Journal of Chromatography, 505 (1990) 191-197
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 255

Note

Supercritical fluid chromatography with light-scattering detection

I. Preliminary results of the analysis of polar compounds with packed columns

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Supercritical fluid chromatography (SFC) does not possess a universal detector that compares with the refractive index detector used in high-performance liquid chromatography (HPLC). The flame ionization detector used in capillary SFC precludes the use of polar additives necessary in the analysis of polar products on packed columns.

A new kind of semi-universal detection has recently been developed in HPLC, which has some significant advantages over refractive index detection. This new type of detector is based on scattered light produced by solid or liquid microparticles resulting from the vaporization of effluents. The signal is directly dependent on the analysed solutes when the eluents are totally evaporated. Light-scattering detection (LSD) is compatible with gradient elution and is easily and rapidly operative. We have constructed an LSD instrument based on this principle, and without the limitations inherent in other available apparatus (Applied Chromatography Systems, Luton, U.K.). It is possible to evaporate aqueous–organic[1,2] and aqueous mobile phases[3] with[4] or without addition of volatile-salt at low temperatures (40°C) and at high flow-rates (3 ml min^{-1}).

LSD is compatible with SFC as it reveals every solid or liquid solute in suspension in the gas phase resulting from the decompression of the supercritical fluid. It is also compatible with polar additives added to the supercritical fluid. We have recently attempted to develop a specific interface for SFC that can be used directly with the light-scattering HPLC detector[5] and have determined the main factors that influence the detection limit and reproducibility in SFC[6].

This preliminary work emphasizes the advantage of this detection method for non-UV-absorbing or UV-absorbing polar solutes. Three classes of either commercially available or laboratory-made solutes are being studied: 0-alkyl glycosides, desulphoglucosinolates and alkylated and non-alkylated polyethylene glycols.

0021-9673/90/$03.50

EXPERIMENTAL

Apparatus

The apparatus used has been described in a previous paper[7]. The following columns were used: 10-μm LiChrospher 100 Diol(125 × 4.6 mm I.D.), 5-μm LiChrosorb CN (150 × 4.6 mm I.D.), both purchased from Merck (Darmstadt, F.R.G.), 7-μm Zorbax CN (150 x 4.6 mm I.D.) purchased from DuPont (Wilmington, DE, U.S.A.), 10-μm μBondapak CN (150 × 3.9 mm I.D.) purchased from Waters Assoc. (Milford, MA, U.S.A.) and 5-μm RSil NO_2 (250 × 4.6 mm I.D.) purchased from RSL (Eke, Belgium).

Chemical and reagents

Carbon dioxide was of B 50 grade (Air Liquide, Paris, France) and methanol of Pestipur grade (SDS, Vitry, France). The solutes (analytical-reagent grade) were dissolved in either methanol-chloroform, pure chloroform or pure methanol, depending of the polarity of the mobile phase. 0-Alkyl β-D-glycosides were purchased from Sigma (St. Louis, MO, U.S.A.) and Aldrich (Strasbourg, France), except for octyl β-D-galactopyranoside and octyl β-D-xylopyranoside[8]. Glucosinolates were synthesized in our laboratory[9]. Polyethylene glycols (PEG), ethylene glycol monohexadecyl ether ($C_{16}E_1$), diethylene glycol monohexadecyl ether ($C_{16}E_2$), tetraethylene glycol monohexadecyl ether ($C_{16}E_4$) and hexaethylene glycol monohexadecyl ether ($C_{16}E_6$) were purchased from Fluka (Buchs, Switzerland). 2,16-Di-*n*-octyl-3,6,9,12,15-pentaoxa-1,17-heptadecanediol ($2C_8E_6$) and 2,5,13,16-tetra-*n*-octyl-3,6,9,12,15-pentaoxa- 1,17-heptadecanediol ($4C_8E_6$) were synthesized in our laboratory".

RESULTS AND DISCUSSION

O-Alkyl glycosides

The analysis of carbohydrates is difficult because of their lack of UV absorbance. However, by means of LSD, an analytical studies of carbohydrates by HPLC or SFC have been carried out with apolar[1,3] and polar[2,7,11,12], stationary phases.

