STABILITY INDICATING ANALYTICAL METHODS (SIAM)

The stability indicating method can be defined as validated quantitative analytical method that can detect the change in the chemical, physical or microbiological properties of the drug substance and drug product with respect to time, and that are specific so that the content of active ingredient and degradation product/impurity can be accurately measured without interference (1). The purpose of stability testing is to provide evidence about the variation of the quality of pharmaceutical products with time under the influence of a variety of environmental factors such as temperature, humidity and light. The stability program also includes the study of product-related factors that influence its quality, for example, interaction of API with excipients, container closure systems and packaging materials. In fixed-dose combination (FDCs) the interaction between two or more APIs also has to be considered.

Potential adverse effects of instability in pharmaceutical products (2)

- Loss of Active Pharmaceutical Ingredient (API)
- Increase in concentration of API
- Alteration in bioavailability
- Loss of content uniformity
- Loss of pharmaceutical elegance and patient acceptability
- Formation of toxic degradation products

  Loss of package integrity

Ideal characteristics of Stability Indicating Analytical method (SIAM)

- It should be capable of separating major API from any degradation product under defined storage condition.
- It should be sensitive to detect and quantify one or more degradation products or impurities or related substances.

Regulatory status of stability-indicating assays

The ICH (International Committee on Harmonisation) guidelines have been incorporated as a law in the Europe, Japan and in the US, but in reality, besides these, other countries are also using them.

The ICH guideline Q1A on Stability Testing of New Drug Substances and Products (3) emphasizes that the testing of those features which are susceptible to change during storage and are likely to influence quality, safety and/or efficacy must be done by validated stability-indicating testing methods. It is also mentioned that forced decomposition studies (stress testing) at temperatures in 10°C increments above the accelerated temperatures, extremes of pH and under oxidative and photolytic conditions should be carried out on the drug substance so as to establish the inherent stability characteristics and degradation pathways to support the suitability of the proposed analytical procedures.

The ICH guideline Q3B entitled 'Impurities in New Drug Products' emphasizes on providing documented evidence that analytical procedures are validated and suitable for the detection and quantitation of degradation products (4). It is also required that analytical methods should be validated to demonstrate that impurities unique to the new drug substance do not interfere with or are separated from specified and unspecified degradation products in the drug product.

The ICH guideline Q6A, which provides note for guidance on specifications (5), also mentions the requirement of stability-indicating assays under Universal Tests/Criteria for both drug substances and drug products. The same is also a requirement in the guideline Q5C on Stability Testing of Biotechnological/Biological Products (6).

The requirement is also listed in World Health Organization (WHO), European Committee for Proprietary Medicinal Products and Canadian Therapeutic Products Directorate's guidelines on stability testing of well-established or existing drug substances and products (7-9). Even the United States Pharmacopoeia (USP) has a requirement listed under 'Stability Studies in Manufacturing', which says that samples of the products should be assayed for potency by the use of a stability-indicating assay (10).

Current ICH guideline on Good Manufacturing Practices for Active Pharmaceutical Ingredients (Q7A), which is under adoption by WHO, also mentions clearly that the test procedures used in stability testing should be validated and be stability- indicating (11).

Development of validated SIAMs those are likely to meet regulatory requirements (12)

Though the requirements with respect to SIAM have been spelt out in regulatory documents, information on the basic steps to be followed for the development and validation of stability-indicating methods is neither provided in the regulatory guidelines nor in the pharmacopoeias. Therefore, the practical steps involved in the development of SIAMs are discussed below:-

Step I: critical study of the drug structure to assess the likely decomposition route(s):
Much information can simply be gained from the structure, by study of the functional groups and other key components. There are definite functional group categories, like amides, esters, lactams, lactones, etc. that undergo hydrolysis (13), other groups like thiols, thioethers, etc. undergo oxidation (14) and compounds like olefins, aryl halo derivatives, aryl acetic acids and those with aromatic nitro groups, N-oxides undergo photodecomposition (15).

For a new congener (new molecule of same chemical series), its degradation chemistry can be easily postulated based on the reported behaviour of other drugs (or the parent compound) in the series.

Step II: collection of information on physicochemical properties:
Before method development is taken up, it is generally important to know various physicochemical parameters like pKa, log P, solubility, absorptivity and wavelength maximum of the drug in question. The knowledge of pKa is important as most of the pH-related changes in retention occur at pH values within ± 1.5 units of the pKa value. The ionization value also helps in selecting the pH of the buffer to be used in the mobile phase (16). The knowledge of log P for the drug and the identified degradation products provides good insight into the separation behaviour likely to be obtained on a particular stationary phase.

Step III: stress (forced decomposition) studies:

The next step in the development of SIAM is the conduct of forced decomposition studies to generate degradation products of the drug. The ICH guideline Q1A suggests the following conditions to be employed: (i) 10°C increments above the accelerated temperatures (e.g. 50°C, 60°C, etc.), (ii) humidity where appropriate (e.g. 75% or greater), (iii) hydrolysis across a wide range of pH values, (iv) oxidation and (v) photolysis.

The hydrolytic degradation of a new drug in acidic and alkaline conditions can be studied by refluxing the drug in 0.1N HCl/NaOH for 8 h. However, in case no degradation or excessive degradation, the conditions can be altered accordingly. In a similar manner, degradation under neutral conditions can be started by refluxing the drug in water for 12 h. Reflux time should be increased/decreased according to the need of degradation. For peroxide degradation (or oxidation), it is suggested to use hydrogen peroxide in the concentration range of 3–30%. The photolytic studies should be carried out by exposure to light, using either a combination of cool white and ultraviolet fluorescent lamps, or one among the xenon and metal halide lamps. Exposure energy should be minimum of 1.2 million lux h fluorescent light and 200 W h/m^2 UV.

Step IV: preliminary separation studies on stressed samples:

The stress samples so obtained are subjected to preliminary analyses to study the number and types of degradation products formed under various conditions. Well-separated and good quality peaks at the outset provide better confidence because of the unknown nature of products formed during stress condition. Selection of proper mobile phase should be done to get resolved degradation peak and the API under study. The detection wavelength should be set, based on the study of spectral behaviour of degraded samples. The results should be critically compared with the blank solutions injected in a similar manner. The observations like the increase or decrease in the API peak should be considered, presence of non-chromophoric degradation product (which can be assessed by LCMS), insolubility of degradation product in the reaction mixture, change in the colour of the reaction mixture, etc. should also be considered.

Step V: final method development and optimization:

Subsequent to preliminary chromatographic studies, the RT and relative retention times (RRT) of all products formed should be tabulated for each reaction condition. In the final

step, a mixture of the reaction solutions is prepared, and subjected again to resolution behaviour study. Resolution in the mixture is studied closely, to see whether the resolution is similar to that obtained in individual samples. This is important to rule out any changes that can happen when reaction solutions of different pH and media (3–30% hydrogen peroxide solution) are mixed. To separate close or co-eluting peaks, the method is optimized, by changing the mobile phase ratio, pH, gradient, flow rate, temperature, solvent type and the column and its type.

Step VI: identification and characterization of degradation products, and preparation of standards

To identify the resolved products, a conventional way is to isolate them and determine the structure through spectral (MS, NMR, IR, etc.) and elemental analysis. However, this approach is tedious and time consuming when multiple degradation products are formed. These days an integrated approach wherein LC-MS or LC-MS-MS is employed to obtain molecular weight and fragmentation information, and further detailed structural information is obtained through LC-NMR analysis. The integrated approach provides rapid and unambiguous identification of several degradation products at one time.

Step VII: validation of SIAMs:

Validation of analytical methods, in general, has been extensively covered in the ICH guidelines Q2(R1) (17) and in the FDA guidelines (18). Overall, there are two stages in the validation of a SIAM. First stage is early in the development cycle when drug substance is subjected to forced decomposition studies and the SIAM is established based on the knowledge of drug degradation behaviour. The second stage, when the SIAM so developed is extended to formulations or other matrices, the emphasis gets limited to just prove the pertinence of the established validation parameters in the presence of excipients or other formulation constituents.

'Specific' and 'Selective' stability-indicating assay methods (10)

'Specific stability-indicating assay method (Specific SIAM)' can be defined as 'a method that is able to measure unequivocally the drug(s) in the presence of all degradation products, excipients and additives, expected to be present in the formulation.'

The 'Selective stability-indicating assay method (Selective SIAM)' on the other hand can be defined as 'a method that is able to measure unequivocally the drug(s) and all

degradation products in the presence of excipients and additives, expected to be present in the formulation.' By this definition, it means that a 'Selective SIAM' is a procedure that is selective to the drug as well its degradation products (separates all of them qualitatively) and is also specific to all the components (measures them quantitatively).

The chromatographic methods, however, can be of both types—'Specific SIAM' as well as 'Selective SIAM'. In case of the former, the method is not fully separative to all components, but does separate the drug equivocally. This normally is a situation where efforts fail to separate degradation products when they are large in number.

Because of the very nature of requirement of separation of multiple components during analysis of stability samples, chromatographic methods have taken precedence over the conventional methods of analysis due to the advantage of greater accuracy and sensitivity for even small quantities of degradation products thus produced. The use of PDA detector to determine peak purity of the active ingredient in stressed samples greatly facilitates the development of stability-indicating assays. The emphasis on the identification of trace impurities and degradants has led to the increased use of hyphenated techniques such as liquid chromatography–mass spectrometry (LC–MS) and liquid chromatography–nuclear magnetic resonance spectroscopy (LC–NMR). The above techniques are often combined with statistical methods like QbD and chemometrics which aid in, to get proper resolution and desired SST parameters for the API as well as the degradation peaks.

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DEVELOPMENT OF STABILITY INDICATING ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF AZELASTINE HYDROCHLORIDE AND BUDESONIDE IN BULK DRUG AND LABORATORY MIXTURE: CHARACTERISATION OF MAJOR DEGRADATION PRODUCTS BY LC-MS/MS TECHNIQUE

5.1. LITERATURE REVIEW

A thorough literature survey for analytical and bioanalytical methods for the estimation of AZ and BD has mentioned in section 3.4 and 4.1 respectively. Few stability indicating analytical methods have also been reported in the literature for AZ and BD either individually or in various combinations, such as stability indicating HPLC methods (1-5), stability indicating TLC method (6), LC-MS characterisation of BD (7) and method for determining alkaline degradant of AZ using membrane selective electrodes (8). No specific stability indicating analytical method has been reported for AZ and BD in a binary mixture.

5.2. EXPERIMENTAL

5.2.1 Instrumentation

Chromatographic separation was performed on Shimadzu (Shimadzu Corporation, Kyoto, Japan) LC system equipped with Shimadzu LC-20AD binary pump, Shimadzu SPD-M20A detector and Rheodyne 7725 injector with fixed loop of 20 µL. Data acquisition and integration was performed using LC Solutions software. Stationary phase used was Oyster C8 column (250 mm length x 4.6 mm i.d., 5µm particle size).

The LC-MS/MS system (Thermo Fisher Scientific LCQ Fleet) coupled with ion trap mass spectrometer and quaternary pump delivery module was used for the analysis. Data acquisition was done on Xcalibur software.

5.2.2. Chemicals and Reagents

The API of AZ was procured from Sigma Aldrich and BD was gifted by Avik Pharmaceuticals Ltd., Vapi Gujarat. HPLC grade MeOH (Spectrochem) and HPLC grade ACN (Spectrochem) were used for HPLC. Double distilled water, AR grade ammoniun formate (Loba Chem) and HPLC grade formic acid (Spectrochem) were

used for preparation of buffer. LC grade ammonium formate (LobaChem) and MilliQ water was used for LCMS analysis.

5.2.3 Chromatographic conditions

Mobile phase A comprised of ammonium formate buffer (10mM) prepared by dissolving 0.63 gm of anhydrous ammonium formate in 1000 ml of double distilled water and adjusted to pH 3 using dilute formic acid. The buffer was filtered with 0.2 µ Nylon membrane filter and degassed by sonication. Mobile phase B was ACN. The gradient elution programme is shown in Table 5.1. The chromatographic elution was carried on oyster C8 column (2.5µ × 4.6mm i.d. × 250mm) at a flow rate of 0.8 mL/min at ambient temperature. MilliQ water and LC grade chemicals were used to prepare formate buffer for LC-MS/MS analysis.

Table 5.1 Gradient Programme

Time (min)	Organic Conc.
0.01	40
5	40
10	60
12	60
13	80
14	80
16	40
20	Stop

5.2.4. Preparation of standard solutions of AZ and BD

Diluent: The mixture of ammonium formate buffer (10mM, pH 3) and ACN in the ratio50:50 was used as diluent for all the solutions. Amber coloured flasks were used for preparation of solutions and degradation study mixtures.

AZ stock solution (1 mg/mL): Accurately weighed 25 mg AZ was transferred in 25 mL volumetric flask, dissolved and then diluted with MeOH up to the mark.

AZ working solution (0.856 mg/mL): The working solution was prepared by transferring 21.4 mL from of AZ stock solution to 25 mL volumetric flask and diluted to the mark with the diluent.

BD stock solution (0.5 mg/mL): Accurately weighed 12.5 mg BD was transferred in 25 mL volumetric flask, dissolved and then diluted with MeOH up to the mark.

BD working solution (0.2 mg/mL): The working solution was prepared by transferring 10 mL from of BD stock solution to 25 mL volumetric flask and diluted to the mark with the diluent.

5.2.5. Approach for development of SIAM

For development of the SIAM for the combination of AZ and BD, the degradation of individual APIs was studied in various conditions, thereafter the conditions were optimised based on the extent of degradation and the formation of degradation products. The optimised conditions were studied for the mixture of APIs and thus a final specific SIAM was developed for the two drugs. The developed SIAM was applied to the laboratory prepare formulation.

5.2.5.1.Preparation of Forced- degradation samples

5.2.5.1.1. Acid- induced degradation

Accurately weighed 5.48 mg AZ and 1.28 mg BD were taken together into a round bottom flask (RBF). The solid mixture was dissolved in minimum amount of MeOH (1 mL). To it 9 mL of 1N HCl was added and the reaction mixture was refluxed at 80ºC for 30 min on oil bath. The degradation was performed in the dark in order to exclude possible degradation effect of light.After 30 min, the reaction mixture was allowed to cool down at room temp., 2.5 mL of aliquot was taken into 10 mL volumetric flask. The sample was neutralized with 1N $NaHCO_3$, the volume was made upto 10 mL with the diluent, filtered with 0.2µ membrane filter and subjected to HPLC analysis. Similarly, blank sample was also prepared without using the API samples and subjected to analysis.

5.2.5.1.2. Base- induced degradation

Accurately weighed 5.48 mg AZ and 1.28 mg BD were taken together into a volumetric flask. The solid mixture was dissolved in minimum amount of MeOH (1 mL). To it 9 mL of 1N NaOH the mixture was kept at room temperature for 1h. The degradation was performed in the dark in order to exclude possible degradation effect of light.After 1 h, 2.5 mL of aliquot was taken into 10 mL volumetric flask. The

sample was neutralized with 1N HCl, the volume was made upto 10 mL with the diluent, filtered with 0.2µ membrane filter and subjected to HPLC analysis. Similarly, blank sample was also prepared without using the API samples and subjected to analysis.

5.2.5.1.3. Peroxide- induced degradation (Oxidation)

Accurately weighed 5.48 mg AZ and 1.28 mg BD were taken together into a RBF. The solid mixture was dissolved in minimum amount of MeOH (1 mL). To it 9 mL of 6% H_2O_2 was added and the reaction mixture was refluxed at 80ºC for 30 minutes on oil bath. The degradation was performed in the dark in order to exclude possible degradation effect of light.After 30 min, the reaction mixture was allowed to cool down at room temp., 2.5 mL of aliquot was taken into 10 mL volumetric flask and heated for 5 min on water bath, so as to remove the H_2O_2.Finally, volume was made upto 10 mL with the diluent, filtered with 0.2µ membrane filter and subjected to HPLC analysis. Similarly, blank sample was also prepared without using the API samples and subjected to analysis.

5.2.5.1.4. Photolytic degradation

For the photolytic stability, the mixture of solid drugs (API), i.e. 5.48 mg AZ and 1.28mg BD, was spread in 1mm thickness on a petri-dish, which was closed (with a glass cover) and exposed to UV light of 5382 LUX and 144UW/cm^2 for 21 days. Degradation solid mixture was taken in 10 mL volumetric mixture, dissolved in 1 mL MeOH and made upto the volume. From this solution, 2.5 mL aliquot was taken and diluted upto 10 mL with the diluent filtered by 0.2 µ membrane filter and analysed by HPLC.

5.2.5.1.5. Neutral hydrolysis

Accurately weighed 5.48 mg AZ and 1.28 mg BD were taken together into a RBF. The solid mixture was dissolved in minimum amount of MeOH (1 mL). To it 9 mL of double distil water was added and the reaction mixture was refluxed at 80ºC for 3 hours on oil bath. The degradation was performed in the dark in order to exclude possible degradation effect of light.After 3 hours, the reaction mixture was allowed to cool down at room temp., 2.5 mL of aliquot was taken into 10 mL volumetric flask. Finally, volume was made upto 10 mL with the diluent, filtered with 0.2µ membrane

filter and subjected to HPLC analysis. Similarly, blank sample was also prepared without using the API samples and subjected to analysis.

5.2.5.1.6. Dry heat induced degradation

For dry heat degradation, the mixture of solid drugs (API), i.e. 5.48 mg AZ and 1.28 mg BD, were placed in oven at 80ºC for 48 h. Degradation solid mixture was taken in 10 mL volumetric mixture, dissolved in minimum amount (1 mL) of MeOH and made upto the volume. From this solution, 2.5 mL aliquot was taken and diluted upto 10 mL with the diluent, filtered by 0.2 µ membrane filter and analysed by HPLC.

5.2.5.2. Preparation of degradation sample of laboratory mixture

The specifications for preparation of the laboratory synthetic mixture are mentioned in Section 3.3. For preparation of degradation sample of lab mixture, 2 mL of prepared laboratory mixture was taken, dissolved in 1 mL MeOH and treated with suitable stressor and the sample was processed as per the conditions stated for standard for various conditions (acid, base and peroxide). For photolytic and dry heat induced degradation, suitable amount (10 mL) of laboratory mixture was taken in a volumetric flask and subjected to stressor treatment as specified for standard API samples. Appropriate dilutions of the degradation samples were then subjected to analysis.

5.2.6. HPLC method validation

Developed Stability indicating RP-HPLC method was validated according to ICH Q2 (R1) guidelines (9) and data complying with the standards were obtained.

The linearity of the HPLC detector response for determination of AZ and BD was evaluated by analysing a series of different concentrations of each compound. The calibration range was established to give accurate, precise and linear results. Seven concentrations were chosen, ranging from 4.28-342.4 µg/mL AZ, and 1-80 µg/mL BD by taking appropriate dilutions from stock solutions and then making up the volume (upto 10 mL) with the diluent and the linearity was determined. The standard mixtures were prepared in the ratio of 4.28:1 for AZ: BD.

For evaluation of the precision estimates, intra-day and inter-day precision were performed (in triplicates) at three concentration levels i.e. 4.28, 85.6 and 342.4 µg/mL for AZ and 1, 20 and 80 µg/mL for BD1 and BD2. The peak areas of the two drugs were calculated for each trial. The experiment was repeated three times in a day for intra-day precision and on three different days for inter-day precision. The precision was calculated in terms of %RSD.

Accuracy was determined by standard addition method at three levels of standard addition *i.e.* 80%, 100%, and 120%. The accuracy concentrations (0, 80%, 100%, 120%) were: 27.4, 49.32, 54.8 and 60.28 µg/mL for AZ and 6.4, 11.52, 12.8 and 14.08 µg/mL for BD. The resulting mixtures were analysed and recovery was calculated.

According to ICH recommendations (9), the approach based on the standard deviation (S.D.) of the y-intercept and the slope was used for determining the limit of detection (LOD) and limit of quantitation (LOQ). Six calibration curves were performed and LOD and LOQ were determined.

Various factors were assessed to check the robustness of the method. The factors such as: pH (2.8, 3.0, 3.2), flow rate (0.7, 0.8, 0.9 mL/min) and initial organic concentration for gradient (39, 40, 41%) were varied and the robustness of method was determined.

The standard stock solutions prepared in the MeOH were kept for 24 h when kept at room temperature and for 48 h when stored in refrigerator (8-25 ºC) and the stability of stock solutions was determined. The reaction mixtures prepared in mobile phase (the final neutralised solutions) were also kept in refrigerator (8-25 ºC) for 24 hours and their stability was also determined.

The specificity of the stability indicating method was determined by analysing the drugs in presence of excipients i.e. in laboratory mixture as well as in the presence of the degradation products.

System suitability parameters such as theoretical plates, symmetry factor and resolution for AZ and BD were calculated for n=6 replicates to study the system suitability of HPLC method.

5.3. RESULTS AND DISCUSSION

5.3.1. RP-HPLC method development

Both drugs showed noticeable degradation after stress oxidation and hydrolysis studies and isocratic elution to resolve all the peaks was not possible. Hence various gradient trials were carried out in the presence of degradation products to separate all the degradation peaks as well as the drug peaks. The trials were carried out using 10 mM ammonium formate buffer pH 3 and ACN. The major separation was to be achieved among some specific peaks viz.: AZ API, AZ peroxide degradation products (DP3 and DP4), BD acid hydrolysis DP (DP1), BD base hydrolysis DP (DP2), BD water hydrolysis DP (DP6), BD peroxide degradation impurity (DP5) and BD peaks (epimers BD1 and BD2). The organic concentration (OC) was varied from 40% to 60%; but the targeted peaks showed good resolution for 40% organic concentration (initial %BI of the gradient) (as stated in Table 5.1). The gradient hold time (for 60% organic as stated in Table 5.1) was varied from 2-10 min. The change in hold time did not had significant effect on the separation of the degradation peaks; however better resolution was obtained for 2 min. The gradient change time (for %BI change from 40% to 60%) was varied from 3-5 min and 5 min gave better results with respect to resolution. The changes in the flow rate proved to be fruitful and finally the optimised gradient elution programme was obtained which could resolve all the targeted four peaks as stated earlier. The efforts were made to separate the epimeric peaks of BD, by altering the change of gradient from 60% OC to upto 95% OC; but a maximum resolution of 0.9 min could be achieved with 80% OC. Some of those trials have been shown in Table 5.2.

