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RESEARCH PAPER

Determination of Tebufenozide and Methoxyfenozide Residues in Vegetables Using Liquid Chromatography-Electrospray Tandem Mass Spectrometry

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Abstract: An analytical method was developed for the determination of tebufenozide and methoxyfenozide residues in vegetables. The procedure involves the extraction of tebufenozide and methoxyfenozide using alkali acetonitrile before solid-phase purification from the matrices. The tebufenozide and methoxyfenozide residues were subsequently determined by an electrospray mass spectrometer after separated by the reversed-HPLC. Mass Spectrum acquisition applied Selected Reaction Monitoring (SRM) to diagnostic transition reactions. The qualitative results were obtained based on the retention time, the precursor ion and two daughter ions, and the quantitative results were based on the intension of the characteristic *m/z* 297 ion and *m/z* 149 ion. The linear range was from 5.0–200 ng ml^{-1} and the correlation coefficients (*r*) were higher than 0.996. The average recovery ranges from 90% to 110% for tebufenozide and 70% to 80% for methoxyfenozide in the replicate sets of vegetable samples fortified with drug concentration of 4.0, 10.0 and 20 μg kg^{-1}, and the relative standard deviations (RSD) were less than 8%. The detection limit and the quantification limit of the method were 1.0 and 4.0 μg kg^{-1}, respectively. This method is quite suitable for determining the residues of tebufenozide and methoxyfenozide in vegetables.

Key Words: Liquid chromatography-electrospray tandem mass spectrometry; Residue; Tebufenozide; Methoxyfenozide; Vegetable

1 Introduction

Tebufenozide and methoxyfenozide are new-generation insecticides. These insecticides induce premature molting and cause the death of insects by mimicking their hormone[1]. Both these insecticides have already been widely used for vegetables planting in China. However, because of the shortage of residue-detecting means, economic loss occurs in vegetables export as a result of excess residues. So an analytical method combining liquid chromatography with electrospray tandem mass spectrometry was developed for the determination of tebufenozide and methoxyfenozide residues in vegetables. Compared with the traditional methods so far employing high-performance liquid chromatography (HPLC)[2–4], which are time-consuming and unable to confirm on-line positive consequence, LC-MS/MS is highly efficient and quite stable. A method for the determination of methoxyfenozide residue in fruits and vegetables by LC-MS/MS was reported in literature[5]. Nevertheless, it was an inconvenient method as more organic solvent expended because of the liquid-liquid extraction. Conversely, solid-phase extraction is efficacious and suitable for routine analysis.

The procedure employed in this study involves the extraction of tebufenozide and methoxyfenozide by alkali acetonitrile before solid-phase purification from the matrices. The tebufenozide and methoxyfenozide residues were subsequently analyzed with electrospray mass spectrometer after separated by the reversed-HPLC. Mass Spectrum acquisition was applied to selected reaction monitoring (SRM) and to two diagnostic transition reactions. On the basis of the selectivity and specificity of SRM, the method for the analysis of tebufenozide and methoxyfenozide was constructed, and the possible mechanism of dissociation in MS was discussed.

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2 Experimental

2.1 Apparatus and Reagents

Surveyor LC system (Thermo, USA); Quantum Ultra triple-quadrupole MS-MS (Thermo-Finnigen); vacuum N_2 evaporator (Zymark); Milli-Q system (Millipore); 12-port SPE vacuum manifold (Supleco).

2.2 Standard preparation

A total of 10.00 mg of tebufenozide and methoxyfenozide was dissolved in a 10 ml volumetric flask and diluted to the required volume with methanol as the stock solution (1.0 mg ml^{-1}). The solutions were kept at –18 °C in black. And fortification standard solution (1.0 μg ml^{-1}) was made by diluting the stock solution. This solution was stable at 0–4 °C for a month. The LC-MS/MS standard system was prepared to 5.0, 10.0, 20.0, 50.0, 100.0 and 200.0 μm l^{-1} six levels diluted with the mobile phase.

