IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
August 2021 Vol.:22, Issue:1

Development and Validation of Reverse Phase HPLC Method for The Quantitative Estimation of Drospirenone in Tablet Dosage Form

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ISSN 2349-7203
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Submitted: 23 July 2021
Accepted: 29 July 2021
Published: 30 August 2021

Keywords: Drosperinone, RP-HPLC, Validation, Stability-indicating, stress degradation, ICH

ABSTRACT

The research work aimed to develop and validate RP- high-performance liquid chromatography (RP-HPLC) stability-indicating method for the determination of drosperinone a synthetic progestin. The reversed-phase chromatographic separation was accomplished using the HPLC system Shimadzo Prominance model L20 HPLC system equipped with UV-Vis detector, C18 column. Isocratic elution was performed using the solvent system acetonitrile: water (65:35 v/v) and UV detection at 220 nm. The simple and rapidly developed method for RP-HPLC analysis of drosperinone was validated with reverence to specificity, selectivity, linearity, accuracy, precision, and robustness as per the ICH guidelines. The linearity was observed in the concentration range of 3-18 µg/mL with a correlation coefficient of 0.9993. The accuracy of the studies was found to be in the range from 99.06 to 100.78%. The relative standard deviation for inter-day precision was lower than 2.0%. The assay of drospirenone was determined in tablet dosage form was found to be within the acceptable limits. Stability studies of drosperinonewere performed in stress conditions namely acidic, alkaline, oxidation, photolysis, and thermal degradations as per the ICH guidelines. The method can be used for quality control analysis of drospirenone in the combined dosage form.

INTRODUCTION

Drospirenone (Dros, **Figure 1**) a synthetic progestin chemically related to 17α-spirolactoneis employed for treatment of scarce levels of estrogen and in contraceptives [1]. As compared to the parent compound spirolactone, drospirenone also shows anti mineralocorticoid and anti-androgen activity and is also effective in menopausal hormonal therapy [2, 3]. Marketed oral contraceptive formulations typically comprise Dros with ethinylestradiol in varying combinations of 20µg ethinylestradiol/3mg drospirenone and 30µg ethinylestradiol/3mg drospirenone [4]. Dros is a fourth-generation oral contraceptive and is reported to possess antimineralocorticoid effects not present in previous generations of oral contraceptives. Its antimineralocorticoid potency was reported to be approximately eight times greater than spironolactone. This activity enhances sodium, chloride, and water excretion, while reducing the excretion of potassium, ammonium, and phosphate [5-7].

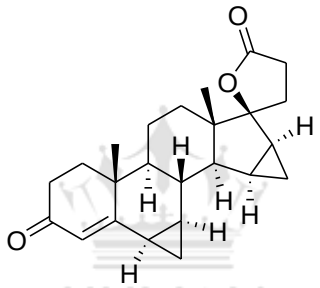

Figure No. 1: Structure of Drospirenone

Literature studies reported for the Dros includes HPLC techniques with either ultraviolet (UV) [8-11], radioimmunoassay (RIA) [12, 13], and tandem mass spectrometry (MS/MS) methods [14, 15] for quantification and pharmacokinetic studies of Dros in combination with drugs and alone in pharmaceutical formulations [9, 14,] and biological fluids [11-15]. From the literature, it is profound that the available RP-HPLC methods are mainly for analyzing drospirenone in various drug combinations in pharmaceutical dosage forms [8-15]. Hence, it was intuited necessary to develop and validate a simple and precise rapid RP-HPLC method for the quantitative determination of Drosin tablet dosage forms. The analytical method developed was validated by ICH guidelines [15, 16]. Forced degradation studies were performed to authenticate the stability-indicating nature of the HPLC method of Dros.

MATERIALS AND METHODS

Reagents and Chemicals:

The solvents acetonitrile, methanol, water and acetic acid used were of HPLC grade (Fisher Scientific, UK). Analytical grade reagents sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), and hydrochloric acid (HCl) were acquired from Scharlau, Spain. Drospirenone standard (purity 100%) was kindly gifted by Cipla Ltd. (Sikkim, India). All the chemicals procured were used as received.

