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Note

Solid sample introduction in preparative high-performance liquid chromatography: separation of diamantanols

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The efficiency of liquid chromatographic separations is partly affected by the method of sample introduction into the column. The usual techniques for injecting dilute samples into analytical high-performance liquid chromatographic (HPLC) columns are by using an injection syringe or by using a loop injector or another type of injection device[1,2]. Also, in preparative liquid chromatography, the injection of a sample solution is used almost exclusively.

The most popular technique in HPLC is to dissolve the sample in the mobile phase[3]. Poor solubility of the sample is probably the main limitation to the requirement to introduce a large amounts of the sample into a column. To increase the sample solubility, it is possible to dissolve it in one of the components of the mobile phase or in another solvent with a chromatographic strength identical with that of the mobile phase but with a different dissolving power[3,4]. One should be careful in choosing the solvent, because the efficiency of the separation depends not only on the nature of the sample itself, but also on the presence of the individual components of the mobile phase[5]. In order to obtain the highest separation efficiency, use of the maximum injection volume of a sample of a given concentration in relation to the column parameters and separation conditions is recommended. If the solubility of the sample in the eluent is low, it is advisable to increase the volume of the column or to use recycling[6,7]. The problem of poor sample solubility can be overcome by evaporating the sample solution on a solid adsorbent. The adsorbent with the sample coated on its surface is then placed in the top part of the column[8,9]. Application of this procedure in preparative dry- or slurry-packed or radially compressed HPLC columns is complicated. It is possible to use some kind of pre-column packed with materials containing samples coated on the surface. The top part of the axially compressed column is easily accessible by disconnecting the upper frit, so that solid sample introduction can be effected simply[10,11].

In this paper, an example of the direct introduction of a solid sample of diamantanols into a preparative HPLC column with an axially compressed bed is described.

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EXPERIMENTAL

Synthesis of mixed diamantanols

A mixture of 1- and 4-diamantanols was prepared according to McKervey and co-workers[12,13] by isomerization of tetrahydrobinor-S in chlorosulphonic acid, using aluminium chloride as the catalyst. The reaction mixture containing 1- and 4-chlorodiamantanes and a small amount of diamantane was hydrolysed with hydrochloric acid in dimethylformamide, yielding a mixture of 1-diamantanol (38.4%), 4-diamantanol (57.5%) and diamantane (4.1%).

Separation of 1- and 4-diamantanol

The mixture of diamantanols was separated into individual compounds on a Chromatospac Prep 100 preparative chromatograph (Jobin Yvon, Longjumeau, France). A 200-g amount of silica gel of irregular shape (particle size 10–20 μm) was packed into a column of 40 mm I.D.; the height of the bed was 270 mm. The adsorbent was obtained from silica gel L-40 (Lachema, Brno, Czechoslovakia) by screening on a Zig-Zag classifier (Alpine, Augsburg, G.F.R.), washing with methanol and drying at 200°C for 5 h before use.

n-Pentane (Reahkim, U.S.S.R.) containing 6, 4 and 2% (w/w) of 2-propanol was used as the mobile phase. The mobile phase was degassed before use by connecting its reservoir to vacuum for 10 min. The flow-rate of the mobile phase was 22–25 ml/min at a pressure of about 200 kPa. A refractive index (RI) detector (Varian, Palo Alto, CA, U.S.A.) was used.

Analysis of fractions

Gas–liquid chromatographic (GLC) analysis of fractions obtained by preparative separation was carried out on a Chrom 4 gas chromatograph (Laboratory Instruments, Prague, Czechoslovakia) at 190°C. The column (1200 × 3 mm I.D.) was packed with 3% of XF-1150 (Applied Science, Oud-Beijerland, The Netherlands) on Chromaton N AW DMCS (Lachema). HPLC analysis was carried out on a Varian 8500 instrument with an RI detector. The column (250 × 4 mm I.D.) was packed with Silasorb, 8 μm (Lachema). The mobile phase was *n*-heptane–2-propanol (96:4, w/w) at a flow-rate of 1 ml/min.

