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Note

Analysis of some trichothecene mycotoxins by liquid chromatography

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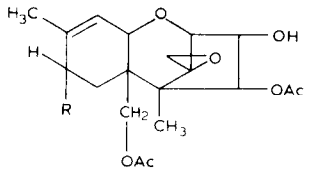
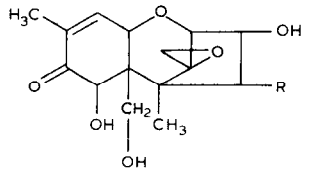
Trichothecene mycotoxins are toxic metabolites of Fusarium and other fungi which are capable of growing on agricultural products before harvest and during storage. Foodstuffs contaminated with these materials can cause severe effects in humans and animals and may result in death[1].

Trichothecenes in foodstuffs and body fluids have been extensively analysed by gas chromatography[2] combined gas chromatography–mass spectrometry[3,4], thin-layer chromatography[5,6], polarography[7], radioimmunoassay[8], and to a limited extent by column liquid chromatography (LC)[9–14]. This paper describes simple LC methods for the analysis of the four trichothecene mycotoxins: T2-toxin; diacetoxyscirpenol (DAS), nivalenol (NIV), and deoxynivalenol (DON).



T2, R = $-OOCCH_2CH(CH_3)_2$

DAS, R = H



NIV, R = –OH

DON, R = H

Analysis of these compounds at trace levels by LC with UV detection is difficult because of their low intensity of absorption and the need to use low wavelengths. The analysis of the toxins containing the conjugated enone (NIV, DON) has however been described[9–11]. T2 and DAS have been analysed at higher levels by using a refractive index detector[12–14]. A more suitable approach would seem to involve the derivatisation of the –OH groups, which are present in all the compounds, with a strongly UV absorbing group. Such an approach has been suggested for T2 and DAS but no experimental details are available[15]. In this study the preparation of the *p*-nitrobenzoyl derivatives of T2, DAS, NIV and DON and their separation by LC is described.

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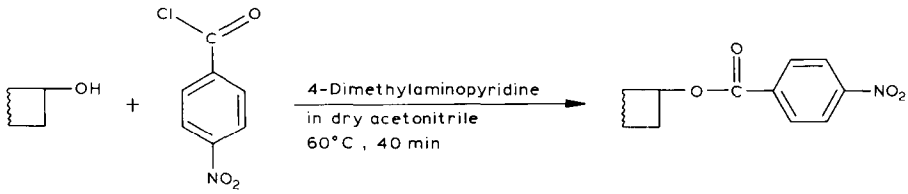

EXPERIMENTAL

Materials

Trichothecenes were obtained from Sigma (U.K.), Mycolabs (U.S.A.) and Wako (Japan).

The LC solvents were prepared from acetonitrile far UV grade (Fisons), organic free water from an Elgastat Spectrum and analytical reagent quality potassium dihydrogen phosphate and phosphoric acid.

The derivatising reagents were *p*-nitrobenzoyl chloride (puriss) and 4-dimethylaminopyridine (purum) from Fluka.

Instrumentation

UV spectra were recorded on a Shimadzu spectrophotometer Model UV-240. LC system comprised: pumps, Waters Model 6000A; detectors, Waters Model 480 and 440; injector, Rheodyne 7125; integrator/recorder, Spectra Physics SP4270, Waters M730 data module; gradient controller, Waters 720 system controller; column, 12.5 × 0.5 cm I.D. packed with 3-μm Hypersil ODS (Hichrom); column heater, Jones Chromatography Model 7910.

Methods

Derivatisation. Solutions containing the trichothecenes in the ng to μg range were dispensed into micro-vials and evaporated to dryness at 60°C under a stream of nitrogen. To the residue were added solutions of 3 mg/ml *p*-nitrobenzoyl chloride (20 μl) and 10 mg/ml 4-dimethylaminopyridine in acetonitrile (10 μl). The tubes were capped with PTFE lined septa and heated at 60°C for 40 min. The handling of the *p*-nitrobenzoyl chloride and preparation of solutions were carried out in a dry box. Calibration solutions were prepared over the range 2–300 ng for NIV and DON and 8 ng–2 μg for T2 and DAS.

Chromatographic analysis. Separation of the underivatised toxins was achieved with a solvent gradient from 5–80% acetonitrile mixed with water at a flow-rate of 1.0 ml/min. Detection was by UV at 220 nm.

