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Stability Indicating RP-HPLC Method Development and Validation of Everolimus in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

Everolimus is semisynthetic derivative of sirolimus, which is isolated from Streptomyces hygroscopicus. A novel reversed phase high performnce liquid chromatography (HPLC) method for determination of the everolimus in the presence of degradation product or pharmaceutical excipients. Seperation was carried out using Cosmosil C18 (250mm x 4. 6ID), column having particle size 5 micron using acetonitrile and methanol mixture, and pH adjusted to 3, a flow rate of 1. 0 mL/min, and ultraviolet detection at 285nm. A retention time nearly 3. 806 min was observed. The calibration curve for everolimus was linear from range of 5 -25 µg/mL with limit of detection and limit of quantitation of 0. 0817 & 0. 2478 µg/mL, respectively. Analytical validation parameters such as selectivity, specificity, linearity, accuracy and precision were evaluated and relative standard deviation value for all the key parameters were less than 2. 0%. The stability-indicating method was developed by exposing the drug to stress conditions of acid and base hydrolysis, oxidation, photodegradation, and thermal degradation; the obtained degraded products were successfully seperated from the APIs. This method was validated in acceptance with ICH guidelines and results within the acceptance criteria.

KEYWORDS: *Everolimus, reversed-phase high performance liquid chromatography, stablity, validation*

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INTRODUCTION:

Everolimus a macrocyclic lactone is a derivative of sirolimus having chain of 2 hydroxyethyl at 40 position of the macrolide ring. It has the potent immunosupressant with antiproliferative effect with more solubility and stability as well as more favourable pharmacokinetics[2, 5]. Everolimus is a mammalian target rapamycin inhibitor that targets the mTOR single transduction complex[7]. The half life of Everolimus shorter than sirolimus and is thus administered twice in daily. Red blood cells is bound from everolimus. The oral bioavalibility of everolimus is generally 16% and 0. 5-1. 0 is peak plasma concentratio. Sirolimus is metabolised by the enzyme CYP-450 and P-glycoprotein is a substrate. Further, everolimus shows significant intra-and inter-subject variation in its pharmacokinetics. Everolimus has a limited therapeutic index and flexible bioavailability[5] . Everolimus is beneficial for the treatment of various type of cancer including The breast carcinoma, renal cell carcinoma, non-small cell lung carcinoma and mantle cell lymphocytes. It is also used for the treatment of kidney cancer [7]. The everolimus dose is 10mg.

Everolimus chemically is dihydroxy-12-[(2*R*)-1-[(1*S*, 3*R*, 4*R*)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl] propan-2-yl]-19, 30-dimethoxy-15, 17, 21, 23, 29, 35-hexamethyl-11, 36-dioxa-4-azatricyclo [30. 3. 1. 0 hexatriaconta-16, 24, 26, 28-tetraene 2, 3, 10, 14, 20-pentone and C53H83NO14 is molecular formula [8].

For the estimation of everolimus single or combination of other drugs of analytical methods are described, such as UV spectrophotometry, HPLC, LC-MS/MS approach, high throughput HPLC technique. The present method was accurate, robust, simple, rapid and precise [3].

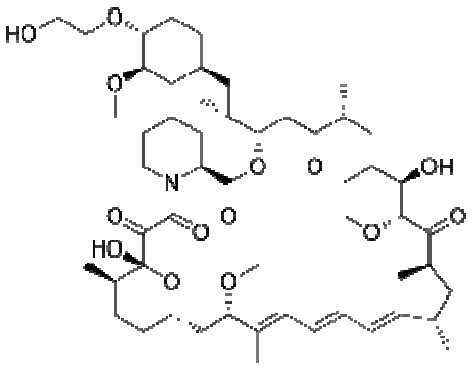

Fig No. 1: Chemical Structure of Everolimus.

MATERIALS AND METHODS

Chromatographic seperation was performed on a HPLC system equipped with UV detector, injection system. Data was analyzed by using HPLC work station software. A cosmosil C18 (250mm x 4. 6ID, Particle size: 5 micron) was used. The flow rate was 1 mL/min using acetonitrile: methanol (60:40) with pH 3 adjusted with mobile phase. Analytical balance used for weighing was shimadzu.

