

# High Performance Liquid Chromatographic Analysis of Almotriptan Malate in Bulk and Tablets

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## ABSTRACT

**Purpose:** A simple RP-HPLC method has been developed and validated for the determination of almotriptan malate (ATM) in bulk and tablets. **Methods:** Chromatographic separation of ATM was achieved by using a Thermo Scientific C18 column. A Mobile phase containing a mixture of methanol, water and acetic acid (4:8:0.1 v/v) was pumped at the flow rate of 1 mL/min. Detection was performed at 227 nm. According to ICH guidelines, the method was validated. **Results:** The calibration curve was linear in the concentration range 5–60 µg/mL for the ATM with regression coefficient 0.9999. The method was precise with RSD <1.2%. Excellent recoveries of 99.60 - 100.80% proved the accuracy of the method. The limits of detection and quantification were found to be 0.025 and 0.075 µg/mL, respectively. **Conclusion:** The method was successfully applied for the quantification of ATM in tablets with acceptable accuracy and precision.

## Introduction

Almotriptan malate (ATM) is a serotonin receptor agonist used in the acute treatment of migraine headache in adults and adolescents aged from 12 to 17 years with or without aura.<sup>1-3</sup> Chemically, ATM is known as 1-[[[3-[2-(Dimethylamino) ethyl]-1H-indol-5-yl] methyl] sulfonyl] pyrrolidine malate (1:1). ATM stimulates specific serotonin receptors in intracranial blood vessels and sensory trigeminal nerves, thereby promote vascular constriction and relieve migraine.

A thorough literature survey has revealed that only a few liquid chromatographic methods are available for the quantification of ATM in bulk, pharmaceutical formulations and biological fluids. Assay of almotriptan in pharmaceutical dosage forms by HPTLC has been reported by Suneetha and Syamasundar.<sup>4</sup> Ravikumar et al. have developed LC-MS method for the determination of almotriptan in human plasma.<sup>5</sup> Nageswara Rao et al. have reported a LC-MS method for determining in vivo metabolites of almotriptan in rat plasma, urine & faeces.<sup>6</sup> Jansat et al. and Fleishaker et al. have reported HPLC method for the determination of almotriptan concentration in human plasma and urine, respectively.<sup>7,8</sup>

Only two HPLC methods with UV detection were reported in the literature for the assay of ATM in pharmaceutical dosage forms. In a method reported by Suneetha and Syamasundar, separation and

quantification of ATM were conducted on Phenomenex Gemini C18 column using potassium dihydrogen phosphate buffer:acetonitrile (80:20) as a mobile phase. The UV detection was performed at 227 nm.<sup>9</sup> Phani kumar and Sunandamma have reported a HPLC method using a Kromosil C18 column with a mobile phase comprising of 1% Triethyl amine: acetonitrile: methanol (05:55:40) and UV detection at 230 nm.<sup>10</sup> The reported HPLC with UV detection methods suffer from the disadvantages such as narrow range of linear response,<sup>9</sup> longer runtime for a single sample,<sup>9,10</sup> preparation of buffer,<sup>9</sup> strict control of pH,<sup>9</sup> and less precise with RSD values greater than 1.5.<sup>10</sup> The present paper deals with the development and validation of a RP-HPLC method with UV detection for the determination of ATM in bulk and tablet dosage forms.

## Materials and Methods

### Instrumentation

Reverse Phase-High Pressure Liquid Chromatography was performed with an isocratic High Pressure Liquid Chromatography system (Shimadzu HPLC class VP series, Shimadzu Corporation, Kyoto, Japan) with two LC-10 AT, VP pumps, variable wavelength programmable UV/Visible detector SPD-10A, VP, CTO-10AS VP column oven, SCL-10A, VP system

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controller. The monitoring software was “class VP series version 5.03” (Shimadzu). Separation of the ATM was achieved by using a 250 mm × 4.6 mm I.D., 5 µm particle size, Thermo Scientific C18 (Phenomenex, Torrance, CA, USA) column under reversed-phase chromatographic conditions.

