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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING RP-HPLC METHOD FOR NABUMETONE

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ABSTRACT

A simple stability-indicating high performance liquid chromatographic method for the assay of nabumetone in the presence of its degradation products was developed using reverse phase Symmetry C-18, 250mm X 4.6mm, 5µm column, in the mobile phase containing phosphate buffer and Acetonitrile**:** Water (80:20 % v/v) at flow rate 1mL/min with UV detection at 230 nm. The retention time was found to be 5.533 min. Validation of an analytical method was established by laboratory studies. Selectivity was validated by subjecting the stock solution of nabumetone to acidic, alkaline, wet, oxidative and thermal degradation. The proposed method was found to be linear at concentration of 1 to 3 µg/mL (R^2=0.999). The limit of detection and limit of quantification was 0.12 µg/mL and 0.37 µg/mL respectively and the method was found to be specific. Method precision and precision of the system was found to be within the limits of the acceptance criteria. Relative standard deviation for precision of the method and precision of the system was found to be 1.4% and 0.4% respectively. The percentage recovery was found 101.31%. The results indicate that there is no interference from excipients for the proposed method, thus making the method simpler, less time consuming and suitable for routine quantitative estimation of nabumetone tablet formulation. As the method could effectively separate the drug from its degradation products, it can be employed as a stability-indicating one.

Keywords: Nabumetone, Stability-indicating HPLC, Stress degradation, Formulations.

INTRODUCTION

The stability-indicating method is defined as validated quantitative analytical method that can detect the change with time in the chemical, physical or microbiological properties of the drug substance and the drug product, that are specific so that the content of active ingredient, degradation can be accurately measured without interference[1]. Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and the excipients in drug product[2].

Nabumetone (Fig. 1) is Anti inflammatory analgesic drug. Nabumetone is a prodrug, which undergoes hepatic biotransformation to the active component, 6- methoxy- 2-naphthylacetic acid (6-MNA) that is a potent inhibitor of prostaglandin synthesis, most likely through binding to the COX-2 and COX-1 receptors.

Nabumetone is a naphthylalkanone. It is a non- selective prostaglandinG/H synthase (cyclooxygenase or COX) inhibitor that acts on bothprostaglandin G/H synthase 1 and 2 (COX-1 and -2). Prostaglandin G/H synthase catalyses the conversion of arachidonic acid to prostaglandin G2 and prostaglandin G2 to prostaglandin H2. Prostaglandin H2 is the precursor to a number of prostaglandins involved in fever, pain, swelling, inflammation, and platelet aggregation. The parent compound is a prodrug that undergoes hepatic biotransformation to the active compound, 6-MNA. The analgesic, antipyretic and anti-inflammatory effects of NSAIDs occur as a result of decreased prostaglandin synthesis.

Although nabumetone is well absorbed from the gastro-intestinal tract, plasma concentrations after oral doses are too small

to be measured as it undergoe rapid and extensive first pass metabolism in liver to the principal active compound 6-MNA and other inactive metabolites. 6-MNA is more than 99 % bound to plasma proteins. 6-MNA diffuses into synovial fluid. It crosses the placenta and is distributed into breast milk. There is considerable interindividual variation in the plasma elimination half life of 6-MNA, especially in the elderly; some reported mean values at steady state include 22 to about 27 h for young adults and about 25 and 34 h in elderly patients. 6-MNA eventually undergoes further metabolism by o-methylation and conjugation. About 80 % of a dose is excreted in urine as inactive or conjugated metabolites and less than 1 % as unchanged 6-MNA.

Literature survey revealed that a few analytical methods have been reported for the estimation of Nabumetone in pharmaceutical dosage forms and in biological fluids by HPTLC[3-4], Ion pair chromatography[5], Simultaneous estimation[6], Spectrophotometric Methods[7], RP-HPLC[8-16], RP-UPLC[17] techniques. The aim of the present study was to conduct forced degradation studies on nabumetone as per ICH guideline Q1A (R2), to establish economic, accurate, specific, repeatable stability-indicating method for determination of nabumetone in presence of its degradation products by using HPLC, to validate the developed methods as per the ICH guidelines Q2 (R1) and to analyzed the marketed formulation of nabumetone.

EXPERIMENTAL

Chemicals and reagents

Pure sample of Nabumetonewas generously supplied by Nosch Labs Pvt Ltd, Hyderabad, India. Nabumetone tablet was purchased from local market. Acetonitrile (HPLC grade), Methanol (HPLC grade), Water (HPLC grade), Concentrated Hydrochloric acid (AR grade), Sodium Hydroxide (AR grade), Hydrogen Peroxide (AR grade) were purchased from SD Fine Chemicals, Mumbai (India). 0.45 µ Nylon filter papers purchased from Pall India Pvt. Ltd, Mumbai (India).