Table 5.2 Trials for optimisation of mobile phase

	Mobile Phase	**AZ, DP3, DP4**	**BD (BD1,BD2), DP5**
1.	ACN: BUFF 60:40 FR=1mL/min	merged (splitted) peak AZ (Rt 4.1) DP3(Rt 4.2) DP4 (Rt 4.3)	merged (splitted) peak BD (Rt 7.2) DP5(Rt 6.9)
2.	Gradient [Time(min)-%ACN] 0-50, 5-50, 8-60, 18-60, 20-40, 22-STOP FR=1 mL/min	merged (splitted) peak AZ (Rt 8.2) DP3(Rt 8.5) DP4 (Rt 8.7) RS1=0.3	DP5(Rt 11.2) BD split peak BD1(Rt 11.6) BD2 (Rt=11.9) RS2= 0.4, RS4= 0.3
3.	Gradient [Time(min)-%ACN] 0-40, 5-40, 8-60, 15-60, 17-40, 20-STOP FR=1 mL/min	merged (splitted) peak AZ (Rt 9.5) DP3(Rt 9.9) DP4 (Rt 10.1) RS1=0.4	DP5(Rt 12.3) BD1(Rt 12.8) BD2 (Rt=13) RS2= 0.4, RS4= 0.5
4.	Gradient [Time(min)-%ACN] 0-40, 5-40, 8-60, 15-60, 17-40, 20-STOP FR=0.8 mL/min	AZ (Rt 10.5) DP3(Rt 11.1) DP4 (Rt 11.4) RS1=0.6	DP5(Rt 16.2) BD1(Rt 16.6) BD2 (Rt=16.8) RS2= 0.7, RS4= 0.6
5.	Gradient [Time(min)-%ACN] 0-40, 5-40, 10-60, 15-60, 16-80, 18-80, 20-40, 22-STOP FR=0.6 mL/min	AZ (Rt 12.8) DP3(Rt 13.7) DP4 (Rt 14.1) RS1=0.9	DP5(Rt 14.7) BD1(Rt 15.7) BD2 (Rt=15.9) RS2= 0.8, RS4= 0.6
6.	Gradient [Time(min)-%ACN] 0-40, 5-40, 8-60, 10-60, 12-80, 15-80, 17-40, 20-STOP FR=0.8 mL/min	AZ (Rt 10.4) DP3(Rt 11.3) DP4 (Rt 12.1) RS1=0.9	DP5(Rt 12.7) BD1(Rt 13.9) BD2 (Rt=14.1) RS2= 1.9, RS4= 0.7
7.	Gradient [Time(min)-%ACN] 0-40, 5-40, 10-60, 12-60, 13-80, 14-80, 16-40, 20-STOP FR=0.8 mL/min	AZ (Rt 10.7) and DP3(Rt 11.4) DP4 (Rt 12.3) RS1=1.6	DP5(Rt 13.6) BD1(Rt 14.3) BD2 (Rt=14.5) RS2= 2.3, RS4= 0.9

RS1=resolution between AZ and DP3, RS2=resolution between DP5 and BD1, RS2=resolution between BD1 and BD2,

5.3.2. Forced degradation studies (10, 11, 12)

The results regarding various degradation studies have been stated as follows:-

5.3.2.1. Acid induced degradation (Acid hydrolysis)

Figure 5.1 and 5.2 shows the degradation peaks (DP) of both drugs in standard mixture and laboratory mixture resp. in 1N HCl, for 30 min, at 80ºC temp. Impurity at 5.6 min i.e. DP1, in the chromatogram is the major acid degradation impurity of BD. AZ was very stable under acidic conditions and did not show any degradation when treated with vigorous conditions of reflux at 5M HCl, 80ºC for 72 hours. Hence the stressor condition at which stability of both could be assessed, was selected as, 1N HCl, for 30 min, at 80ºC temperature.

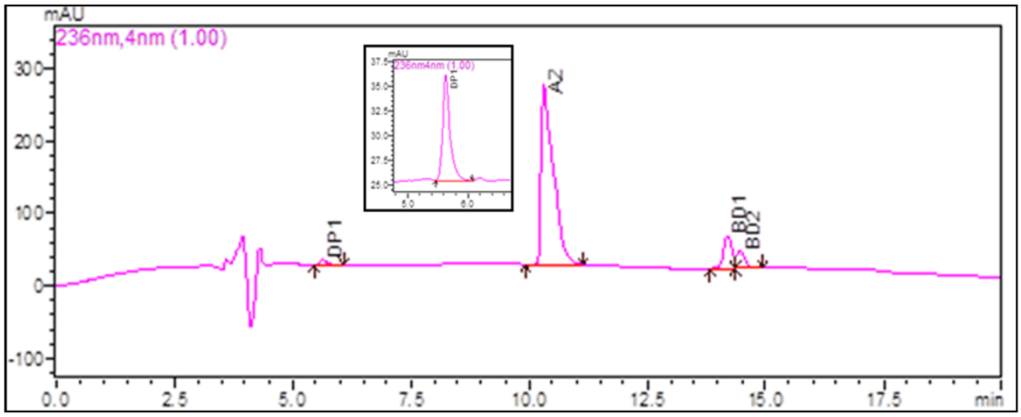


Figure 5.1 Acid degradation of standard mixture of AZ and BD

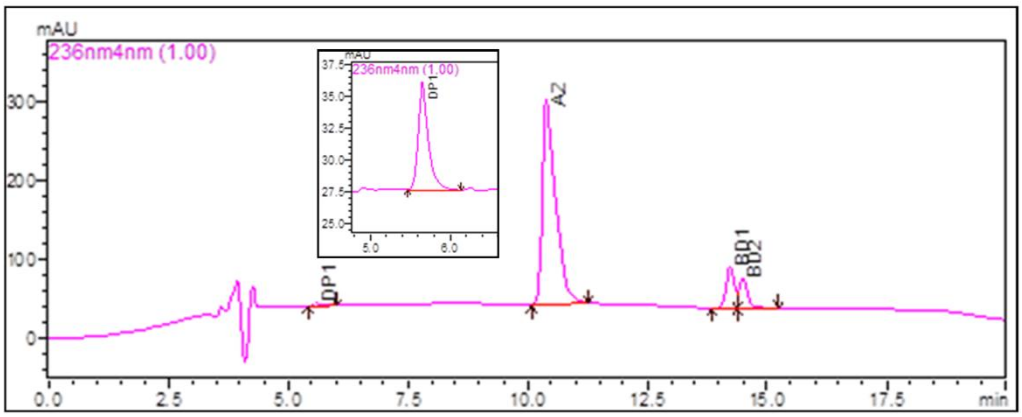


Figure 5.2 Acid degradation of laboratory mixture of AZ and BD

5.3.2.2. Base induced degradation (Base hydrolysis)

BD was very sensitive to alkaline hydrolysis and degraded completely in 1N NaOH in 1 h at room temp. Figure 5.3, 5.4 and 5.5 shows degradation of AZ and BD in 1N NaOH for 30 min in standard mixture, after 1 h in standard mixture and after 1h in laboratory mixture resp. The peak at 7.4 min in Figure 5.4 shows the prominent degradation peak (DP2) of BD. On the other hand, AZ was very stable in basic medium and did not show any degradation in 1M NaOH, for 1 hour at room temperature. As BD degraded completely in 1 hour, the stressor condition at which stability of both could be assessed was selected as 1N NaOH, for 1hour, at room temperature.

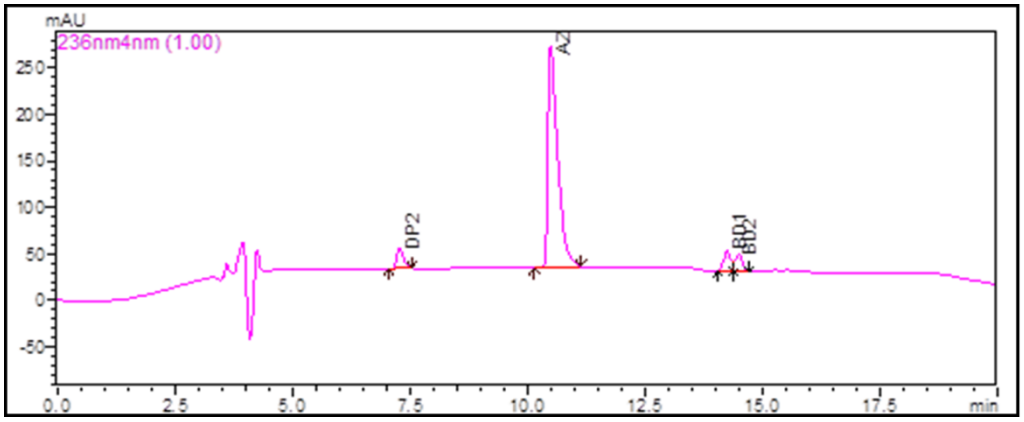


Figure 5.3 Base degradation of standard mixture of AZ and BD (after 30min of duration)

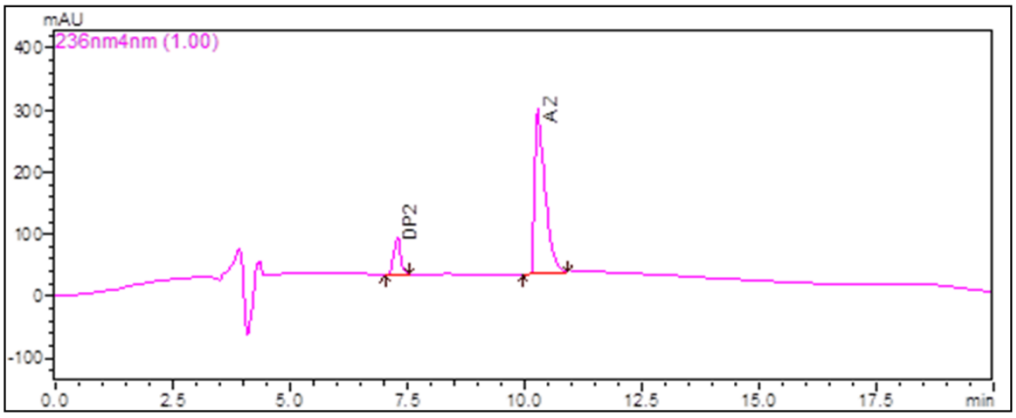


Figure 5.4 Base degradation of standard mixture of AZ and BD (after 1h)

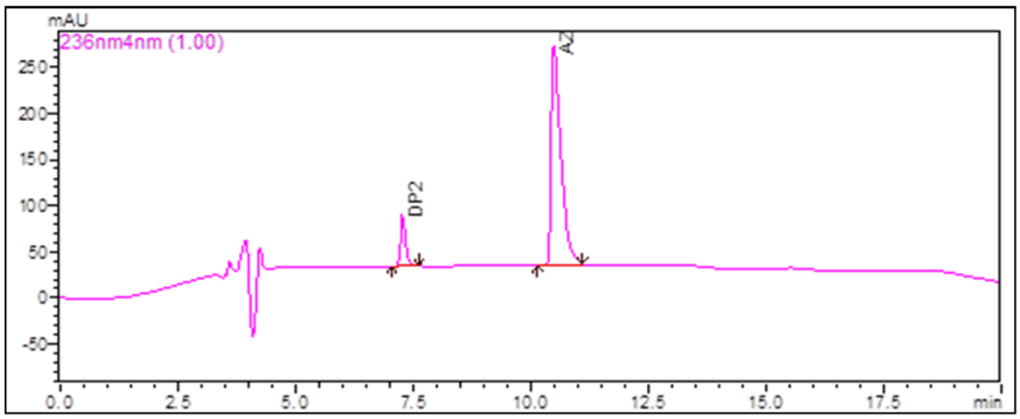


Figure 5.5 Base degradation of laboratory mixture of AZ and BD (after 1 h)

5.3.2.3. Peroxide induced degradation

Both the drugs showed degradation in H_2O_2. The major degradation peaks for both the drugs were obtained in the conditions of 6% H_2O_2 at 80ºC for 30 min. In these conditions two degradation peaks were obtained for AZ (i.e. DP3 Rt=11.4 min and DP4 Rt=12.3 min) and one degradation peak was obtained for BD (DP5 Rt=13.6). In stronger conditions, several small peaks were observed which contributed upto 1-2% of total degradation (which hindered the resolution of main API peaks as well as the DPs). The three major degradation peaks still persisted i.e. DP3, DP4 and DP5. Hence the optimised stressor condition was selected as 6% H_2O_2 at 80ºC for 30 min. Figure 5.6 and 5.7 shows degradation of AZ and BD in 6% H_2O_2 for 30 min in standard mixture and laboratory mixture respectively.

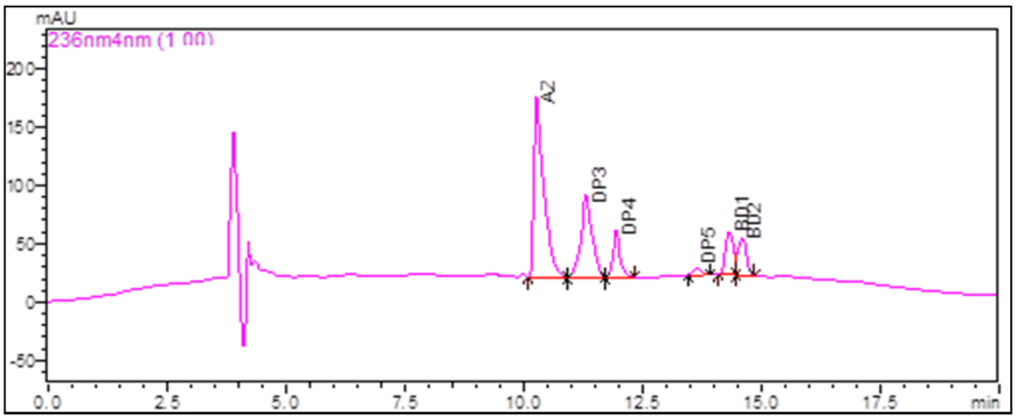


Figure 5.6 Peroxide degradation of standard mixture of AZ and BD

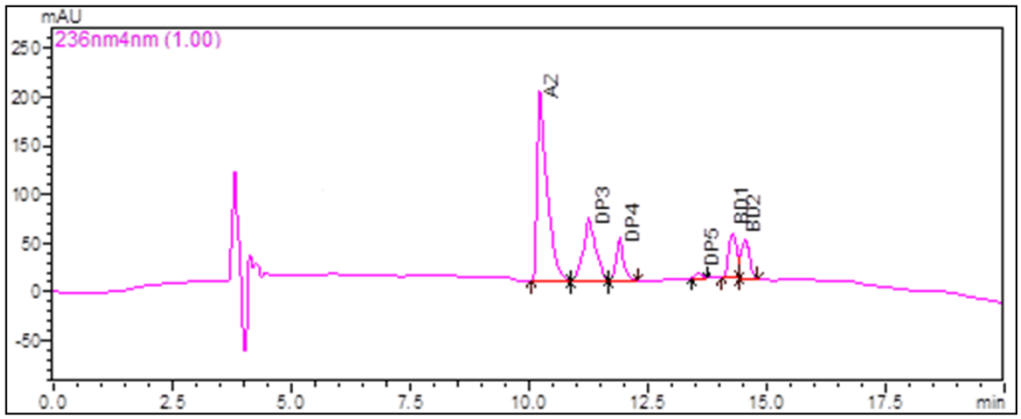


Figure 5.7 Peroxide degradation of laboratory mixture of AZ and BD

5.3.2.4. Photolytic degradation

The photolytic exposure carried out at 5382 LUX and 144 UW/cm^2 for 21 days did not induce significant degradation on the APIs as well as their mixtures. Figure 5.8 and 5.9 shows degradation of AZ and BD in photolytic degradation conditions for standard mixture and laboratory mixture resp. Very small amount of degradation was observed upto 4-5%.

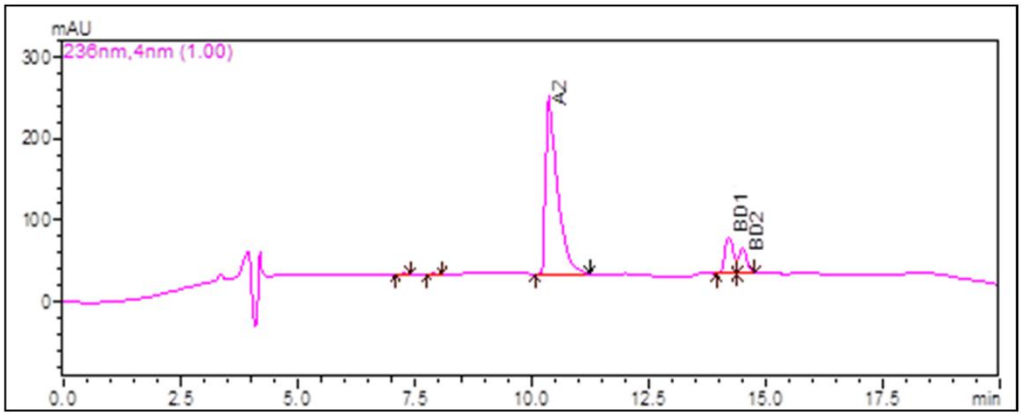


Figure 5.8 Photolytic degradation of standard mixture of AZ and BD

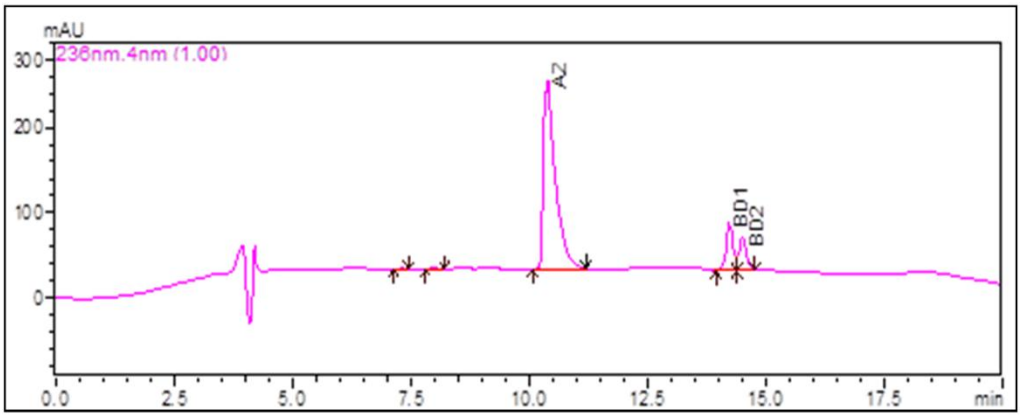


Figure 5.9 Photolytic degradation of laboratory mixture of AZ and BD

5.3.2.5. Neutral hydrolysis

BD showed remarkable degradation in H_2O. The peak at 7.5 min in Figure 5.10 and 5.11 shows the degradation peak of BD (DP6). On the other hand, AZ was very stable under aqueous conditions and did not showed any degradation when treated with vigorous conditions at 80 ºC in double distill water for six hours. As BD was very sensitive to neutral hydrolysis, hence the stressor condition at which stability of both could be assessed was for 3 h, at 80 ºC temp.

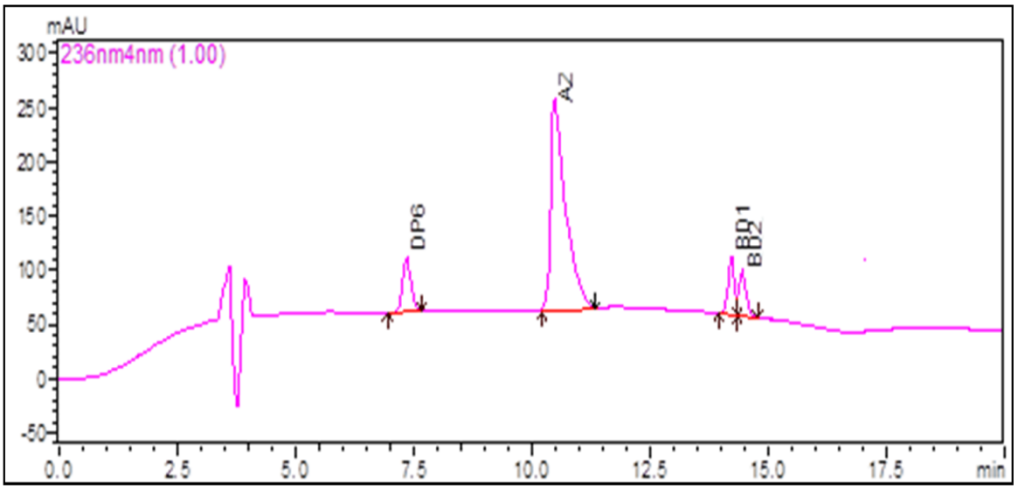


Figure 5.10 Neutral hydrolysis of standard mixture of AZ and BD

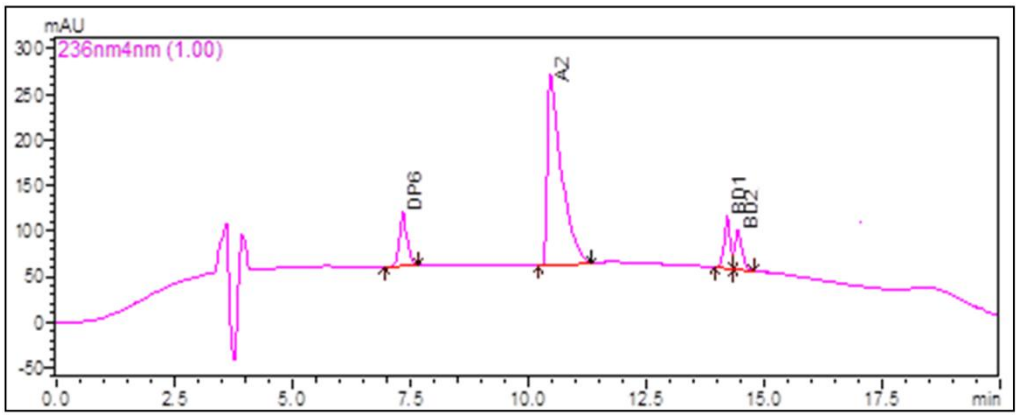


Figure 5.11 Neutral hydrolysis of laboratory mixture of AZ and BD

5.3.2.6. Dry heat induced degradation

The dry heat degradation was carried out at 80 ºC for 14 days for the API mixture. The degradation conditions did not have a significant effect on the APIs. The API of BD had a colour change from white to brown but there was no significant decrease in the peak area and neither any visible degradation peak was observed even after 14 days. Figure 5.12 shows degradation of AZ and BD in dry heat degradation conditions for standard mixture. The formulation was in the form of nasal spray (i.e. aqueous matrix) and hence was not subjected to dry heat degradation.

