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Monitoring of drugs and metabolites in whole blood by restricted-access solid-phase microextraction coupled to liquid chromatography–mass spectrometry

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Abstract

Robust biocompatible solid-phase microextraction (SPME) devices were prepared using various alkyldiol-silica (ADS) restricted-access materials (RAM) as the SPME coating. The ADS-SPME approach was able to simultaneously fractionate the protein component from a biological sample, while directly extracting diazepam and the major metabolites *N*-desmethyldiazepam, oxazepam and temazepam, and overcame the present disadvantages of direct sampling in biological matrices by SPME. The devices were interfaced with an LC–MS system and an isocratic mobile phase was used to desorb, separate, and quantify the analytes. The calculated diazepam, nordiazepam, temazepam, and oxazepam detection limits were 20, 20, 30, and 35 ng/ml in heparinized blood, respectively. The method was confirmed to be linear over the range of 50–1000 ng/ml with an average linear coefficient (R^2) value of 0.996. The injection repeatability and intra-assay precision of the method were evaluated over ten injections at concentrations of 50, 200, and 500 ng/ml, resulting in a R.S.D. of ca. 10%. The robustness of the ADS-SPME device was evaluated for future use in in vivo studies, providing many direct extractions and subsequent determination of benzodiazepines in blood. For the extraction of the peptides angiotensin I, II, and III from blood, a novel restricted access material with cation exchange properties was evaluated. The ion-exchange diol silica improved the extraction efficiency of peptides relative to the conventional ADS material with reversed phase extraction centers.

Keywords: Solid-phase microextraction; Restricted access media; Silica; Alkyldiol; Drugs

1. Introduction

Solid-phase microextraction (SPME) is a sampling and sample preparation technique which was developed over 10 years ago for the analysis of volatile and semivolatile organic compounds analysis in environmental samples [1]. SPME provides many advantages over conventional sampling methods by integrating sample extraction, concentration, and introduction into a single step. SPME has been successfully coupled with gas chromatography (GC), high-performance liquid chromatograph (HPLC), and capillary electrophoresis (CE) and has found numerous applications in many disciplines as indicated by a recent text [2].

Most recently, SPME has been extended to various aspects of biological sample analysis and has been the subject of several reviews [3–6]. The convenient format of SPME devices means the technique can be non-invasive and provide the direct sampling of drugs and metabolites in animals. For example, SPME devices based on polypyrrole films have been used for the in vivo sampling of benzodiazepines in beagles [7]. For these in vivo studies, researchers must overcome difficulties, such as fouling of the SPME fiber by proteins in body fluids during direct extraction. In addition, the application of chemical analysis inside animals requires greater robustness in both the extraction phase and the supporting fiber core than could be obtained with commercial coatings. We present in this study another SPME device based on a restricted-access material (RAM), which can be used for the in vivo and in vitro sampling of benzodiazepines from blood. The properties of this material have been described previously [8–13]. In summary, the pore size of the particles is able to fractionate a sample into the protein matrix and the analyte component. Simultaneous

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with this size exclusion process, low molecular weight compounds are extracted and enriched, via partition, into the phase's interior. It was shown previously that utilizing the ADS particles as a SPME coating can further simplify the extraction process and experimental set-up, while completely eliminating the requirement of extraction solvents [14].

The purpose of this study was to develop a biocompatible SPME coating based on a restricted-access material for the determination of benzodiazepines in blood samples, which has a sufficient robustness to be used for in vivo sampling. Benzodiazepines represent a class of drug compounds administered for a wide range of clinical disorders [15] and the determination often requires sample pretreatment involving tedious and complex pretreatment protocols.

Recently, a new RAM with ion-exchange (XDS) properties was introduced. To the author's best knowledge, this material has only been used in a SPE-cartridge format for the extraction of neuropeptide [16], and basic drugs [17] from serum samples. We present here the first SPME device capable for the extraction of peptides such as angiotensin I, II, and III, from whole blood. Among these peptides, angiotensin II plays the most important role as it is pharmacological active and influences the blood pressure.

2. Experimental

2.1. Materials

All solvents were HPLC grade or better and purchased from Caledon (Georgetown, Canada). The benzodiazepines, shown in Fig. 1, were purchased from Cerilliant (Austin, TX, USA) as 1 mg/ml methanol solutions and stored at 4 °C. [^{3}H] Diazepam was purchased from NEN Life Science Products (Boston, MA, USA) as a 3.454 μg/ml ethanol solution. The specific activity was 82.5 Ci/mmol. The angiotensin peptides and all other chemicals were purchased from Sigma–Aldrich. Deionized water, from a Barnstead/Thermodyne NANO-pure ultrapure water system (Dubuque, IA, USA), was used for dilution of the standards. Stainless steel wires (grade T-304 diameters 1.27 mm) were purchased from Small Parts (Phoenix, AZ, USA). LiChrospher RP-18 alkyldiol-silica (ADS) and XDS 25 μm particles were supplied by Merck (Darmstadt, Germany). An Intel Play QX3 (Santa Clara, CA, USA) digital microscope was used to monitor the coating stability.