Instrumentation

Waters HPLC system equipped with a PU-2080 plus pump and a 2075 absorbance detector having Borwin chromatography software version 1.5 and Rheodyne injector with injection loop capacity 100 µL. All samples were filtered through 0.45µm membrane filter. Mobile phase and sample/standard preparations were degassed using a sonicator.

Chromatographic conditions

Reverse phase high performance liquid chromatographic method was developed on a Symmetry C-18, 250mm X 4.6mm, 5µm column using mobile phase containing phosphate buffer and Acetonitrile**:** Water (80:20 % v/v) at ambient temperature. The elution was carried out isocratically at flow rate of 1mL/min. The UV detector was set at 230 nm.

Preparation of standard solution

Accurately weigh and transfer10 mg nabumetone to 100 mL volumetric flask, dissolved with sufficient quantity of methanol as diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.2 mL of the above stock solution into a 10mL volumetric flask and dilute up to the mark with diluent. . Mix well and filter through 0.45µm filter.

Preparation of sample solution

Accurately transfer the contents of 20 tablets of nabumetone, ground into a fine powder and calculate the average weight. Weigh and transfer the sample equivalent to 10mg ofsample into a 100mL volumetric flask, add about 50 mL of mobile phase as diluent, sonicate to dissolve it completely and filter through 0.45µm filter.

Degradation studies

Standard nabumetone at aconcentration of 40mg/mL was used in all the degradation studies. The standard nabumetone were subjected to stress conditions in 0.1M HCl and 0.1M NaOH, at the temperature 80°C at different time intervals, after completion of the degradation processes, the solutions were diluted with mobile phase. Forthe peroxide degradation studies, standard nabumetone of 40mg/mL was dissolved in 3%hydrogen peroxide and subjected to stress condition at the temperature 60°C at different time intervals. Thermal degradation was performed by exposing solid standard drug to dry heat at 70°C for 70 hrs. Photolytic degradation was performed by exposing solid standard drug to sunlight for 70 hrs. These degraded solutions were withdrawn periodically and subjected to analysis after suitable dilution with mobile phase to get 40µg/mL. 2 µg/mL of thisdegraded solution were injected using the same chromatographic conditions.

Method validation

The analytical method validation was carried out as per ICH method validation guidelines.

The validation parameters addressed were accuracy, precision (intra-day and inter-day), specificity, and linearity, limit of detection, limit of quantitation, robustness and stability of nabumetone in mobile phase. Standard plots were constructed for nabumetone in the range of 20 to 60µg/mL. Accuracy was determined by fortifying the mixture of pre-analysed standard of three unknown concentrations of the drug with the marketed samples.

RESULTS AND DISCUSSION

Development and optimization of the stability-indicating HPLC method

An isocratic method validation was found necessary to optimize the separation of major products formed under various stress conditions. The best resolution was achieved with initial run of phosphate buffer (pH 5.5) and Acetonitrile: water (80:20 % v/v) at a flow rate of 1mL/min, the retention time was observed at 5.533 min.The method worked well with the mixture of degradation solutions and was even applicable to tablet formulation. The typical chromatogram of nabumetone standard was shown in Fig. 2.

Degradation behaviour

HPLC studies on the combination under different stress conditions indicated the following degradation behaviour (Table 1).

Acidic degradation

The standard drug nabumetone at 60°C in 0.1M HCl was found to be degrade 14.61% at 30 min, the degradation product was appeared at retention 5.475 min was identified (Fig.3).

Alkaline degradation

The nabumetone drug was found to be degrade in alkaline hydrolysis of 0.1M NaOH at 60°C and degraded to an extent of 9.65% at 2 hour, the degradation product was appeared at retention5.433 min was identified (Fig. 4).

Wet degradation

There was no degradation found when thenabumetone drug was refluxed with H_2O for 4 h at 100°C and retention at5.425 min was identified (Fig. 5).

Oxidative degradation

The drug nabumetone was found to be labile to hydrogen peroxide at 60°C and 17.46% of the drug was decomposed at 2 hours, the degradation product was appeared at retention 5.592 min was identified (Fig. 6).

Thermal degradation

There was no degradation found when the nabumetone drug was subjecting to dry heat 70°C for 70 hours and at retention 5.592 min was identified (Fig. 7).

Photolytic Degradation

There was no degradation found when the nabumetone drug was exposed to direct sunlight for 70 hours and at retention 5.417 min was identified (Fig. 8).

