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Simultaneous HPLC Estimation of Levonorgestrel and Ethinylestradiol from Tablets

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The present work describes a simple reverse phase HPLC method for the determination of levonorgestrel and ethinylestradiol from tablet formulations. The determination was carried out on a Hypersil, ODS, C-18 (150x4.6 mm, 5 micron) column using a mobile phase of acetonitrile/ water (42:58). The flow rate and runtime were 2.2 ml/min and 7 min, respectively. The eluent was monitored at 210 nm. The method was found to be reproducible, with good resolution between levonorgestrel and ethinylestradiol. The detector response was found to be linear in the concentration range of 10-50 ppm for levonorgestrel and 2-10 ppm for ethinylestradiol.

Wide ranges of steriodal hormones are used for contraception in a variety of formulations such as oral contraceptive pills (tablets), intra uterine devices and subcutaneous implants[1]. Literature survey indicated that HPLC, UV/Vis Spectrophotometer and potentiometry are used as official methods for the analysis of levonorgestrel and ethinylestradiol from tablets and pure drug[2-10]. The reported HPLC methods either lack the sensitivity or tedious, expensive and time consuming. The present investigation is an attempt to develop a highly sensitive, simple, precise and rapid analytical method for the simultaneous estimation of levonorgestrel and ethinylestradiol from tablet formulations.

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Standard samples of levonorgestrel and ethinylestradiol, which were prepared from reference standards procured from British Pharmacopoeia Commission, UK. HPLC grade acetonitrile manufactured by E. Merck was procured from commercial sources. Double distilled water was prepared in the laboratory. Oral contraceptive tablets containing levonorgestrel and ethinylestradiol were used obtained from local market and manufactured in in-house facility.

A Jasco HPLC system comprising a pump (Model: PU-980) with 20 µl loop, a UV/Vis detector (Model: UV-975) and integrator (Model: 807 IT) was used. The column used was

from Merck (Hypersil, ODS, 150x4.6 mm., 5 μ). The mobile phase consisting of acetonitrile and water (42:58) was pumped at a flow rate of 2.2 ml per min with the chart speed of 2 mm/ min. The detection was monitored at 210 nm and the run time was 7 min.

Levonorgestrel and ethinylestradiol (50 mg each) were weighed accurately in to two 100 ml volumetric flasks separately and both standards dissolved in about 30 ml of solvent solution (40 volumes of water and 60 volumes of acetonitrile) each and made volume with the same solvent solution (stock solution). From these stock solutions 10 ml was taken separately in to two 100 ml volumetric flasks and made volume with the solvent solution (50 ppm). In case of levonorgestrel varying amounts (5, 10, 15 and 20 ml) of the above solution (50 ppm) was taken in four different 25 ml volumetric flasks and, the volume was made upto the mark with the solvent solution. 20 microlitres of the solution from each flask was injected two times. In case of ethinylestradiol 20 ml was taken from stock solution (50 ppm) and diluted to 100 ml with the solvent solution (10 ppm). Varying amounts (5, 10, 15 and 20 ml) of the above solution (10 ppm) was taken in four different 25 ml and one 100 ml volumetric flasks respectively and, the volume was made upto the mark with the solvent solution. 20 microlitres of the solution from each flask was injected two times.

Calibration curves were constructed by plotting mean peak areas against the corresponding drug concentrations. The detector response was found to be linear in the concentration range of 10-50 ppm for levonorgestrel and 2-10 ppm for ethinylestradiol.

Twenty oral contraceptive tablets each containing 0.15 mg levonorgestrel and 0.03 mg ethinylestradiol were powdered finely, transferred a quantity equivalent to six tablets in to a 25 ml volumetric flask and added 15 ml of solvent solution. Then heated at 60° for 25 min and shaken for 2 min and cooled to room temperature. Then diluted to volume and filtered through whatman no.1 filter paper. One milliliter of this solution contains 36 ppm levonorgestrel and 7.2 ppm ethinylestradiol. Results of the triplicate analysis are given in Table 1.

This method was validated for statistical parameters i.e. Precision, Accuracy, Specificity, Linearity and Range, Stability of analytical solutions and Ruggedness criteria. Results of the method validation experiments are given in Table 2. This method precision was determined by knowing percentage RSD of means of three replicate solutions of all the three independent samples.

The accuracy of method is determined by adding known amount of standard to that of sample (above and below the normal level) at 3 different levels to cover both above and below (75 to 125%) the normal levels expected in the sample. The normal expected analytical level for the assay of levonorgestrel is about 37.5 μg/ml. (so the study range

TABLE 1: ANALYSIS OF ORAL CONTRACEPTIVE TABLETS CONTAINING LEVONORGESTREL AND ETHINYLESTRADIOL

Formulation	Label Content (mg/tablet)	Amount found (mg/tablet)*	% Drug found*	Amount Found (mg/tablet)**	% Drug found*	Standard Deviation**
Levonorgestrel						
Brand-1	0.15	0.1506	100.40	0.1508	100.53	0.59
Brand-2	0.15	0.1499	99.93	0.1497	99.80	0.55
Brand-3	0.15	0.1484	98.93	0.1482	98.80	0.67
Ethinylestradiol						
Brand-1	0.06	0.0304	101.33	0.0305	101.66	0.60
Brand-2	0.03	0.0298	99.33	0.0297	99.00	0.59
Brand-3	0.03	0.0295	98.33	0.0294	98.00	0.69

All values are average of three determinations. *Determinations of levonorgestrel and ethinylestradiol in pharmaceutical preparations by the official method. **Determinations of levonorgestrel and ethinylestradiol in pharmaceutical preparations by proposed method.

