International Journal of
Medicinal Chemistry & Analysis
e ISSN 2249 – 7587
Print ISSN 2249 - 7595
www.ijmca.com
Research Article

METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF DROSPIRENONE AND ESTETROL BY USING UV-SPECTROSCOPY

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ABSTRACT

Ultraviolet and visible (UV-Vis) absorption spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. Light energy is converted by detectors into electrical impulses that are read out by readout devices. The transmitted radiation strikes the detector, determining the amount of radiation absorbed by the sample. The absorption spectrophotometer's apparatus uses the following types of detectors. Aim of the present study is to develop an accurate, precise, sensitive, selectivity, analytical technique to determine the Drospirenone and Estetrol in pharmaceutical dosage forms. Drospirenone is a progestin used in oral contraceptive pills for the prevention of pregnancy and other conditions. Percentage of RSD for intra-day and inter-day precision studies for both drugs were well within the acceptable range (< 2 %) indicating that the method have excellent repeatability and reproducibility.

Keywords: Ultraviolet and visible (UV-Vis), Absorption spectroscopy, Drospirenone and Estetrol.

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INTRODUCTION

Linearity is the ability of the method to produce test results that are proportional, either directly or by a well-defined mathematical transformation, to the concentration of analyte in samples within a given range [1]. For UV-visible measurements, the usual linear relationship is Beer's law, which states that the absorbance of a solute is directly proportional to its concentration [2].

Access this article online		
Home page: http://ijmca.com/ DOI: http://dx.doi.org/10.21276/ijmca.2022.12.1.1		**Quick Response code**
Received:21.11.23	**Revised**:12.12.23	**Accepted**:01.01.24

A linear calibration curve relating absorbance to concentration should have the form:

A= kc

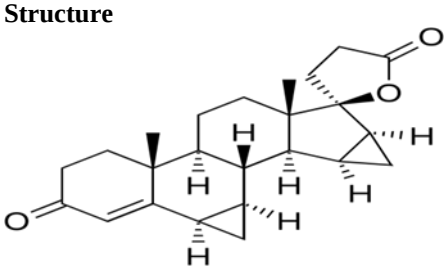
Where A is absorbance, c is concentration, and k is the calibration factor (the slope of the calibration curve). Thus, testing for linearity in effect tests how well our theoretical model (Beer's law) fits the actual measurements.

DRUG PROFILE

DROSPIRENONE

Drospirenone is a progestin used in oral contraceptive pills for the prevention of pregnancy and other conditions. Drospirenone is a synthetic progestin commonly found in the popular oral contraceptive, Yaz in combination with Ethinyl estradiol.

Structure



CAS Number : 67392-87-4
Molecular Weight : 366.4932
Molecular Formula : $C_{24}H_{30}O_3$
Physical State : Solid
Solubility : 0.00225 mg/mL in water
Melting Point : 196-200 °C

Indication

Drospirenone, in combination with ethinyl estradiol or estetrol, is indicated as an oral contraceptive for the prevention of pregnancy [3]. In addition to its use for contraceptive effects, this combination is used to treat moderate acne vulgaris and the symptoms of premenstrual dysphoricdisorder.

Pharmacodynamics

Drospirenone inhibits the maturation of follicles and inhibits ovulation, preventing pregnancy. It has antiandrogen effects, improving acne and hirsutism. When combined with ethinyl estradiol, it has been shown to have favorable effects on the plasma lipid profile.

Mechanism of action

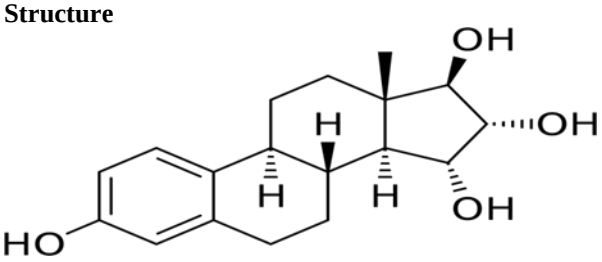
Drospirenone and ethinyl estradiol in combination suppress the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH), preventing ovulation. Other changes induced by this drug which may aid in the prevention of pregnancy include alterations in cervical mucus consistency, hindering sperm movement, and lowering the chance of embryo implantation. Drospirenone is an analog of the diuretic spironolactone, which exerts anti-mineralocorticoid activity, blocking aldosterone receptors, which increases sodium and water excretion [4].