Optimization Detection of Wavelenght

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is one that gives good response for the drugs that are to be detected. For good response, optimization of wavelength was done at different wavelengths by UV detector.

CHROMATOGRAPHIC CONDITIONS

Mobile phase was prepared by mixing 600 mL acetonitrile with 400 ml of methanol and adjusted pH 3. The chromatographic conditions were run as shown table 1.

Preparation of Standard Solution

Accurately 10. 0 mg weighed quantity of Everolimus was transferred to 10. 0 mL volumetric flask. That was dissolved by adding 5. 0 mL mobile phase and then the drug solution was diluted up to the mark with mobile phase to get the stock solution of 1000 µg/mL of Everolimus.

Optimization of Mobile Phase

Based on drug solubility, stability and suitability of drug in different solvents, various mobile phases and compositions were tried to get a good resolution and sharp peak. The standard solution containing drugs were run in different mobile phases.

For selection of mobile phase, various mobile phase compositions containing methanol and water in different ratios were tried. The following mobile phases were tried,

A. Methanol: water [70: 30 % v/v] pH 3
B. ACN: Methanol [50: 50 % v/v] pH 3
C. ACN: Methanol [60: 40 % v/v] pH 3

Each mobile phase was filtered through 0. 45 µm membrane filter and degassed by sonication for 20 min. From the various mobile phases tried, mobile phase containing methanol and water in gradient program was selected, since it gave sharp peaks with symmetry within limits and significant retention times for drugs.

Preparation of Mobile Phase

Prepare mobile phase by taking methanol and water in various proportion ACN: methanol (60: 40) pH3. Mobile phase was filtered through 0. 45µm membrane filter and degassed by sonication for 20 min.

VALIDATION AND SYSTEM SUITABILITY PARAMETERS

LINEARITY

The calibration curve was plotted between concentrations versus peak area over the concentration range of 5-25 µg/ml. An aliquot of each solution were injected under the optimized chromatographic conditions and the chromatograms were recorded. The correlation coefficient (r2) value was found to be 0. 992. The result obtained are shown in table 2

PRECISION

Intraday precision study was carried out by preparing test solution of same concentration and analyzing it at two different times in a day. The same procedure was followed for two different days to determine interday precision. The result was reported as %RSD. The precision result showed a good reproducibility with percent relative standard deviation less than 3, 4. .

ACCURACY

Samples are prepared normally covering 50 % to 150 % of the nominal sample preparation concentration. . For this study, • Prepare three preparation of each 50 %, 100 % and 150 % level and inject in to the chromatography.

- Make the injection lowest concentration to highest concentration.
- Calculate individual recovery, mean recovery and %RSD.

SYSTEM SUITABILITY

System suitability testing is an integral part of many analytical procedures to determine to overall system performance. Calculating the following values used to access overall system performance.

1. Relative retention
2. Theoretical plates
3. Capacity factor
4. Resolution
5. Peak asymmetry
6. Plates per meter

LOD and LOQ

LOD and LOQ determined by the following formula by taking the standard deviation of y intercept and slope from the linearity curves.

LOD=3. 3σ/S

Where, σ = the standard deviation of the y-intercept

S = slope of calibration curve of analyte

LOQ=10σ/S

Where, σ = the standard deviation of the y-intercept

S = slope of calibration curve of analyte.

ROBUSTNESS

The effect of small deliberate change in optimized method was studied by robustness evaluation. To evaluate robustness of the developed method, parameter was deliberately varied. These parameters included variation of flow rate and variation wavelength. Factor selected was changed at two levels, for change in the flow rate in ml/min and change in wavelength in nm. In significant differences in peak areas and less variability in retention time were observed.

RUGGEDNESS

Ruggedness is the study to determine effect of external parameters on the method. To evaluate ruggedness of the developed method, parameter was deliberately varied. These parameter included variation of system, different analyst.

