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Short Communications

Separation of Bile Acids by Paper Chromatography. Bile Acids and Steroids. 3.

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The methods used for the separation of steroids by paper chromatography [1–7] are not suitable for bile acids. The separation of bile acids by reversed phase partition chromatography on columns has recently been developed by Bergström and Sjövall [9,10] with aqueous methanol and chloroform-heptane as phases, whereas Ahrens and Craig [8] have used acetic acid and isopropyl ether-heptane in a counter current extraction procedure.

We have now developed a method with similar solvent systems based on the observation that acetic acid is so firmly bound to filter paper that it can serve as stationary phase. Fig. 1 shows the separation of taurocholic, glycocholic, cholic, hyodesoxycholic and desoxycholic acid. Chenodesoxycholic acid cannot be clearly separated from desoxycholic acid with this method. The specificity of the spraying reagents is shown in Table 1.

In a paper that just appeared Kritchevsky and Kirk [17] report the separation of bile acids by paper chromatography in alkaline media.

Experimental. Solvents: Stationary phase: 70 % acetic acid; moving phase: isopropyl ether: heptane, 7 : 3. The phases are saturated with each other before use.

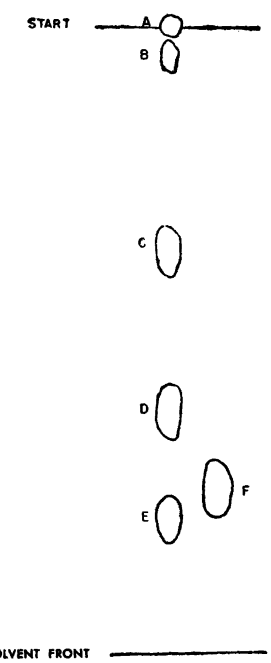

Fig. 1. Separation of taurocholic (A), glycocholic (B), cholic (C), hyodesoxycholic (F) and desoxycholic acid (E). Chenodesoxycholic acid (F) is run separately.
Paper impregnated with 70 per cent acetic acid. Moving phase: isopropyl ether-heptane 7 : 3 saturated with 70 per cent acetic acid.

The filter paper to be used for the chromatography (Whatman No. 1, cut in strips 9 × 40 cm) is wetted with the stationary phase. It is then dried at 90° C for 5 minutes. 25—50 μg of each acid is put on the starting line on an area with a maximum diameter of 0.5 cm. The paper is left

Table 1. Spraying reagents used for the detection of the bile acids.

Acid	Phosphomolybdic acid [11]	Rhodamine B [12]	Phosphoric acid [13]	m-dinitrobenzene [14]	Chlorine+KI+starch [16]	KI+I [15]
Cholic acid	+ + +	+	+ +	−	−	+ +
3, 7-dihydroxy-12-ketocholanic acid	+	+	−	−	−	−
3-hydroxy-7.12-diketocholanic »	−	+	−	−	−	−
3, 7, 12-triketocholanic »	−	+	−	+ +	−	−
Desoxycholic acid	+ +	+	−	−	−	−
3-hydroxy-12-ketocholanic acid	−	+	−	−	−	−
3, 12-diketocholanic »	−	+	−	+ +	−	−
Chenodesoxycholic acid	+ + +	+	−	−	−	−
3-hydroxy-7-ketocholanic acid	−	+	−	−	−	−
3, 7-diketocholanic »	−	+	−	+ +	−	−
Hyodesoxycholic acid	+ +	+	−	−	−	−
Lithocholic acid	−	+	−	−	−	−
3-ketocholanic acid	−	+	−	+ +	−	−
Taurocholic acid	+ + +	−	+ +	−	+ +	−
Glycocholic acid	+ + +	−	+ +	−	+ +	−

in the chromatographic chamber for 2 hours in an atmosphere saturated with vapors from both phases. The vapor equilibrium must be complete before the paper is brought in contact with the moving phase if tailing is to be avoided. Descending chromatography is used. When the chromatogram has been run it is dried at 90° C and the spots developed by some of the methods listed in Table 1.

Spraying reagents *cf.* Table 1.

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