A Comprehensive Perspective on Forced Degradation, Stability and Robustness conditions of Zoledronic acid Impurities by HPLC in Zoledronic Acid Injection

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ABSTRACT

The present study was aimed for forced degradation and establishment of solution stability/Robustness conditions of Zoledronic acid Impurities by reverse phase chromatography in Zoledronic Acid Injection. The separation was achieved by using column Inert Sustain Swift C18 (250 x 4.6 mm,5µ) mobile phase consisted of pH 2.80 triethylamine buffer and methanol in the ratio of (96:4 volume/volume). The flow rate was 0.8mL/min. Zoledronic acid was detected using UV detector at the wavelength of 220nm. Column temperature 30°C and sample temperature ambient and injection volume 20µL, run time 35 minutes. The retention time of Zoledronic acid was noted to be 5.37 min respectively. The method was validated as per ICH guidelines. The proposed method was found to be robust and stable.

Keywords: Zoledronic acid, liquid chromatography, Solution stability, Robustness, Forced degradation studies, validation.

INTRODUCTION

Zoledronic acid is assigned chemically as (1-hydroxy-2-imidazol-1-yl-phosphonoethyl) phosphonic acid monohydrate. It is a white crystalline powder[1].Zoledronic acid, a bisphosphonic acid, is an inhibitor of osteoclastic bone resorption. Biphosphonates are hydrolytically stable analogs of pyrophosphate, which inhibit bone resorption as a consequence of affecting osteoclast and likely osteoblast activity [2].

The therapeutic efficacy of bisphosphonates in disorders of bone turnover has been shown in the treatment of Paget's disease, tumor-induced hypercalcemia (TIH), and multiple myeloma[3]. It is being formulated for the treatment of tumor induced hypercalcemia, bone metastases arising from any cancer, and for the prevention of bone metastases associated with advanced breast cancer and locally advanced prostate cancer [4].

Molecular formula is $C_5H_{10}N_2O_7P_2$ and Molecular weight 272.090 g/mol[5]. The chemical structure of Zoledronic acid shown in Figure1.

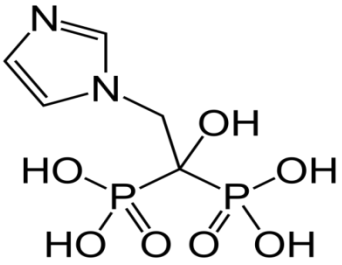


Figure 1: Chemical structure of Zoledronic acid

The literature survey reveals only few methods were reported till date a few HPLC methods[6] were reported for the estimation of Zoledronic acid in pharmaceutical dosage forms[7].

The objective of this study is to develop and validate following parameters like Precision, linearity, Accuracy for the determination of Zoledronic acid impurities in Zoledronic acid in its parenteral dosage form was published and reported by RP-HPLC Method.[8, 9] In continuation with the literature reported[9], following validation parameters like System suitability, Solution stability and Robustness has performed as per ICH guidelines[8].

MATERIALS & METHOD

Chemicals and Reagents

Analytical-grade Triethylamine, Orthophosphoric acid, Methanol, Hydrocholric acid, Sodium Hyroxide, Hydrogen Peroxide and water, reagents and chemicals were procured from Merck Chemicals. Mumbai, India.

Instrumentation

Waters HPLC model: 2695 with PDA, Ultrasonic bath Sonicator, pH Meter (Mettler Toledo) and Analytical Balance (Sartorious) were used in the present study.

Preparation of Buffer: Accurately transfer 2.0 mL of Triethylamine in 1000 mL of water, adjust the pH with orthophosphoric acid to 2.80 ± 0.05, filter and degas prior to use.

Preparation of Mobile phase: Mix 960 mL of buffer, 40 mL of methanol and degas.The flow rate was 0.8mL/min. Zoledronic acid was detected using UV detector at the wavelength of 220nm. Column temperature 30°C and sample temperature ambient and injection volume 20µL, run time 35 minutes. Used mobile phase as diluent.

