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Letter to the Editor

Reversed-phase thin-layer chromatography of estrogen glucuronides

Sir,

Present methodology for separation of estrogen conjugates has not resulted in routine separation of estriol-3-glucuronide and estriol-16-glucuronide [1]. Considerable literature on these methodologies is available. Even present reports of results of high-performance liquid chromatography do not appear to advocate a method for routine use.

During a study of the urinary excretion of estrogen conjugates, a rapid method for separating the glucuronides was developed. Previous gas chromatographic methods required hydrolysis prior to analysis [2]. Radioimmunoassay methods [3], though very sensitive, would detect a single compound per analysis. The one-dimensional thin-layer chromatographic method reported resolves six estrogen conjugates on reversed-phase layers, either C_8 or C_{18}. With this method, the disposal problems of radiolabelled material and the hydrolysis prior to analysis were eliminated. We compare the chromatography of six estrogen conjugates with two mobile phases.

EM Science (Gibbstown, NJ, U.S.A.) or Whatman (Clifton, NJ, U.S.A.) reversed-phase layers, 10 × 10 cm, 250 μm thickness, were washed with methanol prior to use. Synthetic reference compounds obtained from Sigma (St. Louis, MO, U.S.A.), dissolved in ethyl acetate, were applied to the layers, 50—2000 ng per sample. After development, with the mobile phases listed in Table I, plates were air dried for 5 min. For visualization, the developed plates were sprayed with 10% concentrated sulfuric acid in ethanol, and heated at 120°C for 5 min. Chromatograms were scanned with a densitometer (Shimadzu CS910, Columbia, MD, U.S.A.), equipped with a mercury lamp to detect the fluorescence when excited at 366 nm, using a 509-nm cut-off filter.

The R_F values found for the various estrogen glucuronides are listed in Table I. As shown, both the C_8 and C_{18} layers effected satisfactory separations. Use of the 50:50 mobile phase resulted in better resolution of the conjugates than the 45:55 solvent mixture. Separation time was also shorter with it. The sensitivity of this fluorescent assay was below 50 ng per sample. Linear detector response extended to beyond 2000 ng. With this sensitivity, 100-μl

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TABLE I

MOBILITY OF ESTROGEN CONJUGATES

The mobile phase was methanol—0.5% tetramethyl ammonium chloride in each case.

Compound	$R_F \times 100$			
	C_8 Layer		C_{18} Layer	
	50:50	45:55	50:50	45:55
Estrone-3-sulfate	3	2	3	2
Estradiol-17β-glucuronide	16	4	11	6
Estrone-β-glucuronide	34	8	24	8
Estriol-16α-glucuronide	42	12	32	12
Estriol-3-glucuronide	62	34	66	29
Estriol-3-sulfate	49	27	48	24

urine samples could be analyzed routinely from pregnant or non-pregnant subjects.

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