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Development of a GC–MS/MS strategy to determine 15 mycotoxins and metabolites in human urine

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ABSTRACT

The widespread mycotoxins contamination of food commodities has made the monitoring of their levels essential. To overcome the disadvantages of the indirect approach by food analysis, detection of mycotoxin as biomarkers in urine provides a useful and specific data for exposure assessment to these food contaminants. In this work, a sensitive, rapid and accurate method based on gas chromatography-tandem mass spectrometry procedure to determine 15 mycotoxins and metabolites in human urine was optimized and validated taking into consideration the guidelines specified in Commission Decision 2002/657/EC and 401/2006/EC. A salting-out assisted acetonitrile-based extraction was used for sample preparation. The extraction recoveries were in a range of 72–109%, with intra-day relative standard deviation and inter-day relative standard deviation lower than 10% and 13%, respectively for all mycotoxins at 50, 100 and 200 μg/L spiking levels. The limits of quantitation ranged from 0.25 to 8 μg/L. Matrix effect was evaluated and matrix-matched calibration was used for quantitation. The proposed procedure was applied to 10 urine samples collected from children. Mycotoxins were quantified in 30% of samples.

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1. Introduction

Mycotoxins are an heterogeneous group of secondary metabolites of filamentous fungi mainly belonging to *Apergillus*, *Penicillium*, *Alternaria* and *Fusarium* spp. Naturally occurring mycotoxins are of public concern due to their association with a wide range of adverse health effects [1]. In fact, mycotoxins were ranked as the most important chronic dietary risk factors, ahead of synthetic contaminants, plant toxins, and pesticide residues [2]. The diversity of mycotoxins leads to a wide range of acute and chronic toxic effects in animals and humans such as skin irritation, feeding refusal, nausea, vomiting, diarrhea, anemia, hemorrhage, immunosuppression, etc. [3].

Most mycotoxins are chemically stable; they survive storage and processing, and could even remain in cooked food as previously reported in the literature [4]. Thus, to protect consumers from these health risks, many countries have adopted food regulations to limit exposure to mycotoxins [5,6]. Many mycotoxins are metabolized and efficiently excreted. Since high levels of mycotoxins were detected in widely consumed cereals [7], quantifiable amounts of these toxins are expected in urine and it is also possible to find toxin derivatives that result from its biotransformation [8,9]. Thus, in order to understand the possible links between mycotoxins and human disease, it is necessary to measure the exposure of a population to the multiple toxins.

In this line, urine is a very convenient sample for screening because large amounts can be easily and non-invasively collected [10]. Consequently, the development and validation of methods for multi-mycotoxin determination are necessary to assess the levels and frequencies of human mycotoxins exposure [11,12].

Mass spectrometry (MS) and tandem mass spectrometry (MS/MS) have become the most powerful tools for determining multiresidues in several matrices [13]. The use of MS/MS in combination with gas chromatography (GC) or liquid chromatography (LC) is gaining ground providing better sensitivity and confirmation reliability. In fact, the important requirements set by the Commission Decision 2002/657/EC [14] as regards criteria and procedures for the validation of analytical methods is satisfied by MS/MS. In this sense, high dynamic range and good performance reached in selected reaction monitoring (SRM) mode to make the triple quadrupole (QqQ) one of the most widely employed analyzer [15]. Similarly, appropriate sample preparation protocol involving extraction, purification and concentration of the extract is crucial to obtain the desired sensitivity [16]. Sample preparation techniques have been reported in the literature for measuring mycotoxins in urine, such as liquid–liquid extraction [17], solid-phase extraction [18] or immunoaffinity

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columns purification [19]. Salting-out assisted liquid/liquid extraction (SALLE) is also an alternative sample preparation technique based on the salting-out effect to separate water-miscible organic solvents such as acetonitrile [20].

