Available online at www.sciencedirect.com
SCIENCE DIRECT®
Talanta 66 (2005) 194–201
Talanta
www.elsevier.com/locate/talanta

Comparative assessment of solid-phase extraction clean-up procedures, GC columns and perfluoroacylation reagents for determination of type B trichothecenes in wheat by GC–ECD

Francisco M. Valle-Algarra[a], Angel Medina[b], José V. Gimeno-Adelantado[a], Amparo Llorens[b], Misericordia Jiménez[b,*], Rufino Mateo[a]

[a] *Departamento de Química Analítica, Facultad de Química, Universidad de Valencia, Dr. Moliner 50, E-46100 Burjasot, Valencia, Spain*
[b] *Departamento de Microbiología y Ecología, Facultad de Biología, Universidad de Valencia, Edificio de Investigación, Despacho 3.74, Dr. Moliner 50, E-46100 Burjassot, Valencia, Spain*

Received 25 June 2004; received in revised form 27 September 2004; accepted 16 November 2004
Available online 1 January 2005

Abstract

Various solid-phase extraction (SPE) procedures for clean-up, two perfluoroacylation reagents (pentafluoropropionic anhydride (PFPA) and heptafluorobutyric anhydride (HFBA)) and two chromatographic columns (HP-1701 and HP-5) have been assessed comparatively to achieve the determination of type B trichothecenes (deoxynivalenol (DON), nivalenol (NIV), 3- and 15-acetyldeoxynivalenol (3- and 15-ADON)) in wheat grain by gas chromatography (GC)–electron-capture detection (ECD). Spiked wheat samples were extracted with acetonitrile–water (84:16, v/v). Tested SPE procedures were MycoSep 225 column, Florisil and different cartridges prepared in the laboratory with mixtures of various sorbents like alumina, Celite 545, C18, silica and charcoal. We propose MycoSep 225 column, and cartridges made with alumina–charcoal–silica and alumina–charcoal–C18 silica mixtures as clean-up procedures on the basis of recovery values (89.6, 87.3 and 86.1% for deoxynivalenol, respectively, at 1.0 mg/kg spiking level). The two last procedures are less expensive. Pentafluoropropionic anhydride was more stable against moisture and less expensive, while recoveries were similar to those obtained with heptafluorobutyric anhydride. HP-1701 column can separate 3- and 15-acetyldeoxynivalenol derivatives while HP-5 cannot, although this last column provided lower bleed and better sensitivity.

Keywords: Deoxynivalenol; Derivatization; Gas chromatography; Mycotoxins; Nivalenol; Perfluoroacyl derivatives; Solid-phase extraction; Trichothecenes

1. Introduction

Mycotoxins are secondary metabolites produced by certain filamentous fungi. Trichothecenes are sesquiterpenoid mycotoxins, mainly produced by various species of *Fusarium* such as *Fusarium graminearum*, *Fusarium culmorum* or *Fusarium equiseti* [1,2]. Trichothecenes are subdivided into four different groups named using letters from A to D [3,4]. The diversity of trichothecenes leads to a wide range of toxic effects in animals and humans such as feed refusal, vomiting, hemorrhage, anemia and immunosuppression, reduced weight gain, emesis and diarrhoea [5–7]. They are potent inhibitors of protein synthesis that can predispose animals to other diseases and mask the underlying toxicoses [8]. Deoxynivalenol (DON) is the most frequently detected trichothecene in grain samples such as wheat, corn and barley, but nivalenol (NIV), 3- and 15-acetyldeoxynivalenol (3- and 15-ADON) can be also found [9–11]. These mycotoxins are classified as B-type trichothecenes.

Analysis for trichothecenes is a complicated work if specific detection of low levels of the toxins is needed. Sensitivity

* Corresponding author. Tel.: +34 96 3543144; fax: +34 96 3543099.
E-mail address: misericordia.jimenez@uv.es (M. Jiménez).

0039-9140/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.11.007

and accuracy of these methods depend on the ability to isolate toxins selectively from interfering matrix compounds present in natural samples prior to detection. Clean-up procedures are usually used for toxin isolation. They include solid-phase extraction (SPE) with various mixtures of solid phases like charcoal–alumina–Celite 545 [12–14], cation exchange resin [15], alumina–charcoal [16,17], C18 [18], MycoSep 225 column [19–21] and Florisil [22–25]. Supercritical fluid extraction, gel permeation chromatography and immunoaffinity columns have also been used [26–29].

Current reported separation or detection methods for analysis of type B trichothecenes include enzyme-linked immunosorbent assay [30], radioimmunoassay [31], fluorescent minicolumn [32], thin-layer chromatography [33], liquid chromatography (LC) or gas chromatography (GC).

Several authors described various LC analytical methods including direct analysis using ultraviolet detection [34,35], fluorescence detection after derivatization with coumarin-3-carbonyl chloride [36] and post-column derivatization with sodium hydroxide, ammonium acetate and methyl acetoacetate [37]. GC has been the most widely used technique for trichothecene analysis, but it requires derivatization of free hydroxyl groups [38], although methods avoiding derivatization have been published [39]. Two groups of derivatives have been applied, the trimethylsilyl ethers and the perfluoroacyl esters. The fluorinated derivatives can be formed by reaction with pentafluoropropionyl imidazole and heptafluorobutyryl imidazole, trifluoroacetic anhydride, pentafluoropropionic anhydride (PFPA) or heptafluorobutyric anhydride (HFBA) [38,40]. The fluorinated derivatives can be detected selectively at very low levels using negative ion chemical ionization-mass spectrometry or electron-capture detection (ECD) but also positive electron impact ionization or positive chemical ionization have been used [41–43].

