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Keyphrases

Tolbutamide dosage forms—analysis
Degradation products—tolbutamide
TLC—separation
Colorimetric analysis
Ninhydrin, *n*-butylamine—color formation

Determination of Microgram Quantities of Belladonna Alkaloids in Neomycin-Kaolin-Pectin Suspension

By ALFRED BRACEY and GEORGE SELZER

A method combining liquid–liquid extraction and column chromatography has been developed to determine microgram quantities of belladonna alkaloids in kaolin-pectin suspensions. The alkaloids are first separated from the suspension by several ethanol-ether extractions. Interfering substances, such as methylparaben and propylparaben, are eliminated by extracting the alkaloids with chloroform from a basic solution. The alkaloids are further purified by column chromatography. The chloroform extract is passed through an acid siliceous earth column, which retains the alkaloids. The column is made basic with ammonium hydroxide, and the alkaloids are eluted with chloroform. The isolated alkaloids are nitrated, then reduced to the aromatic amine, and finally diazotized and coupled with the Bratton-Marshall reagent for color formation. Recoveries of atropine sulfate added to blank kaolin-pectin suspensions ranged from 92.9 to 99.4 percent.

THE BELLADONNA ALKALOIDS, atropine sulfate, hyoscyamine sulfate, and scopolamine hydrobromide, are often formulated with kaolin and pectin in aqueous suspensions for treatment of intestinal disorders. These preparations may also contain neomycin. The suspensions combine the adsorbent and detoxifying effects of kaolin (which functions in the stomach and small intestines) and pectin (which functions in the large intestines) with the antispasmodic effect of the belladonna alkaloids to help control hypermotility and hypersecretion in the gastrointestinal tract.

Methods for assaying belladonna alkaloids in multicomponent formulations include Koch, Levine, and Zenker's method (1) for belladonna alkaloids in combination with phenobarbital and Woodson's procedure (2) for determining atropine sulfate and hyoscyamine in the presence of methylene blue. However, no procedure has been reported that involves the assay of microgram quantities of belladonna alkaloids in suspension with strong adsorbents such as kaolin.

This paper presents a method of extracting the alkaloids from kaolin–pectin suspensions (3) and determining their total concentration, using the color development procedure of Koch, Levine, and Zenker (1).

Since atropine sulfate and hyoscyamine sulfate are optical isomers, and are not differentiated by this assay procedure, they are considered analytically equivalent. The third alkaloid component of the preparation is scopolamine hydrobromide, which constitutes only 5% of the total alkaloid dose. This compound has been demonstrated to be equivalent to atropine sulfate in the assay procedure except for a small difference in the colorimetric absorptivity, which is made insignificant by its low concentration. Therefore, the belladonna alkaloids are expressed as the equivalent of atropine sulfate.

ASSAY PROCEDURE

Reagents—The following reagents are used: absolute ethanol; ethyl ether; fuming nitric acid (90%); concentrated hydrochloric acid; ammonium

Received August 4, 1967, from the Division of Antibiotics and Insulin Certification, Bureau of Science, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, DC 20204
Accepted for publication October 10, 1967.

hydroxide; chloroform distilled from a mixture of chloroform and 1 *N* sulfuric acid (4:1); 20% sodium hydroxide solution; 0.2 *N* sulfuric acid; 5% sodium nitrite solution; 5% ammonium sulfamate solution; 0.1% *N*-(1-naphthyl)-ethylene diamine dihydrochloride (prepared fresh before use); solid sodium hydrosulfite.

Standard Solution—Atropine sulfate, prepared by stepwise dilution as an aqueous solution containing 0.100 mg./ml. of USP atropine sulfate reference standard.