Validation of method

System suitability

A system suitability test was performed to evaluate the chromatographic parameters before the validation runs. The results of system suitability parameters were given in Table 2.

Specificity

The HPLC chromatograms recorded for the blank tablet and blank tablets exposed to the degradation conditions showed no peaks at the retention time of nabumetone and also the representative chromatograms of stressed samples under various stress conditions showed that nabumetone was well resolved from its degradation products, indicating the specificity of the method.

The HPLC chromatograms recorded for blank, placebo, standard and sample solution showed that nabumetone peak was not affected by diluents and placebo.

Linearity and range

The Figure 10 represents linearity graph for accuracy and precision. The least square line was obtained by plotting the values of amount found versus values of amount added from table no. 4, had a slope of 1.011 and intercept of 0.033. As the value of slope encompassed to 1, value of y- intercept was near to zero and the correlation coefficient was greater than 0.99 indicates that method was linear in the range of 80 to 120%.

Limit of detection (LOD)

The LOD is the lowest limit that can be detected. Based on the S.D. deviation of the response and the slope, the detection limit was found to be 0.12 µg/mL.

Limit of quantitation (LOQ)

The LOQ is the lowest concentration that can be quantitatively measured. Based on the S.D. deviation of the response and the slope, the quantitation limit was found to be 0.37 µg/mL.

Accuracy and precision

The results of intra and inter day variation of nabumetone at three different concentrations levels (80%, 100%, 120%) are depicted in Table4.

The % relative standard deviation for 80%, 100%, 120% was less than 2indicating that the method is accurate and precise.

The mean values of amount estimated of the drug was found to be very close to the amount added and the % RSD values of intra-day were found to be very low indicating acceptable accuracy and precision of the method. The intra and inter-day results at each level were subjected to one way ANOVA and F values at each level were obtained as a ratio of Between Mean Square to the Within Mean Square (F = BMS/WMS). The obtained values were found to be less than the tabulated F (2, 6) at α = 0.05 (Tabulated F value = 5.14). These indicated that there was no significant difference between intra and inter-day variability, suggesting good intermediate precision of the method.

Robustness

In all the deliberately varied chromatographic conditions (e.g., flow rate and UV detectorwavelength), the chromatogram for system suitability showed satisfactory resolution (%RSD <2) with no significant changes in chromatographic parameters as shown in Table 5.

Analysis of marketed products

The validated method was applied for the analysis of nabumetonetablet of strength 500mg. In this case average purity obtained is 102.56% and no interference of impurity peak observed in nabumetone peak. The results of analysis are given in Table 6.

CONCLUSION

This study presents a simple and validated stability-indicating HPLC method for estimation of nabumetone in the presence of degradation products. The developed method is specific, accurate, precise and robust. All the degradation products formed during forced decomposition studies were well separated from the analyte peak demonstrating that the developed method was specific and stability indicating**.** The method could be applied with success even to the analysis of marketed products nabumetone tablet formulation, as no interference was observed due to excipients or other components present.

Table 1: Degradation studies of Nabumetone

Stress condition	Degradation time	Area of peak	% Degradation	% of active drug present
Acidic	30 min	3644359.005	14.61 %	85.39
Alkaline	2 hours	3585028.809	9.65 %	90.35
Wet	4 h	3744947.477	No Degradation	100
Oxidative	2 hours	3252639	17.46 %	82.54
Thermal	70 hours	3640254.19	No Degradation	100
Photolytic	70 hours	4031850.647	No Degradation	100

Table 2: Summary of Validation parameter

System suitability	Result
Theoretical plates	8275.652
Linearity range (µg/mL)	1-3
Retention time	5.533
Co-relation co-efficient	0.999
LOD (µg/mL)	0.12
LOQ (µg/mL)	0.37

Table 3: Recovery study data of Nabumetone

Conc.	Amount added(mg)	Amount found(mg)	% Recovery	% Mean recovery
80%	400	411.04	102.76	101.3
100%	500	505.53	101.11	
120%	600	600.19	100.03	

Table 4: Accuracy and precision studies

Amount added (mg)	Amount found (mg)			Within mean square	Between mean square	F
	Day 1	Day 2	Day 3			
80% 400 mg	407.82	392.00	395.52	47.18	123.61	2.61
	411.72	403.87	393.37			
	413.69	408.26	407.94			
Mean	411.04	401.37	398.94			
S.D.	2.98	8.41	7.86			
% R.S.D.	0.72	2.09	1.97			
100% 500 mg	504.8	505.91	509.30	8.23	11.77	1.42
	505.87	505.77	506.58			
	505.92	512.75	512.37			
Mean	505.53	508.14	509.41			
S.D.	0.63	3.99	2.89			
% R.S.D.	0.12	0.78	0.56			
120% 600 mg	592.08	605.19	605.84	55.66	79.05	1.42
	614.12	612.11	610.58			
	594.37	610.35	610.40			
Mean	600.19	609.21	608.94			
S.D.	12.11	3.59	2.68			
% R.S.D.	2.01	0.59	0.44			

