Journal of Chromatography, 252 (1982) 305–309
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,259

Note

Interference in the gas chromatographic determination of deoxynivalenol in cultures of *Fusarium solani* on corn

A. VISCONTI*
Istituto per lo Studio delle Tossine e Micotossine da Parassiti Vegetali del Consiglio Nazionale delle Ricerche, Via Amendola 197/F, 70126 Bari (Italy)
and
F. PALMISANO
Istituto di Chimica Analitica dell'Università degli Studi, Via Amendola 173, 70126 Bari (Italy)
(First received May 5th, 1982; revised manuscript received August 3rd, 1982)

Deoxynivalenol is a trichothecene toxin produced by certain *Fusarium* species, a causative agent of emesis, feed refusal and growth depression in animals[1]. Recent data[1–4] show that it is found, with increasing frequency, as a natural contaminant in the corn crops of different countries. Adequate analytical methods for the detection of this toxin in naturally contaminated foods and feedstuffs are therefore essential for screenings of deoxynivalenol occurrence in situations of environmental significance[5]. In this context modern polarographic methods such as differential-pulse polarography (DPP) can provide, as recently shown[6], an accurate and sensitive method for the detection of deoxynivalenol in contaminated corn.

Gas chromatography (GC) is, at present, the technique most used for the determination of trichothecenes, even though it is prone to interferences arising from the large number of sample components which can have very similar retention times to that of the toxin being investigated. For example, 1-glycerylmonolinoleate and 1-glycerylmonooleate interfere in the gas–liquid chromatographic (GLC) determination of T-2 toxin in mixed feeds[7], while mixed diglycerides interfere in the determination of T-2 toxin in milk[8]. In both cases the addition of a thin-layer chromatographic (TLC) clean-up step eliminated the interference; this combination of TLC and GLC is, in fact, common practice in trichothecene analysis. However the efficiency of the clean-up TLC procedure in the analysis of fungal-contaminated foodstuffs has not been fully evaluated for trichothecenes other than T-2 toxin and, in particular, has not been evaluated for deoxynivalenol.

This note demonstrates that an erroneous identification of deoxynivalenol in cultures of *Fusarium* spp. on corn can sometimes occur even when the TLC–GLC combination is used, probably because of a peculiar metabolite of the *Fusarium* spp. The use of comparison techniques such as DPP can avoid this problem.

EXPERIMENTAL

GC measurements were performed with a Hewlett-Packard Model 5830 gas

0021-9673/82/0000–0000/$02.75

chromatograph equipped with a 4 ft. × 4 mm I.D. glass column packed with 3% OV-1 on 100–120 mesh Gas-Chrom Q and a flame-ionization detector. Nitrogen was used as carrier gas with a flow-rate of 20 ml min^{-1}. The temperature was programmed from 150 to 280°C at 10°C min^{-1}.

Polarographic measurements were carried out using a PAR 174 A polarographic analyser equipped with a conventional three-electrode cell.

The GC–MS mass spectrometric (MS) apparatus was a Finnigan 4000 gas chromatograph–mass spectrometer coupled to an INCOS data system and equipped with an SE-54 20-m glass capillary.

The reagents used and the procedure for the production of toxins by *Fusarium* spp. on corn have been described elsewhere[6].

Strains of the following *Fusarium* species were assayed: *Gibberrella zeae* (Schw.) Petch (*Fusarium graminearum* Schw.), *Fusarium culmorum* (W. G. Smith) Sacc., *Gibberella intricans* Wollew. [*Fusarium equiseti* (Corda) Sacc.], *Fusarium oxysporum* (Schlecht.) Snyd et Hans., *Nectria haematococca* Berk. et Br. [*Fusarium solani* (Mart.) Snyd et Hans.].

The cultures were extracted with methanol–aqueous sodium chloride, re-extracted with chloroform (after hexane defatting) and cleaned-up on Sep-Pack C_{18} cartridges. Further purification of the extract was accomplished by preparative TLC on Merck precoated silica gel F-254 plates developed with chloroform–methanol–water (90:10:1).

RESULTS AND DISCUSSION

The *Fusarium* cultures were analysed for their deoxynivalenol content by either GC or DPP.

Results from cultures of *F. graminearum*, *F. culmorum*, *F. equiseti* and *F. oxysporum* showed a good correlation between the two techniques (typical results may be found in ref. 6) while in the case of cultures of *F. solani* surprising discrepancies were found. In fact, GC analysis of four cultures separately infected by four different strains of *F.solani* (ITM 131, ITM 132, ITM 133 and ITM 134) gave a deoxynivalenol concentration ranging from 0.8 to 2.9 ppm whereas no deoxynivalenol was detected by DPP.

