J. Sep. Sci. 2001, *24*, 116–122

Emile H.M. Koster,
Chris H.P. Bruins,
Corlien Wemes,
Gerhardus J. de Jong

Department of Pharmaceutical Analysis, University Centre for Pharmacy, P.O. Box 196, 9700 AD Groningen,
The Netherlands

On-fiber derivatization for direct immersion solid-phase microextraction
Part I: Acylation of amphetamine with pentafluorobenzoyl chloride

On-fiber derivatization has been used for solid-phase microextraction (SPME) with gas chromatography in order to increase the extractability and detectability. Amphetamine, which has been used as a model compound, was derivatized with pentafluorobenzoyl chloride that was loaded on the fiber prior to the direct immersion of the fiber into the sample. The extraction performance of amphetamine with and without on-fiber derivatization has been compared. As the derivative possesses properties other than its parent compound, its partitioning between the polydimethylsiloxane coated fiber and sample matrix is different, i.e., with on-fiber derivatization a yield of 55% could be obtained in 55 min while without derivatization a yield of 40% has been obtained after a 105 min extraction. As the derivatization reagent is very suitable for electron capture detection, this detection system gave a factor of about 1 000 better response for the derivative compared to flame ionization detection. Optimization of the method is presented for buffer solutions to show its benefits. Good linearity ($r = 0.9991$) for a 50 pg/mL to 5 ng/mL range has been obtained. The applicability for urine analysis has shortly been demonstrated.

Key Words: Solid-phase microextraction; Amphetamine; Pentafluorobenzoyl chloride; Gas chromatography; Electron capture detection

Ms received: August 9, 2000; accepted: December 12, 2000

1 Introduction

Solid-Phase microextraction (SPME) is a sample preparation technique that was introduced around ten years ago by Arthur and Pawliszyn for the determination of organic compounds in environmental samples [1]. Recently, SPME has been adapted to a broad field of analysis, e. g., for toxicological, pharmaceutical, and biological samples [2]. SPME is based on the partitioning of analytes between a coated fused-silica fiber and the sample. The coated fiber can be directly immersed into the sample [3] or placed in the headspace above the sample [4]. In order to enhance the extraction yield, extraction conditions such as pH, salt concentration and sample agitation should be optimized.

As derivatization of the compound of interest can be performed to alter its properties, it could be used to improve both sensitivity and selectivity of the sample preparation and analysis system. In combination with SPME, derivatization can be performed before, during and after extraction of the compounds from the sample or its gaseous phase [5, 6]. An example of derivatization in urine samples is given by Ugland et al. who described the use of derivatization of designer drugs by alkylchloroformates prior to direct-immersion SPME [7,8]. Derivatization was performed to increase the extractability of the drugs and its robustness with respect to variations in the urine matrix was shown. A reproducible and automated method was developed with detection limits of 50 and 15 ng/mL for amphetamine with nitrogen-phosphorus and mass spectrometric detection, respectively. Nagasawa et al. gave an example of derivatization of amphetamine in the hot injection port of the GC [9]. They showed the influence of derivatization on the detectability of drugs. Headspace extractions from blood samples resulted in linear calibration curves from 0.01 to 2 μg/g using gas chromatography with mass spectrometry.

A special derivatization method is the so-called on-fiber derivatization in which the analytes are derivatized in the fiber coating. There are in fact two types of on-fiber derivatization, (i) the analytes are extracted from the sample after which the fiber is placed in or above the derivatization reagent [10] or (ii) the fiber is first loaded with reagent and then used to extract the compounds of interest from the sample or its headspace. For the latter type, derivatization also influences the extraction performance, as extraction and derivatization take place simultaneously and because

Correspondence: Prof. E.H.M. Koster, Department of Pharmaceutical Analysis, University Centre for Pharmacy, P.O. Box 196, 9700 AD Groningen, The Netherlands.
Fax: +31 50 3637582

