Visible Spectrophotometric Analysis of Almotriptan Malate In Bulk And Formulations

U. Viplava Prasad, M. Syam Bab, B. Kalyana Ramu

ABSTRACT: Two simple and sensitive visible spectrophotometric methods are described for the determination of almotriptan malate in bulk and pharmaceutical preparations based on the formation of colored molecular complex with sodium nitroprusside in presence of hydroxyl amine under alkaline conditions (M_1) or SNP-acetaldehyde reagent (M_2) and exhibiting λ_{max} at 720 nm or 550nm respectively. The Regression analysis of Beer's Law plot showed good correlation in a general concentration range of 8-40µg/ml or 4.0-20µg/ml with correlation coefficient (r= 0.998) for methods M_1 and M_2 respectively. The proposed methods are validated with respect to accuracy, precision, linearity and limit of detection. The suggested procedures are successfully applied to the determination of the drug in pharmaceutical preparation, with high percentage of recovery, good accuracy and precision. The results of analysis have been validated statistically by repeatability and recovery studies. The results are found satisfactory and reproducible. These methods are applied successfully for the estimation of almotriptan in tablet form without the interference of excipients.

KEY WORDS: Almotriptan, Analysis, ACD, Beer's Law, HA, SNP, Tablets

1. 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.

In literature, several analytical methods such as HPLC (Suneetha A etal 2010, Kumar et al 2008) [2],[3], HPTLC (Suneetha A etal 2010) [4], HPLC-MS/MS (Ravi kumar etal 2010) [5], LC-ESI-MS/MS (Nageswera Rao etal 2012) [6], UV Spectrometric (Suneetha A etal 2010)[7],[8] and Fluorometric and Coloricmetric (Razia etal 2011)[9] have been reported for the determination of AM in biological fluids (considerable more) and formulations (less). Even though there is only 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. The main purpose of the present study was to establish a relatively simple, sensitive and validated visible spectrophotometric methods for the determination of AM in pure form and in pharmaceutical dosage forms, since most of the previous methods have been found to be relatively complicated and tedious.

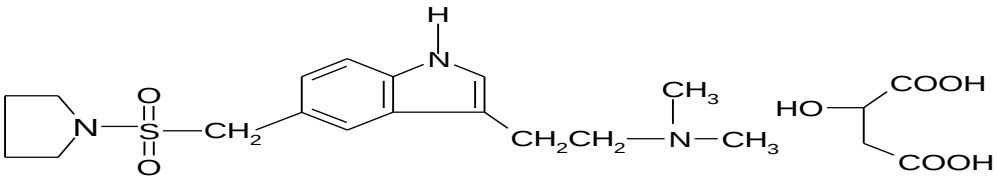

Fig. 1: Chemical structure of Almotriptan malate

The proposed methods are based on the formation of molecular complex of drug with sodium nitroprusside in presence of hydroxylamine hydrochloride under alkaline conditions [10], [11] or SNP-ACD reagent. These methods can be extended for the routine assay of AM formulations.

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Materials & methods (experimental)

5.01. 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-362 was used for pH measurements. All the chemicals used were of analytical grade. AXERT tablets procured from Ortho Mc Nell Pharmaceuticals, USA. All the chemicals used were of analytical grade. SNP (Sd Fine, 0.4%, $1.34x10^{-2}$M, solution prepared by dissolving 400mg of SNP in 100ml distilled water), Hydroxylamine hydrochloride (Loba, 0.4%,$5.75x10^{-2}$M solution prepared by dissolving 400mg of hydroxylamine hydrochloride in 100ml of distilled water), sodium carbonate (Loba,10%,$9.43x10^{-1}$M solution prepared by dissolving 10g of sodium carbonate in 100ml of distilled water), Aqueous solutions of sodium nitroprusside (SNP, E.Merck, 1.0%, $3.35x10^{-2}$M), acetaldehyde (10%), phosphate buffer of pH 8.0(prepared by mixing 30ml of 0.067M potassium hydrogen phosphate and 970ml of 0.067Mdisodium hydrogen phosphate and pH adjusted to 8.0) were prepared for method M_1 and M_2.

Preparation of Standard drug solution: Standard drug solution of AM was prepared by dissolving 20mg of it in 4ml of 0.1M NaOH, followed by dilution to 100ml with distilled water to obtain 200µg/ml solution for both methods.

