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SUPERCRITICAL FLUID CHROMATOGRAPHIC SEPARATION OF LOW- TO MEDIUM-POLARITY COMPOUNDS THAT ARE DIFFICULT TO ELUTE FROM PACKED COLUMNS

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SUMMARY

Supercritical fluid chromatography with flame ionization detection of unsubstituted and substituted heavy carboxylic acids (C_{18}–C_{30}) and of various polymer additives (PAs) is demonstrated. All compounds were easily eluted with carbon dioxide or nitrous oxide from open-tubular capillary columns with non-polar methylsilicone (DB-1) or phenylmethylpolysiloxane (SE-54) stationary phases in less than 20 min. Comparison of the two mobile phases showed that nitrous oxide was not suitable for pressure programming and flame ionization detection due to a severe baseline drift caused by the high background level. On packed C_{18} or cyano bonded phase columns, aliphatic amine (Armostat 400) or amide (Oleamide, Erucamide) PAs as well as carboxylic acids were strongly adsorbed, whereas an alphatic disulphide (Hostanox SE-10) was eluted with the same mobile phases. The peak symmetry was measured and was found to decrease drastically with increasing loads of compounds with polar substituents, compared to a non-polar aliphatic hydrocarbon.

INTRODUCTION

Organic compounds with high molecular weights and polar functional groups traditionally represent an analytical challenge since the requirements of thermal stability limit the use of gas chromatography (GC). The separation of such compounds can in many cases be obtained by liquid chromatography (LC), but there is a lack of sensitive universal LC detectors. In these cases supercritical fluid chromatography (SFC)[1–5] should be considered as an alternative. SFC offers the detection capabilities of GC and transport properties intermediate between those of GC and LC. On packed reversed-phase columns the SFC separation of compounds with polar groups will often require the use of polar modifiers like methanol to deactivate unreacted silanol groups in the stationary phase[6–8]. However, most modifiers, including methanol, are not compatible with flame ionization detection (FID), due to the high background signal generated. To circumvent the use of modifiers, open-tubular capillary columns can be used, since silanol groups are not present in the stationary phase.

In this study, the capillary SFC separation of various compounds of low-to-

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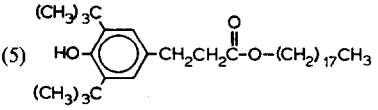
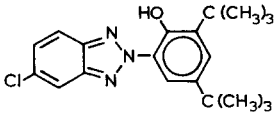
medium polarity have been investigated with carbon dioxide and nitrous oxide as mobile phases. The compounds were substituted and unsubstituted heavy carboxylic acids ($\geqslant C_{18}$) and polymer additives. The SFC separation of lower carboxylic acids has been reported previously[8,10]. Although the overall polarity of long-chain carboxylic acids must be considered as low, the carboxylic group will cause severe adsorption to silanol groups. For example, arachidonic acid was not eluted with 1% methanol in carbon dioxide on a packed C_{18} column[11]. The loadability of a capillary column containing a non-polar stationary phase with carboxylic acids of different polarities has been measured.

"Polymer additives" (PAs) is a term used to denote many classes of compounds with very different chemical and physical properties. Several PAs are usually present in one polymer matrix, and their concentrations vary from 0.01 to 1% (w/w). Some PAs are reactive and decompose partially during polymer processing, and synthetic byproducts from the additive production may also be present in the polymer. Even though most PAs are of limited thermal stability, have high molecular weights and contain polar groups, GC–FID analysis has been evaluated for some applications[12]. The majority of papers published on PA characterization are concerned with antioxidants and UV-stabilizers, which are often well suited for LC analysis with UV detection. Other PAs without chromophores must be detected by the less sensitive and less applicable refractive index (RI) detector[13], unless an LC–mass spectrometric (MS) system is available[14,15]. A need for the characterization of PAs which cannot be analysed by either of the methods mentioned above obviously exists.

