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Research Article

DEVELOPMIENT OF A RP-HPLC METHOD FOR QUALITITIVE AND QUANTITITIVE DETERMINATION OF ALMOTRIPTAN AND ITS STABILITY STUDIES

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ABSTRACT

The aim of the present work was to develop and validate a simple RP-HPLC method for the determination of almotriptan and to carry out forced degradation studies of almotriptan. Chromatographic separation of Almotriptan was achieved by using a C18 column. A Mobile phase containing a mixture of methanol: water (1:1 v/v) was pumped at the flow rate of 1 mL/min. Detection was performed at 229 nm. Validation parameters were evaluated according to the International Conference on Harmonization (ICH) Q2R1 guidelines. Stress degradation studies were carried out at different conditions such as for hydrolytic degradation in acidic, basic and neutral media, oxidative degradation was performed using H_2O_2 and thermal degradation was performed at 70° C. The calibration curve was linear in the concentration range 10-80 µg/mL for the Almotriptan with regression coefficient 0.9999. The method was found to be accurate with 1.611 % RSD. RSD values were found to be 1.6 % in the case of intra-day precision studies, whereas 1.512 % in the case of inter-day precision the limits of detection and quantification were found to be 1.098, 3.32 µg/mL, respectively. The results obtained from the stress testing reveals that Almotriptan drug substance is particularly unstable under acidic, neutral and thermal degradation conditions. From the stability point of view, care should be taken in the manufacturing process and storage of this product in order to avoid degradation; as if the drug is degraded it could result in decrease of therapeutic activity and safety. The method was successfully applied for the quantification purpose and was found to be accurate, precise and rapid for the analysis of Almotriptan.

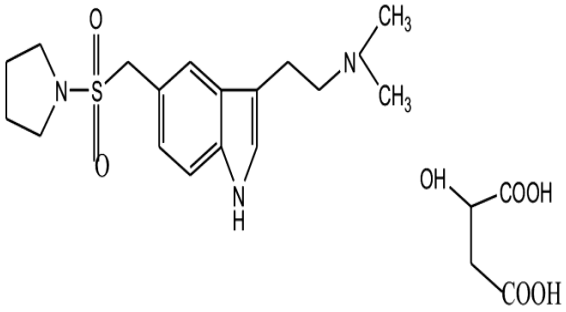
Keywords: Almotriptan, RP-HPLC method development, stability studies and ICH guidelines.

INTRODUCTION

Almotriptan is N, N-dimethyl-2- [5-(pyrrolidin-1-ylsulfonylmethyl)- 1H-indol-3-yl]- ethanamine. Its empirical formula is $C_{17}H_{25}N_3O_2S.C_4H_6O_5$ representing molecular weight of469.56. It is a white to slightly yellow crystalline powder that is soluble in water and sparingly soluble in methanol [1, 2, 3]. Almotriptan is available in market as film coated tablets, coated tablets. The drug is absorbed well orally, with an absolute bioavailability of around 70%.The drug is used to treat severe migraine headaches and vascular headaches; acute treatment of migraine attacks with or without aura.[2,3,4,5] The drug binds with high affinity to 5-HT 1D, 5-HT1B and 5-HT 1F receptors. Because of the particular distribution of the 5-HT 1B/1Dreceptors, Almotriptan basically constricts the human meningeal arteries; therefore it has a limited effect on arteries supplying blood to the brain and little effect on cardiac and pulmonary vessels. Ameliorate migraine through selective constriction of certain intracranial blood vessels, inhibition of neuro peptide release and reduced transmission in trigeminal pain pathway[1, 5, 6, 7.]

The present paper deals with the development, validation of a RP-HPLC method with UV detection for the determination of Almotriptan with stability indicating method

using RP-HPLC for carry out the forced degradation study using different stress condition.



Almotriptan maleate

MATERIAL AND METHODS

INSTRUMENTATION

Reverse Phase-High Pressure Liquid Chromatography was performed with an isocratic High Pressure Liquid Chromatography system (Shimadzu HPLC class) with two LC-10 AT, VP pumps, variable wavelength programmable UV/Visible detector SPD-10A. Separation of the Almotriptan was achieved by using a 250 mm × 4.6 mm I.D, 5 µm particle size, Thermo Scientific C18 (ODS-octa decyl silane) column under reversed-phase chromatographic conditions.

