Simple High-Performance Liquid Chromatographic Assay for Norethindrone–Mestranol in Combination Tablets

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Abstract □ A simple, sensitive, and specific high-performance liquid chromatographic procedure was developed to assay norethindrone–mestranol combination tablets. The method involves a chloroform extraction of a single pulverized tablet. After centrifugation, an aliquot of the supernate was injected into a modular high-performance liquid chromatograph. The effluent from the silica column was monitored serially with a fixed-wavelength UV detector (254 nm) for norethindrone quantitation and a fluorescence detector (230 nm for excitation and 280-nm cutoff filter for emission) for mestranol quantitation. Progesterone was used as an internal standard. The method was employed successfully in content uniformity studies of several brands of commercially available tablets.

Keyphrases □ High-performance liquid chromatography—norethindrone–mestranol tablets, content uniformity studies □ Contraceptives—norethindrone–mestranol tablets, high-performance liquid chromatography, content uniformity studies □ Hormones, synthetic—norethindrone–mestranol tablets, high-performance liquid chromatography, content uniformity studies □ Norethindrone–mestranol combinations—high-performance liquid chromatography, content uniformity studies

Two widely used synthetic hormonal contraceptive steroids are norethindrone and mestranol, and various methods have been reported for their assay. Norethindrone was determined by spectrophotometry (1–3), fluorometry (4, 5), and GLC (3, 6). Mestranol also was determined by spectrophotometry (7), fluorometry (8), and GLC (9, 10). Simultaneous analysis of norethindrone and mestranol in combination tablets was achieved by GLC (11), quantitative TLC (12), and high-pressure liquid chromatography (13). The official compendial method (14) for the combination tablets is an automated method utilizing a spectrophotometer and a fluorometer to quantitate norethindrone and mestranol, respectively.

Each published chromatographic method has certain disadvantages. The TLC method requires scanning the plates at two different wavelengths with intervening spraying. The GLC method results in chromatograms with the undesirable characteristic of widely divergent peak heights for the two steroid components. A similar peak height disparity is observed with the reported high-pressure liquid chromatographic method, and the sample preparation technique sometimes results in the solubilization of excipients that interfere in the chromatography.

The present study reports a rapid high-performance liquid chromatographic (HPLC) method for the simultaneous analysis of norethindrone and mestranol in combination tablets using peak height determinations.

EXPERIMENTAL

Apparatus—The modular high-performance liquid chromatograph consisted of a constant flow pump[1], an automated injector[2], a fixed-wavelength UV detector[3] (254 nm), a fluorescence detector[4] (excitation, 230 nm; emission, 280-nm cutoff filter), and a strip-chart recorder[5] (0.5 cm/min). Stainless steel columns[6] (4.6 mm i.d. × 250 mm), packed with fully porous, irregularly shaped 5-μm silica, were obtained commercially. Sample filtration was achieved by employing a syringe-type 25-mm filter holder[7] and 0.5-μm filters[8]. [An oversized filter (47 mm) was used so that it could be wrapped around the holder to improve the seal.]

Chromatographic Conditions—The mobile phase was ethylene dichloride–butanol–water (97.5:2.4:0.1). A flow rate of 1.3 ml/min was established (1500 psig), and the column was conditioned initially for 16 hr.

Reagents and Materials—Mestranol[9], norethindrone[9], and progesterone[10] were obtained commercially. Solvents were all HPLC grade[11]. Individual stock solutions of mestranol, norethindrone, and progesterone were prepared by dissolving 10, 100, and 400 mg, respectively, in 10 ml of chloroform.

Glassware Preparation—All glassware including pipets and syringes were silanized by immersion into a solution of 5% trimethylchlorosilane in toluene for at least 1 hr. Then the glassware was rinsed with toluene, methanol, water, methanol, and chloroform.