TABLE I

EXAMPLES OF DIFFERENT POLYMER ADDITIVES (PAs)

Compound	*Trade-name*	*MW*	*Use*	*Concentration (%)*
(1) $CH_3(CH_2)_{17}$–S–S–$(CH_2)_{17}CH_3$	Hostanox SE-10	571	Co-stabilizing antioxidant	<0.1
(2) $CH_3(CH_2)_7CH=CH(CH_2)_{11}C(=O)–NH_2$	Erucamide	337	Antiblock agent, slip agent	0.05–0.2 0.02–0.1
(3) $CH_3(CH_2)_7CH=CH(CH_2)_7C(=O)–NH_2$	Oleamide	281	Antiblock agent, slip agent	0.05–0.2 0.02–0.1
(4) $OH(CH_2)_2NH(CH_2)_nNH(CH_2)_2OH$ (n = 12–16)	Armostat 400	228 (n = 12) 334 (n = 16)	Antistatic	<0.2
(5) 	Irganox 1076	531	Antioxidant	0.5–1
(6) 	Tinuvin 327	357	UV absorber	0.2–0.5

In this paper we consider four different PAs known under the trade-names Oleamide, Erucamide, Armostat 400 and Hostanox SE-10 (Table I), none of which can be detected by UV absorption. The compounds belong to different PA classes, have different polarities and are used in lower concentrations than antioxidants and UV stabilizers.

EXPERIMENTAL

Chemicals

All solvents were high-performance liquid chromatography (HPLC) grade from Rathburn (Walkerburn, U.K.). The fatty acids were obtained from Larodan Fine Chemicals (Malmoe, Sweden) and the additives from Statoil (Bamble, Norway). Carbon dioxide, grade 1, and nitrous oxide, medical grade, from AGA Norgas (Oslo, Norway) were purified and filtered on-line by passing the fluid through active carbon and a 0.5-μm Rheodyne stainless-steel filter.

Apparatus

An ISCO μLC-500 syringe pump was equipped with a cooling coil through which was circulated methanol at -15°C during the pump refill procedure. Prior to normal use, the circulation was shut off, and the pump was allowed to return to room temperature, because of increased leakage at the cooled piston seal. The oven and the flame ionization detector of an HP 5790A gas chromatograph were used without modification. The injection system was a Rheodyne 7520 injector with a 0.2-μl sample loop connected to a zero-dead-volume 1/32-in. split-tee (Valco) with a 5–20 cm $\times$ 25 μm I.D. fused-silica capillary serving as the split restrictor. Samples were injected at or slightly above ambient temperature, while splitting occurred under supercritical conditions.

A 20 m $\times$ 100 μm I.D. fused-silica capillary column (J&W Scientific) specially made for SFC, hereafter denoted DB-1, was used for most separations. The column was deactivated, and the stationary phase was a methylsilicone (DB-1) with a film thickness of 0.4 μm. The other column, 5.7 m $\times$ 48 μm I.D., hereafter denoted SE-54, was prepared from FS capillary tubing (SGE) with 5% phenylmethylpolysiloxane (SE-54) as stationary phase. The SE-54 was dissolved in *n*-pentane and statically coated without any preceding surface modification or deactivation of the capillary, to give a film thickness of 0.7 μm. Di(*p*-cumenyl) peroxide was used for cross-linking. The column was purged with nitrogen gas for 2 h and conditioned with supercritical carbon dioxide by simultaneous temperature–pressure programming from 45 to 140°C and 72 to 300 bar in 45 min. Thereafter the column was conditioned at 120°C and 270 bar for 4 h. The DB-1 column was conditioned prior to use by a shorter procedure.

Restriction was accomplished with either a 12–17 cm $\times$ 10 μm I.D. fused-silica capillary or a tapered 10 or 25 μm I.D. capillary of the same length. The tapered restrictors were drawn by hand in a flame, and the tip was gently brushed off. A batch of several restrictors was produced, the flow-rate at 200 bar was recorded and suitable restrictors were selected. A zero-dead-volume internal union (Valco) connected the column and restrictor which was kept at 350°C in the flame ionization detector.