The *p*-nitrobenzoyl derivatives of the toxins were analysed by the direct injection (5 μl) of the derivatising solution. A mobile phase of acetonitrile–aqueous buffer (0.2 *M* potassium dihydrogen phosphate plus 0.2 *M* phosphonic acid) (65:35) was used with a column temperature of 35°C. Solvent flow-rate was 1.0 ml/min; pressure 2000 p.s.i. UV detection was at 254 nm.

RESULTS AND DISCUSSIONS

The separation of the underivatised toxins with low-wavelength detection, Fig. 1, shows that they can all, even T2 and DAS, be handled directly by LC–UV. The sensitivity is not great and the analysis is subject to interference at this low wavelength. The method is however suitable for the analysis of cultures and for following the reaction of the toxins at high concentrations.

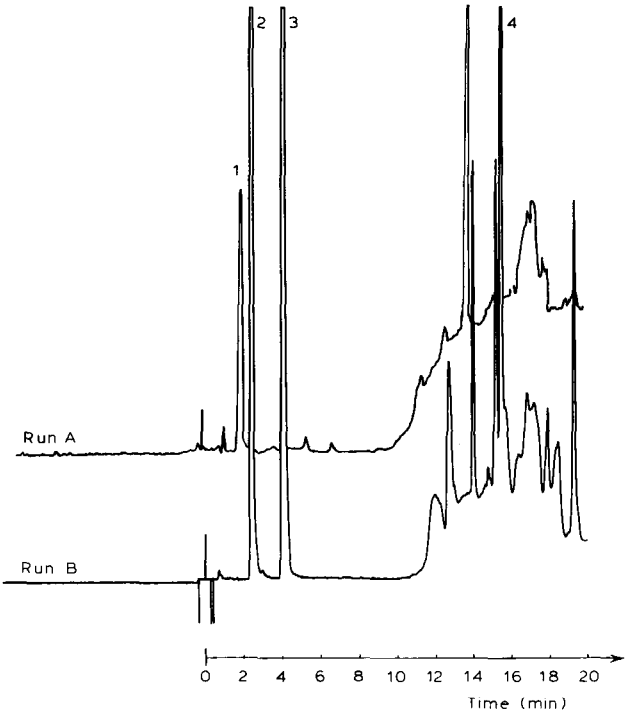

Fig. 1. Chromatogram of trichothecenes using a gradient programme, detection at 220 nm (0.50 a.u.f.s.). Run A: 1 = DAS 75 μg injected in acetonitrile. Run B: 2 = NIV 18 μg; 3 = DON 18 μg; 4 = T2-toxin 55 μg. All injected in methanol–water (10:90). Peaks other than the tabulated toxin peaks are due to the gradient background and impurities.

For the trace analysis of the trichothecenes in foodstuffs the stronger and longer wavelength UV absorption of the *p*-nitrobenzoyl derivatives, Fig. 2, is more suitable. The chromatogram, Fig. 3, shows that all four toxins can be well separated with good peak shape in a single isocratic run. Calibration is linear from the low nanogram detection limit to the limit of the linear range of the detector. The sensitivity to DON and NIV is higher because of the existence of several –OH groups which can be derivatised.

It was found that most samples of *p*-nitrobenzoyl chloride commercially available were of low purity and contained large quantities of *p*-nitrobenzoic acid. Furthermore, the reagent degraded rapidly when opened. The most reliable procedure

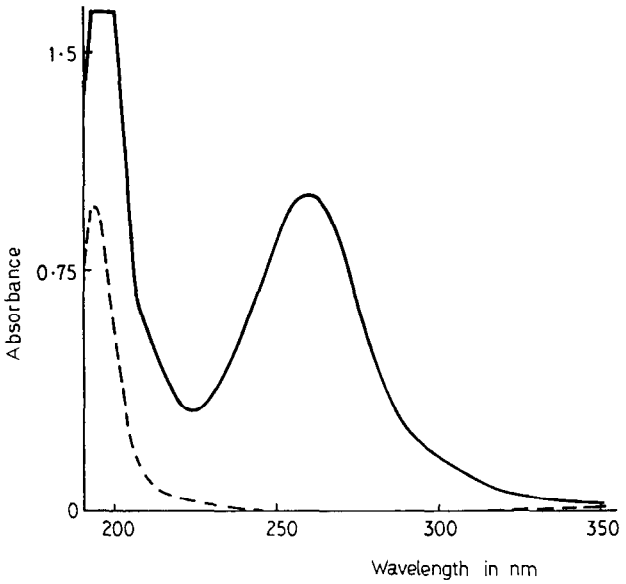

Fig. 2. UV spectra of T2-toxin ($2 \cdot 10^{-6}$ *M*) and its *p*-nitrobenzoate derivative ($8 \cdot 10^{-7}$ *M*).

