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Advancing liquid/liquid extraction through a novel microfluidic device: Theory, instrumentation and applications in gas chromatography

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ABSTRACT

A new chip-based liquid–liquid extraction technique for sample preparation of aqueous samples for GC was developed. Extraction is performed in a segmented flow system with additional mixing provided by an etched channel structure. The dimensions of the device are optimized to allow benefiting of the advantages of chip technology without suffering from the limitations of over-miniaturization. Phase separation is performed with a novel phase separator developed in house. The instrumental set-up is simple. The results obtained for selected test analytes show that the extraction is quantitative (recoveries = 92–110%, RSD < 6%) for a wide range of hydrophobicities (Log $K_{o/w}$ = 0.86–4.79). The performance at different flow rates (0.5–6.0 mL/min) and flow ratios (β = 1–10) was evaluated, confirming the flexibility and the possibility to perform enrichment. The results obtained for a few selected applications demonstrate the suitability of the method to perform quick, simple and reliable sample preparation for analytes of interest in real samples.

1. Introduction

Liquid–liquid extraction (LLE) is one of the most widely used sample preparation techniques for aqueous samples in gas chromatography (GC). In practical use it suffers from a number of important disadvantages. It uses large volumes of aqueous samples and expensive and toxic organic solvents. Moreover, it is laborious, difficult to automate and time-consuming. The development of automated methods for sample preparation of aqueous samples with low solvent and sample consumption is therefore highly desirable.

In the past decades, several miniaturized solvent-free extraction techniques based on sorptive extraction have been successfully developed. An important example is solid-phase microextraction (SPME), a technique nowadays widely used because cheap, simple, easy to couple to a GC and portable for on-site sampling [1–3]. The same principle is used in other designs such as stir-bar sorptive extraction (SBSE) [4–6]. Due to the high donor–acceptor phase ratios these techniques can achieve high enrichment factors. However, the extremely small sorbent amounts lead to a low capacity which can significantly limit the sensitivity [7,8].

Continuous liquid–liquid extraction has also been investigated to minimize manual labor and achieve a better economy with respect to time and solvent consumption. The first example of this approach, based on Flow Injection Analysis (FIA) with segmented flow, was introduced by Karlberg in 1978 [9]. Since then, this technique has shown to be a powerful tool in automated analysis [10–12]. Roeraade [13] was the first to couple a continuous liquid–liquid extraction system on-line to a GC instrument. Since then different examples of automated liquid–liquid extraction coupled on-line to GC have been presented [14–17]. Mixing in these systems is usually obtained by using a knitted reactor, a PTFE tubing manually interlaced to induce turbulent mixing. Phase separation is generally performed using separators based on membranes or gravity [11,18]. Despite the significant progress made, automated LLE-GC so far has not been widely used. One of the weakest points is that the extraction units suffer from limited control and a poor reproducibility.

A great deal of attention has been paid to the use of microfluidic devices to perform continuous liquid extraction on a miniaturized scale [19–22]. In such microdevices extraction is based on molecular diffusion between two laminar flows formed in narrow channels. Excellent reproducibility is obtained due to the very high consistency between chips. The use of microchip-based LLE as a sample preparation method for GC was first presented by Xiao et al. [23]. The authors obtained a good linearity and repeatability, but a high stability and extraction efficiency were achieved only at very

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low flow rates. Similar observations were reported by other authors [24,25]. The poor stability of the laminar flow and the complexity and fragility of the instrumental set-up are due to the extreme miniaturization of these microchip methods. Use of the equipment in the field or its application for high throughput routine analysis is complicated at best [26]. We believe that the use of larger glass wafers applying segmented flow with obstacle-induced mixing is a very interesting alternative to overcome the limitations of such laminar-flow based, extremely miniaturized chip devices. Several studies on the use of microfluidic devices in engineering applications report that segmented-flow systems combine high efficiency with good robustness, allowing the use in applications requiring a high sample throughput [26,27]. When not over-miniaturized, the excellent repeatability and the precise process control of the chip-based extractors would be combined with the flow rate flexibility and good stability typical for segmented flow devices.

In this work we will report the use of a novel, miniaturized liquid–liquid extractor based on segmented flow for sample preparation in GC. The dimensions are optimized to allow benefiting from the advantages of chip technology without suffering from the disadvantages of over-miniaturization. The performance of the method in terms of speed, reliability and efficiency will be studied. Theoretical guidelines are derived to predict extraction yields as a function of the analyte's hydrophobicity ($K_{o/w}$) and the donor- and acceptor flow rates. The results obtained for a few selected applications will be discussed to demonstrate the suitability of the method for real life samples.

2. Theory

One of the most critical aspects of an analytical method is the limit of detection for the target analytes. This is defined by the minimum amount of analyte detectable by the detector used, m_{GC}^{min}. Therefore, the minimum amount that needs to be introduced into the injector is:

$$m_{inj}^{min} = m_{GC}^{min} \cdot SR \quad (1)$$

where SR is the split ratio of the injection here expressed as the total injector flow over the column flow.

When using continuous LLE as a sample preparation method, the minimum concentration in the extraction solvent that is necessary to detect the analyte can therefore be expressed as:

$$C_{org}^{min} = \frac{m_{GC}^{min} \cdot SR}{V_{inj}} \quad (2)$$

where V_{inj} is the injection volume.

During the extraction the analytes initially present in the water sample will distribute between the aqueous and the organic phase. Mass balance considerations require:

$$F_{aq} \cdot C_{aq}^{0} = F_{aq} \cdot C_{aq} + F_{org} \cdot C_{org} \quad (3)$$

where F_{aq} and F_{org} are the aqueous and the organic flow, C_{aq}^{0} is the initial concentration in the aqueous sample and C_{aq} and C_{org} are the final concentrations in the water and the organic phase, respectively. Eq. (3) can be rearranged to:

$$\frac{C_{aq}^{0}}{C_{org}} = \frac{C_{aq}}{C_{org}} + \frac{F_{org}}{F_{aq}} \quad (4)$$

In the equilibrium state Eq. (4) can be expressed as:

$$C_{org}^{eq} = C_{aq}^{0} \cdot \frac{K}{1 + (K/\beta)} \quad (5)$$

where C_{org}^{eq} is the concentration in the organic phase in the equilibrium state, K is the partition coefficient between the water donor