CHEMICALS AND REAGENTS

All the chemicals were of HPLC grade quality. Methanol and water were obtained from SPECTROCHEM PVT. LTD., MUMBAI and THOMAS BEAKER (chemicals) PVT. LTD., MUMBAI and used in the present study.

Preparation of mobile phase

The mobile phase was prepared by mixing methanol and water in the ratio of 1:1 v/v.

Preparation of the standard solution

Accurately weighed 10 mg of the powdered drug Almotriptan was taken in a 10 ml volumetric flask and the prepared solvent HPLC grade methanol: HPLC grade water (1:1) was added up to the mark which gives the concentration of 1000 ppm. From the stock solution 1 ml of the solution was taken in a 10 ml volumetric flask and then it was made up to the mark with the same solvent to prepare the concentration of 100 ppm. From the above solution different aliquots of solution was prepared by taking 1,2,3,4,5,6,7,8 ml was taken in each 10 ml of volumetric flask separately and it was made upto mark with the same solvent to produce 10, 20, 30, 40, 50, 60, 70 and 80 ppm respectively.

Chromatographic conditions

The mobile phase methanol and water (1:1) was pumped at a flow rate of 1 mL/min. It was filtered through 0.45 µm membrane filter and purging was done to remove ant particulate matter and air bubbles if present any. The injection volume was 25 µL and the eluent was monitored at 229 nm. The column temperature was set at 30 ^{0} C.

Calibration curve

A series of working standard solutions prepared above were taken. Twenty µl aliquot of each solution was injected automatically into the column. The peaks were determined at 229 nm. The calibration curve was obtained by plotting concentration in the X- axis against peak area in the Y axis.

METHOD VALIDATION

Accuracy[8,9]

To determine the accuracy of the proposed method was determined in five different sample solutions of same concentration (50%) by analyzing % recovery of Almotriptan by standard addition recovery method. The study carried out by adding the known amount of the sample solution in the standard stock solution. The mean, std. deviation and % RSD were calculated. The results were shown in table.

Precision[9,10]

The precision of the proposed method was assessed by intra-day and inter-day variation studies using only one concentrations of Almotriptan (50 ppm) for several times. During intra-day studies, five sample solutions of each concentration were analyzed on the same day whereas inter-day studies were determined by analyzing five sample solutions of each concentration for 5 consecutive days. The mean, std. deviation and % RSD were calculated. The results were shown in table.

Limit of Detection (LOD) and Limit of Quantification (LOQ)[11,12]

LOD and LOQ were calculated by using the following expressions:

$$LOD = 3.3\ \sigma/S$$
$$LOQ = 10\ \sigma\ /\ S$$

Where “σ” is the standard deviation of the regression line and “S” is the slope of calibration curve.

Robustness[12, 13]

To verify the robustness of the method, three vital experimental variables such as composition of mobile phase, detection wavelength and flow rate were slightly varied.

The analysis was performed by changing the flow rate (1.1 l/min). The data was then subjected to statistical analysis and the results are expressed in mean, standard deviation and %RSD.

Ruggedness[13, 14]

Ruggedness is the degree of reproducibility of the results obtained under a verity of conditions. The method's ruggedness was established by the deter-mination of Almotriptan by changing the flow rate from 1ml/min to 0.9 ml/min. The data was then subjected to statistical analysis and the results are expressed in mean, standard deviation and %RSD.

FORCED DEGRADATION STUDIES

The specificity of the method can be demonstrated through forced degradation studies conducted on the sample using neutral, acid, alkaline, oxidative, thermal degradations. The sample was exposed to these conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient.

1. Degradation in Neutral Condition[15,16]

About 10mg of pure drugs were accurately weighed and taken in 10ml volumetric flasks and dissolved in minimum volume of water. Then the volume was made up to the mark with water and after 15 days interval the solutions were prepared and 25 µl of the sample solutions were injected into the HPLC system and run in the mobile phase.

