Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Journal of Chromatography A, 1054 (2004) 389–395
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Simultaneous detection of type A and type B trichothecenes in cereals by liquid chromatography–electrospray ionization mass spectrometry using NaCl as cationization agent

C. Dall'Asta, S. Sforza, G. Galaverna, A. Dossena, R. Marchelli*

Department of Organic and Industrial Chemistry, University of Parma, Parco Area delle Scienze 17/A, I-43100 Parma, Italy

Available online 11 September 2004

Abstract

A LC/MS method for the simultaneous determination of both type A and type B trichothecenes by using an electrospray ionization (ESI) interface in the positive ionization mode with a single quadrupole analyzer is described. In order to enhance the ionization of both groups of trichothecenes, the sodium ion was used as cationization agent by adding sodium chloride to the eluent. All LC/MS parameters were optimized. The newly developed LC/ESI-MS method was applied to the analysis of a wheat reference material and cereal-based foods and feeds.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Trichothecenes; Cationization

1. Introduction

Trichothecenes constitute the largest group of *Fusarium* mycotoxins. Although the number of trichothecenes identified is large (over 170), only few of them have been detected so far in naturally contaminated cereals and commodities, mainly belonging to type A or type B trichothecenes [1]. Both are tetracyclic sesquiterpenes and both are characterized by the presence of a double bond at C-9,10, a variable number of hydroxy and acetoxy groups and an epoxy-ring at C-12,13: consequently, they are classified as 12,13-epoxy-trichothecenes. Type A trichothecenes have a saturated carbon at C-8, eventually functionalized with an *O*-alkanoyl group and include T-2 and HT-2 toxins, neosolaniol (NEO) and diacetoxyscirpenol (DAS). Type B trichothecenes are characterised by a carbonyl group at C-8 and include, among others, deoxynivalenol (DON), nivalenol (NIV) and their derivatives. The general structures of trichothecenes A and B are reported in Fig. 1.

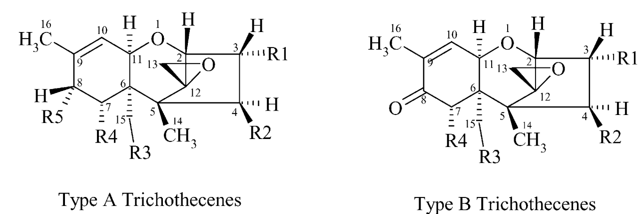
Type A and type B trichothecenes exhibit acute toxicity, causing vomiting and feed refusal; moreover type B trichothecenes are implicated in more chronic toxicoses, resulting in extensive haemorrhage, a general inflammatory response and haematological toxicities. They also inhibit both the protein synthesis and the mitochondrial function in vitro and in vivo and show immunosuppressing effects at low concentrations. In particular, DAS exposure caused suppression in the macrophage phagocytic function [2–4].

In European agricultural commodities type A trichothecenes usually occur less frequently and at lower concentrations than DON, the most diffuse contaminant. The simultaneous occurrence of DON with other *Fusarium* mycotoxins, mainly type B trichothecenes and zearalenone, has been reported for a variety of agricultural commodities [5]. A world-wide survey of DON, NIV and zearalenone, on 500 agricultural samples from 19 countries, reported that 40–50% of the samples were positive to these mycotoxins with an average concentrations of 292 ng/g DON, 267 ng/g NIV and 45 ng/g zearalenone. In particular, DON was found in maize (69%), followed by mixed feed (65%) and wheat (60%) [6].

Despite the high toxicity and the great diffusion of trichothecenes, few countries have established legal regulations

* Corresponding author. Tel.: +39 0521 905410; fax: +39 0521 905472.
E-mail address: rosangela.marchelli@unipr.it (R. Marchelli).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.07.081



Type A Trichothecenes

Type B Trichothecenes

		MW	R_1	R_2	R_3	R_4	R_5
TYPE A TRICHOTHECENES	*T-2 toxin*	466	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$
	HT-2 toxin	424	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
	Diacetoxyscirpenol (DAS)	366	OH	OAc	OAc	H	H
	Neosolaniol (NEO)	382	OH	OAc	OAc	H	OH
TYPE B TRICHOTHECENES	*Nivalenol (NIV)*	312	OH	OH	OH	OH	-
	Deoxynivalenol (DON)	296	OH	H	OH	OH	-
	Fusarenon X (FUSX)	354	OH	OAc	OH	OH	-
	15-acetyl-deoxynivalenol (15-ac-DON)	338	OH	H	OAc	OH	-

Fig. 1. Structures of the main type A and type B trichothecenes.

or recommendations, with recommended maximum levels of DON of 400 and 500 ng/g in food and swine feed [7].

