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Estimation of almotriptan malate in oral film dosage form by RP-HPLC

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ABSTRACT

Almotriptan malate used to treat severe migraine headaches. Almotriptan malate oral films were prepared by solvent casting method. It is available in market as conventional tablets (Axert). A simple, precise, rapid and accurate RP- HPLC method was developed for the percentage drug release estimation of Almotriptan malate in oral film dosage form. An Intertsil C8-3, (250 x 4.6 with 5 microns particle size) and the mobile phase, consisting of 1-octane sulfonic acid sodium salt monohydrate in water adjusting the pH-3.0 with O-Phosphoric Acid: Acetonitrile in ratio of 90:10 v/v & Acetonitrile HPLC Grade: Buffer (70:30 v/v) was used as diluent in the gradient mode. The flow rate was 1.2 ml/min and the effluents were monitored at 228 nm. The retention time was 4.8 minutes for Almotriptan malate. The detector response was linear in the concentration of 2.05 -25.70 µg/mL for Almotriptan malate. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine estimation of percentage drug release in oral film dosage form of Almotriptan malate.

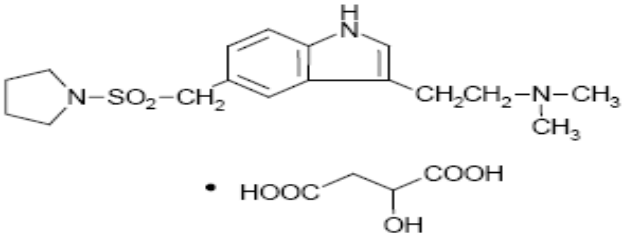
Keywords: Almotriptan malate, RP-HPLC, Estimation of percentage drug release and oral films.

INTRODUCTION

Almotriptan malate, chemically is 1-[[[3-[2-(Dimethylamino)ethyl]-1H-indol-5-yl]methyl]sulfonyl]pyrrolidine (±)-hydroxybutanedioate (1:1) (Figure 1).The Empirical Formula is $C_{17}H_{25}N_3O_2S.C_4H_60_5$ and the Molecular Weight is 469.55 g/mol. Almotriptan malate is classified as a Selective 5-hydroxytryptamine1B/1D (5-HT1B/1D) receptor agonist. It is used for the treatment the acute headache phase of migraine attacks with or without aura. Almotriptan has a high and specific affinity for serotonin 5-HT1B/1D receptors. Binding of the drug to the receptor leads to vasoconstriction of the cranial blood vessels and thus affects the redistribution of cranial blood flow. Almotriptan significantly increases cerebral blood flow and reduces blood flow through extracerebral cranial vessels. Even though it affects cranial blood vessels a single dose of almotriptan (12.5 mg) has no clinically significant effect on blood pressure or heart rate in both young and elderly healthy volunteers. However larger doses seem to slightly increase blood pressure but not beyond clinical relevance [1, 2, 3].

Literature survey reveals few chromatographic methods to determine the Almotriptan malate [4,5] in Tablet dosage form and also in biological fluids. So far, no dissolution methods by liquid chromatography were reported for the percentage drug release estimation of Almotriptan malate in Oral film dosage form at the time of commencement of these investigations. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of percentage drug release of Almotriptan malate in oral film dosage form [6, 7]. A detailed

account of all analytical methods existing for the drug is made to avoid duplication of the method developed. The authors had made some humble attempts, hoping to fulfill and bridge this gap, in succeeding the developing analytical methods, by using HPLC System [8,9]. The results of this labor of love are set forth by developing a simple, precise and accurate reverse-phase HPLC method [10, 11] for the estimation of percentage drug release Almotriptan malate in pharmaceutical oral film dosage forms.



(Figure 1) Almotriptan malate

MATERIALS AND METHODS

Almotriptan malate was obtained as a gift sample from MSN labs pvt ltd, Hyderabad. Acetonitrile and water used were of HPLC grade (Qualigens), 1-octane sulfonic acid sodium salt monohydrate, orthophosphoric acid (85%) and sodium hydroxide were AR grades.

