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A statistical approach to determine fluxapyroxad and its three metabolites in soils, sediment and sludge based on a combination of chemometric tools and a modified quick, easy, cheap, effective, rugged and safe method

Shasha Li[a], Xingang Liu[a,*], Yulong Zhu[a], Fengshou Dong[a], Jun Xu[a], Minmin Li[b], Yongquan Zheng[a,*]

[a] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, PR China

[b] Institute of Agro-products Processing Science and Technology, Chinese Academy of Agricultural Sciences, Beijing 100193, PR China

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ABSTRACT

An effective method for the quantification of fluxapyroxad and its three metabolites in soils, sediment and sludge was developed using ultrahigh performance chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS). Both the extraction and clean-up steps of the QuEChERS procedure were optimised using a chemometric tool, which was expected to facilitate the rapid analysis with minimal procedures. Several operating parameters (MeCN/acetic acid ratio in the extraction solution (i.e., acetic acid percentage), water volume, extraction time, PSA amount, C_{18} amount, and GCB amount) were investigated using a Plackett–Burman (P–B) screening design. Afterward, the significant factors (acetic acid percentage, water volume, and PSA amount) obtained were optimised using central composite design (CCD) combined with the desirability function (DF) to determine the optimum experimental conditions. The optimised procedure provides high-level linearity for all studied compounds with correlation coefficients ranging between 0.9972 and 0.9999. The detection limits were in the range of 0.1 to 1.0 μg/kg and the limits of quantitation (LOQs) were between 0.5 and 3.4 μg/kg with relative standard deviations (RSD) between 2.3% and 9.6% ($n = 6$). Therefore, the developed protocol can serve as a simple and sensitive tool for monitoring fluxapyroxad and its three metabolites in soil, sediment and sludge samples.

1. Introduction

The worldwide need to increase agricultural productivity has led to the extensive use of pesticides. However, it was estimated that more than 99.7% of the applied amount of pesticides are diffused in the environment instead of reaching their target pests [1,2]. Soils, sediments and sludges, as crucial environmental reservoirs, often contain large quantities of pesticides released to the environment where these pesticides undergo biological and physicochemical transformations yielding metabolites [3]. Nevertheless, the number of studies on the occurrence of pesticides and metabolites in these matrices is limited [4–7] possibly due to the large quantities of metabolites produced and the lack of multi-methods for pesticides and particularly for metabolites.

Fluxapyroxad, a second-generation carboxamide fungicides, is a new active ingredient (a.i.) developed by BASF Corporation in 2012 to control a broad spectrum of fungal diseases. 3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxylic acid (C-1), 3-(difluoromethyl)-1*H*-pyrazole-4-carboxylic acid (C-2) and 3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide (C-7) (Fig. S1) are the predominant metabolites of fluxapyroxad in environment samples. These compounds are estimated to be stable towards degradation in soils and/or water/sediment system (half-lives: more than 151 days) [8]. Fluxapyroxad is considered as toxic to aquatic organisms and the metabolite C-1 produces high maternal toxicity at 500 mg/kg bw/day in a developmental range-finding study. Therefore once fluxapyroxad partitions to these environmental compartments, it may accumulate in these matrices resulting in ecotoxicological potential to the underground

* Corresponding authors. Tel.: +86 10 62815938; fax: +86 10 62815938.
E-mail addresses: liuxingang@caas.cn (X. Liu), zhengyongquan@ippcaas.cn (Y. Zheng).

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organisms and other lives ultimately. However, the determination of these chemicals in soils, sediments and sludges is poorly documented. Therefore, it is imperative to develop a reliable analytical method for monitoring fluxapyroxad and its metabolites in soils, sediment and sludge to facilitate the assessment of the risks posed by these chemicals.

The QuEChERS method, introduced by Anastassiades et al. [9], has demonstrated to be an attractive alternative technique for pesticide multiresidue analysis in solid matrices [10]. Nevertheless, the optimisation strategies of the analytical method are commonly based on the study of a one-variable-at-a-time (OVAT) procedure in which only one factor at a time is changed and optimised holding all others fixed. The OVAT method often fails to obtain exact conclusions due to interactions between factors not taken into account. Therefore, it should be replaced by soundly based chemometric approaches [11,12]. This research applied statistical techniques (Plackett–Burman design, central composite design and desirability profile) to identify a stacking modified QuEChERS method for the analysis of fluxapyroxad and its metabolites in soils, sediment and sludge rapidly. The applicability of the proposed method was evaluated to analyse the target compounds in real samples.

2. Experimental

2.1. Reagents and chemicals

Standard fluxapyroxad (99.7% purity) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). The three metabolites C-1 (97% purity), C-2 (97% purity), C-7 (97% purity) were obtained from Boyners Pharmaceutical Co., LTD (Shanghai, China). The standard stock solutions (100 mg/l) of fluxapyroxad and its three metabolites were prepared in pure acetonitrile (MeCN). LC-grade MeCN and methanol were purchased from Sigma–Aldrich (Steinheim, Germany). Sodium chloride (NaCl), anhydrous magnesium sulphate ($MgSO_4$), acetic acid, and MeCN (AR) were purchased from Bei-hua Fine-Chemicals Co. (Beijing, China). Ultra-pure water was obtained from a Milli-Q system (Bedford, MA, USA). PSA (40 μm), C18 (40 μm), and GCB (40 μm) sorbents were purchased from Agela Technologies Inc. (Beijing, China).

