Journal of Chromatography A, 1223 (2012) 84–92

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Applicability of hybrid linear ion trap-high resolution mass spectrometry and quadrupole-linear ion trap-mass spectrometry for mycotoxin analysis in baby food

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ARTICLE INFO

Article history:
Received 18 September 2011
Received in revised form
24 November 2011
Accepted 10 December 2011
Available online 20 December 2011

Keywords:
Orbitrap
QTRAP
MSPD
Mycotoxins
Baby food

ABSTRACT

Recent developments in mass spectrometers have created a paradoxical situation; different mass spectrometers are available, each of them with their specific strengths and drawbacks. Hybrid instruments try to unify several advantages in one instrument. In this study two of wide-used hybrid instruments were compared: hybrid quadrupole-linear ion trap-mass spectrometry (QTRAP®) and the hybrid linear ion trap-high resolution mass spectrometry (LTQ-Orbitrap®). Both instruments were applied to detect the presence of 18 selected mycotoxins in baby food. Analytical parameters were validated according to 2002/657/CE. Limits of quantification (LOQs) obtained by QTRAP® instrument ranged from 0.45 to 45 $\mu g\,kg^{-1}$ while lower limits of quantification (LLOQs) values were obtained by LTQ-Orbitrap®: 7–70 $\mu g\,kg^{-1}$. The correlation coefficients (r) in both cases were upper than 0.989. These values highlighted that both instruments were complementary for the analysis of mycotoxin in baby food; while QTRAP® reached best sensitivity and selectivity, LTQ-Orbitrap® allowed the identification of non-target and unknowns compounds.

1. Introduction

Mycotoxins are regarded as the most serious of natural toxins that can contaminate cereals or derivates [1–3]. Due to the co-occurrence of different toxins in food matrices and their possible synergistic effect in humans, it is absolutely necessary to perform multi-analyte detection methods [4,5]. Moreover, the level of contamination can vary considerably worldwide according to geographical area, region and year and it can range from a few $ng\,g^{-1}$ to several $\mu g\,g^{-1}$ [6]. The different chemical groups of mycotoxins, the complexity of matrices and the low detection limits required increasing the importance of the choice of analytical strategy in this field.

Liquid chromatography-tandem mass spectrometry coupled with triple quadrupole has been widely accepted as the main tool in the identification, structural characterization and quantitative analysis of mycotoxins owing to its superior sensitivity, specificity and efficiency [3,7–9]. However, this mass analyzer is a targeted method that only monitors a relatively large number of analytes defined in advance; in such targeted analyses, signals from all other compounds are ignored [10,11]. As the number of substances to be screened and confirmed is high and not limited, one technique could never be capable sufficient to detect all mycotoxins and related compounds (as metabolites) in one run.

Fortunately, the establishment of directives based on mycotoxins analysis [12–15], validation criteria [16–18] and development of mass spectrometry have growth in parallel way; the use of hybrid instruments could overcome several drawbacks and reach the requirements and robustness data required.

In this work, two widely-used hybrid instruments, QTRAP® and LTQ-Orbitrap®, have been investigated to achieve both accurate and reliable target mycotoxins monitoring in wheat-based baby foods, as well as to find non-target and unknown mycotoxins.

On the one hand, triple quadrupole-linear ion trap-mass spectrometry or QTRAP® was born in the last decade; this instrument is a hybrid linear ion trap triple quadrupole in which the last quadrupole is replaced by a linear ion trap (LIT). The ion trap is capable of 3 levels of fragmentation (MS^3) as well as high sensitivity scan, besides the instrument is able to operate like a triple quadrupole or hybrid running, such as information dependent acquisition (IDA) method [19]. Most often, QTRAP® instrument has been exclusively used as triple quadrupole for mycotoxins analysis [20,21]. The analytical methods developed in these works had basically confirmatory purposes, fulfilling Commission Decision

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doi:10.1016/j.chroma.2011.12.039

2002/657/EC. The methods had several advantages: both of them were rapid, accurate and selective working in triple quadrupole mode, but the applicability of hybrid mode was not studied.

Focus on the analysed matrix, the methods have been commonly applied for the establishment of monitoring programs for mycotoxins analysis in different types of cereals [2,3,19–21]. In the particular case of baby foods, they have been exclusively studied for concrete groups of mycotoxins using triple quadrupole mass spectrometers. For example, the literature shows methods for aflatoxins and ochratoxin A (OTA) [7], as well as fumonisins [8]. Thereby, a multi-mycotoxin method for baby food analysis has not been developed until this moment, and neither the applicability QTRAP® working in hybrid mode has been studied.

