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Note

Confirmation of nivalenol and deoxynivalenol by on-line liquid chromatography–mass spectrometry and gas chromatography–mass spectrometry

Comparison of methods*

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Nivalenol (NV) and deoxynivalenol (DON) are secondary fungal metabolites produced by a number of worldwide-distributed species of fungi imperfecti, such as trichothecium and trochoderma[1]. They represent two mycotoxins in a group of more than 40 compounds, all chemically derived from 12,13-epoxytrichothec-9-ene (scirpen). There is evidence that trichothecenes are responsible for several epidemic outbreaks of severe intoxications in humans and animals, and it is thus important to determine them in foods and feeds in trace amounts.

In our own experiments we realised that thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography with flame ionization or electron-capture detection of TMS- or HFBS-derivatives often lead to false results. Since then, for confirmation, gas chromatography–mass spectrometry (GC–MS) has been applied routinely to all contaminated food samples analysed in our laboratory.

The compatability of HPLC with a mass spectrometer and the direct liquid introduction technique (HPLC–DLI-MS) was made available with the development of microbore-column HPLC[2]. This technique, was used for the first time in combination with negative ion chemical ionization (NICI) in the selected ion monitoring mode to determine the NV and/or DON content of naturally contaminated cereal samples.

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EXPERIMENTAL

Cereal samples (20 g) are extracted with methanol in a blender, defatted with hexane and cleaned up on a florisil column. The trichothecenes are determined by micro liquid chromatography–mass spectrometry (LC–MS) in the negative ion chemical ionization mode, using the concentrate of extracts obtained by the procedure reported previously[3]. Two or three selected ions are monitored. After silylation of an aliquot of the extract, the trimethylsilyl derivatives NV and DON are analysed quantitatively by capillary gas chromatography–mass spectrometry (GC–MS). Here also two or three characteristic ions of the mycotoxins are detected in the selected ion monitoring mode.

Microbore HPLC

Apparatus, Jasco Familic 100 N-Pump with 0.3-μl loop injector; column, PTFE tubing, 16 cm × 0.5 mm I.D. packed with μFine Sil C_{18}-10, particle size 10 μm; transline, all-glass capillary, 0.1 mm I.D., direct liquid introduction (DLI) into ion source through solid sample insertion port. Eluent, acetonitrile–water (50:50); flow-rate, 5 μl/min.

Capillary GC–MS

Apparatus, Finnigan MAT 4023 quadrupole mass spectrometer including gas chromatograph and Incos data systems. GC conditions: J & W Scientific DB 5 fused-silica capillary column (10 m × 0.32 mm I.D.) 0.25 μm film-thickness; carrier gas, helium; flow-rate, 2 ml/min; injector temperature, 250°C; oven temperature, 80°C for 3 min, programmed from 80 to 280°C at 10°C/min, held at 280°C; injected volume, 1 μl, splitless. MS conditions: filament voltage, 70 eV; filament current, 0.3 mA; GC–MS reagent gas, methane; LC–MS mobile phase, acetonitrile–water scanning *m/e* 100–650 in 1.0 sec GC–MS; MID 0.25 sec per mass LC–MS; *m/e* 200–350 in 1.0 sec direct inlet probe (DIP); ion source temperature, GC–MS, 250°C and LC–MS 170°C; ion source pressure, $2 \cdot 10^{-5}$ Torr.

RESULTS

To determine the characteristic ions of the two mycotoxins in the negative ion chemical ionization mode, microgram amounts were analysed using the solid sample inlet port. The resulting mass spectra for the mycotoxin standards are shown in Fig. 1. The fragment ions for the trimethylsilyl derivatives obtained by positive and negative ion chemical ionization GC–MS are plotted in Fig. 2. All the masses that could be relevant for identification and confirmation are listed in Table I. Figs. 3 and 4 represent the mass chromatograms and the total ion current-trace for the selected-ion masses of NV and DON obtained during LC–MS. Fig. 5 shows the selected-ion monitoring plot after injection of a standard mixture of NV and DON in acetonitrile. Although both substances elute from the LC column in one unresolved peak (checked by UV detection at 225 nm), the identification of NV as the more polar (and therefore in reversed-phase LC the first eluting compound) is well demonstrated by a three-ion confirmation at *m/e* 264, 282 and 312.

