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UV-Visible Spectrophotometric Method for Simultaneous Estimation of Dydrogesterone and Estradiol from Bulk and Pharmaceutical Formulation

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BSTRACT:

A simple and precise UV-Visible spectrophotometric method was developed for the simultaneous estimation of Dydrogesterone and Estradiol using methanol as a solvent. The simultaneous estimation was carried out by developing an Absorbance Correction Method wherein at 225 nm both the drugs show good absorbance whereas at 311 nm only Dydrogesterone shows absorbance. Dydrogesterone was found to be linear in the concentration range of 20-140 µg/ml at 225 nm and 5-50 µg/ml at 311 nm and Estradiol was found to be linear in the concentration range of 5-50 µg/ml at 225 nm. The mean % assay of Dydrogesterone and Estradiol in marketed formulation was found as of 100.83 % and 100.51 %, respectively. LOD and LOQ for Dydrogesterone was found to be 0.613 and 1.859 µg/ml, and for Estradiol it was found to be at 0.743 and 2.253 µg/ml respectively. The results % Recovery for Dydrogesterone and Estradiol were observed in the range of 98.25-101.05 % and 98.02-101.73 % respectively. % RSD for precision study was found less than 2% for both the drugs. The developed method can be used for the simultaneous estimation of Dydrogesterone and Estradiol from tablet formulation.

Keywords: Hormone Replacement Therapy, Dydrogesterone, Estradiol, Validation, UV Spectroscopic Method, Absorbance Correction Method.

INTRODUCTION

Menopause, also known as the **Climacteric,** is the time when menstrual periods permanently stop, marking the end of reproduction. It typically occurs between the ages of 45 and 55 years, although the exact timing can vary. Menopause is usually a natural change. It can occur earlier in those who smoke tobacco. Other causes include surgery that removes both ovaries or some type of chemotherapy. At the physiological level, menopause happens because of decrease in the ovaries production of hormones estrogen and progesterone. While typically not needed, a diagnosis of menopause can be confirmed by measuring hormone levels in the blood or urine. Menopause is the opposite of menarche the time when a girls periods start. Recently FDA had launched new era of Hormone Replacement Therapy (HRT), which emphasized on profound long term and short term benefits of HRT for treating Menopausal symptoms[1,2,3].

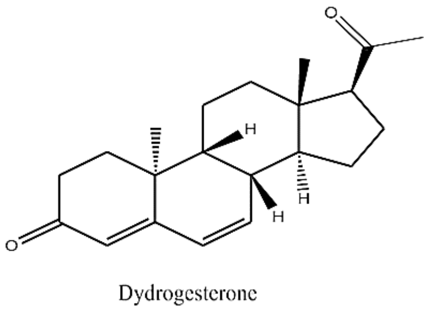

Figure 1: Structure of Dydrogesterone [4]

Dydrogesterone is a potent orally active progesterone receptor agonist that was developed in the 1950s and that has been widely used since the 1960s for menstrual disorders such as premenstrual syndrome, cycle irregularity, endometriosis, threatened miscarriage, habitual miscarriage, and for post-menopausal hormone therapy. Unlike other members of the progestin family, dydrogesterone and its main active metabolite, 20α-hydroxydydrogesterone do not have any clinically relevant agonistic or antagonistic activity on the androgen, estrogen, and glucocorticoid receptors and only have mild anti-mineralocorticoid properties. Safety concerns owing to receptor cross-activation have precluded the use of the majority of the progestins in fertility treatment and pregnancy. Only bio identical progesterone, 17-hydroxy-progesteronecaproate and dydrogesterone are considered to be sufficiently safe for the developing fetus. [5,6]

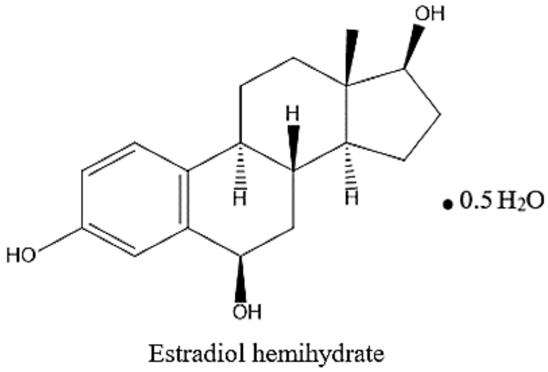

Figure 2: Structure of Estradiol hemihydrate[7]