2. Degradation in Acidic Condition[16,17]

About 10mg of pure drugs were accurately weighed and taken in a 10ml volumetric flask and dissolved in minimum volume of 0.1 N HCL. Then the volume was made up to the mark with 0.1N HCl. and after 15 days interval the solutions were prepared and 25 µl of the sample solutions were injected into the HPLC system and run in the mobile phase.

3. Degradation in Basic Condition[15,17,18]

About 10mg of pure drugs were accurately weighed and taken in a 10ml volumetric flask and dissolved in minimum volume of 0.1 N NaOH. Then the volume was made up to the mark with 1N NaOH and kept at 70^0C and after 15 days interval the solutions were prepared and 25 µl of the sample solutions were injected into the HPLC system and run in the mobile phase.

4. Oxidative Degradation[17,19]

About 10mg of pure drugs were accurately weighed and taken in a 10ml volumetric flask and dissolved in minimum volume of H202 Solution. Then the volume was made up to the mark with H_2O_2 and kept at 70^0C and after 15 days interval the solutions were prepared and 25 µl of the sample solutions were injected into the HPLC system and run in the mobile phase.

5. Thermal Degradation[18,19]

About 100 mg of pure drugs were taken in three separate clean petridishes and subjected to dry heat at 70^0C and and after 15 days interval the solutions were prepared by dissolving with the HPLC mobile phase and 25 µl of the sample solutions were injected into the HPLC system and run in the mobile phase.

RESULTS AND DISCUSSION

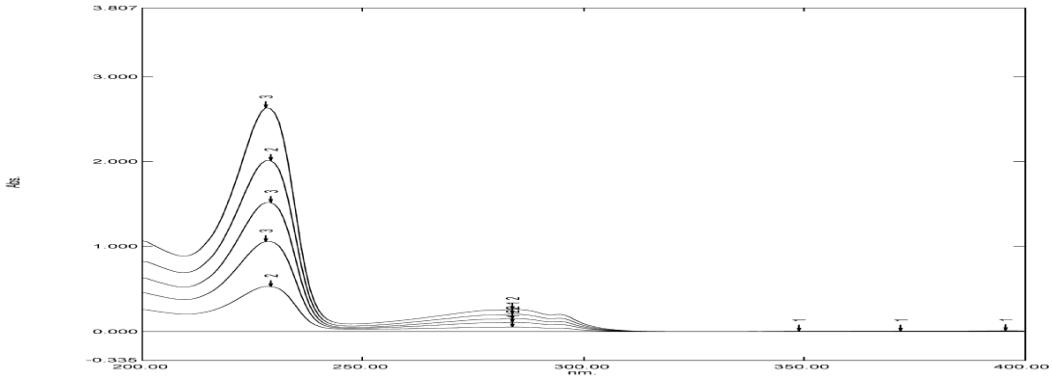
The quantification of the drugs in pharmaceutical dosage forms and biological fluids is necessary during the studies of stability, metabolism, pharmacokinetics, toxicity and quality control studies. For all these investigations, an efficient and validated analytical method is very significantly required. Also a suitable method to verify the stability of the drug is also required to separate the drug from its impurities and to determine the storage condition of the drug.

METHOD DEVELOPMENT

A reversed-phase C18, 250 mm × 4.6 mm I.D., 5 µm particle size column maintained at temperature 30 0 C was used for the separation of Almotriptan using mobile phase methanol: water(1:1). Isocratic elution at the flow rate of 1.0 mL/min has been employed in the present proposed method. Wavelength of 229 nm was chosen to be used for the UV detection. The column is kept at room temperature during the procedure. After this optimization, this method has been used for stability study of Almotriptan.

Forced degradation study was carried out by subjecting the drug to acid (0.1 N HCl) and alkaline (0.1 N NaOH) hydrolysis, thermal, neutral (water) and oxidative degradation (H_2O_2).

Maximum degradation of Almotriptan was observed in neutral condition, followed by decomposition under acidic and thermal condition. Minimum degradation of drug was observed in basic hydrolysis and oxidative condition. Percent degradation of drug under all stressed conditions is included in Table 10.

