4,9%	4,6%	4,7%	4,5%	3,5%	3,8%	0,0%	0,7%	0,1%	6,9%	1,3%	0,5%	1,2%	1,2%	35,7%	1,1%
C8 DS4	8,1%	56,2%	10,3%	8,3%	8,1%	7,1%	4,1%	4,3%	4,2%	2,8%	4,6%	15,2%	3,9%	5,6%	3,8%	3,5%	29,2%	5,0%
C8 DS5	12,5%		8,8%	8,1%	7,4%	7,1%	3,8%	1,4%	5,7%	2,6%	5,5%	8,7%	3,7%	4,6%	3,7%	5,5%	33,7%	2,5%
C8 DS6	15,8%	20,7%	16,4%	11,1%	13,2%	10,6%	8,2%	11,2%	4,5%	6,5%	7,6%	5,7%	6,4%	6,0%	6,4%	4,3%	24,1%	3,0%
C8 DS7	1,6%	8,8%	4,2%	5,5%	5,5%	3,1%	2,9%	5,6%	0,8%	0,8%	4,3%	2,2%	0,9%	1,5%	1,8%	1,4%	8,5%	0,6%
C8 DS8	6,3%	36,8%	6,1%	4,6%	5,2%	4,4%	1,8%	2,7%	1,2%	0,9%	4,6%	9,8%	1,4%	1,7%	1,3%	1,0%	20,8%	1,3%
Phe-Hex DS1	7,7%		9,0%	7,6%	9,0%	9,4%	6,0%	9,5%	5,6%	6,0%	10,3%	12,8%	5,1%	4,7%	7,8%	4,1%	9,7%	5,3%
Phe-Hex DS2	19,8%	12,8%	3,1%	4,2%	3,8%	3,4%	2,3%	2,2%	0,7%	1,7%	4,4%	7,7%	0,4%	2,0%	3,8%	1,4%	15,5%	1,1%
Phe-Hex DS3	8,8%	44,6%	6,6%	8,1%	7,3%	7,0%	3,8%	5,3%	2,0%	3,2%	4,6%	7,9%	3,4%	4,8%	3,7%	2,7%	25,3%	2,4%
Phe-Hex DS4	11,3%	28,5%	16,7%	15,8%	15,5%	15,8%	9,7%	9,5%	8,0%	10,0%	12,6%	13,1%	7,3%	11,4%	12,6%	10,1%	22,2%	1,8%
Phe-Hex DS5	18,5%		9,0%	12,9%	9,9%	10,3%	4,9%	5,6%	6,8%	5,4%	8,4%	9,0%	3,3%	9,5%	10,6%	7,4%	19,2%	2,7%
Phe-Hex DS6	2,6%	6,8%	0,4%	1,4%	1,3%	1,2%	0,9%	3,1%	1,7%	0,9%	1,6%	1,9%	1,0%	1,6%	4,7%	1,8%	5,0%	1,3%
Phe-Hex DS7	4,0%	16,0%	1,4%	1,7%	3,5%	1,9%	2,3%	1,4%	3,5%	1,2%	1,8%	1,7%	2,6%	2,9%	1,4%	2,3%	17,0%	1,8%
Phe-Hex DS8	4,6%	40,7%	16,0%	15,0%	14,8%	15,2%	15,8%	11,3%	13,1%	13,2%	15,1%	13,7%	12,8%	16,0%	16,0%	12,3%	13,8%	6,6%
CN DS1	5,1%		5,1%	6,9%	5,2%	3,1%	4,8%	5,2%	7,0%	6,2%	2,9%	6,3%	6,3%	6,6%	5,5%	5,5%	16,8%	10,7%
CN DS2	0,7%	19,6%	2,7%	4,8%	3,8%	2,7%	2,7%	2,2%	3,5%	3,1%	4,3%	8,9%	1,9%	2,0%	3,3%	2,3%	22,6%	3,1%
CN DS3	7,1%	11,5%	3,4%	5,1%	4,6%	5,4%	1,7%	9,2%	2,0%	1,7%	4,2%	15,0%	0,5%	4,1%	3,2%	1,5%	32,0%	0,4%
CN DS4	6,2%	3,6%	0,4%	0,9%	1,0%	2,2%	2,1%	6,9%	3,3%	1,5%	2,2%	3,5%	2,2%	2,6%	1,3%	3,1%	6,5%	0,2%
CN DS5	5,8%		2,1%	8,7%	4,9%	6,9%	2,5%	2,9%	5,6%	1,9%	1,4%	3,0%	3,4%	3,2%	2,6%	2,4%	28,5%	1,7%
CN DS6	6,6%	9,9%	6,3%	7,8%	7,6%	6,5%	5,8%	8,4%	4,5%	7,0%	5,1%	3,5%	5,0%	5,8%	6,3%	5,7%	26,6%	5,7%
CN DS7	2,7%	26,7%	1,6%	3,6%	2,8%	1,6%	2,2%	3,1%	0,6%	2,2%	1,1%	4,5%	1,2%	3,2%	1,1%	1,6%	7,7%	1,3%
CN DS8	1,7%	23,3%	4,4%	9,6%	5,9%	5,7%	1,1%	8,8%	1,7%	3,2%	1,7%	7,9%	1,6%	1,9%	4,0%	1,0%	25,3%	1,3%
SCX DS1	10,7%		19,0%	15,3%	13,1%	25,4%	12,1%	1,1%	16,0%	19,3%	9,1%	36,0%	19,0%	12,9%	14,9%	13,6%		25,2%
SCX DS2	10,6%		9,1%	6,7%	7,4%	7,8%	4,2%	5,1%	8,2%	10,6%	8,8%	9,5%	12,4%	11,5%	10,7%	11,3%	12,2%	15,8%
SCX DS3	2,2%	20,4%	4,2%	4,8%	5,3%	6,6%	4,0%	1,1%	7,4%	3,7%	2,1%	34,2%	5,6%	4,2%	4,0%	5,4%	7,0%	10,0%
SCX DS4	18,1%		13,9%	11,3%	11,1%	13,2%	8,0%	7,6%	12,4%	13,8%	11,7%	2,1%	18,8%	13,6%	7,9%	14,5%	2,2%	18,7%
SCX DS5	15,7%		1,6%	1,6%	1,5%	2,8%	3,5%	11,9%	5,9%	1,5%	2,9%	12,3%	1,5%	2,2%	1,4%	3,3%	20,8%	7,3%
SCX DS6	4,6%	58,9%	2,4%	2,9%	4,1%	3,1%	3,5%	2,5%	3,5%	0,9%	0,8%	1,6%	1,8%	1,8%	2,9%	3,1%	25,4%	2,9%
SCX DS7	6,9%	3,0%	1,2%	0,8%	0,6%	2,6%	0,9%	5,8%	0,8%	1,3%	2,1%	3,1%	1,8%	0,8%	0,5%	0,8%	4,4%	1,8%
SCX DS8	10,5%	39,9%	4,8%	3,9%	3,9%	4,9%	2,0%	11,3%	3,7%	4,0%	2,2%	8,4%	4,0%	2,4%	0,9%	2,7%	170,2%	6,2%
SIL DS1	2,4%		10,9%	8,9%	9,0%	6,1%	7,0%	3,4%	8,7%	8,3%	5,2%	10,9%	7,4%	6,1%	8,2%	4,8%	9,2%	7,7%
SIL DS2	8,0%	23,8%	1,6%	5,3%	3,2%	2,6%	3,8%	6,1%	2,1%	1,5%	3,1%	13,0%	1,2%	2,6%	0,4%	2,0%	48,5%	1,1%
SIL DS3	2,4%	18,7%	2,9%	3,1%	3,3%	1,8%	4,1%	2,8%	2,0%	1,4%	0,9%	7,7%	1,1%	1,8%	1,9%	1,1%	27,0%	1,9%
SIL DS4	3,1%	12,5%	0,7%	1,5%	2,1%	2,8%	4,1%	1,9%	2,0%	2,1%	1,9%	9,4%	0,5%	2,4%	3,8%	1,4%	20,4%	2,8%
SIL DS5	6,2%		2,8%	4,1%	1,7%	1,8%	2,9%	3,5%	1,6%	3,5%	1,2%	6,2%	2,9%	1,8%	2,0%	0,8%	15,6%	3,8%
SIL DS6	3,7%	17,4%	3,6%	2,6%	1,9%	2,4%	2,0%	2,8%	3,0%	2,5%	4,8%	4,5%	4,0%	3,9%	4,6%	2,8%	87,5%	4,1%
SIL DS7	10,8%	26,2%	2,4%	2,6%	2,7%	1,0%	0,2%	6,0%	0,4%	1,6%	0,3%	8,9%	0,8%	0,1%	1,3%	0,9%	12,8%	3,5%
SIL DS8	7,1%	4,5%	1,0%	3,0%	1,9%	0,7%	1,7%	10,9%	0,6%	0,9%	2,1%	3,9%	2,7%	0,8%	0,8%	0,4%	17,1%	1,6%
C8+CN (3:1) DS1	3,1%		2,5%	4,5%	4,7%	3,4%	4,8%	10,7%	5,3%	2,2%	2,9%	14,7%	1,5%	2,2%	0,3%	3,2%	22,1%	5,9%
C8+CN (3:1) DS2	8,7%	2,5%	2,7%	3,5%	4,0%	2,2%	0,8%	1,7%	1,2%	1,2%	1,8%	6,1%	2,5%	1,2%	1,4%	1,7%	5,5%	3,5%
C8+CN (3:1) DS3	18,8%	4,7%	11,5%	11,5%	11,0%	8,1%	4,5%	7,3%	2,8%	4,1%	5,6%	11,1%	3,3%	4,8%	6,0%	2,8%	8,9%	1,7%
C8+CN (3:1) DS4	6,4%	14,0%	7,8%	7,2%	7,0%	7,5%	4,0%	4,0%	2,4%	4,5%	4,4%	5,6%	2,5%	3,0%	6,6%	4,0%	17,3%	1,0%
C8+CN (3:1) DS5	14,0%	22,7%	9,9%	11,2%	10,9%	10,2%	7,0%	7,3%	8,4%	6,0%	9,3%	7,0%	3,9%	6,8%	8,3%	7,6%	28,1%	4,2%
C8+CN (3:1) DS6	8,4%	12,7%	3,0%	2,3%	3,4%	2,7%	2,2%	2,5%	1,6%	3,0%	3,0%	5,3%	0,1%	1,2%	1,8%	1,3%	17,5%	1,3%
C8+CN (3:1) DS7	3,4%	12,1%	3,5%	2,6%	2,1%	1,8%	1,4%	3,1%	1,0%	1,1%	2,0%	3,7%	0,2%	0,2%	2,6%	1,2%	25,7%	1,9%
C8+CN (3:1) DS8	2,4%	17,1%	5,3%	4,3%	5,2%	3,6%	3,8%	5,0%	3,6%	4,8%	1,5%	8,6%	3,6%	2,7%	2,3%	3,0%	13,3%	1,8%
C8+CN (1:1) DS1	5,7%		6,5%	1,7%	1,7%	4,0%	1,8%	2,3%	5,8%	1,0%	3,3%	4,7%	2,8%	0,6%	1,9%	3,4%	21,5%	4,0%
C8+CN (1:1) DS2	1,2%	28,5%	0,6%	3,7%	2,6%	3,2%	1,5%	2,8%	2,9%	2,7%	0,9%	2,9%	1,6%	2,9%	5,1%	2,1%	11,3%	2,0%
C8+CN (1:1) DS3	12,5%	11,2%	11,4%	9,8%	8,6%	8,3%	3,5%	7,3%	1,9%	2,8%	5,0%	13,0%	2,0%	2,3%	7,1%	1,7%	9,3%	1,6%
C8+CN (1:1) DS4	4,3%	25,4%	10,2%	8,1%	8,4%	6,8%	4,8%	6,2%	2,4%	4,9%	6,3%	3,4%	3,4%	3,9%	5,7%	3,8%	20,0%	3,5%
C8+CN (1:1) DS5	2,3%	18,6%	10,3%	14,5%	13,5%	12,8%	6,7%	3,9%	9,1%	3,4%	9,1%	9,4%	2,3%	8,2%	9,9%	9,0%	12,6%	6,4%
C8+CN (1:1) DS6	1,5%	7,7%	1,8%	2,0%	2,5%	0,5%	1,0%	3,0%	0,4%	2,3%	0,8%	3,7%	1,0%	1,4%	1,2%	1,8%	13,9%	2,4%
C8+CN (1:1) DS7	4,5%	22,1%	2,1%	3,0%	2,1%	2,2%	2,1%	6,9%	2,1%	2,6%	2,2%	4,7%	1,2%	2,3%	2,2%	3,1%	8,5%	2,3%
C8+CN (1:1) DS8	5,6%	8,4%	6,3%	6,4%	5,6%	4,5%	2,8%	6,5%	1,7%	4,1%	2,6%	3,8%	2,4%	2,4%	2,5%	1,1%	10,9%	3,4%
C8+CN (1:3) DS1	13,7%		2,4%	4,1%	3,2%	2,7%	6,2%	7,6%	9,6%	4,2%	2,1%	15,7%	2,7%	4,5%	2,2%	7,6%	23,2%	11,7%
C8+CN (1:3) DS2	1,2%	21,4%	1,5%	1,1%	1,3%	1,4%	0,3%	0,3%	0,9%	1,7%	1,6%	4,2%	1,1%	0,8%	2,2%	1,7%	14,8%	2,6%
C8+CN (1:3) DS3	3,9%	24,3%	2,6%	2,4%	2,2%	2,2%	1,5%	3,4%	1,2%	1,7%	2,0%	2,3%	2,0%	2,3%	2,6%	1,4%	3,4%	1,5%
C8+CN (1:3) DS4	9,2%	42,1%	5,1%	5,8%	4,6%	1,0%	0,9%	7,2%	1,5%	1,3%	1,7%	8,8%	1,7%	0,5%	2,1%	0,8%	10,5%	1,9%
C8+CN (1:3) DS5	12,6%	13,8%	18,8%	11,4%	10,6%	12,7%	10,5%	4,8%	7,9%	4,2%	5,7%	6,7%	4,6%	5,6%	3,2%	3,5%	6,6%	5,4%
C8+CN (1:3) DS6	4,1%	15,8%	3,1%	2,9%	1,4%	2,2%	1,3%	4,8%	0,4%	2,9%	4,5%	11,9%	2,7%	2,4%	3,0%	4,0%	7,3%	1,0%
C8+CN (1:3) DS7	5,0%	9,0%	5,4%	3,8%	3,6%	1,9%	0,6%	2,9%	1,6%	1,8%	4,1%	2,0%	4,0%	0,9%	1,4%	2,2%	15,4%	0,8%
C8+CN (1:3) DS8	3,9%	10,1%	10,7%	2,2%	2,2%	0,8%	3,7%	2,2%	1,1%	1,1%	1,5%	1,6%	1,3%	1,4%	1,4%	1,4%	13,7%	1,5%
C8+SIL (3:1) DS1	4,3%		14,2%	8,8%	10,1%	7,8%	16,5%	1,9%	19,4%	19,8%	3,1%	11,6%	18,4%	9,1%	12,3%	13,2%	20,7%	26,8%
C8+SIL (3:1) DS2	5,0%		4,0%	1,7%	1,6%	3,9%	2,1%	0,8%	6,1%	6,5%	5,8%	8,8%	6,3%	5,5%	5,3%	7,2%	11,0%	8,7%
C8+SIL (3:1) DS3	6,8%	13,3%	1,8%	2,5%	2,2%	2,1%	2,1%	3,4%	2,9%	3,2%	2,6%	7,5%	4,4%	1,9%	3,3%	3,4%	11,7%	4,7%
C8+SIL (3:1) DS4	1,3%	26,3%	5,0%	1,4%	0,9%	3,5%	2,5%	3,4%	4,0%	6,4%	5,2%	6,7%	6,2%	6,4%	5,2%	7,0%	4,4%	9,4%
C8+SIL (3:1) DS5	8,3%	15,4%	6,4%	9,9%	8,6%	11,2%	6,8%	6,0%	9,0%	3,6%	7,8%	2,8%	4,6%	7,6%	8,9%	8,9%	18,9%	5,7%
C8+SIL (3:1) DS6	3,1%	13,4%	2,4%	2,0%	1,7%	2,2%	0,9%	2,7%	1,6%	0,5%	2,6%	0,9%	1,8%	0,5%	3,7%	1,5%	22,5%	2,0%
C8+SIL (3:1) DS7	9,5%	9,9%	1,3%	0,9%	1,4%	2,3%	1,7%	2,9%	0,6%	1,1%	1,8%	3,5%	0,8%	1,6%	6,5%	1,7%	15,7%	0,6%
C8+SIL (3:1) DS8	7,7%	20,0%	1,0%	0,8%	0,3%	2,8%	2,2%	1,1%	1,1%	1,1%	0,9%	4,3%	1,9%	1,2%	3,2%	0,5%	10,6%	2,6%
C8+SIL (1:1) DS1	3,8%		11,0%	10,6%	11,4%	11,4%	14,8%	3,9%	16,3%	10,4%	3,8%	2,2%	6,3%	8,8%	9,3%	9,7%	27,5%	13,9%
C8+SIL (1:1) DS2	5,8%	28,7%	3,5%	5,2%	5,6%	4,9%	3,2%	6,6%	2,8%	4,2%	3,8%	6,5%	2,9%	2,4%	5,9%	3,6%	10,6%	1,6%
C8+SIL (1:1) DS3	4,8%	30,5%	2,0%	1,8%	3,5%	1,3%	2,3%	6,7%	2,3%	3,8%	1,2%	5,8%	2,9%	3,2%	2,5%	2,0%	23,6%	3,7%
C8+SIL (1:1) DS4	9,9%	9,4%	9,7%	10,0%	9,6%	5,9%	6,0%	6,5%	3,7%	5,0%	4,8%	4,9%	2,9%	4,6%	6,8%	3,9%	21,9%	1,8%
C8+SIL (1:1) DS5	8,6%	15,5%	2,8%	4,2%	3,0%	5,5%	4,4%	9,8%	7,4%	2,6%	7,4%	12,4%	2,0%	5,7%	8,4%	6,2%	22,1%	5,7%
C8+SIL (1:1) DS6	8,7%	17,7%	3,0%	1,2%	1,0%	0,9%	0,7%	6,5%	0,8%	0,3%	1,9%	7,4%	1,4%	1,8%	3,5%	2,1%	12,3%	2,2%
C8+SIL (1:1) DS7	15,0%	8,0%	6,9%	5,5%	5,4%	5,6%	4,3%	9,3%	1,3%	4,4%	5,2%	10,1%	3,3%	3,0%	5,8%	2,2%	31,0%	2,0%
C8+SIL (1:1) DS8	0,8%	23,8%	0,9%	4,2%	3,3%	3,1%	2,0%	1,0%	0,5%	1,3%	0,7%	5,5%	1,0%	1,3%	1,1%	2,6%	9,8%	0,5%
C8+SIL (1:3) DS1	3,6%		1,4%	1,8%	2,3%	3,2%	6,2%	4,0%	10,5%	5,3%	3,8%	10,3%	1,7%	2,9%	4,3%	7,1%	37,2%	10,7%
C8+SIL (1:3) DS2	8,1%		4,1%	4,5%	2,5%	4,8%	2,0%	5,2%	0,7%	2,8%	3,6%	3,7%	0,4%	0,7%	1,4%	1,9%	35,4%	0,3%
C8+SIL (1:3) DS3	6,4%	6,9%	4,8%	10,0%	8,5%	4,7%	4,4%	7,1%	1,5%	2,8%	5,8%	6,1%	1,6%	2,4%	4,0%	1,4%	39,6%	3,4%
C8+SIL (1:3) DS4	4,1%		6,2%	7,5%	7,5%	5,7%	5,5%	10,7%	3,3%	4,0%	4,3%	5,4%	3,6%	3,3%	5,0%	1,7%	27,7%	1,9%
C8+SIL (1:3) DS5	8,6%	15,3%	2,9%	3,8%	2,0%	7,4%	6,6%	5,5%	7,1%	2,1%	4,6%	6,4%	2,4%	6,4%	3,1%	7,0%	60,9%	6,6%
C8+SIL (1:3) DS6	3,0%	1,6%	2,4%	3,5%	3,3%	1,4%	1,8%	3,2%	0,7%	3,1%	2,7%	7,9%	0,4%	2,6%	0,8%	1,6%	10,5%	3,2%
C8+SIL (1:3) DS7	7,5%	14,6%	1,8%	2,7%	2,1%	1,6%	3,5%	3,4%	2,3%	0,3%	3,1%	8,1%	0,5%	1,4%	3,0%	2,9%	28,6%	0,7%
C8+SIL (1:3) DS8	7,2%	18,7%	4,2%	5,0%	3,1%	2,5%	3,5%	8,0%	2,0%	2,7%	2,7%	6,7%	2,6%	0,8%	1,5%	1,7%	17,6%	1,3%

Abbreviations used for coatings: octadecyl (C18); octyl (C8); phenyl-hexyl (Phe-Hex); 3-cyanopropyl (CN); benzenesulfonic acid (SCX); and unbonded silica (SIL)
Compositions of desorption solvents: DS1 = acetonitrile/water/formic acid (80/19.9/0.1, V/V); DS2 = acetonitrile/methanol/water/formic acid (40/40/19.9/0.1, V/V); DS3 = methanol/water/formic acid (80/19.9/0.1, V

ketamine	metoprolo	clenbuter	methylphe	zolpidem	cocaine	LSD	bisoprolol	phencyclic	propranol	prednisolo	fentanyl	prednison	buprenorp	butamben	betameth	furosemid	nebivolol	alprazolan	anastrozol
7,0%	8,4%	7,0%	6,0%	3,9%	6,7%	4,2%	7,3%	11,0%	8,3%	6,6%	8,8%	1,4%	12,0%	15,7%	2,6%	14,1%	5,8%	1,5%	2,3%
6,1%	5,9%	6,8%	7,4%	8,2%	6,8%	7,3%	6,7%	7,9%	6,9%	7,6%	8,1%	9,6%	7,6%	7,0%	12,6%	17,6%	7,6%	8,9%	7,3%
1,9%	1,1%	1,0%	0,4%	1,5%	1,6%	2,9%	2,2%	0,8%	2,0%	5,6%	1,7%	2,2%	2,3%	2,5%	2,6%	10,5%	1,9%	1,5%	0,3%
6,2%	5,7%	4,2%	4,9%	3,9%	4,6%	3,7%	5,0%	3,9%	3,7%	3,2%	3,4%	4,7%	2,2%	4,0%	1,2%	15,5%	2,8%	3,4%	4,4%
2,0%	2,6%	3,0%	1,6%	1,2%	0,7%	1,2%	1,3%	2,6%	4,9%	8,3%	1,4%	4,2%	0,2%	9,8%	4,3%	18,6%	1,6%	6,3%	4,0%
3,5%	2,4%	2,0%	2,4%	0,7%	0,9%	0,9%	1,1%	0,7%	1,7%	5,1%	1,4%	1,6%	0,7%	2,0%	1,4%	6,7%	1,7%	1,3%	1,7%
0,3%	2,8%	0,8%	0,8%	1,0%	0,5%	1,0%	1,1%	1,4%	1,3%	4,2%	1,3%	1,0%	1,3%	2,9%	2,6%	5,9%	3,0%	0,2%	1,8%
1,6%	1,3%	0,7%	1,1%	0,6%	1,1%	1,5%	1,7%	0,8%	0,4%	2,6%	1,2%	1,0%	0,7%	5,1%	6,7%	2,6%	0,6%	1,5%	0,9%
3,7%	3,4%	2,7%	2,5%	6,3%	1,9%	5,5%	2,6%	2,2%	5,2%	5,2%	3,9%	2,4%	2,3%	6,0%	2,4%	13,5%	9,5%	1,8%	2,6%
2,8%	3,0%	1,4%	2,3%	1,6%	1,7%	0,5%	1,8%	2,4%	2,1%	1,6%	2,9%	0,8%	2,2%	0,7%	3,0%	4,3%	1,8%	0,3%	1,1%
1,2%	2,5%	1,3%	1,7%	2,2%	3,0%	0,6%	1,9%	2,4%	1,3%	2,8%	1,7%	2,9%	1,4%	2,2%	5,8%	6,6%	2,1%	1,3%	2,3%
2,8%	2,5%	3,4%	2,0%	1,1%	1,7%	2,3%	1,4%	2,0%	2,5%	6,3%	1,7%	2,7%	0,7%	1,8%	4,9%	7,0%	0,2%	0,8%	1,7%
3,7%	4,5%	5,1%	3,3%	5,0%	4,4%	4,1%	4,6%	4,5%	7,0%	4,9%	4,2%	5,6%	5,1%	4,5%	7,9%	3,6%	3,9%	5,1%	4,9%
3,0%	3,9%	3,3%	2,3%	3,0%	2,4%	2,8%	3,3%	2,0%	1,6%	1,4%	3,0%	4,1%	3,0%	5,0%	2,3%	7,3%	3,8%	2,2%	2,0%
1,5%	1,1%	1,6%	0,7%	1,5%	0,3%	1,2%	1,3%	1,1%	2,1%	4,9%	0,7%	3,9%	1,0%	2,6%	4,7%	8,3%	1,6%	1,8%	2,4%
1,6%	1,1%	0,1%	1,3%	0,4%	1,3%	1,2%	1,0%	1,2%	1,2%	4,2%	1,2%	1,7%	1,9%	4,3%	4,2%	4,8%	0,7%	1,5%	3,7%
4,3%	3,1%	5,0%	3,4%	1,4%	3,1%	1,9%	4,9%	7,8%	5,9%	2,4%	6,7%	1,5%	11,3%	17,2%	3,6%	14,7%	3,1%	0,8%	1,9%
0,3%	2,2%	1,2%	0,7%	1,3%	0,5%	0,3%	0,8%	1,1%	0,9%	1,5%	0,6%	1,1%	0,7%	0,6%	3,0%	2,7%	0,6%	1,8%	1,4%
2,2%	1,9%	1,2%	2,0%	1,2%	1,3%	1,3%	1,5%	0,5%	1,9%	3,0%	0,3%	2,2%	0,6%	0,7%	1,9%	9,9%	1,2%	1,0%	2,0%
7,9%	5,2%	4,8%	6,2%	1,3%	1,4%	0,8%	1,2%	1,6%	1,8%	3,4%	2,8%	3,6%	3,3%	3,1%	2,2%	15,1%	3,1%	1,0%	1,3%
2,4%	3,0%	4,5%	4,2%	2,6%	0,2%	2,0%	0,5%	0,7%	1,6%	5,3%	3,5%	1,7%	3,4%	3,4%	3,6%	18,0%	3,5%	1,3%	1,5%
1,3%	1,0%	0,9%	0,9%	1,5%	0,4%	0,4%	0,8%	1,4%	1,6%	3,5%	0,8%	1,5%	1,6%	1,1%	4,1%	1,3%	1,9%	1,4%	1,0%
1,7%	3,1%	1,8%	1,6%	1,3%	1,4%	0,9%	1,5%	1,0%	1,3%	1,4%	1,2%	4,5%	2,0%	2,5%	1,8%	3,0%	1,1%	2,3%	0,8%
11,3%	9,8%	10,8%	10,9%	0,5%	6,5%	0,8%	3,9%	3,7%	2,3%	4,1%	0,8%	2,0%	0,5%	2,5%	3,2%	12,1%	0,4%	1,6%	1,4%
5,0%	6,7%	10,0%	4,2%	4,1%	7,1%	8,4%	7,0%	12,8%	12,8%	4,3%	10,4%	2,6%	14,0%	10,6%	5,9%		8,6%	3,7%	4,2%
3,4%	2,9%	3,8%	2,3%	2,4%	3,2%	3,9%	3,7%	2,8%	3,6%	1,0%	3,2%	4,3%	3,6%	3,9%	5,0%		3,3%	0,6%	0,4%
1,5%	3,4%	2,2%	1,8%	1,4%	0,6%	0,4%	1,8%	2,3%	1,0%	12,0%	1,0%	12,2%	1,9%	1,2%	2,5%		0,7%	2,7%	2,4%
2,2%	2,5%	2,0%	2,1%	1,8%	2,6%	2,5%	2,2%	1,8%	3,0%	3,1%	2,9%	4,0%	2,6%	2,9%	15,5%		2,2%	3,8%	2,1%
3,6%	1,4%	1,6%	1,0%	2,9%	1,6%	4,2%	1,2%	1,0%	3,9%	3,0%	3,3%	2,4%	2,2%	3,3%	5,5%		1,7%	1,2%	1,5%
6,5%	5,8%	4,3%	6,3%	6,0%	5,9%	5,2%	6,1%	6,3%	4,1%	18,9%	5,3%	9,1%	5,6%	5,9%	7,9%		4,8%	6,1%	6,9%
1,1%	3,3%	1,6%	2,1%	0,3%	1,7%	2,5%	1,6%	1,9%	1,5%	10,1%	1,3%	6,3%	1,8%	3,9%	13,3%		1,3%	5,2%	5,2%
3,5%	1,5%	1,4%	1,7%	0,7%	1,0%	1,6%	2,2%	0,5%	0,7%	17,0%	0,9%	15,9%	0,4%	3,1%	18,3%		0,5%	9,9%	10,8%
13,0%	11,4%	13,2%	11,0%	10,3%	13,1%	13,8%	13,8%	17,1%	15,0%	16,7%	16,4%	25,6%	18,9%	24,0%	48,9%		10,4%	3,9%	9,0%
8,9%	7,1%	8,4%	9,4%	10,7%	10,9%	12,9%	7,0%	10,9%	11,8%	7,2%	9,5%	11,7%	9,1%	6,8%	27,2%		7,2%	3,6%	10,5%
4,3%	5,5%	4,0%	3,5%	7,1%	4,8%	8,9%	4,4%	5,0%	5,3%	26,7%	7,3%	20,5%	3,1%	6,9%	18,7%		2,6%	15,9%	12,2%
13,5%	10,6%	11,8%	13,2%	12,2%	15,2%	14,3%	10,4%	13,3%	12,2%	21,2%	11,6%	20,0%	10,2%	10,7%	15,6%		10,5%	6,0%	5,7%
2,6%	2,0%	3,9%	1,6%	2,4%	1,7%	1,8%	2,0%	1,9%	5,7%	11,4%	2,3%	2,0%	2,5%	1,3%	16,3%		4,1%	8,8%	7,3%
1,8%	1,8%	2,1%	3,1%	3,1%	2,8%	2,7%	2,7%	2,8%	3,1%	12,6%	2,7%	17,3%	1,8%	0,2%	19,4%		1,3%	1,9%	8,1%
0,5%	1,0%	1,1%	0,5%	0,8%	1,3%	0,3%	0,5%	0,8%	0,6%	10,2%	0,6%	3,4%	1,1%	1,8%	27,5%		1,7%	5,6%	7,4%
2,5%	4,1%	3,2%	2,7%	2,6%	2,6%	3,1%	2,8%	2,6%	3,5%	9,8%	3,0%	19,0%	2,5%	0,8%	49,7%		2,4%	10,9%	4,7%
7,1%	6,6%	8,6%	4,2%	2,3%	6,2%	5,0%	7,1%	14,0%	11,1%	19,1%	8,9%	31,8%	13,5%	13,3%	26,3%		10,1%	2,1%	6,5%
2,3%	2,5%	2,4%	2,5%	2,5%	2,4%	2,3%	2,2%	3,0%	2,2%	43,1%	2,3%	31,5%	2,7%	3,9%	19,7%		3,2%	3,2%	6,5%
1,0%	1,4%	0,3%	0,9%	0,3%	1,1%	0,6%	0,9%	0,2%	0,7%	11,6%	0,6%	35,1%	1,8%	1,4%	26,5%		0,6%	4,9%	8,9%
2,6%	3,2%	3,2%	2,4%	2,5%	2,5%	3,0%	2,8%	1,8%	1,4%	40,5%	3,5%	12,8%	2,3%	2,5%	11,0%		1,9%	2,7%	6,2%
4,0%	2,1%	1,4%	2,7%	3,6%	4,2%	4,3%	2,1%	2,3%	0,7%	12,1%	4,2%	11,8%	4,6%	3,3%	39,3%		3,0%	4,8%	4,9%
2,9%	3,2%	2,9%	3,2%	2,9%	2,9%	2,7%	3,1%	2,8%	2,6%	19,5%	3,7%	43,0%	3,8%	4,4%	40,0%		1,7%	2,7%	2,2%
0,5%	1,5%	0,7%	1,0%	0,6%	1,8%	2,1%	1,4%	2,0%	1,3%	12,8%	1,5%	51,3%	1,4%	2,3%	31,4%		1,4%	4,2%	3,4%
0,5%	0,7%	0,1%	0,5%	1,1%	0,7%	1,8%	0,6%	1,1%	1,4%	3,9%	1,0%	20,7%	0,3%	2,7%	33,4%		0,6%	7,4%	7,3%
1,5%	4,2%	5,4%	2,8%	3,5%	2,8%	2,0%	6,0%	7,8%	10,3%	3,0%	7,5%	2,6%	9,7%	6,9%	4,2%	6,4%	7,3%	1,3%	1,0%
0,6%	2,4%	0,9%	1,1%	1,4%	2,1%	1,9%	2,1%	2,2%	1,7%	2,7%	1,9%	1,2%	1,2%	0,5%	0,7%	2,9%	0,8%	2,2%	1,7%
2,1%	1,5%	1,7%	1,4%	0,4%	0,6%	2,4%	1,2%	0,6%	1,4%	5,5%	1,2%	5,3%	0,8%	1,3%	2,2%	0,6%	1,1%	0,8%	2,0%
2,0%	2,4%	2,1%	1,5%	0,9%	0,9%	0,7%	1,0%	1,5%	0,1%	4,6%	1,1%	2,6%	1,0%	1,8%	4,8%	1,7%	1,1%	0,6%	0,6%
2,7%	4,9%	5,1%	2,7%	1,0%	1,3%	1,3%	4,1%	4,1%	7,2%	3,0%	2,5%	1,5%	1,3%	7,8%	1,3%	11,2%	3,7%	0,4%	1,0%
1,4%	2,3%	1,7%	1,0%	2,7%	1,2%	1,4%	1,7%	1,0%	0,5%	3,4%	2,0%	3,2%	0,8%	6,2%	3,8%	8,4%	0,8%	2,1%	1,8%
0,6%	2,0%	1,2%	0,1%	1,2%	0,7%	0,2%	0,9%	1,5%	2,7%	1,2%	1,0%	1,4%	0,9%	4,1%	4,2%	2,8%	1,5%	2,1%	0,5%
3,2%	1,2%	2,3%	2,4%	2,6%	3,0%	2,2%	1,9%	1,9%	2,8%	2,9%	2,6%	2,8%	3,6%	5,7%	3,2%	8,4%	3,7%	3,0%	2,3%
3,5%	2,8%	5,2%	2,2%	3,1%	3,2%	3,2%	4,5%	6,8%	12,4%	1,7%	8,4%	0,4%	6,5%	17,9%	1,5%	10,8%	6,4%	2,5%	1,0%
2,2%	0,4%	1,7%	1,7%	2,5%	2,2%	1,9%	1,5%	1,5%	1,8%	2,7%	2,0%	4,6%	1,9%	0,9%	3,4%	1,8%	3,7%	3,7%	1,3%
2,3%	1,7%	1,7%	2,0%	0,5%	1,3%	1,6%	1,3%	0,1%	1,6%	0,8%	1,2%	0,6%	0,9%	1,5%	4,4%	13,1%	0,8%	3,4%	1,7%
1,5%	1,1%	1,3%	1,5%	1,0%	0,5%	0,9%	1,0%	0,7%	0,8%	1,8%	0,9%	1,5%	0,9%	1,9%	6,6%	7,9%	1,0%	0,9%	2,2%
0,8%	5,1%	6,5%	3,2%	0,1%	2,5%	0,6%	2,7%	5,2%	8,3%	4,8%	2,2%	3,5%	2,1%	1,5%	3,5%	10,3%	2,0%	5,9%	0,8%
0,9%	2,5%	1,7%	1,2%	1,3%	1,0%	0,5%	1,1%	1,2%	0,9%	2,4%	0,6%	1,4%	0,5%	4,7%	3,3%	8,1%	1,5%	1,9%	2,0%
2,4%	2,8%	2,4%	1,7%	0,7%	1,5%	0,2%	1,2%	1,3%	2,2%	1,1%	1,7%	1,6%	1,4%	6,3%	4,9%	2,5%	2,1%	0,3%	0,7%
2,2%	2,1%	0,8%	2,5%	1,0%	1,4%	1,3%	0,8%	0,7%	0,9%	1,3%	1,5%	2,9%	0,5%	1,8%	6,7%	1,7%	3,5%	1,4%	0,6%
1,4%	5,8%	8,5%	5,0%	1,5%	6,7%	2,1%	7,3%	9,8%	14,1%	4,8%	9,9%	2,6%	9,6%	22,9%	6,0%	4,4%	13,0%	1,9%	0,9%
1,2%	1,8%	0,1%	0,4%	0,4%	1,7%	0,7%	1,4%	0,4%	2,9%	3,0%	1,0%	2,8%	1,1%	0,2%	5,4%	10,1%	1,7%	1,8%	0,9%
0,2%	1,0%	0,4%	0,9%	0,3%	2,1%	1,3%	0,4%	1,0%	2,0%	3,1%	0,6%	1,9%	1,5%	0,9%	4,4%	10,7%	1,1%	0,6%	1,3%
1,2%	2,0%	0,8%	0,7%	0,8%	0,5%	0,8%	1,6%	0,3%	2,1%	2,6%	2,4%	1,4%	1,0%	1,7%	3,2%	8,1%	1,6%	1,1%	1,6%
2,5%	4,8%	2,9%	3,0%	0,9%	1,8%	1,1%	4,3%	2,1%	5,6%	7,6%	1,0%	0,5%	3,3%	6,1%	7,0%	1,6%	8,0%	6,2%	1,5%
1,4%	2,0%	1,8%	1,3%	1,9%	0,4%	1,5%	0,9%	1,8%	1,6%	7,2%	0,7%	6,1%	0,5%	3,0%	5,8%	9,1%	0,2%	5,8%	2,9%
2,2%	1,4%	0,3%	0,8%	0,8%	1,4%	0,4%	1,4%	1,0%	1,9%	13,0%	2,6%	10,2%	0,6%	5,9%	15,7%	1,5%	2,5%	14,0%	3,5%
1,8%	2,1%	0,3%	0,9%	1,5%	0,7%	1,0%	1,7%	0,3%	0,6%	1,7%	1,1%	0,8%	3,8%	2,5%	4,0%	7,3%	8,6%	5,0%	0,3%
10,9%	9,5%	18,9%	12,4%	12,2%	18,1%	24,5%	13,6%	26,2%	31,1%	2,5%	30,7%	2,2%	31,2%	45,1%	4,9%	8,4%	36,2%	1,1%	1,7%
6,2%	5,8%	5,0%	5,8%	6,6%	8,8%	6,7%	4,8%	7,8%	7,6%	3,4%	6,1%	2,2%	4,0%	4,3%	4,9%	3,5%	7,2%	1,8%	1,3%
2,3%	0,9%	2,6%	2,4%	2,5%	2,6%	2,0%	2,2%	2,1%	1,8%	3,3%	3,0%	2,1%	4,0%	2,5%	6,5%	6,3%	2,7%	3,8%	2,6%
5,9%	3,3%	5,6%	5,8%	5,2%	8,2%	7,4%	3,5%	7,8%	4,9%	5,8%	6,3%	2,7%	4,4%	3,4%	1,6%	10,2%	3,7%	1,5%	1,1%
2,8%	6,2%	5,6%	3,5%	2,2%	5,0%	2,4%	4,7%	6,6%	10,5%	4,8%	3,9%	1,1%	3,0%	9,3%	2,2%	3,0%	4,3%	1,9%	1,2%
0,4%	2,2%	0,5%	1,0%	1,0%	0,5%	0,8%	1,3%	0,9%	2,0%	3,8%	1,3%	4,6%	1,0%	6,4%	3,4%	12,1%	2,9%	0,5%	0,3%
1,2%	0,5%	1,2%	0,7%	1,3%	1,0%	1,3%	0,2%	0,9%	1,6%	1,5%	0,7%	3,1%	0,7%	5,6%	3,0%	6,1%	1,6%	1,1%	1,5%
0,6%	2,4%	0,5%	0,4%	0,9%	0,9%	1,5%	0,8%	0,6%	1,0%	4,0%	0,3%	1,9%	0,6%	4,4%	4,8%	11,1%	1,9%	3,7%	1,2%
3,2%	7,5%	11,7%	5,8%	4,3%	7,5%	3,0%	9,8%	11,0%	17,2%	5,3%	13,2%	3,9%	11,8%	23,3%	2,2%	1,7%	13,4%	2,7%	0,9%
2,5%	3,0%	2,0%	1,7%	0,5%	2,0%	0,6%	1,4%	1,6%	1,8%	5,4%	0,9%	1,5%	0,8%	1,4%	0,9%	6,6%	1,5%	0,1%	2,3%
2,8%	4,2%	1,7%	2,1%	1,9%	2,0%	0,4%	1,1%	0,4%	2,0%	2,6%	1,8%	1,4%	1,3%	1,1%	6,4%	6,8%	0,8%	3,5%	1,0%
2,4%	1,1%	2,3%	2,6%	1,2%	0,7%	0,5%	0,6%	0,4%	0,1%	2,5%	1,2%	3,0%	0,9%	2,4%	1,3%	6,2%	1,1%	2,3%	0,7%
5,7%	2,9%	4,9%	1,5%	1,1%	1,0%	1,7%	3,6%	5,2%	9,1%	8,6%	1,1%	4,4%	1,5%	15,7%	4,9%	5,8%	2,2%	3,9%	3,7%
2,5%	2,5%	1,0%	2,0%	0,8%	1,7%	0,8%	1,2%	1,4%	1,8%	1,8%	1,6%	2,7%	2,3%	4,8%	2,1%	6,7%	1,6%	1,0%	1,8%
1,9%	2,8%	2,2%	2,4%	1,3%	1,8%	1,6%	1,8%	1,8%	3,0%	2,2%	1,7%	4,5%	1,6%	1,5%	1,2%	1,7%	3,1%	2,4%	2,7%
0,3%	0,6%	1,1%	0,3%	1,4%	0,7%	1,1%	0,3%	0,6%	1,0%	1,9%	0,6%	2,2%	1,8%	2,0%	3,5%	6,9%	2,6%	1,6%	0,3%
1,6%	6,1%	8,7%	3,3%	4,1%	5,8%	2,9%	7,7%	11,4%	16,0%	5,6%	11,3%	6,0%	9,9%	24,3%	5,5%	4,5%	16,0%	0,4%	2,8%
0,3%	1,3%	1,3%	1,5%	0,4%	1,6%	1,0%	1,1%	1,1%	0,4%	5,3%	0,4%	1,9%	0,7%	0,8%	8,9%	11,2%	0,9%	1,9%	0,8%
1,2%	2,4%	1,1%	2,1%	2,8%	3,1%	3,9%	1,6%	1,6%	2,4%	1,9%	2,4%	3,7%	2,9%	2,5%	2,6%	15,8%	2,7%	1,4%	1,1%
2,8%	3,1%	2,5%	2,6%	1,9%	1,2%	0,6%	0,9%	1,4%	1,5%	2,3%	1,3%	0,7%	2,0%	2,4%	9,6%	24,4%	2,3%	1,8%	2,9%
2,9%	3,9%	4,9%	2,0%	2,3%	1,8%	1,5%	3,7%	5,1%	8,4%	5,7%	2,2%	3,5%	0,9%	11,3%	3,1%	13,9%	2,5%	3,0%	4,0%
2,0%	1,5%	1,3%	1,1%	2,3%	1,8%	0,4%	1,8%	0,7%	1,0%	4,5%	1,6%	2,5%	0,3%	5,3%	9,8%	5,2%	0,6%	3,3%	2,3%
1,1%	0,9%	1,1%	1,3%	0,3%	0,6%	0,9%	1,3%	1,7%	2,1%	1,2%	0,4%	2,7%	0,4%	2,5%	7,0%	3,8%	2,2%	1,5%	1,7%
1,4%	2,4%	1,0%	1,9%	2,2%	0,9%	1,6%	1,4%	1,0%	0,8%	1,7%	1,2%	6,3%	2,9%	3,3%	7,2%	6,6%	0,6%	1,6%	2,9%

