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Determination of Spirotetramat in Complex Matrices Combining QuEChERs and Solid Phase Extraction Followed by High Performance Liquid Chromatography

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A new sample preparation procedure combining QuEChERs and solid phase extraction was optimized for the determination at trace levels of spirotetramat in complex matrices by high performance liquid chromatography with ultraviolet detector (HPLC-UVD). Seven foods (tea, agaric, celery, leek, citrus, soybean and walnuts) were selected as complex matrices for validating this new method. The target analyte was extracted with acetonitrile and cleaned up by Cleanert TPH and the recoveries of spirotetramat in seven foods were in range of 76.9 to 106.7 % with relative standard deviation of 1.1 to 10.8 % at three spiking levels. The limit of quantification of spirotetramat in tea, agaric, celery, leek, citrus, soybean and walnuts were 0.1, 0.1, 0.2, 0.2, 0.1, 0.1 and 0.05 mg/kg, respectively. The proposed method was applied to determine the residue of spirotetramat in seven samples from regional retailers in Beijing and no spirotetramat was detected.

Keywords: Spirotetramat, QuEChERS, Solid phase extraction, Complex matrix, High performance liquid chromatography.

INTRODUCTION

Recently, pesticide residue in agricultural and sideline products has been of concern to human health[1,2]. Sample preparation such as QuEChERs (Quick, Easy, Cheap, Effective, Rugged and Safe) combined with liquid-liquid extraction (LLE), solid-phase extraction (SPE), dispersive liquid-liquid microextraction (DLLME), dispersive solid-phase extraction (DSPE) and matrix solid-phase dispersion (MSPD) have been reported for extraction of pesticide residues from different samples. The QuEChERs procedure is widely used for multi-residue analysis of pesticides in fruits and vegetables[3], which uses acetonitrile as extraction solvent and dispersive solid phase extraction (DSPE) for cleanup[4] and determined with mass spectrometry (MS). Generally, dispersive solid phase extraction cleanup adsorbents are primary secondary amine (PSA), C18 alkyl-silicone (C18), graphitized carbon black (GCB), florisil, neutral alumina and new materials such as multi-walled carbon nanotubes[5], *etc*. However, the dispersive solid phase extraction cleanup performance was not good enough to remove complex matrices interferences[6,7] such as tea, herbs and soft drinks when the followed determination was not using mass, respectively.

Spirotetramat [ethyl *cis*-3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl carbonate, Fig. 1] is a novel spirocyclic tetramic acid insecticide[8], which affects lipid biosynthesis inhibitor through inhibition of acetyl CoA carboxylase and is transported within both the xylem and phloem[9,10]. Because of its excellent systemic and trans-laminar efficacy, spirotetramat was applied to control a broad spectrum of sucking pests in pepper, chilli, mango, kiwifruit and cotton, *etc.*[11-14]. Owing to no crossing resistance to any other chemical insecticides[15], spirotetramat has been an invaluable new tool to control pests in many crops worldwide. Spirotetramat has no significant impact on arthropods, however, it exhibits a skin-sensitization potential in animals and humans[16,17].

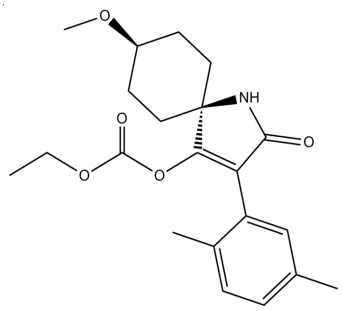

Fig. 1. Chemical structure of spirotetramat

To date, few methods for determination of spirotetramat in environmental samples have been reported. For example, high performance liquid chromatography-photo diode array (HPLC-PDA) was applied for residues analysis of mango and cabbage[18] and high performance liquid chromatography/tandem mass spectrometry (HPLC-MS/MS) was for determination of spirotetramat in some fruits and vegetables[19,20].

The maximum residue limits (MRLs) of spirotetramat in foods have been regulated 0.25-13 mg/kg by US and 0.1-15.0 mg/kg by European Union[19]. However, the residue determination of spirotetramat in complex matrices such as tea, agaric, celery, leek, citrus, soybean and walnuts are not reported in literature.