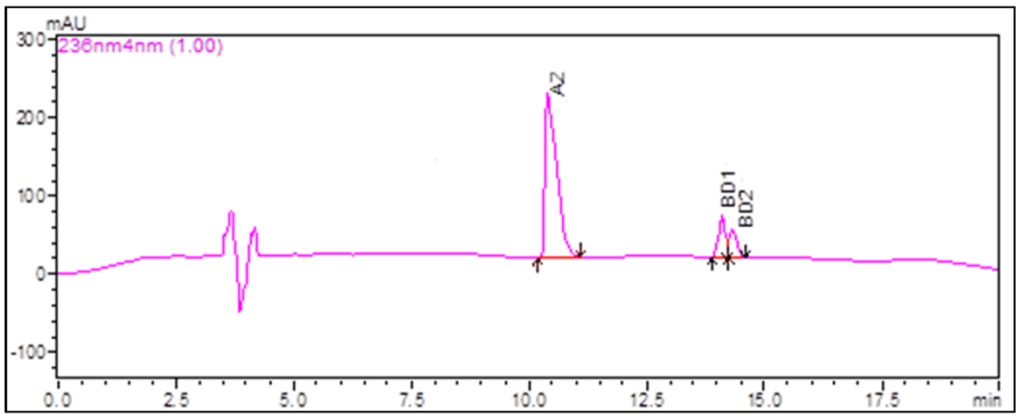


Figure 5.12 Dry heat induced degradation of standard mixture of AZ and BD

5.3.3. Specific stability indicating method

A specific SIAM is the one which resolves the API under study from all its degradation products. Hence the method was developed by combining all the degradation mixture samples. Figure 5.13 shows the blank chromatogram, Figure 5.14 shows the chromatogram of the assay of laboratory mixture and Figure 5.15 shows the chromatogram with combined degradation products of all conditions applied for the laboratory mixture. All the degradation products were well resolved from the main API peaks as seen in Figure 5.15.

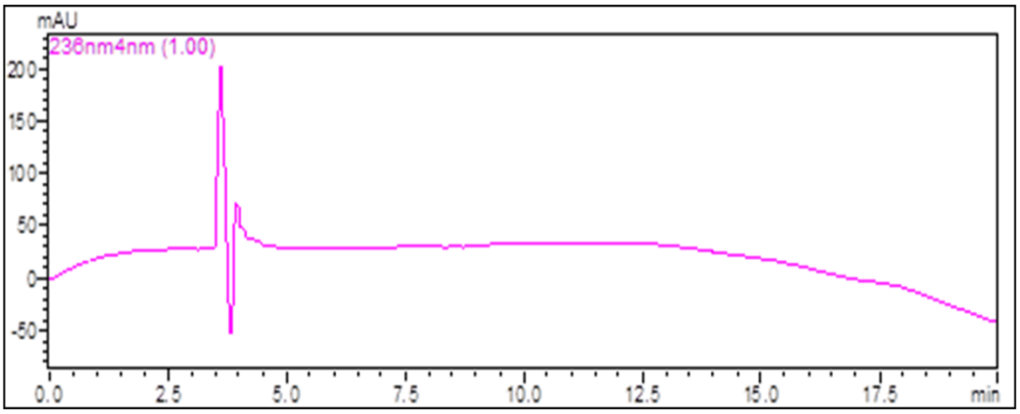


Figure 5.13 Blank chromatogram of combined degradation products

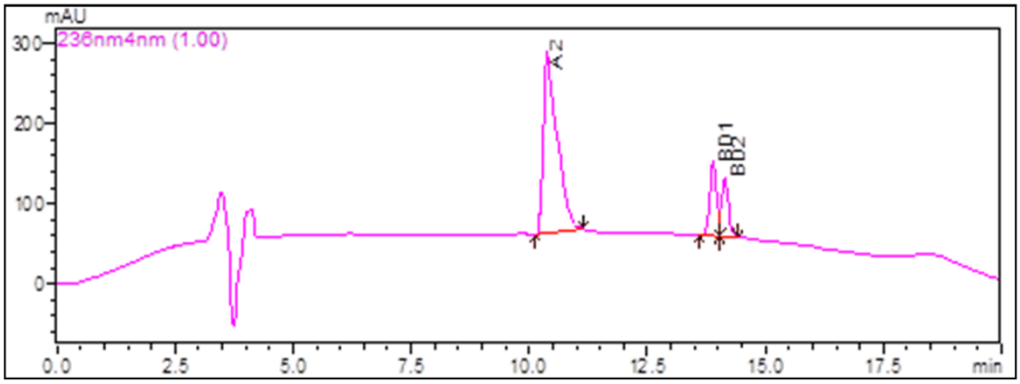


Figure 5.14 Chromatogram of laboratory mixture solution AZ (137 ppm) and BD (32 ppm)

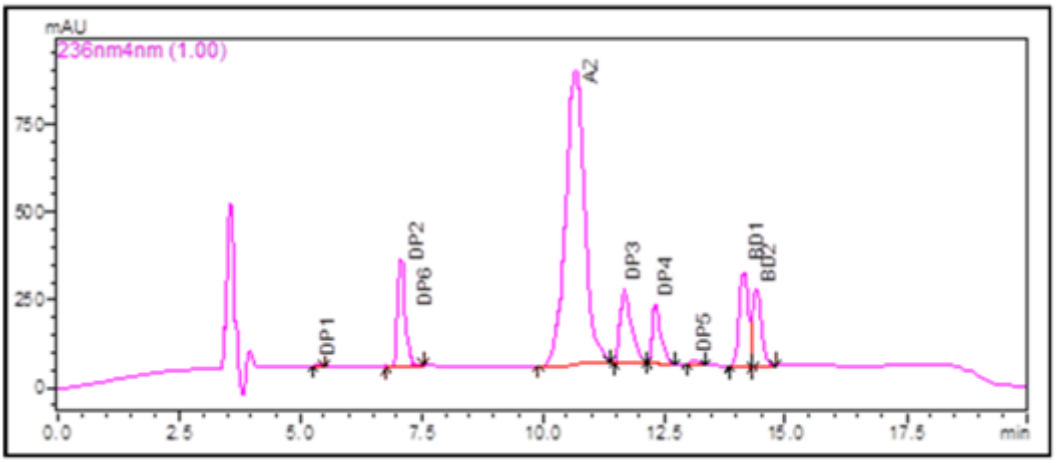


Figure 5.15 Chromatogram showing combined degradation products of all stressors on laboratory mixture

5.3.4. Summary of forced degradation studies

A simple, sensitive, selective, precise and stability-indicating method for determination of AZ and BD in bulk drug and laboratory mixture was developed and validated as per the ICH guidelines (10, 11, 12). AZ was found to be comparatively stable in various conditions like acidic, alkaline, dry heat, wet heat and photolytic whereas BD showed degradation in acidic, alkaline and wet heat conditions. AZ showed degradation only in oxidative condition. However literature reports the degradation of AZ in acid, base and neutral hydrolysis (7). The brief summary of all degradation conditions and their significance in terms of percentage of degradation has been given in Table 5.3.

Table 5.3: Summary of all forced degradation conditions

Degradation Type	Degradation Condition		%Degradation API	
			AZ	BD
Acid hydrolysis	1M HCl, 30 min, 80 ºC	APIM	2.12 ± 0.95	13.31 ± 0.48
		LM	1.25 ± 0.38	10.32 ± 0.7
Base hydrolysis	1MNaOH, 1hour, 80 ºC	APIM	2.05 ± 0.72	100.00 ± 0.0
		LM	1.86 ± 0.56	100.00 ± 0.0
Peroxide	6% H_2O_2,30 min, 80 ºC	APIM	33.68 ± 1.01	12.47 ± 0.47
		LM	29.75 ± 0.64	11.19 ± 0.63
Photolytic	5382 LUX and 144UW/cm^2, 21 days	APIM	6.39 ± 0.47	4.95 ± 0.24
		LM	5.52 ±0.59	3.15 ± 0.64
Neutral hydrolysis	3 hours, 80 ºC	APIM	4.73 ± 0.42	53.11 ± 0.69
		LM	3.97 ±0.58	47.73 ± 0.42
Dry heat	80 ºC, 14days	APIM	3.75 ± 0.41	4.91 ± 0.60

APIM=Standard mixture of AZ+BD

5.3.5. Characterisation of degradation products on the basis of LC-MS/MS analysis

Stress degradation of AZ and BD indicated formation of a number of degradation products. Efforts have been made to understand the degradation mechanism of AZ and BD by LCMS studies. The degradation products thus studied have been stated in Table 5.4.

Table 5.4 Degradation products considered for LC-MS/MS characterisation

Degradation condition	Drug	Rt (min)	Degradation product code
Acid induced degradation	BD	5.6	DP1
Base induced degradation	BD	7.4	DP2
Oxidative degradation	AZ	11.4, 12.3	DP3, DP4
	BD	13.6	DP5
Neutral Hydrolysis	BD	7.5	DP6

The retention time of AZ, BD1 and BD2 were found to be 10.5, 14.3 and 14.5 resp.

5.3.5.1. Acid induced degradation

BD showed 13% degradation by acidic hydrolysis, the degradant, DP1, had retention time at 5.6 min. The LC-MS/MS spectra for DP1 is given in Figure 5.16. The LC-MS studies showed the molecular ion of DP1 at 376 m/z. Further, the LC-MS/MS plot showed the major fragments at 359 m/z and 323 m/z. On the basis of these results the possible structure and the degradation pathway for DP1 has been given in Figure 5.17. The isomeric forms for 358 m/z could not be confirmed.

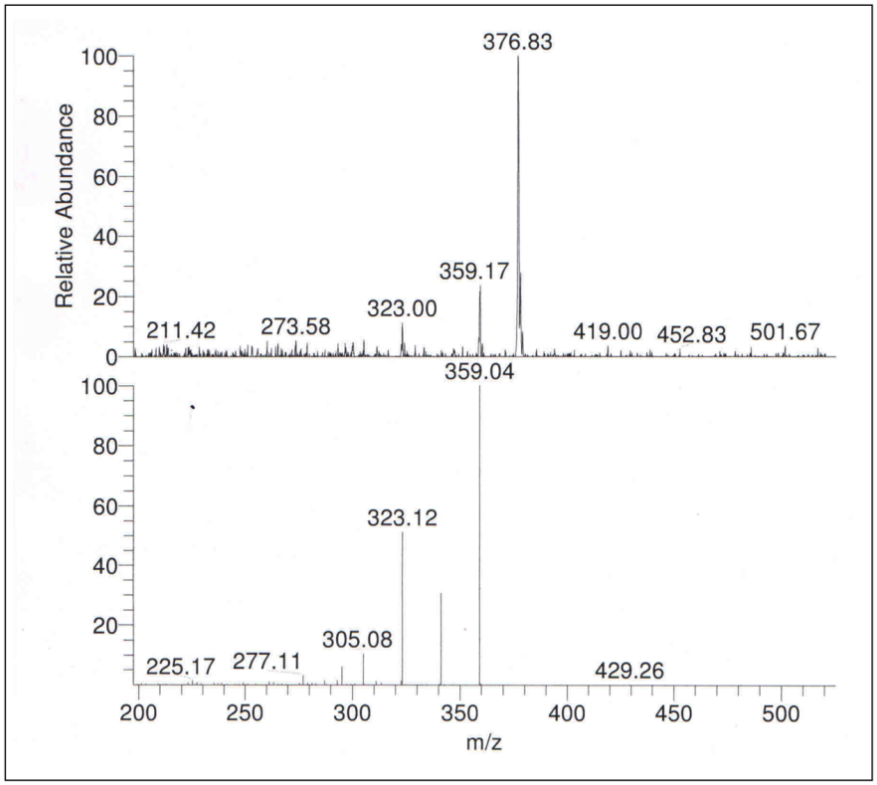


Figure 5.16 LC-MS/MS of acid induced degradation impurity of BD

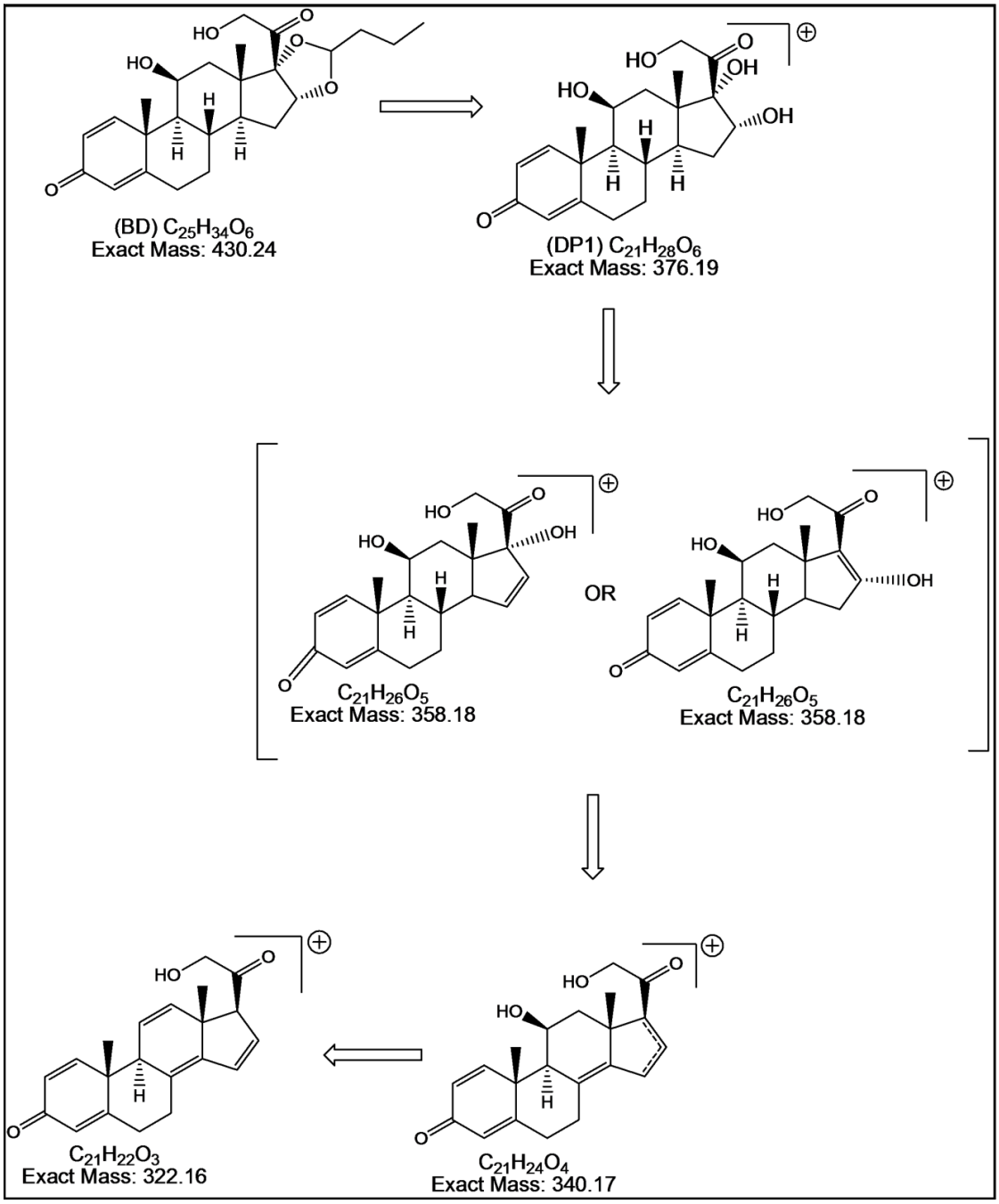


Figure 5.17 The degradation pathway for the formation of DP1 from acid hydrolysis of BD

5.3.5.2. Base induced degradation and Neutral hydrolysis of BD

For BD, the degradation products (DP2 and DP6) obtained by base and neutral hydrolysis were eluted at same Rt i.e. 7.5 min. The LC-MS/MS data for base and neutral hydrolysis, the LC-MS/MS spectra are given in Figures 5.18 and 5.19 resp. Both the degradation products (DP2 and DP6) were similar that was further confirmed by LC-MS data, which showed same m/z ratio of 359 and similar fragmentation pattern. Hence it was concluded that the degradation products (DP2 and DP6) obtained from base hydrolysis and neutral hydrolysis for BD are the same. The structures of the DPs proposed on the basis of the LC-MS/MS fragmentation pattern and their degradation pathway has been shown in Figure 5.20. The fragments of 341 m/z and 323 m/z ratio were similar to that found for the acid degradation product.

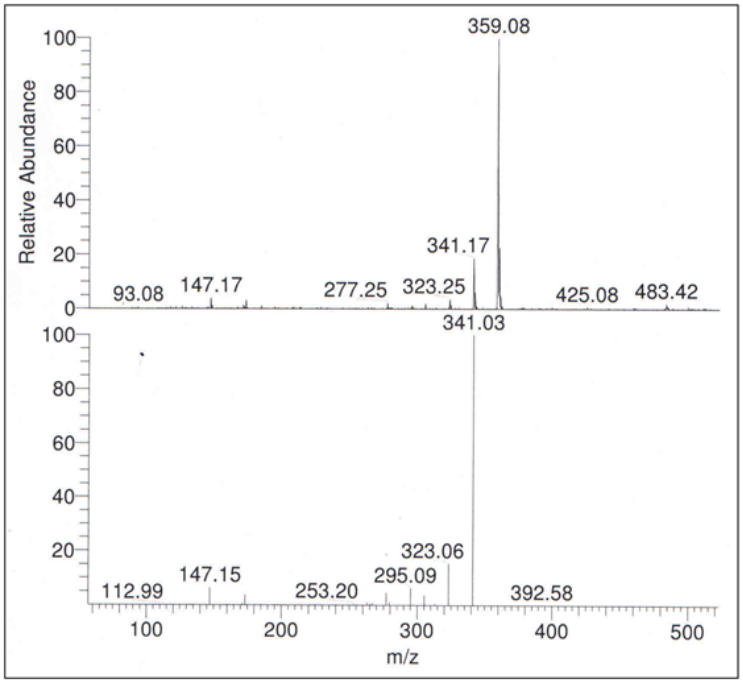


Figure 5.18 LC-MS/MS of base induced degradation impurity of BD (DP2)

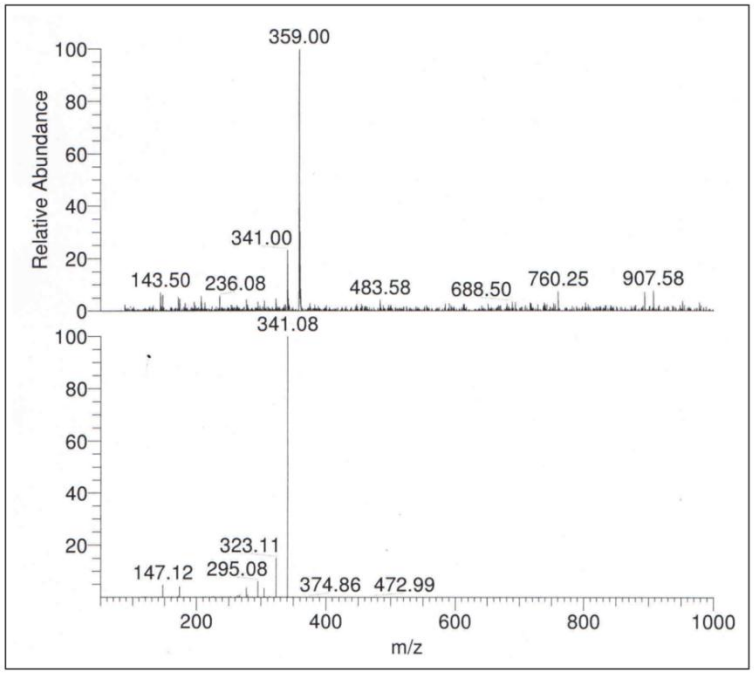


Figure 5.19 LC-MS/MS of neutral hydrolytic degradation impurity of BD (DP6)

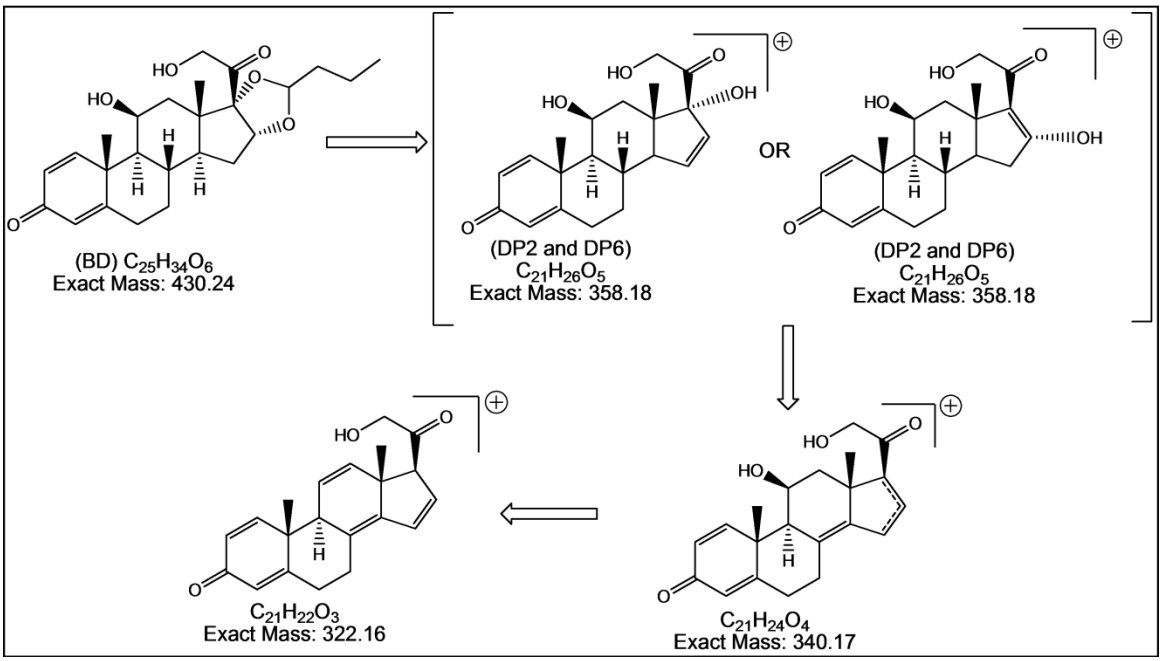


Figure 5.20 The degradation pathway for the formation of DPs from base hydrolysis (DP2) neutral hydrolysis (DP6) of BD

The degradation of BD in alkaline medium has been reported earlier. Bajaj et al (1), have found the mass of alkaline degradation product as 343 $[M+H]^+$ at the conditions

of 85 ºC for 2 h and have also proposed the structure of the degradation product. Jansen et al (3), report the alkaline degradation of BD in nebulizer solutions.

