Single-Tablet Determination of Reserpine at the Microgram Level

Estimation of Reserpine in the Presence of Protoveratrine A and Other Active Drug Components

By WILLIAM J. WEAVER

Mixtures of reserpine and other drugs can be resolved by thin-layer chromatography on silica gel, following extraction from single tablets with chloroform. Quantitation is accomplished by visual inspection, and is accurate to within 10 per cent.

THE PURPOSE of this investigation was to provide a rapid, sensitive, reliable analytical procedure to measure tablet-to-tablet variation of the active ingredients in tablets containing reserpine, protoveratrine A, and hydroflumethiazide.[1] Each tablet contains:

Reserpine	0.125 mg.
Protoveratrine A	0.200
Hydroflumethiazide[2]	50.000

The small amounts of reserpine and protoveratrine A present in single-dosage forms of drugs of this type cannot be determined by conventional analytical procedures because of interferences by other active ingredients which are present in milligram quantities.

Hydroflumethiazide can be determined spectrophotometrically by measuring the ultraviolet absorbance of a filtered methanolic extract of a single pulverized tablet. Reserpine, protoveratrine A, and tablet excipients do not interfere.

The official U.S.P. method (1) for the determination of reserpine involves solvent extraction of the reserpine and subsequent colorimetric determination. The U.S.P. extraction procedure is designed to eliminate degradation products of reserpine. In the procedure described here these purification steps are not required because the degradation compounds do not interfere in the separation and detection.

Protoveratrine A is currently determined in these laboratories by the colorimetric procedure of Craig and Jacobs (2). This procedure requires a reaction time of 16 hr. for samples, standards, and appropriate synthetic tablet blanks. In addition to eliminating these time-consuming operations, the procedure described in this report permits the simultaneous separation and detection of reserpine and protoveratrine A.

Wimer *et al.* (3) have recently reviewed procedures for the separate determination of reserpine and protoveratrine A. Although some of the methods described are extremely sensitive, they are not applicable to formulations which contain certain interfering ingredients. Levine and Fischbach (4, 5) have employed partition and paper chromatography for the separation of the veratrum alkaloids. More recently Montgomery (6) has separated protoveratrines A and B by thin-layer chromatography on cellulose.

This paper describes a thin-layer chromatographic method for the separation of reserpine and/or protoveratrine A from excipients and from other active ingredients present in several commercial tablet dosage forms. Development of the thin-layer plate with the solvent is rapid, requiring only 30 min. The detection reagent, *p*-dimethylaminobenzaldehyde in concentrated sulfuric acid, produces characteristic color reactions with reserpine and protoveratrine A. Graham (7) has investigated the color reactions between the veratrum alkaloids with this and other reagents.

EXPERIMENTAL

Equipment.—Silica Gel G scored-glass Uniplates (20 × 20 cm.) supplied by Analtech, Inc., Wilmington, Del. Reactivate the plate for 30 min. in an oven at 100° immediately before use. Glass developing-

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[1] Marketed as Salutensin tablets by Bristol Laboratories, Division of Bristol-Myers Co., Syracuse, N. Y.
[2] Marketed as Saluron by Bristol Laboratories, Division of Bristol Myers Co., Syracuse, N. Y.

Table I.—Determination of Reserpine in Commercial Samples

Tablet[a]	Active Ingredients	Label Claim, mg./Tablet	Found, Single Tablet Assay, mg.
A	Reserpine Butabarbital Hydrochlorothiazide	0.1	0.09 to 0.11
B	Reserpine Cryptenamine Methyclothiazide	0.1	0.09 to 0.11
C	Reserpine Trichlormethiazide	0.1	0.09 to 0.11
D	Reserpine Nitroglycerin Pentaerythritol tetranitrate	0.07	0.06 to 0.08
E	Reserpine Protoveratrine A Hydroflumethiazide	0.125 0.200	0.112 to 0.137 0.180 to 0.220
F	Reserpine Pyrrobutamine	0.25	0.225 to 0.275

[a] *A*, Butiserpazide -25 (McNeil); *B*, Diutensen R (Neisler); *C*, Metatensin (Lakeside); *D*, Penite (Carnrick); *E*, Salutensin (Bristol); Sandril c̄ Pyronil (Lilly).

