Development of New Visible Spectrophotometric Methods for Quantitative Determination of Almotriptan Malate Using Quinones as Chromogenic Reagents

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Abstract: 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 radical anion with the involvement of basic nitrogen in drug (free base form) (*n*-donor) and chloranilic acid (DHQ) (π-acceptor). The purple colored product exhibits absorption maximum at 544 nm in methanol. The second method (M_2) is based on the interaction of *N*-alkyl vinyl amine formed from the condensation of the free secondary amine group in the drug and acetaldehyde with *p*-chloranil (TQ) to give vinyl amino substituted quinone. The blue colored product exhibits an absorption maximum at 660 nm in dioxane. Beer's law obeyed in the concentration range of 25-125 µg/mL and 20 - 60 µg/mL for method M_1 and M_2 respectively. Commercially available tablets were analyzed; the results obtained by the proposed methods were in good agreement with the labeled amounts. These methods offer the advantages of rapidity, simplicity, sensitivity and normal cost and can be easily applied to resource-poor settings without the need for expensive instrumentation and reagents.

Introduction

Almotriptan malate (AM) (Figure 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]-1*H*-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.
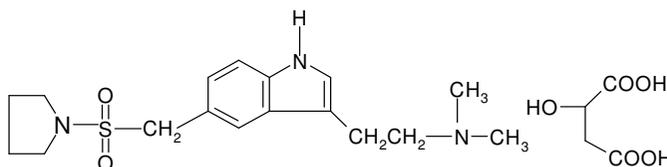
Figure 1. Chemical structure of almotriptan malate

In literature, several analytical methods such as HPLC[2-3], HPTLC[4], LC-ESI-MS/MS[5], UV spectrometric[6,7] and fluorometric and colorimetric[8] have been reported for the determination of AM in biological fluids (considerable more) and formulations (less).

The main purpose of the present study was to establish a relatively simple, sensitive, validated and inexpensive visible spectrophotometric methods for the determination of AM in pure form and in pharmaceutical dosage forms, since most of the previous methods involve sophisticated equipments which are costly and pose problems of maintenance.

A direct chemical analysis based on the reactivity of the intact molecule without cleavage is not frequently encountered. The methods that are based on the charge-transfer complexation are usually rapid and simple to perform π- acceptors (quinones such as 2, 3, 5, 6,-tetrachloro-*p*-benzoquinone) (TQ), DHQ (2,5-dihydroxy 3,6-dichloro-1,4-benzoquinone, chloranilic acid) are known to yield charge-transfer complexes with a variety of electron donors. The present work describes an improved direct simple two analytical procedures that can be applied to quality control laboratories for the analysis of pharmaceutical products containing AM.

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.

Experimental

A Milton roy UV/Visible spectrophotometer model-1201 with 10 mm matched quartz cells was used for all spectral measurements. All the chemicals used were of analytical grade. AXERT tablets procured from Ortho Mc Nell Pharmaceuticals, USA.

Chloranilic acid solution(DHQ, Sd-Fine, 0.1%, 4.785×10^{-3} M prepared by dissoloving 100 mg of chloranilic acid in 20 mL isopropanol initially followed by dilution with methanol to 100 mL), Chloranil (TQ, BDH, 0.1%, 4.067×10^{-3} M prepared by dissolving 100 mg of chloranil in 100 mL of 1,4-dioxane) were prepared and acetaldehyde used directly.

Preparation of standard and sample drug stock solution

One mg mL^{-1} stock solution of AM (in free base form) was prepared by dissolving an accurately weighted quantity of AM (pure or tablet powder) equivalent to 100 mg of it in chloroform and treated with 5 mL of 10% Na_2CO_3 solution, followed by extraction with chloroform (3x15 mL) and dilution to 100 mL with the same solvent. The chloroform extract (50 mL) was evaporated to dryness and the residue was dissolved in 100 mL of methanol to get 500 µg/mL for method M_1 and the other chloroform extract (50 mL) was evaporated to dryness and the residue was dissolved initially in 5 mL DMF and the volume was brought to 50 mL with1, 4-dioxane for method M_2. The working standard solution of AM (200 µg/mL for method M_2) was obtained by appropriately diluting the standard stock solution by using the same solvent.