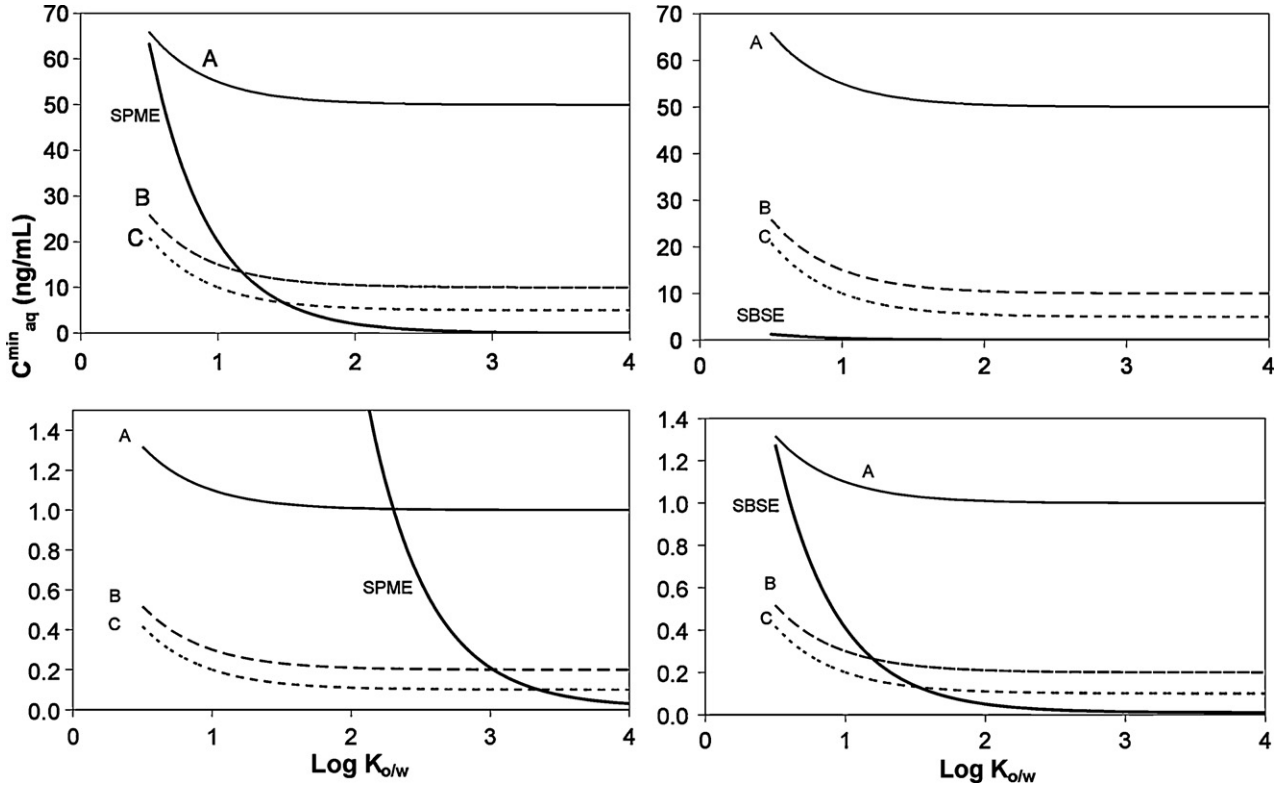

Fig. 1. Theoretical minimum detectable concentrations of analytes in the aqueous sample necessary to assure detection for a minimum detectable amount at the detector of 0.1 ng. LLE in splitless injection (top) and large volume injection (bottom) is compared to SPME and SBSE, respectively. For LLE phase ratios (defined as the ratio between the sample flow and the organic extractant flow) are assumed: 1 (A), 5 (B) and 10 (C). For both SPME and SBSE a sample amount of 10 mL is assumed. For SPME the PDMS volume is 0.5 μL (β = 20,000) whereas for SBSE it is 25 μL (β = 400).

phase and organic acceptor and β is the phase ratio, which here equals the flow ratio (F_{aq}/F_{org}). By combining Eq. (5) with Eq. (2) and rearranging, the minimum detectable concentration in the sample can be expressed as:

$$C_{aq}^{\min} = \frac{m_{GC}^{\min} \cdot SR}{V_{inj}} \cdot \left(\frac{1}{K} + \frac{1}{\beta}\right) \quad (6)$$

The same equation can be used to describe other common equilibrium extraction techniques such as SPME and SBSE. When applied to these techniques β is the ratio of phase volumes as opposed to the ratio of flow rates in continuous LLE. V_{inj} in the case of SPME or SBSE equals the sorbent volume, $V_{sorbent}$. As expected, increasing K values lead to improved detection limits. Also the phase ratio of the system is a crucial parameter. Eq. (6) shows that in general high β values are to be preferred as they lead to low $C_{aq}^{\min}$, as a result of favorable enrichment. This is especially true for compounds with large K. However, very small K values will lead to poor detection limits even at high phase ratios.

To compare continuous LLE with SPME and SBSE, theoretical $C_{aq}^{\min}$ values were calculated using Eq. (6). $K_{o/w}$ values are used for K due to their good similarity to the partition coefficients between water and PDMS [2,4,5]. Fig. 1 shows the results obtained. Continuous LLE with splitless injection is always less sensitive than SBSE and SPME for apolar analytes, whereas with high flow ratio β it can achieve better sensitivity than SPME for polar analytes. When applying large volume injection continuous LLE can achieve better detection limits than SPME for polar analytes already at $\beta = 1$, but it remains always less sensitive for very apolar compounds. In this case a sensitivity comparable to SBSE can be achieved with enrichment.

In the calculations presented above it is assumed that equilibrium is achieved. However, for SPME and SBSE non-equilibrium conditions are often employed. This means that the sensitivity for these two techniques shown in Fig. 1 is overestimated. In the case of continuous LLE, equilibrium is generally easier to achieve due to the large contact area between the organic and aqueous droplets.

3. Experimental

3.1. Chemicals and samples

P.A. grade ethyl acetate, methyl acetate, methyl butyrate, methyl hexanoate, methyl octanoate, methyl decanoate, methyl undecanoate, ethylbenzene and *p*-xylene were purchased from Aldrich (Zwijndrecht, The Netherlands). Methanol, n-pentane, n-hexane and n-heptane were purchased from Biosolve (Valkenswaard, The Netherlands). Sodium hydroxide, 50% aqueous sodium hydroxide and toluene were purchased from Merck (Darmstadt, Germany). Demineralized water (18.2 MΩ cm) was produced by an Arium 611UV Ultrapure Water System (Sartorius Stedim Biotech, Aubagne Cedex, France). The amphetamine sulphate used to prepare standard solutions and spiked urine samples was provided by DSM Resolve (Geleen, The Netherlands). Drug-free urine samples were obtained from healthy volunteers. The amphetamine fortified urine samples were prepared by spiking the drug-free urine with appropriate amounts of a standard solution of amphetamine at 100 μg/mL in 0.1 N sodium hydroxide. The BTEX samples were prepared by adding proper amounts of standard solutions of toluene, ethylbenzene and *p*-xylene (500 μg/mL in methanol) to demineralized water. The chlorinated pesticides samples were prepared spiking demineralized water with a standard solution containing the pesticides (25–240 μg/L in methanol) and adjusting the methanol amount to 2.5% to minimize adsorption.

