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Research Article

SIMPLE RP-HPLC METHOD DEVELOPMENT FOR THE ESTIMATION OF ALMOTRIPTAN MALATE IN NASAL *IN-SITU* FORMULATION

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ABSTRACT

For the determination of Almotriptan malate in bulk and produced nasal *in situ* formulation, a new sensitive and quick HPLC technique was developed and validated according to ICH guidelines. The HPLC analysis was carried out using a waters system with a Thermo Scientific C18 (250 cm 4.6 mm) 5 m column and a mobile phase of acetonitrile: 20Mm KH_2PO_4 in an 80:20 v/v ratio, at a flow rate of 1.0mL/min. The detection was done at a wavelength of 282, and Almotriptan malate had a retention time of 5.658 minutes. The total time spent running was 10.0 minutes. Over the concentration range of 2-10 g/ml, the calibration plot revealed a linear relationship. The LOD and LOQ were 0.12 and 0.35µg, respectively. The accuracy of the proposed method was determined by recovery studies and was found to be near to 100 and % RSD value was found less than 2. The repeatability testing for both standard and sample solutions showed that the method is precise within the acceptable limits. RSD% of the determination of precision was <2%. The proposed method showed excellent linearity, accuracy, precision, specificity, LOD, LOQ, and system suitability results within the acceptance criteria. In addition, the main features of the developed method are low run time and retention time around 5 min.

Keywords: HPLC, Method development, Almotriptan malate, Validation.

1. INTRODUCTION

Almotriptan malate [1], a selective 5-hydroxytryptamine 1B/1D (5-HT1B/1D) receptor agonist, is chemically designated as 1- [[[3- [2-(dimethylamino) ethyl]-1H-indol-5-l]methyl] sulfonyl] pyrrolidine-(±)-hydroxy butanedioate (1:1), whose empirical formula is $C_{17}H_{25}N_3O_2S$-$C_4H_6O_5$, with a molecular weight of 469.56 (fig. 1). Axert tablets contain almotriptan malate, a water-soluble white to slightly yellow crystalline powder used to treat severe migraine headaches. Forner *et al*. [2] and Bosch *et al*. [3] have synthesised almotriptan malate and have published the data in US5565447.

Literature review suggested that, several analytical methods such as HPLC [4-7], HPTLC [8], HPLC-MS/MS [9], LC-ESI-MS/MS [10] are available for the estimation of drug content. Analytical method validation ensures that various HPLC analytical techniques shall give reliable and repeatable results; it is a crucial step in developing new dosage forms as it provides information about accuracy, linearity, precision, detection, and quantitation limits. According to the ICH guideline, "the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose." It is now obligatory in the process of drug development to supply the validation data for the responsible authorities. Guidelines for analysis method validation include ICH and USP guidelines [11-14].

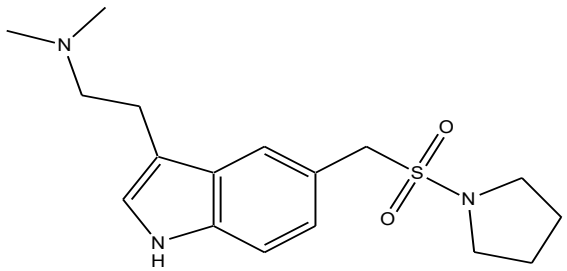


Fig. 1: Structure of Almotriptan malate

2. MATERIAL AND METHODS

2.1. Instrumentation

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing

the generated data. Weighing was done on a Digital Micro Balance (CX-265) manufactured by Citizen Scale (I) Pvt. Ltd.

2.2. Reagents and chemicals

Analytically pure sample of Almotriptan malate was purchased from Sigma Aldrich. Potassium di hydrogen phosphates (AR grade), disodium hydrogen phosphate (AR grade), OPA and acetonitrile (HPLC Grade) was purchased from E. Merck Ltd. Worli, Mumbai, India. All other chemicals used were of analytical grade. Triple distilled water used for whole experiment was generated in-house.

2.3. Selection of mobile phase

Initially, to estimate Almotriptan malate, number of mobile phases in different ratios was tried. Taking into consideration the system suitability parameter like RT, tailing factor, number of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was 20mM KH_2PO_4: acetonitrile (pH 3.5 with ortho-phosphoric acid) in the ratio 20:80 v/v run as isocratic system. The mobile phase was filtered through 0.45 m filter paper and then degassed by Sonication. Flow rate employed for analysis was 1 ml/min.

