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Determination of Six Neonicotinoid Insecticides Residues in Spinach, Cucumber, Apple and Pomelo by QuEChERS Method and LC–MS/MS

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Abstract A modified QuEChERS and LC–MS/MS method has been developed for the simultaneous determination of residues of six neonicotinoids in various crops, including spinach, cucumber, apple and pomelo. The method showed good linearity ($R^2 \geq 0.9995$) and precision (RSD $\leq$ 14.0%). Average recoveries of the six neonicotinoids ranged between 73.7% and 103.8% at spiking levels 0.005, 0.1 and 1 mg kg^{-1}. The LODs and LOQs were in the ranges of 0.20–0.85 μg kg^{-1} and 0.66–2.84 μg kg^{-1}, respectively. The method was satisfactorily validated for the analysis of 50 agricultural samples. Imidacloprid and imidaclothiz were detected at concentration levels ranging from 7 to 5.3 μg kg^{-1}.

Keywords Neonicotinoid · Insecticide · QuEChERS · LC–MS/MS

Neonicotinoid insecticides are a relatively new class of pesticides with novel mode of action. They act as agonists at the insect nicotinic acetylcholine receptors, which plays an important role in synaptic transmission in the central nervous system (Muccio et al. 2006; Nauen et al. 2001). This group of compounds includes nitenpyram, dinotefuran, thiamethoxam, clothianidin, imidacloprid, acetamiprid, thiacloprid and imidaclothiz. Pomelo and spinach are widely cultivated in Asia. These crops are known difficult matrices for residue analysis due to their complex nutrition components (Costel et al. 2012; Zofia et al. 2010). The development of a suitable analytical procedure to separate and evaluate all target compounds is practically important. The maximum residue limits (MRLs) of some neonicotinoid pesticides in products of plant origin have been established by several government agencies to regulate international trade. The United States EPA has established MRLs for neonicotinoid insecticides residues in different kinds of fruits and vegetables in the range 0.02–6 mg kg^{-1} depending on the different pesticides and matrixes (http://www.mrldatabase.com).

In order to monitor and control the compliance of the tolerance level of neonicotinoids, sensitive, accurate and robust analytical methods are necessary. ELISA and HPLC with diode-array detection (DAD) have been reported for screening purpose (Seccia et al. 2008; Watanabe et al. 2007). A study of imidaclothiz residues in cabbage and soil by HPLC was reported recently (Wu et al. 2010). But for the confirmation of suspected positive samples, mass spectrometry coupled with chromatographic separation was one of the most powerful techniques for the residue analysis. Because of the low volatility of neonicotinoids, LC–MS was applied for the determination of these compounds. Four neonicotinoids were determined and validated by LC–MS in fruits and vegetables (Shashi et al. 2004; Ferrer et al. 2005), in honey (Fidente et al. 2005), and drinking water. Some analytical methods use ultra-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) have been reported for determining neonicotinoids in biological samples in recent years (Liu et al. 2010). Xiao et al. (2011) explored utilization of pressurized solvent extraction (PSE) coupled to LC–MS/MS to determine the neonicotinoids in bovine tissues. In the last few years, QuEChERS method has been used for the extraction of a wide variety of compounds and in different matrices, since this approach allows extraction

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of compounds with different physical–chemical properties in a short period of time. However, little was known about the multi-residue analysis of neonicotinoids containing imidaclothiz by LC–MS/MS. In our study, imidaclothiz was chosen for the purpose of comparing with other 5 common used neonicotinoids in this multi-residue determination method. Due to the complexity of the matrixes, the GCB sorbent was used for the clean-up purpose. This paper presents a modified QuEChERS-LC–MS/MS method which allows the simultaneous determination of six neonicotinoid insecticides. The proposed method is efficient, fast and robust.

Materials and Methods

Certified pesticide standards (≥95.0%) for nitenpyram, thiamethoxam, imidacloprid, imidaclothiz, acetamiprid and thiacloprid were purchased from Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA). Acetonitrile and methanol of HPLC grade were obtained from Fisher Chemicals (USA). Ultra-pure water was obtained from a Milli-Q water purification system (Millipore, USA). Acetone (99.5%), magnesium sulfate (98%) and sodium chloride (99.5%) of analytical grade were purchased from Sinopharm Chemical Reagent (Beijing, China). Primary secondary amine (PSA) and graphitized carbon (GCB) sorbent were purchased from Tianjin Bonna-Agela Technologies Inc. The 0.22 μm nylon syringe filters were used to filter the extracts.

