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Determination of 17 pyrethroid residues in troublesome matrices by gas chromatography/mass spectrometry with negative chemical ionization

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ABSTRACT

An analytical method with the technique of QuEChERS (quick, easy, cheap, effective, rugged and safe) and gas chromatography (GC)/mass spectrometry (MS) in negative chemical ionization (NCI) has been developed for the determination of 17 pyrethroid pesticide residues in troublesome matrices, including garlic, onion, spring onion and chili. Pyrethroid residues were extracted with acidified acetonitrile saturated by hexane. After a modified QuEChERS clean-up step, the extract was analyzed by GC-NCI/MS in selected ion monitoring (SIM) mode. An isotope internal standard of trans-cypermethrin-D_6 was employed for quantitation. Chromatograms of pyrethroids obtained in all these matrices were relatively clean and without obvious interference. The limits of detection (LODs) ranged from 0.02 to 6 $\mu g\,kg^{-1}$ and recovery yields were from 54.0% to 129.8% at three spiked levels (20, 40 and 60 $\mu g\,kg^{-1}$ for chili, and 10, 20 and 30 $\mu g\,kg^{-1}$ for others) in four different matrices depending on the compounds determined. The relative standard deviations (RSDs) were all below 14%. Isomerization enhancement of pyrethroids in chili extract was observed and preliminarily explained, especially for acrinathrin and deltamethrin.

1. Introduction

Synthetic pyrethroid insecticides are the major pesticide class in agriculture and public health around the world because of their high insecticidal activity and low mammalian toxicity. Pyrethroids are potent against a wide range of insects at rather low dosages and degrade readily in the environment. However, the widespread use of pyrethroids leads to larger dose of human exposures. It is demonstrated that pyrethroids, especially those with an α-cyano group, are neurotoxicity to mammals mainly by affecting voltage-sensitive sodium channels [1,2]. Many countries and organizations have prescribed strict residue limits for pyrethroid insecticides in agricultural products. Therefore, the analysis of trace pyrethroids in crops, vegetables and fruits has become very important in the area of agriculture and food.

Determination of pyrethroid residues is generally performed on a gas chromatographic (GC) or a high performance liquid chromatographic (HPLC) system. The application of HPLC on pesticide residue analysis is restricted by the low sensitivity of the commonly used UV detector. Galera et al. [3] analyzed nine pyrethroid insecticides by HPLC with post-column photoderivatization and acetonitrile chemiluminescence detection. But the devices and procedures were slightly complicated and cumbersome. As a class of esters, pyrethroids are easy to volatilize due to relatively weak polarity and low boiling point. Many pyrethroids possess one or more electronegative functional groups such as halogen atoms, which are sensitive to electron-captured detector (ECD). Therefore, GC-ECD method is widely used in pyrethroid residue analysis [4–8]. However, when the method is applied to complex matrices, even though rigorous and laborious pre-purification process is carried out until instrumental analysis, reliable qualitative results will be hardly obtained owing to serious matrix interferences. The high sensitivity of mass spectrometry (MS) makes GC–MS the first choice to identify and confirm the results of pyrethroid residue analysis. In GC–MS method, several ionization modes were employed such as electron impact (EI) [7,9,10] and negative chemical ionization (NCI) [5,11–14]. Compared to EI, chemical ionization (CI) source produces less ion fragments and possibly provides molecular weight information. Furthermore, NCI only generates response to electronegative atoms or groups, and therefore has good selectivity to most pyrethroids. Meantime, many interfering impurities

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from column loss or matrix components without any electronegative elements or groups have extremely low MS response in NCI mode. Consequently, signal to noise ratios (S/N) of pyrethroids in NCI are generally higher than in EI. Therefore, GC-NCI/MS is a better choice to analyze pyrethroid residues in complex matrices [15].

Garlic, onion, spring onion and chili are known as troublesome matrices in pesticide analysis. Garlic, onion and spring onion all contain large amount of sulfur-containing compounds which may bring serious matrix effects and significant interferences in MS. Chili contains considerable amount of lipids and pigments which make the separation of pesticides from matrix materials quite difficult. Various extraction and cleanup methods have been applied to improve the pretreatment efficiency for these troublesome matrices, including solid-phase extraction (SPE) [16,17], solid-phase microextraction (SPME) [18], gel permeation chromatography (GPC) [17], matrix solid-phase dispersion (MSPD) [19], supercritical fluid extraction (SFE) [20], etc. Okihashi et al. [21] treated garlic and onion samples in a microwave oven prior to extraction in order to inactivate enzymes. A quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction and cleanup method [22] has become an important technique widely accepted [23,24] in the analysis of multiple pesticide residues in water-rich matrices since it was brought up. The QuEChERS method involves extraction with acetonitrile (MeCN), salting out with sodium chloride and anhydrous magnesium sulfate (anh. $MgSO_4$), and a dispersive solid-phase extraction (DSPE) cleanup step with a small quantity of SPE adsorbents before GC or LC injection. Excellent recovery and repeatability could be obtained in a wide range of pesticides with the technique [25,26]. QuEChERS is an effective, flexible and inexpensive choice in multi-residue analysis of vegetables and fruits.

