DETERMINATION OF TRICHOTHECENE TOXINS IN FOODS AND FEEDS

A. BATA*

Department of Biochemistry and Food Technology, Technical University of Budapest, P.O. Box 91, H-1521 Budapest (Hungary)

A. VÁNYI

Central Veterinary Institute, Tábornoku 2, H-1149 Budapest (Hungary)

and

R. LÁSZTITY and J. GALÁCZ

Department of Biochemistry and Food Technology, Technical University of Budapest, P.O. Box 91, H-1521 Budapest (Hungary)

SUMMARY

Improved gas chromatographic (GC) and high-performance thin-layer chromatographic (HPTLC) methods for the determination of trichothecene toxins in biological materials were developed. For GC, glass capillary columns with 100–120 μm I.D. were used, wetted with SE-52. For HPTLC a benzene–acetone solvent system was employed. Conversion of the trichothecenes to their parent alcohols by transesterification prior to GC respectively HPTLC separation, makes the method suitable for the determination of all naturally occurring trichothecenes.

INTRODUCTION

Mould fungi often occur on foods and feeds and produce the toxic metabolites mycotoxins. In the continental climate fungi belonging to the *Fusarium* species are often components of the infecting mould flora. These fungi produce potent toxins of the sesquiterpenoid and resorcylic acid lactone type. The structures of the most common toxins of the 12,13-epoxytrichothecene type are shown in Fig. 1. At present 40–50 of these derivatives are known of which 6–8, such as T-2 toxin, HT-2 toxin[1], diacetoxyscirpenol[2–18], nivalenol[3], deoxynivalenol[4,5], neosolaniol[6] and zearalenone[7] (Fig. 2) cause most of the known mycotoxic diseases.

Several methods have been used for the detection and determination of mycotoxins, such as thin-layer chromatography (TLC)[8–10], gas chromatography (GC)[11] capillary GC[12,13], GC–mass spectrometry[6] and polarography[14]. TLC is not sufficiently specific to detect these substances at low concentrations. GC on packed or capillary columns is often unreliable because of the many interfering components. The reliability cannot be increased by using a polar–non-polar pair of columns, because polar columns can only be used up to 250°C, and the compounds concerned are not eluted at this temperature. An alternative possibility for the analysis of fu-
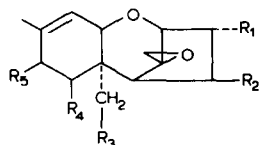
Fig. 1. Structures of naturally occurring trichothecene toxins.

Type A	R_1	R_2	R_3	R_4	R_5
T-2 toxin	OH	OOCCH$_3$	OOCCH$_3$	H	O-isovaleryl
HT-2 toxin	OH	OH	OOCCH$_3$	H	O-isovaleryl
Acetyl T-2 toxin	OOCCH$_3$	OOCCH$_3$	OOCCH$_3$	H	O-isovaleryl
Neosolaniol	OH	OOCCH$_3$	OOCCH$_3$	H	OH
T-2 tetraol toxin	OH	OH	OH	H	OH
Type B					
Diacetoxyscirpenol (DAS)	OH	OOCCH$_3$	OOCCH$_3$	H	H
Monoacetoxyscirpenol	OH	OH	OOCCH$_3$	H	H
Scirpentriol	OH	OH	OH	H	H
Type C					
Nivalenol	OH	OH	OH	OH	=O
Fusarenone-X	OH	OOCCH$_3$	OH	OH	=O
Diacetylnivalenol	OH	OOCCH$_3$	OOCCH$_3$	OH	=O
Deoxynivalenol	OH	H	OH	OH	=O

sariotoxins is described in this paper. This method involves the conversion of these substances to their parent alcohols by transesterification prior to GC separation.