ESTETROL

Description

Estetrol is an estrogen used in combination with drospirenone for oral contraception. Naturally or synthetically produced steroid estrogens have a wide range of pharmaceutical uses ranging from hormonal contraception to the treatment of menopausal symptoms.15 Estetrol (E4) is a native estrogen occurring naturally during pregnancy, but can be synthesized from a plant source and used for contraception. It is more potent and is safer than the synthetic estrogen Ethinylestradiol (EE2) found in 97% of oral contraceptive pills [5].

Structure



CAS Number : 15183-37-6
Molecular Weight : 304.3808
Molecular Formula : C18H24O4
Appearance : Powder
Physical State : Solid
Melting Point : 233-236 0C
pK Values : pKa: 10.33 (Acidic), -3.3 (Basic).

Mechanism of action

Estetrol is a synthetic analogue of a naturally occurring estrogen present during pregnancy, demonstrating selectivity for both estrogen receptor-α (ER-α) and ER-β and suppressing ovulation. Estetrol binds with a low to moderate affinity human estrogen receptor alpha (ER alpha) and ER beta with a preference for ER alpha. Estetrol demonstrates a unique mechanism of action via tissue selective activity [6], showing estrogen receptor agonist activity on the vagina, the uterus and the endometrium, and negative estrogenic activity on breast tissue [7].

MATERIALS AND METHODS

Preparation of diluent solution: Mixture of HPLC grade water and Acetonitrile taken in the ratio 1:1 v/v and sonicated for 15min.

Preparation of Standard stock solutions (142µg/mL&30µg/mL): Accurately Weighed and transferred 14.2mg of Estetrol and 3mg of Drospirenone working Standards into a separate two 100 ml clean and dry volumetric flasks, add 7ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents.

Preparation of Standard working solutions (14.2µg/mL&3.0µg/mL) (100% solution): 1.0ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (14.2µg/ml Estetrol of and 3.0µg/ml of Drospirenone)

Sample Preparation:

Preparation of Sample stock solutions (142µg/mL&30µg/mL): 5 tablets were weighed and

calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100mL volumetric flask, 50mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered.

Preparation of Sample working solutions (14.2µg/mL&3.0µg/mL): From the filtered solution 1.0ml was pipeted out into a 10 ml volumetric flask and made up to 10ml with diluent.

Simultaneous Equation Method Development

Working solutions of both drugs were scanned in the UV range 200–400 nm. The overlay spectra of both drugs were recorded. From overlain spectra, wavelengths 260 nm (of Estetrol) and 338 nm (of LPV) were selected for analysis of both drugs using simultaneous equation method (-260 nm for Estetrol and -338 nm for Drospirenone). Consequently, it may be possible to determine both drugs by the technique of from method or simultaneous equation method. The concentration of drugs x (Estetrol) and y (Drospirenone) in sample solutions were determined by the SE method using the following formula:

$$Cx = \frac{A2\ aY1 - A1\ aY2}{a\ X2\ aY1 - a\ X1aY2}$$

$$Cy = \frac{A1\ a\ X2 - A2\ a\ X1}{a\ X2\ aY1 - a\ X1aY2}$$

where Cx and Cy are the concentration of Estetrol and Drospirenone, A1and A2 are the absorbance of sample solution at 260 nm and 338 nm, respectively, ax1 and ax2 are absorptivity of Estetrol at 260 nm and 344.5 nm, ay1 and ay2 are absorptivity of Drospirenone at 260 nm and 338 nm, respectively [8].

METHOD VALIDATIONPRECISION

The repeatability of the method is established by estimating the % Drug release with respect to label claim for six different sample preparations of same batch. The % RSD of % Drug release of six test solutions was calculated.

Intermediate Precision

Six test solutions of Estetrol and Drospirenone Tablet, were prepared as per the analytical method on different day. These test solutions were analyzed by a different analyst using different UV-Visible spectrophotometer. The % RSD of % drug release results of twelve test solutions (six samples from method precision and six samples from intermediate precision) was calculated.

LINEARITY

Accurately Weighed and transferred 14.2mg of Estetrol and 3mg of Drospirenone working Standards into a 100 ml&100ml clean dry volumetric flasks, add 7ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents.

ACCURACY (% RECOVERY)

Accuracy study was performed by analyzing Esterol and drospirenone test solutions which were prepared by mixing Esterol and drospirenone API with excipient blend.

