Content Uniformity Test for Esterified Estrogen Tablets

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Abstract □ A rapid, automated, analytical procedure for the content uniformity of esterified estrogen tablets is described. The test is based on the measurement of sulfuric acid-induced fluorescence; it uses, as the standard, the batch of esterified estrogen powder used to manufacture the tablets. Data on content uniformity are given, and examples of calibration curves are shown.

Keyphrases □ Estrogen (esterified) tablets—content uniformity test □ Automated analysis—esterified estrogen tablets, content uniformity □ Diagram—automated analyzer, esterified estrogen tablets

The present emphasis on control of tablet content uniformity (1) prompted the search for a rapid and precise test for esterified estrogen tablets. No such procedure is available in the literature, and the assay available in the USP XVIII (2) is much too slow and tedious to be useful as a content uniformity test.

The acid-induced fluorescence of estrogens is a well-known phenomenon (3) and has been useful in automated analytical schemes (4–6). All such assays use a sample of the pure estrogen to generate a calibration curve, which is continuously updated with replicate standards and is used to determine the estrogen content of the samples. Because of the variable composition of esterified estrogens (2), it is not possible to obtain a single material that would be a valid standard for all batches of tablets. A solution to this problem is achieved by using as the standard a sample of the same lot of esterified estrogen powder that is used to prepare the batch of tablets. The esterified estrogen powder to be used was previously assayed by the USP XVIII procedure (2) and found acceptable.

This paper reports on the development of such a content uniformity test.

EXPERIMENTAL

Apparatus—A liquid sampler[1] and proportioning pump[1] are connected to a filter fluorometer[1] as shown in Fig. 1. The excitation filter is a Wrattan 47B[2] and the emission filter is a Wrattan 4[2]. Neutral density filters passing 1, 10, or 25% of the fluorescence are used, depending on the sample size. The water-jacketed mixing coil[3] is modified by the addition of an input line, 1 mm. in diameter, located three coils from the input end of the mixing coil. The fluorometer signal is recorded on a standard single or two-pen recorder[1]. A high-speed kitchen blender[4] equipped with semimicro blades and a stainless steel mixing vessel[5] of 360-ml. capacity with a friction-fit stainless steel cover is used to prepare samples and standards.

[1] Technicon Corp.
[2] Kodak Co.
[3] Technicon Corp., Part No. 114-0209-01.
[4] Waring Co., Winsted, Conn.
[5] Eberbach Co.

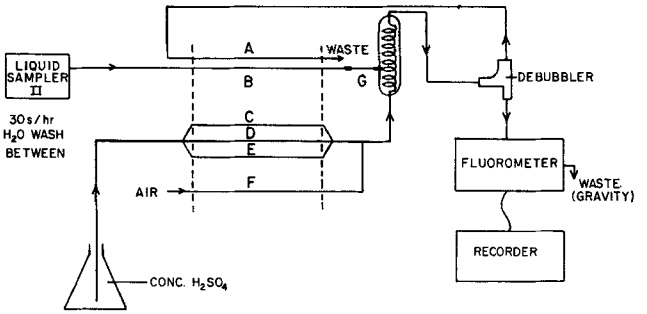

Figure 1—*Autoanalyzer flow diagram for content uniformity of esterified estrogen tablets. Composition and internal diameter (inches) of pump tubes are: A, acid flex, 0.056; B, clear standard, 0.065; C, D, and E, acid flex, 0.110; and F, clear standard, 0.040.*

Reagents—Concentrated sulfuric acid[6] and esterified estrogen powder from the same batch used to manufacture the tablets are used.

Standard Preparation—Esterified estrogen powder from the same lot used to make the tablets is used as the standard for the content uniformity test. Accurately weigh a quantity of esterified estrogen powder exactly equal to 10 times the target amount in a single tablet into the blender vessel, add a 100-ml. aliquot of water, cover, and blend until the contents are homogeneous. Decant and centrifuge the solution until clear. Dilute 6-, 8-, 10-, and 12-ml. aliquots of the clear solution to 100 ml. with water to give solutions corresponding to 60, 80, 100, and 120%, respectively, of the target weight of esterified estrogen powder per tablet.

Sample Preparation—Place a single tablet in the blender, add 100 ml. water, and blend until homogeneous. Filter a portion (about 20 ml.) of the homogenate through filter paper[7], collect in a screw-cap test tube, and cap.

Selection of Neutral Density Filters—Put a portion of the 100% standard solution into the liquid sampler and run it through the system. If the recorder peak height is off scale or less than half-scale deflection, then use the next larger or smaller neutral density filter, respectively. Repeat the procedure until the 100% standard response is approximately 50% of full-scale deflection.

Assay Procedure—The sampling probe of the liquid sampler is aligned to achieve satisfactory aspiration from the sample and wash cups. The fluorometer slit is set at 30× and the liquid sampler at 30 samples/hr. After about 1.0 hr. of conditioning of the flow system using 100% standards, the standards and samples are introduced. The set of four theory standards (60, 80, 100, and 120%) in quadruplicate is sampled initially, and the average fluorescence value is determined for each set. Five samples are then assayed, followed by the 100% standard in duplicate. If the mean of the 100% standards is different from the mean of the 100% standards of the calibration curve by less than 2%, the esterified estrogen powder content of the samples is read directly from the calibration curve. If the difference of the means is greater than 2%, a new calibration curve is drawn, passing through the new 100% standard point and parallel to the original calibration curve. The results of the five samples associated with the adjusted calibration curve are then determined. This procedure corrects for any slight baseline drift.

