Available online at www.sciencedirect.com
SCIENCE DIRECT®

ELSEVIER

Journal of Chromatography A, 1072 (2005) 29–36

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Liquid-phase microextraction based on carrier mediated transport combined with liquid chromatography–mass spectrometry New concept for the determination of polar drugs in a single drop of human plasma

Tung Si Ho*, Jan Leon Egge Reubsaet, Hanne Sofie Anthonsen, Stig Pedersen-Bjergaard, Knut Einar Rasmussen

School of Pharmacy, University of Oslo, P.O. Box 1068, Blindern, N-0316 Oslo, Norway

Available online 18 December 2004

Abstract

Recently, we demonstrated for the first time liquid-phase microextraction (LPME) of polar drugs based on carrier mediated transport. In this new extraction technique, selected analytes were extracted as ion-pairs from small volumes of biological samples, through a thin layer of a water immiscible organic solvent immobilised in the pores of a porous hollow fibre (liquid membrane), and into a μl volume of an acidic aqueous acceptor solution placed inside the lumen of the hollow fibre. In the current paper, this new extraction technique was combined with liquid chromatography–mass spectrometry (LC–MS) for the first time. Carrier mediated LPME was evaluated for several new model drugs ($0.01 < \log P < 1.76$), the sample clean-up aspects were investigated in detail, and this new extraction technique was fully validated for the first time. Extractions were performed from 50 μl of human plasma samples, which provided sufficient material in combination with LC–MS. Sodium octanoate (50 mM) was added to the sample as carrier, 1-octanol (≈15 μl) was used as the liquid membrane in the wall of the hollow fibre, and 50 mM HCl was utilized as acceptor solution in the lumen of the hollow fibre. The addition of carrier to the samples was found to significantly improve extraction recoveries for the polar drugs tested, providing recoveries in the range 16–78%. Validation was accomplished for atenolol and cimetidine. Limits of quantification (S/N = 5) from 50 μl of plasma were 25 and 50 ng/ml for atenolol and cimetidine, respectively. The intra-day precision (R.S.D.) ranged from 7.8 to 17.2% and from 9.5 to 14.1% for atenolol and cimetidine, respectively, and corresponding inter-day precisions (R.S.D.) were within 6.7–1.4% and 7.7–20.3%. The method was linear in the range 25–1500 ng/ml for atenolol ($r = 0.992$), and 50–3500 ng/ml for cimetidine ($r = 0.976$). The accuracy of the method was found to be in range 89.1–99.6% and 83.4–86% for atenolol and cimetidine, respectively. The sample clean-up obtained by carrier mediated LPME was excellent, providing a significantly lower back-ground level in total ion current chromatograms by LC–MS as compared to protein precipitation.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Liquid-phase microextraction; Carrier mediated transport; Drugs; Polar

1. Introduction

For the determination of drugs in biological fluids, a sample preparation step is normally required to isolate and concentrate compounds of interest from the sample matrix, before a final chromatographic or electrophoretic analysis is performed. Several sample preparation techniques have been applied, with liquid–liquid extraction (LLE) and solid phase extraction (SPE) as the most common approaches. LLE is popular because the technique is simple, it provides a high degree of sample clean-up, and extraction selectivity is easily tuned by proper selection of the organic solvent. On the other hand, LLE is difficult to automate, and consumes substantial amounts of hazardous organic solvents. Automation is straightforward with SPE, and this in combination with the availability of a broad spectrum of different sorbents has resulted in widespread use of the SPE-technique. However, sufficient sample clean-up and analyte enrichment may be difficult in some cases with SPE, and

* Corresponding author. Fax: +47 22 85 44 02.
E-mail address: tungsho@farmasi.uio.no (T.S. Ho).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.11.078

also this technique consumes substantial amounts of organic solvents.

To overcome some of the obstacles of LLE and SPE, a recent trend in sample pre-treatment techniques has involved the miniaturisation of LLE by greatly reducing the solvent-to-sample volume ratio, leading to single-drop micro extraction or liquid-phase micro extraction (LPME) [1,2]. In single-drop micro extraction, the extraction phase is a micro drop of a water-immiscible solvent suspended on the tip of a conventional micro syringe, immersed in an aqueous sample solution [2]. Although single-drop micro extraction proved to be a simple, inexpensive, fast, and virtually solvent-free sample pre-treatment technique, problems with drop stability and low sensitivity were often encountered [1,3].

