Comparison of Operational Characteristics of Different Dissolution Testing Systems

E. A. HARDWIDGE ˣ, A. C. SARAPU, and W. C. LAUGHLIN

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Abstract □ Three dissolution apparatus, the rotating basket, the rotating paddle, and the rotating filter–stationary basket, were evaluated for their suitability as production control tools and their relation to blood level studies in dogs. The rotating basket and rotating paddle assemblies were easier to use and less variable than the rotating filter–stationary basket. When relative levels of agitation and the pH of the dissolution medium were held constant, all three apparatus correlated with each other and equally well with the serum drug levels from experimental formulations of an oral hypoglycemic drug after administration to dogs. Such an observed relationship cannot, of course, be used to predict blood levels in other species; however, it does suggest that the choice of one apparatus over another cannot be made *a priori*.

Keyphrases □ Dissolution testing systems—three apparatus compared, suitability for use as production control tools and relation to blood level studies in dogs evaluated □ Apparatus, dissolution testing—three systems compared, suitability for use as production control tools and relation to blood level studies in dogs evaluated

When relative merits of dissolution apparatus design are discussed, there often are no data available to compare results directly with other apparatus in the same laboratory. Thus, differences in parameters such as the dissolution medium or relative levels of agitation, recognized as having profound influence on dissolution results (1), often make direct comparison impossible. Therefore, different dissolution apparatus were evaluated to provide data on comparative operational characteristics under controlled experimental conditions and in a quality control environment.

The rotating basket and rotating paddle are Apparatus 1 and 2, respectively, in the official compendia (2, 3). The rotating filter–stationary basket system was described previously (4, 5).

EXPERIMENTAL

All equipment was obtained commercially. All dissolution tests were conducted on a six-station dissolution apparatus[1]. The rotation speed of the dissolution apparatus was measured with a hand-held digital tachometer[2] in direct friction contact with the rotating basket or rotating paddle shift.

The speed of the rotating filters on the rotating filter–stationary basket apparatus was determined with a digital phototachometer[3] so that measurements could be made on the rotational speed of the filter assembly itself while submerged and in operation. During a test, the dissolution fluid was continuously circulated through flowcells and absorbance was read on a UV spectrophotometer[4]. A peristaltic pump[5] was used to maintain a flow rate of about 40 ml/min. A 60-mesh wire screen was used to filter the solution on the rotating basket and rotating paddle apparatus; the 1-μm stainless steel filter was used on the rotating filter–stationary basket.

Prednisone Tablets (50 mg)—All tests were conducted in 900 ml of deionized water at 37°. Absorbance was read at 244 nm with 1.0-cm flowcells. The apparatus was operated at 25–200 rpm for the rotating basket, at 25–150 rpm for the rotating paddle, and at 150–600 rpm for the rotating filter–stationary basket.

Tetracycline Hydrochloride Capsules (250 mg)—Tests were conducted in 900 ml of deionized water at 37°. Absorbance was read at 268 nm with 0.5-mm flowcells. Since the capsules float in water, a loop of copper wire was used as a weight with the rotating paddle apparatus. This approach was described previously (6). For these capsules, the rotating filter–stationary basket was operated at 600 rpm, the rotating basket was operated at 150 rpm, and the rotating paddle was operated at 100 rpm.

Experimental Hypoglycemic Tablets (500 mg)—All tests were conducted in 900 ml of the dissolution medium at 37°. A tris(hydroxymethyl)aminomethane solution (1:40), adjusted to pH 7.6 with hydrochloric acid, or 0.05 *M* phosphate buffer, pH 7.2, was used. Absorbance was read at 226 nm with 0.5-mm flowcells. Tests were conducted at 600 rpm with the rotating filter–stationary basket apparatus, at 100 rpm with the rotating paddle, and at 150 rpm with the rotating basket.

All dissolution tests were run using a single tablet or capsule in each of the six flasks. On occasion, one spinning filter would stop spontaneously during a rotating filter–stationary basket test. In the 18 tests reported, this problem occurred five times. In this case, the tablet or capsule was dropped from the treatment and the values of the remaining five were used to calculate the average results. In all other cases, reported data are the averages of six tablets.