RESULTS AND DISCUSSION

In capillary supercritical fluid chromatography, precolumn splitting ratios from 1:5 to 1:50 are commonly used to prevent solvent overloading. Injection loops with volumes of 0.06–0.2 μl are commercially available, but in order to fill the loop properly, 1–5 μl of sample solution have to be applied. Together, these two factors may easily result in effective sample splitting ratios of up to 1:1000. If a 0.2-μl injector loop is connected to a 1:50 precolumn split, a sample concentration higher than 1 mg/ml will be required to satisfy a minimum detectable amount of 5 ng. At this concentration, solubility problems may occur at room temperature for high-molecular-weight compounds like heavy carboxylic acids. These circumstances have led us to use reduced splitting ratios (typically 1:5), although this can occasionally cause problems (fluctuating split flow, temporarily or permanently split clogging) usually not mentioned by users of high splitting ratios.

It has previously been observed that a non-polar stationary phase (SE-54) resulted in low chromatographic performance with polar compounds[10]. This effect on the DB-1 stationary phase is clearly seen in Figs. 1 and 2. Fig. 1 illustrates the effect of increasing the column load of 2-methylhexadecanoic acid and of 2-methyleicosanoic acid from 40 to 300 ng. The peaks, initially narrow with some tailing, broaden with pronounced fronting and distortion of the peak maximum at 300 ng. In comparison, an *n*-alkane of comparable molecular weight retains its symmetrical shape even at loads of 200 ng (Fig. 2). These results are quantitatively illustrated in fig. 3, where the asymmetry factor, A_s, at half-height is given as a function of the column load for three components of different polarities. A_s values below 1.0, indicative of fronting and column overload, occur for the more polar hydroxyl- and methyl-substituted eicosanoic acids at 50–60 ng, while 150 ng of the non-polar *n*-

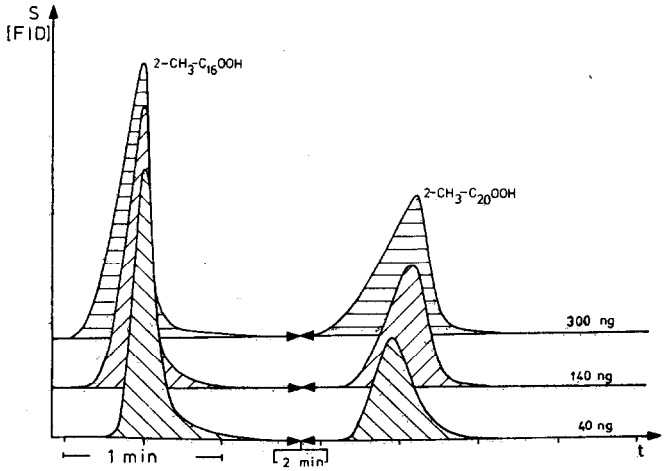


Fig. 1. Peak shape of 2-methylhexadecanoic acid and 2-methyleicosanoic acid at different loads on a non-polar stationary phase. The peak heights and retention times are normalized to the values for the first peak eluted. Conditions: mobile phase, carbon dioxide; temperature, 110°C; pressure, 207 bar; column, DB-1.

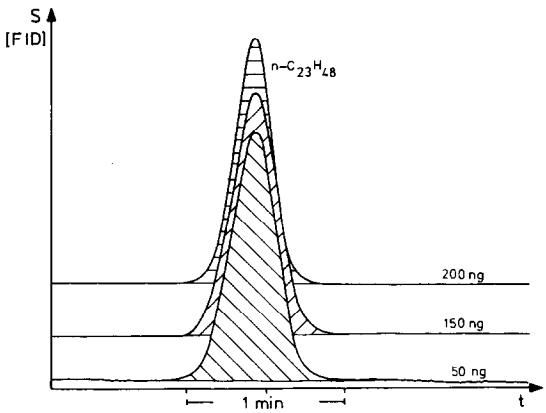


Fig. 2. Peak shape of *n*-tricosane at different loads on a non-polar stationary phase. The peak heights and retention times are normalized. Conditions as in Fig. 1, except pressure, 193 bar.

tricosane can be loaded onto the column before a slight reduction of A_s can be measured.

