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Analysis of phenoxy herbicides in bovine milk by means of liquid–liquid–liquid microextraction with a hollow-fiber membrane

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Abstract

A preconcentration technique, which involves liquid–liquid–liquid microextraction, was developed to determine phenoxy herbicides in bovine milk. A layer of organic phase was impregnated into the pores of a 3.5 cm long porous hollow fiber, while the internal volume of the fiber was filled with NaOH solution (the acceptor solution) that was connected directly to the needle of a microsyringe. The fiber was then immersed into 8 ml of acidified milk sample. When the sample solution was stirred, acidic analytes were extracted into the organic phase and back extracted simultaneously into the alkaline acceptor medium as the analytes were protonated at low pH and deprotonated at high pH. After extracting for a prescribed time, 5 μl acceptor solution was taken back into the syringe and injected directly into a HPLC system for quantification. The analytes were extracted quantitatively from the sample solution into the acceptor solution with a large enrichment factor of 900. Due to its low cost, the hollow-fiber extraction device was disposed of after a single extraction that eliminated the possibility of carry over effects. In addition, because a small volume of organic solvent was required and little waste is generated, the procedure is environmentally friendly, and is compatible with the ''green chemistry'' concept.

Keywords: Liquid–liquid–liquid microextracion; Extraction methods; Milk; Food analysis; Membrane extraction; Hollow-fiber membranes; Phenoxy acids; Pesticides

1. Introduction

Phenoxy herbicides are widely applied to get rid of unwanted plants [1]. They may eventually be present in bovine milk when fodder consumed by cattle is heavily contaminated. A number of papers have reported multiresidue methods for the determination of pesticides in milk. Most of them utilized liquid–liquid extraction (LLE) [2–5] or solid-phase extraction (SPE) [6–8], while the final analysis in most case is accomplished by capillary gas chromatography (GC). The determination of pesticide residues in milk has presented problems because the most common approach has involved total extraction of fat together. In both of LLE and SPE of pesticides from milk, some main drawbacks are involved, e.g. the amounts of solvents and glassware used; the number of manual operations involved, which strongly affect the throughput of residue; centrifugation after each extraction and troublesome emulsions, which sometimes are not easily controlled by centrifugation and addition of ethanol, especially with whole milk.

For the determination of phenoxy acid herbicides, derivatization is always needed before GC analysis [9–12]. Compared with GC, high-performance liquid

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chromatography (HPLC) is a good alternative technique [13–15], in which the separation is achieved without the need of a derivatization step.

Three-phase microextraction was previously developed to extract ionizable and charged compounds from different aqueous samples. Ma and Cantwell [16,17] used an unsupported liquid organic membrane to separate two aqueous phases, the donor phase and the acceptor phase. An ionizable compound, mephentermine, was extracted from the donor phase to the organic phase, then back extracted to the acceptor phase. A similar idea called supported liquid membrane extraction was used by other groups [18–22], where a porous hydrophobic membrane, which immobilizes an organic phase, is sandwiched between two aqueous phases, donor phase and acceptor phase. This approach was further developed by Pálmarsdóttir et al. [23], in which a hollow fiber replaced the ''planar'' membrane. Hollow-fiber membrane has proved useful for enrichment of ionizable and charged species, giving a high degree of cleanup and enrichment of various analytes in different samples [24]. It has been successfully applied to extract drugs from water, human urine or plasma [25–27] and was successfully coupled to capillary electrophoresis and HPLC. It was also used to preconcentrate nitrophenols from water samples before micro-LC analysis [28]. The main idea of this technique is: a thin film of organic solvent is immobilized in the pores of a porous polypropylene hollow fiber; on the exterior of the hollow fiber is the donor aqueous phase, the pH of which is adjusted to make sure the target compound is neutralized; in the internal channel of the hollow fiber is the acceptor aqueous phase, the pH of which is adjusted to ionize the target compounds. With stirring, the neutral compounds in the donor phase are extracted into the organic film on the fiber and then back extracted into the acceptor phase inside the fiber. Due to the high ratio of donor phase volume to acceptor phase, the preconcentration factor can be very high, e.g. around 380-fold enrichment was achieved for nitrophenoles extracted from aqueous samples [28].