Similar detection difficulties were encountered with 0-alkyl glycosides. For these compounds, it is very important to determine the purity or composition of products used such as biological detergents[13], membrane solubilizers or as growth media for RNA and proteins[14]. Different 0-alkyl glucopyranosides and maltosides are commercially available. In order to study the hydrophobic and hydrophilic interactions between solutes and stationary phases, we have synthetized other 0-alkyl derivatives generated from galactopyranoside and xylopyranoside moieties.

Table I gives the retention times of thirteen compounds on cyano- and diol-bonded silicas. Polar interactions are involved because the retentions are mainly dependent on the sugar moiety. For a given carbohydrate, the corresponding O-alkylated derivatives have lower retentions than the non-derivatized solutes. The solute retention times decrease as the percentage of methanol increases. The percentage of methanol, the pressure of the eluent and the stationary phase directly influence the selectivity. Cyano-bonded silica gave smaller retention times than diol-bonded silica but the selectivity was improved (Table I, octyl β-D-gluco- and -galactopyranosides).

TABLE I

RETENTION TIMES OF GLYCOSIDES AND 0-ALKYL GLYCOSIDES ON POLAR PACKED COLUMNS IN SFC AT 40°C

Compound	*Retention time (min)*	
	Column I[a]	*Column 2*[b]
Octyl-β-D-xylopyranoside	3.6	2.0
Hexyl β-D-glucopyranoside	10.0	3.5
Octyl β-D-glucopyranoside	10.9	3.6
Decyl β-D-glucopyranoside	–	3.8
Dodecyl β-D-glucopyranoside	10.7	3.9
Octyl β-D-galactopyranoside	10.9	4.3
Methyl α,β-D-glucopyranoside	14.8	4.45 and 4.73
Methyl a-D-ghtcopyranoside	14.8	4.73
Decyl β-D-maltoside	>30	21.2
Dodecyl α,β-D-maltoside	>30	22.5
α,β-D-xylopyranose	–	4.5
α,β-D-galactopyranose	>30	8.0
a-o-glucopyranose	>30	8.7

[a] LiChrospher diol(125 × 4 mm I.D.), CO,-methanol (93.75:6.25, w/w) 3.9 ml min^{-1}, 145 bar.
[b] Zorbax CN (150 × 4.6 mm I.D.), CO,-methanol (93.0:7.0, w/w). 3.5 ml min^{-1}, 210 bar.

Fig. la confirms the higher selectivity of the Zorbax cyano column compared with the diol column. The chromatogram shows a good separation of α- and β-anomers of methyl D-glucopyranoside and a broad peak with a split top for the *a*- and β-anomer mixture of glucopyranose.

The number of plates calculated for decyl β-D-maltoside gives a reduced plate height of 6.2 at a linear eluent speed of 3.5 mm s^{-1}. This value if lower than that obtained with non-derivatized glycosides using SFC[7] or HPLC[15].

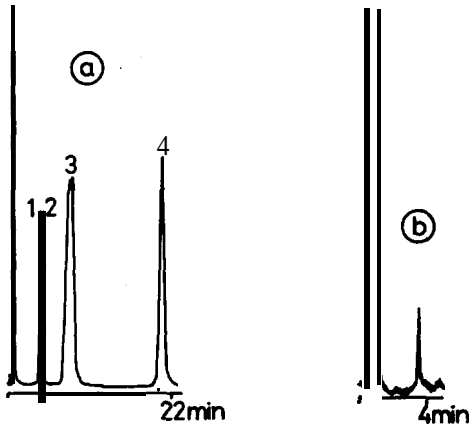

Fig. 1. Chromatograms of glycosides and 0-alkyl glycosides on a polar packed column. Column: Zorbax CN (I 50 × 4.6 mm I.D.). Eluant: CO,-methanol (93.75:6.25, w/w), 3.9 ml min^{-1}, 145 bar. Solutes: (a) 1 = methyl β-D-glucopyranoside, 2 = methyl a-D-glucopyranoside, 3 = α,β-D-glucopyranose, 4 = decyl β-D-maltoside; (b) octyl β-D-glucopyranoside (amount, 100 ng).

