Journal of Chromatography A, 1217 (2010) 1244–1248

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Low-density extraction solvent-based solvent terminated dispersive liquid–liquid microextraction combined with gas chromatography-tandem mass spectrometry for the determination of carbamate pesticides in water samples

Hao Chen [a], Ruiwen Chen [a], Shengqing Li [a,b,*]

[a] Department of Chemistry, College of Science, Huazhong Agricultural University, Wuhan 430070, China
[b] The Supervision, Inspection and Testing Center of Microbial Products Quality (Wuhan), Ministry of Agriculture, China

ARTICLE INFO

Article history:
Received 6 October 2009
Received in revised form
10 December 2009
Accepted 22 December 2009
Available online 4 January 2010

Keywords:
Ionic liquid
Dispersive liquid–liquid microextraction
Carbamate pesticides
Low-density extraction solvent
Gas chromatography-tandem mass spectrometry

ABSTRACT

A simple and fast method of low-density extraction solvent-based solvent terminated dispersive liquid–liquid microextraction (ST-DLLME) was developed for the highly sensitive determination of carbamate pesticides in the water samples by gas chromatography-tandem mass spectrometry (GC-MSMS). After dispersing, the obtained emulsion cleared into two phases quickly when an aliquot of acetonitrile was introduced as a chemical demulsifier into the aqueous bulk. Therefore, the developed procedure does not need centrifugation to achieve phase separation. It was convenient for the usage of low-density extraction solvents in DLLME. Under the optimized conditions, the limits of detection for all target carbamate pesticides were in range of 0.001–0.50 ng mL^{-1} and the precisions were in the range of 2.3–6.8% (RSDs, 2 ng mL^{-1}, $n = 5$). The proposed method has been successfully applied to the analysis of real water samples and good spiked recoveries over the range of 94.5–104% were obtained.

1. Introduction

There is an increasing demand to develop simple and fast sample preparation methods for the determination of pesticide residues in environmental samples. Microextraction-based techniques, such as liquid-phase microextraction (LPME) [1,2], single drop microextraction (SDME) [3,4], solid-phase microextraction (SPME) [5,6], were widely used for carbamate pesticide analysis.

A new procedure termed as dispersive liquid–liquid microextraction (DLLME) has received much attention for sample pretreatment [7,8]. It has a great bounty of applications in the analysis of carbamate pesticides [9–14], organophosphorus pesticides [7,15,16], organochlorine pesticides [17], triclosan [18,19], insecticides [20,21], and some herbicides [22–25]. DLLME provides many advantages of high enrichment factor, simplicity, rapidity, easy to operate, low sample volume, low cost and consumption of organic solvents, and so on.

A ternary system like homogeneous liquid–liquid extraction formed in this developed procedure when an appropriate mixture of extraction and disperser solvents were injected into the aqueous solution. Extraction solvents often used in DLLME were chlorobenzene [7,15,25], carbon tetrachloride [26] and tetrachloroethane [9] with higher density than water. So the micro-droplets of extraction solvents were settled from the aqueous bulk usually by centrifuging the oil-in-water (O/W) emulsion. The instrumental analysis of the sediment was then carried out after the centrifugation. However, many of common liquid–liquid extraction solvents including alkanes, alcohols, ethers, ketons and acetates are less dense than water. The application of these solvents in dispersion-based microextraction like DLLME will be problematic. Saleh et al. reported their resolution to this technical hurdle most recently [27]. They used a deliberately home-designed glass centrifuge vial, which has a conic head and a glass tube fixed on the side of the vial, to explore the possibility of applying low-density organic solvents in ultrasound-assisted emulsification microextraction. After centrifugation, the organic solvents floated on the surface of aqueous samples, lifting-up in the conic head by adding a few microliters of doubly distilled water into the side tube of the vial, were collected prior to gas chromatography analysis.

