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Analytical methodology applicable in dissolution testing of norethindrone–mestranol tablets

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Summary

Simple and sensitive methodology to monitor the dissolution rate of norethindrone–mestranol combination tablets has been developed. The procedure calls for the direct injection of the dissolution medium onto a reverse-phase column and for the monitoring of the eluent serially with ultraviolet and fluorescence detectors. Applicability of the method was demonstrated by carrying out dissolution studies of a combination product in de-aerated water and 30% isopropanol/water.

Introduction

Oral contraceptives have been used widely since their introduction over 20 years ago. Unfortunately until recently, no published analytical method was available that could be used to carry out dissolution studies of these agents. Hirai et al. (1980) developed a spectrofluorometric method to follow the dissolution of ethinyl estradiol in tablets containing norethindrone and ethinyl estradiol. In another report, Sundaresan et al. (1981) described a rapid method to assay concurrently norethindrone and mestranol in tablets. The procedure utilized two HPLC detectors in series and appeared to offer the required sensitivity needed to carry out dissolution studies. However, detailed methodology to perform dissolution studies on norethindrone–mestranol combination tablets has not been published. Based on the approach of Sundaresan et al. (1981), a method has now been developed that permits the simultaneous analysis of both components during the course of dissolution of tablet formulations.

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Materials and methods

Reagents and materials

Mestranol and norethindrone were of USP grade (U.S.P.C, Rockville, MD). Progesterone (lot 87C-0082, Sigma Chemicals, St. Louis, MO) was of reagent grade. All solvents were HPLC grade (Burdick and Jackson Laboratories, Muskegon, MI). Individual stock solutions of mestranol, norethindrone and progesterone were prepared by dissolving 10, 10, and 40 mg in 100 ml of methanol, respectively.

Equipment

A modular high-performance liquid chromatograph was used which consisted of a constant-flow pump (Model M6000A, Waters Assoc., Milford, MA), an automated injector (Model WISP 710A, Waters Assoc., Milford, MA), a fixed wavelength 254 nm ultraviolet detector (Model 440, Waters Assoc., Milford, MA), a fluoreoscence detector set at an excitation wavelength of 230 nm and fitted with a 280 nm cut-off filter for emission (Model FS 970, Schoeffel Instruments, Westwood, NJ) and a strip chart recorder set at a speed of 0.5 cm/min (Model 9176, Varian Instruments, Palo Alto, CA). A commercially available stainless steel HPLC column (4.6 mm id. $\times$ 250 mm) pre-packed with fully porous, irregularly shaped 10 μm silica to which had been chemically bonded an octadecyl group (μ-Bondapak C-18, Waters Assoc., Milford, MA) was used. The mobile phase was a methanol–0.01 M, pH 7, potassium phosphate buffer system (4:1). A flow rate of 1.3 ml/min was established (1500PSIG).

Equilibrium solubility procedure

Equilibrium solubilities were determined by placing excess drug, the appropriate solvent and a magnetic stirring bar into a glass stoppered Erlenmeyer flask. The flask was capped, placed into a water bath maintained at 37 $\pm$ 0.2°C (Circulating System Model 253, CGA C, Chicago, IL) and stirred constantly (Model 1250, Lab-Line Instruments, Melrose Park, IL). Samples were taken approximately every 2 h, filtered (Millipore HA and FH 0.5 μ filters) and assayed by HPLC until no increase in concentration was observed for 3 consecutive samplings.

Dissolution procedure

Dissolution studies were performed using the U.S.P. method II (USP XX) at a paddle speed of 50 rpm. Six tablets were evaluated simultaneously using a custom designed 6-vessel dissolution apparatus meeting all the specifications of the U.S.P. and equipped with a stirring motor controller (Model T2, G.K. Heller, Floral Park, NY). Suitability of the dissolution apparatus was verified with the USP prednisone calibrator (U.S.P.C., Rockville, MD) using the paddle at 50 rpm with de-aerated water as the dissolution medium. Working standard solutions were prepared by diluting the stock solution with the appropriate dissolution medium. The dissolution media employed in the study were de-aerated water and 30% isopropanol/water. A dissolution medium volume of 900 ml at 37°C was used in all the determinations.

After the tablets were dropped (free fall) into the dissolution vessels, the paddles

were lowered, stirring started, and sampling was carried out at 15-min intervals for one hour. At each sampling time, 4 ml of the dissolution media were withdrawn from each vessel, filtered (Millipore HA and FH 0.5 μ filters) and all sample volumes immediately replaced. Exactly 2 ml of the filtrate was transfered to a vial and 5 μl of the internal standard solution was added and mixed. The vials were loaded into the automated injector and the device was programmed to inject a fixed volume depending on the dissolution media (50 μl for 30% isopropanol/water and 100 μl for de-aerated water). The concentrations of the drug entities present in the dissolution media were determined from standard curves prepared by plotting peak height ratios versus the concentrations of the standard solutions.

