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Analysis of polar pesticides in water and wine samples by automated in-tube solid-phase microextraction coupled with high-performance liquid chromatography–mass spectrometry

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Abstract

A simple and sensitive method for the determination of polar pesticides in water and wine samples was developed by coupling automated in-tube solid-phase microextraction (SPME) to high-performance liquid chromatography–electrospray ionization mass spectrometry (HPLC–ESI-MS). To achieve optimum performance, the conditions for both the in-tube SPME and the ESI-MS detection were investigated. In-tube SPME conditions were optimized by selecting the appropriate extraction parameters, especially the stationary phases used for SPME. For the compounds studied, a custom-made polypyrrole (PPY)-coated capillary showed superior extraction efficiency as compared to several commercial capillaries tested, and therefore, it was selected for in-tube SPME. The influence of the ethanol content on the performance of in-tube SPME was also investigated. It was found that the amount of pesticides extracted decreased with the increase of ethanol content in the solutions. The ESI-MS detection conditions were optimized as follows: nebulizer gas, N_2 (30 p.s.i.; 1 p.s.i.=6894.76 Pa); drying gas, N_2 (10 l/min, 350 °C); capillary voltage, 4500 V; ionization mode, positive; mass scan range, 50–350 amu; fragmentor voltage, variable depending on the ions selected. Due to the high extraction efficiency of the PPY coating and the high sensitive mass detection, the detection limits ($S/N = 3$) of this method for the compounds studied are in the range of 0.01 to 1.2 ng/ml, which are more than one order of magnitude lower than those of the previous in-tube SPME–HPLC–UV method. A linear relationship was obtained for each analyte in the concentration range of 0.5 to 200 ng/ml with MS detection. This method was applied to the analysis of phenylurea and carbamate pesticides in spiked water and wine samples.

Keywords: Water analysis; Wine; Food analysis; Extraction methods; Pesticides

1. Introduction

Pesticide analysis in environmental and biological samples has received great attention for many years due to the wide use of pesticides in agricultural and household applications as well as their environmental impact [1–5]. Most analytical methods for pesticide analysis are based on chromatographic techniques, by both gas chromatography (GC) and high-performance liquid chromatography (HPLC). For polar, less volatile and thermally labile pesticides such as phenylureas and carbamates, HPLC is obviously the preferred approach [2,4–9]. A variety of detection methods have been used in HPLC analysis of pesticides, including common UV [1], diode-array [4,6], electrochemical [7] and fluorescence [8]. In

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spite of the high sensitivity of fluorescence detection with post-column derivatization or the robustness of UV detection, mass detection has become widely accepted as the preferred technique for the identification and quantification of pesticides and other polar and thermally labile compounds because of its high sensitivity and selectivity [2,5,9].

In addition to the use of a highly sensitive and selective detection method, selecting an appropriate sample preparation technique is very important for determining small amounts of pesticide residues in complex sample matrices. Several sample preparation techniques, mainly liquid–liquid extraction (LLE) [3] and solid-phase extraction (SPE) [4,5], have been applied for extraction of pesticides from water and other sample matrices. Solid-phase microextraction (SPME), due to its simple, fast, and solvent-less features, has received growing interest for many areas [10–12], including its applications in pesticide analysis (see a recent review in Ref. [1]). In-tube SPME is a relatively new microextraction and preconcentration technique, which can be easily coupled on-line with HPLC for the analysis of less volatile and/or thermally labile compounds [13–23]. This technique, using a coated open tubular capillary as the SPME device, allows for convenient automation of the extraction process, which not only saves the analysis time but also provides better precision relative to off-line manual techniques. In-tube SPME coupled with HPLC–UV has been applied for the analysis of pesticides in water samples [13–16]. However, the sensitivity of the method was limited by the UV detector and the commercial capillary (Omegawax 250) used for extraction [14,15]. Although the sensitivity could be increased by using capillary LC with an on-column focusing technique, it required a much longer analysis time compared with the conventional HPLC analysis [16].

The selection of a suitable coating is the key step in the optimization of an in-tube SPME method. Omegawax 250 was selected in the previous pesticide studies due to its higher extraction ability for polar compounds compared with other commercial capillaries tested [13–17]. However, it has been demonstrated in recent studies that a polypyrrole (PPY)-coated capillary has a superior extraction efficiency toward polar compounds, aromatic compounds, and anionic species compared with the commercial capillaries used [18–23], due to the inherent multifunctional properties of the PPY polymer (base–acid, $\pi-\pi$ and dipole–dipole interactions, ion-exchange, and hydrogen bonding) [24–30]. Therefore, in this work, in-tube SPME of pesticides was re-investigated using a PPY-coated capillary and several commercially available capillaries. In addition, a more sensitive and selective detection method, electrospray ionization (ESI)-MS, was used together with the UV detection method. To determine pesticides in wine and ethanol–water mixtures, the influence of the ethanol content on the performance of in-tube SPME was also investigated.

