

Research Article

DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF DROSPIRENONE AND ETHINYL ESTRADIOL

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ABSTRACT

Simple, accurate and precise spectroscopic technique for simultaneous estimation of drospirenone and ethinyl estradiol in tablet and bulk dosage form by first order derivative technique has been developed. The spectrophotometric technique for estimation of drospirenone and ethinyl estradiol employed first order derivative method for analysis using methyl alcohol as solvent. Drospirenone has absorbance maxima 242nm and ethinyl estradiol has absorbance maxima 218nm these two drugs obey Beer's law in range of 10-50µg/ml concentration for drospirenone and 32-38µg/ml for ethinyl estradiol. The recovery studies determined the accuracy of the proposed technique and the results were established as per ICH guidelines. The results were satisfactory and the technique was used successfully for the estimation of drospirenone and ethinyl estradiol in tablet form without the interference of common excipients.

KEYWORDS: Drospirenone, Ethinyl Estradiol, First Order Derivative Method.

INTRODUCTION

Drospirenone:

Drospirenone is synthesized from Androstenedione and is an analogue of the anti-mineralocorticoid spironolactone. Drospirenone has a similar biochemical and pharmacologic identity as that of endogenous progesterone which is an analogue of spironolactone unlike other progestogens, drospirenone especially regarding anti-mineralocorticoid and anti-androgenic activities. Drospirenone and ethinyl combination is an effective oral contraceptive those are positive effects on lipid levels and weight.

Ethinyl Estradiol:

Ethinyl estradiol also written as 17 α -ethinyl estradiol, ethinylestradiol, ethinylestradiol, ethinylestradiol, is a derivative of 17 β -estradiol the major endogenous estrogen in humans. EE2 is an orally bio active estrogen used in many formulations of combined oral contraceptive pills and is one of the most usually used medications for this purpose [1-6].

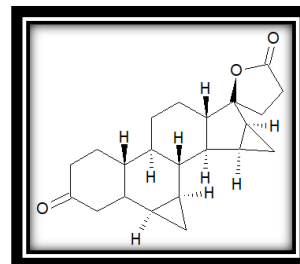


Fig. 1: Drospirenone

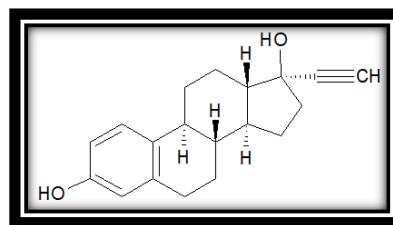


Fig. 2: Ethinylestradiol

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MATERIAL AND METHODS [7-14]

Standard drug Drospirenone and Ethinyl Estradiol: gift sample Chemicals and reagents: Methyl Alcohol (AR Grade).

Instruments: Shimadzu 1800 UV (Shimadzu Japan) spectrophotometer with 1 cm matched quartz cells was used for estimation.

Selection of media: Main criteria of media selection and stability, i.e. drug should be soluble as well as stable for sufficient time in selected media. For present work methyl alcohol has been selected as analytical media.

Preparation of standard stock solution:

The standard stock solution was prepared by transferring 25 mg of drospirenone and ethinyl estradiol in to a two different 25 ml volumetric flask and dissolve with 15ml of methanol and volume made upto 25ml with methanol to get 1000µg/ml and further dilution were made to get 100µg/ml.

Determination of λ max: From the above stock solution of both drospirenone and ethinyl estradiol were pipetted 10ml each and transpired in 25ml two different volumetric flask and volume were made upto 25ml to get 40µg/ml each. The solution was scanned in the UV range 200-400 nm the λ max was found to be 218nm and 254nm for ethinyl estradiol and drospirenone respectively. The spectrum of Drospirenone was recorded.

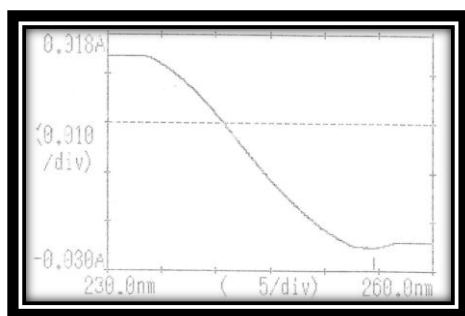


Fig. 3: First order derivative spectrum of Drospirenone of conc. 10µg/ml.

