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Determination of Carbamate and Triazol Pesticides in Soil Using QuEChERS with Liquid Chromatography-Tandem Mass Spectrometry

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A method for the simultaneous determination of 18 carbamate and triazol pesticides in soil using modified QuEChERS combined with liquid chromatography tandem mass spectrometry has been developed. The targets in soil were extracted by shaking extracted with acetone and dichloromethane and the extract was cleaned up by dispersive solid phase extraction, then analyzed by LC-MS-MS in the mode of multiple reactions monitoring with electrospray ionization source. Stable isotope internal standards were used for quantitative analysis. All the correlation coefficients of 18 targets were greater than 0.995. The samples spiked recoveries at a concentration level of 1 μg/kg and 10 μg/kg were from 64.9 to 94.2 % and from 64.7 to 104.7 %, respectively with relative standard differences range from 1.98 through 16.83 % (n = 8). The method detection limits ranged between 0.010 and 0.130 μg/kg. The method is sensitive, simple, low-cost, fast and has been applied to detection of soil samples.

Keywords: Carbamate, Triazol pesticides, QuEChERS, Liquid chromatography-tandem mass.

INTRODUCTION

In the farming process, most of the pesticide has not been exploited by plants directly into the soil, causing the soil, air and water pollutions and creating potential threats on human health and the environment. Carbamate pesticides are a broad-spectrum insecticide and widely used in farming with a high efficiency and short residual period. The triazole pesticides used has also increasing due to effect on crops pathogens and plant growth regulatory function. However, there are evidences that carbamates, triazole pesticides could have a potential health risk of toxicity on human endocrine, Meanwhile the vapour pressure of the carbamates and triazole are low, they will evaporate slowly at normal temperature, which may lead to easy to these residence in soil[1-3]. Therefore, it is necessary to be monitored for carbamate and triazole pesticides in soil.

The gas chromatography (GC) coupled to selective detection system, such as electron-capture detection, nitrogen-phosphorous detection or mass spectrometric detection or liquid chromatography (HPLC) are mainly analytical techniques for pesticides residues analysis[4-9]. GC and GC-MS are not suitable for determination of carbamate and triazole pesticides due to their polar, thermolabile and low vapour pressure, meanwhile HPLC is not also ideal tool for carbamate and triazole pesticide residues analysis because of analytical targets need to be derivatized with low sensitivity and accuracy. In recent years, the liquid chromatography-tandem mass spectrometry (LC-MS-MS) continues to gain popularity for pesticide analysis with most applications focused on non-GC amenable compounds, thermolabile, polar and non-volatile pesticides[10-12]. So it is obvious advantages for carbamate and triazole pesticide residues analysis using LC-MS-MS that targets do not need to be derivatizaed with high sensitivity and accuracy and matrix interference resistance especially in the mode of multiple reaction monitoring (MRM).

However, the residue detection of carbamate and triazole pesticide mainly focus on those of in vegetables, fruits, grains, edible fungus and water at present[4,5,9,10,12]. The report of analysis of carbamate or triazole pesticide in soil is very few and of the simultaneous detection of two kinds of pesticides residue is less. This paper described that simultaneous determination of 18 kinds of carbamate and triazole pesticide along with their degradation products in soil samples by LC-MS-MS in multiple monitoring mode with modified QuEChERS method combined with matrix solid-phase dispersion to rapid extraction and cleanup, in addition to using isotope internal standard to improve quantitation accuracy and realize simple, fast and effective measure for the targets in soil.

EXPERIMENTAL

Agilent 1200 Series HPLC, USA. API 4000 triple quadrupole mass spectrometer with electrospray ion source, AB SCIEX Co. Ltd, USA. 40 mL amber glass bottle with a Teflon-lined film screw cap. The Carbamate dedicated liquid column (4.6 × 150 mm; 5 μm), C_{18} liquid X-bridge (2.1 mm × 3.5 μm; 150 mm) and the phenyl liquid column X-bridge (2.1 mm × 3.5 μm; 150 mm) were purchased from the Waters Co. Ltd, USA.