Recommended procedure/Assay

Method M_1

Aliquots of standard free base AM drug solution (0.5-2.5 mL, 500 µg/mL) in chloroform were delivered into 10 mL graduated tubes. 2.0 mL of (4.785x10^{-3} M) CA in methanol was added and kept aside for 5 minutes. Then the volumes of the contents were made up to the mark with methanol. The absorbance was measured (Figure 2) against a reagent blank at 544 nm within the stability period (30 min.). The amount of the drug was computed from the appropriate calibration graph (Figure 4).
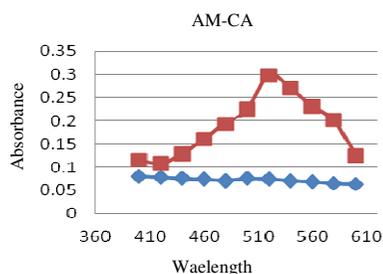
Figure 2. Absorption spectra of AM-CA
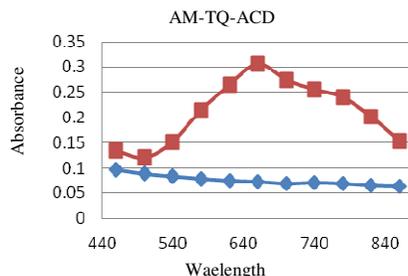
Figure 3. Absorption spectra of AM-TQ-ACD
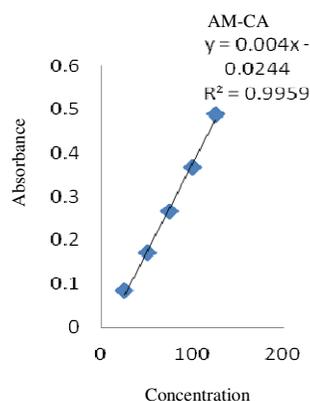
Figure 4. Beer's Law plot of AM-CA (M_1)
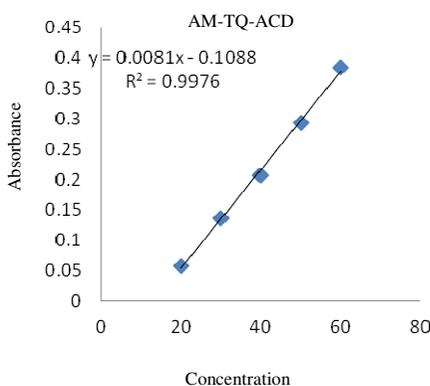
Figure 5. Beer's Law plot of AM-TQ-ACD (M_2)

Method M₂

Aliquots of working standard AM drug solution (200 µg/mL) such as 1.0, 1.5, 2.0, 2.5 mL and 3.0 mL were taken separately in a series of 10 mL calibrated tubes. Then 0.5 mL of acetaldehyde and 1 mL TQ (4.067x10⁻³ M) were added successively and shaken for 2 minutes and kept aside for 15 minutes at room temperature and made up to the mark with 1, 4-dioxane and sonicated for 1 min. The violet colored species was obtained and it was stable for 1 h. The absorbance of the colored species was measured at 660 nm against the reagent blank (Figure 3). The calibration graph (Figure 5) was constructed by plotting the drug concentration versus absorbance.

Results and Discussion

In developing these methods, systematic study 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, volume and strength of CA, TQ, acetaldehyde, stability of colored species and solvent for final dilution of the colored species were studied and the optimum conditions were established. The solvent for final dilution tried with different solvents such as acetinitrile, tetrahydrofuran, ethylene glycol, dimethyl ether, methanol, 1, 4-dioxane. Among these, methanol and 1, 4-dioxane was found to be superior for final dilution for methods M_1 & M_2 respectively. It enhances not only the absorbance but also insert reproducible values for colored species. 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) were calculated and the results are summarized in Table 1. 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	544	660
Beer's law limit, µg/mL	25-125	20-60
Sandell's sensitivity, µg/cm²/0.001 abs. unit	0.028079371	0.019323671
Molar absorptivity (Litre/mole/cm)	16722.5968	24299.73
Regression equation *Y= a +b x		
Intercept (a)	-0.024	-0.108
Slope(b)	0.004	0.008
%RSD	1.03	1.56
% Range of errors(95% Confidence limits)		
0.05 significance level	1.08	1.64
0.01 significance level	1.695	2.57