2.2. Preparation of ADS-SPME devices

The steel wires were cut into 70 mm lengths and thoroughly cleaned by sonication in water, followed by rinsing in pure ethanol and water, respectively. The ADS particles were immobilized on the steel wire using an epoxy binding agent from Supelco (Bellefonte, PA, USA). After preparing a slurry of 150 μl binding agent (previously diluted (1:1) with chloroform) and 100 mg of particles, the slurry was transferred into a 200 μl HPLC microvial. The steel wire was carefully dipped into the microvial containing the slurry and stirred for 30 s followed by curing of the binding agent by heating inside an oven at 200 °C for 2 h. A Hitachi model S-570 (San Jose, CA, USA) scanning electron microscope was used to image the prepared surface of the blank steel wire and the ADS-SPME wires (Fig. 2).

2.3. Conditioning of ADS-SPME device and extraction of [^{3}H] diazepam

The prepared wires were initially conditioned by successively shaking the submerged wires in 2-propranol, methanol, and water for 20 min. The fibers were then stored in a water–methanol (95:5, v/v) mixture until ready to use.

The ADS-SPME and a blank steel wire with polymerized binding agent on the surface were placed in 1.5 ml Eppendorf (Brinkmann Instruments, Mississauga, Canada) plastic micro-centrifuge tubes containing 1.0 ml of [^{3}H] diazepam standard solution (prepared in water) at a concentration of 3.43 ng/ml, followed by agitation on a shaker table for 3 h. Water instead of whole blood was used in this experiment, as we only wanted to investigate the non-specific binding of diazepam to the glue. Matrix interferences from blood may affect the recovery of diazepam from blood, but initially we wanted to estimate the maximum amount of analyte

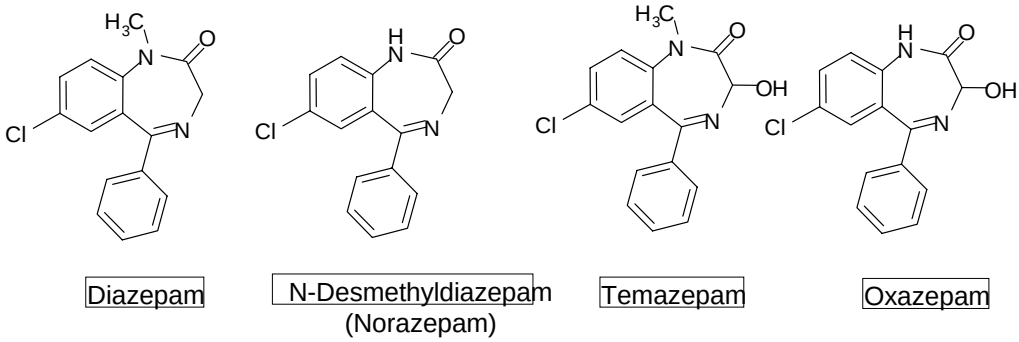

Fig. 1. Structures of benzodiazepines used in this study.

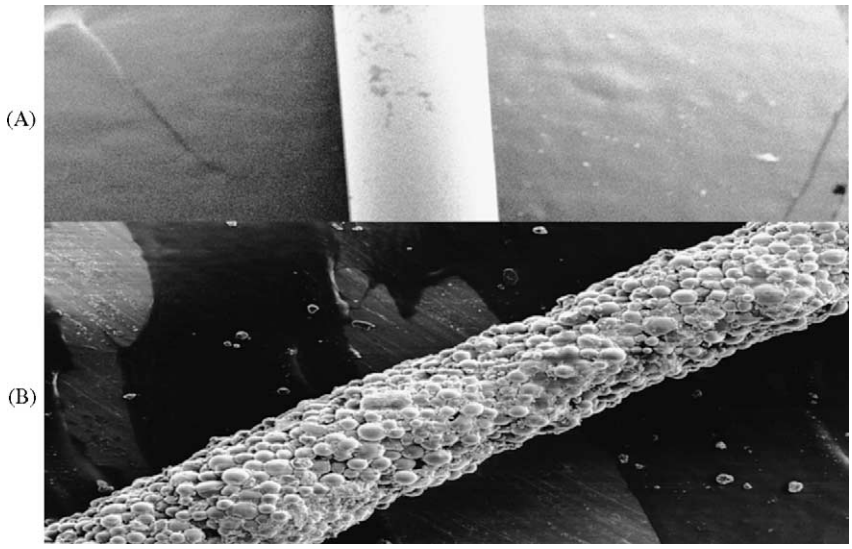

Fig. 2. Scanning electron micrographs of bare silica fiber (A) and ADS-SPME device coating (B). Gold coating overlayer: 30 nm; accelerator voltage: 15 kV.

extracted to see if the capacity of the fiber is high enough for LC–MS analysis.

