A Validated UPLC-MS/MS Multi-mycotoxin Method for Nuts and Cereals for the Official Control in Cyprus within the EU Requirements

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Abstract

The validation of a rapid, reliable and sensitive method for the simultaneous determination of Aflatoxins (AFB₁, AFB₂, AFG₁, AFG₂), Ochratoxin A, Zearalenone, Deoxynivalenol, Fumonisins B₁ and B₂, T-2 and HT-2 toxins, in nuts (peanuts, pistachio and almonds) and cereals (maize and wheat) is reported. The method was developed and validated to fulfill the requirements of the official control of mycotoxins according to EU legislation. This method is based on a single extraction step using an acetonitrile/water mixture followed by the analysis of the diluted crude extract using ultra high performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS). The MS/MS detection was carried out using an electro spray-ionization interface (ESI) in positive ion mode. Matrix-matched calibration was used for the quantification of the mycotoxins, because of the absence of further clean up steps that reduce suppression/enhancement matrix effects. The method performance characteristics were determined after spiking blank samples on multiple levels. The mean recoveries of mycotoxins in spiked nuts ranged from 74.4% to 131.7%, while in cereals ranged from 52.8% to 113.9%. Relative standard deviations were lower than 20.4% for all target mycotoxins. Limits of detection and quantification for nuts and cereals ranged 0.08-30.0 and 0.25-99.0 μg/Kg, respectively.

Keywords: Multiyctoxin analysis; UPLC-MS/MS; Nuts; Cereals; Aflatoxins; Ochratoxin A, Zearalenone; Deoxynivalenol; Fumonisins; T-2 toxin; HT-2 Toxin

Introduction

Mycotoxins are natural chemical contaminants produced as toxic secondary metabolites by some fungal species such as Aspergillus, Penicillium and Fusarium. Mycotoxin formation is affected by several biological factors, by harvesting, storage and processing conditions, insect damage in agricultural crops and finally by climate changes (temperature, humidity) [1,2]. Consumption of food and feed contaminated with mycotoxins can cause severe effects on animal and human health, from allergic responses to cancer and death. Due to their high occurrence, the most common and widely investigated mycotoxins in food and feed, regulated by the European Union legislation [3,4] are: Aflatoxins (AFB₁, AFB₂, AFG₁, AFG₂), Ochratoxin A (OTA), trichothecenes [Deoxynivalenol (DON), T-2 and HT-2 toxins], Fumonisins (FB₁, FB₂) and Zearalenone (ZON) [5-7]. AFs are primarily produced by Aspergillus flavus and Aspergillus parasiticus and are among the most carcinogenic substances known. AFs are significant in nuts, maize and cereal grains [1,8]. OTA has been found in cereals (wheat, barley, oats and maize), spices and dried fruits and is primarily nephrotoxic and considered to be a possible human carcinogen. OTA is produced by Aspergillus ochraceus, Penicillium verrucosum and Penicillium viridicatum. Fumonisins, trichothecenes and ZON are produced by Fusarium species. These mycotoxins are mostly found in cereals, especially in maize [1,8]. Fumonisins have cancer promoting activity and mostly responsible for esophageal carcinoma. ZON can cause oestrogenic effects such as infertility, abortion and cervical cancer. Trichothecenes are related with fatal and chronic toxicoses. They inhibit DNA and protein synthesis, and they are responsible for outbreaks of acute diseases of the digestive system such as nausea, vomiting, diarrhea, dizziness and headache [1,7].

Several analytical (chemical and biochemical) methods have been developed for the determination of individual mycotoxins after immunoaffinity column clean-up [9-15]. Chromatographic methods commonly used for the quantitative determination of mycotoxins in foodstuffs includes thin layer chromatography (TLC) [16], high performance liquid chromatography (HPLC) coupled with ultraviolet (UV), photo diode array (PDA), fluorescence detectors (FLD) or mass spectrometry (MS), and gas chromatography (GC) coupled with electron capture (ECD), flame ionization (FID) or MS detectors [17]. Due to the high toxicity and occurrence of mycotoxins, rapid and reliable screening methods need to be developed for their identification and quantification in foodstuff in order to ensure safety and compliance with the legislation.

