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Original article

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

Łukasz Sobczak [1], Dominika Kołodziej [1], Krzysztof Goryński [*,1]

Bioanalysis Scientific Group, Faculty of Pharmacy, Collegium Medicum in Bydgoszcz at Nicolaus Copernicus University in Toruń, 85-089, Bydgoszcz, Poland

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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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* Corresponding author.
E-mail address: gorynski@cm.umk.pl (K. Goryński).
[1] These authors contributed equally to this work.

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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

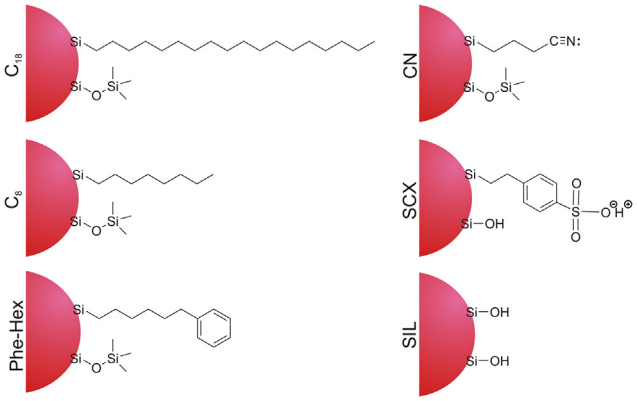

Fig. 1. Chemical structures of ligands bonded with silica particles. C_{18}: octadecyl; C_8: octyl; Phe-Hex: phenyl-hexyl; CN: 3-cyanopropyl; SCX: benzenesulfonic acid; SIL: unbonded silica.

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

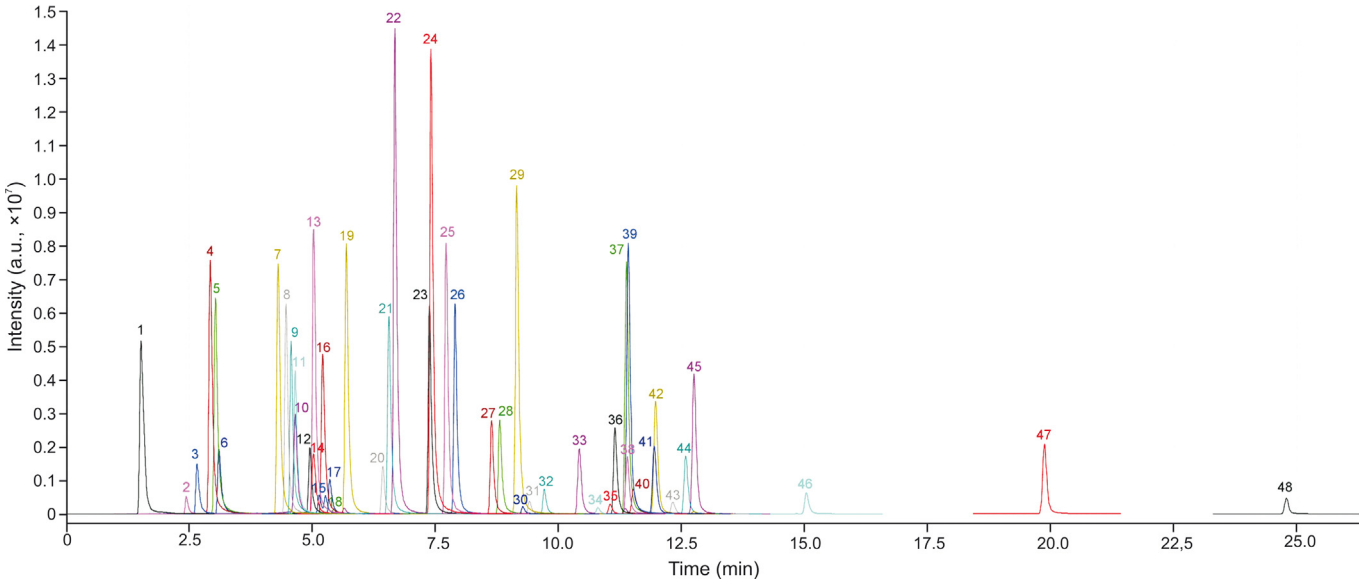

Fig. 2. Chromatogram of analyzed substances under optimum conditions. Order of the peaks: 1: meldonium; 2: psilocybin; 3: morphine; 4: salbutamol; 5: terbutaline; 6: atenolol; 7: fenoterol; 8: nikethamide; 9: carteolol; 10: amphetamine; 11: oxycodone; 12: hydrocodone; 13: methamphetamine; 14: chlorothiazide; 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,