In this study, acetonitrile was used to extract spirotetramat in complex matrices such as tea, agaric, celery, leek, citrus, soybean and walnuts, cleaned up by Cleanert TPH and then analyzed by HPLC-UVD. Therefore, a modified QuEChERS combine with solid phase extraction method for spirotetramat determination in complex matrices was developed.

EXPERIMENTAL

Standard of spirotetramat (purity > 99.6 %), was obtained from Sigma-Aldrich (Shanghai) Trading Co., Ltd., China. HPLC grade acetonitrile, acetone and *n*-hexane were purchased from Fisher Scientific (USA). Analytical reagent grade sodium chloride and anhydrous magnesium sulfate purchased from Sinopharm Chemical Reagent Co., Ltd., China and were heated at 130 °C for over 12 h before use and kept in desiccators. Redistilled water was purified with a Milli-Q system (Millipore, USA). Cleanert TPH (1000 mg, 6 mL) was purchased from Agela Technologies, Tianjin, China. Individual stock standard solution of spirotetramat 1000 mg/L was prepared in acetonitrile and stored in the refrigerator at -20 °C. The working standard solutions were obtained by diluting the stock standard solutions as required with acetonitrile. All standard solutions were stored at -20 °C. Tea, agaric, celery, leek, citrus, soybean and walnuts samples free of spirotetramat were purchased from a local supermarket of Beijing, China. Then the samples were put into a stainless steel blender to be homogenized and stored in a refrigerator at 4 °C before preparation.

Balance 1602MP8-1 (readability 0.1 mg) was purchased from Sartorius AG, Germany. Balance JY2002 (readability 0.01 g) was purchased from the Shanghai Precision & Scientific Instrument Co. Ltd., China. Vortex mixer QL-861 was purchased from the Haimen Qilinbeier Instrument Manufacturing Co. Ltd., Jiangsu, China. Centrifuge TDL-40B was purchased from the Shanghai Anting Scientific Instrument Factory, China. Cleanert TPH (1000 mg, 6 mL) was purchased from Agela Technologies, Tianjin, China. PTFE membrane filter (0.22 μm) was purchased from the Beijing Rui Feng Tong Chuang Analysis Instrument Co. Ltd., China.

Sample preparation: 5 g of homogenized tea and agaric sample or 10 g of homogenized celery, leek, citrus, soybean and walnuts sample 10 g were weighed into 50 mL polypropylene centrifuge tubes with screw caps. Then 5 mL water (only for tea and agaric samples) and 10 mL acetonitrile were added and extracted with a vortex mixer for 1 min. Subsequently, 1 g of sodium chloride and 4 g of anhydrous magnesium sulfate were added to provide a well-defined phase separation. It was vortexed for 2 min and centrifuged at RCF 3802 × g for 5 min. 1 mL of the upper layer (acetonitrile phase) was transferred into 100 mL round bottom flask and vacuum evaporated to dryness and then redissolved with 1 mL acetone/*n*-hexane (v/v, 6/4) for cleanup. For cleanup, solid phase extraction cartridge Cleanert TPH was first activated with 5 mL solvent of acetone/*n*-hexane (v/v, 6/4). Then the 1 mL sample extract was applied to the top of the column and eluted with 6 mL of acetone/*n*-hexane (v/v, 6/4) at the speed of 1 mL/min. The eluate was vacuum evaporated to dryness, redissolved with 1 mL acetonitrile and filtered through 0.22 μm PTFE membrane filter and then analyzed by HPLC-UVD.

Detection method: Determination of spirotetramat was achieved using Shimadzu LC 20 AT (Shimadzu, Japan) equipped with SPD-20A UVD. The spirotetramat was separated with a 5 μm reversed phase SUPELCOSILTM LC-18 (4.6 × 250 mm) column (Sigma-Aldrich Co. LLC, USA) at a wavelength of 240 nm. The mobile phase was a mixture of acetonitrile/water (55/45, v/v) and the injection volume was 20 μL. The column temperature was set at 30 °C with a flow rate of 1 mL/min. Total run time was 20 min and the retention time of spirotetramat was 7.5 min.