EXPERIMENTAL

Chemicals

Mycotoxin standards. Diacetoxyscirpenol (DAS) and zearalenone was purchased from Makor Chemicals (Jerusalem, Israel), T-2 toxin, HT-2 toxin, T-2 tetraol toxin, neosolaniol and scirpentriol were prepared in our laboratory, deoxynivalenol was a generous gift from Dr. Yoshizawa (Kagawa University, Kagawa, Japan) and Dr. Vesonder (Northern Regional Research Center, IL, U.S.A.). Fusarenone-X and nivalenol were a generous gift from Dr. Palyusik (Veterinary Medical Research Institute, Budapest, Hungary).

Reagents and solvents

The materials were trimethylsilylated with Trisil-BT reagent (Pierce, Rockford,
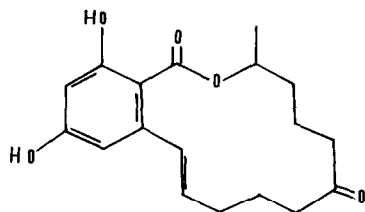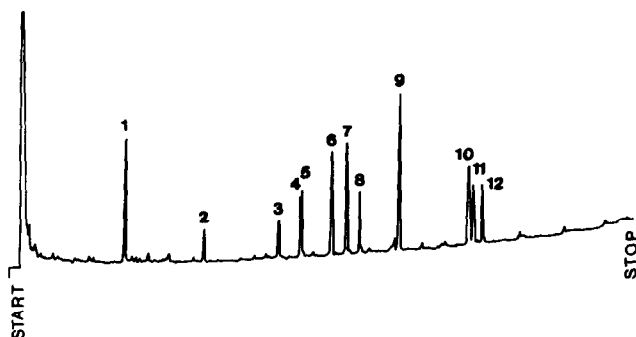

Fig. 2. Structure of zearalenone.

Fig. 3. Gas chromatogram of mycotoxin standards. For conditions, see text. Peaks: 1 = crotocin; 2 = impurity; 3 = scirpentriol; 4 = deoxynivalenol; 5 = monoacetyldeoxynivalenol; 6 = diacetoxyscirpenol; 7 = fusarenon-X; 8 = T-2 tetraol toxin; 9 = neosolaniol; 10 = HT-2 toxin; 11 = T-2 toxin; 12 = zearalenone.

IL, U.S.A.). Sodium methylate reagent was prepared in our laboratory. Other organic solvents were distilled before use.

Apparatus

Kieselgel 10 × 10 cm high-performance TLC (HPTLC) plates were obtained from Merck (Darmstadt, F.R.G.). The solvent system examined was benzene–acetone (1:1). After development, the plate was dried in an air stream and sprayed with 1% 4-(*p*-nitrobenzyl)pyridine solution. After evaporation of the solvent, the plate was heated in an oven at 150°C for 30 min and then sprayed with 10% tetraethylene-pentamine solution. The trichothecene toxins were observed as blue spots on a white background.

A Packard Type 427 gas chromatograph equipped with a flame-ionization detector was used. The capillary wall-coated open-tubular column (18 m × 125 μm I.D.) was prepared according to Grob and co-workers[15,16] and wetted with SE-52 liquid stationary phase, which was immobilized with dibenzoyl peroxide. The temperatures of the injection port and the detector were 250 and 260°C, respectively. The column temperature was programmed from 150 to 270°C at 4°C/min. The carrier gas was hydrogen at a pressure of 0.2 MPa. The splitting ratio was 1:20 and the attenuation was 10^{-11} A. A gas chromatogram of mycotoxin standards is shown in Fig. 3.