HPLC apparatus and Conditions:

Chromatographic separation was accomplishedon Shimadzo Prominence L20 HPLC system equipped with UV-Vis detector, Restex Allure C18 (250mm × 4.6mm i.d., 3 µm particle size) column. Isocratic elution was performed using acetonitrile: water (65:35 v/v) as a solvent system and UV detection at 220 nm. The overall run time of the analysis was around 10 min. and the flow rate was maintained at 1.0 mL/min. 20 µL of the sample was injected into the HPLC system. HPLC analyses were completed at room temperature. All the results were processed by Shimadzu LC Solution software.

Method development

Preparation of mobile phase: The mobile phase was prepared by mixing acetonitrile: water (65:35 v/v). The prepared mixture was filtered and sonicated (30 minutes).

Preparation of Standard Solution: Standard stock solution of Dros was prepared using methanol to obtain a concentration of 1mg/mL. Preparation of stock solution involved accurately weighed Dros standard sample (50 mg) and transferred it into a 50 ml volumetric flask, dissolved in 25ml of methanol. The resultant solution was sonicated (15 min) to dissolve Dros completely and made the volume up to 50 mL with mobile phase to get the stock solution of 1000µg/mL. Later, 1mL of the stock solution was pipetted into a 10mL volumetric flask and diluted with mobile phase to get 100µg/mL strength of Dros. The solution was thoroughly mixed, filtered through 0.45µm membrane paper. The working standard solution of 12 µg/mL was prepared from 1.2 ml of 100µg/mL of Dros in a 10mL volumetric flask. The solution was mixed, filtered through a 0.45µm membrane filter. Aliquots of Dros working standard solutions were transferred into a series of 10 mL volumetric flasks so that the final concentration was in the range of 3-18 µg/mL.

Chromatographic Run: Standard solutions of Droswere loaded into the injector and HPLC parameters were entered as per table1. This method was saved and then the sample was injected and the stop time for each analysis was fixed as 10 min.

Analytical method validation:

As per ICH Guideline (Q2A(R1)), method validation has been performed for the parameters such as specificity, linearity, precision, accuracy, the limit of detection (LOD), the limit of quantification (LOQ), robustness, and system suitability.

System suitability: The assessment for the suitability of the system was done using six (6) drug replicas at a concentration of 12g/ml. It was used to confirm that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be done. The method was evaluated by analyzing the repeatability, retention time, peak area, capacity factor, tailing factor, theoretical plates of the column. The developed method was found to be precise. The standard chromatogram of Dros is presented in Figure 3A and the results of the analysis are summarized in table 1.

Table No. 1: System suitability data of Drosperinone (Dros) (n=6)

Sl. No	Retention time (min)	Mean peak area of Dros	Tailing factor	Number of theoretical plates
1	6.57	22198	1.01	8325
2	6.51	22282	1.03	8174
3	6.33	22174	1.03	8289
4	6.48	22847	1.05	8367
5	6.49	22549	1.03	8288
6	6.5	22749	1.04	8193
Mean	6.48	22466.5	1.031667	8272.667
SD	0.073	265.543	0.012	63.821
%RSD	1.043	1.182	1.16	0.771
Limits	NMT 2%	NMT 2%	NMT 2%	NMT 2%

*Mean of six replicates

Linearity: For evaluation of the calibration graph, a weighted linear regression was performed with nominal concentrations of calibration standards against measured peak areas.

Calibration graph (concentration *vs*. peak area) was constructed at six concentrations levels (3-18 µg/mL). The analytical curve was evaluated on three different days. The slope and y-intercept of the calibration curve was reported in figure 2 and the data for linear regression studies are shown in Table 2.

Table No. 2: Linear regression data for drosperinone (n = 6)

	Statistical Parameters	Values
1	Concentration range	3-18µg/mL
2	Regression equation	y = 964.05x + 10110
3	Correlation coefficient	r^2 = 0.9993