In this study, an analytical method is proposed to determine 17 pyrethroid residues in four troublesome vegetable matrices (garlic, onion, spring onion and chili) based on a modified QuEChERS procedure using acidified MeCN saturated by hexane instead of pure acidified MeCN to improve extraction efficiency, followed by GC–MS with NCI in selected ion monitoring (SIM) mode. The proposed method was properly validated. In addition, isomerization of some pyrethroids occurred in different matrices was investigated.

2. Materials and methods

2.1. Reagents and chemicals

Acetone, *n*-hexane, MeCN and acetic acid (HAc) (HPLC grade) were obtained from Merck (Darmstadt, Germany). Adsorbents primary secondary amine (PSA, 40 μm), graphitized carbon black (GCB, 120/140 mesh, non-porous) and octadecylsilane (C_{18}, end-capped) were purchased from UCT (Bristol, UK). Anh. $MgSO_4$ was supplied by Nanjing Chemical Reagent (Nanjing, China). The high purity nitrogen gas (99.999%) as carrier gas and methane gas (99.99%) as reagent gas were obtained from local suppliers.

17 pyrethroid standards listed in Table 1 and internal standard (IS) trans-cypermethrin-D_6 (100 mg L^{-1} in acetone) were all purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany) with the highest available purity grade (all higher than 90%). Individual standard stock solutions were prepared in acetone at concentration of 1000 mg L^{-1}. Mixed stock standard solution was prepared from individual standard stock solutions and was further diluted to 1 mg L^{-1} as working solution. IS solution was also diluted to 1 mg L^{-1} with acetone as working solution. All standard and working solutions were stored at 4 °C and proved to be stable for at least one month.

2.2. Sample preparation

Definite amount of homogenized sample (10 g for garlic, onion and spring onion, and 5 g for chili) was spiked with 0.2 mL of the 1 mg L^{-1} IS solution (corresponding to 20 μg kg^{-1} in garlic, onion and spring onion, and 40 μg kg^{-1} in chili) before extraction. MeCN acidified with 1% HAc (acidified MeCN) and saturated by hexane was used as extraction solvent. After briefly vortexing the beaker flask containing the sample, 20 mL extraction solvent and 4 g anh. $MgSO_4$ were added. After shaking for 30 min, the mixture was filtered to a 150 mL glass flask. Then, another 20 mL extraction solvent was added to the filtered residue and the extraction procedure was repeated once more. The filtrate was combined and evaporated to dryness with a rotary evaporator (BüCHI, Switzerland), and then redissolved in 2 mL MeCN and transferred to a 10 mL glass tube for the cleanup procedure.

For garlic, onion, spring onion and chili samples, 200 mg PSA was added. Subsequently, different amounts of GCB (100, 50, 150 and 300 mg, respectively) were used according to the content of pigments. That is, GCB was applied until the extract became colorless or almost colorless. In addition, 150 mg C_{18} was added to chili extract to remove oil. The mixture was vortexed for 1 min and then filtered through a 0.45 μm Nylon 66 filter (Jinteng Experiment Equipment, Tianjin, China) prior to GC-NCI/MS analysis.

2.3. GC-NCI/MS analysis

An Agilent 7890A GC (Agilent Technologies, Palo Alto, CA, USA) equipped with a split/splitless injector and a HP-5ms capillary column (30 m × 0.25 mm id, 0.25 μm film thickness, J & W Scientific, Folson, CA, USA) and coupled with an Agilent 5975C mass selective detector was employed. An injection volume of 1 μL was applied in pulsed splitless mode. Temperatures of ion source and quadrupole were both 150 °C. The injector and the transfer line temperatures were 300 °C and 280 °C, respectively. The temperature program of the oven was as follows: initial temperature 100 °C (hold 2 min), increased at 30 °C min^{-1} to 220 °C, then at 1 °C min^{-1} to 225 °C, then at 5 °C min^{-1} to 280 °C, and finally at 20 °C min^{-1} to 300 °C. Characteristic ions used for quantification and identification in SIM mode are listed in Table 1. Data analysis was carried out using Agilent Chemstation software. Calibration curves were set up using five pyrethroid standards at concentrations of 0.025, 0.05, 0.1, 0.2 and 0.4 mg L^{-1} while the concentration of IS was kept at 0.1 mg L^{-1}.

2.4. Recovery study

For recovery test, fortified samples were prepared by adding 100, 200 and 300 μL mixed standard solution into 5 g or 10 g blank samples (detected previously to confirm no pesticides contained), corresponding to 10, 20 and 30 μg kg^{-1} in garlic, onion and spring onion, and 20, 40 and 60 μg kg^{-1} in chili. The spiked samples were left to stand for 30 min before extraction to allow the pesticides to penetrate into the matrix and then processed according to the procedures described in Section 2.2. Recovery value was expressed as the percentage of GC–MS determined concentration against fortified concentration.

