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# DEVELOPMENT OF A VALIDATED FORCED DEGRADATION STUDY FOR CHARACTERIZATION OF CICLESONIDE DEGRADANTS USING LC-MS

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**Keywords:**

Ciclesonide, Oxidative degradation, degradants, Stress conditions

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**ABSTRACT:** The stressed degradation study of Ciclesonide was conducted using various conditions, including acid, base, photolytic, thermal, and oxidative degradation. LC-MS was employed to characterize the degradants formed during these stress conditions. To assess the stability of Ciclesonide under stress, the study followed the guidelines outlined in ICH Q1A (R2). During the analysis, a C18 column (250mm x 4.6ID, 5 Micron) was utilized with a mobile phase of methanol: water (95:05) pH 3, adjusted with Ortho phosphoric acid, flowing at a rate of 0.9 ml/min. The analysis was performed using a PDA detector at an ambient temperature, with a retention time of 7.5 minutes for the drug. UV detection at 225nm was used for quantification. The results revealed the formation of a significant amount of a new compound under the stress conditions of acid, base, photolytic, and thermal degradation. LC-MS data confirmed the presence of stressed impurities of ciclesonide. This method was successfully applied in quality control laboratories for the determination of Ciclesonide, along with its decomposition products.

**INTRODUCTION:** Ciclesonide ([R]-11β, 16α, 17, 21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16, 17-acetal with cyclohexanecarbo-xaldehyde 21- isobutyrate; CIC) as shown in **Fig. 1**. is inhaled into the lungs *via* hydrofluoroalkane-MDI (HFA-MDI), where it is converted by local esterases to its active metabolite, desisobutyryl-ciclesonide (des-CIC). Ciclesonide is a novel inhaled corticosteroid used in the continuous treatment of mild to severe asthma [1]. It works by reducing a swelling and irritation in the airway to allow for easier breathing.

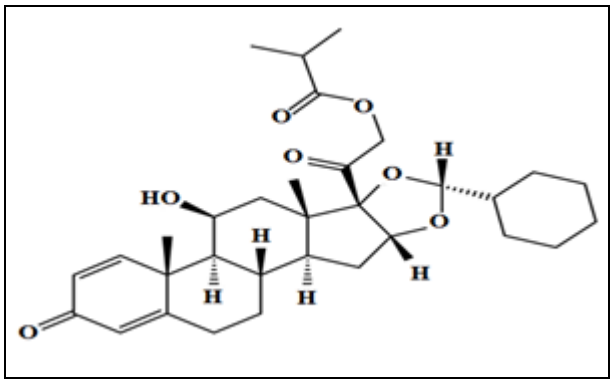

**FIG. 1: CHEMICAL STRUCTURE OF CICLESONIDE**

The therapeutic action of ciclesonide is achieved after the inhaled parent compound (CIC) is cleaved by esterases in the lungs to its active metabolite (des-CIC), a corticosteroid with high receptor affinity and anti-inflammatory activity. In the lung, Ciclesonide is metabolized to its active metabolites which has a more affinity for glucocorticoid receptors. However, Ciclesonide has a different

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structure, it still acts like other inhaled corticosteroids by reducing bronchial hyper-reactivity and inflammation in the airways [2]. The identification, structural elucidation and determining the impurities and degradation product are a prior importance in the course of all phases of the research, development and drug formulation production [3].

To issue 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 the stability testing is done primarily. The study of stressed degradation support for the recognition of achievable degradants, the inherent stability of the drug molecules, possible degradation pathways, and stability indicated analytical procedure validation. It understands the stability of the molecule assist to choose the relevant formulation and package and provide standard storage and shelf life conditions, which is essential for regulatory documentation [4-6].

The literature on the optimized method for rapid estimation of Ciclesonide in bulk and its dosage form (Rotacap) by RP-HPLC proposed that a quick, unadulterated, sensitive, accurate and precise chromatographic method for estimation of Ciclesonide in bulk and capsule formulation when compared with the official method detailed in IP 2007. The official method requires longer time for detection; therefore, there is a need to develop an economic and rapid method for detection of Ciclesonide for routine use [7-9]. In this study, the attempts were made to develop a simple, accurate and precise technique for assessment of Ciclesonide in the presence of its acid, base, photolytic, thermal and oxidative degradation products. By using LC-MS characterization this manuscript outlines the identification of ciclesonide transformation products under stress condition.