The analytical procedures for trichothecenes A and B usually differ in extraction, clean up and chromatographic conditions, on account of the different polarities, depending on which group is to be analysed [8,9]. Thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), enzyme-linked immunosorbent assay (ELISA), capillary gas chromatography (GC) with either electron-capture detection (ECD) or mass-selective detection (MS) are widely used [2,10–17]. The HPLC-UV detection cannot be used for the chromatographic analysis of type A trichothecenes, because these molecules do not present suitable chromophores, while for HPLC-fluorescence it is necessary to use in both cases hydroxyl derivatizing reagents [2,15,17].

LC/MS techniques, in particular with APCI interfaces, have recently been employed for the determination and identification of trichothecenes at trace levels [18,19], by using the positive ionization mode for type A trichothecenes and the negative ionization mode for the type B trichothecenes: in fact, the less polar type A trichothecenes are best detected as positive ions, whereas type B toxins are efficiently detected as negative ions. Typical detection limits for the quantitative analysis of DON in cereals were around 50 ng/g. Very few methods are reported using an electrospray ionization (ESI) interface, probably on account of the poor response of the molecules to the ESI technique, to the difficulty of simultaneously detecting type A and type B trichothecenes and also on account of the increased matrix effects observed with this interface. One of the best method reported the determination of the type B trichothecenes in maize in the negative ion mode by ESI-MS/MS (in the MRM mode) down to few ng/g [20].

The aim of this work is to provide a LC/MS method for the simultaneous determination of both type A and type B trichothecenes with an ESI interface and a single quadrupole analyzer. In order to enhance the ionization of both groups of trichothecenes and to allow the simultaneous detection of type A and type B using a positive ionization mode, the sodium ion was used as cationization agent. The newly developed LC/ESI-MS method was applied to the analysis of a wheat reference material.

2. Experimental

2.1. Chemicals

Trichothecene standards (99% purity, 1 mg) of deoxynivalenol (DON), nivalenol (NIV), T-2 toxin (T-2), fusarenon-X (FUSX), diacetoxyscirpenol (DAS), HT-2 toxin (HT-2), neosolaniol (NEO) and 15-acetyl-deoxynivalenol (15-ac-DON) were obtained from Sigma–Aldrich (Steinheim, Germany). Stock solutions of trichothecene standards were prepared in acetonitrile from the commercial standards (500 μg/ml). Stock solutions containing 10 μg/ml of each standard were prepared in acetonitrile.

Certified reference material T2204 (wheat flour containing deoxynivalenol, 0.894 mg/kg), tested under the supervision of the Food Analysis Performance Assessment Scheme (FAPAS) (DEFRA, Central Science Laboratories, York, UK) was used.

All solvents used were of LC grade from Carlo Erba (Milan, Italy); bidistilled water was produced in our laboratory utilising an Alpha-Q system (Millipore, Marlborough, MA, USA).

2.2. Sample extraction and clean up

Aliquots (25 g) of naturally contaminated samples of finely ground cereals were blended in a high speed blender (Ultraturrax T25, IKA, Stauffen, Germany) with 100 ml of water–acetonitrile (20:80, v/v) for 5 min. After filtering through Whatman No. 4 filter papers, 8 ml of filtrate were eluted through a Mycosep 225 column (Romer Labs, Union, MO, USA). A 4 ml volume of the purified extract was transferred in a vial. The solvent was evaporated to dryness under nitrogen and the residue was dissolved in 1 ml of methanol.

2.3. LC/MS analysis

The preliminary infusion analyses were performed both in positive and negative ion mode by using the following parameters: cone voltage 35 V (positive ion mode) or 22 V (negative ion mode), capillary voltage 3.5 kV (positive ion mode), 3.0 kV (negative ion mode), cone gas (nitrogen) 90 l/h, desolvation gas 470 l/h, source temperature 80 °C, desolvation temperature 150 °C, infusion flow 0.01 ml/min. All the trichothecene standard solutions (3 μg/ml) were prepared by dissolving the proper amount of the toxins in 0.5 ml of water (containing 0.2 mM of NaCl)–methanol solution (1:1 v/v).

The LC/MS system consisted of a 2695 Alliance (Waters co., Milford, MA, USA) equipped with a ZMD single quadrupole mass spectrometer with an electrospray source (Micromass, Manchester, UK).