Sample introduction

A 2–10-ml volume of sample solution (50 mg/ml in isooctane containing 6% of 2-propanol) was injected into the column using a sample reservoir.

Introduction of a solid sample into the column was performed as follows. After preparation of the column by axial compression the pressure was reduced to zero. The top fritted disc of the column was removed from the column and a approximately 1-cm thick silica layer was removed. The sample was placed in a mortar, powdered and mixed with twice the amount of silica and few millilitres of mobile phase. The mixture was homogenized and ground thoroughly, yielding a thick slurry, which was poured into the empty space on the top of the column. The top fritted disc was fitted and the column was compressed again.

RESULTS AND DISCUSSION

The purities of the separated components obtained under different conditions (amount of sample introduced, method of sample introduction, content of 2-propanol in the mobile phase) are given in Table I. Higher purities and higher yields of the separated compounds were achieved when solid sample introduction was used. This method is the most suitable for the purification of the first eluted component of the mixture, as it is nearly always of the highest purity (minimum 98 %). In the separation of a larger amount of sample in one run, it is necessary to collect the second compound later, at about 50 % of the peak height because of contamination of the second compound by the first component. Typical chromatograms of the preparative separation of 1- and 4-diamantanol are presented in Fig. 1.

TABLE I

PURITY OF SEPARATED FRACTIONS

Concentration of 2-propanol in n-pentane in mobile phase (%)	*Amount introduced*		*Purity (%)*	
	In solution	*Solid*	*1-Diamantanol*	*4-Diamantanol*
6	0.1 g/2 ml		98	98
	0.2 g/4 ml		98	98
	0.4 g/8 ml		98	80
	0.5 g/10 ml		90	75
		0.3	98	98
		0.5	98	90 98 (30)*
		1.0	98	80 98 (60)
4		0.5	98	98
		1.0	98	90 98 (15)
		1.5	98	80 98 (40)
		2.0	98	75 90 (50)
2		0.5	98	98
		1.0	98	98
		1.5	98	95 98 (30)
		2.0	98	90 98 (50)
		3.0	98	75 85 (60)
		5.0	See Fig. 2	

* Values in parentheses are percentages of 4-diamantanol peak height when collection of fraction was started.

More detailed information on separation in a heavily overloaded column was obtained by solid sample introduction of 5 g of a mixture of diamantanols. The narrow fractions were collected and analysed. A chromatogram of this preparation and the analyses of some collected fractions are presented in Fig. 2. All fractions obtained from the first peak contain 1-diamantanol only. The concentration profile of the contamination of the second component (4-diamantanol) by 1-diamantanol is also shown.

This kind of overlapping of two separated peaks, when the first component is

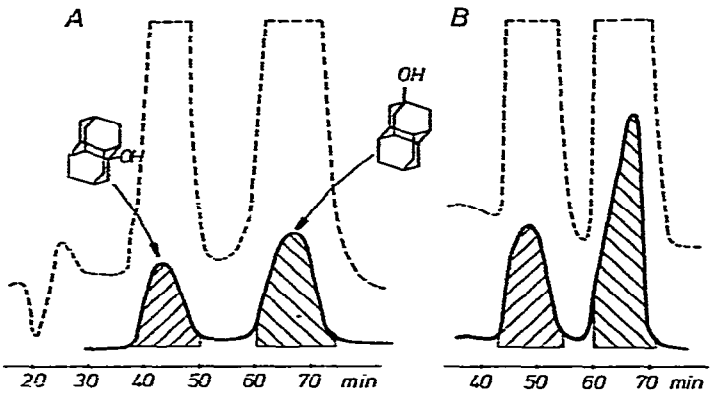

Fig. 1. Preparative chromatograms of separation of 1- and 4-diamantanol. Column, 270 × 40 mm I.D., packed with irregular silica (10–20 μm); mobile phase, *n*-pentane–2-propanol (98:2, w/w); flow-rate, 22 ml/min; detection, RI detector. Amounts introduced in solid state: A, 1 g; B, 2 g.