In order to eliminate the stability problems of single-drop microextraction, a novel micro extraction technique was recently introduced [4–9], where the micro extracting phase (acceptor solution) was placed inside a porous hollow fibre for mechanical protection (hollow fibre LPME). The chemistry of hollow fibre LPME is similar to the chemistry used for extraction with supported liquid membranes (SLM) [10–14], but the techniques differ significantly in terms of instrumentation and operation. In hollow fibre LPME, analytes are extracted from a small volume of a stagnant aqueous sample, through an organic solvent impregnated in the pores of the hollow fibre (liquid membrane), and further into the acceptor solution inside the lumen of the hollow fibre. The use of an aqueous acceptor solution results in a three-phase extraction system. This system is suitable for ionic compounds with hydrophobic properties as their neutral forms are highly soluble in the organic membrane and their ionic forms are highly soluble in the aqueous acceptor solution. However, polar compounds are to large extent excluded because of too low solubility in the liquid membrane.

In order to improve the permeability of polar drugs through the organic liquid membrane, and to expand the application area of hollow fibre LPME, we recently published the first paper on carrier mediated LPME of polar drugs [15]. The principle of this technique is illustrated in Fig. 1. A carrier (hydrophobic ion-pair reagent) is dissolved directly into the sample solution to form hydrophobic ion-pair complexes with the analytes. The ion-pair complexes are extracted into the organic liquid membrane immobilised in the pores of the hollow fibre. In the contact region of the liquid membrane and the acceptor solution, the analytes are released from the ion-pair complex into the acceptor solution, whereas counter-ions (protons) present in a very high concentration in the acceptor solution ion-pairs with the carrier in the contact area, and the new ion-pair complex is back-extracted into the sample. In the sample again, the carrier release the transported counter-ion, potentially form ion-pair with a new analyte molecule, and the cycle may be repeated. Protons are the driving force for the extraction, and a large excess of protons is necessary in order to maintain high extraction recoveries and to prevent the analytes from back-diffusion into the liquid membrane.

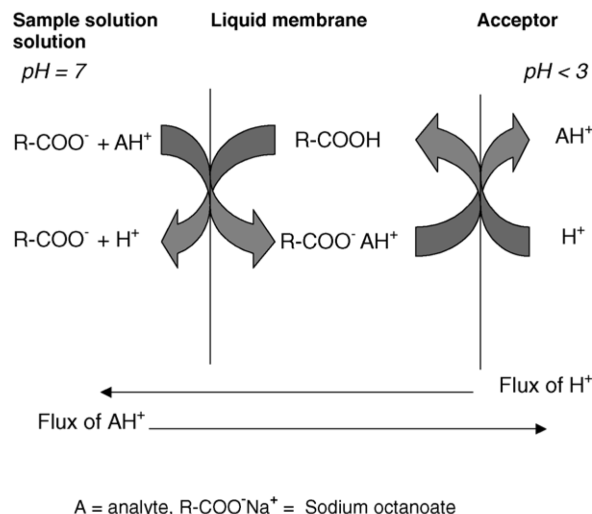

Fig. 1. Working model for carrier mediated extraction.

The first paper on carrier mediated LPME, where the technique was combined with capillary electrophoresis (CE), briefly demonstrated the principle and showed the performance for two polar model drugs [15]. In the present work, we have included several new model drugs of high polarity to demonstrate the performance, and we have combined carrier mediated LPME with liquid chromatography–mass spectrometry (LC–MS) for the first time. In addition, the current paper focus both on the recovery and sample clean-up aspects from a single drop (50 μl) of human plasma, and the new extraction technique has been fully validated for the first time with atenolol and cimetidine as model drugs.

2. Experimental

2.1. Chemicals

Amphetamine, morphine, phenylpropanolamine and practolol were obtained from Norsk Medisinaldepot (Oslo, Norway). Cimetidine, sotalol, atenolol, sodium octanoate and 1-octanol, trifluoroacetic acid and heptanfluorobutyric acid were obtained from Sigma (St. Louis, MO, USA). Hydrochloric acid, sulphuric acid, nitric acid, *ortho*-phosphoric acid, disodium hydrogen phosphate, ammonium acetate and methanol were obtained from Merck (Darmstadt, Germany). Formic acid was obtained from Riedel-De Haen (Hannover, Germany). Acetic acid was obtained from Prolabo (Rohne-Poulenc LTD, Manchester, UK). Drug-free plasma was obtained from Ullevål University Hospital (Oslo, Norway), and from the employers and students at School of Pharmacy (University of Oslo, Norway).

2.2. Standard solutions and biological samples

All solutions were prepared from 1 mg/ml stock solutions of amphetamine, morphine, phenylpropanolamine, cimeti-

dine, sotalol, atenolol and practolol in methanol. The general concentration in all experiments was 500 ng/ml both for samples in pure water and for plasma samples. The concentrations of atenolol and cimetidine in plasma samples for validation were in the range 25–1500 ng/ml and 50–3500 ng/ml, respectively. The concentration of sotalol as internal standard in the plasma samples was 500 ng/ml. All solutions and plasma samples were stored at 5 °C protected from light.

