QUANTITATIVE ASSAY OF ALMOTRIPTAN MALATE IN PURE DRUG AND PHARMACEUTICAL PREPARATIONS USING SIMPLE AND CONVENIENT VISIBLE SPECTROPHOTOMETRIC METHODS

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2. ABSTRACT

Two direct, simple and sensitive visible spectrophotometric methods (M_1&M_2) are described for the assay of almotripan malate in pure and solid dosage forms. The method M_1 involves oxidative coupling of drug with brucine in presence of sodium meta periodate and purple red colored species is formed and exhibits absorption maxima at 520nm. The method M_2 is based on the formation of yellowish brown colored species by the drug with Folin reagent and exhibits absorption maxima at 450nm. Regression analysis of Beer-Lambert plots showed good correlation in the concentration ranges (8.0-24) µg/ml for method M_1, (16-48) µg/ml for method M_2 respectively. The proposed methods are applied to commercial available tablets and the results are statistically compared with those obtained by the reported UV reference method and validated by recovery studies. The results are found satisfactory and reproducible. These methods are applied successfully for the estimation of the almotriptan malate in the presence of other ingredients that are usually present in dosage forms. These methods offer the advantages of rapidity, simplicity and sensitivity and normal cost and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents.

3. Key words: Anti-migraine, BCN-IO_4^-, Folin reagent, Nucleophillic substitution, Oxidative coupling, Statistical analysis, Triptans.

4. INTRODUCTION

Almotriptan malate (AM) (Fig.1) is a selective and potent serotonin 5-hydroxy trytamine1B/1D (5-HT 1B/1D) receptor agonist. It is chemically designated as 1[[[3-[2-(Di methyl amine) ethyl]-1H-indol-5-yl] methyl] sulfonyl] pyrrolidine ± - hydroxy butanedioate[1] (1:1). Its empirical formula is $C_{17}H_{25}N_3O_2S.C_4H_6O_5$ representing molecular weight of 469.56. It is a white to slightly yellow crystalline powder that is soluble in water and sparingly soluble in methanol. Almotriptan is available in market as conventional tablets (AXERT).The drug is absorbed well orally, with an absolute bioavailability of around 70%. The drug is used to treat severe migraine headaches and vascular headaches; acute treatment of migraine attacks with or without aura. The drug binds with high affinity to 5-HT 1D, 5-HT 1B and 5-HT 1F receptors. Because of the particular distribution of the 5-HT 1B/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. Ameliorate migraine through selective constriction of certain intracranial blood vessels, inhibition of neuro peptide release and reduced transmission in trigeminal pain pathway.

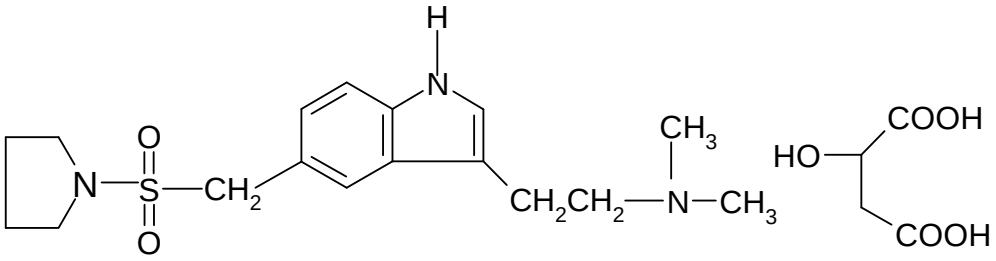


Fig. 1: Chemical structure of Almotriptan malate

In literature, several analytical methods such as HPLC[2-3], HPTLC[4], HPLC-MS/MS[5], LC-ESI-MS/MS[6], UV Spectrometric [7-8] and Fluorometric and Coloricmetric[9] have been reported for the determination of AM in biological fluids (considerable more) and formulations (less). Even though there is one visible spectrophotometric method using TCNQ reported for the determination of the drug they are tedious and less specificity and the functional groups present in the drug not fully exploited. For routine analysis, simple, rapid and cost effective visible spectrophotometric methods are required and preferred. Nevertheless, there still exists a need for development of sensitive accurate and flexible visible spectrophotometric methods for the determination of AM in pharmaceutical preparations. So the authors have made some attempts in this direction and succeeded in developing two methods based on the reaction between the drug and BCN-IO_4^- reagent [10] (M_1) or Folin reagent [11] (M_2) under specified experimental conditions.

