Journal of Chromatography, 191 (1980) 327–331

CHROM. 12,234

Note

Capillary gas chromatographic–mass spectrometric determination of some mycotoxins causing fusariotoxicoses in animals

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The fungal genus *Fusarium* has been noted for an abundance of plant pathogens and toxic and non-toxic natural products. Among the latter, zearalenone (F-2 toxin) and 12,13-epoxytrichothecene derivatives have been regarded as the most important.

Zearalenone is an estrogenic substance, and as such it is responsible for swine mycotoxicoses also known as hyperestrogenism. In the female pig zearalenone poisoning gives rise to hypertrophy and prolapse of vulva, as well as to infertility and reduction of litter size[1]. The 12,13-epoxytrichothecenes (deoxynivalenol, diacetoxyscirpenol, neosolaniol, T-2 toxin, etc.) are closely related sesquiterpenoid compounds. Their structures contain a ring system named trichothecane[2]. Most are severely toxic for both man and animal, causing emesis, refusal of feed, haemorrhagic syndrome and death at low concentrations. Since *Fusarium* produced toxins are naturally occurring feed contaminants, the safety of their evaluation requires improved analytical methods.

Various methods have been employed for mycotoxin detection. Bio-assays[3,4] are sensitive, but not specific and thin-layer chromatography[5,6] (TLC) is neither sensitive nor specific. Gas chromatography (GC) procedures[7–9] are superior to TLC, but may not give specific enough results due to relatively low resolution of the packed columns. We report a new analytical method based on the use of high-resolution glass capillary columns, used together with the powerful and precise identification ability of the mass spectrometer.

EXPERIMENTAL

Materials

Mycotoxin standards: diacetoxyscirpenol was purchased by Makor Chemicals (Jerusalem, Israel), neosolaniol and T-2 toxin were prepared in our laboratory, F-2 toxin and deoxynivalenol were the generous gift of IMC Chemical (Terre Haute, Ill., U.S.A.) and Dr. Yoshizawa, Kagawa University (Kagawa, Japan), respectively. Materials were trimethylsilylated by TRISIL-TBT reagent (Pierce, Rockford, Ill., U.S.A.). Other organic solvents were distilled before use.

Apparatus

A Carlo Erba Fractovap Type 2450 gas chromatograph equipped with a flame ionization detector (FID) was used. The capillary wall-coated open-tubular columns (20 m × 0.256 mm I.D.) were prepared according to Grob and co-workers[10,11] and were coated with OV-101 and OV-17 liquid phases. Theoretical plate number was 5000/m and the peak symmetry and resolution was satisfactory for all components of the Grob *et al.*[12] test mixture. The temperatures of the injection port and detector were 250° and 280°, respectively; column temperature was programmed from 180° to 280° at 3.5°/min. The carrier gas was helium. Both splitless and split mode of injection were used at a split ratio of 1:50. A Finnigan Model 3200 GC–mass spectrometry (MS) data system was used for the identification of the compounds eluted from the GC columns. The column injection system and other GC conditions were as specified above; the temperatures of the separator and the transfer line were 275° and 290°, respectively. Electron energy was 70 eV. The chromatograms shown in Figs. 1–3 were, except otherwise specified, produced by the hard-copy unit of the computer system.

Preparation of feed samples

Samples of 100 or 200 g were ground, moistened with water (approximately 30%) and extracted with ethyl acetate. After filtration, the residue was extracted again with methanol, and the two extracts were combined and concentrated. Partition between light petroleum (b.p. 40–70°) and 33% aqueous methanol made it possible to remove disturbing fatty components. The light petroleum fraction was discarded, the methanolic portion was evaporated to dryness. An aliquot of the extract was silylated and injected into the gas chromatograph.

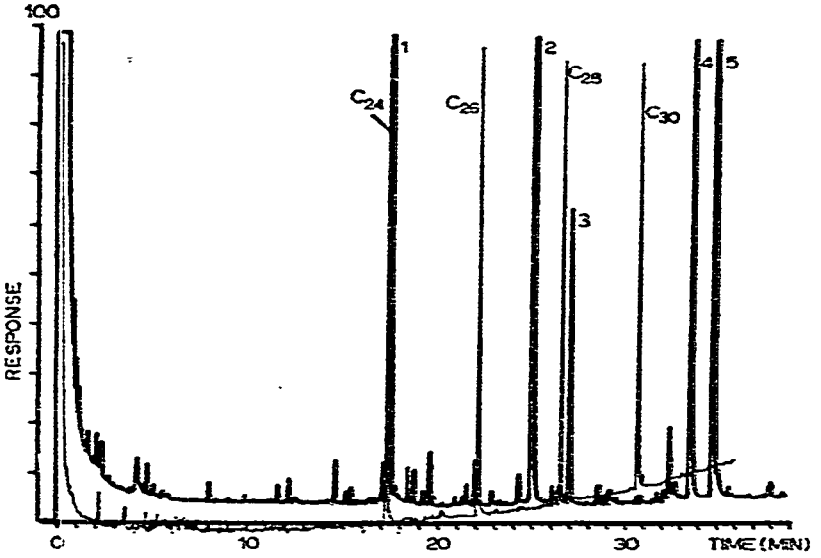

Fig. 1. Separation of standard mycotoxins. Peaks numbered 1–5 (bold line). The chromatogram of mycotoxins was copied upon that of *n*-alkenes (thin line). Column, 20 m long OV-17; linear gas velocity 40 cm/sec; splitting injection. For other conditions see text. Peaks: 1 = deoxynivalenol, 2 = diacetoxyscirpenol, 3 = neosolaniol, 4 = T-2 toxin, 5 = F-2 toxin.