2.3. LC–MS

The LC–MS system consisted of a TSP SCM1000 vacuum de-gasser, TSP SpectraSystem P4000 quaternary gradient pump, a TSP SpectraSystem AS3000 autosampler and a Finnigan LCQ duo ion trap mass spectrometer. Xcalibur® version 1.3 software was used to control this system and to perform data acquisition (all Instrument-Teknikk, Østerås, Norway).

Electrospray (ESI) was used as the ionisation method and was operated in the positive mode. Separations were performed on a Chromsep HPLC column SS 30 mm × 3.0 mm from Varian (Holger, Oslo, Norway). To the analytical column, a BDS-C8 10 mm × 2.0 mm Javelin guard column from Thermo Hypersil-Keystone (Holger, Oslo, Norway) was attached. Both the guard column and the analytical column were equilibrated with LC mobile phase A consisting of 5% methanol in 10 mM formic acid and 10 mM heptanfluorobutyric acid. Prior to injection onto the LC column, the extracts (20 μl) were diluted with 40 μl 10 mM ammonium acetate. An aliquot of 50 μl of the dilute extract was injected by loop injection. All LC separations were carried out at room temperature. Between each analysis, the sample syringe was flushed with 1 ml of water:methanol (50:50). After injection of 50 μl sample, the following gradient program was carried out using 50% methanol in 10 mM formic acid and 10 mM heptanfluorobutyric acid as mobile phase B: from $t = 0.0$ to 8.0 min, the composition of the mobile phase was changed from 95% mobile phase A to 15% mobile phase A. From $t = 8.0$ to 9.0 min, the composition was unchanged. From $t = 9.0$ to 10.0 min, the composition of the mobile phase was changed from 15% mobile phase A to 95% mobile phase A. This composition was kept constant for 6 min. To avoid contamination of the mass spectrometer, the LC effluent was directed to waste during the first 3 min of each chromatographic run.

The following ESI conditions were applied: the spray voltage was 5 kV, sheath and auxillary gas were 40 and 5 arbitrary units, respectively. The other MS settings were: capillary temperature, 250 °C; capillary voltage, 15 V; tube lens offset, 0 V; octapole 1 offset, −4.75 V; lens voltage, −20 V; octapole 2 offset, −8 V; and octapole radio frequency amplitude, 400 V. The number of microscans was set to 2; the maximum injection time, 200 ms. During sample clean-up experiments, full scan in mass range m/z 50–500 was used. In the validation step, SIM at mass $m/z = 253$, 267 and 273 was used. The other experiments, two mass segments in range m/z 132–174 and 250–284 were used.

2.4. Protein precipitation procedure

Human plasma (500 μl) was vortex-mixed with 1 ml cold acetonitrile (stored at −18 °C and used immediately after storage). After 10 min, the sample was centrifuged for 10 min at 1400 rpm. A 750 μl aliquot of the supernatant was transferred to a new vial. The content was dried under a stream of N_2. The residue was resolved in 40 μl of 50 mM HCl and 80 μl of 10 mM ammonium acetate and vortex-mixed for 10 s. The sample was centrifuged again for 10 min at 1400 rpm. A 60 μl aliquot of the supernatant was transferred to a sample vial and placed into the autosampler for LC–MS analysis.

2.5. LPME device

The LPME device has been described in several publications [4–9,15]. In this study, LPME was carried out in 100 μl micro inserts (VWR International, West Chester, PA, USA), which were placed into a conventional 2 ml sample vial (Supelco, Bellefonte, PA, USA) equipped with a screw cap and a silicon septum. Two conventional 0.8 mm o.d. medical syringe needles were inserted through the silicon septum in the screw cap, and the two ends were connected to each other by a 6.5 cm piece of Q3/2 Accurel KM polypropylene hollow fibre (Membrana, Wuppertal, Germany). One medical syringe needle served to introduce the acceptor phase to the lumen of the hollow fibre prior to extraction, whereas the other needle was utilised for collection of the acceptor phase after extraction. The inner diameter of the hollow fibre was 600 μm, the thickness of the wall was 200 μm, and the pore size was 0.2 μm. The acceptor phase volume was 20 μl and the volume of organic phase immobilised in the pores was approximately 18 μl. During extraction, the sample vials were vibrated at 1500 rpm using a Vibramax 100 (Heidolph, Kelheim, Germany).

