Development and validation of stability indicating and force degradation and study of antiretroviral drug

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ABSTRACT

A stability indicating RP-HPLC (Reverse Phase high performance liquid chromatography) method was developed and validated for the determination of Darunavir Hydrate in bulk and tablet dosage form using C18 column CHEMSIL C18 (250 X 4.6 mm), ThermoP4000 with PDA detector in isocratic gradient system integrated high performance liquid chromatographic system with chromquest software. The methanol: acetonitrile: water was selected as mobile phase in ratio of 70: 15:15 and was filtered on membrane filter (0.45µ) at flow rate 0.5 mL/min. The detection was carried out at 263 nm and retention time of Darunavir hydrate was found to be 3.162 min Linearity was observed from 5-30 µg/ml. The correlation coefficient R^2 was 0.997. Darunavir hydrate was subjected to stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation on, and the results showed that it was more sensitive towards basic degradation. The method was validated as per ICH guidelines.

Keywords: RP-HPLC, Darunavir hydrate, Stability, Chromatography.

INTRODUCTION

Darunavir hydrate chemically known as 1*S*,2*R*)-3-[[(4-aminophenyl)sulfonyl](2-methylpropyl)amino]-2-hydroxy-1-(phenylmethyl)propyl]-carbamic acid (3*R*,3a*S*,6a*R*)-hexahydrofuro[2,3-*b*]furan-3-yl ester. A white to off-white hygroscopic powder characterises the physical nature. [1] In aqueous solutions, Darunavir is only slightly soluble. As a result, Darunavir's absorption rate and potentially its extent will depend on the particle size. It has five chiral centers, but the manufacturing method consistently produces only one enantiomer, which is 3R, 3aS, 6aR, 1S, and 2R. The X-ray diffraction analysis has verified the absolute arrangement. Darunavir is produced under commercial synthesis conditions as a crystalline ethanolate (1:1 solvate). [2]

Human immunodeficiency virus (HIV) infection is treated with the HIV protease inhibitor Darunavir in individuals who have previously received antiretroviral therapy. [3] SARS-CoV-2, the corona virus that causes COVID-19, is the subject of research into Darunavir as a potential treatment due to in vitro evidence supporting its capacity to fight this infection. It has been demonstrated to successfully suppress the virus, resulting in noticeably lower rates of morbidity and death. [4]

Stability studies must be conducted in accordance with International Conference on Harmonization (ICH) standards (Q1A) in order to suggest a shelf life for new drug substances and/or drug products. Studies on shelf life are included in a number of regulatory submissions to the FDA. [5] To estimate the shelf life of a drug substance and/or drug product, three different stability tests must typically be conducted: accelerated stability (ACC), intermediate stability (INS), and controlled room temperature (CRT) stability. Accelerated studies last for roughly six months, while studies on intermediate stability and regulated room temperature stability last for roughly 12 to 24 months. When conducting a stability research to ascertain a molecule's intrinsic stability, it is anticipated that the drug substance or drug product will deteriorate or break down and produce other molecules, also referred to as impurities. Various stress conditions are purposefully applied during forced degradation studies to break down the primary compound and produce contaminants, which should separate from the primary compound and from one another. The shelf life of new drug substances and/or drug products is therefore proposed using the results of forced degradation studies, which are used to predict the degradants/decomposed impurities that would occur during stability studies. [6]

MATERIALS AND METHODS

Chemicals

All solvents and chemicals used were HPLC grade i.e. Methanol, Water, Acetonitrile, Orthophosphoric acid, Sodium dihydrogen phosphate monohydrate and Tri ethylamine were purchased from Sigma Aldrich, India.

Equipments

The HPLC analysis of Darunavir Hydrate was performed on a ThermoP4000 with PDA detector in isocratic gradient system integrated high performance liquid chromatographic system with chromquest software. Stationary phase temp maintained at 25°C. The injection volume of sample was 20 µL.

Chromatographic conditions

The compound was separated isocratically on a Chemsil ODS C18 (250 X 4.6 mm), 5 µm column with a mobile phase the methanol: acetonitrile: water was selected as mobile phase in ratio of 70: 15:15 and was filtered on membrane filter (0.45µ) at flow rate 0.5 mL/min at ambient column temperature. The mobile phase was degassed with a Sonicator and filtered through a 0.25-m Millipore filter prior to analysis.

Method Development

Selection and preparation of mobile phase

Different mobile phases with varying amounts of acetonitrile, methanol, and water were tested at various flow rates. With the mobile phase consisting of Methanol: Acetonitrile: Water in a ratio of 70: 15: 15, a good symmetrical peak was discovered. Mobile phase had been produced by mixing 700 ml of methanol in 150 ml each of acetonitrile and water and filtered on membrane filter (0.45µ) to remove dissolve gases solvents were sonicated for 15-30 min. Optimized chromatographic conditions of Darunavir hydrate shown in Table No. 1.