Estradiol is a form of estrogen that regulates multiple functions in human body. It controls ovulation and the development of female sex characteristics, being classically considered a reproductive hormone, due to its well-known role in feedback signaling in the hypothalamic- pituitary-ovarian axis. Estrogens refer to any substance, natural or synthetic, that mimics the effects of the natural hormone. The three major naturally occurring forms of estrogens are estrone, estradiol, and estriol. Among this estradiol is the most potent and prevalent form, although several metabolites also have estrogenic hormonal activity. [8]

The literature survey revealed several analytical methods, such as UV-Visible spectrophotometry[8- 12], Colorimetry[13], HPLC[14-18], Stability Indicating RP-HPLC[19], TLC[20], and Tandem Mass Spectrometry[21] for the estimation of Dydrogesterone and Estradiol hemihydrate either as individual drugs or in combination with other compounds. However, there remains a need to develop simple and rapid spectrophotometric method to estimate these two drugs in combination.

MATERIALS AND METHOD

Instruments:

- UV – Visible Spectrophotometer (Shimadzu – 1700, Software Version – UV Prob 2.32)
- Electronic Weighing Balance (Sartorius- TE-214S)
- Ultrasonicator (GT Sonic- GT-1730QTS)

Chemicals:

Dydrogesterone and Estradiol hemihydrate were received as gift sample from Montage Laboratories Pvt. Ltd, SH 9, Dhandha, Idar Road, Ta: Himstnagar, Dist: Sabarkantha- 383001, Gujarat, India. Whereas market formulation Femoston Conti (DYD 5 mg and Est 1 mg) was purchased from Abbott India Ltd.

Preparation of Standard Stock Solutions

1. **Standard Stock Solution of Dydrogesterone (DYD)**

25 mg of standard DYD was weighed and transferred to 25 ml volumetric flask. DYD was dissolved in methanol by gentle shaking and volume was made up to the mark to obtain final concentration of 1000 µg/ml and labelled as ‘Stock DYD-A’. From the ‘Stock DYD- A’ (1000 µg/ml) solution 2.5 ml of aliquot was transferred into a 25 ml volumetric flask and the volume was made up to the mark with methanol. This solution was labelled as ‘Stock DYD-B’ (100 µg/ml).

2. **Standard Stock Solution of Estradiol (EST)**

25 mg of standard EST was weighed and transferred to 25 ml volumetric flask. EST was dissolved in methanol by gently shaking and volume was made up to the mark up to obtain final concentration of 1000 µg/ml this was labelled as 'Stock EST -A'. From the 'Stock EST -A' (1000 µg/ml), 2.5 ml of aliquot was transferred to 25 ml of volumetric flask and volume was made up to the mark using methanol. This solution was labelled as 'Stock EST-B' (100 µg/ml).

Selection of Analytical Wavelength

From 'Stock DYD-B' solution further 1 ml of aliquot was transferred to 10 ml volumetric flask and the volume was made up to mark with methanol to obtain final concentration of (10 µg/ml). The solution was scanned in the spectrum mode from 200 nm to 400 nm.

From the 'Stock EST-B' (100 µg/ml) solution 0.2 ml of aliquot was pipetted out into 10 ml volumetric flask and the volume was made up to the mark with methanol to get the concentration of 2 µg/ml. The solution was scanned in the spectrum mode from 200 nm to 400 nm. Overlain spectra are shown in Figure 3.

