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Note

General reversed-phase high-performance liquid chromatography procedure for the analysis of oral contraceptive formulations

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Oral contraceptive formulations are for the most part a mixture of two types of biologically active steroids, an estrogen and a progestin; formulations containing only a progestin are also available. They present an interesting problem to the analyst, as the concentration of active ingredient in various formulations may vary over the range 25 μg–5.0 mg per tablet. Furthermore, there are considerable differences between the UV maxima (end absorption to 280 nm) as well as the absorption coefficients of the various drugs used in these preparations.

Oral contraceptive formulations have been quantitatively assayed by a large number of procedures. These include colorimetry[1–3], UV determination after acidic conversion to a UV absorbing compound[4], UV determination of progestin having a natural UV chromophore[4], and fluorometry of estrogens[5,6], with and without prior separation of active ingredients, quantitative thin-layer chromatography (TLC)[7], gas–liquid chromatography (GLC)[8–10] and high-performance liquid chromatography (HPLC)[11–13]. All these procedures are for one particular ingredient or formulation and cannot be used to analyze all possible combinations of progestins and estrogens. However, Johnston[14] has recently described a general procedure using normal-phase HPLC with a variable-wavelength UV detector and a fluorometric detector coupled in series.

This report describes the use of a reversed-phase HPLC system for the simultaneous UV determination of active components in all oral contraceptives on the Canadian market.

EXPERIMENTAL

Apparatus

A modular HPLC system consisting of a SP 8700 solvent delivery system (Spectra-physics, Santa Clara, CA, U.S.A.) operated at 1.50 ml/min, a variable-wavelength UV detector (set at 210 nm, Schoeffel Model SF 770, Westwood, NJ, U.S.A., and a 7000-p.s.i. loop injector (Rheodyne septumless valve injector Model 7125, Berkeley, CA, U.S.A.) (equipped with a 10-μl loop) were used. The column (250 × 4.6 mm I.D.) was octadecylsilane chemically bonded to totally porous spherical microparticulate silica (5–6 μm) (Zorbax ODS, DuPont, Wilmington, DE, U.S.A.).

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Peak retention times and areas were obtained with a reporting integrator (HP3385A automation system, Hewlett-Packard, Avondale, PA, U.S.A.).

Reagents

Ethynyl estradiol, ethynodiol diacetate, mestranol, norethindrone, norethindrone acetate, norethynodrel and norgestrel were USP reference standards. 17α-Ethynyl-4-estrene-3β,17-diol 17.acetate (β-EODA) and 17α-ethynyl-4-estrene-3α,-17-diol 17-acetate (α-EODA) were obtained from norethindrone acetate by reduction with zinc borohydride[15]. 10-β-Hydroperoxynorethindrone was synthesized by oxygen oxidation of norethynodrel[16].

Valerophenone (Aldrich, Milwaukee, WI, U.S.A.) was washed with aqueous hydrochloric acid (1 *N*), followed by aqueous sodium hydroxide solution (1 *N*) and water, then dried over anhydrous sodium sulfate. Acetonitrile and methanol were HPLC grade (J. T. Baker, Phillipsburg, NJ, U.S.A.) and water was double-distilled in glass.

Mobile phase

Acetonitrile and water [filtered through membranes FH 0.2 μm and FA 0.45 μm (Millipore, Bedford, MA, U.S.A.) and degassed (helium bubbling)] were mixed via the solvent delivery system in 60:40 ratio, except for formulations containing ethynodiol diacetate, where a ratio of 75:25 was used.

Internal standard

A solution of valerophenone in methanol–water (4:1) was prepared at a concentration of 25 μl/l.

Standard preparation

Mixed standards of estrogen and progestin were prepared in internal standard solution according to Table I.

