Determination of the Residues of 18 Carbamate Pesticides in Chestnut and Pine Nut by GPC Cleanup and UPLC–MS–MS

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Abstract

A new method using gel permeation chromatography (GPC) cleanup followed by ultra-performance liquid chromatography combined with tandem mass spectrometry (UPLC–MS–MS) has been established for simultaneous determination of 18 carbamate pesticides in nuts (chestnut and pine nut). Recoveries obtained by fortifying nut (spiking at 0.02 mg/kg) range from 70.21% to 89.56%. The proposed method features good sensitivity. Its limits of quantification are low enough to allow pesticide residues to be determined at concentrations below the maximum residue levels legally accepted. The precision, expressed as relative standard deviation, ranges from 2.26% to 4.07%.

Introduction

Carbamate pesticides have become increasingly important in recent years due to their broad spectrum of activity, relatively rapid disappearance, and generally low mammalian toxicity, but because they are inhibitors of acethylcholinesterase, they are considered to be toxic for the environment and for human beings. The detection of their residues in food has caused a great deal of public concern because of carbamate pesticides being used in households and in agriculture on a large number of crops. Analysis involves a number of stages such as extraction, removal of interfering substances from the extract, and determination (1). The regular sample preparation method for the analysis of carbamate pesticides include solid-phase microextraction (SPME) (1–6), solid phase extraction (SPE) (7–13), and gel permeation chromatography (GPC) (14–15). SPME is a simple process, but it is more difficult to choose and to optimize the experimental conditions. SPE are rather complicated processes for sample preparation (3). Big disadvantages of SPE are the large quantities of solvent utilized, the multiple operation steps needed, the preconcentration of the extract required prior to analysis, and the interfering compounds that are more likely to be coextracted (6). GPC appears to be best suited to multi-residue analysis as it affords clean-up of both polar and non-polar pesticides with a single injection on a fully automated system (15). In addition, GPC can clear up material, which is high in oil content. Nuts are a food whose oil content is high, so we chose GPC to clear up nut samples. It is necessary to develop an analytical method with high sensitivity to meet the requirements of carbamate pesticides monitoring. Pesticides are routinely analyzed using gas chromatography (GC), gas chromatography mass spectrometry (GC–MS), high performance liquid chromatography (HPLC), and high performance liquid chromatography combined with mass spectrometry (HPLC–MS). However, because they are nonvolatile and semivolatile, it is difficult or even impossible to analyze such pesticides as carbamates using conventional GC and GC–MS (14). Carbamates pesticides are routinely analyzed using HPLC and HPLC–MS. The purpose of this study is to develop a much more rapid and efficient method than HPLC and HPLC-MS for the simultaneous determination of 18 carbamates pesticides in nut by ultra-performance liquid chromatography combined with tandem mass spectrometry (UPLC–MS–MS) with GPC.

Experimental

Instrument and reagents

The Waters Acquity Ultra-Performance LC combined with Quattro PremierXE tandem mass spectrometry system was applied (Milford, MA). The GPC system consists of J2 Scientific AccuPrep MPS Gel Permeation Chromatography Cleanup System and AccuVap Inline (Columbia, MO), FLX Concentration Systems, and Bio-Beads S-X3 Express column (Bio-Rad, Hercules, CA). Furthermore, T18 basic Ultra-Turrax homogenizer (IKA, Staufen, Germany), LABOROTA 4003 control rotary evaporator (Heidolph, Schwabach, Germany), Keda HC-3518 centrifuge (Hefei, China), and 18780 Reacti-Vap nitrogen evaporator (Thermo Scientific, Rockford, IL) were used.

Acetonitrile (HPLC-grade) was purchased from Fisher (Somerville, New Jersey). Cyclohexane and ethyl acetate (HPLC-

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grade) were purchased from Kermel Chemical Reagent Co., Ltd (Tianjin, China). HPLC-grade water was obtained by the purification of deionized water using a Millipore Mill-Q system (Billerica, MA). The other reagents were analytical-grade.

Individual stock standard solutions of each carbamate pesticide (1.000 mg/mL) were prepared in acetonitrile. Stock standard solution (10 µg/mL) containing all the compounds was prepared from individual standard solution (1.000 mg/mL) by dilution with acetonitrile. Standard solutions (10, 20, 40, 60, 80, and 100 µg/L) were obtained by appropriate dilution of the stock standard solutions (10 µg/mL) in acetonitrile. These solutions were stored at 4°C.

Samples

Whole chestnut and pine nut samples used for this study were collected from local markets. The samples used for recovery and sensitivity studies were previously determined to be free of carbame pesticides.