1615-9306/2001/0202–0116$17.50+.50/0

the derivative is partitioned between sample and fiber, the *K* value of the derivative determining the final extraction yield. Recently Jurando et al. presented an on-fiber derivatization method for the analysis of urine samples [11]. The method describes the headspace extraction of amphetamine and related compounds and subsequently on-fiber derivatization of the analytes with trifluoroacetic anhydride in the headspace of another vial. Quantitation limits of about 10 ng/mL were obtained, again with use of mass spectrometry. Martos and Pawliszyn showed the use of SPME with on-fiber derivatization for water analysis, in which formaldehyde is extracted from the headspace of a sample with a (pentafluorobenzyl)hydroxylamine-loaded polydimethylsiloxane/divinylbenzene coated fiber [12]. A theoretical model has been developed to describe the overall rate of formation of an oxime on a SPME fiber after headspace extraction. In contrast to headspace extraction in which three phases are involved, direct immersion SPME (which is mainly used for the analysis of semi-volatiles in bioanalysis) deals only with two phases. In addition, derivatization reagents can be used for which its usefulness in combination with liquid-liquid extraction already has been proven. Goosens et al. showed the usefulness of pentafluorobenzoyl chloride (PFBCl) as an acylation reagent for primary amines in a continuous two-phase reaction system coupled to gas chromatography (GC) [13].

In our study amphetamine, a drug of abuse, has been used as a model compound in order to investigate on-fiber derivatization for direct-immersion SPME in which extraction and derivatization take place simultaneously. A polydimethylsiloxane (PDMS)-coated fiber was first loaded with PFBCl and subsequently directly immersed into the sample to perform the on-fiber derivatization. In this way derivatization not only influences the chromatographic behavior and the detectability of the amphetamine, but it also influences the partitioning of the analyte between the sample and fiber coating. In order to investigate the influence of derivatization on the extraction performance, SPME of amphetamine was performed with and without derivatization using gas chromatography with flame ionization detection (GC-FID). Finally, gas chromatography with electron capture detection (GC-ECD) has been used to increase the detectability of the derivative.

2 Experimental

2.1 Apparatus and chemicals

The SPME fiber holder for manual use and the 100-μm polydimethylsiloxane (PDMS) fibers were obtained from Supelco (Bellefonte, PA, USA). Stock solutions (1.8 mg/mL) of amphetamine sulfate (Brocacef, Maarsen, The Netherlands) were prepared in ultrapure water. Ultrapure water was obtained by use of an Elga Maxima Ultrapure Water purification system (Salm & Kipp, Breukelen, The Netherlands). Buffer solutions of pH 10 were prepared by dissolving boric acid, purchased from Merck (Darmstadt, Germany), in ultrapure water and adjusting the pH with 1 M sodium hydroxide.

GC-FID system: Hewlett Packard 5890 Series II gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) equipped with split/splitless injector and capillary column (HP-5, 30 m × 0.32 mm i.d., 0.25 μm film thickness). GC-ECD system: Hewlett Packard 6890 Series gas chromatograph equipped with electronic pressure control, split/splitless injector, capillary column (HP-5, 30 m × 0.32 mm i.d., 0.25 μm film thickness) and micro electron capture detection system (μ-ECD). For both GC systems, the column flow rate of the nitrogen carrier gas was 1 mL/min, the injector temperatures 250 °C and detector temperatures were 300 and 325 °C for the FID and μ-ECD, respectively. The amphetamine and its derivative were desorbed from the fiber in the splitless mode for 2.0 min, after which the injector was switched to the split mode (1:50) for the rest of the run. After desorption, the fiber remained in the injector for an additional 15 min in order to remove impurities. The GC oven was kept at 60 °C for 3.0 min after which the temperature was raised at 20 °/min to 215 °C, at 5 °/min to 230 °C and finally at 25 °/min to 290 °C, where it was kept for 5.0 min.

2.2 SPME procedure

In previous studies extraction conditions for lidocaine in buffer and urine, such as fiber coating, time, pH, ionic strength and agitation, were optimized [14, 15]. The main sampling conditions, pH, addition of salt and stirring, were again varied in order to optimize the extraction yield. The influence of salt on the extraction yield was investigated by adding 0.3 g/mL sodium chloride (NaCl, Merck) to the samples and adjusting the pH of the buffer with 1 M sodium hydroxide. One and a half milliliter of solution was transferred into a 2.0-mL vial which contained a 7 × 2 mm magnetic stirring bar, after which the vial was capped immediately. New PDMS fibers were conditioned in the injector of the GC system at 250 °C for 1 h. The fibers were also cleaned daily prior to the first extraction by putting the fiber in the injection port during a whole run in order to ensure that the fiber was clean. For extraction the fiber was inserted through the septum into the vial, i.e., the protective septum piercing needle of the SPME device was pushed through the septum of the sample vial, the plunger was pushed down and the fiber was immersed in the sample. Agitation was performed by a magnetic stirrer (IKA, mini-mr, Staufen, Germany). As there was no indication of the stirring speed (number of revolutions per minute), the

stirrer was operated at a speed which gave a vortex of 0.5 cm in the liquid.