Individual standard neonicotinoids stock solutions of 1,000 mg L^{-1} were prepared in acetonitrile and stored at −20°C in the dark. They were stable over a period of at least 3 months. Standard multicomponent working solutions were prepared by diluting each primary standard solution with the chromatographic mobile phase (methanol/water 25/75, v/v) and were used for spiking samples, preparing matrix-matched calibration standards and studying the linear dynamic range of the LC–MS/MS analysis. Matrix matched calibration standards, which prepared by adding the extract of blank samples, were in the range of 0.005–1 mg L^{-1} for the analyzed compounds. The working solutions were stored in refrigerator at 4°C and in darkness.

Ten (10.0) g previously homogenized samples, spiked with standard multicomponent working solutions, were placed in a 50 mL centrifuge tube and shaken vigorously for 30 s and then vortex (QL-901, Kylin-bell Lab Instruments Co., Ltd, China) for 1 min. After 10 mL acetonitrile was added, the centrifuge tube was capped and agitated on a shaker (HAZ-C, Donglian Electron Technology Co., Ltd., China) for 30 min. After adding 4 g anhydrous magnesium sulfate and 1 g sodium chloride, the sample was mixed vigorously by vortexing for 1 min and centrifuge (TDL-40B, Anke, China) extracted for 5 min at 3,800 r min^{-1}.1 mL of acetonitrile layer was transferred into a 2 mL micro-centrifuge tube containing 3 mg GCB, 50 mg PSA sorbent and 150 mg anhydrous magnesium sulfate. The sample was mixed vigorously by vortexing for 1 min and centrifuge (3K15, Sigma) extracted for 2 min at 6,000 r min^{-1}. Acetonitrile layer was filtered through a 0.22 μm filter membrane and transferred into autosampler vial for LC–MS/MS analysis.

An Agilent 6410 Triple Quadrupole LC/MS (Agilent Technologies, USA), equipped with a degasser and an autosampler, was used for the analysis. Chromatographic separation was achieved on a ZORBAX C_{18} column, 50 mm × 2.1 mm, 1.8 μm (Agilent), with a flow rate of 0.3 mL min^{-1}. The isocratic elution condition employed a mobile phase of methanol and water (v/v = 25:75). The

Table 1 Settings for ion transitions of the selected six neonicotinoids in MRM mode

Compounds	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	Fragmentor (V)	Collision energy (eV)
Nitenpyram	1.27	271	237	100	15
			224	100	15
			126	100	15
Thiamethoxam	1.61	292	211[a]	80	10
			181	80	20
Imidacloprid	2.87	256	209[a]	80	10
			175	80	10
Imidaclothiz	3.51	262	181[a]	80	10
			131	80	10
Acetamiprid	4.75	223	126[a]	80	15
			56	80	15
Thiacloprid	8.26	253	186	120	10
			126[a]	120	15

[a] MS transition used for quantification

inject volume was 5 μL. Nitrogen was used for both nebulizer and collision gas. The drying gas temperature was 350°C with the flow rate 8.0 L min^{-1}. The nebulizing gas pressure was 35 psi. The ions were monitored in positive MRM mode, the optimized settings for each transition are summarized in Table 1. Two or three transitions were measured for the neonicotinoids identification and confirmation among which one was used for quantification. For all transitions, the dwell time was 45 ms.

Results and Discussion

In order to identify and quantify the analytes in real samples at trace levels, the MRM transitions and associated acquisition parameters were optimized for the maximum abundance of fragmented ions under ESI positive mode conditions by infusing standard solutions of the target compounds into the LC–MS/MS. Identification of the parent ion as well as the choice of the ionization mode for each analyte was performed in the full scan mode by recording mass spectra from m/z 50–400. For each analyte the protonated molecular ion $[M + H]^+$ was determined and chosen as precursor ion. Different fragmentor voltages were tested for each precursor ion to find the most suitable settings. Then dissociation was induced and different collision energies were tested in order to find the most abundant product ion. The most sensitive transition in MRM mode was selected for quantification in the screening method. The optimum values for each condition for each compound are summarized in Table 1, and the most intense characteristic MRM transitions were chosen for each analyte. The most important condition to be satisfied for identification of the presence of a target compound is that at least two ion transitions give signals distinguishable from the background ion current when MS/MS detection is performed. Figure 1 shows the typical total ion chromatogram and extracted ion chromatogram of MRM mode for six neonicotinoids at concentration 0.01 mg kg^{-1}.