Table 5: Robustness study of nabumetone

Chromatographic changes		% RSD (Peak area)	Asymmetry	Theoretical plates
Flow rate (ml/min)	0.8	1.68	1.24	6933.32
	1	0.69	1.21	6701.30
	1.2	1.13	1.16	6345.82
UV wavelength (nm)	228	0.86	1.20	6483.33
	230	0.69	1.21	6701.30
	232	0.87	1.20	6286.17

Table 6: Assay result of tablet formulation using proposed method

Amount per tablet (mg)	Mean amount Recovered (mg)	Amount found (%)	Average (%)	±SD	%RSD
500	511.40	102.28	102.56	0.25	0.24
500	511.30	102.66			
500	511.80	102.76			

Table 7: List of abbreviations

Sr. No.	Abbreviation	Full form
1	µg	Microgram
2	mg	Milligram
3	µ	Micron
4	µL	Microliter
5	mL	Millilitre
6	µm	Micro meter
7	mm	Millimeter
8	nm	Nano meter
9	N	Normal
10	λ_{max}	Wavelength of maximum absorbance
11	h	Hours
12	R.T.	Retention time
13	i.d.	Internal diameter
14	r^2	Correlation coefficient
15	RSD	Relative standard deviation
16	SD	Standard deviation
17	CC	Calibration curve
18	BMS	Between mean square
19	WMS	Within mean square
20	CAS	Chemical abstracts service

21	IUPAC	International Union of Pure and Applied Chemistry
22	API	Active pharmaceutical ingredient
23	DP	Drug product
24	SIAM	Stability-indicating assay method
25	UV/VIS Spectrophotometer	Ultra violate/visible spectrophotometer
26	FTIR	Fourier transmission infra-red spectroscopy
27	RP-HPLC	Reverse phase-high performance liquid chromatography
28	HPTLC	High performance thin layer chromatography
29	GC-MS	Gas chromatography-Mass spectrometry
30	LC-MS	Liquid chromatography-Mass spectrometry
31	LC-MS-MS	Liquid chromatography-Tandem mass spectrometry
32	LC-NMR	Liquid chromatography – Nuclear magnetic resonance
33	CE-MS	Capillary electrophoresis- Mass spectrometry
34	ICH	International Conference on Harmonization
35	FDA	Food and Drug Administration
36	US-FDA	United States Food and Drug Administration
37	NSAID	Non-steroidal anti-inflammatory drug
38	DL	Detection limit
39	QL	Quantitation limit

