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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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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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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),

while LTQ-Orbitrap® was connected to Accela LC system (Thermo Scientific, Hemel Hempstead, UK).

2.5. Mass spectrometry conditions

The 3200 QTRAP® mass spectrometer (Applied Biosystems, ABSciex, Foster City, CA, USA) was equipped with a Turbo V™ Ion Spray (ESI) interface. The QTRAP® analyzer combines a fully functional triple-quadrupole and ion trap mass spectrometer within the same instrument. The analyses were performed using Turbo V™ Ion Spray in positive mode. The operation conditions for the analysis in positive ionization mode were the followings: ion spray voltage 5500 V, probe temperature 450 °C, curtain gas 20 (arbitrary units) and GS1 and GS2, 50 and 55 psi, respectively. Nitrogen served as nebulizer and collision gas. Selected reaction monitoring (SRM) experiments were carried out to obtain the maximum sensitivity for the detection of target molecules. The optimization of MS parameters as declustering potential (DP), collision energy (CE) and collision cell entrance potential (CEP) were performed by flow injection analysis for each compound and the values are summarized in Table 1, Supplementary data; entrance potential (EP) and collision cell exit potential (CXP) were set 10 and 4 V, respectively for all analytes. The mass spectrometer was operated in SRM mode and with a unit resolution for Q1 and Q3. For LC–MS/MS analysis, scheduled SRM (sSRM) was used at 50 s of SRM detection window and 1 s of target scan time, in this form was obtained more than 12 data points for all selected mycotoxins. Scheduled SRM is defined as a SRM with the amount of time for detection that surrounds the retention time for each transition.

In order to compare the performance distinctive of two operational modes of the QTRAP® triple quadrupole and triple quadrupole linear ion trap, IDA method was developed. Several experiments were carried out; the first experiment was a SRM which included the most abundant transition of the target compounds. The intensity threshold was set at 700 counts per second (cps); when intensity of the ions was arrived at the minimum, 3 enhanced product ion (EPI) scans (dependent scans) were unleashed at different collision energies (20, 35 and 50 eV). The monitoring of the sSRM ratio and the EPI scan (as an extra-information tool) were used. Analyst® version 1.5.2 software (Applied Biosystems/ABSciex) was used to control and also for data collection and analysis.

LTQ-Orbitrap® XL (Thermo Scientific) is a hybrid LIT–FT mass spectrometer. The linear ion trap (LTQ), part of the hybrid MS system, was equipped with heated electrospray interface (H-ESI), operating in positive ionization mode. Full-scan accurate mass spectra (mass range from 90 to 900 Da) were obtained at high resolution 100,000 full width at half height maximum (FWHM) and processed using Xcalibur v.2.0 and MassFrontier 7.0, both software from Thermo Scientific. The electrospray source conditions were: source voltage 4 kV, heated capillary temperature 275 °C, capillary voltage 30 V and sheat gas and auxiliar gas, 35 and 30 (arbitrary units), respectively. The mass spectrometer was operated in a data-dependent-acquisition (DDA) mode in which both MS and MS^n spectra were acquired specifying parent mass of target compounds. In this mode, the acquisition software probed the MS spectra in real-time on a full scan allowing accurate mass. The accurate mass is capable to find true unknowns since the method does not require any pre-selection of masses. The instrument is initially set to operate in full-scan mode until a parent ion appears to preset the instrument, which switches into the MS^n. The mass resolution was set at 100,000 FWHM for both screening and quantitative analysis. The products ions were generated in the LTQ trap at an optimized collision energy setting of selected mycotoxins. The scan type settings are presented in Table 2, Supplementary data. No exclusion list was used. The total cycle time depends upon the resolution; at a resolution of 100,000 FWHM the total cycle time is about 1 s. The results were used to create a (full-scan) accurate mass, both MS and MS^n, database to enable identification of compound in future screening analysis. The Orbitrap instrument was calibrated using a solution containing caffeine, MRFA, and Ultramark 1621, according to the manufacturer's instructions.

2.6. Validation method for target analysis

2.6.1. QTRAP®

The criteria applied to study the identity of mycotoxins were according to the EU requirements [16]: (i) precursor ion and two transitions were monitored, (ii) the measured retention time of the suspected peak had to correspond to the measured retention time of the standard and finally (iii) the area ratio between the two monitored SRM traces had to be equal in the sample and in the standard or matrix-matched [22]. Moreover in this work, the EPI scan (as an extra-information tool) was carried out for positives samples.

