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Journal of Chromatography B, 739 (2000) 175–182

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Determination of lidocaine in plasma by direct solid-phase microextraction combined with gas chromatography

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Abstract

Direct-immersion solid-phase microextraction (SPME) has been used to extract the local anesthetic lidocaine from human plasma. A simplified model shows the relationship between the total amount of drug in plasma and the amount of drug extracted. The model takes into account that the drug participates between the fiber, sample and proteins. Therefore the model can also be used to obtain a good approximation of the drug–protein binding. Extraction yields of lidocaine in plasma are <1%, and the protein binding of lidocaine was found to be about 74% at pH 9.5. A SPME method has been developed for the determination of the total amount of lidocaine in plasma. The protein binding was reduced by acidification and, subsequently, the sample was deproteinized with trichloroacetic acid. With a 100-μm polydimethylsiloxane-coated fiber and addition of sodium chloride to the sample an extraction yield of about 12% at equilibrium (45 min) has been obtained. The relative standard deviation of this method is <10%. A linear range was found from 25 to 2000 ng ml^{-1} lidocaine in plasma (r=0.998) with a detection limit of 5 ng ml^{-1} in plasma. An extraction yield of about 80% could be obtained after an overnight extraction by use of a 65-μm polydimethylsiloxane–divinylbenzene-coated fiber. If an extraction time of 10 min is used with this fiber, the same yield is obtained as with the single-phase fiber in 45 min. However, the drawback of this mixed-phase fiber is its much shorter lifetime.

Keywords: Solid-phase microextraction; Protein binding; Lidocaine

1. Introduction

Solid-phase microextraction (SPME) has been introduced by Pawliszyn for the extraction of organic compounds in environmental samples [1,2]. Today, this microextraction technique has been applied to a broad field of analysis including food, biological and pharmaceutical samples. SPME can be used for a broad range of analytes in various types of matrices, such as gas, liquid and solid samples. The fused-silica fiber which is coated with a polymer (e.g., polydimethylsiloxane) can be submerged directly into the sample or be placed in the headspace above the sample. After sorption of the analytes, the fiber is desorbed into an analytical system. The fiber is thermally desorbed if SPME is combined with gas chromatography (GC) but it can also be desorbed with liquid and can than be used in combination with either liquid chromatography (LC) or capillary electrophoresis (CE) [3–5].

Recently, the use of SPME for the analysis of drugs in body fluids has become popular, which is expressed by the rapidly increasing number of papers in this field. However, most of these papers demonstrate the applicability of SPME in bioanalysis, but only a few discuss the optimization of the technique

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0378-4347/00/$ – see front matter
PII: S0378-4347(99)00344-8

[6,7]. The determination of drugs in biological fluids is of major concern in the development and use of drugs. In order to measure the total, bound and freely dissolved concentration of drug in blood or plasma, several sample preparation methods such as ultrafiltration, dialysis and liquid–liquid extraction have been developed. Until now, only a few papers describe the use of direct-immersion SPME for plasma analysis [8–10].

In this study, the local anesthetic lidocaine which binds to α_1-acid glycoprotein (α_1-AGP) [11,12] has been chosen as model compound to investigate the use of direct SPME combined with GC for the analysis of the total, bound and freely dissolved amount of drugs in human plasma. Although various concentrations of lidocaine in plasma can be found in the literature, therapeutic plasma concentrations are usually in the range of 2 to 5 μg ml^{-1} [13]. Some theoretical aspects for the calculation of the extracted amount of drug and of the drug–protein binding are also presented. Generally, the applicability of SPME for the determination of drugs in human plasma is illustrated.

2. Experimental

2.1. Apparatus and chemicals

The SPME fiber holder for manual use, the 100-μm polydimethylsiloxane (PDMS) and 65-μm polydimethylsiloxane–divinylbenzene (PDMS–DVB)-coated fibers were obtained from Supelco (Bellefonte, PA, USA). Stock solutions (1 mg ml^{-1}) of lidocaine hydrochloride, purchased from Holland Pharmaceutical Supply (Alphen a/d Rijn, The Netherlands), were prepared in methanol (Lab-Scan, Dublin, Ireland) or ultrapure water. Ultrapure water was obtained by using an Elga Maxima Ultrapure Water purification system (Salm and Kipp, Breukelen, The Netherlands). Buffer solutions of pH 9.5 were prepared by dissolving boric acid, purchased from Merck (Darmstadt, Germany), in ultrapure water and adjusting the pH with 1 *M* sodium hydroxide (Merck).

