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Development Of New Visible Spectrophotometric Methods For Quantitative Determination Of Almotriptan Malate As An Active Pharmaceutical Ingredient In Formulations

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Abstract

Purpose: The aim of the investigation was to see the simple and sensitive visible spectrophotometric methods for the determination of the almotriptan malate in bulk and tablet dosage forms. **Methods**: Two simple, sensitive and cost effective visible spectrophotometric methods (M_1-M_2) were developed for the estimation of almotriptan malate in bulk and dosage forms. The first method (M_1) is based on the formation of blue reduced product by treating drug with Folin Ciocalteu (FC) reagent in the presence of sodium carbonate solution with an absorption maximum of 770nm. The second method (M_2) is based on the complex formation product by drug with 1, 10-phenanthroline in the presence of Fe (III) as an oxidant in phosphoric acid medium with an absorption maximum of 510nm. **Results**: Beer's law obeyed in the concentration range of 4-12µg/ml and 1-5 µg/ml for methodM_1 and M_2 respectively. No interference was observed from the usually existing additives in pharmaceutical formulations and the applicability of the methods was examined by analyzing AXERT tablets containing AM. **Conclusion**: The reported methods for its assay involve sophisticated equipment, which are very costly and pose problems of maintenance. To overcome these problems, the use of visible spectrophotometric technique is justifiable. The statistical data proved the accuracy, reproducibility and the precision of the proposed methods.

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Key words:
Anti migraine, Assay, FC reagent, o- phenanthroline, Spectrophotometer, Tablets.

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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.

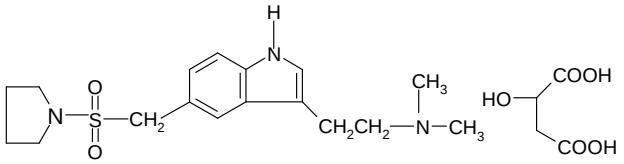


Fig. 1: Chemical structure of Almotriptan malate

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 Colorimetric [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 and quality control analysis. So the authors have made some attempts in this direction and succeeded in developing two methods based on the reaction between the drug and FC reagent [10-11] (M_1) or Fe(III)-1,10-phenanthroline [12] (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.

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. Fe (III) solution (Wilson labs, 0.05%, $3.32x10^{-3}$M prepared by dissolving 50mg anhydrous ferric chloride in 100ml of distilled water), PHEN solution (Merck, 0.2%, $1.10x10^{-2}$M prepared by dissolving 200g of o-phenanthroline in 100ml of distilled water with warming), o-phosphoric acid (Qualigens, $2.0x10^{-2}$M prepared by diluting 1.27ml of o-phosphoric acid to 100ml with distilled water. Ten ml of this stock solution was diluted to 100ml with distilled water) were prepared for method M_2 and commercial available FC reagent (Loba, 2N),

10%Na_2CO_3 (BDH, 9.43x10^{-1}M) solution was used for method M_1.

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- 100 µg/ml & M_2- 50 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: 3.0ml of Standard AM solution was transferred into 25ml calibrated tube. To this 2.5ml of FC (2N) reagent was added. After 3 minutes 7ml of 10% Na_2CO_3 was added. The solutions were mixed and kept at room temperature for 30minutes for complete color development and diluted 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 770nm is the most appropriate wavelength for analyzing AM with suitable sensitivity.

Method M_2: 2.5ml of Standard AM solution was transferred into 25ml calibrated tube. Then 1.5ml of $Fecl_3$ 3.32x10^{-3}M solution and 2.0ml of 1,10PHEN (1.10x10^{-2}M) solution were added successively and total volume in tube was made to 10.0ml with distilled water and kept in boiling water bath for 30 minutes. After cooling to room temperature, 2.0ml of o-phosphoric acid was added and the total volume made up to 25ml 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.3), it was concluded that 510nm is the most appropriate wavelength for analyzing AM with suitable sensitivity.