Herein, we report our experiments facing the obstacle of employing low-density extraction solvents in DLLME. The O/W emulsion is thermodynamically unstable and separated naturally

* Corresponding author at: Department of Chemistry, College of Science, Huazhong Agricultural University, 1 Shizishan St. Hongshan Distric, Wuhan 430070, China. Tel.: +86 27 87287442; fax: +86 27 87287442.
E-mail addresses: hchenhao@mail.hzau.edu.cn (H. Chen), sqingli@mail.hzau.edu.cn (S. Li).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.12.062

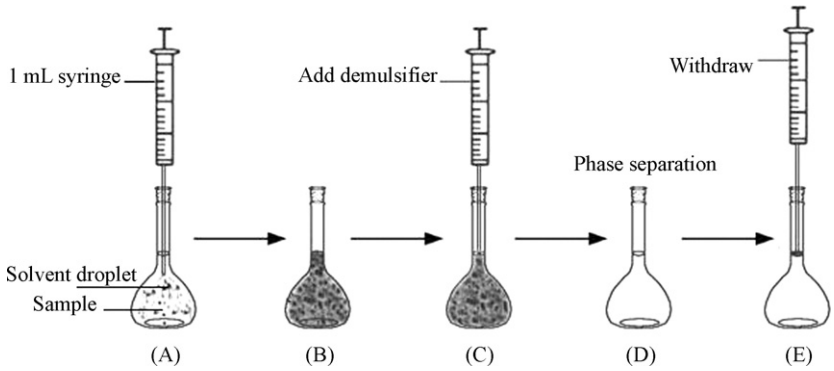


Fig. 1. Schematic procedure of low-density organic solvent-based solvent terminated dispersive liquid–liquid microextraction (ST-DLLME). (A) Injection of extraction solvent and disperser solvent into aqueous sample, (B) formation of emulsion for extraction, (C) addition of terminating solvent to break up the emulsion, (D) phase separation, and (E) collection of low-density extraction solvent in the upper layer.

into their constituent phases in a given sufficient time, as we know. The stability of the tiny extraction droplets in the dispersed system depends on the nature of the emulsion interface, surface electrical charge, and van der Waals forces etc. Such factors as the speed of agitation, temperature, bulk viscosity, and presence of an impurity, can play an important role in the effectiveness of demulsification [28,29]. So in this work, methanol and acetonitrile, usually served as disperser solvents in DLLME, were introduced as chemical demulsifiers to break up the dispersed system considering their characteristics of low surface tension and high surface activity. The emulsion cleared into two phases quickly. In this way, the separation of organic phase from the aqueous bulk was achieved without using centrifugation. A simple and fast method of solvent terminated dispersive liquid–liquid microextraction (ST-DLLME) was developed subsequently. The performance of ST-DLLME, applying low-density toluene and n-octanol as extraction solvents, combined with gas chromatography-tandem mass spectrometry (GC-MSMS), is illustrated for the determination of carbamate pesticides in water samples.

2. Experimental

2.1. Reagent and Materials

All carbamate pesticides (carbofuran, tsumacide, isoprocarb, and pirimicarb) were purchased from National Research Center for Certified Reference Materials (Beijing, China). Stock solutions of 2 mg mL^{-1} for each pesticide were prepared in acetone (analytical grade) and stored at 4 °C. Mixed working solutions with concentrations for each pesticide were prepared daily with water obtained from a Millipore Milli-Q water purification system (Bedford, MA, USA).

All other chemicals and solvents were analytical reagent grade or better. Glassware was cleaned over night in chromic acid and then rinsed with Milli-Q water. Water samples were obtained from Youth Lake and South Lake, Wuhan, China. The aqueous samples were collected in glass bottles and used without previous treatment or filtration. The samples were stored in the dark at 4 °C and analyzed within 24 h of collection.

2.2. Instrumentation

Chromatographic analysis was performed with a Varian CP-3800 gas chromatography equipped with a mass spectrometric detector (Saturn 2200 MSD, Varian, USA). A 1079 injector (with 4.6 mm i.d. glass liner) was used in the splitless mode, and maintained at 250 °C. The separation was achieved on a 30 m × 0.25 mm i.d., 0.25 μm film thickness, FactorFour VF-5MS (5% phenyl/methylsiloxane) fused-silica capillary column. The column temperature was initially held at 100 °C for 1 min, increased at 5 °C min^{-1} to a final temperature of 220 °C. Helium (99.999%, Haipu Beijing, China) was used as carrier gas at a flow rate of 1.0 mL min^{-1}. The MSD transfer-line, manifold and ion trap temperatures were 230, 80 and 160 °C, respectively. Electron impact (EI) ionization was performed at electron energy of 70 eV. The electron multiplier potential was 1500 V. The operation parameters of tandem mass spectrometry were previously optimized for a better detection of carbamate pesticides and the results are given in Table 1.