3.2. Gas chromatography

All GC experiments were performed on two Hewlett Packard (Avondale, PA, USA) 6890 Series GC Systems both equipped with a split/splitless injector. One instrument was coupled to an HP 5973 Mass Selective Detector and the second instrument was equipped with an electron capture detector (ECD) and a flame ionization detector (FID). The hydrogen flow for the FID was produced by a hydrogen generator PG-H_2 Series 3 (Schmidlin-DBS AG, Neuheim,

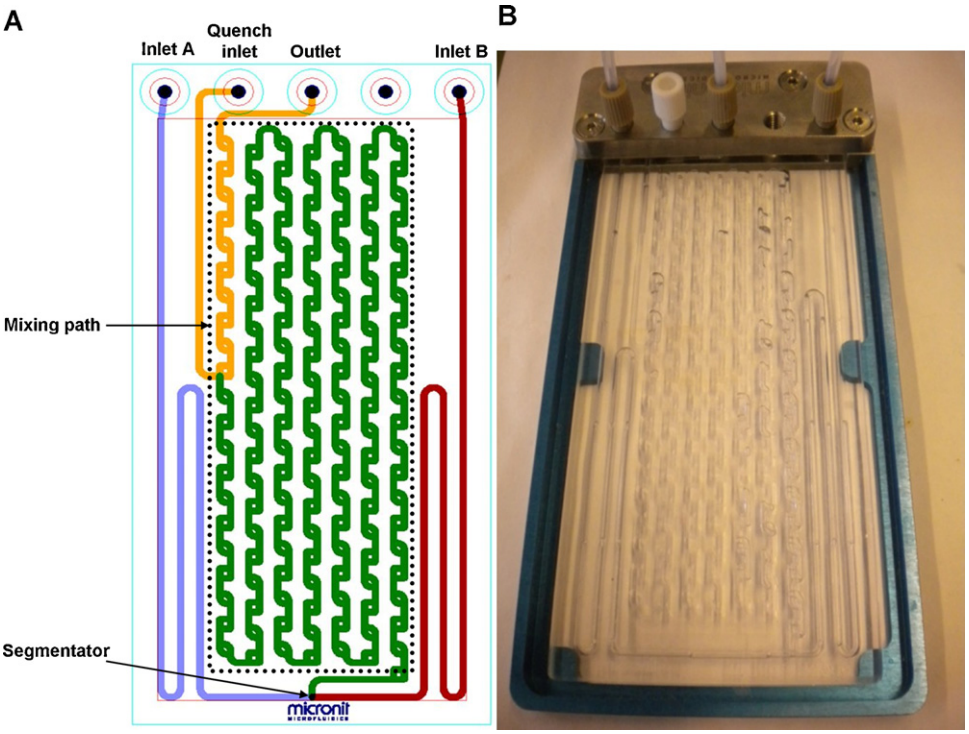

Fig. 2. Schematic representation (A) and photograph (B) of the microextractor.

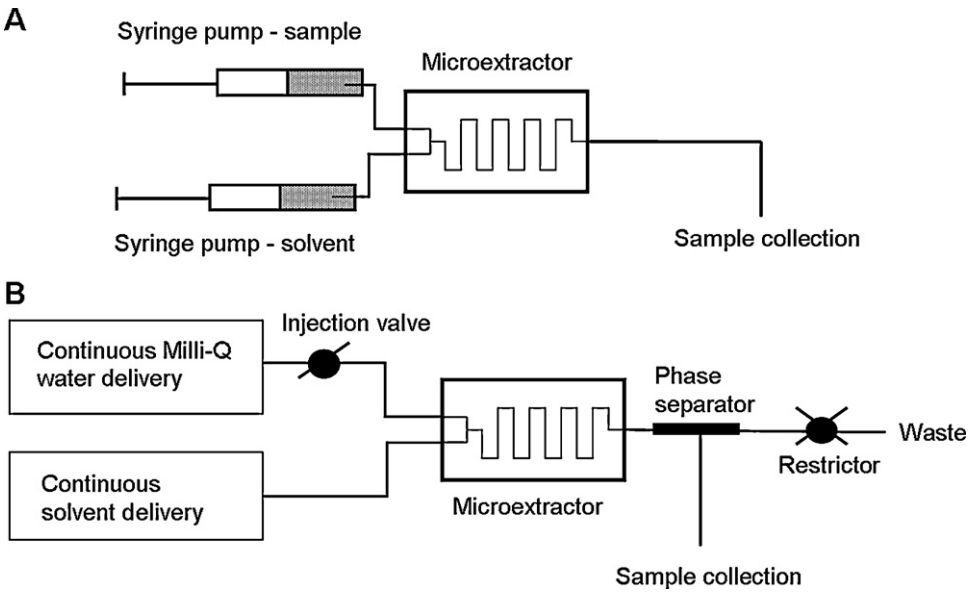

Fig. 3. Schematic representation of the set-ups for continuous introduction LLE (A) and plug-injection LLE (B), respectively.

Switzerland). All experiments were performed on an Rxi-5ms capillary column (length 30 m, i.d. 0.320 mm, film thickness 0.25 μm) from Restek (Bellefonte, PA, USA).

In all experiments an injector temperature of 250 °C and an injection volume of 1 μL were chosen and helium was used as the carrier gas. Test mixtures 1 and 2 (see Section 3.3.2) were injected using a carrier gas flow of 1.5 mL/min in the split mode (split ratio 1:50) and the splitless mode (splitless time 0.75 min), respectively. The oven temperature program started at 40 °C (2 min) and used a heating rate of 20 °C/min to the final temperature of 200 °C. The GC-FID analysis of the amphetamine at high concentrations was carried in the split mode (split ratio 1:50) using a carrier gas flow of 1.3 mL/min. The lower concentrations were detected via GC–MS analysis using a split ratio of 1:10, with data being acquired in SIM mode at *m*/*z* 44. The oven temperature program was the following: 100 °C (0.1 min) to 300 °C at 30 °C/min. The method used for the BTEX analysis applied splitless injection (splitless time 0.5 min) with FID detection at a column flow of 2 mL/min. The temperature program started at 30 °C (2 min) and used a heating rate of 10 °C/min to the final temperature of 120 °C. The analysis of the chlorinated pesticides was performed injecting 2 μL in splitless mode (splitless time 0.75 min) with ECD detection at a carrier gas flow of 1 mL/min. The oven temperature program started at 100 °C (0.2 min) and used a heating rate of 4 °C/min to the final temperature of 250 °C.