Assay—A single tablet was crushed to a fine powder in a folded sheet of weighing paper. The powder was transferred into a 15-ml test tube, and 4 ml of chloroform was added. Then 30 μl of the progesterone internal standard solution was added with a 50-μl syringe[12]. The sample was vortexed for ~30 sec, followed by 5–10 min of standing, and then was vortexed again for 1 min. The samples then were centrifuged and filtered through a 0.5-μm filter. The filtrate was transferred to a silanized vial for subsequent loading into the automated injector, which was programmed to inject 2 μl.

The amounts of mestranol and norethindrone in the tablets were determined from standard curves prepared by plotting peak height ratios (mestranol–progesterone or norethindrone–progesterone) *versus* the concentrations of the direct standards. These direct standards were prepared by spiking, into chloroform, both mestranol and norethindrone and then bringing each to a final volume of 4 ml. The amounts of mestranol and norethindrone spiked were 0 and 0, 30 μg and 0.5 mg, 50 μg and 1.0 mg, 100 μg and 2 mg, 150 μg and 2.5 mg, and 500 μg and 7.5 mg, respectively. In some cases, an additional standard (60 μg of mestranol, 10 mg of norethindrone) was included. To each standard was added 30 μl of the internal standard solution.

Recovery—Five tablets were ground together to a fine powder. Four aliquots, corresponding to the weight of a single tablet, were placed into individual test tubes. Standard mestranol and norethindrone were added to two of these test tubes. The amounts of the standards added were one-half of the tablet strength of each component. All samples then were assayed. This study was conducted on seven different products[13].

Precision—Fifteen tablets of Product F were ground to a fine powder; then 10 aliquots, equivalent to the weight of one tablet, were transferred to 10 test tubes. All samples then were assayed.

Content Uniformity—Seven drug products were assayed for content uniformity.

[1] Model M6000A, Waters Associates, Milford, Mass.
[2] Model WISP 710A, Waters Associates, Milford, Mass.
[3] Model 440, Waters Associates, Milford, Mass.
[4] Model FS970, Schoeffel Instruments, Westwood, N.J.
[5] Model 9176, Varian Instruments, Palo Alto, Calif.
[6] Prepacked HI-EFF Micropart column with Lichrosorb Si60 (5 μm) silica, Applied Science Laboratories, State College, Pa.
[7] Swinnex-25, Millipore Corp., Bedford, Mass.
[8] FHUP-047-00, Millipore Corp., Bedford, Mass.
[9] Reference standard, United States Pharmacopeial Convention, Rockville, Md.
[10] Lot 87C-0082, Sigma Chemical Co., St. Louis, Mo.
[11] Burdick & Jackson Laboratories, Muskegon, Mich.
[12] Hamilton Co., Reno, Nev.
[13] Product A, Norinyl 1 + 50; Product B, Norinyl 1 + 80; and Product C, Norinyl 2/100 (Syntex, Humacao, Puerto Rico). Product D, Ortho-Novum 1/50; Product E, Ortho-Novum 1/80; Product F, Ortho-Novum 2/100; and Product G, Ortho-Novum 10/60 (Ortho Pharmaceutical Corp., Raritan, N.J.).

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Table I—Recovery Data for Seven Commercial Tablets

Product	Assay Results[a] Tablet: Norethindrone, mg	Tablet: Mestranol, μg	Tablet plus Standard[b]: Norethindrone, mg	Tablet plus Standard[b]: Mestranol, μg	Percent Recovery: Norethindrone	Percent Recovery: Mestranol
A 1/50	0.99	50.0	1.46	75.9	96.0	104.0
B 1/80	0.95	79.0	1.48	118.6	106.0	99.0
C 2/100	1.91	97.4	2.90	149.0	98.5	103.0
D 1/50	0.99	50.0	1.46	75.9	94.0	103.7
E 1/80	0.95	78.8	2.44	119.0	98.0	100.5
F 2/100	1.90	97.3	2.90	149.0	98.0	101.4
G 10/60	9.63	56.3	14.65	86.2	100.4	100.0

[a] $n = 2$. [b] Added one-half of tablet strength of each component to the tablets.

