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Determination of neonicotinoid pesticides residues in agricultural samples by solid-phase extraction combined with liquid chromatography–tandem mass spectrometry

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ABSTRACT

This work reports a new sensitive multi-residue liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for detection, confirmation and quantification of six neonicotinoid pesticides (dinotefuran, thiamethoxam, clothiandin, imidacloprid, acetamiprid and thiacloprid) in agricultural samples (chestnut, shallot, ginger and tea). Activated carbon and HLB solid-phase extraction cartridges were used for cleaning up the extracts. Analysis is performed by LC–MS/MS operated in the multiple reaction monitoring (MRM) mode, acquiring two specific precursor-product ion transitions per target compound. Quantification was carried by the internal standard method with D_4-labeled imidacloprid. The method showed excellent linearity ($R^2 \geq 0.9991$) and precision (relative standard deviation, RSD $\leq 8.6\%$) for all compounds. Limits of quantification (LOQs) were 0.01 mg kg^{-1} for chestnut, shallot, ginger sample and 0.02 mg kg^{-1} for tea sample. The average recoveries, measured at three concentrations levels (0.01 mg kg^{-1}, 0.02 mg kg^{-1} and 0.1 mg kg^{-1} for chestnut, shallot, ginger sample, 0.02 mg kg^{-1}, 0.04 mg kg^{-1} and 0.2 mg kg^{-1} for tea sample), were in the range 82.1–108.5%. The method was satisfactorily validated for the analysis of 150 agricultural samples (chestnut, shallot, ginger and tea). Imidacloprid and acetamiprid were detected at concentration levels ranging from 0.05 to 3.6 mg kg^{-1}.

1. Introduction

In recent years, the established regulations regarding the maximum residue levels (MRLs) in commodities have become more and more stringent. The European Union (EU) has set new Directives for pesticides at low levels in vegetables in order to meet these health concerns [1,2]. Neonicotinoid pesticide is a relatively new group of active ingredients with novel modes of action [3]. For their distribution on large areas of agricultural land they could give rise to serious risks for the health and safety of the consumer. In order to address this issue, the European Union (EU) has set maximum residue limits (MRLs) for pesticide residues in tea in the range 0.05–0.1 mg kg^{-1} depending on the imidacloprid, acetamiprid pesticides [3]. Therefore, monitoring of pesticide residues is crucial for proper assessment of human exposure to pesticides through foods.

Although some papers have been found which deal the determination of nicotinoid residues in vegetables, honey, drinking water and bovine milk [4–14], few method [15] has been published for simultaneous determination of six residues of neonicotinoid insecticides in agricultural samples. Among the different pre-treatment approaches, SPE (solid-phase extraction) offers a good compromise between robustness, rapidity, convenience, clean-up efficiency, scope for automation and solvent consumption and is, therefore, ideally suited to routine analysis. However, no simple and fast SPE methods have been reported for simultaneous determination of six residues of neonicotinoid pesticide in agricultural samples by LC–MS or LC–MS/MS. To the best of our knowledge this is the first time that two clean-up extraction steps have been applied to determine six neonicotinoid pesticides simultaneously in agricultural samples (chestnut, shallot, ginger and tea).

Chestnut, shallot, ginger and tea are important agricultural products in China, the commercial cultivation of which receives frequent application of a large number of pesticides throughout the cropping season to control a variety of pests and diseases. To ensure the safety of agricultural products for consumers and to regulate international trade, maximum residue limits for insecticides have been set by the Government agencies and the European Union [16–18].

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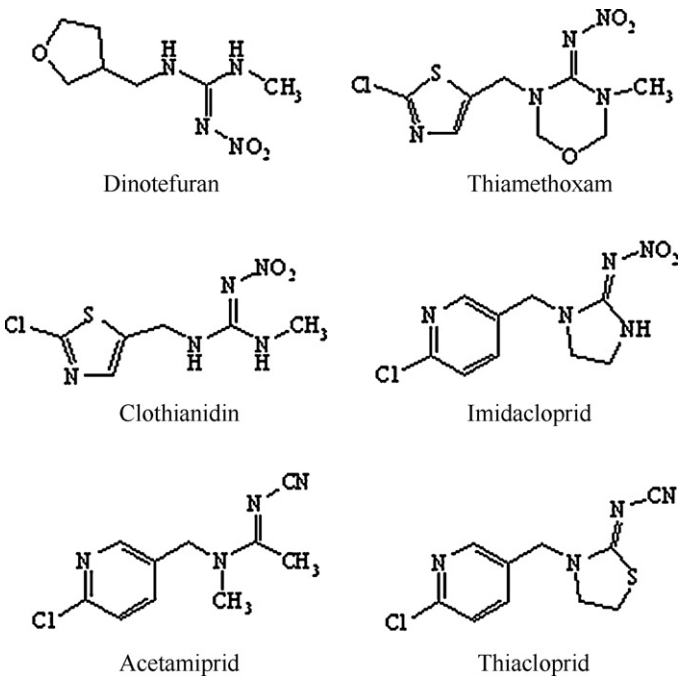

Fig. 1. Chemical structures of the pesticides investigated.

