Journal of Chromatography, 323 (1985) 121–126
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17,344

DESIGN AND CONSTRUCTION OF AN INTERFACE FOR DIRECT LIQUID INTRODUCTION COUPLING OF A MICROBORE HIGH-PERFORMANCE LIQUID CHROMATOGRAPH TO A QUADRUPOLE MASS SPECTROMETER

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SUMMARY

Recently various efforts have been made to transfer the compounds separated by high-performance liquid chromatography into the ion source of a mass spectrometer. One development in such interfacing is the on-line direct liquid introduction of the column effluent.

In this paper we describe the design and construction of a splitless micro-high-performance liquid chromatography–mass spectrometry interface which allows the unrestricted flow of the eluent and jet-formation directly into the ion volume of a quadrupole mass spectrometer. The use of a water-cooling, laser-punctured orifice or diaphragm at the probe tip was not necessary in our experiments. The desolvation of the mobile liquid phase in the transline, an open glass capillary, is controlled only by the selected parameters for ion source temperature, the flow-rate of the chromatographic pump and by the high vacuum of the mass spectrometer.

Tests on different transline materials have been carried out and the results are discussed; applications and mass spectra obtained by this procedure are presented.

INTRODUCTION

Three current developments of interfacing a liquid chromatograph to a mass spectrometer are used in the majority of applications: the moving belt, the thermospray and the direct liquid inlet[1,2]. In our laboratory we have used the direct liquid introduction (DLI) method for the combination of microbore high-performance liquid chromatography–mass spectrometry (HPLC–MS). We have previously reported our results obtained in the detection of the trichothecenes Nivalenol and Deoxynivalenol in the selected ion monitoring (SIM) mode[3].

0021-9673/85/$03.30

EXPERIMENTAL

The LC–MS system used consists of a Jasco Familic 100N micro-HPLC pump and a Finnigan MAT 4023 electron impact (EI)- and chemical ionization (CI)-quadrupole mass spectrometer with an Incos 2300 data-system. The mass spectrometer was modified with the new ion source type of the Finnigan MAT 4500 instrument. Typical LC and MS parameters of our experiments are as follows. LC columns: 16-cm PTFE tubing, 0.5 mm I.D., packed with μ-Fine Sil C_{18}, particle size 10 μm (Jasco), endcapped with a PTFE foam filter to prevent transline- and ion volume contamination with silica. Eluent: acetonitrile–water (50:50, v/v); flow-rate 5 μl/min. Mass spectrometer: CI mode; positive and negative ion detection; source pressure, 0.50 Torr; mass analyser pressure, $2 \cdot 10^{-5}$ Torr; source temperature, 250°C; filament voltage, 70 eV; filament current, 0.3 mA; LC–MS multiple ion detection (MID) scans in 1.75 sec and 2.0 sec per cycle.

Currently we are using a LC–MS interface that was specially designed and manufactured to fit the solid sample probe-inlet of the mass spectrometer. Fig. 1 shows a diagram of this low-cost part made of stainless steel. An open glass capillary (40 cm × 0.1 mm I.D. × 0.45 mm O.D.) is used as the transline. It is inserted into the probe and sealed with ferrules and slit-hexnuts, such as are used by analysts to connect capillary columns to a gas chromatograph. The capillary tip is carefully positioned into the ion volume of the source. The column is coupled to the transline via a short piece of stainless-steel capillary, mantled with tight-fitting PTFE tubing and sealed at the outside with PTFE shrink-tubing.

For the DLI of the HPLC effluent we have tested about fifteen different transline capillaries. In most cases the eluent flow showed a sudden breakdown owing to ice-formation in the desolvation zone of the transline. The ice-crystals were clearly visible after quick disconnection of the transline from the interface-housing. Only two of the capillaries provided the continuous flow necessary for stabilizing the desolvation process in the capillary tip.

We thus conclude that the inner geometry of the capillary in combination with a constant flow-rate of the liquid mobile phase is of major importance for stabilizing the CI conditions of the mass spectrometer, especially as no restriction such as an orifice or diaphragm and no additional control on temperature or pressure was installed in our LC–MS interface.