Due to the relevance of these mycotoxins in food and feed, the aim of this work was to perform a comparative study of different clean-up and derivatization procedures, and GC columns to find a suitable method for determination of these metabolites in a large number of wheat samples. Owing to the variety of existing methods, it is necessary their evaluation and discussion in order to choose the most suitable, easy-to-use, rapid, accurate, and specific method.

2. Experimental

2.1. Chemicals and reagents

Trichothecene standards, including NIV, DON, 15- and 3-ADON were supplied by Sigma (Sigma–Aldrich, Alcobendas, Spain). Toluene was purchased from Panreac (Montcada i Reixac, Barcelona, Spain). Acetonitrile, methanol, chloroform and dichloromethane were purchased from J.T. Baker (Deventer, Holland). All solvents were HPLC grade. Standardized 70–230 mesh aluminium oxide 90 (0.063–0.2 mm particle size) was purchased from Merck (Darmstadt, Germany). C18, Florisil and silica were purchased from Waters (Milford, MA, USA). HFBA, Celite 545 and activated charcoal (Norit) were purchased from Fluka (Sigma–Aldrich, Alcobendas, Spain). Glass microfibre filters (GF/C) and filter papers (Whatman No 4) were from Whatman (Maidstone, UK). PFPA and 4-dimethylaminopyridine (DMAP) were also purchased from Sigma. Pure water was obtained from a Milli-Q apparatus (Millipore, Billerica, MA, USA) and was used when water was required.

2.2. Preparation of standard solutions

Each standard of NIV, DON, 3- and 15-ADON was dissolved in acetonitrile at a concentration of 1.0 mg/ml and stored at −20 °C in a sealed vial until use. Working standards (10.0, 2.0, 1.0, 0.5, 0.25, 0.1, 0.05 and 0.02 μg/ml) were prepared by appropriate dilution of known volumes of the stock solution with acetonitrile and used to obtain calibration curves after derivatization and injection in the chromatographic system.

2.3. Equipment

Rotary evaporator was LaboRota S300 (Resona Technics, Germany) equipped with a refrigeration system Selecta Frigiterm-10 (J.P Selecta, Barcelona, Spain) and a vacuum system Büchi Vac-V500, regulated with a V-800 vacuum controller (Büchi Labortechnik, Flawil, Switzerland). The Ultraturrax T25 high-speed blender and the laboratory mill A-10 were from IKA (Stauffen, Germany).

The GC system was composed of a HP-6890 plus gas chromatograph, equipped with a ^{63}Ni ECD (Hewlett-Packard, Avondale, PA, USA) and an Agilent 7683 Series injector (Agilent Technologies, Waldbronn, Germany). Signals were processed by HP GC ChemStation software Version A.07:01(682) (Hewlett-Packard).

2.4. Extraction

Fifty grams of wheat sample, previously found to be free from type B trichothecenes using the methodology of Mateo et al. [24], was finely ground with a laboratory mill and 5 g of flour was poured into a 100-ml Erlenmeyer flask. Fortification of samples at 0.1 or 1.0 mg mycotoxin/kg level was accomplished at this point by adding appropriate aliquots of each mycotoxin standard solution. Solvent was let to evaporate for 15 min. Then, the flask was capped and shaken carefully to distribute toxins evenly. After adding 40 ml of acetonitrile–water (84:16, v/v), the mixture was blended in a high-speed blender for 10 min. After filtering through Whatman No. 4 filter, the extraction mixture was stored in a tightly closed glass bottle at −20 °C until use.

2.5. SPE clean-up

The following SPE clean-up procedures were assayed.

2.5.1. MycoSep 225 column

Five milliliters of the filtrate sample extract in acetonitrile–water (84:16, v/v) was placed into a MycoSep 225 tube (Romer Laboratories, Union, MO, USA) [21]. The extract was cleaned according to the instructions of the manufacturer. The rubber flange end of the clean up column was pushed slowly in the tube, creating a tight seal between the rubber flange and the glass wall of the culture tube. As the column was pushed harder into the tube, the extract was carefully forced through the frit, one-way valve and packing material. One-milliliter volume of the purified extract was transferred to a vial and concentrated to dryness at 45 °C under gentle stream of nitrogen.

2.5.2. Florisil cartridge

A previous procedure [25] was slightly modified (hexane was used instead of light petroleum). A 30-ml aliquot of filtrate sample extract was loaded into a separatory funnel and was defatted with hexane (3 × 5 ml). Hexane layers were discarded. The defatted sample extract was evaporated in rotary evaporator at 40 °C. The residue was redissolved in 2 × 1 ml of dichloromethane by vortexing for 1 min and transferred to a Florisil cartridge (Waters) that was preconditioned with 2 ml of chloroform–methanol (7:3, v/v) followed by 4 ml of dichloromethane. Trichothecenes were eluted with 5 ml of chloroform–methanol (7:3, v/v) and collected in a 10 ml screw cap vial. The eluate was concentrated to dryness under gentle stream of nitrogen as indicated in Section 2.5.1.

