JOURNAL OF THE TENNESSEE ACADEMY OF SCIENCE

VOLUME 53, NUMBER 3, JULY, 1978

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

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ABSTRACT

The sympathomimetic amines were dissolved in water and ethanol, and treated with bromocresol green on a thin layer of alumina, silica gel and cellulose plates. Systems with two, three and four solvents were tested. The more polar spots were separated satisfactorily.

INTRODUCTION

Thin Layer Chromatography (TLC) is presently considered one of the most suitable techniques for drug analysis. It is fast, requires minimal equipment, and provides highly reliable results (Stahl, 1973). In addition, TLC is especially powerful because of its high sensitivity and resolution. Adequate separations of submicrogram quantities of material may be accomplished in under half an hour (Clarke, 1969).

In a previous report from this laboratory (Shah & Shah, 1976), sympathomimetic amines were separated satisfactorily using a two-solvent system. Rasin et al. (1969) showed that N-2,4 dinitro-phenylsulfenyl derivatives of some sympathomimetic amines are useful in the separation of the amines. The techniques used were ascending and multiple thin layer chromatography techniques on silica gel G or Al_2O_3 G. The best separation is obtained by multiple chromatography in 1:1 benzene-petroleum ether on silica gel G thin layers. The choices of solvent systems for the development of sympathomimetic amines are rather limited. Only systems containing either n-butanol or phenol are in practical use. These solvent systems are acidified either by acetic acid or by hydrochloric acid (Fabini & Konig, 1958a; Wachsmuth & Van Koeckhonen, 1962; Perry & Schroeder, 1963; Weidner & Weiss, 1958). Those most frequently used for paper chromatography (PC) are n-butanol, water, and acetic acid.

In TLC, the most commonly used sorbents are silica gel and cellulose; solvent systems are analogous to those for PC (Choulis, 1967a; DePotter et al., 1965). Sandri-Cavichi et al. (1966) used these systems for the separation of epinephrine and levarternol and for the separation of other adrenergic drugs as well. Halmekoski (1963) in some cases adds molybdate, tungstate or borate ions to the silica gel layer, an action which results in the marked decrease of mobility of compounds with two hydroxyl groups in the ortho position.

The purpose of this study was to develop systems and specific techniques suited for the identification of

some sympathomimetic amines. Chromatograms of five sympathomimetic amines have been developed with different organic solvents and water as the stationary phase. The amines were separated satisfactorily in the four solvent systems examined (Shah & Shah, 1976).

MATERIALS AND METHODS

Reagents

(a) *Solvents:* Nanograde or spectrograde n-butanol, m-xylene, chloroform, ethanol and n-amyl alcohol (Fisher Scientific Co., 1241 Ambassador Blvd., St. Louis, MO 63132); ACS grade glacial acetic acid.

(b) *Bromocresol green:* 0.01%. Dissolve 19 mg bromocresol green (Matheson, Colmen & Bell, Norwood, OH 45212, No. NB 179) in 100 ml ethanol.

(c) *Sympathomimetic amine standard solutions:* Weigh 1 g of each amine (ephedrine hydrochloride, phenylpropanolamine hydrochloride, pyrilamine maleate, pheniramine maleate and chlorpheniramine maleate) into separate 100 ml volumetric flasks. Add 10 ml water and dilute to volume with ethanol (10 mg/ml).

Apparatus

(a) *TLC plates:* (1) Eastman Chromatogram Sheet, scribed silica gel without fluorescent indicator #13197 (scribed with 1 mm separations), 20 x 20 cm, 100 μm thick and cellulose without fluorescent indicator # 13255, 20 x 20 cm, 160 μm thick (Eastman Kodak, Co., Rochester, NY 14650).

(2) *Precoated TLC plates,* Uniplate alumina G, Avicel and silica gel G, 20 x 20 cm, 500 μm thick (Analab, Newark, DE 19711).

(3) *Precoated TLC plates,* silica gel 60 and silica gel F-254 without fluorescent indicator, 20 x 20 cm, 0.25 mm layer thickness (EM Laboratories, Elmsford, NY 10523).

(b) *Developing tanks:* Stainless steel or Glass, 9 x 9 x 4".

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

Procedure

Apply 5 μl standard solutions side by side on alumina, cellulose or silica gel plates 25 cm from bottom and 40 mm apart (do not touch plate with pipet). Let spots dry. Apply 5 μl bromocresol green over each spot and let spots air dry. Amines are indicated by blue spots on greenish yellow background; spots will turn yellow in acetic acid vapors. Carry out chromatograms at room temperature until the solvent front has advanced 10 cm (30 to 45 min). Remove chromatograms from the solvent and dry in an oven at 100°C for ca 3 min. Examine derivatives spots (green-blue) under shortwave UV light. Determine R_f value for each spot.

RESULTS AND DISCUSSION

The two main parameters involved in the identification of amines by TLC are related to detection and R_f values. Many reagents were tried on the amines—bromocresol green, ninhydrine, eriochrom black-T, p-nitrobenzoyl chloride and bromothymol blue. Only bromocresol green was found to react well with amines. The R_f values are only approximate values and are affected by many variables. Therefore, it is absolutely necessary to use several solvent systems and different modes of detection to identify amines.