The DON content of a naturally contaminated wheat kernel sample was con-

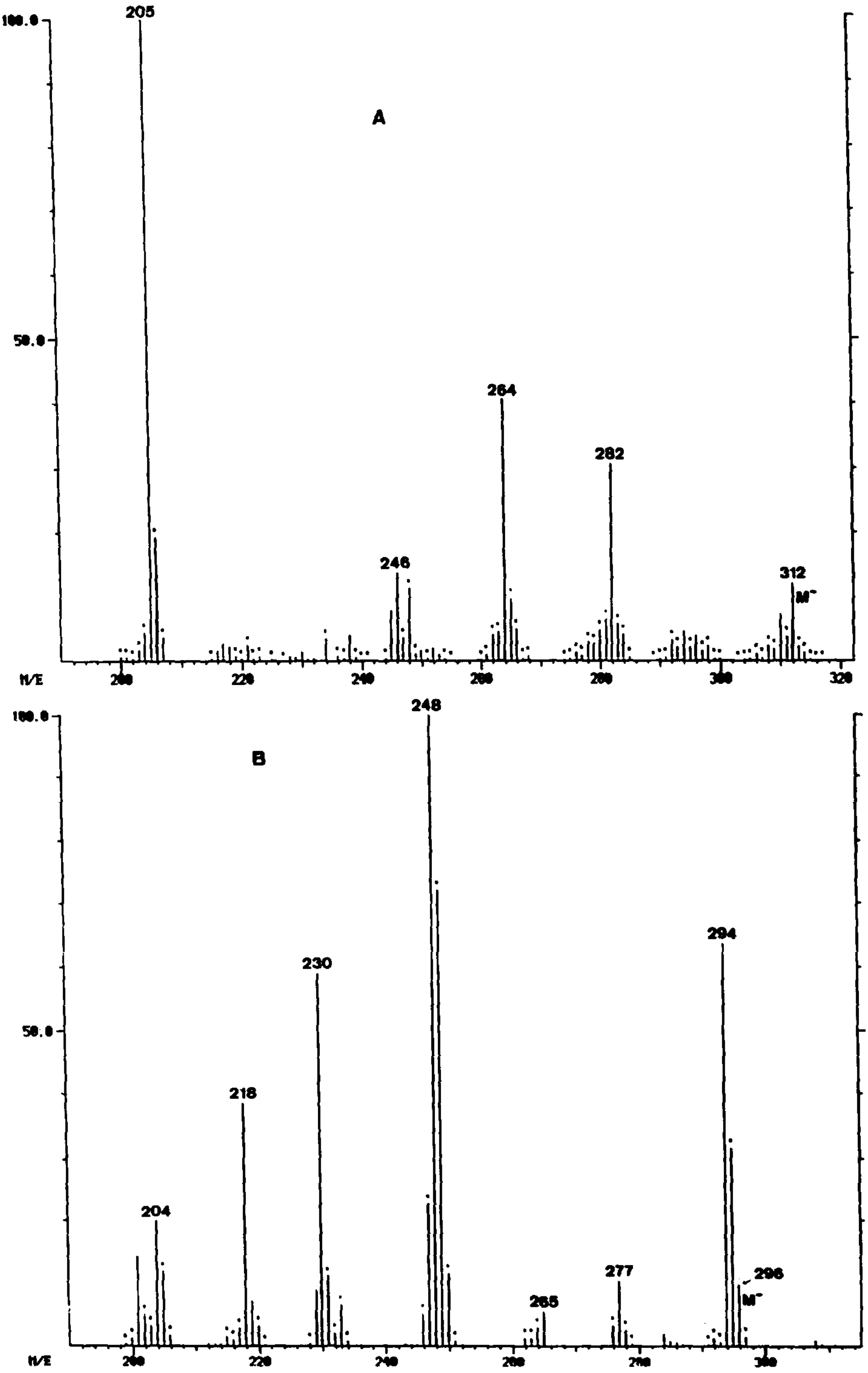


Fig. 1. NICI mass spectra of nivalenol (A) and deoxynivalenol (B) for determination of characteristic ions. Solid sample introduction. Sample amount, 1 μg of each compound (see Table I).