Monomer stock standard solutions of 18 carbamates, triazole pesticides and their degradation products for certified reference materials at a concentration of 100 mg L^{-1}, purchased from the Chemical Metrology & Analytical Science Division, National Institute of Metrology. The carbaryl-D_3, aldicarb-D_3 and tebuconazole-D_6 at a concentration of 100 mg/L used for the isotope internal standards (Germany, Dr. Ehrenstorfer company). Fluconazole-D_4 (Germany, Dr. Ehrenstorfer) and 4-bromo-3,5-xylyl-N-methyl carbamate (BDMC, J & K Chemical Co., Ltd.) at a concentration of 100 mg/L used for surrogate standards. The all of standards should protect from light and stored at -18 °C. Formic acid and ammonium formate (excellent grade, Fluka Company), methanol and acetonitrile (HPLC grade, J & K Chemical Co., Ltd.), acetone and methylene chloride (pesticide residues grade, J & K Chemical Co., Ltd.). The soil samples were collected from grass, watermelon field, wheat field and peach orchard soil near to suburban areas of Beijing respectively and blank soil sample come from no pesticide pollutant soil.

Sample extraction: Weigh 5 g of soil sample into 40 mL amber glass bottle, add 10 μL fluconazole-D_4 and BDMC (1 mg/L) mix surrogate standard, 20 mL acetone-methylene chloride (3:1, V: V) mixture extract solvent. The sample bottle was shook for 60 mins at 230 times/min in oscillator prior to centrifugation for 20 min at 3000 rpm, then 8 mL of the supernatant was transferred to 15 mL polypropylene centrifuge tube with 150 mg PSA, 200 mg GCB and 500 mg of anhydrous magnesium sulfate for cleanup.

Vortex polypropylene centrifuge tubes for 1 min, centrifuged 20 min at 3000 rpm. 4 mL of the supernatant was evaporated to dryness using a gentle stream of nitrogen, restored volume to 1 mL with methanol-water (1:1, V: V), spiked 10 μL of Carbaryl-D_3, aldicarb-D_3, tebuconazole-D_6 mix internal standard at the concentration of 1.0 mg L^{-1}, then determined by LC-MS-MS after filtrating over 0.45 μm PTFE membrane.

Analytical conditions: Waters carbamate dedicated analytical column (4.6 mm × 150 mm; 5 μm), column temperature was set 30 °C. Mobile phase is constituted of A phase water and B phase methanol and its flow rate is of 0.3 mL/min, injection sample volume 40 μL. The gradient elution program: 0-2 min A and B was 50 %, 2-6 min A drop from 50 to 10 %, B to 90 %, 6 to 20 min each phase remains unchanged, 20-23 min A rise of 50 %, B reduced to 50 %, 23 to 30 min two phase remains unchanged.

Electrospray ion source temperature 400 °C, positive ion ionization mode, electrospray ionization voltage IS 5000V, ion source collision gas CAD 68.9 kPa, curtain gas (CUR) 68.9 kPa, atomizing gas GS 137.9 kPa, multiple reaction monitoring mode (MRM).The monitoring ion pairs and mass spectrum parameters are shown in Table-1.

RESULTS AND DISCUSSION

Optimization of extraction conditions for soil sample: QuEChERS (quick, easy, cheap, effective, rugged and safe) is a new sample preparation method for determination of pesticides in fruits and vegetables and published recently as AOAC Method 2007.01, but rarely used in soil samples[11-17]. Its basic analytical procedure is that extracted homogenized samples with acetonitrile buffer solution, centrifuged and cleaned up sample extract by dispersive solid phase extraction and then the supernatant directly injected into LC-MS or GC-MS determination. This paper established a soil rapid extractive method base on QuEChERS, which the carbamates and their degradation products along with triazole pesticides in soil were extracted using shaking extract with organic solvent, the extract was cleaned up by matrix solid-phase dispersion, the supernatant after centrifugation were concentrated and then LC-MS-MS detection., Respectively investigates single and mixed extractive solvent effect on the soil spiked recoveries such as the acetonitrile, methanol, acetonitrile-methanol (2:1, v/v), acetone, acetone-methylene chloride (3:1, v:v). The results optimized are given in Fig. 1. Fig. 1 shows that acetone extraction spiked recoveries of all the targets are generally low, methanol and acetonitrile-methanol mixed solvent of spiked recoveries are also low and acetonitrile spiked recoveries relatively large changes from 48.1 to 125.9 % with the lowest of 48.1 % for asaldicarb sulfone and exceeding 120 % for three hydroxyl carbofuran, aldicarb sulfone, propiconazole and tebuconazole Hexaconazole, but acetone-methylene chloride mixed solvent spiked recoveries were in range of 65.2 to 113.9 % and meet the analytical requirement. Therefore the acetone-methylene chloride (3:1, v:v) was chosen as extractive solvent for extraction of the carbamates and triazole pesticides in soil. The matrix solid-phase dispersion with 150 mg PSA, 200 mg GCB and 500 mg of anhydrous magnesium sulfate for cleanup sample is also simple and fast clean-up approaches for elimination or reduction of interferences of soil matrix. The soil QuEChERS method established overcomes thermal degradation of carbamate, triazole pesticidethe, low recoveries caused using traditional soil sample thermal extraction and realized rapid, easy, low-cost and effective preparation for soil samples.