In the present work, we developed a single-step, selective method for the extraction of acidic herbicides from bovine milk samples using a simple liquid–liquid–liquid microextraction device, which consisted of a disposable, ready-to-use hollow-fiber membrane. Different aspects of the extraction procedure such as the types of organic solvent suitable for the immobilization in the hollow-fiber pores; compositions of the acceptor and donor phases, the extraction time; and magnetic stirring speed were investigated.

2. Experimental

2.1. Liquid–liquid–liquid microextraction (LLLME)

The sample solution was placed in a 10-ml volumetric flask. A 10-μl HPLC syringe (Hamilton, Reno, NV, USA) of 0.8 mm O.D. was used to introduce the acceptor phase and support the hollow fiber, and also acted as an injection syringe for analysis after the extraction. Because the extraction units should be compatible with both aqueous solutions and a broad range of organic solvents, polypropylene was selected as the material for the porous hollow fiber. One end of the Q3/2 Accurel polypropylene hollow fiber (Membrana, Wuppertal, Germany) was flame-sealed. The total length of the fiber was 3.5 cm. The dimensions of the hollow fiber were 600×200 μm I.D. wall thickness; pore size 0.64 μm. Before use each hollow fiber was sonicated for 2 min in acetone to remove any contaminants in the fiber. It was then removed from acetone, and the solvent was allowed to evaporate completely.

Extractions were performed according to the following procedure: a 8-ml aliquot of sample solution (to which varying concentrations of HCl were added) was added to the volume flask, and a 15×6 mm magnetic stirring bar was placed in the solution. Then, 7 μl of the acceptor phase (to which varying concentrations of NaOH were added) was withdrawn using a syringe. The syringe was then inserted into the hollow fiber, and the acceptor solution was introduced into it. The fiber was then immersed in the organic solvent for 10 s for impregnation. The organic solvent filled the pores of the hollow-fiber wall. After impregnation, the fiber (together with the syringe) was put into the donor phase. Magnetic stirring utilizing a MR3001K hotplate stirrer (Heidolph, Kelheim, Germany) was used during the extraction. After extraction, the syringe–fiber assem-

bly was taken out of the solution. Then, 5 μl of the acceptor solution was withdrawn from the fiber, and injected into the HPLC system.

2.2. Reagents and standards

HPLC-grade methanol was bought from Mallinckrodt (Paris, KY, USA); ammonium acetate (NH_4Ac), glacial acetic acid (HAc), 1-octanol and isooctane were bought from Merck (Darmstadt, Germany). Nonane, octanone, butyl acetate and toluene were supplied by Fisher (Fair Lawn, NJ, USA). *n*-Hexyl ether was bought from TCI (Tokyo, Japan). Hydrochloric acid was obtained from J.T. Baker (Philipsburg, PA, USA). Ultrapure water was produced on a Nanopure system (Barnsted, Dubuque, IA).

2,4-Dichlorobenzoic acid (2,4-DCBA, pK_a 2.5 [29]) and 2,4-dichlorophenoxyacetic acid (2,4-D, pK_a 2.87 [29]) were bought from Fluka (Buchs, Switzerland); 2-(2,4,5-trichlorophenoxy)propionic acid (fenoprop, pK_a 4.41 [29]), 2-(4-chlorophenoxy)-2-methylpropionic acid (mecoprop, pK_a 3.10 [29]) and 3,5-dichlorobenzoic acid (3,5-DCBA, pK_a 3.54 [29]) were bought from Aldrich (Milwaukee, WI, USA). They were dissolved in methanol at 1 mg/ml concentration as stock solutions. Mixtures containing each phenoxy acid herbicide at different concentrations in 0.1 *M* NaOH were prepared from the stock solution and used as working solutions.

