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Development of a multi-residue screening method for the determination of pesticides in cereals and dry animal feed using gas chromatography–triple quadrupole tandem mass spectrometry

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Abstract

A multi-residue screening method for simultaneous analysis of 122 gas chromatography amenable pesticides in dry matrices such as cereal grain and certain feedingstuffs was developed. The method entails a simple extraction of re-hydrated sample with acetonitrile followed by a dispersive solid phase extraction (dispersive-SPE) clean-up step prior to the final determination by gas chromatography/triple quadrupole tandem mass spectrometry (GC–MS/MS). Due to complexity of analyzed matrices, two MS/MS transitions were set for each pesticide to eliminate the need for re-analysis of potentially positive samples, and provide unequivocal identification of detected pesticides in accordance with recent guidelines, in a single analytical run. Thus, in the developed GC–MS/MS acquisition method, a total of 216 different multiple reactions monitoring (MRM) transitions were monitored in one set of experimental conditions. To evaluate performance of the method, validation experiments were carried out on wheat grain at three spiking levels (0.01, 0.02 and 0.05 mg kg^{-1}). Additional recovery tests at 0.05 mg kg^{-1} were carried out on several other matrices. The recoveries ranged between 73 and 129% with associated relative standard deviations between 1 and 29% for the majority of pesticides. Limits of detection were less or equal to 0.01 mg kg^{-1} for approximately 68% of pesticides. The applicability of the proposed method to detect and quantify pesticide residues has been demonstrated in the analysis of 136 real samples. Additionally, the method was favorably compared with an acetone extraction method (accepted as a reference method by some of European and U.S. authorities) in the analysis of real samples known to contain pesticide residues.

Keywords: Triple quadrupole tandem mass spectrometry; Gas chromatography; Pesticide residue analysis; Cereals; Animal feed

1. Introduction

Many obvious benefits have been gained from the use of synthetic pesticides in agriculture, but their inappropriate use on crops can result in unacceptably high levels of these compounds in produce. Even when pesticides are applied in accordance with good agricultural practices (GAP), they can leave residues [1]. Therefore, many countries have established legal directives and monitoring programs to control the use of pesticides on agricultural crops, and find out whether the residues are compliant with the statutory maximum residue levels (MRLs) [2].

But screening for traces of pesticides can be an extremely challenging task in the case of matrices such as cereals and dry feedingstuffs due to large quantities of co-extracted components (e.g. starch, proteins, fats) which may adversely affect the method and instrument performance. A vitally important aspect of the determination of pesticide residues in difficult matrices is the application of an efficient extraction followed by a specific final determinative step. An appropriate method should be capable of separating as much as possible the target analytes from other substances that might interfere with the analysis, since recent guidelines imply the necessity of unambiguous confirmation of residues identified in the screening process [3,4].

Unquestionably, tandem mass spectrometry (MS/MS) gives much higher degree of certainty in analyte identification than any single stage mass spectrometry technique, because isobaric

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interferences are avoided and multiple-component spectra can be resolved. Thanks to this, the confirmation of target analytes can be achieved with higher level of confidence. Among the different mass analyzers that can perform tandem mass spectrometry, triple quadrupole mass spectrometers have recently been proposed for the determination of pesticide residues in crops. A remarkable advantage of the triple quadrupoles, in comparison with previously used ion traps [5–17], is the possibility of operating in multiple reaction monitoring mode (MRM) which is a faster scan mode than product ion scan available on the ion traps. Whereas a disadvantageous limitation of the ion traps is their vulnerability to space charge effects, which can degrade the quality of mass spectra, including mass accuracy and resolution. This can be a problem when trace components are analyzed in particularly dirty matrices because the trap fills with a large number of matrix ions [18]. Consequently, with the triple quadrupole mass analyzer more compounds can be spectrometrically resolved in a reduced analysis time [19].

