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Determination of T-2 toxin in cereal grains by liquid chromatography with fluorescence detection after immunoaffinity column clean-up and derivatization with 1-anthroylnitrile

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Abstract

1-Anthroylnitrile (1-AN) has been shown to be an efficient labelling reagent for the determination of T-2 toxin (T-2) by high-performance liquid chromatography (HPLC)–fluorescence detection. This reaction has been used to develop a sensitive, reproducible and accurate method for the determination of T-2 in wheat, corn, barley, oats, rice and sorghum. The method uses immunoaffinity columns containing antibodies specific for T-2 for extract clean-up, pre-column derivatization with 1-AN and HPLC with fluorescence detection for toxin determination. Ground cereal samples were extracted with methanol–water (80:20, v/v), the extracts were purified by immunoaffinity columns and the toxin was quantified by reversed-phase HPLC with fluorometric detection (excitation wavelength 381 nm, emission wavelength 470 nm) after derivatization with 1-AN. Recoveries from the different cereals spiked with T-2 at levels ranging from 0.05 to 1.5 μg/g were from 80 to 99%, with relative standard deviations of less than 6%. The limit of detection was 0.005 μg/g, based on a signal-to-noise ratio of 3:1.

Keywords: Cereals; Sample preparation; Derivatization, LC; Food analysis; Anthroylnitriles; Toxins

1. Introduction

T-2 toxin (T-2) is a highly toxic type-A trichothecene produced by different *Fusarium* species, mainly *F. sporotrichioides*. It occurs occasionally in grains such as wheat, corn, oats, barley, rice, sorghum, beans and cereal-based products, especially in northern temperate climates. T-2 causes outbreaks of haemorrhagic disease in domestic animals and has been shown to be the causative agent of alimentary toxic aleukia (ATA), a fatal disease which occurred in Russia in 1942–47 in a population fed overwintered cereals. Toxicological studies on animals have shown that the immune system is the primary target of T-2, causing changes in leukocyte counts, depletion of selective blood cell progenitors and depression of antibody formation. Exposure to the toxin results in skin pain, pruritis, redness, vesicles, necrosis, epidermal sloughing, nausea, weight loss, vomiting and diarrhea. Severe poisoning results in prostration, weakness, ataxia, collapse, reduced cardiac output, shock, and death [1]. T-2, together with other trichothecenes, has been reported in samples of

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the alleged chemical warfare agent ''yellow rain'' in Southeast Asia [2].

Recently, the JECFA (Joint FAO/WHO Expert Committee on Food Additives) has evaluated the safety of certain mycotoxins in food, emphasizing the toxic effects of T-2 and concluding that ''there is a need for more accurate information on the human exposure to T-2'' [1].

Sensitive and accurate methods for the determination of T-2 in various matrices are highly desirable in order to fulfill the need to protect consumer health from the risk of exposure to the toxin. Analytical methods for the determination of T-2, alone or in combination with other trichothecenes, have been reported, including thin-layer chromatography (TLC) [3,4], enzyme-linked immunoassays (enzyme-linked immunosorbent assay and flow-through membrane-based immunoassay) [3,5,6], gas chromatography (GC) combined with electron-capture detection (ECD), flame ionization detection (FID) or mass spectrometry (MS) [3,7–9], high-performance liquid chromatography (HPLC)–MS [7,10], and supercritical fluid chromatography (SFC)–MS [7]. HPLC with UV or fluorescence detection is not applicable to type-A trichothecenes due of the lack of appropriate chromophores in their structure.

At present, GC with ECD or MS detection and pre-column derivatization (to increase the volatility and sensitivity of the toxins) is the technique mostly used for the quantification of type-A trichothecenes. Recently, a comparative inter-laboratory study on method performance for trichothecene analysis (including T-2) using gas chromatographic methods clearly showed that method improvements are needed with respect to the recovery, accuracy, and precision of the measurements. The main problems are derived from matrix interferences that induce enhancement of the trichothecene response (up to 120%) [11].