The mobile phase consisted of a binary eluent (H_2O:CH_3OH 35:65, v/v) added with NaCl 0.1 mM. The column was a narrowbore C18 Spherisorb S3 ODS-2 (250 mm × 2.1 mm, 3 μm) and the flow rate was 0.2 ml/min. Ninety-five percent of the column flow was splitted by a T-tube before the ESI probe: this value was fixed by using peek tubes of different length inserted in the waste side of the T-tube connected to the ESI probe and measuring in the different cases the splitted volume, until a constant split ratio of 95:5 was reached. The column temperature was set at 35 °C and the injection volume was 5 μl.

The electrospray probe was used in the positive ion mode. The voltage settings used for the LC/MS analyses were the following: cone voltage 30 V, capillary voltage 3.5 kV, cone gas (nitrogen) 100 l/h, desolvation gas 450 l/h, source temperature 100 °C, desolvation temperature 150 °C. The sodiated molecular ions were monitored in the single ion recording (SIR) mode.

The linearity range was tested in the range 100–5000 ng/ml by injection of standards at nine concentration levels (three determinations at each concentration level were performed). Calibration curves were based on the analysis of spiked trichothecene-free wheat samples at six concentration levels (three determination at each level were performed).

The detection limits (LOD) were calculated at a signal-to-noise ratio of 3:1 and were determined by spiking blank wheat samples with proper amounts of each analyte.

3. Results and discussion

3.1. Preliminary infusion ESI-MS experiments

According to the literature data, the best detection of type A trichothecenes was usually obtained using a positive ionization mode [19], whereas the determination of the type B trichothecenes was usually performed in a negative ion mode [18]. The difference is probably due to the ability of the latter toxins to efficiently delocalize a negative charge due to enolate formation.

Preliminary infusion experiments were done on four type A (NEO, DAS, T-2 and HT-2) and four type B trichothecenes (DON, 15-ac-DON, FUSX, NIV) both in positive and negative ionization mode, by dissolving the molecules in water–methanol mixtures. It was actually observed that type A trichothecenes and the type B 15-acetyl-DON showed a high tendency to cationization when observed in the positive ion mode, forming adducts with Na^+ and K^+ ions. In the mass spectra of these trichothecenes, the $[MNa]^+$ and the

