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Effect of the sample solvent and instrument design on the reproducibility of retention times and peak shapes in packed-column supercritical fluid chromatography

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Abstract

Factors affecting the reproducibility of retention times and peak shapes in supercritical fluid chromatography on a cyano-bonded silica packed column have been studied. These included the inclusion of low proportions of modifier in the eluent and the solvent used to inject the analyte. With carbon dioxide as the eluent, polar sample solvents were found to cause residual effects, which changed subsequent separations. These effects were lost when an eluent modifier was present suggesting that they resulted from temporary masking of silanol groups on the silica surface. If the mobile phase was cooled to near the critical point between the oven and a spectroscopic detector, small changes in conditions caused baseline fluctuations, which was considered to be due to changes in the refractive index of the solution.

1. Introduction

Supercritical fluid chromatography (SFC) is now generally accepted as a viable complementary technique to gas–liquid chromatography or high-performance liquid chromatography (HPLC). However, relatively few studies have been reported which examine the reproducibility of retention times or the effect of experimental factors, such as sample preparation, sample solvent or instrument design. This is probably because with a few exceptions, such as the stability control of antipruritic preparations reported by Anton *et al.* [1], SFC methods have not yet been adopted within routine operational or quality control laboratories. Instead they have found their greatest application in research areas or as a sample introduction system for mass spectrometry. One likely reason is that most SFC systems currently in use have been based on existing HPLC or gas chromatography instruments, rather than having been designed specifically for SFC. The resulting methods have not been robust and have proved difficult to adapt for operation with unskilled personnel.

The aim of the present study was to investigate some of the operating parameters and sample preparation practices that might effect the reproducibility of retention times of a typical packed-column system, with particular interest in separations using low proportions of modifier. During an earlier study of the separation of homologous series of phenylalkanols and phenylalkanoic acids [2], although retention times were re-

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producible during a single day, it was difficult to obtain reproducible day-to-day results. It was also noted that there was a considerable change in the retention of phenylalkanols on changing the solvent used for the preparation of the samples.

Packed-column SFC systems, both commercial and laboratory-made are typically derived from dual-pump HPLC instruments by the addition of a cooler to the pump head of the reciprocating pump used for carbon dioxide and of a back-pressure regulation device [3–6]. Early studies by Greibrokk *et al.* [3] reported that over a short term, a packed-column system could give a retention time reproducibility of 1.3% (relative standard deviation, R.S.D.). However, in a later paper [4] they reported some problems at low flow-rates with check valves. Simpson *et al.* [5] reported that the temperature of the cooled pump head was important and that above 1°C the flow-rate was considerably reduced. To avoid the problem of cooling the pump heads, helium head pressures can be employed to deliver the carbon dioxide to the pump as a liquid. However, this method has been reported to result in different retention times to non-pressurised systems and Rosselli *et al.* [7] observed that the changes appeared to depend on the instrument being used. Subsequently, Görner *et al.* [8] suggested that the differences could be caused because helium was soluble in the carbon dioxide and could reduce its density and hence its elution strength.

Another potential source of variation in retention time is the reliability of the composition of the mobile phase. In previous work, we have noted that at low levels of a modifier the selectivity of a separation would be highly sensitive to small changes in concentration [9]. Because of the very low modifier flow-rates that are often needed it might be difficult to maintain a sufficiently high reproducibility of eluent composition with reciprocating pumps. However, in a recent study Morissey *et al.* [10] reported good reproducibility of retention times (0.3–1.75%) during eluent programming up to 10% modifier for the separation of polymer additives. Although it has been reported that cylinders of premixed solvents can be employed to avoid mixing problems, Schweighardt and Mathias [11] found that the composition delivered to the column changes with the extent of usage because of the different volatilities of the carbon dioxide and modifier.

The solvent used to inject the analyte onto the column may be a further potential source of retention variation. In a recent review of injection techniques, Kirschner and Taylor [12] reported that considerable effort had gone into the study of injection methods for capillary SFC because it is relatively easy to overload the column. Large volumes of polar solvents can have a significant effect on retention and solvent elimination methods, such as the work of Brossard *et al.* [13] for waxes, or peak focussing, as described by Bouissel *et al.* [14] for aqueous samples, have been needed to obtain good results. The review also noted that apart from preparative-scale samples, the injection solvent was usually considered to have little effect in packed-column separations [12]. However, Schoenmakers *et al.* [15] found that for a number of liquid crystal components, the retention time was dependent on the sample size suggesting a self-deactivation effect. However, the results were independent of the sample solvent and the effect was not observed with modified eluents.