Preparation of Sample solution: About 20 tablets were pulverized and the powder equivalent to 50mg of AM was weighed and treated with 4x15ml portions of chloroform. The chloroform extract was diluted to 100ml with chloroform. Then 40ml of it was extracted with 3x5ml portions of 0.02M sodium hydroxide and the combined aqueous layers were taken in a 100ml volumetric flask and then diluted to 100ml with distilled water to get 200µg/m for both methods.

Recommended procedure/Assay:

Method M_1: Aliquots of standard AM solution (1.0ml-5.0ml, 200µg/ml) were transferred into a series of 25ml calibrated tubes and the volume in each tube was brought to 5.0ml with distilled eater. One ml each of ($1.324x10^{-2}$M) SNP and ($5.75x10^{-2}$M) hydroxylamine hydrochloride solutions were successively added to each test tube and shaken for 2 minutes. Then 1.0ml of ($9.43x10^{-1}$M) Na_2CO_3 solution was added and further shaken for 15 minutes. The contents were diluted to the mark with distilled water and the absorbencies were measured at 720nm against a reagent blank within the stability period (immediate-120 min). The amount of AM in the sample solution was computed from its calibration graph (Fig.2 showing Beer's Law plot).

Method M_2: Aliquots of standard AM drug solution [0.5-2.5ml, 200µg/ml] were delivered into a series of 25ml calibrated tubes containing 15ml of buffer pH 8.0. Then 1.0ml each of SNP solution and acetaldehyde were added successively and shaken for 2 minutes and kept aside for 15 minutes at room temperature and made up to the mark with distilled water. The purple colored species was obtained and it was stable for 1 hour. The absorbance of the colored species was measured at 550nm against the reagent blank. The amount of AM was computed from its calibration curve (Fig.3 showing Beer's law plot).

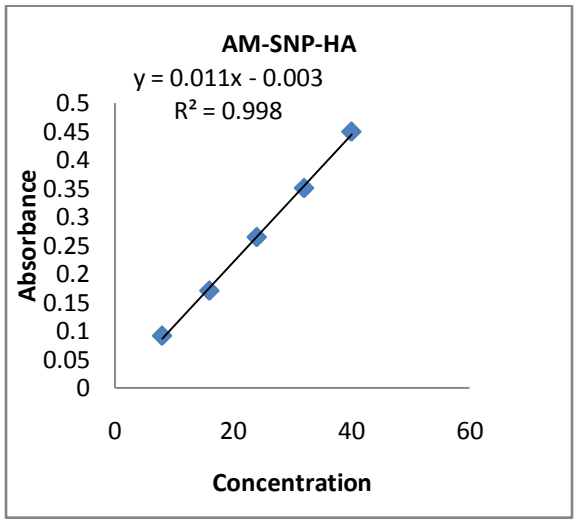


Fig.2: Beer's Law plot of AM-SNP-HA

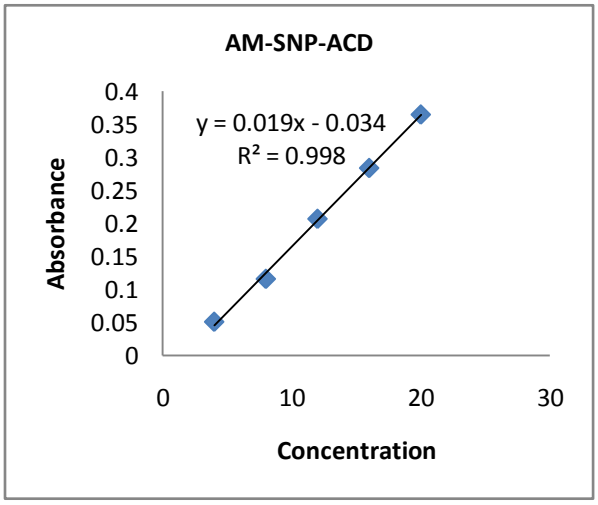


Fig.3: Beer's Law plot of AM-SNP-ACD

6. Result and discussions

In developing these methods, systematic studies of the effects of various parameters were undertaken by varying one parameter at a time and controlling all others fixed. The effect of various parameters such as time, temperature, volume and strength of SNP, NH_2OH, Na_2CO_3, ACD reagents, PH buffer solution and order of addition of reagents on color development and solvent for final dilution of the colored species were studied and the optimum conditions were established. Other water miscible solvents like methanol, ethanol, propan-2-ol and acetonitrile were found to provide no additional advantage. 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 are shown in Table-1.