These test solutions were prepared by adding a quantity of Esterol and drospirenone API to excipient blend to produce three different concentration solutions equivalent to 50%, 100%, and 150% of test concentration.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION(LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of Drospirenone and Estetrol for single-point method were determined by using standard deviation of the response and slope approach as defined in ICH guidelines.

$$LOD = 3.3x\sigma/S \qquad LOQ = 10x\sigma/S$$

ASSAY OF DROSPIRENONE AND ESTETROL

Assay of the marketed formulation was carried out Standard solution and sample solutions were observed separately into the system and Absorbance were recorded and drug present in sample was calculated using before mentioned formula. Average % Assay for Estetrol and Drospirenone obtained was 99.56% and 98.63% respectively [9].

ROBUSTNESS AND RUGGEDNESS

Prepare two test solution of the same lot of Drospirenone and Estetrol in tablet as per analytical method. Measure the absorbance of this solution along with diluent blank solution and systemsuitability solution.

RESULT AND DISCUSSION

SELECTION OF SOLVENT

Drospirenone and estetrol showed solubility in distilled water hence it was selected as thesolvent (diluents) for further studies.

SELECTION OF WAVELENGTH

Standard solution of 142µg/mL of Estetrol exhibited maximum absorbance at 260 nm.

LINEARITY AND RANGE

Preparation of Standard stock solutions (142µg/mL&30µg/mL): Accurately Weighed and transferred 14.2mg of Estetrol and 3mg of Drospirenone

working Standards into a 100 ml&100ml clean dry volumetric flasks, add 7ml of diluent, sonicated for 5 minutes and make up to the final volume with diluents.

Table 1. Linearity and Range

S. No	Pipetted From stock(mL)	Volume offlask (mL)	Concentration in ppm (estetrol)	Concentration In ppm (Drospirenone)	%LinearityLevel
1	0.25	10	3.55	0.75	25
2	0.5	10	7.1	1.5	50
3	0.75	10	10.65	2.25	75
4	0.1	10	14.2	3	100
5	0.25	10	17.75	3.75	125
6	0.5	10	21.3	4.5	150

Table 2. Accuracy table of Estetrol

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	7.1	7.1	100.00	101.16%
	7.1	7.0	98.59	
	7.1	6.97	98.24	
100%	14.2	14.17	99.82	
	14.2	14.22	100.18	
	14.2	14.12	99.47	
150%	21.3	21.35	100.23	
	21.3	21.30	100.00	
	21.3	21.32	100.12	

Table 3. Accuracy table of Drospirenone

% Level	Amount Spiked (ppm)	Amount Recovered (ppm)	% Recovery	Mean % Recovery
50%	0.132	1.48	98.88	99.61%
	0.133	1.49	99.63	
	0.132	1.48	98.88	
100%	0.27	3.03	101.12	
	0.269	3.02	100.75	
	0.27	3.03	101.12	
150%	0.397	4.46	99.13	
	0.394	4.43	98.38	
	0.395	4.44	98.63	

Table 4. Method precision of Esterol

S.No.	Ax1	Ax2	A1	A2
	260.00nm	338.00nm	260.00nm	338.00nm
1	0.581	0.0132	0.597	0.284
2	0.579	0.0131	0.595	0.282
3	0.580	0.0129	0.594	0.283
4	0.579	0.0131	0.592	0.287
5	0.580	0.0130	0.593	0.283
6	0.581	0.0132	0.595	0.285
AVG	0.580	0.013	0.5943	0.2840
SD	0.001	0.0001	0.002	0.002
% RSD	0.15	0.89	0.295	0.630

Table 5. Results of Method precision

Test Solution	% Drug release of Estetrol
1	99.91
2	99.95
3	100.78
4	101.09
5	100.01
6	100.00
Mean	100.29
Standard Deviation (±)	0.51
(%) Relative StandardDeviation	0.51

Table 6. Method precision of Drospirenone

S. No	Ay1	Ay2	A1	A2
	260.00nm	**338.00nm**	**260.00nm**	**338.00nm**
1	0.0139	0.271	0.597	0.284
2	0.0141	0.269	0.595	0.282
3	0.0139	0.268	0.594	0.283
4	0.0138	0.269	0.592	0.287
5	0.0138	0.270	0.593	0.283
6	0.0140	0.271	0.595	0.285
AVG	0.014	0.2697	0.5943	0.2840
SD	0.000	0.0012	0.002	0.002
% RSD	0.84	0.45	0.295	0.630

