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Reduction of extraction times in liquid-phase microextraction

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Abstract

Recently, we introduced a simple and inexpensive disposable device for liquid-phase microextraction (LPME) based on porous polypropylene hollow fibres. In the present paper, extraction times were significantly reduced by an increase in the surface of the hollow fibres. The model compounds methamphetamine and citalopram, were extracted from 2.5 ml of urine, plasma, and whole blood after dilution with water and alkalisation with 125 μl of 2 *M* NaOH though a porous polypropylene hollow fibre impregnated with hexyl ether and into an aqueous acceptor phase consisting of 0.1 *M* HCl. Two commercially available hollow fibres, which differed in surface area, wall thickness and internal diameter, were compared. An increase in the contact area of the hollow fibre with the sample solution by a factor of approximately two resulted in reduction in equilibrium times by approximately the same factor. Thus, the model compounds were extracted to equilibrium within 15 min from both urine and plasma, and within 30 min from whole blood. For the first time LPME was utilised to extract drugs from whole blood, and the extracts were comparable with plasma both with regard to sample clean-up and extraction recoveries. Extraction recoveries for methamphetamine and citalopram varied from 60 to 100% using the two fibres and the different matrices.

Keywords: Extraction times; Liquid-phase microextraction; Methamphetamine; Citalopram

1. Introduction

Membrane-based extraction techniques offer efficient alternatives to classical sample preparation techniques. Advantages of liquid–liquid extraction such as the possibility of tuning the selectivity of the extraction by chemical means are preserved, while disadvantages such as solvent consumption are avoided. Membrane-based extraction techniques also permit high selectivity and high enrichment factors. Different set-ups for membrane-based extraction have been described [1–6], and recently a simple and inexpensive device for liquid-phase microextraction (LPME) based on disposable polypropylene hollow fibres was introduced [7]. In LPME, analytes are extracted from small volumes of biological samples through pores of a porous hollow fibre of polypropylene containing traces of an organic solvent and into a microliter volume of acceptor phase inside the hollow fibre. The LPME concept is compatible both with gas chromatography (GC), high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE). Utilising LPME prior to GC analysis, the acceptor phase inside the hollow fibre is an organic solvent compatible with the GC system, and the analytes are extracted in a two-phase system. In contrast, with LPME in combination with HPLC or CE, the acceptor phase is aqueous for compatibility

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reasons. In the latter case, the analytes are extracted in a three-phase system from aqueous samples through an organic solvent present in the pores of the hollow fibre and into the aqueous acceptor phase. LPME is applicable to neutral compounds with the two-phase system, and to acidic and basic substances utilising either the two- or three-phase concept. With a large volume difference between the sample (1–4 ml) and the acceptor solution (25 μl), analytes may be substantially enriched providing high partition coefficients in the extraction system. In addition to analyte enrichment, LPME provides high selectivity especially utilising the three-phase system, where a simultaneous extraction and back-extraction is performed. Compared with solid-phase microextraction (SPME) [8–13], extraction recoveries from biological matrices obtained after 30–50 min of LPME are high (50–85%). The price of each extraction unit is low (comparable with the price of a solid-phase extraction (SPE) column), and each extraction device is used only for a single extraction. Thus, carry-over effects between extractions are eliminated. As LPME is a miniaturised technique, which requires only a few microlitres of organic solvents, a large reduction in solvent consumption may be achieved compared with traditional sample preparation techniques.

In previous works [7,14–17], a variety of drugs (methamphetamine, benzodiazepines, non-steroid anti-inflammatory drugs and an antidepressant) have been extracted from human urine and plasma, and analysed by GC, HPLC, or CE. Despite the extraction times of typically 30–50 min, a high sample throughput was enabled for LPME by parallel extraction of 20–30 samples.