2.3. HPLC system

Chromatographic analysis was carried out on a Shimadzu (Tokyo, Japan) LC-6A liquid chromatograph system. The column (2 mm×25 cm I.D.) was from Chrompack (Middelburg, Netherlands) with Inertsil-ODS-2 as column packing material. The flow-rate was set at 0.2 ml/min. The column temperature was maintained at 22 °C. The detector was a Shimadzu SPD-6 AV UV–visible spectrophotometric detector set at a wavelength of 240 nm. Chromatographic data was recorded and analysed using a Shimadzu C-R6A Chromatopac integrator. The mobile phase was methanol–water (60:40) (containing 25 m*M* NH_4Ac/HAc).

2.4. Milk extraction

Fresh full-cream milk samples were purchased off the shelf and stored at 4 °C.

A portion of the milk sample (50 ml) was spiked with standard phenoxy acid herbicides to make the final concentration of 10 ng/ml. Then concentrated HCl was added in to make the final concentration of HCl as 0.5 *M*. It was stirred with a glass rod and allowed to equilibrate at room temperature for 5 min. The samples were centrifuged using a Kubota (Tokyo, Japan) 8100 centrifuge for 15 min at 2000 rev./min. After that, the supernatant aqueous layer was decanted to a bottle for later extraction. To another portion of the milk sample (50 ml) was firstly added concentrated HCl as described before. After deproteination, in the supernatant aqueous solution, standard herbicides were spiked to make the final concentrations of 5 and 10 ng/ml. They were extracted by LLLME as described above.

3. Results and discussion

3.1. Optimization of HPLC separation

Due to the low affinity of the acidic herbicides for the C_{18} stationary phase, a mobile phase at low pH (ca. 2.0) is used to suppress the ionization of the analytes during HPLC [30,31]. However, under this condition, the hydrolysis of the modified silica stationary phase can occur. In this study, NH_4Ac/HAc (pH 3.5) buffer was used for improving the separation efficiency of the anionic compounds. We optimized the analytical separation via changes in two factors: concentration of the buffer and the concentration of the methanol in the mobile phase, both of which have great effect on the separation.

The variation of the capacity factor (k') of the herbicides as a function of the concentration of the buffer is shown in Fig. 1. The use of the buffer significantly improved the separation. Replacement of water by buffer in the mobile phase caused suppression of the ionization of the analytes, leading to increased k' values. When buffer was used, the acetate competed with the analytes for the stationary phase. The presence of acetate in the mobile phase also suppressed ionization of the analytes (salting-out

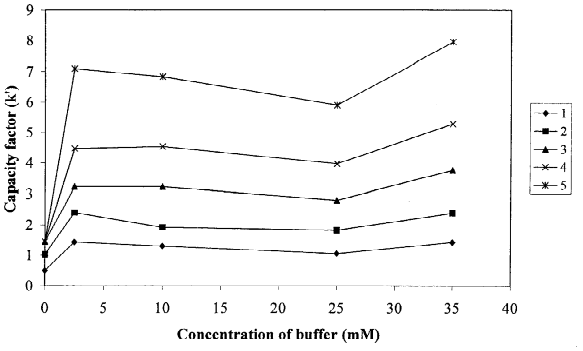

Fig. 1. The effect of concentration of buffer (NH_4Ac/HAc, pH 3.50) in the mobile phase on the capacity factor. Mobile phase: methanol–water (60:40); (1) 2,4-DCBA; (2) 2,4-D; (3) mecoprop; (4) 3,5-DCBA; (5) fenoprop.

