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ACKNOWLEDGMENTS

J. Cornejo gratefully acknowledges the support of the United States–Spanish Joint Committee for Scientific and Technical Cooperation.

This report is Journal Paper 7952, Purdue University Agricultural Experiment Station, West Lafayette, IN 47907.

Quantitative Semiautomated Colorimetric Determination of Thyroid (Iodine) in Thyroid Tablets

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Received October 1, 1979, from the *Food and Drug Administration, Department of Health, Education, and Welfare, Kansas City, MO 64106.* Accepted for publication March 21, 1980.

Abstract □ A faster, simpler, more sensitive, and semiautomated method was developed for the analysis of thyroid in individual thyroid tablets and composite samples. The sample is combusted in a Schoniger flask in an oxygen atmosphere, and the liberated iodine is trapped in a dilute sodium hydroxide solution. The solution is acidified with sulfuric acid (1:1), and the iodine is determined colorimetrically using a suitable spectrophotometer equipped with an automated analyzer. The proposed methodology was applied to tablets containing 32.4–324 mg of thyroid. Recoveries ranged from 90.0 to 125.0% of the amount of iodine added as potassium iodide. The proposed method is sensitive to 2.5 ng of iodine/ml.

Keyphrases □ Thyroid—semiautomated colorimetric analysis in individual tablets □ Iodine—semiautomated colorimetric analysis in individual thyroid tablets □ Colorimetry—analysis, thyroid in individual thyroid tablets

This paper describes a simple method for the determination of thyroid in individual thyroid tablets and composite samples. The method is fast and reliable. It permits more samples to be analyzed in an equivalent amount of time than previously reported methodology (1–3) and gives comparable results when applied to individual thyroid tablets and composite samples.

BACKGROUND

The USP XIX procedure for content uniformity (1) employs an empty sample wrapper, which is combusted and used as the blank preparation. When these sample wrappers are used for the oxygen flask combustion in the developed procedure, high and erratic blanks are recorded. Experiments (4) indicated that the high blanks are due to the black dye incorporated in the sample wrappers and can be minimized by washing the sample wrappers several times with distilled water and drying before use. For reliable results, the blank must have <3 ng of iodine/ml for this procedure.

In the procedure developed by Graham (2), iodine is determined spectrophotometrically as the triiodide ion, following the oxygen flask ignition of Moody *et al.* (3), from a sample equivalent to 30 mg of thyroid. The USP assay (1) utilizes essentially the same chemical transformation but requires a sample equivalent to 1 g of thyroid and a final thiosulfate titration. Both procedures give comparable results.

Luchtefeld (5) developed a semiautomated procedure, which was based on the kinetic procedure of Sandell and Kolthoff (6, 7) for the determination of iodine in the Total Diet market basket. By combining the procedures and making selected modifications, a method has been developed that is faster, simpler, more sensitive, and capable of being automated.

EXPERIMENTAL

Apparatus—The automated analyzer[1] system consisted of a water bath maintained at 40°, heating coils [6.1-m (20-ft) inner coil and 12.2-m (40-ft) outer coil of 1.6-mm i.d. glass tubing], tubing S[2] and T[3], a spectrophotometer[4] (gears must be adjusted to permit chart scan with stationary wavelength at 405 nm), and 10-mm flowcells. The schematic diagram in Fig. 1 shows the coils, tubing, and fittings.

The combustion system utilizes sample wrappers[5] (which must be washed prior to use), sample carriers[6], stoppers[7], 1-liter Schoniger oxygen combustion flasks[8], clamps[9], and an oxygen flask igniter[10].

Sample Wrapper Preparation—The sample wrappers were placed in a beaker and covered with distilled water. The beaker was placed in a sonic vibrator for 10–15 min, and the water was discarded. The procedure was repeated five times to decrease the amount of iodides present in the sample wrappers. Then the wrappers were placed on a paper towel and allowed to dry at room temperature.

Reagents—*Ceric Ammonium Nitrate Solution*—Ceric ammonium nitrate, 8.66 g, was dissolved in 100 ml of water in a 2-liter flask, 56.8 ml of sulfuric acid (1:1) was added, and the solution was cooled and diluted to 2 liters with water.

Arsenous Acid Solution—Arsenic trioxide, 15 g, was dissolved in 500 ml of water (made basic with 10 ml of 50% NaOH) by heating on a steam bath. The solution was cooled, 56.8 ml of sulfuric acid (1:1) was added, and the solution was cooled and diluted to 2 liters with water.

