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Research Article

Method Development and Validation of Drospirenone in Bulk and Pharmaceutical Dosage Form by Stability Indicating RP-HPLC Method Studies.

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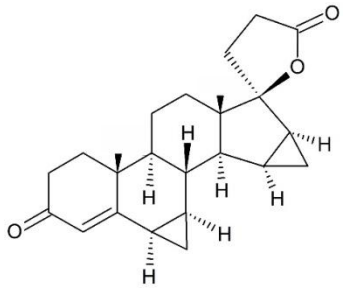
ABSTRACT
To assess the stability of Drospirenone under stress conditions, it was subjected to acidic, alkaline, oxidative, thermal and light degradation according to ICH guideline Q1A (R2). The analysis was carried out on C18 Thermo Hypersil BDS (250×4.6×5 mm) column, using Ammonium acetate: Acetonitrile (70:30) pH6.8-7.2 as mobile phase with flow rate 1ml/min and analysis was performed using PDA detector at ambient temperature where 3.15min was retention time of the drug .The Linearity, precision and accuracy was found to be acceptable over the concentration range of 10 to 60 ug/ml of Drospirenone. The correlation coefficient was 0.987. Drospirenone was found to be more sensitive to alkaline hydrolysis and somewhat stable to acidic degradation. The peaks of degraded products were resolved from the pure drug with significant variation in their retention time values. The method was effectively applied to the determination of Drospirenone with decomposed products in Quality control laboratories.

KEYWORDS
Drospirenone, degradation, stress conditions.

1. INTRODUCTION



Chemically Drospirenone is 6β, 7β, 15β, 16β-dimethylene-3-oxo-17α-pregn-4-ene-21,17carbolactone. Drospirenone is a synthetic progestin that is an analog to spironolactone. It is present in number of birth control formulations. Drospirenone be different from other synthetic progestins as its pharmacological outline in preclinical studies shows it to be closer to the natural progesterone. As such Drospirenone has anti-mineralocorticoid properties, counteracts the estrogen–stimulated activity of the rennin–angiotensin-aldosterone system, and is not androgenic[1].Stability testing is done primarily to provide the evidence that the drug substance or the drug product maintains its essential features of quality, identity, purity and strength (within acceptable ranges) throughout the time in which, it is expected to remain safe for further processing or human consumption. Study of Stressed Degradation support for the identification of feasible degradants, the inherent stability of the drug molecules, possible degradation pathways and stability indicated analytical procedure validation. Understanding the stability of the molecule support to select the appropriate formulation and package and provide standard storage and shelf life conditions, which is essential for regulatory documentation[2-5].

The literature on the analytical methods used for the estimation of Drospirenone suggests that most widely high performance liquid chromatography (HPLC) techniques have been published for quantification and pharmacokinetic studies of Drospirenone mostly in combination with ethinylestradiol or other drugs in pharmaceutical formulations and biological fluids[6-9]. In the present study, attempts were made to develop a simple, accurate and precise method for estimation of Drospirenone in the presence of its degradation products. This manuscript outline the development and validation of the stability indicated isocratic RP-HPLC method for the determination of Drospirenone in the presence of degradation products according to the ICH guidelines.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Drospirenone was supplied as a gift sample by Swapnroop Drugs and Pharmaceuticals, Aurangabad, Maharashtra, India. Methanol, Acetonitrile, Ammonium acetate, double distilled water was purchased from Merk, India. All other chemicals and materials used are of analytical grade.

2.2. Apparatus and Instruments

Table 1. Apparatus and Instruments used for Method.

Sr. No.	Name	Model	Manufacturer
1	HPLC	2080	Thermo
	Detector	PDA	Thermo
	Gradient	Quaternary	NA
2	UV-Visible Double Beam Spectrophotometer	3200	Labindia
3	Sonicator	Thermostatic	Labindia
4	Weighing Balance	1mg	Shimadzu

2.3. Selection of chromatographic conditions

Selection of HPLC method is depends upon the character of the sample, its molecular weight and solubility. The chromatographic variables such as mobile phase, flow rate and solvent ratio were studied. The resultant chromatograms were recorded and the chromatographic parameters such as asymmetry, selectivity and sensitivity were selected for estimation.