The proposed methods for AM determination have many advantages over other analytical methods due to its rapidity, normal cost and environmental safety. Unlike HPLC, HPTLC procedures, the instrument is simple and is not costly. Economically, all the analytical reagents are inexpensive and available in any analytical laboratory. These methods can be extended for the routine quality control analysis of pharmaceutical products containing AM.

5. MATERIALS & METHODS (EXPERIMENTAL)

Apparatus and chemicals

A Milton Roy UV/Visible spectrophotometer model-1201 with 10mm matched quartz cells was used for all spectral measurements. A Systronics digital pH meter mode-361 was used for pH measurements. All the chemicals used were of analytical grade. AXERT tablets procured from Ortho Mc Nell Pharmaceuticals, USA. Aqueous solution of brucine (Loba, 0.2%, $506.7x10^{-3}$M prepared by dissolving 200mg of brucine initially in minimum amount of 0.16M sulphuric acid and then made up to 100ml with distilled water), sodium metaperiodate (BDH, 0.2%, $9.35x10^{-3}$M prepared by dissolving 200mg of sodium metaperiodate in 100ml distilled water and standardized iodometrically) and sulphuric acid (Qualigens,1.2M prepared by diluting 126ml of conc. H_2SO_4 to 100ml of distilled water initially, followed by diluting to 1000ml with distilled water) were prepared for method M_1.

Folin reagent (NQS) solution (Loba, 0.5%, $1.92x10^{-2}$M prepared by dissolving 500mg of NQS in 100 ml of distilled water), pH 8.0 buffer solution (prepared by mixing 30ml of potassium hydrogen phosphate (0.067M) and 970ml of disodium hydrogen phosphate (0.067M) and the pH of the solution was adjusted to 8.0) were prepared for method M_2.

Preparation of Standard stock solution: The standard stock solution (1mg/ml) of AM was prepared by dissolving 100mg of AM in 100 ml distilled water. The working standard solutions of AM were obtained by appropriately diluting the standard stock solution with the same solvent (M_1- 200 µg/ml & M_2- 400 µg/ml). The prepared stock solution was stored at 4° C protected from light. From this stock solution, a series of standards were freshly prepared during the analysis day.

Preparation of Sample solution: About 20 tablets were weighed to get the average tablet weight and pulverized. The powder equivalent to 100mg of AM was weighed, dispersed in 25ml of Isopropyl alcohol, sonicated for 15 minutes and filtered through Whatman filter paper No 41.The filtrate was evaporated to dryness and the residue was dissolved as under standard solution preparation.

Determination of wavelength maximum (λ_{max}):

Method M_1: The 3.0ml of working standard solution of AM (200µg/ml) was taken in 25ml calibrated tube. To this, 3.0ml brucine, 1.5ml of $NaIO_4$ solution and 2.0ml of sulphuric acid were added successively and the volume was brought up to 10ml with distilled water and kept in boiling water bath for 15min. for complete color

development .The solution was cooled to room temperature and the volume was made up to the mark with distilled water. In order to investigate the wavelength maximum, the above colored solution was scanned in the range of 400-660 nm UV-Visible spectrophotometers against a reagent blank. From the absorption spectra (Fig.2), it was concluded that 520 nm is the most appropriate wavelength for analyzing AM with suitable sensitivity.

