International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.5, No.2, pp 459-466, April-June 2013

Development And Validation Of Hplc Method For The Determination Of Almotriptan Malate In Bulk And Tablet Dosage Forms

Petikam Lavudu[1], Avula Prameela Rani[2], Chandra Bala Sekaran[3]*

[1]Department of Pharmaceutical Biotechnology, Vishnu Institute of Pharmaceutical Education and Research, Narsapur, Andhra Pradesh-500072.
[2]University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Guntur, Andhra Pradesh-522510.
[3]Department of Biotechnology, Jagarlamudi Kuppuswamy Choudary College, Guntur, Andhra Pradesh-522006.

***Corres author: balumphil@gmail.com**
Tel: +91-9848834447

Abstract: A simple and rapid high-performance liquid chromatographic method has been developed and validated for determination of the almotriptan malate in bulk and tablets. Separation was achieved in a Thermo Scientific C18 column using a mobile phase consisting of chloroform, methanol and acetic acid (80: 15: 5 v/v). An isocratic method with a flow rate of 1mL/min was used. The developed method was sensitive, precise, accurate, robust and linear over the concentration range of 1-80 µg/mL, with a limit of detection and a limit of quantification of 0.015 and 0.045 µg/mL, respectively. The developed method was found to be suitable and reproducible for analysis of almotriptan malate in tablets.
Keywords: Almotriptan malate, HPLC, Method development, Validation, Tablets.

Introduction:

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

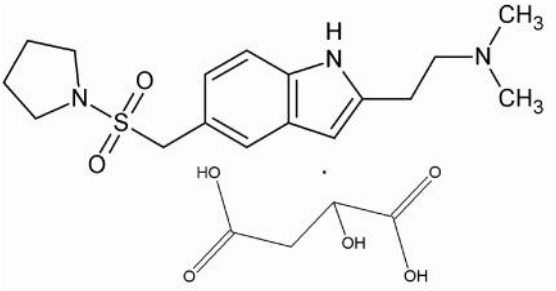

Figure 1. Structure of almotriptan malate

Literature survey reveals a few chromatographic methods for determination of ATM in bulk, in pharmaceutical formulations and in biological fluids. Suneetha and Syamasundar applied HPTLC method for the assay of ATM in pharmaceutical dosage forms[4]. Ravikumar K. *et al*. presented an LC-MS method for the quantitative determination of ATM in human plasma[5]. Nageswara Rao *et al.* estimated invivo metabolites of almotriptan in rat plasma, urine & feces using LC-MS[6]. HPLC method was reported by Jansat *et al.* and Fleishaker *et al*. for the analysis of ATM in human plasma and urine, respectively[7,8]. The reported chromatographic methods are not suitable for the routine analysis of ATM as it involves drawbacks like tedious sample preparation, time consumption and requirement of an expensive detector. Determination of ATM in pharmaceutical dosage forms by HPLC method with UV detection has also been reported[9,10]. The reported HPLC with UV detection methods have many disadvantages, like narrow range of linear response[9], longer runtime for a single sample[9,10] , preparation of buffer[9] , strict control of pH[9], and is less precise with RSD values greater than 1.5.[10] Other methods reported for the determination of ATM in formulations include UV spectrophotometry[11] and visible spectrophotometry[12]. The spectrophotometric methods suffer from disadvantages like use of expensive reagent[12], lesser sensitivity[11,12], lack of selectivity[11,12] and narrow linear range of response[11].

The present work is aimed at developing a simple, sensitive, rapid, accurate and precise HPLC method, which would overwhelm the difficulties encountered in the reported HPLC and spectrophotometric methods, for the quantification of ATM in bulk and tablet dosage forms.

Experimental:

Apparatus:

The present work was performed on a 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 controller. Shimadzu class VP series version 5.03 was used for data acquisition. The column used was Thermo Scientific C18 column (250 mm × 4.6 mm I.D., 5 µm particle size, Phenomenex, Torrance, CA, USA).