The matrix-matched calibration curves were used for effective quantitative determinations. The linearity in the response was calculated using standard solutions and matrix-matched solutions were prepared by spiking wheat-based baby food presentations in triplicate at six concentrations levels into the analytical range: from the limit of quantification (LOQ) to 100 times this LOQ. The matrix effect (ME) was calculated for each mycotoxin in baby food, as the percentage of the matrix-matched calibration slope (B) divided by the slope of the standard calibration in solvent (A); the ratio ($B/A \times 100$) is defined as the matrix effect (ME%). A value of 100% indicates that there is no absolute matrix effect. There is signal enhancement if the value is >100% and signal suppression if the value is <100%.

Recoveries ($n = 10$) were carried out by spiking wheat-based baby food at LOQ concentration level and 100 times LOQ. The precision of the method (% RSD), was estimated by the repeated analysis ($n = 10$) of a spiked wheat-based baby food at LOQ and 100 times LOQ during the same day (intra-day) and on different five days (inter-day). In order to compare the sensitivity of SRM Scheduled™ modes, the limits of detection (LODs) were calculated using spiked baby food. The LODs were determined as the lowest mycotoxin concentration whose qualified transition (q) presented a signal-to-noise ratio ($S/N \geq 3$. The quantification limits (LOQ) were determined as the minimum detectable amount of analyte with a $S/N \geq 10$ for the quantified transition (Q) (Table 1).

2.6.2. LTQ-ORBITRAP® XL

Validation of the method was performed following directive and guide on that subject [16,17]. The following parameters were studied: confirmation of identity, specificity/selectivity, linearity, limit of detection (LOD), lower limit of quantification (LLOQ), precision as repeatability and within-lab reproducibility, process efficiency and recovery.

Confirmation of identity was based on the following criteria: (i) the measured accurate mass of $[M+H]^+$ or $[M+NH_4]^+$ must fit the theoretical accurate mass with a mass tolerance set at ±5 ppm. (ii) Fragment ions obtained by ion trap must be present and their relative abundances with respect to $[M+H]^+$ or $[M+NH_4]$ ion must coincide with those of a calibration standard within ±15%. (iii) The retention time window was set to ±2% from that of a calibration standard.

Linearity was evaluated using standard solutions and matrix-matched calibrations by analyzing in triplicate six concentrations levels between LLOQ and 100 times LLOQ. Matrix effects were studied as previous section. Other analytical parameters, such as limits of detection (LODs) and lower limits of quantification (LLOQs), were determined empirically by analyzing a series of decreasing

Table 1
Maximum levels (ML) for selected mycotoxins in baby food according to EC1881/2006, EC1126/2007 and EC165/2010 Commission Regulations and limit of detection (LOD), limit of quantification (LOQ) and lowest limit of quantification (LLOQ) expressed as $\mu g\,kg^{-1}$, obtained by different mass analyzers.

Mycotoxins	ML Baby food[a]	QTRAP *Scheduled* SRM		QTRAP® IDA method (EPI mode)		ORBITRAP® Full scan DDA	
		LOD	LOQ	LOD	LOQ	LOD	LLOQ
NIV		12	45	60	150	40	70
DON	200	5	15	12	30	15	30
3-ADON		4	12	10	30	15	35
DAS		1.5	4	4	12	10	25
HT-2		1.5	3.5	3	12	7	18
T-2		0.8	2.5	2.5	8	5	12
FB1	200 ($FB_1 + FB_2$)	10	30	30	60	32	55
FB2		12	36	30	65	30	60
FB3		10	30	30	60	32	65
ZEN	20	2	8	7	20	8	18
ZOL		2	6	5	20	10	25
BEA		1	3	3	8	5	12
AFB1	0.1	0.2	0.45	0.5	2	3	7
AFB2		0.25	0.75	0.8	3	4	8
AFG1		0.25	0.75	0.8	3	5	8
AFG2		0.25	0.75	0.8	3	4	8
STER		0.5	1.5	0.8	3	5	9
OTA	0.5	0.15	0.45	0.5	2.25	3	7

[a] Maximum level (ML) [12–14].

concentrations of the wheat baby food in multiple replicates ($n = 3$), using three different calibration lines. The LOD was the concentration at which the analyte response could be identified with relative standard deviation (% RSD) and mean relative error (MRE) (MRE% defined as [measured concentration − nominal concentration/nominal concentration × 100]) > 20% and ≤30%. The LLOQ was defined as the lowest concentration that could be quantified with RSD% and the absolute value of MRE% ≤ 20% [23]. LODs and LLOQs are listed in Table 1. The recovery experiments were carried out by spiking the sample in ten replicates at two concentration levels, LLOQ level and 100 times LLOQ level. In the same way, precision of the method was determinate in fortified wheat powered baby food at LLOQ level and 100 times LLOQ level and calculated as RSD of measurements in ten replicates during the same day and five non-consecutive days.

