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Simultaneous determination of carbamate insecticides and mycotoxins in cereals by reversed phase liquid chromatography tandem mass spectrometry using a quick, easy, cheap, effective, rugged and safe extraction procedure

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ABSTRACT

A simple, sensitive and reliable analytical method was developed for the simultaneous determination of 22 carbamate insecticides and 17 mycotoxins in cereals by ultra high performance liquid chromatography electrospray ionization tandem mass spectrometry (UHPLC–ESI-MS/MS). Carbamates and mycotoxins were extracted from cereal samples using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) procedure without any further clean-up step. The extract was diluted with water containing 0.1% formic acid and 5.0 mM ammonium acetate, and analyzed by LC–MS/MS on a Waters Acquity BEH C_{18} column with water (0.1% formic acid, 0.50 mM ammonium acetate)/methanol as mobile phase with gradient elution. Matrix-matched calibration was used for quantification. Blank samples (rice, wheat and corn) were fortified at 5, 10 and 50 μg/kg except for five zearalenonic compounds at 25, 50 and 250 μg/kg, and recoveries were in the range of 70–120%. Relative standard deviations were lower than 20% in all cases. The LOQ values were in the range of 0.20–29.7 μg/kg. The method is suitable for the simultaneous determination of carbamate insecticides and mycotoxins in cereals. The total time required for the analysis of one sample, including sample preparation, was about 35 min.

1. Introduction

Cereals are the most important crops in the world for human diet. Among the cereals, corn, wheat and rice are the most important. However, the consumption of cereals is not free from the risk of exposure to harmful compounds, such as pesticides and mycotoxins [1]. In order to protect human health, the maximum residue limits (MRLs) of pesticides and mycotoxins have been established in food by the European Union, China, etc. [2–5].

Carbamate insecticides are widely used in agricultural environments to protect crops against a range of pests; whereas mycotoxins are secondary metabolites of fungal origin, and they were found in different relevant food crops, especially in cereals and cereal products. To control and monitor the occurrence of carbamate insecticides and mycotoxins in food, it is necessary to develop accurate analytical methods for their identification and quantification. Undoubtedly, multi-residue analytical methods are the best strategy for monitoring purposes. They allow for the analysis of a number of compounds in a single operation, as well as decrease the cost of analysis. Therefore, a large number of multi-residue analytical methods have been established for the determination of carbamate insecticides and mycotoxins since the 1980s [6–13]. Especially in recent years, many liquid chromatography tandem mass spectrometry (LC–MS/MS) methods have been developed for simultaneous determination of multi-class mycotoxins in food [13–23].

However, the confirmatory method has not been reported for simultaneously determine carbamate insecticides and mycotoxins in cereals. In the area of analysis of pesticides and mycotoxins, only three papers have been focused on a simultaneous determination in food. Lacina et al. developed a LC–MS/MS method with QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method to evaluate 22 mycotoxins and 222 pesticides in cereals [24]. Mol et al. developed a generic extraction method for contaminants to evaluate 36 mycotoxins and 136 pesticides in food and feed, and proposed three new extractions/"dilute-and-shoot" type methods [1]. However, the LC–MS/MS methods developed by Lacina et al. and Mol et al. often were chosen as a kind of screening method. Recently, Aguilera-Luiz et al. have successfully developed a confirmatory LC–MS/MS method to determine 42 pesticides and six mycotoxins in milk samples [25].

It is well known that LC with fluorescence detection after derivatization was the most widely used quantitative method

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for carbamate insecticides and mycotoxins in the past due to their physical chemical properties, such as polarity [26,27]. Now, LC–MS/MS is an excellent alternative technique for the analysis of polar substances like carbamate insecticides and mycotoxins, because no derivatization step is required and higher selectivity and sensitivity can be acquired. To reduce the cost of sample analysis and to increase sample throughput, it is essential to develop simultaneous determination method for carbamate insecticides and mycotoxins by LC–MS/MS. Electrospray ionization (ESI) positive ion mode had been used to analyze carbamate insecticides and mycotoxins, but factors, that can affect ESI efficiency and chromatographic behavior include mobile phase composition and type of column, have to studied again to determine simultaneously carbamate insecticides and mycotoxins with good sensitivity. QuEChERS method was originally developed for the extraction of pesticides from fruits and vegetables [29], has been employed for the extraction of mycotoxins in many matrices [19]. Nevertheless, its application in simultaneous determination of pesticides and mycotoxins is still very scarce, especially in cereals.

Here we developed a simple confirmatory LC–MS/MS method for the simultaneous determination of carbamate insecticides and multiclass mycotoxins in corn, wheat and rice with reverse phase system. To achieve the goal, a simple pretreatment procedure based on QuEChERS was established. The final optimized method was validated for selectivity, linearity, trueness, precision, limit of detection (LOD), and limit of quantification (LOQ).

