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Review

Bioanalysis of drugs by liquid-phase microextraction coupled to separation techniques

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Abstract

The demand for automation of liquid–liquid extraction (LLE) in drug analysis combined with the demand for reduced sample preparation time has led to the recent development of liquid-phase microextraction (LPME) based on disposable hollow fibres. In LPME, target drugs are extracted from aqueous biological samples, through a thin layer of organic solvent immobilised within the pores of the wall of a porous hollow fibre, and into an μl volume of acceptor solution inside the lumen of the hollow fibre. After extraction, the acceptor solution is subjected directly to a final analysis either by high performance liquid chromatography (HPLC), capillary electrophoresis (CE), mass spectrometry (MS), or capillary gas chromatography (GC) without any further treatments. Hollow fibre-based LPME may provide high enrichment of drugs and excellent sample clean-up, and probably has a broad application potential within the area of drug analysis. This review focuses on the principle of LPME, and recent applications of three-phase, two-phase, and carrier mediated LPME of drugs from plasma, whole blood, urine, and breast milk.

Keywords: Liquid-phase microextraction; Hollow fibres; Drug analysis; Plasma; Whole blood; Urine; Breast milk

Contents

1. Introduction

During the last 10 years, some interest has been focused on the miniaturising of analytical liquid–liquid extractions (LLE). The major idea behind this has been to facilitate automation, to speed up extractions, and to reduce the consumption of organic solvents. Miniaturised liquid–liquid extraction, or liquid-phase microextraction (LPME), was first introduced in 1996, and was based on a droplet of organic solvent hanging at the end of a micro syringe needle [1–4]. The organic micro droplet was placed into the aqueous sample, and the analytes were extracted into the organic droplet (micro extract) based on passive diffusion. Following extraction, the organic droplet was withdrawn into the syringe, the syringe was transferred to a capillary gas chromatograph (GC), and the micro extract was injected into the GC. In addition, LPME was performed in a three-phase system where ionic analytes in their neutral form were extracted from aqueous samples, through a thin layer of an organic solvent on the top of the sample, and into an aqueous micro droplet (micro

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extract) placed at the tip of a micro syringe [5,6]. In the latter, pH was selected to ionise the analytes to maximise partition coefficients and to prevent back-extraction into the organic phase again. In the three-phase system providing an aqueous micro extract, high performance liquid chromatograph (HPLC) was typically used in the final chromatographic analysis.

In both two- and three-phase LPME based on hanging droplets, high preconcentration may be achieved for analytes with high partition coefficients because they are transferred by passive diffusion from a relatively large sample volume (1–5 ml) and into a micro extract of typically 5–50 μl. In addition, the consumption of organic solvent is low, and especially in the three-phase mode including two simultaneous extractions, excellent clean-up has been observed even from biological samples. Unfortunately, LPME based on hanging droplets is not very robust [7], and the droplets may be lost from the needle tip of the syringe during extraction. This is especially the case when samples are stirred effectively to speed up the extraction process.

In order to develop a more robust format for LPME, Pedersen-Bjergaard and Rasmussen recently introduced an alternative concept for LPME based on the use of disposable low-cost porous hollow fibres made of polypropylene [8–24]. In this LPME device, the micro extract is contained within the lumen of a porous hollow fibre, and consequently, the micro extract is not in direct contact with the sample solution. Analytes are extracted through an organic liquid immobilised within the pores of the hollow fibre before they are trapped in the protected micro extract. Samples may be stirred or vibrated effectively without any loss of micro extract into the sample solution. Thus, hollow fibre-based LPME is a more robust and reliable alternative for LPME. The chemistry of hollow fibre-based LPME is similar to the chemistry used for extraction with supported liquid membranes (SLM) [25–29], but the techniques differ significantly in terms of instrumentation and operation. SLM is a flowing system with a pump, which continuously feed the membrane with fresh sample. Thus, SLM is an instrumental sample preparation technique, and each membrane is normally used for a large number of extractions. On the other hand, in hollow fibre-based LPME, both the sample and the extracting phase are stagnant, the membrane (hollow fibre) is used only for a single extraction, and no instrumentation like pumps are required for the sample processing. Thus, with LPME, a large number of samples may be processed simultaneously for instance in a 96-well system.

In the period from 1999 since the first publication on hollow fibre-based LPME, a few other groups have worked with related concepts in the field of drug analysis [7,19,30–32], and these efforts are reviewed in the present paper together with the work carried out in the authors laboratory. The review is focused on the different extraction principles, on applications of three-phase, two-phase, and carrier-mediated LPME within drug analysis, and on future directions of this promising sample preparation technique.

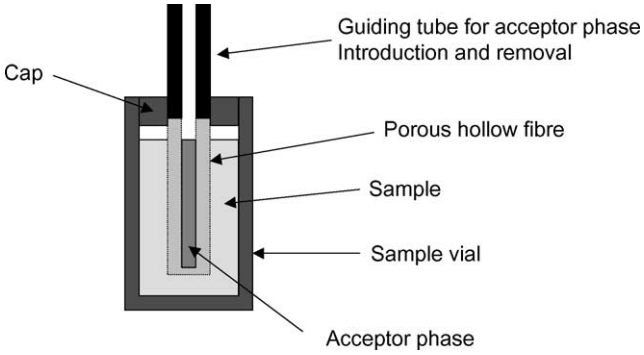

Fig. 1. Principle of LPME.

2. Principle

The basic principle of hollow fibre-based LPME is illustrated in Fig. 1, demonstrating the latest technical set-up used in the authors laboratory. The aqueous sample is filled into a sample vial, and a piece of a porous polypropylene hollow fibre is placed within this sample. The bottom end of the hollow fibre is closed, and the top of the fibre is connected to a guiding tube for a micro syringe to introduce and remove the acceptor phase from the lumen of the fibre. The volume of aqueous sample is typically within 100 μl to 4 ml depending on the application, and the length of the hollow fibre is normally 1.5–8 cm. Before extraction, the hollow fibre has been soaked in an organic solvent to immobilise the solvent in the pores of the wall of the hollow fibre (organic phase), and the lumen of the fibre has been filled with acceptor solution from a micro syringe. Excess solvent on the outside of the fibre has been removed by ultra-sonification. The solvents used as organic phase are immiscible with water and of low volatility to ensure that it remains within the pores during extraction with no leakage to the biological samples. The organic solvent forms a thin layer within the wall of the hollow fibre, which typically has a thickness of 200 μm, and the total volume of organic solvent immobilised in the fibre wall is typically in the range 15–20 μl. For acidic and basic analytes, pH within the sample is adjusted to a value where they are deionised to improve their extractability into the organic phase. During extraction, the fibre is placed in the sample solution within the sample vial. The analytes are transferred by passive diffusion from the aqueous sample, through the organic phase in the pores of the hollow fibre, and further into the acceptor solution placed inside the lumen of the hollow fibre. To speed up this process, extensive agitation or stirring of the sample is applied. After extraction, the acceptor solution is collected by a micro syringe and directly transferred to a chromatographic or electrophoretic system. The acceptor solution may be the same organic solvent as immobilised in the pores of the wall, resulting in a two-phase system where the analyte (A) is collected in an organic phase:

$$A_{Sample} \leftrightarrow A_{Organic\ acceptor}$$

As discussed in more detail later, two-phase LPME may be applied for analytes with a high solubility in non-polar organic solvents. The acceptor solution may be directly analysed with capillary GC, or may be evaporated and reconstituted in an aqueous medium for injection in HPLC or CE.

Alternatively, the acceptor solution may be another aqueous phase providing a three-phase system, where the analytes (A) are extracted from an aqueous sample, through the thin film of organic solvent in the wall of the hollow fibre, and into an aqueous acceptor solution:

$$A_{Sample} \leftrightarrow A_{Aqueous\ acceptor} \leftrightarrow A_{Organic\ acceptor}$$

This extraction mode is limited to basic or acidic analytes with ionisable functionalities. For the extraction of basic compounds, pH in the sample has to be adjusted into the alkaline region to promote their extraction into the organic phase, whereas pH in the acceptor solution should be low to promote high extraction efficiency from the organic phase and into the acceptor phase. For acidic analytes in contrast, pH in the sample should be low and an alkaline acceptor solution should be utilised within the lumen of the fibre. Following extraction, the aqueous acceptor solution is directly injectable in HPLC or CE without any further treatments.

The above mentioned two- and three-phase LPME systems are both based on passive diffusion where extraction requires high partition coefficients from the sample an into the acceptor phase. However, for highly polar analytes, partition coefficients into water immiscible organic solvents are low, and consequently their extractability in two- and three-phase LPME is very poor. In these situations, hollow fibre-based LPME may be accomplished in a carrier-mediated mode [23,24], where a carrier is added to the sample solution as illustrated in Fig. 2. The carrier, which is a relatively hydrophobic ion-pair reagent providing acceptable water solubility, forms ion-pairs with the analytes followed by extraction of the ion-pair complexes into the organic phase in the pores of the hollow fibre. In the contact region of the organic phase and the acceptor solution, the analytes are released from the ion-pair complex into the acceptor solution, whereas

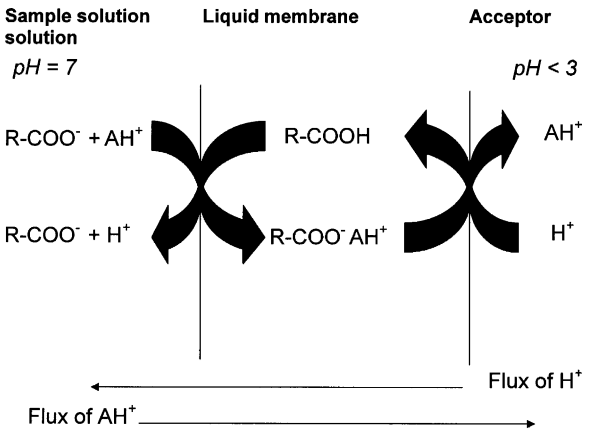

Fig. 2. Principle of carrier-mediated LPME.

counter-ions 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, form ion-pair with a new analyte molecule, and the cycle is repeated. For basic analytes, the carrier may typically be a carboxylic acid with an appropriate hydrophobic moiety (like octanoic acid), pH in the sample is adjusted to ensure that the analytes are present in their ionised state, and pH in the acceptor solution is low to ensure that (1) the carrier is not trapped within this phase and (2) a sufficient amounts of protons are present to serve as counter-ions.

3. LPME based on three-phase extractions

The different drugs extracted by three-phase LPME are summarised in Table 1. The first report on hollow fibre-based LPME utilised three-phase extraction with methamphetamine as a model drug [8]. This paper showed for the first time that extraction of drugs from complicated biological samples through an organic film in the wall of a hollow fibre and into an acceptor phase was possible in a totally stagnant system. Surprisingly, extraction times were relatively short (<45 min) and recoveries were high taking the high phase ratio into account; the analytes were extracted from 2.5 ml of biological sample and into only 25 μl of acceptor phase. The paper suggested a new and reliable solution to LPME, and served as the basis for the rest of the work reported in this review. Extractions were performed from 2.5 ml volumes of both plasma and urine. Three different solvents (*n*-octanol, 2-octanone, and dihexyl ether) were tested as organic phase. These are not typical solvents for liquid extraction, but they all provided a relatively low polarity and low volatility, which are important in LPME to ensure high stability within the pores of the fibre. Thus, all the solvents remained immobilized within the fibre during extraction, with no apparent loss into the sample solution. *n*-Octanol was preferred because it provided the highest recovery for methamphetamine. The experiment with the different solvents highlighted an important issue in three-phase LPME; extraction performance depends on the organic phase, since both recoveries and extraction speed is affected by the partition coefficients from the sample to the organic phase, and from the organic phase to the acceptor phase. As acceptor phase, 25 μl of 0.1 M HCl was used; also the acceptor phase chemistry affected the extraction performance, and lower concentrations of HCl resulted in lower recovery, whereas higher HCl concentrations were avoided due to compatibility problems with CE used for subsequent separation purposes. For the sample, addition of NaOH to a final concentration of 0.1 M was found to be optimal. This served to effectively deionise methamphetamine within the biological samples. In order to speed up extractions, stir bars were added to each sample, and stirring was conducted at 400 rpm. In subsequent work, however, this procedure has been replaced with strong vibration or shaking for conve-

Table 1
Application overview for three-phase LPME of drugs from human biological samples