The chromatogram in Fig. lb illustrates the detection limit, giving a signal-to-noise ratio > 10 for an injection of 100 ng (20 μl of a 5 ppm solution of octyl β-D-glucopyranose). Larger sample injection volumes permit the determination of 0-alkyl glycosides at levels as low as 1 ppm.

Desulphoglucosinolates

Studies on glucosinolates or desulphoglucosinolates are extremely important as these compounds are found in various plant materials (rape, lupin, etc.) and also cause physiological effects in animals such as inappetence and goitrogenic effects[16]. Stuctures of desulphoglucosinolates are shown in Fig. 2.

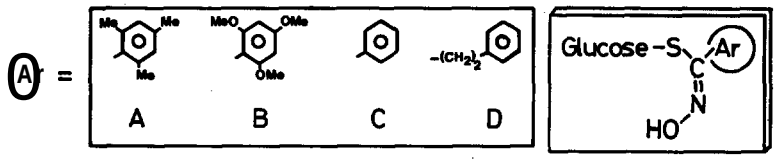

Fig. 2. Structures of desulphoglucosinolates. Me = CH_3.

We have been studying the synthesis, extraction from raw materials and analysis of natural and artificial glucosinolates and desulphoglucosinolates. Preliminary results for desulphoglucosinolates obtained using SFC are reported in Table II. Three desulphoglucosinolates, A, B and C, not yet found in plants and one natural desulphoglusinolate, D (trivial name gluconasturtin), were synthesized[9] (see Fig. 2). UV detection or LSD can be used for the analysis of these compounds.

The retentions of desulphoglucosinolates are generally higher than that of glucopyranose. The selectivities are dependent on the nature of the stationary phase and on the composition of the mobile phase. Nitro-bonded silica is more selective for desulphoglucosinolates, especially B and D. This result corroborates the results obtained with non-derivatized glycosides'.

The retention times of desulphoglucosinolates A, B and C were lower (A and C) or higher (B) than that of the natural desulphoglucosinolate D. Consequently, A, B

TABLE II

RETENTION TIMES OF DESULPHOGLUCOSINOLATES ON POLAR PACKED COLUMNS IN SFC AT 40°C

Compounds	*Retention time (min)*	
	Column 1[a]	Column 2[b]
A	10.7	6.2
B	15.9	12.3
C	14.6	7.4
D	15.7	7.6
o-Glucose	8.1	7.1

[a] LiChrospher diol (125 × 4 mm I.D.), CO_2-methanol (90:10, w/w), 4.1 ml min^{-1}, 180 bar.
[b] RSil NO, (250 × 4.6 mm I.D.), CO_2-methanol (89.3:10.7 w/w), 4.6 ml min^{-1}, 245 bar.