HPLC with fluorescence detection generally gives high sensitivity, selectivity and repeatability of measurements. Several specific labelling reagents are currently available for hydroxyl groups to form fluorescent esters [12–17]. However, their application to trichothecene analysis failed due to either low reactivity or the presence of interfering peaks in the chromatogram [17]. Recently, a method for the determination of T-2, HT-2 toxin, neosolaniol and diacetoxyscirpenol in cereal cultures of *Fusarium sporotrichioides* by solid-phase extraction (SPE) column clean-up and HPLC with fluorescence detection has been reported [17,18]. The method was based on derivatization with coumarin-3-carbonyl chloride, a laboratory-synthesized reagent previously reported by Cohen and Boutin-Muma in the esterification of T-2, HT-2 toxin, T-2 triol and T-2 tetraol [19]. Although the method had good sensitivity, its applicability to cereal samples showed low toxin recoveries [20].

The use of immunoaffinity columns for purification of extracts has already been applied with success to the analysis of several mycotoxins in various food matrices [21–25]. Rapid clean-up procedures and clean extracts due to the specificity of the antibody are obtained. The aim of this work was to find a commercially available fluorescent labelling reagent for T-2 and develop a sensitive, reproducible and accurate method for its determination in cereals by HPLC with fluorescence detection after immunoaffinity column clean-up and pre-column derivatization. A laboratory method validation and optimized conditions for the derivatization reaction are described.

2. Experimental

2.1. Chemicals and materials

Acetonitrile, methanol (both HPLC grade) and toluene (for organic residue analysis) were purchased from Mallinckrodt Baker (Milan, Italy). Ultrapure water was produced by a Millipore Milli-Q system (Millipore, Bedford, MA, USA). T-2 toxin (T-2) and 4-dimethylaminopyridine (DMAP) were purchased from Sigma–Aldrich (Milan, Italy) and 1-anthroyl cyanide (1-anthroylnitrile, 1-AN) from Wako (Neuss, Germany). T-2 immunoaffinity columns (T-2 TAG) were obtained from Vicam (Watertown, MA, USA). Glass microfibre filters (Whatman GF/A) and paper filters (Whatman No. 4) were obtained from Whatman (Maidstone, UK).

2.2. Preparation of standard and reagent solutions

T-2 stock solution was prepared by dissolving the

solid commercial toxin in acetonitrile (1 μg/μl). T-2 standard solutions for HPLC calibration or spiking purposes were prepared by dissolving adequate amounts of the stock solution, previously evaporated to dryness under a nitrogen stream, in acetonitrile.

DMAP and 1-AN solutions were prepared in toluene at concentrations of 0.325 and 0.3 μg/μl, respectively. T-2 and reagent solutions were stored at −20 °C and warmed to room temperature before use.

2.3. Apparatus

The HPLC apparatus consisted of a Perkin-Elmer (Norwalk, CT, USA) Series 200 LC binary pump equipped with a Rheodyne (Cotati, CA, USA) Model 7125 injection valve, a Jasco (Tokyo, Japan) FP-1520 fluorometric detector and a Turbochrom 4.0 data system (Perkin-Elmer). The analytical column was a C_{18} reversed-phase Symmetry (150×4.6 mm, 5 μm particles) (Waters, Milford, MA, USA) preceded by a Rheodyne guard filter (0.5 μm). The mobile phase consisted of a mixture of acetonitrile–water (80:20, v/v) eluted at a flow-rate of 1.0 ml/min. The excitation and emission wavelengths of the fluorometer were set at 381 and 470 nm, respectively.

2.4. Fluorescence labelling of T-2 toxin

Two commercially available fluorescent reagents for modifying alcohols, 9-anthroylnitrile (9-AN) (Molecular Probes, Eugene, OR, USA) and 1-anthroylnitrile (1-AN), were tested for labelling T-2 in order to make it detectable by HPLC with fluorometric detection. Due to its high efficiency for a variety of acylation reactions, the base DMAP was used as catalyst for the reaction.

The derivatization conditions were optimized by investigating different reaction solvents, reagent molar ratios, temperatures and reaction times. In order to prevent possible hydrolysis, aprotic solvents such as methylene chloride, acetonitrile and toluene were investigated at different reaction temperatures and times. Reactions were carried out with methylene chloride at 25 °C, with acetonitrile at 25 and 50 °C and with toluene at 25, 50, 70 and 80 °C in the range from 1 min to 3 h (triplicate experiments). These experiments were performed with a reagent molar ratio of 1:10:20 (T-2/1- or 9-AN/DMAP).