2.4. Optimization of chromatographic parameters

Optimization in HPLC is the process of finding a set of conditions that adequately analyze the quantification of the analyte with acceptable accuracy, precision, sensitivity, specificity, cost, ease, and speed of analysis.

Table 2. Parameters used for method development.

Parameters	Description
Column C18	Thermo Hypersil BDS (250×4.6×5 mm)
Mobile phase	Ammonium acetate: Acetonitrile (70:30) pH 6.8-7.2
Injection volume	20µl
Flow rate	1 ml/min
Detector	PDA
Wavelength	220nm
Column Temperature	40°C
Auto Sampler Temperature	25°C
Run Time	15 min

2.5. Optimization of mobile phase

For selection of mobile phase, several modifications including change in compositions of mobile phase and column temperature modification were tried but the resolution was not found to be satisfactory. Finally, mobile phase contain Ammonium acetate: Acetonitrile (70:30) pH 6.8-7.2 found to give best resolution. Previous to analysis; the mobile phase was filtered through a 0.45µ nylon filter, and then degassed ultrasonically for 15 min.

2.6. Selection of internal standard

After observing retention behavior of several drugs Estradiol was selected as an internal standard. It was found to give good resolution, accuracy and precision of quantitative results.

2.7. Preparation of standard drug solutions

Accurately 0.48mg/ml Drospirenone solution in methanol was prepared and sonicate till achieve complete solubility of Drospirenone. Further pipette out 5ml (2.4mg/ml) of stock solution of Drospirenone (0.24mg/ml).Appropriate aliquots of the standard stock solution were transferred into series of volumetric flask for further dilutions of 10, 20, 30, 40, 50, 60 mcg/ml.

2.8. Preparation of sample drug solution

Tablets of marketed sample (Crisanta from Cipla,3mg/tablet) was transfer into a 50 ml of volumetric flask; 25 ml of methanol was added, sonicate it for 10 minutes for getting 100% solubility and made up volume with methanol (stock solution). Used this solution for further readings.

2.9. Chromatographic Run

Stock standard solutions of above dilutions were filled in Auto sampler unit of Thermo RP-HPLC after optimization.

2.10. Relative recovery

The relative recovery was calculated using drospirenone samples from the pharmaceutical formulation and spiked with standard drospirenone solution and internal standard. The assay method was implemented as per routine procedure. Relative recovery was calculated by comparing standard assay value.

2.11. System suitability parameters

System suitability parameters were analyzed on freshly prepared standard stock solution of Drospirenone the drug was injected into the chromatographic system under the optimization of chromatographic conditions. Parameters that were studied to evaluate the suitability of the system were,

1. Number of theoretical plates.
2. Calibration curve.
3. Tailing Factor.
4. Resolution Factor.
5. Retention Time.
6. Selectivity.

2.12. Linearity

The calibration curve with six concentration points for Drospirenone was sufficiently linear in the concentration range between 10-60 ug/ml. The linear least-square regression equation was y= 8456.x+48894 with correlation coefficient 0.987.

2.13. Stress degradation

2.13.1. Preparation of Solutions

Standard Solutions of Base, Acid, Peroxide solutions were prepared in the following manner.

2.13.1.1. Preparation of 1N Sodium Hydroxide

Sodium hydroxide (NaOH) 20gm was dissolved in distilled water and made up to 100ml to get 5N NaOH solution.1N solution was prepared by taking 20ml from 5N NaOH solution and diluting up to 100ml.

2.13.2. Preparation of 1N Hydrochloric acid

Concentrated Hydrochloric acid (HCl) 8.50ml was pipette out and dilute with distilled water up to 100ml, to get 5N HCl solution. From 5N solution, 1N HCl was prepared by pipetting out 20 ml and diluted up to100ml.

2.13.3. Preparation of 3% Hydrogen peroxide

Hydrogen peroxide solution (30%w/v H2O2) 10ml was pipette out and the volume made upto 100ml to get 3% hydrogen peroxide solution.

2.14. Forced degradation conditions

2.14.1. Sample Preparations

Forced degradation studies of the Drospirenone were conducted as per ICH guidelines. For every stressed condition of the drug, four samples were generated.

1. Blank solutions stored in normal conditions.
2. Blank subjected to Stressed conditions in the same manner as like that of the drug.
3. Zero time sample containing drug solution.
4. Drug solutions were subjected to stress treatment.