2. Experimental

2.1. Chemicals and reagents

Pyrrole (98%) (Aldrich, Canada) was distilled before use. *N*-Methylpyrrole (99%) (Aldrich) was used as received. Ferric perchlorate [$Fe(ClO_4)_3 \cdot 6H_2O$] and perchloric acid (70%) were used as received (BDH, Toronto, Canada). Six phenylurea pesticide standards (diuron, fluometuron, linuron, monuron, neburon, and siduron) and six carbamates (barban, carbaryl, chlorpropham, methiocarb, promecarb, propham) were obtained from Chem Service (West Chester, PA, USA). They were of $\geq$98% purity and used as received. Acetonitrile, ethanol and methanol (HPLC grade) were obtained from EM science (Gibbstown, NJ, USA). Pure water was obtained from a Barnstead/Thermodyne NANO-pure ultrapure water system (Dubuque, IA, USA). Surface water was obtained from Laurel Creek, Waterloo, Canada. Private residential well water was obtained from Cambridge, Canada. Municipal tap water was taken from the tap in our lab (Waterloo, Canada). A white wine (Hochtaler, Andres Wines) was purchased from a local liquor store.

Individual standards for each compound with concentrations of 2 mg/ml were prepared using methanol as a solvent. A standard stock mixture with a concentration of 0.2 mg/ml for each compound was prepared in methanol. Water samples (spiked with 20, 40, and 100 ng/ml of each compound) were freshly prepared before experiments by spiking the standard stock solution or diluted solutions into pure

water, surface water, well water, and tap water. The aqueous samples for calibrations, limit of detection, and linearity tests were prepared by spiking the standard mixture or diluted mixtures into pure water or water samples to reach appropriate concentration levels. Methanol concentration was kept equal to or lower than 1% in the solutions. A wine sample was analyzed directly or after a 10-fold dilution with pure water, the results obtained from spiked wine samples were compared with those of non-spiked wine samples and water samples.

2.2. Instrument and analytical conditions

All experiments were carried out on an Agilent 1100 series HPLC system coupled with an atmospheric pressure ESI mass spectrometer and a variable-wavelength UV detector (Agilent Technologies, Palo Alto, CA, USA). A Supelcosil LC-18 column (5 cm×4.6 mm, 5 μm particle size) from Supelco (Bellefonte, PA, USA) was used for the separation under room temperature. Mobile phases consisted of acetonitrile and water (each of them contained 0.05% formic acid). For the separation of phenylurea pesticides, the ratio of acetonitrile–water was kept at 40:60 for the first 4.5 min and then the content of acetonitrile was increased linearly to 60% at 7 min and held at this ratio for the rest of the run. The flow-rate was 0.5 ml/min. For the separation of carbamates, mobile phase ratio of acetonitrile–water was kept at 55:45 with a flow-rate of 0.5 ml/min. The wavelength used for UV detection was 245 nm for phenylureas and 225 nm for carbamates according to the previous studies [13–16]. The optimized ESI-MS conditions were as follows: nebulizer gas, N_2 (30 p.s.i.; 1 p.s.i.=6894.76 Pa); drying gas, N_2 (10 l/min, 350 °C); capillary voltage, 4500 V; ionization mode, positive; mass scan range, 50–350 amu; fragmentor voltage, variable depending on the ions selected. The monitoring ions selected for each analyte and the corresponding fragmentor voltages used are listed in Table 1.

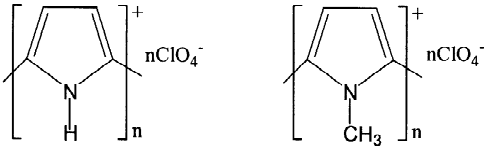
2.3. Preparation for the custom-made capillaries

Polypyrrole (PPY) or poly-*N*-methylpyrrole (PMPY) film was coated on the inner surface of a fused-silica capillary (60 cm, 0.25 mm I.D.) by a chemical polymerization method described previously [18,21]. Briefly, the inner surface polymer coating was prepared by first passing the monomer solution (pyrrole or *N*-methylpyrrole in isopropanol, 50% v/v) through the capillary with the aid of nitrogen gas to form a thin layer of monomer on the capillary inner surface, and then allowing oxidant solution (0.2 *M* ferric perchlorate in 0.4 *M* perchloric acid) to flow through the capillary in the same way as for the monomer. The polymer was formed by oxidative reactions when the oxidant reagent reached the monomer in the capillary. The above procedure was referred to as one coating cycle, which could be repeated several times (four times in this study) to increase the coating thickness. The capillary was first cleaned with acetone and then dried with N_2 before it was coated. During polymerization, the color of the capillary changed gradually from yellow to black, indicating the formation of PPY or PMPY on the inner wall of the capillary. The PPY- or PMPY-coated capillary was then washed with methanol for 2 min and dried by purging with nitrogen. Finally, it was coupled to a HPLC system as outlined in the next section, conditioned with mobile phase and checked with a blank solution before use. The structures of PPY, PMPY and the pesticides studied are given in Fig. 1.