In order to establish the stability of 1-AN in toluene, 0.1 μg T-2 was reacted (as reported in Section 2.6) with 1-AN solutions stored in amber vials at different temperatures (−20, 4 and 25 °C) for different periods. To test the stability of the T-2 derivative, different amounts of T-2 (from 0.1 to 1.5 μg) were derivatized with 1-AN and the reaction mixture was reconstituted in mobile phase and stored in the dark and under light at room temperature. At selected time intervals, 20 μl of the reaction mixture was injected into the HPLC apparatus.

2.5. Sample preparation and immunoaffinity clean-up

Fifty grams of cereal samples (wheat, corn, barley, oats, rice and sorghum), finely ground by a Model MLI-204 Bühler mill (Bühler, Milan, Italy), were weighed into a blender jar and extracted with 100 ml methanol–water (80:20, v/v) by blending at high speed for 2 min with a Sorvall Omnimixer. The mixture extract was centrifuged for 5 min at 3000 *g* at 4 °C and the supernatant was filtered through filter paper. Ten millilitres of the filtrate were collected and mixed with 40 ml distilled water. The diluted extract was filtered through a glass microfibre filter and the filtrate collected. For barley, an additional centrifugation step (5 min, 3000 *g*) was necessary before filtration through the glass microfibre filter. Ten millilitres of diluted extract (equivalent to 1.0 g sample) were passed through the T-2 immunoaffinity column at a flow-rate of about one drop per second, followed by 10 ml distilled water at one to two drops per second. T-2 was then eluted with 1.5 ml methanol and collected in a 4-ml screw-cap amber vial. The eluted extract was evaporated under a stream of air at ca. 50 °C in a heating block and the dried residue derivatized with 1-AN as reported below.

2.6. Derivatization procedure and HPLC determination

Fifty microlitres of DMAP solution followed by 50 μl of 1-AN reagent were added to the residue. The vial was closed and mixed by vortex for 1 min. The mixture was left to react for 15 min at 50 °C in a

heater block and then cooled in ice for 10 min. The whole volume of the reaction mixture was dried under a stream of air at ca. 50 °C and reconstituted with 1000 μl mobile phase. Twenty microlitres of reconstituted reaction mixture were injected into the chromatographic apparatus by a full loop injection system.

Quantification of toxin was performed by measuring peak areas at the T-2 derivative retention time, and comparing them with the relevant calibration curve in the range 0.01–1.5 μg/g T-2.

For calibration curve and linearity response of the T-2 derivative–peak area, aliquots of T-2 standard solutions (equivalent to 0.01–10 μg T-2) were placed in screw-cap amber vials and the solvent evaporated to dryness at about 50 °C under a stream of nitrogen before the derivatization reaction.

2.7. Identity of T-2 anthroyl ester

The identity of T-2 anthroyl ester [T-2-(1-AN)] was confirmed by HPLC–MS using a 1050-Ti chromatographic system and a UV detector set at 254 nm (Hewlett-Packard, Palo Alto, CA, USA) interfaced to an API 165 mass spectrometer equipped with a turbo-ionspray interface (Perkin-Elmer Sciex, Norwalk, CT, USA).

T-2-(1-AN) (derived from the reaction of 1 mg T-2) was purified and isolated using a Merck silica gel 60 preparative TLC plate (20×20, 500 μm) with toluene–acetone (88:12, v/v) as eluting solvent ($R_F = 0.60$). Aliquots of T-2 anthroyl ester solution were injected and eluted at a flow-rate of 1.0 ml/min through a Symmetry column (150×4.6 mm, 5 μm particles) in isocratic mode with an acetonitrile–ammonium acetate solution (10 m*M*) (80:20, v/v) as mobile phase. Splitting of the HPLC flow was performed to allow just 200 μl to enter the turbo-ionspray interface. The mass spectrometric conditions (positive chemical ionization) were: nebulizer gas (air) 1.5 l/min, curtain gas (nitrogen) 1.4 l/min, desolvation gas (nitrogen) temperature 300 °C, mass range 200–750 u, scan time 2 s, needle voltage 5500 V, ring voltage 220 V, and orifice voltages 20 or 180 V in two different experiments.