Fig. 1 shows, as an example, the gas chromatogram of the silylated extract of a culture of *F. solani* ITM 134 (left side) compared to that of the standard toxin (right side). The peak with a retention time of 10.91 min could indicate the presence of deoxynivalenol in the sample. However the same extract when submitted to polarographic analysis gave a signal whose peak potential [in a Britton Robinson buffer (pH 8)] was −1.45 V *vs.* standard calomel electroce (SCE), *i.e.* 60–70 mV more negative than the expected value. The pH dependence of this wave was also completely different from that found previously[6] for deoxynivalenol, as shifts of *ca.* 60 mV per unit change in the pH of the buffer (instead of the expected value of *ca.* 20 mV) were found. The absence of deoxynivalenol in the sample was ascertained by addition of an authentical sample of this material whereupon a new peak occurred at −1.46 V *vs.* SCE (pH of buffer = 11.5), *i.e.* 200 mV more positive than the peak shown (under the same conditions) by the sample extract. On the other hand, the failure to detect deoxynivalenol by DPP cannot be ascribed to the technique not being sensitive

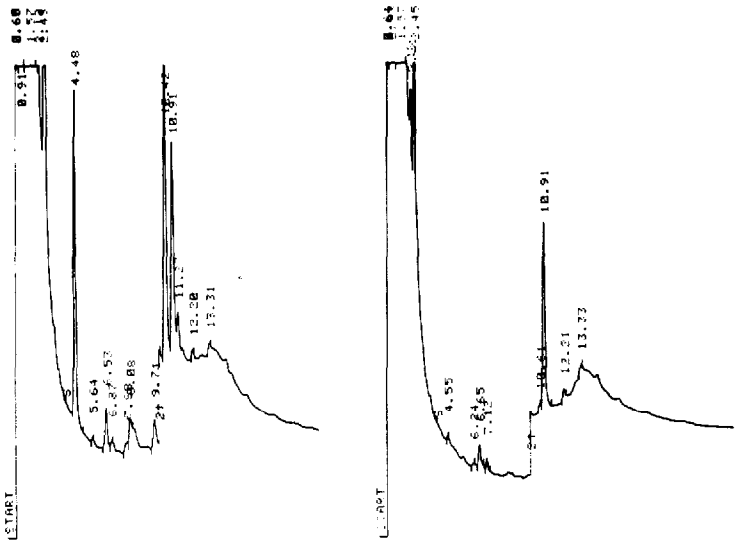

Fig. 1. Gas chromatogram of (left) the trimethylsilylated extract of a culture of *Fusarium solani* ITM 134 and (right) the trimethylsilylated deoxynivalenol standard.

enough because, as previously shown[6], its detection limit is more than one order of magnitude lower than the lowest toxin concentration indicated by the GC method.

The sample investigated by either DPP or GC was further submitted to analysis by GC–MS in order to resolve the previous conflicting results. As is evident from Fig. 2, the electron-impact (EI) spectra of the substance eluted at the same retention time as the trimethylsilyl ether–deoxynivalenol system (upper spectrum) and of the

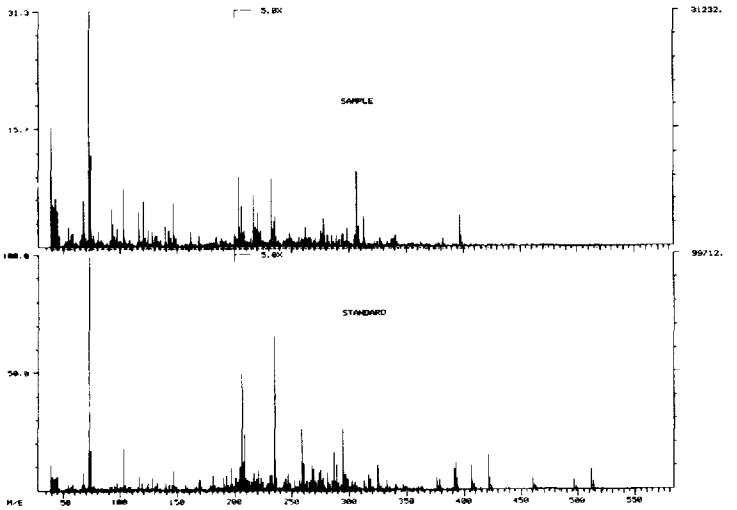

Fig. 2. EI mass spectra of the trimethylsilated deoxynivalenol standard (lower spectrum) and of the derivatized interfering compound eluted at the same retention time as deoxynivalenol (upper spectrum). Electron energy: 70 eV. Source temperature: 220°C.

trimethylsilyl ether–deoxynivalenol system itself (lower spectrum) are clearly different. The specific key fragments (*m/e* 512, 422 and 235) of deoxynivalenol were also absent when the selected ion monitoring mass chromatogram of the silylated extract from the culture of *F. solani* ITM 134 was recorded in the EI mode. The above results were furtherly confirmed by the mass chromatogram of the *m/e* 512 ion run in the negative chemical-ionization (CI) mode. As expected, this technique gave much less fragmentation (Fig. 3), had a sensitivity more than one order of magnitude higher than the EI ionization and was less prone to interferences from the sample matrix.