GC analysis was performed with a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with split/splitless injector, flame ionization detection (FID) system and a capillary column (HP-5, 30 m×0.32 mm I.D., 0.25 μm film thickness). The column flow-rate of the nitrogen carrier gas was about 1 ml min^{-1}. The temperature of the injector was 250 or 220°C, that of the detector 300°C. The fibers were desorbed 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 8 min to avoid carry-over effects, after which it was removed. The GC oven was kept at 80°C for 3.0 min after which the temperature was raised by 20°C min^{-1} to 215°C, by 5°C min^{-1} to 230°C and finally by 25°C min^{-1} to 290°C, where it was kept for 5.0 min.

2.2. SPME procedures

New fibers were conditioned in the injector of the GC system as follows: PDMS fibers at 250°C for 1 h and PDMS–DVB fibers at 220°C for 1 h because their lifetime is prolonged at lower temperature. The fibers were also cleaned every day prior to the first extraction by putting the fiber in the injection port during a whole run of the GC system.

In a previous study the extraction conditions for lidocaine in buffer and urine, such as fiber coating, time, pH, ionic strength, temperature and agitation, were optimized [6]. The same conditions were used for the plasma extractions. The influence of salt on the extraction yield was investigated by adding sodium chloride (NaCl, Merck) to the samples and adjusting the pH to 9.5. Plasma was spiked with the stock solution and diluted with borate buffer (pH 9.5) to obtain the desired concentrations of lidocaine and protein.

Deproteinization of plasma containing lidocaine was performed by acidifying 2 ml of plasma with 50 μl hydrochloric acid (37%, Merck) to disturb the lidocaine protein binding after which 150 μl trichloroacetic acid (TCA, Merck) was added to denature the proteins. Subsequently, the samples were centrifuged at 5600 rpm for 15 min. The supernatant (1 ml) was then mixed with 1 ml borate buffer (0.5 *M*, pH 9.5) and 0.6 g NaCl, after which the pH was

adjusted to 9.5 with 1 *M* sodium hydroxide. One milliliter of the solution was transferred into a 1.5-ml vial which contained a 7×2 mm magnetic stirring bar, after which the vial was capped immediately. Subsequently 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 submerged 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 about 0.5 cm in the liquid. The extraction time was 45 min unless mentioned otherwise.

After extraction the fibers were thermally desorbed in the injector of the GC system. In order to determine the extraction yield of SPME the GC systems had to be calibrated. This was carried out by preparing a 1 mg ml^{-1} stock solution of lidocaine in methanol and making standard solutions by dilution; 1 μl of these standard solutions was injected.

Fitting of the time–sorption profile and the curve for the dilution of plasma was performed with Microsoft Excel version 7.0.

3. Results and discussion

3.1. Influence of proteins

Fig. 1a shows the time–sorption profile of lidocaine (500 ng ml^{-1}) in 1:1 buffered plasma (pH 9.5) extracted with a 100-μm PDMS-coated fiber. An extraction time of about 45 min is sufficient, i.e., the extraction yield is more than 95% of the maximal achievable yield under conditions that are similar to those used in a previous study [6]. Under these conditions, an extraction yield of only about 0.4% could be achieved. The extraction yield of lidocaine in buffer under comparable conditions is about 1.0% which indicates that about 40% of lidocaine is freely dissolved and about 60% is bound to plasma proteins. However, this rough approximation is only based on the free dissolved amount of lidocaine, i.e., disturbance of the drug–protein equilibrium by the extraction is neglected.

In order to investigate the influence of proteins on the extraction yield plasma was diluted with buffer. The protein content was varied from its original value to one-tenth of it, i.e., the number of binding sites in a fixed sample volume has been decreased. After dilution the plasma samples were spiked with