Up to date, a few multi-mycotoxin methods for determining mycotoxin and their metabolites in human urine have been described in the literature [17,18,21,22]. In response, a simple optimized analytical procedure for the simultaneous determination of 15 mycotoxins and metabolites, namely de-epoxydeoxynivalenol, deoxynivalenol, 3-acetyldeoxynivalenol, fusarenon-X, diacetoxyscirpenol, nivalenol, neosolaniol, HT-2, T-2, zearalanone, α-zearalanol, ß-zearalanol, zearelenone, α-zearalenol, and ß-zearalenol in human urine is described herein. This method merges the advantages of a solvent extraction at high ionic strength followed by dispersive solid phase extraction with the sensitivity of a gas chromatography tandem mass spectrometry (GC–MS/MS) technique. Selected compounds have been determined in SRM mode, using a state of the art triple quadrupole analyzer reaching a chromatographic separation by 17 min total run. To the best knowledge of the authors, the paper here presented is the first application of GC–QqQ–MS/MS to evaluate multiple mycotoxins in human urine. In addition, the optimized validated analytical procedure was used to evaluate the occurrence of target mycotoxins and metabolites in 10 urine samples.

2. Material and methods

2.1. Standards

Mycotoxin standards and metabolites namely de-epoxydeoxynivalenol (DOM-1), deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), fusarenon-X (FUS-X), diacetoxyscirpenol (DAS), nivalenol (NIV), neosolaniol (NEO), HT-2, T-2, zearalanone (ZAN), α-zearalanol (α-ZAL), ß-zearalanol (ß-ZAL), zearelenone (ZON), α-zearalenol (α-ZOL), and ß-zearalenol (ß-ZOL) were obtained from Sigma-Aldrich (St. Louis, USA). Individual stock solutions of all analytes were prepared at the same concentration (1000 mg/L) in methanol. The stock solutions were diluted with acetonitrile in order to obtain the appropriate multi-compounds working standard solutions (50 mg/L). All standards were stored in darkness and kept at −20 °C until the GC–MS/MS analysis.

2.2. Chemical, reagents and other material

The derivatization reagent composed of BSA (N,O-bis(trimethylsilyl)acetamide)+TMCS (trimethylchlorosilane)+TMSI (N-trimethylsilyimidazole) (3:2:3) was purchased from Supelco (Bellefonte, USA). Sodium dihydrogen phosphate and disodium hydrogen phosphate, used to prepare phosphate buffer, were acquired from Panreac Quimica S.L.U. (Barcelona, Spain). Picric acid (moistened with water, ≥98%) and creatinine (Crea) standard were supplied by Sigma-Aldrich (St. Louis, USA) whereas sodium hydroxide was acquired from BDH Prolabo—VWR International (Barcelona, Spain).

All solvents, acetonitrile, hexane and methanol (HPLC grade) were purchased from Merck KGaA (Darmstadt, Germany). Anhydrous magnesium sulfate (thin powder) was obtained from Alfa Aesar GmbH & Co (Karlsruhe, Germany); sodium chloride was purchased from Merck and C18-E (50 µm, 65A) was purchased from Phenomenex (Torrance, USA).

2.3. Apparatus

For simultaneous determination of all mycotoxins, a GC–MS/MS method was developed for their separation and detection. A GC system Agilent 7890A coupled with an Agilent 7000A triple quadrupole mass spectrometer with inert electron-impact ion source and an Agilent 7693 autosampler (Agilent Technologies, Palo Alto, USA) were used for MS/MS analysis. Chromatographic separation was achieved on a HP-5MS 30 m × 0.25 mm, 0.25 µm capillary column.

Aliquots of 1 µL of sample extracts were injected into the gas chromatograph in splitless mode at 250 °C by a programmable temperature vaporization (PTV) inlet employing helium as carrier gas at fixed pressure of 20.3 psi. The oven temperature program was initially set at 80 °C, and the temperature was increased to 245 °C at 60 °C/min. After a 3 min hold time, the temperature was increased to 260 °C at 3 °C/min and finally to 270 °C at 10 °C/min and then held for 10 min. All analytes eluted within 17 min, reaching the requirement for a high throughout determination.

Quantitation data were acquired at SRM mode and the mass spectrometer operated in electrospray ionization (EI) mode. The transfer line and source temperatures were 280 and 230 °C, respectively. The EI energy used was 70 eV as in that region the maximum abundance was observed. The collision energies varied from 5 to 20 eV, depending on the precursor and product ions. The analysis was performed with a filament-multiplier delay of 3.50 min. The collision gas for MS/MS experiments was nitrogen, and the helium was used as quenching gas, both at 99.999% purity supplied by Carburos Metálicos S.L. (Barcelona, Spain). The dwell times also varied from 5 to 35 eV. Data was acquired and processed using the Agilent Masshunter version B.04.00 software.

