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A SIMPLE AND RAPID VALIDATED STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF DROSPIRENONE IN A PHARMACEUTICAL PRODUCT

Sirajunisa Talath*, Sunil Dhaneshwar
Department of Pharmaceutical Chemistry, RAK College of Pharmaceutical Sciences, RAK Medical and Health Sciences University, Ras Al Khaimah, UAE.

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ABSTRACT

The main objective of the present research work was to develop and validate a simple reversed-phase high performance liquid chromatography (RP-HPLC) stability-indicating method for the determination of synthetic progestin drospirenone. The chromatographic separation was performed by using the instrument Shimadzo Prominance model L20 HPLC system equipped with SPD 20A prominence UV-Vis detector, RESTEX allure C18 (250mm × 4.6mm i.d., 3 µm particle size) column. Isocratic elution was performed using methanol: water (65:35 v/v) as solvent and UV detection at 247 nm. The RP-HPLC method developed for analysis of drospirenone was validated with respect to specificity, selectivity, linearity, accuracy, precision and robustness as per the ICH guidelines. The linearity for developed method was perceived in the concentration range of 3-18 µg/mL with the correlation coefficient of 1.0. The percentage accuracy of drospirenone ranged from 99.06 to 100.62%. 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 limits. Drospirenone was subjected to stress conditions such as neutral, acidic, alkaline, oxidation and photolysis degradations as per ICH guidelines. The peaks of degradation products were found to be resolved effectively from the standard drug peak and hence this method can be used for quality control assay of drospirenone. The degradation studies revealed that the drug was found to degrade maximum (74.27%) in alkaline degradation conditions followed by oxidative degradation conditions (36.41). The drug was highly resistant towards neutral, acidic and photolytic degradation conditions.

Corresponding author
Dr. Sirajunisa Talath
Department of Pharmaceutical Chemistry,
RAK College of Pharmaceutical Sciences,
RAK Medical & Health Sciences University, Ras Al Khaimah,
PO Box. 11172, United Arab Emirates
restalath@gmail.com, sirajunisa@rakmhsu.ac.ae
+971 7 226 9000; +971 2269 996.

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INTRODUCTION

Forced degradation is a process that involves the degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. The ICH guidelines states that stress testing is intended to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways and to validate the stability indicating procedures used [1]. In the present research work, the aim our study was the development and validation of the stability indicating analytical method for the synthetic progestin drospirenone.

Drospirenone (Dros, Figure 1) is a synthetic progestin which is structurally related to 17α-spirolactone and is most widely used as oral contraceptive [2]. Comparing to its parent compound spirolactone, drospirenone also displays antimineralocorticoid and antiandrogenic activity and is used in menopausal hormonal therapy [3, 4]. The combined oral contraceptive of Dros with ethinylestradiol is available in the formulations containing 20µg ethinylestradiol/3mg drospirenone and 30µg ethinylestradiol/3mg drospirenone [5]. Drospirenone is a fourth generation oral contraceptive and is reported to possess antimineralocorticoid effects that are not present in previous generations of oral contraceptives. Its antimineralocorticoid potency is reported to be approximately eight times greater than spironolactone [6]. This activity enhances sodium, chloride, and water excretion, while reducing the excretion of potassium, ammonium, and phosphate [7].

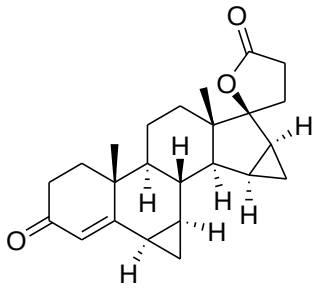


Figure 1: Structure of drospirenone

The literature on the analytical methods used for the quantitative studies of Dros suggests that most widely high performance liquid chromatograph (HPLC) techniques coupled with either ultraviolet (UV) [8-11], radioimmunoassay (RIA) [12, 13] and tandem mass spectrometry (MS/MS) methods [14, 15] have been published for quantification and pharmacokinetic studies of Dros mostly in combination with ethinylestradiol or other drugs in pharmaceutical formulations [9, 14,] and biological fluids [11-15].