The objective of this work was to develop a rapid, sensitive and accurate LC–MS/MS method for determining six nicotinoid insecticides, dinotefuran, thiamethoxam, clothiandin, imidacloprid, acetamiprid and thiacloprid, in agricultural samples, following a single extraction with the activated carbon and Oasis HLB SPE cartridges. The low MRLs (maximum residue limits) have fostered the development of more powerful sensitive analytical methods to meet the requirements in complex samples, such as food. In this sense, liquid chromatography–tandem mass spectrometry (LC–MS/MS) with triple quadrupole in multiple reaction monitoring (MRM) mode has become so far, the most widely used technique for the quantitation of (polar) pesticides in food as reported extensively in the literature [19–31]. Matrix effects arising during the LC–MS/MS analysis of agricultural samples were evaluated by comparing solvent and matrix-matched calibration curves at different agricultural samples matrix concentrations. The average recoveries, measured at three concentration levels (0.01 mg kg^{-1}, 0.02 mg kg^{-1} and 0.1 mg kg^{-1} for chestnut, shallot, ginger, 0.02 mg kg^{-1}, 0.04 mg kg^{-1} and 0.2 mg kg^{-1} for tea sample), were in the range 82.1–108.5% for the six compounds tested with relative standard deviations below 7.9%.

2. Experimental

2.1. Chemicals and reagents

Certified standards of pesticides and D_4-labeled imidacloprid (purity > 98%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Common names and structures of the six neonicotinoids evaluated here are shown in Fig. 1.

HPLC grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Formic acid (purity 98%, *w/w*) was from Sigma–Aldrich (Steinheim, Germany). The water used was purified with a Milli-Q water purification system from Millipore (Bedford, MA, USA). Cartridges used for solid-phase extraction were Oasis HLB (500 mg per 6 mL; Waters, Milford, MA, USA) and activated carbon (500 mg per 6 mL; Supecol, USA).

Individual standard pesticide stock solutions (600 mg L^{-1}) were prepared in methanol and stored at −20 °C in the dark. They were stable over a period of at least three months. Standard multi component solution (10 μg mL^{-1}) was prepared by diluting each primary standard solution with the chromatographic mobile phase (acetonitrile/water (0.1% formic acid) 30:70, v/v) and was used for spiking agricultural samples, for preparing matrix-matched calibration standards in agricultural samples blank and for studying the linear dynamic range of the LC–MS/MS analysis. Matrix matched calibration standards were prepared by adding to extract agricultural blank samples appropriate volumes of standard working solution were linear over the range 4–100 μg L^{-1} for the analyzed compounds. The D_4-labeled imidacloprid internal standard was used to ensure accuracy of the MS response and was added to each standard to give a final concentration of 40 μg L^{-1}. The standard solutions were stored under refrigerator conditions (4 °C) and protected from light; under these conditions the standard solutions are stable for at least 1 month.

2.2. Sample extraction

The agricultural samples (chestnut, shallot, ginger and tea all obtained from market) were freeze-dried for approximately 24 h (Labconco, USA) to a constant mass and ground to a homogenous powder using a ZM 100 ultra centrifugal mill (ring sieve 0.5 mm) (Retsch, Haan, Germany) under liquid nitrogen protection. The milled powder was stored at 4 °C prior to use. Accurately weighed respectively 1.0 g of homogenized chestnut, shallot and ginger sample and 0.5 g of homogenized tea sample was taken into a 50-mL polypropylene centrifuge tube and 1 mL water was added and soaked for about 30 min. Then 0.4 mL D_4-labeled imidacloprid (100 ng mL^{-1}) and 10 mL acetonitrile were placed in centrifuge tube and the tube was capped. The sample was shaken vigorously for 30 s then vortex mixed for 1 min. This extraction process was repeated three times with 10 mL acetonitrile and the solvent extracts were then combined. The organic layer was then separated, dried under anhydrous sodium sulfate for 30 min, and then concentrated to about 4 mL under vacuum at 50 °C.

2.3. Solid-phase extraction

Solid-phase extraction was performed with the activated carbon and Oasis HLB SPE cartridges. Before use the activated carbon cartridge, the cartridge was conditioned with 5 mL of acetonitrile. The samples were percolated through the cartridges and left to flow through under the action of gravity. The cartridges were eluted with 5 mL of acetonitrile and the eluate was evaporated to dryness on a water bath at 50 °C under a flow of nitrogen by use of an N-Evap evaporator (Organomation, Berlin, MA, USA). The dried extract was reconstituted in 10 mL water, vortex mixed for 60 s.

Before use the Oasis HLB SPE cartridge, the cartridge was conditioned with 5 mL of methanol and 5 mL of ultra-pure water. The reconstituted samples were percolated through the cartridges and left to flow through under the action of gravity. The cartridge was vacuum-dried for 3 min, then the retained pesticides were eluted with 2 × 5 mL of methanol and the eluate was collected in a test tube. The eluent was reduced to dryness by vacuum rotary evaporation on a water bath at 50 °C. The residue was reconstituted in 2 mL of mobile phase. The final solution was filtered through a 0.22 μm PTFE syringe filter (Millex LCR, Millipore, Milford, MA, USA) before LC–MS/MS analysis.

2.4. LC–MS/MS system and operating conditions

An Agilent 1200 Series LC system (Agilent Technologies, Waldbronn, Germany) consisting of a solvent degassing unit, a quaternary pump, an autosampler and a thermostatted column

Table 1
Common name, MS/MS transitions and instrument conditions of each pesticide.

