

unidentified amino compounds⁸. Moreover, the sensitivity of cystine to slight changes in buffer pH^{4,9} also applies to the other diamino-dicarboxylic acids mentioned. Accordingly, workers interested in definitive separation of compounds from this area of the ion-exchange chromatogram would do well to consider a form of gradient elution².

The separation of *meso*- from DL-cystine has been noted^{10,4}. However, it was of interest that *meso*- and DL-homocystine are not separated. It was interesting that the addition of one methylene group to just one side of the —S—S— group as in the asymmetrical disulfide of cysteine and homocysteine also prevents resolution of the optical isomers.

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A rapid paper chromatographic separation of estrogens*

Although there are many methods for the separation of estrogens¹, most procedures have limited applicability because effective separation of the three common estrogens is difficult in a single system or because of the prolonged chromatographic time required to move estriol from the origin. By modifying the well-tested chromatographic systems of ZAFFARONI², it is possible to obtain good separation of the estrogens in a short period of time.

The stationary phase is modified by the addition of formic acid to increase its acidity. Whatman No. 1 filter paper was used; Skellysolve B was substituted for hexane. All solvents were purified by standard techniques and redistilled prior to use. The results are given in Table I.

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TABLE I

Mobile phase	Stationary phase Formamide: methanol: formic acid (v/v/v)	R_F values			
		Estriol	Estradiol	Estrone	Equilenin
Skellysolve B-benzene	1:1:0			0.30	0.22
Benzene	1:1:0	—	0.3	0.65	—
	1:1:0	—	—	0.53	0.50
	1:1:1/2	0	0.13	0.35	—
	1:1:2	0	0.22	0.50	—
Benzene-chloroform	1:1:1	0	0.74	0.95	—
	1:1:1 1/2	0	0.74	0.95	—
	1/2:1:1	0.03	0.43	0.75	—
Chloroform	1:1:1/2	0.1	0.56	0.67	—
	1:1:1	0.02	0.60 ^a	0.60 ^a	—
	1:1:1 + 1				
	part water	0.03	0.67 ^a	0.67 ^a	—

^a R_F values are not precise because a definite separation could not be detected.

Reference to the data confirms that best separations are obtained when the mobile phase is either benzene or chloroform. The addition of formic acid (1/2 part) increased the mobility of the acidic estriol and still permitted adequate separation of the other two common estrogens. The effect of formic acid on the less polar estrogens was not so obvious but the separation of estrone from estradiol is readily accomplished by most systems. No attempt was made to modify the systems for the separation of estrone from equilenin and equilin because it was found that the usual Zaffaroni Skellysolve B-benzene system, when overrun for six hours, resulted in adequate separation. The three steroids moved 19, 13 and 17 cm respectively from the origin.

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