Semiautomated Method for Determining Diethylstilbestrol in Low Dosage Tablet Formulations

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Abstract □ A semiautomated analytical system was developed for the assay of diethylstilbestrol in tablet preparations. The automated methods are similar in principle to the USP manual procedure. The heart of the assay lies in the use of a coiled quartz capillary tubing with reflectorized backing as the irradiation cell. This method is suitable for concentrations of 0.5 mg. or greater active ingredient per tablet.

Keyphrases □ Diethylstilbestrol—analysis in low dosage tablet formulations, semiautomated method □ Tablets, low dosage diethylstilbestrol formulations—semiautomated method □ Analysis—diethylstilbestrol in low dosage tablet formulations, semiautomated method

A semiautomated method was developed for the determination of diethylstilbestrol in low dosage tablets. The method is an extension of the manual assay procedure reported by Goodyear *et al.* (1) in 1954, with the inclusion of techniques described by Banes (2, 3) and Doyle *et al.* (4). The method is similar to the procedure found in USP XVIII (5) and has been demonstrated to yield the same assay results as the USP XVIII method for tablets and raw materials. The continuous flow irradiation technique is similar to that previously reported by Skeggs and Hochstrasser (6), Grasshoff (7), Heinicke *et al.* (8), Love and McCoy (9), and Dowd *et al.* (10).

EXPERIMENTAL

Equipment—The analytical train consisted of the following modules: Technicon Liquid Sampler II, Technicon Proportionating Pump III, a spectrophotometer[1] with 5-mm. flow cell, and a recorder[2]. A 435-w. lamp[3] served as the irradiation source of UV light. The irradiation cell[4] was a quartz capillary tubing of 2-mm. i.d. and 4-mm. o.d. The capillary tubing, of 6.0-ml. liquid capacity, was glass-blower bent into a compact grid 160 mm. long per turn [15.8 cm. (6.25 in.)] (Fig. 1). The capillary was backed with an aluminum reflector to intensify the illumination. The position of the capillary was then adjusted with the lamp on and a sample flowing at steady state. The final adjustment was made so that the yellow chromophore was essentially completely produced by the time the sample passed through the first 25% of the capillary tubing. A mere visual examination, using safety glasses to avoid eye injury, proved adequate for this adjustment. A hood was also employed. The hood served to remove ozone and helped to carry heat away from the irradiation cell.

Reagents—For the 50% ethanol–water reagent, add 500 ml. of SD 3A ethanol to 500 ml. of deionized water in a 1-l. volumetric flask. Mix well but do not dilute to volume.

For the pH 9.7–9.8 buffer (as measured in 50% ethanol), dissolve 5 g. of anhydrous dipotassium hydrogen phosphate in 500 ml. of deionized water in a 1-l. volumetric flask. Add 500 ml. of SD 3A ethanol slowly and with stirring. Do not dilute to volume.

Standards—Dissolve approximately 5 mg. of the reference standard *trans*-diethylstilbestrol, weighed accurately, in 100 ml. of 50% ethanol–water in a 100-ml. volumetric flask. Use ultrasonics for 15 sec. to aid dissolution. Approximately once a month, test the linearity of the assay system. For the linearity test, use approximate weights of 7, 5, 3, and 1 mg. weighed accurately. Dissolve these in the same manner as the 5-mg. reference standard.

Sample Preparation—The individual tablet or core of enteric tablet was weighed. The formulation was then thoroughly ground with a mortar and pestle. The ground formulation was then transferred to a suitable volumetric flask so that the final concentration in the flask would be 0.5 mg./10 ml. Deionized water equal to half the labeled capacity of the flask was added by pipet. Ultrasonic vibration for 15 sec. was employed. Pure alcohol equal to half the labeled capacity of the flask was then added by pipet. The flask was diluted to volume with 50% ethanol–water and allowed to stand for 30 min. prior to assay. The contents of the flask were then filtered through filter paper[5] (medium porosity), and the filtrate was placed into sampling cups of 5-ml. capacity.

Table I—Effect of Excipients on Assay when Added to Standard Diethylstilbestrol

Excipient	Milligrams	Assay, %
Talc	52	100.2
	102	100.2
	216	100.2
Magnesium stearate	20	100.0
	48	100.0
	106	100.2
Starch	62	100.8
	125	100.0
	260	100.0
Lactose	11	99.8
	27	100.2
	51	99.8
	99	100.0