The fiber was removed and rinsed twice by total immersion in water and placed into scintillation vials containing 20 ml of Ecolume scintillation cocktail (a proprietary mixture of linear alkylbenzene, phenylxylylethane, non-ionic surfactants, 2,5-diphenyloxazole, and *p-bis*(*o*-methylstyryl)benzene). The vials were vigorously shaken and counted in a Beckman–Coulter (Fullerton, CA, USA) model LS1701 scintillation counter for 5 min. This removed most of the labeled diazepam from the fiber coating as determined by subsequent recounting of the fiber in fresh scintillation cocktail.

2.4. Instrumentation and analytical conditions

A Hewlett-Packard (Palo Alto, CA, USA) HPLC system (model 1100) complete with autosampler and multiple-wavelength UV detector ($\lambda = 230$ nm) coupled to an Agilent mass-selective detector equipped with electrospray ionization (ESI) source was used with a Supelcosil C_{18} column (5.0 cm × 4.6 mm i.d.; 5 μm particle size) from Supelco. A LiChrosorb RP-18 guard column (1 cm × 4.6 mm) from Supelco was installed at the inlet of the chromatographic column for protection of the analytical column. A SPME–HPLC interface was constructed as previously described [14] using a Valco zero volume tee from Chromatographic Specialties (Brockville, Canada). Elution of the extracted compounds from the ADS-SPME wire and separation by the reverse phase HPLC column was accomplished with switching the six-port injection valve to redirect the water–methanol (50:50, v/v) mobile phase over the fiber surface at a flow rate of 0.6 ml/min. The flow was introduced directly into the ESI interface. The capillary voltage was set to 3 kV, drying gas temperature was 300 °C, dry gas flow was 10 l/min, nebulizer pressure was set to 50 p.s.i. (1 p.s.i. = 6894.76 Pa). For diazepam, *N*-desmethyldiazepam, oxazepam, and temazepam the quasi molecular ions 285, 271, 301, and 287 were monitored in the selected ion monitoring (SIM) mode.

Elution of the peptides from the XDS–SPME wires on the analytical column (Supelco RP-18, 15 cm × 4 mm, 5 μm) was performed using gradient elution. Solvent A consisted of 0.01 mM NH_4COOH (pH 3.3)–acetonitrile (ACN) (75:25, v/v) and solvent B was 0.01 mM NH_4COOH (pH 6.7)–ACN (50:50, v/v). The gradient was started with 100% of solvent A and solvent B was increased to 100% within 15 min. The 1 ml/min flow was split 1:1 by a conventional Valco T-piece before introduction into the MS. The capillary voltage was set to 4.5 kV, drying gas temperature was 300 °C, dry gas flow was 13 l/min and nebulizer pressure was set to 18 p.s.i. For Angiotensin I, II, and III the ions 546, 648 (double charged), and 884, respectively, were monitored in the SIM mode.

2.5. Preparation of blood samples

Blood samples were collected from a drug free healthy volunteer and filled into 7 ml Vacutainer tubes (Franklin Lakes, NJ, USA) containing sodium heparin. The tubes were shaken for 2 min. One hundred and fifty microlitres of the heparinized blood were transferred into a 2.0 ml amber sample vial containing a 200 μl micro-insert. The four benzodiazepines were directly spiked into the blood over a range of 0.1–2 μg/ml. The ADS-SPME wire was directly submerged into 1.5 ml of the blood. The ADS-SPME device was rinsed twice by total immersion in water before interfacing to the LC–MS system for desorption and separation of the extracted analytes.

2.6. Preparation of phosphate-buffered saline (PBS) buffer

Eight grams NaCl, 0.2 g KCl and 1.4 g Na_2PO_4, 0.24 g KH_2PO_4 were dissolved in 800 ml of deionized water. The pH was adjusted with 0.1 M HCl to pH 7.4 and the final volume was adjusted to 1 l.

3. Discussion

3.1. Immobilization of ADS material

The chemical and physical properties of the ADS material have been previously discussed in the literature [12]. In summary, the porous ADS particles possess a hydrophilic electroneutral diol exterior surface to prevent protein adsorption, and a variable inner surface (cation exchange or alkyl hydrophobic bonded phases, such as C_4, C_8, C_{18}) that is responsible for extraction of the target compounds. Immobilization of the material onto a stainless steel wire provided a SPME coating whereby the inert outer layer protected the coating from contamination by proteins, while allowing direct and multiple extractions of blood.

