Journal of Applied Sciences

ISSN 1812-5654

science alert

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Journal of Applied Sciences 10 (16): 1802-1805, 2010
ISSN 1812-5654

Application of H-Point Standard Additions Method for Simultaneous Determination of Levonorgestrel and Ethinylestradiol in Oral Contraceptives LD

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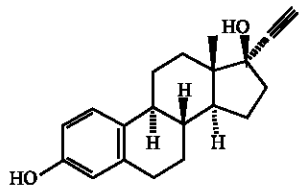
Abstract: The H-Point Standard Addition Method (HPSAM) has been applied for simultaneous determination of levonorgestrel (LEV) and ethinylestradiol (ETE) in contraceptive LD pill. LEV and ETE can be determined simultaneously in the range of 0.50-5.00 and 0.20-8.00 μg mL^{-1}, respectively with satisfactory accuracy and precision. The proposed method has successfully been applied for the simultaneous determination of both compounds in several synthetic samples and Iranian commercial low-dose oral contraceptives. A Limit of Detection (LOD) of LEV and ETE was obtained 0.011 and 0.010 μg mL^{-1}, respectively for synthetic samples. The standard error (E_r) of LEV and ETE was obtained 3.33 and 4.16%, respectively. This procedure for simultaneous determination of LEV and ETE is sensitive and easy to perform.

Key words: Levonorgestrel, ethinylestradiol, simultaneous determination, HPSAM, spectrophotometry

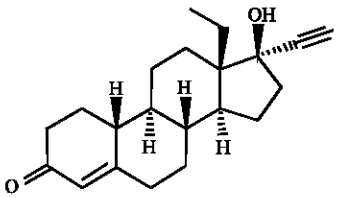
INTRODUCTION

Ethinylestradiol (ETE) is a semisynthetic estrogen female sex hormone, which is present at a very low dosage level (30 μg $tablet^{-1}$) in combination with levonorgestrel (LEV), an orally active synthetic progestin which is present at a level of 1-4 times that of the estrogen. The structure of LEV has a characteristic D^4-3-Keto group in A-ring with a different chromophoric power to ETE. Oral contraceptives have had an enormous positive impact on public health for the past three decades and, overall, there has been a remarkably low incidence of troublesome side-effects.

ETE:



LEV:



At present three types of oral contraceptives are available. In the sequential type, estrogen is administered alone for the first week, followed by a lower dosage of the estrogen in conjuction with progestogen for the remainder of the course. In the second type, commonly used, both estrogen and progestogen are present in the tablets (as either a single dose or in three different doses). In the progestogen type, progestogen alone is administered.

Several studies on the determination of contraceptive agents including Pollow *et al.* (1974) and Verna *et al.* (1975), the use of radioactively labeled derivatives, Penzes and Oertel (1972), dansyl or other flurescent derivatives, Penzes and Oertel (1972) and Eldawy *et al.* (1975), spectrophotometry or photometry, Graham and Kenne *et al.* (1973) and Lisboa and Strassner (1975) gel or column chromatography, Nevado *et al.* (1997) two techniques PLS and PCR, Prabhakar and Deshpande (1999) and Sun *et al.* (2009) high performance liquid chromatography-tandem mass spectrometry, but these methods are complicated. These methods are also time-consuming, expensive, laborious and require considerable experimental skill for good results and need separation procedures. There has been report by Berzas (1997) of the determination of LEV and ETE by derivative spectrophotometry. There is no published example of H-Point standard addition method (HPSAM) having been employed for simultaneous determination of levonorgestrel and ethinylestradiol and the results of this method have been better than the derivative spectrophotometry.

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The simultaneous determination of these drugs by the use of traditional spectrophotometry techniques is difficult as, their absorption spectra overlap and the superimposed curves not suitable for quantitative evaluation. Gustafsson and Fallgren (1983) and Bosch-Reig and Campins-Falco (1988) outlined the fundamentals of HPSAM which is a simple bivariate chemometric technique. The greatest advantage of HPSAM is that, it can remove errors resulting from the presence of an interfering and blank reagent. So, HPSAM can determine the two components simultaneously (Bosch-Reig *et al*., 1990). HPSAM is applied to selected wavelengths where the analytical signals due to one of the species (interferent) is constant and for another one (analyte) to be different as much as possible. Bosch-Reig *et al*. (1992) presented by plotting the analytical signal versus added analyte concentration, two straight lines are obtained that have a common point with coordinates H ($-C_H$, A_H), where $-C_H$ is the unknown analyte concentration and A_H the analytical signal due to interferent species.

