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Short Communication

Analysis of *Fusarium* mycotoxins by supercritical fluid chromatography with ultraviolet or mass spectrometric detection

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ABSTRACT

Supercritical fluid chromatography (SFC) on packed high-performance liquid chromatographic columns combined with ultraviolet and moving belt (MB)-mass spectrometry (MS) was applied to the separation and identification of some *Fusarium* mycotoxins in *F. roseum* liquid culture extracts. Mycotoxins observed included the trichothecenes 4-deoxynivalenol (DON), isoDON, 3-acetylDON, 3,15-diacetylDON, calonectrin, 15-deacetylcalonectrin, and 7-hydroxyisotrichodermol, as well as culmorin, culmorone and sambucoin. SFC separations were rapid; retention times were typically less than about 6 min. Detection limits for a variety of mycotoxin standards analyzed by SFC–MB-MS ranged from 10 to 250 mg.

INTRODUCTION

The utility of supercritical fluid chromatography (SFC) on capillary and packed columns with flame ionization and single wavelength ultraviolet (UV) detection for the separation of a variety of *Fusarium* mycotoxins was demonstrated recently [1]. These detectors provide only partial information on the nature of a particular mycotoxin by virtue of congruence of retention times with those of standards. Further characterization of individual mycotoxin constituents requires additional information. The SFC–MS analysis of only a few trichothecene mycotoxins (standards only) on capillary columns has been reported [2–4]. This study was initiated to determine the efficacy of coupling packed column SFC with a full scan UV detector or, via moving belt (MB) interface, with a mass spectrometer (MS) for the characterization of natural mixtures of mycotoxins (see Fig. 1 for

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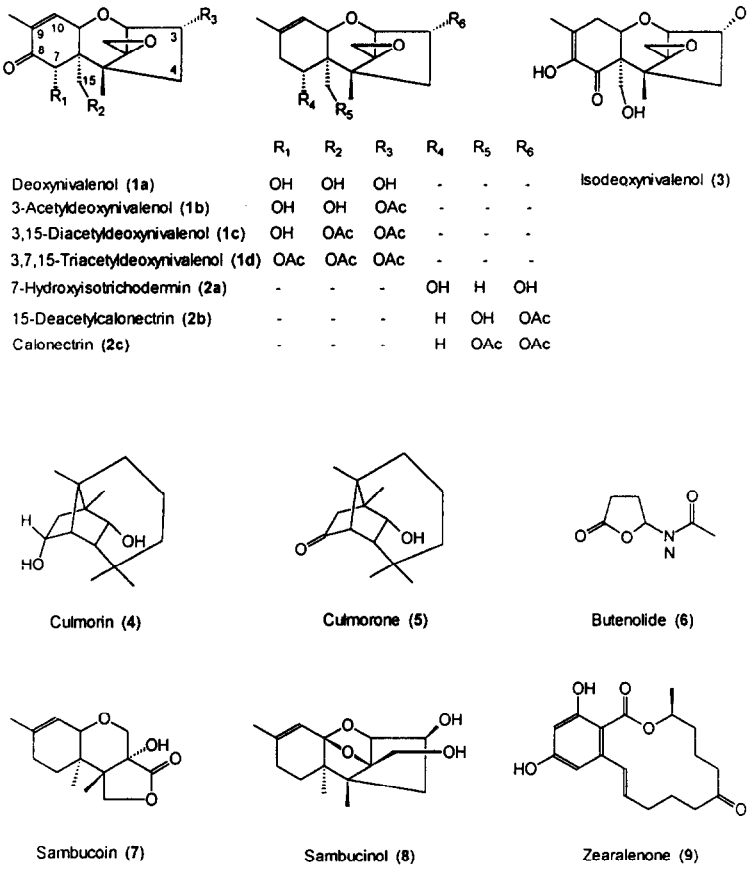

Fig. 1. Structures of some *Fusarium* mycotoxins.

structures of typical *Fusarium* mycotoxins) isolated from several *Fusarium roseum* liquid culture extracts.

MATERIALS AND METHODS

Mycotoxins and reagents

Mycotoxin standards were obtained from liquid cultures of various *Fusarium* spp. (prepared in the Plant Research Centre laboratories, Ottawa, Canada). Natural mixtures of mycotoxins were obtained from liquid culture extracts of *F. roseum*. All reagents and solvents were of analytical-reagent grade. Instrument-grade carbon dioxide supplied in cylinders with a dip tube (BOC, London, UK) and glass-distilled methanol were used as eluents.