2.4. In-tube SPME

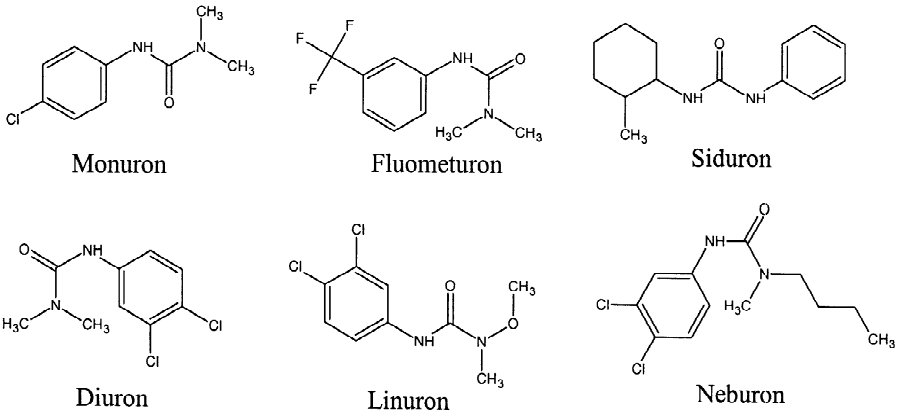
In-tube SPME is an on-line extraction process that is achieved through the repeated steps of moving a sample into and out of a coated capillary using an autosampler. Since the technique of coupling automated in-tube SPME to HPLC has been described in detail in previous reports [16–18], it will not be discussed here. A schematic illustration for the in-tube SPME–HPLC–ESI-MS system can be found in Refs. [17,18]. To compare the extraction efficiencies of different capillary stationary phases, a PPY-coated capillary, a PMPY-coated capillary and the following commercial capillaries (from Supelco) were tested under the same conditions: Omegawax 250 (0.25 μm film thickness, 0.25 mm I.D.), Supel-Q PLOT (thickness unknown, 0.32 mm I.D.), Supelcowax (0.1 μm film thickness, 0.25 mm I.D.), SPB-1 (0.25 μm film thickness, 0.25 mm I.D.), SPB-5 (0.25 μm film thickness, 0.25 mm I.D.), and a retention gap capil-

A. Polypyrrole (PPY) and poly-*N*-methylpyrrole (PMPY)



PPY PMPY

B. Phenylurea pesticides



C. Carbamate pesticides

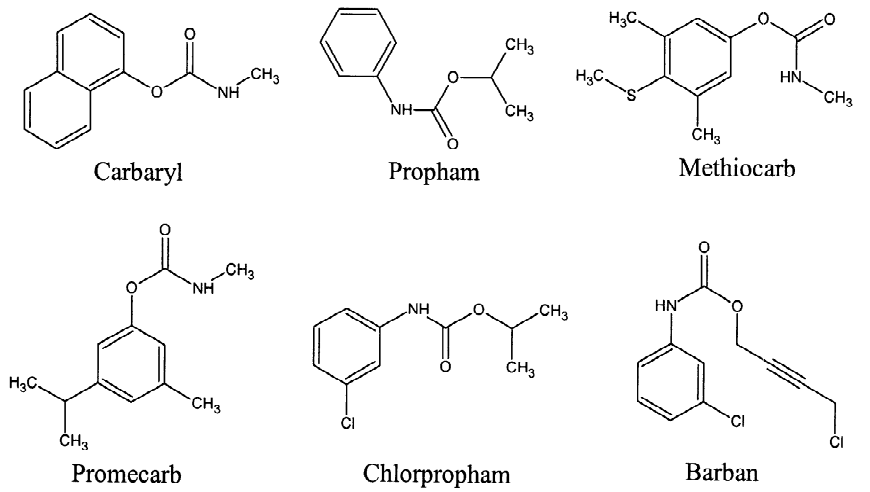


Fig. 1. Structures of PPY, PMPY and the pesticides studied.

lary (a polar silica tubing, 0.25 mm I.D., which was also used as host capillary to make PPY-coated capillary). In addition, the effects of coating thickness and ethanol content in sample solutions on extraction efficiency were also investigated to optimize the extraction conditions.

2.5. Safety considerations

The pesticides tested are toxic and, therefore, should be handled carefully in a fumehood, using appropriate protective clothing. They should be stored in a tightly sealed container in a cool dry place.

3. Results and discussion

3.1. Separation and detection

The results showed that both pesticide mixtures could be separated under the conditions listed in Section 2. To select the monitoring ion for each of the compounds, ESI mass spectra under positive ion detection mode were analyzed by liquid injection. The following mass detection conditions were optimized, including fragmentor voltage, capillary voltage, nebulizer gas pressure, drying gas flow-rate and temperature. Table 1 lists the ions selected for each compound and the corresponding fragmentor voltages used for the selected ions.