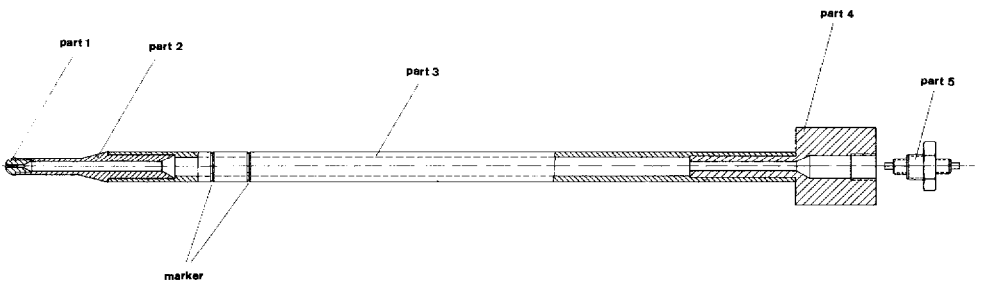

Fig. 1 Diagram of the stainless-steel LC–MS interface probe. Part 1: probe tip with 0.5-mm pinhole; part 2: connecting piece with narrowing cone; part 3: tube with marker for probe-insertion; part 4: socket for fixing-screw; part 5: fixing-screw for capillary positioning and sealing.

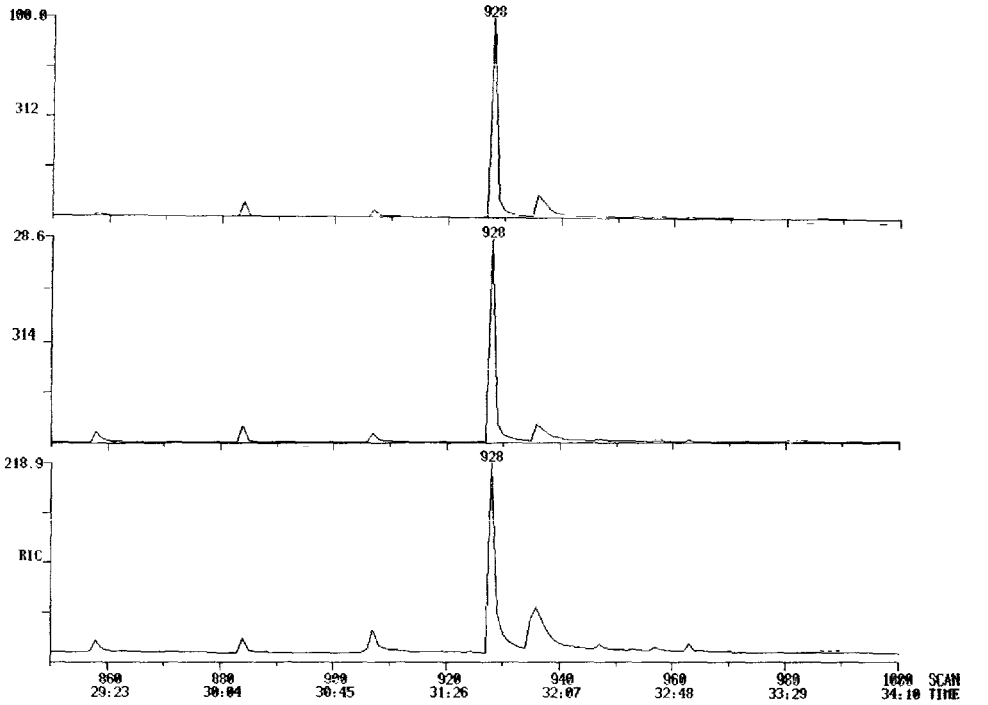

Fig. 2. Negative ion chemical ionization LC–MS analysis of aflatoxins B_1 and B_2. MID reconstructed ion chromatogram (RIC) and mass chromatograms for the selected ion masses of m/z 312 (M^-) for aflatoxin B_1 and m/z 314 (M^-) for aflatoxin B_2. Sample, 30 ng of each mycotoxin.