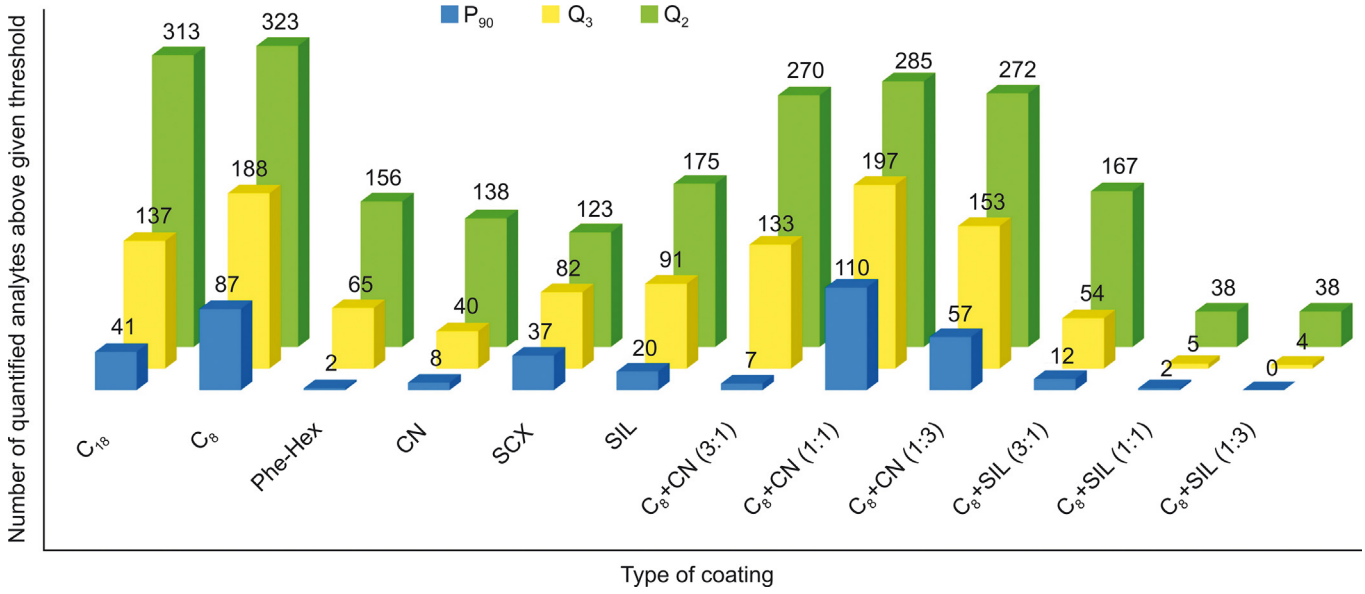

Fig. 3. Summarized number of quantified analytes in the 2nd quartile (Q_2), 3rd quartile (Q_3), and 90th percentile (P_{90}) for each coating type.

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** (0.005)
	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.