3.2. Optimization of in-tube SPME conditions

The extraction efficiency in SPME can be evaluated by determining the amount of analyte extracted by the coating. For coatings that extract analytes based on absorption, the amount of analytes extracted can be expressed as [10]:

$$n_A = K_A V_f V_s C_A^o / (K_A V_f + V_s) \quad (1)$$

where n_A is the amount of analyte A extracted by the coating at equilibrium, V_s and V_f are the volumes of the sample solution and coating, respectively, C_A^o is the initial concentration of the analyte in the sample, and K_A is the partition coefficient. For porous coatings that extract analytes by adsorption (such as Supel-Q porous-layer open tubular (PLOT) and PPY coating [22]), the amount of analyte extracted by the coating can be expressed as follows [31]:

$$n_A = K_A V_f V_s C_A^o (C_{f\,max} - C_{fA}^{\infty}) / [V_s + (K_A V_f (C_{f\,max} - C_{fA}^{\infty})] \quad (2)$$

where $C_{f\,max}$ is the maximum concentration of active sites on the coating, C_{fA}^{∞} is the equilibrium concentration of the analyte on the coating, and K_A is the adsorption equilibrium constant. Other terms are the same as in Eq. (1).

Since it is difficult to use the above equations to obtain n_A as some of the terms like K_A, $C_{f\,max}$, C_{fA}^{∞} and V_f are often unknown or difficult to measure, the amount of analyte extracted (n_A) by an SPME coating is often determined experimentally with the following equation:

Table 1
Ions selected for each pesticide and the corresponding fragmentor voltages (V_f) used in ESI-MS detection

Carbamate	M_w	m/z and ions selected	V_f (V)	Phenylurea	M_w	m/z and ions selected	V_f (V)
Carbaryl	201	202 $[M+H]^+$	30	Monuron	198	199 $[M+H]^+$	60
		145 $[M+H-CH_3NCO]^+$	60			221 $[M+Na]^+$	70
Propham	179	120 $[C_6H_5NCO+H]^+$	90			72 $[C_3H_6NO]^+$	100
		138 $[M+H-C_3H_6]^+$	60	Fluometuron	232	233 $[M+H]^+$	60
Methiocarb	225	226 $[M+H]^+$	30			72 $[C_3H_6NO]^+$	100
		169 $[M+H-CH_3NCO]^+$	60	Diuron	232	233 $[M+H]^+$	60
Promecarb	207	208 $[M+H]^+$	30			72 $[C_3H_6NO]^+$	100
		151 $[M+H-CH_3NCO]^+$	60	Siduron	232	233 $[M+H]^+$	60
Chlorpropham	214	154 $[M-C_3H_7OH]^+$	90			255 $[M+Na]^+$	120
		172 $[M-C_3H_6]^+$	60	Linuron	248	249 $[M+H]^+$	60
Barban	258	258 $[M]^+$	30	Neburon	274	275 $[M+H]^+$	50
		178 $[M+H-81]^+$	60			297 $[M+Na]^+$	70

$$n_A = FA = (m/A_d)A \quad (3)$$

where n_A is the amount (mass) of analyte extracted by SPME, F is the detector response factor which can be calculated by comparing the amount of analyte (m) injected and the area counts (A_d) obtained by liquid injection ($F = m/A_d$), A is the response (area counts) obtained by SPME. Therefore, the extraction efficiencies of different coatings for the same analyte can be evaluated by comparing the n_A values obtained by SPME experiments under the same extraction conditions.

In this work, to optimize the in-tube SPME conditions for pesticide analysis, the following parameters were investigated by coupling in-tube SPME to HPLC, including the stationary phase of the SPME capillary, capillary length, coating thickness, extraction time profile (the number of draw/eject cycles for each extraction), sample matrix and pH, and desorption condition. However, only the influence of capillary coatings and coating thickness on extraction will be discussed in this paper, because the results obtained for other parameters in this work are similar to those obtained and discussed in the previous studies [15,18,20]. In this work, a 60-cm-long capillary was selected and an extraction time-profile of 15 draw/eject cycles (30 μl for each cycle at a flow-rate of 100 μl/min) was used in all in-tube SPME experiments. No buffer solutions or salts were used since they did not influence extraction efficiency significantly towards the studied pesticides [14–16]. The analytes were desorbed with mobile phases by switching the six-port valve to the INJECT position.

