



A reliable screening of mycotoxins and pesticide residues in paprika using ultra-high performance liquid chromatography coupled to high resolution Orbitrap mass spectrometry



Ingars Reinholds^{a, b, *}, Iveta Pugajeva^a, Vadims Bartkevics^{a, b}

^a Institute of Food Safety, Animal Health and Environment "BIOR", Lejupes iela 3, Riga, Latvia

^b University of Latvia, Faculty of Chemistry, Kr.Valdemara iela 48, Riga, Latvia

ARTICLE INFO

Article history:

Received 19 May 2015

Received in revised form

6 September 2015

Accepted 10 September 2015

Available online 14 September 2015

Keywords:

Paprika

Mycotoxins

Pesticides

UHPLC-Orbitrap-HRMS

UHPLC-QqQ-MS/MS

Correlation analysis

ABSTRACT

In this study, an ultra-high performance liquid chromatography (UHPLC) coupled to a high resolution Orbitrap mass spectrometry (Orbitrap-HRMS) was demonstrated as a promising technique in high-throughput method development for the routine analysis and contamination control of mycotoxins and pesticide residues in spices. The method was applied for the analysis of fifty ground paprika samples containing blends of sweet and hot paprika harvested in Brazil and China. The efficiency and detection sensitivity of the used UHPLC-Orbitrap-HRMS technique were compared to the results obtained using a triple quadrupole tandem mass spectrometric detector (UHPLC-QqQ-MS/MS). The values of recovery (75–120%) and repeatability (8–15%) for both methods, calculated as the average ($n = 5$) from the results of spiked ($10\text{--}500\ \mu\text{g kg}^{-1}$), paprika samples, were in good conformity to the relevant EU guidelines. The high resolution of the used Orbitrap-HRMS technique provided a better sensitivity for quantitative determination of several pesticide contaminants in paprika, compared to the results obtained by the QqQ-MS/MS method and were comparable in case of mycotoxins. The results of analysis demonstrated the ubiquitous presence of three mycotoxins (fumonisin B₁, ochratoxin A, and sterigmatocystin) and twelve pesticide residues in paprika. The concentrations of determined contaminants were below the MRLs set by the Regulations of the European Union with exception of iprovalicarb, which violated the EU MRL in two samples of hot paprika. In addition, a notable difference in the concentration of fumonisin B₁ was determined depending on the harvest period (2009–2013), reaching the maximum concentrations of $33\ \mu\text{g kg}^{-1}$ in sweet paprika and $140\ \mu\text{g kg}^{-1}$ in hot paprika. There was no significant correlation found between the determined mycotoxin contamination levels and the pesticide residues, with the sole exception of decreased fumonisin B₁ content in samples with an elevated concentration of metalaxyl fungicide.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Spices and herbs are important food flavourings, colourants, dietary supplements, and have prominent antioxidant and antimicrobial roles in herbal medicine and cosmetics (Peppin & Pappagallo, 2014; Santos et al., 2011). Environmental pollution and chemical contamination during the growth and storage of agricultural products, such as bell fruits of *Capsicum* spp. (paprika, cayenne peppers, and chili), harvested in tropical or sub-tropical

conditions, has been a serious concern to human health (Iqbal, Asi, Zuber, Akhtar, & Saif, 2013). Aflatoxins (AFs) and ochratoxin A (OTA), produced by fungi such as *Aspergillus* and *Penicillium*, are among the most hazardous pollutants causing carcinogenic, hepatotoxic, and neurotoxic effects (Almela et al., 2007; Da Rocha, Freire, Maia, Guedes, & Rondina, 2014). The occurrence of aflatoxin B₁ (AFB₁) and OTA is controlled according to the European Union (EU) Regulation No. 165/2010, which sets the maximum limits (MLs) for AFB₁ and the total AFs in dried fruits of *C. spp.* to $5\ \mu\text{g kg}^{-1}$ and $10\ \mu\text{g kg}^{-1}$, respectively (EC, 2010). The ML for OTA in dried paprika has been $15\ \mu\text{g kg}^{-1}$ since 01.01.2015 (EC, 2012a).

