Journal of Chromatography, 368 (1986) 374–380
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 939

Note

Comparison of column phases for separation of derivatized trichothecenes by capillary gas chromatography

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(Received June 5th, 1986)

The trichothecenes are a group of secondary fungal metabolites chemically derived from 12,13-epoxytrichothec-9-ene and formed by species of *Fusarium* and certain other genera of fungi[1]. Over 100 trichothecenes have now been identified, although only a few, in particular deoxynivalenol (DON), nivalenol (NIV), T-2 toxin (T-2), HT-2 toxin (HT-2) and diacetoxyscirpenol (DAS), have so far been detected as natural contaminants in cereal grains[1–3]. Analytical methods for trichothecenes have been reviewed[4,5] and gas chromatography (GC) of derivatized trichothecenes with electron-capture (ECD) or mass spectrometric (MS) detection is favoured for quantitation[4].

Capillary GC has been used by several laboratories for determination of up to six trichothecenes in grains and feeds[2,3,6–16]. For multiple trichothecene analysis capillary GC is essential for their separation from interferences, particularly if ECD is used. Only one of the published papers on capillary GC[3] includes all of the five trichothecenes (DON, NIV, T-2, HT-2 and DAS) referred to above. Also little comparative work on trichothecene separation on different GC phases has been done with either capillary or packed columns[2,8,17,18]. In order to obtain basic information on the separation of DON, NIV, 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), fusarenone-X (FX) (type B trichothecenes) and T-2, HT-2, DAS, neosolaniol (NS), and 4-monoacetoxyscirpenol (MAS) (type A trichothecenes) for multitrichothecene method development, we have compared their behaviour as heptafluorobutyrate (HFB) and trimethylsilyl (TMS) (emphasizing type B trichothecenes) derivatives on six fused-silica capillary columns with stationary phases ranging in polarity from SE-30 (100% methylpolysiloxane) to DB-225 (cyanopropylmethyl–methylphenylpolysiloxane, 50:50). Response of TMS derivatives of type A trichothecenes to ECD is known[3,19] to be much poorer than for TMS derivatives of type B trichothecenes, which possess a conjugated 8-carbonyl group; nevertheless type A trichothecenes were tested on five of the columns as TMS ethers.

EXPERIMENTAL

DON, 3-ADON, 15-ADON[20], and 7,8-dihydroxycalonectrin (DHCAL)[21], were from the Chemistry and Biology Research Institute (Agriculture Canada, Ot-

0021-9673/86/$03.50

TABLE I

TEMPERATURE PROGRAMS FOR CAPILLARY GC OF TRICHOTHECENE DERIVATIVES

Column	*HFB*		*TMS*	
	*Ramp 1**	*Ramp 2*	*Ramp 1**	*Ramp 2***
SE-30	30°C/min to 190°C (held 2 min)	2°C/min to 240°C (held 3 min)	30°C/min to 210°C (held 21 min)	30°C/min to 240°C (held 14 min)
SE-54	30°C/min to 140°C (held 5 min)	4°C/min to 220°C (held 2 min)	30°C/min to 180°C (held 12 min)	5°C/min to 220°C (held 12 min)
DB-1701	30°C/min to 175°C (held 2 min)	3°C/min to 220°C (held 10 min)	30°C/min to 200°C (held 12 min)	10°C/min to 220°C (held 13 min)
DB-17	50°C/min to 125°C (held 5 min)	4°C/min to 220°C (held 10 min)	30°C/min*** to 190°C (held 11 min)	10°C/min to 220°C (held 17 min)
DB-210	30°C/min to 200°C (held 3 min)	1°C/min to 220°C (held 8 min)	30°C/min to 195°C (held 14 min)	20°C/min to 220°C (held 25 min)
DB-225	50°C/min to 155°C (held 5 min)	4°C/min to 220°C (held 10 min)	25°C/min to 200°C (held 12 min)	–

* Following an initial period at 70°C for 1 min (80°C for SE-54 and HFB derivatives).
** For inclusion of type A trichothecenes.
*** 50°C/min used for data shown in Fig. 5.

tawa, Canada), NIV, FX, and NS were purchased from Wako Chemicals (Dallas, TX, U.S.A.); MAS[22] was from C. J. Mirocha (University of Minnesota, St. Paul, MN, U.S.A.); T-2 was obtained from Myco-Lab (Chesterfield, MO, U.S.A.; and HT-2 and DAS were purchased from Makor Chemicals (Jerusalem, Israel). Preparation of stock solutions and formation of HFB and TMS derivatives were carried out as previously described[11]. Amounts injected were generally 10–100 pg (10–20 times more for TMS ethers of type A trichothecenes). After 1 day there was a marked loss in response for NIV and NS HFB derivatives, but all other derivatives (HFB and TMS) could be kept for several days (at −15°C).

