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Determination of trichothecenes in beer by capillary gas chromatography with flame ionisation detection☆

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Abstract

In 1999 and 2000, high concentrations of deoxynivalenol (DON) were found in wheat and breakfast cereals sold on the Dutch market. Besides wheat other grains can be contaminated with trichothecenes. Using these grains in the brewing process, the produced beer could be contaminated with trichothecenes. A previously developed GC–FID method to determine trichothecenes in wheat was made applicable for the determination of trichothecenes (DON, nivalenol, 3-acetyldeoxynivalenol, fusarenon X, T-2 toxin, HT-2 toxin, diacetoxyscirpenol and neosolaniol) in beer. Surveys were carried out in November 2000 and March 2001 to investigate the presence of trichothecenes in beers marketed in the Netherlands. In only three of the 51 beer samples low quantities of DON were found, ranging from 26 to 41 μg/l. Besides DON, no trichothecenes were found in the beers at levels above the limit of quantification (25 μg/l). All positive samples were from so-called top fermentation beers.

Keywords: Trichothecenes; Beer; DON; NIV; 3-AcDON; FusX; T-2; HT-2; DAS; NeoSol; GC; FID

1. Introduction

Trichothecenes are secondary metabolites produced by several fungal genera, but mainly by *Fusarium* species. Growth of *Fusarium* species and toxin production can occur at relatively low temperatures on agricultural commodities in the field or during storage. Therefore, trichothecenes are commonly found in cereals from moderate climatic zones (Egmond & Speijers, 1999).

Deoxynivalenol (DON) is the most commonly found trichothecene all over the world and is associated with various adverse health effects in animals and humans. The most prominent toxic effects of DON are growth retardation and immunotoxic effects.

In 1999, high concentrations of DON [and nivalenol (NIV)] were found in breakfast cereals sold on the Dutch market (DON levels up to 2600 μg/kg). A survey was carried out in the Netherlands in the first half of 1999 and 2000 to determine the quantities of trichothecenes in (imported) wheat (1998 and 1999 harvests). In about 40% of the wheat samples the temporary DON limit of 500 μg/kg for cleaned wheat, effective in the Netherlands, was exceeded. Besides DON, no other trichothecenes were found (Schothorst & Jekel, 2001). The presence of trichothecenes however is not only limited to wheat but can also be present at high concentrations in other grains (Eskola, Parikka, & Rizzo, 2001; Placinta, D'Mello, & Macdonald, 1999). If these grains are used in the brewing process, trichothecenes can contaminate the produced beer. (Niessen, Böhm-Schrami, Vogel, & Donhauser, 1993).

Trichothecenes have frequently been found in beers from different countries. (Molto, Samar, Resnik, Martinez, & Pacin, 2000; Scott, 1996, 1997; Scott, Kanhere, & Weber 1993; Won-Bo, Jin-Cheaol, Jeong-Ah, & Yin-Won, 1997).

As beer consumption can be rather high, contaminated beer could therefore contribute substantially to the daily intake of trichothecenes, especially DON (Eriksen & Alexander, 1998).

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The objective of the present study was to make a previously developed GC–FID method to determine trichothecenes in wheat (Schothorst & Jekel, 2001), applicable for the determination of trichothecenes [DON, NIV, 3-acetyldeoxynivalenol (3-AcDON), fusarenon X (FusX), T-2 toxin (T-2), HT-2 toxin (HT-2), diacetoxyscirpenol (DAS) and neosolaniol (NeoSol)] in beer.

Surveys were carried out in the Netherlands in November 2000 and March 2001 to determine the quantities of trichothecenes in beers marketed in the Netherlands. The results of these surveys are presented.

2. Experimental

2.1. Chemicals and reagents

All reagents were of analytical grade, unless otherwise specified. Trichothecenes standards were purchased from Sigma, and the internal standard α-chloralose was purchased from Dr. Ehrenstorfer Reference Materials. A combined solution containing 5 μg/ml of the internal standard and each of the trichothecenes in acetonitrile was prepared from the standards. This solution was kept refrigerated and was stable for at least 2 years. (Pettersson, 2000). An internal standard solution of 0.5 μg/ml in water was prepared from the standard. This solution was kept at room temperature and was stable for at least 1 year.