The overwhelming influence of various pathogens, weeds, and insects on plant illnesses and reduction in yields determines the inevitable need for application of various pesticides (insecticides,

* Corresponding author. Institute of Food Safety, Animal Health and Environment "BIOR", Lejupes iela 3, Riga, Latvia.

E-mail address: Ingars.Reinholds@bior.lv (I. Reinholds).

fungicides, herbicides, etc.) with subsequent risk of their leftover accumulation in produce and in the environment (Van Boxstael et al., 2013). The maximum residue limits (MRLs) of pesticides used in the EU are strictly regulated (EC, 2005).

High-performance liquid chromatography coupled to a fluorescence detector (HPLC-FLD) is the most widespread method for the analysis of mycotoxins (Kunsagi & Stroka, 2014). Elevated concentrations of OTA and AFB₁ have been found in more than 40% of paprika samples produced in the EU countries (Zhao, Yuan, Zhang, & Yue, 2014). Within the field of spice contamination analysis, multi-mycotoxin determination based on the triple quadrupole tandem mass spectrometry (QqQ-MS/MS) technique (Boonzaaijer, Van Osenbruggen, Kleinnijenhuis, & Van Dongen, 2008; Desmarchelier et al., 2014) is of increasing importance. A notably high contamination with B-type fumonisins (FBs) has been found in chili and cayenne peppers (Waskiewicz, Beszterda, Bocianowski, & Goliński, 2013).

The spices of paprika are controlled for highly-persistent non-polar pesticide residues, monitored by gas chromatography (GC) coupled to QqQ-MS/MS or time-of-flight (TOF-MS) detectors (Fernandes et al., 2014), and the various polar pesticides are assessed by UHPLC coupled to QqQ-MS/MS (Masiá, Blasco, & Picó, 2014; Zhu, Zhao, Nan Feng, & Han Kim, 2014) and Orbitrap-HRMS (Albert, Kramer, Scheeren, & Engelhard, 2014) detection techniques.

There is a high demand for multi-component analysis applicable to the multiple types of contamination in spices and herbs. The wide range of molecular mass, polarity, acid-base behaviour of contaminants, and the matrix effects (e.g., essential oils, pigments, etc.) may interfere with the proper clean-up, extraction, and detection of contaminants, especially in complex matrices such as spices and herbs (Lacina et al., 2012).

The application of HRMS technologies has significantly enhanced the capabilities for multi-component analysis of contaminants in food products, with the increasing availability of Orbitrap mass spectrometers (MS). In 2012, Kaufmann et al. demonstrated the comparable efficiency in terms of performance (e.g., precision, sensitivity, selectivity, and accuracy) of QqQ-MS/MS and Orbitrap-MS detection techniques in multi-pesticide analysis. The resolutions of 35,000 and, especially, 70,000 are most frequently recommended for accurate detection of contaminants in complex matrices containing pigments and essential oils (Rajski, Gómez-Ramos, & Fernández-Alba, 2014).

To our best knowledge, the use of Orbitrap technology for the throughput multi-component determination of mycotoxins and pesticide residues in paprika and other spices has been previously less described in the literature. There are only a few recent investigations dealing with the analysis of rather uncomplicated matrices, unlike those of condiments. In 2012, De Dominicis, Commissati, and Suman evaluated the contamination of bakery ingredients by determination of sixteen target pesticides, twelve mycotoxins, and eight antibiotics. However, the scope of compounds targeted for analysis (especially pesticides) was rather limited, compared to the EU legislation (EC, 2012b).

The present study describes the development and use of a high throughput multi-component method for the screening of mycotoxins and pesticides in dried paprika. The method applied a rapid one-stage extraction method and high-resolution Orbitrap-HRMS detection system. The efficiency in terms of recovery and sensitivity of the elaborated procedure was compared to the results obtained using the conventional QqQ-MS/MS detection technique. To our best knowledge, the presented study on the application of the Orbitrap-HRMS based detection technology is the first research applying multi-component analysis of pesticide residues and mycotoxins in paprika.