Preparation of Impurity Stock Solution: Weigh and transfer around 2.0 mg of Imidazole RS and 2.0 mg of Imidazole-1-yl-ethanoic acid RS (RC-A) separately into

a 50 mL volumetric flask containing 15 mL diluent dissolve and dilute to volume up to the mark with diluent.

Preparation of System Suitability Solution:

Weigh and transfer around 4 mg of ZA into a 5 mL volumetric flask containing 2.5 mL diluent dissolve and add 0.5 mL of each impurity stock solution to the volumetric flask, dissolve and dilute to volume up to the mark with diluent.

Reference standard solution for RS:

Weigh transfer around 4.0 mg of ZA standard into a 5 mL volumetric flask containing 2.5mL diluent dissolve and dilute up to the mark with diluent and mix. Transfer 0.5 mL of this solution into a 50 mL volumetric flask and volume dilute up to the mark with diluent to obtain a solution of known concentration 0.01 mg/ mL.

System Suitability[8]

As per methodology, injected Blank (diluent) diluted standard for six times into HPLC system. Retention times of Imidazole, RC-A and Zoledronic acid are respectively 3.501, 4.661, and 5.415.

Bench top stability of standard & test preparation[8]:

Design:

Performed the stability study of diluted standard solution and sample preparation, by keeping them on bench top and established the solution stability.

Table 1: System suitability and Standard solution stability at RT and 2-8 °C, Mobile phase stability results

	Similarity factor		Resolution		% RSD
	Parameter RT	2-8 °C	Imidazole-RS & RC-A	RC-A & Zoledronic acid	
Initial	NA	NA	5.68	3.35	1.25
Day-1	0.99	1.00	5.64	3.36	1.12
Day-2	0.98	0.99	5.69	3.35	0.85
Acceptance	0.98 to 1.02		NLT 2.0	NLT 2.0	NMT 5.0