2.2. Sampling

November 2000 and March 2001, 51 beer samples (one bottle each), consisting of 49 different brands, were collected in local stores in the Bilthoven area in the Netherlands and kept at room temperature in the dark until analysis. The beers originated from Germany (9), Belgium (9), Ireland (1) and the Netherlands (32). Table 3 summarises the origin and type of the collected beers.

2.3. Extraction

A 10-ml portion of a degassed (ultrasonic) beer sample to which 5 ml internal standard was added was put on a ChemElute® (Varian) column and eluted with 100 ml of ethylacetate. The extract was rotary evaporated (70 °C) under reduced pressure. The dry residue was redissolved in 4 ml acetonitrile/water (84 + 16, v/v). With this procedure the recovery for nivalenol is very poor (Table 2). In order to achieve also acceptable recoveries for nivalenol, the ChemElute® column can be eluted with 250 ml ethylacetate/methanol (95 + 5, v/v).

2.4. Clean-up and derivatisation

The samples were cleaned up with Mycosep® 227 (consists of various adsorbents, including charcoal, Celite and ion-exchange resins) and Mycosep® 216 (consists of charcoal) columns (Romer Labs.). The stationary phase of a Mycosep® 227 column was repacked above the packing material of a Mycosep® 216 column (Fig. 2). Before use, the combined column was washed three times with 5 ml acetonitrile/water (84 + 16, v/v). The concentrated extract (4 ml) was put onto the combined Mycosep® column. The trichothecenes were

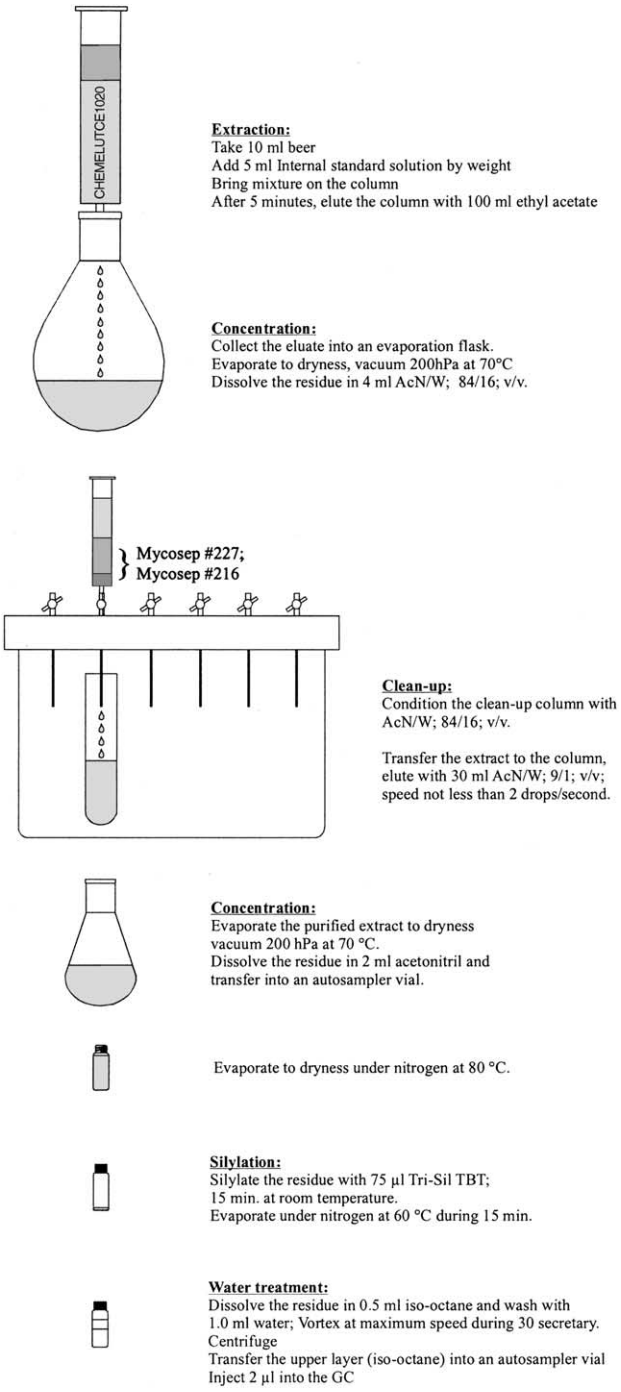

Fig. 1. Sample preparation procedure for determination of trichothecenes in beer.