3.3. Microreactor-based LLE

3.3.1. Microextractor

The device used to perform the liquid–liquid extractions was a system originally designed as a microreactor for parallel chemical reactions, provided by Micronit (Enschede, The Netherlands). A photograph and schematic of the device are shown in Fig. 2.

The device consists of a glass-wafer (14.6 cm × 7.3 cm × 0.4 cm, total volume 3.4 mL) into which channels (0.7–1.5 mm) are etched. The chip has three inlets and one outlet. When used as a microreactor two of the inlets are used to introduce the reagents while the third inlet, situated close to the outlet, can be used to introduce a quenching reagent. In our use as microextractor this third inlet was not used. The device is placed into a Hastelloy connection block with aluminium frame (Micronit). This grants robustness to the chip and allows to connect the device to peripheral equipment such as solvent and sample delivery units. The split-and-recombine mixing structure of the chip ensures that, when immiscible liquids are introduced, a well-controlled segmented flow is generated at the T-intersection where the two streams encounter. This flow

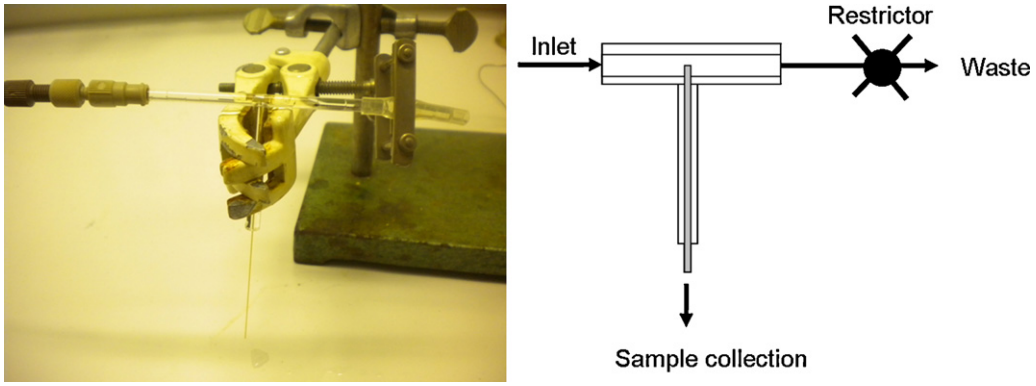

Fig. 4. Photograph (left) and schematic representation (right) of the phase separator developed.

goes through the mixing path where the channel structure provides efficient contact between the aqueous and organic segments and enhances internal circulation in the flow segments. In engineering literature the discrete liquid segments in segmented flow systems are normally referred to as "slugs" [26–28]. In this work we will also address them with this term.

3.3.2. Instrumental set-ups

Two different instrumental modes were used in this work, continuous introduction LLE and plug-injection LLE. Schematic drawings of the two systems are shown in Fig. 3.

When the volume of sample available was large, continuous introduction LLE could be performed (Fig. 3A). The aqueous sample and the organic solvent were loaded into interchangeable Fortuna Optima glass syringes with clear-glass-barrels (Poulten and Graf, Wertheim, Germany). They were delivered into the microextractor by two KDS210 syringe pumps (KD Scientific, Holliston, MA, USA). The connections of the syringes with the extractor were made with 1/4 in. OD PEEK tubes and flat-bottom connections. The microextractor was first rapidly flushed with demineralized water and the organic solvent at the desired flow-rate ratio in order to eliminate any air present. Once bubble-free, the syringe with the water was replaced with a syringe containing the sample. The system was operated until a steady plateau concentration was reached in the accepting organic solvent flow. At that point it was possible to sample from the organic flow for GC analysis. This set-up was employed to perform the fundamental studies on the performance of the micro-extractor.

When only a limited sample volume was available an alternative mode, here referred to as plug-injection LLE (Fig. 3B), was used. In this mode a constant water flow is delivered by a pump and the sample is introduced as a discrete plug. This second set-up was built using two HP-1050 Series Quaternary HPLC pumps (Hewlett Packard, Avondale, PA, USA) and a Rheodyne six-port valve (IDEX, Wertheim-Mondfeld, Germany) installed in the water line. The loop was manually loaded with the sample using a syringe. A discrete plug of sample could so be introduced via the six-port valve into the continuously flowing stream of clean water. The sample loop volumes employed were 0.5, 1.25 and 2.4 mL, respectively. Two HPLC columns (25 cm, 4.6 mm, 5 μm) were used to provide the backpressure necessary to ensure good performance of the pumps. This set-up was employed for all real samples studied (Section 4.2).

In both set-ups the connections between the different parts of the device were assembled reducing as much as possible the length of the tubing. This was done to minimize the risk of adsorption of analytes and to avoid the addition of extra dead volume to the system. Furthermore, limiting the total volume of the system reduced the required amount of expensive/toxic solvents.

3.3.3. Phase separator

Phase separation was performed using a novel separator designed and developed in house. A schematic representation and a picture of the prototype assembled are shown in Fig. 4.

A fused silica capillary coated with 5% diphenyl/95% dimethylpolysiloxane (length 9 cm, i.d. 0.10 mm, film thickness 0.40 μm) was inserted perpendicularly into a glass tube (length 5 cm, o.d. 5 mm, i.d. 3 mm) through a small hole, the opening being positioned in the centre of the tube. For improved mechanical strength the capillary was supported by a second glass tube glued to the first one. This support tube had a very small internal diameter, matching the external diameter of the capillary. The system was connected with the outlet of the extractor using 1/4 in. OD PEEK tubes and fingertight connections. An adjustable flow restrictor was used to create the backpressure necessary to generate flow through the capillary. Due to the low polarity of the stationary phase non-polar organic solvents could go through the capillary to be collected into a vial, whereas the aqueous phase was repelled and delivered to waste.

Table 1
Composition of the standard mixtures used to evaluate the performance of the extractor.

	Component	Log $K_{o/w}$	Conc. (μg/mL)
Test mixture 1	Methyl acetate	0.37	101
	Methyl butyrate	0.86	90
	Ethyl acetate	1.36	70
Test mixture 2	Methyl hexanoate	2.34	2.30
	Methyl octanoate	3.32	0.90
	Methyl decanoate	4.30	1.60
	Methyl undecanoate	4.79	0.70

3.3.4. Test solutes

The analytes chosen for the fundamental studies are an ethyl ester and several methyl esters of fatty acids (FAMEs) with increasing $K_{o/w}$. Two aqueous test mixtures were prepared by direct weighing and dilution. Methanol was added to the water phase as organic modifier at 5% (v/v) to eliminate the risk of adsorption. The analytes and the details about their concentrations are listed in Table 1. n-Pentane and n-heptane were used as organic extractant for test mixture 1 and 2, respectively.