For the past years, multi-target methods for the simultaneous detection and quantification of different, co-occurring mycotoxins have been developed to replace the single analyte methods. Most of these methods are based on the combination of high- or ultra-high-performance liquid chromatography with tandem [18-21] or high-resolution [22,23] mass spectrometry. A major advantage of LC-MS-based multi-target methods is the elimination of the need for sample derivatization, in addition with the high selectivity and sensitivity. Furthermore, such multi-target methods are suitable for the analysis of highly variable mycotoxin concentrations. Mycotoxins present a great diversity in their physicochemical properties, therefore the optimization of an effective and efficient extraction procedure is of great importance. Mixtures of water with high amounts of methanol or acetonitrile (>70 %) are appropriate as extraction solvents for most mycotoxins. However, for fumonisins, higher extraction recoveries are achieved when the water proportion is increased and/or the pH of the solvent is decreased [19]. For the cleanup, multi-target methods use simple “dilute-and-shoot” approaches [19-20,24,25], solid phase extraction [21,26,27], immunoaffinity columns [28,29] and, more recently QuEChERS methodology (Quick, Easy, Cheap, Effective, Safe, Reliable) [30,31].
Rugged and Safe) [23,29]. However, multi-analyte methods with very simple sample preparation are leading to matrix effects which have to be considered for the quantification. In some cases these multi target methods are used just for semiquantitative screening purposes, due to the fact that such methods require extensive validation which is time- and cost-consuming.

In Cyprus, the occurrence of AFs, OTA, DON, T-2, HT-2, FB₁, FB₂, and ZON in locally produced and imported foodstuffs were monitored and controlled systematically and effectively, using single analyte methods with immunoaffinity column clean up [30-32] since 1990. The aim of this study was to develop and validate a simple, fast UPLC-MS/MS multianalyte method based on a single extraction step without any clean up steps for the simultaneous determination of 11 mycotoxins. The method was validated to fulfill the requirements for the official control of mycotoxins according to the relevant EU legislation [3,33,34].

Materials and Methods

Samples and sampling plan

Samples of nuts (peanuts, almonds and pistachio) and cereals (wheat and maize) were collected at critical control points (import, primary storage and market) by the Competent Authority (Health Services of Ministry of Health of Cyprus) according to a sampling plan, within the multiannual control plans. It has preventing nature in order to prohibit unfit products entering the Cyprus and EU market and fulfills the requirements of the relevant EU legislation [33-36]. This sampling plan is based on the categorization and prioritization of the risk i.e. known safety and violation problems, toxicity or severeness of risks, increased consumption, especially by high risk or vulnerable population groups (e.g. children). From each lot (15000-40000 Kg) of imported nuts, three aggregated samples (20 Kg each) are collected for enforcement (laboratory samples), defence and referee purposes, respectively. Usually 1 Kg laboratory sample is collected from the market. The laboratory sample was analysed as slurry, which was prepared with the homogenization of the sample with the appropriate amount of water in room temperature. Homogenization was done using a high shear mixer, Silverson EX, producing a slurry with nut pieces of approximately 2-3 mm. Peanuts were prepared as 1 part sample to 1 part water, whilst almonds and pistachios were prepared as 1 part sample to 1.5 parts water. On the other hand, for each lot of imported cereal samples (~50000 Kg), three aggregated samples (1-10 Kg) are collected and the laboratory sample was grounded with grinding mills to produce a homogeneous particle size before analysis. Dry milling was performed using an ultra centrifugal mill, Retsch ZM200, with 0.2-1.0 mm sieves. All samples before milling were kept under dark and dry conditions for a short period in order to avoid mycotoxin production. The samples after wet or dry milling were kept in the refrigerator until analysis.