After extraction the fibers were thermally desorbed in the injector of the GC system. In order to determine the extraction yield of amphetamine the GC system had to be calibrated. This was performed by preparing a 1.0 mg/mL stock solution of amphetamine in methanol (Lab-Scan, Dublin, Ireland) and making standard solutions by dilution; 1 μL of these standard solutions was injected. Fitting of the time-sorption curves was performed with Microsoft Excel 7.0.

2.3 On-fiber derivatization

A few microliters of pentafluorobenzoyl chloride (Sigma-Aldrich, Dorset, United Kingdom) were transferred into a 4.5 mL screwcap vial and closed immediately with a septum-containing cap. The needle of the SPME device was pushed though the septum and the fiber was exposed to the PFBCl vapor for 15 s. After this the fiber was withdrawn from the reagent vial and directly immersed into the buffered amphetamine solutions for 55 min as described previously. After extraction the fiber was placed in a GC liner with a small internal diameter (2 mm) for five min at ambient temperature through which a nitrogen stream of 1 L/min was passed in order to reduce the excess of reagent on the fiber. Subsequently the fiber is thermally desorbed in the GC system. In order to determine the extraction yield of SPME with on-fiber derivatization, the response of the detection system for the derivative had to be obtained. This was performed by preparing a 1.0 μg/mL stock solution of amphetamine in ethyl acetate containing an excess of PFBCl and preparing standard solutions of the amphetamine derivative by dilution; 1 μL of these standard solutions was injected.

3 Results and discussion

3.1 SPME of amphetamine

In our study amphetamine has been used as a model compound as in the literature many details can be found about SPME of this drug as described in the introduction. The extraction of amphetamine from buffer solutions was optimized in order to compare it with the on-fiber derivatization in which extraction and derivatization take place simultaneously. Furthermore, the optimized extraction conditions could also be used to obtain an impression about the performance of on-fiber derivatization in which derivatization would take place after extraction. Amphetamine was adsorbed by the Teflon coated stirring bar, which led to irreproducible yields and carry-over effects, i. e., blank buffer solutions were contaminated with amphetamine from the stirring bars although they were thoroughly rinsed with water and ethanol before reuse. Moreover, because the adsorption onto the stirring bar competes with the sorption of amphetamine by the PDMS-coated fiber, lower extraction yields and non-linear calibration curves were found. In order to prevent these effects, subsequent experiments were performed with home-made glass-coated stirring bars.

As in previous optimization studies [14, 15] the extraction yield was optimized by varying the pH over a range of 8–10. Due to the acid dissociation constant of amphetamine (pK_a 9.9 [16]), the highest extraction yield was obtained at pH 10. A higher pH was not used as this could damage the fiber, i. e., according to information of the manufacturer the 100-μm PDMS-coated fiber is stable between pH 4–10. The addition of sodium chloride to the sample increased the extraction yield by a factor of about 10 as also observed in refs. 14 and 15. Therefore, a NaCl concentration of 0.3 g/mL, which is close to saturation, is used.

In order to obtain a time-sorption profile, extractions were performed for 5–90 min. **Figure 1** shows the time-sorption curve of amphetamine from buffer solutions under optimum conditions (pH 10, 0.3 g/mL NaCl). Fitting the curve with the mathematical model $n_{f,t} = n_{f,e}\,[1 - e^{(-at)}]$ [15, 17] resulted in an equilibrium time of 105 min, i.e., the extraction yield ($n_{f,t}$) ≧ 95% of the maximum yield ($n_{f,e}$). The extraction yield at the optimized conditions for a 1.5 mL sample is about 40%. The limit of detection (LOD) defined as three times the blank peak at the same retention time as amphetamine is 10 ng/mL. Further validation of the method without derivatization was outside the scope of this paper.

