Journal of Chromatography, 394 (1987) 429–441
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1196

TECHNIQUES AND APPLICATIONS IN SUPERCRITICAL FLUID CHROMATOGRAPHY

T. GREIBROKK*, B. E. BERG, A. L. BLILIE, J. DOEHL, A. FARBROT and E. LUNDANES
Department of Chemistry, University of Oslo, P.O. Box 1033, Blindern, 0315 Oslo 3 (Norway)

SUMMARY

Supercritical fluid chromatography (SFC) has undergone much development in the past 5 years. Important to this progress is the introduction of capillary SFC and improved detection capabilities connected with the use of small-bore columns. However, a better understanding of the possibilities of SFC, compared with other chromatographic techniques, is still desirable. This paper gives a realistic view of the present possibilities by discussing three limitations of SFC.

The first aspect is solubility, particularly of polar solutes, in various fluids. A short overview, with some examples, is given of the properties of common mobile phases and the implications of the addition of modifiers. The second aspect is connected with the currently available instrumentation. The properties of the major pumping systems and the most common restrictors are briefly evaluated, in conjunction with the mobile phase and the size of the column. The third limitation is concerned with injection on to capillary columns, which requires relatively concentrated sample solutions. In order not to overload the columns, split injection is usually applied. Different ways of obtaining splitless injection in SFC are discussed, and a few results from preliminary studies are presented.

INTRODUCTION

It is generally acknowledged that supercritical fluids are attractive as mobile phases in chromatography owing to their low viscosity and a high diffusivity relative to liquids. Varying the density is the main way of controlling retention in supercritical fluid chromatography (SFC), analogous to varying the temperature in gas chromatography (GC). Additional means of controlling retention in SFC include varying the temperature or the mobile phase composition. Based on the diffusion coefficients, it is easy to show that the highest efficiency per unit time is obtained by GC, followed by SFC and high-performance liquid chromatography (HPLC)[1]. However, this comparison requires similar columns, either packed or open capillaries. As the state of the art today includes open capillaries in SFC and packed columns in HPLC, the relevance of such comparisons is arguable. Standard packed columns with 3–5 μm particles will theoretically find their equivalence in open tubes with a diameter

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of 8–13 μm, or possibly slightly larger owing to the higher density drop over a packed column in SFC[2]. As these diameters are currently out of range for practical SFC, the claims for high efficiency per unit time could well be moderated. In our opinion, the detection capabilities of SFC are much more important than efficiency per unit time, at least with open-tubular columns.

Gouw and Jentoft[3] estimated that gas chromatography could be used directly on 15% of all known compounds. Even assuming an expansion of the GC range by a factor of 2 by employing appropriate derivatization techniques, an estimated 60–80% of all compounds are in direct need of other methods, such as HPLC or SFC. The main deficiency of HPLC, in our opinion, is the absence of a simple, sensitive universal detector, like the flame ionization detector in GC. Not all chromatographers agree with this view, emphasizing the need for selective detection more than universal detection. Selectivity is naturally more important when the analytical problem is to determine low concentrations of one component in a complex sample, irrespective of the remainder of the sample. However, in the characterization of crude oil and petroleum fractions, of other oils and waxes, in fingerprint analysis and in the industrial control of many raw materials and products, the need for a universal, mass-sensitive detector is obvious. Compared with HPLC, the advantage of SFC is the ability to use the universal flame ionization detector, in addition to some of the selective GC detectors, in addition to most HPLC detectors and mass spectrometers.

The main limitation of SFC lies in the limited solubility of compounds with polar groups in fluids such as carbon dioxide. Another limitation has been the slow progress in the commercial availability of various instrument parts, and a third limitation is connected with injection on to capillary columns. In this paper we discuss some implications of these three limitations in view of the progress that has been made recently.

INSTRUMENTS

Instrument 1

One instrument contained an ISCO μ-LC 500 syringe pump for fluid delivery. A cooling tube was coiled around the pump cylinder. Cold methanol at −15°C was pumped through the tube during filling. Cooling is essential in order to be able to fill the pump cylinder with liquid carbon dioxide. Without cooling, a large part of the volume was filled with gas. The filling procedure, which takes 45 min, was performed at the end of each day in order to allow the cylinder to return to room temperature. This procedure resulted in fewer leaks and better pressure control than what was obtained with constant cooling of the cylinder. With new piston seals, the loss of fluid overnight from the cylinder was not measurable. After a few months of use the seals had to be replaced.