TABLE 2: RESULTS OF METHOD VALIDATION EXPERIMENTS OF LEVONORGESTREL AND ETHINYLESTRADIOL

Performance parameters		Results	Acceptance limit
Precision	Levonorgestrel	1.94%	NMT 2.0% RSD
	Ethinylestradiol	1.99%	
Accuracy	Levonorgestrel	3.17%	% Bias NMT 5%
	Ethinylestradiol	1.69%	
Specificity	Levonorgestrel	0.782%	No Interference due to placebo (RSD NMT 2.0%)
	Ethinylestradiol	0.734%	
Linearity (Regression Coefficient –r)	Levonorgestrel	Linear (r=0.999601)	Linear NLT 0.999%
	Ethinylestradiol	Linear (r=0.999897)	
Stability of analytical solutions (Normal conditions)	Levonorgestrel	0.81%	NMT 2.0% RSD
	Ethinylestradiol	0.66%	
Stability of analytical solutions (In a dark refrigerator)	Levonorgestrel	0.52%	NMT 2.0% RSD
	Ethinylestradiol	0.73%	
Ruggedness	Levonorgestrel	0.55%	NMT 2.0% RSD
	Ethinylestradiol	0.60%	

was 25, 37.5 and 50 µg/ml) and ethinylestradiol is about 7.5 µg/ml (so the study range was 5, 7.5 and 10 µg/ml).

The specificity of assay method of levonorgestrel in levonorgestrel and ethinylestradiol tablet was studied by spiking levonorgestrel at the expected content in to the placebo preparations. The specificity of assay method of ethinylestradiol in levonorgestrel and ethinylestradiol tablet was studied by spiking ethinylestradiol at the expected content in to the placebo preparations.

The linearity of analytical method was studied by analysing response of standard with predetermined concentration range, linearity curve was plotted for response areas against the concentration of the solution. Regression coefficient was calculated using above plot. For levonorgestrel, prepared solutions within concentration range of 10 to 50 µg/ml at 5 constant consecutive concentration levels. i.e. 10, 20, 30, 40 and 50 µg/ml. For ethinylestradiol, prepared solutions within concentration range of 2 to 10 µg/ml at 5 constant consecutive concentration levels. i.e. 2, 4, 6, 8 and 10 µg/ml. The regression coefficient of area of above consecutive concentrations was calculated.

The stability of analytical solutions of the method

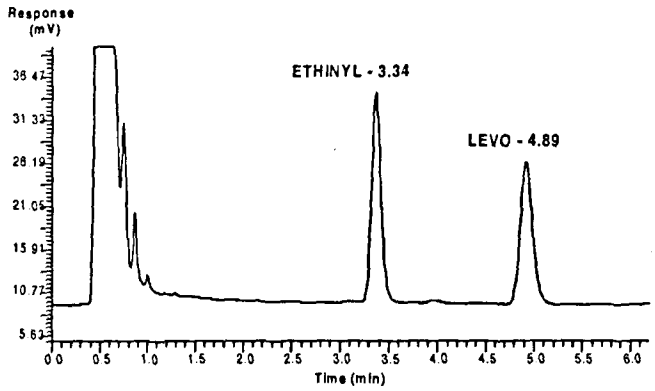

Fig. 1: A typical Chromatogram of levonorgestrel and ethinylestradiol.

***The retention times of levonorgestrel and ethinylestradiol were same in standard and sample.**

studied by a series of samples and standards were prepared and analysed immediately. They were stored at normal lab conditions and in a dark refrigerator, then reanalysed 120 h later against freshly prepared standard solutions.

The ruggedness of analytical method for levonorgestrel and ethinylestradiol in assay determination was studied by the analysing the samples by two sets. (i.e. different analysts , different reagents and solutions, different days and different instruments).

A typical chromatogram obtained in the present investigation is shown in fig. 1. The developed method was compared with the reference method 7. The results obtained were summarized in Table 1. Prior to the analysis, the method was subjected to system suitability tests. The resolution factor was found to be 6.55, which indicated that there is good resolution between levonorgestrel and ethinylestradiol. This method is highly sensitive than official method to estimate ethinylestradiol, which usually contains very low dose in tablet formulations. It requires less time for estimation than official method.

The statistical parameters in method validation studies for precision, accuracy, specificity, stability of analytical solutions and ruggedness were justified the validity of the proposed method. The results of the assay and method validation studies given in Table 1 and 2 have shown that the method is simple, accurate and precise and, non-interference from tablet excipients.

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