3. Results and discussion

3.1. Validation study

The methods were validated according to the criteria specified in Commission Decision 2002/657/EC for quantitative confirmation method [16]. The specificity of the methods was demonstrated by the analysis of "blank" baby food samples (samples without analytes) and fortified samples (samples on a mixture of mycotoxins at known concentration was spiked).

Characteristic values of performance, including limits of detection (LODs) and quantification (LOQs), recoveries; intra-day and inter-day precision were obtained in fortified samples at appropriate concentration level by ten replicates. The LODs and LOQs in $\mu g\,kg^{-1}$ for the methods are summarized in Table 1. The analytical parameters of the methods are summarized in Table 2 for QTRAP® and Table 3 for LTQ-Orbitrap®. These limits were between 3 and 20 fold better by QTRAP® instrument when sSRM method was used: this implies that for each compound one specific product ion was selected for quantifying and a second product ion was used for confirmation. The principle to the *scheduled* SRM is to monitor these transitions increasing the time that is available for acquiring one data point. It was therefore observed an enhancement of the selectivity and consequently improvement on LODs and LOQs.

These limits are an important point in this work, since maximum levels (MLs) established for mycotoxins in baby foods are more restrictive than other foodstuff [12–15]. In this way, QTRAP® system reached these MLs for all selected mytcotoxins when mass analyzer worked in sSRM mode with the exception of AFB_1. When the instrument were working in hybrid mode (IDA method) reached LODs and LOQs, which were higher than in sSRM mode, and this mode did not fulfil established MLs for OTA and aflatoxins. LTQ-Orbitrap® system did not fulfil MLs for aflatoxins and OTA.

For this reason, some authors have preferred to limit to a particular group of mycotoxins. For example, a sensible analytical method has been recently developed for AFs and OTA in baby food using ultra high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). Remarkable LOQs were reached lower than $25\,ng\,kg^{-1}$ and excellent accuracy was obtained [7]. On the other hand, a method for fumonisins was successfully developed reaching low LOQs: $2\,\mu g\,kg^{-1}$ for FB_1 and FB_2, and $5\,\mu g\,kg^{-1}$ for FB_3 [8]. Usually, a multi-mycotoxin method is a compromise of several parameters. The structural variability of mycotoxins is the main problem for their simultaneously extraction and detection.

In our research, this compromise was observed in different ways. LOQs were fulfilled for selected mycotoxins except AFB_1 when QTRAP® worked as a triple quadrupole and AFB_1 and OTA when QTRAP® worked in hybrid mode. As the number of compounds is increased, more complex is the analytical method. However, the LOQs reached in this study were according to recent multi-mycotoxins methods which were applied to cereals [2,3,19–21].

Focus on Orbitrap®, there is not available bibliography about baby food analysis. One study compared the Orbitrap® technology with triple quadrupole instrument [10]. This work carried out the validation for triple quadrupole instrument, but it was not validated the method for Orbitrap®. Even so, the LODs for Orbitrap® ranged from 4 to $2000\,\mu g\,kg^{-1}$. The authors concluded that the high resolution full scan method could be used for screening unknown compounds, while the best sensitivity and quantification were obtained by triple quadrupole.

Step by step, new analytical methods have appeared in this way. These methods are completely focused on Orbitrap® technology; beer was the starting point and two multi-mycotoxin methods were developed. The first method used Exactive Orbitrap®; in this case the LLOQs ranged from 0.5 to 65 μg/L [24]. The second one was developed using the LTQ-Orbitrap®; LLOQs ranged from 12 to 155 μg [23]. The difference between these instruments is the presence of LIT, which could be used to confirm the compounds by

Table 2
QTRAP® validation parameters: matrix effect, low and high recovery levels (values (%)) and relative standard deviations (RSD, %) given in brackets calculated at two concentration levels (μg kg^{-1}).

