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Automated dynamic liquid–liquid–liquid microextraction followed by high-performance liquid chromatography-ultraviolet detection for the determination of phenoxy acid herbicides in environmental waters

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Abstract

Automated dynamic liquid–liquid–liquid microextraction (D-LLLME) controlled by a programmable syringe pump and combined with HPLC-UV was investigated for the extraction and determination of 5 phenoxy acid herbicides in aqueous samples. In the extraction procedure, the acceptor phase was repeatedly withdrawn into and discharged from the hollow fiber by the syringe pump. The repetitive movement of acceptor phase into and out of the hollow fiber channel facilitated the transfer of analytes into donor phase, from the organic phase held in the pore of the fiber. Parameters such as the organic solvent, concentrations of the donor and acceptor phases, plunger movement pattern, speed of agitation and ionic strength of donor phase were evaluated. Good linearity of analytes was achieved in the range of 0.5–500 ng/ml with coefficients of determination, $r^2 > 0.9994$. Good repeatabilities of extraction performance were obtained with relative standard deviations lower than 7.5%. The method provided up-to 490-fold enrichment within 13 min. In addition, the limits of detection (LODs) ranged from 0.1 to 0.4 ng/mL ($S/N = 3$). D-LLLME was successfully applied for the analysis of phenoxy acid herbicides from real environmental water samples.

Keywords: Dynamic extraction; Liquid–liquid–liquid microextraction; Automation; Liquid-phase microextraction; Hollow fiber membranes; Water analysis; Phenoxy acid herbicides

1. Introduction

Phenoxy acid herbicides are a major class of herbicides employed in agricultural and forestry applications to control the growth of different unwanted plants. Due to their large-scale application, the existence of phenoxy acid herbicides in soils or environmental waters has attracted attention [1–9]. Although they generally have low mammalian toxicity, impurities and high dosages may cause teratogenic effects in rodents [8]. Furthermore, several recent studies have demonstrated the occurrence of phenoxy acid herbicide metabolites in surface water and groundwater [10–12]. Therefore, the presence of phenoxy acid herbicides in environmental samples should be monitored, especially in the aquatic environment, due to their persistent and polar character.

For the determination of phenoxy acid herbicides with gas chromatographic (GC) analysis [1–6], a prior derivatization step is necessary because of their low volatility and high polarity. In recent years, capillary electrophoresis (CE) has been introduced for the analysis of phenoxy acid herbicides [7,13] because of its very high chromatographic efficiency. However, the high detection limit and the rather complex optimization procedure has limited its usage. Compared with GC and CE, high-performance liquid

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chromatography (HPLC) is a good alternative technique [8,9,14], in which separation is achieved without the need of a derivatization step and relatively low detection limits can be obtained.

Liquid phase microextraction (LPME) [15–20] has been shown to be a viable alternative sample preparation method to conventional liquid–liquid extraction (LLE) because it is simple, cost-effective and has high extraction efficiency. Liquid–liquid–liquid microextraction (LLLME) [14,20] is one type of LPME. In this procedure, porous polypropylene hollow fiber with impregnated organic solvent within its wall pores is used as an interface between the donor (sample) and acceptor (held within the fiber channel) phases. Dynamic LPME was previously reported by He and Lee [21,22], in which a commonly used microsyringe was used as a microseparatory apparatus for extraction. The "dynamic" appellation refers to the repeated withdrawal into and discharge from the syringe barrel of the sample solution, which facilitates more rapid mass transfer. Dynamic LPME was shown to provide a larger enrichment factor within a shorter time than the static mode. Yet another LPME approach, dynamic headspace (HS) LPME was developed and successfully applied for the extraction of chlorobenzenes in soil [23]. Although the dynamic mode of the procedures mentioned above was shown to be fast and highly efficient, the manual manipulation of the syringe plunger made operation tedious. Additionally, the repeatability of the procedure was relatively poor. Based on the considerations above, a programmable syringe pump was introduced for automating the extraction to overcome these problems [24]. In automated hollow fiber-protected dynamic LPME, a piece of membrane hollow fiber serves as sample holder [25,26]. The programmable syringe pump was employed for withdrawing and discharging the aqueous sample in and out the hollow fiber. This approach provided higher enrichment factor and better reproducibility. Dynamic three-phase microextraction (D-LLLME) was later introduced for the extraction of aromatic amines [27], in which a hollow fiber unit as usual served as the "protector" of the organic solvent while the microsyringe barrel held the acceptor phase. With the repeated movement of the syringe plunger afforded by a programmable syringe pump, the renewable organic film and aqueous sample plug were formed inside the hollow fiber. D-LLLME provided significantly better extraction efficiency as well as reproducibility compared to conventional static LLLME.