The drug was stressed to maximum condition where degradation of 5-20% occurred. The drug was declared as stable, if no degradation occurred after 30 days of stress conditions.

2.15. Acidic and Alkaline Degradation

The initial concentration of Drospirenone at 480µg/ml was used for all stress degradation studies. One ml of standard stock solution of Drospirenone was mix with 1 ml of 0.1 M HCl, 1 M HCl, 0.1 M NaOH, 1 M NaOH, and 0.01 M NaOH in 10 ml volumetric flask. Samples were allowed to keep at 80ºC for 30 minutes to observe the degradation of Drospirenone. The resulted samples were neutralized by suitable amounts of NaOH or HCl and injected to the HPLC system subsequent to dilution by mobile phase.

2.16. Oxidative degradation

The oxidative degradation was performed by mixing 2 ml of standard Drospirenone solution and 8 ml of 1% or3% hydrogen peroxide were transferred to a 10 ml volumetric flask and kept at room temperature or 80ºC.

2.17. Thermal and Light Degradation

Drospirenone was spread into a thin layer watch glass and it was exposed to light (visible and UV) and heat 80ºCand for 5 days.

A standard stock solution of Drospirenone was collected (0.48µg/ml) and chromatographed.

Along with Drospirenone degradation in every degradation conditions was determined. HPLC chromatogram of acid, base, photolytic, thermal, oxidative degradation mixture were represented in the Fig.5

3. RESULTS AND DISCUSSION

3.1. Preparation of mobile phase

After trial of several mobile phases, mobile phase was prepared by mixing ammonium acetate: acetonitrile in the ratio of (70:30) pH 6.8-7.2 and was filtered and degassed.

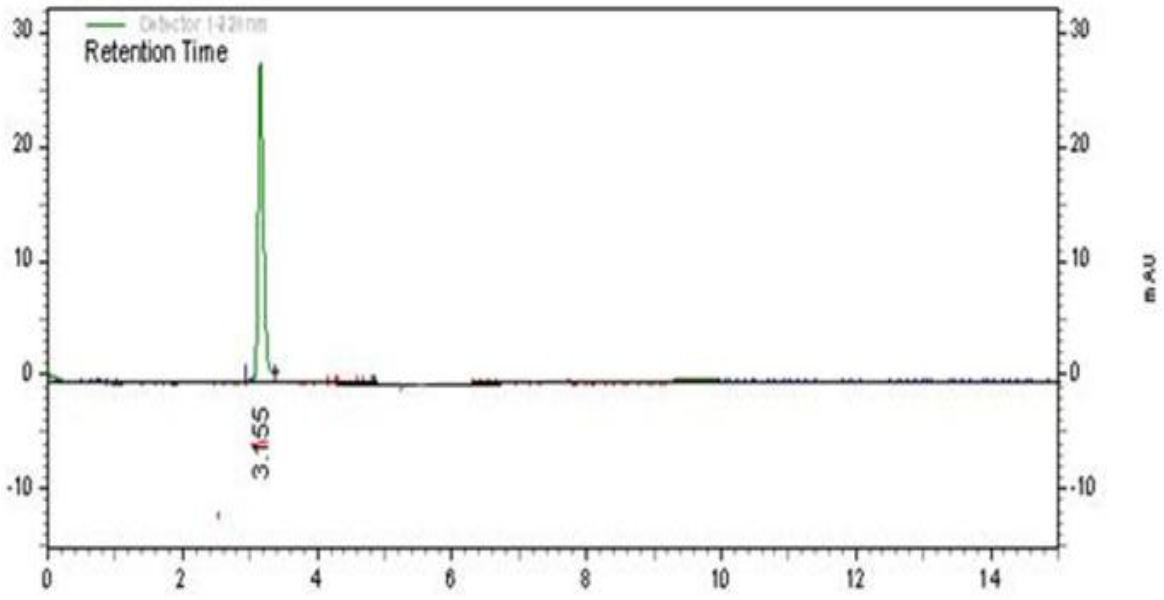


Figure 1. Chromatograph of Drospirenone.

