Journal of Chromatography A, 1390 (2015) 28–38

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Sorption of amitriptyline and amphetamine to mixed-mode solid-phase microextraction in different test conditions

Hester Peltenburg[a,*], Steven T.J. Droge[a], Joop L.M. Hermens[a], Ingrid J. Bosman[b]

[a] Institute for Risk Assessment Sciences, Utrecht University, P.O. Box 80177, 3508 TD Utrecht, The Netherlands
[b] Netherlands Forensic Institute, P.O. Box 24044, 2490 AA The Hague, The Netherlands

ARTICLE INFO

Article history:
Received 3 October 2014
Received in revised form 17 January 2015
Accepted 20 February 2015
Available online 28 February 2015

Keywords:
Solid-phase microextraction
Mixed-mode fiber
C18/SCX
Cationic drugs
Cation-exchange
Bio-analysis

ABSTRACT

A solid-phase microextraction (SPME) method based on a sampler coating that includes strong cation groups (C18/SCX) is explored as a rapid direct sampling tool to detect and quantify freely dissolved basic drugs. Sampling kinetics, sorption isotherms and competitive effects on extraction yields in mixtures were tested for amphetamine and the relatively large/hydrophobic tricyclic antidepressant amitriptyline. Both compounds are >99% ionized at pH 7.4 but their affinity for the C18/SCX fiber is markedly different with distribution coefficients (D_{fw} values) of 2.49 ± 0.02 for amphetamine and 4.72 ± 0.10 for amitriptyline. Typical changes in electrolyte homeostasis that may occur in biomedical samples were simulated by altering pH and ionic composition (Na^+ and K^+ concentrations). These changes were shown to affect C18/SCX sorption affinities of the tested drugs with less than 0.2 log units. At relatively low fiber loadings (<10 mmol/L coating) and at all tested exposure times, linear sorption isotherms were obtained for both compounds but at aqueous concentrations of the individual drugs corresponding to concentrations in blood that are lethal, sorption isotherms became strongly nonlinear. Competition effects within binary mixtures occurred only if combinations of aqueous concentrations resulted in total fiber loadings that were in the nonlinear range of the SPME sorption isotherm for the individual compounds. We also compared sorption to the (prototype) C18/SCX SPME coating with analogue (biocompatible) C18 coated SPME fibers. C18/SCX fibers show increased sorption affinity for cationic compounds compared to C18 fibers, as tested using amitriptyline, amphetamine and trimethoprim. Surprisingly, sorption affinity of these ionized compounds for the C18 SPME fibers were within 1 log unit of the C18/SCX SPME fibers. This shows that the strong cation exchange groups within the C18/SCX coating only has a relatively small contribution to the total sorption affinity of cationic compounds. Also the role of negatively charged silanol groups in both the C18 and C18/SCX coating seems small, as anionic diclofenac species sorbed strongly to the C18 fiber. Ionized organic species seem to be substantially adsorbed to the high surface area of C18 in SPME types using porous silica based coatings.

1. Introduction

Solid-phase microextraction (SPME) was developed in the early 1990s by Arthur and Pawliszyn [1], and is a simple partition-based extraction technique with several advantages over the conventional sampling methods. New developments and applications of SPME are published on a regular basis, for instance on newly available coatings [2] and on automation of the sampling method for high-throughput analysis [3]. SPME sampling has unique characteristics, such as the yield of clean concentrated extracts from heterogeneous matrices [4,5] and negligible depletion of the system that allows for repeated sampling without disturbance of that system [6,7]. At present, SPME has been validated for many different bioanalytical and forensic studies, including *ex vivo* and *in vivo* sampling for a variety of pharmaceuticals [5,8–18]. Of particular interest are minimally invasive studies into the biokinetics of pharmaceuticals and drugs. The challenge here is that the majority of drugs of interest in screening procedures are polar ionizable chemicals, of which most exist largely as ionic species at physiological conditions, while conventional SPME sampling has largely focused on extracting neutral compounds, or neutral fractions of ionizable compounds, and may have an insufficient yield for ionized polar drugs.