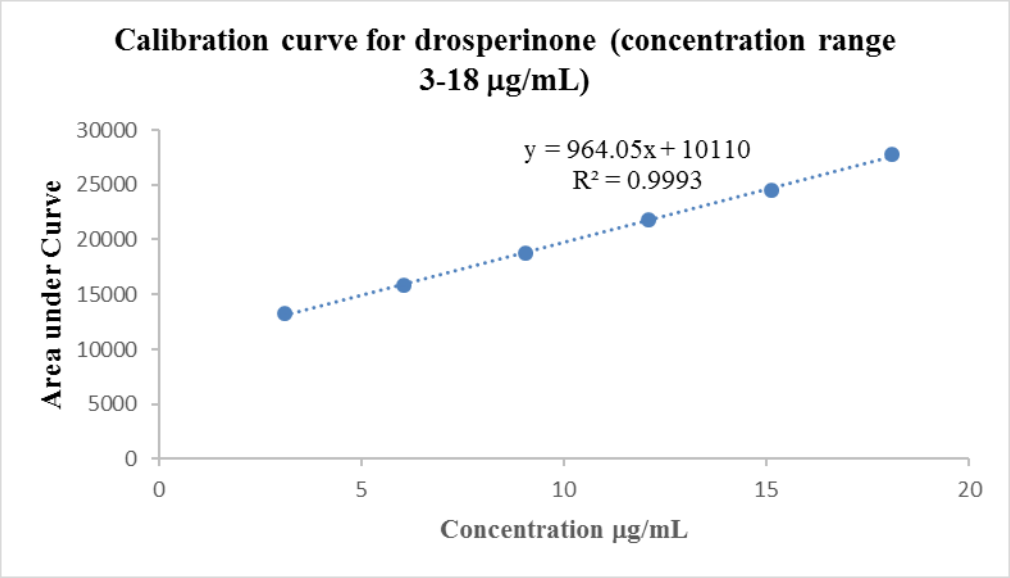

Figure No. 2: Calibration curve for drosperinone (concentration range 3-18 µg/mL)

Sensitivity: The sensitivity for Dros in terms of Limit of quantification (LOQ) and Limit of detection (LOD)were calculated from the standard deviation (SD) of response and slope of the curve (S) using the equations: LOD=3.3(SD/S) and LOQ=10(SD/S), according to ICH guidelines. The LOD was found to be 0.20µg and LOQ was to be 0.75 µg.

Precision: The precision of the analytical method was studied by analyzing multiple samples of the homogeneous sample. The precision is expressed as standard deviation or relative standard deviation. The precision of the analytical method for the sample dros was demonstrated intra-day and intra-day variation studies.

Intra-day precision: In the intra-day studies, six injections of a standard solution of dros (6 µg/mL, 9 µg/mL, 12 µg/ml L) were injected into the RP-HPLC system at different time intervals within a day. % RSD was calculated and represented in table 3.

Inter-day precision: In the inter-day studies, six injections of a standard solution of dros(6 µg/mL, 9 µg/mL, 12 µg/ml L) were injected into the RP-HPLC system at different time intervals within a day. % RSD was calculated and represented in table 3.

Table No. 3: Results of Intraday and interday Precision for Drosperinone (n=6)

Concentration (µg/mL)	Day 1			Day 3		
	* Mean peak area for Dros	SD	RSD (%)	PA area for Dros	SD	RSD (%)
3.09	13260.66	95.67	0.721	13357.66	98.76	0.739
6.03	15868.66	166.39	1.05	15899.66	148.803	0.925
9.02	18756.55	173.15	0.994	18956.55	186.210	0.982
12.04	21772.87	249.19	1.14	21886.87	234.12	1.07
15.09	24533.5	191.57	0.781	24743.5	187.114	0.781
18.10	27745.43	224.09	0.807	27879.43	237.331	0.851

*Mean of six replicates

Accuracy: The accuracy of the method was determined by calculating recoveries of the drug by the method of standard addition. A known amount of standard drug corresponding to 50%, 100%, and 150% of the label claim was added to prequalified sample solution and the amounts of drug were estimated by measuring the peak areas and the results of the study is represented in table 4.

Table No. 4: Results of Accuracy studies for Drosperinone (n=6)

Concentration (µg/mL)	*Mean peak area for Dros	SD	RSD (%)	*Drug found	%Recovery
6.04	15239.17	136.67	0.896	5.946	99.1
12.	22257.34	227.92	1.02	12.01	100.25
18	27286.73	268.77	0.984	18.11	100.61

*Mean of six replicates

Robustness: To evaluate the robustness of the method the influence of small deliberate variation of analytical parameters on the retention times of drospirenone was studied. The parameters selected were the effect of acetonitrile in mobile phase composition (63 and 67%), flow rate (0.8 and 1.2 mL/min), and wavelength (218 and 222 nm). Only one parameter was changed while the others were kept constant. Results of the study are summarized in table 5.