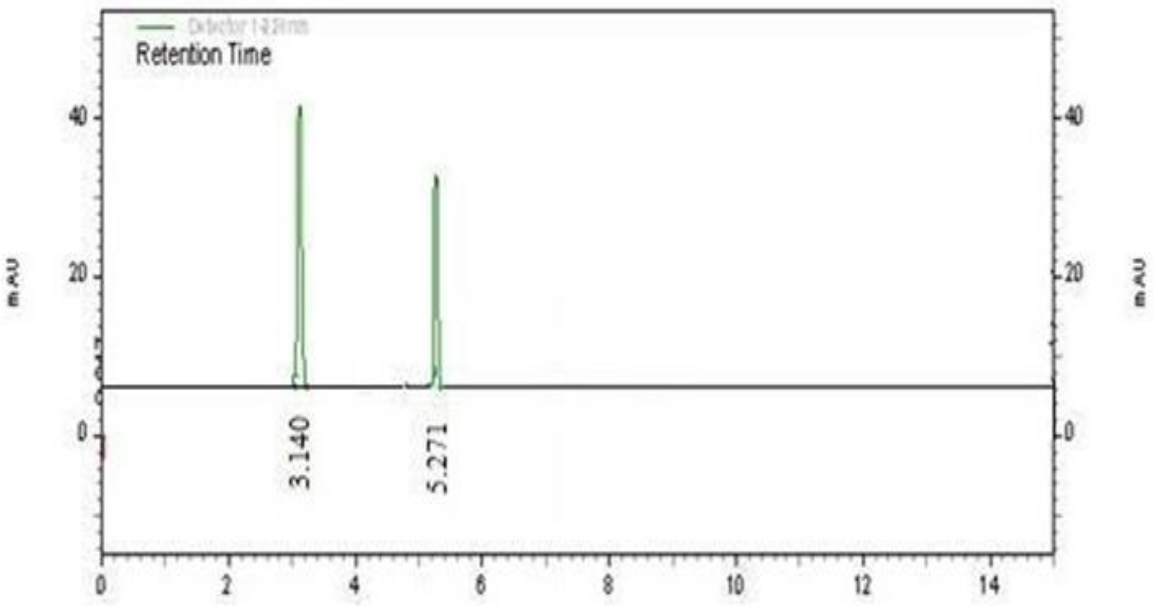


Figure 2. Chromatograph of Drospirenone with internal standard Estradiol.

Table 3. Linearity study of Drospirenone in physical mixture.

Sr. No.	Concentration (µg ml^{-1})	Peak Area
1.	10	171936
2.	20	193864
3.	30	278413
4.	40	374103
5.	50	474601
6	60	576311

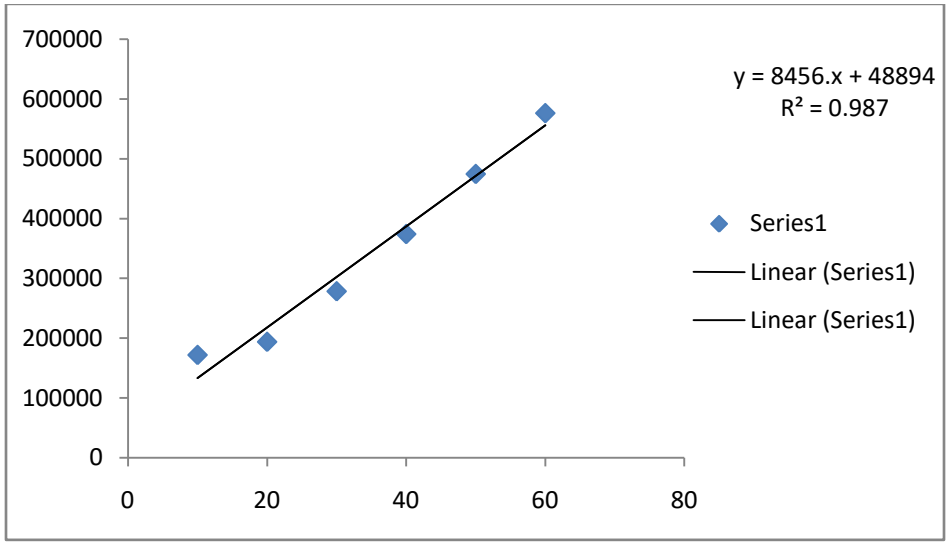


Figure 3. Calibration curve for Drospirenone.