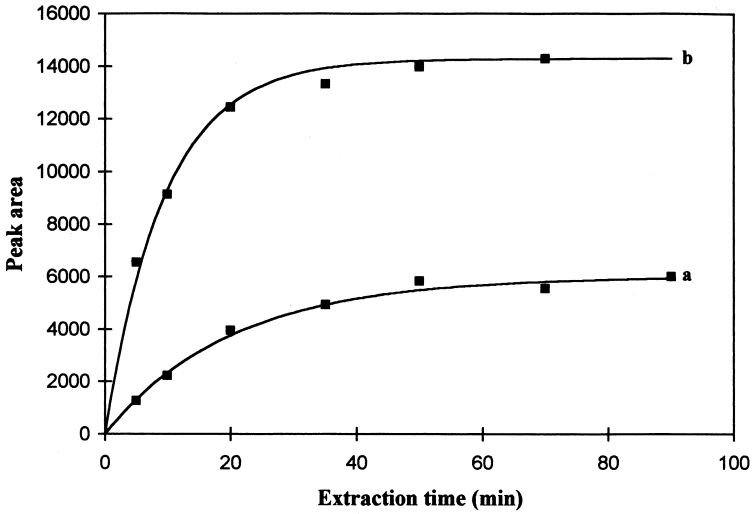

Fig. 1. Time–sorption profiles for the extraction of lidocaine (500 ng ml^{-1}) from plasma samples after 1:1 buffering to pH 9.5 (a) without and (b) with deproteinization.

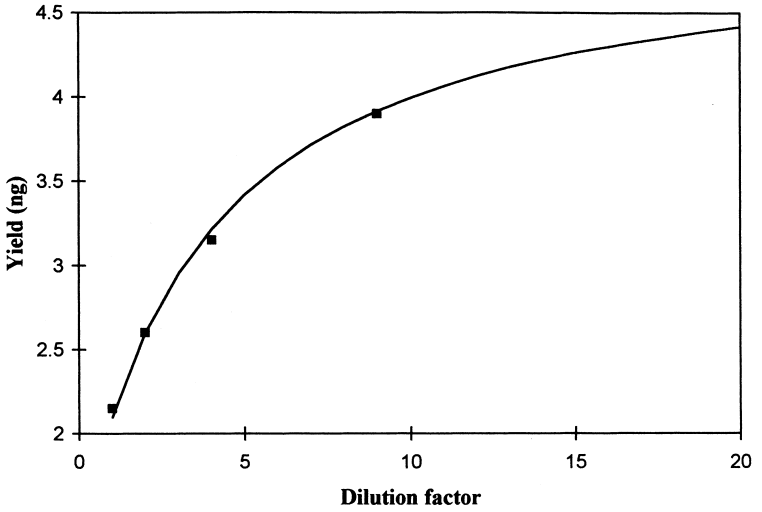

Fig. 2. Amount of lidocaine extracted from plasma after dilution with buffer pH 9.5 in different ratios. The concentration of lidocaine is constant (500 ng ml^{-1}).

lidocaine to obtain a 500 ng ml^{-1} solution. The results are shown in Fig. 2 where the extracted amount of lidocaine is plotted against the dilution factor. The amount increases with a decreasing protein content. The influence of proteins on the extraction yield can be described by (see Appendix):

$$n_f = \frac{K_f V_f n_0}{K_f V_f + K_p N_p^0 + V_{pl}} \quad (1)$$

where n_f is the amount of drug in the fiber coating, K_f is the fiber–matrix partition constant, n_0 is the total amount of drug in the sample, K_p is the protein-association equilibrium constant, N_p^0 is the number of available sites for protein–drug binding and V_f and V_{pl} are the volume of the fiber coating (0.628 μl) and the plasma sample, respectively.

After performing extractions of lidocaine from buffer solutions in order to determine K_f, which turned out to be 16.1, Eq. (1) can be used to fit the curve in Fig. 2. Taken into account that the number of binding sites in a fixed sample volume is reduced by the dilution, a mean value for the product of K_p and N_p^0 was found, which turned out to be 2.8 ± 0.1. As this value is directly proportional to the quotient of the amount of lidocaine bound to protein and the amount of freely dissolved lidocaine in the 1 ml plasma sample (see Eq. (A.1)), a mean drug–protein binding of 74% has been found under the given conditions. Furthermore, there was no significant difference in the product of K_p and N_p^0 calculated at each point of the curve, which indicates that it was justified to use Eq. (1), i.e., $N_p^0 - N_b = N_p^0$.

Although the calculated protein binding for lidocaine is in accordance with values found in the literature [11–15] it should be kept in mind that in this experiment the sample pH is 9.5 instead of 7.0 to 7.5 as it is in human body. Another pH not only changes the concentration of neutral lidocaine and, therefore, the extraction yield, but it can probably also change the interaction between the drug and α_1-AGP as protein structures are dependent of pH. Moreover, the concentration of α_1-AGP in plasma can fluctuate which results in different protein binding values [11,16].