In this work, automated D-LLLME was applied for the analysis of phenoxy acid herbicides, in combination with HPLC. The movement of the acceptor phase in the hollow fiber was controlled automatically by a programmable syringe pump. Factors such as the extraction organic solvent, concentrations of the donor phase and the acceptor phase, plunger movement pattern, stirring speed, and salt added were investigated. Finally, environmental water samples were extracted and analyzed to demonstrate the feasibility of the procedure.

2. Experimental

2.1. Standards and reagents

The Accurel Q3/2 polypropylene hollow fiber membrane (600-μm I.D., 200-μm wall thickness, 0.2-μm pore size) was bought from Membrana (Wuppertal, Germany). Ammonium acetate was bought from Ajax (Sydney, Australia). Acetic acid was obtained from Fluka (Buchs, Switzerland). Sodium chloride was purchased from GCE (Chula Vista, CA, USA). NaOH and 1-octanol were from Merck (Darmstadt, Germany). HCl and ethyl acetate were obtained from J.T. Baker (Philipsburg, NJ, USA). Toluene, hexane, n-nonane and HPLC-grade methanol were supplied by Fisher (Loughborough, UK). *n*-Octane was bought from Acros (New Jersey, NJ, USA). Ultrapure water was produced on a Nanopure (Barnstead, Dubuque, IA, USA) water-purification system.

2,4-Dichlorobenzoic acid (2,4-DCBA) and 2,4-dichlorophenoxyacetic acid (2,4-DCPA) were provided by Fluka. 2-(2,4-Dichlorophenoxy)-propionic acid (2-(2,4-DCPPA)) was bought from TCI (Tokyo, Japan). 3,5-Dichlorobenzoic acid (3,5-DCBA) and 2-(2,4,5-trichlorophenoxy)-propionic acid (fenoprop) were purchased from Aldrich (Milwaukee, WI, USA). Stock solutions (1 mg/mL of each analyte) were prepared separately in methanol and stored at 4 °C. A fresh standard sample was prepared by spiking ultrapure water with the five analytes at known concentrations (50 ng/mL of each analyte) every week during the optimization exercise.

Environmental water samples were collected from a pond situated in a botanic garden and drain near a children's playground. Samples were stored at 4 °C after collection. They were filtered through a 0.45-μm hydrophilic polytetrafluoroethylene membrane filter (Millipore, Billerica, MA, USA) prior to extraction.

2.2. Instrumentation

Analysis of herbicides was performed on a Waters (Milford, MA, USA) HPLC system. The chromatographic system consisted of a Rheodyne (Cotati, CA, USA) 77251 injector equipped with a 20-μL sample loop, a Waters 1525EF binary pump, and a Waters 2487 UV–vis spectrophotometric detector. Data was collected and processed by Empower version 5.0 (Waters) data analysis software.

A column (250 mm × 2 mm I.D.) from Phenomenex (Torrance, CA, USA) packed with BuckySep-RP was used. A flow-rate 0.1 mL/min was applied and a column temperature of 22 °C was maintained. The mobile phase was methanol–100 mM ammonium acetate (63:37, v/v; pH 5). The detection wavelength was 240 nm.

2.3. Apparatus

A Harvard Apparatus (Holliston, MA, USA) PHD 2000 syringe pump was used for extraction together with a 10-μL microsyringe with a flat needle tip (SGE, Sydney, Australia).

The hollow fiber was ultrasonically cleaned in acetone for 30 min before it was heat-sealed at one end. The approximate internal volume of each hollow fiber segment (2.8-cm length) was about 6 μL which was suitable for the amount of extraction solvent used in this work.