Compound	Sample	Organic phase	Acceptor phase	Extraction time (min)	Reference
Aminophenyl ethanol	Urine	*n*-octanol	0.1 M HCl	50	[30]
Amitriptyline	Whole blood	Dodecyl acetate	0.2 M HCOOH	30	[22]
Amphetamine	Whole blood, urine	Dihexyl ether	0.01 M HCl	15–30	[14,18]
Atenolol	Urine	*n*-octanol	0.1 M HCl	50	[30]
Citalopram	Plasma	Dihexyl ether	Phosphate pH 2.75	15–45	[12,13,20]
	Whole blood	Dihexyl ether	0.1 M HCl	30	[13,22]
	Breast milk	Siloxane[a]	0.01 M HCl	60	[21]
Clomipramine	Whole blood	Dodecyl acetate	0.2 M HCOOH	30	[22]
Doxepine	Whole blood	Dodecyl acetate	0.2 M HCOOH	30	[22]
Fluoxetine	Whole blood	Dodecyl acetate	0.2 M HCOOH	30	[22]
Fluvoxamine	Breast milk	Siloxane[a]	0.01 M HCl	60	[21]
	Whole blood	Dodecyl acetate	0.2 M HCOOH	30	[22]
Haloperidol	Plasma, urine	Dihexyl ether	0.01 M HCl	15–45	[16,18]
Ibuprofen	Urine	Dihexyl ether	0.1 M NaOH	45	[9]
Ketoprofen	Urine	Dihexyl ether	0.1 M NaOH	45	[9]
MBDB[b]	Whole blood, urine	Dihexyl ether	0.01 M HCl	15	[14]
MDA[c]	Whole blood, urine	Dihexyl ether	0.01 M HCl	15	[14]
MDEA[d]	Whole blood, urine	Dihexyl ether	0.01 M HCl	15	[14]
MDMA[e]	Whole blood, urine	Dihexyl ether	0.01 M HCl	15	[14]
Methadone	Plasma, urine	Dihexylether	0.01 M HCl	15–45	[16,18]
Methamphetamine	Plasma, urine	*n*-octanol	0.1 M HCl	30–45	[8,10,13]
	Whole blood	Dihexyl ether	0.1 M HCl	15–30	[13,14]
Mianserin	Plasma	Dihexyl ether	0.01 M HCl	45	[17]
	Breast milk	Siloxane[e]	0.01 M HCl	60	[21]
	Whole blood	Dodecyl acetate	0.2 M HCOOH	30	[22]
Naproxen	Urine	Dihexyl ether	0.1 M NaOH	45	[9,10]
Norephedrine	Urine	*n*-octanol	0.1 M HCl	50	[30]
Paroxetine	Breast milk	Siloxane[e]	0.01 M HCl	60	[21]
	Whole blood	Dodecyl acetate	0.2 M HCOOH	30	[22]
Pethidine	Plasma	Dihexyl ether	0.01 M HCl	15–30	[18]
Pindolol	Urine	*n*-octanol	0.1 M HCl	50	[30]
Promethazine	Plasma, urine	Dihexylether	0.01 M HCl	15–45	[16,18]
Steroid glucuronides	Urine	*n*-octanol	0.25 M NH_3	60	[19]
Trimipramine	Whole blood	Dodecyl acetate	0.2 M HCOOH	30	[22]

[a] Polyphenyl-methylsiloxane.
[b] *n*-methyl-1-(3,4-methylene-dioxyphenyl)-2-butanamine.
[c] 3,4-methylenedioxyamphetamine.
[d] 3,4-methylenedioxyethylamphetamine.
[e] 3,4-methylenedioxymethamphetamine.

nience and in order to avoid potential contamination of the samples from the stir bars. With the optimised conditions, the recovery for methamphetamine was approximately 75% both from plasma and urine after 45 min of extraction, and with a phase-ratio between sample volume and acceptor phase volume of 100, methamphetamine was enriched by a factor of 75 in both cases. In addition to this, very clean extracts were observed both from plasma and urine, with only a few other peaks found by CE with UV-detection at 200 nm. This is illustrated in Fig. 3 for drug free urine and urine spiked with methamphetamine. The three-phase nature of the system tuned for basic compounds combined with a high volume ratio between sample and acceptor solution (discussed below) was the principal reasons for the excellent sample clean-up.

In a subsequent paper, attention was switched to acidic drugs [9]. The nonsteroid anti-inflammatory drugs ibuprofen, naproxen, and ketoprofen were selected as model compounds, and extractions were performed from urine samples. Again, dihexyl ether, *n*-octanol, and 2-octanone were evaluated as organic membranes, with the former providing the highest recoveries for this class of compounds. For the acceptor phase, 0.01 M NaOH was found to be sufficient when extracting from pure water samples. However, from urine, recoveries were lower, and an increased level of NaOH did not improve recoveries. However, by addition of 25% methanol to 0.01 M NaOH, recoveries became comparable with water extractions. Extraction of acidic drugs from urine revealed that several other compounds were co-extracted, and the addition of methanol served to improve the solubility capacity of the acceptor solution. In a similar manner, extractions from water required only addition of HCl to a level of 0.1 M in the sample, whereas urine samples required a 1 M HCl level to effectively extract the acidic drugs. Under optimised conditions, naproxen was extracted with a 82% recovery from urine. Thus, the paper concluded that also acidic drugs may be successfully extracted by three-phase LPME. This con-

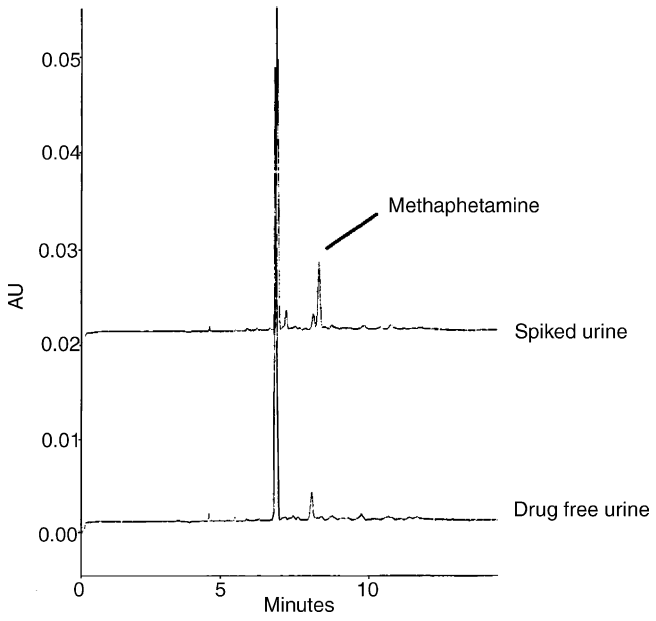

Fig. 3. Three-phase LPME and CE–UV of 100 ng/ml of methamphetamine in human urine. Reprinted with permission [8].