Assay of Marketed Formulation

20 tablets of Everolimus marketed formulation (Evermil 10) were taken, weighed individually and crushed into fine powder. Average weight of tablet sample (equivalent to 10 mg of Everolimus) were weighed and transferred to 100mL volumetric flask & diluent was added to make up the volume. Sonicate for 10 min with occasional swirling. The above solution was filtered through 0. 45µm membrane filter, the prepared stock solution is of 100 µg/ml.

FORCED DEGRADATION STUDY:

To perform the forced degradation studies, 10 mg of everolimus drug was subjected to acidic, alkaline, neutral, oxidizing, thermal and photolytic conditions. For acidic degradation, 1 ml of stock solution was added to 1 ml of 2 N HCl and heated under reflux at 60º for 30 min. The mixture was neutralized by the addition of 2 N NaOH. For alkaline degradation, 1 ml of stock solution was added to 1 ml of 2 N NaOH and heated under reflux at 60º for 30 min. The mixture was neutralized by the addition of 2 N HCl. For neutral degradation, 1 ml of stock solution under reflux at 60º for 6 h. For degradation under oxidizing conditions, 1 ml of stock solution was added to 1 ml of 20% hydrogen peroxide (H2O2) at 60º for 30 min. For thermal degradation, the powdered drug was exposed at 105º for 6 h. For photolytic degradation, the powdered drug was exposed to sunlight for 7 days. The above solutions were subjected to different stress conditions to determine whether any peaks arise from the degraded excipients. After completion of the treatments, the solutions were left to return to room temperature, diluted with diluent to obtain 100 µg/ml solution. 20 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample. The purity of drug peak obtained from the stress sample was measured by UV detector.

RESULT AND DISCUSSION

Optimization conditions of chromatography were performed to obtain the good resolution, good peak shape and peak parameter (theoretical plates). For the selection of mobile phase initially methanol: water, acetonitrile: methanol (50:50) has been tried in different proportions and different column but it gave poor base peak and also poor system suitability parameters. Finally, acetonitrile: methanol (60:40)mixture was tried at pH 3 at a flow rate of 1 ml/min was found to be satisfactory and good system suitability parameters. The everolimus drug component was measured with UV detector at 285nm. The average retention time for everolimus was 3. 806 min, cut down on overall time of sample analysis and the method was more cost effective as it utilizes very less quantity of mobile phase. The number of theoretical plate was found to be 8441, which indicates efficient performance of the column. The developed HPLC method was validated as per ICH guidelines

The method was linear in the range of 5-25 µg/ml for everolimus with correlation coefficient of 0. 992. Precision was calculated as intraday and interday variation for everolimus. The %RSD for intraday precision and interday precision for everolimus were found to be 0. 33 and 0. 50, which indicate the method was precise with %RSD less than 2%. The method was specific as no interference observed when the drugs were estimated in presence of excipients.

Accuracy was determined by calculating the % recovery. The method was found to be accurate with % recovery between 99. 98%-100. 1% and the % mean recovery was found to be 100. 02% for everolimus, which indicate the method is accurate. The limit of detection and limit of quantification for everolimus was found to be 0. 0814 and 0. 2478 µg/ml, which indicate the sensitivity of the method. Selectivity of the method was demonstrated by the

absence of any interfering peaks at the retention time of the drug. Validated method was applied for the determination of everolimus in commercial formulations. The % assay for everolimus was found to be 99. 86% indicating good compliance with the label claim.

A simple, linear, precise, accurate, selective, sensitive and rapid RP-HPLC method with UV detection for everolimus in pharmaceutical dosage form has been developed and validated. The method has been found best than from few methods reported, because of use of a less economical and readily available mobile phase, lack of extraction procedures. Degradation products produced as a result of stress conditions did not interfere with detection of everolimus and the assay method can thus be regarded as stability indicating. The method would be extensively used for the estimation of everolimus in bulk and pharmaceutical formulation.

