Scientific paper

Comparison of Micelle-Mediated Extraction and Diazotized 2,4-Dimethoxyaniline Methods for the Simultaneous Determination of Carbamate Insecticides by HPLC–UV

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Abstract

The feasibilities of two strategies for the simultaneous analysis of five carbamate insecticides (i.e. propoxur, carbofuran, carbaryl, isoprocarb, and promecarb) by high performance liquid chromatography (HPLC) have been investigated. The first method is the preconcentration strategy based on micelle-mediated extraction (MME) using sodium dodecyl sulfate. The second strategy uses chemical derivatization with 2,4-dimethoxyaniline (DMA) reagent before subjecting to HPLC. The parameters affecting the analysis were optimized. For MME, the optimum conditions were 4.0% (w/v) SDS in the presence of 5.0 mol L^{-1} HCl. Meanwhile, the DMA derivatization condition was 1.5 mmol L^{-1} DMA, 7 mmol L^{-1} $NaNO_2$, and 50 mmol L^{-1} HCl, and 250 mmol L^{-1} NaOH for the hydrolysis of carbamates before their derivatization. The target carbamates and their derivatives were monitored at wavelengths of 270 and 380 nm for the MME and DMA derivatization methods, respectively. The capability of each developed method was compared in terms of separation time and limits of detection (LODs). The results show that the five studied insecticides were successfully separated within 8 min (DMA) and 27 min (MME), respectively. LODs of the insecticides obtained from DMA (0.01–0.04 mg L^{-1}) were lower than those obtained from MME (0.1–0.7 mg L^{-1}). The proposed DMA method is versaltile and superior to MME for the analysis of carbamate insecticides in tap water samples.

Keywords: Carbamate insecticides; 2,4-dimethoxy aniline; micelle-mediated extraction; high performance liquid chromatography; water sample

1. Introduction

Carbamates include a group of insecticides containing carbamic acid as a functional group. Nowadays, carbamates (such as carbaryl, carbofuran, and propoxur) are widely used in agriculture for the protection of fruit and vegetable crops from pests. They are used instead of organochlorine compounds because of their selective insecticidal properties and lower persistence in the environment.[1–3] However, the increasing or inappropriate use of these carbamates causes serious effects on the environment and poses a risk to consumers because the carbamates exhibit high acute toxicity and they are suspected of being carcinogens and mutagens.[4,5] Therefore, reliable, sensitive, simple, rapid, and effective analytical methods are needed for monitoring the pesticide residues in samples.

Several analytical methods have been proposed for the separation and determination of carbamate residues in various food samples.[2,3,6–13] Due to their physical and chemical properties, such as thermal instability and polarity, carbamates are difficult to analyze using gas chromatography (GC) without the time-consuming process of derivatization. The preferred analytical technique for analysis of these insecticides is high performance liquid chromatography (HPLC) employing ultraviolet (UV),

photodiode array (PDA),[11] fluorescence (FL),[3,12,13] chemiluminescence (CL)[14,15] or mass spectrometric (MS) detections.[2,6–8,10] Although LC–MS has been accepted for single and multi-residue analysis of the pesticides because of its sensitivity and selectivity; it is still expensive in terms of instrumentation and running cost. Post-column derivatization prior to FL or post-column photolysis before CL detection can enhance sensitivity and provide good selectivity in the determination of carbamates, but it needs more complex flow manifolds and expensive post-column systems.[3,11,12,13,14,15] Reversed phase-HPLC (RP-HPLC) with UV and PDA detection has been accepted as a simple and reliable technique for the determination of carbamates in a range of samples. However, the use of direct UV detection for the target pesticides is lacking in sensitivity. To overcome the problem, two strategies can be used for improving the sensitivity by either a preconcentration or a derivatization strategy. A promising preconcentration technique using surfactants such as Triton X-114 non-ionic surfactant[16] (so called cloud-point extraction (CPE) or micelle-mediated extraction (MME)) has been recently presented to preconcentrate the analytes before analysis by HPLC–UV. Another alternative technique is based on derivatization of carbamates with proper reagents and spectrometric detection of its azo-dye products. The use of active derivatizing agents which are 2-naphthylamine-1-sulfonic acid (ANSA),[17] *p*-aminophenol (PAP),[18] *p*-nitroaniline (PNA),[19] before spectrophotometry has also recently developed. However, these reagents have some limitations for developing diazotization and their analyses. ANSA can be used only for single analysis of carbaryl,[17] whereas unstable absorbance and large values of a blank after chemical derivatization especially using PAP are frequently obtained.[19,20] To our knowledge, the application of CPE or MME and analysis based on derivatization for simultaneous determination of carbamates is limited. There is a work published on acid-induced MME using anionic surfactant for preconcentration before fluorescence spectrophotometric determination of carbaryl and 1-naphthol in water samples.[21] Meanwhile, the method based on derivatization using 4-aminoantipyrine (AP) reagent coupled to HPLC has also been reported for single analysis of carbofuran.[22]

