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Multiresidue method for high-performance liquid chromatography determination of carbamate pesticides residues in tea samples

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A multiresidue method was developed to determine 19 carbamate pesticides in tea samples. Optimizations of different parameters, such as the type of extraction solvents, clean-up cartridges, and elution solvents were carried out. The developed method used acetonitrile as extraction solvent, amino cartridge for adsorbents and acetone-*n*-hexane as the eluting solution. Nineteen carbamate residues were then analyzed by high-pressure liquid chromatography (HPLC) with fluorescence detector. The present results showed good linearity by correlation coefficients of more than 0.9999 for all analyses. Limits of detection and quantification varied from 0.0005–0.023 mg L⁻¹, 0.008–0.077 mg L⁻¹, respectively. Recoveries of sixteen carbamate pesticides ranged from 65% to 135% at the spiked level of 0.5, 1.0 and 2.0 mg L⁻¹. The relative standard deviations were lower than 20% and coefficient of variations were lower than 15%. The results indicate that the proposed method provides an effective multi and trace level screening determination of carbamate pesticides residues for tea samples.

Keywords: Carbamate pesticides; tea; multiresidue analysis.

Introduction

Carbamate pesticides are widely used on a large number of crop protections, and are increasingly used instead of organochlorine and organophosphorus pesticides. Carbamate pesticides have been available commercially since the 1950s; they have become increasingly important in recent years, due to their broad spectrum of biological activity, high insect toxicity but a generally low toxicity toward warm-blooded species. Therefore, they are widely applied to control the insects, mites, and nematodes in agricultural products.^[1]

The action of carbamate pesticides are binding to the enzyme acetylcholinesterase, disrupting nerve function, resulting in paralysis and death. As a result, they are considered toxic for the environment and for human beings. A large number of carbamate pesticides are presently used in crop plantations, and pose a potential risk to human

health.^[2] Therefore, in recent years continuous monitoring of these compounds on crops has become very important for public health.

Multi-residue methods (MRMs) are known to be cost-effective techniques for pesticide residues analysis. Government agencies of most countries have recently established MRMs and set up monitoring protocols for pesticide residues on agriculture products. Nevertheless, MRMs analysis generally consists of a series of laborious steps, such as the pesticide extraction with organic solvents, clean up by cartridges, concentration, and chromatographic separation and identification. There has been an increasing need of analytical methods for identification and quantification of pesticide residues in tea. Almost all the common analytical methods to measure organophosphorus, pyrethroid and organochlorine residues in tea are based on the use of chromatographic techniques, mainly gas chromatography (GC) and have been employed extensively.^[3–5] Carbamate pesticides and their degradation products or derivatives, however, are polar, no-volatile and thermally unstable, that can not be directly analyzed by GC.^[6] Since the 1970s, high-performance liquid chromatography (HPLC) has become a preferred choice for the determination of carbamate pesticides in crops, fruits, vegetables, foodstuffs and environment

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bodies.^[4,6–8] So far to our knowledge, there has been no literature reporting the MRM with high-pressure liquid chromatography (HPLC) to detect carbamate pesticides residues for tea samples. The aim of this paper was to develop an effective MRM for simultaneous determination of carbamate pesticides in tea samples using HPLC. In addition, the optimization of solid-phase extraction (SPE) was also assessed in the MRM.

Materials and methods

Reagents

All pesticide standards were divided to two groups, group A (aldicarb sulfoxide, oxamyl, 3-OH carbofuran, aldicarb, metolcarb, thiodicarb, carbofuran, carbaryl, isoprocarb, and fenobucarb) and group B (aldicarb sulfone, methomyl, butocarboxin, 3-keto carbofuran, propoxur, meobal, macbal, methiocarb and promecarb). The chemicals were obtained from Riedel de-Haën (Hannover, Germany) with purity of 98.6–99.9%. All the solvents and chemicals used in this study were of analytical grade from Merck (Germany).

Calibration, limits of detection and quantification

A standard stock solution (1000 mg L^{-1}) was prepared in acetonitrile and stored at -20°C . The working solutions required for preparing a standard curve (0.05, 0.1, 1.0, 2.5, 5.0 and 10 mg L^{-1}) were prepared daily from the standard stock solution by serial dilutions. All measurements were performed in three replicates for each level was analyzed; calibration curves were calculated by linear least-squares regression using peak areas.

The limits of detection (LODs) and quantification (LOQs) with this procedure were defined as the concentrations of each of the pesticides in tea samples (expressed as mg L^{-1}) that gave signals of 3 and 10 times the noise, respectively.^[9,10]

Recovery of pesticides from fortified samples

Tea samples spiked with 0.5, 1.0 and 2.0 mg L^{-1} carbamate pesticides were used for quantification analysis to evaluate the recovery (%), and for determining the coefficient of variation (CV) of each carbamate pesticide. The recovery assays were replicated three times.

Extraction

The extraction and cleaning procedure was modified from (1999).^[11] Samples of 10 grams of tea powder were homogenized with 40 mL distilled water, and were extracted with 50 mL or 100 mL of different extraction solvents (acetonitrile, dichloromethane, acetone, and *n*-hexane) by homoge-

nization for 1 min with the polytron. Extracts were filtered through a Whatman GF/C 110 mm filter paper with vacuum pump, transferred to flasks, and then added tested extraction solvent to 100 mL. Immediately after addition of 15 g NaCl and shaking for 1 min, the upper 10 mL extraction solvent layer were collected, and evaporated to dryness with nitrogen gas. The residue was then dissolved in 5 mL acetone-dichloromethane (1/9 (v/v)). The following clean-up was accomplished with an amino cartridge (Agilent Technologies, USA) rinsed with 5 mL acetone-dichloromethane (1/9 (v/v)). The concentrated extract (in acetone-dichloromethane, 1/9 (v/v)) was transferred to the top of the cartridge, and the elution was collected carefully. Four 8 mL of the acetone-dichloromethane was continuously added twice to the cartridge and collected together. Collected eluent was dried by nitrogen gas, dissolved again in 1 mL acetonitrile, and filtered through a $0.2 \mu\text{m}$ membrane before HPLC injection.

Cartridge studies

The extracted dried residue was dissolved in a 5 mL mixture (acetone-dichloromethane, 1/9 (v/v)). Recovery analysis during the clean-up step was evaluated with five types of cartridges including octyl (Agilent Technologies, USA), octadecyl (Agilent Technologies, USA), florisil (J. T. Baker, USA), silica gel (J. T. Baker, USA), and amino (Agilent Technologies, USA). The cartridges were rinsed with 5 mL of elution solution (acetone-dichloromethane, 1/9 (v/v)). Concentrated extract residue was loaded onto the top of the cartridge, and the elution was collected carefully. And 4 mL of the elution solution was added twice (8 mL in total) to the cartridge and the eluent was collected together. Collected residue eluent was dried by nitrogen gas, dissolved in 1 mL acetonitrile, and passed through $0.2 \mu\text{m}$ membrane filters before HPLC injection.