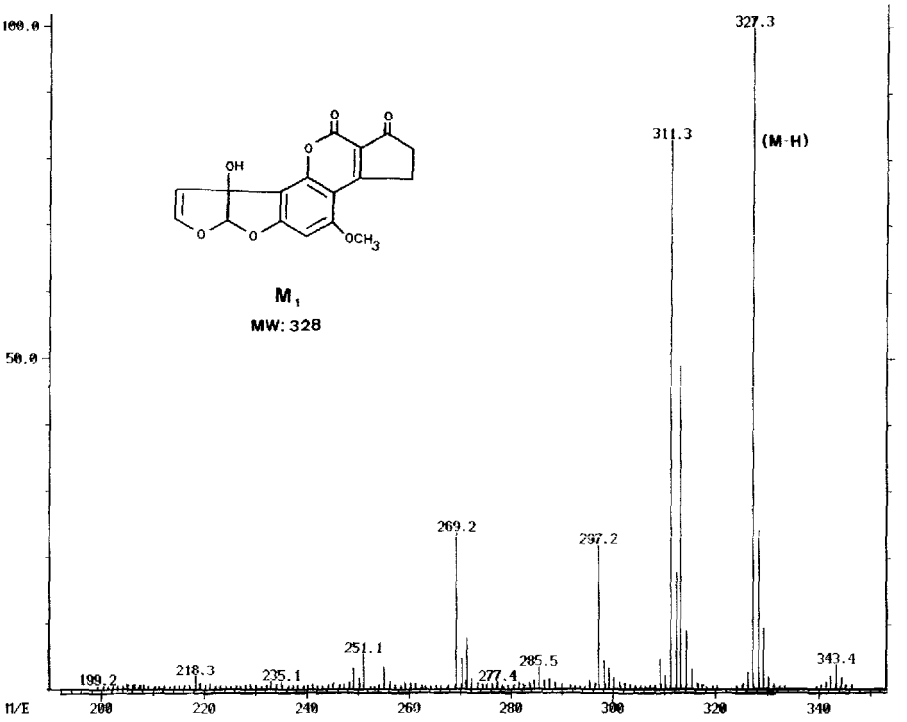

Fig. 3. Negative ion chemical ionization mass spectrum of aflatoxin M_1. Source temperature, 230°C; sample, 500 ng; solid sample introduction.