3.2. Optimization of the extraction method

3.2.1. Salt addition

The extraction yield of lidocaine can be increased by using the well-known ''salting-out effect'' [6,17]. The influence of salt on the extraction yield was investigated by adding various amounts of NaCl to the 1:1 buffered plasma solution (pH 9.5). The results (not shown here) demonstrate that there is no logarithmic relationship between the extracted

amount and the NaCl concentration as it should be according to the ''salting-out theory'' [6]. The extraction yield increased only from about 0.4% in absence of NaCl to 0.6% at 0.3 g ml^{-1} NaCl. In buffer solutions the extraction yield can be increased by a factor 10 by this salt concentration which indicates the disturbance of the salting-out effect by the plasma matrix. A possible reason for this phenomenon could be that the fraction of lidocaine bound to protein and thus also the free fraction of lidocaine is changed by the addition of NaCl because the solubility of lidocaine (which is mainly in its neutral form at pH 9.5) in the plasma is lowered, i.e., both the extraction and protein binding equilibria are shifted by salting-out. At high NaCl concentrations the samples become slightly turbid probably due to the shielding of the protein charges which results in structure loss and therefore precipitation of the proteins including bound or enclosed lidocaine. As a result, both the amount of lidocaine available for extraction and the NaCl concentration are decreased which results in a lower yield. As will be illustrated below the ''normal'' salting-out effect can be obtained after the removal of the proteins.

3.2.2. Deproteinization

Because plasma proteins diminish the extraction yield, which consequently results in a relatively high detection limit, they should be removed prior to extraction for the determination of the total drug concentration. During deproteinization with TCA a considerable part of lidocaine was lost. In order to investigate the cause of this, a combination of hydrochloric acid and NaCl has been used to denature the proteins under milder conditions. Some lidocaine is removed from the sample together with the proteins if acid is added after salt to the plasma sample. However, if salt is added to the plasma after the acid, less drug is lost, i.e., the extraction yield is 1.3% when acid is added first and 1.0% when the order is reversed. This indicates that the interaction between lidocaine and protein is influenced by acidification before denaturation of the proteins takes place. If the plasma samples were spiked with lidocaine after deproteinization of the plasma, a yield of 1.5% could be obtained which is higher than the yield that is obtained if plasma is spiked before deproteinization and thus this indicates that there is still some loss of lidocaine during the deproteinization process. The extraction yield in plasma after deproteinization is somewhat higher than the yield in buffer solutions, but this can be explained by the presence of NaCl (about 0.05 g ml^{-1}) which was added to the plasma samples for the precipitation of the proteins.

The time–sorption profile of lidocaine (500 ng ml^{-1}) in plasma after deproteinization and 1:1 buffering (pH 9.5) is shown in Fig. 1b. The results show that an extraction time of 45 min is again sufficient. If Fig. 1a and b are compared mathematically {the curves were fitted with the general formula $n_{f,t}=n_{f,e}[1-e^{(-a\cdot t)}]$ [18,19]}, it shows that the slope of the curve for plasma after removal of the proteins is steeper than that of the protein containing plasma, which is probably caused by the difference in viscosity of the samples. After disturbing lidocaine–protein binding and protein removal the extraction yield could be increased by salt addition. The yield obtained after deproteinization could be increased up to 11.7% by adding NaCl (0.25 g ml^{-1}). This increase in yield corresponds with the result that was obtained in buffer solutions. The extraction yields under the various conditions are summarized in Table 1.

3.2.3. Fiber coating

Higher extraction yields were obtained with a 65-μm PDMS–DVB-coated fiber after deproteinization, buffering (pH 9.5) and adding NaCl (0.25 g ml^{-1}). A time–sorption profile of lidocaine (500 ng ml^{-1}) in protein-free plasma was measured. The extraction yield is increasing almost linear with the extraction time to about 50% at 75 min. An overnight extraction of about 16 h resulted even in a yield of about 80%, which indicates the high affinity of lidocaine for this fiber. However, for this high yield a longer extraction time is needed than for the PDMS-coated fiber to obtain the maximal achievable yield. The yield can still be increased from about 12 to 30% if an extraction time of 45 min is used. On the other side, with this fiber shorter extraction times can be used without a loss in yield, i.e., a 10 min extraction with the PDMS–DVB fiber results in the same yield as can be obtained with the PDMS fiber after a 45 min extraction. The drawback of the PDMS–DVB fiber is that its lifetime in either plasma