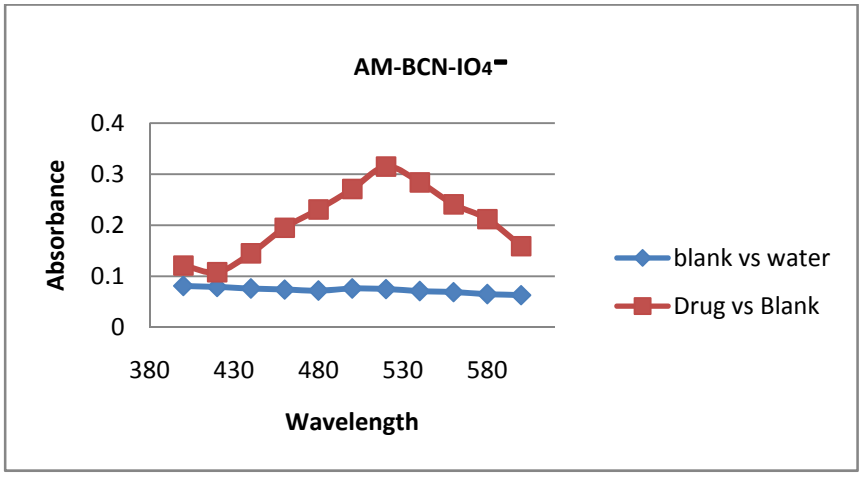


Fig.2: Absorption spectra of AM-BCN-IO_4^-

Method M_2: The 3.0 ml of working standard solution of AM (400µg/ml) was taken in 25ml standard flask. To this, 1.0ml of folin reagent ($1.092x10^{-2}$M), 5.0 ml of buffer pH 8.0 and 1.5ml of distilled water were added and kept aside for 15 min for complete color development. Then the volume was made up to 25 ml using distilled water and sonicated for 1 min. In order to investigate the wavelength maximum, the above standard stock solution was scanned in the range of 360-560nm by UV-Visible spectrophotometer. From the spectra (Fig.3), it was concluded that 450nm is the most appropriate wavelength for analyzing AM with suitable sensitivity.

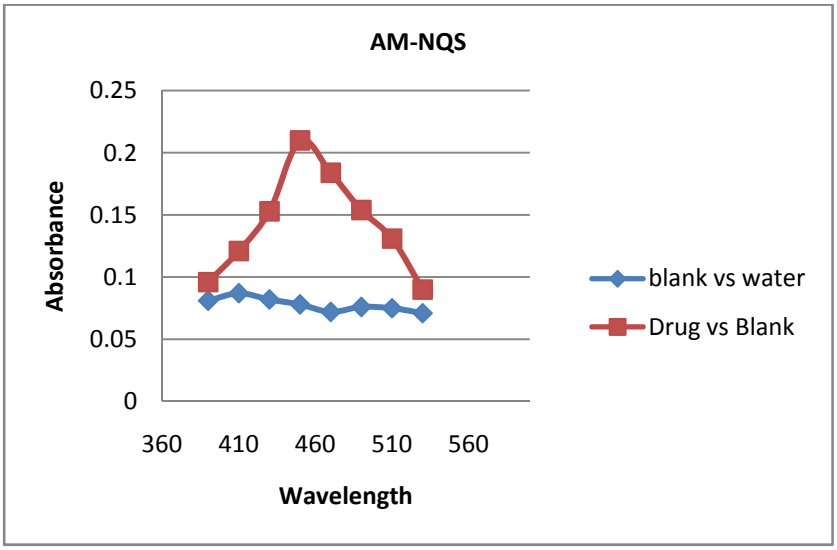
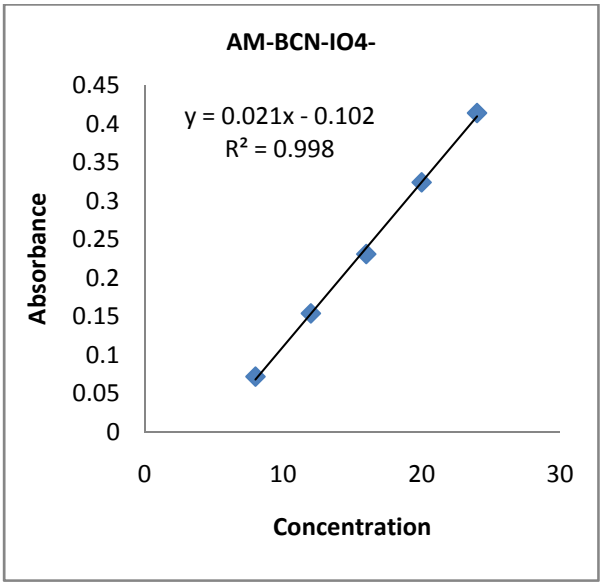


Fig.3: Absorption spectra of AM-NQS

Preparation of calibration curve: Aliquots of the standard AM solution [1.0-3.0ml, 200µg/ml (method M_1), 400µg/ml (method M_2)] were placed in a series of 25ml standard flask. Then 3.0ml brucine, 1.5ml of $NaIO_4$ solution and 2.0ml of sulphuric acid were added successively and the volume was brought up to 10ml with distilled water and kept in boiling water bath for 15min. for complete color development. The solution was cooled to room temperature and the volume was made up to the mark with distilled water (method M_1) or 1.0ml of folin reagent ($1.092x10^{-2}$M), 5.0 ml of buffer pH 8.0 and 1.5ml of distilled water were added and kept aside for 15 min for complete color development. Then the volume was made up to 25 ml using distilled water

and sonicated for 1 min. (methodM_2) The absorbance was measured at 520nm (method M_1) or 450 nm (method M_2) against a reagent blank within the stability period (5minutes to 30min). The calibration graph was constructed by plotting the drug concentration versus absorbance (Fig.4&5). The amount of drug was computed from its calibration graph.



