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Analytical Methods

Application of an HPLC–MS/MS method for mycotoxin analysis in commercial baby foods

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ABSTRACT

This article describes the validation of an analytical method for the detection of 21 mycotoxins in baby food. The analytical method is based on the simultaneous extraction of selected mycotoxins by matrix solid-phase dispersion (MSPD) followed by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) using a hybrid triple quadrupole-linear ion trap mass spectrometer (QTRAP®). Information Dependent Acquisition (IDA), an extra confirmation tool for samples that contain the selected mycotoxins, was used. The matrix effects were evaluated, and the corrections for the matrix effects were performed using two calibration approaches: external matrix-matched calibration and internal standard calibration. Matrix-matched calibration was ultimately used for accurate quantification, and the recoveries obtained were generally higher than 70%. The analytical method was applied to the analysis of 35 samples of commercial baby foods. No sample exceeded the maximum limit (ML) fixed by the European Union for these mycotoxins in baby food. However, this survey highlighted the occurrence of mycotoxins in cereal-based infant foods.

1. Introduction

Although breast milk is the major food source for the period of infancy, the gradual replacement of exclusive milk feeding by complementary foods from the fourth month of life onwards is very important, according to paediatric guidelines (Briefel, Reidy, Karwe, & Devaney, 2004; Forrest & Riley, 2004). Cereals are one of these complementary foods.

Mycotoxins are widely regarded as the natural toxins that can cause the most serious contamination of these cereals (FAO, 2004). This situation becomes more worrisome because several of these mycotoxins are stable throughout the processing of the foodstuffs and can survive intact in the final products (Bullerman & Bianchini, 2007). Infants and children are considered to be more susceptible to these toxins than adults because of their lower body weight, higher metabolic rate, and lower ability to detoxify the mycotoxins (Sherif, Salama, & Abdel-Wahhab, 2009).

Bearing in mind the risks associated with mycotoxin intake by infants, the European Union has set a very low limit for the presence of mycotoxins in infant food (EU, 2006a, 2007, 2010). However, validated methods for the analysis of mycotoxins in baby food are scarce and there is currently a growing interest in the development of reliable detection systems for mycotoxins in this food commodity.

The analytical methods that are developed and reported in the literature are not usually directly applied to baby food, but the methods that have been developed achieve good performance at low concentration levels and are also sufficient for baby food analysis (Beltrán, Ibáñez, Sancho, & Hernández, 2009; Ren et al., 2007). Only a few papers specifically focus on baby food (D'Arco, Fernández Franzón, Font, Damiani, & Mañes, 2008; Lombaert et al., 2003). In Table 1 Supplementary data, an overview of several validated LC methods for the analysis of mycotoxins in baby food is presented.

Liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) is the technique of choice for multi-mycotoxin analysis because of its versatility, specificity, and selectivity. Until recently, triple quadrupole (QqQ) LC–MS/MS equipment has been the most widely employed equipment for the performance of quantitative mycotoxin analysis (Beltrán et al., 2009; D'Arco et al., 2008; Ren et al., 2007). Although the sensitivity, selectivity and efficiency of QqQ are excellent, the qualitative information needed to support the structural elucidation of the compounds is lost (Hernández et al., 2005). This liability could be overcome with the hybrid mass spectrometer QTRAP®, which is appropriate for both quantification and confirmation (Gros, Petrovic, & Barceló, 2009; Martínez Bueno et al., 2007).

In previous research (Rubert, Soler, & Mañes, 2010, 2011), matrix solid-phase dispersion (MSPD) extraction procedures have been developed and reported for the legislated mycotoxins. As a follow-up to these previous studies, the objective of this work was the development of a fast, selective and sensitive mycotoxin

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analytical method based on MSPD extraction followed by LC–MS/MS using a 3200 QTRAP® instrument applied to mycotoxins in baby food. To our knowledge, an MSPD method (followed by QTRAP® mass spectrometry) is a technique that has scarcely appeared in the literature as a routine analytical technique in the mycotoxin field and baby food analysis appears to be an unresolved issue for the analysis of these natural contaminants.

2. Experimental section

2.1. Reagents and chemicals

Acetonitrile and methanol were supplied by Merck (Darmstadt, Germany). The dispersant used for MSPD was octadecyl silica (C_{18}) (50 μm), bonded silica from Analisis Vinicos S.L. (Tomelloso, Spain).

Deionised water (>18 MΩ cm^{-1} resistivity) was purified using the Milli-Q® SP Reagent water system plus from Millipore Corp. (Bedford, MA, USA). All solvents were passed through a 0.45 μm cellulose filter purchased from Scharlau (Barcelona, Spain). Analytical grade formic acid (purity >98%), and ammonium formate were obtained from Panreac Quimica S.A.U. (Barcelona, Spain).

The standards of aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), ochratoxin A (OTA), sterigmatocystin (STER), α-zearalenol (ZOL), zearalenone (ZEN), nivalenol (NIV), deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), fusarenon X (FUS-X), neosolaniol (NEO), diacetoxyscirpenol (DAS), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2) and beauvericin (BEA) were purchased from Sigma Aldrich (Madrid, Spain). T-2 and HT-2 toxins, aflatoxin M_1 (AFM_1) and deepoxy-deoxynivalenol (DOM-1) stock solutions (in acetonitrile) were obtained from Biopure referenzsubstanzen GmBH (Tulln, Austria). Fumonisin B_3 (FB_3) was supplied by the PROMEC unit (Programme on Mycotoxins and Experimental Carcinogenesis, Tygerberg, South Africa).