2.3 Extraction and purification

A total of 5.00 g fresh vegetables were accurately weighed into a centrifuge tube. Then, 4 ml 0.1 M NaOH and 15 ml acetonitrile saturated with hexane were added into the tube. After vortex-mixing, the samples were ultrasoniced for 10 min and centrifuged. The supernatant was filtered into the clean tubes. Another 8 ml acetonitrile saturated with hexane were added to the same as above. A total of 4-ml acetonitrile was added to wash the filter paper after filtration. Proper NaCl solid was added into the tubes. After vortex-mixing and centrifuging, acetonitrile was demixed from water. The upper organic layer was collected into a 50-ml round-bottom flask. Then the extract was evaporated to dryness in a water bath at 40 °C. And 3 ml hexane was added to dissolve samples for solid phase extraction (SPE). The active carbon column was washed with 3 ml acetone, and vacuum was applied until the column was dry. Then, the column reached equilibrium with 3 ml hexane. The sample was added to the column before hexane had gone, and the solution was collected simultaneously. The column was eluted with 5 ml 8:2 (v/v) hexane/acetone mixtures after the column were dried again. A flow rate of 1 ml min^{-1} was established. The elution solvent was removed from the extracts with an N_2 evaporator in a water bath at 50 °C, and the residue was redissolved in 1 ml 1:1 (v/v) methanol solution. The sample was mixed on a vortex mixer, filtered through a 0.45 μm Teflon filter, and transferred into LC vials for HPLC/ESI-MS/MS analysis.

If the sample is vegetable powder, it could be extracted with acetonitrile twice followed with evaporation. The rest is the same as above.

An accurate amount of tebufenozide and methoxyfenozide were added to the weighted vegetable for the spiked experiment. After 10 min at room temperature, the exact solution was added. The rest is the same as described above.

2.4 Chromatogram and mass spectrum conditions

The HPLC column was a Waters SunFire C_{18} (150 mm × 2.1 mm × 3.5 μm); the temperature of column was room temperature; 0.1% formic acid (A) and methanol (B) were the mobile phase; The gradient conditions were set as follow: 0 min, 20% B; 3.0 min, 90% B; 8.2 min, 90% B; 8.5 min, 20% B. The flow rate: 0.25 ml min^{-1}; aliquots: 20 μl.

ESI: positive ion mode; source temperature: 360 °C; ionization voltage: 4.8 kV; monitoring mode: SRM. The following traces were monitored (Table 1).

Table 1 Precursor ions and product ions of tebufenozide and methoxyfenozide in the positive ion mode

Chemicals	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (eV)
Tebufenozide	353	297	10
		133	14
Methoxyfenozide	369	313	10
		149	14

2.5 Evaluation of data

The content of residues of tebufenozide and methoxyfenozide were calculated by interpolating the standard curve, which was determined by the peak areas of standard solutions quantified by the precursor ions *m/z* 297 and *m/z* 149, respectively.

3 Results and discussion

3.1 Optimized conditions of extraction

The acetonitrile saturated with hexane as the extract solution was easy to salt out and enhances the distribution of tebufenozide and methoxyfenozide in acetonitrile layer at the same time. The extraction efficiency of acetonitrile saturated with hexane was compared with acetonitrile, it is found that the recovery of the former was 30% higher than the latter. Filter paper would absorb some tebufenozide and methoxyfenozide, leading to unsteady or low recoveries. Therefore, a proper procedure is required for washing the filter paper.

Because of plenty of pigments in vegetables such as spinage, the color of the solution was often very dark after extraction. If analyzed directly after evaporation, the pigment would stay and contaminate the machine, which contributes to the decrease of SNR and this affects accuracy. Thus, it is necessary for the samples to go through the active carbon column. In SPE purification, some residues would go through the column with

hexane. Three types of eluting solution were contrasted, which were 8:2, 6:4, 4:6 (v/v) of hexane/acetone mixtures. The results show that the higher the ratio of hexane, the higher its SNR was. As a result, 8:2 hexane/acetone mixtures were chosen as the eluting solution, and both the sample solution and eluting solution were collected.

The LC ESI-MS/MS chromatograms of tebufenozide and methoxyfenozide in the spiked white cauliflower are shown in Fig.1.

Fig.1 LC ESI-MS/MS chromatograms of tebufenozide (A) and methoxyfenozide (B) in spiked sample

3.2 MS/MS conditions and mechanism of dissociation

In the light of the EC/657 regulation of EU, there are two requirements for confirming the residues by mass spectrum. One is the presence of four identifiable points; the other is that the abundance ratio of the character ions is identical to that of the standards. For MS/MS, one precursor ion (1 point) and two product ions (1.5 points × 2) meet that demand. The character ions of tebufenozide and methoxyfenozide are 353/297, 353/133 and 369/313, 369/149, respectively. On the other hand, the abundance ratio of the character ions of residues from the spiked sample is almost equal to that of the standards. Thus it also can be confirmed. The MS spectra of standards are shown in Fig.2.