Even though parallel LPME extractions have ensured high throughput, a reduction in extraction time would be favourable. Therefore, in the present work attempts to reduce the extraction times have been made by increasing the contact area of the hollow fibre with the sample solution. The polypropylene hollow fibre utilised previously [7,14–17], was compared with a second commercially available polypropylene hollow fibre where the contact area was increased by a factor of approximately two. The comparisons of the hollow fibres were made by simultaneous extractions of methamphetamine and citalopram from pure standard solutions prepared in water, and from human urine, and plasma. In addition, LPME of drugs from whole blood was presented for the first time in the present paper. Citalopram and methamphetamine were chosen as model compounds as it has been demonstrated that both substances may be extracted efficiently utilising the standard LPME device [14,16]. After extraction, all extracts were analysed by CE and compared with emphasis on extraction times, extraction recoveries and sample clean-up.

2. Experimental

2.1. Chemicals

Methamphetamine (>99%) was obtained from Norsk Medisinaldepot (Oslo, Norway), and citalopram hydrobromide was from H. Lundbeck (Copenhagen, Denmark). Sodium hydroxide, sodium acetate, and hydrochloric acid, all of analytical grade, and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany). Hexyl ether was obtained from Sigma (St Louis, MO), and acetic acid (99.8%) of analytical grade was purchased from Prolabo (Rohne-Polenc, Manchester, UK). All aqueous solutions were prepared with water purified with an EASYpure RO system (Barnstead, Dubuque, IA).

2.2. Standard solutions and biological samples

Standard solutions of methamphetamine and citalopram in water, urine, plasma, and whole blood were prepared by dilution from a 10 μg/ml solution of methamphetamine and citalopram in water. The 10 μg/ml solution was prepared from a 1 mg/ml stock solution of methamphetamine in water and a 1 mg/ml stock solution of citalopram in methanol. All solutions were stored at 5°C protected from light.

2.3. Porous hollow fibres

Two different kinds of polypropylene hollow fibres were used. The fibres differed in internal diameter, wall thickness, length, and pore size. One of the hollow fibres was the Q3/2 Accurel KM® polypropylene hollow fibre (Akzo Nobel, Wuppertal, Germany) used previously [7,14–17]. The other fibre was chosen due to its enlarged contact area with the

sample solution and was a Plasmaphan P1LX® polypropylene hollow fibre (Akzo Nobel). The Q 3/2 Accurel KM hollow fibre had an inner diameter of 600 μm, the thickness of the wall was 200 μm, and the pore size was 0.2 μm. An 8-cm piece of this hollow fibre was used providing an acceptor phase volume of approximately 25 μl. The Plasmaphan P1LX hollow fibre had an inner diameter of 330 μm, the thickness of the wall was 150 μm, and the pore size was 0.4 μm. A 27-cm piece of this hollow fibre was used providing an acceptor phase volume of approximately 17 μl.

2.4. Liquid-phase microextraction device

The disposable LPME device is illustrated in Fig. 1 [14]. The sample solution was filled into a 4-ml amber vial with a screw top/silicone septum (Supelco, Bellefonte, PA). Two syringe needles (Terumo, Leuven, Belgium) were inserted through the silicon septum; one served to introduce the acceptor solution into the hollow fibre prior to extraction while the second needle was utilised for collection of the acceptor solution after extraction. The ends of the two needles were connected to the polypropylene hollow fibre. Different needles were used depending on the hollow fibre utilised. Two 0.8-mm O.D. syringe needles were utilised together with the Q3/2 Accurel KM fibre while two 0.4-mm O.D. syringe needles were utilised together with the Plasmaphan P1LX fibre.

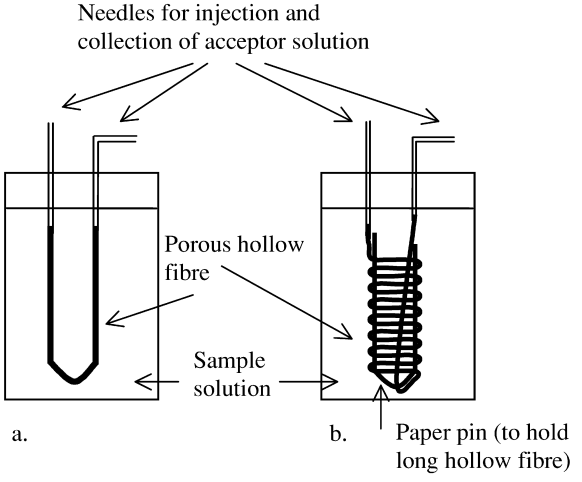

Fig. 1. Diagram of: (a) the original LPME unit [14] and (b) the modified LPME unit.