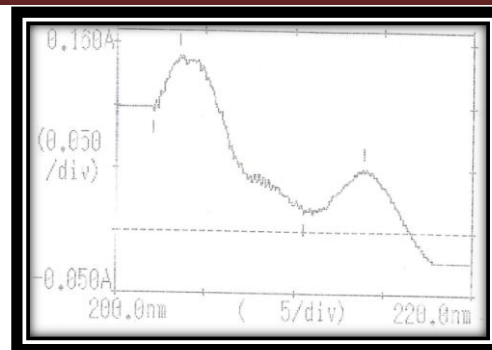


Fig. 4: First order derivative spectrum of conc. 10µg/ml. Ethinyl Estradiol

Study of Beer-Lambert's Law: From the above stock solution of drospirenone I have taken 2.5ml, 5ml, 7.5ml, 10ml and 12.5ml in five different 25ml volumetric flask and made the volume upto 25ml to get conc. of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml. These all were scanned at 254nm and the spectrum were recorded. Similarly for ethinyl estradiol taken 8ml, 8.5ml, 9ml and 9.5ml from above stock solution of ethinyl estradiol and transferred in 25ml different volumetric flask and made the volume upto 25ml with methanol to get concentration of 32µg/ml, 34µg/ml, 36µg/ml and 38µg/ml. These concentrations were scanned at 218nm and spectrum was recorded.

Table No. 1: Standard calibration table for Drospirenone at 254nm

Sr. No.	Conc. of Drospirenone (µg/ml)	Absorbance at 254nm
1	10	-0.025
2	20	-0.056
3	30	-0.082
4	40	-0.109
5	50	-0.130

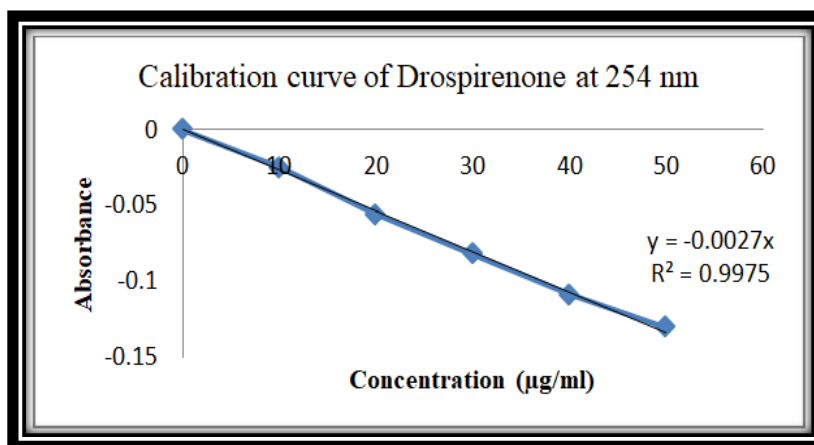


Fig. 5: Calibration curve of Drospirenone at 254nm

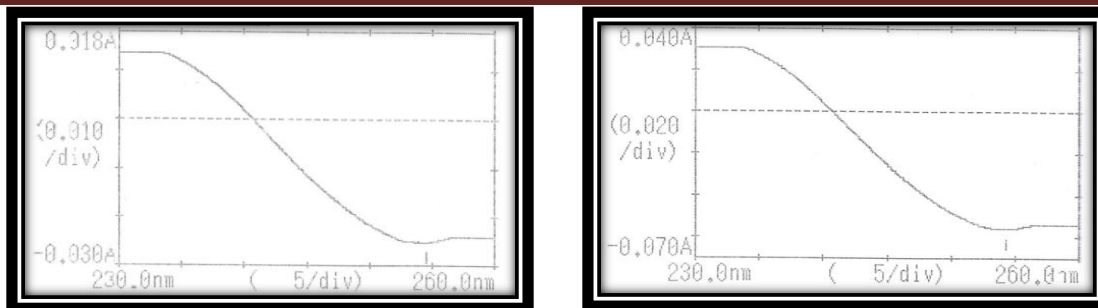


Fig. 6 & 7: First order spectrum of Drospirenone of conc. 10µg/ml and conc. 20µg/ml