A 5 μL microsyringe model 75N for sample introduction was purchased from Hamilton (Hamilton, Bonaduz, Switzerland). 5 mL volumetric flasks (Tianbo, Tianjin, China) were used as extraction vessel. Emulsification process was performed using a 1.00 mL blunt tip microsyringe (Feige, Shanghai, China). The final upper level extraction solvent volume was checked by a 50 μL blunt tip microsyringe.

2.3. ST-DLLME procedure

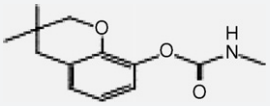
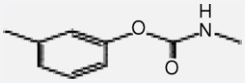
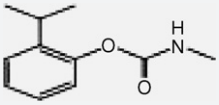
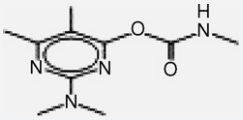
Fig. 1 shows the schematic procedure of the ST-DLLME. An aliquot of 5 mL water sample was placed in a 5 mL volumetric flask. A mixture of 15 μL organic solvent and 0.50 mL disperser solvent was injected rapidly into the sample solution through the 1.00 mL syringe. An emulsion (water, extraction solvent, disperser solvent) was formed in the volumetric flask. After a setting time, another 0.5 mL of disperser solvent, served as the demulsifier, was injected into the top surface of the aqueous bulk to break up the emulsion. Then the emulsion cleared into two phases quickly. The upper layer was collected using a single-use glass capillary and the volume of the light phase was checked. One microliter of the organic phase was transferred immediately by the 5 μL syringe into the GC injection port for analysis.

3. Results and discussion

3.1. Optimization of solvent terminated dispersive liquid–liquid microextraction

In order to obtain the optimized extraction condition and high extraction efficiency, several factors including the type of extraction and disperser solvents, the volume of extraction and disperser solvents, extraction time, and pH were studied and optimized. The recovery (R, %) of the analyte was the parameter used to evaluate the influence of the factors on the performance of the ST-DLLME.

Table 1
Tandem MS method for determination.

Pesticides	Structures	Parent ion (Da)	Product ion (Da)	REV[a] (eV)
Carbofuran		164	122	40
Tsumacide		108	91	35
Isoprocarb		121	91	35
Pirimicarb		166	72	30

[a] The resonance excitation energy used in MSMS mode.

The extraction recovery (R) was calculated using the following equation:

$$R = \frac{C_{us}V_{us}}{C_0V_{aq}} \times 100$$

where C_{us} and C_0 are the concentration of analyte in the upper layer (with volume as V_{us}) and the initially aqueous samples (with volume as V_{aq}), respectively. The calculation of C_{us} was conducted by the direct injection of the carbamate pesticides standard solutions with concentrations in the range of 0.1–200 μg mL^{-1}.

3.1.1. Extraction solvent

The selection of an appropriate extraction solvent is of great importance to the optimization of the ST-DLLME. Four low-density solvents (toluene, cyclohexane, n-hexane, octanol) differing in polarity and water solubility were tested for this purpose. It is necessary to add an excess amount of solvent to recover an equal volume of different extraction solvents in the upper layer for comparison. Therefore, a series of sample solution were studied by using 14.5 μL toluene, 19.8 μL n-hexane, 15.3 μL cyclohexane, 17.1 μL n-octanol, in according to their solubility in water. The final volume of the upper layer remained at 15 μL level. The choice of solvent was based on the following factors: extraction efficiency, reproducibility and the GC behavior. Five replicate tests were performed for each solvent. Toluene, followed by cyclohexane, n-hexane and octanol, has the highest extraction efficiency as shown in Fig. 2. It seems the ring structure and aromatic group of organic solvents benefit the extraction of the selected pesticides, which have aromatic group in the molecular structure (given in Table 1). In this respect, toluene was selected for subsequent experiments.