The method was validated for linearity, detection and quantification limits, selectivity, accuracy and precision. The calibration was performed by use of matrix-matched calibration standards prepared as described in experimental section in order to compensate for the matrix effect including signal suppression or enhancement. The matrix effect of the present method was investigated by comparing standards in solvent with matrix-matched standards. Table 2 summarizes the analytical results including slope, R^2 and matrix effect values calibrated by using slope ratio method in four matrices. Good linearity of the response was found for all pesticides at concentrations within the tested interval, with linear correlation coefficients (R^2) above 0.9995. The matrix effect values of six neonicotinoids in four matrices are between 0.474 and 0.893.The LODs and LOQs were defined as the concentration with a signal-to-noise ratio (S/N) of 3 and 10 using the less intensive ion transition for each analyte. This parameter was determined by analysis of a series of decreasing concentrations of the spiked sample in multiple replicates. The LODs and LOQs values obtained for six neonicotinoids were in the ranges of 0.20–0.85 μg kg^{-1} and 0.66–2.84 μg kg^{-1}, respectively. The accuracy and precision of the method were assessed using fruit and vegetable samples fortified with three different levels (0.005, 0.1, and 1 mg kg^{-1}). The recovery, repeatability and reproducibility were determined by the relative standard deviation (RSD). The repeatability RSD_r (intra-day precision) was measured by comparing standard deviation of the recovery percentages spiked samples run the same day. The reproducibility RSD_R (inter-day precision) was determined by analyzing spiked samples for three alternate days. Good corrected recoveries were obtained for each of the six neonicotinoids at all fortification levels as shown in Table 3. The average recoveries ranged between 73.7% and 103.8% with intra-day RSD_r values between 1.7% and 9.9%. Inter-day repeatability was found satisfactory for the six compounds under survey ($RSD_R \leq 14.0\%$).

Fig. 1 LC–MS/MS total ion chromatogram and extracted ion chromatogram (MRM mode) of the six neonicotinoids, 0.01 mg kg^{-1} ▶

The effective of this method in measuring trace levels of neonicotinoids was checked by analyzing 50 commercially available agricultural samples (spinach, cucumber, apple and pomelo). These samples produced by different producing areas were purchased in local supermarkets. Imidacloprid and imidaclothiz were detected at concentration levels ranging from 0.007 to 0.053 mg kg^{-1}. However, none of the other target compounds left residues at detectable levels.

In conclusion, a simple, rapid and sensitive QuEChERS-LC–MS/MS method has been successfully applied to the determination of six neonicotinoid insecticides residues (nitenpyram, thiamethoxam, imidacloprid, imidaclothiz, acetamiprid and thiacloprid) in spinach, cucumber, apple and pomelo. The method developed shows satisfactory validation parameters in terms of linearity, low limits, accuracy and precision. The average recovery in all matrixes for each neonicotinoids ranged between 73.7% and 103.8%. The uncertainty associated to the analytical method, expressed as RSD, was lower than 14% for all compounds tested in all matrixes. The calculated LOQs (0.66–2.84 μg kg^{-1}) were much lower than the established MRLs. The proposed work is a reference for establishing a national standard for routine controls in China.