Sample extraction

Accurately weighed 2.000 g samples in a 50-mL centrifuge tube were added with 20 mL acetonitrile, homogenized for 1 min, and then centrifuged for 5 min at 40,000 rpm. The supernatants were made to pass through a glass funnel with 5 g sodium sulfate and collected in a 250-mL evaporation flask, re-homogenized in the centrifuge tube with 20 mL acetonitrile, re-centrifuged, and then transferred to the previously mentioned glass funnel before the extracts were combined, which were then placed in a water bath of 40ºC and evaporated to dryness on a rotary evaporator for cleanup.

Process of GPC cleanup

The concentrated extracts were dissolved using 5 mL of cyclohexane–ethyl acetate mixture (1:1, v/v), transferred to a 10-mL volumetric flask, rinsed the evaporation flask with 2 mL of cyclohexane–ethyl acetate mixture (1:1, v/v) twice, and transferred to the previously mentioned 10-mL volumetric flask before diluting to volume with cyclohexane–ethyl acetate mixture (1:1, v/v) and mixed well. The sample solutions were filtered into a 10-mL test tube and cleaned up based on the following conditions by GPC. Mobile phase was cyclohexane–ethyl acetate mixture (1:1, v/v); flow rate 4.7 mL/min; injection volume 5 mL; starting collecting time 8.2 min; stopped collecting time 14.2 min. The eluted portions of 8.2–14.2 min were collected in a sample vial and then blown to dryness with nitrogen gas, the precipitate of which were then dissolved in 1 mL of 10 mM ammonium acetate–acetonitrile mixture (9:1, v/v) before being submitted for determination by UPLC–MS–MS.

Table I. Gradient Conditions

Time (min)	Flow rate (mL/min)	%A (Acetonitrile)	%B (10mM ammonium acetate)
0	0.3	10	90
2	0.3	50	50
4	0.3	60	40
5	0.3	90	10
7	0.3	100	0
8	0.3	100	0
9	0.3	10	90

UPLC–MS–MS

The column used was a Hss T_3 (2.1 mm × 50 mm, 1.8 µm). The mobile phase was 10 mM NH_4AC–acetonitrile mixture, and a gradient program were used at a flow rate of 0.3 mL/min. Table I shows the gradient conditions. UPLC injection volume was 10 µL. MS detection was performed with an electrospray interface operating in the positive ionization mode for each target compound. In addition to the specific cone voltage and collision energies for each compound, the capillary voltage was 3 kV; RF lens voltage 0.5 V; source temperature 110°C; desolvation temperature 350ºC; Nitrogen was used as nebulizing, desolvation, and cone gas. The flow rate of the desolvation gas was set to 500 L/h, and that of the cone gas was set to 20 L/h.

Table II. MS–MS Conditions Used For the Detection

Pesticide	Precursor ion (m/z)	Product ion (m/z)	t_R (min)	Settle time (s)	Cone voltage (V)	Collision energy (eV)
Aldicarb sulfxide	207.20	88.80	1.07	0.100	18.00	15.00
		132.00		0.100	18.00	6.00
Oxamyl	220.30	71.80	1.33	0.100	18.00	15.00
		89.70		0.100	18.00	1.00
Aldoxycarb	240.30	85.80	1.34	0.100	15.00	19.00
		223.00		0.100	15.00	7.00
Methomyl	163.20	87.70	1.47	0.100	18.00	7.00
		105.90		0.100	18.00	7.00
3-OH-carbofuran	238.30	163.20	1.81	0.100	25.00	18.00
		181.00		0.100	25.00	10.00
Aldicarb	191.10	88.70	2.33	0.100	15.00	12.00
		116.10		0.100	15.00	3.00
Propoxur	210.30	92.90	2.67	0.050	20.00	23.00
		111.00		0.050	20.00	14.00
Carbofuran	222.10	122.90	2.72	0.070	25.00	22.00
		164.90		0.070	25.00	10.00
Carbaryl	202.00	127.00	2.81	0.100	21.00	28.00
		144.80		0.100	21.00	15.00
Pirimicarb	239.20	71.90	2.81	0.030	18.00	19.00
		182.00		0.030	18.00	15.00
Ethiofencarb	226.30	106.80	2.93	0.070	18.00	18.00
		164.10		0.070	18.00	6.00
Isoprocarb	194.30	94.80	3.11	0.050	22.00	10.00
		136.90		0.050	22.00	8.00
Methiocarb	226.30	121.00	3.54	0.100	20.00	17.00
		169.10		0.100	20.00	9.00
Fenobucarb	208.40	94.90	3.56	0.100	20.00	15.00
		151.90		0.100	20.00	8.00
Fenothiocarb	254.40	72.00	4.57	0.200	20.00	18.00
		160.10		0.200	20.00	8.00
Benfuracarb	411.10	102.00	5.71	0.100	25.00	28.00
		195.20		0.100	25.00	22.00
Furathiocarb	383.10	195.00	5.73	0.100	30.00	18.00
		252.00		0.100	30.00	15.00
Carbosulfan	381.20	118.00	6.67	0.100	10.00	22.00
		160.00		0.100	10.00	13.00

