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Sorption of structurally different ionized pharmaceutical and illicit drugs to a mixed-mode coated microsampler

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ABSTRACT

The mixed-mode (C18/strong cation exchange-SCX) solid-phase microextraction (SPME) fiber has recently been shown to have increased sensitivity for ionic compounds compared to more conventional sampler coatings such as polyacrylate and polydimethylsiloxane (PDMS). However, data for structurally diverse compounds to this (prototype) sampler coating are too limited to define its structural limitations. We determined C18/SCX fiber partitioning coefficients of nineteen cationic structures without hydrogen bonding capacity besides the charged group, stretching over a wide hydrophobicity range (including amphetamine, amitriptyline, promazine, chlorpromazine, trifluprom azine, difenzoquat), and eight basic pharmaceutical and illicit drugs (pKa > 8.86) with additional hydrogen bonding moieties (MDMA, atenolol, alprenolol, metoprolol, morphine, nicotine, tramadol, verapamil). In addition, sorption data for three neutral benzodiazepines (diazepam, temazepam, and oxazepam) and the anionic NSAID diclofenac were collected to determine the efficiency to sample non-basic drugs. All tested compounds showed nonlinear isotherms above 1 mmol/L coating, and linear isotherms below 1 mmol/L. The affinity for C18/SCX-SPME for tested organic cations without H—bond capacities increased with longer alkyl chains, ranging from logarithmic fiber-water distribution coefficients (log D_{fw}) of 1.8 (benzylamine) to 5.8 (trifluprom azine). Amines smaller than benzylamine may thus have limited detection levels, while cationic surfactants with alkyl chain lengths >12 carbon atoms may sorb too strong to the C18/SCX sampler which hampers calibration of the fiber-water relationship in the linear range. The log D_{fw} for these simple cation structures closely correlates with the octanol-water partition coefficient of the neutral form ($K_{ow,N}$), and decreases with increased branching and presence of multiple aromatic rings. Oxygen moieties in organic cations decreased the affinity for C18/SCX-SPME. Log D_{fw} values of neutral benzodiazepines were an order of magnitude higher than their log $K_{ow,N}$. Results for anionic diclofenac species (log $K_{ow,N}$ 4.5, pKa 4.0, log D_{fw} 2.9) indicate that the C18-SCX fiber might also be useful for sampling of organic anions. This data supports our theory that C18-based coatings are able to sorb ionized compounds through adsorption and demonstrates the applicability of C18-based SPME in the measurement of freely dissolved concentrations of a wide range of ionizable compounds.

1. Introduction

Solid-phase microextraction (SPME) is a simple, passive sampling technique [1]. This technique has been evolving rapidly in the last decade, with innovations in coatings or extraction phases used [2–4], changes in experimental set-ups to allow for high-throughput sampling [5], and expanding to other fields of application including forensics [6], biomedical analysis [7] and *in vivo* sampling [8].

One of the recent advances in SPME is the use of so-called "mixed-mode" coatings. These coatings employ a mixture of two extraction mechanisms, thereby increasing analyte coverage. The C18/SCX fiber, consisting of a hydrophobic phase (C18) and strong cation exchange sites (SCX), is one of these "mixed-mode" coatings. The first publication on this fiber showed increased metabolite coverage (log K_{ow} ranging between −3 to 7) compared to other SPME coatings in an untargeted metabolomic profiling study in human plasma [9]. The authors later showed that this SPME coating could

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also be used *in vivo*, when it was applied in mice [10], pigs [11,12] and rats [13]. The major benefit of the C18/SCX fiber is the relative high sorption affinity for neutral hydrophilic compounds, such as amino acid analogues [14], neurotransmitters [15] and glucuronide drug conjugates [16]. These studies show the high sensitivity of C18/SCX SPME in metabolomics.

Additionally, steps have been made to elucidate the sorption mechanism of the C18/SCX fiber. Using cationic amphetamine [17,18] and cationic amitriptyline [18], a large number of variables have been identified that can influence sorption to the C18/SCX fiber. In general, the C18/SCX fiber shows increased sorption affinity for ionizable compounds compared to more conventional coatings such as polyacrylate, and over a wide pH range (pH 2–10) [17,19]. Although C18/SCX fibers are not yet commercially available, these coatings could provide useful sampling tools in clinical application, where ionized or ionizable compounds are numerous, and for *in vivo* sampling, where matrix-modifying steps to ensure a large neutral fraction are impossible or undesirable.

Although the C18/SCX coating apparently has high sensitivity for cationic drugs, current data is limited to a few compounds. The chemical applicability domain of the mixed-mode SPME as a passive sampler depends to a large extent on the range of sorption affinities; not too high to (i) deplete systems, (ii) readily saturate the sampler, and/or (iii) hamper calibration of the fiber in the linear range, and not too low to meet adequate detection limits at relevant concentration ranges. Here, we present data on the sorption of various structurally different compounds to the C18/SCX fiber. We studied the sorption of a number of ionized amines and ammonium compounds with different alkyl chain lengths, to assess the influence of amine class and hydrophobicity. Additionally, we studied a large set of basic pharmaceuticals and illicit drugs with a pKa > 8 containing additional polar moieties besides the charged group, as well as 3 neutral benzodiazepines and the acidic non-steroidal anti-inflammatory drug (NSAID) diclofenac (pKa 4.0).