From the literature, it is palpable that the available RP-HPLC methods are mainly for analyzing drospirenone with other drug combinations in pharmaceutical dosage forms [8-15]. Therefore, it was felt necessary to develop and validate a simple, precise and rapid RP-HPLC method for the quantitative determination of drospirenone in the presence of its degradation products or other pharmaceutical excipients. The analytical method developed was validated as suggested by ICH guidelines [1, 16]

MATERIALS AND METHODS

REAGENTS AND CHEMICALS:

Methanol and water used were of HPLC grade (Fisher Scientific, UK). Sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2) and hydrochloric acid (HCl) were obtained from Scharlau, Spain. Drospirenone standard (purity 100%) was kindly gifted by Cipla Ltd. (Sikkim, India). All the chemicals procured were of analytical grade and used as received.

HPLC APPARATUS AND CONDITIONS:

Chromatographic separation was achieved by using the instrument Shimadzo Prominance model L20 HPLC system equipped with SPD 20A prominence UV-Vis detector, RESTEX allure C18 (250mm × 4.6mm i.d., 3 µm particle size) column. Isocratic elution was performed using methanol: water (65:35 v/v) as solvent and UV detection at 247 nm. The overall run time of the analysis was generally 10 minutes and the flow rate was 1.0 mL/min. 20 µL of sample was injected into the HPLC system. All the analyses were carried out at room temperature. Results were acquired and processed by Shimadzu LC Solution software.

METHOD DEVELOPMENT

Preparation of the mobile phase:

The mobile phase was prepared by mixing methanol: water (65:35 v/v). The solution was filtered through 0.45µm nylon filter paper after sonication for 15 minutes.

Preparation of Standard Solution:

Standard stock solution of Dros was prepared using methanol to obtain a concentration of 1 mg/mL. The procedure involved accurately weighed 10 mg of Dros standard sample and transferred into a 10 mL volumetric flask, dissolved in 5mL of methanol. The resultant solution was sonicated for 15 minutes to dissolve the drug completely and made the volume up to 10mL with methanol to get the primary stock solution of 1000 µg/mL. Further 1mLof the above stock solution was pipetted out into a 10mL volumetric flask and diluted up to the 10 mL with mobile phase to get the 100 µg/mL. The solution was mixed well and filter through 0.45µm nylon filter paper. The working standard solution (12 µg/mL) was prepared by taking 1.2 mL of 100 µg/mL in 10 mL volumetric flask and diluted up to 10 mL with methanol. The solution was mixed well and filter through 0.45µm nylon filter paper. Aliquots of the suitable 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.

Analytical method validation:

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

System suitability:

The assessment for the suitability of the system was done using six (06) drug replicas at concentration of 12 µg/mL. It was used to confirm that the resolution and reproducibility of the chromatographic system is adequate for the analysis to be done. The method was assessed by analyzing the repeatability, retention time, peak area, capacity factor, tailing factor, theoretical plates of the column. The method was found to be precise and specific. A typical chromatogram of drospirenone is shown in Figure 3A and the results of the analysis are summarized in table 1.

Table 1: System suitability data of Drospirenone (Dros) (n=6).

Sl. No	Retention time (min)	*Mean peak area of Dros	Tailing factor	Number of theoretical plates	Capacity factor
1	6.48	9148.5	1.01	21325	3.28
2	6.49	9152.3	1.03	21274	3.27
3	6.505	9151.2	1.031	21289	3.29
4	6.495	9147.3	0.998	21300	3.32
5	6.493	9149.8	0.989	21288	3.3
6	6.485	9149.7	1	21393	3.29
Mean	6.491	9149.8	1.009667	21311.5	3.29
SD		17.98	0.015944	43.40852	0.017
%RSD		0.2	1.58	0.203693	0.523

*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 is shown in table 2.

Table 2: Linear regression data for Drospirenone (n = 6).

	Statistical Parameters	Values
1	Concentration range	3-18µg/mL
2	Regression equation	-1.9666 + 0.00015x
3	Correlation coefficient	1.00

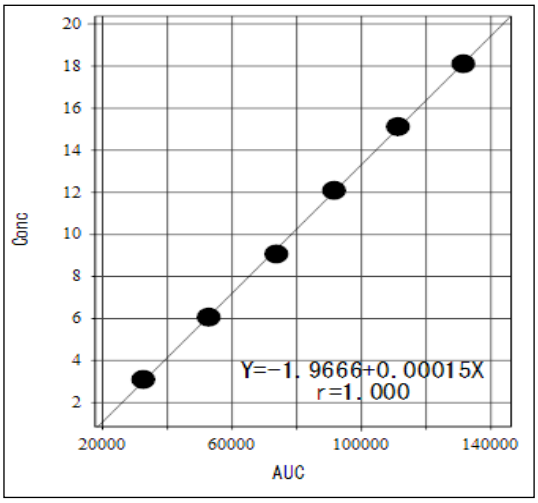


Figure 2: Calibration curve for Drospirenone (concentration range 3-18 µg/mL).