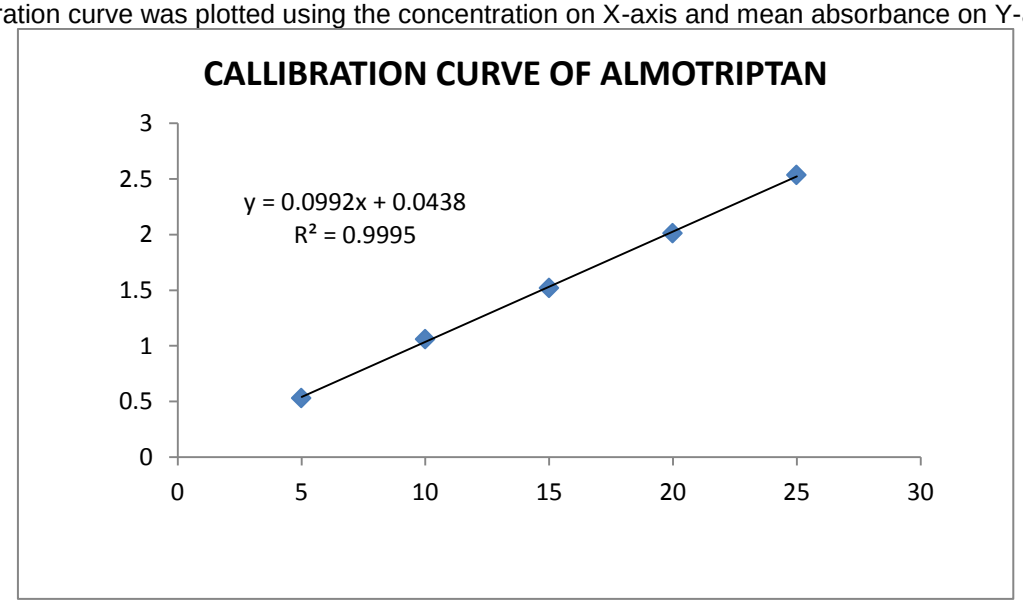


Overlay Spectra of Almotriptan by Uv-Visible Spectroscopy
Using Solvent Methanol: Water (1:1)
This spectra showing ƛ max (maximum wavelength) is about 229 nm

Table 1: Calibration Table of the Uv-Visible Spectroscopic Method for Almotriptan

Concentration (PPM)	Absorbance
5	0.531
10	1.061
15	1.519
20	2.013
25	2.535

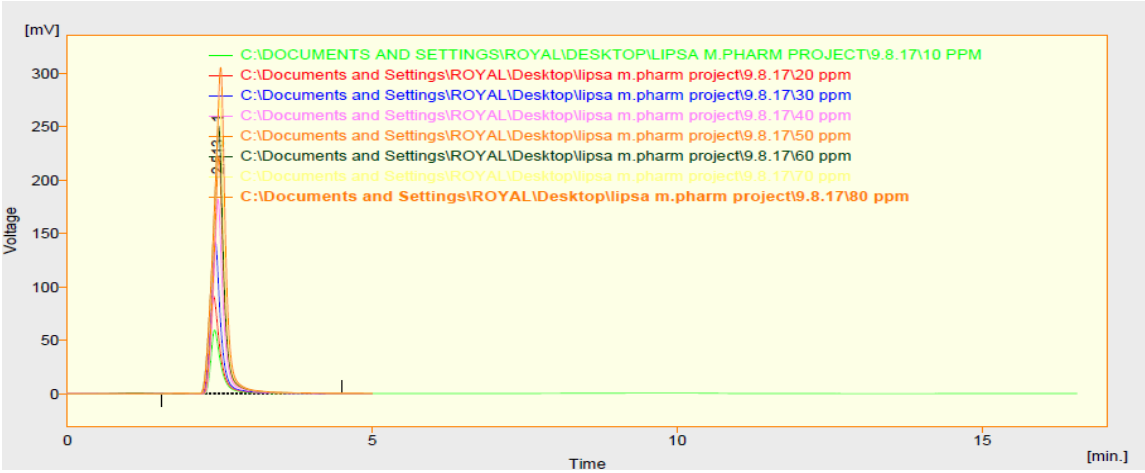
A calibration curve was plotted using the concentration on X-axis and mean absorbance on Y-axis.