2. Materials and methods

2.1. Chemicals and materials

SPME fibers with mixed-mode coating (C18/propylsulfonic acid; C18/SCX) are prototype fibers provided by Supelco, Sigma Aldrich (Bellefonte, PA, USA). A schematic representation of the C18/SCX fibers is provided in the Supporting information, Fig. S1. These fibers are produced in a nearly identical way as commercially available biocompatible C18-SPME fibers. A comparison between extractions with C18/SCX-SPME and C18-SPME is published elsewhere [18]. The C18/SCX fibers consisted of a 3 cm piece of nitinol wire with a diameter of 202 μm of which 1.5 cm contains the SPME coating, with an average thickness of 45 μm (fiber volume 524 nL), delivered without the hypodermic needle used in the biocompatible C18-SPME fiber. Both C18 and propylsulfonic acid (2–2.5% sulfur loading) are bonded on porous HPLC column grade silica material (3 μm particles, mean pore size 100 Å, total surface area ~450 $m^2 g^{-1}$), which is then bound to the wire with a biocompatible polymeric binder (Supelco, pers.comm.). Phosphate buffered saline (PBS; pH 7.4) consisted of 138 mM NaCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4 and 2.7 mM KCl (all Merck, Darmstadt, Germany) dissolved in Milli-Q water (18.2 MΩ cm, Millipore, Amsterdam, The Netherlands). Some compounds were tested at pH 6.3 to ensure that >99% was present as the charged species. Tests at pH 6.3 were carried out using a 10 mM phosphate buffer with 50 mg/L NaN_3 and NaCl, to a total ionic strength of 150 mM Na^+. Ammonia solution (25%) was obtained from Merck, trifluoroacetic acid was obtained from Sigma Aldrich (Zwijndrecht, The Netherlands). Methanol and acetonitrile were HPLC-grade (Bio-Solve, Valkenswaard, The Netherlands). A list of all test compounds including molecular structures is given in the Supporting information, Table S2.

2.2. SPME procedure

Test solutions with different concentrations of analyte were made in glass vials by spiking buffer using stock solutions in methanol, ensuring methanol fractions of <1%. Each compound was evaluated separately, to avoid any competitive adsorption processes in mixtures [18]. During SPME fiber exposure, samples were placed on a roller mixer (40 rpm). After equilibrium was reached, fibers were transferred to vials containing 120 μL desorption fluid. Equilibrium times were either determined empirically or fibers were exposed for at least 18 h. Fibers were wiped gently using a paper tissue to remove any buffer droplets before placing them in desorption fluid. Desorption fluid for all compounds consisted of 90% acetonitrile and 10% Milli-Q water with 0.1% NH_3 (of end volume), with a resulting pH of 11. After desorption and removal of the fiber, desorption solution was acidified to pH 2–3 using 60 μL 0.1 M HCl, to approximate the pH of the mobile phase [17]. To reuse the fibers, they were pooled after use, kept in desorption fluid overnight and subsequently stored in 50/50 methanol/Milli-Q at room temperature. A second desorption step showed that carry-over was 1–3% [17]. Fiber blanks (in triplicate) were incorporated in every experiment, using buffer solutions that had not been spiked to confirm the absence of carry-over between experiments. Since these fibers are intended for single use, they were checked regularly to monitor changes in sorption capacity after repeated use. If sorption capacity was decreased, new fibers were used for the next experiment. C18/SCX SPME fibers show excellent repeatability and reproducibility, as previously described [18]. Decreases in sorption capacity are usually only seen after exposure of the fibers to pH > 8 for >24 h [18]. Twenty consecutive exposures of three C18/SCX fibers to 3.6 μM of amitriptyline showed a RSD of 9.4% between all fiber concentrations and an RSD of 4.5% between all triplicates [18].

Optimization of the SPME conditions, such as the desorption parameters, has been performed in previous research [17]. However, further optimization to increase the extraction efficiency of the SPME method is not in the scope of this paper. Constant agitation is achieved using roller mixers (Stuart SRT9), and this agitation speed is kept equal for all analytes investigated. Sample pH was chosen so that all analytes are predominantly present in their cationic form, usually at pH 7.4. Extraction time was not optimized as all experimental work was performed at equilibrium.

For the linear alkyl amines, which were analyzed using LC–MS/MS, fibers were desorbed using 90% acetonitrile and 10% Milli-Q water with 0.1% trifluoroacetic acid (of end volume) with a pH around 2 [20], as linear alkyl amines are volatile in their neutral form (pH > 8.5). Aqueous samples for analysis were prepared by transferring 200 μL of the aqueous phase to 600 μL of this acidic desorption fluid. The resulting sample was mixed by repeated pipetting using the same pipette tip, to minimize loss of analyte to the tip. The acidic desorption fluid contained the tertiary amine N,N-dimethyldecylamine (N(C)(C)-C10) as internal standard for the linear alkyl lamines to account for deviations in ionization efficiency in the LC–MS/MS analysis.

The data in this paper has not been published previously, with exception of amphetamine [17], amitriptyline [18], diazepam [21], tramadol [21] and C12-DEA [19].

2.3. HPLC and LC–MS/MS parameters

All pharmaceuticals were analyzed using HPLC with either UV or fluorescence detection. Only the linear alkyl amines were analyzed