Content calculation

The pesticide content in matrix (c_m, µg/kg) was obtained by equation $c_m = 2 \times c_v \times v \times 1/m$, in which c_v (µg/L) was corresponding concentration calculated from the calibration curve, v was sample volume (mL) before UPLC analysis, and m was quantity of matrix samples (kg). Because injection volume of GPC was half, so pesticides content was two times the results. In this study, v = 1 mL, m = 2 g; so we obtained c_m (µg/kg) = c_v (µg/L)

Table III. Linear Equation and the r^2 of 18 Carbamates*

	a		b		
Pesticide	Mean (n = 6)	RSD (%)	Mean (n = 6)	RSD (%)	Correlation Coefficient (r^2)
Aldicarb Sulfxide	528.01	3.83	6374.13	15.14	0.9918
Oxamyl	153.70	7.41	4650.40	8.60	0.9905
Aldoxycarb	23.15	6.25	456.12	9.47	0.9922
Methomyl	127.33	8.21	4748.95	7.72	0.9902
3-OHcarbofuran	368.15	6.01	9060.99	8.96	0.9991
Aldicarb	25.51	10.85	538.40	11.67	0.9936
Propoxur	211.94	9.88	4477.70	15.34	0.9907
Carbofuran	457.67	6.28	7712.45	11.26	0.9925
Carbaryl	1689.55	10.71	40796.01	15.50	0.9916
Pirimicarb	4.73	3.13	344.69	6.63	0.9905
Ethiofencarb	133.56	6.10	2884.41	2.31	0.9912
Isoprocarb	11.38	5.47	190.28	10.65	0.9924
Methiocarb	19.78	7.06	372.52	11.38	0.9953
Fenobucarb	30.96	3.28	475.27	9.76	0.9946
Fenothiocarb	14.71	6.97	296.89	5.98	0.9918
Benfuracarb	1139.04	8.35	41608.30	14.23	0.9911
Furathiocarb	653.16	5.81	24977.40	8.92	0.9911
Carbosulfan	181.20	2.33	2269.12	12.33	0.9904

* The linear range was all between 10–100 µg/L for 18 carbamates. Linear equation was Y = aX + b, and mg/L was the unit of X.

Table IV. LOD, LOQ, and MRL*

Pesticide name	Molecular weight	LOD (µg/kg)	LOQ (µg/kg)	MRL (mg/kg)
Aldicarb sulfxide	206.2	0.056	0.188	–†
Oxamyl	239.3	0.021	0.070	0.50‡
Aldoxycarb	219.3	0.002	0.007	–
Methomyl	162.2	0.001	0.003	0.05§
3-OHcarbofuran	237.3	0.008	0.028	–
Aldicarb	190.1	0.001	0.004	0.50§
Propoxur	221.1	0.148	0.495	0.05§
Carbofuran	209.3	0.020	0.065	0.10§
Carbaryl	238.2	0.085	0.285	1.00§
Pirimicarb	201.0	0.087	0.291	1.00‡
Ethiofencarb	225.3	0.124	0.414	5.00‡
Isoprocarb	193.3	0.048	0.137	–
Methiocarb	225.3	0.091	0.305	–
Fenobucarb	207.4	0.130	0.434	–
Fenothiocarb	253.4	0.052	0.175	–
Benfuracarb	410.1	0.007	0.022	0.50§
Furathiocarb	382.1	0.025	0.085	–
Carbosulfan	380.2	0.025	0.085	0.05§

* LOD = Limits of Detection; LOQ = Limits of Quantification; MRL = Maximum Residue Limit.
† No MRL references for nuts and similar foods found.
‡ Allowed by Korea Food & Drug Administration (16).
§ Allowed by European Commission Directive (17).