Preparation of Standard Stock solution

Darunavir, precisely weighed at 100 mg, was dissolved in mobile phase to produce a standard stock solution (Stock solution-I, 1000 mcg/ml), which was then diluted with mobile phase to a volume of 100 ml. 10 ml of stock solution-I was diluted to 100 ml with mobile phase (Stock

solution-II, 100 mcg / ml). 1 ml of stock solution-II was taken in 10 ml standard flask diluted to 10 ml with mobile phase to get the concentration 10 mcg / ml.

Preparation of Calibration Curve

Appropriate volume of aliquots from standard Darunavir stock solutions was transferred to a series of 10 ml capacity of volumetric flasks. Mobile phase was used to adjust the volume to the desired concentrations, which ranged from 5 to 30 g/ml. At a maximum wavelength of 263 nm, the absorbance spectra of each solution were quantified against a mobile phase blank. The obtained absorbance values are plotted against the concentration to get the calibration graph. The correlation value and regression equation were calculated. Darunavir's validation metrics were calculated according to ICH standards, and they included range, linearity, accuracy, precision, ruggedness, LOD, and LOQ.

Method Validation

The developed method was verified by evaluating linearity, accuracy, precision, robustness, ruggedness, detection limit, quantification limit and stability. Coefficient of variation and relative errors of less than 2% were considered acceptable, except for the quantification limit, for which these values were established at 2%. [8]

Linearity

The stock solutions were diluted to get the final concentration of 5, 10, 15, 20, 25, 30 µg/mL for Darunavir hydrate. Each level solution was injected into the chromatographic system and the peak area was measured. Peak area versus concentration was plotted on a graph (concentration on the X-axis, peak area on the Y-axis), and the correlation coefficient was determined. [9]

Accuracy

A single set of various standard addition techniques was used to test the method's accuracy at concentration levels of 20%, 50%, and 100%, with the difference between the spiked value and true found value being compared afterwards. [10]

Precision

The area for each of the five injections of the standard solution (10 g/mL) was measured in HPLC. The precision of the assay was also determined in terms of intra and inter-day precision. It was discovered that the %RSD for the region of five duplicate injections was within the predetermined range. [11]

Robustness

Analysis of aliquots from homogenous lots with varying physical parameters, such as flow rate and mobile phase composition, as well as temperature variations, which may differ, were used to test the robustness of the suggested technique. [12]

Ruggedness

The test solutions were prepared as per test method and injected under variable conditions. Ruggedness of the method was investigated by different analyst. [13]

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of the developed method were determined by analysing progressively low concentration of the standard solution using the developed methods. The concentration of the analyte at which a measurable reaction occurs is known as the LOD (signal to noise ratio 3.3). The limit of quantification (LOQ) refers to the analyte concentration at which a reaction can be precisely measured (signal to noise ratio of 10). [14]

The LOD is determined by the formula

$$LOD = 3.3SD / Slop$$

LOQ is determined by the following formula:

$$LOQ = 10SD / Slop$$

Force Degradation studies

An initial concentration of Darunavir hydrate at 5µg/ml was used for all stress degradation studies. A 10 ml volumetric beaker was filled with two ml of the Darunavir hydrate stock standard solution (25 g/ml). Then 1 M HCl, 0.1 M HCl, 1 M NaOH, and 0.01 M NaOH were added to each flask to reach the volume and the flasks were kept at room temperature or 35ºC to study the degradation of Darunavir hydrate. The resulted solutions were neutralized by appropriate amounts of NaOH or HCl and injected to the HPLC system after dilution to 25 µg/ml by mobile phase. For oxidative degradation, 2 ml of stock standard solution of Darunavir hydrate and 8 ml of 3% hydrogen peroxide were transferred to a 10 ml volumetric flask and kept at room temperature or 35ºC. For thermal and light degradation, a solid sample of Darunavir hydrate was spread in a thin layer in a watch glass and exposed to heat (70ºC) and light (visible and UV) for 5 days. The HPLC apparatus was then given an injection of a standard solution that had been prepared at a concentration of 25 g/ml in the mobile phase. Darunavir hydrate degradation was

assessed in all degradation conditions using a newly prepared standard solution of Darunavir hydrate (25 g/ml) that was also injected into the HPLC system.

The results of the stress degradation of Darunavir hydrate in different conditions depicted in Table. Darunavir hydrate degraded slowly in 0.1 M hydrochloric acid. The peak area of Darunavir hydrate was decreased about 44% after 1 h exposure 60-90 ºC. Two new peaks were generated between the retention times of 1 and 2 min. [15]

RESULTS AND DISCUSSION

Method development by RP-HPLC

Determination of Darunavir Hydrate λ_{max} by UV spectrophotometer

For the UV spectrophotometer-based estimation of Darunavir hydrate [ICH Q2B], a thorough, precise, and accurate method has been devised. Spectrum of Darunavir Hydrate in mobile phase Methanol: Acetonitrile: Water (60:15:15 v/ v/v) was recorded on UV spectrophotometer. Recorded UV spectrum is shown in figure no. 2.

Darunavir Hydrate showed maximum absorbance at wavelength 263 nm when analyzed by UV spectroscopy.

Method Validation

Linearity

Linearity study for Darunavir Hydrate was carried out in range 5-30 µg/ml. The results of linearity were tabulated in table no. 2.

