Steroid Tablet Assay Involving Automated Sample Preparation and Blue Tetrazolium Reaction

By WILLIAM F. BEYER

Automated homogenization, chloroform extraction, and blue tetrazolium assay of hydrocortisone, methylprednisolone, and prednisolone tablets are described. The coefficients of variation for the determination of the three steroids were 1.39, 1.51, and 2.33 per cent, respectively. Tablets are analyzed at a rate of 20/hr. with Technicon's solid preparatory unit and proportioning pump in conjunction with a commercially available spectrophotometer and a strip chart recorder.

THE MANUAL assay of steroid tablets by the blue tetrazolium procedure requires somewhat time-consuming techniques. Directions of U.S.P. XVII (1) and N.F. XII (2) require disintegration of tablets in water and extraction with chloroform. Aliquots of chloroform are then evaporated, and steroids are redissolved in alcohol. Color is developed by the reduction of blue tetrazolium to form the formazan in a solution buffered with tetramethylammonium hydroxide. After standing 90 min. in the dark, color is read at about 525 mμ. Automation of certain aspects of this procedure was described by Greely *et al.* at the New York Academy of Science Conference on Automation in January 1965 (3). The need for a system capable of doing many single tablet assays and the requirement of tablet content uniformity by U.S.P. XVII and N.F. XII for specified tablets led to the development of a completely automated system. Details of the procedure and assay data are the subject of this report.

EXPERIMENTAL

Equipment.—Solid preparatory unit (Solidprep) proportioning pump and 37° water bath.[1] Spectrophotometer and recorder.[2]

Reagents.—3A alcohol;[3] blue tetrazolium, 0.1% in 3A alcohol; chloroform, analytical grade; distilled water with 0.5 ml. of polyoxyethylene lauryl ether[4] added to each 5 gal.; tetramethylammonium hydroxide, 10% aqueous solution diluted 1:10 with 3A alcohol, filtered before use.

Standards.—Accurately weighed quantities of hydrocortisone, methylprednisolone, and prednisolone reference standards are dissolved in 75% 3A alcohol so that each 10.0 ml. contains an amount of steroid equivalent to each tablet.

Tablets.—Steroid tablets are placed in sample cups with 10.0 ml. of 75% 3A alcohol. Cups are covered and allowed to stand for 1 hr. or until tablets disintegrate.

Procedure.—Reagents are pumped using the manifold flow system shown in Fig. 1, and instrumental units are standardized to give a zero base line. The Solidprep is adjusted to deliver 75 ml. of distilled water with polyoxyethylene lauryl ether and mixer speed positioned at No. 4 setting. Approximately 4 cups of the appropriate standard are placed in the sampler head, followed by cups of disintegrated tablets. Standards are placed thereafter at regular intervals to minimize reagent changes and instrumental variation. Samples are automatically homogenized in the blender assembly of the Solidprep. Steroids contained in dilute alcohol are then automatically extracted with chloroform using 3 (6 in.) extraction coils. Blue tetrazolium and tetramethylammonium hydroxide are added directly to the chloroform, and the reaction mixture passes through 2 small mixing coils placed in a 37° water bath. Absorbance of the solution is measured at 524 mμ using a 1.0-cm. flow cell and automatically recorded on a strip chart recorder.

Calculations are made using corresponding absorbances of standards and tablet samples.

RESULTS AND DISCUSSION

Preliminary studies were made indicating that chloroform solutions of steroid could be used satisfactorily in the blue tetrazolium reaction. Aspiration of sample directly from the blender of the Solidprep and extraction with chloroform was carried out initially; however, owing to irregular

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[1] Technicon, Chauncey, N. Y.

[2] Hitachi, Perkin-Elmer 139 spectrophotometer with 1.0 cm. Thomas flow cell No. 9120-NO5, Sargent SRL recorder, A. H. Thomas Co., Philadelphia, Pa.

[3] One hundred gallons of ethanol denatured with 5 gal. of methanol (U. S. Industrial Chemicals Co., New York, N. Y.) constitutes 3A alcohol.

