Determination of Herbicide Propisochlor in Soil, Water and Rice by Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) Method Using by UPLC-ESI-MS/MS

Xiaohu Wu, Jun Xu, Xingang Liu, Fengshou Dong, Yanbing Wu, Ying Zhang, and Yongquan Zheng

Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Key Laboratory of Integrated Pest Management in Crops, Ministry of Agriculture, Beijing 100193, P.R. China. E-mail: yongquan_zheng@yahoo.com.cn

Introduction

Propisochlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-[1-methylethoxy) methyl] acetamide) with the commercial name of pulebao, is a chloroacetanilide herbicide produced by the company Nitrokéemia 2000 (Hungary) (Figure 1).

It is an important pre-emergent herbicide used to control some broad leaf and annual grass weeds in soybean, peanut, cotton, corn and rice fields. When absorbed through the roots and shoots just above the seed of the target weeds, it acts as a growth inhibitor by suppressing synthesis of protein. However, because of lack of data of propisochlor, it can not conclude that propisochlor is safe to human healthy, food and environment. So the European Union prohibited the registration of propisochlor from 2012. Therefore, a simple, quick and reliable analytical method for the confirmation and quantification of propisochlor has become important for food and environmental safety.

Numerous methods have been published on the determination of propisochlor residues in corn, soybeans, rice and other samples by GC-ECD, GC-NPD, HPLC and GC-MS. However, an efficient analytical method for the determination of propisochlor using ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) has not been developed. And UPLC has led to a higher resolution and sensitivity and a shorter analysis time. In MS/MS, the use of multiple reaction monitoring (MRM) mode results in a significant decrease in detection limits due to an increased signal-to-noise ratio. UPLC in combination with tandem MS has been shown to be a more robust analytical tool for pesticide residue analysis in different matrices.

Some sample preparation methods such as liquid-liquid extraction (LLE), solid-phase extraction (SPE), accelerated solvent extraction (ASE) and solid-phase micro-extraction (SPME) were reported to be used for the extraction of propisochlor, in which these methods are the large quantities of solvent utilized, the multiple operation steps needed, and special materials required and expensive equipment required. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method is an important sample preparation methodology for pesticide residue analysis that was developed in 2003. This methodology is based on the extraction of pesticides from the sample with acetonitrile. Removal of residual water and clean-up are performed simultaneously by using a rapid procedure, called dispersive solid-phase extraction, in which anhydrous magnesium sulfate (MgSO₄) and primary-secondary amine (PSA) sorbent are added before determination, reducing analysis cost, labour, waste, and glassware and increasing sample throughput. This method, owing to many advantages over traditional techniques, has been introduced recently as an attractive alternative method for sample preparation.

Therefore, this paper describes a simple and effective QuEChERS extraction procedure and UPLC-MS/MS technique to determine propisochlor in water, soil and rice (stalks, rice and rice hull). After validation, this method was used in

Figure 1. Chemical structure of propisochlor.

These authors contributed equally to this work.
routine analysis of propisochlor in food and environmental monitoring.

Experimental

Chemicals and Reagents. The analytical propisochlor standard (99.1% purity) was purchased from Shenyang Kefa New Technology Development Company. Propisochlor (30% WP) was obtained from the pesticide factory of Institute of Plant Protection, Chinese Academy of Agricultural Sciences. HPLC grade acetonitrile was purchased from Sigma-Aldrich (Steinheim, Germany), Ultra-pure water was obtained from a Milli-Q system (Bedford, MA, USA). Analytical grade acetonitrile and sodium chloride (NaCl) for pesticide residue analysis were purchased from Beihua Fine-Chemicals Co. (Beijing, China). Analytical-grade MgSO\(_4\) was purchased from Sinopharm Chemical Reagent Co. Ltd (Beijing, China). PSA, GCB and 0.22-μm nylon syringe filters were purchased from Agela Technologies Inc. (Tengda, Tianjin, PRC).

Preparation of Standard Solutions. The stock solution of propisochlor (100 mg/L) was prepared in acetonitrile and serially diluted to produce working solutions of 0.005, 0.01, 0.05, 0.1, 0.5 and 1 mg/L in acetonitrile. All solutions were stored in a refrigerator at −20°C until use.

