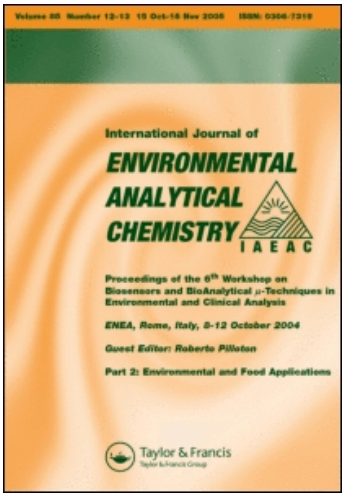

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Dispersive liquid-liquid microextraction followed by high-performance liquid chromatography for the determination of three carbamate pesticides in water samples

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Dispersive liquid–liquid microextraction followed by high-performance liquid chromatography for the determination of three carbamate pesticides in water samples

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A simple, rapid and efficient method, dispersive liquid–liquid microextraction (DLLME) in conjunction with high-performance liquid chromatography (HPLC), has been developed for the determination of three carbamate pesticides (methomyl, carbofuran and carbaryl) in water samples. In this extraction process, a mixture of 35 μL chlorobenzene (extraction solvent) and 1.0 mL acetonitrile (disperser solvent) was rapidly injected into the 5.0 mL aqueous sample containing the analytes. After centrifuging (5 min at 4000 rpm), the fine droplets of chlorobenzene were sedimented in the bottom of the conical test tube. Sedimented phase (20 μL) was injected into the HPLC for analysis. Some important parameters, such as kind and volume of extraction and disperser solvent, extraction time and salt addition were investigated and optimised. Under the optimum extraction condition, the enrichment factors and extraction recoveries ranged from 148% to 189% and 74.2% to 94.4%, respectively. The methods yielded a linear range in the concentration from 1 to 1000 $\mu g\,L^{-1}$ for carbofuran and carbaryl, 5 to 1000 $\mu g\,L^{-1}$ for methomyl, and the limits of detection were 0.5, 0.9 and 0.1 $\mu g\,L^{-1}$, respectively. The relative standard deviations (RSD) for the extraction of 500 $\mu g\,L^{-1}$ carbamate pesticides were in the range of 1.8–4.6% ($n = 6$). This method could be successfully applied for the determination of carbamate pesticides in tap water, river water and rain water.

Keywords: dispersive liquid–liquid microextraction; high-performance liquid chromatography; carbamate pesticides; water samples

1. Introduction

Carbamate pesticides, a kind of broad-spectrum pesticides, are derived from carbamic acid and used worldwide against insects, fungi and weeds [1]. Many people have paid attention to the poisons and the residues of them in food and water, because the amount of these pesticides used is becoming bigger and bigger. At present, many countries have formulated strict limits about carbamate pesticide residues in water. They are on the priority lists released by the US Environment Protection Agency (EPA), which has set a maximum allowed concentration of 40 $\mu g\,L^{-1}$ in tap water [2].

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Also, New Zealand legislation has set a maximum allowed carbofuran concentration of $8\,\mu g\,L^{-1}$ in tap water [3].

Due to the low concentrations and complex matrices, carbamate pesticides in water samples are not directly analysed with conventional methods such as high-performance liquid chromatography (HPLC) or gas chromatography (GC). Up to now, there have been many reports on the application of a preconcentration and separation technique prior to the analysis of carbamate pesticides such as liquid–liquid extraction (LLE) [4], supercritical fluid extraction (SFE) [5], solid-phase extraction (SPE) [6,7], solid-phase microextraction (SPME) [8,9] and liquid-phase microextraction (LPME) [10,11]. Recent researches have been directed towards efficient, economical and miniaturised sample preparation. As a result, SPME and LPME have been developed. SPME is a solvent-free process that includes simultaneous extraction and preconcentration of analytes from the aqueous samples or the headspace of the samples. However, SPME is expensive, its fibre is fragile and sample carry-over could be a problem [12,13]. LPME has been developed as an alternative extraction technique. This method provides analytes extraction using only a few microlitres of organic solvent. But, this method has some disadvantages as follows: fast stirring speed tends to break up the organic drop; extraction is time-consuming and equilibrium could not be attained during a short time in most cases [14].