Elution studies

Evaluation of elution solution was performed with different types of solvent mixtures (acetone-dichloromethane, methonal-dichloromethane, *n*-hexane-dichloromethane, and acetone-*n*-hexane) and an amino cartridge. The ratio of each elution mixtures were 1/9 (v/v). Concentrated extract was loaded to the top of the cartridge rinsed with 5 mL of mixture. The elution was collected. For elution volume analysis, different volumes (1 mL, 2 mL, 4 mL) of the elution mixtures were continuously added twice to the cartridge (in total 2 mL, 4 mL, and 8 mL respectively), and the eluted solutions were collected together. The collected eluent was again dried by nitrogen gas, dissolved in 1 mL acetonitrile and filtered through a $0.2 \mu\text{m}$ membrane before HPLC injection.

Sample analysis

The present developed MRM were practically used for market tea samples analysis. Six thousand and forty eight tea samples were collected by the Agriculture and Food Agency (AFA), Council of Agriculture from 12 tea plantation areas of Taiwan (Yilan, Taipei, Tayoyuan, Hsinchu, Miaoli, Taichung, Nantou, Yunlin, Chiayi, Kaohsiung, Taitung and Hualien) from 2004 to 2005.

The samples were ground to a powder in a mortar with a pestle, then homogenized and stored at -20°C before analysis. Samples were analyzed within four weeks after collection.

Sample extraction and clean up procedure were conducted as the previously optimized and described by using an amino cartridge (Agilent Technologies, USA). The purified residues were reconstituted with 1 mL acetonitrile and filtered through a $0.2\ \mu\text{m}$ membrane prior to HPLC analysis.

Apparatus and experimental conditions

Analysis of carbamate pesticides was performed on an Agilent Liquid Chromatograph (Model 1100, Agilent Technologies, USA), equipped with a quaternary pump, an auto sampler, a fluorescence detector, column thermostat and a post-column deliver reactor (Pickering 5100X). Separation was carried out with a reversed phase column (C18, $5\ \mu\text{M}$ spherical, $4.6 \times 250\ \text{mm}$, Astec, USA). Column oven temperature was kept at 40°C , and the sample injection volume was $10\ \mu\text{L}$. Mobile phases for gradient elution were distilled water (mobile phase A) and acetonitrile (mobile phase B) delivered at a constant flow rate of $1.0\ \text{mL min}^{-1}$. The gradient program of the mobile phase was as follows: 70% A and 30% B at initial, 60% A and 40% B at 5 min, 35% A and 65% B at 6 min, 40% A and 60% B at 20 min, 30% A and 65% B at 23 min and then hold 2 minutes. The post-column reactor utilized sodium hydroxide and o-phthalaldehyde/thiofluor reagents and delivered at 100°C by post-column deliver reactor. Detection of pesticides as fluorescent derivatives was carried out using 330 and 460 nm as wavelengths for excitation and emission, respectively.

Results and discussion

Chromatogram of carbamate pesticides

Figure 1 showed chromatograms of the separation for 19 anilities in standard solution of carbamate pesticides. The chromatograms revealed a sharp and symmetrical peak for each standard molecule, as well as with tea sample. The retention times for all compounds investigated are listed in Table 1. A good separation was obtained in a relatively short running time, the first retention time was 3.91 min for aldicarb sulfoxide and the final retention time was 22.98 min for promecarb. The chromatograms result suggested

that detection of all nineteen carbamate pesticides can be done within 25 min by the HPLC.

Linearity

Condition of uniform variance over the linear range was checked. All measurements were performed over five different levels ranging between 0.05 and $10.00\ \text{mg L}^{-1}$ (three replicates for each level were analyzed). Calibration curves were calculated by linear least-squares regression using peak areas. The results are summarized in Table 1, the calibration curves were found to have good linearity by correlation coefficients (r^2) of more than 0.999 in all analyses, indicating that the linear regression method can answer the concentrations of the analyses under study, within the concentration range investigated.

LODs and LOQs

LODs for 19 carbamate pesticides investigated were at low-nanogram per milliliter levels, with the range of $0.0005\ \text{mg L}^{-1}$ and $0.023\ \text{mg L}^{-1}$ (Table 1). The highest LODs were $0.023\ \text{mg L}^{-1}$ for aldicarb and carbofuran, and the lowest LODs for were $0.0005\ \text{mg L}^{-1}$ for isoprocarb and fenobucarb. In LOQs the highest was $0.077\ \text{mg L}^{-1}$ for carbofuran and the lowest were $0.008\ \text{mg L}^{-1}$ for meobal and macbal (Table 1).

The LODs and LOQs data obtained in the present experimental conditions were all satisfactory, being 10–100 times lower than maximum residue limits (MRLs) of the carbamate pesticides required by the regulation of Taiwan, the European Union (EU), and Japan (Table 2).^[12–15] These data confirm the usefulness of our method in detecting the carbamate pesticide residues in tea samples.

Recovery test and coefficient of variations (CV) of tea samples

The recovery, relative standard deviations (RSD) and CV of nineteen carbamate pesticides from tea samples spiked with 0.5 – $2\ \text{mg L}^{-1}$ were listed in Table 3. In tea spiked with $2\ \text{mg L}^{-1}$, 17 carbamate pesticides were detected and the recoveries were within the range of 65% to 135%. In the same recoveries ranges we also detected sixteen and seventeen carbamate pesticides spiked with levels of $1\ \text{mg L}^{-1}$ and $0.5\ \text{mg L}^{-1}$, respectively. It might be due to the high volatility during the nitrogen drying processed under 35°C , aldicarb sulfoxide was not detected within the spiking range.

Good results were obtained with the mean recoveries of the carbamate pesticides that fell within the recommended range of 65% to 135% by EPA method 531.2,^[16] suggesting that this method is suitable for the residue analysis. Table 3 also showed levels of RSD for the carbamate pesticides. Almost all the carbamate pesticides RSD were lower than 20%, expected in a tea sample spiked with 0.5 – $2\ \text{mg L}^{-1}$.