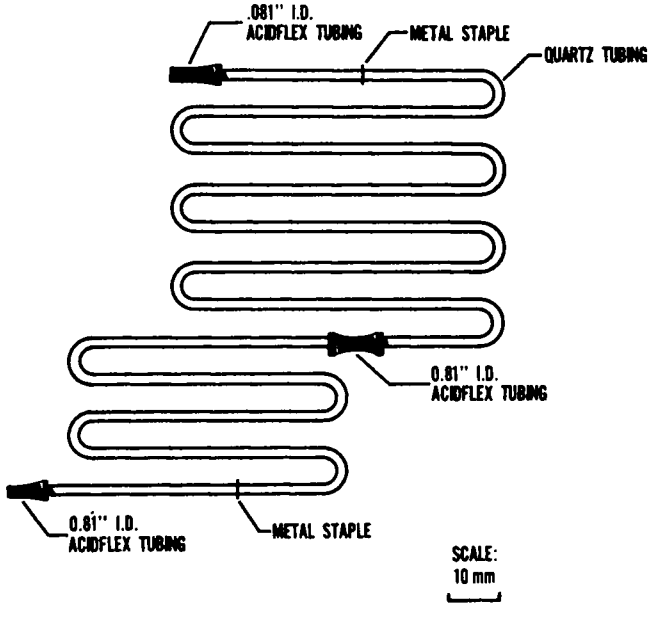

Figure 1—*Irradiation coil design for determination of diethylstilbestrol.*

[1] Turner 330.
[2] Beckman.
[3] Hanovia type 2, serial 7420, equipped with tube 727723.
[4] General Electric, Willoughby, Ohio.
[5] SS 560.

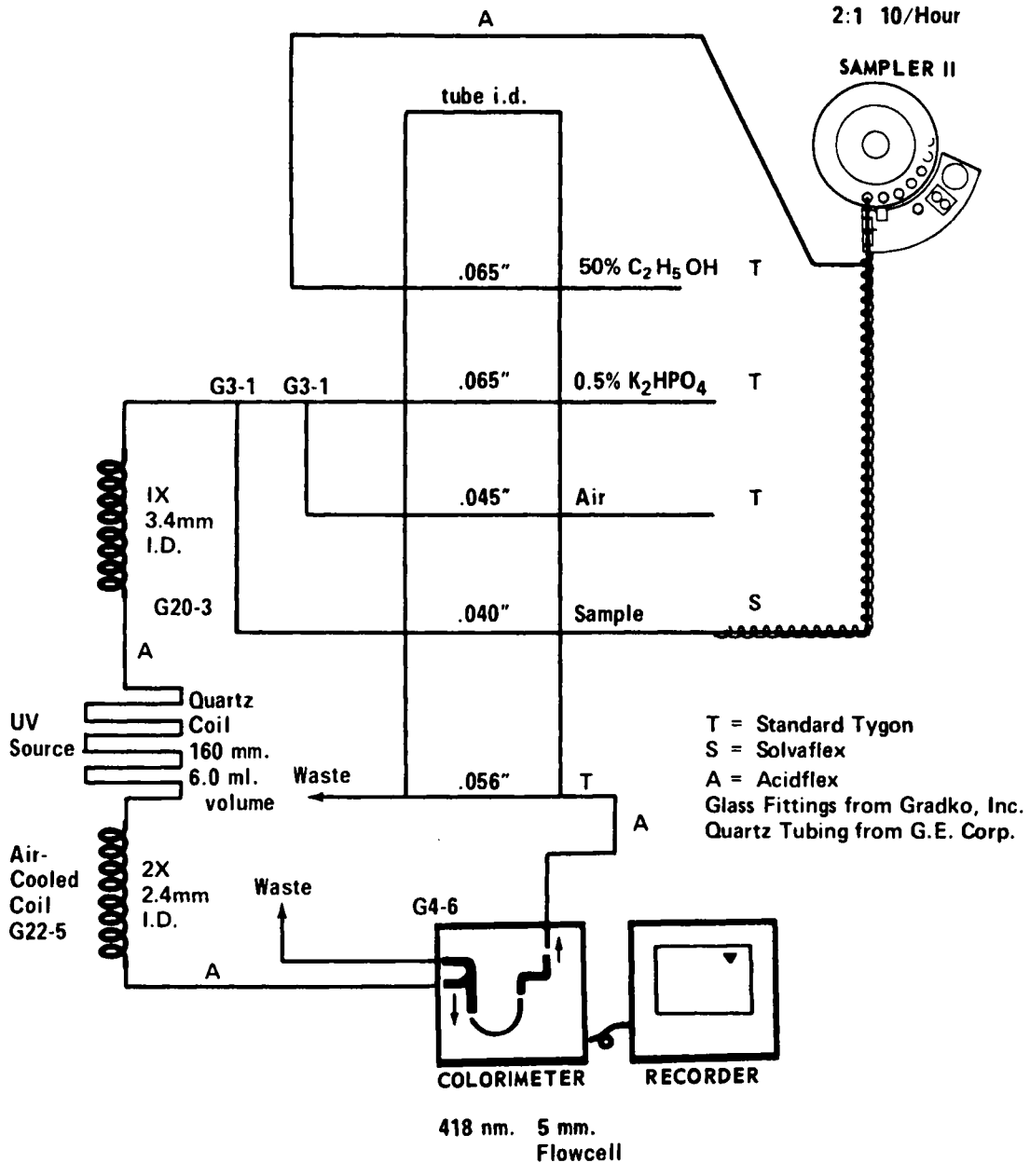

Figure 2—*Autoanalyzer schematic for determination of diethylstilbestrol.*

Procedure—A schematic diagram of the automated system is shown in Fig. 2. A typical assay run is shown in Fig. 3.