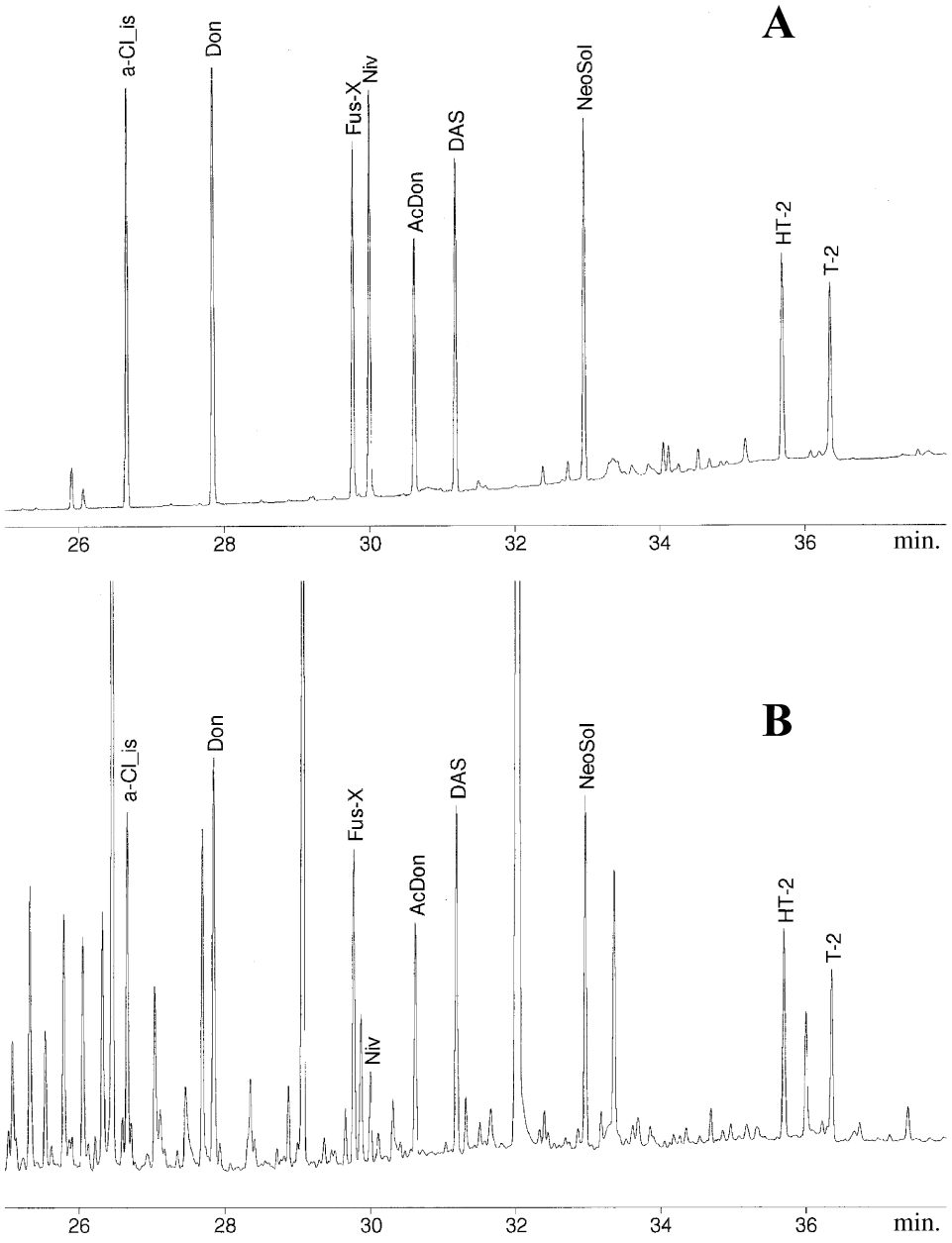

Fig. 2. Chromatograms of a silylated standard solution of trichothecenes (A) and of a silylated beer sample with addition of standard solution (B).

