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Short Communication

Determination of patulin by capillary gas chromatography of the heptafluorobutyrate derivative

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ABSTRACT

Formation and capillary gas chromatography of patulin heptafluorobutyrate is reported for the first time. The derivative was identified by gas chromatography–mass spectrometry ($M^+ = 350$). Electron-capture detector response for patulin heptafluorobutyrate was linear in the range 0.05 to 0.5 ng. This sensitive and reproducible derivatization technique was applied to the determination of patulin in apple juice by capillary gas chromatography–electron-capture detection. The overall method recovery averaged 84% and ≤ 10 μg/l could be detected.

INTRODUCTION

Patulin (Fig. 1) is a toxic mold metabolite produced by several species of *Aspergillus* and *Penicillium* and by *Byssochlamys nivea* [1]. It is frequently found in apple juice [1–3] and is regulated in that product in several European countries at a tolerance level of 50 μg/kg [4].

Although liquid chromatography (LC) is currently the method of choice for determination of patulin in apple juice and other foods [2,5], there are numerous methods utilizing gas chromatography (GC). Most of these involve derivatization of patulin to its trimethylsilyl (TMS) ether, for which both electron-capture detection (ECD) [6–9] and mass spectrometry (MS) [3,10–13] offer sub-nanogram sensitivity not

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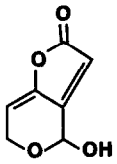

Fig. 1. Chemical structure of patulin.

possible with flame ionization detection [14–17]. Other derivatives of patulin that have been used for GC analysis are the acetate and chloroacetate [16–20], but the lowest reported detection limit was only 12 ng (for the chloroacetate by ECD [16]). Derivatization of patulin to the trifluoroacetate or heptafluorobutyrate (HFB) with trifluoroacetic anhydride and heptafluorobutyric anhydride, respectively, was not satisfactory, according to Bergner-Lang *et al.* [20]. We nevertheless report here for the first time the formation and capillary GC–ECD of patulin HFB, a highly sensitive analytical technique which was applied to the determination of patulin in apple juice.

EXPERIMENTAL

Derivatization of patulin

Patulin working standard solution (10.0 μg/ml chloroform) (200 μl) was evaporated to dryness under nitrogen in a 3.7-ml vial. The HFB derivative was formed by a procedure similar to that used for trichothecenes [21]. After addition of toluene–acetonitrile (95:5) (500 μl) and heptafluorobutyrylimidazole (HFBI) (25 μl), the vial was closed with a PTFE-lined screw cap, mixed 20–30 s on a vortex mixer, and heated in a heating block at 60°C for 10–15 min. After cooling to room temperature (it was later found that derivatization occurred within 1 min without heating), 5% sodium bicarbonate solution (1.0 ml) and toluene–acetonitrile (95:5) (500 μl) were added, shaken 45–60 s on the vortex mixer, and the layers allowed to separate (if the top layer was not clear, additional mixing for 15–20 s was necessary). Using an adjustable Eppendorf pipet, 250 μl of the top layer was added to *n*-heptane (4.75 ml) containing 50 ng hexachlorobenzene (HCB)/ml as internal standard in an 11-ml vial. The vial was closed with a PTFE-lined screw cap and shaken 5 s on the vortex mixer. This solution contained patulin HFB at a concentration corresponding to 0.1 μg patulin/ml; 2.0 μl (or another suitable volume) were injected into the gas chromatograph.

Gas chromatography

Capillary GC was carried out on a Varian 6000 gas chromatograph equipped with an autosampler, a split–splitless injector (250°C) operating in the splitless mode, a J&W Scientific DB-5 fused-silica capillary column (30 m × 0.32 mm I.D., 0.25 μm film thickness), and a ^{63}Ni electron-capture detector (at 350°C). Column temperature programs used are given in the captions to Figs. 2 and 3. The carrier gas was helium (34.6 cm/s) and the make-up gas was nitrogen (20 ml/min). Attenuation was 64 at range 10.

Gas chromatography–mass spectrometry

The mass spectrum of patulin HFB was recorded on a VG Micromass ZAB-2F mass spectrometer operated at 1500 resolution and 70 eV electron energy and interfaced with a Varian 3700 gas chromatograph equipped with splitless injection (180°C) and a 25 m × 0.25 mm I.D. DB-5 fused-silica capillary column (0.25 μm film thickness). The column was programmed from 80°C (after 1 min) to 220°C at 5°C/min. The amount of patulin injected (as HFB) was 80 ng.