3.2 SPME with on-fiber derivatization

3.2.1 Standard solutions of the derivative

As there was no standard available for the pentafluorobenzoyl amide, amphetamine was dissolved in ethyl acetate containing an excess of PFBCl in order to prepare the derivative. A calibration curve has been obtained by performing direct injections of the prepared derivative solutions into the GC-FID system. The results of the in-solution derivatization showed that the reaction was fast, i.e., within 1 min all the amphetamine was converted into the derivative, and longer derivatization times did not give rise to an increase of the peak area of the derivative. Reactions were performed in ethyl acetate as PFBCl reacts with methanol which resulted in many additional peaks in the chromatogram and for which, as a consequence, more reagent was needed. Because the μ-ECD system is very sensitive it was impossible to inject standard solutions of the derivative as these solutions contained a too high concentration of reagent which disturbed the detection system. As the extraction yield was known from the

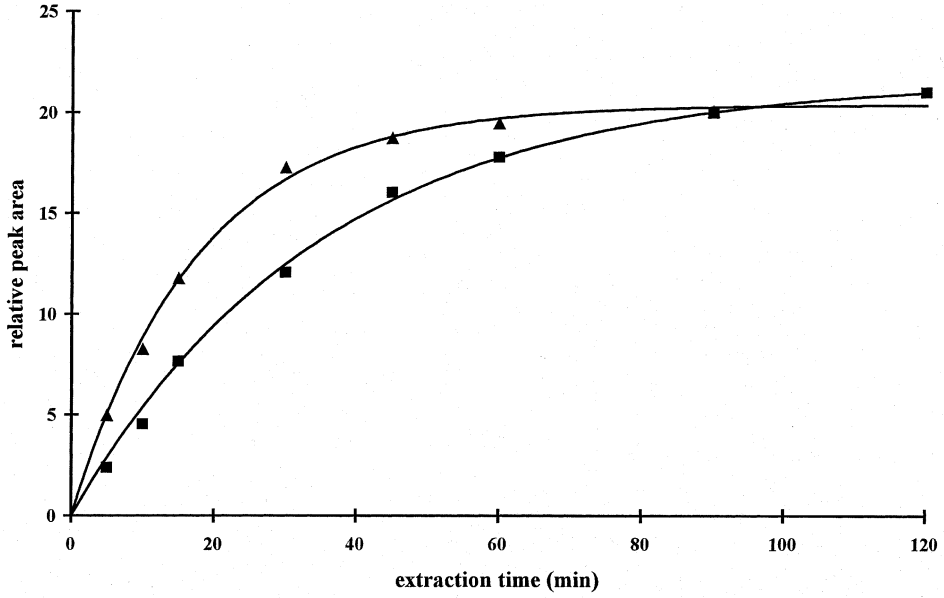

Figure 1. Time-sorption profiles for SPME-GC-FID of amphetamine (■) and on-fiber derivatization of amphetamine (▲); SPME: 500 ng/mL amphetamine in buffer pH 10, 0.3 g/mL NaCl; SPME with on-fiber derivatization: 15 s PFBCl, 50 ng/mL amphetamine in buffer pH 10, 0.3 g/mL NaCl, 5 min nitrogen (1 L/min).

experiments with GC-FID, it was not necessary to perform direct injections for calibration of the GC-ECD.

3.2.2 Optimization

In order to obtain an excess of reagent on the fiber before extraction, the 100-μm PDMS-coated fiber was placed in a vial containing PFBCl vapor. The sorption time of the reagent was varied and subsequent extractions were performed. A loading time of 15 s proved to be long enough to have an excess of reagent on the fiber, as no amphetamine was detected after extraction of 1.5 mL buffer solution containing 200 ng/mL amphetamine. The influence of the pH on the extraction yield was investigated by varying the pH of the borate buffer from 8 to 10. As expected the yield increased with the pH giving the highest yield at pH 10, which is in agreement with the results for the two-phase derivatization obtained by Goosens et al. [13]. Also the influence of NaCl on the on-fiber derivatization-extraction yield was investigated. As found in previous studies [14, 15] and for the extraction of amphetamine (see above), the extraction yield of the derivative increased with a factor of about 10 by adding NaCl (0.3 g/mL).

