

CHROM. 11,647

Note

Separation of cyclodextrins by high-performance liquid chromatography

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(Received November 28th, 1978)

Several chromatographic methods have been described for the separation of cyclodextrins¹ (cycloamyloses consisting of α -1–4 linked glucose units): partition chromatography on cellulose², adsorption chromatography on charcoal³ and Sephadex G-15⁴, as well as gel chromatography on polyacrylamide^{5,6}. Recently, we published a method suitable both for analytical and for preparative separation of α -, β - and γ -cyclodextrin (α -CD, β -CD and γ -CD, consisting of six, seven and eight glucose units, respectively)⁷.

However, there is no high-performance method for rapid chromatographic analysis of samples containing α -CD, β -CD and γ -CD. On the basis of our renewed investigations summarized below, we found high-performance liquid chromatography (HPLC) on μ Bondapak-carbohydrate column packing to be very suitable for this purpose.

It is to be noted that some oligosaccharides were determined recently by HPLC on μ Bondapak-carbohydrate using β -CD as an internal standard⁸.

EXPERIMENTAL

The α -CD, β -CD and γ -CD were the products of Chinoin (Budapest, Hungary).

Liquid chromatography was performed using a Waters Model ALC/GPC 201 chromatograph equipped with an U6K injector, a R 400 differential refractometer and a column (30 cm \times 4 mm I.D.) packed with μ Bondapak-carbohydrate (μ -silica with an amine functional group) and thermostatted at 25°. All this apparatus was obtained from Waters (Königstein, G.F.R.).

The sample solution contained α -CD, β -CD, γ -CD (each 10 mg/ml) and glucose as an internal standard (5 mg/ml) in distilled water. Acetonitrile-water mixtures (25–35 vol. % water) were used for the elution.

RESULTS

Acetonitrile–water mixtures (25–35 vol.% water) were found to be very suitable solvents for developing the chromatograms of cyclodextrins. Fig. 1 shows how the retention factors, $k' = (t_R - t_0)/t_0$, where t_R = retention time of a given peak and t_0 = dead time, depended on the composition of this eluent.

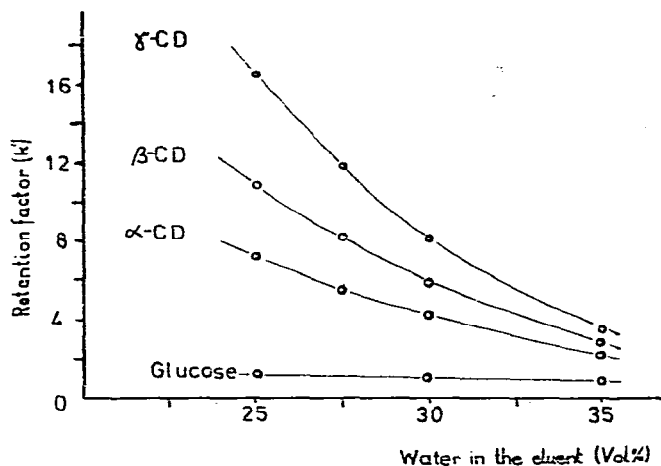


Fig. 1. Plot of the retention factors (k') of cyclodextrins (α -CD, β -CD, γ -CD) and glucose vs. the concentration (vol.%) of water in the acetonitrile–water eluent. Column: 30 cm \times 4 mm I.D., packed with μ Bondapak–carbohydrate. Flow-rate, 2 ml/min; temperature, 25°.

The retentions of α -CD, β -CD and γ -CD as well as of the internal standard glucose significantly differed from each other and samples containing these components could be effectively separated under the conditions used. As may be deduced from Fig. 1, the selectivity can be altered (increased or reduced) by changing the composition of the acetonitrile–water eluent.

For example, Fig. 2 shows a chromatogram illustrating the high resolution and

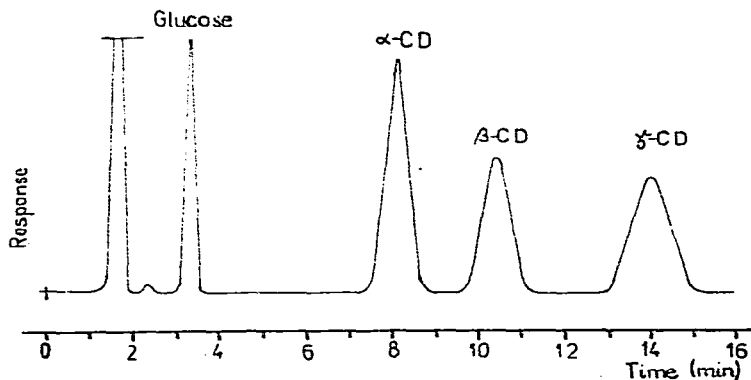


Fig. 2. A chromatogram illustrating the separation of cyclodextrins (α -CD, β -CD, γ -CD) and glucose by HPLC on μ Bondapak–carbohydrate. Column, flow-rate and temperature as in Fig. 1. Detector: differential refractometer. Eluent: acetonitrile–water (70:30, v/v). Sample size: 15 μ l injector volume, dissolved in water

rapid separation of cyclodextrins and glucose by HPLC on μ Bondapak-carbohydrate column packing. With the appropriate calibration, the chromatograms could also be evaluated quantitatively on the basis of the peak areas.

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