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 the ICH guidelines. The LOD was found to be 0.16 µg/mL and LOQ was to be 0.75 µg/mL.

Precision:

The precision of the analytical method was studies by analyzing multiple sample of 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 by intra-day and intra-day variation studies.

Intra-day precision:

In the intra-day studies, six injections of standard solution of Dros (3-18 µg/mL) were injected into the RP-HPLC system at different time intervals within a day. % RSD was calculated for the each analysis was calculated and summarized in table 3.

Inter-day precision:

In the inter-day studies, six injections of standard solution of Dros (3-18 µg/mL) were injected into the RP-HPLC system at three different days. % RSD was calculated for the each analysis was calculated and summarized in table 3.

Table 3: Results of Intraday and Interday Precision for Drospirenone (n=6).

Concentration	Day 1			Day 3		
(µg/mL)	* Peak area for Dros	SD	RSD (%)	*Peak area for Dros	SD	RSD (%)
3.10	3260.66	45.67	1.40	3321.4	56.59	1.704
6.07	5268.66	36.39	0.691	5274.17	48.803	0.925
9.07	7356.5	73.15	0.994	7365.75	86.210	1.170
12.09	9172.87	49.19	0.536	9149.28	62.707	0.685
15.12	11133.5	91.57	0.822	11147.05	87.114	0.781
18.11	13145	124.09	0.944	13145.31	137.331	1.044

*Mean of six replicates.

Accuracy:

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

Table 4: Results of Accuracy studies for Drospirenone (n=6).

Concentration (µg/mL)	*Peak area for Dros	SD	RSD (%)	*Drug found	%Recovery
6	5239.17	54.67	0.688	5.943	99.06
12	9257.34	47.29	0.511	11.934	99.45
18	13286.73	168.77	1.27	18.112	100.62

*Mean of six replicates.

Robustness:

In order to evaluate the robustness of the method, the influence of small and deliberate variation of analytical parameters on the retention times of drospirenone was studied. The parameters selected were the effect of methanol in the mobile phase composition (63 and 67 %), flow rate (0.8 and 1.2 mL/min) and wavelength (245 and 249 nm). Only one parameter was changed while the others were kept constant. Results of the study are summarized in table 5.

Table 5: Results of Robustness studies for Drospirenone (n=6).

Condition	Modification	*Peak area for Dros	SD	RSD (%)
Mobile phase composition	63:37	9133.65	53.98	0.591
(methanol: water) 65:35	67: 33	9199.17	56.98	0.6194
Flow rate	0.8 mL	9149.38	58.15	0.635
(1mL/min)	1.2 mL	9177.52	63.42	0.691
Wavelength 247nm	245 nm	9148.15	65.15	0.712
	249 nm	9152.83	67.33	0.7356

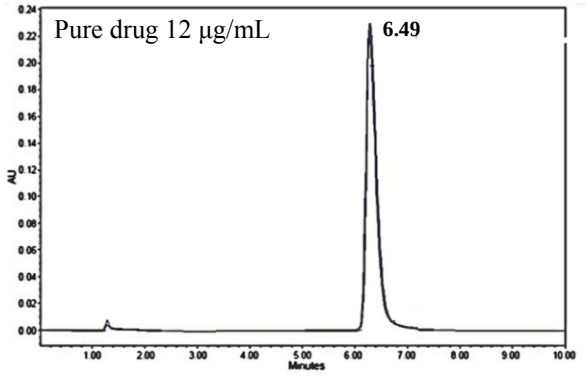
*Mean of six replicates.

Analysis of Marketed Formulations:

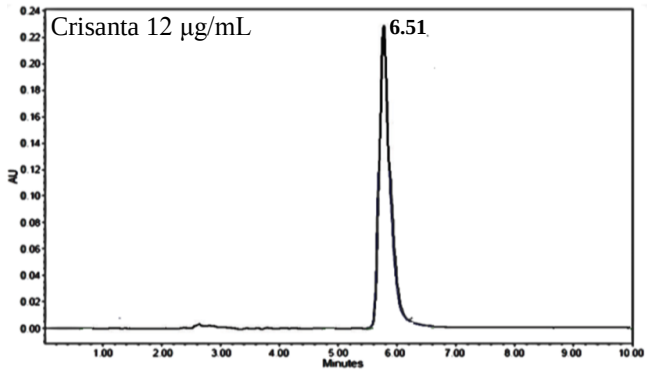
Two different brands of drospirenone tablets (Crisanta and Yasmin; Label claim 3mg drospirenone) were used to determine the drug content. Twenty tablets from respective marketed formulations were accurately weighed; their average weight was determined and finely powdered. An aliquot of powder equivalent to the weight of 10 mg was accurately weighed and transferred into a 10 mL volumetric flask and dissolved completely with methanol. The resulting solution was sonicated for 15 min to enable complete dissolution of Dros and filtered using 0.45µm membrane filter paper. This sample solution had a concentration of 1000 µg/mL. Further 1mL of the above stock solution was pipetted out into a 10mL volumetric flask and diluted up to the 10 mL with mobile phase to get the concentration of 100µg/mL. The working standard solution of 12µg/mL was prepared by diluting 1.2 ml of above stock solution upto 10 mL with methanol. These solutions were filtered through a 0.45 µm membrane filter before injections. The resulting solution was subjected to chromatographic analysis in triplicate. The drug peak area was referred to linear regression equation to get the sample concentration and nominal % of label claim. Chromatograms are shown in the figures: 3B-3C and the percent recovery data is summarized in table 6.

Table 6: Assay Results of the Drospirenone tablets (n=6).

	Tablet brand names	Label claim (mg)	Amount recovered (mg)	% Recovery
1	Crisanta	3	2.979	99.30
2	Yasmin	3	3.085	102.83



A.



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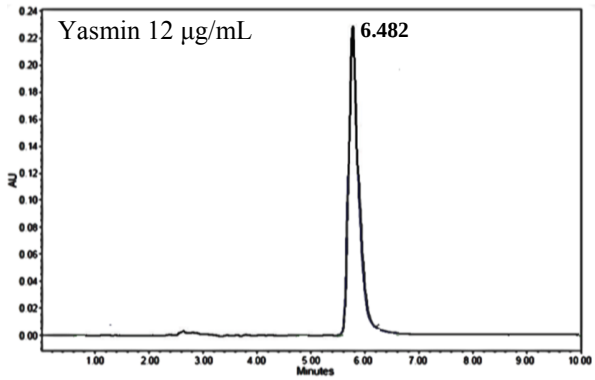

C.

Figure 3: Typical chromatograms of Drospirenone (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 [1, 16]. The stability of Dros was determined by subjecting it to neutral, acidic, alkaline, oxidative and photolytic conditions in order 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 concentration of 12µg/mL were prepared using methanol and filtered before injection. The chromatograms of the study are shown in Figures 4A-4E and percent drug degraded are displayed in the table 7.

Degradation in Neutral Condition:

Dros sample 12µg/mL was treated with methanol for 30 min in a thermostat maintained at 80 ºC. At different time intervals, solutions (20 µl) of the sample was injected into the HPLC system. The chromatogram recorded is presented in Figure 4A.

Acidic degradation:

Acidic degradation was performed by treating the Dros solution (12 µg/mL) with 0.1 N hydrochloric acid for 30 min in a thermostat maintained at 80 ºC, cooled and diluted with mobile phase as per the requirement before injecting in to the HPLC system. The chromatogram recorded is presented in Figure 4B.

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 diluted with mobile phase as per the requirement before injecting in to the HPLC system. The chromatogram recorded is presented in Figure 4C.

Oxidative degradation:

Oxidative degradation was performed by treating 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 in to the HPLC system. The chromatogram recorded is presented in Figure 4D.

Photolytic degradation:

The drug Dros 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 12 µg/mL and 20µL was injected into the HPLC system. The chromatogram recorded is presented in Figure 4E.

Table 7: Results of Stress degradation studies for Drospirenone (n=6).

Sl. No	Stress condition	Mean peak area	% Drug recovered	% Drug degraded
1	Neutral	9167.04	100	0
2	Acidic	9213.56	97.45	2.55
3	Alkaline	9169.74	25.73	74.27
4	Oxidative	9184.92	63.59	36.41
5	Photolytic	9179.28	99.67	0.33