Mycotoxin	Matrix effects[a] (%)	Intra-day[d]		Inter-day[e]	
		Low level[b]	High level[c]	Low level[b]	High level[c]
NIV	65	81 (9)	75 (10)	80 (12)	78 (12)
DON	75	83 (7)	87 (7)	84 (8)	82 (8)
3-ADON	60	75 (8)	71 (5)	72 (7)	75 (4)
DAS	70	74 (7)	79 (3)	73 (6)	74 (7)
HT-2	83	79 (8)	73 (5)	74 (7)	74 (7)
T-2	85	71 (6)	72 (4)	73 (5)	73 (11)
FB1	95	95 (10)	92 (14)	94 (16)	93 (15)
FB2	98	91 (12)	93 (15)	95 (15)	92 (14)
FB3	96	95 (10)	95 (15)	94 (12)	93 (16)
ZEN	80	76 (5)	73 (4)	75 (5)	74 (9)
ZOL	77	78 (7)	75 (9)	76 (6)	73 (6)
BEA	68	71 (8)	69 (5)	73 (5)	74 (9)
AFB1	49	73 (4)	70 (8)	77 (7)	78 (11)
AFB2	52	77 (4)	73 (9)	74 (6)	76 (12)
AFG1	56	72 (5)	69 (7)	75 (4)	73 (9)
AFG2	55	78 (5)	75 (7)	73 (7)	72 (10)
STER	69	71 (5)	72 (6)	72 (4)	71 (6)
OTA	81	78 (6)	72 (4)	72 (5)	75 (9)

[a] ME %: slope matrix matched sample/slope standard in solvent × 100.
[b] Low level: LOQ level (μg kg^{-1}).
[c] High level: 100 times LOQ level (μg kg^{-1}).
[d] Number of replicates: 10.
[e] Different days: 5.

fragmentation study. In our study the LLOQs were ranged from 7 to 70 μg kg^{-1} being LLOQs according to recent works [23–25].

The calibration curves for each compound by both methods were established using matrix-matched from LOQ to 100 times LOQ for QTRAP® and LLOQ to 100 times LLOQ for LTQ-Orbitrap® system. Linear regression analysis was performed by plotting peak area ratios versus analyte concentrations using a least-square linear regression mode. The linearity was acceptable for all analytes in the whole range of tested concentrations, as proved the correlation coefficients (r) upper than 0.991 values for all curves in the case of the QTRAP® mass spectrometer and upper than 0.989 for the LTQ-Orbitrap® system.

Matrix effects calculated in percentages, as it has previously been described above, were similar on both instruments (Tables 2 and 3), although ME (%) were slightly higher on LTQ-Orbitrap® system than on QTRAP® instrument. In the first one, the most striking fact was the enhancement observed in fumonisins, while in the second one was curious the suppression resulted in the detection of aflatoxins. These matrix effects should be compensated by using appropriate calibration method. In this study, on both methods, external matrix-matched calibration showed to be effective in compensation of matrix effects.

The intra-day and inter-day precisions of the methods were evaluated on spiked wheat-based baby food at two different concentration levels (LOQ and 100 times LOQ). The RSD values for

Table 3
LTQ-ORBITRAP XL validation parameters: matrix effect, low and high recovery levels (values (%)) and relative standard deviations (RSD, %) given in brackets calculated at two concentration levels (μg kg^{-1}).

Mycotoxin	Matrix effect[a] (%)	Intra-day[d]		Inter-day[e]	
		Low level[b]	High level[c]	Low level[b]	High level[c]
NIV	60	77 (10)	81 (11)	78 (10)	78 (12)
DON	63	83 (11)	78 (9)	81 (10)	81 (9)
3-ADON	59	84 (11)	80 (14)	81 (9)	82 (9)
DAS	62	74 (9)	72 (8)	73 (11)	78 (9)
HT-2	63	71 (8)	72 (9)	71 (7)	76 (11)
T-2	69	68 (10)	69 (10)	71 (8)	68 (11)
FB1	112	79 (11)	82 (11)	77 (19)	81 (12)
FB2	110	89 (15)	86 (14)	83 (18)	85 (15)
FB3	123	82 (12)	86 (12)	72 (11)	77 (12)
ZEN	79	75 (11)	77 (13)	73 (14)	71 (12)
ZOL	88	75 (8)	76 (8)	71 (15)	75 (12)
BEA	64	69 (15)	66 (18)	70 (13)	70 (19)
AFB1	60	72 (7)	78 (7)	69 (6)	77 (9)
AFB2	54	67 (10)	70 (11)	73 (13)	71 (9)
AFG1	51	71 (8)	73 (7)	74 (12)	72 (9)
AFG2	56	70 (8)	73 (9)	69 (11)	71 (10)
STER	66	69 (11)	73 (6)	72 (12)	71 (9)
OTA	77	71 (6)	70 (12)	78 (9)	73 (8)

[a] ME %: slope matrix matched sample/slope standard in solvent × 100.
[b] Low level: LLOQ level (μg kg^{-1}).
[c] High level: 100 times LLOQ level (μg kg^{-1}).
[d] Number of replicates: 10.
[e] Different days: 5.