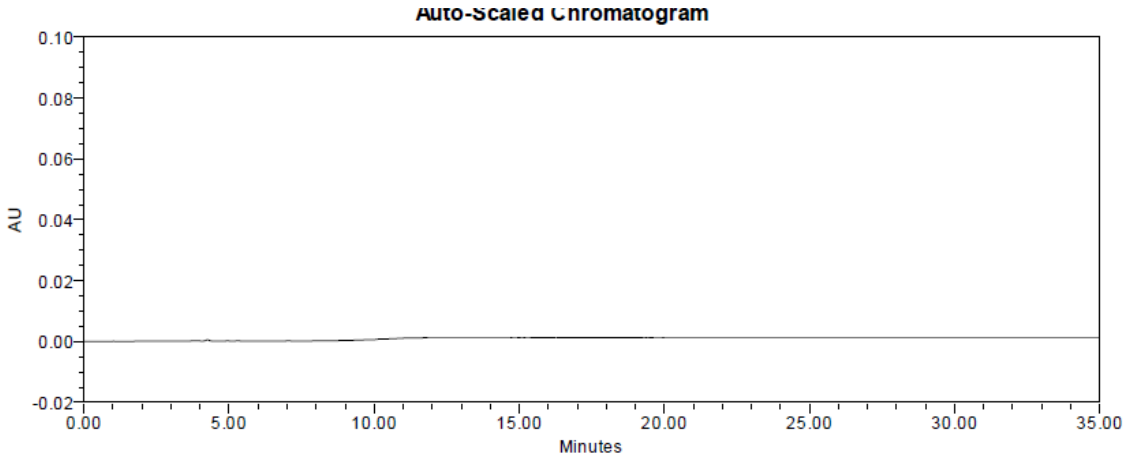
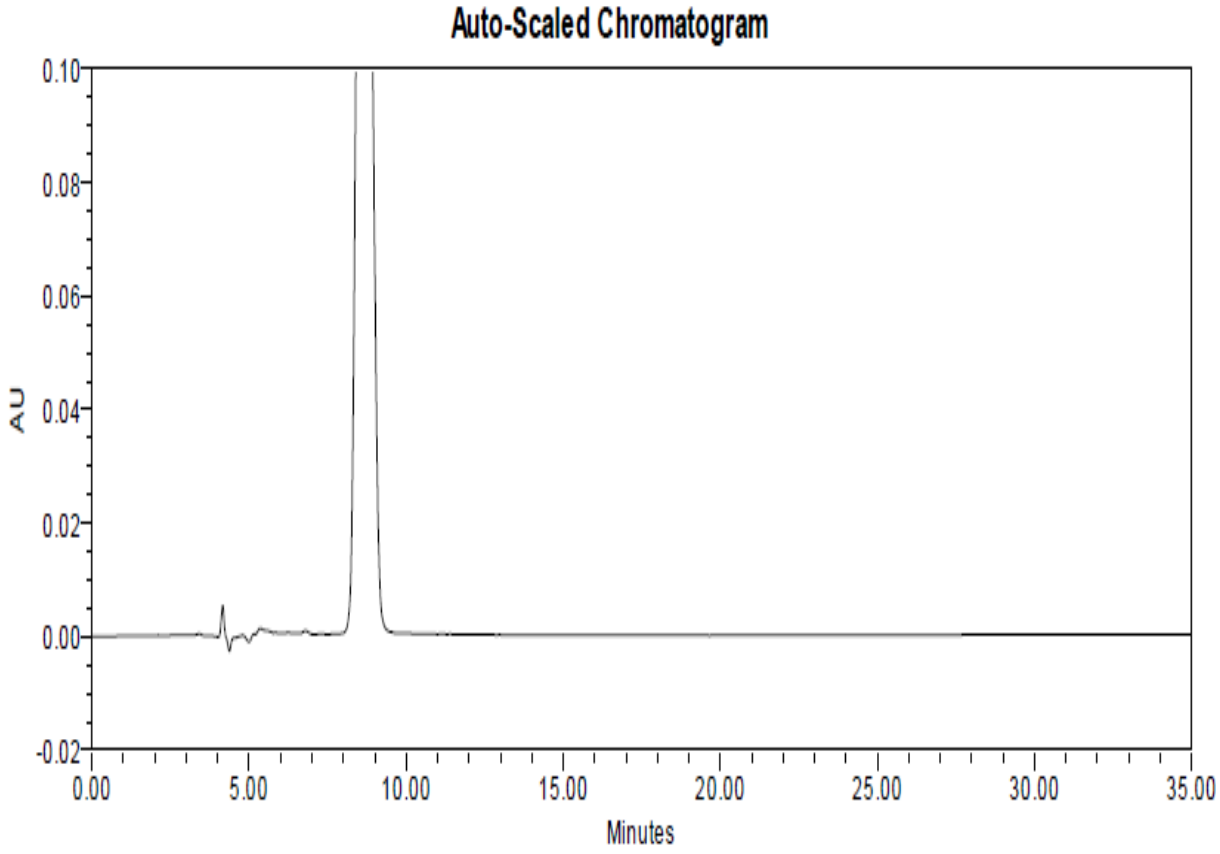