Pesticides	Transition mass (*m/z*)[a]	DP (V)	CE (V)	Transition mass (*m/z*)	DP (V)	CE (V)
Dinotefuran	203.2 → 129.0	55	18	203.2 → 113.2	55	16
Thiamethoxam	292.1 → 211.0	61	19	292.1 → 181.0	61	33
Clothianidin	250.1 → 169.0	61	19	250.1 → 131.9	61	25
Imidacloprid	256.0 → 209.3	62	22	256.0 → 175.2	62	22
Acetamiprid	223.1 → 126.1	68	31	223.1 → 56.0	68	37
Thiacloprid	253.1 → 126.1	75	34	253.1 → 90.2	75	56
D_4-labeled imidacloprid	260.1 → 213.1	67	26	260.1 → 179.2	67	26

[a] MS–MS transition used for quantification.

compartment was used in the LC–MS/MS system. Separation of the analytes was achieved on a ZORBAX Eclipse XDB-C_8 column (150 mm × 4.6 mm i.d., 5 μm) with a column oven temperature of 30 °C. The mobile phase consisted of two eluents, namely, solvent A (ultrapure water with 0.1% formic acid) and solvent B (acetonitrile), delivered at a flow rate of 0.4 mL min^{-1}. Gradient elution employed with the ratio of A:B varied as follows: 0 min, 70:30; 3.5 min, 30:70; 8 min, 30:70; 10 min, 70:30; 16 min, 70:30.

An API 4000 Qtriple-quadrupole mass spectrometer (Applied Biosystems, Concord, Ontario, Canada) was operated with a Turbo Ion Spray interface in positive ion mode. Analyst 1.4.2 software (Applied Biosystems) was used for the control of equipment, data acquisition, and analysis. The source optimization of each neonicotinoid pesticides and the IS was tuned by introducing the analyte into the mass spectrometer through direct infusion via a syringe pump (Harvard Apparatus, Holliston, MA, USA) at a flow rate of 10 μL min^{-1}. Finally, the instrument was operated with the ion spray voltage set at +4.8 kV and the heater gas temperature at 540 °C. Additionally, we used a nebulizer gas (Gas 1) of 0.289 MPa, a heater gas (Gas 2) of 0.31 MPa, a curtain gas (CUR) of 0.172 MPa, and a collision gas (CAD) of 0.041 MPa. All gases used were nitrogen. The dwell time for each transition was 200 ms. Prior to injection, the needle of the injector was rinsed thoroughly in the injection port with a mixture of methanol: H_2O 50:50 (v/v) before and after each injection to minimise potential carryover. The instrument was operated in positive ion electrospray mode during LC separation in the multiple reaction monitoring (MRM) modes. In this work, the most intense characteristic MRM transitions chosen for each analyte and surrogate standard and Table 1 lists the precursor, daughter ions monitored, declustering potential (DP) and collision energy (CE) of each pesticide.

2.5. Method validation

Method quantification was based on peak area and was performed using internal standard calibration curve obtained from analyzing calibration standards. The limits of quantifications (LOQs) were calculated as signal-to-noise ratio of 10 (S/N = 10).

To evaluate possible interferences encountered in the method, the selectivity of methods was verified by analyzing 20 blank samples of different agricultural samples (chestnut, shallot, ginger and tea blank samples were in quintuplicate). For matrix calibration curves, agricultural samples were fortified with working standard solutions and the matrix calibration curves were linear over the range 4–100 μg L^{-1} for the analyzed compounds. The samples were analyzed on three different days and the linearity of calibration curves was expressed by the correlation coefficient.

The repeatability was determined by fortifying six blank agricultural samples at each of three concentration levels (0.01 mg kg^{-1}, 0.02 mg kg^{-1} and 0.1 mg kg^{-1} for chestnut, shallot, ginger sample, 0.02 mg kg^{-1}, 0.04 mg kg^{-1} and 0.2 mg kg^{-1} for tea sample) with the analyzed compounds. The samples were analyzed on the same day with the same instrument and the same operators and the relative standard deviation (RSD) were calculated as repeatability.

The within-laboratory reproducibility was determined by fortifying another two sets of blank agricultural samples at the same concentration levels of analyzed compounds as for the repeatability and analyzing on two different days with the same instrument and the different operators. The overall RSD was calculated as within-laboratory reproducibility.

The percentage recovery was evaluated in the same experiment as repeatability by comparing the mean measured concentration with the fortified concentration of the agricultural samples.

3. Results and discussion

3.1. Optimisation of MS parameters and MRM transitions

Preliminary experiments were conducted with the purpose of finding the best instrumental conditions that would allow unambiguous identification of the analytes in real samples at trace levels. Single compound standard solutions (400 ng mL^{-1}) prepared in methanol:H_2O 50:50 (v/v), in the presence or absence of formic acid, were introduced into the MS at a flow rate of 10 μL min^{-1} using a syringe pump (Harvard Apparatus, Australia). 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 to 500 in positive mode. For ESI (+) mode, formic acid substantially promoted the formation of $[M+H]^+$ parent ions, leading to better sensitivity, as well as better resolution and peak shape.