The fluids were pumped through a purifier column (500 × 7 mm I.D.) filled with active carbon (Alfa Products, Danvers, MA, U.S.A.). The purifier was replaced after 20 kg of liquid carbon dioxide, or whenever another fluid was installed. Shut-off valves (SSI 02-0120; Scientific Systems, State College, PA, U.S.A.), mounted at the inlet and outlet of the pump and after the purifier column, allowed the remainder of the system to be held at the equilibrium pressure during pump filling, column replacement, tank replacement, etc.

Samples were injected with a Rheodyne 7520 injector, equipped with a 0.2-μl rotor. The injector was installed on top of the column oven, thermally insulated from the oven in order to be able to heat the column independently of the injector. A 50-μm I.D. fused-silica tube connected the injector with the column. The injector connection, which used graphitized Vespel ferrules (100/0.4-VG 2; Applied Science Labs., Deerfield, IL, U.S.A.), withstood pressures of at least 400 bar. A three-way union tee (Valco ZT 5) from VICI (Schenkon, Switzerland) connected the capillary with the column and with a 25-μm fused-silica capillary for splitting of the injected sample volume. The splitting ratios were regulated by varying the length of the capillary. The outlet of the capillary was placed inside the column oven in order to reduce the amount of solute deposition and plugging at the outlet. With large splitting ratios, *e.g.*, 1:50, the high flow-rates through the capillary counteracted the deposition of sample components. With small splitting ratios, however, the splitting ratio could not be maintained constant at the low flow-rates used with capillary columns. The fused-silica capillaries were connected to the union tee by means of 1/32-in. Vespel tubing adapters of 0.5 mm bore (FS 5) from Chrompack (Middelburg, The Netherlands). With splitless injections the side-flow was stopped by installing a capillary plug or by a stop-flow valve.

The column used for most experiments was a 20 m $\times$ 100 μm I.D. fused-silica capillary with a 0.4-μm DB1 bonded methyl silicone film from J & W Scientific (Rancho Cordova, CA, U.S.A.). The pre-column used for splitless injection was a 30 m $\times$ 50 μm I.D. fused-silica capillary from Chrompack. The two columns were connected by a three-way 1/32-in. union tee (Valco ZT 5). The side-stream of the union was plugged except for pre-column venting experiments. The end of the column was connected to the fused-silica restrictor by a 1/32-in. Valco union (ZU 5T FS4).

The tapered fused-silica restrictors were obtained by drawing a 10-μm capillary in a flame. The end of the restrictor was positioned 3–5 mm below the jet in the flame ionization detector. A Hewlett-Packard 5790 A gas chromatograph was used without any modifications to the column oven and detector.

Instrument 2

This instrument was used with packed columns and with a thermoionic detector. The modifications to the Waters Assoc. (Milford, MA, U.S.A.) Model 6000A reciprocating piston pump have been described elsewhere[4]. The fluid was purified as on instrument 1 with active carbon and a shut-off valve was inserted after the purifier.

A dual-stem three-way valve (SSI) was mounted after the shut-off valve. This valve allowed the major part of the fluid to be recirculated through the pump, a minor part being delivered to the injector and the (narrow-bore) column. A 1/16-in. union tee (Valco) allowed the fluid to be mixed with a modifier, pumped by an LDC/Milton Roy microMetric™ metering pump or by a Waters Assoc. Model 590 pump. A PDM 3.350 pulse damper (Orlita, Giessen, F.R.G.) was installed between the union tee valve and the injector. The modifier was mixed with the fluid in the pulse damper. The injector (Valco CI 4W), which had a 60-μl rotor installed, was kept at room temperature. A stainless-steel column (150 $\times$ 0.5 mm I.D.) was packed with 4-μm Nova-Pak C_{18}, obtained from Waters Assoc. Another column (250 $\times$ 1.3 mm I.D.) contained 8-μm CP-Spher C_{18} from Chrompack. The restrictor was made by crimping a 100-μm platinum tube from Goodfellow Metals (Cambridge, U.K.).

A Hewlett-Packard 5700 A gas chromatograph equipped with a thermionic detector was used without modification to the column oven and detector.