In the present study, we aimed to study the feasibility of employing two different developed strategies, i.e. acid-induced MME using sodium dodecyl sulfate (SDS) and the derivatization method using 2,4-dimethoxyaniline (DMA) reagent for improving detection and simultaneous analysis of carbamate insecticides. DMA reagent has some advantages over the other reagents such as high stability of the derivatives (up to 68 h) and high molar absorptivity ($\sim 3 \times 10^4$ L mol^{-1} cm^{-1}).[23] The experimental parameters affecting the performance of each method were optimized. The selected method was then applied for analysis of target pesticides in water samples.

2. Experimental

2. 1. Chemicals and Reagents

All reagents were of analytical reagent grade. All carbamate insecticide standards (carbaryl or CBR, carbofuran or CBF, propoxur or PPX, isoprocarb or IPC, and promecarb or PMC) were purchased from Sigma-Aldrich (Seelze, Germany). The stock 1000 mg L^{-1} standards of carbamate insecticides were prepared by dissolving each pesticide in methanol. Sodium dodecyl sulfate (SDS) was purchased from BDH (Poole, England). Concentrated HCl was obtained from Carlo Erba (Val de Reuil, France). The solution of 20 mM 2,4-dimethoxy aniline (DMA) (Fluka, Japan) was prepared by dissolving an appropriate amount in a little volume of methanol first and then adjusting the final volume with water. The aqueous solutions of 145 mM $NaNO_2$ (Riedel-de Haën, Geramany), 1 M HCl, and 5 M NaOH (Carlo Erba, France) were also prepared for coupling diazotization reaction. Methanol, LC grade, (Labscan Asia Co. Ltd., Bangkok, Thailand) and glacial acetic acid (Carlo Erba, Val de Reuil, France) were used for mobile phase preparation. Aqueous solutions were prepared with deionized water from the RiO_s^{TM} Type I Simplicity 185 model with the resistivity of 18.2 MΩ cm (Millipore, Massachusetts, USA).

2. 2. Instrumentations

Chromatographic separation was performed on a Waters Tiger LC System (Waters, Massachusetts, USA) that include a 20 μL sample loop and UV detector (200–380 nm). The Clarity software (Waters) was used for data acquisition. A C18 – Nova Pak (3.9 mm × 150 mm, 5 μm) column (Waters, Massachusetts, USA) was used. A Kokusan (Biomed group Co. Ltd., Bangkok, Thailand) centrifuge was used for phase separation.

2. 3. Micelle-Mediated Extraction

An aliquot of sample or standard solution (5.0 mL) was mixed with 0.4 g SDS (ca. 8.0 %, *w/v*), followed by the addition of 4.0 mL of ca. 5.0 mol L^{-1} HCl. This solution was homogeneously mixed for 1 min using a magnetic stirrer. The solution was placed into a 15 mL centrifuge tube and then left at room temperature for 5 min to equilibrate. After centrifugation for 10 min at 3500 rpm, it was kept in an ice bath for 10 min. The surfactant-rich upper phase (gelatinous phase) was removed from the aqueous solutions by a spatula. At room temperature, the gelatinous phase returned to the liquid state. The surfactant-rich phase was then diluted with methanol (~ 300 μL) and water to the volume of 5.00 mL. Before being injected into HPLC, the extract solutions were filtered through a 0.45 μm membrane filter. All the experiments were per-formed in triplicate.