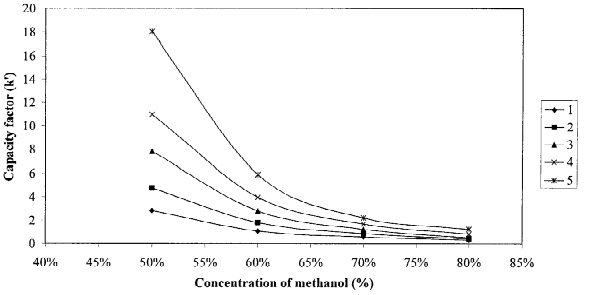

Fig. 3. The effect of concentration of methanol in the mobile phase on the capacity factor k'. The concentration of buffer in the mobile phase was 25 m*M*. (1) 2,4-DCBA; (2) 2,4-D; (3) mecoprop; (4) 3,5-DCBA; (5) fenoprop.

effect). Reinforcement and competition of the latter two factors caused a drop in the k' values initially, then an increase when the salt concentration of the buffer continued to increase, as shown in Fig. 1. At a concentration of 2.5 m*M* buffer, all the compounds could be separated completely. However, a higher concentration of 25 m*M* gave the lowest k' values without compromising the separation. Thus, 25 m*M* was accepted as optimum in the mobile phase composition in the interest of a reasonable analysis time. It was also found that k' increased with the dissociation constant (pK_a) of the compounds. As shown in Fig. 2, there was a linear relationship between $k'$with the corresponding pK_a of the compounds.

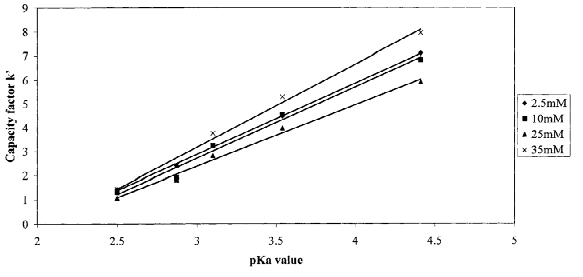

Fig. 2. The relationship between $\log k'$ and pK_a of the compounds. Mobile phase: methanol–water (60:40) with different concentrations of buffer.

Fig. 3 illustrates the effect of concentration of methanol in the mobile phase on the k' values. The results are as expected for reversed-phase HPLC. In the interest of a reasonable analysis time and satisfactory separation, 60% methanol was selected as the optimum composition.

3.2. Effect of organic solvent

The type of solvent immobilized within the pores of the hollow fiber is of great importance in order to achieve efficient analyte preconcentration. Six organic solvents, namely 1-octanol, *n*-hexyl ether, isooctane, toluene, butyl acetate and nonane, were investigated for their effect on enrichment. Isooctane and nonane showed poor extraction of the compounds; *n*-hexyl ether was capable of extracting 2,4-DCBA only. On the other hand, mecoprop could be extracted by toluene, and 2,4-DCBA and 2,4-D by butyl acetate. The solvents thus have different extraction selectivities for different acidic herbicides. Among the six types of organic solvents tested, only 1-octanol could extract all five herbicides and the enrichment factor (EF, which is defined as the ratio between the final analyte concentration in the acceptor phase and the initial concentration of analyte within the sample) was relatively higher than any other organic solvent studied. This is probably due to its relatively higher polarity and its greater affinity for the acidic herbicides. On the basis of these

experiments, 1-octanol was considered as the best solvent and was therefore used for subsequent experiments.

3.3. Compositions of donor and acceptor phases

The compositions of donor and acceptor phases are very important parameters that affect the enrichment efficiency in LLLME. With the hollow fiber impregnated with 1-octanol, a series of experiments were conducted to optimize the compositions of both the donor and acceptor solutions. For all of the experiments, the microextraction was accomplished for 20 min at 500 rev./min stirring speed with HCl in the donor phase and NaOH in the acceptor phase. For the donor phase, the concentration of HCl was varied between 0.01 and 1.0 M; at the same time, the concentration of NaOH also varied between 0.01 and 1.0 *M*. The results are shown in Table 1.