2.6. Carrier mediated LPME

A 50 μl volume of the sample solution (human plasma or water sample) was filled into the micro insert followed by 50 μl of 50 mM sodium octanoate dissolved in 25 mM phosphate buffer adjusted to pH 7.0. The hollow fibre was dipped for 5 s in 1-ocnanol, followed by ultra sonification for 15 s in a water bath to remove excess of the solvent. Subsequently, 20 μl of 50 mM HCl (acceptor phase) was injected into the lumen of the hollow fibre with a micro syringe. The fibre was placed in the sample in the 100 μl micro insert, and during extraction, the sample vials were vibrated at 1500 rpm. After extraction, the total volume of acceptor phase (20 μl) was flushed into a 200 μl micro insert and diluted with 40 μl of 10 mM ammonium acetate. An aliquot of 50 μl of the dilute extract was injected by loop injection for LC–MS analysis.

2.7. Calculations

All the calculations of this work were similar to that used in our previous report [15].

3. Results and discussion

In our first paper on carrier mediated LPME [15], amphetamine, morphine and practolol were used as model compounds to demonstrate the principle. In the present paper, phenylpropanolamine, cimetidine, sotalol, and atenolol were included in addition as polar model drugs. The structures of the different model drugs are shown in Fig. 2. In an initial experiment, the effect of the carrier was studied for the new model compounds in the original extraction system [15] as shown in Table 1. Also data for amphetamine, morphine and practolol were included for comparison, where amphetamine was studied as a representative for a more hydrophobic substance. As seen from the table, extraction recoveries were improved substantially when octanoic acid was added as carrier to the samples, and all the drugs were extracted with recoveries between 16 and 78% from pure water. This observation further supported that carrier mediated LPME may be a future approach for polar drugs. In addition, also extractions from plasma samples were successful for the new model drugs, which supported that carrier mediated LPME may provide acceptable efficiency also for plasma samples. Recovery values in plasma were slightly lower than from pure water samples. Although the polar drugs are only slightly bound to plasma proteins, plasma interaction may partially be the reason for this observation. In addition, the extractability from plasma samples may be affected by a somewhat higher viscosity or by a minor inhibition of the extraction process by matrix components. Nevertheless, carrier mediated LPME provided surprisingly high recoveries of polar drugs from human plasma samples.

Basically, the extraction system used in the rest of this work was similar to that used in our first publication on carrier mediated LPME [15]. Thus, no optimization of the carrier or the liquid membrane was accomplished in the present work. However, because the system in the current work was coupled with LC–MS and not CE, an optimization of the acceptor solution was performed.

3.1. Evaluation of acceptor phases for carrier mediated LPME

Basically, the acceptor solution should be compatible with the LC–MS system, and it should provide a strong proton gradient from the acceptor solution to the sample. The protons release the analytes at the liquid membrane/acceptor interface by counter-ion exchange, and are co-transported through the liquid membrane and into the sample solution. Carrier mediated transport, therefore, leads to a continuous loss of protons from the acceptor solution to the sample solution as the analytes are enriched in the acceptor solution. A sufficiently large excess of protons in the acceptor solution is necessary both to provide extraction and to prevent back extraction of analytes as ion-pairs with the carrier. In addition, the analytes are highly soluble in the acidic acceptor solution were they are trapped as salts.

In order to study different acids as proton sources, both mineral acids and organic acids were tested as acceptor solution. Acceptor solutions made of 10, 50, and 100 mM of HCl, 50 mM H_2SO_4, 50 mM HNO_3, 100 mM HCOOH, 100 mM CH_3COOH and 50 mM trifluoroacetic acid were evaluated. Extraction recoveries of the model drugs with different acceptor phases are presented in Table 2. Acceptor phases made of relatively high concentrations of the strong mineral acids such as 50 mM HCl, 100 mM HCl, 50 mM HNO_3, and 50 mM H_2SO_4 provided the highest analyte recoveries. On the other hand, low concentrations of the strong acids and all solutions with the week organic acids provided significantly lower extraction recoveries. This observation is in accordance with the theory that strong proton gradients is an important driving force in carrier mediated LPME.