2.3.1. Preparation of standard stock solution

Accurately weighed 10mg of Almotriptan was transferred into 50 ml volumetric flasks and dissolved in 10 ml of 6.6 pH phosphate buffer, then volume was made up to 50 ml with 6.6 pH phosphate buffer and vortex to get complete dissolution of drug. Allowed to stand aside for few minute, Concentration of Almotriptan was 200µg/ml (Stock- A).

2.3.2. Preparation of Sub Stock Solution

Five ml of solution was taken from stock-A of Almotriptan transferred into 10 ml volumetric flask separately and diluted up to 10 ml with diluent to give concentration of 100µg/ml (Stock-B).

2.3.3. Preparation of different solutions

A 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with (6.8 pH phosphate buffer). This gave the solutions of 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml for drug.

2.4. Linearity and calibration graph

To establish the linearity of analytical method, a series of dilution ranging from 2-25 µg/ml was prepared. All the solutions were filtered through 0.2µm membrane filter and injected, chromatograms were recorded at 282nm and it was repeated thrice. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

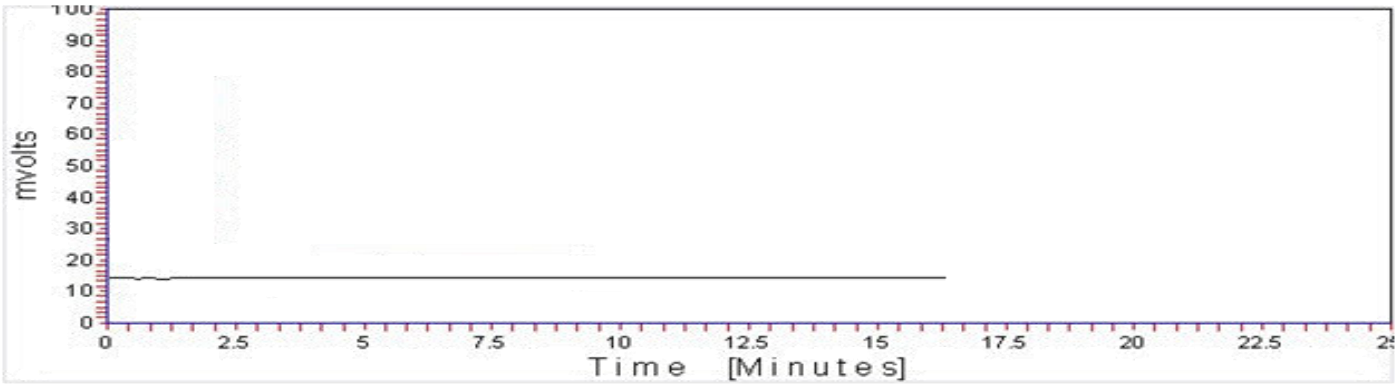
2.5. Validation of developed method

2.5.1. Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample [15]. The calibration plot was contracted after analysis of five different concentrations (from 2 to 10µg/ml) and areas for each concentration were recorded three times, and mean area was calculated. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration table 1.

2.5.2. Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and matrix components (fig. 2).



