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Spectrophotometrc Methods for the Determination of Ambrisentan Using Charge Transfer Reagents

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Abstract

The color developing reaction between ambrisentan and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) or 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone (CLA) was successfully employed in the development of two simple and sensitive spectrophotometric methods (M1 and M2) for the determination of ambrisentan in its pharmaceutical dosage forms.The methods are based on the charge transfer reaction of ambrisentan with DDQ (M1) or CLA (M2), to give colored radical anions. The colored products are measured at 560 nmin methanol and at 520 nm in acetonitrile for the methods M1 and M2, respectively.Under the optimized reaction conditions, Beer's law is obeyed in the range of 5–50 μg ml^{-1} for both the methods. The limit of detection, limit of quantification, molar absorptivity and Sandell's sensitivity were also reported for both the methods. Intra- and inter-day precision and accuracy of the methods were satisfactory. The proposed method was successfully applied to the quantification of ambrisentan in its tablet dosage forms with good accuracy and precision.

Key words: *Ambrisentan, Charge Transfer Reaction, Method Development, Validation.*

Introduction

Ambrisentan (ABN) is an orally active and highly selective endothelin A-receptor antagonist [1-4]. It is approved by the US Food and Drug Administration in 2007 for the treatment of pulmonary arterial hypertension to improve exercise capacity and delay clinical worsening.

To the best of our knowledge, the quantification of ABN is not official in any pharmacopoeias. An HPLC method for the determination of ABN enantiomers has been reported by Douša

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and Gibala [5]. HPLC–positive ion electrospray tandem mass spectrometry method has also been used in the assay of ABS in rat plasma [6]. There are few reports on the use of visible spectrophotometry in the determination of ABN [7, 8]. However, the reported HPLC methods are costly, laborious and time consuming. The spectrophotometric methods suffer from lack of sensitivity and requires extraction step. Therefore, the need for a simple, sensitive and economicalmethod is obviousfor the analysis of ABN in tablets. The aim of this study was to develop two simple, sensitive and extraction free visible spectrophotometric methods for the determination of ABN in tabletdosage forms. DDQ [9-12] and CLA [11-13] have been used as charge transferreagents for the determination of many pharmaceutical compounds. The reaction between ABN and these reagents has not been investigated so far. Therefore, the present study was devoted to explore DDQ and CLA as charge transfer reagents in the development of simple, sensitive and extraction free spectrophotometric methods for the determination of ABN in tablets.

Experimental

Materials

All the reagents were of analytical grade. ABN was obtained and used as received. DDQ, CLA, methanol and acetonitrile were obtained from Merck, Mumbai, India. DDQ (0.1%) solution was prepared by dissolving 100 mg of DDQ in 100 ml of methanol. CLA (0.5%) solution was prepared by dissolving 500 mg of CLA in 100 ml of acetonitrile.Letairis tablets (Gilead Sciences, Inc., CA, US) are labeled to contain 10 mg of ABN per tablet.

Stock and working standard solutions of ambrisentan

A stock standard solution containing 1 mg ml^{-1} of ABN was prepared in methanol for method M1 and in acetonitrile for method M2. Working standard solution equivalent to 250 µg ml^{-1} of ABN was prepared by appropriate dilution of stock solution withmethanol and acetonitrile for methods M1 and M2, respectively.

Tablet sample solution

Twenty tablets were weighed accurately and finely powdered. An accurately weighed powder equivalent to 50 mg ABN was transferred into a 50 ml beaker and dissolved in 10 ml of methanol (method M1) or in 10ml of acetonitrile (method M2). After 10 minutes of continuous shaking, the solution was filtered into a 50 ml of volumetric flask through Whatmann No 1 filter paper and was diluted to 50 ml with the respective solvents, to obtain a stock solution with a concentration of 1mg ml^{-1}.

Methods

Method M1 (Chargetransfer complexation with DDQ)

Different aliquots (0.2–2.0 ml) of working

standard ABN solution were transferred into a series of 10 ml volumetric flasks. The total volume was adjusted to 2.0 ml by adding adequate quantity of methanol. To each flask was then added 2.0 ml of 0.1% DDQ solution. The content was mixed well and kept aside for 15 min. The flasks were made up to 10 ml with methanol and the absorbance of each solution was measuredat 560 nm against the reagent blank.

Method M2 (Charge transfer complexation with CLA)

Different aliquots (0.2–2.0 ml) of working standard ABN solution were transferred into a series of 10 ml volumetric flasks. The total volume was adjusted to 2.0 ml by adding sufficient quantity of acetonitrile. To each flask was then added 2.0 ml of 0.5% CLA solution and the content was mixed well. The flasks were made up to 10 ml with acetonitrile.The absorbance of each solution was measured at 520 nm against the reagent blank.

