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Short Communication

Automation of a clean-up procedure for determination of trichothecenes in cereals using a charcoal–alumina column

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ABSTRACT

Automation of the clean-up procedure for trichothecenes on a charcoal–alumina column is described. Standard high-performance liquid chromatographic equipment was used for the clean-up step. An acetonitrile–water (84 + 16, v/v) extract of the sample was cleaned up on a column packed with charcoal–alumina–Celite, which was washed with acetonitrile between each sample. The eluates were collected directly in reaction vials and evaporated to dryness. The residual water was removed azeotropically with benzene. The sample was derivatized with 1-(trimethylsilyl)imidazole and analysed by capillary gas chromatography with electron-capture detection.

INTRODUCTION

Mycotoxins, being secondary metabolites of fungi, have caused many welldocumented cases of toxicosis following consumption of fungus-contaminated cereals [1–4]. Probably the trichothecenes are the most important group produced by the genus *Fusarium*. According to studies in many countries, deoxynivalenol is the toxin that most often occurs, but other trichothecenes have also frequently been reported [1–5]. An analytical method capable of screening large numbers of samples is of great importance when cereals and feed products are to be controlled and when factors that affect the growth of *Fusarium spp.* and toxin formation are to be established.

Thin-layer (TLC) [6,7], high-performance liquid (HPLC) [8–10] and supercritical fluid chromatography (SFC) [11] have been used for the determination of trichothecenes, but gas chromatography (GC) with electron-capture (EC) or mass spectrometric (MS) detection are probably the methods that are most commonly used [12–14].

Several clean-up procedures have been published. Most of them include a liquid–liquid extraction step and purification of the extract on a Florisil or silica gel column and different evaporation steps [15–18]. These procedures are time consuming and difficult to automate. Romer *et al.* [19] presented a clean-up method using a charcoal–alumina column. The method was later modified [7–8] and the columns can today be purchased from Romers Labs. [20]. The column can be used for most of the group A and B trichothecenes. The sample is extracted with

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acetonitrile–water, the extract is transferred directly to a charcoal–alumina column, and the trichothecenes are eluted with the same solvent mixture. The column has to be connected to a vacuum system to obtain sufficient flow through the column. The first vacuum systems described were for only one column [7,8,20], but subsequently manifolds for the simultaneous connection of up to six columns have been used in many laboratories. The large elution volumes, however, restrict the number of columns that can be connected to the manifold.

The aim of this work was to automate the clean-up procedure of trichothecenes using the charcoal–alumina column so that large series of samples can be run simultaneously.

EXPERIMENTAL

Reagents and apparatus

Trichothecene standard solution was obtained from Romers Labs. and contained 100 μg/ml each of deoxynivalenol (DON), fusarenon-X (F-X), nivalenol (NIV), neosolaniol, diacetoxyscirpenol (DAS), T-2 and HT-2 in acetonitrile. 3-Acetyl-DON was purchased from Sigma. A stock solution which contained 2 μg/ml of each compound in acetonitrile was prepared.

1-(Trimethylsilyl)imidazole (TMSI) (Fluka) was used as a derivatization agent. Acetonitrile was of HPLC grade and hexane was of Distol grade, both obtained from Fisons.

The samples were extracted with a universal flask shaking machine from Edmund Bühler, Type SM 2.5.

The equipment used for the clean-up procedure consisted of a Perkin-Elmer Series 10 pump, one of the pumps on a Perkin-Elmer Series 2 delivery system and a Perkin-Elmer ISS-101 autoinjector equipped with a 2-ml preparative loop. A Rheodyne six-port valve (Model 7000) and a Gilson fraction collector (Model 203) were controlled by an Omega-2 (Perkin-Elmer) data system via a time relay. The clean-up column was a Chromguard cartridge (50 mm × 3 mm I.D.) with a snap-open–snap-shut holder (Chrompack). The cartridge was packed with Darco G-60 activated carbon, 100–325 mesh (Aldrich), neutral aluminium oxide 90 active, 0.063–0.200 mm (Merck), and Celite, reagent grade (Supelco), in the proportions 2.33:1.67:1 (w/w/w).

GC analysis was performed on a Varian Model 3400 gas chromatograph equipped with a ^{63}Ni electron-capture detector, a splitless injector and a Model 8100 autosampler.

All chromatograms were recorded on a Varian Star data system. The capillary GC fused-silica column was 30 m × 0.32 mm I.D., coated with 0.25-μm SPB-1. The carrier gas was helium at 9 p.s.i., and the make-up gas was nitrogen. The temperatures of the injector and detector were 280 and 300°C, respectively. The oven temperature was 60°C for 2 min, then increased to 210°C at 30°C/min, to 225°C at 1°C/min and to 250°C at 20°C/min, the final temperature being maintained for 20 min.

Procedure

A 25-g amount of the ground sample was extracted for 1 h with 125 ml of acetonitrile–water (84:16, v/v). Some of the extract was filtered through a folded filter-paper and 0.5 ml of the extract was injected into an HPLC system, shown in Fig. 1. The trichothecenes were eluted for 5 min with acetonitrile–water (84:16, v/v) before the column was washed for 5 min with acetonitrile. The column was then conditioned for about 2 min with acetonitrile–water before a new sample was injected. The flow-rate of both pumps was 1.0 ml/min. The eluates were collected directly in 5-ml reaction vials, which were subsequently placed in a heating module (60°C), and the eluates were evaporated to dryness under a stream of nitrogen. A 1-ml volume of benzene was added to each vial and mixed on a Whirlimixer for 30 s, and thereafter the mixture was again evaporated to dryness. The vials were sealed with screw-caps until room temperature was obtained. The residue was dissolved in 100 μl of TMSI. The vials were sealed and mixed on a Whirlimixer before being heated at 80°C for 30 min for derivatization. A 500-μl volume of hexane was added and mixed on a Whirlimixer. The hexane phase was extracted with 1 ml of water, which was discarded, and then dried with sodium sulphate. A 0.5-μl volume of the extract was injected into the GC system.

