Pharmaceutical Research, Vol. 4, No. 1, 1987

Report

Automated Stability-Indicating High-Performance Liquid Chromatographic Assay for Ethinyl Estradiol and (Levo)norgestrel Tablets

Van D. Reif,[1,2] W. Mark Eickhoff,[3] John K. Jackman,[4] and Nicholas J. DeAngelis[1]

Received June 30, 1986; accepted September 17, 1986

An automated high-performance liquid chromatography (HPLC) assay for ethinyl estradiol and norgestrel or levonorgestrel in oral contraceptive tablets was developed. Tablets were prepared for on-line injection using a solid sampler and segmented continuous flow techniques. The active components were separated from tablet excipients, impurities, and degradation products on reversed-phase C8 and C18 columns by elution with water–acetonitrile–methanol (45:35:15). A UV detector connected in series with a fluorometric detector measured the UV absorbance of levonorgestrel and norgestrel at 240 nm and the fluorescence of ethinyl estradiol at 310 nm (excitation at 210 nm). The method employed computer control of the injection system and solid sampler for synchronization of the chromatographic and segmented flow streams. The method is applicable for content uniformity and stability testing at a rate of eight samples per hour.

KEY WORDS: automated high-performance liquid chromatography (HPLC); (levo)norgestrel–ethinyl estradiol tablets; segmented continuous flow automated analysis; automated sample preparation; stability-indicating assay.

INTRODUCTION

Various analytical procedures have been described for the analysis of ethinyl estradiol (1) and norgestrel (2a) in oral contraceptive tablets. The official method (1) employs an ultraviolet spectrophotometric determination of 2a after alcoholic extraction and an acid-induced colorimetric analysis of 1 after multiple sample extractions. Sensitive high-performance liquid chromatography (HPLC) methods have been described (2–4) for 1 and 2a in tablets. However, these methods required time-consuming extractions for sample preparation, and specificity in the presence of degradation products was not demonstrated. Procedures employing automated sample preparation with continuous segmented flow analysis and fluorescent or colorimetric detection have been reported for 1 (5,6) and 2a (7,8). However, with these procedures the specificity for the analyte in the presence of degradation products, dyes, tablet coatings, or the other active component is often questionable. A combination of continous flow analysis with HPLC has the advantage of both specificity and automated sample preparation (9,10). The techniques for employing this type of combination have been reviewed by Burns and co-workers (11,12). This report describes a totally automated, precise, and specific HPLC procedure for simultaneous determination of ethinyl estradiol and norgestrel or levonorgestrel, 2b, in oral contraceptive tablets. A computer interface was used to control HPLC injection valves and system timing. The HPLC system has been adopted as the official method for levonorgestrel and ethinyl estradiol tablets (13).

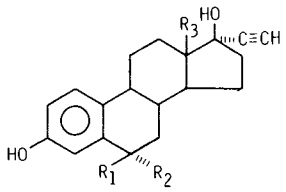
MATERIALS AND METHODS

Instrumentation

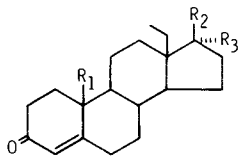
The liquid chromatograph was equipped with a DuPont Model 870 pump and two detectors connected in series, a Laboratory Data Control Spectromonitor III UV detector set at 240 nm and a Schoeffel FS970 fluorescence detector set with excitation at 210 nm and no filter in the emission path (photomultiplier window: 80% transmission at 310 nm). The fixed-loop septumless injector (Rheodyne Model 7125) with a 100-μl loop was pneumatically activated by a two-three way solenoid valve (Rheodyne Model 7163-022). The reverse-phase column (DuPont Zorbax C8, 7 μm, 150 × 4.6-mm i.d., or Hypersil ODS, 3–5 μm, 100 × 4.6-mm i.d.) was preceded by a 40 × 4.6-mm-i.d. C-135 Uptight precolumn (Upchurch Scientific, Inc.), dry-packed with silica gel 60 (230–400 mesh) from EM Reagents. The automatic sample preparation components, a SOLIDprep II, a Proportioning Pump III, a Continuous Tape filter (reverse phase with 591C paper), and tubing, were obtained from Technicon (Fig. 1).

[1] Analytical Research and Development Division, Wyeth Laboratories, Inc., P.O. Box 8299, Philadelphia, Pennsylvania 19101.
[2] To whom correspondence should be addressed.
[3] Present address: Smith Kline French Laboratories, 600 Allendale Road, King of Prussia, Pennsylvania 19406.
[4] Present address: Department of Engineering, Pennsylvania State University, State College, Pennsylvania 16801.