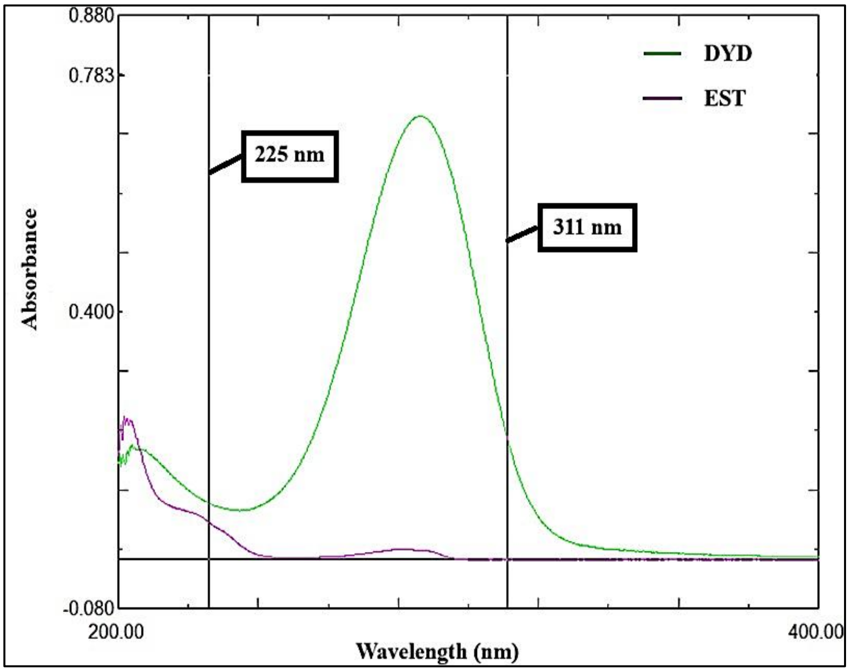

Figure 3: Overlain spectra of DYD (10 µg/ml) and EST (2 µg/ml) in Methanol

Calibration Curve for DYD and EST

The stock solutions of DYD were diluted to get concentration range of 5-50 µg/ml and 20-140 µg/ml and absorbance of these solutions were measured at 311 and 225 nm respectively. While for EST the stock solution was diluted to get the concentration range of 5-50 µg/ml and absorbance of these solutions were measured at 225 nm. Calibration curves were plotted for these drugs as shown in the Figure 4, 5 and 6.

