Analysis of *Fusarium* mycotoxins by gas chromatography–Fourier transform infrared spectroscopy

J. Christopher Young[*,a], David E. Games[b]

[a] *Plant Research Centre, Agriculture Canada, Ottawa K1A 0C6, Canada*
[b] *Mass Spectrometry Research Unit, Department of Chemistry, University College of Swansea, Singleton Park, Swansea SA2 8PP, Wales, UK*

Abstract

The Fourier transform infrared (FTIR) spectra of selected *Fusarium* mycotoxins of various structure types were determined. Absorptions were observed for the following functionalities: hydroxyl at 3625–65 cm^{-1} and 3482 cm^{-1}, the latter being associated with a 7α-hydroxyl adjacent to an 8-carbonyl in *keto* trichothecenes; carbonyl at 1760–6 cm^{-1} for 5-membered rings and at 1695–8 cm^{-1} for those conjugated to a single C=C in a six-membered ring; acetate carbonyl at 1765 cm^{-1} and acetate C–O at 1220–9 cm^{-1}; and C=C at 1680 cm^{-1}. Gas chromatography combined with FTIR and mass spectrometry was applied to the identification of some mycotoxins in a *F. roseum* liquid culture extract.

1. Introduction

Mycotoxins are secondary fungal metabolites formed during the growth of certain fungi when environmental conditions of moisture, temperature and host are suitable. There is considerable interest in the analysis of these compounds because they can occur in food products and are toxic to humans and animals and plants [1]. The *Fusarium* spp. produce an impressive diversity of secondary metabolites (see Fig. 1).

Analysis of mycotoxins typically involves extraction from the sample matrix followed by some preliminary cleanup, chromatographic separation [thin-layer, gas (GC) or high-performance liquid (HPLC)] and detection [2]. Some of the *Fusarium* mycotoxins can be analyzed directly by GC, while others require derivatization. Confirmation of identity usually requires comparison of at least two independent properties, such as chromatographic retention time (or R_F) and a spectrum. One of the most powerful combinations is the coupling of GC with mass spectrometry (MS), which frequently enables direct identification of known compounds and location and partial identification of new compounds. Infrared (IR) spectroscopy has traditionally been restricted to functional group identification in pure samples. However, the recent availability of Fourier transform infrared spectroscopy (FTIR) detectors that can be coupled to GC effluents gives additional dimensions to the analysis of complex mixtures [3–6]. The IR spectra of the individual components of a complex mixture can be recorded as they elute, and, as with MS, such spectra also can be
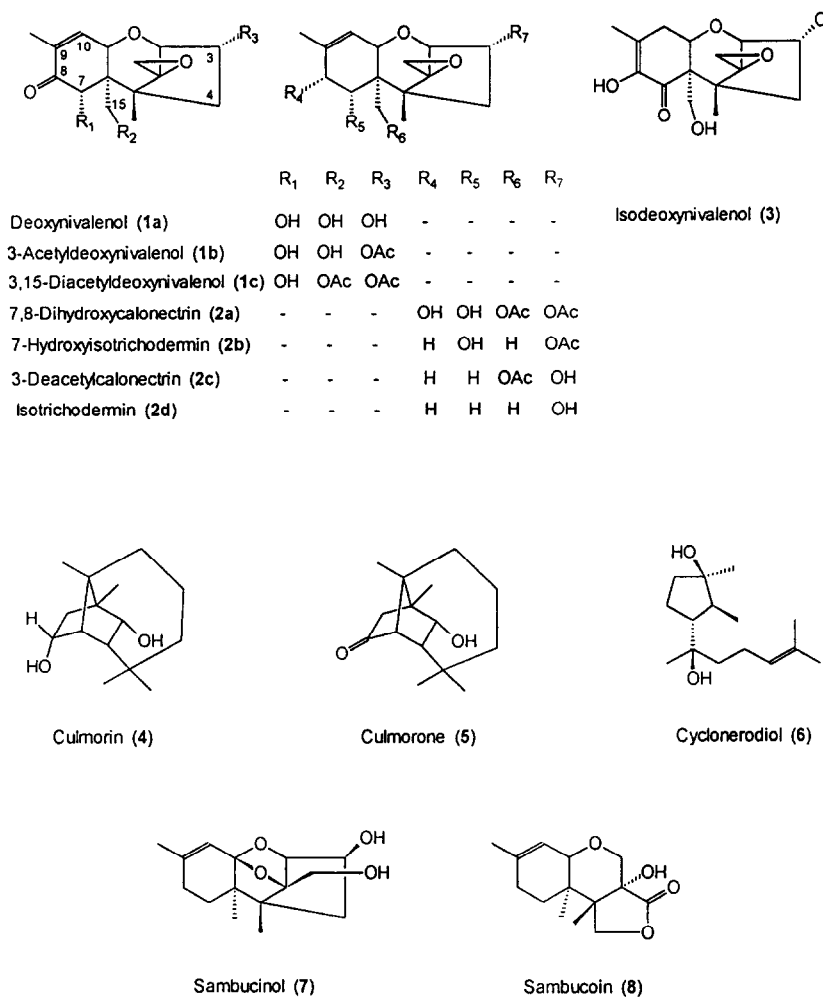
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* Corresponding author.