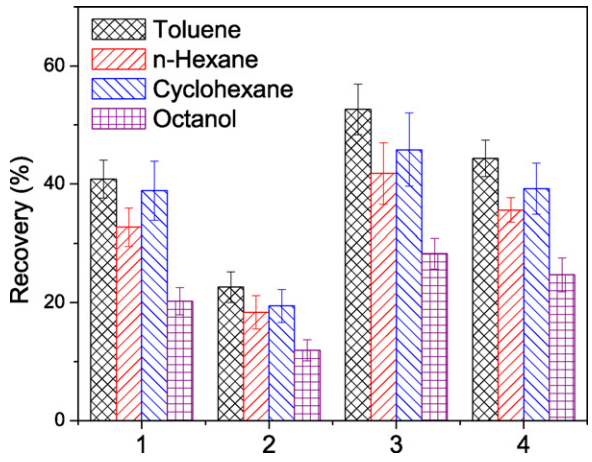


Fig. 2. Effect of extraction solvent on the extraction recovery. Conditions: sample volume, 5 mL; spiked concentration, 2 ng mL^{-1}; extraction solvent volume, 30 μL; disperser and terminating solvent, 1.0 mL acetone; extraction time, 10 min; n = 5.

3.1.2. Disperser solvent and terminating solvent

The organic solvent will disperse into the aqueous bulk as tiny droplets when it is rapidly injected into the water sample. The miscibility of disperser in both organic and water is the main point of selection for the emulsification of extraction solvent. Therefore, acetone, acetonitrile and methanol were introduced not only as the disperser to accelerate the dispersion of extraction solvent but also as the demulsifier to break up the O/W emulsion. The effect of these solvents on the performance of ST-DLLME was investigated.

To simplify the process of selection, the tested solvent was divided into two equal parts. One served as disperser was mixed with the extraction solvent and injected into the aqueous sample. After certain minutes, the other part performed as terminating solvent was injected into the dispersed system to end off the extraction. It was observed that the emulsion first enhanced with the addition of the terminating solvent and then separated quickly into two layers in 10–15 s. It means the new procedure does not need centrifugation to separate the organic phase in this manner. The analyte in the upper layer was determined by GC-MSMS and the obtained results are shown in Fig. 3. Acetonitrile was used as the disperser and terminating solvent in the following experiments since the recovery is higher with it than with acetone and methanol.

3.1.3. Volumes of extraction solvent, disperser and terminating solvent

During the dispersive liquid–liquid microextraction process, volumes of extraction and disperser solvents are essential factors, which can influence the occurrence of the emulsion state and determine the extraction performance. To examine the effect of extraction solvent volume, series volumes of 30, 40, 50, and 60 μL, were evaluated. Different extraction volumes would result in various volumes of upper phase. Therefore, the final volume of

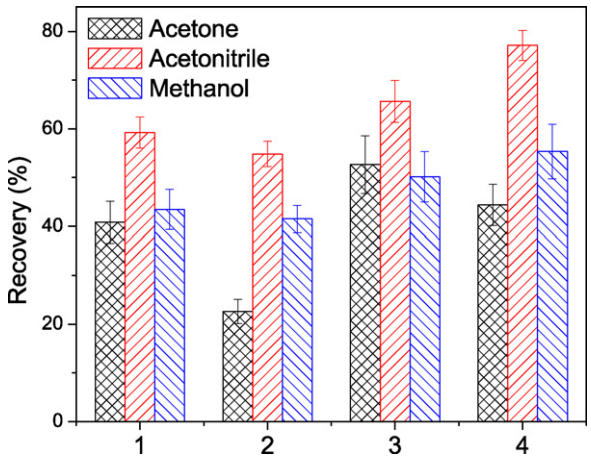


Fig. 3. Effect of disperser and terminating solvent on the extraction recovery. Conditions: sample volume, 5 mL; spiked concentration, 2 ng mL^{-1}; extraction solvent, 30 μL toluene; total volume of disperser and terminating solvent, 1.0 mL; extraction time, 10 min; $n = 5$.

the upper layer was checked using a 50 μL blunt tip microsyringe and then 1.0 μL extraction solvent was introduced into the GC-MS instrument. Based on the experimental results observed in Fig. 4, 50 μL toluene was adopted for further use.

In order to study the influence of disperser and terminating solvent volume on the extraction efficiency, 50 μL toluene solved in five different volumes of acetonitrile, 0.4, 0.5, 0.6, 0.8 and 1.0 mL, were conducted to the ST-DLLME. Same volumes of acetonitrile were added respectively into the aqueous bulk as the terminating solvent to stop the extraction after certain minutes. Therefore, the total volumes of acetonitrile used as disperser and terminating solvent were 0.8, 1.0, 1.2, 1.6 and 2.0 mL, respectively, in this study. As Fig. 5 shows, higher extraction efficiency was obtained using 1.0 mL acetonitrile. Thus, 1.0 mL (i.e. 0.5 + 0.5 mL) acetonitrile was chosen in this work.

