Analysis of Drug Mixtures Containing Antihistamines by Quantitative Thin-Layer Chromatography

By J. C. MORRISON and L. G. CHATTEN

A method is described which utilizes the application of thin-layer chromatographic techniques to the analysis of antihistamines in multicomponent pharmaceuticals. The procedure is relatively rapid and convenient and requires no expensive equipment. Tablet and capsule excipients do not interfere. Quantitative measurements are accomplished by spraying the developed plate and measuring the area of the spot.

THE WIDE-SPREAD USE of antihistaminic preparations has resulted in the development of several analytical techniques for the assay of these compounds in pharmaceutical preparations. The "United States Pharmacopeia" (1) utilizes nonaqueous titrimetry for the quantitative determination of the crystalline antihistamines, but prefers analyses by ultraviolet spectrophotometry for the commercial products. The 1963 edition of "The British Pharmacopoeia" (2) also employs nonaqueous techniques for the crystalline materials, but methods for the products vary widely from spectrophotometry to the rather obsolete Kjeldahl procedure. None of the procedures in either compendium are of value in the analysis of multicomponent pharmaceuticals.

A report by Clair and Chatten (3) presented a nonaqueous technique which is an accurate and convenient means of assaying single-component antihistaminic products, but is of no value where the antihistamines are combined with other nitrogenous bases.

Since its introduction by Stahl (4), thin-layer chromatography has found an increasing number of applications in the separation and estimation of inorganic, organic, and biological mixtures. Within the past few years, several papers have been published which describe quantitative thin-layer techniques (5–7). Usually these have involved either the elution of the substance from the adsorbant and its subsequent estimation by chemical analyses (8) or the careful measure of the area of the spot from which the concentration of the substance can be calculated. By this latter technique Purdy and Truter (9) have shown in thin-layer chromatography that the square root of the area of the spot is directly proportional to the logarithm of the weight of the material present. These workers developed a procedure which separated and quantitatively determined mixtures of organic compounds and inorganic cations.

Since antihistamines are combined with a wide variety of other medicinal agents in multicomponent products, their isolation and estimation from such mixtures frequently is time consuming; the results leave much to be desired. One of the great advantages of thin-layer chromatography is its rapidity. Hence, the purpose of this present investigation was to determine whether this technique could be utilized in the analysis of multicomponent antihistaminics.

EXPERIMENTAL

Materials and Apparatus.—Glass plates (200 × 200 mm.) and glass developing tanks (12-in. diameter, 12 in. deep) lined with solvent saturated filter paper were used.

Adsorbant.—The adsorbant was silica gel G (Cave & Co.).

Solvent.—Glacial acetic acid, A.C.S.: distilled water (20:80) served as the solvent.

Spray Reagent.—Five grams of ceric sulfate was dissolved in 10 ml. of concentrated sulfuric acid. Distilled water was added to 100 ml.

Preparation of Plates.—The plates were coated with a layer of adsorbant 250 mμ thick according to the method of Stahl (4). The slurry was prepared by mixing 25 Gm. of silica gel G with 50 ml. of 20% 1.2 dimethoxyethane in distilled water. This gave a smooth, even dispersion of silica gel which was not liable to flake or crack.

Standards.—One-hundred milligrams of the drug was dissolved in 25 ml. of water. All standards were freshly prepared. Compounds used were methapyrilene hydrochloride (I), chlorpheniramine maleate (II), diphenhydramine hydrochloride (III), promethazine hydrochloride (IV), pyrilamine maleate (V), and *l*-phenylephrine hydrochloride (VI). All drugs were supplied by manufacturers and used without further purifications.