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. 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		
M₁	Tablet-1	6.25	6.23 ±0.024	1.58	1.92	6.21±0.034	99.62±0.39
	Tablet-2	12.5	12.43 ±0.78	0.15	3.71	12.44±0.15	99.41±0.63
M₂	Tablet-1	6.25	6.19±0.046	2.66	1.83	6.21±0.034	99.02 ± 0.73
	Tablet-2	12.5	12.35±0.079	1.41	3.66	12.44±0.15	98.77 ± 0.63

*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 10 mg added to the pre analyzed sample (average of three determinations). Reference method (reported UV method) using methanol (λ_{max}=227 nm)*

Chemistry of colored species:

Method M₁

The interactions of any of the investigated compounds with poly halo and poly cyano quinone π-acceptors in non polar solvents was found to produce colored charge-transfer complexes with low molecular absorptivity values. In polar solvents such as acetonitrile or methanol or 1, 4-dioxane, complete electron transfer from donor to the acceptor moiety takes place with formations of intensity colored radical ions with higher molar absorptivity values according to the following scheme.

$$D: + \quad A \quad \underset{\longleftarrow}{\overset{\text{Polar solvent}}{\longrightarrow}} \quad (D\text{-}A) \quad \underset{\longleftarrow}{\overset{}{\longrightarrow}} \quad A^{\cdot-} + D^{\cdot+}$$
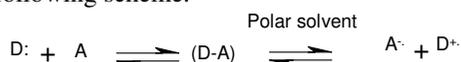
The position of equilibrium depends on the donor, the acceptor and the solvent. The dissociation of the D-A complex is promoted by donors of low ionization potential, acceptors of high electron affinity and solvents with strong solvating powers. Tertiary nitrogen of AM permits for the development of colored radical ion in the present investigation.

Method M₂

Henbest and his co-workers[9,10] found that many secondary or primary amines react with TQ and acetaldehyde. The *N*-alkyl vinyl amine obtained by condensing the amine with acetaldehyde reacts with TQ to give vinyl amino substituted quinone to give blue color. The proposed method exploits structural features aromatic secondary amine of the AM molecule due to the presence of cyclic imino group in indole portion. The nature of colored species formation with TQ-acetaldehyde reagent is initial *N*-alkyl vinyl amine formation with acetaldehyde then followed by formation of colored *N*-alkyl vinyl amino substituted quinones with T Q has been assumed in the scheme (Figure 6).

*sec. or ter. Nitrogen of drug involve in ion radical formation with quinone derivatives
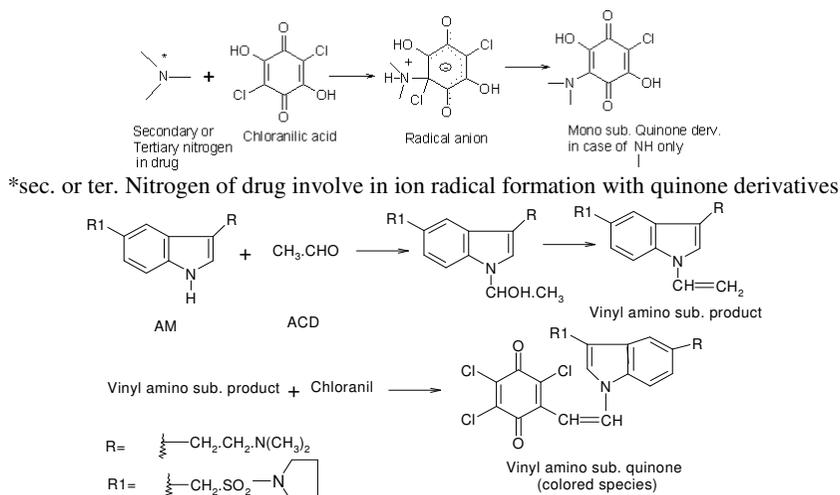
Figure 6. Scheme of the reactions for method M_1&M_2

Conclusion

The reagents utilized in the proposed method are normal cost, readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The proposed visible spectrophotometric methods possesses reasonable precision, accuracy and are 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.

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