A Novel Technique For The Simultaneous Analysis Of Drosperinone And EstetrolIn Bulk And Tablet Dosage Form By Rp- Uplc

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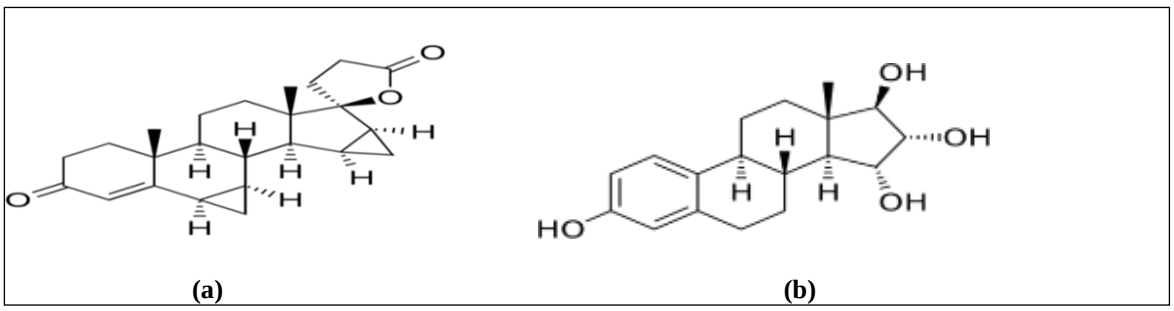
ABSTRACT
A convenient, precise, consistent, and robust quantitative approach was established for separation and simultaneous quantification of drosperinone and estetrol in bulk and tablet dosage form. The chromatographic separation was carried out by using CSH C18(50x 2.1mm, 1.7□) column at a temperature of 30°C and by using isocratic separationmode with a flow rate of 0.3ml/min. The system was operated by using mobile phase 0.01N kh_2po_4 buffer and acetonitrile(60:40v/v) with a TUV detector at a detection wavelength of 240nm. Drosperinone and Estetrol retention time was found to be 1.87min and 1.46min respectively. The recommended method was validated for linearity, accuracy, precision, robustness as per the ICH guidelines and the method found to be acceptable. The stress degradation studies were developed and found to be satisfactory. Results shows that the proposed method was precise,simple, robust and suitable for routine analysis.

Keywords: Drosperinone, Estetrol, ICH guideline(international conference of harmonization), stress degradation,CSH (charge surfaced hybrid),Reverse phase ultra performance chromatography.

INTRODUCTION

Drosperinone is an progestin medication mainly used for birth control pills and also in menopausal hormonal therapy.[1] Drosperinone is an antimineralocorticoid spironolactone which was synthesized from androstenone.[4] Drosperinone, is an analogue of spironolactone, whose biochemical and pharmacologic profiles are similar to endogenous progesterone[3].Chemically it is (6R,7R,8R,9S,10R,13S,14S,15S,16S,17S) 1, 3',4',6,6a, 7,8,9,10,11, 12,13,14,15,15a,16-Hexadecahydro -10,13- dimetylspiro-[17H- dicyclopropa[6,7:15,16]cyclopenta[a]phenantrene- 17,2'(5'H)-furan]-3,5'(2H)-dione[5].Estetrol is also known as oestetrol (E4) is a weak steroid estrogen hormone which is detectable mainly during pregnancy as it is a natural hormone mainly it is used in combination with Drosperinone as a oral contraceptive pill[3]. Chemically Estetrol is(1*R*,2*R*,3*R*,3a*S*,3b*R*,9b*S*,11a*S*)-11a-Methyl- 2,3,3a,3b,4,5,9b,10,11,11a-decahydro-1*H* cyclopenta[*a*]phenanthrene-1,2,3,7-tetrol.[11]

Literature survey shows few HPLC methods[6],and UPLC[11] methods are available for Drosperinone and ethinyl estradiol in combination and alone.so an attempt was made for simultaneous and method development of drosperinoneand estetrol in combination to develop a sensitive and reproducible method.