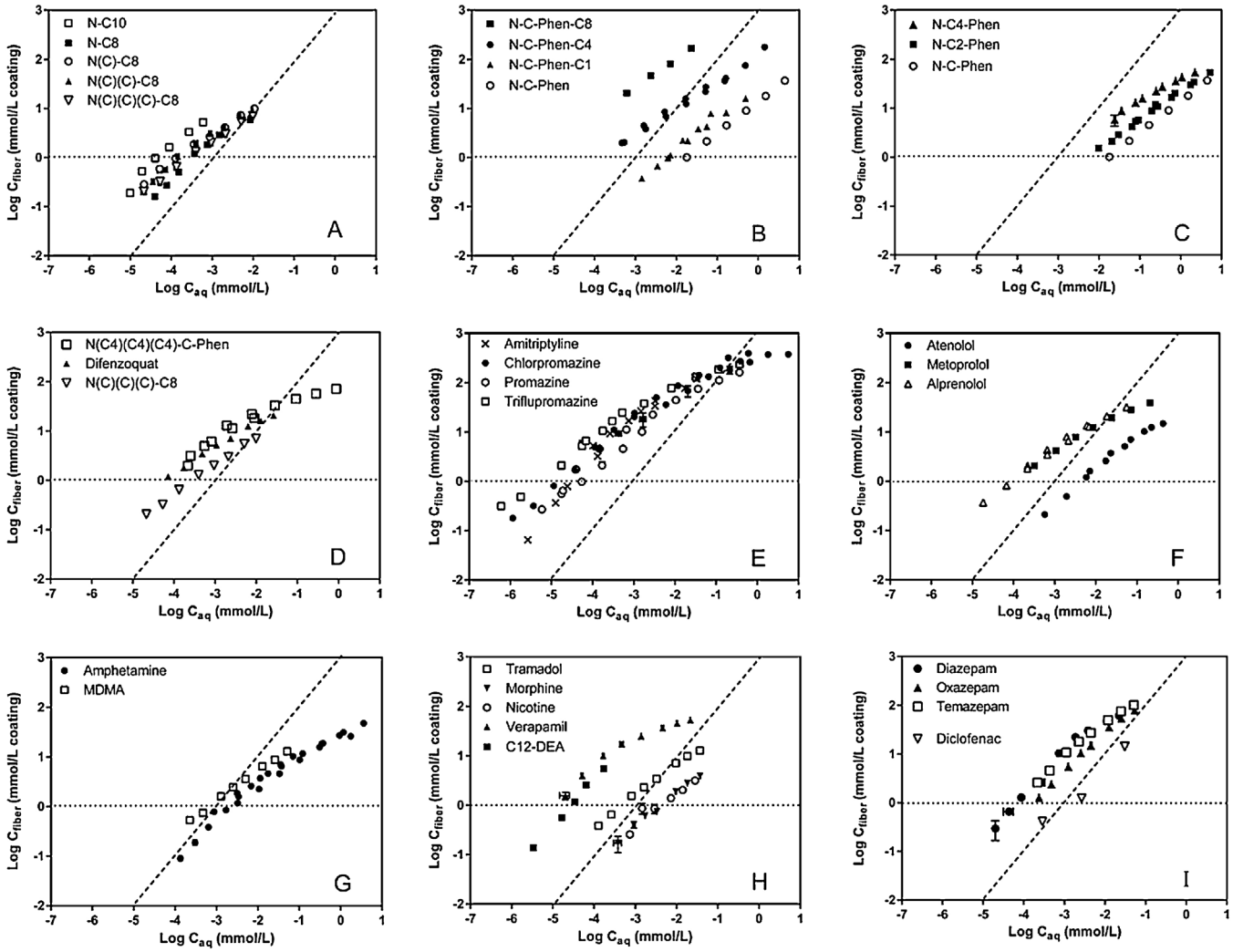

Fig. 1. Sorption isotherms to the C18/SCX fiber exposed in PBS medium for 48–96 h. Broken lines indicate a linear relationship between concentrations on C18/SCX fibers and dissolved concentrations with a D_{fw} of 1000 (log D_{fw} of 3). Horizontal dotted lines indicate sorbed concentrations of 1 mmol/L C18/SCX which are used to calculate log D_{fw} values. Graphs (A–D) represent organic cations without oxygen containing H—bonding functional groups. Graph (E) are tricyclic antidepressant bases (also organic cations without oxygen containing H—bonding functional groups). Graph (F) are beta-blocker bases, graph (G) amphetamine bases, graph (H) analgesic bases, the basic calcium channel blocker (verapamil), and the cationic surfactant lauryl diethanolamine (C12-DEA), graph (I) benzodiazepines and the acidic NSAID diclofenac. Data for amphetamine is taken from Ref. [17], data for amitriptyline from Ref. [18], data for diazepam and tramadol are from [21], data for C12-DEA is from Ref. [19], all with publisher permission. X- and Y-axes were kept identical to enable easy comparison.

using LC–MS/MS. For all equipment and parameters used, see the Supporting information, Tables S3 and S4.

2.4. Quantification and data analysis

The concentration of analyte on the fiber (further named fiber concentrations) as well as the concentration of analyte remaining in the buffer solution (further named aqueous concentrations) were always measured. Sorption coefficients were calculated using the aqueous concentration after exposing the fiber instead of the initial concentration. A calibration curve made from aqueous concentrations before exposure was used to calculate the remaining aqueous concentration after exposure and calculate depletion using a mass balance approach. A calibration curve in acidified desorption fluid was used to calculate the concentration in fiber desorption samples and to confirm the mass balance. Calibration curves for the linear alkyl amines were made in the previously described acidic desorption fluid, with *N*,*N*-dimethyldecylamine added as internal standard to account for drift of the overall LC–MS/MS signal. For the lowest aqueous concentrations of amitriptyline, promazine, chlorpromazine and triflupromazine, quantification was not possible as these aqueous concentrations were below the LOQ of the current HPLC-UV method. To still establish a sorption isotherm at these concentrations, the mass balance approach was used to calculate the aqueous concentration after exposure, assuming negligible sorption to the vial surfaces (as was shown by complete mass balances obtained at all other tested concentrations).