Fig. 1. Chromatograms for: A: standard mixture of pesticides group A containing of 1 mg kg^{-1} of: 1. aldicarb sulfoxide, 2. oxamyl, 3. 3-OH carbofuran, 4. aldicarb, 5. metolcarb, 6. thiodicarb, 7. carbofuran, 8. carbaryl, 9. isoprocarb, 10. fenobucarb; B: standard mixture of pesticides group B with 1 mg kg^{-1} of: 1. aldicarb sulfone, 2. methomyl, 3. butocarboxin, 4. 3-keto carbofuran, 5. propoxur, 6. meobal, 7. macbal, 8. methiocarb, 9. promecarb; C: tea sample spiked with standard mixture group A; D: tea sample spiked with standard mixture group B; E: blank tea sample. Analysis condition is shown in “Apparatus and conditions” section.

The RSD values were in agreement with that recently reported in the EU guidelines.^[9]

The CV values were calculated in Table 3, almost all the carbamate pesticides CV were lower than 15%, as was ex-

pected in a tea sample spiked with 0.5 mg L^{-1} . We found that butocarboxin and 3-OH carbofuran with the CV were higher than 15%, a similar result was also found in a tea sample spiked with 1 mg L^{-1} and 2 mg L^{-1} of butocarboxin

Table 1. Linearity, calibration curves, limits of detection (LODs), and limits of quantification (LOQs) for the carbamate pesticides under study.

Pesticide	RT (min)	Linear range (mg L ⁻¹)	Parameters of linearity			LOD mg L ⁻¹	LOQ mg L ⁻¹
			r ²	Slope	Intercept		
Group A							
Aldicarb sulfoxide	3.912	0.05–10	0.9999	9.794 × 10 ¹	0.101	0.009	0.028
Oxamyl	4.962	0.05–10	0.9999	9.941 × 10 ¹	−0.497	0.008	0.026
3-OH Carbofuran	7.018	0.05–10	0.9999	9.222 × 10 ¹	0.059	0.008	0.026
Aldicarb	10.971	0.05–10	0.9999	1.104 × 10 ²	0.572	0.023	0.076
Metolcarb	13.113	0.05–10	0.9999	1.355 × 10 ²	2.042	0.017	0.057
Thiodicarb	14.204	0.05–10	0.9999	1.078 × 10 ²	1.373	0.021	0.069
Carbofuran	15.244	0.05–10	0.9999	1.030 × 10 ²	0.632	0.023	0.077
Carbaryl	18.981	0.05–10	0.9999	1.587 × 10 ²	0.699	0.015	0.050
Isoprocab	18.865	0.05–10	0.9999	1.108 × 10 ²	0.521	0.0005	0.016
Fenobucarb	21.981	0.05–10	0.9999	1.023 × 10 ²	−6.463	0.0005	0.018
Group B							
Aldicarb sulfone	5.062	0.05–10	0.9994	9.498 × 10 ¹	0.726	0.014	0.046
Methomyl	5.814	0.05–10	0.9997	1.267 × 10 ²	2.187	0.010	0.034
Butocarboxin	9.798	0.05–10	0.9997	5.097 × 10 ¹	0.481	0.022	0.074
3-Keto Carbofuran	10.844	0.05–10	0.9998	6.682 × 10 ¹	2.777	0.014	0.048
Propoxur	14.819	0.05–10	0.9995	1.080 × 10 ²	3.937	0.011	0.035
Meobal	16.296	0.05–10	0.9995	1.197 × 10 ²	3.728	0.003	0.008
Macbal	16.906	0.05–10	0.9996	1.245 × 10 ²	3.430	0.002	0.008
Methiocarb	22.147	0.05–10	0.9996	9.166 × 10 ¹	4.830	0.003	0.010
Promecarb	22.984	0.05–10	0.9997	1.043 × 10 ²	3.139	0.003	0.010

and 3-OH carbofuran, respectively. According to GARP (Group of Analysts of Residues of Pesticides),^[17] the acceptable values of variation coefficient are lower than 15%. However, the US-EPA can accept values of variation coefficient up to 30%.^[18] Thus, the obtained values from extraction and cleanup procedure of this study are reliable for routine analysis of carbamate pesticides in tea.

Effects of extraction solvents

In trace analysis, when residue levels are close to the limit of sensitivity of the instrument, the trace matrix components can interfere with determination processes, so the

extraction and the elution solvents should be adequately selected. A number of studies indicate that liquid-liquid extraction (LLE) methods have been used to extract carbamate pesticides from several vegetable matrices, and the most frequently employed solvents are dichloromethane and *n*-hexane.^[19]

Because of the different structural characteristics and polarity of the carbamate pesticides, it is important to optimize the extraction conditions. Several extraction solvents for analysis recoveries were further evaluated in the present study (Table 4). There were significant differences in extracting efficiency among the extraction solvents for most of carbamate pesticides in tea samples. Comparing four solvents *n*-hexane had the lowest recoveries with all the recovery values below 65%. It might be due to that carbamate pesticides were less soluble in *n*-hexane than in other three kinds' solvents. There were fourteen and thirteen carbamate pesticides on a range of recoveries between 65% and 135% when acetonitrile and acetone were used as an extraction solvent respectively. On the other hand, if dichloromethane was used as extraction solvent only four carbamate pesticides showed the recovery range of 65% to 135%.

Thus, acetonitrile or acetone revealed to be an optimal extraction solvent for the carbamate pesticides extraction in tea samples. Considering the whole average recovery values of all tested carbamate pesticides and the nitrogen dryness process, acetonitrile was selected as the extraction solvent in the following experiments.

Table 2. The maximum residue levels of carbamate pesticides (mg kg⁻¹) regulated in different areas.

Pesticide	Taiwan	European Union	Japan
Aldicarb	NE	0.05	NE
Carbaryl	2.0	NE	1.0
Carbofuran	1.0	0.2	0.2
Carbosulfan	NE	0.1	NE
Macbal	NE	NE	10.0
Methomyl	1.0	0.1	20.0
Thiabendazole	NE	0.1	NE
Thiodicarb	NE	NE	25.0
Propoxur	NE	0.1	NE

NE: Not established.

Table 3. Recoveries of carbamate pesticides from the tea samples spiked at different concentration levels.