/V); DS4 = acetonitrile/·-propanol/methanol/water/formic acid (30/25/25/19.9/0.1, v/V); DS5 = acetonitrile/water/ammonium hydroxide (80/19.9/0.1, v/V); DS6 = acetonitrile/methanol/water/ammonium hydroxide (4

stanozolol	methadon	boldenone	clonazepa	nandrolon	methandi	flunitraze	cannenone	THC-COOH	THC
2,6%	11,9%	3,2%	3,2%	5,1%	2,9%	3,9%	3,0%	4,2%	12,1%
5,9%	8,4%	7,4%	7,7%	9,2%	6,7%	6,9%	7,0%	9,1%	11,6%
3,8%	2,8%	1,4%	0,7%	3,2%	1,4%	1,0%	1,2%	2,8%	2,4%
3,3%	2,4%	3,5%	3,2%	3,8%	2,7%	2,3%	1,2%	5,5%	4,1%
2,9%	2,7%	4,5%	4,8%	4,7%	2,8%	4,0%	3,6%	4,7%	1,3%
1,1%	0,9%	0,9%	0,9%	3,0%	0,5%	0,5%	1,2%	4,7%	8,0%
2,1%	1,5%	1,4%	0,1%	2,5%	1,1%	1,3%	1,7%	1,4%	2,9%
1,5%	1,5%	2,5%	0,9%	2,0%	1,3%	0,9%	2,2%	1,9%	2,7%
8,8%	4,4%	2,0%	2,0%	2,1%	2,6%	1,0%	5,8%	3,4%	20,9%
1,3%	1,8%	2,7%	1,9%	3,5%	1,5%	1,8%	1,7%	1,0%	4,4%
2,1%	2,1%	3,8%	2,3%	3,4%	2,4%	3,7%	3,1%	1,4%	8,6%
2,2%	1,6%	1,2%	1,5%	1,9%	2,3%	1,8%	1,9%	2,7%	10,0%
6,2%	4,8%	4,6%	4,8%	5,6%	5,7%	4,6%	6,0%	11,5%	15,0%
4,6%	3,6%	1,8%	2,1%	4,2%	2,5%	1,9%	1,5%	2,0%	5,4%
1,0%	1,5%	1,6%	1,7%	1,4%	1,2%	2,0%	1,6%	2,1%	2,2%
2,6%	2,0%	1,2%	0,5%	2,2%	2,6%	0,8%	2,3%	2,6%	2,9%
1,0%	10,2%	1,0%	0,9%	0,4%	1,5%	1,5%	1,7%	5,7%	4,8%
5,0%	0,5%	2,1%	1,1%	1,6%	1,8%	1,0%	0,8%	5,0%	2,5%
2,4%	0,6%	0,7%	0,6%	2,0%	0,8%	0,7%	1,0%	2,1%	2,7%
1,0%	3,6%	1,7%	1,9%	1,1%	1,0%	1,4%	1,7%	3,1%	4,1%
4,0%	2,0%	2,7%	1,2%	1,9%	2,2%	2,7%	2,3%	2,2%	3,1%
1,5%	0,8%	0,2%	2,5%	0,9%	1,1%	1,9%	1,0%	0,6%	3,6%
3,6%	0,9%	0,5%	1,5%	1,0%	0,3%	0,6%	2,0%	1,2%	4,3%
3,0%	1,5%	0,9%	2,9%	3,2%	0,2%	1,8%	1,1%	5,2%	7,0%
2,0%	15,0%	4,5%	4,2%	3,2%	3,6%	4,5%	0,5%	9,1%	21,6%
1,6%	4,2%	0,7%	0,7%	3,9%	1,1%	1,1%	2,5%	2,7%	0,5%
4,4%	1,4%	2,3%	2,4%	3,2%	1,9%	2,6%	2,3%	6,6%	1,9%
6,4%	2,7%	3,5%	4,9%	6,1%	3,8%	3,9%	4,4%	10,4%	7,0%
3,5%	0,9%	0,8%	0,9%	6,0%	1,7%	1,6%	2,8%	2,8%	4,2%
3,4%	5,4%	4,9%	6,8%	10,8%	5,2%	5,6%	5,3%	1,7%	3,4%
3,7%	2,1%	6,3%	5,9%	3,8%	3,3%	4,3%	1,6%	5,9%	9,1%
2,5%	1,5%	9,3%	10,3%	11,3%	9,7%	10,4%	6,6%	12,3%	12,0%
8,1%	17,2%	7,4%	11,7%	13,6%	7,3%	8,7%	15,1%	57,2%	63,0%
3,9%	10,1%	7,1%	7,0%	9,6%	11,4%	7,8%	10,5%	31,5%	11,4%
7,6%	4,7%	21,0%	17,6%	13,7%	15,1%	16,1%	11,8%	16,5%	17,8%
3,7%	11,9%	3,9%	2,8%	9,8%	4,6%	2,6%	3,8%	40,8%	9,9%
3,4%	1,4%	6,1%	5,5%	17,4%	8,6%	5,5%	7,9%	30,1%	8,0%
4,5%	2,1%	10,4%	5,1%	13,4%	7,2%	5,5%	8,8%	21,5%	11,6%
8,4%	1,4%	7,1%	5,2%	5,5%	4,1%	5,2%	8,5%	22,4%	6,7%
8,8%	2,1%	9,4%	8,4%	13,3%	9,0%	11,4%	11,2%	5,7%	9,4%
3,0%	14,1%	16,3%	8,0%	7,2%	10,8%	6,1%	9,4%	17,6%	76,6%
8,6%	3,1%	6,8%	5,3%	2,9%	3,0%	5,1%	5,2%	16,0%	8,2%
8,1%	0,3%	1,8%	3,1%	7,6%	8,0%	4,3%	2,9%	24,2%	19,0%
15,7%	2,2%	7,1%	4,1%	12,7%	4,6%	4,0%	2,6%	39,2%	30,2%
18,3%	1,3%	4,0%	5,9%	8,1%	5,6%	6,3%	6,5%	11,0%	18,5%
7,1%	3,2%	3,7%	1,9%	4,7%	3,5%	0,8%	2,5%	14,6%	14,6%
5,9%	1,7%	7,3%	5,8%	4,5%	11,8%	6,8%	2,9%	23,1%	3,4%
3,5%	1,5%	5,4%	7,3%	11,7%	6,6%	5,6%	8,1%	9,8%	3,0%
8,9%	10,7%	1,9%	1,4%	3,7%	0,1%	0,8%	1,0%	8,6%	88,2%
0,2%	2,3%	0,6%	0,6%	2,6%	0,9%	1,6%	3,0%	1,5%	3,8%
6,5%	1,7%	1,1%	2,1%	0,9%	1,1%	1,8%	0,6%	5,3%	1,0%
6,9%	1,6%	0,3%	1,9%	1,2%	1,1%	1,0%	1,2%	3,0%	3,8%
3,6%	4,0%	0,6%	1,4%	2,2%	1,8%	1,4%	1,4%	7,9%	5,7%
1,6%	1,8%	0,9%	1,3%	2,6%	0,5%	0,8%	1,6%	2,6%	2,5%
3,3%	2,4%	1,2%	0,8%	2,4%	0,3%	1,4%	0,9%	3,2%	2,0%
1,0%	2,8%	4,1%	3,3%	1,4%	2,6%	2,6%	1,9%	5,4%	0,9%
8,7%	11,2%	1,1%	2,7%	0,6%	1,4%	1,3%	1,4%	4,9%	30,8%
3,9%	1,7%	4,6%	3,8%	3,7%	2,8%	2,9%	2,4%	4,4%	1,4%
3,9%	0,4%	1,1%	3,0%	2,4%	1,9%	2,2%	0,8%	3,1%	7,8%
5,2%	1,3%	1,2%	1,4%	2,5%	0,9%	0,6%	0,7%	2,2%	3,7%
4,8%	4,5%	4,4%	3,6%	5,1%	3,5%	3,7%	2,7%	7,4%	7,7%
2,4%	1,2%	0,3%	2,0%	1,2%	1,6%	0,4%	1,2%	2,7%	7,3%
2,4%	1,5%	1,6%	0,9%	5,3%	1,2%	1,3%	1,5%	11,9%	8,2%
2,7%	0,4%	2,0%	1,5%	2,5%	2,0%	1,6%	0,4%	4,5%	4,0%
2,6%	14,6%	2,3%	3,1%	1,2%	2,1%	1,8%	1,5%	11,9%	40,0%
3,3%	0,8%	1,1%	1,5%	3,3%	1,5%	0,5%	0,9%	0,4%	2,3%
1,0%	1,2%	2,1%	0,8%	3,1%	1,2%	0,5%	0,5%	1,7%	3,6%
4,1%	1,5%	1,0%	1,2%	0,1%	2,5%	1,1%	3,4%	3,6%	5,0%
0,5%	2,1%	2,3%	3,2%	0,4%	1,9%	3,6%	0,8%	6,9%	5,0%
1,7%	2,6%	3,9%	5,0%	2,7%	3,0%	5,5%	2,4%	3,1%	5,1%
3,3%	2,0%	10,6%	11,1%	5,0%	6,1%	12,2%	3,3%	7,6%	5,4%
0,4%	1,1%	3,5%	2,2%	0,8%	3,2%	4,9%	2,6%	11,0%	9,0%
10,9%	41,9%	2,4%	2,7%	4,0%	1,9%	3,8%	13,9%	2,0%	18,6%
1,5%	7,5%	1,4%	1,6%	3,1%	1,9%	2,3%	2,1%	5,8%	13,1%
3,1%	3,3%	2,2%	1,4%	2,3%	2,6%	1,8%	2,5%	2,9%	9,5%
2,1%	7,9%	1,3%	3,1%	1,6%	2,1%	1,0%	2,0%	3,2%	2,5%
5,7%	6,5%	1,5%	1,2%	4,2%	1,9%	1,6%	1,2%	2,5%	2,3%
3,0%	2,3%	1,4%	0,6%	0,9%	2,8%	1,4%	2,5%	2,6%	5,6%
2,0%	1,3%	2,3%	2,2%	1,2%	0,6%	1,9%	2,2%	3,2%	2,9%
2,4%	0,5%	0,5%	2,6%	1,9%	0,4%	1,0%	1,0%	3,5%	7,5%
4,7%	17,7%	2,3%	2,1%	1,6%	1,8%	2,1%	2,1%	2,5%	11,4%
1,1%	0,9%	2,5%	1,5%	1,8%	3,2%	1,5%	2,6%	14,2%	3,6%
1,7%	1,9%	2,4%	2,1%	0,9%	1,4%	1,9%	2,8%	5,3%	4,2%
7,6%	1,1%	1,2%	2,2%	1,3%	0,2%	0,9%	2,7%	4,3%	8,7%
8,5%	6,5%	5,7%	5,0%	4,4%	3,2%	4,3%	3,4%	6,7%	6,6%
4,6%	2,9%	0,8%	2,5%	2,8%	1,9%	1,3%	1,4%	4,8%	0,7%
2,9%	3,6%	0,4%	1,7%	2,3%	2,1%	2,3%	1,0%	9,1%	7,2%
3,4%	1,5%	0,5%	0,6%	3,5%	0,4%	1,6%	0,9%	4,3%	6,9%
0,8%	18,3%	1,4%	0,2%	2,3%	2,2%	3,2%	2,2%	6,0%	20,9%
3,0%	0,6%	2,3%	0,8%	0,8%	2,7%	1,0%	1,9%	2,8%	6,7%
3,9%	2,3%	1,7%	3,2%	1,9%	2,7%	1,8%	0,9%	4,0%	13,6%
2,3%	0,8%	2,2%	2,3%	1,0%	2,8%	4,0%	0,8%	4,7%	5,0%
9,0%	4,6%	2,8%	2,3%	2,8%	3,4%	3,8%	4,8%	12,4%	5,3%
0,8%	0,6%	1,7%	2,5%	0,8%	1,8%	1,9%	2,3%	14,6%	5,3%
2,6%	2,7%	0,6%	2,5%	2,8%	1,5%	1,7%	2,3%	14,5%	4,9%
4,8%	1,5%	1,5%	2,1%	1,1%	2,1%	1,8%	4,9%	11,5%	3,9%

)/40/19.9/0.1, V/V); DS7 = methanol/water/ammonium hydroxide (80/19.9/0.1, V/V); and DS8 = acetonitrile/-propanol/methanol/water/ammonium hydroxide (30/25/25/19.9/0.1, V/V).

Table S4. Reproducibility, n=4.

substance	RSD
meldonium	11,3%
psilocybin	10,7%
morphine	16,4%
salbutamol	15,1%
terbutaline	12,2%
atenolol	13,8%
fenoterol	6,1%
nikethamide	23,4%
carteolol	7,7%
oxycodone	6,5%
amphetamine	10,1%
chlorothiazide	18,8%
hydrocodone	5,9%
methamphetamine	7,5%
methylhexanamine	13,8%
MDMA	6,1%
hydrochlorothiazide	27,4%
strychnine	6,6%
ketamine	7,2%
metoprolol	7,5%
clenbuterol	5,2%
methylphenidate	5,4%
zolpidem	5,8%
cocaine	3,1%
LSD	4,9%
bisoprolol	6,0%
phencyclidine	4,0%
propranolol	8,4%
prednisolone	16,9%
fentanyl	4,8%
prednisone	24,0%
buprenorphine	4,9%
ibutamoren	12,1%
betamethasone	11,7%
furosemide	21,1%
nebivolol	16,0%
alprazolam	14,3%
anastrozole	4,6%
stanozolol	18,8%
methadone	4,8%
boldenone	4,0%
clonazepam	5,8%
nandrolone	4,4%
methandienone	3,9%
flunitrazepam	2,8%
canrenone	2,4%
THC-OH	8,8%

molecules

MDPI

Article

Benefits of Innovative and Fully Water-Compatible Stationary Phases of Thin-Film Microextraction (TFME) Blades

Łukasz Sobczak, Dominika Kołodziej and Krzysztof Goryński *

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Abstract: Octadecyl (C_{18}) groups are arguably the most popular ligands used for preparation of solid phase microextraction (SPME) devices. However, conventional C_{18}-bonded silica particles are not fully compatible with the nearly 100% aqueous composition of typical biological samples (e.g., plasma, saliva, or urine). This study presents the first evaluation of thin-film SPME devices coated with special water-compatible C_{18}-bonded particles. Device performance was assessed by extracting a mixture of 30 model compounds that exhibited various chemical structures and properties, such as hydrophobicity. Additionally, nine unique compositions of desorption solvents were tested. Thin-film SPME devices coated with C_{18}-bonded silica particles with polar end-capping groups (10 µm) were compared with conventional trimethylsilane end-capped C_{18}-bonded silica particles of various sizes (5, 10, and 45 µm) and characteristics. Polar end-capped particles provided the best extraction efficacy and were characterized by the strongest correlations between the efficacy of the extraction process and the hydrophobicity of the analytes. The results suggest that the original features of octadecyl ligands are best preserved in aqueous conditions by polar end-capped particles, unlike with conventional trimethylsilane end-capped particles that are currently used to prepare SPME devices. The benefits associated with this improved type of coating encourage further implementation of microextractraction as greener alternative to the traditional sample preparation methods.

Keywords: sample preparation; thin-film microextraction; solid phase microextraction; octadecyl; polar end-capped particles; liquid chromatography; TFME; SPME; C18; HPLC-MS/MS

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1. Introduction

Ever since liquid chromatography moved towards its current leading position among available analytical methods, both sample preparation and chromatographic separation were performed predominantly with octadecylsilane (ODS, C_{18})-bonded particles. Joseph Jack Kirkland, one of the early pioneers in high-performance liquid chromatography (HPLC) stated that "C18-based silanes were readily available at that time and reasonable in cost" [1] when the method was initially developed, and since then, "tradition" paved their way to their present status. Regardless of circumstantial beginnings, C_{18}-bonded particles are often superior to any alternative in many applications and are thus likely to remain popular. However, evolution in this field is occurring. Novel supports with improved particle shapes, chemistry, and increased purity; end-capping that mitigates undesired interactions; and incorporating additional functional groups as end-capping agents or as complementary functional ligands are a few of the more recent technological advances in this field of study. Thus, polar end-capped, polar embedded, positively charged surface, or mixed-mode (containing ion-exchanging groups) chemistries are readily available in contemporary octadecylsilane HPLC and UHPLC columns.

For sample preparation, methods such as solid phase extraction (SPE) or solid phase microextraction (SPME) also strongly rely on octadecyl (C_{18}) chains as the most popular chemistry that has been successfully used in combination with liquid chromatography. This

fact is perhaps best highlighted by the currently available SPME devices that are designed for direct immersion into liquid samples. Such solutions are particularly sought when so-called "green" extraction procedures are desired or during the analysis of thermally unstable and non-volatile compounds that cannot be analysed with gas chromatography. In such cases, C_{18}-based coatings are one of only two options that are marketed as SPME devices and are compatible with liquid chromatography; the other option is PDMS/DVB (polydimethylsiloxane/divinylbenzene). SPME is considered a "greener" alternative to concurrent sample preparation techniques. In accordance with the principles of Green Analytical Chemistry, microextraction methods reduce organic solvents consumption, and combine sample collection, extraction and analyte enrichment above required concentration into a single step [2–4]. Additionally, the method is characterized by low energy consumption, low laboratory waste production, and device reusability, while still enabling performance comparable or superior to the traditional methods [5]. C_{18}-coated SPME tips and fibers are already popular around the world, as indicated by the publications of many research groups within the last 2 years [6–9]. C_{18} is also one of most popular coating types for the development of new SPME solutions, such as thin-film microextraction (TFME) blades. Although just recently commercialized, the format of TFME had already been shown to be useful in high-throughput analysis of biological fluids due to its physical stability, long-term reusability, and high degree of reproducibility [10,11]. Additionally, TFME is characterized by a greater surface area, which increases the extraction efficacy (yield), and thin layer(s) of the coating that makes attainment of thermodynamic equilibrium of the process easier compared to commercially available SPME tips or fibers. Concurrently, the format of the blades allows extraction from common 96-well deep well plates, which enables full or partial automation of the extraction process and yields a large improvement in sample throughput [12].

TFME blades with C_{18} coatings were first used to extract four benzodiazepines from urine and phosphate-buffered saline (PBS) in 2009 [13]. Many other applications of the simultaneous extraction of diverse analytes from complex biological matrices have been reported. Examples include extraction of benzodiazepines from plasma and PBS [14], 49 doping agents from saliva [15], a study of repaglinide metabolism [16], and obtaining fish tissue extracts [17]. C_{18} coatings are also often subjected to comparisons with different coating chemistries in various applications. Selected publications on this subject from the last eight years compare four coatings with regard to the extraction of 110 doping agents from urine [18]; five coatings with regard to the extraction of phenolic compounds from wine, grapes, and berries [19]; seven coatings in metabolomic processes [20]; two coatings with regard to the extraction of abused drugs from plasma, blood, and urine [21]; two coatings with regard to the extraction of beta-blockers and bronchodilators from plasma and urine [11]; and eight chemistries with regard to the systemic evaluation of thin-film microextraction coatings [22]. The results of these studies often demonstrated that C_{18} is the most suitable coating type for a given application.

A common strategy used to increase the efficacy of TFME blades is an implementation of chemistries theoretically more suited for the use with aqueous samples than the conventional C_{18}. Most popular solutions include mixed-mode C_{18} (octadecyl with strong cationic exchanger of benzenesulfonic acid) [18,19], polar enhanced (with unspecified weak anionic exchanger) polystyrene-divinylbenzene (PS-DVB) [18–20,22–24], hydrophilic-lipophilic balance (HLB) [11,20–22], and phenylboronic acid (PBA) [18–20,22,23] coatings. However, the selection of optimal chemistry is highly dependent on target analytes and their proprieties, with each coating characterised by its unique advantages and limitations. For example, PS-DVB coatings were more efficient than PBA coatings for extraction of diverse small molecules from plasma and PBS. In addition, they did not require preconditioning, unlike PBA coatings [23]. PS-DVB coatings were also superior to C_{18}, mixed-mode, or PBA coatings in terms of number of doping agents extracted from urine samples and their amounts, but also were characterized by greater carry-over, what prevented their use for anti-doping control purposes [18]. In another study, divinylbenzene (DVB) coatings were

selected over the considered C_{18} coatings for the extraction of six anti-inflammatory and antibacterial drugs from water samples. The reason stated by the authors was DVB's known suitability for extraction of small molecules from water [25]. Bearing structural similarity to DVB, the HLB particles comprised poly(divinylbenzene-co-N-vinylpyrrolidone) were purposely designed to exert both hydrophobic and hydrophilic type interactions [26] and thus, provide a universal stationary phase for simultaneous extraction of chemically diverse analytes. The benefits of HLB TFME coatings, such as an excellent wettability in aqueous conditions [21], even without preconditioning, were demonstrated for the extraction of nine quaternary ammonium compounds (with mixed hydrophobic-hydrophilic nature) from buffered water [27]. Evaluated by several authors, HLB coatings were superior to the compared alternatives for extraction of small molecules from aqueous media. The examples include better efficacy and lower carry-over than the C_{18} and PS-DVB coatings for the extraction of 25 prohibited substances from plasma [28], greater efficacy than C_{18} for extraction of eight drugs from plasma and urine [11], and greater efficacy than DVB for extraction of six chlorination by-products from hot tub water [26].

However, one fundamental aspect concerning silica-based C_{18} particles has yet to be addressed by researchers. While the stationary phase (coating) is initially wetted in the SPME method during the preconditioning step via insertion into a water–water miscible organic solvent mixture that allows stationary phase wetting to occur under atmospheric pressure [29], the subsequent step of extraction is also performed in a water-based matrix (e.g., plasma, saliva, or urine) under atmospheric pressure. According to the Washburn equation, a pressure of approximately 100 bars (10 MPa) would be required for C_{18}-bonded silica particles with 100 Å pores (a commonly used dimension) to remain wet in pure water [30]. Therefore, just as in liquid chromatography, solid phase collapse (dewetting, or chain folding) [1,29] may occur at this stage, potentially altering the efficacy and physicochemical nature of the extraction process.

This study reports the first known attempt to investigate this topic by introducing fully water-compatible C_{18}-based stationary phase end-capped with polar groups as a coating of thin-film microextraction blades. A comparison of the performance of these polar and conventional trimethylsilane (TMS) end-capped particles of different sizes is also presented.

2. Results and Discussion

2.1. Data Quality

This study evaluated four types of SPME coatings and nine different compositions of desorption solvents; in total, 36 unique conditions were tested. Each coating–desorption solvent combination was tested in triplicate. Due to the diverse nature of samples present in different solvents (e.g., unique ionization intensity in electrospray ion source), every result was stacked against the mean value (n = 4) that was recorded for the reference sample prepared with a mixture of analytes spiked into the corresponding type of desorption solvent.

Several calibration runs in the expected concentration range of 5–100 ng/mL were performed in all acetonitrile-based, isopropanol-based, and methanol-based desorption solvents. The resulting 7-point calibration curves always provided coefficients of determination exceeding $R^2 = 0.9912$ for every analyte with a $1/a^2$ weighting (see Table S1 in Supplementary Materials for more details). Additionally, no problems with carry-over or poor peak shapes were found with any of the analytes, and good reproducibility of the results was achieved. Relative standard deviation (RSD) was below 10% for over 98.6% of the results (median RSD = 3.1%) and did not exceed 15% at any time.

Analysis of the signal recorded for internal standards spiked into desorption solvents confirmed the stability of the detector throughout the experiment. The relative standard deviations in each desorption solvent tested were 4.1–10.1% (median = 5.5%) for oxycodone D_3, 2.9–8.4% (median = 3.8%) for cocaine D_3, 8.9–12.5% (median = 10.8%) for alprazolam D_5, and 3.8–14.9% (median = 5.0%) for THC-COOH D_3.

2.2. Statistical Analysis

Normality was assessed within the dataset for results determined by desorption with the same type of desorption solvent using the Shapiro–Wilk test. The initial results conformed to a normal distribution for every desorption solvent composition except for one, DS3n comprised methanol/water (80/20, *v*/*v*). Following log-transformation, a normal distribution was found in all groups; the testing null hypothesis confirmed the normal distribution of the results. Each group contained an equal number of results, and analysis of variance (ANOVA) performed with the Levene test confirmed the null hypothesis and homoscedasticity of the data. Fulfilment of these conditions allowed us to apply parametrical tests, such as the Pearson correlation coefficient or one-way ANOVA, which are vulnerable to deviations from normality. A one-way ANOVA showed no significant differences in the average values of the dependent variable within the analysed groups.

2.3. Quantitative Results (Extraction Efficacy)

With three out of four types of coatings, the desorption solvent labelled as DS3a and composed of methanol/water/formic acid (80/19.9/0.1, *v*/*v*) yielded the best efficacy, except with the 5 µm particles, which also performed worst in this evaluation, where DS1b containing isopropanol/water/ammonium hydroxide (80/19.9/0.1, *v*/*v*) was the most effective. Methanol used as a solvent has been reported to increase steric repulsion of bulky solutes better than acetonitrile [31,32] which may explain the more effective desorption observed with this type of solvent. Steric repulsion is also determined by the density of the ligands bonded with the particle [31]; therefore, the "more crowded" 5 µm particles may have prevented bulky solutes from being intercalated between hydrocarbon chains during the extraction and thus excluded this mechanism from influencing desorption efficacy.

As mentioned before, the poorest extraction efficacy was achieved with coatings prepared with the 5 µm particles. With desorption to the best performing desorption solvent type (DS1b), the extraction efficacy exceeded the median recorded for the entire dataset only in 12 out of 30 analytes (40%), and only a single analyte (methadone) extraction yield was in an upper quarter of the results. With no analyte, the best extraction efficacy was achieved with this type of coating.

With the three remaining coatings, the 10 µm particles yield extraction efficacies above the median for 28 out of 30 analytes (hydrocodone and oxycodone were the exceptions), within the 3rd quartile for 18 analytes (60%); however, the best extraction efficacy was not recorded with this type of coating after desorption to the most effective desorption solvent (DS3a).

The largest tested particles (45 µm) performed much better, and extraction efficacy exceeding the median value was recorded for every single analyte after desorption to DS3a. Additionally, 28 results in the 3rd quartile were recorded, as well as the largest extraction efficacy of the eight analytes.

With Phenomenex® Synergi™ Hydro-RP, which are the only particles end-capped with polar functional groups, results above the median were achieved for all analytes after desorption to DS3a. One less result in the 3rd quartile was recorded than for the 45 µm particles (27 out of 30); however, concurrently, the largest extraction efficacy was recorded for more than double the number of analytes (18 out of 30, or 60%).

Figure 1 shows the number of results above the median (upper half), in the 3rd quartile (upper quarter) and the number of best results for the most effective coating–desorption solvent combinations for each coating type. The results of statistical analysis for every tested coating–desorption solvent combination are shown in the Supplementary Materials Table S2.

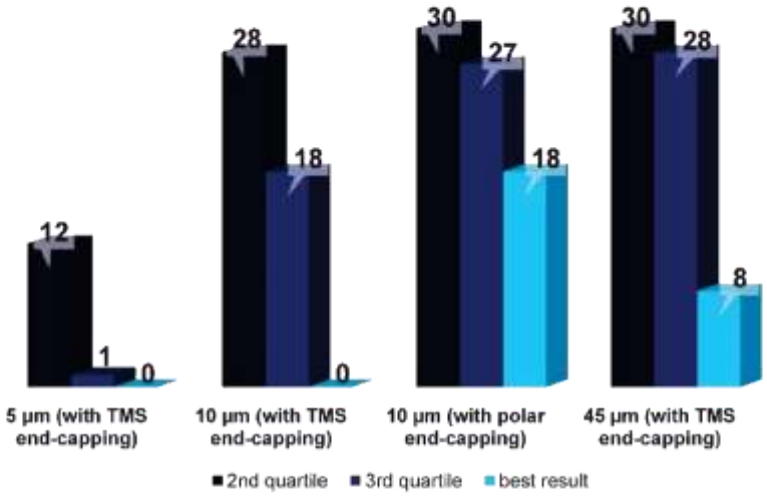

Figure 1. Number of results above the median (2nd quartile), in the 3rd quartile, and the number of best results recorded for the most effective coating–desorption solvent combinations for each coating type. Results for 5 µm particles after desorption to DS1b comprised isopropanol/water/ammonium hydroxide (80/19.9/0.1, *v*/*v*) are shown; results for the other particles after desorption to DS3a comprised methanol/water/formic acid (80/19.9/0.1, *v*/*v*) are shown.

With regard to the top extraction yields for a given analyte, most results (18 = 60% of all) were recorded for the 10 µm particles with polar end-capping groups (all cases after desorption to DS3a) and for the 10 µm particles with TMS end-capping in 2 cases (1 with DS3n and 1 with DS3b). For the 45 µm particles, 10 cases were recorded (8 after desorption to DS3a, 1 with DS1b, and 1 with DS2a). No such results were achieved with the 5 µm particles. The optimal extraction efficacies and optimal conditions for every tested analyte are shown in Table 1, and the comprehensive results with all extraction yields and corresponding relative standard deviations for every analyte are shown in Table S3 in the Supplementary Materials. Median extraction efficacy, extraction efficacy determining the 3rd quartile, as well as top extraction efficacy for given substance are also provided in Table S3 in Supplementary Materials for easier interpretation of the results.

The impact of the physicochemical characteristics of the stationary phases on their extraction performance in this study can be assessed based on the three coatings prepared with TMS end-capped particles. The larger particles performed better than the smaller ones (45 µm > 10 µm > 5 µm). However, this seemed to be an outcome of larger specific surface areas, that were associated with the particles' sizes (490 m^2/g > 381 m^2/g > 338 m^2/g). In addition, the extraction performance was corelated with the particles' pore widths. Generally, particles with narrower pores have more crowded ligands [33] (therefore, a greater specific surface area). In this study, pores' size decreased with an increasing particles' size (116 Å for 5 µm particles, 104 Å for 10 µm particles, and 64 Å for 45 µm particles), and so increased the particles' performance. A similar pattern could also be observed for other particle types, such as HLB, where particles with 13 Å pores performed better than particles with 71 Å or 80 Å pores [26]. As noted by the authors, the increased porosity is of an advantage for the extraction of small molecules. With similar pore sizes (71 Å and 80 Å), larger particles performed better for all tested analytes (30–60 µm > 5 µm) [26]. More details on the particles used in this study are shown in Table 2.

Table 1. Optimal extraction efficacies [%] and conditions. Relative standard deviations [%] given in brackets, $n = 3$, substances arranged by retention order.

Substance	Extraction Efficacy with 10 µm Polar End-Capped Particles and Desorption to DS3a	Optimal Extraction Conditions	
		Extraction Efficacy	Stationary Phase + Desorption Solvent
fenoterol	48.0 (6.7)	65.1 (2.2)	45 µm + DS3a
carteolol	71.0 (3.2)	85.8 (3.0)	45 µm + DS3a
oxycodone	52.4 (8.2)	76.1 (3.5)	45 µm + DS3a
hydrocodone	82.4 (3.4)	93.5 (2.1)	45 µm + DS2a
ketamine	97.8 (1.8)	102.1 (1.9)	45 µm + DS3a
remifentanil acid	63.7 (4.4)	83.9 (1.8)	45 µm + DS3a
metoprolol	90.2 (2.9)	95.1 (3.3)	45 µm + DS3a
6-acetylcodeine	112.5 (2.1)	117.6 (5.7)	10 µm + DS3b
methylphenidate	85.7 (1.4)	94.4 (1.6)	45 µm + DS3a
zolpidem	125.5 (2.5)	10 µm polar end-capped particles + DS3a	
cocaine	102.4 (0.6)	102.8 (0.6)	45 µm + DS1b
LSD	115.0 (4.4)	10 µm polar end-capped particles + DS3a	
melatonin	69.1 (4.8)	89.5 (2.4)	45 µm + DS3a
bisoprolol	120.9 (3.2)	10 µm polar end-capped particles + DS3a	
phencyclidine	114.5 (2.4)	10 µm polar end-capped particles + DS3a	
cortisol	122.9 (5.2)	10 µm polar end-capped particles + DS3a	
buprenorphine	117.0 (4.1)	10 µm polar end-capped particles + DS3a	
alprazolam	120.7 (4.7)	10 µm polar end-capped particles + DS3a	
anastrozole	124.4 (1.6)	10 µm polar end-capped particles + DS3a	
methadone	118.5 (4.3)	10 µm polar end-capped particles + DS3a	
11-deoxycortisol	131.3 (7.4)	10 µm polar end-capped particles + DS3a	
boldenone	131.1 (2.7)	10 µm polar end-capped particles + DS3a	
clonazepam	115.3 (1.3)	10 µm polar end-capped particles + DS3a	
agomelatine	122.8 (3.5)	10 µm polar end-capped particles + DS3a	
methandienone	128.1 (3.0)	10 µm polar end-capped particles + DS3a	
flunitrazepam	124.5 (2.2)	10 µm polar end-capped particles + DS3a	
androstenedione	129.5 (2.9)	10 µm polar end-capped particles + DS3a	
canrenone	131.5 (4.5)	139.9 (1.9)	10 µm + DS3n
progesterone	127.9 (4.8)	10 µm polar end-capped particles + DS3a	
THC-COOH	119.0 (4.3)	10 µm polar end-capped particles + DS3a	

Please note that the extraction efficacies above 100% are due to the evaporation of organic solvents during the desorption step, which occurred under the controlled temperature in open bed configuration of 96-well plates. Desorption solvents compositions: DS1b = isopropanol/water/ammonium hydroxide (80/19.9/0.1, *v*/*v*); DS2a = acetonitrile/water/formic acid (80/19.9/0.1, *v*/*v*); DS3a = methanol/water/formic acid (80/19.9/0.1, *v*/*v*); DS3n = methanol/water (80/20, *v*/*v*); DS3b = methanol/water/ammonium hydroxide (80/19.9/0.1, *v*/*v*).

Table 2. Characteristics of particles used to prepare TFME coating.

Particle Type	Nominal Particle Size [µm]	Measured Particle Size [µm]	Pore Diameter [Å]	Specific Surface Area [m^2/g]	Total Carbon [%]	Surface Coverage [µmole/m^2]	Compatible pH Range
NUCLEODUR® C_{18} Htec	5	N/A	116	338	18 *	N/A	1–11
Luna® C18(2)	10	8.37	104	381	16.38	3.01	1.5–10
Synergi™ Hydro-RP	10	7.6	88	449	18.9	2.37	1.5–7.5
CHROMABOND® C_{18}ec	45	33	64	490	14 *	N/A	2–8

* According to the information provided in product catalogue.

2.4. Relationship between Extraction Efficacy and Analyte Characteristics

This study evaluated thin-film microextraction devices coated with immobilized octadecyl-bonded silica particles. However, silica surfaces can never be entirely bonded with ligands. Due to steric impedance, it is estimated that below half of the silanol groups present on silica surfaces are bonded with octadecylsilane molecules [1], and part of these silanols becomes permanently inaccessible to end-capping agents such as TMS during

synthesis [34,35]. This incomplete substitution of silanols causes multiple interactions between the stationary phase (coating) and analytes to affect the extraction efficacy. This phenomenon also explains why no single parameter of the analyte can be perfectly correlated with extraction yield (i.e., Pearson's coefficient of exactly r = 1). Several physicochemical properties of the analytes were selected by the authors as potentially affecting extraction, and the correlation of these parameters with the efficacy of the process was investigated. All physicochemical descriptors used for calculations are available in online databases and are shown in Supplementary Materials Table S4.

As expected from a coating prepared with hydrophobic octadecyl-bonded particles, there were two-way significant and strong positive correlations (Pearson's coefficient values of $r > 0.5$) between the extraction efficacy and hydrophobicity of the analytes. Expressed as the logarithm of the partition coefficient (logP) or the distribution coefficient (logD), the hydrophobicity of the analytes was computed using several programs. logP values were calculated with the ACD/Labs [36], ALOGPS [37,38], ChemAxon [37,38], and XlogP3.0 [39] programs. logD values at pH 7.4 were calculated with ACD/Labs [36]. Out of this group of descriptors, logD at pH 7.4 was characterized by the highest correlations with extraction efficacy. Given that logD considers whether the molecule is ionizable or not (and majority of the analytes extracted in this study are), these results are not surprising, and the extractions were performed from PBS, which has a nominal pH value of 7.4. Of the four coatings investigated in this study, one prepared with the 45 µm particles exhibits the lowest correlations. The median correlation coefficient value was r = 0.533 (0.495–0.618, $p \leq 0.005$, $n = 8$). Greater correlations were observed for smaller particle sizes with median value of r = 0.698 (0.615–0.769, $p < 0.001$, $n = 9$) for the 10 µm particles and r = 0.756 (0.612–0.796, $p < 0.001$, $n = 9$) for the 5 µm particles. The largest correlations occurred with the 10 µm particles with polar end-capping with a median value of r = 0.767 (0.646–0.787, $p < 0.001$, $n = 9$). Stronger correlations were also found in the most efficient desorption solvents, achieving maximum values with the 10 and 45 µm particles (both with DS3a) and near-maximum values for the 5 (r = 0.743 in DS1b vs. r = 0.796 in DS1a) and 10 µm particles with polar end-capping (r = 0.784 in DS3a vs. r = 0.787 in DS1b).

Other investigated analyte parameters included the polar surface area [Å^2], which was computed with ACD/Labs [36], Cactvs 3.4.6.11 [39], and ChemAxon [37,38] programs; the polarizability [Å^3], which was computed with ACD/Labs [36], and ChemAxon [37,38]; pKa, which was computed with ChemAxon [37,38]; and the number of hydrogen acceptor and donor spots, which were computed with ACD/Labs [36], Cactvs 3.4.6.11 [39] and ChemAxon [37,38]. Of this group of descriptors, two-way significant positive correlations were only observed between the extraction efficacy of the 45 µm particles and the pKa value (strongest acidic) of the analytes. The median coefficient value was r = 0.593 (0.551–0.602, $p \leq 0.01$, $n = 5$). However, two-way significant negative correlations were present with the extraction yield and pKa value (strongest basic) for the 5 µm particles with a median value of r = −0.488 (−0.545–−0.486, $p \leq 0.009$, $n = 3$), the 10 µm particles with a median value of r = −0.516 (−0.517–−0.502, $p \leq 0.007$, $n = 4$), and the 10 µm polar particles with a median value of r = −0.513 (−0.525–−0.497, $p \leq 0.007$, $n = 3$). Correlations between the extraction efficacy and number of donor spots for hydrogen bonds were also negative for all particle types. Stronger correlations were observed with data from ChemAxon (number of pairs = 28 analytes) than with data from the ACD/Labs and Cactvs programs ($n = 30$ both). With the 5 µm particles, the median correlation value was r = −0.537 (−0.601–−0.480, $p \leq 0.01$, $n = 8$). With the 10 µm particles, r = −0.544 (−0.643–−0.484, $p \leq 0.009$, $n = 9$). With the 10 µm polar particles, r = −0.529 (−0.563–−0.493, $p \leq 0.008$, $n = 9$). With the 45 µm particles, r = −0.611 (−0.698–−0.560, $p \leq 0.002$, $n = 9$).

All of the two-way significant correlations discussed above are summarised in Table 3.

Table 3. Two-way significant correlations [Pearson's r values] discussed in Section 2.4. *p* Values given in brackets.

Parameter	Desorption Solvent	10 µm Polar End-Capped Particles	5 µm Particles	10 µm Particles	45 µm Particles
logD at pH = 7.4 [36]	DS1a	0.780 (0.000)	0.796 (0.000)	0.768 (0.000)	0.576 (0.001)
	DS1n	0.767 (0.000)	0.794 (0.000)	0.752 (0.000)	0.568 (0.001)
	DS1b	0.787 (0.000)	0.743 (0.000)	0.677 (0.000)	0.547 (0.002)
	DS2a	0.782 (0.000)	0.728 (0.000)	0.708 (0.000)	0.513 (0.004)
	DS2n	0.739 (0.000)	0.756 (0.000)	0.698 (0.000)	0.495 (0.005)
	DS2b	0.731 (0.000)	0.763 (0.000)	0.641 (0.000)	0.498 (0.005)
	DS3a	0.784 (0.000)	0.792 (0.000)	0.769 (0.000)	0.618 (0.000)
	DS3n	0.646 (0.000)	0.612 (0.000)	0.615 (0.000)	
	DS3b	0.726 (0.000)	0.683 (0.000)	0.629 (0.000)	0.518 (0.003)
pKa (strongest acidic) [37,38]	DS1a				0.602 (0.004)
	DS1b				0.551 (0.010)
	DS2a				0.593 (0.005)
	DS2b				0.565 (0.008)
	DS3b				0.600 (0.004)
pKa (strongest basic) [37,38]	DS1a		−0.486 (0.009)	−0.515 (0.005)	
	DS2a	−0.525 (0.004)		−0.517 (0.005)	
	DS2n	−0.497 (0.007)	−0.488 (0.008)	−0.502 (0.007)	
	DS3a	−0.513 (0.005)	−0.545 (0.003)	−0.516 (0.005)	
H donors [37,38]	DS1a	−0.493 (0.008)	−0.480 (0.010)	−0.489 (0.008)	−0.590 (0.001)
	DS1n	−0.498 (0.007)	−0.525 (0.004)	−0.531 (0.004)	−0.621 (0.000)
	DS1b	−0.544 (0.003)	−0.585 (0.001)	−0.621 (0.000)	−0.619 (0.000)
	DS2a	−0.526 (0.004)	−0.536 (0.003)	−0.513 (0.005)	−0.611 (0.001)
	DS2n	−0.529 (0.004)	−0.540 (0.003)	−0.556 (0.002)	−0.560 (0.002)
	DS2b	−0.554 (0.002)	−0.535 (0.003)	−0.619 (0.000)	−0.663 (0.000)
	DS3a	−0.494 (0.008)		−0.484 (0.009)	−0.562 (0.002)
	DS3n	−0.559 (0.002)	−0.538 (0.003)	−0.544 (0.003)	−0.584 (0.001)
	DS3b	−0.563 (0.002)	−0.601 (0.001)	−0.643 (0.000)	−0.698 (0.000)

Desorption solvents compositions: DS1a = isopropanol/water/formic acid (80/19.9/0.1, *v*/*v*); DS1n = isopropanol/water (80/20, *v*/*v*); DS1b = isopropanol/water/ammonium hydroxide (80/19.9/0.1, *v*/*v*); DS2a = acetonitrile/water/formic acid (80/19.9/0.1, *v*/*v*); DS2n = acetonitrile/water (80/20, *v*/*v*); DS2b = acetonitrile/water/ammonium hydroxide (80/19.9/0.1, *v*/*v*); DS3a = methanol/water/formic acid (80/19.9/0.1, *v*/*v*); DS3n = methanol/water (80/20, *v*/*v*); DS3b = methanol/water/ammonium hydroxide (80/19.9/0.1, *v*/*v*).

3. Materials and Methods

3.1. Particles Used to Prepare TFME Coatings

In this study, thin-film microextraction coatings were prepared using 4 different types of silica particles bonded to octadecyl ligands and end-capping groups. Macherey Nagel™ NUCLEODUR® C_{18} Htec (henceforth referred to as "5 µm particles"), Phenomenex® Luna® C18(2) ("10 µm particles"), and Macherey-Nagel™ CHROMABOND® C_{18}ec ("45 µm particles") were all end-capped with trimethylsilane, and Phenomenex® Synergi™ Hydro-RP ("10 µm polar end-capped particles") was end-capped with a polar ethanol group [40]; see Figure 2 for visualization.

Additionally, apart from the differences in end-capping type, important particle parameters included the particles' size, pore diameter, specific surface area, and total carbon load (see Table 2 for more details).

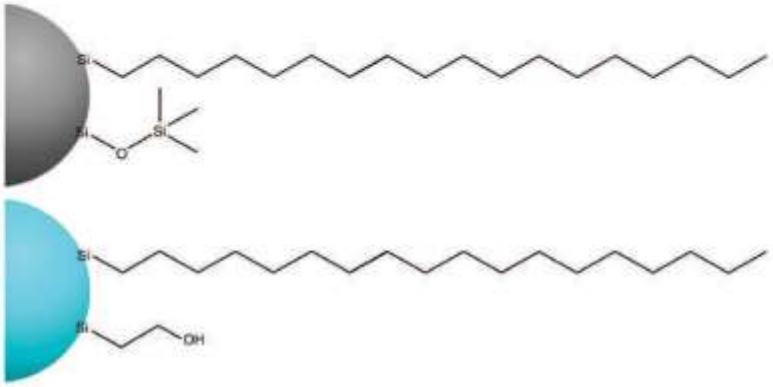

Figure 2. Octadecyl-bonded silica particles with trimethylsilane (**top**) and with polar ethanol (**bottom**) end-capping groups.