The most sensitive transition in MRM mode was selected for quantification in the screening method. A minimum of three identification points are required to meet the identification performance criteria defined by the EU Commission for quantitative mass spectrometric detection. Using LC–MS/MS to monitor one precursor ion and two daughter ions 'earns' four identification points (1 for the parent ion and 1.5 for each daughter ion) and therefore fulfils these criteria.

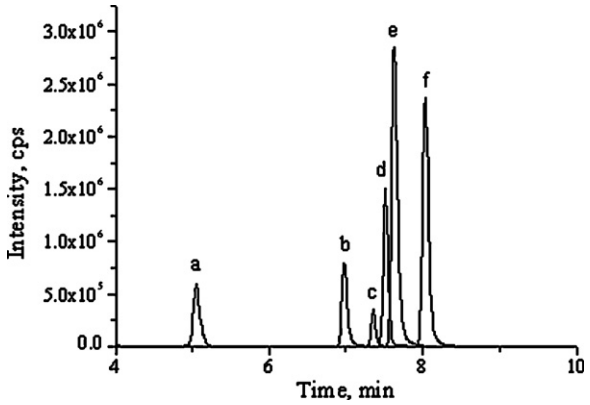

Fig. 2. LC–MS/MS total ion chromatogram (MRM mode) of a ginger sample spiked with 0.01 mg kg^{-1} of the six pesticides. Dinotefuran (a), thiamethoxam (b), clothianidin (c), imidacloprid (d), acetamiprid (e), thiacloprid (f), imidacloprid-D_4 (d).

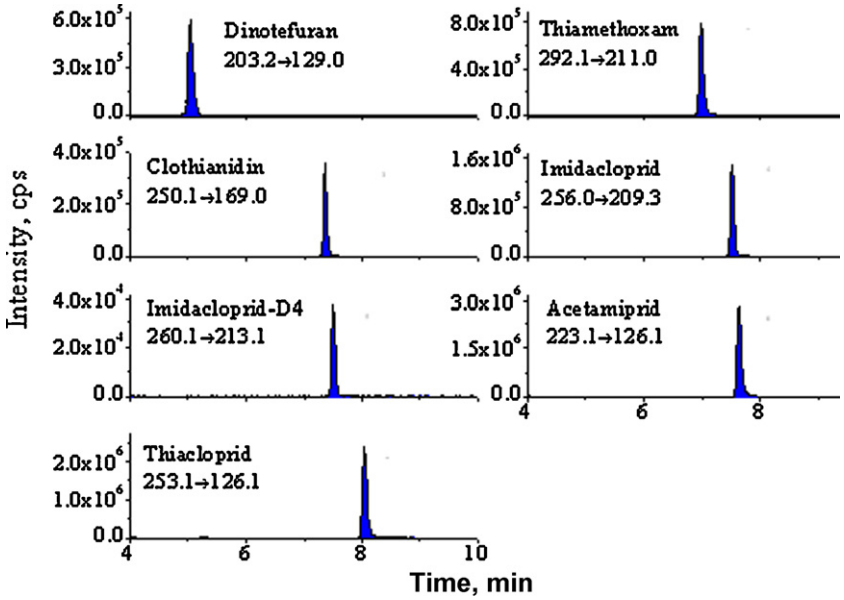

Fig. 3. Typical MRM profile of a ginger sample at 0.01 mg kg^{-1}.

To choose the transitions in the MRM mode, different parameters were studied. The precursor and the product ions of each compound were selected. The last parameter optimized was the collision energy; different values were tested (30, 40, 50, 55 and 60 V). In Table 1 we summarize the optimum values for each condition for each compound. The optimization was then done following the normal optimization procedure. In this work, the most intense characteristic MRM transitions were chosen for each analyte and surrogate standard and Table 1 lists the precursor and daughter ions monitored.

Total ion chromatogram (TIC) of all six ions monitored from a 0.01 mg kg^{-1} spiked ginger sample was satisfactory, except