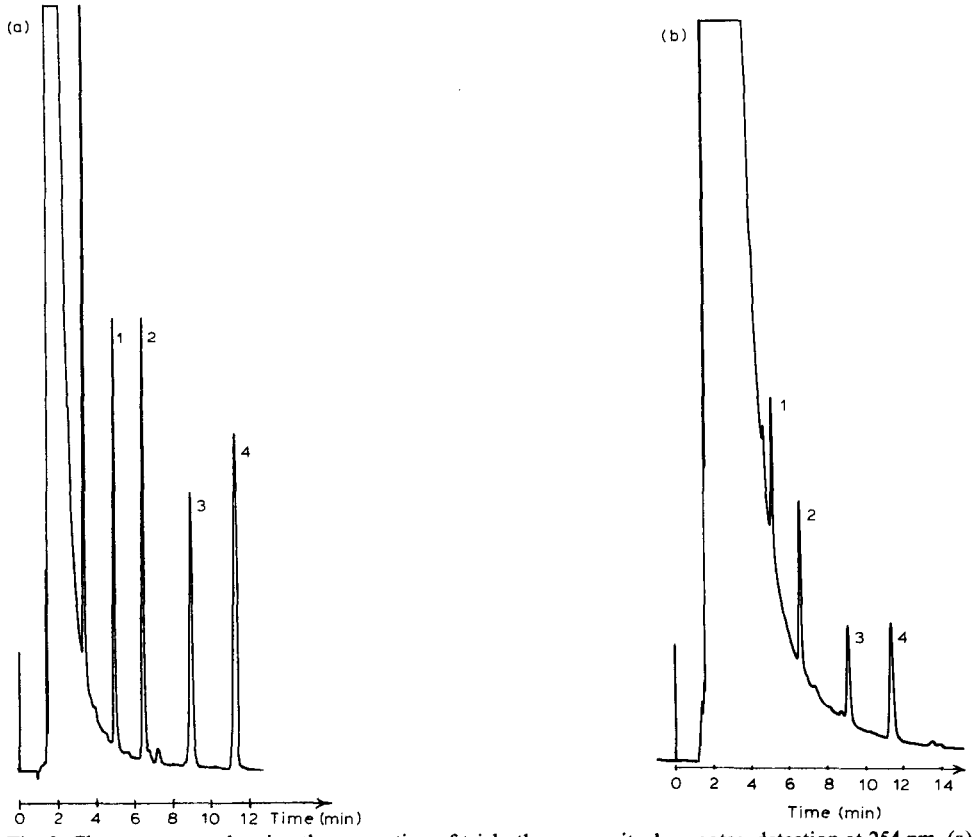

Fig. 3. Chromatogram showing the separation of trichothecene *p*-nitrobenzoates, detection at 254 nm. (a) 0.2 a.u.f.s. Peaks: 1 = DAS 600 ng; 2 = DON 166 ng; 3 = T2-toxin 800 ng; 4 = NIV 116 ng. (b) 0.02 a.u.f.s. Peaks: 1 = DAS 25 ng; 2 = DON 6.3 ng; 3 = T2-toxin 30 ng; 4 = NIV 6.3 ng.

was to prepare sealed ampoules of the reagent and carry out all operations in a dry box. The toxin derivatives were however quite stable to hydrolysis with normal laboratory handling for several days.

Phosphate buffer was included in the mobile phase to ensure the elution of the *p*-nitrobenzoic acid as a sharp peak. With a simple mobile phase of acetonitrile–water alone the acid was eluted as a series of broad peaks which interferred with subsequent chromatograms.

The esterification catalyst, 4-dimethylaminopyridine, has been used previously in the derivatisation of sterically hindered alcohols[16] and in this work it was found to give reliable results.

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