## MATERIALS AND METHODS:

**Chemical and Reagents:** Ciclesonide was supplied as a gift sample by Cipla Ltd. Maharashtra, India. Methanol, Ortho phosphoric acid and double distilled water was purchased from RAP Analytical lab, India and the further chemicals used are of analytical grade.

**Apparatus and Instruments:** Used UV-Visible double beam spectrophotometer (Model-UV2012). For the chromatographic separation HPLC system (3000 Series), auto-sampler was used. Ultrasonic cleaner of Spectra Lab India was used.

**Selection of Analytical Wavelength:** The spectra of Ciclesonide at λ max 225 nm, when overlapped, allowed for the use of methanol as a solvent for chromatographic estimation of the drug.

**Preparation of sample for HPLC and LC-MS Analysis:** The stressed sample of Ciclesonide were diluted with 10 ml of mobile phase before injection into the HPLC. Before HPLC and LC-MS analysis all the samples were purified by filtration through 0.22 um membrane filter.

**Selection of Chromatographic Condition:** HPLC method selection is depends on the character of the sample, solubility and its molecular weight. The chromatographic variables like mobile phase, flow rate and solvent ratio were studied. The resultant chromatograms were recorded and the chromatographic variables like asymmetry, selectivity, and sensitivity were selected for evaluations.

**Chromatographic Parameters Optimization:** The optimization in HPLC is the procedure of finding a set of conditions that appropriately analysed the quantification of the analyte with sustainable accuracy, precision, specificity, sensitivity, cost, ease, and speed of analysis.

**Method Validation:** In accordance with the guidelines set by the International Council for Harmonisation (ICH), the proposed RP-HPLC method underwent validation to serve as a stability-indicating assay for evaluating the stability of Ciclesonide under various forced degradation conditions. The validation of the developed method aimed to ensure the reliability of the analysis results across different parameters, including linearity, range, accuracy, precision, robustness, limit of quantification (LOQ), limit of detection (LOD), and specificity. To assess linearity, triplicate sequential dilutions of Ciclesonide (ranging from 10 to 50 µg/mL) were analysed in a methanol: water (95:5) mixture. Accuracy was evaluated through recovery studies, where the standard solution was spiked into previously tested

samples. Additionally, three replicates of Ciclesonide at three different concentrations (10, 15, 20 µg/ml) were analysed within a single day to calculate the percentage relative standard deviation (%RSD). Inter-day variation studies were performed by analysing three replicates of Ciclesonide at various concentrations over three consecutive days, and %RSD was calculated to assess precision. The robustness of the method was examined by introducing variations in the flow rate (0.9, 1, and 1.1 ml) and the acetonitrile content in the organic phase by 1%. LOD and LOQ were determined using the signal-to-noise ratio method to establish the lower limits of detection and quantification, respectively. To verify the method's specificity, the drug sample was subjected to prescribed stress conditions. By following these rigorous validation procedures, the RP-HPLC method has been established as a reliable tool for stability-indicating assays and forced degradation studies of Ciclesonide.

**Mobile Phase Optimization:** For the mobile phase selection, the various modifications, involved change in composition of mobile phase and column temperature modification, were tried, but the satisfactory resolution was not found. Eventually, the mobile phase contains methanol: water (95:05) pH 3 found to be best resolution.

**Standard Drug Solution Preparation:** Weigh accurately 10mg Ciclesonide added and diluted it up to 10ml with the help of mobile phase solvent. This was 50ppm solution of drug.

**Stress Degradation:**

**Preparation of Sample:** As per the International Council for Harmonization (ICH) guidelines, a forced degradation study was conducted for Ciclesonide. Four samples were generated to evaluate the drug's stability under stressed conditions.

1. In the normal condition, blank solutions were stored.
2. Similar to the drug, blank samples were subjected to stressed conditions in the same manner.
3. A zero-time sample containing the drug solution was prepared.
4. The drug solution was exposed to stress conditions for treatment.

**Acid Degradation:** 10mg of drug was dissolved in 10ml of 0.1N HCl and the mixture was condensed for 15 min. After that 500ul sample was taken from mixture and diluted it up to 10ml with the help of mobile phase solvent.

**Base Degradation:** 10mg of drug was dissolved in 10ml of 0.1N NaOH and the mixture was condensed for 15 min. After that 500ul sample was taken from mixture and diluted it up to 10ml with the help of mobile phase solvent.