	R₁	R₂	R₃	R₄	R₅	R₆	R₇
Deoxynivalenol (1a)	OH	OH	OH	-	-	-	-
3-Acetyldeoxynivalenol (1b)	OH	OH	OAc	-	-	-	-
3,15-Diacetyldeoxynivalenol (1c)	OH	OAc	OAc	-	-	-	-
7,8-Dihydroxycalonectrin (2a)	-	-	-	OH	OH	OAc	OAc
7-Hydroxyisotrichodermin (2b)	-	-	-	H	OH	H	OAc
3-Deacetylcalonectrin (2c)	-	-	-	H	H	OAc	OH
Isotrichodermin (2d)	-	-	-	H	H	H	OH

Isodeoxynivalenol (3)

Culmorin (4)

Culmorone (5)

Cyclonerodiol (6)

Sambucinol (7)

Sambucoin (8)

Fig. 1. Structures of some *Fusarium* mycotoxins.

compared with those in IR data bases for confirmation of identity.

This is the first study that reports on the FTIR spectra of *Fusarium* mycotoxins and illustrates the combined application of GC–FTIR and GC–MS to the analysis of a *F. roseum* liquid culture extract.

2. Experimental

2.1. Mycotoxins and reagents

Mycotoxin standards were obtained from liq-

uid cultures of various *Fusarium* spp. (prepared in the Plant Research Centre laboratories, Ottawa, Canada). Purity was established by HPLC and nuclear magnetic resonance analyses. A natural mixture of mycotoxins was obtained from a liquid culture extract of *F. roseum* according to the method of Greenhalgh *et al.* [7]. Standards were dissolved in analytical-reagent grade methanol at a concentration of 1 mg/ml.

2.2. Gas chromatography–Fourier transform infrared (GC–FTIR) analysis

GC–FTIR analyses were conducted on a Hew-

lett-Packard 5890A/GC–5965B/IRD (Hewlett-Packard, Avondale, PA, USA). A J&W Scientific DB5 30 m × 0.25 mm I.D. column with 0.26 μm film (J&W Scientific, Folsom, CA, USA) was used in the splitless mode with a purge delay of 30 s, injector 250°C, and the column was temperature programmed from 100–280°C at 15°C min⁻¹. Transfer lines were at 280°C.