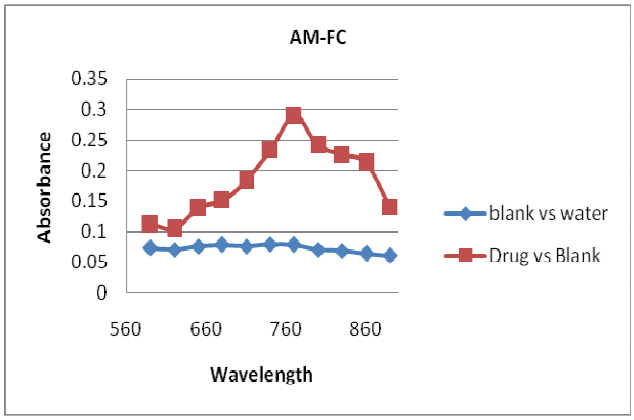


Fig.2: Absorption spectra of AM-FC system

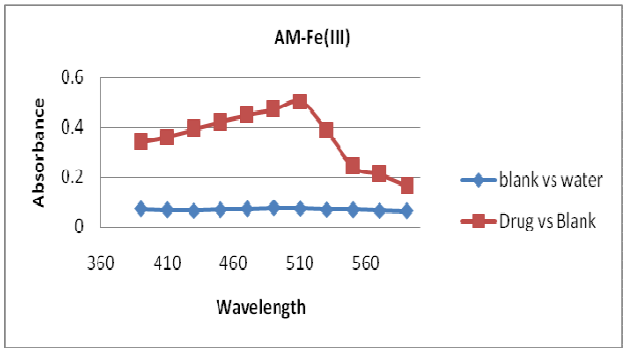


Fig.3: Absorption spectra of AM-Fe(III)-1,10-PTL system

Preparation of calibration curve:

Method M_1: Aliquots of Standard AM solution (1.0-3.0ml, 100µg/ml) were transferred into a series of 25ml calibrated tubes. To each tube 2.5ml of FC (2N) reagent was added. After 3 minutes 7.0ml of 9.43x10^{-1}M Na_2CO_3 was added. The solutions were mixed and kept at room temperature for 30minutes for complete color development and then diluted to the mark with distilled water. The absorbance was

measured at 770nm against a reagent blank prepared simultaneously. The amount of drug was computed from its calibration graph (Fig.4).

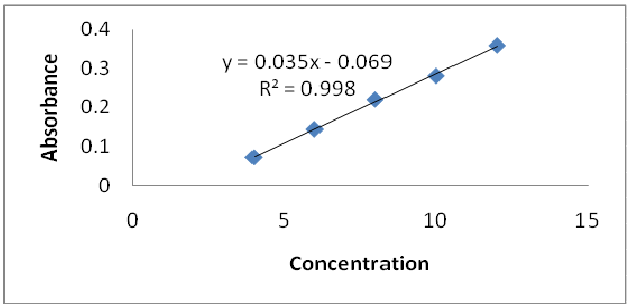


Fig.4. Beer's Law Plot of AM-FC system

Method M_2:

Aliquots of Standard AM solution (0.5-2.5ml, 50 µg/ml) were transferred into a series of 25ml calibrated tubes. Then 1.5ml of $Fecl_3$ ($3.32x10^{-3}$M) solution and 2.0ml of 1,10-PHEN ($1.10x10^{-2}$M) solution were added successively and total volume in tube was made to 10.0ml with distilled water and kept in boiling water bath for 30minutes. After cooling to room temperature, 2.0ml of o-phosphoric acid was added and the total volume made up to 25ml with distilled water. The absorbances of the colored complex solution were measured after 5 minutes before 60minutes at 510nm against the reagent blank prepared similarly. The content of the drug computed from the appropriate calibration graph (fig.5).