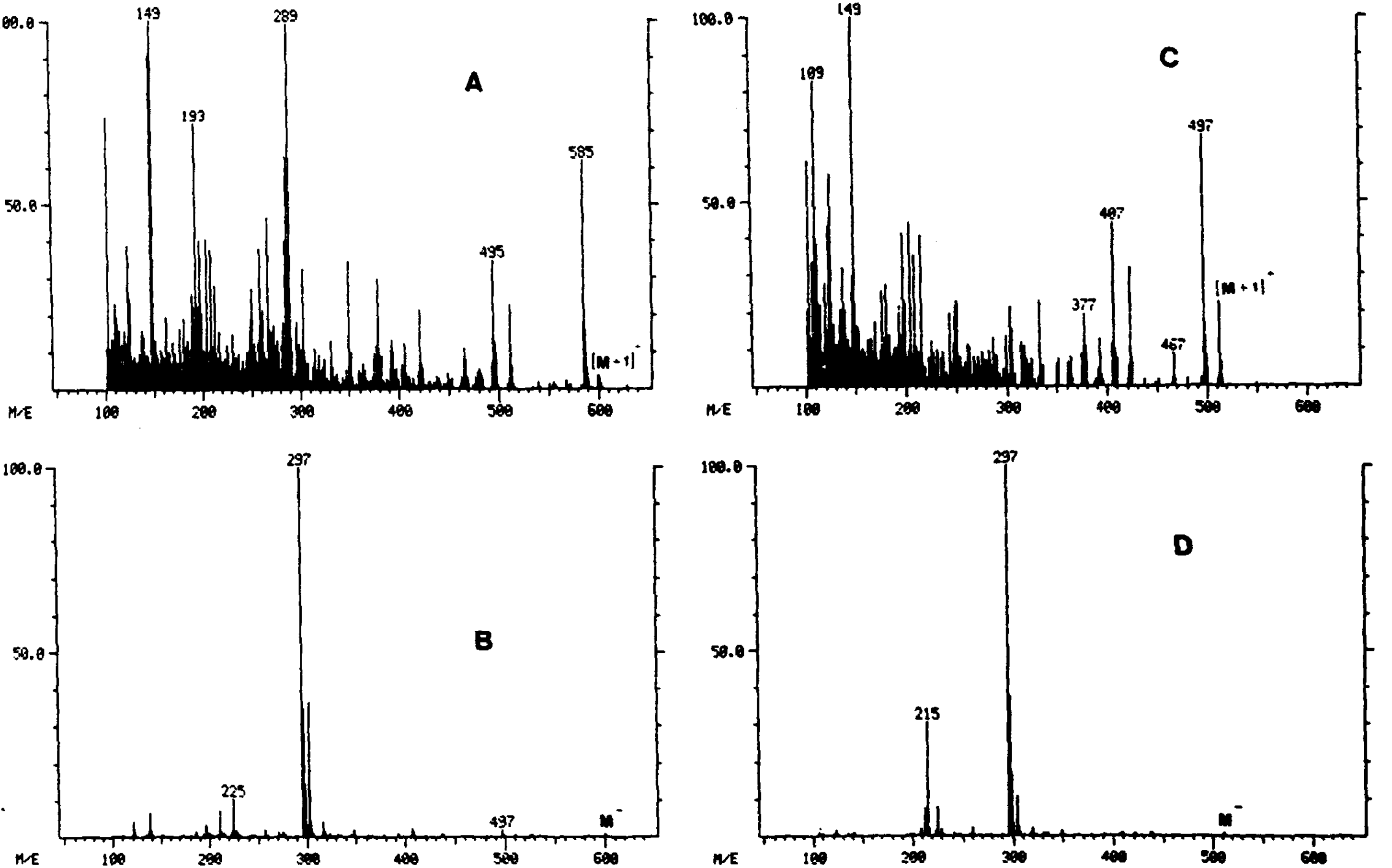
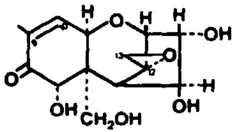
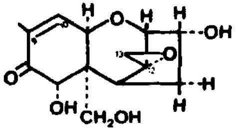
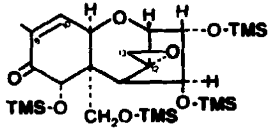
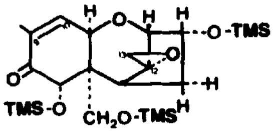


Fig. 2. GC–MS chemical ionization mass spectra of trimethylsilyl derivatives of (A) nivalenol, CI positive ion; (B) nivalenol, CI negative ion; (C) deoxynivalenol, CI positive ion; (D) deoxynivalenol, CI negative ion (see Table I).