In the present study a very simple, sensitive, selective and low cost HPSAM for simultaneous determination of LEV and ETE is described. The method has been successfully applied for simultaneous determination of LEV and ETE in synthetic samples and real samples.

MATERIALS AND METHODS

Apparatus: UV-Vis scanning absorbance spectra were recorded on a Bio-TEK Kon 922 double beam UV-Vis scanning spectrophotometer equipped with quartz cells.

Reagents: All the reagents used were of analytical reagent grade chemicals unless otherwise stated double distilled water was used throughout the experiment. LEV and ETE were obtained from Iran Hormone Laboratories (Tehran, Iran, 2009).

A stock solution of ETE (20.0 μg mL^{-1}) was prepared by dissolving 2.00 mg of ETE in ethanol (Merk) and diluted to 100 mL with the same solvent. LEV stock solution (10.0 μg mL^{-1}) was prepared by dissolving 1 mg of LEV in ethanol and diluting to 100 mL.

Procedure

Individual calibration: The concentration range of ETE and LEV was between 0.20-20.0 and 0.50-15.00 μg mL^{-1} respectively. The absorbencies were measured at 237 and 224 nm against a reagent blank for LEV and ETE, respectively.

H-Point standard addition method: For each measurement, 3 mL of the reagent solution was transferred to the spectrophotometer cell and the absorbance of this solution was zeroed at 210, 241 nm before injecting the analyte(s). Samples containing different amounts of LEV and ETE were prepared and known amounts of LEV were added and H-Point graphs were constructed. The concentration of LEV was evaluated from C_H and that of ETE was evaluated by the calibration method with a single standard the ordinate value of the H-Point (A_H). For the analysis of real sample, ten commercially available tablets (Iran hormone) containing LEV and ETE were weighted and ground and the solution was prepared by dissolving a certain amount of grounded tablets in the ethanol (Merk) solutions and filtering the solution. The study was carried out in 3 months. The procedure was repeated for some mixtures to show applicability of the method.

RESULTS AND DISCUSSION

Levonorgestrel and ethinylestradiol formed contraceptive LD pill. Figure 1 clearly shows the overlapping absorption spectra of LEV and ETE so each compound interferes with the analytical determination of the other. Therefore simultaneous determination of LEV and ETE is possible using HPSAM.

H-Point standard addition method: For selection of appropriate wavelengths for application HPSAM, the following principles were followed. At two selected wavelengths, the analyte signals must be linear with the concentration, the interferent signals must be remain equal, even if the analytical concentrations are changed and the analytical signals of the mixture composed from the analyte and the interferent should be equal to the sum of the individual signals of the two straight lines obtained at λ_1 and λ_2 must be as large as possible in order to get good accuracy and sensitivity.

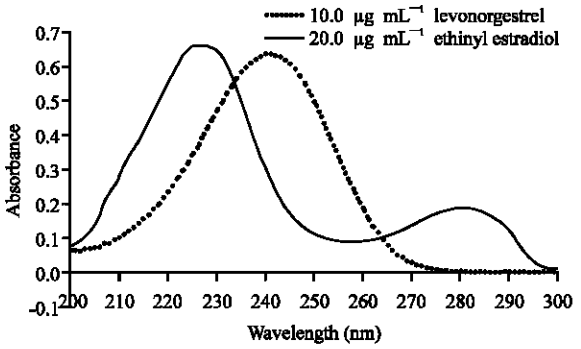

Fig. 1: Absorption spectra of 10.0 μg mL^{-1} levonorgestrel and 20.0 μg mL^{-1} ethinyl estradiol

Table 1: Results of several experiments for the analysis of LEV and ETE in synthetic samples by HPSAM

		Present (μg mL^{-1})		Found (μg mL^{-1})		Recovery (%)	
A-C equation	R^2	LEV	ETE	LEV	ETE	LEV	ETE
$A_{210} = 0.0062C_i+0.1258$	0.9996	8.00	5.00	7.96	4.92	99.50	98.40
$A_{241} = 0.0394C_i+0.2892$	0.9988						
$A_{210} = 0.0101C_i+0.1106$	0.9989	6.00	4.00	6.30	4.30	105.0	107.5
$A_{241} = 0.0455C_i+0.2630$	0.9996						
$A_{210} = 0.0071C_i+0.1056$	0.9987	7.00	4.00	6.60	4.29	94.28	107.2
$A_{241} = 0.0430C_i+0.2598$	0.9997						
$A_{210} = 0.0109C_i+0.0318$	0.9995	4.00	2.00	4.26	2.20	106.5	108.0
$A_{241} = 0.0467C_i+0.1112$	0.9997						
$A_{210} = 0.0085C_i+0.0338$	0.9990	3.00	4.50	2.95	4.36	99.45	96.88
$A_{241} = 0.0414C_i+0.1138$	0.9989						
$A_{210} = 0.0083C_i+0.0128$	0.9996	2.00	5.00	1.96	4.85	98.00	97.00
$A_{241} = 0.0422C_i+0.0940$	0.9994						
$A_{210} = 0.0100C_i+0.0264$	0.9996	1.50	4.50	1.44	4.40	96.00	97.77
$A_{241} = 0.0443C_i+0.0758$	0.9997						
RSD%				0.97	0.98		