2. Experimental

2.1. Chemicals and samples

The samples of the phenylalkanols and decanophenone were of laboratory grade from a range of suppliers. Carbon dioxide was of laboratory grade from British Oxygen Company and solvents were of HPLC grade from Fisons Scientific Equipment.

2.2. Equipment

The supercritical fluid separations were carried out using a JASCO (Tokyo, Japan) system, consisting of a 880 PU pump with cooled pump head for the delivery of carbon dioxide at 2 ml

min^{-1} and a PU-980 pump for the delivery of modifier. For low levels of modifier (< 1%), a customised Acurate microflow processor (LC Packings, Amsterdam, Netherlands) [16] was used to split the flow from the modifier pump. The eluent was mixed in a SP8500 dynamic mixer (Spectra-Physics) and passed to a cyano Capcell SG120 column (150 mm × 4.6 mm; Shiseido, Yokohama, Japan) in a 860-CO column oven. The peaks were detected using a 875-UV ultraviolet detector fitted with a high-pressure flow cell and a 880-81 back-pressure regulator. The chromatograms were recorded using a Jones JCL6000 chromatographic data system software on an Elonex 386SX computer. Samples (5 μl) were injected using a 7125 valve (Rheodyne, Cotati, CA, USA) fitted with a 20-μl loop.

3. Results and discussion

In a recent study of the separation of phenylalkanols and phenylalkanoic acids [2], there was concern that the results showed poor reproducibility. Although for a packed-column system it might be assumed that similar precision to a HPLC separation should be achievable, SFC may be regarded as inherently less robust because of the high sensitivity of the eluent strength to temperature and pressure as well as composition. The need for a restrictor or back-pressure regulator introduces an additional component, which might cause problems as it can suffer from blocking or icing-up on decompression of the eluent. The diversity of SFC designs, different pumps, different back pressure systems, and restrictors or pressure vent valves, could cause a particular systems to differ to a greater or lesser extent than another so that previous claims may not be a useful guide to expected results.

3.1. Retention reproducibility

To determine the reproducibility for typical analytes with supercritical carbon dioxide as the eluent, the retentions of benzyl alcohol, 3-phenylpropanol, 5-phenylpentanol and decanophenone in isooctane were determined on a cyano-bonded silica column over a 7-day period. The experiment ran continuously for two days (six injections) then was turned off for a weekend and restarted for three further days (twelve injections) (Fig. 1). The same instrument settings, carbon dioxide flow-rate of 2 ml min^{-1}, back-pressure regulator at 150 bar and column temperature of 60°C, were used in both runs. The exit gas flow-rate, pump-head coolant temperature and ambient temperature were monitored throughout the runs.

The retention times of the analytes (Table 1) showed significant variations with R.S.D.s of 4.8–6.6%. The relative retention times compared to decanophenone as an internal standard were much better with a R.S.D. of 1.1–1.5%. This suggested that the principal variations were in the retentive capacity of the system rather than in the selectivity of the separation. However, the mean retention times for decanophenone (mean $t_R = 8.85$ min, S.D. 0.15) from the first two days were markedly different from the results for the second period (mean $t_R = 9.52$ min, S.D. 0.65). The corresponding

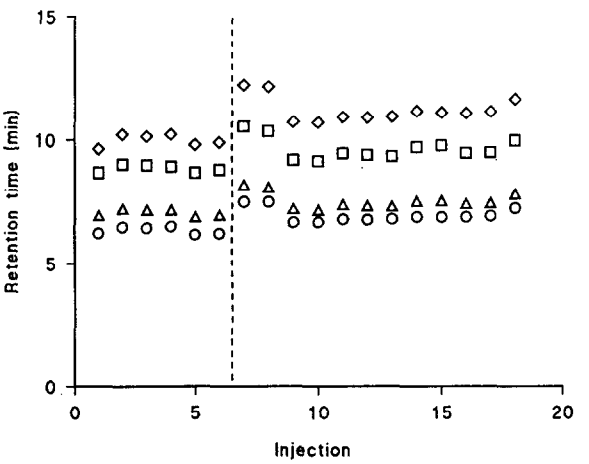

Fig. 1. Reproducibility of retention times using SFC over a 7-day period. Conditions: column, cyano Capcell; eluent, carbon dioxide; temperature, 60°C; pressure, 150 bar. Analytes: ○ = benzyl alcohol; △ = 3-phenylpropanol; □ = decanophenone; ◇ = 5-phenylpentanol. The system was run for six assays over two days, turned off for two days (marked by the dotted line), and then run for a further twelve assays over three days.