2.4. Sample preparation

All urine samples were first centrifuged at 4000 rpm for 5 min. A 10 mL portion of the centrifuged urine was then used for analysis. 5 mL of acetonitrile was added to the urine samples followed by the addition of 4 g of $MgSO_4$ and 1 g of NaCl prior to be shaken vigorously and centrifuged for 3 min at 4000 rpm. Then the upper layer was submitted to a dispersive solid phase extraction (d-SPE) with a mixture of 900 mg of $MgSO_4$ and 300 mg of C18 and centrifuged for 1 min at 1500 rpm. Finally the extract was evaporated to dryness under nitrogen flow.

The dry extract was added with 50 µL of BSA+TMCS+TMSI (3:2:3) and the sample was left for 30 min at room temperature. The derivatized sample was diluted to 200 µL with hexane and mixed thoroughly on a vortex for 30 s. Then the diluted derivatized sample was added with 1 mL of phosphate buffer (60 mM, pH 7) and the upper layer (hexane phase) was transferred to an autosampler vial for the chromatographic analysis.

2.5. Sampling

Sampling was carried out in a primary school located in Valencia, Spain. Ten urine samples from children (from 8 to 11 years old) were randomly collected in June 2013. A written and approved informed consent was obtained from the progenitors of all volunteers. The informed consent for the mycotoxin evaluation in urine was in accordance to the Helsinki Declaration on ethical principles for medical research involving human subjects.

All urine samples were obtained from urine recipients and frozen for 6 h after collection. The samples with undetectable levels of mycotoxins were used for spiking and recovery studies.

2.6. Method validation

Commission Decision 2002/657/EC [14] and 401/2006/EC [23] were used as guidelines for the validation studies. All the parameters were evaluated by spiking blank samples. Samples were spiked and left to equilibrate over night before the analysis. For identification purposes, retention times of mycotoxins in standards and samples were compared at tolerance of ±0.5%. Moreover, in accordance with

the 2002/657/EC Decision, the relative ion intensity of analytes studied in the standard solution and the spiked samples at the concentration levels used for the calibration curve were compared.

Method performance characteristics such as linearity, limits of detection (LOD), limits of quantitation (LOQ), matrix effect, extraction recovery, repeatability and reproducibility were evaluated for all tested mycotoxins.

2.7. Creatinine analysis

Creatinine urinary levels were determined based on a spectrophotometric method slightly modified [17]. In summary, 3.5 mM picric acid was reacted with 1000 mM NaOH to form alkaline picrate. This solution was stored in the dark in an amber glass recipient. Alkaline picrate (1 mL) was reacted with 1 mL of diluted urine (1/10, v/v, in ultrapure water). The optical density was measured at 500 nm after 30 min using a Shimadzu mini 1240 spectrophotometer. Mycotoxin urinary concentrations were correlated to the creatinine content of a sample expressed as μg/g creatinine.

3. Results and discussion

3.1. Optimization of the GC–MS/MS conditions

The concentrations of the mycotoxins and/or metabolites in urine samples often occur in low μg/L; hence it is important to optimize the GC–MS/MS method to reach the required levels. Due to regulatory requirements for confirmatory methods, MS/MS instrumentation was selected in order to fulfill the rule of identification points. Each compound was identified and several characteristic fragment ions were obtained as described below. In the present manuscript, GC–MS/MS optimization of 6 metabolites namely DOM-1, ZAN, α-ZAL, ß-ZAL, α-ZOL and ß-ZOL were described for the first time. The optimization of MS/MS method consisted of (i) acquisition of respective MS spectra in full scan mode (*m/z* 50–650 mass range); (ii) selection and fragmentation of appropriate precursor ions; (iii) product ion scans at different collisions energies (CEs) 5, 10, 15 and 20 eV to obtain the best product ion transition signal and different dwell times (Dts) 5, 15, 25 and 35 ms to provide a good peak shape; and (iv) fine tuning of CE and Dt in SRM mode. Fig. 1 shows the optimization of collision energies and dwell times carried out in the studied metabolites. Once the final choices were made, the SRM conditions were tested in mixed