Data was plotted and analyzed using GraphPad Prism 6 for Windows. All samples were prepared in triplicate, unless specified otherwise. Data are plotted as mean ± standard deviation in both x- (measured aqueous concentrations after fiber exposure) and y-direction (measured fiber concentrations). Fiber-water sorption coefficients (log D_{fw}) are obtained by extrapolation of log linear curve to a sorbed concentration of 1 mmol/L fiber coating (at logY = 0). Although the porous coating material on the C18/SCX fibers represents a specific surface area rather than a bulk sorbent volume, a fiber coating volume of 524 nL was used as calculated according the average thickness of the fiber coating and the diameter of the nitinol wire, following Refs. [17–19].

Table 1
Fiber-water distribution coefficients (D_{fw}) and octanol-water partition coefficients of organic cations: simple structures containing only C, H and N.

Compound #	Amine type	Name[a]	Abbreviation[b]	Log D_{fw}[c]	Log $K_{ow,N}$ selected[d]
Same carbon chain length, different amine type					
1	NH_3^+ (1°)	1-octanamine	N–C8	3.39	3.06
2	NH_2^+ (2°)	*N*-methyl-1-octanamine	N (C)–C8	3.83	3.29
3	NH^+ (3°)	*N*,*N*-dimethyl-1-octanamine	N (C) (C)–C8	3.78	3.78
4	N^+ (4°)	*N*,*N*,*N*-trimethyl-1-octaminium	N (C) (C) (C)–C8	3.52	–
Primary amines, different carbon chains					
5	NH_3^+	1-decanamine	N–C10	4.27	4.12
6	NH_3^+	4-phenyl-1-butanamine	N–C4–Phen	2.79	2.36
7	NH_3^+	amphetamine[e]	N–C3–Phen	2.64	1.81
8	NH_3^+	2-phenylethanamine	N–C2–Phen	2.26	1.46
9	NH_3^+	1-phenylmethanamine	N–C–Phen	1.76	1.09
10	NH_3^+	1-(4-methylphenyl) methanamine	N – C – Phen – C1	2.25	1.55
11	NH_3^+	1-(4-butylphenyl) methanamine	N–C–Phen–C4	3.97	3.14
12	NH_3^+	1-(4-octylphenyl) methanamine	N–C–Phen–C8	–	5.27
Quaternary amines					
13	N^+	*N*-benzyl-*N*,*N*-dibutyl-1-butanaminium chloride	N (C4) (C4) (C4)–C–Phen	4.28	–

[a] Structures of the test chemicals are presented in Table S2 of the Supporting information.
[b] Abbreviation is based on the structure.
[c] Sorption coefficients including 95% confidence interval are presented in Table S5 of the SI file.
[d] K_{ow} values are taken from different sources (see Table S6 of the SI file).
[e] Amphetamine is a drug, but because of its simple structure it is included in this data set. The C3 moiety contains a branched methyl group.

Table 2
Fiber-water distribution coefficients (D_{fw}) and octanol-water partition coefficients of pharmaceutical and drugs. These organic cations containing C, H, N and in most cases H—bond donor and acceptor groups. Also, one anionic and three neutral compounds are included.

Compound #	Amine type	Name[a]	No. Of H—bond donor (D) and acceptor (A)[b]	Log D_{fw}[c]	Log $K_{ow,N}$ selected[d]
Tertiary amines, tricyclic compounds					
14	NH^+	Amitriptyline	–	4.51	4.92
15	NH^+	Promazine	1 A	4.56	4.55
16	NH^+	Chlorpromazine	1 A	4.84	5.41
17	NH^+	Triflupromazine	1 A	5.37	5.54
Diverse compounds					
18	NH_2^+	MDMA	2 A	3.19	2.15
19	NH^+	Tramadol	1 D, 2 A	3.31	2.51
20	NH^+	Morphine	2 D, 3 A	2.38	0.89
21	NH^+	Nicotine	1 A	2.43	1.17
22	NH^+	Verapamil	5 A	4.94	3.79
23	NH^+	C12-DEA[e]	2 D, 2 A	4.55	4.69
24	N^+	Difenzoquat[e]	–	4.29	–
Secondary amines, beta blockers					
25	NH_2^+	Atenolol	2 D, 3 A	2.34	0.16
26	NH_2^+	Metoprolol	1 D, 3 A	4.03	1.88
27	NH_2^+	Alprenolol	1 D, 2 A	4.07	3.10
Neutral compounds, benzodiazepines					
28	Neutr.	Diazepam	2 A	4.15	2.82
29	Neutr.	Temazepam	1 D, 2 A	4.02	2.19
30	Neutr.	Oxazepam	2 D, 3 A	3.67	2.24
Anionic compound					
31	COO—	Diclofenac	1 D, 2 A	2.76	4.51

[a] Structures of the test chemicals are presented in Table S2 of the Supporting information.
[b] H—bond donor and acceptor moieties were taken from [25].
[c] Sorption coefficients including 95% confidence interval is presented in Table S5 of the SI file.
[d] K_{ow} values are taken from different sources (see Table S6 of the SI file).
[e] C12-DEA is a surfactant, difenzoquat is a pesticide.

3. Results

3.1. Normalizing the sorption affinity to C18/SCX at an equal chemical activity of 1 mmol/L coating

Before comparing sorption affinities between chemicals, we had to normalize the sorption affinity to a similar sorbed concentration because all currently tested compounds display nonlinear sorption isotherms over wide concentration ranges, as previously reported by us [17] and others [22–24]. For all of the tested cationic and neutral compounds, C18/SCX-SPME sorption isotherm data span at least two orders of magnitude of aqueous concentrations. The anionic diclofenac was only tested at three concentrations in order to get comparable C18/SCX sorption data to measurements on the C18 fiber reported in [18]. All tested cationic and neutral compounds showed nonlinear isotherms above sorbed fiber concentrations of 10 mmol/L coating (Fig. 1). As we have discussed before [18], the C18-based SPME coatings are produced using highly porous silica particles for which adsorption is the main sorptive process. Apparently at loadings around 10 mmol/L, sorption sites reach critical levels where competition effects reduce the partition coefficients of both neutral and ionic compounds. Sorption eventually reaches a maximum loading (readily visualized for the dataset on the tricyclic antidepressant chlorpromazine, Fig. 1). Below fiber concentrations of 1 mmol/L coating, however, the slopes of the isotherms for all compounds are all close to a value of 1 on logarithmic scale plots, suggesting that sorption is a linear process at these sorbent loadings. Since nearly all compounds were measured at, or

Table 3
Fragment values for fiber –water partitioning (K_{fw}) and octanol-water partition coefficient (see details in Table S7 of the SI).

