DEVELOPMENT AND VALIDATION OF A RELIABLE LC-MS/MS METHOD FOR THE QUANTITATIVE ANALYSIS OF PESTICIDE RESIDUES IN SOIL

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Abstract: In order to carry out the multi-residue determination of the pesticides in the soil the liquid chromatography coupled with tandem mass spectrometry with a triple quadrupole analyzer was applied. The basis of the method is the modified version of the environmentally friendly QuEChERS extraction. The validation parameters (linearity, recovery, precision, matrix effects, limit of detection and limit of quantification) were set in accordance with the SANTE/12682/2019. The obtained results show that the applied method represents the safe and reliable determination method for the cyprodinil, fenpropimorph, prochloraz and tebuconazole residues in the soil.

Key words: validation, soil, pesticide residues, LC-MS/MS

Introduction

The plant protection products are widely used around the world, while the pesticide demand is continuously increasing in order to maintain the quality production and high agricultural yields (Sarkar et al., 2021). The most important pesticides` advantages are the ease of use, wide variety and faster results compared with the other pest control methods. However, the negative effects towards the environment should not be disregarded. The low pesticides` degradability has led to their presence in water, soil and food (Baloš et al., 2020). The pesticides have various effects on the beneficial microflora in the soil, such as the nitrification inhibition and soil fertility reduction. In addition, the pesticides that seep into rivers from the cropland soils and reach the lakes or the sea via the surface and groundwater affect a variety of life forms. The pesticides` application is carried out directly to the soil surface and into the soil, on the plant, or on the seed in the form of seed spraying. A significant part of the pesticides thrown onto the plant surface falls into the soil and moves in the soil over time depending on the soil type (physico-chemical properties), solubility, permanence and the climate factors (Balkan, 2021).

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One of the most reliable modern analytical methods is liquid chromatography tandem-mass spectrometry (LC–MS/MS), having advantages of both an accurate identification and a quantification of pesticide residues in different matrices (Vuković et al., 2021).

The objective of this study was to developed the multi-residue method with the high sensitivity, accuracy and precision to meet the SANTE/12682/2019 standard for the determination of fenpropimorph, cyprodinil, prochloraz and tebuconazole (Figure 1) residues in the soil by the modified QuEChERS extraction and the SPE clean-up using the liquid chromatography coupled with tandem mass spectrometry.
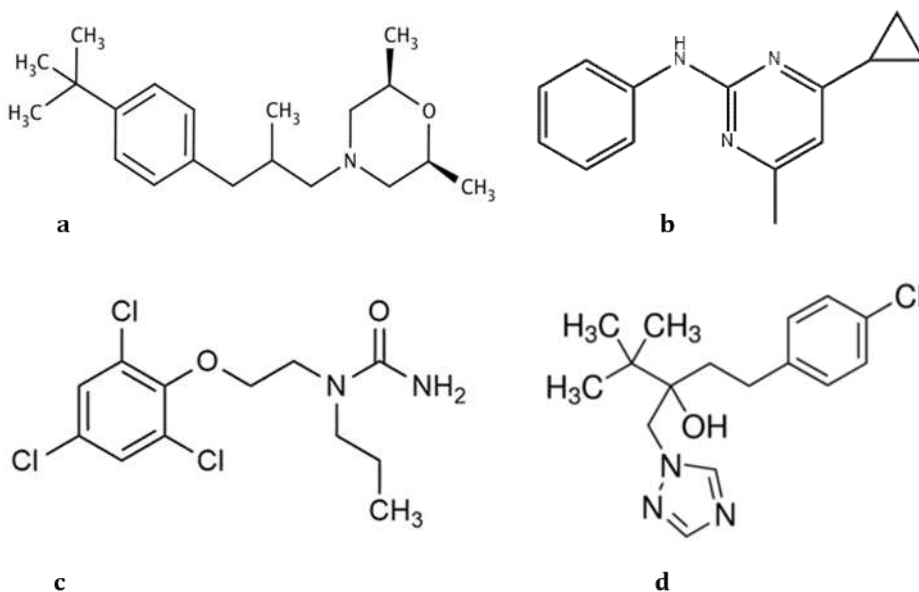

Figure 1. The structural formulas of the a) fenpropimorph, b) cyprodinil, c) prochloraz and d) tebuconazole.

Material and methods

Chemicals and apparatus. Fenpropimorph, cyprodinil, prochloraz and tebuconazole reference standards were obtained from Dr. Ehrenstorfer. The Carbofuran-D3 was used as the internal standard (Dr. Ehrenstorfer). The mixture working standard solution was prepared at 1.0 and 10 μg/mL with acetonitrile and stored in the dark at -20 °C. Acetonitrile and methanol were analytical grade (J.T. Baker), as well as the formic acid (Fisher Scientific UK).

LC-MS/MS with electrospray ionization. 6410 Agilent Technologies. The separation was performed using a Zorbax Eclipse XDBC18 column (50 mmx4.6mm id 1.8 μm.) at 25 ˚C. The mobile phase (0.4 mL/min): methanol with 0.1% formic acid and 0.1% formic acid in water in the gradient mode. Total run was 30 min. The injection volume was 5 μL. The instrument uses MassHunter software version B.06.00 for the quantitation and confirmation of target ions.

Method validation. The recovery was determined according to the SANTE/12682/2019. The recovery was obtained by spiking the soil samples in the concentrations 0.01 and 0.1 μg/mL in three replicates. The limit of detection (LOD) was estimated in the MRM mode analysis as the lowest concentration level that yielded S/N (S-signal, N-noise) ratio of five. The calibration was done at five concentration levels (0.01, 0.02, 0.05, 0.1 and 0.2 μg/mL).