2.4. Automated D-LLLME procedure

The syringe pump was programmed based on: (1) refill speed; (2) sampling volume (the volume of the acceptor phase withdrawn into the microsyringe); (3) dwell time (length of time the acceptor phase remains in the microsyringe); (4) infusion speed; (5) sampling volume (the volume of the acceptor phase infused into the hollow fiber); (6) dwell time (length of time the acceptor phase remains in the hollow fiber); (7) restart. Briefly, D-LLLME consists of the following steps: (1) A 10-mL sample solution was placed in a sample vial with a 15 mm × 6 mm magnetic stirring bar; (2) The sample vial was placed on the magnetic stirrer/hotplate; (3) A 6-μL portion of acceptor phase was withdrawn into the microsyringe; (4) The syringe needle was then inserted into open end of the hollow fiber, and the acceptor solution was introduced into it; (5) The fiber was immersed in organic solvent for 5 s for impregnation of its wall pores; (6) The fiber together with the syringe needle was placed in the donor phase and microsyringe was fixed on the syringe pump; (7) A piece of aluminum foil (Diamond, Richmond, Virginia, USA) was used to cover the sample vial in order to prevent or reduce evaporation of the organic solvent; (8) The magnetic stirrer and the syringe pump were then simultaneously switched on, and the pump program was activated; (9) After extraction, the syringe needle/hollow fiber was removed from the sample solution, and the extract withdrawn into the syringe. The hollow fiber was discarded. The 5 μL analyte-enriched extract was injected directly into the HPLC. A fresh hollow fiber was used for each extraction. Triplicate analysis was performed in the optimization experiments.

3. Results and discussion

3.1. Optimization of D-LLLME

3.1.1. Selection of organic solvent

The type of solvent immobilized within the pores of the hollow fiber is very important in order to obtain satisfactory enrichment factor (the ratio between the equilibrium analyte concentration in the acceptor phase and the initial concentration in the sample solution). Six types of organic solvent were investigated. These were: 1-octanol (dielectric constant: 10.30), toluene (dielectric constant: 2.38), ethyl acetate (dielectric constant: 6.08), *n*-octane (dielectric constant: 1.948), hexane (dielectric constant: 1.89) and *n*-nonane (dielectric constant: 1.97) [28]. In these experiments, about 9-min extractions from solutions containing 50 ng/mL phenoxy acid herbicides dissolved in 0.01 M HCl were performed at a stirring speed of 73 rad/s (ca. 700 rpm; 1 rpm = 0.10472 rad/s). The acceptor phase was 6.0 μL 0.1 M NaOH. The pump pattern was: a plunger speed of 0.5 μL/s, and a dwell (pause) time of 4 s. The cycle was repeated 20 times. Ethyl acetate, *n*-octane and *n*-nonane demonstrated poor extraction of the target anlytes. Hexane was capable of extracting 2,4-DCPA only. Toluene was able to extract all compounds except for 2,4-DCBA. Only 1-octanol could extract all target analytes and the enrichment factors (about 130-fold) were higher than for the other five organic solvents. It is likely that the relatively higher polarity of 1-octanol (dielectric constant: 10.30) is the main reason for this observation. Based on the results above, 1-octanol was selected as organic solvent in subsequent experiments.

3.1.2. Concentrations of the donor and acceptor phases

The pH of the donor phase and the acceptor phase play important roles in D-LLLME. Experiments were conducted to optimize the concentrations of both the donor phase HCl and the acceptor phase NaOH. All experiments were carried out with stirring speed at 73 rad/s and with 1-octanol as the solvent. No salt was added to the donor solution. The syringe pump conditions were as before (number of cycles: 20; dwell time: 4 s; plunger speed: 0.5 μL/s). The concentrations of HCl and NaOH were varied from 0.01 to 1.00 M, respectively. The results are exhibited in Table 1. As can be seen, it is obvious that the concentrations of HCl and NaOH had different influence on the extraction efficiency. When NaOH concentration was increased from 0.01 to 1.00 M, the enrichment factors of 2,4-DCBA and 2,4-DCPA increased. However, the enrichment factors of 3,5–DCBA and fenoprop exhibited the opposite trend. For 2,4-DCBA (pK_a 2.68) and 2,4-DCPA (pK_a 2.64), a relatively more basic solution was needed for extracting them from the organic phase. For 3,5-DCBA (pK_a 3.54) and fenoprop (pK_a 4.41), a slightly less basic solution was preferred. The optimum enrichment factors of target analytes were obtained at 0.10 M HCl in general. On the basis of the above observations, a combination of 0.1 M HCl and 0.01 M NaOH under which optimum enrichment factors of the five herbicides were achieved was chosen as the donor and the acceptor phases, respectively.

