Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1023 (2004) 57–66
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Quantification of ochratoxin A in foods by a stable isotope dilution assay using high-performance liquid chromatography–tandem mass spectrometry

Michael Lindenmeier, Peter Schieberle, Michael Rychlik*

Institut für Lebensmittelchemie der Technischen Universität München, Lichtenbergstr. 4, D-85748 Garching, Germany

Received 19 June 2003; received in revised form 1 October 2003; accepted 3 October 2003

Abstract

A stable isotope dilution assay (SIDA) was developed for quantification of the mycotoxin ochratoxin A (OTA) by using [$^2H_5$]-OTA as internal standard. The synthesis of labelled OTA was accomplished by acid hydrolysis of unlabelled OTA and subsequent coupling one of the products, ochratoxin α, to [$^2H_5$]-L-phenylalanine. The mycotoxin was quantified in foods by LC-tandem MS after extraction with buffers containing [$^2H_5$]-OTA and clean-up by immuno affinity chromatography or by solid phase extraction on silica. The method showed a sufficient sensitivity with a low detection and quantification limit of 0.5 and 1.4 μg/kg, respectively, and good precision in inter-assay studies showing a CV ($n = 3$) of 3.6%. The analysis of certified reference materials resulted in a low bias of 2.1% from the certified values and revealed excellent accuracy of the new method. To prove the suitability of SIDA, OTA was quantified in a number of food samples and resulted mainly in not detectable OTA contents. However, three samples of raisins exceeded the legal limit of 10 μg/kg and highlighted the need for further controlling the contamination with the mycotoxin.

Keywords: Stable isotope dilution; Food analysis; Mycotoxins; Ochratoxin A

1. Introduction

The mycotoxin ochratoxin A (7-carboxy-5-chloro-8-hydroxy-3,4-dihydro-3*R*-methylisocoumarin-L-β-phenylalanine, OTA) is produced by several species of the fungal genera *Penicillium* (e.g. *veridicatum*) and *Aspergillus* (e.g. *ochraceus*). In particular cereals [1], coffee [2], grape products [3] and liquorice products [4] are frequently contaminated with OTA due to inappropriate conditions during growth, storage and manufacture of the raw material and subsequent invasion by the before mentioned moulds.

As OTA is a potent hepato and nephrotoxin [5] and is clearly associated with a kidney disease referred to as Balkan Endemic Nephropathy [6], there is general consensus that contamination of foods has to be controlled thoroughly. Food intake calculations and surveys of blood plasma concentrations in some European countries revealed that at least one-third of the acceptable daily intake (ADI) [7] is covered by the mean intake in countries such as Sweden or Germany [8,9]. Therefore, the European Union has set OTA limits for cereal products, cereals and raisins of 3, 5 and 10 μg/kg, respectively [10].

The most frequently used methods to analyze OTA in foods are HPLC with fluorescence detection (LC/FD) and enzyme-linked immunosorbent assays (ELISA) [11]. Whereas the latter are mainly suited for screening purposes, validation studies revealed that LC/FD suffers from several constraints. In a collaborative study of OTA quantitation in pig liver recovery values differed widely between 43 and 128% [12]. Similarly, for barley [13] and wheat bran [14] low recoveries of 56 and 70%, respectively, were found.

Recently we reported on the excellent accuracy of stable isotope dilution assays (SIDA) for quantification of the mycotoxin patulin [15] or the vitamins of the folate group [16] by employing isotopomers of the analytes as internal standards. This enabled an optimal compensation for losses of the analytes in all analytical steps. The aim of the current study was, therefore, to develop a SIDA for OTA and to verify the accuracy of the new method by analyzing standard reference materials.

* Corresponding author. Tel.: +49-89-289-13255; fax: +49-89-289-14183.
E-mail address: michael.rychlik@ch.tum.de (M. Rychlik).

0021-9673/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.10.004

2. Materials and methods

2.1. Materials and reagents

The following compounds were obtained commercially from the sources given in parentheses: acetic acid, acetonitrile, ethyl acetate, formic acid, *n*-hexane, hydrochloric acid, methanol, L-phenylalanine, toluene, trifluoracetic acid (Merck, Darmstadt; Germany); chloroform, L-phenylalanine methylester, thionyl chloride (Aldrich, Steinheim, Germany); [$^2H_5$]-L-phenylalanine (CDN isotopes, Quebec, Canada); Sep-Pak C18 Cartridges, Sep-Pak Silica Cartridges (Waters, Eschborn, Germany); Mycosep OTA Cartridges; Ochraprep Cartridges (Coring Systems Diagnostik, Gernsheim, Germany). Crystalline ochratoxin A was purchased from Sigma (Deisenhofen, Germany). Two certified reference materials CRM 471 (wheat flour blank) and CRM 472 (wheat flour contaminated) were obtained from the Community Bureau of Reference of the European Commission (Standard, Measurement & Testing Programme, Brussels, Belgium).

All solvents were of gradient quality. The food samples were purchased from local markets.

PBS-buffer (pH 7,3) was prepared by dissolving 2.9 g Na_2HPO_4, 0.2 g KH_2PO_4, 8 g NaCl, 0.2 KCl in 1 l water and adjusting the solution with HCl to pH 7.3.

2.2. Synthesis of [$^2H_5$]-OTA

Deuterated OTA was prepared by hydrolyzing unlabelled OTA and coupling one of the hydrolysis products to [$^2H_5$]-L-phenylalanine using a modified procedure by van der Merwe et al. [17], Steyn and Holzapfel [18] and Rousseau et al. [19].

2.2.1. Ochratoxin α (2)

Ochratoxin A (**1**; 15 mg, 37.1 μmol) was suspended in aqueous hydrochloric acid (18 mol/l, 50 ml) and refluxed for 48 h in an atmosphere of nitrogen. The homogeneous mixture was then cooled to room temperature and extracted with chloroform (3 ml × 20 ml). After drying the organic phase over anhydrous Na_2SO_4, removal of the solvent gave 5-chloro-3,4-dihydro-8-hydroxy-3-methylisocoumarin-7-carboxylic acid (ochratoxin α **2**; 9.5 mg, 37.0 μmol).