Instrumentation and LC-MS/MS Analytical Conditions. Chromatographic separation was carried out on a Waters Acquity UPLC binary solvent manager, an Acquity UPLC manager, and an Acuity cartridge heater equipped with a Waters Acquity UPLC BEH Shield RP18 column (100 × 2.1 mm, 1.7 μm particle size; Milford, MA, USA). This column is packed with a C18 reverse-phase bound to an ethylene-bridged hybrid (BEH) substrate. The mobile phases, which were composed of ultrapure water as mobile phase A and acetonitrile as mobile phase B, were pumped at a flow rate of 0.3 mL min\(^{-1}\). The gradient elution was: 0-0.5 min, 90-40% A; 0.5-3.0 min, 40-10% A; 3.0-3.1 min, 10-90%; then held at 90% A for 2.0 min. Separation and stabilization were achieved in 5.1 min. The column was kept at 45°C and the temperature in the auto-sampler was set at 5°C, the injection volume was 5 μL.

Analysis of propisochlor was conducted on a triple-quadrupole mass spectrometer (TQD, Waters Crop.) using the multiple reaction monitoring (MRM) mode and positive ESI mode. The nebulizer gas was 99.95% nitrogen, and the collision was 99.999% argon with a pressure of 2 × 10\(^{-4}\) mbar in the T-wave cell. The conditions were typically as follows: The capillary voltage was set at 3.0 kV, and the cone voltage was 30 V; the source temperature and desolvation temperature were held at 120°C and 350°C, respectively; The cone and desolvation gas were set at a flow of 50 and 500 L h\(^{-1}\) respectively; 284 (m/z) was selected as the precursor ion, and its quantitative and qualitative product ions were 73 (m/z) and 224 (m/z), respectively; when the collision energies were 13 V and 10 V, respectively. Figure 2 shows characteristic fragmentation pattern of propisochlor (MW 283.8). For UPLC analysis, Masslynx NT v.4.1 (Waters) software was used to process quantitative data obtained from the calibration standards and samples. Under the described conditions, the retention time of propisochlor was approximately 1.84 min.

QuEChERS Extraction and Purification. The soil, water, rice stalks, rice, and rice hull were collected from the rice trial field. After collection, the soil samples were air-dried at room temperature, homogenized, and passed through a 2-mm sieve, and the rice hull samples were separated from rice by a threshing machine. Rice, rice hull and rice stalk samples were chopped and homogenized by high speed homogenization, respectively.

Water Samples. The 10 mL water samples were weighed into a 50-mL Teflon centrifuge tube and 20 mL of acetonitrile were added. The tubes were vortexed for 4 min and allowed to stand for 15 min at room temperature. Then 5 g NaCl were added and immediately vortexed vigorously for 1 min and centrifuged for 5 min at RCF 2077 g. Then, the treated samples were filtered through 0.22 mm Nylon syringe filters for UPLC-MS/MS determination.

Soil Samples and Rice Samples. Soil sample (10 g) or rice sample (10 g) respectively was weighed into a 50-mL Teflon centrifuge tube and 20 mL of acetonitrile were added. The tubes were vortexed for 4 min and allowed to stand for 15 min at room temperature. Then 4 g MgSO\(_4\) and 2 g NaCl were added. The tubes were vortexed and immediately vortexed vigorously for 1 min and then centrifuged for 5 min at RCF 2077 g. Then, 1.5 mL of the upper layer (acetonitrile) was transferred into a 2.0 mL micro-centrifuge tube waiting for cleanup.

