

Development and Validation of Stability Indicating RP HPLC method for the estimation of armodafinil and Characterization of its base Degradation Product by LC-MS/MS

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Abstract— The present work focuses on the development and validation of a rapid, selective and reliable stability indicating reverse phase High Performance Liquid Chromatographic method for the quantitative analysis of Armodafinil (ARM). Armodafinil is a eugeroics drug used for treatment of narcolepsy and shift work sleep disorder and also as an adjunctive treatment for obstructive sleep apnea. The separation was carried out on a Hibar Purospher C₁₈ (250 mm × 4.6 mm; 5μ) column by using 0.01 M ammonium formate (pH 4.5, Adjusted with acetic acid) and methanol as mobile phase in the ratio of 45:55 % v/v. The flow rate was maintained at 1 mL/min and the eluents were monitored at 220 nm. Stress studies were carried out with 1 mg/mL of the drug solution, starting with mild conditions and followed by intrinsic conditions to achieve sufficient degradation. Armodafinil underwent extensive degradation under basic hydrolysis condition. The unknown degradation product formed (DP I; 6.59±0.2 min) under the basic degradation condition was separated on a semi preparative HPLC, characterized by UPLC-Q-TOF and its fragmentation pathway was proposed. The proposed structure of the degradation product was confirmed by HRMS analysis. The developed stability indicating LC method was validated with respect to accuracy, precision, specificity/selectivity and linearity. The degradation product was characterized and reported.

Keywords- Armodafinil; LC-MS/MS; degradation; Validation.

I. INTRODUCTION

The stress testing of pharmaceuticals has long been recognized as an important part in the drug development process. They help in demonstrating the specificity of stability indicating methods and to determine the degradation pathways and degradation product of the active ingredients. These methods are also useful in the investigation of the chemical and physical stability of the crystal forms, the stereo chemical stability of the drug substances & drug product, mass balance issues and for differentiating drug substances related

degradation products in the formulations. The focus on these degradation products are basically due to various health and safety considerations. A subset of these impurities may be genotoxic in nature posing additional safety concern leading to carcinogenicity and genetic mutations.

Armodafinil (ARM), 2-[(R)-(diphenyl methyl) sulfinyl] acetamide, falls under the category of eugeroics. It is used for the treatment of narcolepsy and shift work sleep disorder and also as an adjunctive treatment for obstructive sleep apnea. [1].

Few analytical methods are available for the determination of ARM in bulk and pharmaceutical dosage form by UV [2] and HPLC [3]. Two LC-MS/MS methods were reported for the analysis of ARM in human plasma [4, 5]. Jennifer *et al* have reported the chiral analysis of d and l modafinil in human plasma and their application to pharmacokinetic studies [6]. Enantioselective separation of modafinil was carried out and quantified by capillary electrophoresis [7]. Rao *et al* reported an RP-HPLC method for the separation and determination of related substances of modafinil [8] and also an enantioselective HPLC method for the resolution of synthetic intermediates of armodafinil and their related substances [9]. Wei Wang *et al* have reported the enantiomeric separation and determination of the enantiomeric impurity of armodafinil by capillary electrophoresis with sulfobutyl ether β cyclodextrin as chiral selector [10]. Mona Darvish *et al* had reported a study on a possible interaction between quetiapine and armodafinil in patients with schizophrenia: An open label multiple dose study [11]. Deepti Jain and Pawan K Basniwal had reported the intrinsic stability of armodafinil hydrochloride by forced degradation and impurity profiling [12].

However, this article deals with the development and validation of an isocratic RP HPLC method for the quantification of ARM in bulk drug, its validation as per ICH guidelines [13], semi preparative isolation of the base

degradation product formed and to elucidate the degradation pathway and plausible structure of the degradation product.

II. EXPERIMENTAL

A. Instrumentation

A Waters HPLC system equipped with 1515 solvent delivery system, 2487 dual wavelength absorbance detector and a Rheodyne 7725i manual injector with 20 μ l loop volume were used to carry out the stress degradation studies.