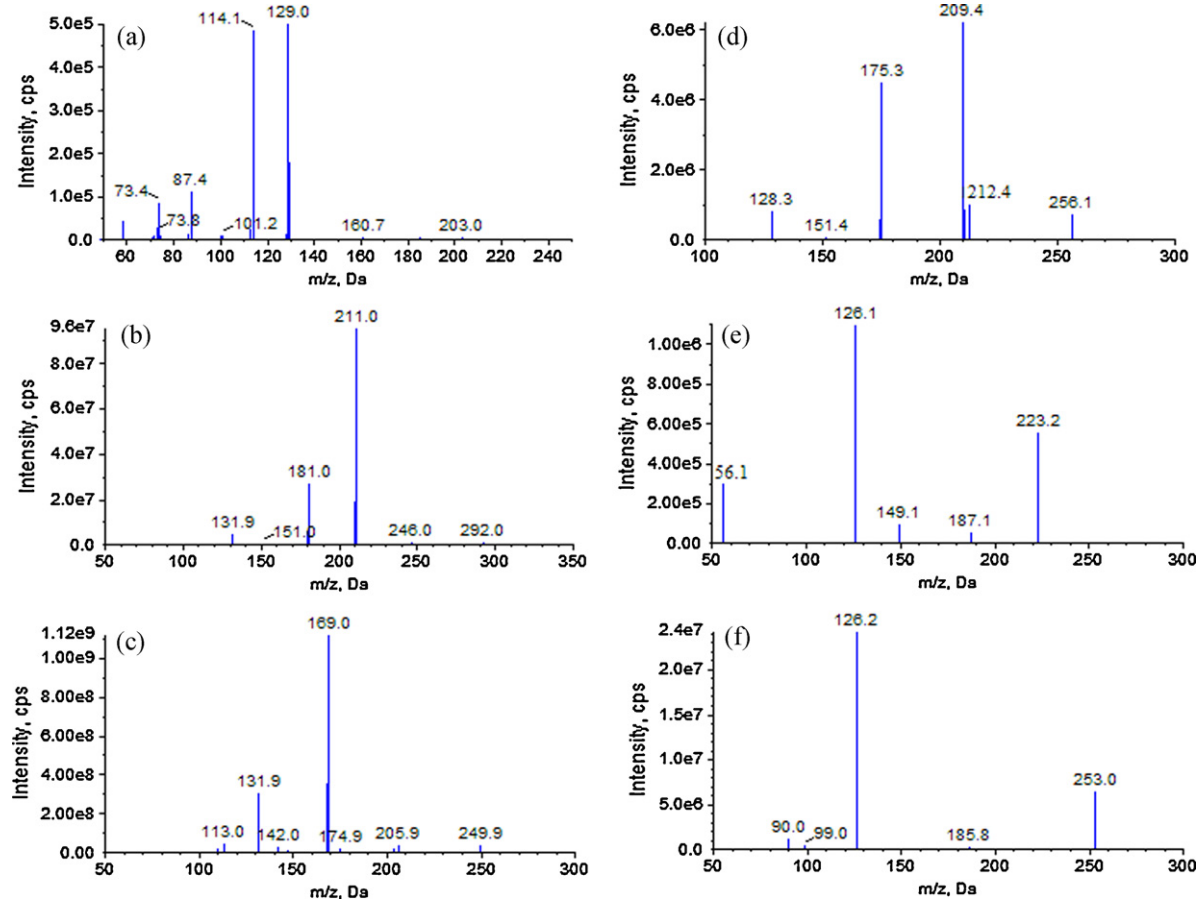

Fig. 4. ESI-MS/MS product scan spectrum of dinotefuran (a), thiamethoxam (b), clothianidin (c), imidacloprid (d), acetamiprid (e), and thiacloprid (f).