Table 1
Reproducibility of retention in packed-column SFC

Compound	Retention time (min)			Relative retention time		
	Mean	S.D.	R.S.D. (%)	Mean	S.D.	R.S.D. (%)
Benzyl alcohol	6.77	0.39	5.81	0.721	0.009	1.25
3-Phenylpropanol	7.32	0.35	4.85	0.780	0.009	1.15
Decanophenone	9.38	0.53	5.67	1.000		
5-Phenylpentanol	10.81	0.72	6.63	1.152	0.018	1.54

Conditions: column, cyano Capcell SG120; eluent, carbon dioxide; flow-rate, 2 ml min^{-1}; pressure, 150 bar; temperature, 60°C; detection, 254 nm. Retentions, relative retentions compared to decanophenone, standard deviations (S.D.) and relative standard deviations (R.S.D.) were based on 18 measurements over 7 days.

mean relative retention times for 5-phenylpentanol changed from 1.13 to 1.16. It therefore appeared that both the selectivity and the absolute retentions were changing and the effects of a number of potential variables in the system were examined.

There was concern that the flow-rate delivered by the pump, and hence the flow through the column, might be affected by density changes in the liquid carbon dioxide in the pump head. The coolant temperature of the pump head was therefore deliberately altered between −12°C to + 1°C. Apart from a large increase in retention at the highest temperature when the carbon dioxide might not be completely condensed [5], there was only a small increase in retention times with increasing temperature. Over the range of temperatures observed in the reproducibility test, from −10.5 to −12.1°C, this effect would have a negligible effect. This study does identify a potential source of variation between systems as often the pump head temperature is not controlled as carefully as the column temperature. The eluent gas flow-rate exiting the column, pressure drop across the column and ambient temperature were also recorded but no systematic correlations could be obtained with changes in retention times. It was concluded that there were no obvious instrumental causes for the poor reproducibility.

3.2. Low modifier flow-rates

In capillary SFC the addition of a modifier to the eluent primarily alters the properties of the mobile phase and thus a significant proportion is required for the effect to be apparent. In contrast, in packed-column SFC, a marked change occurs with even very small amounts of modifier suggesting a surface effect on the stationary phase [17,18]. The present study had initially set out to examine separations with low percentages of modifier. However, in order to introduce 1.0% modifier into a carbon dioxide rate of 1 ml min^{-1} a flow-rate of 10 μl min^{-1} of modifier is required, which is at or smaller than the specification of many reciprocating pumps. In previous work, an attempt to prepare a eluent of 2% methanol in carbon dioxide eluent for supercritical fluid extraction had been made using a pump, which was claimed to have the capability of 10 μl min^{-1}. When the eluent flow was examined using an ultraviolet spectroscopic detector at 254 nm it gave a flat baseline. However, if the detection wavelength was changed to 205 nm, it was clear that modifier flow was only occurring during the final part of each piston stroke. The pump was therefore delivering regular but discrete pulses of modifier to the carbon dioxide. Even the introduction of a stirred mixing chamber with a volume of 2.5 ml was unable to even out the variation in the composition. It was felt that this problem was probably being accentuated by the compressibility of the carbon dioxide eluent and relatively high pressures being used. As soon as the flow-rate from the modifier pump diminished slightly, the pressurised carbon dioxide backed up the modifier inlet tube, effectively stopping the flow. Similar problems probably also occur in dual-pump HPLC separations, although it is rare

for such low proportions of a minor component to be employed in reversed-phase separations, except in the early stages of a gradient. In normal-phase chromatography, the much easier approach of a premixed eluent is usually adopted as there is rarely a requirement for gradient elution.