(a) **(b)**

Figure 1: structure of(a)- Drosperinone,(b) Estetrol

MATERIALS AND METHODS

Chemicals

UPLC grade water, acetonitrile, potassium dihydrogen orthophosphate purchased from Thermo Fischer Scientific Pvt.Ltd. The drug samples were attained from Spectrum labs Ltd.,India.

Instrumentation
UPLC system model no (1200 series, make: Waters)equipped with quaternary pumps with TUV detector and Auto sampler by Empower 2.0. UV-VIS spectrophotometric(PG Instruments),and coordinated quartz cells incorporated with the UV of 6.

Standard solution preparation
3mg of drosperinone and 14.2mg of estetrol working standard solution was weighed into 50ml volumetric flask. To this 10ml of diluent (water:ACN) was added and finally make up to volume by using diluent to get 60µg/ml of drosperinone and 284µg/ml of estetrol. From this 1ml was taken in to 10ml volumetric flask and further made up to volume with diluent to get 6µg/ml of drosperinone and 28.4µg/ml estetrol.

Preparation of test solution
10 tablets of olanzapine and samidorphan was weighed and powdered. Then from the powder weight equivalent to 1tablet was transferred into a clean 100ml volumetric flask, 50ml of diluents added and the solution was sonicated for25 min. Finally made up volume to get 30µg/ml of drosperinone and142µg/ml of estetrol with diluent. To 10ml of volumetric flask, 2ml of above solution was added and finally volume was made to get 6µg/ml of drosperinone and28.4µg/ml estetrol with diluent.

Method validation
The developed method was validated as per ICH guide lines and various parameters like linearity, precision ,accuracy robustness ,LOD&LOQ ,stress degradation studies were evaluated.

System suitability
systems accuracy is verified by calculating systems suitability. some parameters like USP tailing, USP plate count and %RSD were evaluated by injecting six replicate injections and all were found to be within the acceptable limit.

Specificity
This method is performed by separately injecting blank,placebo,standard sample containing Drosperinone andestetrol. Interference was not observed and the method was found to be specific.

Precision
The precision is the degree of reproducibility under the same conditions. Intraday precision is caluculated within oneday and interday precision was calculated between days. The %RSD of drosperinone and estetrol for six standard solutions were calculated and the method was precise for both drugs.

Accuracy
Accuracy is the degree of veracity. In this method samples were taken in triplicate by spiking at three different levels 50%,100%,150% of the targeted concentration of drugs. From this mean percent recovery was calculated bycomparing the difference between spiked value and actual value.

Linearity
In this method series of standard six solutions were taken in the concentration of 1.5-9 µg/ml of drosperinone and7.1-42.6 µg/ml of estetrol. A standard plot was constructed between concentration versus mean peak area in µg/mlto know the best fit line of both drugs.

Robustness
Robustness was performed by small deliberate changes in the parameters like flow rate, mobile phase compositionand column temperature and no change was identified in the method. %RSD was within the limits.

LOD&LOQ
Detection limit(LOD) and quantitation limit(LOQ) was calculated based upon signal to noise ratio and the formula isa follows

$$LOD = 3.3 \times \sigma / s, \quad LOQ = 10 \times \sigma / s$$

Forced degradation studies
100mg of standard drug of olanzapine and samidorphan was mixed with 100ml of 2N hydrochloric acid, 2N sodium hydroxide and 20% hydrogen peroxide for acidic, basic and oxidation studies respectively. For neutral study the solution was refluxed in water for 1hr at 60 ºC. For photolytic and thermal studies standard drug solutions were kept in UV for 1 day at 258nm and for heat in an oven 105^0C for 2hr.Percentage degradation was calculated by comparingthe peak area response of both drugs with calibration curve results.