Reagents and chemicals

The HPLC and MS grade solvents like methanol (MeOH) and acetonitrile (ACN) were purchased from Aldrich, Germany. Toluene:Acetic Acid, 99:1 v/v), ZON, DON, T-2 and HT-2 (in ACN), FB₁ and FB₂ (in ACN:H₂O, 50:50 v/v) were prepared by dissolving the solid pure substance in the appropriate solvent and were stored at -18°C until use. The concentration of the stock solution was measured by UV spectroscopy (Shimadzu, UV-1700) according to AOAC Official methods [10-12]. Working standard solutions of known concentrations were prepared by the appropriate dilution of the stock solution. For the spiking of AFs and OTA a mixture of the four AFs (1 ng µL⁻¹) and a solution of OTA (1 ng µL⁻¹) were prepared in ACN and MeOH, respectively. For the spiking of all other mycotoxins the stock solutions were used. For the preparation of the calibration curves, four mixtures/solutions were prepared: the four AFs in ACN (0.01-1.0 ng µL⁻¹), OTA in MeOH (0.01-2.0 ng µL⁻¹), DON, T-2 and HT-2 in ACN (0.3-100.0 ng µL⁻¹), and ZON, FB₁, and FB₂ in ACN (0.25-40 ng µL⁻¹).

Extraction

The extraction method was based on an earlier published study [20] and was carried out as follows: 40g slurry (1:1 w/v) or 50g slurry (1:1.5 w/v) were mixed with 60 mL acetonitrile, and the mixture was shaken for 2 h in a horizontal shaker. After filtration, 1 mL of the extract was taken and was diluted in 3 mL of water (in the case of 1:1 w/v slurry) or 2.55 ml (in the case of 1:1.5 w/v slurry). The solution was filtered using a 0.45μm membrane syringe filter (Agilent, USA) and was directly injected into the UPLC-MS/MS system. The extraction method described above was used for the preparation of ground samples, however in this case 25 g were mixed with 100 ml of a mixture of acetonitrile/water (70:30 v/v).

Instrumentation

The analysis of the 11 mycotoxins was carried out using a UPLC system (Acquity I-Class, Waters, Milford, MA, USA) coupled with a triple quadrupole mass spectrometer (Xevo TQ-S, Waters Micromass, Manchester, UK) using an orthogonal Z-spray-electrospray interface (ESI). The chromatographic separation was attained using an Acquity UPLC BEH C18 analytical column (2.1 mm × 100 mm, 1.7 μm particle size, Waters), at a flow rate of 0.4 mL min⁻¹, with an injection volume of 20 μL. The HPLC and MS grade solvents like methanol (MeOH) and acetonitrile (ACN) were purchased from Aldrich, Germany. Toluene for residue analysis (99.8%) was purchased from Lab-Scan, Ireland. Formic Acid (mass spectrometry, ~98%) was purchased from Fluka, Germany. Acetic Acid (Glacial, 100%) was purchased from Merck, Germany. The standards of AFs, OTA, DON, ZON, FB₁, FB₂, T-2 and HT-2 were obtained from Sigma-Aldrich, Germany. Pure water was obtained from Millipore water purification system (Milli-Q 185 Plus).

Preparation of standard solutions

Standards of AFs (in Toluene:ACN, 9:1 v/v), OTA (in Toluene:Acetic Acid, 99:1 v/v), ZON, DON, T-2 and HT-2 (in ACN), FB₁ and FB₂ (in ACN:H₂O, 50:50 v/v) were prepared by dissolving the solid pure substance in the appropriate solvent and were stored at -18°C until use. The concentration of the stock solution was measured by UV spectroscopy (Shimadzu, UV-1700) according to AOAC Official methods [10-12]. Working standard solutions of known concentrations were prepared by the appropriate dilution of the stock solution. For the spiking of AFs and OTA a mixture of the four AFs (1 ng µL⁻¹) and a solution of OTA (1 ng µL⁻¹) were prepared in ACN and MeOH, respectively. For the spiking of all other mycotoxins the stock solutions were used. For the preparation of the calibration curves, four mixtures/solutions were prepared: the four AFs in ACN (0.01-1.0 ng µL⁻¹), OTA in MeOH (0.01-2.0 ng µL⁻¹), DON, T-2 and HT-2 in ACN (0.3-100.0 ng µL⁻¹), and ZON, FB₁, and FB₂ in ACN (0.25-40 ng µL⁻¹).

