# Spectrophotometric Methods for simultaneous Estimation of Ethinylestradiol and Levonorgestrel in Tablets

P.R. MISHRA, A. NAMDEO AND N.K. JAIN*
Department of Pharmaceutical Sciences
Dr. H.S. Gour University, Sagar

Accepted 19 February 1999
Received 20 June 1998

**Two simple and convenient simultaneous spectrophotometric methods for determination of ethinylestradiol and levonorgestrel in a combination dosage form were developed. These are based on native maxima of ethinylestradiol and levonorgestrel at 281 and 244 nm respectively when chloroform was used as a solvent. Both the drugs obey Beer's law in the concentration range employed for these methods. The results of analysis have been validated statistically and by recovery studies.**

Ethinylestradiol (EE) is an estrogen. Chemically it is 19-nor-17-pregna-13,5 (10)-triene-20-yl-17-β diol and often employed in a combination for the treatment of postmenopausal deficiency states, irregular menses and in contraception[1]. The USP[2] descibes a HPLC method while IP[3] describes titrimetric method for its analysis. Levonorgestrel (LN), (-) isomer of norgestrel, is chemically (13-β ethyl-17-β hydroxy-18, 19-dinor-17-pregn-4-ene-20-yn-3-one). The IP[4] describe a HPLC method while BP[5] describes titrimetric method for its analysis.

High performance thin layer chromatography has been reported for the determination of LN and EE[6]. High performance liquid chromatography has also been reported[7] for separation and analysis of LN, EE, Hydrocortisone and 5-Fluorouracil. No simple spectrophotometric method is reported so far for the simultaneous estimation of LN and EE though their combination is available in the market as contraceptive pills. Therefore a simple and convenient method for simultaneous estimation of EE and LN using simultaneous equation and multicomponent mode of analysis in UV-visible spectrophotometer was developed for tablet dosage form.

A Shimadzu ultraviolet/visible recording spectrophotometer (model-1601) with wavelength accuracy of ± 0.5 nm and spectral band width of 3 nm was used. Matched quartz cells of 10 mm pathlength were employed. Reagent used was Chloroform (Qualigens, Mumbai). Ethinylestradiol and Levonorgestrel were received as gift samples from Wyeth Laboratories, Mumbai.

The first method is based on the native ultraviolet absorbance maxima of ethinylestradiol and levonorgestrel in chloroform. The stock solutions of 100 mcg/ml of EE and LN respectively were prepared in chloroform. These were mixed in definite ratio as cited in Table 1 to get the mixed standard and scanned over the range of 300 to 220 nm in the multicomponent mode using two sampling points as 281 and 244 nm. These two sampling wavelengths were determined from overlain spectra of ethinylestrdiol and levonorgestrel. The overlain spectra of five mixed standards are presented in Fig-1.

Twenty five tablets each of two batches containing (EE-0.05 mg and LN-0.25 mg) were crushed and homogenised separately, powder equivalent to 1 mg EE was accurately weighed and transferred in a 100 ml volumetric flask, dissolved in chloroform with intermittent shaking. The solution was filtered through whatman filter paper no. 42, to get 10 mcg/ml of EE. The sample solutions were scanned over the range 300 nm to 220 nm in multicomponent mode and concentrations of each component were estimated by analysis of spectral data of sample solution with respect to mixed standard. This procedure was repeated four times and results shown in Table 2 were statistically significant.

Recovery studies carried out gave satisfactory results which are compiled in Table 3.

*For correspondence

Table 1 - Concentration of levonorgestrel and ethinylestradiol in mixed standards

| Drug | | Sample No. 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| EE | (mcg/ml) | 10 | 20 | 30 | 40 | 50 |
| LN | (mcg/ml) | 50 | 40 | 30 | 20 | 10 |

Table 2 - Analysis of formulation by proposed method

| Tablet sample | Label claim (mg/tab) | | % of label claim found ±S.D. | | | |
|---|---|---|---|---|---|---|
| | | | Method I | | Method II | |
| | LN | EE | LN | EE | LN | EE |
| I | 0.25 | 0.05 | 96.24±0.43 | 97.62±0.91 | 97.71±0.75 | 97.68±0.58 |
| II | 0.25 | 0.05 | 97.46±0.83 | 95.21±0.76 | 97.92±0.89 | 98.69±0.65 |

Table 3 - Recovery Study Data

| S.No. | Amount of drug added (mcg/ml) | | % Recovery | | | |
|---|---|---|---|---|---|---|
| | | | Method I | | Method II | |
| | LN | EE | LN | EE | LN | EE |
| 1. | 2 | 2 | 101.4 | 100.1 | 99.3 | 99.7 |
| 2. | 4 | 4 | 100.7 | 99.0 | 101.2 | 98.5 |
| 3. | 6 | 6 | 98.5 | 99.5 | 98.3 | 100.7 |
| 4. | 8 | 8 | 99.2 | 100.5 | 98.9 | 101.5 |

A set of two simultaneous equations was developed in second method using molar absorptivity coefficient of the two drugs determined at 281 and 244 nm. The values for the absorptivity coefficient are given below :-

$$A_1 = (1.985\ C_1 + 20.277\ C_2) \times 10^3$$

$$A_2 = (2.995\ C_1 + 1.9023\ C_2) \times 10^3$$

Where $C_1$ and $C_2$ are the concentrations of ethinylestradiol and levonorgestrel respectively in moles/L. in the sample solution. $A_1$ and $A_2$ are the absorbances of sample solution measured at 244 and 281 nm respectively. $1.985 \times 10^3$ and $20.27 \times 10^3$ are molar absorptivities at 244 nm of ethinylestradiol and levonorgestrel while $2.995 \times 10^3$ and $1.902 \times 10^3$ are molar absorptivities at 281 nm of ethinylestradiol and levonorgestrel respectively. The molar absorptivities reported are mean of five independent determinations. By substituting values of $C_1$ in equation 2 the numerical value of $C_2$ can be determined. The overlain spectra of ethinylestradiol and levonorgestrel are given in Fig. 2.