Apparatus—(*a*) An acid trap column is prepared by placing a small wad of glass wool at the juncture of the barrel and the stem of a chromatographic tube about 150 mm. long and 20 mm. in diameter. The column was packed with 5.0 Gm. of chromatographic siliceous earth[1] thoroughly mixed with 4.0 ml. of 0.2 *N* sulfuric acid, transferred to the column in several portions, and packed firmly with a tamping rod after each addition. (*b*) Filtering tube consisting of a wad of glass wool at the juncture of the barrel and the stem of a tube about 250 mm. long and 40 mm. in diameter. (*c*) Colorimeter or spectrophotometer

Extraction of Alkaloids—Place a 60-ml. aliquot of the suspension containing about 250 mcg. of alkaloids into a 500-ml. glass-stoppered conical flask. Add 100 ml. of ethanol and 4.0 ml of hydrochloric acid. Stopper tightly and place on a mechanical shaker for 30 min. Add 240 ml. of ether and shake vigorously. Add 10 ml. of ammonium hydroxide, swirl gently, and then shake well. Allow the suspended material to settle. Decant the supernatant through the filtering tube, collecting the filtrate in an 800-ml. beaker. Extract the sample a second time by adding 10.0 ml. of water and 50 ml. of ethanol to the flask. Stopper and shake well. Add 120 ml. of ether and shake. Add 2.0 ml. of ammonium hydroxide, swirl gently, and then shake vigorously. Allow the suspended material to settle and pour the supernatant through the filtering tube, combining the filtrates. Add 50 ml. of ethanol and 120 ml. of ether; then shake vigorously. Add 1.0 ml. of ammonium hydroxide, swirl gently, then shake, and allow suspended material to settle. Pour the supernatant into the filtering tube, followed by as much of the residue as possible. Finally, rinse the flask with 25 ml. of ether and pour it into the filtering tube. After the tube drains, acidify the combined filtrate with hydrochloric acid until it is acid to pH paper and evaporate it to about 50 ml. on a warm steam bath under a gentle stream of air.

Transfer the aqueous residue quantitatively to a 125-ml. separator. Adjust the solution to about pH 9 with 20% sodium hydroxide and immediately extract with one 50-ml. portion and three 30-ml. portions of chloroform. Pass each extract through the acid trap column. Add 2.0 ml. of ammonium hydroxide to the column, followed by 125 ml. of chloroform, and collect the alkaloid eluate in a 250-ml. beaker. Evaporate the eluate to about 30 ml. and transfer it quantitatively to a 50-ml. graduated glass-stoppered centrifuge tube. Add about four drops of hydrochloric acid to the tube, stopper, and shake. Evaporate to dryness by placing the tube in a hot water bath under a gentle stream of air.

[1] Celite 545, Johns-Manville Corp., New York, N. Y.

A procedural blank, consisting of 60 ml. of water, and a procedural standard, consisting of 200 mcg. of atropine sulfate in 60 ml. of water, are treated as samples throughout the assay.

Color Development—Add 1.0 ml. of fuming nitric acid to each tube containing the chloroform residue and to a reagent blank tube. Stopper each tube loosely, place on a steam bath with test tube clamps, and heat vigorously for 30 min. Remove the tubes from the steam bath and to each one add 10 ml. of water, 2.0 ml. of ammonium hydroxide, and about 50 mg. of sodium hydrosulfite. Shake each tube gently to dissolve the sodium hydrosulfite and allow to stand at room temperature for 20 min. Add 5.0 ml. of 5% sodium nitrite and 2.0 ml. of hydrochloric acid. Shake each tube gently and place in an ice-water bath for 30 min. Slowly add 10.0 ml. of 5% ammonium sulfamate in 5-ml. portions, and shake the tube gently after each addition to dispel the nitrous oxide fumes. Add 4.0 ml. of freshly prepared 0.1% solution of *N*-(1-naphthyl)-ethylenediamine dihydrochloride. Allow 30 min. for color development before diluting to the 50.0-ml. mark with water. Determine the absorbance at 550 mμ against the reagent blank. Correct the absorbance of the standard and sample for that of the procedural blank.

RESULTS

The recovery of atropine sulfate added to blank preparations containing all the ingredients except the alkaloids ranged from 92.9 to 99.4%, as shown in Table I. The amount of atropine sulfate added was approximately equivalent to the belladonna alkaloid concentration in commercial preparations. As shown in Table II, the assay results for commercially available preparations ranged from 90.3 to 106.5% of label claim. These batches represent formulations ranging in date of production from recent to those

TABLE I—RECOVERY OF ATROPINE SULFATE FROM KAOLIN-PECTIN SUSPENSIONS

Sample	Mfr.	Atropine Sulfate Added, mg./30 ml.	Atropine Sulfate Recovered, mg./30 ml.	% Recovery
1	I	0.1000	0.0985	98.5
2	I	0.1000	0.0929	92.9
3	I	0.1000	0.0940	94.0
4	II	0.1000	0.0994	99.4
5	II	0.1000	0.0971	97.5