### **Chemicals and Reagents**

All the chemicals were of HPLC grade quality. Milli-Q-water was obtained from Merck Specialties Private Ltd, Hyderabad, India and used right throughout the process. Methanol and glacial acetic acid were obtained from Sd fine Chem Limited, Mumbai, India. Pharmaceutical grade ATM was obtained from Matrix laboratories, Hyderabad, India. Branded ATM products (Axert tablets, Ortho-McNeil-Janssen Pharmaceuticals, Inc. USA), labeled to contain 6.25 and 12.5 mg of ATM were purchased and used in the present study.

### **Preparation of Mobile Phase**

The mobile phase was prepared by mixing methanol, water and glacial acetic acid in the ratio of 4:8:0.1 v/v.

### **Preparation of Standard Drug Solutions**

For the stock standard solution (1 mg/mL), an exactly weighed 100 mg of ATM was dissolved and diluted to the volume with the mobile phase in a 100 mL volumetric flask. Working standard solutions equivalent to 5 to 60 µg/mL of ATM were prepared by appropriate dilution of the stock standard solution with the mobile phase.

### **Preparation of Tablet Extract**

Twenty tablets were weighed and finely powdered. A precisely weighed portion of the powder equivalent to 100 mg of ATM was extracted with mobile phase. The extract was transferred to a 100 mL volumetric flask and made up to the mark with the mobile phase. The solution was filtered through a 0.45 µm membrane filter. The tablet extract was appropriately diluted with mobile phase to obtain a concentration of 60 µg/mL.

### **Chromatographic Conditions**

The mobile phase [methanol, water and acetic acid (4:8:0.1 v/v)] was pumped at a flow rate of 1 mL/min. It was filtered through 0.45 µm membrane filter and degassed before using with a helium sparge for 15 min. The injection volume was 20 µL and the eluent was monitored at 227 nm. The column temperature was 25±1°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 in triplicate. The peaks were determined at 227 nm. The mean peak area versus concentrations of almotriptan were plotted to obtain the calibration curve. The seven concentrations of the working standard solution were subjected to

regression analysis to calculate regression equation, slope, intercept and regression coefficient. The concentration of the drug was calculated from the calibration curve or the regression equation.

### **Assay in Tablet Dosage Forms**

A 20 µl aliquot of tablet extract (60 µg/mL) was injected into the HPLC system in triplicate. The peaks were determined at 227 nm. The concentration of the drug in tablet dosage form was calculated from the calibration curve or the regression equation.

### **Method Validation**

The proposed method was validated for system suitability, linearity, limit of detection, limit of quantification, precision, accuracy, selectivity, robustness, ruggedness and stability of drug.

### **System Suitability**

The study of system suitability was done to validate the sufficiency of the reproducibility of chromatographic system for the analysis of ATM. This was carried out by five replicate analyses of the drug at a concentration of 60 µg/mL. The proposed method was evaluated by analyzing the parameters like retention time, peak asymmetry, theoretical plates, plates per meter and height equivalent to theoretical plate.

### **Linearity**

For the assessment of linearity, determination of ATM was done at seven concentration levels (5, 10, 20, 30, 40, 50 and 60 µg/mL). Twenty µl aliquot of each concentration solution was injected automatically into the column in triplicate and the peak areas were recorded.

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

LOD and LOQ were calculated by using the following expressions:

$$\text{LOD} = 3.3s / S$$

$$\text{LOQ} = 10s / S$$

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

### **Precision and Accuracy**

The precision and accuracy of the proposed method was assessed by intra-day and inter-day variation studies using three different concentrations of ATM (5, 30 and 60 µg/mL). 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 three consecutive days.

### **Recovery Studies**

Recovery studies were performed using the standard addition method. For this study, a known amount of pure drug was added to the preanalyzed sample

solution. Mean percent recovery of the ATM was determined.

