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Stress Degradation Studies of Almotriptan Tablets by a Validated Stability- Indicating Liquid Chromatographic Method

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ABSTRACT

Almotriptan is an anti-migrain drug prescribed to treat severe migraine headaches and vascular headaches and for acute treatment of migraine attacks with or without aura. In the present study, degradation behavior of almotriptan was studied by subjecting the drug to various International Conference on Harmonization-recommended stress conditions. Also, a stability indicating high-performance liquid chromatography method was established for analysis of the drug in the presence of various degradation products. An acceptable separation of the drug and its degradation products was achieved on a C-18 column at 40°C using a mobile phase comprised of methanol, acetonitrile and acetic acid in the ratio of 75:20:5 (v/v/v) at a flow rate of 1 mL/min. The detection wavelength was 231 nm. The method was validated for linearity, precision, accuracy, selectivity, specificity, and robustness. The method was found to be linear over a concentration range of 20–120 µg/mL (n = 6). The value of slope was found to be 3933.6x ppm with correlation coefficient of 0.9996 and relative standard deviation (RSD) of 0.5673%. RSD values ranged from 1.651% to 0.5670% in the case of intra-day precision studies, whereas the values ranged from 1.0470% to 0.3843% in the case of inter-day precision. Validation parameters were evaluated according to the International Conference on Harmonization (ICH) Q2R1 guidelines. Using HCl, NaOH, H₂O₂, thermal, UV radiation and water performed the forced degradation studies. Almotriptan is more sensitive towards oxidative degradation condition. The developed method was successfully applied for the quantification and hyphenated instrumental analysis. The results obtained from the stress testing reveal that almotriptan drug substance is particularly unstable under acidic, alkaline and thermal degradation conditions. Therefore, care should be taken in the manufacturing process and storage of this product in order to avoid degradation, since if the drug is degraded it could result in decrease of therapeutic activity and safety.

Keywords: Almotriptan, HPLC, Stress testing, Stability-indicating, Acidic, Neutral, Alkaline, Oxidation, Photolysis, Tablets, Forced degradation

INTRODUCTION

A forced degradation or stress testing studies are the intentional degradation of the API and/or DP to an appropriate extent by means of various stress conditions include acidic, alkaline, neutral hydrolysis conditions, pH, temperature, light, oxidizing agents, and mechanical stress [1-3]. Forced degradation studies are designed to generate product-related variants and develop analytical methods to determine the degradation products formed during accelerated pharmaceutical studies and long-term stability studies. Any significant degradation product should be evaluated for potential hazard and the need for characterization and quantitation [4,5].

Almotriptan is a selective agonist of 5-HT_{1B/D} receptors (Figure 1) [6]. The therapeutic activity of almotriptan for the treatment of migraine headache can most likely be attributed to the agonist effects at the 5-HT_{1B/D} receptors on intracranial blood vessels and sensory nerves of the trigeminal system, which result in cranial vessel constriction, and inhibition of pro-inflammatory neuropeptide release. Due to presence of the indole moiety it is susceptible to undergo degradation. Almotriptan binds with high affinity to 5-HT_{1D}, 5-HT_{1B} and 5-HT_{1F} receptors and has weak affinity for 5-HT_{1A} and 5-HT₇ receptors, with no significant affinity or pharmacological activity at 5-HT₂, 5-HT₃, 5-HT₄, 5-HT₆, alpha or beta adrenergic, adenosine (A₁ and A₂), angiotensin - (AT₁ and AT₂), dopamine (D₁ and D₂), endothelia (ETA and ETB) or tachykinin (NK₁, NK and NK₃) binding sites. The IUPAC name is 1[[[3-[2-(Dimethyl amine) ethyl]-1Hindol5yl] methyl] sulfonyl] pyrrolidine hydroxybutanedioate (1:1). The empirical formula is C₁₇H₂₅N₃O₂S.C₄H₆O₅, representing a molecular weight of 469.56.

In the literature, various analytical methods analytical methods such as RP-HPLC and spectrometry methods have been reported for the determination of almotriptan in formulations. A stability-indicating assay method helps in establishing the inherent stability of a drug, providing assurance on detection changes in identity, purity, and potency of the product. To date, no report is available on the stability studies of almotriptan in bulk under the various stress conditions (acidic, neutral, alkaline, oxidative, thermal, and photolytic stress) specified in the ICH guidelines for stress testing of a drug substance. Using the reversed-phase high performance liquid chromatography (RP-HPLC) method, degradation studies were performed on the drug after exposing the drug to various stress conditions [7-11].