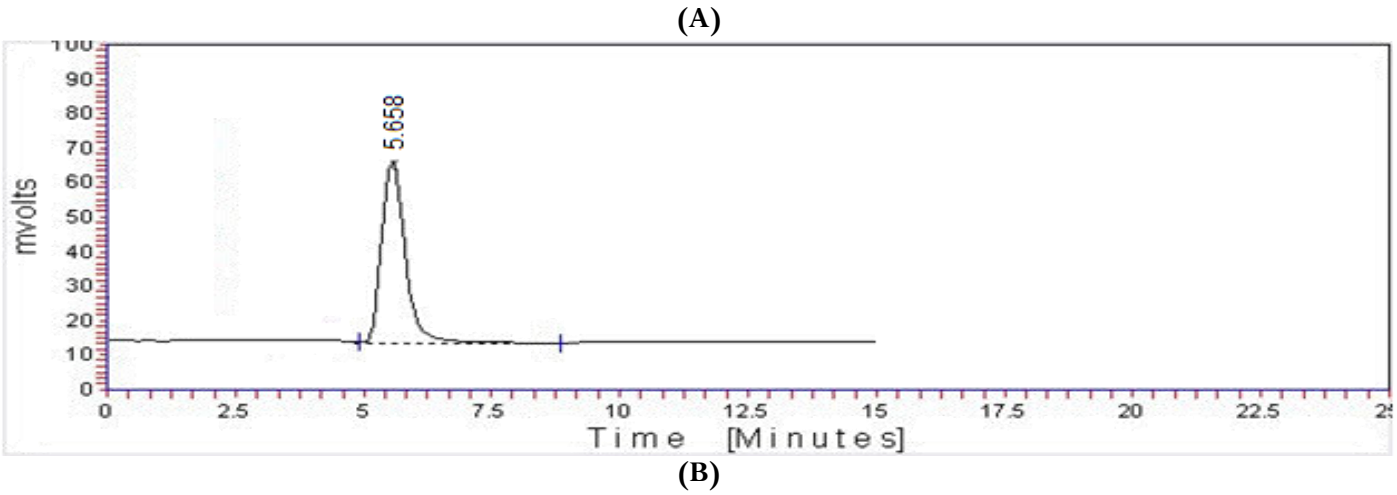


Fig. 2: Chromatograms of (A) Blank mobile phase (B) Almotriptan malate (5µg/ml) as reference substances

2.5.3. Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre-analysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed (table 2).

Table 1: Response ration data for linearity of Almotriptan

Replicates	Concentration (µg/ml)	Mean AUC	Response Ratio
Rep-1	2	115.089	57.545
Rep-2	4	238.502	59.626
Rep-3	6	337.756	56.293
Rep-4	8	455.140	56.893
Rep-5	10	568.515	56.852
		Mean	57.545
		SD	1.299
		%RSD	2.261

**Value of five replicate and three concentrations*

Table 2: Results of recovery study

% Level	% Mean ± SD*
80%	99.62±0.246
100%	98.42±0.118
120%	99.58±0.393

**Value of three replicate and three concentrations*

2.5.4. Precision

2.5.4.1. Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 2, 4, 6, 8 and 10 µg/ml for Almotriptan indicates the precision under the same operating condition over short interval time.

2.5.4.2. Intermediate Precision

2.5.4.2.1. Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days in three replicate at five concentrations (table 3).

2.6. Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve (table 4).

Table 3: Statistical data for precision

Statistical parameter	Mean*	S.D*	R.S.D*
Repeatability	98.178	0.102	0.104
Intermediate Precision (I) (A day to day)	98.525	0.065	0.066
(II) Analyst to Analyst	99.25	0.098	0.092

**Mean of 5 determinations (three replicates at five concentration level)*

Table 4: LOD and LOQ

Name	LOD (µg/ml)	LOQ (µg/ml)
Micafungin	0.12	0.35

2.7. Assay of nasal in house formulation

For analysis of the *in situ* nasal formulation, weight equivalent to 10 mg of Almotriptan malate was transferred to 10 ml volumetric flask and dissolved in diluents. The solution was shakenvigorously for 20 min and filtered through Whattman filter paper no.41, then

volume was made up to mark with diluents. From the above solution, 1 ml of solution was taken and diluted to 10 ml with diluents to get a solution containing 100µg/ml. From the above solution, 1 ml of solution was taken and diluted to 10 ml with diluents to get a solution containing 10µg/ml of Almotriptan malate. The amounts of Almotriptan malate in injection were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with formulation.

3. RESULTS AND DISCUSSION

HPLC method was developed and validated for the estimation of Almotriptan malate in prepared *in-situ* formulation. The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and estimated into the UV and the results were recorded. The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD less than 2, indicate the accuracy of method.

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and % RSD less than 2 indicate the precision of method. The results of the analysis of tablets formulation were reported. The assay value of drugs was close to 100, SD and % RSD less than 2 indicate the no interference of excipients in the estimation of drugs.

Table 5: Assay of tablet formulation

S. No.	Parameter	Almotriptan
1.	Mean	99.25
2.	S. D.	0.145
3.	% RSD	0.124

Mean of five determinations

4. CONCLUSION

The proposed HPLC method was validated as per the International Conference on Harmonisation (ICH) Q2B Guidelines, and was found to be applicable for routine quantitative analysis of Almotriptan malate by HPLC in pharmaceutical dosage form. The results of linearity, precision, accuracy and specificity, were proved to be within the limits. The method provides selective quantification of Almotriptan malate with no interference from other formulation excipients. The proposed method was highly reproducible, reliable, rapid, robust and specific. Therefore, a high percentage of recovery and the run time of less than seven minutes allow its application for the routine determination of Almotriptan malate in the pharmaceutical dosage form.

Conflict of interest

None declared

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