From Table 1, it can be seen that the composition of donor and acceptor phases had different effects on different compounds. On the whole, the EF for 2,4-DCBA and 2,4-D increased with the increase in the concentration of NaOH in the acceptor phase, while the EF for 3,5-DCBA and fenoprop exhibited the opposite effect. On the other hand, the EF for mecoprop did not change significantly with the variation in the NaOH concentration (except when at 0.1 *M* HCl was used). It is noted that the concentration difference of HCl and NaOH also had some effect on the EF. A big difference, e.g. 0.01 *M* HCl and 1.00 *M* NaOH, or 1.00 *M* HCl and 0.01 *M* NaOH, was not beneficial to the extraction. A possible reason is that the highly acidic (0.5 and 1.0 *M*) donor solution leached from the donor solution (8 ml) into the acceptor solution (7 μl). When the base concentration in the acceptor solution was low (0.01 *M*), the base was neutralized by the acid; hence, the efficiency of the extraction was very poor. As shown in Table 1, the compounds could not be

Table 1
The effect of composition of donor and acceptor phases on the enrichment factors of LLLME

Concentration of HCl	Compound	Concentration of NaOH			
		0.01 M	0.10 M	0.50 M	1.00 M
0.01 M	2,4-DCBA	57	80	176	41
	2,4-D	55	78	127	38
	Mecoprop	53	55	50	50
	3,5-DCBA	53	34	14	21
	Fenoprop	62	33	13	11
0.10 M	2,4-DCBA	60	87	163	54
	2,4-D	64	80	102	47
	Mecoprop	89	83	90	55
	3,5-DCBA	74	61	37	26
	Fenoprop	87	50	31	19
0.50 M	2,4-DCBA	–	84	128	341
	2,4-D	–	83	119	299
	Mecoprop	–	72	76	78
	3,5-DCBA	–	74	39	24
	Fenoprop	–	61	31	14
1.00 M	2,4-DCBA	–	52	80	50
	2,4-D	–	58	47	39
	Mecoprop	–	77	75	68
	3,5-DCBA	–	51	37	26
	Fenoprop	–	44	22	14

–, lower than the LOD.

concentrated at 0.01 *M* NaOH, with 0.5 and 1.0 *M* HCl.

Based on the maximum EF of all five compounds, 0.5 *M* HCl in the donor phase and 0.1 *M* NaOH in acceptor phase were selected as the optimum concentrations. NaCl was added to the donor solution to study the possibility of salting-out effect. No significant increase in enrichment was achieved when 5, 10 and 20% NaCl were used.

3.4. Effect of stirring speed

Stirring is mainly applied to accelerate the kinetics of extraction. With stirring, the analyte molecules are able to pass through the interfacial layer of the hollow fiber more easily and more analyte molecules can be extracted. As a result, the enrichment efficiency increased with stirring speed. Hence, the stirring speed was optimized for the extraction. The extraction experiment was performed on the aforementioned standard mixture solution under the optimum conditions. Fig. 4 illustrates the effect of extraction stirring speed on the EF using 2,4-D as an example. The EF increased with the stirring speed until 1250 rev./min, which is the highest speed that could be achieved by the magnetic stirrer. In terms of stirring requirements, LLLME with a hollow fiber as a supporter is superior to LLLME with an unsupported liquid drop at the end of needle [16], during which the drop is liable to be lost under great agitation. The extraction operation is easier to handle and can tolerate high speed agitation. The latter factor helps to improve the stability and repeatability of the extraction.

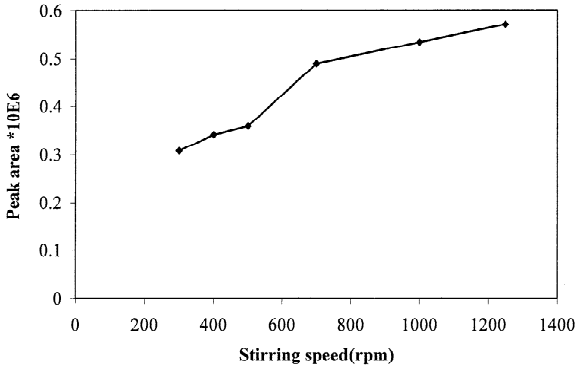

Fig. 4. The effect of stirring speed on the extraction efficiency. Conditions: 1-octanol as the impregenation solvent; 0.5 *M* HCl in the donor phase and 0.1 *M* NaOH in the acceptor phase; extraction time 20 min. 2,4-D was used as an example.