During our work on SFC characterization of PAs on packed columns, we observed that compounds containing amido or amino groups like Oleamide (3, Table I), Erucamide (2, Table I) and Armostat 400 (4, Table I) were not eluted from the two bonded phase columns investigated, 150 mm × 1.2 mm I.D., 4-μm C_{18} silica (Novapak) and 30 mm × 2.1 mm I.D., 5-μm cyano silica (Brownlee), with carbon

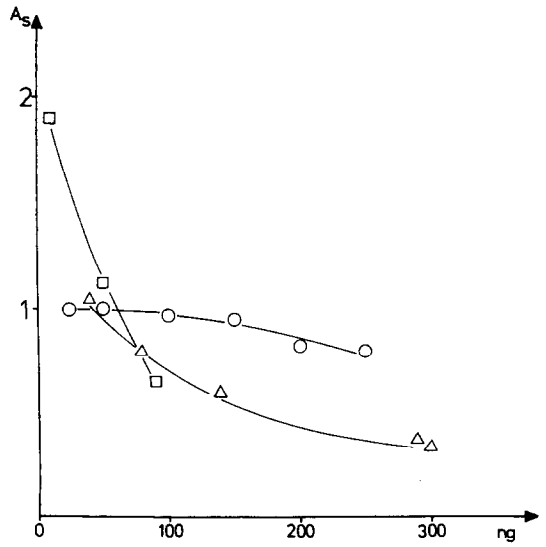


Fig. 3. The asymmetry factor, A_s, at half-height for compounds of different polarities as a function of the sample amount injected onto a column with a non-polar stationary phase. Conditions as in Fig. 1, except pressure 193 bar (○, *n*-tricosane), 207 bar (△, 2-methyleicosanoic acid) and 241 bar (□, 2-hydroxyeicosanoic acid). Molecular weights: 324 (*n*-tricosane), 326 (2-methyleicosanoic acid) and 328 (2-hydroxyeicosanoic acid).

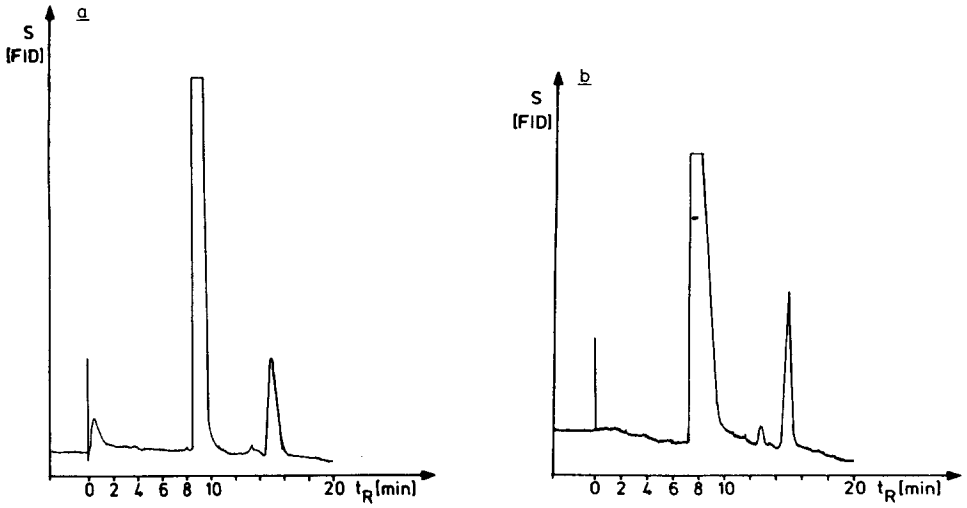


Fig. 4. Separation of Oleamide on the DB-1 column with carbon dioxide (a) and nitrous oxide (b) as the mobile phase. Conditions: temperature, 80°C; pressure, 193 bar.