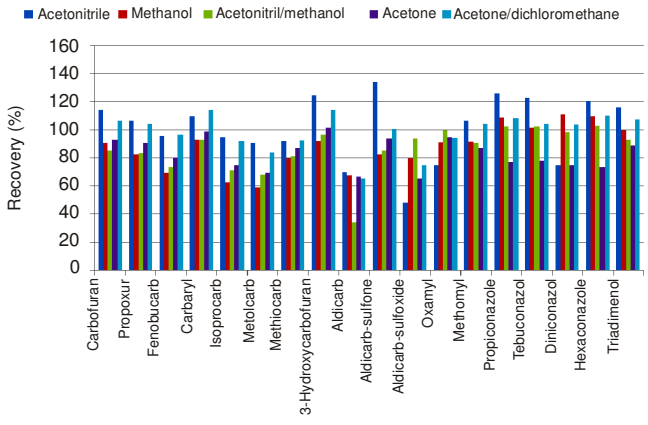

Fig. 1. Effect of the solvent of soil QuEChERS extraction on spiked recoveries of target compounds

TABLE-1
MS PARAMETERS OF TARGET COMPOUNDS

Targets	Molecular weight	Parent ion (*m/z*)	Daughter ion (*m/z*)	DP (V)	EP (V)	CE (V)	CXP (V)
Carbofuran	221.1	222.1	123.2[a]	60	10	30	15
		$[M + H]^+$	165.1	60	10	18	15
Fenobucarb	209.2	210.2	111.0[a]	52	6	20	12
		$[M + H]^+$	168.1	52	6	11	12
Isoprocarb	207.3	208.3	95.1[a]	60	6	20	8
		$[M + H]^+$	152.1	60	6	12	12
Mercaptodimethur	201.1	202.2	145.1[a]	54	6	15	14
		$[M + H]^+$	127.1	54	6	40	11
Aldicarb	193.1	194.0	95.1[a]	59	7	35	18
		$[M + H]^+$	137.1	59	7	13	25
Aldicarb-sulfoxide	165.1	166.1	109.1[a]	55	6	15	21
		$[M + H]^+$	94.1	55	6	42	18
Methomyl	225.3	226.2	169.1[a]	60	7	14	12
		$[M + H]^+$	121.1	60	7	27	12
Tebuconazole	237.2	238.2	220.1[a]	66	6	9	20
		$[M + H]^+$	181.1	66	6	16	15
Hexaconazole	190.1	213.0	116.1[a]	50	10	16	10
		$[M + Na]^+$	89.0	50	10	23	8
Carbofuran	222.2	223.2	148.1[a]	65	6	13	13.5
		$[M + H]^+$	86.0	65	6	22	17
Fenobucarb	206.2	229.2	166.1[a]	50	10	16	12
		$[M + Na]^+$	132.0	54	6	10	12
Isoprocarb	219.2	242.1	72.1[a]	55	10	32	7
		$[M + Na]^+$	121.1	55	10	19	10
Mercaptodimethur	162.2	163.1	88.0[a]	45	7	14	9
		$[M + H]^+$	106.0	45	7	14	10
Aldicarb	341.1	342.1	159.1[a]	80	6	40	15
		$[M + H]^+$	69.1	80	6	36	13.5
Aldicarb-sulfoxide	307.1	308.2	70.1[a]	80	6	50	13.5
		$[M + H]^+$	125.1	80	6	50	12.5
Methomyl	325.1	326.1	70.1[a]	90	7	56	14
		$[M + H]^+$	159.1	90	7	45	16
Tebuconazole	313.2	314.2	70.1[a]	80	6	45	12
		$[M + H]^+$	159.0	80	6	45	12
Hexaconazole	295.1	296.1	70.2[a]	38	6	45	12
		$[M + H]^+$	279.1	27	6	7	20
Carbaryl-D3	204.1	205.2	145.1[a]	54	6	14	14
		$[M + H]^+$	127.1	54	6	37	13
Aldicarb-D3	193.1	216.1	89.0[a]	55	8	23	17
		$[M + H]^+$	116.1	55	8	17	23
Tebuconazole-D6	313.1	314.2	72.1[a]	80	6	50	14
		$[M + H]^+$	125.1	80	6	50	14
Fluconazole-D4	310.2	311.2	242.2[a]	72	6	23	16
		$[M + H]^+$	223.1	72	6	26	22
BDMC	257.0	258.1	201.1[a]	65	6	15	15
		$[M + H]^+$	122.2	65	6	30	12