3.2. *Preparation of TFME Blades*

Thin-film microextraction blades were prepared following a protocol that was previously described by Mirnaghi et al. [10] with certain adjustments including reduction of the drying temperature (from 180 °C to 110 °C) to avoid thermal damage to the coating particles [41].

Pre-cut metal blades (PAS Technology Deutschland GmbH, Magdala, Germany) were etched in concentrated hydrochloric acid (Fluka™, Honeywell International Inc., Charlotte, NC, USA) for 60 min in an ultrasonic bath. The etched blades were cleaned using distilled water and then dried in an oven (150 °C for 30 min).

Next, the blades were covered with 10 layers of previously prepared biocompatible coating with a nitrogen operated sprayer (see Figure 3). After applying each layer, the blades were dried in an oven (110 °C for 3 min). Each type of prepared coating consisted of C_{18}-bonded silica particles dispersed in an N,N-dimethylformamide solution of polyacrylonitrile. For every 1.000 g of particles, 7.739 g of N,N-dimethylformamide (Sigma-Aldrich®, Merck KGaA, Darmstadt, Germany) and 0.421 g of polyacrylonitrile (Aldrich®, Merck KGaA, Darmstadt, Germany) were used.

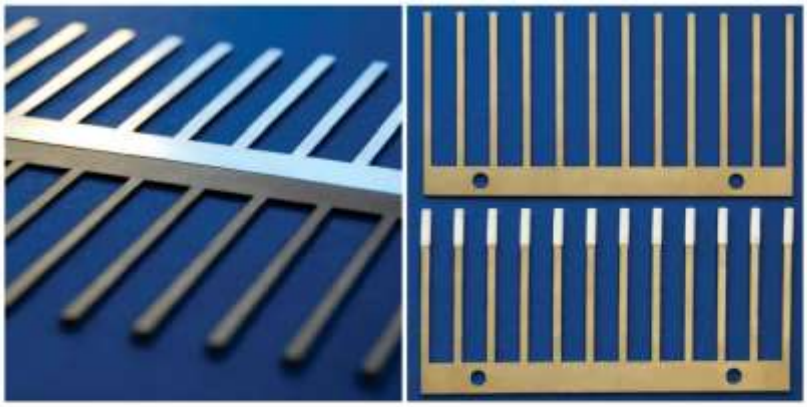

Figure 3. TFME Blades. **Left**: Metal surface before (top) and after (bottom) etching in hydrochloric acid. **Right**: TFME blade before (top) and after (bottom) applying coating.

3.3. Reference Standards

Analytical standards of 30 structurally diverse small molecules, such as therapeutic drugs, endogenous hormones, drugs of abuse, doping agents, and their metabolites, were used in this study. The full list presented in alphabetical order includes: 6-acetylcodeine, 11-deoxycortisol, 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), agomelatine, alprazolam, anastrozole, androstenedione, bisoprolol, boldenone, buprenorphine, canrenone, carteolol, clonazepam, cocaine, cortisol, fenoterol, flunitrazepam, hydrocodone, ketamine, lysergic acid diethylamide (LSD), melatonin, methandienone, methadone, methylphenidate, metoprolol, oxycodone, phencyclidine, progesterone, remifentanil acid, and zolpidem.

More details about the reference standards used in this study, including their suppliers, are shown in Supplementary Materials Table S5.

3.4. Preparation of the Samples

A testing mixture was prepared by spiking phosphate-buffered saline with stock solutions of all reference substances (to achieve 50 ng/mL concentration). The prepared testing solution was aliquoted to the 96-well DeepWell™ Plates (Nunc™, Thermo Fisher Scientific Inc., Waltham, MA, USA) and used for extractions. A single lot of testing mixture was used throughout the experiment, and all extractions were performed simultaneously to minimize any possible inconsistencies in the results.

A mixture of 4 deuterium-labelled standards (alprazolam D_5, cocaine D_3, oxycodone D_3, and THC-COOH D_3) was added at a 3 ng/mL concentration to every type of desorption solvent as an internal standard.

3.5. Extraction Protocol

All extractions were performed in 96-well plates using plate-compatible SH10 Heater-Shaker (Ingenieurbüro CAT, M. Zipperer GmbH, Ballrechten-Dottingen, Germany). The extraction protocol included: 1st preconditioning (1.5 mL methanol/water (50/50, *v*/*v*), 1 h, 850 rpm agitation); 2nd preconditioning (1 mL methanol/water (50/50, *v*/*v*), 1 h, 850 rpm agitation); 1st rinse (1.5 mL water, 5 s, no agitation); extraction (1 mL of testing mixture (50 ng/mL in PBS), 2.5 h, 850 rpm); 2nd rinse (1.5 mL water, 5 s, no agitation); and desorption (1 mL of desorption solvent, 2 h, 850 rpm). The temperature during the entire protocol was monitored and kept at 18.8 °C. Nine different variants of desorption solvents were used, and their compositions are shown in Table 4. Each variant was spiked with a mixture of deuterium-labelled reference standards at a concentration of 3 ng/mL.

Table 4. Composition of desorption solvents.

Desorption Solvent	Composition
DS1a	IPA/W/FA (80/19.9/0.1, *v*/*v*)
DS1n	IPA/W (80/20, *v*/*v*)
DS1b	IPA/W/AH (80/19.9/0.1, *v*/*v*)
DS2a	ACN/W/FA (80/19.9/0.1, *v*/*v*)
DS2n	ACN/W (80/20, *v*/*v*)
DS2b	ACN/W/AH (80/19.9/0.1, *v*/*v*)
DS3a	MeOH/W/FA (80/19.9/0.1, *v*/*v*)
DS3n	MeOH/W (80/20, *v*/*v*)
DS3b	MeOH/W/AH (80/19.9/0.1, *v*/*v*)

Chemicals used: ACN = acetonitrile (LC-MS grade; CHROMASOLV™, Honeywell International Inc., Charlotte, NC, USA); AH = ammonium hydroxide (LC-MS grade; Fluka™, Honeywell International Inc., Charlotte, NC, USA); FA = formic acid (LC-MS grade; Optima™, Fisher Chemical, Thermo Fisher Scientific Inc., Waltham, MA, USA); IPA = isopropanol (LC-MS grade; CHROMASOLV™, Honeywell International Inc., Charlotte, NC, USA); MeOH = methanol (LC-MS grade; CHROMASOLV™, Honeywell International Inc., Charlotte, NC, USA, W = water (LC-MS grade; LiChrosolv®, Merck KGaA, Darmstadt, Germany).

Additionally, the reference samples were transferred to the unoccupied wells of the 96-well plates prior to the desorption step. Therefore, the samples and the reference samples

were affected equally by the evaporation of the desorption solvents. Such an approach excluded the impact of the evaporation on the results.

3.6. HPLC-MS/MS Method

Samples were analysed with a Shimadzu LCMS-8060 triple quadrupole (Shimadzu Corporation, Kyoto, Japan) system equipped with an Agilent InfinityLab Poroshell 120 EC-C18 analytical column (3 × 100 mm, 2.7 µm) and guard column (3 × 5 mm, 2.7 µm) (Agilent, Santa Clara, CA, USA) using a method previously optimized and described by Sobczak and Goryński [42]. Thus, the column was maintained at 25.0 °C, an injection volume of 0.2 µL was used, and separation was performed in gradient elution mode. Mobile phases consisted of water (LC-MS grade; LiChrosolv®, Merck KGaA, Darmstadt, Germany) and acetonitrile (LC-MS grade; CHROMASOLV™, Honeywell International Inc., Charlotte, NC, USA), both with an addition of 0.1% formic acid (LC-MS grade; Optima™, Fisher Chemical, Thermo Fisher Scientific Inc., Waltham, MA, USA). A full list of retention times and monitored precursor–product ion(s) transitions is shown in Table S6 in Supplementary Materials.

3.7. Statistical Analysis

The dataset was analysed with IBM SPSS Statistics for Windows, Version 26.0. (IBM Corp, Armonk, NY, USA).

4. Conclusions

Thin-film microextraction blades coated with silica particles containing polar end-capping groups provided the best extraction efficacy out of all evaluated types of octadecyl-bonded particles. This type of coating is also characterized by the strongest correlations between the efficacy of the extraction process and hydrophobicity of the analytes, despite not having the highest density of hydrophobic octadecyl ligands. For example, while the hydrophobicity parameter (*H*) of Synergi™ Hydro-RP particles containing polar end-capping groups is higher than the *H* parameter of an average octadecyl particle (according to the hydrophobic-subtraction model [43,44]), the *H* of these particles is lower than the *H* of NUCLEODUR® C_{18} Htec particles [44]. This was not reflected by the results of this study, where the less hydrophobic polar end-capped particles provided better extraction efficacy of the hydrophobic analytes. This suggests that the extraction of small molecules from aqueous samples with the conventional TMS end-capped octadecyl-bonded particles differs from the theoretical expectations. Moreover, in the hydrophobic-subtraction model, polar end-capping groups closely resemble TMS groups [45]; thus, this difference in end-capping type does not provide a satisfactory explanation for the correlations observed with the tested particles. Therefore, one may hypothesize that the extraction yields of TMS end-capped particles are below the theoretical optimal performance due to immersion in a water-based matrix, with which they are not compatible. Such a situation could not occur with water-compatible polar end-capped particles that retained their original characteristics throughout extraction. This reasoning seems to elucidate the benefits of using fully water-compatible stationary phases for extraction from aqueous samples, and was reflected by the results of this study.

Additional investigation is required to assess whether water-compatible polar end-capping can improve extraction efficacy with ligands other than the octadecyl groups that were evaluated in this study. Some examples of stationary phases that are relatively popular in liquid chromatography and susceptible to so-called stationary phase collapse include octyl (C_8) and phenyl-hexyl. These chemistries could significantly benefit from the incorporation of polar end-capping groups. However, if that were confirmed, then a significant breakthrough in solid phase microextraction could be achieved by preserving all original ligand traits, which may result in improved extraction efficacy. This process may then broaden the range of successfully used liquid chromatography-compatible coatings for future SPME devices and promote methods' use as a green alternative for

sample preparation enabling low organic solvent consumption without compromising the extraction yield.

Supplementary Materials: The following are available online, Table S1. Coefficients of determination (R^2) determined during calibration runs in different desorption solvents.; Table S2. Number of results in each category for every stationary phase—desorption solvent combination.; Table S3. Extraction efficacies [%] for each stationary phase—desorption solvent combination.; Table S4. Essential physicochemical proprieties of the analysed substances.; Table S5. List of reference standards in alphabetical order.; Table S6. Monitored precursor—product ion(s) transitions.

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Sample Availability: Not applicable.

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Supplementary Material for:

Benefits of innovative and fully water-compatible stationary phases of thin-film microextraction (TFME) blades

by Łukasz Sobczak, Dominika Kołodziej and Krzysztof Goryński

List of contents:

Table S1. Coefficients of determination (R^2) determined during calibration runs in different desorption solvents. *Substances arranged by retention order.*

substance	coefficient of determination (R^2) in desorption solvent composition:		
	isopropanol/water (80/20, v/v)	acetonitrile/water (80/20, v/v)	methanol/water (80/20, v/v)
fenoterol	0.9996	0.9999	0.9982
carteolol	0.9993	0.9996	1.0000
oxycodone	0.9991	0.9985	0.9984
hydrocodone	0.9992	0.9998	0.9991
ketamine	0.9989	0.9998	1.0000
remifentanil acid	0.9993	0.9984	0.9995
metoprolol	1.0000	0.9998	0.9980
6-acetylcodeine	0.9995	0.9998	1.0000
methylphenidate	0.9999	0.9998	0.9995
zolpidem	1.0000	0.9991	1.0000
cocaine	0.9994	0.9999	0.9989
LSD	0.9999	0.9999	0.9973
melatonin	0.9999	0.9997	0.9996
bisoprolol	0.9998	0.9999	0.9997
phencyclidine	0.9998	0.9993	0.9991
cortisol	0.9978	0.9994	0.9998
buprenorphine	0.9996	0.9992	0.9999
alprazolam	0.9975	0.9997	0.9989
anastrozole	1.0000	0.9997	0.9984
methadone	0.9982	1.0000	0.9999
11-deoxycortisol	0.9944	0.9999	0.9978
boldenone	1.0000	0.9981	0.9988
clonazepam	0.9991	0.9976	0.9991
agomelatine	0.9989	0.9998	0.9999
methandienone	0.9993	0.9999	0.9980
flunitrazepam	0.9998	0.9999	0.9999
androstenedione	0.9990	0.9991	0.9999
canrenone	0.9919	0.9912	0.9999
progesterone	0.9999	0.9998	0.9999
THC-COOH	0.9978	0.9998	1.0000

Table S2. Number of results in each category for every stationary phase – desorption solvent combination. *Table arranged by the type of stationary phase.*

stationary phase + desorption solvent combination	number of results:		
	above median (in Q_2)	in 3rd quartile (Q_3)	best results
5 µm + DS1a	2	-	-
5 µm + DS1n	-	-	-
5 µm + DS1b	12	1	-
5 µm + DS2a	5	-	-
5 µm + DS2n	-	-	-
5 µm + DS2b	6	-	-
5 µm + DS3a	-	-	-
5 µm + DS3n	1	1	-
5 µm + DS3b	10	1	-
10 µm + DS1a	22	17	-
10 µm + DS1n	6	1	-
10 µm + DS1b	28	6	-
10 µm + DS2a	29	13	-
10 µm + DS2n	28	13	-
10 µm + DS2b	23	1	-
10 µm + DS3a	28	18	-
10 µm + DS3n	14	5	1
10 µm + DS3b	28	8	1
10 µm with polar end-capping + DS1a	10	2	-
10 µm with polar end-capping + DS1n	3	-	-
10 µm with polar end-capping + DS1b	9	5	-
10 µm with polar end-capping + DS2a	20	15	-
10 µm with polar end-capping + DS2n	20	8	-
10 µm with polar end-capping + DS2b	-	-	-
10 µm with polar end-capping + DS3a	30	27	18
10 µm with polar end-capping + DS3n	5	1	-
10 µm with polar end-capping + DS3b	3	2	-
45 µm + DS1a	24	14	-
45 µm + DS1n	14	4	-
45 µm + DS1b	29	26	1
45 µm + DS2a	25	12	1
45 µm + DS2n	23	12	-
45 µm + DS2b	18	10	-
45 µm + DS3a	30	28	8
45 µm + DS3n	15	7	-
45 µm + DS3b	20	12	-

Desorption solvents compositions: DS1a = isopropanol/water/formic acid (80/19.9/0.1, v/v); DS1n = isopropanol/water (80/20, v/v); DS1b = isopropanol/water/ammonium hydroxide (80/19.9/0.1, v/v); DS2a = acetonitrile/water/formic acid (80/19.9/0.1, v/v); DS2n = acetonitrile/water (80/20, v/v); DS2b = acetonitrile/water/ammonium hydroxide (80/19.9/0.1, v/v); DS3a = methanol/water/formic acid (80/19.9/0.1, v/v); DS3n = methanol/water (80/20, v/v); DS3b = methanol/water/ammonium hydroxide (80/19.9/0.1, v/v).

Table S3. Extraction efficacies [%] for each stationary phase – desorption solvent combination (SP + DS). *Relative standard deviations [%] given in brackets, n=3, substances arranged by retention order.*

SP + DS ↓ / → substance	fenoterol	carteolol	oxycodone	hydrocodone	ketamine	remifentanil acid	metoprolol	6-acetylcodeine	methylphenidate	zolpidem	cocaine	LSD	melatonin
median extraction efficacy	**35.3**	**61.2**	**48.1**	**68.8**	**76.0**	**59.3**	**75.5**	**99.6**	**69.3**	**103.5**	**85.5**	**97.2**	**56.7**
3rd quartile extraction efficacy	**45.2**	**71.2**	**60.3**	**81.6**	**87.0**	**70.3**	**84.9**	**104.5**	**80.3**	**105.8**	**94.3**	**101.1**	**66.9**
top extraction efficacy	**65.1**	**85.8**	**76.1**	**93.5**	**103.1**	**83.9**	**95.1**	**117.6**	**94.4**	**135.5**	**102.8**	**115.0**	**89.5**
10 µm + DS1a	38.3 (8.3)	60.2 (6.8)	47.8 (7.7)	65.6 (6.6)	75.0 (4.1)	59.8 (8.2)	79.8 (3.5)	93.3 (2.1)	68.5 (3.9)	106.2 (2.3)	83.7 (2.9)	93.8 (2.6)	57.6 (6.7)
10 µm polar + DS1a	35.7 (8.1)	55.6 (7.4)	40.4 (3.1)	66.5 (5.0)	73.5 (4.2)	46.3 (4.5)	71.2 (9.0)	89.2 (4.6)	65.3 (7.1)	101.7 (3.4)	78.1 (5.6)	92.5 (4.4)	50.9 (2.4)
5 µm + DS1a	25.5 (5.3)	40.0 (6.9)	29.4 (10.4)	45.5 (5.3)	59.4 (2.9)	36.4 (6.0)	54.0 (2.2)	82.4 (1.1)	48.3 (4.9)	99.2 (3.2)	67.0 (1.7)	88.1 (2.0)	43.7 (3.2)
45 µm + DS1a	60.1 (6.8)	79.5 (6.3)	72.4 (6.5)	90.2 (3.3)	94.3 (3.5)	77.4 (5.3)	89.5 (7.9)	89.1 (3.4)	87.8 (6.3)	102.8 (3.4)	90.3 (4.6)	100.5 (3.5)	83.5 (6.2)
10 µm + DS1n	30.8 (8.1)	58.5 (5.7)	41.3 (6.6)	61.2 (1.9)	72.9 (2.6)	53.6 (4.5)	68.7 (4.0)	91.9 (1.8)	61.7 (2.0)	100.4 (0.1)	80.9 (2.8)	81.6 (2.9)	48.8 (2.3)
10 µm polar + DS1n	28.5 (5.4)	49.7 (1.3)	33.3 (5.0)	58.7 (4.7)	70.3 (3.1)	43.6 (7.0)	62.1 (4.1)	86.7 (2.4)	59.0 (3.0)	98.1 (0.9)	75.6 (1.9)	83.0 (4.8)	41.0 (7.6)
5 µm + DS1n	23.1 (3.1)	39.5 (1.7)	29.3 (2.5)	43.9 (5.2)	59.4 (1.8)	34.3 (6.1)	52.4 (2.6)	77.4 (0.8)	47.6 (5.4)	92.2 (2.2)	67.6 (2.0)	75.1 (5.8)	35.1 (6.8)
45 µm + DS1n	47.8 (1.6)	71.7 (5.7)	59.5 (4.4)	81.4 (2.3)	88.7 (1.6)	69.0 (0.9)	80.9 (3.7)	95.6 (2.2)	80.9 (5.2)	99.7 (1.2)	95.1 (2.5)	88.7 (0.2)	63.7 (1.9)
10 µm + DS1b	38.8 (12.8)	70.4 (8.2)	60.8 (10.1)	76.0 (6.2)	84.6 (4.8)	68.1 (8.4)	80.9 (7.2)	99.6 (3.3)	79.8 (6.5)	104.0 (4.8)	95.0 (2.8)	95.1 (4.3)	59.6 (10.6)
10 µm polar + DS1b	27.2 (5.4)	51.8 (9.5)	39.7 (7.9)	62.4 (2.7)	77.4 (5.4)	44.4 (5.6)	66.1 (3.4)	94.9 (0.5)	65.1 (2.3)	103.0 (1.1)	88.7 (0.4)	94.4 (1.2)	44.5 (4.4)
5 µm + DS1b	34.0 (14.3)	61.0 (9.4)	51.1 (11.4)	65.9 (9.0)	79.0 (5.5)	55.9 (8.7)	74.8 (18.7)	94.0 (2.5)	71.0 (9.0)	100.2 (1.8)	89.3 (4.3)	93.3 (3.5)	55.9 (9.6)
45 µm + DS1b	53.5 (2.5)	88.0 (2.7)	76.1 (2.5)	92.0 (0.9)	98.9 (1.1)	81.2 (0.5)	98.4 (3.2)	105.9 (2.3)	94.3 (2.2)	106.8 (1.2)	102.8 (0.6)	101.5 (1.7)	77.1 (0.8)
10 µm + DS2a	44.8 (8.2)	69.2 (1.5)	56.6 (3.9)	75.1 (2.2)	83.9 (1.9)	69.5 (4.7)	84.2 (3.4)	100.2 (0.5)	74.0 (1.8)	105.0 (2.6)	92.0 (1.0)	98.5 (3.0)	64.0 (4.5)
10 µm polar + DS2a	33.3 (6.1)	52.5 (3.6)	40.5 (5.5)	67.4 (3.3)	80.8 (1.9)	46.9 (2.0)	70.9 (5.6)	99.4 (0.7)	66.7 (3.8)	108.0 (2.8)	85.2 (1.9)	101.2 (1.4)	52.2 (3.4)
5 µm + DS2a	42.4 (10.5)	61.4 (8.5)	48.4 (7.1)	67.2 (5.5)	74.2 (5.2)	58.7 (7.5)	75.4 (5.5)	90.4 (3.1)	66.5 (8.0)	98.5 (1.7)	83.2 (4.7)	91.5 (3.1)	58.9 (7.5)
45 µm + DS2a	58.2 (3.8)	80.4 (5.5)	71.4 (5.6)	93.5 (3.1)	93.3 (1.0)	79.8 (2.8)	90.1 (2.5)	101.6 (2.3)	88.0 (2.9)	105.6 (2.6)	93.0 (2.0)	99.8 (2.3)	78.5 (5.2)
10 µm + DS2n	40.2 (7.8)	65.0 (3.0)	57.7 (4.4)	77.8 (2.7)	85.8 (1.9)	70.2 (4.8)	81.7 (4.6)	102.6 (1.8)	76.4 (3.6)	109.2 (1.6)	94.6 (4.4)	106.8 (1.7)	68.3 (3.9)
10 µm polar + DS2n	32.6 (5.3)	53.7 (7.7)	43.3 (5.6)	69.1 (2.7)	82.1 (0.8)	51.1 (0.9)	73.4 (6.5)	99.7 (1.5)	67.8 (6.7)	108.6 (2.8)	85.9 (5.7)	105.5 (2.5)	52.3 (2.3)
5 µm + DS2n	28.6 (3.5)	49.5 (5.6)	38.1 (8.3)	58.0 (5.8)	68.1 (4.5)	47.2 (6.1)	63.3 (8.5)	87.0 (3.2)	56.3 (5.7)	97.9 (4.7)	77.7 (6.9)	90.8 (5.1)	47.4 (5.3)
45 µm + DS2n	53.3 (5.7)	76.9 (5.6)	64.9 (1.9)	90.0 (0.8)	93.4 (1.6)	79.1 (1.3)	85.1 (1.5)	104.1 (0.7)	85.8 (1.1)	105.6 (0.7)	96.5 (2.2)	104.1 (3.5)	77.3 (1.2)

SP + DS ↓ / → substance	bisoprolol	phencyclidine	cortisol	buprenorphine	alprazolam	anastrozole	methadone	11-deoxycortisol	boldenone	clonazepam	agomelatine	methandienone	flunitrazepam	androstenedione	canrenone	progesterone	THC-COOH
median extraction efficacy	**97.8**	**88.0**	**96.4**	**91.6**	**99.9**	**97.7**	**96.7**	**101.9**	**102.1**	**83.3**	**96.5**	**100.8**	**96.9**	**101.4**	**104.4**	**98.5**	**79.5**
3rd quartile extraction efficacy	**101.6**	**94.5**	**101.4**	**95.2**	**104.5**	**104.1**	**101.3**	**109.3**	**107.9**	**99.4**	**104.1**	**106.4**	**103.8**	**106.8**	**111.9**	**103.8**	**86.6**
top extraction efficacy	**120.9**	**114.5**	**122.9**	**117.6**	**120.7**	**126.4**	**118.5**	**131.3**	**131.1**	**115.3**	**122.6**	**126.1**	**124.5**	**129.5**	**138.9**	**127.9**	**119.8**
10 µm + DS1a	104.0 (0.9)	96.8 (1.6)	105.4 (2.7)	100.0 (2.6)	103.9 (2.4)	104.8 (1.8)	106.9 (2.0)	120.7 (1.3)	109.7 (0.6)	101.7 (1.5)	104.7 (1.0)	113.0 (1.8)	106.8 (1.9)	111.6 (2.4)	113.7 (3.1)	111.7 (2.1)	97.7 (5.4)
10 µm polar + DS1a	95.4 (4.1)	90.5 (4.5)	97.2 (5.1)	94.0 (2.7)	96.4 (5.4)	94.7 (3.6)	100.2 (3.5)	106.6 (1.5)	102.2 (4.9)	87.5 (5.6)	94.3 (3.7)	101.3 (4.2)	93.5 (3.1)	102.5 (3.3)	103.0 (2.7)	102.2 (2.6)	51.0 (1.5)
5 µm + DS1a	89.5 (2.6)	82.0 (2.8)	92.3 (3.8)	90.0 (8.7)	93.7 (3.3)	87.2 (4.6)	99.3 (3.3)	97.3 (4.0)	97.9 (1.3)	83.1 (3.8)	90.5 (3.8)	96.6 (3.5)	90.5 (2.9)	104.3 (2.5)	101.5 (3.8)	97.1 (9.6)	76.0 (9.1)
45 µm + DS1a	102.8 (5.2)	99.2 (4.6)	106.1 (3.2)	87.5 (4.4)	104.1 (2.7)	103.8 (5.9)	101.1 (3.1)	101.0 (4.0)	105.1 (2.8)	103.8 (2.8)	104.1 (3.4)	105.1 (1.7)	104.2 (3.6)	105.8 (1.7)	103.9 (2.3)	95.8 (2.5)	71.4 (2.4)
10 µm + DS1n	94.6 (4.5)	89.5 (4.9)	93.2 (3.1)	95.3 (1.5)	98.6 (2.9)	94.2 (1.0)	100.6 (5.9)	104.9 (6.7)	100.6 (2.2)	92.0 (1.8)	98.6 (2.0)	99.0 (2.3)	96.6 (1.7)	101.8 (3.1)	100.0 (4.1)	100.4 (2.1)	85.9 (2.9)
10 µm polar + DS1n	90.3 (0.7)	82.7 (2.7)	90.5 (1.6)	93.5 (1.5)	94.0 (0.9)	88.4 (3.5)	96.4 (5.3)	105.3 (7.5)	97.4 (0.8)	84.9 (3.7)	90.8 (0.8)	96.6 (1.9)	90.6 (4.1)	98.5 (3.4)	97.5 (2.4)	95.8 (1.4)	80.6 (5.2)
5 µm + DS1n	80.5 (2.5)	77.7 (4.0)	80.1 (1.8)	84.6 (1.4)	85.5 (3.6)	78.8 (5.4)	92.3 (5.0)	86.0 (3.4)	88.2 (1.5)	73.7 (1.2)	81.9 (0.9)	86.8 (3.0)	83.1 (1.6)	93.5 (3.8)	95.1 (1.4)	89.5 (3.8)	73.8 (9.2)
45 µm + DS1n	96.7 (3.1)	93.1 (4.7)	97.2 (1.9)	81.6 (4.8)	98.1 (1.9)	96.7 (1.1)	97.2 (3.9)	94.7 (4.6)	98.1 (0.7)	94.6 (0.5)	98.9 (1.2)	98.6 (1.7)	96.2 (2.2)	97.8 (1.7)	95.4 (2.3)	88.3 (3.8)	67.1 (2.3)
10 µm + DS1b	103.5 (3.6)	103.4 (5.6)	101.3 (4.9)	98.2 (2.3)	104.4 (3.2)	101.4 (5.1)	109.2 (2.9)	102.9 (6.2)	104.9 (5.7)	98.2 (3.3)	99.7 (4.7)	100.9 (4.8)	103.5 (5.8)	106.6 (2.7)	103.8 (7.7)	101.8 (3.8)	80.0 (5.6)
10 µm polar + DS1b	97.7 (1.8)	96.3 (2.3)	96.1 (4.7)	103.1 (6.0)	100.8 (5.1)	94.9 (0.7)	106.7 (4.0)	100.1 (8.2)	104.8 (0.1)	87.6 (2.4)	92.6 (1.4)	100.1 (1.7)	94.8 (2.3)	103.3 (2.3)	105.3 (5.0)	105.5 (3.4)	92.9 (13.7)
5 µm + DS1b	96.8 (3.1)	94.2 (4.2)	95.7 (4.2)	95.2 (2.5)	97.4 (3.7)	95.5 (1.6)	105.1 (4.3)	105.2 (2.0)	100.2 (1.1)	90.8 (3.6)	97.5 (1.4)	101.5 (2.5)	97.1 (3.8)	105.3 (2.1)	103.0 (1.9)	100.8 (2.5)	77.0 (3.0)
45 µm + DS1b	107.8 (2.8)	105.8 (3.4)	110.2 (1.4)	92.7 (5.0)	108.3 (3.3)	106.0 (1.7)	107.3 (5.7)	115.0 (2.3)	110.3 (5.4)	101.8 (2.7)	109.5 (1.3)	108.3 (3.1)	109.3 (2.5)	107.9 (3.9)	107.2 (6.4)	98.6 (3.7)	74.8 (5.0)
10 µm + DS2a	105.4 (3.0)	92.8 (1.2)	106.3 (1.8)	97.1 (2.5)	103.2 (3.4)	108.4 (2.5)	96.4 (1.8)	130.9 (3.3)	110.0 (4.4)	102.3 (2.3)	107.0 (1.1)	130.1 (3.3)	107.8 (2.0)	134.4 (3.5)	113.3 (1.3)	106.4 (2.2)	89.7 (1.1)
10 µm polar + DS2a	102.4 (1.2)	93.0 (2.2)	102.0 (5.6)	96.2 (3.3)	104.3 (3.5)	104.0 (1.3)	93.8 (8.7)	120.5 (1.2)	112.1 (1.5)	97.7 (1.2)	109.6 (1.0)	118.0 (8.6)	105.1 (2.3)	126.5 (1.8)	116.5 (1.1)	111.5 (1.9)	96.3 (2.6)
5 µm + DS2a	95.7 (3.6)	87.7 (4.5)	91.8 (3.7)	87.2 (1.3)	90.3 (1.1)	96.3 (3.0)	94.2 (1.7)	103.0 (4.4)	99.2 (5.0)	86.5 (4.0)	94.0 (3.7)	98.4 (2.2)	95.9 (2.4)	103.3 (2.9)	103.9 (3.8)	98.3 (0.1)	78.4 (1.3)
45 µm + DS2a	102.6 (2.2)	94.0 (1.4)	100.8 (2.3)	86.2 (1.8)	103.3 (8.0)	107.4 (5.0)	95.3 (4.0)	101.6 (5.8)	107.3 (5.0)	103.3 (2.8)	104.0 (1.9)	103.9 (2.1)	104.8 (2.0)	104.5 (2.4)	105.6 (4.3)	94.5 (3.4)	71.8 (5.3)
10 µm + DS2n	99.4 (2.4)	85.6 (4.8)	103.3 (3.7)	94.7 (0.4)	107.9 (2.2)	105.4 (2.3)	86.8 (6.0)	105.6 (3.0)	108.6 (1.4)	100.8 (2.5)	108.2 (0.7)	109.7 (2.2)	103.6 (1.8)	107.6 (3.0)	107.4 (2.0)	106.9 (3.1)	89.3 (3.6)
10 µm polar + DS2n	97.9 (4.7)	81.9 (4.2)	99.2 (5.0)	94.7 (1.7)	106.3 (3.3)	106.3 (2.5)	87.7 (5.0)	106.5 (2.4)	107.7 (1.6)	91.7 (0.9)	105.5 (2.2)	108.9 (2.6)	102.5 (1.8)	108.7 (2.2)	110.5 (1.4)	108.9 (1.8)	86.3 (1.7)
5 µm + DS2n	87.8 (7.5)	77.6 (6.5)	87.8 (4.8)	82.4 (4.4)	94.3 (3.9)	90.4 (5.4)	84.6 (8.2)	94.8 (4.0)	95.2 (5.5)	80.1 (5.8)	91.4 (6.1)	97.3 (5.0)	91.1 (5.2)	101.0 (3.0)	94.7 (6.1)	92.1 (4.1)	77.6 (3.3)
45 µm + DS2n	96.6 (2.7)	81.4 (5.0)	98.2 (3.2)	86.7 (1.4)	105.9 (3.0)	102.3 (5.1)	81.6 (2.6)	103.7 (4.5)	102.5 (2.9)	99.0 (2.2)	101.6 (5.3)	105.6 (3.2)	100.7 (1.8)	105.5 (2.8)	100.0 (5.5)	93.6 (8.7)	71.2 (4.6)

SF + DS ↓ / substance →	fenoterol	carteolol	oxycodone	hydrocodone	ketamine	remifentanil acid	metoprolol	6-acetylcodeine	methylphenidate	zolpidem	cocaine	LSD	melatonin	bisoprolol	phencyclidine
30 µm + DS2b	29.8 (10.5)	68.2 (5.5)	57.7 (7.3)	74.6 (6.4)	84.8 (5.3)	68.4 (8.3)	84.2 (5.5)	100.0 (3.5)	76.9 (6.3)	102.2 (3.7)	94.1 (5.6)	101.3 (3.3)	62.8 (6.8)	102.7 (4.5)	92.9 (5.9)
10 µm polar + DS2b	21.4 (3.0)	52.0 (5.1)	37.8 (4.1)	62.2 (2.5)	73.7 (4.0)	46.0 (2.7)	69.3 (5.2)	89.3 (3.8)	62.0 (4.7)	97.2 (3.7)	82.6 (5.5)	94.7 (3.0)	48.0 (4.5)	93.2 (3.5)	82.3 (4.3)
5 µm + DS2b	23.9 (10.5)	51.7 (5.6)	39.6 (4.0)	58.3 (5.8)	72.2 (3.4)	48.3 (8.0)	68.4 (6.3)	90.3 (1.6)	58.5 (4.0)	100.1 (2.2)	82.3 (1.2)	97.2 (2.0)	51.4 (6.9)	93.7 (2.2)	86.0 (1.5)
45 µm + DS2b	35.0 (9.5)	78.0 (8.1)	68.3 (8.0)	87.1 (4.0)	93.5 (2.8)	77.0 (4.0)	88.7 (3.1)	99.9 (1.6)	84.9 (5.1)	100.8 (1.5)	98.0 (3.3)	102.2 (0.6)	75.7 (5.7)	98.5 (3.9)	88.8 (4.2)
30 µm + DS1a	40.5 (9.1)	63.6 (8.5)	47.0 (8.8)	68.5 (5.7)	79.1 (5.7)	61.3 (8.8)	82.5 (6.5)	100.8 (5.3)	71.7 (7.4)	113.7 (3.3)	96.2 (4.4)	100.9 (2.3)	59.1 (9.6)	109.4 (3.6)	103.7 (5.9)
10 µm polar + DS1a	48.0 (6.7)	71.0 (3.2)	52.4 (8.2)	82.4 (3.4)	97.8 (1.8)	63.7 (4.4)	98.2 (2.9)	112.3 (2.1)	85.7 (1.4)	125.3 (2.3)	102.4 (0.6)	115.0 (4.4)	69.1 (4.8)	120.9 (3.2)	114.5 (2.4)
5 µm + DS1a	32.4 (8.5)	49.4 (4.6)	36.3 (5.4)	50.3 (6.6)	67.8 (4.9)	45.4 (9.2)	62.1 (3.8)	79.9 (1.3)	55.6 (6.3)	93.3 (1.5)	70.5 (3.8)	82.2 (2.3)	50.9 (6.8)	85.5 (2.6)	82.3 (2.6)
45 µm + DS1a	65.1 (2.2)	85.8 (5.0)	76.1 (3.5)	92.3 (2.6)	102.1 (1.9)	83.9 (1.8)	95.1 (5.5)	105.7 (0.2)	94.4 (1.6)	109.5 (0.6)	100.0 (1.5)	105.5 (0.7)	83.5 (2.4)	108.6 (0.7)	104.7 (1.3)
30 µm + DS3n	35.6 (12.3)	52.8 (9.6)	41.0 (11.3)	69.1 (8.1)	71.6 (6.4)	62.0 (10.9)	64.9 (4.3)	108.2 (1.7)	55.4 (8.4)	107.4 (2.4)	71.2 (5.3)	100.3 (1.4)	38.8 (12.3)	98.2 (2.1)	78.0 (2.1)
10 µm polar + DS3n	29.8 (8.2)	43.9 (2.3)	32.6 (8.4)	65.8 (4.3)	68.5 (6.1)	45.8 (8.7)	56.9 (5.5)	102.9 (3.5)	53.1 (5.2)	105.4 (1.8)	68.2 (2.1)	99.6 (2.5)	12.6 (6.2)	94.7 (3.2)	75.1 (1.4)
5 µm + DS3n	32.2 (3.7)	43.8 (5.0)	34.1 (3.6)	57.8 (1.8)	68.6 (0.8)	49.6 (3.0)	54.9 (6.3)	87.9 (1.5)	47.4 (6.3)	89.4 (1.3)	58.9 (3.7)	86.6 (2.8)	34.7 (1.7)	83.0 (4.1)	63.3 (5.8)
45 µm + DS3n	54.9 (1.5)	68.2 (4.2)	61.2 (4.5)	82.3 (3.7)	82.3 (3.2)	79.8 (3.3)	75.6 (4.5)	110.5 (3.8)	78.1 (2.2)	103.8 (2.4)	75.9 (2.6)	102.4 (3.3)	54.5 (2.2)	97.9 (2.1)	78.5 (2.1)
30 µm + DS3b	43.9 (6.1)	71.9 (6.3)	60.1 (5.7)	79.0 (3.0)	86.5 (4.8)	70.3 (6.2)	87.5 (3.1)	117.6 (5.7)	80.1 (4.8)	100.0 (3.8)	97.6 (6.0)	98.2 (4.2)	68.7 (6.9)	99.9 (4.9)	95.4 (5.9)
10 µm polar + DS3b	27.8 (1.6)	30.6 (7.4)	37.0 (8.5)	61.0 (4.7)	72.8 (6.3)	45.6 (6.9)	64.4 (7.4)	109.9 (1.5)	62.3 (5.4)	97.7 (1.3)	82.5 (5.8)	92.5 (3.1)	44.8 (7.6)	92.0 (3.9)	84.9 (5.4)
5 µm + DS3b	34.3 (1.9)	61.8 (4.3)	49.0 (4.3)	69.7 (5.4)	77.5 (3.2)	58.3 (5.1)	78.7 (4.1)	108.7 (1.8)	70.4 (4.0)	99.8 (3.0)	89.5 (3.4)	95.4 (3.2)	59.6 (1.7)	96.3 (3.4)	88.5 (6.0)
45 µm + DS3b	46.6 (5.1)	76.4 (4.0)	66.5 (2.8)	89.8 (1.4)	98.6 (2.0)	73.9 (2.3)	86.7 (2.7)	108.5 (2.7)	94.8 (2.6)	101.0 (1.4)	96.1 (0.8)	99.5 (1.4)	74.1 (4.0)	99.4 (3.1)	89.9 (2.0)

SF + DS ↓ / substance →	cortisol	buprenorphine	alprazolam	anastrozole	methadone	11-deoxycortisol	boldenone	clonazepam	agomelatine	methandienone	flunitrazepam	androstenedione	canrenone	progesterone	THC-COOH
30 µm + DS2b	97.6 (5.5)	92.2 (5.5)	102.6 (3.6)	99.2 (4.8)	97.0 (3.8)	102.2 (4.8)	104.5 (4.1)	96.3 (4.4)	97.7 (4.6)	99.5 (3.2)	101.0 (4.9)	102.6 (3.2)	102.0 (3.0)	97.0 (3.8)	79.1 (2.3)
10 µm polar + DS2b	94.1 (4.5)	83.5 (5.4)	94.8 (1.9)	89.9 (3.4)	90.8 (5.0)	95.8 (1.7)	99.3 (5.5)	82.4 (2.1)	95.2 (4.2)	98.0 (1.8)	91.1 (2.6)	96.0 (2.7)	96.1 (4.4)	96.3 (7.8)	77.0 (6.5)
5 µm + DS2b	95.3 (0.8)	90.9 (4.5)	98.3 (1.6)	93.6 (0.4)	98.7 (2.8)	106.2 (6.8)	108.8 (3.2)	87.5 (1.4)	95.5 (0.9)	101.0 (2.0)	93.7 (1.1)	103.4 (1.1)	102.5 (1.0)	98.8 (3.2)	79.8 (2.0)
45 µm + DS2b	96.8 (0.5)	83.6 (1.3)	101.3 (1.1)	100.7 (2.0)	89.3 (3.0)	98.3 (0.9)	101.4 (2.8)	97.3 (2.7)	101.5 (0.4)	100.6 (2.2)	99.7 (1.2)	100.8 (1.9)	98.1 (4.3)	91.9 (2.8)	70.3 (4.3)
30 µm + DS1a	112.8 (1.4)	105.9 (2.8)	109.8 (6.1)	112.3 (4.2)	110.8 (4.6)	116.2 (3.5)	117.5 (4.4)	105.7 (3.5)	113.5 (4.2)	115.7 (2.3)	113.0 (5.2)	119.9 (2.4)	120.3 (1.4)	116.2 (0.7)	106.4 (0.9)
10 µm polar + DS1a	123.8 (3.2)	117.0 (4.3)	120.7 (4.7)	124.4 (1.8)	118.5 (4.3)	131.3 (7.4)	131.1 (2.7)	115.3 (1.3)	122.8 (3.3)	128.1 (3.0)	124.5 (2.2)	129.5 (2.9)	131.5 (4.5)	127.9 (4.8)	119.0 (4.3)
5 µm + DS1a	91.0 (2.2)	86.6 (3.4)	90.3 (4.0)	92.5 (2.6)	98.0 (0.8)	101.1 (2.7)	96.9 (2.4)	85.9 (3.9)	93.5 (3.2)	97.8 (1.0)	92.6 (3.5)	100.2 (1.9)	102.8 (5.4)	98.5 (4.4)	78.9 (6.5)
45 µm + DS1a	130.7 (2.3)	92.2 (2.4)	101.3 (0.8)	116.7 (1.5)	103.4 (1.5)	129.1 (0.4)	114.0 (2.3)	108.9 (1.8)	113.3 (1.7)	133.1 (0.3)	112.4 (0.4)	134.4 (2.7)	114.0 (5.8)	107.8 (2.8)	83.2 (9.0)
30 µm + DS3n	74.7 (1.6)	93.5 (3.6)	98.0 (2.7)	99.4 (3.4)	90.9 (6.1)	85.0 (2.8)	108.7 (2.7)	90.5 (3.4)	76.8 (2.1)	94.6 (3.5)	86.0 (3.7)	103.8 (2.6)	139.9 (1.9)	99.9 (2.1)	84.0 (4.3)
10 µm polar + DS3n	69.9 (3.8)	89.2 (4.3)	93.8 (3.8)	90.2 (5.8)	93.7 (7.9)	83.6 (3.7)	102.4 (0.8)	83.0 (3.7)	71.8 (2.5)	91.7 (2.7)	81.0 (2.1)	100.0 (3.9)	133.7 (4.1)	95.7 (2.8)	76.0 (2.4)
5 µm + DS3n	62.4 (2.3)	77.0 (3.3)	79.5 (3.1)	81.5 (1.5)	76.4 (6.9)	71.2 (2.7)	89.3 (2.6)	73.6 (3.7)	63.2 (2.1)	78.0 (3.0)	72.2 (1.2)	87.8 (2.2)	117.8 (2.6)	85.7 (1.9)	68.4 (2.3)
45 µm + DS3n	74.0 (4.3)	83.5 (2.2)	93.2 (3.0)	97.3 (2.5)	90.1 (4.1)	84.3 (3.5)	103.0 (4.0)	92.5 (3.2)	74.6 (1.6)	87.7 (3.8)	84.8 (2.6)	98.8 (1.8)	131.7 (4.1)	94.1 (2.1)	75.4 (3.0)
30 µm + DS3b	97.1 (0.7)	93.1 (3.9)	101.9 (3.8)	100.7 (2.5)	101.9 (4.4)	98.6 (4.7)	101.3 (4.3)	96.8 (4.5)	101.6 (3.6)	103.3 (5.0)	101.6 (3.9)	104.4 (3.7)	105.4 (2.8)	101.2 (4.8)	83.7 (5.9)
10 µm polar + DS3b	86.7 (0.6)	90.5 (2.4)	94.3 (0.6)	89.5 (1.8)	96.7 (3.9)	92.9 (2.3)	94.0 (0.6)	83.2 (3.4)	92.2 (2.5)	93.3 (3.0)	91.6 (2.7)	97.1 (1.5)	100.6 (2.4)	96.0 (2.2)	87.5 (3.0)
5 µm + DS3b	92.4 (2.3)	87.7 (6.7)	98.8 (0.6)	94.1 (2.8)	96.6 (3.5)	96.5 (3.6)	98.0 (2.8)	89.3 (2.9)	94.4 (2.0)	100.8 (2.7)	95.8 (1.9)	99.0 (2.2)	101.3 (4.4)	99.8 (5.0)	81.9 (9.0)
45 µm + DS3b	93.8 (0.3)	82.5 (4.7)	104.8 (1.1)	98.1 (0.8)	95.8 (3.5)	97.8 (6.3)	98.8 (2.1)	95.1 (0.8)	99.2 (1.0)	101.8 (1.0)	100.3 (1.0)	101.8 (0.8)	100.8 (2.1)	94.3 (2.0)	74.1 (2.6)

Description solvents compositions: DS1a = isopropanol/water/formic acid (80/19.9/0.1, v/v); DS1n = isopropanol/water (80/20, v/v); DS1b = isopropanol/water/ammonium hydroxide (80/19.9/0.1, v/v); DS2a = acetonitrile/water/formic acid (80/19.9/0.1, v/v); DS2n = acetonitrile/water (80/20, v/v); DS2b = acetonitrile/water/ammonium hydroxide (80/19.9/0.1, v/v); DS3a = methanol/water/formic acid (80/19.9/0.1, v/v); DS3n = methanol/water (80/20, v/v); DS3b = methanol/water/ammonium hydroxide (80/19.9/0.1, v/v).