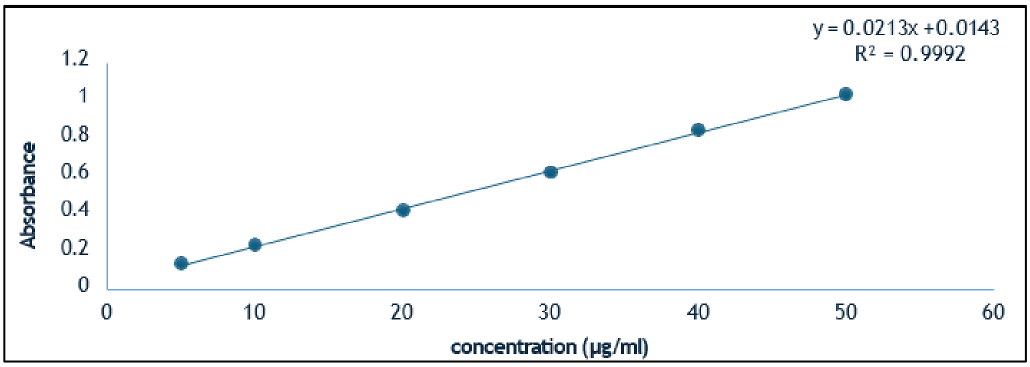

Figure 4 Calibration Curve of DYD (5-50 µg/ml) at 311nm in Methanol

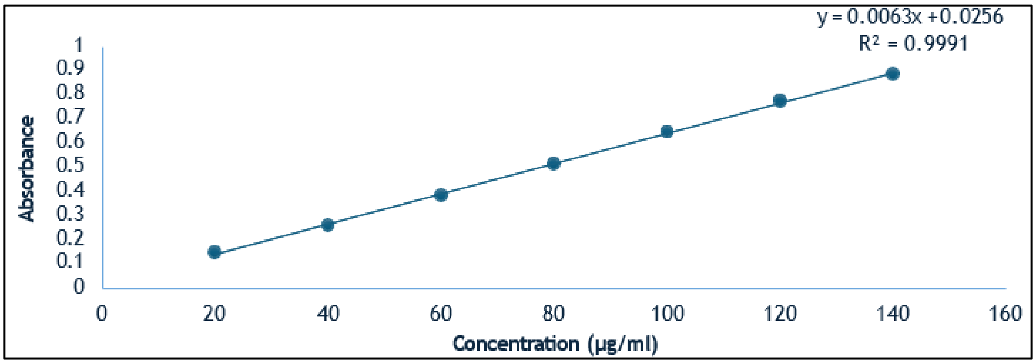

Figure 5: Calibration Curve of DYD (20-140 µg/ml) at 225 nm in Methanol

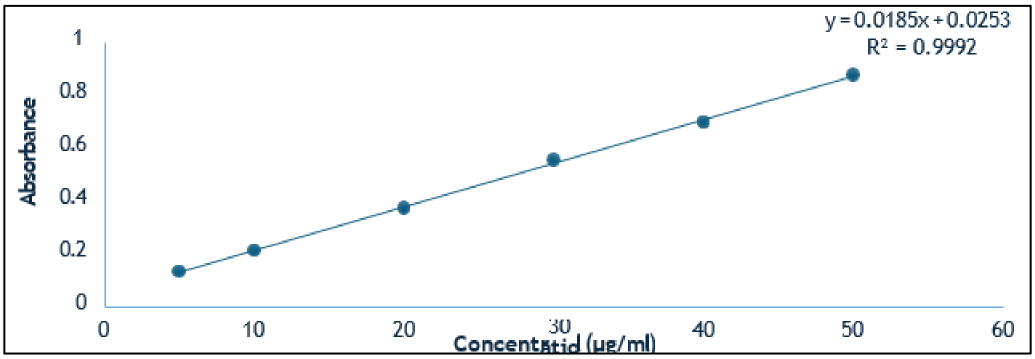

Figure 6 Calibration Curve of EST (5-50 µg/ml) at 225 nm in Methanol

Table 1: Linear Regression analysis for DYD and EST

Drugs Parameter	**DYD**		**EST**
Wavelength	311 nm	225 nm	225 nm
Beer's Law Limit (µg/ml)	5-50 µg/ml	20-140 µg/ml	5-50 µg/ml
Correlation Coefficient (R^2)	0.9992	0.9991	0.9992
Slope	0.0213	0.0063	0.0185
Intercept	0.0143	0.0256	0.0253
A(1%,1cm)	213	63	185
LOD (µg/ml)	0.613	3.148	0.743
LOQ (µg/ml)	1.859	9.541	2.253

In this method, two wavelengths 225 nm (λ1) and 311 nm (λ2) were selected in such a way that absorbance of EST is nearly zero at the analytical wavelength of DYD (311 nm). The concentrations of two drugs in mixture were calculated by using following equations:

$$C_{DYD} = A_1/ax_1 \quad C_{EST} = (A_2 - ax_2 C_{DYD})/ay_1$$

Where, C_{DYD} and C_{EST} are the concentrations of DYD and EST respectively. A_1 and A_2 are the absorbances of the mixture at 311 nm and 225 nm respectively. ax_1 and ax_2 are the Absorptivities of DYD at 311 nm and 225 nm respectively; whereas, ay_1 is the Absorptivity of EST at 225 nm.

Analysis of Tablet Formulation

Twenty Tablets of DYD and EST (Femoston Conti, Abbott India Limited) (DYD-5 mg and EST-1 mg) were weighed, average weight was calculated and crushed to obtain fine powder. An accurately weighed tablet powder equivalent to about 10 mg of DYD (2 mg of EST) was transferred to 25 ml volumetric flask and sonicated for 20 minutes with methanol for extraction of drugs and the volume was made up to the mark with methanol. The resulting solution was filtered through Whatman filter paper no. 41, few ml of filterate was discarded, and rest of solution was used as 'Sample Stock A'. (400 µg/ml of DYD, 80 µg/ml of EST).

From the 'Sample Stock A' solution (400 µg/ml of DYD, 80 µg/ml of EST), 0.5 ml of the aliquot was pipetted out and transferred to a 10 ml volumetric flask. The volume was made to the mark with methanol to obtain a solution with final concentration of 20 µg/ml of DYD and 4 µg/ml of EST. The solution was scanned from 200 to 400 nm. The absorbance was taken at 311 nm and 225 nm. The % Assay was calculated using A (1%, 1cm) value of DYD and EST. The results are shown in Table 2.