Table 4. Statistical data of calibration curves of drospirenone (n=6) Parameters.

Parameters	Results
Linearity range	10-60 µg/ml
Regression equation	Y= 8456.x+48894
Standard deviation of slope	2.250
Relative standard deviation of slope (%)	0.1139
Standard deviation of intercept	2.250
Correlation coefficient (r2)	0.987
Limit of quantification (LOQ)	0.024 µg/ml
Limit of detection (LOD)	0.0008 µg/ml

3.2. Accuracy and precision

The CV values for the within-day and between-day were less than 1.4% (Standard as per ICH) which confirms proposed method is precise. There is no change after day difference.

Table 5. Precision and accuracy of the method for determination of Drospirenone.

(3 sets for 3 days) Concentration added (µg/ml)	**Concentration found (µg/ml)**	**CV (%)**	**Error (%)**
Within day (n = 3)			
10.00	9.976±0.4	0.00029	-0.9996
30.00	29.976±0.42	0.00035	-0.9996
60.00	59.97±0.21	0.00017	-0.9998
Between day (n = 3)			
10.00	9.976±0.04	0.00029	-0.9996
30.00	29.976±0.31	0.00035	-0.9996
60.00	59.97±0.48	0.00017	-0.9998

3.3. Robustness

Robustness of the proposed method was performed by deliberately changing chromatographic conditions, the effect of pH variation (±0.2) and also mobile phase composition (±2 ml) was studied on chromatographic parameters. The variations did not have significant effect on chromatographic resolution

3.4. Analysis of pharmaceutical product

The proposed HPLC method was applied for the determination of Drospirenone in tablets. Drospirenone amount was shown to be 3.05±0.01 mg which is in agreement with the labeled amount (3.00 mg).

3.5. Recovery

The % recovery was calculated with standard addition method. The acquire recovery was 100.5±0.5 as well as no interferences were observed due to excipients at the retention time of Drospirenone.

Table 6. System suitability parameters.

Parameters	**Found**	**Acceptable limits**
USP theoretical plates (n = 6)	2105	N>1500
USP tailing factor (n = 6)	1.31	T<1.5
Repeatability (tR) (n = 6)	0.39	RSD<1%
Repeatability (peak area)(n = 6)	0.96	RSD<1%

