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Extractive Spectrophotometric Determination of Ambrisentan

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ABSTRACT

***Purpose*:** Ambrisentan (ABS) is an antihypertensive drug used in the treatment of pulmonary atrial hypertension. The survey of literature for ABS revealed only two spectrophotometric methods for its quantification. The reported methods lack the sensitivity. This study is aimed at developing two sensitive extractive spectrophotometric methods for the determination of ABS in bulk and in tablets. ***Methods*:** The proposed methods are based on the formation of colored chloroform extractable ion-pair complexes of ABS with methylene blue (MB method) and safranine O (SO method) in buffered solution at pH 9.8. The extracted complexes showed maximum absorbance at 525 and 515 nm for methylene blue and safranine O, respectively. ***Results*:** In both the methods, the calibration curve was linear from 1–15 μg mL^{-1} of drug. Apparent molar absorpitivities were 1.7911 x 10^5, 2.3272 x 10^5 L mol^{-1} cm^{-1}; Sandell's sensitivities were 0.0215, 0.0162 μg cm^{-2}; LOD were 0.182, 0.175 μg mL^{-1}; LOQ were 0.551, 0.531 μg mL^{-1} for methods MB and SO, respectively. The relative standard deviation and percent recovery ranged from 0.206–1.310% and 99.0–101.5%, respectively. ***Conclusion*:** The results demonstrate that the proposed methods are sensitive, precise, accurate and inexpensive. These methods can easily be used for the assay of ABS in quality control laboratories.

Introduction

Ambrisentan (ABS), a non-peptide, is a highly selective endothelin-1 type A receptor antagonist.[1-5] ABS belongs to antihypertensive class of drugs and used in the treatment of pulmonary atrial hypertension in patients with WHO Class II or III symptoms. Endothelin is a peptide that constricts blood vessels and elevates blood pressure. ABS blocks the effects of endothelin-1 and thus decreases blood pressure in lungs. The thickening of blood vessels in the lungs and heart is also inhibited by ABS. ABS is chemically known as (2S)-2-(4,6-dimethylpyrimidin-2-yloxy)-3-methoxy-.3,3- diphenylpropionic acid (Figure 1).

To the best of our knowledge, the assay of ABS is not official in pharmacopoeias. Due to the vital significance of the ABS, the development of a sensitive, simple and fast method for its quantification is of significant need. The detailed survey of literature revealed that very few methods have been reported for the estimation of ABS. Douša and Gibala developed and validated highperformance liquid chromatography (HPLC) method for the determination of ABS enantiomers. Enantioseparation was achieved on Chiralcel OZ-3R (cellulose 3-chloro-4-methylphenylcarbamate) using mixture of 20 mM sodium formate (pH 3.0) with acetonitrile (55:45; *v/v*).[6] Ramakrishna et al. reported a HPLC–positive ion electrospray tandem mass spectrometry method for the quantification of ABS in plasma using armodafinil as internal standard.[7] This method was applied to quantify ABS concentration in a rodent pharmacokinetic study.

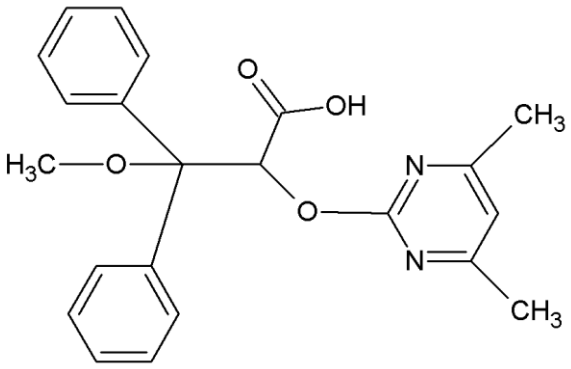

Figure 1. Structure of ambrisentan.

Spectrophotometric method of analysis is extensively used in the analysis of drugs in pharmaceutical formulations owing to its good sensitivity, selectivity and cost effectiveness. Ambrisentan can be estimated by using UV spectroscopy in tablets. But the selectivity of the method is less because the interference from the tablet excipients increases in UV region. Two visible spectrophotometric methods have been reported for the assay of ABS by Vinaya Kumar et al.[8] The first method is based on the reaction between ABS and 1,2-naphthoquinone-4-sulphonate.[8] The second method is

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based on the oxidation of ABS by ammonium metavanadate in presence of H_2SO_4.[8] Beer's law is obeyed in the concentration ranges between 10-60 and 10-50 µg/mL ABS for 1,2-naphthoquinone-4-sulphonate (first method) and ammonium metavanadate (second method), respectively. The reported visible spectrophotometric methods are associated with lack of sensitivity.