Table S4. Essential physicochemical proprieties of the analysed substances. *Substances arranged by retention order.*

substance	monoisotopic mass [Da] [PubChem 2.1][1]	logP [XLogP3.0][1]	logP [ALOGPS][2,4]	logP [ChemAxon][1,3]	logP [ACD/Labs][4]	logD pH=7.4 [ACD/Labs][4]	polar surface area [Å²] [Cactvs 3.4.6.11][1]	polar surface area [Å²] [ChemAxon][1,3]	polar surface area [Å²] [ACD/Labs][1]	polarizability [Å3] [ChemAxon][1,3]	polarizability [Å3] [ACD/LABS][4]	pKa (strongest acidic) [ChemAxon][1,3]	pKa (strongest basic) [ChemAxon][1,3]	H acceptors [Cactvs 3.4.6.11][1]	H donors [Cactvs 3.4.6.11][1]	H acceptors [ChemAxon][1,3]	H donors [ChemAxon][1,3]	H acceptors [ACD/Labs][4]	H donors [ACD/Labs][1]
fenoterol	303.147058	2.0	1.36	1.47	0.89	-0.45	93.0	92.95	93	31.75	33.7	8.85	9.63	5	5	5	5	5	5
carteolol	292.178693	1.0	1.05	1.42	1.35	-0.24	70.6	70.59	71	32.79	32.3	13.41	9.76	4	3	4	3	5	3
oxycodone	315.147058	1.2	1.04	1.03	1.67	0.45	59.0	59.00	59	32.79	33.0	13.57	8.77	5	1	5	1	5	1
hydrocodone	299.152144	2.2	2.13	1.96	1.83	0.17	38.8	38.77	39	32.05	32.3	18.00	8.61	4	0	4	0	4	0
ketamine	237.092042	2.2	2.69	3.35	2.18	2.07	29.1	29.10	29	24.97	26.1	18.78	7.45	2	1	2	1	2	1
remifentanil acid	362.184172	-0.7	N/A	N/A	1.58	-1.14	87.2	N/A	87	N/A	38.1	N/A	N/A	6	1	N/A	N/A	7	1
metoprolol	267.183444	1.9	1.80	1.76	1.79	-0.25	50.7	50.72	51	31.90	30.6	14.09	9.67	4	2	4	2	4	2
6-acetylcodeine	341.162708	1.7	N/A	N/A	2.09	1.12	48.0	N/A	48	N/A	36.6	N/A	N/A	5	0	N/A	N/A	5	0
methylphenidate	233.141579	0.2	1.47	2.25	2.55	0.26	38.3	38.33	38	26.21	26.4	N/A	9.09	3	1	2	1	3	1
zolpidem	307.168462	2.5	3.15	3.02	3.07	3.06	37.6	37.61	38	35.06	37.1	N/A	5.65	2	0	2	0	4	0
cocaine	303.147058	2.3	1.97	2.28	3.08	1.22	55.8	55.84	56	32.36	32.2	N/A	8.85	5	0	3	0	5	0
LSD	323.199762	3.0	3.30	2.28	2.74	2.60	39.3	39.34	39	37.54	38.5	17.02	7.98	2	1	2	1	4	1
melatonin	232.121178	0.8	1.42	1.15	0.96	1.74	54.1	54.12	54	25.65	26.8	15.80	-1.60	2	2	2	2	4	2
bisoprolol	325.225308	1.9	2.30	2.20	2.14	0.12	60.0	59.95	60	38.50	36.7	14.09	9.67	5	2	5	2	5	2
phencyclidine	243.198700	3.6	5.31	4.49	4.89	3.66	3.2	3.24	3	29.66	30.4	N/A	10.56	1	0	1	0	1	0
cortisol	362.209324	1.6	1.79	1.28	1.43	1.66	94.8	94.83	95	39.45	37.9	12.59	-2.80	5	3	5	3	5	3
buprenorphine	467.303559	5.0	4.53	3.55	3.43	3.48	62.2	62.16	62	53.11	52.1	7.50	12.54	5	2	5	2	5	2
alprazolam	308.082874	2.1	2.23	2.37	2.50	2.63	43.1	43.07	43	32.22	35.0	18.30	5.08	3	0	3	0	4	0
anastrozole	293.164046	2.1	2.31	3.03	0.97	2.68	78.3	78.29	78	31.97	35.7	N/A	2.00	4	0	4	0	5	0
methadone	309.209264	3.9	4.14	5.01	4.20	2.80	20.3	20.31	20	36.28	38.0	19.79	9.12	2	0	2	0	2	0
11-deoxycortisol	346.214409	2.5	2.97	2.58	2.74	2.67	74.6	74.60	75	38.86	37.3	12.59	-3.30	4	2	4	2	4	2
boldenone	286.193280	3.5	3.08	3.36	3.50	3.31	37.3	37.30	37	33.26	32.9	18.86	-0.88	2	1	2	1	2	1
clonazepam	315.041069	2.4	2.76	3.15	2.34	2.53	87.3	87.28	87	29.59	32.2	11.89	1.86	4	1	4	1	6	1
agomelatine	243.125929	2.7	2.83	2.04	2.27	3.02	38.3	38.33	38	27.18	29.0	15.96	-0.94	2	1	2	1	3	1
methandienone	300.208930	3.6	3.55	3.64	4.04	3.76	37.3	37.30	37	35.09	34.8	18.86	-0.53	2	1	2	1	2	1
flunitrazepam	313.086269	2.1	2.20	2.55	1.44	2.03	78.5	78.49	78	29.60	32.4	N/A	1.70	5	0	4	0	6	0
androstenedione	286.193280	2.7	2.93	3.93	2.90	2.90	34.1	34.14	34	33.20	32.4	19.03	-4.80	2	0	2	0	2	0
canrenone	340.203845	2.7	2.79	3.60	2.99	2.50	43.4	43.37	43	38.67	37.8	N/A	-4.80	3	0	2	0	3	0
progesterone	314.224580	3.9	3.58	4.15	4.04	3.72	34.1	34.14	34	37.26	36.1	18.47	-4.80	2	0	2	0	2	0
THC-COOH	344.198759	6.3	5.24	5.14	6.21	3.07	66.8	66.76	67	39.41	38.4	4.21	-4.90	4	2	4	2	4	2

References:
1 PubChem database, https://pubchem.ncbi.nlm.nih.gov/, (accessed March 31, 2021).
2 DrugBank database, https://www.drugbank.com/, (accessed March 31, 2021).
3 The Human Metabolome Database, https://hmdb.ca/, (accessed March 31, 2021).
4 ChemSpider database, http://www.chemspider.com/, (accessed March 31, 2021.

Table S5. List of reference standards in alphabetical order.

substance	reference standard	grade	manufacturer
(-)-11-nor-9-carboxy-delta9-THC (THC-COOH)	MeOH solution 1 mg/mL	CRM (primary standard)	LGC
6-acetylcodeine	ACN solution 1 mg/mL	CRM (primary standard)	Cerillant
11-deoxycortisol	MeOH solution 1 mg/mL	CRM (primary standard)	Cerillant
agomelatine	MeOH solution from powder 1 mg/mL	N/A	TRC
alprazolam	MeOH solution 1 mg/mL	CRM (primary standard)	LGC
anastrozole	MeOH solution from powder 1 mg/mL	reference standard (100%)	LGC
androstenedione	ACN solution 1 mg/mL	CRM (primary standard)	Cerillant
bisoprolol	MeOH solution 1 mg/mL	CRM (primary standard)	LGC
boldenone	MeOH solution from powder 1 mg/mL	analytical standard (≥ 98%)	VETRANAL™ (Sigma-Aldrich)
buprenorphine	MeOH solution 1 mg/mL	CRM (primary standard)	Cerillant
canrenone	MeOH solution from powder 1 mg/mL	HPLC (≥ 97%)	Sigma
carteolol	MeOH solution from powder 1 mg/mL	USP reference standard (100%)	USP
clonazepam	MeOH solution 1 mg/mL	CRM (primary standard)	Cerillant
cocaine	ACN solution 1 mg/mL	CRM (primary standard)	LGC
cortisol	MeOH solution from powder 1 mg/mL	N/A	N/A
fenoterol	MeOH solution from powder 1 mg/mL	reference standard (99.9%)	LGC
flunitrazepam	MeOH solution 1 mg/mL	CRM (primary standard)	LGC
hydrocodone	MeOH solution 1 mg/mL	CRM (primary standard)	Cerillant
ketamine	MeOH solution 1 mg/mL	CRM (primary standard)	LGC
lysergic acid diethylamide (LSD)	ACN solution 1 mg/mL	CRM (primary standard)	LGC
melatonin	MeOH solution from powder 1 mg/mL	TLC (≥98%)	Sigma
methandienone	1,2-dimethoxyethane solution 1 mg/mL	CRM (primary standard)	Cerillant
methadone	MeOH solution 1 mg/mL	CRM (primary standard)	Cerillant

methylphenidate	MeOH solution 1 mg/mL	CRM (primary standard)	LGC
metoprolol	MeOH solution 1 mg/mL	CRM (primary standard)	LGC
oxycodone	MeOH solution 1 mg/mL	CRM (primary standard)	LGC
phencyclidine	MeOH solution 1 mg/mL	CRM (primary standard)	LGC
progesterone	MeOH solution from powder 1 mg/mL	N/A	N/A
remifentanil acid	ACN solution 100 µg/mL	CRM (primary standard)	Cerillant
zolpidem	MeOH solution 1 mg/mL	CRM (primary standard)	LGC
deuterium-labelled internal standards			
alprazolam D_5	MeOH solution 100 µg/mL	CRM (primary standard)	Cerillant
cocaine D_3	ACN solution 100 µg/mL	CRM (primary standard)	Cerillant
oxycodone D_3	MeOH solution 100 µg/mL	CRM (primary standard)	Cerillant
(-)-11-nor-9-carboxy-delta9-THC D_3 (THC-COOH D_3)	MeOH solution 100 µg/mL	CRM (primary standard)	Cerillant

Table S6. Monitored precursor – product ion(s) transitions. *Substances arranged by retention order.*

substance	retention time [min]	precursor ion [m/z]	product ions [m/z]:		
			1	2	3
fenoterol	4.156	304.00	107.15	135.15	286.10
carteolol	4.499	293.00	237.15	202.15	74.10
oxycodone D_3	4.532	319.00	301.20	244.10	259.20
oxycodone	4.567	316.10	298.20	241.20	256.10
hydrocodone	4.914	300.00	199.15	171.10	128.15
ketamine	5.711	237.90	125.05	220.10	207.15
remifentanil acid	6.755	363.00	113.10	146.20	214.15
metoprolol	6.499	267.90	116.15	74.15	72.10
6-acetylcodeine	6.911	342.00	225.15	165.15	197.20
methylphenidate	6.765	234.00	84.15	56.10	91.10
zolpidem	7.537	307.90	235.15	236.20	263.20
cocaine	7.501	303.90	182.20	82.10	105.10
cocaine D_3	7.503	307.00	185.20	77.10	85.20
LSD	7.844	323.90	223.20	208.15	207.10
melatonin	8.048	232.90	174.20	130.15	159.10
bisoprolol	8.018	326.20	116.20	74.05	72.10
phencyclidine	8.828	244.00	91.05	86.10	159.20
cortisol	9.490	363.20	121.00	327.20	105.00
buprenorphine	9.891	468.10	55.15	396.25	414.25
alprazolam D_5	11.420	314.10	210.20	286.10	279.25
alprazolam	11.484	308.90	205.15	281.15	274.10
anastrozole	11.506	294.00	225.20	210.20	115.05
methadone	11.622	310.00	265.15	105.10	77.05
11-deoxycortisol	11.617	347.15	97.10	109.05	79.05
boldenone	12.042	287.00	121.20	135.20	77.15
clonazepam	12.062	316.00	270.10	214.05	207.15
agomelatine	12.210	244.00	185.20	170.10	141.10
methandienone	12.691	301.20	121.05	149.25	77.00
flunitrazepam	12.851	313.90	268.15	239.10	183.10
androstenedione	14.570	287.30	97.05	109.05	78.95
canrenone	15.153	341.00	107.15	187.25	235.15
progesterone	17.880	315.00	97.10	109.10	297.30
THC-COOH D_3	19.956	348.00	330.20	302.25	196.25
THC-COOH	19.982	345.00	327.10	299.25	193.30

9. Nowe rozwiązania w mikroekstrakcji (część 2): Zastosowanie technologii druku 3D oraz polimerów adsorpcyjnych

Kolejnymi aspektami, których udoskonalenie może korzystnie wpłynąć na popularyzację danej metody przygotowania próbek są m.in.: łatwość wykonania oraz niski koszt. W przypadku metody TFME, jej zastosowanie jest stosunkowo proste, a urządzenia ekstrakcyjne można wykorzystać wielokrotnie (w związku z czym koszt zastosowania tej metody jest relatywnie niski) [98]. Niestety, sama produkcja urządzeń TFME jest żmudnym i czasochłonnym procesem [107], który w dodatku jest stosunkowo kosztowny.

Częściowym rozwiązaniem tego problemu jest produkcja tańszych zamienników niektórych komponentów urządzeń mikroekstrakcyjnych z wykorzystaniem technologii druku 3D. Jednak zupełnie przełomowa może być możliwość wykorzystania w dziedzinie preparatyki próbek najnowszych osiągnięć druku 3D, m.in. wprowadzenia nowych polimerowych materiałów do druku 3D (w postaci tzw. filamentów). W szczególności zaś, obiecujące perspektywy ma zastosowanie materiałów powszechnie stosowanych w chemii analitycznej jako adsorbenty (np. poliamidy [113-116]) do wykonania urządzeń ekstrakcyjnych wyłącznie z wykorzystaniem druku 3D. Jest to rozwiązanie zarówno proste w realizacji, jak i mało kosztowne, a także wysoce zautomatyzowane (a więc również wysoce powtarzalne).

Innowacyjne badania nad zastosowaniem druku 3D do przygotowania urządzeń ekstrakcyjnych zostały zaprezentowane w ramach następujących publikacji:

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Article

Polyamide Noncoated Device for Adsorption-Based Microextraction and Novel 3D Printed Thin-Film Microextraction Supports

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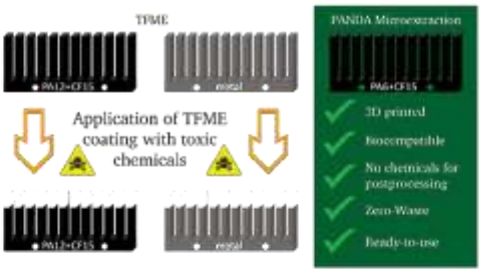
ABSTRACT: Polyamide noncoated device for adsorption-based microextraction (PANDA microextraction) is a brand new, easy to prepare, environmentally friendly, inexpensive, and efficient sample preparation method created entirely with the use of 3D printing. The proposed method is based on the extractive proprieties of the unmodified polyamide and carbon fiber blends and is compared with the highly selective thin-film microextraction (TFME). In addition, 3D printing was used to simplify the process of TFME. Prototype sample preparation devices were evaluated by the extraction of oral fluid spiked with 38 small molecules with diverse chemical natures, such as lipophilicity in the log *P* range of 0.2−7.2. The samples were analyzed by high-performance liquid chromatography coupled with tandem mass spectrometry. The results indicate that chemically and thermally resistant 3D printed supports can be successfully used as a cost-saving, environmentally friendly solution for the preparation of TFME devices, alternative to the conventional metal supports, with only marginal differences in the extraction yield (mean = 4.0%, median = 1.8%, range = 0.0−22.3%, n = 38). Even more remarkably, in some cases, the newly proposed PANDA microextraction method exceeded the reference TFME in terms of the extraction efficacy and offered excellent sample cleanup as favorable matrix effects were observed (mean = −8.5%, median = 7.5%, range = −34.7−20.0%, n = 20). This innovative approach paves the road to the simplified sample preparation with the use of emerging extractive 3D printing polymers.



3D printing emerged in the late 1980s when Charles Hull patented the Standard Tessellation Language (with the .stl file format) for the transmission and processing of 3D data files to a self-prepared prototype of a 3D printer based on stereolithography (SLA).[1] However, it was only after Michael Cima and Emanuel Sachs incorporated fused deposition modeling (FDM), the invention of Scott Crump,[2] into their 3D printing system that the technology was fast-tracked to mainstream use. FDM owes its success to its affordability and compatibility with an unparalleled plethora of polymers that are readily prepared as spooled filaments. The described method relies on heating the filaments to their melting point and applying the semisolid polymers layer by layer to create the designed prototype. In addition, FDM is appreciated for providing good reproducibility, as well as for the chemical and mechanical resistance of the final products. Another significant benefit is the ability to freely and instantly modify the shape and size of the prototype, all at the relatively low cost of the commercially available filaments. This multitude of benefits has resulted in the rapid expansion of FDM 3D printing into new fields, including analytical chemistry and sample preparation.[3−5]

Most often, sample preparation is a critical part of the analytical protocol and is necessary for the attainment of high-quality and unbiased results. Thus, the benefits of implementing 3D printing into analytical methodology are rapidly gaining increasing interest, well mirrored by the number of studies indexed by the phrase "3D printed" in the Web of Science database. However, papers published on the extraction devices that were prepared exclusively by the 3D printing method are still scarce. Some especially interesting examples of the fully 3D printed prototypes include the study of Su et al., who demonstrated the application of polyacrylate for the binding of trace elements in seawater to the solid-phase extraction (SPE) preconcentrator,[6] an idea further continued by the authors with various polyurethane-based prototypes.[7] Another research group proposed 3D printed LAYFOMM-60 (CC-Products, Germany) as a stationary phase for the extraction of small molecules.[8] LAYFOMM-60 is a polyurethane-based thermoplastic containing water-soluble polyvinyl alcohol (PVA) that needs to be eluted with water after printing, for example, to increase the surface porosity. Published applications include the extraction of the antidiabetic drug

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glimepiride,[8] extraction of endo- and exogenous steroids from plasma and phosphate-buffered saline,[9,10] and extraction of arylpiperazine derivates of anxiolytic drugs.[11]

Another interesting idea was pursued with polybutylene terephthalate (PBT), a type of 3D printable thermoplastic material. Although not 3D printed by the authors of this study, it was proposed as a supporting material for coating with microextraction stationary phases due to its good chemical resistance and biocompatibility.[12] PBT fibers and blades were coated with a polyacrylonitrile hydrophilic lipophilic balance (PAN-HLB) stationary phase and evaluated for the extraction of 17 doping agents from blood plasma, urine, and whole blood with good results. However, until recently, there were significant impediments that prevented the straightforward implementation of 3D prototyping in the development of microextraction-based sample preparation methods, especially with regular FDM 3D printers. The reason for these impediments was simple, yet no obvious solution was available at the time. As established through extensive method development, a preferred method for the application of microextraction coatings is spray painting, the results of which are superior to dipping or brush painting,[13] but the preparation protocols necessitate the use of high temperature (at least 110 °C)[14] for curing the sprayed coatings. Therefore, this requirement of good thermal resistance and good chemical resistance to the strong organic solvents that are used in the process, such as *N*,*N*-dimethylformamide (DMF), significantly hindered the 3D prototyping of the microextraction supports due to the lack of compatible and 3D printable materials. For example, thin-film microextraction (TFME) supports 3D printed from PBT would not be able to withstand the temperature of 125 °C that is used for coating with PAN-HLB[12] or the heat wave encountered when entering the oven because of the diminished heat deflection temperature (HDT). Fortunately, this obstacle can now be overcome with recently commercialized thermoplastics such as carbon fiber-reinforced polyamides (PA + CF). These emerging biocomposites can be obtained from lignocellulosic biomass[15] and decomposed with gentle solvent treatment utilizing nonhazardous reagents,[16] ensuring their sustainability in addition to their already proven biocompatibility.[17,18] Moreover, neat polyamide 6 was previously reported as a stationary phase used in SPE columns for on-line sample preparation preceding the instrumental analysis[19,20] and for the preparation of headspace solid-phase microextraction fibers.[21,22] The versatility and applicability of this polymer were additionally demonstrated by the authors through its application for the determination of bisphenol A contaminants in environmental waters,[23] various insecticides in soil and waters,[24] ochratoxin A in beer,[25] and resveratrol in wines.[26] Remarkably, polyamide 6 provided nearly superior results in comparison with the acclaimed octadecyl (C_{18}) stationary phase.[24] In addition, the superiority of the 3D structures prepared with polyamide 6 over the corresponding 2D structures was shown for the extraction of chlorobenzenes.[22] However, it should be underlined that although polyamide 6 was first synthesized in 1938,[27] all of the aforementioned studies used electrospun fibers, and until recently, the polyamides were not available as 3D printing filaments.

Moreover, the introduction of polyamides as 3D printing filaments enables pursuing more environmentally aware interests, parallel to the focus on developing and improving the analytical solutions. The principles of green analytical chemistry, emphasizing aspects such as organic solvent consumption reduction, design enabling degradation, and process sustainability, may now be impeccably implemented by combining the benefits of 3D printing and microextraction sample preparation techniques. Microextraction methods such as TFME facilitate low-volume sample analysis by combining extraction with preconcentration (occurring during the desorption step) into a single analytical protocol. Reduced sample loading with microextraction methods results in decreased organic solvent consumption in comparison with concurrent sample preparation techniques.[28,29] Additionally, the portability and biocompatibility of microextraction methods grants unparalleled ability to perform direct on-site sampling in environmental research or in vivo sampling in medical studies, allowing simultaneous sampling and sample preparation.[30] With ex vivo applications, biocompatibility is not only a trendy catch phrase but also has a direct impact on extraction efficacy. With biocompatible microextraction methods, extraction of the analytes from complex matrices such as blood, oral fluid, or plasma without coextraction of undesired macromolecules is possible[31] owing to the absence of peptide and protein adsorption to the stationary phase.[32,33] These characteristics, in conjugation with low laboratory waste production and potential for reusability of the extraction devices,[34] demonstrate the unambiguous benefits of green analytical chemistry resulting from the replacement of the traditional sample preparation methods with microextraction while still offering comparable extraction performance.[28,29]

Furthermore, direct adsorption of the analytes to biocompatible 3D printed microextraction devices prepared with sustainable biocomposites without additional laborious pre- or postprocessing offers an unprecedented opportunity to capitalize on the benefits of microextraction techniques while simultaneously eliminating the use of any harmful reagents. For comparison, the preparation of relatively green TFME coatings still regrettably requires the use of highly toxic concentrated hydrochloric acid and DMF, a potential carcinogen and teratogen.[13]

Building upon these possibilities, we aimed to fulfill the following goals:

(1) obtain affordable and biocompatible 3D printed support for TFME devices, characterized by good chemical and thermal resistance;

(2) prepare efficient and sustainable extraction devices with 3D printed biocomposites, sparing laborious pre- or postprocessing with harmful chemicals.

Two promising blends of polyamides (nylons) with carbon fiber were selected based on their biocompatibility, high HDT, and sustainable production: polyamide 6 + carbon fiber 15% (PA6 + CF15) and polyamide 12 + carbon fiber 15% (PA12 + CF15). To the best of our knowledge, the present study introduces 3D printed TFME supports and 3D printed ready-to-use polyamide noncoated device for adsorption-based microextraction (PANDA microextraction), which do not require pre- or postprocessing with any hazardous chemical agents, for the first time.

EXPERIMENTAL SECTION

Preparation of Microextraction Devices. The blades used as supports for the TFME coatings were prepared from precut metal sheets (PAS Technology, Germany) and by 3D printing with a FDM method. All the supports had equal

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dimensions and shapes of 96-well-compatible 12-pin blades to ensure equal areas of the applied TFME coatings. Devices in the newly proposed PANDA microextraction format were prepared exclusively with 3D printing and had the same shape and size as the TFME supports.

The 3D designs were prepared in Blender version 2.82 (Free Software Foundation, Inc.) as .stl files, then sliced and converted to printer-compatible .gcode files in PrusaSlicer (Prusa Research, Czech Republic), and prototyped with a Prusa i3 MK2 printer (Prusa Research, Czech Republic) from two different types of polyamide and carbon fiber blends: 1.75 mm PA6 + CF15 (Spectrum Industrial, Spectrum Group, Poland) and 1.75 mm PA12 + CF15 (Fiberlab, Fiberlogy, Poland). The printer was fitted with a double-sided textured polyetherimide (PEI) powder-coated spring steel sheet (Prusa Research, Czech Republic) and ruby nozzle (BROZZL, Schimautz GmbH, Austria) for a 0.4 mm E3D V6 hot end. As recommended by the manufacturer, the PA6 + CF15 filament was conditioned for 2 h in an oven set at 75 °C before use. The following parameters were used for the printer: 15% linear infill on the pins of the prototypes, 15% 45° triangular infill for the remaining part of the prototypes, a heat bed temperature of 90 °C, a nozzle temperature of 260 °C, a height of 0.2 mm for the first layer, and a height of 0.05 mm for the remaining layers. A three-layer skirt outline was used. The printing speeds were 20 mm s^{-1} for the first layer, 45 mm s^{-1} for perimeters, 25 mm s^{-1} for small perimeters, 80 mm s^{-1} for solid infill, 40 mm s^{-1} for top solid infill, 30 mm s^{-1} for bridges, and 40 mm s^{-1} for the gap fill.

The metal blades were etched in concentrated hydrochloric acid (Fluka, Honeywell) for 60 min in an ultrasonic bath to increase their surface porosity. After cleaning with distilled water, the blades were dried in an oven set at 150 °C for 30 min.

A TFME coating was prepared by dispersing 10 μm C_{18}-bonded silica particles with polar end-capping groups (Synergi Hydro-RP, Phenomenex) in DMF (Sigma-Aldrich, Merck Group) solution of PAN (Aldrich, Merck Group). One centimeter of the coating was applied on the tips of the blades, each consisting of 10 layers of coating slurry, utilizing a nitrogen-operated sprayer and a previously established protocol.[13] Each layer was dried for 3 min in an oven set at 110 °C immediately after application. This temperature was previously determined to be optimal for the process.[14]

Extraction Method. An extraction device was created by combining eight 12-pin blades to form a 96-pin brush compatible with 96-well 2 mL DeepWell plates (Nunc, Thermo Scientific). The experiments were performed with a semiautomatic plate-compatible benchtop SH10 Heater-Shaker (Ingenieurbüro CAT, Germany). Protocol included preconditioning in methanol/water (50/50, v/v; 1.5 mL, 60 min, 850 min^{-1} agitation); first rinse with water (1.5 mL, 5 s, no agitation); extraction from spiked oral fluid (1 mL, 2.5 h, 850 min^{-1} agitation); second rinse with water (1.5 mL, 5 s, no agitation); and desorption to methanol/water/formic acid (80/19.9/0.1) containing deuterium-labeled reference standards at 5 μg L^{-1} concentration (1 mL, 2 h, 850 min^{-1} agitation). Formic acid (Optima, Fisher Chemical), methanol (CHROMASOLV, Honeywell), and water (LiChrosolv, Merck Group) were all LC−MS-grade reagents. All experiments were performed in quadruplicate.

HPLC-MS/MS Method. The extracts were analyzed by high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) on a Shimadzu LCMS-8060 triple quadrupole. The chromatographic method for the Agilent InfinityLab Poroshell 120 EC-C18 column (3 × 100 mm, 2.7 μm) fitted with a guard column (3 × 5 mm, 2.7 μm) was based on gradient elution with acetonitrile (CHROMASOLV, Honeywell; LC−MS grade) and water (LiChrosolv, Merck; LC−MS grade) as the mobile phases and was previously used for the separation of similar solutes.[35] The gradient program began with 10% acetonitrile maintained for 0.5 min, succeeded by a linear increase to 100% at 26 min mark; 100% acetonitrile was maintained for 3 min, followed by rapid drop to 10% for column re-equilibration for the next 6 min. In total, the gradient program took 35 min per sample. Both mobile phases contained 0.1% formic acid, the total flow rate was 300 μL min^{-1}, the injection volume was 0.7 μL, and the column temperature was maintained at 25.0 °C. The retention times and precursor−product ion transitions are listed in Table S1 in the Supporting Information.

Oral Fluid Collection and Reference Standards. Oral fluid samples were obtained from two healthy volunteers (female aged 24 and male aged 27) in accordance with applicable regulations. These volunteers declared no previous use of the analyzed substances. The samples were pooled together to obtain a uniform matrix and spiked with a mixture of 38 reference standards, each at a 50 μg L^{-1} concentration. The spiked matrix was mixed on a benchtop shaker and stored for 60 min at room temperature to allow drug−protein binding.

Reference standards of the 38 various small molecules (log *P* calculated with the XLogP3.0 program is in the range of 0.2−7.2, and molecular masses are in the range of 149.12−528.24 Da)[36] were purchased from LGC Standards (LGC Poland) and Sigma-Aldrich (Sigma-Aldrich Poland) as ready-to-use 1 g L^{-1} stock solutions or prepared by dissolving the powder in LC−MS-grade methanol. Deuterium-labeled reference standards of the 20 analytes were purchased from the same suppliers as 100 mg L^{-1} stock solutions or prepared from powder. A full list of reference standards is presented in Table S2 in the Supporting Information.

RESULTS AND DISCUSSION

This study compared the newly proposed format of PANDA microextraction with three TFME devices. Each of these TFME devices comprised three elements: a support (for the application of the coating layers), a PAN binder, and C_{18}-bonded silica particles. Water-compatible polar end-capped particles were used for this study as they were previously determined to be more suitable for the aqueous samples than the conventional trimethylsilane end-capped particles.[37] Three materials were tested as TFME supports: conventional precut metal, PA6 + CF15, and PA12 + CF15. In addition, the isolated impact of every element of the TFME devices on the extraction efficacy was investigated. Extensive results for all analytes and every factor further disclosed in this paper can be found in Table S3 in the Supporting Information.

Data Quality. All of the 38 analyzed small molecules were successfully extracted and quantified with the HPLC-MS/MS method using both TFME and PANDA microextraction as the sample preparation techniques. The linearity and sensitivity of the HPLC-MS/MS system were verified with calibration runs of a drug-spiked desorption solvent, which resulted in at least 7-point calibration curves. The mean coefficient of determination calculated with $1/a^2$ weighting was R^2 = 0.9998. The

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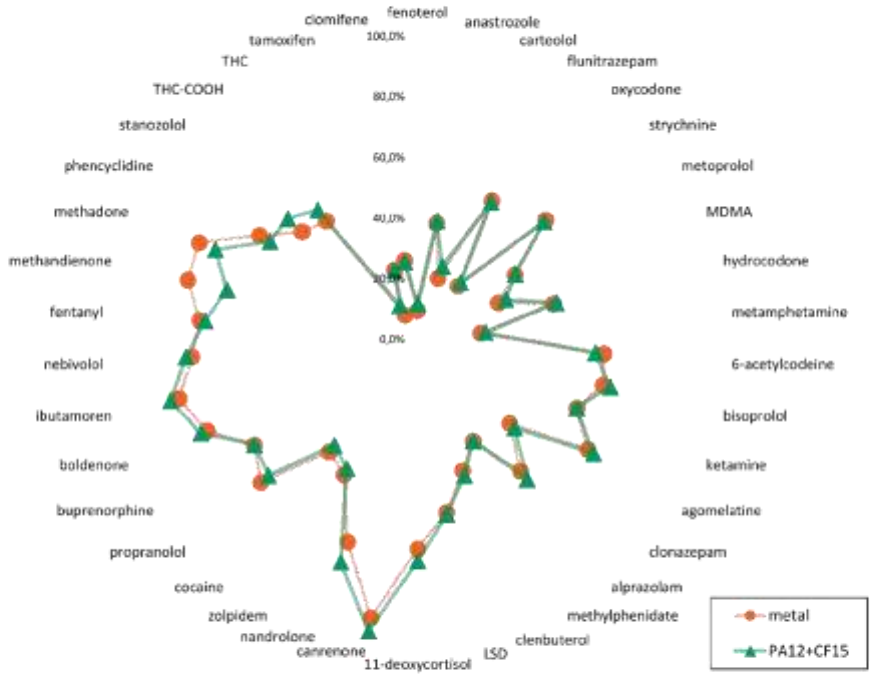

Figure 1. Extraction efficacies of TFME devices prepared with different support materials. Substances arranged clockwise by their log *P* value.

lowest recorded values were R^2 = 0.9974 for nandrolone and R^2 = 0.9981 for stanozolol. The calibration runs were performed in the 1−75 μg L^{-1} concentration range for every analyte with the exception of three steroid drugs (canrenone, nandrolone, and stanozolol), for which the quantification range was 5−75 μg L^{-1}. Lower ends of the ranges were compared with previously reported limits of quantification (LOQs) for TFME methods,[38−41] with similar or superior results obtained in this study. Full comparison is presented in Table S4 in the Supporting Information. The stability of the instrument was monitored by the system suitability test (SST) samples run in duplicate at regular intervals of 10 samples. The mean relative standard deviation (RSD) for 30 consecutive SST samples was 5.7% (median = 5.3%, min = 3.4%, max = 10.5%, *n* = 38).

Adsorption to Noncoated TFME Supports. The method of 3D printing with FDM was shown to be perfectly suited for the preparation of TFME supports. Due to the small diameter of the extrudate that is squeezed through the nozzle, small objects such as pins of the TFME blades are composed of several parallel bundles of molten and resolidified filaments. This structure provides a porous but highly reproducible surface that does not require preprocessing with concentrated hydrochloric acid before application of the TFME coating.

Neither metal nor PA12 + CF15 adsorbs the analytes well, and this trait is desirable for the support materials. In contrast, PA6 + CF15 provides good extraction efficacy and is described in subsequent paragraphs as an alternative extraction device (PANDA microextraction) rather than a support material.

For both the metal and PA12 + CF15, the amount of the extracted analyte (from nonspecific binding) was on average just 2.8% (*n* = 38). For PA12 + CF15, the extracted amounts were in the range of 0.4−13.1% (*n* = 31), but only in the case of nine analytes were they sufficient for quantification. In the case of the metal, the extracted amounts were in the range of 0.0−21.7% (*n* = 33), but these amounts were sufficient only for the quantification of 12 of the analytes. However, two drugs (nebivolol and stanozolol) were obvious outliers contributing to the significant increase in the observed mean values. For comparison, the median values of the extracted amounts were only 0.7% (*n* = 31) for the metal and 1.2% (*n* = 33) for PA12 + CF15. Such compound-specific fluctuations, present only for a few of the analytes, exclude transfer of the small fraction of the original sample (a droplet) on the extraction device as an explanation for these results.

Adsorption to noncoated surfaces is, however, dependent on the analytes' hydrophobicity. Below a log *P* value of 2.7 (*n* = 24),[36] no recorded result was above the LOQ for the PA12 + CF15, and only two such results were observed for the metal [for ibutamoren (2.6%) and strychnine (2.3%)]. Above a log *P* value of 4.5 (*n* = 6),[36] every analyte can be extracted, allowing its quantification with both noncoated supports (although with relatively poor efficacy).

Adsorption to the PAN Binder. PAN is widely used as a biocompatible binder for immobilizing particles comprising the stationary phase of TFME devices. As such, it exhibits only weak adsorptive properties toward small molecules. Therefore, as expected, the extraction efficacies of the TFME supports coated with PAN (without C_{18}-bonded particles) were marginal.

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Table 1. Extraction Efficacies of Fenoterol and THC from Oral Fluid with Selected Microextraction Devices[a]

	extraction substance			
device	PANDA microextraction (noncoated PA6 + CF15)	C_{18}-coated TFME on the PA6 + CF15 support	C_{18}-coated TFME on the PA12 + CF15 support	C_{18}-coated TFME on the metal support
fenoterol	28.6% (1.8%)	19.0% (4.0%)	11.3% (2.0%)	9.2% (1.3%)
THC	12.9% (4.9%)	11.2% (3.1%)	12.2% (7.2%)	8.7% (5.4%)

[a]Corresponding relative standard deviations given in parentheses.

For PAN-coated metal supports, the mean extracted amount was just 3.1%. Out of 38 analytes, 3 were not detected, and 23 were below their LOQs. Therefore, only 12 analytes could be quantified with a mean extraction yield of 7.5%.

PAN-coated PA12 + CF15 supports delivered similar results. The mean extracted amount was 2.4%, with 9 analytes below the limit of detection (LOD), 20 below the LOQ, and only 9 analytes extracted in quantifiable amounts with a mean extraction yield of 5.1%.

In contrast, the PAN-coated PA6 + CF15 supports were characterized by significantly greater extraction efficacies with an average of 11.1%. No analytes were below the LOD, and only one (oxycodone) was below the LOQ. However, it should be noted that noncoated PA6 + CF15 (PANDA microextraction) exhibited significant adhesion of the small molecules, and the PAN coating only decreased the extraction efficacy by an average of 10.3% (min = 0.9%, max = 29.9%, n = 38). One centimeter of the coating was applied. Therefore, a small fraction of the support was immersed in the sample during extraction due to the applied agitation.

Once again, it was evident that 2 out of 38 analytes were outliers prone to nonspecific binding regardless of the contact surface. For stanozolol (log P = 4.5),[36] the amount extracted with the PAN-coated metal was 18.2% (and 18.0% for noncoated metal), with a PAN-coated PA12 + CF15 extracted amount of 8.6%—falling below the LOD (and 11.1% for noncoated PA12 + CF15). For nebivolol (log P = 3.0),[36] the extracted amount with the PAN-coated metal was 25.4% (21.7% for noncoated metal) and with the PAN-coated PA12 + CF15, the extracted amount was 10.3% (13.1% for noncoated PA12 + CF15).

Impact of the TFME Support Material on the Extraction Efficacy. All compared materials (metal and both PA + CF blends) were shown to be equivalent alternatives as supports for TFME coatings, providing very similar extraction efficacies and good reproducibility of the results.

The mean TFME efficacy with the metal support was only 0.3% greater than that with the PA6 + CF15 support (median = −0.8%, min = −12.8%, max = 25.0%, n = 38) and only 1.0% smaller than that with the PA12 + CF15 support (median = −0.1%, min = −22.3%, max = 20.7%, n = 38).

The mean differences in the extraction efficacies were 5.1% (median = 3.3%, min = 0.1%, max = 25.0%, n = 38) between the metal and PA6 + CF15 and only 4.0% (median = 1.8%, min = 0.0%, max = 22.3%, n = 38) between the metal and PA12 + CF15. Therefore, the PA12 + CF15 supports provided results more similar to those of the metal supports than those of the PA6 + CF15 supports. Figure 1 demonstrates the equivalence of the extraction efficacies recorded with both metal and PA12 + CF15 TFME supports. Few of the distinct exceptions, for which an above-average differences between both support materials could be observed, include more hydrophobic analytes such as synthetic opioid methadone (difference in the extraction efficacy = 5.9%, log P = 3.9)[36] and three anabolic steroids: stanozolol (difference = 6.4%, log P = 4.5),[36] nandrolone (difference = 7.1%, log P = 2.6),[36] and methandienone (difference = 13.3%, log P = 3.6).[36]

The repeatability of the results recorded with all compared types of supports was very good, and only nonsignificant variations were observed. The mean RSD value for the metal support was 3.0% (median = 2.9%, min = 0.7%, max = 7.8%, n = 38); for PA6 + CF15, the mean RSD = 2.7% (median = 2.8%, min = 1.1%, max = 7.1%, n = 38); and the most favorable mean RSD value of less than 2.7% (2.68%) was recorded for PA12 + CF15 (median = 2.5%, min = 0.9%, max = 7.2%, n = 38).

PANDA Microextraction—Efficacy, Linearity, and Repeatability. In this study, all analytes could be sufficiently extracted (i.e., above their levels of quantification) by PANDA microextraction with very good reproducibility (mean RSD = 2.6%, median = 2.4%, min = 0.6%, max = 5.8%, n = 38). These low RSD values, lower than the numbers recorded for TFME devices, result from evading the necessity of manually spray-painting the TFME coatings.

Remarkably, in addition to its versatility in allowing sufficient extraction of all the analytes in this study, PANDA microextraction exceeds TFME devices with C_{18} coatings in terms of extraction efficacies of fenoterol (log P = 2.0)[36] and Δ^9-tetrahydrocannabinol (THC; log P = 7.0).[36] See Table 1 for details.

The extraction efficacies for the remaining 36 substances were in the range of 4.9−60.9%, but most importantly, they were always sufficient for the quantification of every analyte from a relatively small injection volume of 0.7 μL and without any additional sample processing (such as evaporation of the solvent for preconcentration of the sample). This potentially allows the application of this method for the extraction of less stable analytes.

In comparison with the C_{18}-coated TFME, PANDA microextraction provides adsorption of the analytes by the hydrophobic, hydrogen bonding, and dipole−dipole type interactions, rather than exclusively by the hydrophobic-type interactions such as octadecyl functional groups.[42] Nevertheless, both extractive phases are best suited for the extraction of similar substances, specifically the hydrophobic multicyclic structures [with boldenone (log P = 3.5), canrenone (log P = 2.7), ibutamoren (log P = 1.3), methandienone (log P = 3.6), nandrolone (log P = 2.6), nebivolol (log P = 3.0), and propranolol (log P = 3.0) as mutual examples].[36] In the case of PANDA microextraction, the best extraction efficacies were recorded for the substances in the log P range of 1.3−5.0 (with a mean log P value of 3.2, n = 10). With TFME, the best extraction efficacies were observed for analytes in the log P range of 1.3−4.0 (mean = 3.0, n = 10). PANDA microextraction performed worse only for the most hydrophilic ones of the target molecules, with the log P values in the range of 0.2−2.3 (mean = 1.8, n = 10). No such trend could be

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observed for TFME, with the worst results being for molecules with the log P values in the wide range of 1.0−7.2 (mean = 3.4, n = 10). Therefore, PANDA microextraction provided more consistent coverage of the analytes likely to the several unique adsorption mechanisms.

Adsorption and desorption to the PA6 + CF15 surface were determined as linear processes by preparing calibration curves (5−8 points, depending on extraction efficacy) from drug-spiked oral fluid samples with PANDA microextraction sample preparation in conjugation with HPLC-MS/MS analysis. The resulting coefficient of determination values, calculated with $1/a^2$ weighting, were in the R^2 = 0.9539−0.9995 range (mean = 0.9776, median = 0.9809, n = 36).

PANDA Microextraction and TFME—Sample Cleanup (Matrix Effect). In addition, sample cleanup provided by the PANDA microextraction method was compared with that of the TFME devices prepared with three alternative support materials under comparison in this study. The degree of sample cleanup was assessed based on the differences in the signal intensities of 20 deuterium-labeled internal standards (ISs) spiked to the desorption solvent. One batch of the spiked desorption solvent was used for all extractions and preparation of the SST sample. Therefore, it was possible to demonstrate a direct relationship between the differences observed in signals measured for ISs after extraction with a given device type and for SST (mean value from four SST samples, both preceding and succeeding the extracted samples). As all extraction protocols were uniform, the degree of sample cleanup provided by a given microextraction device was the only variable accounting for the differences observed in the IS signal intensities. The degree of sample cleanup affects MS detection and is generally referred to as the matrix effect.[43] In this study, negative matrix effect values signify signal suppression, while positive values result from signal enhancement.