For peak purity and LC/MS studies, a Shimadzu HPLC system equipped with LC 20 AD pump, SPD-M20A PDA detector, ESI-quadrupole mass spectrometer equipped with an SIL-20AC auto sampling units were used. The separation was carried out on a Hibar C₁₈ (250 \times 4.6 mm, id, 5 μ m) column. The data acquisition and processing were performed using Lab Solutions software. LC-MS/MS studies and accurate mass measurements were performed using 1290 infinity UHPLC-quadrupole-time of flight (Q-TOF) mass spectrometer (Agilent Technologies, USA) equipped with ESI source with a mass range and resolution from 50-3200 amu and 40000 FWHM respectively. The data acquisition was performed using Analyst[®] QS software.

B. Chemicals

Armodafinil was obtained as gift sample from Wockhardt Pharmaceuticals, Aurangabad, India. HPLC grade methanol (Qualigens Fine Chemicals, India) and HPLC water (Milli-Q water purification system, France) were used for analysis. Analytical reagent grade of ammonium acetate, acetic acid and sodium hydroxide were procured from S. D. Fine chemicals (Mumbai, India).

C. High Performance Liquid Chromatography Conditions

The separation of the degradation samples were carried out on a Hibar C₁₈ column (250 \times 4.6 mm, id, 5 μ m) with a mobile phase consisting of Ammonium acetate (0.01M; pH 4.5) and methanol in an isocratic elution mode (45:55% V/V) at room temperature (16-20°C). The flow rate was maintained at 1.0 mL/min. The sample injection volume was 20 μ L and eluents were monitored at 220 nm. Semi-preparative isolation of armodafinil degradation product was carried out with same mobile phase on Zorbax C₁₈ (250 \times 9.0 mm, id, 5 μ m) with a sample injection volume of 100 μ L and the flow rate was maintained at 5.0 mL/min.

D. Mass spectrometry conditions for MS/MS

For MS/MS analysis 10 μ L of all the samples were injected directly into the source by flow injection mode using Ammonium acetate (0.01M; pH 4.5) and methanol in the ratio of 45:55 %v/v as mobile phase at a flow rate of 0.5 mL/min. The mass spectrum was recorded in ESI positive mode. Ultra-high purity nitrogen and helium were used as curtain and collision gas, respectively. The typical ion source conditions were: nebulizer gas, 60 psi; dry temperature, 325°C; dry gas, 5.0 mL/min; capillary voltage, 5kV; capillary current, 81.787 nA; vaporizer temperature, 400°C and dwell time, 200 ms. For the collision induced dissociation (CID) experiments, the

precursor ion was selected using quadrupole analyzer and product ions were analyzed by time of flight analyzer. HRMS data acquisition was performed by the following source conditions: capillary voltage, 5 kV; declustering potential (DP) and the collision energy (CE) were -60 V and -10 V, respectively; focusing potential, 220 V; resolution 40,000 (FWHM).

E. Forced Degradation Study

Forced degradation studies of armodafinil was carried out under acid, alkaline, oxidative, thermal and photolytic stress conditions as per ICH guidelines [14]. A stock solution of ARM (1 mg/mL) was prepared by dissolving the appropriate amount in a suitable diluent. The stress was induced at a concentration of 100 μ g/mL; aliquots of samples were withdrawn at different time intervals, neutralized to pH 5-7, suitably diluted to obtain 10 μ g/mL and injected in the optimized chromatographic conditions against appropriate blank for control and stress conditions. Prior to analysis the aliquots of the samples were stored in refrigerator at 4°C. Prior to HPLC and LC-MS/MS analysis, the sample solutions were filtered through 0.45 μ m membrane filter.

F. Solution Stability

The primary stock solution of ARM was suitably diluted in the mobile phase and injected periodically into the column against blank to study the stability of the solution.

G. Preparation of Solutions for Stress Studies

100 mg of the ARM was weighed accurately and transferred to a 100 mL volumetric flask, a small quantity of diluent was added to dissolve the drug and the volume was made up with the diluent (Water: Methanol 45:55 %v/v). Stress degradation solutions were prepared by diluting 2.5 mL of the above solution to 25 mL with HCl (1N), NaOH (0.1N), water, diluent and hydrogen peroxide (6%) respectively for hydrolysis and oxidation. Photo degradation of the sample in solid state was performed under hot sunlight.