2. Materials and methods

2.1. Reagents

Aflatoxin B_1 (AFB_1, 99%), aflatoxin B_2 (AFB_2, 99%), aflatoxin M_1 (AFM_1, 99%), aflatoxin M_2 (AFM_2, 99%), aflatoxin G_1 (AFG_1, 99%) and aflatoxin G_2 (AFG_2, 99%), DON (98%), T-2 (98%), OTA (99%), fumonisin B_1 (FB_1, 97%) and fumonisin B_2 (FB_2, 97%) were from Alexis Biochemicals (San Diego, USA). HT-2 (98%) was from Biopure Corporation (Tulin, Austria). α-Zearalanol (α-ZAL, 98%), β-zearalanol (β-ZAL, 98%), α-zearalenol (α-ZOL, 98%), β-zearalenol (β-ZOL, 98%), zearalenone (ZAN, 98%) were from National Measurement Institute of Australia (Sydney, Australia). Carbamate reference standards (purity higher than 98%) were from Dr. Ehrenstofer (Augsburg, Germany). Water was purified with a Milli-Q reverse osmosis system (Millipore, Milford, MA, USA). Methanol (LC grade) and acetonitrile (LC grade) were from Fisher Chemicals (Fairlawn, USA). Formic acid was from Tedia Company Inc. (Fairfield, USA). Acetic acid and ammonium acetate were analytical grade and purchased from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Standard solutions

Stock standard solutions of individual compounds (100 μg/mL) were prepared by exact weighing of the compound followed by dissolution in 100 mL (carbamate insecticides) or 10 mL (mycotoxins) of acetonitrile, and stored at −18 °C in the dark. Three multi-compound working solutions (1.25, 2.50, and 12.5 μg/mL for zearalenonic compounds and 0.25, 0.50 and 2.50 μg/mL for the rest of the compounds) were prepared by diluting stock standard solutions with acetonitrile.

2.3. Chromatographic conditions

A Waters Acquity UPLC instrument (Milford, MA, USA) was used in the present experiment. Separation was carried out on an Acquity BEH C_{18} column (2.1 mm × 100 mm, 1.7 μm) maintained at 30 °C. The mobile phase consisted of solvent A (0.1% formic acid–0.50 mM ammonium acetate in water) and solvent B (methanol). Initial gradient conditions were set to 15% B and held for 1.5 min before incorporating a linear gradient increasing to 85% B at 7.5 min and held for 1.5 min. At 10.1 min the gradient was programmed to initial conditions to reequilibrate the column for 1.9 min (total run time 12 min). The flow rate was 0.20 mL/min. The injection volume was 10 μL in full loop injection mode.

2.4. Mass spectrometry conditions

Detection was carried out by a Waters Xevo™ TQ triple-quadrupole MS fitted with ESI probe operated in the positive ion mode except for α-ZOL and β-ZOL in the negative ion mode. The following parameters were optimal: capillary voltage, 2500 V; ion source temperature, 150 °C; desolvation gas temperature, 500 °C; desolvation gas flow rate, 1000 L/h of nitrogen. Detection was carried out in multiple reaction monitoring (MRM) mode. Argon was used as the collision gas, and the collision cell pressure was 3.2 mbar. Other parameters are shown in Table 1.

2.5. Sample preparation

A 5 g of homogenous representative sample was weighed in a 50 mL plastic centrifuge tube and 20 mL of methanol/water/acetic acid (74.25:24.75:1) were added. The samples were extracted in an ultrasonic water bath (300 mm × 150 mm × 150 mm, Kunshan Ultrasonic Instrument Co. Ltd., Jiangshu, China) for 10 min at room temperature. After addition of 1 g of NaCl and 5 g of $MgSO_4$, the mixture was shaken vigorously for 1 min. To separate aqueous and organic phase, the sample was centrifuged at 8000 rpm for 3 min. An aliquot of the upper organic phase (2.0 mL) was diluted with 2 mL of water containing 0.1% formic acid and 5 mM ammonium acetate. Prior to final instrumental analysis, sample solution was passed through the 0.20 μm filter (Jinteng, Tianjin, China).

2.6. Confirmation criteria

For confirmation of carbamate insecticides and mycotoxins in cereals, the following three criteria had to be met: (i) the retention time was within 2.5% of the external standard solution; (ii) the signal-to-noise ratio (S/N) for each diagnostic ion shall be ≥3:1; and (iii) the relative abundance of two transitions in the samples was within an acceptable range relative to the average external standards according to the European SANCO guideline 10684/2009 for LC–MS/MS methods [28].

2.7. Method validation

The validation study was performed on the basis of the European SANCO guideline 10684/2009 [28]. Analytical characteristics evaluated were linearity, selectivity, sensitivity, mean recovery (as a measure of trueness) and intra-day and inter-day precision (expressed as relative standard deviation, RSD).

Linearity was studied using matrix-matched standards, and analyzed each of them in triplicate at six concentrations (1.0, 2.0, 5.0, 10, 25 and 100 μg/L for zearalenonic compounds, 0.05, 0.25, 1.0, 2.0, 10 and 25 μg/L for carbamates except for methomyl, indoxacarb, pirimicarbdesmethyl, aldicarb sulfone and aldicarb sulfoxide, 0.20, 0.50, 1.0, 2.0, 10 and 25 μg/L for the rest of the compounds).

To verify the absence of interfering substances around the retention time of carbamate insecticides and mycotoxins, 10 blank samples for each kind of sample were analyzed.

LODs of the method were estimated with respect to signal of the chromatographic peak of analyte (signal to noise peak to peak ratio >3) in fortified samples at the lowest concentration, LOQs were