Recently, Assadi and co-workers reported a novel microextraction technique named 'dispersive liquid–liquid microextraction' (DLLME) [15,16]. It is based on a ternary component solvent system like homogeneous liquid–liquid extraction (HLLE) [17] and cloud point extraction (CPE) [18]. In this process, a cloudy solution is formed in the test tube when an appropriate mixture of extraction and disperser solvent is rapidly injected into an aqueous sample containing analytes. The cloudy state results from the formation of fine droplets of the extraction solvent which is dispersed in the sample solution. After centrifugation, the fine droplets are sedimented at the bottom of the conical test tube. Thus, the analytes concentrated in the sedimented phase can be determined by conventional analytical methods. The advantages of the DLLME technique are of simplicity of operation, rapidity, low cost, high recovery and enrichment factor (EF). With the development of DLLME, its application has been extended to determine organic [19–23] and inorganic [24–26] compounds in water samples.

The goal of the present work was to study the possibility of using DLLME combined with HPLC for the analysis of carbamate pesticides in water samples. The influence of the different experimental parameters on the extraction efficiency is described and discussed in this work. This method was employed to investigate the levels of the target species in several real water samples.

2. Experimental

2.1 *Standard solution and reagents*

Methomyl (98.4% purity), carbofuran (98.5% purity) and carbaryl (98.7% purity) were purchased from Pesticide Research Institute (Shanghai, China) and their structures are listed in Figure 1. All of the reagents used as extraction solvent in this experiment (chlorobenzene, tetrachloroethane, carbon tetrachloride, carbon disulphide, dichloromethane and chloroform) were of analytical grade and distilled at least three times. Acetonitrile, methanol, acetone and ethanol (HPLC grade) were used as disperser solvents. Doubly distilled water was used throughout this work.

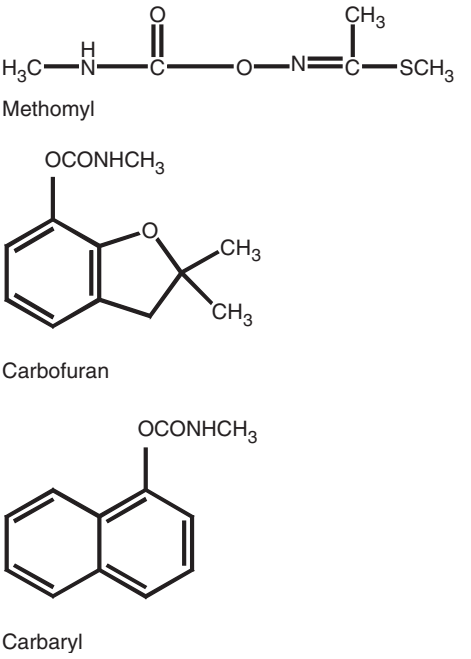

Figure 1. Structures of methomyl, carbofuran and carbaryl.

The individual stock standard solutions were prepared in methanol at a concentration of 100 μg mL^{-1} and stored at 4°C. The standard working solutions were daily prepared by dilution of stock standard solution with distilled water to the required concentrations.

Tap water was collected fresh from our laboratory. Rain water was obtained from Henan University of Technology, Zhengzhou, China. River water samples were collected from Yellow River, Zhengzhou, China. All of the water samples were filtered through 0.45 μm filter membrane immediately after sampling and stored in brown bottles at 4°C.

2.2 *Instrumentation*

A HPLC system consisted of a LC-10ATvp pump, a SPD-10Avp ultraviolet detector (Shimadzu, Japan) and a 7725i injector valve with a 20 μL sample loop (Rheodyne, USA). A VP–ODS column (250 × 4.6 mm, 5 μm particle size) was used for separation. The mobile phase was a mixture of methanol and water (80 : 20, v/v) delivered at a flow rate of 0.8 mL min^{-1} and the UV detector was set at a wavelength of 230 nm. The phase separation was conducted with a centrifuge (Jintan Huafeng Instrument Co. Ltd., Changzhou, China).

2.3 *DLLME procedure*

In the DLLME, 5.0 mL aqueous solution containing analytes was added to a 10 mL test tube with conical bottom. Acetonitrile (1 mL) as disperser solvent containing 35 μL chlorobenzene (extraction solvent) was injected rapidly into the sample solution using

a 1.0 mL syringe (Kehua Co. Ltd., Shanghai, China) and then the mixture was gently shaken. A cloudy solution (water, acetonitrile and chlorobenzene) was formed in the test tube and the cloudy state was stable for a long time. After centrifugation for 5 min at 4000 rpm, extraction solvent was sedimented at the bottom of the conical test tube (about 25 μL). Twenty microlitre of sedimented phase was removed using a microsyringe and injected into the HPLC system for analysis.