3.5. Effect of extraction time

Because there are two liquid–liquid interfaces (i.e. donor phase–organic phase, organic phase–acceptor phase) in the extraction system, it is supposed that solute molecules need time to pass through these interfaces. Extraction time was therefore another important factor influencing the extraction efficiency that was considered. The extraction experiment was performed on a standard mixture solution in 0.5 *M* HCl (10 ng/ml of each herbicide). The acceptor phase was 0.1 *M* NaOH, the impregnation solvent was 1-octanol, and the stirrer speed was fixed at 1250 rev./min. The extraction time was 5–70 min. The results are shown in Fig. 5. The EF increased rapidly with the extraction time up to 20 min, and increased more gradually between 20 and 60 min. It almost leveled out after 60 min. Based on this, 60 min was selected as the optimum extraction time. Although the extraction time was relatively long, a large number (~10) of samples could be extracted simultaneously due to the simplicity and the low cost of the extraction device.

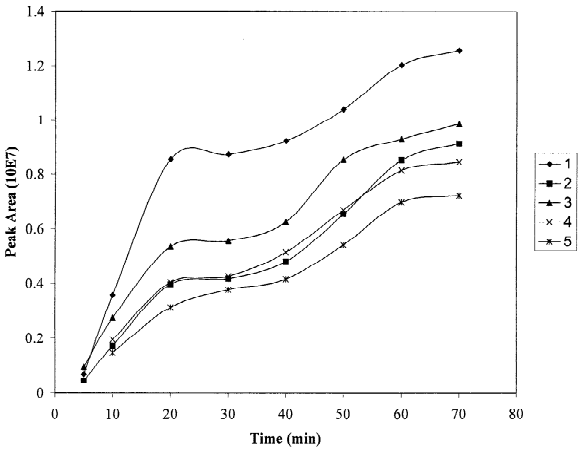

Fig. 5. The effect of extraction time on the enrichment of analytes. Conditions: 1-octanol as the impregnation solvent; 0.5 *M* HCl in the donor phase and 0.1 *M* NaOH in the acceptor phase; stirring speed 1250 rev./min. (1) 2,4-DCBA; (2) 2,4-D; (3) mecoprop; (4) 3,5-DCBA; (5) fenoprop.

3.6. Extraction efficiency

On the basis of the experiments discussed above, optimum LLLME of herbicides was obtained by utilizing a 3.5 cm porous hollow fiber immobilized with 1-octanol, a donor solution of 0.5 *M* HCl, an acceptor solution of 0.1 *M* NaOH, at a stirring speed of 1250 rev./min and with an extraction time of 60 min. Under these optimum extraction conditions, the enrichment factor could be high as 950-fold, as shown in Table 2.

3.7. Quantitative aspects

To evaluate the practical applicability of the proposed LLLME, the repeatability, linearity and limits of detection were investigated using the optimum conditions. The performance of this method is shown in Table 2. It can be seen that the RSD was smaller than 7.1% based on the peak areas for six replicates. The linearity was evaluated within the range 1–200 ng/ml. Each analyte exhibited good linearity with regression coefficient, $r^2>0.9965$. The limits of detection (LODs, 0.5 ng/ml for all) for the acidic herbicides were calculated at a signal-to-noise ratio of 3.