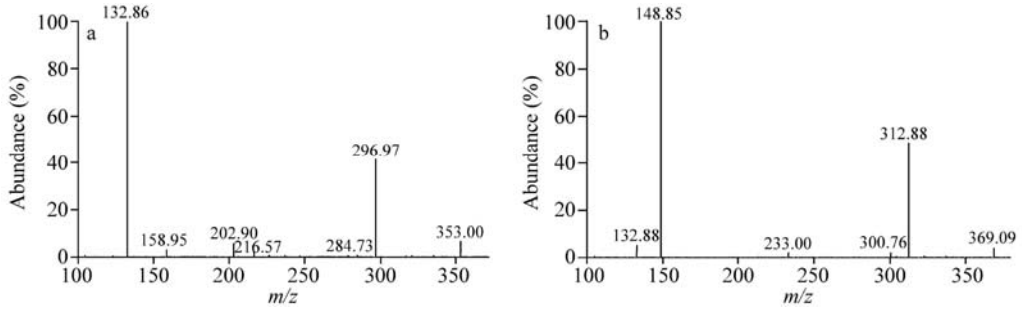

Fig.2 MS spectra of standards of tebufenozide (a) and methoxyfenozide (b)

According to the result, the possible mechanism of dissociation was discussed taking tebufenozide as an example. It is shown in Fig.3. The dissociation mechanism of methoxyfenozide will be similar to that of tebufenozide because of their similar structures.

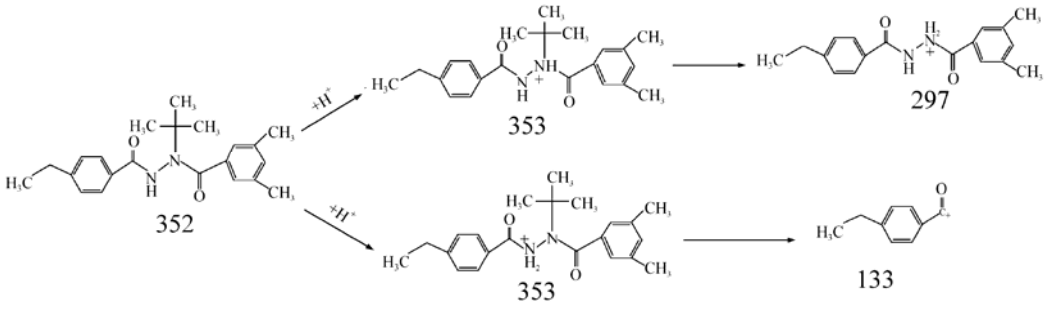

Fig.3 Reaction process of tebufenozide precursor ion and product ions

3.3 Linear range, standard curves, and detection limits

Tebufenozide and methoxyfenozide have good linearity in the range of 5.0–200 ng ml^{-1}. The linear equations are y = 928601x – 563640 and y = 1439740x – 575171, with correlation coefficients 0.9962 and 0.9991, respectively. The detection limit is 1.0 μg kg^{-1} according to S/N = 3. And the quantitative limit is 4.0 μg kg^{-1} by analyzing spiked vegetables.

3.4 Recoveries and RSD

Tebufenozide and methoxyfenozide were analyzed in replicate sets of vegetable samples fortified with drug

concentrations of 4.0, 10.0 and 20 μg kg^{-1}, and were determined by the peak areas of standard solutions quantified by the precursor ions *m/z* 297 and *m/z* 149, respectively. Matrix effects were found in spinage, shallot, white cauliflower, and so on.

Statistics in experiment and after calibration are shown in Table 2. The average recovery after calibration for tebufenozide ranges from 90% to 110% and for methoxyfenozide from 70% to 80%, while the RSD is less than 8%, which satisfy routine analysis. This proves the applicability of this method for routine analysis.

Table 2 Recovery and precision of the method

	Tebufenozide sipked levels (μg kg^{-1})						Methoxyfenozide sipked levels (μg kg^{-1})					
	4.0		10.0		20.0		4.0		10.0		20.0	
	Original	Amended	Original	Amended	Original	Amended	Original	Amended	Original	Amended	Original	Amended
Found concentration (μg kg^{-1})	1.89	4.21	4.88	10.84	8.18	18.17	1.66	3.31	3.90	7.80	6.94	13.88
	1.98	4.40	4.90	10.90	7.63	16.96	1.71	3.41	3.75	7.50	7.20	14.39
	1.98	4.39	4.52	10.04	8.46	18.79	1.62	3.24	4.07	8.14	7.71	15.41
	2.30	5.10	4.60	10.22	8.41	18.69	1.61	3.21	3.71	7.41	7.27	14.53
	2.20	4.89	4.93	10.96	8.42	18.72	1.51	3.01	4.06	8.12	7.26	14.51
	1.95	4.34	4.72	10.48	8.27	18.38	1.54	3.09	3.82	7.64	7.68	15.35
Recovery (%)	51.2	113.8	47.6	105.7	41.2	91.5	40.2	80.3	38.8	77.6	36.7	73.3
RSD (%)	7.8		3.6		3.8		4.5		4.0		4.0	

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