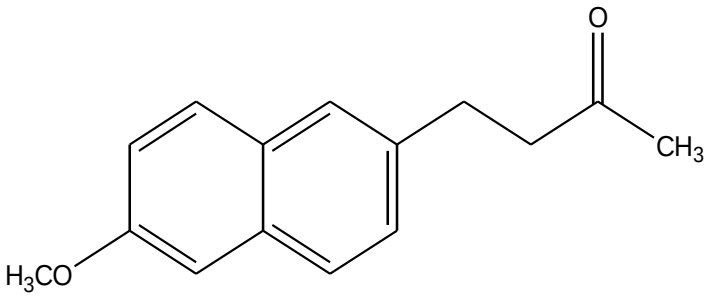

Fig. 1: Chemical structure of Nabumetone

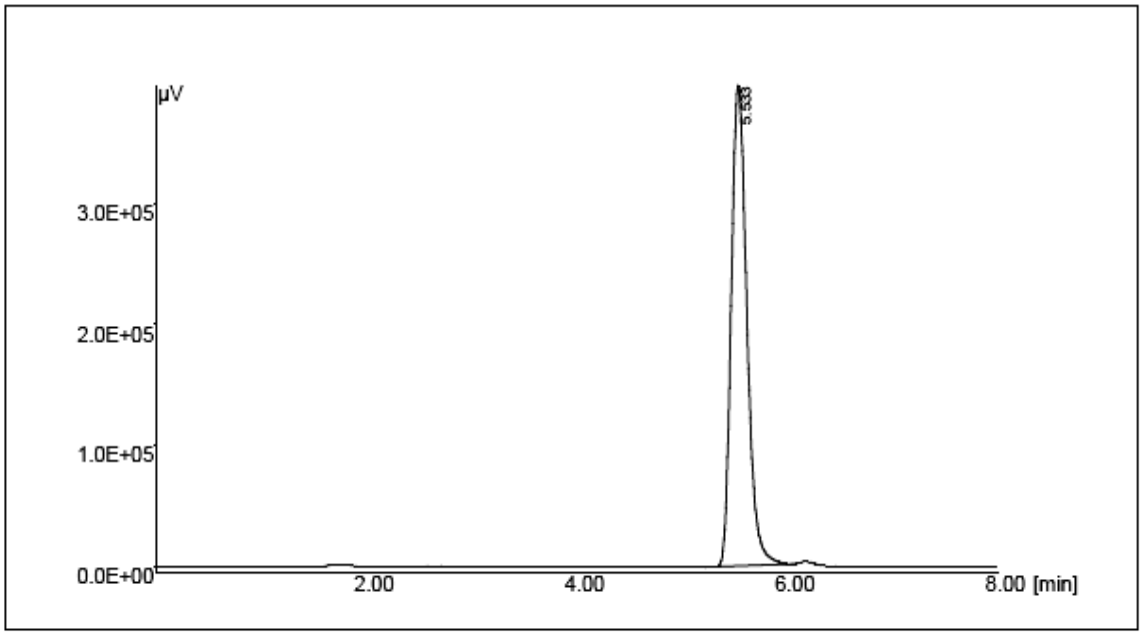

Fig. 2: Typical chromatogram of nabumetone

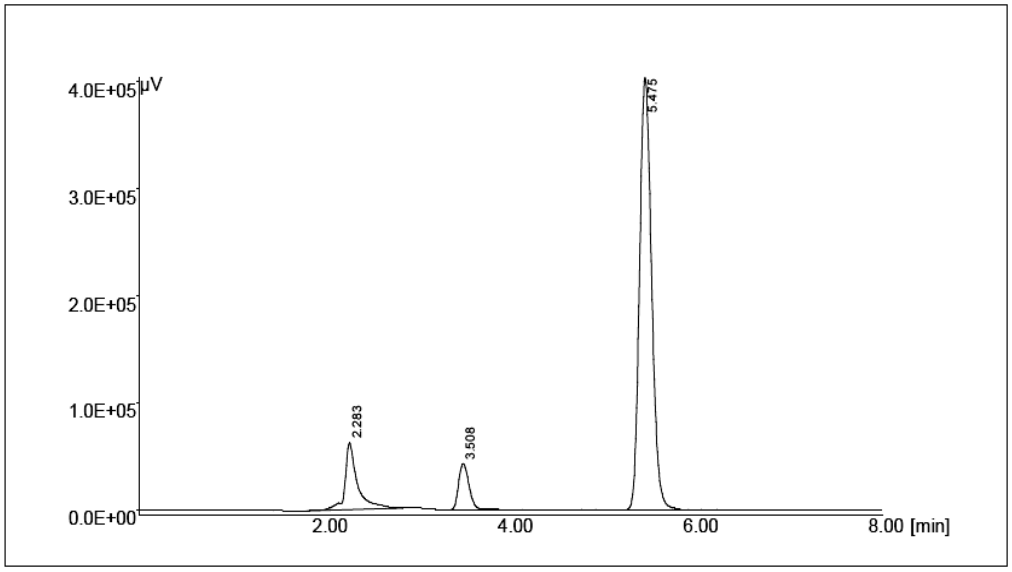

Fig. 3: Typical HPLC chromatogram recorded during acidic degradation