SOLUBILITY IN SUPERCRITICAL FLUIDS

Relatively few data are available on the solubility of individual organic compounds in supercritical fluids[5,6]. In chromatographic terms, however, compounds that are expected to be suitable for adsorption chromatography and gel permeation chromatography (GPC) with non-polar eluents, should also be suitable for SFC in carbon dioxide or in supercritical alkanes. In addition, a variety of compounds that frequently give elution problems in adsorption chromatography may also be eluted by SFC in carbon dioxide[7–10]. Silyated polysaccharides with molecular weight up to 7000 have been purified[11], and even an underivatized sugar[12].

Compounds with molecular weights of several hundreds to several thousands can generally be separated better by SFC than by GPC owing to the narrow range of distribution coefficients in exclusion chromatography. For thermally stable non-polar compounds, supercritical alkanes are particularly useful owing to their high critical tempertures, which increase the solubility. Coronene, for example, is essentially insoluble in liquid pentane, but was chromatographed easily in supercritical pentane at 210°C[13]. Another large hydrocarbon, ovalene, with ten condensed rings, was sixteen times more soluble in supercritical pentane than in liquid dichloromethane[13]. Thus, for many high-molecular-weight compounds, the solubility in the mobile phase may be a smaller problem than finding a suitable solvent for the sample. Al-

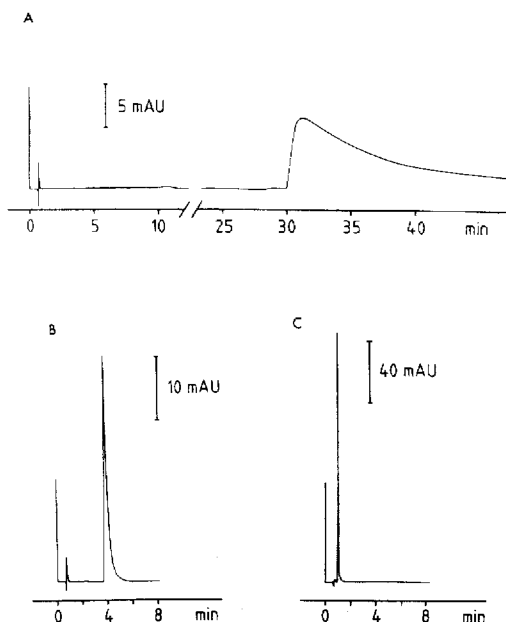

Fig. 1. 2,7-Dinitro-9-fluorenone, eluted with (A) 0%, (B) 1% and (C) 10% methanol in carbon dioxide at 40°C and 160 bar from a 250 × 1.4 mm I.D. column packed with 8-μm CP-Spher C_{18}. Detector, UV; restrictor, platinum.

though it has been claimed that high-temperature GC can compete favourably with SFC for the separation of polymers[14], certain problems with high-temperature GC are difficult to avoid, *i.e.*, discrimination in the injector or accumulation of non-eluted components at the column inlet, and thermal degradation at 400–500°C.

With polar solutes, the choice in SFC is between the addition of polar modifiers or the use of fluids of higher polarity. Polar modifiers in SFC have been studied mainly with packed columns, where the deactivating effect on the porous particles may be difficult to separate from the solubility effect in the mobile phase[15,16]. By adding the modifier in different concentrations (Fig. 1), deactivation is obtained at modifier concentrations too low to have a significant effect on solubility. At higher concentrations the effects of increased solubility are similar, with reduced retention and improved peak symmetry. With open-tubular capillary columns, the addition of smaller amounts of methanol was shown to have little impact[17], whereas higher concentrations of methanol, 2-propanol and acetonitrile decreased the retention considerably[18]. Unfortunately, the use of modifiers makes the use of flame ionization detection (FID) almost impossible. Although levels of up to 1% of methanol in carbon dioxide have been reported to be compatible with FID at reduced sensitivity[19], detectors that cause few problems with modifiers are based on UV absorbance and fluorescence. Major applications with these detectors are in the separation of oligomers. Other detectors that tolerate the addition of modifiers are selective GC detectors, such as the thermionic detector (Fig. 2). Two modifiers that give a low background with FID are water and formic acid. Water should be restricted to systems that cannot be corroded or damaged, avoiding hydrolysable stationary phas-