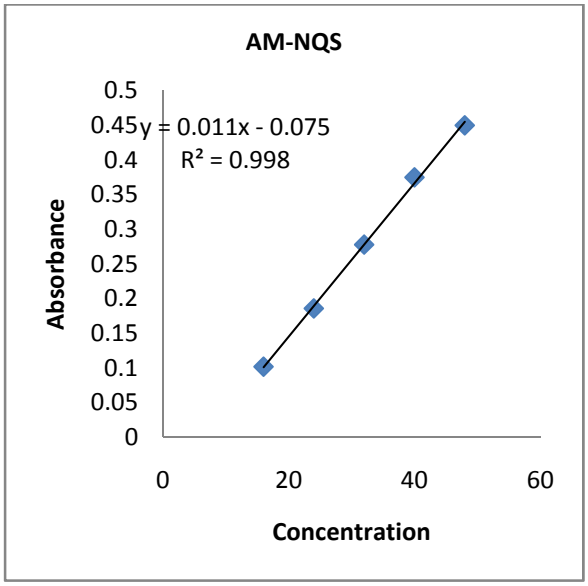


Fig.4: Beer's Law plot of AM-BCN-IO_4^- Fig.5: Beer's Law plot of AM-NQS

6. RESULTS AND DISCUSSION

In the present investigation, the presence of cyclic imino group in indole moiety of AM permits the development of visible spectrophotometric methods for its determination through the oxidative coupling reaction with BCN-IO_4^- reagent (M_1) or the nucleophillic substitution with folin reagent (M_2).

Optimum operating conditions used in the procedure were established adopting variation of one variable at a time (OVAT) method. The effect of various parameters such as time, volume and strength of reagents, the order of addition of reagents, pH buffer solutions and solvent for final dilution of the colored species were studied. The other oxidants such as Fe (III), Cr (IV), IO_3^-, and $S_2O_8^{-2}$ were tried in place of $NaIO_4$ and found to be inferior incase of method M_1. Distilled water was found to be best solvent for final dilution. Other water miscible solvents like methanol, ethanol, propan-2-ol and acetonitrile have no additional advantage in increasing the intensity of the color in both methods. The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation, (calculated from the six measurements containing 3/4th of the amount of the upper Beer's law limits), Regression characteristics like standard deviation of slope (S_b), standard deviation of intercept (S_a), standard error of estimation (S_e) and % range of error (0.05 and 0.01 confidence limits) were calculated and the results are summarized in Table-1.

Commercial formulations containing AM were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically by the t-and F-test and found not to differ significantly. As an additional demonstration of accuracy, recovery experiments were performed by adding a fixed amount of the drug to the pre analyzed formulations at three different concentration levels. MS Excel Software-2007 used for calculations and graphs. These results are summarized in Table-2.

Chemistry of colored species: In method M_1, the dimethoxy benzene nucleus of brucine is attacked by IO_4^- with the formation of o-quinone (bruciquinone) which in turn undergo nucleophillic attack on the most electron-rich position of the coupler (cyclic imino group in indole moiety of AM) to give 1-monosubstituted bruciquinone derivative(purple red colored species) . In method M_2, yellowish brown colored species (N-alkyl amino napthaquinone) was formed by replacement of the sulphonate group of the napthaquinone sulphonic acid by a secondary amino group of drug. The formation of colored species with these reagents may be assigned through above analogy as shown in Scheme (Fig.6).