Fragment	Fragment value for sorption to C18/SCX fiber	Fragment	Fragment value for partitioning to octanol (neutral compound)[d]
$-NH3^+$	-0.51 ± 0.59[b]	$-NH_2$	−1.41
$-NH2^+$ (C)	-0.03 ± 0.55[b]	−NH (C)	−0.95
$-NH^+$ (C)(C)	-0.08 ± 0.55[b]	−N (C)(C)	−0.72
$-N^+$ (C)(C)(C)	-0.34 ± 0.55[b]		n.a.[e]
CH_2 (aliphatic)[a]	0.48 ± 0.07[c]	CH_2 (aliphatic)	0.49
CH (aromatic)	0.29 ± 0.09[c]	CH (aromatic)	0.23

[a] No distinction is made between CH, CH_2 and CH_3.
[b] Fragment value is not significant (see text for explanation).
[c] Fragmentvalue is significant ($p < 0.01$).
[d] Derived from EpiSuite [28].
[e] Fragmentvalue for quaternary nitrogen not available.

Table 4
Comparison of sorption isotherm parameters for difenzoquat, nicotine and verapamil using ion-exchange membranes [24] or C18/SCX fibers.

Compound	This study			Oemisch et al.		
	K_F(10 μM)	n_F(0.01–1 mmol/L)	log D_{fw} at 1 mmol/L	K_F(10 μM)	n_F(1–100 μM)	log $K_{IEM/water}$ at 1 mmol/kg
difenzoquat	3.21	0.53	4.29	4.40	0.74	5.23
nicotine	2.25	0.57	2.43	2.70	0.75	2.93
verapamil	4.55	0.87	4.94	2.32	0.08	7.12

Distribution coefficients (log $K_{IEM/water}$ for the ion-exchange membranes and log D_{fw} for the C18/SCX fibers) are calculated at C_{aq} of 10 μM using the Freundlich equation with exponent n_F over the tested dissolved concentration range, and at a constant sorbed concentration of 1 mmol/L or mmol/kg.

close to, sorbed fiber concentrations of 1 mmol/L coating, we could fit the logarithmic fiber-water distribution coefficient (log D_{fw}) at 1 mmol/L coating (Tables 1 and 2), with exception of compound #12 (N–C–Phen–C8). Log D_{fw} was estimated using a Freundlich fit of the data below 10 mmol/L coating, thus only incorporating data in the linear concentration range. Only compound #12 (N–C–Phen–C8) was not measured below 10 mmol/L coating, so this compound was excluded from the calculations.

3.2. C18/SCX sorption affinity of organic cations with only C and H atoms

Sorption affinities to the C18/SCX fiber were determined for several series of cationic C_xH_yN structures that lack hydrogen bonding moieties besides the amine or ammonium. Compared to series of more complex pharmaceutical test compounds, these simple cationic structures allow for a more straightforward evaluation of the influence on the C18/SCX sorption affinity of (i) amine type (comparing 1°, 2°, 3° octylamines and 4° octyltrimethylammonium), (ii) different alkyl chain lengths (alkylbenzylamines), (iii) presence of an aromatic ring, (iv) alkyl chain branching. This set of simple cations further allowed us to study the relationship between sorption affinities and simple molecular descriptors, as a framework to compare and predict the affinities of more complex organic cation structures.

Table 1 lists compounds with relatively simple structures with only C, H and N atoms. Using this small data set allows for a tentative estimation of fragment values for the contribution of simple molecular moieties, such as aliphatic carbon units, aromatic carbon units, and charged nitrogen moieties, to the fiber-water sorption coefficient. Based on multiple linear regression of data for the eleven amines, fragment values were estimated (see Table 3 and Table S7). Fragment values for the different nitrogen head groups were not significant because each fragment for the head group occurs only once in the data set. Still – using the octylamines – there is a clear trend in the influence of amine type on sorption affinities, in the order $2° \approx 3° > 4° > 1°$, with a difference of 0.4 log units between 2° and 1° octylamines (Table 1). Interestingly, 4° octyltrimethylammonium has three methyl groups attached to the nitrogen atom, but does not have the highest sorption affinity of the linear alkyl amines as would be expected based on the contribution of an additional methyl group. Quaternary ammonium compounds may show lower sorption than expected, as the charge delocalization around the nitrogen atom can be unfavorable in sorption processes. Since these simple amines were tested as >99.9% ionic species, it is unlikely that the fraction of neutral species contributed to sorption to the C18/SCX coating.

The trend in fragment values for D_{fw} follows the trend in $K_{ow,N}$ based fragments for neutral nitrogen head groups (see Table 3). Clearly, the values for the N entities for sorption to the C18/SCX are much higher than values of the same fragments for the octanol-water system because of the lack of electrostatic interactions in octanol [26].