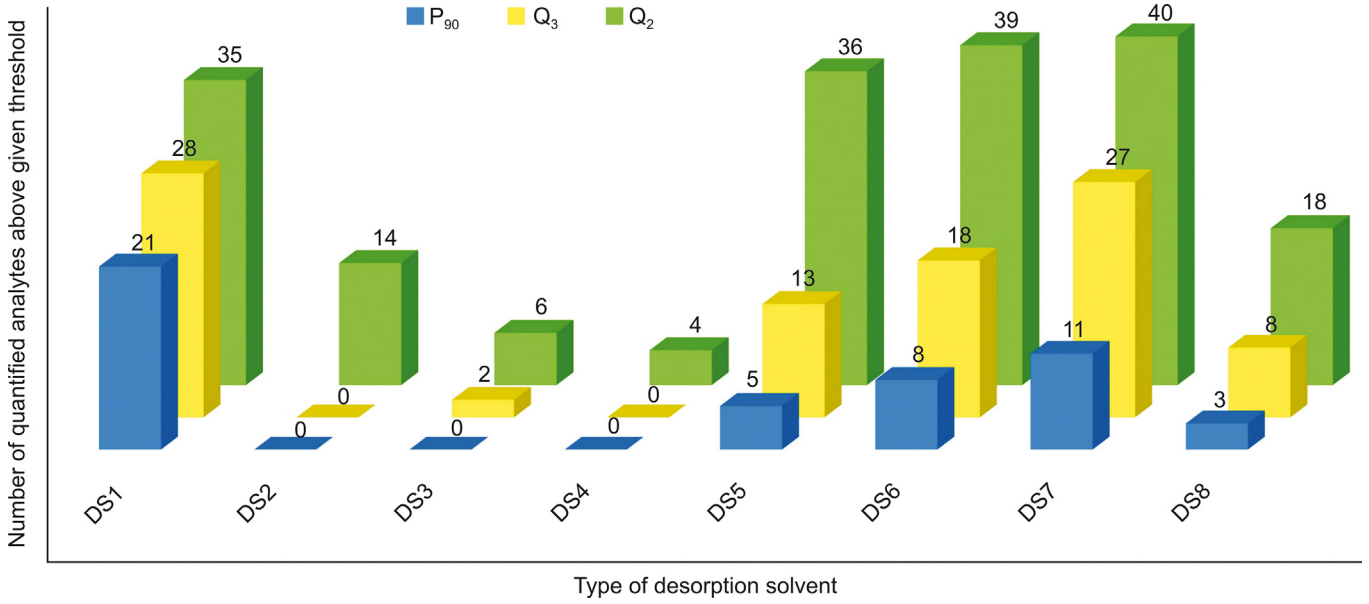

Fig. 4. Number of quantified analytes in the Q_2, Q_3, and P_{90} for the C_8 + CN (1:1) coating and every desorption solvent tested. Compositions of desorption solvents: DS1 = acetonitrile:water:formic acid (80:19.9:0.1, *V/V/V*); DS2 = acetonitrile:methanol:water:formic acid (40:40:19.9:0.1, *V/V/V/V*); 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.