dioxide or nitrous oxide as the mobile phases. As previously mentioned, arachidonic acid was also strongly retained on a C_{18} column, and was not eluted within reasonable pressure and time limits. On the other hand, the disulphide Hostanox SE-10 (1, Table I), as well as the aromatic compounds Irganox 1076 and Tinuvin 327 (5,6, Table I), were readily eluted. Since the three substituents contributing most strongly to retention on silica adsorption systems are amido, carboxy and amino groups[16], these results are consistent with the assumption that the observed retention is mainly caused by active, unreacted silanol groups in the bonded phase material.

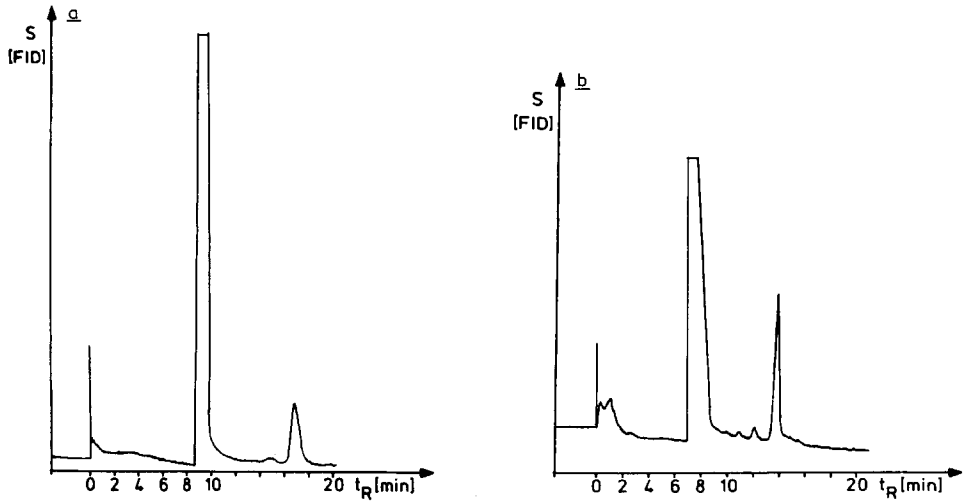


Fig. 5. Separation of Erucamide on the DB-1 column with carbon dioxide (a) and nitrous oxide (b) as the mobile phases. Conditions: temperature, 80°C; pressure, 207 bar.