Serum Level Studies—Sixteen male beagle dogs were employed in a 4 × 4 crossover design. The dogs were fasted overnight and orally administered one 0.5-g oral hypoglycemic tablet. Blood samples were withdrawn just prior to dosing and at 2, 4, 6, 8, 12, 24, and 48 hr after

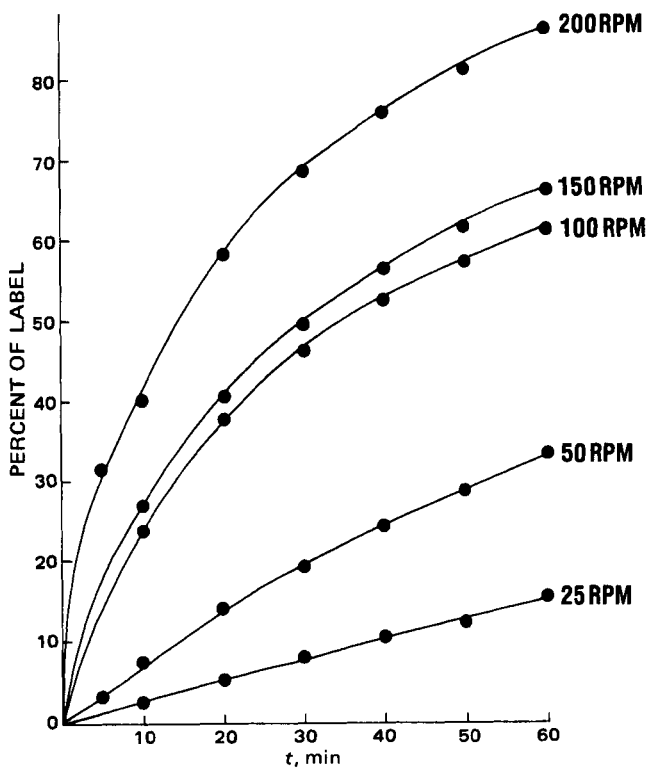

Figure 1—*Dissolution profile for 50-mg prednisone tablets with varying rotation speed in the rotating basket apparatus.*

[1] Hansen model 72B-115 or Coffman Industries model 7401 rotating filter–stationary basket apparatus.
[2] Biddle model 9970.
[3] Pioneer model DT-9600.
[4] Beckman Kintrac VII or Beckman 25-7.
[5] Harvard model 1210.

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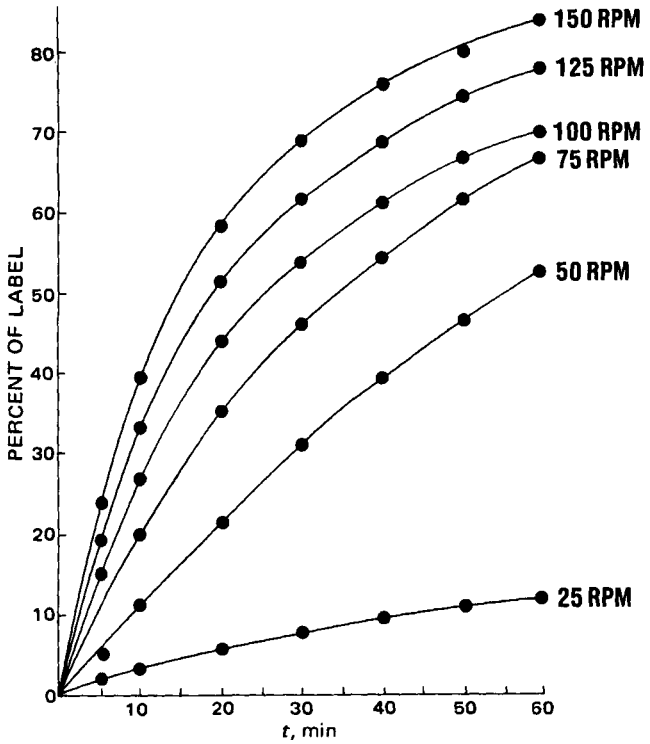

Figure 2—*Dissolution profile for 50-mg prednisone tablets with varying rotation speed in the rotating paddle apparatus.*

dosing. Average individual serum peak levels were observed at 7 hr, and the half-life of elimination for this drug was approximately 16 hr.

