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Journal of Chromatography A, 1152 (2007) 184–192
JOURNAL OF CHROMATOGRAPHY A
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Review

Developments in single-drop microextraction

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Available online 13 November 2006

Abstract

Single-drop microextraction (SDME) has become a very popular liquid-phase microextraction technique because it is inexpensive, easy to operate and nearly solvent-free. Essentially, SDME combines extraction (and conceivably, cleanup) and concentration in a minimum number of steps, and thereafter, direct extract introduction into an analytical system. In this review, in order to encourage further development of SDME, we focus on its recent developments in its various guises. Its applications when used in combination with different analytical techniques, such as gas chromatography, high-performance liquid chromatography, inductively-coupled plasma mass spectrometry, capillary electrophoresis, mass spectrometry and electrothermal atomic absorption spectrometry, are summarized. SDME does have some limitations, and these are also discussed as well. Finally, an outlook on the future of the technique is given.

Keywords: Single-drop microextraction; Liquid-phase microextraction; Solvent-minimized extractions

Contents

1. Introduction

Despite substantial technological advances in the analytical field, most instruments cannot handle complex sample matrices directly and, as a result, a sample-preparation step is commonly involved in an analytical procedure. The main aim of sample preparation is to clean up, and concentrate the analytes of interest, while rendering them in a form that is compatible with the analytical system. Liquid–liquid extraction (LLE) is the classical sample pretreatment method to achieve this objective, and remains a popular choice. However, it is time-consuming, tedious and uses large amounts of potentially toxic organic solvent that is usually expensive because of its high purity (necessary for analytical applications). LLE can be automated to some extent, but this is not often practiced. Another popular sample preparation approach is solid-phase extraction (SPE). Although it uses much less solvent than LLE, usage can still be considered significant, and normally an extra step of concentrat-

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doi:10.1016/j.chroma.2006.10.073

ing the extract down to a small volume is needed. SPE can be automated but this entails complexity and therefore additional cost.

It is considerable challenge to come up with more direct sample preparation procedure that is simple (preferably one-step), affordable and economical (obviating the need for sophisticated apparatus or equipment), can be performed at a miniaturized scale (leading to reduction in solvent and material usage), and be automated to some degree (a desirable feature, but not completely necessary for reasons mentioned previously—complexity and capital expenditure).

Solid-phase microextraction (SPME), now a commercial product, was developed by Pawliszyn's group [1]. It is an innovative solvent-free procedure that has gained tremendous popularity. It satisfies most of the desired characteristics of a sample preparation technique mentioned above, and has been used for wide range of applications, particularly in environmental, biological and pharmaceutical analyses. It is portable, simple to use, relatively fast, and can be automated and coupled online to analytical instrumentation. Most people, if not all of them, would agree to the assertion that the advantages of SPME far outweigh the qualities of LLE and SPE. However, the coated fibers may be considered to be expensive, and for some applications, have limited lifetimes. Additionally, automated SPME systems (primarily coupled to gas chromatography, GC) are expensive and normally out of the reach of most laboratories.

An alternative miniaturized sample preparation approach emerged in the mid-to-late 1990s. Liquid-phase microextraction (LPME), as its name suggests, makes use of only a small amount of solvent for concentrating analytes from aqueous samples [2]. It overcomes many of the disadvantages of LLE as well as some of those of SPME (e.g. independence of a commercial source and sample carryover); it is simple, fast and is characterized by its affordability, and reliance on widely-available apparatus.

In LPME, extraction normally takes place between a small amount of a water-immiscible solvent and an aqueous phase containing the analytes of interest. The acceptor phase can be directly immersed in or suspended above the sample (for headspace extraction). The volume of the receiving phase is in the microlitre or submicrolitre range. In this way, high enrichment factors are obtainable owing to the high sample volume-to-acceptor phase volume ratio. Since the extraction medium is in the form of a single drop, this type of LPME has been termed single-drop microextraction (SDME).

SDME, characterized by its simplicity of operation and use, has attracted considerable attention in recent years. Psillakis and Kalogerakis [3] have written an overview of its basic extraction principles as well as advances from its advent to about 2002. The present review concentrates on updated developments and applications of SDME. It covers almost all the publications related to the procedure from the beginning. In addition, some limitations and an outlook on further developments are discussed.

2. Scope of this review

From the introduction of SDME in 1996 [4], hitherto, different modes of SDME have been developed, catering to various analytical applications, such as direct immersion (DI)-SDME, headspace (HS)-SDME and continuous-flow microextraction (CFME). Based on these various implementations, different approaches have been taken by researchers to improve selectivity, introduce a degree of automation, expand the application range of the procedure, and make it compatible with more analytical techniques. For example, ionic liquids (ILs) have recently been investigated as SDME extractant solvents [5–9] (see below). These are generally considered to be environmentally-friendlier solvents with unique characteristics (e.g. no effective vapor pressure, adjustable viscosity and immiscibility in water and other organic solvents) that may be tuned by changing the combination of different anions and cations. ILs are considered potentially attractive alternative extractant phases that may enhance analyte selectivity in DI- and HS-SDME. They may also be compatible with a wide range of techniques such as high-performance liquid chromatography (HPLC), atomic absorption spectrometry and cold-vapor atomic fluorescence spectrometry.

The focus in this review is on applications. It is felt that the basic theory of SDME has been given adequate treatment elsewhere (see for example [3,10–12]), and so will not be considered here.

2.1. Direct immersion (DI)-SDME (static mode) versus dynamic LPME

In 1996, Liu and Dasgupta [4] reported a drop-in-drop system to extract sodium dodecyl sulfate. In this report, a 1.3-μL microdrop of a water-immiscible organic solvent was immersed into a large flowing aqueous drop to accomplish the extraction process. At almost the same time, Jeannot and Cantwell [10] introduced a procedure that they termed solvent microextraction in which the extraction medium was a droplet (8 μL) of 1-octanol held at the end of a Teflon rod and suspended in a stirred aqueous sample solution. After extraction for a prescribed time, the Teflon rod was withdrawn from the aqueous solution; the organic phase was sampled with a microsyringe and injected into a GC system for analysis. In this work, the authors also proposed equilibrium and kinetic theories to explain this new mode of microextraction.