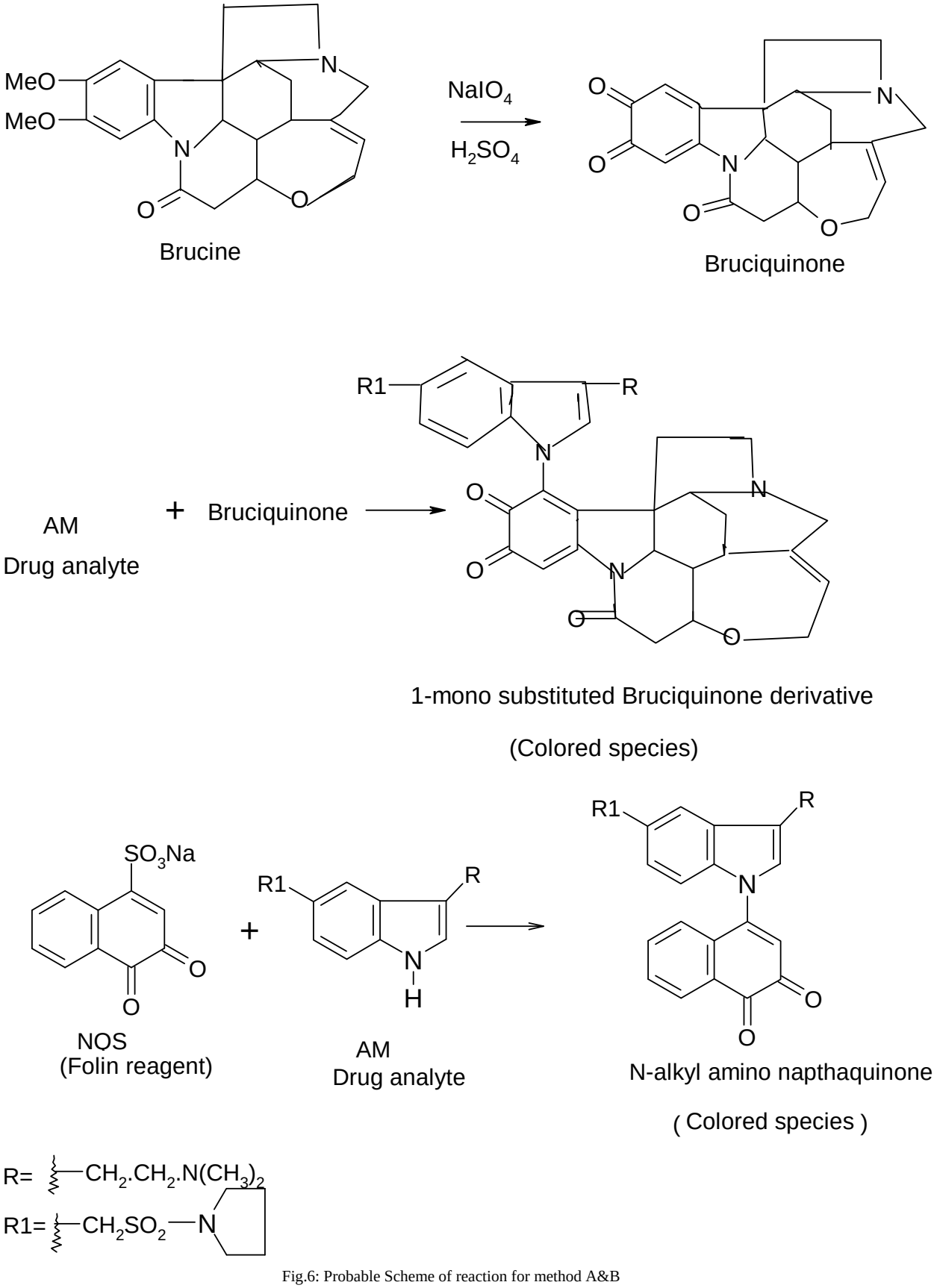


Fig.6: Probable Scheme of reaction for method A&B

7. CONCLUSION

The proposed methods applicable for the assay of drug, the advantage of wider range under Beer's law limits, validated as per ICH guide lines and possess reasonable precision, accuracy, and simple, sensitive. These methods can be extended for the routine assay of AM formulations.