4. Results and discussion

4.1. System evaluation

The extraction efficiency of the device was evaluated using the methyl/ethyl ester mixtures described in Section 3.3.4. The continuous LLE mode was used because of its simplicity and the large availability of sample. Phase separation was performed manually immediately after the liquid left the extractor chip.

4.1.1. Extraction yield versus hydrophobicity

The relationship between hydrophobicity and extraction yield was studied in a first series of experiments. The extraction yield was calculated as the percentage of analyte transferred to the organic phase. A low recovery is an obvious consequence of unfavorable partition coefficients. However, it can also be caused by the equilibrium not being reached as a result of poor performance of the extractor. In these first experiments a total flow rate of 1 mL/min with a flow ratio of 1 was selected. The results obtained are shown in Fig. 5. As can be seen from this figure good recoveries (92–110%)

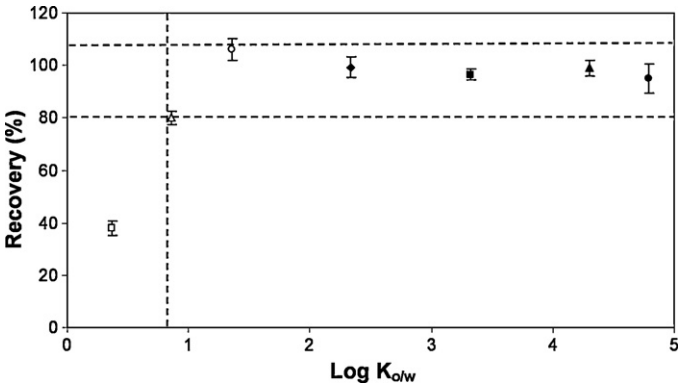

Fig. 5. Recovery obtained with the new microextractor-based LLE at a total flow rate of 1.0 mL/min and a flow ratio of 1. RSD% are calculated on $n = 3$. Analytes: methyl acetate (□ Log $K_{o/w}$ = 0.37), methyl butyrate (△ Log $K_{o/w}$ = 0.86), ethyl acetate (○ Log $K_{o/w}$ = 1.36), methyl hexanoate (♦ Log $K_{o/w}$ = 2.34), methyl octanoate (■ Log $K_{o/w}$ = 3.32), methyl decanoate (▲ Log $K_{o/w}$ = 4.30) and methyl undecanoate (● Log $K_{o/w}$ = 4.79).

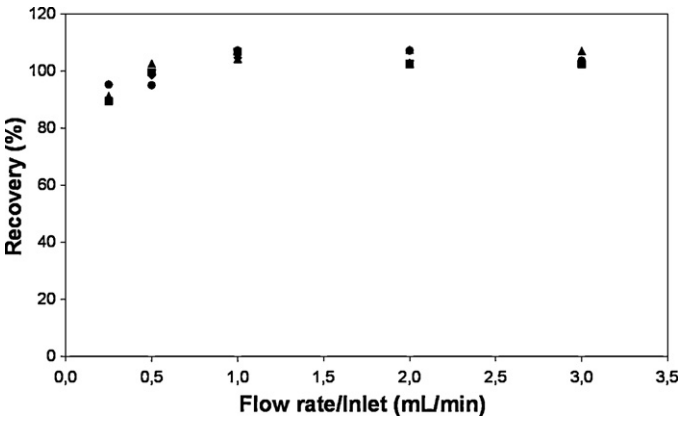

Fig. 6. Effect of flow velocity on the recovery using the microextractor at a flow ratio of 1. Analytes: methyl hexanoate (♦ Log $K_{o/w}$ = 2.34), methyl octanoate (■ Log $K_{o/w}$ = 3.32), methyl decanoate (▲ Log $K_{o/w}$ = 4.30) and methyl undecanoate (● Log $K_{o/w}$ = 4.79).

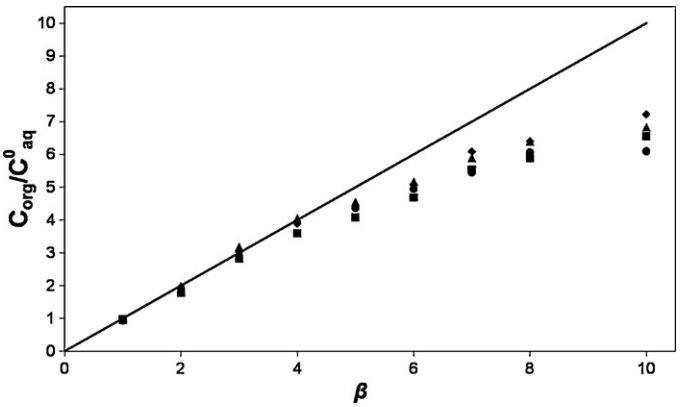

Fig. 7. Enrichment. Recovery obtained at different flow ratios β. Test mixture: methyl hexanoate (♦ Log $K_{o/w}$ = 2.34), methyl octanoate (■ Log $K_{o/w}$ = 3.32), methyl decanoate (▲ Log $K_{o/w}$ = 4.30) and methyl undecanoate (● Log $K_{o/w}$ = 4.79). The organic flow is 0.5 mL/min while the sample flow is increased from 0.5 to 5 mL/min. The solid line represents the theoretical behavior (Eq. (7)).

were obtained for compounds with Log $K_{o/w}$ values above approximately 1.4, while for more hydrophilic analytes the recoveries were lower. The good recoveries obtained for non-polar compounds indicate that the low recoveries for the more polar analytes are due to their unfavorable partition coefficients and are therefore an intrinsic property of the acceptor/donor system and do not reflect poor performance of the extractor. Due to the favorable acceptor volume our microextractor-based method is quantitative (>80%) for compounds with Log $K_{o/w}$ > 1, showing an improvement in the extraction yields of polar analytes when compared to SPME and SBSE.

4.1.2. Influence of flow rates

The actual distribution of the analytes between the aqueous sample and organic acceptor flows is determined by two factors: the thermodynamic equilibrium distribution and the extent to which equilibrium is reached. Exhaustive mass transfer or full equilibration are not always necessary. Well-controlled non-equilibrium conditions can be applied when using proper calibration.