From the calibration curve it was found that it shows linearity in the range of **5–20 µg/m**l with regression coefficient **0.999.**

Table 2: Optimized Chromatographic Condition of Almotriptan For RP-HPLC (Method Development)

Parameter	Condition
Stationary phase (column)	ODS C-18 (250 x 4.6 mm, packed with 5 micron)
Mobile phase	HPLC Grade Methanol: HPLC grade water(1:1)
Flow rate(ml/min)	1 ml/min
Run time (min)	10 min
Column temperature(°C)	30
Volume of injection (µl)	25
Detection wavelength (nm)	229 nm
Drug Rt(min)	2.5
LOD	1.098 µg/ml
LOQ	3.32 µg/ml



Overlay chromatogram of almotriptan by HPLC

Table 3: Calibration Table of the RP-HPLC Method For almotriptan

Concentration (PPM)	Peak area
10	658.119
20	1352.791
30	1965.634
40	2593.069
50	3111.45
60	3722.491
70	4331.253
80	4864.815

A calibration curve was plotted using the concentration on X-axis and peak area on Y-axis.

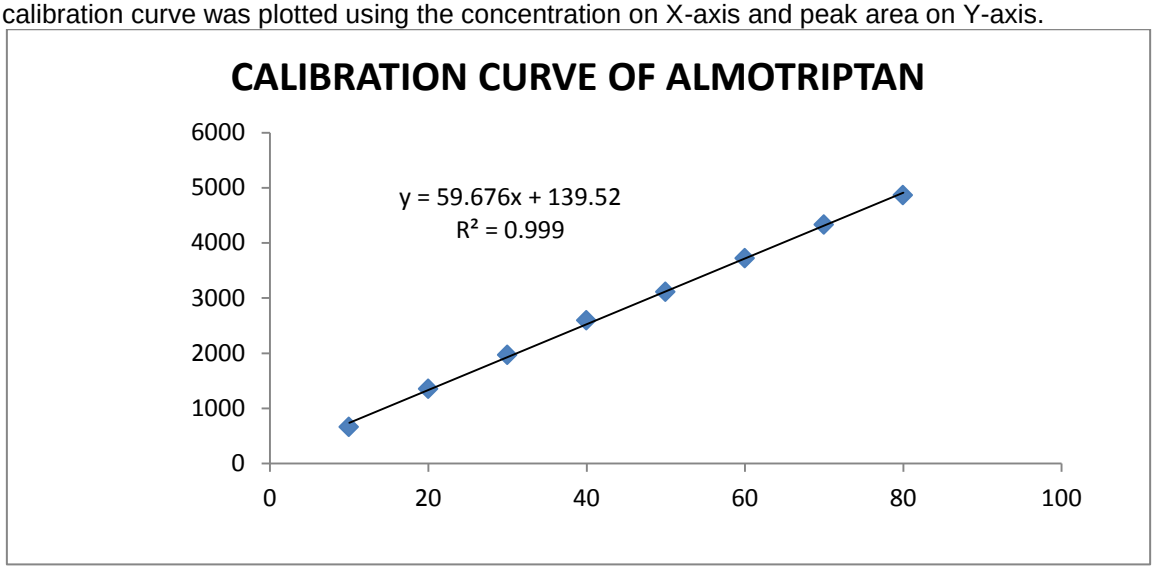


Table 4: Accuracy Data of RP-HPLC method for almotriptan

S. No	Conc. (PPM)	Peak area	Calculated Conc.	Statistical Parameter
1.	50	3201.32	51.31	Mean=50.51 Std. Deviation=0.814 %RSD=1.611
2.	50	3166.939	50.73	
3.	50	3078.76	49.25	
4.	50	3196.935	51.23	
5.	50	3111.45	49.80	
6.	50	3161.844	50.65	