For all of the compared microextraction devices, low average matrix effects were observed and only sporadically exceeded ±20% for certain drugs. All matrix effect values can be found in Table S5 in the Supporting Information. With regard to the C_{18}-coated TFME devices, devices with metal support provided a mean matrix effect of −10.3% (median = −10.3%, min = −17.1%, max = −1.8%, n = 20); with the PA6 + CF15 support, the mean value was −15.3% (median = −14.7%, min = −47.3%, max = 0.5%, n = 20); and with the PA12 + CF15 support, the mean value was −10.5% (median = −11.3%, min = −19.0%, max = −3.5%, n = 20). For PANDA microextraction, the mean matrix effect was −8.5%, with a median value of −7.5% and a range of −34.7−20.0%, n = 20.

Utilizing the matrix effect to correct for the extraction efficacies of the 20 matching pairs (analyte—the IS of the analyte), one may observe that the differences between the extraction efficacies of the TFME devices prepared with metal and alternative support materials decreased even further than previously described. For the C_{18}-coated PA6 + CF15, the mean difference decreased from 4.1% (median = 2.8%, min = 0.2%, max = 15.9%, n = 20) to 3.5% (median = 2.7%, min = 0.7%, max = 11.0%, n = 20). With the PA12 + CF15 support, the difference decreased from 2.7% (median = 1.4%, min = 0.1%, max = 17.4%, n = 20) to 2.4% (median = 1.3%, min = 0.2%, max = 13.0%, n = 20). Thus, additional argument for the preparation of TFME coatings with the PA12 + CF15 supports is given as its performance is similar to that prepared with the conventionally used metal supports.

Further Discussion. Undoubtedly, the most important part of a microextraction device is its extractive surface. PANDA microextraction, prepared entirely by 3D printing from a sustainable PA6 + CF15 blend, allows extraction by direct adsorption of the analytes to its surface. Moreover, no pre- or postprocessing with chemicals is required. Minor postprocessing only involves cutting out the remnants of the idle printer head movements (ca. 3 min, see Figure 2).

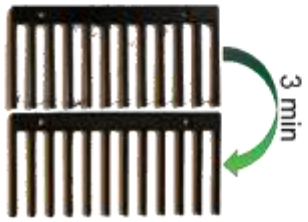

Figure 2. Easy and chemical-free preparation process of PANDA microextraction.

Similarly, microextraction supports 3D printed from PA12 + CF15 are ready for application of the coating without prior etching in hydrochloric acid and the laborious cleaning procedure and as a result, providing cost and time savings, as well as diminished environmental impact of the preparation process.

For comparison, the preparation of conventional TFME devices required approximately 2 h for preparation of the supports, 1 h for application of the coatings, and additional time for postprocessing of the coatings after spray painting, in total over 4 h.[13] In addition to the time consumed, the average cost of a single 12-pin TFME blade prepared for this study was 40.5 $ (33.5 $ for the coating slurry and 7 $ for the metal support) and harmful reagents such as concentrated hydrochloric acid and DMF were used in the process. With 3D printed polyamide-based TFME supports, the overall preparation time was reduced by approximately 1 h 40 min (from ca. 2 h to ca. 20 min for the preparation of the supports), and the cost was limited by 6.8 $ per single TFME blade (from 7 $ to just 0.2 $). With the presented savings, over 99% of the remaining cost is down to the cost of the coating itself. It is also worth mentioning that thanks to the identical shape, chemical and thermal resistance, as well as the adhesive and porous surface, the 3D printed supports can be chemically functionalized with any given type of microextraction coatings, just like the conventional metal supports.

In contrast to TFME, the complete process of PANDA microextraction took only 20 min (17 min for prototyping and 3 min for postprocessing), instead of over 4 h. The entire postprocessing method was chemical-free and comprised a simple single step of cutting out the remnants of the idle printer head movements. The total cost of PANDA microextraction preparation was 0.2 $ (for 0.63 m of the filament to create 2.04 g prototype of the 12-pin blade), over 200 times less than 40.5 $ for a single TFME blade. However, it must be emphasized that despite relatively high preparation/purchase costs, TFME devices are reusable, dividing the initial investment per multiple samples extracted. Additionally, less laboratory waste is generated than with alternative (e.g., protein precipitation or liquid−liquid extraction) sample

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preparation methods. Just like TFME, PANDA microextraction can potentially also be reused multiple times as no degradation of the device occurs during extraction or desorption with the proposed extraction protocol. If necessary, it can also be recycled without hazardous solvents.[16] Similar to the TFME,[13,38−41] PANDA microextraction can also be operated in semi- or fully automated high-throughput mode. Owing to the use of 96-well plates and two benchtop shakers, in this study, up to 192 samples could be processed simultaneously, resulting in less than 2 min preparation time per sample. It should also be stressed that FDM 3D printing, used to prepare both PANDA microextraction and polyamide-based TFME supports, is considered a zero-waste method due to the complete use of substrate materials (in these cases, the filament) and the lack of generated byproducts.

CONCLUSIONS

The present study demonstrates the benefits associated with the implementation of 3D printing in analytical sample preparation. For the first time, alternatives to the costly metal supports of TFME devices are proposed. In addition, a promising new PANDA microextraction format is introduced. These advances were materialized utilizing novel carbon fiber-reinforced polyamide biocomposites, which are both sustainable[15,16] and biocompatible.[17,18]

Both TFME and PANDA microextraction methods are compatible with 96-well plates, allowing the simultaneous processing of multiple samples. The new TFME supports prepared with PA12 + CF15 are equivalent to the conventionally used metal supports. However, their introduction helps preserve the environment, financial resources, and time. In turn, PANDA microextraction provides not only a reduction in production costs (ca. 200 times) and time savings (over 12 times) but also excellent sample cleanup, good extraction efficacy, and reproducibility—in the case of some analytes, these qualities were superior even to those of the established and highly selective TFME method. PANDA microextraction, prepared with a PA6 + CF15 biocomposite, is ready to use after prototyping and only a brief postprocessing step, which is a significant improvement over the polyurethane-based LAYFOMM-60 first proposed for 3D printed extraction devices. According to the recommendations of the manufacturer, LAYFOMM-60 requires a 2−4 day preconditioning protocol to elute water-soluble PVA before it is ready to use, especially to minimize matrix effects when samples are analyzed by HPLC-MS. PANDA microextraction only requires cutting out the remnants of the idle printer head movements. Therefore, the entire preparation process is free of any reagents (particularly, concentrated hydrochloric acid and DMF). In addition to the previously mentioned benefits and savings, PANDA microextraction can be shared as a ready-to-print file and prepared with a portable 3D printer on-site immediately before its use. No specialized laboratory equipment or good technical skills are necessary for the process. Unlike spray painting of handmade TFME coatings, with its outcome highly dependent on the practical and manual experience of the personnel preparing the device (10 layers of dense coating slurry are applied on both sides of the supports with a hand sprayer), additional validation steps are needed to ensure adequate repeatability of the product.

As demonstrated in this study, PANDA microextraction offers unique advantages that can be applied in sample preparation for numerous structurally diverse small molecules: doping agents, endogenous hormones, prohibited substances, and therapeutic drugs. In addition, biocompatibility enables direct application in in vivo studies (e.g., saliva sampling) and simplifies the analytical protocol, possibly allowing analysis of the most labile analytes. Ultimately, this study introducing brand new sample preparation method paves the road to the future application of emerging extractive 3D printing polymers to encourage a new direction in general analytical chemistry.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.1c03672.

Monitored precursor−product ion(s) transitions; list of reference standards in the alphabetical order; extraction efficacies [%] of evaluated microextraction devices and their elements; comparison of the presented method with previously published limits of quantification [in $\mu g\ L^{-1}$] for TFME methods; and matrix effects [%] determined for the evaluated microextraction devices and their elements (PDF)

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All authors contributed equally to this work. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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Supporting Information for

Polyamide Noncoated Device for Adsorption-Based Microextraction and Novel 3D Printed Thin-Film Microextraction Supports

by Dominika Kołodziej, Łukasz Sobczak, and Krzysztof Goryński*

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Table of Contents:

Table S1. Monitored precursor – product ion(s) transitions.

substance	retention time [min]	precursor ion [m/z]	product ions: 1 [m/z]	CE [V]	2 [m/z]	CE [V]	3 [m/z]	CE [V]
fenoterol	4.156	304.00	107.15	-31	135.15	-18	286.10	-14
carteolol	4.499	293.00	237.15	-16	202.15	-21	74.10	-23
oxycodone D_3	4.532	319.00	301.20	-20	244.10	-30	259.20	-27
oxycodone	4.567	316.10	298.20	-19	241.20	-29	256.10	-26
hydrocodone D_3	4.964	303.00	199.10	-31	171.15	-40	128.00	-55
hydrocodone	4.914	300.00	199.15	-30	171.10	-40	128.15	-55
methamphetamine D_5	5.023	154.90	92.10	-20	91.10	-20	121.20	-14
methamphetamine	5.036	150.00	91.10	-21	65.10	-41	119.20	-15
MDMA D_5	5.227	198.90	165.10	-14	107.15	-24	135.20	-20
MDMA	5.242	194.00	163.10	-13	105.10	-24	133.10	-20
strychnine	5.430	334.90	184.10	-37	156.20	-46	129.15	-55
ketamine D_4	5.732	242.00	129.05	-28	224.10	-15	211.10	-15
ketamine	5.711	237.90	125.05	-26	220.10	-14	207.15	-14
metoprolol	6.499	267.90	116.15	-19	74.15	-22	72.10	-20
6-acetylcodeine	6.911	342.00	225.15	-27	165.15	-47	197.20	-31
clenbuterol D_9	6.648	286.00	204.10	-18	268.20	-12	169.05	-30
clenbuterol	6.681	277.10	203.05	-16	132.10	-28	168.15	-30
methylphenidate	6.765	234.00	84.15	-20	56.10	-46	91.10	-46
zolpidem D_6	7.517	314.00	235.10	-36	236.20	-29	263.20	-27
zolpidem	7.537	307.90	235.15	-36	236.20	-28	263.20	-26
cocaine	7.501	303.90	182.20	-20	82.10	-30	105.10	-33
cocaine D_3	7.503	307.00	185.20	-20	77.10	-55	85.20	-30
LSD D_3	7.878	327.00	226.20	-25	210.20	-44	208.15	-29
LSD	7.844	323.90	223.20	-24	208.15	-28	207.10	-42
bisoprolol	8.018	326.20	116.20	-19	74.05	-26	72.10	-25
phencyclidine D_5	8.801	249.00	96.15	-32	86.15	-15	164.25	-14
phencyclidine	8.828	244.00	91.05	-30	86.10	-15	159.20	-14
propranolol D_7	8.912	267.00	189.20	-18	117.20	-19	161.20	-26
propranolol	8.980	260.10	116.20	-19	183.10	-18	155.20	-26
fentanyl D_5	9.325	342.10	188.20	-24	105.10	-39	103.05	-55
fentanyl	9.364	337.00	188.20	-23	105.15	-37	103.10	-55
buprenorphine	9.891	468.10	55.15	-54	396.25	-39	414.25	-35
buprenorphine D_4	9.895	472.10	59.15	-52	400.20	-42	415.25	-37
ibutamoren	10.604	529.00	267.10	-21	91.15	-52	263.15	-18
nebivolol	11.348	406.10	151.10	-31	123.10	-42	103.10	-55
alprazolam D_5	11.420	314.10	210.20	-42	286.10	-27	279.25	-27
alprazolam	11.484	308.90	205.15	-28	281.15	-40	274.10	-26
anastrozole	11.506	294.00	225.20	-22	210.20	-34	115.05	-55
stanozolol	11.745	329.10	81.15	-46	95.15	-41	121.10	-38
methadone D_3	11.585	313.10	268.15	-16	105.05	-28	77.15	-55
methadone	11.622	310.00	265.15	-15	105.10	-27	77.05	-55
11-deoxycortisol	11.617	347.15	97.10	-30	109.05	-32	79.05	-50
boldenone	12.042	287.00	121.20	-22	135.20	-15	77.15	-55
clonazepam	12.062	316.00	270.10	-25	214.05	-38	207.15	-34
clonazepam D_4	12.108	319.90	274.15	-26	218.15	-37	211.20	-34
agomelatine	12.210	244.00	185.20	-16	170.10	-27	141.10	-47
nandrolone	12.528	275.10	109.10	-27	239.10	-18	257.30	-17
methandienone	12.691	301.20	121.05	-24	149.25	-15	77.00	-55
flunitrazepam	12.851	313.90	268.15	-27	239.10	-34	183.10	-53
flunitrazepam D_7	12.861	321.10	275.15	-27	246.15	-37	245.10	-37
clomifene	15.133	406.10	100.15	-25	72.10	-35	58.15	-39
tamoxifen	15.607	372.00	72.10	-24	70.10	-46	129.15	-27
canrenone	15.153	341.00	107.15	-30	187.25	-21	235.15	-25
canrenone D_6	15.204	347.20	107.20	-31	189.20	-24	91.10	-54
THC-COOH D_3	19.956	348.00	330.20	-17	302.25	-20	196.25	-27
THC-COOH	19.982	345.00	327.10	-16	299.25	-21	193.30	-26
THC D_3	24.938	317.80	195.05	-19	235.95	-10	85.05	-35
THC	24.968	315.00	193.10	-22	123.10	-32	259.15	-19

Substances arranged by retention order. CE = collision energy.

Table S2. List of reference standards in alphabetical order.

reference standard	stock solution	grade	manufacturer
(+/-)-3,4-methylenedioxy methamphetamine (MDMA)	MeOH solution 1 g L^{-1}	CRM (primary standard)	LGC
6-acetylcodeine	ACN solution 1 g L^{-1}	CRM (primary standard)	Cerilliant
11-deoxycortisol	MeOH solution 1 g L^{-1}	CRM (primary standard)	Cerilliant
(-)-11-nor-9-carboxy- Δ^9-tetrahydrocannabinol (THC-COOH)	MeOH solution 1 g L^{-1}	CRM (primary standard)	LGC
agomelatine	MeOH solution from powder 1 g L^{-1}	N/A	TRC
alprazolam	MeOH solution 1 g L^{-1}	CRM (primary standard)	LGC
anastrozole	MeOH solution from powder 1 g L^{-1}	reference standard (100%)	LGC
bisoprolol	MeOH solution 1 g L^{-1}	CRM (primary standard)	LGC
boldenone	MeOH solution from powder 1 g L^{-1}	analytical standard (≥ 98%)	VETRANAL™ (Sigma-Aldrich)
buprenorphine	MeOH solution 1 g L^{-1}	CRM (primary standard)	Cerilliant
canrenone	MeOH solution from powder 1 g L^{-1}	HPLC (≥ 97%)	Sigma
carteolol	MeOH solution from powder 1 g L^{-1}	USP reference standard (100%)	USP
clenbuterol	DMSO solution 1 g L^{-1}	CRM (primary standard)	LGC
clomifene	MeOH solution from powder 1 g L^{-1}	analytical standard	Sigma-Aldrich
clonazepam	MeOH solution 1 g L^{-1}	CRM (primary standard)	Cerilliant
cocaine	ACN solution 1 g L^{-1}	CRM (primary standard)	LGC
fenoterol	MeOH solution from powder 1 g L^{-1}	reference standard (99.9%)	LGC
fentanyl	MeOH solution 1 g L^{-1}	CRM (primary standard)	LGC
flunitrazepam	MeOH solution 1 g L^{-1}	CRM (primary standard)	LGC
hydrocodone	MeOH solution 1 g L^{-1}	CRM (primary standard)	Cerilliant
ibutamoren	MeOH solution from powder 1 g L^{-1}	HPLC (≥ 98%)	LGC
ketamine	MeOH solution 1 g L^{-1}	CRM (primary standard)	LGC
lysergic acid diethylamide (LSD)	ACN solution 1 g L^{-1}	CRM (primary standard)	LGC
methadone	MeOH solution 1 g L^{-1}	CRM (primary standard)	Cerilliant
methandienone	1,2-dimethoxyethane solution 1 g L^{-1}	CRM (primary standard)	Cerilliant
methamphetamine	MeOH solution 1 g L^{-1}	CRM (primary standard)	LGC
methylphenidate	MeOH solution 1 g L^{-1}	CRM (primary standard)	LGC
metoprolol	MeOH solution 1 g L^{-1}	CRM (primary standard)	LGC
nandrolone	ACN solution 1 g L^{-1}	CRM (primary standard)	LGC
nebivolol	MeOH solution from powder 1 g L^{-1}	HPLC (≥ 98%)	Sigma-Aldrich
oxycodone	MeOH solution 1 g L^{-1}	CRM (primary standard)	LGC
phencyclidine	MeOH solution 1 g L^{-1}	CRM (primary standard)	LGC
propranolol	MeOH solution 1 g L^{-1}	CRM (primary standard)	LGC
stanozolol	ACN solution 1 g L^{-1}	CRM (primary standard)	LGC
strychnine	MeOH solution from powder 1 g L^{-1}	HPLC (≥ 98%)	Sigma-Aldrich
tamoxifen	MeOH solution from powder 1 g L^{-1}	analytical standard (≥ 98%)	Sigma-Aldrich
(-)-Δ^9-tetrahydrocannabinol (THC)	MeOH solution 1 g L^{-1}	CRM (primary standard)	LGC
Zolpidem	MeOH solution 1 g L^{-1}	CRM (primary standard)	LGC
deuterium-labelled internal standards			
(+/-)-3,4-methylenedioxy methamphetamine D_5 (MDMA D_5)	MeOH solution 100 mg L^{-1}	CRM (primary standard)	Cerilliant
(-)-11-nor-9-carboxy- Δ^9-tetrahydrocannabinol D_3 (THC-COOH D_3)	MeOH solution 100 mg L^{-1}	CRM (primary standard)	Cerilliant
alprazolam D_5	MeOH solution 100 mg L^{-1}	CRM (primary standard)	Cerilliant
buprenorphine D_4	MeOH solution 100 mg L^{-1}	CRM (primary standard)	Cerilliant
canrenone D_6	MeOH solution from powder 100 mg L^{-1}	99.85%	LGC
clenbuterol D_9	MeOH solution 100 mg L^{-1}	CRM (primary standard)	Cerilliant
clonazepam D_4	MeOH solution 100 mg L^{-1}	CRM (primary standard)	Cerilliant
cocaine D_3	ACN solution 100 mg L^{-1}	CRM (primary standard)	Cerilliant
fentanyl D_5	MeOH solution 100 mg L^{-1}	CRM (primary standard)	Cerilliant
flunitrazepam D_7	MeOH solution 100 mg L^{-1}	CRM (primary standard)	LGC
hydrocodone D_3	MeOH solution 100 mg L^{-1}	CRM (primary standard)	Cerilliant
ketamine D_4	MeOH solution 100 mg L^{-1}	CRM (primary standard)	LGC
lysergic acid diethylamide D_3 (LSD D_3)	ACN solution 100 mg L^{-1}	CRM (primary standard)	Cerilliant
methamphetamine D_5	MeOH solution 100 mg L^{-1}	CRM (primary standard)	Cerilliant
methadone D_9	MeOH solution 100 mg L^{-1}	CRM (primary standard)	Cerilliant
oxycodone D_6	MeOH solution 100 mg L^{-1}	CRM (primary standard)	Cerilliant
phencyclidine D_5	MeOH solution 100 mg L^{-1}	CRM (primary standard)	Cerilliant
propranolol D_7	MeOH + 5% 1M HCl solution 100 mg L^{-1}	CRM (primary standard)	Cerilliant
(-)-Δ^9-tetrahydrocannabinol D_3 (THC D_3)	MeOH solution 100 mg L^{-1}	CRM (primary standard)	Cerilliant
zolpidem D_6	MeOH solution 100 mg L^{-1}	CRM (primary standard)	Cerilliant

Substances arranged by retention order. MeOH = methanol; ACN = acetonitrile; CRM = certified reference material.

Table S3. Extraction efficacies [%] of evaluated microextraction devices, and their elements.

substance \ support	TFME blades with C_{18} coating			TFME blades with no coating			TFME blades with PAN-only coating		
	PA6+CF15	PA12+CF15	metal	PA6+CF15 (PANDA Microextraction)	PA12+CF15	metal	PA6+CF15	PA12+CF15	metal
fenoterol	19.0 (4.0)	11.3 (2.0)	9.2 (1.3)	28.6 (1.8)	0.7 (9.7) *	0.4 (42.6) *	18.8 (5.8)	0.6 (10.4) *	0.3 (8.3) *
carteolol	23.3 (3.9)	25.1 (3.4)	20.9 (2.2)	12.2 (1.7)	0.7 (9.1) *	0.5 (48.6) *	5.2 (7.3)	0.6 (14.7) *	0.4 (8.7) *
oxycodone	5.9 (3.1)	23.2 (3.8)	21.7 (7.8)	2.1 (2.8)	0.6 (12.9) *	0.4 (35.5) *	1.2 (9.3) *	0.6 (14.3) *	0.4 (11.2) *
hydrocodone	35.9 (3.7)	47.4 (2.3)	46.4 (4.1)	6.3 (3.7)	0.8 (13.1) *	0.6 (37.3) *	3.0 (7.4)	0.8 (15.7) *	0.6 (11.2) *
methamphetamine	21.1 (2.9)	22.5 (2.9)	20.9 (5.5)	5.7 (1.1)	0.6 (8.9) *	0.5 (41.1) *	3.1 (8.1)	0.6 (12.2) *	0.4 (8.1) *
MDMA	30.0 (2.9)	31.8 (3.7)	29.1 (4.5)	10.2 (1.3)	0.8 (8.4) *	0.7 (35.7) *	5.2 (7.0)	0.8 (13.2) *	0.6 (8.6) *
strychnine	46.4 (3.1)	56.6 (3.5)	57.6 (3.3)	16.2 (1.7)	< LOD	2.3 (14.8)	7.6 (8.8)	< LOD	2.4 (17.3)
ketamine	56.7 (2.2)	57.3 (3.7)	57.5 (4.4)	8.8 (2.2)	0.9 (2.5) *	0.6 (33.6) *	4.1 (6.8)	0.9 (17.3) *	0.6 (8.8) *
metoprolol	28.6 (3.3)	38.5 (4.1)	38.4 (1.7)	5.1 (2.1)	0.6 (13.2) *	0.5 (53.0) *	2.7 (8.2)	0.6 (22.1) *	0.4 (22.0) *
6-acetylcodeine	36.7 (1.4)	59.0 (1.9)	61.7 (2.9)	5.8 (2.5)	0.7 (7.9) *	0.7 (31.3) *	2.8 (4.0)	0.7 (18.3) *	0.8 (12.8) *
clenbuterol	45.3 (3.3)	47.8 (2.4)	46.2 (4.7)	21.2 (0.8)	1.0 (4.9) *	0.9 (22.5) *	10.6 (8.4)	1.0 (12.7) *	0.9 (13.6) *
methylphenidate	38.5 (2.3)	38.5 (2.7)	38.6 (3.8)	4.9 (2.6)	0.4 (9.8) *	0.4 (29.9) *	2.4 (9.6)	0.5 (18.9) *	0.5 (12.5) *
zolpidem	48.2 (2.1)	48.9 (1.7)	51.1 (3.0)	16.2 (1.2)	< LOD	1.2 (9.2) *	6.2 (6.0)	< LOD	1.3 (22.8) *
cocaine	43.5 (3.6)	44.8 (3.5)	47.5 (4.6)	5.2 (3.4)	0.5 (6.7) *	0.4 (32.4) *	2.8 (9.5)	0.6 (14.3) *	0.5 (8.5) *
LSD	57.9 (2.3)	59.1 (2.1)	58.3 (2.4)	23.6 (2.3)	0.9 (12.8) *	1.0 (17.9) *	9.0 (5.5)	0.9 (13.0) *	1.1 (23.4) *
bisoprolol	61.9 (3.2)	65.6 (1.2)	63.1 (3.1)	6.0 (1.4)	0.8 (2.7) *	0.6 (41.7) *	3.0 (10.9)	0.7 (11.6) *	0.5 (7.2) *
phencyclidine	58.5 (2.8)	58.2 (2.4)	62.2 (2.9)	17.4 (2.8)	0.9 (8.6) *	1.0 (14.5) *	9.2 (5.9)	1.1 (18.0) *	1.7 (11.8) *
propranolol	62.4 (3.7)	66.8 (1.5)	70.2 (3.1)	32.9 (2.2)	2.8 (4.9)	2.8 (5.1)	22.0 (8.0)	2.5 (15.8)	3.7 (13.3)
fentanyl	70.2 (2.7)	70.4 (1.6)	72.1 (2.2)	27.1 (2.3)	2.0 (10.2) *	2.9 (3.1)	14.3 (5.3)	2.2 (20.6)	3.8 (21.0)
buprenorphine	62.1 (2.5)	64.3 (1.6)	64.3 (0.7)	34.6 (2.9)	6.3 (4.8)	3.3 (10.5)	18.9 (7.9)	6.8 (23.8)	5.2 (6.0)
ibutamoren	84.0 (2.2)	84.1 (2.1)	81.2 (2.9)	36.4 (2.6)	1.2 (5.9) *	2.6 (14.9)	15.4 (8.0)	< LOD	4.1 (10.2)
nebivolol	77.3 (1.8)	76.7 (1.0)	74.7 (0.7)	60.9 (4.3)	13.1 (5.6)	21.7 (9.8)	43.3 (5.2)	10.3 (18.6)	25.4 (5.2)
alprazolam	58.3 (2.0)	58.9 (2.8)	55.4 (3.5)	10.9 (5.8)	< LOD	< LOD	4.1 (2.3)	< LOD	1.3 (13.3) *
anastrozole	39.3 (3.1)	39.2 (1.8)	38.5 (3.7)	7.1 (0.6)	< LOD	< LOD	2.7 (12.6)	< LOD	0.8 (15.3) *
stanozolol	59.0 (2.6)	58.2 (4.7)	51.8 (3.7)	45.7 (3.6)	11.1 (15.6)	18.0 (10.5)	35.6 (19.3)	8.6 (13.2) *	18.2 (8.9)
methadone	73.1 (3.0)	72.8 (1.5)	78.7 (2.3)	26.9 (1.5)	1.9 (14.4) *	3.8 (4.2)	15.7 (8.0)	3.1 (31.0)	5.9 (14.3)
11-deoxycortisol	74.9 (3.0)	73.5 (2.7)	69.4 (1.9)	28.6 (2.2)	< LOD	0.0*	10.6 (9.8)	< LOD	< LOD
boldenone	80.7 (2.3)	78.0 (1.1)	75.9 (2.1)	32.2 (2.8)	1.8 (7.2) *	< LOD	12.9 (9.1)	1.6 (5.3) *	< LOD
clonazepam	46.1 (2.8)	43.6 (2.7)	41.3 (2.2)	24.4 (3.0)	1.3 (16.6)	0.5 (27.2) *	9.2 (3.9)	1.1 (12.1) *	0.4 (10.3) *
agomelatine	72.8 (2.3)	69.4 (2.6)	67.1 (2.0)	42.4 (1.6)	4.4 (5.0)	0.6 (40.8) *	19.3 (6.1)	3.5 (14.4)	0.6 (5.5) *
nandrolone	83.8 (7.1)	78.0 (3.2)	70.9 (2.9)	33.5 (1.8)	< LOD	0.0*	15.1 (15.7)	0.0*	0.0*
methandienone	85.6 (1.6)	64.8 (1.5)	78.1 (0.8)	31.5 (1.8)	1.4 (9.2) *	< LOD	12.7 (8.3)	< LOD	0.8 (8.3) *
flunitrazepam	54.0 (1.1)	51.0 (4.7)	51.5 (3.5)	11.8 (2.5)	< LOD	< LOD	4.6 (6.4)	< LOD	< LOD
clomifene	28.4 (1.6)	25.5 (3.5)	26.0 (3.0)	24.7 (5.3)	6.9 (11.1)	8.6 (6.7)	16.0 (7.3)	5.5 (13.9)	7.8 (6.7)
tamoxifen	25.0 (1.9)	23.5 (3.5)	23.8 (2.6)	20.0 (3.9)	5.3 (11.5)	8.3 (7.3)	12.5 (7.0)	4.4 (12.7)	7.8 (6.3)
canrenone	100.0 (1.8)	98.1 (2.5)	93.5 (1.9)	56.7 (3.3)	2.9 (10.9) *	0.0	26.9 (4.4)	2.7 (10.6) *	< LOD
THC-COOH	53.8 (1.7)	53.6 (0.9)	49.0 (2.1)	15.3 (3.0)	2.3 (8.7)	2.5 (13.2)	5.8 (3.5)	1.9 (12.7) *	3.5 (9.1)
THC	11.2 (3.1)	12.2 (7.2)	8.7 (5.4)	12.9 (4.9)	11.1 (6.5)	2.4 (23.1)	7.8 (7.6)	8.0 (8.3)	2.4 (6.3)

Corresponding relative standard deviation values [%] given in parentheses, substances arranged by retention order, $n = 4$, *indicates results below the established LOQ (5 μg L^{-1} for canrenone, nandrolone, and stanozolol; 1 μg L^{-1} for all other substances).

Table S4. Comparison of the presented method with previously published limits of quantification [in µg L^{-1}] for Thin-Film Microextraction methods.

reference	this study	Boyacı, et al., *Anal. Chim. Acta* **2014**, *809*, 69-81.	Vasiljevic et al., *Rapid Commun. Mass Spectrom.* **2019**, *33*, 1423-1433.		Goryński et al., *J. Pharm. Biomed. Anal.* **2016**, *127*, 147-155.		Reyes-Garcés et al., *J. Chromatogr. A* **2014**, 1374, 40-49.
matrix / substance	oral fluid	urine	urine	plasma	urine	plasma	plasma
6-acetylocodeine	1	5	-	-	-	-	-
anastrozole	1	5	-	-	-	-	-
bisoprolol	1	5	-	-	-	-	0.5
buprenorphine	1	5	-	-	-	-	-
canrenone	5	5	-	-	-	-	-
clenbuterol	1	15	-	-	-	-	0.5
cocaine	1	5	-	-	-	-	-
fenoterol	1	1	-	-	0.23	0.39	-
fentanyl	1	5	0.5	1	-	-	-
hydrocodone	1	-	25	2.5	-	-	-
methadone	1	-	2.5	10	-	-	-
methamphetamine	1	5	-	-	-	-	0.5
metoprolol	1	5	-	-	-	-	0.25
nandrolone	5	10	-	-	-	-	-
oxycodone	1	5	25	25	-	-	-
propranolol	1	5	-	-	-	-	1
stanozolol	5	10	-	-	-	-	0.5
strychnine	1	10	-	-	-	-	1
THC	1	5	-	-	-	-	-

Substances arranged in alphabetical order.

Table S5. Matrix effects [%] determined for evaluated microextraction devices, and their elements.

substance \ support	TFME blades with C_{18} coating			TFME blades with no coating			TFME blades with PAN-only coating		
	PA6+CF15	PA12+CF15	metal	PA6+CF15 (PANDA Microextraction)	PA12+CF15	metal	PA6+CF15	PA12+CF15	metal
oxycodone	-47.3 (1.9)	-3.6 (2.2)	-1.8 (0.9)	-33.5 (2.3)	10.0 (4.0)	20.3 (1.0)	-19.4 (2.4)	12.5 (6.4)	11.1 (2.0)
hydrocodone	-27.2 (4.4)	-6.3 (3.5)	-7.4 (2.1)	-27.4 (2.0)	8.7 (3.7)	16.9 (1.3)	-9.0 (4.7)	13.7 (6.9)	8.2 (4.6)
methamphetamine	-15.7 (3.1)	-11.0 (1.7)	-8.9 (3.5)	-7.0 (3.1)	6.9 (1.1)	18.4 (0.4)	6.3 (3.5)	10.5 (6.4)	9.7 (3.4)
MDMA	-15.3 (1.6)	-7.7 (2.2)	-5.9 (1.5)	-7.1 (2.3)	11.3 (4.1)	17.7 (1.3)	8.3 (2.8)	15.4 (5.5)	13.1 (4.3)
ketamine	-14.2 (1.8)	-11.7 (2.4)	-11.8 (2.2)	-2.6 (2.1)	7.1 (3.7)	16.9 (2.1)	8.6 (2.7)	8.5 (6.0)	8.2 (2.1)
clenbuterol	-27.5 (2.0)	-12.8 (0.8)	-10.5 (1.1)	-25.4 (2.4)	9.1 (0.5)	13.3 (4.4)	-13.5 (5.1)	9.6 (6.7)	6.9 (1.8)
zolpidem	-13.5 (2.4)	-13.5 (1.2)	-9.8 (2.6)	-7.8 (2.7)	4.4 (2.6)	10.8 (1.8)	4.8 (5.0)	9.7 (4.7)	8.8 (2.8)
cocaine	-17.4 (2.6)	-17.6 (1.9)	-15.5 (3.7)	-9.1 (2.6)	6.4 (2.8)	12.5 (1.8)	2.1 (5.0)	9.2 (5.1)	6.8 (3.5)
LSD	-20.3 (2.2)	-17.3 (2.5)	-14.0 (3.4)	-13.1 (2.8)	1.3 (1.1)	8.6 (1.9)	-0.2 (4.1)	5.2 (4.8)	6.8 (1.6)
phencyclidine	-17.3 (3.3)	-17.5 (1.7)	-12.9 (1.5)	-16.7 (1.6)	-1.1 (3.2)	5.0 (1.0)	-5.3 (4.8)	3.9 (3.9)	1.2 (2.1)
propranolol	-25.0 (3.1)	-13.2 (3.1)	-12.9 (4.3)	-14.7 (3.4)	8.7 (6.2)	23.2 (3.5)	1.8 (3.6)	9.0 (5.3)	10.1 (3.0)
fentanyl	-7.0 (1.4)	-8.3 (1.5)	-10.1 (1.3)	-6.9 (2.8)	4.3 (3.4)	11.1 (1.6)	8.4 (5.4)	11.8 (4.9)	8.5 (1.2)
buprenorphine	-10.8 (5.9)	-6.3 (1.6)	-5.5 (3.0)	-8.8 (4.2)	11.6 (2.4)	20.7 (1.4)	9.3 (2.4)	13.0 (5.5)	11.0 (3.0)
alprazolam	-7.9 (4.3)	-4.2 (2.1)	-14.2 (5.0)	10.0 (4.3)	14.7 (5.1)	19.7 (6.1)	22.5 (6.0)	16.6 (5.5)	13.6 (7.0)
nactludone	-20.3 (3.5)	-19.0 (0.7)	-17.1 (2.5)	-14.5 (4.0)	3.9 (2.3)	9.5 (1.7)	2.5 (2.9)	6.5 (5.3)	3.9 (3.9)
clonazepam	-9.2 (1.7)	-12.3 (3.1)	-12.6 (0.8)	-0.2 (3.6)	1.9 (6.5)	14.1 (5.4)	13.8 (3.2)	2.6 (3.4)	0.5 (2.0)
flunitrazepam	-6.5 (2.4)	-13.8 (1.8)	-7.2 (3.1)	4.3 (3.6)	0.7 (1.6)	12.7 (3.0)	11.1 (4.3)	1.5 (6.0)	8.4 (5.1)
canrenone	0.5 (2.7)	-4.4 (6.5)	-11.3 (2.0)	20.0 (5.0)	27.1 (1.5)	20.2 (7.8)	22.2 (9.2)	12.7 (1.5)	9.6 (5.8)
THC-COOH	-2.4 (1.1)	-3.5 (1.5)	-6.6 (0.9)	7.7 (2.7)	13.1 (2.4)	16.9 (1.9)	18.9 (6.2)	17.1 (3.8)	11.3 (2.2)
THC	-1.2 (0.5)	-5.3 (1.9)	-9.7 (2.0)	6.2 (3.8)	9.2 (3.9)	10.5 (1.8)	12.0 (4.0)	15.4 (6.5)	9.9 (4.5)

Corresponding relative standard deviation values [%] given in parentheses, substances arranged by retention order, $n = 4$.

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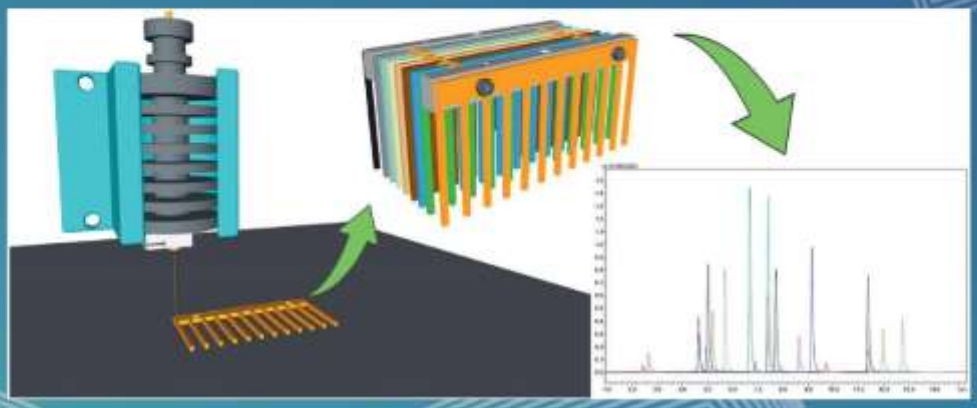

Featured Article

Innovative, simple, and green: A sample preparation method based on 3D printed polymers

From Dominika Kołodziej, Łukasz Sobczak, Krzysztof Goryński

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Innovative, simple, and green: A sample preparation method based on 3D printed polymers

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ABSTRACT

The present study evaluates the capability of fifteen 3D printed thermoplastic polymers as novel stationary phases for the extraction of forty-three physicochemically diverse analytes from fortified human oral fluid samples. Prototype extraction devices were prepared in 96-well plate-compatible format using fused deposition modeling 3D printer. The sample preparation was performed with 5-step protocol utilizing 96-well plates and semiautomated benchtop shaker. All resulting extracts were analyzed via high-performance liquid chromatography (operated in reversed-phase gradient elution mode) and tandem mass spectrometry (with electrospray ionization and triple quadrupole mass spectrometer). Exceptionally favorable results were observed for three polymer types: polyamide 6 (reinforced with 15% carbon fiber), LAYFOMM-60 (polyurethane with water-soluble polyvinyl alcohol), and S-FLEX 90A (thermoplastic polyurethane). Furthermore, this study also introduces an automated and repeatable 3D printing method for the fast fabrication of high-throughput, and highly selective sample preparation devices, most of which are ready-to-use without any additional processing or chemical functionalization. As such, the proposed printing method represents a significant step towards the introduction of novel polymeric stationary phases for analytical sample preparation, thus providing laboratory personnel with a method that is safer and more convenient, while minimizing negative environmental impacts.

1. Introduction

Over one hundred years have passed since Leo Hendrik Baekeland was granted a patent for the first synthetic polymer, Bakelite, in 1909 [1]. Following this breakthrough, polymers rapidly began to impact all aspects of everyday life; this was especially true in the sciences, including analytical chemistry. As a result, laboratories now have a considerable array of polymeric products to choose, from common expendables and instrumentation parts, all the way to the latest stationary phases used for sample preparation [2–6].

The tremendous significance of polymers in modern analytical chemistry is most clearly seen not only in the abundance of new studies examining these compounds, but also in the unparalleled creativity and diversity of these works. Some of the most promising recent studies have proposed acrylonitrile butadiene styrene (ABS) nanofibers as new sorbents for the determination of polycyclic aromatic hydrocarbons (PAHs) produced by the combustion of fossil fuels [7]. The implementation of ABS as stationary phase for headspace thin-film microextraction (HS-TFME) was possible thanks to its mechanical and chemical resistance, as well as its porosity and the presence of nitrile (–CN) functional groups on its surface [8]. Carbon fiber has also been proposed as an environmentally friendly sorbent that complies with the principles of green analytical chemistry [9,10]. In prior studies, organic cotton fiber was thermally processed into carbon fiber for the determination of chlorophenols in human urine [11] and isoflavones in environmental water samples [12] via dispersive solid-phase extraction (DSPE). Elsewhere, poly-ε-caprolactone (PCL) nanofibers have been proposed for the extraction of parabens from problematic matrices, particularly those that are rich in proteins. Indeed, findings have shown that the use of PCL nanofibers in online solid-phase extraction (SPE) protocols can enable the selective extraction of analytes (i.e., without coextraction of macromolecules) [13]. Other studies have demonstrated polystyrene's

Abbreviations: PANDA Microextraction, Poly Amide Non-coated Device for Adsorption-based Microextraction; FDM, fused deposition modeling; TFME, thin-film microextraction; SPME, solid-phase microextraction; PA6 + CF15, *Polyamide 6 reinforced with 15 % carbon fiber.*

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considerable potential as a thin-film microextraction (TFME) membrane for the extraction of methadone from urine [14], as a solid-phase microextraction (SPME) sorbent for the separation of pesticides from honey [15], and in microextraction by packed sorbent (MEPS) for the isolation of microbial metabolites in blood serum [16]. However, in addition to its excellent adsorptive properties, polystyrene offers good mechanical resistance. This feature enabled its use in the knitting of imprinted acrylate microspheres and the production of a selective SPME sorbent, which both outperformed commercial fibers for the extraction of parabens from water samples [17].

Currently, much research attention is being devoted to the implementation of polyamides (nylons) in analytical procedures. These polymers are considered particularly promising, thanks to a combination of favorable features, such as: excellent mechanical and chemical resistance; green chemistry-compatible synthesis from lignocellulose biomass [18]; and a recycling process that not only does not require harmful reagents [19], but that also reinforces the mechanical and chemical durability of the resultant polymer [20]. However, polyamides' ability to provide the selective adsorption of targeted analytes is the main feature motivating attempts to incorporate them into sample preparation protocols. Benedé et al. proposed the use of polyamide 6-coated paper for the extraction of six penicillin-derived antibiotics from saliva [21], while other researchers have also employed polyamide 6 as a sorbent for the extraction of estrogens from saliva and urine [22]. Polyamide's advantages have been further demonstrated in several successful environmental sample preparation applications, including the extraction of phenols and chlorophenols [23], bisphenol A [24], and estrogens [25]. Recently, polyamide 6 has also emerged as a novel stationary phase for food analysis, with researchers having examined its use for the extraction of resveratrol from wines [26] and ochratoxin A from beer [27].

From the above summary, it is apparent that a broad array of applications have been proposed for a vast selection of polymers. However, the existing literature lacks work that comprehensively and directly compares these adsorptive polymers, especially when applied as stationary phases for the extraction of small molecules. The present study addresses this research gap by providing experimental data relating to the extraction efficacy, extraction repeatability, and degree of sample clean-up achieved using various adsorptive polymers for sample preparation. The presented results are supported by a theoretical discussion detailing the molecular mechanisms of small molecule adsorption to enable the informed selection of polymers for future analytical applications. The primary benefit of this study is that it establishes the background essential to enabling the implementation of adsorptive polymers into specialized analytical procedures. For example, in a recent breakthrough study [2], we applied 3D printing using a fused deposition modeling (FDM) technique to fabricate a biocompatible polymeric sample preparation device from polyamide 6 reinforced with 15% carbon fiber. The device – known as "PANDA Microextraction" (PolyAmide Non-coated Device for Adsorption-based Microextraction) –was the first fully 3D printed device to be prepared from a chemically unmodified and non-proprietary polymer that had previously only been used as a sorbent in non-3D-printed sample preparation methods, usually with excellent results. Prior studies wherein 3D printing was employed for the extraction of small molecules have exclusively used the porous LAYFOMM-60 (CC Products, Germany) which has been shown to be an effective stationary phase for the extraction of the anti-diabetic drug, glimepiride [6], various anxiolytic drugs [28], and steroids [29]. Thus, the development of PANDA Microextraction has opened up the tremendously rich selection of readily available polymers for use as novel stationary phases – all thanks to the increasingly popular 3D printing technology.

The importance of this achievement should not be overshadowed by the numerous examples of polymers that have been employed as stationary phases, or the popularity of commercially available polydimethylsiloxane (PDMS)-based microextraction devices, as the use of 3D printing technology offers an unprecedented opportunity for prototype customization, including format, shape, and size modifications. Without a doubt, the unrestricted freedom to adjust surface texture is an essential advantage of 3D printing. For example, Bagheri et al. found that the use of 3D structures enabled an 8–10 fold improvement in the extraction yield of chlorobenzenes compared to the corresponding 2D structures [30]. Given that reprocessing enhances the mechanical strength of polymers [20,31,32], it is critical to highlight the fact that the FDM 3D printing process involves heating the polymers to their melting point before resolidifying them to form a predesigned prototype. Moreover, these benefits are also accompanied by significantly shorter preparation times and highly reduced financial costs.