Increasing the flow rates means shorter residence times in the chip and therefore could lead to poor extraction yields if equilibrium is not reached. On the other hand, in segmented flow systems high flow rates result in shorter aqueous and organic slugs with increased internal circulation, enhancing the driving force for mass transfer [26–28]. The influence of the flow rates on the extraction yield is therefore difficult to predict. Here it was investigated experimentally using different flow rates at a constant flow ratio of 1. The results are shown in Fig. 6. It can be observed that, for all test compounds studied, very good recoveries (89–107%) are obtained at all flow rates tested. No significant influence of the polarity of the analyte was observable (with Log $K_{o/w}$ > 2.34). The residence times in the chip ranged from around 7 to less than 0.5 min. Effective mixing apparently takes place leading to complete extraction even at short residence times, indicating that fast extractions can be performed. Fig. 6 also shows that at high flow rates recovery is slightly higher for all four analytes, confirming that mixing improves at higher flow rates.

The residence time in the chip is affected also by the length of the mixing path. This is fixed and could not be tuned. However, a preliminary assessment of its influence on the extraction yields was carried out by delivering the organic solvent via the inlet situated close to the end of the mixing path, normally kept closed. This way the mixing path was reduced to about 7% of its total length. The recovery obtained this way was 70% compared to the use of the full mixing path. This shows that the size of the extractor can be reduced to minimize the sample- and organic solvent consumption without compromising the extraction efficiency.

4.1.3. Enrichment

The detection limit of the method is a critical aspect for many applications. One of the biggest advantages of the miniaturized or solvent-free techniques developed in recent years is that they can often combine the extraction with an enrichment step. The possibility to achieve pre-concentration with our chip-based LLE was investigated.

The enrichment factor obtained for a given analyte at certain flow setting follows from Eq. (5). When K is very large, this equation can be simplified to:

$$\frac{C^{eq}_{org}}{C^{0}_{aq}} \approx \beta \tag{7}$$

Therefore, for high K values the concentration in the organic phase in equilibrium will increase linearly with the flow ratio between sample and extraction solvent. In practice unfortunately the range of flow rate ratios that can be applied will be limited. At very high or very low ratios the required segmented flow pattern might not be established.

The possibility to perform extraction combined with preconcentration was investigated in a separate set of experiments. We varied the flow ratio by increasing the sample flow rate at constant organic flow. The results obtained are shown in Fig. 7. Quantitative recoveries are obtained for β values up to 4. At higher ratios the recovery is gradually reduced. This can be explained by the fact that the large slugs of sample obtained at high aqueous-over-organic flow ratios are characterized by a low internal circulation, negatively affecting mass transfer between the two phases and preventing equilibrium from being reached. This phenomenon has been described by Okubo et al. [26]. However, as previously stated it is not always necessary to be at equilibrium. In fact Fig. 7 shows that the detection limits obtained for high β, where the deviation from the equilibrium situation was the largest, were still better than at low β where equilibrium was achieved.

A way to reach equilibrium is to increase the residence time by employing lower flow rates. Lower flow rates might on the other hand lead to a poorer extraction efficiency due to a decrease of the slugs' internal circulation. In order to evaluate whether the first or the latter contribution was more significant, different flow rates were tested at a constant flow ratio of 10 for which a strong deviation from linearity was previously obtained. At lower

Table 2
Influence of the flow rates on the recovery obtained for test mixture 2 at a constant flow ratio of 10.

Component	Log $K_{o/w}$	F_{aq}:F_{org} (mL/min)			
		5.0:0.5	2.5:0.25	1.0:0.1	0.5:0.05
Methyl hexanoate	2.34	72.2	76.6	82.1	99.9
Methyl octanoate	3.32	65.6	74.9	79.0	98.0
Methyl decanoate	4.30	68.2	80.1	83.2	101.3
Methyl undecanoate	4.79	61.0	87.4	91.1	102.8

total flow rates the recoveries increased significantly for all compounds and became quantitative again for the flow combination 0.5:0.05 mL/min (see Table 2). This result confirms that the loss of efficiency at higher flow ratios was due to the limited residence time in the chip, which did not allow equilibrium to be reached. When increasing the extraction time by using lower flow rates full equilibrium was again obtained with an enrichment factor up to 10. These results are in contrast with what was previously observed for a flow ratio of 1, where high flow velocities proved to enhance the efficiency slightly (Section 4.1.2). Apparently, when the slugs are of comparable size mass transfer occurs faster whereas longer extraction times are required if small organic slugs are used to extract large sample slugs. This confirms that the slugs' relative dimensions play a very important role regarding the time required to obtain complete mass transfer [26].

From the above results it is clear that very high enrichment factors comparable to those of solvent-free methods like SPME and SBSE are not possible. However, this is true for all on-line liquid–liquid extraction techniques so far developed, which generally involve limited or no enrichment. Additionally, obtaining efficient phase separation and recovering the organic phase can be a practical issue at flow ratios exceeding 5 or 10 [11,16]. Fortunately, as already discussed in Section 2, by applying large volume injection methods excellent detection limits can also be obtained in liquid extraction even when using relatively high organic-over-water ratios [29].

4.1.4. Elution profile in the plug-injection mode

In the continuous extraction mode as applied above, the sample is continuously fed into the extractor and the analyte concentration in the organic acceptor stream will reach a continuous, stable plateau. It is then possible to collect the sample at any interval. In the plug-injection mode the sample is introduced as a discrete plug into a continuous water stream. The combination of the loop size and the aqueous and organic flows determines the width and shape of the plug profile in the organic stream at the outlet of the microextractor. When using this extraction mode a representative fraction for analysis can be taken at the maximum of the extraction band. The location of this maximum is difficult to predict from theory but can be easily determined experimentally.

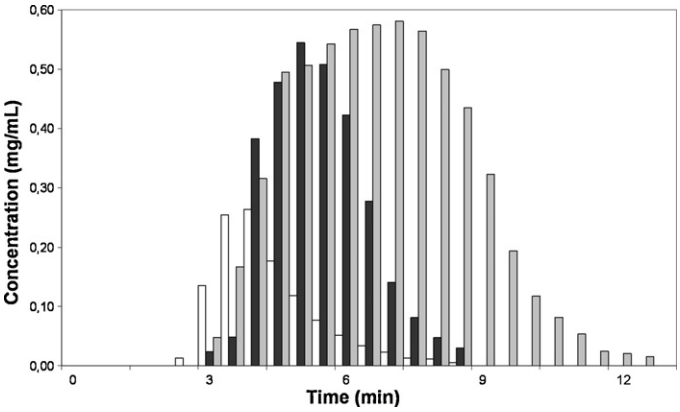

Fig. 8. Extraction elution profiles for a methyl hexanoate solution (0.58 mg/mL, 5% v/v methanol) with plug-injection LLE using a total flow rate of 1.0 mL/min and a flow ratio of 1. Three different sample volumes are shown: 0.5 mL (white blocks), 1.25 mL (black blocks) and 2.4 mL (grey blocks), respectively.