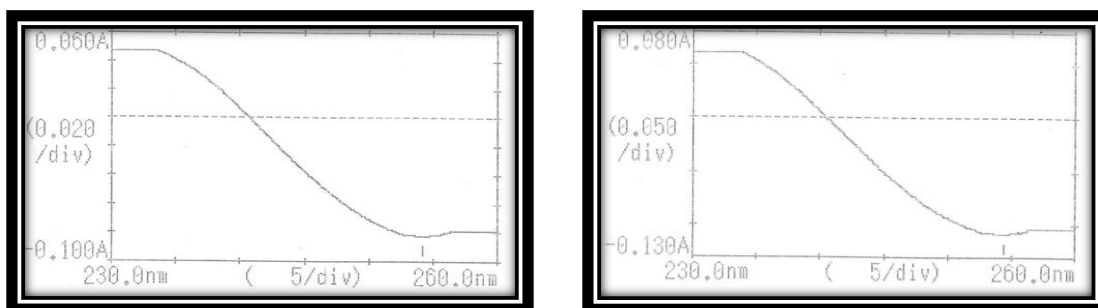


Fig. 8 & 9: First order spectrum of Drospirenone of conc. 30µg/ml and conc. 40µg/ml

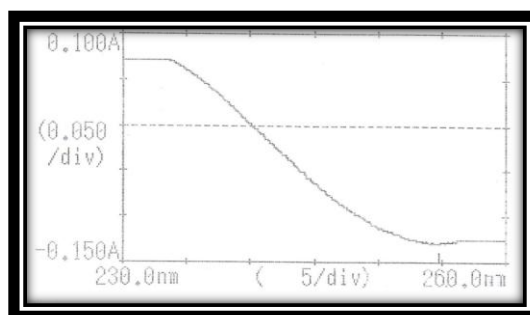


Fig. 10: First order spectrum of Drospirenone of conc. 50µg/ml

Table No. 1: Standard calibration table for Ethinyl Estradiol at 218nm

Sr. No.	Conc. of Ethinyl Estrdiol (µg/ml)	Absorbance at 218nm
1	32	0.139
2	34	0.145
3	36	0.165
4	38	0.172

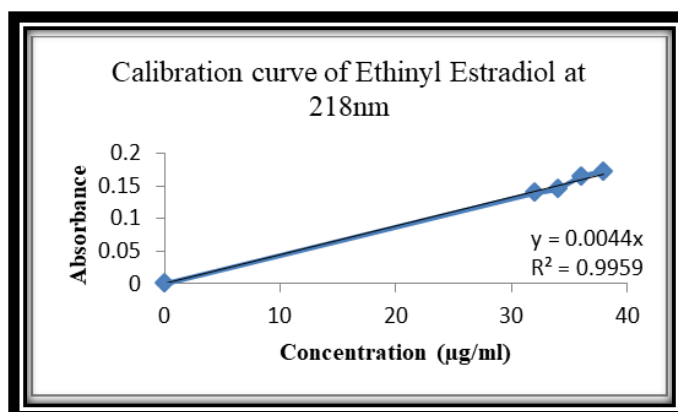


Fig. 11: Calibration curve of Ethinyl Estradiol at 218nm.

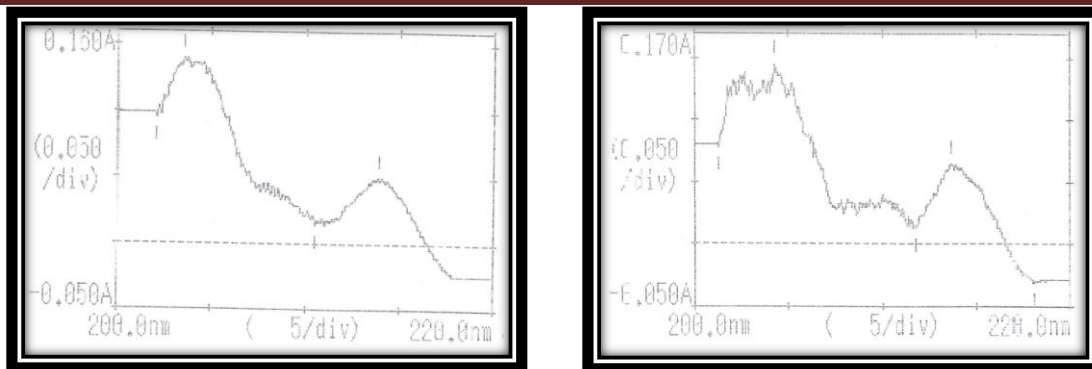


Fig. 12 & 13: First order spectrum of Ethinyl Estradiol of conc. 32µg/ml and conc. 34µg/ml.