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10 μL. Mobile phase consisting of A (H2O, 0.1% HCOOH) and B (ACN, 0.1% HCOOH) was used. The following time programmed gradient was applied: initial condition 90% for A, which was linearly decreased to 10% in 6 min; then at 6.1 min it was returned to the initial condition (90% A) and remained constant until the time reached 8 mins. The column temperature was set at 40°C. ESI-MS/MS was performed in a multiple reaction monitoring mode (MRM) at positive polarity, at a capillary voltage of 3 kV, a desolvation temperature of 400°C, a source temperature of 150°C, a desolvation gas flow 800 L h⁻¹, a variable cone voltage, a cone gas flow of 800 L h⁻¹, all under nitrogen atmosphere. Operating in the MS/MS mode, the collision gas in this case was argon with a pressure of approximately 4×10⁻³ mbar in the collision cell (0.15 eV). The applied cone voltages and collision energies for each mycotoxin are summarized in Table 1. Automatic dwell times between 0.03 and 0.11 s were used. MassLynx and TargetLynx (MassLynx v. 4.1, Waters, Manchester, UK) software were used for data acquisition and processing.

Method validation

Method validation was carried out for the 11 mycotoxins in nuts (peanuts, almonds, pistachio) and cereals (maize, wheat), according to the requirements of the accreditation standard EN ISO/IEC 17025:2005 and the performance criteria of the Commission Regulation (EC) 401/2006, by analyzing six or more replicates of spiked samples of the matrices mentioned above. The method applies external quantification. Linearity of the method was evaluated by taking at least five matrix-matched standard solutions which were analyzed in duplicate in the following ranges: 0.01-1 ng mL⁻¹ for AFs, 0.01-2 ng mL⁻¹ for OTA, 0.03-0.1 ng mL⁻¹ for DON, 0.03-0.1 ng mL⁻¹ for FB₁, FB₂, and ZON, 0.3-100 ng mL⁻¹ for T-2 and HT-2. Recoveries were determined by analyzing the spiked blank samples at several spiking levels as shown in Tables 2-6. Intraday precision (expressed as relative standard deviation in %) was calculated from the results generated by the repeatability conditions, whilst interday precision was calculated from the results generated by the reproducibility conditions. The limit of detection (LOD) and limit of quantification (LOQ) were estimated by analyzing the spiked samples at the lowest concentration. The whole method is already accredited by the Greek Accreditation Body, ESYD. The laboratory in addition to internal quality control using spiked samples, also applies external quality control by successful participation in a proficiency test (PT) organized by FAPAS. The results of the participation in FAPAS PT 04246 (maize) are shown below: AFB₁ (z-score: 0.8), OTA (z-score: 0.2), DON (z-score: 0.4), ZON (z-score: 0.5), FB₂ (z-score: -0.1), FB₁ (z-score: 1.3). Total FBs (z-score: 0.7), T-2 (z-score: 0.1), HT-2 (z-score: -1.2) and SUM T-2 / HT-2 (z-score: -0.4).

Results and Discussion

UPLC-MS/MS optimization

Mixtures of two different solvents namely A (water + formic acid (0.1%)) and B (acetoniitrile + formic acid (0.1%)) at different flow rates (0.2-0.4 mL min⁻¹) were used for the elution of 11 mycotoxins in UPLC-MS/MS. The best chromatograms for all mycotoxins with the lowest noise were obtained using a mobile phase at a flow rate of 0.4 mL min⁻¹ with the gradient elution program mentioned above. Full scan and MS/MS spectra of the analytes were obtained from the infusion.