Linearity graph of Darunavir Hydrate showed equation of line y = 23.249 x with R^2 = 0.9973. Hence the method is linear concentration range 5-30µg/ml.

Accuracy

The recovery experiment was carried out in the standard addition method. Accuracy studies were performed at concentration 20%, 50% and 100% .i.e. (2, 5 and 10 µg/ml). Obtained chromatogram and recovery results are shown in table no. 3.

The mean recoveries ranged between 97.82 and 99.82%, it was discovered. The recovery outcome shows the accuracy of the suggested approach.

Precision

Intermediate Precision: (Repeatability) In HPLC, the area was measured for each of the five injections of the standard solution. The results are tabulated in Table no. 4.

Darunavir Hydrate's RSD values were determined to be 0.573% (Table No. 5). It was discovered that the %RSD for the region of five duplicate injections was within the predetermined range. Low values indicating that method is repeatable table no.3.

Intermediate Precision: (Reproducibility)

Five injections of the standard solution were performed, and the areas of each injection were quantified using HPLC. It was discovered that the %RSD for the region of five duplicate injections was within the predetermined range. Two analysts as per test method conducted the study. Results are tabulated in table no. 5.

Limit of Detection and Limit of Quantification

By analyzing the standard solution at increasingly lower concentrations while using the developed methods, the LOD and LOQ of the method were established.

Darunavir Hydrate's limit of detection was established to be 5 g/ml (signal-to-noise ratio 3). The lowest limit of quantification was determined to be 25 g/ml.

Robustness

a) Effect of variation in flow rate

In order to ascertain the impact of flow rate variation, a research was carried out. The flow rate was adjusted between 0.8 and 1.2 ml/min. The results are tabulated in the table no. 6. It was assessed how flow rate variance affected things. Because of the asymmetry and retention duration RSD% were within allowable ranges for flow variation. The acceptable flow rate should therefore range from 0.8 ml to 1.2 ml.

b) Effect of variation of mobile phase composition

A research was done to ascertain the impact of varying the mobile phase ratio by altering the mobile phase ratio. The mobile phase's organic content was adjusted to a range of 2% v/v. The results are tabulated in the table no. 7. The impact of changing the composition of the mobile phase was assessed. As the % RSD of retention time and asymmetry were within limits for variation in mobile phase composition.

Force degradation study

Acidic Condition:

The drug peak and the degradation peak both peaked at 1.947 min in the acidic hydrolysis findings. When the drug was kept in 0.1 M HCl, 18.9% of drug degradation was visible in the peak region. The results are tabulated in the figure no. 4 and table no. 8.

Basic Condition:

Darunavir hydrate upon alkaline degradation in 1 M NaOH up to 30 min underwent percentage degradation is 79.6 % in the above condition. The results are tabulated in the figure no. 5 and table no. 9.

Photolytic Condition:

Darunavir hydrate did not degrade after it was kept under direct sunlight. No peak other than the drug peak discovered in the chromatogram of that sample. The results are tabulated in the figure no. 6 and table no. 10

Thermal 70º C Condition:

Darunavir hydrate did not degrade after it was kept under thermal condition at 70º C. No peak other than the drug peak discovered in the chromatogram of that sample. The results are tabulated in the figure no. 7 and table no. 11.

Thermal 60º C Condition:

Darunavir hydrate did not degrade after it was kept under thermal condition at 60º C. No peak other than the drug peak discovered in the chromatogram of that sample. The results are tabulated in the figure no. 8 and table no. 12.

Thermal 90º C Condition:

Darunavir hydrate did not degrade after it was kept under thermal condition at 90º C. No peak other than the drug peak discovered in the chromatogram of that sample. The results are tabulated in the figure no. 9 and table no. 13.

Oxidative Condition:

.In the oxidative degradation study, Darunavir hydrate showed no degradation after exposure. The results are tabulated in the Figure No. 10 and Table No. 11.

Discussion

The method was effectively validated in the optimized conditions, the validation parameters were within the ranges, and the linearity in this RP-HPLC method was between 5 and 30 g/ml. The LOD and LOQ of Darunavir hydrate were discovered to be 5 g/ml and 25 g/ml, respectively, using this chromatographic technique. In the present investigation, Darunavir hydrate was subjected to its stability studies under different conditions as per the ICH guidelines. Darunavir hydrate was studied for neutral hydrolytic degradation, and it was discovered that there was no deterioration under neutral conditions.

Conclusion

In acidic condition drug was found degraded upto 76% while in basic conditions, Darunavir hydrate discovered more labile. The drug was degraded about 80% after 30 min exposure to 1 M NaOH at room temperature. The degradation was slower under exposure to 0.01 M NaOH and 55% degradation was observed after 15 min at room temperature. Darunavir hydrate was discovered to decay in 1% H_2O_2 to an extent of 15% after 1 hour, with a new peak appearing at a retention time of about 2.4. More degradation was observed by using 3% H_2O_2 at room temperature. Darunavir hydrate bulk powder was stable under exposure to heat, UV light, and visible light and no significant degradation was observed.