TABLE I

SOME CHEMICAL AND PHYSICAL DATA ON NIVALENOL AND DEOXYNIVALENOL AND THEIR CORRESPONDING TRIMETHYLSILYL DERIVATIVES

	Chemical structure	*Molecular weight*	*Characteristic ions obtained by*		
			Solid sample probe NICI mode	*GC–MS*	
				Positive ion CI mode	*NICI mode*
Nivalenol		312	264,282,312	–	–
Deoxynivalenol		296	248,294,295,296,265	–	–
Nivalenol trimethylsilyl derivative		600	–	289,585,511	297,303,298,600
Deoxynivalenol trimethylsilyl derivative		512	–	497,423,513	297,296,305,512

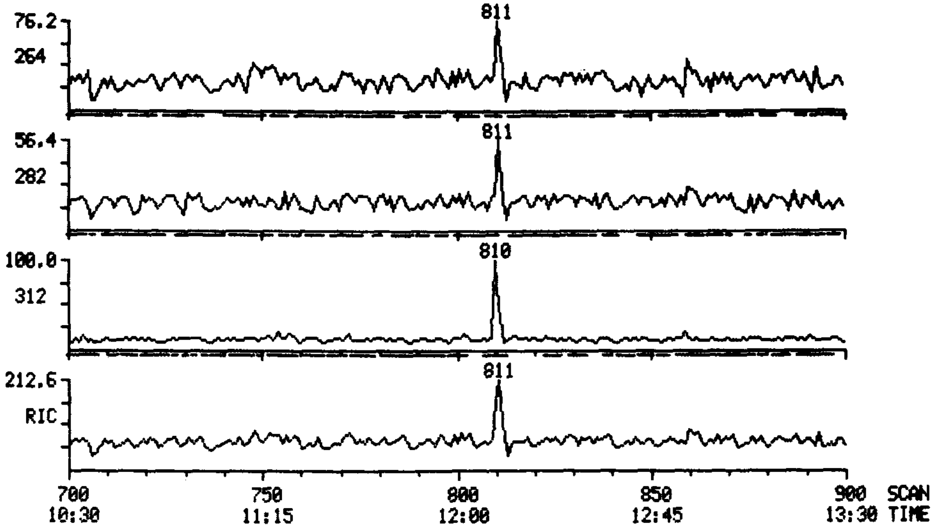


Fig. 3. Micro LC–MS multiple ion detection (MID) reconstructed ion chromatogram (RIC) and mass chromatograms of nivalenol standard, 50 ng. Time in min:sec.

firmed by LC–MS, prior to derivatization and quantification by GC–MS. The absence of NV was verified by the mass chromatogram of the characteristic fragment ion at *m/e* 282 in Fig. 6. The contamination level of the sample was 40 μg/kg, determined by GC–MS. Quantification is achieved by an internal standard method: in our experiments, 2,2′,3,3′,4,5-hexachlorobiphenyl serves as an internal standard. A calibration curve is established fore the peak-area ratio of the mycotoxins and the chlorinated biphenyl *versus* the amount of mycotoxin present.

Fig. 7 shows an example of quantitative GC–MS analysis of a wheat bran sample containing both NV and DON.

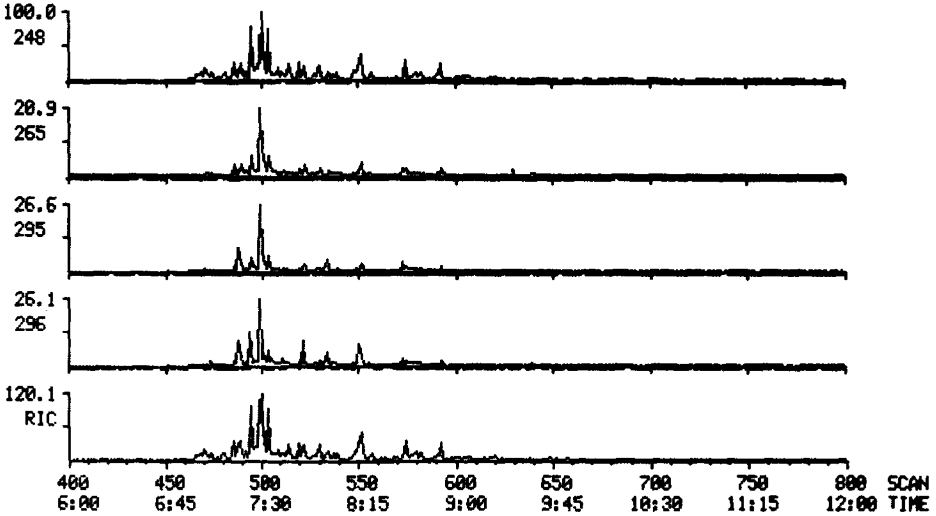


Fig. 4. Micro LC–MS MID RIC and mass chromatograms of deoxynivalenol standard, 50 ng.