The mass obtained for the BD alkaline degradation product (DP2) in present study, is different from the reported one as the conditions at which degradation is reported in reference (1) are vigorous as compared to the present work.

5.3.5.3. Peroxide induced degradation

5.3.5.3.1. Characterisation for oxidative degradation product of AZ

The oxidative degradation of AZ gives two degradation products DP3 and DP4 with the retention times 11.4 and 12.3 min resp. The LC-MS/MS spectra for DP3 and DP4 has been given in Figure 5.21 and 5.22 resp. The LC-MS data gave the m/z as 398.17 and 398.33 for DP3 and DP4 resp. The LC-MS fragmentation was same for both DPs (DP3 and DP4) and based on that the possible structures and degradation scheme for DP3 and DP4 are shown in Figure 5.23.

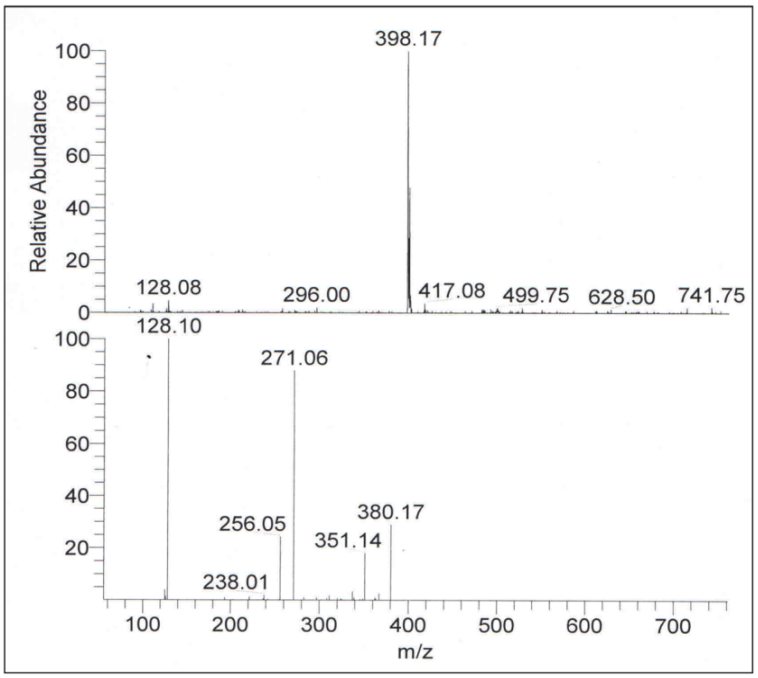


Figure 5.21 LC-MS/MS of peroxide induced degradation product of AZ (DP3)

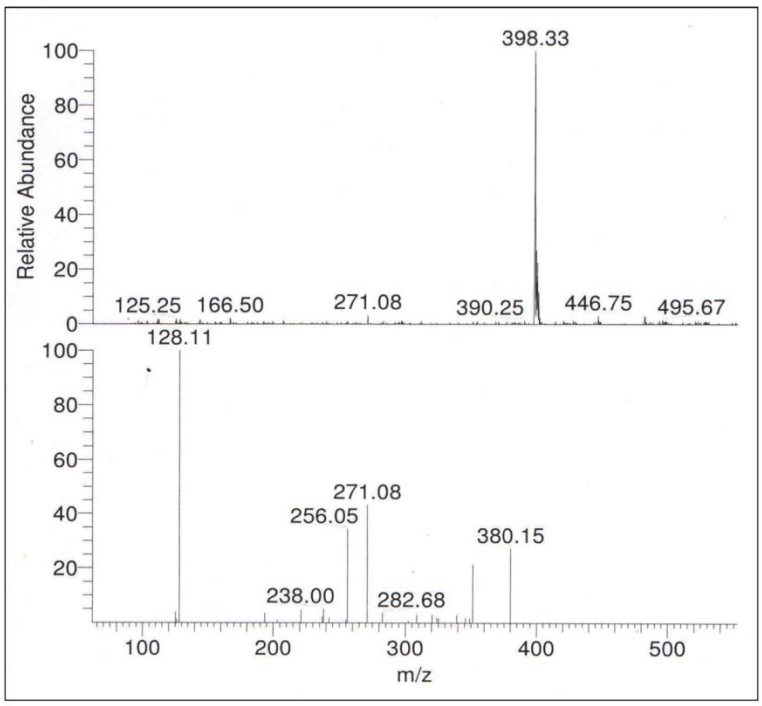


Figure 5.22 LC-MS/MS of peroxide induced degradation product of AZ (DP4)

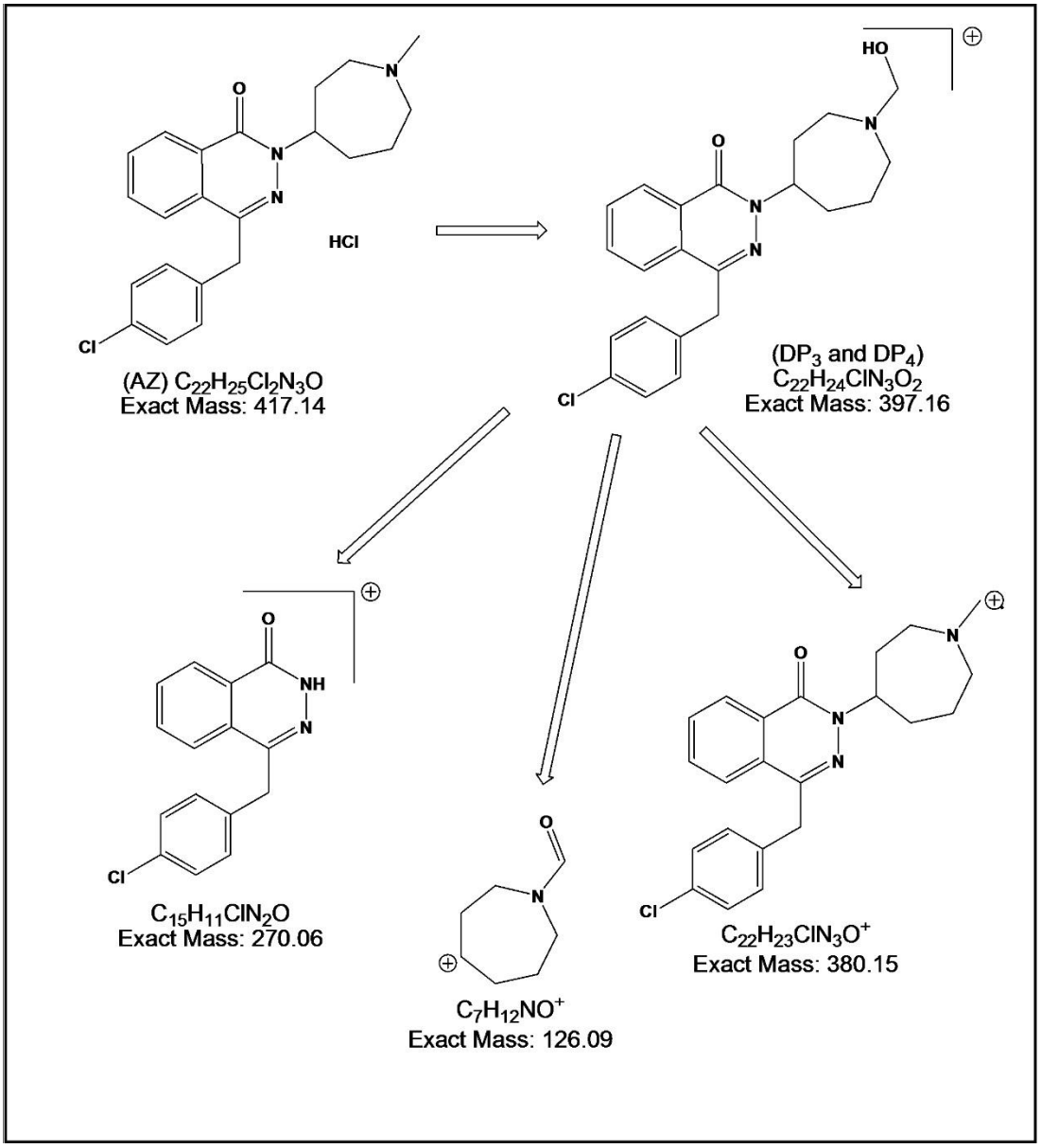


Figure 5.23 The degradation pathway for the formation of DPs (DP3 and DP4) from oxidative degradation of AZ

The degradation of AZ in peroxide has been reported by Yamada et al (7), where two degradation peaks of mass 369 $[M+2H]^{++}$ and 408 $[M+Na]^{+}$, have been obtained from the peroxide degradation at the conditions of 9% v/v H_2O_2, 80 ºC for 10-40 min. The masses obtained in the literature are quite different from the results obtained in the present study.

5.3.5.3.2. Characterisation for oxidative degradation product of BD

The oxidative degradation of BD gives a degradation product DP5 at the retention time 13.6 min. The LC-MS/MS spectra for DP5 is given in Figure 5.24. The LC-MS data gave the m/z as 417 for DP5. Based on that the possible structure and degradation scheme for DP5 is shown in Figure 5.25.

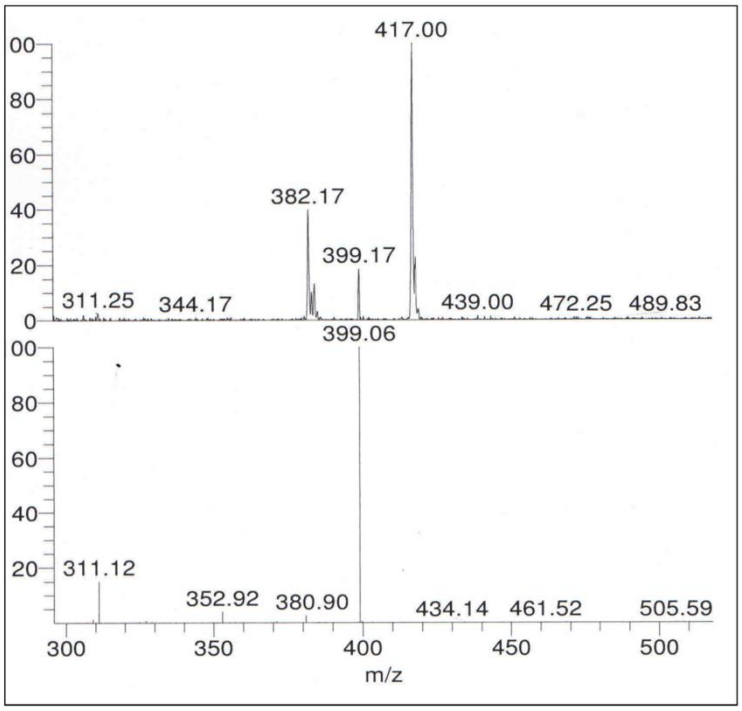


Figure 5.24 LC-MS/MS of peroxide induced degradation impurity of BD (DP5)

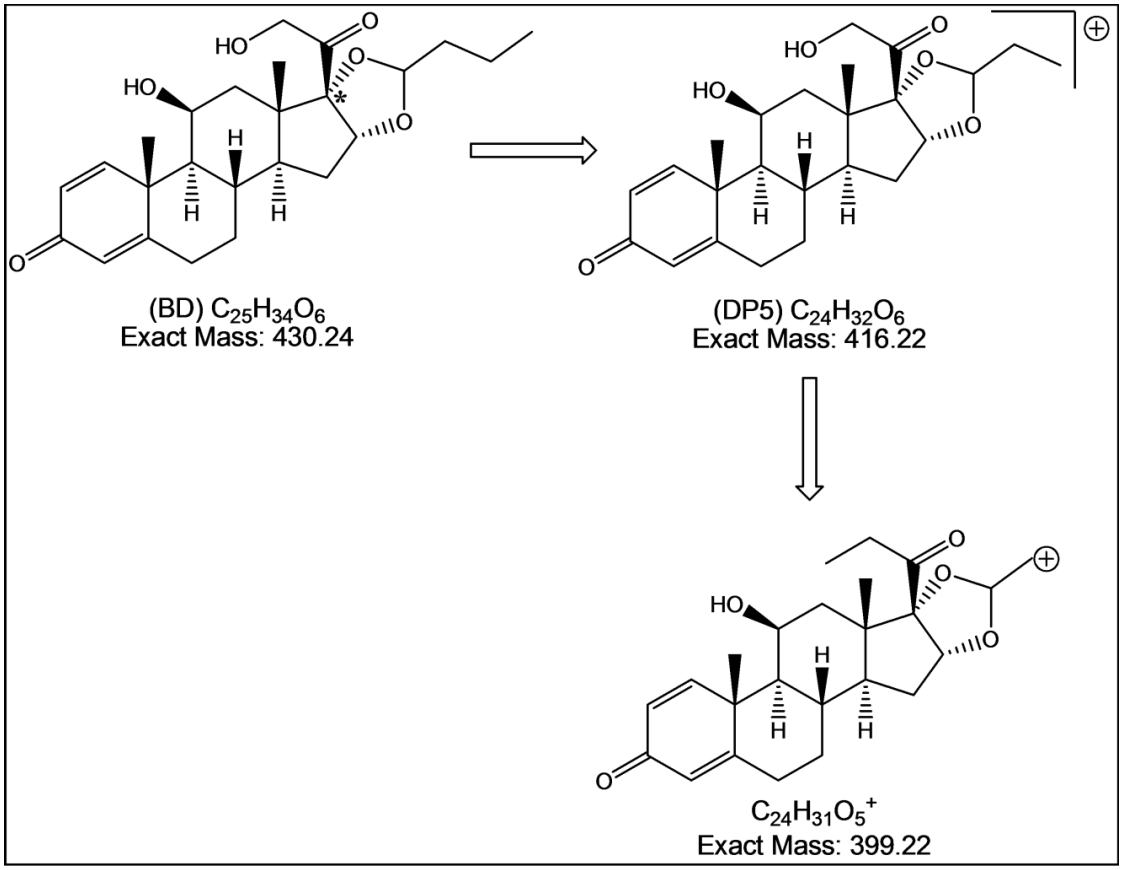


Figure 5.25 The degradation pathway for the formation of DP5 from oxidative degradation of BD

5.3.5.4. Epimers of BD

The two peaks of BD with somewhat poor resolution (0.8-0.9) were obtained in the gradient method thus developed. Literature reveals the evidence of the epimeric peaks of BD (2). Hence LC-MS/MS analysis was carried out for the two peaks for their epimeric confirmation. As seen in the LC-MS/MS figures (Figure 5.26 and 5.27 resp.), the two peaks of BD i.e. BD1 and BD2, had same mass fragmentation and hence it was concluded that the two peaks are the epimers of BD. The structure of BD with its epimeric centre is given in Figure 5.28.

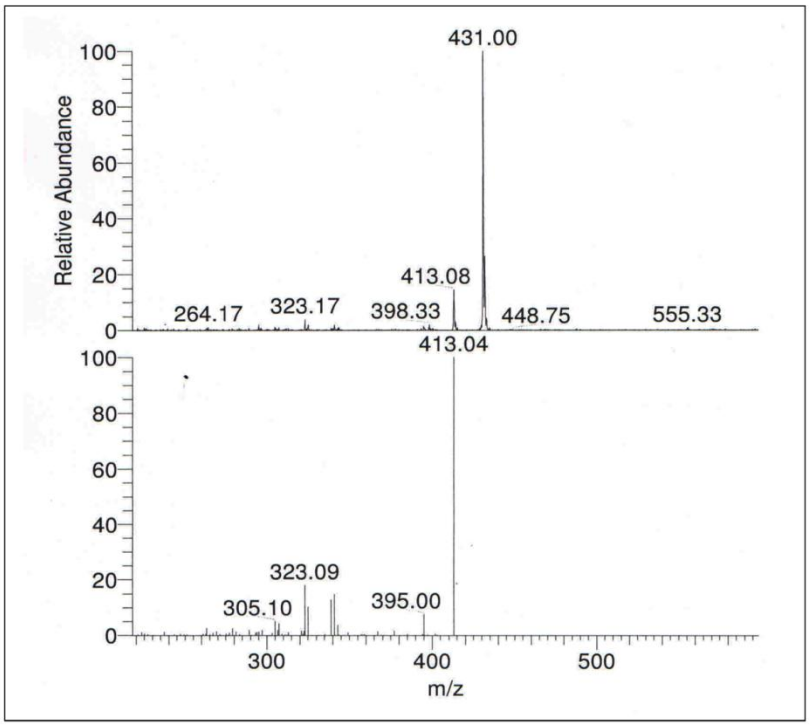


Figure 5.26 LC-MS/MS spectra of BD1 peak

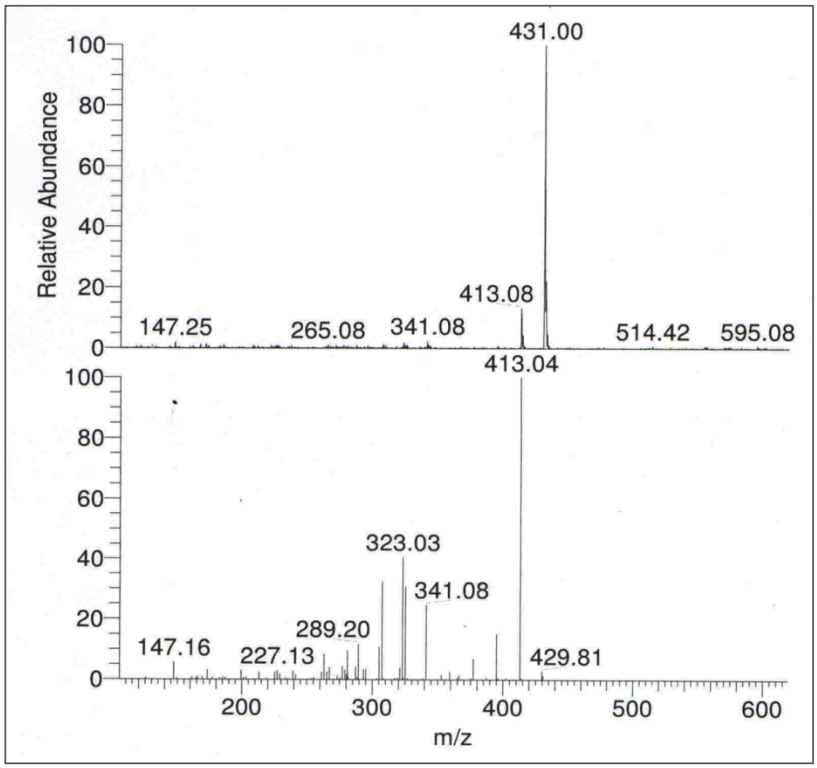


Figure 5.27 LC-MS/MS spectra of BD2 peak

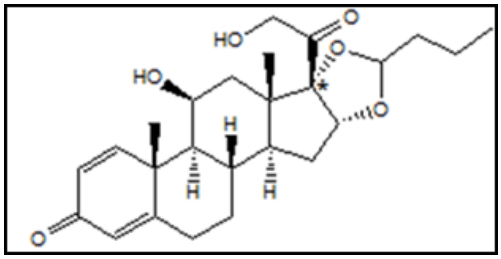


Figure 5.28 Structure of BD (mass= 430) showing its epimeric centre(*)

5.3.6. Summary of validation parameters of the developed SIAM

Retention time for AZ, BD1 and BD2 (BD epimers) was found to be 10.5 min and 14.3 and 14.7 min respectively. The overlay chromatograms and the calibration curves plotted for linearity of AZ and BD are shown in Figures 5.29 and 5.30 respectively. The regression statistics have been given in Table 5.5. The precision results in terms of % RSD, the accuracy results in terms of the percentage recovery± % RSD and the LOD and LOQ values are given in Table 5.5. The method was found to be robust for the pH change of ±0.2 units and flow rate change of ±0.1 mL/min and for initial %B of gradient (±1%). The results are shown in Table 5.5.

The standard solutions prepared in the MeOH exhibited no chromatographic changes and no significant change in the peak area was observed for 24 h when kept at room temperature and for 48 h when stored in refrigerator (8-25 ºC). No additional peak was found in the chromatogram which indicated the stability of the standard solutions under study. The reaction mixtures samples were also found to be stable for 24 h in refrigerator.

