Determination of B-trichothecenes in Wheat by Post Column Derivatisation Liquid Chromatography with Fluorescence Detection (PCD-HPLC-FLD)

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Abstract

B-trichothecenes are one of the most common contaminants of cereals in Europe. Therefore, the use of fast and accurate methods is necessary to measure contamination levels and observe regulatory limits.
At the moment, mostly gas chromatographic (GC) methods are used but HPLC-UV methods are also employed. Clean-up is commonly done either with immunoaffinity or Mycosep® columns.
In the Christian Doppler Laboratory for Mycotoxin Research we have established an alternative HPLC method with post column derivatisation (PCD) as an alternative to existing chromatographic methods. This PCD-HPLC-FLD method uses a Mycosep® clean-up and allows the simultaneous detection and quantification of deoxynivalenol, nivalenol, fusarenon X, 3-acetyldeoxynivalenol and 15-acetyl-deoxynivalenol. A validation with wheat gave for deoxynivalenol a limit of quantification ten times below the drafted European Union guideline level (500 µg.kg^{-1}) and a limit of detection of 8 µg.kg^{-1}. The relative standard derivation for DON was 10% (n=30). The obtained mean recovery rate for DON was 90% in a range from 50 to 1000 µg.kg^{-1}.

Keywords: B-trichothecenes, post column derivatisation

Introduction

B-trichothecenes, possessing a carbonyl function at C-8, are a sub group of over 140 known trichothecenes. Deoxynivalenol (DON) and nivalenol (NIV) are the most common trichothecene contaminants in the world. 3-acetyldeoxynivalenol (3-AcDON) and 15-acetyldeoxynivalenol (15-AcDON) are intermediates occurring during fungal mycotoxin production.
For health and safety reasons, different countries have established guidelines and maximum levels for DON. These vary from 500 $\mu g.kg^{-1}$ to 2000 $\mu g.kg^{-1}$ for cereals. In the European Union a guideline level of 500 $\mu g.kg^{-1}$ for cereal products has been discussed in a draft commission recommendation (1).
Different chromatographic methods for B-trichothecenes analysis have been published. An overview is given in (2). The most common methods combine a clean up with Mycosep® or immunoaffinity (IAC) columns with gas chromatography (GC), either with electron capture (ECD) or a mass sensitive detector (MSD). These methods have a limit of detection (LOD) between 1 and 100 $\mu g.kg^{-1}$.

Published HPLC methods, either with MS or UV detection have a LOD of 1 and 500 $\mu g.kg^{-1}$ respectively. Sano et al. published an HPLC-FLD method using a two step post column derivatisation (3). In the first step the B-trichothecenes are decomposed under alkaline conditions into formaldehyde (4, 5). In the second step the formaldehyde is transformed into a fluorescent lutidine derivative by means of a Hantzsch reaction (6). Whereas Sano describes the analytical feasibility of this method, Lepschy (7) focuses on its suitability for routine analysis. In this work, these methods have been modified to enable the simultaneous detection of five B-trichothecenes.

Materials and Methods

10 g of ground sample were extracted for 90 min with 40 mL of acetonitrile/water (84+16) in an Erlenmeyer flask on an orbital shaker. After filtering the extract, the tube of the Mycosep® column (#227) was filled up to ¾. The column was pressed down until about 4.5 mL of the purified extract were obtained. 4 mL were taken and dried under a constant stream of nitrogen. The residue was dissolved in 300 µL water and filtered.

Analyses were performed with an Hewlett Packard HP1090 HPLC system. 200 µL of the sample were injected. Chromatographic separation was carried out with a Phenomenex® Luna C18(2) (250 x 3.00 mm, 5 µm) column with a flow rate of 0.5 $mL.min^{-1}$ at 35 °C. A gradient elution from 90+10 water/acetonitrile to 80+20 water/acetonitrile was applied.

A PCX 5200 unit (Pickering Laboratories, Mountain View, USA) was employed for post column derivatisation. 0.1 M sodium hydroxide solution and a solution of 30 mM methyl acetoacetate and 2 M ammonium acetate were prepared. The flow rates of the two reagents were adjusted to 0.2 $mL.min^{-1}$. The reactor temperature was set to 115 °C.
For the preparation of standard solutions the selected trichothecenes were weighed

out separately and dissolved in acetonitrile. Concentrations were about 25 $\mu g.ml^{-1}$. A standard solution of all five toxins was prepared by mixing equal amounts of each standard. This solution was used for spiking and the external calibration. For spiking, the appropriate amount was pipetted to 10 g of the sample. The samples stood overnight in the extractor hood for solvent removal.
Statistical data was obtained by using Validata™ (8). By means of this program the limits of detection (LOD) and limits of quantification (LOQ) were calculated according to the guidelines of the German norm DIN 32645 (May 1994). In this norm the LOD and LOQ are derived from Y-intercept and its confidence interval after linear regression.