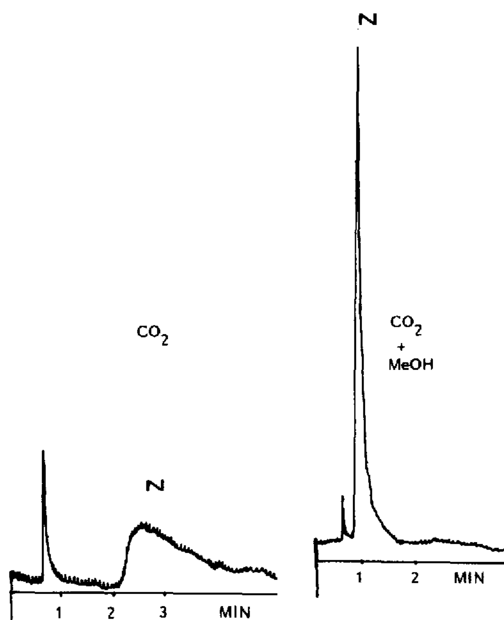

Fig. 2. 4-Nitroaniline, eluted with 0% and 7% methanol (MeOH) in carbon dioxide at 70°C and 230 bar from a 150 × 0.5 mm I.D. column packed with 4-μm Nova-Pak C_{18}. Detector, nitrogen–phosphorus; detector temperature, 425°C; bead voltage, 18.5 V; restrictor, platinum.

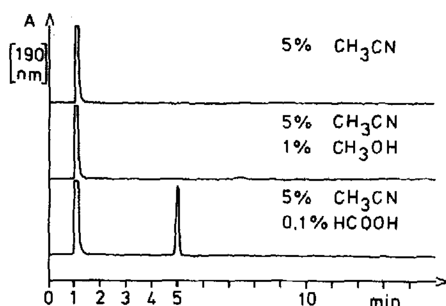

Fig. 3. Arachidonic acid injected with various modifiers added to carbon dioxide into a CP-Spher C_{18} column (250 × 1.3 mm I.D.) at 50°C and 140 bar. Detector, UV at 190 μm; restrictor, platinum.

es[20]. Preliminary studies of the solubility in carbon dioxide modified with water indicate that the effects that can be obtained are small[21].

Mixtures of formic acid (0.5%) and carbon dioxide have been utilized to separate classes of hydrocarbons[19]. A striking effect on the adsorption of arachidonic acid is shown in Fig. 3, which also demonstrates the compatibility of carbon dioxide with low-wavelength UV detection.

The alternative to modifiers is to use fluids with better solvent properties than carbon dioxide, such as nitrous oxide or ammonia. The difference in solubility in carbon dioxide and nitrous oxide is not large for many compounds[17], with some exceptions, such as high-molecular-weight components of petroleum[21]. Unfortunately, the combustion of nitrous oxide in the flame ionization detector increases the detection limits owing to a large background current[22]. Supercritical ammonia is an interesting candidate as a good solvent for polar compounds[5]. So far, however, the chemical reactivity of ammonia and the environmental problems have resulted in few applications.

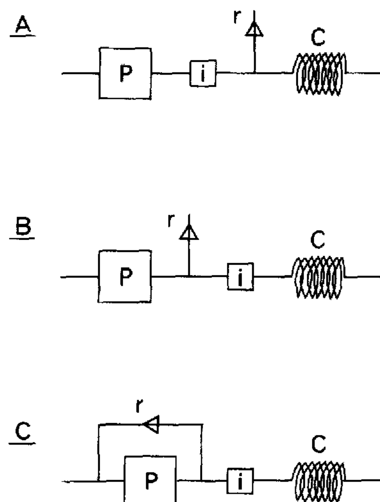

Fig. 4. Different splitting arrangements: (A) injector split (sample to waste); (B) mobile phase split (fluid to waste); (C) closed-loop fluid split. P = pump, i = injector, r = restrictor, C = column.