The previous in-tube SPME work on pesticide analysis showed that Omegawax was the best capillary among the commercial GC capillary columns tested [13–16]. However, it has been found recently that a PPY-coated capillary has better extraction efficiency than the Omegawax towards aromatic compounds, polar compounds and anionic species [18–23]. Therefore, the effect of different capillary coatings on pesticide extraction was evaluated further in this work, using the custom-made PPY and PMPY capillaries and the commercially available capillaries. The amounts of analytes extracted by in-tube SPME under the same conditions were calculated by Eq. (3) and are listed in Table 2. As in the previous pesticide studies [13–16], the three commercial capillaries, SPB-1, SPB-5 and Supelcowax, did not show good extraction efficiency for the pesticides studied, therefore, their results are not included in Table 2. For phenylurea pesticides, PPY, PMPY and Supel-Q PLOT capillaries showed better extraction efficiency than Omegawax as shown in Table 2, indicating their potential application for in-tube SPME. Similar results were also obtained for carbamates (data not shown). The high extraction efficiencies of these coatings might be explained in part by the high surface areas of their porous surface structures [22,23]. Since the PPY-coated capillary had the best extraction efficiency for the analytes among all the tested capillaries, it was selected for further study.

Table 2
Comparison of the extraction efficiencies for phenylureas obtained by in-tube SPME with different capillary coatings

Compound[a]	Detector response[b] F ($\times 10^{-5}$)	Amount of analyte extracted (ng)[c]					Extraction yield (%)[d]				
		Host	PMPY	PPY	Supel-Q	Omegawax	Host	PMPY	PPY	Supel-Q	Omegawax
Monuron	0.27	1.4	35.5	52.9	52.3	6.5	0.7	17.7	26.5	26.2	3.3
Fluometuron	0.21	2.6	33.6	48.6	45.5	6.8	1.3	16.8	24.3	22.7	3.4
Diuron	0.63	2.4	48.8	66.8	63.9	21.3	1.2	24.4	33.4	31.9	10.7
Siduron	0.17	2.2	43.7	53.8	52.1	12.8	1.1	21.8	26.9	26.0	6.4
Linuron	1.44	1.3	42.3	53.4	36.4	15.0	0.7	21.1	26.7	18.2	7.5
Neburon	0.52	4.4	50.9	67.1	46.0	36.8	2.2	25.5	33.6	23.0	18.4

[a] Sample contains 200 ng/ml each of the analytes.

[b] A 10-μl (2-ng) sample solution was directly injected to obtain the detector response factor (see Eq. (3)).

[c] A 1-ml sample was used for in-tube SPME. The amount of each pesticide extracted was calculated by Eq. (3).

[d] Extraction yields (%) are the percentages of extracted amounts of pesticides per initial amounts (200 ng) in the 1-ml sample solution using in-tube SPME.

The effect of coating thickness on extraction is one of the most important parameters for SPME [10,11]. Since the thickness of PPY coating could be increased easily and gradually by increasing the number of PPY coating cycles (see Section 2), it provided the unique opportunity to systematically study the effect of coating thickness on extraction. It is expected from Eq. (2) that the amount of analyte extracted will increase when the coating thickness increases. For porous coatings such as PPY coating, increasing the coating thickness means not only the increase of the total coating volume (V_f), but also the increase of the total surface area ($C_{f\ max}$). Therefore, the extraction efficiency of a thick coating will be greater than a thinner coating. This expectation has been confirmed recently by both the PPY coating SPME and the scanning electron microscopic studies [20,22,23]. As shown in Table 3, the extraction efficiency for phenylurea pesticides increased gradually with the increase of the PPY coating thickness, which is consistent with results obtained previously [18–20,22,23]. Similar results were also obtained for the carbamate pesticides (data not shown). However, it is difficult to apply more than four coating cycles, because the polymer film formed can block the capillary when the coatings get thicker during polymerisation. Therefore, the maximum number of coating cycles used in this work is four as shown in Table 3.

To determine pesticides in wine or other ethanol–water-containing food products [32], the effect of ethanol content in sample solutions on extraction was studied. As shown in Table 4, the amount of pesticides extracted decreases with the increase of ethanol content in the solutions. For example, the extracted amount (mass) for each pesticide was decreased up to 90% in a solution containing 40% ethanol relative to a solution having no ethanol. Similar results were also obtained in a recent study on the determination of pesticides in food simulants using a fiber SPME method [32]. Because of this effect, the content of ethanol or methanol (which has a similar effect as ethanol) in the sample solutions should be kept to the lowest level or at least kept at the same level in order to achieve reproducible results. In addition, internal calibration or a standard addition method must be used for quantification.

The extraction time for a 15-cycle extraction was about 12 min, therefore, the whole analysis for the pesticides including extraction, desorption, separation and detection (UV and MS) could be accomplished automatically within 25 min by the developed method. The analyte carryover (or memory) effect was not observed because the analytes were desorbed completely by the mobile phase flow and the extraction capillary was washed continually with mobile phase during analysis.