3.1.3. Optimization of the pattern of the syringe pump plunger movement

In this automated D-LLLME process, the extraction was performed by automatically manipulating the plunger repeatedly in and out of the microsyringe barrel. Each cycle of the extraction includes the withdrawal and discharge of acceptor phase with two pauses (dwell time) in between. The analytes were then extracted rapidly from the aqueous samples to the organic solvent and back-extracted into a second aqueous solution (acceptor phase). The syringe pump plunger pattern, including number of cycles, dwell time and plunger speed which are important parameters for the automated D-LLLME efficiency, were studied.

Table 1
Effect of concentrations of the donor phase and the acceptor phase on automated D-LLLME enrichment factors[a]

Concentration of NaOH	Compound	Concentration of HCl			
		0.01 M	0.10 M	0.50 M	1.00 M
0.01 M	2,4-DCBA	240 (2.7%)[b]	409 (5.2%)	86 (2.5%)	76 (4.9%)
	2,4-DCPA	190 (3.3%)	353 (4.9%)	107 (3.7%)	89 (3.4%)
	2-(2,4-DCPPA)	260 (4.0%)	457 (7.2%)	99 (4.7%)	140 (7.5%)
	3,5-DCBA	359 (3.5%)	477 (3.6%)	147 (5.7%)	251 (6.2%)
	Fenoprop	297 (5.1%)	438 (3.4%)	88 (4.3%)	81 (6.1%)
0.10 M	2,4-DCBA	174 (5.7%)	151 (6.5%)	204 (4.9%)	115 (7.2%)
	2,4-DCPA	157 (3.3%)	138 (2.6%)	325 (6.4%)	91 (6.2%)
	2-(2,4-DCPPA)	192 (4.3%)	163 (5.7%)	207 (7.4%)	85 (5.8%)
	3,5-DCBA	198 (6.9%)	166 (4.9%)	227 (8.2%)	118 (2.6%)
	Fenoprop	161 (4.3%)	135 (5.7%)	190 (3.8%)	109 (4.5%)
0.50 M	2,4-DCBA	298 (5.3%)	231 (6.2%)	417 (5.2%)	175 (4.8%)
	2,4-DCPA	201 (6.7%)	189 (5.3%)	204 (7.3%)	114 (6.8%)
	2-(2,4-DCPPA)	212 (4.8%)	188 (4.5%)	230 (5.7%)	159 (5.2%)
	3,5-DCBA	203 (6.3%)	190 (7.8%)	233 (6.9%)	122 (6.4%)
	Fenoprop	137 (5.9%)	143 (6.8%)	185 (8.3%)	112 (7.4%)
1.00 M	2,4-DCBA	127 (7.5%)	198 (6.3%)	114 (8.4%)	246 (7.4%)
	2,4-DCPA	122 (6.2%)	145 (5.4%)	65 (3.2%)	151 (4.3%)
	2-(2,4-DCPPA)	86 (5.3%)	151 (5.7%)	84 (6.4%)	151 (7.2%)
	3,5-DCBA	132 (8.2%)	144 (4.2%)	92 (5.9%)	178 (6.2%)
	Fenoprop	91 (4.3%)	109 (5.9%)	69 (6.2%)	82 (7.2%)

[a] The experiments were performed in triplicate.
[b] RSD.