2.5. Isomerization of pyrethroids

Chili samples from two different locations (Chili 1 and Chili 2) were used to study the isomerization of pyrethroids. Matrix extracts were prepared by the modified QuEChERS method described in Section 2.2, that is, 5 g of homogenized chili was extracted twice with extraction solvent and then evaporated to dryness. The remaining was redissolved in 2 mL MeCN or acidified MeCN and processed throughout the cleanup procedure. 10 μL of

Table 1
SIM target and qualifier ions for GC–MS, and calibration curves of 17 pyrethroid pesticides.

Pesticides	Retention time/min	Characteristic ions[a]	Linear range/mg L^{-1}	Correlation coefficient (r^2)
Tefluthrin	5.11	241*, 382, 205, 243	0.025–0.4	0.9999
Fenfluthrin	5.34	207*, 209, 171	0.025–0.4	0.9999
Transfluthrin	5.60	207*, 209, 171	0.025–0.4	0.9999
Allethrin	6.96	167*, 134, 168	0.025–0.4	0.9997
Prallethrin	7.23	167*, 132, 168	0.025–0.4	0.9999
Tetramethrin	12.04, 12.28	331*, 165, 332	0.025–0.4	0.9991
Bifenthrin	12.31	386*, 241, 387, 205	0.025–0.4	0.9987
Fenpropathrin	12.51	141*, 142, 143	0.025–0.4	0.9998
Lambda-cyhalothrin	13.86, 14.23	241*, 205, 243	0.025–0.4	0.9997
Acrinathrin	14.24, 14.46	333*, 305, 334 167	0.025–0.4	0.9995
Permethrin	15.57, 15.82	207*, 390, 209, 171	0.025–0.4	0.9966
Cyfluthrin	16.77, 16.96, 17.10, 17.19	207*, 171, 209	0.025–0.4	0.9997
Cypermethrin	17.36, 17.56, 17.70, 17.79	207*, 171, 209	0.025–0.4	0.9994
trans-Cypermethrin-D_6	17.50, 17.72	213*, 215, 177	0.025–0.4	
Flucythrinate	17.81, 18.20	243*, 244, 245	0.025–0.4	0.9996
Fenvalerate	19.12, 19.52	211*, 213, 212	0.025–0.4	0.9996
Fluvalinate	19.57, 19.71	294*, 296, 502	0.025–0.4	0.9997
Deltamethrin	20.15, 20.50	297*, 295, 505, 217	0.025–0.4	0.9994

[a] Quantification ions were marked with asterisk.

the 10 mg L^{-1} mixed standard solution of 17 pyrethroids was added to 990 μL chili extract to reach a concentration of 0.10 mg L^{-1}. Pyrethroid solution in diluted matrix extract was prepared by adding 10 μL of the 10 mg L^{-1} mixed standard solution and 100 μL matrix extract (redissolved in MeCN) to 890 μL MeCN, which means the matrix concentration was approximately diluted 10 times. Standard solutions for the study of isomerization were prepared by diluting the 10 mg L^{-1} mixed standard solution to 0.10 mg L^{-1} with MeCN or acidified MeCN.

3. Results and discussion

3.1. Modification of conventional QuEChERS

The classical QuEChERS method involves extraction with MeCN or acidified MeCN, partition between MeCN and aqueous phase after addition of sodium chloride and anh. $MgSO_4$, and a DSPE cleanup step with a small quantity of SPE adsorbents (PSA, GCB and/or C_{18}). In order to improve the extraction performance, acidified MeCN saturated by hexane was employed as the extraction solvent instead of pure acidified MeCN. To evaluate the extraction efficiency of two different solvents mentioned above, garlic samples spiked at 10 μg kg^{-1} were extracted using pure acidified MeCN and hexane-saturated acidified MeCN respectively, and processed throughout the whole sample preparation procedures. Recoveries of pyrethroids extracted with both solvents are shown in Fig. 1. It could be concluded that acidified MeCN saturated with hexane was superior to pure acidified MeCN. This may be explained that hexane has weaker polarity than MeCN, and therefore the extraction efficiency of pyrethroids from matrices was increased with hexane-saturated MeCN whose polarity is closer to pyrethroids than pure MeCN. In addition, hexane-saturated MeCN was conducive to remove water subsequently due to the lower hydrophilicity.

To learn the loss of analytes during sample preparation, recoveries of pyrethroids during extraction and cleanup steps in garlic were determined respectively. Recoveries of extraction were between 57.2% and 72.9%, which indicated the efficiency of extracting all 17 pyrethroids from garlic was acceptable. The loss during cleanup step was very small with the recoveries ranged from 82.4% to 119.3%.

Besides, as shown in Fig. 2, the GC-NCI/MS chromatograms of these four sulfur-rich or heavily lipidic matrices were quite "clean". None serious interfering peaks were observed around the retention time of each compound analyzed. This was credited to the high selectivity of NCI, and necessarily, the effective cleanup procedure. It could be reasonably inferred that the modified QuEChERS method was suitable for the determination of pyrethroid pesticides in these troublesome matrices (Table 2).