2.8. Immunoaffinity column capacity

The capacity of the T-2 immunoaffinity columns was determined by comparing (duplicate measurements) the amount of T-2 added to the immunoaffinity column with the amount bound. Different amounts of T-2, from 0.05 to 5.0 μg, were added to the immunoaffinity column by loading 10 ml (equivalent to 1.0 g matrix) of blank wheat diluted extract spiked with the corresponding amount of T-2.

2.9. Recovery experiments

Recovery experiments were performed in quadruplicate by spiking blank cereal samples with T-2 at levels of 0.05, 0.2, 0.5, 1.0 and 1.5 μg/g. Spiked samples were left overnight to allow solvent evaporation prior to sample extraction.

3. Results and discussion

3.1. Fluorescence labelling of T-2 toxin for HPLC analysis

Both 1- and 9-anthroylnitrile reacted with the hydroxyl group of T-2 under mild conditions to form the corresponding esters. The T-2-(1-AN) derivative gave a higher fluorescence intensity than the T-2-(9-AN) derivative (up to 15 times) in all experiments. Fig. 1 shows a scheme of the reaction of T-2 with 1-AN.

Excitation spectra of the T-2-(1-AN) derivative showed maximum excitation wavelengths at 238, 254 and 381 nm. The latter wavelength was chosen as the excitation wavelength due to its specificity. The maximum emission wavelength was found to be 470 nm.

The identity of the T-2-(1-AN) derivative (M_r 670.4) was determined by HPLC–MS (positive chemical ionization). At a low orifice voltage (20 V), major ions with m/z 671.4 $[M+H]^+$ (base peak) and m/z 205.3 $[M+H-(T\text{-}2)]^+$, and additional ions with m/z 693.3 $[M+Na]^+$ and m/z 688.2 $[M+NH_4]^+$, confirmed the formation of the T-2-(1-AN) derivative. At a higher orifice voltage (180 V), a single major peak (m/z 205.3) due to declustering of the anthroylnitrile fragment was observed (see Fig. 1).

Toluene was selected as the best reaction solvent due to the highest fluorescence intensity of the peak relevant to the T-2-(1-AN) derivative under the same experimental conditions. The reaction rate increased

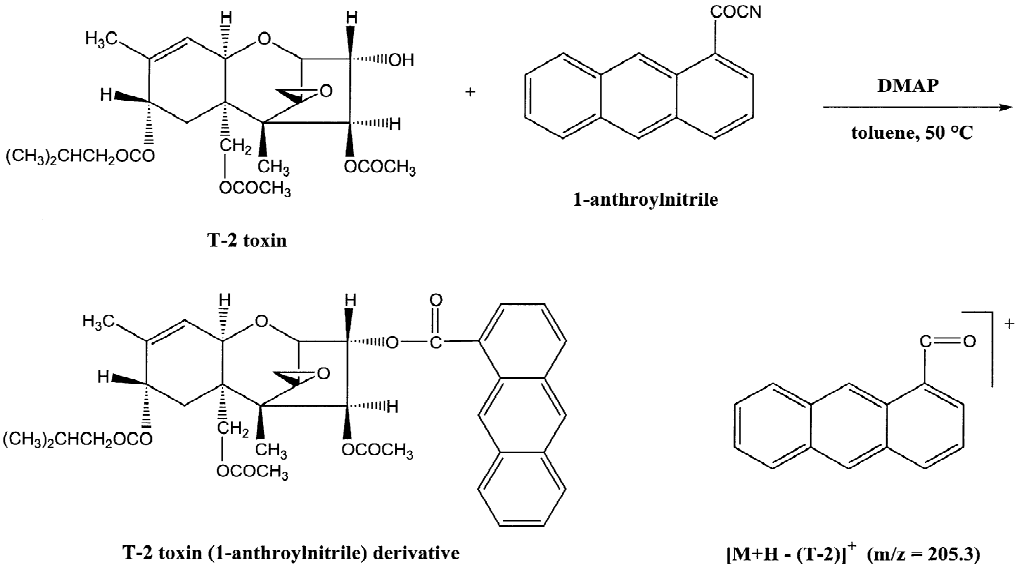

Fig. 1. Scheme of the derivatization reaction of T-2 toxin with 1-anthroylnitrile. The major ion of the T-2 toxin (1-anthroylnitrile) derivative with m/z 205.3, obtained by HPLC–MS (positive chemical ionization), is shown at the bottom right.