Results and Discussion

Optimization of GPC cleanup variables

Because most nuts are high in oil content, olive oil, methomyl, and benfuracarb were chosen to optimize the collection condition for the pesticide fraction from GPC system. The solution (5 mL), obtained by dissolving methomyl, benfuracarb, and olive oil in cyclohexane–ethyl acetate mixture (1:1, v/v), was injected into the GPC column at 4.7 mL/min. The molecular mass of methomyl is the least, and the benfuracarb is biggest in the pesticides chosen. Molecular masses of pesticides were between 163.2–411.1 whereas that of lipids ranged from 600 to 1500. Hence, the larger lipid molecules that are too big to pass through the pores of polymer beads are not retained, and they are the first to be eluted from the column. As can be seen in Figure 1, the fat fraction was eluted between 4–8 min. On the other hand, the carbamates pesticides were detected between 8.2–14.2 min. No lipids fraction was detected over the chromatographic separation of the pesticides.

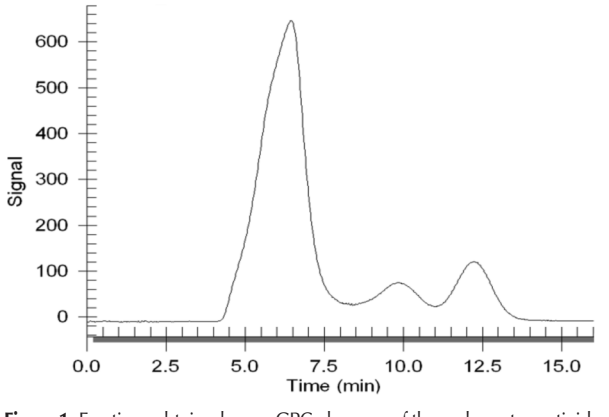

Figure 1. Fractions obtained upon GPC clean-up of the carbamate pesticides as determined by spectrophotometry at 254 nm. The first peak: fats; the second peak: benfuracarb; the third peak: methomyl.

Table V. Recovery and Precision (RSD) Obtained From Different Samples Spiked with 20 µg/kg (n = 10)

	Chestnut		Pine nut	
Pesticide	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Aldicarb Sulfxide	88.17	2.83	89.56	3.26
Oxamyl	83.23	3.56	85.62	4.07
Aldoxycarb	80.11	3.23	81.79	3.68
Methomyl	80.19	3.05	79.21	3.01
3-OHcarbofuran	72.94	2.93	75.17	2.85
Aldicarb	84.79	3.91	85.52	3.74
Propoxur	74.21	3.31	83.84	3.52
Carbofuran	71.85	3.10	70.93	2.26
Carbaryl	71.13	2.51	78.18	3.09
Pirimicarb	70.89	3.24	70.79	2.91
Ethiofencarb	71.81	2.45	80.06	2.31
Isoprocarb	79.16	3.61	75.04	3.26
Methiocarb	73.41	2.86	75.31	2.52
Fenobucarb	71.38	3.67	72.08	3.91
Fenothiocarb	70.21	2.30	74.28	2.76
Benfuracarb	71.01	2.35	73.12	2.64
Furathiocarb	74.72	3.21	75.46	2.74
Carbosulfan	73.30	2.43	70.89	2.82

