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Note

Preparative high-performance liquid chromatography using detection by thermospray mass spectrometry

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There are many situations wherein preparative chromatographic procedures are made more difficult because of the lack of an on-line detector to monitor the separation process. Examples are where the substrate to be separated lacks a chromophore, in the use of UV-opaque solvents, and in situations wherein detector baseline distortions are encountered when gradient elution is used with refractive index detection. Thermospray mass spectrometry (MS)[1], in total ion current mode, is a sensitive general liquid chromatographic (LC) detector; however, it is a destructive method. With thermospray MS, the use of a chromatographic effluent splitter, made with a simple tee and a flow restrictor on one leg, and the mass spectrometer on the other leg, has limitations because the back-pressure of the thermospray vaporizer is variable with temperature, and by occlusion with use, thus causing the split ratio to vary. We describe a device that can be adjusted to changing conditions and that allows 75% to 93% of a chromatographic eluent to be collected, with the balance sent to the mass spectrometer. We illustrate its use in the separation of one of the three isomers of di-O-cyclohexylidene-1,2,3,4,5,6-[$^2H_6$]*myo*-inositol from a crude reaction mixture.

EXPERIMENTAL

The thermospray system used was a Vestec (Houston, TX, U.S.A.) Model 210 liquid chromatograph–mass spectrometer operated with a Teknivent (St. Louis, MO, U.S.A.) Vector 1 data system. Two Shimadzu (Tokyo, Japan) Model LC-6A pumps controlled by a Shimadzu Model SCL-6A gradient mixing unit were used to supply the chromatographic elution buffer. Chromatographic characterization of the system was carried out on a 25 × 0.46 cm, 5 μm particle diameter Supelcosil (Supelco, Bellefonte, PA, U.S.A.) ODS analytical column at a flow-rate of 0.8 ml/min. The separations for collection were carried out on a Beckman (Berkeley, CA, U.S.A.) Ultrasphere 5 μm, 25 × 1 cm ODS preparative-scale column operating at a flow-rate of 3 ml/min.

The construction of the effluent splitter used with the Vestec thermospray LC–MS system is shown schematically in Fig. 1. The effluent from the column is first divided with a low-dead-volume stainless-steel tee, the splitter tee in Fig. 1 (part

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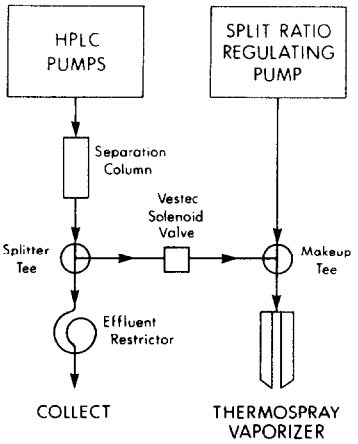

Fig. 1. Splitter flow system.

U-428, Upchurch Scientific, Oak Harbor, WA, U.S.A.), the sample-collecting leg of which was connected to an effluent restrictor made of 45 cm of 100 μm I.D. × 1/16 in O.D. stainless-steel tubing. The other leg of the tee was connected to the thermospray inlet solenoid valve. The LC–MS side of the solenoid valve was connected to a second low-dead-volume tee, the makeup tee, by 5 cm of 100 μm I.D. tubing. One leg of the second tee was supplied with water, at flow-rates that determined the split ratio, by a Waters (Milford, MA, U.S.A.) Model 9000 pump (the split ratio regulating pump in Fig. 1). The remaining leg of the makeup tee carries the minor portion of the split to the vaporizer of the thermospray mass spectrometer.

The need for a continuously monitored HPLC effluent splitter arose in the separation of the products of the acid-catalyzed reaction of deuterium-labeled *myo*-inositol with 1-ethoxycyclohexene in which three di-O-cyclohexylidene ketals are formed, the 1,2:4,5-, 1,2:3,4- and 1,2:5,6-isomers. The reaction was carried out according to Garegg *et al.*[2] except for the use of 100 mg of [$^2H_6$]*myo*-inositol (MSD Isotopes, St. Louis, MO, U.S.A.). After washing the reaction mixture to remove *p*-toluenesulfonic acid, it was taken to dryness and dissolved in methanol. The methanolic solution was chromatographed, 3 mg per run, on the large ODS column eluted with a linear gradient of 25 min duration and a flow-rate of 3 ml/min, starting with methanol–water (1:1) and ending with methanol–water (8:2).