Procedure for tablets

The tablet sample solution prepared in the section "Tablet sample solution'' was diluted appropriately with methanol (method M1) or with acetonitrile (method M2) quantitatively to obtain a concentration of 250 μg ml^{-1} of ABN. This solution was analyzed by following the procedures of the methods M1 and M2 described above.

Results and discussion

Charge transfer complex (electron donor–electron acceptor complex) is formed by a transfer of electronic charge from the electron donor (having adequately low ionization potential) to the electron acceptor (having adequately high electron affinity) [14]. The formation of charge transfer complex can be rapidly assessed for its validity as a simple quantitative analytical method for many pharmaceutical substances which can behave as electron donors [9-13]. In the present work CLA and DDQ are used as charge transfer reagents. The proposed methods (M1 and M2) are based on the formation of charge-transfer complexes between the ABN as an n-donor and DDQ (M1) or CLA (M2) as *pi*-acceptor. The products exhibited absorption maxima at 560 nm for DDQ and 520 nm for CLA. The colored products formed in methods M1 and M2 are due to the formation of DDQ radical anion and CLA radical anion, respectively. The radical anions are produced as the result of transfer of electrons from ABN to DDQ in methanol solvent system(M1) or from ABN to CLA in acetonitrile solvent system (M2).

Optimization of experimental variables

The experimental parameters such as concentration of charge transfer reagents,type of organic solvents and reaction time affecting the intensity of the colored products formed

in the methods M1 and M2 were studied and optimized to obtain the maximum color intensity.

Effect of concentration of charge transfer reagents

To investigate the effect of volume of charge transfer reagent (0.1% DDQ–M1; 0.5% CLA–M2) for color development, different volumes (0.5-4.0ml) were mixed with 1ml of ABN (25µg ml^{-1}). The results reveal that the addition of 2.0 ml of charge transfer reagent (0.1% DDQ–M1; 0.5% CLA–M2) gave the highest absorbance, which remained constant up to 4.0 ml. Therefore, 2.0 ml of the charge transfer reagent (0.1% DDQ–M1; 0.5% CLA–M2) was chosen for the determination of the drug all through the experiment.

Effect of reaction time

The optimum reaction time for the development of colored charge transfer complexes at room temperature was studied. It was found that 15 minutes of standing time is required for the complete color formation in method M1 whereas the color development was instantaneous in method M2. The color formed was stable for at least 3 hrs in both methods.

Effect of diluting solvent

The effect of diluting solvents such as methanol, acetonitrile, chloroform and dichloromethane were investigated to obtain the maximum color intensity. In the case of method M1, better results were achieved in methanol medium whereas acetonitrile was selected as the suitable solvent for method M2, yielding maximum absorbance.

Method validation

The proposed methods (M1 and M2) were validated as per the ICH guidelines [15].

Calibration curve

Calibration curve for the quantification of ABN by its reaction with DDQ (M1) or CLA (M2) was constructed by plotting the absorbances as a function of the corresponding concentrations. The regression analysis using the method of least square was performed to calculate the slope, intercept and regression coefficient. The regression equation was

$A = 0.0160 + 0.0213C$ ($R^2 = 0.9991$) – M1

$A = 0.0106 + 0.0178C$ ($R^2 = 0.9993$) – M2

Where A is the absorbance, C is the concentration of ABN in µg ml^{-1} and R^2 is the regression coefficient. The results are summarized in Table 1.The results proved the linearity of the proposed methods.

Sensitivity

The sensitivity of the proposed methods was determined by calculating molar absorptivity, Sandell's sensitivity, limit of detection and limit of quantification. The results are summarized in Table 1. The results revealed the high sensitivity of the proposed methods.

Table 1. Linearity, regression equation and sensitivity for the reaction of ABN with DDQ and CLA

Parameter	DDQ(M1)	CLA(M2)
Beer's Limit (μg ml^{-1})	5-50	5-50
Molar Absorbtivity (L $mole^{-1}$ cm^{-1})	1.044 x 10^5	8.476 x 10^4
Sandell's sensitivity (μg cm^{-2}/0.001 Absorbance unit)	3.62x 10^{-3}	4.46x 10^{-3}
LOD (μg ml^{-1})	0.126	0.152
LOQ (μg ml^{-1})	0.383	0.458
Regression equation (A= mC + I)$^{\$}$		
Slope (m)	0.0213	0.0178
Intercept (I)	0.0160	0.0106
Regression coefficient (r^2)	0.9991	0.9993

$^{\$}$A = mC + I, where A is the absorbance and C is the concentration of drug in μg ml^{-1}.