Experimental Procedure

Almotriptan was obtained as a gift sample from Natco Pharma Ltd, Hyderabad, India. The branded formulation of almotriptan was procured from the local market. Analytical reagent (AR) NaOH and H₂O₂ were purchased from S.D. Fine-Chem. Ltd., Mumbai, India. HCl, acetonitrile, methanol, and orthophosphoric acid (HPLC grade) was from Merk India (Mumbai). All other chemicals were of analytical grade.

Almotriptan stock standard solution

Accurately weighed 100 mg of almotriptan powder was transferred to a 100-mL volumetric flask; to this, 30 mL of water was added, then the mixture was sonicated for 40 s. The final volume was made up with water, and the resulting solution was vortexed for 1 min (1 mg/mL).

Instrumentation and chromatographic conditions

Chromatography was performed by using a LC-10 ATVP Shimadzu pump (Japan) equipped with a SPD-10 A VP UV-visible detector. The data was processed using Spinchrom Software. The separations were carried out using a C-8 RP column (Thermo Hypersil, BDS, 150 Å~ 4.6 mm, S-5 μ), which was operated at 40°C. The mobile phase comprised of methanol, acetonitrile and acetic acid in the ratio of 75:20:5 (v/v/v). The flow rate was adjusted at 1 mL/min, and the wavelength of detection was 231 nm. Specificity testing was done on a Waters (Milford, MA) Delta 600 HPLC equipped with a 600 controller pump, 2996 photodiode array (PDA) detector, and a degasser module. Empower 2 Software was used for data acquisition and processing. For the investigation of photo stability of the drug, a stability chamber (KBF 240, WTB Binder, Tuttlingen, Germany) equipped with light sources was used, as defined under option 2 of the ICH guideline Q1B. In this chamber, the combination of two black lights OSRAM L73 lamps and four white fluorescent OSRAM L20 lamps formed the light bank. The spectral distribution of black light lamps was between 345 and 410 nm with a maximum output set at 365 nm, whereas the output of white fluorescent lamps was similar to that specified in ISO 10977 (1993). Both UV and visible lamps were put on simultaneously. The chamber was maintained at 40°C and 75% relative humidity (RH). The samples were exposed for a total period of 10 days. Thermal degradation of the drug was investigated using a hot air oven maintained at 60°C for 15 days.

Mobile phase selection

In order to select a suitable mobile phase for the analysis of almotriptan, various combinational ratios of various solvents were tried on the basis of trial and error. Considering the system suitability parameters viz., tailing factor, number of theoretical plates, retention time etc. and the mobile phase found to be most suitable for analysis was acetonitrile, methanol and acetic acid in the ratio of 20:75:05 (v/v/v). The mobile phase was filtered through 0.45 μ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 mL/min and the concentration was detected at 231 nm.

Preparation of standard solution

Ten tablets of almotriptan 2.5 mg were weighed and powdered. A portion equivalent to 10 mg of almotriptan was accurately weighed and transferred to a 10 ml volumetric flask, sufficient amount of mobile phase was added to dissolve it and volume was made upto to 10 mL. Aliquots of stock solution were further diluted with mobile phase up to 10 mL to get concentration of 20, 40, 60, 80, 100, and 120 - μg/ml for the linearity study [12-16].

Forced decomposition (stress) studies

Forced decomposition studies were performed under different conditions as mentioned in ICH Q1A (R2) [17,18].

Acid hydrolysis

Forced degradation in acid media was performed by adding an aliquot of stock solution (1 mg/ml) of almotriptan to 10 ml each of methanol and 0.1 M HCl and refluxing the mixture at 60°C for approximately six hours. The solution was then left to reach room temperature, neutralized to pH 7 by the addition of 0.1 M NaOH, and diluted to 100 ml with the mobile phase so as to get a final concentration of 10 µg/ml.

Alkaline hydrolysis

Alkali - induced, forced degradation was performed by adding an aliquot of stock solution (1 mg/ml) of almotriptan to 10 ml each of methanol and 0.1 M NaOH, and refluxing the mixture at 60°C for approximately six hours. The solution was then left to reach room temperature, neutralized to pH 7 by addition of 0.1 M HCl, and diluted to 100 ml with the mobile phase, so as to get a final concentration of 10 µg/ml.

Oxidative degradation

To study the effect of oxidizing conditions, an aliquot of stock solution (1 mg/ml) of almotriptan was added to 10 ml of 30% H₂O₂ solution and the mixture was refluxed at 60°C for approximately six hours. The solution was left to reach room temperature and diluted to 100 ml with the mobile phase, so as to get a final concentration of 10µg/ml.