Remarkably, all the polymers discussed in this section [7,8,11–17, 21–27] are now commercially available as 3D printing filaments that are compatible with the most popular FDM method. Furthermore, the number of available chemistries is constantly increasing, owing to the introduction of new polymers and new blends of existing ones. The majority of these emerging polymers have yet to be introduced to analytical chemistry, especially as new stationary phases that could be perfectly fit for sample preparation methods based on solid extraction phases. In the light of this exciting opportunity, we elected to pursue materialization of this innovative idea. The present study comprises a complex evaluation of the extractive properties of fifteen 3D printing filaments featuring eleven unique chemical compositions by applying them to extract forty-three model compounds from fortified oral fluid samples.

2. Materials and methods

2.1. Preparation of the 3D printed extraction devices

All 3D printed extraction devices were prepared in the form of 12-pin blades, similar to those used in TFME [33], that were compatible with commercial 96-well plates. The prototype models were prepared in Blender 2.82 3D creation suite (Free Software Foundation, Inc.) and saved as.stl files. While the developed devices were similar to the commercial metal TFME blades available from PAS Technology Deutschland GmbH, some minor modifications were made, such as broadening the entire prototype by 0.8 mm (relative to the y axis) and increasing the side length (thickness) of each pin from 0.625 mm to 2 mm. Broadening the prototypes minimized the contraction of the polymers after 3D printing and ensured compatibility with the format of 96-well plates, while increasing the side length of the pins provided additional extractive area, thus improving extraction efficacy.

Next, the prepared 3D model was sliced into ten 0.2 mm layers using PrusaSlicer (Prusa Research, Czech Republic). At this stage, a selection of distinctive 3D printing parameters (e.g., heat bed temperature and extruder temperature) were integrated into the printer-compatible. gcode files, which were prepared individually for each polymer type. Finally, the 3D models were prototyped using a Prusa i3 MK3S 3D printer (Prusa Research, Czech Republic). A detailed list of the printing parameters is available in Table S1 in the Supporting Information. Depending on the polymer type, the printer was fitted with either a brass or ruby nozzle (both from BROZZL, Brozzl GmbH, Austria) for a 0.4 mm E3D V6 hotend and double-sided textured polyetherimide (PEI) powder-coated spring steel sheet (Prusa Research, Czech Republic), or a smooth double-sided PEI-coated spring steel sheet (Prusa Research, Czech Republic).

Given the diversity of the evaluated polymers and the accompanying differences in 3D printing parameters, such as the broad range of printing temperatures (190–267 °C), we anticipated that the prepared prototypes could contract to varying degrees. To address this issue, each of the 3D printed extraction devices was measured after cooling down, and the contraction coefficient was determined based on the relative error calculated using the actual and anticipated dimensions. For the majority of the polymers, the contraction coefficient was within the

preestablished ±1% limit, with the lowest contraction being observed for the polyamides and the highest contraction being observed for polypropylene (PP) and high impact polystyrene (HIPS). Consequently, the revised models for these polymers were prepared by rescaling the original models during slicing. The updated prototypes were always within ±1% of the size-range limit.

2.2. Preparation of the samples

The target analytes selected for this study consisted of 43 small molecules containing a broad range of physicochemical properties, unique chemical structures, molecular masses between 135.10 Da and 528.24 Da, and logP values between −2.1 and 7.1 [34]. All reference standards, as well as 20 deuterium-labelled internal standards, were purchased as ready-to-use stock solutions from LGC Standards (LGC, Poland) and Merck (Merck Group, Poland), or were prepared by dissolving pure analytical standards in LC-MS-grade methanol (CHROMASOLV™, Honeywell International Inc., USA). Detailed information about the reference standards used in the work is available in Table S2 of the Supporting Information.

Two volunteers (one 25-year-old female and one 28-year-old male), who were naïve to the analyzed drugs were recruited as sample donors. First, oral fluid was collected from each participant into 15 mL polypropylene test tubes (as such method was previously determined not to bias the attained results [35]), and then pooled together to form a uniform matrix. Collection process was conducted in accordance with the applicable regulations. Pooled oral fluid was then spiked with reference standards to obtain a concentration of 50 ng/mL. Finally, the drug-spiked matrix was conditioned for 60 min at room temperature using a semi-automated benchtop shaker to encourage drug-protein binding. The pH value of the samples was not adjusted.

2.3. Extraction protocol

Extractions were performed in quadruplicate in 2 mL 96-well DeepWell™ plates (Nunc™, Thermo Fisher Scientific Inc., USA) using a semi-automated benchtop SH10 Heater-Shaker (Ingenieurbüro CAT, M. Zipperer GmbH, Germany) in an airconditioned laboratory maintained at 20.0 °C. The five-step extraction protocol included: preconditioning with methanol/water (50/50, v/v; 1.5 mL, 60 min, 850 rpm agitation); rinse with water (1.5 mL, 5 s, no agitation); extraction from oral fluid samples (1 mL, 2.5 h, 850 rpm agitation); rinse with water (1.5 mL, 5 s, no agitation); and desorption to methanol/water/formic acid (80/19.9/0.1, v/v/v) spiked with 20 isotope-labelled internal standards at 5 ng/mL (1 mL, 2 h, 850 rpm agitation). LC-MS-grade formic acid (Optima™, Thermo Fisher Scientific Inc., USA), methanol (CHROMASOLV™, Honeywell International Inc., USA), and water (LiChrosolv®, Merck Group, Poland) were used in all of the above steps.

Methanol/water/formic acid (80/19.9/0.1, v/v/v) was chosen as elution medium for all analytes and polymers. This decision was based on previous studies, where it proved to be both effective [2], and arguably the most versatile out of all compared desorption solvents [36]. However, in such conditions, those analytes that have a strong interaction with the polymers would not be sufficiently desorbed and could be present at low concentrations in the eluates.

2.4. Instrumental analysis

The obtained extracts were analyzed with a Shimadzu LCMS-8060 triple quadrupole consisting of a high-performance liquid chromatograph coupled with an electrospray ion source and tandem mass spectrometer (Shimadzu Corporation, Shim-Pol A.M. Borzymowski, Poland). The samples extracted with each polymer type were tested in quadruplicate using an analytical method based on methods previously employed for the separation of the same or similar solutes [2,33,35,36]. In brief, the protocol consisted of reversed-phase gradient elution chromatographic separation on an Agilent InfinityLab Poroshell 120 EC-C18 analytical column (3 × 100 mm, 2.7 μm) with a guard column (3 × 5 mm, 2.7 μm; both columns from Agilent, USA). LC-MS-grade acetonitrile and water (LiChrosolv®, Merck Group, Poland), both containing 0.1% formic acid (Optima™, Thermo Fisher Scientific Inc., USA), were used as mobile phases. Separations were performed at 25.0 °C with a total flow rate of 300 μL/min and a volume of 0.7 μL, for a total run time of 35 min per sample. More details on HPLC-MS/MS retention times and precursor – product-ion transitions can be found in Table S3 in the Supporting Information.

2.5. Method validation

The HPLC-MS/MS method used in this study was previously developed by our research group for the quantification of various prohibited substances in human-derived samples (oral fluid, plasma, and urine) prepared with thin-film microextraction technique.

The original analytical protocol was successfully subjected to full method validation in accordance with the latest guidelines of the European Medicines Agency (EMA) [37] and the Food and Drug Administration (FDA) [38]. Drug-free biomatrices were purchased from Lee Biosolutions™ Inc, USA, and collected from drug-naïve volunteer in accordance with the applicable regulations. All samples (excluding zero and blank samples) were prepared by spiking the drug-free matrices with a mixture of reference standards, to achieve predefined concentrations. The matrices were either pooled from multiple donors, or obtained from a single donor – depending on the specific criteria for each validation experiment. In particular, the investigated parameters of the method included: selectivity (signal assessment for blank samples); linearity (three 7-point calibration curves, prepared on 3 different days, with 4–5 replications per each point); sensitivity; accuracy and precision (at 4 different concentration levels, both intra and extra-day); recovery of the extraction method; instrumental carry-over (measured after injecting the highest concentration sample); matrix effect (assessed with blank samples from 6 unique individuals and water, with all samples spiked after the extraction at two concentration levels); sample stability before the extraction (stored at room temperature (20 °C) for 24–48 h, refrigerated at 4 °C for 5–7 days, and after 3 freeze/thaw (−20 °C/20 °C) cycles); and stability of the extracts at HPLC autosampler temperature (4 °C) – all stability experiments were performed at 2 different concentrations. All samples were analyzed in randomized order, and at least 2 precursor – product transitions were used per each analyte for tandem mass spectrometry detection. Additionally, the instrument was controlled by a system suitability test (SST) using analytes other than the drugs under study, and the retention times were monitored for any shifts. The presented study used the same instrumental method as the previously validated protocol, and the only difference was the use of an alternative sample preparation method (TFME was replaced with 3D-printed extraction devices). Therefore, only a partial validation was performed for this study (as per requirements of the validation guidelines [37,38]). Partial validation included assessment of the precision and accuracy of the sample preparation method. For these experiments, a reference adsorptive polymer (polyamide 6 reinforced with 15% carbon fiber) [2] was used to extract 33 model compounds from the fortified oral fluid sample (in 50 ng/mL concentration). In addition, some of the key HPLC-MS/MS system parameters were verified for the 43 reference standards used in this study. Investigated HPLC-MS/MS parameters included linearity of the signal-to-concentration response increase within a working range (1–75 ng/mL, resulting in 8-point calibration curves), and repeatability of the attained results (within 20 measurements of a 50 ng/mL concentration).

3. Results and discussion

In this study, we evaluated fifteen unique 3D printed sample preparation devices, and as such this study comprises the most

comprehensive analysis to date in this innovative field of analytical science. A full list of the polymers used in this work is presented in Table 1.

The selected 3D printing filaments included several replicates of the most abundant polymer types obtained from different manufacturers, including ABS, polyethylene terephthalate glycol-modified (PET-G), and polylactic acid (PLA). The inclusion of multiple specimens of the same polymer allowed us to cross-check the attained results and evaluate how the unique procedures and additives used during filament production impacts the device's eventual adsorptive properties towards small molecules.

The prototype devices were prepared in a format compatible with commercial 96-well plates (see Fig. 1) to enable high-throughput sample preparation (below 2 min extraction time per sample in this study), with sampling being followed by highly selective instrumental analysis via high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS).

3.1. *Initial assessment of the polymers*

Due to incomplete and often inconclusive information about the chemical resistance of the evaluated polymers to typical HPLC solvents, an initial assessment was conducted to verify their compatibility with certain chemicals, including the methanol-based (80%) desorption solvent containing 0.1% formic acid. None of the tested polymers showed any visible sign of degradation after being immersed in this solution for 2 h at ambient temperatures (ca. 22.0 °C) and 850 rpm agitation. These conditions were employed to mimic the desorption step of the extraction protocol.

3.2. *Quality of results attained with HPLC-MS/MS system and partial validation of the sample preparation method*

Repeatability of the HPLC-MS/MS system was monitored by 20 measurements of the reference sample containing a mixture of all the evaluated analytes (each in 50 ng/mL concentration). The results obtained were repeatable with calculated relative standard deviation (RSD) values within 3.8–19.3% range (mean = 7.1%, median = 6.1%, n = 43). The only outlier with RSD above 15% was morphine (19.3%). The signal-to-concentration response increase of the instrument was assessed

Table 1
List of evaluated 3D printing polymers.

#	Filament	Polymer Type	Manufacturer
1	ABS	acrylonitrile butadiene styrene	Fiberlogy, Fiberlab
2	ABS PRO	acrylonitrile butadiene styrene	N/A
3	HIPS	polystyrene (high impact)	N/A
4	LAYFOMM-60	polyurethane – polyvinyl alcohol	FORMLAY, LAY-FILAMENTS™, CC-Products
5	PA6 + CF15	polyamide 6 + carbon fiber (15%)	Spectrum Industrial, Spectrum Group
6	PA12 – CF15	polyamide 12 + carbon fiber (15%)	Fiberlogy, Fiberlab
7	PC-PBT	polycarbonate + polybutylene terephthalate	Polymaker™ Industrial
8	PET-G	polyethylene terephthalate glycol-modified	Fiberlogy, Fiberlab
9	PET-G	polyethylene terephthalate glycol-modified	Prusa Research
10	PLA	polylactic acid	Filament PM
11	PLA EASY	polylactic acid	Fiberlogy, Fiberlab
12	PLA PEARL	polylactic acid	N/A
13	PMMA	polymethyl methacrylate	FDD Filament
14	PP	polypropylene	Renkforce
15	S-FLEX 90A	polyurethane (thermoplastic)	Spectrum Filaments, Spectrum Group

Polymers are listed in alphabetical order. N/A – no details available.

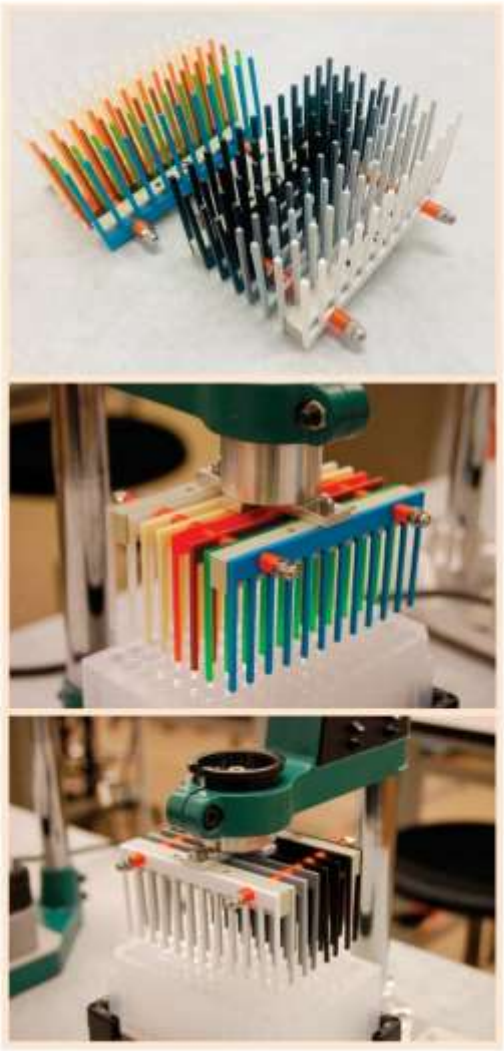

Fig. 1. Prototype 3D printed polymeric extraction devices in a 96-well-plate-compatible format.

in the 1–75 ng/mL range. As a result, the linear working range was ascertained within the boundaries corresponding to extraction efficacies of 2–150%. All results below 1 ng/mL were reported in this study as results below the limit of quantification (LOQ). The resulting 8-point calibration curves, calculated with $1/a^2$ weighting, delivered high coefficient of determination values in the R^2 = 0.9814–1.0000 range (mean = 0.9982, median = 0.9994, n = 43). All the calculated regression curve equations delivered positive slope values, what indicated that in each case there was a positive linear relationship between the increase of signal and the increase of analyte concentration. Discussed results are available in Table S4 in the Supporting Information.

Partial validation of sample preparation method included testing

method's precision and accuracy. In terms of precision, the evaluated method resulted in RSD values in 1.2–9.5% range (mean = 4.3%, median = 4.3%, n = 33). No results were above the 15% threshold. Therefore, the evaluated method was proven to be precise. In terms of accuracy, the recorded RSD values were in 1.5–31.0% range (mean = 9.4%, median = 7.3%, n = 33). Several RSD values were above the 15% threshold, for example: buprenorphine (15.5%), tamoxifen (20.2%), metoprolol (21.2%), nandrolon (23.5%), hydrocodone (26.4%), and Δ^9-tetrahydrocannabinol (THC; 31.0%). These results suggest matrix effect derived from oral fluid samples, and demonstrate the necessity of signal correction. Full results of the partial validation are available in Table S5 in the Supporting Information.

3.3. Quantitative results

The comprehensive quantitative results of this study, including extraction efficacies and the corresponding relative standard deviation (RSD) values for all analytes and all polymer types, are available in Table S6 in the Supporting Information. All RSD values were calculated from corrected signals to diminish bias of the results originating from any external factors. All results were corrected using ratios created by stacking the mean signal measured for the extracted samples against the mean signal measured for the reference sample (i.e., analytes mixture in 50 ng/mL concentration in desorption solvent). The reference sample was measured in quadruplicate with each batch of samples extracted with the same polymer type. Additional assessment of signal suppression/enhancement (so called matrix effect [39]) was conducted using deuterium-labelled internal standards spiked into the desorption solvent. Results based on 20 matching analyte – internal standard pairs are available in Table S7 in Supporting Information.

As shown in Fig. 2, three polymer types clearly outperformed the others in terms of number of analytes successfully quantified, when used as an extraction phase.

Of the analyzed polymers, polyamide 6 reinforced with 15% carbon fiber (PA6 + CF15) provided the best performance, enabling the quantification of 40 out of 43 target small molecules, while LAYFOMM-60 was a close second with 39 of 43 small molecules, and S-FLEX 90A (Spectrum Group, Poland) was third with 25 analytes extracted. Each of these polymers in discussed in detail below, with special emphasis on the physicochemical attributes governing their adsorption of small molecules. In addition, polymers providing exceptionally favorable results will be discussed as the most promising candidates for future research.

3.4. Polyamide 6 reinforced with 15% carbon fiber

While PA6 + CF15 was the only filament type to require thermal conditioning (80 °C for 2 h) prior to 3D printing, this pretreatment step served to enhance its mechanical properties by increasing its surface roughness. This increased surface roughness helped to decrease transverse stresses between individual layers of the 3D print, thus producing a more durable prototype [40]. As noted above, the PA6 + CF15 prototype provided the broadest coverage for the targeted small molecules, successfully enabling the extraction and quantification of 40 of 43 analytes. Moreover, the extracted compounds possessed remarkable physicochemical diversity (with hydrophobicities ranging from logP 0.2 to 7.1, and molecular masses ranging from 135.10 Da to 528.24 Da [34]), suggesting this polymer's potential future use for a variety of unique applications [2]. The only analytes that could not be sufficiently extracted with this polymer were the more polar structures, namely, meldonium (logP = −2.1), nikethamide (logP = 0.3), and heroin (logP = 1.5) [34].

The highest extraction efficacies for the PA6 + CF15 prototype were recorded for stanozolol (60.4%, RSD = 8.1%, n = 4), nebivolol (58.2%, RSD = 1.2%, n = 4), higenamine (50.9%, RSD = 0.9%, n = 4), agomelatine (50.5%, RSD = 4.3%, n = 4), and buprenorphine (48.6%, RSD = 3.8%, n = 4). The apparent dissimilarities between the basic physicochemical properties of these molecules (e.g., their hydrophobicities, molecular masses, polar surface areas, pK_as, number of hydrogen-bonding donor and acceptor sites, or types of functional groups) further highlights the universal potential of polyamide 6-based prototypes for the extraction of small molecules. All of the aforementioned descriptors of the analytes are available in Table S8 in the Supporting Information. The observed versatility of the PA6 + CF15 prototype was the result of several unique interactions between functional groups and the analytes (hydrophobic, hydrogen bonding, and dipole–dipole) created by the polyamide 6 [41].

In addition to favorable extraction efficacies and broad analyte coverage, the PA6 + CF15 prototype provided a very repeatable extraction process with low RSD values (mean = 5.3%, median = 4.3%, min = 0.9%, max = 32.9%, n = 40), as well as good selectivity with low matrix effects (mean = 6.0%, median = 2.5%, min = −3.3%, max = 38.4%, n = 19). The prototype's RSD exceeded the 15% threshold only twice (17.8% for atenolol and 32.9% for morphine), while the matrix effects only breached the ±20% limit for two analytes (+22.0% for hydrocodone and +38.4% for morphine).

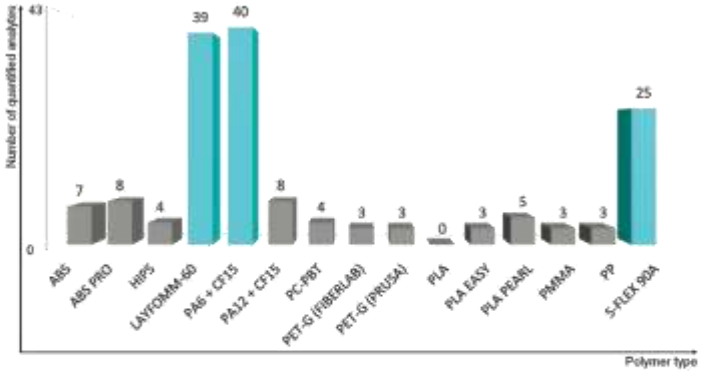

Fig. 2. Number of analytes (out of 43) quantified after extraction with each polymer type. Polymers are arranged in alphabetical order.

3.5. LAYFOMM-60

In contrast to the PA6 + CF15 prototype, the LAYFOMM-60 device required treatment after 3D printing. This post-processing step entailed rinsing the polyvinyl alcohol with water (following manufacturer's instructions, three 24 h conditioning cycles were performed with water exchange after each cycle), resulting in a highly porous surface that greatly expanded the prototype's adsorptive area [42]. Once this process had been completed, the LAYFOMM-60 prototype was assessed for its extraction efficacy.

Combined with this post-processing step, the LAYFOMM-60 prototype provided the second best analyte coverage, quantifying 39 of 43 target analytes. As with the PA6 + CF15 prototype, the four unquantified analytes tended to be more polar, including meldonium (logP = −2.1), nikethamide (logP = 0.3), atenolol (logP = 0.2), and salbutamol (logP = 0.3). The LAYFOMM-60 prototype demonstrated particularly good extraction efficacies for nebivolol (88.2%, RSD = 3.8%, n = 4), stanozolol (82.4%, RSD = 2.7%, n = 4), buprenorphine (72.9%, RSD = 10.3%, n = 4), propranolol (56.2%, RSD = 8.3%, n = 4), fentanyl (55.3%, RSD = 7.8%, n = 4), tamoxifen (55.2%, RSD = 13.4%, n = 4), agomelatine (54.4%, RSD = 5.0%, n = 4), and nandrolone (53.2%, RSD = 9.2%, n = 4). For this prototype, analyte adsorption was achieved through the hydrophobic, hydrogen bonding, dipole–dipole, and π–π type interactions provided by the polyurethane.

LAYFOMM-60 was also characterized by repeatable extraction with low RSD values (mean = 9.6%, median = 8.8%, min = 2.3%, max = 29.1%, n = 39) and good selectivity with low matrix effects (mean = 8.8%, median = 9.5%, min = −45.8%, max = 28.6%, n = 20). Exceptions to this trend included the RSD values for hydrocodone (15.2%), terbutaline (15.9%), THC (16.4%), morphine (16.8%), and metoprolol (29.1%). In addition, significant signal suppression was observed for THC (matrix effect = −45.8%), while signal enhancements were recorded for amphetamine (matrix effect = +21.2%), hydrocodone (+25.8%), and morphine (+28.6%). Some of these results, which were generally less favorable than those obtained with the PA6 + CF15 prototype, may have resulted from the incomplete elution of polyvinyl acid during the preconditioning process (i.e., by allowing the device to soak in water for 4 days with daily water changes, per manufacturer specifications).

3.6. S-FLEX 90A

S-FLEX 90A is the commercial name of a thermoplastic polyurethane (TPU) with a 90A score on the Shore Hardness Scale. Unlike the PA6 + CF15 and LAYFOMM-60 polymers, 3D printed prototypes made from S-FLEX 90A do not require pre- or post-processing. Furthermore, S-FLEX 90A is also relatively user friendly, as its high elasticity helps to avoid damage to the prototype during agitation-aided sample preparation, which can sometimes pose a problem with more brittle polymers, such as polymethyl methacrylate (PMMA) and polyamide 12 reinforced with 15% carbon fiber (PA12 + CF15), or with certain functionalized silica particle-based stationary phases used in conventional microextraction devices.

Similar to LAYFOMM-60, the adsorption of small molecules to S-FLEX 90A is mediated by the hydrophobic, hydrogen bonding, dipole–dipole, and π–π type interactions enabled by the polyurethane [42–44]. However, the porosity of the S-FLEX 90A prototype was not enhanced after 3D printing; as a result, the observed performance of these two polymers differed considerably for some analytes.

The S-FLEX 90A prototype enabled the successful determination of 25 of 43 analytes. As with the LAYFOMM-60 prototype, the S-FLEX 90A device was best suited for the extraction of very non-polar analytes, with the best results being recorded for stanozolol (logP = 4.5), THC (logP = 7.0), and tamoxifen (logP = 7.1). In particular, the highest extraction efficacies were observed for stanozolol (89.5%, RSD = 20.1%, n = 4), tamoxifen (66.6%, RSD = 19.6%, n = 4), buprenorphine (65.1%, RSD = 5.5%, n = 4), and agomelatine (51.7%, RSD = 4.9%, n = 4).

The RSD values indicated that the S-FLEX 90A device provided repeatable extraction (mean = 9.4%, median = 7.6%, min = 3.1%, max = 24.3%, n = 25), while the recorded matrix effects confirmed its selectivity (mean = 12.1%, median = 15.0%, min = −18.2%, max = 35.7%, n = 20). Rare outliers with RSD values exceeding the 15% threshold included clonazepam (15.4%), tamoxifen (19.6%), stanozolol (20.1%), and THC (24.3%). While significant signal suppression (matrix effect below −20%) was not observed, signal enhancement (matrix effects exceeding +20%) was present in the cases of heroin (+22.1%), clenbuterol (+24.1%), and hydrocodone (+35.7%).

3.7. Other polymers

Apart from the polyamide 6 (reinforced with 15% carbon fiber) and polyurethane-based devices discussed above, prototypes were also fabricated using ABS, PA12 + CF15, PC-PBT, PET-G, PLA, PMMA, PP, and HIPS. The findings indicated that each of these polymers provided extraction efficacies that enabled the successful quantification of selected analytes. Overall, 14 of the 15 tested polymers provided results above the LOQs established in this study. The sole exception to this trend was 1 of the 3 tested PLA-based devices. The two most hydrophobic substances were successfully extracted by every type of polymer; specifically, the extraction efficacies for tamoxifen (logP = 7.1) ranged from 3.2% to 66.6% (n = 14), and from 2.4% to 27.5% (n = 14) for THC (logP = 7.0). The findings further indicated that many of the tested polymers were suitable for extraction of other hydrophobic analytes. For instance, with the exception of PP and plain PLA, all of the polymers were able to extract nebivolol (logP = 3.0) with efficacies ranging from 2.2% to 72.9% (n = 13). Similarly, with the exception of PET-G (both types), PLA (2 of 3 types), and PMMA, all of the tested polymers provided extraction efficacies ranging from 2.2% to 72.9% (n = 10) for buprenorphine (logP = 5.0).

Notably, inconsistencies were observed among the devices made from the same type of polymers sourced from different manufacturers. For instance, the differences between the two types of PET-G were marginal, with a maximal recorded difference of merely 0.8% (n = 3) and a relative error of 0.21. Similarly, the maximal difference in extraction efficacy between the two types of ABS was only 3.7% (n = 7), with a relative error of only 0.34. However, significant differences in the number of analytes quantified after sample preparation were observed among the 3 types of PLA-based prototypes (i.e., 5 *vs* 3 *vs* 0). Thus, using these polymers for sample preparation without accounting for the subtle differences in their chemical constitution (and resulting adsorptive properties) could prove problematic. For example, while the PLA-PEARL device provided extraction efficacies of 12.4% (RSD = 2.1%) and 13.1% (RSD = 3.1%) for tamoxifen and nebivolol, respectively, the plain PLA device was unable to extract these substances above the LOQ.

3.8. Further discussion

The main advantage of 3D printed sample preparation devices is the enormous flexibility they provide to adjust the chemistry of the extraction phase and shape of the device to create tailored extraction devices in-lab that are suited to a specific analyte or application. Furthermore, the proposed methodology is rather inexpensive, as prices of 3D printing filament spools used in this study (750 g) were within 18 $–85 $ range. It should also be emphasized that the overall cost and associated workload are predominantly affected by the type of targeted small molecule. With some analytes, relatively basic polymers such as ABS or S-FLEX 90A were able to provide results that were comparable to those of more expensive and time-consuming alternatives requiring pre- or post-processing. It is also essential to highlight the fact that, in this study, prototyping a single sample preparation device only required 4.42 g of 3D printing filament (1.48 m of the spool). Thus, a single spool of filament could be sufficient to create around 170 prototypes that

corresponds to a cost within 0.1 $–0.5 $ range (per unit). The printing process took only 26 min per device and was fully automated after loading the filament into the printer. Upon the completion of the 3D printing, some of the prototypes had to be stripped of the polymer strands left by idle printer head movements. This procedure took little time (3–5 min per prototype), was undemanding, and could be performed with basic pliers. However, this step can be eliminated by using a more advanced 3D printer (with enclosed print chambers), as these printers offer significantly higher print resolution and leave no remnants of the polymer on the final device. Thus, the use of a more advanced 3D printer can save preparation time and improve the repeatability of the devices.

Without a doubt, the need to thermally precondition the PA6 + CF15 filament or elute polyvinyl alcohol (PVA) from LAYFOMM-60 prototypes is inconvenient. Nevertheless, compared to the methods used to fabricate conventional microextraction sample preparation devices, which usually involve numerous harmful reagents, the proposed 3D printing method provides numerous clear benefits, particularly in relation to the safety of laboratory personnel and reduced environmental impact. As such, polyamide-based adsorptive polymers such as PA6 + CF15 are especially appealing materials for future research. Not only can these polymers be produced and recycled in a fully sustainable manner [18,19], but no chemical waste is generated during either process. Furthermore, the use of the FDM 3D printing method supports the zero-waste principle of green analytical chemistry, as it only consumes the portion of polymer required to fabricate the sampling device, while leaving the remainder for future use with no negative consequences (e.g., spoilage).

Ultimately, the ability to easily implement 3D printed prototypes into existing analytical protocols – as was demonstrated in this study – and the unrestricted customization offered by 3D printing opens up an entire vista of new and exciting opportunities in modern analytical chemistry.

4. Conclusions

This study constitutes, by far, the most comprehensive comparison of 3D printed thermoplastic polymers with adsorptive properties used for the extraction of small molecules. The selected polymers included most chemistries currently available for FDM 3D printing that are compatible with basic 3D printers, and, as such, it should result in readily available information for analytical scientists who are interested in enhancing their research with 3D printing techniques at a relatively minimal cost. However, many of the polymers examined in this work had never previously been trialed as extractive phases, nor had their compatibility with popular analytical methods, such as HPLC separation or mass spectrometry detection, been assessed. This work addressed this gap in the literature by applying 15 polymer-based prototypes to extract a large and representative group of 43 model compounds from fortified human oral fluid in order to assess their extraction efficacy and selectivity for the target analytes.

The results showed that the prototypes fabricated from polyamide 6 (PA6 + CF15) and two polyurethane-based polymers (LAYFOMM-60 and S-FLEX 90A) significantly outperformed the other tested prototypes. The firm structure of polyamide 6 proved advantageous, as it contributed to the PA6 + CF15 device's excellent extraction repeatability (mean RSD value of 5.3%, compared to 9.6% and 9.4% for LAYFOMM-60 and S-FLEX 90A, respectively). Most likely, this result was due to the more consistent kinetics maintained between the 3D printed PA6 + C15 extraction device and the sample, as the device's firm structure allowed for more effective sample agitation, which is needed to eliminate the detrimental depletion zone of slow analyte diffusion throughout the stationary layer of liquid sample that gathers around the extraction probes [45]. On the contrary, the more flexible polyurethane-based polymers were less susceptible to damage during the fabrication of the sample preparation devices and sample analysis. However, uneven sample agitation during extraction resulted in the decreased repeatability of the process.

It should further be emphasized that the tested polymers (particularly polyamides [46], carbon fiber [47], and polyurethanes [48]) are entirely biocompatible and biodegradable, largely due to the presence of amide groups. These amide groups are responsible for the effective mechanisms of small-molecule adsorption (i.e., via hydrogen bonding, dipole–dipole, and π–π type interactions), and they also mimic natural peptide bonds to avoid triggering immune responses in living donors. This biocompatibility is an essential feature of the tested polymers, as it allows them to be safely implemented for the analysis of any given biological sample, especially in *in vivo* studies.

The use of the proposed 3D printing technique to prepare extraction devices offers some key advantages. First, it enables a nearly endless selection of surfaces and shapes for prototypes, thus allowing the produced devices to meet even the most specific demands of various applications. Moreover, the costs associated with this method are remarkably low, with the cost of single prototype ranging from 0.1 $ to 0.5 $. Furthermore, prototyping with the FDM 3D printing method is very convenient, as it significantly reduces the time and effort required to prepare a extraction device [2]. This benefit was demonstrated in the present study, as it only took 26 min to produce a ready-to-use product using the proposed fully automated preparation process. Thus, these advantages can enable the fabrication of a large number of selective sample preparation devices in a short time with relatively minimal cost and labor. At the same time, the proposed 3D printing method offers a feasible means of producing customized devices for more intricate applications, while still offering excellent reproducibility due to its automation capability. In addition, no prior expertise in 3D modelling is necessary, as the prepared printable datafile can be shared with fellow analysts and used to prepare prototypes on any given FDM 3D printer, thanks to the universal file formats.

Finally, it is worth emphasizing that the demonstrated extraction efficacies and selectivity towards the targeted small molecules were achieved using basic and commercially available 3D printing polymers, without any additional chemical functionalization or extensive optimalization of the extraction protocols. Therefore, while this study is a valuable guide to the use of 3D printed polymers for the extraction of small molecules, further research is required to fully exploit the potential of this emerging technique in analytical chemistry.

Credit author statement

Dominika Kołodziej[#]: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Visualization, Validation, Writing – original draft; review & editing; Łukasz Sobczak[#]: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Visualization, Validation, Writing – review & editing; Krzysztof Goryński: Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing. [#]These authors contributed equally.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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Supporting Information for

Innovative, simple, and green: A sample preparation method based on 3D printed polymers

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Table S1. 3D printing parameters used for preparation of extraction devices.

#	filament	recommended printing temperature [°C]	recommended heat bed temperature [°C]	actual printing temperature [°C]	actual heat bed temperature [°C]	working sheet type
1	ABS	250–265	90–110	260	105 → 110 [a]	textured
2	ABS PRO	220–240	80	255	105 → 110 [a]	textured
3	HIPS	220–240	100	220	100 → 110 [a]	smooth
4 [b]	LAYFOMM-60	220–230	< 50	225	60	textured
5 [c]	PA6 + CF15	235–260	80–100	260	90	textured
6	PA12 + CF15	255–270	90–110	260	90	textured
7	PC-PBT	260–280	100–115	265	105 → 110 [a]	textured
8	PET-G (Fiberlab)	230–250	90	240	90	textured
9	PET-G (Prusa)	250	70–90	230 → 240	85 → 90 [a]	textured
10	PLA	200–220	60	215	60	smooth
11	PLA EASY	200–230	50–70	215	57	smooth
12	PLA PEARL	190–220	< 60	215	60	smooth
13	PMMA	230–250	60–90	267	110	smooth
14	PP	210–260	90–130	190	100 → 110 [a]	smooth
15	S-FLEX 90A	200–230	50–70	220	50	textured

Polymers arranged in alphabetical order.

[a] Indicates temperature increase after printing the initial layer.

[b] 3D printed prototype conditioned by soaking in water for 4 days (with daily water change), to elute water-soluble polyvinyl alcohol.

[c] Filament conditioned in oven at 80 °C for 2 h before printing.

Table S2. List of the reference standards.

substance	full name	form	grade (purity)	brand
6-acetylcodeine	6-acetylcodeine	1 mg/mL acetonitrile solution	certified reference material	Cerillant®, Merck Group
agomelatine	agomelatine	1 mg/mL methanol solution (prepared from pure substance)	analytical standard	Toronto Research Chemicals
alprazolam	alprazolam	1 mg/mL methanol solution	certified reference material	LGC Standards
amphetamine	(+\-)-amphetamine	1 mg/mL methanol solution	certified reference material	LGC Standards
amphetamine D_5	(+\-)-amphetamine D_5 (label on ring)	100 µg/mL methanol solution	certified reference material	Cerillant®, Merck Group
anastrozole	anastrozole	1 mg/mL methanol solution (prepared from pure substance)	reference standard (100%)	LGC Standards
atenolol	atenolol	1 mg/mL acetonitrile solution	certified reference material	LGC Standards
bisoprolol	bisoprolol (fumarate)	1 mg/mL methanol solution	certified reference material	LGC Standards
boldenone	boldenone	1 mg/mL methanol solution (prepared from pure substance)	analytical standard (≥ 98%)	VETRANAL® (Sigma-Aldrich, Merck Group)
buprenorphine	buprenorphine	1 mg/mL methanol solution	certified reference material	Cerillant®, Merck Group
buprenorphine D_4	buprenorphine D_4	100 µg/mL methanol solution	certified reference material	Cerillant®, Merck Group
carteolol	carteolol (hydrochloride)	1 mg/mL methanol solution (prepared from pure substance)	USP reference standard (100%)	United States Pharmacopeia
clenbuterol	clenbuterol (hydrochloride)	1 mg/mL DMSO solution	certified reference material	LGC Standards
clenbuterol D_9	clenbuterol D_9 (hydrochloride)	100 µg/mL methanol solution	certified reference material	Cerillant®, Merck Group
clonazepam	clonazepam	1 mg/mL methanol solution	certified reference material	Cerillant®, Merck Group
clonazepam D_4	clonazepam D_4	100 µg/mL methanol solution	certified reference material	Cerillant®, Merck Group
cocaine	cocaine	1 mg/mL acetonitrile solution	certified reference material	LGC Standards
cocaine D_3	cocaine D_3	100 µg/mL acetonitrile solution	certified reference material	Cerillant®, Merck Group
fenoterol	fenoterol (hydrobromide)	1 mg/mL methanol solution (prepared from pure substance)	reference standard (99.9%)	LGC Standards
fentanyl	fentanyl	1 mg/mL methanol solution	certified reference material	LGC Standards
fentanyl D_5	fentanyl D_5	100 µg/mL methanol solution	certified reference material	Cerillant®, Merck Group
heroin	heroin	1 mg/mL acetonitrile solution	certified reference material	LGC Standards

substance	full name	form	grade (purity)	brand
heroin D_9	heroin D_9	100 µg/mL acetonitrile solution	certified reference material	Cerillant®, Merck Group
higenamine	higenamine (hydrochloride)	1 mg/mL methanol solution (prepared from pure substance)	HPLC (≥ 95%)	Sigma®, Merck Group
hydrocodone	hydrocodone	1 mg/mL methanol solution	certified reference material	Cerillant®, Merck Group
hydrocodone D_3	hydrocodone D_3	100 µg/mL methanol solution	certified reference material	Cerillant®, Merck Group
ibutamoren	ibutamoren (mesylate)	1 mg/mL methanol solution (prepared from pure substance)	analytical standard (≥ 98%)	Cayman Chemicals
ketamine	ketamine (hydrochloride)	1 mg/mL methanol solution	certified reference material	LGC Standards
ketamine D_4	ketamine D4 (hydrochloride)	100 µg/mL methanol solution	certified reference material	LGC Standards
LSD	lysergic acid diethylamide	1 mg/mL acetonitrile solution	certified reference material	LGC Standards
LSD D_3	lysergic acid diethylamide D_3	100 µg/mL acetonitrile solution	certified reference material	Cerillant®, Merck Group
MDMA	(+\-)-3,4-methylenedioxy methamphetamine	1 mg/mL methanol solution	certified reference material	LGC Standards
MDMA D_5	(+\-)-3,4-methylenedioxy methamphetamine D_5	100 µg/mL methanol solution	certified reference material	Cerillant®, Merck Group
meldonium	meldonium (dihydrate)	1 mg/mL methanol solution (prepared from pure substance)	Ph.Eur. reference standard	European Directorate for the Quality of Medicines & HealthCare
methadone	(+\-)-methadone	1 mg/mL methanol solution	certified reference material	Cerillant®, Merck Group
methadone D_3	(+\-)-methadone D_3	100 µg/mL methanol solution	certified reference material	Cerillant®, Merck Group
methamphetamine	(+\-)-methamphetamine	1 mg/mL methanol solution	certified reference material	LGC Standards
methamphetamine D_5	(+\-)-methamphetamine D_5	100 µg/mL methanol solution	certified reference material	Cerillant®, Merck Group
methandienone	methandienone	1 mg/mL 1,2-dimethoxyethane solution	certified reference material	Cerillant®, Merck Group
methylphenidate	methylphenidate (hydrochloride, racemic)	1 mg/mL methanol solution	certified reference material	LGC Standards
metoprolol	metoprolol (tartrate)	1 mg/mL methanol solution	certified reference material	LGC Standards
morphine	morphine	1 mg/mL methanol solution	certified reference material	LGC Standards
morphine D_6	morphine D_6	100 µg/mL methanol solution	certified reference material	Cerillant®, Merck Group
nandrolone	nandrolone	1 mg/mL acetonitrile solution	certified reference material	LGC Standards

substance	full name	form	grade (purity)	brand
nebivolol	nebivolol (hydrochloride)	1 mg/mL methanol solution (prepared from pure substance)	HPLC (≥ 98%)	Sigma®, Merck Group
nikethamide	nikethamide	1 mg/mL methanol solution (prepared from pure substance)	analytical standard (99%)	Aldrich, Merck Group
oxycodone	oxycodone	1 mg/mL methanol solution	certified reference material	LGC Standards
oxycodone D_3	oxycodone D_3	100 µg/mL methanol solution	certified reference material	Cerillant®, Merck Group
phencyclidine	phencyclidine	1 mg/mL methanol solution	certified reference material	LGC Standards
phencyclidine D_5	phencyclidine D_5	100 µg/mL methanol solution	certified reference material	Cerillant®, Merck Group
propranolol	propranolol (hydrochloride)	1 mg/mL methanol solution	certified reference material	LGC Standards
propranolol D_7	propranolol D_7 (label on ring)	100 µg/mL methanol + 5% 1M HCl solution	certified reference material	Cerillant®, Merck Group
salbutamol	salbutamol (sulfate)	1 mg/mL methanol solution	certified reference material	LGC Standards
stanozolol	stanozolol	1 mg/mL acetonitrile solution	certified reference material	LGC Standards
strychnine	strychnine (hydrochloride)	1 mg/mL methanol solution (prepared from pure substance)	analytical standard (≥ 98%)	Sigma®, Merck Group
tamoxifen	tamoxifen	1 mg/mL methanol solution (prepared from pure substance)	analytical standard (> 98%)	Sigma-Aldrich, Merck Group
terbutaline	terbutaline (hemisulfate)	1 mg/mL methanol solution (prepared from pure substance)	reference standard (99.9%)	LGC Standards
THC	(-)-Δ^9-tetrahydrocannabinol	1 mg/mL methanol solution	certified reference material	LGC Standards
THC D_3	(-)-Δ^9-tetrahydrocannabinol D_3	100 µg/mL methanol solution	certified reference material	Cerillant®, Merck Group
THC-COOH	(-)-11-nor-9-carboxy-Δ^9-tetrahydrocannabinol	1 mg/mL methanol solution	certified reference material	LGC Standards
THC-COOH D_3	(+\-)-11-nor-9-carboxy-Δ^9-tetrahydrocannabinol D_3	100 µg/mL methanol solution	certified reference material	Cerillant®, Merck Group
zolpidem	zolpidem (tartrate)	1 mg/mL methanol solution	certified reference material	LGC Standards
zolpidem D_6	zolpidem D_6	100 µg/mL methanol solution	certified reference material	Cerillant®, Merck Group

Substances arranged in alphabetical order.