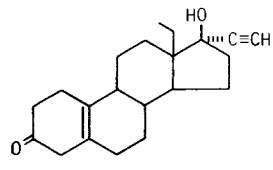
0724-8741/87/0200-0054$05.00/0



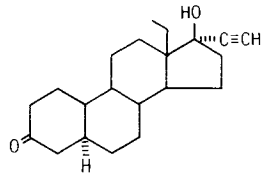
1, R_1 = H R_2 = H R_3 = CH_3
3, R_1, R_2 = =O R_3 = CH_3
4, R_1, R_2 = =O R_3 = CH_2CH_3
5, R_1 = H R_2 = OH R_3 = CH_3
6, R_1 = OH R_2 = H R_3 = CH_3



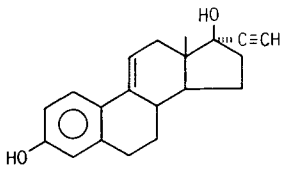
2a, 2b, R_1 = H R_2 = OH R_3 = C≡CH
7, R_1 = H R_2, R_3 = =O
8, R_1 = H R_2 = OH R_3 = H
11, R_1 = OH R_2 = OH R_3 = C≡CH



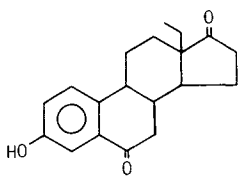
9



10



12



13

Scheme I

A laboratory computer system, consisting of a Hewlett Packard Model 1000 Minicomputer, Computer Automated Laboratory System (CALS) software from Computer Inquiry Systems, Inc. (CIS), and a CIS Mark IV Digimetry Unit data coupler, were used for data acquisition and reduction. The injection-switching valve was computer controlled via an AC power controller interface (obtained in-house from Mr. Gus Shaw). This interface converted 5-V transistor-transmitted logic (TTL) signals, generated by the data coupler, to 115-V AC signals. A relay attached to the dwell-function switch of the solid sampler was also computer controlled via the power controller interface so that the program disk could be temporarily stopped during processing. This was necessary because chromatographic runs were longer than 6 min, the longest cycle time permitted by the sampler program disk.

Chemicals

Compounds **1** and **2a** were obtained as USP Reference Standards, and **2b** was obtained in-house. The following compounds were prepared in-house by the Chemistry Division using published procedures: **3** and **4** (14), **5** (15), **6** (16), **7** and **8** (17,18), **9** (17–19), **10** (20), and **11** (21). Dehydro ethinyl estradiol, **12**, was isolated from degraded **1** (22). The procedure for **4** (14) was used to prepare **13** from **7**. IR (KBr): 3340 (OH), 1716 (C=0), 1680 (C=0) cm^{-1}. MS (70 eV): *M/Z* 298 (M^+), 269 ($M-C_2H_5$). UV max (ethanol): 222, 256, 326 nm. UV max (ethanol/NaOH): 243 nm.

Automated HPLC Method

Solutions. Acetonitrile–methanol–water (35:15:45) was used for the mobile phase, the homogenization diluent, and the diluent for all other solutions. Assay standard solutions were formulated to give an amount of **1** and **2a** or **2b** equal to the label claim per tablet in 1 ml. Content uniformity standard solutions were formulated at one-fifth of the assay standard solution concentration. The standard solution concentrations ranged from 0.006 to 0.04 mg/ml for **1** and 0.01 to 0.3 mg/ml for **2a** or **2b**. The system suitability test solution contained **1** (3 μg/ml) and **2a** (5 μg/ml). The injection-point test solution contained about 100 μg/ml of **2a**.

System Suitability. The UV detector was set at 215 nm, and 100 μl of the test solution was chromatographed. The resolution factor (23) between **1** and **2a** was ≥3.0. Unsuitable resolution could be improved by a slight increase in the water concentration in the mobile phase.

Injection Point. The system was assembled as shown in Fig. 1, except the postfilter sample line and debubbler line were connected to the flow cell of a UV spectrophotometer (Technicon) set at 240 nm. A 5-ml aliquot of the injection-point test solution was placed in several sampler cups. The sampler parameters were set as in Procedure, except a single 50-ml diluent volume was used. The test solution cups were processed, and the injection point was defined as the location of the sampler control disk when the norgestrel peak reached 1 min of steady state (9). The injection point was between 4 and 42.5 on the control disk and occurred during the homogenization cycle of the subsequent cup.