Application of Drugs.—Three solutions of each drug were prepared: a standard solution, a dilution

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TABLE I.—CHROMATOGRAPHIC DATA ON SELECTED DRUGS

Drug	Spray	Spot, Color	Spot, Shape	Min. Detectable Quantity, mcg.	R_f in 20% Acetic Acid
Methapyrilene hydrochloride	Ceric sulfate	Grey	Elliptical	15	0.38
Chlorpheniramine maleate	Ceric sulfate	White	Elliptical	25	0.27
Diphenhydramine hydrochloride	Ceric sulfate	Yellow	Circular	15	0.55
Promethazine hydrochloride	Ceric sulfate	Pink	Circular	10	0.51
Pyrilamine maleate	Ceric sulfate	Purple	Elliptical	15	0.35
l-Phenylephrine hydrochloride	Ceric sulfate	Brown	Circular	15	0.83
Ephedrine hydrochloride	Ninhydrin	Pink	Circular	15	0.59
Phenindamine tartrate	Ceric sulfate	Grey	Elliptical	15	0.35

TABLE II.—QUANTITATIVE RESULTS ON SINGLE DRUGS AND BINARY MIXTURES

	Amount Added, mcg.	Recovered, ±	Recovered, %	Determinations, No.	Area, mm.²
Individual Drugs					
Thenylene HCl	25	25.18 ± 2.17	100.72	23	164
	50	48.86 ± 3.57	97.72	23	247
Chlorpheniramine maleate	25	24.93 ± 2.27	99.72	35	136
	50	50.13 ± 3.57	100.26	26	271
Diphenhydramine HCl	25	25.78 ± 1.76	103.12	23	170
	50	52.46 ± 2.47	104.92	29	179
Promethazine HCl	25	25.20 ± 1.60	100.80	29	160
	50	50.29 ± 4.01	100.58	24	175
Pyrilamine maleate	25	25.43 ± 1.69	101.72	25	184
	50	51.71 ± 4.61	103.42	21	216
l-Phenylephrine HCl	25	25.48 ± 1.34	101.92	28	161
	50	50.76 ± 4.59	101.52	27	180
Binary Mixtures					
Thenylene HCl	25	24.99 ± 1.44	99.96	25	164
l-Phenylephrine HCl	25	25.61 ± 1.59	102.44	25	161
Chlorpheniramine maleate	25	25.04 ± 1.58	100.16	26	136
l-Phenylephrine HCl	25	25.46 ± 1.41	101.84	26	161
Diphenhydramine HCl	25	24.61 ± 1.60	98.44	28	170
l-Phenylephrine HCl	25	25.51 ± 1.81	102.04	28	161
Promethazine HCl	25	25.32 ± 1.83	101.28	25	160
l-Phenylephrine HCl	25	25.89 ± 1.88	103.56	25	161
Mepyramine maleate	25	24.94 ± 1.50	99.76	29	184
l-Phenylephrine HCl	25	25.54 ± 1.71	102.16	29	161

prepared from the standard, and the unknown. Fifteen microliters (3 mcg./ml.) of the diluted standard, 20 µl. (4 mcg./ml.) of the standard, and 25 µl. (5 mcg./ml.) of the unknown were applied to the plates from self-filling lambda pipets calibrated to deliver accurately known volumes. The size of the spot of application of these solutions was about 10 mm. or less. The spots were applied 20 mm. from the edge of the plates and were allowed to develop over 150 mm. from the starting line after the tanks had equilibrated overnight.

Detection of Spot Area.—When the solvent reached the finishing line, the plates were removed, dried for 30 minutes at room temperature, and sprayed with ceric sulfate solution. They were developed at 120° for 2 hours.

Measurement of Spot Area.—The area of the spot was outlined carefully with a dissecting needle. A sheet of transparent paper was superimposed on the spot and the area traced. Sheets of acetate viewfoil, on which circles and ellipses of known area had been imprinted, were superimposed on the paper and the area calculated. Areas could be added or subtracted from the circles or ellipses by placing a sheet of millimeter graph paper under the viewfoil and calculating the specific area to be determined.

RESULTS AND DISCUSSION

The investigation fell into three parts: (*a*) to ascertain whether the concentration of single drugs could be related directly to their spot area and to determine the maximum and minimum quantities of the drugs that could be employed; (*b*) to separate and estimate the concentration of individual drugs in synthetic binary mixtures; and (*c*) to apply this technique to the analysis of antihistamines in various pharmaceutical preparations.

When the developed plates were sprayed with the ceric sulfate solution, each drug gave a characteristic color on a faint yellow background. The lower limits of detection, in micrograms, varied with each drug. The values quoted in Table I are the minimum quantities necessary for quantitative area measurement. All areas were marked out following the removal of the plate from the oven because the spots faded slightly with time. In general, the smaller the initial area of application of the solutions, the more uniform the area of the developed spot. In practice, the area of application should not exceed 10 mm. It was also advantageous to insure that the unknown solution had a slightly higher concentration than the reference standard.