RESULTS AND DISCUSSION

Criteria for evaluation of these apparatus were based on their suitability as production control tools and their potential ability to produce correlations with blood level data. Effects of changes in agitation speed on dissolution, ease of operation in a quality control environment, sensitivity to production and formulation changes, and variability were all considered.

The prednisone tablets and tetracycline capsules were used primarily to examine relative agitation and variability. The oral hypoglycemic tablets provided data for comparison of dog blood levels with dissolution test results.

Effect of Rotation Speed—The three dissolution apparatus all agitate the solution with a rotating device, *i.e.*, a basket, paddle, or filter. To compare the effect of changes in rotation speed for each device, the dissolution of 50-mg prednisone tablets, known to have a relatively long dissolution time, was studied. Use of these tablets thus made it possible to run the dissolution test for a long enough period to assure that differences observed would be valid.

Each apparatus generated a family of curves that appear similar in shape (Figs. 1–3). With the basket and paddle apparatus, the dissolution rate increased with increasing rotational speed. However, while similar behavior with the rotating filter device was observed from 150 to 300 rpm, little change in the dissolution rate occurred from 350 to 600 rpm. The plateauing of the apparent level of agitation for the rotating filter in that range is surprising and unexplained at this time.

Direct comparison of agitation levels for the three apparatus is possible when the percent of label claim dissolved at a given time, 30 min, is plotted as a function of the revolutions per minute (Fig. 4). Thus, values of 75–125 rpm for the rotating paddle, 100–150 rpm for the rotating basket, and 350–600 rpm for the rotating filter–stationary basket gave approximately equivalent agitation since they gave about 50% of label claim in solution at 30 min. This comparison is based on the results obtained for the 50-mg prednisone tablet results only; however, it provides good initial values for comparison with other dosage forms, and large differences would not be anticipated.

Variability—During the comparisons, it was important to observe the variability obtained with the three apparatus. Variability is defined as the relative standard deviation of observed dissolution rates for the

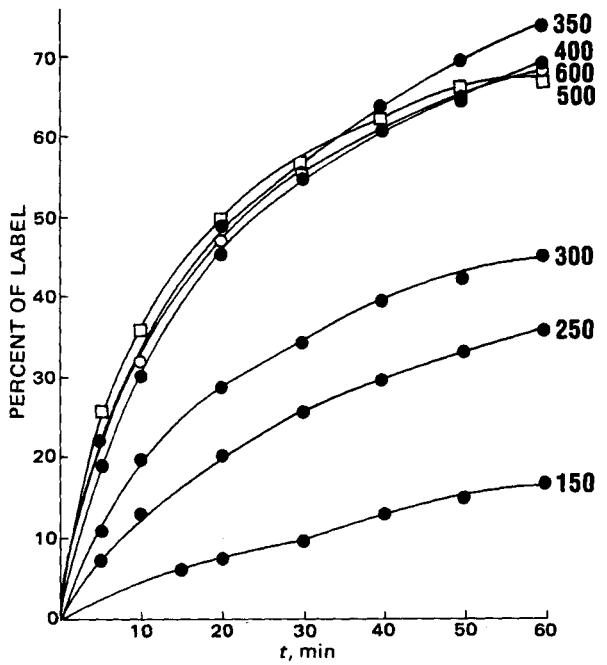

Figure 3—*Dissolution profile for 50-mg prednisone tablets with varying rotation speed in the rotating filter–stationary basket apparatus.*

dosage forms studied simultaneously in a single dissolution test. This parameter is a measure of within-run reproducibility. With the percent dissolved at 30 min for the 50-mg prednisone tablets (Figs. 1–3) as a test point, the observed relative standard deviation was calculated and plotted as a function of rotation speed for each apparatus. These data (Fig. 5) show that the rotating filter–stationary basket generated significantly greater variability than either the rotating basket or the rotating paddle. This variability was very pronounced at intermediate speeds (*i.e.*, 300–350 rpm) with the rotating filter–stationary basket apparatus.