3. Results and discussion

3.1 *Optimisation of DLLME*

There are several factors that affect the extraction process including extraction solvent, disperser solvent, volume of extraction solvent and disperser solvent, salt addition and extraction time. The optimisation was carried out with sample solutions of 500 $\mu g\,L^{-1}$ for each analyte. The chromatographic peak area was used to evaluate the extraction efficiency. All experiments were performed three times and the average of the results was used in plotting of curves or in tables.

3.1.1 *Selection of extraction solvent*

The selection of an appropriate extraction solvent is important for the DLLME process. The extraction solvent has to meet four requirements: (1) to have higher density than water, (2) to have good chromatographic behaviour, (3) to have a higher extraction efficiency to interest analytes and (4) to be immiscible with water. Among the solvents with density higher than water, CH_2Cl_2 (1.32 $g\,mL^{-1}$), $CHCl_3$ (1.47 $g\,mL^{-1}$), $C_2H_2Cl_4$ (1.60 $g\,mL^{-1}$), CCl_4 (1.59 $g\,mL^{-1}$), CS_2 (1.26 $g\,mL^{-1}$) and C_6H_5Cl (1.11 $g\,mL^{-1}$) were studied. A series of sample solution were studied by using 1.0 mL methanol and 20.0 μL different kinds of extraction solvents. In the case of CH_2Cl_2 and $CHCl_3$ as extraction solvents, a two-phase system was not observed and there was no sedimented phase at the bottom of the test tube after centrifugation. It is probably due to higher solubility of these solvents in water than the other tested solvents. The peak of $C_2H_2Cl_4$ interfered the peak of carbofuran and carbaryl seriously. According to the results in Figure 2, chlorobenzene showed higher extraction efficiency than the other solvents. This is probably owing to the interaction between the benzene ring of chlorobenzene and that of analytes. Therefore, chlorobenzene was selected as the extraction solvent for subsequent experiments.

3.1.2 *Selection of disperser solvent*

In DLLME process, disperser solvent should be miscible with both sample solution (aqueous phase) and extraction solvent (organic phase), and have good chromatographic behaviour. Therefore, methanol, acetonitrile, acetone and ethanol were tested as disperser solvent and their effect on the performance of DLLME was investigated. A series of sample solutions were studied using 1.0 mL of methanol, acetonitrile, acetone and ethanol containing 20.0 μL chlorobenzene. The peak of acetone interfered with the peak of methomyl. Figure 3 shows that acetonitrile was the most suitable disperser solvent compared to others. Thus, acetonitrile was chosen as the disperser solvent in this work.

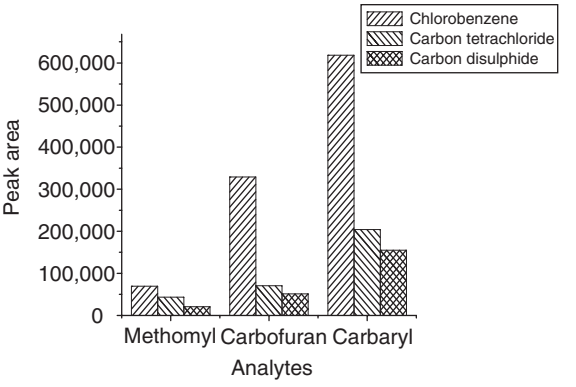

Figure 2. Effect of extraction solvent on extraction efficiency. Extraction conditions: 5.0 mL water sample; 20 μL extraction solvent; 1.0 mL disperser solvent (methanol).

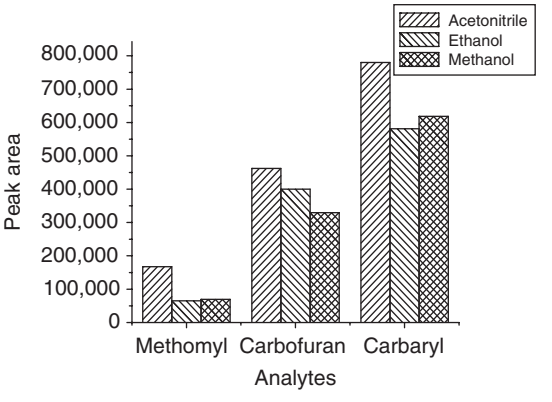

Figure 3. Effect of disperser solvent on extraction efficiency. Extraction conditions: 5.0 mL water sample; 1.0 mL disperser solvent; 20 μL extraction solvent (chlorobenzene).