with increasing temperature and time, although, at 80 °C, a splitting of the peak relevant to the derivative was observed, suggesting possible decomposition of the derivative. The optimum reaction temperature and time were 50 °C and 15 min, respectively. A longer time did not increase the peak intensity. Similar results were also obtained at 25 °C when the reaction was left for 45 min. No difference in fluorescence intensity was observed when anhydrous toluene was used. The use of ultra-high-purity toluene (i.e. for organic residue analysis) improved the chromatogram profile, whereas additional and interfering peaks were observed with ACS toluene and toluene for HPLC (J.T. Baker).

After testing different molar ratios of toxin–derivatizing reagent–catalyst (triplicate experiments) to improve the reaction yield, the optimum conditions in terms of sensitivity and repeatability of the derivatization reaction were found with a ratio of 1:20:40 (T-2/1-AN/DMAP). Under these conditions, no purification of the reaction mixture was necessary before injection into the HPLC apparatus. Previous studies showed that an excess of reagent and base with respect to the toxin was necessary to increase the reactivity [20].

Fig. 2 shows chromatograms for the ''blank'' reaction mixture (containing only 1-AN and DMAP) and the mixture from the derivatization reaction of 0.5 μg of T-2. The peak relevant to the T-2-(1-AN) derivative was well resolved from unreacted reagents and impurities derived from the labelling reaction.

A good linearity response (peak area–injected amount) was observed in the range 0.01–10.00 μg of derivatized T-2 ($r = 0.9993$). The repeatability (relative standard deviation) of the reaction was less than 3% ($n = 4$) for the derivatization of different amounts of T-2 (from 0.01 to 1 μg). The lower detectable amount of derivatized T-2 was 5 ng (signal-to-noise ratio 3:1).

Experiments to test the stability of 1-AN in toluene showed no significant difference in the peak intensity of T-2-(1-AN) derivatives up to 1 week after preparation of the solution and storing at different temperatures (−20, 4 and 25 °C). A decrease in peak intensity (about 20%) was observed when the solution was kept at room temperature for 8 days after preparation. Reagent solutions were stable for 1 week at room temperature and for at least 1 month at +4 °C and 1 year at −20 °C after preparation.

Experiments to test the stability of T-2 anthroyl ester showed no decrease in the fluorescence intensity of the T-2 derivative up to 5 days for solutions stored in the dark and under light. The high stability

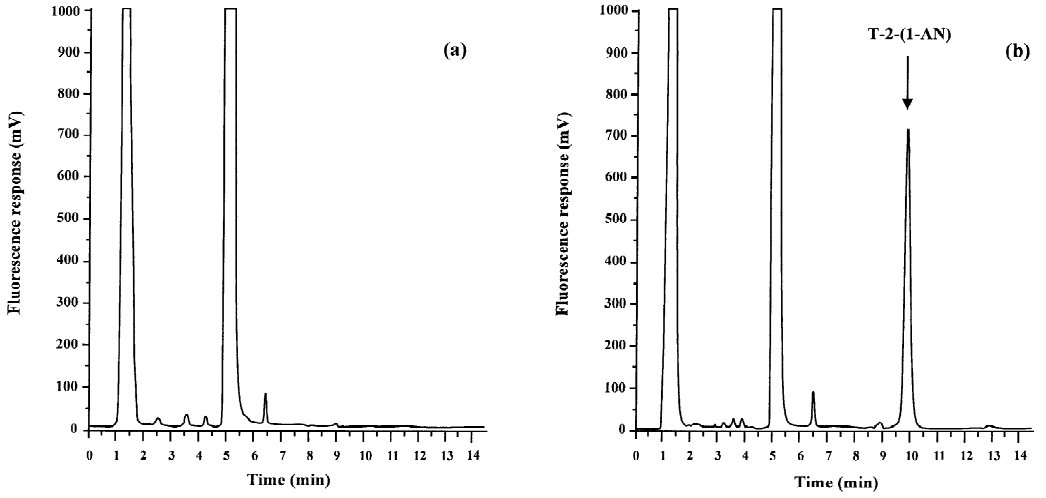

Fig. 2. Chromatograms for (a) a ''blank reaction mixture'' (1-AN + DMAP) and (b) a mixture from a derivatization reaction with 0.5 μg of T-2 toxin. Chromatographic conditions are reported in Sections 2.3 and 2.6.

of the derivatives allows the use of automatic samplers coupled with HPLC for the analysis of a large number of samples.