Figure 2: Typical chromatogram of Blank



\Figure 3: Typical chromatogram of Placebo

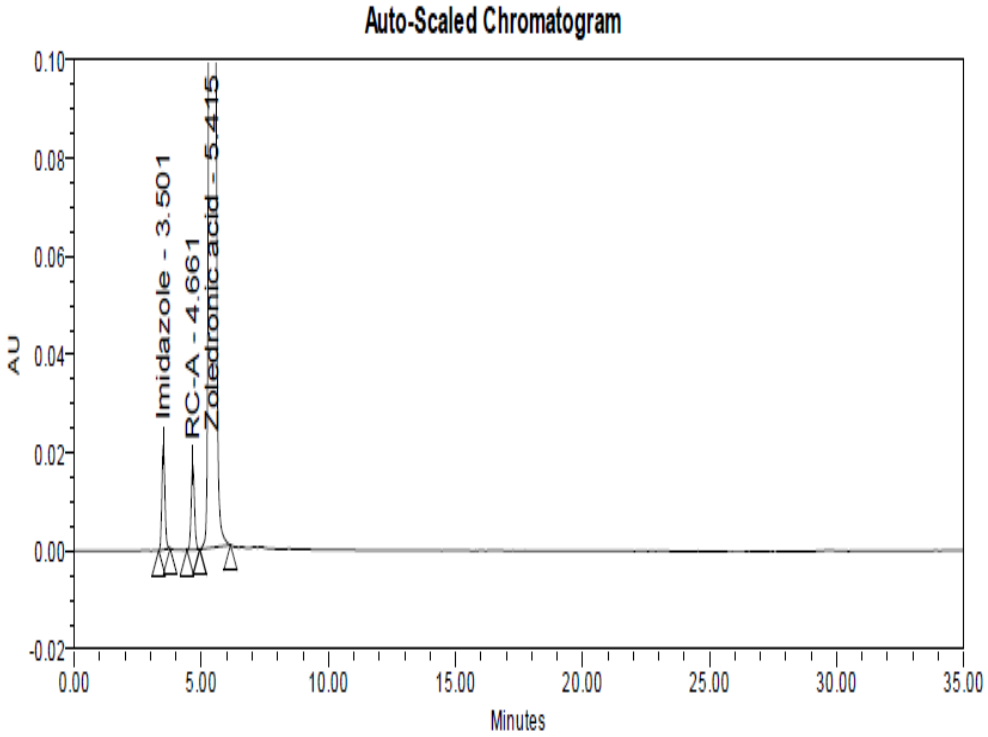


Figure 4: Typical chromatogram of SSTTable 2: Solution Stability of Sample Solution at room temperature

Table 3: Solution Stability of Sample Solution at 2-8 °C (Cold room)

Parameter	Initial	Day-1	Day-2	Initial	Day-1	Day-2
Imidazole-RS	0.591	0.567	0.575			
Difference	NA	0.034	0.016			
RC-A	0.608	0.586	0.570			
Difference	NA	0.022	0.038			
Maximum unknown	0.232	0.219	0.221			
Difference	NA	0.013	0.011			
Total impurity	1.431	1.372	1.366			
Difference	NA	0.059	0.065			
parameter						
Imidazole-RS				0.591	0.558	0.564
Difference				NA	0.033	0.027
RC-A				0.608	0.576	0.572
Difference				NA	0.032	0.036
Maximum unknown				0.232	0.228	0.224
Difference				NA	0.004	0.008
Total impurity				1.431	1.362	1.360
Difference				NA	0.069	0.071

Acceptance criteria:

- The system suitability should pass
- The similarity factor for standard in the range of 0.90 to 1.10
- The individual impurity should not differ more than 0.05 % and total impurities not more than 0.1 % from initial value.

The above results reveal that the standard solution is stable up to 2 days at room temperature and 2-8 °C and sample is stable up to 24 hours at room temperature and 2-8°C.

Robustness[8]:

Effect of Variation in buffer pH change[8]:

Design:

Analyzed system suitability preparations as per the methodology at low buffer pH (2.6 pH) and high buffer pH (3.0 pH).

Conclusion:

Table: 4 System suitability comparisons Variation in buffer pH change

Flow rate-mL/min	Resolution		% RSD
	Imidazole-RS & RC-A	RC-A & Zoledronic acid	
Low pH(2.6)	4.35	2.33	2.1
As Such(2.8)	5.75	3.45	1.4
High pH(3.0)	4.29	2.28	2.1
Acceptance	NLT 2.0	NLT 2.0	NMT 5.0

Conclusion: The above results reveal that the method is robust at low buffer pH (2.6 pH) and high buffer pH (3.0 pH).

Effect of Variation in Flow rate[8]:

Design:

Analyzed system suitability preparations as per the methodology at low column flow (0.7 mL/min) and high flow (0.9 mL/min).

Table: 5 System suitability comparison data of flow rate variation

Flow rate-mL/min	Resolution		% RSD
	Imidazole-RS & RC-A	RC-A & Zoledronic acid	
Low flow	6.1	3.6	0.8

As such	5.61	3.35	1.0
High flow	5.67	3.33	0.6
Acceptance	NLT 2.0	NLT 2.0	NMT 5.0

Conclusion:

The above results reveal that the method is robust at flow between 0.7 mL/min and 0.9 mL/min.