Sodium Sulfate Solution—To prepare the sodium sulfate solution, 60 ml of 50% NaOH was transferred to a 2-liter flask with 1 liter of water. Sulfuric acid (1:1), 100 ml, was added cautiously. The solution was cooled and diluted to 2 liters with water.

Sodium Chloride Solution—The sodium chloride solution was a 0.5% aqueous solution.

Standard Preparation—To prepare the standards, 0.1308 g of certified ACS grade potassium iodide[11] was dissolved in water, and the solution was diluted to 1 liter to give a concentration of 0.1 mg of iodine/ml. This solution was diluted with the sodium sulfate solution to give a concentration of 0.1 μg of iodine/ml. Standards containing 2.5, 5.0, 10.0, 15.0, 20.0, and 25 ng of iodine/ml were prepared from the resulting solution and the sodium sulfate solution. These solutions are used for the standard curve and should be prepared fresh daily.

[1] AutoAnalyzer, liquid sampler 1, and proportioning pump 1, Technicon Instruments, Tarrytown, N.Y.
[2] Solvaflex. Technicon Instruments, Tarrytown, N.Y.
[3] Tygon, Plastics and Synthetics Division, Norton Co., Tallmadge, Ohio.
[4] Model DK-2A, Beckman Instruments, Fullerton, Calif.
[5] Block, Catalog No. 6414-F70, Arthur H. Thomas Co., Philadelphia, Pa.
[6] Ogg, Catalog No. 6514-F45, Arthur H. Thomas Co., Philadelphia, Pa.
[7] Thomas-Ogg, Catalog No. 6514-F45, Arthur H. Thomas Co., Philadelphia, Pa.
[8] Catalog No. 4980, Arthur H. Thomas Co., Philadelphia, Pa.
[9] Thomas-Ogg, No. 35, Arthur H. Thomas Co., Philadelphia, Pa.
[10] Thomas-Ogg, model 11, Arthur H. Thomas Co., Philadelphia, Pa.
[11] Fisher Scientific Co., Pittsburgh, Pa.

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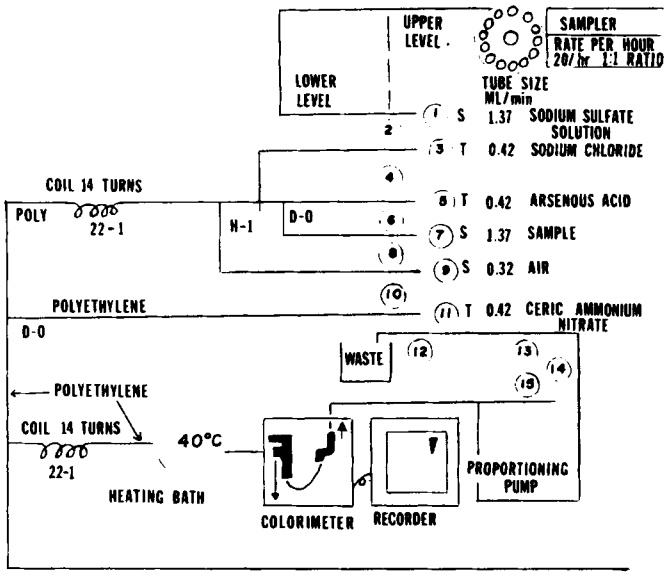

Figure 1—*Flow diagram of semiautomated system for determination of iodine in thyroid tablets.*

Sample Preparation—An amount of tablet or composite sample equivalent to 12–40 mg of thyroid was weighed accurately on a washed sample wrapper. The wrapper was folded, and the sample was transferred to the sample carrier. Thirty milliliters of 0.5 *N* NaOH was placed in a 1-liter Schoniger combustion flask, and the flask was deaerated with a rapid oxygen stream for 4 min with shaking. The mouth of the flask was moistened with water, and the stopper containing the sample was placed immediately in the flask and clamped securely. The joint of the flask around the stopper was moistened with water, and the sample was ignited with the igniter.

After combustion was completed, the flask was shaken vigorously for 1 min and then was allowed to stand for a minimum of 15 min with intermittent shaking. The solution in the flask was transferred quantitatively with water to a 200-ml volumetric flask containing 1 ml of sulfuric acid (1:1), and the solution was diluted to volume with water. Then a 4-ml aliquot was transferred to a 100-ml volumetric flask, and the solution was diluted to volume with the sodium sulfate solution.