2.3. Gas chromatography–mass spectrometric (GC–MS) analysis

GC–MS analyses were conducted on a Hewlett-Packard 5971A mass selective detector in the electron ionization (EI) mode at 70 eV. A J&W Scientific DB5 30 m × 0.25 mm I.D. column with 0.26 μm film was used in the splitless mode with a purge delay of 30 s, injector 250°C, and the column was temperature programmed from 100–280°C at 15°C min⁻¹.

Identity of components in the *F. roseum* extract was established by comparison of observed FTIR and MS spectra to those in spectral data bases as well as congruence of chromatographic retention times with those of the corresponding standards.

3. Results and discussion

3.1. Fourier transform infrared spectra of standards

Table 1 summarizes the observed FTIR and reported literature value absorption bands for the *Fusarium* mycotoxins examined. Very sharp hydroxyl band absorptions were observed in the 3625–3665 cm⁻¹ and 3482 cm⁻¹ regions. The absorption at 3482 cm⁻¹ was associated with a 7α-hydroxyl adjacent to an 8-carbonyl [*e.g.* in 4-deoxynivalenol (DON) (**1a**)]. This assignment was supported by the observed reduction in and disappearance of the 3665 cm⁻¹ absorption band when going from DON to 3-acetylDON (**1b**) and 3,15-diacetylDON (**1c**), respectively. The assign-

ment of the 3482 cm⁻¹ band to an α-hydroxy-ketone in trichothecenes has not been reported before. Bands in this region have been observed, when measured as a film or KBr pellet (see ref. 8, pp. 203, 209, 215), however they tend to be obscured by the general broad hydroxyl absorption. The carbonyls in a five-membered ring appeared at 1760–1767 cm⁻¹, while those conjugated to a single double bond in a six-membered ring appeared at 1695–1698 cm⁻¹. There were two sharp absorption bands associated with acetates: one at 1765 cm⁻¹ attributed to the carbonyl and another at 1220–1229 cm⁻¹ attributed to the C–O bond. A very strong C=C absorption for *iso*-4-deoxynivalenol (IDON) (**3**) was observed at 1642 cm⁻¹ whereas those in sambucinol (**7**) and sambucoin (**8**) at 1680 cm⁻¹ were quite weak. The 9,10 double bond absorptions in the trichothecenes studied were generally masked by the overlapping carbonyl absorptions.

Differences in absorption frequencies and band shapes were noted in all cases between those determined by GC–FTIR and those by "classical" methods (*e.g.* in a KBr pellet, as a thin film on NaCl, or in a Nujol mull). GC–FTIR hydroxyl band absorptions were very sharp, compared with the generally very broad absorptions reported by other techniques, and were shifted higher by up to 200 cm⁻¹ into the 3625–3665 cm⁻¹ region. Carbonyl absorptions were shifted higher by 25 and 10 cm⁻¹ for those in a five-membered ring or conjugated to one double bond in a six-membered ring, respectively. In acetates, the carbonyl absorption was shifted higher by about 25 cm⁻¹ while that for the C–O absorption was shifted lower by about 10 cm⁻¹. Welti [12] noted that the IR bands associated with stretching and deformation vibrations shift to higher and lower frequencies, respectively, when measured in the vapour state as compared with those obtained in the liquid or solid states.

3.2. Application of GC–FTIR to analysis of a Fusarium extract

One of the advantages of GC–MS is the ability

Table 1
Summary of Fourier transform infrared spectra of selected *Fusarium* mycotoxins