Assay preparation

Not less than twenty tablets were weighed and finely powdered. An amount of powder equivalent to one tablet was accurately weighed into a 15-ml PTFE-lined, screw-capped culture tube. Internal standard was added according to Table I. The tube was capped and vigorously shaken on a vortex type mixer (Rotary evapo-mix, Buchler Instruments, New York, NY, U.S.A.) for 30 min. The tube was then centrifuged to obtain a clear solution.

Content uniformity preparation

One tablet was transferred to a 15.ml PTFE-lined, screw-capped culture tube. Internal standard solution was added according to Table I. The procedure was then as described in Assay preparation.

Recovery study

Synthetic formulation mixtures were fabricated as follows: sufficient inert material [lactose–corn starch–polyvinylpyrrolidone–calcium stearate (73:20:5:1) 5 g] was added to 10 ml of alcohol containing a sufficient, accurately weighed amount of the

TABLE I

ASSAY INSTRUCTIONS FOR ORAL CONTRACEPTIVES

Formulation composition	*Strength (mg)*	*Vol. Int. Std. sol. to be added (ml)*	*Standard concentration (mg/ml)*
Ethynodiol diacetate/ ethynyl estradiol	1.0 0.05	2.0	0.5 0.025
Ethynodiol diacetate/ mestranol	1.0 0.1	2.0	0.5 0.05
Norethindrone/ mestranol	1.0 0.05	2.0	0.5 0.025
Norethindrone/ mestranol	1.0 0.08	2.0	0.5 0.040
Norethindrone/ mestranol	1.0 0.035	2.0	0.5 0.018
Norethindrone acetate/ ethynyl estradiol	1.0 0.05	2.0	0.5 0.025
Norethindrone acetate/ ethynyl estradiol	2.5 0.050	5.0	0.5 0.010
Norethynodrel/ mestranol	2.5 0.1	6.0	0.42 0.017
Norethynodrel/ mestranol	5.0 0.075	5.0	1.0 0.015
Norethynodrel/ mestranol	9.85 0.150	10.0	1.0 0.015
Norgestrel/ ethynyl estradiol	0.5 0.05	2.0	0.25 0.025
Norgestrel/ ethynyl estradiol	0.3 0.03	2.0	0.15 0.015
Norethindrone/ ethynyl estradiol	1.0 0.035	2.0	0.5 0.018
Norethindrone/ ethynyl estradiol	0.5 0.035	2.0	0.25 0.018

estrogen and of the progestin to give the same amount of active ingredients per 100 mg of mixture as in commercial formulations (Table I).

Following evaporation of the alcohol, a 100-mg portion of synthetic mixture was treated as described under Assay preparation.

Procedure

Aliquots (10 μl) of standard preparation and sample preparation were successively injected into the chromatograph. The area ratios of the estrogen and of the progestin to the internal standard were calculated. The quantities of active ingredients

per tablet were calculated using the following formula:

$$Cu = V \cdot C_s \cdot \frac{R_u}{R_s} \cdot \frac{W_t}{W_u}$$

where Cu = active ingredient per tablet (mg), C_s = concentration of active ingredient in standard preparation (mg/ml), R_u = area ratio of active ingredient to internal standard in sample preparation, R_s = area ratio of active ingredient to internal standard in standard preparation, W_u = weight of sample taken (mg), W_t = average weight per tablet (mg), and V = volume of internal standard solution added.

RESULTS AND DISCUSSION

All chromatograms (Figs. 1 and 2) were as expected with respect to the shape of the peaks, and complete baseline resolution was achieved between solvent front,

Fig. 1. Analysis of norethynodrel–mestranol formulation. Mobile phase: acetonitrile–water (60:40). Peaks: A = 10 β-hydroperoxynorethindrone; B = ethynyl estradiol; C = norethindrone; D = norethynodrel, E = internal standard; F = mestranol.