RESULTS AND DISCUSSION

The ratio of sample diverted to collection *versus* that sent to the mass spectrometer was monitored with the column effluent pumped at 3 ml/min and the split ratio regulating pump operating at various rates. The split ratio was measured by comparing the elution of adenosine at the collection leg of the splitter with that at the mass spectrometer. The amount of adenosine diverted to the collection leg was measured at 254 nm using an LKB (Cambridge, U.K.) Ultrospec K UV–VIS spectrometer, while that entering the mass spectrometer was measured at *m/z* 268 (MH^+). At a split ratio regulating pump flow-rate of 0.1 ml/min at the makeup tee, the collected

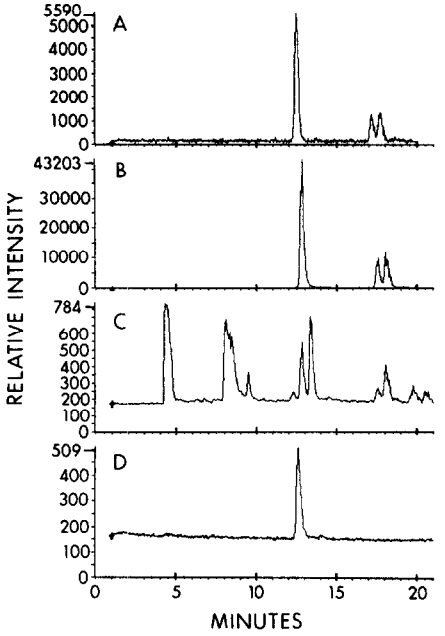

Fig. 2. HPLC separation of bis(cyclohexylidene) isomers of [$^2H_6$]*myo*-inositol from a preparative reaction with detection by MS. (A) Reconstructed mass chromatogram of *m/z* 347 (MH^+) obtained by LC–MS of the mixture on an analytical HPLC column connected directly to the LC–MS system. The peak at 12.5 min is 1,2:4,5-di-O-cyclohexylidene-[$^2H_6$]*myo*-inositol. (B) As A but with the mixture separated on the preparative-scale column and with the use of the splitter assembly. The later retention times relative to A result from the use of the large column. (C) Reconstructed total ion current mass chromatogram of the reaction mixture separated on the preparatory-scale column with the splitter. The 1,2:4,5-di-O-cyclohexylidene isomer elutes at 12.8 min barely separated from two unknowns. (D) Total ion current chromatogram of the collected and purified 1,2:4,5-di-O-cyclohexylidene-[$^2H_6$]*myo*-inositol, rechromatographed on the analytical column.

amount was 93%, which is the recovery of adenosine from the column without the splitter assembly in place (*i.e.*, 100% for pratical purposes). Intermediate flow-rates gave split ratios that bore a linear relationship to those values.

In Fig. 2A is shown the reconstructed mass chromatogram of *m/z* 347 (MH^+) from the washed reaction mixture containing the mixed isomers of the di-O-cyclohexylidene derivatives of deuterated *myo*-inositol. The peaks at *m/z* 347 are, in order of elution, the 1,2:4,5-isomer at 12.5 min, with the 1,2:3,4- and 1,2:5,6-isomers only partially resolved at 17–18 min. Fig. 2A was made using the ODS analytical column without a splitter and with a flow-rate of 0.8 ml/min. Fig. 2B is the *m/z* 347 reconstructed mass chromatogram of the same material separated on the preparative-scale ODS column with the splitter operating. As can be seen, no degradation of the separation results from the use of the preparative column or the splitter. The slightly different retention times of the analytical and preparative columns are due to differences in the chromatographic conditions. Fig. 2C is the total ion chromatogram (*i.e.*, non-selective detection) of the reaction mixture separated by the preparative-scale column showing the complexity of the mixture and the presence of an unknown eluting at 13.5 min, close to the desired 1,2:4,5-isomer. Fig. 2D is the reconstructed

total ion chromatogram of the collected 1,2:4,5-isomer, run on the analytical-size column, which is free of the unknown at 13.5 min as well as the other substances in the mixture.

In conclusion, we have described an effluent splitter suitable for use with a thermospray LC–MS system which enables the collection of non-chromophore-bearing substrates as well as gradient separations to be carried out by LC–MS. The collection of deuterium-labeled 1,2:4,5-di-O-cyclohexylidene-*myo*-inositol from a complex reaction mixture with > 90% efficiency illustrates the close match between detector and collection for the collection of closely eluting samples.

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