<table>
<thead>
<tr>
<th>Mycotoxins</th>
<th>Conc. Range (μg Kg⁻¹)</th>
<th>Repeatability a  R ± RSDr (%)</th>
<th>Reproducibility a  R ± RSDR (%)</th>
<th>LOD (μg Kg⁻¹)</th>
<th>LOQ (μg Kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B₁</td>
<td>0.25-16.0</td>
<td>108.6 ± 6.9 (0.6)</td>
<td>99.3 ± 5.1 (5.0)</td>
<td>0.20</td>
<td>0.65</td>
</tr>
<tr>
<td>Aflatoxin B₂</td>
<td>0.25-16.0</td>
<td>108.1 ± 4.9 (0.6)</td>
<td>98.3 ± 4.8 (5.0)</td>
<td>0.20</td>
<td>0.65</td>
</tr>
<tr>
<td>Aflatoxin G₁</td>
<td>0.25-16.0</td>
<td>113.9 ± 3.8 (0.6)</td>
<td>101.1 ± 3.9 (5.0)</td>
<td>0.21</td>
<td>0.68</td>
</tr>
<tr>
<td>Aflatoxin G₂</td>
<td>0.25-16.0</td>
<td>107.5 ± 3.8 (0.6)</td>
<td>97.3 ± 1.8 (5.0)</td>
<td>0.20</td>
<td>0.65</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>0.24-16.0</td>
<td>71.1 ± 13.2 (0.6)</td>
<td>98.1 ± 4.2 (3.0)</td>
<td>0.13</td>
<td>0.43</td>
</tr>
<tr>
<td>Fumonisin B₁</td>
<td>5.0-640</td>
<td>n.a.</td>
<td>105.2 ± 7.4 (500)</td>
<td>30.0</td>
<td>99.0</td>
</tr>
<tr>
<td>Fumonisin B₂</td>
<td>5.0-640</td>
<td>n.a.</td>
<td>101.1 ± 5.3 (500)</td>
<td>8.0</td>
<td>26.1</td>
</tr>
<tr>
<td>Zearealenone</td>
<td>5.0-640</td>
<td>104.8 ± 5.7 (10.0)</td>
<td>93.2 ± 4.9 (100)</td>
<td>3.1</td>
<td>10.2</td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>5.0-800</td>
<td>95.7 ± 5.4 (20.0)</td>
<td>99.4 ± 1.3 (750)</td>
<td>7.7</td>
<td>25.3</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>5.0-800</td>
<td>103.8 ± 6.2 (20.0)</td>
<td>107.5 ± 6.7 (100)</td>
<td>3.4</td>
<td>11.3</td>
</tr>
<tr>
<td>HT-2 toxin</td>
<td>5.0-800</td>
<td>112.7 ± 6.8 (10.0)</td>
<td>103.7 ± 2.1 (100)</td>
<td>6.3</td>
<td>20.8</td>
</tr>
</tbody>
</table>

Table 2: Average recovery values (R), relative standard deviation (RSD), LODs and LOQs (μg Kg⁻¹) obtained for the eleven mycotoxins in maize.