H. Acid/Base Hydrolysis

Stress solutions of 100 μ g/mL were prepared in 1N HCl and 0.1N NaOH at room temperature and allowed to stand for 24 hrs. Further to increase the extent of degradation, the standard solution (100 μ g/mL) prepared in 1N HCl and 0.1N NaOH were refluxed at 50° and 60°C for 24 hrs. Samples were withdrawn at regular time intervals, neutralized with 10% NaOH for acid hydrolysis samples and 10% HCl for base hydrolysis samples. They were then diluted ten times with the mobile phase prior to analysis with the optimized chromatographic conditions.

I. Degradation in presence of water and diluent

To assess the stability of ARM in the presence of water and the diluent used for the studies, degradation studies were also carried out in the presence of water and the diluent (Water: Methanol 45:55 %v/v) used. Stress solutions of 100 μ g/mL were prepared in water and diluent at room temperature and allowed to stand for 24 hrs. Further to increase the extent of

degradation, the standard solution (100 µg/mL) prepared in water and diluent were refluxed at 50° and 60°C for 24 hrs. Samples were withdrawn at regular time intervals, diluted ten times with the mobile phase prior to analysis with the optimized chromatographic conditions.

J. Oxidation

Stress solutions of 100 µg/mL were prepared in 6% hydrogen peroxide at room temperature and allowed to stand for 24 hrs. Further to increase the extent of degradation, the standard solution (100 µg/mL) prepared in 6% hydrogen peroxide were refluxed at 50° and 60°C for 24 hrs. Samples were withdrawn at regular time intervals, diluted ten times with the mobile phase prior to analysis with the optimized chromatographic conditions.

K. Photo Degradation

For photo degradation studies, a known quantity of ARM was weighed in a petridish and exposed to hot sunlight for 72 hrs. The samples withdrawn at regular time intervals were diluted ten times with the mobile phase prior to analysis with the optimized chromatographic conditions.

L. Purification of Alkali Degradation Product (PD-I)

The target impurity (DP-I) with retention time of 6.59±0.2 min was separated by isocratic elution mode using Zorbax RP C₁₈ (250 × 9.0 mm, id, 5µm) stationary phase and ammonium acetate (0.01M; pH 4.5): Methanol (45:55% v/v) as mobile phase at a flow rate of 5 mL/min. The eluents were monitored at 220 nm and the runtime was 25 min. The isolated compound was re-confirmed by chromatographic analysis, the purity of DP-I was found to be 99.8% and this sample was used for mass analysis (HR – MS/MS).

III. RESULTS AND DISCUSSION

A. Optimisation of Chromatographic Conditions

Initial separation of ARM was carried out on a Purospher C₁₈ column (150 × 4.6 mm id, 3µm) and methanol/water as mobile phase by varying the organic phase from 80% to 40%. ARM was well resolved but with tailing. Hence to obtain a symmetric peak the aqueous phase was replaced with buffer. Ammonium acetate buffer (0.01M)/Methanol was used at different ratios such as 30:70, 40:60, 45:55 and 50:50. Based on the retention time, a ratio of 45:55% v/v was optimized to achieve retention at about 8.42 ± 0.2 min. In order to obtain better theoretical plates, trials were performed by varying the pH of the aqueous phase from 2.5 to 4.5. A pH of 4.5 was optimized for the analysis.

Hence a better resolution between the ARM and degradation products was achieved with Hibar purospher C₁₈ (250 × 4.6 mm id, 5µm) column as stationary phase and ammonium acetate (0.01M, pH 4.5 adjusted with acetic acid)/methanol (45:55% v/v) as mobile phase in isocratic elution mode. The flow rate was maintained at 1 mL/min, the injection volume was 20 µL and the eluents were monitored at 220 nm with 20 min as runtime for each analysis. The chromatogram of the standard ARM (10 µg/mL) is shown in

Fig: 1. The method showed good separation between the degradation products formed during the degradation studies under various degradation conditions and ARM. The retention time of the major degradation product formed in acid and base degradation study was 6.59 ± 0.2 min (DP-I). The resolution among DP-I, DP-II and ARM was significant and was found to be more than 2. The results were tabulated in table-1. For LC-MS/MS and HRMS studies, the same method was used as HPLC, without the replacement of buffer.