Table No. 5: Results of Robustness studies for Drosperinone (n=6)

Condition	Modification	*Mean Peak area for Dros	SD	RSD (%)
Mobile phase composition	63:37	22133.65	233.36	1.05
(Acetonitrile : water) 65:35	67: 33	22199.17	228,77	1.03
Flow rate	0.8 mL	22149.38	238.15	1.07
(1mL/min)	1.2 mL	22177.52	223.42	1.01
Wavelength	218 nm	22148.15	235.15	1.06
220 nm	222 nm	22152.83	227.33	1.03

*Mean of six replicates

Analysis of Marketed Formulations: We have selected two different brands of drospirenone tablets namely Crisanta and Yasmin(Label claim 3mg drospirenone) to determine the drug content. Twenty tablets from respective marketed formulations were accurately weighed; their average weight was determined and finely powdered. The tablet powder equivalent to the weight of 10 mg of Dros was accurately weighed and transferred into a 10 ml volumetric flask. It was completely dissolved with methanol. The resulting solution was sonicated for 15 min and filtered using a 0.45µm membrane filter. The concentration of test sample solutions was 1000 µg/ml. It was further diluted up to the 10 ml with mobile phase to get the 100µg/mL. The solution was mixed well and filtered through a 0.45µm membrane filter. The working test solution of 12µg/mL was prepared by diluting 1.2 ml of the above stock solution up to 10ml with the mobile phase. These solutions were filtered through a 0.45 µm membrane filter before injecting into the chromatographic column. All the chromatographic analyses were performed in replicates of six. The drug peak area was referred to linear regression equation to get the sample concentration and nominal % of label claim. The chromatogram is shown in figure: 3B-3C and the assay results are described in table 6.

Table No. 6: Assay Results of the Drosperinone tablets (n=6)

	Tablet brand names	Label claim (mg)	Amount recovered (mg)	% Recovery
1	Crisanta	3	2.98	99.33
2	Yasmin	3	3.02	100.66

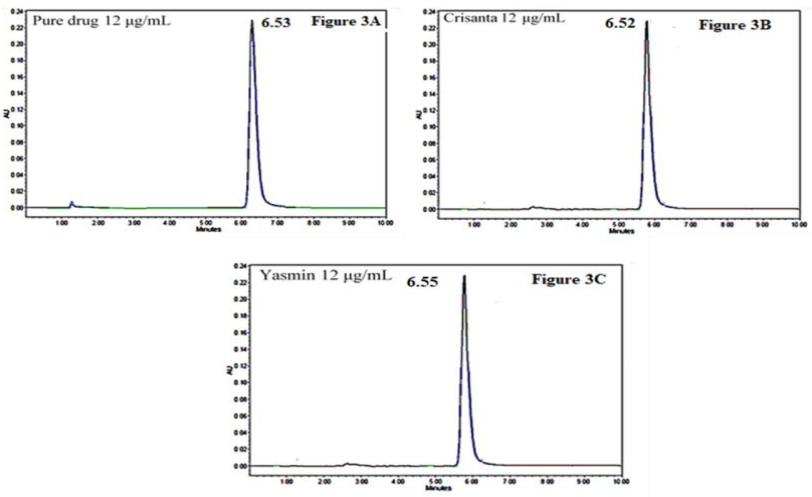

Figure No. 3: Typical chromatograms of Drosperinone (12 µg/mL) (A) pure drug; (B) Crisanta (label claim: 3 mg); (C) Yasmin (label claim: 3 mg).

Forced degradation solutions: Forced degradation studies were performed to evaluate the stability-indicating properties and specificity of the method [15, 16]. The stability of the drug Dros was determined by subjecting it to oxidative, alkaline, acidic, neutral, and photolytic conditions to accelerate conditions favorable to degradation. The stress solutions were prepared from a solution of 1 mg/mL and subjected to heating (80°C). Solutions at a concentration of 12µg/mL were prepared using purified water and filtered before injection. The drug peak area was referred to linear regression equation to get the sample concentration and nominal % of label claim. The HPLC chromatograms are shown in figures: 4A-4E and the percent recovery data are summarized in table 7.