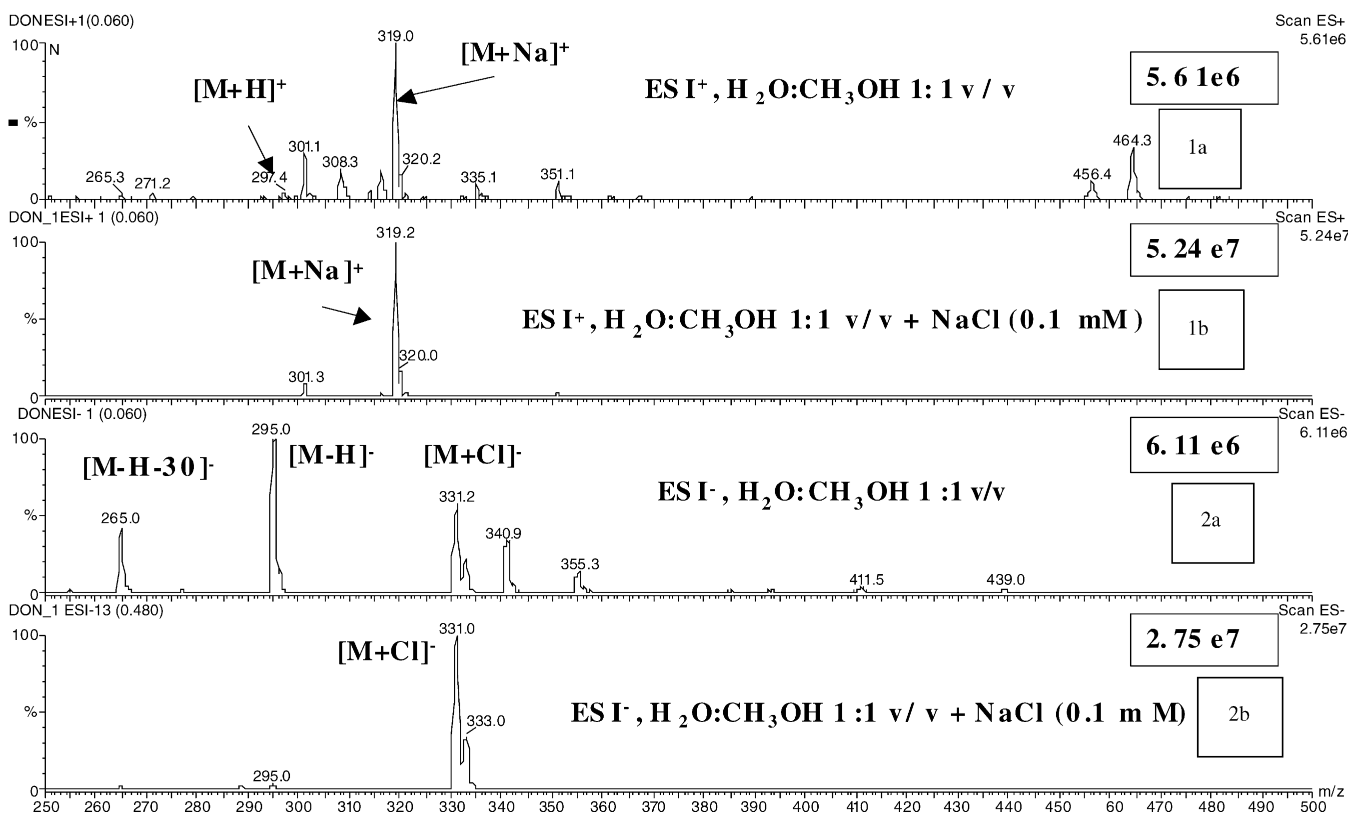


Fig. 2. Mass spectra of DON obtained in the presence (1b and 2b) and in the absence (1a and 2a) of 0.1 mM NaCl, using both positive (1a and 1b) and negative (2a and 2b) ionization modes. In the high right corner of each chromatogram the intensity of the highest peak is reported.

