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Simultaneous determination of type-A and type-B trichothecenes in barley samples by GC–MS

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ABSTRACT

A validated method for the simultaneous determination of eight type-A and type-B trichothecenes in barley has been applied to the analysis of 44 samples from the 2007 harvest in Navarra (Spain). The procedure included simultaneous extraction of trichothecenes with acetonitrile and water (84:16), clean up with Multisep® columns, derivatization of the extract and GC–MS analysis. During method validation, selectivity, linearity, precision and accuracy, limits of detection and quantification and recovery were evaluated. Recovery ranged from 92.0 to 101.9% (RSD < 15%), except for nivalenol (NIV) (63.1%), and the limits of detection and quantification ranged from 0.31 to 3.87 μg kg^{-1} and from 10 to 20 μg kg^{-1}, respectively. The higher occurrence was found for deoxynivalenol (DON) (89% of the samples), although at concentrations below the maximum permitted level. The calculated dietary intakes of DON, NIV and the sum of T-2 and HT-2 were below the TDI values proposed. Two or more trichothecenes were present in 41% of the samples and so, the mycotoxin co-occurrence, and therefore synergic or additive effects, should be taking into account when determining permitted levels or risk assessment.

1. Introduction

Mycotoxins are toxic secondary metabolites produced by several fungal species growing on many agricultural commodities and processed food (Bennett and Klich, 2003). Trichothecenes constitute a mycotoxin family mainly produced by fungi of the *Fusarium* genus that grow in the field and contaminate different foodstuffs. They appear as natural contaminants in cereal grains such as wheat, barley, oat, maize, rice, and derived products, such as bread, malt and beer (Pronk, Schothorst and van Egmond, 2002).

There are more than 170 known trichothecenes (Krska, Baumgartner and Josephs, 2001), which can be classified into four categories (A, B, C and D) according to their chemical structure. Type-A trichothecenes are characterized by the presence of hydrogen, a hydroxyl or an ester group at C-8 position (R5 substituent in Fig. 1), whereas type-B trichothecenes have a carbonyl group in this position. The most important trichothecenes, due to their toxicity and their world-wide prevalence are T-2 and HT-2 toxins, diacetoxyscirpenol (DAS) and neosolaniol (NEO) (type-A trichothecenes); and deoxynivalenol (DON), nivalenol (NIV), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON) and fusarenon-X (FUS-X) (type-B trichothecenes) (Pronk, Schothorst and van Egmond, 2002). The chemical structure of trichothecenes analysed in this study are shown in Fig. 1.

Several surveys have shown toxicological and immunological effects on farm animals, produced by trichothecenes after ingestion of mould-contaminated cereal grains. The main symptoms observed, especially for DON, are skin and gastrointestinal irritation or necrosis, haematological disorders, diarrhoea, vomiting and feed refusal, decreased body weight gain, damage to the haematopoietic systems in bone narrow, spleen, thymus and lymph nodes and immunological alterations (Pronk, Schothorst and van Egmond, 2002). These compounds are potent inhibitors of protein and DNA and RNA synthesis (Rotter, Prelusky and Pestka, 1996). With regard to human diseases, these compounds have been related to several poison outbreaks such as alimentary toxic aleukia (ATA) and Akakaby-bio or red mould disease (Miller, 2008).

Trichothecenes levels in different matrices vary from μg kg^{-1} up to mg kg^{-1} depending on toxin, matrix, climatic conditions, as well as other factors. Since toxins can never be completely

Abbreviations: 3-ADON, 3-acetyldeoxynivalenol; 15-ADON, 15-acetyldeoxynivalenol; CLO, α-Chloralose; DAS, Diacetoxyscirpenol; DON, Deoxynivalenol; FUS-X, Fusarenon-X; GC–MS, Gas chromatography with mass spectrometry detection; IS, Internal standard; LOD, Limit of detection; LOQ, Limit of quantification; NEO, Neosolaniol; NIV, Nivalenol; PFPA, Pentafluoropropionic anhydride; SIM, Selected-ion monitoring; TDI, Tolerable daily intake; UV, Ultraviolet detection.