Thermal degradation

To study the effect of temperature, approximately 50 mg almotriptan was stored at 100°C in a hot air oven for 24 hours. It was then dissolved in 10 ml of methanol and the volume was adjusted to 50 ml with the mobile phase. The above solution was further diluted with the mobile phase, to give a solution of final concentration equivalent to 10 µg/ml of almotriptan

Photolysis

To study the effect of UV light, approximately 50 mg of almotriptan was exposed to short and long wavelength UV light (254 nm and 366 nm, respectively) for 24 hours, and then dissolved in 10 ml of methanol. The volume was made up by the mobile phase in a 50 ml volumetric flask, and then 1 ml of stock solution was further diluted with the mobile phase to give a solution of final concentration equivalent to 10 µg/ml of almotriptan.

Twenty microliters of the resulting almotriptan solution was injected into the HPLC system and the chromatograms were recorded. The stability samples were analyzed using a PDA detector to determine the peak purity.

Method validation

Linearity and range: A calibration curve of almotriptan was prepared in water for the establishment of linearity. For the same, a stock solution of the drug (1 mg/mL) was prepared in water. The concentrations ranging from (20–120 µg/mL) were prepared after suitable dilution (n = 6). The samples were filtered through a 0.45-µ-membrane filter prior to HPLC analysis. All

the determinations were done in triplicate. The linearity plots were constructed, and linear regression was applied on the data [19,20].

Precision: To determine the repeatability (intra-day precision) and intermediate precision (inter-day precision) of the method, the almotriptan solution at nominal standard concentration (100 µg/mL) and sample solution (100 µg/mL) were analyzed in six replicates on the same day (intra-day precision) and daily for six times over a period of three days (inter-day precision). The results were expressed as % RSD of the measurements [21].

Accuracy: Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (20%, 40%, and 60%) of the pure drug to the pre-analyzed formulation. The solutions were prepared in triplicates and the % recovery was calculated.

Specificity and selectivity: The specificity of the method was established through the study of resolution factors of the drug peak from the nearest resolving peak and also among all other peaks [22].

LOD and LOQ: The LOD and LOQ were determined at signal-to-noise ratios of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations.

Robustness: Robustness of the method was investigated by varying the chromatographic conditions, such as, change of flow rate ($\pm 10\%$), organic content in the mobile phase ($\pm 2\%$), wavelength of detection ($\pm 5\%$), and pH of the buffer in the mobile phase ($\pm 0.2\%$). Robustness of the developed method was indicated by the overall % RSD between the data, at each variable condition.

Solution stability: The solution stability was carried out by storing standard solutions of almotriptan in tightly capped volumetric flasks at -20°C for seven days. These solutions were assayed after seven days against fresh samples.

RESULT AND DISCUSSION

Forced degradation study was carried out by subjecting the drug to acid and alkaline hydrolysis, chemical oxidation and photolytic conditions and its chromatograms were shown in Figure 2. The forced degradation studies of almotriptan tablet formulation was done on stress degradation by using 0.1N HCl and product degradation was found to be 21.84% for 8hrs, stress degradation by hydrolysis under alkaline conditions by using 0.1 N NaOH was found to be 27.84% for 8hrs, Dry heat induced degradation was done by using 90°C temperature was found to be 27.18% for 48 hours. Using hydrogen peroxide 30% did oxidative degradation and product degradation was found to be 20.62% for 6hrs. Photolytic degradation was found to be Photolytic degradation (VIS) 71.57% for 48 hrs and Photolytic degradation (UV) 33.3% for 48 hours [23-27].

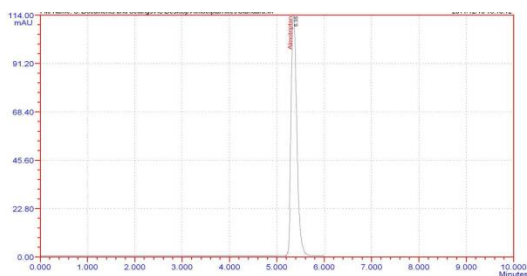
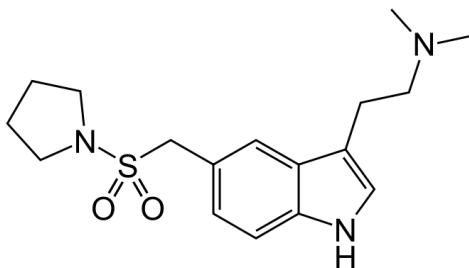
Maximum degradation of almotriptan was observed in Photolytic degradation (VIS), followed by decomposition under Photolytic degradation (UV), alkaline and thermal photolysis. Minimum degradation of drug was observed in acid hydrolysis and oxidative conditions. Percent degradation of drug under all stressed conditions is included in Table 1.