The results of the HPLC method validation have been summarised in Table 5.5.

Table 5.5 Summary of validation parameters

Parameters	AZ	BD1	BD2
Calibration range (µg/mL)	4.28-342.4	1-80	1-80
LOD (µg/mL)	0.63	0.45	0.60
LOQ (µg/mL)	1.90	1.37	1.82
Regression equation	y = 51293x – 175923	y = 25726x + 3003.1	y = 21969x + 246464
Correlation coefficient (r^2)	0.9992	0.9998	0.9992
Accuracy	Percentage recovery ± SD		
80%	98.79 ± 0.61	98.86 ± 1.02	100.05 ± 0.47
100%	100.60 ± 0.85	99.80 ± 0.36	98.80± 0.94
120%	98.47 ± 1.01	99.30 ± 1.54	99.57 ± 0.71
Precision		%RSD	
Intraday	0.40	1.40	0.26
Interday	0.44	1.46	0.66
Robustness		Mean± SD	
pH	10.69±0.04	14.32±0.07	14.72±0.03
Flow rate	10.73±0.15	14.39±0.12	14.74±0.09
Organic conc.	10.74±0.07	14.35±0.06	14.73±0.05

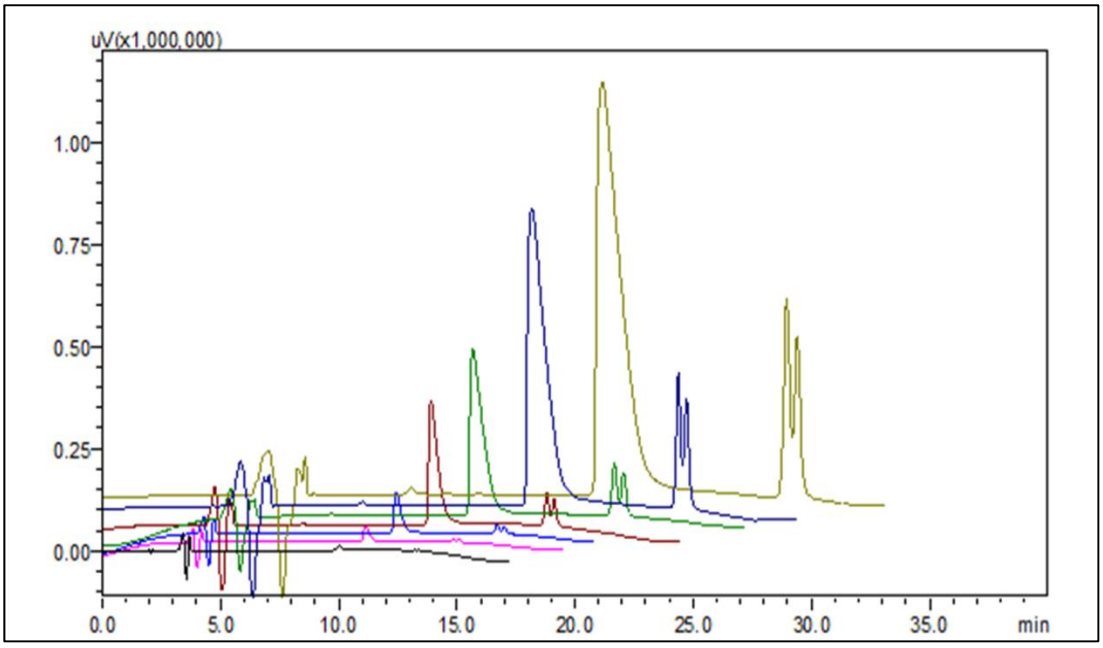


Figure 5.29 Overlay chromatogram showing AZ (1st peak) and BD (2nd peak for epimers of BD i.e. BD1 and BD2)

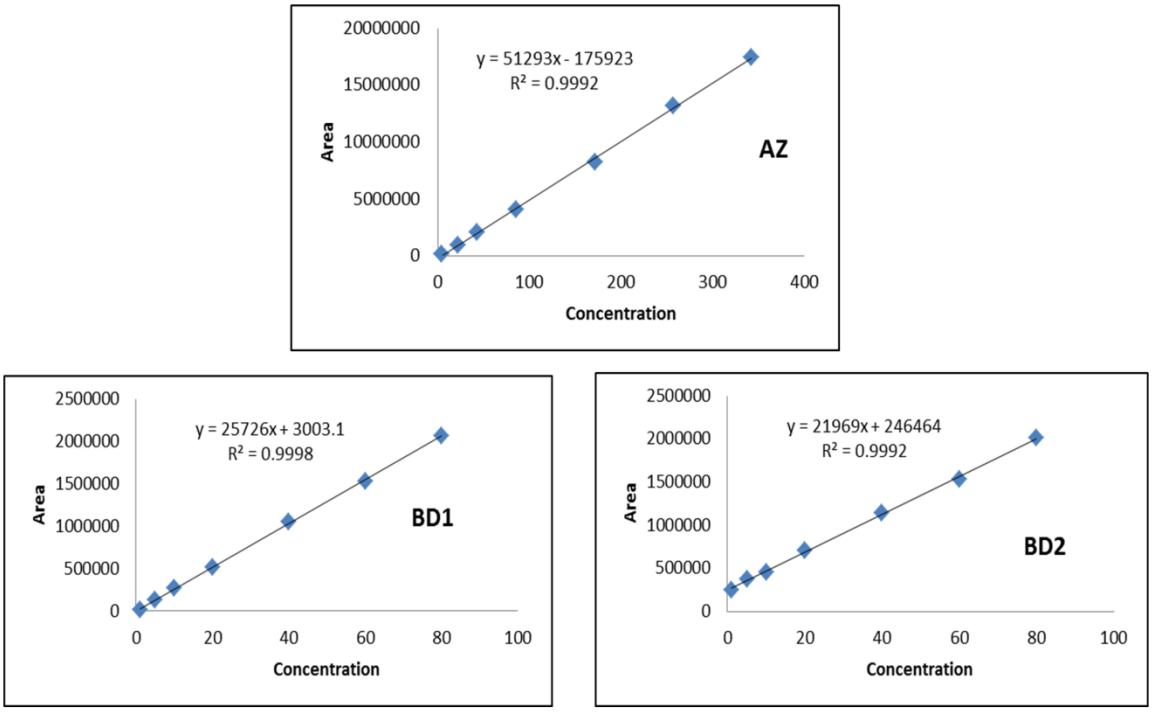


Figure 5.30 Calibration graphs of AZ, BD1 and BD2

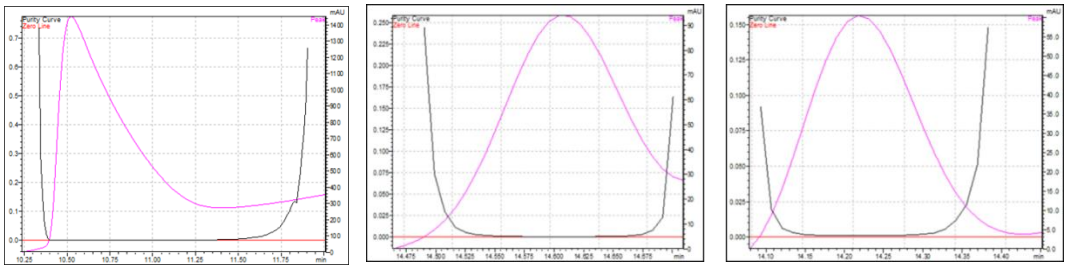
Satisfactory results were obtained for the system suitability of HPLC method as shown in Table 5.6.

Table 5.6 System Suitability Parameters for the developed HPLC method

Parameters	AZ	BD1	BD2
Retention Time	10.68 ± 0.08	14.32 ± 0.16	14.54 ± 0.06
Theoretical Plates	4352.20 ± 1.77	42186.84 ± 1.35	45857.34 ± 1.43
Tailing factor	1.39 ± 0.03	1.03 ± 0.13	1.39 ± 0.05
Resolution	5.96 ± 0.53	1.73 ± 0.12	0.92 ± 0.08

Mean±standard deviation for n=6 replicates

The peak purity curves have been shown in Figure 5.31 and the peak purity results are given in Table 5.7.



AZ BD1 BD2

Figure 5.31 The peak purity curves of AZ, BD1 and BD2

Table 5.7 Peak Purity results

Drug Name	Peak Purity Index	Threshold
AZ	1.0000	0.9999
BD1	0.9999	0.9994
BD2	0.9999	0.9989

5.4. CONCLUSION

A specific stability indicating RP-HPLC method was developed for estimating AZ and BD in presence of their degradation products. A well resolved chromatogram for AZ and BD in presence of its six major degradation products could be obtained. The method was successfully validated for linearity, accuracy, precision, LOD and LOQ.

Hence, the developed stability indicating analytical RP-HPLC method could be successfully applied for estimation of AZ and BD in bulk and laboratory mixture. The characterisation of degradation products was done successfully on the basis of the LC-MS data and the structures of major degradation impurities alongwith the structure of its fragments were revealed.

5.5. REFERENCES

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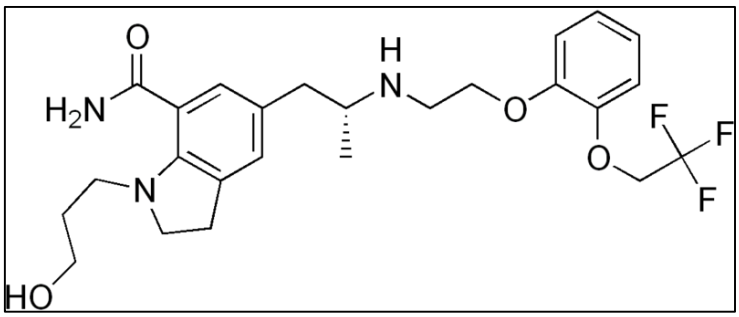
DEVELOPMENT OF STABILITY INDICATING ANALYTICAL METHOD FOR SILODOSIN IN BULK DRUG AND FORMULATION: DEGRADATION KINETICS AND CHARACTERISATION OF MAJOR DEGRADATION PRODUCTS BY LC-MS/MS TECHNIQUE

6.1. SELECTION OF DRUG

Silodosin (SDN) is a highly selective third generation α1A-adrenoceptor antagonist, approved by FDA in 2008 for the treatment of benign prostatic hyperplasia (BPH) (1). As not much data on stability was available at the time of undertaking the study, the drug was selected for developing a stability indicating analytical method and to study the degradation products in detail. While the research was being conducted several research papers were published reporting the degradation profiles of Silodosin. In the present study the degradation of Silodosin was carried out to get more insight about the degradation products, their formation pathways and their kinetics.

6.2. DRUG PROFILE (2)

SILODOSIN (SDN)



a. Category : Selective alpha-1 adrenergic receptor antagonist

b. Molecular formula : $C_{25}H_{32}F_3N_3O_4$

c. Molecular Weight : 495.53

d. Nomenclature: 1-(3-Hydroxypropyl)-5-[(2R)-2-({2-[2-(2,2,2-trifluoroethoxy) phenoxy]ethyl}amino)propyl]-7-indolinecarboxamide

e. Physicochemical Properties:

i. Description: White crystalline powder

i. Solubility: Freely soluble in MeOH, ACN

ii. log P:3.04

iii. pKa: 8.77

iv. Melting Point: 108-112^{0}C

f. Official Status: Official in USP and BP.

6.3. MARKETED FORMULATION

Marketed formulation: RAPILIF Capsule (IPCA LAB. LTD) was used for analysis. Each capsule consists of 4 mg or 8 mg Silodosin.

6.4. LITERATURE REVIEW

Several methods have been described in the literature for the determination of Silodosin, such as: spectrophotometric methods (3, 4, 5); visible spectrophotometric method (6, 7); spectrofluorimetric methods (8); voltammetric method (9); HPTLC method (10); HPLC methods (11-14); bionalytical method by LC-MS/MS (15), LC method for determination of the enantiomeric purity of Silodosin (16) and RPHPLC method for determination of Silodosin in combination with Dutaseride (17). Stability indicating HPLC method (18) and UPLC method (19, 20) are available in the literature for SDN. The comparison and variability in these literatures have been stated in Table 6.1. The data was found to be varied in different works with respect to the extent of degradation hence it was thought of further investigation of the degradation study of SDN and hence its products. So the present study was carried out.

Table 6.1 Comparison of literature data for SIAM of SDN

Literature Ref. No. (Type of method)	Degradation conditions	% degradation observed	Other information
18. (RP-HPLC)	Acid (0.1N HCl, 10 min , RT	84.88	--
	Base (0.1N NaOH, 10 min, RT)	87.64	
	Peroxide (10% H_2O_2, 10 min, RT)	82.69	
19. (UPLC)	Acid (1N HCl)	3.97	(i) 4 process related impurities. (ii) SDN IMP 4 structure same as std SDN (but IUPAC different).
	Base (1N NaOH)	4.8	
	H_2O_2 (3%)	Not mentioned	

20. (UPLC)	Acid (0.1N HCl, 90ºC, 7h)	4.63	4 process related impurities
	Base (0.1N NaOH, 90 ºC, 7h)	0.27	
	Peroxide (0.05% H_2O_2, 10 min, RT)	14.82	
	Thermal (105 ºC, 2 days)	4.64	

6.5. EXPERIMENTAL

6.5.1. Instrumentation

Chromatography was performed on Shimadzu (Shimadzu Corporation, Kyoto, Japan) chromatographic system equipped with Shimadzu LC-20AD and Shimadzu PDA-M20A Diode Array Detector. Samples were injected through a Rheodyne 7725 injector valve with fixed loop at 20 µL. Data acquisition and integration was performed using LC Solution software (Shimadzu Corporation, Kyoto, Japan). Separation and quantitation were made on Phenomenex RPC18 column (5µm × 250mm × 4.6mm i.d.).

The LC-MS/MS analysis was carried for the identification of degradation products. The LC-MS/MS system (Thermo Fisher Scientific LCQ Fleet) coupled with ion trap mass spectrometer and quaternary pump delivery module was used for the analysis. Data acquisition was done on Xcalibur software.

6.5.2. Materials and reagents

Silodosin was purchased from Hangzu Dayang, Co., China. The formulation of Rapilif capsule (IPCA laborataries), procured from local pharmacy, was used for analytical application HPLC grade MeOH and ACN (Spectrochem), potassium dihydrogen phosphate (AR grade, LobaChem), triethylamine (HPLC grade, Spectrochem), ammonium acetate (AR grade, LobaChem) were used for the analysis. AR grade hydrochloric acid (Merck), Sodium hydroxide (SDFineChem), hydrogen peroxide (Spectrochem) were used for degradation purpose.

6.5.3. Chromatographic conditions

For RPHPLC, phosphate buffer (0.01 M) was prepared by dissolving 1.36 g of anhydrous potassium orthophosphate (KH_2PO_4) in 1 L of previously filtered double distilled water and the pH was adjusted to 6.5 with the help of triethylamine. The elution was carried out with mobile phase composed of the mixture of ACN and 0.01M phosphate buffer (pH 6.5) in the ratio of 65:35. All determinations were performed at ambient temperature. The flow rate was 1 ml/min. The injection volume was 20 µL and detection wavelength was 220nm.A Thermo C18 column (150 mm× 4.6 mm i.d. ×5 µm particle size) was used as the stationary phase.

For LC-MS/MS, 10 mM ammonium acetate (pH 6.5) was prepared by dissolving 0.77 g of ammonium acetate in 1000 mL Milli Q water and the pH was adjusted to 6.5 with acetic acid. The mixture of ammonium acetate (pH 6.5) and ACN in a 65:35 (v/v) ratio was used as the mobile phase and as diluent for sample preparation. The flow rate was 1.0 ml/min. The elution was carried out at ambient temperature. A Thermo C18 column (150 mm× 4.6 mm i.d. ×5 µm particle size) was used as the stationary phase.

6.5.4. Preparation of Standard solution:

SDN standard solution (1mg/mL): Accurately weighed 25 mg of SDN was taken in 25mL volumetric flask and diluted with MeOH up to the mark.

SDN working solution (0.1 mg/mL): 2.5 mL aliquot from the standard solution was taken in 25 mL volumetric flask and diluted with mobile phase upto the mark.

Appropriate aliquots were taken from the working stock solution, in 10 mL volumetric flask and diluted up to the mark with mixture of mobile phase to give final concentration from1-100 µg/mL of SDN.

6.5.5. Preparation of forceddegradation samples

6.5.5.1. Acid induced degradation (Acid hydrolysis)

An accurately weighed 8 mg of SDN API & capsule powder (equivalent to 8 mg SDN) were taken in RBF separately and 10 mL, 1 M HCl was added to it separately. This mixture was refluxed for 6 h at 80ºC on oil bath for acidic degradation. The

forced degradation in acid media was performed in the dark in order to exclude possible degradation effect of light. The degradation sample was then cooled to room temp. From this mixture, 1mL aliquot was taken, neutralised with 1M NaOH and the final volume was made upto 10 mL with the mobile phase. The degradation sample was filtered by 0.2 µ membrane filter and analysed by HPLC.The capsule powder of SDN equivalent to 8 mg of drug was taken and the degradation sample was prepared in a similar manner as that for the standard.

6.5.5.2. Base induced degradation (Base hydrolysis)

An accurately weighed 8 mg of SDN API & capsule powder (equivalent to 8 mg SDN) were taken in RBF separately and 10 mL of 1 M NaOH was added and solution was refluxed for 6 h at 80ºC on oil bath. The forced degradation in basic media was performed in the dark in order to exclude possible degradation effect of light. The degradation sample was then cooled to room temp. From this mixture, 1mL aliquot was taken, neutralised with 1M HCl and the final volume was made upto 10 mL with the mobile phase. The degradation sample was filtered by 0.2 µ membrane filter and analysed by HPLC. The capsule powder of SDN equivalent to 8 mg of drug was taken and the degradation sample was prepared in a similar manner as that for the standard.

6.5.5.3. Peroxide induced degradation

An accurately weighed 8 mg of SDN API & capsule powder (equivalent to 8 mg SDN) were taken in RBF separately and 10 mL, 3% H_2O_2 was added to it separately. This mixture was refluxed for 6 h at 80ºC on oil bath for oxidation degradation. The forced degradation hydrogen peroxide media was performed in the dark in order to exclude possible degradation effect of light. The degradation sample was then cooled to room temp. From this mixture, 1mL aliquot was taken, heated on a water bath for 5 min and the final volume was made upto 10 mL with the mobile phase. The degradation sample was filtered by 0.2 µ membrane filter and analysed by HPLC. The capsule powder of SDN equivalent to 8 mg of drug was taken and the degradation sample was prepared in a similar manner as that for the standard.

6.5.5.4. Dry heat induced degradation

For dry heat degradation, the 8 mg API was placed in oven at 80ºC for 14 days. The degradation sample was taken in 10 mL volumetric mixture, dissolved in MeOH and

made upto the volume. From this solution, 1 mL aliquot was taken and diluted upto 10 mL with the diluent, filtered by 0.2 µ membrane filter and analysed by HPLC. The capsule powder of SDN equivalent to 8 mg of drug was taken and the degradation sample was prepared in a similar manner as that for the standard.

6.5.5.5. Neutral hydrolysis

An accurately weighed 8 mg of SDN API was taken in RBF separately and 10 mL double distil water was added to it. This mixture was refluxed for 6 hours at 80ºC on oil bath for neutral hydrolysis. The degradation sample was then cooled to room temp. From this mixture, 1mL aliquot was taken and the final volume was made upto 10 mL with the mobile phase. The degradation sample was filtered by 0.2 µ membrane filter and analysed by HPLC. The capsule powder of SDN equivalent to 8 mg of drug was taken and the degradation sample was prepared in a similar manner as that for the standard.

6.5.5.6. Photolytic degradation

For the photolytic stability the solid drug (API) was spread in 1mm thickness on a petridish which was closed (with a glass cover) and exposed to UV light of 5382 LUX and 144UW/cm^2 for 14 days. 1mg of SDN was taken in volumetric flask and the final volume was made upto 10 mL with the mobile phase. The degradation sample was filtered by 0.2 µ membrane filter and analysed by HPLC. The capsule powder of SDN equivalent to 8 mg of drug was taken and the degradation sample was prepared in a similar manner as that for the standard.

6.5.6. HPLC method validation

Developed Stability indicating RPHPLC method was validated according to ICH Q2 (R1) guidelines (21) and data complying with the standards were obtained.

The linearity of the HPLC detector response for determination of SDN was evaluated by analysing a series of different concentrations of each compound. The suitable calibration range was established to give accurate, precise and linear results. Appropriate aliquots were withdrawn from the stock solution and seven concentrations were chosen, ranging from 1-100 µg/mL SDN and the linearity was determined.

For evaluation of the precision estimates, repeatability and intermediate precision were performed at three concentration levels i.e. 10, 40 and 80 µg/mL (in triplicates). The peak areas were calculated for each level and the precision was determined in terms of % RSD. The experiment was repeated three times in a day for intra-day precision and on three different days for inter-day precision.

Accuracy was determined by standard addition method at three levels of standard addition *i.e.* 80%, 100%, and 120%. The concentrations (total concentration) for accuracy (0, 80, 100, 120 %) were 40, 72, 80, 88 µg/mL. The resulting mixtures were analysed in triplicates and results obtained and the percentage recovery was determined.

For the determination of limit of detection (LOD) and limit of quantitation (LOQ), the approach based on the standard deviation (S.D.) of the y-intercept and the slope was used Calibration curve was repeated for six times and the standard deviation (SD) of the intercepts was calculated.

Various factors were assessed to check the robustness of the method. The factors such as: organic concentration (63, 65, 66), pH (6.3, 6.5, 6.8) and flow rate (0.8, 1.0, 1.2 mL/min) were varied and the robustness of method was determined.

The standard solution of SDN (100 ppm) was prepared in the MeOH and kept for 24 h at room temperature and for 6 days stored in refrigerator (8-25 ºC) and the stability of stock solution was determined. The reaction mixtures prepared in mobile phase (the final neutralised solutions) were also kept in refrigerator (8-25 ºC) for 12 h and their stability was also determined.