Acidic degradation: Acidic degradation was performed by treating the Dros solution (12µg/mL) with 0.1 N hydrochloric acids for 30 min in a thermostat maintained at 80 ºC, cooled and then the stressed sample was neutralized and diluted with mobile phase as per the requirement before injecting into the HPLC system.

Alkaline degradation: Alkaline degradation was performed by treating the Dros solution (12µg/mL) with 0.1 N sodium hydroxide for 30 min in a thermostat maintained at 80 ºC, cooled and then the stressed sample was neutralized and diluted with mobile phase as per the requirement before injecting into the HPLC system.

Oxidative degradation: Oxidative degradation was performed by treating the Dros solution (12µg/mL) with 3 % H_2O_2 for 30 min in a thermostat maintained at 80 ºC, cooled, and then the stressed sample was diluted with mobile phase as per the requirement before injected into the HPLC system.

Photolytic degradation: The drug was exposed to direct sunlight for 7 days. The stock solution was prepared using the procedure described above. The solution obtained was further diluted to obtain a concentration of 12µg/ml and 20µL was injected into the HPLC system.

Table No. 7: Results of Stress degradation studies for Drosperinone (n=6)

Sl. No	Stress condition	Mean peak area	% Drug recovered	% Drug degraded
1	Neutral	22362.04	100	0
2	Acidic	22519.56	96.45	3.55
3	Alkaline	22186.74	26.83	73.16
4	Oxidative	21874.92	62.55	37.45
5	Photolytic	22179.28	99.22	0.88

