IMPROVED EVAPORATIVE LIGHT-SCATTERING DETECTION FOR SUPERCRITICAL FLUID CHROMATOGRAPHY WITH CARBON DIOXIDE–METHANOL MOBILE PHASES

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SUMMARY

The coupling of evaporative light-scattering detection (LSD) with packed-column supercritical fluid chromatography (SFC) using a carbon dioxide–methanol mixture as the mobile phase was studied. A new column–detector interface is described which allows decompression and nebulization of the mobile phase and efficient evaporation of the methanol. In order to economize on experiments, the main parameters which influence the detector response were studied using factorial designs. Within the investigated range, the best detection conditions were deduced. The detection limit of LSD coupled with SFC was improved, permitting the sensitive analysis of solutes with no chromophoric or fluorophoric groups such as fatty acids and fatty alcohols.

INTRODUCTION

In a previous paper, we reported the use of light-scattering detection (LSD) coupled with packed-column supercritical fluid chromatography (SFC)[1]. The comparison between capillary- and packed-column SFC has already been discussed[2,3]. Obviously, packed columns exhibit higher efficiency per unit time than capillary columns and separations can be transposed directly from analytical or preparative liquid chromatography (LC) to SFC. Further, a conventional liquid chromatograph can easily be converted into a supercritical fluid chromatograph[4]. Nevertheless, in SFC, elution of polar solutes from a packed column with a carbon dioxide mobile phase often requires polar modifiers such as methanol[5,6]. Therefore, the use of a flame ionization detector is proscribed and there is a need for a universal detector when SFC is performed with polar modifiers.

In the previous paper we demonstrated the potential of LSD as a quasi-universal detection method for SFC with carbon dioxide–methanol mixtures. Further investigations were required in order to improve both the nebulization and the evap-

oration steps before detection. In fact, difficulties arose from the cooling effect that occurred during the decompression of the mobile phase and the resulting aggregation of solutes.

Therefore, this paper describes a new interface that we constructed to enhance heat transfer and to elucidate the phenomena that occur during the decompression and modifier evaporation.

The use of factorial designs[7-9] allowed the effective study of the detector response through the variation of the main parameters affecting the detector response within a range depending on the solutes of interest.

EXPERIMENTAL

The chromatographic system, described in detail elsewhere[1,4], consisted of a Model 303 reciprocating pump (Gilson, Villiers-le-Bel, France) and a Model 8500 syringe pump (Varian, Palo Alto, CA, U.S.A.) for the carbon dioxide and the methanol, respectively, a Model 802 dynamic mixing chamber (Gilson), a heated bath for temperature control, a Rheodyne 7120 sample injector with a 4-μl injection loop and a Model DDL 10, light-scattering detector (Cunow, Clichy, France) (Fig. 1).

Pressure control was provided by a 10–20-cm length of 75-μm I.D. fused-silica tubing cemented in a 500-μm I.D. stainless-steel tube in order to hold it in the interface and to improve heat transfer between the brass holder and the fused-silica restrictor (Fig. 2).
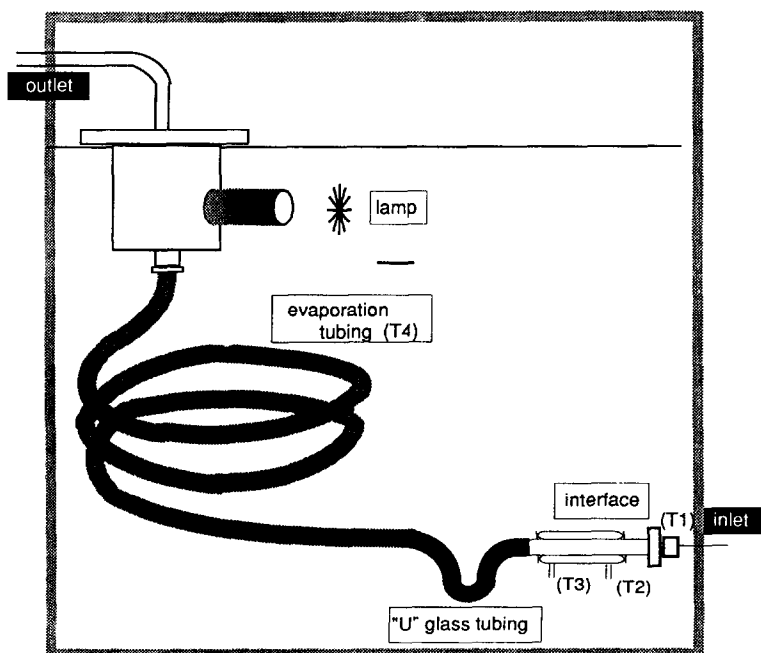