The immobilization of the ADS particles was accomplished by adhering the particles onto a cleaned stainless steel wire. Several tests to monitor the physical ruggedness of the coating were performed and the stability of the coating was monitored under a digital microscope. Two binding agents, Supelco epoxyglue and Loctite 349 (Rocky Hill, CT, USA) were evaluated and optimized on their ability to physically maintain the ADS coating throughout the SPME experiments. Therefore, to test the physical robustness of the fiber, the ADS-SPME wire was wiped with a tissue several times. The binding agent must also be chemically stable in the various organic solvents and pHs of the mobile phase since the presence of background peaks, resulting from the breakdown of the binding agent, could interfere with the analyte's determination. The chemical stability of the ADS-SPME wires was evaluated by sonication in methanol for 10 min. Finally the heat stability of the coating was tested, as the fiber has to be sterilizeable for in vivo use. The ADS coated wires were placed in 10 ml glass tubes filled with 5 ml of PBS buffer. The tubes were placed in an autoclave chamber and sterilized at 125 °C for 30 min. Overall, the epoxyglue provided the most uniform and robust bonding of the ADS particles to the steel wires. As shown in Fig. 2, scanning electron micrographs (SEM) of blank steel wire (a) and ADS-SPME (b) wires were recorded for comparison purposes. The confirmation of the ADS particles immobilized on the fiber over a fairly uniform coating was obvious. The SEM data was used to estimate an average coating thickness of 50 μm.

3.2. ADS-SPME device characterization

The extraction mechanism of the ADS-SPME coating was via absorption, as the analytes can partition into the C_{18} stationary phase of the inner pores. The C_{18} extraction process is non-competitive (in comparison to adsorption) and the amount of analyte extracted from a sample is independent of the matrix composition. Absorption occurs if analyte can partition in alkyl bonded layer. Many publications are devoted to the study of the structure, confirmation, mobility, and solvation of bonded alkyl chains [18–23]. It has been shown that alkyl chains bonded on silica surface have a noticeable conformational freedom [21–23] and a certain degree of solvation [24,25] which supports a partitioning model [26,27]. The extraction performance of the ADS-SPME device was validated using [^{3}H] diazepam and liquid scintillation detection. Scintillation counting was chosen due to its simplicity, speed, and sensitivity. The reproducibility of the coating was tested with five independently prepared ADS-SPME devices. The fibers were submerged in a 3.43 ng/ml standard [^{3}H] diazepam solution (in 95:5 water:methanol) for 3 h on a shaking bed. The fiber was then removed from the solution, washed twice by total immersion in water–methanol (95:5) and placed in a scintillation vial containing 20 ml of scintillation cocktail. The vials were vigorously shaken and counted in triplicate with the liquid scintillation counter for 5 min. The average value of the three counts was considered as the final counting result. In addition, a steel wire with polymerized binding agent on the surface (control) was evaluated in the 3.43 ng/ml standard [^{3}H] diazepam solution and an ADS-SPME device evaluated in a water–methanol (95:5) solution (blank). Tables 1 and 2 show the scintillation results, and its reproducibility, respectively. The amount of diazepam binding to the blank wire control fiber was determined to be negligible. However, the ADS-SPME coating on the wire's surface was successful in extracting a significant portion of the diazepam from the sample. The diazepam penetrated into the porous structure of the ADS and was absorbed by the C_{18} extraction phase. The preparation of the ADS-SPME

Table 1
[^{3}H] Diazepam scintillation response comparison between ADS-SPME devices and control

Fiber	Scintillation counts
Blank	110
ADS-SPME	45649

Table 2
Reproducibility of 5 ADS-SPME fiber coating synthesis for the extraction ($n = 3$) of a 1 μg/ml diazepam PBS-buffer solution

Fiber	Area counts
1	3523710
2	4117640
3	3722390
4	3214530
5	4239154
R.S.D. (%)	10