RESULTS AND DISCUSSION

Method development for optimized chromatographic condition

By using charge surfaced hybrid column C_{18} (50x 2.1mm, 1.7□.) the best chromatographic separation was obtained at a flow rate of 0.3ml/min with a wavelength of 240nm. Mobile phase comprising of 0.01N kh_2po_4 buffer and acetonitrile(60:40)v/v) was taken. By following the above conditions, optimized chromatogram was obtained by evaluating the column efficiency parameters. The results of the conditions were depicted in figure 2

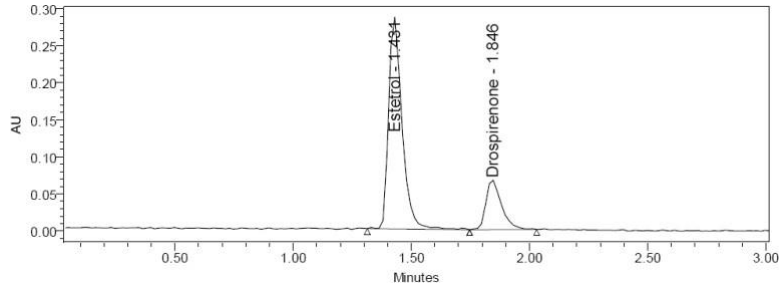


Fig 2 :Standard chromatogram

Method validationsystem suitability

System suitability was tested from standard solutions by injecting six times and chromatograms were recorded .Fromthe chromatograms Retention time (Rt), number of theoretical plates (N) and tailing factor (T) are evaluated for six replicate injections The results were given in table 1.

Table 1: System suitability results

Parameters	**Drosperinone**	**Estetrol**
Retention time	1.872	1.447
Theoretical plate	3887	2695
Tailing factor	1.39	1.36

Precision

The precision of the method was evaluated by injecting six samples for interday precision on different days and for intraday precision on the same day. The results were depicted in table 2.

Table 2: Results of Intraday and interday precision

S.no	Intra day precision		Interday precision	
	Drosperinone	Estetrol	Drosperinone	Estetrol
1	292696	1100931	288871	1070312
2	286925	1102156	295507	1074103
3	286310	1087289	298128	1085031
4	291277	1106597	287415	1106145
5	288265	1085288	291531	1061302
6	290449	1064623	285804	1097693
Mean	289320	1091147	291209	1082431
Std.Dev	2546.0	15545.8	4806.7	17115.5
%RSD	0.9	1.4	1.7	1.6

Specificity

No interference was observed and the standard and sample peaks were identical.

Limit of detection and limit of quantification

The values of LOD and LOQ for drosperinone was found to be 0.04 µg/ml and 0.12 µg/ml .Foe estetrol LOD andLOQ values was found to be 0.44 µg/ml and 1.34 µg/ml respectively.

Accuracy

Recovery studies were conducted in triplicate by spiking at different levels(50%,100%,150%). The results were shownin table 3

Table 3 : Accuracy for olanzapine and samidorphan

Level	Amt added		Amt recovered		%recovery		Mean % recovery	
	DSP	EST	DSP	EST	DSP	EST	DSP	EST
	3	**14.2**	3.01	**14.3**	100.35	100.60		

50%	3	**14.2**	2.95	**14.3**	98.42	100.90	99.34	100.73
	3	**14.2**	2.98	**14.3**	99.26	100.70		
100%	6	**28.4**	6.03	**28.4**	100.51	99.90	100.25	100.10
	6	**28.4**	5.98	**28.7**	99.67	101.20		
	6	**28.4**	6.03	**28.2**	100.57	99.20		
150%	**9**	**42.6**	8.99	**42.9**	99.88	100.60	99.85	100.00
	9	**42.6**	8.98	**42.1**	99.72	98.90		
	9	**42.6**	9	**42.8**	99.96	100.50		

Linearity

By analyzing a series of standard solutions in the ratio of 1.5-9µg/ml for Drospirenone, & 7.1-42.6µg/ml for Estetrol.A graph was plotted between mean peak area versus concentration . the results were shown in fig 3. and table4.