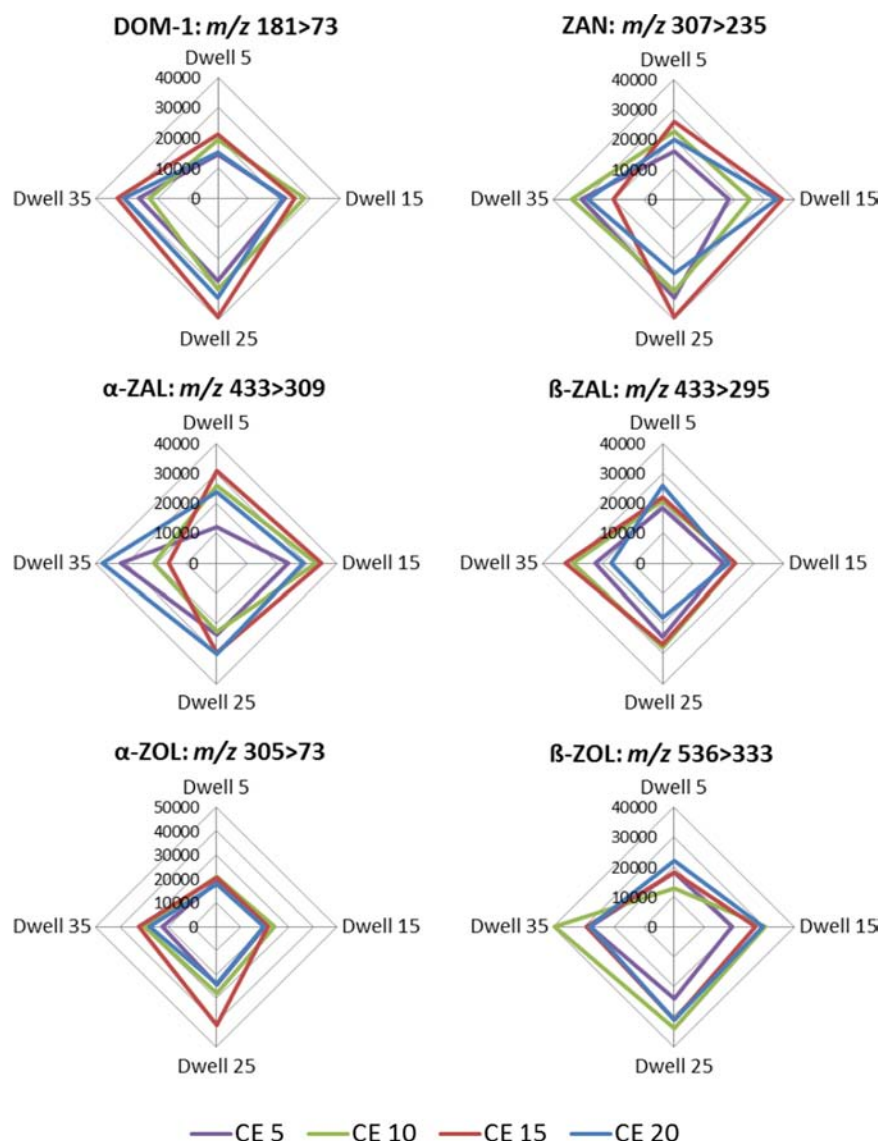

Fig. 1. Optimization of collision energy (CE) and dwell time for the quantitation transition.

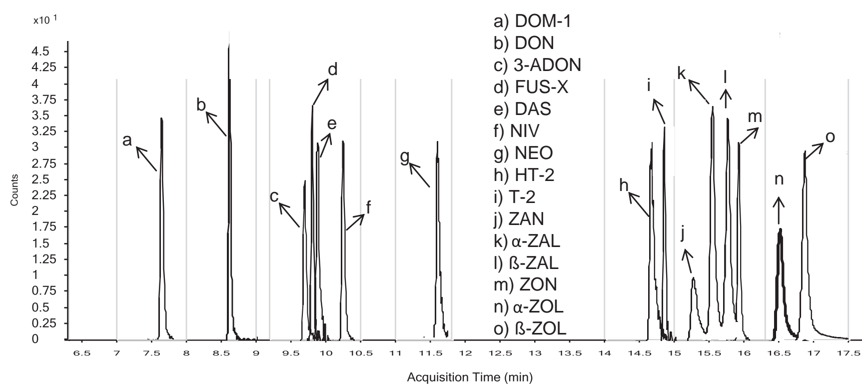

Fig. 2. SRM chromatogram of blank urine sample spiked with a mixture of the studied mycotoxins at 25 μg/L.