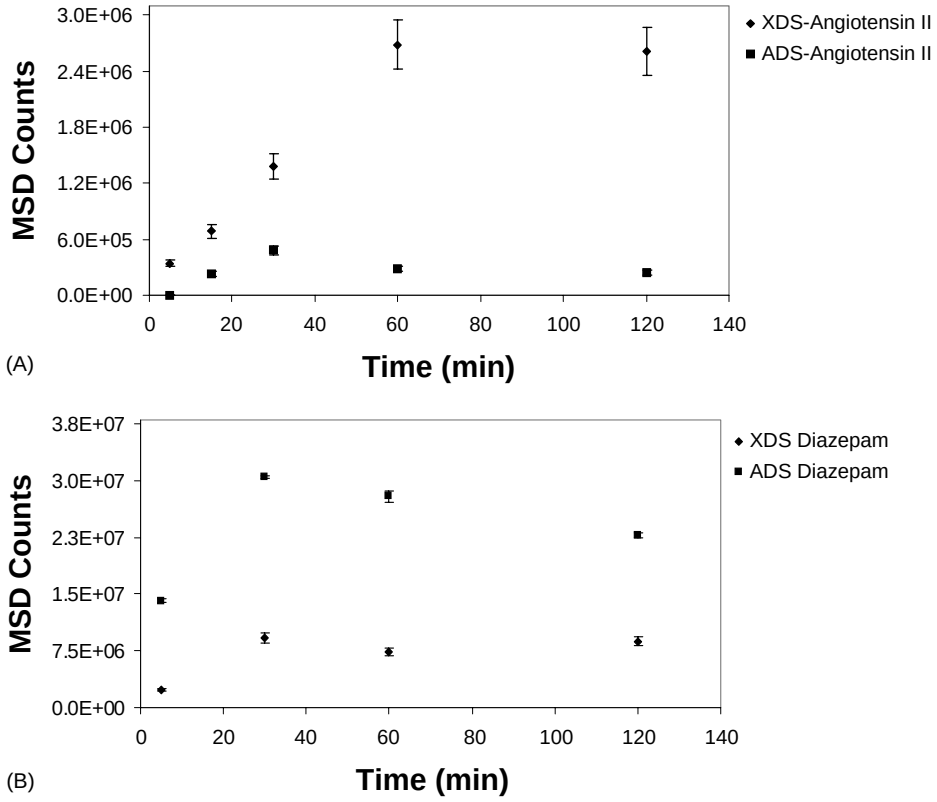

Fig. 3. Extraction time profile of 1 μg/ml angiotensin II (A) and diazepam (B) from PBS buffer using ADS- and XDS-SPME coatings.

devices and the extraction procedure was determined to be reasonably reproducible with a R.S.D. value of <11%.

For a convenient analysis of diazepam and metabolites in blood, the fiber has to be interfaced to the LC–MS system. A modified SPME–LC–MS interface and desorption and separation conditions for the benzodiazepines have been discussed before [14].

An ADS-SPME–LC–MS extraction time profile for diazepam and metabolites was obtained using 1 μg/ml diazepam, *N*-desmethyldiazepam, temazepam, and oxazepam standard samples (prepared in PBS buffer) and extracting them for progressively longer periods of time. This profile was useful to confirm the ADS-SPME wire's ability to extract diazepam and metabolites, and to determine the maximum sensitivity, reached at equilibrium, under the specified experimental conditions. Because intravenous blood flow is variable, in vitro extractions were conducted under equilibrium conditions from static (non-stirred) samples to allow for a 'worst case' scenario of sample agitation. As the extraction profile shown in Fig. 3 indicates, an increase in the amount of diazepam and the peptide angiotensin II extracted with increasing exposure of the ADS-SPME device to the standard solution. This trend eventually reaches a plateau, indicating equilibrium conditions after 30 min so all extractions were performed with an extraction time of 30 min. Based on SPME theory, it is well known that any movement in the sample will result in a shorter extraction time and so at 30 min, extraction is independent on blood flow rate and will provide maximum sensitivity. Although the time required to reach equilibrium was long, shorter periods (such as 5–15 min) provided a measurable amount of analyte extracted and could be used in future experiments to reduce the total analysis time.

The amount of analyte extracted by the ADS-SPME device (via absorption) is linearly proportional to the sample's concentration [14]. To confirm a linear extraction response, an ADS-SPME device was used to extract diazepam and metabolites from blood over a concentration range of 25–1000 ng/ml for diazepam and 50–1000 ng/ml for the metabolites ($n = 6$). The calibration curves demonstrated excellent linearity, with a R^2 value of 0.996. As the fibers are to be used for in vivo sampling, no further optimization of the extraction conditions, such as sample pH or salt adjustment, were done. However, previous SPME studies using neutral coatings (such as C_{18}) for the extraction of benzodiazepines have confirmed optimal extraction conditions near a physiological pH [28,29].

3.2.1. Sterilization

Before introducing a sampling device into the vein of an animal, the device has to be sterile. In order to confirm the ability of the ADS-SPME wires for in vivo extractions, a 1 μg/ml diazepam spiked blood sample was extracted and analyzed ($n = 6$) before and after the fibers had been sterilized in PBS buffer for 30 min at 125 °C as described earlier. The results are shown in Table 3 and illustrate that the

Table 3
Comparison of extraction efficiency before and after sterilization

	Peak area	R.S.D. (%)
Before sterilization	15741055	7
After sterilization	159030000	9

sterilization process did not influence the extraction properties of the ADS-SPME device.