SFC PUMPS

Syringe pumps, reciprocating piston pumps and diaphragm pumps are currently used to deliver the mobile phase in SFC. Syringe pumps dominate owing to the low noise levels and high reliability. The piston pumps that have been modified most recently[23] so far cannot be recommended for use with capillary columns. The low column flow-rates require split-flow systems, which are difficult to maintain with good reproducibility if the splitting point is located after the injector (Fig. 4). If the splitting occurs prior to the injector, the consumption of fluid becomes extremely high with pressure programmes unless the splitting ratio is variable, which again is difficult to reproduce at the flow-rates required for capillary columns. The third possible splitting arrangement (Fig. 4) is to use a closed-loop system, whereby the split-out fluid is returned to the pump. With current instrumentation, the splitting ratio is difficult to adjust precisely.

The conclusion is that with standard-sized packed columns and UV or fluorescence detectors which allow high flow-rates, the choice of the pump is not critical. For the low flow-rates with capillary columns, however, syringe pumps are better suited than currently available reciprocating pumps. As the pressure-feedback system of reciprocating pumps is normally constructed for liquids of low compressibility, the use of more compressible fluids has resulted in higher pulsation noise with pressure control than with flow control, at least with the present instrumentation. Syringe pumps, on the other hand, should not be used with flow control, owing to the compressibility of the large volumes of fluid and also the risk of leaks at the piston seal. At constant pressure, leaks will not affect the column flow, and leaks do unfortunately occur at the piston seals of syringe pumps[23]. Another factor is that the compression

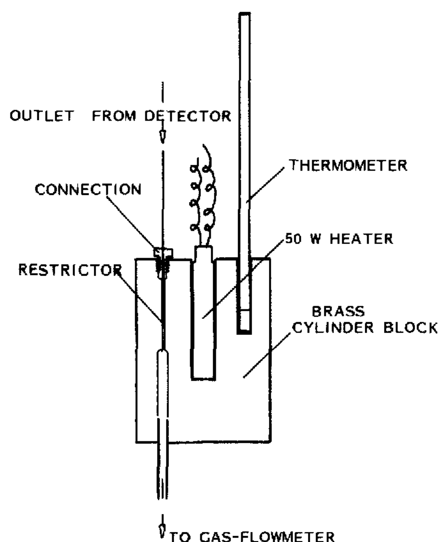

Fig. 5. Heating arrangement for testing a stainless-steel restrictor (thermal expansion restrictor). The restrictor is inserted into a 2-mm heating channel drilled through the brass block and locked at the top with a 1/16-in. nut and ferrule. Gas flow-rates were measured by connecting the outlet to a flow meter.

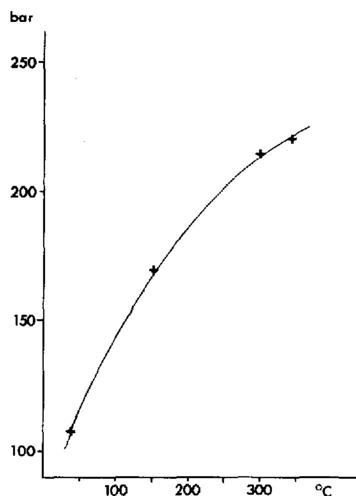

Fig. 6. Pressure–temperature relationship at a pump flow-rate of 0.2 ml/min of carbon dioxide for the thermal expansion restrictor (made of stainless steel).

time of a pressure gradient depends on the volume of fluid remaining in the pump cylinder. With the present syringe pumps, this time could vary by a factor of 2.

RESTRICTORS IN SFC

The requirements on the restrictors used in SFC differ according to whether they are located prior to or after the detector.

Post-detector restrictors

Variable back-pressure regulators[24] are used in conjunction with UV, IR or fluorescence detectors. Another variable restrictor, which is not based on adjustable valves, is shown in Fig. 5. The restrictor consists of a crimped steel capillary placed in a heated metal block. Increasing temperatures reduce the flow, and allow pressure variations of 100–200 bar at constant flow (Fig. 6). By using a restrictor material with a higher coefficient of thermal expansion the pressure limits could probably be expanded.