Pesticide	Spiked level (mg L ⁻¹)		
	0.5	1.0	2.0
Group A ^a			
Oxamyl	78.5 ^b ± 6.7 ^c (8.5) ^d	75.9 ± 6.5 (8.6)	65.5 ± 5.7 (8.7)
3-OH Carbofuran	41.9 ± 12.3 (29.3)	36.2 ± 7.3(6.8)	47.5 ± 14.7(30.9)
Aldicarb	116.5 ± 11.8 (10.2)	93.2 ± 3.2 (3.4)	94.8 ± 3.6 (3.8)
Metolcarb	140.4 ± 16.4 (11.7)	91.0 ± 5.7(6.2)	100.0 ± 4.2(4.2)
Thiodicarb	102.2 ± 14.0 (13.7)	69.0 ± 3.1 (4.5)	82.0 ± 3.8 (4.7)
Carbofuran	119.7 ± 13.4 (11.2)	115.5 ± 1.2(1.0)	96.9 ± 3.5 (3.6)
Carbaryl	187.8 ± 9.7 (5.2)	125.7 ± 4.6(3.6)	111.1 ± 3.2(2.9)
Isoprocab	84.0 ± 8.5 (10.1)	98.5 ± 5.0 (5.1)	81.4 ± 3.3 (4.0)
Fenobucarb	115.5 ± 7.6(6.6)	89.4 ± 3.8(4.2)	89.4 ± 4.3(4.8)
Group B			
Aldicarb sulfone	86.7 ± 3.4(3.9)	88.6 ± 6.9 (7.8)	67.1 ± 3.8(5.7)
Methomyl	95.9 ± 8.1(8.4)	88.8 ± 6.3 (7.1)	74.6 ± 3.8(5.1)
Butocarboxin	94.8 ± 19.8 (20.9)	62.9 ± 11.7(18.5)	76.1 ± 6.1 (8.0)
3-keto Carbofuran	118.1 ± 15.2 (12.8)	102.2 ± 4.5(4.4)	87.5 ± 4.4 (5.0)
Propoxur	88.5 ± 12.1 (13.7)	115.3 ± 2.2(1.9)	85.2 ± 5.2 (6.1)
Meobal	85.1 ± 8.7 (10.2)	98.0 ± 5.4 (5.6)	82.4 ± 4.7 (5.7)
Macbal	103.1 ± 2.5(6.2)	137.8 ± 7.4(5.4)	103.5 ± 5.4(5.2)
Methiocarb	92.8 ± 6.4(6.9)	94.4 ± 4.2 (4.4)	95.1 ± 5.1 (5.3)
Promecarb	64.1 ± 4.3(6.7)	87.4 ± 2.8 (3.2)	103.5 ± 6.4(6.2)

^a Aldicarb sulfoxide was not detected.^b Recovery (%) = 100% × (Level of carbamate pesticides in spiked sample level of carbamate pesticides in blank sample) / Level of carbamate pesticides in spiked tea sample.^c Relative standard deviation (RSD).^d Coefficient of variations (CV, %) are shown in parenthesis.

All values represent the average for three determinations.

Table 4. Recoveries of carbamate pesticides from the tea samples with different extraction solvents.

Pesticide	Extraction		Solvent	
	Acetonitrile	Dichloromethane	Acetone	n-Hexane
Group A ^a				
Oxamyl	95.9 ^b ± 0.3 ^c (0.3) ^d	74.1 ± 0.5 (0.7)	77.1 ± 0.2 (0.2)	ND
3-OH Carbofuran	55.6 ± 0.4(0.6)	50.8 ± 0.6 (1.1)	79.7 ± 0.4 (0.4)	ND
Aldicarb	60.7 ± 0.5 (0.9)	34.2 ± 0.1 (0.3)	37.3 ± 0.1 (0.2)	4.0 ± 0.0 (0.8)
Metolcarb	84.6 ± 0.6 (0.8)	52.1 ± 0.4 (0.7)	55.4 ± 0.1 (0.1)	9.8 ± 0.1 (0.9)
Thiodicarb	85.1 ± 0.3 (0.3)	57.9 ± 0.5 (0.8)	60.8 ± 0.3 (0.4)	3.1 ± 0.3 (10.2)
Carbofuran	103.9 ± 0.4 (0.4)	59.6 ± 0.3 (0.6)	65.7 ± 0.2 (0.3)	11.8 ± 0.3 (2.5)
Carbaryl	112.8 ± 0.8(0.7)	69.7 ± 0.3 (0.4)	79.5 ± 0.3 (0.3)	12.4 ± 0.0 (0.3)
Isoprocab	102.0 ± 0.6(0.6)	61.0 ± 0.1 (0.2)	75.8 ± 0.4 (0.5)	20.0 ± 0.1 (0.4)
Fenobucarb	99.9 ± 1.0 (1.0)	63.1 ± 0.4 (0.6)	63.7 ± 0.2 (0.3)	36.9 ± 1.7 (4.7)
Group B				
Aldicarb sulfone	91.6 ± 0.3 (0.3)	85.7 ± 1.8 (2.0)	106.6 ± 0.2 (0.2)	ND
Methomyl	85.4 ± 0.2 (0.2)	63.7 ± 0.1 (0.2)	82.0 ± 0.2 (0.2)	ND
Butocarboxin	68.7 ± 0.1 (0.2)	55.4 ± 0.1 (0.3)	69.8 ± 0.1 (0.1)	5.0 ± 0.0 (0.5)
3-keto Carbofuran	55.7 ± 0.3 (0.5)	43.6 ± 0.3 (0.6)	65.9 ± 0.2 (0.2)	2.8 ± 0.1 (5.2)
Propoxur	95.9 ± 0.3 (0.3)	67.9 ± 0.3 (0.4)	97.6 ± 0.1 (0.1)	10.1 ± 0.1 (1.4)
Meobal	55.2 ± 0.1 (0.2)	41.5 ± 0.1 (0.3)	62.0 ± 0.7 (1.1)	8.1 ± 0.1 (0.6)
Macbal	71.3 ± 0.3 (0.4)	51.9 ± 0.1 (0.1)	80.5 ± 0.8 (1.0)	10.2 ± 0.1 (0.9)
Methiocarb	73.3 ± 0.3 (0.4)	49.6 ± 0.3 (0.6)	67.6 ± 0.1 (0.2)	14.3 ± 0.4 (2.9)
Promecarb	79.5 ± 0.3 (0.3)	50.6 ± 0.3 (0.6)	66.8 ± 0.3 (0.5)	15.5 ± 0.4 (2.9)

^a Aldicarb sulfoxide was not detected.^b Recovery (%) = 100% × (Level of carbamate pesticides in spiked sample level of carbamate pesticides in blank sample) / Level of carbamate pesticides in spiked tea sample.^c Relative standard deviation (RSD).^d Coefficient of variations (CV, %) are shown in parenthesis.

All values represent the average for three determinations.

Table 5. Recoveries of carbamate pesticides from the tea samples with different extraction volumes.