Effect of Variation in Column Oven Temperature[8]:

Design:

Analyzed system suitability preparations as per the methodology at low column oven temperature (25°C) and high column temperature (35°C).

Table: 6 System suitability

Oven Temperature	Resolution		% RSD
	Imidazole-RS & RC-A	RC-A & Zoledronic acid	
25°C	5.49	2.45	0.20
30°C	5.66	3.36	1.11
35°C	5.64	3.42	1.25
Acceptance	NLT 2.0	NLT 2.0	NMT 5.0

Conclusion:

The above results reveal that the method is robust at column oven temperature between 25°C to 35°C variations.

Effect of Variation in Organic phase composition in the Mobile Phase[8]:

Design:

Analyzed system suitability preparations as per the methodology by changing the Organic

Phase composition in the Mobile Phase

Table: 7 System suitability

Buffer:MeoH (v/v)	Resolution		% RSD
	Imidazole-RS & RC-A	RC-A & Zoledronic acid	
970:30	4.26	2.10	1.15

960:40	5.65	3.25	1.11
950:50	4.25	2.22	1.25
Acceptance	NLT 2.0	NLT 2.0	NMT 5.0

Forced Degradation Studies[8]

Study Design:

Applied the stress conditions to the samples and then injected into HPLC System.

Results:

Table: 8 Interference from Degradation process in Blank

Name of Condition	Stress Condition	Interference at RT of (Yes/No)		
		RC-A	**Imidazole RS**	**Zoledronic acid**
Acid	Stressed with 0.2N HCl for 1 Hour at 60 °C	No	No	No
Base	Stressed with 0.2N NaOH for 1 Hour at 60°C in Oven	No	No	No
Peroxide	Stressed with 1 mL of 30 % H2O2 solution for 1 Hour at 60°C	No	No	No
Thermal	Stressed for about 6 Hours at 105 °C in Oven	No	No	No
Water	Added 1 mL of Water Stressed for 1 Hour at 60°C in Oven	No	No	No

Table: 9 Interference from Degradation process in Placebo

Name of Condition	Stress Condition	Interference at RT of(Yes/No)		
		RC -A	**Imidazole RS**	**Zoledronic acid**

Acid	Stressed with 0.2N HCl for 1 Hour at 60 °C	No	No	No
Base	Stressed with 0.2N NaOH for 1 Hour at 60°C in Oven	No	No	No
Peroxide	Stressed with 1 mL of 30 % H2O2 solution for 1 Hour at 60°C	No	No	No
Thermal	Stressed for about 6 Hours at 105 °C in Oven	No	No	No
Water	Added 1 mL of Water Stressed for 1 Hour at 60°C in Oven	No	No	No

Acceptance criteria: Peaks due to blank and placebo should not show any interference at the retention time of Zoledronic acid and Impurities.

Table: 10 Acid degradation

Condition	Stressed with 0.2N HCl for 1 Hour at 60 °C
% Assay	91.2
Imidazole-RS	NA
RC-A	NA
SMU	7.728
Net degradation	8.011