Stress conditions	Degradation studies (Hrs.)	AM	
		% Assay	% Degradation
Control		99.9	-----
Acid hydrolysis	8	78.06	21.84
Base hydrolysis	8	72.06	27.84
Hydrolytic	8	78.50	21.4
Oxidative	6	79.28	20.62
Thermal	48	72.72	27.18
Photolytic(VIS Light)	48	80.5	19.5
Photolytic (UV Light)	48	66.6	33.3

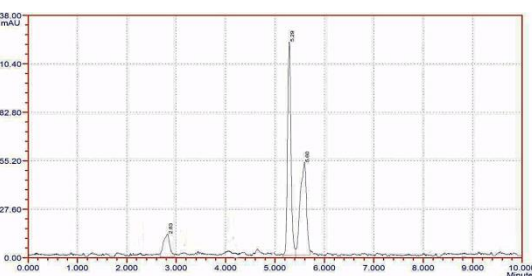
Table 1: Forced degradation study results.

Method validation

The method was validated with respect to parameters like linearity, precision, accuracy, specificity and robustness (Figure 1).



Standard chromatogram



Base degradation

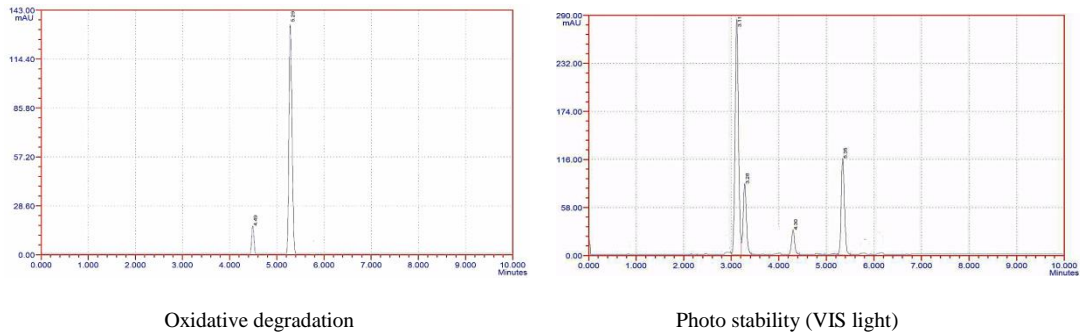
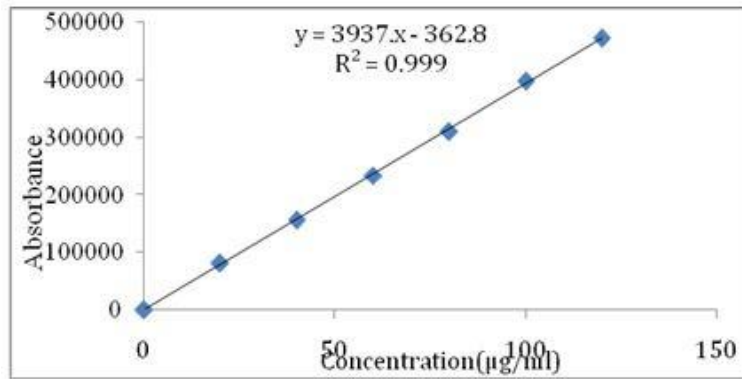


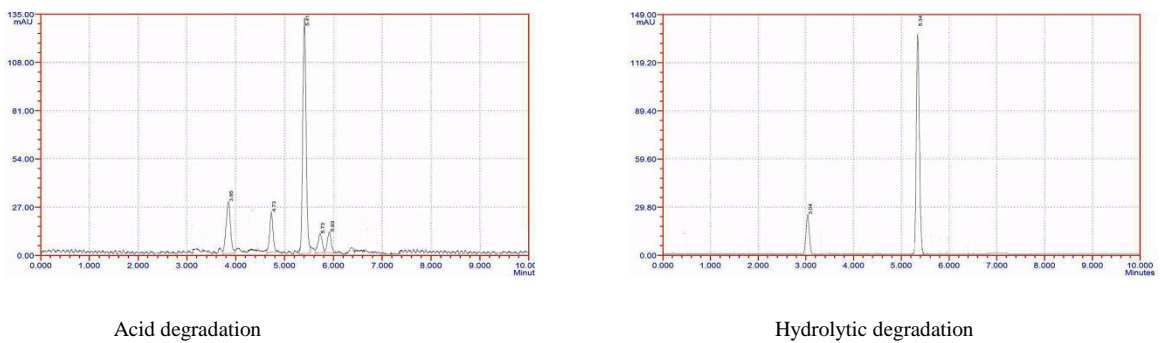
Figure 1: Structure of Almotriptan

Linearity

The response for this drug was strictly linear in the concentration range between 20–120 µg/mL. The equation of the line was $y = 3933. \times -221.9$. A very high correlation of 0.9996 was obtained with RSD of 0.5673% was shown in Tables 2 and 3 (Figure 2).