3.2. Analytical method for T-2 toxin in cereals

The developed derivatization procedure was applied to the determination of T-2 in different cereal grains (wheat, corn, barley, oats, rice and sorghum) after purification of the extracts by immunoaffinity columns. Immunoaffinity columns specific for T-2 determination (T-2 TAG), commercially available from Vicam and designed for using with a fluorometer, were used in the procedure for T-2 determination in cereals by HPLC–fluorescence detection.

Methanol–water (80:20, v/v) was selected for the best performance for the extraction of T-2 from the various cereals analyzed in this study, after testing different methanol–water ratios (35:65, 50:50 and 80:20, v/v). Acetonitrile–water (84:16, v/v) or methanol–water in different volumetric ratios are usually used for the extraction of trichothecenes from grains [1,4]. Generally, the use of acetonitrile in the extraction mixture limits the amount of extract that can be loaded onto the immunoaffinity column in a reasonable time due to denaturation of the antibody in the presence of low levels of acetonitrile (3–5%) compared with methanol (15–20%). Consequently, methanol–water was chosen as the extraction solvent, also due to its reduced potential toxicity.

A dilution factor of 1:5 (v/v) with distilled water before loading the extract onto the immunoaffinity column was found satisfactory, providing good sensitivity and accuracy. Lower recoveries were found when a dilution factor of 1:1 or 1:2 was used instead of 1:5 in an attempt to increase the sensitivity of the method, whereas no difference was found with a dilution factor of 1:10. To evaluate the performance of the immunoaffinity column with respect to matrix interferences, different volumes of diluted blank wheat extract (1:5, v/v), spiked with 1 μg/g T-2, were loaded onto the column (triplicate measurements). Optimal conditions were found with 10 ml of diluted extract. A slowing of extract elution and a reduction in recoveries were observed when larger volumes (up to 20 ml) were loaded onto the column.

The capacity of T-2 columns was found to be about 1.6 μg of T-2. Above this level, no increase in the fluorescence response was observed, indicating the saturation of T-2 binding sites. T-2 recoveries from the column below the saturation level were greater than 93%. Considering the column capacity, the range of applicability of the method for different cereals was from 0.005 to 1.5 μg/g of T-2.

Results of the recovery experiments (quadruplicate measurements) for the full analytical procedure carried out on different cereal grains spiked with T-2

Table 1
Recoveries from blank cereals spiked with T-2 toxin at different levels

Spiking level (μg/g)	Recovery, % (RSD, %)					
	Wheat	Corn	Barley	Oats	Sorghum	Rice
0.05	98.5 (5.7)	90.4 (1.5)	98.7 (2.7)	90.5 (4.1)	80.6 (1.2)	95.7 (1.5)
0.2	90.4 (3.1)	81.3 (5.6)	96.0 (2.8)	83.0 (2.2)	82.8 (2.2)	88.7 (0.8)
0.5	91.3 (2.7)	82.8 (4.7)	94.6 (3.1)	82.0 (1.6)	80.6 (1.3)	94.8 (5.1)
1.0	94.2 (2.8)	80.4 (5.7)	93.0 (2.2)	86.0 (3.6)	82.4 (4.5)	85.7 (3.3)
1.5	87.9 (0.8)	82.5 (4.8)	88.7 (4.6)	85.5 (2.9)	80.4 (5.3)	83.8 (4.3)

RSD, relative standard deviation ($n=4$ replicates).