Chemicals and reagents:

HPLC grade quality Chloroform, Methanol and Acetic acid were purchased from Sd fine chem Limited, Mumbai, India. Milli-Q-water was obtained from Merck Specialties Private Ltd, Hyderabad, India. Milli-Q-water was used all the way through the process. Matrix laboratories (Hyderabad, India) kindly gifted Pharmaceutical grade ATM. Axert tablets (Ortho-McNeil-Janssen Pharmaceuticals, Inc. USA) Limited labeled to contain 6.25 and 12.5 mg of ATM were purchased.

Chromatographic conditions:

Mobile phase consists of a mixture of chloroform, methanol and acetic acid (80: 15: 5 *v/v*). The mobile phase was degassed with a helium sparge for 15 min prior to use. The run time was 10 min at a flow rate of 1 mL/min, detector wavelength 257 nm. The column temperature was 25±1^{0}C. The injection volume was 20 µl.

Stock and Standard Solutions:

Stock standard solution (1 mg/mL) of ATM was prepared in mobile phase. Working standard solution (100 µg/mL) was prepared by diluting 10 mL stock standard solution to 100 mL with the same solvent.

General procedure:

Aliquots of the working standard solution of ATM were transferred into 10 mL volumetric flasks and the solutions were made up to volume with mobile phase to give final concentrations of 1 , 5, 10, 20, 40, 60, 80 µg/mL. An aliquot (20 µL) of each concentration solution was injected into the column in triplicate after filtration using 0.45 µm membrane filter. The peak areas were recorded at 257 nm. The calibration curve was prepared by plotting peak areas *versus* drug concentrations (µg/mL). Alternatively, the corresponding regression equation was derived.

Procedure for tablets:

Twenty Axert tablets were weighed and pulverized. A weighed quantity of the powder equivalent to 100 mg of ATM was transferred into a small conical flask and extracted with 30 mL of mobile phase. The extract was filtered into a 100 mL volumetric flask and completed to the mark with the same solvent. The tablet extract

was suitably diluted with mobile phase to give a final concentration of 60 µg/mL. The procedure described under "general procedure" was applied. The nominal concentration of the tablet was calculated either using the calibration curve or the regression equation.

Results and Discussion:

Method development:

Chromatographic conditions (mobile phase composition, type of column, column temperature, flow rate and detection wavelength) were optimized to achieve efficient separation, sharp peak shape and a short run time per analysis of ATM. The optimization experiments were carried out by varying one parameter at a time and keeping the others constant. A mixture of chloroform, methanol and acetic acid was used as mobile phase. Different ratios of chloroform, methanol and acetic acid were tried. It was observed that a mixture consisting of chloroform, methanol and acetic acid in the ratio of 80: 15: 5 *v/v* was most suitable for good separation, elution and peak shape. The results of optimization experiments have confirmed that the Thermo Scientific C18 column (250 mm × 4.6 mm I.D., 5 µm particle size) maintained at ambient temperature (25±1^{0}C) was suitable for the fast separation and elution of ATM. At the flow rate of 1 mL/min, symmetric and well shaped peak was obtained. Hence, the flow rate of 1 mL/min was selected for the present investigation. Wavelength of 285 nm was selected for the detection. Under the optimized chromatographic conditions symmetric and sharp peak of ATM was obtained at retention time of 2.742 min. A typical chromatogram of ATM is shown in Figure 2.