Compound [a]	Absorption maxima (cm^{-1})				Literature
	Hydroxyl	Carbonyl	Acetate	C=C	
3-Acetyl-4-deoxynivalenol (1b)	3664, 3482	1695	1767, 1231		
	3465 [b]	*1685*	*1745*		Ref. 8 [c]
Calonectrin (2d)			1765, 1232		
			1745, 1240		Ref. 9 [d]
Culmorin (4)	3652				
	3340				Ref. 9 [d]
Culmorone (5)	3653	1767			
	3420	*1743, 1725*			Ref. 9 [d]
Cyclonerodiol (6)	3644				
	3425				Ref. 10 [e]
3-Deacetylcalonectrin (2c)	3626		1765, 1227		
4-Deoxynivalenol (1a)	3646, 3481	1698			
	3470, 3430,	*1680*			Ref. 8 [c]
iso-4-Deoxynivalenol (3)	3588, 3476	1690		1642	
3,15-Diacetyl-4-deoxynivalenol (1c)	3482	1698	1767, 1220		
	3455	*1685*	*1745, 1240*		Ref. 9 [d]
7,8-Dihydroxycalonectrin (2a)	*3540*		*1735, 1265*		Ref. 9 [c]
			1715, 1252		
7-Hydroxyisotrichodermol (2b)	3637				
Sambucinol (7)	3660, 3570			1680	
	3350			*1675*	Ref. 11 [c]
Sambucoin (8)	3605	1760		1678	
	3380	*1735*		*1760*	Ref. 11 [c]
iso-Trichodermol (2e)	3625				

[a] See Fig. 1 for structures.
[b] Literature values in italics.
[c] Determined in KBr pellets.
[d] Determined as thin film on NaCl plates.
[e] Method of determination not reported.

to conduct retrospective selected-ion monitoring of acquired data, which can result in simplification of chromatograms and aid in the identification of individual components. The possibility for computerized comparison of acquired spectra with those in a library for identification purposes also exists. GC–FTIR can offer similar advantages, as illustrated below.

Fig. 2a shows the GC–FTIR total response chromatogram of a *F. roseum* extract. Examination of the FTIR spectrum (Fig. 3a) of the major component A revealed an absorption at 3652 cm^{-1} indicative of the presence of a hydroxyl group. A library search gave an excellent match

with the FTIR spectrum of culmorin (4). The mass spectrum [13] of this component, obtained by GC–MS using the same column, confirmed the FTIR structural assignment.

The second major component in the GC–FTIR total response chromatogram, peak E, was next examined and its infrared spectrum (Fig. 3e) showed the presence of at least two hydroxyl groups (3644 and 3481 cm^{-1}) and a conjugated carbonyl (1695 cm^{-1}). A library search showed an excellent match with DON (1a) and this assignment was confirmed by the mass spectrum [13]. A search of the chromatogram for peaks with particular absorption frequencies was used
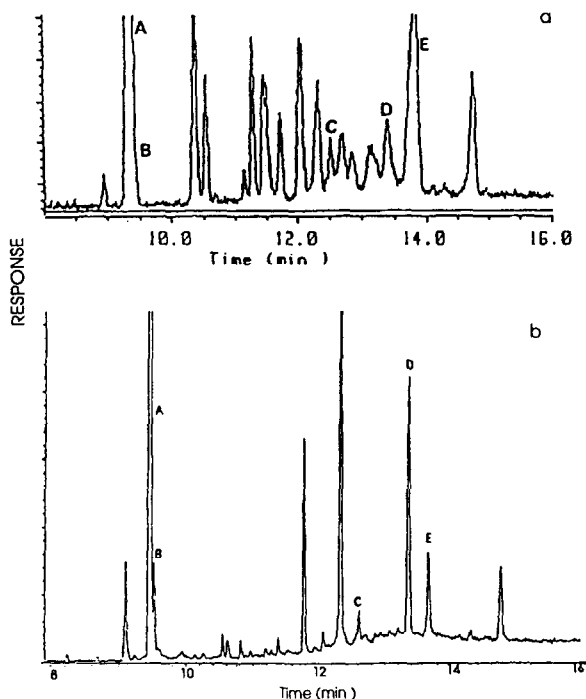
Fig. 2. Gas chromatogram of a *Fusarium roseum* liquid culture extract. Separation by GC on a 30 m × 0.25 mm I.D. DB5 column temperature programmed from 100–280°C at 15°C/min. (a) Detection by Fourier transform infrared spectrocopy; (b) detection by electron ionization mass spectrometry. For peaks A–E, see Fig. 3.