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LIST OF TABLES

Table 1. Optimized chromatographic conditions of Darunavir hydrate

Parameters	Conditions
Column:	CHEMSIL ODS-C18 (250 mm X 4.6 mm), 5µm column
Flow Rate:	1 ml/min
Injection Volume:	20 µL
Column Temperature:	Ambient
Wavelength	263 nm
Run time	10 min
Mobile phase	Methanol: Acetonitrile: Water 70: 15:15

Table 2. Results for Darunavir Hydrate Linearity by RP-HPLC

Sr. no.	Concentration(µg/ml)	Peak Area (mv)
1	05	135.245
2	10	245.789
3	15	354.132
4	20	476.234
5	25	580.113
6	30	680.567
Correlation coefficient		**0.9973**

Table 3. Results for Darunavir Hydrate Accuracy

Sr. No	Drug / Formulation	Percentage recovery			Mean	S.D.	%RSD
		20%	50%	100%			
1.	Bulk	97.82	98.72	99.82	98.78	1.001	0.33

Table 4. Results of Darunavir Hydrate for intermediate precision (Repeatability)

Injection number	Area (mv)	Mean	S.D.	%RSD
1	233.656	232.85	1.33	0.573
2	233.228			
3	230.832			
4	232.324			
5	234.255			

Table 5. Results of Darunavir Hydrate for intermediate precision (reproducibility)

Parameter	% Assay
Mean*	98.13
SD	0.346
% RSD*	0.35

Table 6. Results for Darunavir Hydrate Robustness by RP-HPLC (variation in flow rate)

Sr. No	Flow rate (ml/min)	Peak Area (mv)	Robustness results
			USP Tailing
1	0.8	229.56	1.49
2	1.0	230.412	1.51
3	1.2	228.94	1.52
SD			**0.74**
%RSD			**0.25**

Table 7. Robustness results for Darunavir Hydrate by RP-HPLC (variation in mobile phase composition)

Sr. No	Mobile phase composition(v/v)	Peak Area	Robustness results
			USP Tailing
1	Mobile phase +2%	229.620	1.5
2	Mobile phase -2%	230.521	1.4
%RSD			0.315

Table 8. Acidic Condition

Retention Time	Area	Area Percent	Integration Code	Theoretical Plates (USP)	Resolution (USP)
1.947	602149	13.58	VV	NA	NA
3.137	596848	21.04	VV	NA	NA
7.21	11353	3.27	VV	NA	NA

Table 9. Basic Condition

Retention Time	Area	Area Percent	Integration Code	Theoretical Plates (USP)	Resolution (USP)
3.298	610401	18.41	VV	NA	NA
7.371	12031	2.71	VV	NA	NA

Table 10. Photolytic Condition

Retention Time	Area	Area Percent	Integration Code	Theoretical Plates (USP)	Resolution (USP)
3.155	586060	19.39	VV	NA	NA

Table 11. Thermal 70º C Condition

Retention Time	Area	Area Percent	Integration Code	Theoretical Plates (USP)	Resolution (USP)
3.174	243161	13.79	VV	NA	NA
5.941	273109	10.71	VV	NA	NA

Table 12. Thermal 60º C Condition

Retention Time	Area	Area Percent	Integration Code	Theoretical Plates (USP)	Resolution (USP)
3.174	210444	9.1	VV	NA	NA

Table 13. Thermal 90º C Condition

Retention Time	Area	Area Percent	Integration Code	Theoretical Plates (USP)	Resolution (USP)
1.520	432110	7.21	VV	NA	NA

Table 11. Oxidative Condition

Retention Time	Area	Area Percent	Integration Code	Theoretical Plates (USP)	Resolution (USP)

3.140	498700	6.215	VV	NA	NA
5.271	778121	14.45	VV	NA	NA

Table 12. The results of the stress degradation tests on Darunavir hydrate bulk powder using different conditions

Stress test condition	Solvent	Temperature	Time	% of Darunavir hydrate
Acidic	1 M HCl 1 M HCl 0.1 M HCl	Room temperature 35ºC 35ºC	30 min 30 min 1 h	81.1 24.5 66.4
Basic	1 M NaOH 0.01 M NaOH	Room temperature Room temperature	30 min 15 min	20.4 44.1
Oxidative	3% H_2O_2 3% H_2O_2 1% H_2O_2	Room temperature 35ºC 35ºC	30 min 30 min 1 h	93.8 18.9 81.1
Photolytic UV light Visible light	Solid form Solid form	Room temperature Room temperature	5 days 5 days	100.3 99.9
Heat	Solid form	90ºC 70ºC 60ºC	5 days	100.1