Supercritical fluid chromatography

SFC analyses were conducted with supercritical carbon dioxide containing 10% methanol on a Hewlett-Packard 1084B liquid chromatograph (LC) (Hewlett-Packard, Avondale, PA, USA) modified for SFC [5] at (1) 60°C and pressure 250 bar at a flow-rate of 2 ml/min on a 250 × 2 mm I.D. stainless-steel column of 3-μm Hypersil connected to a Spectra-Physics scanning ultraviolet detector or (2) at 50°C and pressure 315 bar at a flow-rate of 4 ml/min on a 250 × 4.6 mm I.D. stainless-steel column of Spherisorb Amino 3 μm connected via a LC–MS moving belt (MB) interface (VG Analytical, Wythenshawe, UK) to a VG-7070E (VG Analytical, Manchester, UK) mass spectrometer operating in the electron ionization (EI) mode at 70 eV or in the chemical ionization (CI) mode with ammonia or methane.

The liquid carbon dioxide and pump heads were cooled to −25°C using a Neslab RTE-4Z refrigerated bath (Neslab Instruments, Newington, NH, USA).

RESULTS AND DISCUSSION

Supercritical fluid chromatography–ultraviolet detection of Fusarium mycotoxins

Fig. 2 shows the three dimensional SFC chromatogram for UV detection, from 200–325 nm, of extract I from a *Fusarium roseum* liquid culture. This chromatogram implies the presence of at least two major compounds with UV spectra beyond end absorption. Full spectrum scans of the two major peaks, at 4.93 and 5.13 min, were congruent with those for iso-4-deoxynivalenol (IDON) (**3**) and 4-deoxynivalenol (DON) (**1a**), respectively, and occurred at the same retention times as standards. Confirmation of structure assignment was assured by examination of their respective mass spectra (see below). Fig. 2 also reveals that there are no other major UV absorbing components.

Depending upon the UV absorption cut off of the solvent system used for traditional high-performance liquid chromatography (HPLC), detection at low wavelengths (at or below 200 nm) may not be possible and components with only low wavelength absorption would be missed.

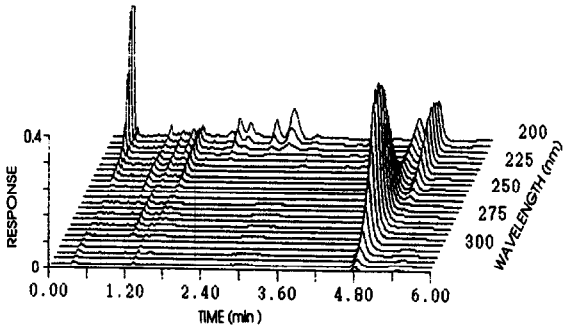

Fig. 2. Three-dimensional chromatogram of *Fusarium roseum* liquid culture extract I. Separation by SFC with eluent supercritical CO_2 containing 10% methanol at 60°C and pressure 250 bar at a flow-rate of 2 ml/min on 250 × 2 mm I.D. 3-μm Hypersil column. Detection by UV spectrometry from 200 to 325 nm.

SFC with CO_2 and methanol modifier is only limited by the range of the detector. The chromatogram at 200 nm (Fig. 2) clearly indicates the presence of other components with low wavelength absorption. However, end absorption spectra are of little diagnostic value. Since *Fusarium* mycotoxins without UV spectra are known, other methods of detection are required in order to discover the presence of such components.

Supercritical fluid chromatography–mass spectrometric detection of Fusarium mycotoxins

The SFC effluent was linked, via a moving-belt interface, to a mass spectrometer operated under EI conditions. The retention times and detection limits of a variety of *Fusarium* mycotoxin standards were determined. The results summarized in Table I show that SFC–MB-MS detection limits were in the range of 10–250 ng. These are higher than those observed under GC-MS conditions [6].

SFC–MS analysis of Fusarium roseum culture extracts

Reanalysis of extract I by SFC–MS under EI

TABLE I

DETECTION LIMITS OF SELECTED *FUSARIUM* MYCOTOXINS ANALYZED BY SUPERCRITICAL FLUID CHROMATOGRAPHY–MOVING BELT INTERFACE-MASS SPECTROMETRY

SFC with eluent supercritical CO_2 containing 10% methanol at 50°C and a pressure of 316 bar at a flow-rate of 4 ml/min on 250 × 4.6 mm I.D. stainless-steel column of Spherisorb Amino 3 μm. Detection on a magnetic sector VG 7070E mass spectrometer operating in the electron ionization mode.