eluted with 30 ml of acetonitrile/water (90 + 10, v/v), and the cleaned extract was rotary evaporated (70 °C) under reduced pressure. The dry residue was re-dissolved in 2 ml acetonitrile and transferred to an autosampler vial. The extract was evaporated to dryness under nitrogen at 80 °C with a heating block. Seventy-five microlitres of tri-sil TBT [trimethylsilylimidazole [-bis(trimethylsilyl)acetamide-trimethylchlorosilane (3:3:2), Pierce] was added to the dry residue. After the autosampler vial was flushed with N_2, it was closed, and the extract was derivatised to trimethylsilyl (TMS) ethers in 15 minutes at room temperature. The autosampler vial was opened, and the extract was evaporated for 15 min at 60 °C under nitrogen. The residue was re-dissolved in 0.5 ml *iso*-octane and washed with 1.0 ml water. The mixture was centrifuged, and the *iso*-octane layer was transferred to a new autosampler vial.

The sample preparation procedure for determining the amounts of trichothecenes in beer is shown schematically in Fig. 1.

The final extract was analysed for trichothecenes by GC with FID under the experimental conditions specified in Table 1.

2.5. Equipment

Table 1 summarises the GC–FID equipment and the experimental conditions. In the case of a positive finding,

Table 1
Test conditions for determining trichothecenes in beer

Test GCsystem	Gas chromatograph, Fisons 8160 with Fisons AS 800 autosampler
Column type	CP-Sil 19 CB (7% cyanopropyl-, 7% phenyl-, 86% dimethylpolysiloxane), 60 m, 0.25 mm i.d., 0.15μm film thickness (Chrompack) with 1.5 m, 0.53 mm i.d. retention gap (723 560.25, Machery-Nagel).
Carrier gas	Hydrogen at 125 kPa. Linear velocity: 33.7 cm/sec at initial temperature (115 °C)
Internal standard	α-Chloralose
Injector	Cold on-column
Injection volume	2 μl
Detection	Flame ionisation detector (FID) at 300 °C
Calculation	Internal standard
Column temperature programme	4 min at 115 °C 6 °C/min to 300 °C 10 min at 300 °C
Confirmation column temperature programme	
	4 min at 115 °C 20 °C/min to 220 °C 6 °C/min to 300 °C 10 min at 300 °C

Table 2
Results of repeatability and recovery experiments (spiking level about 190 μg/l for all trichothecenes)

	Recovery ($n=5$)		
	Mean (%)	S.D.	RSD (%)
Extraction with 100 ml ethylacetate			
DON	92.0	3.0	3.2
FusX	97.8	2.3	2.4
NIV	24.1	1.7	7.1
3-AcDON	102.0	2.7	2.7
DAS	108.3	7.4	6.9
NeoSol	111.4	3.7	3.3
HT-2	110.7	7.1	6.4
T-2	105.9	6.6	6.2
Extraction with 250 ml ethylacetate/methanol			
DON	87.9	5.6	6.3
FusX	85.5	6.3	7.4
NIV	83.3	8.4	10.1
3-AcDON	81.3	14.7	18.0
DAS	93.4	4.9	5.2
NeoSol	93.2	2.3	2.5
HT-2	94.0	4.2	4.5
T-2	96.8	1.8	1.9

a second injection was done with another column temperature programme. This way a different elution profile was obtained, which made it is possible to eliminate false positives.

