Short communication

Use of modifier as trapping fluid in preparative supercritical fluid chromatography

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Abstract

A commercial supercritical fluid chromatograph has been adapted for preparative SFC. A simple fractionating and collection system which can use the liquid supercritical fluid modifier as a trapping fluid is described. The fractionation of polycarboxylic acid mixtures is used to illustrate the technique. Acids, isolated and obtained in this way, were of a purity close to 100%. The effect on recovery rates of using additional liquid collection solvent in the collection vessels and of surrounding them with a dry ice jacket was investigated.

Keywords: Preparative chromatography; Trapping methods; Modifiers; Carboxylic acids

1. Introduction

Supercritical fluid chromatography as a preparative separation technique [1–6] has several advantages over liquid chromatography. These include the high diffusivity and low viscosity of supercritical fluids which often allow rapid and efficient separations and the ability to change solvent strength not only by a compositional change but by altering the temperature and pressure which permits separations of many types of compounds. In addition, the gaseous or volatile nature of many fluids used for SFC at room temperature and pressure means that the separated solutes can readily be recovered from the collected fractions by depressurisation.

The technique of preparative SFC consists of four main stages: introduction of the sample into the mobile phase stream; chromatographic separation; non-destructive detection of separated components; and collection of resulting fractions.

Sample collection can be performed in a number of ways: at high pressure; at atmospheric pressure; adsorption on to a solid and subsequent elution; and dissolution in a liquid solvent. High-pressure collection was initially reported by Jentoft and Gouw [1]. This method employed gas eluent collection tubes contained in a vessel pressurised to high pressure using nitrogen gas. The nitrogen pressure maintained the liquid-like density of the collected carbon dioxide mobile phase which contained isolated solute until collection was complete; then gradual depressurisation allowed precipitation of the collected solutes from their carbon dioxide solutions. More recently, Bartle et al. [6] have isolated separated solutes in an eluting supercritical mobile phase by using liquid nitrogen to freeze and trap portions of the fluid. Perrut and Jusforgues [2] reported collection in high-pressure separators from which solutes were sub-

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sequently obtained by depressurisation. Many methods of fraction collection at atmospheric pressure have been described. These include the extensive investigations of Saito and co-workers [5,7], and the fractionation [4] of a range of samples using SFC coupled with thin-layer chromatography. A specially shaped glass collection device which uses the post-restrictor decompression of the carbon dioxide mobile phase to solidify the eluent has also been described [4]. Isolation of tocopherols from wheatgerm by direct collection of the depressurised mobile phase has been reported [7]. Other collection methods include solid-phase adsorption of the separated solutes followed by their elution with a liquid solvent [8], and the dissolution of the solute in a liquid solvent added to the mobile phase between detection and depressurisation [9].

In this paper, a simple fractionating and collection system for use in SFC is described; mixtures of polycarboxylic acids were successfully chromatographed using analytical and semi-preparative columns and fractions with a purity approaching 100% were collected. The effect of a dry-ice cooling jacket and the use of a collection solvent on the percentage recovery of the acids has been investigated.

2. Experimental

2.1. The preparative SFC chromatograph

Preparative SFC was carried out on a modified Hewlett-Packard G1205A SFC system, with a Hewlett-Packard Series 1050 multiple-wavelength ultraviolet detector. The instrument was modified in three ways: an injection valve was fitted which permitted the use of external sample loops up to 200 μl volume; a post-restrictor splitting valve was added to permit the collection of eluted fractions; and modifications were carried out to allow 10 mm I.D. columns to be used with a mobile phase flow up to 5 ml/min.

To modify the internal sample loop valve, a six-way stator and rotor (for valve Model 7010) was obtained from Rheodyne (Cotati, CA, USA), to replace the existing four-way parts. The internal sample loop disk was removed, filed flat and polished after cutting off the calibrated loop tubing.

This disk was then replaced back to front in the valve housing and acted as a back support for the new six-way rotor. The new rotor and stator were then fitted and adjusted and an external sample loop installed. This modification, equivalent to the installation of a replacement six-part external loop injector, is illustrated in Fig. 1.