Preparation of samples

A sample of 20 g was extracted first with 200 ml of ethyl acetate for 2 h, then with 200 ml of methanol for 2 h. The two extracts were evaporated and combined and the residue was dissolved in 20 ml of methanol–water (4:1) and shaken with 20 ml of light petroleum (b.p. 60–70°C). The light petroleum fraction was discarded and the methanol fraction was dried over anhydrous sodium sulphate and then evaporated. The residue was dissolved in 2 ml of benzene–acetone (9:1) and placed on a 10 × 1 cm I.D. column, packed with silica gel (Kieselgel 60, 0.200–0.063 mm; Merck). The column was washed with 20 ml of benzene and the toxins were eluted with 20 ml of benzene–acetone (1:1). The eluate containing the toxins was divided into two parts and evaporated to dryness. The samples were analysed by TLC or GC, either

untreated or after transesterification with 100 μl of sodium methylate and neutralization with hydrochloric acid–methanol. All samples were silylated with 100 μl of Trisil-BT reagent (10 min, 60°C) for GC.

RESULTS AND DISCUSSION

The R_F values of fusariotoxins occurring in nature[17] are shown in Table I. Toxins of type A give, after transesterification, T-2 tetraol ($R_F = 0.20$) those of type B give scirpentriol ($R_F = 0.16$) and those of type C give nivalenol ($R_F = 0.20$). When tested by capillary GC, the same toxins eluted without tailing. The GC retention indices are also given in Table I.

Fig. 4 shows the chromatogram of a wheat sample. The peak with a retention time of 21.63 min corresponds to the neosolaniol-TMS and that of 25.97 min to T-2 toxin-TMS. T-2 tetraol toxin is eluted at a retention time of 19.80–19.90 min, where only a small peak due to some trace components is seen.

Fig. 5 shows the chromatogram of the same sample after transmethylation. It can be seen that a high peak is present at a retention time of about 19.85 min, corresponding to tetraol-TMS formed by hydrolysis of neosolaniol and T-2 toxin. The original peak of neosolaniol-TMS disappeared, but a smaller peak remained at the position of T-2 toxin-TMS. This means that a compound not sensitive to hydrolysis was present, increasing the apparent amounts of T-2-toxin-TMS. The amount of this interfering compound reached 30% of the total amount of T-2 toxin-TMS. Therefore, there is a possibility, when using a method without transmethylation, that T-2 toxin may appear to be detected in a sample actually free of this toxin,

TABLE I

R_F VALUES OF FUSAROTOXINS IN A 1:1 BENZENE–ACETONE SOLVENT SYSTEM AND RETENTION INDICES ON AN SE-52 COLUMN

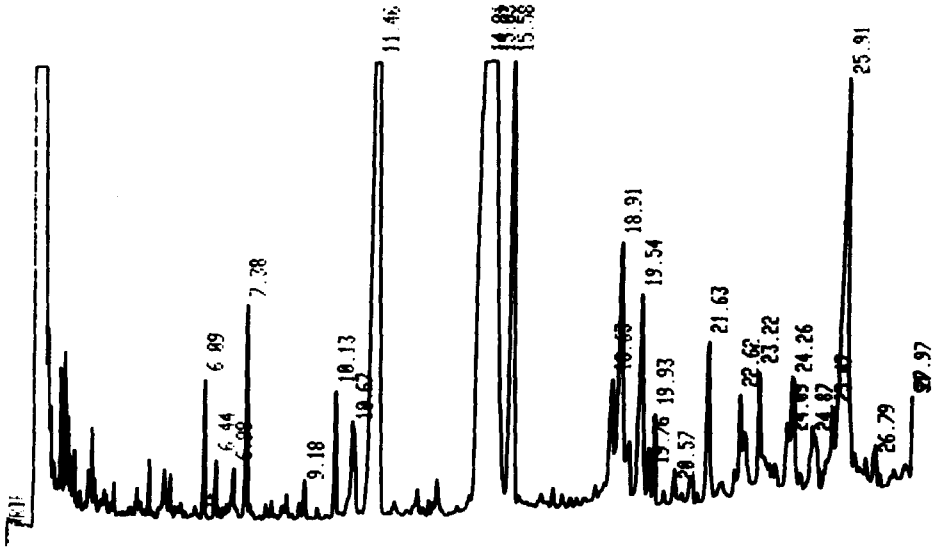| Type	Toxin	TLC R_F values	GC retention index
A	T-2 toxin	0.59	2822
	HT-2 toxin	0.35	2809
	Neosolaniol	0.42	2652
	Monoacetyl neosolaniol	0.40	–
	T-2 tetraol toxin	0.20	2547
B	Diacetoxyscirpenol	0.55	2421
	Monoacetoxyscirpenol	0.37	2317
	Scirpentriol	0.16	2260
C	Diacetylnivalenol	0.54	–
	Fusarone-X	0.41	2485
	Nivalenol	0.20	–
	Deoxynivalenol	0.33	2323
–	Crotocin	0.62	–
–	Zearalenone	0.67	2867