[4] Marketed as Brij 35 by Atlas Chemical Industries Wilmington, Del. Technicon reagent, No. AR-110-62.

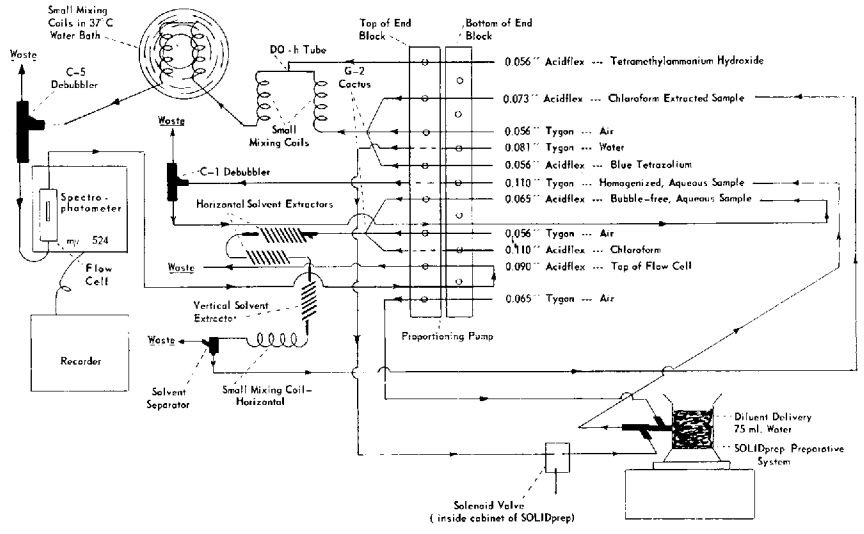

Fig. 1.—Manifold - flow diagram for steroid tablets using AutoAnalyzer Solidprep, solvent extractors and separator, and proportioning pump, in conjunction with a spectrophotometer and strip chart recorder. Transmission lines carrying air or aqueous solutions consist of Tygon tubing, those carrying alcohol or chloroform are acidflex.

sampling, a debubbler was introduced, and a bubble-free sample was withdrawn and extracted. The use of the first two extractors in a horizontal position and the last vertical produced an even pumping action and bubble pattern. Attaching a small horizontal mixing coil to the last extractor permitted satisfactory separation of chloroform and water phases.

On occasion, small acidflex lines would not aspirate reagents until primed with a syringe containing the same solution. Minimal difficulty was encountered when the tubes were placed exactly as shown in Fig. 1. Positioning polyethylene end blocks of the manifold so that the tubes were secured by the rounded ends of the tracts instead of under the projections prevented the acidflex tubes from being sheared. With acidflex tubing larger than 0.090 in., Technicon N-4 nipples had to be used, since pressure from pumping and the action of chloroform on the lines caused a separation of the acidflex tubing from smaller nipples. Wherever possible, acidflex tubing was attached directly to glass fittings.

Following construction of a satisfactory manifold

TABLE I.—PERCENTAGE RECOVERIES OF STEROIDS USING AUTOMATED EXTRACTION AND BLUE TETRAZOLIUM REACTION

Hydrocortisone, 5.0 mg.	Methylprednisolone, 4.0 mg.	Prednisolone, 5.0 mg.
97.6	98.3	100.9
100.4	100.6	102.6
100.6	99.2	98.9
100.0	101.1	95.1
98.4	102.0	96.8
100.4	102.0	101.9
100.0	100.3	100.0
100.0	99.2	101.1
102.6	98.1	100.4
101.0	98.7	101.1
98.4		102.2
Coefficients of Variation		
1.39%	1.51%	2.33%

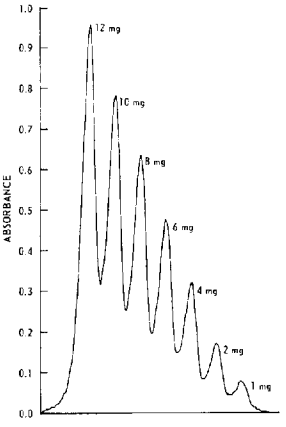

Fig. 2.—Absorbance – concentration recording of hydrocortisone using automatic extraction and blue tetrazolium reaction. Steroid is contained in 10.0 ml. of 75% alcohol, absorbance is measured at 524 mμ, and a 1.0-cm. flow cell is used.