Mass balance	99.21
Purity Angle	0.434
Purity Threshold	1.278
Peak purity	PASS

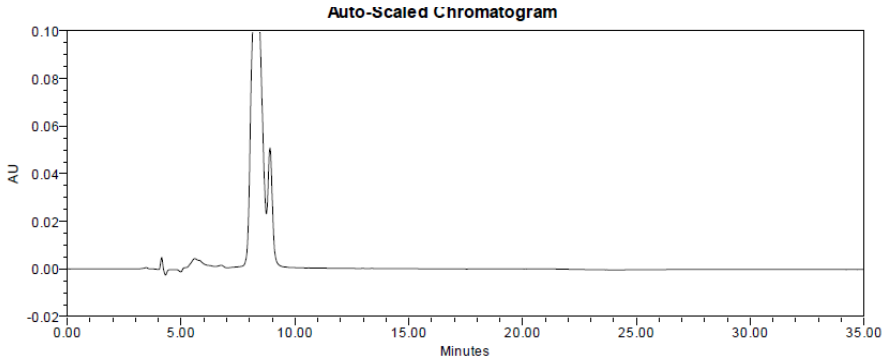


Figure: 5 Typical chromatogram of Acid stress Placebo

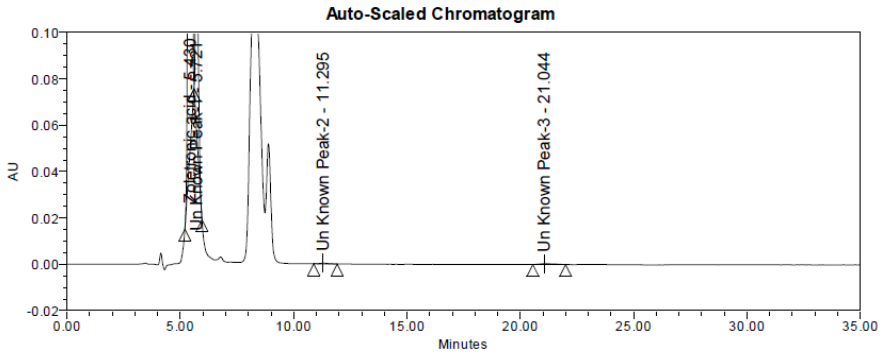


Figure: 06Typical chromatogram of Acid stress Sample

Table: 11 Base degradation:

Condition	Stressed with 0.2N NaOH for 1 Hour at 60°C in Oven
% Assay	98.8
Imidazole-RS	NA
RC-A	NA
SMU	0.167

Net degradation	0.412
Mass balance	99.21
Purity Angle	5.324
Purity Threshold	20.075
Peak purity	PASS

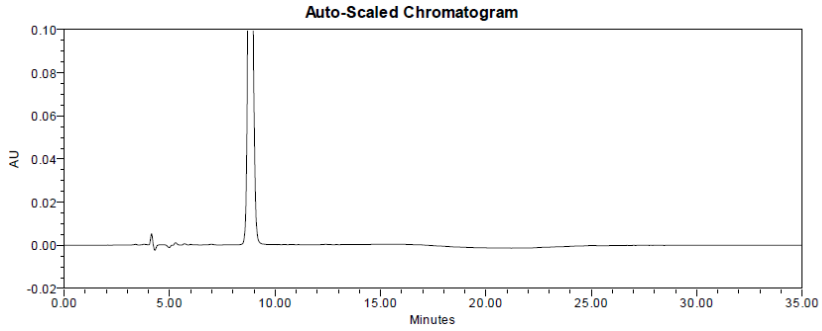


Figure: 07 Typical chromatogram of Base stress Placebo

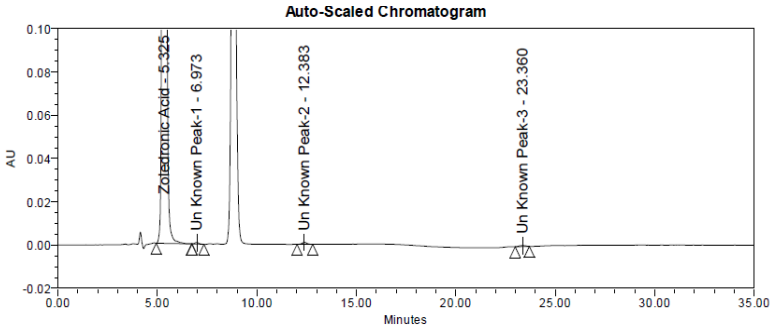


Figure: 08 Typical chromatogram of Base stress Sample

Table: 12 Peroxide degradation:

Condition	Stressed with 1 mL of 30 % H_2O_2 solution for 1 Hour at 60°C
% Assay	93.2
Imidazole-RS	0.116
RC-A	NA

SMU	1.307
Net degradation	2.401
Mass balance	95.6
Purity Angle	7.335
Purity Threshold	18.001
Peak purity	PASS