In this work we investigated the elution profiles with different loop sizes (0.5, 1.25 and 2.4 mL) and total flow rates (1.0, 1.5 and 2.0 mL/min) at a constant flow ratio of 1. A solution of methyl hexanoate in water with 5% methanol at 0.58 mg/mL was used as sample. Extraction was performed using n-heptane. The resulting outlet flow was collected in narrow fractions. The test compound was then quantified in each of the fractions by GC-FID. The elution profiles were reconstructed by plotting the concentrations found versus time or eluted volume. The sample volume seemed to be the most critical parameter effecting shape and dilution, whereas the flow rate showed little influence. Fig. 8 summarizes the results obtained for the three loops at a total flow rate of 1 mL/min. As can be seen the elution profiles are broadened blocks and they show a tendency to tail, in particular for the smallest loop volume. It is evident that dilution is largest for the smallest loop. For the 2.4 mL loop the final sample band reaches a plateau with no dilution between 6 and 8 min. The proper sampling window can easily be determined. In this case in order to maximize the sensitivity of the method it seems convenient to use the 2.4 mL loop and collect the sample in the interval 6–8 min from sample introduction. It is noteworthy that when sampling a discrete slice there is no actual need to wait for the entire sample plug to be eluted before injecting the following sample. In this way the sample throughput can be significantly increased.

4.2. Applications

The extraction method described was applied to a set of applications to assess its feasibility. In applying the method a number of choices have to be made. The selection of the plug-injection mode against continuous extraction is based on practical aspects. For example, when the available sample volume is limited, plug-injection is preferred. The selection of the total flow rate and flow ratios is also important and must be made to fulfill the sensitivity and throughput requirements of the particular application.

In this work we chose to use the plug-injection mode because of its easier automation. Based on the results obtained during the evaluation of the sample elution profiles, we decided to use a 2.4 mL loop and a flow of 0.5 mL/min per inlet to achieve a dilution-free situation and to maximize the sensitivity. Water-free extracts were collected in a vial from the phase separator in the time window 6–8 min after the injection.

4.2.1. Amphetamine in urine samples

The control of illicit substances of abuse has become very important because of their widespread use and the correlated risks for health and social behavior. Urine is the matrix of choice for screening and identification of many of these drugs because the concentration levels of the compounds in the urine are relatively high in this matrix and sample pretreatment is straightforward. Amphetamine is one of the most common recreational drugs. In this work we assessed the applicability of our automated LLE method to detect amphetamine in urine samples. Since this compound is largely excreted in the native form, only the amphetamine itself was measured [30].

A first assessment of the extraction efficiency for the target compound was performed on standard solutions of amphetamine in 0.1 N sodium hydroxide at 100 μg/mL. The very basic pH was necessary in order to deprotonate the amphetamine and facilitate transfer into the organic acceptor phase. Toluene was selected as the organic extraction solvent because it was known to pro-

Table 3
Extraction of toluene, ethylbenzene and *p*-xylene from aqueous samples with Vortex LLE and our microextractor-based LLE. The recoveries shown are obtained for an aqueous solution at 450 μg/L in each analyte (5% methanol, v/v). RSD% are calculated on $n = 3$. The linearity data for the microextractor are recorded in the concentration range 0.45–4.5 μg/mL.

Component	Log K_o/w	Vortex	Microextractor			
		Recovery (%)	Recovery (%)	Slope	Intercept	R^2
Toluene	2.54	75.0 (±1.6)	73.9 (±1.6)	7.5518	−0.4785	0.9995
Ethylbenzene	3.03	83.2 (±2.9)	74.9 (±1.0)	6.9622	−0.1313	0.9978
p-Xylene	3.09	68.7 (±1.5)	60.2 (±1.3)	6.2695	0.3473	0.9976

vide good extraction for this analyte [31]. Quantitative recovery and good precision (101.0% ± 3.1, $n = 3$) were achieved. This result is consistent with those of the model experiment with the fatty acid esters. The polarity of the amphetamine (Log $K_{o/w} = 1.79$) is within the hydrophobicity range for which complete extraction was obtained.

Amphetamine-containing urine samples at abuse-alert level (0.5 μg/mL) were prepared by spiking fresh drug-free urine. 10 mL of the urine were then brought to pH 12 by adding two droplets of 50% aqueous sodium hydroxide. The samples were diluted 1:1 with demineralized water and filtered through a 0.45 μm syringe filter prior to extraction to eliminate any risk of clogging the extractor channel. After an extraction the system was flushed for 5 min at 1 mL/min per inlet before introducing the next sample. Blanks acquired between samples confirmed the absence of memory effects. Although a slight emulsion formation occurred in the urine/toluene segmented flow, efficient phase separation was successfully achieved. No trace of urine was detected in the organic vial after separation. The recovery obtained was 73% (±3, $n = 5$). The experiment was repeated on a standard solution of amphetamine in water with a concentration equal to that in the urine samples after dilution. The recovery obtained now was 99%, indicating matrix effects to be the cause of the non-quantitative recovery. The linearity of the extraction from urine was also assessed using spiked samples in the concentration range 0.5–10 μg/mL. The results obtained show a very good linearity ($R^2 = 0.9992$) and confirm the suitability of this approach for quantitative analysis in this application. The limit of detection of the method, defined as S/N = 3, was estimated to be 0.01 μg/mL.