Positive APCI-MS: *m/z* (%) = 257 (100), 239 (80), 259 (40), 241 (35).

2.2.2. [$^2H_5$]-L-Phenylalanine methyl ester (4)

[$^2H_5$]-L-phenylalanine (**3**; 100 mg, 588 μmol) was dissolved in a mixture of thionyl chloride in methanol (10%, 100 ml), then cooled to 0 °C for 1 h and subsequently kept for another 24 h at room temperature. Rotary evaporation of the solution at room temperature gave the title compound as a white solid (90 mg, 489 μmol).

Positive APCI-MS: *m/z* (%) = 309 (100), 185 (25), 370 (20).

2.2.3. [$^2H_5$]-Ochratoxin A methyl ester (5)

Ochratoxin α (**2**; 5 mg, 19.5 μmol) was dissolved in thionyl chloride (8 ml) and heated under reflux for 2 h. After evaporating the thionyl chloride under reduced pressure, the resulting ochratoxin α chloride was taken up in dry pyridine (1 ml) and cooled to 0 °C. [$^2H_5$]-L-phenylalanine methylester (25 mg) in dry pyridine (0.5 ml) was slowly added to the mixture and left at room temperature for 4 h. Subsequently, water (15 ml) was added to the mixture, which was then extracted with chloroform (3 × 10 ml). After washing the organic phase successively with hydrochloric acid (2 mol/l, 2 × 10 ml) aqueous sodium hydrogen carbonate (0.1 mol/l, 2 × 10 ml) and water (2 × 10 ml), the organic layer was dried with $CaCl_2$. Rotary evaporation gave **5** as a solid (1 mg, 2.4 μmol).

Positive ESI-MS: *m/z* (%) = 384 (100), 423 (65), 386 (27), 424 (20), 425 (17), 257 (16), 363 (14).

2.2.4. [$^2H_5$]-Ochratoxin A (6)

[$^2H_5$]-Ochratoxin A methyl ester (**5**; 500 μg, 1.2 μmol) was dissolved in methanol (1.5 ml) and stirred with NaOH (0.8 ml, 1 mol/l) at room temperature for 2 h. The mixture was then acidified with aqueous hydrochloric acid (2 mol/l) to pH 3–4 and extracted with chloroform (3 × 10 ml). The organic phase was dried over $CaCl_2$, concentrated in vacuo to 2 ml and purified by preparative LC/FD.

Purification of the [$^2H_5$]-OTA (**6**) was accomplished by injecting 100 μl of the raw solution on a Nucleosil RP18 column (250 mm × 10 mm i.d., 5 μm, Macherey-Nagel, Düren, Germany) eluted with a mobile phase consisting of variable mixtures of aqueous formic acid (0.1%, solvent A) and acetonitrile (solvent B). The gradient started at 0% B and was programmed within 20 min to 70% B. Then, the content of B was raised to 100% within 2 min and maintained for further 8 min before being brought back to the initial mixture. The eluting [$^2H_5$]-OTA peak was detected by fluorescence detection (excitation 333 nm, emission 460 nm) and pooled from 20 runs. The pooled purified solution was rotary evaporated to dryness and gave [$^2H_5$]-OTA as a white solid (197 μg, 0.488 μmol, 95% purity by HPLC-UV).

Positive ESI-MS: *m/z* (%) = 409 (100), 257 (58), 411 (40), 239 (37).

2.3. Preparation and determination of the concentration of standard solutions

Stock solutions were prepared by dissolving OTA (labelled as well as unlabelled) in methanol. Concentration of OTA was determined by UV spectrometry at 333 nm using the molar extinction coefficient 5550 $M^{-1}\,cm^{-1}$ reported by Becker and co-workers [20]. The UV spectrometer U-2000 (Hitachi, Berks, GB) was calibrated using potassium dichromate [21]. The stock solutions were checked spectrophotometrically revealing stability of OTA at −18 °C over a period of several months.

2.4. Sample preparation and clean-up

2.4.1. Solid phase extraction (SPE) on silica

SPE on silica was performed as detailed in the official collection of test methods according to article 35 of the German food law [22]. Briefly, samples (20 g or 20 ml) were mixed with aqueous hydrochloric acid (2 mol/l, 30 ml), aqueous $MgCl_2$ (0.4 mol/l, 50 ml) and toluene (100 ml) containing [$^2H_5$]-OTA (100 ng). After stirring for 1 h at room temperature, the mixture was centrifugated and the supernatant organic phase was subjected to SPE using an 12-port vacuum manifold (Alltech, Bad Segeberg, Germany). The SPE-cartridge (Sep-pak Vac RC Silica, 500 mg, Waters, Milford, MA, USA) was preconditioned with toluene (10 ml), then the sample extract (50 ml) was applied and the cartridge was washed with *n*-hexane (20 ml), toluene/acetone (95 + 5, v/v, 20 ml) and toluene (5 ml). Finally, ochratoxin A was eluted with a mixture of toluene and acetic acid (9 + 1, v/v, 20 ml).

2.4.2. Extraction and immuno affinity (IA) clean-up

Solid samples (20 g) were suspended in aqueous sodium carbonate (200 ml, 1%) containing [$^2H_5$]-OTA (100 ng) and stirred for 30 min. Subsequently, the mixtures were filtered and the filtrate (60 ml) was passed through the IA column (Ochraprep P13B, Rhone Diagnostics, Glasgow, Scotland). Liquid samples (60 ml) were diluted with PBS-buffer pH 7.3 (60 ml) before application on the IA column. After application of the extract (60 ml), the IA cartridge was washed with aqueous methanol (20%) and OTA was eluted with methanol/acetic acid (98 + 2, v/v, 1.5 ml) and water (1.5 ml). The solvent was evaporated in vacuo and the residue taken up in methanol (250 μl).

Each sample was analysed in triplicate by LC/MS–MS as described below.

2.5. LC/MS–MS

The samples (50–100 μl) were analyzed on a spectra series HPLC system (Thermo Separation Products, San Jose, CA, USA) equipped with an Aqua C-18 reversed phase column (250 mm × 4.6 mm; 5 μm, Phenomenex, Aschaffenburg, Germany) coupled to an UV-Detector and an LCQ ion-trap mass spectrometer (Finnigan MAT, Bremen, Germany).