Analysis of apple juice

Apple juice was extracted with ethyl acetate and cleaned up on a silica gel column according to the official method of the Association of Official Analytical Chemists [22,23]. Extracts were first analysed by two-dimensional thin-layer chromatography (TLC) [23]. The extract from 45 g apple juice was then evaporated, derivatized, and analysed by GC–ECD as described above, using the column temperature program given in the caption to Fig. 3 for both sample and patulin standard and quantitating by peak-area ratios.

RESULTS AND DISCUSSION

Patulin HFB had a retention time of 11.8 min under the column programming conditions described in Fig. 2. The peak was identified by GC–MS, with a molecular ion at m/z 350, ions at m/z 197 ($C_3F_7CO^+$) and 169 ($C_3F_7^+$) that confirmed the

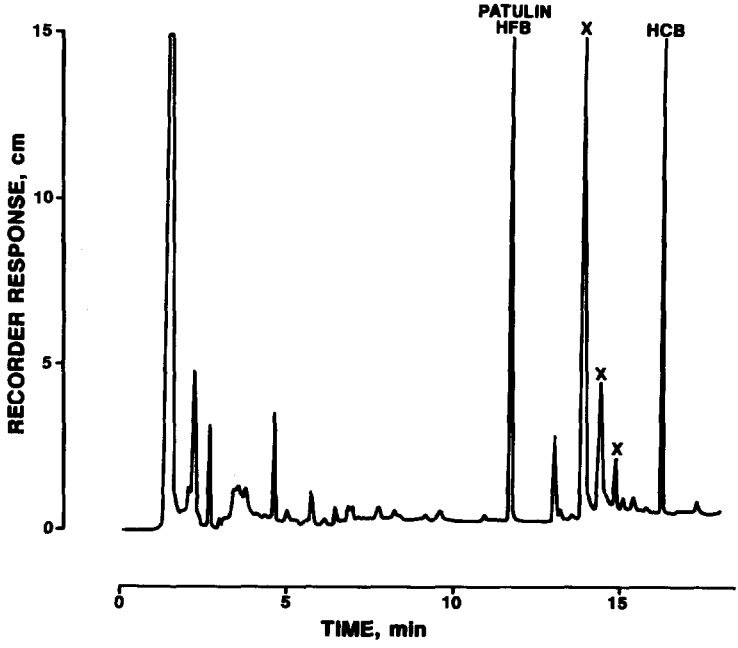

Fig. 2. Gas chromatogram of patulin HFB (0.2 ng patulin injected). Peaks marked × arise from the HFBI reagent; hexachlorobenzene (HCB) is internal standard. Column temperature program: 80°C (1 min), 5°C/min to 140°C, 20°C/min to 210°C.

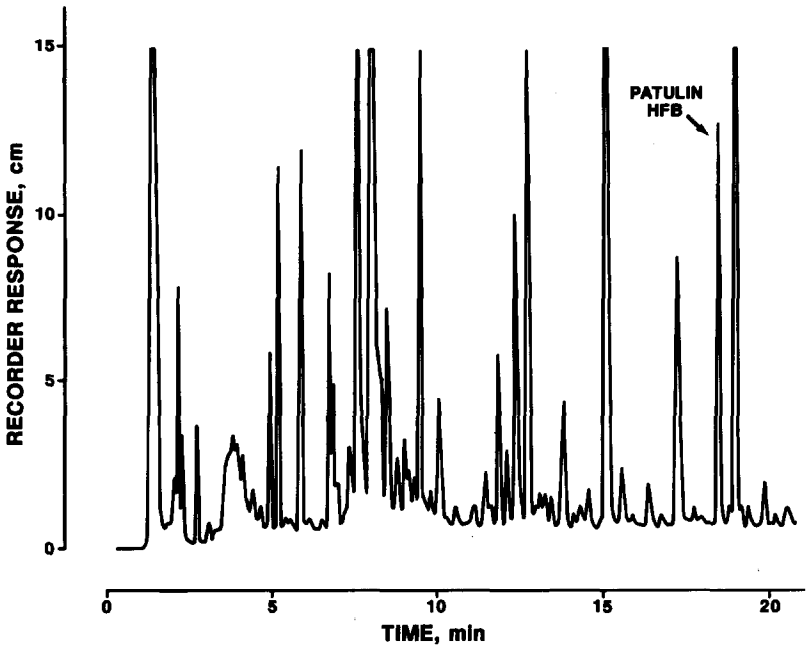

Fig. 3. Gas chromatogram of HFBI-derivatized extract of apple juice estimated to contain 38 μg patulin/l. Column temperature program: 80°C (1 min), 2°C/min to 120°C.

presence of the HFB moiety, and other ions at *m/z* 153, 136, 125, 110, 97, 82, 71, 69, 55 and 53 that were consistent with a patulin-H moiety [24,25] (Fig. 4). The later eluting peaks in the chromatogram were from the reagent blank; the two major ones had molecular ions at *m/z* 461 and 370.