Pre-detector restrictors

Most detectors used in SFC, such as flame ionization thermionic detector, flame photometric and light-scattering detectors[25] and the mass spectometer, require the restrictor to be positioned at the detector inlet. So far all the restrictors have been fixed, but the thermal expansion restrictor can at least in theory be installed in such detectors. The higher temperatures that would be required to dissolve large solutes are also required to avoid spiking, rendering the restrictor principle attractive. One disadvantage with metal restrictors is the danger of corrosion with nitrous oxide as the mobile phase, which was evident with both steel and platinum restrictors at high temperatures[22]. Platinum restrictors, which have been made by crimping the end of a narrow (100-μm) tube[4], are good heat conductors and give no spiking. With a

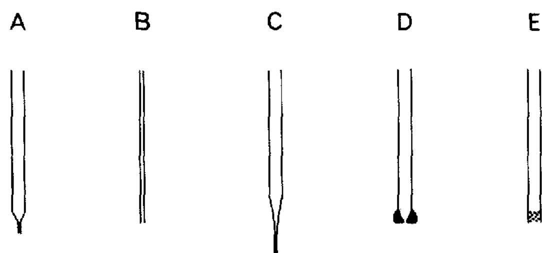

Fig. 7. Current SFC restrictors at detector inlets: (A) metal (platinum, stainless steel), crimped; (B) fused silica, long, 5–10 μm I.D.: (C) fused silica, tapered, drawn in flame; (D) fused silica, melted and opened by abrasion; (E) ceramic frit in fused silica.

flow-rate of carbon dioxide of 100 μl/min or more, the platinum restrictors have given no problems. At the low flow-rates used with capillary columns (1–30 μl/min), the platinum restrictors are unfortunately very difficult to make, at least by the simple crimping process.

In order to avoid solute precipitation and spiking, the restrictor should be short and made from a heat-conducting material. The long fused-silica restrictors (Fig. 7) that have been used fairly extensively do not satisfy any of these requirements. In order to reduce the length of the restriction zone, fused-silica capillaries can be robot-drawn in a flame to make tapered restrictors[26], or the capillary can be closed in the flame and opened by abrasion to give a conical end[27] (Fig. 7). The recent ceramic frit restrictor contains numerous small restrictions[7], which prevent plugging by a single particle. Whether there is a risk of plugging the tiny capillaries in the frit by non-volatile solutes remains to be seen.

With various restrictors that are slowly becoming commercially available, one of the major obstacles to the more common use of SFC will disappear. It is to be hoped that the days will soon be gone when reproducible restrictors do not exist.

INJECTION IN SFC

Without modifications, standard micro-scale HPLC injectors are used in SFC at ambient temperatures or in heated compartments. By using a timing device some injectors can introduce only a fraction of the sample from the loop on the column[28]. Although many applications do not require injection above room temperature, examples to the contrary include polymers which often need elevated temperatures in order to remain in solution. In the literature, instruments are often described with the injector in the column oven, a configuration that generally cannot be recommended, as the injector temperature easily becomes too high for volatile sample solvents when temperatures are increased in order to optimize a column separation. The result of partial vaporization of the solvents during injection is a complete loss of reproducibility. Accordingly, the injector and the column ought to be heated in separate compartments.

The smallest volumes that can be injected through current micro-scale HPLC injectors are 60–200 nl. With packed narrow-bore columns and sample solvents with little detector response, such volumes create no problems (Fig. 8). With open-tubular

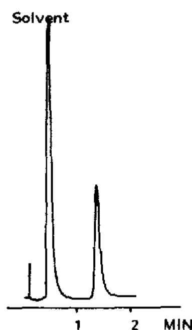

Fig. 8. Injection of tri-(2',4-di-*tert.*-butylphenyl) phosphite (20 ng), corresponding to 1 ng of P, in acetone (60 nl) with thermionic detection (nitrogen–phosphorus). Signal-to-noise ratio, 200:1; column, 150 × 0.5 mm I.D. Nova-Pak C_{18} with carbon dioxide at a pump speed of 1.5 ml/min and a net column flow-rate of approximately 35 μl/min (closed-loop splitting). Pressure, 200 bar; detector temperature, 400°C; bead voltage, 19 V; restrictor, platinum.

columns of I.D. 50–100 μm and thin films of stationary phase, however, these volumes usually cause gross column overloading. So far the solution to the problem has been to split the injection. With splitting ratios of 1:20 or 1:50, microlitres of sample are required in order to introduce nanolitres on to the column. With only a few nanolitres transferred to the column, the sample needs to be relatively concentrated in order to obtain a signal from the detector, and this requirement for relatively concentrated solutions is a major weakness of open capillary SFC today.