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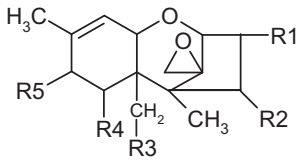

	TRICHOTHECENES	R1	R2	R3	R4	R5
Type-A	T-2 toxin (T-2)	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$
	HT-2 toxin (HT-2)	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
	Diacetoxyscirpenol (DAS)	OH	OAc	OAc	H	H
	Neosolaniol (NEO)	OH	OAc	OAc	H	OH
Type-B	Deoxynivalenol (DON)	OH	H	OH	OH	=O
	3-Acetyldeoxynivalenol (3-ADON)	OAc	H	OH	OH	=O
	15-Acetyldeoxynivalenol (15-ADON)	OH	H	OAc	OH	=O
	Nivalenol (NIV)	OH	OH	OH	OH	=O
	Fusarenon X (FUS-X)	OH	OAc	OH	OH	=O

Fig. 1. Chemical structure of type-A and type-B trichothecenes.

removed from the food supply and they are potential health risks for humans and animals, the European Union has implemented regulations for some of them. In the case of DON in cereals and derived products the maximum permitted level in unprocessed cereals has been established at 1250 μg kg^{-1} (European Commission, 2006a). With regard to T-2 and HT-2, the European Commission has stated that the presence of these toxins can be of concern for public health. Therefore, the development of reliable and sensitive methods, collection of more occurrence data and more investigations/research in the factors involved in the presence of T-2 and HT-2 toxin in cereals and cereal products, are necessary and of high priority (European Commission, 2006a). Regulations with respect to these toxins are still being considered. Discussion limit for the sum of T-2 and HT-2 was 100 μg kg^{-1} for unprocessed cereals and cereals products (Meneely, Ricci, van Egmond and Elliott, 2010).

The co-occurrence of different trichothecenes (type-A and type-B) in one same foodstuff, could originate at least additive effects on human or animal health; however, the knowledge regarding this aspect is still scarce. In order to be able to monitor several toxins, it is necessary to develop analytical methods for their simultaneous analysis that meets the regulatory requirements. This question is a great challenge for chemical analysts due to the heterogeneity of food matrices and the physicochemical characteristics of the toxins (Köppen, Koch, Siegel, Merkel, Maul and Nehls, 2010).

Several analytical methods for the determination and quantification of trichothecenes in food and feed commodities have been described in the reference literature, which have subsequently been reviewed by some researchers (Krska and Molinelli, 2007; Krska, Baumgartner and Josephs, 2001; Meneely, Ricci, van Egmond and Elliott, 2010; Langseth and Rundberget, 1998; Lattanzio, Pascale and Visconti, 2009). However, most of these studies are based on the extraction and clean up of a single mycotoxin (usually DON) or a group of related mycotoxins (type-A or type-B), instead of the simultaneous analysis of type-A and type-B trichothecenes. In Spain, methods have recently been reported for the determination of DON and NIV in corn-based food products (Castillo, Montes, Navarro, Segarra, Cuesta and Hernández, 2008), for the simultaneous analysis of five type-A and -B trichothecenes (T-2, HT-2, DAS, DON and NIV) in wheat flour (Sospedra, Blesa, Soriano and Manes, 2010), and for the determination of DON, T-2 and HT-2 in cereal-based food (Cano-Sancho et al., 2010).

Some surveys regarding the presence of trichothecenes in cereals have been carried out on cereals from Europe (Biselli and Hummert, 2005), Finland (Eskola, Parikka and Rizzo, 2001), Lithuania (Keblys, Flaoyen and Langseth, 2000) and Norway (Langseth and Elen, 1997; Langseth and Rundberget, 1999), but to the knowledge of the authors, this is the first time that the co-occurrence of eight type-A and type-B trichothecenes has been studied in barley samples from Spain.

In this paper, a sensitive and validated method was developed and successfully applied to the simultaneous analysis of eight type-A and type-B trichothecenes in 44 barley samples collected in Navarra (Spain) during 2007 harvest. The procedure involves one simple extraction process with acetonitrile and water for all of the analytes, and the clean up of the extract with Multisep® columns. The samples were then derivatized to be analyzed by GC–MS.

2. Experimental

2.1. Chemical and reagents

Trichothecenes standards were purchased from Fluka (Schnelldorf, Germany) as certified reference materials and pentafluoropropionic anhydride (grade derivatization 99%) from Aldrich (Schnelldorf, Germany). Acetonitrile HPLC grade, α-chloralose, imidazole, sodium bicarbonate and sodium sulphate anhydrous were supplied by Sigma–Aldrich (St. Quentin Fallavier, France) and pro-analysis grade toluene by Panreac (Barcelona, Spain). Millipore type I water was obtained daily from a Milli-Q water-purifying system. Multisep® 227 Trich + columns were purchased from Romer Labs® (Tulln, Austria).