Chromatography Instrument Quantitative HPLC was performed on liquid Chromatograph, Waters separation 2996, PDA detector module equipped with automatic injector with injection volume 10 µl. An Intertsil, C8-3 Column (250 x 4.6 mm i.d; particle size 5 µ) was used. The HPLC system was equipped with Empower 2 Software. The column was maintained at 30^{o} C and eluted under isocratic conditions over 10.0 min at a flow rate of 1.2 ml/min.

HPLC Conditions Weigh accurately 0.5 g of 1-octane sulfonic acid sodium salt monohydrate and add 5 mL of orthophosphoric acid (85%) in 1000 mL of milli –Q water and adjust to a pH of 3.0 with 10 N sodium hydroxide solution. Filter this solution through 0.45 µ filter and sonicate to degas.

Mobile Phase – A: Buffer: Acetonitrile (90:10 v/v)
Mobile Phase –B: Buffer: Acetonitrile (30:70 v/v)
Preparation of mobile phase: Mobile phase –A and Mobile phase –B (72:28 v/v)

Preparation of the Primary Standard/Stock Drug Solution Weigh and transfer Almotriptan malate standard equivalent 26 mg of almotriptan into 50 mL colored volumetric flask, add 60 mL of diluents sonicate to dissolve for 5 minutes and dilute to volume with diluent. Further dilute 4 mL of above solution to 100 mL with dissolution medium.

Dissolution parameters
Dissolution medium: pH 6.8 Phosphate buffer
Volume: 300 mL
Apparatus: Paddle
Speed: 50 RPM
Temperature: 37^{0}C ± 0.5°C
Time points: 1, 2,3,5,7 and 10 mins

Preparation of Sample solution Arrange the dissolution apparatus as per above dissolution conditions, add one film to each of the six vessels and run the system for 10 minutes. Withdraw 10 mL of sample at the end of specific time interval and discard first 5 mL of the filtrate through 0.45 µ Nylon filter, and collect sample solutions directly into vials from each of the bowl.

Linearity Aliquots of standard Almotriptan malate stock solution was taken in 50 ml volumetric flasks and diluted up to the mark with the dissolution medium. From the above standard stock solutions diluted to different

concentrations such that the final concentrations of Almotriptan malate was in the range of 2.07 -31.07 µg/mL. Each of these drug solutions (10 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 228 nm and the calibration graph was obtained by plotting peak area versus concentration in µg/mL of Almotriptan malate **(Figure: 2)**. The plot of peak areas of each sample against respective concentration of Almotriptan malate was found to be linear in the range of 2.07 -31.07 µg/mL with correlation coefficient of 0.9998. Linear regression least square fit data obtained from the measurements are given in **Table I**. The regression characteristics, such as slope, intercept & % RSD were calculated for this method and given in **Table I.**

Accuracy Accuracy was evaluated in triplicate by addition of three different amounts of Almotriptan malate, to a previously analyzed sample and comparing the amounts of analytes recovered with the amounts added. The amounts added were equivalent to 50, 100, and 150% of the amount originally present. %Recovery and RSD (%) were calculated for amount added. From the data obtained, it is obvious that the method is remarkably accurate, which ensures that this method produces reliable results as depicted in **Table: II**.

Precision The precision of the method was ascertained, separately from the peak area obtained by actual determination of six replicas of a fixed amount of the drug and formulation.

The HPLC system was set up, describing chromatographic conditions, mentioned as above and following the system equilibration of the working standard solution containing 20.5 µg/mL of Almotriptan , by injecting six times and recording the response peak areas. The precision was repeated with the formulated sample for the same concentrations by injecting the working sample solutions containing 20.8 µg/mL. The test sample was processed six times for the response of peak area. The % Relative Standard Deviation (RSD) were calculated and presented in **Tables: III & IV** respectively.

Method Applicability The present developed method was evaluated by applying to Oral film dosage forms for the estimation of Almotriptan malate by our research group.