The mobile phase consisted of variable mixtures of trifluoroacetic acid in water (0.05%; solvent A) and trifluoroacetic acid in methanol (0.05%: solvent B), at a flow of 0.8 ml/min. Gradient elution started at 60% B maintained for 2 min, followed by raising the concentration of B linearly to 100 within 4 min. After maintaining these conditions for 5 min the concentration of B was brought back within 4 min to the initial mixture and the column equilibrated for 1 min.

To ensure an adequate spray stability, the column effluent was diverted to waste during the first 8 min of the gradient programme. The mass spectrometer was operated in the positive electrospray mode using selected-reaction monitoring (SRM) with the mass transitions (*m/z* precursor ion/*m/z* product ion) 404/358 for OTA and 409/363 for [$^2H_5$]-OTA, respectively. The spray voltage was set to 5.0 kV, the capillary temperature to 200 °C and the capillary voltage to 32.0 V. The maximum ionization time was set to 50 ms and the MS–MS transition was measured using three microscans in order to obtain reproducible peak areas. For maximum sensitivity the isolation width of the parent ion was adjusted to 1 Da and the isolation width of the product ion was set to 1 Da in order to detect the product ion most selectively. The sheath and auxiliary gas flow rates were set to 80 and 20% of their maximum flow rates, respectively.

Flow injection analysis was performed by injecting pure solutions of the compounds to be analyzed into the MS at a flow of 8 μl/min.

Atmospheric pressure ionization (APCI) was performed by using the APCI interface of the LCQ ion-trap mass spectrometer. The temperature of the vaporizer tube was 450 °C and the corona discharge needle was supplied with a voltage of −3 kV, the discharge current was 5 μA. The capillary temperature was 150 °C and the capillary voltage −4 V. The nitrogen flows were 57 and 43% of their maximum flow rates for sheath and auxillary gas, respectively.

2.6. Calibration and quantitation

Solutions of unlabelled and labelled OTA were mixed in nine mass ratios ranging from 0.11 to 9 to give a total OTA concentration of 0.5 μg/ml. LC/MS–MS analysis of each mixture (20 μl) was performed in triplicate as outlined before. The calibration curve was constructed from these results and revealed a linear response of the peak area ratios to the mass ratios of unlabelled to labelled OTA between the mass ratios 0.2 and 9. The equation for the regression line was $y = 0.960x + 0.053$ ($r^2 = 0.9996$), where x is the peak area ratio in the trace MS–MS 404/358 to that in the trace MS–MS 409/363 and y is the mass ratio of unlabelled to labelled OTA.

Contents C of OTA in foods were computed using the following equation:

$$C = \left(\frac{A_{\text{OTA}}}{A_{\text{d-OTA}}} \times 0.960 + 0.053 \right) m_{\text{d-OTA}}$$

where A_{OTA} is the area of unlabelled OTA in trace MS–MS 404/358; $A_{\text{d-OTA}}$ the area of labelled OTA in trace MS–MS 409/363; $m_{\text{d-OTA}}$ is the amount of added labelled OTA.

2.7. Detection and quantitation limits

Detection (DL) and quantitation limits (QL) were determined using a wheat flour devoid of OTA. The following amounts of OTA (unlabelled as well as labelled compounds) were added: 0.5, 1.0, 3.0 and 5 μg/kg. Extraction and SPE sample clean-up was continued and LC/MS–MS analysis was conducted as outlined above. Each addition assay was

performed in triplicate and DLs as well as QLs were calculated according to Hädrich and Vogelgesang [23]. In short, a calibration graph of measured versus added OTA amounts was plotted and both the lower and the upper 95% confidence intervals were included. Considering this graph, DL is the concentration calculated from the maximum height of the 95% confidence interval at the zero addition level. QL is the addition level for which the lower 95% confidence limit equals the upper 95% confidence limit of the addition level at the DL [24].

2.8. Stability of deuterium labelled standards to protium–deuterium exchange

[$^2H_5$]-OTA was stirred for 30 min in aqueous sodium carbonate (200 ml, 1%) and subjected to IA clean-up and LC/MS–MS as detailed above.

3. Results and discussion

3.1. Synthesis of isotopomeric ochratoxin A

In the past there have been two attempts to synthesize radioactively labelled OTA to be used in metabolic studies [19,25]. Both approaches consisted of a metathesis by hydrolyzing OTA and subsequent coupling of the resulting isocoumarin derivative ochratoxin α (OTα) to labelled L-phenylalanine. Following the route reported by Rousseau et al. [19], we transformed ochratoxin α to NHS-OTα and attempted to purify the latter intermediate by HPLC. However, NHS-OTα only appeared in minor amounts, which made the isolation ineffective. Therefore, we chose the acid chloride method to activate OTα. As already described by Steyn and Holzapfel [18], OTα was converted by addition of thionyl chloride into OTα chloride, which was then reacted with [$^2H_5$]-phenylalanine methyl ester, hydrolysis of which in sodium hydroxide provided [$^2H_5$]-OTA in a total yield of 4.9%. A survey of the complete synthetic route is displayed in Fig. 1.

Flow injection electrospray mass analysis of the synthesized material shown in Fig. 2 revealed an isotopic purity of 99.7% and a shift of the molecular mass of 5 Da corresponding to the introduction of five deuteriums by using labelled phenylalanine as reactant. This mass shift was also apparent in collision-induced dissociation (CID) experiments on the respective protonated molecules. As evident from Fig. 3, the MS–MS spectrum revealed a conceivable signal corresponding to a loss of formic acid from $[M+1]^+$.