2. 4. 2,4-Dimethoxy Aniline Diazotization Procedure

The coupling diazotized DMA reagent solution (solution I) was prepared by adding the solutions in the follow-ing order: 1.5 mmol L^{-1} DMA, 7 mmol L^{-1} $NaNO_2$, and 50 mmol L^{-1} HCl. Appropriate volumes of sample or standard carbamate solution were hydrolyzed with 250 mmol L^{-1} NaOH (solution II). The resultant hydrolysis product of phenolates was coupled with the diazotized DMA reagent to give orange-red colored compounds by adding the solution I into the solution II. After the reaction had completed (10 min), the solution was filtered through 0.45 μm membrane filter before subjecting to the HPLC system.

2. 5. Chromatographic Separation Conditions

For separation of carbamates after MME, the experiments were carried out using gradient elution of methanol and 0.1 % (*v/v*) acetic acid at a flow rate of 0.7 mL min^{-1}. The separation column was kept at room temperature. The detection wavelength was set at 270 nm. Meanwhile, the separation of the derivatized carbamates (DMA method) was performed at 25 °C using gradient elution of MeOH and 5 mmol L^{-1} acetate buffer (pH 5.0) at a flow rate of 0.7 mL min^{-1}. The detection was monitored at 380 nm. The gradient profiles for both methods are summarized in Table 1.

3. Results and Discussion

3. 1. Optimization Conditions for MME

Two crucial parameters affecting the efficiency of extraction of acid-induced micelle-mediated extraction include (i) the concentration of anionic surfactant and (ii) the amount of hydrochloric acid (HCl) which were investigated. Other parameters, such as vortex time and centrifugation time are expected to have little effect on the extraction efficiency and recoveries.[21,24]

In this study, SDS was used because it gives a clear homogeneous surfactant-rich phase.[21] The effect of concentration of SDS on the extraction efficiency was studied in the range 0.10–0.50 g per 5 mL sample (data not shown). Peak areas of the studied carbamate increased as the SDS content increased to 0.40 g but rapidly decreased beyond this level. In addition, the proportion of the surfactant-rich phase volume (V_{SRP}) showed a linear relationship to the SDS content. High V_{SRP} results in decreasing response of target analytes after extraction. To obtain highest response, 0.40 g SDS was chosen in this study as the optimum value for further experiments.

The effect of HCl content on the extraction was observed for study. The highest peak areas (data not shown) for all the studied insecticides were obtained at 4.0 mL (ca. 5.0 mol L^{-1} HCl) per 5 mL aqueous sample solution. Beyond this point, peak areas of the target insecticides were decreased. It was observed that the V_{SRP} decreased with increasing HCl content, as also reported by other researchers.[25–27] To summarize, the optimum conditions for coacervation extraction was 0.4 g SDS, 4.0 mL of ca. 5.0 mol L^{-1} HCl and centrifugation at 3500 rpm for 10 min. Under these conditions, the pH of the surfactant-rich phase was about 1–2, too low to be directly injected into the chromatographic system. Thus, it was necessary to dissolve the surfactant-rich phase in 300 μL methanol and water (adjusted to 5-mL final volume) to reduce viscosity and increase the pH of the solution being injected to the HPLC.

3. 2. Optimization Conditions of Diazotization Procedure

In general, diazotization procedure is carried out in the following two steps; (1) hydrolyzation of carbamates in alkaline medium (NaOH solution) and (2) derivatization of hydrolyzed carbamates with derivatizing agent. In this study, the effect of mixing order of reagents (DMA, $NaNO_2$, and HCl) was firstly investigated and the effect of their concentrations as well as NaOH con-

Table 1. Chromatographic conditions used in DMA and MME methods for the separation of the target compounds

	MME	**DMA**
Mobile phase	(A) 0.1% (v/v) acetic acid (B) MeOH	(A) 5 mM sodium acetate pH 5.0 (B) MeOH
Time program	0–10 min, 40% B 10–25 min, 70% B 25–30 min, 100% B	0–2 min, 78% B 2–6 min, 80% B 6–7 min, 80% B 7–8 min, 90% B 8–10 min, 90% B
Flow rate (mL min^{-1})	0.7	0.7
Detection wavelength (nm)	270	380

tent subsequently studied on the derivatization of carbamates.