2.5. Liquid-phase microextraction procedure

Extractions were performed according to the following scheme: a 2.5-ml sample solution was filled into the vial. The solution was rendered alkaline with 0.125 ml of 2 *M* NaOH and diluted to 4 ml with water. A new length of hollow fibre was placed between the two needle ends and the hollow fibre with enlarged contact area was coiled up around a paper pin. For impregnation, the fibres were dipped for 2 s in hexyl ether. Subsequently, 20 s of ultrasonification in a water bath removed the excess solvent. After impregnation, 25 μl of 0.1 *M* HCl (acceptor solution) was injected into the Q3/2 Accurel KM hollow fibre with a microlitre syringe, while the Plasmaphan P1LX hollow fibre was filled with an excess of 0.1 *M* HCl. Subsequently, the hollow fibres were placed in the sample solutions for LPME. During extraction, the solution was vibrated at 1500 rpm using a Vibramax 100 (Heidolph, Kelheim, Germany). After extraction, the acceptor solution was flushed into a 200-μl vial/insert for the capillary electrophoresis instrument by applying a small pressure with a 5-ml syringe on the inlet needle of the hollow fibre. Each piece of hollow fibre was used only for a single extraction.

2.6. Capillary electrophoresis

Capillary electrophoresis was performed with a MDQ instrument (Beckman, Fullerton, CA, USA) equipped with a UV detector. Separations were accomplished in a 75 μm I.D. fused-silica capillary (BGB Analytik AG, Anwil, Switzerland) with an effective length of 30 cm (total length of 40.2 cm). The separation buffer was 50 m*M* acetate adjusted to pH 4.6 with concentrated acetic acid. Before analysis the separation buffer was filtered through a 0.45-μm filter (Minisart RC 25, Sartorius AG, Göttingen, Germany). The instrument was operated at 15 kV, generating a current level of approximately 62 μA. All samples were introduced by hydrodynamic injection at 0.5 p.s.i. for 5 s. Detection was accomplished at 200 nm utilising a 100×800 μm slit.

2.7. Calculation of enrichment factors and extraction recoveries

The enrichment factor (EF) was defined as the ratio between the final analyte concentration $C_{a,final}$ in the sample extract (acceptor phase) and the initial concentration of analyte $C_{s,initial}$ within the sample:

$$EF = C_{a,final}/C_{s,initial} \quad (1)$$

The enrichment factor was determined by peak area measurements and by calibration with standard solutions containing the drugs at a 100-fold higher concentration level than in the samples. The extraction recovery (ER) was defined as the percentage of the total analyte amount $n_{s,initial}$ (originally present in the sample) which was transferred to the extract (acceptor phase) at the end of the extraction ($n_{a,final}$):

$$ER = (n_{a,final}/n_{s,initial}) \times 100\%$$

$$= (V_a/V_s)EF \times 100\% \quad (2)$$

where V_a and V_s are the volumes of acceptor solution and sample solution (donor solution), respectively. The extraction recovery was determined from EF, V_a and V_s as shown in Eq. (2).

3. Results and discussion

3.1. Basic principle

The basic drugs methamphetamine and citalopram were selected as model compounds (analytes) in the present work and were extracted from 2.5 ml volumes of aqueous standard solutions, human urine, human plasma, and human whole blood. After alkalinisation and dilution, the analytes were extracted through the porous polypropylene hollow fibre impregnated with organic solvent (hexyl ether) and into the acidic acceptor solution. The extraction into the organic solvent was facilitated by the reduced solubility of the analytes in the sample solution, and further extraction into the acceptor phase was promoted by the high solubility of the analytes in the dilute hydrochloric acid inside the hollow fibre. Inside the hollow fibre the analytes were ionised, and hence prevented from re-entering the organic solvent.

