Thin-Layer Chromatography of Ergot Alkaloids in Pharmaceutical Preparations

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A simple thin-layer chromatographic procedure based on the use of alkaline silica gel as adsorbant and chloroform–methanol (9:1) as developing solvent is presented for identifying and appraising the purity of a number of ergot alkaloids occurring in pharmaceutical dosage forms. The application of this method to the detection of other active ingredients often present in these products is also described.

MANY thin-layer and paper chromatographic procedures have been described for the separation and identification of ergot alkaloids and their derivatives. Partition paper chromatography in particular has been recommended for the examination of some of these alkaloids of pharmaceutical interest (1, 2) and is included in the U.S.P. XVII. A thin-layer method (3) has been recently described for a few pharmaceutical preparations, while a number of procedures have been reported for ergot alkaloids in general (4–9).

The purpose of this communication is to report a simple thin-layer chromatographic technique which has been applied successfully for some time in the Regional Laboratories, Food and Drug Directorate, for the characterization and analysis of ergot alkaloids and a number of other drugs frequently occurring along with these compounds in pharmaceutical formulations.

EXPERIMENTAL

Thin-Layer Chromatographic Plates.—Prepare with standard equipment for thin-layer chromatography. To obtain 5 plates 20 × 20 cm., mix 25 Gm. of Silica Gel G (Merck) with 50 ml. of 0.1 *N* NaOH. Apply the mixture to plates (layer thickness about 250 μ) and activate immediately before use by heating at 110° for 30 min.

Solvent Systems.—Chloroform–methanol (9:1 v/v) (4) and isopropanol–ammonia (25%)–chloroform (45:10:45 v/v) (10).

Spray Reagents.—(*a*) (van Urk)—Dissolve 1 Gm. of dimethylaminobenzaldehyde in 50 ml. of concentrated hydrochloric acid, and add 1 drop of 9% aqueous ferric chloride solution. (*b*) Iodoplatinate—Prepare aqueous solution of $H_2PtCl_6 \cdot 6H_2O$ (0.3%) and KI (6%), and mix equal volumes just before use. (*c*) Vanillin–Sulfuric Acid—Five per cent vanillin in concentrated sulfuric acid (w/v). (*d*) Mercurous Nitrate—Prepare a 1% aqueous solution of $HgNO_3 \cdot H_2O$ immediately before use, and add 1 drop of concentrated nitric acid.

Standard Reference Solutions.—Weigh the following quantities of standards and make to 10 ml. with methanol: ergot alkaloids, atropine, homatropine, hyoscyamine, or scopolamine (2.0 mg.); diphenylpyraline, dimenhydrinate, or cyclizine (10.0 mg.); phenobarbital, barbital, allylbarbituric acid, or pentobarbital (20.0 mg.); phenacetin, or caffeine (30.0 mg.).

Preparation of Samples.—*Bulk Drugs.*—Process as described for standard reference solutions.

Tablets.—Mix a quantity of finely powdered tablet material containing 0.5 to 2.0 mg. of ergot alkaloid with sufficient methanol to obtain a concentration of active ingredient of about 1 mg./5 ml. For tablets containing belladonna alkaloids, suspend a quantity of powdered material, containing approximately 250 mcg. of belladonna alkaloid, in 10 ml. of 1% aqueous tartaric acid and stir for 10 min. Make alkaline with ammonia and extract with three 10-ml. portions of ether. Dry the combined ether extracts over anhydrous sodium sulfate and evaporate to dryness. Dissolve the residue in 1 ml. of methanol.

TABLE I.—CHROMATOGRAPHIC DATA OF COMPOUNDS EXAMINED

Medicinal	R_f Value[a]	Sample Size, mcg.	Color Observed
Ergonovine	0.19	2	Blue[f]
Methylergonovine	0.23	2	Blue[f]
Methysergide	0.35	2	Blue[f]
Dihydroergotamine	0.45	2	Blue[f]
Ergotamine	0.51	2	Blue[f]
LSD	0.61	2	Blue[f]
Aci-dihydroergotamine	0.31	2	Blue[f]
Ergonovinine	0.36	2	Blue[f]
Ergotaminine	0.76	2	Blue[f]
Atropine	0.05	2	Bluish-purple[g]
Hyoscyamine	0.05	2	Bluish-purple[g]
Homatropine	0.07	2	Bluish-purple[g]
Diphenylpyraline	0.32	10	Bluish-purple[g]
Scopolamine	0.39	2	Bluish-purple[g]
Dimenhydrinate	0.45	10	Bluish-purple[g]
Cyclizine	0.60	10	Bluish-purple[g]
Phenacetin	0.67	30	Purple[h]
Caffeine	0.70	30	Purple[h]
Phenobarbital	0.64[b]	20	Gray[i]
Barbital	0.67[c]	20	Gray[i]
Allylbarbituric acid	0.71[d]	20	Gray[i]
Pentobarbital	0.73[e]	20	Gray[i]

[a] Solvent system chloroform–methanol (9:1). [b, c, d, e] R_f values 0.29, 0.40, 0.54, and 0.61, respectively, with isopropanol–ammonia–chloroform solvent system. [f] With van Urk. [g] With iodoplatinate. [h] With iodoplatinate followed by vanillin-sulfuric acid. [i] With mercurous nitrate.