Synthetic samples by HPSAM

The best wavelength pair of 210 and 241 nm were chosen with smallest error. Several synthetic samples with different concentration ratios of LEV and ETE were analyzed using HPSAM.

LEV can be considered as the analyte and ETE as interferent. Under optimum conditions, determination of LEV and ETE was carried out using HPSAM. The concentration of interferent was calculated in each test solution by the calibration method with a single standard and the ordinate value of the H-point (A_H). Several synthetic mixtures with different concentrations of LEV and ETE were analyzed by using the suggested method.

Limits of Detection (LOD) and Quantitation (LOQ): LODs were about 0.010 and 0.011 μg mL^{-1} for LEV and ETE and the LOQs were estimated to be about 0.034 and 0.032 μg mL^{-1}, respectively.

Recovery was calculated and mean relative error (Er%) for LEV and ETE was obtained -0.28 and 2.11%, respectively. The recoveries were between 96.88 and 108.0% for ETE and between 94.28 and 106.5% for LEV for the wavelengths studied. The results are given in Table 1.

These results show that the method is effective for the simultaneous determination of ETE and LEV by HPSAM. H-Point standard addition plots for several synthetic samples are also shown in Fig. 2. It is clear from Fig. 2 that concentration of LEV (C_H) is independent of the concentration of ETE and absorbance proportional to ETE concentration (A_H) is also independent of LEV concentration.

Peak purity: Peak purity was obtained for both LEV and ETE by overlay of the spectra captured at the apex, upslope and downslope and no interference was noted for ETE and LEV.

Table 2: Comparison between high performance liquid chromatography (HPLC) and h-point standard addition method (HPSAM) in the assays of commercial formulations (average of three determinations)

	Label claim/tablet (mg)		Label claim (HPSAM)		Label claim (HPLC)	
Commercial formulation	LEV	ETE	LEV	ETE	LEV	ETE
Ovocept LD	0.15	0.03	99.5	100	98	99

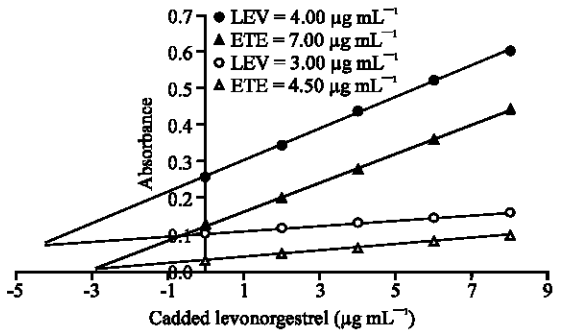

Fig. 2: Plots of HPSAM for simultaneous determination of LEV and ETE in different concentrations

Application to pharmaceutical formulations: The proposed method was applied to the determination of LEV and ETE in a commercially available tablet (Iran Hormone) containing 0.150 mg LEV and 0.030 mg ETE. For comparison between these results and real contents in industry, sample was sent there and had been determined by HPLC chromatography. The relative differences between HPLC and HPSAM results were between ±2% for LEV and ETE determinations in all oral contraceptives. The results of this analysis are summarized in Table 2.

As it is shown LEV and ETE can be determined with satisfactory accuracy and precision in pharmaceutical preparations. Good correlations between the two methods were found.

CONCLUSION

In this study, an accurate and simple method was proposed for the simultaneous determination of LEV and ETE in pharmaceuticals without the use of any expensive instrument is achieved. This reduces the cost of applied method.

No extraction step is required as determination has been done and hence the use of organic solvents is avoided. Most of the organic solvents that are being used for extraction are classified as toxic and environmental pollutants and some have been listed as carcinogenic by the US Environmental Protection Agency.

Standard addition can be used in the proposed method and matrix effects can be removed easily, therefore, this method can be used for resolving binary mixtures in the complex samples with unknown matrices.

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