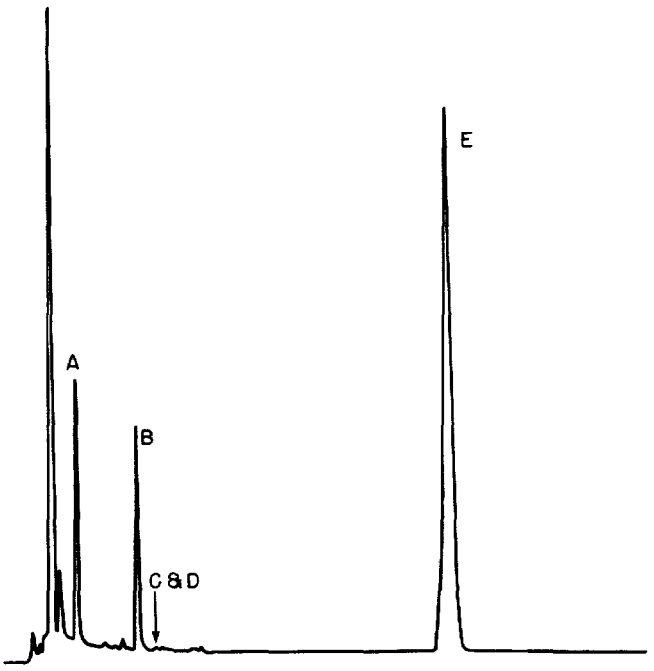

Fig. 2. Analysis of ethynodiol diacetate–ethynyl estradiol formulation. Mobile phase: acetonitrile–water (75:25). Peaks: A = ethynylestradiol; B = internal standard; C = β-EODA: D = norethindrone acetate; E = ethynodiol diacetate.

TABLE II

CAPACITY FACTORS OF COMPOUNDS OF INTEREST AND RESOLUTION FACTORS FOR EACH PAIR OF PEAKS

Compound	*Acetonitrile–water (60:40)*		*Acetonitrile–water (75:25)*	
	k'	R_s	k'	R_s
10β-Hydroperoxy norethindrone	1.44		–	
		3.85		
Ethynyl estradiol	3.07		1.65	
		1.72		1.54
Norethindrone	3.86		2.26	
		3.40		4.22
Norethynodrel	5.67		–	
		0.33		
Norgestrel	5.88		–	
		4.30		
Valerophenone	8.49		3.91	
		2.59		2.75
β-EODA*	10.26		4.67	
		0.96		0.29
Norethindrone acetate	11.02		4.83	
		3.24		1.16
Mestranol	13.61		5.44	
		0.24		1.04
α-EODA*	13.79		5.95	
		–		11.09
Ethynodiol diacetate	–		15.28	

* Identified according to their elution order (see ref. 19).

TABLE III

STANDARD CURVES FOR COMPOUNDS OF INTEREST

Compound	*Range (μg injected)*	*Curve*	*Correlation coefficient*
Ethynodiol diacetate	2.0–12.0	$0.741x + 0.072$	0.9997
Ethynyl estradiol	0.04–2.4	$4.559x - 0.022$	0.9996
Mestranol	0.035–1.5	$4.816x - 0.007$	0.9997
Norethindrone	0.8–5.0	$0.908x - 0.013$	0.9996
Norethindrone acetate	1.0–6.0	$0.744x - 0.006$	0.9998
Norethynodrel	0.4–12.0	$0.989x - 0.010$	0.9999
Norgestrel	0.5–3.0	$0.729x - 0.007$	0.9999

main components in each commercial formulation, their potential impurities or degradation products and the internal standard, except as noted below.

Table II shows capacity factors (k') and resolution between each pair of eluted compounds) R_s) using the described mobile phases, produced by the overlapping resolution maps (ORM) optimization techniue[17]. A factor of 1.25 between two peaks was considered to represent complete resolution. Only one formulation failed this basic requirement; however, Snyder and Kirkland[18] established that a resolution of 1.0 introduced less than 1% error to the major peak when either peak height or peak area was used. The worst case occurred in ethynodiol diacetate–mestranol formulations, in which mestranol is resolved from ethynodiol diacetate degradation products norethindrone acetate by 1.16 and α-EODA by 1.04.