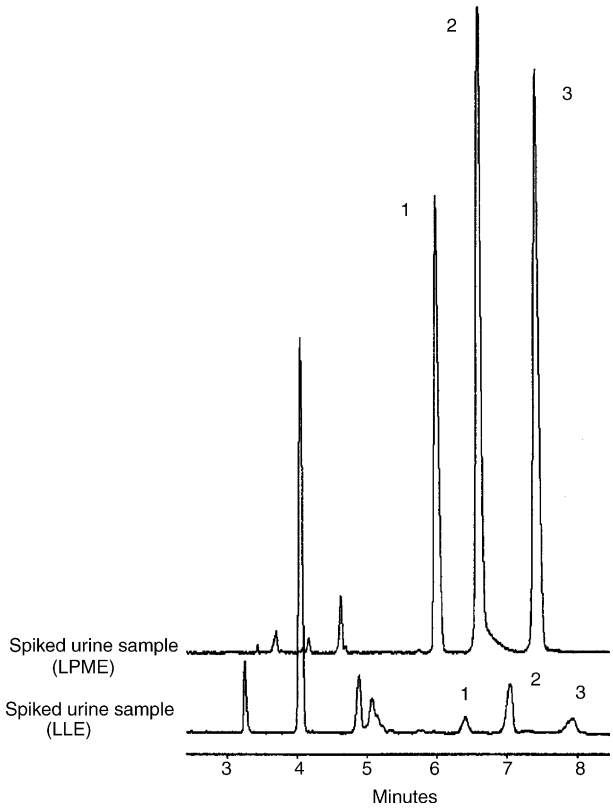

Fig. 4. Three-phase LPME (upper electropherogram) and three phase LLE (lower electropherogram) combined with CE–UV of 100 ng/ml of promethazine (peak 1), methadone (peak 2), and haloperidol (peak 3) in human urine. Reprinted with permission [16].

clusion has been supported by a recent study on three-phase LPME of different steroid glucuronides from urine [19]. In this case, *n*-octanol was selected as the organic phase, and the acceptor solution was 0.25 M NH_3 containing 20% (v/v) methanol. NH_3 was selected in order to be compatible with LC–MS, whereas methanol was added to avoid capacity problems in the acceptor phase.

Because three-phase LPME may be an alternative to traditional LLE, a fundamental study was carried out comparing the two techniques in terms of recovery, enrichment, and sample clean-up [16]. It was found that LPME was much more sensitive to the magnitude of partition coefficients than LLE, because LPME is carried out with a very high volume ratio between sample and acceptor solution. Thus, whereas LLE may be accomplished with relatively large volumes of solvent to compensate for poor partition coefficients, LPME suffered from low recoveries either if the partition coefficient from the sample to the organic phase, or the partition coefficient from the organic phase to the acceptor phase, was low. The application area of three-phase LPME is therefore inferior compared to LLE, but for good three-phase LPME candidates, preconcentration values are much higher in LPME than LLE. In addition, because three-phase is more selective in nature, it also provides higher sample clean-up that LLE. Even in comparison with LLE with back-extraction, which itself is known to be a very efficient clean-up from biological samples, three-phase LPME is superior as illustrated in Fig. 4 for the extraction of promethazine, methadone, and haloperidol from urine. The limitations of the application area for three-phase LPME may be regarded as a disadvantage, but, as we will discuss later, it is complemented both by two-phase LPME and by carrier-mediated LPME, which all together may cover a very broad range of compounds within the future.

Two publications focused on the extraction of the antidepressant drug citalopram [12,13]. In addition to adding a new compound to the list of drugs extracted by three-phase LPME, the papers addressed several important fundamental issues. In both papers, dihexyl ether was utilised as the organic phase for optimal recovery. As acceptor phase, both 0.1 M HCl and 0.02 M phosphate buffer pH 2.75 were utilised. Their extraction performance was almost equal, and this was further supported by a paper dedicated to the development of acceptor phases [15]; both phosphate buffers with a high buffer capacity and solutions of strong acids with a very low pH provides a strong acceptor phase for extraction of basic drugs. Validation was carried out for citalopram from plasma samples utilising an internal standard close in structure to the antidepressant, and this revealed that calibration graphs were linear in the range 20–1000 ng/ml, and within-day repeatability was better than 11% RSD. The validation data were acceptable even if the extraction units were prepared manually. Two interesting aspects were tested in connection with citalopram; first, extractions were even performed with high success from whole blood samples, and secondly, extraction speed was improved utilising a longer fibre with a reduced internal diameter. The first experiment was a major surprise and revealed

that three-phase LPME was directly compatible even with whole blood samples. Thus, the only pre-extraction step was to adjust pH by the addition of NaOH. This should preferably be done by a relative large volume of NaOH solution to simultaneously reduce the viscosity of the sample since the latter influence on the extraction speed. The excellent compatibility with whole blood is a very attractive feature, which is not shared by sample preparation techniques like solid-phase extraction (SPE) and solid-phase micro extraction (SPME). The second experiment revealed that the extraction speed in LPME is dependant on the surface area of the fibre, and this should be maximised as much as practically possible in future constructions of LPME equipment.

Based on the above mentioned fact that three-phase LPME of basic drugs from plasma resulted in very clean extracts, it was questioned if the technique may be directly interfaced with a mass spectrometer in a flow injection system, eliminating a time-consuming chromatographic or electrophoretic separation. This was evaluated in a paper utilising different amphetamines as model drugs, and both whole blood and urine were evaluated as sample matrices [14]. The amphetamines were extracted for only 15 min from alkaline samples through dihexyl ether as the organic phase and into 25 μl of 0.01 M HCl as the acceptor phase. Subsequently, the acceptor phase was directly injected into a flow-injection system interfaced with an atmospheric-pressure chemical ionisation MS, where the analysis of each micro extract was completed within 1 min. Because of excellent sample clean-up, serious ion suppression effects were not observed although the micro extract entered the mass spectrometer as a small plug without any separation. Several of the amphetamines were identified correctly in unknown whole blood and urine samples even down to the low ng/ml level. Quantitative measurements were not included in the paper, but most probably, some chromatographic separation is needed in order to provide highly reliable quantitative data.