Unfortunately, the most LC–MS friendly acceptor solutions consisting of either HCOOH or CH_3COOH provided the lowest recoveries. To maximize recovery, it was decided to continue with 50 mM HCl as the acceptor solution. Since it was undesirable to inject 50 mM HCl directly into the LC–MS

Table 1
Effect of carrier addition in LPME

Additions to 50 μl of sample solution	Recovery (%)*						
	Amphetamine	Phenylpropanolamine	Cimetidine	Morphine	Sotalol	Atenolol	Practolol
Extraction from pure standard solutions							
50 μl of 0.25 mM NaOH, pH 13*	82	16	16	15	5	3	6
50 μl of 25 mM phosphate buffer, pH 7**	19	17	18	19	6	3	5
50 μl of 50 mM sodium octanoate in 25 mM phosphate buffer***, pH 7	78	65	73	59	29	16	39
Extraction from human plasma							
50 μl of 50 mM sodium octanoate in 25 mM phosphate buffer***, pH 7	58	52	58	49	26	14	37

The solution (50 μl) containing 500 ng/ml of each analyte was added to (*), (**), and (***). Extraction time: 60 min; organic membrane: 1-octanol; acceptor phase: 50 mM HCl; $n = 4$; R.S.D. < 15%.

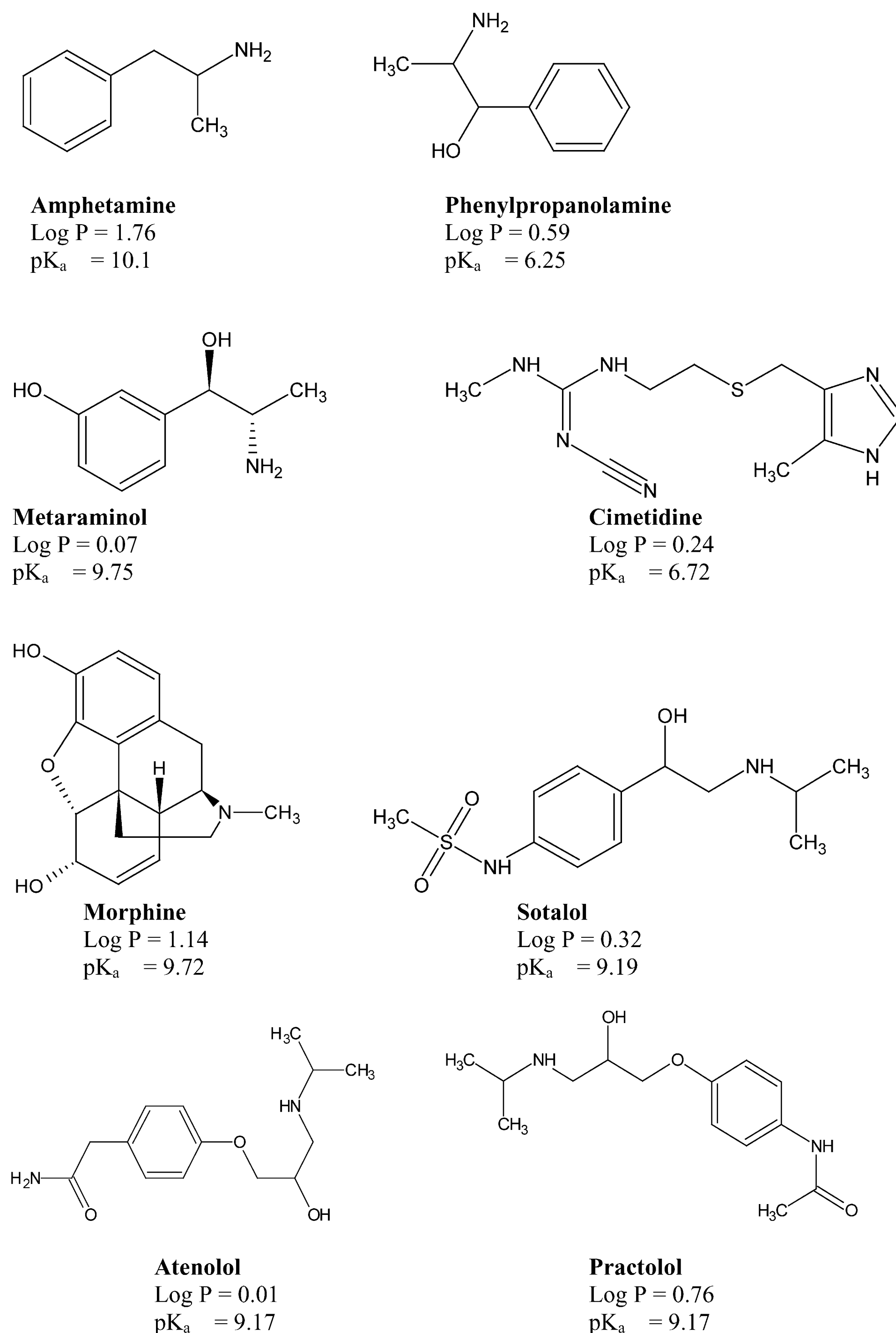

Fig. 2. Structure, pK_a and log P of the tested substances.

system, each 20 µl of acceptor solution was diluted with 40 µl of 10 mM ammonium acetate before the final analysis.