Although considered as one of the most powerful techniques, there are not many references in the literature to the use of gas chromatography/triple quadrupole tandem mass spectrometry in the analysis of pesticides residues. Garrido Frenich et al. [19] studied the potential of GC with triple quadrupole MS/MS for the multi-residue analysis of pesticides in vegetables, validated the method on cucumbers [20], then expanded the scope of the method, and validated on strawberries [21]. Other examples are the determination of priority pesticides in baby foods [22], xenoestrogens (pesticides, PCBs and polybrominated diphenyl ethers) in human breast tissues [23], organochlorine and organophosphorous pesticides in animal liver [24], and meat samples [25,26]. Also, in a previous work of this author, low-pressure gas chromatography/triple quadrupole tandem mass spectrometry (LP-GC–MS/MS) conditions were developed for 78 pesticides but validation data were generated for only 12 pesticides in tomato [27].

The main objective of the present work was to investigate the potential of GC coupled with a triple quadrupole mass analyzer for sensitive and reliable screening for a large number of pesticides in dry samples such as cereals and feedingstuffs. The special attention was devoted to the optimization of triple quadrupole MS/MS operating parameters because to the knowledge of this author, there was neither a detailed specification nor discussion on settings these parameters, and their impact on peak shapes and sensitivity in literature. Furthermore, none of the above quoted references has reported analysis of more than one hundred pesticides using two transitions for each of them to enable quantification and confirmation of detected residues, simultaneously. For preparation of sample extracts prior to the final GC–MS/MS analysis, a miniaturized acetonitrile-based extraction followed by a dispersive solid phase extraction clean-up step (QuEChERS) was evaluated [28–32]. Additionally, extraction efficiency of the QuEChERS method was compared with that of a classical acetone extraction method in the analysis of real samples known to contain pesticide residues.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile (super gradient) was purchased from Labscan (Dublin, Ireland). Acetone (for residue analysis) was purchased from S. Witko (Łódź, Poland). Anhydrous magnesium sulphate (reagent grade), sodium citrate tribasic dihydrate (ACS reagent), and disodium hydrogen citrate sesquihydrate (pure) were all purchased from Sigma–Aldrich (Steinheim, Germany). Toluene (for residue analysis) and formic acid (ACS grade) were purchased from Merck (Darmstadt, Germany). Sodium chlorine was purchased from POCH (Gliwice, Poland). Bondesil PSA (40 μm) bulk sorbent was purchased from Candela (Warszawa, Poland) and C18 (50 μm) bulk sorbent was purchased from Anaserwis (Baranowo, Poland).

2.2. Analytical standards

Certified pesticide standards were purchased from Dr. Ehrenstorfer (Ausburg, Germany) and were of purity above 90% (most of them at least 98%). For formothion and triazophos analytical standards of purity 74 and 81%, respectively, were available. Triphenylphosphate, TPP (I.S.) was purchased from Sigma–Aldrich (Steinheim, Germany). Individual pesticide stock solutions were prepared at concentrations of about 1000 $\mu g\,mL^{-1}$. Purity was included in the calculation of actual concentration of each standard solution. Of these stock solutions, a single composite mixture of all pesticides was prepared, of which subsequent dilutions to obtain working standards were made. The single composite mixtures at appropriate concentrations were used to calibrate the GC/MS/MS system and spike samples in recovery experiments.

2.3. GC–MS/MS conditions

Determinations were performed using a CP-3800 gas chromatograph (Varian Inc., Middelburg, Netherlands). The system was equipped with electronic flow control (EFC), a 1079 universal capillary injector, and a CP-8400 autosampler. The injector temperature was held at 250 °C for 1.5 min during injection, then programmed at 200 °C min^{-1} to 300 °C which was held for 20 min. A split ratio was initially set at 20:1, at 0.01 min the split vent was closed until 1.5 min to ensure complete sample transfer, then the split ratio was held at 100:1 until 20 min, and finally reduced to 20:1. The injector liner was fitted into a CarboFrit plug (Restek, Bellefonte, USA), and the Carbofrit was conditioned prior to use [10]. Sample extracts (5 μL) were injected in toluene. Separations were performed on a DB-5 MS 30 m × 0.25 mm × 0.5 μm column (J & W, Folsom, USA) protected by a 2 m × 0.53 mm guard column of uncoated fused silica at the inlet end. Helium was used as a carrier gas at a flow rate of 1.2 mL min^{-1}. The column was held at 80 °C for 3 min after injection then programmed at 30 °C min^{-1} to 150 °C, then programmed to 300 °C at 10 °C min^{-1} which was held for 10 min.