Fig. 1. Schematic diagram of the light-scattering detector. T1 = Heated restrictor holder; T2 = heating wire cemented on the glass jacket; T3 = thermoregulated glass jacket; T4 = heated evaporation tube.
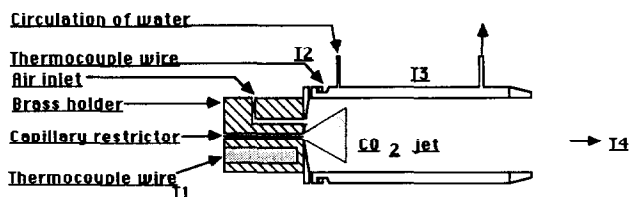
Fig. 2. Schematic diagram of the interface between the column and the light-scattering detector.

In order to reduce freezing during the expansion of the mobile phase, the outlet of the restrictor was cut with care using a special tool for optical fibre cutting (Mini-cut M 125-2; ATI; Courcouronnes, Evry, France).

The new device used as the interface between the column and the detector is shown in Fig. 2. The restrictor was installed in the brass part, which can be heated to 250°C through a thermocouple wire connected to an external power supply. An air supply was provided because addition of nebulization make-up gas was reported to enhance the evaporation rate[10].

Because the aerosol formation directly influences the detector response[11–13], we used a glass piece to watch nebulization and the carbon dioxide–methanol jet. To provide efficient heating during the expansion of the mobile phase, a heating wire was cemented at the top of the glass jacket where the expansion takes place. Hot water was circulated through the glass jacket to ensure modifier evaporation. The outlet of this straight glass tube was connected through a glass U-tube to the evaporation tube. The U-tube was used to collect any modifier that might condense.

Air tightness between all parts was ensured by O-ring PTFE seals. The restrictor was sealed by cementing the stainless-steel holder into the brass. Care must be taken to prevent air intake because of its water content: with bad sealing, freezing of water occurred and dry-ice appeared at the outlet of the restrictor. Consequently, solutes aggregated and the noise increased considerably. This effect was also reduced with a properly cut restrictor.

All experiments were carried out with a 15 × 0.46 cm I.D. column packed with Zorbax ODS (5–7 μm) (DuPont, Wilmington, DE, U.S.A.) or LiChrosorb RP-18 (5 μm) (Merck, Darmstadt, R.F.G.).

The responses of docosanol and palmitic acid were studied. Their properties are reported in Table I. Solutions were prepared in HPLC-grade hexane. Carbon dioxide (standard quality) was purchased from L'Air Liquide (Paris, France). Methanol (Prolabo, Paris, France) was of HPLC grade.

TABLE I

CHEMICAL PROPERTIES OF THE TEST SOLUTES

Solute	Molecular weight (daltons)	Melting point (°C)	Boiling point (°C)
Docosanol	326.6	71	180 (0.22 mmHg)
Palmitic acid	256.4	63	161 (1 mmHg)

RESULTS AND DISCUSSION

   In our previous study[1], the interface used necessitated a high temperature of the brass holder and evaporation tubing (*ca.* 80°C) in order to evaporate the methanol succesfully before the detection of polar compounds such as carbohydrates or trigly-cerides. This represents a major drawback for the detection of thermolabile or volatile solutes, as they could decompose or evaporate. Therefore, in order to enhance the evaporation yield while reducing the temperature required for the evaporation of the solutes, we used a new interface (Fig. 2) which provided three different heated zones.
   In addition to the temperature of the interface, it was necessary to investigate the influence of parameters that we expected would alter detector response[1] (Table II) by measuring the peak area of the test compounds. In a first step, the influence of these parameters was studied with a low content of methanol in the mobile phase (<2.4%) to define the initial conditions.
   The following parameters were found to not affect the LSD response within the range studied.

