Research Article

NEW RP - HPLC METHOD DEVELOPMENT AND VALIDATION FOR ANALYSIS OF ALMOTRIPTAN

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ABSTRACT
A simple, rapid and precise reverse phase high performance liquid chromatography method was developed for the analysis of Almotriptan. Chromatographic separation of Almotriptan was performed by using a kromosil C_{18} column (250 x 4.6mm, 5 μm) as stationary phase with a mobile phase comprising of 1% Triethyl amine : acetonitrile:methanol: 05:55:40 (v/v) at a flow rate of 1.0ml/min and UV detection at 230nm. The linearity of Almotriptan is in the range of 10 ppm to 60 ppm. The limit of detection for Almotriptan was found to be 0.05ppm. The proposed method was found to be accurate, precise and rapid for the analysis of Almotriptan.

Keywords: Almotriptan, accurate, precise, linearity.

INTRODUCTION
Almotriptan is N,N-dimethyl-2- [5-(pyrrolidin-1-ylsulfonylmethyl)- 1H-indol-3-yl]- ethanamine prescribed to treat the acute treatment of the headache phase of migraine attacks with or without aura. Almotriptan is a selective and potent serotonin 5-HT1B/1D agonist. Because of the particular distribution of the 5-HT1B/1D receptors, 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.
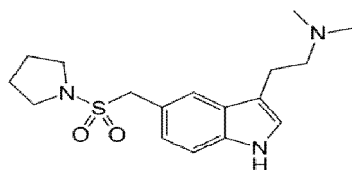

Fig. 1: Structure of Almotriptan

MATERIALS AND METHODS
Methanol, Acetonitrile, Tri ethyl amine, Methanol used was analytical grade. Chromatographic separation was performed with PEAK high performance liquid chromatography having LC-P7000 isocratic pump, equipped with PEAK LC-UV7000 variable wavelength detector. Chromatograms and data were recorded by means of PEAK Chromatographic Software version 1.06.

Preparation of standard solution
10mg of Almotriptan was taken in a 100ml volumetric flask and 100ml of mobile phase was added to obtain 100 ppm of Almotriptan standard solution.

Chromatographic Conditions

Mobile phase	:	Triethyl amine (5%), acetonitrile(55%), methanol(40%)
P^H	:	6.2
Analytical Column	:	Kromosil C_{18} column (250mm x 4.6mm) 5μ
UV detection	:	230nm
Flow rate	:	1.0ml/min.
Injection Volume	:	20μl
Temperature	:	Ambient
Run time	:	10 min.
Retention time	:	6.0 min.

Linearity

In order to check the linearity for the developed method, solutions of five different concentrations ranging from 10ppm-60ppm were prepared. The chromatograms were recorded and the peak areas were given in table-1.A linear relationship between areas versus concentrations was observed in about linearity range. This range was selected as linear range for analytical method development of Almotriptan. Linearity graph was shown in figure: 2

Precision (Repeatability)
2 mg/ml standard solution was prepared to calculate the precision for the developed method. The prepared solution was injected into injector at same concentrations and same chromatographic conditions. The chromatograms were recorded.

Limit of Quantification (LOQ) and Limit of Detection (LOD)
The LOQ and LOD were established at a signal to noise ratio. The LOD of Almotriptan is 0.05ppm. The LOQ of Almotriptan is 0.15.

RESULTS AND DISCUSSIONS
The Reverse Phase High Performance Liquid Chromatography method was developed a stability indicating assay method. Pure drugs chromatogram was run in different mobile phases containing methanol, acetonitrile, THF, and different buffers like potassium dihydrogen phosphate, sodium dihydrogen phosphate, Ortho phosphoric acid in different volumes ratios. Different columns like C_8, C_{18}, phenyl,

cyano with different dimensions were used. Then retention time and tailing factor were calculated. Finally Tri ethyl amine: acetonitrile:methanol: 05:55:40 v/v and Kromosil C_{18} analytical column was selected which gave a sharp and symmetrical peak with 1.87 tailing. Calibration graph was found to be linear at range 10 ppm to 60 ppm. Five different concentrations of Almotriptan in range given above were prepared and 20μl of each concentration injected in HPLC. The slope (m) and intercept (c) obtained were found to be 4085.444 and 6798.828.The correlation of coefficient (r^2) obtained was found to be 0.9989. It was observed that the concentration range showed a good relationship. The limit of detection for Almotriptan was found to be 0.05pm and the limit of quantification was found to be 0.15ppm. It proves the sensitivity of method. The low values of standard deviation and coefficient of variation at each level of the recovery experiment indicate high precision of the method.

CONCLUSION
The RP-high performance liquid chromatographic method for the analysis of Almotriptan from their formulations was found to be accurate and precise. Thus, the proposed HPLC method can be successfully applied for the routine quality control analysis of Almotriptan formulations.
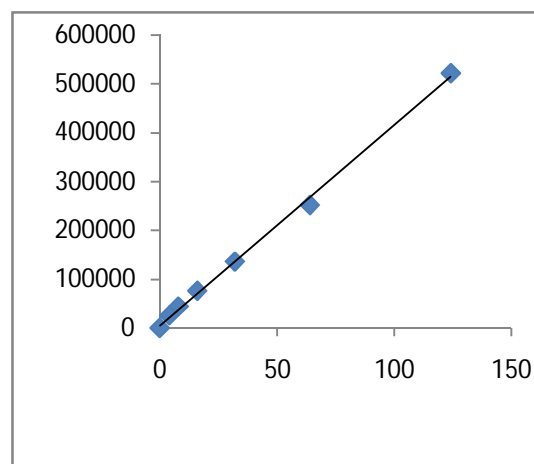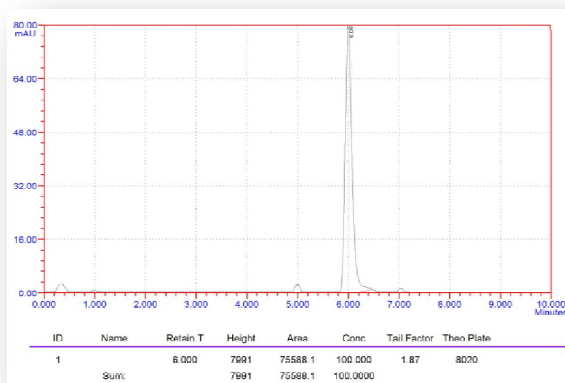

Fig. 2: Linearity graph for Almotriptan

Fig. 3: Typical chromatogram

Table 1

Linearity level	Concentration ppm	Area
1	10	25279
2	20	43855
3	30	75588
4	40	135670
5	50	251753
6	60	521571

Table 2: Precision study

Day	Mean area	R.S.D
1	551249	1.586
2	567430	1.612
3	569325	1.647

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