Compound	Detection limit (ng)
3-Acetyldeoxynivalenol (**1b**)	100
Butenolide (**6**)	20
Culmorin (**4**)	50
4-Deoxynivalenol (**1a**)	150
Sambucinol (**7**)	30
Triacetyldeoxynivalenol (**1c**)	10
Zearalenone (**9**)	250

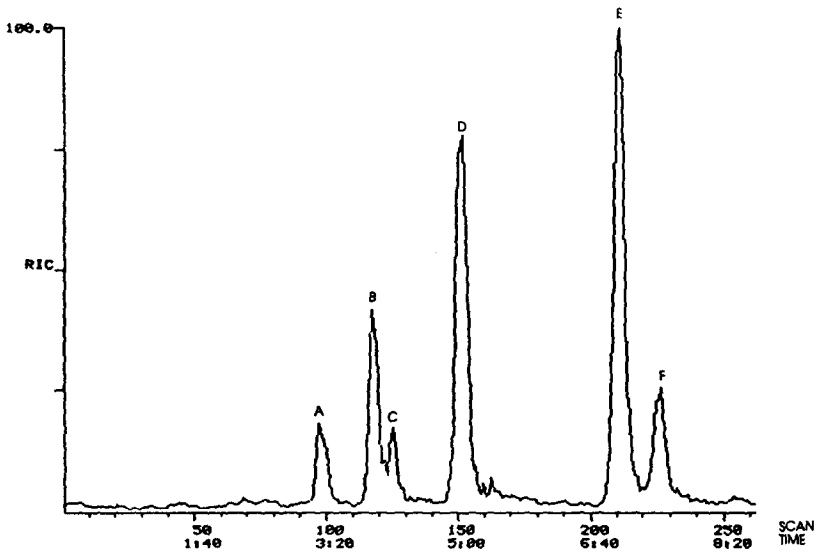

Fig. 3. Total ion mass spectrometric chromatogram of *Fusarium roseum* liquid culture extract I. Separation by SFC with eluent supercritical CO_2 containing 10% methanol at 50°C and pressure 315 bar at a flow-rate of 4 ml/min on 250 × 4.6 mm I.D. 3-μm Spherisorb Amino column. Moving belt interface with MS detection in EI mode. A = culmorin (**4**); B = unknown of molecular mass 342; C = 7-hydroxyisotrichodermol (**2a**); D = unknown of molecular mass 266; E = iso-4-deoxynivalenol (**3**); F = 4-deoxynivalenol (**1a**). Time in min.

conditions (chromatogram illustrated in Fig. 3) revealed the presence of at least six components. Examination of the MS of each component and comparison with those of standards led to the following peak assignments; A = culmorin (**4**), C = 7-hydroxyisotrichodermin (**2a**), E = IDON (**3**) and F = DON (**1a**). Since the MS of the remaining unknown components did not give a clear indication of molecular mass, the analysis was repeated under chemical ionization (CI) conditions by using methane and ammonia. Minor components B and D were shown, by these analyses, to have molecular masses of 342 and 266, respectively. The methane CI chromatogram (not shown) revealed that culmorin (**4**) (peak A) gave a significantly greater relative response under these conditions.

Two additional *F. roseum* extracts were analyzed by SFC–MS under EI and methane and ammonia CI conditions. Fig. 4 shows the SFC–CI-MS (ammonia) chromatogram for extract II; EI MS revealed components G and H to be culmorone (**5**) and sambucoin (**8**), respectively.

The SFC–EI-MS chromatogram for extract III, depicted in Fig. 5, suggested the presence of three major components. However detailed MS deconvolution analysis showed the first peak (I, J) to be a partially resolved mixture of calonectrin (**2c**) and 3,15-diacetyl-4-deoxynivalenol (**1c**). The remaining major components were identified as K = 15-deacetyl-calonectrin (**2b**) and L = 3-acetyl-4-deoxynivalenol (**1b**).

Although the sensitivity of SFC–MB-MS is less than that for GC–MS, there are several advantages. One is the ability to chromatograph thermally unstable or non-volatile substances without the need for derivatization prior to detection. Another is the speed of analysis. For example, the components of extracts II and III were completely eluted in less than 3 min (Figs. 4 and 5) whereas the analysis of the same extracts by GC–MS required about 12 min [6]. Although the peak widths are slightly broader than those observed by capillary GC, they are much narrower than those obtained from conventional HPLC [1].