Temperature of restrictor heater (T_1)
   The carbon dioxide velocity was too high during the decompression step to allow effective heat exchange between the restrictor holder and the mobile phase; the calculated linear velocity of the carbon dioxide at the outlet of the restrictor equalled the speed of sound, as reported by Bally and Cramers[14]. The effect of the restrictor holder temperature on the detector response as reported previously[1] was provided by heating the inlet of evaporator tubing in close contact with the heated piece of brass.

Carbon dioxide flow-rate
   The carbon dioxide flow-rates were selected because they were known to give a nearly maximum response[1]. However, as we used the same restrictor for all mea-surements, it was necessary to choose two close levels to avoid too much variation in the chromatographic conditions because of the dependence of density on carbon dioxide flow-rate.

TABLE II

INITIAL CONDITIONS: LEVELS OF THE STUDIED PARAMETERS

Parameter	Level (−)	Level (+)
A. Photomultiplier power supply (PM)	4	6
B. Temperature of brass holder (T_1) (°C)	35	50
C. Content of methanol (%, w/w)	1.4	2.8
D. Carbon dioxide liquid flow-rate (ml min^{-1})	5.5	6.5
E. Temperature of glass jacket (T_3) (°C)	40	50
F. Temperature of thermocouple wire (T_2) (°C)	40	62
G. Temperature of evaporation tubing (T_4) (°C)	35	50
H. Temperature of the mobile phase (T_{MP}) (°C)	40	60
I. Restrictor position in brass holder	Out	Close to the outlet

Temperature of the glass tubing (T₂, T₃)

This tubing was added to ensure fast evaporation of the modifier in order to prevent its condensation on the walls of the evaporation tubing and to compensate for the cooling effect that occurs during the expansion of the carbon dioxide. The low modifier contents in the mobile phase (< 2.8% w/w) are probably the reason why this parameter had no influence during these experiments.

To achieve the maximum sensitivity, the following parameters must be considered: the restrictor outlet must be placed outside the brass holder, otherwise the detector background increases substantially; most of the modifier was evaporated in the glass jacket, hence heating of the evaporation tubing (T4) was not required otherwise the detector response would be lowered owing to partial evaporation of solutes; the power supply of the photomultiplier must be set at range 4 or 5; as Fig. 3 shows,
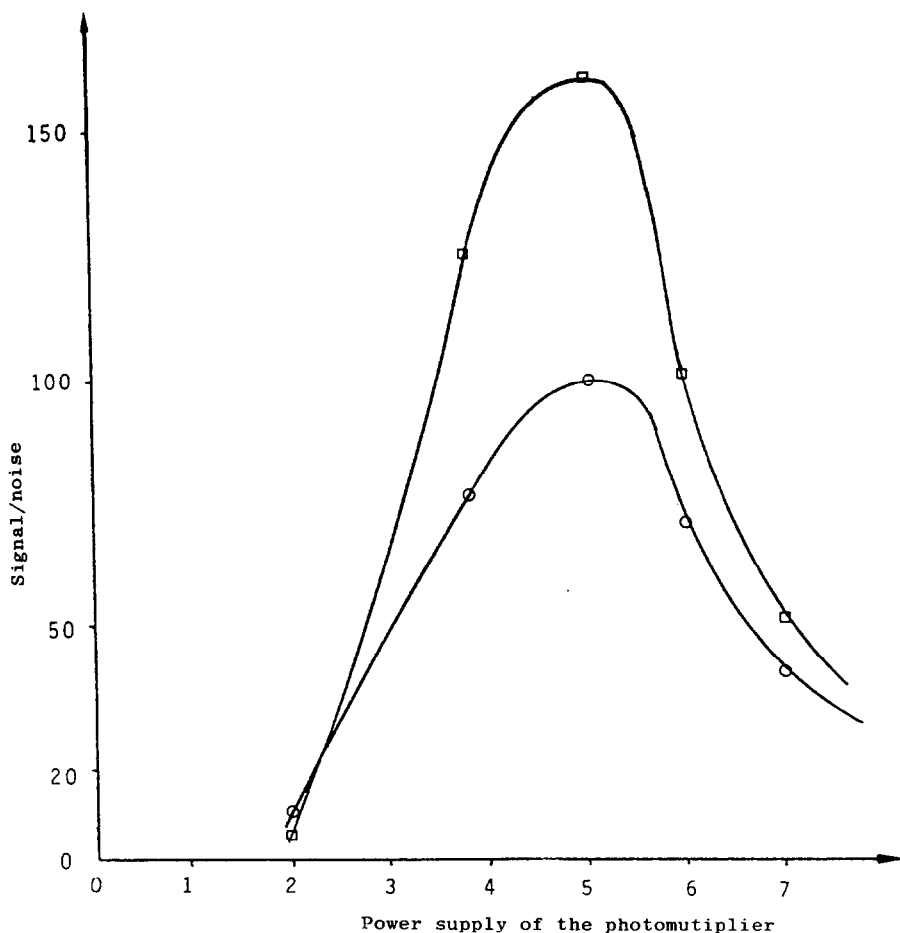