The specificity of the method was determined by analysing the formulation as well as analysing the drug alongwith the degradation products and the method was checked for interference of any peaks affecting the estimation of SDN.

System suitability parameters such as theoretical plates, symmetry factor and resolution for SDN were calculated for n=6 replicates to study the system suitability of HPLC method.

6.5.7. Degradation kinetics of SDN

6.5.7.1. Acid induced degradation kinetics

For acid degradation kinetic study, accurately weighed 10 mg of SDN was dissolved in 10 mL HCl (of respective concentrations in which the kinetic study was to be performed) and the kinetic study was performed at three concentration levels *i.e.* 0.5 M, 1.0 M and 1.5 M of HCl and at three temperature levels *i.e.* 80º C, 90 ºC and 100 ºC, protected from light. From the degradation sample solution, 1 mL aliquot was taken in 10 mL volumetric flask, at proper time interval, neutralised with NaOH and made upto the mark with mobile phase. The resultant solution was further appropriately diluted with mobile phase to get final concentration of 50 ppm.

6.5.7.2. Peroxide induced degradation kinetics

For peroxide degradation kinetic study, 10 mg of SDN was dissolved in 10 mL H_2O_2 (of respective concentrations in which the kinetic study was to be performed) and the kinetic study was performed at three concentration levels *i.e.* 0.5 %, 1.0 % and 1.5 % v/v of H_2O_2 and at three temperature levels *i.e.* 80º C, 90 ºC and 100 ºC, protected from light. From the degradation sample solution, 1 mL aliquot was taken in 10 mL volumetric flask, at proper time interval, heated for 2 min on water bath and made upto the mark with mobile phase. The resultant solution was further appropriately diluted with mobile phase to get final concentration of 50 ppm.

6.6. RESULT & DISCUSSION

6.6.1. Method development

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. In the present study drug solutions of 10 µg/mL of SDN was prepared in MeOH. This drug solution was then scanned in the UV region of 200-400 nm and the spectrum was recorded (Figure 6.1). The drug showed appreciable absorbance at 220 nm and hence this was selected as the analytical wavelength.

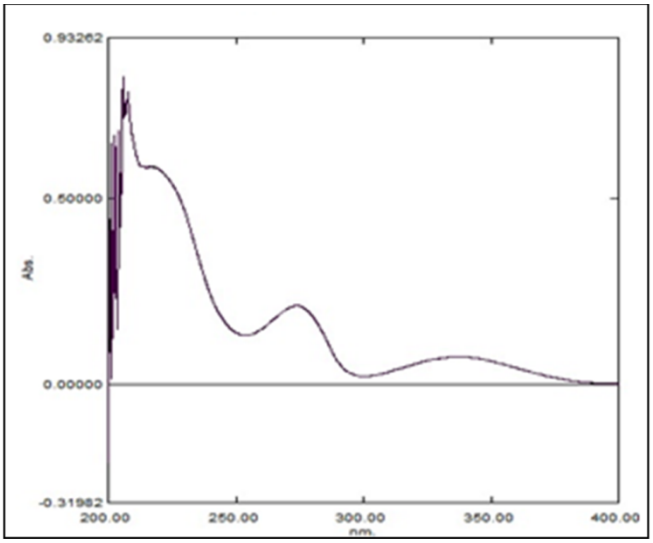


Figure 6.1 Zero order spectra of SDN (10 ppm)

To optimize the chromatographic conditions, the effect of chromatographic variables such as composition of mobile phase, pH of mobile phase and flow rate were studied. The resulting chromatograms were recorded and the chromatographic parameters such as asymmetric factor, resolution and theoretical plates were calculated. The conditions that gave the best resolution, symmetry and theoretical plate were selected for estimation. The results of these trials are reported in Table 6.2.

Table 6.2 Optimisation of HPLC conditions

TRIALS	Rt (min)	SDN Peak shape
Water (pH 6.1): MeOH 50:50	21	Broad peak
Water (pH 6.1): ACN 40:60	9.7	Broad and shouldering
Water (pH 6.1): ACN 30:70	9.6	Less broad and no shouldering
Water (pH 6.1): ACN 50:50	10.2	Slightly broad
10 mM Phosphate Buffer (pH 6.1) : ACN 50:50	7.6	Slightly broad and fronting
10 mM Phosphate Buffer (pH 6.1): ACN 40:60	8.9	Sharp peak and fronting
10 mM Phosphate Buffer (pH 6.1): ACN 35:65	9.8	Sharp peak
pH 3 10 mM Phosphate Buffer : ACN 35:65		Spitted, broad and asymmetric peak

pH 5.5 10 mM Phosphate Buffer : ACN 35:65	6.9	Sharp peak, fronting increased
pH 6.5 Phosphate Buffer : ACN 35:65 For LCMS analysis	9.8	Sharp and symmetric peak
10 mM formate Buffer : ACN 35:65	6.0	Spitted and asymmetric peak
10 mM Ammonium acetate (pH 6.5) : ACN 35:65	9.6	Sharp peak

Besides quantification of SDN, determination of possible degradation products is of importance during the development of pharmaceutical formulations. To analyze SDN together with its possible degradation products, reverse phase LC in combination with PDA detector was developed. The studies suggested that a mobile phase containing phosphate buffer at slightly acidic pH value favoured the peak shape of SDN as well the degradation peaks, on the column to achieve a reasonable retention and resolution. The final optimised conditions for HPLC method of SDN are given in Table 6.3 and the optimised chromatogram of standard solution is given in Figure 6.2.

Table 6.3 Optimised HPLC parameters

Parameters	Optimised Value
Column	Thermo C18 (150mm x 4.6mm i.d.x5µ particle size)
Mobile phase	ACN:10 mM Phosphate buffer pH 6.5 (pH adjusted with triethylamine) =65:35(%v/v)
Flow rate	1.0 mL/min
Retention time	9.86 min
Detection wavelength	220nm
Needle wash	Mobile phase
Column temperature	Ambient

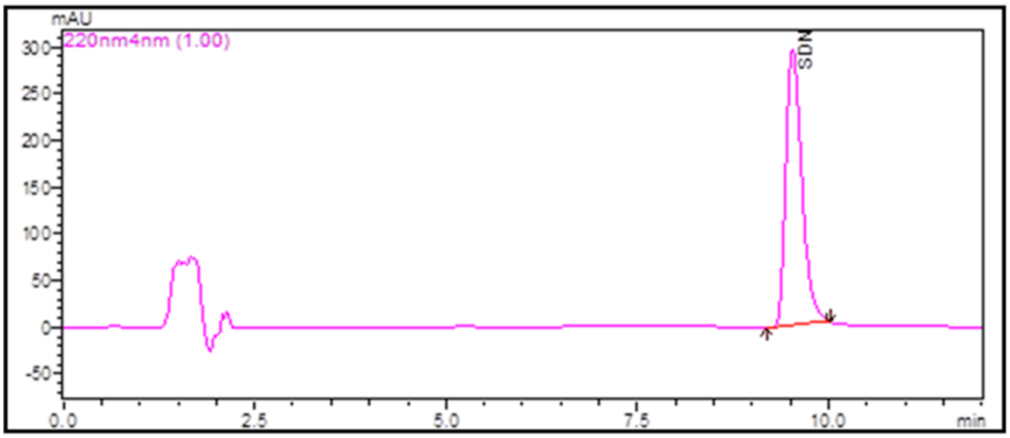


Figure 6.2 Chromatogram of standard solution of SDN (80 ppm)

6.6.2. Forced Degradation studies

6.6.2.1. Acidic induced degradation

The chromatograms of acid (1 M HCl) degraded samples of SDN at 80ºC for 6 h, showed the degradation peak, other than the SDN peak at retention time (Rt) 3.4 min. The chromatograms for acid degradation of SDN in API and formulation have been shown in Figure 6.3 and 6.4 respectively.

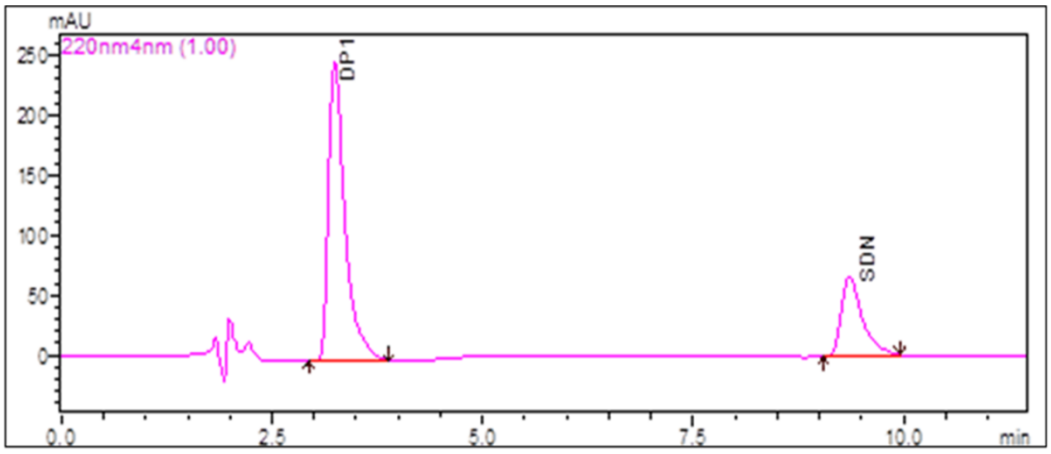


Figure 6.3 Acid degradation of standard solution of SDN

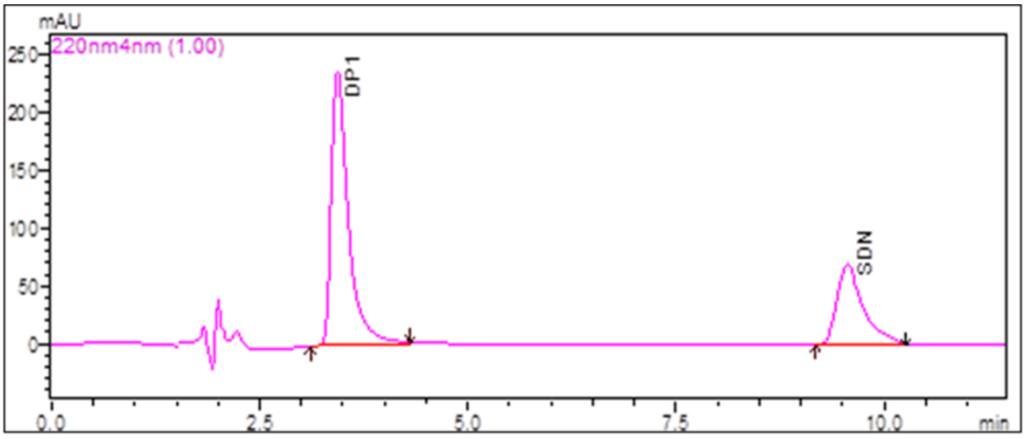


Figure 6.4 Acid degradation of marketed formulation of SDN

6.6.2.2. Alkaline induced degradation

The chromatograms of base (1 M NaOH) degraded samples of SDN at 80ºC for 6 h, showed the degradation peak, at retention time (Rt) 7.17 min. The chromatograms for base degradation of SDN in API and formulation have been shown in Figure 6.5 and 6.6 respectively.

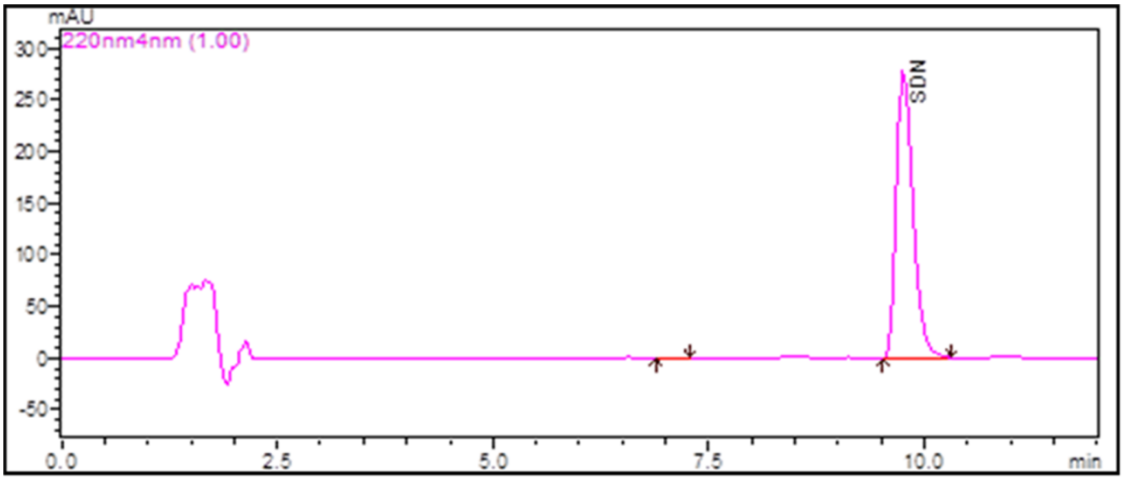


Figure 6.5 Base degradation of standard solution of SDN

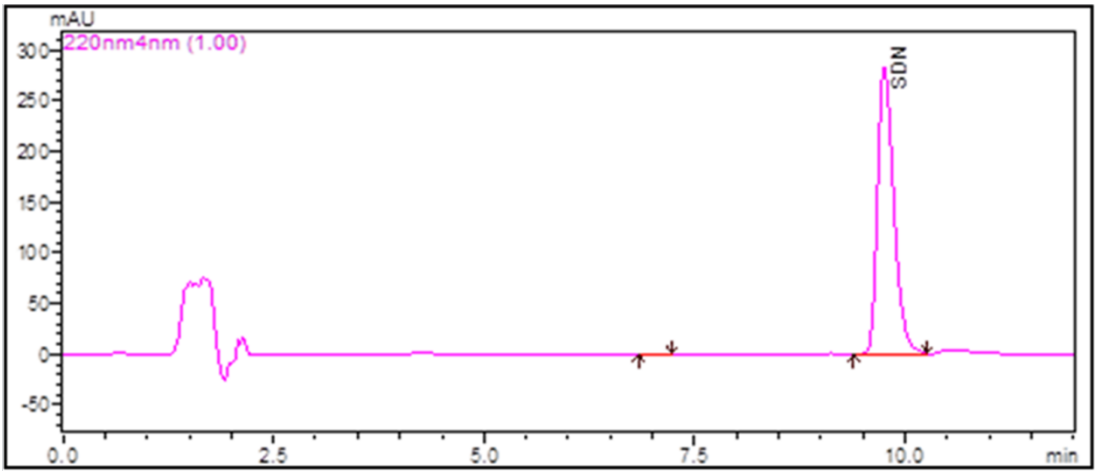


Figure 6.6 Base degradation of marketed formulation of SDN

6.6.2.3. Peroxide degradation

The chromatograms of peroxide (3% H_2O_2) degraded samples of SDN at 80ºC for 6 h, showed the degradation peaks, other than the SDN peak at retention time (Rt) 4.1 and 5.6 min. The Figure 6.7 shows the blank chromatogram for H_2O_2 sample. The chromatograms for peroxide degradation of SDN in API and formulation have been shown in Figure 6.8 and 6.9 respectively.

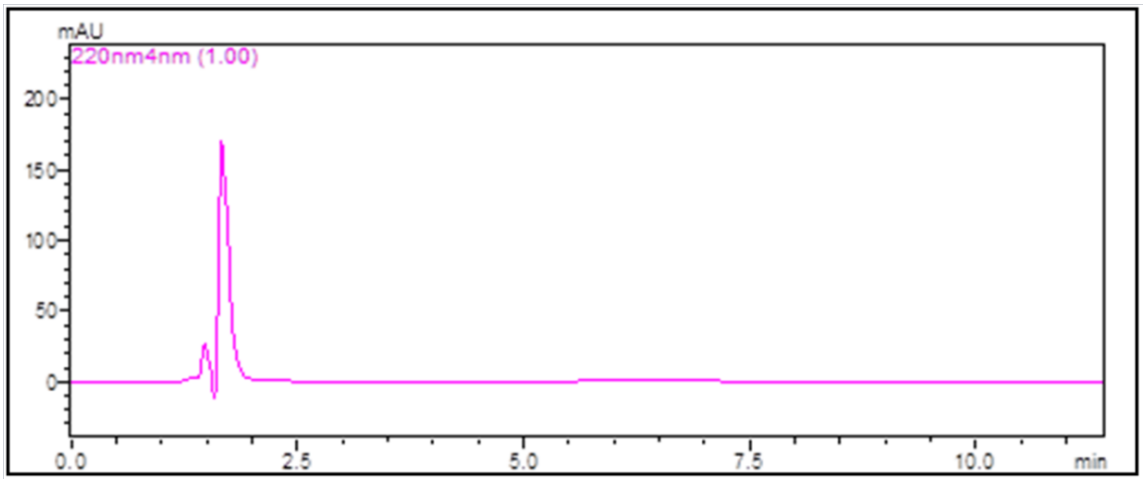


Figure 6.7 Blank chromatogram

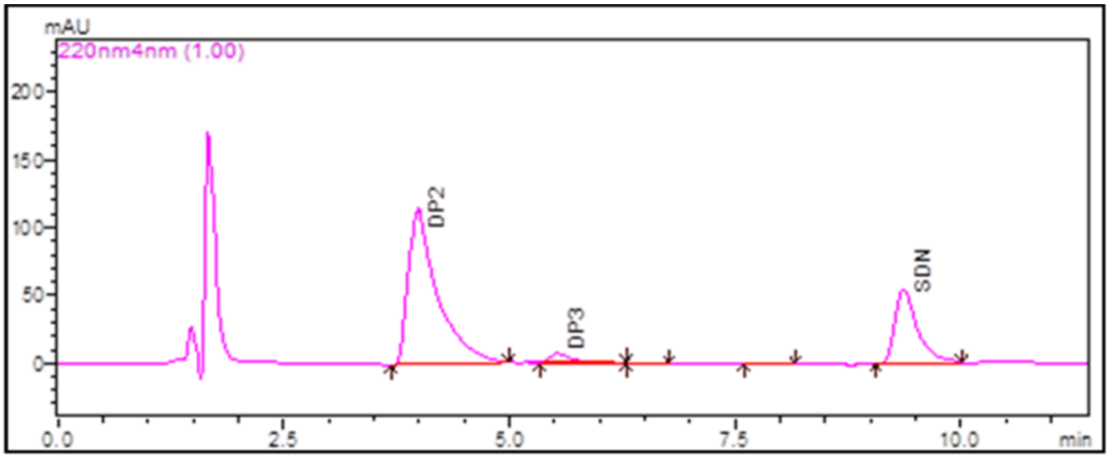


Figure 6.8 Peroxide degradation of standard solution of SDN

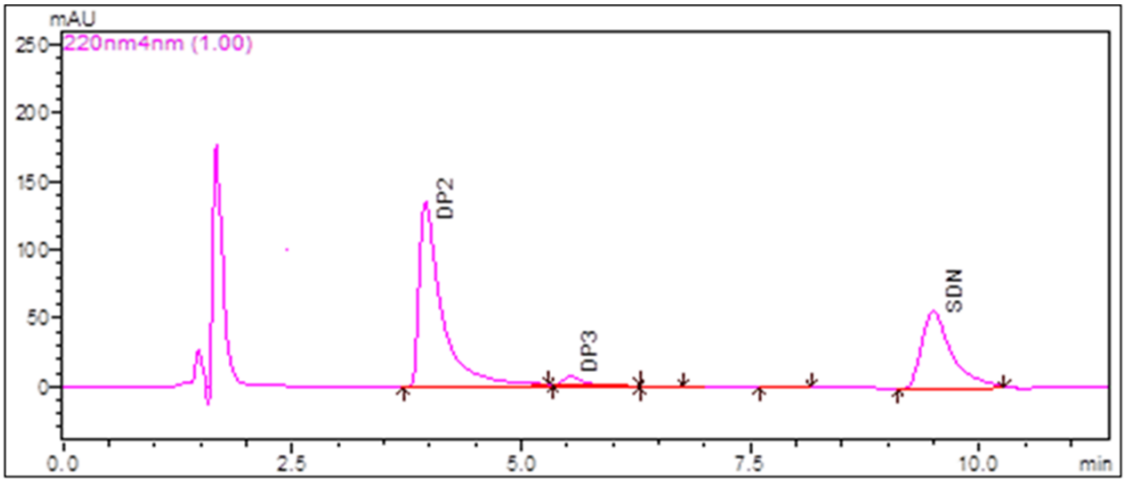


Figure 6.9 Peroxide degradation of marketed formulation of SDN

6.6.2.4. Dry heat degradation

The chromatograms of dry heat degradation samples of SDN, at 80ºC for 9 h, showed no significant degradation peaks, other than the SDN peak. The chromatograms for dry heat degradation of SDN in API and formulation have been shown in Figure 6.10 and 6.11 respectively.