Twenty five tablets each from two batches were weighed and finely powdered. An accurately weighed powder sample equivalent to 1 mg of ethinylestradiol was transferred in a 100 ml volumetric flask and dissolved in 30 ml of chloroform by intermittent shaking. The solution was then filtered through whatman filter paper No. 42 and

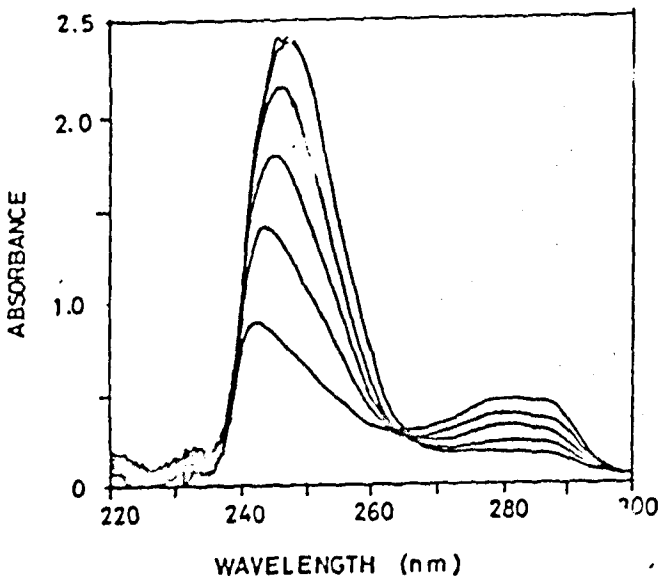


**Fig. 1 : Overlain spectra of mixed standards of Levonorgestrel and Ethinylestradiol**

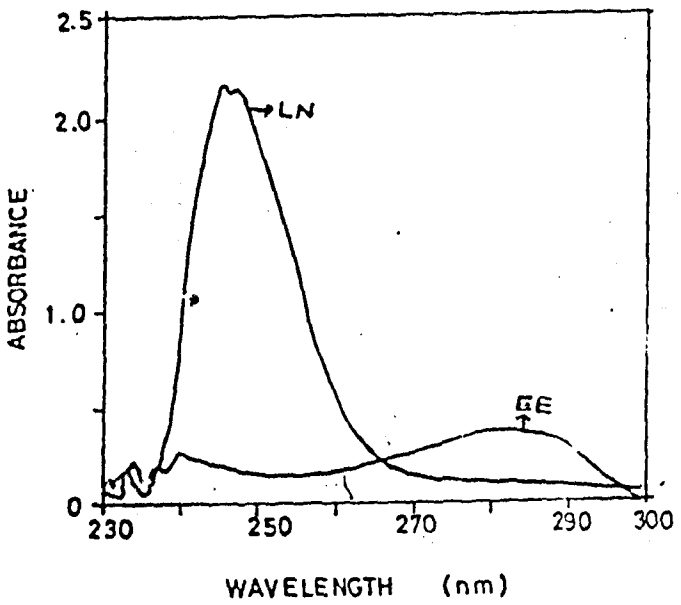


**Fig. 2 : Overlain spectra of Levonorgestrel and Ethinylestradiol**

the volume was made up to get a final concentration of 10 mcg/ml of ethinylestradiol. Absorbance of this sample solution was recorded at 244 and 281 nm as $A_1$ and $A_2$ respectively and concentrations of the two drugs in the sample solution were determined using above equations. The results of four repetitions are presented in Table 2. Substantial results were obtained when recovery studies were carried out which are presented in Table 3.

For routine analysis these proposed methods will find significant importance as they are simple, convenient and reproducible. Interference among the components can be reduced using five mixed standards and two sampling wavelengths. The values of standard deviation were low and recovery was close to 100% indicating reproducibility of the method. The only limitation with multiwavelength method is the feasibility on the instrument having this software. The simultaneous determination of this mixture was also tried by first derivative spectroscopy, in which LN and EE showed zero absorbances at 242 nm and 229 nm respectively. Where as EE gave prominent absorbance at 242 nm, the absorbance of LN at 229 nm was very low in magnitude, therefore this method was not successful for this combination.

## ACKNOWLEDGEMENTS

Thanks are due to Council of Scientific and Industrial Research for providing Senior Research fellowship to Prabhat R. Mishra and Alok Namdeo and Wyeth Laboratories, Mumbai for providing Ethinylestradiol and Levonorgestrel as gift sample.

## REFERENCES

1. Martindale, The extra Pharmacoepia 31st Edn. The Royal Pharmaceutical Society, London, 1996, 2082.
2. **The United States Pharmacopoeia/National Formulary** XXIII, Mack Publishing Co., Easton, 1995, 638.
3. **Indian Pharmacopoeia,** 4th Edn, Vol-I, Govt, of India, Controller of Publications, New Delhi, 1996, 301.
4. **Indian Pharmacopoeia,** 4th Edn, Vol-I Govt. of India, Controller of Publications, New Delhi, 1996, 434.
5. **The British Pharmacopoeia,** Vol-I Her Majesty, Stationary office, London 1993, 382.
6. Amin, M. and Hassenbach, M., **Analyst,** 1979, 104, 407.
7. Friend, D., Catz, P. and Heller, J., **J. Control Rel.** 1989, 9, 35.