a. Expressed as quantitative fragmention

Column selection: Chromatographic column is one of the important parameters that influence on separation and retention of targets. This paper examines properties of separation and retention of the target utilizing X-bridge C_{18} column (Waters, 2.1 mm × 3.5 µm; 150 mm), X-bridge phenyl column (Waters, 2.1 mm × 3.5 µm; 150 mm) and carbamate dedicated analytical column (Waters, 3.9 mm × 5 µm; 150 mm). C_{18} column is widely used as reversed phase chromatographic column, Phenyl column be more helpful for the retention of polar compounds with weak polarity, Carbamate analytical column is specific object for separation and analysis of carbamate pesticides. The separation and retention of the targets on the three kinds of analytical columns are shown as in Fig. 2a, 2b and 2c, respectively. Most of the target objects has strong hold on C_{18} column (Fig 2a), their chromatographically peaks were sharp, symmetrical, but retentions of aldicarb-sulfone, aldicarb-sulfoxide and oxamyl were more weaker and their chromatographic peak broadening, their mass response intensity is very poor. Fig. 2b shows that capacity of retention and separation of the phenyl column for targets are very similar to C_{18} column, the peak width and analytical sensitive of aldicarb-sulfone, aldicarb-sulfoxide and oxamyl do not be improved. Fig. 3c indicates separation capable of carbamate special chromatographic column for the targets with high mass responds was best in all the column, especially that aldicarb-sulfone, aldicarb-sulfoxide and oxamyl's peaks has more sharp, symmetry and

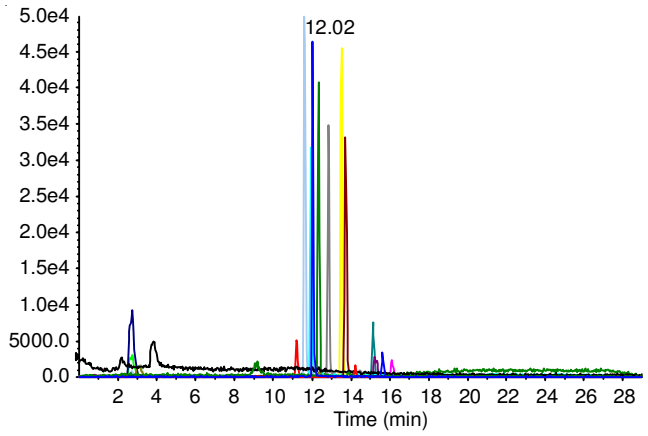

Fig. 2a. Retention of 18 carbamate and triazol pesticides on Waters X-bridge C_{18} columns. (2.1 mm × 3.5 µm;150 mm)

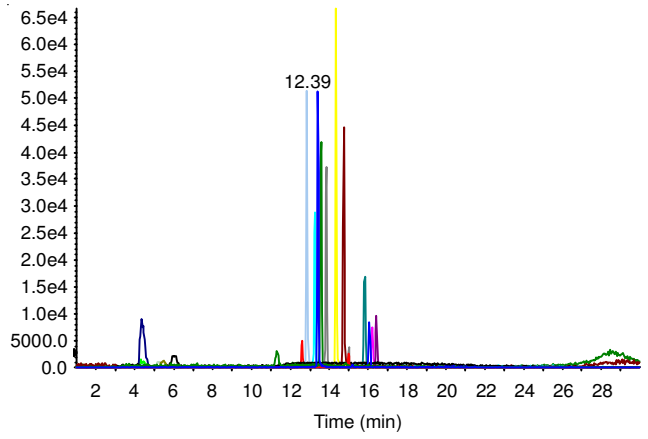

Fig. 2b. Retention of 18 carbamate and triazol pesticides on. Waters X-bridge phenyl columns. (2.1 mm × 3.5 µm; 150 mm)

and the analytical sensitivity were improved significantly. So the carbamate dedicated column was chosen as the analytical column of targets.

