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Determination of pesticide residues in samples of green minor crops by gas chromatography and ultra performance liquid chromatography coupled to tandem quadrupole mass spectrometry

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ABSTRACT

A method was developed for pesticide analysis in samples of high chlorophyll content belonging to the group of minor crops. A new type of sorbent, known as ChloroFiltr, was employed for dispersive-solid phase extraction cleanup (dispersive-SPE) to reduce the unwanted matrix background prior to concurrent analysis by gas chromatography and ultra-performance liquid chromatography coupled to tandem quadrupole mass spectrometry (GC–MS/MS and UPLC–MS/MS). Validation experiments were carried out on green, unripe plants of lupin, white mustard and sorghum. The overall recoveries at the three spiking levels of 0.01, 0.05 and 0.5 mg kg^{-1} fell in the range between 68 and 120% (98% on average) and 72–104% (93% on average) with relative standard deviation (RSD) values between 2 and 19% (7% on average) and 3–16% (6% on average) by GC–MS/MS and UPLC–MS/MS technique, respectively. Because of strong enhancement or suppression matrix effects (absolute values $>20\%$) which were exhibited by about 80% of the pesticide and matrix combinations, acceptably accurate quantification was achieved by using matrix-matched standards. Up to now, the proposed method has been successfully used to study the dissipation patterns of pesticides after application on lupin, white mustard, soya bean, sunflower and field bean in experimental plot trials conducted in Poland.

1. Introduction

A particular crop may be called “minor” if such small amounts are grown that it will provide a limited market for pesticides. If the crop is considered to be of low economic importance at a national level, the pesticide manufacturers have little interest to do expensive research and development work needed for the registration of pesticides for use on the crop, and as a consequence of such a situation, the crop will have limited options for protection against pests and pathogens. Therefore, there are little data, or effectively no data available, about efficacy and residue behaviour of candidate pesticides having potential to be applied for the protection of minor crops. For authorization of pesticides on minor crops, or for minor use, it is preferable to explore other possibilities for determining the efficacy and crop safety of pesticides than those based on the amount of data required for authorization on major crops [1,2].

Although minor crops are grown in relatively small amounts compared with major crops, they are of substantial economic importance in many countries. Which crops are minor largely depends on the specific country and region. In Poland, the list of the plants classified as minor crops is published by the Ministry of Agriculture and Rural Development, and it comprises various crop groups including vegetables, fruit and berry plants, industrial plants and cereals, herbaceous plants and forest nurseries plants [3]. In this work, we focus on the analysis of pesticide residues in green crops of various plants including lupin, white mustard, soya, sunflower, and field bean by using gas chromatography and ultra performance liquid chromatography coupled with tandem quadrupole mass spectrometry (GC–MS/MS and UPLC–MS/MS)-based methods.

It must be highlighted, that the majority of up to now published pesticide residue methods were mainly focused on analysis of less complicated matrices such as fruit and vegetables [4]. The main reason might be the fact that fresh fruits and vegetables are consumed in larger amounts than other crops, so there is a risk for high intake of pesticide residues, especially when they are present above their legislative maximum residue levels (MRLs) [5]. However, another reason is that multiresidue pesticide analysis in matrices of high chlorophyll content is more difficult owing to matrix interferences and complicated extraction procedures [6]. In pesticide analysis, green matrices, high in chlorophyll, represent a particular challenge due to a massive load of co-extractives in the extract. The co-extracted

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chlorophyll is one of the most problematic matrix interferences in pesticide residue analysis because of its non-volatile characteristics. It may hinder identification of the pesticides of interest by contamination of the chromatographic systems, causing downtime, and reducing the overall analytical performance [7,8].

The use of graphitized carbon black (GCB) as a sorbent has been shown to be effective for removal of co-extracted chlorophyll and other pigments from extract of plant crops [9–15]. On the other hand, GCB is well-known to adsorb pesticides with planar functionality leading to unsatisfactory recoveries of a number of pesticides susceptible to this adsorption, e.g. chlorothalonil, cyprodinil, fenazaquin, mepanipyrim, pirymethanil, prochloraz, quinoxifen, quintozene and thiabendazole [16–18].

The main objective of this work was to investigate the possibility of application of a new type of sorbent, known as ChloroFiltr, in order to reduce the content of chlorophyll from extracts of green plants belonging to the category of minor crops, and thereby reduce the unwanted background while not affecting the recovery of the target pesticides. For the final determination, concurrent analyses were carried out by using a programmable temperature vaporization injector (PTV) gas chromatography–tandem quadrupole mass spectrometry (GC–MS/MS) and ultra-performance liquid chromatography–tandem quadrupole mass spectrometry (UPLC–MS/MS) to achieve improved selectivity and high accuracy. Comprehensive method validation was carried out to evaluate fitness for the intended application, and it involved determination of recovery, precision, linearity, assessment of matrix effects and estimation of measurement uncertainty.

The developed and validated method was applied to the study of dissipation patterns of pesticides in experimental plot trials after application to minor crops including lupin, white mustard, soya bean, field bean and sunflower.