**Photolytic Degradation:** 10mg of drug was dissolved in 10ml of mobile phase solvent and sample was kept photolytically at room temp. for 24 hr.

**Thermal Degradation:** 10mg of drug was dissolved in 10ml of mobile phase solvent and sample was kept thermally at room temp. for 24 hr.

**Oxidative Degradation:** The process of oxidative degradation involved the addition of 2 ml of a standard Ciclesonide solution to 8 ml of 3% hydrogen peroxide. This mixture was carefully transferred to a 10 ml volumetric flask and subsequently maintained at room temperature.

**Postulation Profile of Forced Degradation Products of Ciclesonide:** Forced degradation studies of the bulk drug were conducted under appropriate solid-state and solution-state stress conditions, following the guidelines outlined by the International Council for Harmonisation (ICH) [10]. The stock solution was utilized to assess the stability-indicating property and specificity of the proposed method. Before injection, samples were withdrawn at relevant intervals, neutralized (if required for acid and alkali hydrolysis), and then diluted with the appropriate solvent. The total chromatographic run time was set at approximately twice the retention time of the drug peak. LC-MS studies of sample preparation:

1. 10mg of drug was dissolved in 10ml of 0.1N HCl and the mixture was condensed for 15 min. After that 500ul sample was taken from the mixture and diluted it up to 10ml with the help

of mobile phase solvent. This was a 50ppm solution of drug.

2. Same procedure was performed for 0.1N NaOH.
3. 10mg of drug was treated with 3% H2O2 and the mixture was kept at room temperature for 24 hrs. Then the remaining procedure was performed as same given in acid degradation studies.
4. 10mg of drug was kept in the oven for 24 hrs.
5. 10mg drug was kept under UV light for 24 hrs. and the remaining procedure was performed the same.

**RESULTS AND DISCUSSION:** The relevant physical and chemical properties of Ciclesonide were extracted from existing literature. The analytical method was developed to determine the initial reversed-phase HPLC chromatographic conditions, including the mobile phase, detection wavelength, and sample preparation procedure. Several trials were conducted to explore different ratios of methanol and water, aiming to optimize the chromatographic conditions. After extensive experimentation, the mobile phase comprising methanol and water in a ratio of 95:05 v/v, with a flow rate of 0.9 ml/min, injection volume of 20 µl, run time of 12.14 min, and column temperature set at 30°C, was identified as the most suitable chromatographic condition for the entire study.

Under these optimized conditions, Ciclesonide was eluted, forming symmetrical peak shape, achieving satisfactory resolution, and providing a suitable analysis time, with a retention time of approximately 7.5 minutes in **Fig. 2** and selected maximum wavelngth at 225 nm in **Fig. 3** respectively.