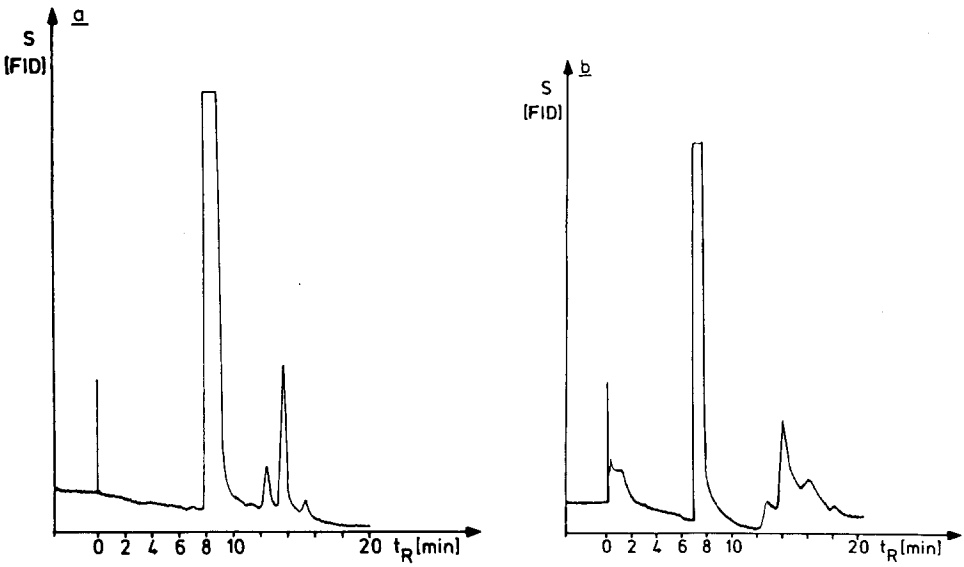


Fig. 6. Separation of Hostanox SE-10 on the DB-1 column with carbon dioxide (a) and nitrous oxide (b) as the mobile phases. Conditions: temperature, 80°C; pressure, 241 bar.

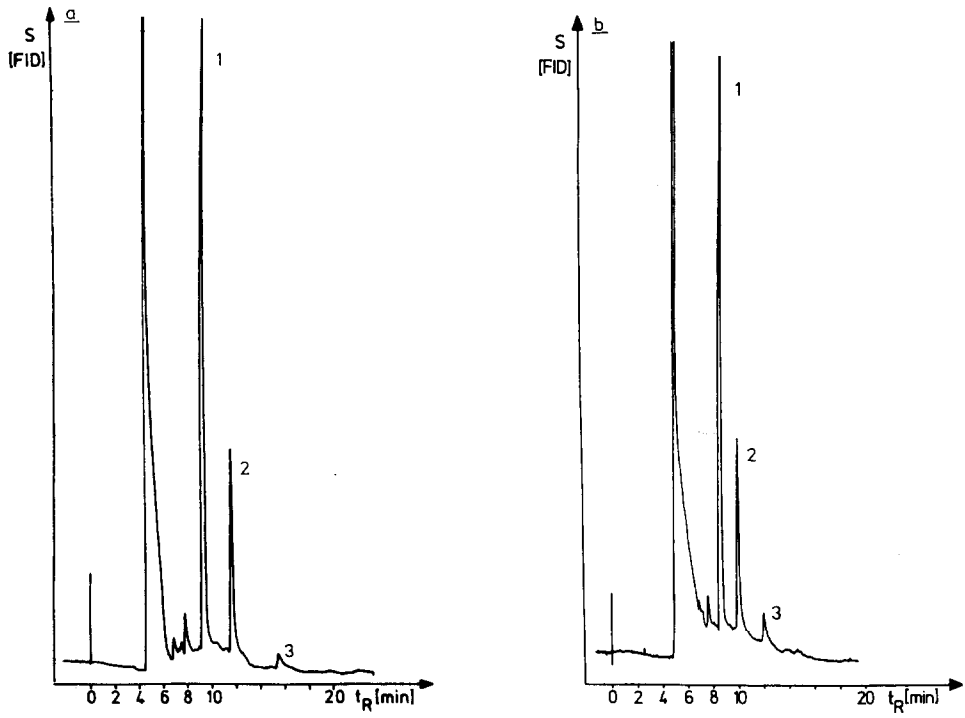


Fig. 7. Separation of Armostat 400 on the DB-1 column with carbon dioxide (a) and nitrous oxide (b) as the mobile phases. The peak numbers refer to Table II. Conditions: temperature, 110°C; pressure, 221 bar.

By using open-tubular capillary columns, PAs as well as carboxylic acids could be eluted. In Figs. 4–7, chromatograms of four PAs (1–4, Table I) with carbon dioxide and nitrous oxide as mobile phases are compared. The PAs are usually highly soluble in solvents like dichloromethane, and sample amounts of 0.2–0.4 μg could therefore be transferred to the column after injection. The column temperature and pressure were kept constant when changing the mobile phase, thus the density was slightly higher with nitrous oxide.