3.3. Blood analysis

3.3.1. Benzodiazepines

The heterogeneity of biological samples complicates sample analysis as the direct injection of the sample into a chromatographic system is prohibited by the presence of many contaminates and interferents. Therefore, sample preparation and cleanup approaches such as liquid–liquid extraction [30,31] or solid-phase extraction (SPE) [32–34] have been developed. Although, the undesirable solvent requirements of these approaches have been eliminated with SPME [29,35,36] the biocompatibility of the commercially available SPME fibers is often poor. In contrast, the ADS-SPME device was able to directly fractionate the protein component from the hydrophobic analytes in the sample without requiring solvents or complicated instrumentation like the commercial use of ADS precolumns. For example, column switching between the ADS and analytical columns requires a dual pump and valve system, while the ADS-SPME devices are easily adapted to a standard single LC–MS instrument for greater simplicity and reduction in solvent use.

A simple and isocratic ADS-SPME–LC–MS method was developed for the extraction and analysis of benzodiazepines in blood samples. The blood samples were spiked over a range of concentrations (20–1000 ng/ml) with the four benzodiazepine compounds. Fig. 4 represents a typical chromatogram for an ADS-SPME fiber extraction of a 50 ng/ml benzodiazepine spiked blood sample. It shows the successful extraction of the free and pharmacologically active form [37] of the benzodiazepines by the ADS-SPME device from blood, followed by the LC–MS elution and detection of all compounds. Fig. 4 illustrates the ability of the ADS-SPME coating to provide a clean extract from this complicated matrix. Allowing the ADS fiber to remain in the SPME–HPLC interface over the duration of the chromatographic run can also minimize the presence of sample carry over. Furthermore, throughout the analysis, blanks were run periodically to ensure the absence of contaminants from sample carry over.

Calibration curves were constructed over a range of 25–1000 ng/ml for the four compounds. As shown in Table 4, excellent linearity was observed for all benzodiazepines in blood (average $R^2 = 0.996$). The detection limit for each compound was determined at a concentration where the signal/noise ratio was equal to three and these calculated concentrations have also been included in Table 4. For diazepam, *N*-desmetyldiazepam, oxazepam, and temazepam detection limits of 20, 20, 30, and 35 ng, respectively could be obtained. It is worth noting that these concentrations resemble the free concentration of diazepam and metabolites in blood, confirming the suitability of the approach for in vivo drug and metabolite screening.

The reproducibility of the developed method was determined with ten injections of a 50, 250, and 1000 ng/ml spiked blood sample. This injection repeatability was calculated as a R.S.D. for each benzodiazepine MS peak area and the average value for all compounds was determined to be 5.2%. The intra-assay precision was determined with repeated analysis of a sample that has been independently prepared, over 1 day, yielding an average R.S.D. of 10.1% and the between-day variance was determined to be 12.4%.

The ADS-SPME coating was based on a very robust material. The ADS material has been previously validated with biological samples for over 2000 injections [38]. Although, the ADS-SPME device was not subjected to as many injections in this study, its stability was evaluated for over 15 analyses with minimal loss of performance and confirmed the suitability of the fiber for simple and direct extraction of benzodiazepines from a biological sample. Non-specific binding on the outer layer of the particles due to the binding agents leads to a reduced reusability. It is worth noticing that crude blood samples contain red blood cells and that the

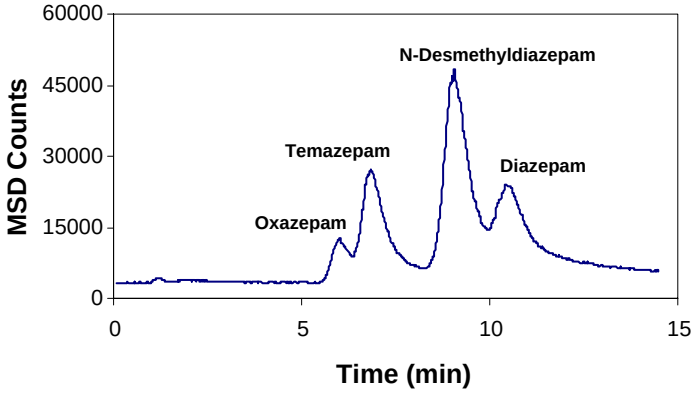

Fig. 4. ADS-SPME–LC–MS–SIM chromatogram for benzodiazepine spiked blood (benzodiazepine concentration = 50 ng/ml).

Table 4
Analytical figures of merit for the ADS-SPME–LC–MS method

Compound	Linear dynamic range (ng/ml)	R^2	Slope	Intercept	LOD (ng/ml)	Sample R.S.D. (%)			
						50 ng/ml	250 ng/ml	1000 ng/ml	Average
Oxazepam	50–1000	0.999	220.0	8905	35	8	6	12	9
Temazepam	50–1000	0.999	1396	16531	30	12	8	16	12
N-Desmethyldiazepam	25–1000	0.992	2875	173584	20	9	7	15	10
Diazepam	25–1000	0.994	3794	57160	20	10	12	15	12

hemolysis of these red blood cells may increase the interferences in the matrix compared to plasma or serum samples. Using thicker wires (diameter 2.5 mm), less binding agent, and more effective fiber washing regimes, a stable coating could be obtained that could be at least used 100 times for blood analysis.