Rice Stalks Samples and Rice Hull Samples. Rice stalk sample (5 g) or rice hull sample (5 g) was weighed into a 50-mL Teflon centrifuge tube and 20 mL of acetonitrile were added. The tubes were vortexed for 4 min and allowed to stand for 15 min at room temperature. Then 4 g MgSO\(_4\) and 2 g NaCl were added. The tubes were vortexed and immediately vortexed vigorously for 1 min and then centrifuged for 5 min at RCF 2077 g. Then, 4 mL of the upper layer (acetonitrile) was transferred into a 50 mL graduated glass tube, and
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The matrix effects of the target analytes may result in positive or negative responses compared with those produced by solvent solutions, and may greatly affect the method’s accuracy. The occurrence of matrix-induced effects depends on whether or not the extracts contain compounds that will significantly influence the quantity of ionized analyte molecules of reaching the MS/MS path. Therefore, the matrix effect on MS detector of this method using PSA sorbent was studied in five different matrices at

0.005, 0.01 and 0.05 mg/kg spiked levels by comparing standards in solvent with matrix-matched standards in triplicate. The mean relative responses obtained from different sample matrices at different concentrations were shown in Figure 3. From the results of mean relative responses (response matrix/response solvent), the signal reduction that was detected were in the ranges of 0.73-0.99%, 0.46-0.69%, 0.77-0.91% from soil, water and rice hull, respectively, and the signal enhancement in rice stalks ranged from 1.01 to 1.15. Therefore, calibration was performed by external matrix-matched standards to eliminate the matrix effect and to obtain a more realistic determination in this study.

Validation of the Method.

Linearity, LODs and LOQs: The calibration curves obtained for propisochlor (from 0.005 mg/L to 1 mg/L) in different matrices were shown in Table 1. Satisfactory linearities were obtained, where the correlation coefficients (R²) were higher than 0.99 in all cases.

The Limits of detection and quantification (LOD and LOQ), defined as the lowest concentration that the analytical process can reliably differentiate from background levels, were estimated for spiked samples (0.005 mg/kg) based on an S/N of 3:1 and 10:1. As shown in Table 1, the LODs of propisochlor ranged from 0.03 µg/kg to 0.12 µg/kg, and LOQs ranged from 0.1 µg/kg to 0.4 µg/kg in different matrices, which were lower than that of the published methods. These LOQs were also far below the Maximum Residue Limit (MRL) of propisochlor (0.01 mg/kg and 0.05 mg/kg in grains by Hungary and Republic of Korea respectively, 0.1 mg/kg by the EU in soybean). Moreover, there was no MRL for propisochlor in rice. This method may be helpful to establish MRL for propisochlor and monitor it in routine applications.

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Matrix Effects. The matrix effects of the target analytes may result in positive or negative responses compared with those produced by solvent solutions, and may greatly affect the method’s accuracy. The occurrence of matrix-induced effects depends on whether or not the extracts contain compounds that will significantly influence the quantity of ionized analyte molecules of reaching the MS/MS path. Therefore, the matrix effect on MS detector of this method using PSA sorbent was studied in five different matrices at

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food control.

**Recovery, Repeatability and Reproducibility:** Validation of the method was performed in terms of recovery studies before analysis of unknown samples. The recovery and relative standard deviations (RSDr) of propisochlor for water, soil, rice stalks, rice hull, and rice samples were listed in Table 2. The mean recoveries ranged from 78.8% to 87.3% with RSDr of 2.4% to 11.9% for soil, 78.7% to 87.5% with RSDr of 1.1% to 13.9% for water, 83.0% to 94.9% with RSDr of 4.7% to 10.9% for rice, 77.7% to 85.3% with RSDr of 1.4% to 11.9% for rice stalks, and 73.7% to 86.6% with RSDr of 3.4% to 13.5% for rice hull. Figure 3 shows chromatograms of propisochlor standard and rice sample at 0.005 mg/Kg. The results suggested that extraction and clean-up procedure could be suitable for routine analysis of propisochlor in experimental matrices.

The repeatability of the instrument was determined by analyzing the rice spiked at 0.005 mg/Kg. The sample was injected 10 times, and the RSD values obtained for peak areas and retention times by UPLC/MS/MS were 2.6% and 0.18%, respectively. The precision of the method was deter-

