

Chromatographic separation of 17-hydroxycorticosteroids on Sephadex LH-20

It has been reported that sugars¹ and some organic acids² can be separated by partition chromatography on ion exchange resins, and the use of a moving phase with a lower polarity has permitted the separation of 17-hydroxycorticosteroids on cation exchange resins, Dowex 50W-X4³ and partially esterified Amberlite IRC-50⁴.

It has also been shown by NYSTROM AND SJÖVALL⁵ that methylated Sephadex can be used as the stationary phase for the separation of steroids. It was suggested by these authors that this separation was based partly on liquid-liquid partition between a mobile phase and a stationary gel-solvent phase. A hydrophobic gel resembling methylated Sephadex is now available commercially under the name of Sephadex LH-20, and its use for the separation of steroid sulfate has been reported⁶.

In the present paper, the successful separation of some 17-hydroxycorticosteroids is described utilizing Sephadex LH-20 as the stationary phase and a less polar solvent mixture (ethanol-benzene-*n*-hexane-water, 50:350:80:3.3, by volume) as the moving phase.

Sephadex LH-20 was classified by the sedimentation method⁷ in 30% aqueous ethanol and the particles of 40-90 μ were collected. They were first washed with 10 volumes of 99% ethanol and then with 20 volumes of the moving phase. The washed Sephadex was suspended in two volumes of the moving phase and poured into a chromatographic tube and allowed to settle. After about 200 ml of the eluent has been passed through, the column was ready for use.

The sample was dissolved in 0.5 ml of a mixture of ethanol, benzene and water (30:210:1, by volume) and then 0.2 ml of a mixture of *n*-hexane and carbon tetrachloride (5:1, by volume) was added with mixing. This solution was applied to the column and overlaid carefully with the eluent and elution was performed with the same eluent. The effluent was collected in fractions of 20 drops in test tubes using a drop count type automatic fraction collector. 17-Hydroxycorticosteroids were analyzed by the Porter-Silber reaction or by the ultraviolet absorption at 240 m μ . Each

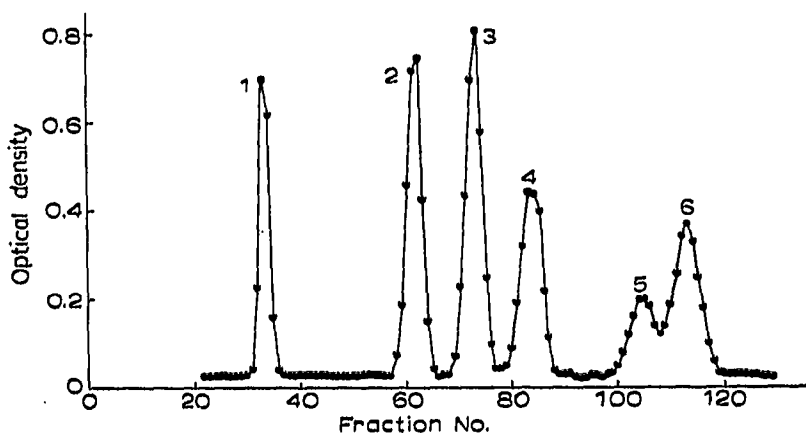


Fig. 1. Elution of standard samples of various 17-hydroxycorticosteroids. The compounds in the order of their elution from the column are: 17 α ,21-dihydroxypregn-4-ene-3,11,20-trione (1), 11 β ,17 α ,21-trihydroxypregn-4-ene-3,20-dione (2), 3 α ,17 α ,21-trihydroxy-5 β -pregnane-11,20-dione (3), 6 β ,17 α ,21-trihydroxypregn-4-ene-3,20-dione (4), 3 α ,11 β ,17 α ,21-tetrahydroxy-5 α -pregnan-20-one (5) and 3 α ,11 β ,17 α ,21-tetrahydroxy-5 β -pregnan-20-one (6). Column size: 0.5 \times 60 cm. Fraction size: 20 drops. Flow rate: 3 fractions per hour.

fraction was allowed to evaporate at room temperature and the residue was dissolved in 2 ml of ethanol for measurement of ultraviolet absorption. For estimation of these steroids with Porter-Silber reagent, the residue was dissolved in 0.5 ml of 60% aqueous ethanol and then 1.0 ml of Porter-Silber reagent was added and mixed. The mixture was allowed to stand at room temperature for 15 h and the optical density was measured at 410 m μ . The recovery of steroids was 75 to 80%. The elution pattern is shown in Fig. 1. The column could be used repeatedly.

Since the solutes distributed themselves between the less polar outer liquid phase and the more polar swollen stationary phase which is in equilibrium with the liquid phase, the elution sequence of 17-hydroxycorticosteroids resembled that in straight phase partition chromatography. This type of partition chromatography, which utilizes synthetic cross-linked polymer of medium polarity as the stationary phase and a less polar solvent mixture consisting of smaller amounts of polar components (e.g., ethanol and water) and larger amounts of less polar components (e.g., benzene and *n*-hexane) as the moving phase, seems to be useful for the separation of neutral organic compounds of moderate and higher polarity.

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The prediction of R_F values

Several theoretical studies have appeared in the chromatography literature. A large number of specific solutions to particular problems are also available. Very few studies have been made using both approaches simultaneously. A plea is made here for more studies applied to the practical problems of the selection of solvents, the determination of the conditions of chromatography, and the presentation of data.

Chromatography is essentially a stochastic process and the statistical knowledge of estimation and prediction could be applied with success here. The aim of this investigation is to show the possibilities of predicting the R_F value of a compound, given a set of conditions.

The physical factors influencing R_F values have been investigated quite extensively on filter paper¹⁻⁴. CONSDEN, GORDON AND MARTIN⁴ called attention to the factors paper, temperature, quantity of material, extraneous substances, degree of

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