Received June 25, 1965, from the Pharmaceutical Chemistry Division, Food and Drug Directorate, Ottawa, Ontario, Canada.
Accepted for publication August 9, 1965.
Presented to the Scientific Section, A.PH.A., New York City meeting, August 1964.
The authors are indebted to Dr. Leo Levi for his interest in this study.

Suppositories.—Dissolve one suppository in 25 ml. of petroleum ether, centrifuge, remove the supernatant, and wash the residue with another portion of petroleum ether. Dissolve the residue in sufficient methanol to give an ergot alkaloid concentration of 1 mg./5 ml. For suppositories containing belladonna alkaloids, dissolve one suppository in 25 ml. of petroleum ether and extract with three 5-ml. portions of 1% tartaric acid. Make the aqueous extractions alkaline with ammonia and proceed as described for tablets containing belladonna alkaloids.

Chromatographic Procedure.—For each component to be identified, spot a volume of sample solution containing the optimum amount of active ingredient (Table I) and 10 µl. of the corresponding standard solution at an adjacent location. For the estimation of isomerization products present in the ergot alkaloid, spot 25 µl. of sample (containing about 5 mcg. of ergot alkaloid) and 1, 2, and 3 µl. of standard solution at adjacent locations. These volumes contain 0.2, 0.4, 0.6 mcg. of the alkaloid and correspond to 4, 8, and 12%, respectively, of the amount of sample spotted.

Equilibrate a suitable jar with the chromatographic solvent for about 1 hr. using a lining of filter paper to ensure efficient solvent equilibration. Insert the plates and allow the solvent to rise about 15 cm. above the starting line. The average time required for development is 33 min. Examine the plate under ultraviolet light: all the ergot alkaloids, except dihydroergotamine, fluoresce blue. Phenacetin, if present, appears as a faint purple spot. Other compounds are not visible at the concentrations used.

For color observations, cover the chromatogram with glass plates so that only areas containing spots to be developed are exposed, and spray with the appropriate reagent (Table I). Repeat the process for the detection of other compounds using appropriate spray reagents. Identify the components present by observation of their R_f values and comparison with those of the reference standards. Any secondary blue spots showing up in the chromatogram of ergot alkaloid represent impurities. Estimate the amount of such impurities by comparing their intensities in the sample chromatogram containing 25 µl. of solution with those of the adjacent standard solutions containing known amounts of reference standard.

For samples containing barbiturates, repeat the chromatographic procedure using the methanol–ammonia–chloroform solvent system and identify the drugs by comparison of their R_f values with those of standard samples.

DISCUSSION

The R_f values of compounds examined by the described procedure are shown in Table I, while the scope of the method is demonstrated in Table II. The following advantages are offered by the technique. Solutions of alkaloidal salts may be spotted directly since the corresponding bases are liberated by the alkaline adsorbant. Thus, the necessity of prior conversion of the salts to free bases followed by solvent extraction is eliminated; product decomposition is minimized since alkaloidal salts are less susceptible to isomerization than their free bases (no isomerization was detected during chromatography, but faint auxilliary or secondary red and yellow spots were observed in the chromatograms of solutions kept for a few days before analysis); minute amounts (2 mcg.) of ergot alkaloid are required for analysis; impurities may be semiquantitatively estimated by visual comparison with standards; sample preparation is simple and rapid. Other compounds commonly encountered in dosage forms with ergot alkaloids, particularly with ergotamine, may be readily identified by the same general method.

TABLE II.—LABELED COMPOSITION OF PREPARATIONS EXAMINED

Active Ingredient	Product No. 1	2	3	4	5	6	7	8	9	10
Ergotamine	x	x	x	x	x	x	x	x		
Dihydroergotamine									x	
Ergot extract										x
Belladonna alkaloids						x	x			
Homatropine										x
Hyoscyamine					x					
Scopolamine								x	x	
Cyclizine		x								
Dimenhydrinate			x							
Diphenylpyraline				x						
Caffeine	x	x	x	x	x	x	x			x
Phenacetin							x			
Allylbarbituric acid								x	x	
Barbital								x	x	
Pentobarbital					x	x				
Phenobarbital								x	x	x

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