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PAPER CHROMATOGRAPHY OF BILE ACIDS¹

by

David Kritchevsky and Martha R. Kirk

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The separation of bile acids by paper chromatography has been investigated in this laboratory and two solvent systems which give different, reproducible R_f values for several acids have been found. The two systems are n-propyl alcohol-ammonia-water 90:2:8 and n-propyl alcohol-ethanolamine-water 90:5:5. Of the two systems, the latter concentrates the moving material into a smaller area and is, therefore, preferable for identification or separation.

Using this solvent mixture, we have been able to achieve separation of various mixtures of these bile acids. Although the R_f values of dehydrocholic, cholic, and norcholic acids are close together, we have been able to separate mixtures of desoxycholic, dehydrocholic, and cholic acids, and of desoxycholic, dehydrocholic, and norcholic acid. In these experiments we have generally observed two distinct spots of the two acids whose R_f values are close together; in some cases, however, they merge to give one spot. All experiments were carried out using 50 γ of material; mixtures contained 50 γ of each component.

For identification of the bile acids, a 15% phosphoric acid spray, slightly different from that originally proposed by Neher and Wettstein²,

(2) R. Neher and A. Wettstein, *Helv. Chim. Acta*, 34, 2278 (1951)

was used. The acids appeared as brown or red spots in white light, or displayed a greenish-yellow or pink fluorescence in ultra violet light,

The results are tabulated below:

TABLE I
R_f VALUES FOR BILE ACIDS

	P-M-W* 90:5:5	P-A-W 90:2:8	E-A-W 90:2:8	P-A-W 5:2:3
Desoxycholic Acid	0.92	0.74	0.66	0.95
Dehydrocholic Acid	0.65	0.47	0.65	0.89
Cholic Acid	0.71	0.52	0.71	0.94
Norcholic Acid	0.69	0.51	0.70	0.94
Triformyl Norcholic Acid	0.92	0.68	0.75	0.94

* P = n-Propyl Alcohol; M = Monoethanolamine; A = Ammonia; W = Water

TABLE II
SEPARATIONS

<u>Mixture</u>	<u>R_f Values</u>
Desoxycholic/dehydrocholic/cholic	0.95/0.65/0.72
Desoxycholic/dehydrocholic/norcholic	0.92/0.62/0.73

EXPERIMENTAL

The organic solvents were distilled prior to use. All mixtures are by volume as given. Whatman #1 paper was used throughout.

The material to be chromatographed was applied to a spot about 2 cm. in diameter on a 4 by 50 cm. strip of filter paper. Descending chromatography was used and after the solvent front had advanced 25-35 cm. from the origin, the strips were removed from the chromatographic chamber (a 7 by 50 cm. test tube) and air

dried. Prior to spraying, the strips were dried at 80° C. for 15 minutes. The spray solution was prepared by mixing 10 parts of 85% phosphoric acid with 25 parts each of water and 95% ethanol. After the papers were sprayed, they were kept at 90° C. for 20 minutes. Generally, cholic and norcholic acids showed up as red or brick colored spots and occasionally one of the other acids appeared as a red spot. In ultraviolet light (Model SL Mineralight, Ultra-Violet Products, Inc., South Pasadena, California) desoxycholic acid exhibited a pink fluorescence and the other acids exhibited a greenish-yellow fluorescence. When larger quantities of these acids were used (100 - 200 γ) they all gave colored spots in white light as well as appearing more readily in the ultraviolet.

The R_f values were measured from the foremost point of the origin to the leading edge of the spot. The solvent mixtures which included ammonia tended to give some streaking, whereas with ethanalamine spots about 15 mm. in diameter were obtained.

All R_f values represent the average of a number of experiments.

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