Fig. 3. Signal-to-noise ratio plotted as a function of the power supply of the photomultiplier. Column, 15 × 0.46 cm I.D. Zorbax ODS (5–7 μm); mobile phase, carbon dioxide–methanol (97:3, w/w); flow-rate, 4.5 ml min⁻¹ (0°C); temperature, 30°C; column inlet pressure, 295 bar; interface, fused-silica capillary tubing (10 cm × 75 μm I.D.). Detector requirements: air, 3.7 l min⁻¹ (detection cell); T_3 (glass tubing), 30°C; T_4 (evaporation tubing), 25°C. Solutes: 730 ng each of (○) docosanol and (□) palmitic acid.
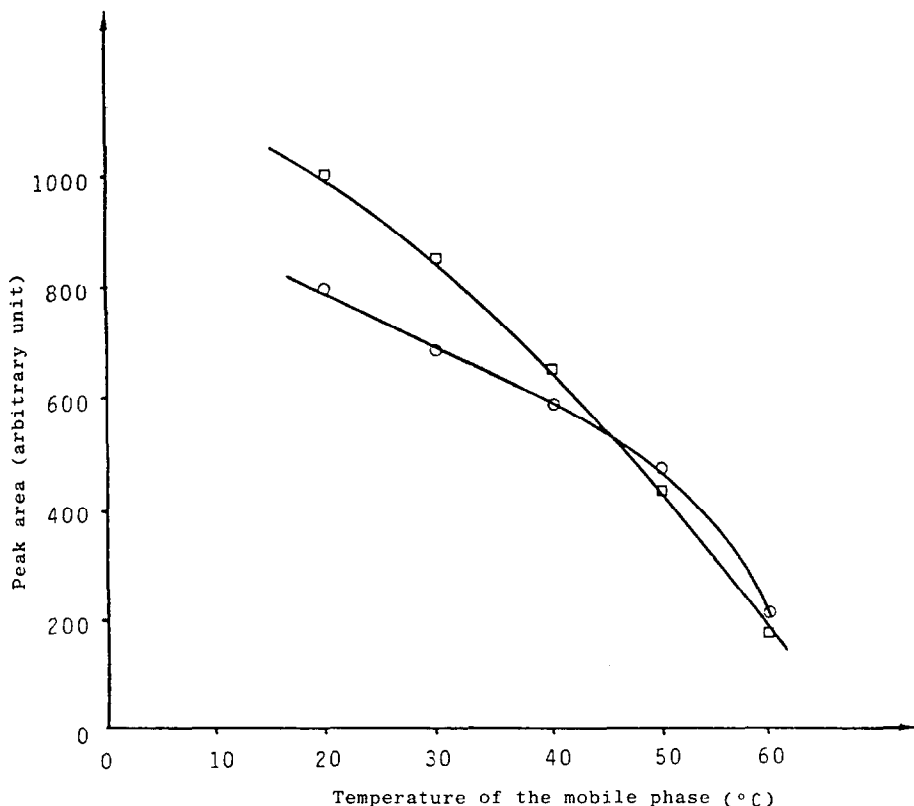
Fig. 4. Response of the light-scattering detector as a function of the temperature of the mobile phase. Column, 15 × 0.46 cm I.D., LiChrosorb Si 60 RP-18 (5 μm); mobile phase, carbon dioxide–methanol (97.2:2.8, w/w); flow-rate, 5.4 ml min^{-1}; inlet column pressure, 180 bar; interface, fused-silica capillary tubing (12 cm × 75 μm I.D.). Detector requirements as in Fig. 3. Solutes: 600 ng each of (○) docosanol and (□) palmitic acid.