LIST OF FIGURES

Figure 1. Structure of Darunavir hydrate

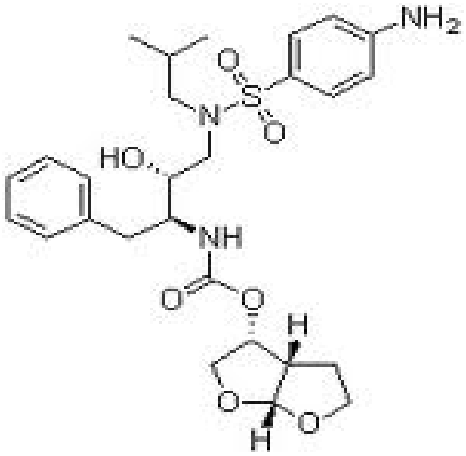

Figure 2. UV Spectrum of Darunavir Hydrate

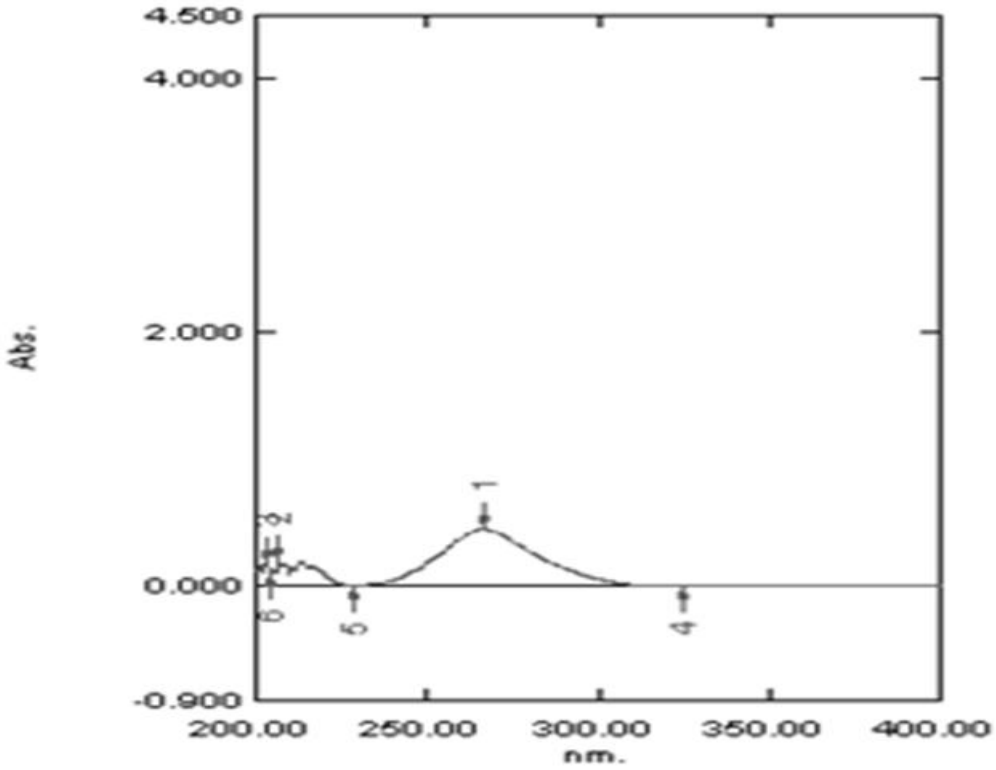