2.5.3. Alumina–charcoal–Celite 545 mixture

'Made-in-laboratory' cartridges were prepared using 5-ml sterile plastic syringes. A glass microfibre filter was placed at the bottom. Then, a mixture of packing bed was poured on it. It was made of a first layer of 0.1 g of Celite 545 and a second layer of 1.5 g of a homogenised mixture containing alumina–charcoal–Celite 545 (5:7:3, w/w/w). Another glass microfibre filter was placed on the bed top. Then, it was pressed tightly but carefully with a plunger. A 30-ml aliquot of filtrate sample extract was loaded into a separatory funnel and defatted with hexane (3 × 5 ml) [13]. The hexane layers were discarded and the defatted sample extract was passed through the prepared cartridge. Most of trichothecenes went off with the solvent while interfering substances were retained in the cartridge. The filtrate was collected in a flask. Then, the cartridge was rinsed with 20 ml of acetonitrile–water (84:16, v/v) to elute the trichothecenes remaining in the solid phase. The purified extract and the rinsing liquid were combined and evaporated at 40 °C in rotary evaporator. The residue was redissolved in 2 × 1 ml of acetonitrile–water (84:16, v/v) and transferred to a 4-ml vial. The extract was concentrated to dryness under nitrogen as described in Section 2.5.1.

2.5.4. Alumina–charcoal

A solid-phase extraction cartridge was prepared as indicated previously (see Section 2.5.3), but the packed material consisted of 1.015 g of a homogeneous mixture containing alumina–charcoal (100:15, w/w). Mixtures of these materials were previously used but the ratios were 75:70 (w/w) [16] and 100:3 (w/w) [17]. Three milliliters of sample extract was passed through the prepared column. The filtrate was collected in a vial. Then, the cartridge was rinsed with 2 ml of acetonitrile–water (84:16, v/v) and the rinsing liquid was combined with the filtrate. The rinsing volume was slightly different from the volume used in the literature [16,17]. The mixture was concentrated to dryness under nitrogen as indicated in Section 2.5.1.

2.5.5. Alumina–charcoal–C18 silica

Three different procedures were tested. Cartridges were prepared as indicated previously (Section 2.5.3), but the packed material consisted of:

(a) 1.18 g of alumina–charcoal–C18 silica (75:3:40, w/w/w) [44,45]. Three milliliters of sample extract was passed through the cartridge. The cleaned filtrate was collected in a vial and concentrated to dryness as indicated in Section 2.5.1.
(b) 1.16 g of alumina–charcoal–C18 silica (75:1:40, w/w/w). Five milliliters of sample extract was passed through the cartridge. The cleaned filtrate was collected in a vial and concentrated to dryness as indicated previously.
(c) The same mixture indicated in the preceding paragraph (b). Three milliliters of sample extract was passed through the prepared cartridge and collected in a vial. The cartridge was rinsed with 2 ml of acetonitrile–water (84:16, v/v). The eluate was collected in the same vial and the purified extract was concentrated to dryness in the usual way (Section 2.5.1).

2.5.6. Alumina–charcoal–silica

A cartridge was prepared as indicated previously (Section 2.5.3), but the packed material consisted of 1.55 g of a homogeneous mixture containing alumina–charcoal–silica (90:1.5:5, w/w/w). Three milliliters of sample extract was passed through the prepared cartridge and collected in a vial. Then, the cartridge was rinsed with 2 ml of acetonitrile–water (84:16, v/v). These liquids were combined in the same vial and concentrated to dryness in the usual way (Section 2.5.1).

2.6. Derivatization for GC–ECD determination

One hundred microliters of a 2 mg/l solution of DMAP in toluene–acetonitrile (80:20, v/v) and 50 μl of HFBA (or PFPA) were added to each dry extract in a screw cap vial. After capping tightly, the reaction mixture was heated at 60 °C for 60 min in an aluminium heater block. After the mixture had cooled, 1 ml of a 3% (w/v) aqueous solution of $NaHCO_3$ was added and the vial was vortexed for 15 s. The two layers were allowed to separate. The top (organic phase) layer was transferred to a GC autoinjector vial and analyzed by GC–ECD as pointed out below.

2.7. GC–ECD analysis

The GC–ECD determination was carried out using two different sets of chromatographic conditions. In both cases, 1.0 μl of solution was injected in splitless mode. The temperatures of the injection port and the detector were 250 and 300 °C, respectively. The first procedure used a fused-silica capillary column HP-5 [5% methyl phenyl siloxane (30 m × 0.32 mm i.d., 0.25 μm film thickness, Agilent Technologies)]. The oven temperature program was: 90 °C held for 1 min, 40 °C/min to 160 °C, 1.5 °C/min to 182 °C, 5 °C/min to 240 °C, and then 40 °C/min to 275 °C, held for 2 min. Helium at a constant pressure of 42.1 kPa was used as carrier gas. The second procedure used a fused-silica capillary column HP-1701 [14% cyanopropyl phenyl methyl polysiloxane (30 m × 0.25 mm i.d., 0.15 μm film thickness, Agilent Technologies)]. The oven temperature program was 90 °C held for 1 min, 40 °C/min to 160 °C, 5 °C/min to 173 °C, 2 °C/min to 195 °C, 5 °C/min to 240 °C, and then 40 °C/min to 270 °C held for 1 min. Helium at a constant pressure of 103 kPa was used as carrier gas.