Gas chromatography

The apparatus consisted of a Varian Aerograph Model 3700 equipped with a J&W Scientific on-column capillary injector for wide bore (0.32 mm I.D.) columns, make-up gas conversion adapter, and a ^{63}Ni electron-capture detector (temperature 300°C); J&W fused-silica capillary columns (in approximately increasing order of polarity): SE-30 and SE-54 standard phase (respectively 30 m and 14 m × 0.312 mm I.D., 0.25 μm film thickness), Durabond (DB)-1701 and DB-17 (15 m × 0.33 mm I.D., 0.25 μm film thickness), DB-210 (15 m × 0.32 mm I.D., 0.50 μm film thickness), and DB-225 (15 m × 0.33 mm I.D., 0.25 μm film thickness), with helium carrier gas, flow-rate 2.1 ml/min and nitrogen make-up gas, flow-rate 27.3 ml/min. Column temperature programming varied with the column and derivative (Table I).

RESULTS AND DISCUSSION

The variation of retention times of ten trichothecene HFB derivatives with capillary column phase is shown in Fig. 1 and three chromatograms are shown as

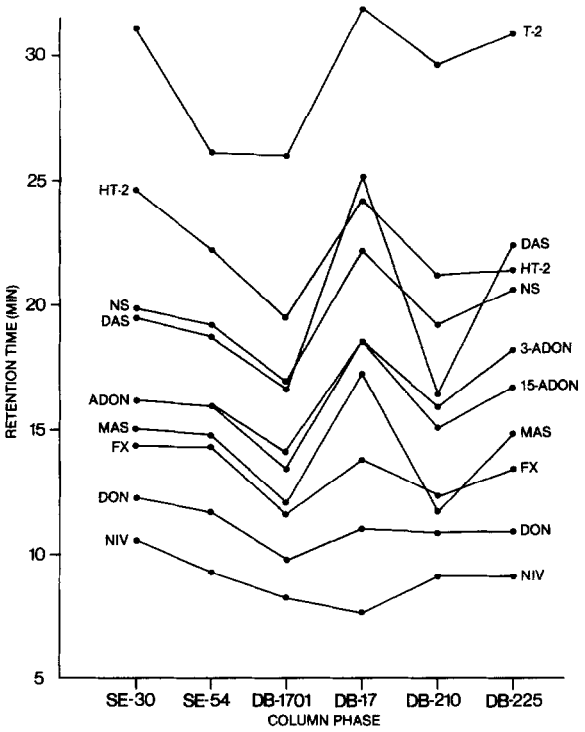

Fig. 1. Plot of retention times (min) of ten trichothecene HFB derivatives *versus* column phase of approximately increasing polarity.

examples in Figs. 2–4 (an additional trichothecene, DHCAL, was included for DB-225). The elution order does not change with column polarity for NIV, DON, FX, 3-ADON, NS, HT-2 and T-2 HFB derivatives. However, DAS has either a longer or shorter retention time than NS and HT-2 going from DB-1701 to DB-225; previously, DAS and HT-2 were not separable on an OV-17 packed column[18]. MAS elutes faster than FX only on DB-210, which could be related to the trifluoropropyl grouping present in that phase. 3-ADON and 15-ADON are separated as HFB derivatives on three of the four most polar capillary columns.

Variation of elution order of the TMS derivatives with column polarity is more dramatic than for the HFB derivatives, even for just the five type B trichothecenes (Figs. 5–8). From the data on SE-30, SE-54, DB-1701, DB-17, and DB-210 columns, it is also apparent that the total separation picture for the five type B and five type A trichothecene TMS ethers is markedly affected by column type (Figs. 6–8). DB-1701 (chromatogram not shown) differed from DB-210 in that MAS eluted in between FX and NIV forming an almost unresolved peak and that 15-ADON and 3-ADON were not resolved, making this the least useful column for type B trichothecenes. On DB-17, although DAS, NS, HT-2 and T-2 TMS ethers eluted last in a similar pattern to the DB-210 and DB-1701 chromatograms, there were reversals in the elution order for the type B trichothecenes (Fig. 5) and MAS came in between FX and 3-ADON, only partially resolved from the latter (chromatogram not shown).