One disadvantage of the aforementioned methods is that extraction and injection have to be performed separately, using different apparatus. In order to overcome this shortcoming, Jeannot and Cantwell [11] introduced the idea of using a microsyringe as the organic solvent holder instead of a Teflon rod. A microlitre of organic solvent was first withdrawn into a microsyringe, and then the needle of the microsyringe was passed through the sample vial septum and immersed in the liquid sample. A droplet of organic solvent was suspended at the tip of the syringe needle in a stirred aqueous sample, as shown in Fig. 1. After extraction, the organic phase was withdrawn back into the microsyringe, which was then used directly for injecting the sample into a GC system. The microsyringe served as both the solvent holder and the GC sample injector. Thus, extraction and extract injection could be carried out with a single device. This represents a desirable convenience of the microextraction operation. The authors also provided more detailed insights into the theory of the procedure.

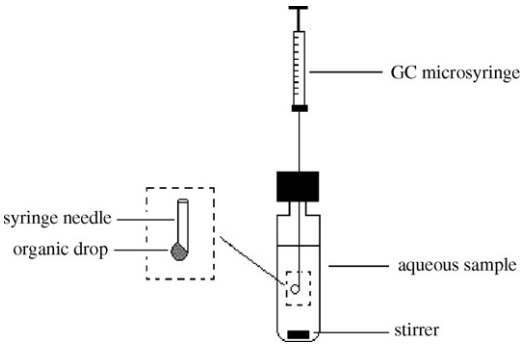

Fig. 1. Schematic of direct immersion single-drop microextraction.

In order to improve extraction efficiency further, He and Lee [13] developed what they termed dynamic LPME (it should be noted from the outset that this is not strictly SDME since a "drop" configuration is not involved). In this study, the microsyringe again played a dual role, as a microseparatory apparatus for extraction as well as a GC sample injector. Basically, the aqueous sample was withdrawn into the microsyringe barrel preloaded with an organic solvent such as toluene (see Fig. 2a). The sequence of sample withdrawal and expulsion was: withdrawal of aqueous sample (3 μL) over 2 s; waiting (dwell) time of 3 s to allow extraction of analytes into a thin film of organic solvent formed along the wall of the barrel as the bulk organic solvent was withdrawn back up towards the back of the barrel (as depicted in Fig. 2b); and expulsion of the sample over 2s (recombination of the bulk solvent and the organic thin film); a dwell time of 3 s. This cycle was repeated 20 times within 3 min (see Fig. 2c). The enriched toluene was then injected into the GC instrument for analysis. As compared to the static mode (organic drop held at the needle tip), dynamic LPME featured the repeated movement of the microsyringe plunger that allowed the formation of the thin organic film that facilitated the mass transfer of analytes from the sample, and considerable agitation of the two liquid phases that also enhanced extraction. Dynamic LPME provided a higher enrichment factor (~27-fold) than the static mode (~12-fold) for some chlorinated benzenes. In another study, the same authors evaluated the parameters influential to this mode of extraction more systematically [14]. Subsequently, they used a programmable syringe pump [15] to automate the repetitive process of withdrawing the sample into, and expelling it from, the microsyringe barrel. Even higher enrichment (>280-fold) for the determination of polycyclic aromatic hydrocarbons (PAHs) in environmental water was achieved.

In a subsequent study, Myung et al. [16] controlled the movement of the syringe plunger with a computer. The automated device was used in the extraction and determination of benzene ethylamine derivatives in biological liquids, and showed good validation results, apart from the enhanced convenience.

One limitation of DI-SDME is the instability of the droplet at high stirring speeds. In general, however, a high stirring speed enhances extraction. To obviate the problem caused by elevated stirring speeds, a 1-μL microsyringe (instead of a more common 10-μL one) with some modification of its tip was employed by Ahmadi et al. [17] for extraction. In their work, 0.9 μL of carbon tetrachloride was used as the extractant solvent; the sample was stirred at 1300 revolutions/min (rpm) for 40 min without any addition of salt. By making use of almost the maximum volume of the microsyringe, the repeatability of drop volume and injection was improved. The modification of the needle tip enlarged its cross-sectional area, resulting in greater adhesion between the tip and the drop. With this modification, the organic drop was able to withstand a higher stirring speed, up to 1700 rpm, and enhanced enrichment factors ranging from 540 to 830 for organophosphorus pesticides were observed.

2.2. Headspace (HS)-SDME

HS-SDME in which the organic droplet is held above the aqueous sample solution is most suitable for the consideration of volatile or semi-volatile analytes (see Fig. 3). The first report on HS-SDME came out in 2001 [18], and the approach continues to gain attention in recent years [19–52] owing to its advantages. For instance, this method allows rapid stirring of the sample solution with no adverse impact on the stability of the droplet. Additionally, as in HS-SPME, non-volatile matrix interferences are reduced, if not eliminated. In this mode, the analytes are distributed among three phases, the water sample, headspace and organic drop. Aqueous phase mass transfer is the rate determining step in the extraction process as explained

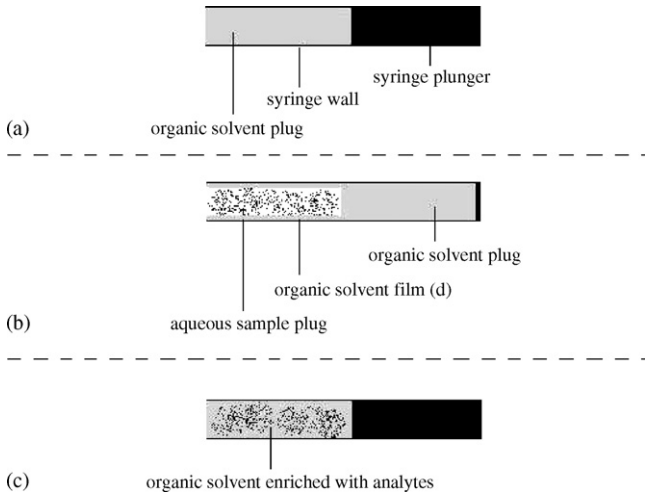

Fig. 2. Operation of dynamic liquid-phase microextraction.

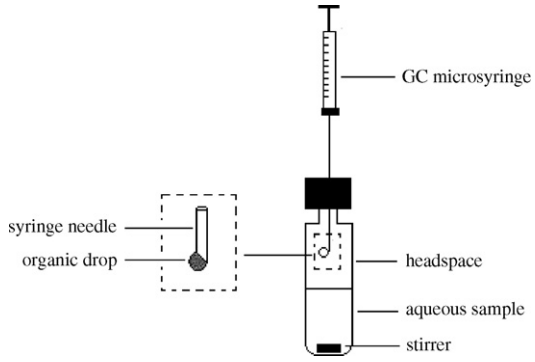

Fig. 3. Schematic of headspace single-drop microextraction.

by Theis et al. [18]. Hence, a high stirring speed of the sample solution facilitates mass transfer among the three phases.