3.2. LC/MS–MS

Separation of OTA from main interferences in food samples was achieved on a RP-18 column at a gradient

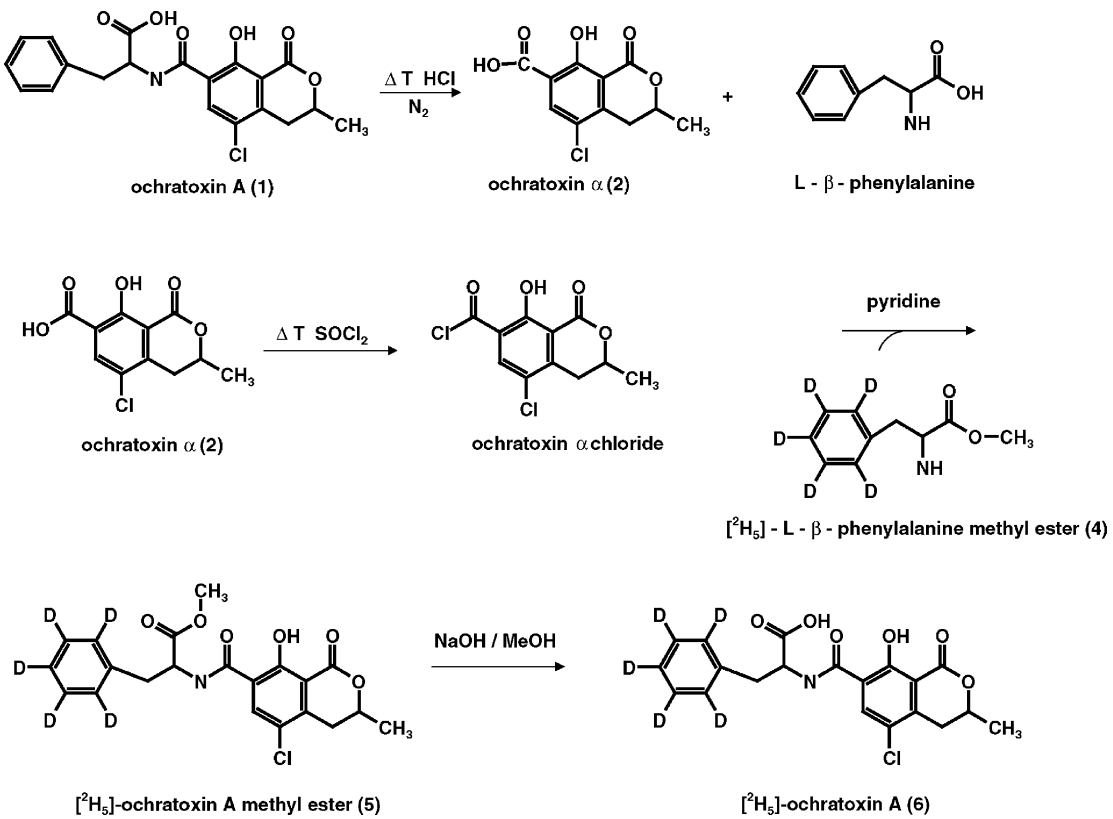

Fig. 1. Reaction scheme leading to [$^2H_5$]-ochratoxin A (**6**).

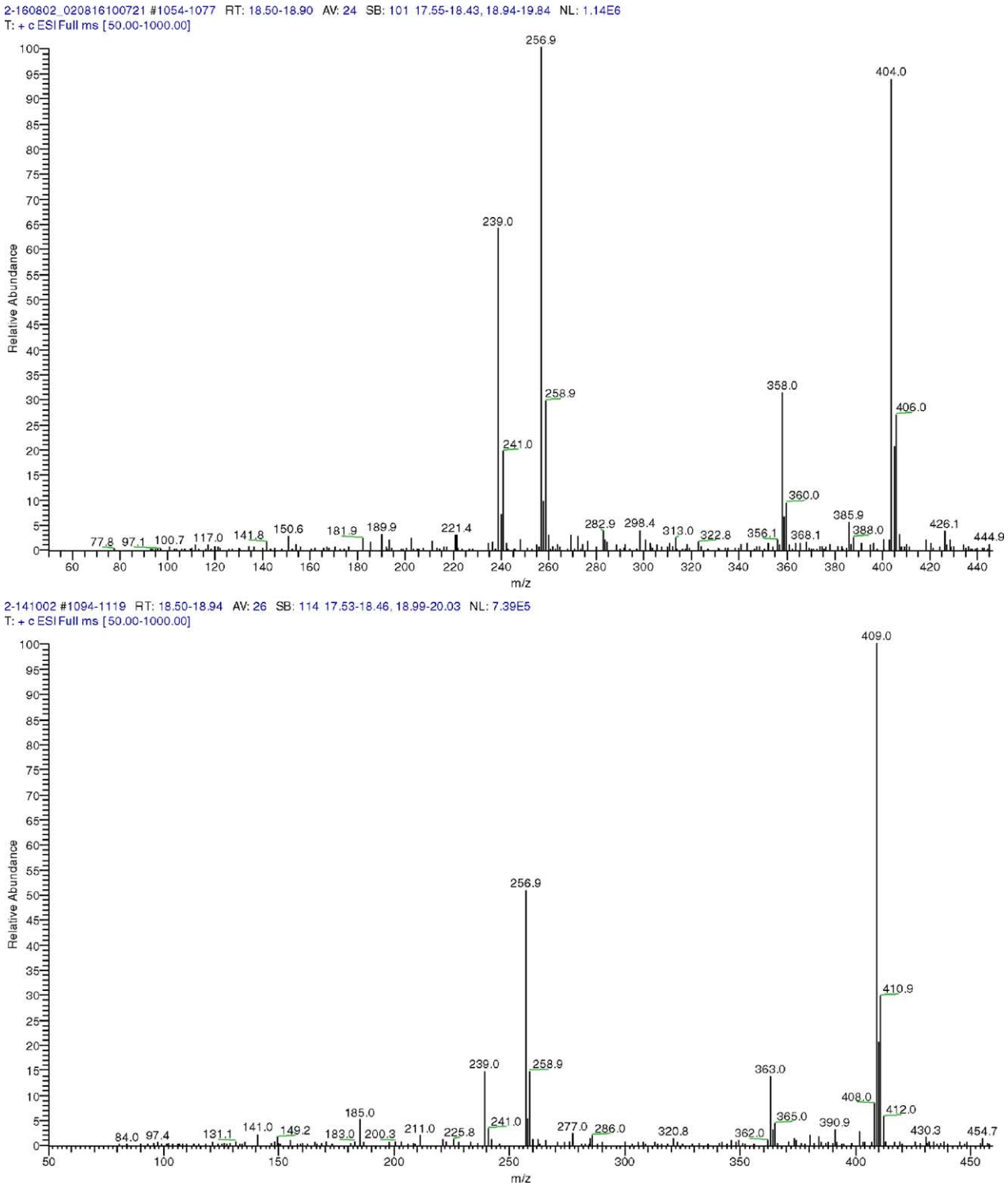

Fig. 2. LC-ESI(+)-mass spectrum of ochratoxin A (above) and [$^2H_5$]-ochratoxin A (below).

consisting of variable mixtures of methanolic trifluoroacetic and aqueous trifluoroacetic acid. LC/MS of standard solutions of mixtures of the isotopomeric OTAs revealed suitable peak shapes and enabled to differentiate unlabelled OTA from its labelled analogue by monitoring the mass traces of $[M + 1]^+$at *m/z* 404 and *m/z* 409, respectively. Analoguously, the isotopomers could be distinguished in the LC/MS–MS mode by monitoring the ions resulting from the loss of formic acid from the respective protonated molecules.

