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Injection of large volumes of aqueous solutions in capillary supercritical fluid chromatography and sample preconcentration by multiple injections

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ABSTRACT

The possibility of using water or aqueous solutions as the solvent in capillary supercritical fluid chromatography was successfully demonstrated. Large volumes (up to 1 μl) of aqueous sample solutions were injected. Sample preconcentration was performed by means of multiple injections of aqueous sample solutions. The solutes were trapped at the beginning of the column at low density (high temperature and low pressure) and eluted using a density programme. The method can be applied to trace analysis. It proved to be linear in the range examined. Flame ionization detection was used for the studies. As this technique is not sensitive to water, no solvent peak appears, which may be an advantage for certain applications. The influence of water injections on the column performance and the reproducibility of injection was investigated.

INTRODUCTION

The properties of the solvent are known to play an important role in supercritical fluid chromatography (SFC) [1,2]. Very complex solubility phenomena may occur during injection, as combinations of a supercritical fluid, neat or mixed with solvent, a subcritical liquefied gas, solvent and solutes may be present simultaneously in the injector and the column head. The band-broadening effect of solvents, which is well known in LC, has also been demonstrated in SFC. Split peaks result from the injection of excessive volumes.

Although peak splitting may be caused by various phenomena in the injector or the column inlet, an important mechanism in SFC seems to be incomplete mixing of the injection solvent with the fluid. Part of the sample is eluted with the solvent plug until complete mixing is obtained on the column, while the remainly is slightly retained by partitioning in the first part of the column [3]. Additionally, the column diameter in open-tubular column SFC is about five times smaller than in, *e.g.*, open-tubular gas chromatography (GC). With an allowable injection volume in SFC of approximately one tenth of that in GC, a five to ten times more concentrated sample must be introduced into the column in order to obtain comparable quantitative levels. Such a sample is then introduced into the column dissolved in a solvent that often has better solubilizing properties for the solute than does the mobile phase under the injection conditions. There is, nevertheless, sometimes a need for large injection volumes, *e.g.*, in trace analysis.

Various splitless methods that allow the injection of up to 1 μl have been reported. Most of the methods are based on the separation of the solutes from the solvent. This includes the retention gap technique [4,5], solvent venting [5–8] and backflushing [9]. In contrast, Hirata and co-workers [10–12] developed a mixing method and indicated that solute focusing can be greatly faciliated by dilution of the

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solvent with carbon dioxide. By combination of solvent venting and dilution, injection of 100-μl volumes on to a 100 μm I.D. column became possible [13]. An advantage of the various venting techniques and solvent backflushing is the almost complete elimination of the solvent. With 10–15-m open-tubular columns, the solvent often takes more than 10 min to elute, which contributes significantly to the analysis time. Additionally, a broad solvent peak may interfere with the determination of early eluting peaks.

In this paper we describe the injection of large volumes (up to 1 μl) of aqueous sample solutions. The influence of water injections on the column and detector performance and the reproducibility of injection were studied. Sample preconcentration was performed by means of multiple injections of the same sample solution. The solutes were trapped at the beginning of the column at low density (high temperature and low pressure) and eluted using a density programme [14]. The linearity of this accumulation procedure was investigated.

EXPERIMENTAL

Instrumentation

A Lee Scientific (Salt Lake City, UT, USA) Series 600 SFC instrument was used with flame ionization detection (FID). The detector was kept at 375°C. The pump was cooled by circulating a water–ethylene glycol mixture at 7°C using a Haake (Karlsruhe, Germany) refrigerating unit. Carbon dioxide (Carbagas, Basle, Switzerland) was used as the mobile phase. Sample was introduced using a Rheodyne (Cotati, CA, USA) Model 7520 injector. Commercially available sample rotors with 0.2- and 1.0-μl loops were used. A 9 m × 50 μm I.D. SB Cyanopropyl-25 open-tubular capillary column (Lee Scientific) with a film thickness of 0.25 μm was used as an analytical column. A frit restrictor (25 cm × 50 μm I.D. (Lee Scientific) was used to maintain the pressure. Data collection and reporting were performed on a Perkin Elmer Class 2000 system.