#### Selectivity

Selectivity was assessed by examining peak interferences from excipients present in the tablet dosage forms and components of mobile phase. This was done by comparing the chromatograms of blank and tablet extract with the pure drug.

#### Robustness

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 at the deliberately varied experimental conditions by taking two different concentrations of ATM (5 and 60  $\mu\text{g/mL}$ )

#### Ruggedness

The method's ruggedness was established by the determination of ATM at two different concentrations (5 and 60  $\mu\text{g/mL}$ ). This was performed by two different analysts.

#### Solution Stability

To assess the stability of ATM in mobile phase, the sample solutions (5 and 60  $\mu\text{g/mL}$ ) were prepared in the mobile phase and stored at room temperature for 48

hr. The sample solutions were assayed at an interval of every 12 hr for 48 hr.

#### 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 drug quality control. For all these investigations, an efficient and validated analytical method is very significantly required.

#### Method Development

A reversed-phase C18, 250 mm  $\times$  4.6 mm I.D., 5  $\mu\text{m}$  particle size column maintained at ambient temperature was used for the separation of ATM. Regarding the mobile phase, a mixture of methanol, water and glacial acetic acid was used. In order to improve the separation and peak symmetry, the mobile phase composition was varied until optimum composition was chosen (4:8:0.1 v/v). Isocratic elution at the flow rate of 1.0 mL/min has been employed in the present proposed method. Wavelength of 227 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 the assay of ATM. A good separation with satisfactory resolution has been obtained (Figure 1).

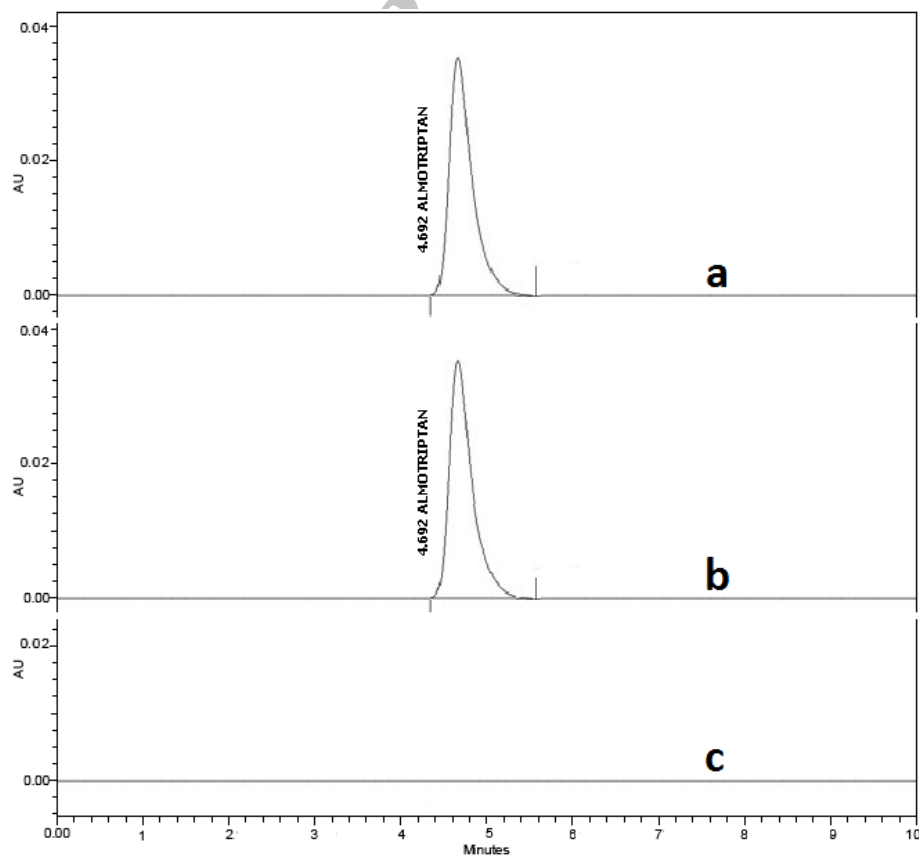


Figure 1. Representative chromatogram of pure ATM (a), tablet extract (b) and blank mobile phase.