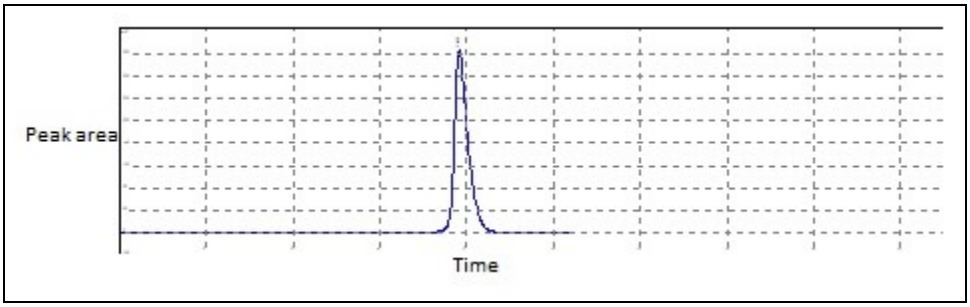


**FIG. 2: CHROMATOGRAM OF CICLESONIDE 50PPM**

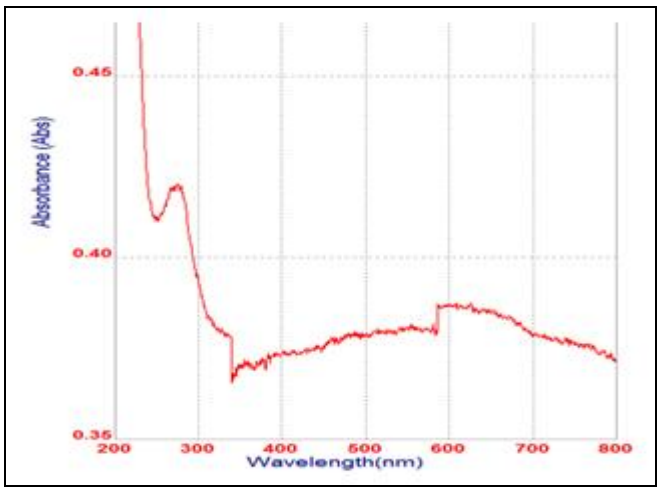


**FIG. 3: UV SPECTRA OF CICLESONIDE**

**TABLE 1: PARAMETERS USED FOR METHOD DEVELOPMENT**

| Sr. no. | Parameters | Description |
|---|---|---|
| 1 | Column C18 | Cromasil |
| 2 | Mobile phase | Methanol: Water (95:05) pH 3 |
| 3 | Injection volume | 20 ul |
| 4 | Flow rate | 0.9 ml/min |

| | | |
|---|---|---|
| 5 | Detector | PDA |
| 6 | Wavelength | 225 nm |
| 7 | Run time | 12.14 min |

**TABLE 2: ELEMENTAL COMPOSITION OF STRESS STUDIES**

| Ciclesonide and DI | Proposed formula | Observed mass | Calculated mass | Error |
|---|---|---|---|---|
| Thermal | $C_{32}H_{42}O7$ | 541.237 | 541 | -0.0010 |
| Acidic | $C_{32}H_{43}O_7$ | 541.329 | 541 | -0.0002 |
| Basic | $C_{30}H_{42}O_6$ | 498.633 | 498 | -0.0011 |
| Photolytic | $C_{31}H_{39}O_7$ | 523.187 | 523 | -0.0003 |

**TABLE 3: ELEMENTAL COMPOSITIONS OF CICLESONIDE DEGRADATION PRODUCT**

| Ciclesonide | Rt (min) | Proposed formula | Observed mass | Calculated mass | Error | MS/MS fragment ions |
|---|---|---|---|---|---|---|
| $H_2O_2$ | 1.05 | $C_{32}H_{43}O6$ | 523.1 | 524 | -0.0010 | 523.1, 435.1, 393, 322.9,263,209 |
| Thermal | 1.06 | $C_{32}H_{43}O_6$ | 523.1 | 523 | -0.0002 | 523.1, 435.1, 393, 323,277, 172.9, 121.2, Degradation Product-I |
| Acid | 1.07 | $C_{30}H_{41}O7$ | 523.2 | 523 | -0.0013 | 523.2, 435.3, 393,323.1, 277.1, 224.9, 83.3, Degradation Product-I ,II |
| Base | 0.98 | $C_{18}H_{39}O_6$ | 350.9 | 351 | -0.0009 | 350.9, 200.8, Degradation Product-III |
| Photolytic | 1.06 | $C_{32}H_{43}O_6$ | 523.2 | 523 | -0.0003 | 523.2,435.1,393,323,277, 173.1, 121.2, Degradation Product-I,II |

The linearity of the method was established through a linear regression equation with a correlation coefficient (R2) of 0.9989. A calibration graph was plotted, correlating concentration with the corresponding area in the chromatogram. Accuracy was thoroughly examined at all levels, and excellent recoveries of Ciclesonide ranging from 99.02% to 101.07% were achieved for various added concentrations, meeting the accuracy criteria with a calculated % RSD of 0.02. The precision of the method was evaluated through repeatability measurements, determining the quantity of Ciclesonide in six different replicated solutions. The repeatability result showed a determined quantity of 100.89% with a % RSD of 0.008. Intermediate precision was also calculated, yielding values close to 100% (101.77% and 101.71%) with % RSD less than one (1.044 and 1.055) at three different concentration levels. The method's robustness was analyzed by varying the mobile phase composition and flow rate. The results indicated robustness, as insignificant differences in peak areas and less variability in retention time were observed. LOD and LOQ were determined using the signal-to-noise ratio method. All the experimental parameters confirmed the specificity of the developed method for the analysis of Ciclesonide. The rate of degradation in the basic medium was found to be significant, indicating that Ciclesonide was highly labile to base degradation. After subjecting Ciclesonide to a reaction in 0.1 N NaOH at 60°C for 15 minutes, two major degradation products were detected with peaks at 4.2 minutes and 3.5 minutes, as shown in the chromatogram in **Fig. 4.**