3. Results and discussion

3.1. SPE clean-up procedures

The clean-up methods for type B trichothecenes in wheat that were tested in this work, provided very different recoveries depending on the procedure and the type of SPE cartridge. The results are in Table 1. It can be observed that the best recoveries were obtained with MycoSep 225, alumina–charcoal–C18 silica (procedure (c)) and alumina–charcoal–silica cartridges. Following these protocols, the extract can be transferred directly to the vial for concentration and further derivatization, without other previous step. Consequently, this process is usually relatively easy and fast. Among these three procedures, the MycoSep 225 column provided good results in terms of recovery for DON, 3- and 15-ADON. In the case of NIV, the best results were obtained with the alumina–charcoal–C18 silica cartridge following procedure (c) (i.e. with cartridge rinsing). The packing material of MycoSep 225 contains several sorbents including charcoal, Celite 545, and ion-exchange resin. Other authors who used this type of column [21] reported 88 and 70% recovery rates in a wheat sample spiked with 0.400 mg/kg of DON and NIV, respectively, which are similar to our data. The main disadvantage of this column with respect to 'made-in-laboratory' cartridges containing alumina–charcoal–C18 silica or alumina–charcoal–silica is its high cost. When 'made-in-laboratory' cartridges were used, recoveries varied between roughly 73–87% for DON, 102–110% for NIV and 58–70% for 3- and 15-ADON depending on the applied clean-up procedure, and reproducibility was similar to that obtained with MycoSep 225 column. Other authors [44,45] have reported higher recovery rates (nearly 100%) for DON in barley samples spiked at 0.5 and 1.0 mg/kg using alumina–charcoal–C18 silica.

Of the three studied clean-up procedures carried out with alumina–charcoal–C18 silica cartridges (Section 2.5.5a–c), the best recoveries (closest to 100%) were obtained by procedure (c), obtaining acceptable repeatability (R.S.D. = 9.7–11.1%) and less interfering peaks on the baseline.

Trichothecene signals appeared when cartridges containing alumina and charcoal (but no Celite 545) were used. Quite good results were obtained using alumina–charcoal–Celite 545 (5:7:3, w/w/w) cartridge with previous defatting of extract with hexane. After removing fat components from the matrix and after loading the sample, trichothecenes were eluted off the cartridge, although not completely. The elution was carried out with a great amount of solvent (acetonitrile–water, 84:16, v/v), which provokes co-elution of other compounds that can interfere. In addition, solvent evaporation of eluate in rotary evaporator should be made at very low pressure due to its high water content, which might lead to analyte losses. Other authors using alumina–charcoal–Celite 545 cartridge with defatting [14] in a corn extract fortified with 80 ng of mycotoxin/ml extract reported to obtain recovery rates of 94, 94, and 81% for DON, NIV, and 3-ADON, respectively.

As in the previous case, a considerable amount of water has to be evaporated before passing the extract through the Florisil cartridge. In spite of it, recoveries for 3- and 15-ADON were comparable to those obtained with alumina–charcoal–C18 silica or alumina–charcoal–silica cartridges. Nevertheless, low recovery (average 55.1%) was obtained for DON. Analyses carried out by other authors [23] in barley samples spiked with 67 μg of DON and 3-ADON/g using Florisil show recovery values of 75.8 and 82%, respectively.

High recoveries for all analytes (>100%) were obtained with the cartridge that contained alumina–charcoal, but interfering peaks were also obtained along with the trichothecenes, which led to very high recoveries, especially in the case of DON. This mixture was unsuitable for clean-up of these compounds. Some authors [16] who used this packing material found recovery values of 108, 60, 65 and 69% for DON, NIV, 3- and 15-ADON, respectively, in corn spiked with 0.250 mg toxin/kg.

The SPE procedures that can be chosen in terms of best reliability, low cost and less analysis time are those involving alumina–charcoal–C18 silica (procedure (c)) and alumina–charcoal–silica cartridges. Both methods have been compared, by further experiments involving trichothecene recovery determination. Results obtained in these experiments are shown in Tables 1 and 2. These two SPE cartridges were compared using a HP-1701 column after derivatization with PFPA of a wheat sample that had been spiked with 1.0 and 0.1 mg of each trichothecene/kg sample (Tables 1 and 2). The results of Table 2 are very similar to those shown in Table 1. Nevertheless, more irregular

Table 1
Influence of the clean-up procedure on the recovery of type B trichothecenes (spiked at 1.0 mg/kg level) in wheat using HP-5 column

SPE method	Trichothecene							
	DON		NIV		15-ADON		3-ADON	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
MycoSep 225 column	89.6	8.3	75.6	7.8	75.1	8.9	72.6	9.4
Florisil cartridge	55.1	16.0	70.7	21.3	68.2	10.4	66.6	8.0
Alumina–charcoal–Celite 545 (5:7:3, w/w/w) (with previous defatting of extract)	42.2	34.4	47.3	16.2	29.0	14.3	21.8	16.7
Alumina–charcoal	131.9	58.0	121.0	25.5	103.9	28.5	108.5	43.3
Alumina–charcoal–C18 silica								
(a)	72.8	10.2	102.7	11.9	64.1	10.6	61.3	12.4
(b)	81.0	9.6	110.3	13.2	57.6	14.1	59.7	14.0
(c)	86.1	11.1	101.8	9.8	69.4	11.0	65.3	9.7
Alumina–charcoal–silica	87.3	19.3	105.7	20.8	69.9	14.9	68.4	19.4

Only one mycotoxin was spiked in each recovery experiment. Derivatization was carried out with PFPA ($n = 5$). (a), (b) and (c): see the three different procedures described in Section 2.5.5 for this type of packing material. ND, below detection limit.