Table II.—Comparison of Methods for Determination of Reserpine and Protoveratrine A in Tablets Containing Reserpine, Protoveratrine A, and Hydroflumethiazide[1]

Sample	Reserpine, mg./Tablet			Protoveratrine A, mg./Tablet		
	Label	U.S.P.	TLC-Range[a]	Label	Craig & Jacobs (2)	TLC Range[a]
1	0.125	0.122	0.112 to 0.137	0.200	0.197	0.180 to 0.220
2	0.125	0.121	0.112 to 0.137	0.200	0.204	0.180 to 0.220
3	0.125	0.120	0.112 to 0.137	0.200	0.192	0.180 to 0.220
4	0.125	0.124	0.112 to 0.137	0.200	0.194	0.180 to 0.220

[a] Acceptable concentration limits have been established as ±10% of label claim.

tanks (25.5 cm. in diameter, 25.5 cm. deep) lined with solvent-saturated filter paper. Hamilton, No. 701-N, 10-μl. syringe. Fisher 125-ml. chromatographic indicator spray flask.

Solvent.—Prepare 30 vol. % acetone, ACS, in chloroform, ACS, to serve as the developing solvent.

Spray Reagent.—Dissolve 500 mg. of *p*-dimethylaminobenzaldehyde, Eastman, in 50 ml. of concentrated sulfuric acid.

Stock Protoveratrine A Standard.—Weigh 90 mg. (±0.1 mg.) of standard protoveratrine A into a 10-ml. volumetric flask. Dissolve and dilute to volume with chloroform. This solution is stable for 1 week.

Stock Reserpine and Protoveratrine A Standard.—Weigh 11.25 mg. (±0.1 mg.) of reserpine U.S.P. into a 10-ml. low-actinic volumetric flask. Pipet 2.0 ml. of the stock protoveratrine A standard solution into the flask containing the dry reserpine. Dilute to volume with chloroform. Prepare fresh daily.

Working Reserpine and Protoveratrine A Standard.—Dilute 2.0 ml. of the stock reserpine and protoveratrine A standard to 10.0 ml. with chloroform in a low-actinic volumetric flask. Prepare just prior to use. This solution contains 1.125 mcg. reserpine and 1.8 mcg. of protoveratrine A/5 μl., and 1.35 mcg. of reserpine, and 2.16 mg. of protoveratrine A/6 μl. These amounts represent reserpine and protoveratrine A corresponding to 90% and 110%, respectively, of the theoretical reserpine and protoveratrine A content in 5 μl. of the final sample concentrate.

Preparation of Sample and Application to TLC Plate.—Pulverize one Salutensin tablet, and transfer quantitatively to a 50-ml. glass-stoppered, low-actinic conical flask. Add 30 ml. of chloroform, and shake 15 min. in a mechanical shaker. Filter the chloroform extract through Whatman No. 30 filter paper into a 50-ml. serum vial protected from direct light with aluminum foil. Wash the flask and filter paper with two consecutive 2-ml. portions of chloroform. Evaporate the chloroform extract to dryness under a stream of nitrogen, then dissolve the residue in the vial with 0.50 ml. of chloroform. Stopper the vial, and swirl to ensure complete solution of the active ingredients.

Employing a 10-μl. syringe, spot consecutively on a thin-layer plate about 10 mm. apart and 20 mm. from the bottom edge of the plate: 5.0 μl. of the working standard, 5.0 μl. of the sample concentrate, and 6.0 μl. of the working standard. Maintain the diameter of the spot at 3 to 5 mm. Allow the spots to air-dry. Develop the plate with the solvent to a distance of 10 cm. from the origin.