Previous reports have required extraction times of 30–50 min to obtain maximum extraction recoveries [7,14–17]. In order to improve the LPME technique further, the present paper focused on reduced extraction times utilising a hollow fibre with enlarged contact area. By reducing the internal diameter of the hollow fibre from 600 to 330 μm and by a corresponding increase in the length from 8 to 27 cm, the contact area of the hollow fibre was increased by a factor of approximately two. Comparison of LPME with the two different fibres was accomplished both from pure standard solutions in water, and from urine, plasma, and whole blood. The matrices were chosen mainly because of their importance in drug monitoring (urine, plasma and whole blood) and reflected a broad and complicated range with respect to matrix chemistry.

3.2. Modification of technical set-up

The technical set-up used in recent publications was modified to handle the hollow fibre with enlarged contact area. A diagram of the original LPME device is shown in Fig. 1a utilising the 8 cm long piece of hollow fibre with small contact area. Because the length of the hollow fibre with enlarged contact area was 27 cm, the fibre was coiled up on a paper pin (Fig. 1b) to fit within the conventional 4-ml vial. Syringe needles with smaller O.D. were used in order to fit inside the hollow fibre with enlarged contact area. Using the original LPME device (Fig. 1a), the acceptor solution was filled into the hollow fibre with a microlitre syringe. In the modified device (Fig. 1b), the hollow fibre was filled with excess of acceptor solution using a 5-ml syringe. The volume of the acceptor solution in the modified device was determined to be 17 μl, while the volume was 25 μl using the original device.

3.3. Effects of changing the hollow fibre

Enrichment factors after extraction with the two hollow fibres were plotted versus extraction time for each matrix as shown in Fig. 2a–d. For all of the matrixes, a reduction in equilibrium times was observed utilising the hollow fibre with enlarged