B. Validation

The developed method was validated as per ICH (Q2) guidelines with respect to selectivity, specificity, linearity, accuracy, precision, robustness, limit of detection (LOD)/Limit of Quantification (LOQ). [13].

- **Specificity:** The samples of forced degradation studies were used to demonstrate the specificity and selectivity of the developed stability indicating analytical method. The study employed the samples generated during the degradation of ARM during photo degradation (exposure to sunlight, 72 hrs), Hydrolysis (1M HCl - at room temperature, Reflux at 50° and 60°C for 24 hrs, 0.1M NaOH - room temperature, Reflux at 50° and 60°C for 24 hrs, Water - at room temperature, Reflux at 50° and 60°C for 24 hrs) and oxidation (6% Hydrogen peroxide, at room temperature, Reflux at 50° and 60°C for 24 hrs). The degradants formed were adequately separated and well resolved from ARM, thus demonstrating the specificity and selectivity of the developed stability indicating analytical method. The peak purity was found to be greater than 99%.
- **Linearity and Range:** The linearity of the developed method for ARM was studied in the range of 0.5–35 µg/mL at nine different concentrations. Samples were analyzed in triplicate at nine concentrations. The linear regression equation and correlation coefficient were $Y = 64717x + 901$ and 0.9987 respectively demonstrating the linearity of the method. The results are tabulated in table-2.
- **Accuracy:** The accuracy of the method was measured in terms of recovery studies using standard addition method at 80, 100 and 120% levels of the sample concentration. The results were carried out in triplicate and the recoveries of the drug was calculated from the difference in peak areas of the fortified and unfortified samples. Excellent recoveries were observed between 98-102%. (Table-2)
- **Precision:** The intra and inter day precision was assessed using three quality control samples in the linearity range. Six replicates were analysed to determine the intraday precision. The inter day precision of the method was determined by repeating the procedure six times over three days. The % RSD values for the intra and inter day precision studies were <2%, indicating that the developed analytical method is precise. (Table-2)

- **Limit of detection and Limit of Quantification:** The sensitivity of the analytical method was expressed in terms of LOD and LOQ which represent the concentration of analyte that would yield a signal to noise ratio of 3 for LOD and 10 for LOQ. The LOD and LOQ were found to be 0.1 and 0.3 $\mu\text{g/mL}$ respectively. This was confirmed by preparing and analyzing these predicted concentrations in triplicate with the optimized chromatographic conditions. (Table-2)
- **Robustness & Ruggedness:** The robustness of the method was determined by making small but deliberate changes in the method parameters such as change in flow rate (± 0.2 mL) and pH (± 0.1 units). The resolutions between the closely eluting peaks were greater than 2 demonstrating that the method is robust. The ruggedness of the method was checked by different analyst on different instruments (Shimadzu and Agilent HPLC). The critical parameters were assessed and found to be within the limits indicating the ruggedness of the developed analytical method.

C. Forced degradation studies

- **Acid induced Degradation:** ARM on treatment with 1N HCl at room temperature for 24hrs showed 6.20% degradation. To enhance the rate of degradation ARM on treatment with 1N HCl was refluxed for 24 hrs at 50° and 60°C, which showed 13.82 and 34.33% degradation. One degradant (DP I) was observed at the Rt of 6.49 min under all the conditions. The area % of the degradant was 1.59, 1.70 and 2.63 respectively when exposed to 1N HCl at RT (room temperature), reflux at 50° and 60°C for 24 hrs respectively. The assay % of ARM after 24 hrs in 1N HCl at RT, reflux at 50° and 60°C was 98.32%, 99.28 % and 75.44% respectively (Table-3). The chromatogram of acid induced degradation at room temperature is shown in Fig. 2.
- **Base induced Degradation:** When ARM was subjected to basic degradation with 0.1N NaOH at room temperature showed 99.91% degradation. Also ARM was subjected to intrinsic degradation by refluxing at 50° and 60°C for 24 hrs. One degradant (DP I) eluted at Rt of 6.42 min when the sample was subjected to base hydrolysis at room temperature for 24 hrs. Whereas under reflux conditions the drug completely degraded with the formation of two degradation products at the retention times of 6.42 min and 9.34 min. The area % of the degradant DP I was 99.89% and assay % of ARM was 0.11% at room temperature. Under intrinsic conditions (reflux at 50° and 60°C for 24 hrs) the area % of degradants DP I and DP II, were found to be 98.49, 0.58% and 97.66, 2.63% respectively at 50° and 60°C. Also the assay % of ARM was found to be 0.11 and 0.014% during reflux at 50° and 60°C for 24 hrs (Table-3). The

chromatogram of base induced degradation is shown in Fig. 3.