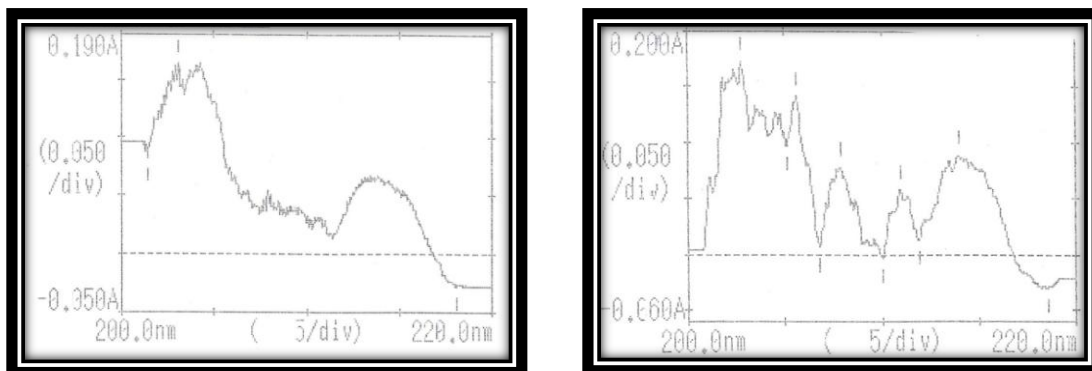


Fig. 14 & 15: First order spectrum Ethinyl Estradiol of conc. 36µg/ml and conc. 38µg/ml.

Optical Parameters for the Calibration curve:

The optical Parameters of the calibration curves are given below.

Table No. 3: Optical and regression Parameters of the Calibration Curve obtained by first derivative method.

Parameters	Drospirenone µg/ml	Ethinyl Estradiol µg/ml
Linearity range (µg/ml)	10-50	32-38
Slope	-0.00263	0.00446
Intercept	-0.0015	-0.00084
Regression coefficient (r ²)	0.997	0.995

Validation of proposed method: Estimation of drug from dosage form: (Tablet assay study)

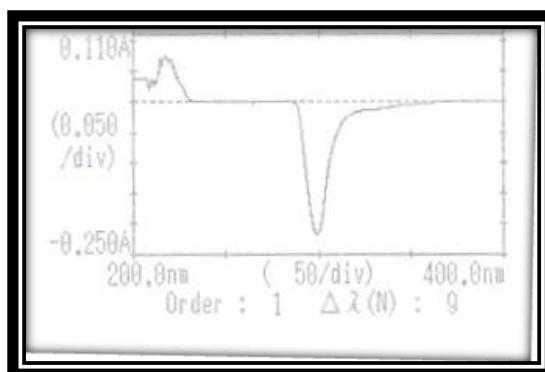


Fig. 16: Combined spectra of Drospirenone and Ethinyl Estradiol in tablet dosage form.

Assay of Ethinyl Estradiol performed as below:

Three hundred thirty four tablets containing ethinyl estradiol and drospirenone were taken and weighed and made powder form and quantity of powder equivalent to 10mg of Ethinyl Estradiol and transferred in 10ml volumetric flask and

made the volume up to 10ml with methanol. Further dilution was made to get 50µg/ml. These concentrations were scanned at wavelength 218nm for first order derivative mode with n=5. Absorbance was compared with standard.

Assay of Drospirenone performed as below:

Similarly as that of Ethinyl Estradiol four tablets containing Ethinyl Estradiol and Drospirenone were taken, weighed and made powder form. The quantity of powder equivalent to 10mg of drospirenone taken and transferred in 10ml of volumetric flask and volume were made upto 10ml with methanol. Further dilution was made to get 50µg/ml. These concentrations were

scanned at 254nm wavelength for first order derivative mode with n=9. Absorbance was compared with standard.

The results and statistical parameters of both Drospirenone and ethinyl estradiol in tablet analysis are shown below,

Table No. 4: Assay of ethinyl estradiol in tablet dosage form by first order derivative method.