3.3. Method performance

Due to the high sensitivity of mass detection and the high extraction efficiency of PPY coating, lower detection limits ($S/N = 3$) were obtained for all the pesticides studied (Tables 5 and 6) compared with

Table 3
Effect of the PPY coating thickness on the extraction efficiency for the phenylureas

Compound	Amount of analyte extracted (ng)[a,b]					Extraction yield (%)[c]				
	0-PPY	1-PPY	2-PPY	3-PPY	4-PPY	0-PPY	1-PPY	2-PPY	3-PPY	4-PPY
Monuron	1.4	6.4	15.0	35.5	52.9	0.7	3.2	7.5	17.7	26.5
Fluometuron	2.3	5.3	15.3	33.6	48.6	1.2	2.6	7.7	16.8	24.3
Diuron	2.4	12.1	26.4	48.8	66.8	1.2	6.1	13.2	24.4	33.4
Siduron	2.2	9.7	22.3	43.7	53.8	1.1	4.9	11.2	21.8	26.9
Linuron	1.3	6.7	14.5	42.3	53.4	0.7	3.3	7.2	21.1	26.7
Neburon	4.4	16.1	27.6	50.9	67.1	2.2	8.0	13.8	25.4	33.6

[a] The coating thickness of PPY increases from 0-PPY (coating cycle, without coating) to 4-PPY (coating cycles). Detector response factor (F) and other conditions are the same as in Table 2.

[b] A 1-ml sample was used for in-tube SPME. The amount of each pesticide extracted was calculated by Eq. (3).

[c] Extraction yields (%) are the percentages of extracted amounts of pesticides per initial amounts (200 ng) in the 1-ml sample solution using in-tube SPME.

Table 4
Effect of ethanol contents in sample solutions on the extraction efficiency for the phenylurea pesticides

Compound[a]	Amount of analyte extracted (ng)[b] at the following ethanol contents (%)				Extraction efficiency decrease (%)[c] at the following ethanol contents (%)			
	0	10	20	40	0	10	20	40
Monuron	52.9	41.4	33.0	5.1	0	22	38	90
Fluometuron	48.6	43.5	32.1	6.2	0	10	34	87
Diuron	66.8	57.9	50.6	10.1	0	13	24	85
Siduron	53.8	41.0	33.5	6.1	0	24	38	89
Linuron	53.4	46.6	40.8	8.9	0	13	24	83
Neburon	67.1	55.8	50.0	14.4	0	17	26	79

[a] Detector response factor (F) and other conditions are the same as in Table 2.
[b] A 1-ml sample was used for in-tube SPME. The amount of each pesticide extracted was calculated by Eq. (3).
[c] The percentages of the decreased amount of analyte extracted in ethanol-containing solutions as compared with the amount of analyte extracted in a solution having no ethanol.

Table 5
Linear regression data, detection limits (DL) and precision (RSD) obtained for phenylureas by SPME–LC with two detection methods[a]

Compounds	PPY-coated capillary in-tube SPME–HPLC–ESI-MS				PPY-coated capillary in-tube SPME–HPLC–UV			
	Regression equation	Correlation (R^2)	DL (ng/ml)	RSD (%)	Regression equation	Correlation (R^2)	DL (ng/ml)	RSD (%)
Monuron	$y = 89149x + 89042$	0.9993	0.03	3.1	$y = 0.965x + 1.866$	0.9996	2.1	2.1
Fluometuron	$y = 86567x + 140045$	0.9998	0.03	3.3	$y = 0.673x + 0.390$	0.9999	4.5	4.7
Diuron	$y = 40971x - 44166$	0.9974	0.08	4.5	$y = 0.730x - 1.114$	0.9987	3.7	3.6
Siduron	$y = 121358x + 70201$	0.9992	0.01	2.6	$y = 0.684x - 0.600$	0.9993	4.2	4.4
Linuron	$y = 14990x - 8951$	0.9995	0.32	5.3	$y = 0.959x + 0.505$	0.9979	2.2	2.6
Neburon	$y = 88949x + 17039$	0.9957	0.03	4.2	$y = 0.833x + 2.524$	0.9986	3.1	3.7

[a] Regression equations were obtained from calibration curves by plotting the peak area counts against analyte concentrations; number of data points: eight points ($n = 3$ for each point). Detection limits (DL) were determined with $S/N = 3$. RSD (%) was calculated from a sample containing each analyte at 20 ng/ml ($n = 5$).

the previous in-tube SPME studies [13–15]. For most of the pesticides, the limits of detection of the PPY in-tube SPME–MS method are more than one order of magnitude lower than those of the previous in-tube SPME–HPLC–UV studies [13–15]. In this work, UV detection was carried out together with

Table 6
Linear regression data, detection limits (DL) and precision (RSD) obtained for carbamates by SPME–LC with two detection methods[a]