Compared with HS-SPME, HS-SDME appears to have similar capabilities in terms of precision and speed of analysis; however, the latter offers two distinct advantages. Firstly, intuitively, the choice of solvents is wider, if not virtually unlimited, as compared to the limited number of phases currently available for SPME. Secondly, the cost of solvent (on the basis of several microlitres) is negligible compared to the cost of commercially available SPME fibers. (It should be noted that in-house coating of fibers has been attempted successfully [53,54], but the process is non-trivial.) However, use of SDME for headspace analysis seems relatively difficult, because solvents with relatively lower vapor pressures would be preferred. Yet, the most suitable solvents for GC should have relatively high vapor pressures. The difficulty with the latter solvents is clear: they would evaporate too quickly in the headspace during extraction. Thus, in reality, the choice of suitable solvents is limited, particularly if the vapor pressure of the solvent rather than its extraction efficiency is the primary consideration.

To overcome the above disadvantages of HS-SDME, Shen and Lee [19] proposed dynamic SDME for headspace extraction. Extraction took place within the microsyringe barrel, so like conventional dynamic LPME described above. (Strictly speaking, like the original dynamic LPME referred to earlier, an organic drop was not involved. The procedure is included in this discussion for the purpose of demonstrating the progression made by automating the extraction process that obviates the problem of drop instability caused by volatility issues.) The operation steps involved were as follows: (1) 2 μL of organic solvent was withdrawn into the microsyringe; (2) the microsyringe needle was pushed through the headspace sample vial septum, with the needle tip then held over the liquid sample; (3) 5 μL of gaseous sample was withdrawn at 1.0 μL/s by pulling back the plunger, which was then immediately reset to its original mark. After a waiting time of 5 s, the cycle was repeated. Each extraction consisted of several such cycles. As in conventional dynamic LPME, when the syringe plunger was withdrawn, a thin organic solvent film (OSF) was generated on the inner syringe wall as the gaseous sample was drawn in. The analytes in the vapor, chlorobenzenes in this case, partitioned between the OSF and the gaseous phase. When the syringe plunger was depressed to expel the vapor, the chlorobenzenes-enriched OSF recombined with the bulk organic solvent. This operation could significantly increase the surface area of the interface by using a very small amount of organic solvent, helpful to increase the extraction efficiency. Again, in comparison to conventional HS-SDME, the selection of extractant solvent is more flexible. Based on this procedure, some applications have been reported, for example, the determination of acetone in human blood [20] as well as panaxynol (a bioactive compound) and essential oils in traditional Chinese medicines (TCMs) [21,22].

Due to the imprecision of manually-operated dynamic HS-SDME [19], as described in the preceding paragraph, semiautomatic dynamic HS-SDME was suggested by Saraji [23] with a view of providing greater reproducibility with more convenience operationally. This semiautomatic apparatus consisted of a variable-speed stirring motor attached to a circular plate, which was connected to the syringe plunger. Rotation of the plate activated the movement of the syringe plunger. Unfortunately, the hoped-for great improvement in precision did not materialise. The reported relative standard deviations (RSDs) were from 5.5 to 9.3% for ethanol, 2-propanol, *tert*-butanol, 1-propanol, 2-butanol, 1-butanol, 2-pentanol and 1-pentanol, except for methanol (16.4%). In the manual procedure [19], RSDs for chlorobenzenes were between 3.62 and 9.26%.

Recently, Ouyang et al. [24] developed fully-automated HS-SDME, using the CTC Combipal autosampler. All the operational parameters involved in this process could be precisely and conveniently controlled by the autosampler. Additionally, a new kinetic calibration method to correct for the matrix effects in applications was devised, and demonstrated with the analysis of BTEX (benzene, toluene, ethylbenzene and xylenes) in orange juice.

Zhang and co-workers [25,26] used a suspended solvent microdrop containing derivatization agent to perform HS-SDME of aldehydes, followed by GC–mass spectrometry (MS) analysis. Since sample extraction, concentration and derivatization were carried out in a single step, the method provided a simple, rapid, low-cost and efficient approach. The procedure was employed for the analysis of the aforementioned analytes in waste water [25] and blood [26]. The same group of workers also described the combination of microwave-assisted extraction with HS-SDME for paeonol (a bioactive compound) analysis in TCMs [27].

Very recently, multiple headspace (MHS)-SDME was evaluated and validated for the extraction and quantitative determination of styrene in polystyrene [28]. The procedure involved several consecutive extractions from the same sample vial; thus, in theory, the extraction could continue until all the analytes were removed from the sample, resulting in complete recovery. This appears to be the first SDME procedure applied to the direct extraction of volatiles from solid matrices.

A new organic-free mode of headspace SDME termed headspace water-based LPME was proposed by Lee's group [29] recently. A sodium hydroxide aqueous solution instead of a high-boiling point organic solvent was used as the acceptor solvent to extract volatile or semivolatile ionizable compounds (phenols). This permitted the use of capillary electrophoresis (CE) in the determination of the analytes. High enrichment factors were obtained in this study. Bendicho's group [30–34] has also carried out a series of investigations on headspace sequestration of a hydride by an aqueous single-drop as the acceptor phase after its first report by Chamsaz et al. [35]. They focused on the utility of this mode of SDME as a preconcentration method for electrothermal atomic absorption spectrometry (ETAAS) (see below).

2.3. Continuous-flow microextraction (CFME)

CFME evolved from conventional SDME, and was first described by Liu and Lee in 2000 [55], as illustrated in Fig. 4. Briefly, an aqueous sample (typically of total volume 3 mL or less) was pumped continuously at a constant flow rate

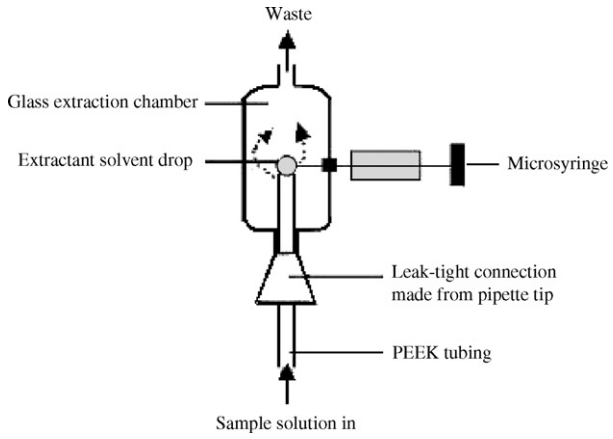

Fig. 4. Schematic of continuous-flow microextraction.