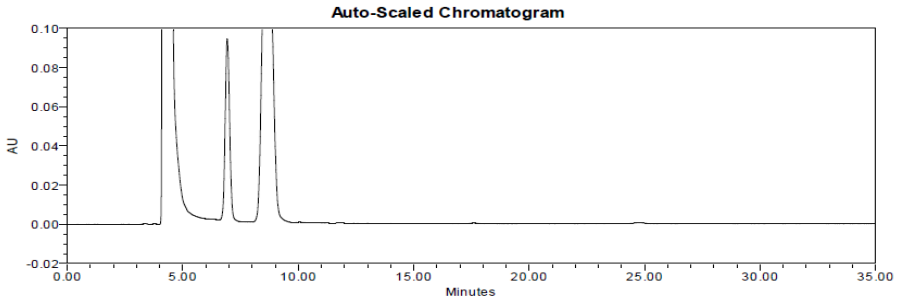


Figure: 09 Typical chromatogram of H_2O_2 stress Placebo

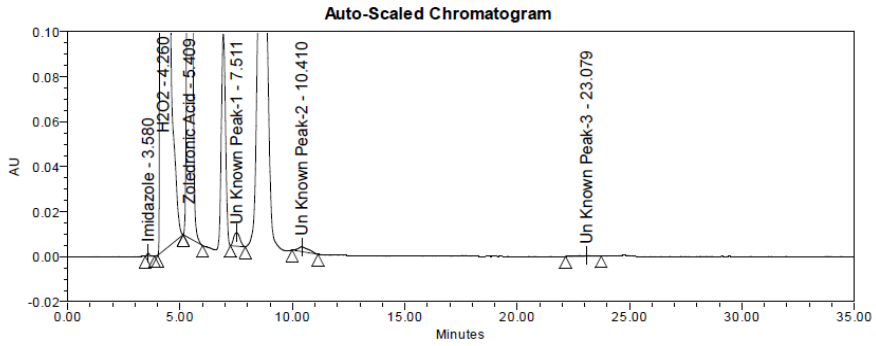


Figure: 10 Typical chromatogram of H_2O_2 stress sample

Table: 13 Thermal degradation:

Condition	Stressed for about 6 Hours at 105 °C in Oven
% Assay	100.9
Imidazole-RS	NA
RC-A	NA

SMU	0.219
Net degradation	0.302
Mass balance	101.2
Purity Angle	29.809
Purity Threshold	90.000
Peak purity	PASS