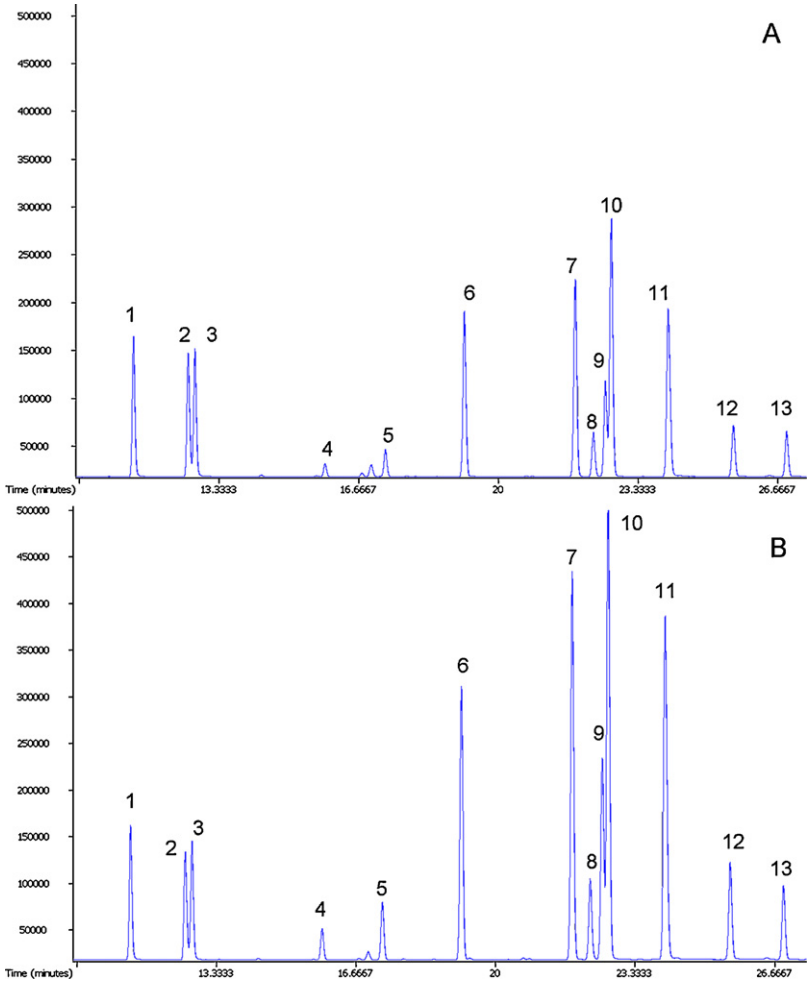

Fig. 9. Comparison between the chromatograms obtained extracting an aqueous solution containing 13 chlorinated pesticides (10–100 μg/L, 5% v/v methanol) with our microextractor (A) and Vortex (B). Peaks: 1) α-BHC; 2) β-BHC; 3) Lindane; 4) Heptachlor; 5) Aldrin; 6) Heptachlor exo-epoxide; 7) Dieldrin; 8) 4,4′-DDE; 9) 2,4′-DDD and 4,4′-DDD; 10) Endrin; 11–12) 2,4′-DDT and 4,4′-DDT; 13) Endrin ketone (Endrin decomposition product).

Conventional LLE was performed to provide reference data. Here 0.8 mL of fortified urine and 0.8 mL of toluene were Vortex-extracted for 5 min. One significant drawback observed for Vortex extraction was the pronounced formation of emulsions. The samples had to be centrifuged for 5 min at 3500 rpm to obtain a clear phase separation. For some of the Vortex samples this process was not sufficient and even repeated centrifugation for 15 min did not give clear phase separation, resulting in the loss of several samples. During the experiments performed with our chip-based method, this problem was absent. The recovery obtained in Vortex extraction was 81.0%. The recovery of our method was 90% compared to Vortex extraction.

As a consequence of the moderate miniaturization the sample amount required and the solvent consumption, in the order of few milliliters, are still larger than in small scale LLE like in-vial Vortex LLE. However, the device used is a prototype and additional design optimization to further decrease the chip volume without losing efficiency is not only desirable but also possible. Besides, significant advantages over Vortex extraction are the possibility to achieve semi- or complete automation and to combine on-line phase separation. This way high sample throughout can be achieved and problems such as the formation of emulsions which would require an additional step like centrifugation can be eliminated, reducing the analysis time and minimizing manual intervention.

4.2.2. Volatile aromatic compounds in water

BTEX are emitted into the environment from an extensive variety of sources related to human activity such as different industrial processes. They are well-known for their harmful influence on human health. The maximum allowable levels for these compounds for drinking water according to the EPA are 5 μg/L for benzene, 700 μg/L for ethylbenzene and 1000 μg/L for toluene and xylene, respectively. The efficiency of our method for the detection of toluene, ethylbenzene and *p*-xylene was evaluated.

The sample extracted was a 5% methanol aqueous solution containing toluene, ethylbenzene and *p*-xylene at a concentration of 450 μg/L. n-Hexane was used as the extraction solvent. Conventional LLE was performed as the reference method. Here 0.8 mL of fortified water and 0.8 mL of hexane were Vortex extracted for 5 min. The linearity of our method was also tested. The results obtained are shown in Table 3. As can be seen good recoveries and linearity were obtained for all three analytes. The extraction yields obtained using the microextractor were consistent with the reference method for toluene, while for ethylbenzene and *p*-xylene an efficiency loss of about 10% is seen when compared with conventional LLE.

4.2.3. Organochlorinated pesticides in water

Nowadays a great deal of attention is paid to the detection and quantification of chlorinated pesticides in drinking and surface water. In this work we assessed the efficiency of our extraction method to detect several chlorinated pesticides in water.

The sample used was an aqueous solution containing 13 different chlorinated pesticides at different concentration levels (10–100 μg/L). Different amounts of methanol (2–5%, v/v) were tried in order to minimize adsorption. The best results were obtained for the sample with 2.5% of modifier. n-Hexane was used as the extraction solvent and Vortex extraction was performed as the reference method. Here 0.8 mL of fortified water and 0.8 mL of hexane were Vortex extracted for 5 min. The chromatograms obtained with the two methods respectively are shown in Fig. 9. As can be observed, for the first three peaks (α-BHC, β-BHC and Lindane) the results obtained with our method are consistent with those obtained with the reference method (100–109%). This was expected since these compounds have a $\text{Log}\,K_{o/w}$ of 4.26, which is within the hydrophobicity range for which complete extraction was previously obtained (Section 4.1.1). On the other hand, for the remaining pesticides ($\text{Log}\,K_{o/w}$ = 5.45–6.79) the recovery is clearly lower for our method (41–59% when compared to Vortex). A possible explanation is that for the more unpolar and highly chlorinated analytes adsorption can occur either on the glass surface of the microextractor or/and on the tubing. Nevertheless, the sensitivity of the method is still good enough to allow detection of all compounds at the concentration levels investigated.

5. Conclusions

A new chip-based liquid–liquid extraction system with segmented flow for the extraction of aqueous samples in GC was successfully developed. The use of a chip grants excellent repeatability and process control. Moreover, the manual labor and the solvent consumption are reduced compared to classic LLE. Miniaturization is moderate in order to avoid suffering from the limitations typical of microfluidic devices. The instrumental set-up is simple and mechanically strong. This simplicity and robustness, combined with the stability typical of segmented flow, allows the use for automated operation and/or use in the field. The prototype of a new phase separator was also evaluated and used in series with the microextraction system, giving good results in terms of reliability, efficiency and flexibility.

The results obtained show that the microextractor has a wide application range in terms of hydrophobicity of the target analytes, performing (semi-)quantitative extraction for $\text{Log}\,K_{o/w}$ values from 1 to 5 and higher. The device allows using a wide range of flow rates from 0.5 to 6.0 mL/min. With these flow rates the extraction time can be minimized to achieve a high sample throughput with no decrease in efficiency. Enrichment factors up to 10 can be obtained simply by tuning the acceptor and donor flow rates. Good results were obtained using the method for drug detection in urine and for the analysis of toxic compounds in water samples. This convincingly demonstrates the applicability of the microextractor in different fields.

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