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STRUCTURE ELUCIDATION OF OXIDATIVE DEGRADATION PRODUCT OF DROSPIRENONE

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Keywords:

Drospirenone, Stress conditions, Oxidative degradation, LC

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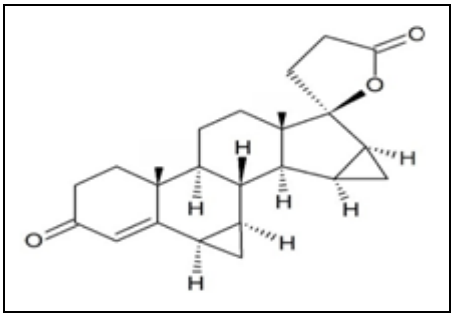
ABSTRACT: Stressed degradation study of Drospirenone in H_2O_2 and characterization of degradants by IR, NMR, LC-MS was done. Stressed degradation study of Drospirenone in H_2O_2 and characterization of degradants by IR, NMR, and LC-MS was done. To evaluate the stability of Drospirenone under stress conditions, it was subjected to oxidative 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) pH 6.8 as mobile phase with flow rate 1ml/min and analysis was done using PDA detector at an ambient temperature where 3.15 min was retention time of the drug. The Linearity, precision, and accuracy were found to be satisfactory over the concentration range of 10 to 60 µg/ml of the drug. The correlation coefficient was 0.987. Drospirenone was found to degrade in 1% H_2O_2 to the extent of 19% after 1 h. More degradation was observed by using 3% H_2O_2 at 80 ºC. Interestingly, in the applied conditions, the new compound was found out in a significant amount with oxidative stress conditions. FT-IR, NMR, LC-MS data demonstrated that the oxidative stressed impurity of Drospirenone (Biphenyl moiety) is reported. The method was effectively applied to the determination of Drospirenone with decomposed products in quality control laboratories.

INTRODUCTION: Drospirenone is 6β, 7β, 15β, 16β- dimethylene- 3- oxo- 17α-pregn-4-ene-21, 17 carbolactone. 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

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analytical procedure validation. It understands 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 ethinyl estradiol or other drugs in pharmaceutical formulations and biological fluids [6-13]. In the present study, attempts were made to develop a simple, accurate, and precise method for estimation of Drospirenone in the presence of its oxidative degradation products. This manuscript outlines the identification of Drospirenone transformation products under oxidative stress conditions through IR, NMR, and LC-MS characterization.

MATERIALS AND METHODS:

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.

Apparatus and Instruments: An HPLC system Thermo (2080), autosampler and PDA detector (Thermo) was used for chromatographic separation. UV-Visible Double Beam Spectrophotometer Lab India (3200) was used. Sonicator of Thermostatic Lab India, Weighing balance of Shimadzu was used.

Selection of Chromatographic Conditions: The selection of the HPLC method 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.

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 1: 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

Optimization of Mobile Phase: For the selection of the 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, the mobile phase contains ammonium acetate: Acetonitrile (70:30) pH 6.8-7.2 found to give the best resolution. Previous to analysis; the mobile phase was filtered through a 0.45µ nylon filter, and then degassed ultrasonically for 15 min.

Selection of Internal Standard: After observing the 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.

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

Preparation of Sample Drug Solution: Tablets of the marketed sample (Crisanta from Cipla, 3 mg/tablet) was transfer into a 50 ml of the 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). Stock solution was used for further readings.

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

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.

Stress Degradation:

Sample Preparations: Forced degradation study of the Drospirenone was conducted as per ICH guidelines. For 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.

Oxidative Degradation: The oxidative degradation was performed by mixing 2 ml of standard Drospirenone solution and 8 ml of 1% or 3% hydrogen peroxide were transferred to a 10 ml volumetric flask and kept at room temperature or 80 ºC.