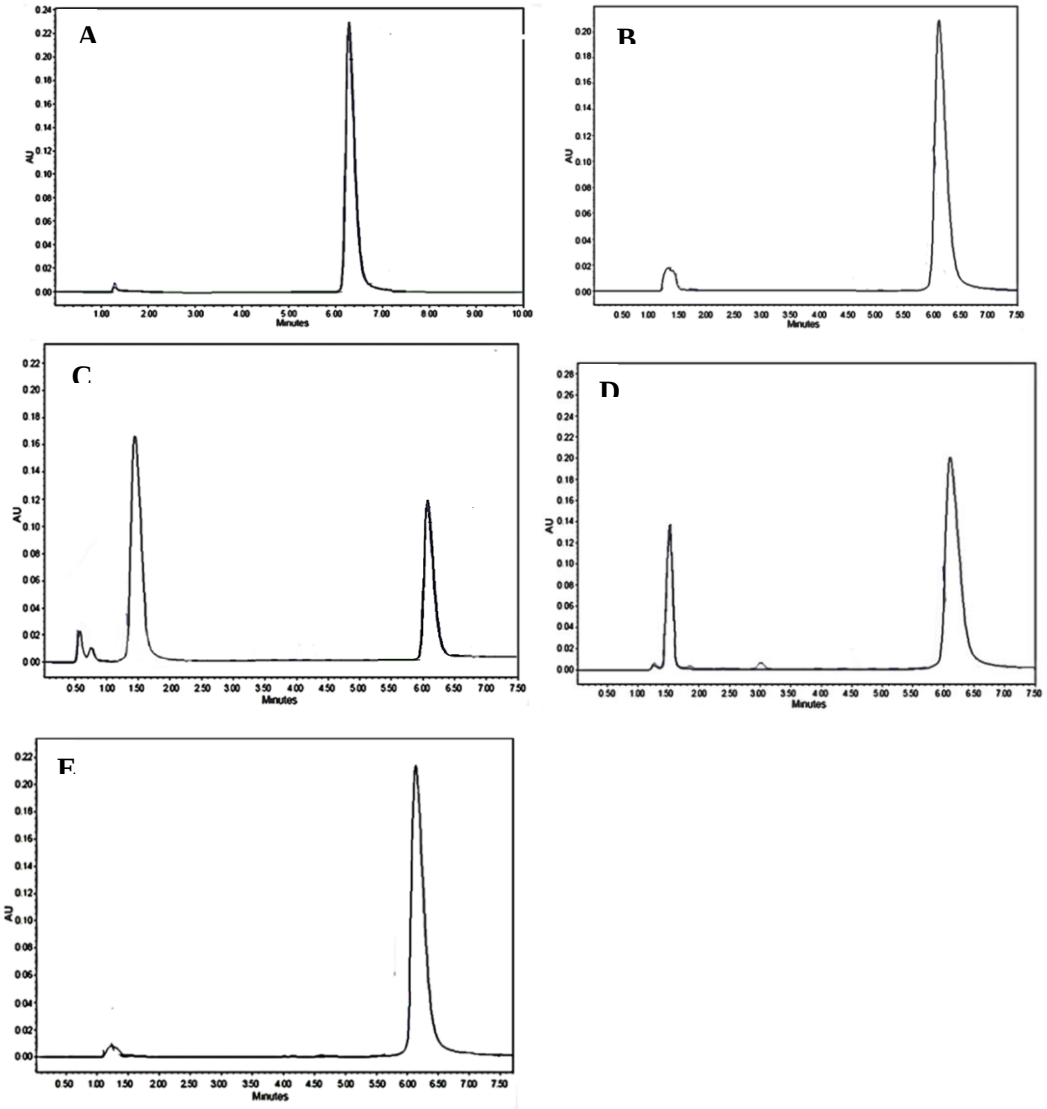

Figure 4: **HPLC chromatogram of Drospirenone (12µg/mL) after exposure to: (A) Neutral degradation; (B) Acid hydrolysis (0.1N hydrochloric acid for 30 min 80 ºC); (C) alkaline hydrolysis (0.1N sodium hydroxide for 30 min 80 ºC); (D) Oxidative degradation (3% H_2O_2 for 30 min in a thermostat maintained at 80 ºC); (E) photolytic degradation.**

RESULTS AND DISCUSSION

The RP-HPLC method development and validation to analyze and quantify Drospirenone was performed according to the specification parameters described in International Conference on Harmonization (ICH-1996). [1, 16, 17]

HPLC method development and optimization:

The aim of the present work was at developing a simple, precise and accurate stability-indicating RP-HPLC method to estimate drospirenone in the tablet dosage form. Numerous trails were performed for apposite selection of column and mobile phase for the HPLC method development. Based on the trail data, the most appropriate column selected was the RESTEX allure C18 column (250mm × 4.6mm i.d., 3 µm particle size). The mobile phase used was methanol: water (65:35 v/v) and the wavelength 247nm as the drug exhibited good absorbance at this wavelength. The flowrate of 1mL/min, overall run time 10 minutes and injection volume 20µl was used. The retention time of Dros was found to be 6.49 minutes. A typical chromatogram of the Dros is shown in the Figure 3A. The various parameters considered for HPLC method validation in the present study was system suitability, specificity, range and linearity, limit of detection, limit of quantification, accuracy, precision, ruggedness and robustness. Replicate (n=6) injections were made to ensure reproducibility and accuracy of measurements.