Pesticide	Extraction 50 mL	Volume 100 mL
Group A ^a		
Oxamyl	129.7 ^b ± 0.7 ^b (0.5) ^d	72.8 ± 0.9 (1.3)
3-OH Carbofuran	81.7 ± 1.8(2.1)	49.0 ± 0.4 (0.8)
Aldicarb	107.9 ± 1.0 (0.9)	51.7 ± 0.3 (0.7)
Metolcarb	135.0 ± 1.1 (0.8)	66.3 ± 0.3 (0.5)
Thiodicarb	116.0 ± 0.6 (0.5)	63.3 ± 0.4 (0.7)
Carbofuran	165.2 ± 1.3 (0.8)	84.1 ± 0.5 (0.6)
Carbaryl	171.1 ± 1.3 (0.7)	88.1 ± 0.3 (0.3)
Isoprocarb	161.1 ± 1.4 (0.8)	75.7 ± 0.4 (0.5)
Fenobucarb	168.2 ± 1.4 (0.9)	80.0 ± 0.5 (0.6)
Group B		
Aldicarb sulfone	166.7 ± 1.5 (0.9)	93.3 ± 0.8 (0.8)
Methomyl	140.2 ± 1.0 (0.7)	76.4 ± 0.6 (0.8)
Butocarboxin	136.4 ± 1.0 (0.8)	62.0 ± 0.9 (1.4)
3-keto Carbofuran	123.8 ± 1.1 (0.9)	67.3 ± 0.5 (0.8)
Propoxur	185.1 ± 2.0 (1.1)	85.1 ± 0.7 (0.8)
Meobal	171.6 ± 1.4 (0.8)	78.5 ± 0.7 (0.9)
Macbal	187.5 ± 1.4 (0.7)	93.3 ± 0.8 (0.8)
Methiocarb	167.8 ± 1.5 (0.9)	76.4 ± 0.6 (0.8)
Promecarb	166.5 ± 1.4 (0.9)	62.0 ± 0.9 (1.4)

^a Aldicarb sulfoxide was not detected.

^b Recovery (%) = 100% × (Level of carbamate pesticides in spiked sample level of carbamate pesticides in blank sample) / Level of carbamate pesticides in spiked tea sample.

^c Relative standard deviation (RSD).

^d Coefficient of variations (CV, %) are shown in parenthesis.

All values represent the average for three determinations.

Effects of extraction solvent volumes

The second parameter studied was the different volumes of extraction solvent for the extraction of the pesticides. Comparison with different extraction solvent volumes (50 mL and 100 mL) in carbamate pesticides analysis of tea samples is described in Table 5. The results showed that the recoveries of these two extraction volumes were above 65%, except that aldicarb sulfoxide was not detected.

When used 50 mL acetonitrile to extract the tea samples, there were eight carbamate pesticides on a range of recoveries between 65% and 135%, and 10 carbamate pesticides on a range of recoveries between 165.2% and 187.5%. On the other hand, under 100 mL acetonitrile extraction there were 13 carbamate pesticides on a range of recoveries between 65% and 135%, and five carbamate pesticides on a range of recoveries between 49.0% and 63.3%.

In this study, the extraction solvent volumes of 100 mL acetonitrile were efficient in extracting carbamate pesticide residues in tea samples.

Solid phase extraction (SPE)

SPE has been increasingly used in pesticides analysis, mainly for sample clean-up. Several papers have described

the determination of pesticides in fruit, vegetables,^[18] and water^[20,21] by use of solvent extraction and solid-phase extraction. SPE columns not only offer the potential of simplifying the purification of the initial extract and reducing the amount of solvent consumed, but also decrease matrix effects that influence the analysis and the detection of studied compounds.

The physical and chemical properties of pesticides and adsorbents used in SPE devices vary widely. The SPE extraction procedure compared in this work was based on non-polar cartridge (octyl, octadecyl), moderately polar cartridge (florisil, silica gel), and polar cartridge (amino). Table 6 illustrates the recoveries obtained with the five types of cartridges. By using amino cartridge there were sixteen carbamate pesticides that could be detected with recoveries ranged from 65% to 135%. The other adsorbents showed relative low recoveries. For florisil or octyl cartridges there were 14 and 13 carbamate pesticides that could be detected within the range of 65% to 135%, and particularly for silica gel cartridge there were only 12 carbamate pesticides that could be detected within the range of 65% to 135%. Aldicarb sulfoxide was not detected by all five cartridges tested, perhaps due to its polarity and volatility. It was also found that the recovery of 3-OH carbofuran was very low (36.2%) by using an amino cartridge. In other four type cartridges 3-OH carbofuran was even not detected.

To our knowledge, no comprehensive data on the SPE procedure for carbamate pesticides analysis in tea samples has been documented. The present study demonstrated amino cartridge to be an appropriate adsorbent for a routine residues analysis of carbamate pesticides in tea samples.

Effects of elution solvents

After the sorbent type of SPE was determined, efficiency of different elution solvents for the amino cartridge was further evaluated. The recovery values of different elution mixture solvents are shown in Table 7. Using the *N*-hexane dichloromethane and acetone-dichloromethane mixture as elution solvents, the recoveries of sixteen and fifteen carbamate pesticides were in the range above 65%, respectively. On the other hand, only four carbamate pesticides had recoveries above 65% when using methanol-dichloromethane as elution solvents. None of recoveries of carbamate pesticides by acetone-*n*-hexane elution exceeded 30%, suggesting that acetone-*n*-hexane was not a suitable elution solvent for the amino cartridge SPE clean up of tea sample extractions.

Effects of elution volumes

The other parameter studied was the elution volume for the amino cartridge SPE clean up process. Resultant recovery values for elution volumes of 2 mL, 4 mL and 8 mL are shown in Table 8. Better recoveries were obtained with 4 mL and 8 mL of *n*-hexane-dichloromethane, with sixteen

Table 6. Recoveries of carbamate pesticides from the tea samples with different packing materials of cartridges.