Detection of Spots.—Remove the plates after solvent development, and allow to air-dry. Holding the sprayer 30 cm. from the plate, spray the plates lightly with the spray reagent until green spots become visible. Place the plates in an oven at 100° for 5 to 8 min. Remove the plates from the oven, and observe the positions of the spots. Protoveratrine A is indicated by an elongated, purple spot, R_f = 0.1 to 0.2. Reserpine appears as a greenish-black spot, R_f = 0.3 to 0.35.

Quantitation.—Estimate the concentrations of reserpine and protoveratrine A in the sample by comparing the sizes and intensities of the reserpine and protoveratrine A sample zones with those

obtained from the standards. Distinct differences can be noted between the standards at the 90% and 110% concentrations.

RESULTS

The method has been applied to six commercial dosage forms containing reserpine and other active ingredients. The results are shown in Table I. (The active ingredients, rauwolfia serpentina and protoveratrines A and B,[3] were also separated on a thin-layer plate. Reserpine and the protoveratrines were not quantitated against standard reserpine and protoveratrine mixtures.) The commercial tablets were obtained on the open market in bottles of 50 or 100, and single tablets of each were used for assay. None of the active ingredients or excipients present in the tablets interfered in the determination of reserpine.

Table II shows a comparison of results obtained from the determination of reserpine and protoveratrine A in tablets containing reserpine, protoveratrine A, and hydroflumethiazide[1] by the proposed single tablet method *versus* the U.S.P. method (1) for reserpine and the protoveratrine A procedure of Craig and Jacobs (2), using multiple-tablet extracts.

DISCUSSION

Sensitivity.—From 2 to 6 μl. of the working standard solution was spotted on a plate. The plate was developed and sprayed with the reagent. The lower limits of reserpine and protoveratrine A that can be detected are 0.9 and 1.44 mcg., respectively.

Interferences.—Reserpine was hydrolyzed in an alkaline medium according to the method of Neuss (8). The products, reserpic acid and trimethoxybenzoic acid, were dissolved in chloroform at a concentration equivalent to that of the working standard solution and chromatographed in the usual fashion. Reserpic acid and trimethoxybenzoic acid do not react with the reagent. When the plate is exposed to ultraviolet light, faint fluorescent spots are observed at the origin.

When the standard stock solution of reserpine and protoveratrine A is exposed to bright light for approximately 4 hr., diluted, and chromatographed, fluorescent spots are observed at the origin and at an R_f of 0.5 to 0.58. These spots are not found on a developed plate when freshly prepared, light-protected standard solutions are chromatographed. Excipients and active components found in the seven formulations reported above do not interfere.

[3] Marketed as Rauprote tablets by The Vale Chemical Co., Inc., Allentown, Pa.

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Determination of Hydrocortisone and Hydrocortisone Acetate in Antibiotic Mastitis Preparation

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A modified column partition chromatographic method has been developed for the determination of hydrocortisone and hydrocortisone acetate in antibiotic mastitis preparations. A sodium bicarbonate trap under a methyl alcohol–water stationary phase in Celite is used with two mobile phases. First, the interfering oil is eluted with methylene chloride–isooctane, then the steroid to be assayed by the blue tetrazolium method is eluted with methylene chloride. This procedure shows a marked improvement over the U.S.P. XVII hydrocortisone ointment method in the removal of interferences and results in satisfactory assays for the two steroids in mastitis formulations. Recoveries of the steroids added to blank mastitis preparations ranged from 90.3 to 100.9 per cent. The average percentage of recovery for hydrocortisone and hydrocortisone acetate was 96.9 and 98.2, respectively.

Corticosteroids have been incorporated in a large number of drug preparations, primarily for their anti-inflammatory activity. Two of the most common, hydrocortisone and hydrocortisone acetate, are often used in ointments and oils for the treatment of mastitis in dairy animals. These mastitis preparations frequently contain procaine penicillin G and a vegetable oil, *e.g.*, peanut oil, and may contain, in addition, one or more of the following: dihydrostreptomycin sulfate, neomycin sulfate, polymyxin B sulfate, sulfamerazine, sulfamethazine, sulfathiazole, sulfanilamide, methylpara-

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