To determine if this was a general problem, four further commercial HPLC pumps were tested for the addition of modifier to an SFC system, two dual-head pumps and two master/slave systems, each with a specified minimum flow-rate capability of 10 or 1 μl min^{-1}. Acetone was used as the test modifier and its concentration in the column eluent was monitored using a spectroscopic detector set at 260 nm. The pumps were assessed using a packed column at 50°C, a carbon dioxide flow-rate of 4 ml min^{-1} and a back-pressure regulator setting of 200 or 100 bar. All the pumps gave a pulsating signal for the modifier (for example see Fig. 2). The magnitude of the pulsation was dependent on the volume of the pump head. As the specified modifier flow-rates decreased the signal for the acetone decreased in each case. However, the magnitude of the signals was not proportional to the nominal flow-rates suggesting that the eluent composition differed from the value that had been set. Although one pump stood out as giving a particularly low pulsation, its flow stopped completely below 15 μl min^{-1}.

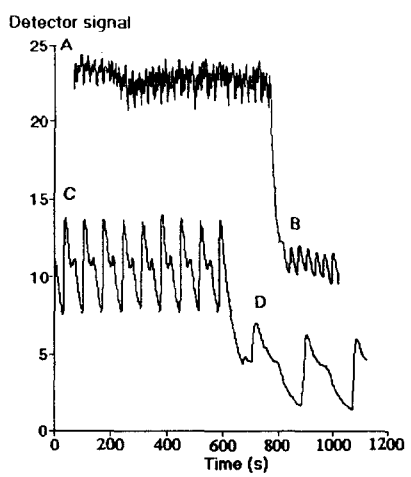

Fig. 2. Test of reciprocating pumps for the addition of modifier to carbon dioxide. Conditions; carbon dioxide flow-rate, 4 ml min^{-1}; modifier, acetone; back-pressure, 200 bar; spectroscopic detection, 260 nm. Pumping systems: A, modifier pump with 10 μl pump head volume set at 50 μl min^{-1} of modifier; B, as A set at 20 μl min^{-1}; C, modifier pump with 100 μl pump head volume set at 50 μl min^{-1} modifier; D, as C set at 20 μl min^{-1}.

Clearly none of these pumps could be used at the 0.1% level with any confidence and these results must raise questions about published work, which has claimed to use similar levels without confirmation of the eluent composition. Two alternatives can be used, either syringe pumps or flow splitting. The former was ruled out because of the cost (4–5 times greater than a reciprocating pump). Studies were therefore carried out using a commercial capillary flow splitter [16]. As these are normally intended for work against a relatively low back-pressure in a liquid chromatography system, a specially modified version was provided by the manufacturer, which was designed to work against the higher pressure in SFC. To achieve a split with this system it is necessary to have a certain pressure drop across the splitter and thus the inlet pressure must be raised significantly about the column head pressure.

The principal problem with the splitter was that it was difficult to calibrate the system *in situ*. The outlet flow for a given inlet flow could be easily measured when the unit was not connected to the SFC system but in use the flow would differ because of the high back-pressure of the eluent in the SFC system, which might typically range from 100 to 400 bar. As methanol lacks a chromophore, it could not easily be measured directly at these low levels in the carbon dioxide eluent. Because the split ratio is dependent on the viscosity of the modifier, methanol cannot be replaced by an alternative solvent with a chromophore. Instead the flow-rates with no back-pressure were measured and these were used as nominal maximum flow-rates. At an input flow of methanol of 0.3 ml min^{-1} and an inlet pump pressure of 160 bar the splitter delivered 4.3 μl min^{-1} and at 0.7 ml min^{-1} and 375 bar, it delivered 8.6 μl min^{-1}.

When the splitter was employed in a SFC system, for the same modifier inlet flow a higher pressure was recorded suggesting that there was

increased resistance to flow. There was no evidence of fluctuations in composition in the outlet flow. As well as being unable to determine the exact flow-rate, two further problems were encountered with this system. Firstly only a narrow nominal outlet range of 4.3 to 10.2 μl min^{-1} modifier could be obtained before the maximum pressure setting of the input pumping system was exceeded. Secondly the range of modifiers which could be used was limited by their viscosity. Solvents such as isopropanol gave no outlet flow even when used without a back-pressure.

This system was used in the subsequent work in the paper but modifier proportions of less than 0.5% must be regarded as nominal maximum values.