Table 5: Inter Day Precession Data of the RP-HPLC method for almotriptan

S. NO	Conc. (PPM)	Peak Area	Calculated Concentration	Statistical Parameter
1.	50	3076.347	49.21	Mean=50.49 std. Deviation=0.767 %RSD=1.512
2.	50	3195.076	51.21	
3.	50	3152.439	50.49	
4.	50	3129.654	50.12	
5.	50	3163.25	50.68	
6.	50	3198.76	51.27	

Table 6: Intra Day Precession Data of the RP-HPLC method for almotriptan

S. No	Conc. (PPM)	Peak area	Calculated Concentration	Statistical Parameter
1.	50	3199.876	51.29	MEAN=50.42 std. Deviation=0.815 %RSD=1.6
2.	50	3095.764	49.55	
3.	50	3192.43	51.17	
4.	50	3086.768	49.40	
5.	50	3138.987	50.27	
6.	50	3172.657	50.84	

Calculation of LOD and LOQ

Table 7: LOD AND LOQ data for RP-HPLC method of almotriptan

Conc. (PPM)	Peak area (Reading 1)	Peak area (Reading 2)	Peak area (Reading 3)	Average
1.	65.76	98.52	105.78	90.02
2.	150.53	153.32	153.655	152.50
3.	181.57	215.67	204.77	200.67
4.	281.76	287.12	260.96	276.61
5.	321.45	348.54	322.56	330.85
6.	385.91	416.54	363.91	388.78
7.	465.56	495.98	434.56	465.36
8.	534.43	555.62	490.76	526.93
9.	583.43	634.56	528.78	582.25

Limit of detection (LOD) = 3.3σ/S = 3.3 × 20.71/62.23 =1.098

Limit of quantification (LOQ) = 10 σ/S = 10 × 20.71/62.23 =3.32

Calculation of ruggedness & robustness (using different flow rate)

Table 8: Ruggedness Data for RP-HPLC method of almotriptan

SL. No.	Conc.	Flow Rate	Peak area	Cal. Conc.	Statistical Parameter
1.	50	0.9 ml/min	3176.935	50.90	Mean= 49.76 std. Deviation= 0.683 %RSD= 1.37
2.	50	0.9 ml/min	3073.654	49.17	
3.	50	0.9 ml/min	3061.432	48.96	
4.	50	0.9 ml/min	3111.45	49.80	
5.	50	0.9 ml/min	3122.567	49.99	
6.	50	0.9 ml/min	3108.342	49.75	

Table 9: Robustness Data for RP-HPLC method of almotriptan

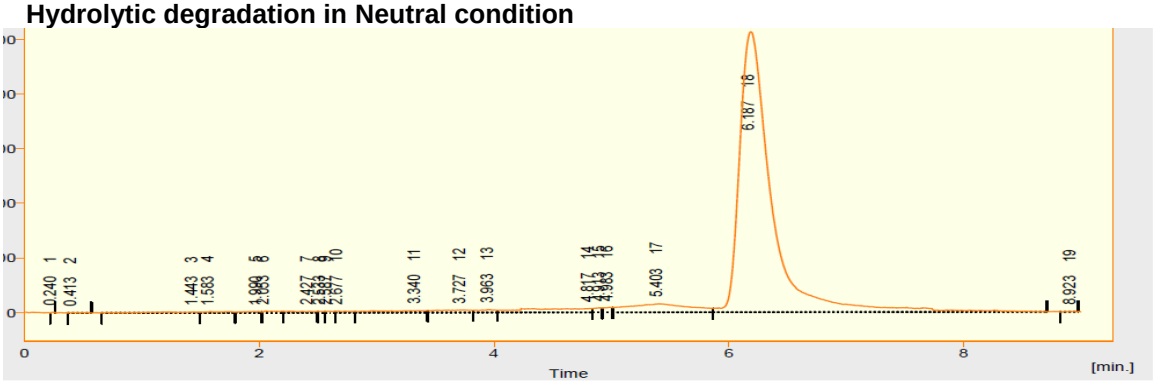
SL. No.	Conc.	Flow Rate	Peak area	Cal. Conc.	Statistical Parameter
1.	50	1.1 ml/min	3112.567	49.82	MEAN=50.805 std. deviation= 0.687 %RSD=1.35
2.	50	1.1 ml/min	3131.654	50.14	
3.	50	1.1 ml/min	3183.23	51.01	
4.	50	1.1 ml/min	3195.76	51.21	
5.	50	1.1 ml/min	3181.65	50.98	
6.	50	1.1 ml/min	3221.67	51.65	