Method validation: The evaluation of the analytical curve's linearity was done based on injections of the standard solutions prepared in organic solvent acetonitrile at the concentrations 0.05, 0.1, 0.2, 0.5, 1, 2, 5 mg/kg, where this sequence was injected six times (n = 6). Calibration was performed of the average peak areas, calibration curve equation and the determination coefficients (R^2) and linear ranges were determined for spirotetramat at three different levels. A spiked recovery method was based on the accuracy and precision data obtained *via* the recovery determinations in 7 complex matrices. The method LOQ was defined as the lowest validated spike level meeting the requirements of a recovery within the range 70-110 % and a relative standard deviation less than 20 %.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions: Mobile phase composition is an important parameter in adjusting retention time, selectivity and peak shape in HPLC separation[21]. To avoid matrix interference, different proportions of acetonitrile/water (70/30, 60/40, 55/45, 40/60, 30/70) were tested and we found that well-shaped and high response peaks could be achieved when the mobile phase was set at 55/45 (acetonitrile/water, v/v). The flow rate was maintained at 1 mL/min and the column temperature was set at 30 °C. Detected wavelength was selected at 240 nm and the relative retention time of spirotetramat was 7.5 min, with well-shaped peaks and high selectivity. The typical HPLC chromatograms of spirotetramat of standard solution are shown in Fig. 2.

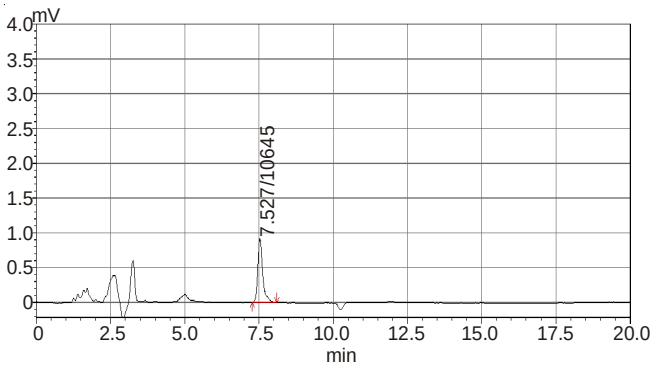

Fig. 2. Typical chromatogram of spirotetramat (0.5 mg/L)

Optimization of sample preparation: One objective of this study was to establish a modified QuEChERS method for spirotetramat in tea, agaric, celery, leek, citrus, soybean and walnuts samples. QuEChERS procedure is widely used for analysis of pesticides in fruits and vegetables[3] and the fruits and vegetables have certain moisture. Tea and agaric were comparatively dry matrices; therefore, it is very common to add a volume of water to the samples to increase the water content and to make the dry samples more accessible by the extraction solvent. And 5 mL of water and 10 mL of acetonitrile and were chosen for tea and agaric extraction. Celery, leek, citrus, soybean and walnuts could be adequately extracted by 10 mL of acetonitrile.

In this study, dispersive solid phase extraction cleanup with PSA (40 µm), C18 (40-60 µm), GCB (30-90 µm), florisil and multi-walled carbon nanotubes were compared with no clean-up (no sorbent) in terms of method recovery and interference from the study matrix. Therefore, 50 mg of PSA, 50 mg of C18, 50 mg of florisil, 20 mg of GCB and 10 mg of multi-walled carbon nanotubes were added to 1 mL spirotetramat standard solution (1 mg/kg), respectively. After pretreatment and analysis, the recoveries of spirotetramat were 91.6, 96.7, 95.7, 95.7 and 84.2 %. The results showed these sorbents was satisfactory for the cleanup of spirotetramat, however, these sorbents was not satisfactory for matrix interference such as tea, agaric, celery and leek. Therefore, solid phase extraction was investigated for cleanup spirotetramat in complex matrices. Cleanert TPH (1000 mg, 6 mL) was tested for the cleanup of spirotetramat. Using 6 mL of acetone/ *n*-hexane (v/v, 7/3, 6/4, 5/5, 4/6, 3/7) as elution solvents, the results showed that recoveries of spirotetramat were 64.4, 93.2, 87.9, 53.9 and 42.1 %, respectively. In addition, elution curve was done and the recovery of spirotetramat was above 90 % by only using 6 mL of acetone/*n*-hexane (v/v, 6/4). Finally, cleanert TPH was chosen for the cleanup tea, agaric, celery, leek, citrus, soybean and walnuts.