Table 1
Extraction yield of lidocaine (500 ng ml^{-1}) under various conditions (100-μm PDMS-coated fiber, 1 ml sample, pH 9.5)

Sample	Extraction yield (%)
Buffer	1.0
Buffered plasma (1:1)	0.4
Buffered plasma (1:1) and 0.3 g ml^{-1} NaCl	0.6
Spiked plasma, deproteinization[a]	1.0
Spiked plasma, deproteinization[b]	1.3
Plasma, spiked after deproteinization[b]	1.5
Spiked plasma, deproteinization[b] and 0.25 g ml^{-1} NaCl	11.7

[a] Deproteinization was performed by adding 0.1 g ml^{-1} NaCl to the plasma and then acidifying for the precipitation of the proteins after which the sample is centrifuged; the supernatant is buffered (1:1).

[b] Deproteinization was performed by acidifying the plasma sample and then adding 0.1 g ml^{-1} NaCl for the precipitation of the proteins after which the sample is centrifuged; the supernatant is buffered (1:1).

or buffer samples is much shorter than that of a PDMS fiber which can be used for more than 300 extractions. About 25 extractions can be performed with the PDMS–DVB fiber before the coating bursts from the silica core or before the fiber drops out the stainless steel tubing to which it is connected with epoxy glue for its mechanical strength.

3.3. Validation of the SPME–GC method

For the validation of the determination of lidocaine in plasma, the PDMS-coated fiber was used because of its longer lifetime. In order to avoid the presence of an unknown NaCl concentration, TCA was used for deproteinization in stead of NaCl. Spiked plasma samples were acidified with hydrochloric acid and deproteinized with TCA, the supernatant was buffered and NaCl was added according to the procedure described in Experimental. Chromatograms of the SPME–GC analysis of blank and spiked plasma samples are shown in Fig. 3. The blank plasma, Fig. 3a, contains an interfering peak which is caused by the buffer. The precision of the determination of lidocaine in human plasma samples including deproteinization and SPME–GC were satisfactory. The relative standard deviation (RSD, $n=5$) of the whole method at 500 ng ml^{-1} is $\leq 10\%$ which is about three-times as high as that obtained for SPME–GC of buffer solutions using PDMS-coated fibers.

In order to obtain a calibration curve, plasma was spiked with lidocaine (1.25–4000 ng ml^{-1}), proteins were removed, supernatant was diluted (1:1) with buffer, NaCl was added (0.3 g ml^{-1}), pH was adjusted to 9.5 and 1 ml sample was extracted with a 100-μm PDMS-coated fiber for 45 min. A linear range from 25 to 2000 ng ml^{-1} lidocaine in plasma

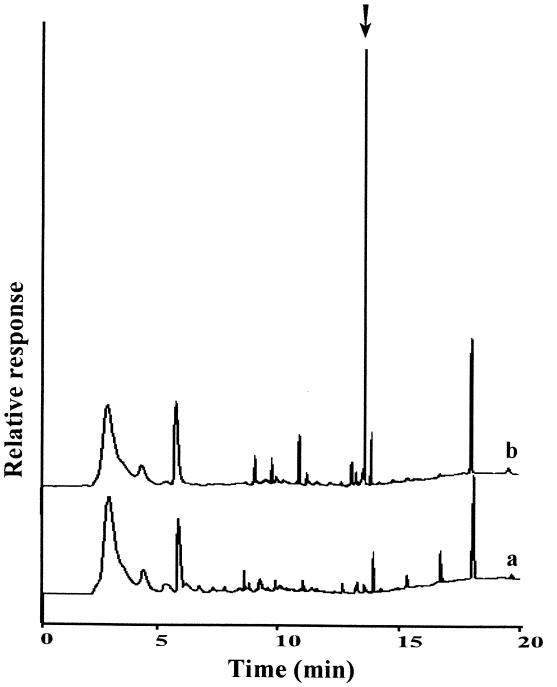

Fig. 3. Chromatograms of the SPME–GC analysis of (a) blank plasma and (b) spiked plasma (1 μg ml^{-1} lidocaine) after deproteinization, 1:1 buffering to pH 9.5 and addition of 0.3 g ml^{-1} NaCl. Extraction was carried out with a 100-μm PDMS-coated fiber for 45 min.