Table 2
Influence of selected SPE clean-up procedures on the recovery of type B trichothecenes (spiked at 1.0 and 0.1 mg/kg levels) in wheat using HP-1701 column

SPE cartridge	Spiking level (mg/kg)	Trichothecenes							
		DON		NIV		15-ADON		3-ADON	
		Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
Alumina–charcoal–C18 silica	1.0	82.6	9.6	98.4	8.4	64.2	18.5	76.8	7.4
	0.1	98.3	25.8	95.6	19.7	125.1	30.5	120.2	39.1
Alumina–charcoal–silica	1.0	98.4	23.0	109.3	10.9	67.0	21.3	85.9	26.8
	0.1	65.9	36.5	94.1	28.4	55.8	29.8	60.2	34.9

The sample was spiked with a mixture of all four mycotoxins. Derivatization was carried out with PFPA ($n = 5$).

baseline and greater number of neighbour peaks were noticed working with alumina–charcoal–silica cartridge. This behaviour caused that the R.S.D.s of trichothecene recoveries were higher than those attained when clean-up was done with alumina–charcoal–C18 silica cartridge. Table 2 shows that at 0.1 mg/kg spiking levels recoveries were higher (except for NIV) than values obtained at 1.0 mg/kg spiking level with the alumina–charcoal–C18 silica cartridge (procedure (c)).

Fig. 1 shows two chromatograms of a mixture of DON, NIV, and 3-ADON derivatized with HFBA (Fig. 1A) or PFPA (Fig. 1B) and separated using HP-5 column. Limits

Table 3
Limits of detection (LOD), coefficients of determination (r^2) and slopes of the linear calibration lines for type B trichothecenes as pentafluoropropionyl and heptafluorobutyryl derivatives separated by GC–ECD with HP-5 and HP-1701 capillary columns

Trichothecene	Column	Derivatization reagent					
		PFPA			HFBA		
		LOD (mg/kg)	r^2	Slope (l/mg)	LOD (mg/kg)	r^2	Slope (l/mg)
DON	HP-5	0.007	0.9993	248254	0.004	0.9964	386268
	HP-1701	0.016	0.9982	204359	0.007	0.9977	229096
NIV	HP-5	0.017	0.9975	146493	0.020	0.9962	123060
	HP-1701	0.028	0.9900	110919	0.039	0.9902	103728
3-ADON	HP-5	0.010	0.9995	203877	0.010	0.9990	211380
	HP-1701	0.016	0.9974	188763	0.012	0.9991	199473
15-ADON	HP-5	0.011	0.9991	175328	0.012	0.9987	163728
	HP-1701	0.018	0.9963	134815	0.014	0.9974	134635

Trichothecene range: 0.006–0.625 mg/l. LOD were calculated using alumina–charcoal–C18 silica (procedure (c)) to clean-up a blank wheat sample. PFPA, pentafluoropropionic anhydride; HFBA, heptafluorobutyric anhydride.