Drug	Label claim (mg/tab)	Amount found (mg/tab)	% of label claim	Mean %	SD	CV
Ethinyl Estradiol	0.030	0.0301	100.33	100.16	0.2520	0.0025
	0.030	0.0302	100.66			
	0.030	0.0300	100.00			
	0.030	0.0300	100.00			
	0.030	0.0300	100.00			
	0.030	0.0300	100.00			

Table No. 5: Assay of drospirenone in tablet formulation by first order derivative method.

Drug	Label claim (mg/tab)	Amount found (mg/tab)	% of label claim	Mean %	SD	CV
Drospirenone	3.00	3.05	101.66	100.44	1.2263	0.0122
	3.00	2.95	98.33			
	3.00	2.98	99.33			
	3.00	3.02	100.66			
	3.00	3.03	101.00			
	3.00	3.05	101.66			

Accuracy (Recovery Test): Accuracy was studied by recovery experiments. To do recovery experiments we have to add known amount of standard ethinyl estradiol in tablet powder. The recovery was performed at three levels 80, 100 and 120 of Ethinyl Estradiol of standard concentration. The recovery samples were prepared. The solution were then analyzed, and percentage recoveries were calculated using formula.

Similarly for Drospirenone we have to do the recovery experiments by adding known amount of standard drospirenone drug to the tablet powder. Same as that of the Ethinyl Estradiol we prepared three concentrations 80, 100 and 120 of standard. The recovery samples were prepared. The solution was then analyzed and the percentage recoveries were calculated by using following formula.

$$\% \text{ Recovery} = \frac{\text{Observed amount of compound in sample}}{\text{Amount of all compound present in sample}} \times 100$$

The recovery values are summarized in following tables:

Table No. 6: Results of accuracy parameter of Ethinyl Estradiol for lynoral tablet 0.01mg.

Level of % Recovery	Amount present (µg/ml)	Amount of standard added (µg/ml)	Total amount recovered (µg/ml)	% Recovery	% mean Recovery	SD	CV
80	0.01	0.05	0.0601	100.16	100.21	0.0719	0.0007
80	0.01	0.05	0.0602	100.33			
80	0.01	0.05	0.0601	100.16			
100	0.01	0.10	0.1101	100.09	100.15		
100	0.01	0.10	0.1102	100.18			
100	0.01	0.10	0.1102	100.18			
120	0.01	0.15	0.1601	100.06	100.12		
120	0.01	0.15	0.1603	100.18			
120	0.01	0.15	0.1602	100.12			

Table No. 7: Results of accuracy parameters Drospirenone

Level of % Recovery	Amount present (µg/ml)	Amount of standard added (µg/ml)	Total amount recovered (µg/ml)	% Recovery	% mean Recovery	SD	CV
80	3.00	2.5	5.51	100.18	100.24	0.2112	0.0021
80	3.00	2.5	5.52	100.36			
80	3.00	2.5	5.51	100.18			
100	3.00	3.00	6.02	100.33	100.05		
100	3.00	3.00	5.98	99.66			
100	3.00	3.00	6.01	100.16			
120	3.00	3.5	6.51	100.15	100.04		
120	3.00	3.5	6.49	99.84			
120	3.00	3.5	6.51	100.15			

Precision Study: For Ethinyl Estradiol the dilution were made to get concentration of 25(µg/ml) and scanned at wavelength 218nm in first order derivative mode by four different analyst using same laboratory and same instrument. The precision values of Ethinyl Estradiol are given below.

Similarly for Drospirenone we made dilution of 25(µg/ml) and scanned at wavelength of 254nm in first order derivative mode by four different analyst using same laboratory and same instrument and the precision values of drospirenone are given below.