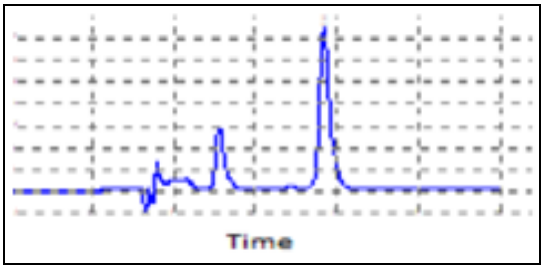


**FIG. 4: CHROMATOGRAM OF CICLESONIDE IN BASE DEGRADATION**

In an acid medium, 10% degradation was observed when Ciclesonide solution was refluxed with 0.1 M HCl at 60°C for 10 minutes, resulting in the formation of a degradation product at a retention time of 3.6 minutes in HPLC, as depicted in the chromatogram in **Fig. 5**.

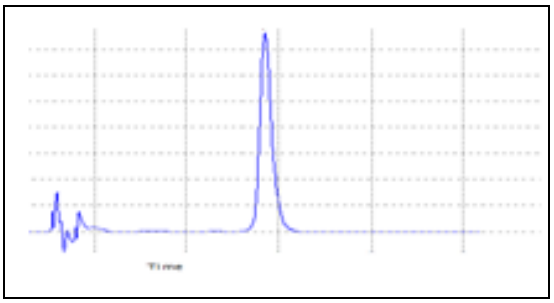

**FIG. 5: CHROMATOGRAM OF CICLESONIDE IN ACID DEGRADATION**

Furthermore, Ciclesonide was found to be susceptible to photochemical degradation and thermal degradation after being exposed to photolysis for 24 hours in **Fig. 6** and sunlight for 24 hours in **Fig. 7**, respectively. To identify the degradation impurities of Ciclesonide, an optimized LC-MS method was applied.

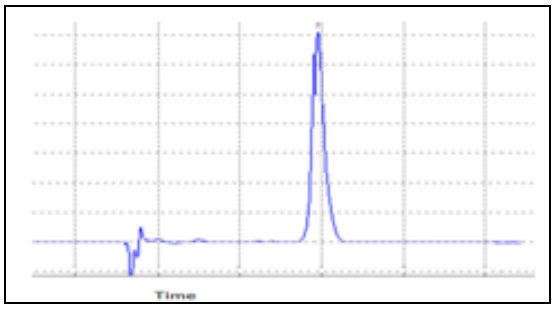

**FIG. 6: CHROMATOGRAM OF CICLESONIDE IN PHOTOLYTIC DEGRADATION**

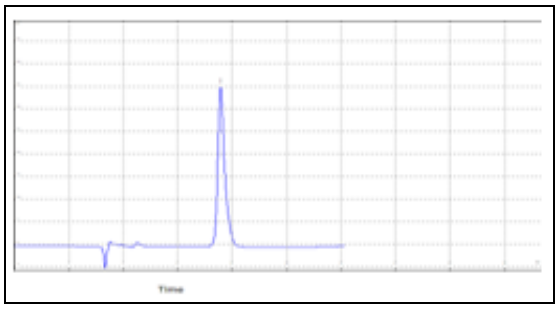

**FIG. 7: CHROMATOGRAM OF CICLESONIDE IN THERMAL DEGRADATION**

The LC-MS total ion chromatograms (TIC) of H2O2 are shown in **Fig. 8A** the drug formed a degradation product at m/z 541.23 under thermal conditions in **Fig. 8B**, m/z 541.32 under acidic conditions in **Fig. 8C**, m/z 498 under basic conditions in **Fig. 8D**, and m/z 541.35 under photolytic conditions in **Fig. 8E**. Using accurate mass measurements, all these degradation products were structurally elucidated via LC-MS.