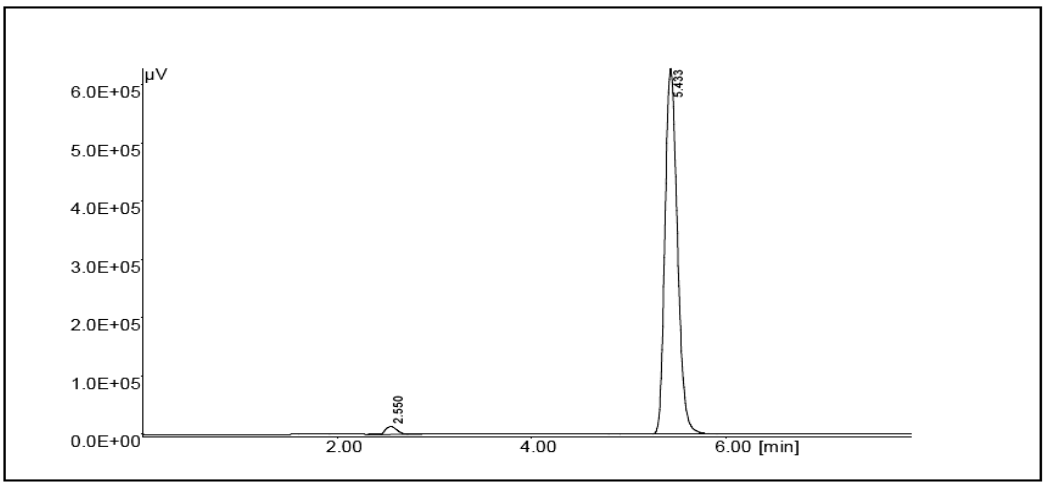

Fig. 4: Typical HPLC chromatogram recorded during alkaline degradation

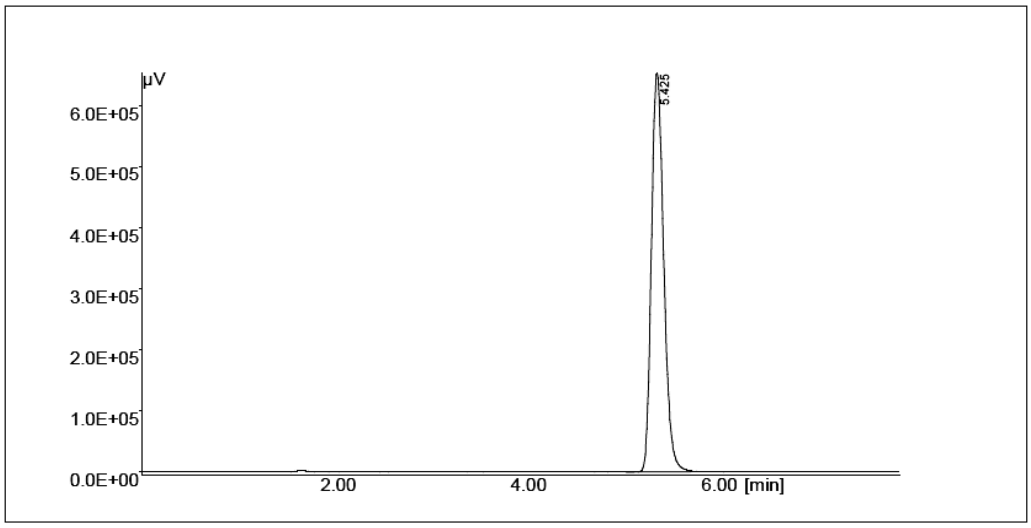

Fig. 5: Typical HPLC chromatogram recorded during wet degradation

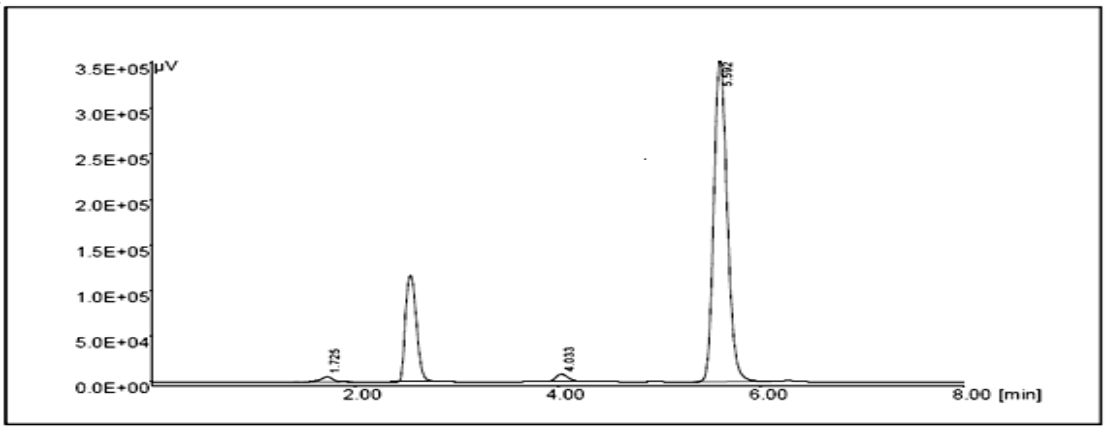

Fig. 6: Typical HPLC chromatogram recorded during oxidative degradation