ACKNOWLEDGEMENT

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TABLES:

Table 1: Chromatogrphic condition-

Mobile phase	ACN: methanol (60:40)pH3
Selection of column	Cosmosil C18 (4. 6mm x 290mm, Particle size: 5µm)
Injection volume	20 Ml
Flow rate	1. 0 ml/min
Column temperature	Room Temperature
Detection wavelength	285nm
Retention time	3. 8 min

Table 2: Data of calibration curve of everolimus

Sr. No.	Conc. (µg/ml)	Area
1	5	2015565
2	10	4232656
3	15	6012665
4	20	8165656
5	25	10165658

Table 3: Precision Data for intraday precision

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	5	2015858	2026114. 67	10028. 85	0. 494979
2	5	2026587			
3	5	2035899			
4	10	6026389	6025413	20808. 78	0. 34535
5	10	6014555			
6	10	6035295			
7	15	10156559	10174269. 7	15992. 86	0. 157189
8	15	10178595			
9	15	10187655			

Table-4: Data for interday precision

Sr. No.	Conc. (µg/mL)	Area	Mean	SD	%RSD
1	5	2012548			
2	5	2036557	2035352. 33	22226. 4982	1. 09202214
3	5	2056952			
4	15	6015897			
5	15	6036456	6022649. 33	11957. 8775	0. 19854846
6	15	6015595			
7	25	10154588	10180209	23758. 8565	0. 2333828
8	25	10184525			
9	25	10201514			

Table 5: Accuracy

Level of addition	Standard Added (mg/ml)	conc. (mg/ml)	Total conc. (mg/ml)	Area obtained*	Stud Area	Drug Recovered (mg/ml)	%Recovery
50%	5	10	15	6016555	6012665	15. 009705	100. 064697
	5	10	15	6016584		15. 009777	100. 065179
	5	10	15	6017856		15. 01295	100. 086334
100%	10	10	20	8165918	8165656	20. 000642	100. 003209
	10	10	20	8163256		19. 994122	99. 9706086
	10	10	20	8163258		19. 994127	99. 9706331
150%	15	10	25	10168921	10165658	25. 008025	100. 032098
	15	10	25	10163956		24. 995814	99. 9832574
	15	10	25	10169827		25. 010253	100. 041011

Table 6: Data for system suitability parameter

Sr. No.	conc. (mg/ml)	Retention Time (min)	Theoretical Plates	Asymmetry Factor
1	15	3. 8	8487	1. 25
2	15	3. 7	8552	1. 24
3	15	3. 8	8462	1. 25
4	15	3. 7	8359	1. 23
5	15	3. 9	8252	1. 24
6	15	3. 8	8539	1. 25
Mean		3. 783333333	8441. 83333	1. 2433333
SD		0. 075277265	115. 691688	0. 008165
%RSD		1. 989707452	1. 37045691	0. 6566997

Table 7: Results of LOD and LOQ

Drugs	LOD (µg/ml)	LOQ (µg/ml)
Everolimus	0. 0. 814	0. 2478

Table 8: Data for robustness study

Sr. No	Parameter	Condition	Area	Mean	SD	%RSD
1	Change in Flow rate (ml/min)	0. 9	6015326	6017787	2200. 74	0. 03657
2		1	6018469			
3		1. 1	6019566			
1	Change in Wavelength (nm)	283	6021526	6021026	7131. 64	0. 11845
2		285	6027895			
3		287	6013658			

Table:9 Data for ruggedness study

Sr. No	Analyst	Conc. (µg/ml)	Area	Mean area*	SD	% RSD
1	Analyst-I	15	6018456	6019759	1477. 98072	0. 02855216
			6019456			
			6021365			
2	Analyst-II	15	6030215	6022570. 67	8870. 07251	0. 14728051
			6024652			
			6012845			

Table:10 Result of Assay

Sr. No.		Area of Standard	Area of degraded Sample	% Assay
1	**Vermeil 10**	6012665	6004486	99. 86

Table:11 Results of Forced degradation study

	Acid stress	Alkali stress	Peroxide stress	Thermal stress	Photolytic stress
% Recovered	84. 23%	89. 64%	80. 85%	95. 65%	98. 15%
% Degradation	15. 77%	10. 36%	. 19. 15%	4. 35%	1. 85%

FIGURE:

Fig 1: UV Spectra of Everolimus

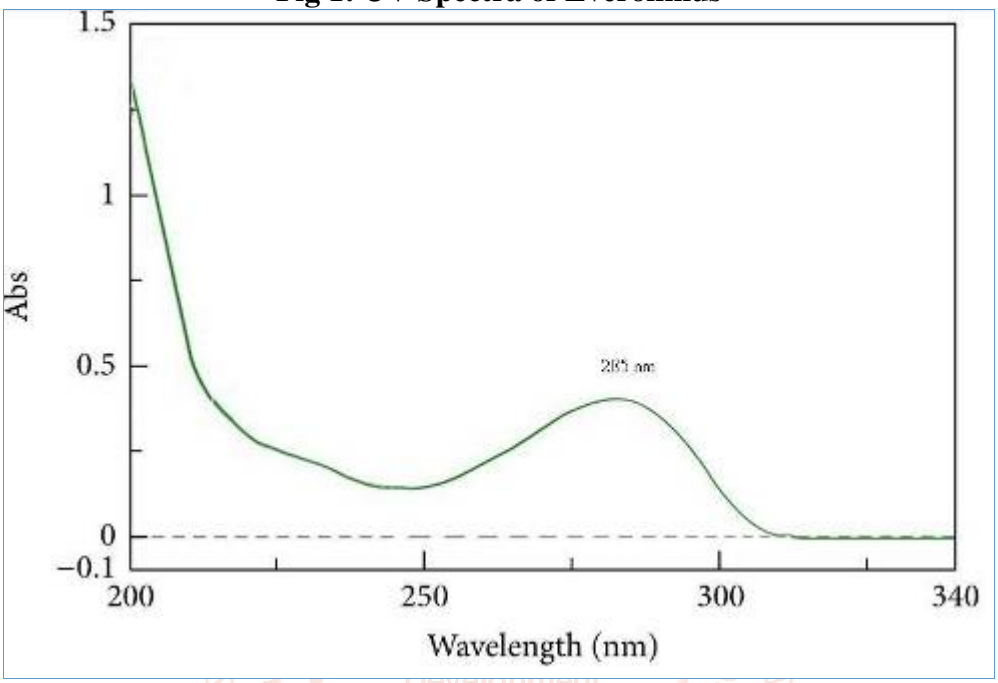


Fig 2: Chromatogram of everolimus