Postulation Profile of Forced Degradation Products of Drospirenone: None of the degradation product could be isolated from the reaction solutions by solvent extraction or crystallisation**.**

Hence, key stress oxidative solutions were subjected to LC-MS analysis for profiling and identification of the degradation products. LC-MS was utilised to obtain the molecular weight and fragmentation information using ESI interface in the positive ion mode.

Complete postulation of probable degradation products that can be obtained for Drospirenone was done as preliminary step, to understand the degradation behaviour of the drug molecule. Scheme of pathways followed by the drug to understand the liability under various stress conditions was obtained.

The key stress samples were treated to remove mobile phase matrices and other interferences before LC-MS analysis.

Composition of LC-MS unstressed drug spectrum with spectral scan of key stress solutions will lead to profiling the impurities and degradation products that may have formed during stress.

Sample Preparation for LC-MS Studies: 3% of H_2O_2 was added to a solution of Drospirenone (500ug/ml standard Drospirenone in methanol) in proportion of 1:1. The reaction mixture was kept in dark to avoid photo- oxidation effects and maintained at room temperature. After the reaction the solution was heated carefully for 10 min in water bath to eliminate excess of peroxide and arrest further degradation. The oxidative key stress solution was cooled and later diluted and assessed for LC-MS study.

RESULTS AND DISCUSSION:

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.

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 **Fig. 1, 2** and **Table 1**.

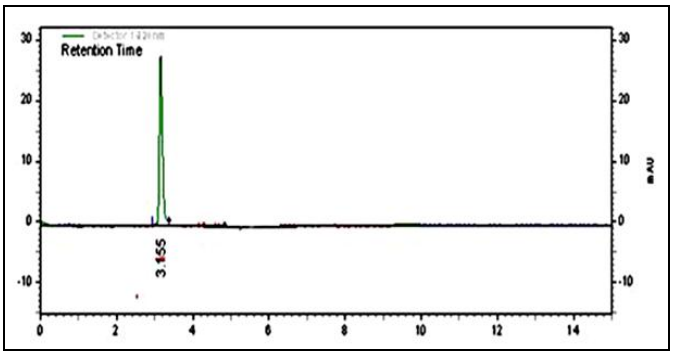

FIG. 1: CHROMATOGRAM OF DROSPIRENONE

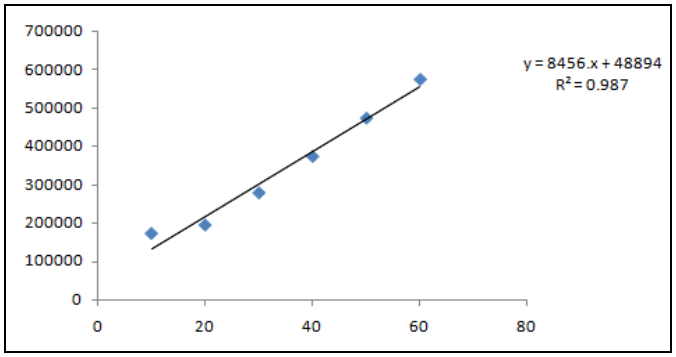

FIG. 2: CALIBRATION CURVE FOR DROSPIRENONE

TABLE 1: 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

System Suitability Parameters: System suitability parameters were analyzed on the freshly prepared standard stock solution of Drospirenone; the drug was injected into the chromatographic system under the optimization of chromatographic conditions **Table 2**.

Accuracy and Precision: Precision of the assay was determined in relation to repeatability (intraday) and intermediate precision (Interday) at three levels of concentration. The CV values for the within-day and between-day were less than 1.4% (Standard as per ICH), which confirms the proposed method is precise. There is no change after a day difference.

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 a significant effect on the chromatographic resolution

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).

Recovery: The % recovery was calculated with a 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 2: 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%

Oxidative Degradation: Drospirenone was found to degrade in 1% H_2O_2 to the extent of 19% after 1 h. More degradation was observed by using 3% H_2O_2 at room temperature or 80 ºC **Fig. 3** and **Table 3**.