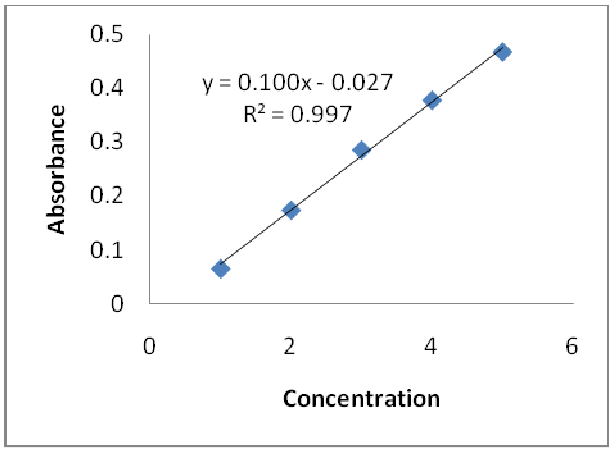


Fig.5. Beer's Law Plot of AM-Fe(III)-1, 10-PTL system

RESULTS AND DISCUSSION

Optimum operating conditions used in the procedure were established by 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 and solvent for final dilution of the colored species were studied. In method M_1, Na_2CO_3 preferred among other bases like NaOH or Pyridine as they were found to be inferior. 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 $3/4^{th}$ 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.

Table 1: Optical characteristics, precision and accuracy of the proposed methods

Parameters	Method M_1	Method M_2
λ_{max}(nm)	770	510
Beer's law limit (µg/ml)	4- 12	1-5
Sandell's sensitivity (µg/cm²/0.001 abs. unit)	0.001447964	0.000420536
Molar absorptivity (Litre/mole/cm)	324289.875	1116574.55
Regression equation (Y) *= a +b x		
Intercept (a)	-0.069	-0.027
Slope(b)	0.035	0.100
%RSD	0.9545	1.217
% Range of errors(95% Confidence limits) 0.05 significance level 0.01 significance level	 1.002 1.57	 1.277 2.03

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

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.

Table-2: Analysis of AM in pharmaceutical formulations

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	Tablet-1	6.25	6.154±0.073	1.02	4.67	6.21±0.034	98.46±1.17
	Tablet-2	12.5	12.30±0.102	1.24	2.18	12.44±0.15	98.40±0.818
M_2	Tablet-1	6.25	6.24±0.0165	1.01	4.196	6.21±0.034	99.77 ± 0.26
	Tablet-2	12.5	12.44 ± 0.069	0.002	4.73	12.44±0.15	99.53 ± 0.55

* Tablet- 1 and Tablet-2: 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 UV reference method. 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}=227nm).

Chemistry of color species:

Method M_1: The color formation by Folin- Ciocalteu reagent with AM may be explained basing on the analogy with the reports of earlier workers. The mixed acids in the FC preparation are the final chromogen and involve the following chemical species.

$3H_2O.P_2O_5.13WO_3.5MoO_3.10H_2O$

And

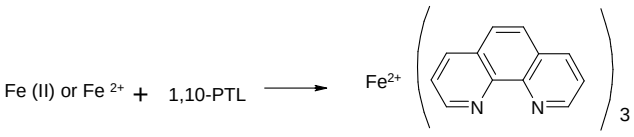
$3H_2O.P_2O_5.14WO_3.4MoO_3.10H_2O$

AM probably effects a reduction of the 1,2 or 3 oxygen atoms from tungstate and /or molybdate in FC preparation (phosphomolybdo tungstate), thereby producing one or more several reduced species which have characteristic intense blue color.

Method M_2: AM drug exhibits reducing property due to the presence of functional moieties vulnerable to oxidation selectively with oxidizing agents such as Fe (III) under controlled experimental conditions. When treated with known excess of oxidant, AM undergoes oxidation, giving products of oxidation (inclusive of reduced form of oxidant, Fe II from Fe III) besides unreacted oxidant. It is possible to estimate the drug content colorimetrically, which is equivalent to either reacted oxidant or reduced form of oxidant formed. The reduced form of Fe III (i.e. Fe II) has a tendency to give a colored complex on treatment with 1, 10-PTL.

AM + Fe (III) → oxidation products + Fe (II) + un reacted Fe (III)

Fe (III) + o-phosphoric acid → Complex (un reactive)



Fe(II)-1,10-PTL Complex

Scheme for Method M_2

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.

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