Figure 3. Linearity graph for Darunavir Hydrate

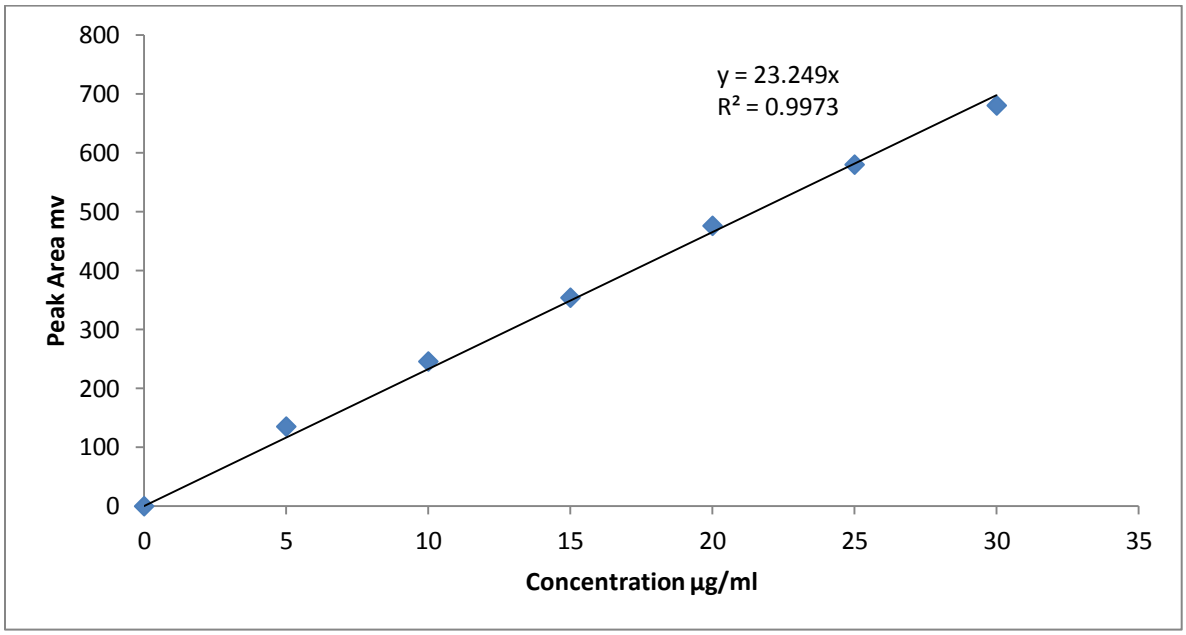

Figure 4. Acidic Condition

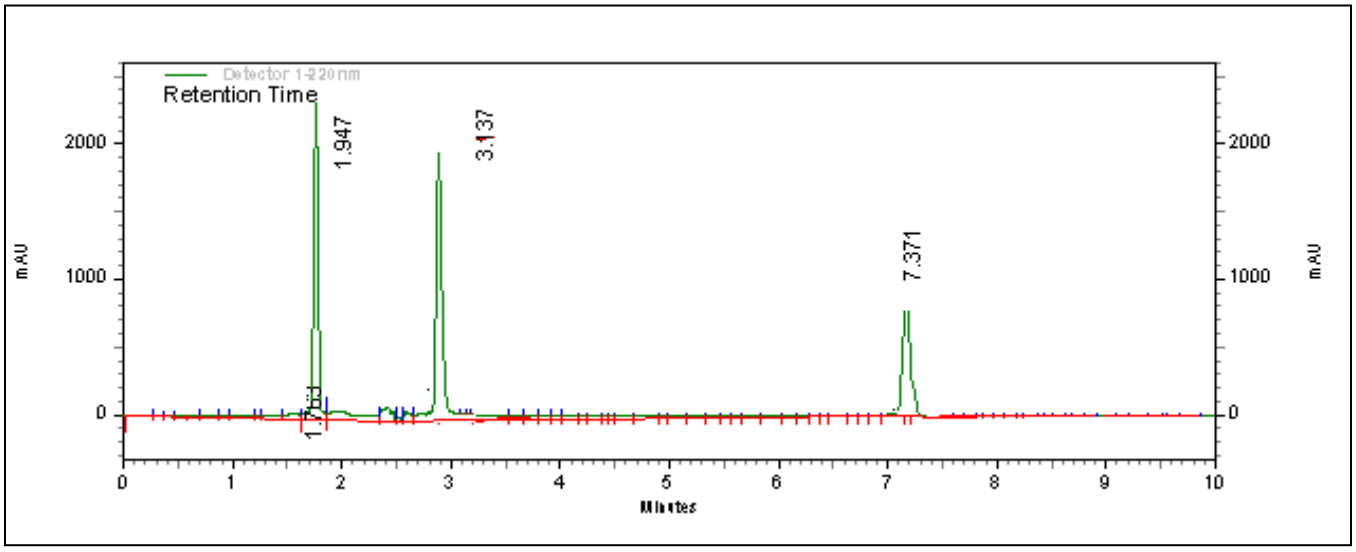

Figure 5. Basic Condition