Table 7. Results of Method precision of Drospirenon

Test Solution	% Drug release of Drospirenon
1	100.36
2	100.33
3	100.00
4	99.82
5	99.86
6	100.00
Mean	100.06
Standard Deviation (±)	0.23
(%) Relative Standard Deviation	0.23

Table 8. Intermediate precision of Esterol

S.No.	Ax1	Ax2	A1	A2
	260.00nm	**338.00nm**	**260.00nm**	**338.00nm**
1	0.579	0.0130	0.597	0.284
2	0.577	0.0131	0.595	0.282
3	0.579	0.0132	0.594	0.283
4	0.578	0.0129	0.592	0.285
5	0.576	0.0130	0.593	0.283
6	0.577	0.0131	0.595	0.285
AVG	0.578	0.0131	0.5943	0.2837
SD	0.0012	0.0001	0.0018	0.0012
% RSD	0.21	0.80	0.29	0.43

Table 9. Results of intermediate precision of Esterol

Test Solution	% Drug release of Estradiol and Drospirenone
1	101.46
2	101.05

3	100.29
4	101.53
5	101.10
6	101.42
Mean	101.14
Standard Deviation (±)	0.46
(%) Relative Standard Deviation	0.46

Table 10. Intermediate precision of Drospirenone

S.No.	Ay1	Ay2	A1	A2
	260.00nm	**338.00nm**	**260.00nm**	**338.00nm**
1	0.0136	0.267	0.597	0.284
2	0.0137	0.266	0.595	0.282
3	0.0135	0.269	0.594	0.283
4	0.0138	0.268	0.592	0.287
5	0.0140	0.267	0.593	0.283
6	0.0137	0.268	0.595	0.285
AVG	0.014	0.2675	0.5943	0.2840
SD	0.000	0.0010	0.002	0.002
% RSD	1.26	0.39	0.295	0.630

Table 11. Results of intermediate precision of Drospirenone

Test Solution	% Drug release of Drospirenone
1	100.73
2	100.72
3	100.25
4	100.00
5	100.49
6	100.71
Mean	100.48
Standard Deviation (±)	0.30
(%) Relative Standard Deviation	0.30

Table 12. Result of twelve test solution of Estetrol and Drospirenine in Tablet

Analysis performed during method precision study By Analyst 1 on system 1 on day 1		
Same column	**% Drug release of Esterol**	**Drug release of Drospirenone**
1	99.91	100.36
2	99.95	100.33
3	100.78	100.00
4	101.09	99.82
5	100.01	99.86
6	100.00	100.00
Analysis performed during intermediate precision study By Analyst 2 on system 2 and on day 2		
Test Solution	**% Drug release of Esterol**	**Drug release of Drospirenone**
7	101.46	100.73
60	101.05	100.72
9	100.29	100.25
10	101.53	100.00
11	101.10	100.49
12	101.42	100.71
Mean of twelve samples	100.72	100.27
Standard Deviation (±)	0.64	0.34
(%) Relative Standard	0.64	0.34

Table 13. Assay table of Estetrol

S. No	Amount present in mg	Amount present in %
1	14.16	99.746
2	14.13	99.500
3	14.08	99.188
4	14.19	99.895
5	14.19	99.929
6	14.07	99.114
Mean	14.14	99.562
S. D	0.050	0.353
%RSD	0.35	0.35

Table 14. Assay table of Drospirenone

S. No	Amount present in mg	Amount present in %
1	2.974	99.14
2	2.965	98.84
3	2.956	98.52
4	2.950	98.34
5	2.950	98.32
6	2.961	98.70
Mean	2.959	98.64
S. D	0.009	0.316
%RSD	0.32	0.32

Table 15. Summary Table

S.No	Parameter	Esterol	Drospirenone
1	Absorption maxima (nm)	260	338
2	Beer's Law Limit (mcg/ml)	3.55.21.3	1.75-4.5
3	Slope	0.040	0.089
4	Intercept	0.004	0.002
5	Correlation coefficient	0.999	0.998
6	Regression equation	y = 0.04x + 0.004	0.089x + 0.002
7	LOD (mcg/ml)	0.33	0.07
8	LOQ (mcg/ml)	1.0	0.2