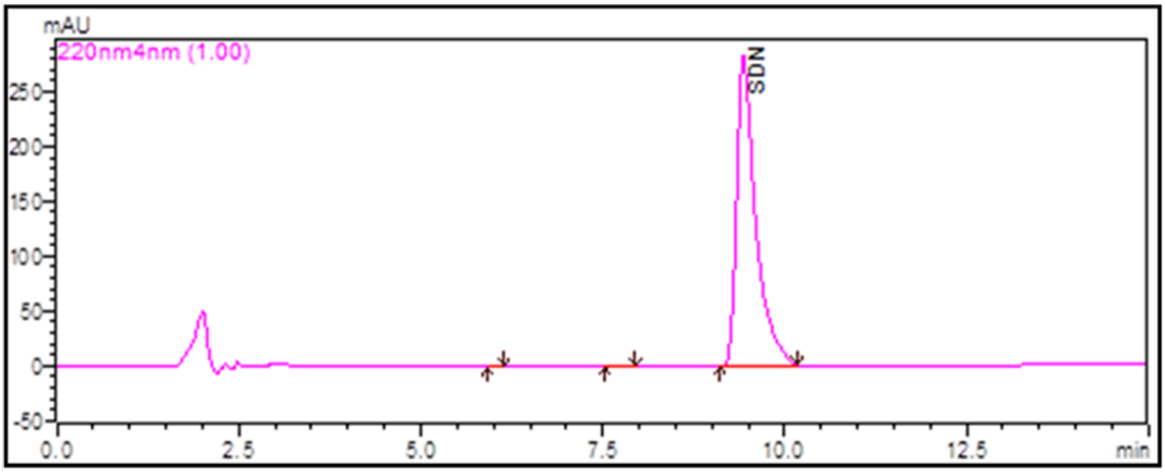


Figure 6.10 Dry heat degradation of SDN API

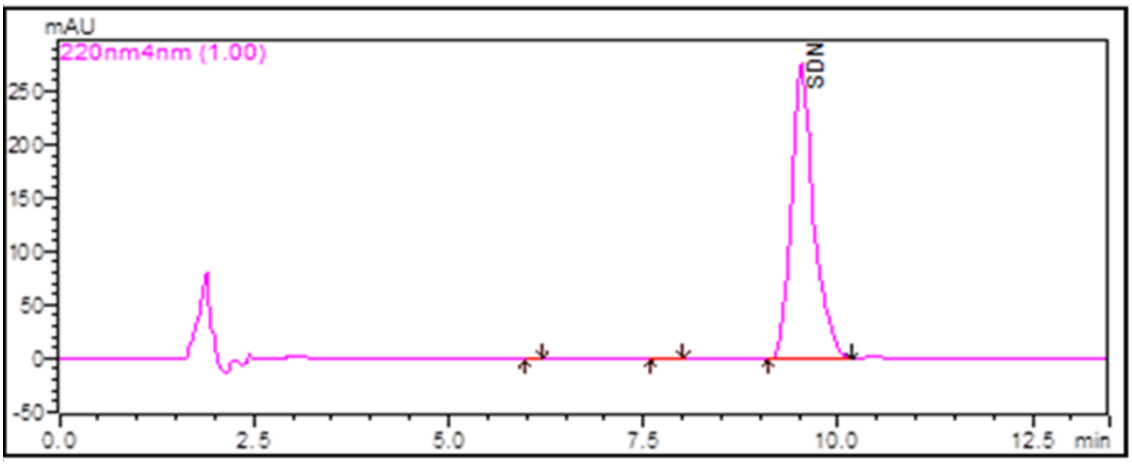


Figure 6.11 Dry heat degradation of marketed formulation of SDN

6.6.2.5. Neutral hydrolysis

The chromatograms of neutral hydrolysis degradation samples of SDN, at 80ºC for 6 h, showed no significant degradation peaks, other than the SDN peak. The chromatograms for neutral hydrolysis of SDN in API and formulation have been shown in Figure 6.12 and 6.13 respectively.

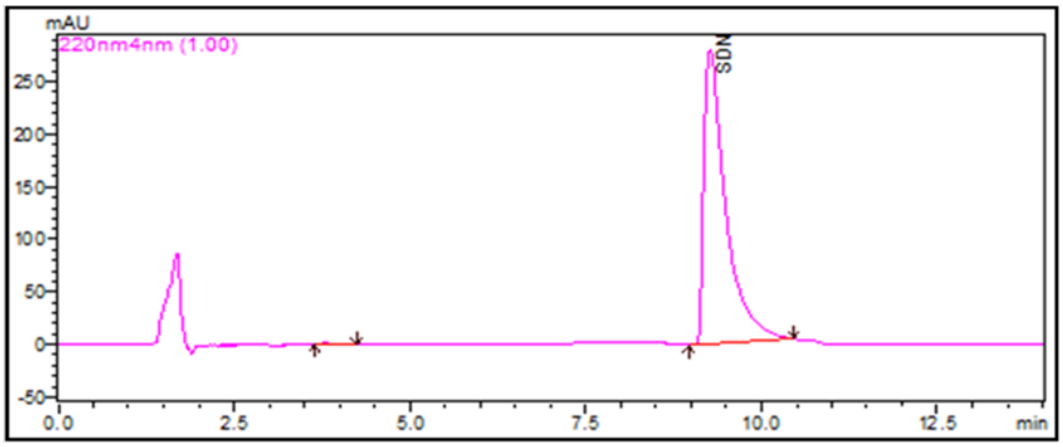


Figure 6.12 Neutral hydrolysis of standard solution of SDN

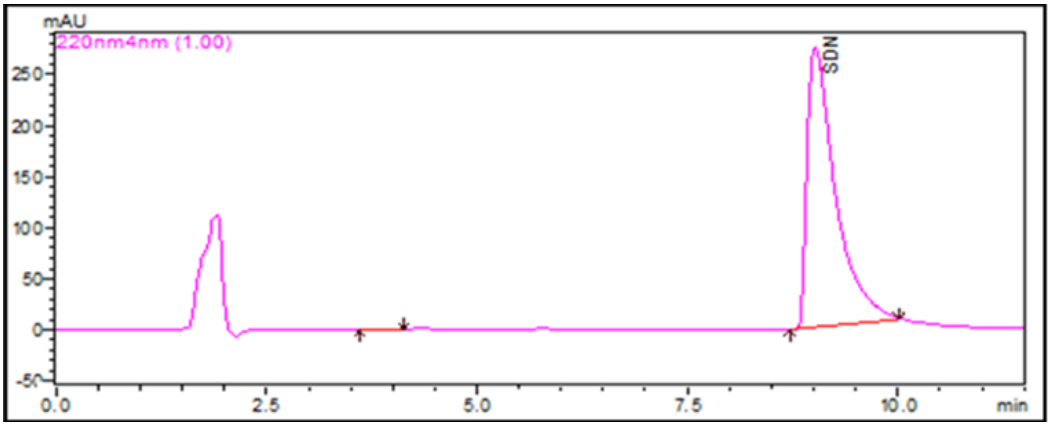


Figure 6.13 Neutral hydrolysis of marketed formulation of SDN

6.6.2.6. Photolytic degradation

The chromatograms of photolytic degradation samples of SDN showed no significant degradation peaks, other than the SDN peak. However the area of SDN was reduced by 10-12% which showed that there may be some non-chromophoric products that may not be visible in HPLC. The chromatograms for photolytic degradation of SDN in API and formulation have been shown in Figure 6.14 and 6.15 respectively.

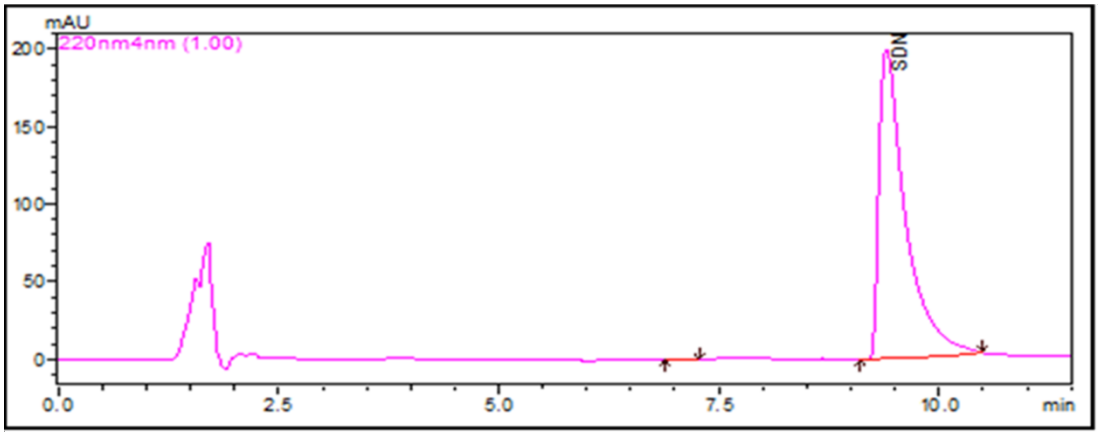


Figure 6.14 Photolytic degradation of standard solution of SDN

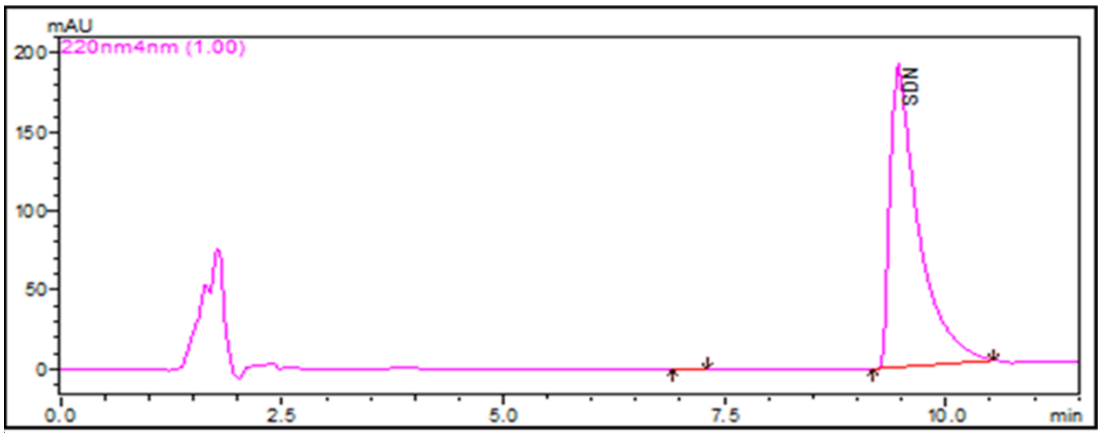


Figure 6.15 Photolytic degradation of marketed formulation of SDN

6.6.3. Summary of Degradation studies

A simple, sensitive, selective, precise and stability-indicating method for determination of SDN both as bulk drug and from pharmaceutical formulation was developed and validated as per the ICH guidelines (22, 23, 24). Results of forced degradation study of SDN are listed in Table 6.4. The blank chromatogram of SDN for degradation mixture has been shown in Figure 6.16 and the combined chromatogram of SDN alongwith its major degradation products (i.e. acid and peroxide degradation product) has been shown in Figure 6.17. Degradation products resulting from the forced degradation studies did not interfere with the detection of SDN and the method is thus stability-indicating one. SDN significantly degraded in acidic and peroxide conditions. SDN was almost somewhat stable in alkaline, dry heat, wet heat and photolytic condition. Due to high degradation of SDN in oxidative and acid hydrolysis condition, kinetic investigation of degradation was performed.

Table 6.4 Summary of degradation study

Sr. No.	Sample exposure conditions		RTvalue of degradation products (min.)	% Degradation
1	Acid, 1M HCl (80ºC, 6Hr)	API	3.43	82.66
		Capsule	3.45	81.74
2	Base, 1M NaOH (80ºC, 6Hr)	API	5.32, 5.82	2.72
		Capsule	5.33, 5.80	2.13
3	3 % H_2O_2 (80ºC, 6Hr)	API	4.12, 5.6	79.31
		Capsule	4.13, 5.65	77.08
4	Photostability (21 days)	API	No visible degradation peaks observed	11.95
		Capsule		10.26
5	Wet heat (80ºC, 6Hr)	API		1.68
		Capsule		1.46
6	Dry heat (80ºC,9Hr)	API		3.77
		Capsule		2.58

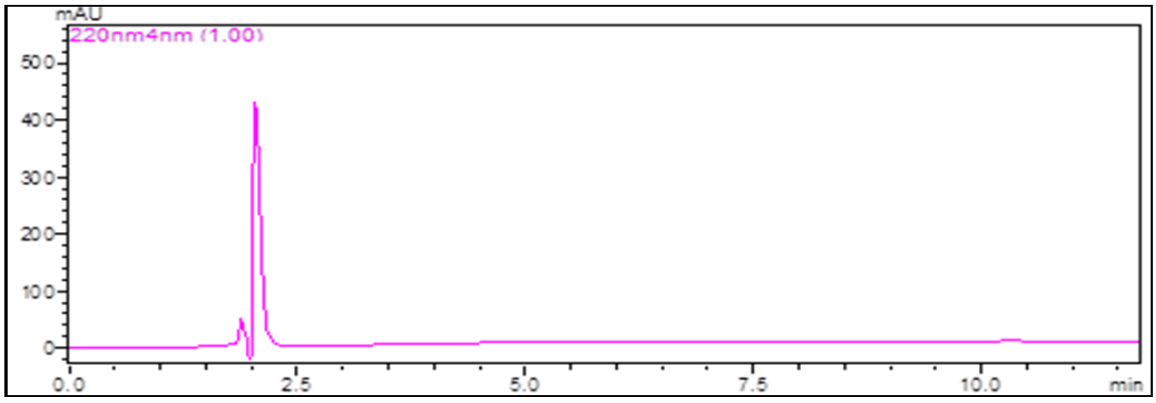


Figure 6.16 Blank chromatogram for mixture

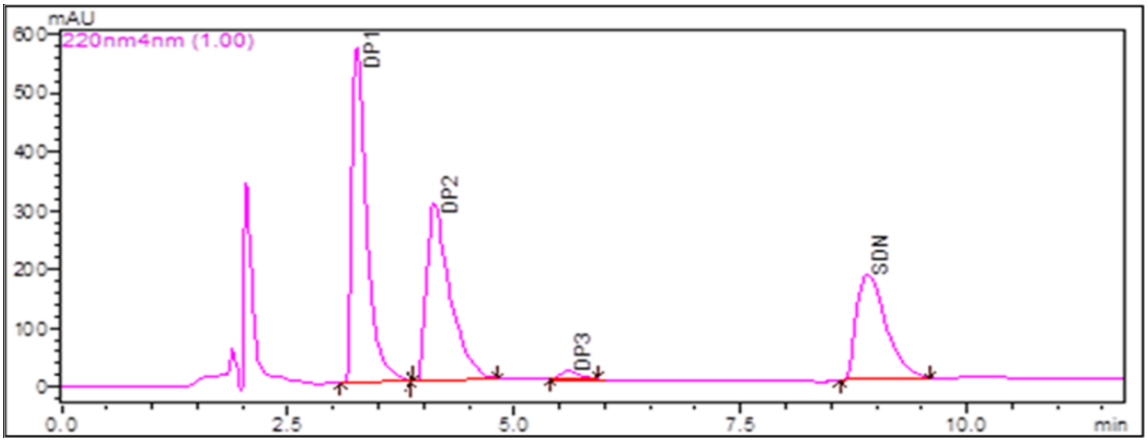


Figure 6.17 Chromatogram of SDN-Degradation Product spiked solution of SDN

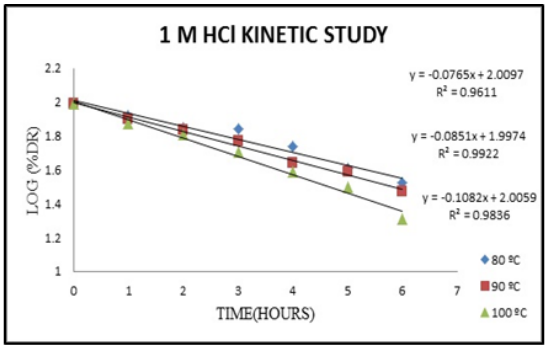
6.6.4. Kinetic studies of acid and peroxide degradation

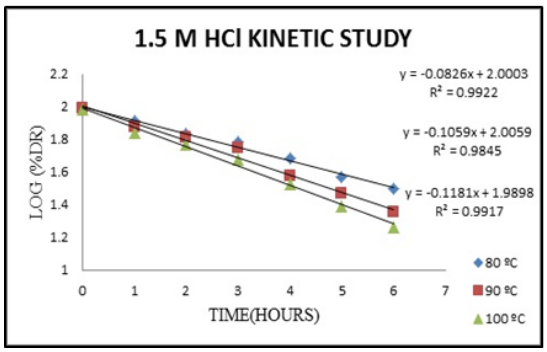
The method of accelerated testing of pharmaceutical products based on the principles of chemical kinetics was used to measure the stability of the drug under these conditions**.** The degradation kinetics of SDN for acid hydrolysis and oxidation was investigated in various concentrations and temperatures.

A regular decrease in the concentration of SDN with increasing time intervals as well as successive increase in temperature was observed in acidic as well as oxidative conditions. At the selected temperatures (80, 90 and 100ºC for acid and oxidation) the degradation process followed first order kinetics which can be found out from the values of rate constant (K) and regression coefficient (R^2) for various reaction orders were obtained from the slopes of the straight lines at each temperature as stated in Table 6.5 for acid degradation kinetics and Table 6.6 for peroxide degradation kinetics. The kinetic degradation plots for acid and peroxide degradation have been shown on Figure 6.18 and 6.19 resp.

Table 6.5 Results for acid degradation kinetics of SDN

Acid degradation kinetics						
HCl	K			R^2		
Conc.	80 ºc	90 ºc	100 ºc	80 ºc	90 ºc	100 ºc
Zero order						
1M	-10.547	-11.165	12.331	0.9776	0.9776	0.9786
1.5M	-10.997	-12.294	-12.468	0.9857	0.9754	0.9589
2M	-11.879	-12.513	-12.656	0.9817	0.9626	0.9405
First order						
1M	-0.0765	-0.0851	-0.1082	0.9611	0.9922	0.9836
1.5M	-0.0826	-0.1059	-0.1181	0.9922	0.9845	0.9917
2M	-0.1016	-0.116	-0.1213	0.9851	0.9805	0.9724
Second order						
1M	0.0059	0.0038	0.0032	0.8802	0.9509	0.9045
1.5M	0.0071	0.0055	0.0036	0.9182	0.921	0.9491
2M	0.0073	0.0067	0.0052	0.9325	0.9302	0.92





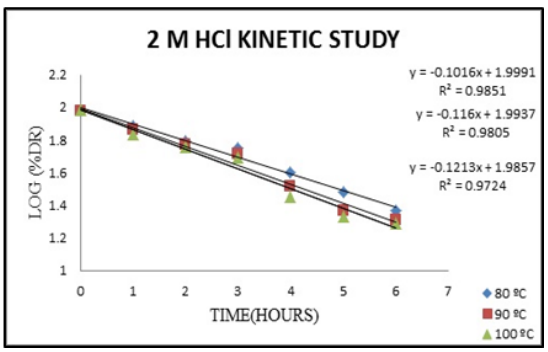


Figure 6.18 Kinetic study plots for acid degradation of SDN

(for first order kinetics)

Table 6.6 Results for peroxide degradation kinetics of SDN

Peroxide degradation kinetics						
H_2O_2 Conc.	K			R^2		
	80 ºc	90 ºc	100 ºc	80 ºc	90 ºc	100 ºc
Zero order						
0.5%	-20.198	-22.236	-23.484	0.9838	0.9771	0.9571
1%	-22.515	-23.412	-24.928	0.9819	0.969	0.9594
1.50%	-25.782	-25.666	-26.096	0.9884	0.9651	0.9366
First order						
0.5%	-0.1396	-0.1648	-0.1864	0.9885	0.9895	0.982
1%	-0.1676	-0.1865	-0.2139	0.9838	0.9925	0.9931
1.5%	-0.2233	-0.2309	-0.2594	0.9745	0.9914	0.9966
Second order						
0.5%	0.0088	0.0071	0.0054	0.9205	0.9362	0.9513
1%	0.0113	0.0088	0.0073	0.9244	0.9478	0.9174
1.5%	0.017	0.013	0.012	0.9125	0.9157	0.8737

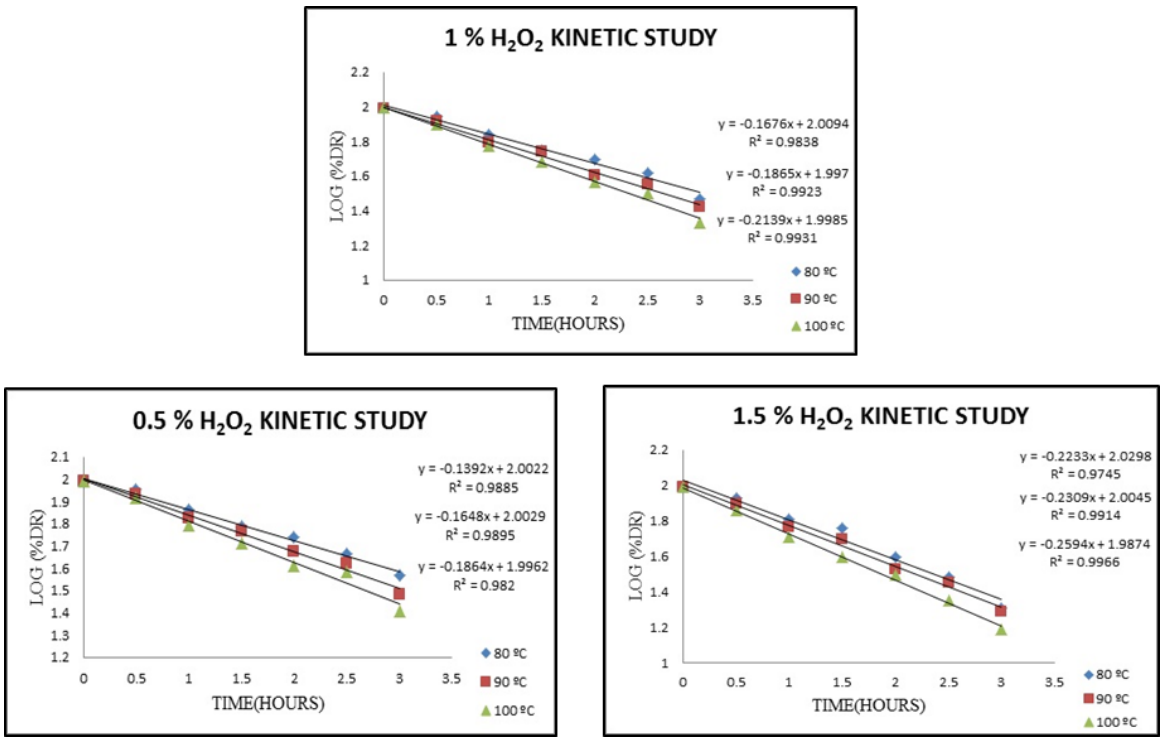


Figure 6.19 Kinetic study plots for peroxide degradation of SDN (for first order kinetics)

The data obtained from first-order kinetics treatment were further subjected to fitting to the Arrhenius equation i.e.

$$\log K = \log A - Ea/2{:}303RT$$

where K is the rate constant, A is the frequency factor, Ea is the activation energy (kcal/mol), R is the gas constant (1.987 cal/deg/ mol), and T is absolute temperature.