<p>| Table 2. Recoveries (n = 5, percent) and RSD (percent) for propisochlor from different matrices in three spiked levels |</p>
<table>
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<tr>
<th>Sample</th>
<th>Spiked level (mg Kg⁻¹)</th>
<th>Intra-day (n = 5)</th>
<th>Inter-day (n = 15)</th>
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<tr>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Average</td>
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<td></td>
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<td>recoveries (%)</td>
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<tr>
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<td></td>
<td>0.05</td>
<td>87.3</td>
<td>8.7</td>
</tr>
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<td>Rice</td>
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<tr>
<td></td>
<td>0.01</td>
<td>88.3</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>88.3</td>
<td>10.9</td>
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<tr>
<td></td>
<td>0.01</td>
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</tr>
<tr>
<td></td>
<td>0.05</td>
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</tr>
<tr>
<td></td>
<td>0.01</td>
<td>73.7</td>
<td>3.6</td>
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<tr>
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<td>0.05</td>
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</table>

| Table 3. Half-life and other statistical parameters for propisochlor dissipation in the rice field conditions |
| Matrix     | Sample location | Regression equation | Correlation coefficient (r) | Half-life (days) |
| Rice stalks| Hunan          | C=1.0089e⁻⁰.⁴⁰⁷⁸t  | 0.8668                       | 1.7 |
|            | Anhui          | C=0.3212e⁻².¹²²t  | 0.7282                       | 5.7 |
|            | Guangxi        | C=0.5461e⁻¹.¹⁴⁴⁷t | 0.9549                       | 4.8 |
| Water      | Hunan          | C=0.9186e⁻⁰.⁴⁹⁴⁷t  | 0.8104                       | 1.5 |
|            | Anhui          | y=0.451e⁻¹.⁶³³⁷x  | 0.9779                       | 1.0 |
|            | Guangxi        | C=1.3662e⁻⁰.⁶⁶⁷t  | 0.9859                       | 1.0 |
| Soil       | Hunan          | C=0.0656e⁻¹.²⁰³⁷t  | 0.8776                       | 2.3 |
|            | Anhui          | C=0.0561e⁻¹.³⁵⁵⁸t  | 0.9845                       | 1.9 |
|            | Guangxi        | C=0.0889e⁻².²²³t  | 0.9717                       | 3.1 |

Figure 4. UPLC-MS/MS ion chromatograms of (a) propisochlor standard, (b) blank rice sample and (c) rice sample at 0.005 mg/Kg.
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Repeatability and reproducibility were determined by comparing standard deviation of the recovery percentages of spiked samples run the same day. The reproducibility RSD was determined with analyzing spiked samples for 3 different days by three operators. The reproducibility ranged from 3.3% to 12.7%, as summarized in Table 2.

Application to Field-treated Samples: A gradual and continuous dissipation of propisochlor residue in water, soil and rice stalks was observed as a function of time after application. The rate equation was calculated from the first-order rate equation: $C = C_0 e^{-kt}$. The half-lives and other statistical parameters of the propisochlor residue dissipation were calculated from the experimental data and summarized in Table 3. The initial concentrations of propisochlor in water 2 h after application were 1.631 mg/kg in Hunan, 0.341 mg/kg in Anhui and 1.113 mg/kg in Guangxi, respectively, which declined to 0.005 mg/kg, 0.003 mg/kg and 0.004 mg/kg after 14 days respectively. The dissipation rates were more than 98% by the 14th day after treatment. The half-lives of propisochlor in water were 1.5 days in Hunan, 1.0 day in Anhui and Guangxi. And the half-lives of propisochlor in soil and rice stalks were from 1.9 to 3.1 days and from 1.7 to 5.7 days, respectively.

Conclusion

A UPLC-MS/MS method for the trace analysis of propisochlor in water, soil and rice stalks (stems, rice and rice hull) were developed in this study. The developed method combined with acetonitrile extraction followed by the dispersive-SPE purification showed satisfactory validation parameters in terms of linearity, lower limits, accuracy and precision, which is also rapid, simple and sensitive for monitoring of propisochlor residue in rice. The degradation dynamics was also studied and the results showed that the decline of propisochlor in rice stalks, soil and water fit a first-order decay process. The half-lives of propisochlor ranged from 1.7 days to 5.7 days in rice stalks, from 1.0 day to 1.5 days in water and from 1.9 days to 3.1 days in soil. This study offered an effective residue analysis method for propisochlor in food and environment.

Acknowledgments. This work was supported by Nature Science Foundation of China (NSFC, 31000863; 31171879) and National Basic Research Program of China (2009CB119000).

References