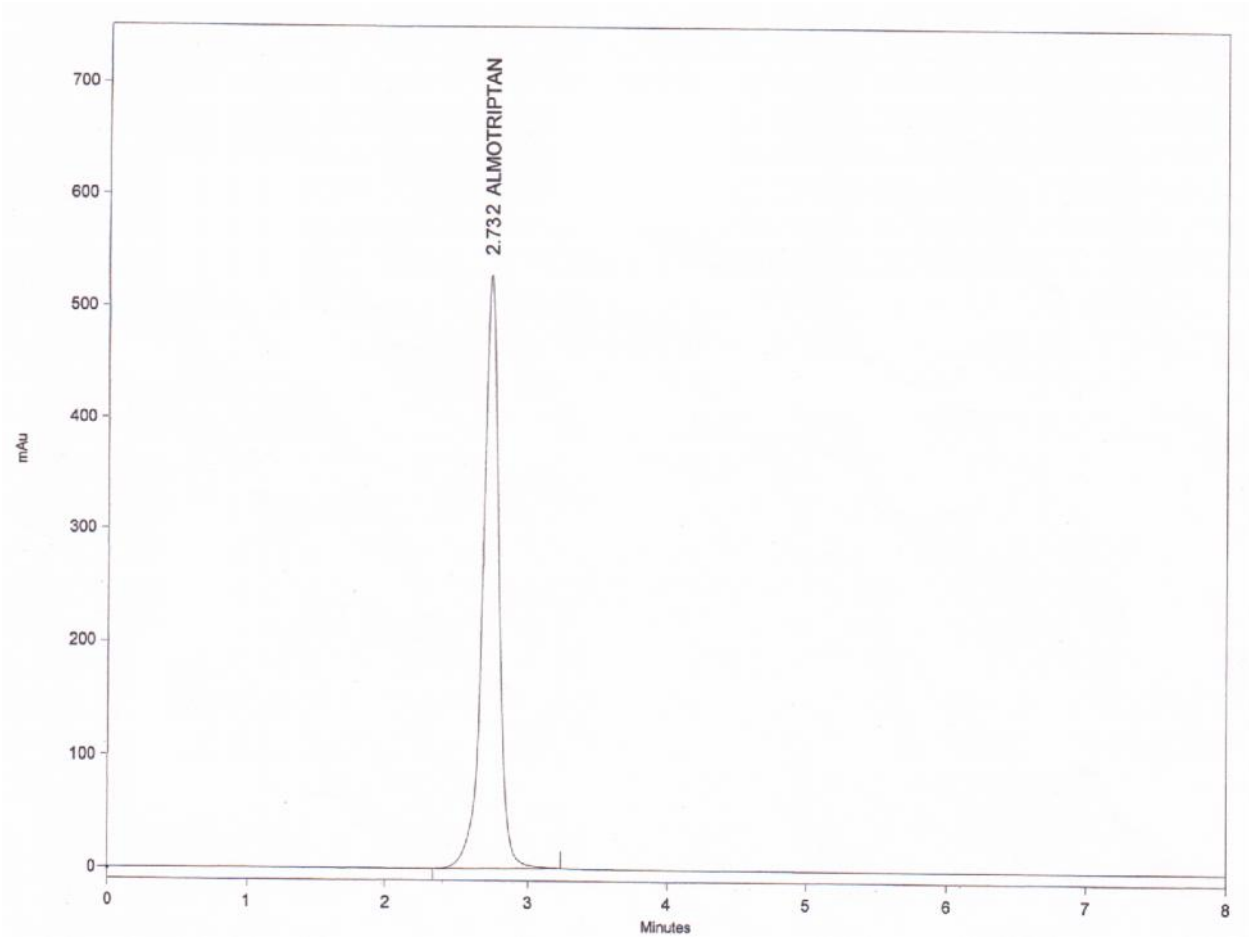

Figure 2. Chromatogram of standard ATM (60 µg/mL)

Method validation:

According to ICH guidelines[13], the developed chromatographic method was validated for system suitability, selectivity, linearity, limit of detection & quantitation, accuracy, precision and robustness.

System suitability

To assess system suitability of the method, the retention time, peak asymmetry, theoretical plates, plates per meter and height equivalent to theoretical plates, of five replicate injections of ATM (60 µg/mL) were used and the RSD values were calculated in each case. The results are summarized in Table 1. The values were within limits which confirmed that the reproducibility of the system is adequate for the analysis to be performed.