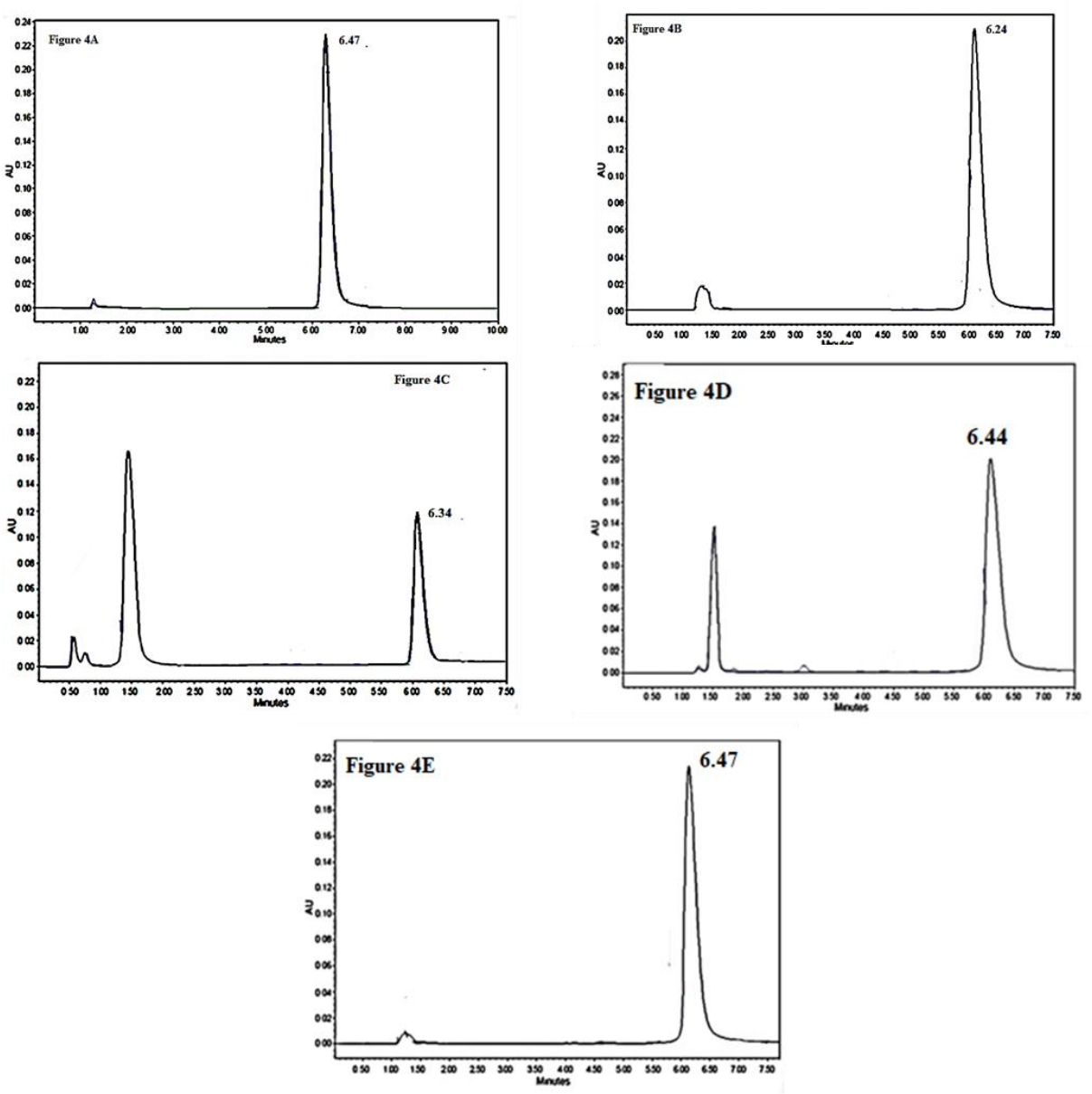

Figure No. 4: HPLC chromatogram of drosperinone (12µg/mL) after exposure to **(A)** Neutral degradation; **(B)** Acid hydrolysis (0.1 N hydrochloric acid for 30 min 80 ºC); **(C)** alkaline hydrolysis (0.1 N sodium hydroxide for 30 min 80 ºC)**; (D)** Oxidative degradation (3 % H_2O_2 for 30 min in a thermostat maintained at 80 ºC)**; (E)** photolytic degradation.

RESULTS AND DISCUSSION

In the present study, RP-HPLC method development and validation to report the analysis and quantification of drosperinone were performed as per the specification parameters described in International Conference on Harmonization (ICH-1996).

HPLC method development and optimization:

The present work aimed to develop a simple, precise, and accurate stability-indicating RP-HPLC method to estimate Dros in the tablet dosage form. Several trials were performed for the appropriate selection of the mobile phase for the RP-HPLC method development. Based on the trial data the most appropriate mobile phase selected was acetonitrile: water (65:35 v/v) and the wavelength set at 220 nm as the drug Dros exhibited good absorbance at this wavelength. The flow rate of 1mL/min, the overall run time was 10 minutes and an injection volume of 20 µL was used. The retention time of Dros was found to be 6.5 minutes. A typical chromatogram of the drospirenone is shown in Figure 3A. The various parameters considered for HPLC method validation in the present study were system suitability, specificity, range and linearity, the limit of detection (LOD), the limit of quantification (LOQ), accuracy, precision, ruggedness, and robustness. Replicate injections (n=6) were prepared to ensure reproducibility and accuracy of measurements.

Method validation:

System suitability:

The assessment for the suitability of the system was carried out using six (n = 6) replicas of Dros at a concentration of 12g/mL. It was used to endorse that the resolution and reproducibility of the chromatographic system were adequate for the sample analysis. The various parameters measured include repeatability, retention time, peak area, tailing factor, theoretical plates (Tangent) of the column. The tailing factor showed less than 2, the capacity factor was more than 2 and the theoretical plates were more than 2000. The average retention time was 6.491 minutes and the %RSD of peak area was 0.20%. The values for system suitability parameters showed the feasibility of this method for routine pharmaceutical applications. The results of system suitability tests are shown in table 1.

Linearity:

The calibration curve for Dros with good linearity was obtained in the concentration range 3-18 µg/mL. The corresponding linear regression equation was y = 964.05x + 10110 and the correlation coefficient for calibration curve was 0.9993 (Figure 2 andTable 2).The results showed good linearity between the peak area and analyte concentration. The HPLC chromatograms for Dros acquired from the standard solution and various tablet formulations are displayed in Figures 3A-3C.

Sensitivity:

LOD is the ability of the analytical method to detect the lowest concentration of the analyte. LOQ is the lowest concentration of the analyte with acceptable precision and accuracy. It can be calculated based on the signal-to-noise ratio. The LOD and LOQ for Dros were found to be 0.20 µg/mL and 0.75 µg/mL, respectively.