3.1.3 *Effect of extraction solvent volume*

In order to study the effect of extraction solvent volume on the extraction efficiency, different volumes of chlorobenzene (25.0–85.0 μL at 10 μL interval) and a constant volume of dispersive solvent (acetonitrile, 1.0 mL) were tested. According to Figure 4, with the increase of chlorobenzene volume (25.0–35.0 μL), the peak areas of three analytes increased; however, increasing the volume of chlorobenzene (35.0–85.0 μL), the peak areas of three analytes decreased. The decrease was due to the increase in the volume of sedimented phase when increasing the volume of chlorobenzene, so the concentration of analytes decreased in the sedimented phase. As a result, 35.0 μL chlorobenzene was used as extraction solvent for further experiments.

3.1.4 *Effect of disperser solvent volume*

For obtaining optimised volume of acetonitrile, experiments were performed by using different volumes of acetonitrile containing 35.0 μL chlorobenzene. Figure 5 shows that the peak areas of analytes increased with increase of the volume of acetonitrile

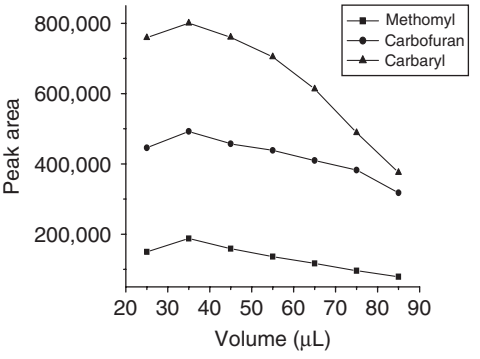

Figure 4. Effect of the volume of chlorobenzene on extraction efficiency. Extraction conditions: 5.0 mL water sample; 1.0 mL disperser solvent (acetonitrile); chlorobenzene as extraction solvent.

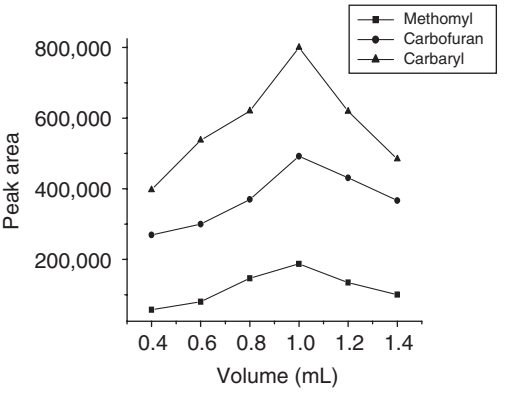

Figure 5. Effect of the volume of acetonitrile on extraction efficiency. Extraction conditions: 5.0 mL water sample; 35 μL extraction solvent (chlorobenzene); acetonitrile as disperser solvent.

from 0.4 to 1.0 mL. Decrease in peak areas was observed when the volume of acetonitrile exceeded 1.0 mL. It was found that, at low volumes of acetonitrile, the cloudy solution was not formed completely, so the extraction efficiency of analytes was low; while, at high-volume acetonitrile, the solubility of chlorobenzene in aqueous solution was increased, therefore, the extraction efficiency decreased. According to the results, 1.0 mL acetonitrile was selected as optimal volume.

3.1.5 *Effect of extraction time*

Extraction time is one of the most important factors in most of the extraction procedures, especially in microextraction techniques such as SPME and LPME. In DLLME, extraction time is defined as an interval time between the time of injecting mixture of disperser solvent and the extraction solvent and before centrifuging. For evaluating this parameter, different extraction times (0–60 min at 10 min interval) were studied. The experimental results demonstrated that extraction time had no significant effects on the extraction efficiency of analytes. In DLLME, the surface area between extraction solvent and aqueous phase is infinitely large after formation of cloudy solution. Thus, the transfer