(0.05 mL/min, or above) using an HPLC solvent delivery system, into a 0.5-mL glass extraction chamber via a connecting PEEK tubing. After the chamber had been filled with the sample solution, with the surplus flowing to waste through an outlet, the required volume of an organic extraction drop (1–5 μL) was introduced into the system via a conventional HPLC injection valve. The drop then traveled to the outlet of the PEEK tubing (within the chamber) when it remained attached. The sample solution was continuously pumped "around" the drop, allowing analytes to be extracted efficiently. At the end of the extraction, a microsyringe needle was introduced into the chamber to collect some of the extract for analysis. High concentration factors ranging from 260- to 1600-fold were achieved within 10 min of extraction for trace nitroaromatics and chlorobenzenes. In combination with GC-electron capture detection (ECD), the procedure allowed analytes to be detected at femtogram/mL levels.

CFME differs from other extraction approaches that a drop of solvent fully and continuously makes contact with a fresh and flowing sample solution. Both diffusion and molecular momentum result from mechanical forces contribute to its effectiveness. With the use of an HPLC injection valve, precise control of the solvent drop size could be achieved while introduction of undesirable air bubbles was avoided. Another advantage was that because of the high preconcentration factor that can be achieved, smaller volumes of aqueous samples were needed for extraction. Liang and co-workers [56] also used CFME in combination with GC-ECD to preconcentrate halohydrocarbons in water; the limits of detection (LODs) in the range of 0.001–0.02 μg/L. The same authors have also coupled HPLC with CFME to determine phoxim, an organophosphorus pesticide in lake water [57]. They obtained an LOD of 5 ng/mL. Recently, He and Lee [58] reported the combination of CFME with HPLC to extract and determine the widely-used organonitrogen and organophosphorus pesticides, simazine, fensulfothion, etridiazole, mepronil and bensulide.

Xia et al. [59,60] made some modification to the basic CFME setup and developed a recycling-flow system, in which the "waste" from the chamber was returned to the sample vial. This allowed further reduction in the sample size (and non-attended operation since there was no danger of the sample vial running dry). This is further discussed below.

3. Applications

3.1. SDME with GC and HPLC

Since water-immiscible solvents are generally used in SDME, the preferred technique for the analysis of extracts is GC. More than 60% of the published applications of SDME are coupled with GC. The versatility of SDME-GC is seen in relation to the variety of applications in many areas, as depicted in Table 1, which is updated to 2006.

In general, HPLC is a widely used versatile separation and quantification technology. However, there have been only a few papers reporting the use of this technique following SDME. SDME is more suited for volatile or semivolatile analytes that are of a relatively more hydrophobic nature, necessitating the use of immiscible extracting solvents. This intuitively disqualifies HPLC from being considered. Moreover, a smaller volume of the extracting solvent is usually chosen for the consideration of stability during SDME with stirring; for most HPLC injections, however, a sample size of more than 2 μL is preferred. It is of course important that the selected organic extraction phase be compatible with the HPLC mobile phase. This limits the choice of solvents and is not usually realized, however, and an extra step of solvent exchange and extract reconstitution is needed before analysis [58].

Ma and Cantwell [80] attempted to address the aforementioned problem by performing SDME (or solvent microextraction, as the original technique was termed by the authors) with an organic solvent, followed by back-extraction into an aqueous solution. In their method, 30 μL of *n*-octane membrane acted as the transferring or intermediary phase between the two aqueous phases (sample and final accepting phase), which was confined inside a small Teflon ring and held over 1.60 mL of an aqueous sample phase (maintained at pH 13). The receiving aqueous drop (at pH 2.1) of 0.50 or 1 μL was suspended in this liquid membrane from the tip of a microsyringe needle. Here, basic analytes methamphetamine, mephentermine, methoxyphenamine and 2-phenylethylamine were considered. When the sample solution was stirred, the basic analytes were extracted into the organic membrane phase, and then back-extracted into the final accepting aqueous drop. Since they were neutral at high pH, the analytes could be easily transferred from the high-pH aqueous solution to the organic membrane. Additionally, because they were easily protonated at low pH, they could then be back-extracted to the low pH aqueous drop from the intermediary organic phase. After a 10-min extraction at a stirring speed of 2000 rpm, the microdrop was withdrawn into the syringe needle and injected directly into a reversed-phase HPLC system for quantification. Using the same setup (described by the authors as LPME with back-extraction), also in combination with reversed-phase HPLC analysis, Zhao and Lee [81] achieved >100-fold enrichment factors for six alkyl- and chlorophenols, with LODs ranging from 0.5 to 2.5 μg/L.

The conventional two-phase SDME has been attempted for HPLC in any case. Liang et al. [82] determined phoxim (a biologically-active compound) in water samples by reversed-