In GC, splitless injection or cold on-column injection makes the introduction of large volumes of dilute solutions possible. In HPLC, analogous effects can be obtained by injecting in solvents of low solvent strength, particularly with reversed-phase systems. Peaden *et al.*[29] examined on-column injection at ambient pressure in SFC and concluded that sample concentration at the column inlet appeared to be feasible, but that the overall performance of the injector was less favourable. Since then, progress on injection in SFC seem to have been limited to the introduction of time-controlled injection[28], which has improved peak shapes and also improved the reproducibility, but has had no effect on the concentration problem. In theory, there are several ways of achieving sample concentration at the column head in SFC. One is simply to inject at a density and a temperature where the solvent, but not the solutes, elutes rapidly through the column, as illustrated in Fig. 9 with injection at the equilibrium pressure of carbon dioxide. The result was a considerable improvement compared with injection at higher pressure, when the solute peaks completely disappeared under the 200-nl acetone peak. The solvent separation could, of course, be improved and discrimination of components is evident. The discrimination is due to lower solubility of the higher acids in carbon dioxide. At an injector temperature just under the boiling point of acetone, the solubility was increased and the discrimination disappeared (Fig. 10). The peak splitting of the two lowest acids, which is a result of too high an injector temperature, disappeared when the temperature was lowered.

Figs. 9 and 10 illustrate the opportunities, but also the limitations, of injection at low densities. One apparent disadvantage of this technique is the time-consuming

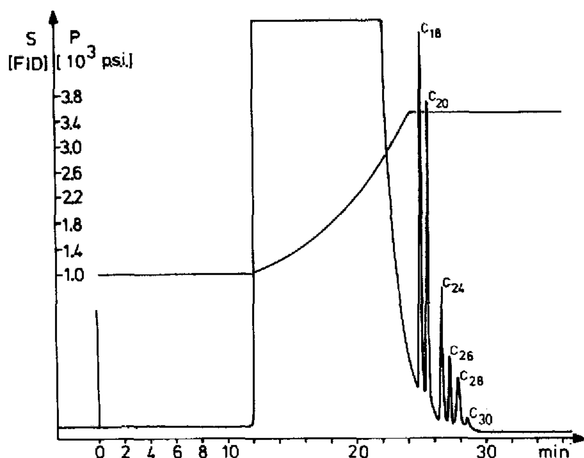

Fig. 9. Splitless injection of fatty acids (0.2 μg each) in acetone (0.2 μl) in carbon dioxide into a 20 m × 100 μm I.D. J & W DB-1 column. Injector temperature, 20°C; column temperature, 110°C; detector temperature, 350°C; restriction, tapered fused silica. FID = Flame ionization detector.

elution of the solvent, due to the low linear flow-rate. With splitting after the column, the linear flow can be increased at the low-density stage. After-column splitting would probably not be compatible with thick films, however, as dissolved solvent is expected to elute slowly with peak tailing.

The column temperature is another factor to be considered. When a C_{23} *n*-alkane in 200 nl of *n*-hexane was injected onto the column without splitting, the solute peak was completely covered by the solvent. With a 1:7 splitting ratio, a partial separation of solvent and solute was obtained at 50°C (Fig. 11). At 100°C, the solvent eluted much faster, the solute being less affected (Fig. 11).

Without splitting, the *n*-C_{23} peak did not separate from the solvent, even with a pressure gradient. The question is whether a system can be constructed whereby

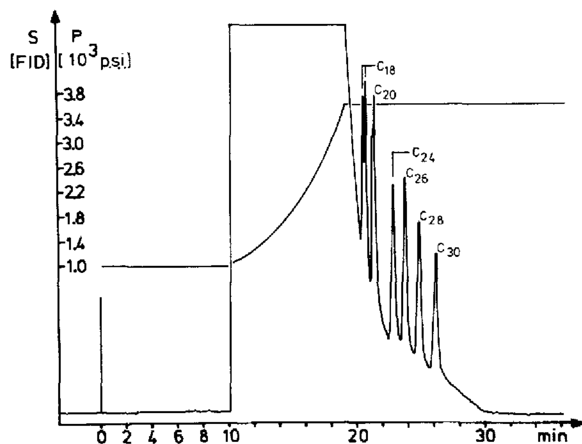

Fig. 10. Splitless injection of fatty acids as in Fig. 9, except injector temperature, 55°C and column temperature, 140°C.