Dissolution 10 µl of sample solution (Almotriptan oral film – 6.25 mg) was injected into the injector of liquid chromatograph. The retention time was found to be 4.8 min for Almotriptan malate. The amount of drug present per oral film was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in **Table II**.

Recovery Studies Accuracy was determined by recovery studies of Almotriptan malate; known amount of standard was added to the pre-analyzed sample and subjected to the proposed HPLC analysis. Results of recovery studies are shown in **Table II**. The study was done at three different concentration levels.

RESULTS AND DISCUSSION

HPLC Method Development and Optimization[11] In response to lack of simple, reliable and easy-to-use method for the determination of Almotriptan malate concentrations in Oral films, a gradient RP-HPLC method was developed for quantification of Almotriptan malate. We examined several HPLC method variables with respect to their corresponding effects on the result of analysis. To optimize the chromatographic conditions, different combinations of Acetonitrile & O-Phosphoric Acid Buffer adjusting the pH 4.0, pH 3.0 with Sodium hydroxide, Methanol & O-Phosphoric Acid Buffer adjusting the pH-4.0 with Sodium hydroxide. Mobile Phase A 0.5 g of 1-octane sulfonic acid sodium salt monohydrate O-Phosphoric Acid Buffer with pH-3.0 and Acetonitrile (90:10 v/v) Mobile Phase B 0.5 g of 1-octane sulfonic acid sodium salt monohydrate O-Phosphoric Acid Buffer with pH-3.0 and Acetonitrile (70:30 v/v) was promisingly preferred, because it resulted in greater resolution of Almotriptan after several preliminary investigatory runs, compared with other mobile phases. The other parameters in this factorial design were different column, temperature, variation in flow rate, detection wavelength, buffer pH variation in mobile phase and volume of injection. At 228 nm, ʎ max was observed and there is no interferences. Under these conditions, the analyte peaks were well defined and free from tailing. Considering the whole body of the data obtained from this extensive study, the set of conditions indicated earlier in this article was selected for further validation. Typical chromatogram (Std & Working Sample) of Almotriptan malate has been shown in **Figure: 3 & 4.**

Parameters that were studied to evaluate the suitability of the system were discussed and presented in **Table V**.

Method Validation Tests Recommended method validation characteristics including Method precision (RSD, %), Method accuracy (Recovery % and RSD, %) and Linear range (Correlation Coefficient were investigated systematically.

Linearity The plot of peak areas of each sample against respective concentrations were found to be linear, in the range of 2.07- 31.07 µg/ml for Almotriptan malate with correlation coefficient of 0.9997 (**Table: 1)**. Linear regression least square fit data obtained from the measurements are given in **Table: 1.** The regression characteristics, such as slope and intercept were calculated for this method and given in **Table I.** The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in **Table: 1.**These results show that there was an excellent correlation between peak areas and analyte concentration.

Accuracy Recovery of the individual substances at 50%, 100%, and 150% of specified concentrations were between 98.4% -101.2%, which proves the accuracy of the method. From these data, RSD was always less than 1%, which indicates it is obvious that the method is remarkably accurate, produces reliable results (**Table: II**)

Precision The low value (<1%) of RSD indicates the repeatability of the method. These data indicate a considerable degree of precision and reproducibility for the method both during one analytical run and between different runs (**Table: III & IV**).

Robustness Robustness was studied out to evaluate the effect of small but deliberate variations in the chromatographic conditions at three different levels, i.e. –2, 0, +2. To determine the robustness of this method, the experimental conditions were deliberately altered at three different levels and retention time and chromatographic response were evaluated. One factor at a time was changed to study the effect. Variation of the columns, mobile phase flow rate by 10% of actual flow, mobile phase pH by ±0.2 units (pH 2.8 and pH 3.2) had no significant effect on the retention time and chromatographic response of the method, indicating that the method was robust. The results are shown in **Table: VI**.

Specificity No evidence of signals, in the corresponding times of the chromatogram were monitored as a sign of potential interfering peaks, were found when the pharmaceutical formulations were tested. Hence, this method can be used reliably for the estimation of respected active pharmaceutical ingredients in a variety of dosage forms.