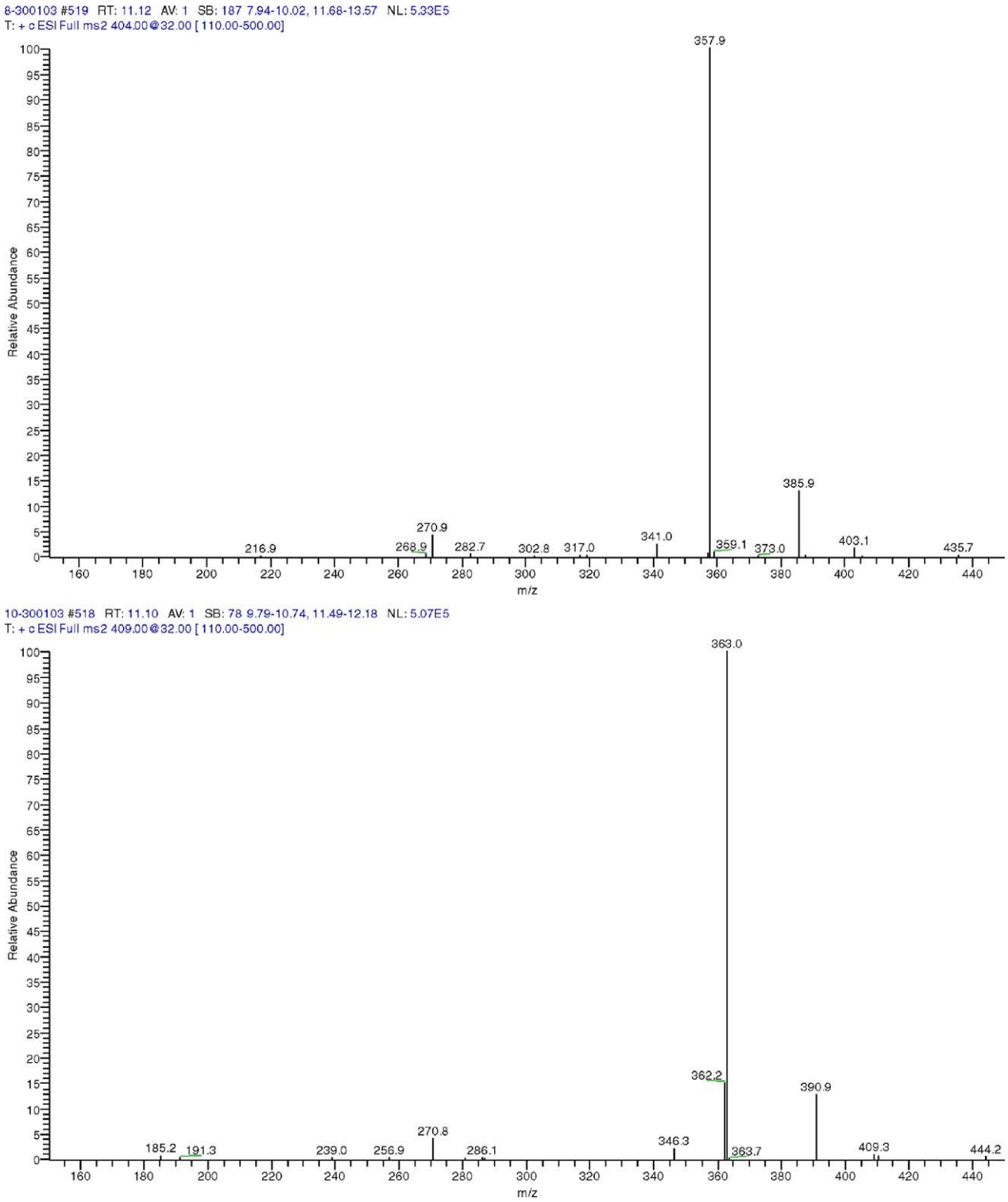

Fig. 3. LC-ESI(+)/MS–MS spectrum of ochratoxin A (above) and [$^2H_5$]-ochratoxin A (below). Precursor ions were the protonated molecules.

3.3. Calibration

To enable calculation of mass ratios from intensity ratios of OTA isotopomers in their respective mass traces, a calibration function was determined by analyzing mixtures of OTA and [$^2H_5$]-OTA standard solutions the mass ratios of which ranging between 1:9 and 9:1. Plotting the area ratios against the mass ratios revealed a linear calibration function with the respective equation showing a r^2 of 0.9996. This behaviour was expected, as the labelled OTA material

is nearly devoid of unlabelled OTA residues and no spectral overlap due to natural isotopomers in unlabelled OTA is likely to occur as the mass shift between the isotopomers is as high as 5 Da. Linearity and identical response factors were observed in LC/single stage MS and LC/tandem MS mode, as well.

3.4. Sample purification

According to the literature, sample clean-up can be achieved by the following methods: solid phase extraction (SPE) either on (a) silica or on (b) reversed-phase cartridges, or (c) on anion exchange columns or (d) by immuno affinity chromatography. In preliminary studies reversed-phase cartridges revealed low recovery and the anion exchange extracts showed significant interferences during LC/MS–MS. Therefore, a direct comparison only between silica cartridges and IAC was carried out using the matrixes wheat, coffee and red wine.

