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IDENTIFICATION OF SOME SYMPATHOMIMETIC AMINES BY THIN LAYER CHROMATOGRAPHY (TLC)

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ABSTRACT

The sympathomimetic amines were dissolved in 0.1N HCl or ethanol and water, and treated with p-nitrobenzoyl chloride on a thin layer of alumina, silica gel and ITLC fiber paper. Two component solvent systems were used for development. Amines in pharmaceuticals may generally be separated by using two component solvent systems.

INTRODUCTION

An analyst is often faced with the problem of having to choose a paper or thin layer chromatographic system, from the hundreds that have been used, for the identification of basic drugs. The most important features of individual systems to be considered are (a) speed, (b) sensitivity, (c) reproductibility, (d) distribution of chromatographic values over the useful range of the system and (e) correlations between systems when more than one is used. A compromise between all the above factors must be obtained to choose the better systems. In a previous report from this Laboratory (Shah & Shah, 1976) sympathomimetic amines were separated satisfactorily using two and four component solvent systems.

Hanson and Alm (1962) have reported the separation of some diphenylamine derivatives of thin layers of silicic acid. Yasuda (1964) has identified, by two dimensional thin-layer chromatography employing silica gel G., the products from diphenylamine in PBX-9404, as well as those prepared by treatment of diphenylamine and 2-nitro diphenylamine with N_2O_4. The use of glass fibre paper impregnated with 0.1M KH_2PO_4 has also been reported for separation of epinephrine and norepinephrine (Stern, et. al. 1967). For the separation of epinephrine from levarterenol, n-butanol-pyridine-water (4:1:1) has been used. Much better results have been achieved by using hexane-acetone (5:1) after the substances had been acetylated; however, acetylated levarterenol gave two spots.

Phenylethylamine may be detected with isatin as the primary amine group reacts with isatin. The sensitivity of this reaction and the resulting color is substantially influenced by the presence of a hydroxyl group in the aliphatic chain. The color resulting from the ninhydrin reaction is not always more sensitive with a primary amino group than with a secondary amine. For example the primary amines, amphetamine, metamphetamine and tuaminoheptan, do not react with ninhydrin. To detect ephderine, it is necessary to heat the chromatogram to 130-140°C (Hiller, 1961). A similar reaction to that with ninhydrine can be carried out with Folin's Reagent, which does react with amphetamines and results in the formation of differently colored spots with different amines. Sympathomimetic of the primary amine type can also be detected by their reaction with the sodium salt of the 1,2-naphthoquinone sulfonic acid. For compounds containing a secondary amino group, the reaction with dithiocarbamate is recommended.

The purpose of this study was to develop a good solvent component system, a detecting reagent and specific techniques suited for the identification of some sympathomemetic amines. We have developed chromatograms of five sympathomimetic amines with different organic solvent system and with water as the stationary phase. The amines were separated satisfactorily with the two component solvent systems examined (Shah & Shah, 1977).

METHOD

Reagents

(a) *Solvents:* Spectrograde benzene, n-butanol, n-hexane, isopropanol, ethyl acetate, ethanol (Fisher Scientific Co., 1241 Ambassador Blvd., St. Louis, Mo. 63132).

(b) *p-nitrobenzoyl chloride:* 0.4%. Dissolve 400 mg p-nitrobenzoyl chloride (Eastman Kodak Co., Rochester, NY 14650, No p-499) in 10 ml ethyl acetate and dilute to 100 ml with n-hexane.

(c) *Sympatomimetic amine standard solutions:* Weigh 1 gm of each amine (ephedrine-hydrochloride, phenylpropanolamine hydrochloride, chlorpheniramine maleate, pheniramine maleate and pyrilamine maleate) into separate 100 ml volumetric flasks. Add 0.1N HCl and dilute to volume with 0.1N HCl (10mg/ml).

Apparatus

(a) *TLC fiber sheet:* Gelman ITLC fiber SAF (instant thin layer chromatogram, salicylic acid fluorescent media, Gelman Instrument Co., Ann Arbor, MI 48106). Fiber sheet can be used without activation.