TABLE I: LINEAR REGRESSION DATA OF CALIBRATION CURVES

Parameter	Almotriptan malate
Concentration range(µg/mL)	2.07 -31.07
Slope (m)	152964
Intercept (Y)	25361
STEYX	46771
Correlation coefficient (r)	0.9997
Linear regression (r^2)	0.9996

TABLE II: ASSAY & RECOVERY ACCURACY STUDIES OF ALMOTRIPTAN MALATE IN ORAL FILM DOSAGE FORMS

Film formulation	Amount claim (mg/film)	Amount Obtained (mg)* by proposed method	** % Recovery by the Proposed method
	Almotriptan malate	Almotriptan malate	Almotriptan malate
1)150%	9.375	9.254	98.70
2)100%	6.25	6.21	99.36
3)50%	3.125	3.14	100.48
Average Mean	6.25	6.20	99.56

**Average of three determinations*

ACCURACY PARAMETER	ALMOTRIPTAN MALATE
Assay (150%)	148.06%
Assay (100%)	99.36 %
Assay (50%)	50.24%

Table: III: Precision of Recommended Procedure Using API- {Almotriptan malate} & its Oral Film

Sr. No	Inj. No	Name of the Standard Drug & Conc. (20.5 µg/mL)	Retention time in minutes	Peak Area	Name of the Sample Drug & Conc. (20.5 µg/mL)	Retention time in minutes	Peak Area
		API (Almotriptan malate)			Formulation (Oral Film)		
1	1	Almotriptan malate	4.83	4440728	Almotriptan malate oral film	4.92	4478625
2	2	Almotriptan malate	4.85	4450124	Almotriptan malate oral film	4.86	4485445
3	3	Almotriptan malate	4.79	4448777	Almotriptan malate oral film	4.88	4481253
4	4	Almotriptan malate	4.93	4450128	Almotriptan malate oral film	4.93	4471458
5	5	Almotriptan malate	4.88	4460009	Almotriptan malate oral film	4.75	4492582
6	6	Almotriptan malate	4.75	4470998	Almotriptan malate oral film	4.78	4489245
7	Mean		4.84	4453461	Mean	4.853333	4483101
8	Standard Deviation		0.06	10552.12	Standard Deviation	0.073666	7643.841
9	% RSD		1.32	0.23	% RSD	1.517842	0.170503

Table IV: Validation Summary / System Suitability:

PARAMETER	ALMOTRIPTAN MALATE (Standard API drug)	Formulation (Oral Film)
Theoretical Plates(N)	9689	9752
Tailing factor	1.02	1.07
Retention time(min)	4.75	4.839
Area	4453258	4429388

Table V: Results from testing of the Robustness of the method

Condition Studied in Robustness	Modification In analysis	Parameter Fixation	Mean Peak Area ± S.D	% RSD (Peak Area)	Mean Retention Time (in min) ± S.D	% RSD (Retention time)
			Almotriptan malate	Almotriptan malate	Almotriptan malate	Almotriptan malate
Column(s) Intertsil C8-3	Xterra RP8, & Hypersil BDS C18	Standard	5491643 ± 37237.97	0.6	4.96 ± 0.051	0.62
		Sample	5578310 ± 53665.63	0.9	4.89 ± 0.068	0.84
Flow rate (1.2 ml/min)	1.08 ml/min & 1.32 ml/min	Standard – Increase	4353258 ± 30237.97	0.4	4.71 ± 0.045	0.49
		Standard - Decrease	4553201 ± 21234.69	0.2	4.92 ± 0.056	0.40
		Sample- Increase	4453217 ± 29223.14	0.5	4.75 ± 0.031	0.40
		Sample- Decrease	4644783 ± 26536.48	0.4	4.98 ± 0.019	0.31
pH (3.0)	2.8 & 3.2	Standard - Increase	4231270 ± 20417.0	0.3	4.76 ± 0.009	0.34
		Standard - Decrease	4621298 ± 31419.7	0.3	4.94 ± 0.013	0.58
		Sample - Increase	43029380 ± 19416.18	0.3	4.70 ± 0.011	0.21
		Sample - Decrease	4641201 ± 38019.27	0.3	4.93 ± 0.037	0.65