Table 1. System suitability studies

Parameter	Value	RSD (%)
Retention Time (t) (Min)	2.732	0.017
Peak area	4696444	0.857
Theoretical Plates (n)	4782	0.890
Plates per Meter (N)	19128	1.177
Height equivalent to theoretical plate (HETP) (mm)	$5.2x10^{-7}$	1.056
Peak asymmetry	0.820	1.011

Selectivity:

The selectivity of the method was established by comparing the chromatograms of standard ATM (60 µg/mL) and those of tablet extract (60 µg/mL) & blank mobile phase. The chromatograms obtained for the tablet extract (Figure 3) and the blank mobile phase (Figure 4) do not show any peak with a similar retention time to that of the standard ATM (Figure 2). Therefore, the absence of interferences of the excipients in tablet dosage forms and components of mobile phase, was observed. Then, it was concluded that the developed method is selective.

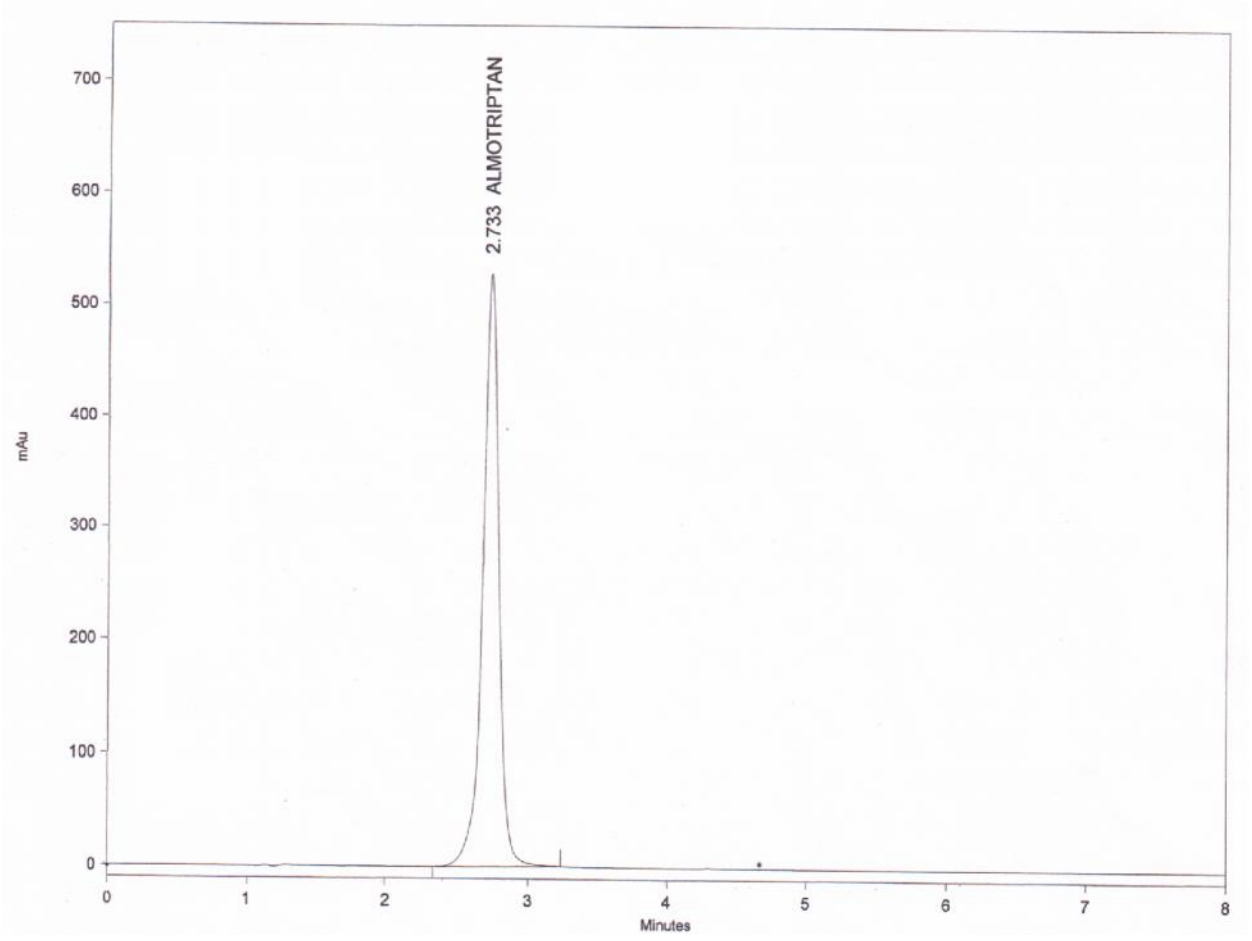

Figure 3. Chromatogram of ATM tablet extract (60 µg/mL)

