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

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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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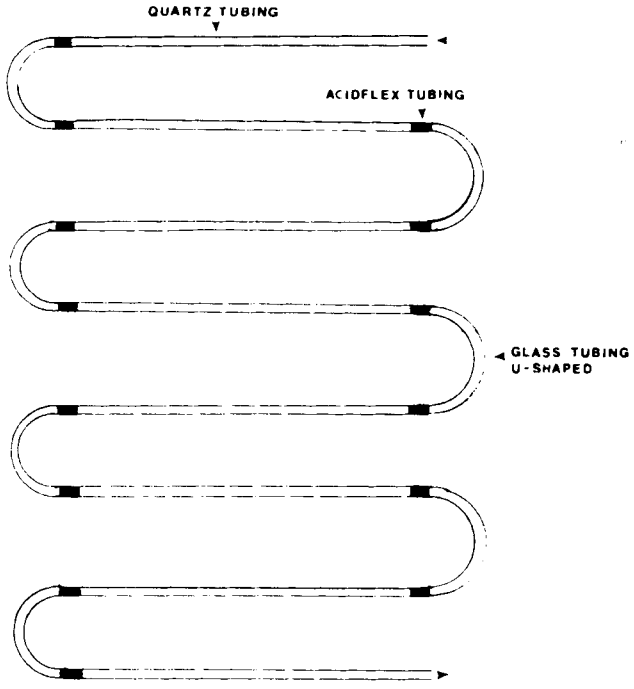

Figure 1—*Section of quartz irradiation coil. The coil consists of 22 37-cm lengths of quartz tubing, 2 mm i.d. × 3 mm o.d., connected by tubing and glass U-tubes. The coil is formed into top and bottom grids, each having 11 lengths of quartz tubing. The grids are shifted out of line to maximize exposure to UV light.*

parameters were: sample aperture, 3; reference aperture, 2; and full-scale recording, 110.

The UV irradiation assembly consisted of clear quartz tubing[4], 2 mm i.d. × 3 mm o.d. and ~9 m long, made into a coil as described by Hussey *et al.* (3) and connected by tubing[5] and glass U-tubes[6] (Fig. 1), and a 15-w germicidal lamp[7] mounted ~10 cm above the quartz coil. Aluminum foil was placed under the coil to provide a reflective background. The entire UV irradiation assembly was covered with aluminum foil for maximum internal reflectance and to prevent leakage of harmful UV radiation.

Reagents—The alcohol used was 95% ethanol.

To prepare 0.05 *M* alcoholic dibasic potassium phosphate, 8.7 g of anhydrous dibasic potassium phosphate was dissolved in 500 ml of water, and the solution was diluted to 1 liter with alcohol.

The 1 *N* HCl was prepared by diluting 83 ml of concentrated hydrochloric acid to 1 liter with water.

For the alcoholic hydrochloric acid, 83 ml of concentrated hydrochloric acid was diluted to 500 ml with water, and the mixture was diluted to 1 liter with alcohol.

The 1 *N* NaOH was prepared by diluting 50 ml of 50% (w/v) NaOH to 1 liter with water.

For the alcoholic phosphoric acid solution, 250 ml of alcohol was added to 60 g of 85% H_3PO_4, and the mixture was diluted to 1 liter with water. The strength of this reagent was adjusted, if necessary, to obtain the correct pH after irradiation.

To prepare the pyrocatechol solution, 20 g of pyrocatechol was dissolved in 500 ml of water, and 178 ml of hydrochloric acid and 250 ml of alcohol were added. The solution was mixed and diluted to 1 liter with water.

The organic solvent was prepared by adding 200 ml of *n*-butanol to 1 liter of isooctane with mixing. *n*-Butanol and distilled-in-glass isooctane that are suitable for spectrophotometry and chromatography were used.

Diethylstilbestrol Standard Solution—Approximately 25 mg of diethylstilbestrol USP reference standard was weighed into a 250-ml volumetric flask. The standard was dissolved and diluted to volume with the alcoholic dibasic potassium phosphate. A 10.0-ml aliquot of the stock standard solution was transferred to a 100-ml volumetric flask and diluted to volume with the alcoholic dibasic potassium phosphate; this solution was stable for at least 10 days. To assay tablets containing <1 mg of diethylstilbestrol, a 10.0-ml aliquot of the stock standard solution was placed in a 100-ml volumetric flask, 40 ml of the alcoholic dibasic potassium phosphate reagent was added, and the mixture was diluted to volume with the alcoholic hydrochloric acid.