Fig.No:6.1 UV Spectrum of Estetrol at 260 nm

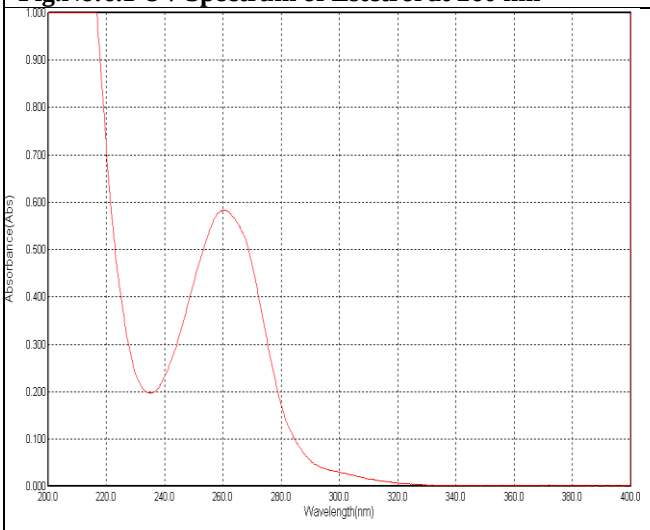


Fig.No:6.2 UV Spectrum of Drospirenone at 338 nm

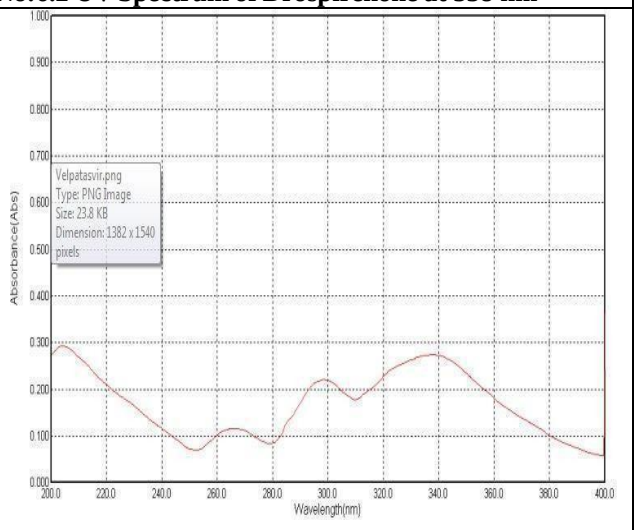


Fig.No:6.3 Estetrol linearity plot

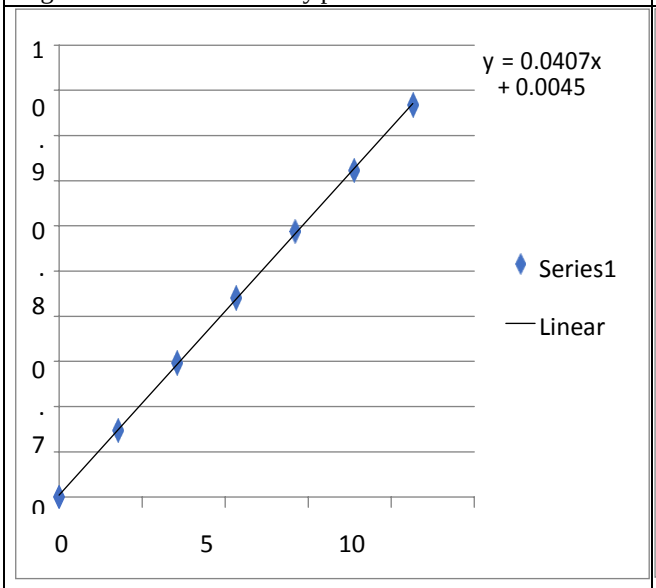
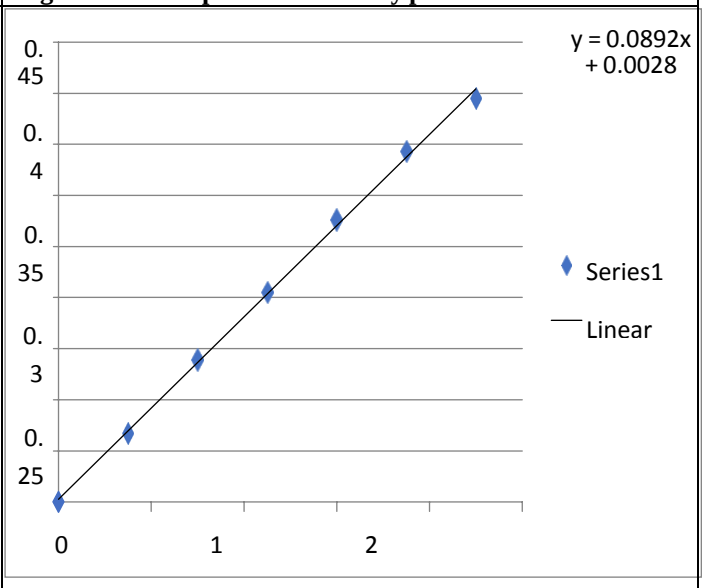