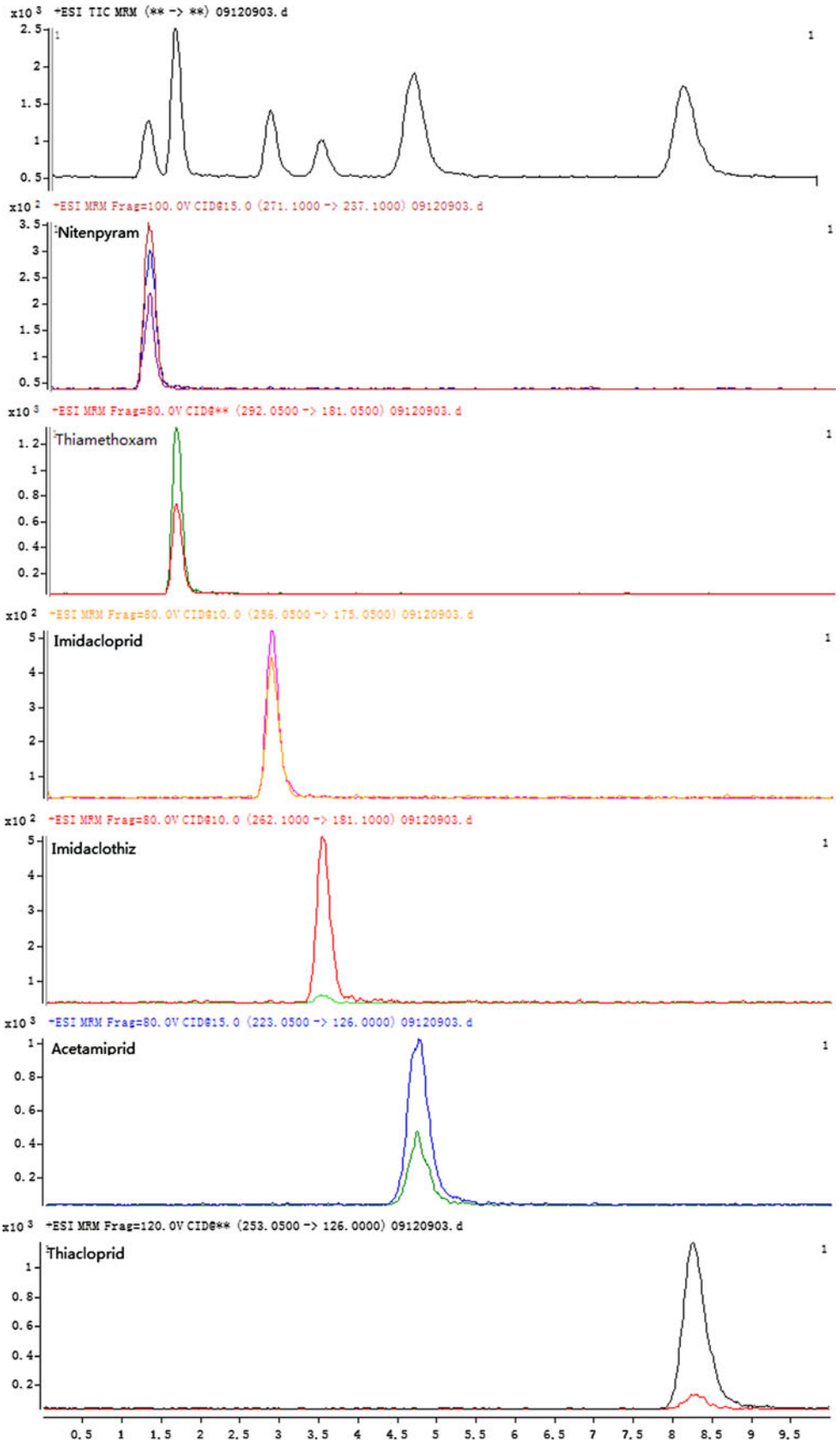
x10 3 +ESI TIC MRM (** -> **) 09120903.d
x10 2 +ESI MRM Frag=100.0V CID@15.0 (271.1000 -> 237.1000) 09120903.d
Nitenpyram
x10 3 +ESI MRM Frag=80.0V CID@** (292.0500 -> 181.0500) 09120903.d
Thiamethoxam
x10 2 +ESI MRM Frag=80.0V CID@10.0 (256.0500 -> 175.0500) 09120903.d
Imidacloprid
x10 2 +ESI MRM Frag=80.0V CID@10.0 (262.1000 -> 181.1000) 09120903.d
Imidaclothiz
x10 3 +ESI MRM Frag=80.0V CID@15.0 (223.0500 -> 126.0000) 09120903.d
Acetamiprid
x10 3 +ESI MRM Frag=120.0V CID@** (253.0500 -> 126.0000) 09120903.d
Thiacloprid
0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8 8.5 9 9.5