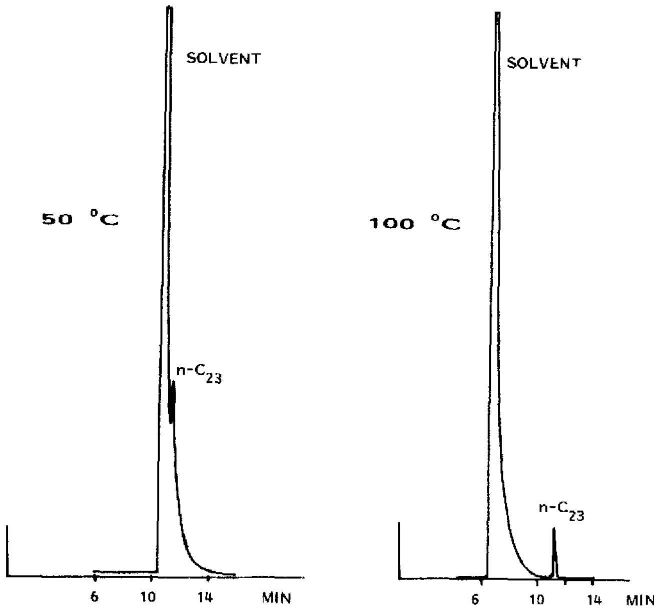

Fig. 11. Split injection of C_{23} *n*-alkane (0.3 μg) in *n*-hexane (0.2 μl) in carbon dioxide at 195 bar into the column specified in Fig. 9. Splitting ratio, *ca*. 1:7; injector temperature, 24°C; detector temperature, 325°C; restrictor, tapered fused silica; column temperature, 50 and 100°C.

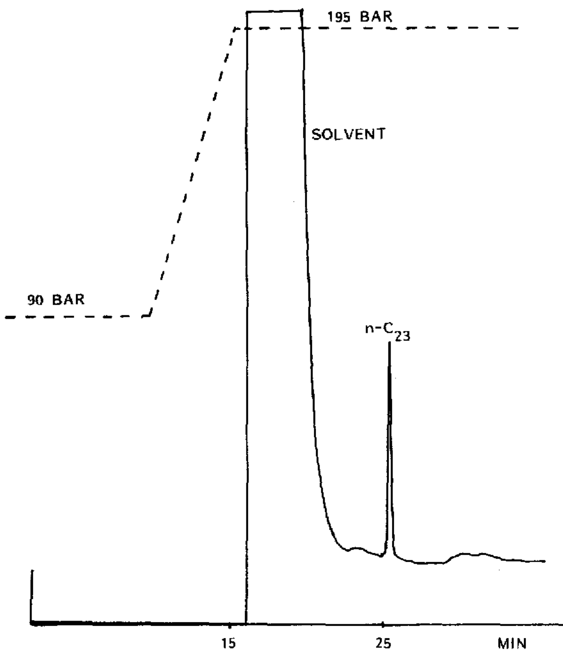

Fig. 12. Splitless injection of C_{23} *n*-alkane (0.3 μg) in *n*-hexane (0.2 μl) into a 30 m × 50 μm I.D. uncoated pre-column connected to the column specified in Fig. 9. Injector temperature, 24°C; column temperature, 50°C; detector (flame ionization) temperature, 325°C; restrictor, tapered fused silica.

the major part of the solvent is separated from the solutes at the inlet of the column, causing no interference with a solute band concentration at the column inlet. In our opinion, the best way to achieve this is to use a pre-column with a low k' value compared with the main column. Owing to the limited availability of columns with various films, the combination of a narrow thin-film pre-column with a thicker film main column has not yet been studied. However, even an uncoated pre-column had a considerable effect on the separation of solvent and solute (Fig. 12). The results from other splitless arrangements, including pre-column solvent venting, will be presented in a later publication.

ACKNOWLEDGEMENTS

Financial support was received from Statoil, Norway, through the Norwegian Academy of Sciences and from the Norwegian Council for Scientific and Industrial Research (NTNF). Three narrow-bore columns were packed and donated by Waters Assoc. (Milford, MA, U.S.A.).

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