in the exploration for additional components. Fig. 4 shows the results obtained with searching for hydroxyl absorptions. Trace a (3655–3665 cm^{-1}) shows the secondary hydroxyls of culmorin (**4**) at 9.37 min and DON (**1a**) at 13.84 min while in trace b the hydroxyl α to the carbonyl in DON (3465–3485 cm^{-1}) is readily identified as is an additional component (D) at 13.40 min. Component D also showed hydroxyl absorptions at 3588 and 3640 cm^{-1}, a carbonyl absorption at 1690 cm^{-1} and a very strong C=C absorption at 1642 cm^{-1}. A library search of the FTIR spectrum of this component (Fig. 3d) gave an excellent match with IDON (**3**) and its mass spectrum [13] was also consistent with this structural assignment.

Examination of chromatograms for C=O and C=C frequencies (Fig. 5) confirmed the presence of DON (**1a**) (trace b, 1690–1700 cm^{-1}) and

IDON (**3**) (trace c, 1635–1650 cm^{-1}). In trace a (1760–1770 cm^{-1}), the presence of a minor component (B) containing a carbonyl was noted at the retention time for culmorin (A). Examination of the GC–MS total ion chromatogram (Fig. 2b) of the extract showed a minor peak eluting shortly after that of culmorin. Retrieval of the FTIR spectrum for this component from the major peak resulted in a spectrum (Fig. 3b), which showed the presence of a hydroxyl group (3652 cm^{-1}) and a carbonyl group (1767 cm^{-1}) and some similarity in the "fingerprint region" (800–1500 cm^{-1}) with that of the spectrum of culmorin (Fig. 3a). Its mass spectrum [13] showed a molecular ion at *m/z* 236, two mass units less than culmorin. This information suggested that one of the hydroxyl groups in culmorin is changed to a ketone. A library search of the FTIR spectrum gave an excellent match with culmorone (**5**) and the mass spectrum [13] is in agreement with this structural assignment.

Acetylated compounds are common in *Fusarium* species and they have FTIR absorptions in both the C=O (1760–1770 cm^{-1}) and C–O (1218–1235 cm^{-1}) ranges. Study of the chromatograms for these ranges (Fig. 6) showed a component (C) with an elution time of 12.52 min. A library search of its FTIR spectrum (Fig. 3c) gave a good match with 3-deacetylcalonectrin (**2c**) and the mass spectrum [13] was in agreement with this structure.

A comparison of the FTIR and MS chromatograms (Figs. 2a and 2b, respectively), obtained by using the same column, reveals not unexpectedly differences in FTIR and MS response factors for individual components, especially for DON and IDON. Quantitative analyses using these detectors must therefore take individual response factors into consideration. Because FTIR detection is less sensitive than that by MS [14], larger quantities had to be injected, which resulted in a loss of chromatographic resolution *e.g.* for components A and B.