Table 1
Applications of SDME combined with GC

Compounds	Real sample	Extractant solvent	Volume of organic solvent (μL)	Stirring speed (rpm)	Extraction time (min)	Detector	LOD	Ref.
Organophosphorus pesticides	Farm water	Carbon tetrachloride	0.9	1300	40	FPD	0.001–0.005 μg/L	[17]
Acetone	Human blood	Toluene	2	1100	–	MS	6.5 nM	[20]
Panaxynol	TCM	*n*-Octanol	5	–	–	MS	2 μg/g	[21]
Essential oil	TCM	Cyclohexane	1	–	–	MS	–	[22]
Alcohols	Beer	*n*-Octanol	0.8	1500	9.5	MS	1–97 μg/L	[23]
Aldehydes	Waste water	Decane	1	700	4	MS	0.08–0.32 ppb	[25]
Aldehydes	Human blood	Decane	2	1100	6	MS	0.12–0.16 nM	[26]
Paeonol	TCM	*n*-Octanol	1	700	4	MS	2.0 μg/g	[27]
Styrene	Solid	Butylacetate	2	–	5	MS	0.1–15 μg	[28]
Alcohols	Beer	Ethylene glycol	1	600	15	FID	3.8–52 μg/mL	[36]
Volatile organic compounds	Water	*n*-Hexadecane	1	1200	6	FID	0.72–5 μg/L	[37]
BTEX	Engine oils	*n*-Hexadecane	1	–	3	FID	–	[38]
PAHs	Water	1-Butanol	3	400	12	FID	4–41 μg/L	[39]
Organotins	Water and soil	Decane	2		1	MS	3 ng/L	[40]
Halocarbons	Ground and tap water	*n*-Octanol	1	1000	10	μECD	0.002–0.374 μg/L	[41]
Trihalomethanes	Drinking water	*n*-Octanol	1	800	10	ECD	0.15–0.4 μg/L	[42]
2-Butoxyethanol	Color samples	Benzyl alcohol	3	400	15	FID	0.5 μg/mL	[43]
Fatty acids	Blood plasma	*n*-Butyl phthalate	2	800	45	FID	0.02–0.08 μg/mL	[44]
Chlorobenzenes	Tap and well water	Toluene	2.5	1000	5	MS	0.003–0.031 μg/g	[45]
Geosmin	Lake water	1-Hexanol	1.5	600	15	MS	0.8, 3.3 ng/L	[46]
66 Volatile compounds	TCMs	*n*-Dodecane	0.8	–	20	MS	–	[47]
Halocarbons	Tap water	Tridecane	2	800	11	μECD	0.003–0.146 μg/L	[48]
Volatile solvents	Pharmaceutical product	*n*-Octanol	3	600	20	FID	0.2–2.0 mg/L	[49]
Acetone	Blood	Decane	2	1100	6	MS	2 nM	[50]
Volatile compounds	TCMs	Benzyl alcohol	1	–	20	MS	–	[51]
Chlorobenzenes	Tap water, sea water, waste water	Dodecane	2.5	500	5	ECD	0.1–0.3 μg/L	[52]
Nitroaromatic explosives	Ground water	Toluene	1	400	15	MS	0.08–1.3 μg/L	[57,61]
Amino acids	Urine	Chloroform:toluene (3:1, v/v)	1.5	200	5	MS	0.26–68 ng/mL	[62]
Amino acids	Urine	Chloroform:toluene (3:1, v/v)	1.5	200	5	FID	0.010–0.025 μg/mL	[62]
Anisaldehyde	Human urine and blood serum	Toluene	0.5	360	5	MS	2–5 ng/mL	[63]
Carbonyl compounds	Human blood	*n*-Octanol	2	1100	8	MS	0.24–0.62 nM	[64]
Chemical warfare agents	Water	Dichloromethane: tetrachloromethane (3:1, v/v)	1	300	30	MS	75–10 μg/L	[65]
Endosulfan	Tap and surface water	Isooctane	1.5	800	20	ECD	0.01 μg/L	[66]
Organophosphorous pesticides	Surface water	Toluene	1.5	800	15	MS	0.010–0.073 μg/L	[67]
Dialkyl phthalate esters	Food simulant	Dichloromethane: hexanene:toluene (7:3:0.5, w/w/w)	2	600	25	FID	0.03–0.4 μg/L	[68]
Iodine	Pharmaceuticals, iodized salt, milk powder and vegetables	*iso*-Octanol	1	300	15	MS	0.9998–10 ng/L	[69]
Phenols	River water	Hexyl acetate	2.5	250	15	MS	4–61 ng/L	[70]
Phenols	River water	Butyl acetate	2.5	600	10	MS	5–22 ng/L	[71]
Amphetamines	Urine	Chloroform	2	–	8	PDHID	0.015–0.05 μg/mL	[72]
Organochlorine pesticides	Tap water and reservoir water	*n*-Hexane	3	400	25	ECD	0.02–0.2 μg/L	[73]
Methoxyacetophe-none	–	Toluene	0.5	360	5	MS	1 ng/mL	[74]
Fungicides	Natural water and wine	Toluene	1.6	800	15	μECD	0.006–0.01 μg/L	[75]
Organophosphorus pesticides	Juice	Toluene	1.6	400	15	FPD <5 μg/L		[76]
Chloroacetanilide herbicides	Natural, tap and river water	Toluene	1.6	400	15	μECD	0.0002–0.114 μg/L	[77]
Bisphenol A	River water	Toluene	4	1000	90	MS	2 pg/mL	[78]
Organophosphorus pesticides	Lake water and fruit juice	Toluene	1.5	600	20	FPD	0.21–0.56 ng/mL	[79]

Note: FPD = flame photometric detection; MS = mass spectrometry; FID = flame ionization detection; ECD = electron-capture detection; μECD = microelectron capture detection; PDHID = pulsed discharge helium ionization detection.

phase HPLC-diode array detection after SDME. They used a 2.5 μL drop of hexane under high stirring speed (600 rpm). The LOD was as low as 10 ng/mL. The work on CFME combined with reversed-phase HPLC by the same group has already been referred to earlier in this narrative [57]. In this latter case, a relatively large volume (3 μL) of organic solvent was used as extracting phase, enabling the combination with HPLC, for reasons of requiring a larger extract size mentioned previously. For the same compound, phoxim, the LOD could be lowered to 5 ng/mL.

Gioti et al. [83] employed SDME to preconcentrate pseudohypericin, hypericin and hyperforin in urine. One microlitre of the organic solvent drop (*n*-octanol:chloroform, 7:3, v/v) was used as the extracting organic phase. After extraction, the organic solvent was transferred into a microvial and diluted with methanol up to 30 μL, then injected into HPLC. LODs were calculated to be from 3 to 12 ng/mL. However, since the extractant was diluted to make it more compatible with the HPLC mobile phase (95% methanol:5% phosphate buffer (at pH 2)), the enrichment factor was compromised.

In a more recent study [84], *o*-dibutyl phthalate, with good compatibility with the reversed-phase HPLC mobile phase used in this instance, was selected as the extractant solvent. This method was successfully applied to the determination of anaesthetics in biological fluids. LODs (lower than 0.05 μg/mL) were as good as previously reported GC methods.

As mentioned above, ionic liquids (ILs) have been used as solvents in SDME. Jiang and co-workers [5–9] demonstrated that ILs such as 1-alkyl-3-methylimidazolium hexafluorophosphate ([CnMIM][PF_6]) (n = to number of carbon atom) can be employed as extractant phase with high selectivity. Compared to *n*-octanol, a larger volume drop of the IL (3–10 μL) could be utilized that was sustainable for a longer extraction time (30 min). This led to better extraction efficiency. Moreover, the extract in the IL could be directly analyzed by HPLC. For PAHs, enrichment factors of between 42- and 166-fold were achieved. In addition, formaldehyde [6], phenols [7] and chlorinated anilines [8] were also studied using the same IL as extractant solvent, followed by HPLC analysis. In other similar work [9], the same workers studied the extraction capability of [CnMIM][PF_6] (n = 4 or 8) for a series of BTEX, PAHs, phthalates, phenols, aromatic amines, herbicides, organotin, and organomercury followed by analysis by different types of detection techniques. ILs were demonstrated by the authors to be compatible with many detection techniques, apart from HPLC, such as atomic absorption spectrometry and cold-vapor atomic fluorescence spectrometry.