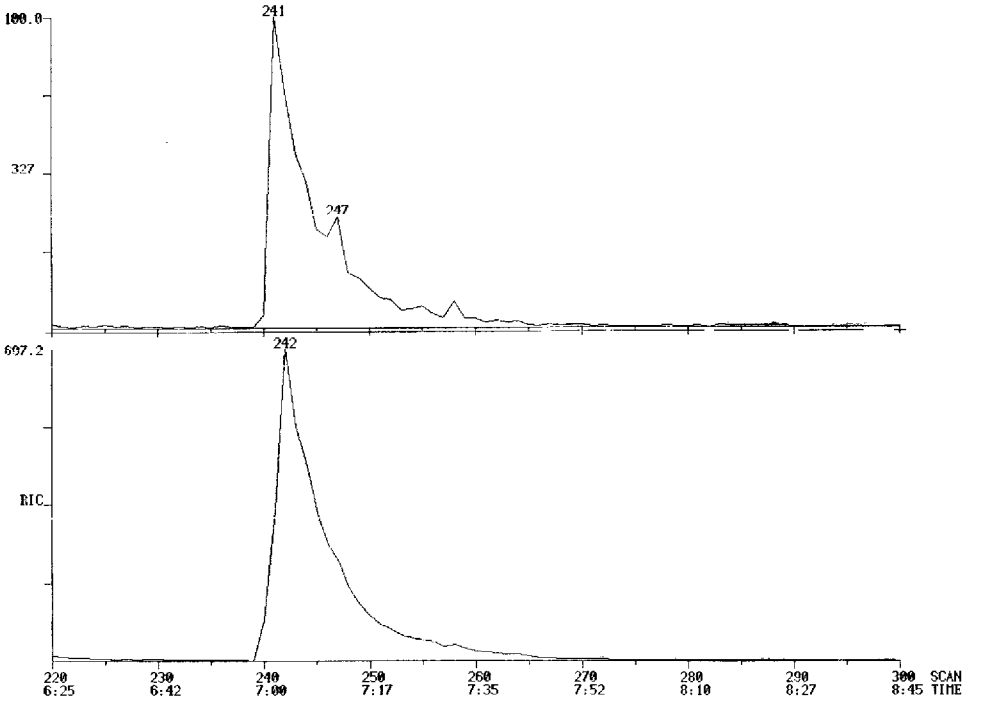

Fig. 4. Negative ion chemical ionization LC MS Analysis of aflatoxin M_1 (MW 328). MID RIC and mass chromatogram for the selected ion mass m/z 327. Sample, 30 ng.

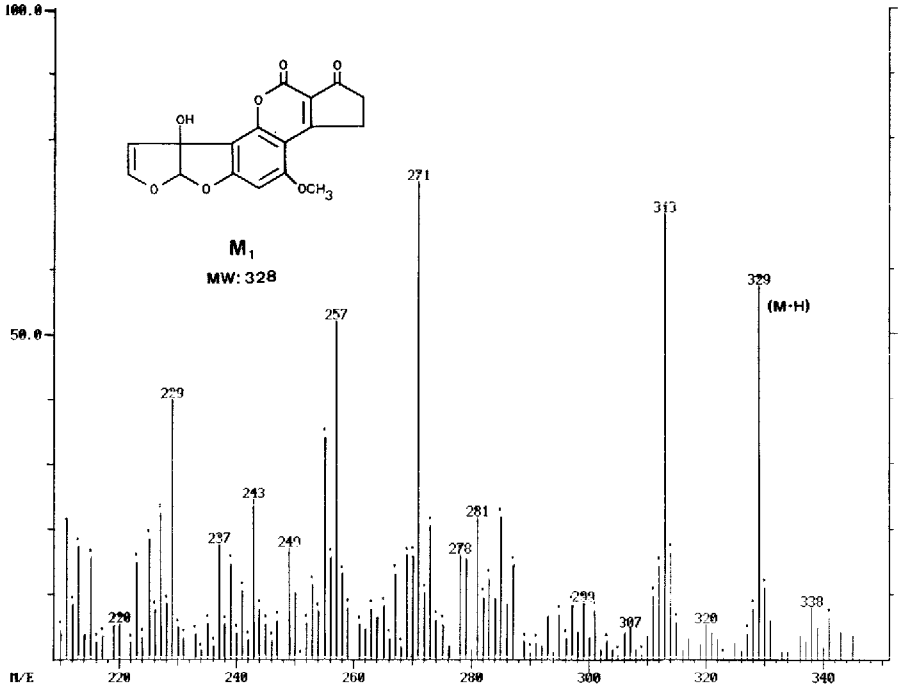

Fig. 5. Positive ion chemical ionization mass spectrum of aflatoxin M_1. Source temperature, 230°C; sample, 500 ng; solid sample introduction.

RESULTS AND DISCUSSION

The results we achieved by this simple coupling technique are illustrated in Figs. 2–6. Fig. 2 shows the MID reconstructed ion chromatogram and the mass chromatograms for the negative ions of aflatoxins B_1 and B_2 in the SIM mode. Owing to the poor performance of the microbore HPLC column, both compounds eluted in one unresolved peak, which was verified by UV detection. They are separately detected by monitoring their quasi-molecular ions at *m/e* 312 (M^-) for aflatoxin B_1 and at *m/e* 314 (M^-) for aflatoxin B_2. The injected amount of each mycotoxin was 30 ng. The retention times recorded in the figures do not represent the true retention of the compounds, as in the case of aflatoxins B_1 and B_2 we applied multi-injections in a long run and for aflatoxin M_1 the start of the aquisition and activation of the filament was delayed. This was done to prolong the life of the filament. The actual retention times for the aflatoxins B were 8.4 min and for aflatoxin M_1 14.1 min.