The derivatization reaction was highly reproducible. When 2.0 μg patulin was derivatized and the equivalent of 0.2 ng injected, mean integrator area counts for a duplicate series of injections from 6 reactions were 202 818 and 200 172 with coefficients of variation (C.V.) of 1.9 and 4.0%, respectively. By comparison, mean area counts for 0.1 ng HCB used as internal standard were 324 634 (n = 6, C.V. 2.1%) and 316 456 (n = 6, C.V. 3.4%). The patulin HFB derivative was stable in n-heptane solution for up to 35 h at room temperature.

The ECD response for patulin HFB was linear in the range 0.05 ng to 0.5 ng patulin. In fact, it would be possible to detect and measure considerably less than 0.05 ng patulin, in view of the detector range and attenuation used in this study. Thus, the HFB derivative is probably more sensitive for ECD than the TMS derivative, for which the minimum determinable amount of patulin was reported as 0.1 ng, albeit on a packed column [7,9]. Sensitivity appears to be similar to that of the TMS derivative determined by capillary GC–MS [3] and is achieved with less expensive and more readily available instrumentation. By comparison, the lowest level of patulin reported to be detectable by LC was 1 ng [5].

The HFB derivatization technique was applied to determination of patulin in apple juice. For this analysis, a GC temperature program with a slower heating rate

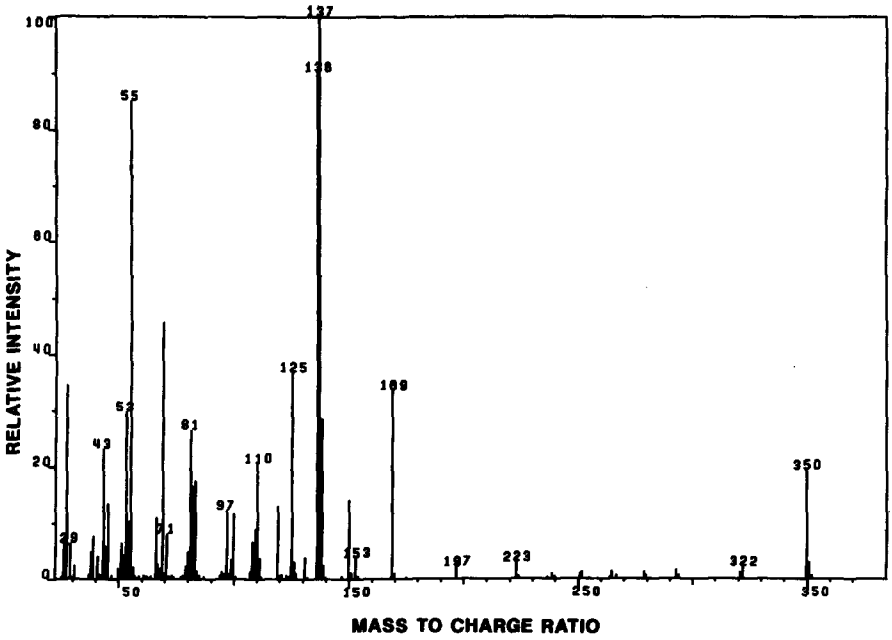

Fig. 4. Mass spectrum of patulin HFB after separation by GC.

was preferable and the retention time for patulin HFB was 18.7 min (Fig. 3). Two naturally contaminated samples determined to contain 78 and 38 μg/l by TLC were found to contain 99 and 38 μg/l, respectively, when the extracts were analysed by GC–ECD. The detection limit by GC was $\leqslant 10$ μg/l. Recovery of patulin from apple juice spiked at 100 μg/l averaged 85% ($n = 6$, range 73–95%); in a second set of experiments (where derivatization was carried out at room temperature), the mean recovery was 83% ($n = 6$, C.V. 6.3%).

In conclusion, GC–ECD of patulin HFB is a sensitive and reproducible procedure for quantitative determination of patulin that can be used for analysis of apple juice.

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