Most of the three-phase LPME extractions discussed above included extraction times of typically 30–45 min. The reason for these relatively long extraction times has been to ensure extraction equilibrium where no further gain in analyte recovery is obtained following prolonged extraction times. For many applications, 30–45 min of extraction is acceptable since a large number of samples may be extracted simultaneously. However, it may be relevant to reduce extraction times in some cases, and in one paper three-phase LPME was investigated under non-equilibrium conditions [18]. With amphetamine, pethidine, prometazine, methadone, and haloperidol as model compounds, comparison was made between 15 and 45 min extractions from plasma. In general, extraction recoveries were about 30% higher after 45 min of extraction than for 15 min, but validation data on linearity and precision were almost comparable for the two different extraction times. Thus, with a 30% decrease in the analytical signal as the only disadvantage, rapid extractions may be performed under non-equilibrium conditions apparently without loss of performance. In addition to this important finding, the paper also discussed the aspects of strong drug–protein interactions in plasma samples. For some drugs, the pre-extraction change of pH to deionise the analytes was sufficient to suppress protein interactions, and high recoveries comparable with those from pure water samples were obtained without any further efforts. However, for some drugs, addition of small (5% v/v) or large (50% v/v) amounts of methanol to plasma samples was required to effectively suppress the protein interactions. For method development, this has to be tested for the compounds of interest since no clear correlation between reported protein-binding values and the need for methanol was observed.

In the papers discussed so far in this review, attention was focused on urine, plasma, and whole blood samples, and all of these matrices were directly compatible with three-phase LPME. As mentioned earlier, the only pre-treatment of these samples was to adjust pH. To evaluate more biological matrices, three-phase LPME from breast milk has also been reported with paroxetine, fluvoxamine, mianserin, and citalopram as model compounds [21]. Following a simple pH-adjustment to deionise the analytes, very low recoveries were obtained, and this was attributed to strong interactions between the drugs and lipid as well as proteins present in the milk. Because the content of lipid and proteins may vary significantly from milk sample to milk sample, also the recoveries varied significantly, complicating quantitative analysis considerably. Therefore, it was found that removal of the lipid-layer on the top of the milk samples was required in order to eliminate recovery variations from sample to sample. In conclusion, breast milk seems to be the only biological matrix of the four tested so far requiring pre-extraction treatments.

Most three-phase LPME publications have involved capillary electrophoresis (CE) as the final method of analysis. However, in a recent publication, the technique was evaluated in combination with LC–MS [22], which is becoming the standard analytical tool in many laboratories working with drug analysis. In combination with LC–MS, an acceptor phase of 0.2 M formic acid was used for compatibility reasons. This acceptor phase was somewhat inferior to HCl, but it provided acceptable recoveries (9–52%) for the nine antidepressant drugs selected as model compounds. Dodecyl acetate was used as a highly stable and efficient organic phase, and extractions were performed both from plasma and whole blood samples. Due to the high sensitivity of LC–MS, it was possible to detect the drugs at the low ng/ml level even from sample volumes as small as 50 μl (single drop analysis). Quantitative data were examined for potential ion suppression effects, but these were absent due to the excellent clean-up properties of three-phase LPME. The repeatability was studied, and RSDs were better than 20% when extractions were performed from 50 μl samples, and were further improved when sample volumes were increased to 500 μl. From this it was concluded that inaccuracies in the liquid handling became a significant contribution to the RSDs when sample volumes of only 50 μl were used. The paper also demonstrated that under standard conditions, where the extraction

conditions are not carefully optimised for each compound, recovery values within a group of drugs may vary substantially because of differences in their partition coefficients [22]. This has to be considered during future method development.

Within the field of drug analysis, chiral analysis is highly important. In two recent publications, three-phase LPME was combined with chiral CE systems to individually determine concentrations of different enantiomers [17,20]. Both mianserin and citalopram were investigated, and either dihexyl ether or dodecyl acetate were utilised as organic phases. Because of high enrichment, the drug enantiomers were detected within their therapeutically relevant concentration levels down to the low ng/ml with CE, although the latter provides relatively low concentration sensitivity. Both publications included validation data, which further supported that three-phase LPME provides acceptable linearity, precision, and accuracy for practical work, especially taking into account that all extraction units were prepared manually.

All the publications reviewed above have been made in the laboratory of the authors of this review [8–18,20–24], or in close contact with us [19]. A few other groups have been involved in three-phase LPME, but their efforts have mainly been within environmental analysis. This has been reviewed recently [33], and is behind the scope of this paper. However, a recent publication reported on three-phase LPME of 2-amino-1-phenylethanol, norephedrine, pindolol, and atenolol from urine. The drugs were extracted from alkaline samples, through *n*-octanol as the organic phase, and into 5 μl of 0.1 M HCl as acceptor phase. One interesting aspect in this paper is that relatively high recoveries were obtained in spite of the polar nature of the analytes. Thus, compounds with log *P* values (octanol–water partition coefficients) down to approximately 1 may in some cases be extracted by three-phase LPME. A second interesting aspect was that the acceptor phase was analysed by capillary electrophoresis utilising on-column stacking. With this concept, the analytes were enriched by a factor of approximately 110.