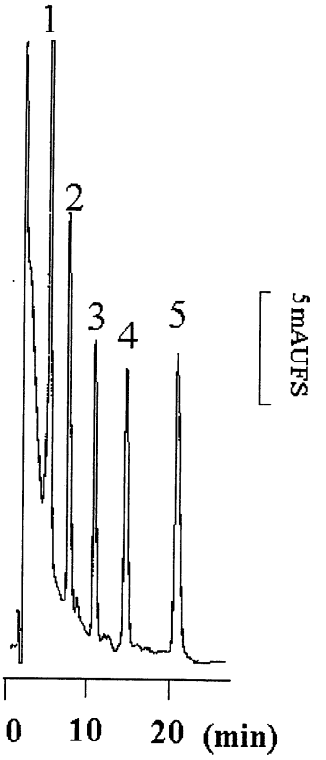

Fig. 6. Chromatography of phenoxy acid herbicides extracted from milk sample spiked at 10 ng/ml. Extraction conditions: 1-octanol as the impregnation solvent; 0.5 *M* HCl in donor phase; 0.1 *M* NaOH in acceptor phase; extracted for 60 min at 1250 rev./min. Peak identification: (1) 2,4-DCBA; (2) 2,4-D; (3) mecoprop; (4) 3,5-DCBA; (5) fenoprop.

3.8. Analysis of herbicides in milk

Finally, the LLLME technique was tested to preconcentrate phenoxy acid herbicides from fresh milk samples. Addition of concentrated HCl caused the milk to curdle. The proteins were coarsely discarded by centrifuging at 2000 rev./min for 15 min. One portion of the milk sample was spiked with a standard mixture of the herbicides to a final concentration of 10 ng/ml before deproteination.

Table 2
Performance of LLLME

Compound	Enrichment factor	RSD% ($n=6$)	Linear range (ng/ml)	LOD (ng/ml)	Recovery (%)	
					5 ng/ml[a]	10 ng/ml[a]
2,4-DCBA	952	4.56	1.0–200	0.5	76.8	89.5
2,4-D	689	4.75	1.0–200	0.5	77.0	88.6
Mecoprop	312	6.33	1.0–200	0.5	74.6	88.3
3,5-DCBA	269	6.96	1.0–200	0.5	72.1	85.3
Fenoprop	261	7.02	1.0–200	0.5	70.8	84.0

LLLME conditions: 0.5 *M* HCl in donor phase, 0.1 *M* NaOH in acceptor phase, extraction time 60 min; extraction stirring speed 1250 rev./min.

[a] The final concentration of each analyte after spiking in milk.

Another portion was spiked with herbicides after deproteination such that one sample contained 5 ng/ml, and the other, 10 ng/ml, of each of each the analytes. As illustrated in Fig. 6, the herbicides were effectively preconcentrated from the milk samples. Even though there were fat, some remaining proteins and other acid compounds present in the milk sample, the recoveries from the sample spiked after deproteintation were >70%, as shown in Table 2. The recoveries from the sample spiked before deproteination were lower than those from the sample spiked after deproteination. This suggests that the proteins could retain some of the herbicides, thus making them unavailable for extraction. Nevertheless, with internal standardisation, it should still be possible to perform quantitative analysis satisfactorily on untreated milk directly using the procedure developed. It is clear that the method is a simple and selective one to preconcentate phenoxy acid herbicides from milk. Multi centrifugation steps are avoided and there are no problems associated with emulsion.

4. Conclusion

This study has demonstrated the high-performance of liquid–liquid–liquid microextraction of phenoxy acid herbicides from aqueous and milk samples utilizing a porous polypropylene fiber. There are two major advantages for this technique: Firstly, it is very simple to prepare the milk sample for analysis, no complicated defatting and deproteinization procedures are needed. Secondly, after extraction, the extract was injected into the HPLC directly. This is more convenient than GC methods that require derivatization of the analytes. Very effective sample clean-up and up to 950-fold enrichment factor could be achieved. Due to the simplicity and the low cost of the extraction device, the hollow-fiber can be discarded after each extraction to avoid carryover and cross-contamination. This serves to maintain high reproducibility and repeatability. Due to the protection afforded by the hollow fiber, the acceptor phase can tolerate considerably high speed stirring. We have demonstrated that LLLME with a hollow-fiber membrane is an effective method to enrich phenoxy acid herbicides from bovine milk prior to direct HPLC analysis.