- **Degradation in presence of water and diluent:** When ARM was treated with water and diluent (Methanol: water, 55:45% w/w) for 24 hrs at room temperature, no degradation was observed. Further the samples were also studied under intrinsic conditions of refluxing with water and diluent at 50° and 60°C for 24 hrs. No degradation products were observed under intrinsic conditions. With water the assay % of ARM was found to be 99.03, 98.22 and 96.97% respectively at room temperature, reflux at 50° and 60°C for 24 hrs. Whereas with the diluent assay % of ARM was found to be 99.72, 96.88 and 94.63% respectively at room temperature, reflux at 50° and 60°C for 24 hrs (Table-3). The chromatograms of the water and solvent induced degradation are shown in Fig. 4, 5.
- **Oxidative degradation:** When ARM was treated with 6% H_2O_2 for 24 hrs at room temperature, only 5% of degradation was observed and the assay of ARM was found to be 96.32%. To enhance the rate of degradation ARM was treated with 6% H_2O_2 and was refluxed at 50° and 60°C for 24 hrs. No degradation products were observed and the assay % of ARM was found to be 94.97 and 94.11% respectively when refluxed at 50° and 60°C for 24 hrs (Table-3). The chromatograms of the oxidative degradation are shown in Fig. 6.
- **Photo degradation:** When ARM was exposed to hot sunlight for 72 hrs (hot sunny light equivalent to 18 times of 1.2 million lux hrs) 11.56% of degradation was observed with the formation of one degradation product at Rt of 6.79 min. The area % of the degradant (DP I) was 0.07 % and the % assay of ARM was found to be 99.93% (Table-3). The chromatograms of photo degradation are shown in Fig. 7.

D. Degradation behavior of Armodafinil

From the degradation studies carried out under acid/base/neutral hydrolysis, oxidation and photolytic conditions, it was observed that the drug undergoes extensive degradation under base hydrolysis. Only one degradation product (DP-I) was formed under acid hydrolysis and photolytic conditions. But under intrinsic conditions of base hydrolysis two degradation products were observed (DP-I, DP-II). The retention of DP-I was 6.59 ± 0.2 min and DP-II was found to be 9.34 ± 0.2 min. Since the % of degradation under base hydrolysis was about 99%, the DP-I was isolated from the base hydrolysis samples by subjecting them to semi preparative HPLC conditions. The chromatographic and system suitability parameters such as Rt, RRT, peak purity, resolution, tailing factor, theoretical plates and asymmetry factor for ARM and DP-II are reported in Table-1.

E. Characterization studies by LC-MS/MS studies

The isolated target impurity (DP-I) was subjected to LC-MS/MS analysis. (Fig.8)

The hydrolysis of ARM under basic condition yielded two degradation products (DP-I, DP-II) in the presence of 0.1N NaOH at room temperature for 24 hrs. Since DP-I was the major degradation product, it was further isolated for chemical characterization. The molecular ion peak of DP-I appeared at an m/z of 261 (M+3H). The formation of this ion may be due to the hydrolysis of the amide moiety (release of Ammonia) leading to the formation of aromatic aldehyde. The major MSⁿ fragment ions of DP-I were observed at m/z 218 and 169. The m/z at 218 [M+2H; 2-((diphenyl methyl) hydroxyl) sulfonium ion] may be formed due to the loss of -CH₂CHO ion from m/z 261. Further elimination of the sulphonyl group may lead to the formation of the base peak at m/z 169 [M+2H; di phenyl methylum ion]. Based on the MSⁿ fragmentation studies DP-I and the formation of base peak at m/z 169, the DP-I was further identified as 2-(benzhydryl sulphonyl) aldehyde which is also supported by the literature [12]. The plausible structure of DP-I, and the fragmentation pathway are shown in Fig. 9.