TABLE II—DETERMINATION OF BELLADONNA ALKALOIDS IN COMMERCIAL SAMPLES[a] LABEL CLAIM 0.1296 mg./30 ml.[b]

Mfr.	Sample[c]	Amt. Found, mg./30 ml.	% Label Claim
I	1	0.1320	101.8
I	2	0.1380	106.5
I	3	0.1290	99.5
I	4	0.1170	90.3
II	1	0.1170	90.3
II	2	0.1170	90.3
II	3	0.1190	93.0
II	4	0.1190	93.0

[a] The active ingredient formulation of these products is as follows: atropine, sulfate 0.0192 mg./30 ml.; hyoscyamine sulfate, 0.1037 mg./30 ml.; scopolamine hydrobromide, 0.0065 mg./30 ml. [b] Weight of belladonna alkaloid expressed as the equivalent weight of atropine sulfate. [c] Aqueous belladonna alkaloid, neomycin, kaolin-pectin suspensions.

TABLE III—DETERMINATION OF INTERFERENCE OF BLANK FORMULATION AS ATROPINE SULFATE EQUIVALENT

Blank[a]	Mfr.	Apparent Atropine Sulfate, mg./30 ml.	% Interference[b]
1	I	0.0016	1.2
2	II	0.0054	4.1

[a] The commercial blank preparation contains all the ingredients of belladonna alkaloids neomycin suspension except the alkaloids. [b] The percent of interference is relative to the 0.1296 mg./30 ml. label claim of alkaloids in commercial preparation.

nearing expiration date. In Table III is shown the interference of a blank preparation from each manufacturer in terms of apparent atropine sulfate. By the method presented, this interference was found to be 1.2 and 4.1%.

DISCUSSION

The liquid extraction column chromatography (4) method described here permits the isolation and quantitative determination of belladonna alkaloids in kaolin-pectin suspensions. Conventional extraction methods were ineffective because of the strong adsorptive properties of kaolin and the strong tendency of this type of preparation to form an unmanageable emulsion with organic solvents. Therefore, the first phase of the assay consists of a desorption procedure (3), in which ethanol, water, and ether are used to remove all of the soluble ingredients from the suspension. After evaporation of most of the organic portion of the extract, the aqueous portion is adjusted to pH 9 with 20% sodium hydroxide and the alkaloids are extracted with chloroform, leaving behind most of the methyl and propylparaben preservatives. If tropic acid is present most of it will also be retained by the aqueous phase. Tropic acid is one of the principal degradation products of the belladonna alkaloids and the portion of the molecule responsible for the color reaction. The alkaloids are further purified by column chromatography. The column retains the alkaloids while traces of methylparaben, propylparaben, and tropic acid are washed through. Then the purified alkaloids are eluted and determined colorimetrically by the method of Koch, Levine, and Zenker (1).

An aqueous kaolin suspension containing only kaolin and atropine sulfate at concentrations equivalent to that of commercial preparations was centrifuged to remove the kaolin and a portion of the supernatant assayed according to the color development procedure. Duplicate determinations yielded recoveries of 17.2 and 17.5% (or 82.8 and 82.5% adsorption). The recovery for a similar preparation extracted by the proposed procedure and assayed colorimetrically was 99.7%. One of the problems encountered in developing this method was the relatively high absorbance of the procedural blank. This high blank was found to be caused by impurities in the chloroform. Attempts were made to purify the chloroform by filtering it through columns containing either silica gel or aluminum oxide, without satisfactory improvement. Reagent grade chloroform gave a procedural blank with an absorbance of about 28% of that from a 200 mcg. atropine sulfate standard. The column-purified chloroform gave procedural blanks with absorbances of about 10% of the same standard. However, when the chloroform was purified according to the distillation method of Koch, Levine, and Zenker (1), the procedural blank had an absorbance of about 5% of that from a 200-mcg. atropine sulfate standard.

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Keyphrases

Belladonna alkaloids, neomycin, kaolin, pectin suspension
Microanalysis—belladonna alkaloids
Liquid–liquid extraction—ethanol–ether
Column chromatography—separation
Colorimetric analysis