Sample Preparation—For tablets containing 1 mg or more of diethylstilbestrol, individual tablets were disintegrated or weighed composites were dispersed in an accurately measured volume of the alcoholic dibasic potassium phosphate reagent to give a concentration of 0.01 mg/ml. Samples were placed in an ultrasonic generator for ~15 min with intermittent swirling to ensure tablet disintegration. Enteric-coated tablets were sonicated until visual examination indicated no trace of intact tablet particles when the sample was swirled. Up to 30 min was required for some products, depending on the enteric-coating formulation. The alcoholic dibasic potassium phosphate reagent was used as the wash solution for the automatic analyzer.

For tablets containing <1 mg of diethylstilbestrol, individual tablets were disintegrated or weighed composites were dispersed in an accurately measured volume of the alcoholic dibasic potassium phosphate reagent to give half of the final volume of the sample solution. The diethylstilbestrol from the tablet material was dissolved by ultrasonic treatment as described. An accurately measured equal volume of the alcoholic hydrochloric acid reagent was added to give a final diethylstilbestrol concentration of 0.01 mg/ml. The solution was allowed to stand until the precipitate had settled. For the automatic analyzer, a wash solution of dibasic potassium phosphate reagent–alcoholic hydrochloric acid reagent (1:1) was used.

Procedure—The automated system was assembled as shown in Fig. 2. The first manifold (Pump A) is the extraction manifold. The second manifold (Pump B) is the irradiation and fluorescence manifold.

The automated system was equilibrated as follows. With the fluorescence manifold pump turned off, the proper reagents were pumped through the extraction manifold. When the B4 fitting on the fluorescence manifold was filled with aqueous and organic layers, the fluorescence manifold pump was turned on and all of the reagents were pumped through the system. Approximately 30 min was allowed for system equilibration. The pH of the phosphate solution was checked after irradiation by collecting, on pH paper, a few drops from the waste line off the C3 resample fitting. A pH of 7–8 was desired.

Portions of the prepared sample and standard solutions were placed in 8.5-ml polystyrene cups on the sampler, which was set to a rate of 20 samples/hr and a sample-to-wash ratio of 2:1. The sampling pattern was three cups of standard, five cups of sample, one cup of standard, five cups of sample, *etc.* Two cups of standard were placed at the end of each run. In the calculations, the first two and the last standard peaks were disregarded.

In the automatic analyzer, the sample was withdrawn, segmented with air, and acidified with 1 *N* HCl. Isooctane–butanol was added and mixed, and the aqueous and organic phases were separated. The organic phase, which contained the drug, was resampled, segmented with air, and mixed with 1 *N* NaOH. The aqueous phase, which then contained the diethylstilbestrol, was separated and mixed with alcoholic dibasic potassium phosphate and phosphoric acid. The solution was irradiated in a quartz coil under a germicidal lamp. The solution was resampled, mixed with 2% pyrocatechol in 2 *N* HCl and alcohol, and heated at 70°. The solution, which then contained a fluorophore of the diethylstilbestrol irradiation product, was cooled and passed through the fluorometer. The fluorescence was measured at an excitation wavelength of 335 nm and an emission wavelength of 410 nm. Typical peaks from the analyzer are shown in Fig. 3.

RESULTS AND DISCUSSION

Four diethylstilbestrol standard solutions having concentrations of 50, 100, 150, and 200 μg/ml were passed through the analyzer. A graph of the fluorescence intensities of these solutions *versus* concentration resulted in a straight line that passed through the origin.

A sampling rate of 25 cups/hr was tested with standard solutions. However, the coefficient of variation for the system increased to 2.00% at 25 cups/hr from 0.60% at 20 cups/hr. Moreover, the percentage of steady state (defined as 100 times the ratio of the sample peak intensity to the steady-state signal intensity) achieved by the peaks decreased from 94.4 to 84.0%.

A composite sample of enteric-coated, 1-mg tablets was prepared from a commercial product. Twenty weighed portions of this composite, each equivalent to a single tablet, were assayed by the semiautomated method. The coefficient of variation obtained was 1.09% (Table I). In addition,

[4] W. A. Sales, Deerfield, Ill.
[5] Acidflex, Technicon Corp., Tarrytown, N.Y.
[6] No. 116-0223, Technicon Corp., Tarrytown, N.Y.
[7] No. E-15763, Crescent Manufacturing Co., Philadelphia, Pa.