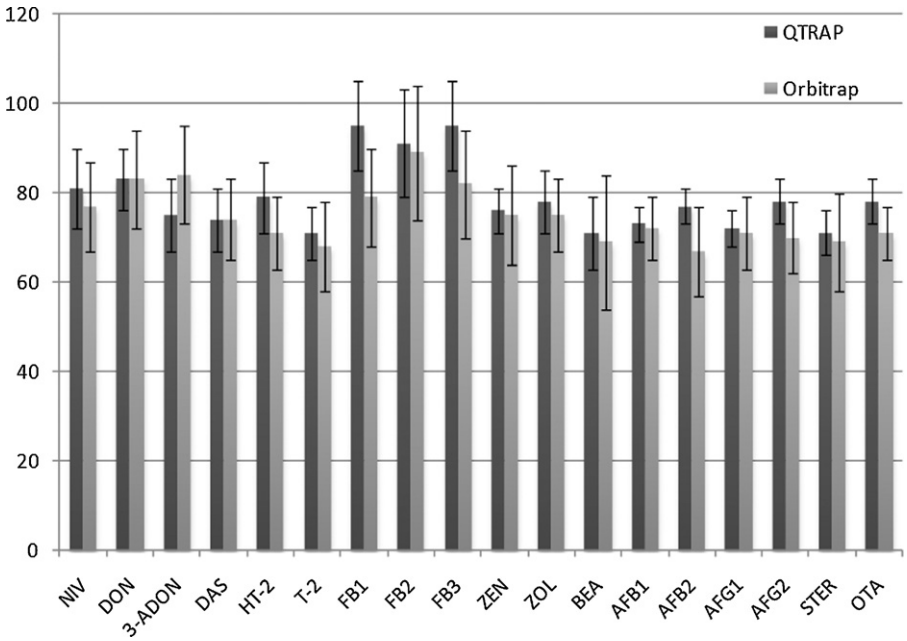

Fig. 1. Recoveries (%) and RSDs (*y*-error bars) at the LLOQ concentration level (LTQ-Orbitrap) of selected mycotoxins in baby food obtained by QTRAP and LTQ-ORBITRAP instruments.