3.2. Method validation

The present work was validated according to the European SANCO guidelines and the ISO/ECC 17025 norm.

3.2.1. Linearity and limit of detection (LOD)

GC retention behaviors and NCI/MS responses of tefluthrin, fenfluthrin and transfluthrin significantly differed from IS trans-cypermethrin-D_6 presumably because there are four or five fluorine atoms on benzene ring in their molecules resulting in higher polarity. Thus these three pyrethroids were not calibrated with IS while the rest 14 pyrethroids were all calibrated with IS. Pyrethroids

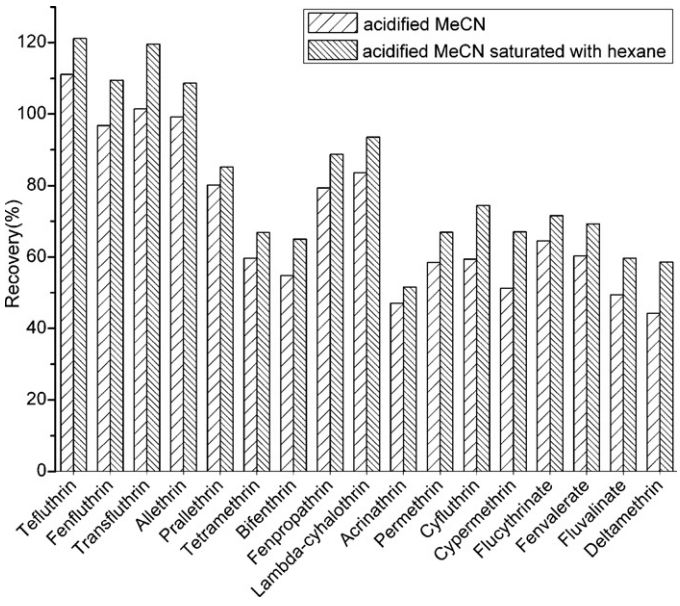

Fig. 1. Effect of extract solvent on the recovery of 17 pyrethroids spiked at 10 μg kg^{-1} in garlic. Recovery of each pyrethroid was calculated by solvent-based calibration without IS.