Results and discussion

Because of the relative insolubility of these steroids in water (Shroff and Mayer, 1975; Higuchi et al., 1979), the ability to carry out the dissolution studies in another medium in which the drugs should be much more soluble was desirable. Hydroalcoholic solvent systems have been suggested as dissolution media for highly water-insoluble drugs (Pharmacopoeial Forum, 1980). Preliminary studies showed that the solubility of mestranol and norethindrone could be dramatically increased in isopropanol–water solvent systems. In order to have high solubility without excessive isopropanol, a system of 30% isopropanol–water was considered. To ascertain the feasibility of using a 30% isopropanol–water solvent system, the equilibrium solubilities of the two substances in that system at 37°C were determined and found to be 140 and 680 μg/ml for mestranol and norethindrone, respectively. These values are considerably higher than the equilibrium solubilities for mestranol and norethindrone in water which were determined to be 0.58 and 8.8 μg/ml, respectively.

To follow the dissolution of combination products of low dosage strength, very sensitive assay procedures are necessary. Using a modification of the methodology of Sundaresan et al. (1981), a procedure capable of quantitation down to 10% of tablet strength of both steroids dissolved in 900 ml of dissolution media has been developed. The methodology permits the direct injection of the dissolution medium into the HPLC. The ability to inject the filtered dissolution media directly is a major advantage and favors the use of this method as a routine dissolution monitoring technique.

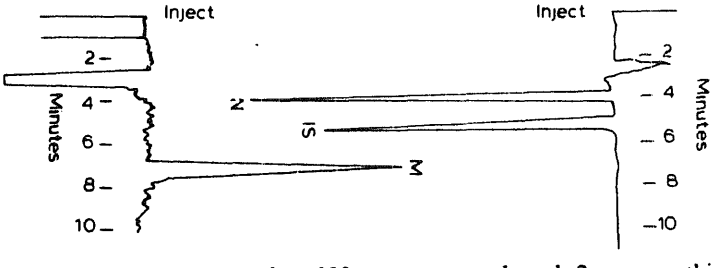

Fig. 1. Chromatogram of a 100 μg mestranol and 2 mg norethindrone tablet in de-aerated water dissolution medium at 60 min (norethindrone = N; internal standard = IS; mestranol = M).

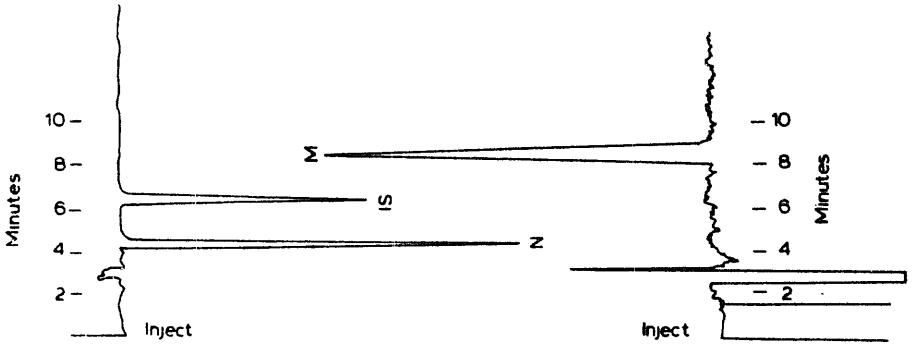

Fig. 2. Chromatogram of a 100 μg mestranol and 2 mg norethindrone tablet in 30% isoproponal–water dissolution medium at 30 min (norethindrone = N; internal standard = IS; mestranol = M).

Chromatograms obtained in the determination of the dissolution profile of a tablet (100 μg mestranol and 2 mg norethindrone) in de-aerated water and in 30% isopropanol–water are shown in Figs. 1 and 2. The retention times for norethindrone, progesterone (the internal standard) and mestranol were 4.2., 6.0 and 8.6 min, respectively. No interferences from any tablets' excipients were observed in either medium.

Standard linear calibration curves were obtained for direct standards dissolved in either water or 30% isopropanol–water with concentrations equivalent to 10–100% of labeled dosage strength. Recovery studies were performed by assaying dissolution media to which had been added known amounts of norethindrone and mestranol. The analysis was carried out on samples drawn from the dissolution vessels at 0 and 60 min. Recoveries from the aqueous and hydroalcoholic media were found to be in the range of 95–107%.