It can be seen from the results in Figure 1 that the order of mixing reagents in the diazotization reaction affects the responses of most carbamate derivatives. The addition of DMA reagent into the mixture solution of HCl and $NaNO_2$ ($HCl–NaNO_2–DMA$) provided higher responses for all carbamates studied than that of $HCl–NaNO_2$ or $NaNO_2–HCl$ added into the reagent solution of DMA. Therefore, the solution of HCl and $NaNO_2$ was firstly prepared and then added to the DMA reagent to form diazonium ions before derivatizating with carbamates in alkaline medium (NaOH was used in this study). The proposed reaction of DMA and hydrolyzed carbamates (carbaryl is the representative analyte) is demonstrated in Figure 2. Although the detection can be carried out in the visible region (absorption spectra not shown) at around 460 nm as the maximum absorption wavelength (λ_{max}) for most analytes, another λ_{max} in the UV region at 380 nm was used in this study which still obtained high molar absorptivity and low noise background.

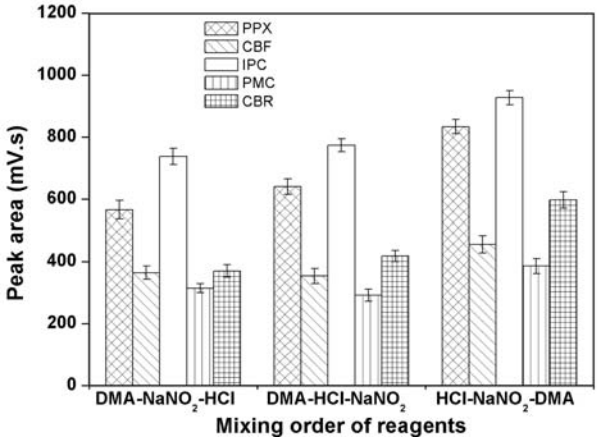

Figure 1: Effect of order of reagent mixing for diazotization reaction of the studied carbamates (5 mg L^{-1} each pesticide) with 2,4-dimethoxy aniline (DMA).

The investigation of DMA concentration was done in the range 0.5–2.0 mmol L^{-1} (Figure 3a). It was clearly seen that the responses of carbamate derivatives increased with the DMA concentration up to 1.5 mmol L^{-1} and rapidly decreased beyond that point, except for PMC and CBR. Subsequently, the effect of $NaNO_2$ concentration was studied in the range of 4–15 mmol L^{-1} (Figure 3b). The responses of IPC, PPX, and CBF were slightly decreased after the $NaNO_2$ was higher than 7 mmol L^{-1}, while CBR showed the highest response at