Table II—Content Uniformity of Seven Commercial Tablets

Product	Mean (*CV*, %): Norethindrone, mg	Mean (*CV*, %): Mestranol, μg	Range: Norethindrone, mg	Range: Mestranol, μg
A 1/50	1.02 (1.2)	50.0 (2.5)	0.99–1.03	47.4–51.8
B 1/80	1.01 (2.4)	77.2 (2.8)	0.97–1.04	72.9–80.9
C 2/100	1.85 (1.4)	95.5 (1.8)	1.80–1.88	92.6–97.2
D 1/50	0.96 (2.0)	49.2 (2.2)	0.94–0.99	47.0–50.1
E 1/80	0.94 (2.6)	78.6 (2.2)	0.92–1.00	74.6–80.1
F 2/100	1.96 (2.0)	99.1 (1.8)	1.91–2.02	97.6–101.8
G 10/60	9.56 (0.9)	56.3 (1.0)	9.42–9.65	55.1–57.0

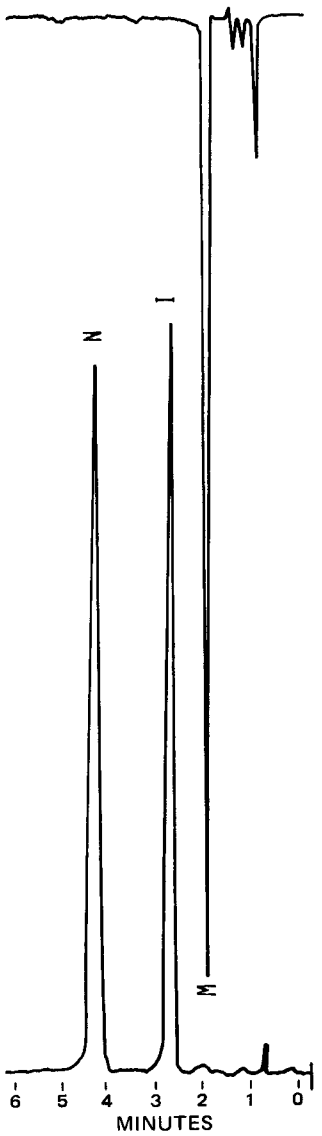

Figure 1—*Chromatogram obtained from HPLC assay of Product F. Column effluent was monitored for mestranol (M) using a fluorescence detector (1.0 μA range sensitivity) and for internal standard (I) and norethindrone (N) using an absorbance detector (0.05 aufs).*

RESULTS AND DISCUSSION

The simultaneous analysis of combination drug products that contain widely different amounts of active components presents unique problems. All of the components of the drug product must be extracted quantitatively, and the detection techniques must give adequate sensitivity for even the lowest level component.

In norethindrone–mestranol tablets, the components are present in ratios from 12:1 to 160:1. Since the solubilities of both components were high in chloroform, this solvent was used for the extraction. The key to resolving the problem of adequate sensitivity for mestranol, the low level component, was the observation that estrogens possess native fluorescence (15) whereas norethindrone does not fluoresce (4). Thus, it seemed plausible to develop an HPLC method that employed a fluorescence detector and an absorbance detector to monitor the column effluent. Initial studies showed that excellent resolution of the tablet components could be obtained using a microparticulate silica column.

A chromatogram obtained in the analysis of Product F is shown in Fig. 1. By using two detectors, it was possible to optimize the response for each steroid component. The retention times for mestranol, progesterone (the internal standard), and norethindrone were 4.0, 5.6, and 8.8 min, respectively.

Initial studies indicated that drug adsorption onto the glassware was occurring. To overcome this problem, all glassware was silanized before use.