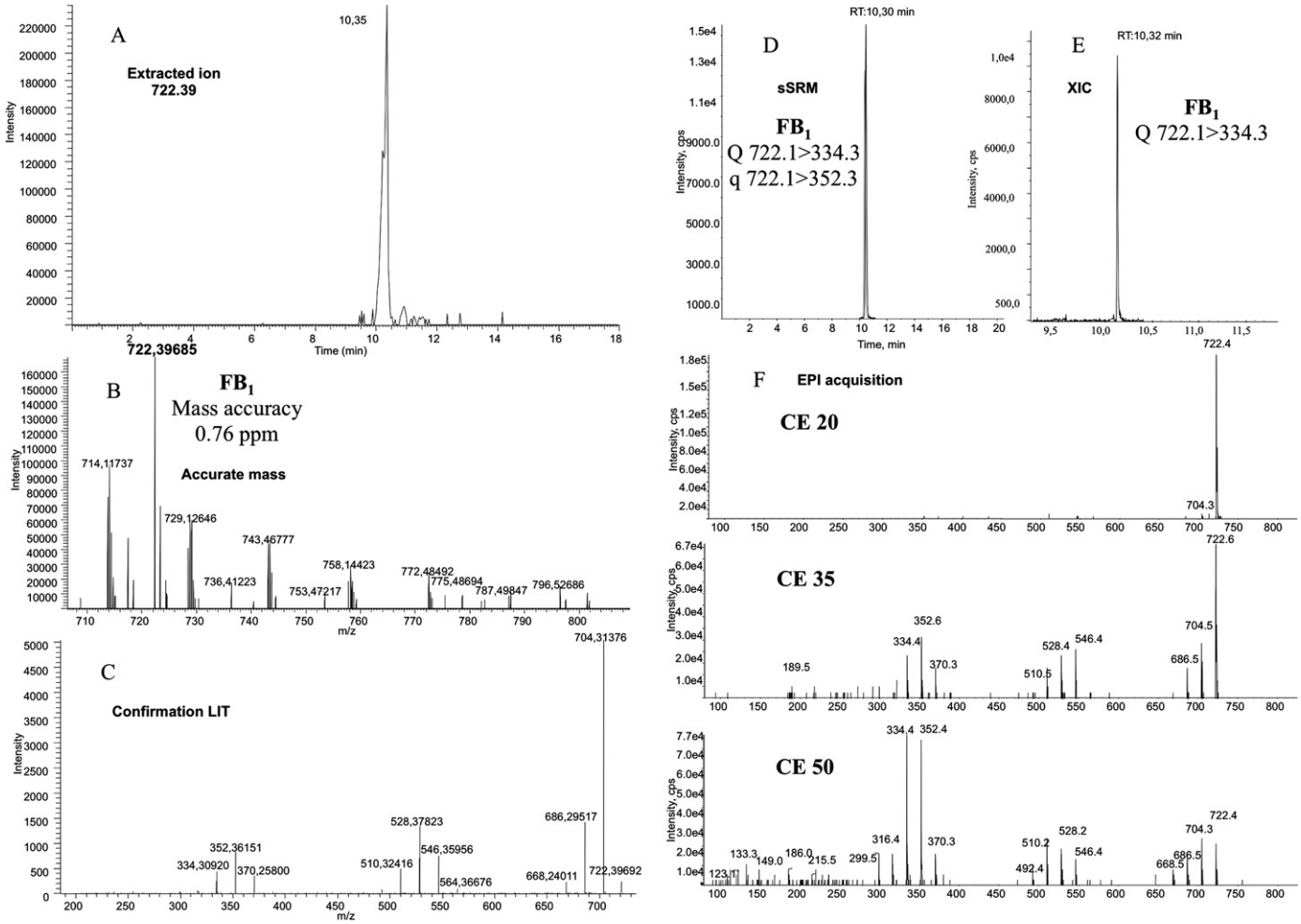

Fig. 2. Analysis of FB1 mycotoxin in a spiked wheat-based baby food at 75 μg kg-1 by LTQ-Orbitrap® (A–C) and by QTRAP® (D–F). Extracted ion 722.39 (A), accurate mass FB1 (B) and confirmation by ion trap (C). Schedule SRM transitions (D), XIC from the TIC (E) and EPI spectrum at different collision energy voltage (F).