Table 1
Experimental conditions of the optimized GC–MS/MS method for the selected mycotoxins and metabolites.

Compound	t_R (min)	SRM transitions (m/z)	CE [a](eV)	Dwell time (ms)	Ratio Q/q (RSD)
DOM-1	7.63	181 > 73	15	25	41.6 (3.2)
		391 > 271	15	35	
DON	8.60	392 > 259	10	25	41.6 (3.2)
		407 > 197	10	25	
3-ADON	9.68	392 > 287	5	35	47.5 (12.3)
		467 > 147	10	25	
FUS-X	9.73	450 > 260	10	35	11.9 (7.0)
		450 > 245	20	35	
DAS	9.85	350 > 229	15	35	56.9 (10.3)
		378 > 124	10	25	
NIV	10.15	289 > 73	15	35	29.6 (2.7)
		379 > 73	15	35	
NEO	11.68	252 > 195	10	25	40.6 (4.3)
		252 > 167	15	35	
HT-2	14.73	347 > 157	10	25	86.7 (7.8)
		347 > 185	10	25	
T-2	14.80	350 > 259	10	25	81.9 (5.8)
		350 > 229	15	35	
ZAN	15.15	307 > 235	15	25	59.9 (7.2)
		449 > 335	10	25	
α-ZAL	15.45	433 > 309	20	35	26.1 (4.9)
		433 > 295	20	35	
ß-ZAL	15.68	433 > 295	15	35	82.2 (9.7)
		307 > 73	10	35	
ZON	15.95	462 > 151	10	25	76.9 (3.9)
		462 > 333	10	25	
α-ZOL	16.45	305 > 73	15	25	12.7 (10.7)
		305 > 289	15	20	
ß-ZOL	16.82	536 > 333	10	35	66.1 (8.4)
		536 > 446	15	20	

[a] Collision energy.

sample extracts spiked or not with the mycotoxins under study in order to evaluate the sensitivity and selectivity of the method. An example of the chromatogram of urine spiked with the mixture of all target mycotoxins at 25 μg/L is shown in Fig. 2.

Retention time in standards and samples must agree, so restrictive tolerances were set. Excellent repeatability in terms of retention times (RSD < 0.5%) was observed and thus fulfilling the tolerance of ±0.5% considered for quantitation purpose as specified in Commission Decision 2002/657/EC. Table 1 shows the optimized GC–MS/MS parameters for all target mycotoxins.

3.2. Method performance

The suitability of the quantitation method for urinary mycotoxin levels was evaluated by a validation study. Table 2 shows the figures of merit of the proposed method, namely, linearity, extraction recovery, repeatability, reproducibility, LODs and LOQs and matrix effect (as signal suppression/enhancement, SSE).

The linearity of the MS/MS method was established by eight calibration points within a concentration range from LOQ to 250 μg/L. Each calibration point was obtained as the mean of three injections. Correlation between the response and the amount of analytes was verified by plotting signal intensity against analyte concentrations. Good linearity was achieved in all cases with regression coefficients higher than 0.990. Calibration curves were checked at the end of the analysis to assess the response drift of the method.

The specificity and selectivity of the method relies on the chromatographic retention time of each analyte and on the SRM transition used. Selectivity and specificity were assessed by recognizing both quantitation (Q) and confirmation (q) transitions of each mycotoxin at the same concentration levels as used for the construction of the calibration curve. In addition the ion ratio, defined as the ratio between both transitions (Q/q), of the real samples had to be in agreement with the ion ratio of the matrix-matched calibration curve to confirm a finding. The average ion ratios are listed in Table 1.

The sensitivity of the method was assessed by the limits of detection and quantitation. The LODs and LOQs were calculated as the lowest matrix-matched calibration providing signal-to-noise ratios greater than 3 and 10, respectively, at both Q and q transitions and matching the intensity ratio observed for the particular compound in the standard solution. LOD and LOQ obtained from 0.12 to 4 μg/L and from 0.25 to 8 μg/L, respectively showed the suitability of the developed method for the determination of trace amounts of the selected mycotoxins in urine samples.