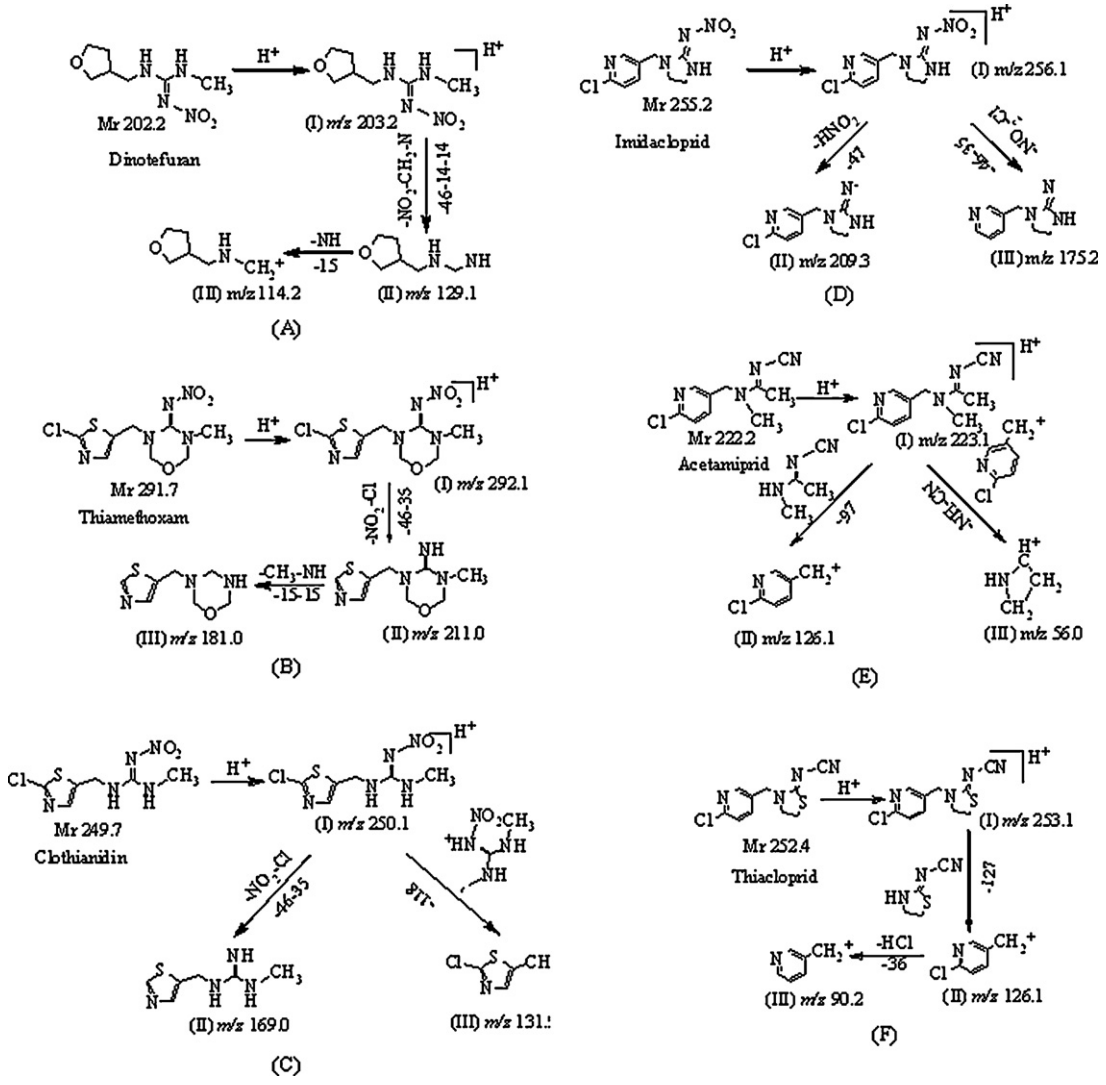

Fig. 5. Proposed fragmentation pattern with tentative structures for the product ions of dinotefuran (A), thiamethoxam (B), clothianidin (C), imidacloprid (D), acetamiprid (E), and thiacloprid (F).

for imidacloprid and acetamiprid, which were poorly resolved (Fig. 2). However they could be easily identified and quantified on multiple reaction monitoring (MRM) mode due to different pseudo-molecular and fragment ions. Analysis of blank samples revealed no traces of the pesticides studied. The typical chromatograms of individual MRM transitions for six pesticides in ginger extracts at concentration 0.01 mg kg^{-1} are shown in Fig. 3.

The protonated molecular ion $[M+H]^+$ was the base peak in the spectra of six compounds in the positive ion mode. The ESI-MS/MS product scan spectrum of the six compounds (Fig. 4a–f) was acquired under the conditions described above. In Fig. 4a (Dinotefuran), fragments at *m/z* 203.0, 129.0 and 114.1 were observed. Since there are no reports on these neonicotinoid pesticides, these might derive from $[M+H]^+$, $[M+H-NO_2-CH_2-N]$, $[M+H-NO_2-CH_2-N-NH]$, respectively. In Fig. 4b (Thiamethoxam), fragments at *m/z* 292.0, 211.0 and 181.0 were observed, these might derive from $[M+H]^+$, $[M+H-NO_2-Cl]$, $[M+H-NO_2-Cl-NH-CH_3]$, respectively. In Fig. 4c (Clothianidin), fragments at *m/z* 249.9, 169.0 and 131.9 were observed, these might derive from $[M+H]^+$, $[M+H-NO_2-Cl]$, $[M+H-NH-C-NH-NH-NO_2-CH_3]$, respectively. In Fig. 4d (Imidacloprid), fragments at *m/z* 256.1, 209.3 and 175.2 were observed, these might derive from $[M+H]^+$, $[M+H-HNO_2]$, $[M+H-NO_2-Cl]$, respectively. In Fig. 4e (Acetamiprid), fragments at *m/z* 223.1, 126.1 and 56.1 were observed, these might derive from $[M+H]^+$, $[M+H-CH_3-CN-N-C-NH-CH_3]$, $[M+H-NH-CN-Cl-C-N-CH-C-CH-CH-CH_2]$, respectively. In Fig. 4f (Thiacloprid), fragments at *m/z* 253.0, 126.1 and 90.0 were observed, these might derive from $[M+H]^+$, $[M+H-CN-N-C-S-CH_2-CH_2-NH]$, $[M+H-CN-N-C-S-CH_2-CH_2-NH-HCl]$, respectively. The fragmentation pattern is proposed among with tentative structures for the observed main product ions (Fig. 5A–F).

In general, the use of tandem mass spectrometry allows analysis without complete chromatographic separation of analytes since it is uncommon to find molecules that elute at the same retention time and share the same MS/MS transition. However, a certain degree of separation is necessary in order to enable programming of various MRM transitions into different time segments along the chromatogram.

3.2. Extraction and SPE clean up

Extraction solvent and extraction method were investigated to obtain reasonable experimental results and satisfactory efficiency. One positive tea sample (Imidacloprid and acetamiprid concentration levels were 0.5 and 0.7 mg kg^{-1}) was used in extraction experiment.

The selection of solvent is very important when extracting multi-residues. In solvent selection experiments, ethyl acetate, acetonitrile, and cyclohexane were studied. Cyclohexane could not extract the six polar pesticides because of its low polarity and the recoveries of all analytes were no more than 21.5%. Next, the choice was made between ethyl acetate and acetonitrile. The recoveries of all analytes were higher than 80.2% when acetonitrile was used and the recoveries of all analytes were about 58.7% when ethyl acetate was used. The results showed that acetonitrile was the most effective solvent. In order to obtain higher recovery, 1 mL water was first added and soaked for about 30 min. Then D_4-labeled imidacloprid and acetonitrile were placed.

The efficiency of extraction of neonicotinoid pesticides by vortex was compared with that obtained by ultrasonic extraction. The frequency of ultrasonic extraction was 37 kHz and the power of ultrasonic extraction was 400 W. Yields of neonicotinoid pesticides from tea samples by use of vortex were little higher than those achieved by use of ultrasonic extraction, and the extraction time was much less than ultrasonic extraction, so vortex was used in the experiments.