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

Parameters	Method M_1	Method M_2
λ_{max}(nm)	720	550
Beer's law limit (µg/ml)	8-40	4-20
Sandell's sensitivity (µg/cm^2/0.001 abs. unit)	0.003622642	0.002318841
Molar absorptivity (Litre/mole/cm)	129618.125	202497.75
Regression equation (Y) *= a +b x		
Intercept (a)	-0.003	-0.034
Slope(b)	0.011	0.019
%RSD	1.20	1.14
% Range of errors(95% Confidence limits)	1.26	1.19
0.05 significance level	1.98	1.88
0.01 significance level		

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

Tablets containing AM were successfully analyzed by the proposed methods. The values obtained by the proposed and reference method (reported UV method in methanol, λ_{max} 227nm) 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. These results are summarized in Table-2. The ingredients usually present in formulations of AM did not interfere with the proposed analytical methods.

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	6.23±0.046	1.16	1.85	6.21±0.034	99.69±0.74
	Tablet-2	12.5	12.54±0.09	0.71	2.69	12.44±0.15	100.31±0.74
M_2	Tablet-1	6.25	6.19±0.058	1.04	2.89	6.21±0.034	99.09 ± 0.92
	Tablet-2	12.5	12.43 ± 0.15	0.59	1.07	12.44±0.15	99.42 ± 1.25

* Tablet- 1 and Tablet-2: AXERT tablets of Ortho Mc Nell Pharmaceuticals, USA

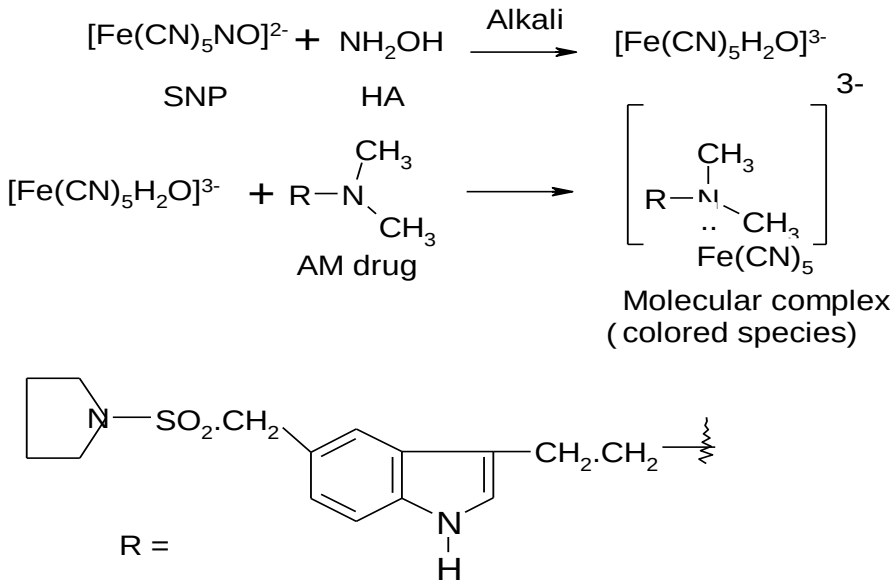
**Average ± Standard deviation of six determinations, the t- and f-values refer to comparison of the proposed method with UV reference method. Theoretical values at 95% confidence limits t =2.57 and F = 5.05.

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

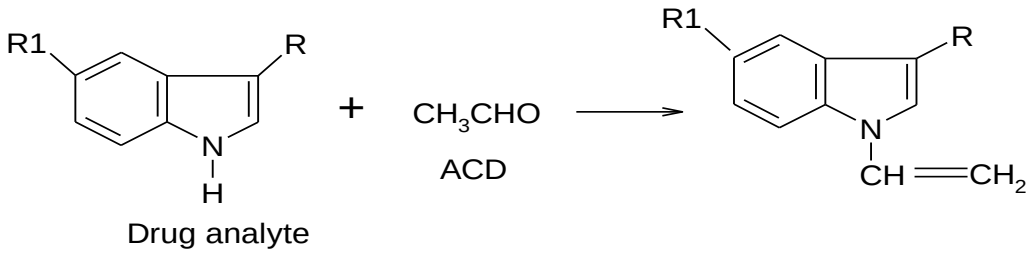
Reference method (reported UV method) using methanol (λ_{max}=227nm).