3.1.4. Extraction time

In this experiment, extraction time means the time interval from the beginning of the dispersion and its end just before addition of the terminating solvent. The effect of extraction time was examined in the range of 2.5–30 min. As results clearly shown, extraction time has no significant effect on the recoveries of these four carbamates, because the rate of extraction in DLLME is extremely fast [8]. The result revealed that 2.5 min of extraction time was enough to achieve high extraction efficiency. It should be noted that shorter extraction time might increase variation among tests. In the following experiments, the extraction time of 10 min was adopted to get maximum recovery of the carbamate pesticides.

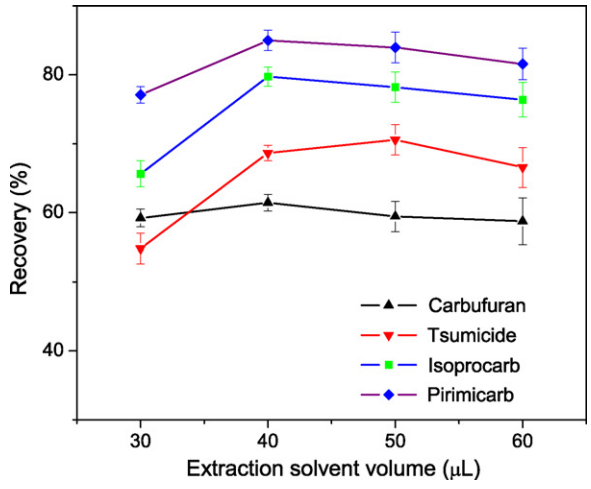


Fig. 4. Effect of extraction solvent volume on the extraction recovery. Conditions: sample volume, 5 mL; spiked concentration, 2 ng mL^{-1}; extraction solvent, toluene; disperser and terminating solvent, 1.0 mL acetonitrile; extraction time, 10 min; $n = 5$.

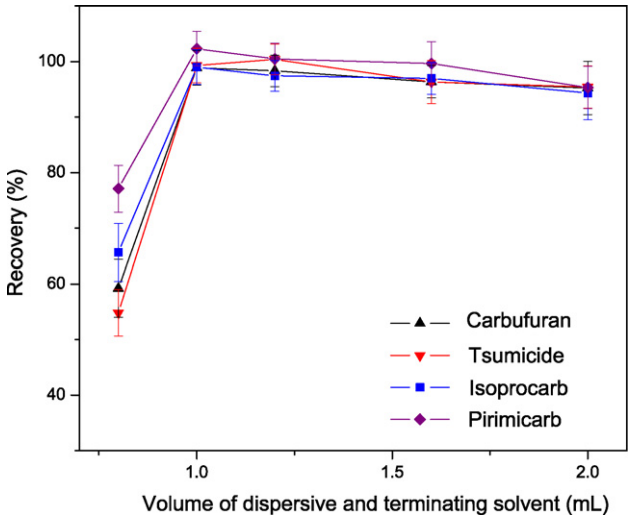


Fig. 5. Effect of disperser and terminating solvent volume on the extraction recovery. Conditions: sample volume, 5 mL; spiked concentration, 2 ng mL^{-1}; extraction solvent, 50 μL toluene; disperser and terminating solvent, acetonitrile; extraction time, 10 min; $n = 5$.

Table 2
LOD, Linear range, (LR) correlation coefficients (r^2), and RSD of the method ($n = 5$).

Pesticides	LOD (ng mL^{-1})	LR (ng mL^{-1})	r^2	RSD (%)
Carbofuran	0.008	0.02–20	0.9938	4.6
Tsumicide	0.050	0.10–20	0.9988	3.7
Isoprocarb	0.001	0.005–5	0.9963	2.3
Pirimicarb	0.008	0.02–20	0.9995	6.8

3.1.5. pH

The pH of the extracted solution is expected to induce significant impact on the extraction. In order to examine this parameter, experiments were carried out with the pH of the original aqueous samples varying from 3.0 to 7.0. The pH value above 7.0 was not tested since degradation of the carbamate pesticides may occur under the alkaline condition. The obtained results displayed that the sample pH had no notable effect on the recovery. The carbamate pesticides tend to form neutral molecular at low pH, which have good affinity to the non-polar solvent. That would improve the extraction efficiency. On the other hand, however, it was found that the performance of dispersion decreased to some extent at pH lower than 5.0. Hence, pH value of 7.0 was the reasonable compromise for this purpose.