Table 10: Data showing % of Degradation of almotriptan by RP-HPLC

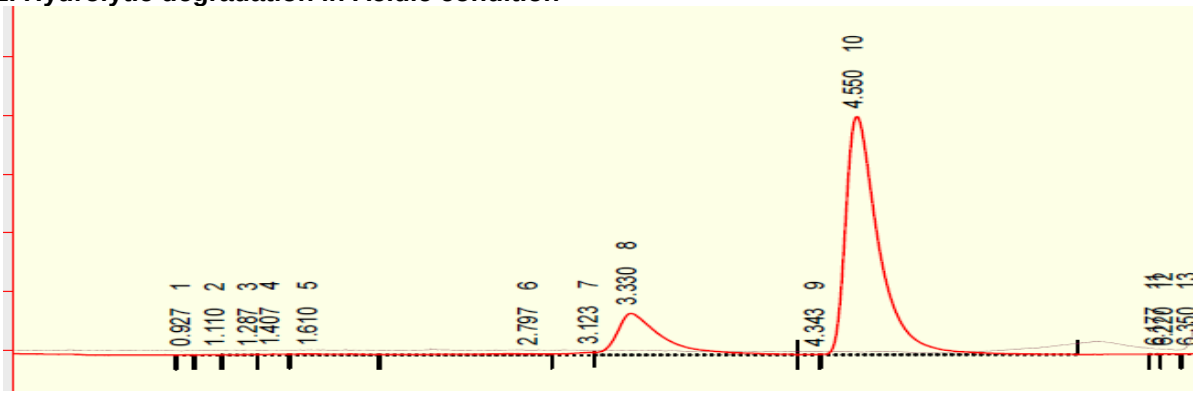
Condition	% degradation
Neutral	3.33%
Acidic	2.56%
Basic	1.02%
Oxidative	1.01%
Thermal	2.21%

Degradation peaks

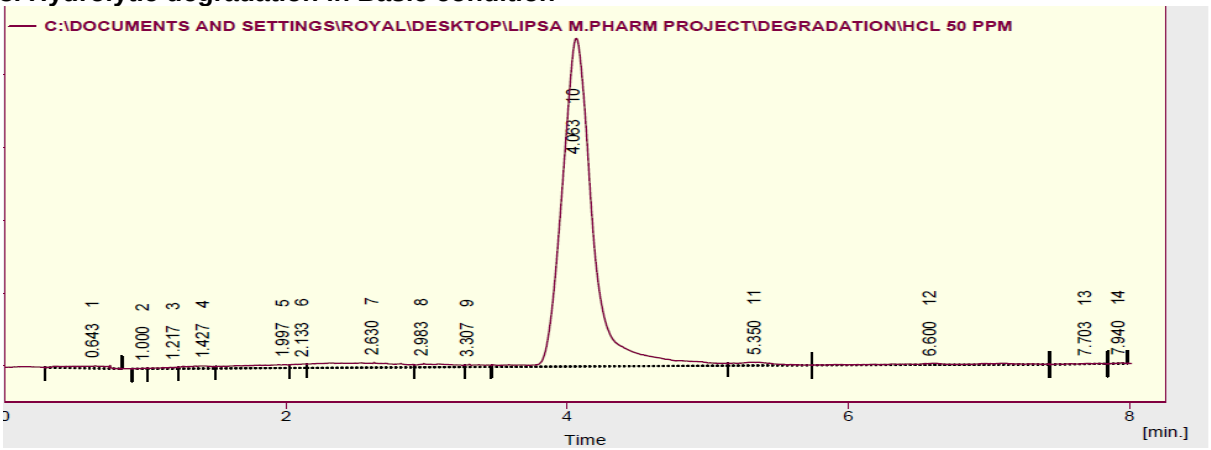
1. Hydrolytic degradation in Neutral condition