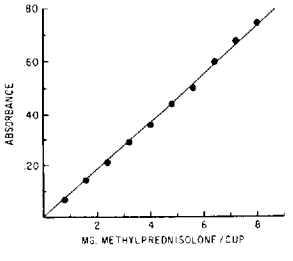

Fig. 3.—Beer's law plot for methylprednisolone.

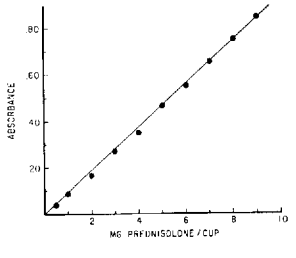

Fig. 4.—Beer's law plot for prednisolone standard.

and optimizing conditions for the blue tetrazolium reaction, the precision of the automated procedure was determined. Replicate standards of hydrocortisone, methylprednisolone, and prednisolone in 75% 3A alcohol were analyzed, giving coefficients of variation of 1.39, 1.51, and 2.33% for the respective steroids. Table I gives results of this study. The precision for the procedure is considered quite satisfactory, considering that this represents the entire assay error from sample preparation, addition of water diluent, homogenization, chloroform extraction, blue tetrazolium reaction, to recording of absorbance peaks. The entire procedure is completed in approximately 8 min. After the first sample, however, additional results are recorded every 3 min.

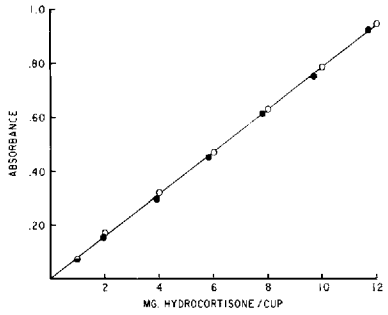

Fig. 5.—Beer's law plot for hydrocortisone originating from standard and tablets. Key: O, hydrocortisone standard; ●, hydrocortisone in pulverized tablet.

Figure 2 shows a recording of various quantities of hydrocortisone standard in 75% 3A alcohol. It can be noted that absorbance is a linear function of concentration. Figures 3 and 4 show that Beer's law is obeyed for methylprednisolone and prednisolone.

To test the recovery of hydrocortisone from varying amounts of 5.0-mg. tablets, 50 tablets were weighed and reduced to a fine powder. Accurately

Table II.—Recovery of Steroids from Various Quantities of Powdered Tablets Using Automated Extraction and Blue Tetrazolium Reaction

Steroid Added from Powdered Tablet, mg.	Amt. of Std. Added, mg.	Total Amt. of Steroid Present, mg.	Amt. of Steroid Recovered, mg.	Recovery, %
		Hydrocortisone Tablets + Std.		
0.98	4.00	4.98	5.00	100.4
1.99	3.00	4.99	5.18	103.8
3.01	2.00	5.01	5.00	99.8
4.00	1.00	5.00	4.73	94.6
		Methylprednisolone Tablets + Std.		
0.78	3.20	3.98	3.87	97.2
1.58	2.40	3.98	3.94	99.0
2.15	1.60	3.75	3.75	100.0
3.19	0.80	3.99	3.87	97.0
		Prednisolone Tablets + Std.		
1.05	4.00	5.05	4.81	95.2
2.17	3.00	5.17	4.98	96.3
3.19	2.00	5.19	5.09	98.1
4.28	1.00	5.28	5.16	97.7

Table III.—Steroid Tablet Assays Using Automated Extraction and Blue Tetrazolium Reaction and Comparisons with a Manual Blue Tetrazolium Procedure