Blank Preparation—An empty sample wrapper was subjected to the same procedure as described for sample preparation.

Determinative Step—The automated analyzer pump manifold was set up as shown in Fig. 1. From the water bath, which was maintained at 40 ± 1°, the solution passed through a 10-mm flowcell in a suitable spectrophotometer adjusted to maintain a constant wavelength at ~405 nm and to scan the chart at or near the slowest speed. The reference beam contained an empty 10-mm flowcell.

The instrument was zeroed by placing Tubes 1, 3, 5, and 11 (Fig. 1) in a container of distilled water. The water was pumped through the automated analyzer pump manifold through a 10-mm flowcell in the sample compartment of the spectrophotometer for 30 min. Then the tubes were removed from the water and placed in the following solutions: Tube 1, sodium sulfate solution; Tube 3, sodium chloride solution; Tube 5, arsenous acid solution; and Tube 11, ceric ammonium nitrate solution. The solutions were pumped through the automated analyzer as in zeroing the instrument to establish a baseline (usually from 0.7 to 0.9 absorbance unit). A standard curve (Fig. 2) was obtained by placing the iodine standard solutions in successive cups using a rate of 20/hr. The sample blank and the samples were run in the same manner as the standards.

The iodine content of the samples was determined from the standard curve. Figure 3 shows typical signal peaks used to construct the iodine standard curve.

The percent of iodine per tablet based on the label declaration of thyroid was calculated from:

$$\% \text{ iodine} = \frac{\frac{(S - B)}{1{,}000{,}000} \times DF \times ATW \times 100}{SW \times D} \qquad \text{(Eq. 1)}$$

where S is the nanograms of iodine found in the sample from the standard curve, B is the nanograms of iodine found in the blank from the standard curve, DF is the dilution factor of the sample (5000), ATW is the average tablet weight (grams), 1,000,000 is the factor converting nanograms of iodine to milligrams, SW is the sample weight (grams), and D is the label declaration of thyroid per tablet (milligrams). If the label declaration of thyroid is given in grams per tablet instead of milligrams per tablet, 64.8 mg should be taken as equal to 1 gr.

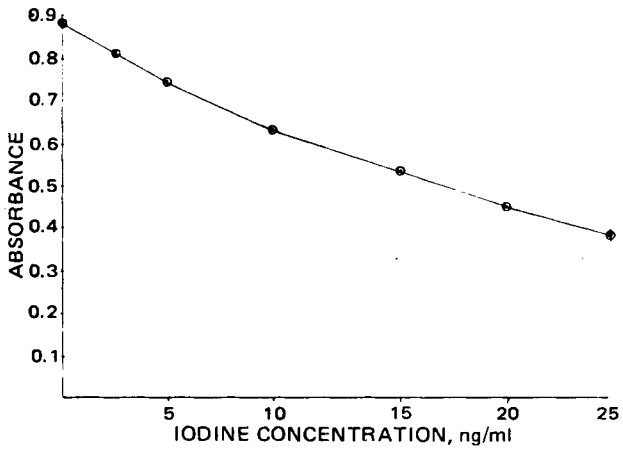

Figure 2—*Standard curve for semiautomated determination of iodine.*

RESULTS

The efficiency of the method was determined by adding known amounts of iodine to the 0.5 *N* NaOH in the combustion flask before the sample combustion and calculating the percent recoveries (Table I).