3.3.2. Peptide analysis

Two different coatings (ADS and XDS) have been prepared for the extraction of peptides. As no blank blood samples without these peptides are available and since the biocompatibility of the material has been demonstrated before, a PBS buffer solution was spiked instead. As shown in Fig. 5A, the XDS showed much higher extraction efficiency for the extraction of angiotensin II than the conventional ADS material with RP-extraction centers. This result is even more profound considering the ionization efficiency of angiotensin II is much lower when compared to diazepam and the peptides can become adsorbed by glass, which decreases the solution's concentration. Improved extraction efficiencies for neutral molecules, such as diazepam, were possible with the neutral ADS coating, as seen in Fig. 5B.

The peptides are desorbed from the XDS-device at low pH (pH 3). At low pH the terminal amine group of the peptides is protonated and therefore they would coelute on a RP-column. In order to obtain a good separation, a pH gradient is run, allowing the separation of the three peptides, as seen in Fig. 3. The detection limit for the determination of angiotensin in blood was 50 ng/ml using this method. The method's linearity was between 50 and 500 ng/ml in PBS buffer. Further improvements and validation of the method are underway.

4. Conclusions

A novel ADS-SPME device was developed for the direct extraction, desorption and LC–MS determination of benzodiazepines and peptides in whole blood. The ruggedness and stability of the device was evaluated in addition to its sterilization capability. There was no requirement to precipitate proteins from the sample prior to extraction, therefore minimizing sample preparation time and eliminating potential sample preparation artifacts. The binding capacity, extraction efficiency and reproducibility of the device were suitable for MS determination over a wide range of concentrations in blood. The resultant biocompatible ADS-SPME device was reusable, simple to use and eliminated the requirement of extraction solvents.

The utilization of the ADS material for other classes of drugs [8,39] 40, ensures the potential versatility and suitability of this approach. More fundamentally, the extraction phase located inside the pores of the coating can be designed towards the class of compounds under analysis. We have shown that phases with C_{18} functional groups are more suitable for the extraction of neutral drugs like benzodiazepines, while the phases with cation exchange properties are more suitable for the extraction of charged compounds like peptides. Mix mode fibers (containing a mixture of both

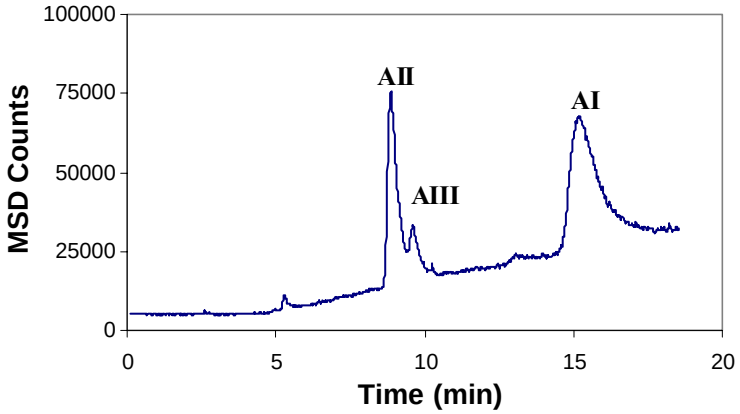

Fig. 5. XDS-SPME–LC–MS–SIM chromatogram for angiotensin I, II, III spiked PBS-buffer (angiotensin concentration = 500 ng/ml).

hydrophobic and cation exchange properties) are also possible to target a wider class of analytes.

The coupling of the XDS–SPME device to a reversed phase column combine the properties of ion exchange and reversed phase separation and allows therefore orthogonal separation of peptides. Studies are presently underway in our laboratories to improve the performance of the devices for the determination of drug and peptide concentrations in vivo (in the circulating blood of an animal) and thereby eliminating the need to draw blood. Improved sensitivity for blood peptide analysis using modern LC–MS–MS instrumentation will allow the determination of angiotensin II in blood with the necessary sensitivity (10 pg/ml) and will be an attractive alternative to radioimmunoassays.