Procedure. The apparatus was assembled as shown in Fig. 1. The sampler program disk (10/hr) was set as follows: homogenization, 4 to 42.5; sample, 43 to 81.5; content uniformity diluent (volume 1 × 50 ml), 1.5 to 3.5; and assay diluent (volume 2 × 50 ml), 1.5 to 3.5 and 5.5 to 5.7. Homogenizer speed was 6 with stir speed off. A 5-ml aliquot of standard solution was pipetted into each standard cup. One tablet was placed in each sample cup for content uniformity testing and five tablets per cup were used for the assay.

A computer acquisition method (Table I) was used to acquire data, control the HPLC injection valve, and implement sampler dwell via the data coupler. The dwell time was

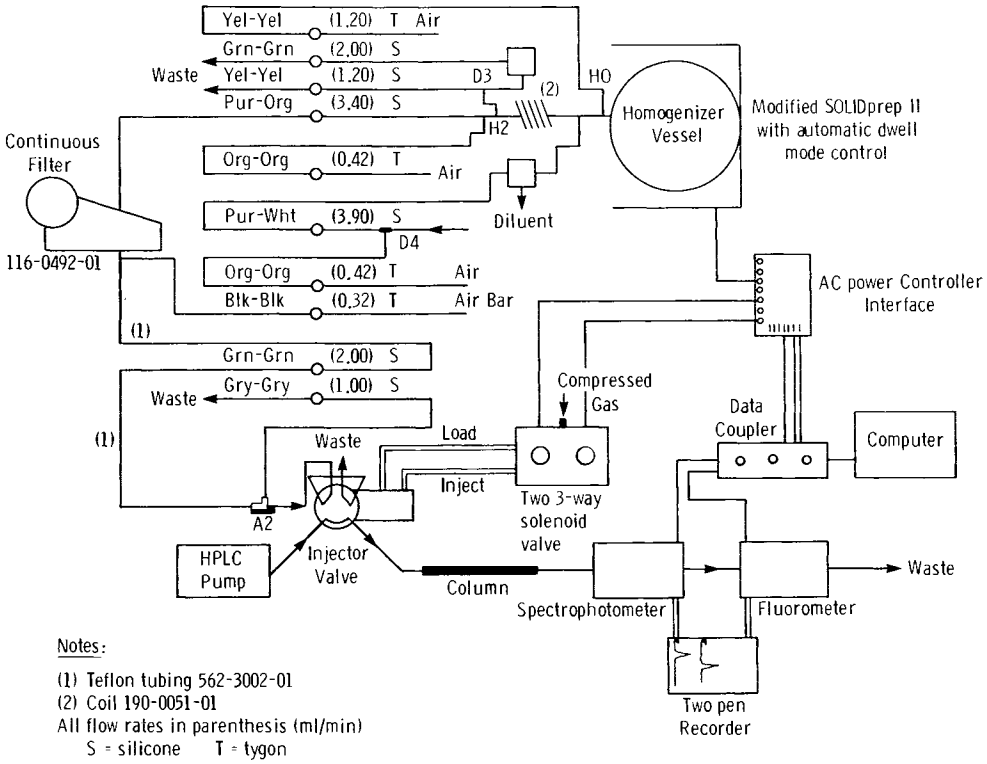


Fig. 1. Flow diagram for analysis system.

calculated as the chromatographic run time in excess of 6 min. To begin a run, the solid sampler was started, and at the injection point of the first cup, the acquisition method was initiated via the data coupler. As each successive data file was opened by the computer, the acquisition method was automatically repeated.

RESULTS AND DISCUSSION

The procedure was suitable for tablets containing 30–40 μg of 1 and 50–300 μg of **2a** or **2b**. A typical sample chromatogram is shown in Fig. 2. The recovery and reproducibility data (Table II) indicated that the procedure is accurate and reproducible. In a further check of extraction of active from the tablet matrix during homogenization, content uniformity results for three lots of tablets were found to be equivalent by both the automated and the manual sample preparation procedures (Table II). Both sample preparation procedures also gave equivalent results for assay of highly stressed tablets (Table II). Typical excipients, such as lactose, microcrystalline cellulose, starch, povidone, polacrilin potassium, magnesium stearate, talc, sucrose, calcium carbonate, and dyes, did not interfere with the procedure. Linearity was checked by assaying standard solutions which re-

Table I. A Typical Computer Acquisition Method[a,b]

				Explanation
1. SR, AV CT, RD TH	3	15	5.00	Chromatographic signal sampling rate
2. TIME, CODE, VALUE	.10	1		Start chromatographic data acquisition
3. TIME, CODE, VALUE	.10	10	1	Inject mode
4. TIME, CODE, VALUE	.10	10	3	Start Dwell mode
5. TIME, CODE, VALUE	.20	10	−1	Release Inject mode current
6. TIME, CODE, VALUE	2.10	10	−3	Stop Dwell mode
7. TIME, CODE, VALUE	2.11	10	2	Load Sample mode
8. TIME, CODE, VALUE	2.21	10	−2	Release Load mode current
9. TIME, CODE, VALUE	7.80	0	.20	Stop data acquisition, wait 0.2 min before next data file
10. ANALYSIS SVC, METHOD GC	6			Data analysis parameters

[a] Chromatographic run time, 8 min; dwell time, 2 min.