Figs. 3 and 5 show the CI spectra of aflotoxin M_1 in negative and positive ion detection mode, obtained by the solid sample introduction technique. The quasi-molecular ions at *m/e* 327 (M − H) and at *m/e* 329 (M + H), respectively, were selected for the detection of the mycotoxin by on-line LC–MS analysis. The resulting MID RICs and mass chromatograms are shown in Figs. 4 and 6.

With our method we have been able to detect several compounds with a molecular weight of above 300 in the lower nanogram range. It is applicable to extracts from food samples, as was reported earlier[3]. It should also be applicable to the

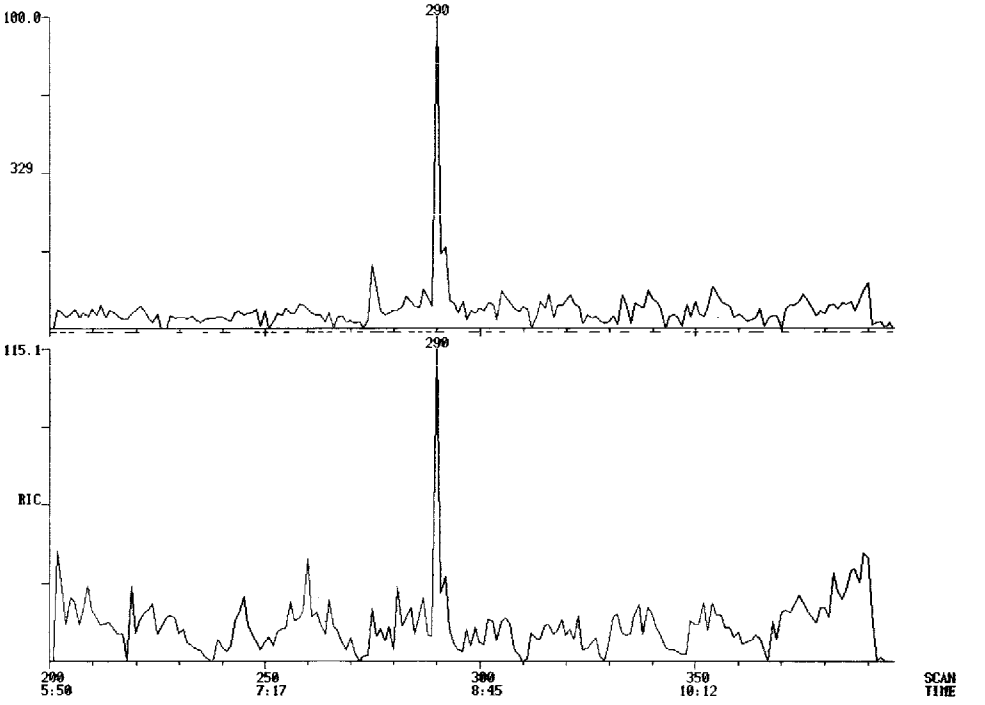

Fig. 6. Positive ion chemical ionization LC–MS analysis of aflatoxin M_1 (MW 328). MID RIC and mass chromatogram for the selected ion mass *m/z* 329. Sample, 30 ng.

confirmation of compounds that are isolated or fractionated by other chromatographic procedures, *e.g.* preparative HPLC[4] or preparative thin-layer chromatography.

We plan to introduce a stainless-steel capillary transfer line into the interface and we hope to overcome the problems observed using some glass and fused-silica capillaries. The quantification of contaminants determined by on-line LC–MS methods with the use of other than labelled internal standards, which are often not available, is still of major interest to many analysts. We shall attempt to improve knowledge in this area.

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