A major contributor to the high variability of the rotating filter–stationary basket probably is the tendency of the spinning filter to wobble occasionally. Where a spinning filter had developed a noticeable wobble,

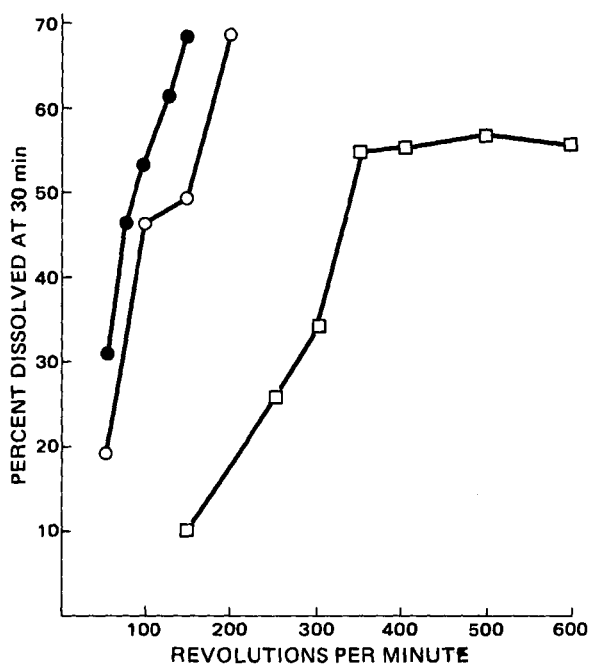

Figure 4—*Comparison of dissolution rate* versus *rotation speed for three dissolution apparatus using 50-mg prednisone tablets. Key:* ●, *rotating paddle;* ○, *rotating basket; and* □, *rotating filter–stationary basket.*

Table I—Summary of 250-mg Tetracycline Hydrochloride Capsule $T_{50\%}$ Values

Manufacturer	Rotating Filter–Stationary Basket (600 rpm)	Rotating Basket (150 rpm)	Rotating Paddle (100 rpm)
A	5.8 (33)[a]	7.1 (22)	5.0 (12)
B	4.1 (17)	5.6 (21)	5.6 (36)
C	5.5 (25)	6.5 (16)	5.6 (26)

[a] Values in parentheses are percent relative standard deviations.

the dissolution rate in that particular flask increased qualitatively. No attempt was made to quantitate these effects, but wobble occurred more often at the intermediate speeds and the apparatus operated more smoothly at higher speeds.

The only other study in which the variability of the rotating filter–stationary basket was compared directly to another apparatus using the same formulation, dissolution medium, and relative level of agitation was reported by Shah *et al.* (4). They compared dissolution results for the rotating filter–stationary basket and the rotating basket. A single six-place dissolution run of an unidentified tablet sample was reported for each apparatus. The observed relative standard deviations were 3.28 and 41.7% for the rotating filter–stationary basket and rotating basket, respectively. For the tablets examined, the rotating basket test gave considerably greater variability than the rotating filter–stationary basket.

The formulation dependence of such a result may be important since the rotating basket test was operated at an unusually high level of agitation (300 rpm), well outside the operational limits of the USP test. The high relative standard deviation reported (4) for the rotating basket in this case is not typical of results obtained when drug products are tested by compendial dissolution methods. On the other hand, the variability reported (4) for the rotating filter–stationary basket is much less than that reported for the capsules and tablets in the present work. The differences may be attributable to the choice of test samples or differences in apparatus performance.

Capsule Dissolution Testing—To test the relative behavior of the three dissolution apparatus with capsules, 250-mg tetracycline hydrochloride capsules produced by three different manufacturers were obtained. Results are presented in Table I as $T_{50\%}$ in each case. The three methods gave comparable dissolution rates and did not demonstrate significant differences in dissolution behavior between the capsules. Furthermore, the relative standard deviations for all three methods were nearly the same, unlike the results with the prednisone tablets. Based on the observed variability for tablets plotted in Fig. 5, it appears that the rotating basket and rotating paddle gave relatively higher variability with the capsules than with the tablets. The rotating filter–stationary basket apparatus gave uniformly high variability for both capsules and tablets.

Ease of Use—The rotating basket and rotating paddle apparatus were comparable in the ease of use in a quality control environment. Of the three apparatus, the rotating filter–stationary basket was the most difficult to operate and clean. It was necessary to dismantle the entire filter assembly for cleaning, which involves manipulation of the seals and several other parts. Some of these parts are relatively fragile, particularly the pilot tube, a glass capillary on which the filter assembly rotates. Breakage of two pilot tubes occurred during this study.