4. LPME based on two-phase extractions

While LPME based on three-phase extraction has been discussed in a relatively large number of publications, only limited information is current available for drugs with two-phase LPME [7,10,11,16,31,32]. The different drugs extracted by two-phase LPME are summarized in Table 2. In all of these publications, the extracts were analysed by capillary gas chromatography. In the first report, the two benzodiazepines diazepam and prazepam were extracted from human plasma and urine along with *N*-desmethyldiazepam, which is a phase I metabolite of diazepam [11]. Direct LPME on the raw plasma samples resulted in relatively low extraction recoveries due to the high protein-binding of benzodiazepines (approximately 98%). However, addition of 200 μl methanol to 3 ml of plasma was found to effectively suppress these interactions, and no other pre-extraction efforts were required with respect to plasma samples. For urine, the major problem was pH variations from sample to sample, and in order to overcome this problem, a relatively strong phosphate buffer was added to the urine samples prior to extraction. Selection of the organic solvent for the pores and the lumen of the fibre was carefully optimised, because the solvent should provide both high extractability for the analytes and an acceptable medium for injection into the GC-system. Based on these criteria, a 1:1 (v/v) mixture of butyl acetate and *n*-octanol was selected for urine. With this solvent combination, *N*-desmethyldiazepam was recovered by 69%, and with a 25 μl volume of acceptor phase, *N*-desmethyldiazepam was enriched by a factor of 97. For plasma samples, the mixture of butyl acetate and *n*-octanol failed. Acceptor phase was partly lost during extraction, probably because of the emulsifying nature of plasma. Thus, for plasma samples, a 1:1 (v/v) mixture of *n*-octanol and dihexyl ether was found appropriate, and provided recovery and enrichment values of, respectively, 68 and 82% for diazepam. A typical chromatogram obtained by GC with nitrogen–phosphorous detection (NPD) is shown in Fig. 5 for a drug-free plasma sample spiked with 5 nmol/ml of the three benzodiazepine compounds. The extracts were remarkable clean within a broad retention time window. A preliminary validation with home-made extractions units revealed linear calibration graphs, intra-day and inter-day RSDs below 10–12%, and accuracy data in the range 1–12% relative errors. In addition, due to the excellent analyte enrichment, the compounds were easily detected at their clinical concentrations by GC–NPD analysis.

In addition to benzodiazepines, attention has been focused on extraction of 11-norΔ^9-tetrahydrocannabinol-9-carboxylic acid (THC–COOH) from urine, which is the major metabolite found from abuse of marijuana [7]. Unfortunately, this compound decarboxylates above 80 °C, and consequently it should be derivatised prior to GC-analysis. This was performed in a very elegant way by adding *N*,*O*-

Table 2
Application overview for two-phase LPME of drugs from human biological samples

Compound	Sample	Organic phase	Acceptor phase	Reference
Cocaine	Saliva, urine	Chloroform	Chloroform	[31,32]
Diazepam	Plasma, urine	*n*-octanol	*n*-octanol	[10,11]
Methadone	Plasma, urine	Dihexyl ether	Dihexyl ether	[16]
Prazepam	Plasma, urine	*n*-octanol	*n*-octanol	[10,11]
Promethazine	Plasma, urine	Dihexyl ether	Dihexyl ether	[16]
Tetrahydrocanna-binolcarboxylic acid	Urine	*n*-octane	*n*-octane	[7]

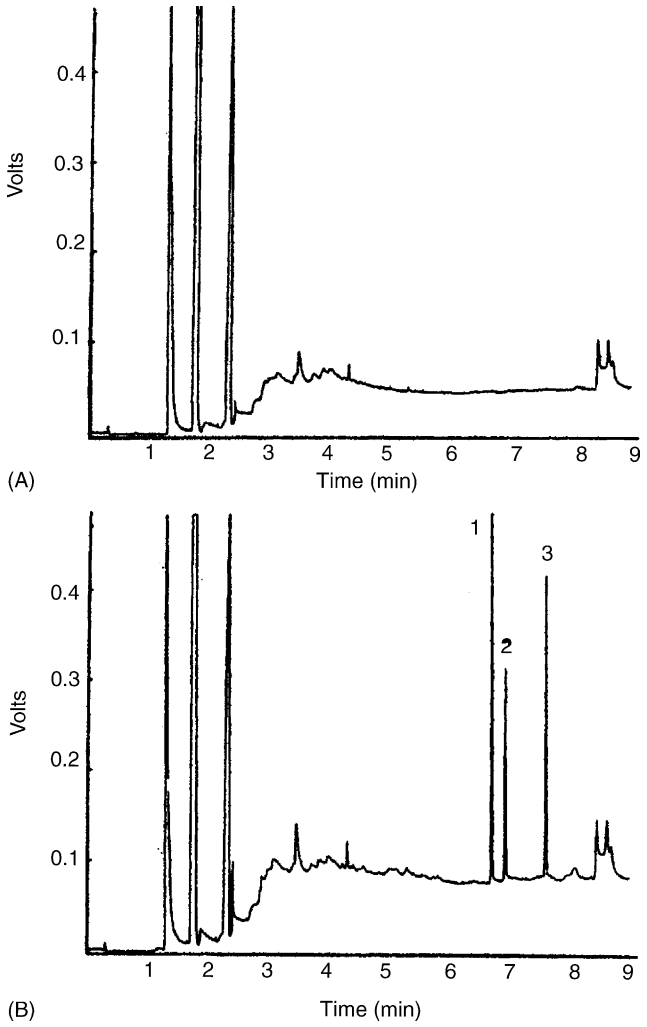

Fig. 5. Two-phase LPME and GC–NPD of drug free plasma (upper chromatogram) and plasma spiked with 5 nmol/ml of diazepam (peak 1), *N*-desmethyldiazepam (peak 2), and prazepam (peak 3). Reprinted with permission [11].

bis(trimethylsilyl)trifluoroacetamide (BSTFA) as a derivatisation reagent directly to the acceptor solution. Thus, the analyte was derivatised at the same time as it was extracted from the urine samples and into the acceptor phase. A 1:1 (v/v) mixture of BSTFA and octane was found to be the optimal acceptor phase for extraction and derivatisation. The pores of the hollow fibres were immobilised with pure octane, since the BSTFA is instable in contact with water. Two different extraction schemes were tested: (1) direct extraction of THC–COOH from acidified samples and (2) ion-pair formation with tetramethylammonium hydrogen sulphate and subsequent extraction from alkaline samples. The latter concept was found to provide better recoveries and better RSD data, and the reason for this was attributed to the higher ionic strength of system (2), which reduced solvent leakage into the sample. Extractions and derivatisation were performed for only 8 min. The recoveries were relatively low (2–3%), but this was probably due to the very mild derivatisation conditions (8 min at room temperature); normally BSTFA derivatisations are carried out at 100 °C for at least 20 min. Two-phase LPME with simultaneous derivatisation is a highly interesting concept, which should be further evaluated in the near future.