TABLE I

RETENTION INDEX VALUES OF SOME *FUSARIUM* TOXINS

Values were calculated from a programmed-temperature run.

Toxin name	*Retention index*
Deoxynivalenol	2404
Diacetoxyscirpenol	2717
Neosolaniol	2817
T-2 Toxin	3133
Zearalenone (F-2 toxin)	3199

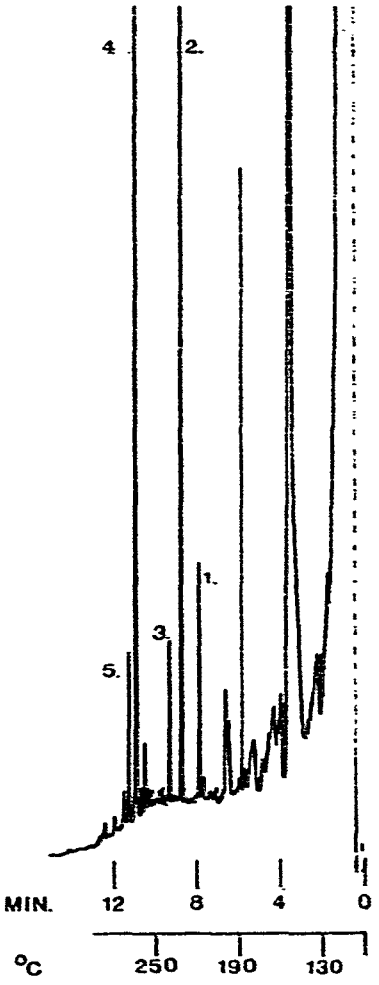

Fig. 2. Separation of 5–50 ng standard mycotoxins. Column, 10 m long OV-101; carrier gas hydrogen; linear gas velocity, 40 cm/sec; splitless injection. Peaks as in Fig. 1.

RESULTS AND DISCUSSION

Naturally occurring *Fusarium* toxins[13] can be detected in animal feeding stuff samples also by capillary gas chromatography, because in appropriately prepared columns the toxin peaks are sharp, tailing free and not diminished by absorption loss (Fig. 1.).

Retention index values can also be calculated from a programmed-temperature run[14]. These values are shown in Table I. These calculations were used in routine work as an additional identification tool before submission of the samples for GC–MS determination.

With adequately prepared columns, the material tension of the peaks was high enough in the ion source to evaluate the mass spectrum. The toxin standards and samples analysed by us resulted in equally qualitative as well as quantitative reproducible mass spectra, conforming those reported in the literature[15,16].

The capillary technique has the advantage that the use of shorter columns makes possible a considerable reduction of the analysis time, and at the same time the separation of the components is superior to that obtainable with packed columns (Fig. 2.). If a 20 m long column is employed with temperature programme, the analysis time may be as long as 35–40 min, but the resolution capacity becomes considerably improved.

The sentivity of the procedure is high enough for the detection of 50–100 ppb feed levels of toxin or 5–50 ng per injection (Fig. 2.). According to our own experience, the splitting injection mode should be preferred for routine determinations or with relatively high concentrations (above 1 ppm) of toxin, while the splitless system meets the requirements of MS and of the detection of low levels of toxin.

The high resolution of the capillary column accounts for complete separation of the toxins not only from one another, but also from contaminants (Fig. 3). Up to now only a limited number of samples (one mixed feed sample, three feeding

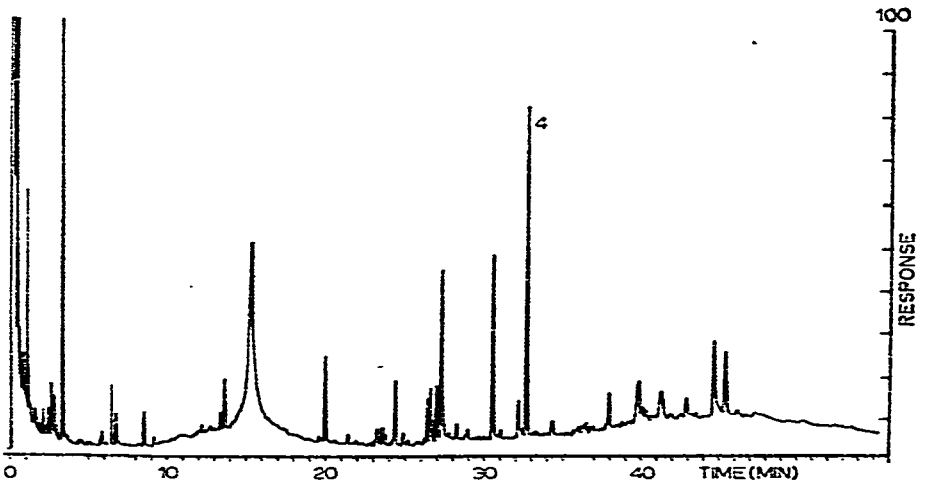

Fig. 3. Detection of T-2 toxin (4) in a corn sample. Defatted extract without further purification. Column, 20 m long OV-17; linear gas velocity, 40 cm/sec; splitless injection. For other conditions, see text.

stuff (corn) samples and a few culture extracts) have been available for analysis. All samples could be analysed preceding clean-up, *e.g.* ferric hydroxide gel or cupric carbonate depigmentation, column- or TLC purification, etc. Thus use of the new procedure not only economizes time and expense, but also minimizes toxin loss, and thereby improves the sensitivity of determination.

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