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Simultaneous Estimation Of Ethinyl Estradiol And Levonorgestrel From Transdermal Patches By HPLC

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The present work describes a simple reverse phase HPLC method for the determination of ethinyl estradiol (ETES) and levonorgestrel (LN) from transdermal patches. The determination was carried out on a Phenomenex, C-18, 250 x 4.6 mm, 10 micron column using a mobile phase of acetonitrile/ water (55:45). The flow rate was 1.2 ml/minute. The eluent was monitored at 225 nm. Quantitation was done using an external standard. The method was found to be reproducible, with good resolution between ETES and LN. The detector response was found to be linear in the concentration range of 1-5 ppm for ETES and 5.25 ppm for LN. The sensitivity of the method was found to be 0.1 µg/ml.

A wide range of steriodal hormones are used for contraception in a variety of formulations such as oral contraceptive pills, intra uterine devices and subcutaneous implants[1]. Efficient contraception can be achieved when the steroidal hormones, the estrogens and the progestins are used in combination[2]. The combination of the semisynthetic estrogen, ethinyl estradiol (ETES) and the synthetic progestin, levonorgestrel (LN), in low concentrations, is frequently used in oral contraceptive preparations[3-5].

Literature survey indicated that UV, HPLC fluorimetry and potentiometry are used as official methods for the analysis of ETES and LN from tablets[6-9]. Transdermal drug delivery is a relatively new concept. Transdermal therapeutic systems (TTS) for drugs like scopolamine, nitroglycerine, estradiol, clonidine and nicotine are available abroad commercially, though they are not yet available in India[10-13]. TTS containing steroids namely LN and combination of estradiol and LN which are to be used as contraceptives are patented in USA, though they have not reached the market yet[14-15]. TTS are still under research and not commercialized in India. Literature reveals that a lot of work is being carried out in various research institutions and pharmaceutical companies. However, there is no analytical method reported for the simultaneous determination of ETES and LN from transdermal patches containing various adjuvants. The present investigation is an attempt to develop a simple, precise and rapid analytical method for the simultaneous estimation of ETES and LN from transdermal patches.

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EXPERIMENTAL

Instrumentation

A Tosoh HPLC system comprising a reciprocating pump with 10 µl loop, a UV-Visible detector, UV-8010 and systems instrument corporation integrator (SIC-21) was used. The column used was from Phenomenex, USA, (Hypersil-10, C-18, 250 x 4.6 mm, 10 micron). The mobile phase consisting of acetonitrile/water (55:45) was pumped at a flow rate of 1.2 ml/min. with the chart of 1 cm/min. The detection was monitored at 225nm.

Materials

Standard samples of ETEs and LN were procured from Wyeth Laboratories Limited, Mumbai, as a generous gift.

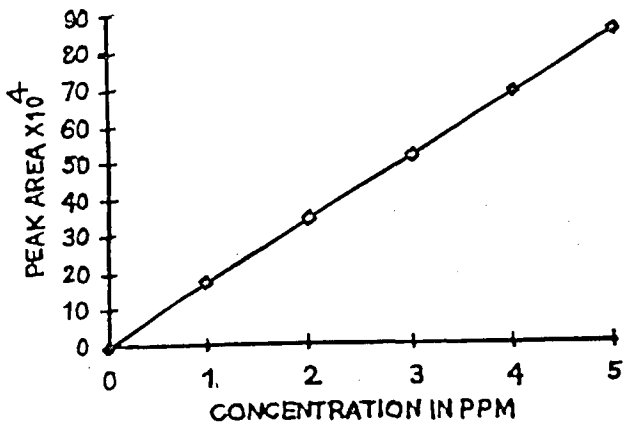


Fig -1 Linearity Plot of Ethinyl Estradiol

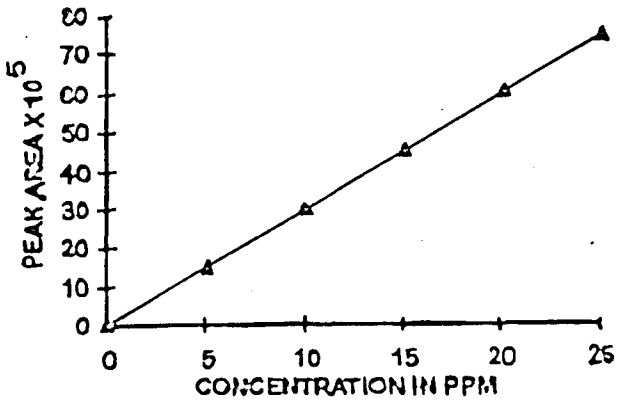


Fig -2. Linearity plot of Levonorgestrel

HPLC grade methanol and acetonitrile manufactured by S. D. Fine Chemicals were procured from commercial sources. Double distilled water was prepared in the laboratory. Transdermal patches containing ETEs and LN were prepared in the laboratory.