A blank urine sample extract, from a urine sample previously analyzed to confirm the absence of target mycotoxins, was analyzed ($n=5$) to study signals obtained from the matrix and to evaluate possible sample interferences. The good specificity of the technique makes possible to have no signal at all in the blank urine for any of the mycotoxins.

Co-eluting matrix components can negatively influence the accuracy and quantitative methods through ion suppression or enhancement in the ion source; thus the effects of a possible matrix mismatch were assessed. The matrix effect for each analyte is defined as the percentage of the matrix-matched calibration slope (A) divided by the slope of the standard calibration in solvent (B). The ratio ($A/B \times 100$) is defined as the absolute matrix effect. A value of 100% indicates that there is no absolute matrix effect whereas values higher than a 100 indicates signal enhancement and values below 100% indicates signal suppression. Significant signal suppression (SSE) was observed (from 6% to 36%) between the slopes of the calibration lines meaning that matrix

Table 2
Overview of the extraction recovery, repeatability and reproducibility (Rec (RSD), %), limits of detection (LODs) and quantitation (LOQ) and signal suppression/enhancement (SSE) for the studied analytes.

Proposed biomarkers	Correlation coefficient (r)	Rec (intraday RSD, %)[a]			Rec (interday RSD, %)[a]			LOD (μg/L)	LOQ (μg/L)	SSE (%)
		Low level (50 μg/L)	Medium level (100 μg/L)	High level (200 μg/L)	Low level (50 μg/L)	Medium level (100 μg/L)	High level (200 μg/L)			
DOM-1	0.990	84 (2)	92 (3)	90 (5)	87 (6)	81 (8)	91 (4)	0.25	0.50	20
DON	0.996	96 (4)	89 (6)	93 (2)	93 (8)	97 (10)	88 (9)	0.12	0.25	23
3-ADON	0.992	92 (5)	96 (1)	84 (9)	102 (6)	94 (4)	96 (11)	0.25	0.50	27
FUS-X	0.992	95 (3)	89 (4)	84 (6)	83 (6)	94 (13)	90 (6)	2	4	12
DAS	0.998	89 (4)	94 (3)	83 (2)	94 (10)	98 (11)	95 (8)	1	2	35
NIV	0.996	87 (3)	82 (7)	95 (2)	90 (7)	94 (7)	85 (4)	0.50	1	6
NEO	0.999	93 (5)	98 (7)	106 (7)	98 (5)	106 (3)	109 (11)	0.25	0.50	36
HT-2	0.999	96 (4)	105 (5)	102 (4)	102 (10)	93 (11)	92 (8)	1	2	28
T-2	0.998	102 (6)	91 (1)	89 (1)	96 (8)	104 (9)	93 (6)	0.50	1	8
ZAN	0.993	72 (7)	75 (6)	79 (6)	77 (10)	79 (11)	80 (8)	4	8	36
α-ZAL	0.998	79 (5)	83 (9)	74 (10)	78 (6)	82 (12)	77 (8)	4	8	28
ß-ZAL	0.997	77 (8)	74 (6)	89 (9)	75 (12)	77 (9)	83 (12)	4	8	33
ZON	0.991	81 (5)	87 (3)	96 (5)	83 (12)	89 (5)	79 (8)	3	6	23
α-ZOL	0.995	88 (2)	81 (8)	93 (8)	98 (6)	80 (12)	84 (4)	1	2	19
ß-ZOL	0.991	80 (6)	78 (7)	83 (5)	78 (9)	84 (8)	79 (9)	2	4	14

[a] $n=3$.

Table 3
DON performance characteristics of five LC–MS/MS-based multibiomarker methods in human urine compared with the here reported GC–MS/MS methodology.

Reference	[17]	[20]	[21]	[27]	[28]	Present study
Recovery (%)	92	65	88	107	77	96
$RSD_{r\ (\%)}$	16	20	6	3	n.i.	4
$RSD_{R\ (\%)}$	19	22	8	5	n.i.	8
LOD (μg/L)	2.85	0.5	4	10	0.45	0.12
LOQ (μg/L)	5.7	1.7	13	35	1.51	0.25
Extraction and clean up	LLE-SPE	SALLE	-[a]	IAC	SPE-IAC	SALLE-dSPE
No. of detected mycotoxins	18	12	14	11	7	15
Total elution time (min)	15	15	15	9	n.i.	17

RSD_r: intra-day relative standard deviation; RSD_R: inter-day relative standard deviation n.i.: not indicated.