Table 2: Results of Market Formulation

Analyte	Label Claim	Mean % Assay	% RSD
DYD	5 mg	100.83 ± 0.8782	0.8709
EST	1 mg	100.51 ± 1.3385	1.3316

Validation

The proposed method was validated in accordance to ICH guidelines. Accuracy of the method was determined using recovery study by standard addition method at three different levels 80%, 100% and 120% of assay concentration and percentage recovery were calculated. Precision of the method was determined using Intermediate precision: Intra-day precision, Inter-day precision, Variation by different analyst and Repeatability study. LOD and LOQ was determined by following formula.

$$\mathrm{LOD} = 3.3 \times \frac{\textit{Standard Deviation of y–Intercepts of Six Calibration curves}}{\textit{Average Slope of Six Calibration Curves}}$$

$$\mathrm{LOQ} = 10 \times \frac{\textit{Standard Deviation of y–Intercepts of Six Calibration curves}}{\textit{Average Slope of Six Calibration Curves}}$$

RESULTS AND DISCUSSION

To develop absorbance correction method for simultaneous estimation of DYD and EST, the drug solutions were prepared in suitable concentration (10 µg/ml of DYD and 2 µg/ml of EST) in methanol. These solutions were scanned from 200-400 nm and overlay spectra was observed for selection of analytical wavelength. It was observed that at 311 nm DYD was showing good absorbance where EST is showing zero absorbance. So, 311 nm was selected as an analytical wavelength for DYD. The concentration of DYD was determined as a single drug estimation using A1% 1 cm value as 213. To determine DYD absorbance at 225 nm, A=abc equation was used and absorbance of DYD at 225 nm was calculated using A1% 1cm value as 63. From the total absorbance at 225 nm absorbance due to DYD was nullified to get absorbance because of EST alone. Further EST concentration was determined using A1% 1cm value as 185 at 225 nm.

Using this method, the absorbance of marketed formulation (Femoston Conti) (DYD-5 mg and EST-1 mg) was measured at 225 nm and 311 nm and amount of drugs present were found using A1%1cm values of the respected drugs at their analytical wavelength are given in Table 4. Mean percentage assay of DYD and EST were found as 100.83 % and 100.51 % respectively.

The developed absorbance correction method for simultaneous estimation of DYD and EST was validated as per ICH guidelines and was found linear in the concentration range of 5-50 µg/ml for DYD with R^2 value as 0.9992 at 311 nm and that for EST the method was linear in the concentration range of 5-50 µg/ml with R^2 value as 0.9992 at 225 nm. As the R^2 value is more than 0.999 the method showed satisfactory linearity in the selected range. Accuracy was determined by standard addition method (80%, 100% and 120% of the assay level), % recoveries of DYD and EST were found to be in the range of 98.25-101.05 % and 98.02-101.73 % respectively. Precision of the method was determined using intra-day precision, inter-day precision using 20, 30, 40 µg/ml for DYD and 10, 20, 30 µg/ml for EST. It was found that %RSD for all these studies and both the drug were less than 2%. Variation of the results using two different analytes was determined by Performing assay (n=3). As calculated values of F-test and t-test were found less than the tabulated values hence no significant difference was observed between the results of two analysts. LOD and LOQ of DYD were found to be 0.613 and 1.859 µg/ml at 311 nm, respectively. LOD and LOQ value of EST were found to be 0.743 and 2.253 µg/ml at 225 nm, respectively.

CONCLUSION

A simple, precise, and sensitive UV-spectrometric method utilizing the absorbance correction technique has been developed for the simultaneous estimation of Dydrogesterone and Estradiol Hemihydrate.

The developed method was rigorously validated according to ICH guidelines, and the results of the assay and validation studies were satisfactory. Therefore, this method can be successfully applied for routine analysis of Dydrogesterone and Estradiol Hemihydrate in marketed formulations within the pharmaceutical industry.

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