Operating conditions

Samples were introduced by direct injection on to the analytical column. The density was kept low (0.17 g/ml; oven temperature 200°C; pressure *ca.* 135 bar) during injection. Under these conditions the solubility of the solutes is decreased and trapping with peak focusing is achieved on the top of the column [5,15].

After a 5-min hold (for the 1-μl loop the hold time was increased to 20 min), the density was programmed to 0.49 g/ml at a rate of 0.04 g/ml·min (oven temperature 200°C). By increasing the density, the solutes are redissolved in the mobile phase and chromatographed through the column.

For sample accumulation several successive injections were performed (time interval *ca.* 3 min) at low density (0.17 g/ml) before the SFC density programme was started.

Sample solution

A 14.6-mg amount of PCO 400 (benzopyran derivative) was dissolved in 25 ml of water.

RESULTS AND DISCUSSION

The miscibility of the injection solvent with the mobile phase is very important in order to avoid split solute peaks [2]. For the injection of aqueous solutions of PCO 400 a high operating temperature was chosen (oven temperature 200°C) in order to promote the solubility of water in carbon dioxide.

Examples of chromatograms obtained by single and multiple injections of aqueous PCO 400 sample solutions are given in Figs. 1 and 2. Water does not influence the performance of the system, even if large amounts (*e.g.*, 1 μl) are injected.

As the detector is not sensitive to water, no solvent peak is observed. This may be an advantage for certain applications, *e.g.*, if early eluting peaks are covered by the solvent peak.

The peak shape is influenced by the density and, more important, the polarity of the sample solvent [2,3]. Both properties affect the miscibility of the sample solvent with the mobile phase. Therefore, the peak shapes may vary if different solvents are used for the analysis of the same solute. PCO 400 samples (0.58 mg/ml) were injected with the 1-μl loop using either pure ethanol or water as solvent (Fig. 3). Whereas slight fronting of the PCO 400 peak appears when ethanol is used as the solvent, slight tailing is observed with water under the same conditions. In both instances, however, the peak shape is acceptable. With water as solvent virtually no solvent peak is visible compared with ethanol.

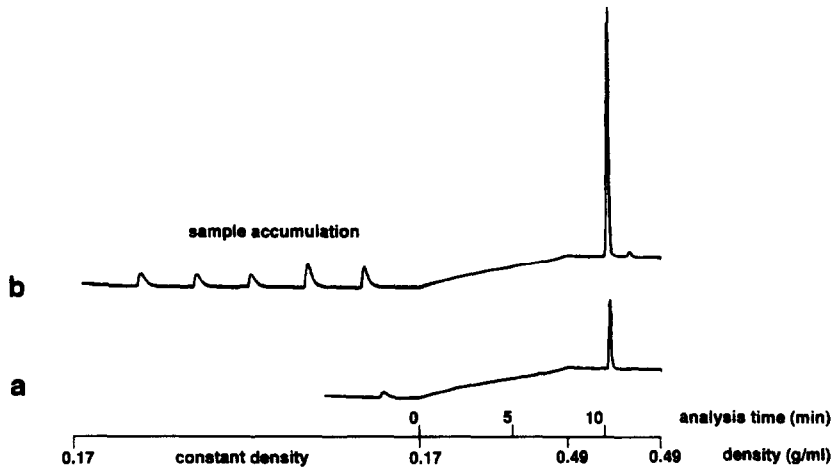

Fig. 1. Direct injection of aqueous PCO 400 solutions. (a) 116 ng of PCO 400 in water, obtained by one injection; (b) 580 ng of PCO 400 in water, obtained by five successive injections of the same sample solution. Conditions: 9 m × 50 μm I.D. SB-Cyanopropyl-25 fused silica column (0.25-μm film thickness), 25 cm × 50 μm I.D. frit restrictor, 0.2-μl sample loop, injection time 1.8 s, CO_2 at 200°C, linear density programme from 0.17 to 0.49 g/ml at 0.04 g/ml·min after an initial isopycnic period of 5 min (solute trapping).