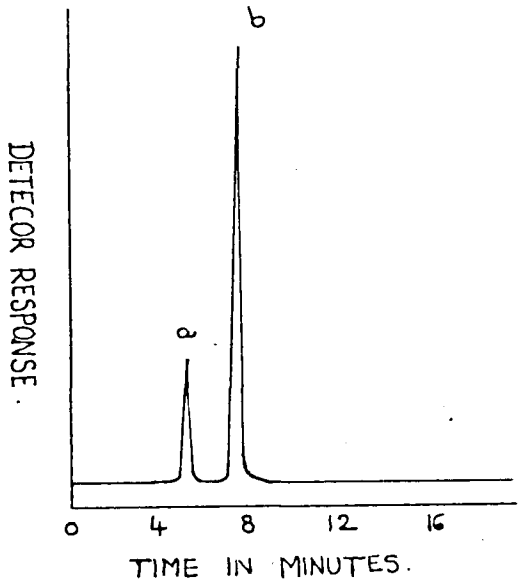
Preparation of standard curves

ETES (10 mg) and LN (50 mg) were weighed accurately. A standard stock solution containing 10 ppm ETES and 50 ppm LN was prepared in methanol. Varying amounts (1-5 ml) of the above standard stock solution were taken in five different 10 ml volumetric flasks and the volume was made upto the mark with the mobile phase. Ten microlitres of the solution from each flask was injected seven 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 1-5 ppm for ETES and 5-25 ppm for LN. The linearity plots for ETES and LN are given in fig. 1 and fig. 2.

Assay Procedure

In a typical assay procedure, ten transdermal patches each containing 0.2 mg ETES and 1 mg LN were extracted with 100 ml of methanol for 24 hours. Initially two 40 ml portions of methanol were used for extraction (for a duration of 8 h and 16 h). They were combined and the volume was made upto 100 ml. One ml of this solution was diluted with the mobile phase to give a solution containing 2 ppm ETES



a = Ethinyl Estradiol, b = Levonorgestrel

Fig -3. A typical Chromatogram of ETES and L.N.

and 10 ppm LN. The drug content was estimated at three concentration levels, namely, 2, 3 and 4 ppm for ETEs and 10, 15 and 20 ppm for LN, respectively. Estimation was carried out using an external standard. Results of the triplicate analysis are given in table 1.

Recovery studies

Recovery studies were carried out by using the method of standard addition to test the accuracy, precision and reproducibility of the proposed method. A fixed amount of the preanalysed sample was taken and three different

Table - 1: Analysis of Transdermal Patches Containing ETES and LN

S.No.	Concentration (ppm)	Label Content (mg/patch)	Amount found (mg/patch)	Standard deviation	Coefficient of variation	% Drug found
	ETES					
1.	2	0.2	0.1969	0.0016	0.8006	98.45
2.	3	0.2	0.1976	0.0029	1.4450	98.80
3.	4	0.2	0.1981	0.0022	1.1110	99.05
	LN					
4.	10	1	0.9892	0.0161	1.6249	98.92
5.	15	1	0.9900	0.0133	1.3456	99.00
6.	20	1	0.9959	1.2440	1.2490	99.59

Table - 2: Results of Recovery Experiments of ETES and LN

Sample	Drug content (μg/ml)	Concentration of standard drug added (μg/ml)	Amount of Drug found (μg/ml)	% recovery
	ETES			
0	2	0	1.9756	98.78
1	2	1	3.0058	100.19
2	2	2	3.9597	98.99
3	2	3	4.9380	98.75
	LN			
0	10	0	9.9996	99.99
1	10	5	14.950	99.66
2	10	10	19.906	99.53
3	10	15	24.990	99.94

concentrations of the standard drug were added to make four different samples. Each sample was injected seven times. The percent drug recovered was calculated. Results of the recovery experiments are given in table 2.

RESULTS AND DISCUSSION

A typical chromatogram obtained in the present investigation is shown in fig. 3. Prior to the analysis, the method was subjected to system suitability tests. The resolution factor was found to be 8.152 which indicated that there is good resolution between ETES and LN. In the reproducibility studies for retention times, the standard deviation and coefficient of variation (%) for ETES were found to be 0.0052 and 0.1 and the standard deviation and coefficient of variation (%) for LN were found to be 0.0059 and 0.1, respectively. The low statistical parameters indicate good precision of the proposed method.

The results of the linearity studies indicated that there is a highly significant positive linear correlationship, for both the drugs in the concentration range of 1.5 ppm for ETES and 5.25 ppm for LN. The results of the assay and recovery studies given in tables no. 1 and 2 have shown that the method is simple, accurate and precise. The percent recovery obtained indicated non-interference from patch excipients.

ACKNOWLEDGEMENTS

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