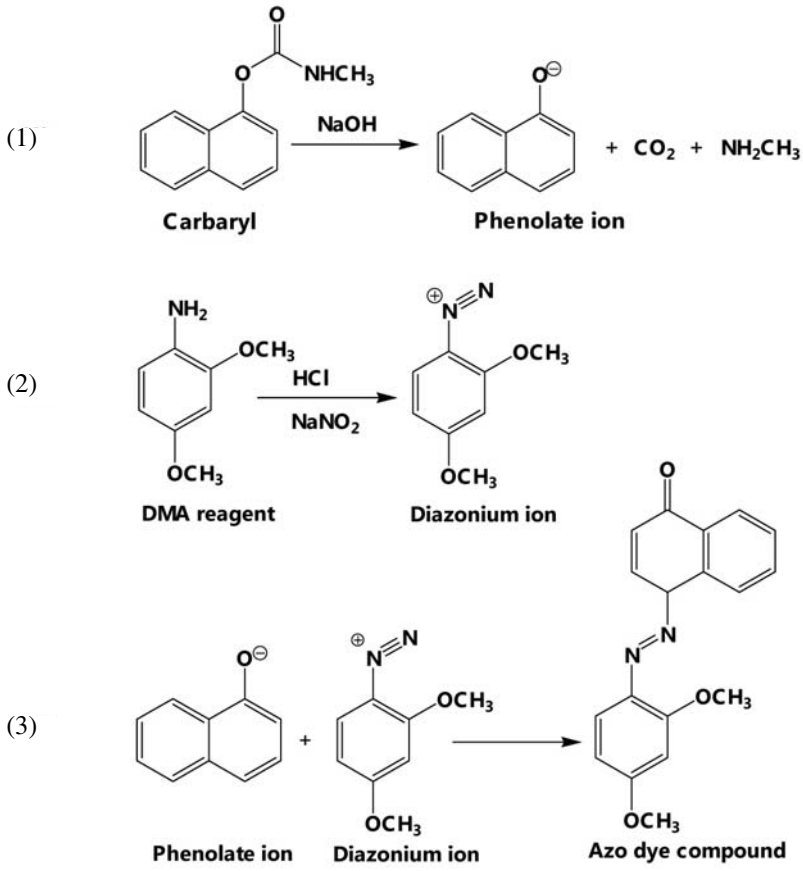

Figure 2: Possible proposed mechanism of diazotization reaction of carbaryl (as representative carbamate) with 2,4-dimethoxyaniline (DMA) reagent. Reaction steps: 1, hydrolysis of pesticides with NaOH to form phenolate species; 2, formation of diazonium ion; 3, coupling reaction of phenolate ion with diazonium ion.

the $NaNO_2$ concentration of 11 mmol L^{-1}. PMC was less affected by the reagent concentrations in the studied range. To compromise, 7 mmol L^{-1} $NaNO_2$ was chosen as the optimum value. Meanwhile, HCl was also studied in the range 25–100 mmol L^{-1} (Figure 3c). The results show that the responses of most analytes studied were the highest at 50 mmol L^{-1}. This observation was except for CBR, which gave the highest signal at 75 mmol L^{-1}. HCl at 50 mmol L^{-1} was then selected to compromise the sensitivity of all studied derivatives. Lastly, the developed diazotization reaction is carried out in alkaline medium (strong pH), in order to hydrolyze the carbamates to form phenolates before derivatization. In this study, the NaOH range 50–500 mmol L^{-1} was evaluated (Figure 3d). As the results show, decreased responses of carbamate derivatives were ob-served at higher concentrations of NaOH added to the system. Only CBR was found with increasing sensitivity as the NaOH increased up to 350 mmol L^{-1}. To provide high signals for the studied analytes, 250 mmol L^{-1} NaOH (corresponding to pH around 12.5) was chosen through-out the experiments.

To summarize the optimum conditions for derivatization, the reaction was carried out in the following conditions: 1.5 mmol L^{-1} DMA, 7 mmol L^{-1} $NaNO_2$, 50 mmol L^{-1} HCl, and 250 mmol L^{-1} NaOH at pH 12.5. Under these selected conditions, the molar ratio of DMA: $NaNO_2$:HCl was about 1:5:33.

3. 3. Comparison of the Analytical Performances and Method Validations

Under the optimum conditions for each studied method (i.e., DMA and MME), the obtained chromatograms of the studied pesticides are demonstrated in Figure 4. It was observed that the order of elution of the studied pesticides after derivatization (DMA method) is different from that of the MME method, especially for IPC, PMC and CBR. Higher peak responses of most studied compounds and their shorter separation times are also observed.

To evaluate the performance of the studied methods, some analytical characteristics of the developed DMA and MME methods were investigated along with their method validations. The results are summarized in Table 2. It can be seen that the diazotized DMA method provided greater sensitivity (calibration's slope comparison) by up to 84 times compared to the MME method. A good relationship between peak area versus concentration for each carbamate was also obtained with the coefficient of determination (R^2) higher than 0.99. Precisions in terms of intra-day (10 injections) and inter-day (triplicate injections in 5 days) performance were investigated by injecting several determinations of mixture standards at a concentration of 3.0 mg L^{-1} each under the optimum derivatizing conditions. The results show good precision in terms of the relative standard deviations (RSDs) of peak area and retention time (t_R), which are lower than 1.6 %