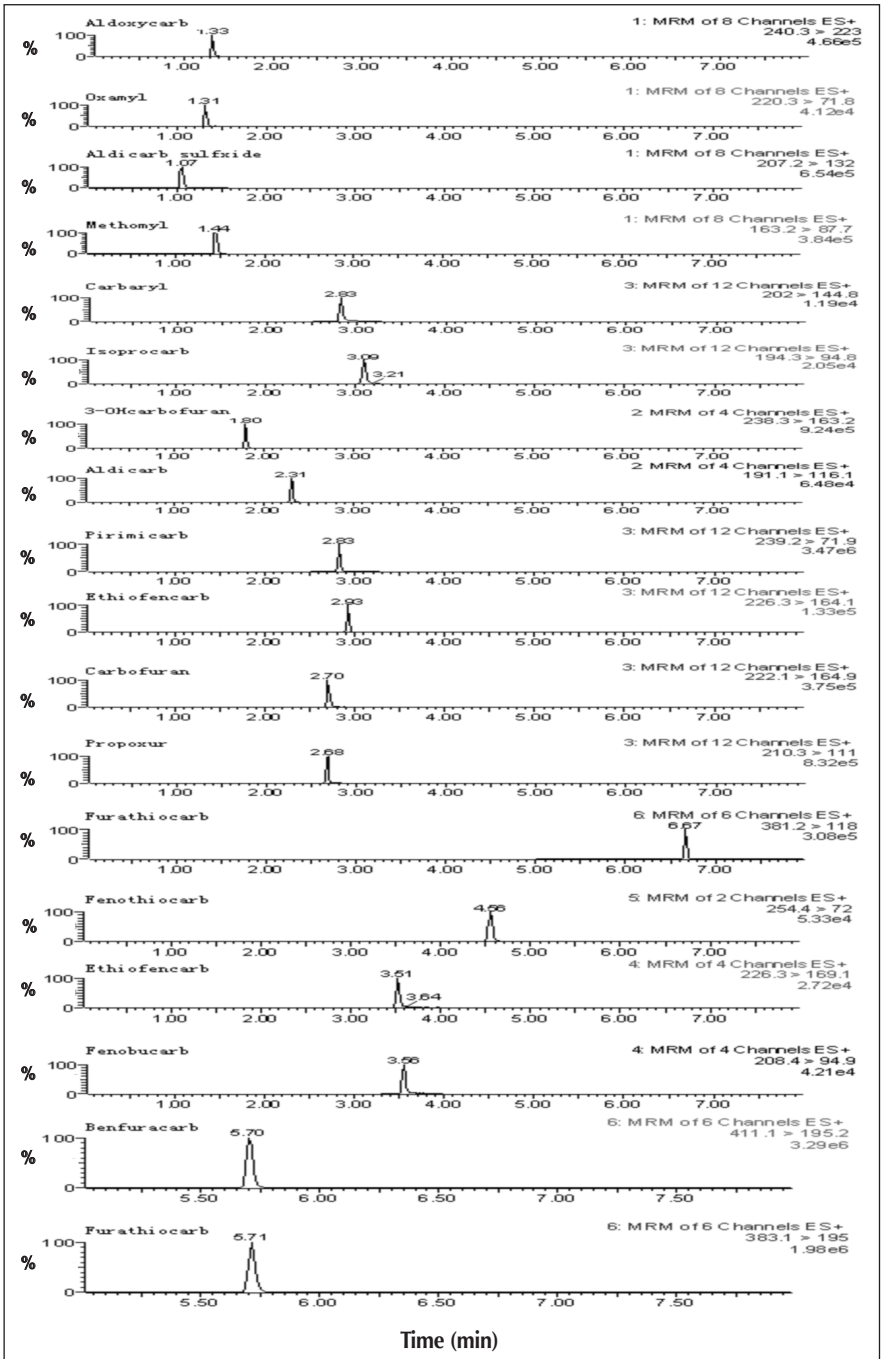

Figure 2. Typical chromatogram of a standard mixture solution of the studied pesticides.

Optimization of UPLC–MS–MS conditions

Monitoring conditions were optimized for each pesticide. Table II shows the MS–MS conditions used for the detection. The chromatogram of the standard pesticides obtained is shown in Figure 2.

Validation of the method

The linearity was determined using the calibration curve obtained with concentrations of 10, 20, 40, 60, 80, and 100 µg/L. The results showed good linearity with the correlation coefficients $r \geq 0.99$ (Table III). The slopes and intercepts of calibration curves for different carbamate pesticides were very different. It suggests that molecular structure of the individual pesticide have remarkable influence on its response on UPLC–MS–MS analysis and detection. This also explains the big difference of the limits of detection (LOD) and quantitation (LOQ) for different carbamate pesticides (Table IV).

LOD is considered as the minimum concentration of analyses that generated a response three times greater than the noise level of the detection system. LOQ is considered as the minimum concentration of analyses that generated a response 10 times greater than the noise level of the detection system. LOD and LOQ were calculated from the chromatogram in this study. Compared to legally accepted value, LOD and LOQ obtained with the developed method were obviously lower (Table IV). It suggests that the proposed method features good sensitivity. Its LOQs are low enough to allow pesticide residues to be determined at concentrations below the maximum residue levels legally accepted.

The accuracy and precision of the previously mentioned method were investigated by the analysis of chestnut and pine nut spiked at 20 µg/kg (Table V). Recoveries ranged from 70.21% to 89.56%. The precision, expressed as relative standard deviation (RSD), ranged from 2.26% to 4.07%. These results demonstrate that the developed method has good precision and accuracy.

The developed method was verified by Jilin Border Inspection and Quarantine Bureau, Yunnan Border Inspection and Quarantine Bureau, and four other laboratories. There was no statistical difference on 95% confidence level between the data obtained by different laboratories.

Conclusions

A method for the GPC clean-up and determination of 18 carbamate pesticides in nut by UPLC–MS–MS was developed. The GPC technique was found to substantially simplify the removal of fat matter relative to other sample treatments. The method has good recovery, reproducibility, and low limits of quantification. Its limits of quantification are much lower than the maximum residue levels legally accepted.

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