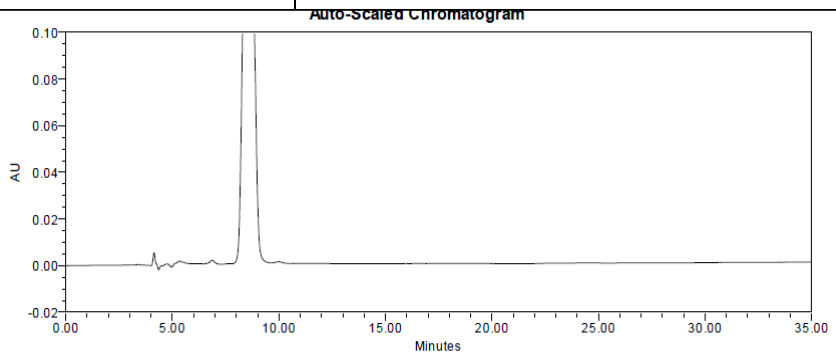


Figure: 11 Typical chromatogram of Thermal stress Placebo

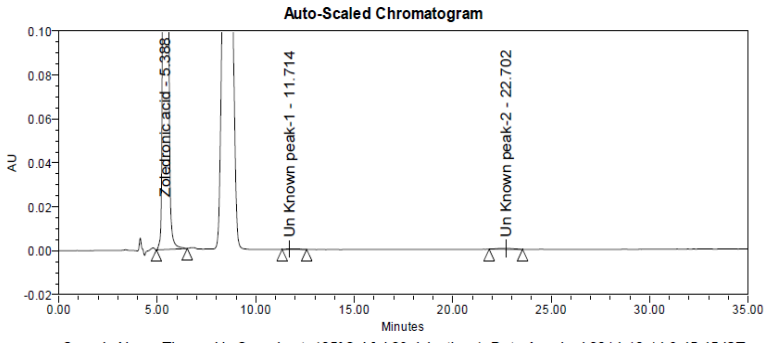


Figure: 12 Typical chromatogram of Thermal stress Sample

Table: 14 Water degradation

Condition	Added 1 mL of Water Stressed for 1 Hour at 60°C in Oven
% Assay	97.2
Imidazole-RS	NA

RC-A	NA
SMU	0.164
Net degradation	0.380
Mass balance	97.6
Purity Angle	1.591
Purity Threshold	5.072
Peak purity	PASS

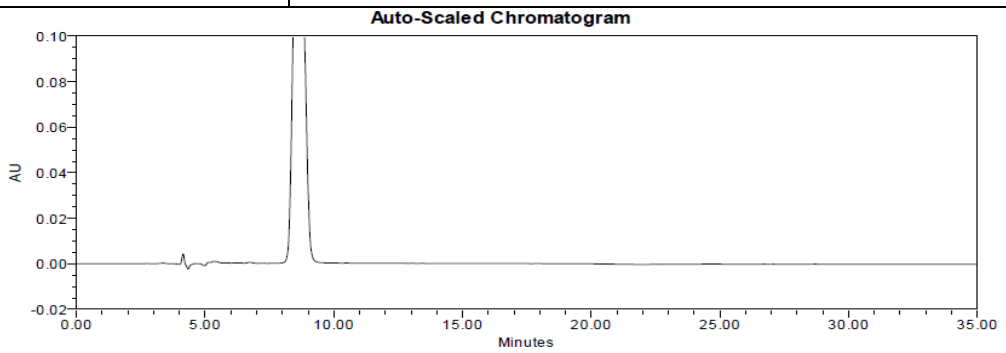


Figure: 13 Typical chromatogram of H_2O stress Placebo

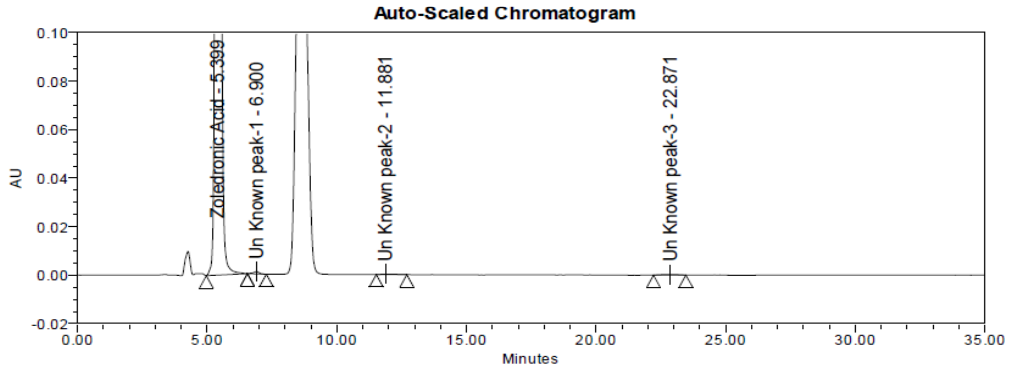


Figure: 14 typical chromatogram of H_2O stress sample

- Mass balance of all stressed samples should not be less than 90 % and should not be more than 110 %.
- Peak purity of Active should pass as per spectral data analysis.

Conclusion:

The above results reveal that the method is specific.

Conclusion:

Forced degradation studies were done stress conditions like acid, base, peroxide, thermal and water. Results obtained from the degradation studies reveal that method is specific. The present analytical method was validated as per defined protocol and it meets the specified acceptance criteria. Hence, it was concluded that the analytical method is robust and stable. The above results reveal that the method is robust at Organic phase composition in the Mobile Phase variations. Hence, the present analytical method proved as stability indicating and can be used for regular analysis and its intended purpose.

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