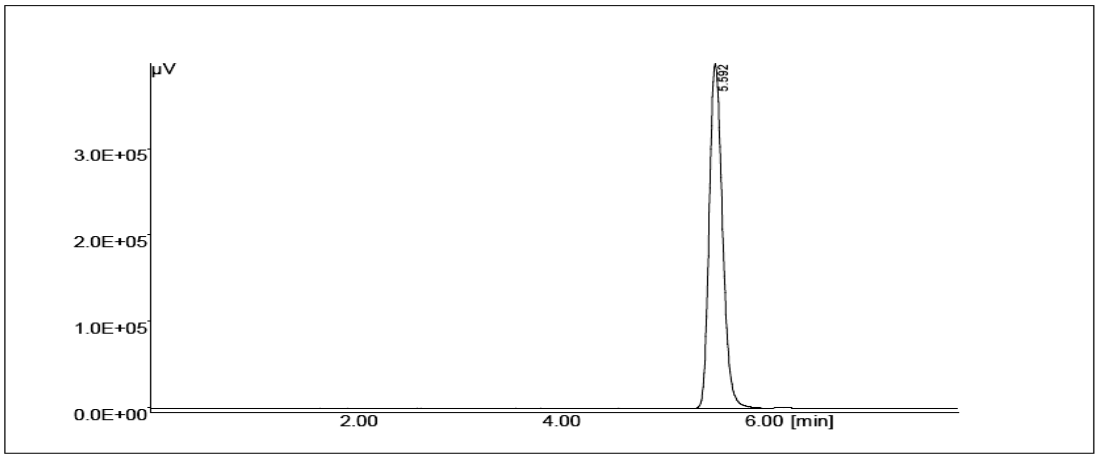

Fig. 7: Typical HPLC chromatogram recorded during thermal degradation

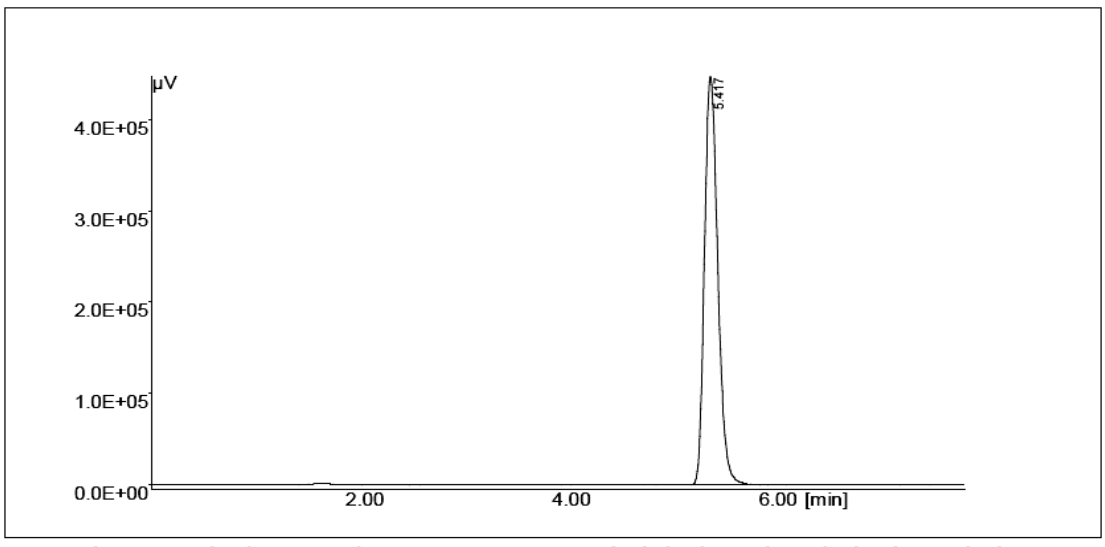

Fig. 8: Typical HPLC chromatogram recorded during Photolytic degradation

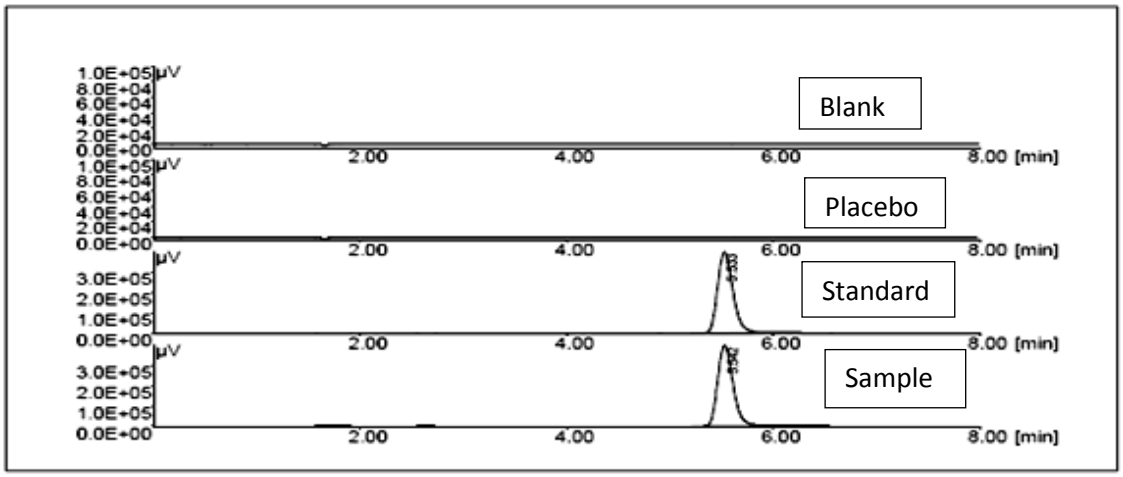