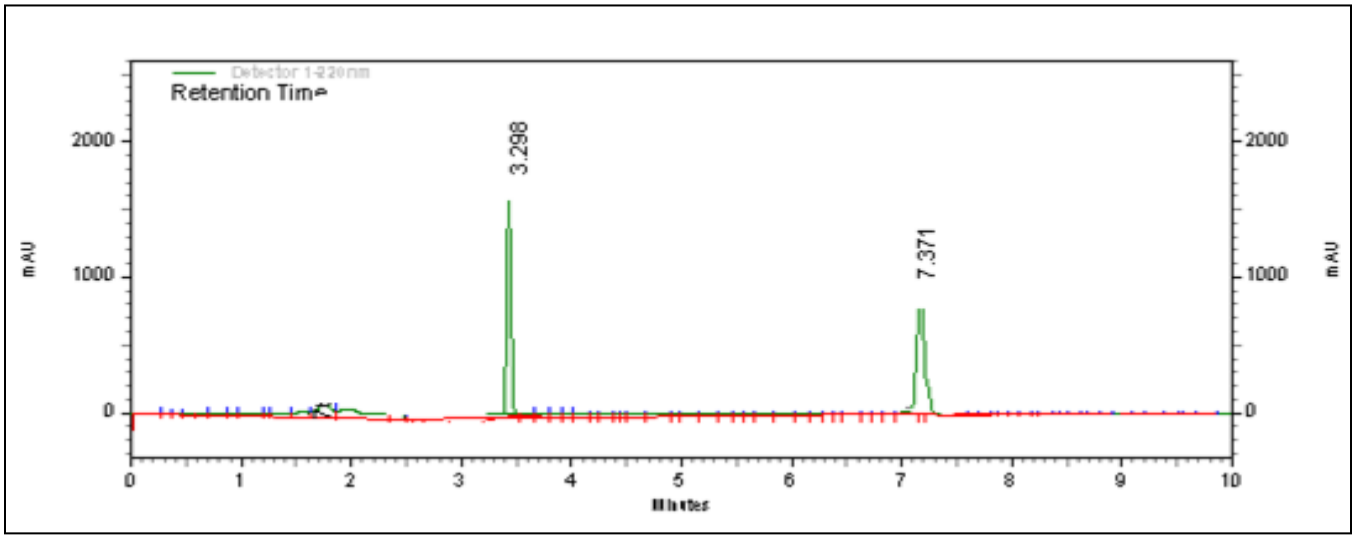

Figure 6. Photolytic Condition

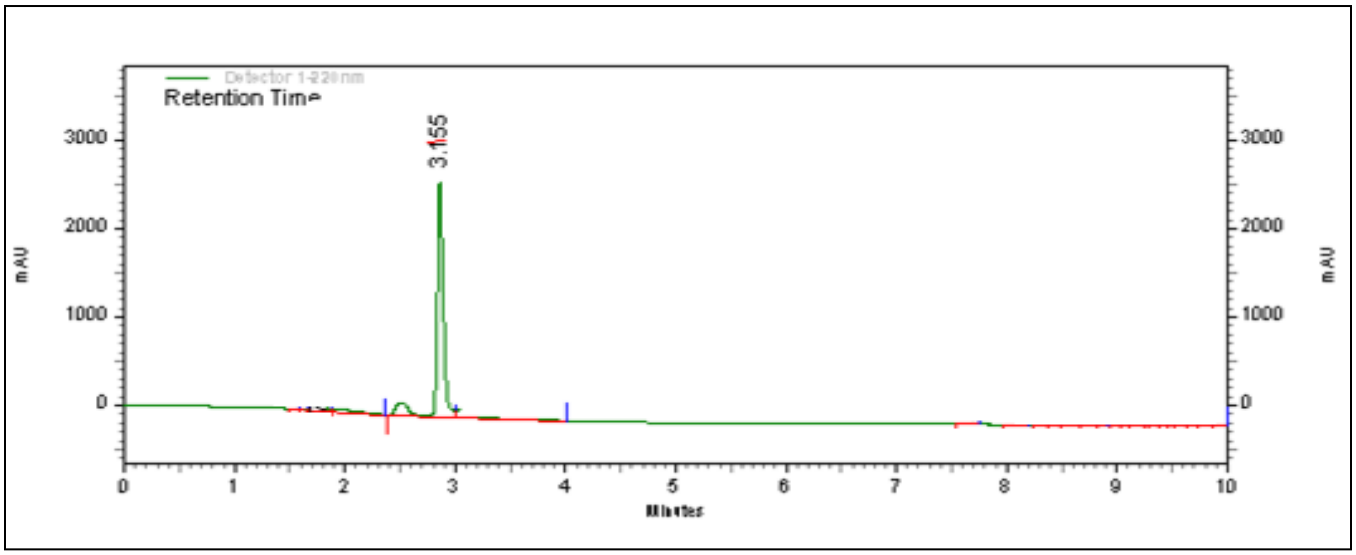

Figure 7. Thermal 70º C Condition

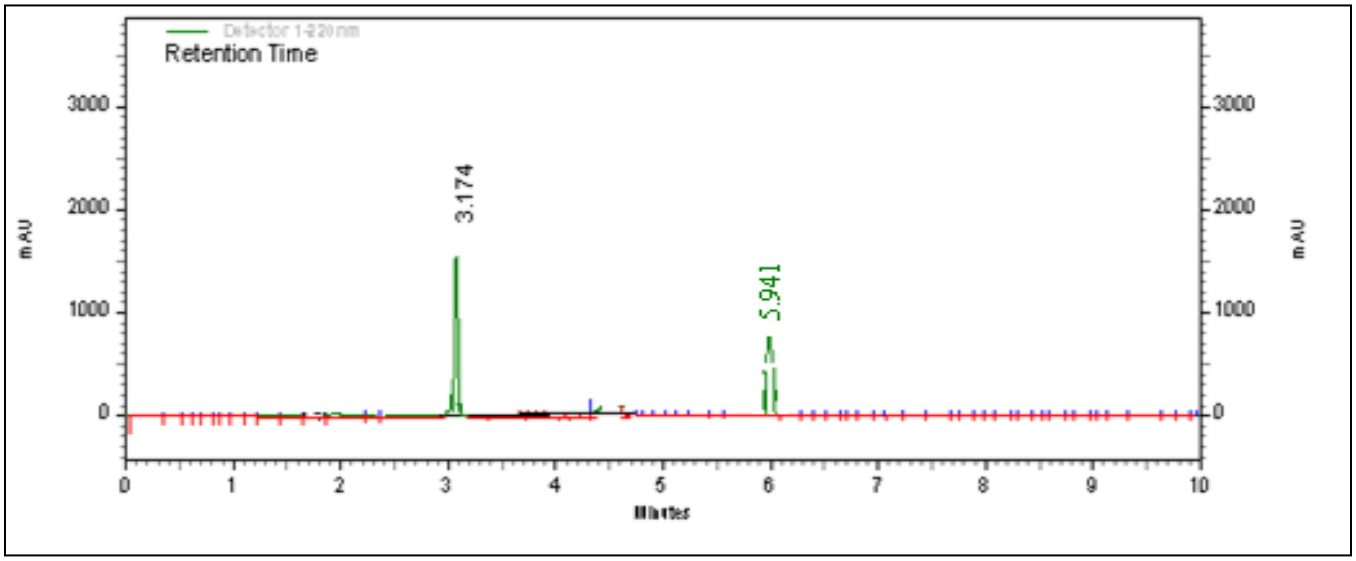