<table>
<thead>
<tr>
<th>Mycotoxins</th>
<th>Conc. Range (μg Kg⁻¹)</th>
<th>Repeatability a  R ± RSDr (%)</th>
<th>Reproducibility a  R ± RSDR (%)</th>
<th>LOD (μg Kg⁻¹)</th>
<th>LOQ (μg Kg⁻¹)</th>
</tr>
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<tr>
<td>Aflatoxin B₁</td>
<td>0.25-16.0</td>
<td>103.7 ± 5.1 (0.5)</td>
<td>101.0 ± 8.1 (2.0)</td>
<td>0.16</td>
<td>0.52</td>
</tr>
<tr>
<td>Aflatoxin B₂</td>
<td>0.25-16.0</td>
<td>100.0 ± 6.7 (0.5)</td>
<td>99.8 ± 3.8 (2.0)</td>
<td>0.15</td>
<td>0.5</td>
</tr>
<tr>
<td>Aflatoxin G₁</td>
<td>0.25-16.0</td>
<td>112.3 ± 8.1 (0.5)</td>
<td>104.6 ± 3.7 (2.0)</td>
<td>0.17</td>
<td>0.56</td>
</tr>
<tr>
<td>Aflatoxin G₂</td>
<td>0.25-16.0</td>
<td>91.3 ± 7.3 (0.5)</td>
<td>102.7 ± 7.0 (2.0)</td>
<td>0.14</td>
<td>0.46</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>0.4-32.0</td>
<td>113.0 ± 4.7 (0.5)</td>
<td>103.8 ± 4.1 (3.0)</td>
<td>0.17</td>
<td>0.56</td>
</tr>
<tr>
<td>Fumonisin B₁</td>
<td>5.0-640</td>
<td>52.8 ± 3.3 (10.0)</td>
<td>82.2 ± 7.3 (500)</td>
<td>1.6</td>
<td>5.3</td>
</tr>
<tr>
<td>Fumonisin B₂</td>
<td>5.0-640</td>
<td>66.2 ± 1.5 (10.0)</td>
<td>98.1 ± 4.7 (500)</td>
<td>2</td>
<td>6.6</td>
</tr>
<tr>
<td>Zearealenone</td>
<td>5.0-640</td>
<td>110.5 ± 2.7 (10.0)</td>
<td>101.9 ± 4.4 (750)</td>
<td>3.4</td>
<td>11.1</td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>5.0-800</td>
<td>104.6 ± 2.3 (25.0)</td>
<td>99.7 ± 2.1 (760)</td>
<td>7.9</td>
<td>26.2</td>
</tr>
<tr>
<td>T-2 toxin</td>
<td>5.0-800</td>
<td>107.7 ± 9.6 (25.0)</td>
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</tr>
</tbody>
</table>

a Spiking levels (μg Kg⁻¹) are given in the brackets
n.a.: not applicable
of individual 1 μg mL⁻¹ acetonitrile solutions (without matrix) of each compound at a flow rate of 20 μL min⁻¹ combined with the mobile phase at a flow rate of 0.09 mL min⁻¹. Experiments were carried out with ESI ionization in positive mode due to the satisfactory fragmentation patterns of all mycotoxins. Full-scan mass spectra were acquired in order to obtain at least one precursor ion and the optimum cone voltage. Furthermore, product ion scan at different collision energies was carried out to determine the most abundant product ion for each compound for quantification, while the second least abundant transition (target or confirmatory) ion was used for identification by calculating the ratio of the target ion to the quantification ion. Consequently, the MRM parameters (transitions, cone voltages, collision energies) were obtained in positive-ion mode, as listed in Table 1. Figure 1 shows a UPLC-MS/MS total ion chromatogram of the 11 mycotoxins in a matrix-matched maize standard solution, and Figures 2 and 3 show the chromatograms of a spiked wheat sample and a spiked pistachio sample, respectively.

The evaluation of the chromatographic process resulted in the reduction of the analysis time, achieving a detection of all mycotoxins in less than 5 min. However, it was not possible to avoid the co-elution of analytes. In the MRM mode, the transition of the most abundant product transition (quantitative) ion was selected for quantification, while the second least abundant transition (target or confirmatory) ion was used for identification by calculating the ratio of the target ion to the quantification ion. Consequently, the MRM parameters (transitions, cone voltages, collision energies) were obtained in positive-ion mode, as listed in Table 1. Figure 1 shows a UPLC-MS/MS total ion chromatogram of the 11 mycotoxins in a matrix-matched maize standard solution, and Figures 2 and 3 show the chromatograms of a spiked wheat sample and a spiked pistachio sample, respectively.