Moreover, dynamic seals on the top of the filter assembly at the point where the pilot tube enters the rotating filter assembly were defective at the start of the study and had to be replaced. They were leaking and admitting solid particles into the continuous flow stream as the dissolution medium was pumped without passing through the filter. After replacement, the problem did not recur. The time required for setup and breakdown on a per run basis was considerably greater for the rotating filter–stationary basket system compared to the rotating paddle and rotating basket apparatus.

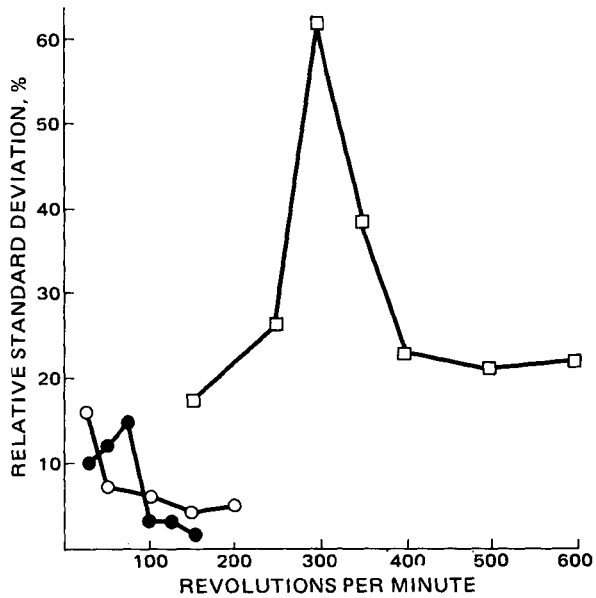

Figure 5—*Observed apparatus variability as a function of rotation speed (50-mg prednisone tablets). Key: ●, rotating paddle; ○, rotating basket; and □, rotating filter–stationary basket.*

Dissolution Rates and Serum Level Data—Relative serum level data in dogs were obtained previously in these laboratories for four experimental lots of an oral hypoglycemic drug intentionally formulated to give a range of dissolution results (Table II). Each apparatus gave $T_{50\%}$ values of approximately 30 min for Lot C at pH 7.6 and significantly longer times for Lot D. The trend to lower serum drug levels with increased dissolution rate became highly significant ($p < 0.05$) for Lot D, which had a dissolution $T_{50\%}$ of greater than 80 min at pH 7.6 for all three apparatus.

The observations that there was a range of dissolution rates over which drug absorption was not significantly affected and that there was some critical value beyond which absorption was affected were noted previously (7). The correlation coefficients tabulated provide only a rough guide for comparison in such a case but do, nevertheless, indicate that all three apparatus give essentially equivalent results. When all lots were retested in pH 7.2 buffer and the correlation coefficients were recalculated, differences were noted. For example, the rank-order exchange of Lots B and C observed for the rotating filter–stationary basket apparatus results in an improved apparent correlation. Such results emphasize the importance of the dissolution medium on observed dissolution rates. To be valid, direct comparisons of one apparatus with another must be made using the same dissolution medium and approximately equivalent agitation.

SUMMARY AND CONCLUSIONS

Production Control—Of the three apparatus studied, the rotating

Table II—Canine Serum Levels and Dissolution $T_{50\%}$ Values for Oral Hypoglycemic Experimental Lots (500 mg)

Lot	Rotating Filter–Stationary Basket (600 rpm)		Rotating Paddle (100 rpm)		Rotating Basket (150 rpm)		$AUC_{0\text{-}48hr}$[a], μg-hr/ml	*AUC* Differences[b] ($p \leq 0.05$)
	pH 7.6[c]	pH 7.2[d]	pH 7.6	pH 7.2	pH 7.6	pH 7.2		
A	2.0 (17)[e]	2.0 (17)	1.6 (10)	2.4 (3)	2.5 (7)	4.1 (11)	4250	A > D
B	40.8 (33)	21.1 (43)	29.3 (26)	14.4 (52)	26.4 (24)	13.6 (21)	4120	B > D
C	33.5 (28)	32.1 (62)	30.9 (16)	16.3 (38)	30.3 (15)	20.8 (36)	3706	None
D	94.5 (17)	61.5 (53)	85.2 (25)	38.6 (9)	83.8 (15)	33.6 (31)	3017	D < A,B
Correlation coefficient, $T_{50\%}$ *versus AUC*	−0.92	−0.98	−0.95	−0.96	−0.96	−0.97		

[a] Area under the serum concentration–time curve. [b] Tukey's multiple comparison test. [c] With 0.21 *M* tris(hydroxymethyl)aminomethane buffer. [d] With 0.05 *M* phosphate buffer. [e] Values in parentheses are percent relative standard deviations for each run.

basket and rotating paddle were relatively easy to use, reliable, and amenable for routine use in a quality control environment. The rotating filter–stationary basket apparatus was more difficult to operate, required more time per test, and generally gave greater variability.