Table 3
Origin and occurrence of DON in beer

Country of origin	Type of beer	Concentration (μg/l)[b]
The Netherlands-1[a]	Pilsener	–
The Netherlands-2[a]	Pilsener	–
The Netherlands-3	Pilsener	–
The Netherlands-4	Pilsener	–
The Netherlands-5	Pilsener	–
The Netherlands-6	Pilsener	–
The Netherlands-7	Pilsener	–
The Netherlands-8	Pilsener	–
The Netherlands-9	Pilsener	–
The Netherlands-10	Pilsener	–
The Netherlands-11	Pilsener	–
The Netherlands-12	Pilsener	–
The Netherlands-13	Pilsener	–
The Netherlands-14	Pilsener	–
The Netherlands-15	Pilsener	–
The Netherlands-16	Bock	–
The Netherlands-17	Bock	–
The Netherlands-18	Ale	–
The Netherlands-19	Ale (Trappist)	–
The Netherlands-20	Ale	–
The Netherlands-21	Ale	–
The Netherlands-22	Ale	–
The Netherlands-23	Lager	–
The Netherlands-24	Non-alcoholic	–
The Netherlands-25	Non-alcoholic	–
The Netherlands-26	Non-alcoholic	–
The Netherlands-27	Non-alcoholic	–
The Netherlands-28	Low-alcoholic	–
The Netherlands-29	Low-alcoholic	–
The Netherlands-30	Low-alcoholic	–
The Netherlands-31	White	41
The Netherlands-32	White	–
Germany-1[a]	Pilsener	–
Germany-2[a]	Pilsener	–
Germany-3	Pilsener	–
Germany-4	Pilsener	–
Germany-5	Pilsener	–
Germany-6	Pilsener	–
Germany-7	Pilsener	–
Germany-8	Alt	–
Germany-9	White	–
Belgium-1	White	36
Belgium-2	Ale	–
Belgium-3	Ale	–
Belgium-4	Ale	–
Belgium-5	Ale	26
Belgium-6	Ale	–
Belgium-7	Ale	–
Belgium-8	Ale	–
Belgium-9	Ale	–
Ireland	Ale	–

[a] The Netherlands 1 and 2 and Germany 1 and 2 are the same brand.
[b] Below limit of quantification (25 μg/l).

3. Method validation

The validation of the GC–FID method for the determination of trichothecenes in wheat has been

described in detail in a previous paper (Schothorst & Jekel, 2001). Compared with the earlier-described method for the determination of trichothecenes in beer the most significant changes are in the extraction procedure. Therefore a supplementary validation has been carried out. The following parameters were investigated; the limit of detection, the limit of quantification and the recovery.

A chromatogram of a silylated standard solution of trichothecenes is presented in Fig. 2, together with a chromatogram of a silylated beer sample with addition of standard solution

3.1. Limit of detection and limit of quantification

With a test portion of 10 ml of the beer sample, the limit of detection for the tested trichothecenes is 8 μg/l at a signal-to-noise ratio of 3. The limit of quantification for the tested trichothecenes is 25 μg/l at a signal-to-noise ratio of 9.

3.2. Recovery

Recovery experiments were done by adding standards of the trichothecenes to a blank beer sample. The level at which standards were added to the test portion was about 190 μg/l. Different beer samples were analysed on five separate days. For both extraction methods the results are summarised in Table 2.

4. Results and discussion

The GC–FID method described was used to determine the trichothecene content of 51 beer samples collected in November 2000 and March 2001.

During the analytical sessions, duplicate determinations and recovery experiments were performed. Blanks (determination without test portion) were performed at regular intervals. The results of these quality assurance experiments complied with the performance characteristics established for the GC–FID method (Schothorst & Jekel, 2001).

The results of determining the trichothecene content of the beer samples are summarised in Table 3. DON was the only trichothecene found in the analysed beer samples. NIV, 3-AcDON, FusX, T-2, HT-2, DAS and NeoSol were not detected in the samples.

In only three of the 51 beer samples low quantities of DON were found ranging from 26 μg/l to 41 μg/l. All positive samples were so-called top fermentation beers. None of the beers exceeded the temporary tolerance limit in the Netherlands for DON of 500 μg/kg.

Based on the earlier mentioned results we conclude that, at least for the period covered by the survey, the contribution of DON from beers to the total DON intake is negligible. However, all the beers were probably prepared from grains harvested in 2000, a year with a relatively low incidence of DON contamination. In years with high incidences of DON contaminated grains, the situation could be totally different.

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