Acknowledgements

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References

[1] N.Q. Li, H.K. Lee, Anal. Chem. 72 (2000) 3077.
[2] A.D. Muccio, M. Rizzica, A. Ausili, I. Camoni, R. Dommarco, F. Vergori, J. Chromatogr. 456 (1988) 143.
[3] V. Cerkvenik, D.Z. Doganoc, J. Jan, Food Tech. Biotech. 38 (2000) 155.
[4] P.J. John, N. Bakore, P. Bhatnagap, Environ. Int. 26 (2001) 231.
[5] S.M. Waliszewski, V.T. Pardio, K.N. Waliszewski, Sci. Total Environ. 208 (1997) 127.
[6] F.J. Schenck, J. Wagner, Food Addit. Contam. 12 (1995) 535.
[7] A. Lagana, A. Marino, G. Fago, Chromatographia 41 (1995) 178.
[8] J. Manes, G. Font, Y. Pico, J. Chromatogr. 642 (1993) 195.
[9] J.A. Field, K. Monohan, Anal. Chem. 67 (1995) 3357.
[10] S.N. Chatfield, M.Y. Croft, T. Dang, E.J. Murby, G.Y.F. Yu, R.J. Wells, Anal. Chem. 67 (1995) 945.
[11] A. Balinovo, J. Chromatogr. A 728 (1996) 319.
[12] M. Vink, J.M. Van der Poll, J. Chromatogr. A 733 (1996) 361.
[13] I. Turnes, I. Rodríguez, C.M. García, R. Cela, J. Chromatogr. A 743 (1996) 283.
[14] H. Lee, T.E. Peart, R.L. Hong-You, J. Chromatogr. 636 (1993) 263.
[15] S. Fingler, V. Drevenkar, Z. Vasilic, Mikrochim. Acta 11 (1987) 163.
[16] M.H. Ma, F.F. Cantwell, Anal. Chem. 70 (1998) 3912.
[17] M.H. Ma, F.F. Cantwell, Anal. Chem. 71 (1999) 388.
[18] G.A. Audunsson, Anal. Chem. 58 (1986) 2714.
[19] J.Å. Jönsson, L. Mathiasson, Trends Anal. Chem. 11 (1992) 106.
[20] R.E. Majors, LC–GC Int. 8 (1995) 375.
[21] N.C. Van De Merbel, J.J. Hageman, U.A.Th. Brinkman, J. Chromatogr. 634 (1993) 1.
[22] J. Trocewicz, J. Chromatogr. A 725 (1996) 121.
[23] S. Pálmarsdóttir, E. Thordarson, L.E. Edhom, J.Å. Jönsson, L. Mathiasson, Anal. Chem. 69 (1997) 1732.
[24] Y.Z. Luo, J. Pawliszyn, Anal. Chem. 72 (2000) 1058.
[25] S. Pedersen-Bjergaard, K.E. Rasmussen, Anal. Chem. 71 (1999) 2650.

[26] K.E. Rasmussen, S. Pedersen-Bjergaard, M. Krogh, H.G. Ugland, T. Gronhaug, J. Chromatogr. A 873 (2000) 31.
[27] S. Pedersen-Bjergaard, K.E. Rasmussen, Electrophoresis 21 (2000) 579.
[28] L. Zhu, L. Zhu, H.K. Lee, J. Chromatogr. A 924 (2001) 407.
[29] J.A. Dean, Handbook of Organic Chemistry, McGraw-Hill, New York, 1987.
[30] J.M. Sanchis-Mallols, S. Sagrado, M.J. Medina-Hernandez, R.M.V. Camanas, E. Bonet-Domingo, J. Liq. Chromatogr. Rel. Technol. 21 (1998) 1871.
[31] F. Sanchez-Rasero, M.B. Matallo, E. Romero, G. Dios, A. Pena, J. Liq. Chromatogr. Rel. Technol. 21 (1998) 2211.