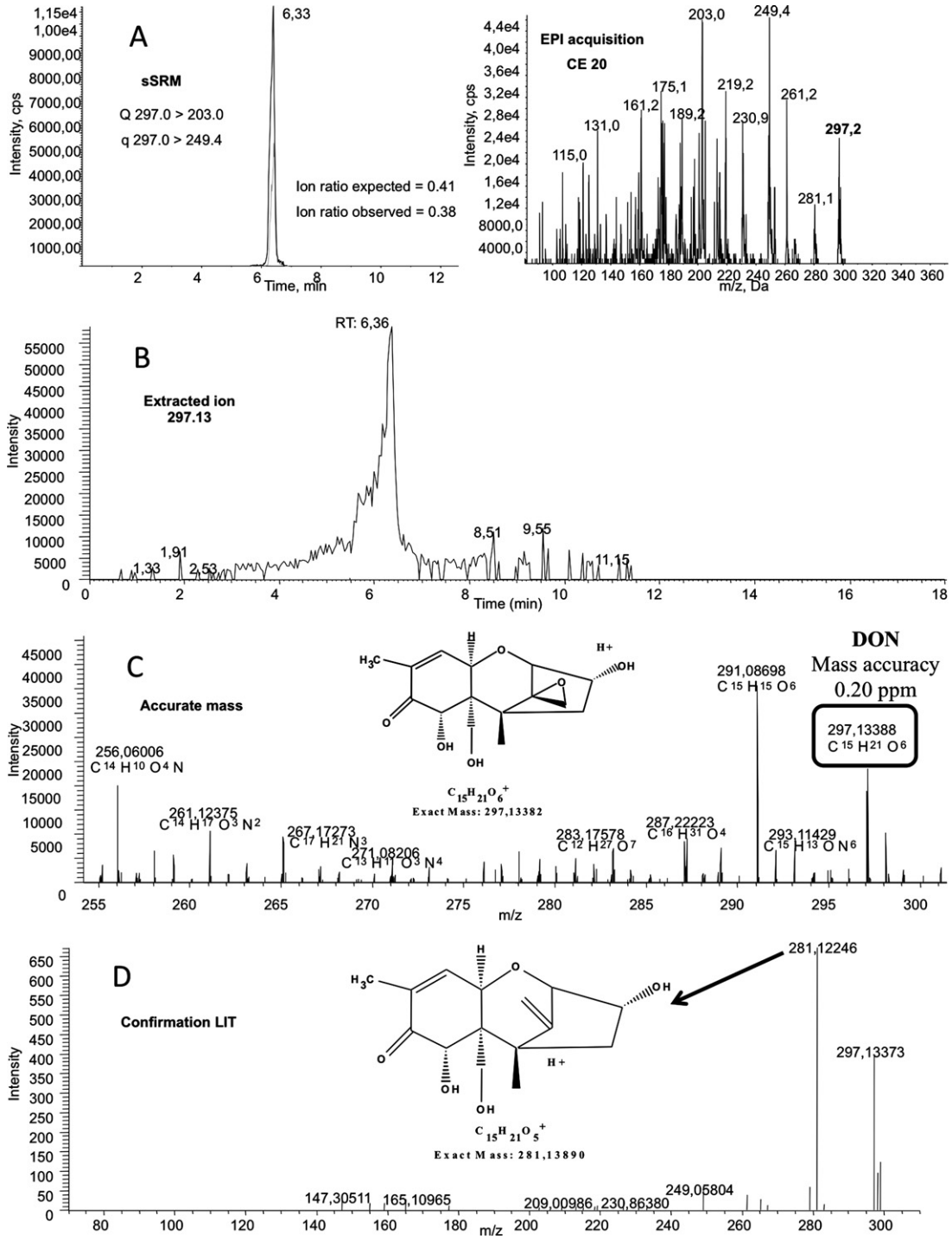

Fig. 3. Positive sample: DON. Figure (A) shows the sSRM chromatogram and EPI acquisition mode (20 eV) obtained in QTRAP®. Figures (B–D) show detection and confirmation using LTQ-Orbitrap®. Figure extracted ion 297.13 (B), accurate mass DON (C) and confirmation by linear ion trap (D).

intra-day analyses were in the range of 3–15% and the RSD for inter-day values ranged from 4 to 16%, showing good reproducibility for QTRAP® instrument (Table 2). In the same way, using the LTQ-Orbitrap® mass spectrometer, RSD values for intra-day analyses were in the range of 6–18% and the RSD for inter-day values ranged between 6 and 19% (Table 3). Although the RSD values obtained by LTQ-Orbitrap® technology were slightly higher than for QTRAP®, they were considered satisfactory.

The mean recovery values at LOQ and 100 times LOQ spiked levels ranged between 71–95% and 69–95%, respectively by QTRAP® (Table 2) and between 67 and 89% for LLOQ concentration level and 66–86% for 100 times LLOQ concentration level using LTQ-Orbitrap® (Table 3). Fig. 1 shows the recoveries and RSDs obtained at LLOQs concentration level (Table 1) of selected mycotoxins in baby food obtained by QTRAP® and LTQ-Orbitrap® instruments, showing acceptable and very similar values for both instruments.

In light of these results, soft differences were observed between the compared analyzers. When the linearity was studied, QTRAP® provided a slightly better linear response than LTQ-Orbitrap®. In the same way, LTQ-Orbitrap® evidenced higher matrix effects;

this fact can be explained since it is generally assumed that the specificity afforded by sSRM mode discriminates between target mycotoxins and matrix components. Results showed that accuracy was better in the QTRAP® system. The recoveries obtained were similar in both systems, which is normal due to the recoveries mainly depends on the extraction procedure and not on the determination systems. However, the differences in the RSDs between both analyzers gave an idea of the quantification accuracy.

To demonstrate the differences and similarities in the mass spectra, Fig. 2 depicts the analysis of FB_1 mycotoxin in a spiked wheat-based baby food at 75 $\mu g\,kg^{-1}$ by QTRAP® and by LTQ-Orbitrap®. On the one hand, the LTQ-Orbitrap® is able to acquire a full-scan (A), as well as fragmented ions under data-depending acquisition (C), which can be acquired in a single Orbitrap mass spectrum [22,26,27]. However, QTRAP® product ion mass spectra are generated using Q1 as a resolving RF/DC transmission quadrupole to select the precursor ion of interest. This precursor ion is then accelerated into the pressurized collision cell inducing fragmentation and the resulting fragment and residual precursor ions are transmitted into the Q3 linear ion trap (LIT) where they are mass selectively scanned out toward the detector while the Q3 LIT is performing the mass scan ions can be accumulated in Q0 further enhancing instrument duty cycle. This scan is referred as an EPI scan (F) [28].