Results and Discussion

In a 30 min HPLC run with a gradient from 90+10 water/ACN to 80+20 water/ACN a sufficient separation of the B-trichothecenes could be achieved (see chromatogram, figure 1). Due to the post column derivatisation and the fluorescence detection a cleaner chromatogram with less interferences compared to an absorbance detection was obtained, which made quantification and identification easier, more sensitive and more accurate.

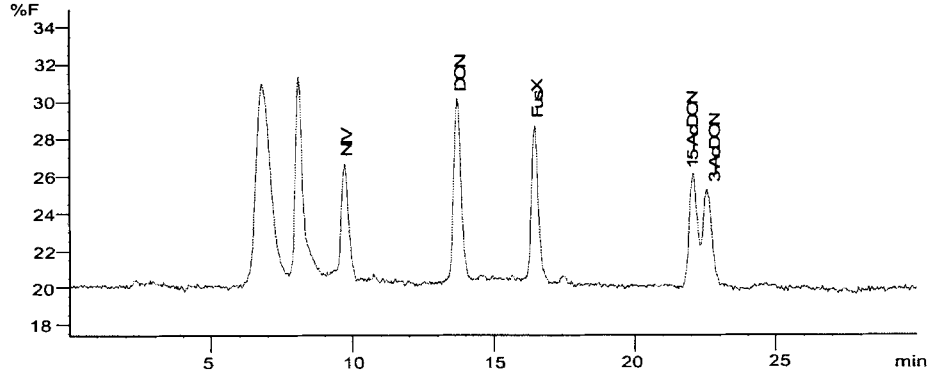

Figure 1: Chromatogram of a wheat sample fortified with 125 $\mu g.kg^{-1}$

Validation data were obtained by comparing a calibration row with six levels and five measured repetitions with samples also spiked at six levels and measured in five repetitions. The recovery rate was obtained by plotting the measured values of the spiked samples against the expected values. The slope of the resulting correlation line corresponds with the recovery rate (see figure 2).

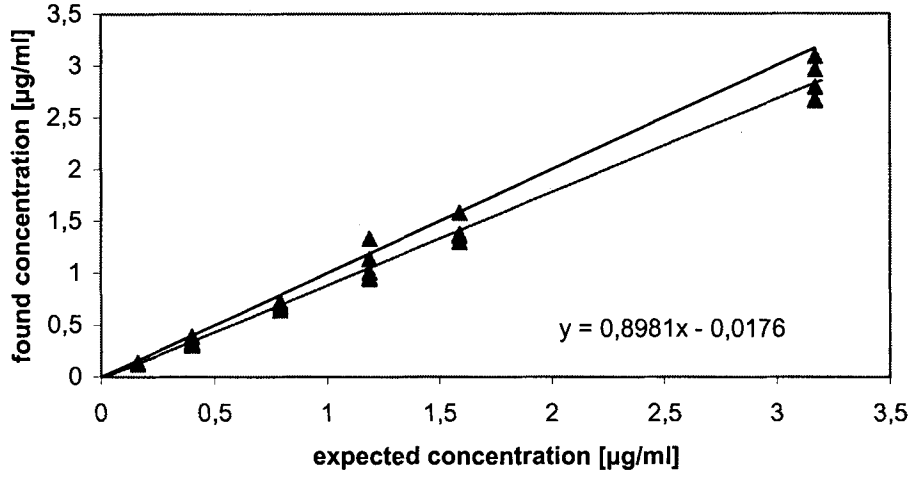

Figure 2: Determination of the recovery rate for DON in wheat (n = 30)

The fast and easy clean-up with the # 227 Mycosep® columns provided a mean recovery rate of 90% (n = 30) for DON in the range from 50 to 1000 µg.kg^{-1}. The method achieved a limit of quantification (LOQ) more then ten times below the expected regulatory limit (table 1).

Compared to an immunoaffinity clean-up this method allows the simultaneous detection and quantification of the five most important B-trichothecenes. The clean up with the single step Mycosep® columns is also faster and less complicated.

On the one hand side commonly used GC methods are more time consuming compared to the presented HPLC-FLD method. On the other hand, HPLC methods using UV-detection are usually less sensitive and more matrix dependent. GC runs are longer than HPLC runs and also the derivatisation to make the B-trichothecenes volatile enough for GC analysis can not be automated easily. Furthermore, strong matrix effects are observed by using GC-ECD.

Toxin	LOD [µg.kg^{-1}]	LOQ [µg.kg^{-1}]	Recovery [%][a]	RSD of procedure [%]
NIV	16	57	54	6
DON	8	29	90	10
FusX	12	40	78	7
15-AcDON	13	44	85	13
3-AcDON	12	43	77	9

[a] mean recovery in the range from 50 to 1000 µg.kg^{-1}

Table 1: Validation data for wheat (n=30)

To sum up, it can be concluded that this PCD-HPLC-FLD method is suitable for routine analysis of type B-trichothecenes in cereals and has advantages compared to commonly used methods. In support of the method is the low LOQ and the financial benefits from a less time consuming method. Of course these financial benefits have to offset the purchase of the post column derivatisation equipment and training of the staff, which is important as handling of the equipment needs practice.

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