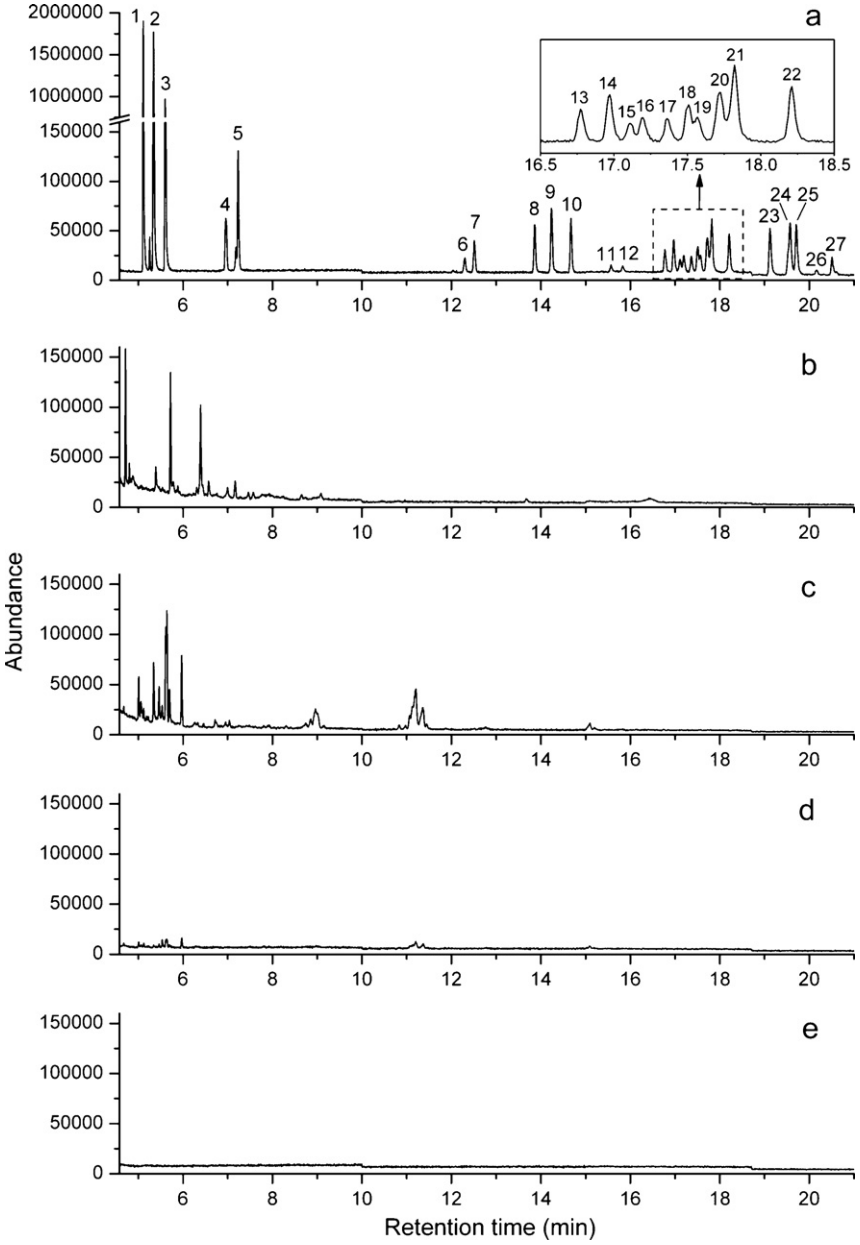

Fig. 2. Total ion chromatograms (TICs) of mixed standard solution (0.1 μg mL^{-1}) (a), blank matrix solutions of garlic (b), onion(c), spring onion (d) and chili (e). Peaks: 1. Tefluthrin; 2. Fenfluthrin; 3. Transfluthrin; 4. Allethrin; 5. Prallethrin; 6. Tetramethrin (2), Bifenthrin; 7. Fenpropathrin; 8. Lambda-cyhalothrin (1); 9. Lambda-cyhalothrin (2), Acrinathrin (1); 10. Acrinathrin (2); 11. Permethrin (1); 12. Permethrin (2); 13. Cyfluthrin (1); 14. Cyfluthrin (2); 15. Cyfluthrin (3); 16. Cyfluthrin (4); 17. Cypermethrin (1); 18. Cypermethrin (2), trans-Cypermethrin-D_6 (1); 19. Cypermethrin (3); 20. Cypermethrin (4), trans-Cypermethrin-D_6 (2); 21. Flucythrinate (1); 22. Flucythrinate (2); 23. Fenvalerate (1); 24. Fenvalerate (2), Fluvalinate (1); 25. Fluvalinate (2); 26. Deltamethrin (1); 27. Deltamethrin (2). The number in parenthesis is to distinguish among the isomers of each compound. Peak of tetramethrin (1) was not assigned because this peak was very tiny.

which have more than one peak owing to the existence of diasteroisomers were calibrated and quantified by area sum of isomers peaks.

Linear range was determined using pyrethroid standard solutions. Excellent linearity of calibration curves was achieved in the range of 0.025–0.4 mg L^{-1}, and the values of correlation coefficient (r^2) of the linear regression were higher than 0.996 for all pyrethroids (Table 1).

Limits of quantitation (LOQs) of the method were determined using the extracts of spiked samples at levels approaching the limits as the references. LOQ and LOD were calculated on the basis of ten and three S/N, respectively. Due to the high S/N in NCI, extremely low LODs and LOQs of pyrethroids in all four troublesome matrices were achieved in the range of 0.02–6 μg kg^{-1} and 0.08–20 μg kg^{-1}, respectively (Table 3). The LOQ and LOD of permethrin were relatively higher because permethrin is less electronegative and hence generated lower NCI/MS response than other pyrethroids. The LODs of pyrethroids in GC-NCI/MS were much lower than those in vegetables and fruits achieved by GC-ECD (15 μg kg^{-1} for orange sample) [6], and those for garlic samples were lower than the results of three pyrethroids determined in the same matrix obtained by GC and ion trap MS in selective ion storage (SIS) mode (0.08, 0.13 and 0.11 mg kg^{-1} for beta-cypermethrin, fenvalerate and deltamethrin, respectively) [16]. Moreover, the sensitivity can far satisfy the requirement of maximum residue limits (MRLs, 0.01–5.0 mg kg^{-1}) set by European Union (EU), United States Environmental Protection Agency (EPA) and Japan.

Table 2
Recoveries of 17 pyrethroids spiked in garlic at different steps of sample preparation.

Pesticides	Recovery/%[a]		
	Extraction	Cleanup	Total sample preparation
Tefluthrin	60.2	111.6	64.8
Fenfluthrin	63.0	110.3	68.8
Transfluthrin	65.7	114.4	73.3
Allethrin	65.3	107.4	69.1
Prallethrin	65.9	111.6	72.5
Tetramethrin	67.8	112.5	70.0
Bifenthrin	60.0	96.8	53.7
Fenpropathrin	64.4	119.3	73.4
Lambda-cyhalothrin	70.0	113.2	73.8
Acrinathrin	58.2	97.9	56.7
Permethrin	59.6	108.1	63.3
Cyfluthrin	59.8	112.5	63.4
Cypermethrin	57.2	108.3	56.1
Flucythrinate	67.8	104.1	68.8
Fenvalerate	67.4	105.1	66.1
Fluvalinate	62.6	95.5	56.3
Deltamethrin	72.9	82.4	57.7

[a] Calculated by solvent-based calibration without IS.