3.2. Sample clean-up considerations of carrier mediated LPME

In several previous LPME publications [4–9], extractions were performed from different biological fluids such as urine, plasma, whole blood and human milk. These reports showed that LPME without carriers provided high sample clean-up from the different biological matrices. In our first report on carrier mediated LPME, the results from CE-analysis indicated that high sample clean-up also was accomplished when a carrier is added to the LPME system. However, CE is not very sensitive, and the results were not compared with alternative sample preparation methods. Therefore, the

Table 2
Extraction recovery utilizing different acceptor solutions

Acceptor solution (20 μl)	Recovery (%)						
	Amphetamine	Phenylpropanolamin	Cimetidine	Morphine	Sotalol	Atenolol	Practolol
10 mM HCl	34	47	35	44	21	11	31
50 mM HCl	78	65	57	59	29	16	39
100 mM HCl	76	68	59	57	30	14	42
50 mM H_2SO_4	77	68	60	56	30	18	46
50 mM HNO_3	71	60	52	54	28	16	41
50 mM TFA	8	10	9	9	15	11	12
400 mM HCOOH	10	10	9	8	7	4	11
400 mM CH_3COOH	5	7	6	9	5	2	6

The solution (50 μl) containing 500 ng/ml of each analyte was added 50 μl of 25 mM phosphate buffer pH 7.0. Extraction time: 60 min; organic membrane: 1-octanol; $n = 4$; R.S.D. < 15%.

sample clean-up aspects of carrier mediated LPME was further investigated in this report with LC–MS. Blank human plasma extracts obtained by carrier mediated LPME were analyzed by full scan LC–MS, and these were compared with total ion current (TIC) signals from conventional protein precipitation of blank human plasma. Fig. 3A shows the TIC chromatogram of full scan LC–MS obtained from injection of pure water. Fig. 3B and C demonstrate similar chromatograms obtained by carrier mediated LPME and protein precipitation of blank human plasma, respectively. The same scales for signal intensity and time were used for the three chromatograms. The plasma extract achieved from carrier mediated LPME provided significantly higher sample clean-up than protein precipitation. From the chromatogram obtained by protein precipitation (Fig. 3C), a high abundance of matrix components eluted as a broad peak before 4 min. Some of the polar model compounds eluted in the same area of the chromatogram, and ion suppression from the matrix may seriously affect their analyte signals. On the other hand, from the chromatogram obtained by carrier mediated LPME, the abundance of matrix components was significantly lower, although the base line level was slightly elevated as compared to the injection of pure water. The experiment suggested that the excellent sample clean-up properties of LPME may be preserved although a carrier is added to the system.

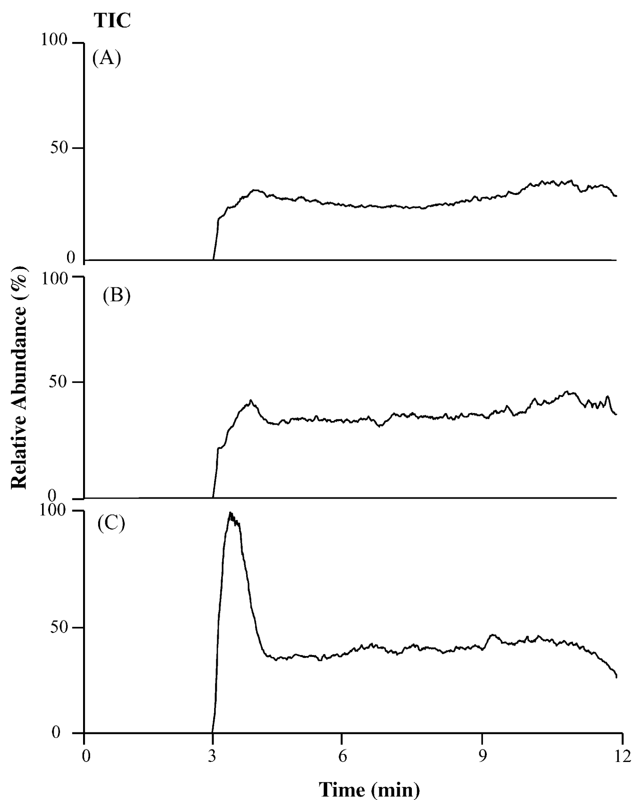

Fig. 3. Full scan MS (m/z 50 − 500) of pure water (A), blank extract after carrier mediated LPME from human plasma (B), and blank after protein precipitation from human plasma (C).