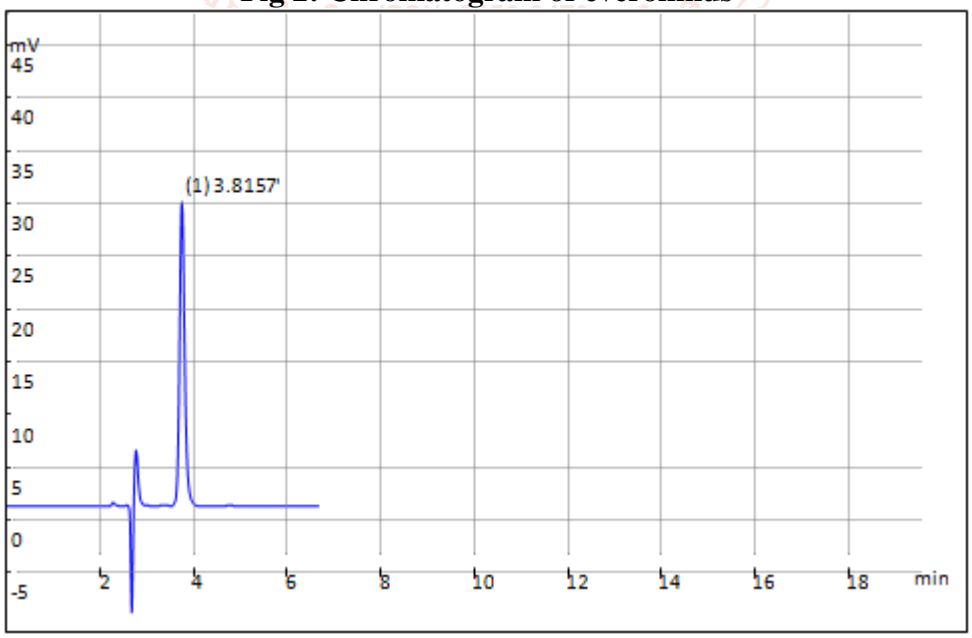
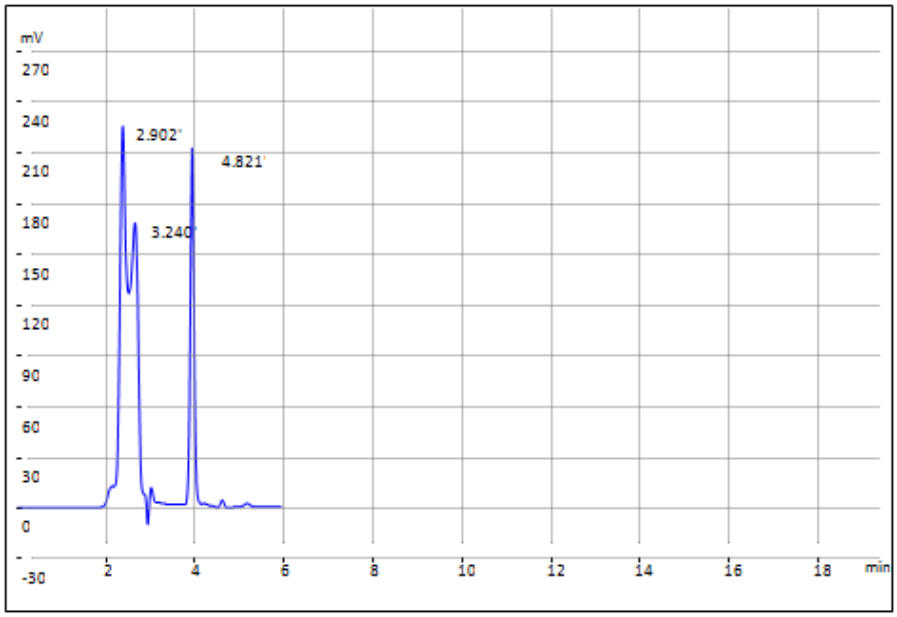
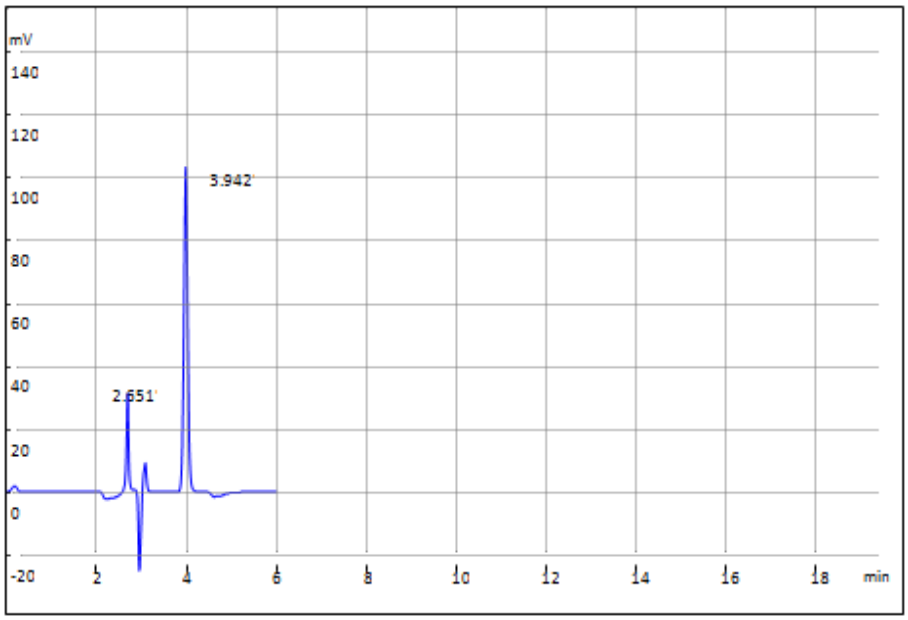