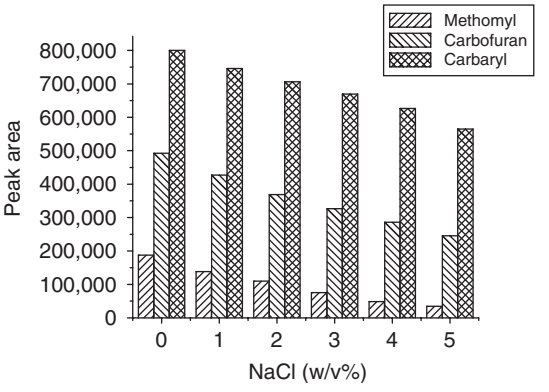

Figure 6. Effect of addition of NaCl on extraction efficiency. Extraction conditions: 5.0 mL water sample volume; 35 μL chlorobenzene; 1.0 mL acetonitrile.

of analytes from aqueous phase to extraction phase is fast, and equilibrium state is achieved quickly. The short extraction time is the most important advantage of DLLME technique as reported before [15,16]. In this extraction process, time-consuming step was centrifugation of sample solution, which was about 5 min.

3.1.6 *Salt addition*

For investigating the influence of ionic strength on the extraction efficiency, various experiments were performed by adding different amount of NaCl (0–5% (w/v)) with other experimental conditions being constant. It was found that the volume of sedimented phase increased with the increase of the concentration of NaCl, which was due to the decrease in solubility of the extraction solvent in aqueous phase in the presence of salt. As shown in Figure 6, with addition of salt, a reverse effect on extraction efficiency occurred because of increase in the volume of sedimented phase. In this study, all the extraction experiments were carried out without salt.

3.2 *Evaluation of DLLME method*

3.2.1 *Features of this method*

The characteristic calibration data listed in Table 1 were obtained under optimised conditions and typical chromatogram obtained by the extraction from one sample of water is presented in Figure 7. As can be seen, good linearities were observed in the range of 1–1000 $\mu g\,L^{-1}$ for carbofuran and carbaryl, 5–1000 $\mu g\,L^{-1}$ for methomyl, with the correlation coefficient (R^2) ranging from 0.9987 to 0.9998. The limits of detection (LOD) was based on the signal-to-noise ratio of 3 and varied from 0.1 to 0.9 $\mu g\,L^{-1}$. The repeatability (relative standard deviations, RSD) was evaluated on six replicate experiments at 500 $\mu g\,L^{-1}$ concentration and was in the range of 1.8–4.6%. The EF and extraction recovery (ER%) were high ranging from 148 to 189 and from 74.2% to 94.4%, respectively. EF and ER% were calculated from Equations (1) and (2).

$$\mathrm{EF} = \frac{C_{\mathrm{sed}}}{C_0}, \tag{1}$$

Table 1. Feature of the DLLME method.

Compound	Linear range ($\mu g\,L^{-1}$)	R^2	LOD ($\mu g\,L^{-1}$)	RSD (%)	EF	ER(%)
Methomyl	5–1000	0.9996	0.5	1.8	172	85.7
Carbofuran	1–1000	0.9998	0.9	3.7	148	74.2
Carbaryl	1–1000	0.9987	0.1	4.6	189	94.4

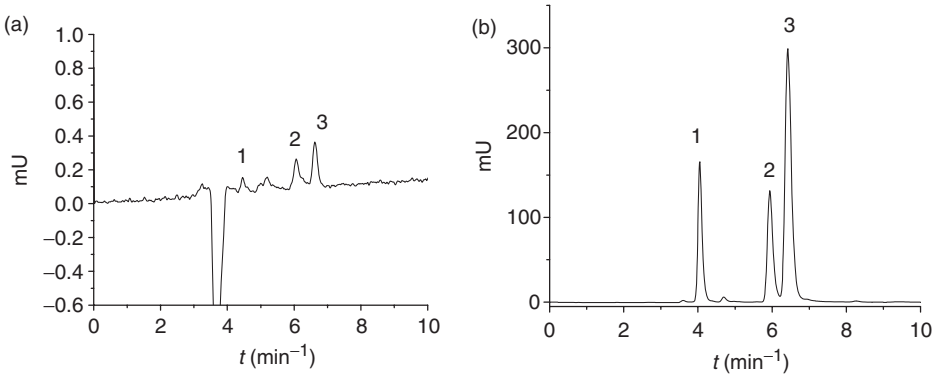

Figure 7. Chromatograms obtained for the aqueous solutions consisting $500\,\mu g\,L^{-1}$ of each analyte before (a) and after (b) using DLLME under the optimum conditions. Peak: (1) methomyl; (2) carbofuran and (3) carbaryl.