References

[1] N. Reyes-Garcés, E. Gionfriddo, G.A. Gómez-Ríos, et al., Advances in solid phase microextraction and perspective on future directions, Anal. Chem. 90 (2018) 302–360.
[2] K. Goryński, A critical review of solid-phase microextraction applied in drugs of abuse determinations and potential applications for targeted doping testing, Trends Analyt. Chem. 112 (2019) 135–146.
[3] N. de Giovanni, D. Marchetti, A systematic review of solid-phase microextraction applications in the forensic context, J. Anal. Toxicol. 44 (2020) 268–297.
[4] C.L. Arthur, J. Pawliszyn, Solid phase microextraction with thermal desorption using fused silica optical fibers, Anal. Chem. 62 (1990) 2145–2148.
[5] D. Vuckovic, R. Shirey, Y. Chen, et al., *In vitro* evaluation of new biocompatible coatings for solid-phase microextraction: Implications for drug analysis and *in vivo* sampling applications, Anal. Chim. Acta. 638 (2009) 175–185.
[6] M. Sajid, M. Khaled Nazal, M. Rutkowska, et al., Solid phase microextraction: Apparatus, sorbent materials, and application, Crit. Rev. Anal. Chem. 49 (2019) 271–288.
[7] I. Bruheim, X.C. Liu, J. Pawliszyn, Thin-film microextraction, Anal. Chem. 75 (2003) 1002–1010.
[8] F.S. Mirnaghi, D. Hein, J. Pawliszyn, Thin-film microextraction coupled with mass spectrometry and liquid chromatography-mass spectrometry, Chromatographia 76 (2013) 1215–1223.
[9] E. Gionfriddo, E. Boyacı, J. Pawliszyn, New generation of solid-phase microextraction coatings for complementary separation approaches: A step toward comprehensive metabolomics and multiresidue analyses in complex matrices, Anal. Chem. 89 (2017) 4046–4054.
[10] D. Vuckovic, E. Cudjoe, D. Hein, et al., Automation of solid-phase microextraction in high-throughput format and applications to drug analysis, Anal. Chem. 80 (2008) 6870–6880.
[11] E. Cudjoe, D. Vuckovic, D. Hein, et al., Investigation of the effect of the extraction phase geometry on the performance of automated solid-phase microextraction, Anal. Chem. 81 (2009) 4226–4232.
[12] F.S. Mirnaghi, Y. Chen, L.M. Sidisky, et al., Optimization of the coating procedure for a high-throughput 96-blade solid phase microextraction system coupled with LC-MS/MS for analysis of complex samples, Anal. Chem. 83 (2011) 6018–6025.
[13] F.S. Mirnaghi, M.R.N. Monton, J. Pawliszyn, Thin-film octadecyl-silica glass coating for automated 96-blade solid-phase microextraction coupled with liquid chromatography-tandem mass spectrometry for analysis of benzodiazepines, J. Chromatogr. A 1246 (2012) 2–8.
[14] T. Vasiljevic, G.A. Gómez-Ríos, F. Li, et al., High-throughput quantification of drugs of abuse in biofluids via 96-solid-phase microextraction-transmission mode and direct analysis in real time mass spectrometry, Rapid Commun. Mass Spectrom. 33 (2019) 1423–1433.
[15] V. Bessonneau, E. Boyaci, M. Maciazek-Jurczyk, et al., *In vivo* solid phase microextraction sampling of human saliva for non-invasive and on-site monitoring, Anal. Chim. Acta 856 (2015) 35–45.
[16] Ł. Sobczak, D. Kołodziej, K. Goryński, Benefits of innovative and fully water-compatible stationary phases of thin-film microextraction (TFME) blades, Molecules 26 (2021), 4413.
[17] K. Goryński, A. Kiedrowicz, B. Bojko, Development of SPME-LC-MS method for screening of eight beta-blockers and bronchodilators in plasma and urine samples, J. Pharm. Biomed. Anal. 127 (2016) 147–155.
[18] F.S. Mirnaghi, J. Pawliszyn, Development of coatings for automated 96-blade solid phase microextraction-liquid chromatography-tandem mass spectrometry system, capable of extracting a wide polarity range of analytes from biological fluids, J. Chromatogr. A 1261 (2012) 91–98.
[19] N. Reyes-Garcés, B. Bojko, J. Pawliszyn, High throughput quantification of prohibited substances in plasma using thin film solid phase microextraction, J. Chromatogr. A 1374 (2014) 40–49.
[20] E. Boyacı, K. Gorynski, A. Rodriguez-Lafuente, et al., Introduction of solid-phase microextraction as a high-throughput sample preparation tool in laboratory analysis of prohibited substances, Anal. Chim. Acta 809 (2014) 69–81.
[21] J.-W. Liu, K. Murtada, N. Reyes-Garcés, et al., Systematic evaluation of different coating chemistries used in thin-film microextraction, Molecules 25 (2020), 3448.
[22] A.A. Aly, T. Górecki, Green approaches to sample preparation based on extraction techniques, Molecules 25 (2020), 1719.
[23] K.M. Billiard, A.R. Dershem, E. Gionfriddo, Implementing green analytical methodologies using solid-phase microextraction: A review, Molecules 25 (2020), 5297.
[24] National Survey on Drug Use and Health, Substance Abuse and Mental Health Services Administration (SAMHSA), 2018. https://www.samhsa.gov/data/report/2018-nsduh-detailed-tables. (Accessed 13 May 2021).
[25] European Drug Report 2020: Trends and Developments, European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). https://www.emcdda.europa.eu/publications/edr/trends-developments/2020. (Accessed 13 May 2021).
[26] Anti-doping testing figures, The World Anti-Doping Agency (WADA). https://www.wada-ama.org/en/resources/laboratories/anti-doping-testing-figures-reports. (Accessed 13 May 2021).
[27] Prohibited list, World Anti-Doping Agency (WADA). https://www.wada-ama.org/en/resources/science-medicine/prohibited-list-documents. (Accessed 13 May 2021).
[28] Ł. Sobczak, K. Goryński, 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 145 (2020) 7279–7288.
[29] N. Reyes-Garcés, M.N. Alam, J. Pawliszyn, The effect of hematocrit on solid-phase microextraction, Anal. Chim. Acta 1001 (2018) 40–50.
[30] D.V. McCalley, Comparison of conventional microparticulate and a monolithic reversed-phase column for high-efficiency fast liquid chromatography of basic compounds, J. Chromatogr. A 965 (2002) 51–64.
[31] K. Croes, A. Steffens, D.H. Marchand, et al., Relevance of π-π and dipole-dipole interactions for retention on cyano and phenyl columns in reversed-phase liquid chromatography, J. Chromatogr. A 1098 (2005) 123–130.
[32] L.R. Snyder, J.W. Dolan, P.W. Carr, The hydrophobic-subtraction model of reversed-phase column selectivity, J. Chromatogr. A 1060 (2004) 77–116.
[33] N.S. Wilson, M.D. Nelson, J.W. Dolan, et al., Column selectivity in reversed-phase liquid chromatography I. A general quantitative relationship, J. Chromatogr. A 961 (2002) 171–193.
[34] N.S. Wilson, M.D. Nelson, J.W. Dolan, et al., Column selectivity in reversed-phase liquid chromatography II. Effect of a change in conditions, J. Chromatogr. A 961 (2002) 195–215.
[35] N.S. Wilson, J.W. Dolan, L.R. Snyder, et al., Column selectivity in reversed-phase liquid chromatography III. The physico-chemical basis of selectivity, J. Chromatogr. A 961 (2002) 217–236.
[36] P.W. Carr, J.W. Dolan, U.D. Neue, et al., Contributions to reversed-phase column selectivity. I. Steric interaction, J. Chromatogr. A 1218 (2011) 1724–1742.
[37] D.H. Marchand, P.W. Carr, D.V. McCalley, et al., Contributions to reversed-phase column selectivity. II. Cation exchange, J. Chromatogr. A 1218 (2011) 7110–7129.
[38] P.W. Carr, J.W. Dolan, J.G. Dorsey, et al., Contributions to reversed-phase column selectivity: III. Column hydrogen-bond basicity, J. Chromatogr. A 1395 (2015) 57–64.
[39] D. Stoll, P. Boswell, HPLC Columns database. http://hplccolumns.org/database/compare.phps. (Accessed 13 May 2021).
[40] 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.
[41] J.C. Ma, D.A. Dougherty, The cation–π interaction, Chem. Rev. 97 (1997) 1303–1324.
[42] C.F. Poole, H. Ahmed, W. Kiridena, et al., Contribution of steric repulsion to retention on an octadecylsiloxane-bonded silica stationary phase in reversed-phase liquid chromatography, Chromatographia 62 (2005) 553–561.
[43] C.F. Poole, W. Kiridena, C. DeKay, et al., Insights into the retention mechanism on an octadecylsiloxane-bonded silica stationary phase (HyPURITY C18) in