In addition to benzodiazepines and THC–COOH, two-phase LPME has also been used for the screening of cocaine in human urine and saliva [31,32]. In these reports, hollow fibres made of polypropylene were compared with fibres made of polyvinyldene difluoride, with the former providing highest recoveries with chloroform as extraction solvent. This finding is interesting since the literature contains very little information about alternatives to polypropylene for the fibre material. With chloroform, extraction times as short as 3 min were used for urine (8 ml samples) and 10 min for saliva (2 ml samples). This provided acceptable recoveries to address real life concentration levels, whereas longer extraction times resulted in poor recovery of the extraction solvent from the fibre due to partial evaporation. Also in this case validation was accomplished, and addition of an internal standard was found to be crucial in order to obtain acceptable validation data. A comparison was made between results obtained with hollow fibre LPME and LPME based on the hanging drop concept. The former was found to be more reliable and produced significantly lower RSD values. In addition, hollow fibre-based LPME provided substantially higher extraction recoveries, primarily because this technique enabled strong vibration or stirring of the samples without loss of acceptor phase.

In a recent report, two-phase LPME was compared with conventional LLE [16]. As discussed above for three-phase LPME, the partition coefficient between the sample phase and the acceptor phase should be high also in two-phase LPME in order to obtain acceptable recoveries. The practical consequence of this is that LLE methods for analytes with high partition coefficients are successfully transferred to two-phase LPME, whereas polar analytes will fail in two-phase LPME. On the other hand, two-phase LPME will provide higher selectivity since it discriminates more polar analytes. This is illustrated in Fig. 6, where methadone and promethazine were extracted from human urine by both LPME and LLE.

5. LPME based on carrier-mediated extractions

Two- and three-phase LPME extractions are complementary and, together, cover a broad range of analytes. The basic requirement is that the analytes of interest can be extracted into the organic phase. If it is difficult to extract the analyte from this hydrophobic environment into another aqueous phase, two-phase LPME should be selected. This is typically the case for relatively hydrophobic compounds with no acidic or basic groups. If the chemical nature of the analyte allows further extraction into a new aqueous phase, three-phase LPME may be used. This is the case for hydrophobic

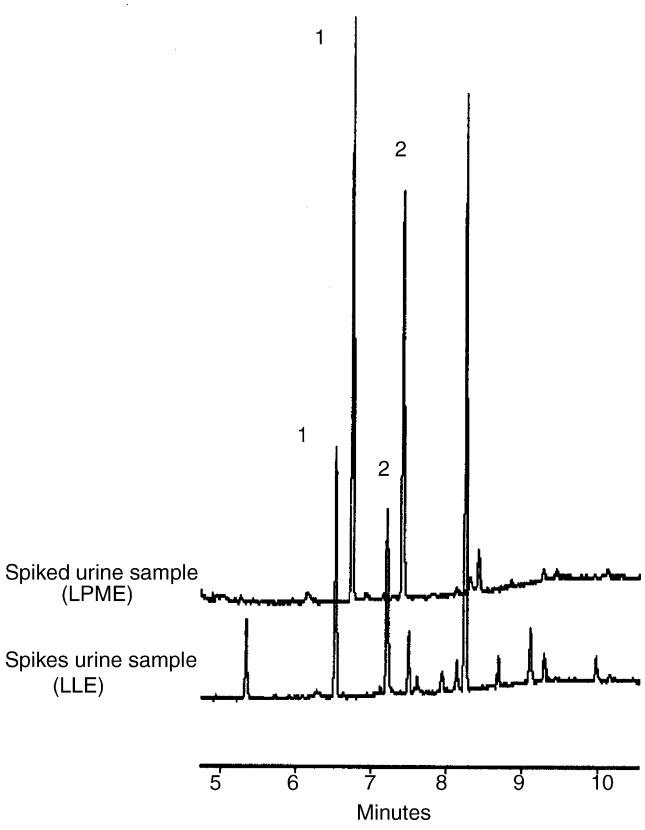

Fig. 6. Two-phase LPME (upper chromatogram) and two-phase LLE (lower chromatogram) combined with GC–FID of 2.5 μg/ml of methadone (peak 1) and promethazine (peak 2) in human urine. Reprinted with permission [16].

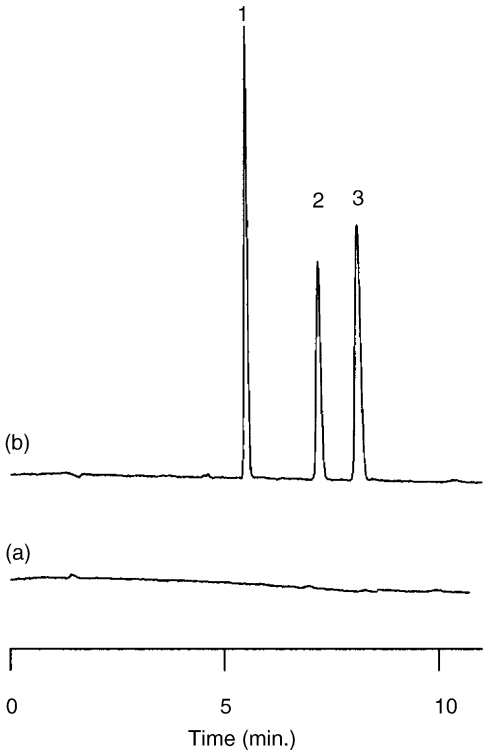

Fig. 7. Carrier-mediated LPME and CE–UV of (a) drug free plasma plasma and (b) plasma spiked with 16 μg/ml of amphetamine (peak 1), morphine (peak 2), and practolol (peak 3). Reprinted with permission [23].

compounds containing either acidic or basic functionalities. Unfortunately, very polar compounds can not be extracted by either technique because such type of analytes will have too low an affinity for the organic phase in the pores of the hollow fibre. In this case, ion-pair reagents can be added to the sample, as in carrier-mediated LPME [23,24].

In the first paper, morphine and practolol were selected as model compounds because they were poorly extracted by three-phase LPME. For plasma and urine, pH was adjusted to 7.0 with a phosphate buffer to ensure that the drugs were ionised in the sample. Then, octanoic acid was added to a final concentration of 25 mM. The ion pairs formed had a sufficient hydrophobic character to effectively enter the organic phase (*n*-octanol). As the acceptor solution, 50 mM HCl was used. The strongly acidic nature of this phase ensured that octanoic acid did not leak into the acceptor phase; in addition, the high concentration of protons maintained an efficient pumping system for the analytes into the acceptor phase. During release of the drug molecules at the interface between the organic and acceptor phases, protons were utilised as counter-ions. Subsequently, non-ionized octanoic acid was back-extracted into the sample for a new cycle.