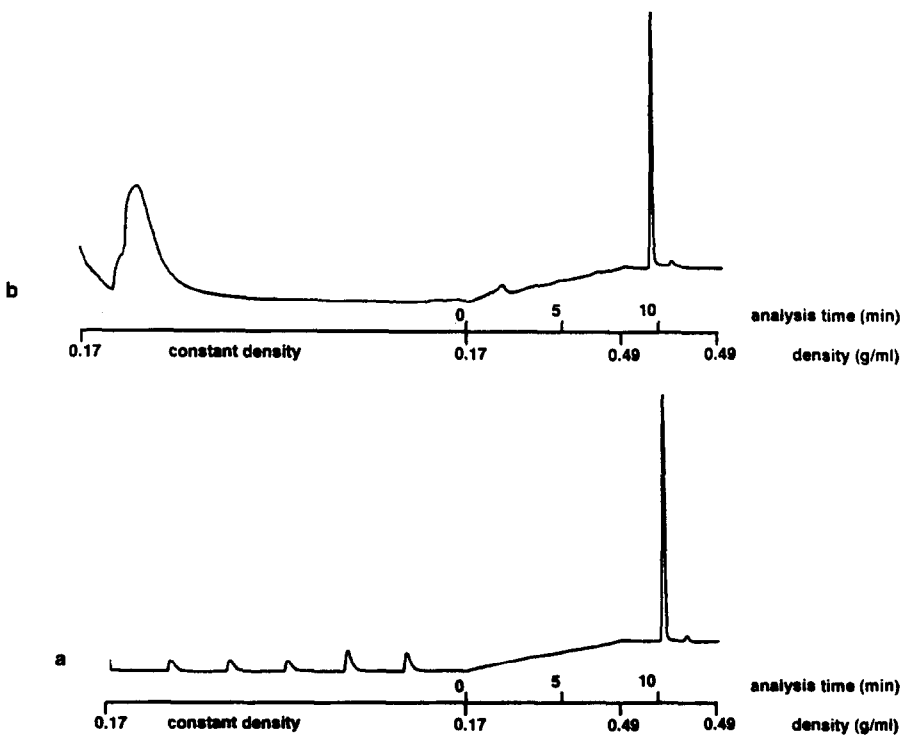

Fig. 2. Direct injection of aqueous PCO 400 solutions with different loops. (a) Sample accumulation with five successive injections with the 0.2-μl sample loop; (b) one injection with the 1.0-μl sample loop. Conditions as in Fig. 1b except for a slightly shorter restrictor for (b).