TABLE I. SYSTEM SUITABILITY PARAMETERS OF ARMODAFINIL AND ITS DEGRADATION PRODUCT DP-I

comp ound	System suitability parameters						
	Rt (min)	RRT	PPI	Rs	Tf	As	N
DP-I	6.59 ±0.2	0	0.99	0	0.98	1.05	13966
ARM	8.41 ±0.2	1.28	0.99	2.25	0.97	0.96	14238

TABLE II. VALIDATION SUMMARY

Validation summary	
Accuracy (% Mean Recovery ± SD; %RSD)	
Concentration spiked level 1 ^x	99.76±0.45; 0.58
Concentration spiked level 2 ^x	99.88±0.75; 0.67
Concentration spiked level 3 ^x	99.02±0.64; 0.54
Linearity	
Linearity range	0.5-35 µg/ml
Slope	64717
Intercept	901
Correlation coefficient	0.9987
Precision	
Intraday precision (% Mean Recovery ± SD; %RSD)	
Concentration spiked level 1 ^y	10.05±0.03; 0.31
Concentration spiked level 2 ^y	19.93±0.20; 1.02
Concentration spiked level 3 ^y	29.98±0.19; 0.62
Interday precision (% Mean Recovery ± SD; %RSD)	
Concentration spiked level 1 ^y	10.04±0.05; 0.49
Concentration spiked level 2 ^y	19.97±0.36; 1.82
Concentration spiked level 3 ^y	29.96±0.19; 1.37
Sensitivity	
Limit of Detection	0.100 µg/ml
Limit of Quantification	0.300 µg/ml

concentration -level 1 = 10 µg/ml; concentration -level 2 = 20 µg/ml; concentration -level 3 = 30 µg/ml; ^x Average of three determinations; ^y Average of six determinations

TABLE III. DEGRADATION DATA OF ARMODAFINIL

Stress condition	Degradation data of Armodafinil			
	No of degradants	Retention time (min)	% Degradation at t _R	% Assay of ARM
1N HCl (RT, 24 hrs)	1	DP-I: 6.49	1.59	98.32
1N HCl (50°C, 24 hrs)	1	DP-I: 6.49	1.70	99.28
1N HCl (60°C, 24 hrs)	1	DP-I: 6.82	2.63	75.44
0.1N NaOH (RT, 24 hrs)	1	DP-I: 6.42	88.16	9.52
0.1N NaOH (50°C, 24 hrs)	2	DP-I: 6.42 DP-II: 9.34	98.49 0.58	0.11
0.1N NaOH (60°C, 24 hrs)	2	DP-I: 6.42 DP-II: 9.33	97.66 2.63	0.014
Water (RT, 24 hrs)	-	-	-	99.03
Water (50°C, 24 hrs)	-	-	-	98.22
Water (60°C, 24 hrs)	-	-	-	96.97
Diluent (RT, 24 hrs)	-	-	-	99.7
Diluent (50°C, 24 hrs)	-	-	-	96.88
Diluent (60°C, 24 hrs)	-	-	-	94.63
6% H ₂ O ₂ (RT, 24 hrs)	-	-	-	98.43
6% H ₂ O ₂ (50°C, 24 hrs)	-	-	-	94.97
6% H ₂ O ₂ (60°C, 24 hrs)	-	-	-	94.11
Sunlight (72 hrs)	1	6.71	0.63	97.36

TABLE IV. ELEMENTAL COMPOSITION OF DP-I IN MS/MS SPECTRA

Analyte	Elemental composition of DP-I in MS/MS spectra		
	Observed ion mass	Proposed formula	Calculated mass
DP-I	261.1044	C ₁₅ H ₁₄ O ₂ S	261.0949
	218.1887	C ₁₃ H ₁₂ OS	218.0765
	169.9697	C ₁₃ H ₁₂	170.1096