A standard linear calibration curve was obtained for direct standard solutions equivalent to 30–100 μg of mestranol/tablet and 0.5–10 mg of norethindrone/tablet. These ranges span those found in all commercially available tablets. The recovery studies were performed on spiked samples of seven drug products. The results (Table I) show excellent recovery in all cases. No interferences from any tablet excipient were observed in the resulting chromatograms. Precision was determined by multiple analyses of aliquots of a Product F composite. The means and coefficients of variation for the assay of mestranol and norethindrone were 101 μg ± 1.8% and 1.94 mg ± 2.0%, respectively. These results demonstrate excellent assay precision. An additional estimate of the precision was obtained by the evaluation of multiple direct standard curves. The values for the slopes were 0.0140 ± 1.6% (coefficient of variation) for mestranol and 0.5543 ± 1.25% for norethindrone.

The USP XX specified that coated tablets and tablets containing 50 mg or less of active ingredient must pass a content uniformity test. The newly developed HPLC assay was used in content uniformity studies of the same seven products examined in the recovery studies. The results established that the batches of the seven formulations met the compendial requirements (Table II). The methodology will be modified and used in planned dissolution studies.

REFERENCES

(1) J. Y. P. Wu, *J. Assoc. Off. Anal. Chem.*, **57**, 747 (1974).

(2) *Ibid.*, **58**, 75 (1975).

(3) A. P. Shroff and J. Grodsky, *J. Pharm. Sci.*, **56**, 460 (1967).

(4) T. James, *ibid.*, **59**, 1648 (1970).

(5) *Ibid.*, **61**, 1306 (1972).

(6) O. D. Boughton, R. Bryant, W. J. Ludwig, and D. L. Timma, *J. Pharm. Sci.*, **55**, 951 (1966).

(7) J. Y. P. Wu, *J. Assoc. Off. Anal. Chem.*, **54**, 617 (1971).

(8) M. P. Short and C. T. Rhodes, *Can. J. Pharm. Sci.*, **8**, 26 (1973).

(9) A. P. Shroff and R. E. Huettemann, *J. Pharm. Sci.*, **57**, 882 (1968).

(10) B. A. Lodge, *Can. J. Pharm. Sci.*, **5**, 74 (1970).

(11) G. Moretti, G. Cavina, G. Chiapetta, I. Fattori, M. Petrella, and V. Pompi, *Boll. Chim. Farm.*, **116**, 463 (1977).

(12) A. P. Shroff and C. J. Shaw, *J. Chromatogr. Sci.*, **10**, 509 (1972).

(13) P. Helboe and M. Thomsen, *Arch. Pharm. Chemi Sci. Ed.*, **6**, 57 (1978).

(14) "The United States Pharmacopeia," 20th rev., United States Pharmacopeial Convention, Rockville, Md., 1980, p. 557.

(15) D. Duggan, R. Bowman, B. Brodie, and S. Udenfriend, *Arch. Biochem. Biophys.*, **68**, 1 (1957).

Optical Crystallographic Properties of Drugs of Abuse: Commonly Used Amine Street Drugs

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Received July 1, 1980, from the **School of Pharmacy, University of Wyoming, Laramie, WY 82071*, and the ‡*School of Pharmacy, University of Washington, Seattle, WA 98195.* Accepted for publication November 21, 1980.

Abstract □ The optical crystallographic properties of the diliturate derivatives of amine drugs found in illicit street drug preparations known as "white crosses," "mini-bennies," or "whites" were determined. The crystallographic properties, especially the crystal morphology, extinction angles, and indexes of refraction, identify the drug substances found in the white cross suite. These data can be used with UV and IR spectroscopic and chromatographic evaluations for drug identification.

Keyphrases □ Drugs of abuse—substance identification by optical crystallographic properties of diliturate derivatives of amine drugs □ Optical crystallography—substance identification of diliturate derivatives of amine drugs □ Amphetamines—drugs of abuse, identification of drug substance by optical crystallographic properties

Optical chemical crystallography is a physical method for rapid drug identification (1–3). Microchemical properties of amine drug salts have been determined with various reagents (4), but few studies (5, 6) concentrated on the optical crystallographic properties.