3.3. Validation of carrier mediated LPME

For the first time, a full validation of carrier mediated LPME was performed. The validation was performed for atenolol and cimetidine, and sotalol was utilized as internal standard. Fig. 4A and B show the representative chromatograms obtained by SIM after carrier mediated LPME from human plasma of cimetidine and atenolol. The results are summarized in Table 3. Extraction standard curves were constructed using six concentration levels in spiked human plasma, ranging from 25 to 1500 ng/ml for atenolol, and from 50 to 3500 ng/ml for cimetidine. These concentration ranges covered the typical therapeutic concentrations of atenolol (100–1000 ng/ml) and of cimetidine (250–3000 ng/ml) in plasma [16]. As shown in Table 3, the plasma standard curve for atenolol was found to be linear in the range of 25–1500 ng/ml with a correlation coefficient of 0.992, and the plasma standard curve for cimetidine was found to be linear in the range of 50–3500 ng/ml with a correlation coefficient 0.976. The limits of quantification (LOQ) were calculated at a signal to noise ratio of 5 [17]. The LOQs of atenolol and cimetidine were found to be 25–50 ng/ml, respectively. These LOQ values were well below the therapeutic concentrations normally found in patient samples, and demonstrated that carrier mediated LPME from only 50 μl of human plasma may be sufficient when the technique is combined with LC–MS.

Table 3
Validation data of atenolol and cimetidine from human plasma sample

Drug	Concentration (ng/ml)	Intra-day precision R.S.D. (%, $n = 6$)	Inter-day precision R.S.D. (%, $n = 18$)	Accuracy (%, $n = 6$)	LLOQ (ng/ml, S/N > 5)	Linear range (ng/ml)	r
Atenolol	25	17.2	19.4	89.1	25	25–1500	0.992
	250	10.7	15.0	99.6			
	1000	7.8	6.7	95.9			
Cimetidine	50	9.5	20.3	83.4	50	50–3500	0.976
	500	14.1	13.5	86			
	2000	12.7	7.7	86.8			

Sotalol as internal standard.

Table 4
Extraction recovery from different human plasma samples

Sample	Recovery (%)						
	Amphetamine	Phenylpropanolamin	Cimetidine	Morphine	Sotalol	Atenolol	Practolol
Plasma 1	64	56	52	40	24	14	38
Plasma 2	57	52	43	46	20	12	32
Plasma 3	60	48	50	52	25	13	33
Plasma 4	54	49	46	46	21	15	34
Plasma 5	52	53	41	56	19	11	31
Plasma 6	45	41	48	49	21	16	39
Plasma 7	55	46	52	46	19	13	34
Plasma 8	53	50	42	50	20	11	32
Plasma 9	66	44	39	47	28	17	40
Plasma 10	59	55	41	42	21	13	43
Plasma Ullevål	58	52	58	49	26	14	37
R.S.D.(%)	7.8	7.4	10.6	8.4	11.7	9.3	8.6

The solution (50 μl) containing 500 ng/ml of each analyte (plasma) was added 50 μl 25 mM phosphate buffer pH 7.0. Extraction time: 60 min; organic membrane: 1-octanol; acceptor phase: 50 mM HCl; $n = 4$; R.S.D. < 15%.

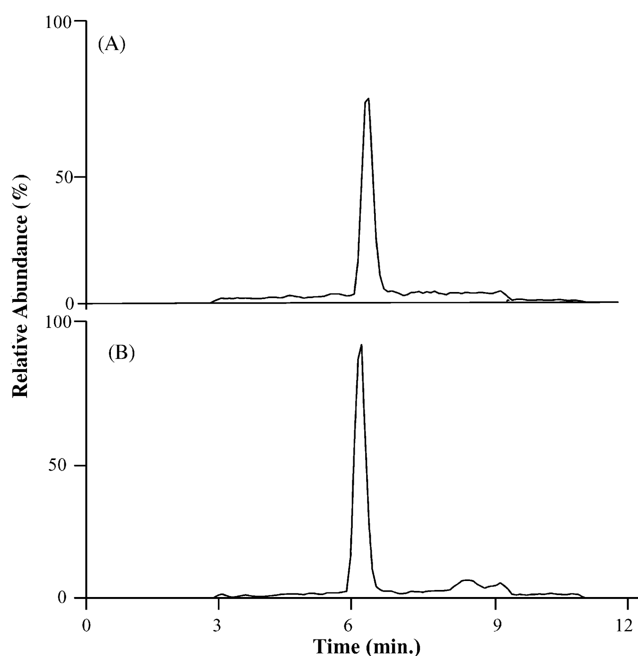

Fig. 4. The representative chromatograms obtained by SIM after carrier mediated LPME from human plasma spiked with 500 ng/ml cimetidine (A) and 250 ng/ml atenolol (B).