2.2. Instrumentation

The analysis of the four compounds was conducted on a Waters ACQUITY UHPLC system (Milford, MA) equipped with a Waters Acquity UHPLC HSS T3 column (2.1 mm × 100 mm, 1.8 μm particle size) the temperature of which was kept at 40 °C. The mobile phase comprised MeCN (A) and 0.02% (v/v) acetic acid in water (B), which were pumped at a flow rate of 0.45 ml/min. The gradient program was as follows: 0 min, 10% A; 1.5 min, 15% A; 2.5 min, 90% A; 2.6 min, 10% A; 5 min, 10%. The injection volume was 3 μl. The compounds were eluted in the following order: fluxapyroxad (3.12 min), C-1 (1.89 min), C-2 (1.17 min) and C-7 (1.36 min). The eluted compounds were then monitored using a triple–quadrupole mass-spectrometer (TQD, Waters Crop., Milford, MA, USA) equipped with an electrospray ionisation (ESI) source. The multiple reaction monitoring (MRM) mode was used for each analyte. The parameters for the MRM transitions, cone voltage and collision energy were listed in Table S1.

2.3. Sample preparation

The preparation and physicochemical characteristics of the soils, sediment and sludge are shown in the supplementary materials.

An aliquot (5.0 g) of dried, finely homogenised sample was weighed into a 50 ml Teflon centrifuge tube. Next 4.2 ml water and 5 ml MeCN (containing 1.2% (v/v) acetic acid) were added. The mixtures were shaken vigorously for 5 min by a shaker. Subsequently, 2 g $MgSO_4$ and 1 g NaCl were added into the mixtures. The tubes were shaken for 2 min. After centrifuging the tubes at 2811 × *g* for 5 min, a 1.5 ml aliquot was transferred into a single-use centrifuge tube containing 50 mg C_{18} and 150 mg anhydrous $MgSO_4$. The tubes were vortexed for 30 s and then centrifugation was performed at 2400 × *g* for 5 min. After centrifugation, an aliquot of 1 ml supernatant was withdrawn into a single-use 5 ml centrifuge tube to be dried under a gentle nitrogen stream until dryness and was then reconstituted with 1 ml MeCN and water (10:90, v/v). The tube was vortexed for 1 min, and the extract was filtered through a 0.22-μm nylon syringe filter and transferred to an autosampler vial for UHPLC–MS/MS injection.

2.4. Experimental design

In this work, P–B and CCD designs combined with DF were used for searching the best experimental conditions for fluxapyroxad and its metabolites simultaneously. The experimental matrix designs were conducted and results were evaluated using Statsoft Statistica 8.0 (2007 Edition, Tulsa, USA).

3. Results and discussion

3.1. UHPLC–MS/MS optimisation

The optimisation of MS/MS parameters of fluxapyroxad and its metabolites was automatically performed using IntelliStart software in ESI positive and negative modes. Higher responses were achieved for C-7 in positive mode and for fluxapyroxad, C-1 and C-2 in negative mode. All compounds showed abundant $[M-H]^-$ ions ($[M+H]^+$ for C-7). The optimum MS/MS conditions for fluxapyroxad and its metabolites were listed in Table S1.

For chromatographic optimisation, working solution prepared using MeCN, MeCN–acetic acid aqueous solution (10:90, V/V), MeCN–water (10:90, V/V) were injected in the instrument. The retention behaviour of C-1, C-2 and the peak shape of C-7 were improved using MeCN–acetic acid aqueous solution and MeCN–water both of which didn't exhibit significant difference. So MeCN–water (10:90, V/V) was selected. Furthermore, different mobile phases (MeCN and methanol) with different additives (acetic acid and ammonium acetate) were assayed. The data showed that acidification of the LC eluent greatly increased the peak shape of C-1 and C-2. Then, different buffer concentration of acetic acid aqueous solutions ranged from 0.02% to 0.2% were investigated. The best separation and ionisation of the target analytes was acquired using a mixture of MeCN–0.02% acetic acid aqueous solution.

3.2. Optimisation of the QuEChERS procedure

In this study, an experimental P–B design (Table S2) was built to identify the main factors affecting the responses among numerous variables. The effects of 11 factors (six selected real factors and five dummy factors) were investigated in 12 runs with three replicates for the central point to estimate the experimental error (pure error) [13]. Analysis of variance (ANOVA) was performed to examine the main effects using a *t*-test with a 95% probability [14]. The effects of the studied factors in the P–B design were then illustrated in standardised Pareto charts (Fig. 1), which indicate that the PSA amount (negative), the volume of water (positive) and the percentage of acetic acid (positive) were the most significant variables. The amounts of GCB and C_{18} were another two significant factors with negative effects on fluxapyroxad and positive effects on C-2, respectively. Therefore the GCB amount and C_{18} amount