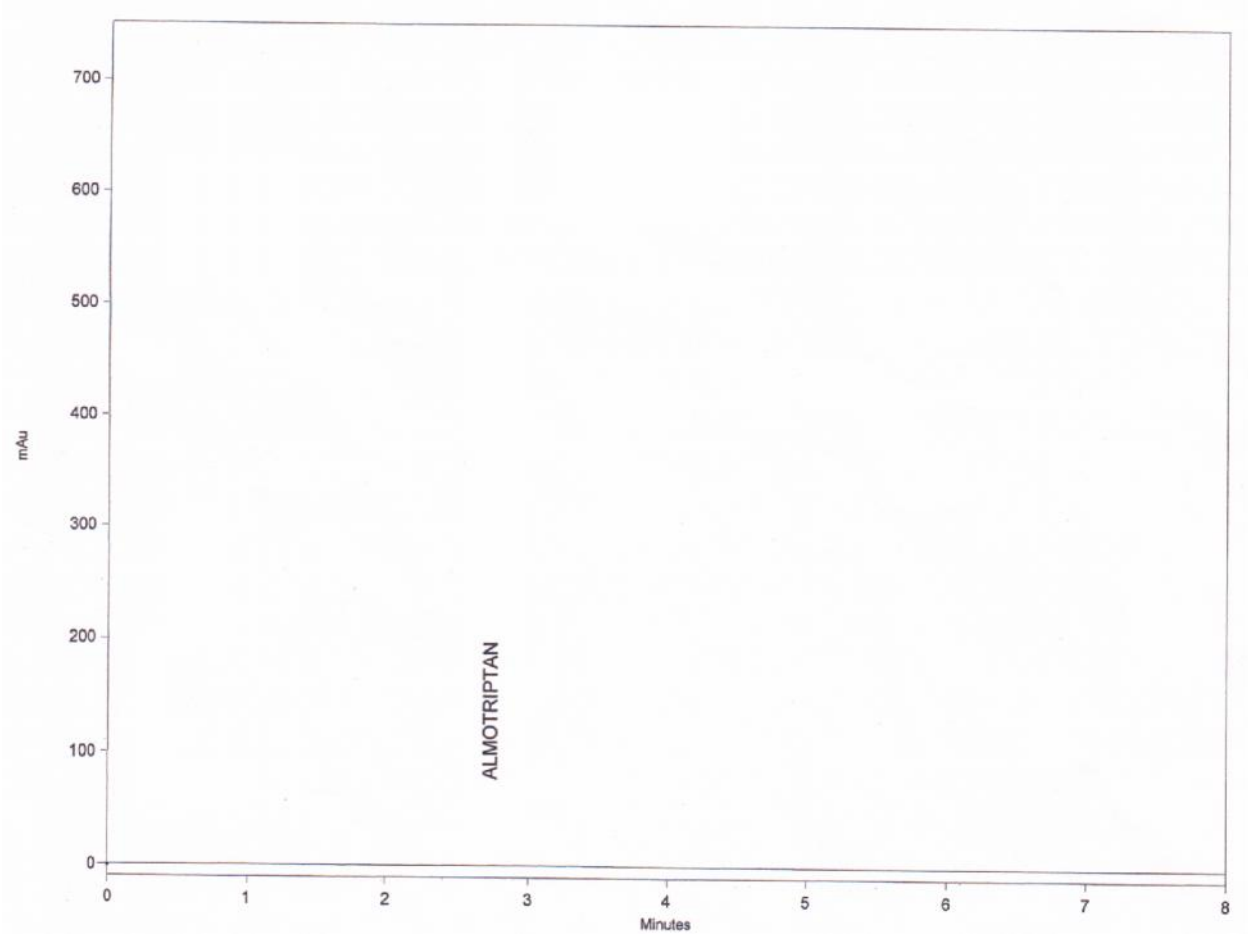

Figure 4. Chromatogram of blank mobile phase

Linearity:

Good linear relationship was confirmed between the ATM peak areas *vs.* ATM concentrations over a range of 1–80 µg/mL. The linear regression equation of the peak versus drug concentration (µg/mL) showed the regression coefficient R^2 =0.9995 (y = 33618 x + 25433, where y and x are peak areas and concentration of ATM in µg/mL, respectively).