3.2. SDME with inductively-coupled plasma-mass spectrometry (ICP-MS)

Hitherto, the overwhelming majority of work on SDME has been focused on organic compounds. There have been some attempts to extend the procedure to inorganic analytes. With the development of electrothermal vaporization (ETV), trace sample introduction to ICP-MS can be realized easily. The combination of SDME with ETV-ICP-MS can be an attractive technique for inorganic analytes with this possibility of injecting microlitre sample volumes. Xia et al. [59,60] reported some early studies in this area. With the assistance of 8-hydroxyquinoline as additive in a drop of chloroform as extractant solvent, free Al could be quantitatively extracted with high selectivity while Al_0 remained in the sample. Extracting for 8 min gave an enrichment factor of up to 210-fold. The LOD was as low as 3.3 pg/mL. The procedure was applied effectively to the determination of Al species in natural water, coffee and tea infusions [59].

The same authors also proposed the combination of recycling-CFME with low temperature ETV-ICP-MS for the determination of trace Be, Co, Pd and Cd in biological matrices [60]. In this case, benzoylacetone was employed as both extractant and chemical modifier for the purpose of enrichment and high selectivity. The LODs for Be, Co, Pd and Cd were 0.072, 0.56, 0.83 and 0.16 pg/mL, respectively. For the conventional (single-cycle mode) CFME, the LODs were higher (these were, for Be, Co, Pd and Cd, respectively, 0.12, 0.99, 1.5 and 0.27 pg/mL).

3.3. SDME with ETAAS

In 2003, Chamsaz et al. [35] reported a preliminary study on the combination of HS-SDME with ETAAS for the analysis of inorganic compounds. HS-SDME was demonstrated to be an efficient extraction technique for analyzing arsenic in real samples, like tap water and washing machine powder, with a relatively high pre-concentration factor, greatly increased sensitivity and lower detection limit. The method was simple and fast, with the overall time of extraction and determination for each sample taking only 8 min. The LOD of arsenic was calculated to be 45 pg/mL.

Later on, as already alluded to above, Bendicho's group published a series of papers in which they reported on headspace sequestration of a hydride by an aqueous drop as a preconcentration method. For example, the presence of Pd^{2+} in the suspended drop was demonstrated to be highly effective for sequestration of the arsine formed, a preconcentration factor of 70 being achieved for As(III). The LODs of As(III), Se(IV) and Sb(III) were 0.1, 0.15 and 0.2 ng/L, respectively [30,34]. When using in situ photogeneration of Se vapors in the presence of organic acids, the determination of selenium could be as low as pg/mL [33]. The method has also been optimized for preconcentration of other hydride-forming elements. An aqueous drop (3 μL) containing Pd(II) or Pt(IV) (50 and 10 mg/L, respectively) was used for headspace microextraction of methylmercury hydride (MeHgH) with a 40-fold enrichment factor. The LODs of methylmercury was 5 and 4 ng/mL (as Hg) with Pd(II) or Pt(IV) as trapping agents, respectively [32].

3.4. SDME with CE

Considering the general incompatibility of the extracting organic phase in conventional SDME with the normal running buffer in CE, the extract is not directly analyzable by this technique. However, as Ma and Cantwell [80] and Zhao and Lee [81] have shown with reversed-phase HPLC, to eliminate the need

for solvent evaporation and then constitution of the extract with a more suitable solvent, an in situ back-extraction into the final aqueous receiving solution, should allow the use of CE. Having such a three-phase system involves some careful manual manipulation of the extraction system. Choi et al. [85] realized more directly the combination of SDME (although referred to as LPME by the authors) with CE. A drop of basic aqueous phase was hung at the separation capillary inlet tip, covered with *n*-octanol as a thin organic film as the intermediary transfer phase. Then this two-phase droplet was placed into an acidic aqueous sample solution (comprising acidic compounds fluorescein and fluorescein isothiocyanate) for extraction. Although an interesting approach, the operation seems cumbersome, and appears inconvenient to be accomplished on a commercial CE instrument. Nevertheless, enrichment factors of three orders of magnitude were obtained after a 30-min extraction.

In another CE application, Fang et al. [86] combined online back-extraction with field-amplified sample injection following SDME. The analytes of interest were first extracted by DI-SDME. Four microlitres of chloroform was used as solvent. After extraction, this phase was transferred to a sample vial and "sealed" with a 40-μL water plug for back-extraction and CE injection. The latter two operations were carried out by first placing the tip of the capillary carefully in the aqueous phase. Under high voltage, the equilibrium between chloroform and water phase was disturbed. As the analytes migrated into the capillary from the aqueous phase, more analytes moved into the water from the chloroform extract, achieving back-extraction into the running buffer, with the help of the applied voltage. The procedure was applied to analyze cocaine and thebaine in a urine sample. The linear range approached three orders of magnitude and LODs were in the range of 2–10 ng/mL. However, the need to position the capillary tip carefully indicated the operation difficulty associated with this method.

More recently, as already mentioned above, water-based headspace LPME (true SDME mode) was developed for extracting several nitro-, chloro- and alkylphenols with subsequent analysis by CE [29]. In this case, because a sodium hydroxide solution was used as the extractant phase, direct extract introduction into a CE system was possible. The LODs achieved were low, between 0.001 and 0.003 μg/mL. This extraction system was completely organic solvent-free, inexpensive and convenient, and may be applied to other volatile or semivolatile ionizable compounds.

3.5. SDME-MS

Although MS is a common detection system used with GC for SDME applications, a more direct combination of SDME with MS is much less common. Recently, Sun et al. [87] developed HS-SDME with matrix-assisted laser desorption/ionization (MALDI)-Fourier transform (FT)-MS as a fast and effective method for the determination of 25 volatile basic components in tobacco. The small drop size of the extractant in SDME was considered to be advantageous because it was compatible with MALDI-MS sampling requirements. Here, a solution of 2,5-dihydroxylbenzoic acid dissolved in 1 mL of water/acetone (1:1, v/v) containing 1% trifluoroacetic acid was selected as the solvent. The work demonstrated the feasibility of applying the technique to complex samples without need of a chromatographic separation step.

In another MALDI-MS application, very recently, Sudhir et al. [88] introduced gold nanoparticles to the SDME solvent to extract peptides. The analysis was subsequently performed with atmospheric pressure MALDI-MS. Gold nanoparticles were dispersed into toluene with the help of tetraalkylammonium bromide. Then, a 0.8-μL drop containing the gold nanoparticles was used to extract Methionine and Leucine peptides. The extraction proceeded based on the surface charge of the gold nanoparticles and the isoelectric point (pI) of the peptides. Several SDME conditions, i.e. extraction time (1 min), sample agitation or stirring rate (240 rpm), and sample pH (>pI of the respective analytes) were optimized. Using this method, the lowest detectable concentrations were 0.2 and 0.17 μM for Met- and Leu-enkephalin peptides, respectively. The efficacy of the procedure was demonstrated with a urine sample. The interplay of nanoscience and bioscience via microextraction in this example is interesting.