Method validation:

System suitability:

The assessment for the suitability of the system was carried out using six (06) replicas of the drug Dros at concentration of 12µg/mL. It was used to confirm that the resolution and reproducibility of the chromatographic system is adequate for the analysis to be performed. The parameters measured were repeatability, retention time, peak area, capacity factor, 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 of retention time was 6.491 minutes and the %RSD of peak area was 0.20%. The values for system suitability parameters demonstrated feasibility of this method for routine pharmaceutical application. The results of system suitability tests are shown in the table 1 and the chromatogram is shown in Figure 3A.

Linearity:

The calibration curve for Dros with good linearity was obtained over the concentration range 3-18 µg/mL. The corresponding linear regression equation was y = -1.9666 + 0.00015x and the correlation coefficient for calibration curve was 1.00 (Figure 2 and Table 2). Good linearity was found between the peak area and analyte concentration. The typical HPLC chromatograms for Dros acquired from the standard solution and various tablet formulations are displayed in the Figures 3A-3C.

Sensitivity:

LOD is the ability of 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 0.16 µg/mL and 0.75 µg/mL, respectively.

Precision:

The precision of the proposed method was determined by performing standard solution assay on same day (intra-day) and on 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 their RSD (%). For day 1 (one) precision studies, the RSD (%) values for the five different concentrations of Dros was observed in the range of 0.536–1.40 while for day 3 (three) precision studies the RSD (%) range was 0.685-1.704. 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:

In order to ascertain the accuracy of the proposed HPLC method, recovery studies were carried out by adding known amounts of Dros corresponding to three concentration levels: 50%, 100%, and 150% of the label claim and the results of the recovery studies are displayed table 4. Percent RSD for Dros was found to be in the range 0.511-1.27% and the percentage recovery was 99.06-100.62%. The results of the recovery test indicate that the method is highly accurate.

Robustness:

The robustness of the analytical method was determined by the consistency of the peak height and peak shape with the deliberate small changes made in the experimental conditions. It is a measure of its capacity to retain unaffected by small, but deliberate variations in method conditions and provides an indication of its reliability during normal usage [17]. To determine the robustness of the proposed method, the following variations were made in the analytical method developed: percentage of methanol in the mobile phase (63% and 67%), wavelength (245 and 247 nm), flow rate (0.8 and 1.2 ml/min). The results obtained (Table 5) from assay of the test solutions were not affected by varying the conditions and were in accordance with the results for original conditions. The % RSD value of assay determined for the same sample under original conditions and robustness conditions was less than 2.0% (0.59%-0.73%) 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 (Crisanta and Yasmin, Label claim 3 mg), the results of the assay is shown in table 6. The HPLC chromatogram for the representative samples of tablet dosage are shown in Figure 3B-3C. The percentage recovery of the drug was in the range 99.30% - 102.83%. The assay result showed that this 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 [1, 16]. The stability of Dros was determined by subjecting it to neutral, acidic, alkaline, oxidative and photolytic conditions in order to accelerate conditions favorable to degradation. The results of the degradation studies are displayed in the table 7 and the chromatograms for the studies in the 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 2.55% (Table 7; Figure 4B). In alkaline stress conditions, the drug sample Dros was found to be very labile. The percent drug degradation observed after exposure to 0.1 M NaOH for 30 min at 80°C was 74.27 %. A new peak at the retention time of about 1.65 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 36.41% (Table no. 7). The chromatogram Figure 4D, showed the presence of a new peak at the retention time of about 1.56 minutes.

CONCLUSIONS

The present stability indicating method was based on the use of RP-HPLC with UV- spectrophotometric detection and was best suited for the determination of Drospirenone. The method developed was found to be simple, rapid, sensitive and economic. 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 (74.27%) and oxidative degradation (36.41%) and the method is specific for the estimation of Drospirenone 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 in pharmaceutical formulations.

Authors' Statements

Competing Interests

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

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