Pesticide	Type of cartridge				
	Florisil	Amino	Octyl	Octadecyl	Silica gel
Group A ^a					
Oxamyl	23.4 ^b ± 1.2 ^c (4.9) ^d	75.9 ± 6.5 (8.6)	ND	ND	67.9 ± 8.2 (12.1)
3-OH Carbofuran	ND	36.2 ± 7.3 (6.8)	ND	ND	ND
Aldicarb	94.7 ± 6.7 (7.1)	93.2 ± 3.2 (3.4)	81.2 ± 2.3 (2.8)	82.5 ± 4.4 (5.4)	95.8 ± 23.8(24.9)
Metolcarb	97.4 ± 1.5 (1.5)	91.0 ± 5.7 (6.2)	81.9 ± 3.4 (4.1)	81.2 ± 3.5 (4.4)	71.2 ± 16.9(23.8)
Thiodicarb	43.2 ± 3.8 (8.9)	69.0 ± 3.1 (4.5)	55.9 ± 0.4 (0.7)	58.3 ± 2.6 (4.4)	70.6 ± 5.1 (7.2)
Carbofuran	78.2 ± 3.5 (4.5)	115.5 ± 1.2(1.0)	70.9 ± 2.1 (3.0)	70.0 ± 5.0 (7.1)	ND
Carbaryl	123.6 ± 8.5(6.8)	125.7 ± 4.6(3.6)	123.5 ± 2.5 (2.0)	121.8 ± 7.3 (6.0)	ND
Isoprocab	84.4 ± 6.8 (8.1)	98.5 ± 5.0 (5.1)	85.0 ± 1.4 (1.6)	83.1 ± 4.7 (5.7)	68.6 ± 19.6(28.6)
Fenobucarb	85.7 ± 5.6 (6.6)	89.4 ± 3.8 (4.2)	90.7 ± 1.9 (2.1)	89.7 ± 3.0 (3.3)	80.0 ± 10.7(13.4)
Group B					
Aldicarb sulfone	51.3 ± 6.9 (13.4)	88.6 ± 6.9 (7.8)	ND	ND	66.6 ± 22.7 (34.1)
Methomyl	66.2 ± 4.4 (6.6)	88.8 ± 6.3 (7.1)	ND	ND	70.2 ± 2.6 (3.8)
Butocarboxin	90.6 ± 3.0 (3.3)	62.9 ± 11.7 (18.5)	78.3 ± 7.9 (10.1)	78.1 ± 3.9 (5.0)	98.5 ± 7.4 (7.5)
3-keto Carbofuran	98.4 ± 13.8 (14.1)	102.2 ± 4.5 (4.4)	79.0 ± 6.7 (8.5)	80.2 ± 4.8 (18.4)	125.0 ± 15.3 (12.2)
Propoxur	85.7 ± 6.2 (7.3)	115.3 ± 2.2 (1.9)	104.3 ± 4.1 (3.9)	103.8 ± 6.8 (6.6)	ND
Meobal	88.2 ± 7.1 (8.1)	98.0 ± 5.4 (5.6)	87.7 ± 4.4 (5.0)	88.2 ± 4.6 (5.2)	ND
Macbal	133.4 ± 9.6(7.2)	137.8 ± 7.4 (5.4)	134.3 ± 5.3 (4.0)	131.0 ± 3.1 (2.4)	ND
Methiocarb	86.3 ± 2.7 (3.1)	94.4 ± 4.2 (4.4)	88.3 ± 4.6 (5.2)	88.4 ± 3.6 (4.1)	88.5 ± 5.8 (6.5)
Promecarb	82.0 ± 3.4 (4.1)	87.4 ± 2.8 (3.2)	87.2 ± 3.8 (4.4)	87.7 ± 7.1 (8.1)	84.1 ± 3.6 (4.2)

^a Aldicarb sulfoxide was not detected.^b Recovery (%) = 100% × (Level of carbamate pesticides in spiked sample level of carbamate pesticides in blank sample) / Level of carbamate pesticides in spiked tea sample.^c Relative standard deviation (RSD).^d Coefficient of variations (CV, %) are shown in parenthesis.

All values represent the average for three determinations.

Table 7. Recoveries of carbamate pesticides from the tea sample with different cartridge elution solvents.

Pesticide	Elution solvent			
	Acetone-dichloromethane (1/9)	Methonal-dichloromethane (1/9)	N-hexane-dichloromethane (1/9)	Acetone-hexane (1/9)
Group A ^a				
Oxamyl	86.6 ^b ± 0.5 ^c (0.6) ^d	76.1 ± 0.4 (0.6)	84.6 ± 0.5 (0.6)	ND
3-OH Carbofuran	51.1 ± 0.3 (0.7)	46.7 ± 0.4 (0.9)	92.6 ± 0.6 (0.7)	0.9 ± 0.1 (9.3)
Aldicarb	54.8 ± 0.4 (0.7)	37.4 ± 0.3 (0.5)	51.2 ± 0.6(1.2)	6.1 ± 0.2 (3.1)
Metolcarb	75.5 ± 0.6 (0.7)	53.3 ± 0.2 (0.4)	63.6 ± 0.4 (0.6)	18.5 ± 0.4 (2.2)
Thiodicarb	75.2 ± 0.5 (0.7)	55.8 ± 0.3 (0.5)	72.3 ± 0.6 (0.8)	ND
Carbofuran	88.6 ± 0.7 (0.8)	61.2 ± 0.3 (0.5)	78.2 ± 0.6 (0.7)	16.7 ± 0.3 (2.0)
Carbaryl	97.6 ± 0.8 (0.8)	67.4 ± 0.2 (0.3)	84.6 ± 0.4 (0.5)	17.2 ± 0.2 (1.4)
Isoprocab	87.8 ± 0.7 (0.8)	58.2 ± 0.2 (0.3)	74.3 ± 0.3 (0.3)	29.4 ± 0.5 (1.6)
Fenobucarb	83.8 ± 0.9 (1.1)	56.5 ± 0.3 (0.6)	70.1 ± 0.3 (0.5)	30.4 ± 0.5 (1.6)
Group B				
Aldicarb sulfone	79.1 ± 1.8 (2.3)	77.6 ± 1.0 (1.3)	95.8 ± 0.6 (0.6)	ND
Methomyl	84.7 ± 0.3 (0.4)	66.8 ± 0.3 (0.5)	84.8 ± 0.3 (0.4)	1.0 ± 0.1 (5.9)
Butocarboxin	76.7 ± 0.4 (0.6)	60.1 ± 0.4 (0.7)	73.1 ± 0.3 (0.4)	7.7 ± 0.1 (1.7)
3-keto Carbofuran	61.7 ± 0.4 (0.7)	52.3 ± 0.6 (1.2)	68.9 ± 0.3 (0.5)	7.4 ± 0.3 (3.8)
Propoxur	87.3 ± 0.5 (0.5)	64.9 ± 0.2 (0.4)	81.5 ± 0.7 (0.9)	16.8 ± 0.3 (2.0)
Meobal	69.7 ± 0.7 (1.0)	54.6 ± 0.6 (1.1)	76.6 ± 0.8 (1.0)	19.9 ± 0.5 (2.7)
Macbal	79.9 ± 0.5 (0.6)	61.8 ± 0.5 (0.8)	90.6 ± 0.7 (0.8)	23.6 ± 0.6 (2.6)
Methiocarb	79.8 ± 0.5 (0.6)	58.6 ± 0.5 (0.9)	77.3 ± 0.7 (0.8)	24.3 ± 0.5 (2.0)
Promecarb	81.1 ± 0.4 (0.5)	59.2 ± 0.4 (0.8)	74.9 ± 0.9 (1.2)	27.6 ± 0.6 (2.2)

^a Aldicarb sulfoxide was not detected.^b Recovery (%) = 100% × (Level of carbamate pesticides in spiked sample level of carbamate pesticides in blank sample) / Level of carbamate pesticides in spiked tea sample.^c Relative standard deviation (RSD).^d Coefficient of variations (CV, %) are shown in parenthesis.