In case of wheat and red wine, performance of silica and IA clean-up was quite similar. However, regarding the silica extracts of coffee, the peaks of isotopomeric OTAs were obscured by background compounds as shown in Fig. 4. In contrast to this, the IA extracts were devoid of interferences and displayed a well shaped and clearly separated [$^2H_5$]-OTA peak (Fig. 5). It can, therefore, be assumed that IA chromatography is the most effective clean-up procedure in OTA analysis.

3.5. Stability of deuterium labelled standards to protium–deuterium exchange

As the labelled OTA contained five deuteriums, a protium–deuterium (H–D) exchange during the course of analysis would result in systematic errors during quantification. In order to exclude H–D-exchange, labelled OTA was stirred in extraction buffer and passed through IAC. The resulting eluate then was analysed by LC/MS–MS and compared with the untreated [$^2H_5$]-OTA solution, which contained 0.5% unlabelled material. After sample treatment, the degree of unlabelled material averaged at 0.6%, which proved that no H–D-exchange did occur.

3.6. Limits of detection and quantification

As we detailed in case of SIDA development for the vitamins of the folate group [16] and pantothenic acid [24] as well as for the mycotoxin patulin [15], the method proposed by Hädrich and Vogelgesang [23] is best suited to consider (i) losses during extraction and clean-up, (ii) background noise due to matrix interferences and (iii) data scattering in low concentration ranges for the determination of detection (DL) and quantitation limits (QL).

In analogy, we calculated the DL from the confidence interval of a calibration line prepared by spiking wheat flour devoid of OTA with variable amounts of the analyte.

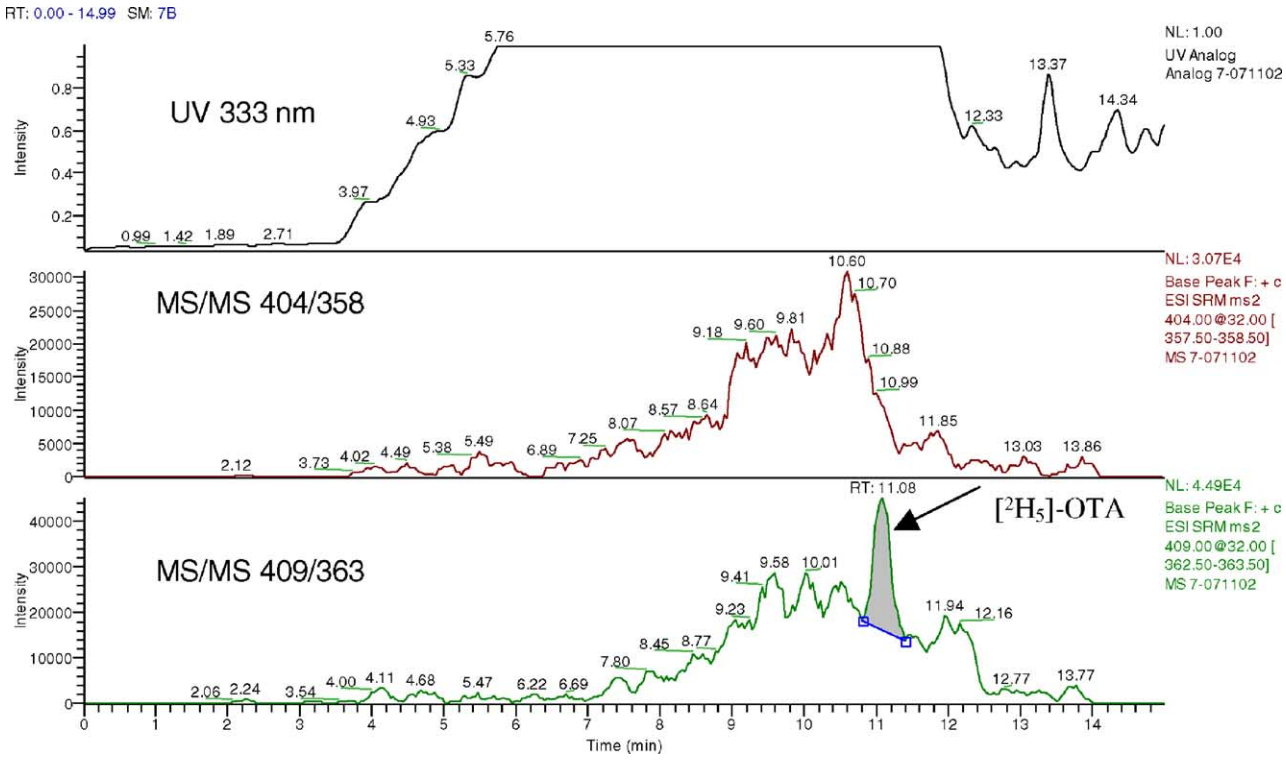

Fig. 4. LC-ESI(+)/MS–MS of a coffee extract not containing OTA after clean-up on a silica SPE cartridge. The internal standard [$^2H_5$]-ochratoxin A in trace MS–MS 409/363 is obscured by matrix interferences, unlabelled ochratoxin A in trace MS–MS 404/358 cannot be unambiguously confirmed or excluded.