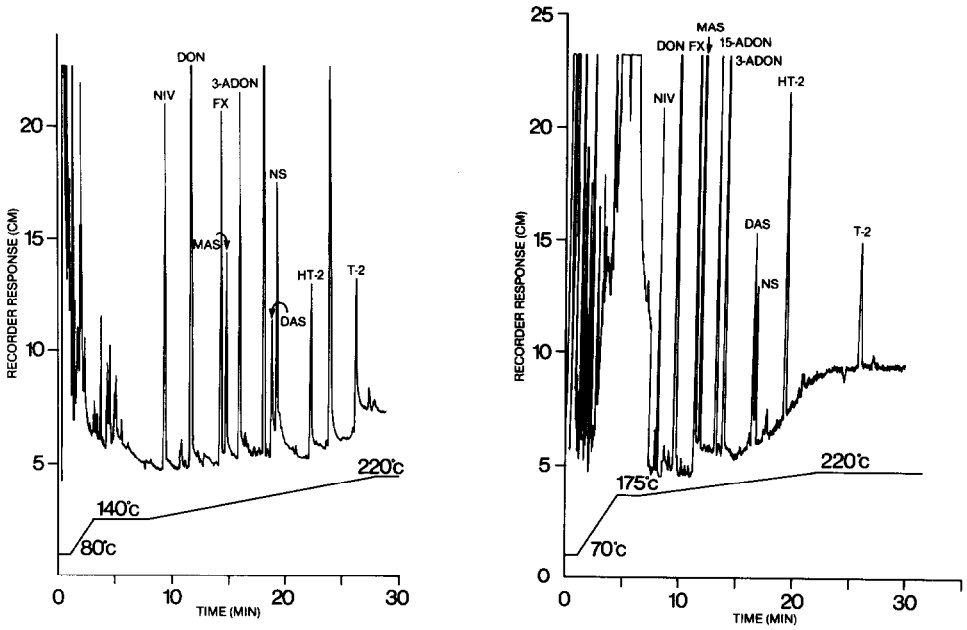

Fig. 2. Capillary GC of trichothecene HFB derivatives on SE-54; *ca.* 50 pg each of NIV, DON, FX, MAS, 3-ADON and HT-2 and 100 pg each of DAS, NS and T-2 injected. 3-ADON and 15-ADON are not separable on this column. Attenuation 64 × 10^{-12} A/mV.

Fig. 3. Capillary GC of trichothecene HFB derivatives on DB-1701; 20 pg of each injected, except for DAS and T-2 (40 pg). Attenuation 64 × 10^{-12} A/mV.

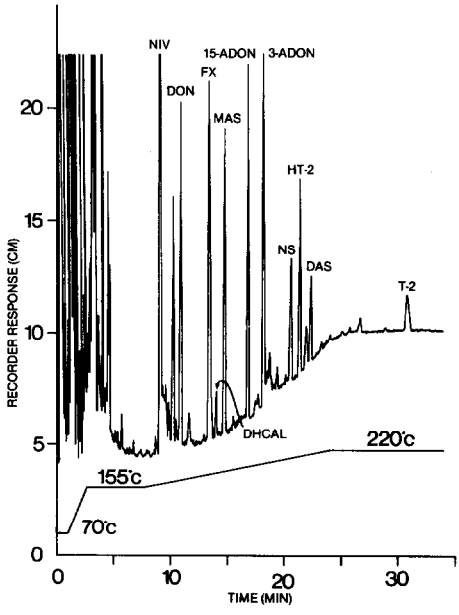

Fig. 4. Capillary GC of trichothecene HFB derivatives on DB-225; 20 pg of each injected, except for DAS and T-2 (40 pg) and DHCAL (qualitative). Attenuation 64 × 10^{-12} A/mV.

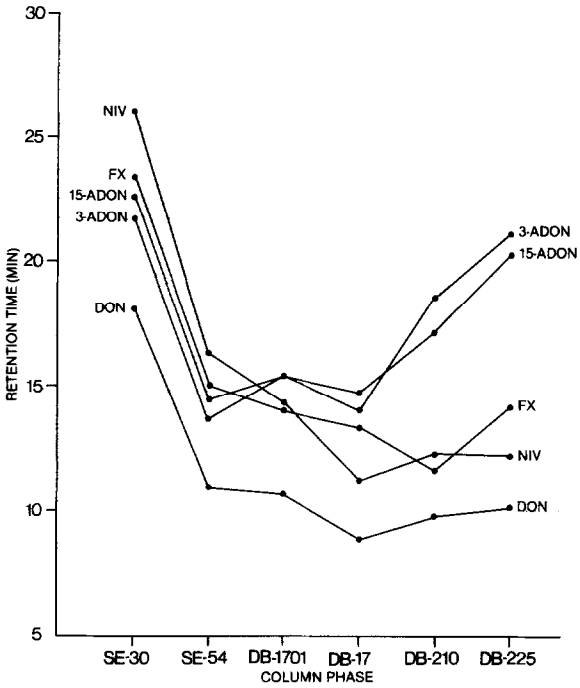

Fig. 5. Plot of retention times (min) of TMS derivatives of five type B trichothecenes *versus* column phase of approximately increasing polarity.