The present study reports the optical crystallographic data and constants for the diliturate derivatives of amine drugs found in illicit street drug preparations entitled "white crosses" or "mini-bennies." These preparations are so termed because of the physical shape of the small, white, cross-scored tablets purported to contain 2–8 mg of *d*-methamphetamine (7, 8). However, other drugs have been freely substituted for dextroamphetamine in these street preparations since the 1970 Drug Enforcement Administration Controlled Substances Act made the amphetamines difficult to procure for the street market (8).

EXPERIMENTAL

Materials—The drugs used to prepare the diliturate derivatives were obtained from pharmaceutical manufacturers and chemical supply

Table I—Optical Properties of Drug Diliturates

Derivative[a] (Optic Sign)	System[b]	Optical Orientation	Refractive Indexes			2V by Nomogram[c] Method	Elongation	Habit	Extinction Angle
			α	β	γ				
dl-Amphetamine (−)	O	Obtuse	1.470	1.645	1.698	53°	(±)	Acicular	Parallel
Dextroamphetamine (−)	O	Obtuse	1.471	1.653	1.704	52°	(±)	Tabular	Parallel
dl-Chlorpheniramine (−)	M	Inclined obtuse	1.512	1.682	1.732	52°	(±)	Lamellar	15°
Diphenhydramine (−)	M	Inclined acute	1.582	1.608	1.624	75°	(+)	Tabular	42°
l-Ephedrine (−)	O	Optic normal	1.544	1.619	1.655	66°	(−)	Lamellar	Parallel
dl-Ephedrine (−)	T	Inclined optic axis	1.537	1.662	1.731	67°	(±)	Tabular	38°
Mephentermine (−)	M	Inclined optic normal	1.488	1.659	1.688	40°	(−)	Acicular	27°
dl-Methamphetamine (−)	M	Inclined obtuse	1.482	1.654	1.656	8°	(−)	Acicular	24°
d-Methamphetamine (−)	M	Acute	1.545	1.648	1.705	70°	(±)	Tabular	33°
Methapyrilene (−)	M	Inclined obtuse	1.548	1.689	1.723	48°	(±)	Lamellar	33°
Papaverine (−)	T	Inclined optic normal	1.493	1.742	1.785	41°	(+)	Prismatic	27°
Phentermine (−)	O	Obtuse	1.495	1.665	1.688	36°	(−)	Acicular	Parallel
Phenylephrine (−)	T	Inclined obtuse	1.520	1.664	1.752	70°	(±)	Prismatic	42°
dl-Phenylpropanolamine (lath) (−)	O	Obtuse	1.461	1.678	1.708	36°	(−)	Lath	Parallel
dl-Phenylpropanolamine (lam) (−)	O	Obtuse	1.471	1.663	1.685	34°	(−)	Lamellar	Parallel
Propoxyphene (−)	M	Inclined obtuse	1.495	1.618	1.658	55°	(±)	Acicular	15°
Pseudoephedrine (−)	O	Optic normal	1.520	1.622	1.640	36°	(+)	Prismatic	Parallel
Dilituric acid (−)	M	Inclined obtuse	1.388	1.684	>1.785	50° est.	(−)	Tabular	9°

[a] The authors acknowledge supplies of *dl*-chlorpheniramine (Chlortrimeton, Schering), mephentermine (Wyamine, Wyeth), methapyrilene (Histadyl, Lilly), phentermine (Ionamine, Penwalt), propoxyphene (Darvon, Lilly), and pseudoephedrine (Sudafed, Burroughs Wellcome) and express their appreciation to the manufacturers who supplied the amine salts.

[b] O = orthorhombic, M = monoclinic, and T = triclinic. [c] Determined by the method of Hartshorne and Stuart (11).

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