Fragment values for aliphatic carbon and for aromatic carbon in a phenyl group were significant: 0.48 ± 0.06 for aliphatic carbon and 0.29 ± 0.07 for an aromatic carbon (Table 3). These values of 0.48 and 0.29 are very similar to fragment values for partitioning between water and octanol for neutral compounds: 0.49 for an aliphatic CH_2 fragment and 0.23 for an aromatic carbon atom (Table 3). The difference in these fragment values for aliphatic and aromatic carbon atoms is related to differences in molecular volume or surface area of an aliphatic hydrocarbon chain versus an aromatic hydrocarbon. The van der Waals surface area (SA) of hexane (aliphatic C6) and benzene (aromatic C6) are 178 and 110 $Å^2$, respectively [27]. The ratio in SA of benzene versus hexane of 0.62 is similar as the ratio in fragment values ($C_{aliphatic}/C_{aromatic}$) for D_{fw} of 0.60. In addition to the influence of surface area, the hydrogen bond accepting character of an aromatic ring may also have a slight influence on sorption of aromatic compounds.

The D_{fw} of tributylbenzylammonium (compound #13) is lower than predicted via these fragments. The reason is likely the extensive charge delocalization on all branches on the amine (by one phenyl and three C4 chains) and the more bulky structure of this molecule. It also shows considerably lower affinity to C18/SCX as

the simple aromatic amines. Amphetamine only has 1 branched methyl unit, and fits closely to the relationship observed for all simple aromatic amines.

3.3. C18/SCX sorption affinity of pharmaceuticals and drugs with additional hydrogen bonding moieties

To study the sorption of more polar cations to the C18/SCX fiber, we used a set of 14 different pharmaceutical and illicit drugs. Most of them are >98% cationic at test pH, with the exception of diazepam, oxazepam, temazepam (all neutral), and diclofenac (>99% anionic). Table 2 lists all log D_{fw} values (at a C_{fiber} of 1 mmol/L coating) for these more polar compounds. We found relatively high sorption affinities for all compounds tested. For the $C_XH_YN^+$ amine amphetamine, the C18/SCX fiber was shown to have increased sorption affinity compared to polyacrylate fibers [17], and this was also shown for a more hydrophobic cationic surfactant lauryl diethanolamine (C12-DEA) [19]. Haftka et al. measured sorption affinity of chlorpromazine to polyacrylate fibers at pH 7 and found log D_{fw} values of 3.12 [29], while the C18/SCX fiber displays a 50-fold higher sorption affinity for chlorpromazine (log D_{fw} = 4.84 at pH 6.3, see Table 2).

When the log D_{fw} (at <1 mmol/L C18/SCX) values are plotted against the molecular weight (M_w) of the tested compounds, as shown in the Supporting information Fig. S8, the organic cations with polar groups have a considerably lower affinity to the C18/SCX material than the alkylbenzylamine cations. The following discussion of the sorption data to the C18/SCX fiber and the effects of chemical structure on sorption is based on a comparison with octanol-water partition coefficients of the neutral form of the compounds (log $K_{ow,N}$). Of course log $K_{ow,N}$ is typically used to describe the hydrophobicity driven sorption behavior of neutral compounds, and typically log $K_{ow,N}$ accounts for differences due to the presence of aliphatic carbon chains and aromatic rings and many polar functional groups. Experimentally derived log $K_{ow,N}$ values are available for the neutral form of several of our compounds (Tables 1 and 2), and can be predicted with limited accuracy for the remaining set of compounds, e.g. using the EPIsuite algorithm or the ACD Labs software (see Table S6 in the SI file). Using log $K_{ow,N}$, however, precludes the analysis of quaternary ammonium compounds, since there is no neutral form for these organic salts. Since experimental log $K_{ow,N}$ values are not available for several simple $C_xH_yN^+$ amines, ACD labs estimates are used. For the set of organic cations, log $K_{ow,N}$ is a useful descriptor of the organic cations without an oxygen containing H—bond donor/acceptor group, as shown in Fig. 2. The tricyclic compounds agree well with the other simple amines based on their log $K_{ow,N}$. Apparently the additional sulfur atom and chlorine or fluorine atoms do not lead to a significant increase in the sorption to the C18/SCX fiber.

When these groups of organic cations are combined they give a strong simple regression for all amines, only disregarding amines with oxygen containing H—bonding functionality:

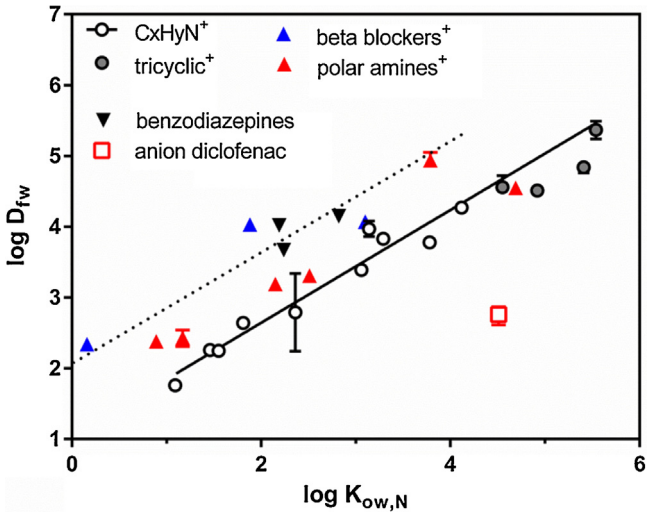

Fig. 2. Relationship between octanol-water partitioning of neutral species (ACD Labs estimates for $C_xH_yN^+$ amines, experimental values for all others) and linear sorption affinity to the C18/SCX coating of the corresponding cationic species. $C_xH_yN^+$ compounds are from Table 1, with exception of the quaternary amines (compounds 4 and 13, as these have no log $K_{ow,N}$) and compound 11 (as log D_{fw} could not be extrapolated accurately). The polar amines are listed in Table 2, compounds 18–23. The dotted line indicates a 10x higher sorption affinity to the C18/SCX fiber compared to the log $K_{ow,N}$ relationship (line = 0.80·log $K_{ow,N}$ + 2.05).