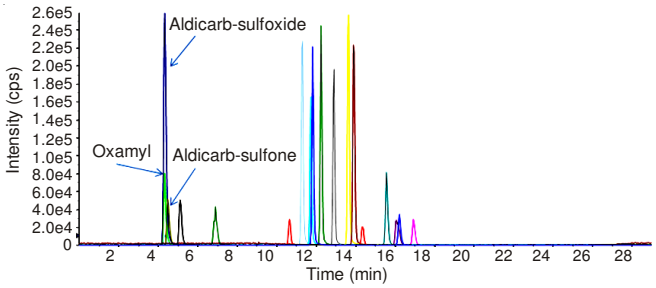

Fig. 2c. Retention of 18 carbamate and triazol pesticides. On carbamate special analytical column (3.9 mm × 5 µm; 150 mm)

MS parameters optimization: In multiple monitoring mode the mass parameters were optimized respectively and the optimal results were listed below Table-1. The optimal experimental was conducted on the liquid chromatography with flow injection (FIA) sampling. The ion source parameters of electrospray ionization voltage, ion source temperature (TEM), curtain gas (CUR), atomization gas (GS), heated auxiliary gas are also optimized. Fig 3 is chart of targets ionization efficiency at different ion source temperature.

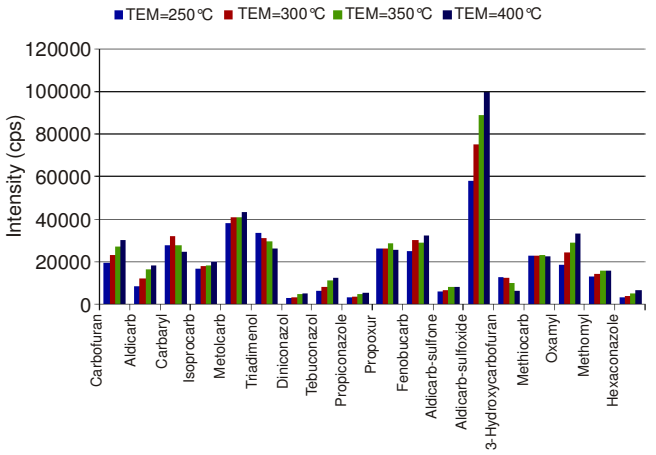

Fig. 3. Effect of ion source temperature on the ionization efficiency of target compounds

TABLE-2
LINEAR RANGES, REGRESSION EQUATION AND CORRELATION COEFFICIENTS (R^2) OF TARGET COMPOUNDS

Target	Linear range (µg/L)	Linear equation	Correlation coefficient (R^2)	Method Detection Limits (µg/kg)
Carbofuran	0.1-100	y = 0.9927x+0.0135	0.9999	0.010
Propoxur	0.1-100	y = 0.7924x-0.0095	0.9991	0.020
Fenobucarb	0.1-100	y = 1.4403x-0.0659	0.9980	0.025
Carbaryl	0.1-100	y = 1.0581x-0.0001	0.9986	0.030
Isoprocarb	0.1-100	y = 0.9545x-0.0239	0.9996	0.020
Metolcarb	0.1-100	y = 1.3277x-0.0528	0.9989	0.060
Mercaptodimethur	0.1-20	y = 0.8937x+0.0317	0.9967	0.025
3-Hydroxycarbofuran	0.5-100	y = 0.2795x-0.0284	0.9958	0.120
Aldicarb	0.1-100	y = 0.7502x-0.0321	0.9997	0.025
Aldicarb-sulfone	0.1-10	y = 0.2450x+0.0056	0.9995	0.010
Aldicarb-sulfoxide	0.1-10	y = 4.9287x+0.0418	0.9997	0.030
Oxamyl	0.2-20	y = 1.1285x-0.0068	0.9987	0.075
Methomyl	0.2-10	y = 0.4174x+0.0133	0.9991	0.130
Propiconazole	0.1-100	y = 0.8749x-0.1068	0.9956	0.025
Tebuconazole	0.1-100	y = 1.2944x-0.0881	0.9981	0.015
Diniconazole	0.1-100	y = 0.4856x-0.0701	0.9954	0.040
Hexaconazole	0.1-100	y = 0.5812x-0.0481	0.9967	0.035
Triadimenol	0.1-100	y = 0.2806x-0.0021	0.9961	0.015

x. The target concentration/internal standard concentration, y. The target peak area/internal standard peak area