All values represent the average for three determinations.

Table 8. Recoveries of carbamate pesticides from the tea samples with different elution volumes.

Pesticide	Elution volume		
	2 mL	4 mL	8 mL
Group A ^a			
Oxamyl	66.1 ^b ± 0.6 ^c (0.9) ^d	84.4 ± 0.5 (0.6)	104.3 ± 0.5 (0.5)
3-OH Carbofuran	31.7 ± 1.6 (5.2)	31.3 ± 1.1 (3.4)	70.4 ± 1.5 (2.2)
Aldicarb	59.2 ± 0.3 (0.5)	57.1 ± 0.5 (0.8)	61.8 ± 0.3 (0.5)
Metolcarb	67.8 ± 0.8 (1.2)	67.3 ± 0.3 (0.5)	76.7 ± 0.3 (0.4)
Thiodicarb	66.6 ± 0.3 (0.5)	65.4 ± 0.4 (0.7)	74.6 ± 0.4 (0.5)
Carbofuran	86.7 ± 0.6 (0.7)	86.1 ± 0.5 (0.6)	95.8 ± 0.6 (0.6)
Carbaryl	90.3 ± 0.6 (0.6)	88.3 ± 0.4 (0.4)	99.9 ± 0.4 (0.4)
Isoprocarb	77.1 ± 0.7 (0.9)	78.7 ± 0.3 (0.4)	87.0 ± 0.4 (0.4)
Fenobucarb	81.1 ± 0.6 (0.8)	81.4 ± 0.3 (0.4)	92.2 ± 0.4 (0.5)
Group B			
Aldicarb sulfone	63.3 ± 0.7 (1.0)	84.2 ± 0.5 (0.6)	95.9 ± 0.6 (0.7)
Methomyl	67.8 ± 0.2 (0.3)	73.1 ± 0.4 (0.6)	75.1 ± 0.4 (0.5)
Butocarboxin	66.7 ± 0.2 (0.4)	68.7 ± 0.5 (0.7)	64.9 ± 0.7 (1.1)
3-keto Carbofuran	52.6 ± 0.9 (1.7)	65.9 ± 0.3 (0.5)	67.4 ± 0.9 (1.4)
Propoxur	80.9 ± 0.5 (0.6)	83.3 ± 0.6 (0.8)	85.0 ± 0.5 (0.6)
Meobal	80.4 ± 0.7 (0.8)	80.4 ± 0.8 (1.0)	85.0 ± 0.9 (1.1)
Macbal	90.5 ± 0.7 (0.8)	91.6 ± 0.8 (0.9)	94.7 ± 0.9 (1.0)
Methiocarb	74.4 ± 0.4 (0.5)	80.3 ± 0.5 (0.6)	76.8 ± 0.4 (0.5)
Promecarb	72.9 ± 0.3 (0.4)	76.6 ± 0.4 (0.6)	77.4 ± 0.4 (0.5)

^a Aldicarb sulfoxide was not detected.

^b Recovery (%) = 100% × (Level of carbamate pesticides in spiked sample level of carbamate, pesticides in blank sample) / Level of carbamate pesticides in spiked tea sample.

^c Relative standard deviation (RSD).

^d Coefficient of variations (CV, %) are shown in parenthesis.

All values represent the average for three determinations.

carbamate pesticides in a range higher than 65%. When 8 mL of acetone-dichloromethane was used as the elution solvent, only the recoveries of aldicarb and butocarboxin were less than 65%, which were 61.8% and 64.9%, respectively. Using 4 mL of acetone-dichloromethane as the elution solvent, the recoveries under 65% were 31.3% and 57.1% for 3-OH carbofuran and aldicarb, respectively. But using 2 mL of acetone-dichloromethane the recoveries of four analytes were above 65%.

The results suggest that 8 mL of n-hexane-dichloromethane was sufficient for the amino cartridge clean up of tea samples in carbamate pesticides analysis.

Evaluation the carbamate pesticides in tea sample

The developed protocol was practically applied for a dietary exposure assessment of carbamate pesticides in made-tea samples by our laboratory. This study was undertaken between 2004 and 2005 as part of the tea pesticide residue monitoring program. We collected 4,133 made-tea samples from tea processors and markets around almost all Taiwan area. Tea samples were analyzed by the present developed multiresidue pesticides analysis method with optimum conditions as described above. In total 19 carbamate pesticides residues could be monitored for tea sam-

ples by the developed method. The results are expressed in Table 9, and the percentages of positive samples were relatively low. Only two kinds of carbamate pesticides residues, carbaryl and carbofuran, were detected in tea samples. Carbaryl was the most frequently detected carbamate pesticides in the tea samples, from 2004 to 2005, and the frequency was 0.086% to 0.384%, respectively. Carbofuran was only detected in 2005, the frequency was 0.043%. The detected residues level of carbaryl ranged from 0.01 mg kg⁻¹ to 1.39 mg kg⁻¹ for samples between 2004 and 2005, and the level of carbofuran was 0.01 mg kg⁻¹ in 2005. In Taiwan these two kinds of carbamate pesticides have been legally recommended to control insects on tea bushes. The present detected residue levels of carbaryl and carbofuran were lower than their correspondent maximum residues levels (MRLs) regulated by the Taiwan government. The other carbamate pesticides residues were not detected from these samples. That may be due to the dissipation of carbamate pesticides during the growth, processing and roasting.^[22] The surveyed result is similar to that of our previous reports for tea fresh-leaf samples.^[23] The data also suggest that the residues of carbamate pesticides on field-sprayed tea plants can be dissipated almost completely when good agriculture practice (GAP) is followed by tea farmers.

Table 9. Levels of carbamate pesticides residues detected in made-tea samples from Taiwan during 2004 to 2005.

Pesticide	2004 ^a		2005	
	No. of positive samples	Concentration range (mg kg ⁻¹)	No. of positive samples	Concentration range (mg kg ⁻¹)
Aldicarb	ND ^b	0	ND	0
Carbaryl	7 ^c (0.384%) ^d	0.17–1.39	2 (0.086%)	0.01–0.02
Carbendazim	ND	0	ND	0
Carbofuran	ND	0	1 (0.043%)	0.01
Carbosulfan	ND	0	ND	0
Isoprocab	ND	0	ND	0
Macbal(XMC)	ND	0	ND	0
Methiocarb	ND	0	ND	0
Methomyl	ND	0	ND	0
Metolcarb	ND	0	ND	0
Thiodicarb	ND	0	ND	0
Thiabendazole	ND	0	ND	0
Propoxur	ND	0	ND	0

^aSurveyed samples in 2004 and 2005 year were 1820 and 2313, respectively.