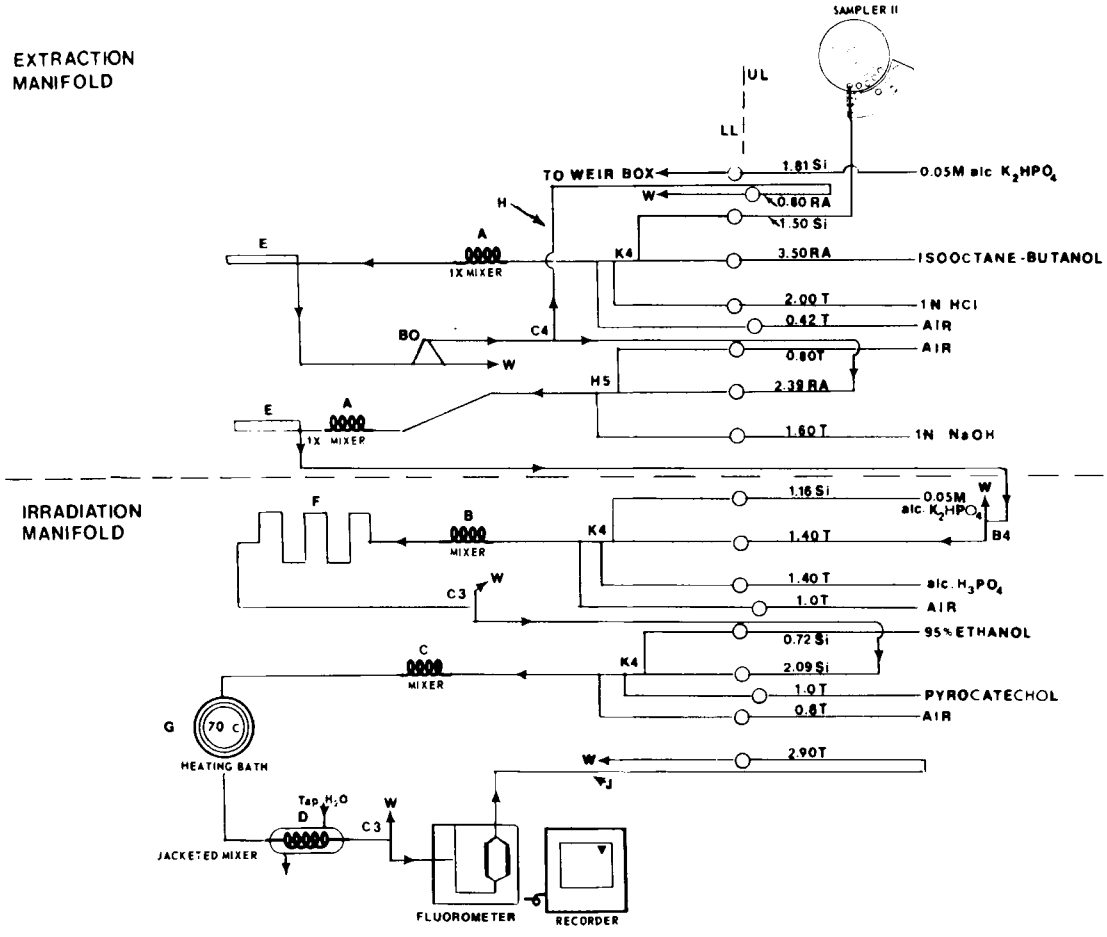

Figure 2—*Flow diagram for the determination of diethylstilbestrol in tablets. Key: A, 1× mixer, 7 turns, 1.0 mm i.d.; B, 1× mixer, five turns, 2 mm i.d.; C, 1× mixer, 14 turns, 2 mm i.d.; D, jacketed mixer, 14 turns, 2.4 mm i.d.; E, delay (settling) coil, 5.5 turns, 2.4 mm i.d.; F, irradiation assembly, consisting of quartz coil (Fig. 1) and 15-w UV lamp; G, heating bath, containing 12-m (40-ft) glass coil, 1.6 mm i.d., set at 70°; H, polyethylene tubing, 0.82 mm (0.034 in.) i.d.; and J, Tygon tubing, 1.59 mm (0.0625 in.) i.d. Transmission tubing is glass, 2 mm i.d. For the pump tubes, RA is red Acidflex, T is Tygon, and Si is silicon; W indicates flow to waste reservoir.*