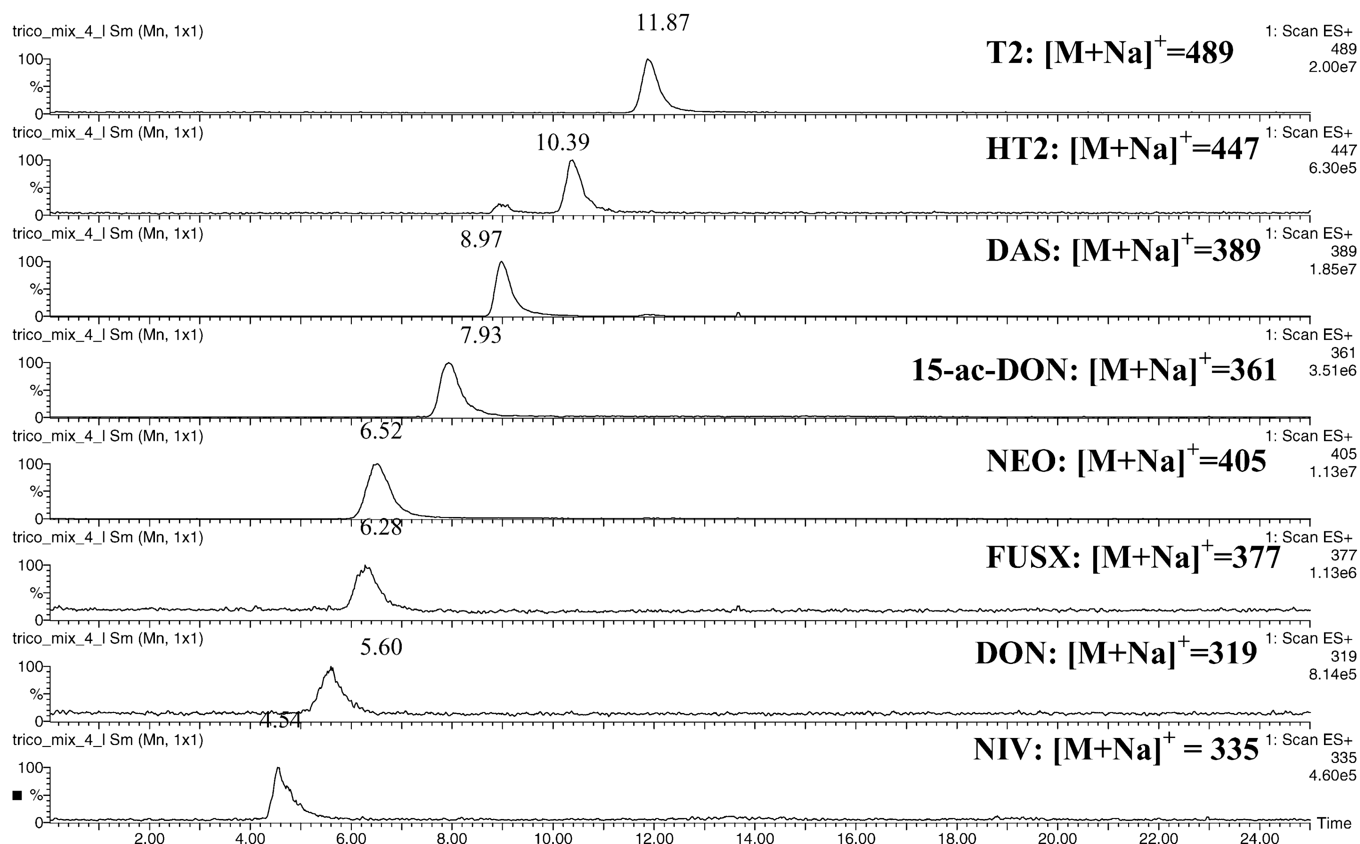


Fig. 3. Chromatogram of a standard mixture of trichothecenes with the molecular mass of the sodiated molecular ions monitored (concentration of each trichothecene: 3 μg/ml) (column: Spherisorb S3 ODS 2 narrowbore, 250 mm × 2.1 mm; eluent: H_2O–CH_3OH 35:65 + 0.1 mM NaCl; flow: 0.2 ml/min).

$[MK]^+$ ions were usually more abundant than the molecular ions $[MH]^+$. The other type B trichothecenes, on the other side, showed indeed poor response in positive polarity and were best detected as negative ions. Therefore, on account of the differences observed, the simultaneous detection of both type A and type B trichothecenes in the positive ion mode was not feasible. Moreover, also for type A trichothecenes, the intensity of $[MNa]^+$ and $[MK]^+$ adducts was not very reproducible, so the LC/MS analysis based on these ions was lacking of precision.

In order to allow a simultaneous detection of type A and type B trichothecenes using the most sensitive positive ionization mode and to control the formation of the adducts, we decided to use NaCl as additive to the solvent in order to assist the positive ionization. The mass spectra of the four type A (NEO, DAS, T-2 and HT-2) and of the four type B trichothecenes (DON, 15-ac-DON, FUSX, NIV) were recorded in the absence and in the presence of NaCl 0.1 mM.

After the addition of NaCl, the spectra of type A trichothecenes showed a gain in sensitivity and the disappearance of any peak other than the $[MNa]^+$ adduct. Moreover, also with type B trichothecenes, the addition of NaCl was very effective: the presence of the $[MNa]^+$ adduct really improved the ESI+ detection, whereas the simultaneous presence of a very high $[MCl]^-$ adduct in ESI− spectra could be used as a qualitative control for the identification of the compounds. The ESI+ and ESI− mass spectra obtained for DON (the most diffuse type B trichothecene) in the presence or in the absence of NaCl are reported in Fig. 2.

Also LiCl was assayed as cationization agent, but it was found to be slightly less effective than NaCl. In order to allow the quantitative determination of the analytes using NaCl as additive, it was necessary to found the optimal ionization parameters ensuring the stability of the observed sodium adducts. Preliminary infusion experiments were performed using a solution of each toxin in CH_3OH:H_2O 1:1 added of an equal volume of a water solution of NaCl 0.2 mM. Three parameters (desolvation temperature, capillary and cone voltages) were investigated and every parameter was optimised for the analysis. The desolvation temperature was changed from 80 to 300 °C; the capillary voltage range was investigated from 2.2 to 5 kV, whereas for the cone voltage the range of investigation was 20–180 V. All sodium adducts were shown to be very stable in nearly all conditions, as it was expected, being sodiated adducts less susceptible to fragmentation than the protonated counterparts. In the case of DON, as example, the sodiated molecular ion was always the only peak observed in the spectrum at increasing cone

Table 1
Linear calibration parameters obtained for each toxin (Concentration levels: 100, 250, 400, 800, 1000, 2000 ng/g added to a toxin-free wheat sample)

	Retention time (min)	Slope	Intercept	r^2
NIV	4.5	2.1×10^3	3.7×10^3	0.98
DON	5.6	1.4×10^3	2.7×10^3	0.98
FUSX	6.3	1.3×10^3	2.4×10^3	0.97
NEO	6.5	2.8×10^3	5.3×10^3	0.99
15-ac-DON	7.9	2.9×10^3	3.3×10^3	0.99
DAS	8.9	5.9×10^3	6.5×10^3	0.99
HT-2	10.4	3.9×10^3	2.2×10^3	0.99
T-2	11.9	3.0×10^3	4.1×10^3	0.99

Chromatographic conditions: C18 Spherisorb S3 ODS-2 narrowbore column (250 mm × 2.1 mm, 3 μm); H_2O:CH_3OH 35:65 + NaCl 0.1 mM mobile phase, flow: 0.2 ml/min.