Table 4 : Linearity data

Analyte	Linearity range(µg/ml	Calibration curveequation	Correlation coefficient
Drosperinone	1.5-9 µg/ml	Y=48697.x+6866	0.999
Estetrol	7.1-42.6 µg/ml	Y=38864.x+20329	0.999

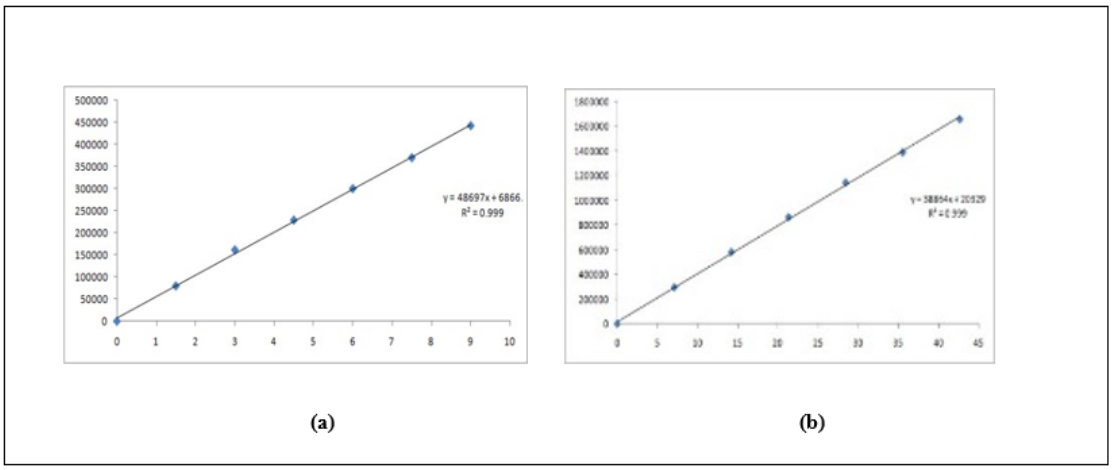


Fig 3.....Linearity plot of(a)- drosperinone,(b)estetrol

Robustness

By slight changes in flow rate, mobile phase, temperature the %RSD was found to be not more than 2,hence the developed method specifies to be robust. The results were summarized in the table5.

Tab 5 : Results of robustness

S.no	Condition	Drosperinone		Estetrol	
		Peak area	%RSD	Peak area	%RSD
1	FR 1	263257	1.1	984816.6	1.6
2	FR 2	314377	1.4	1189123	1.3
3	MP 1	264644	1.7	1001499	1.5
4	MP 2	290192	0.9	1049388	1.0
5	CT 1	304595	1.6	1098223	1.4
6	CT 2	269383	1.6	981927	1.3

FR1- flow rate minus(0.27ml/min),FR2- flow rate plus(0.33ml/min), MP1-mobile phase minus(buffer 65:35 ACN) ,MP2-mobile phase plus(buffer 55:45 ACN ,CT1-column temperature minus (27°C) , CT2-column temperature plus(33°C)

Stress degradation studies

As per ICH(Q2 R1)degradation studies like acid, base, oxidation, thermal, and neutral investigations were carried out and all were within the acceptance criteria. The results were shown in Table...

Tab 6 : Results of degradation studies

	Estetrol	**Drosperinone**

Degradation condition	% of undegraded	% of degraded	% of undegraded	% of degraded
Acid	95.57	4.43	95.73	4.27
Base	95.72	4.28	95.9	4.1
Oxidation	94.24	5.76	94.36	5.64
Dryheat	97.45	2.55	97.23	2.77
UV	98.15	1.85	98.54	1.46
Water	99.4	0.6	99.48	0.52

Assay

In this method standard and sample solutions were injected seperately in to the chromatographic system and the chromatograms were recorded.The amounts of drosperinone and estetrol estimated were found to be 100.34% and99.95% .This shows that the developed method is accurate.

CONCLUSION:

The proposed UPLC method was unique and developed for quantification of drosperinone and estetrol in bulk and in pharmaceutical formulation. The method was found to be specific,accurate satisfactory as all the validation parameterswere within the acceptance criteria. The stress degradation studies were conducted for both drugs and the method wasstable and accurate. The developed method was stable and effective and can be used for drug testing in routine analysis.

CONFLICT OF INTEREST

None.

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