3.2.2. Recovery and precision

Recovery tests were performed at 10, 20 and 30 μg kg^{-1} fortification levels of each pyrethroid in garlic, onion and spring onion, and at 20, 40 and 60 μg kg^{-1} fortification levels in chili. Recoveries of all 17 pyrethroids ranged from 59.8% to 126.0% in garlic, 62.6% to 129.8% in onion, 77.5% to 123.3% in spring onion, and 54.0% to 124.0% in chili, and relative standard deviations (RSDs) were all below 14% (Table 4). Good Recoveries were obtained in the range of 70–130% except for fenvalerate and deltamethrin in onion and chili.

As a well-known knowledge, matrix-matched calibration is the most effective and accurate way to deal with matrix effect and analyte loss during sample preparation. However, the preparation of matrix-matched calibration solutions is quite inconvenient and time consuming. With the solvent-based IS calibration, acceptable recoveries and RSDs were obtained for all 17 pyrethroids and no serious matrix effects were observed. Therefore, the solvent-based calibration was employed in this method.

3.3. Isomerization of pyrethroids

Synthetic pyrethroid pesticides could be divided into two types on the basis of the existence of an α-cyano group. Type I pyrethroids do not contain α-cyano group in their molecules and thus only have two chiral centers generated by the cyclopropane ring. Therefore, type I pyrethroids have four stereoisomers, including two diasteroisomers. Type II pyrethroids have an additional asymmetric carbon atom due to the α-cyano group. Therefore, type II pyrethroids such as acrinathrin and deltamethrin have eight stereoisomers, including four diasteroisomers. At most two peaks for type I and four peaks for type II pyrethroids were expected on chromatograms for the reason that only diasteroisomers could be separated on non-chiral GC columns according to the difference of physicochemical properties.

Pyrethroids may undergo isomerization under the exposure to heat, light or organic solvent [27]. It was reported by Maštovská and Lehotay [28] and You and Lydy [29] that isomerization of pyrethroids differed in various matrices and solvents, and under different GC conditions. It was also confirmed that isomerization of pyrethroids was suppressed in acidic matrices or solvents, which could be explained that acid blocked the epimerization reaction that occurred by means of α-proton exchange with polar solvent [29]. But none of these researches mentioned the situation of isomer conversion in basic matrices or solvents.

Isomerization of pyrethroids was investigated during our GC analysis. Isomer ratios of all pyrethroids remained basically the same in garlic, onion and spring onion matrices as in standard solutions prepared in MeCN. At the same time, significantly different phenomenon of isomer conversion of several type II pyrethroids was observed in chili. Compared with those in pure solvent and other matrices studied, ratios of the major isomers in chili decreased, which means isomerization of these pyrethroids was accelerated by matrix components of chili. The maximum change of isomer ratios befell acrinathrin and deltamethrin, with a decrease of 27% and 30% in the ratio of major isomers respectively. Chili contains large amount of basic compounds such as capsaicin. It could be speculated that such basic components induced α-proton exchange reaction resulting in isomer conversion.

Another chili sample produced from a different location (Chili 2) was used to confirm the experiment result of isomerization. Similar result to Chili 1 was obtained in Chili 2 except the degrees of isomerization were slightly smaller than those in Chili 1. It could be explained that the content of basic components in chili varied with different production locations and different variety of capsicums (Table 5).

The influences of acidification and matrix concentration were also studied. According to the isomer ratios listed in Table 6, HAc added in matrix-matched solution could hardly neutralize the

Table 3
LODs and LOQs of pyrethroid pesticides in different matrices (μg kg^{-1}).

Pesticides	LOD				LOQ			
	Garlic	Onion	Spring onion	Chili	Garlic	Onion	Spring onion	Chili
Tefluthrin	0.06	0.02	0.03	0.06	0.2	0.08	0.09	0.2
Fenfluthrin	0.09	0.06	0.08	0.1	0.3	0.2	0.3	0.3
Transfluthrin	0.2	0.2	0.1	0.4	0.8	0.8	0.4	1.3
Allethrin	2.0	2.7	1.2	1.0	6.7	9.1	4.0	3.3
Prallethrin	1.5	2.0	1.0	0.5	5.0	6.7	3.3	1.7
Tetramethrin	2.0	3.0	1.2	2.0	6.7	10	4.0	6.7
Bifenthrin	1.1	2.0	1.5	1.2	3.6	6.7	5.0	4.0
Fenpropathrin	2.0	2.1	2.0	1.0	6.7	7.1	6.7	3.3
Lambda-cyhalothrin	1.2	1.8	0.9	0.5	4.0	5.9	3.0	1.8
Acrinathrin	0.6	0.6	0.3	1.2	2.0	2.0	1.0	4.0
Permethrin	6.0	5.0	5.0	5.5	20	17	17	18
Cyfluthrin	2.0	1.5	1.5	1.3	6.7	5.0	5.0	4.4
Cypermethrin	2.5	2.3	1.5	1.1	8.3	7.7	5.0	3.6
Flucythrinate	1.0	1.7	0.6	0.9	3.3	5.6	2.0	3.1
Fenvalerate	1.2	2.5	0.4	0.5	4.0	8.3	1.4	1.5
Fluvalinate	1.2	2.0	0.4	2.6	4.0	6. 7	1.2	8.7
Deltamethrin	2.5	2.5	1.5	2.4	8.3	8.3	5.0	8.0