The relationship in Eq. (1) illustrates again that cationic species sorb stronger than expected to the porous C18/SCX material on the mixed mode SPME fibers, taking into account that these ionized compounds also display a very high aqueous solubility. Similar conclusion can be drawn from the fragment values in Table 3. It is also interesting to note that six polar compounds are well predicted with the log $K_{ow,N}$ relationship, while three are substantial outliers that sorb much stronger to the C18/SCX fiber than predicted by the log $K_{ow,N}$ of the neutral form. The three cationic beta-blockers have a similar backbone, containing multiple polar groups, but with different substitutions on the aromatic ring: atenolol (log $K_{ow,N}$ 0.16, methylamide in para-position) and metoprolol (log $K_{ow,N}$ 1.88, methoxyethyl in para-position) sorb a factor 15 and 30, respectively, stronger to the C18/SCX material than predicted by eq. 1, while the more hydrophobic beta-blocker alprenolol (log $K_{ow,N}$ 3.10, vinyl group in ortho-position) differs only by a factor of 3. The only other polar organic cation that differs more than a factor of 5 is the large calcium channel blocker verapamil (log $K_{ow,N}$ 3.79, two aromatic rings, 4 ethers). The other polar organic cations nicotine (heterocyclic nitrogen), MDMA (methylenedioxy), tramadol (hydroxy and methoxy units), morphine (complex polycyclic diol) all sorb up to a factor 3 stronger when compared to the regression based on simple $C_xH_yN^+$ amines. The cationic surfactant C12-DEA

$$\mathrm{LogD_{fw,cation}(at\ <1\ mmol/LC18/SCX)} = 0.80(\pm0.07)\cdot\mathrm{logK_{ow,N}} + 1.05(\pm0.19),\ \mathrm{n} = 10,\ \mathrm{R^2} = 0.946,\ \mathrm{sy.x(standarddeviationoftheresiduals,\ as(SS/df)^{0.5})} = 0.213 \quad (1)$$

For three neutral benzodiazepine compounds, sorption affinity to the C18/SCX fiber does not appear to be readily predictable based on (experimentally derived) log $K_{ow,N}$ values alone. Diazepam, oxazepam and temazepam are structurally very similar but do show significant differences in sorption affinity. Compared to diazepam, temazepam has an extra OH group, which causes a decrease in sorption affinity of approximately 0.1 log units (though not significant), while experimental log $K_{ow,N}$ values differ by 0.6 log units. Oxazepam contains the same OH group but is also demethylated, further decreasing the C18/SCX sorption affinity by 0.3 log units, while experimental log $K_{ow,N}$ values do not differ between oxazepam and temazepam (Table 2).

appears to follow the sorption as predicted based on log $K_{ow,N}$, while containing 2 ethanol groups.

Other physicochemical descriptors than log $K_{ow,N}$ may be sought to derive an overall polyparameter relationship for organic cations, which includes the sorption values of atenolol, verapamil and metoprolol, to predict the linear sorption affinity to C18/SCX. For instance, Difilippo and Eganhouse [30] combined sorption affinities of hydrophobic organic compounds to polydimethylsiloxane (PDMS) coated fibers and were able to predict sorption through a polyparameter linear solvation energy relationship (LSER), using parameters related to the refractive index, polarizability and hydrogen bonding capacities in addition to the molecular volume. A

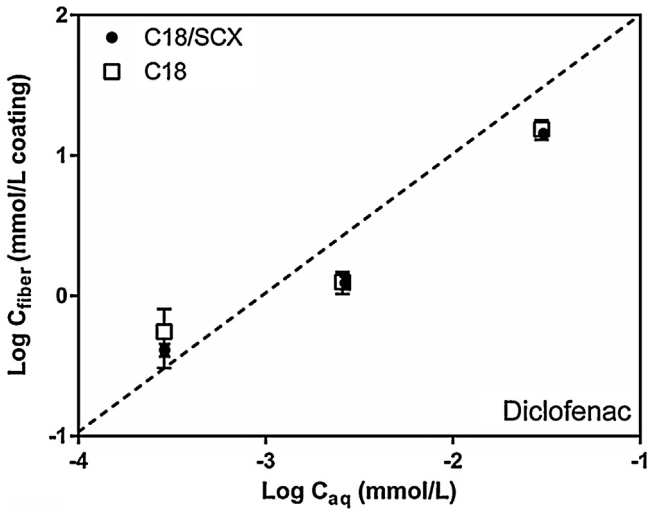

Fig. 3. Comparison of sorption of diclofenac to the C18/SCX and C18 fiber. Exposure time was 72 h. Dotted line indicates linearity.

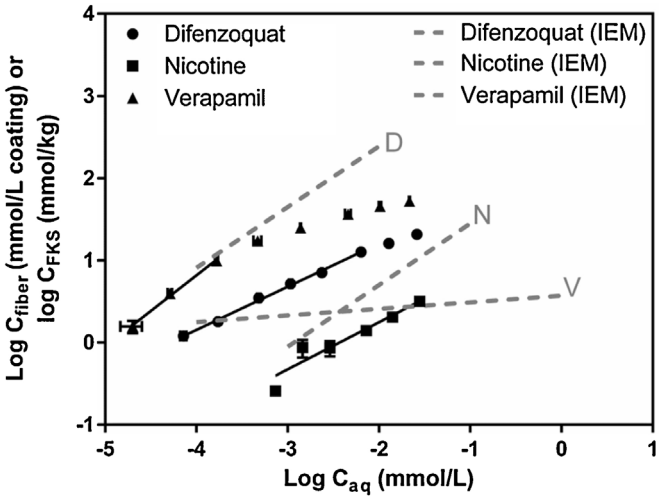

Fig. 4. Comparison of sorption isotherms for difenzoquat, nicotine and verapamil to the C18/SCX fiber and ion-exchange membranes [24].

similar compilation was made by Endo et al. [31] for a diverse set of neutral organic compounds, where sorption to polyacrylate coated SPME fibers could be predicted through polyparameter linear free energy relationship (PP-LFER) models. Limitation of these polyparameter predictions is that they only seem to be applicable to sorption of neutral compounds to neutral SPME coatings, and the required experimental molecular descriptors are (i) not equally relevant, and likely even different for charged chemicals, and (ii) none are available for the neutral form of the tested chemicals in our study.