A solution of these five sympathomimetic amines in ethanol showed one spot when treated with bromocresol green and chromatographed in the chloroform-ethanol solvent systems. The reagent itself gives one spot in this system. Table 1 indicates the R_f values of standards in the two-solvent systems. Kodak scribed silica gel was the best plate for the identification of amines in this system. Ephedrine hydrochloride, pyrilamine maleate and pheniramine maleate can be identified from the other amine standards.

A solution of these amines in ethanol showed one spot when treated with bromocresol green and chromatographed in the isopropanol-water-acetic acid and cyclohexane-benzene-diethylamine solvent systems. By using these systems, specific amines cannot be identified.

A solution of these amines in ethanol showed one and two spots when treated with bromocresol green and chromatographed in the n-butanol-m-xylene-water-acetic acid and n-amyl alcohol-m-xylene-water-acetic acid solvent systems, while the reagent gives only one spot. Table 2 indicates the R_f values of standards in the four-solvent systems. Uniplate avicel and Kodak cellulose were the best plates for the identification of amines in these system. Ephedrine hydrochloride, phenylpropanolamine hydrochloride, pyrilamine maleate and chlorpheniramine maleate can be identified in the latter system. Identification was made by comparison with amine standards.

TABLE 1: *R_f values of some sympathomimetic amines in two-solvent systems, after treatment with bromocresol green (45 minute developing time; all standards 10 mg/ml in ethanol).*

Compound	Solvent System S_1			Solvent System S_2			Solvent System S_3		
	Kodak	EM 60	EM F-254	Kodak	EM 60	EM F-254	Kodak	EM 60	EM F-254
Ephedrine hydrochloride	0.45	0.19	0.29	0.35	0.10	0.17	0.36	0.11	0.19
Phenylpropanolamine hydrochloride	0.43	0.16	0.26	0.29	0.09	0.13	0.32	0.09	0.16
Pyrilamine maleate	0.70	0.41	0.51	0.55	0.28	0.40	0.64	0.33	0.47
Pheniramine maleate	0.60	0.22	0.31	0.53	0.17	0.24	0.55	0.19	0.29
Chlorpheniramine maleate	0.62	0.23	0.32	0.50	0.20	0.26	0.56	0.21	0.31
Reagent	—	0.07	0.08	—	—	—	—	—	—

S_1 = chloroform + ethanol (80 + 20)
S_2 = chloroform + ethanol (83 + 17)
S_3 = chloroform + ethanol (85 + 15)

Kodak = Kodak scribed silica gel
EM 60 = EM Laboratories silica gel 60
EM F-254 = EM Laboratories silica gel F-254

TABLE 2: *R_f values of some sympathomimetic amines in four-solvent systems, after treatment with bromocresol green (30 minute developing time; all solutions mixed in 250 ml separatory funnel for ca. 5 minutes, aqueous layer discarded and organic layer used for TLC).*

Compound	Solvent System S_4				Solvent System S_5				Solvent System S_6				Solvent System S_7			
	Ks	U	Kc	EM	Ks	U	Kc	EM	Ks	U	Kc	EM	Ks	U	Kc	EM
Ephedrine hydrochloride	0.23	0.42	0.42	0.14	0.31	0.67	0.53	0.18	0.18 0.27	0.43	0.48	0.14	0.30	0.63	0.56	0.18
Phenylpropanolamine hydrochloride	0.18 0.25	0.30	0.32	0.16	0.35	0.45	0.41	0.20	0.07 0.11	0.32	0.36	0.15	0.35	0.43	0.43	0.21
Pyrilamine maleate	0.06 0.13	0.82	0.70	0.09	0.19	0.91	0.77	0.13	0.07 0.11	0.80	0.78	0.09	0.18	0.88	0.85	0.13
Pheniramine maleate	0.06 0.14	0.79	0.68	0.07	0.19	0.89	0.75	0.13	0.07 0.15	0.78	0.77	0.06	0.17	0.86	0.82	0.11
Chlorpheniramine maleate	0.11 0.14	0.85	0.79	0.10	0.22	0.94	0.84	0.13	0.17 0.26	0.86	0.88	0.09	0.22	0.92	0.92	0.13
Reagent	0.05 —	0.16	—	0.07	0.12	0.29	0.20	0.13	0.07 —	0.17	0.18	0.08	0.11	0.26	0.27	0.13

S_4 = n-butanol + m-xylene + water + acetic acid (15 + 85 + 10 + 40)
S_5 = n-butanol + m-xylene + water + acetic acid (20 + 80 + 10 + 40)
S_6 = n-amyl alcohol + m-xylene + water + acetic acid (15 + 85 + 10 + 40)
S_7 = n-amyl alchohol + m-xylene + water + acetic acid (20 + 80 + 10 + 40)

Ks = Kodak scribed silica gel
U = Uniplate avicel
Kc = Kodak cellulose
EM = EM Laboratories silica gel F-254

CONCLUSION

The more polar spots have R_f values which are approximately the same, and the less polar spots have different R_f values, thus producing separation. By using two- and four-solvent systems, we can identify some amines in the presence of others.

ACKNOWLEDGMENT

The authors acknowledge with thanks the use of research facilities at the Woodson-Tenent Laboratories, Inc., to undertake these investigations.

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