Table 2
Limits of detection of type A and type B trichothecenes (calculated as S/N = 3 in spiked wheat samples)

		LOD (ng/g)
Type A	NEO	20
	DAS	20
	T-2	50
	HT-2	20
Type B	DON	45
	NIV	50
	15-ac-DON	20
	FUSX	40

voltages from 30 to 100 V, although with different intensity. The optimised conditions for the ionization are reported in Section 2.

3.2. LC/ESI-MS analysis

The ability of NaCl to assist the positive ionization of the analytes was used to develop a LC/ESI-MS method for the simultaneous detection of type A and type B trichothecenes. A Spherisorb S3 ODS2 (2.1 mm × 250 mm, 3 μm) narrowbore column and a water–methanol (35:65, v/v) mobile phase were used in the isocratic mode. The eluent was modified by addition of 0.1 mM NaCl with the ionization parameters previously established.

Under the conditions applied, a good separation of the four type A trichothecenes (NEO, DAS, T2, HT2) and of the four type B trichothecenes (DON, NIV, FUSX and 15-ac-DON) was obtained as shown in Fig. 3.

The linearity was tested in the concentration range between 100 and 5000 ng/ml, using a standard solution of the eight trichothecenes: all the analytes showed good regressions ($r^2 > 0.98$) and the standard calibration was reproducible. Calibration curves were obtained by addition of the proper amount of each toxin to a blank wheat sample (six concentration levels: 100 ng/g–250 ng/g–400 ng/g–800 ng/g–1000 ng/g–2000 ng/g). The calibration parameters obtained for every trichothecene are reported in Table 1.

The detection limits (LOD, S/N = 3/1) obtained for all the analytes are reported in Table 2.

The limits are, in all cases, similar to those obtained by LC/APCI-MS analysis [2,17,18]. The repeatability of the method was obtained by injection of a standard mixture at three concentration levels (each injection was performed in triplicate): the method showed a good precision with a variation coefficient of 5%.

It should be underlined that, by applying this method, a qualitative confirmation was also possible for type B

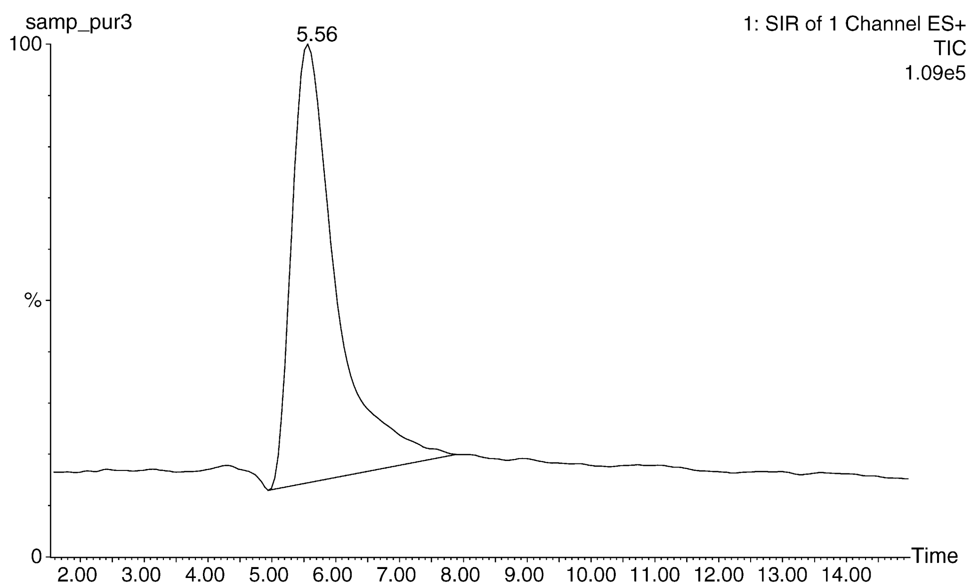


Fig. 4. Chromatogram of a sample extracted from wheat reference material (FAPAS certified) containing 894 ng/g of DON, obtained by monitoring the sodiated molecular ion at m/z 319.