The first parameter for optimization was the number of cycles. The experiments were conducted by varying this number from 5 to 60 with dwell time at 4 s and plunger speed at 0.50 μL/s. The other experimental conditions were: 1-octanol as organic solvent, 0.1 M HCl as donor phase, 0.01 M NaOH as acceptor phase, stirring speed at 73 rad/s, without sodium chloride in the sample solution. Results are shown in Fig. 1. For 2,4-DCBA, 2,4-DCPA and fenoprop, the enrichment factors increased continuously when the number of cycles increased from 5 to 20, but continuously decreased with further increase in this number from 20 to 60. For 2-(2,4-DCPPA) and 3,5-DCBA, the trend exhibited by the enrichment factors were similar. However, the maximum extraction efficiency was achieved when the number of cycles was 30. D-LLLME is an equilibrium-based extraction, like

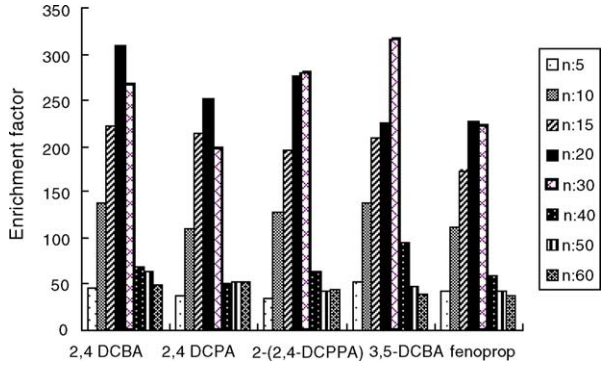

Fig. 1. Effect of number of extraction cycles on the extraction efficiency of D-LLLME (spiked with 50 ng/mL of each compound).

static LLLME. The amount of analytes extracted is related to the mass transfer of analyte from the aqueous donor phase to the organic solvent, and then to the aqueous acceptor phase. The extraction time increased with the increase in number of cycles. This is a possible reason why better extraction efficiency was achieved when this number was increased from 5 to 20 or 30. However, the loss of extraction solvent as a result of dissolution in the water possibly led to the decrease in extraction efficiency with further increase of cycles. Since only a small volume of organic solvent was impregnated in the wall pores of the hollow fiber, the effect of solvent depletion was more severe with increasing extraction cycles [24]. On the whole, 20 was used as the optimum number of cycles based on the above discussion.

Different dwell times ranging from 2 to 7 s were investigated on the extraction efficiency at 20 cycles. The other experimental parameters were kept the same as in the preceding paragraph. As shown in Fig. 2, the best extraction efficiency for all the target analytes was obtained at a dwell time of 3 s.

Lastly, syringe plunger speeds were optimized. The experiments were carried out by varying plunger speeds from 0.1 to 0.5 μL/s (at 20 cycles) and dwell time fixed at 3 s. The results are shown in Fig. 3. It is clear that the highest enrichment factors were achieved when the syringe plunger speed was 0.3 μL/s, possibly because of a compromise between the formation of a thicker organic film on the wall of the hollow fiber and the shortened extraction time with the increase of plunger speed. As described previously [22,23,26,29], the faster the movement of the syringe plunger, the thicker the organic film.

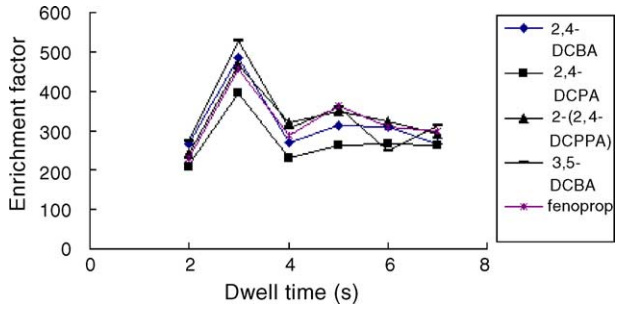

Fig. 2. Effect of dwell time on the extraction of anaytes (spiked with 50 ng/mL of each compound) by D-LLLME.

When the plunger speed was decreased, although the equilibrium between the organic solvent and aqueous solvent could be reached at a longer time, however, a thinner film limited the extraction of the amount of analytes [22,26]. This is the possible reason why better extraction efficiency was achieved when the plunger speed was increased from 0.1 to 0.3 μL/s. However, with the further increase of plunger speed from 0.3 to 0.5 μL/s, lower enrichment factors were observed. This may be because the time allowed for mass transfer is the dominant factor which limits the attainment of equilibrium when the film formed is relatively thick with the relatively high plunger speed [22]. Decreasing the plunger speed movement speed allowed more time for mass transfer and as a result more analytes were extracted into the organic solvent and then to the acceptor phase. In addition to this, a heterogeneous organic solvent film might have been formed by fast plunger movement, which would affect the extraction efficiency [23]. Thus, 0.3 μL/s was chosen as the optimum plunger speed.