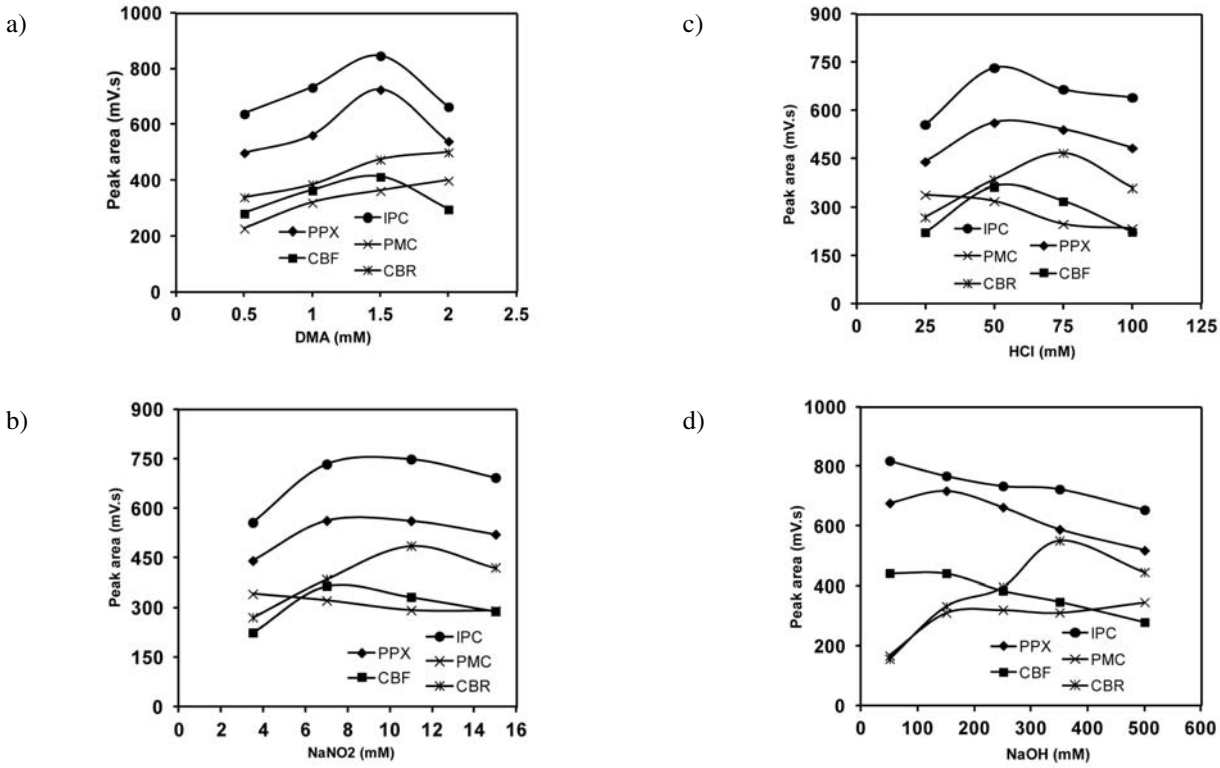

Figure 3: Effect of concentration of reagents for diazotization reaction of carbamates (5 mg L^{-1} each pesticide): (a) DMA, (b) $NaNO_2$, (c) HCl, and (d) NaOH.