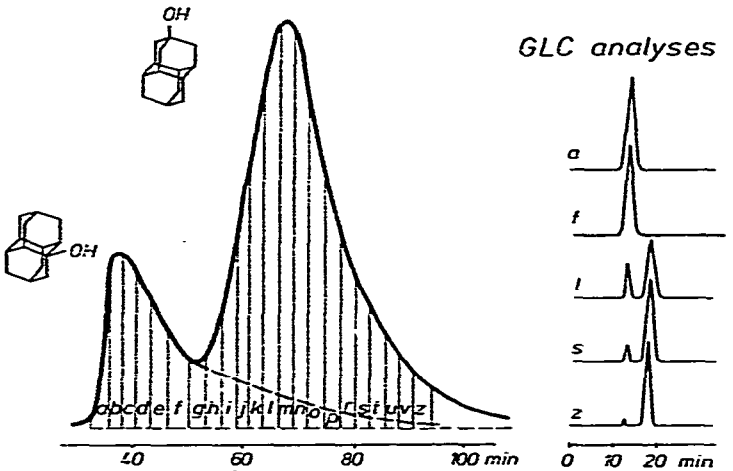

Fig. 2. Preparative separation of a mixture of diamantanols and GLC analyses of some fractions. Preparative conditions as in Fig. 1. Amount of sample introduced in the solid state, 5 g. Analytical conditions as in Fig. 3B.

pure while the second is more or less contaminated by the earlier eluted compound, differs from the "symmetrical separation" published previously[3]. The questions remain of what the effect of the non-linear adsorption isotherm is and what the contribution of the solid sample introduction method itself is.

The influence of the mobile phase composition on the separation of diamantane derivatives was studied earlier[14]. It was found that the separation selectivity of 1- and 4-diamantanol is higher when *n*-heptane–diethyl ether ($\alpha = 3$) instead of *n*-heptane–2-propanol ($\alpha = 2.1$–2.4) is used as the mobile phase. In our work, *n*-pentane–2-propanol instead of *n*-heptane–diethyl ether was used as the mobile phase, for two reasons: the regeneration of an *n*-pentane–2-propanol mixture is very simple, and the efficiency of separation is higher when *n*-heptane is replaced with *n*-pentane[15].

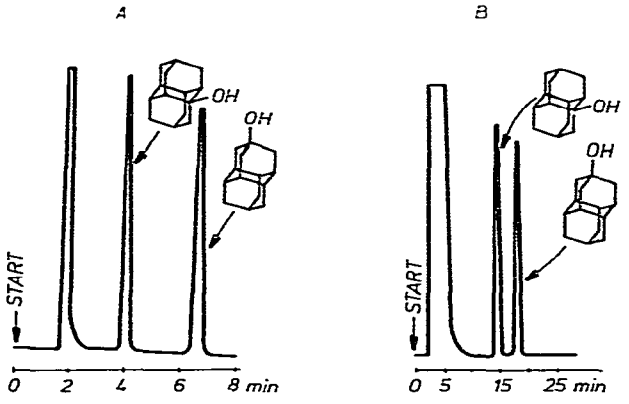

Fig. 3. Analytical chromatograms of diamantanols. (A) HPLC separation. Column, 250 × 4 mm I.D., packed with Silasorb, 8 μm; mobile phase, *n*-heptane–2-propanol (96:4, w/w); flow-rate, 1 ml/min; detection, RI detector. (B) GLC separation. Column, 1200 × 3 mm I.D., packed with Chromaton N AW DMCS coated with 3% XF 1150; temperature, 190°C.

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