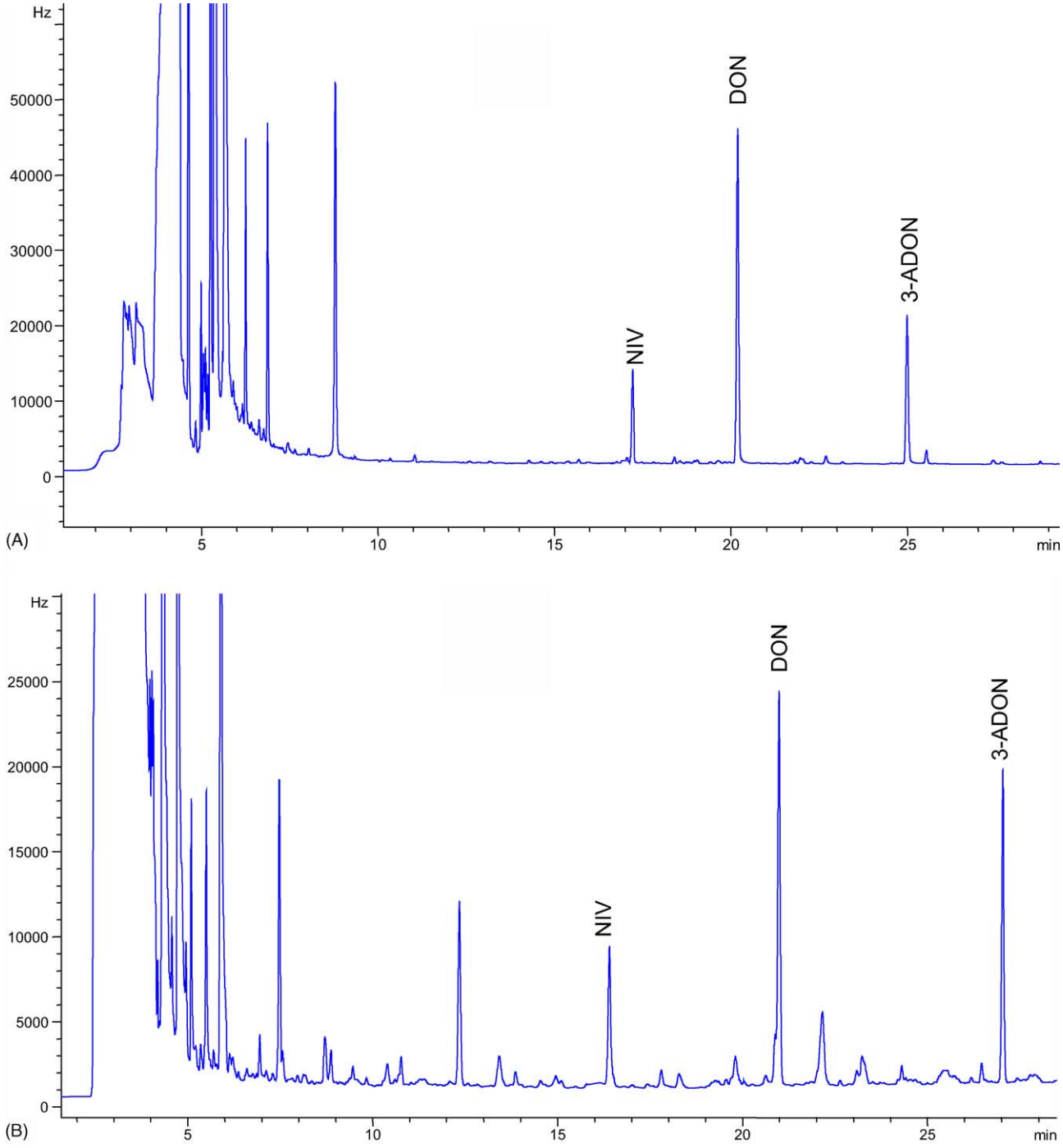

Fig. 1. Chromatograms of a mixture of DON, NIV, and 3-ADON (1 ng of each mycotoxin/μl) derivatized with (A) HFBA and (B) PFPA. Clean-up: alumina–charcoal–C18 silica cartridge (procedure (c)). Column: HP-5. Chromatographic conditions: see Section 2.

of detection (LOD) (analyte concentrations that provide signals equal to blank signal plus 3S.D. of blank signal) [46] were practically the same for NIV, 3- and 15-ADON, regardless of the derivatization reagent using clean-up on alumina–charcoal–C18 silica cartridge (Table 3). In the case of DON, it was observed that for the same amount of standard, signal obtained with HFBA was higher than signal obtained with PFPA. Thus, the former reagent provides higher sensitivity (higher slope) (Table 3). This difference in sensitivity agrees with the limits of detection obtained using these two reagents.

Calibration lines were linear in the 0.006–0.625 mg/l range for both types of derivatives although the coefficient of determination was better when PFPA was used as derivatization reagent. HFBA is a more expensive reagent, has less stability against moisture and trichothecene recoveries

were not significantly better. Therefore, PFPA was selected as derivatization reagent for further analysis of type B trichothecenes.

Detection of 3- and 15-ADON cannot be done satisfactorily when HP-5 (non-polar) column was used, because these compounds, after being derivatized, have the same retention time. This is a disadvantage of this column. In order to solve this problem, analysis was achieved with HP-1701 (low/mid polar) column, which provided different retention times for these compounds and should be used to differentiate these trichothecenes.

The chromatograms of a wheat sample contaminated with type B trichothecenes, using both types of columns, are shown in Fig. 2. Two well-separated peaks were obtained for each 3- and 15-ADON using HP-1701 column. All the analyzed trichothecenes were separated in this column, but