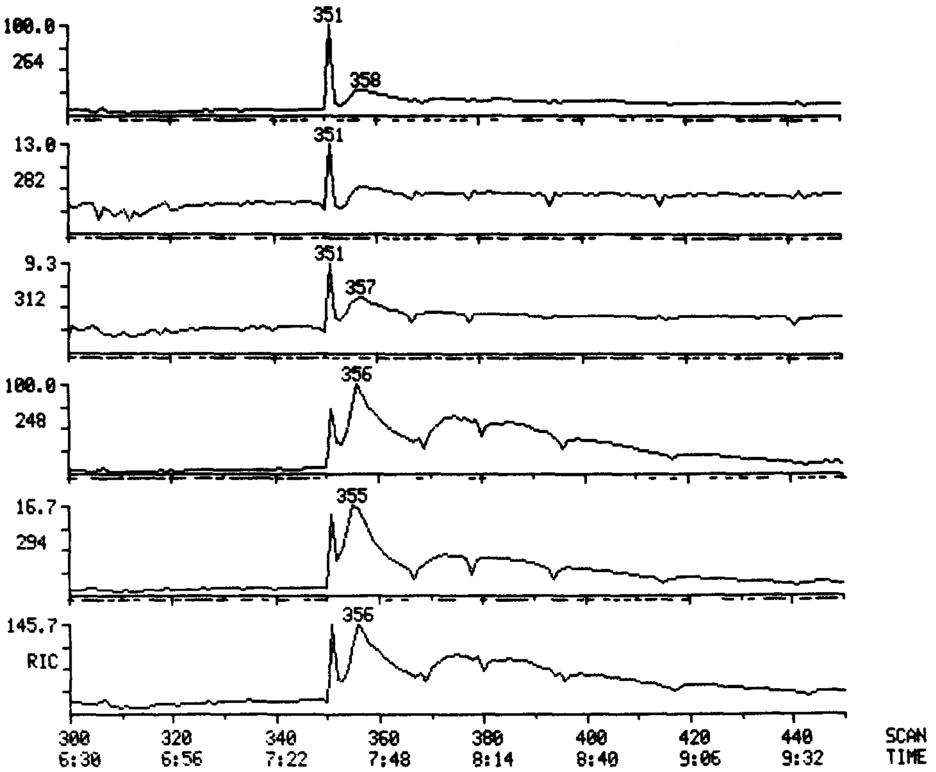


Fig. 5. Micro LC–MS MID RIC and mass chromatograms of a standard mixture of nivalenol and deoxynivalenol, 200 μg/ml each compound. Selected ions for nivalenol are *m/z* 264, 282 and 312, and for deoxynivalenol *m/z* 248 and 294.

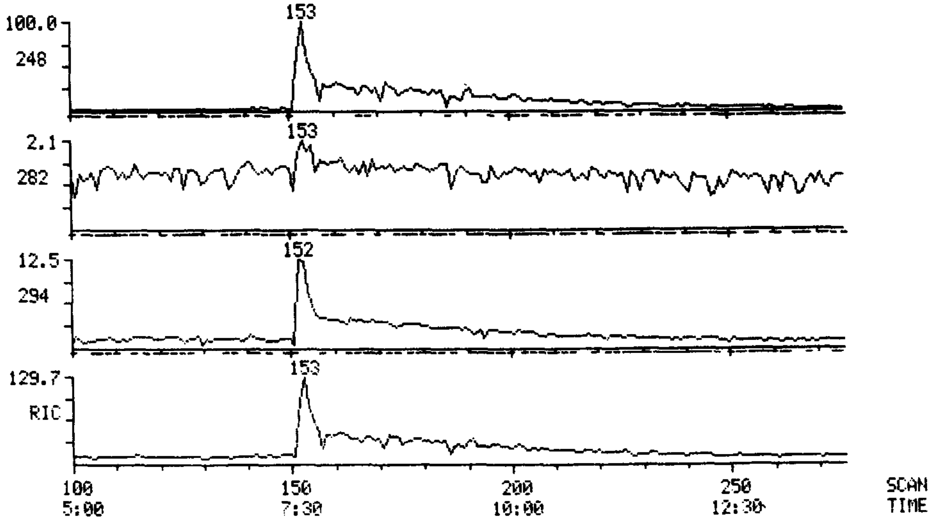


Fig. 6. Micro LC–MS MID RIC and mass chromatograms of a wheat kernel sample, contaminated with 40 μg/kg of deoxynivalenol, as determined by capillary GC–MS in the NICI mode. The ion monitoring for *m/z* 282 was used to exclude the presence of nivalenol.