Table No. 8: Determination of precision of Ethinyl Estradiol for first order derivative method

Sample Number	Assay of Ethinyl Estradiol as % of Labelled amount			
	Analyst-1	Analyst-2	Analyst-3	Analyst-4
1	99.97	99.92	99.94	99.95
2	100.15	100.25	100.05	100.11
3	99.89	99.97	99.93	100.13
4	100.17	99.87	99.82	100.25
5	100.14	100.10	99.95	99.95
6	99.82	100.15	99.94	99.85
Mean %	100.02	100.04	99.93	100.04
SD	0.1373	0.1341	0.0666	0.1350
CV	0.0013	0.0013	0.0006	0.0013

Table No. 9: Determination of precision of Drospirenone for first order derivative method

Sample Number	Assay of Drospirenone as % of Labelled amount			
	Analyst-1	Analyst-2	Analyst-3	Analyst-4
1	100.15	99.95	99.89	100.14
2	99.20	100.07	100.14	100.25
3	100.25	100.12	100.35	100.35
4	100.50	100.15	100.29	99.89
5	100.15	100.25	100.12	99.95
6	100.05	99.85	99.85	100.05
Mean %	100.05	100.06	100.10	100.10
SD	0.4051	0.1316	0.1857	0.1610
CV	0.0040	0.0013	0.0017	0.0014

RESULTS

The standard solution of Drospirenone and ethinyl estradiol in methanol (10µg/ml each) subjected to a scan at the wavelength of 200nm to 400nm at first order derivative order mode and the first order derivative spectra was taken at n=9 for drospirenone and n=5 for ethinyl estradiol using shimadzu 1800 spectronic UV visible spectrophotometer. λ_{max} of drospirenone and ethinyl estradiol were found to be at 254nm and 218nm respectively. Therefore, 254nm for drospirenone and 218nm for ethinyl estradiol was selected as λ_{max} for the present study figure 3 and 4. The calibration curve of drospirenone and ethinyl estradiol were found to be linear in the range of 10-50µg/ml shown in figure 4-8 and 32-38µg/ml at 242nm and 218nm shown in figure 10-13 respectively.

For the determining the practicability of the developed technique for the assessment of commercially available brands of medicinal formulations, the technique was initially attempted on bulk drugs in their synthetic sample and concentration were estimated. Then the technique was subjected to the assay of tablets in marketed dosage brands and adequate results were attained within the acceptable limits as per the content of the label claim for ethinyl estradiol and drospirenone Table 4 and 5.

The recovery experiments were conducted by adding known amounts to tablet. The recovery was performed at three level 80, 100 and 120% of drospirenone and ethinyl estradiol standard concentration. Three samples were prepared for each recovery level. The solution were then analyzed and the percentage recoveries were found to be satisfactory within the

acceptable limits as per the content of the label claim for marketed tablet ethinyl estradiol and drospirenone respectively Table 6 and 7.

The newly developed method was validated as per the ICH guidelines and parameters. The novel method for the estimation of drospirenone and ethinyl estradiol was subjected to different validation parameters like specificity and selectivity in presence of formulation additives and excipients, studied for linearity and range at different levels of concentration and calibration standards where the determination range was optimized, accuracy was proved by recovery studies at different concentration levels, precision for ethinyl estradiol and drospirenone were established through the analysis of samples by four different analyst using same instrument and same laboratory Table 8 and 9. The method was developed successfully for drospirenone and ethinyl estradiol in its combined dosage forms by first order derivative method.

DISCUSSION

The drugs were subjected to analysis after getting appropriate dilutions of standard solutions, applied on both bulk drugs and formulations availed from market. The authors claim that the method shows linearity in the employed range with satisfactory accuracy and precision. Quantification of drugs simultaneously in combined preparations is generally accomplished by separating the contents using chromatographical techniques like High Performance Liquid Chromatography. Based on the above result, aim of the present study was an effort for the development of analytical techniques for the estimation of selected combined drugs present in their synthetic bulk mixtures and multi-component formulations for cost effective routine analysis like dissolution studies, determination of drugs in biological fluids, simultaneous release studies, and simultaneous kinetic studies etc

The advantage chief of the above work is its simplicity, because the instrument described is easy to handle. The other advantage is its applicability for the routine analysis for various routine investigations like dissolutions studies, rate determinations studies, release studies, Pharmacokinetic studies, bioavailability studies and other common day to day evaluations. Another application of this technique is its cost-effectiveness and it is the primary advantage over high performance liquid chromatographic methods of analytical investigation. The method employs methyl alcohol as the only solvent and no other reagent is required. The method utilizes very limited number of apparatus, i.e. routine laboratory glassware's used for dilutions purposes whereas the chromatographic techniques demand costly reagents, solvents and chemicals.