Table 4
Recoveries of pyrethroid pesticides at three spiked levels in different matrices.[a]

Pesticides	Garlic			Onion			Spring onion			Chili		
	Standard spiked/μg kg^{-1}											
	10	20	30	10	20	30	10	20	30	20	40	60
Tefluthrin	121.1 (9)	106.1 (9)	102.8 (8)	105.4 (4)	98.2 (4)	92.1 (2)	102.7 (3)	95.2 (4)	89.4 (2)	81.9 (1)	81.1 (2)	79.6 (2)
Fenfluthrin	109.6 (7)	100.9 (7)	96.4 (7)	108.9 (1)	98.9 (4)	91.3 (2)	100.6 (3)	91.5 (2)	85.6 (2)	84.5 (3)	83.4 (2)	81.5 (3)
Transfluthrin	119.5 (9)	113.9 (8)	109.0 (6)	104.4 (2)	100.8 (5)	92.0 (2)	102.5 (2)	92.5 (4)	88.2 (4)	85.3 (3)	82.2 (2)	80.3 (5)
Allethrin	123.3 (8)	122.0 (4)	118.8 (6)	129.1 (5)	127.6 (5)	129.8 (4)	110.4 (6)	107.8 (5)	111.9 (6)	114.0 (3)	124.0 (7)	115.9 (4)
Prallethrin	118.9 (5)	120.5 (6)	112.5 (8)	106.5 (6)	122.4 (2)	116.5 (6)	108.0 (6)	109.5 (5)	114.2 (6)	103.1 (6)	118.7 (6)	118.6 (4)
Tetramethrin	100.4 (6)	106.4 (6)	91.3 (8)	95.4 (2)	104.1 (10)	106.9 (5)	123.3 (6)	108.1 (4)	107.5 (3)	103.6 (7)	108.2 (3)	94.5 (1)
Bifenthrin	94.1 (12)	81.5 (11)	70.3 (6)	77.0 (7.)	75.8 (10)	72.0 (7)	90.7 (5)	87.7 (7)	86.4 (7)	103.3 (2)	86.6 (4)	76.9 (1)
Fenpropathrin	121.2 (4)	119.0 (9)	99.3 (7)	89.8 (10)	87.0 (1)	78.5 (6)	102.7 (2)	106.6 (5)	105.0 (4)	80.4 (4)	88.4 (7)	83.2 (3)
Lambda-cyhalothrin	126.0 (2)	109.6 (8)	95.5 (8)	96.9 (4)	82.2 (4)	82.0 (6)	106.9 (2)	105.4 (6)	108.0 (2)	87.9 (10)	120.4 (6)	114.5 (4)
Acrinathrin	77.0 (9)	65.4 (10)	59.6 (5)	86.0 (4)	75.1 (6)	76.8 (3)	92.6 (2)	92.4 (4)	92.0 (3)	107.0 (3)	102.0 (3)	101.2 (1)
Permethrin	112.4 (6)	109.3 (6)	107.1 (5)	117.3 (10)	109.9 (4)	102.7 (2)	93.9 (6)	87.6 (2)	103.4 (7)	104.3 (4)	109.7 (4)	99.5 (6)
Cyfluthrin	106.7 (10)	99.7 (6)	89.5 (5)	89.8 (12)	88.2 (2)	86.1 (4)	96.4 (4)	102.1 (3)	99.8 (3)	105.0 (3)	97.9 (2)	92.5 (2)
Cypermethrin	100.1 (12)	98.6 (12)	87.2 (4)	92.4 (2)	84.3 (7)	82.6 (6)	91.6 (7)	96.5 (4)	96.2 (4)	89.5 (12)	90.1 (7)	91.7 (5)
Flucythrinate	98.8 (6)	87.4 (6)	76.0 (7)	103.9 (3)	89.9 (2)	87.4 (7)	91.5 (4)	93.9 (3)	92.5 (3)	118.5 (2)	116.5 (3)	110.6 (2)
Fenvalerate	85.8 (8)	74.7 (8)	64.9 (4)	81.6 (3)	69.5 (3)	65.7 (6)	82.8 (2)	83.6 (5)	83.5 (8)	65.3 (7)	54.0 (6)	56.6 (2)
Fluvalinate	97.9 (5)	87.9 (7)	75.0 (5)	91.2 (5)	77.8 (1)	76.3 (3)	90.3 (4)	93.7 (3)	92.7 (2)	79.9 (8)	77.8 (4)	74.9 (3)
Deltamethrin	97.8 (10)	75.6 (10)	69.8 (13)	72.5 (4)	62.6 (10)	64.2 (14)	77.5 (4)	78.2 (2)	78.9 (8)	80.0 (12)	64.2 (6)	59.3 (5)

[a] $n = 6$, that is, six same samples were fortified at each spiked level. Values in parentheses are RSDs (%).

effect of basic components. When the matrix concentration was diluted ten times, enhancement of isomerization decreased compared to that in original chili extracts.