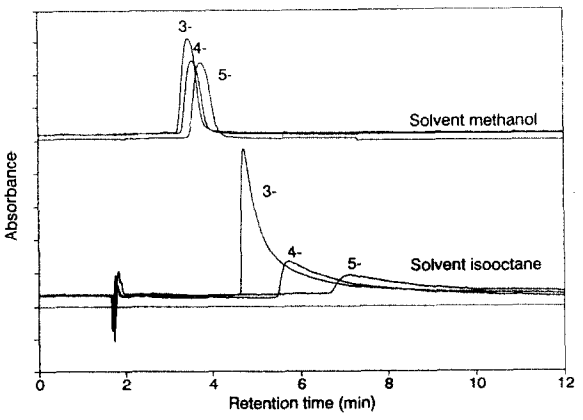

Fig. 3. Separations of 3-phenylpropanol, 4-phenylbutanol and 5-phenylpentanol injected as solution in isooctane and methanol. Conditions: column, cyano Capcell; eluent, carbon dioxide; temperature, 60°C; pressure, 160 bar; detection, 254 nm.

3.3. Solvent effects

In many cases, with alkyl-bonded silica-based packed columns, the primary mode of retention in SFC is normal-phase interaction with the underlying silanols so that the column behaves in very much a normal-phase mode [19,20]. Under these conditions, the separations can potentially be influenced by the solvent used to prepare the sample if it can interact strongly with the stationary phase. These interactions might also alter the retention of subsequent analytes if the solvent remained on the silica and masked active groups on the surface.

Preliminary experiments using a cyano-bonded silica had suggested that solvent effects were occurring with the phenylalkanols as there were considerable differences in the retentions of 3-phenylpropanol, 4-phenylbutanol and 5-phenylpentanol, when injected as samples in isooctane (t_R = 4.8, 5.8 and 7.1 min, respectively) or in methanol (t_R = 3.5, 3.7 and 3.9 min, respectively) into carbon dioxide at 60°C and 160 bar. The peak shapes were also markedly different, which suggested that when the samples were injected as solutions in isooctane they were interacting strongly with the stationary phase (Fig. 3). Although the cyano-bonded column material used in this study is reported to be prepared by coating the silica surface with a polymer [21], it has previously been found to shown strong interactions with analytes in SFC [19].

The experiment was then repeated with a series of phenylalkanols from benzyl alcohol to 5-phenylpentanol using both carbon dioxide and 0.2% methanol modified carbon dioxide as the eluent (Table 2). The retentions generally increased with the molecular mass, as in the earlier study [2], but in each case the samples injected in isooctane were more highly retained than those injected in methanol. The difference between the solvents was reduced in the methanol-modified eluent. The retention of the phenylalkanols appeared to be more effected by the sample solvent than by the proportion of modifier in the mobile phase. When the chromatograms were examined, isooctane was found to cause a solvent disturbance peak but the methanolic solutions showed no signal suggesting that the methanol might have been adsorbed onto the column.

To determine the effect of different solvents, samples of 2-phenylethanol and 4-phenylbutanol as solutions in methanol, isopropanol, tetrahydrofuran (THF) and hexane were chromatographed using an increasing proportion of methanol as a modifier in carbon dioxide (Table 3 and 2-phenylethanol, Fig. 4). At low levels of modifier there were marked differences between

Table 2
Effect of sample solvent on phenylalkanols in the presence and absence of modifier

	Capacity factor			
	0% Methanol		0.21% Methanol	
	Sample solvent		Sample solvent	
	Isooctane	Methanol	Isooctane	Methanol
Benzyl alcohol	–	1.12	1.35	0.85
2-Phenylethanol	1.40	0.84	1.56	0.85
3-Phenylpropanol	1.81	1.24	1.60	1.17
4-Phenylbutanol	2.31	1.22	1.84	1.30
5-Phenylpentanol	3.50	1.31	2.25	1.64

Conditions: column, cyano Capcell SG120; eluent, carbon dioxide; flow-rate, 2 ml min^{-1}; column outlet pressure, 160 bar; temperature, 60°C; detection, 254 nm.