CONCLUSION

From the experimental studies it can be concluded that first order derivative methods are developed for the simultaneous estimation of drospirenone and ethinyl estradiol in their combined dosage form. The Proposed methods for selected drugs were found to be accurate and precise. However, this method is more reproducible. The results and the statistical parameters demonstrate that the proposed UV spectrophotometric method is simple, rapid, specific, accurate and precise. The most striking features of spectrophotometric method are their simplicity and rapidity. Result of validation parameters demonstrated that these analytical procedures are

suitable for its intended purpose and meets the criteria defined in ICH Q2/B.

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REFERENCES:

1. Effat S, Seyed MM and Maliheh BT. A Stability indicating HPLC Method for the Determination of Drospirenone in pharmaceutical Dosage Forms. *Res J Pharm Biol Chem Sci* **2014**;5(6):1-6.
2. Patel RC, Rathod DK, Rajesh KS, Patel VS. RP-HPLC Method Development and Validation for Estimation of Drospirenone and Ethinyl Estradiol in Bulk and Combined dosage form. *Pharmagene* **2013**;1(1):15-20.
3. Shrikant W, Santhakumari B, Chandewar AV. Development and validation of a simple and sensitive RP-HPLC method for simultaneous estimation of drospirenone and ethinyl estradiol in combined tablet dosage form. *Int J Pharm Pharm Sci* **2012**;4(5): 466-470.
4. Maryam N, Fariborz K, Manijeh M, Masoud M. Improved HPLC Method for Determination of Four PPIs, Omeprazole, Pantoprazole, Lansoprazole and Rabeprazole in Human Plasma. *J Pharm Pharm Sci* **2010**;13(1):1-10.
5. Satyanarayana B, Vasudev P. Hydrolytic Degradation Study of Lansoprazole, Identification, Isolation and Characterisation of Base Degradation Product. *Am J Anal Chem* **2015**;6:145-155.
6. Shoyeb A and Vani R. Stability indicating method development and validation for simultaneous estimation of lansoprazole and domperidone in bulk and its pharmaceutical dosage form by RP-HPLC. *World J Pharm Pharm Sci* **2015**;4(1):656-665.
7. Idrees F, Momani A, Majdoleen HR. Validation of HPLC and FIA Spectrophotometric Methods for the Determination of Lansoprazole in Pharmaceutical Dosage Forms and Human Plasma. *Am J Anal Chem* **2010**;1:34-39.
8. Janardhanan VS, Rajappan M, and Kannappan V. Stability-indicating HPLC method for the simultaneous determination of pantoprazole, rabeprazole, lansoprazole and domperidone from their combination dosage forms. *Int J Drug Dev & Res* **2011**;3(4):323-335.
9. Anil Kumar SM, Nagraj JG, Siddalinga Swamy MS. Validated Spectrophotometric Methods for the Simultaneous Estimation of Domperidone and Lansoprazole in Bulk Drug and Pharmaceutical Formulation. *Int J PharmTech Res* **2012**;4(2):828-834.
10. Anil kumar A, Venkataramana K, Narasimharaju CH and Sudhakararao G. A simple UV Spectrophotometric method for determination of Lansoprazole in bulk and pharmaceutical dosage forms. *ijpcbs* **2012**;2(4):524-528.
11. Muthu Kumar S, Selva Kumar D, Rajkumar T, Udhaya Kumar E, Suba Geetha A and Dinesh Diwedi. Development and validation of RP-HPLC method for the

- estimation of Lansoprazole in tablet dosage form. J Chem Pharm Res **2010**;2(6):291-295.
12. R. Vani. Stability indicating method development and validation for simultaneous estimation of lansoprazole and domperidone in bulk and its pharmaceutical dosage form by RP-HPLC. World J Pharm Pharm Sci 4(1):656-665.
 13. Cakiroglu Y, Vural B, Isgoren S. The effects of drospirenone ethinyl estradiol and drospirenone ethinyl estradiol metformin on ovarian ultrasonographic markers, body fat mass index, leptin, and ghrelin. Arch Gynecol Obstet **2013**;288(1):213-220
 14. Bird ST, Delaney JAC, Etminan M, Brophy JM, and Hartzema AG. drospirenone and non-fatal venous thromboembolism: is there a risk difference by dosage of ethinyl-estradiol?. J thrombhaemost **2013**;11:1059-68.

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