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References

[1] R.G. Belardi, J. Pawliszyn, J. Water Pollut. Res. Can. 224 (1989) 179.
[2] J. Pawliszyn (Ed.), Applications of Solid Phase Microextraction, Royal Society of Chemistry, Cambridge, UK, 1999.
[3] W. Mullett, J. Pawliszyn, J. Sep. Sci. 26 (2003) 251.
[4] G. Theodoridis, E.H.M. Koster, G.J. de Jong, J. Chromatogr. B 745 (2000) 49.
[5] H. Lord, J. Pawliszyn, J. Chromatogr. A 902 (2000) 17.
[6] N.H. Snow, J. Chromatogr. A 885 (2000) 445.
[7] H. Lord, M. Walles, R. Grant, B. Incledon, B. Fahie, J. Pawliszyn, J. Anal. Chem. (2003) in press.
[8] C. Mislanova, A. Stefancova, J. Oravcova, J. Horecky, T. Trnovec, W. Lindner, J. Chromatogr. B 739 (2000) 151.
[9] G. Lamprecht, T. Kraushofer, K. Stoschitzky, W. Lindner, J. Chromatogr. B 740 (2000) 219.
[10] R. Lauber, M. Mosimann, M. Buhrer, A.M. Zbinden, J. Chromatogr. 614 (1994) 69.
[11] A. El Mahjoub, C. Staub, J. Chromatogr. B 742 (2000) 381.
[12] K.S. Boos, C.H. Grimm, Trends Anal. Chem. 18 (1999) 175.
[13] Z.X. Yu, D. Westerlund, K.S. Boos, J. Chromatogr. B 704 (1997) 53.
[14] W.M. Mullett, J. Pawliszyn, Anal. Chem. 74 (2002) 1081.
[15] O.H. Drummer, J. Chromatogr. B 713 (1998) 201.
[16] K. Wagner, T. Miliotis, G. Marko-Varga, B. Gyoergy, K.K. Unger, Anal. Chem. 74 (2002) 809.
[17] P. Chiap, O. Rbeida, B. Christiaens, P. Hubert, D. Lubda, K.S. Boos, J. Crommen, J. Chromatogr. A 975 (2002) 145.
[18] H. Poppe, J. Chromatogr. A 656 (1993) 19.
[19] M. Jaroniec, J. Chromatogr. A 656 (1993) 37.
[20] P.W. Carr, J. Li, A.J. Dallas, D.I. Eikens, L.C. Tan, J. Chromatogr. A 656 (1993) 113.
[21] D.W. Sindorf, G.E. Maciel, J. Am. Chem. Soc. 105 (1983) 3767.
[22] L.C. Sander, J.B. Callis, L.R. Field, Anal. Chem. 55 (1983) 3767.
[23] K.L. Rowlen, J.M. Harris, Anal. Chem. 55 (1983) 1068.
[24] M. Ho, J.E. Pemberton, Anal. Chem. 70 (1998) 4915.
[25] A. Yu Fadeev, G.V. Lisichkin, V.K. Runov, S.M. Staroverov, J. Chromatogr. 558 (1991) 31.
[26] I. Rustamov, T. Farcas, F. Ahmed, F. Chan, R. Lobrutto, H.M. McNair, Y. Vkazakevich, J. Chromatogr. A 913 (2001) 49.
[27] M. Pursch, L.C. Sander, K. Albert, Anal. Chem. 68 (1996) 4107.
[28] H. Yuan, Z. Mester, H. Lord, J. Pawliszyn, J. Anal. Toxicol. 24 (2000) 718.
[29] K. Jinno, M. Taniguchi, M. Hayashida, J. Pharm. Biomed. Anal. 17 (1998) 1081.
[30] J. Darius, P. Banditt, J. Chromatogr. B 738 (2000) 437.
[31] J. Zweigenbaum, K. Heinig, S. Steinborner, T. Wachs, J. Henion, J. Hennion, Anal. Chem. 71 (1999) 2294.
[32] H. Inoue, Y. Maeno, M. Iwasa, R. Matoba, M. Nagao, Forensic Sci. Int. 113 (2000) 367.
[33] G. Casamenti, R. Mandrioli, C. Sabbioni, F. Bugamelli, V. Volterra, M.A. Raggi, J. Liq. Chromatogr. Related Technol. 23 (2000) 1039.
[34] M.W.J. van Hout, R.A. de Zeeuw, G.J. de Jong, J. Chromatogr. A 858 (1999) 117.
[35] K. J Reubsaet, H.R. Norli, P. Hemmersbach, K.E. Rasmussen, J. Pharm. Biomed. Anal. 18 (1998) 667.
[36] Y. Luo, L. Pan, J. Pawliszyn, J. Microcolumn Sep. 10 (1998) 193.
[37] J.K. Seydel, K.J. Schaper, Pharmacol. Ther. 15 (1982) 131.
[38] R.E. Majors, K.S. Boos, C.H. Grimm, D. Lubda, G. Wieland, LC GC 14 (1996) 554.
[39] W. R Baeyens, G. Van der Weken, J. Haustraete, E. Smet, Biomed. Chromatogr. 14 (2000) 61.