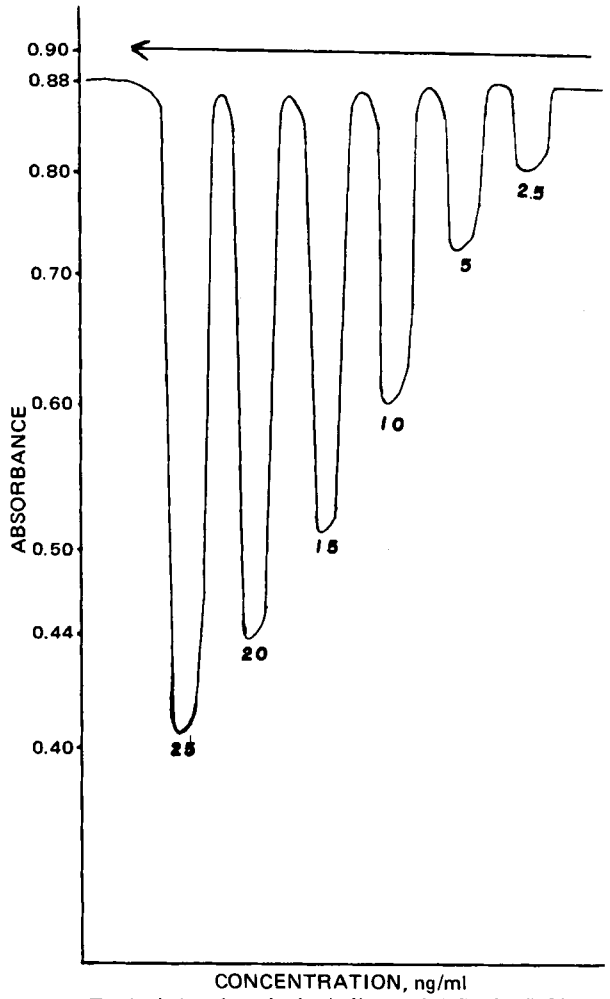

Figure 3—*Typical signal peaks for iodine at 2.5, 5, 10, 15, 20, and 25 ng/ml. Peaks are scanned from right to left.*

Table I—Recovery of Iodine Added to Composite Samples

Sample	Composite	Added, ppm	Found, ppm	Recovery, %
A, 32.4 mg/tablet	1	200	230	115
	2	400	410	102
	3	400	410	102
	4	400	490	122
	5	500	570	114
	6	600	550	92
Average				108
B, 64.8 mg/tablet	1	200	210	105
	2	200	190	95
	3	200	250	125
	4	400	460	115
	5	600	590	98
Average				108
A-1, 64.8 mg/tablet	1	400	360	90
B-1, 120 mg/tablet	1	500	460	92
C-1, 180 mg/tablet	1	222.2	244.5	110
D-1, 240 mg/tablet	1	425.5	468	110
E-1, 300 mg/tablet	1	375	400	107

Four concentrations of uncoated thyroid tablets labeled in milligrams (32.4, 64.8, 130, and 180 mg) and five concentrations of uncoated thyroid tablets labeled in grains (1, 2, 3, 4, and 5 gr) were assayed by the proposed procedure for individual tablets and composite samples. The results were compared to those obtained from the USP assay (1) on the composite samples and are in close agreement (Table II).

DISCUSSION

The primary considerations in developing this semiautomated procedure for content uniformity of individual tablets were its reliability, sensitivity, speed, and simplicity. The proposed procedure addresses all of these concerns.

While this procedure was being developed, much experimentation was required to determine the cause of high blanks. Blanks obtained by using the sample wrappers as received were high, inconsistent, and erratic. In some instances, the absorbance of the blanks indicated that more iodine was present than in the samples; therefore, the quantity of thyroid in the thyroid tablets could not be determined with any accuracy.

Table II—Comparison of Individual Thyroid Tablets and Composite Samples Assayed by the Proposed Procedure and USP (1) Assay for Percent of Labeled Amount of Thyroid

Sample	Composite Sample, %	Individual Tablet Assay[a], %	USP Composite Assay, %
A, 32.4 mg/tablet			
Mean	0.200	0.206	0.216
SD	±0.019[b]	±0.019	±0.005[b]
B, 64.8 mg/tablet			
Mean	0.207	0.214	
SD	±0.008[b]	±0.009	
C, 130 mg/tablet			
Mean	0.213		0.214
SD	±0.007[b]		±0.002[b]
D, 180 mg/tablet			
Mean	0.208		
SD	±0.008[b]		
A-1, 64.8 mg/tablet			
Mean	0.209[c]	0.216	0.200
SD		±0.017	±0.002[d]
B-1, 130 mg/tablet			
Mean	0.200[c]	0.204	0.203
SD		±0.016	±0.002[d]
C-1, 180 mg/tablet			
Mean	0.195[c]	0.198	0.204
SD		±0.012	±0.002[d]
D-1, 240 mg/tablet			
Mean	0.203[c]	0.212	0.204
SD		±0.012	±0.004[d]
E-1, 300 mg/tablet			
Mean	0.214[c]	0.207	0.205
SD		±0.007	±0.005[d]

[a] Assay of 10 individual tablets. [b] $n = 6$. [c] One determination of composite samples A-1, B-1, C-1, D-1, and E-1 was performed using the proposed methodology. [d] $n = 3$.