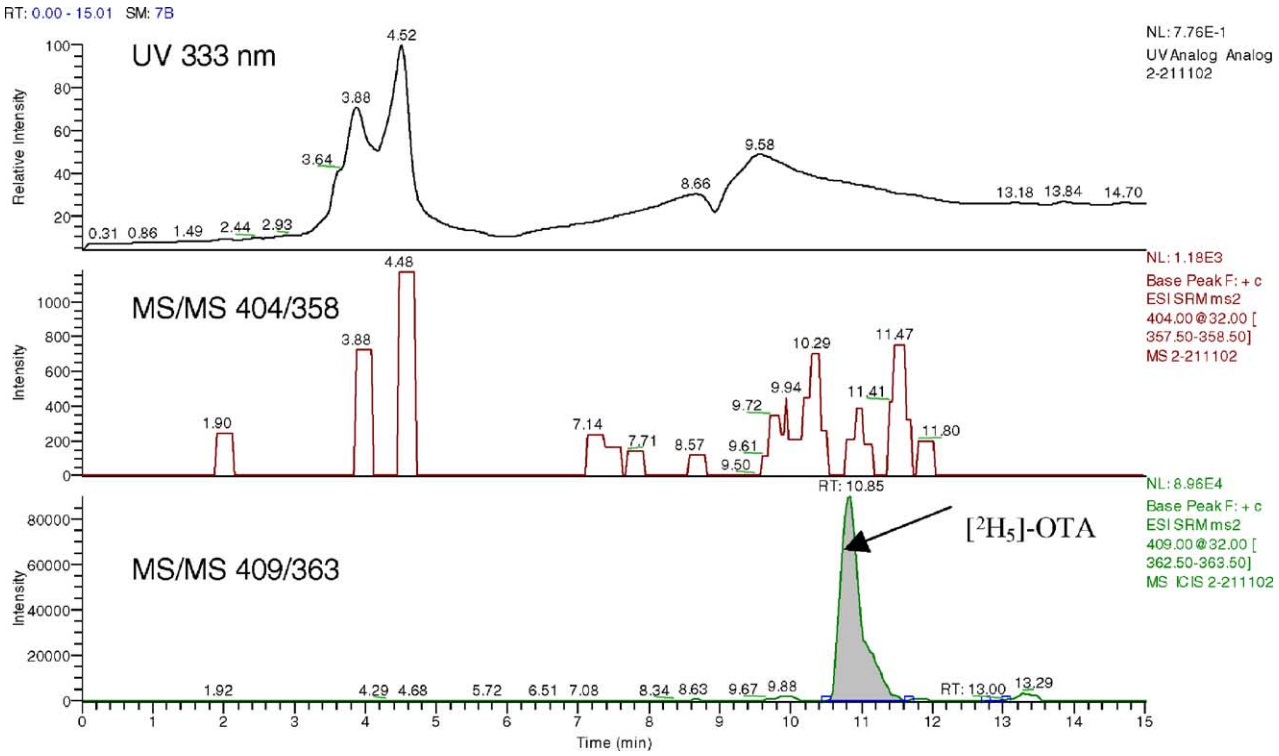

Fig. 5. LC-ESI(+)/MS–MS of a coffee extract not containing OTA after clean-up by immuno affinity chromatography. The internal standard [$^2H_5$]-ochratoxin A is unambiguously detected in trace MS–MS 409/363, unlabelled ochratoxin A in trace MS–MS 404/358 is not present.

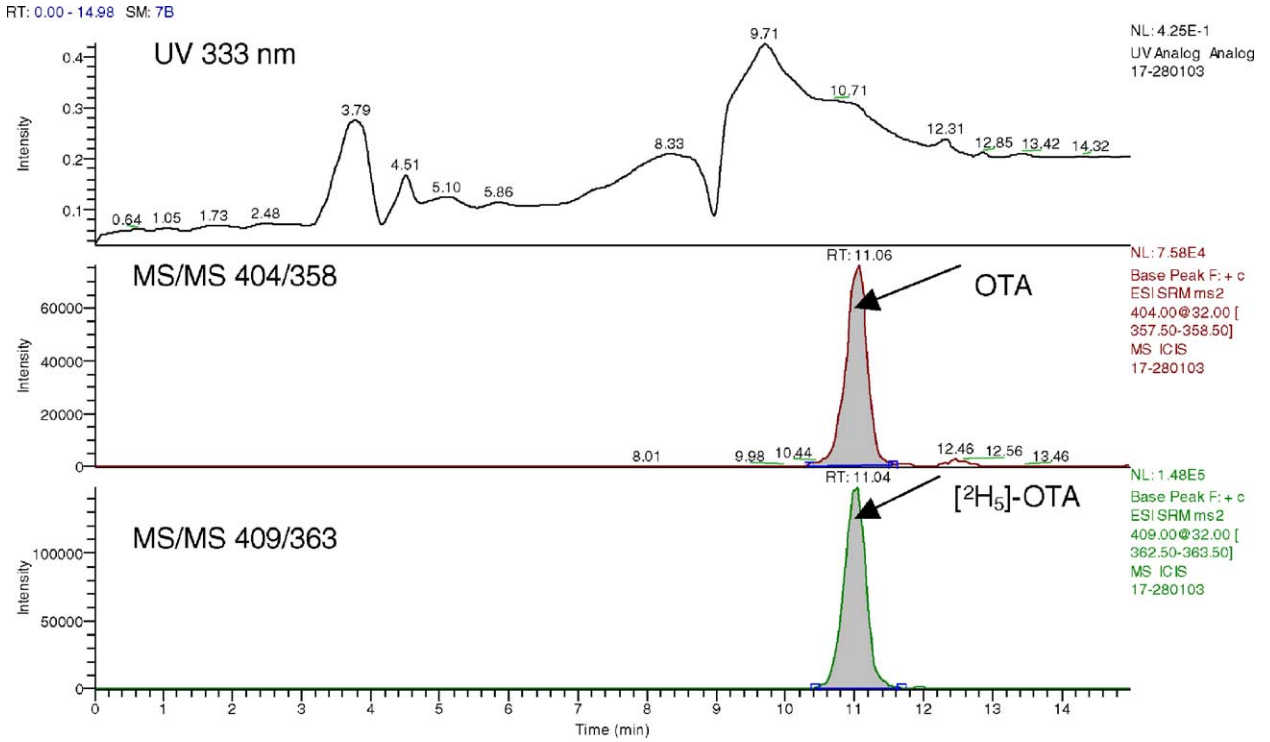

Fig. 6. LC-ESI(+)/MS–MS of certified reference material CRM 472 wheat flour after clean-up on an immuno affinity cartridge.

Table 1
Performance data of the stable isotope dilution assays (SIDA) for ochratoxin A based on the analyses of certified reference material CRM 472

Detection limit	0.5 μg/kg
Quantification limit	1.4 μg/kg
Inter-assay coefficient of variation	3.6% ($n = 3$)
Certified OTA content of CRM 472	8.2 ± 1.0 μg/kg
Quantified OTA content of CRM 472	8.0 ± 0.3 μg/kg
Bias	2.1%
Recovery (addition level 3 μg/kg) ± standard deviation	105.4 ± 3%

Addition experiments revealed a DL of 0.5 and a QL of 1.4 μg/kg for OTA. These data proved the SIDAs to be sensitive enough to quantify OTA contents even below the EU limits for foods. Moreover, the DL of SIDA was in the same order of magnitude as those of LC/FD methods ranging between 0.04 and 0.9 μg/kg [26]. As sample size for liquids may exceed multiply that of solids, the DL for liquid samples is even lower and can be estimated to 0.1 μg/kg.