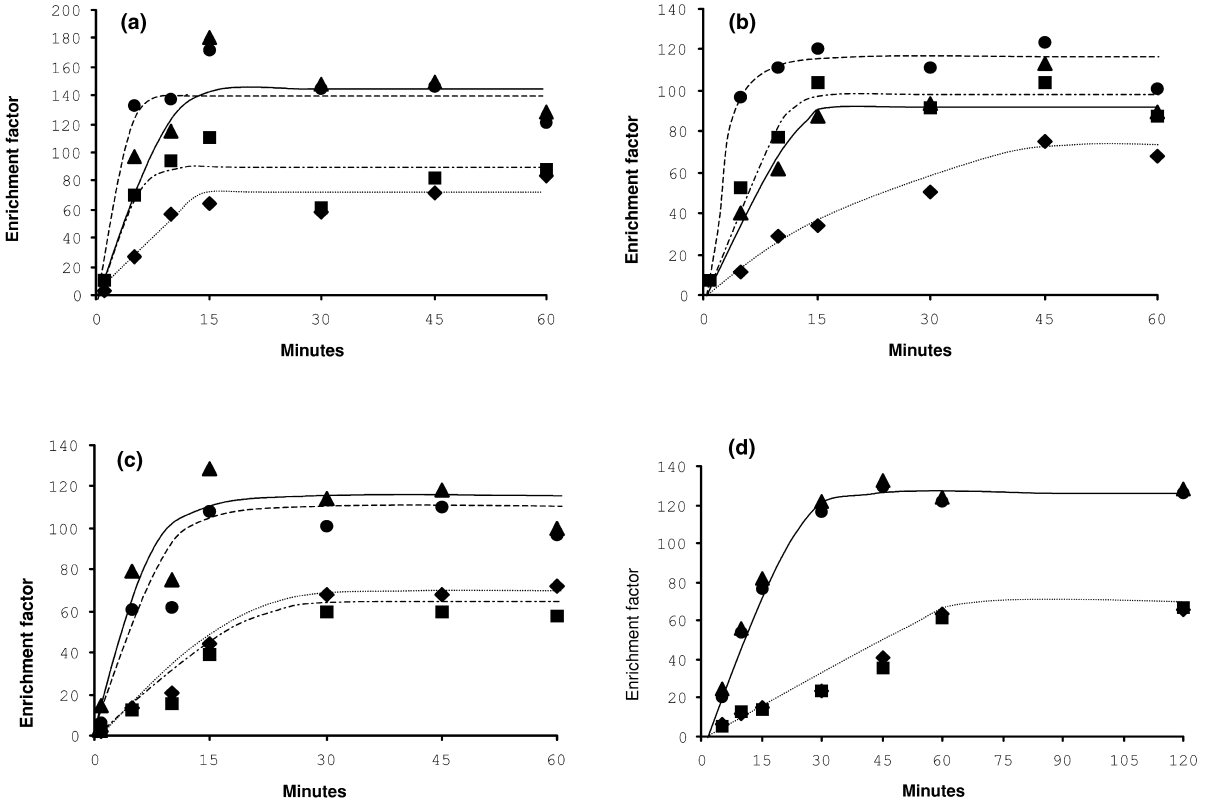

Fig. 2. Enrichment factors of methamphetamine and citalopram using the two different hollow fibres versus extraction time. Methamphetamine (♦) and citalopram (■) extracted with the hollow fibre with small contact area, and methamphetamine (▲) and citalopram (●) extracted with the hollow fibre with enlarged contact area from (a) water, (b) urine, (c) plasma and (d) whole blood. Based on three replicates.

contact area. The effect was small for water samples, but pronounced for the biological samples where equilibrium times were reduced by a factor of approximately two. The reduction in equilibrium times utilising the hollow fibre with enlarged contact area was probably a result of an increase in the membrane area (contact area) and a reduction in the wall thickness. The less pronounced reduction of equilibrium times in the pure water samples may indicate that in this matrix, other parameters were rate-limiting. The smaller internal diameter of the hollow fibre with enlarged contact area may also have influenced the equilibrium times.

The reduction in extraction times utilising the hollow fibre with enlarged contact area resulted in extraction times of 15 min or less for methamphetamine and citalopram in water, urine and plasma, and of 30 min for the more viscous matrix whole blood. As the extraction times have been the rate-limiting step of the sample preparation process even though parallel extraction of 20–30 samples have been performed, a reduction in this time by a factor of two resulted in an increase in the sample throughput.

The enrichment factors obtained utilising the fibre with enlarged contact area were higher than the enrichment factors utilising the original fibre (95–145 and 60–105, respectively). Due to the difference in acceptor volume, different maximum enrichment factors were expected with the two fibres. The fibre with enlarged contact area had a maximum enrichment factor of 147 using the 2.5-ml sample solution and an acceptor solution of 17-μl, while the original fibre had a maximum enrichment factor of 100 using

the 2.5-ml sample solution and an acceptor solution of 25-μl. Extraction recoveries utilising the two different fibres varied between 60 and 100% for the original hollow fibre and between 64 and 99% for the fibre with enlarged contact area (Table 1).

3.4. Matrix considerations

In the previous LPME publications [7,14–17], extractions have been performed from water, urine and plasma samples. In the present work, LPME of whole blood was included. Whole blood samples required no extra pre-treatment prior to LPME. Comparison of the electropherograms from the plasma and whole blood samples showed that LPME provided high clean-up in both cases (Fig. 3c,d). In addition, the degree of enrichment was comparable for human plasma and whole blood (Fig. 2c,d). Hence with LPME, sample preparation of whole blood samples is as easy and efficient as from plasma samples, despite the complex composition of whole blood. Due to the high viscosity of whole blood, though, extraction equilibrium times were twice as long and 30 min was required to reach equilibrium (Fig. 2c,d).

Analysis of the extracts by CE showed extremely clean electropherograms for all extracts (Fig. 3). Only urine extracts showed a few additional peaks. The high selectivity is one of the advantages of LPME, and is especially important when extractions are performed from complex biological matrices.