Table S3. Selected HPLC-MS/MS parameters.

substance	retention time [min]	precursor ion [m/z]	product ions [m/z]		
			1	2	3
meldonium	1.496	147.00	58.10	59.15	
morphine D_6	2.342	292.00	152.15	153.10	181.05
morphine	2.356	286.10	152.10	201.15	165.10
salbutamol	2.632	240.00	148.10	222.25	166.20
terbutaline	2.745	226.00	152.10	107.05	125.10
atenolol	2.814	267.00	145.10	190.20	74.15
higenamine	3.819	272.00	107.05	255.20	161.10
fenoterol	4.154	304.00	107.15	135.15	286.10
nikethamide	4.279	178.90	108.05	80.10	72.10
carteolol	4.490	293.00	237.15	202.15	74.10
amphetamine D_5	4.491	141.10	96.10	124.15	95.10
oxycodone D_3	4.521	319.00	301.20	244.10	259.20
amphetamine	4.545	135.90	91.10	119.15	65.10
oxycodone	4.556	316.10	298.20	241.20	256.10
hydrocodone D_3	4.895	303.00	199.10	171.15	128.00
hydrocodone	4.900	300.00	199.15	171.10	128.15
methamphetamine D_5	4.951	154.90	92.10	91.10	121.20
methamphetamine	4.967	150.00	91.10	65.10	119.20
MDMA D_5	5.156	198.90	165.10	107.15	135.20
MDMA	5.173	194.00	163.10	105.10	133.10
strychnine	5.355	334.90	184.10	156.20	129.15
ketamine D_4	5.674	242.00	129.05	224.10	211.10
ketamine	5.702	237.90	125.05	220.10	207.15
metoprolol	6.487	267.90	116.15	74.15	72.10
clenbuterol D_9	6.578	286.00	204.10	268.20	169.05
clenbuterol	6.616	277.10	203.05	132.10	168.15
methylphenidate	6.745	234.00	84.15	56.10	91.10
6-acetylcodeine	6.892	342.00	225.15	165.15	197.20
heroin D_9	6.934	379.00	272.20	212.15	335.20
heroin	6.971	370.10	165.15	268.20	211.10
zolpidem D_6	7.451	314.00	235.10	236.20	263.20
cocaine D_3	7.468	307.00	185.20	77.10	85.20
cocaine	7.473	303.90	182.20	82.10	105.10
zolpidem	7.510	307.90	235.15	236.20	263.20
LSD D_3	7.821	327.00	226.20	210.20	208.15
LSD	7.827	323.90	223.20	208.15	207.10
bisoprolol	8.010	326.20	116.20	74.05	72.10
phencyclidine D_5	8.736	249.00	96.15	86.15	164.25
phencyclidine	8.782	244.00	91.05	86.10	159.20
propranolol D_7	8.868	267.00	117.20	189.20	161.20
propranolol	8.940	260.10	116.20	183.10	155.20
fentanyl D_5	9.266	342.10	188.20	105.10	103.05
fentanyl	9.305	337.00	105.15	188.20	103.10

substance	retention time [min]	precursor ion [m/z]	product ions [m/z]		
			1	2	3
buprenorphine D_4	9.863	472.10	59.15	400.20	415.25
buprenorphine	9.870	468.10	55.15	396.25	414.25
ibutamoren	10.577	529.00	267.10	91.15	263.15
nebivolol	11.328	406.10	151.10	123.10	103.10
alprazolam	11.451	308.90	205.15	281.15	274.10
anastrozole	11.465	294.00	225.20	210.20	115.05
methadone D_3	11.574	313.10	268.15	105.05	77.15
methadone	11.593	310.00	265.15	105.10	77.05
stanozolol	11.610	329.10	81.15	95.15	121.10
clonazepam D_4	11.971	319.90	274.15	218.15	211.20
boldenone	11.996	287.00	121.20	135.20	77.15
clonazepam	12.026	316.00	270.10	214.05	207.15
agomelatine	12.170	244.00	185.20	170.10	141.10
nandrolone	12.378	275.10	109.10	239.10	257.30
methandienone	12.636	301.20	121.05	149.25	77.00
tamoxifen	15.676	372.00	72.10	70.10	129.15
THC-COOH D_3	19.895	348.00	330.20	302.25	196.25
THC-COOH	19.929	345.00	327.10	299.25	193.30
THC D_3	24.776	317.80	195.05	235.95	85.05
THC	24.802	315.00	193.10	123.10	259.15

Substances arranged by retention time.

Table S4. Results of HPLC-MS/MS system assessment: repeatability (relative standard deviations (RSDs)), coefficients of determination (R^2), and constituents of regression curve equations determined during calibration runs.

substance	RSD (n = 20)	R^2 (n = 8)	regression curve equation	
			slope	intercept
meldonium	7.6%	0.9996	3.3E-11	-1.1E-13
morphine	19.3%	0.9814	4.2E-10	1.2E-11
salbutamol	6.5%	0.9999	1.7E-11	1.9E-17
terbutaline	3.8%	0.9994	2.7E-11	-1.1E-13
atenolol	11.3%	0.9991	1.9E-10	-9.7E-13
higenamine	5.7%	0.9999	6.3E-11	-1.2E-13
fenoterol	5.5%	0.9994	1.5E-11	-5.7E-14
nikethamide	6.3%	0.9999	4.0E-11	-1.3E-14
carteolol	6.1%	0.9992	3.7E-11	-1.5E-13
amphetamine	10.0%	0.9994	1.8E-10	-1.0E-12
oxycodone	9.0%	0.9954	9.3E-11	-1.1E-12
hydrocodone	6.1%	0.9972	3.7E-10	-3.5E-12
methamphetamine	5.4%	0.9991	9.5E-12	-4.9E-14
MDMA	5.8%	0.9999	3.0E-11	-6.4E-14
strychnine	7.0%	0.9991	5.6E-10	-3.3E-12
ketamine	6.8%	0.9993	3.4E-11	-1.5E-13
metoprolol	5.3%	0.9999	4.2E-10	-7.8E-13
6-acetylcodeine	8.5%	0.9959	1.3E-10	-1.4E-12
clenbuterol	5.7%	0.9995	4.6E-11	-2.0E-13
methylphenidate	5.7%	1.0000	4.4E-12	2.7E-15
heroin	10.0%	0.9934	6.2E-09	-8.6E-11
zolpidem	5.9%	0.9999	7.8E-12	-2.0E-14
cocaine	6.3%	0.9997	1.3E-11	-4.8E-14
LSD	5.5%	0.9988	2.3E-11	8.0E-14
bisoprolol	9.3%	0.9999	2.7E-11	-5.4E-14
phencyclidine	6.0%	1.0000	2.3E-11	1.9E-14
propranolol	6.3%	0.9984	1.3E-10	-8.1E-13
fentanyl	5.4%	0.9994	6.1E-12	-2.7E-14
buprenorphine	7.9%	0.9993	2.9E-10	-1.4E-12
ibutamoren	5.6%	0.9981	1.7E-10	-1.3E-12
nebivolol	9.6%	0.9999	1.1E-10	2.9E-13
alprazolam	5.7%	0.9899	6.6E-10	8.5E-12
anastrozole	7.3%	1.0000	9.1E-11	-3.6E-14
stanozolol	9.7%	0.9939	1.2E-08	-1.7E-10
methadone	5.8%	1.0000	5.0E-12	-5.5E-15
boldenone	5.2%	0.9921	2.0E-09	-3.2E-11
clonazepam	8.3%	1.0000	2.3E-10	-1.5E-13
agomelatine	5.4%	0.9997	1.5E-10	3.0E-13
nandrolone	7.2%	0.9992	7.7E-08	6.9E-11
methandienone	4.3%	0.9999	1.2E-09	-1.0E-12
tamoxifen	5.2%	0.9999	1.5E-12	-2.8E-15
THC-COOH	6.0%	1.0000	4.1E-10	-2.2E-13
THC	8.1%	0.9999	2.3E-09	-9.1E-14

Substances arranged by retention order.

Table S5. Results (RSD values) of partial validation of sample preparation method with reference adsorptive polymer type.

substance	precision (n = 4)	accuracy (n = 3)
fenoterol	9.5%	8.9%
carteolol	4.4%	10.5%
oxycodone	7.7%	1.5%
hydrocodone	7.3%	26.4%
MDMA	4.5%	5.9%
strychnine	5.1%	11.4%
ketamine	5.0%	3.5%
metoprolol	8.4%	21.2%
6-acetylcodeine	1.8%	7.3%
clenbuterol	2.0%	12.7%
methylphenidate	5.5%	2.9%
zolpidem	2.9%	6.7%
cocaine	3.6%	2.3%
LSD	2.2%	3.7%
bisoprolol	3.0%	3.0%
phencyclidine	3.6%	2.5%
propranolol	1.2%	6.4%
fentanyl	3.2%	1.6%
buprenorphine	3.8%	15.5%
ibutamoren	4.7%	2.7%
nebivolol	1.2%	15.0%
alprazolam	1.6%	8.1%
anastrozole	1.9%	12.7%
stanozolol	8.1%	7.7%
methadone	3.5%	2.0%
boldenone	5.2%	4.3%
clonazepam	5.5%	5.2%
agomelatine	4.3%	2.3%
nandrolone	6.2%	23.5%
methandienone	4.4%	9.3%
tamoxifen	3.0%	20.2%
THC-COOH	1.7%	11.5%
THC	4.9%	31.0%

Substances arranged by retention order, polymer type = polyamide 6 reinforced with 15% carbon fiber.

Table S6. Extraction efficacies [%] of the evaluated polymers.

polymer / substance	ABS	ABS PRO	HIPS	LAYFOMM -60	PA6 + CF15	PA12 + CF15	PC-PBT	PET-G (Fiberlab)	PET-G (Prusa)	PLA	PLA EASY	PLA PEARL	PMMA	PP	S-FLEX 90A
meldonium	0.1* (26.6)	0.2* (19.8)	0.1* (9.7)	0.6* (25.0)	0.7* (4.3)	0.4* (10.1)	0.1* (19.4)	0.2* (33.7)	0.1* (22.2)	0.1* (17.9)	0.2* (13.2)	0.1* (37.6)	0.3* (18.1)	0.2* (5.5)	0.3* (12.9)
morphine	0.2* (70.0)	0.1* (39.6)	0.2* (21.5)	2.4 (16.8)	8.5 (32.9)	0.5* (12.9)	0.1* (29.9)	0.2* (41.1)	0.1* (41.4)	0.2* (38.1)	0.2* (69.3)	0.2* (15.6)	0.3* (23.9)	0.2* (18.3)	0.4* (47.3)
salbutamol	0.2* (24.2)	0.2* (16.1)	0.1* (5.3)	1.2* (21.9)	7.2 (3.0)	0.5* (19.5)	0.1* (37.4)	0.2* (23.4)	0.1* (20.8)	0.1* (13.4)	0.2* (10.5)	0.1* (39.4)	0.3* (16.9)	0.2* (17.7)	0.3* (15.9)
terbutaline	0.2* (32.0)	0.2* (21.0)	0.1* (16.5)	2.1 (15.9)	16.3 (3.6)	0.5* (9.5)	0.1* (23.2)	0.2* (33.5)	0.1* (23.3)	0.1* (19.5)	0.2* (8.5)	0.1* (30.0)	0.3* (22.8)	0.2* (24.3)	0.3* (18.2)
atenolol	0.2* (23.2)	0.2* (18.9)	0.1* (27.0)	1.0* (21.1)	5.0 (17.8)	0.4* (17.0)	0.1* (54.4)	0.2* (43.5)	0.1* (23.6)	0.1* (26.1)	0.2* (28.4)	0.2* (31.9)	0.3* (18.2)	0.2* (47.4)	0.3* (35.6)
bigenamine	0.3* (12.2)	0.2* (25.8)	0.2* (27.0)	16.4 (8.4)	50.9 (0.9)	0.6* (14.2)	0.1* (41.1)	0.3* (25.7)	0.2* (15.6)	0.2* (14.5)	0.3* (10.9)	0.2* (26.4)	0.3* (19.5)	0.2* (24.1)	0.8* (4.6)
fenoterol	0.2* (22.9)	0.2* (11.9)	0.2* (22.6)	9.7 (5.8)	20.4 (9.5)	0.5* (15.9)	0.1* (22.5)	0.2* (26.7)	0.1* (23.1)	0.2* (21.7)	0.2* (13.3)	0.2* (19.5)	0.3* (38.2)	0.2* (6.3)	0.5* (24.8)
nikethamide	0.1* (49.3)	0.2* (31.4)	0.1* (37.0)	1.4* (18.3)	1.3* (4.1)	0.4* (23.4)	0.1* (30.2)	0.1* (33.4)	0.1* (43.3)	0.1* (10.5)	0.1* (20.2)	0.1* (10.8)	0.3* (16.6)	0.2* (14.7)	0.6* (11.4)
carteolol	0.2* (28.9)	0.2* (28.5)	0.1* (34.4)	2.7 (11.2)	12.9 (4.4)	0.5* (19.8)	0.1* (38.3)	0.2* (24.6)	0.1* (27.6)	0.1* (40.5)	0.2* (33.4)	0.1* (34.8)	0.3* (23.4)	0.2* (22.3)	0.4* (20.7)
oxycodone	0.2* (34.9)	0.3* (31.6)	0.1* (12.8)	5.0 (7.5)	3.3 (9.2)	0.5* (15.5)	0.1* (34.3)	0.2* (26.2)	0.1* (29.3)	0.1* (49.6)	0.2* (24.3)	0.2* (42.2)	0.4* (27.4)	0.2* (27.5)	2.1 (5.6)
amphetamine	0.2* (74.6)	0.3* (25.3)	0.1* (124.7)	3.5 (12.0)	7.2 (7.7)	0.5* (15.9)	0.1* (76.7)	0.1* (73.6)	0.2* (75.8)	0.1* (77.6)	0.1* (71.0)	0.2* (76.5)	0.3* (31.5)	0.2* (40.9)	0.3* (28.6)
hydrocodone	0.2* (119.4)	0.1* (118.9)	0.1* (23.8)	5.2 (15.2)	5.5 (7.3)	0.5* (15.1)	0.2* (116.8)	0.1* (116.5)	0.0* (200.0)	0.2* (66.7)	0.2* (34.9)	0.1* (15.4)	0.3* (39.9)	0.2* (67.8)	1.1* (9.8)
methamphetamine	0.2* (6.9)	0.3* (6.9)	0.1* (27.1)	4.0 (13.1)	7.2 (5.4)	0.5* (7.3)	0.1* (30.7)	0.2* (28.7)	0.1* (6.0)	0.1* (40.7)	0.1* (31.8)	0.1* (41.6)	0.2* (22.1)	0.2* (25.6)	0.3* (16.7)
MDMA	0.2* (37.3)	0.3* (24.0)	0.1* (16.9)	6.5 (10.9)	10.2 (4.5)	0.5* (18.0)	0.1* (47.1)	0.2* (17.1)	0.1* (17.1)	0.1* (26.4)	0.2* (24.0)	0.2* (40.3)	0.3* (29.4)	0.2* (18.3)	0.4* (12.7)
strychnine	0.3* (19.3)	0.7* (30.8)	0.1* (62.3)	22.1 (12.9)	15.4 (5.1)	0.5* (36.4)	0.2* (35.7)	0.2* (27.6)	0.2* (6.2)	0.1* (12.0)	0.2* (45.2)	0.3* (57.6)	0.2* (44.2)	0.2* (35.6)	4.3 (14.5)
ketamine	0.4* (21.2)	0.4* (4.2)	0.1* (29.5)	22.3 (8.2)	9.6 (5.0)	0.6* (12.5)	0.1* (25.4)	0.2* (49.0)	0.1* (24.2)	0.1* (20.1)	0.1* (38.3)	0.1* (26.4)	0.3* (36.1)	0.3* (12.3)	15.9 (5.2)
metoprolol	0.2* (77.9)	0.2* (31.1)	0.1* (32.4)	3.8 (29.1)	5.7 (8.4)	0.4* (27.6)	0.1* (32.5)	0.2* (43.8)	0.1* (29.6)	0.1* (40.2)	0.1* (32.1)	0.1* (22.8)	0.3* (23.3)	0.1* (86.0)	0.3* (21.8)
6-acetylcodeine	0.4* (42.7)	0.5* (33.5)	0.1* (27.0)	15.2 (9.0)	8.2 (1.8)	0.4* (18.6)	0.1* (30.6)	0.2* (35.4)	0.1* (68.3)	0.1* (35.3)	0.2* (24.0)	0.2* (42.4)	0.3* (30.1)	0.2* (35.6)	4.8 (11.6)
clenbuterol	0.3* (42.0)	0.5* (26.3)	0.1* (40.6)	34.8 (7.9)	20.9 (2.0)	0.6* (14.4)	0.2* (17.5)	0.2* (18.5)	0.1* (42.5)	0.1* (55.0)	0.2* (24.9)	0.4* (11.9)	0.3* (23.8)	0.2* (21.9)	1.5* (12.7)
methylphenidate	0.4* (26.0)	0.4* (5.1)	0.1* (17.4)	9.8 (9.9)	9.4 (5.5)	0.5* (12.1)	0.1* (11.5)	0.2* (24.7)	0.1* (21.5)	0.1* (26.7)	0.2* (17.5)	0.2* (10.8)	0.3* (27.6)	0.2* (7.5)	1.5* (10.3)
heroin	0.1* (84.6)	0.2* (47.5)	0.1* (72.5)	6.0 (15.0)	not detectable	0.2* (25.3)	0.1* (70.4)	0.0* (118.9)	0.1* (116.3)	0.0* (116.8)	0.1* (35.1)	0.1* (55.0)	0.1* (133.6)	0.1* (126.6)	1.1* (23.2)
zolpidem	0.3* (16.3)	0.2* (24.5)	0.2* (23.5)	12.7 (7.4)	25.6 (2.9)	0.6* (10.3)	0.2* (32.0)	0.2* (39.1)	0.2* (23.3)	0.1* (11.5)	0.2* (17.3)	0.2* (21.8)	0.3* (17.7)	0.2* (7.2)	10.7 (4.6)

polymer / substance	ABS	ABS PRO	HIPS	LAYFOMM 60	PA6 + CF15	PA12 + CF15	PC-PBT	PET-G (Fiberlab)	PET-G (Prusa)	PLA	PLA EASY	PLA PEARL	PMMA	PP	S-FLEX 90A
cocaine	0.3* (15.4)	0.4* (20.2)	0.1* (27.3)	12.1 (11.3)	8.9 (3.6)	0.5* (7.7)	0.1* (31.6)	0.2* (26.0)	0.1* (32.6)	0.1* (29.3)	0.2* (6.2)	0.1* (32.3)	0.3* (19.2)	0.2* (20.8)	5.7 (6.6)
LSD	0.3* (25.6)	0.5* (17.1)	0.1* (10.3)	26.6 (5.1)	33.9 (2.2)	0.8* (15.3)	0.1* (32.5)	0.2* (39.0)	0.1* (36.0)	0.2* (9.3)	0.2* (17.9)	0.2* (24.2)	0.3* (16.3)	0.2* (15.1)	17.3 (4.3)
bisoprolol	0.2* (30.0)	0.2* (17.5)	0.1* (11.8)	3.5 (13.4)	7.6 (3.0)	0.6* (12.4)	0.1* (29.5)	0.2* (25.6)	0.1* (34.2)	0.1* (39.0)	0.2* (12.1)	0.2* (22.6)	0.3* (21.0)	0.2* (22.3)	0.3* (12.8)
phencyclidine	1.5* (21.2)	1.9* (5.3)	0.3* (5.2)	33.3 (8.8)	27.2 (3.6)	0.9* (13.1)	0.3* (16.4)	0.2* (23.5)	0.2* (9.8)	0.2* (18.4)	0.2* (9.1)	0.2* (21.1)	0.3* (13.8)	1.8* (12.6)	8.2 (5.3)
propranolol	1.6* (33.1)	2.3 (9.2)	0.3* (20.1)	56.2 (8.3)	44.5 (1.2)	1.9* (9.9)	0.4* (28.1)	0.4* (17.5)	0.2* (30.4)	0.2* (24.4)	0.5* (19.2)	1.0* (14.4)	0.5* (32.1)	0.3* (16.8)	3.6 (9.8)
fentanyl	3.9 (26.8)	5.9 (12.7)	0.4* (6.6)	55.3 (7.8)	34.4 (3.2)	1.4* (12.8)	0.6* (11.6)	0.4* (25.1)	0.3* (20.8)	0.2* (11.8)	0.4* (6.2)	0.9* (11.8)	0.5* (18.3)	0.4* (8.5)	44.7 (3.1)
buprenorphine	4.9 (10.1)	5.8 (11.8)	3.0 (13.4)	72.9 (10.3)	48.6 (3.8)	7.1 (5.0)	2.2 (23.0)	1.4* (17.4)	1.3* (6.4)	0.8* (14.5)	1.2* (7.6)	2.4 (9.3)	1.6* (8.8)	2.6 (12.0)	65.1 (5.5)
ibutamoren	0.5* (19.6)	0.6* (11.4)	0.3* (32.7)	21.5 (8.8)	44.2 (4.7)	0.8* (17.6)	0.4* (5.4)	0.4* (42.9)	0.3* (22.2)	0.2* (14.5)	0.4* (18.9)	0.6* (16.5)	0.5* (16.4)	0.2* (26.6)	11.1 (8.1)
nebivolol	10.8 (11.8)	12.1 (10.8)	7.0 (8.5)	88.2 (3.8)	58.2 (1.2)	9.5 (15.2)	9.7 (7.6)	3.8 (26.8)	3.0 (15.0)	0.9* (6.0)	2.1 (15.1)	13.1 (3.1)	2.0 (12.7)	1.5* (17.0)	41.3 (12.3)
alprazolam	0.1* (39.9)	0.2* (25.1)	0.1* (5.8)	11.2 (3.6)	15.6 (1.6)	0.4* (35.7)	0.1* (24.8)	0.2* (23.4)	0.1* (107.2)	0.1* (115.7)	0.2* (51.7)	0.1* (46.6)	0.1* (69.1)	0.1* (40.8)	9.3 (14.0)
anastrozole	0.1* (44.1)	0.2* (44.4)	0.1* (29.2)	15.4 (3.4)	11.7 (1.9)	0.5* (7.2)	0.1* (54.4)	0.2* (28.5)	0.1* (30.6)	0.1* (49.8)	0.1* (26.2)	0.1* (64.7)	0.2* (41.0)	0.2* (27.3)	13.4 (5.6)
stanozolol	2.3 (31.7)	3.7 (29.0)	1.4* (23.3)	82.4 (2.7)	60.4 (8.1)	16.0 (16.2)	1.8* (23.1)	1.7* (20.3)	1.0* (62.2)	0.2* (69.2)	1.6* (35.7)	2.5 (25.7)	0.9* (42.7)	0.6* (98.2)	89.5 (20.1)
methadone	2.7 (27.7)	4.0 (16.0)	0.4* (12.6)	48.3 (10.2)	34.7 (3.5)	1.5* (23.6)	0.6* (11.1)	0.5* (18.3)	0.3* (10.9)	0.2* (7.3)	0.5* (13.9)	0.8* (8.8)	0.5* (23.6)	0.5* (12.8)	10.6 (8.2)
boldenone	0.3* (15.5)	0.2* (70.4)	0.2* (42.8)	44.9 (4.6)	39.3 (5.2)	1.2* (6.9)	0.1* (84.0)	0.2* (39.4)	0.1* (88.0)	0.1* (115.7)	0.2* (29.4)	0.1* (73.0)	0.4* (39.9)	0.1* (69.3)	41.0 (6.1)
clonazepam	0.3* (37.6)	0.2* (29.8)	0.1* (24.8)	47.3 (5.8)	18.8 (5.5)	0.6* (21.8)	0.1* (21.6)	0.2* (24.2)	0.1* (79.1)	0.1* (32.4)	0.1* (25.7)	0.2* (21.9)	0.2* (31.5)	0.1* (30.3)	14.8 (15.4)
agomelatine	0.7* (24.9)	0.6* (35.5)	0.1* (115.7)	54.4 (5.0)	50.5 (4.3)	3.9 (4.2)	0.2* (126.0)	0.1* (141.1)	0.1* (200.0)	not detectable	0.1* (200.0)	0.3* (76.8)	0.2* (71.7)	0.1* (117.3)	51.7 (4.9)
nandrolone	1.2* (77.4)	1.1* (65.9)	1.2* (53.3)	53.2 (9.2)	37.8 (6.2)	2.4 (8.3)	1.0* (91.0)	0.8* (76.3)	0.9* (74.8)	0.7* (120.2)	0.7* (116.3)	1.4* (44.9)	1.1* (80.3)	0.7* (121.0)	48.0 (7.6)
methandienone	0.4* (26.9)	0.5* (25.9)	0.2* (72.8)	45.2 (5.8)	35.8 (4.4)	0.9* (15.0)	0.2* (27.3)	0.2* (45.0)	0.1* (37.7)	0.2* (37.8)	0.3* (12.7)	0.2* (25.7)	0.4* (17.5)	0.2* (26.1)	43.1 (4.6)
tamoxifen	26.3 (11.9)	24.6 (3.7)	7.8 (2.2)	55.2 (13.4)	35.5 (3.0)	11.5 (21.6)	8.7 (8.0)	3.8 (23.6)	3.2 (7.0)	1.9* (12.1)	4.0 (25.6)	12.4 (2.1)	3.3 (7.9)	6.7 (2.0)	66.6 (19.6)
THC-COOH	1.6* (13.5)	0.8* (23.0)	1.6* (3.4)	31.6 (2.3)	19.1 (1.7)	3.0 (11.3)	1.2* (9.7)	1.2* (15.8)	0.9* (7.0)	0.5* (8.9)	1.1* (7.0)	0.9* (16.3)	0.9* (10.2)	1.5* (6.7)	17.0 (9.3)
THC	10.9 (1.8)	7.2 (7.3)	6.9 (4.5)	15.5 (16.4)	18.0 (4.9)	22.3 (6.1)	4.8 (8.8)	3.0 (14.4)	2.6 (5.1)	1.2* (13.0)	2.4 (8.6)	5.9 (5.4)	3.3 (6.8)	4.2 (4.6)	27.5 (24.3)

Relative standard deviations [%] given in brackets, n = 4, substances arranged by retention order.

* Indicates result below the established LOQ (1 ng/mL).

Table S7. Matrix effects [%] recorded for the evaluated polymers.

polymer / substance	ABS	ABS PRO	HIPS	LAYFOMM -60	PA6 + CF15	PA12 + CF15	PC-PBT	PET-G (Fiberlab)	PET-G (Prusa)	PLA	PLA EASY	PLA PEARL	PMMA	PP	S-FLEX 90A
morphine	41.7	8.7	59.2	28.6	38.4	−0.7	39.0	24.1	43.8	−12.8	52.7	45.4	−8.3	−6.7	7.1
amphetamine	10.9	15.1	24.3	21.2	6.3	7.7	17.5	8.5	8.8	11.0	11.6	15.4	15.9	4.6	15.8
oxycodone	14.3	29.9	20.2	14.5	12.0	30.5	8.5	11.1	6.2	17.6	11.8	16.0	23.3	10.7	19.9
hydrocodone	20.2	31.4	25.1	25.8	22.0	42.2	14.5	25.7	15.7	29.1	21.0	21.2	28.6	16.2	35.7
methamphetamine	9.9	22.1	17.5	13.0	6.1	7.1	13.6	9.2	5.3	7.3	10.4	15.8	14.0	4.8	18.7
MDMA	10.4	20.5	18.7	16.8	5.8	11.4	14.0	6.5	8.3	18.9	9.0	16.5	17.9	10.3	20.0
ketamine	9.4	11.4	11.9	13.1	1.5	0.0	8.7	8.2	2.9	7.5	4.1	10.2	14.5	2.2	16.2
clenbuterol	14.8	18.5	24.1	14.3	14.4	22.7	12.4	13.8	9.0	19.3	13.9	16.4	16.8	8.0	24.1
heroin	7.3	3.6	16.8	18.2	N/A	13.6	12.7	−0.5	7.5	8.5	17.9	18.3	12.3	−4.9	22.1
zolpidem	10.3	17.5	12.0	9.6	6.3	1.9	6.3	3.7	6.7	9.6	8.5	9.8	13.7	3.1	16.5
cocaine	11.8	11.0	10.6	7.9	1.0	−4.0	5.8	6.4	2.8	6.7	5.4	7.1	9.7	4.5	13.7
LSD	4.8	2.3	0.5	3.5	0.5	−2.1	−4.9	0.9	−6.1	−1.6	−1.7	−7.0	3.6	−4.1	8.5
phencyclidine	5.5	13.2	11.5	7.1	2.5	−2.9	7.0	5.9	4.0	10.7	6.5	7.0	12.8	0.9	14.2
propranolol	17.2	17.4	21.7	9.4	0.8	15.9	6.3	6.0	3.9	10.4	9.4	18.6	14.0	7.6	6.3
fentanyl	10.6	18.3	10.6	9.2	3.7	−3.7	1.1	9.1	0.9	13.9	1.7	7.7	17.6	6.1	16.2
buprenorphine	3.8	9.7	15.8	6.7	−1.0	−4.3	4.9	4.4	6.9	10.6	4.3	13.4	13.1	4.1	−16.3
methadone	4.6	12.8	10.0	6.9	0.7	−0.9	2.3	2.5	2.8	8.5	4.6	6.4	11.9	2.7	11.5
clonazepam	2.8	10.4	5.1	5.9	−2.2	−11.7	−3.6	−0.8	−3.3	6.6	−2.9	−2.2	10.7	0.8	6.8
THC-COOH	5.3	11.6	6.9	−9.4	−1.1	−6.6	−0.6	3.9	−1.6	5.8	1.3	4.3	7.2	0.8	3.8
THC	7.9	11.7	12.9	−45.8	−3.3	−3.9	3.9	5.3	−2.1	8.0	2.1	5.2	11.6	−0.8	−18.2

n = 4, substances arranged by retention order.

Table S8. Selected physicochemical descriptors of the analytes.

substance	monoisotopic mass [Da] [a]	logP [b]	polar surface area [Å²] [c]	pK_a [d] (*strongest acidic*)	pK_a [d] (*strongest basic*)	H-bonding sites [c] acceptors	H-bonding sites [c] donors
6-acetylcodeine	341.1627	1.7	48.0	N/A	N/A	5	0
agomelatine	243.1259	2.7	38.3	15.96	−0.94	2	1
alprazolam	308.0829	2.1	43.1	18.30	5.08	3	0
amphetamine	135.1048	1.8	26.0	N/A	10.01	1	1
anastrozole	293.1640	2.1	78.3	N/A	2.00	4	0
atenolol	266.1630	0.2	84.6	14.08	9.67	4	3
bisoprolol	325.2253	1.9	60.0	14.09	9.67	5	2
boldenone	286.1933	3.5	37.3	18.86	−0.88	2	1
buprenorphine	467.3036	5.0	62.2	7.50	12.54	5	2
carteolol	292.1787	1.0	70.6	13.41	9.76	4	3
clenbuterol	276.0796	2.2	58.3	14.06	9.63	3	3
clonazepam	315.0411	2.4	87.3	11.89	1.86	4	1
cocaine	303.1471	2.3	55.8	N/A	8.85	5	0
fenoterol	303.1471	2.0	93.0	8.85	9.63	5	5
fentanyl	336.2202	4.0	23.6	N/A	8.77	2	0
heroin	369.1576	1.5	65.1	N/A	9.10	6	0
higenamine	271.1208	2.2	72.7	8.75	9.55	4	4
hydrocodone	299.1521	2.2	38.8	18.00	8.61	4	0
ibutamoren	528.2406	1.3	130.0	N/A	N/A	7	2
ketamine	237.0920	2.2	29.1	18.78	7.45	2	1
LSD	323.1998	3.0	39.3	17.02	7.98	2	1
MDMA	193.1103	2.2	30.5	N/A	10.14	3	1
meldonium	146.1055	−2.1	52.2	4.14	N/A	3	1
methadone	309.2093	3.9	20.3	19.79	9.12	2	0
methamphetamine	149.1204	2.1	12.0	N/A	10.21	1	1
methandienone	300.2089	3.6	37.3	18.86	−0.53	2	1
methylphenidate	233.1416	0.2	38.3	N/A	9.09	3	1
metoprolol	267.1834	1.9	50.7	14.09	9.67	4	2
morphine	285.1365	0.8	52.9	10.26	9.12	4	2
nandrolone	274.1933	2.6	37.3	19.28	−0.88	2	1
nebivolol	405.1752	3.0	71.0	13.52	8.90	7	3
niketamide	178.1106	0.3	33.2	N/A	3.61	2	0
oxycodone	315.1471	1.2	59.0	13.57	8.77	5	1
phencyclidine	243.1987	3.6	3.2	N/A	10.56	1	0
propranolol	259.1572	3.0	41.5	14.09	9.67	3	2
salbutamol	239.1521	0.3	72.7	10.12	9.40	4	4
stanozolol	328.2515	4.5	48.9	16.23	2.86	2	2
strychnine	334.1681	1.9	32.8	N/A	N/A	3	0
tamoxifen	371.2249	7.1	12.5	N/A	8.76	2	0
terbutaline	225.1365	0.9	72.7	8.86	9.76	4	4
THC	314.2246	7.0	29.5	9.34	−4.90	2	1
THC-COOH	344.1988	6.3	66.8	4.21	−4.90	4	2
zolpidem	307.1685	2.5	37.6	N/A	5.65	2	0

Substances arranged in alphabetical order.

[a] PubChem Database. Available online: https://pubchem.ncbi.nlm.nih.gov/ (accessed on 9 July, 2022).

[b] **XLogP3.0**; PubChem Database. Available online: https://pubchem.ncbi.nlm.nih.gov/ (accessed on 9 July, 2022).

[c] **Cactvs 3.4.6.11**; PubChem Database. Available online: https://pubchem.ncbi.nlm.nih.gov/ (accessed on 9 July, 2022).

[d] **ChemAxon**; DrugBank Database. Available online: https://www.drugbank.com/ (accessed on 9 July, 2022) and: D.S. Wishart, Y.D. Feunang, A. Marcu, et al., HMDB 4.0—The Human Metabolome Database for 2018, Nucleic Acids Res. 46 (2018) D608–D617.

10. Wnioski

Zaprezentowane w przedstawionej rozprawie doktorskiej innowacje umożliwiają realizację wszystkich założonych przed rozpoczęciem badań celów. Do kluczowych spośród wyznaczonych zadań należały:

- Zastosowanie śliny jako tzw. alternatywnego materiału do badań;
- Udoskonalenie etapu przygotowywania próbek z wykorzystaniem metod mikroekstrakcyjnych;
- Wdrożenie zupełnie nowych rozwiązań w dziedzinie preparatyki próbek.

Na początkowym etapie badań potwierdzono możliwość stosowania obiecującego materiału biologicznego jakim jest ślina, do oznaczania substancji niedozwolonych. Jednocześnie wskazano na wady dostępnych obecnie urządzeń do pobierania próbek śliny, przede wszystkim na wysokie straty niektórych spośród badanych substancji.

- Aby w przyszłości wdrożyć ślinę jako podstawowy materiał do badań, **niezbędne wydaje się być opracowanie udoskonalonych urządzeń do pobierania jej próbek**.

Podczas dalszych badań udoskonalono kolejny (po pobieraniu próbki) etap protokołu analitycznego, jakim jest jej oczyszczanie. Zastosowano w tym celu TFME, czyli technikę należącą do wysoce selektywnych metod mikroekstrakcyjnych. W ramach rozwoju metody TFME wprowadzono szereg istotnych innowacji w postaci nowych rodzajów faz ekstrakcyjnych.

- Wykazano **korzyści ze stosowania faz ekstrakcyjnych złożonych z połączenia grup oktylowych (C_8) oraz 3-cyjanopropylowych (CN), w stosunku 1:1**. Takie połączenie zapewnia skuteczniejszą (wyższy odzysk analitów) oraz bardziej powtarzalną ekstrakcję (mniejsza zmienność pomiędzy wynikami) zróżnicowanych chemicznie związków (względem pozostałych 11 zastosowanych w tym porównaniu faz ekstrakcyjnych). Podstawą zwiększonej skuteczności faz ekstrakcyjnych składających się z cząsteczek C_8 i CN jest

wprowadzenie dodatkowego (przykładowo względem popularnych faz stacjonarnych składających się wyłącznie z grup C_{18}) mechanizmu ekstrakcji. Oprócz podstawowego mechanizmu wiązania analitów, jakim są w tym przypadku oddziaływania hydrofobowe, mieszanka C_8 i CN dzięki obecności grup CN zapewnia również odziaływania typu π−π oraz dipol−dipol.

- Udowodniono również **korzystny dla procesu ekstrakcji z próbek wodnych** (np. śliny) **wpływ stosowania polarnych** (etanolowych) **grup pomocniczych**. Polarne grupy pomocnicze, w porównaniu do zwyczajowo stosowanych grup trimetylosililowych (TMS, ang. *trimethylsilyl group*), nie tylko zabezpieczają powierzchnię fazy ekstrakcyjnej przed występowaniem niepożądanych (niespecyficznych) oddziaływań pomiędzy fazą ekstrakcyjną a analitami (co może to być źródłem utraty powtarzalności procesu ekstrakcji), ale także utrzymują cząsteczki wody w pobliżu odpowiadających za ekstrakcję ugrupowań hydrofobowych. Jest to korzystne zarówno z powodu zapobiegania zjawisku tzw. zapadania się hydrofobowych grup funkcyjnych w środowisku wodnym (dochodzi wówczas do obniżenia skuteczności ekstrakcji), jak i ze względu na umożliwienie skuteczniejszego wiązania hydrofilowych analitów obecnych w wodzie.

- Wykazano także, że przy przygotowywaniu urządzeń TFME **korzystniejsze jest stosowanie cząsteczek o mniejszej wielkości wewnętrznych porów**. Jest to cecha istotniejsza niż wielkość cząsteczek wykorzystywanych do przygotowania faz ekstrakcyjnych. Wynika to z większej gęstości grup funkcyjnych występującej wewnątrz ciaśniejszych porów, co przekłada się bezpośrednio na wzrost właściwej powierzchni ekstrakcyjnej cząsteczek.

Z wykorzystaniem techniki druku 3D zaproponowano alternatywną metodę przygotowywania wewnętrznej struktury (podpór pokrywanych fazą ekstrakcyjną) urządzeń TFME, zachowując jednocześnie kompatybilność z wysokoprzepustowym formatem 96-dołkowych płytek.

- Do wykonania odpornych termicznie oraz chemicznie podpór TFME wykorzystano nowy rodzaj termoplastycznego (możliwego do druku 3D)

polimeru jakim jest **poliamid 12 wzmocniony włóknem węglowym**. W porównaniu do stali nierdzewnej (tradycyjnie stosowanej do przygotowania urządzeń TFME), **jest to rozwiązanie tańsze, szybsze w przygotowaniu oraz eliminujące stosowanie szkodliwych odczynników** takich jak stężony kwas chlorowodorowy.

Jednak przede wszystkim, technika druku 3D umożliwia wykonanie kompletnych (od razu gotowych do użycia) urządzeń ekstrakcyjnych w jednym prostym kroku. Jest to możliwe dzięki wykorzystaniu powszechnie dostępnych i relatywnie mało kosztownych materiałów charakteryzujących się właściwościami adsorpcyjnymi.

- **Za pomocą druku 3D z wykorzystaniem poliamidu 6 wzmocnionego włóknem węglowym przygotowano innowacyjne urządzenie do ekstrakcji** substancji drobnocząsteczkowych ze złożonych materiałów biologicznych. Otrzymane w ten sposób narzędzie do przygotowania próbek charakteryzowało się kluczowymi zaletami metod mikroekstrakcyjnych, w szczególności takimi jak duża powtarzalność procesu ekstrakcji, wysoki stopień oczyszczenia próbki (mało nasilony efekt matrycy), a także kompatybilność z wysokoprzepustowym standardem 96-dołkowych płytek. Jednocześnie względem referencyjnej metody TFME uzyskano znaczną redukcję kosztów (około 200-krotną) oraz czasu (ponad 12-krotną) przygotowania urządzeń ekstrakcyjnych.

- Podobnie jak w przypadku urządzeń TFME z fazami ekstrakcyjnymi złożonymi z połączenia cząsteczek C_8 i CN, **za korzystne właściwości wydrukowanych z poliamidu 6 urządzeń ekstrakcyjnych odpowiada wykorzystanie kilku komplementarnych mechanizmów ekstrakcji**: oddziaływania hydrofobowe, odziaływania typu dipol−dipol oraz wiązania wodorowe.

- Nowo opracowaną metodę przygotowania próbek nazwano PANDA microextraction (ang. *polyamide noncoated device for adsorption-based (microextraction)*).

Ponadto dokonano kompleksowej analizy przygotowanych z wykorzystaniem druku 3D urządzeń ekstrakcyjnych stworzonych z 11 zróżnicowanych polimerów charakteryzujących się właściwościami adsorpcyjnymi.

- Podczas porównania **potwierdzono zalety wykorzystania poliamidu 6 jako bardzo obiecującego polimeru do produkcji urządzeń ekstrakcyjnych**. Urządzenia wykonane z poliamidu 6 charakteryzowały się wysokim odzyskiem badanych substancji oraz doskonałą powtarzalnością procesu ekstrakcji. Wysoka powtarzalność wyników została uzyskana dzięki sztywnej strukturze poliamidu 6 wzmocnionego włóknem węglowym, co wpłynęło na poprawę właściwości kinetycznych procesu ekstrakcji.

- Ponadto **korzystne wyniki zapewniły również urządzenia wydrukowane z różnych rodzajów poliuretanu** (LAYFOMM-60 i S-FLEX 90A). Polimer ten umożliwia ekstrakcję szerokiego spektrum analitów dzięki połączeniu aż 4 komplementarnych mechanizmów oddziaływań międzycząsteczkowych. Są to oddziaływania hydrofobowe, odziaływania typu dipol−dipol, odziaływania typu π−π oraz wiązania wodorowe.

- Dodatkową korzyść stanowi **brak negatywnego wpływu stosowanych do produkcji urządzeń ekstrakcyjnych polimerów na środowisko**. Zarówno poliamid 6, poliuretan, jak również i włókno węglowe są materiałami możliwymi do uzyskania z przetworzonych resztek organicznych, a także możliwymi do ponownego przetworzenia bez wykorzystania szkodliwych odczynników. Zarazem są to materiały odporne na działanie podstawowych odczynników laboratoryjnych.

Reasumując, szczególnie interesującym kierunkiem rozwoju przyszłych metod analitycznych, spełniającym specyficzne wymagania analiz antydopingowych, kontroli stosowania środków odurzających oraz terapeutycznego monitorowania leków jest **zastosowanie druku 3D do produkcji urządzeń ekstrakcyjnych**. Wśród najbardziej obiecujących polimerów adsorpcyjnych stosowanych w tym celu znajdują się poliamid 6 i jego połączenia z włóknem węglowym, a także różne rodzaje poliuretanu. Co więcej, wszystkie wymienione powyżej polimery są materiałami biokompatybilnymi, co sprawia, że **możliwe jest ich wykorzystanie do badań prowadzonych na żywych organizmach, w tym do badań prowadzonych na człowieku**. Tym samym możliwe staje się stworzenie w przyszłości otrzymanych za pomocą druku 3D urządzeń łączących etapy pobierania próbki oraz jej oczyszczania na potrzeby późniejszej analizy instrumentalnej. Umożliwiałoby to m.in. istotne udoskonalenie procesu pobierania próbek śliny – co jak wspomniano na początku tego rozdziału wydaje się być niezbędne dla upowszechnienia stosowania tego wysoce obiecującego materiału biologicznego do badań.

11. Streszczenie

Stosowanie środków odurzających i substancji dopingujących jest problemem towarzyszącym ludzkości od czasów starożytnych. Jednakże dopiero od stosunkowo niedawna postęp naukowy umożliwił kontrolę ich stosowania, a zarazem skuteczną walkę z tym procederem. Obecnie zwalczaniem zjawiska dopingu w sporcie zajmuje się przede wszystkim Światowa Agencja Antydopingowa, która do przeprowadzania badań wykorzystuje wyłącznie próbki krwi oraz moczu.

Badania zaprezentowane w przedłożonej rozprawie doktorskiej mają na celu popularyzację wykorzystania śliny jako obiecującego materiału biologicznego do kontroli stosowania substancji zakazanych. Jednak w szczególności koncentrują się na wprowadzeniu innowacji w dziedzinie przygotowania próbek do analiz laboratoryjnych. Przedmiotem badań jest zarówno udoskonalenie obecnie stosowanych metod mikroekstrakcyjnych, jak również wdrożenie nowych rozwiązań.

W ramach przedstawionych badań dokonano kompleksowej analizy wszystkich dostępnych komercyjnie urządzeń do pobierania próbek śliny (15 różnych rodzajów). Porównano również 12 rodzajów faz ekstrakcyjnych urządzeń mikroekstrakcyjnych oraz 11 rodzajów adsorpcyjnych polimerów stosowanych do przygotowania innowacyjnych urządzeń ekstrakcyjnych przy zastosowaniu techniki druku 3D. Wiele spośród wymienionych rozwiązań nie było przedtem wykorzystywanych w dziedzinie chemii analitycznej. Do prowadzonych badań stosowano szeroki panel (30−49) wyselekcjonowanych i reprezentatywnych substancji wzorcowych oraz próbki śliny pochodzące od ochotników. Analizy instrumentalne prowadzono przy zastosowaniu wysokosprawnej chromatografii cieczowej oraz tandemowej spektrometrii mas.

