

STUDIES ON THE POLYAMIDE THIN LAYER CHROMATOGRAPHY OF ESTROGENS AND RELATED COMPOUNDS

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I. Migration rates of estrogens and elution capacity of solvents on polyamide thin-layer chromatography

Thin-layer chromatography on polyamide (Nylon 6) was applied to the separation of estrone, estradiol and estriol, by using twenty-three kinds of single solvent and three kinds of binary solvent mixture.

The R_f values of estrogens with a few exceptions depended on hydrogen-bonding parameter (γ values) and solubility parameter (δ values) of the single solvent used. The R_f values became larger with increasing γ values in solvent systems with their values above 5. Solvent systems with both γ and δ values above 10 caused desorption and the thin-layer of polyamide hardly showed separation ability.

For better resolution of estrogens, the suitable developing solvent systems would be protophilic solvents with γ values above 5 or aprotic polar solvents with γ values below 5. A mixture of these or the aprotic polar solvents with an addition of a small amount of protic solvent with δ value above 10 would be also useful.

II. Polyamide thin-layer chromatography of estrogen related compounds and influence of chemical structure on migration rates

Thin-layer chromatography on polyamide (Nylon 6) has been examined for twenty estrogens and its related compounds including hexestrol and diethylstilbestrol by using thirteen kinds of binary solvent systems as the developments. R_f values for six kinds of low polar compounds, such as 3-deoxy- or methoxy-estra-1,3,5(10)-triene derivatives, went above 0.8 by use of the binary developing systems composed of either aprotic solvents and a small amount of methanol, or aprotic solvents and acetone. On the other hand, migration rates of the other hydroxy-compounds of estratriene and stilbene derivatives decreased with the increase of the number of hydroxyl or double bond. The relatively better separation of these compounds could be accomplished with such solvent systems as hexane-acetone (3:2v

/v) and benzene-acetone (3:1).

To investigate the influence of the chemical structure of estrogens on their migration rates, R_m increments (ΔR_m values) were calculated for several functional groups on the basis of the mean R_f values of a pair of estrogens having the common nucleus. The representative ΔR_m values obtained in the specific developing solvents are ; +1.30, +0.82 (2-OH); +1.10 (3-OH); +0.65, +0.55 (16 α -OH); +0.38 (16 β -OH); 0.22 (3-OH \rightarrow 2-OH); +0.38 (16 β -OH \rightarrow 16 α -OH); +0.10 (Δ^7); +0.29 ($\Delta^{6,8}$). From these values, it seems that phenolic and secondary hydroxyl groups mainly contribute to the adsorption of these estrogens on polyamide.

III. The effect of amide group-density of nylon on the polyamide chromatography of estrogens

Three estrogens were developed with protic and aprotic solvent by three Nylon tic plates. The order of R_f values are found to be estrone > estradiol > estriol and Nylon 6 > 66 > 610, for both protic and aprotic solvent systems.

The latter order may be arisen from amide group-density in polyamide which have different hydrophilic and hydrophobic properties. The R_m values of estrogens have a linear relationship with the water contents of Nylon regained by immersing in water and by equilibrating in atmosphere. Liquid chromatography was applied to Nylon 66 and 11. The retention volumes of the estrone and estradiol obtained with the hydrophobic Nylon 11 were bigger than those obtained with the hydrophilic Nylon 66. Using Nylon 11, it was found that estriol had the smallest retention volume of the three estrogens.

The fact that R_f values decreased with more hydrophobic Nylon are contrary to the expected R_f values from the hydrogen bonding of amide group-density in polyamide. So, it was thought that there was an interaction between samples and the hydrophobic methylene chain, the latter's hydrophobicity increased with decreasing amide density.

From the above data, we would point out that apart from hydrogen bonding of polyamide and estrogen, the hydrophobic interaction also plays an important role in polyamide sorption process.

Reference:

- 1) Hsing-Chien Hsiu: JAPNN ANALYST, 20 1026-1031 (1971)
- 2) Hsing-Chien Hsiu: *ibid*, 23 1226-1231 (1974)
- 3) to be published.