Figure 4: Calibration Curve of the Almotriptan malate (PML) by RP-HPLC

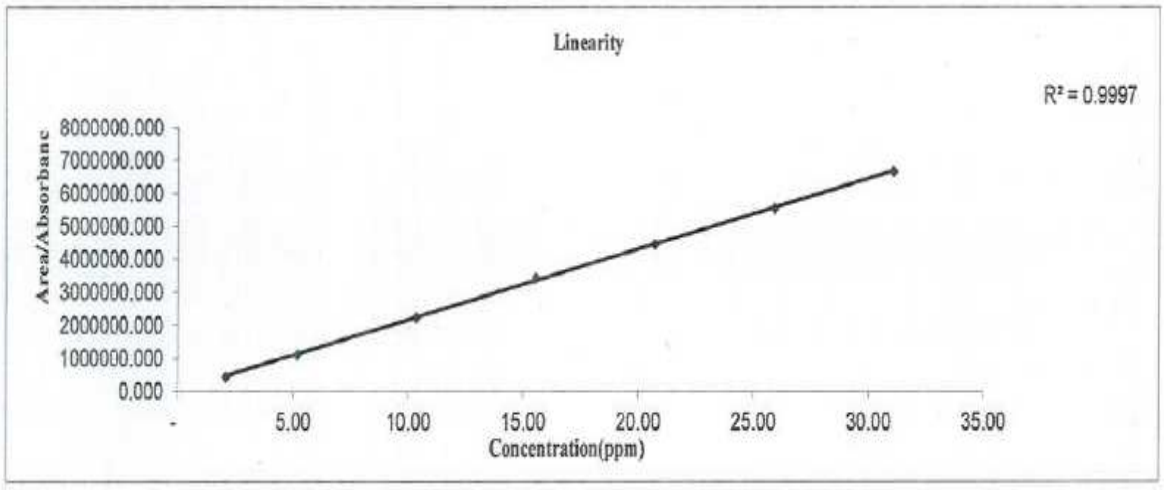
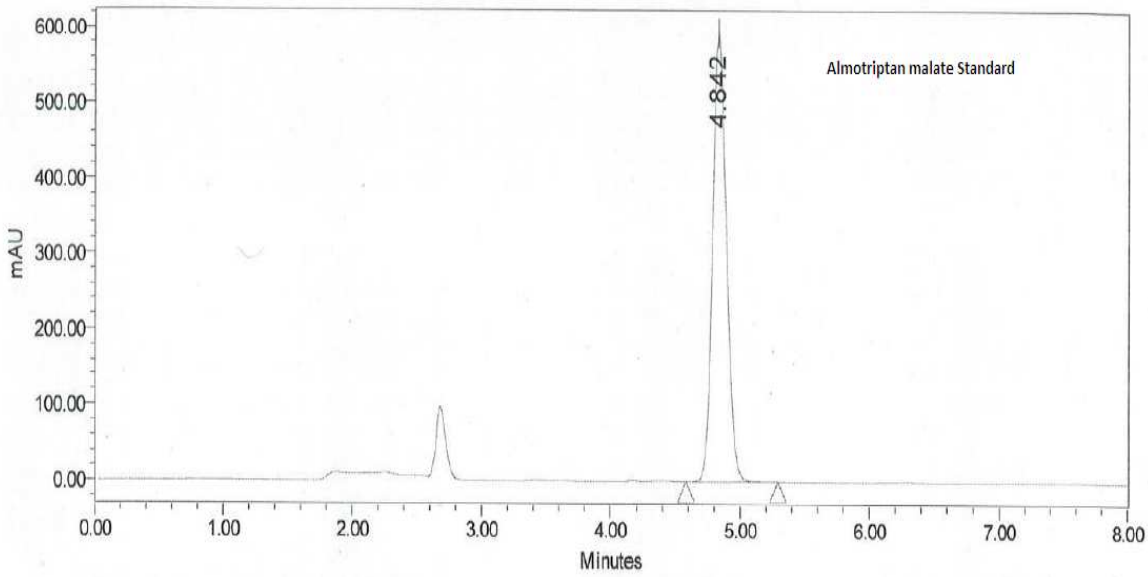