On the other hand, hybrid linear ion trap-high resolution mass spectrometry or LTQ-ORBITRAP® has recently appeared combining Orbitrap analyzer with an external accumulation device such as a linear ion trap, making possible multiple levels of fragmentation (MS^n) for the elucidation of analyte structure. The use of the LTQ Orbitrap allows high-quality accurate mass and acquisition of MS^n spectra [22,23]. Focus on mycotoxin analysis by Orbitrap® technology, it has not been commonly used for routine analysis. It could be due to this technology is recent, even so it has been just applied to cereals and beer [23–25]. However, this technology has been never applied to baby food analysis and it has not been evaluated against other hybrid instrument. Previous work carried out a first approach for determining 31 mycotoxins in grain comparing triple quadrupole with Orbitrap instrument [10]. The authors concluded that one of the major advantages of the high resolution full scan method is the possibility of screening unknown compounds, however the best sensitivity was obtained with triple quadrupole instrument.

This paper highlights the advantages, limitations and applicability of these two instruments and their validation to be applied for mycotoxins analysis in baby food. Since our knowledge, it is the first time that these two hybrid instruments (in the hybrid mode detection) are compared in the field on mycotoxins analysis in this food matrix.

2. Materials and methods

2.1. Reagents and materials

Acetonitrile and methanol were supplied by Merck (Darmstadt, Germany). Solid-phase used for matrix solid-phase dispersion (MSPD) extraction was Sepra C18-E (50 μm, 65 Å) endcapped silica-based C_{18} from Phenomenex (Torrance, USA). Deionized water (>18 MΩ cm^{-1} resistivity) was purified using Milli-Q® SP Reagent water system plus from Millipore Corp. (Bedford, USA). All solvents were passed through a 0.45 μm cellulose filter purchased from Scharlau (Barcelona, Spain). Analytical grade reagent 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), OTA, sterigmatocystin (STER), α-zearalenol (ZOL), zearalenone (ZEN), nivalenol (NIV), deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), diacetoxyscirpenol (DAS), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), beauvericin (BEA) were purchased from Sigma Aldrich (Madrid, Spain). T-2 toxin (T-2) and HT-2 toxin (HT-2) stock solutions (in acetonitrile) were purchased 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 500 μg mL^{-1} were prepared in acetonitrile and STER, ZOL, ZEN, NIV, DON, 3-ADON, FB_1, FB_2, BEA were prepared at the same concentration in methanol. Stock solutions of DAS, FB_3, T-2 and HT-2 at 100 μg mL^{-1} were prepared in acetonitrile. All these standard solutions were kept in safety 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 (wheat-based) were purchased from different stores from Valencia (Spain) and Cork (Ireland) and kept at −20 °C in a dark and dry place. A wide range of brands and retailers, including pharmacies, supermarkets and smaller shops, were covered in order to ensure that the survey was representative of the baby food industry. The entire commercial samples were homogenized, and 200 g of subsample was collected in a plastic bag and stored under the same conditions until analysis [15]. A total of 25 samples of wheat-based baby foods were bought and analysed.

2.3. Extraction procedure

Sample preparation was optimized in a previous study [3]. A MSPD extraction method was applied to wheat-based baby foods. Samples (200 g) were prepared using an Oster® food processor (Professional Series Blender model BPST02-B00), mixing the sample thoroughly. Homogenized and representative portions of 1 g were weighed and placed into a glass mortar (50 mL) and were gently blended with 1 g of C_{18} for 5 min using a pestle, to obtain a homogeneous mixture. The homogeneous mixture was introduced into a 100 mm × 9 mm i.d. glass column, and eluted dropwise with 15 mL of elution solvent which was a mixture of acetonitrile/methanol (50/50, v/v) at 1 mM ammonium formate by applying a slight vacuum. Then, the extract was 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 methanol/water (50/50, v/v) and filtered through a 13 mm/0.22 μm nylon filter purchased from Membrane Solutions (Texas, USA) before their injection into the liquid chromatography tandem mass spectrometry (LC–MS/MS) system.

For the preparation of fortified samples, 1 g of "blank" samples (sample in which it was corroborated before the analysis that no analytes were present) were spiked with 0.1 mL of a working mixture of mycotoxins at the appropriate concentration. Then, spiked samples were left to stand 3 h at room temperature before the extraction to allow the evaporation of the solvent and to establish equilibration between the mycotoxins and baby food sample. Ten replicates were prepared for each spiking level.

2.4. General chromatographic conditions and HPLC instrumentation

Separation of analytes was performed with a reversed-phase analytical column (Gemini C_{18}, 150 mm, 2 mm i.d, 5 μm; Phenomenex) maintained at 35 °C. As mobile phase, 5 mM ammonium formate and 0.1% formic acid in water (A) and 5 mM ammonium formate in methanol (B) were used. The gradient was as follows: at the start 5% of solvent B and after the percentage of solvent B was linearly increased to 95% in 10 min. The percentage of solvent B was kept for 5 min. Finally, the column was equilibrated to initial conditions for 10 min. The flow rate was 200 μl min^{-1} and the injection volume was 10 μl.

The 3200 QTRAP® mass spectrometer was coupled to Agilent 1200 chromatograph (Agilent Technologies, Palo Alto, CA, USA),