3.5. Validation

Validation of LPME-CE of methamphetamine in water and of citalopram in plasma utilising the hollow fibre with small contact area has been performed earlier [14,16]. In this paper the hollow fibres with enlarged contact area were briefly validated with respect to repeatability, linearity, detection limit and quantification limit of citalopram in whole blood. Methamphetamine (100 ng/ml) was used as internal standard. First, repetitive extractions ($n=6$) of citalopram (100 ng/ml) were performed. After correction of the absolute peak areas of citalopram with the internal standard the results varied within 13.3% RSD. The RSD of citalopram utilising this hollow fibre was slightly higher than the RSD observed using the hollow fibre with small contact area [16]. This may be a result of the more complex extraction set-up of this longer hollow fibre (Fig. 1). In addition different internal standard and biological matrix were used in this experiment. The standard curve for citalopram was linear from 20 to 1000 ng/ml ($r=0.9964$). The excellent linearity was obtained in an extended range compared to the therapeutic window (20–110 ng/ml; [18]) and indicated no capacity problems due to acceptor phase saturation during LPME in this new set-up with less acceptor phase. The limit of quantification ($S/N=10$) was estimated to be 6.5 ng/ml and the limit of detection ($S/N=3$) was 2.0 ng/ml for citalopram with UV detection at 200 nm. These limits were lower than the quantification and detection limits obtained in the previous work determining citalopram in plasma utilising LPME-CE [16], mainly due to a larger volume of sample solution (2.5 vs. 1 ml).

4. Conclusions

In the present work, attention was focused on equilibrium times in LPME. Two different polypropylene hollow fibres differing in the contact area

Table 1
Extraction recoveries (%) of citalopram and methamphetamine from water, human urine, human plasma and humane whole blood after extraction to equilibrium

	Hollow fibre with small contact area (Q3/2 Accurel KM)		Hollow fibre with enlarged contact area (Plasmaphan P1LX)	
	Methamphetamine	Citalopram	Methamphetamine	Citalopram
Water	70	90	99	95
Urine	70	100	64	78
Plasma	70	60	78	75
Whole blood	65	65	85	85