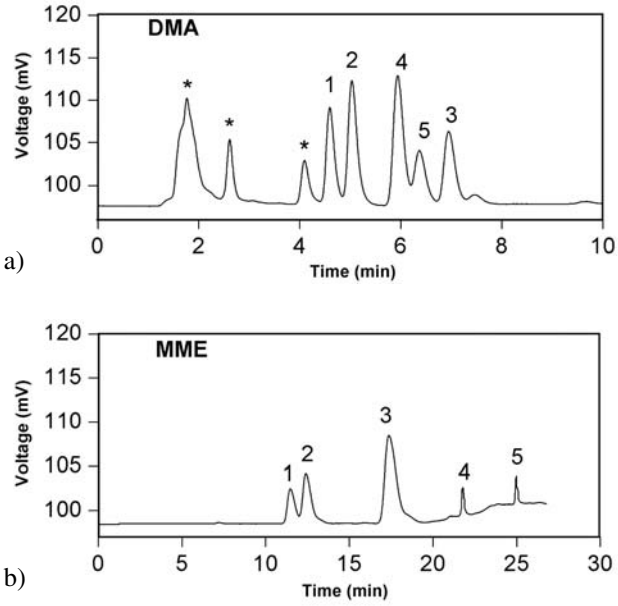

Figure 4: Typical chromatograms of the studied carbamates obtained from (a) the diazotized DMA method and (b) micelle-mediated extraction. Conditions: (a) 1.0 mg L^{-1} pesticide each, detection at 380 nm; (b) 3.0 mg L^{-1} for PPX, CBF, and CBR, and 6.0 mg L^{-1} for IPC and PMC, detection at 270 nm. Peak assignments: 1, PPX; 2, CBF; 3, CBR; 4, IPC; 5, PMC, and asterisks (*) are reagent peaks.

for t_R and lower than 1.2 % for peak area (Table 2). The limit of detection (LOD) obtained from the DMA method (Table 3) based on a signal-to-noise ratio of 3:1, was in the range of 0.01 (IPC) – 0.04 (PMC) mg L^{-1}, while the MME method provided LOD in the range 0.1 (CBR) – 0.7 (IPC & PMC) mg L^{-1}. The results show that the DMA method can provide higher sensitivity than MME in this study by the factor of ca. 3.3 (CBR) – 70 (IPC). The LOD obtained from the DMA method was also compared to that from our previous work studying Triton X-114 non-ionic surfactant for cloud-point extraction (CPE).[16] It can be seen that the proposed DMA method gave LODs comparable to the CPE method for most studied carbamates. Surprisingly, lower LODs for IPC and PMC obtained from the DMA method were 30 and 3 fold, respectively, compared to that of the CPE method. Moreover, baseline separation of the studied carbamates by the DMA method was successfully achieved in around a 3 times shorter time. This phenomenon may be attributed to target carbamate derivatives having higher polarity than the native forms. On the other hand, MME using anionic SDS in this study cannot provide preconcentration of the analytes due to much dilution of the strong acidic surfactant-rich phase before HPLC analysis. To avoid this problem and obtain high preconcentration factor, MME or CPE using non-ionic surfactants such as Triton X-114 instead of anionic SDS surfactant has been recommended.[16] Based on the results above, the chemical derivatization method can be used for improving sensitivity (by elevated molar absorptivity) for all target analytes in the same run and benefits for short-time analysis. In this study, the DMA derivatization method was further used for water analysis and to investigate the accuracy of the method.

3. 4. Application of Proposed DMA Method to Tap Water Samples

Tap waters were selected for accuracy test by investigating the recovery of the spiked standard carbamtes (0.5, 1.0, and 5.0 mg L^{-1} for each pesticide). The results are summarized in Table 4. Good recoveries were obtained in the range 86.7–111.8% (on average) with the relative standard deviation (RSD) lower than 7%.

4. Conclusion

This study demonstrates that the determination of five carbamates by HPLC after their derivatization with DMA is superior to acid-induced MME–HPLC in terms of sensitivity (lower LODs) and short analysis time. Derivatization of carbamates before the determination by HPLC is a simple and straightforward method to enhance the sensitivity for detection. It can be used as an alternative method for the analysis of carbamate insecticides in samples.

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Table 2. Analytical performances of the proposed DMA method and MME