Another effect of the addition of salt is the excess of PFBCl remaining on the fiber after extraction, i. e., this excess is much higher than without the addition of salt to the buffer solution. Due to high concentrations of reagent, the performance of the capillary GC column decreased as active sites in the first part of the column were formed. With the used setup it was not possible to determine exactly how much of the reagent remained on the fiber as the reagent gives rise to several peaks in the chromatogram. The amount of reagent left on the fiber after derivatization resulted in a very large peak at the beginning of the chromatogram (t_R = 5.3 min) and many other smaller peaks in the 5–12 min region. In order to reduce the amount of reagent introduced in the GC system, the fiber was placed into a nitrogen stream (1 L/min) after the on-fiber derivatization. **Figure 2** depicts the amount of reagent (peak at 5.3 min) and derivative on the fiber after reduction of the excess of reagent by nitrogen for a certain time. As shown the amount of derivative is constant as the excess of reagent is reduced when the fiber is exposed to the nitrogen stream for a longer time. The amount of PFBCl (peak at 5.3 min) on the fiber after 5 min nitrogen is about 2.0% and that after 60 min nitrogen approximately 0.3% of the amount of reagent on the fiber after 15 s loading and reaction with amphetamine during 30 min extraction. The amount of reagent left on the fiber without removal by nitrogen is not shown in Figure 2.

The desorption of the derivative from the fiber was investigated, by varying the splitless time of the injection system from 0.5 to 5 min. The peak area of the derivative did not significantly increase if splitless times longer than 2.0 min were used. Therefore the desorption time of the derivative was set at 2.0 min which is similar to that for the desorption of amphetamine.

Figure 1 shows the time-sorption curve of amphetamine from buffer solutions in combination with on-fiber derivati-

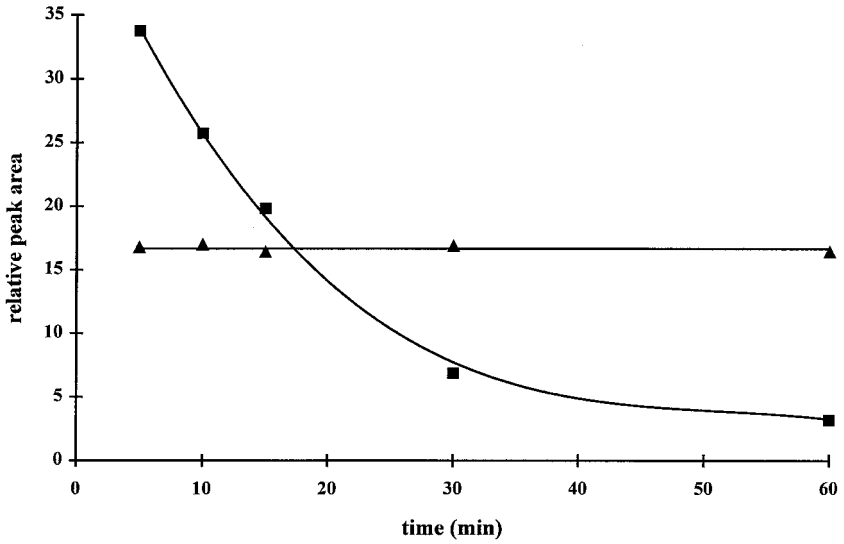

Figure 2. Amount of PFBCl represented by peak at t_R = 5.3 min (■) and amount of amphetamine derivative (▲) after on-fiber derivatization and placing the fiber in a nitrogen flow (1 L/min). Conditions: 15 s PFBCl, 50 ng/mL amphetamine in buffer pH 10, 0.3 g/mL NaCl, 30 min extraction. The PFBCl peak at 5 min is only about 2% of the original amount (see text).

zation under the optimized conditions as described above. Because the response of the detection system is higher for the derivative than for amphetamine, a lower concentration (50 ng/mL) was analyzed in order to obtain comparable peak areas. An equilibrium time of 55 min was found using the same mathematical model as described previous for amphetamine. Compared to the extraction of amphetamine without derivatization, this equilibrium time is much shorter which shows that the derivatization influences the kinetics of the extraction. An extraction yield of 55% could be obtained at optimized conditions for a 1.5 mL sample which is only slightly higher than that obtained for amphetamine without derivatization. Combination of the higher *K* value (higher extraction yield) and shorter equilibrium time makes extraction at non-equilibrium conditions attractive for SPME with on-fiber derivatization, e.g. an extraction time of 20 min resulted in a yield of 18 and 37% for SPME and SPME with on-fiber derivatization, respectively. The results seem to be in contradiction with those shown by Dewulf et al. [18], i. e., they found a linear relationship between the *K* value and the equilibrium time. However, because in the present case the fiber is loaded with reagent, the properties of the fiber coating may also have been changed. In fact a modified fiber was used during the extraction which can have an substantial effect on the extraction yield as has been described by Krogh et al. who used an 1-octanol modified polyacrylate-coated fiber for the extraction of diazepam from plasma [19].