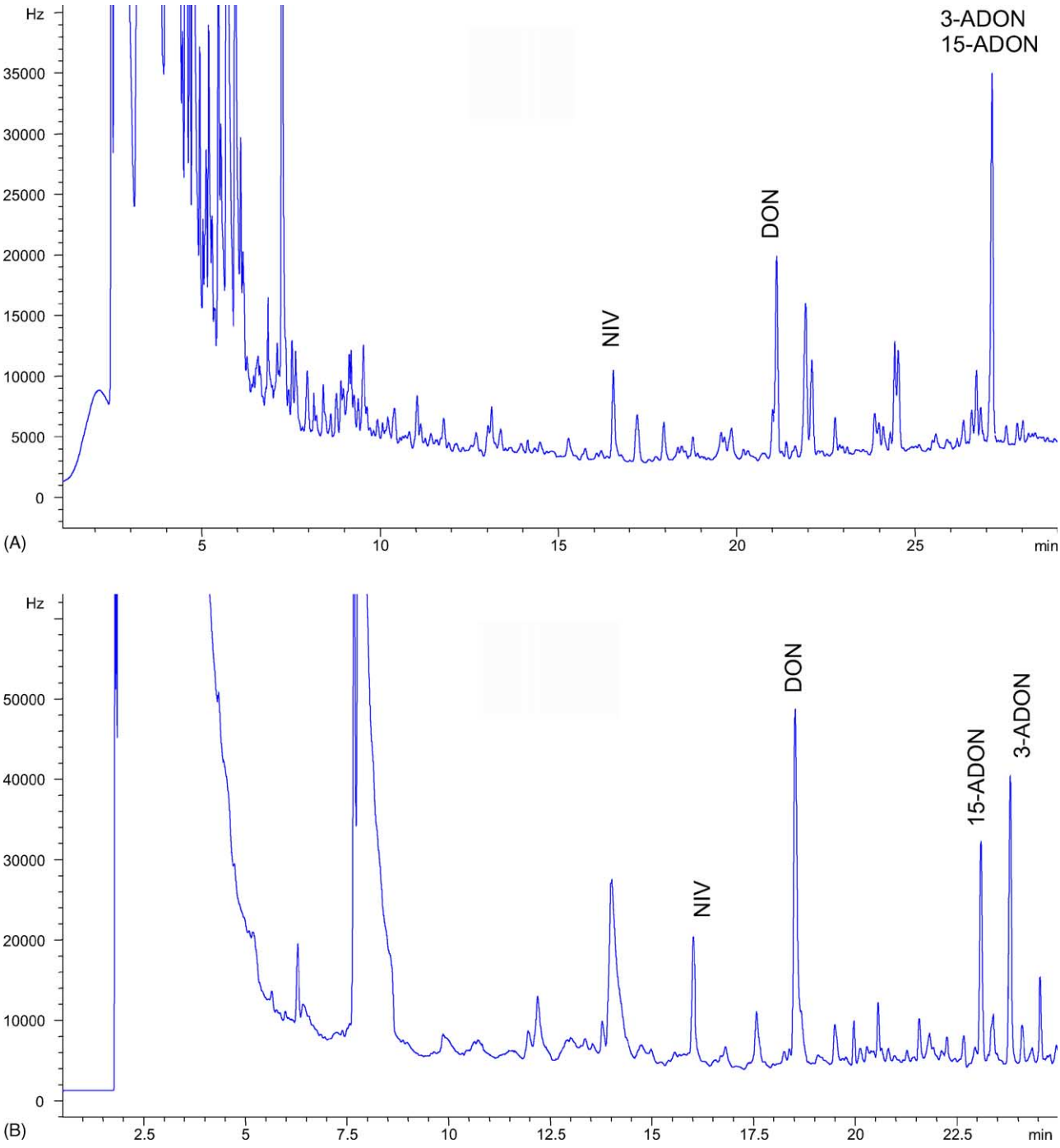

Fig. 2. Chromatograms of a blank wheat sample spiked with (A) 1.0 mg/kg of each DON, NIV, 3- and 15-ADON, derivatized with PFPA, and separated by HP-5 column and (B) 2.5 mg/kg of each of the same trichothecenes, derivatized with PFPA and separated by HP-1701 column. Clean-up: alumina–charcoal–C18 silica cartridge (procedure (c)). Chromatographic conditions: see Section 2.

the limits of detection (Table 3) were, for all toxins, superior to those obtained with HP-5 column.

4. Conclusions

MycoSep 225 column and cartridges containing alumina–charcoal–C18 silica or alumina–charcoal–silica offered the best results for analysis of type B trichothecenes in wheat with similar reproducibility. Clean-up was performed in a simple step and was more effective than other assayed procedures. Although MycoSep 225 provided the best recoveries for DON, this commercial column is more expensive than 'made-in-laboratory' cartridges and provided lower recoveries for NIV.

HP-5 capillary column shows low bleed and sensitivity for the trichothecene derivatives is greater when separate in it; thus, it is preferable for analysis of naturally contaminated samples that usually present low contamination levels. However, the use of HP-1701 column is needed to confirm the possible detection of 3- and 15-DON. This column can also be helpful (especially if GC–MS facility is unavailable) for confirmation of the other type B trichothecenes due to the different retention times they exhibit as a result of the increased polarity of HP-1701 with respect to HP-5. PFPA is preferred to HFBA as a derivatization reagent because of better stability against moisture, lower cost and similar sensitivity of trichothecene derivatives, except perhaps for DON.

Acknowledgements

The authors wish to thank financial support from the Spanish "Ministerio de Ciencia y Tecnología" (Project AGL-2001-2974-C05-01) and from the Valencian Government (Conselleria de Cultura, Educació i Esport). The authors thank to Ms. Laila Aledón Andújar for her technical assistance.