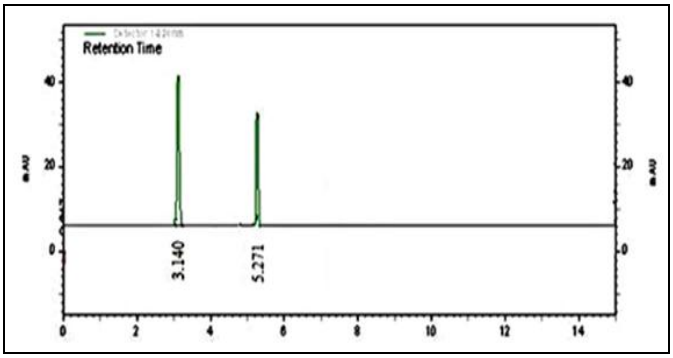

FIG. 3: CHROMATOGRAM DROSPIRENONE IN OXIDATIVE STRESS CONDITION

TABLE 3: THE RESULTS OF THE STRESS DEGRADATION TESTS ON DROSPIRENONE USING OXIDATIVE STRESS CONDITIONS

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

Characterization of Degradation Product by Mass Spectrometry, IR, and NMR Spectroscopy: Fig. 4, 5, 6, 7 and **Table 4**.

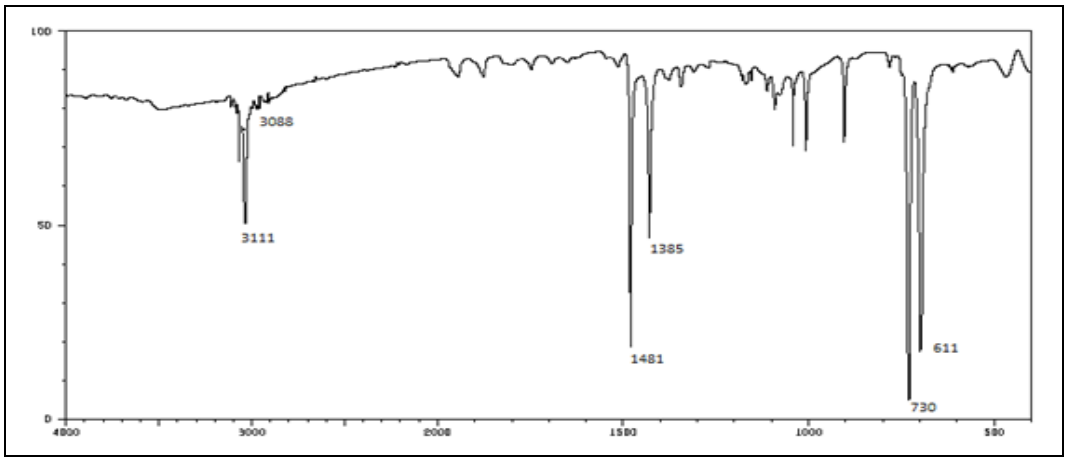

FIG. 4: REPRESENTATION OF FTIR SPECTRUM OF DEGRADATION PRODUCT

TABLE 4: INTERPRETATION OF IR

S. no.	Lit. Value (cm^{-1})	Obs. Value (cm^{-1})	Indication
1	3000-3700	3111 cm^{-1}	-CH (S) Stretch
2	2700-3300	3088 cm^{-1}	-CH (w) Stretch
3	1200-1500	1481 cm^{-1}	-C=C- (S) Bending
4	1200-1500	1385 cm^{-1}	-C-C- (w) Bending
5	Fingerprint Region	730 cm^{-1}, 611cm^{-1}	-C-H- (S) Stretch,