The nature of other components in this extract is currently being investigated and these techniques are being applied to a series of other *Fusarium* extracts enabling identification of a wide range of mycotoxins.
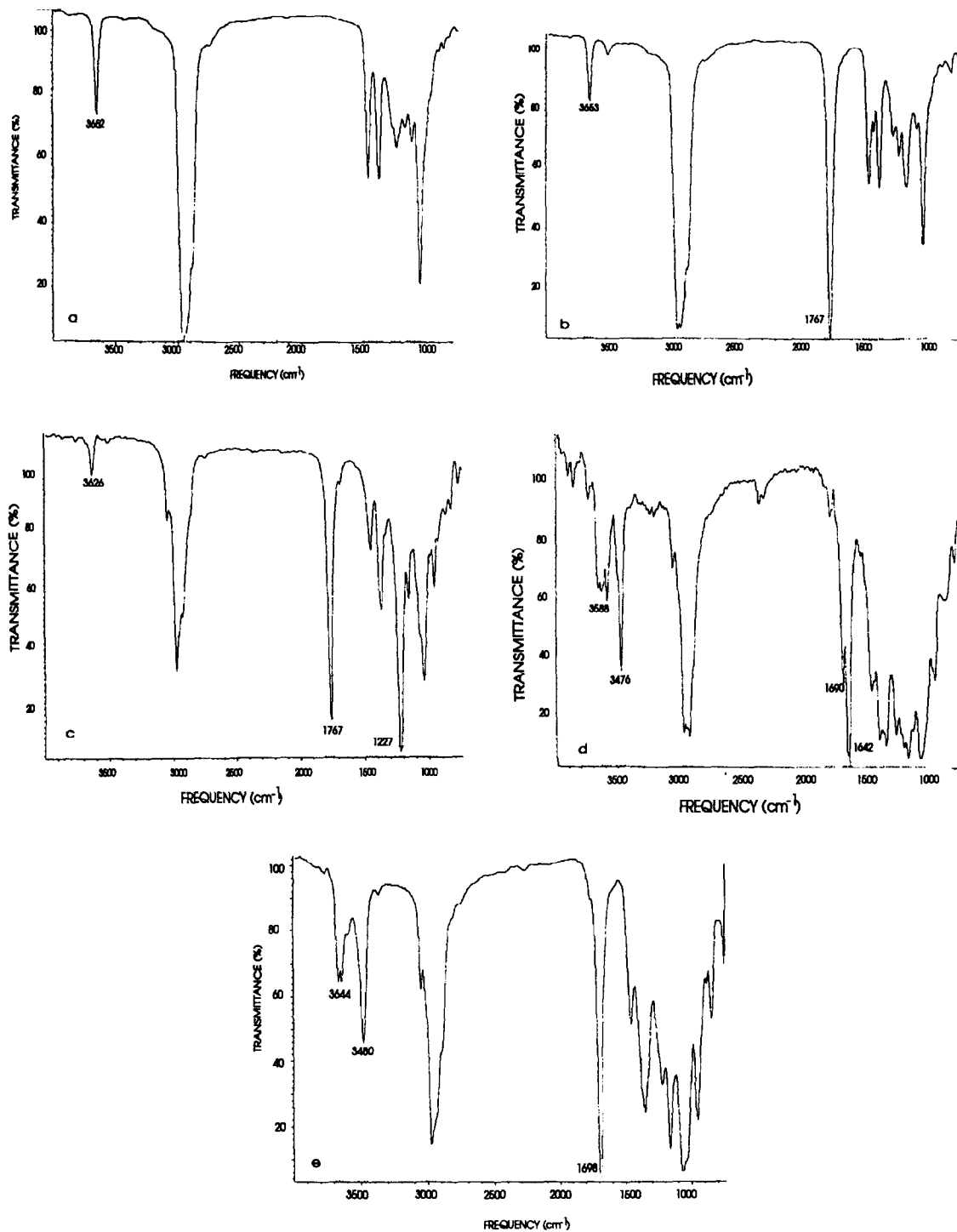
Fig. 3. FTIR spectra (a) of component A, culmorin (4); (b) of component B, culmorone (5); (c) of component C, 3-deacetylcalonectrin (2c); (d) of component D, iso-4-deoxynivalenol (3); and (e) of component E, 4-deoxynivalenol (1a).
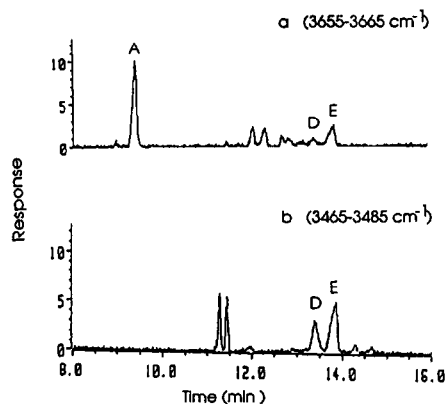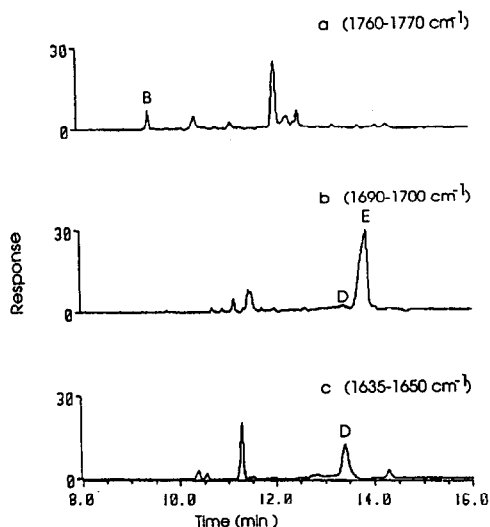
Fig. 4. Gas chromatogram of a *Fusarium roseum* liquid culture extract. Chromatographic conditions as in Fig. 2. Detection by Fourier transform infrared spectroscopy at selected wavelengths characteristic of hydroxyl functionalities: (a) 3655–3665 cm^{-1}; (b) 3465–3485 cm^{-1}.

