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Application of Glass Fiber Chromatography for Separation of Some Neutral and Phenolic Steroid Sulfates[1]

Our continued interest in the metabolism of steroid sulfates led to the development of a rapid technique for the separation of dehydroepiandrosterone sulfate and androstenediol 3-sulfate on silica gel impregnated glass fiber sheets (ITLC-SG, Gelman Instrument Company). The chromatographic behavior on ITLC-SG of several other steroid sulfates was also determined.

Steroid sulfates were extracted from tissue fractions as described earlier (1). The final residue, dissolved in absolute methanol, was applied as 0.05 ml aliquots at 0.5 cm intervals. The chromatographic procedure was similar to that described for free steroids (2), using two developments with chloroform/acetone/acetic acid 110/35/6 (v/v). It took 25 min for the solvent front to travel 16 cm. Standards were detected either by spraying with methylene blue reagent as described by Crepy *et al.* (3), except that the reagent was not diluted with acetone, or by spraying with sulfuric acid/ethanol 1/1 (v/v) and heating for 15 min at 110°C. Detection and elution of radioactive samples were performed as described (2). Steroid sulfates could be solvolyzed directly on the ITLC-SG sheets by exposing them to an atmosphere of HCl/dioxane 90/10 (v/v) for 3 hr (4) and the resulting free steroids eluted for further studies.

As is shown in Table 1 all of the neutral 17-hydroxy-3-monosulfate

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TABLE 1
Chromatographic Behavior of Steroid Sulfates on Silica Gel Impregnated Glass Fiber Sheets [a,b]

Systematic name	Abbreviation	R_s[c]	Mobility, cm
Estra-1,3,5(10)-trien-17-one-3β-yl sulfate	Estrone-S	1.00	11.6
Estra-1,3,5(10)-trien-17β-ol-3β-yl sulfate[d]	Estradiol-3-S	0.80	9.3
Estra-1,3,5(10)-trien-3β,17β-yl disulfate	Estradiol-3,17-S	0.00	0.0
Estra-1,3,5(10)-trien-16α-ol-17β-ol-3β-yl sulfate[d]	Estriol-3-S	0.18	0.26
Androst-5-en-17β-ol-3β-yl sulfate	Androstenediol-3-S	0.70	8.1
Androst-5-en-17-one-3β-yl sulfate	Dehydroepiandrosterone-S	0.89	10.3
5α-Androstan-17β-ol-3β-yl sulfate	Androstanediol-3-S	0.72	8.3
5α-Androstan-17-one-3β-yl sulfate	Epiandrosterone-3-S	0.91	10.6
Pregn-5-en-17α-ol-20-one-3β-yl sulfate[e]	17α-OH Pregnenolone-3-S	0.73	8.5
Pregn-5-en-20-one-3β-yl sulfate[e]	Pregnenolone-S	0.96	11.1
Androst-4-en-3-one-17β-yl sulfate	Testosterone-S	0.72	8.4

[a] Silica gel impregnated glass fiber sheets, Gelman Instrument Co.

[b] Solvent: chloroform/acetone/glacial acetic acid 110/35/6 v/v. Two developments.

[c] Reference compound = estrone-S.

[d] We are indebted to H. Fex, A. B. Leo, Halsingborg, Sweden, for a gift of these estrogen sulfates.

[e] These steroid sulfates were kindly furnished by Dr. Robert Jaffe.

steroids are well separated from their 17-keto analogs or from pregnenolone-S. The tailing observed with 17α-OH-pregnenolone-3-S by Pierrepoint (5), using thin-layer chromatography, did not occur in our studies.

The method is also applicable to the separation of the 3-monosulfate esters of estrone, estradiol, and estriol as well as the disulfate ester of estradiol. The latter remained at the origin. Free steroids moved with the solvent front and therefore any traces of these that might have remained in the butanol extract were thus separated from the sulfate esters.

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