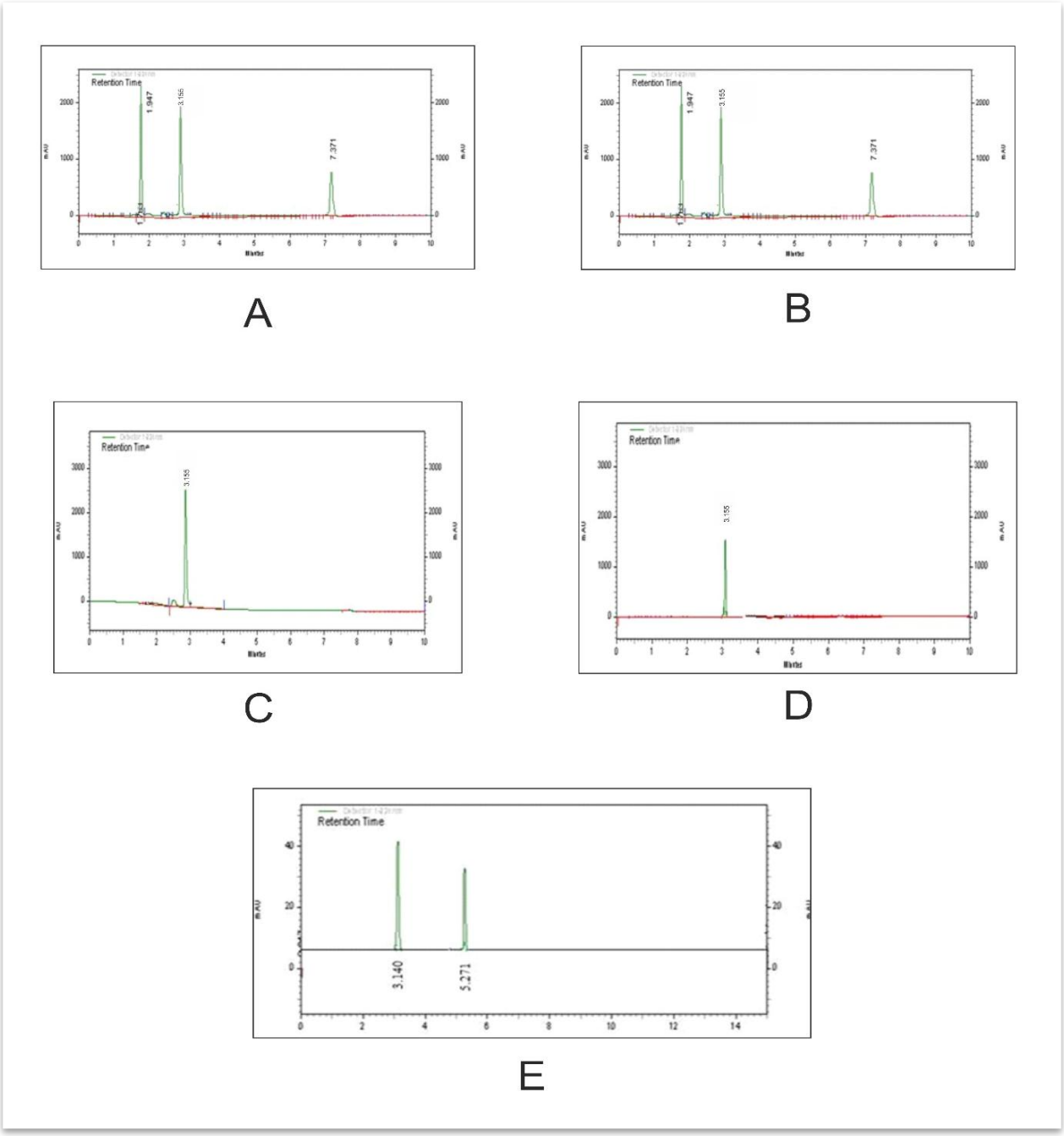


Figure 2. Chromatograph of Drospirenone with A. Base Hydrolysis B. Acid hydrolysis mixture C. Photolytic degradation D. Thermal degradation E. Oxidation degradation.

Table 7. The results of the stress degradation tests on Drospirenone bulk powder using different conditions.

Stress test condition	Solvent	Temperature	Time	% of Drospirenone	% of degradation product

Acidic	1 M HCl	Room Temperature	30 min	80.1	19.9
	1 M HCl	80^0	30 min	25.5	74.5
	0.1 M HCl	80^0	1 h	65.4	34.6
Basic	1 M NaOH	Room temperature	30 min	21.5	78.5
	0.01 M NaOH	Room temperature	15 min	45.0	55.0
Oxidative	1% H_2O_2	80ºC	1 h	80.3	19.7
	3% H_2O_2	Room temperature	30 min	93.8	6.2
	3% H_2O_2	80ºC	30 min	20.0	80.0
Photolytic					
UV light	Solid form	Room temperature	5 days	100.0	0
Visible light	Solid form	Room temperature	5 days	99.9	0.1
Heat	Solid form	90ºC	5 days	99.5	0.5
		70ºC	5 days	99.5	0.5
		60ºC	5 days	99.5	0.5

In acidic conditions drug start degraded in 1M HCL after 30min up to 20%, which has been calculated by observing HPLC behavior of sample injected. Basic conditions show maximum degradation after analysis of HPLC peaks which was up to 78.5% least value. Drospirenone was found to degrade in 1% H2O2 to an extent of 19% after 1h. More degradation was observed by using 3% H2O2 at room temperature or 80ºC.Minimal degradation of drug substance was observed in photolytic and heat conditions.

4. CONCLUSION

The present stability indicating method was based on the use of RP-HPLC with PDA detection and best suited for the determination of Drospirenone. The proposed method is simple, fast, accurate and precise. The method developed was validated as per ICH guidelines as per ICH Q2A/B. The lesser values of % RSD indicate the method be precise and accurate. From the forced degradation studies it can be concluded that the drug was labile for alkaline and acidic hydrolysis. Minimal degradation of drug substance was observed in photolytic and heat conditions. The proposed method is specific for the estimation of Drospirenone in presence of its degradation products and impurities and able to be applied for routine analysis of Drospirenone in laboratories of Quality control.

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