[b] Code 10 indicates operation of one of seven outputs from the data coupler. The output channel is designated by the value.

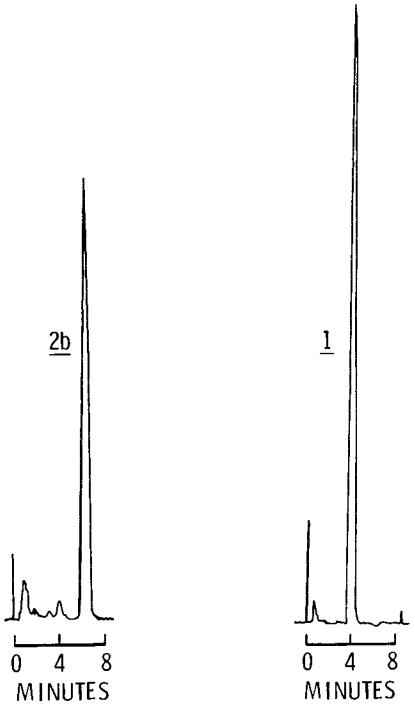


Fig. 2. Typical assay chromatogram.

Table III. Chromatographic Separation of Impurities and Degradation Products

	Compound	HPLC retention time (min) Column 1[a]	Column 2[b]
5	(6-α-hydroxy EE)	1.8	1.7
6	(6-β-hydroxy EE)	2.3	1.8
3	(6-keto EE)	2.3	1.8
11	(10-hydroxy norgestrel)	2.3	1.8
4	(18-methyl-6-keto EE)	3.1	2.0
13	(18-methyl-6,17-diketo)	3.3	2.0
12	(9,11-dehydro EE)	3.8	3.6
1	(EE)	4.2	4.1
8	(4-en-17-ol)	4.8	5.5
7	(4-en-17-one)	5.6	5.8
2a	(norgestrel)	5.9	6.2
2b	(levonorgestrel)	5.9	6.2
9	(5-ene)	9.2	9.7
10	(dihydronorgestrel)	10.2	10.3

[a] Hypersil ODS, 3–5 μm, 4.6 × 100 mm, 1.0 ml/min.
[b] Zorbax C8, 7 μm, 4.6 × 150 mm, 1.5 ml/min.

Table II. Recovery/Reproducibility[a] and Comparative Data[b,c] by Two Methods

Determination (sample storage conditions)	Ethinyl estradiol (1) Manual[d]	Ethinyl estradiol (1) Automated[e]	Levonorgestrel (2b) Manual[d]	Levonorgestrel (2b) Automated[e]
Recovery/reproducibility (initial)[f,g]	—	101 ± 0.8	—	101 ± 0.8
Recovery/reproducibility (initial)[h,g]	—	101 ± 1.0	—	101 ± 1.1
Recovery/reproducibility (initial)[i,g]	—	—	—	99 ± 0.6
Reproducibility (initial)[j,k]	—	99 ± 1.1	—	99 ± 1.3
Recovery/reproducibility (initial)[l]	—	100 ± 0.3	—	100 ± 0.2
Content uniformity (initial)[m]	98 ± 1.3	97 ± 1.7	99 ± 0.6	98 ± 1.8
Content uniformity (initial)[j]	99 ± 2.8	98 ± 1.7	99 ± 3.3	97 ± 1.1
Content uniformity (initial)[j]	98 ± 1.3	97 ± 1.7	99 ± 0.6	98 ± 1.8
Assay (3 days/105°C/100% RH)[m]	85	89	88	90
Assay (5 days/UV)[m]	100	101	100	101
Assay (3 days/105°C/100% RH)[n]	78	79	72	73
Assay (3 days/105°C/100% RH)[j]	87	92	76	78
Assay (5 days/UV)[j]	97	99	99	98
Assay (6 months/ambient temperature)[j]	101	99	105	103
Assay (3 months/45°C)[j]	96	97	98	97
Assay (3 months 35°C/80% RH)[j]	96	98	98	98