Table II shows that most of the drugs when applied in concentrations of 25 or 50 mcg. can be estimated with an accuracy of approximately 3%. Diphenhydramine hydrochloride appears to be an exception to this when applied alone. However, when this antihistamine was applied in combination with *l*-phenylephrine, the percentage recovery was improved considerably. In binary mixtures, the recoveries of all other antihistamines were highly satisfactory. Figure 1 depicts a classical plate which has been developed and sprayed in the manner described in this paper.

The formula employed to calculate the areas was as follows (9):

$$\log W = \log W + \left(\frac{\sqrt{A} - \sqrt{A_s}}{\sqrt{A_d} - \sqrt{A}}\right) \log d$$

where W = weight of the unknown, W_s = weight of the drug in the standard solution, d = the factor involved in diluting the standard to the weaker standard solution, A = area, in mm.^{2}, of the "unknown" spot, A_s = area, in mm.^{2}, of the "standard" spot, and A_d = area, in mm.^{2}, of the "diluted standard" spot.

The pharmaceutical preparations were assayed conveniently by the proposed technique, and the results are reported in Table III. The recoveries are within the limits of experimental error for thin-layer chromatography. However, it was essential to vary the concentration of the solution applied to the plates from one drug to another in a way such that the solution of a particular drug being estimated contained at least the minimum detectable quantity. A classical example would be Histadrina capsules which contain 8 mg. of ephedrine hydrochloride and 25 mg. of methapyrilene hydrochloride. In determining the methapyrilene content, five capsules were dissolved in water, filtered, and made up to 25 ml. Five microliters of this solution and 5 μl. of a 3–5 times dilution of it were applied to the plate. Twenty micrograms in 5 μl. of methapyrilene hydrochloride was applied as the standard. Similar adjustments were made to estimate the ephedrine content.

Attempts were made to obtain comparative analysis of the commercial samples by the procedure reported by Clair and Chatten (3). In every instance, no satisfactory visual or potentiometric end point was observed; undoubtedly, the complexity of the products was the major contributing cause. The nonaqueous technique can only be considered to be satisfactory for certain single-component products, as previously reported (3). The present method has the advantage of being applicable to single or multicomponent products.

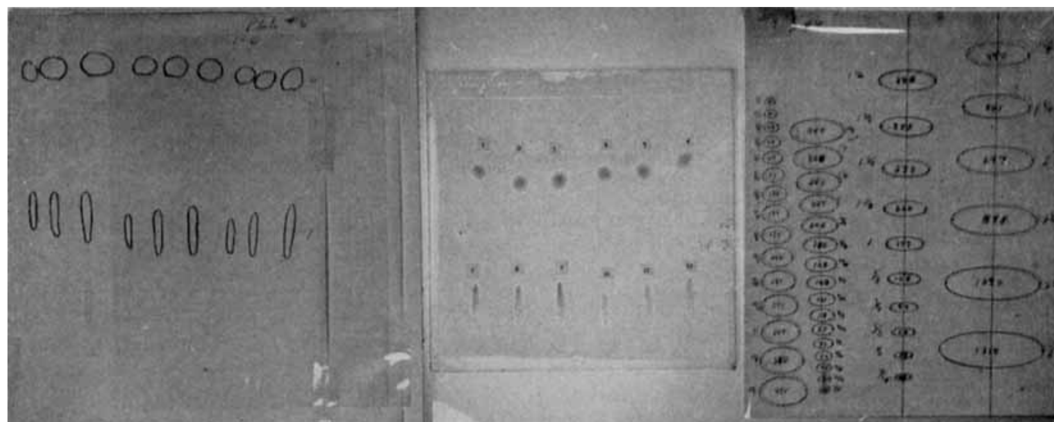

Fig. 1.—Developed chromatoplate with acetate viewfoil used in area measurement. Spots 1–6, *l*-phenylephrine hydrochloride; spots 7–9, thenylene hydrochloride; spots 10–12, pyrilamine maleate.