Inter-day precision and accuracy were both studied at three concentration levels and with six replications for each, and intra-day precision was carried out on three consecutive days at three concentration levels with eighteen replications each. As can be seen from Table 3, the intra- and inter-day precision for atenolol in plasma were in the range 7.8–17.2% R.S.D. and 6.7–19.4% R.S.D., respectively. The intra- and inter-day precision for cimetidine in plasma were in the range 9.5–14.1% R.S.D. and 7.7–20.3% R.S.D., respectively. The accuracy for atenolol and cimetidine from plasma sample were in the range 89.1–99.6% and 83.4–86%, respectively. The validation data were complied with regulatory requirements [17], although slightly better results may be expected from traditional extraction techniques like LLE and SPE. This may be due to two reasons; the sample size was only 50 μl and all extractions were made with home-built extraction units. Thus, a small increase in sample size and development of commercial equipment with narrow specifications in terms of fibre characteristics most probably will give excellent validation data in the future.

3.4. Test for matrix effects in carrier mediated LPME

Plasma samples consists of a large number of endogenous components [18], and some compositional variations may

be expected from person to person. In order to be a robust extraction technique, extraction recoveries should not be affected significantly by these matrix variations, and this was studied in the experiment summarized in Table 4. Eleven different drug-free plasma samples were spiked with the seven model drugs to an individual concentration of 500 ng/ml. Each plasma sample was extracted in three replicates by carrier mediated LPME, and recovery values from sample to sample were compared. As demonstrated in Table 4, extraction recoveries from sample to sample varied within the range 7.4–11.7%. These variations were considered to be within the experimental inaccuracies of the set-up taking the validation into consideration, and demonstrated that carrier mediated LPME of the selected model drugs was not sensitive to matrix variations of human plasma samples.

4. Conclusion

In the present work, we have for first time combined carrier mediated liquid-phase micro extraction (LPME) with liquid chromatography–mass spectrometry for the determination of polar drugs in a single drop of human plasma. Carrier mediated LPME was found to provide acceptable extraction recoveries and excellent sample clean-up from human plasma samples. Validation data with the current extraction equipment, which was home-built, were fully acceptable, and due to a combination of high recoveries of carrier mediated LPME and excellent sensitivity of LC–MS, 50 μl of human plasma was sufficient to determine the selected model drugs in their therapeutically relevant concentrations. In addition, carrier mediated LPME was found not to be sensitive to matrix variations from different human plasma samples. In conclusion, the present work has demonstrated that carrier mediated LPME may be a new extraction technique in future drug analysis. As a consequence of this, work is in progress to characterize the extractability of more polar drugs and to establish a more detailed theoretical understanding of carrier mediated LPME.

References

[1] E. Psillakis, N. Kalogerakis, Trends Anal. Chem. 21 (2002) 53.
[2] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 69 (1997) 235.
[3] E. Psillakis, N. Kalogerakis, J. Chromatogr. A 938 (2001) 113.
[4] S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chem. 71 (1999) 2650.
[5] T.G. Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 909 (2001) 87.
[6] A. Bjørhovde, T.G. Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chim. Acta 491 (2003) 155.
[7] H.G. Ugland, M. Krogh, K.E. Rasmussen, J. Chromatogr. B 749 (2000) 85.
[8] T.S. Ho, S. Pedersen-Bjergaard, K.E. Rasmussen, Analyst 127 (2002) 608.
[9] T. Kuuranne, T. Kotiaho, S. Perdersen-Bjergaard, K.E. Rasmussen, A. Leinonen, S. Westwood, R. Kostiainen, J. Mass Spectrom. 38 (2003) 16.
[10] G. Audunsson, Anal. Chem. 58 (1986) 2714.
[11] G. Nilvé, G. Audunsson, J.Å. Jönsson, J. Chromatogr. 471 (1989) 151.
[12] J.Å. Jönsson, L. Mathiasson, Trends Anal. Chem. 11 (1992) 106.
[13] J.Å. Jönsson, L. Mathiasson, J. Chromatogr. A 902 (2000) 205.
[14] J.Å. Jönsson, L. Mathiasson, J. Sep. Sci. 24 (2001) 495.
[15] T.S. Ho, T.G. Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 998 (2003) 61.
[16] M. Schulz, A. Schmoldt, Pharmazie 52 (1997) 12.
[17] FDA Guidance for Industry: Bioanalytical Methods Validation for Human Studies, Center for Drug Evaluation and Research, US Department of Health and Human Services, December 1998.
[18] A.J. Vander, J.H. Sherman, D.S. Luciano, Human Physiology, sixth ed., 1994, p. 396.