at different levels are reported in Table 1. Within the spiking range 0.05–1.5 μg/g, the average recoveries ranged from 80 to 99% with a within-laboratory relative standard deviation (RSD_r) of less than 6% for all spiking levels. The limit of detection of the method was 0.005 μg/g, based on a signal-to-noise ratio of 3:1. Recovery and precision data were good for all cereals, especially for wheat and barley. However, for barley, an additional centrifugation step was necessary before immunoaffinity clean-up because of the formation of a cloudy suspension after dilution of the extract with water. Recovery and repeatability values of the method are much better than those established by the CEN (European Committee for Standardization) working group ''Biotoxin'' for criteria for the acceptance of a analytical method for T-2, i.e. recoveries between 60 and 120% and RSD_r <40% for T-2 concentrations in the range 0.05–0.25 μg/g, and recoveries between 60 and 110% and RSD_r <30% for concentrations >0.25 μg/g [26].

Chromatograms of a blank wheat sample and of the same sample spiked with T-2 at levels close to those found in naturally contaminated wheat are shown in Fig. 3. Similar chromatogram profiles were obtained for corn, barley, oats, rice and sorghum extracts.

With respect to the HPLC method using coumarin-3-carbonyl chloride (synthesized in the laboratory) and SPE clean-up for the determination of type-A trichothecenes [17–20], the present method has the advantage of using a commercially available labelling probe giving a stable and highly fluorescent derivative under mild reaction conditions. Moreover,

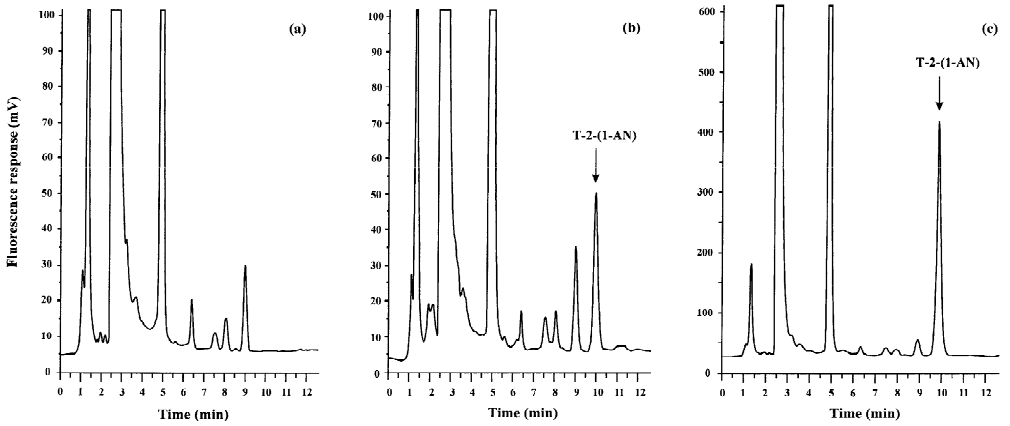

Fig. 3. Chromatograms of wheat samples: (a) blank (<0.005 μg/g T-2); (b) blank sample spiked with T-2 at 0.05 μg/g (T-2 found 0.048 μg/g) and (c) 0.5 μg/g (T-2 found 0.450 μg/g). Peaks due to residual 1-AN and its corresponding hydrolysis product occur at retention time of about 1 and 5 min. Chromatographic conditions are reported in Sections 2.3 and 2.6.

the previously reported method showed low T-2 recoveries (54.6–69.5%) when applied to cereal samples [20], not fulfilling CEN criteria for T-2 method acceptability [26]. The use of immunoaffinity columns gives cleaner extracts, due to the specificity of the antibody, and a rapid clean-up procedure, making analysis less time-consuming.

At present, no official method for the determination of T-2 is available, although some methods have been tested in comparative studies [11,27]. Mycosep column (Romer Labs., Washington, USA) clean-up and GC–ECD or GC–MS is the technique commonly used for quantification of trichothecenes, including T-2; however, this technique is not reliable in terms of accuracy, repeatability and reproducibility. The present method allows the determination of T-2 at ng/g (ppb) levels in various cereals with good accuracy and precision and is appropriate for quantifying the toxin at levels that can occur in naturally contaminated cereal samples.