3.2.3 Validation

The optimized conditions of the on-fiber derivatization have been used in order to validate the SPME method. The linearity of the SPME-GC-FID method was investigated by varying the concentration of amphetamine in buffer over a 2–200 ng/mL range. Good linearity (r = 0.9995) was obtained for the whole measured range. The inter-day RSD of the method was 5.2% (at 25 ng/mL, n = 7), the LOD 2 ng/mL. A chromatogram of the SPME-GC-FID analysis of amphetamine with on-fiber derivatization is shown in **Figure 3**.

As the use of PFBCl derivatization in combination with ECD is obvious because the reagent contains five fluorine atoms and the detection system is particularly sensitive to halogen-containing molecules, GC-ECD was used to improve the detectability of the derivative. Although in principle the ECD is also more selective no cleaner chromatograms were obtained as the reagent gave many other products that could be detected. The present reagent is 99% pure (according to the label) but can still contain compounds that give a high detector response. It should be kept in mind that next to the peaks of the reagent itself also reaction products (formed by reaction with water) are present in the chromatogram.

The calibration curve of amphetamine with on-fiber derivatization and GC-ECD proved to be linear ($y = 0.05173x + 3.35825$, $r = 0.9991$) over the range of 50 pg/mL to 5 ng/mL. The LOD, defined as blank peak

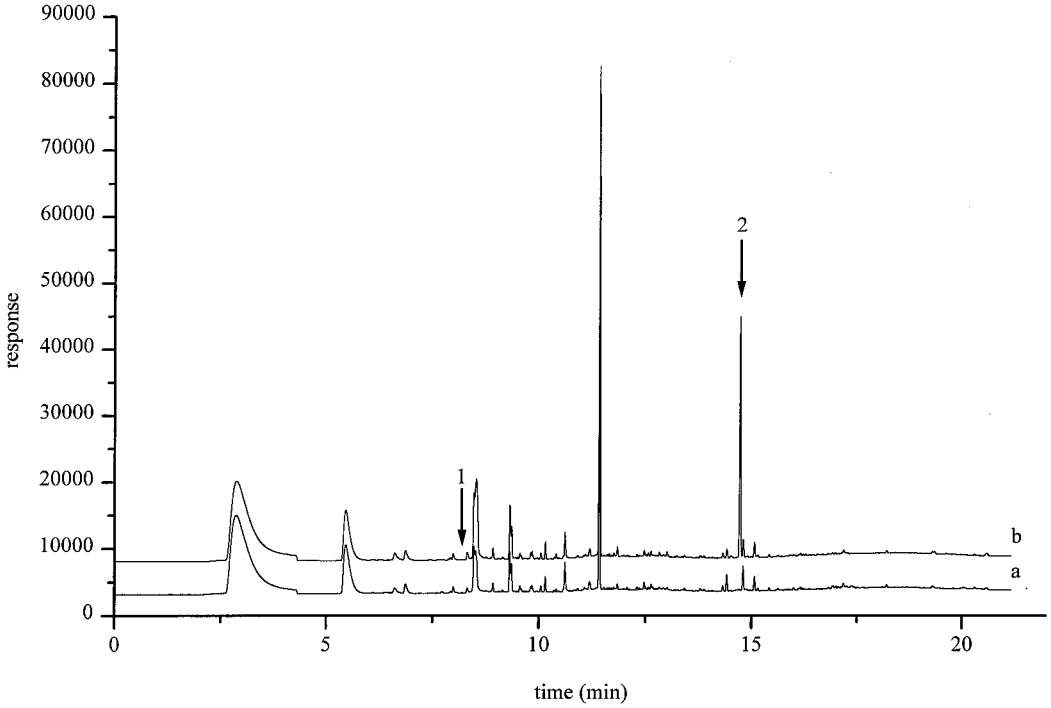

Figure 3. SPME-GC-FID chromatograms of blank borate buffer pH 10, 0.3 g/mL NaCl (a) and 25 ng/mL amphetamine in borate buffer pH 10, 0.3 g/mL NaCl (b) after on-fiber derivatization. The place indicated by arrow 1 is retention time of amphetamine, peak indicated by arrow 2 is amphetamine derivative.