We have previously described the sampling process of the basic drug amphetamine (pK_a 9.9 [19]) from physiological medium using

* Corresponding author. Tel.: +31 30 253 3631; fax: +31 30 253 5077.
E-mail address: H.Peltenburg@uu.nl (H. Peltenburg).

http://dx.doi.org/10.1016/j.chroma.2015.02.065
0021-9673/

a newly developed "mixed-mode" coating (C18/SCX), consisting of hydrophobic C18 chains and embedded strong cation exchange groups (propylsulfonic acid) coated on porous silica [20]. This C18/SCX fiber was shown to extract ionized amphetamine with a much higher yield than other, neutral polymer coatings such as polydimethylsiloxane (PDMS) and polyacrylate (PA). The same benefit of this C18/SCX fiber over conventional SPME coatings was recently shown for a cationic surfactant [21]. Also, this surfactant displayed a much higher affinity to the C18/SCX coating than amphetamine. This indicates that the chemical application range of C18/SCX possibly includes small and polar, but also relatively hydrophobic basic compounds, and that the impact of competing ionic drugs for binding to the C18/SCX coating needs to be examined. A comparable custom-made mixed-mode SPME coating already showed increased metabolite coverage compared to other conventional fibers in an untargeted metabolomics profiling study [22]. This mixed-mode fiber was consequently applied in three *in vivo* global metabolomics studies: in the blood of mice [23], in liver and lung tissue of pigs [24] and in the brain of rats [25]. These studies show that coatings with ion-exchange phases may especially provide high yields for small organic cations, where the neutral fraction has insufficient affinity to sorb to classic SPME coatings. However, the sorption process is dependent on several factors such as pH and the concentration of other (inorganic) cations such as Na^+ and K^+ [20], which may compete for sorption to the C18/SCX coating. Although these variables have been studied in equilibrated systems, other factors that influence sorption to C18/SCX coatings, such as the influence of uptake kinetics and the effects on sorption affinity in the presence of other charged drugs in mixtures are still not completely understood. The effects of these variables and test conditions need to be further investigated for a proper application of the C18/SCX sampling method for basic drugs in biological samples.

Our previous work was based on equilibrium sampling of amphetamine in simple physiological buffers to compare the C18/SCX fiber to other SPME coatings [20]. Detailed insight into the characteristics and mechanisms of the extraction process is needed before it can be applied in toxicity or forensic studies. Having these insights is relevant for the relatively new C18/SCX coating where more complex interactions of ionized chemicals with the fiber coating may occur. The current study investigates the influence of pH, ionic composition, temperature, exposure time and agitation on the sorption to the C18/SCX fiber, both for amphetamine as well as amitriptyline. These factors need to be carefully characterized as they can influence sorption to the fiber during applications in *in vitro* and *in vivo*. After death, especially, pH and ionic composition may change and this may influence sorption to the fiber. Postmortem, blood pH drops from 7.4 to around 5.5 due to an accumulation of acidic glucose metabolites [26]. The ionic composition of blood changes as Na^+ concentration decreases and K^+ concentration increases due to failure of the Na^+/K^+/ATPase pump [27]. Furthermore, the C18/SCX fiber was exposed to mixtures of amphetamine and amitriptyline to study potential competition effects on sorption affinities within mixtures. Another topic that is specifically addressed in this study is the comparison of the affinity of ionized drugs to the mixed mode (C18/SCX) coating to their affinity for analogue C18 coated SPME fibers that lack the SCX functionality. Such a comparison may provide a more refined rationale on which interactions govern extraction efficiencies to mixed-mode SPME coatings. This study leads to general knowledge that can easily be utilized in more applied studies with these two chemicals, but also in studies with other positively ionized compounds.