TABLE III.—QUANTITATIVE ANALYSIS OF PHARMACEUTICALS BY THIN-LAYER CHROMATOGRAPHY

Trade Prepn.	Amount per Tablet Labelled mg.	Amount per Tablet Recovered, ±	Recovered, %	Determinations, No.
Histadrina (capsules)				
Ephedrine HCl	8.00	7.79 ± 0.46	97.42	30
Methapyrilene HCl	25.00	26.12 ± 2.75	104.48	30
Romilar CF (capsules)				
Phenindamine tartrate	6.50	6.64 ± 0.73	102.15	30
l-Phenylephrine HCl	5.00	4.90 ± 0.37	98.96	30
Thenylene A.P.C. (capsules)				
Methapyrilene HCl	25.00	25.05 ± 2.87	100.20	30
Chlor-tripolon (tablets)				
Chlorpheniramine maleate	4.00	3.86 ± 0.30	96.42	29
Histadyl (capsules)				
Methapyrilene hydroxybenzoyl benzoate	25.00	23.63 ± 2.48	94.52	29
Romilar CF full strength (capsules)				
Chlorpheniramine maleate	1.00	0.94 ± 0.09	94.32	30
l-Phenylephrine HCl	5.00	5.09 ± 0.36	101.73	30

provided the respective components separate sufficiently sharp to permit measurement on the plate.

Determining a satisfactory solvent system can be done qualitatively by finding the R_f values of each component on a silica gel coated glass microscope slide. A 250-ml. beaker covered with aluminum foil makes a satisfactory developing chamber. Development takes about 20 minutes and, in this way, a large number of solvents can be examined rapidly.

In their report, Purdy and Truter (9) estimated all area measurements by using millimeter graph paper. This method is tedious and can result in eye strain. Using viewfoil on which circles and ellipses of known areas are imprinted is much more rapid and convenient. When standard areas were checked by both methods, the resulting differences were 3% or less.

The proposed method is conveniently applicable to pharmaceutical preparations. For example, tablet excipients and diluents do not interfere with the separation of the active constituents or their delineation. In addition, several assays can be performed on multicomponent dosage forms in a normal 8-hour day.

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Novel Decarboxylative *N*-Alkylation Reaction Resulting from Controlled Pyrolysis of Procaine

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2-Diethylaminoethyl *p*-aminobenzoate is unstable at elevated temperatures and evolves carbon dioxide, 2-diethylaminoethanol, and *N,N*-diethyl-*N'*-phenylethylenediamine. The pharmacological properties of the diamine correlate with some of the actions of systemic procaine.

AN INCREASED interest has been evidenced in the pharmacological properties of systemic procaine. One active pursuit has been the resolution of conflicting clinical observations reported by various investigators in studies of its long-term administration to humans. Another aspect centers upon the inability of correlating useful clinical effects with the drug's rapid *in vivo* metabolism by hydrolysis to *p*-aminobenzoic acid and 2-diethylaminoethanol. Intravenous procaine has been found effective for the relief of herpetic pain and postherpetic neuralgia (1). Efforts to solve some of the uncertainties include searches for active metabolites and the identification of significant impurities which may be present initially in commercial drug preparations.

With this in mind, a novel decarboxylative *N*-alkylation reaction of procaine which yields carbon dioxide and *N,N*-diethyl-*N'*-phenylethylenediamine is reported. The process was first encountered upon subjecting the ester to moderately elevated temperatures in a study of its manufacture. At temperatures above 200°, preferably in the range 225–235° and under pressures of 20–25 mm., the compound evolves carbon dioxide and easily condensable vapors consisting of a mixture of the diamine and 2-diethylaminoethanol. The aminoalcohol is formed by intermolecular amine-ester self-condensation and is separated readily from the diamine by fractional distillation.

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Production of the diamine appears to be initiated intramolecularly by attack of the nucleophilic ternary ester nitrogen atom upon the procaine acyl carbonium ion (I). After loss of carbon dioxide, the aniline anion (II), the result of a proton shift to the ring, and the diethylaminomethylcarbonium ion (III) combine to give *N,N*-diethyl-*N'*-phenylethylenediamine (IV).