TABLE-3
SPIKED RECOVERIES IN SOIL AND METHOD DETECTION LIMITS OF TARGET COMPOUNDS

Target	Spiked level 1 (1 μg/kg)		Spiked level 2 (10 μg/kg)		
	Detected value (μg/kg)	Recovery (R/%)	Detected value (μg/kg)	Recovery (R/%)	RSD (%, n = 8)
Carbofuran	0.88	87.8	10.47	104.7	5.42
Propoxur	0.80	79.8	9.29	92.9	8.71
Fenobucarb	0.67	67.4	8.97	89.7	10.98
Carbaryl	0.71	70.7	9.23	92.3	8.11
Isoprocarb	0.75	74.5	8.69	86.9	9.69
Metolcarb	0.68	68.1	7.82	78.2	8.17
Mercaptodimethur	0.76	76.4	9.88	98.8	10.10
3-Hydroxycarbofuran	0.87	86.9	10.33	103.3	6.32
Aldicarb	0.72	71.5	8.74	87.4	3.71
Aldicarb-sulfone	0.93	93.1	9.97	99.7	2.27
Aldicarb-sulfoxide	0.66	65.9	6.47	64.7	4.22
Oxamyl	0.94	94.2	9.07	90.7	5.29
Methomyl	0.92	91.9	9.48	94.8	1.98
Propiconazole	0.69	68.7	9.28	92.8	9.28
Tebuconazole	0.68	68.2	8.36	83.6	7.88
Diniconazole	0.72	71.7	8.87	88.7	12.88
Hexaconazole	0.65	64.9	8.85	88.5	14.84
Triadimenol	0.75	75.4	10.58	105.8	16.83

Method validation: Prepared five concentration levels targets standards with internal standards and surrogates standards at a concentration of 10 μg L^{-1}, then deterrmined by LC-MS-MS under the optimized analysis conditions. The linear range, the linear correlation coefficient and regression equations obtained are shown in Table-2. The results show that in addition to aldicarb-sulfone, aldicarb-sulfoxide and methomyl linear range of 0.1-10 μg L^{-1}, methiocarb, oxamyl linear range of 0.1-20 μg L^{-1}, the other targets the linear range were between 0.1-100 μg L^{-1}, the linear correlation coefficient R^2 of all targets were greater than 0.995. The targets spiked recoveries and precisions are listed in Table-3. The results showed that recoveries matrix spiked at 1 μg kg^{-1} and 10 μg kg^{-1} ranged from 64.9 to 94.2 % and 64.7 to 104.7 %, respectively and relative standard deviations (RSDs) ranged between 1.98 to 16.43 % (n = 8) with method detection limits ranged from 0.010 μg kg^{-1} to 0.130 μg kg^{-1}.

Determination of real soil samples: The optimized procedure was applied to analyze carbamate and their degradation products and triazole pesticide in soil that collected from grass, watermelon field, wheat field and peach orchard soil near to suburban areas of Beijing. The propoxur and triadimenol were detected in all samples with the concentration of propoxur in range of 0.23-0.42 μg kg^{-1} and triazole alcohols range between 0.01-0.17 μg kg^{-1}. The ablealdicarb were also found in watermelon field and wheat field with at the concentration of 0.09-0.12 μg kg^{-1} and higher diniconazole was detected in grass at the concentration of 5.08 μg kg^{-1}, therefore it is an important to monitor pesticides residues of carbamate and triazole in soil.

Conclusion

The soil QuEChERS method established is simple, economical, fast and suitable for the sample preparation of thermally labile organic pollutants and provides a new way for the large numbers of soil sample analysis.

The analytical method using LC-MS MS in multiple monitor mode may overcomes the defects that carbamate and their degradation products need be derived, low sensitivity, matrix interference and thermal degradation using HPLC or GC for determination of targets in soil. The method detection limits ranged from 0.010 to 0.130 μg kg^{-1} with recoveries matrix spiked ranging from 64.9 to 94.2 % and 64.7 to 104.7 % at 1 μg kg^{-1} and 10 μg kg^{-1}, respectively, meanwhile relative standard deviations (RSDs) ranging between 1.98 to 16.43 % (n = 8). The method established is sensitive, accurate, rapid, effective, rugged and safe.

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