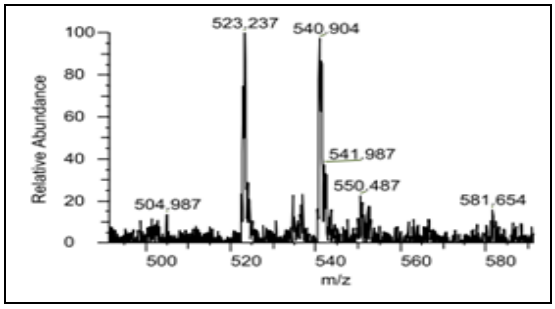


**FIG. 8: (A) $H_2O_2$ LC-MS**

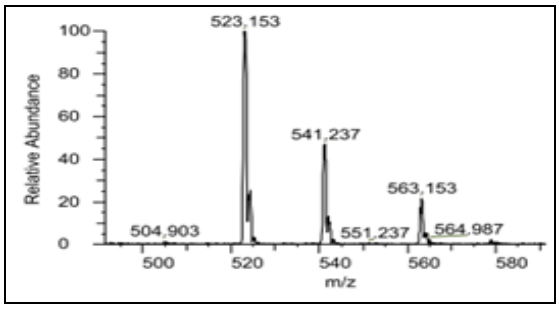


**FIG. 8: (B) THERMAL LC-MS**

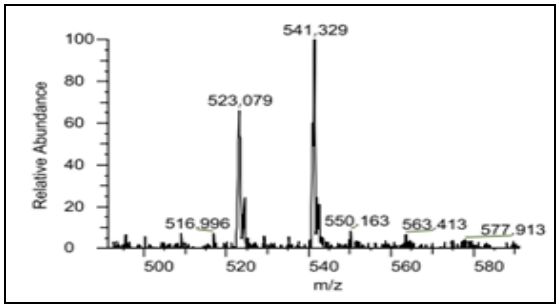


**FIG. 8: (C) ACID LC-MS**

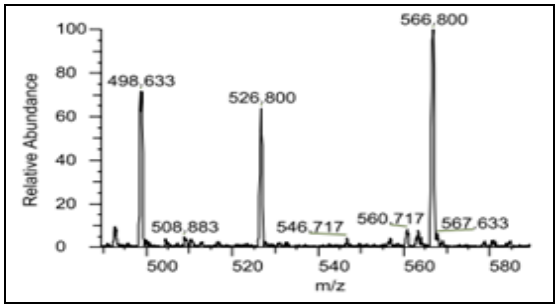


**FIG. 8: (D) BASE LC-MS**

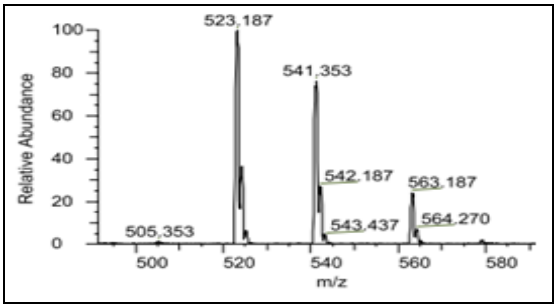


**FIG. 8: (E) PHOTOLYTIC DEGRADATION**

**FIG. 8: (A) LC-MS TIC OF $H_2O_2$, B) LC-MS TIC OF THERMAL DEGRADATION C) LC-MS TIC OF ACID DEGRADATION D) LC-MS TIC OF BASE DEGRADATION E) LC-MS TIC OF PHOTOLYTIC DEGRADATION**

The proposed structures of degradation impurities of Ciclesonide and their elemental compositions are presented in **Table 2** and in **Fig. 8**, respectively. The fragmentation analysis of Ciclesonide degradants was conducted using product ion scan via LC-MS. During the stress studies, three degradation products (D1-D2) were observed for Ciclesonide. Among these products, D1 was formed under acidic, photolytic, and thermal conditions, while D2 was generated under basic conditions. Notably, complete degradation occurred for basic hydrolysis after 72 hours. LC-MS analysis was performed to identify the degradation products of acid, thermal, photolytic, and base hydrolysis. The total ion chromatograms of the degradants exhibited molecular ions at m/z 541.3, m/z 541.23, m/z 541.35, and m/z 498.63, respectively, confirming the presence of the respective degradation products. No neutral and oxidation products were observed for the drug sample after exposure to water, 80 ºC, and $H_2O_2$, indicating the drug's stability under neutral and hydrogen peroxide stress conditions. The mass-to-charge (m/z) values of all degradants and their corresponding fragmentation ions are illustrated in **Fig. 9A** to **E**. This analysis confirms the structural elucidation of degradation impurities for Ciclesonide **Fig. 10.** Considering that the typical adulteration tolerance level usually falls within the range of 0.1% to 1.0%, the identification of impurities below the 0.1% level was not deemed mandatory, except in cases where these potential impurities are likely to be toxic or exceptionally potent.