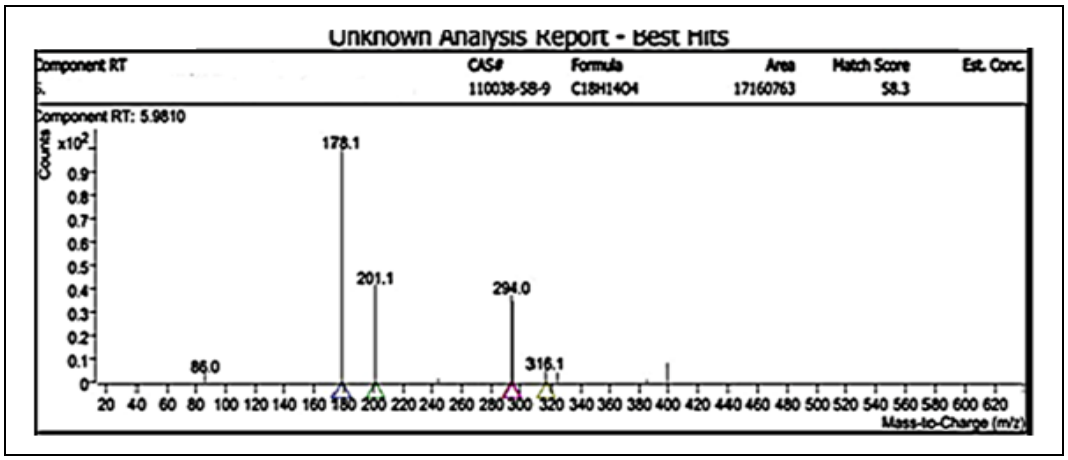

FIG. 5: REPRESENTATION OF LC-MS SPECTRUM OF DEGRADATION PRODUCT

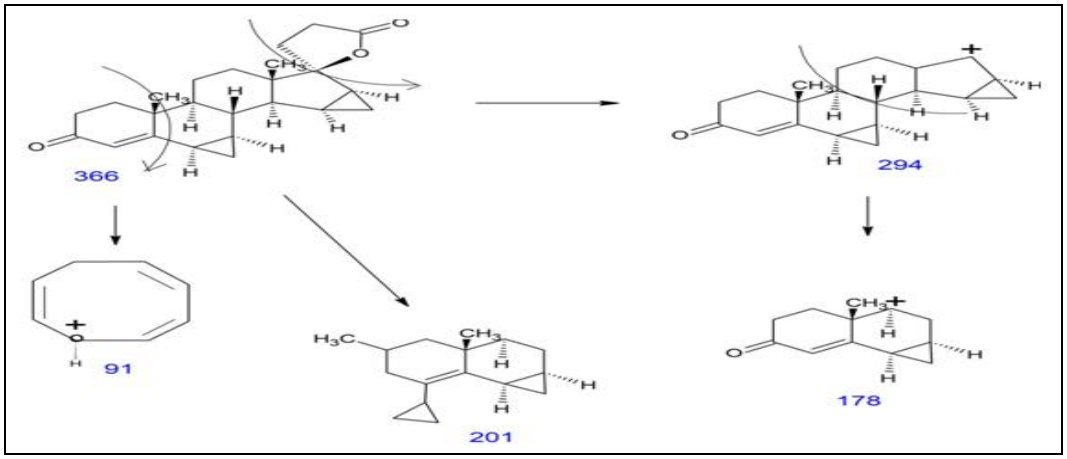

FIG. 6: POSTULATED PATHWAYS OF DEGRADATION OF DROSPIRENONE

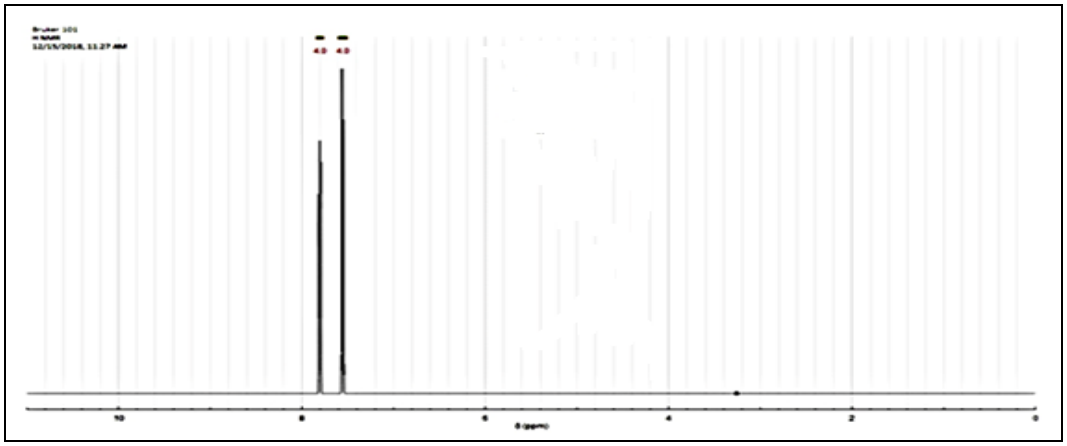

FIG. 7: REPRESENTATION OF H1 NMR SPECTRUM OF DROSPIRENONE DEGRADATION PRODUCT

^{1}H NMR: δ 3.45 (2H, d, *J* = 5.5 Hz), 7.01 (1H, d, *J* = 8.6, 0.5 Hz), 7.38-7.49 (3H, d, 7.43, *J* = 8.0, 6.9, 1.8, 0.5 Hz), 7.44 (1H, d, *J* = 8.6, 1.5, 0.5 Hz), 7.44 (1H, d, *J* = 8.6, 6.9, 1.8, 0.5 Hz), 7.59 (1H, d, *J* = 8.0, 1.8, 1.5, 0.5 Hz), 7.84 (1H, d, *J* = 8.6, 1.8, 0.6, 0.5 Hz).

Discussion of Postulation Profile of Forced Degradation Product of Drospirenone: Taking in to account that the typical impurity tolerance level generally ranges between 0.1% to 1.0% and that identification of impurities lower than the 0.1% level was not considered to be necessary unless the potential impurities are likely to be strangely potent or toxic. The stress studies of Drospirenone revealed the presence of forced degradation products above the identification threshold limit; hence it was essential to identify the potential forced degradants in the stress samples using hyphenated techniques similar to LC-MS. The complete LC-MS spectral scan for Drospirenone stressed samples are given in **Fig. 5**.

A closer study of the drug molecule revealed the major degradation pattern that it could probably follow, and a postulation was drawn. The complete postulation for the degradation route of Drospirenone is given in **Fig. 6**. Based on the literature, various degradation