the solvents in each case. Generally the retentions decreased with increasing polarity of the solvent. The differences were reduced as the proportion of modifier in the eluent increased and above 0.5% modifier each of the solvents gave the same retention times suggesting that all the silanols were effectively masked. These results suggested that the more polar solvents were contributing to the deactivation of the stationary phase. The extents of the interactions can be related to the effectiveness of the solvents as modifiers in SFC. Blilie and Greibrokk [22] found that hexane had little effect on retention but the alcohols and THF reduced the interaction with the stationary phase. Berger and Deye [18] reported that on cyano and other polar columns, less polar modifiers such as THF and acetonitrile gave poorer peak shapes than

Table 3
Effect of different sample solvents on retention times with different proportions of methanol as modifier in the eluent

Compound	Solvent	Retention time (min)				
		Methanol (%)				
		0.0	0.21	0.43	1.0	2.0
2-Phenylethanol	Methanol	2.39	2.49	2.41	2.25	2.01
	Isopropanol	2.97	2.93	2.49	2.21	2.04
	THF	2.99	3.14	2.50	2.21	2.05
	Hexane	3.16	3.31	2.47	2.21	2.04
4-Phenylbutanol	Methanol	3.06	3.11	2.99	2.65	2.30
	Isopropanol	4.20	3.69	3.06	2.62	2.36
	THF	4.10	4.57	3.05	2.61	2.35
	Hexane	4.12	3.31	3.13	2.63	2.35

Conditions: column, cyano Capcell SG120; eluent, carbon dioxide with methanol modifier; flow-rate, 2 ml min^{-1}; column outlet pressure, 151 bar; temperature, 60°C; detection, 254 nm.

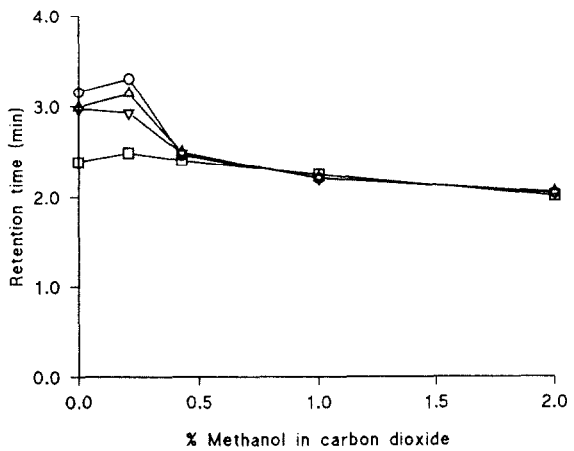

Fig. 4. Effect of sample solvent on retention time of 2-phenylethanol. Conditions as in Fig. 3: eluent, carbon dioxide containing different proportions of methanol as modifier. Sample solvents: ○ = hexane; △ = THF; ▽ = isopropanol; □ = methanol.

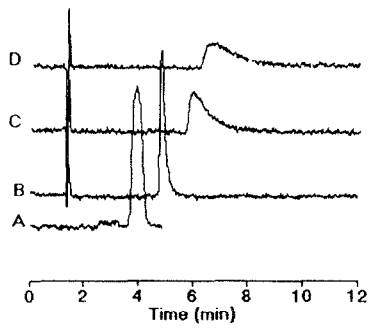

Fig. 5. Separation of 5-phenylpentanol injected successively in different solvents. Solvents: A = methanol; B, C and D = sequential injections in isooctane. Conditions as in Fig. 3.

polar additives, such as methanol. This reflected results by Levy and Ritchey [23] who found that the effect of modifiers and level of effective complete deactivation depended on the nature of the analyte and stationary phase.

There was concern that the interaction of the sample solvent would effectively represent a temporary but uncontrolled modification of the stationary phase activity, which might influence subsequent samples even if these were in a low-polarity solvent. A series of studies was carried out to determine the persistence of the solvent effect on retention. When the injection of a sample of 5-phenylpentanol in methanol was directly followed by a sample in isooctane, the retention time of the analyte from the second solution was slightly longer but the peak shape was much better (Fig. 5). Further injections in isooctane showed a steady reversion to the typical isooctane sample retention time and peak shape. These results suggested that two effects were occurring. Firstly, the initial methanol injection had deactivated the silica surface and this reduced tailing in subsequent injections. This effect continued until sufficient carbon dioxide had passed through the column to wash out the methanol and regenerate the active sites. Secondly, because the first isooctane injection gave a sharper peak for 5-phenylpentanol than the peak from the methanol injection, the sample solvent appeared to be having a direct effect on the band broadening. The methanol solvent was acting as a strong eluent causing rapid elution and hence band broadening until significant mixing occurred with the carbon dioxide mobile phase. The less polar isooctane solvent acted as a weak eluent on the deactivated column giving sharp peaks. Similar band broadening caused by a sample solvent, which is a stronger eluent than the mobile phase, is well recognised as a problem in HPLC [24] but does not appear to have been reported previously in packed-column SFC.