Table 2 Analytical data for the six neonicotinoids using the proposed method

Compounds	Matrix	R^2	Matrix effect[a]	LOD (μg kg^{-1})	LOQ (μg kg^{-1})
Nitenpyram	Spinach	0.9997	0.631	0.35	1.18
	Cucumber	0.9999	0.633	0.39	1.29
	Apple	0.9999	0.636	0.36	1.20
	Pomelo	1.0000	0.693	0.38	1.27
Thiamethoxam	Spinach	0.9999	0.474	0.35	1.16
	Cucumber	1.0000	0.485	0.33	1.09
	Apple	0.9996	0.477	0.32	1.06
	Pomelo	1.0000	0.491	0.36	1.19
Imidacloprid	Spinach	0.9997	0.862	0.37	1.23
	Cucumber	0.9998	0.877	0.35	1.16
	Apple	0.9999	0.869	0.35	1.18
	Pomelo	1.0000	0.881	0.81	2.69
Imidaclothiz	Spinach	0.9997	0.945	0.85	2.84
	Cucumber	0.9995	0.952	0.82	2.73
	Apple	0.9997	0.959	0.84	2.79
	Pomelo	1.0000	0.969	0.74	2.48
Acetamiprid	Spinach	0.9997	0.980	0.37	1.23
	Cucumber	0.9999	0.980	0.36	1.19
	Apple	0.9999	0.983	0.39	1.31
	Pomelo	1.0000	0.098	0.36	1.20
Thiacloprid	Spinach	6.0000	0.974	0.29	0.97
	Cucumber	0.9997	0.980	0.20	0.66
	Apple	0.9998	0.981	0.25	0.82
	Pomelo	1.0000	0.983	0.29	0.95

[a] slope matrix/slope solvent

Table 3 Recoveries (%), repeatability (RSD_r, %) and reproducibility (RSD_R, %) values of the neonicotinoids at spiking levels 0.005, 0.1 and 1 mg kg^{-1} (n = 5)

Compounds (mg kg^{-1})	Spike level	Spinach (%)			Cucumber (%)			Apple (%)			Pomelo (%)		
		Mean%	RSD_r%	RSD_R%	Mean%	RSD_r%	RSD_R%	Mean%	RSD_r%	RSD_R%	Mean%	RSD_r%	RSD_R%
Nitenpyram	0.005	73.7	8.9	13.9	77.7	6.4	10.7	90.7	8.0	6.5	80.0	8.2	8.3
	0.1	82.1	7.1	5.7	83.4	4.4	5.1	84.3	5.1	4.3	91.9	5.9	5.3
	1	81.3	1.7	2.3	86.6	3.7	2.5	95.9	6.8	2.2	90.2	5.5	1.3
Thiamethoxam	0.005	88.9	8.6	9.3	99.8	5.7	7.4	89.2	9.8	11.8	85.2	7.5	8.9
	0.1	81.1	5.2	6.7	90.6	7.7	4.8	90.9	8.7	5.9	98.7	6.7	1.1
	1	77.4	6.2	1.5	101.5	2.3	0.9	99.6	5.4	1.3	95.9	6.6	1.1
Imidacloprid	0.005	82.3	6.3	11.5	93.2	4.2	6.5	88.7	5.4	10.2	93.8	5.4	10.5
	0.1	86.2	2.5	9.4	96.9	8.0	6.5	90.8	3.0	5.9	94.0	5.5	0.9
	1	87.4	4.6	2.4	103.8	2.5	2.0	101.4	5.0	1.1	97.1	3.8	2.7
Imidaclothiz	0.005	90.4	9.9	10.3	85.7	7.1	10.7	86.4	5.4	14.0	90.7	5.5	11.6
	0.1	86.7	5.6	3.8	98.1	4.3	5.8	87.8	5.9	6.5	89.9	6.6	4.1
	1	91.6	6.9	1.9	95.4	4.5	2.0	99.4	3.0	1.7	96.2	3.6	1.8
Acetamiprid	0.005	95.6	1.8	13.9	94.3	3.5	10.7	89.2	3.4	10.2	85.8	2.8	7.8
	0.1	96.0	4.6	7.4	93.7	3.5	5.3	89.5	2.8	4.7	88.7	6.6	2.4
	1	92.6	3.4	1.4	98.2	3.1	1.0	95.7	8.2	0.8	89.6	2.2	1.8
Thiacloprid	0.005	75.5	6.6	12.8	83.3	9.7	12.5	82.4	6.8	12.5	88.3	9.6	9.0
	0.1	90.3	7.7	5.7	81.0	9.0	6.4	85.8	7.4	5.5	92.5	9.7	0.8
	1	86.4	7.8	1.8	92.0	7.1	0.8	94.2	7.5	1.7	81.6	3.7	2.3

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