Fig. 4. Gas chromatogram of a feed sample. For conditions, see text. Peaks: 21.63 min, neosolaniol; 25.91 min, T-2 toxin.

owing to the interference from other compounds. For example, for the wheat sample shown in Figs. 4 and 5, an amount of 0.35 ppm of T-2 toxin may be determined and this wheat should be classified as not conforming to the quality criteria because of the health hazard.

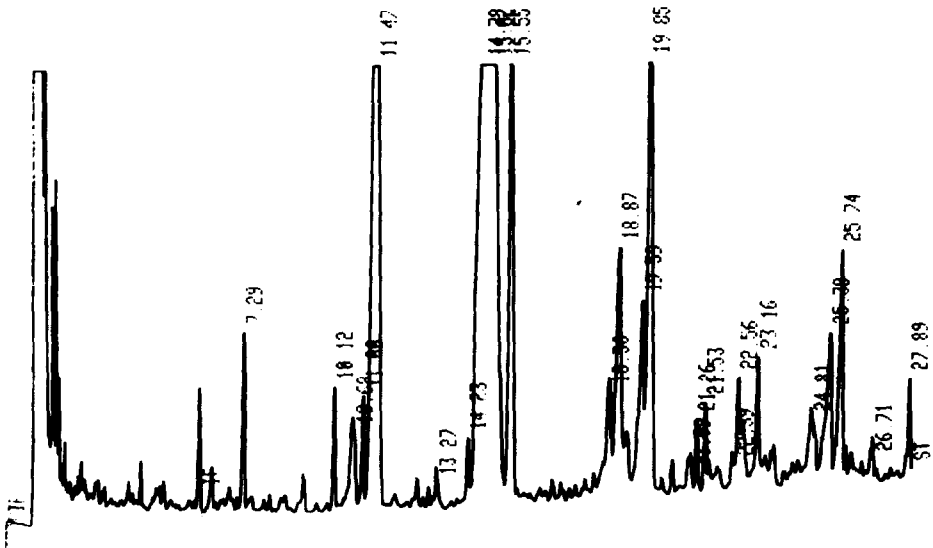Such problems may also occur with other grains such as maize, oats, barley

Fig. 5. Gas chromatogram of feed sample after transesterification. For conditions, see text. Peak at 19.85 min, T-2 tetraol toxin.

and soya. Bearing in mind the facts mentioned above, it can be stated that the method using transmethylation is needed in order to avoid incorrect determinations. The same is so if other apolar columns, *e.g.,* OV-1 or OV-17 are used.

The sensitivity of the method is fairly good. A 50 ppb* concentration of toxin may be detected, corresponding to 3–5 ng per injected sample. The lowest concentration of this group of fusariotoxins causing biological effects in animals lies in the range 100–500 ppb. Higher concentrations have a more drastic influence on animals and a concentration of 1–5 ppm may be lethal. The modified method described here gives the possibility of more confident detection of fusariotoxins.

A further advantage of the method is that only two or three fusariotoxins are produced in pure form as standards (T-2 toxins, diacetoxyscirpenol, nivalenol). Information is needed on the total amount of other fusariotoxins and the method presented here is suitable for this purpose, as in one step it gives information on the total amount of more than ten fusariotoxins.

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* Throughout this article, the American billion (10^9) is meant.