Table III—Comparison of Analytical Data with and without Solvents Used to Reduce the Amount of Iodine in the Sample Wrappers

Sample	Solvent	Absorbance	Iodine Found, μg
1	Water	0.765	0.1
2	Acetic Acid	0.410	20.8
3	Ethanol	0.382	22.6
4	Methanol	0.351	25.0
5	None		
	Control (1)	0.535	12.4
	(2)	0.515	13.7

A series of four experiments (4) was designed to check experimentally the apparatus as well as the solutions and sample wrappers. The analysis of these results indicated that the high iodine blanks were caused by the black dye incorporated in the paper used to wrap the samples.

The sample wrappers were washed with distilled water, alcohol, methanol, acetic acid, chloroform, acetone, benzene, and heptane in glass beakers of convenient size. The solvents were decanted from the wrappers, and all wrappers were dried first on a steam bath under a current of air and then in a convection oven at 105° (except the wrappers from the water wash, which were allowed to dry at room temperature).

The wrappers from the chloroform, acetone, benzene, and heptane washes were brittle after drying and were discarded. The remaining wrappers (one from each of the water, acetic acid, ethanol, and methanol washes) and two unwashed sample wrappers (controls) were assayed by the proposed procedure, and the amount of iodine remaining in the wrappers was calculated from a standard iodine curve. The results of the analysis (Table III) indicate that the methanol, ethanol, and acetic acid were contaminated with iodine; distilled water was the solvent of choice for decreasing the amount of iodine in the sample wrappers to give negligible iodine blanks.

Several factors influence the determination of iodine in thyroid tablets. One is the increase in absorbance as the temperature is increased. This phenomenon implies that the standards and the sample should be run at the same temperature. This effect also was observed and demonstrated by Moran (8). Another factor is the ionic strengths of the cerium(IV) and arsenic(III) solutions. These solutions should be prepared and maintained as proposed in this procedure if the analysis is to be valid and useful. A previous study (9) supported these views. The final factor is the tubing (S and T); it should be changed when the absorbances of the standard iodine solutions change significantly while the other parameters remain the same.

The reaction rate is not directly proportional to the iodine concentration; therefore, a calibration curve with a slight curvature is obtained (Fig. 2).

In developing this procedure for the analysis of thyroid in thyroid tablets, 0.25 *N* NaOH was used first as the absorbing solution to trap the liberated iodine after the oxygen flask combustion. Low recoveries of iodine were obtained. The next attempt was with 0.5 *N* NaOH, and the recoveries were much improved (Table IV). This contrast in recoveries probably is due to the lower sodium hydroxide concentration not ab-

Table IV—Comparison of Percent Recovery of Iodine Added to Composite Samples Using Two Concentrations of Sodium Hydroxide

Sample	Sodium Hydroxide Concentration, *N*	Composite	Added, ppm	Found, ppm	Recovery, %
A, 32.4 mg/tablet	0.25	1	400	280	70
		2	400	284	71
		3	400	300	75
	0.5	1	400	410	102
		2	400	410	102
		3	400	490	122
B, 64.8 mg/tablet	0.25	1	200	118	59
		2	200	124	62
		3	200	150	75
	0.5	1	200	210	105
		2	200	190	95
		3	200	250	125

sorbing all of the iodine released from the oxygen flask combustion of the tablets.

The combustion apparatus and glassware must be scrupulously cleaned before and after use with distilled water, dilute sodium hydroxide (0.1–0.5 *N*), and again with distilled water to prevent contamination of iodine adsorbed onto these surfaces.

The proposed procedure was not used on coated thyroid tablets, but it should work if a homogeneous sample is obtained.

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ACKNOWLEDGMENTS

The author thanks Ronald G. Luchtefeld, Food and Drug Administration, Kansas City, Mo., for constructing the semiautomated iodine system used.

Semiautomated Method for Analysis of Enteric-Coated and Plain Coated Diethylstilbestrol Tablets

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Received December 13, 1979, from the **National Center for Drug Analysis, Food and Drug Administration, 1114 Market Street, St. Louis, MO 63101*, and the ‡*Food and Drug Administration, Seattle, WA 98174.* Accepted for publication March 19, 1980.