In this study was observed an interesting difference between instruments in terms of qualitatively different products ions obtained and relative abundances of these fragments. This can be explained considering the different mechanisms of ion isolation and fragmentation, previously explained. Although the fragment ions obtained were the same in both instruments, their abundances were not the same. In fact, in the LTQ-Orbitrap® spectra it was observed that the main fragment ion for FB_1 was m/z 704.3136 that corresponds to the lost of water molecule. It can be assumed that the fragmentation mechanism in the LTQ-Orbitrap® is softer than in the QTRAP®, whose main fragments were m/z 334.4 and m/z 352.4.

3.2. Application to baby food samples

Once the proposed methods were optimized and validated, the two mass analyzers were applied for monitoring 18 mycotoxins in a total of 25 commercial baby-food samples. All the samples were analysed by HPLC-QTRAP®-MS and HPLC-LTQ-Orbitrap®-MS. Samples in which mycotoxins were detected (positive samples), an extra confirmation tool was carried out by IDA method.

After the analysis of all the samples, only one sample was positive for DON. Fig. 3 shows the chromatogram in both instruments. Fig. 3A shows the chromatogram in sSRM, showing two selected transitions and the ion ratio expected and observed according to EU guidelines [16]. This analyzer allowed obtaining a spectrum of second generation, EPI method, increasing the number of identification points (IPs) and the identification was unambiguous (Fig. 3A). The calculated concentration was $60.1 \pm 3.8\ \mu g\,kg^{-1}$ ($n = 5$).

This sample was also analysed by the LTQ-Orbitrap® and DON was detected. Fig. 3B shows an extract ion of DON, showing adequate retention time and calculated concentration was $57.8 \pm 5.3\ \mu g\,kg^{-1}$ ($n = 5$), insignificant difference with QTRAP® quantification was observed. The error mass was 0.2 ppm (Fig. 3C) confirming the presence of this mycotoxin in this sample. Besides, the ion-trap showed DON pathway for the sample completely equal to the standard obtained (Fig. 3D). DON could be fragmented by the ion-trap generating the deepoxy-deoxynivalenol metabolite known as DOM-1, meat the loss of epoxy group.

In order to enlarge the capability of the method, a simple strategy, described in previous work [23], is followed for the identification of target and non-target analytes in the samples. At this regard it should be pointed out that sample preparation itself implies some selection of recovered analytes.

In this context, all the samples were analysed on looking for emergent micotoxins, such as enniatins A, B, A_1, B_1 and fusaproliferin as it has been done in a previous work [23]. MassFrontier 7.0 was used as complementary software in order to identify these compounds and unknowns in the 25 samples. No one non-target mycotoxin was found in any sample.

4. Conclusions

Two hybrid instruments were checked to analyse mycotoxins from baby foods. On the one hand, QTRAP® working in sSRM mode allowed a reliable quantification of 18 mycotoxins from wheat-based baby food. Besides, QTRAP® working in full mass rang and using IDA method that permitted to develop EPI mode, could improve identification and confirmation, decreasing slightly LOQ levels respect sSRM mode.

On the other hand, LTQ-Orbitrap® has the ability to perform quantitative target and non-target analysis using full-scan FTMS in the instrument and it allows simultaneously target analysis in LIT. The ultra-high resolution mass was therefore used to identify target and non-target mycotoxins and LIT was valuable for analyte confirmation. Thereby, all the samples were analysed by HPLC-LTQ-Orbitrap® in order to find the presence of other non-targets mycotoxins as enniatins and fusaproliferin.

No one of non-target mycotoxin was found in the samples.

In conclusion, QTRAP® instrument is more suitable for quantitative purposes and it allows extra information by IDA methods for unambiguous identification. It allows an increase of identification points. Nevertheless, LTQ-Orbitrap® has other advantage: in addition to quantification of mycotoxins from baby food, the ultra-high resolution mass could identify non-target and unknowns mycotoxins. This potential comes from the ultra-high resolution mass allowing an exact mass accuracy. Moreover, this instrument allows a retrospective data analysis, which means that from Full-Scan it could be studied. Thereby, the extracted ion chromatogram of a specific analyte could be processed after the chromatogram has been acquired.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.12.039.

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