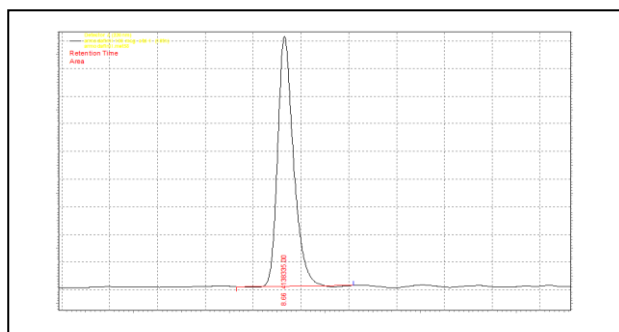


Fig 1: Typical Chromatogram of standard Armodafinil

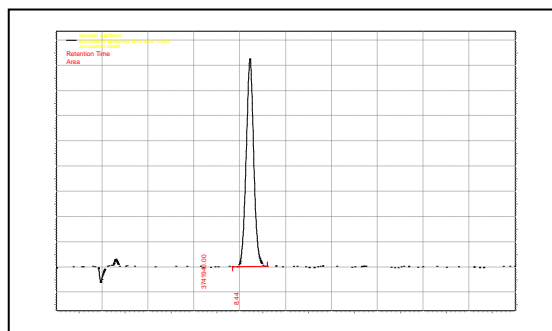


Fig 4: Chromatogram of neutral degradation sample with water at room temperature after 24 hrs

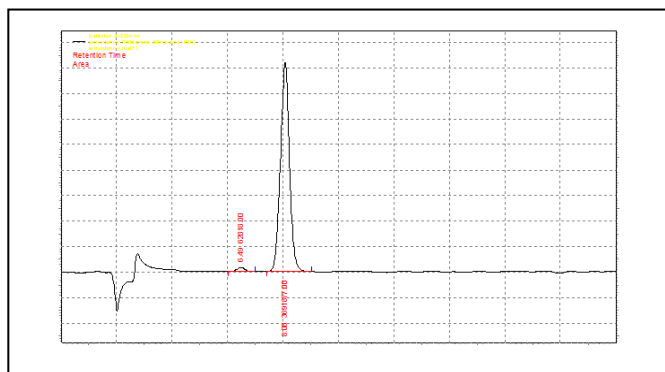


Fig 2: Chromatogram of Acid degradation sample with 1N HCl at room temperature after 24 hrs

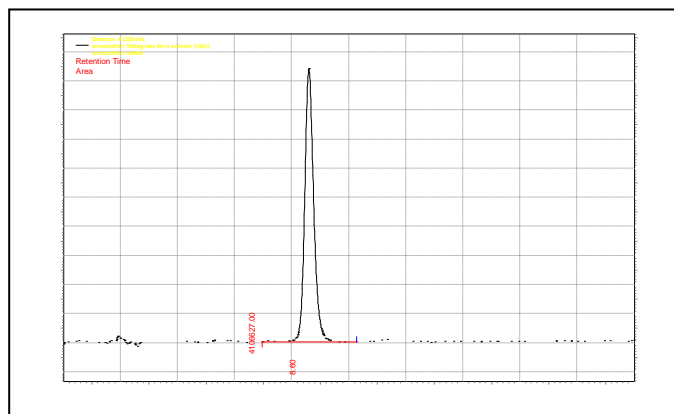


Fig 5: Chromatogram of neutral degradation sample with diluent at room temperature after 24 hrs

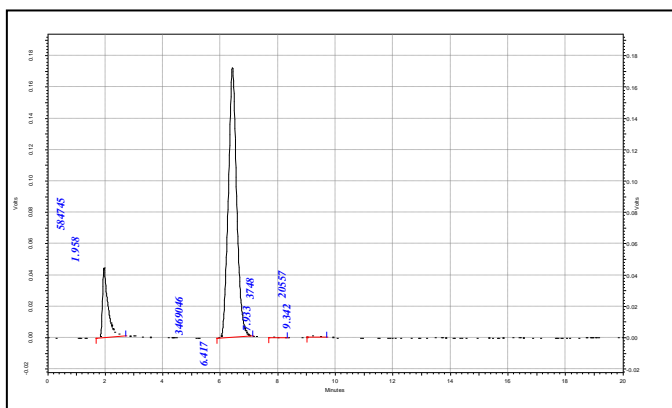


Fig 3: Chromatogram of base degradation sample with 0.1N NaOH at room temperature after 24 hrs