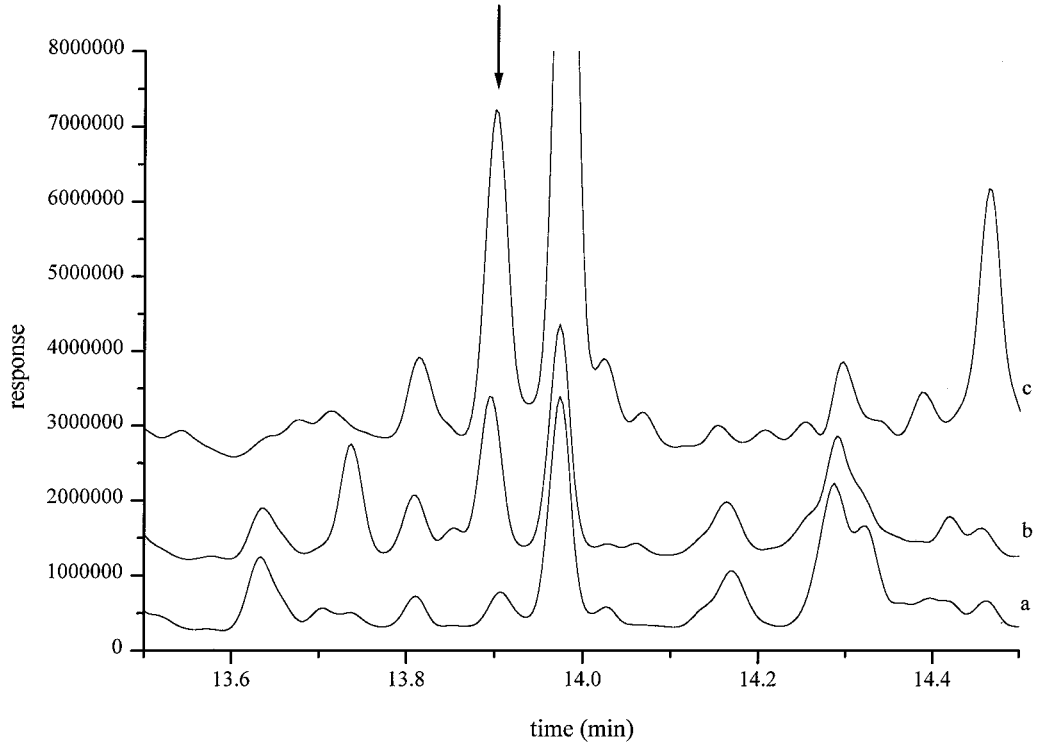

Figure 4. Enlargement of the 13.5 to 14.5 min region of the SPME-GC-ECD chromatograms of blank borate buffer pH 10, 0.3 g/mL NaCl (a), 100 pg/mL amphetamine in borate buffer pH 10, 0.3 g/mL NaCl (b) and 500 pg/mL amphetamine in 1:1 buffered urine pH 10, 0.3 g/mL NaCl (c) after on-fiber derivatization. The peak indicated by the arrow is amphetamine derivative.

plus tree times its standard deviation, is 50 pg/mL. At this very low level, which is about a factor 200 lower than obtained with GC-FID for amphetamine, even small carry-over effects may cause fluctuations in the blank peak. In addition, the amount of reagent on the fiber after placing the fiber in the nitrogen stream to remove the excess of PFBCl is difficult to control and should therefore be standardized. The inter-day RSD ($n = 9$) of the on-fiber derivatization GC-ECD method was 10.0% at the 100 pg/mL level.