Figure 8. Thermal 60º C Condition

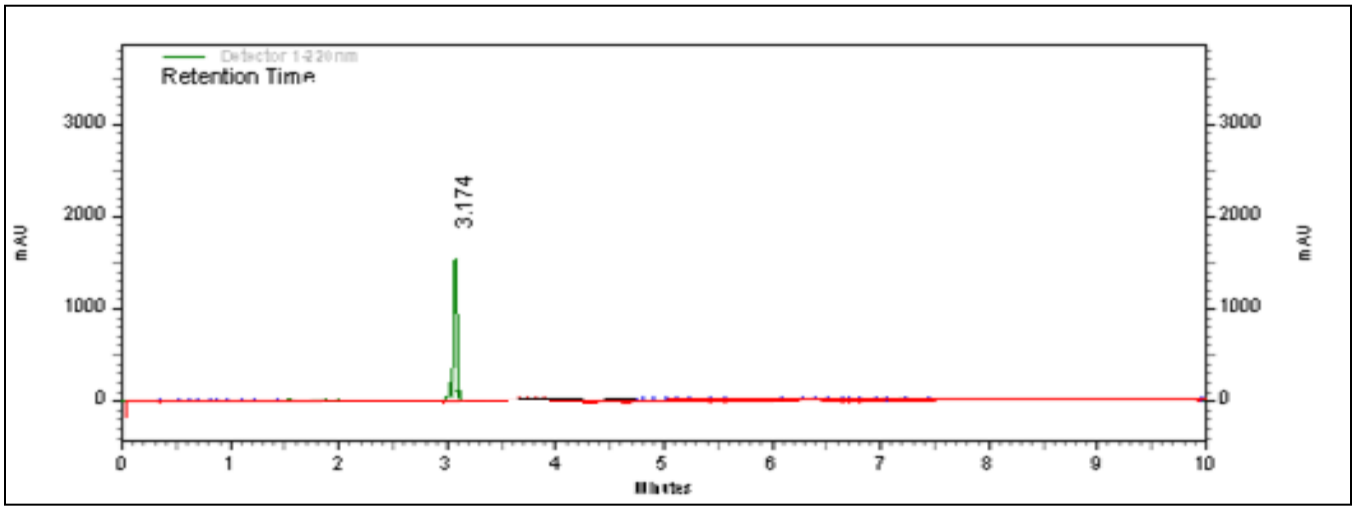

Figure 9. Thermal 90° C Condition

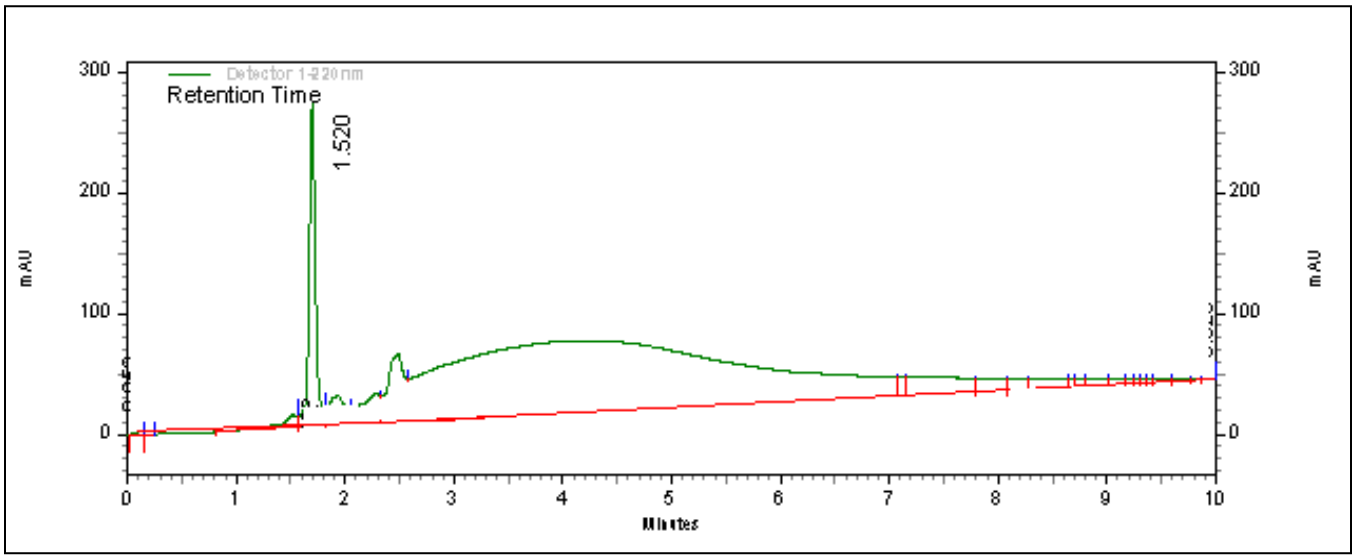

Figure 10. Oxidative Condition