2. Material and methods

2.1. Chemicals and reagents

Acetonitrile and acetone (for residue analysis) were obtained from Witko (Łódź, Poland). Anhydrous magnesium sulphate (reagent grade) and Supel Que Citrate (EN) tubes containing 4 g magnesium sulphate, 1 g sodium chloride, 0.5 g sodium citrate dibasic sesquihydrate, 1 g sodium citrate tribasic dehydrate, water with 0.1% formic acid (LC–MS Chromasolv) and methanol with 0.1% ammonium acetate were obtained from Sigma-Aldrich Sp.z o. o. (Poznań, Poland). Enviro Clean extraction tubes containing 900 mg magnesium sulphate, 300 mg PSA and 150 mg ChloroFiltr were obtained from UCT (Bristol, PA, USA). Deionized water of resistivity 18.2 MΩ cm was prepared with a Milli-Q Plus system from Millipore (Billerica, USA).

2.2. Pesticide analytical standards

Certified pesticide standards and internal standards triphenylphosphate (TPP) and simazine-d10 were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Stock solutions (approximately 1000 μg mL^{-1}) were prepared in acetone. Purities of the standards were accounted for when calculating the concentration of each stock solution. A blend stock solution of all pesticides at a concentration of 10 μg mL^{-1} was prepared in acetone. The working standards at 0.005, 0.01, 0.02, 0.05, 0.2, 0.5 and 1.0 μg mL^{-1} were prepared by diluting this stock solution with acetonitrile and 0.1% ammonium acetate in methanol/0.1% formic acid in water (1:1, v/v) for the GC–MS/MS and UPLC–MS/MS analysis, respectively.

Matrix-matched standards were prepared differently for GC–MS/MS and UPLC–MS/MS analysis. For the GC–MS/MS analysis, a volume (1.5 mL) of the standard at appropriate concentration was evaporated under nitrogen and reconstituted in acetonitrile sample extract at a concentration of 0.5 g mL^{-1}. While for the UPLC–MS/MS analysis, a volume (1 mL) of the standard at appropriate concentration and 2 mL acetonitrile sample extract at a concentration of 0.5 g mL^{-1} were evaporated under nitrogen then reconstituted in 1 mL methanol with 0.1% ammonium acetate/water with 0.1% formic acid (1:1, v/v).

2.3. GC–MS/MS conditions

The GC–MS/MS analysis of was carried out using a Varian CP-3800 gas chromatograph coupled with a Varian 1200 triple quadrupole mass spectrometer (Varian Inc., Middelburg, The Netherlands). The analyte separation was obtained on a DB-5 30 m × 0.25 mm × 0.5 μm capillary column, protected by a deactivated guard column (2 m × 0.53 mm). Helium (purity 99.9999%) at a flow rate of 1.2 mL min^{-1} was used as the carrier gas. The column oven temperature programme was as follows: 80 °C (held for 3 min), programmed at 30 °C $min^{-1}$to 150 °C, then programmed at 10 °C min^{-1} to 300 °C (held for 10 min). Large volume injection (LVI) with programmed temperature vaporization (PTV) was used. The injection volume was 10 μL of sample extract in acetonitrile using a 100 μL syringe. The injector temperature programme was as follows: 70 °C (held for 0.5 min), programmed at 200 °C $min^{-1}$to 300 °C (held for 15 min). The injector split ratio was initially set at 100:1, the splitless mode was enabled at 0.5 min, at 4 min the split ratio was set at 50:1, and it was reduced to 20:1 at 10 min.

The mass spectrometer was operated in electron impact ionization mode (EI, 70 eV) with the filament current of 50 μA and electron multiplier voltage at 300 V above the voltage determined by automatic tuning with perfluorotribulylamine (PFTBA). The manifold ion source and transfer line temperatures were 40, 270 and 290 °C, respectively. The collision gas (argon, 99.9998% purity) was set at the collision cell pressure of 1.7 mTorr. Multiple reaction monitoring (MRM) transitions and other acquisition parameters can be found in supplementary data included with this article. Instrument control, data acquisition and evaluation was performed by using a Varian MS Workstation software, version 6.6.

2.4. UPLC–MS/MS conditions

The UPLC–MS/MS analysis was carried out using a Waters ACQUITY UPLC ultra-performance liquid chromatography system (Milford, USA) coupled with a triple quadrupole mass spectrometer (Waters Inc., Micromass, Quattro Premier XE). The nebulizer and desolvation gas was obtained from a nitrogen generator model NM30-LA (Peak Scientific, Renfrewshire, Scotland, UK). The analyte separation was achieved using a BEH C18 100 mm-× 2.1 mm × 1.7 μm UPLC column protected by a VanGuard Pre-Column 5 mm × 2.1 mm × 1.7 μm. The temperature of the column was thermostated at 40 °C. The column was eluted with the mobile phase: water with 0.1% formic acid (A) and methanol with 0.1% ammonium acetate (B) at the flow rate of 0.3 mL min^{-1} using gradient mode. Gradient was programmed to increase the amount of B from an initial content of 10–100% in 6 min, held for 1 min and returned to the initial conditions (10% B) in 1 min (from 7 to 8 min), held for 1 min. The total run time was 9 min. Sample extract volumes of 5 μL were injected into the system. The temperature of the autosampler was thermostated at 25 °C.

For the MS/MS data acquisition, the interface conditions were optimized for maximum intensity of the precursor ions: nebulizer and desolvation gas (nitrogen) flows were 100 L h^{-1} and 700 L h^{-1}, respectively, source block and desolvation temperatures were 120 °C