Arrhenius plot of lnK values versus 1/T was obtained, which was found to be linear in the temperature range. The slopes of the linear plots gave the value of -Ea/2.303R (activation energy) for the kinetics of acidic and oxidative degradation processes (Table 6.7). The Arrhenius plots of acid and peroxide degradation kinetics are shown in Figure 6.20. The $t_{1/2}$ values for both the degradation studies were also found out using the formula $t_{1/2}$ =ln2/K and the values are given in Table 6.7.

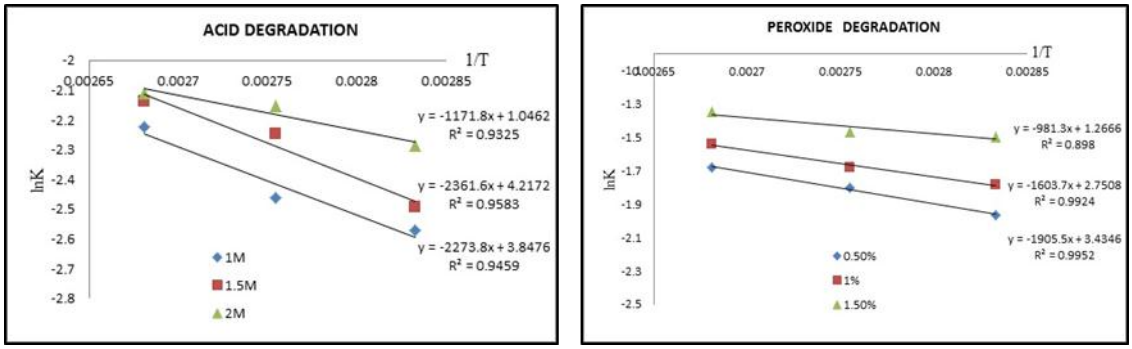


Figure 6.20 The activation energy plots for acid and peroxide degradation of SDN

Table 6.7 The results of degradation kinetics

Degradation condition	$t_{1/2}$ (min)			Activation energy (kJ/mole)
HCl conc.	Acid degradation kinetics			
1M	9.06	8.39	6.82	
1.5M	8.15	6.55	5.98	12.44
2M	6.41	5.87	5.71	
H_2O_2 conc.	Peroxide degradation kinetics			
0.5%	4.97	4.14	3.10	
1%	4.21	3.72	3.00	16.09
1.5%	3.72	3.24	2.67	

6.6.5. Characterisation of degradation products on the basis of LC-MS/MS analysis

The major degradation products that were formed from the acid and peroxide degradation of SDN i.e. DP1, DP2 and DP3 were analyzed by LC-MS/MS and efforts have been made to understand the degradation mechanism of SDN. The degradation products thus studied have been stated in Table 6.8.

Table 6.8 Degradation products considered for LC-MS/MS characterisation

Degradation condition	Rt (min)	Degradation product code
Acid induced degradation	4.2	DP1
Oxidative degradation	3.2, 5.0	DP2, DP3

6.6.5.1. Acid induced degradation

The LC-MS/MS spectra of the acid induced degradation product of SDN (DP1), is given in Figure 6.21. The acid hydrolysis of SDN resulted to the formation of degradation product, DP1 at retention time 4.2 min, which gave the molecular ion at 497 m/z. Based on that, the structure proposed for DP1 and degradation pathway has been given in Figure 6.22.

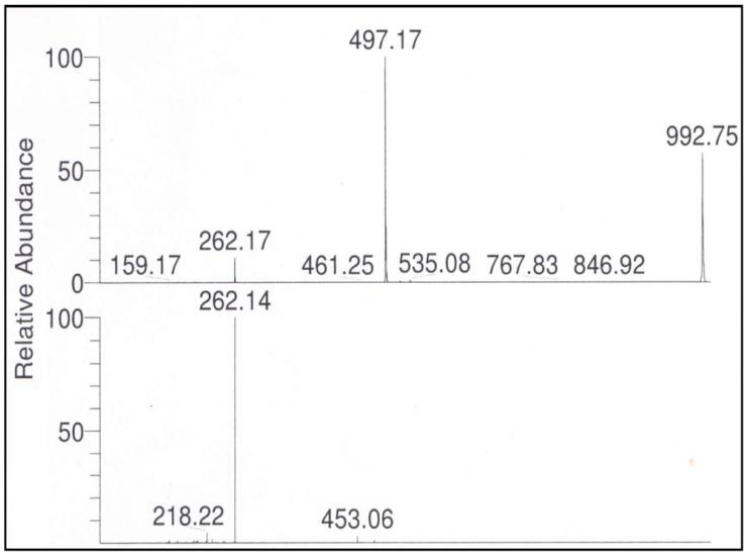


Figure 6.21 LC-MS/MS spectra for acid degradation peak of SDN (DP1)

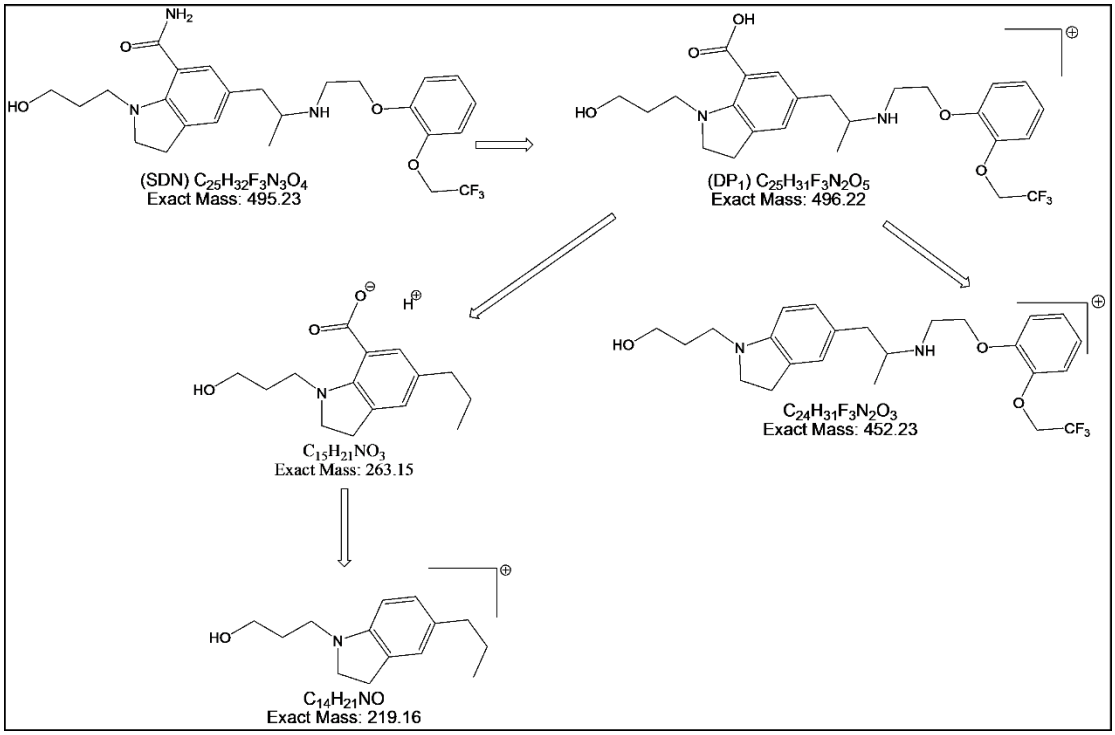


Figure 6.22 The proposed structure and degradation pathway for DP1

6.6.5.2. Peroxide degradation product

The LC-MS/MS fragmentation pattern for the peroxide induced degradation peak (DP2 and DP3) has been shown in Figure 6.23 and 6.24 resp. Based on that the structure for the degradation products and the pathway has been given in Figure 6.25 and 6.26. The LC-MS analysis of peroxide degradation product (DP2, DP3) revealed that the mass of the two degradation peaks is 324.17 (Rt =3.2, DP2) and 325.17 (Rt=5, DP3). Based on the LC-MS/MS fragmentation the possible structure of the DPs and the degradation pathway is given in Figure 6.25 and 6.26 for DP2 and DP3 resp.

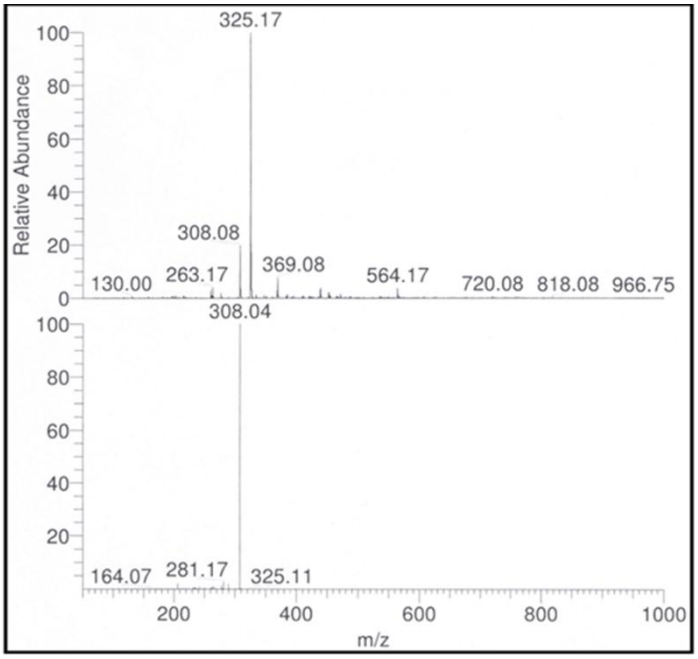


Figure 6.23 LC-MS/MS spectra of DP2

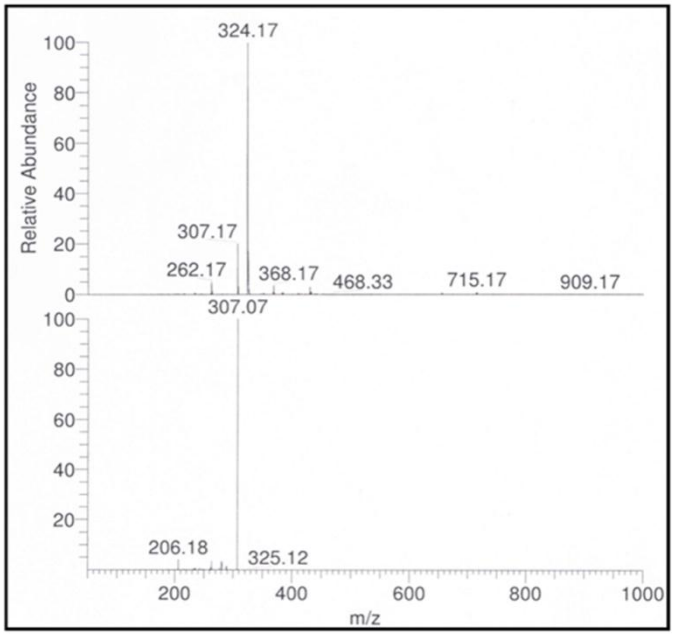


Figure 6.24 LC-MS/MS spectra of DP3

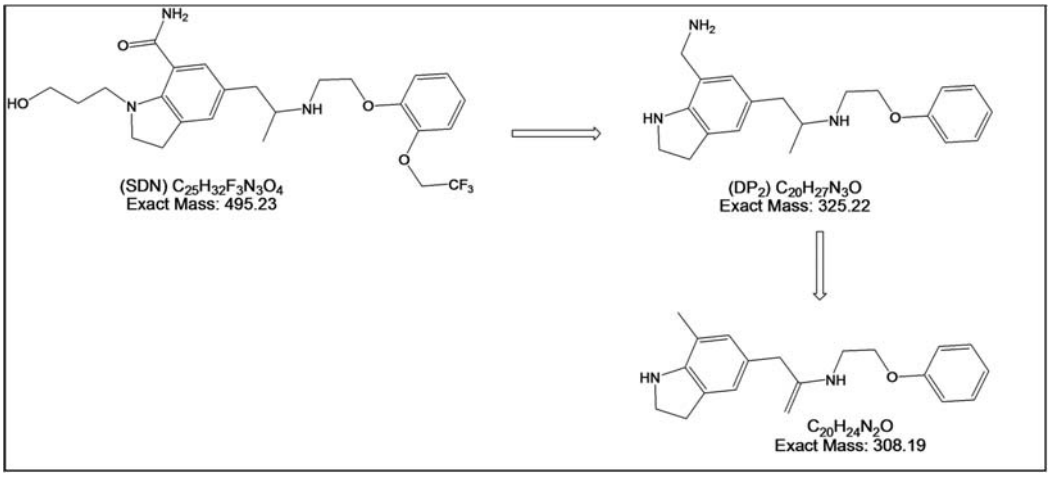


Figure 6.25 The proposed structure for DP2 and its proposed degradation pathway

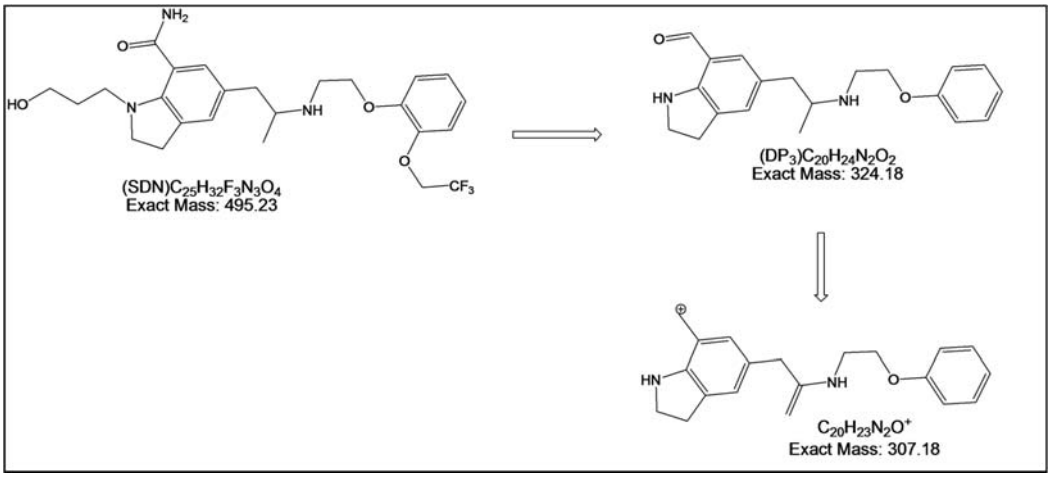


Figure 6.26 The proposed structure for DP3 and its proposed degradation pathway

6.6.6. Summary of validation parameters

The calibration curve constructed for SDN was linear over the concentration range of 1-100 µg/mL. Figure 6.27 shows the overlain chromatogram of the linearity series of SDN and Figure 6.28 shows the calibration plot. The precision has been reported in terms of average %RSD of intra-day and inter-day measurements for determination of SDN in Table 6.9. The low value of %RSD confirms the precision of the method. Percentage recovery greater than 98.99 % with low SD, justified the accuracy of the method. The method was found to be robust for the parameters viz. pH, flow rate and

percentage of organic solvent. The average value of SD for determination of SDN was less than 2 % which revealed the robustness of the method. The solution of SDN was found to be stable at room temperature for 24 h and for 6 days in refrigerator. No additional peak as well as no significant change in the peak area was found in the chromatogram indicating the stability of SDN in the sample solution.

The summary of all the validation parameters has been shown in Table 6.9.

Table 6.9 Summary of validation parameters

Parameters	SDN
Calibration range (µg/mL)	1-100
LOD (µg/mL)	0.29
LOQ (µg/mL)	0.90
Regression equation	y = 21915x+ 136.44
Correlation coefficient (r^2)	0.9999
Accuracy	%Recovery±SD
80%	99.14 ± 0.56
100%	99.11 ± 0.68
120%	98.99 ± 0.40
Precision	%RSD
Intraday	0.71
Interday	1.03
Robustness	Mean±SD
pH	9.87±0.0071
Flow rate	9.78±0.89
Organic conc.	9.90±0.67

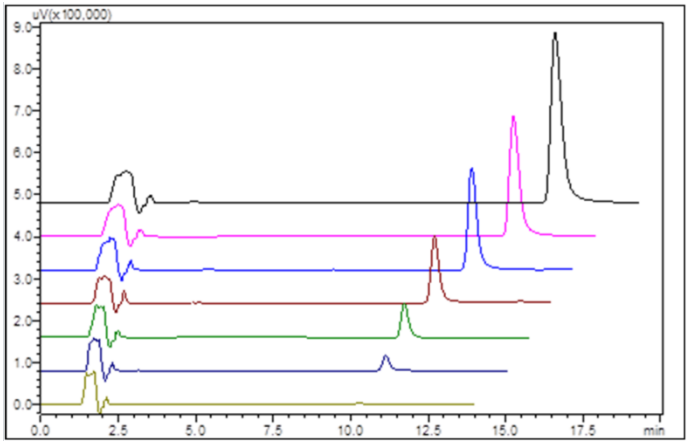


Figure 6.27 3D view of different concentrations of SDN (1-100ppm)

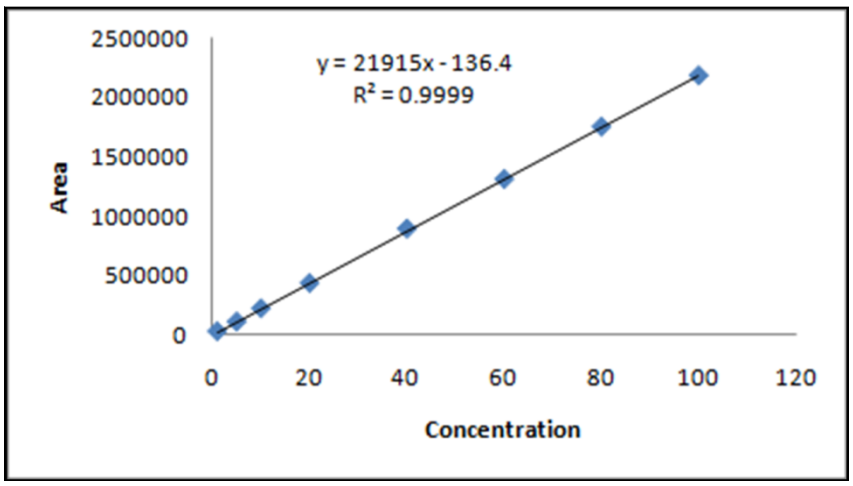


Figure 6.28 Calibration curve of SDN [Peak area vs Conc. (µg/mL)]

The results of assay and forced degradation studies indicated the specificity of HPLC method for SDN. There was no interference of excipients as well as the degradation peaks as shown in the Figure 6.29 and 6.17 which represent the chromatogram of sample solution (formulation) and the combine chromatogram of SDN with its degradation peaks resp.

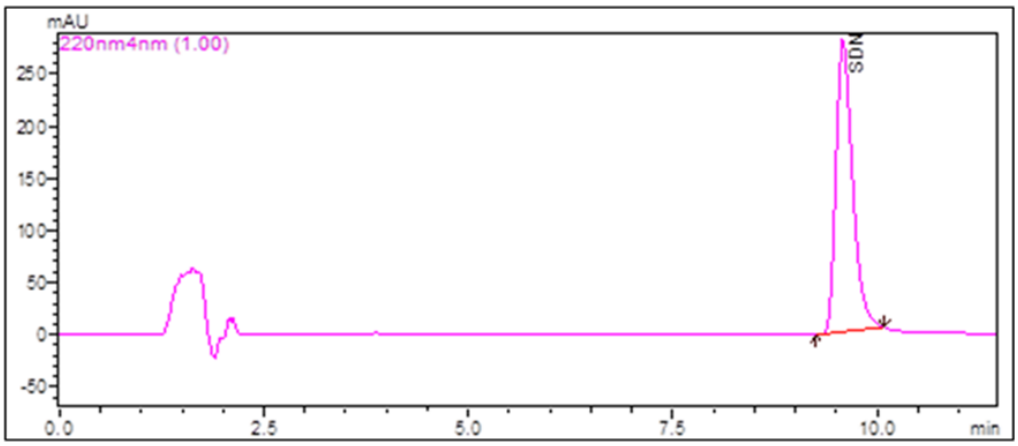


Figure 6.29 Chromatogram of sample solution for marketed formulation

The results for system suitability parameters calculated for HPLC method are given in Table 6.10. The peak purity curve has been shown in Figure 6.30 and the data for peak purity are given in Table 6.11.

Table 6.10 System suitability parameters

Parameters	Values
Retention Time (min)	9.87 ± 0.66
Tailing factor	1.33 ± 1.05
Resolution	28.07 ± 0.97
Theoretical plate	11022.05 ± 1.30
Capacity factor	5.08 ± 1.34

Results for n=6 replicates ± RSD

Table 6.11 Peak Purity results

Drug Name	**Peak Purity Index**	**Threshold**
SDN	1.0000	0.9999

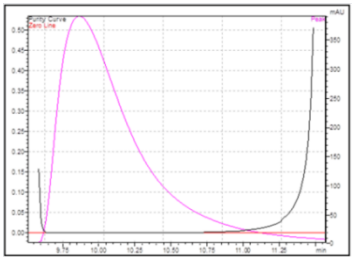


Figure 6.30 The peak purity curve of SDN

6.7. CONCLUSION

A specific stability indicating analytical RP-HPLC method was developed for Silodosin. The degradation of SDN was studied under various conditions and the two conditions in which significant degradation was identified was studied in detail. The degradation kinetics of these two conditions i.e. acid and peroxide was successfully carried out. The attempts were made to characterize the acid and peroxide degradation products on the basis of LC-MS/MS results.

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