[a] Recovery/reproducibility, average percentage label claim ± relative SD (N = 5 determinations).
[b] Content uniformity, average percentage label claim ± relative SD (N = 10 compressed tablets).
[c] Assay—% label claim, sugar-coated tablets.
[d] Assay—10 tablets/100 ml sample solution; content uniformity—1 tablet/10 ml sample solution, detection of **1** and **2b** at 215 nm.
[e] Assay and recovery/reproducibility, 5 tablets/sample cup; content uniformity, 1 tablet/sample cup.
[f] Label claim, 30 μg **1** and 300 μg **2a**.
[g] Synthetic mixtures of actives and excipients for compressed tablets were assayed.
[h] Label claim, 30 μg **1** and 150 μg **2a**.
[i] Label claim, 75 μg **2a**.
[j] Label claim, 30 μg **1** and 50 μg **2b**.
[k] Compressed tablets.
[l] Sugar-coated placebo tablets spiked with **1** and **2b**; label claim, 40 μg **1** and 125 μg **2b**.
[m] Label claim, 30 μg **1** and 125 μg **2b**; RH, relative humidity.
[n] Label claim, 40 μg **1** and 75 μg **2b**.

sulted in injection concentrations ranging from 0.5 to 2.5 μg/ml of 1 and 1–30 μg/ml of **2a**. The chromatographic response was linear to the highest concentration tested. An analysis rate of eight samples per hour, which included the total sample preparation time, was obtained. The rate-limiting factor was the chromatographic run time of 8 min. A sample rate of up to 20/hr could be obtained when a nonstability-indicating HPLC system with a shorter run time was used.

The specificity of the HPLC assay was demonstrated by the separation of potential impurities and degradation products from the intact active components on two different columns as shown in Table III. The compounds **3**, **5**, **6**, and **12** are potential degradation products of ethinyl estradiol, whereas **4**, **7–11**, and **13** are potential degradation products and/or impurities of norgestrel.

Acknowledgment

This paper was presented in part at the Pharmaceutical Analysis and Control Section, APhA Academy of Pharmaceutical Sciences meeting in Philadelphia, October 1984.

REFERENCES

1. *The United States Pharmacopeia*, 21st rev., United States Pharmacopeial Convention, Rockville, Md., 1984, p. 747.
2. K. R. Bagon and E. W. Hammond. *Analyst* **103**:156–161 (1978).
3. A. M. Bond, I. D. Heritage, and M. H. Briggs. *Anal. Chim. Acta* **138**:35–45 (1982).
4. M. A. Johnston. *J. Chromatogr.* **216**:269–278 (1981).
5. A. J. Khoury and L. J. Cali. *J. Pharm. Sci.* **56**:1485–1489 (1967).
6. W. F. Beyer. *J. Pharm. Sci.* 57:1415–1418 (1968).
7. L. F. Cullen, J. G. Rutgers, P. A. Lucchesi, and G. J. Papariello. *J. Pharm. Sci.* 57:1857–1864 (1968).
8. P. M. Short and C. T. Rhodes. *Steroids* **16**:217–224 (1970).
9. J. C. MacDonald. *Am. Lab.* 9:69–76 (1977).
10. J. W. Dolan, J. R. Gant, N. Tanaka, R. W. Giese, and B. L. Karger. *J. Chrom. Sci.* **16**:616–622 (1978).
11. D. A. Burns, *Res. Dev.* 4:22–32 (1977).
12. D. A. Burns, J. L. Fernandez, J. R. Gant, and A. L. Pietrantonio. *Am. Lab.* **11**:79–89 (1979).
13. *The United States Pharmacopeia*, 21st rev., Suppl. 1, United States Pharmacopeial Convention, Rockville, Md., 1984, p. 1739.
14. H. Hofmeister, H. Laurent, and R. Wiechert. *Chem. Ber.* **106**:723–726 (1973).
15. P. N. Rao. *Steroids* **23**:173–183 (1974).
16. M. L. Cotter, S. D. Levine, R. Mallory, and C. Shaw. *Tetra. Lett.* 1939–1942 (1978).
17. G. A. Hughes and H. Smith. U.S. Patent 3,959,322 (1976).
18. H. Smith *et al. J. Chem. Soc.* 4472–4489 (1964).
19. F. B. Colton, U.S. Patent 2,725,389 (1955).
20. D. C. DeJongh and J. D. Hribar. *Steroids* **11**:649–666 (1968).
21. T. J. Foell, R. W. Rees, and H. Smith. U.S. Patent 3,412,112 (1968).
22. Unpublished results.
23. *The United States Pharmacopeia*, 21st rev., United States Pharmacopeial Convention, Rockville, Md., 1984, p. 1230.