Fig:3 Chromatograms of stress condition of everolimus

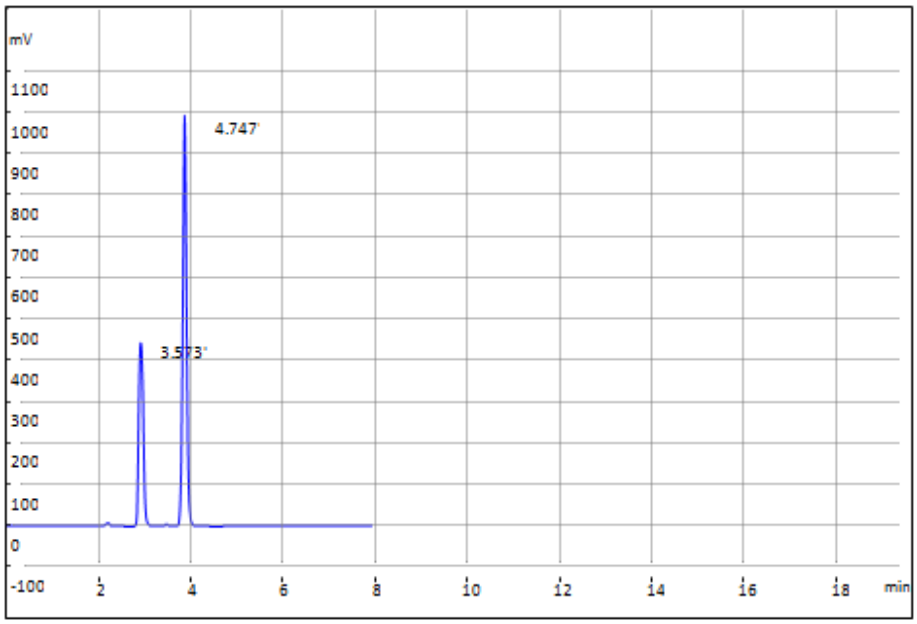
A. Acidic degradation



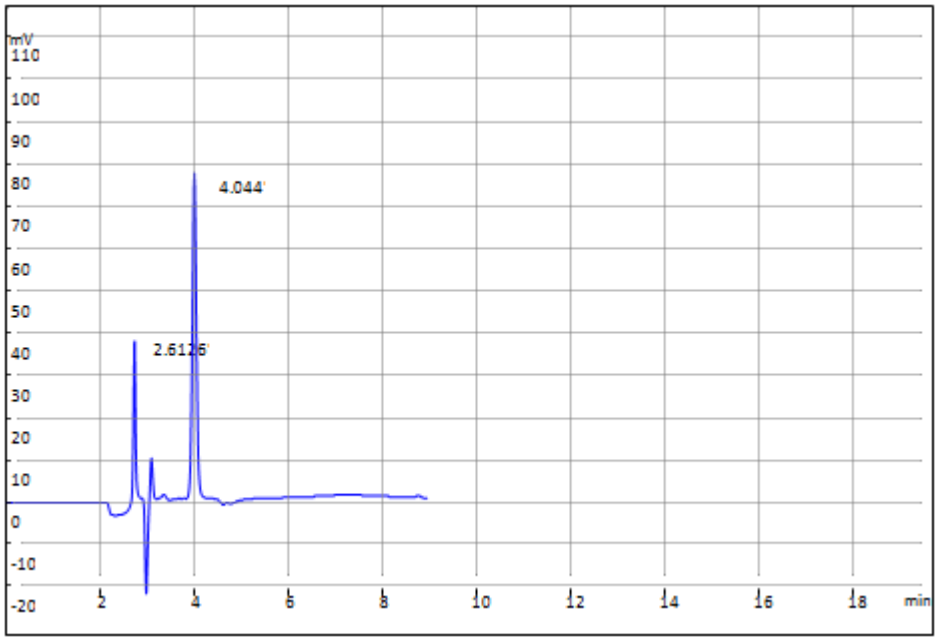
B. Alkaline degradation



C. Peroxide degradation



D. Thermal degradation



E. Photolytic degradation