Chromatograms of the two amides Oleamide and Erucamide are illustrated in Figs. 4 and 5. With nitrous oxide as the mobile phase, the capacity factor increased, and the peak shape improved for both components compared to carbon dioxide. On the other hand, separation of the sample components of the disulphide Hostanox SE-10 was lost with nitrous oxide, whereas carbon dioxide gave three well resolved peaks (Figs. 6). The reason is thought to be a solubility problem of this particular compound in supercritical nitrous oxide. Immediately following injection a baseline disturbance caused by a temporary clogging of the split restrictor was observed. Neither the split performance nor the resolution was improved by reducing the injected amount from 0.2 to 0.02 μg. With Armostat 400 (Fig. 7) several components were separated using either carbon dioxide or nitrous oxide. The selectivity and capacity factors for three sample components are compared in Table II. The capacity factors are reduced with nitrous oxide, while the selectivity is not significantly changed.

Whereas samples containing single PAs or series with one to three homologous carboxylic acids (Figs. 8 and 9) could be eluted under isobaric conditions, broader distributions of carboxylic acids required a pressure programme (Fig. 10) to shorten the analysis time. However, it was difficult to utilize pressure programming with nitrous oxide as mobile phase. A pressure increase from 138 to 207 bar at 110°C caused an increase in the FID baseline level of 59 mV with nitrous oxide, while the corresponding value at the same detector attenuation was 1 mV with carbon dioxide. The problem can be partly solved by increasing the pressure prior to the appearance of the first sample peak, but detecting the sample components under isobaric conditions. An initial pressure gradient is necessary to shorten the analysis time. As shown in Fig. 11, the baseline drift was still pronounced.

TABLE II

SELECTIVITY COEFFICIENTS, α, AND CAPACITY FACTORS, k', FOR THREE COMPONENTS FROM THE SFC SEPARATION OF ARMOSTAT 400 WITH CARBON DIOXIDE AND NITROUS OXIDE AS THE MOBILE PHASES

Conditions as given in Fig. 7. Index numbers refer to components in Fig. 7.

	Carbon dioxide		*Nitrous oxide*	
	3000 p.s.i.	*3200 p.s.i.*	*3000 p.s.i.*	*3200 p.s.i.*
k' (1)	1.32	1.08	1.23	0.76
α (1,2)	1.44	1.44	1.41	1.36
k' (2)	1.90	1.55	1.73	1.02
α (2,3)	1.53	1.55	1.43	1.39
k' (3)	2.91	2.40	2.47	1.42

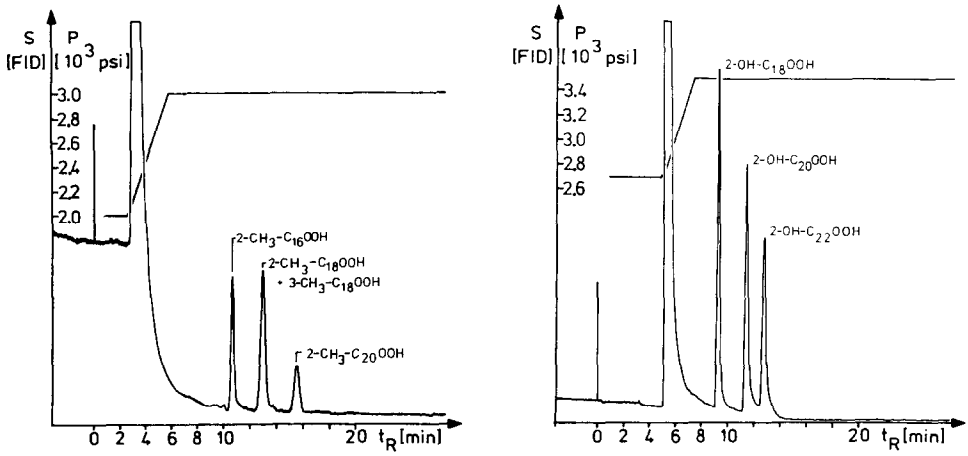


Fig. 8. Separation of methyl-substituted carboxylic acids (*ca.* 50 ng of each compound were injected). The sample was dissolved in acetone. Other conditions as in Fig. 1.