Precision:

The precision of the proposed method was determined by performing a standard solution assay on the same day (intra-day) and three different days (inter-day). The precision of the method was evaluated by performing six independent determinations of the standard Dros solutions of five different concentrations (3-18 µg/mL) and calculating RSD (%). For day 1 (one) precision studies, the RSD (%) values for the five samples of Dros were observed in the range of 0.721–1.14 while for day 3 (three) precision studies the RSD (%) range was 0.739-1.07. The results of intra-day and interday precision studies are reported in Table 3. The low RSD values indicate that the method is precise.

Accuracy:

To ascertain the accuracy of the proposed HPLC method, recovery studies were performed by adding known amounts of Dros corresponding to three concentration levels: 50%, 100%, and 150% of the label claim, and the results of recovery studies are displayed in table 4. The percent RSD values for Dros were observed in the range 0.896-1.02 % and the percentage recovery was 99.1 - 100.61 %. The results of the recovery test studies designate that the method is highly accurate.

Robustness:

The robustness of the present analytical method was determined by the consistency of the peak height and peak shape with deliberate small changes made in the experimental conditions [17]. To determine the robustness of the proposed method, the following variations were made in the developed analytical method: percentage of acetonitrile in the mobile phase (63% and 67%), wavelength (218 and 222 nm), flow rate (0.8 and 1.2 ml/min). The results acquired (Table 5) from the assay of the test solutions were found to be unaffected by changes in the conditions. The % RSD value of assay determined for the same

sample under original conditions and robustness conditions was less than 2.0% (1.01 %- 1.07 %) indicating that the method is robust.

Analysis of Marketed Formulations:

The proposed validated method was applied for the quantification of Dros in two different tablet dosage forms namely Crisanta and Yasmin (Label claim 3 mg), the results of the assay are shown in table 6. The HPLC chromatogram for the representative samples of tablet dosage is shown in Figure 3B-3C. The percentage recovery of the drug was in the range of 99.10 - 100.61%. The assay result revealed that the developed RP-HPLC method was sensitive and specific for the quantitative analysis of Dros in the tablet dosage form. No significant interference was observed from excipients commonly used in the formulation.

Forced degradation study:

Forced degradation studies were performed to evaluate the stability-indicating properties and specificity of the method and to identify the possible degradation products of the drug Dros [15, 16]. The stability of Dros was determined by subjecting it to oxidative, alkaline, acidic, neutral, and photolytic conditions to accelerate conditions favorable to degradation. The results of the degradation studies are displayed in table 7 and the chromatograms for the studies in figures 4A-4E. From the degradation studies it was observed that Dros was stable to neutral and photolytic degradation while it showed slight degradation in acid hydrolysis (0.1N HCl for 30min at 80°C), the percent drug degraded was 3.55 % (Table 7; Figure 4B). In alkaline stress conditions (0.1 N NaOH for 30min at 80°C), the drug sample was found to be very labile, exhibiting percent drug degradation as73.15 %. A new peak at the retention time of about 1.2 min was appeared in the chromatogram (figure no. 4C).In oxidative degradation studies (3 % H_2O_2 for 30 min at 80 ºC), the percent Dros degraded was about 37.45 % (Table no. 7). The chromatogram Figure 4D showed the presence of a new peak at the retention time of about 1.2 minutes.

CONCLUSIONS:

The present stability-indicating method was based on the use of RP-HPLC with UV-spectrophotometric detection as the method for the determination of drosperinone. The RP-HPLC method developed was found to be simple, rapid, sensitive. The analytical conditions and the solvent developed resulted in good resolution and proved to be economical. The method developed was validated as per ICH guidelines for method validation defined in ICH

Q2A/B. The lower values of % RSD indicate the method is precise and accurate. From the forced degradation studies, it can be concluded that the drug was labile for alkaline hydrolysis (73.16 %) and oxidative degradation (37.45%) and the method is specific for the estimation of Dros in presence of its degradation products and impurities. The simplicity of the method allows its use in quality control laboratories for routine analysis of drospirenone.

Authors' Statements

Competing Interests

The authors declare no conflict of interest in the publication of the paper.

ACKNOWLEDGMENTS

The authors thank Dr. Padma GM Rao, Dean, RAKCOPS; Dr. S. Gurumadhva Rao, President, RAKMHSU, for their constant encouragement and providing facilities. We also thank Cipla Ltd. (Sikkim, India), for providing the gift sample of standard drospirenone.

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