Compounds	PPY-coated capillary in-tube SPME–HPLC–ESI-MS				PPY-coated capillary in-tube SPME–HPLC–UV			
	Regression equation	Correlation (R^2)	DL (ng/ml)	RSD (%)	Regression equation	Correlation (R^2)	DL (ng/ml)	RSD (%)
Carbaryl	$y = 66753x + 232642$	0.9993	0.04	2.7	$y = 4.498x + 17.985$	0.9983	0.38	2.3
Propham	$y = 32888x + 107280$	0.9998	0.08	4.3	$y = 0.623x - 4.015$	0.9995	4.6	3.8
Methiocarb	$y = 59018x + 213654$	0.9974	0.05	3.6	$y = 0.724x + 2.700$	0.9985	3.4	2.9
Promecarb	$y = 144595x + 491057$	0.9992	0.01	3.4	$y = 0.393x + 0.737$	0.9996	8.0	5.0
Chlorpropham	$y = 17246x + 221013$	0.9995	0.41	5.8	$y = 0.431x + 4.101$	0.9935	7.4	4.2
Barban	$y = 5603x + 10710$	0.9957	1.2	6.3	$y = 0.366x + 0.617$	0.9997	8.2	4.6

[a] Regression equations were obtained from calibration curves by plotting the peak area counts against analyte concentrations; number of data points: eight points ($n = 3$ for each point). Detection limits (DL) were determined with $S/N = 3$. RSD (%) was calculated from a sample containing each analyte at 20 ng/ml ($n = 5$).

MS detection for comparison. The detection limits obtained by UV detector are also listed in these tables, which are lower than those of the previous in-tube SPME method due to the higher extraction efficiency of PPY compared with Omegawax [13–15]. The precision of the method varies depending on the analytes tested, their concentrations and the detectors used (see Tables 5 and 6). The calibration curves were constructed by comparing peak area counts against analyte concentration ranging from 0.5 to 200 ng/ml. A linear relationship was obtained for each analyte in the concentration range of 0.5 to 200 ng/ml for MS detection and 2 to 200 ng/ml for UV detection, as shown in Tables 5 and 6.

Recent studies have demonstrated that the stability of the PPY coating for in-tube SPME is comparable to or better than the commercial coatings tested [18–23]. In this work, one single PPY-coated capillary was used for all the extraction experiments (except for the coating thickness experiments), and no significant changes in its extraction performance were observed after hundreds of extractions during the 2-month period of study. The reproducibility obtained from five different capillaries coated with the same thickness of PPY was satisfactory, with the differences in their extraction efficiency ranging from 3 to 10% for a sample containing 100 ng/ml phenylureas.

It should be noted that the two groups of pesticides were studied in two separate runs in this work based on the published separation methods, in order to compare the results obtained in this work with those of the previous studies under the same conditions [14,15]. However, it is possible to analyze both groups of pesticides simultaneously in one run, especially when using MS detection under selected ion monitoring (SIM) mode due to the selectivity of the MS detector. For UV detection, separation and detection (wavelength) conditions have to be optimized further in order to separate and detect all the pesticides in one run.

3.4. Analysis of pesticides in water and wine samples

Tap water, surface water and well water samples spiked with different amounts of the pesticides were analyzed by the method and the results were compared with those of non-spiked water and pure water samples. No pesticides studied were detected from the non-spiked water samples using this method. As shown in Fig. 2, by detecting the selected analyte ions (Table 1) in the SIM mode and summing the signals, the MS detection method provided greater selectivity for the carbamates studied compared with the UV detection method. For tap water and surface water samples, the UV detector recorded large impurity peaks in front of the analyte signals. These peaks might influence the determination of pesticides when a complete separation could not be achieved. Although MS detection did not provide significant improvement (compared with UV) in sensitivity for chlorpropham (peak 5) and barban (peak 6), it

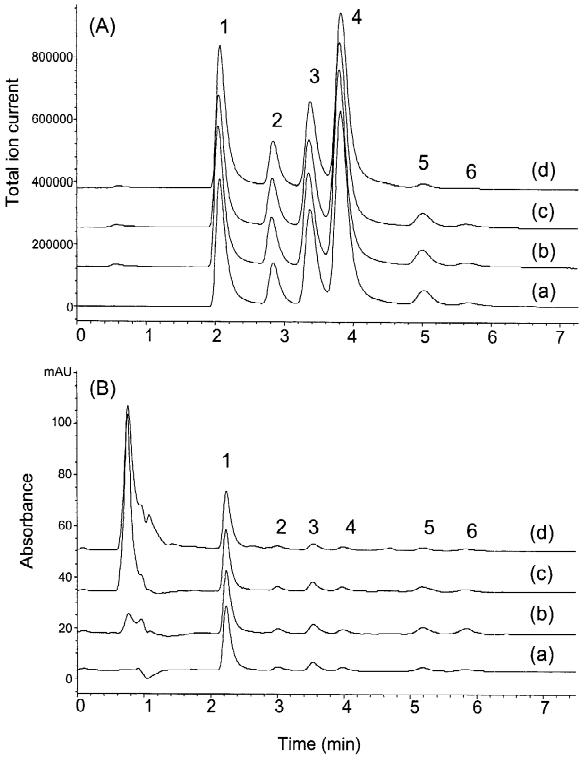


Fig. 2. Chromatograms of the six carbamates spiked into the different water samples obtained by (A) in-tube SPME–HPLC–ESI-MS under the total selected ion monitoring (SIM) mode and (B) in-tube SPME–HPLC–UV. Twenty ng each of the pesticides were spiked in each of the 1-ml water samples. Water samples: (a) pure water; (b) well water; (c) tap water; (d) surface (lake) water. Peak identification: 1, carbaryl; 2, propham; 3, methiocarb; 4, promecarb; 5, chlorpropham, and 6, barban.