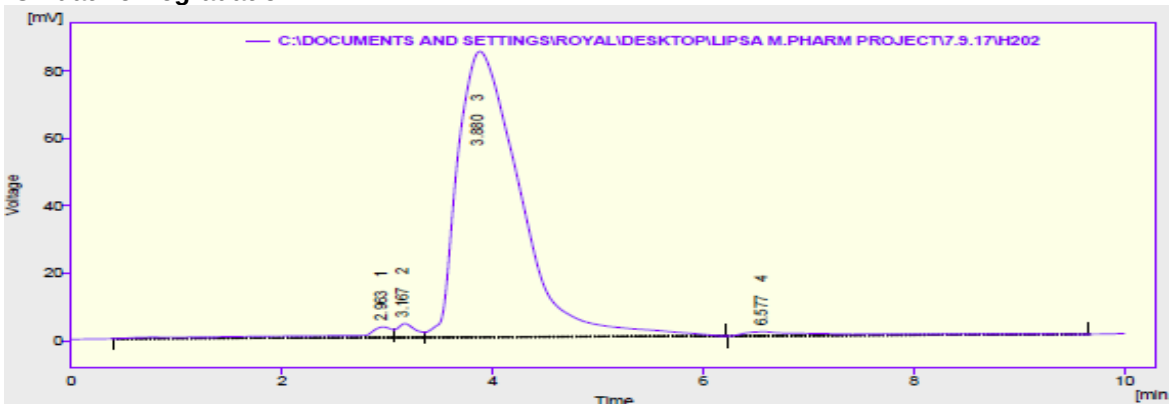
2. Hydrolytic degradation in Acidic condition



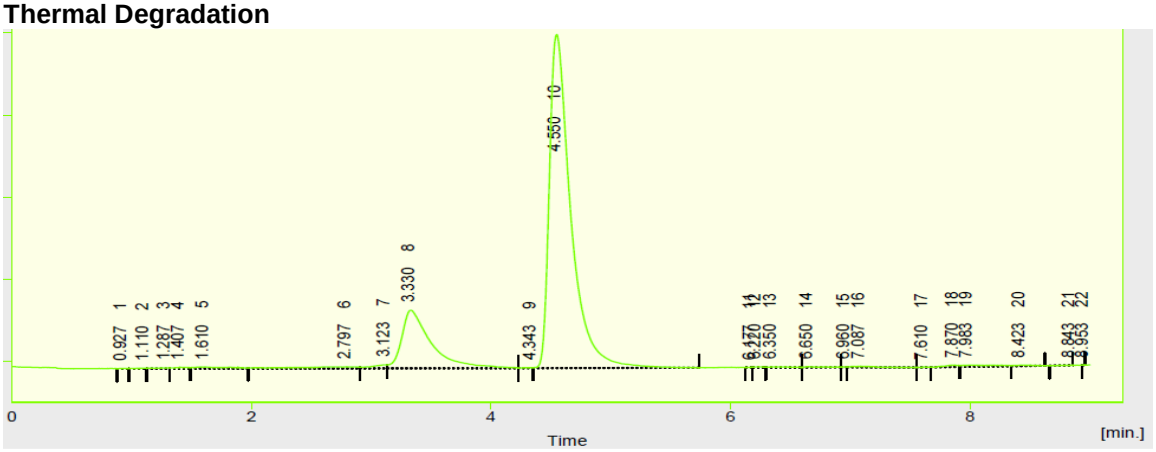
3. Hydrolytic degradation in Basic condition



4. Oxidative Degradation



5. Thermal Degradation



CONCLUSION

The proposed HPLC method was found to be simple, precise, and accurate and validated as per ICH guidelines and rapid for the estimation of Almotriptan. The mobile phase is simple to prepare, inexpensive solvent where it has the ability to separate these drugs from their degradation products and related substances. Hence, this method can be easily and conveniently adopted for routine analysis of Almotriptan in quality control laboratories.

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