In a more extensive study, five 5-μl samples of 5-phenylpentanol in methanol were injected at 1-min intervals onto the column with carbon dioxide as the mobile phase. These injections were used to calculate the peak height on a methanol-deactivated column. After a delay of 10 min, a 5-μl sample of 5-phenylpentanol in isooctane was injected. As the 5-phenylpentanol was eluted, a further sample in isooctane was injected to monitor the continuing changes with time. These injections were repeated until the peak heights were nearly constant. The full experiment was then repeated using delays of 15, 20 and 30 min between the methanol solutions and the first isooctane solution. The heights of the peaks for 5-phenylpentanol were monitored as they were a good guide to the peak shapes. For each series of injections there was a sys-

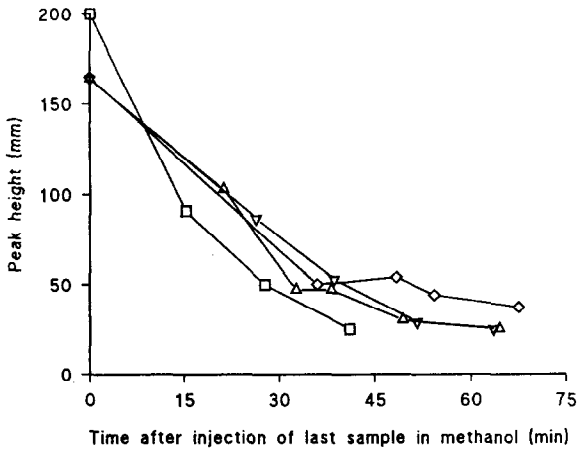

Fig. 6. Change in peak heights for 5-phenylpentanol with time. Peak height at 0 min is the mean of five injections of a solution in methanol. These were followed after: (□) 10 min; (△) 15 min; (▽) 20 min and (◇) 30 min by a series of sequential injections of a solution of 5-phenylpentanol in isooctane. Conditions as in Fig. 3.

tematic and nearly exponential decrease in peak height with time after the methanolic samples (Fig. 6), which continued for over 45 min. Although the broadening occurred most rapidly for the set of runs that started after only 15 min, the results were variable and there did not appear to be a significant correlation between the waiting time before injecting the first isooctane sample and the peak shape after a particular time. This suggested that the reactivation of the column occurred at a similar rate irrespective of the number of isooctane samples that had been examined. Thus the primary mode of reactivation appeared to be the slow elimination of the methanol from the column by the carbon dioxide.

The effect of an injection of a sample as a solution in methanol can therefore persist for a considerable time even after that particular sample has eluted. Importantly, the residual methanol on the column can have a significant effect on any subsequent polar samples injected in a less polar solvent altering both their peak shapes and retention times. A related prolonged retention of a polar additive was exploited by Berger and Deye [25] for the separation of phenols. They loaded a diol-bonded silica column with trifluoroacetic acid and found that it still behaved as a deactivated column even after washing with methanol. Other researchers [26] have found that SFC columns can be conditioned by the repeated injection of basic analytes to deliberately coat the active sites. The observation that the retention times of some analytes can vary with injection size can be considered to be a form of self deactivation [15].

Despite these observations, the deactivation effect of a polar sample solvent does not appear to have been widely reported in SFC and the preparation of samples for packed-column SFC is rarely mentioned. However, the effect is not new in chromatography and frequently occurs in normal-phase HPLC using non-polar mobile phases. In that case, traces of a polar solvent can disrupt the separation and change selectivity and resolution [27]. It can also take a considerable time to reactivate the column. Consequently, low proportions of a polar modifier are frequently included in the mobile phase to improve the stability of the system [27,28].