Uzyskane wyniki potwierdziły możliwość stosowania śliny jako materiału do badań, jednocześnie uwydatniły konieczność udoskonalenia metod pobierania jej próbek. Wykazano także korzyści ze stosowania nowych rodzajów faz ekstrakcyjnych, zwłaszcza złożonych z połączenia grup oktylowych i 3-cyjanopropylowych (1:1), a także zalety stosowania polarnych grup pomocniczych dla faz ekstrakcyjnych używanych w urządzeniach mikroekstrakcyjnych. Najważniejsze osiągnięcie stanowiło jednak wprowadzenie zupełnie nowej metody przygotowania próbek – PANDA microextraction – możliwe dzięki stworzeniu urządzeń ekstrakcyjnych wyłącznie za pomocą druku 3D przy użyciu poliamidu 6 wzmocnionego włóknem węglowym.

12. Summary

The prevalence of psychoactive substances misuse, as well as chasing unfair advantage by the means of doping in sports, are burdening mankind since ancient times. However, lately thanks to the novel scientific advancements we are able to control and effectively counter the aforementioned issues. As of now, the World Anti-Doping Agency – which is spearheading the efforts to combat doping in sports – controls the competitors by exclusively testing blood and urine samples.

Research presented in the following doctoral thesis aims at providing arguments for the use of oral fluid as an excellent testing specimen for controlling the misuse of prohibited substances. However, the special emphasis is put on introduction of new devices for analytical sample preparation. The scope of this goal ranges from improving the current devices used in microextraction methods, to establishing the brand new sample processing method.

The presented study involved in-depth evaluation of all commercially available devices for oral fluid sample collection (in total 15 different devices). Moreover, investigated sample preparation devices involved as many as 12 different chemistries of the microextraction devices, as well as 11 unique adsorptive polymers used for fabrication of the innovative extraction devices prepared exclusively with 3D printing technique. Many of these devices were introduced to the analytical chemistry for the very first time. Major part of the research was conducted with oral fluid samples collected from volunteers and with a broad (30−49) selection of model compounds (that were physicochemically diverse, as well as highly relevant to the actual applications). For laboratory testing, the methods of high-performance liquid chromatography and tandem mass spectrometry were used.

The attained results confirmed applicability of oral fluid as testing specimen. However, at the same time they highlighted the great demand for the improved sample collection methods. Moreover, the studies presented the benefits of using novel microextraction chemistries, especially comprised of mixed octyl and 3-cyanopropyl (1:1) -bound particles, as well as entitled with polar end-capping groups. Yet undoubtably, the greatest amongst the presented achievements was the introduction of a brand new sample preparation method – PANDA microextraction – accomplished exclusively with a 3D printing technique from carbon fiber-reinforced polyamide 6.

13. Piśmiennictwo

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14. Załączniki

14.1. Oświadczenia współautorów o udziale w publikacjach:

Bydgoszcz, 17.05.2023

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Oświadczam, że w pracy:

> Sobczak, Ł.; Goryński, K. Evaluation of swabs from 15 commercially available oral fluid sample collection devices for the analysis of commonly abused substances: Doping agents and drugs of abuse. *Analyst* **2020**, *145*, 7279–7288. DOI: 10.1039/d0an01379j

Mój wkład polegał na: zaproponowaniu tematyki badań; opracowaniu metodologii badań; zebraniu dokumentacji; przeprowadzeniu badań; opracowaniu danych; walidacji wyników; wizualizacji danych; napisaniu manuskryptu; wprowadzaniu uwag i poprawek do manuskryptu

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Oświadczam, że praca:

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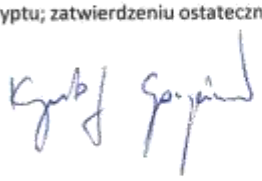
powstała w związku z realizacją badań w ramach autorskiego projektu nr LIDER/44/0164/L-9/17/NCBR/2018, na który otrzymałem finansowanie z NCBiR i którym kierowałem oraz zatrudniałem z pieniędzy grantu współautora publikacji. Jednocześnie deklaruję, że wszystkie badania wykorzystane w publikacji w całości sfinansowane zostały z kierowanego przeze mnie grantu.

Mój wkład polegał na: zaproponowaniu tematyki badań; opracowaniu metodologii badań; przeprowadzeniu badań; pozyskaniu zasobów i funduszy do realizacji pracy; zarządzaniu projektem; nadzorze nad projektem; wprowadzaniu uwag i poprawek do manuskryptu; zatwierdzeniu ostatecznej wersji artykułu; autor korespondencyjny.



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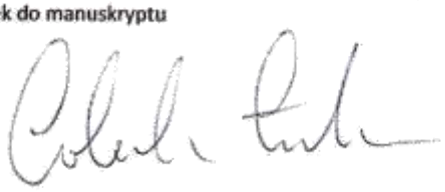
Oświadczam, że w pracy:

> Sobczak, Ł.; Kołodziej, D.; Goryński, K. Modifying current thin-film microextraction (TFME) solutions for analyzing prohibited substances: Evaluating new coatings using liquid chromatography. *J. Pharm. Anal.* **2022**, *12*, 470–480. DOI: 10.1016/j.jpha.2021.12.007

Mój wkład polegał na: zaproponowaniu tematyki badań; opracowaniu metodologii badań; zebraniu dokumentacji; przeprowadzeniu badań; opracowaniu danych; walidacji wyników; wizualizacji danych; napisaniu manuskryptu; wprowadzaniu uwag i poprawek do manuskryptu



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Mój wkład przy pisaniu pracy polegał na: zaproponowaniu tematyki badań; opracowaniu metodologii badań; przeprowadzeniu badań; pozyskaniu zasobów i funduszy do realizacji pracy; zarządzaniu projektem; nadzorze nad projektem; wprowadzaniu uwag i poprawek do manuskryptu; zatwierdzeniu ostatecznej wersji artykułu; autor korespondencyjny.

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Oświadczam, że w pracy:

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Oświadczam, że praca:

> Sobczak, Ł.; Kołodziej, D.; Goryński, K. Benefits of Innovative and Fully Water-Compatible Stationary Phases of Thin-Film Microextraction (TFME) Blades. *Molecules* **2021**, *26*, 4413. DOI: 10.3390/molecules26154413

powstała w związku z realizacją badań w ramach autorskiego projektu nr LIDER/44/0164/L-9/17/NCBR/2018, na który otrzymałem finansowanie z NCBiR i którym kierowałem oraz zatrudniałem z pieniędzy grantu współautorów publikacji. Jednocześnie deklaruję, że wszystkie badania wykorzystane w publikacji w całości sfinansowane zostały z kierowanego przeze mnie grantu.

Mój wkład polegał na: opracowaniu metodologii badań; pozyskaniu zasobów i funduszy do realizacji pracy; zarządzaniu projektem; nadzorze nad projektem; wprowadzaniu uwag i poprawek do manuskryptu; zatwierdzeniu ostatecznej wersji artykułu; autor korespondencyjny.

Bydgoszcz, 17.05.2023

mgr Dominika Kołodziej
Katedra Technologii Chemicznej Środków Leczniczych
Wydział Farmaceutyczny, Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy
Uniwersytet Mikołaja Kopernika w Toruniu
ul. Jurasza 2, 85-089 Bydgoszcz

Oświadczam, że w pracy:

> Kołodziej, D.; Sobczak, Ł.; Goryński, K. Polyamide Noncoated Device for Adsorption-Based Microextraction and Novel 3D Printed Thin-Film Microextraction Supports. *Anal. Chem.* **2022**, *94*, 2764–2771. DOI: 10.1021/acs.analchem.1c03672

Mój wkład polegał na: zaproponowaniu tematyki badań; opracowaniu metodologii badań; zebraniu dokumentacji; przeprowadzeniu badań; opracowaniu danych; wizualizacji danych; napisaniu manuskryptu; wprowadzaniu uwag i poprawek do manuskryptu

Bydgoszcz, 17.05.2023

mgr Łukasz Sobczak
Katedra Toksykologii i Bromatologii
Wydział Farmaceutyczny, Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy
Uniwersytet Mikołaja Kopernika w Toruniu
ul. Jurasza 2, 85-089 Bydgoszcz

Oświadczam, że w pracy:

> Kołodziej, D.; Sobczak, Ł.; Goryński, K. Polyamide Noncoated Device for Adsorption-Based Microextraction and Novel 3D Printed Thin-Film Microextraction Supports. *Anal. Chem.* **2022**, *94*, 2764–2771. DOI: 10.1021/acs.analchem.1c03672

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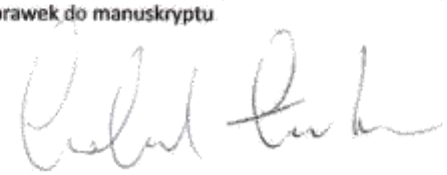

Bydgoszcz, 13.04.2023

dr Krzysztof Goryński
Wydział Technologii i Inżynierii Chemicznej
Politechnika Bydgoska im. J.J. Śniadeckich
ul. Seminaryjna 3
85-326 Bydgoszcz

Oświadczam, że praca:

> Kołodziej, D.; Sobczak, Ł.; Goryński, K. Polyamide Noncoated Device for Adsorption-Based Microextraction and Novel 3D Printed Thin-Film Microextraction Supports. *Anal. Chem.* **2022**, *94*, 2764–2771. DOI: 10.1021/acs.analchem.1c03672

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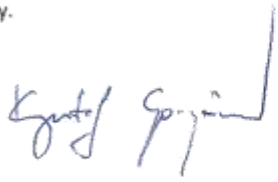

Bydgoszcz, 17.05.2023

mgr Dominika Kołodziej
Katedra Technologii Chemicznej Środków Leczniczych
Wydział Farmaceutyczny, Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy
Uniwersytet Mikołaja Kopernika w Toruniu
ul. Jurasza 2, 85-089 Bydgoszcz

Oświadczam, że w pracy:

> Kołodziej, D.; Sobczak, Ł.; Goryński, K. Innovative, Simple, and Green: A Sample Preparation Method Based on 3D Printed Polymers. *Talanta* **2023**, *257*, 124380. DOI: 10.1016/j.talanta.2023.124380

Mój wkład polegał na: zaproponowaniu tematyki badań; opracowaniu metodologii badań; zebraniu dokumentacji; przeprowadzeniu badań; opracowaniu danych; walidacji wyników; wizualizacji danych; napisaniu manuskryptu; wprowadzaniu uwag i poprawek do manuskryptu

Bydgoszcz, 17.05.2023

mgr Łukasz Sobczak
Katedra Toksykologii i Bromatologii
Wydział Farmaceutyczny, Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy
Uniwersytet Mikołaja Kopernika w Toruniu
ul. Jurasza 2, 85-089 Bydgoszcz

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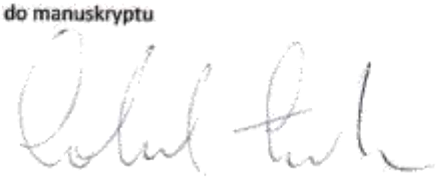

Bydgoszcz, 13.04.2023

dr Krzysztof Goryński
Wydział Technologii i Inżynierii Chemicznej
Politechnika Bydgoska im. J.J. Śniadeckich
ul. Seminaryjna 3
85-326 Bydgoszcz

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> Kołodziej, D.; Sobczak, Ł.; Goryński, K. Innovative, Simple, and Green: A Sample Preparation Method Based on 3D Printed Polymers. *Talanta* **2023**, *257*, 124380. DOI: 10.1016/j.talanta.2023.124380

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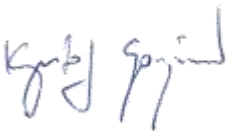
Mój wkład przy pisaniu pracy polegał na: pozyskaniu zasobów i funduszy do realizacji pracy; zarządzaniu projektem; nadzorze nad projektem; wprowadzaniu uwag i poprawek do manuskryptu; zatwierdzeniu ostatecznej wersji artykułu; autor korespondencyjny.



14.2. Zgody na prowadzenie badań:

1/2016

KUJAWSKO – POMORSKI
WOJEWÓDZKI INSPEKTOR FARMACEUTYCZNY
w BYDGOSZCZY

Bydgoszcz, dn. 01.02. 2016 r.

WIFBY-KK.857.2.4.2016

DECYZJA

Na podstawie art.35 ust.2, ust.4 pkt2, ust.5 i 6 ustawy z dnia 29 lipca 2005 r. o przeciwdziałaniu narkomanii (t.j. Dz.U. z 2012r. poz. 124 ze zm.) w związku z §5 i 12 Rozporządzenia Ministra Zdrowia z dnia 09 listopada 2015r. w sprawie wydawania zezwoleń na wytwarzanie, przetwarzanie, przerabianie, przywóz, dystrybucję albo stosowanie w celu prowadzenia badań naukowych środków odurzających, substancji psychotropowych lub prekursorów kategorii 1 (Dz.U. z 2015r poz. 1951) oraz art. 104 i 107 § 4 ustawy z dnia 14 czerwca 1960 r. Kodeks postępowania administracyjnego (t.j. Dz.U. z 2016 r., poz. 23) po rozpatrzeniu wniosku z dnia 17.12.2015r (data wpływu 05.01.2016r.)

Kujawsko – Pomorski Wojewódzki Inspektor Farmaceutyczny
w Bydgoszczy

udziela zezwolenia jednostce naukowej:

UNIWERSYTET MIKOŁAJA KOPERNIKA W TORUNIU

na stosowanie, w zakresie działalności statutowej przez Uniwersytet Mikołaja Kopernika w Toruniu Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy **Katedra i Zakład Farmakodynamiki i Farmakologii Molekularnej,** Wydział Farmaceutyczny, ul. Dr A. Jurasza 2, 85-089 Bydgoszcz, substancji psychotropowych grupy I-P, II-P, III-P, IV-P i środków odurzających grupy I-N, II-N i IV-N w celu prowadzenia badań naukowych tj. opracowanie nowej analitycznej metody równoległego oznaczania wielu grup leków i środków dopingujących stosowanych przez sportowców, kierowców pojazdów mechanicznych i młodzież szkolną („dopalacze") w standardowych i alternatywnych matrycach biologicznych.

Wyżej wymienione grupy środków odurzających i substancji psychotropowych będą stosowane w postaci roztworów wzorcowych. Przewidywane zużycie na poziomie 50-100mg miesięcznie, każdego z zakupionych środków.

Podmiot prowadzący badania zobowiązany jest:

1. Przechowywać posiadane środki odurzające i substancje psychotropowe w sposób zabezpieczający przed kradzieżą lub zniszczeniem, w tym podczas transportu,
2. prowadzić ewidencję w/w substancji zgodnie § 12 Rozporządzenia Ministra Zdrowia z dnia 09 listopada 2015r. w sprawie wydawania zezwoleń na wytwarzanie, przetwarzanie, przerabianie, przywóz, dystrybucję albo stosowanie w celu prowadzenia

badań naukowych środków odurzających, substancji psychotropowych lub prekursorów kategorii 1,

Na podstawie art. 107 § 4 Kodeksu postępowania administracyjnego odstąpiono od uzasadnienia decyzji.

POUCZENIE

Od niniejszej decyzji służy stronie odwołanie do Głównego Inspektora Farmaceutycznego w Warszawie za pośrednictwem Kujawsko-Pomorskiego Wojewódzkiego Inspektora Farmaceutycznego w Bydgoszczy w terminie 14 dni od jej doręczenia.

Kujawsko - Pomorski
Wojewódzki Inspektor Farmaceutyczny
w Bydgoszczy
mgr farm. Zofia Wrzesińska

Otrzymują:
1.Wnioskodawca – 2 egz
2. a/a

Adnotacja:
Dokonano zapłaty opłaty za złożenie wniosku o wydanie zezwolenia w wysokości 750.00 zł w dniu 30 grudnia 2015 r. na konto Wojewódzkiego Inspektoratu Farmaceutycznego nr 75-1010-1078-0078-9422-3100- 0000.
Podstawa prawna: art. 39 ust. 8 i 9 pkt 1 ustawy z dn. 29 lipca 2005 r. o przeciwdziałaniu narkomanii (t.j. Dz.U. z 2012 r. poz. 124)1

Uniwersytet Mikołaja Kopernika w Toruniu

Collegium Medicum im L. Rydygiera w Bydgoszczy

KOMISJA BIOETYCZNA

Ul. M. Skłodowskiej-Curie 9, 85-094 Bydgoszcz, tel.(052) 585-35-63, fax.(052) 585-38-11

KB 651/2018 Bydgoszcz, 25.09.2018r.

Działając na podstawie art.29 Ustawy z dnia 5 grudnia 1996 roku o zawodzie lekarza (Dz.U. z 1997 r. Nr 28 poz. 152 (wraz z późniejszymi zmianami), zarządzenia Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania komisji bioetycznych (Dz.U.Nr 47 poz.480) oraz Zarządzeniem Nr 21 Rektora UMK z dnia 4 marca 2009 r. z późn. zm. w sprawie powołania oraz zasad działania Komisji Bioetycznej Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im Ludwika Rydygiera w Bydgoszczy oraz zgodnie z zasadami zawartymi w ICH – GCP

Komisja Bioetyczna przy UMK w Toruniu, Collegium Medicum w Bydgoszczy

(skład podano w załączeniu), na posiedzeniu w dniu **25.09.2018 r.** przeanalizowała wniosek, który złożył kierownik badania:

dr n. farm. Krzysztof Goryński
Katedra Farmakodynamiki i Farmakologii Molekularnej
Collegium Medicum w Bydgoszczy

z zespołem w składzie

- prof. dr hab. n. med. Jacek Kubica, dr n. farm. Krzysztof Goryński, dr n. med. Ewa Obońska, mgr farmacji Łukasz Sobczak,

w sprawie badania:

„Nowoczesne rozwiązania technologiczne dedykowane szybkiemu wykrywaniu wybranych leków i związków drobnocząsteczkowych w ślinie - grant LIDER IX w ramach finansowania NCBiR."

Po zapoznaniu się ze złożonym wnioskiem i w wyniku przeprowadzonej dyskusji oraz głosowania Komisja podjęła

Uchwałę o pozytywnym zaopiniowaniu wniosku

w sprawie przeprowadzenia badań, w zakresie określonym we wniosku pod warunkiem:

- poinformowania uczestników badania o celu oraz zakresie badań i uzyskania od każdego z nich osobnej, pisemnej, świadomej zgody na udział w badaniu, zgodnie z obowiązującymi przepisami, datowanej najpóźniej na moment rozpoczęcia badania a nie wcześniej niż data uzyskania z Komisji Bioetycznej zgody na takie badanie;
- zachowania tajemnicy wszystkich danych, w tym danych osobowych pacjentów, umożliwiających ich identyfikację w ewentualnych publikacjach;
- zapewnienia, że osoby uczestniczące w eksperymencie badawczym nie są ubezwłasnowolnione, nie są żołnierzami służby zasadniczej, nie są osobami pozbawionymi wolności, nie pozostają w zależności służbowej, dydaktycznej lub innej z prowadzącym badanie;
- sugerujemy uzyskanie podpisu uczestnika badania pod informacją o badaniu, lub sporządzenie formularza informacji i świadomej zgody na udział w badaniu na jednej kartce.

Jednocześnie informujemy, iż „Zgoda na udział w badaniu" winna zawierać m.in.: imię i nazwisko badanej osoby; Nr historii choroby pacjenta (L.ks.gł. Oddziału/Poradni) oraz datę i podpis badanej osoby, a także

klauzule, że uczestnik badania wyraża zgodę na przetwarzanie danych osobowych dotyczących realizacji tematu badawczego, z wyjątkiem publikacji danych osobowych.

Kierownik badania zobowiązany jest do przechowywania wszystkich dokumentów dotyczących badania przez okres dwudziestu lat.

Zgoda obowiązuje od daty posiedzenia (25.09.2018 r.) do końca 2021 r.

Wydana opinia dotyczy tylko rozpatrywanego wniosku z uwzględnieniem przedstawionego projektu; każda zmiana i modyfikacja wymaga uzyskania odrębnej opinii. Wnioskodawca zobowiązany jest do informowania o wszelkich poprawkach, które mogłyby mieć wpływ na opinię Komisji oraz poinformowania o zakończeniu badania.

Od niniejszej uchwały podmiot zamierzający przeprowadzić eksperyment medyczny, kierownik zakładu opieki zdrowotnej, w której eksperyment medyczny ma być przeprowadzony, mogą wnieść odwołanie do Odwoławczej Komisji Bioetycznej przy Ministrze Zdrowia, za pośrednictwem Komisji Bioetycznej przy Collegium Medicum im. L. Rydygiera w Bydgoszczy, w terminie 14 dni od daty otrzymania niniejszej Uchwały.

Prof. dr hab. med. Karol Śliwka

Przewodniczący Komisji Bioetycznej

Otrzymuje:
dr n. farm. Krzysztof Goryński
Katedra Farmakodynamiki i Farmakologii Molekularnej
Collegium Medicum w Bydgoszczy

Dorobek naukowy:

I. Publikacje niebędące przedmiotem rozprawy doktorskiej:

1. Sobczak, Ł.; Goryński, K. Leki z apteki a doping w sporcie. *Farm. Pol.* **2018**, *74*, 199–205.

Punktacja: ministerialny wykaz czasopism punktowanych: 8

2. Sobczak, Ł.; Goryński, K. Pharmacological Aspects of Over-the-Counter Opioid Drugs Misuse. *Molecules* **2020**, *25*, 3905. DOI: 10.3390/molecules25173905

Punktacja: IF: 4,412; ministerialny wykaz czasopism punktowanych: 140

Suma punktacji prac nieobjętych rozprawą doktorską:
IF: 4,412; ministerialny wykaz czasopism punktowanych: 148

Łączna punktacja wszystkich prac:
IF: 42,545; ministerialny wykaz czasopism punktowanych: 768

II. Udział w konferencjach – plakaty

1. **Temat:** Co ślina na włókno przyniesie..., czyli udoskonalenie techniki oznaczania popularnych substancji odurzających i psychotropowych w płynie pochodzącym z jamy ustnej z zastosowaniem metody SPME-LC-MS
   Autorzy: Łukasz Sobczak, Krzysztof Goryński, Michael Pasek, Barbara Bojko
   Konferencja: Zjazd Zimowy Sekcji Studenckiej Polskiego Towarzystwa Chemicznego
   Miejsce i data: Bydgoszcz, 8-9 grudnia 2017

2. **Temat:** New solutions applied in oral fluid drug testing: fine-tuning and optimization of the SPME-LC-MS method
   Autorzy: Łukasz Sobczak, Barbara Bojko, Krzysztof Goryński
   Konferencja: MSACL 2019 EU. The 6th European Congress & Exhibits: Mass Spectrometry: Applications to the Clinical Lab
   Miejsce i data: Salzburg, Austria, 22-26 września 2019

3. **Temat:** Novel Sample Preparation Methods for Prohibited Substances Analysis – Which Criteria are Most Important for Analysis?
   Autorzy: Krzysztof Goryński, Łukasz Sobczak, Miłosz Różański, Paulina Goryńska, Arkadia Ciepłuch, Marcin Stachowiak, Sławomir Goryński, Bartosz Sadowski, Janusz Pawliszyn, Barbara Bojko
   Konferencja: PITTCON 2020 Conference & Expo: The Clear Advantage
   Miejsce i data: Chicago, USA, 1-5 marca 2020

4. **Temat:** Dobór grup funkcyjnych fazy stacjonarnej w metodzie mikroekstrakcji do fazy stałej (SPME) do oczyszczania próbek stosowanych w kontroli antydopingowej
   Autorzy: Łukasz Sobczak, Arkadia Ciepłuch, Marcin Stachowiak, Krzysztof Goryński
   Konferencja: ANALIZA ZAGADNIENIA, ANALIZA WYNIKÓW - WYSTĄPIENIE MŁODEGO NAUKOWCA, Edycja II
   Miejsce i data: Kraków, 1-2 kwietnia 2020 (online)

5. **Temat:** Zwalidowana metoda analityczna do wykrywania i oznaczania w ślinie substancji odurzających i środków dopingujących przy wykorzystaniu mikroekstrakcji do fazy stałej oraz wysokosprawnej chromatografii cieczowej sprzężonej z tandemową spektrometrią mas (SPME-HPLC-MS/MS)
   Autorzy: Łukasz Sobczak, Michael Pasek, Krzysztof Goryński
   Konferencja: DOKONANIA NAUKOWE DOKTORANTÓW, VIII EDYCJA
   Miejsce i data: Kraków, 27-28 kwietnia 2020 (online)

6. **Temat:** MIKROEKSTRAKCJA DO FAZY STAŁEJ (SPME) – metoda przygotowania próbek z zastosowaniem formatu włókien
   Autorzy: Łukasz Sobczak, Krzysztof Goryński
   Konferencja: DOKONANIA NAUKOWE DOKTORANTÓW, VIII EDYCJA
   Miejsce i data: Kraków, 27-28 kwietnia 2020 (online)

7. **Temat:** Analysis of prohibited substances from saliva: comparison few extraction phases applied in sample preparation
   Autorzy: Krzysztof Goryński, Łukasz Sobczak, Marcin Stachowiak, Paulina Goryńska, Sławomir Goryński
   Konferencja: 1st Virtual Festival of Life and Earth Sciences
   Miejsce i data: Online, 20-26 lipca 2020

8. **Temat:** The importance of using correct sampling procedures to obtain meaningful and representative analysis results
   Autorzy: Paulina Goryńska, Krzysztof Goryński, Łukasz Sobczak, Sławomir Goryński, Katarzyna Kuhn
   Konferencja: 1st Virtual Festival of Life and Earth Sciences
   Miejsce i data: Online, 20-26 lipca 2020

9. **Temat:** Sample preparation with thin-film microextraction (TFME) as reliable and reusable method for drugs of abuse analysis: validated TFME-LC-MS/MS protocol for oral fluid, plasma, and urine
   Autorzy: Łukasz Sobczak, Dominika Kołodziej, Krzysztof Goryński
   Konferencja: QUO VADIS Life Sciences
   Miejsce i data: Opole 23-27 czerwca 2021 (online)

III. Udział w konferencjach – referaty

1. **Temat:** Saliva drug testing by SPME-LC-MS – a promising complement to traditional antidoping control?
   Autorzy: Łukasz Sobczak, Krzysztof Goryński, Michael Pasek, Janusz Pawliszyn, Barbara Bojko
   Konferencja: iMEDIC 2017 2nd International Medical Interdisciplinary Congress
   Miejsce i data: Bydgoszcz, 10 czerwca 2017

2. **Temat:** Solid phase microextraction coupled to high sensitive LC-MS/MS system in analysis of prohibited substances from saliva - new insights
   Autorzy: Krzysztof Goryński, Łukasz Sobczak, Michael Pasek, Paulina Goryńska, Vincent Bessonneau, Janusz Pawliszyn, Barbara Bojko
   Konferencja: ExTech 2017. 19th International Symposium on Advances in Extraction Technologies
   Miejsce i data: Santiago de Compostela, Hiszpania, 27-30 czerwca 2017

3. **Temat:** SPME as sample preparation technique applied in prohibited substances analysis from various matrices - promising tool for doping control laboratories?
   Autorzy: Krzysztof Goryński, Łukasz Sobczak, Michael Pasek, Paulina Goryńska, Janusz Pawliszyn, Barbara Bojko
   Konferencja: MSACL 2017 EU. The 4th Annual European Congress of the Association for Mass Spectrometry: Applications to the Clinical Lab
   Miejsce i data: Salzburg, Austria, 10-14 września 2017

4. **Temat:** Ślina w analizie antydopingowej - mit, czy nowe możliwości w kierunku szybkiej kontroli?
   Autorzy: Krzysztof Goryński, Łukasz Sobczak, Michael Pasek, Paulina Goryńska, Janusz Pawliszyn, Barbara Bojko
   Konferencja: XXIII Naukowy Zjazd Polskiego Towarzystwa Farmaceutycznego Farmacja w Polsce: perspektywy nauki i zawodu
   Miejsce i data: Kraków, 19-22 września 2017

5. **Temat:** Novel sample preparation techniques applied in prohibited substances analysis from various matrices: which criteria are most important for analysis?
   Autorzy: Krzysztof Goryński, Miłosz Różański, Łukasz Sobczak, Paulina Goryńska, Janusz Pawliszyn, Barbara Bojko
   Konferencja: 1st Annual North American Mass Spectrometry Summer School
   Miejsce i data: Madison, USA, 6-9 sierpnia 2018

6. **Temat:** Oral fluid testing for the detection and quantification of beta-blockers by highly sensitive SPME-LC-MS method
   Autorzy: Łukasz Sobczak, Michael Pasek, Barbara Bojko, Krzysztof Goryński
   Konferencja: iMEDIC 2019 4th International Medical Interdisciplinary Congress
   Miejsce i data: Bydgoszcz, 1 czerwca 2019

7. **Temat:** Evaluating of the saliva sampling devices for the determination of pharmacologically active compounds: old and new solutions
   Autorzy: Krzysztof Goryński, Łukasz Sobczak, Sławomir Goryński, Bartosz Sadowski
   Konferencja: PITTCON 2020 Conference & Expo: The Clear Advantage
   Miejsce i data: Chicago, USA, 1-5 marca 2020

8. **Temat:** Porównanie komercyjnych urządzeń do pobierania próbek śliny w aspekcie zastosowania w kontroli antydopingowej przy użyciu wysokosprawnej chromatografii cieczowej sprzężonej z tandemową spektrometrią mas (HPLC-MS/MS)
   Autorzy: Łukasz Sobczak, Bartosz Sadowski, Krzysztof Goryński
   Konferencja: ANALIZA ZAGADNIENIA, ANALIZA WYNIKÓW - WYSTĄPIENIE MŁODEGO NAUKOWCA, Edycja II
   Miejsce i data: Kraków, 1-2 kwietnia 2020 (online)

9. **Temat:** Wpływ dostępnych komercyjnie zestawów do pobierania próbek śliny na oznaczanie popularnych substancji odurzających
   Autorzy: Łukasz Sobczak, Bartosz Sadowski, Krzysztof Goryński
   Konferencja: DOKONANIA NAUKOWE DOKTORANTÓW, VIII EDYCJA
   Miejsce i data: Kraków, 27-28 kwietnia 2020 (online)

10. **Temat:** Udoskonalenie metody preparatyki próbek zawierających substancje odurzające z wykorzystaniem mikroekstrakcji do fazy stałej (SPME)
    Autorzy: Łukasz Sobczak, Arkadia Ciepłuch, Marcin Stachowiak, Krzysztof Goryński
    Konferencja: DOKONANIA NAUKOWE DOKTORANTÓW, VIII EDYCJA
    Miejsce i data: Kraków, 27-28 kwietnia 2020 (online)

11. **Temat:** A comparative study of saliva sampling devices for the determination of pharmacologically active compounds
    Autorzy: Sławomir Goryński, Łukasz Sobczak, Bartosz Sadowski, Paulina Goryńska, Katarzyna Kuhn, Krzysztof Goryński
    Konferencja: 1st Virtual Festival of Life and Earth Sciences
    Miejsce i data: Online, 20-26 lipca 2020

12. **Temat:** Determining the most important criteria of sampling and sample preparation applied in prohibited substances analysis from saliva
    Autorzy: Krzysztof Goryński, Łukasz Sobczak, Arkadia Ciepłuch, Marcin Stachowiak, Sławomir Goryński, Katarzyna Kuhn, Dominika Kołodziej
    Konferencja: 4th International Caparica Christmas Conference on Sample Treatment 2020
    Miejsce i data: Caparica, Portugalia, 30 listopada - 3 grudnia 2020 (online)

13. **Temat:** Impact of sample collection device type on analyte concentration for the HPLC-MS/MS analysis of commonly abused substances from oral fluid
    Autorzy: Łukasz Sobczak, Bartosz Sadowski, Krzysztof Goryński
    Konferencja: 4th International Caparica Christmas Conference on Sample Treatment 2020
    Miejsce i data: Caparica, Portugalia, 30 listopada - 3 grudnia 2020 (online)

14. **Temat:** Advances in microextraction sample preparation methods – recent developments and appealing perspectives for the future
    Autorzy: Łukasz Sobczak, Dominika Kołodziej, Krzysztof Goryński
    Konferencja: iMEDIC 2021 5th International Medical Interdisciplinary Congress
    Miejsce i data: Bydgoszcz, 5 czerwca 2021 (online)

15. **Temat:** Preparation of alternative 3D printed thin-film microextraction (TFME) supports
    Autorzy: Dominika Kołodziej, Łukasz Sobczak, Krzysztof Goryński
    Konferencja: iMEDIC 2021 5th International Medical Interdisciplinary Congress
    Miejsce i data: Bydgoszcz, 5 czerwca 2021 (online)

16. **Temat:** 3D printed polyamide and carbon fiber blends as an alternative support for thin-film microextraction (TFME) devices or an alternative stationary phase for extraction of small molecules
    Autorzy: Dominika Kołodziej, Łukasz Sobczak, Krzysztof Goryński
    Konferencja: QUO VADIS Life Sciences
    Miejsce i data: Opole, 23-27 czerwca 2021 (online)

17. **Temat:** New water-compatible C_{18} coating of thin-film microextraction (TFME) devices is superior over conventional C_{18} coatings in extraction of small molecules from aqueous samples
    Autorzy: Łukasz Sobczak, Dominika Kołodziej, Krzysztof Goryński
    Konferencja: QUO VADIS Life Sciences
    Miejsce i data: Opole, 23-27 czerwca 2021 (online)

18. **Temat:** Novel Water-compatible Type of Stationary Phase for Thin-film Microextraction (TFME) of Small Molecules from Aqueous Samples
    Autorzy: Łukasz Sobczak, Dominika Kołodziej, Krzysztof Goryński
    Konferencja: 37th International Symposium on Microscale Separations and Bioanalysis
    Miejsce i data: Boston, USA, 12-15 lipca 2021 (online)

19. **Temat:** Preparation of Thin Film Microextraction (TFME) devices with 3D printing technology: alternative supports and novel extractive phases from polyamide with carbon fiber
    Autorzy: Dominika Kołodziej, Łukasz Sobczak, Krzysztof Goryński
    Konferencja: 5th International Caparica Christmas Conference on Sample Treatment 2021
    Miejsce i data: Caparica, Portugalia, 15-18 listopada 2021

20. **Temat:** Thin Film Microextraction (TFME) demonstrated as effective and reusable sample preparation method for quantification of prescription-only medicines: validated TFME-HPLC-MS/MS protocol for oral fluid, plasma, and urine samples
    Autorzy: Łukasz Sobczak, Dominika Kołodziej, Krzysztof Goryński
    Konferencja: 5th International Caparica Christmas Conference on Sample Treatment 2021
    Miejsce i data: Caparica, Portugalia, 15-18 listopada 2021

IV. Nagrody

1. **Nagroda:** nagroda za 1 miejsce (referat)
   Temat: Saliva drug testing by SPME-LC-MS – a promising complement to traditional antidoping control?
   Autorzy: Łukasz Sobczak, Krzysztof Goryński, Michael Pasek, Janusz Pawliszyn, Barbara Bojko
   Konferencja: iMEDIC 2017 2nd International Medical Interdisciplinary Congress
   Miejsce i data: Bydgoszcz, 10 czerwca 2017

2. **Nagroda:** nagroda za 3 miejsce w wydziałowym konkursie prac magisterskich
   Temat: Opracowanie metody analitycznej do oznaczania w ślinie wybranych substancji zakazanych w sporcie metodą mikroekstrakcji do fazy stałej sprzężonej z chromatografią cieczową i spektrometrią mas
   Autorzy: Łukasz Sobczak, Krzysztof Goryński (opiekun naukowy)
   Miejsce i data: Bydgoszcz, 26 października 2017

3. **Nagroda:** nagroda publiczności w wydziałowym konkursie prac magisterskich
   Temat: Opracowanie metody analitycznej do oznaczania w ślinie wybranych substancji zakazanych w sporcie metodą mikroekstrakcji do fazy stałej sprzężonej z chromatografią cieczową i spektrometrią mas
   Autorzy: Łukasz Sobczak, Krzysztof Goryński (opiekun naukowy)
   Miejsce i data: Bydgoszcz, 26 października 2017

4. **Nagroda:** nagroda za 2 miejsce (poster)
   Temat: Co ślina na włókno przyniesie..., czyli udoskonalenie techniki oznaczania popularnych substancji odurzających i psychotropowych w płynie pochodzącym z jamy ustnej z zastosowaniem metody SPME-LC-MS
   Autorzy: Łukasz Sobczak, Krzysztof Goryński, Michael Pasek, Barbara Bojko
   Konferencja: Zjazd Zimowy Sekcji Studenckiej Polskiego Towarzystwa Chemicznego
   Miejsce i data: Bydgoszcz, 8-9 grudnia 2017

5. **Nagroda:** nagroda za 3 miejsce (referat)
   Temat: Oral fluid testing for the detection and quantification of beta-blockers by highly sensitive SPME-LC-MS method
   Autorzy: Łukasz Sobczak, Michael Pasek, Barbara Bojko, Krzysztof Goryński
   Konferencja: iMEDIC 2019 4th International Medical Interdisciplinary Congress
   Miejsce i data: Bydgoszcz, 1 czerwca 2019

6. **Nagroda:** nagroda w konkursie krótkich wystąpień (Lightning Talk)
   Temat: New solutions applied in oral fluid drug testing: fine-tuning and optimization of the SPME-LC-MS method
   Autorzy: Łukasz Sobczak, Barbara Bojko, Krzysztof Goryński
   Konferencja: MSACL 2019 EU. The 6th European Congress & Exhibits: Mass Spectrometry: Applications to the Clinical Lab
   Miejsce i data: Salzburg, Austria, 22-26 września 2019

7. **Nagroda:** najlepszy poster

   Temat: Dobór grup funkcyjnych fazy stacjonarnej w metodzie mikroekstrakcji do fazy stałej (SPME) do oczyszczania próbek stosowanych w kontroli antydopingowej

   Autorzy: Łukasz Sobczak, Arkadia Ciepłuch, Marcin Stachowiak, Krzysztof Goryński

   Konferencja: ANALIZA ZAGADNIENIA, ANALIZA WYNIKÓW - WYSTĄPIENIE MŁODEGO NAUKOWCA, Edycja II

   Miejsce i data: Kraków, 1-2 kwietnia 2020 (online)

8. **Nagroda:** nagroda za 1 miejsce (referat)

   Temat: Advances in microextraction sample preparation methods – recent developments and appealing perspectives for the future

   Autorzy: Łukasz Sobczak, Dominika Kołodziej, Krzysztof Goryński

   Konferencja: iMEDIC 2021 5th International Medical Interdisciplinary Congress

   Miejsce i data: Bydgoszcz, 5 czerwca 2021 (online)

9. **Nagroda:** nagroda za 2 miejsce (referat)

   Temat: Preparation of alternative 3D printed thin-film microextraction (TFME) supports

   Autorzy: Dominika Kołodziej, Łukasz Sobczak, Krzysztof Goryński

   Konferencja: iMEDIC 2021 5th International Medical Interdisciplinary Congress

   Miejsce i data: Bydgoszcz, 5 czerwca 2021 (online)

10. **Nagroda:** nagroda za 1 miejsce (referat)

    Temat: 3D printed polyamide and carbon fiber blends as an alternative support for thin-film microextraction (TFME) devices or an alternative stationary phase for extraction of small molecules

    Autorzy: Dominika Kołodziej, Łukasz Sobczak, Krzysztof Goryński

    Konferencja: QUO VADIS Life Sciences

    Miejsce i data: Opole, 23-27 czerwca 2021 (online)

11. **Nagroda:** nagroda w konkursie krótkich wystąpień (Excellent Shotgun Communication Prize)
    Temat: Thin Film Microextraction (TFME) demonstrated as effective and reusable sample preparation method for quantification of prescription-only medicines: validated TFME-HPLC-MS/MS protocol for oral fluid, plasma, and urine samples
    Autorzy: Łukasz Sobczak, Dominika Kołodziej, Krzysztof Goryński
    Konferencja: 5th International Caparica Christmas Conference on Sample Treatment 2021
    Miejsce i data: Caparica, Portugalia, 15-18 listopada 2021

V. Granty

1. **Główny wykonawca części analitycznej grantu**
   SALIVA - Nowoczesne rozwiązania technologiczne dedykowane szybkiemu wykrywaniu wybranych leków i związków drobnocząsteczkowych w ślinie finansowanego przez Narodowe Centrum Badań i Rozwoju w ramach umowy numer LIDER/44/0164/L-9/17/NCBR/2018 programu Lider IX
   Lata: 2019–2021

2. **Grant wyjazdowy:** MSACL 2019 EU Young Investigator Educational Grant
   Laureat: Łukasz Sobczak
   Konferencja: MSACL 2019 EU. The 6th European Congress & Exhibits: Mass Spectrometry: Applications to the Clinical Lab
   Miejsce i data: Salzburg, Austria, 22-26 września 2019

VI. Kursy, szkolenia i warsztaty

1. **Temat:** Szkolenie z obsługi oprogramowania LabSolutions LCMS oraz konserwacji i obsługi zestawu z omówieniem sposobów rozwiązywania napotykanych problemów, związanych z obsługą chromatografu cieczowego sprzężonego ze spektrometrem mas SHIMADZU LCMS-8060
   Miejsce i data: Bydgoszcz, 24-26 kwietnia 2019

2. **Temat:** Everything You Wanted to Know about Internal Standards But Were Too Afraid to Ask
   Miejsce i data: Online, 28 maja 2019

3. **Temat:** Szkolenie aplikacyjne dotyczące pracy z systemem SHIMADZU LCMS-8050/8060 oraz oprogramowaniem LabSolutions LCMS (ver. 5.xx) zorganizowanym przez firmę SHIM-POL A.M. Borzymowski
   Miejsce i data: Kraków, 5-7 czerwca 2019

4. **Temat:** Where Ultra LC Performance Meets Selectivity: NEW Kinetex Core-Shell PS C18
   Miejsce i data: Online, 26 czerwca 2019

5. **Temat:** LC-MSMS 101: Getting Started with Quantitative LC-MS/MS in the Diagnostic Laboratory
   Miejsce i data: Salzburg, Austria, 22-24 września 2019 (MSACL 2019 EU 6th European Congress & Exhibition)

6. **Temat:** MALDI-MS technology combined with automated interpretation software, a solution adapted to high throughput screening
   Miejsce i data: Salzburg, Austria, 22-24 września 2019 (MSACL 2019 EU 6th European Congress & Exhibition)

7. **Temat:** Robustness and reliability of the novel pre-analytical filtration system CLAM-2030 and LCMS-8050
   Miejsce i data: Salzburg, Austria, 22-24 września 2019 (MSACL 2019 EU 6th European Congress & Exhibition)

8. **Temat:** Rethinking the capability and workflows in clinical metabolomics and biomarker research using the LCMS-9030 Q-TOF technologies
   Miejsce i data: Salzburg, Austria, 22-24 września 2019 (MSACL 2019 EU 6th European Congress & Exhibition)

9. **Temat:** Advancing analytical workflows for prohibited substance detection in equine athletes
   Miejsce i data: Online, 18 marca 2020

10. **Temat:** HPLC Workshop – Good Practice and Troubleshooting
    Miejsce i data: Online, 15 kwietnia 2020

11. **Temat:** Oh no! Not another boring webinar on column lifetime?
    Miejsce i data: Online, 29 kwietnia 2020

12. **Temat:** Your Favourite C18 is not a Method Development Toolkit
    Miejsce i data: Online, 6 maja 2020

13. **Temat:** Chiral HPLC Introduction – Method Development and Applications
    Miejsce i data: Online, 20 maja 2020

14. **Temat:** Role of Column Morphology, and Bonded Phase in Reversed-Phase Selectivity
    Miejsce i data: Online, 26 maja 2020

15. **Temat:** HILIC – A Systematic Approach
    Miejsce i data: Online, 3 czerwca 2020

16. **Temat:** HPLC Column Care
    Miejsce i data: Online, 10 czerwca 2020

17. **Temat:** The Basics of Solid Phase Extraction
    Miejsce i data: Online, 17 czerwca 2020

18. **Temat:** Sample Preparation: Liquid/Liquid Extraction, Supported Liquid Extraction, Protein Precipitation and Phospholipid Depletion
    Miejsce i data: Online, 24 czerwca 2020

19. **Temat:** Troubleshooting SPE
    Miejsce i data: Online, 21 lipca 2020

20. **Temat:** Strategies for Improving Resolution in Chromatography
    Miejsce i data: Online, 28 lipca 2020

21. **Temat:** UHPLC Small Particle Solutions for Big Chromatography Challenges
    Miejsce i data: Online, 22 września 2020

22. **Temat:** HPLC System Optimization
    Miejsce i data: Online, 24 września 2020

23. **Temat:** Reversed Phase Method Developement: Mobile Phase Selection & Selectivity Consideration
    Miejsce i data: Online, 8 października 2020

24. **Temat:** Overview of Sample Preparation Techniques for Biological Samples
    Miejsce i data: Online, 27 października 2020