provided much higher sensitivity than UV detection for most of the carbamates studied. Using MS detection, higher sensitivity and selectivity were also obtained in phenylurea pesticide analysis compared with UV detection. Therefore, only the results obtained by MS detection are shown in Fig. 3. Using PPY in-tube SPME–HPLC, the recoveries (relative to spiked pure water samples) of the spiked analytes from well water samples are close to those obtained from pure water samples (95–104%). However, the relative analyte recoveries are 10 to 18% lower from tap water and surface water samples compared with the results obtained from pure water samples. This is likely due to the sample matrices. Therefore, the standard addition method was used in all the analysis. The sample matrix effect became more obvious when analyzing wine samples directly, because the spiked analyte signals from wine samples were much lower compared with those from pure water samples. There are significant concentrations of other components as well as 11% ethanol in the wine samples, which influence not only the extraction efficiency of SPME but also the ESI-MS and UV detection for the analytes. After appropriate dilutions of the sample (10 times dilution, for example), sample matrix effects could be reduced significantly [19]. In this work, therefore, wine samples were analyzed after a 10-fold dilution. As shown in Fig. 4, MS detection again produced better sensitivity and selectivity than

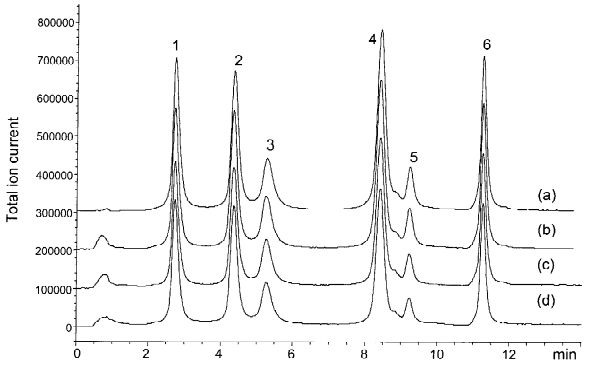


Fig. 3. Chromatograms of the six phenylureas spiked into the various water samples obtained by in-tube SPME–HPLC–ESI-MS in the SIM mode. Twenty ng each of the pesticides were spiked in each of the 1-ml samples. Water samples: (a) pure water; (b) well water; (c) tap water; (d) surface (lake) water. Peak identification: 1, monuron; 2, fluometuron; 3, diuron; 4, siduron; 5, linuron; 6, neburon.

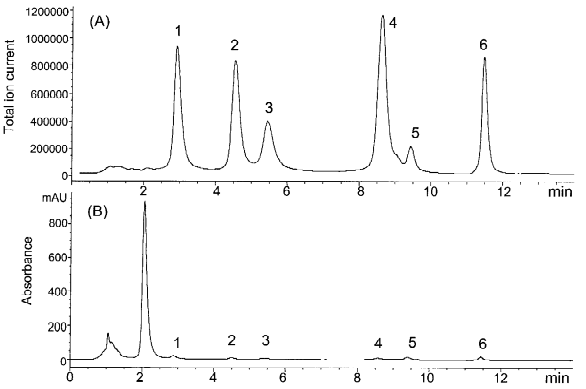


Fig. 4. Chromatograms of the six phenylurea-spiked wine sample obtained by (A) in-tube SPME–HPLC–ESI-MS in the SIM mode and (B) in-tube SPME–HPLC–UV. Eighty ng each of the pesticides were spiked into the 1-ml sample and then the sample was diluted 10 times before analysis. Peak identifications as in Fig. 3.

UV detection for analysis of the spiked phenylureas in wine samples. Similar results were also obtained for carbamates (data not shown). The analyte recoveries (relative to the spiked pure water samples) from spiked wine samples are between 89.2 and 96.9%.

4. Conclusions

The high extraction efficiency of the PPY coating for polar pesticides from water and wine samples has been demonstrated. Compared with the previous studies of in-tube SPME–HPLC–UV, higher sensitivity and selectivity have been achieved by the method developed in this work, due to the combination of the high extraction efficiency of PPY and the high sensitivity and selectivity of ESI-MS detection. This PPY-coated capillary in-tube SPME–HPLC–MS method can be extended to the analysis of other polar compounds with further optimization based on the properties of analytes studied.

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