Fig. 9. Separation of hydroxyl-substituted carboxylic acids. Conditions as in Fig. 1 except pressure, 241 bar.

For rough quantitation and characterization of PAs, a shorter column with reduced phase ratio, β (= volume of mobile phase/volume of stationary phase), will reduce the analysis time. Fig. 12 shows a chromatogram of Armostat 400 on the SE-54 column (β = 17) operated at a linear velocity of 6–7 cm/s (methane at 50°C, 193 bar). Compared to the DB-1 column (β = 41) at 2–3 cm/s (Fig. 7), a considerable reduction in analysis time was obtained at the cost of some resolution.

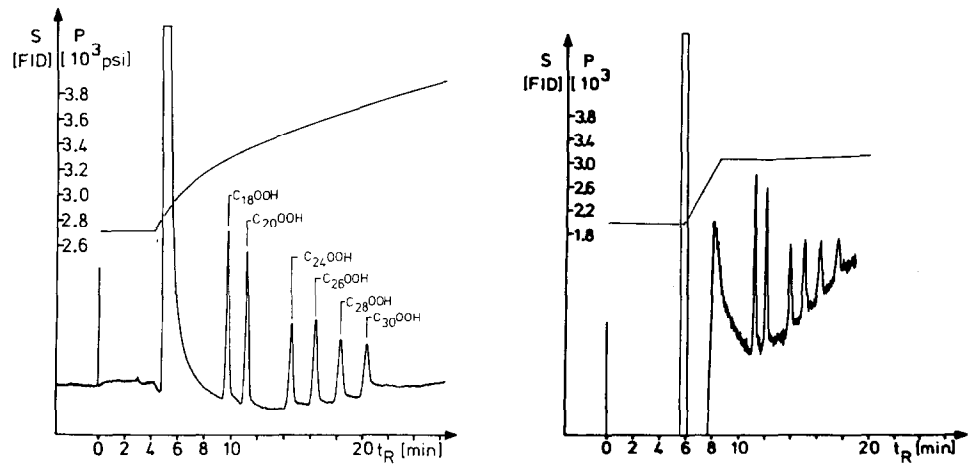


Fig. 10. Separation of C_{18}–C_{30} carboxylic acids (*ca.* 50 ng of each) on the DB-1 column with carbon dioxide as the mobile phase. The sample was dissolved in acetone. Conditions: temperature; 110°C, pressure as indicated.

Fig. 11. Separation of C_{18}–C_{30} carboxylic acids (*ca.* 50 ng of each) on the DB-1 column with nitrous oxide as the mobile phase. The sample was dissolved in acetone. Conditions: temperature, 110°C; pressure as indicated.

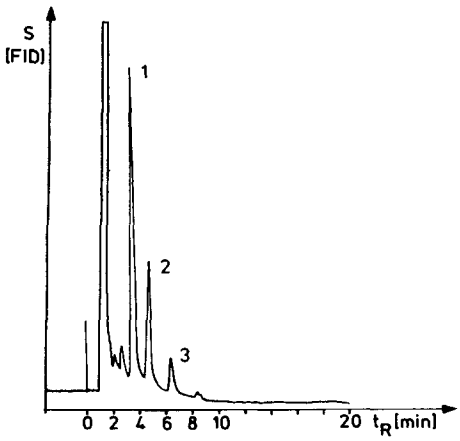


Fig. 12. Separation of Armostat 400 on the SE-54 column with carbon dioxide as the mobile phase. Conditions: temperature, 110°C; pressure, 221 bar. The peak numbers refer to Table II.

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