Steuer *et al.* [29] have reported that, if the mobile phase composition was altered in SFC, the system stabilised much more rapidly than in HPLC (10–20 column volumes compared to over 300 volumes). However, the present study suggests that subsequent samples can still be affected and this effect could be a contributor to poor reproducibility in some SFC separations. For example, samples containing traces of moisture even in apparently low-polarity solvent could also have an effect on subsequent retentions although this was not tested.

3.4. Baseline noise

During this work, an attempt was made to work at lower pressures so that a less dense mobile phase could be examined. However, at 100 bar the baseline of the detector response became very unstable and acceptable results could not be recorded (Fig. 7, part A). Careful investigation suggested that the noise was caused by the detector rather then the pumping or column system. Surprisingly, the baseline stabil-

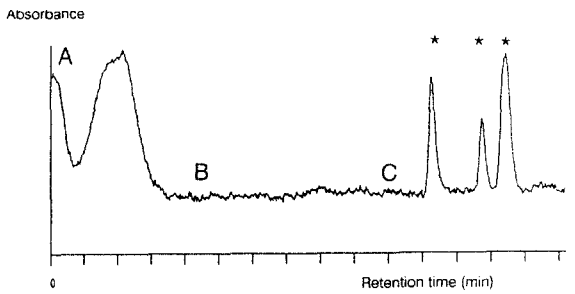

Fig. 7. Effect of temperature fluctuations in the connecting tubing on the baseline of spectroscopic detector at low eluent pressures. Conditions: column, cyano Capcell; eluent, carbon dioxide; temperature, 60°C; pressure, 100 bar. Detector response at 260 nm: A, background signal with no precautions; B, background signal with cooling to 0°C between oven and detector; C, effect of holding the connecting tubing between two fingers at *.

ised on increasing the pressure even though this would put more mechanical strain on the system.

As in many converted HPLC systems, the detector flow cell in the present chromatograph was external to the column oven and was effectively at ambient temperature. Heat loss from the connecting tubing carrying the eluent to the detector would cause the eluent to cool from the oven temperature of 60°C. As a result, the eluent in the detector flow cell would probably be near to the critical point particularly at low pressures. Under these circumstances the refractive index of carbon dioxide is very susceptible to even small changes in the temperature {typical values at 1500 p.s.i. (*ca.* 103 bar), $n = 1.1580$ at 37°C and 1.0587 at 71°C [30]}. Thus even small changes in the temperature, such as those caused by sunlight or drafts near the connecting tubing, would cause significant changes in the refractive index. This would result in changes in the path of the light through the detector flow cell and baseline noise. The present system was so sensitive at 100 bar that significant changes in the baseline could be produced by holding the connecting tubing between two fingers (Fig. 7, part C). Once the problem was identified, the noise could be almost eliminated by cooling the eluent between the oven and detector in an icebath so that it was subcritical and was therefore not as sensitive to small changes in the conditions (Fig. 7, part B). This approach was more successful than attempting to maintain the connecting tubing at the oven temperature. Although there was a heat-exchanger coil built into the detector prior to the flow cell, it appeared that this was insufficient to stabilise the temperature. However, the detector was originally designed for HPLC use, where large temperature changes or such a high sensitivity of the refractive index of the eluent to the conditions in the flow cell are rarely found.

4. Conclusions

Although there have been a number of claims of high reproducibility for SFC separations, these are not always easy to replicate. Many of the problems observed in the present study can be attributed to deactivation effects of the active surface of the stationary phase. These will be more serious for polar analytes which are being retained partly by a normal-phase mechanism and will be worsened if polar solvents are used to prepare the sample solutions. Although the reactivation of the column will be faster in SFC than in normal-phase HPLC, the retention and peak shapes of subsequent injections may still be affected for a significant time. The effects can be reduced by the introduction of a modifier into the eluent to mask the silanol sites but a consequence can be the loss of retention and possibly of selectivity from the column, although the peak shapes will often improve. These studies emphasise that a normal-phase type of interaction is often a significant contributor to packed-column SFC retentions.

The importance of the solvent used to prepare sample for SFC, experimental limitations in the preparation of eluents containing low proportions of modifier, and of sensitivity of the refractive index of the eluent to conditions in the detector flow cell near the critical point were also identified as problems in packed-column SFC. Some of these effects have been recognised by manufacturers and equipment specifically designed to work with supercritical fluids is now becoming available.

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6. References

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