3.1.4. Effect of the stirring speed

Agitation is a critical parameter for D-LLLME since the extraction efficiency is enhanced with faster stirring speed which permits the continuous exposure of the extraction surface to fresh aqueous sample. As depicted in Fig. 4, extraction efficiency improved with the increase of stirring speed and highest enrichment factors were obtained for most of analytes at a stirring speed of 73 rad/s. On the other hand, the enrichment factors decreased with a stirring speed of 104 rad/s or higher speed. This is probably due to the formation of air bubbles, generated on or near the fiber surface, which decreased the amount of analytes extracted into the organic solvent. On the basis of the above study, 73 rad/s was used as the optimum stirring speed.

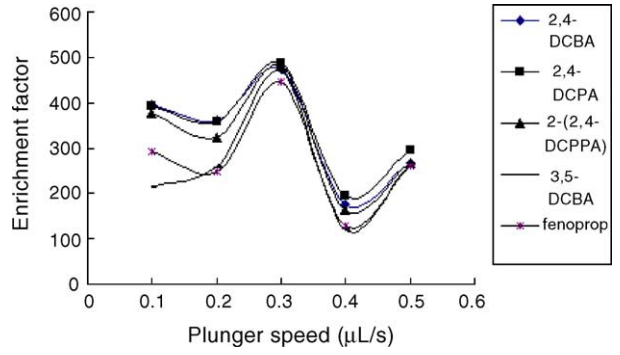

Fig. 3. Effect of syringe plunger speed on the extraction efficiency of D-LLLME (spiked with 50 ng/mL of each compound).

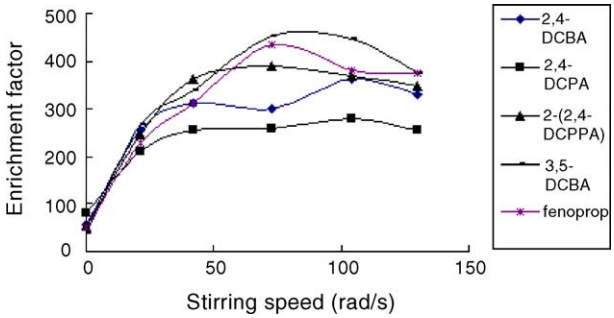

Fig. 4. Effect of stirring speed on the extraction of analytes (spiked with 50 ng/mL of each compound) by D-LLLME.

3.1.5. Effect of ionic strength of sample solution

The addition of salt often improved the extraction of analytes in conventional liquid–liquid extraction, solid-phase microextraction and LPME through the salting-out effect. It has also been observed that no change of extraction efficiency or even salting-in effect (decreasing extraction efficiency with the addition of salt) was achieved when salt was added. In the present work, no significant increase in extraction efficiency was obtained when 50–250 g/L sodium chloride were employed (results not shown).

3.2. Extraction efficiency

Based on the experiments above, optimum D-LLLME of phenoxy acid herbicides was achieved by using 1-octanol as organic solvent, 0.1 M HCl as the donor phase, 0.01 M NaOH as the acceptor phase at a stirring speed of 73 rad/s without salt. Additionally, 20 was employed as the number of cycles with dwell time at 3 s and plunger speed at 0.3 μL/s. As shown in Table 2, the enrichment factor of 490-fold could be obtained within an extraction time of 13 min.