Linearity of response *versus* concentration was studied for all active ingredients in oral contraceptive preparations (Table III). All standard curves were found to be linear in the concentration ranges studied and passing close to the origin. Their correlation coefficients were nearly ideal (≥ 0.9996).

Detection sensitivities were very good, all compounds being detectable in the 1–5-ng range (Table IV).

Methanol–water (4:1) was preferred as the extracting solvent to mobile phase, pure methanol or acetonitrile because of its ability to assist in disintegration. The mobile phase separated into phases when high concentrations of polar excipients (*e.g.* lactose) were present in the formulation.

TABLE IV

MINIMUM LIMITS OF DETECTABILITY OF ORAL CONTRACEPTIVE DRUGS (4 × BASELINE)

Compound	*Limit of detection (ng)*
Ethynodiol diacetate	5
Ethynyl estradiol	1
Mestranol	1
Norethindrone	2
Norethindrone acetate	2
Norethynodrel	1
Norgestrel	2

TABLE V

RECOVERY STUDY

Formulation	*Added (mg)*	*Found (μg)*	*Recovery (%)*	*C.V. (n = 10)*
Ethynodiol diacetate/ ethynyl estradiol	1.015 0.0519	1.009 0.051	99.3 98.3	0.7 1.3
Ethynodiol diacetate/ mestranol	1.015 0.100	1.009 0.099	99.3 99.3	0.7 1.0
Norethindrone/ mestranol	1.016 0.0527	0.985 0.0522	96.9 98.9	1.3 1.4
Norethindrone acetate/ ethynyl estradiol	1.011 0.0507	1.008 0.0503	99.7 99.2	1.3 1.1
Norethynodrel/ mestranol	2.508 0.103	2.492 0.105	99.4 101.6	1.5 1.1
Norgestrel/ ethynyl estradiol	0.5005 0.0500	0.515 0.0518	103.0 103.6	2.2 2.1

Table V shows the accuracy of the procedure for synthetic mixtures prepared to correspond to formulations studied. Recovery was excellent in all cases, ranging from 96.9 to 103.6% of added amounts, with relative standard deviation better than or equal to 2.2%.

Quantitative analytical results of commercial formulations are listed in Table VI. All results are within compendial limits (90–110%). Coefficients of variation were good (<2.6%). Means of the content uniformity test agree well with assay results; differences are randomly distributed between −3.9% and 4.3%.

TABLE VI

COMMERCIAL FORMULATION ANALYSIS

Formulation	*Labelled (mg)*	*Assay**		*Content uniformity*	
		Mean (%)	*R.S.D. (%)*	*Mean (%)*	*R.S.D. (%)*
Ethynodiol diacetate/ ethynyl estradiol	0.5 0.05	98.1 94.9	0.8 0.6	95.7 93.6	1.0 1.2
Ethynodiol diacetate/ mestranol	1.0 0.10	93.3 96.4	1.0 0.9	94.2 96.0	2.7 3.1
Norethindrone/ mestranol	1.0 0.08	100.6 104.6	2.5 1.0	100.5 105.1	1.8 1.8
Norethindrone acetate/ ethynyl estradiol	1.0 0.05	98.1 99.6	1.9 2.6	99.6 97.5	2.5 3.3
Norethynodrel/ mestranol	2.5 0.1	100.8 107.4	1.4 1.5	99.0 103.5	2.6 4.4
Norgestrel/ ethynyl estradiol	0.3 0.03	99.8 97.2	2.5 1.0	102.5 101.5	1.9 1.2

* In triplicate.

CONCLUSION

The accuracy, reproducibility and specificity of this HPLC procedure make it an excellent quantitative assay and content uniformity method for the analysis of oral contraceptive formulations.

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