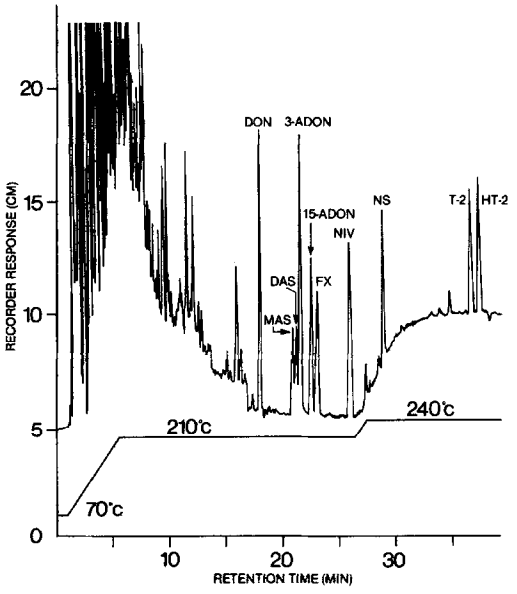

Fig. 6. Capillary GC of trichothecene TMS ethers on SE-30; *ca.* 30 pg each of DON, 3-ADON, 15-ADON, FX and NIV and 1000 pg each of MAS, DAS, NS, T-2 and HT-2 injected. Attenuation 64 × 10^{-12} A/mV.

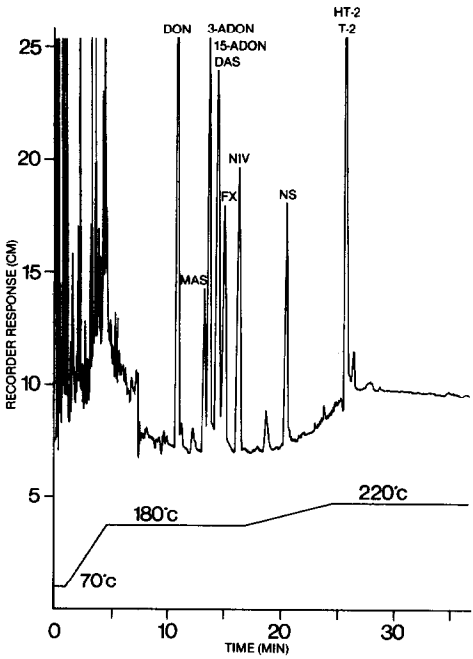

Fig. 7. Capillary GC of trichothecene TMS ethers on SE-54; 40 pg each of DON, 3-ADON, 15-ADON, FX and NIV and 400 pg each of MAS, DAS, NS, HT-2 and T-2 injected. Attenuation 64 × 10^{-12} A/mV.

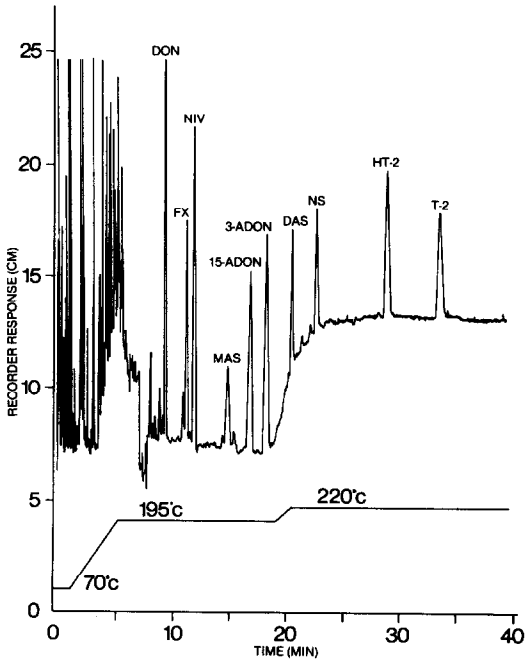

Fig. 8. Capillary GC of trichothecene TMS ethers on DB-210; 30 pg each of DON, FX, NIV, 15-ADON and 3-ADON and 600 pg of MAS, DAS, NS, HT-2 and T-2 injected. Attenuation 64 × 10^{-12} S/mV.

We found that T-2 and HT-2 TMS ethers were not separated on SE-54; they were separated on SE-30 and DB-210 but with opposite order of elution. Bata *et al.*[2] reported a minimal separation of T-2 and HT-2 TMS ethers on an SE-52 capillary column. Other workers have separated them on OV-101 and OV-1 capillary columns[3,16] but surprisingly, considering these are both methylsilicone phases, the reported elution orders were opposite for those two columns. The overall order of separation for DON, DAS, FX, NIV, T-2 and HT-2 TMS ethers on the OV-1 capillary column reported by Karppanen *et al.*[3] agrees with our present data on SE-30. In practice, TMS derivatization is not favoured for determination of type A trichothecenes by ECD because of lower sensitivity relative to type B trichothecenes[3,19].

Work is in progress to determine the best column for determination of selected type A and type B trichothecenes in corn and wheat.

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