Serum Level Data—Dissolution times measured with all three apparatus correlated equally well with serum drug levels in dogs for the four experimental hypoglycemic lots. None of these three apparatus gave substantially better correlations when other test conditions, such as the dissolution medium and relative agitation levels, were kept constant. These data cannot be used, of course, to prove or disprove ability to predict a relation between dissolution and blood levels in other species. On the contrary, since there is a high degree of correlation of dissolution results between apparatus when experimental conditions are kept as similar as possible, claims that one apparatus or another is *a priori* superior for prediction of *in vivo* behavior should be critically assessed.

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High-Pressure Liquid Chromatographic Analysis of Estrogens in Pharmaceuticals by Measurement of Their Dansyl Derivatives

ROBERT W. ROOS

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Abstract □ A high-pressure liquid chromatographic method is described for the analysis of estrogens in pharmaceutical tablet and injectable dosage forms. In general, the estrogens are isolated, an internal standard is added, the dansyl derivatives are formed, and the dansyl estrogen solution is injected into a liquid chromatograph. Linear response is experienced between the mass of estrogen and the ratio of the estrogen peak height to the internal standard peak height, using a microparticle silica column and chloroform–*n*-heptane mobile phases. With fluorometric measurement, limits of detectability for ethinyl estradiol and estradiol were 0.04 and 0.05 ng, respectively. Methyltestosterone, an androgen in combination with ethinyl estradiol, was analyzed simultaneously. Commercial pharmaceutical preparations containing estrone, ethinyl estradiol, and estradiol were analyzed by the proposed method. The results indicate the method to be sensitive, reasonably precise (<2%), and accurate in the analysis of estrogen in dosage forms.

Keyphrases □ Estrogens, various—high-pressure liquid chromatographic analyses of dansyl derivatives, pharmaceutical preparations □ High-pressure liquid chromatography—analyses, dansyl derivatives of various estrogens, pharmaceutical preparations □ Dansyl derivatives—various estrogens, high-pressure liquid chromatographic analyses, pharmaceutical preparations

Dansyl chloride, 5-(dimethylamino)-1-naphthalenesulfonyl chloride (I), is a useful reagent for the production of fluorescent derivatives (fluorogenic labeling) with several functional groups, including primary and secondary amines, imidazoles, and phenols. However, since I can decompose to yield dansyl hydroxide (actually a sulfonic acid), dansyl dimethylamide, and other compounds (1) under the conditions used for derivatization, analyses involving I usually include a procedure to separate the dansyl derivative from any other fluorescent compounds present in the solution.

The ability of various classes of compounds to form dansyl derivatives that can be detected at low levels is advantageous. Some analytical applications were reviewed by Seiler and Wiechmann (1). Recently, high-pressure liquid chromatographic (HPLC) methods for the analysis of carbamate insecticides (2), hydroxybiphenyls (3), and barbiturates (4) used dansyl derivatives and demonstrated the value of this approach.

Better methods of analysis for the determination of estrogens in pharmaceutical dosage forms are needed (5, 6). Penzes and Oertel (7, 8) described the TLC separation of the dansyl derivatives of estrone, estradiol, and estriol, and Fishman (5) introduced a conventional fluorescence method for some estrogens using dansyl estrogen derivatives.

This study was conducted to determine the utility of the formation of dansyl derivatives of estrogens in an HPLC analytical procedure. The procedure was adapted satisfactorily to the analysis of estradiol, ethinyl estradiol, and estrone in pharmaceutical dosage forms. Ethinyl estradiol, the estrogen receiving the most attention owing to its small dose, is frequently found in combination with a progestin (oral contraceptive) and an androgen such as methyltestosterone. Fortunately, some nonestrogen steroids are separated from the dansyl estrogens, so they can be analyzed with the same column. A simultaneous method for

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