8. ACKMOWLEDGEMENTS

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9. REFERENCES

[1] Maryadele JON. The Merck Index 14 th ed., Merck &Co Inc., New Jersey, 2006.
[2] Suneetha A and Syamsundar B. A validated RP-HPLC method for estimation of almotriptan maleate in pharmaceutical dosage form. Journal of the Chinese Chemical society. 57 (5A); 2010: 1067-1070.
[3] Kumar AP, Ganesh VRL, Subba Rao DV, Anil B, Venu gopal rao B, Hari Krishna VS. A validated RP-HPLC method for determination of process related impurities in almotriptan maleate API. Journal of Pharmaceutical & Biomedical Analysis. 46(4); 2008: 792-798.
[4] Suneetha A and Syamsundar B. Development and validation of HPTLC method for the estimation of almotriptan maleate in tablet dosage form. Indian Journal of Pharmaceutical Sciences. 72(5); 2010: 629-32.
[5] Ravi kumar K, Reddy BS Ch, Babu rao Ch, Chandra sekhar KB. Method development & validation of almotriptan maleate in human plasma by HPLC-tandem mass spectrophotometry: Application to pharmacokinetic study. Scientia Pharmaceutica http//dx. Doi. Org/10.3797/2010 Sci. Pharm./1112-01.
[6] Nageshwar rao R, Guruprasad K, Gangu naidu Ch, Raju B, Srinivas R. LC_ESI-MS/MS determination of in vivo metabolites of almotriptan maleate in rat plasma, urine and feces: application to pharmokinetics. Journal of Chromatography B Analytical Technol Biomedical Life Sciences. 891-892; 2012: 44-51.
[7] Suneetha A and Syamsundar B. New simple UV spectrophotometric method for estimation of almotriptan maleate in bulk and pharmaceutical dosage form. Asian journal of Research in Chemistry. 3(1); 2010: 142-144.
[8] Suneetha A, Kathirvel S, Prathyusha A and Anusha K. Spectrophotometric estimation of almotriptan maleate in bulk and pharmaceutical formulations by multivariateTechnique. Scientificipca. Org/paper/2011/09/13/201109131246120A. docx.
[9] Ramzia I. EI-Bagary, Mohammed NG, Nasr HA. Fluorimetric and colorimetric methods for the determination of some anti -migraine drugs. Journal of Chemical and Pharmaceutical Research. 3(4); 2011: 304-314.
[10] 10.D.L Massart, B.G.M Vandegingtc, S.M Perming, Y. Michotte and L. Kaufman, Chemometrics, A text book, Elsevier, Amsterdam, 283 (1988).
[11] D.H Rosenblatt, P. Hlinka, J. Epstein, Anal. Chem., 27, 1290 (1955).

TABLE 1: OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF PROPOSED METHODS

Parameter	**Method M_1**	**Method M_2**
λ_{max}(nm)	520	450
Beer's law limit(µg/ml)	8-24	16-48
Sandell's sensitivity (µg/cm^2/0.001 abs. unit)	0.002770563	0.004604317
Molar absorptivity (Litre/mole/cm)	169481.8125	101982.5625
Correlation coefficient	0.998	0.998
Regression equation (Y)*		
Intercept (a)	-0.102	-0.075
Slope(b)	0.021	0.011
%RSD	1.86	1.05
% Range of errors(95% Confidence limits)		
0.05 significance level	1.95	1.10
0.01 significance level	3.06	1.73

*Y = a + b x, where Y is the absorbance and x is the concentration of AM in µg/ml

TABLE-2 ANALYSIS OF ALMOTRIPTAN MALATE IN PHARMACEUTICAL FORMULATIONS BY PROPOSED AND REFERENCE METHODS.

Method	*Formulations	Labeled Amount (mg)	Found by Proposed Methods			Found by Reference Method ± SD	#% Recovery by Proposed Method ± SD
			**Amount found ± SD	t	F		
M_1	AXERT TABLET	6.25	6.20 ± 0.034	0.896	1.01	6.21± 0.034	99.23 ± 0.54
		12.5	12.39 ± 0.072	0.773	4.42	12.44± 0.15	99.17 ± 0.574
M_2	AXERT TABLET	6.25	6.19 ± 0.051	2.27	2.23	6.21± 0.034	98.96 ± 0.808
		12.5	12.24 ± 0.310	2.14	4.23	12.44± 0.15	97.96 ± 2.48

* AXERT tablets of Ortho Mc Nell Pharmaceuticals, USA

**Average ± Standard deviation of six determinations, the t- and f-values refer to comparison of the proposed method with reference method. (UV). Theoretical values at 95% confidence limits t =2.57 and f = 5.05.

Recovery of 10mg added to the pre-analyzed sample (average of three determinations).

Reference method (reported UV method) using methanol (λ_{max}=227 nm).