the best signal-to-noise ratio was obtained at range 5.

Surprisingly, the temperature of the mobile phase was found to have a large effect on the detector response (Fig. 4). A large decrease in the detector response occurred when the temperature of the mobile phase was increased. This effect can probably be related to the relationship between the molar enthalpies of mixing (H_M^E), recently reviewed by Christensen *et al.*[15] for carbon dioxide–alkane mixtures: along isobars, small positive H_M^E below the lower critical temperature are transformed into large negative H_M^E as the temperature rises, then to large positive H_M^E near the upper critical temperature, and finally they diminish until they resemble those observed below the lower critical temperature. If similar changes in H_M^E occur during carbon dioxide–methanol mixing, as the temperature varies in the chromatograph, exothermic or endothermic effects cause increases or decreases in the temperature of the mobile phase. Consequently, a different behaviour can be expected during the chromatographic process, including the decompression step. A knowledge of H_M^E values when mixing carbon dioxide and methanol could be very useful in understanding these phenomena.

In a second step, in order to expand the potential of SFC–LSD for the analysis of polar compounds, we investigated the LSD response with mobile phases containing up to 4.8% (w/w) of methanol using a 2^5 factorial design in order to economize on experiments and to investigate the interactions between parameters.

A thorough study of factorial designs is beyond the scope of this paper, as they were only used as a tool for our purposes. We built our approach on the data published by Sado and Goupy[7], Feinberg and Ducauze[8] and Miller and Miller[9].

We assigned each parameter two levels (Table III). In the matrix of experiments, the signs indicate the level of parameters for each measurement. The effects reported in Table IV were deduced from the measurements using the matrix: the signs of the column corresponding to the parameter of interest were associated with the responses (reported in Table III) to calculate the algebraic sum of responses. Then, the sum was divided by the number of experiments to give the effect of the parameter. A significant negative or positive value indicates a decrease or an increase, respectively in the detector response, when the parameter is set to the $(+)$ level instead of the $(-)$ level with the level of the other parameters being fixed.

As the measurements were repeated five times, an analysis of variance was done[8]. Thus, we were able to correlate a significant effect with parameters by calculating the ratio (F) between the variance caused by the parameter (or interaction) investigated and the residual variance (Table IV). The effect was considered to be significant from Snedecor's table when $F > 7.71$.

In addition to the temperatures of the interface and the mobile phase, we also investigated the effect of air as nebulization make-up gas flowing around the restrictor outlet[10]. Other parameters were selected according to the previous conclusions.

The influence of the parameters studied is summarized in Table V as a function of the methanol content in the mobile phase. In all instances, when air was added the detector response was $2 - 5$ times lower, depending on the levels of the other parameters.

For a low methanol content (2.4%), the other parameters had no effect or lowered the response when they were set to the $(+)$ level instead of the $(-)$ level; low temperatures of the interface and the mobile phase allow total evaporation of the mobile phase. When the temperature was increased, solute vaporization could no longer be neglected and consequently a decrease in detector response occurred.

For a high methanol content (4.8%), without air, the maximum response was determined when the temperature of the glass jacket (T_3) or the mobile phase temperature was set at the $(+)$ level for low levels of the other parameters only. When air was added, the maximum response occurred for maximum heating of only the interface. These results are consistent with the previous conclusions. Obviously, the highest methanol content in the mobile phase requires supplementary heating. However, these results also emphasize the large effect of interactions; when another temperature was elevated in addition to T_3, the response decreased.