(b) *Glass Plates:*
  (1) Precoated glass plates for TLC (EM Laboratories, Elmsford, NY 10523). Silica gel 60F-254, #5775, 20 x 20 cm, 0.25 mm layer thickness.
  (2) Precoated TLC plates—Uniplate Alumina G, 20 x 20 cm, 500 μm thick (Analab, Newark, Del 19711).

(c) *Developing Tanks:* Stainless Steel or Glass, 9 x 9 x 4"

(d) *Ultraviolet (UV) light:* Black-Ray UVL-22 (Ultraviolet Products, Inc., San Gabriel, CA 91778), 115v, 60 cycles, equipped with short and long wavelengths.

Procedure

Apply 5 μl standard solutions on plate and fiber sheet 25 mm from bottom and 40 mm apart (do not touch plate with pipet). Let spot air dry Over each spot apply 5 μl p-nitrobenzoyl chloride reagent and let spot air dry Develop chromatogram sheet to line 10-12 cm above spot Remove chromatogram sheet and dry in 100°C oven ca 1 min Examine derivatives spots (yellow) under short UV light Determine Rf value for each spot

RESULTS AND DISCUSSION

A solution of these five sympathomimetic amines in 0.1N HCl gives one or two spots when treated with p-nitrobenzoyl chloride and chromatographed in the

benzene-ethyl acetate, isopropanol-ethyl acetate and n-butanol-ethyl acetate solvent systems. The p-nitrobenzoyl chloride gives no or one spot in these systems (Number of spots in the TLC experiments depend upon solvent system, unpaired electron in the compound and detecting reagent). Uniplate Alumina G (Analab) was the best plate for the identification of amines in these systems because spots are completely separated without tailing; such is not the case when using ITLC fiber sheet and EM Lab silica gel 60F-254, #5775. Table 1 indicates the R_f values of standards in the two component solvent systems.

CONCLUSION

By using this two component solvent system (benzene + ethyl acetate 20+80), we can identify ephedrine hydrochloride, phenylpropanolamine hydrochloride, chlorpheniramine maleate and pheniramine maleate or pyrilamine maleate from other amine standards.

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TABLE 1. *R_f values of some sympathomimetic amines in two solvent system[a], after treatment with p-nitro benzoylchloride.*

Compound[b]	S_1		S_2	S_3	S_4	S_5*		S_6*		S_7*	
	c	d	c	c	c	e		e		e	
1	0.84	0.13, 0.76	0.50	0.87	0.88	0.41,	0.95	0.34,	0.97	0.39,	0.96
2	0.90	0.14, 0.92	0.57	0.93	0.93	0.51,	0.98	0.39,	0.98	0.46,	0.99
3	0.75	0.13, —	0.51	0.75	0.76	0.09,	0.42	0.07,	0.37	0.38	—
4	0.72	0.13, —	0.44	0.71	0.69	0.09,	0.45	0.07,	0.39	0.37	—
5	0.90	0.06, —	0.74	0.92	0.90	0.13,	0.43	0.10,	0.38	0.34	—
Reagent	—	0.12 —	—	—	—	—	—	—	—	—	—

[a] 30 min developing time.

[b] 1. ephedrine hydrochloride, 2. phenylpropanolamine hydrochloride, 3. chlorpheniramine maleate, 4. pheniramine-maleate, 5. pyrilamine maleate (all standards 10 mg/ml 0.1N HCl).

[c] Uniplate Alumina G (Analab), [d] ITLC fiber sheet, [e] EM lab silica gel 60F-254 (#5775).

Solvent Systems:—S_1 = benzene + ethyl acetate (40+60), S_2 = benzene + ethyl acetate (70+30), S_3 = benzene + ethyl acetate (30+70), S_4 = benzene + ethyl acetate (20+80), S_5 = isopropanol + ethyl acetate (60+40), S_6 = isopropanol + ethyl acetate (30+70), S_7 = n-butanol + ethyl acetate (60+40).

* one hour developing time.

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