Fig. 5. Gas chromatogram of a *Fusarium roseum* liquid culture extract. Chromatographic conditions as in Fig. 2. Detection by Fourier transform infrared spectroscopy at selected wavelengths characteristic of carbonyl functionalities: (a) 1760–1770 cm^{-1}; (b) 1690–1700 cm^{-1} and double bonds: (c) 1635–1650 cm^{-1}.

Fig. 6. Gas chromatogram of a *Fusarium roseum* liquid culture extract. Chromatographic conditions as in Fig. 2. Detection by Fourier transform infrared spectroscopy at selected wavelengths characteristic of acetate functionalities: (a) 1760–1770 cm^{-1}; (b) 1210–1230 cm^{-1}.

useful for characterization of *Fusarium* mycotoxins. The ability to monitor chromatograms at particular frequencies in the IR has proved to be very useful for the location and identification of minor components, particularly those trichothecenes with a 7α-hydroxyl adjacent to an 8-carbonyl as in DON (sharp absorption band at 3482 cm^{-1}) or those with an acetate functionality (two bands at 1765 and 1228 cm^{-1}). The use of GC–FTIR together with GC–MS provides a very powerful combination for the identification of a variety of mycotoxins that represent diverse structure types in fermentation extracts. These combined techniques should also have considerable utility for the identification of such components in food and feed products.

4. Conclusions

Although GC–FTIR may be a less sensitive technique than GC–MS, it always gives a stable spectrum pattern [14] and thus gives reliable spectra with consistent absorption frequencies
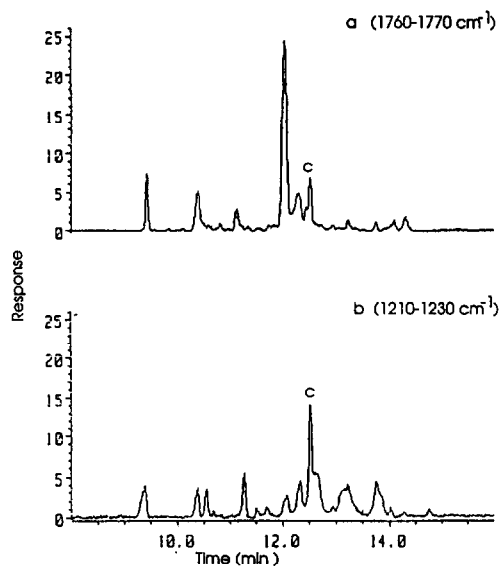
5. Acknowledgements

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