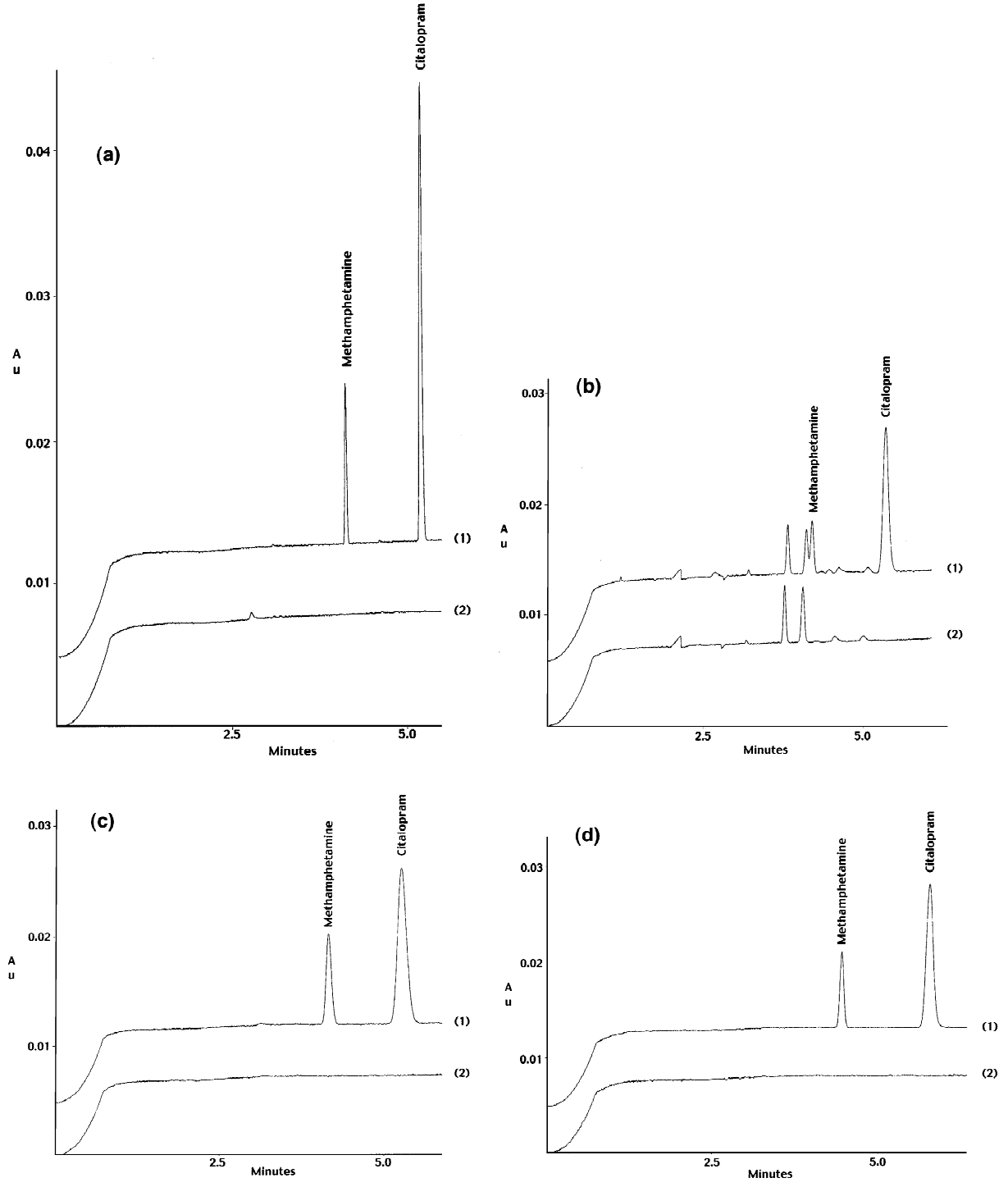

Fig. 3. Electropherograms of LPME extracts from: (a) water, (b) urine, (c) plasma and (d) whole blood after extraction with the modified LPME device to equilibrium (extracts from: (1) samples spiked with 100 ng/ml methamphetamine and citalopram, and (2) blank samples).

with the sample solution were compared. Utilising the hollow fibre with enlarged contact area, extraction times from urine, plasma, and whole blood samples were reduced by a factor of two. The reduction in extraction time enabled LPME of the model compounds in water, urine, and plasma samples to equilibrium within 15 min. This work also demonstrated the successful extraction of whole blood samples by LPME within 30 min, providing extracts comparable with those of plasma regarding sample clean-up and extraction recovery.

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References

[1] J.Å. Jönsson, L. Mathiasson, Trends Anal. Chem. 18 (1999) 325.
[2] J.Å. Jönsson, L. Mathiasson, Trends Anal. Chem. 18 (1999) 318.
[3] N.C. van de Merbel, J. Chromatogr. A 856 (1999) 55.
[4] Q. Yang, A.J. Tomlinson, S. Naylor, Anal. Chem. 71 (1999) A183.
[5] J.Å. Jönsson, L. Mathiasson, J. Chromatogr. A 902 (2000) 205.
[6] B.M. Cordero, J.L. Pérez Pavón, C. García Pinto, M.E. Fernández Laespada, R. Carabias Martínez, E. Rodríguez Gonzalo, J. Chromatogr. A 902 (2000) 195.
[7] K.E. Rasmussen, S. Pedersen-Bjergaard, M. Krogh, H.G. Ugland, T. Grønhaug, J. Chromatogr. A 873 (2000) 3.
[8] H.G. Ugland, M. Krogh, K.E. Rasmussen, J. Chromatogr. B 701 (1997) 29.
[9] S. Ulrich, J. Martens, J. Chromatogr. B 696 (1997) 217.
[10] Y. Luo, L. Pan, J. Pawliszyn, J. Microcol. Sep. 10 (1998) 193.
[11] S. Ulrich, S. Kruggel, H. Weigmann, C. Hiemke, J. Chromatogr. B 731 (1999) 231.
[12] H.G. Ugland, M. Krogh, K.E. Rasmussen, J. Pharm. Biomed. Anal. 19 (1999) 463.
[13] M. Moeder, S. Schrader, M. Winkler, P. Popp, J. Chromatogr. A 873 (2000) 95.
[14] S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chem. 71 (1999) 2650.
[15] S. Pedersen-Bjergaard, K.E. Rasmussen, Electrophoresis 21 (2000) 579.
[16] T.G. Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 909 (2001) 87.
[17] H.G. Ugland, M. Krogh, K.E. Rasmussen, J. Chromatogr. B 749 (2000) 85.
[18] L. Bjerkenstedt, L. Flyckt, K.F. Overö, O. Lingjærde, Eur. J. Clin. Pharmacol. 28 (1985) 553.