reversed-phase liquid chromatography, J. Chromatogr. A 1115 (2006) 133–141.
[44] P. Nikitas, A. Pappa-Louisi, P. Agrafiotou, New insights on the retention mechanism of non-polar solutes in reversed-phase liquid chromatographic columns, J. Chromatogr. A 1034 (2004) 41–54.
[45] ChemSpider database, Royal Society of Chemistry. http://www.chemspider.com. (Accessed 13 May 2021).
[46] PubChem database, National Library of Medicine (NLM), National Center for Biotechnology Information (NCBI). https://pubchem.ncbi.nlm.nih.gov. (Accessed 13 May 2021).
[47] J.M. Herrero-Martınez, A. Méndez, E. Bosch, et al., Characterization of the acidity of residual silanol groups in microparticulate and monolithic reversed-phase columns, J. Chromatogr. A 1060 (2004) 135–145.
[48] E. Lesellier, C. West, A. Tchapla, Classification of special octadecyl-bonded phases by the carotenoid test, J. Chromatogr. A 1111 (2006) 62–70.
[49] F. Gritti, G. Guiochon, Heterogeneity of the adsorption mechanism of low molecular weight compounds in reversed-phase liquid chromatography, Anal. Chem. 78 (2006) 5823–5834.
[50] J. Dai, P.W. Carr, Effect of mobile phase anionic additives on selectivity, efficiency, and sample loading capacity of cationic drugs in reversed-phase liquid chromatography, J. Chromatogr. A 1216 (2009) 6695–6705.
[51] F. Gritti, G. Guiochon, Effect of the density of the C_{18} surface coverage on the adsorption mechanism of a cationic compound and on the silanol activity of the stationary phase in reversed phase liquid chromatography, J. Chromatogr. A 1132 (2006) 51–66.
[52] J. Nawrocki, The silanol group and its role in liquid chromatography, J. Chromatogr. A 779 (1997) 29–71.
[53] F. Gritti, G. Guiochon, Adsorption mechanism in reversed-phase liquid chromatography: Effect of the surface coverage of a monomeric C_{18}-silica stationary phase, J. Chromatogr. A 1115 (2006) 142–163.