It was stated that isomer ratios changed significantly after GC maintenances, e.g. replacing the contaminated liner and cutting initial portion of the deteriorated GC column, and assumed that isomerization depended on the active sites of the GC inlet system [28,29]. In the present study, pyrethroids dissolved in pure solvent and chili extract were both determined before and immediately after the GC liner was replaced and the front part of the GC column was cut, but no obvious changes were seen in either solution. The maximum variation appeared in acrinathrin dissolved in chili extract with a 3% increase in the ratio of major isomer after GC maintenance, which was relatively small compared to the decrease of major isomer ratio in chili extract mentioned above. That is, the change in amount of active sites had minor contribution to isomer conversions. Isomerization of type II pyrethroids in chili extract should be mainly attributed to basic components inducing α-proton exchange.

Table 5
Comparison of isomerization of pyrethroid pesticides in pure solvent and matrix extract.

Pesticides	Isomer ratio/%[a]				
	MeCN[b]	Garlic[c]	Onion[c]	Spring onion[c]	Chili[c]
trans-Cypermethrin-D_6 (IS)	50:50	50:50	50:50	50:50	50:50
Tetramethrin	23:77	25:75	25:75	25:75	20:80
Lambda-cyhalothrin	47:53	52:48	46:54	47:53	49:51
Acrinathrin	17:83	17:83	18:82	17:83	44:56
Permethrin	49:51	44:56	44:56	50:50	57:43
Cyfluthrin	26:37:17:20	27:38:17:18	25:37:17:21	27:38:16:19	24:29:19:28
Cypermethrin	29:25:26:20	29:26:25:20	28:27:24:21	28:26:25:21	26:25:23:26
Flucythrinate	54:46	54:46	54:46	53:47	60:40
Fenvalerate	69:31	68:32	66:34	68:32	60:40
Fluvalinate	48:52	49:51	49:51	49:51	53:47
Deltamethrin	21:79	20:80	20:80	22:78	51:49

[a] Calculated by peak area ratio of each isomer under the same GC condition.
[b] Average of peak area ratio of each isomer in 5 standard solutions with a concentration range of 0.025–0.4 mg L^{-1}.
[c] Average of peak area ratio of each isomer in 18 spiked samples used in recovery test.

Table 6
Effects of sample variety, acidification and matrix concentration on isomerization of pyrethroid pesticides.

Pesticides	Isomer ratio/%*					
	MeCN		Chili 1 extract			Chili 2 extract
	Original	0.1% HAc	Original	Diluted 10 times	0.1% HAc	Original
Tetramethrin	24:76	25:75	20:80	21:79	19:81	20:80
Lambda-cyhalothrin	47:53	45:55	48:52	51:49	47:53	47:53
Acrinathrin	18:82	18:82	47:53	39:61	45:55	34:66
Permethrin	47:53	58:42	53:47	53:47	57:43	59:41
Cyfluthrin	27:36:18:19	28:37:16:19	24:29:20:27	23:30:18:29	23:29:19:29	24:30:19:27
Cypermethrin	30:23:26:21	31:25:24:20	27:24:24:25	29:22:26:23	27:24:23:26	27:23:27:23
Flucythrinate	55:44	56:44	60:40	61:39	61:39	62:38
Fenvalerate	69:31	69:31	62:38	62:38	62:38	62:38
Fluvalinate	48:52	47:53	53:47	53:47	48:52	52:48
Deltamethrin	23:77	21:79	53:47	46:54	49:51	49:51

* Obtained under the same GC condition and calculated by peak area ratio of each isomer.

4. Conclusions

A simple, rapid and effective QuEChERS-GC-NCI/MS method was developed for the simultaneous determination of 17 pyrethroid pesticides in troublesome matrices: garlic, onion, spring onion and chili. Better extraction efficiency was obtained by amending classical QuEChERS method using hexane-saturated acidified MeCN as extraction solvent instead of pure acidified MeCN. Due to the high sensitivity and selectivity of NCI, low LODs were achieved in the range of 0.02–6 $\mu g\,kg^{-1}$, which had the advantage of other published methods dealing with these troublesome matrices and could meet the requirements of residue analysis well. Satisfactory accuracy and precision were also acquired with recoveries ranged from 70% to 130% except for fenvalerate and deltamethrin in onion and chili and RSDs below 14%. Isomerization of pyrethroids in different solutions and matrices were also discussed. Basic matrix components in chili extracts accelerated the isomerization of type II pyrethroids, resulting in the decrease of major isomer ratios compared to pure solvent and other matrices. This finding would be important information for the analysis and agricultural use of pyrethroids.

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