($r = 0.998$) was found. The detection limit, defined as the concentration of lidocaine in plasma which corresponds with three-times the blank peak, is 5 ng ml^{-1}.

4. Conclusions

A direct-immersion SPME–GC method has been developed for the analysis of the free, protein-bound and total amount of lidocaine in human plasma. Plasma proteins diminish the extraction yield which indicates the disturbance of the extraction process. Both the sample–protein and sample–fiber equilibria have to be taken into account. SPME can be used to obtain a good approximation of the plasma protein binding. It was found that lidocaine is for about 74% bound to protein which corresponds to literature values. However, it should be kept in mind that the extraction conditions can influence the drug–protein interaction.

It was shown that deproteinization is necessary for the determination of the total amount of lidocaine in plasma. Sodium chloride can be added to the protein-free sample for a considerable increase of the extraction yield. An extraction yield of about 12% has been obtained with a 100-μm PDMS-coated fiber. The detection limit for the determination of lidocaine in plasma with SPME–GC–FID is in the low ng ml^{-1} range. When nitrogen–phosphorus detection or mass spectrometry is used, selectivity and sensitivity can probably be enhanced. With the use of a 65-μm PDMS–DVB fiber, extraction yields could be increased to about 80%, although very long extraction times are needed. However, with this fiber higher extraction yields can be obtained in the same time. The drawback of the mixed-phase fiber is its limited lifetime.

SPME is a simple and promising sample pretreatment method for biological samples and also seems very suitable for ''on-site'' sampling. A careful optimization of the SPME step is needed because the composition of the sample can have a strong influence on the extraction process. Protein binding of drugs is a special aspect which has a main impact on the extraction yield. On the other side, SPME can be used for protein-binding studies.

Acknowledgements

The authors wish to thank J. Brands from Sigma–Aldrich (Zwijndrecht, The Netherlands) for the gift of the SPME fibers.

Appendix

The adsorption of drugs by proteins can be described by the process in which drugs are bound to a number of specific binding sites on the protein [8,16]. If assumed that in real samples the total number of binding sites in plasma is much larger than the amount of drugs bound to the proteins, the association equilibrium constant, K_p, can be calculated by:

$$K_p = \frac{C_b}{C_{fr} C_p} = \frac{n_b V_{pl}}{n_{fr} N_p^0} \quad \text{(A.1)}$$

where C_b is the concentration of drug bound to plasma proteins, C_{fr} is the concentration of drug freely dissolved in the plasma matrix, C_p is the concentration of protein, n_b is the amount of drug bound to the proteins, n_{fr} is the amount of drug freely dissolved in the plasma matrix, V_{pl} is the volume of the plasma sample and N_p^0 is the number of available sites for drug binding. Exactly, $N_p^0 - N_b$ (N_b is the number of drug molecules bound to the proteins) has to be used in Eq. (A.1) but this can be replaced by N_p^0 in the absence of drugs or in the presence of small amounts of drugs. If the concentrations of the bound and freely soluble drug are expressed in mol l^{-1} the number of available binding sites should be expressed in mol.

SPME is an equilibrium process in which analytes partition between the sample matrix and the polymer coating. For direct-immersion SPME of plasma samples, n_{fr} can be expressed as:

$$n_{fr} = \frac{V_{pl} n_f}{K_f V_f} \quad \text{(A.2)}$$

where n_f is the amount of drug in the fiber coating, V_f is the volume of the fiber coating and K_f is the fiber–matrix partition constant. Combining Eqs. (A.1) and (A.2) results in:

$$n_b = \frac{K_p N_p^0 n_f}{K_f V_f} \quad (A.3)$$

During extraction, the total amount of drug, n_0, remains constant:

$$n_0 = n_f + n_{fr} + n_b \quad (A.4)$$

Therefore an equation for the calculation of the extracted amount of drug can be obtained by combining Eqs. (A.2–A.4):

$$n_f = \frac{K_f V_f n_0}{K_f V_f + K_p N_p^0 + V_{pl}} \quad (A.5)$$

This equation shows that there is a linear relationship between the extracted amount and the total amount of drugs in the plasma sample if the number of binding sites is much larger than the number of bound drug molecules and the equilibrium process of the drug–protein interaction is considered to be fast. A much simpler expression for the extracted amount has been obtained than that given by Ulrich and Martens [8].