2. Materials and methods

2.1. Reagents and chemicals

HPLC grade acetonitrile and methanol were purchased from Merck-Millipore (Darmstadt, Germany). ACS grade formic acid ($\geq 96.0\%$) and ammonium formate (99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure deionised water was generated by a Millipore Milli-Q™ system (Billerica, MA, USA). A buffer-salt mixture consisting of 1 g trisodium citrate dihydrate, 1 g sodium chloride, 0.5 g disodium hydrogen citrate sesquihydrate, and 4 g of anhydrous magnesium sulphate was obtained from Phenomenex (Torrance, CA, USA). Disposable Ultrafree® PVDF membrane filters (0.22 μm) were obtained from Merck, Millipore Ltd.

2.2. Mycotoxin and pesticide standards

Standards of eleven mycotoxins, such as aflatoxin B₁ (AFB₁), ochratoxin A (OTA), fumonisin B₁ (FB₁), citrinin (CIT), deoxynivalenol (DON), zearalenone (ZEN), HT-2 toxin (HT₂), T-2 toxin (T₂), nivalenol (NIV), sterigmatocystin (STERIG), and fusarenon X (FusX) were purchased from Romer Labs Biopure (Tulln, Austria). The reference standards of 134 pesticides were purchased from AccuStandard (New Haven, USA) and Dr. Ehrenstorfer (Germany). Information on the targeted pesticides and their MRLs in paprika is provided in the [supplementary Table S1](#).

Stock solutions of all compounds at 1 mg mL⁻¹ were prepared by dissolving of 10 mg of solid substance in 10 mL of acetonitrile. The mix of working standard solutions of all compounds (pesticides or mycotoxins) at 10 $\mu\text{g mL}^{-1}$ concentrations was prepared by dissolving 100 μL of each stock solution of pesticides with concentration of 1000 $\mu\text{g mL}^{-1}$ in 10 mL volumetric flask with acetonitrile and stored at $-20\text{ }^\circ\text{C}$ no longer than 1 month. The working standard solutions of mycotoxins and the solutions of pesticides were used for spiking blank samples at the appropriate concentrations (10, 50, 100, 250, and 500 $\mu\text{g kg}^{-1}$). For quality assurance, the control samples with known standard concentrations (100 $\mu\text{g kg}^{-1}$) were also analysed in each sample batch.

2.3. Samples for analysis

A total of 50 paprika samples packaged in air-tight plastic bags, each of 100 g weight, were provided in 2014 by collaborating company FUCHS Gewürze GmbH (Germany) and stored in the dark at room temperature until processing. The samples consisted of steam treated blend powders of sun dried hot ($n = 14$) and sweet ($n = 36$) paprika grown in Brazil and China. The paprika samples represented three different growing seasons: harvested in 2009 ($n = 34$), 2010 ($n = 5$), and 2013 ($n = 11$).

2.4. Sample preparation

An EU version of buffered QuEChERS extraction method was used to obtain organic extracts of mycotoxins and pesticides for analysis (EN 15662, 2008). The samples were obtained already in the ground form ($<1.50\text{ mm}$) and used without further pre-treatment. Each sample ($2 \pm 0.1\text{ g}$) was weighed into a 50 mL polypropylene centrifuge tube. In the cases of calibration and quality control samples, the standard solutions were added at the appropriate spiking level. Then, deionised water (10 mL) and acetonitrile (10 mL) were both added, followed by a salt mixture consisting of 4 g of magnesium sulphate, 1 g of sodium chloride, 1 g of trisodium citrate dihydrate, and 0.5 g of disodium hydrogen citrate sesquihydrate. The samples were vortexed for 1 min

Download English Version:

<https://daneshyari.com/en/article/6390382>

Download Persian Version:

<https://daneshyari.com/article/6390382>

[Daneshyari.com](https://daneshyari.com)