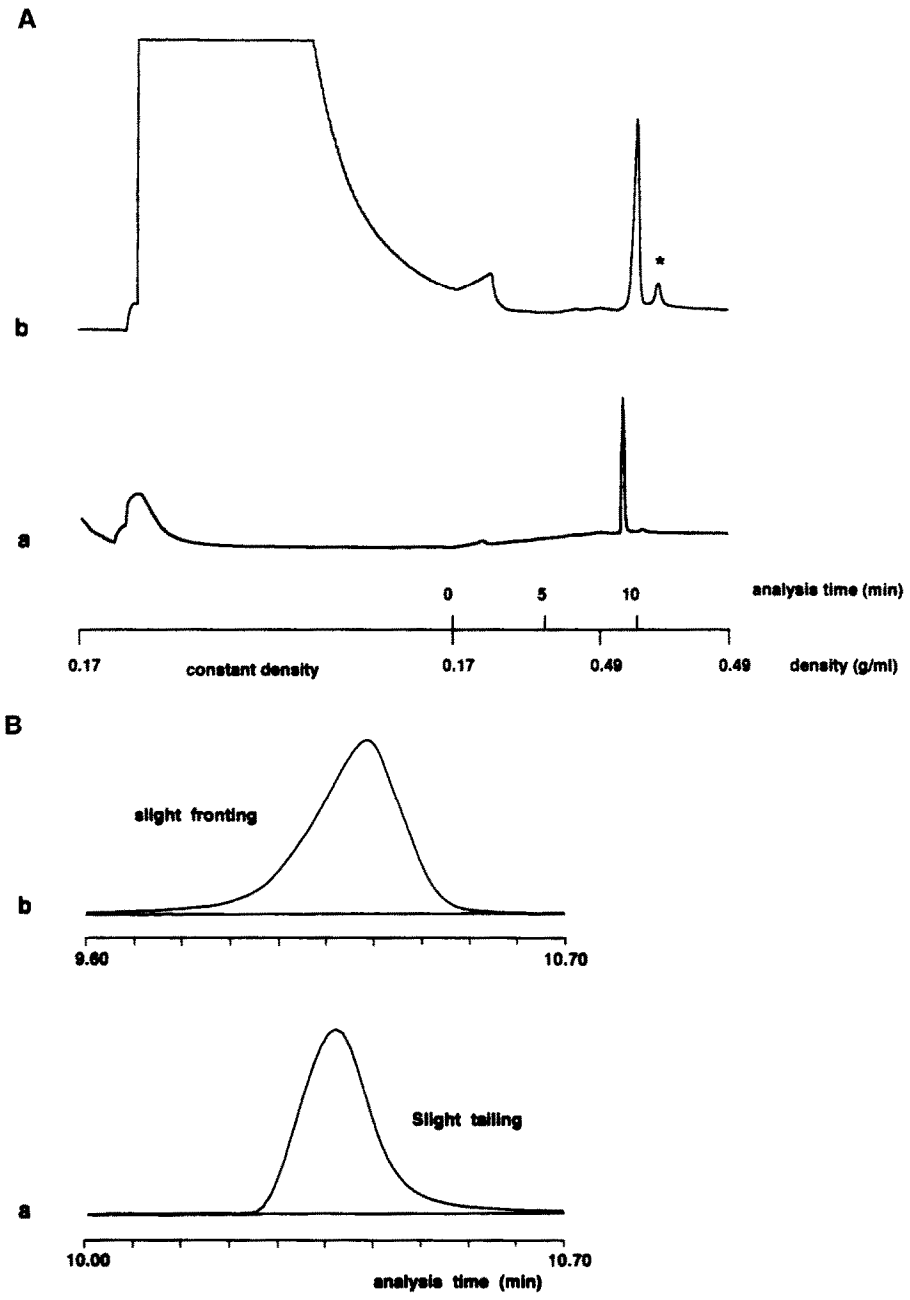

Fig. 3. (A) Direct injection of PCO 400 solutions with different solvents: (a) water and (b) ethanol. Conditions: 9 m × 50 μm I.D. SB-Cyanopropyl-25 fused silica column (0.25-μm film thickness), 25 cm × 50 μm I.D. frit restrictor (slightly shorter for (a), 1.0-μl sample loop, injection time 40 s, CO_2 at 200°C, linear density programme from 0.17 to 0.49 g/ml at 0.04 g/ml·min after an initial isopycnic period of 20 min. The asterisk indicates a degradation product of PCO 400. (B) Influence of the solvent on the peak shape (1.0-μl sample loop): (a) water; (b) ethanol. Conditions as in (A).

TABLE I

RELATIVE STANDARD DEVIATION OF THE ABSOLUTE PEAK AREA OF PCO 400 (0.58 mg/ml) IN DIFFERENT SOLVENTS AND WITH DIFFERENT LOOPS

Loop (μl)	Solvent	R.S.D. of the absolute peak area (%)
1.0	Ethanol	3.1 (n = 4)
	Water	7.3 (n = 9)
0.2	Ethanol	6.7 (n = 5)
	Water	10.6 (n = 6)

The small peak that is observable with the aqueous solution is due to residual ethanol which was used to rinse the syringe. With ethanol the solvent peak is broad and may cover early eluting peaks.

The detector response is influenced by the solvent. For a 1-μl injection of PCO 400 in ethanol (0.58 mg/ml) an average peak area (n = 4) of *ca.* 10^6 (arbitrary units) was found for the PCO 400 peak, whereas under the same conditions with water as solvent an area of only *ca.* $3.25 \cdot 10^5$ (average of nine injections) was observed. The decrease in sensitivity may be caused by quenching of the flame of the detector owing to the large amount of water injected. Detector parameters such as make-up gas flow must therefore be optimized in order to achieve optimum sensitivity.

The relative standard deviation (R.S.D.) of the absolute peak areas was determined for injections with the 1.0- and 0.2-μl loops (Table I). The values obtained are, in our experience, representative for direct injection and well within the range reported in the literature for this injection technique [4]. For both solvents the R.S.D.'s are lower for the 1.0- than the 0.2-μl loop.

No decrease in the performance of the system was observed even when water was used as the solvent over a long period of time. The peak shape and retention time did not change after *ca.* 340 injections of aqueous PCO 400 solutions (Fig. 4).