4. SDME limitations and outlook

SDME has emerged as a viable sample preparation approach with which one could obtain generally acceptable analytical data. It can and has been shown to be routinely applicable to real world samples. Due to its simplicity, ease of implementation, and insignificant startup cost, SDME is accessible to virtually all laboratories. To be sure, it has some limitations, for example: (a) in its most basic (direct immersion) mode it requires careful and elaborate manual operation because of the problem of drop dislodgment and instability; (b) since more complex matrixes will compromise the stability of the solvent drop during extraction, an extra filtration step of the sample solution is usually necessary; this problem can be alleviated by carrying out HS-SDME; (c) notwithstanding the acceptable analytical performance mentioned above, the sensitivity and the precision of SDME methods need further improvement. The main issue lies with the adverse consequences of prolonged extraction time and fast stirring rate, since they may result in drop dissolution and/or dislodgement; and (d) SDME is not yet suitable as a routinely applicable online preconcentration procedure. Although some progress has been made to automate SDME, cost considerations will mean that the approach will not be widely accessible.

What other efforts can be made to further development of SDME? Based on the strictly defined criterion of the technique (namely, involvement of a "solvent drop") a specially-designed holder to better stabilize the solvent may be devised. This potentially could improve reproducibility and precision. As already mentioned above, the use of a low-volume microsyringe obviated dead-volume problems and gave improved performance. New materials with which to fabricate syringe needles that work just as well as stainless steel for sample introduction but which provide better adhesion with the extractant solvent, could be designed. This will also be useful to the continuous-flow mode of SDME. Also, a mixture of various organic solvents or addi-

tion of some additives (ion-pair, chelating and chiral agents) to the extractant drop can be expected to enhance selectivity and expand the range of SDME applications. In addition, novel solvents can continue to be exploited, such as ionic liquids, or mixtures of them, or mixtures with other, more conventional solvents.