Table 3
Quantitative data on trichothecenes (given in ng/g) obtained by LC/MS (and LC-fluorescence)

	DON	15-ac-DON[b]	NIV	FUSX	DAS	T-2	HT-2	NEO
Wheat A	<100[a] (40 ± 1)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Wheat B	n.d.	n.d.	<100[a] (70 ± 3)	n.d.	<100[a] (75 ± 3)	n.d.	n.d.	n.d.
Wheat C	n.d.	n.d.	189 ± 1 (150 ± 4)	n.d.	n.d.	n.d.	n.d.	n.d.
Wheat D	125 ± 2 (100 ± 3)	n.d.	n.d.	n.d.	<100[a] (95 ± 2)	<100[a] (30 ± 4)	n.d.	n.d.
Wheat E	<100[a] (50 ± 2)	n.d.	n.d.	n.d.	n.d.	233 ± 1 (200 ± 5)	n.d.	n.d.
Wheat F	<100[a](80 ± 3)	n.d.	n.d.	<100[a] (75 ± 5)	n.d.	n.d.	n.d.	n.d.
Wheat G	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Wheat H	n.d.	n.d.	n.d.	n.d.	180 ± 4 (130 ± 4)	n.d.	n.d.	n.d.
Wheat I	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Wheat J	355 ± 2 (320 ± 8)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Wheat K	n.d.	<100[a,b] (n.d.)[b]	110 ± 1 (150 ± 6)	n.d.	n.d.	n.d.	n.d.	n.d.
Wheat L	<100[a] (20 ± 4)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Wheat M	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Wheat N	n.d.	n.d.	n.d.	210 ± 1 (210 ± 6)	n.d.	n.d.	n.d.	n.d.
Maize feed A	n.d.	n.d.	n.d.	n.d.	847 ± 5 (830 ± 5)	n.d.	n.d.	n.d.
Maize feed B	314 ± 2 (300 ± 6)	184 ± 6[b] (85 ± 3)[b]	251 ± 2 (250 ± 4)	229 ± 1 (250 ± 2)	n.d.	160 ± 2 (150 ± 6)	n.d.	n.d.
Maize feed C	568 ± 1 (560 ± 6)	n.d.	320 ± 2 (300 ± 2)	311 ± 1 (300 ± 7)	n.d.	n.d.	178 ± 2 (150 ± 4)	n.d.
Maize feed D	n.d.	n.d.	n.d.	n.d.	376 ± 1 (380 ± 6)	389 ± 1 (360 ± 6)	200 ± 2 (230 ± 4)	n.d.
Maize feed E	<100[a] (55 ± 3)	n.d.	n.d.	n.d.	n.d.	256 ± 1 (240 ± 9)	n.d.	n.d.
Maize feed F	289 ± 2 (250 ± 6)	231 ± 4[b] (98 ± 4)[b]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Maize feed G	n.d.	n.d.	n.d.	n.d.	n.d.	234 ± 2 (259 ± 2)	319 ± 2 (310 ± 8)	n.d.

For comparison, the quantitative results by LC-fluorescence (obtained according to ref. [17]) are also reported among parentheses (in ng/g). Every sample was prepared in triplicate (n.d. = not detected).

[a] Calibration curves were calculated starting from 100 ng/g.

[b] The discrepancies are due to the fact that the LC/MS method did not allow the separation of 15-ac-DON from 3-ac-DON, which were then detected and quantified together, while the LC-fluorescence method allows to separately quantify the two acetylated derivatives.

trichothecenes, in particular for the most common DON, by ESI− determination of the $[MCl]^-$ ion.

3.3. Application to real samples

In order to evaluate the possibility of using the present method for the quantitative analysis of real samples, the procedure was first applied to a certified wheat reference material naturally contaminated with DON (certified contamination level: 894 ng/g; satisfactory range: 603–1185 ng/g). The wheat sample was extracted using the procedure normally applied to the analysis of trichothecenes in cereals, by extracting the blended sample with a water–acetonitrile mixture and purifying the extract through a Mycosep column, as described in Section 2.

The chromatogram obtained for the reference material is reported in Fig. 4.