^bNot detected.

^cNumber of detected.

^dDetection rate (%) in parenthesis.

Conclusion

The work shows an efficient HPLC-based multiresidue method with good extraction and SPE cartridge clean-up step for carbamate pesticide residues in tea samples. The amino cartridge for adsorbent was superior to other cartridges in terms of the high extraction efficiency, and reproducibility. The method allows determination of 0.0005–0.023 mg L⁻¹ levels of carbamate pesticide residues in tea samples. Overall results indicate that the presented method has satisfactory reproducibility, recovery, and accuracy for nineteen carbamate pesticides analysis in tea samples. Results of practical survey of carbamate pesticides residues in tea samples of Taiwan suggest that residue levels were relatively low, and posed low risk to consumer health as well.

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References

- [1] Krause, R.T. Liquid chromatographic determination of Nmethylcarbamate insecticides and metabolites in crops. *J. AOAC. Int.* **1985**, *68*, 726–733.
- [2] Dittus, K.L.; Hillers, V.H. Consumer trust and behavior related to pesticides. *Food Technology* **1993**, *47*, 87–89.
- [3] Tsumura, Y.; Wads, I.; Fujiwara, Y.; Nakamura, Y.; Tonogai, Y.; Ito, Y. Simultaneous determination of 13 synthetic pyrethroids and their metabolite, 3-phenoxybenzoic acid, in tea by gas chromatography. *J. Agric. Food Chem.* **1994**, *42*, 2922–2925.
- [4] Nakamura, Y.; Tonogai, Y.; Tsumura, Y.; Ito, Y. Analysis of pyrethroid residues in vegetables, fruits, grains, beans and green tea leaves. Application to pyrethroid residue monitoring studies. *J. Assoc. Off. Anal. Chem.* **1993**, *76*, 1348–1361.
- [5] Jaggi, S.; Sood, C.; Kumar, V.; Ravindranath, S. D.; Shanker, A. Leaching of pesticides in tea brew. *J. Agric. Food Chem.* **2001**, *49*, 5479–83.
- [6] Liu, C.H.; Mattern, G.C.; Yu, X.; Rosen, R.T.; Rosen, J.D. Multiresidue determination of nonvolatile and thermally labile pesticides in fruits and vegetables by thermospray liquid chromatography/mass spectrometry. *J. Agric. Food Chem.* **1991**, *39*, 718–723.
- [7] Yang, S.S.; Goldsmith, A.; Smetena, I. Recent advances in the residue analysis of N-methylcarbamate pesticides. *J. Chromatogr A* **1996**, *754*, 3–16.
- [8] Wang, N.; William, L.B. Determination of carbamate, urea, and thiourea pesticides and herbicides in water. *Anal. Chem.* **2001**, *73*, 997–1006.
- [9] European Union (EU). Quality control procedures for pesticide residues analysis (doc. SANCO/10476/2003). (available on line at http://europa.eu.int/comm/food/plant/protection/resources/publications_en.htm.) (verified March 20, 2007).
- [10] Chasin, A.A.M.; Nascimento, E.S.; Ribeiro-Neto, L.M.; Siqueira, M.E.P.B.; Andraus, M.H.; Salvadori, M.C.; Fernicola, N.A.G.; Gorni, R.; Salcedo, S. Validation of analytical methodologies for toxicological analysis. *Rev. Bras. Toxicol.* **1998**, *11*, 1–6.
- [11] Anonymous. Method of test for pesticides residues in foods-multiresidue analysis (II) No.13570–2, N6276–2. Taiwan, 1999.
- [12] Tzeng, D.D., Ed. The maximum residues levels in main export countries for Taiwan agriculture products. Agriculture and Food Agency: Nantou, Taiwan, **2005**; 275–287.
- [13] Council of European Committees (EC). Council Directive 76/895/EC of 23 November 1976 relating to the fixing of maximum

- levels for pesticide residues in and on fruit and vegetables. Official J. **1976**, p. 26–31.
- [14] EC. Council Directive 90/642/EEC of 27 November 1990 on the fixing of maximum levels for pesticide residues in and on certain products of plant origin, including fruit and vegetables. Official J. **1990**, p. 71–79.
- [15] Codex Alimentarius Commission. *Codex maximum residue limits for pesticides*. (available on line at <http://apps.fao.org/servlet/org.fao.waicent.Codex>. FAO 1990–1998.) (verified March 20, 2007).
- [16] Environmental Protection Agency (EPA). Method 531.1. http://www.ultrasci.com/Docs/500/531_1.PDF (accessed March 20, 2007).
- [17] Group of Analysts of Residues of Pesticides (GARP). Andef Validation of analytical methodologies for determination of pesticides residues. Brazilia: Ministry of Agriculture: Bras, Brazil, 1997.
- [18] Gou, J.; Tragas, C.; Lord, H.; Pawliszyn, J. On-line coupling of in-tube solid phase microextraction (SPME) to HPLC for analysis of carbamates in water samples: Comparison of two commercially available autosamplers. *J. Microcol.* **2000**, *12*, 125–134.
- [19] Tomsej, T.; Hajslova, J. Determination of benzoylurea insecticides in apples by high-performance liquid chromatography. *J. Chromatogr. A.* **1995**, *704*, 513–517.
- [20] Araoud, M.; Douki, W.; Rhim, A.; Najjar, M. F.; Gazzah, N. Multiresidue analysis of pesticides in fruits and vegetables by gas chromatography-mass spectrometry. *J. Environ. Sci. Health, Part B*, **2007**, *42*, 179–187.
- [21] Crescenzi, C.; Di Corcia, A.; Guerriero, E.; Samperi, R. Development of a multiresidue method for analyzing pesticide trace in water based on solid-phase extraction and electrospray liquid chromatography mass spectrometry. *Environ. Sci. Technol.* **1997**, *31*, 479–488.
- [22] Wu, C.C.; Chu, C.; Wang, Y.S.; Lur, H.S. Dissipation of carbofuran and carbaryl on Oolong tea during tea bushes, manufacturing and roasting processes. *J. Environ. Sci. Health, Part B*, **2007**, *42*, 669–675.
- [23] Wu, C.C. Study on the residual of carbamate insecticides in the fresh tea leaves in Taiwan. *Taiwan Tea Research Bulletin.* **2001**, *20*, 43–52.