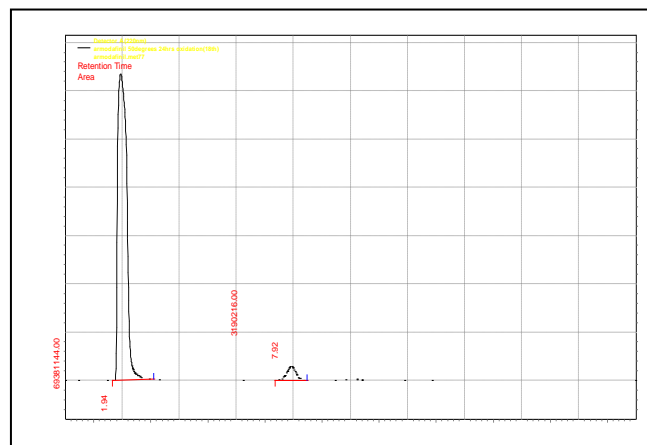


Fig 6: Chromatogram of oxidative degradation sample with 6% H₂O₂ at room temperature after 24 hrs

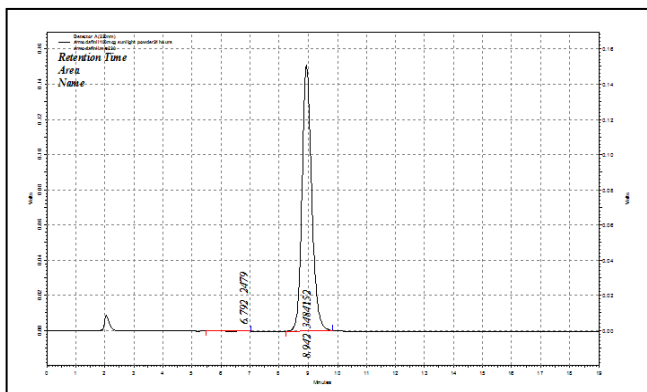


Fig 7: Chromatogram of oxidative degradation sample with 6% H₂O₂ at room temperature after 24 hrs

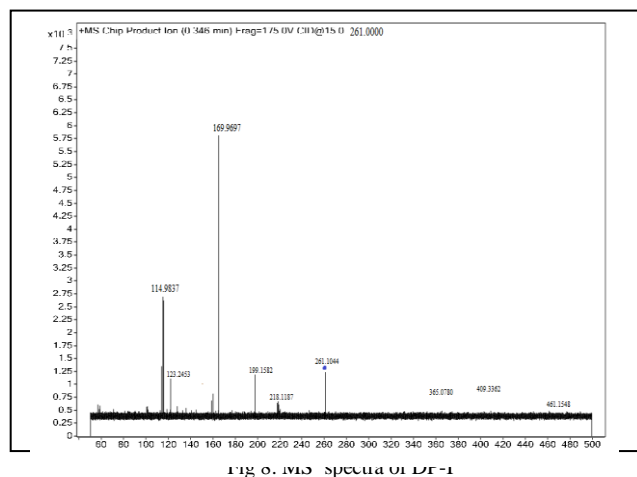


Fig 8. MS spectra of DP-I

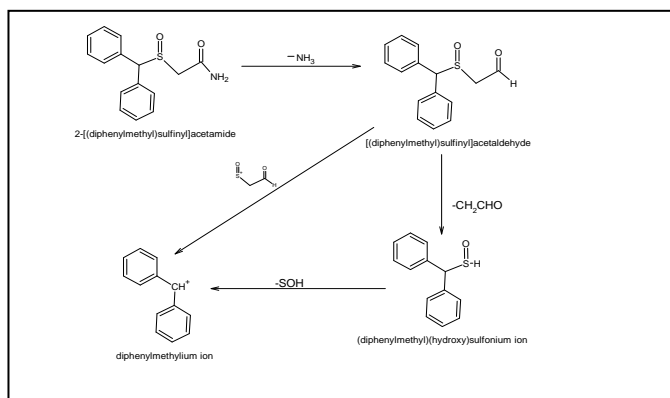


Fig 9: Plausible degradation pathway of base degradation product DP-I

IV. CONCLUSION

A simple, sensitive and specific stability indicating RP HPLC method was developed for the analysis of ARM in bulk drug and validated as per ICH guidelines. One degradation product was formed during the base degradation studies and it was well resolved from the API by the proposed LC method. The proposed structure of the base degradation product was characterized by MSⁿ studies and further confirmed by HR-MS data and accurate mass measurements.

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