Over all, the optimized ST-DLLME conditions were 50 μL toluene within 0.5 mL acetonitrile for dispersion; 0.5 mL acetonitrile for termination; extraction time 10 min; sample pH 7.

3.2. Analytical performance

The optimum experimental conditions were used to assess the applicability of the proposed method for quantitative determination of target analytes by GC-MS. A series of experiments were designed for obtaining linear ranges, precision, detection limits and other characteristics of the method. Standard solutions with analytes over the concentration range of 0.02–20 ng mL^{-1} ($n = 5$) were served to the method to perform calibration. The calculated calibra-

Table 3
Reproducibility and recovery of the method ($n = 5$).

	Youth lake water				South lake water			
	Added ($ng\,mL^{-1}$)	Found ($ng\,mL^{-1}$)	Recovery (%)	RSD (%)	Added ($ng\,mL^{-1}$)	Found ($ng\,mL^{-1}$)	Recovery (%)	RSD (%)
Carbofuran	–	nd	–	–	–	nd	–	–
	0.50	0.487	97.4	4.2	0.50	0.491	98.2	4.5
	1.00	0.981	98.1	3.1	1.00	0.990	99.0	2.7
Tsumicide	–	nd	–	–	–	nd	–	–
	0.50	0.492	98.4	5.3	0.50	0.494	98.7	6.2
	1.00	0.979	97.9	3.6	1.00	0.994	99.4	4.2
Isoprocarb	–	nd	–	–	–	nd	–	–
	0.50	0.518	104	3.9	0.50	0.507	101	4.0
	1.00	1.026	103	2.4	1.00	1.045	104	2.7
Pirimicarb	–	nd	–	–	–	nd	–	–
	0.50	0.473	94.5	6.8	0.50	0.487	97.3	5.2
	1.00	0.975	97.5	4.2	1.00	0.983	98.3	5.1

nd, not detected.

tion curves gave a high level of linearity for all target analytes with correlation coefficients (r^2) ranging between 0.9938 and 0.9995 (Table 2). The precisions, obtained by performing five replicates at the concentration of $2\,ng\,mL^{-1}$, were in the range of 2.3–6.8%. The limits of detection for all target carbamate pesticides were calculated by comparing the signal-to-noise-ratio (S/N) of the lowest detectable concentration to the S/N ratio of three. As shown in Table 2, limits of detection values obtained in this work are found to vary between 0.001 and $0.050\,ng\,mL^{-1}$ for the four carbamate pesticides.

3.3. Analysis of real water samples

In order to validate the applicability of the method, the determination of carbamate pesticides (carbofuran, tsumacide, isoprocarb, pirimicarb) in water samples was carried out with the proposed method. Reproducibility and recovery experiments were performed at two concentration levels of 0.5 and $1.0\,ng\,mL^{-1}$ for each pesticide. Table 3 lists the experimental results and no carbamate pesticide was found in the real water samples. The reproducibility of the method was obtained with RSD ranging in 2.4–6.8% and the recoveries of pesticides tested were between 94.5 and 104% at all spiked levels. The results demonstrate that the method is a reliable technique for analysis of trace carbamate pesticides in environmental water samples.

4. Conclusions

In the present study, a simple and fast method of solvent terminated dispersive liquid-liquid microextraction (ST-DLLME) has been developed for the highly sensitive determination of carbamate pesticides in the water samples by GC-MS. The developed method was convenient for the usage of low-density extraction solvents such as toluene, cyclohexane and octanol in DLLME. The new procedure of ST-DLLME is distinguished from the normal DLLME method that it does not need centrifugation to separate the organic phase. In this work, an aliquot of acetonitrile was used as the demulsifier to break up the oil-in-water (O/W) emulsion and to end off the extraction process. The analytical results confirm that the proposed approach is feasible for the fast determination of carbamate pesticides in water samples.

Acknowledgements

Financial supports from the National Natural Science Foundation of China (No. 20705009) and the Research Fund for New Teachers of Higher Education of China (No. 20070504039) are gratefully acknowledged.