where C_{sed} and C_0 are the concentration of analyte in sedimented phase and initial concentration of analyte in aqueous sample, respectively. The C_{sed} was obtained from calibration graph of direct injection of carbamate pesticides standard in the range of $0.5–25\,\mu g\,mL^{-1}$.

$$ER\% = \frac{V_{sed}C_{sed}}{V_{aq}C_0} \times 100 = \frac{V_{sed}}{V_{aq}} EF \times 100, \quad (2)$$

where V_{sed} and V_{aq} are the volume of sedimented phase and aqueous sample, respectively.

3.2.2 *Real water samples analysis*

Tap water, river water and rain water were analysed using the proposed method. The results showed that the concentration of three carbamate pesticides were all below detectable level among the above-mentioned samples. Recovery testing was carried out with 25, 40 and $60\,\mu g\,L^{-1}$ for three analytes spiked to the water samples. As listed in Table 2, the relative recoveries for three carbamate pesticides in tap water, river water and rain water were in the range of 84.5–102.8%, 87.2–102.3% and 86.3–104.4%, respectively. It demonstrated that the matrices in samples had little effect on the proposed method.

3.2.3 *Comparison of DLLME with other methods*

This method was compared with other reported methods such as SPE, SPME and LPME for the extraction of carbamate pesticides in water samples. As shown in Table 3,

Table 2. Determination of three analytes in real water samples.

	Tap water			River water			Rain water		
Compound	Found[a] ($\mu g\,L^{-1}$)	RR[b] (%)	RSD (%)	Found ($\mu g\,L^{-1}$)	RR (%)	RSD (%)	Found ($\mu g\,L^{-1}$)	RR (%)	RSD (%)
Methomyl	22.8	91.2	2.6	21.8	87.2	2.4	22.5	90.0	3.2
	36.5	91.3	3.4	37.4	93.5	4.1	37.1	92.8	5.5
	60.2	100.3	3.8	56.4	94.0	4.6	58.2	97.0	5.1
Carbofuran	23.1	92.4	4.8	24.5	98.0	3.1	26.1	104.4	2.7
	33.8	84.5	4.4	35.8	89.5	5.8	34.5	86.3	5.9
	58.5	97.5	5.8	61.4	102.3	4.4	57.8	96.3	2.9
Carbaryl	22.4	89.6	3.2	23.6	94.4	6.9	24.1	96.4	4.6
	40.8	102.0	5.4	39.1	97.8	6.1	37.8	94.5	5.6
	61.7	102.8	6.4	60.8	101.3	5.1	58.4	97.3	6.1

Notes: [a]Water samples were spiked with 25, 40 and 60 $\mu g\,L^{-1}$ for three analytes, respectively.
[b]RR: Relative recovery.

Table 3. Comparison of DLLME with other methods.

Methods	Linear range ($\mu g\,L^{-1}$)	LOD ($\mu g\,L^{-1}$)	RSD (%)	Extraction time	Reference
SPE–GC–MS	0.4–1160	0.1	<6	60 min	[6]
SPME–HPLC–UV	5–10,000	1.0	1.7–5.3	25 min	[9]
LPME–GC–MS	1–400	0.2–0.8	4.9–7.8	20 min	[10]
LPME–HPLC–UV	10–100	1.0–5.0	2.0–6.2	20 min	[11]
DLLME–HPLC–UV	5–1000	0.1–0.9	1.8–4.6	A few seconds	This work

in comparison with other extraction methods, extraction time in DLLME is very short, and extraction equilibrium can be achieved quickly (a few seconds). Linear range, LOD and RSD for the proposed method are comparable with and in some cases are better than those of the other methods. This indicates that DLLME combined with HPLC is an efficient method for the extraction and determination of carbamate pesticides in water samples.

4. Conclusion

In the present study, DLLME combined with HPLC method has been used for the determination of carbamate pesticides in water samples. The optimum conditions of the developed DLLME were obtained. The method was also applied for the extraction of carbamate pesticides from tap water, river water and rain water with satisfactory relative recovery and low RSD. As compared with the other sample preparation methods for extraction and determination of carbamate pesticides, this method offered some advantages such as rapidity, sensitivity, simplicity of operation and low cost.

Acknowledgements

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