Linearity graph of Almotriptan.



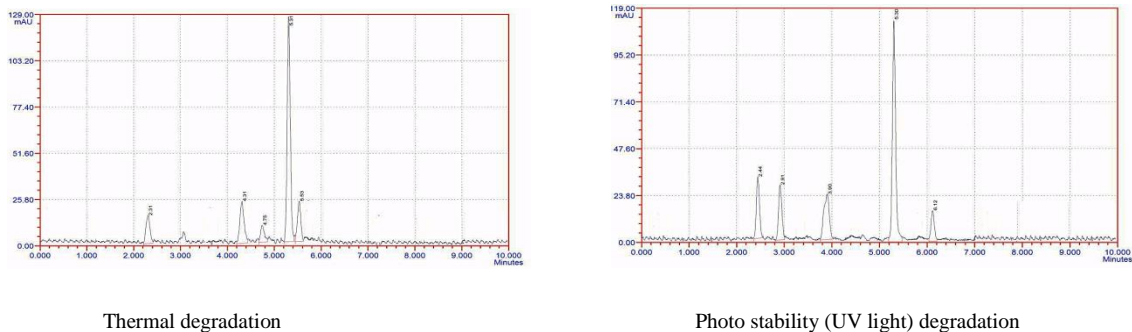


Figure 2: Degradation of Almotriptan.

Table 2: Forced degradation study results.

Stress conditions	Degradation studies (Hrs.)	AM	
		% Assay	% Degradation
Control	-----	99.9	-----
Acid hydrolysis	8	78.06	21.84
Base hydrolysis	8	72.06	27.84
Hydrolytic	8	78.50	21.4
Oxidative	6	79.28	20.62
Thermal	48	72.72	27.18
Photolytic(VIS Light)	48	80.5	19.5
Photolytic (UV Light)	48	66.6	33.3

Table 3: Linearity.

S. No	Concentrations µg/ml	Area of the peak
1	20	80521.4
2	40	156326
3	60	234462
4	80	310114
5	100	397319
6	120	472596

Precision

Table 4 shows the results of intra and inters –day precision studies. From the intra and day studies, the % RSD value was found to be in the range of 1.6510% to 0.5670%: whereas, in the case of inter-day studies, the values ranged from 1.0470% to 0.3843%.

Table 4: Intra and Inter day precisions.

S.NO	Concentration (µg/ml)	Intra-day precision mean n = 3	% Amount calculated	% RSD	Inter-day precision mean n = 3	% Amount calculated	% RSD
1	15	14.83	98.87	1.651	15.03	100.2	1.047
2	30	29.96	99.87	0.408	30.03	100.1	0.4078
3	45	44.93	99.84	0.567	45.06	100.1	0.3843

Accuracy

The data obtained from the recovery studies is represented in Table 5. The recovery was in the range of 97.65% to 101.67%.

Table 5: Data recovery studies.

Time	AM	
	% Assay	% Difference
Initial	99.29	---
12 th hr	98.71	0.58
18 th hr	98.89	0.4
24 th hr	98.68	0.61

Robustness

The method was found to be robust by varying the temperature of column as shown in Table 6.

Table 6: Robust to vary the temperature of column.

S. No	Variations	Chromatographic parameters	
		Tailing factor	Theoretical plates
1	34% of methanol in the mobile phase	1.14	9638

2	24% of methanol in the mobile phase	1.88	8854
3	Flow rate at 0.9ml/min	0.96	6240
4	Flow rate at 1.1ml /min	1.11	9685
5	pH change of mobile phase	1.16	9677
6	pH change of mobile phase	1.39	9942

CONCLUSION

The proposed method described a new RP-HPLC were developed and validated as per ICH guidelines, the standard deviation and % RSD calculated for the proposed method are low, indicating high degree of precision of the method. The recovery study performed show the high degree of accuracy and has the use of inexpensive solvent where it has the ability to separate these drugs from their degradation products, related substance; excipients found in tablet dosage forms and can be applied, for the routine analysis in quality control laboratory.

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