Fig.NO:6.4 Drospirenone linearity plot



Accuracy

Accuracy study was performed by analyzing Esterol and drospirenone test solutions which were prepared by mixing Esterol and drospirenone API with excipient blend. These test solutions were prepared by adding a quantity of Esterol and drospirenone API to excipient blend to produce three different concentration solutions equivalent to 50%, 100%, and 150% of test concentration.

Acceptance criteria: Mean recovery at each concentration level should be between 98.0% and 102.0%.

The system suitability criteria were found to meet the pre-established acceptance criteria as perthe analytical method. (Refer to Table – 11 for system suitability result). The results of accuracystudy obtained are presented.

Precision

The repeatability of the method is established by estimating the % Drug release with respect to label claim for six different sample preparations of same batch. The % RSD of % Drug release of six test solutions was calculated.

Acceptance criteria: % RSD of the results of six test solutions should not be more than 2.0%.

The repeatability of the method is established by estimating the % Drug release with respect to label claim for six different sample preparations of same batch. The results of average % Drug release obtained from six test solutions preparations are presented.

Intermediate Precision:

Six test solutions of Estetrol and Drospirenone Tablet, were prepared as per the analytical method on different day. These test solutions were analyzed by a different analyst using different UV-Visible spectrophotometer. The % RSD of % drug release results of twelve test solutions (six samples from method precision and six samples from intermediate precision) was calculated.

Acceptance criteria: % RSD of the results of twelve test solutions (six of method precision and six of intermediate precision) should not be more than 2.0%.

The system suitability criteria were found to meet the pre-established acceptance criteria as perthe analytical method. (Refer to Table -7 for system suitability results). The results of drug release obtained from six test solutions are presented in Table - 8. % RSD of drug release results from method precision and intermediate precision (12 results) are presented.

Remark: The analysis was carried out on six test solutions of the same lot of the drug productby two different analysts using two different equipments within the same laboratory on two different days. The % RSD of the twelve drug release results (six of method precision and six from intermediate precision) is found to be more than 2.0%. Thus, the method is found to be rugged and precise.

Assay of the marketed formulation was carried out Standard solution and sample solutions were observed separately into the system and Absorbance were recorded and drug present in sample was calculated using before mentioned formula. Average % Assay for Estetrol and Drospirenone obtained was 99.56% and 98.63% respectively.

SUMMARY

Simple, precise and accurate UV spectrophotometric method were developed and validated as per ICH guidelines for the estimation of Estetrol and Drospirenone in tablet dosage form. From the solubility profile, distilled water was chosen as a common solvent for the estimation of Estetrol and Drospirenone. The sample solution of 30µg/mL and 142µg/mL of std stock solution of Estetrol and Drospirenone in distilled water prepared and the solution was scanned in UV region in wavelength range from 260-338 nm by using distilled water as a solvent. The overlay spectra of Estetrol and Drospirenone was recorded. From the spectra, Estetrol and Drospirenone shows maximum absorbance at 260-338 nm.

CONCLUSION

Percentage of RSD for intra-day and inter-day precision studies for both drugs were well within the acceptable range (< 2 %) indicating that the method have excellent repeatability and reproducibility. The percentage relative standard deviation for precision and accuracy was found to be low, which indicates that the method has considerable accuracy and precision. Percent recovery for Estradiol and Drospirenone was found in the range of 101.16 % to 99.61 % with standard deviation well below 2 indicating accuracy of the method. Recovery greater than 98% with the low standard deviation justifies the accuracy of the method. Intra-day and Inter-day precision studies were carried out by analyzing tablet formulation, by this method. The results are in good agreement with the label claim. The proposed method is found to be simple, precise, accurate and sensitive and therefore, can be used as a quality control tool for the estimation of drug from their dosage form in quality control laboratory.

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