Selectivity of the method was evaluated by comparing the chromatographic peak widths at half-heights for norethindrone and mestranol direct standards to chro-

TABLE I

INTRA-DAY PRECISION DATA FOR MESTRANOL–NORETHINDRONE STANDARDS

Spiked concentrations (μg/ml)		Experimental concentrations (μg/ml)						Mean concentrations (± % C.V.)	
		Run I		Run II		Run III			
Mes	Nor	Mes	Nor	Mes	Nor	Mes	Nor	Mes	Nor
0.010	0.25	0.010	0.28	0.010	0.27	0.012	0.29	0.011 (±11)	0.28 (±3.6)
0.030	0.75	0.030	0.78	0.031	0.76	0.028	0.76	0.030 (±5.1)	0.77 (±1.5)
0.050	1.00	0.052	0.97	0.049	0.98	0.049	0.97	0.050 (±3.5)	0.95 (±4.6)
0.070	2.50	0.069	2.43	0.069	2.45	0.068	2.45	0.069 (±0.8)	2.44 (±0.5)
0.090	5.00	0.094	5.02	0.089	4.98	0.092	5.03	0.092 (±2.7)	5.01 (±0.5)

Mes = mestranol; Nor = norethindrone. Solvent = 30% isopropanol–water.

TABLE 2

INTER-DAY PRECISION—LINEAR EQUATIONS[a] OF STANDARD CURVES (±S.D.) FOR NORETHINDRONE AND MESTRANOL IN BOTH DISSOLUTION MEDIA

Drug	Dissolution medium [b]	
	De-aearated water (n=5)	30% isopropanol–water (n=4)
Norethindrone	y=4.38 (±0.32)x+0.020 (±0.005)	y=8.48 (±0.29)x+0.003 (±0.024)
Mestranol	y=0.63 (±0.03)x+0.008 (±0.015)	y=1.44 (±0.13)x+0.008 (±0.044)

[a] y=mx+b where 'y' equals chromatographic peak height ratio of drug to internal standard, 'm' equals slope of standard curve and 'x' equals concentration of drug.

[b] Quantity of internal standard added and detector settings were different for the assays of samples taken from the two dissolution media.

matographic peak widths for the drugs from solutions of individual tablets (several brands) containing additional amounts of pure standards. The peak widths at half-height were constant. Intra- and inter-day precision was determined by the multiple analysis of standards and daily standard curves, respectively. Intra-day precision for norethindrone and mestranol (Table 1) was estimated by calculating the mean percent coefficient of variations for standards of mestranol (4.6%) and norethindrone (2.1%). The equations for the standard curves with standard deviations for norethindrone and mestranol, listed in Table 2, provide an estimate of the inter-day precision.

Utility of the method was demonstrated by developing dissolution profiles for a tablet containing 100 μg mestranol and 2 mg norethindrone (Ortho-Novum 100/2)

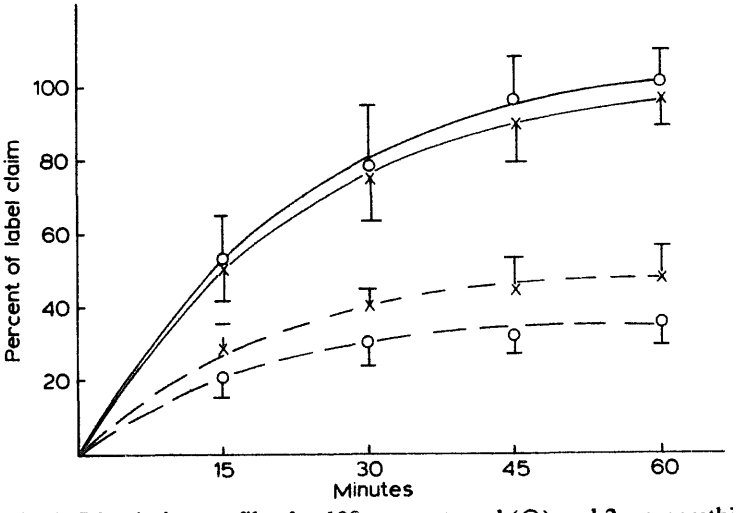

Fig. 3. Dissolution profiles for 100 μg mestranol (○) and 2 mg norethindrone (×) combination tablet in 900 ml de-aerated water (- - - - -) or 30% isopropanol–water (———) at 37°C by the paddle method. For studies in de-aerated water, n=24; for studies in 30% isopropanol–water, n=12; mean±1 S.D.

in both de-aerated water and 30% isopropanol–water as the dissolution media (Fig. 3). As expected because of the lipophilicity of the drugs, the dissolution in the hydroalcoholic medium was much faster and more complete than in de-aerated water. It was observed, however, that even in de-aerated water, 90% of the amount of the drugs present in the tablet dissolved after extensive stirring (4 h at 50 rpm and half-four at 200 rpm). Further studies to define the dissolution characteristics of mestranol–norethindrone combination products are under way and will be reported later.

In conclusion, an analytical method has been developed that has the required sensitivity and simplicity to be used in dissolution studies of norethindrone–mestranol combination tablets.

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