Limit of detection (LOD) and Limit of quantification (LOQ):

The limit of detection and limit of quantification were determined based on the standard deviation between response and slope of the curve at lowest concentrations. The LOD and LOQ were found to be 0.015 and 0.045 µg/mL, respectively. LOD and LOQ values show that the sensitivity of the method is satisfactory.

Precision and Accuracy:

Precision and accuracy was studied by intra- and inter-day assay. Intra-day assay was performed by injecting five standard solutions of three different concentrations (2, 40 and 75 µg/mL) on the same day and inter-day assay was performed by injecting the same solutions for three consecutive days. Relative standard deviation and percent recovery was then calculated to represent precision and accuracy, respectively. The low RSD and excellent recovery values (Table 2) confirmed that the method is sufficiently precise and accurate.

Table 2. Precision and accuracy data

ATM taken (µg/mL)	ATM found (µg/mL) ± SD[a]	RSD (%)	Recovery(%)
Intra-day assay			
2	2.05±0.016	0.780	102.50
40	39.96±0.134	0.335	99.90
75	75.02±0.598	0.797	100.03
Inter-day assay			
2	1.96±0.025	1.275	98.00
40	40.05±0.362	0.904	100.13
75	74.96±0.642	0.856	99.94

[a]Average of five determinations

Robustness:

To access the robustness of the proposed methods, minor changes were made in mobile phase composition, flow rate and detection wavelength. The analysis was performed at deliberately varied experimental conditions by taking two different concentrations of ATM (2 and 75 µg/mL). The results are summarized in Table 3. The low values of RSD indicate the method is robust enough to withstand the small variations in the chromatographic conditions.

Table 3. Robustness

Parameter	ATM taken (µg/mL)	ATM found (µg/mL) ± SD[d]	% RSD
Mobile phase[a]	2	1.96±0.024	1.224
	75	74.95±0.186	0.248
Flow rate[b]	2	1.98±0.013	0.656
	75	75.03±0.257	0.342
Detection wavelength[c]	2	2.04±0.039	1.666
	75	74.94±0.391	0.521

[a]Chloroform, methanol and acetic acid ratios (*v/v*): 81:14:5, 80: 15: 5, 79:16:5
[b]Flow rate (mL/min) – 0.9, 1.0 and 1.1
[c]Wavelength (nm) – 256,257 and 258
[d]Average of three determinations

Application of the proposed method:

The method developed was applied to tablet dosage forms (Axert tablets containing 6.25 and 12.5 mg ATM) for determining their ATM content. The results obtained were compared with those given by the reference UV spectrophotometric method. Statistical analysis of the results (Table 4) obtained from proposed and reference methods revealed no significant difference between the two methods with reference to accuracy and precision as revealed by Student's t-test and variance ratio, F-test.

Table 4. Evaluation of Almotriptan malate in tablets

Formulation	Method	Labelled Claim(mg)	Found ± S.D[b]	RSD (%)	Recovery (%)
Axert [a]	Reference	6.25	6.24 ± 0.014	0.224	99.93
		12.50	12.50 ± 0.024	0.194	100.09
	Proposed	6.25	6.23±0.048	0.770	99.68
		12.50	12.51±0.086	0.687	100.08

[a] Ortho-McNeil-Janssen Pharmaceuticals, Inc. USA
[b]Average of five determinations

Accuracy of the developed method was further determined by standard addition technique. In the standard addition technique, known quantity of standard ATM was added to the sample solution previously analysed and then analysed by the developed method. Percentage recoveries and relative standard deviations are given in Table 5. From the facts given in Table 5, it is apparent that the method is highly accurate and appropriate for the intended use.