DISCUSSION

Two limitations were encountered in the development of this assay. First, tablets of dosage less than 0.25 mg. could not be assayed, owing to sample handling and volume considerations. Second, tablets containing visible dyes, such as core-coated (or finished) enterics, could not be assayed.

The introduction of water prior to alcohol in the tablet dissolution was necessary for complete diethylstilbestrol recovery. Lactose, magnesium stearate, talc, and starch were examined separately and together, both in the presence of diethylstilbestrol and alone, and were shown not to interfere (Table I).

Occasional noise found in the system was traced to aged acidflex tubing. The acidflex tubing (used for its opaqueness) was then changed every 2 weeks as a precaution.

The position of the UV radiation source was the most critical aspect of the analysis. The position needed to be arranged so that the yellow chromophore production occurred in the first 25% of the quartz irradiation coil. Hold time in the coil is approximately 3 min. Visual checking of the chromophore formation, using safety glasses, proved just as adequate as checking the rise-time to steady state and was easier.

Table II—Reproducibility of Assay, Composite Tablets[a]

Day of Assay	Formulation 1, mg./Tablet	Formulation 2, mg./Tablet
19	—	0.248
18	0.501	0.254
	—	0.254
17	—	0.254
15	0.496	—
	0.495	—
2	0.494	—
1	0.496	—
	0.495	—

[a] Relative standard deviation for Formulation 1 was 0.52%; for Formulation 2, 1.20%.

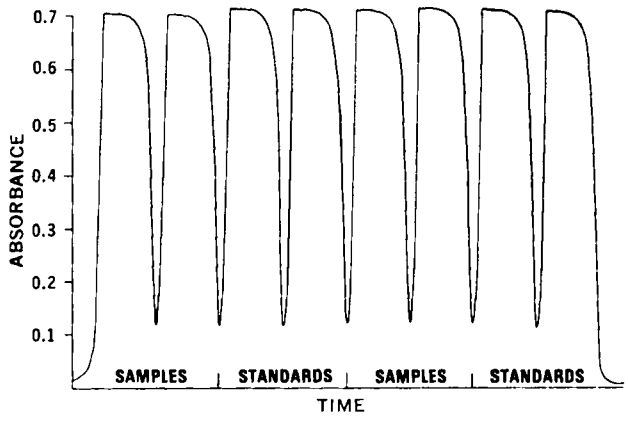

Figure 3—*Typical autoanalysis recording.*

Because of the time required for chromophore formation and wash in the assay, a sampling of 10/hr. was determined to be the maximum rate allowable.

RESULTS

Standard Curves—The absorbances, at 418 nm., of four solutions in the concentration range 0–60 mcg./ml. were measured. A plot of the four values gave a straight line passing through the origin.

Reproducibility of Standards—The reproducibility of the method was checked. The coefficient of variation was 0.005 on standards run as samples (or 0.5% expressed as relative standard deviation).

Reproducibility of Samples—Seven dosage forms, all containing magnesium stearate, starch (and/or paste), lactose, and talc, were automated. The standard deviation for all dosage forms was essentially the same. The method gave day-by-day reliability as well. Table II indicates the typical reproducibility of the assay over 19 days. Not all formulations were run on the same day. Table III compares the manual procedure of USP XVIII to the automated procedure. In this comparison, all samples equivalent to one tablet or core of enteric tablet were weighed from composite grinds to eliminate nonhomogeneity from the comparison. The method has since been employed on a unit dose basis for all seven dosage forms mentioned.

SUMMARY

A semiautomated method for the determination of diethylstilbestrol in tablet dosage forms was described. The automated procedure proved reliable and provided a threefold reduction in assay man-hours.

Table III—Correlation of Automated Method to Manual Method, Composite Tablets

Dosage Form	Strength, mg.	Lot	Manual	Automated
Tablet	0.250	1	0.250	0.242
		2	0.252	0.251
Tablet	0.500	1	0.490	0.482
		2	0.497	0.497
Tablet	1.0	—	1.00	1.04
Tablet	25.0	—	24.8	24.7
Enteric core	0.250	—	0.246	0.250
Enteric core	1.0	—	1.01	1.02
Enteric core	5.0	—	4.90	4.91

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