[6] Du Pont Yellow Label.
[7] Whatman No. 1.

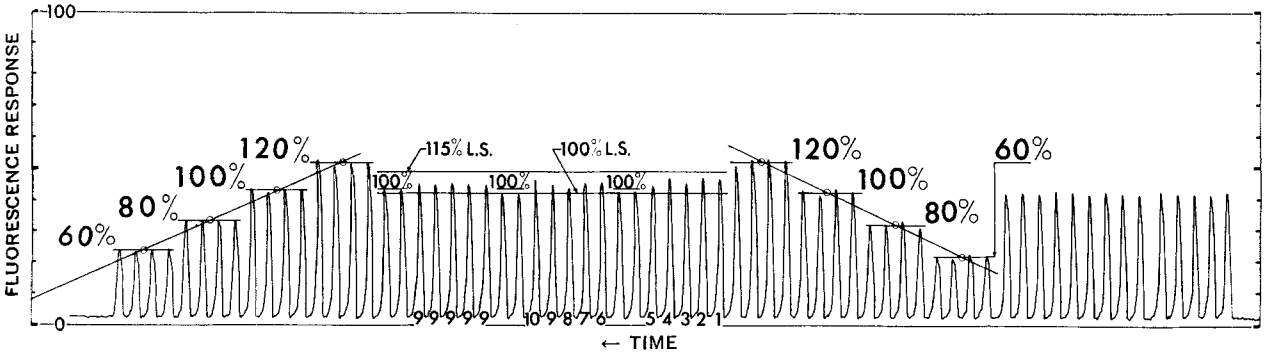

Figure 2—*Typical autoanalyzer curves showing linearity of the fluorescence response of esterified estrogen powder standards and the content uniformity of typical tablets. Peaks 1–10 are individual tablet results. Tablet 9 was run repeatedly to demonstrate precision. Target strength for all tablets is 107.5% L.S.*

RESULTS AND DISCUSSION

An example of a typical recorder output is shown in Fig. 2 and demonstrates the linearity of the calibration curve. Typical recorder peaks of production tablets and repeated runs of a single-tablet solution are also shown. The precision of the test is shown by the 95% confidence interval (106.6 ± 0.44%) calculated from the six replicate determinations of a single-tablet extract. The tablets contain a 7.5% overage which is reflected in the result. Typical results for a production batch of esterified estrogen tablets are given in Table I and in Fig. 2. All results are within the USP limits of 85–115% of label claim.

The validity of this test is based on the fact that the esterified estrogen powder used as the standard was previously assayed for estrone sulfate, equilin sulfate, and total estrogen sulfates by the USP XVIII procedure and found acceptable. Therefore, based on the content uniformity test results and the USP assay, it is possible to control adequately the absolute amounts of estrone and equilin sulfates present in a tablet, even though the fluorescence response may include substances in the esterified estrogen powder other than estrone and equilin sulfates. The variation of fluorescence response between lots of esterified estrogen powder has no effect on the validity of the uniformity test, because each batch of esterified estrogen powder is used as its own standard. A comparison of fluorescence responses of several batches of esterified estrogen powder is shown in Table II. The response of D is much higher than that of A, but the estrogen sulfate content, as measured by the USP XVIII assay, is within 2% of Sample A. This indicates that appreciable variation in the kind and/or quantity of unspecified material in esterified estrogen powder can occur. It was found that the fluorescence response of intentionally degraded tablets is significantly less than the response for undegraded tablets, but correlation of the results with the USP assay showed that the uniformity test overestimates the extent of degradation.

It should be possible to use this test to control the content uniformity of conjugated estrogen tablets (2) as well as esterified estrogen tablets, since the active principle differs only in quantity and not in type.

Table I—Typical Content Uniformity Results of a Production Batch of Esterified Estrogen Tablets

Tablet	Percent Target	Tablet	Percent Target	Tablet	Percent Target
1	101.4	11	97.2	21	97.7
2	97.7	12	100.9	22	94.9
3	101.4	13	93.0	23	96.7
4	99.5	14	93.0	24	99.7
5	97.7	15	94.9	25	93.0
6	99.5	16	101.4	26	94.9
7	99.5	17	101.4	27	97.7
8	101.4	18	94.4	28	97.7
9	96.7	19	102.8	29	98.8
10	96.7	20	102.3	30	95.3

Mean = 97.9% of target

Table II—Variation of Sulfuric Acid-Induced Fluorescence of Esterified Estrogen Powder USP

Lot	Percent Response[a]	Percent Estrogen Sulfate[b]
A	—	—
B	99.5	100.1
C	99.6	102.7
D	108.3	101.8

[a] Results are expressed as percent of Lot A. [b] The USP XVIII assay for total conjugated estrogens was used. The results are expressed as percent of Lot A.

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