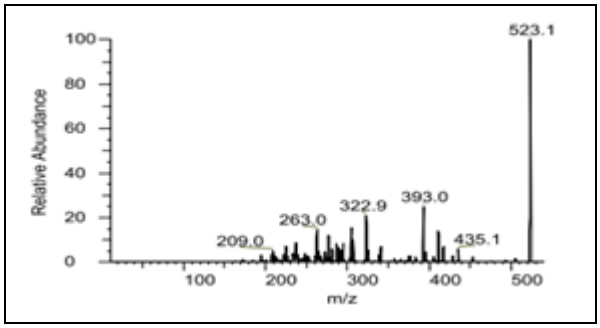


**FIG. 9: (A) H2O2 LC-MS**

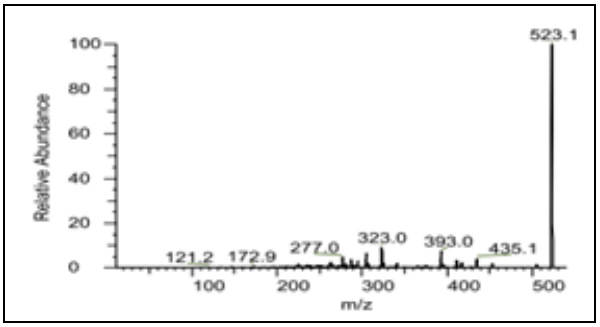


**FIG. 9: (B) THERMAL LC-MS**

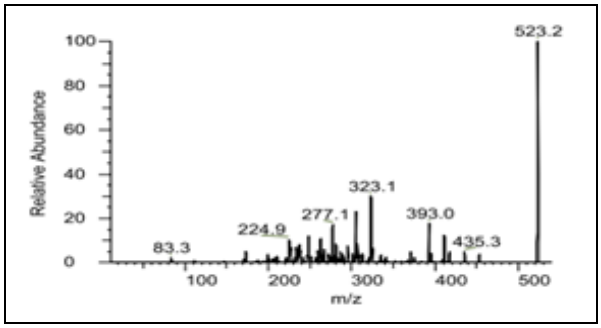


**FIG. 9: (C) ACID DEGRADATION**

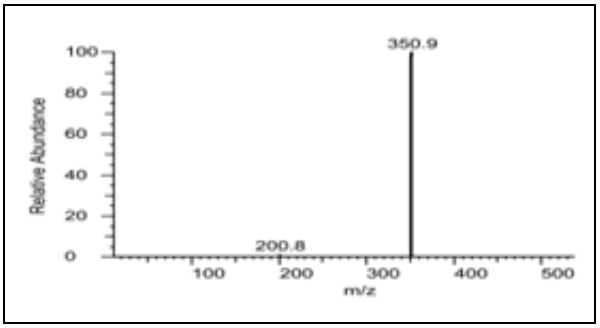


**FIG. 9: (D) BASE DEGRADATION**

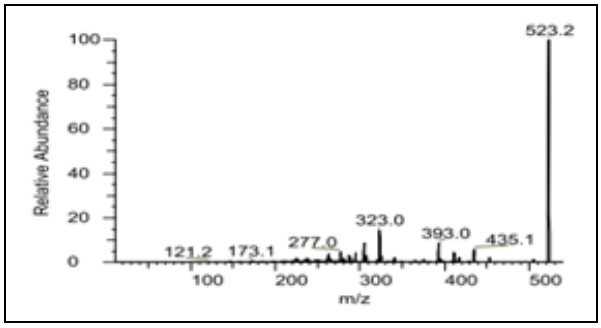


**FIG. 9: (E) PHOTOLYTIC DEGRADATION**

**FIG. 9: (A) MASS FRAGMENTATION PATTERN OF $H_2O_2$, (B) MASS FRAGMENTATION PATTERN OF THERMAL DEGRADATION (C) MASS FRAGMENTATION PATTERN OF ACID DEGRADATION (D) MASS FRAGMENTATION PATTERN OF BASE DEGRADATION (E) MASS FRAGMENTATION PATTERN OF PHOTOLYTIC DEGRADATION PRODUCTS**