Sample preconcentration was performed by means of multiple injections of aqueous solutions of PCO 400. The solutes were trapped at the beginning of the column at low density (high temperature and low pressure) and eluted with a density programme. Up to 10 × 0.2 μl and 12 × 1.0 μl volumes of PCO 400 in water (0.58 mg/ml) were injected on to the

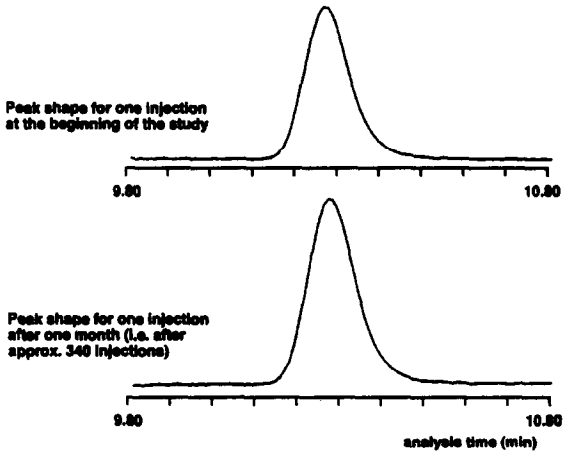

Fig. 4. Peak shape before and after the experiments with aqueous PCO 400 solutions (0.2-μl sample loop).

column. The linearity of the accumulation procedure was studied for an aqueous PCO 400 solution in the range of 116 ng PCO 400 (corresponding to one individual injection) up to 928 ng (corresponding to eight successive injections).

A linear correlation (correlation coefficient r = 0.995) between the amount injected and the peak area detected was found in the range examined (Fig. 5). The column efficiency decreased slightly when the multiple injection technique was used. Whereas for a single injection with the 1.0-μl loop *ca.* 38 000 theoretical plates per meter (based on the PCO 400 peak) were achieved, only *ca.* 30 000 theoretical plates per meter were obtained when the same amount of sample was analysed by injecting 5 × 0.2 μl. Some peak broadening was observed with the multiple injection technique, probably because solute focusing was not optimum. Solute focusing might be improved by using the multiple injection technique in combination with an appropriate focusing device.

The multiple injection technique may be applied to trace analysis. The amount of sample to be analysed can be increased without increasing the injection volume or the concentration of the sample solution.

CONCLUSIONS

Water can be successfully used as a solvent in capillary SFC. Under appropriate operating condi-

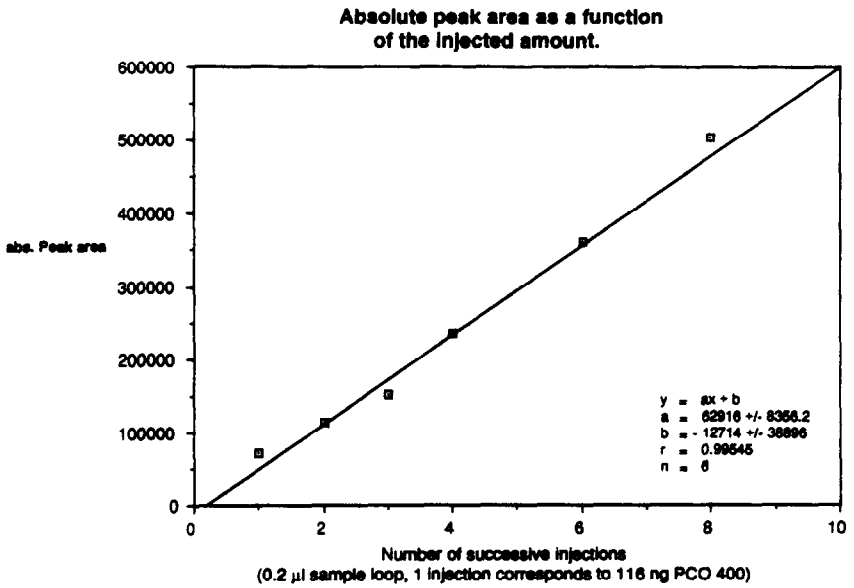

Fig. 5. Linearity of the accumulation procedure.

tions no miscibility problems between the solvent and mobile phase occur which might affect the performance of the system.

Sample preconcentration by means of multiple injections can be used to increase the amount of sample to be analysed. This technique may be very useful in trace analysis.

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