a single determination on each of 10 days was performed on the same composite to test the day-to-day reproducibility of the system, and the coefficient of variation obtained was 0.59%. The individual standard solutions used in the tests were assayed by comparison to freshly prepared standard solutions. All of the standard solutions were stoppered and stored on a bench top, exposed to room light and to changes in temperature and humidity. These solutions were reexamined after 8 days, and no decomposition was observed.

Table I—Reproducibility Data for 1-mg Diethylstilbestrol Enteric-Coated Tablets Using the Semiautomated Method [a]

	Reproducibility	
	Same Day	Day-to-Day
	102.4	102.0
	101.3	102.2
	100.1	102.3
	101.1	102.2
	104.6	102.3
	102.3	102.4
	100.6	102.2
	100.8	103.2
	99.9	101.0
	100.9	101.3
	101.3	
	101.2	
	102.2	
	99.6	
	100.7	
	101.9	
	100.2	
	101.8	
	101.1	
	101.3	
Mean (20)	101.3	(10) 102.1
SD	1.11	0.60
CV, %	1.09	0.59

[a] Results are reported as percent of label declaration.

Seventy-six cups of a solution prepared from a composite of plain coated tablets were passed through the system. No serious evaporation effects were observed. However, since the samples were dissolved in a 50% alcoholic solution and the sampler was set at 20 cups/hr, the cups were filled and the trays were loaded just before analysis.

Enteric-coated tablets usually are difficult to disintegrate. Normally, an enteric-coated tablet must be crushed or the outer shell must be cracked before the solvent is added; otherwise, tablet disintegration with an ultrasonic generator proceeds slowly and a uniform dispersion of tablet material is not obtained. Use of alcoholic dibasic potassium phosphate as a solvent proved successful for the disintegration of enteric-coated diethylstilbestrol tablets. With the aid of an ultrasonic generator, this solvent disintegrated the tablets into a fine dispersion; the outer shell disintegrated, the tablet core slowly disintegrated, and complete tablet disintegration occurred in a reasonable period. The task of cracking the outer shell of each tablet before solvent addition thus was eliminated.

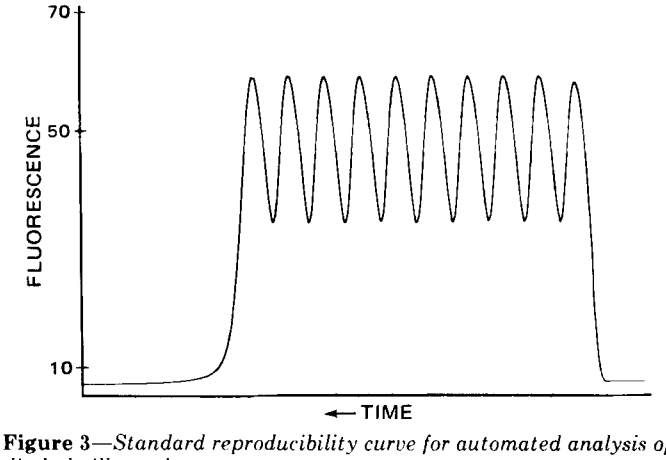

Figure 3—*Standard reproducibility curve for automated analysis of diethylstilbestrol.*

Table II—Comparison of the Semiautomated Method with the USP XIX Method for Assay of Diethylstilbestrol Tablets [a]

Dosage	Semiautomated	USP
Plain coated, 5 mg	96.0	96.5
Plain coated, 5 mg	100.0	99.8
Plain coated, 1 mg (mean)	99.3 (5)	98.6 (2)
CV, %	0.32	
Enteric coated, 5 mg (mean)	100.2 (5)	97.7 (2)
CV, %	0.48	
Enteric coated, 1 mg (mean)	99.2 (5)	98.7 (1)
CV, %	0.54	
Enteric coated, 0.25 mg (mean)	99.0 (5)	95.6 (2)
CV, %	0.68	
Enteric coated, 0.1 mg (mean)	99.8 (5)	86.1 (2)
CV, %	1.24	

[a] Results are reported in percent of label declaration. Numbers of samples analyzed are given in parentheses.