Table 5. Results of standard addition technique

Labeled claim (mg)	Pure drug added (mg)	Found ± S.D[$]	RSD (%)	Recovery (%)
6.25	3	9.28±0.054	0.581	100.33
12.50	6.25	18.69±0.194	1.037	99.68

[$]Average of five determinations

Conclusion:

An HPLC method was developed and validated for the estimation of ATM in bulk drug and in tablets. The developed method is simple, selective, sensitive, accurate, precise and robust. The recovery studies suggested non-interference of excipients in the assay. Hence, the developed method can be used for the quality control of the ATM and for routine analysis of the ATM in their tablet dosage forms.

Acknowledgements:

One of the authors, P. Lavudu, expresses his gratitude to the management of Vishnu Institute of Pharmaceutical Education & Research, Narsapur and Principal, University College of Pharmaceutical sciences, Acharya Nagarjuna University, Guntur for providing research facilities.

References:

1. Chen L.C. and Ashcroft D.M., Meta-analysis examining the efficacy and safety of almotriptan in the acute treatment of migraine, Headache., 2007, 47, 1169-1177.
2. Keam S.J., Goa K.L. and Figgitt D.P., Spotlight on almotriptan in migraine. CNS Drugs, 2002, 16, 501-507.
3. Dodick D.W., Oral almotriptan in the treatment of migraine: safety and tolerability Headache, 2001, 41, 449-455.
4. Suneetha A. and Syamasundar B., Development and validation of HPTLC method for the estimation of almotriptan malate in tablet dosage form, Indian J. Pharm. Sci., 2010, 72, 629–632.
5. Ravikumar K., Balasekhara Reddy C., Babu Rao C. and Chandrasekhar K.B., Method development and validation of almotriptan in human plasma by HPLC tandem mass spectrometry: Application to a pharmacokinetic study, Sci. Pharm., 2010, 80, 367-378.
6. Rao R.N., Guruprasad K., Naidu Ch. G., Raju B. and Srinivas R., LC–ESI-MS/MS determination of in vivo metabolites of almotriptan in rat plasma, urine and feces: Application to pharmacokinetics, J. Chroma. B, 2012, 891-892, 44-51.
7. Jansat J.M., Martinez-Tobed A., Garcia E., Cabarrocas X. and Costa J., Effect of food intake on the bioavailability of almotriptan, an antimigraine compound, in healthy volunteers: an open, randomized, crossover, single-dose clinical trial, Int. J. Clin. Pharmacol. Ther., 2006, 44, 185-190.
8. Fleishaker J.C., Ryan K.K., Jansat J.M., Carel B.J., Bell D.J., Burke M.T. and Azie N.E., Effect of MAO-A inhibition on the pharmacokinetics of almotriptan, an antimigraine agent in humans, Br. J. Clin. Pharmacol., 2001, 51, 437-441.
9. Suneetha A. and Syama Sundar B., A validated RP HPLC method for estimation of almotriptan malate in pharmaceutical dosage form, J. Chinese Chem. Soc., 2010, 57, 1067-1070
10. Phani kumar V. and Sunandamma Y., New RP - HPLC method development and validation for analysis of almotriptan, Int. J. Res. Pharm. Chem., 2011, 1, 542-545
11. Suneetha A. and Syama Sundar B., New simple UV Spectrophotometric method for estimation of almotriptan malate in bulk and pharmaceutical dosage forms, Asian J. Res. Chem., 2010, 3, 142-145.

12. El-Bagary R.I., Mohammed N.G. and Nasr H.R., Fluorimetric and colorimetric methods for the determination of some antimigraine drugs, J. Chem. Pharm. Res., 2011, 3, 304-314.
13. Validation of Analytical Procedures; Methodology, International Conference on Harmonization (ICH), Text and Methodology Q2(R 1), Complementary Guideline on Methodology dated 06 November 1996, incorporated in November 2005, London.

*****