Lot	Automated Procedure: Tablets, No.	Automated Procedure: mg. Steroid/Tablet (± Coefficient of Variation)	Manual Method[a]
	Hydrocortisone Tablets, 5.0 mg./Tablet		
1	10	4.79 ± 3.14%	4.93
2	15	4.93 ± 4.25%	5.04
3	15	5.22 ± 3.91%	5.10
4	15	5.08 ± 4.23%	4.75
5	12	4.52 ± 1.74%	4.90
	Methylprednisolone Tablets, 4.0 mg./Tablet		
6	15	3.91 ± 2.53%	3.96
7	10	3.99 ± 4.27%	4.10
8	15	3.90 ± 3.28%	3.85
9	12	4.00 ± 2.25%	3.90
10	12	3.91 ± 1.79%	4.03
	Prednisolone Tablets, 5.0 mg./Tablet		
11	15	4.89 ± 3.93%	4.83
12	10	5.08 ± 2.95%	5.00
13	10	5.19 ± 2.52%	5.12
14	10	5.20 ± 3.11%	5.17
15	10	5.14 ± 1.95%	4.96

[a] In some instances, the AutoAnalyzer was used for the blue tetrazolium reaction.

weighed quantities of the powder were placed in 10.0 ml. of 75% 3A alcohol and allowed to stand approximately 1 hr. Hydrocortisone standards approximating the steroid content of powder samples were also prepared and analyzed. When absorbances of hydrocortisone standard and powdered tablets are plotted, a single line (Fig. 5) can be drawn through absorbance values for both standard and tablets. Covariance analyses of the data were performed, and no significant differences in results were found.

Studies were performed to determine the efficiency of the automated procedure by adding varying amounts of powdered tablets to a particular standard. Table II shows that recoveries were good.

Assays of steroid tablets were carried out for 5 lots of each particular steroid by the automated procedure and compared to the method essentially as directed by U.S.P. XVII. The data in Table III show good agreement. The automated procedure is suggested for single-tablet assays and should be of value when complying with content-uniformity tests of U.S.P. XVII and N.F. XII.

After completion of this report, several modifications in the automated procedure were made that are considered worthy of reporting. Tetrabutylammonium hydroxide is substituted for tetramethylammonium hydroxide, and filtration of this reagent is no longer a requirement. A small glass wool in-line filter is placed in the flowing stream just prior to the cell debubbler, eliminating the possibility of eroded tubing and other particles entering into the cell.

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Design and Operation of a Laboratory Glass Spray Drier

By JOHN D. TOPHAM

A spray drier made from borosilicate glass is described, which has been used to dry streptomycin without loss of activity and also other pharmaceuticals. Its advantages over other spray driers are: low cost, complete vision of the drying process, and its ease of adaptation to produce sterile powders.

MANY PAPERS have been published on the spray drying of pharmaceuticals (1–5) since the erection of a spray drier at Manchester University in 1939 (6). So far, to the author's knowledge, no work has been published on the spray drying of antibiotics, although one manufacturer has installed a spray drier for this purpose. All the spray driers which are used in industry are made of metal, which is not a satisfactory material when solutions of substances, which are very sensitive to oxidation, are to be dried. Since glass is used to replace metal in the apparatus described, oxidative discoloration does not take place when streptomycin is dried in the apparatus. Also, it is difficult to observe the drying process if metal apparatus is used. These 2 considerations, plus cheapness, persuaded the author to design a spray drier made of borosilicate glass.[1]

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[1] An application has been made to patent the apparatus.

Temperature.—Borosilicate glass softens at 700° and will crack as a result of thermal shock, if sudden temperature fluctuations take place. Consequently, at no time during the operation of the drier should a temperature of 500° be exceeded. In fact, 320° is the maximum temperature to which the apparatus, described in this article, has been subjected. The temperature range within which the spray drier has been used is 140–220°. The normal operating temperature was 160°.

Wetting.—The contact angle between glass and water is zero. Consequently, any drops of solution which come into contact with the walls of the apparatus will adhere. This difficulty has been overcome by silicone coating the apparatus, using a 2% solution of dimethyldichloro-silane in carbon tetrachloride.

Sealing of Joints.—A film of silicone grease was applied to most joints which were then clipped together using simple metal clips.

Spray.—After considerable experimentation it was found that the best spray was produced using an atomizer device constructed from standard laboratory glassware (Fig. 1). This was con-