4. Conclusions

1-AN has been shown to be an efficient labelling reagent for the determination of T-2 by HPLC–fluorescence detection due to its high reactivity towards hydroxyl groups. The conditions of the derivatization reaction have been optimized to obtain a stable fluorescent T-2 derivative. The use of immunoaffinity columns for extract clean-up allows the purification of T-2 from different cereal grain extracts. The proposed HPLC method uses commercially available products, is simple to perform and shows laboratory performances superior to other published methods for the determination of T-2 in contaminated cereal samples in terms of sensitivity, accuracy and precision.

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References

[1] WHO Food Additives Series 47, FAO Food and Nutrition Paper 74, Presented at the 56th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), WHO, Geneva, 2001, p. 557.
[2] C.J. Mirocha, R.A. Pawlosky, K. Chatterjee, S. Watson, W. Hayes, J. Assoc. Off. Anal. Chem. 66 (1983) 1485.
[3] R. Krska, S. Baumgartner, R. Josephs, Fresenius J. Anal. Chem. 371 (2001) 285.
[4] M.W. Trucksess, A.E. Pohland (Eds.), Mycotoxin Protocols, Methods in Molecular Biology, Vol. 157, Humana Press, Totowa, NJ, 2001, p. 115.
[5] J. Park, F. Chu, J. AOAC Int. 79 (1996) 465.
[6] L. Sibanda, S. De Saeger, C. Van Peteghem, J. Grabarkiewicz-Szczesna, M. Tomczak, J. Agric. Food Chem. 48 (2000) 5864.
[7] W. Langseth, T. Rundberget, J. Chromatogr. A 815 (1998) 103.
[8] R.C. Schothorst, A.A. Jekel, Food Chem. 73 (2001) 111.
[9] M. Schollenberger, U. Lauber, H.T. Jara, S. Suchy, W. Drochner, H.-M. Muller, J. Chromatogr. A 815 (1998) 123.
[10] U. Berger, M. Oehme, F. Kuhn, J. Agric. Food Chem. 47 (1999) 4240.
[11] H. Petterson, W. Langseth, BCR Information, Project Reports EUR 20285/1 EN (Part I) and EUR 20285/2 EN (Part II), European Commission, Brussels, 2002.
[12] A. Takadate, M. Irikura, T. Suehiro, H. Fujino, S. Goya, Chem. Pharm. Bull. 33 (1985) 1164.
[13] J. Goto, N. Goto, F. Shamsa, M. Saito, S. Komatsu, K. Suzaki, T. Nambara, Anal. Chim. Acta 147 (1983) 397.
[14] M. Takahashi, M. Nagashima, S. Shigeoka, M. Nishijima, K. Kamata, J. Chromatogr. A 775 (1997) 137.
[15] I. Kubo, S. Komatsu, J. Chromatogr. 362 (1986) 61.
[16] A. Metori, A. Ogamo, Y. Nakagawa, J. Chromatogr. 622 (1993) 147.
[17] M. Jiménez, J.J. Mateo, R. Mateo, J. Chromatogr. A 870 (2000) 473.
[18] J.J. Mateo, R. Mateo, M. Jiménez, Int. J. Food Microbiol. 72 (2002) 115.
[19] H. Cohen, B. Boutin-Muma, J. Chromatogr. 595 (1992) 143.
[20] J.J. Mateo, R. Mateo, M.J. Hinojo, A. Llorens, M. Jiménez, J. Chromatogr. A 955 (2002) 245.
[21] P.M. Scott, M.W. Trucksess, J. AOAC Int. 80 (1997) 941.
[22] A. Visconti, M. Pascale, J. Chromatogr. A 815 (1998) 133.
[23] A. Visconti, M. Pascale, G. Centonze, J. Chromatogr. A 864 (1999) 89.
[24] A. Visconti, M. Pascale, G. Centonze, J. Chromatogr. A 888 (2000) 321.
[25] M. Solfrizzo, A. De Girolamo, A. Visconti, Food Addit. Contam. 18 (2001) 227.
[26] CEN (European Committee for Standardization), TC275-WG5, Biotoxin, Doc. No. 195, CEN Report CR 13505, Brussels, 1999.
[27] S. De Saeger, L. Sibanda, A. Desmet, C. Van Peteghem, Int. J. Food Microbiol. 75 (2002) 135.