The declared (894 ng/g) and the determined (848 ng/g) DON concentrations are really consistent, with an accuracy value as error percentage $E = 5\%$ and a very low z-score value ($z = 0.32$). The recovery calculated for the certified reference material was 95% at the contamination level.

Finally, several cereal-based samples (wheat for human consumption and maize-based feeds) were extracted and analyzed according to the method here reported. For comparison, they were also analyzed by LC-fluorescence, after derivatization with coumaroyl chloride, according to the method recently reported by our group [17]. Results are reported in Table 3.

The data found by LC/MS and by LC-fluorescence are in fair agreement and, being every sample independently prepared and injected in triplicate, show the good precision of both methods. Quantitative results found by LC/MS are often slightly higher than those found by LC-fluorescence: this could be due to an incomplete derivatization reaction with coumaroyl chloride. Trichothecene contamination in the samples analyzed was generally low or absent, except in feeds, which resulted usually more contaminated than wheat for human consumption.

4. Conclusions

The use of NaCl to assist the ionization of both type A and type B trichothecenes allowed for the first time the simultaneous determination of both groups by LC/ESI-MS in the positive ionization mode, improving the detectability of 8 trichothecenes. Being the cationization reproducible and forming stable adducts, it was also possible to use NaCl as ionization assistant for quantitative analyses.

For all toxins the limits of detection were found to be similar to those reported for the detection of type A and type B trichothecenes by using the LC/APCI-MS technique.

The developed method has been successfully applied to the analysis of a certified reference material (wheat), obtaining a very good accuracy with a recovery of 95%. The method was applied to the analysis of cereal-based foods and feeds obtaining results in good agreement with LC-fluorescence detection.

Acknowledgements

This project was supported by a grant by Ministero della Salute (Rome, Italy). Progeo s.r.l. (Masone, Italy) is gratefully acknowledged for having provided the cereal samples.

References

[1] Y. Ueno, Trichothecenes—chemical, biological and toxicological aspects, in: Development in Food Science, vol. 4, Elsevier, Amsterdam, 1983.
[2] H.S. Hussein, J.M. Brasel, Toxicology 167 (2001) 101.
[3] E. Creppy, Toxicol. Lett. 127 (2002) 19.
[4] A. Gutleb, E. Morrison, A. Murk, Environ. Toxicol. Pharmacol. 11 (2002) 309.
[5] M. Schollenberger, H. Terry Jara, S. Suchy, W. Drochner, H.M. Mueller, Int. J. Food Microbiol. 72 (2002) 85.
[6] T. Tanaka, A. Hasegawa, S. Yamamoto, U.S. Lee, Y. Suigiura, Y. Ueno, J. Agric. Food Chem. 36 (1988) 979.
[7] R. Krska, S. Baumgartner, R. Josephs, Fresenius J. Anal. Chem. 371 (2001) 285.
[8] R. Krska, J. Chromatogr. A 815 (1998) 49.
[9] Z. Radova, K. Holadova, J. Hajslova, J. Chromatogr. A 829 (1998) 259.
[10] W. Langseth, T. Rundberget, J. Chromatogr. A 815 (1998) 103.
[11] J.C. Young, D.E. Games, J. Chromatogr. A 653 (1993) 374.
[12] S. Croteau, D.B. Prelusky, H. Locksley Trenholm, J. Agric. Food Chem. 42 (1994) 928.
[13] T. Moller, H. Gustavsson, J. AOAC Int. 75 (1992) 1049.
[14] R.C. Schothorst, A.A. Jekel, Food Chem. 73 (2001) 111.
[15] R.D. Josephs, R. Krska, M. Grasserbauer, J.A.C. Broekaert, J. Chromatogr. A 795 (1998) 297.
[16] M. Schoellenberg, U. Lauber, H.T. Jara, S. Suchy, W. Drochner, H.M. Mueller, J. Chromatogr. A 815 (1998) 123.
[17] C. Dall'Asta, G. Galaverna, A. Biancardi, M. Gasparini, S. Sforza, A. Dossena, R. Marchelli, J. Chromatogr. A 1047 (2004) 241.
[18] E. Razzazi-Fazeli, J. Bohm, W. Luf, J. Chromatogr. A 854 (1999) 45.
[19] E. Razzazi-Fazeli, B. Rabus, B. Cecon, J. Bohm, J. Chromatogr. A 968 (2002) 129.
[20] A. Lagana, R. Curini, G. D'Ascenzo, I. De Leva, A. Faberi, E. Pastorini, Rapid Commun. Mass Spectrom. 17 (2003) 1037.