## Validation

### System Suitability

The system suitability parameters, like retention time, peak asymmetry, theoretical plates, plates per meter and height equivalent to theoretical plate were calculated. From the results (Table 1), it was observed that all the values are within the limits.

**Table 1.** System suitability studies.

Parameter	Value	RSD (%)
Retention Time (t) (Min)	4.692	0.052
Theoretical Plates (n)	4832.82	1.06
Plates per Meter (N)	16100	1.113
Height equivalent to theoretical plate (HETP) (mm)	$6.2 \times 10^{-7}$	1.021
Peak asymmetry	1.16	1.071
RSD: Relative standard deviation		

### Linearity

Linear relationship was observed by plotting concentrations of the drug against peak areas. ATM exhibited linear response in the concentration range, 5 to 60  $\mu\text{g/mL}$ . The corresponding linear regression equation was  $y = 53532x + 39.216$  with regression coefficient ( $R^2$ ) 0.9999.

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

LOD and LOQ of the proposed method were determined as s/S ratio 3.3 for LOD and 10 fold for LOQ. The low values of LOD and LOQ, that is, 0.025 and 0.075  $\mu\text{g/mL}$ , respectively suggest the high sensitivity of the method.

### Precision and Accuracy

The relative standard deviation for intra-day and inter-day analysis was in the range of 0.129 to 0.823 % and 0.822 to 1.170 %, respectively. Mean recovery of ATM using the proposed method was in the range of 99.60-100.30 (intra-day analysis) and 99.76-100.80 (inter-day analysis). The results point out the precision and accuracy of the method developed (Table 2).

**Table 2.** Precision and accuracy of the method.

Assay type	Concentration of ATM ( $\mu\text{g/mL}$ )		RSD (%)	Recovery (%)
	Taken	Recovered $\pm$ SD*		
Intra-day	5	$4.98 \pm 0.041$	0.823	99.60
	30	$30.09 \pm 0.052$	0.175	100.30
	60	$60.04 \pm 0.092$	0.129	100.06
Inter-day	5	$5.04 \pm 0.059$	1.170	100.80
	30	$29.93 \pm 0.298$	0.995	99.76
	60	$59.95 \pm 0.493$	0.822	99.91
*Average of five determinations; SD: Standard deviation; RSD: Relative standard deviation				

### Recovery Studies

Preanalyzed sample solution was spiked with pure ATM and then the total amount of ATM was determined by the proposed method. The results (Table 3) showed that the mean recovery and relative standard deviation were in the range of 99.97-100.09 % and 0.799-0.973%, respectively. From the recovery study it was apparent that the proposed method is very accurate for quantitative determination of ATM in tablet dosage form. The excipients and additives commonly present in the tablet dosage forms did not interfere in the assay.

**Table 3.** Results of recovery studies.

Concentration of ATM (mg)			RSD (%)	Recovery (%)
Tablet	Spiked	Recovered $\pm$ SD*		
6.25	3.0	$9.248 \pm 0.090$	0.973	99.97
12.50	6.25	$18.766 \pm 0.150$	0.799	100.09
* Average of five determinations , SD: Standard deviation, RSD: Relative standard deviation				

### Selectivity

The selectivity of the proposed method demonstrated that excipients in the tablet dosage forms and components of mobile phase did not interfere with the drug peak. In addition, the well shaped peaks also signify the selectivity of the proposed method (Figure 1).

### Robustness

Robustness of the proposed method was assessed by making small changes in the composition of mobile phase, detection wavelength and flow rate. The small variations in any of the variables did not significantly affect the results (Table 4). The lower values of RSD (<1.3%) demonstrate the excellent robustness of the proposed method.