The 13.5 – 14.5 min part of the SPME-GC-ECD chromatograms of amphetamine with on-fiber derivatization is shown in **Figure 4**. Although the chromatogram obtained for the on-fiber derivatization method contains many peaks arising from the derivatization reagent, the enlargement of blank, spiked buffer, and spiked urine (Figure 4) shows that small amounts of derivative (pg/mL level) can be determined. Preliminary results of the analysis of urine samples gave an impression about the potential of the system for biological samples. The peak of the derivative in spiked urine (Figure 4.c) proved to be lower compared to that for the analysis of spiked buffer. Due to the presence of other primary amines in urine, the excess of PFBCl loaded on the fiber seems to be critical and can therefore result in a lower peak of the amphetamine derivative. The difference in response is currently under investigation. As far as the selectivity is concerned, the blank chromatogram of urine is still dominated by peaks caused by the reagent

4 Conclusions

The potential of on-fiber derivatization for direct-immersion SPME-GC in which amphetamine is simultaneously extracted and derivatized has been demonstrated. The sampling conditions such as sample pH, the addition of salt and sampling time have been optimized to improve the extraction and on-fiber derivatization performance. In comparison with the extraction of amphetamine, higher extraction yields could be obtained in a shorter extraction time but the main gain is the better detectability of the derivative. The combination of extraction and derivatization was found to be simple, reproducible, and linear over at least two orders of magnitude. Detection limits in the low pg/mL range were obtained with GC-ECD which is more than sufficient for real samples. The applicability of the method for the analysis of biological samples is currently under investigation and the influence of the matrix on the on-fiber derivatization is being studied. Moreover, methods in which on-fiber derivatization is performed after the extraction will be compared with that of simultaneous reaction and extraction. Further optimization and validation of the urine analysis will be published in the near future. Other derivatization reactions will also be investigated, e. g., the labeling of analytes with fluorophores for SPME-liquid chromatography with fluorescence detection.

References

[1] C.L. Arthur, J. Pawliszyn, *Anal. Chem.* **1990**, *62*, 2145.

[2] G. Theodoridis, E.H.M. Koster, G.J. de Jong, *J. Chromatogr. B.* **2000**, *745*, 49.

[3] D. Louch S. Motlagh, J. Pawliszyn, *Anal. Chem.* **1992**, *64*, 1187.

[4] Z. Zhang, J. Pawliszyn, *Anal. Chem.* **1993**,*65*, 1843.

[5] M.-L. Bao, F. Pantani, O. Griffini, D. Burrini, D. Santianni, K. Barbieri, *J. Chromatogr. A.* **1998**, *809*, 75.

[6] L. Pan, J. Pawliszyn, *Anal. Chem.* **1997,** *69*, 196.

[7] H.G. Ugland, M. Krogh, K.E. Rasmussen, *J. Chromatogr. B.* **1997**, *701*, 29.

[8] H.G. Ugland, M. Krogh, K.E. Rasmussen, *J. Pharm. Biomed. Anal.* **1999**, *19*, 463.

[9] N. Nagasawa, M. Yashiki, Y. Iwasaki, K. Hara, T. Kojima, *Forensic Sci. Int.* **1996**, *78*, 95.

[10] M.-R. Lee, R.-J. Lee, Y.-W. Lin, C.-M. Chen, B.-H. Hwang, *Anal. Chem.* **1998**, *70*, 1963.

[11] C. Jurado, M.P. Giménez, T. Soriano, M. Menéndez, M. Repetto, *J. Anal. Toxicol.* **2000**, *24*, 11.

[12] P.A. Martos, J. Pawliszyn, *Anal. Chem.* **1998**, *70*, 2311.

[13] E.C. Goosens, M.H. Broekman, M.H. Wolters, R.E. Strijker, D. de Jong, G.J. de Jong, U.A.Th. Brinkman, *J. High Resol. Chromatrogr.* **1992**, *15*, 242.

[14] E.H.M. Koster, N.S.K. Hofman, G.J. de Jong, *Chromatographia* **1998**, *47*, 678.

[15] E.H.M. Koster, C. Wemes, J.B. Morsink, G.J. de Jong, *J. Chromatogr. B.* **2000**, *739*,175.

[16] *Clarke's Isolation and Identification of Drugs*, A.C. Moffat, J.V. Jackson, M.S. Moss, B. Widdop (eds), The Pharmaceutical Press, London 1986, p. 349.

[17] E.H.M. Koster, G.J. de Jong, *J. Chromatogr. A.* **2000**, *878*, 27.

[18] J. Dewulf, H. Van Langenhove, M. Everaert, *J. Chromatogr. A.* **1997**, *761*, 205.

[19] M. Krogh, H. Grefslie, K.E. Rasmussen, *J. Chromatogr. B.* **1997**, *689*, 357.