Ready-to-use cartridges, filled with a diatomaceous earth material, have been already used in place of the usual liquid–liquid partitioning with solvents, to extract pesticides from food [32–34]. In the developed extraction step we used activated carbon and Oasis HLB SPE cartridges, to extract and clean-up in two steps with acetonitrile and methanol the neonicotinoid insecticides from agricultural samples, obtaining very clean eluants.

Ethyl acetate, acetonitrile and methanol, being suitable solvents for a wide polarity range of target compounds, were initially considered for HLB SPE elution. Lower recovery rates (the recoveries of all analytes were about 53.2%) were observed in the case of ethyl acetate used as elution solvent compared to methanol and acetonitrile for the tested compounds. Although satisfactory results (the recoveries of all analytes were higher than 85.8%) were obtained with both methanol and acetonitrile, the former provided better overall recoveries and was selected as the elution solvent when HLB SPE cartridges were used.

3.3. Matrix effects

Matrix effects (generally recognized as a suppression or enhancement of the analytical signal due to co-eluting matrix components) have been widely studied and recognized as a source of error in quantitative LC–MS/MS analysis of food samples [35]. In this study, the use of matrix-matched calibration standards was done to compensate for the matrix effect, i.e., signal suppression or enhancement of studied pesticides in matrix solution. The matrix effect, expressed as the signal from the pesticide in matrix compared to the signal in solvent was tested in all matrices. Table 2 shows matrix effects for every tested pesticide at the concentration level of 10 ng mL^{-1} in tea. The recoveries calculated by solvent calibration curve were not so good. Dinotefuran, imidacloprid and thiacloprid displayed the enhancement of the signal, and thiamethoxam, clothianidin, and acetamiprid displayed the suppression of the signal. Thiamethoxam and acetamiprid were significantly affected by the matrix components; their recoveries were 22 and 68%, respectively. On contrary, the recoveries calculated by matrix solution calibration curve were very good (97–107%). Therefore, for an accurate quantification, the use of matrix-matched standards is required.

Table 2
Tea matrix solution affected selected ion.

Compounds	Transitions	[a]Recovery%	[b]Recovery%
Dinotefuran	203.2/129.0	118	106
Thiamethoxam	292.1/211.0	22	97
Clothianidin	250.1/169.0	91	107
Imidacloprid	256.0/209.3	109	103
Acetamiprid	223.1/126.1	68	102
Thiacloprid	253.1/126.1	115	105

[a] Solvent calibration curve.
[b] Matrix solution calibration curve.

3.4. Method validation

3.4.1. Linearity of calibration standards

The concentrations of the analytes in the samples were calculated by matrix calibration using internal standards. It was evident from the results that more reproducible results were obtained using imidacloprid-D_4 as internal standard for quantification of analyzed substances. Calibration curves with $1/x$ weighting were plotted for each individual analytes. The matrix calibration curves were linear over the range 4–100 μg L^{-1} for the analyzed compounds. The correlation coefficients were between 0.9991 and 0.9995 (Table 3). The

Table 3
Retention time, calibration equations and correlation coefficients of each pesticide.

Pesticide	Agricultural sample	Retention time (min)	Calibration equations	Correlation coefficients (R^2)
Dinotefuran	Chestnut	5.1	$Y = 75.4X + 0.0882$	0.9995
	Shallot		$Y = 144X + 0.517$	0.9994
	Ginger		$Y = 72.3X - 0.00474$	0.9992
	Tea		$Y = 36.8X + 0.0293$	0.9992
Thiamethoxam	Chestnut	7.0	$Y = 36.6X$-0.00908	0.9991
	Shallot		$Y = 64X + 0.0737$	0.9994
	Ginger		$Y = 45.1X$-0.0152	0.9995
	Tea		$Y = 15.8X$-0.011	0.9995
Clothianidin	Chestnut	7.3	$Y = 24.4X + 0.0168$	0.9992
	Shallot		$Y = 25.8X + 0.0244$	0.9993
	Ginger		$Y = 26X + 0.00474$	0.9991
	Tea		$Y = 10.2X + 0.00862$	0.9992
Imidacloprid	Chestnut	7.51	$Y = 148X + 0.142$	0.9993
	Shallot		$Y = 134X + 0.0872$	0.9995
	Ginger		$Y = 174X + 0.114$	0.9991
	Tea		$Y = 72.6X$-0.00649	0.9993
Acetamiprid	Chestnut	7.7	$Y = 393X + 0.785$	0.9995
	Shallot		$Y = 430X + 0.332$	0.9994
	Ginger		$Y = 479X + 0.748$	0.9992
	Tea		$Y = 272X + 0.382$	0.9991
Thiacloprid	Chestnut	8.1	$Y = 270X + 0.457$	0.9991
	Shallot		$Y = 307X + 0.267$	0.9993
	Ginger		$Y = 329X + 0.384$	0.9993
	Tea		$Y = 186X + 0.0901$	0.9994

Table 4
Mean recoveries and repeatability of the developed method at three concentration levels with LOQs ($n = 6$).