The pesticides extraction from the spiked soil samples was carried out using a modified QuEChERS method described by Đurović Pejčev et al. (2019).

Results and discussion

The fragmentation of the H$^+$ molecular ion obtained by the LC-MS/MS in the positive electrospray ionization (ESI+) of the cyprodinil, fenpropimorph, prochloraz and tebuconazole is given in Table 1, as well as the average recoveries. The selected reaction monitoring mode (SRM) was carried out to obtain the maximum sensitivity for each pesticide detection, while for the pesticide confirmation two SRM transitions were used, taking into account the matching of the retention time (Rt). The target ion transition with the highest intensity (primary ion transition) was used for the quantitation, whereas the second target ion transition was used for the confirmation.

Table 1. The precursor ions, product ions, collision energies (CE), fragmentation energy (Frag) and Rt of the investigated pesticides

Pesticide	Precursor ion (m/z)	Product ions (m/z)	CE (V)	Frag (V)	Rt (min)
Cyprodinil	226.0	93.0 108.0	10 15	167	17.75
Fenpropimorph	304.3	147.1 130.0	10 25	100	15.90
Prochloraz	367.0	308.0 109.1	15 30	150	18.89
Tebuconazole	302.1	70.0 125.0	20 15	120	19.23
Carbofuran-D3	225.1	123.0 165.0	15 10	100	9.67

The limits of detection (LOD) were calculated from a signal-to-noise ratio of 5.0, using the chromatogram of the spiked sample at the lowest concentration level. The limit of quantification (LOQ) was set at 0.01 mg/kg. The blank soil extracts were used for the estimation of the background noise of the chromatographic analysis.

The linearity was evaluated using the calibration curves at 0.01, 0.02, 0.05, 0.1 and 0.2 µg/mL prepared in matrix blank extract (soil). Each calibration point was obtained by 3 repeated injections. The linear ranges and correlation coefficients (R^2) were summarized in Table 2. For all the studied pesticides, the correlation coefficients were above 0.99 indicating a good linearity. This result shows that the method was linear within the specified concentration range.

Table 2. The calibration data

Pesticide	Regression equation	R^2
Cyprodinil	y= 7.238997x+0.0352	0.9995
Fenpropimorph	y= 6.677314x-0.020931	0.9999
Prochloraz	y= 3.494007x+0.005265	0.9992
Tebuconazole	y= 6.164773x+0.128867	0.9998

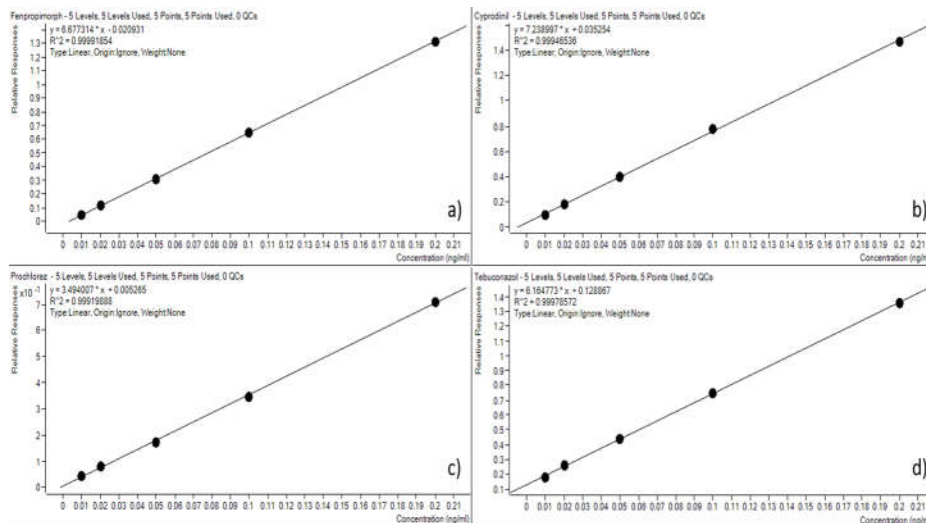The obtained calibration curves are shown in Figure 2.

Figure 2. The calibration curves of the a) fenpropimorph, b) cyprodinil, c) prochloraz and d) tebuconazole

The obtained average recovery for the investigated pesticides were in accordance with the SANTE/12682/2019, namely in the range from 70 to 120%. For cyprodinil the average recovery was 92.4±12.11%, while for fenpropimorph was 90.3±9.47%. The average recovery for prochloraz was 89.7±9.81% and 86.9±10.24% for tebuconazole.

The precision of the method, expressed as repeatability (% RSDr, relative standard deviation), was evaluated by analyzing the soil samples spiked at 0.01 and 0.1 mg/kg. The RSDr of developing method was under the 12.11% (RSDr<13%) which is within the established value of 20%.

Conclusion

In this study an analytical method based on the modified QuEChERS extraction combined with the LC-MS/MS analysis was developed for the determination of fenpropimorph, cyprodinil, prochloraz and tebuconazole residues in the soil according to the SANTE/12862/2019. The developed method demonstrated the appropriate validation parameters in terms of linearity, limit of detection and quantification, high accuracy and precision. Owing to the MS/MS detection, the method gave the good sensitivity and selectivity for the fenpropimorph, cyprodinil, prochloraz and tebuconazole determination in the soil. This method is suitable for the fenpropimorph, cyprodinil, prochloraz and tebuconazole quantitative analyses in the soil samples.

Acknowledgment

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