The stock solutions of aflatoxins (AFs) and OTA at a concentration of 500 μg mL^{-1} were prepared in acetonitrile and stock solutions of STER, ZOL, ZEN, NIV, DON, 3-ADON, 15-ADON, FUS-X, NEO, FB_1, FB_2 and BEA were also prepared at a concentration of 500 μg mL^{-1} but in methanol. Stock solutions of FB_3, DAS, T-2 and HT-2 at a concentration of 100 μg mL^{-1} were prepared in acetonitrile. The internal standards (ISs) were AFM_1 (for AFs) at 0.05 μg mL^{-1} and DOM-1 (for trichothecenes) at 0.150 μg mL^{-1}. Both of these solutions were prepared by dilution of individual stock solutions in methanol.

All solutions were kept in secure conditions at −20 °C.

All other working standard solutions were prepared immediately before use by diluting the stock solution with methanol:water (50:50) (v/v).

2.2. Samples

Baby food samples were purchased and kept at −20 °C under dark and dry conditions. A wide range of brands and retailers, including pharmacies, supermarkets and smaller shops of Valencia (Spain), were covered to ensure that the survey was a representative study. The entire commercial sample was homogenised and a subsample of 200 g of the retail packing was collected in a plastic bag and kept at −20 °C in a dark and dry place until analysis. A total of 35 commercial baby foods, including breakfast foods, savoury products and dessert-cereal products, were bought and analysed between March 2010 and July 2010. Samples were classified according to the way the product is presented as the following: (i) powdered baby food (i.e., multi-cereals, rice, maize, wheat, oats, with fruits, with nuts, with honey, with chocolate), (ii) puréed baby food (cereals and fruit) and (iii) liquid "ready-to-eat" baby food (cereals, fruit juice and milk).

2.3. Extraction procedure

Sample preparation was performed according to a previous study (Rubert et al., 2011). Baby food subsamples (200 g) were mixed thoroughly using an Oster® food processor (Professional Series Blender model BPST02-B00). Portions of 1 g were placed into a glass mortar (50 mL) and gently blended with 1 g of C_{18} for 5 min using a pestle to obtain a homogeneous mixture. This homogeneous mixture was introduced into a 100 mm × 9 mm i.d. glass column and eluted dropwise with 15 mL of a mixture of acetonitrile:methanol (50:50) (v/v) and 1 mM ammonium formate by applying a slight vacuum. The extract was then transferred to a 25 mL conical tube and evaporated to dryness at 35 °C with a gentle stream of nitrogen using a multi-sample Turbovap LV Evaporator (Zymark, Hoptkinton, USA). The residue was reconstituted to a final volume of 1 mL with a mixture of methanol:water (50:50) (v/v) and filtered using a 13 mm/0.22 μm nylon filter purchased from Membrane Solutions (Texas, USA) before the injection of the prepared samples into the LC–MS/MS system.

For fortified samples (a sample enriched with a known amount of the analyte to be detected) (EU, 2002), 1 g of "blank" sample (sample in which it was corroborated that no analyte was present) was spiked with 0.2 mL of a working mixture of the mycotoxins at the appropriate concentration and 0.05 mL of the IS mixture at an appropriate concentration as described above. Spiked samples were then left to stand for 3 h at room temperature before the extraction to allow the solvent to evaporate and to establish equilibration between the spiked mycotoxins and the baby food samples. Ten replicates were prepared at each spiking level.

2.4. Instrumentation

LC–tandem MS analyses were conducted on a system consisting of a Agilent 1200 chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to a 3200 QTRAP® mass spectrometer (Applied Biosystems, AB Sciex, Foster City, CA, USA) equipped with a turbo ionspray electrospray ionisation (ESI) interface. The QTRAP® analyser combines a fully functional triple quadrupole and an ion trap mass spectrometer within the same instrument. Separation of analytes was performed using a Gemini-NX (Phenomenex, 150 mm × 4.6 mm, 5 μm of particle size) LC-column preceded by a guard column utilising the same packing material. The flow rate was set to 0.8 mL min^{-1}, and the oven temperature was 40 °C, with eluent A water (mobile phase A) slightly acidified with 0.1% formic acid and 5 mM ammonium formate and eluent B (mobile phase B) methanol with 5 mM ammonium formate. The elution gradient started with 0% of eluent B, increased to 100% in 10 min, decreased to 80% in 5 min and, finally, decreased to 70% in 2 min. During the subsequent 6 min, the column was cleaned and readjusted to the initial conditions and equilibrated for 7 min. The volume of the injections was 20 μL.

The analyses were performed using the Turbo V® ionspray in positive ionisation mode (ESI+). The operating conditions for the analysis were the following: ion spray voltage, 5500 V; curtain gas, 20 (arbitrary units); GS1 and GS2, 55 and 65 psi, respectively; probe temperature (TEM), 500 °C. Nitrogen served as the nebuliser and collision gas. SRM experiments were performed to obtain the maximum sensitivity for the detection of target molecules. The optimisation of MS parameters as declustering potential (DP), collision energy (CE) and collision cell entrance potential (CEP) was performed by flow injection analysis for each compound; entrance potential (EP) and collision cell exit potential (CXP) were set at 10 and 4 V, respectively, for all analytes. The MS was operated in