Finally, using these conclusions, the detection limit (signal-to-noise ratio $= 3$) measured with docosanol $(k' = 0.8)$ and octadecanol $(k' = 1.2)$ was 12 ng. Such a low value has never been reported before.

TABLE III

2^5 FACTORIAL DESIGN: MATRIX OF EXPERIMENTS, LEVELS OF PARAMETERS AND RESPONSES

Level	Parameter				
	$T_2(°C)$ (temperature of thermocouple wire)	$T_3(°C)$ (temperature of glass jacket)	$T_{MP}(°C)$ (temperature of the mobile phase)	CH_3OH (%) (methanol content in the mobile phase)	Air ($l\ min^{-1}$) (air flow-rate)
Level ($-$)	30	30	30	2.4	0
Level ($+$)	50	60	50	4.8	2

Experiment No.	T_2	T_3	T_{MP}	CH_3OH	Air	Peak area of docosanol ($\times\ 10^{-3}$)	Peak area of palmitic acid ($\times\ 10^{-3}$)
1	$-$	$-$	$-$	$-$	$-$	1130	1328
2	$+$	$-$	$-$	$-$	$-$	1082	1194
3	$-$	$+$	$-$	$-$	$-$	944	993
4	$+$	$+$	$-$	$-$	$-$	886	905
5	$-$	$-$	$+$	$-$	$-$	1237	934
6	$+$	$-$	$+$	$-$	$-$	770	604
7	$-$	$+$	$+$	$-$	$-$	683	388
8	$+$	$+$	$+$	$-$	$-$	746	461
9	$-$	$-$	$-$	$+$	$-$	84	85
10	$+$	$-$	$-$	$+$	$-$	204	207
11	$-$	$+$	$-$	$+$	$-$	1130	1111
12	$+$	$+$	$-$	$+$	$-$	1193	1146
13	$-$	$-$	$+$	$+$	$-$	1250	1320
14	$+$	$-$	$+$	$+$	$-$	1368	1492
15	$-$	$+$	$+$	$+$	$-$	824	662
16	$+$	$+$	$+$	$+$	$-$	826	595
17	$-$	$-$	$-$	$-$	$+$	269	320
18	$+$	$-$	$-$	$-$	$+$	260	303
19	$-$	$+$	$-$	$-$	$+$	267	283
20	$+$	$+$	$-$	$-$	$+$	229	252
21	$-$	$-$	$+$	$-$	$+$	260	227
22	$+$	$-$	$+$	$-$	$+$	197	185
23	$-$	$+$	$+$	$-$	$+$	190	107
24	$+$	$+$	$+$	$-$	$+$	190	125
25	$-$	$-$	$-$	$+$	$+$	274	316
26	$+$	$-$	$-$	$+$	$+$	355	361
27	$-$	$+$	$-$	$+$	$+$	508	603
28	$+$	$+$	$-$	$+$	$+$	352	395
29	$-$	$-$	$+$	$+$	$+$	182	174
30	$+$	$-$	$+$	$+$	$+$	183	175
31	$-$	$+$	$+$	$+$	$+$	205	184
32	$+$	$+$	$+$	$+$	$+$	234	214