Only a few SPME experiments are needed to obtain a good approximation of the drug–protein binding. First of all extractions have to be performed in buffer or deproteinized plasma to determine K_f. Once this value is known, Eq. (A.5) can be used to calculate $K_p N_p^0$ by one plasma extraction. Subsequently, Eq. (A.1) can be used to calculate the quotient of the bound and free amount of drug in plasma and, so, drug–protein binding.

If plasma is diluted with buffer and the extracted amount of drug is plotted against the dilution factor, Eq. (A.5) can be used to fit the curve which results in a mean value of $K_p N_p^0$ and thus also a mean value for the drug–protein binding. The advantage of the determination of protein binding with a dilution curve at constant drug concentration is that it shows if the assumption that the number of binding sites is much larger than the number of bound drug molecules is valid. For each point in the curve the value for $K_p N_p^0$ can be calculated which should be constant although the ratio N_p^0:n_0 changes. However, if the number of binding sites is limited, only a relatively low amount of drug can be bound to the plasma protein, i.e., above a certain concentration the freely dissolved amount of drug is approximately equal to the total amount of drug in the sample. Thus at high drug concentrations, it can be assumed that $N_p^0 - N_b \approx 0$. In consequence the amount of drug extracted can be calculated by:

$$n_f = \frac{K_f V_f n_0}{K_f V_f + V_{pl}} \quad (A.6)$$

which is the commonly used equation for direct-immersion SPME.

References

[1] C.L. Arthur, J. Pawliszyn, Anal. Chem. 65 (1990) 2145.
[2] R. Eisert, K. Levsen, J. Chromatogr. A 733 (1996) 143.
[3] D. Louch, S. Motlagh, J. Pawliszyn, Anal. Chem. 64 (1992) 1187.
[4] J. Chen, J. Pawliszyn, Anal. Chem. 67 (1995) 2530.
[5] C.W. Whang, J. Pawliszyn, Anal. Commun. 35 (1998) 353.
[6] E.H.M. Koster, N.S.K. Hofman, G.J. de Jong, Chromatographia 47 (1998) 678.
[7] M. Krogh, H. Grefslie, K.E. Rasmussen, J. Chromatogr. B 689 (1997) 357.
[8] S. Ulrich, J. Martens, J. Chromatogr. B 696 (1997) 217.
[9] M. Krogh, K. Johansen, F. Tønnesen, K.E. Rasmussen, J. Chromatogr. B 673 (1995) 299.
[10] W.H.J. Vaes, E. Urrestarazu Ramos, H.J.M. Verhaar, W. Seinen, J.L.M. Hermens, Anal. Chem. 68 (1996) 4463.
[11] S. O'Byrne, M.G. Barry, W.C.J. Collins, P. O'Connor, Clin. Pharmacokinet. 24 (1993) 183.
[12] G.T. Tucker, R.N. Boyes, P.O. Bridenbaugh, D.C. Moore, Anesthesiology 33 (1970) 287.
[13] A.C. Moffat, J.V. Jackson, M.S. Moss, B. Widdop (Eds.), Clark's Isolation And Identification of Drugs, The Pharmaceutical Press, London, 1986, p. 705.
[14] Y. Shen, J.A. Jönsson, L. Mathiasson, Anal. Chem. 70 (1998) 946.
[15] P.A. Routledge, in: F. Belpaire, M. Bogaert, J.P. Tillement, R. Verbeeck (Eds.), Proceedings of an International Symposium held on the Occasion of the Centenary of the Heymans Institute of Pharmacology, Academia Press, Gent, 1990, p. 207.
[16] R. Verbeeck, in: F. Belpaire, M. Bogaert, J.P. Tillement, R. Verbeeck (Eds.), Proceedings of an International Symposium held on the Occasion of the Centenary of the Heymans Institute of Pharmacology, Academia Press, Gent, 1990, p. 121.
[17] J. Dewulf, H. Van Langenhove, M. Everaert, J. Chromatogr. A 761 (1997) 205.
[18] J. Ai, Anal. Chem. 69 (1997) 1230.
[19] E.H.M. Koster, G.J. de Jong, submitted for publication.