Pesticide	Agricultural sample	Recovery (%) (RSD%)			LOQs (mg kg^{-1})	MRLs (mg kg^{-1})
		Spiking level (mg kg^{-1})				
		0.01	0.02	0.1		
Dinotefuran	Chestnut	95.1(5.1)	104.2(4.5)	90.2(4.9)	0.01	–
	Shallot	88.5(7.5)	93.4(6.3)	104.5(6.7)		–
	Ginger	85.3(6.2)	91.2(7.2)	106.4(4.2)		–
Thiamethoxam	Chestnut	84.1(7.9)	90.2(4.8)	103.7(5.5)		0.02[a]
	Shallot	86.8(6.1)	92.7(6.5)	105.4(4.2)		0.02[a]
	Ginger	88.1(8.5)	89.5(4.2)	96.4(4.1)		0.02[a]
Clothianidin	Chestnut	85.7(8.2)	105.9(7.4)	96.1(5.2)		0.02[a]
	Shallot	85.1(7.4)	106.8(6.5)	93.5(5.8)		0.02[a]
	Ginger	82.1(8.6)	106.8(6.5)	94.8(3.6)		0.02[a]
Imidacloprid	Chestnut	83.8(8.2)	91.3(7.5)	95.4(6.4)		0.1[a]
	Shallot	85.7(5.9)	92.4(4.5)	94.3(3.7)		0.1[b]
	Ginger	83.6(6.5)	105.4(5.1)	108.5(4.1)		0.1[a]
Acetamiprid	Chestnut	85.4(6.8)	105.1(5.3)	96.2(3.5)		–
	Shallot	84.8(7.6)	91.5(6.1)	95.2(3.8)		0.2[a]
	Ginger	86.7(6.4)	105.6(5.3)	93.8(3.9)		0.1[b]
Thiacloprid	Chestnut	90.2(7.1)	92.3(6.2)	106.7(4.8)		–
	Shallot	88.5(5.9)	91.2(4.1)	95.4(6.3)		–
	Ginger	83.5(8.3)	107.5(6.5)	106.1(5.5)		–
Pesticide	Agricultural sample	Recovery (%) (RSD%)			LOQs (mg kg^{-1})	MRLs (mg kg^{-1})
		Spiking level (mg kg^{-1})				
		0.02	0.04	0.2		
Dinotefuran	Tea	86.8(2.7)	101.5(5.4)	102.7(5.7)	0.02	–
Thiamethoxam		93.5(6.8)	97.8(5.2)	106.1(3.3)		20[a]
Clothianidin		89.2(3.9)	100.7(5.2)	102.2(2.7)		0.05[b]
Imidacloprid		89.4(5.2)	95.0(3.2)	97.7(5.2)		0.05[b]
Acetamiprid		93.4(7.9)	88.2(7.6)	95.1(3.9)		50[a]
Thiacloprid		84.3(6.9)	96.8(4.5)	104.3(5.4)		30[a]

[a] MRLs of Japan.
[b] MRLs of EU.

limits of quantifications (LOQs) were calculated as signal-to-noise ratio of 10 (S/N = 10), and was 0.01 mg kg^{-1} for chestnut, shallot, and ginger and 0.02 mg kg^{-1} for tea sample. Quantification was performed based on calibration plots using the peak area of the most intense transition of the analyte.

3.4.2. Assay selectivity

The selectivity was evaluated by the analysis of 5 blank samples of different tea samples. No interfering peaks from endogenous compounds were found in the retention time of the target analytes for tea samples.

3.4.3. Precision and accuracy

Intra-day precision was examined by analysis of the same standard solutions at three different concentrations (high, medium, and low) on the same day; inter-day precision was determined by analysis of the same solutions on three different days. RSD values were 1.72 and 1.96%, respectively. Method accuracy was evaluated by recovery studies, using "blank" tea samples labeled as organically produced and confirmed as not containing the target pesticides using the proposed method. "Blank" chestnut, shallot, and ginger samples were spiked with the appropriate amounts of the target compounds at three concentration levels, 0.01, 0.02 and 0.1 mg kg^{-1}. "Blank" tea samples were spiked with the appropriate amounts of the target compounds at three concentration levels, 0.02, 0.04 and 0.2 mg kg^{-1}. The pesticide contents were determined by use of the corresponding calibration plot and recovery was calculated from the ratio of the amounts detected to those added. Average recovery was in the range 82.1–108.5% (Table 4) and with relative standard deviations below 8.6%, indicative of the good recovery and precision of the method.

3.5. Real samples

A survey on residues of six selected pesticides in commercially available agricultural sample was performed. 150 agricultural samples (chestnut, shallot, ginger and tea) of different brands, produced by different domestic companies, were purchased in local supermarkets and analyzed following the proposed method. Imidacloprid and acetamiprid were detected at concentration levels ranging from 0.05 to 3.6 mg kg^{-1} in agricultural samples (chestnut, shallot, ginger and tea). It can be concluded that the presence and levels of this pesticides in agricultural samples should be a matter of concern.

4. Conclusion

The method described in this paper provides reliable quantitative analysis of six pesticide residues in agricultural samples. It has been validated for different agricultural samples matrices as chestnut, shallot, ginger and tea. Although every agricultural sample showed matrix effect that was influencing the analyte signal, it was successfully eliminated using matrix-matched standards. Recoveries were in the range 82.1–108.5%. Repeatability of the method, expressed as the relative standard deviation, was below 8.6%. The method quantification limit was 0.01 mg kg^{-1} for chestnut, shallot, ginger and 0.02 mg kg^{-1} for tea sample, lower than MRLs set by the EU and Japan, indicating that the method is suitable for quantification of six selected pesticides in agricultural samples.

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