Abstract □ A semiautomated fluorometric method for the analysis of enteric-coated and plain coated diethylstilbestrol tablets is presented. To eliminate interferences from tablet excipients, diethylstilbestrol is extracted into an organic solvent and then into a basic aqueous solution. After UV irradiation, a product of diethylstilbestrol is formed from which a fluorophore is produced chemically. The fluorescence is measured at an excitation wavelength of 335 nm and an emission wavelength of 410 nm. The coefficient of variation measured for the semiautomated procedure was 0.59%. Assay results agreed well with the USP procedure for tablets containing >1 mg of diethylstilbestrol. Tablet dyes and excipients interfered in the USP procedure, which yielded low results for tablets containing <1 mg of diethylstilbestrol. Standard recovery data and assays of tablet composites showed that dyes and other excipients do not interfere with the semiautomated procedure.

Keyphrases □ Diethylstilbestrol—semiautomated fluorometric analysis of enteric-coated and plain coated tablets □ Fluorometry—semiautomated analysis of enteric-coated and plain coated diethylstilbestrol tablets □ Estrogens—diethylstilbestrol, semiautomated fluorometric analysis of enteric-coated and plain coated tablets

Diethylstilbestrol is one of several synthetic estrogens whose use has been the subject of many clinical studies. The estrogenic activity of this stilbenediol derivative was discovered in 1938 (1). In recent years, diethylstilbestrol has been administered for estrogen replacement therapy and treatment of mammary carcinoma and as a contraceptive.

BACKGROUND

The USP (2) content uniformity requirement for diethylstilbestrol tablets increased the analytical workload of many pharmaceutical laboratories. The individual tablet assay described in the monograph is tedious and, because of excipient interference, cannot be used to assay enteric-coated tablets containing <1 mg of diethylstilbestrol. The semiautomated method developed by Hussey *et al.* (3) is similar in principle to the USP XIX procedure (2) but is limited to the analysis of plain tablets and the cores of enteric-coated tablets of dosage levels of >0.25 mg.

The semiautomated method described in this report utilizes a fluorometric determinative step to achieve greater sensitivity for the analysis of low dosage tablets. After UV irradiation of diethylstilbestrol, a fluorophore is produced quantitatively by reducing the irradiation product (4) with an alcoholic hydrochloric acid solution containing 2% pyrocatechol and heating to form a phenanthrenediol. The pyrocatechol increases the fluorescence intensity slightly, which adds to the overall stability of the automated system.

Use of the irradiation product itself as the fluorophore for analysis was investigated by Goodyear and Jenkinson (5). Excipients in enteric coatings were reported to cause some interference, and the fluorescence intensity of the irradiation product without the addition of acid and without heating was not sufficient for quantitation of low dosage tablets in the automated system. However, an intense fluorophore, 3,6-dihydroxy-9,10-diethylphenanthrene, is produced when the UV irradiation product is heated with an alcoholic hydrochloric acid solution containing 2% pyrocatechol. Umberger *et al.* (6) performed a similar reduction by heating the UV irradiation product with acid and bisulfite in alcohol solution to form the phenanthrenediol.

The need to analyze low dosage, enteric-coated diethylstilbestrol tablets from various manufacturers prompted the development of this semiautomated system. The method utilizes the principles and materials of the methods cited, and an extraction step was added to eliminate interferences from tablet excipients.

EXPERIMENTAL

Principles—A solution of diethylstilbestrol in 0.05 *M* alcoholic dibasic potassium phosphate was acidified with 1 *N* HCl and extracted with isooctane–butanol. The drug in the organic phase then was extracted with 1 *N* NaOH. The extract was mixed with phosphoric acid and dibasic potassium phosphate, and the mixture was irradiated with UV light. The irradiation product was reacted with a 2% solution of pyrocatechol in 2 *N* HCl at 70°. The fluorophore thus produced was measured at an excitation wavelength of 335 nm and an emission wavelength of 410 nm.

Apparatus—The automated analyzer[1] system consisted of a sampler, two proportioning pumps, a heating bath at 70°, a fluorometer with a primary[2] and a secondary[3] filter, and a recorder utilizing linear chart paper to record the relative fluorescence intensity. Typical fluorometer

[1] AutoAnalyzer with sampler II, proportioning pump I, and fluorometer II, Technicon Corp., Tarrytown, N.Y.

[2] No. 72786, maximum transmittance at 335 nm, Beckman Instruments, Fullerton, Calif.

[3] No. 5113, 3.8 mm, maximum transmittance at 410 nm, Corning Glass Works, Corning, N.Y.

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