**Table 4.** Robustness of the method.

Experimental parameter	Concentration of ATM ( $\mu\text{g/mL}$ )		RSD (%)
	Taken	Recovered $\pm$ SD <sup>§</sup>	
Mobile phase*	5	$5.05 \pm 0.021$	0.415
	60	$59.97 \pm 0.324$	0.540
Detection wavelength**	5	$5.02 \pm 0.036$	0.717
	60	$59.90 \pm 0.466$	0.777
Flow rate***	5	$4.96 \pm 0.063$	1.270
	60	$60.01 \pm 0.514$	0.856
*Methanol, water and acetic acid ratios (v/v): 3.9:8.1:0.1, 4:8:0.1, 4.1:7.9:0.1			
**Wavelength (nm) – 226,227 and 228			
***Flow rate (mL/min) – 0.9, 1.0 and 1.1			
§Average of three determinations			
SD- Standard deviation			
RSD- Relative standard deviation			

### Ruggedness

Ruggedness of the method was performed by analyzing 5 and 60 µg/mL of ATM by two different analysts keeping same experimental conditions. The results are presented in Table 5. The lower values of RSD (<1.0%) confirm the ruggedness of the proposed method.

**Table 5.** Ruggedness of the method

Analyst	Concentration of ATM (µg/mL)		SD**	RSD (%)
	Taken	Recovered*		
I	5	4.96	±0.042	0.841
II	5	5.02		
I	60	59.90	±0.056	0.093
II	60	59.98		

\* Average of five determinations, \*\* Standard deviation for two values, SD: Standard deviation, RSD: Relative standard deviation

### Solution Stability

Relative standard deviation for assay of ATM during solution stability was <1.0% (Table 6). The results from solution stability experiments confirmed that ATM in the mobile phase was stable for up to 48 hr.

**Table 6.** Stability of ATM in mobile phase

Time (hr)	Concentration of ATM (µg/mL)		SD**	RSD (%)
	Taken	Recovered*		
12	5	5.05	±0.044	0.880
24	5	5.02		
36	5	4.98		
48	5	4.95		
12	60	60.05	±0.062	0.103
24	60	59.95		
36	60	59.97		
48	60	59.90		

\* Average of five determinations, \*\* Standard deviation for four values, SD: Standard deviation, RSD: Relative standard deviation

### Application to Tablet Dosage Forms

The proposed method was effectively applied for the quantification of ATM in two different dosage forms. The results, shown in Table 7, are in good agreement with those obtained with the reference UV spectrophotometric method.<sup>11</sup>

### Conclusion

The proposed HPLC method was found to be simple, precise, accurate and rapid for the estimation of ATM in bulk and in its dosage forms. The mobile phase is simple to prepare. The sample recoveries in all tablet dosage forms were in good agreement with their

respective labelled claim and suggested noninterference of tablet excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of ATM in quality control laboratories.

**Table 7.** Results of analysis of ATM in tablets

Formulation	Method	Concentration of ATM (mg)	
		Tablet	Recovered ± SD**
Tablet*	Reference	6.25	6.246 ± 0.014 % R=99.93 % RSD=0.224
		12.50	12.504 ± 0.024 % R=100.09 % RSD=0.194
	Proposed HPLC method	6.25	6.249 ± 0.054 % R=99.98 % RSD=0.863 t Value <sup>§</sup> = 1.19 F value <sup>§§</sup> =3.29
		12.50	12.490 ± 0.120 % R=99.92 % RSD=0.960 t Value <sup>§</sup> = 1.11 F value <sup>§§</sup> =4.66

\*Axert tablets, Ortho-McNeil-Janssen Pharmaceuticals, Inc. USA  
 \*\* Average of five determinations  
 %R – Percentage recovery  
 % RSD – Percentage relative standard deviation  
 § Tabulated t-value at 95% confidence level is 2.306  
 §§ Tabulated F- value at 95 % confidence level is 6.390

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### Conflict of Interest

The authors declare there is no Conflict of interest in the content of this study.

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