Precision and accuracy

Three different concentrations of ABN (within Beer's law limits) were analyzed in five replicates by the proposed methods during the same day (intra-day precision) and three consecutive days (inter-day precision). Standard deviation (SD) and relative standard deviation (RSD) were calculated. The SD and RSD values of intra-day and inter-day studies for ABN showed that the precision of the proposed methods were adequate (Table 2). Accuracy was evaluated as percentage recovery and percentage relative error between the measured mean concentrations and nominal concentrations for ABN. As summarized in Table 2, accuracy was satisfactory.

Table 2. Assessment of precision and accuracy of the proposed methods for ABN determination

Method	ABN (μg ml^{-1})		% RSD	% Recovery	% Error
	Taken	Found^a ± SD			
Intra-day analysis					
DDQ (M1)	5	5.04 ± 0.018	0.357	100.80	0.80
	25	24.97 ± 0.129	0.516	99.88	0.12
	50	49.95 ± 0.301	0.602	99.90	0.10
CLA (M2)	5	5.02 ± 0.042	0.836	100.40	0.40
	25	24.95 ± 0.197	0.789	99.80	0.20
	50	49.96 ± 0.402	0.804	99.92	0.08
Inter-day analysis					
DDQ (M1)	5	4.95 ± 0.064	1.29	99.00	1.00
	25	24.96 ± 0.142	0.568	99.84	0.16
	50	50.03 ± 0.421	0.841	100.06	0.06
CLA (M2)	5	4.97 ± 0.037	0.744	99.40	0.60
	25	24.93 ± 0.201	0.806	99.72	0.28
	50	50.01 ± 0.414	0.827	100.02	0.02

^{a}Average of five determinations

Robustness

The robustness of the proposed methods was examined by making small deliberate changes in the experimental parameters at two different concentration levels (5 and 50µg ml-1). The experimental parameters selected were:

Method M1

Volume of DDQ (2.0 ± 0.2ml)

Reaction time (15 ± 2 min)

Method M2

Volume of CLA (2.0 ± 0.2 ml)

The percentage recovery and relative standard deviation values are calculated (Table 3). The results indicate acceptable robustness of the proposed methods.

Table 3. Assessment of robustness of the proposed methods for ABN determination

Parameter	ABN (µg ml^{-1})	% Recovery[a]	% RSD
DDQ (M1)			
Volume of DDQ (2.0 ± 0.2 ml)	5	100.8	0.833
	50	99.94	1.148
Reaction time (15 ± 2 min)	5	100.8	0.781
	50	99.94	0.823
CLA (M2)			
Volume of CLA (2.0 ± 0.2 ml)	5	98.6	0.973
	50	99.92	0.778

[a]Average of three determinations

Recovery Study

Recovery experiments were carried out by the standard addition method in order to study the accuracy of the proposed methods and to check the interference from excipients used in the tablet dosage forms. The recovery study was performed by addition of the known amounts of ABN to preanalyzed solution of tablets at three different concentration levels (50, 100 and 150 % of labeled claim). The total amount of the drug was once again determined by the proposed methods. The percentage recovery was calculated. The results were shown in Table 4 which indicated the accuracy of the proposed methods was not affected by the excipients.

Table 4. Recovery data of the proposed methods for ABN determination

Method	ABN in tablet (mg)	Pure ABN added (mg)	Total found[a] (mg) ± SD	% Recovery
DDQ (M1)	10	5	14.95	99.66
	10	10	20.03	100.15
	10	15	25.01	100.04
CLA (M2)	10	5	14.93	99.53
	10	10	20.03	100.15
	10	15	24.95	99.80

[a]Average of five determinations

Application of the Proposed Methods

The proposed methods were applied to tablets containing ABN. The results (Table 5) indicate the high accuracy of the proposed methods for the determination of the ABN. The proposed methods have the advantage of being almost free from interferences by excipients in tablets.

Table 5. Determination of ABN in tablets by the proposed methods

Commercial product	Method	Found[a] ± SD	% Recovery
Letairis-10mg/tablet	DDQ (M1)	9.98	99.80
	CLA (M2)	9.94	99.40

[a]Average of five determinations

Conclusion

Two visible spectrophotometric methods have been developed and validated for the estimation of ABN using charge transfer reagents, DDQ and CLA. The developed methods can be concluded as simple, sensitive, accurate and precise. These methodscan be easily applied to the tablet dosage forms without interference from the excipients.The methods are useful for its routine application in quality control laboratories for analysis of ABN.

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