The predominantly anionic compound diclofenac shows substantial sorption to the C18/SCX fiber, which is surprising as it was expected that the anionic species are repulsed from the (presumably) negatively charged C18/SCX surface. Based on its log $K_{ow,N}$, diclofenac indeed sorbs a factor 64 lower than predicted by the organic cation regression of Eq. (1). At pH 7.4, only 0.04% of diclofenac is present in the neutral form. Comparison of the C18/SCX fiber and C18 fiber (without strong cation exchange sites) shows equal sorption of diclofenac to both fiber types (Fig. 3). The strong cation exchange groups in the C18/SCX coating do not appear to inhibit sorption of anionic diclofenac. However, as sorption of diclofenac is lower than the predicted sorption based on log $K_{ow,N}$, which could indicate that both the C18 and the C18/SCX coating contain sorbent material that repulses anions. We have previously hypothesized that deprotonated free silanol groups might contribute to the sorption of cations, and counteract the sorption of anions [17,18]. However, to be able to predict sorption behavior of anionic compounds, the data set on these compounds should be extended.

3.4. Comparison of C18/SCX fibers with cation-exchange membranes

Passive sampling devices such as polar organic chemical integrative samplers (POCIS) are already used to sample ionizable chemicals in aqueous environments such as rivers and sewage treatment plants [32,33]. Although POCIS samplers can apply various polymers optimized to sorb certain types of dissolved chemicals, the polymer sorbent is typically used as an unsaturable sink, allowing for time integrated analysis of dissolved solute concentrations. The C18/SCX fiber is an equilibrium-based sampling tool that is more suitable in lab-scale partitioning studies and clinical applications. In comparison to this application of C18/SCX fibers, the use of ion-exchange membrane strips as equilibrium based sampling devices was recently evaluated for several ionized compounds [24]. A cation exchange membrane (IEM) was used to study the sampler affinity in HBSS buffer (pH 7.4) for the cationic compounds nicotine, difenzoquat and verapamil. Here, we studied the sorption affinity of these compounds to the C18/SCX fiber and compared sorption affinities and Freundlich slopes (n_F) of both passive sampling devices (Fig. 4).

Using the IEM, good results were obtained for difenzoquat and nicotine. However, sorption of verapamil resulted in a nearly constant concentration in the IEM at any water concentration tested, reflected by the Freundlich slope of 0.08 [24]. According to the authors, the IEM was already saturated at the lowest water concentration tested. However, this is inconsistent with the total ion-exchange capacity of these membranes, which is reported at 1200 mmol/kg. Using the C18/SCX fiber, good results were obtained for all three compounds. Freundlich slopes are somewhat more nonlinear than those obtained with the IEM, with exception of verapamil (Table 4). As the cation-exchange capacity of the C18/SCX fibers (~400 mmol/L coating) is a factor three lower than that of the IEM, sorption of cationic compounds to the C18/SCX fiber starts to level off at a lower fiber loading compared to the IEM.

For both passive sampling materials, molecular size and structural geometry could influence the accessibility of the ion-exchange sites. It is likely that sorption of verapamil to the IEM is limited by its large molecular size, thereby limiting the occupation of all cation-exchange sites. This compound has more predictable sorption to the C18/SCX fiber, as highlighted by the higher Freundlich coefficient. This could be the result of the highly porous nature of the C18/SCX coating, making it more accessible for larger compounds. This porosity can also have disadvantages, such as fouling of the device in protein-containing samples. A good example is fouling with bovine serum albumin (BSA). This fouling effect is larger for the IEM at low BSA concentrations, but larger for the C18/SCX fiber at high BSA concentrations [21].

4. Conclusion

The C18/SCX fiber has previously shown to be capable of extracting cationic compounds. Here, the data set for sorption of cationic compounds is expanded. In addition, sorption of three neutral compounds and one anionic compound is incorporated. As all compounds show sorption to the C18/SCX fiber, this strongly supports our hypothesis that ionized compounds sorb to C18-based SPME coatings through adsorptive processes. The strong

cation exchange groups in the C18/SCX fiber increase sensitivity of this fiber for cationic compounds, but could also make the C18/SCX fiber more prone to competitive effects of salts, especially for polar cations with relatively low sorption affinities (e.g. log $D_{fw} < 2$). This makes modeling sorption of ionizable compounds to the C18/SCX fiber difficult. However, there is a clear linear relationship between molecular weight and sorption affinity for alkyl amines and aromatic amines without oxygen-containing H—bonding groups. Moreover, this relationship also exists for all $C_xH_yN^+$ cations based on log $K_{ow,N}$, facilitating the expectations for the calibration feasibility of the C18/SCX fiber for related organic cation structures, e.g. cationic surfactants. More polar compounds, i.e. cations with oxygen-containing H—bonds, sorb as strong as or stronger to the C18/SCX fiber than predicted based on this log $K_{ow,N}$ relationship.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2016.04.017.

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