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Separation of Bile Acids with Reversed Phase Partition Chromatography

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In connection with an investigation of the intermediate metabolism of bile acids a method was needed to separate the bile acids quantitatively on a small scale. In earlier work chromatography of different types of esters of bile acids on alumina have been used [1–4].

The excellent method of Howard and Martin [5] for the separation of higher fatty acids by "reversed-phase partition chromatography" prompted us to try to find a solvent system suitable for the separation of the bile acids with this technic. The hydroxylated bile acids are too insoluble in the stationary phases that gave the best results with the higher fatty acids.

The best results have so far been obtained with chloroform containing ten per cent heptane as the stationary phase and aqueous methanol as the descending phase. The addition of heptane increased the stability of the columns, which can be used for several consecutive runs.

The columns contained 4.5 g hydrophobic Hyflo-Supercel to which 4 ml of the stationary phase was added. The material was then slurried in the aqueous phase giving a column of the approximate dimensions 12 × 110 mm.

A typical separation of a mixture of cholic, deoxycholic and lithocholic acid in 58 per cent methanol is shown in figure 1. The acids appear in the effluent in the order mentioned and at the same place as when each acid was run alone.

Decreasing the methanol concentration from 58 to 55 per cent with an unchanged stationary phase delayed the appearance of the band of deoxycholic acid from 38 ml to approximately 50 ml (*cf.* Figs. 1 and 2).

Increasing the proportion of heptane in the stationary phase or increasing the strength of the aqueous methanol resulted in an earlier appearance of the acids.

The effect of varying the amounts of bile acids used on the same column is exemplified in Figure 2. The cholic acid band appeared at the same place

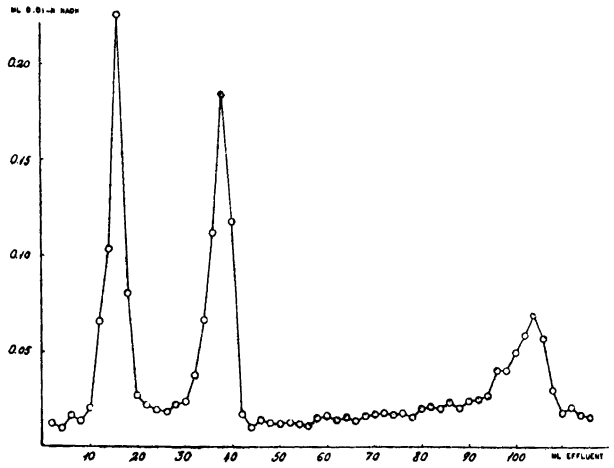

Fig. 1. The separation of a mixture of cholic acid (2.53 mg), deoxycholic acid (3.01 mg) and lithocholic acid (2.56 mg). Stationary phase: 4 ml chloroform-heptane (9 + 1) supported on 4.5 g hydrophobic kiselguhr. Moving phase: 58 % (%$_v$) aqueous methanol.

(15—20 ml) when varying the amount between 2 and 20 mg and the deoxycholic acid appeared at approximately the same place irrespective of this variation in the amount of cholic acid.

This method will thus be easily adaptable for many preparative problems when it is desirable to separate and identify the different components of a mixture of bile acids. As the recoveries are generally about 70—90 per cent the method can also be used to determine the composition of mixtures in a semiquantitative way.

The separation of other bile acids is being studied.

EXPERIMENTAL

The columns were prepared essentially as described by Howard and Martin. The kiselguhr (Hyflo-Supercel, Johns Manville Co.) was made hydrophobic by storage in a dessicator containing dimethyl-dichlorosilane or a lowboiling mixture of chlorosilanes (British Thomson-Houston Export Co., Ltd., Rugby). It was then washed with methanol, dried at 100° and stored in a closed vessel. The two phases were saturated with one another by shaking in a separatory funnel. — All work was done at room temperature (18—20°).

For analytic runs 4 ml of the stationary phase was added to 4.5 g of hydrophobic kiselguhr in a 50 ml Erlenmeyer flask and worked with a spatula until

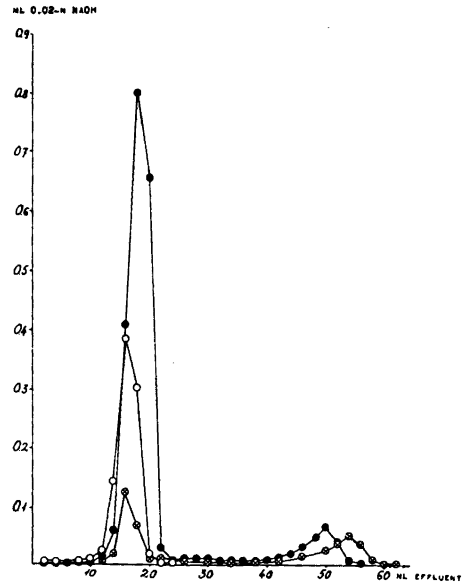

Fig. 2. Chromatography of mixtures of cholic and deoxycholic acid.

1. × — × *cholic acid 2.69 mg, deoxycholic acid 2.95 mg.*
2. ○ — ○ » » *9.50* » » » — »
3. ● — ● » » *20.0* » » » *2.30* »

Stationary phase: see fig. 1. Moving phase: 55 % (%v) aqueous methanol.

homogeneous. Enough of the other phase was then added to make a slurry that could be poured into the chromatographic tube, i.d. 12 mm, height about 400 mm with a narrow tube with a stop cock at the lower end. The slurry was homogenized with a plunger and then allowed to settle under weak suction until the height was about 10—12 cm.

When the column was almost dry at the top, the suction was disconnected and the substance to be tested, dissolved in 2—4 ml of the moving phase, was carefully added. When the solution had disappeared into the column the wall of the tube was washed twice with one ml of the solvent.

The moving phase was then added to a height of about 20 cm above the top of the column and maintained there with a separatory funnel with the tip of the stem at this height.

The rate was then regulated to about 2 ml per 10 minutes with the stopcock of the column.

The effluent was either collected and titrated as described by Howard and Martin [5] or alternatively collected in test tubes with an automatic fraction collector. After addition of bromthymol blue the contents of the tubes were then titrated in the tubes with a Rehberg microburette with a slow stream of nitrogen bubbling through the solution. With appropriate illumination the latter simple and time saving method was found to give as good results as the former one.

SUMMARY

A method for the separation of cholic, deoxycholic and lithocholic acids by reversed phase partition chromatography has been described.

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