Fig. 9: Representative chromatogram for specificity

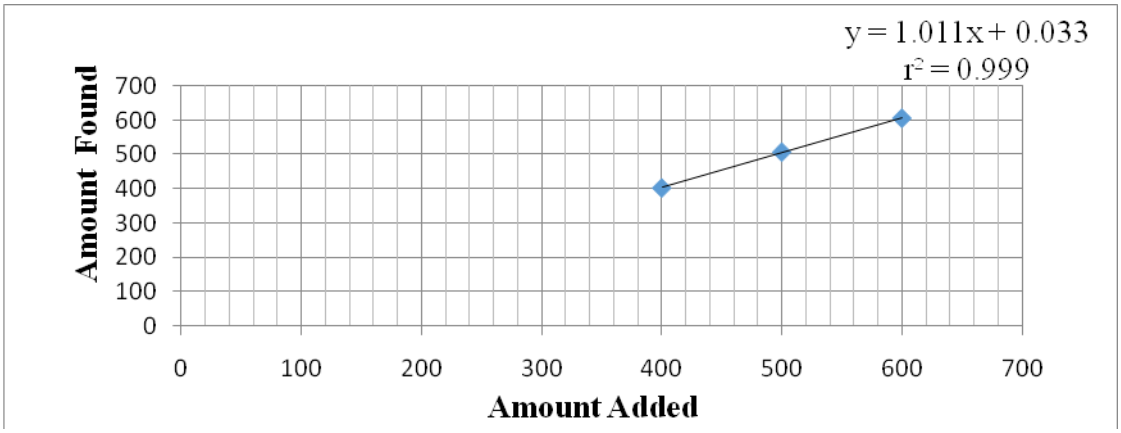

Fig. 10: Linearity graph for accuracy and precision

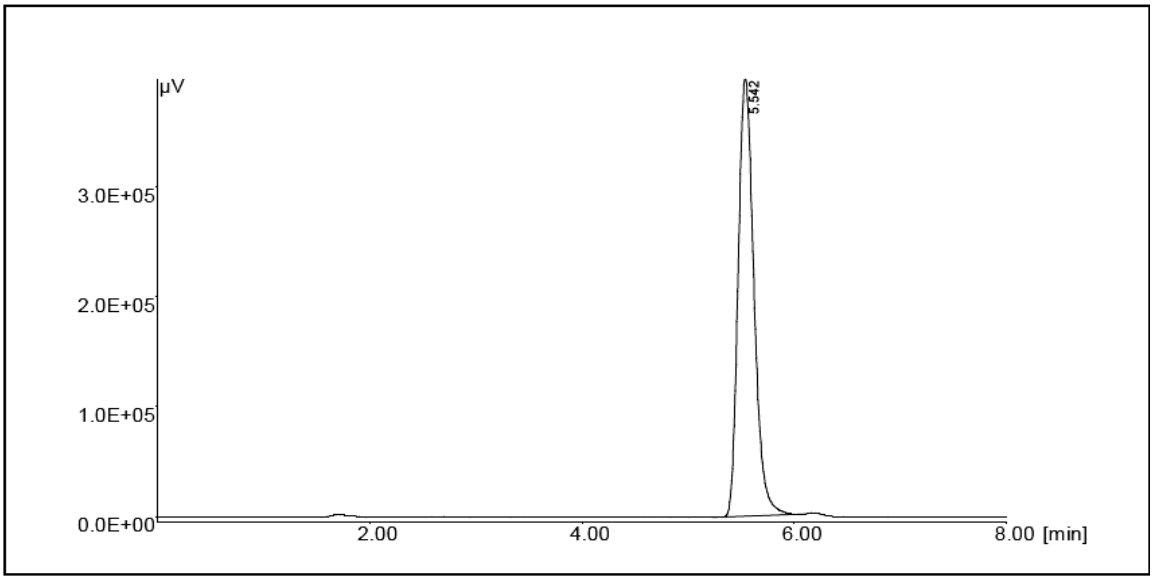

Fig. 11: Representative chromatogram of marketed formulation

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