Analyte	Linear equation	Linearity (mg L^{-1})	R^2	Intra-day (%RSD), n = 10		Inter-day (%RSD), n = 3 × 5	
				t_R	Peak area	t_R	Peak area
PPX	Y = 141.9 X + 17.5	0.08–10	0.9980	0.90	1.32	1.07	1.94
	(Y = 9.9X + 0.4)[a]	*(0.70–10)*	*(0.9999)*				
CBF	Y= 72.8X + 5.5	0.07–10	0.9970	0.91	1.97	1.45	2.94
	(Y=16.9X + 0.1)	*(0.50–10)*	*(0.9992)*				
IPC	Y= 155.7X +9.7	0.05–10	0.9970	0.91	1.69	1.83	3.23
	(Y =1.8X + 1.0)	*(1.00–10)*	*(0.9984)*				
PMC	Y = 96.3X + 5.5	0.08–10	0.9990	0.88	1.43	1.74	2.80
	(Y =1.5X + 0.2)	*(1.00–10)*	*(0.9946)*				
CBR	Y = 111.7X + 16.5	0.07–10	0.9980	0.86	3.09	1.67	3.11
	(Y = 50.3X + 1.3)	*(0.30–10)*	*(0.9995)*				

[a] The values in parentheses were obtained from MME.

Table 3. Comparison of limit of detection (LOD) and separation time of the studied carbamates obtained from the proposed methods and literature

Insecticide	LOD (mg/L)		Sensitivity improvement[a]	LOD (mg/L) Triton X-114–CPE[16]
	MME	DMA		
PPX	0.3	0.02	15	0.01
CBF	0.3	0.03	10	0.01
IPC	0.7	0.01	70	0.3
PMC	0.7	0.04	17.5	0.1
CBR	0.1	0.03	3.3	0.005
Separation time (min)	26	8	–	27

[a] It was calculated based on the ratio of LODs obtained from MME and DMA methods.

Table 4. Recovery of the studied carbamates spiked in tap water samples obtained from the DMA method (n = 3)

Insecticide	Spiked (mg/L)	Determined (mg/L)	Recovery (%), n = 3	
			Mean	RSD (%)
PPX	0	ND	–	–
	0.5	0.476	95.2	4.5
	1.0	0.867	86.7	5.0
	5.0	4.471	89.4	3.3
CBF	0	ND	–	–
	0.5	0.452	90.5	6.8
	1.0	0.986	98.6	4.5
	5.0	4.792	95.8	5.6
IPC	0	ND	–	–
	0.5	0.559	111.8	5.4
	1.0	1.058	105.8	3.9
	5.0	5.523	110.5	4.1
PMC	0	ND	–	–
	0.5	0.467	93.4	5.5
	1.0	0.999	99.9	3.2
	5.0	4.685	93.7	5.9
CBR	0	ND	–	–
	0.5	0.471	94.2	5.5
	1.0	1.005	100.5	1.9
	5.0	4.442	88.8	5.7

ND: not detected

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Povzetek

Raziskovali smo pripravnost dveh strategij za hkratno analizo petih karbamatnih insekticidov (propoksur, karbofuran, karbaril, izoprokarb in promekarb) s tekočinsko kromatografijo visoke ločljivosti (HPLC). Pri prvi metodi je predkoncentracija osnovana na micelarni ekstrakciji (MME) s pomočjo natrijevega dodecilsulfata (SDS). Pri drugem postopku gre za derivatizacijo z reagentom 2,4-dimetoksianilinom (DMA) pred HPLC analizo. Optimizirali smo dejavnike, ki vplivajo na analizo. Pri MME so bili optimalni pogoji 4,0 % (w/v) SDS v prisotnosti 5,0 mol L^{-1} HCl. Pogoji za derivatizacijo z DMA pa so bili: 1,5 mmol L^{-1} DMA, 7 mmol L^{-1} $NaNO_2$ in 50 mmol L^{-1} HCl, ter še 250 mmol L^{-1} NaOH za hidrolizo karbamatov pred derivatizacijo. Tarčne karbamate ter njihove derivate smo spremljali pri valovnih dolžinah 270 nm za MME in 380 nm za DMA derivatizacijsko metodo. Uporabnost obeh razvitih metod smo primerjali s pomočjo ločbenega časa in meje zaznave (LOD). Pet preiskovanih insekticidov se je pri DMA uspešno ločilo v 8 min, pri MME pa v 27 min. LOD za insekticide so bile nižje pri DMA (0,01–0,04 mg L^{-1}) kot pri MME (0,1–0,7 mg L^{-1}). Predlagana DMA metoda je uporabna in boljša od MME za analizo karbamatnih insekticidov v modelnih vzorcih vodovodne vode.