In order to allow collection of the separated fractions as they elute from the column, a six-way splitter valve (Upchurch, Oak Harbor, WA, USA), was installed into the mobile phase stream immediately after the restrictor, where fluid expansion occurs. The valve was connected to the restrictor with a 15-cm length of 200 μm I.D. PEEK tubing (Alltech Assoc. Applied Science, Carnforth, Lancashire, UK). This narrow diameter was chosen to minimise the post-restrictor dead volume before splitting occurs. To each of the six outlets of the valve, 5-cm lengths of PEEK tubing with internal diameter of 700 μm were attached and the free ends placed in vented vials which could be held in dry ice (Fig. 2). A small quantity (2–5 ml) of methanol could be added to the vials to aid collection. This
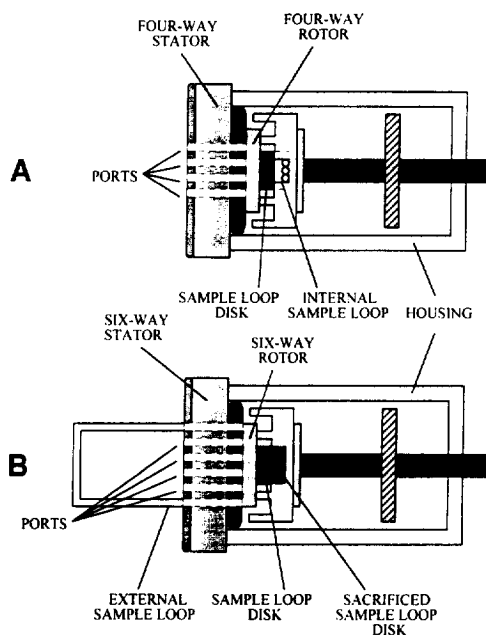

Fig. 1. Schematic diagram of the injection valve modification to permit fitting of a large volume external sample loop. (A) Unmodified internal loop valve; (B) valve modified to take an external loop.
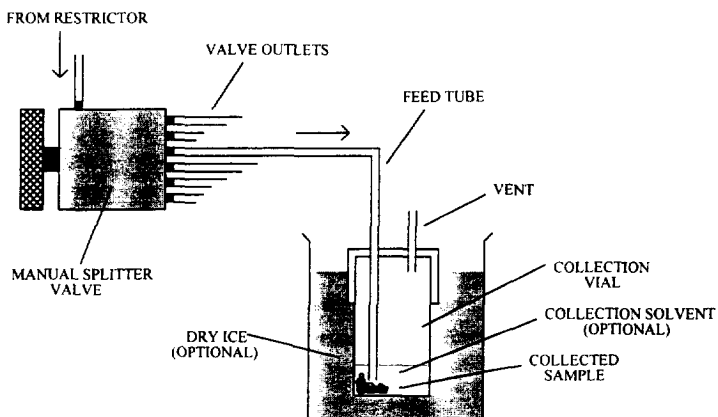
Fig. 2. Schematic diagram of the collection method from the post-restrictor splitter valve.

wider bore tubing was employed to help prevent blocking due to precipitation of solutes upon mobile phase expansion. In addition, the wider bore has the effect of reducing the linear velocity of the expanded mobile phase prior to collection. This prevents the condensed modified/added solvent being ejected through the vent and makes collection a smoother process.

To allow increased quantities to be separated from a single chromatographic injection, a column with a diameter wider than the 4.6-mm I.D. columns typically used was installed. A 10-mm column consisting of a PEEK inner and screw thread end fittings, shrouded in a stainless-steel sleeve was installed with zero dead volume connectors allowing the 10-mm column and standard 4.6-mm column (with 1/16 in. stainless-steel end fittings; 1 in.=2.54 cm) to be readily interchanged.

2.2. Supercritical fluid chromatography

Columns used for preparative SFC were obtained from Phase Separations (Deeside, UK) and contained 5-μm particles of silica: S5 W in 10 cm×4.6 mm (P/N 830112); 10 cm×10 mm (P/N 159789). In order to allow elution of the larger polyacids from these columns, the conditions for the separation of the carboxylic acids required 2% (v/v) trifluoroacetic acid (TFAA) as additive in the mobile phase modifier (i.e. for a 10% methanol-modified mobile phase, the mobile phase as a whole contains 0.2% TFAA). The optimised separation conditions were: fluid

pressure 100 atm (1 atm=101 325 Pa); oven temperature 40°C; mobile phase modifier (10% methanol containing 2% TFAA). For the 4.6-mm column, a 20-μl sample loop was used with an injection period of 4 s which ensured that the entire loop contents were transferred to the column. A flow-rate of 1 ml/min was employed. Sample loops up to a maximum of 200 μl were used with the 10-mm column, thus requiring an injection period of up to 15 s. A flow-rate of 5 ml/min was used with this column to give an approximately equal mobile phase linear velocity [4] to that used with the 4.6-mm column so that the results from each column could be compared. A 1:1:1:1 solution (concentrations 5%, w/w) of the four acids was injected in this way. The detection wavelength was 290 nm to ensure that the response of the detector remained linear with increasing concentration.