Method validation: The linear calibration curve was obtained for spirotetramat by plotting the average peak area against the concentration. The range of the 7 point calibration curve varied from 0.05 to 5 mg/kg. The calibration curves showed good linearity with typical correlation coefficient (R^2) higher than 0.99. The linear equation was y = 25134x + 34.655. The limit of detection (LOD) was expressed as the concentration of the matrix-matched standard which can perform a signal-to-noise (S/N) ratio of 3:1. The LOD of spirotetramat in tea, agaric, celery, leek, citrus, soybean and walnuts were 0.03, 0.03, 0.05, 0.05, 0.03, 0.03 and 0.01 mg/kg, respectively. The limit of quantification (LOQ) for each matrix was considered as the lowest spiked level of spirotetramat. The LOQs of spirotetramat in tea, agaric, celery, leek, citrus, soybean and walnuts were 0.1, 0.1, 0.2, 0.2, 0.1, 0.1 and 0.05 mg/kg, respectively. The main goal of the recovery experiment is to determine the method accuracy, *via* comparison of the real concentration of spirotetramat measured by performing the complete procedure with the known pesticide concentration initially added to the matrix. The method precision is expressed as the repeatability (relative standard deviation, %) of the recovery determinations at the three different spiking levels and each level was done five times (n = 5). In this study, a spiked recovery method was applied in which standard solution was spiked in 7 complex matrices at three levels. A total of five replicate measurements were performed for each concentration level. Table-1 shows the results of recovery at three different spiked concentration levels. Average recovery 76.9 to 106.7 % were obtained for spirotetramat in all 7 matrices and relative standard deviation is less than 10.8 %.

TABLE-1
AVERAGE RECOVERIES OF SPIROTETRAMAT IN TEA, AGARIC, CELERY, LEEK, CITRUS, SOYBEAN AND WALNUT AT THREE SPIKING LEVELS

Matrices	Fortification level (mg/kg)	Mean recovery[a] (%)	Relative standard deviation (%)
Tea	0.1	98.4 ± 1.3	1.3
	1	94.4 ± 6.3	6.7
	2	92.4 ± 3.9	4.2
Agaric	0.1	104.0 ± 7.1	6.8
	1	85.6 ± 4.3	5.0
	2	90.9 ± 4.5	4.9
Celery	0.2	93.6 ± 3.3	3.5
	1.0	90.3 ± 1.4	1.6
	2.0	91.3 ± 1.4	1.3
Leek	0.2	106.7 ± 4.6	4.3
	1.0	86.4 ± 3.6	4.3
	2.0	79.0 ± 4.8	6.2
Citrus	0.1	101.3 ± 6.2	6.1
	0.5	99.4 ± 6.7	6.7
	1	99.0 ± 2.4	2.7
Soybean	0.1	96.3 ± 10.4	10.8
	0.5	87.7 ± 3.3	3.8
	1	78.4 ± 2.9	3.7
Walnut	0.05	88.5 ± 3.3	3.7
	0.5	86.6 ± 1.4	1.7
	1	76.9 ± 0.8	1.1

[a] n = 5 replicates

Application to real samples: The proposed method was applied to determine the residue of spirotetramat in tea, agaric, celery, leek, citrus, soybean and walnuts from regional retailers in Beijing. Thirty samples for each vegetable were collected from markets and no spirotetramat was detected. The most important reason is that typical sample contained spirotetramat has not been obtained since this insecticide is seldom used in Beijing. So further study will be focussed on more samples from different regions.

Conclusion

In this paper, a modified QuEChERS method in combination with solid phase extraction and HPLC-UVD was developed for analysis of spirotetramat in tea, agaric, celery, leek, citrus, soybean and walnuts, which was satisfactory qualitatively as well as quantitatively. The quantification limits achieved were below the maximum residue levels established in US regulation. This method showed reliable validation performances and good cleanup effects, which was efficient and accurate for analysis of spirotetramat in complex samples.

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