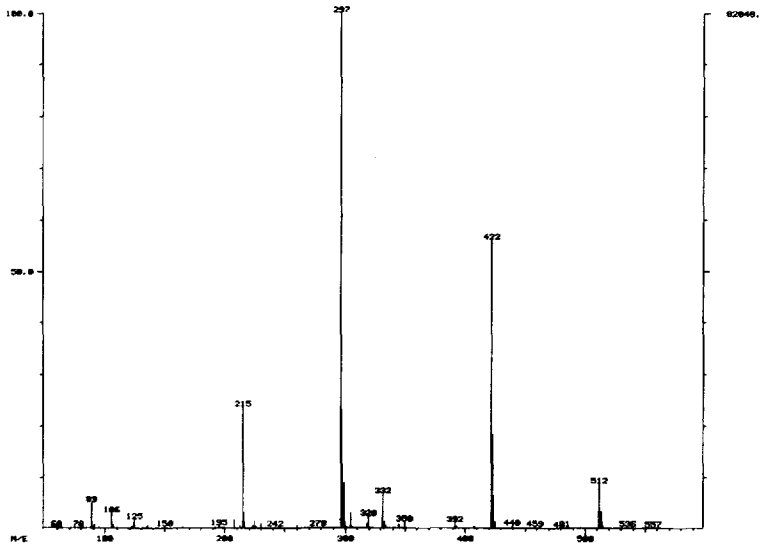

Fig. 3. Methane CI mass spectrum of the trimethysilylated deoxynivalenol standard. Electron energy: 70 eV. Source temperature: 220°C. Ionization chamber pressure: $5 \cdot 10^{-5}$ Torr.

These results show that the gas chromatographic peak obtained at 10.91 min can be ascribed to an interfering substance and agrees with mycological data[9] which indicate that until now *F. solani* is a "non-producer" of deoxynivalenol. Therefore the clean-up TLC method does not prove satisfactory for *F. solani*-infected corn. The experimental evidence that similar interference effects were not found when the same materials, methods and techniques were applied to other *Fusarium* cultures seems to indicate that the interfering substance may be a peculiar metabolyte of the *F. solani*. Further knowledge on the fungal metablism is needed for an unambigous identification of this metabolite.

This note should focus attention on the complexity of this matter and in particular on the problems of erroneous identification which can arise with complex matrices such as fungal-contaminated foodstuffs. The use of different analytical techniques is always recommended for solving such particularly complex problems; electroanalytical techniques such as DPP can offer cheap, sensitive and interference-free complementary methods for the determination of trichothecenes.

ACKNOWLEDGEMENTS

Professor A. Bottalico and P. G. Zambonin are gratefully acknowledged for their interest in the work. We thank the Finnigan AG of Basel for access to their GC–MS apparatus.

REFERENCES

1 S. V. Pathre and C. J. Mirocha, *J. Amer. Oil Chem. Soc.*, 56 (1979) 820.
2 R. F. Vesonder and A. Cigler, *Eur. J. Appl. Microbiol. Biotechnol.*, 8 (1979) 237.
3 A. Bottalico, P. Lerario, S. Frisullo and A. Visconti, in G. Cirilli (Editor), *La Contaminazione degli Alimenti con Micotossine*, Segreteria Simposi, Milan, 1982, p. 51.
4 A. Bottalico, P. Lerario and A. Visconti, *Phytopath. medit.*, 20 (1981) 1.
5 R. M. Eppley, *J. Amer. Oil Chem. Soc.*, 56 (1979) 824.
6 F. Palmisano, A. Visconti, A. Bottalico, P. Lerario and P. G. Zambonin, *Analyst (London)*, 106 (1981) 992.
7 C. J. Mirocha, S. V. Pathre and J. Behrens, *J. Ass. Offic. Anal. Chem.*, 59 (1979) 221.
8 G. J. Collins and J. D. Rosen, *J. Ass. Offic. Anal. Chem.*, 62 (1979) 1274.
9 C. J. Mirocha, S. V. Pathre and C. M. Christensen, in T. D. Willie and L. G. Morehouse (Editors), *Mycotoxic fungi, Mycotoxins, Mycotoxicoses: an Encyclopedic Handbook*, Vol. 1, Marcel Dekker, New York, 1977, p. 365.