2.3. Operation of trapping device

As separated fractions elute from the column, they pass through the detector and immediately into the restrictor where depressurisation of the mobile phase occurs. The expanded phase (carrying the separated sample component) then passes into the splitter valve which can divert the eluent to any one of the six collection vessels. The delay between detection and passage into the valve is negligible due to the narrow tubing used and the reduced phase density with consequent high linear velocity. On reaching the cooled collection vessel some of the carbon dioxide

mobile phase freezes/condenses on to the sides of the vessel together with condensed modifier and separated sample. Carbon dioxide remaining as a vapour escapes through the vent. Upon completion of fraction collection (monitored by observing the detector response) the valve is switched to the next vessel. Removal and gentle warming of the vessel containing sample/modifier/carbon dioxide results in vaporisation of the remaining solidified mobile phase and leaves a solution of the separated sample in modifier plus any additional collection solvent (if added). To avoid a safety hazard, the vent of the collection vial should be large enough to avoid the build-up of excessive pressure in the vial.

2.4. Reagents and materials

Gases employed for the chromatography were obtained from British Oxygen (BOC, Guildford, Surrey, UK) and were of CP grade purity. All cylinders were vapour withdrawal. Methanol for mobile phase modification was purchased from Fisons (Loughborough, UK) and was of HPLC grade. Standard laboratory reagents and materials used were obtained from various suppliers and were of analytical grade or better. Benzoic acid (analytical grade) and n-hexane (HPLC grade) were purchased from Fisons. Trifluoroacetic, phthalic, 1,2,4-benzene

tricarboxylic and 1,2,4,5-benzenetetracarboxylic acids were obtained from Aldrich (Poole, UK).

Solutions (1:1:1:1) of the four carboxylic acids in methanol (5%, w/w, in each acid) were prepared by ultrasonication for approximately 1 h.

3. Results and discussion

SFC with a 4.6-mm I.D column permitted separation of up to 1 mg of the individual acids, injected via a 20-μl volume sample loop. On a larger diameter 10-mm I.D column, a 200-μl loop could be used to separate 10 mg of each acid in a single injection over 15 s (Fig. 3). Although the detector response was saturated during peak elution the baseline was still clear enough for the switching point to be determined.

The recoveries of each of the four acids obtained with neither dry ice jacket nor solvent, and with dry ice jacket alone, with solvent alone, and with both dry ice jacket and collection solvent were determined by drying each of the collected acid fractions resulting from a single injection and evaporation to constant weight in an oven at 40°C. This was done in triplicate for each collection method and the mean mass of each acid recovered together with the standard deviation (σ) of the mean mass was calcu-
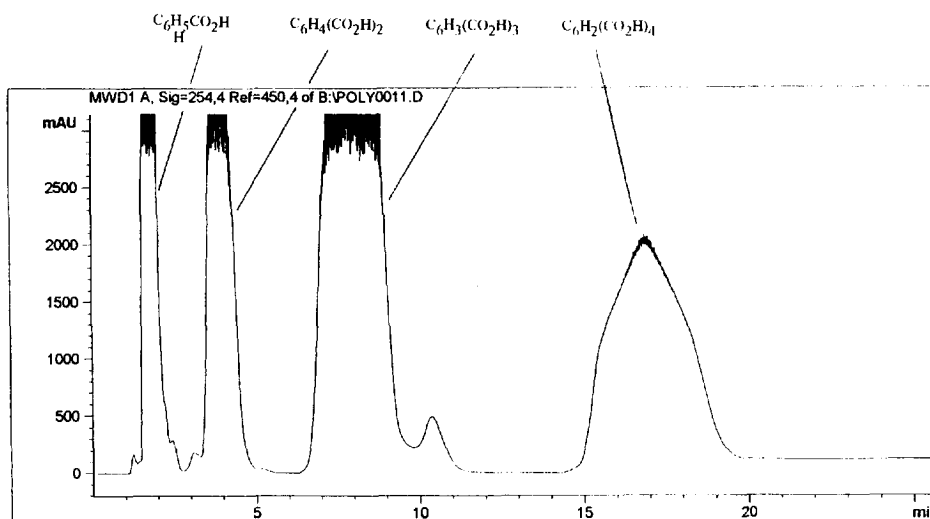