[a] Dilute and shoot.

effect is present and thus quantitation should be conducted with matrix-matched calibration standards in order to have reliable and accurate results.

Recovery and precision, expressed as percentage relative standard deviation (% RSD), were determined by analyzing urine samples at three different concentrations, 50, 100 and 200 μg/L. Blank sample extracts were employed in method's trueness and precision. Intra-day precision data were obtained from three analyses performed on one day; inter-day data were tested on three different working days within 20 days. Precision studies showed that the method was repeatable ($RSD < 10\%$) and reproducible ($RSD < 13\%$). Satisfactory results in terms of recoveries ($n=9$) were found. The fifteen studied analytes showed recoveries results inside the range 72–109%. The results obtained were in agreement with the recoveries accepted by the Commission Regulation (EC) no. 401/2006.

The key performance characteristics documented through the above outlined validation protocol also met the criteria established in SANCO document no. 12495/2011 [24]. Validation results here reported are in agreement with other analytical methodologies previously reported for the determination of several mycotoxins in human urine (Table 3). For some mycotoxins, an improvement of the validation parameters such as recovery range, intra- and inter-day precision and limits of detection and quantitation were obtained. Thus, according to the obtained results, the developed method seems robust and suitable for its purpose, and can be an alternative method for the determination of mycotoxins and their metabolites in human urine.

Table 4
Summary of the analytes found in the analyzed urine samples.

Samples	Parameter	Biomarker	
		DOM-1	**DON**
Urine ($n=10$)	Incidence	1/10	3/10
	% positive samples	10	30
	Mean positive (μg/g Crea)	1.3	7.4
	Maximum (μg/g Crea)	1.3	21.1

3.3. Application to real samples

The optimized and validated analytical procedure was used to evaluate the occurrence of the target analytes in 10 children urine samples. Table 4 summarizes the results obtained. Of the 15 mycotoxins and metabolites detectable by the here proposed method, only two analytes, namely DOM-1 and DON, were quantified in the urine samples assayed. 3 out of 10 urine samples were found positive at least with one mycotoxin. Results showed that DOM-1 was detected in one urine sample at 1.3 μg/g creatinine, whereas DON was quantified in 3 out of 10 urine samples at average concentrations of 7.4 μg/g creatinine. GC–MS/MS chromatograms of one naturally contaminated urine sample in which co-occurred DOM-1 and DON at 1.3 and 21.1 μg/g creatinine, respectively are shown in Fig. 3.

3.4. Extrapolation of the DON urinary data

Urinary contamination data can be employed to estimate an exposure assessment approach to contaminants. As an example, urinary DON and DOM-1 levels ($\sum DON_{eq\ (\mu g)}/g$ Crea) here reported from one child urine sample were used to assess DON exposure and compared with the established DON provisional maximum tolerable daily intake (PMTDI) set at 1 μg/kg bw [25]. On the basis of an assumed of 1.5 L daily urine volume for children (V_{urine}), an average body weight of 35 kg and an estimated DON excretion rate (Exc_r) of 72% [26], the amount of DON probable daily intake (DON PDI) was roughly calculated as indicated in the following equation:

$$\text{DON PDI} = \frac{\left[\sum(\text{DON}_{eq\ (\mu g)}/g\ \text{Crea})\right] \cdot \text{CI} \cdot V_{urine(L)} \cdot 1/\text{Exc}_{r\ (\%)}}{\text{Body weight(kg)}}$$