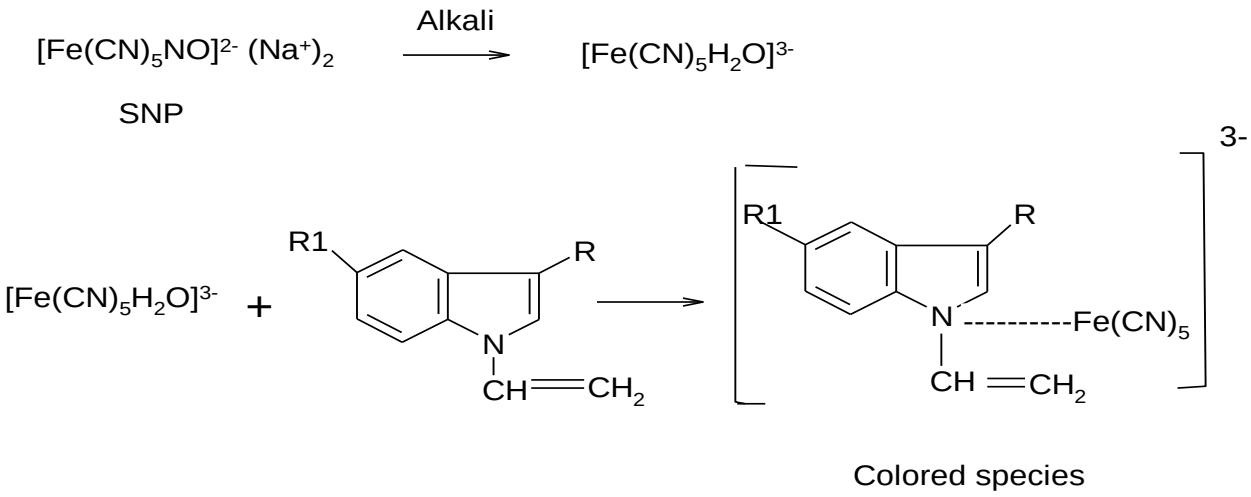
Chemistry of colored species: Method M_1: In the present investigation, AM drug functions as a donor due to the presence of aliphatic tertiary nitrogen present in side chain in indole portion. Sodium nitroprusside in the presence of hydroxylamine and alkali exists as aquoferrocyanide $[Fe(CN)_5H_2O]^{3-}$. In a general way it may be expected that the electron transfer depends upon the extent of delocalization of the donor and acceptor metal orbitals of the intervening ligands. From this stand point, ligands such as water and ammonia, which contain single bonds, are expected to be much less effective in conducting electrons between metal ions than unsaturated ligands such as CN^- whose complexes are characterized by high degree of covalency and electron delocalization.

Method M_2: Cullies and Waddington [12] found that many secondary but not primary or tertiary amines react with sodium nitroprusside and acetaldehyde under mild alkaline conditions. Wolfe and Swine hart [13] have reported the formation of $[Fe(CN)_5H_2O]^{3-}$ in aqueous solution of sodium nitroprusside. The proposed method exploit structural features cyclic imino group in indole which behaves like secondary amine of the AM molecule. The nature of colored species formation with sodium nitroprusside-acetaldehyde reagent is initial N-alkyl vinyl amine formation with acetaldehyde then followed by formation of colored inner molecular complex with sodium nitroprusside has been assumed in the scheme. Based on the analogy, the probable sequence of reactions is presented in scheme (Fig.4)



Scheme for method M_1





Where R= $-CH_2.CH_2.N(CH_3)_2$

R1= $-CH_2.SO_2-N$

7. Conclusion

The reagents utilized in the proposed methods are normal cost and readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The proposed analytical method is validated as per ICH guide lines and possess reasonable precision, accuracy, simple, sensitive and can be used as alternative methods to the reported ones for the routine determination of AM depending on the need and situation.

8. Acknowledgements

The authors (MS Bab & B.K. Ramu) are very much thankful to University Grants Commission, New Delhi for providing financial assistance under the Teacher Fellow Ship.

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