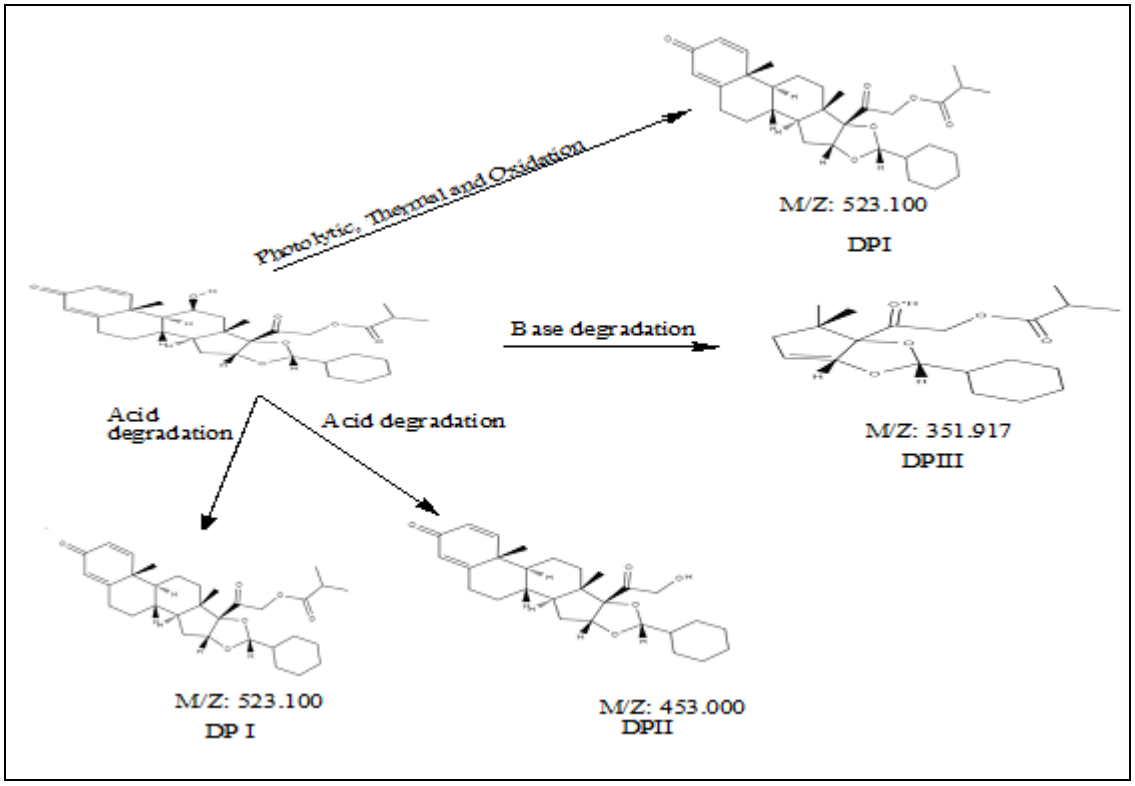


**FIG. 10: DEGRADATION PATHWAY OF CICLESONIDE**

In the stress studies conducted on Ciclesonide, the presence of forced degradation products exceeding the recognition threshold limit was revealed. Therefore, it became imperative to employ hyphenated methods, such as LC-MS, to identify the possible forced degradants in the stress samples.

**CONCLUSION:** An LC-MS method for the quantification of Ciclesonide was successfully developed and validated. The method demonstrated excellent performance, showing selectivity, linearity, accuracy, precision, recovery, robustness, and stability. Its sensitivity allowed for quantitative detection of the analyte in pharmaceutical preparations.

The degradation behavior of Ciclesonide was investigated under various stress conditions, including acid, base, oxidation, photolysis, thermal, and neutral conditions. The results indicated that Ciclesonide was found to be unstable in acid, base, photolytic, and thermal stress conditions. During the study, a total of three unknown degradants were identified and characterized using accurate mass measurements through LC-MS. This developed method holds potential for a wide range of applications, including checking the quality of Ciclesonide in stability samples, routine analysis, quality control, stability studies, and suitability for therapeutic drug monitoring (pharmacokinetic or bioequivalence studies) in pharmaceutical formulations containing Ciclesonide. Furthermore, the method can be useful in further investigations and characterization of other process-related impurities, as well as confirming the identity of degradation products formed. In the future, research possibilities include synthesizing and developing reference standards to monitor their presence in the stability samples, specifically focusing on the identified degradation products. This approach ensures the reliability and validity of the method for future applications.

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

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