References

[1] J. Zhang, H.K. Lee, J. Chromatogr. A 1117 (2006) 31.
[2] H.Y. Xie, Y.Z. He, W.E. Gan, G.N. Fu, L. Li, F. Han, Y. Gao, J. Chromatogr. A 1216 (2009) 3353.
[3] H. Chen, R.W. Chen, R. Feng, S.Q. Li, Chromatography 70 (2009) 165.
[4] M. Saraji, N. Esteki, Anal. Bioanal. Chem. 391 (2008) 1091.
[5] D.W. Lachenmeier, U. Nerlich, T. Kuballa, J. Chromatogr. A 1108 (2006) 116.
[6] Y. Zhang, J. Zhang, Anal. Chim. Acta 627 (2008) 212.
[7] S. Berijani, Y. Assadi, M. Anbia, M.R.M. Hosseini, E. Aghaee, J. Chromatogr. A 1123 (2006) 1.
[8] M. Rezaee, Y. Assadiab, M.R.M. Hosseinia, E. Aghaee, F. Ahmadi, S. Berijani, J. Chromatogr. A 1116 (2006) 1.
[9] G.H. Wei, Y.Y. Li, X.D. Wang, J. Sep. Sci. 30 (2007) 3262.
[10] L.Y. Fu, X.J. Liu, J. Hu, X.N. Zhao, H.L. Wang, X.D. Wang, Anal. Chim. Acta 632 (2009) 289.
[11] Q.H. Wu, X. Zhou, Y.M. Li, X.H. Zang, C. Wang, Z. Wang, Anal. Bioanal. Chem. 393 (2009) 1755.
[12] Z.M. Liu, X.H. Zang, W.H. Liu, C. Wang, Z. Wang, Chin. Chem. Lett. 20 (2009) 213.
[13] L.J. He, C.J. Wang, Y.J. Sun, X.L. Luo, J. Zhang, K. Lu, Int. J. Environ. Anal. Chem. 89 (2009) 439.
[14] K. Farhadi, M.A. Farajzadeh, A.A. Matin, J. Sep. Sci. 32 (2009) 2442.
[15] E.C. Zhao, W.T. Zhao, L.J. Han, S.R. Jiang, Z.Q. Zhou, J. Chromatogr. A 1175 (2007) 137.
[16] M. Garcia-Lopez, I. Rodriguez, R. Cela, J. Chromatogr. A 1166 (2007) 9.
[17] W.C. Tsai, S.D. Huang, J. Chromatogr. A 1216 (2009) 5171.
[18] J.H. Guo, X.H. Li, X.L. Cao, Y. Li, X.Z. Wang, X.B. Xu, J. Chromatogr. A 1216 (2009) 3038.
[19] R. Montes, I. Rodriguez, E. Rubi, R. Cela, J. Chromatogr. A 1216 (2009) 205.
[20] Y. Liu, E.C. Zhao, W.T. Zhu, H.X. Gao, Z.Q. Zhou, J. Chromatogr. A 1216 (2009) 885.
[21] Q.H. Wu, Y.P. Li, C. Wang, Z.M. Liu, X.H. Zang, X. Zhou, Z. Wang, Anal. Chim. Acta 638 (2009) 139.
[22] Q.H. Wu, C. Wang, Z.M. Liu, C.X. Wu, X. Zeng, J.L. Wen, Z. Wang, J. Chromatogr. A 1216 (2009) 5504.
[23] R.S. Zhao, C.P. Diao, Q.F. Chen, X. Wang, J. Sep. Sci. 32 (2009) 1069.
[24] Q.X. Zhou, L. Pang, G.H. Xie, J.P. Xiao, H.H. Bai, Anal. Sci. 25 (2009) 73.
[25] D. Nagaraju, S.D. Huang, J. Chromatogr. A 1161 (2007) 89.
[26] J. Xiong, B. Hu, J. Chromatogr. A 1193 (2008) 7.
[27] A. Saleh, Y. Yamini, M. Faraji, M. Rezaee, M. Ghambarian, J. Chromatogr. A 1216 (2009) 6673.
[28] Y.H. Kim, J. Ind. Eng. Chem. 5 (1999) 22.
[29] P. Canizares, F. Martinez, C. Jimenez, C. Saez, M.A. Rodrigo, J. Hazard. Mater. 151 (2008) 44.