3.7. Accuracy

3.7.1. Trueness

To check trueness of SIDA, two certified reference materials (CRM) from the Community Bureau of Reference (BCR) of the European Commission were analyzed. The CRMs consisted of wheat flour, one of which was certified to contain 8.2 ± 1.0 (CRM 472) and in the other of which the OTA content was certified to be below the detection limit of 0.6 μg/kg (CRM 471). These data had been calculated from the results of nine European laboratories using LC/fluorescence detection and fulfilling the performance criteria reported by Wood et al. [26].

SIDA of the reference materials resulted in an OTA content of 8.0 ± 0.3 for CRM 472, the MS–MS chromatogram of which is displayed in Fig. 6. In CRM 471, no OTA was detected above the detection limit of 0.5 μg/kg as mean of triplicate analyses. Thus, the bias from the certified reference value of CRM 472 was as low as 2.1% and proved the trueness of the presented method.

3.7.2. Precision and recovery

Inter-assay precision was evaluated by extracting CRM 472 three times within 2 weeks and revealed a coefficient of variation of 3.58%. Recovery was determined by adding unlabelled OTA to wheat flour devoid of the mycotoxin at an addition level of 3 μg/kg in triplicate and quantifying the OTA content by SIDA giving a value of 105.4%. The aforementioned validation data are summarized in Table 1.

3.8. Quantification of OTA in food

Of those foods in which OTA is most likely to occur, wheat flour, coffee, liquorice, beer, wine, and some spices were quantified after clean-up by IA columns.

The majority of foods analyzed did not contain OTA above its DL. However, in soluble coffee, OTA was detectable, but not quantifyable. Higher concentrations were found in mulled wine (n.d. −3.3 μg/kg), nutmeg powder (1.8 μg/kg), and raisins (n.d. −29.8 μg/kg). In the latter products, eight out of nine samples contained detectable contents of the mycotoxin, of which three samples (CV of each sample less than 3.2%) exceeded the legal limit of 10 μg/kg.

4. Conclusion

The validation data of the SIDA presented here revealed excellent accuracy and sensitivity of the new method for all analyzed samples.

Of all analyzed foods, the majority contained OTA below DL. However, three samples of raisins were found to exceed the legal limit and may, therefore, be a risk for consumers' health. As the survey is not representative due to low sample numbers, a broader survey would be necessary to evaluate the actual hazard due to OTA consumption.

Due to the three-dimensional specifity of LC/MS–MS, SIDA offers the perspective to be suited for clinical matrices, for which the alternative methods show discernable drawbacks. Therefore, a method comparison to the latter methodologies for matrices such as blood plasma or urine is under way.

References

[1] J. Bauer, M. Gareis, J. Vet. Med. B 34 (1987) 613.
[2] M. Blanc, A. Pittet, R. MunozBox, R. Viani, J. Agric. Food Chem. 46 (1998) 673.
[3] P. Majerus, H. Otteneder, Dtsch. Lebensm. Rundsch. 92 (1996) 388.
[4] P. Majerus, M. Max, H. Klaffke, R. Palavinskas, Dtsch. Lebensm. Rundsch. 96 (2000) 451.
[5] T. Kuiper-Goodman, P.M. Scott, Biomed. Environm. Sci. 2 (1989) 179.
[6] K. Hult, R. Plestina, V. Habazin-Novak, B. Radic, S. Ceovic, Arch. Tox. 51 (1982) 313.
[7] T. Kuiper-Goodman, Food Addit. Contam. 13 (1996) 53.
[8] A. Thuvander, T. Moller, H.E. Barbieri, A. Jansson, A. C. Salomonsson, M. Olsen, Food Addit. Contam. 18 (2001) 696.
[9] H. Rosner, B. Rohrmann, G. Peiker, Arch. Lebensmittelhyg. 51 (2000) 104.
[10] European commission EC No. 466/2001.
[11] E. Märtlbauer, G. Terplan, Arch. Leb. Hyg. 39 (1988) 133.
[12] A.C. Entwisle, K. Jorgensen, A.C. Williams, A. Boenke, P.J. Farnell, Food Addit. Contam. 14 (1997) 223.
[13] M. Gareis, Arch. Lebensmittelhyg. 50 (1999) 83.
[14] K. Larsson, T. Möller, J. AOAC Int. 79 (1996) 1102.
[15] M. Rychlik, P. Schieberle, J. Agric. Food Chem. 47 (1999) 3749.
[16] A. Freisleben, P. Schieberle, M. Rychlik, Anal. Bioanal. Chem. 376 (2003) 149.
[17] K.J. van der Merwe, P.S. Steyn, L. Fourie, J. Chem. Soc. 1965 (1965) 7083.
[18] P.S. Steyn, C.W. Holzapfel, Tetrahedron Lett. 23 (1967) 449.
[19] D. Rousseau, G. Slegers, C. Van Peteghem, A. Claeys, J. Labelled Compd. Radiopharm. 21 (1984) 429.
[20] M. Becker, P. Degelmann, M. Herderich, P. Schreier, H.U. Humpf, J. Chromatogr. A 818 (1998) 260.

[21] AOAC, Official Methods of Analysis of AOAC International, 16th ed., AOAC International, Gaithersburg, Maryland, 1995 (Method 970.44).
[22] Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin. Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG, Beuth Verlag, Berlin 1999 (Method 15.00-1/1).
[23] J. Hädrich, J. Vogelgesang, Dtsch. Lebensm. Rundsch. 95 (1999) 428.
[24] M. Rychlik, J. Agric. Food Chem. 48 (2000) 1175.
[25] K. Hult, J. Labelled Compd. Radiopharm. 23 (1986) 801.
[26] G.M. Wood, S. Patel, A.C. Entwisle, A. Boenke, Food Addit. Contam. 13 (1996) 519.