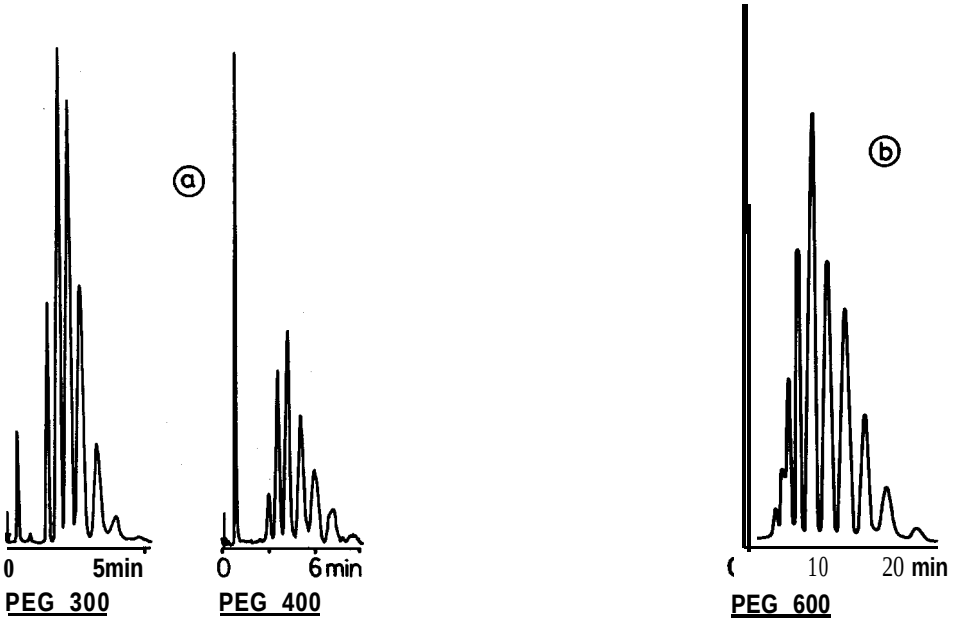

Fig. 3. Chromatograms of polyethylene glycol (PEG) 300, 400 and 600 on polar packed columns. (a) Column: LiChrosper Diol(125 × 4 mm I.D.). Eluent: CO,-methanol (95.2:4.8, w/w). 3.4 ml min^{-1}, 4000 p.s.i. (b) Column: LiChrospher Diol (125 × 4 mm I.D.). Eluent: CO,-methanol (90.9:9.1, w/w), 1.8 ml min^{-1}, 2500 p.s.i.

and C can be used as internal standards in glucosinolate analysis as they have never been identified in natural glucosinolate mixtures and therefore could not lead to incorrect quantitative analyses.

One of the major interests in LSD is the possibility of obtaining a mass response within a simple solute class[1,3,17]. This is advantageous for the quantitative analysis of complex glucosinolate mixtures compared with UV detection, which largely depends of alkyl or aryl substituents.

It has been shown that UV detection in SFC, when compared with UV detection in HPLC, provides quantitative results which are less precise". We have observed the same loss of precision in the SFC–LSD[12] analysis of sugars.

TABLE III

RETENTION TIMES (min) OF ALKYLATED POLYETHYLENE GLYCOLS ON A μBONDAPAK CN COLUMN

Column: μBondapak CN (150 × 3.9 mm I.D.), CO,-methanol (97.1:2.9, w/w). 4.15 ml min^{-1}, 224 bar.

Compound	*Retention time (min)*	*Compound*	*Retention time (min)*
$C_{16}E_1$	*0.7*	$C_{16}E_6$	*7.2*
$C_{16}E_2$	*0.8*	$4C_8E_6$	*3.8*
$C_{16}E_4$	*1.4*	$2C_8E_6$	*7.1*

Straight and derivative polyethylene glycols

Non-ionic detergent such as PEG or alkylated PEG are very used in various fields. Our laboratory is interested in the specific synthesis of non-UV-absorbing derivatives of PEG in order to produce reference compounds usable for the characterization and identification of complex commercially available PEG mixtures. Commerical PEG 300,400 and 600 have been analysed both by HPLC[19,20] and SFC using capillary columns and with parameter programming (density–pressure[21] and temperature[22]).

The chromatograms in Fig. 3 illustrate some preliminary results obtained on packed columns. They show an isocratic SFC elution using a diol column and a carbon dioxide-methanol mobile phase. From the shape of the chromatogram, it is easy to recognize the oligomer distribution.

Table III illustrates the important role of the polar ethoxy group in determining the retention time. With a constant alkyl chain length (C_{16}) the retention times increase with increase in the ethylene oxide number: $C_{16}E_6 > C_{16}E_4 > C_{16}E_2 > C_{16}E_1$. With a constant number of ethylene oxide groups, the retention times decrease with increase in the number of alkyl chains: $2C_8E_6 > 4C_8E_6$. A more detailed study will attempt to elucidate the different factors (e.g., alkyl chain length and alkyl chain number) involved in the retention of these compounds.

CONCLUSION

SFC of polar compounds using polar packed columns needs a polar modifier to the carbon dioxide supercritical fluid. Examples of separations of three classes of compounds have been shown. LSD afford a universal detection method that is compatible with SFC using a polar modifier, and opens up a new field of potential applications of such analyses.

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