pathways of Drospirenone were theoretically postulated, and preliminary stress degradation studies gave the idea about the degradation behavior of the drug molecule. It clearly stated the formation of major impurities expected for Drospirenone

The molecular weight of Drospirenone is 366.493 mg of Drospirenone. On in-depth observation, five-membered rings detached, so that probable impurity at m/z 294 was obtained, whereas the impurity at m/z 91 is observed due to probably six-membered rings becomes seven-membered. From m/z 294 possibilities of the detachment of 6 membered rings hence fragment shown at m/z 201.

LC-MS, IR, NMR analysis of forced degradation product of Drospirenone showed the presence of impurity. The structure of this forced degradation product has been postulated in the degradation scheme and this oxidative impurity (biphenyl) of Drospirenone, which is newly reported.

CONCLUSION: FT-IR, NMR, HPLC-LC-MS method have been used to monitor the Oxidative degradation product of Drospirenone. Drospirenone was found to degrade in 1% H_2O_2 to the extent of 19% after 1 h; more degradation was observed by using 3% H_2O_2 at 80 ºC. FT-IR, NMR, HPLC-LC-MS data demonstrated that the oxidatively stressed impurity of Drospirenone (Biphenyl moiety) is reported.

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CONFLICTS OF INTEREST: The authors declare that they have no conflict of interest.

ETHICAL APPROVAL: This article does not contain any studies with human participants or animals performed by any of the authors.

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