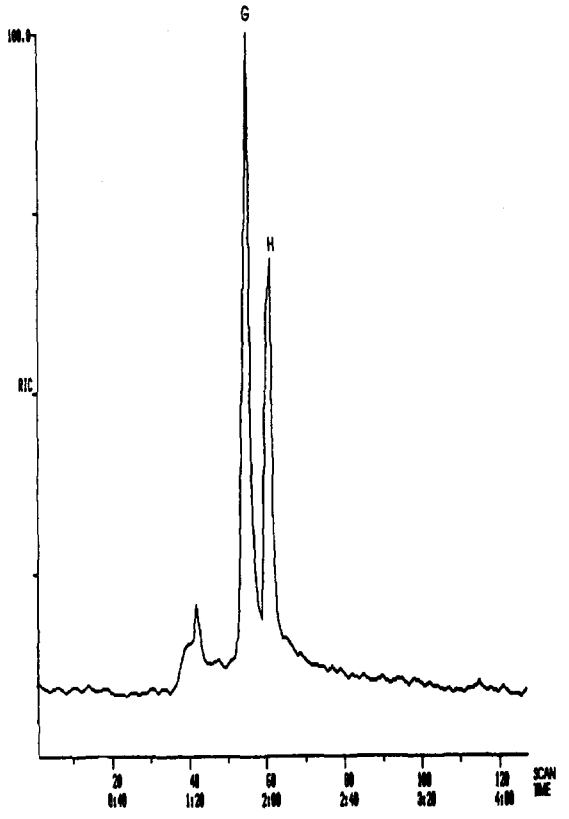

Fig. 4. Total ion mass spectrometric chromatogram of *Fusarium roseum* liquid culture extract II. Separation by SFC with eluent supercritical CO_2 containing 10% methanol at 50°C and pressure 315 bar at a flow-rate of 4 ml/min on 250 × 4.6 mm I.D. 3-μm Spherisorb Amino column. Moving belt interface with MS detection in Cl-NH_3 mode. G = culmorone (**5**); H = sambucoin (**8**). Time in min.

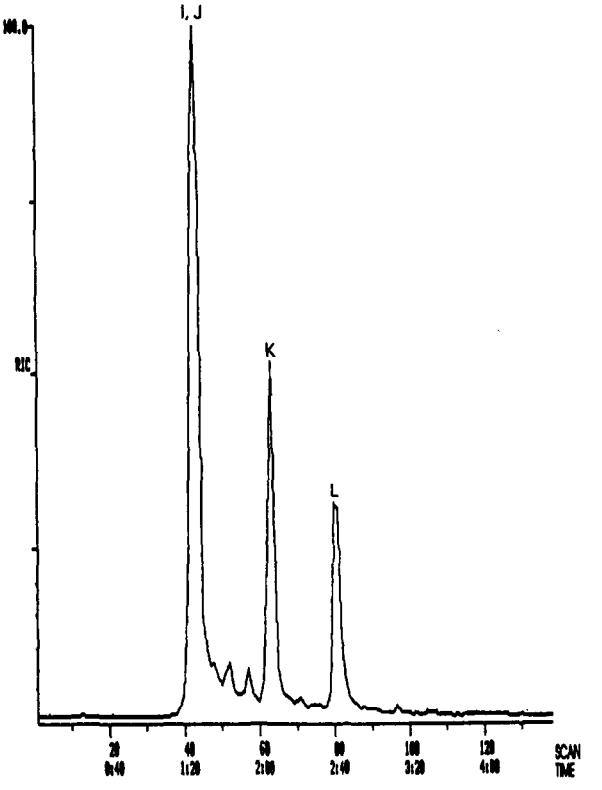

Fig. 5. Total ion mass spectrometric chromatogram of *Fusarium roseum* liquid culture extract III. Separation by SFC with eluent supercritical CO_2 containing 10% methanol at 50°C and pressure 315 bar at a flow-rate of 4 ml/min on 250 × 4.6 mm I.D. 3-μm Spherisorb Amino column. Moving belt interface with MS detection in EI mode. I = calonectrin (**2c**); J = 3,15-diacetyl-4-deoxynivalenol (**1c**); K = 15-deacetylcalonectrin (**2b**); L = 3-acetyl-4-deoxynivalenol (**1b**). Time in min.

CONCLUSIONS

The full scanning UV detector can be used in conjunction with SFC for the characterization of *Fusarium* mycotoxins that have distinctive UV absorption patterns. For these and non-UV absorbing substances, the coupling of SFC with MS via the moving belt interface can provide unequivocal identification. Compared with conventional HPLC and GC, packed column SFC also offers faster analysis times.

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