3.3. Method validation

The repeatability, linearity and limits of detection (LODs) were investigated under optimized conditions and the results are shown in Table 2. As can be seen, good linearity of response of each analyte was observed in the range of 0.5–500 ng/mL with coefficients of determination (r^2) higher than 0.9994. The LODs (between 0.1 g/mL and 0.4 ng/mL) were calculated at a signal-to-noise ratio (S/N) of 3. In comparison with literature values [14] relating to the static LLLME of phenoxy acid herbicides in water, D-LLLME provided relatively lower LODs except for 2-(2,4-DCPPA). The relative standard deviations (RSDs) were smaller than 7.5%

Table 2
Performance of automated D-LLLME

Compound	Enrichment factor	RSD% ($n = 6$)	Linear range (ng/mL)	Coefficient of determination (r^2)	Recovery (%)		LOD[a] (ng/mL)	LOD (ng/mL)
					5 ng/mL[b]	10 ng/mL[b]		
2,4-DCBA	467	7.5	0.5–500	0.9997	94 ± 4.2%	104 ± 6.3%	0.1	0.5[c]
2,4-DCPA	384	5.6	1.0–500	0.9999	112 ± 3.0%	111 ± 5.7%	0.3	0.5[c]
2-(2,4-DCPPA)	448	6.4	1.0–500	0.9994	109 ± 6.7%	115 ± 4.3%	0.3	–[d]
3,5-DCBA	490	6.5	1.0–500	0.9999	109 ± 6.8%	96 ± 7.7%	0.4	0.5[c]
Fenoprop	451	3.9	1.0–500	0.9999	111 ± 5.2%	102 ± 4.2%	0.3	0.5[c]

Automated D-LLLME conditions: organic solvent: 1-octanol; donor phase: 0.1 M HCl; acceptor phase: 0.01 M NaOH; number of extraction cycles: 20; dwell time: 3 s; syringe plunger speed: 0.3 μL/s; extraction stirring speed: 73 rad/s; ionic strength (sodium chloride concentration): 0 g/L.

[a] LODs of automated D-LLLME calculated from $S/N = 3$.

[b] The final concentration of each analyte after spiking in ultrapure water, the experiments were performed in triplicate.

[c] LODs of static LLLME of water sample [14].

[d] Not available.

based on the peak areas for six replicates. The recoveries for ultrapure water sample spiked at 5 ng/mL of each analyte, were 94% (2,4-DCBA), 112% (2,4-DCPA), 109% (2-(2,4-DCPPA)), 109% (3,5-DCBA), and 111% (fenoprop). Recoveries were 104% (2,4-DCBA), 111% (2,4-DCPA), 115% (2-(2,4-DCPPA)), 96% (3,5-DCBA), and 102% (fenoprop) when extraction of ultrapure water spiked at 10 ng/mL of each analyte was performed.

3.4. Extraction of herbicides in environmental waters

The D-LLLME technique was used for extracting phenoxy acid herbicides from environmental waters: pond water situated in a botanic garden and playground drain water. There were no target analytes detected in the drain water. In pond water, 47 ng/mL of 2, 4-DCPA (Fig. 5A) was determined by the standard addition method. Its presence was confirmed by spiking the five herbicides into the sample and reanalyzing it (Fig. 5B and C). Both water samples were spiked with the herbicide standards at various concentrations in order to assess matrix effects. Results of relative recoveries (defined as the ratios of HPLC peak areas of the respective spiked water sample extracts to spiked ultrapure water extracts) and RSDs of two water samples in triplicate are shown in Table 3 when 5 and 10 ng/mL herbicide standards were added in the samples, respectively. The relative recoveries ranged from 85 to 107% for all phenoxy acid herbicides. This means that the matrix had little effect on automated D-LLLME.