RESULTS AND DISCUSSION

Romer [20] found that 50 ml of elution solvent, in addition to 5–10 ml of the sample extract, was required to obtain optimum recovery of the seven

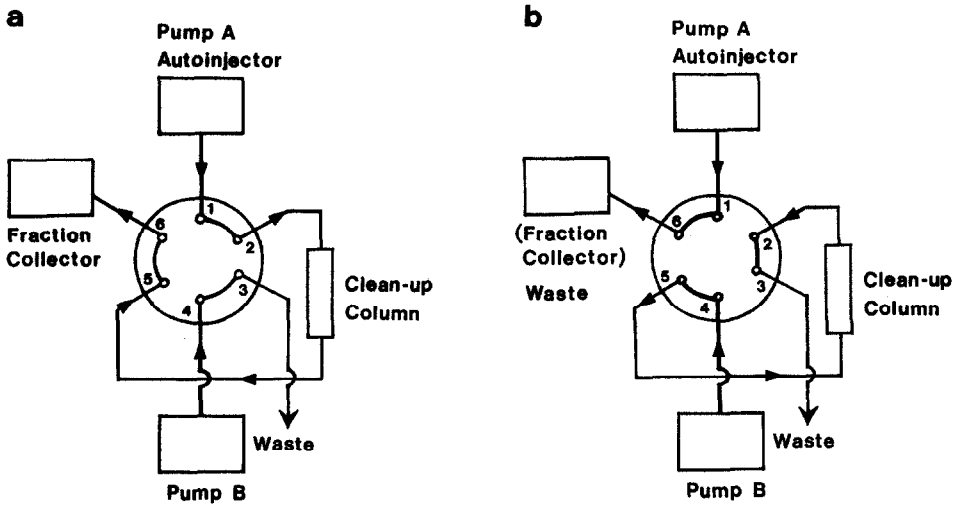

Fig. 1. Flow diagram of the Rheodyne six-port valve. (a) Direction of flow during collection of the eluent; (b) direction of flow during washing of the column.

most commonly analysed trichothecenes with a 21 × 19 mm I.D. charcoal-alumina column, while 22.5 ml were used in our laboratory with a 40 × 0.9 mm I.D. column. In both instances the eluent had to be evaporated to dryness in a rotary evaporator. This operation is very time consuming, as only one flask can be run at the same time. Loss of sample may also occur in connection with evaporation, because trichothecenes are easily adsorbed on the glass walls [15].

The total elution volume can be reduced to about 10 ml by using the same cartridges as commonly used for 1-ml solid-phase extraction columns (5 × 0.55 mm I.D.). By using HPLC clean-up columns, such as the Chromguard cartridge, the elution volume can be reduced to 5 ml or less, which is the volume of the reaction vials normally used for derivatization. No extra evaporation step or quantitative transfer of extract from one vial to another is then needed.

Reducing the column diameter results in a lower capacity of the column. The detection limit of trichothecenes measured by GC is, however, mostly limited by other impurities in the extract injected. Therefore, although only 0.5 ml of sample extract was injected in this procedure, which corresponds to 0.1 g of the sample, a detection limit of about 20 μg/kg was obtained, which is satisfactory for most purposes.

Replacement of the vacuum manifold with an HPLC pump or similar equipment renders automation of the procedure possible. A more uniform flow through the column is also ensured. The pressure above the column was between 100 and 150 psi with a flow-rate of 1 ml/min. Standard HPLC equipment was used in this work for the clean-up step, except that the normal injector loop was replaced with a larger preparative loop. The fraction collector was also slightly modified so that all steps could be controlled by the data system via a time relay. Special holes for the reaction vials were also made in the rack.

The clean-up column cartridge was dry-packed with carbon, alumina and Celite, which were mixed well in the correct proportions before use. The trichothecenes were eluted with acetonitrile–water (84 + 16, v/v). By washing with acetonitrile between each injection, one cartridge could be used for about ten samples before it had to repacked. Some of the contaminants remained on the column and restricted the lifetime of the cartridge. By using a cartridge column with a snap-open–snap-shut holder, a new cartridge can be inserted within 1 min. Much time is saved by reducing the time-consuming job of packing columns.

Nitrogen was used for evaporation to prevent decomposition. It is important that no water remains in the vials to ensure complete derivatization. Omission of the addition of benzene sometimes caused poor recoveries, even when the residue appeared dry, probably because water was occluded in the residue. Benzene forms an azeotrope with water, and thereby ensures the removal of most of the water. The vials were sealed during cooling to avoid adsorption of water. Addition of as much as 100 μl of TMSI was necessary to ensure complete derivatization. The charcoal–alumina column does not remove many of the more polar contaminants. The washing of the hexane phase at the end of the procedure does, however, ensure the removal of many of these components [21].

At least two recovery tests were carried out for the group B trichothecenes for each set of samples analysed. An 80- or 160-ng amount of each of the trichothecenes was added to an 1-ml aliquot of sample extract. The same clean-up and GC method was used for these extracts as for the others. The recovery for 50 spiked samples was 94% (σ_{n-1} = 11) for DON, F-X and NIV and 81% (σ_{n-1} = 20) for 3-acetyl-DON. About 500 samples have been analysed by the method described.

CONCLUSIONS

An automated clean-up procedure capable of screening a large number of samples for trichothecenes has been developed. A two- to threefold increase in the number of samples analysed was observed in our laboratory when the traditional clean-up method with 9 mm I.D. columns and vacuum manifold was replaced with the proposed method.

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