For morphine and practolol, carrier-mediated LPME provided recoveries of, respectively, 57 and 45%, while the corresponding values for urine were 52 and 46%. The recoveries are surprisingly high, and suggest a strong potential for this type of extractions in drug analysis. For both sample types, clean extracts were obtained as illustrated in Fig. 7 for plasma. Carrier-mediated LPME also provided excellent linearity of the selected drugs in the range 1–25 μg/ml.

The fundamental aspects of carrier-mediated LPME were studied in the next paper [24], which was focused on extractions from pure water, but also demonstrated that amphetamine, phenylpropanol amine, metaraminol, cimetidine, sotalol, and atenolol may be extracted by carrier-mediated LPME. Substantial differences in extraction kinetics were observed; the most hydrophobic drugs were extracted to equilibrium in less than 30 min whereas the more hydrophilic drugs required more time. Surprisingly, all drugs were quantitatively extracted if the extraction time was 24 h. Naturally, extraction times as long as 24 h are not acceptable for analytical purposes, but the experiments demonstrate that the pumping system of carrier-mediated LPME transports all analytes to the acceptor solution provided that the acceptor phase contains sufficient protons for back-extraction of octanoic acid.

More work will have to be carried out to show that carrier-mediated LPME provides acceptable validation data for quantitative applications, and more drugs and carriers should be studied to document that the technique is generally applicable for hydrophilic drugs.

6. Conclusions and future directions

The present paper has reviewed the current literature on hollow fibre-based LPME coupled to chromatographic techniques. Although only a limited number of publications have emerged, the technique appears to be very attractive. Extraction units are inexpensive to built, and are used only for a single extraction to avoid cross contamination problems. The consumption of organic solvents is almost eliminated, and the technique is compatible both with whole blood, plasma, and urine samples. The technique may provide medium to high recoveries, high analyte enrichment, and excellent sample clean-up from biological samples. In addition, extractions may be finished in less than 45 min for most applications. Due to the simplicity of the extraction units, many samples may be processed in parallel providing a high sample throughput. In addition, with exactly the same extraction units, both three-phase LPME, two-phase LPME, and carrier-mediated LPME may be performed, providing a high degree of flexibility.

Future work should be focused on extraction of more drugs to further support that LPME is an alternative for a broad range of applications, and that validation data is comparable with existing methods based on LLE, SPE, and SPME. Especially for carrier-mediated LPME, substantial research has to be done. Hopefully, these research activities will be complemented by commercial equipment, which currently is under development.

References

[1] M.A. Jeannot, F. Cantwell, Anal. Chem. 68 (1996) 2236.
[2] M.A. Jeannot, F. Cantwell, Anal. Chem. 69 (1997) 235.
[3] V. He, H.K. Lee, Anal. Chem. 69 (1997) 4634.
[4] L. Zhao, H.K. Lee, J. Chromatogr. A 919 (2001) 381.
[5] M. Ma, F. Cantwell, Anal. Chem. 70 (1998) 3912.
[6] M. Ma, F.F. Cantwell, Anal. Chem. 71 (1999) 388.
[7] K.E. Kramer, A.R.J. Andrews, J. Chromatogr. B 760 (2001) 27.
[8] S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chem. 71 (1999) 2650.
[9] S. Pedersen-Bjergaard, K.E. Rasmussen, Electrophoresis 21 (2000) 579.
[10] K.E. Rasmussen, S. Pedersen-Bjergaard, M. Krogh, H. Grefslie Ugland, T. Grønhaug, J. Chromatogr. A 873 (2000) 3.
[11] H. Grefslie Ugland, M. Krogh, K.E. Rasmussen, J. Chromatogr. B 749 (2000) 85.
[12] T.G. Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 909 (2001) 87.
[13] T.G. Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. B 760 (2001) 219.
[14] T. Grønhaug Halvorsen, S. Pedersen-Bjergaard, J.L.E. Reubsaet, K.E. Rasmussen, J. Sep. Sci. 24 (2001) 615.
[15] S. Pedersen-Bjergaard, T. Si Ho, K.E. Rasmussen, J. Sep. Sci. 25 (2002) 141.
[16] T.S. Ho, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 963 (2002) 3.
[17] S. Andersen, T. Grønhaug Halvorsen, S. Pedersen-Bjergaard, J. Chromatogr. A 963 (2002) 303.
[18] T.S. Ho, S. Pedersen-Bjergaard, K.E. Rasmussen, Analyst 127 (2002) 608.
[19] T. Kuuranne, T. Kotiaho, S. Pedersen-Bjergaard, K.E. Rasmussen, A. Leinonen, S. Westwood, R. Kostiainen, J. Mass Spectrom. 38 (2003) 16–26.
[20] S. Andersen, T. Grønhaug Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, L. Tanum, H. Refsum, J. Pharm. Biomed. Anal. 33 (2003) 263.
[21] A. Bjørhovde, T. Grønhaug Halvorsen, K.E. Rasmussen, S. Pedersen-Bjergaard, Anal. Chim. Acta 491 (2003) 155.
[22] T. Grønhaug Halvorsen, S. Pedersen-Bjergaard, J.L.E. Reubsaet, K.E. Rasmussen, J. Sep Sci. 26 (2003) 1520.
[23] T.S. Ho, T. Grønhaug Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 998 (2003) 61.
[24] T.S. Ho, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A (submitted for publication).
[25] G. Audunsson, Anal. Chem. 58 (1986) 2714.
[26] G. Nilvé, G. Audunsson, J.Å. Jönsson, J. Chromatogr. 471 (1989) 151.
[27] J.Å. Jönsson, L. Mathiasson, Trends Anal. Chem. 11 (1992) 106.
[28] J.Å. Jönsson, L. Mathiasson, J. Chromatogr. A 902 (2000) 205.
[29] J.Å. Jönsson, L. Mathiasson, J. Sep. Sci. 24 (2001) 495.
[30] L. Hou, X. Wen, C. Tu, H.K. Lee, J. Chromatogr. A 979 (2002) 163.
[31] L.S. de Jager, A.R.J. Andrews, Analyst 126 (2001) 1298.
[32] L. de Jager, A.R.J. Andrews, Anal. Chim. Acta 458 (2002) 311.
[33] K.E. Rasmussen, S. Pedersen-Bjergaard, Trends Anal. Chem. 23 (2004) 1.