TABLE IV

2^5 FACTORIAL DESIGN: CALCULATED EFFECT OF THE PARAMETERS AND CORRESPONDING F VALUE

Parameters	Docosanol		Palmitic acid	
	Effect	F	Effect	F
T_2	−11.5	7.5	−13.5	3.2
T_3	9	2.4	−20.5	11.1
T_{MP}	5	3.2	−61	70.3
CH_3OH	5	3.2	13.5	2.9
Air	−318	4762	−287.5	1544
T_2T_3	5	0.1	1.5	2.1
T_2T_{MP}	−8	0.3	4.5	4.9
T_2CH_3OH	27	8.2	21	33
T_2Air	−1.5	0	0.5	0.6
T_3T_{MP}	−106	285	−123.5	542
T_3CH_3OH	76	99.5	124	273
T_3Air	3	17.9	31.5	1
$T_{MP}CH_3OH$	55	179	98	137
T_{MP}Air	−60	15	−61	183
CH_3OHAir	32	12.1	25.5	50
$T_2T_3T_{MP}$	26	3	17.5	21.6
$T_2T_3CH_3OH$	−29	20	−32.5	39
T_2T_3Air	−16	2	−9.5	14.3
$T_2T_{MP}CH_3OH$	11	0.6	5	4.9
T_2T_{MP}Air	−14	1.9	9.5	12.5
T_2CH_3OHAir	−23	11.1	−25	24
$T_3T_{MP}CH_3OH$	−91	240	−113.5	374
T_3T_{MP}Air	93	193	100.5	426
T_3CH_3OHAir	−50	21.3	−34	118
$T_{MP}CH_3OH$Air	−86	293	−124	341
$T_2T_3T_{MP}CH_3OH$	−13	1.5	−9.5	8.4
$T_2T_3T_{MP}$Air	−4	0.4	4.5	1
$T_2T_3CH_3OH$Air	14	5.4	15.1	9.5
$T_2T_{MP}CH_3OH$Air	−4	0.7	6	0
$T_3T_{MP}CH_3OH$Air	91	193	113.5	322
$T_2T_3T_{MP}CH_3OH$Air	24	9.2	22.5	27.5

APPLICATIONS

Fig. 5 shows the chromatogram of docosanol and palmitic acid used as test solutes during the study of the detector response.

The coupling of SFC with LSD can be used to advantage for the analysis of fatty alcohols and fatty acids, as demonstrated in Figs. 6 and 7; such compounds are very difficult to elute from a packed column when only carbon dioxide is used as the mobile phase (*i.e.*, with flame-ionization detection[10]). One can deduce from these chromatograms that the LSD response varies as a function of volatility, for the operating conditions in Fig. 6, tetradecanol started to evaporate and consequently gave a lower signal than the other solutes; moreover, the LSD response is a function of the particle diameter in the detection cell, which is related to the concentration of the solutes in the effluent before the nebulization step[13]. Consequently, a correction

TABLE V

2⁵ FACTORIAL DESIGN: EFFECT OF THE PARAMETERS AS A FUNCTION OF METHANOL
CONTENT

$0 =$ no significant effect; $\uparrow$ and $\downarrow =$ increase and decrease, respectively, in detector response.

Methanol content (%)	Air	Temperature of the thermo-couple wire (T_2)	Temperature of the glass tubing (T_3)	Temperature of the mobile phase (T_{MP})	Temperature of the glass jacket / mobile phase
2.4	With	0	$\downarrow$	$\downarrow$	$\downarrow$
	Without	0	$\downarrow$	$\downarrow$	$\downarrow$
4.8	Without	0	$\uparrow$	$\uparrow$	$\downarrow$
	With	0	$\uparrow$	$\downarrow$	$\downarrow$

factor was proposed in order to take into account the retention of the solutes for
peak-area calculation[10]. This permitted its influence to be partially reduced but more
investigations are required on the main parameters (molecular weight, volatility, re-
fractive index, retention, etc.) that affect the LSD response. The retention dependence
of the LSD response is not a major drawback as a calibration graph is required for
quantitative analysis because of the non-linear relationship between the LSD re-
sponse and concentration[1,11,13,16–20].

　　With the improved sensitivity we obtained, LSD can be regarded as a sensitive
detection method for SFC when the analysis of this type of solute must be performed
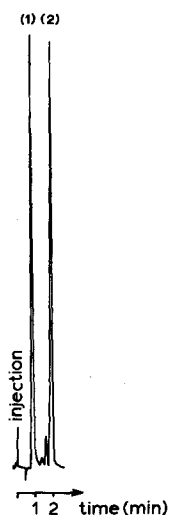on packed columns.