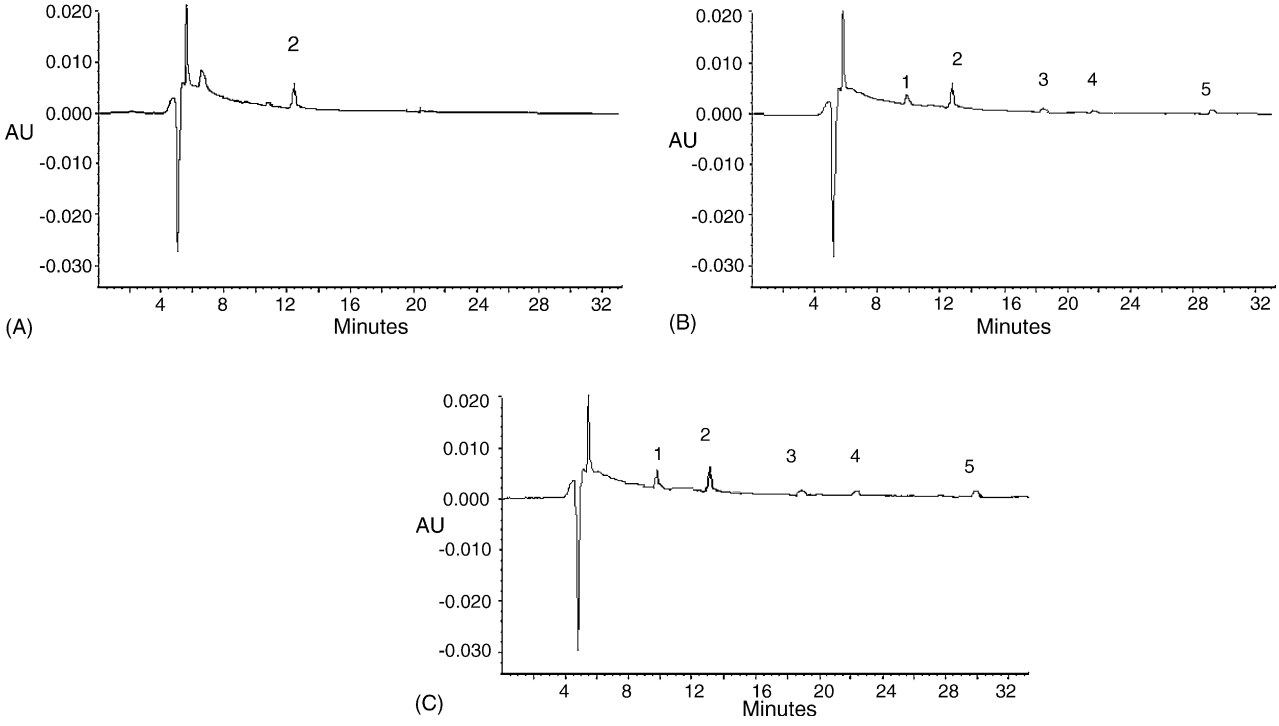

Fig. 5. HPLC-UV chromatograms of pond water situated in a botanic garden extracted by automated D-LLLME. (A) Blank pond water sample, (B) pond water sample spiked with 5 ng/mL of each analyte, (C) pond water sample spiked with 10 ng/mL of each analyte. Peaks: 1 = 2,4-DCBA, 2 = 2,4-DCPA, 3 = 2-(2,4-DCPPA), 4 = 3,5-DCBA, 5 = fenoprop. For HPLC conditions, see Section 2.

Table 3
Results of analysis of phenoxy acid herbicides in spiked environmental water samples

Compound	Drain water				Pond water			
	5 ng/mL		10 ng/mL		5 ng/mL		10 ng/mL	
	Recovery[a] (%)	RSD% (n = 3)	Recovery[a] (%)	RSD% (n = 3)	Recovery[a] (%)	RSD% (n = 3)	Recovery[a] (%)	RSD% (n = 3)
2,4-DCBA	99	5.6	95	8.3	100	6.7	102	4.7
2,4-DCPA	90	7.5	95	7.4	NC[b]	6.3	NC[b]	5.6
2-(2,4-DCPPA)	94	4.9	102	5.7	107	8.2	100	4.9
3,5-DCBA	88	6.4	95	5.6	102	4.5	103	6.5
fenoprop	88	5.9	89	4.8	94	5.3	85	6.8

[a] n = 3.
[b] Not considered since this analyte was detected in pond water.

4. Conclusions

The combination of automated hollow fiber-protected D-LLLME with HPLC-UV has been successfully employed for the analysis of 5 phenoxy acid herbicides. Good linearity, sensitivity and relative recoveries were achieved. In addition to this, automated D-LLLME provided up to 490-fold enrichment within 13 min, a very attractive feature of the technique that is probably not achievable by any single-step extraction procedure. The LODs for the five herbicides studied were in the range of 0.1–0.4 ng/mL. This study demonstrated automated D-LLLME could be applied to real world analysis of environmental water samples (the general concentration of phenoxy acid herbicides in water samples is 0.01–2 ng/mL [4]) without the need for reconstitution of analytes before injection for HPLC analysis. Further investigations are now under the way to extend the procedure to "dirtier" (e.g. slurry) samples.

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