A white precipitate formed in the coils when the enteric-coated tablets from one manufacturer, which contained <1.0 mg of diethylstilbestrol, were assayed. The inner coating of the tablet contained a plasticizer that precipitated when treated with acid on the extraction manifold. The problem was solved by addition of the alcoholic hydrochloric acid reagent to the sample, which previously had been treated with the alcoholic dibasic potassium phosphate reagent, *before* sampling on the automatic analyzer. The precipitate formed and was allowed to settle, and a portion of the supernate then was placed in a cup and sampled.

The extraction manifold was designed to eliminate excipient interference. A mixture of isooctane and butanol was selected as the organic solvent; butanol was added to isooctane to achieve increased polarity for complete extraction of diethylstilbestrol. Dyes present in the outer shells of the enteric-coated tablets remained in the aqueous layer and proceeded to the waste reservoir.

In initial experiments, a coil made from polytetrafluoroethylene[8] tubing was used in the irradiation assembly. However, this tubing was unsuitable; pulsing occurred in the coil because of the presence of air between sample and wash segments. A quartz irradiation coil similar to that described by Hussey *et al.* (3) resulted in a more even flow and more complete irradiation. The extraction step provided a cleaner solution for irradiation and decreased the possibility of interferences in the fluorometric measurement. A 70° temperature in the heating bath for the reaction with pyrocatechol produced the maximal fluorescence.

Results from the semiautomated method compared closely with results from the USP XIX content uniformity method (2) when ground composites of enteric-coated and plain coated 1-mg diethylstilbestrol tablets were analyzed. Ten determinations by the semiautomated method on a ground composite of enteric-coated tablets resulted in a mean of 102.1% of the label declaration and a coefficient of variation of 0.59%. Five determinations on the same composite by the USP method gave a mean of 102.5% of the label declaration and a coefficient of variation of 1.31%. Twenty determinations of a ground composite of plain coated tablets by the semiautomated method resulted in a mean of 95.4% of the label declaration and a coefficient of variation of 0.66%. Five determinations on the same composite by the USP method resulted in a mean of 96.0% of the label declaration and a coefficient of variation of 0.91%.

Composites of enteric-coated and plain coated tablets, obtained from four manufacturers and labeled as containing 0.1–5 mg of diethylstilbestrol, were analyzed by the semiautomated and USP assay methods. Results agreed closely for plain coated and enteric-coated tablets containing 1 mg or more of diethylstilbestrol (Table II) but were low when enteric-coated tablets containing <1 mg of diethylstilbestrol were assayed by the USP method. The large amount of tablet material required for the assay of low dosage, enteric-coated tablets interfered with the USP extraction procedures. Two major problems were encountered: emulsions occurred in the extraction step, and tablet dyes interfered with the color produced by irradiation. These problems were not encountered in the semiautomated method.

A recovery experiment was performed on solutions containing diethylstilbestrol reference standard plus tablet dye and excipients using the semiautomated and manual assay procedures. Two standard solutions, each containing dye to simulate a sample solution of a 0.1-mg diethylstilbestrol tablet, were analyzed by the semiautomated method, and a standard recovery of 100.7% was obtained from each. Two similar standard solutions, containing both dye and excipients, were analyzed by the semiautomated method and gave standard recoveries of 100.8 and 100.6%. The automated analysis of two additional standard solutions of diethylstilbestrol and dye, each equivalent to a sample solution of a 0.25-mg diethylstilbestrol tablet, yielded standard recoveries of 100.1 and 100.3%. A standard solution of diethylstilbestrol, assayed by the USP method, gave a 100.8% standard recovery at a level of 0.1 mg of diethylstilbestrol/tablet. However, a standard solution corresponding to the same diethylstilbestrol level but containing dye and excipients resulted in recovery of only 92.4%. These results indicated that tablet excipients that gave problems in the USP method did not interfere in the semiautomated procedure.

The addition of an extraction and a fluorometric determination to a previously published automated method has made it possible to assay low dosage, enteric-coated tablets without interference from tablet dyes and other excipients. The semiautomated method is reproducible, accurate, and precise and was used to analyze 1500 enteric-coated and 800 plain coated diethylstilbestrol tablets in a survey for content uniformity.

[8] Teflon.

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