References

[1] J. Olson, T. Börjesson, T. Lundstedt, J. Schnürer, Int. J. Food Microbiol. 72 (2002) 203.
[2] M.J. Sweeney, A.D.W. Dobson, Int. J. Food Microbiol. 43 (1998) 141.
[3] J.E. Smith, C.W. Lewis, J.G. Anderson, G.L. Solomons, Report EUR 16048 EN, European Commission, Directorate-General XII, Brussels, 1994.
[4] Y. Ueno (Ed.), Trichothecenes—Chemical, Biological and Toxicological Aspects, Elsevier, Amsterdam, 1983.
[5] M.W. Trucksess, D.E. Ready, M.K. Pender, C.A. Ligmond, G.E. Wood, S.W. Page, J. AOAC Int. 79 (1996) 883.
[6] B.A. Rotter, D.B. Prelusky, J.J. Pestka, J. Toxicol. Environ. Health 48 (1996) 1.
[7] U. Berger, M. Oehme, F. Kuhn, J. Agric. Food Chem. 47 (1999) 4240.
[8] D.B. Prelusky, B.A. Rotter, R.G. Rotter, in: J.D. Miller, H.L. Trenholm (Eds.), Mycotoxins in Grain, Compounds Other than Aflatoxins, Eagan Press, St. Paul, Minn, 1994, p. 359.
[9] M.W. Trucksess, F. Thomas, K. Young, M.E. Stack, W.J. Fulgueras, S.W. Page, J. AOAC Int. 78 (1995) 631.
[10] T. Tanaka, S. Yamamoto, A. Hasegawa, N. Aoki, J.R. Besling, Y. Sugiura, Y. Ueno, Mycopathologia 110 (1990) 19.
[11] E. Razzazi-Fazele, J. Böhm, W. Luf, J. Chromatogr. A 854 (1999) 45.
[12] P.A. Cunniff (Ed.), Official Methods of Analysis, 16th ed., AOAC International, Gaithersburg, MD, 1998, 4th revision, Method No. 986.17.
[13] M.M. Mossora, S. Adams, J.A.G. Roach, M.W. Trucksess, J. AOAC Int. 79 (1996) 1116.
[14] W. Langseth, P.E. Clasen, J. Chromatogr. 603 (1992) 290.
[15] D.R. Lauren, R. Greenhalgh, J. AOAC Int. 70 (1987) 479.
[16] S.M. Croteau, D.B. Prelusky, H.L. Trenholm, J. Agric. Food Chem. 42 (1994) 928.
[17] C. Hsueh, Y. Liu, M.S. Freund, Anal. Chem. 71 (1999) 4075.
[18] L.K. Prom, R.D. Horsley, B.J. Steffenson, P.B. Schwarz, J. Am. Soc. Chem. 57 (1999) 60.
[19] M. Keblys, A. Flaoyen, W. Langseth, Acta Agric. Scand. Sect. B, Soil Plant Sci. 50 (2000) 155.
[20] R.C. Schothorse, A.A. Jekel, Food Chem. 73 (2001) 111.
[21] W. Langseth, A. Bernhoft, T. Rundberget, B. Kosiak, M. Gareis, Mycopathologia 144 (1999) 103.
[22] T. Möller, H. Gustavsson, J. AOAC Int. 75 (1992) 1049.
[23] Y. Onji, Y. Aoki, N. Tani, K. Umebayashi, Y. Kitada, Y. Dohi, J. Chromatogr. A 815 (1998) 59.
[24] J.J. Mateo, A. Llorens, R. Mateo, M. Jiménez, J. Chromatogr. A 918 (2001) 99.
[25] R.M. Black, R.J. Clarke, R.W. Read, J. Chromatogr. 388 (1987) 365.
[26] R. Krska, J. Chromatogr. A 815 (1998) 49.
[27] F. Kotal, Z. Radová, Czech. J. Food Sci. 20 (2002) 63.
[28] L.M. Cahill, S.C. Kruger, B.T. McAlice, C.S. Ramsey, R. Prioli, B. Kohn, J. Chromatogr. A 859 (1999) 23.
[29] Z. Radová, K. Holadová, J. Hajslová, J. Chromatogr. A 829 (1998) 259.
[30] R.C. Sinha, M.E. Savard, R. Lau, J. Agric. Food Chem. 43 (1995) 1740.
[31] J.J. Park, F.S. Chu, J. AOAC Int. 79 (1996) 465.
[32] W.C. Gordon, L.J. Gordon, J. AOAC Int. 73 (1990) 266.
[33] A.W. Schaafsma, R.W. Nicol, M.E. Savard, R.C. Sinha, L.M. Reid, G. Rottinghaus, Mycopathologia 142 (1998) 107.
[34] C.M. Maragos, R.D. Plattner, J. Agric. Food Chem. 50 (2002) 1827.
[35] F. Walter, B. Meier, J. AOAC Int. 81 (1998) 741.
[36] M. Jiménez, J.J. Mateo, R. Mateo, J. Chromatogr. A 870 (2000) 473.
[37] A. Sano, S. Matsutani, M. Suzuki, S. Takitani, J. Chromatogr. 410 (1987) 427.
[38] W. Langseth, T. Rundberget, J. Chromatogr. A 815 (1998) 103.
[39] P.A. D'Agostino, L.R. Provost, D.R. Drover, J. Chromatogr. 367 (1986) 77.
[40] K.F. Nielsen, U. Thrane, J. Chromatogr. A 929 (2001) 75.
[41] D.W. Hewetson, C.J. Mirocha, J. AOAC 70 (1987) 647.
[42] S. Gravesen, P.A. Nielsen, R. Iversen, K.F. Nielsen, Environ. Health Perspect. 107 (Suppl. 3) (1999) 505.
[43] T. Tanaka, A. Yoneda, S. Inoue, Y. Sugiera, Y. Ueno, J. Chromatogr. A 882 (2000) 23.
[44] B.K. Tacke, H.H. Casper, J. AOAC Int. 79 (1996) 472.
[45] C.J. Mirocha, E. Kolaczkowski, W. Xie, H. Yu, H. Jelen, J. Agric. Food Chem. 46 (1998) 1414.
[46] J.C. Miller, J.N. Miller, in: E. Horwood (Ed.), Estadística para Química Analítica, Addison-Wesley Iberoamerican, Wilmington, DE, 1993, p. 99.