References

[1] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[2] K.E. Rasmussen, S. Pederson-Bjergaard, Trends Anal. Chem. 23 (2004) 1.
[3] E. Psillakis, N. Kalogerakis, Trends Anal. Chem. 21 (2002) 53.
[4] H. Liu, P.K. Dasgupta, Anal. Chem. 68 (1996) 1817.
[5] J.-F. Liu, G.-B. Jiang, Y.-G. Chi, Y.-Q. Cai, Q.-X. Zhou, J.-T. Hu, Anal. Chem. 75 (2003) 5870.
[6] J.-F. Liu, J.-F. Peng, Y.-G. Chi, G.-B. Jiang, Talanta 65 (2005) 705.
[7] J.-F. Liu, Y.-G. Chi, G.-B. Jiang, C. Tai, J.-F. Peng, J.-T. Hu, J. Chromatogr. A 1026 (2004) 143.
[8] J.-F. Peng, J.-F. Liu, G.-B. Jiang, C. Tai, M.-J. Huang, J. Chromatogr. A 1072 (2005) 3.
[9] J.-F. Liu, Y.-G. Chi, G.-B. Jiang, J. Sep. Sci. 28 (2005) 87.
[10] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 68 (1996) 2236.
[11] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 69 (1997) 235.
[12] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 69 (1997) 2935.
[13] Y. He, H.K. Lee, Anal. Chem. 69 (1997) 4634.
[14] Y. Wang, Y.C. Kwok, Y. He, H.K. Lee, Anal. Chem. 70 (1998) 4610.
[15] L. Hou, H.K. Lee, J. Chromatogr. A 976 (2002) 377.
[16] S.-W. Myung, S.-H. Yoon, M. Kim, Analyst 128 (2003) 1443.
[17] F. Ahmadi, Y. Assadi, S.M.R. Milani Hosseini, M. Rezaee, J. Chromatogr. A 1101 (2006) 307.
[18] A.L. Theis, A.J. Waldack, S.M. Hansen, M.A. Jeannot, Anal. Chem. 73 (2001) 5651.
[19] G. Shen, H.K. Lee, Anal. Chem. 75 (2003) 98.
[20] C. Deng, N. Li, X. Wang, X. Zhang, J. Zeng, Rapid Commun. Mass Spectrom. 19 (2005) 647.
[21] C. Deng, X. Yang, X. Zhang, Talanta 68 (2005) 6.
[22] C. Deng, N. Yao, A. Wang, X. Zhang, Anal. Chim. Acta 536 (2005) 237.
[23] M. Saraji, J. Chromatogr. A 1062 (2005) 15.
[24] G. Ouyang, W. Zhao, J. Pawliszyn, Anal. Chem. 77 (2005) 8122.
[25] C. Deng, N. Yao, N. Li, X. Zhang, J. Sep. Sci. 28 (2005) 2301.
[26] N. Li, C. Deng, X. Yin, N. Yao, X. Shen, X. Zhang, Anal. Biochem. 342 (2005) 318.
[27] C. Deng, N. Yao, B. Wang, X. Zhang, J. Chromatogr. A 1103 (2006) 15.
[28] E. Hansson, M. Hakkarainen, J. Chromatogr. A 1102 (2006) 91.
[29] J. Zhang, T. Su, H.K. Lee, Anal. Chem. 77 (2005) 1988.
[30] S. Fragueiro, I. Lavilla, C. Bendicho, Spectrochim. Acta Part B 59 (2004) 851.
[31] S. Fragueiro, I. Lavilla, C. Bendicho, J. Anal. Atom. Spectrom. 19 (2004) 250.
[32] S. Gil, S. Fragueiro, I. Lavilla, C. Bendicho, Spectrochim. Acta Part B 60 (2005) 145.
[33] R. Figueroa, M. García, I. Lavilla, C. Bendicho, Spectrochim. Acta Part B 60 (2005) 1556.
[34] S. Fragueiro, I. Lavilla, C. Bendicho, Talanta 68 (2006) 1096.
[35] M. Chamsaz, M.H. Arbad-Zavar, S. Nazari, J. Anal. Atom. Spectrom. 18 (2003) 1279.
[36] A. Tankeviciute, R. Kazlauskas, V. Vickackaite, Analyst 126 (2001) 1674.
[37] A. Przyjazny, J.M. Kokosa, J. Chromatogr. A 977 (2002) 143.
[38] J.M. Kokosa, A. Przyjazny, J. Chromatogr. A 983 (2003) 205.
[39] S. Shariati-Feizabadi, Y. Yamini, N. Bahramifar, Anal. Chim. Acta 489 (2003) 21.
[40] V. Colombini, C. Bancon-Montigny, L. Yang, P. Maxwell, R.E. Sturgeon, Z. Mester, Talanta 63 (2004) 555.
[41] X. Li, X. Xu, X. Wang, L. Ma, Int. J. Environ. Anal. Chem. 84 (2004) 633.
[42] R.-S. Zhao, W.-J. Lao, X.-B. Xu, Talanta 62 (2004) 751.
[43] Y. Yamini, M. Hojjati, M. Haji-Hosseini, M. Shamsipur, Talanta 62 (2004) 265.
[44] L. Tan, X.P. Zhao, X.Q. Liu, H.X. Ju, J.S. Li, Chromatographia 62 (2005) 305.
[45] L. Vidal, A. Canals, N. Kalogerakis, E. Psillakis, J. Chromatogr. A 1089 (2005) 25.
[46] H. Bagheri, A. Salemi, J. Sep. Sci. 29 (2006) 57.
[47] J. Cao, M. Qi, Y. Zhang, S. Zhou, Q. Shao, R. Fu, Anal. Chim. Acta 561 (2006) 88.
[48] T. Zhang, X. Chen, Y. Li, P. Liang, Chromatographia 63 (2006) 633.
[49] X. Wang, T. Jiang, J. Yuan, C. Cheng, J. Liu, J. Shi, R. Zhao, Anal. Bioanal. Chem. 385 (2006) 1082.
[50] L. Dong, X. Shen, C. Deng, Anal. Chim. Acta 569 (2006) 91.
[51] L. Fang, M. Qi, T. Li, Q. Shao, R. Fu, J. Pharmaceut. Biomed. 41 (2006) 791.
[52] M. Khajeh, Y. Yamini, J. Hassan, Talanta 69 (2006) 1088.
[53] C. Dietz, J. Sanz, C. Cámara, J. Chromatogr. A 1103 (2006) 183.
[54] W.M. Mullett, J. Pawliszyn, J. Sep. Sci. 26 (2003) 251.
[55] W. Liu, H.K. Lee, Anal. Chem. 72 (2000) 4462.
[56] Y. Li, T. Zhang, P. Liang, Anal. Chim. Acta 536 (2005) 245.
[57] L. Guo, P. Liang, T. Zhang, Y. Liu, S. Liu, Chromatographia 61 (2005) 523.
[58] Y. He, H.K. Lee, J. Chromatogr. A 1122 (2006) 7.
[59] L. Xia, B. Hu, Z. Jiang, Y. Wu, L. Li, R. Chen, J. Anal. Atom. Spectrom. 20 (2005) 441.
[60] L. Xia, B. Hu, Z. Jiang, Y. Wu, Y. Liang, Anal. Chem. 76 (2004) 2910.
[61] E. Psillakis, N. Kalogerakis, J. Chromatogr. A 938 (2001) 113.
[62] Y.C. Fiamegos, C.G. Nanos, C.D. Stalikas, J. Chromatogr. 813B (2004) 89.
[63] B.-M. Liu, P. Malik, H.-F. Wu, Rapid Commun. Mass Spectrom. 18 (2004) 2059.
[64] N. Li, C. Deng, N. Yao, X. Shen, X. Zhang, Anal. Chim. Acta 540 (2005) 317.
[65] M. Palit, D. Pardasani, A.K. Gupta, D.K. Dubey, Anal. Chem. 77 (2005) 711.
[66] M.C. López-Blanco, S. Blanco-Cid, B. Cancho-Grande, J. Simal-Gándara, J. Chromatogr. A 984 (2003) 245.
[67] D.A. Lambropoulou, E. Psillakis, T.A. Albanis, N. Kalogerakis, Anal. Chim. Acta 516 (2004) 205.
[68] R. Batlle, C. Nerín, J. Chromatogr. A 1045 (2004) 29.
[69] P. Das, M. Gupta, A. Jain, K.K. Verma, J. Chromatogr. A 1023 (2004) 33.
[70] M. Saraji, M. Bakhshi, J. Chromatogr. A 1098 (2005) 30.
[71] H. Bagheri, A. Saber, S.R. Mousavi, J. Chromatogr. A 1046 (2004) 27.
[72] C. Casari, A.R.J. Andrews, Forensic Sci. Int. 120 (2001) 165.
[73] L. Zhao, H.K. Lee, J. Chromatogr. A 919 (2001) 381.
[74] H.-F. Wu, J.-H. Yen, C.-C. Chin, Anal. Chem. 78 (2006) 1707.
[75] Y. Liu, E. Zhao, Z. Zhou, Anal. Lett. 39 (2006) 2333.
[76] E. Zhao, L. Han, S. Jiang, Q. Wang, Z. Zhou, J. Chromatogr. 1114A (2006) 269.
[77] E. Zhao, W. Shan, S. Jiang, Y. Liu, Z. Zhou, Microchem. J. 83 (2006) 105.
[78] M. Kawaguchi, R. Ito, N. Endo, N. Okanouchi, N. Sakui, K. Saito, H. Nakazawa, J. Chromatogr. A 1110 (2006) 1.
[79] Q. Xiao, B. Hu, C. Yu, L. Xia, Z. Jiang, Talanta 69 (2006) 848.
[80] M. Ma, F.F. Cantwell, Anal. Chem. 71 (1999) 388.
[81] L. Zhao, H.K. Lee, J. Chromatogr. A 931 (2001) 95.
[82] P. Liang, L. Guo, Y. Liu, S. Liu, T.-Z. Zhang, Microchem. J. 80 (2005) 19.
[83] E.M. Gioti, D.C. Skalkos, Y.C. Fiamegos, C.D. Stalikas, J. Chromatogr. A 1093 (2005) 1.
[84] M. Ma, S. Kang, Q. Zhao, B. Chen, S. Yao, J. Pharmaceut. Biomed. 40 (2006) 128.
[85] K. Choi, Y. Kim, D.S. Chung, Anal. Chem. 76 (2004) 855.
[86] H. Fang, Z. Zeng, L. Liu, D. Pang, Anal. Chem. 78 (2006) 1257.
[87] S. Sun, Z. Cheng, J. Xie, J. Zhang, Y. Liao, H. Wang, Y. Guo, Rapid Commun. Mass Spectrom. 19 (2005) 1025.
[88] P.-R. Sudhir, H.-F. Wu, Z.-C. Zhou, Anal. Chem. 77 (2005) 7380.