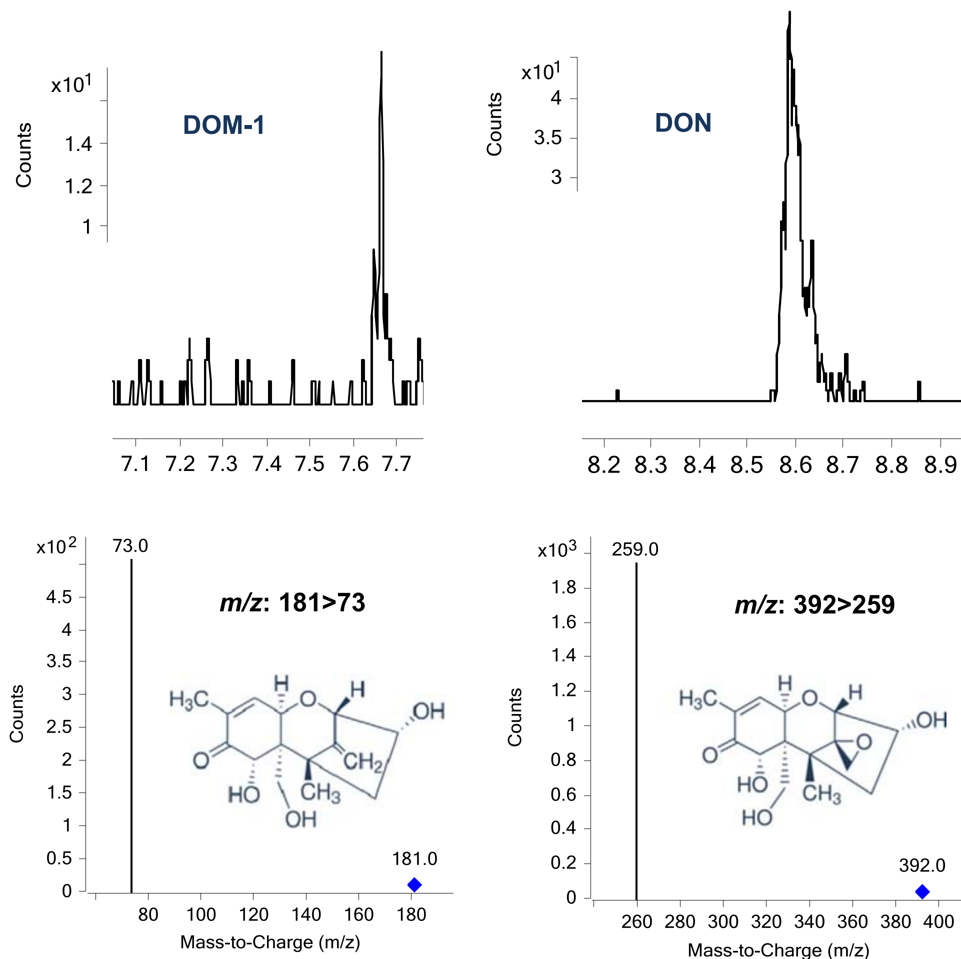

Fig. 3. GC–MS/MS chromatograms of a child urine sample naturally contaminated with DOM-1 and DON at 1.3 and 21.1 μg/g creatinine, respectively

where CI is the creatinine index (obtained from Section 2.7) expressed as g Crea/L urine.

Taken into account the above considerations, a DON probable daily intake of 1.39 μg/kg bw was calculated. Then, a risk characterization, expressed as % PMTDI, was obtained by comparing the DON PDI to DON PMTDI. DON daily intake represented 139% of the established PMTDI which exceeded the tolerable limits suggested by the Scientific Committee on Food [25]. That could indicate a possible health risk scenario. Thus, further studies should focus on bio-monitoring of mycotoxin contamination to properly understand the extent of exposure and to propose intervention strategies to reduce potentially associated health risks.

4. Conclusions

The present study was conducted to produce a sensitive, rapid and accurate method to determine 15 mycotoxins and metabolites in human urine. As urine sampling is non-invasive technique, mycotoxin analysis in this matrix is a promising alternative as exposure biomarker. The determination of the analytes in a GC–MS/MS system working in SRM mode allowed an accurate determination of even (ultra)trace levels of mycotoxins due to the triple quadrupole MS analyzer. It was, together with the use of an easy and cheap extraction procedure and the benefits from its application, the most important features of this analytical procedure. The method performance fulfilled the EU guideline standardized in the Commission Decision 2002/657/EC and 401/2006/EC. The recoveries of all target analytes were within the acceptable range of 72–109% and precision studies conducted at three spiking levels were $\leq 13\%$. Under the optimized conditions the LOQs were in the range of 0.25–8 μg/L. Thus, the analytical strategy optimized in this study represents a reliable tool for rapid quantitation of mycotoxins and their metabolites in urine. The here proposed method was used to evaluate the occurrence of the selected mycotoxins in 10 children urine samples. Occurrence of mycotoxins was detected in 30% of samples.

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