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Method validation

Method validation was performed in terms of selectivity, linearity, accuracy, repeatability, reproducibility, LODs and LOQs. The presence of matrix components can affect the ionisation of the target compounds, reducing or enhancing the response compared with the pure compounds dissolved in solvents. Furthermore, this matrix effect is strongly depended on the mycotoxin and the matrix, and consequently for the analysis of different mycotoxins in several matrices, one matrix-matched calibration should be prepared for each matrix in order to obtain accurate results. Matrix-matched standards were prepared by spiking blank extract samples at different concentration levels. Peak area was selected as peak response and good linearity was found for all the mycotoxins, with the determination coefficients being higher than 0.98 for low concentration levels and higher than 0.99 for high concentration levels.

Accuracy and precision were estimated by means of recovery experiments at different spiking levels. The method was validated for the five food matrices selected in this work (maize, wheat, peanuts, almonds and pistachio), by spiking blank samples at different concentration levels. The results obtained were satisfactory in all five matrices, with most of the recoveries being between 70-110% and relative standard deviation (RSD) below 20% (Tables 2-6), that fulfils the requirements established by the European Union legislation [33]. In particular, the mean recoveries of mycotoxins were within the acceptable recovery range, which depends on the concentration of the analyte. According to Commission Regulation (EC) No 401/2006, the acceptable recovery values for AFs and OTA range between 50-120%, for fumonisins, DON and ZON range between 60-120% and for T-2 and HT-2 range between 60-130%. Repeatability and reproducibility for recovery studies were evaluated at one or more concentration levels, by performing six replicates for each level (Tables 2-6). It was observed that repeatability (RSDr) and reproducibility (RSDR) were much lower than 20% for all the mycotoxins and matrices evaluated, the exception being T-2 toxin in almonds that had an RSD value of 20.4%. The above results were similar to those of other studies in the literature [8,18,19,25,27,29] obtained using different sample preparation procedures and instrumentation.

The lowest level validated for each compound with satisfactory precision and recovery was used for the estimation of LOD and LOQ.

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Figure 3: UPLC-MS/MS chromatogram for the eleven mycotoxins in a spiked pistachio sample. Spiking level in brackets.

multiple analysis of a blank sample containing a very low concentration of DON. In particular, the lowest LODs and LOQs obtained for AFs and OTA ranged between 0.1 and 0.8 μg Kg⁻¹ for all matrices, whereas higher values were achieved for the other mycotoxins, where LOD and LOQ values ranged from 1.5-30.0 and 5.0-99.0 μg Kg⁻¹, respectively. In all cases, LOQs were always far below (10 times or more) the maximum residue limits of the European regulations [3,4], indicating the suitability of the method for the determination of trace concentration of these compounds. Tables 2-6 show the LODs and LOQs estimated for all eleven mycotoxins in the five matrices.

The selectivity of the method was evaluated by the analysis of blank samples. The absence of any chromatographic signal at the same retention time as the target compounds indicated the absence of chemical or matrix interferences. Identification of the mycotoxins was carried out by the comparison of the ion ratios of the two transitions (RTWs), defined as the retention time ± 5%, and confirmation was carried out by searching in the appropriate retention time windows for matrix-matched standards.

Conclusions

A selective, sensitive, rapid and reliable multi-target method involving a liquid-solid extraction, followed by the dilution of the crude extract and analysis using an ultra high performance liquid chromatography coupled to tandem mass spectrometry with triple quadrupole was employed for the determination of AFs, OTA, ZON, DON, FB₁, FB₂, T-2 and HT-2 toxins. The dilution of the extract was necessary for the reduction of matrix-effects, whereas matrix-matched calibration was used to correct the remaining matrix effects. Method performance characteristics were determined after spiking blank samples of nuts and cereals on multiple levels. The mean recoveries of mycotoxins in spiked nuts and cereals, as well as the relative standard deviations and limits of detection and quantification were in agreement with the requirements of the relevant EU legislation for official control of mycotoxins.

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References