2. Materials and method

2.1. Chemicals and materials

Solid-phase microextraction fibers with mixed-mode (C18/propylsulfonic acid; C18/SCX) coating were prototype fibers provided by Supelco, Sigma Aldrich (Bellefonte, PA, USA). The fibers were 3 cm pieces of nitinol wire (202 μm diameter) with 1.5 cm of coating at an average thickness of 45 μm (total fiber volume 524 nL). Both C18 and propylsulfonic acid are bonded on porous HPLC column grade silica material which is then bound to the wire with a biocompatible polymeric binder (Supelco, pers.comm.). Analogous SPME-LC fiber probes (functional group C18) were purchased from Sigma Aldrich (Zwijndrecht, The Netherlands). These C18 fibers (fiber volume 520 nL) also consist of a 1.5 cm coating at an average thickness of 45 μm, coated on nitinol wire (200 μm diameter). Amphetamine hydrochloride was purchased from Spruyt Hillen, IJsselstein, The Netherlands. Amitriptyline hydrochloride and diclofenac sodium were from Sigma Aldrich. Trimethoprim was from Dr. Ehrenstorfer (Augsburg, Germany). Phosphate buffered saline (PBS) consisted of 138 mM NaCl, 8 mM of Na_2HPO_4, 1.5 mM of 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). Calcium dichloride (Sigma Aldrich) was used to make Dulbecco's PBS (DPBS) [28], *i.e.* PBS containing 0.9 mM Ca^{2+}. Buffers of different pH were either phosphate buffers (H_3PO_4 and NaH_2PO_4 between pH 2 and 4, NaH_2PO_4 and Na_2HPO_4 between pH 6 and 8, and Na_2HPO_4 and Na_3PO_4 above pH 11), acetate buffers (CH_3COOH and CH_3COONa between pH 4 and 6) or carbonate buffers ($NaHCO_3$ and Na_2CO_3 between pH 8 and 11). Borate buffer pH 10 consisted of H_3BO_3 adjusted to pH 10 with NaOH. All salts were from Merck or Sigma Aldrich. Ammonia solution (25%) was obtained from Merck. Methanol and acetonitrile were HPLC-grade (BioSolve, Valkenswaard, The Netherlands).

2.2. SPME procedure

Test solutions were spiked from stock solutions in methanol, ensuring methanol fractions of <1%. During SPME fiber exposure, samples were either placed on a roller mixer for agitated sampling (40 rpm) or kept on the lab table during static sampling. These roller mixers (Stuart SRT9) are specially designed to provide a gentle but effective agitation, which is needed for future work with cells or proteins. After a certain exposure time, fibers were transferred to vials containing 120 μL desorption fluid. Fibers are wiped gently to remove any droplets of buffer before placing them in desorption fluid. For C18/SCX fibers, this consisted of 90% acetonitrile and 10% Milli-Q water with 0.1% NH_3 (of end volume) with a pH around 11 to extract the neutral base. The fibers were desorbed for more than 96% within 15 min (Supporting Information, Fig. S1). After taking out the desorbed fibers, the desorption solutions were acidified to pH 2–3 using 60 μL 0.1 M HCl, to approximate the mobile phase [20]. C18 fibers were desorbed for a minimum of 30 min in 90% acetonitrile and 10% water (180 μL), according to the instructions of the manufacturer. To re-use the fibers for several experiments, they were pooled after use, kept in desorption fluid overnight and then stored in 50/50 methanol/water until the next experiment. Fiber blanks were incorporated in every experiment in triplicate from exposure solutions that had not been spiked to confirm that no carry-over existed between experiments. Sorption isotherms for amphetamine were shown to be reproducible for fibers from different batches (Supporting Information, Fig. S2). Sorption isotherms at pH 7.4 of amphetamine comparing the same fibers new and after twenty experiments showed a small decrease of 0.1–0.4 log units