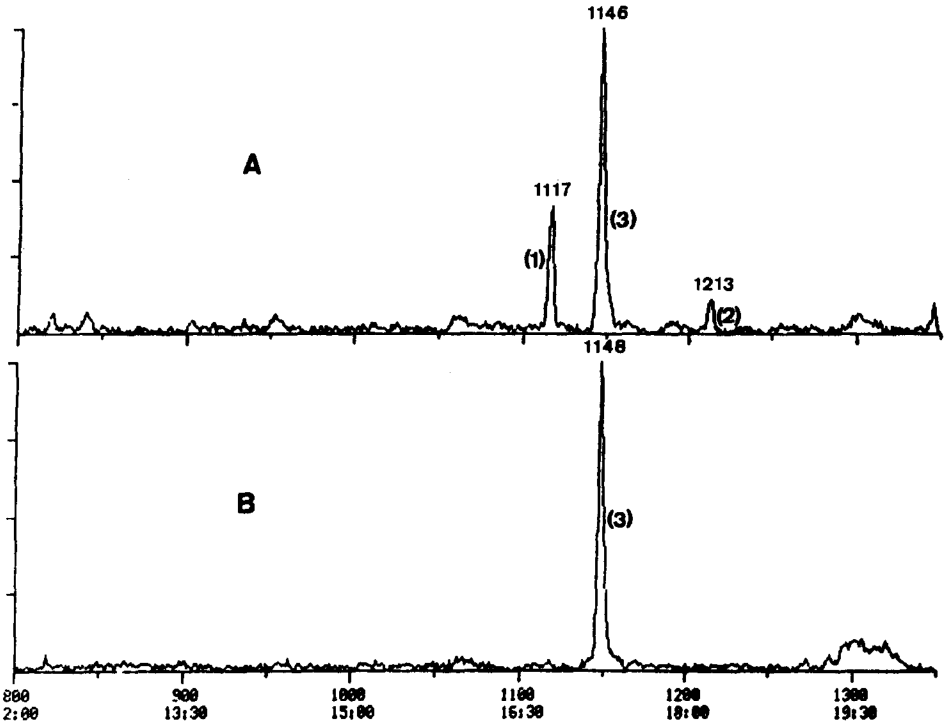


Fig. 7. Capillary GC–MS MID RIC of the masses *m/z* 297, 303, 305, 341, 512 and 600. Mass *m/z* 341 is the selected ion of the internal standard, 2,2′,3,3′4,5-hexachlorobiphenyl. Peaks: 1 = deoxynivalenol-TMS; 2 = nivalenol-TMS; 3 = internal standard. (A) Silylated wheat bran extract from sample containing 76 μg/kg of deoxynivalenol and 50 μg/kg of nivalenol. (B) Uncontaminated wheat flour extract.

CONCLUSIONS

Under the experimental conditions described in this paper, we found the combination of direct liquid introduction HPLC–MS in the negative ion chemical ionisation mode and selected-ion monitoring to be a useful tool to confirm the identity of nivalenol and deoxynivalenol in food sample extrcts. It is especially applicable to extracts that showed positive results in TLC or HPLC.

The minimum detectable concentrations of the LC–MS method were of the order of 5–10 μg/kg, which is very near the sensitivity obtained by the GC–MS method presented elsewhere in detail[3].

Quantification, as in GC–MS, using an internal standard method would only be possible with appropriate accuracy if all the parameters of the LC–MS system are stable under optimized conditions.

There are still limiting factors to the performance of the mass spectrometer, such as impurities in the highly concentrated extracts and fluctuations in the eluent transfer from LC into the ion source and these can lead to a big decrease in the sensitivity of the instrument.

If the technical problems can be solved, LC-DLI–MS will also become a rapid routine method for the identification and quantification of nivalenol and deoxynivalenol in foods and feeds.

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