Fig. 3. SFC separation of 10 mg of each of benzene polycarboxylic acids. Conditions: 10 cm×10 mm I.D. column packed with 5 μm S5W silica. Mobile phase: 10% methanol, 0.2%, TFA in CO_2; at 100 atm and 40°C; flow-rate 5 ml/min.

lated. In addition, by using the volume of acid injected for fractionation (200 μl) and the density of the 1:1:1:1 solution (0.95±0.02 g/ml), the quantity of each acid injected on to the chromatograph could be calculated. This value was then used to calculate the fractional recovery for each acid isolated using each collection method. Resulting mean and fractional recoveries are included in Table 1. Errors are appended and are quoted as ±2σ.

The data contained in Table 1 shows that the best method of trapping is the use of liquid collection solvent together with dry ice jackets around the collection vials. With neither a solvent nor a dry ice jacket, the recoveries are quite low. Approximately one quarter of the mass of each acid was not trapped. A possible explanation is that the expanded carbon dioxide–methanol fluid is an aerosol as it emerges from the feed tube and enters the collection vial so that some product is swept out through the vent (Fig. 2) before complete solute condensation occurs. By bubbling the fluid through a small quantity of solvent in the vial, the recoveries are drastically improved, most of the product (97%) being trapped by this addition. The recoveries obtained when solvent is present are very similar to those obtained when a dry ice jacket is used in the absence of additional solvent. This method tends to result in a build up of frozen carbon dioxide mobile phase on the inside of the collection vessel which evaporates when the vessel is removed and allowed to reach room temperature. Recoveries approach 100% when both a dry ice jacket and collection solvent are used together. Although the solubility of the acids in the

collection solvent is likely to be decreased by the reduced temperature, which could have a detrimental effect on the trapping efficiency, the solute trapping effect brought about by the partial freezing of the mobile phase appears to outweigh this effect.

The purities of the resulting acids were determined by dissolving the collected acid fractions in approximately 2 ml of methanol and re-analysing each solution using the 4.6-mm I.D. column together with the 20-μl sample loop. In all cases the purities were determined to be close to 100%.

The use of supercritical fluid modifier as trapping fluid in SFC appears to be a promising technique for isolating modifier soluble solutes. The key advantage of the trapping method described here is the fact that commercial SFC equipment can be simply modified. The technique is, furthermore, equally applicable to trapping either microgram or milligram quantities. It can be used to trap solutes with a very high purity and with a high yield providing the resolution is adequate. Partially resolved components can be isolated with high purity but with a reduced fractional recovery by "cutting out" the overlapping sections of the separation.

Acknowledgments

Table 1
Mean recoveries during preparative SFC of organic acids with different trapping procedures

Trapping methods		Mean mass recovered (mg) (fractional recovery in parentheses)			
Collection solvent	Dry ice jacket	Benzoic acid	Phthalic acid	Benzene tricarboxylic acid	Benzene tetracarboxylic acid
No	No	6.9±0.2 (0.73±0.03)	6.7±0.2 (0.71±0.03)	7.2±0.2 (0.76±0.03)	7.1±0.2 (0.75±0.03)
Yes	No	9.2±0.2 (0.97±0.03)	9.3±0.2 (0.98±0.03)	9.2±0.2 (0.97±0.03)	9.1±0.2 (0.96±0.03)
No	Yes	9.3±0.2 (0.98±0.03)	9.3±0.2 (0.98±0.03)	9.4±0.2 (0.99±0.03)	9.3±0.2 (0.98±0.03)
Yes	Yes	9.4±0.2 (0.99±0.03)	9.5±0.2 (1.00±0.03)	9.4±0.2 (0.99±0.03)	9.5±0.2 (1.00±0.03)

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