(1) (2)

injection

1 2   time (min)

Fig. 5. SFC–LSD chromatogram of (1) palmitic acid and (2) docosanol used as test compounds for
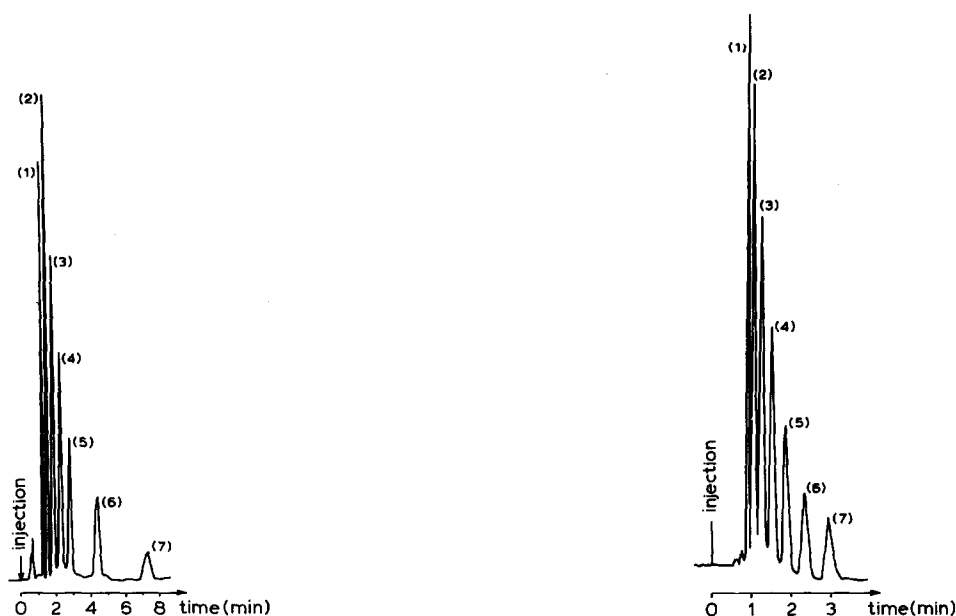optimization. Conditions as in Fig. 3.

Fig. 6. SFC–LSD chromatogram of fatty alcohols. Column, 15 × 0.46 cm I.D., Zorbax ODS (5–7 μm); mobile phase, carbon dioxide–methanol (98.7:1.3, w/w); flow-rate, 5.6 ml min^{-1}; inlet column pressure, 290 bar; interface, fused-silica capillary tubing (12 cm × 75 μm I.D.). Detector requirements: air, 3.7 l min^{-1} (detection cell); T_3 (glass tubing), 30°C; T_4 (evaporation tubing), 30°C. Solutes: 1 = tetradecanol; 2 = hexadecanol; 3 = octadecanol; 4 = eicosanol; 5 = docosanol; 6 = hexacosanol; 7 = triacontanol. Amount injected, 700 ng, except tetradecanol, 1.4 μg.

Fig. 7. SFC–LSD chromatogram of fatty acids. Column, 15 × 0.46 cm I.D., Zorbax ODS (5–7 μm); mobile phase: carbon dioxide–methanol (97.6:2.4, w/w); flow-rate, 3.9 ml min^{-1}; inlet column pressure, 200 bar; interface, fused-silica capillary tubing, 12 cm × 75 μm I.D. LSD requirements: air, 3.7 l min^{-1} (detection cell); T_3 (glass tubing), 30°C; T_4 (evaporation tubing), 30°C. Solutes: 1 = lauric acid; 2 = myristic acid; 3 = palmitic acid; 4 = stearic acid; 5 = arachidic acid; 6 = docosanoic acid; 7 = tetracosanoic acid. Amount injected, 700 ng of each solute.

CONCLUSION

With the new interface design between the column and the light-scattering detector, the sensitivity of SFC-LSD was enhanced by a factor of 4 in comparison with our previous results while the temperatures of the interface and evaporation tubing were significantly reduced even with a methanol content of up to 5% in the mobile phase. Hence the gain in sensitivity is a real improvement because the risk of partial evaporation or degradation of solutes before the detection stage is also greatly reduced.

Factorial designs were used to plan and to economize on measurements. Obviously, the conclusions must be considered with care as they concern a limited range of variation of the parameters. Without further experiments, a linear relationship must be assumed between the two levels of the parameters we studied. Nevertheless, the 2^5 factorial design demonstrated their potential as we investigated interactions between parameters which were found to influence greatly the detector response. It permitted

the use of LSD under conditions such that the best sensitivity was obtained for this type of compound.

Various applications in the field of carbohydrate and triglyceride analysis are under investigation to demonstrate the potential and advantages of SFC–LSD.

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