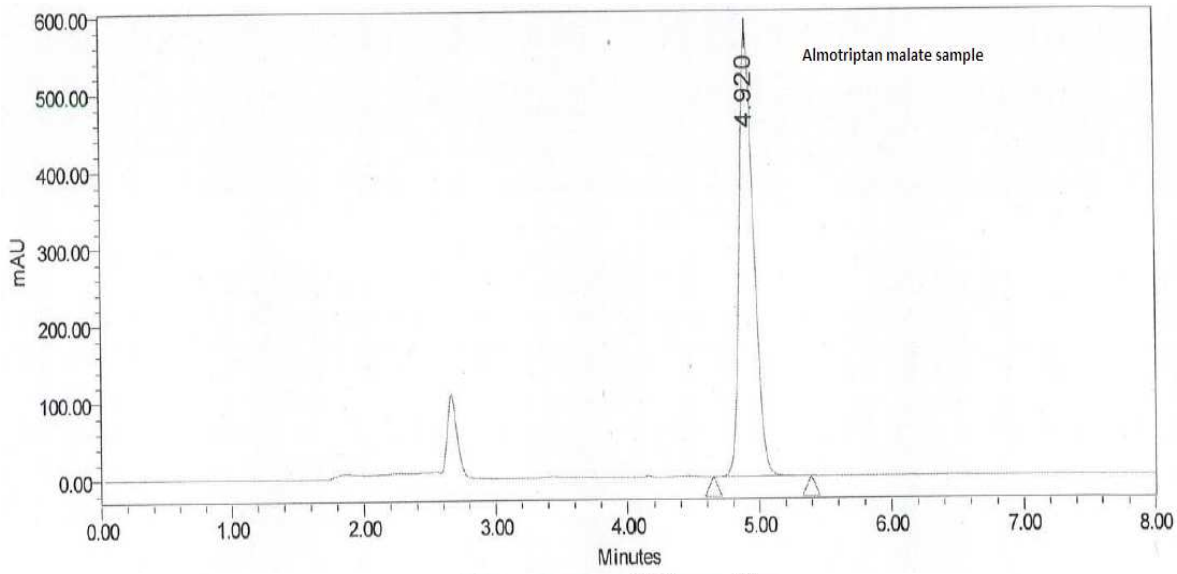
Figure 5: Typical Chromatogram of Almotriptan malate (Standard) by RP-HPLC



Component Results

	RT	Area	EP Plate Count	Symmetry Factor	Resolution
1	4.842	4430331	9756	1.07	

Figure 6: Typical Chromatogram of Almotriptan malate (Test Sample)



Component Results

	RT	Area	EP Plate Count	Symmetry Factor	Resolution
1	4.920	4415631	9521	1.08	

A simple and easily available HPLC method was developed in this study for the estimation of percentage drug release of Almotriptan malate in Oral films. The main advantages of this method are its considerably shorter run times, easy-to-use and its simplicity. All of these properties are very important in practice, particularly when a large number of samples are to be analyzed. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the oral films. The results of validation tests were, collectively, indicative for a method with a relatively wide linear range, acceptable precision and accuracy and practically reliable sensitivity. The method enables simple, selective, sensitive, and specific analysis of Almotriptan malate and can be used for routine analysis in pharmaceutical quality control within a short time.

CONCLUSION

In the present investigation the estimation of percentage drug release in Almotriptan malate oral films was successfully developed by using RP-HPLC. The proposed method was found to be simple, fast, robust, more precise and accurate under given experimental conditions. Therefore the developed method can be used for routine analysis of estimation of percentage drug release Almotriptan malate in oral film dosage forms.

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