Methylene blue[9-12] (MB) and safranin O[12-15] (SO) have been used as ion-pair complexing dyes in the development of extractive spectrophotometric method for the determination of many pharmaceutical compounds. The present study is aimed to investigate the ion-pair complexation of ABS with MB (MB method) and SO (SO method) at pH 9.8, and employment of this reaction in the development of two new simple and sensitive extractive spectrophotometric methods for the determination of ABS in bulk and in its pharmaceutical dosage forms.

Materials and Methods

Instrumentation

A systronics (Ahemadabad, India) digital double beam UV-Visible spectrophotometer, model Visiscan-167 with 1 cm matched quartz cell is used for the spectral and absorbance measurements. A Shimazdu (Tokyo, Japan) electronic weighing balance, model BL 220 H is used for weighing the samples.

Chemicals and Reagents

Standard Sample and Tablets

ABS Standard was kindly donated by MSN laboratories, Hyderabad, India. Letairis tablets (Gilead Sciences, Inc., CA, US) labeled to contain 10 mg ABS per tablet were employed in the present study.

Stock and Working Standard Solutions

The ABS stock solution (1 mg/mL) was prepared by dissolving 100 mg of the ABS in 20 mL of 0.1 N NaOH (Fisher Scientific, Mumbai, India) and then diluted to 100 mL with distilled water. The ABS stock solution was diluted with distilled water to get working concentration of 100 µg/mL ABS for MB and SO methods.

Dye Solutions (0.1% MB and 0.05% SO)

Aqueous solutions of 0.1% MB (Fisher Scientific, Mumbai, India) and 0.05% SO (Sdfine-Chem limited, Mumbai, India) were prepared for methods MB and SO, respectively.

Buffer

Ammonia-ammonium chloride buffer solution (pH 9.8) was prepared by mixing 7 g of ammonium chloride (Sdfine-Chem limited, Mumbai, India) with 56.8 mL of 25% liquor ammonia (Merck, Mumbai, India) and diluted to 100 mL with distilled water and pH was adjusted to 9.8.

General Procedure

To a set of 125 mL separating funnels, aliquot volumes (0.1-1.5 mL) containing the ABS in the working concentration range of 1-15 µg/mL were transferred. The volume in each funnel was adjusted to 1.5 mL with 0.1 N NaOH. To each funnel 2 mL of buffer (pH 9.8) followed by 1 mL dye solution [0.1% MB in MB method or 0.05 % SO in SO method] were added and mixed well. The funnels were shaken vigorously with 5 mL of chloroform (Merck, Mumbai, India) for 2 min. The funnels were allowed to stand at room temperature for the clear separation of the two phases. The separated colored organic phase was transferred into a 10 mL volumetric flask, made up to the mark with chloroform and mixed well. The absorbance of the colored organic phase was measured at 525 and 515 nm against the corresponding reagent blank for methods MB and SO, respectively. In both the methods, the calibration graphs were constructed by plotting the absorbance versus the final concentration of the ABS (µg/mL). On the other hand, the corresponding regression equation was derived.

Procedure for Letairis Tablets

The content of twenty tablets of Letairis was weighed. An exactly weighted portion equivalent to 100 mg ABS was transferred into a beaker and then 25 mL of methanol (Merck, Mumbai, India) was added. The solution was shaken for 20 minutes. The solution was filtered using Whatman No. 1 filter paper. The filtrate was evaporated to dryness on a water bath. Then, the residue was transferred into a 100 mL calibrated flask containing 20 ml of 0.1 N NaOH and mixed well. The beaker was washed with few ml of 0.1 N NaOH to make use of the residue completely without any wastage. The washings were transferred to the flask and the volume was made up to the mark with distilled water. Suitable aliquots of the ABS solution (100 µg/mL) were used for analysis and treated as described in the above methods MB and SO. The recovery of ABS was calculated from either the corresponding linear regression equation or the calibration curve.

Validation of the Proposed Methods

According to ICH guidelines,[16] the proposed methods were validated for linearity, sensitivity, precision, accuracy and robustness.

For assessment of linearity, determination of ABS was done at six concentration levels (1, 3, 6, 9, 12, and 15 µg/mL) by the proposed methods. Least square regression analysis was carried out for calculating the slope, intercept and regression coefficient values.

The sensitivity parameters such as molar absorptivity, Sandell's sensitivity, limit of detection (LOD) and limit of quantification (LOQ) for ABS in each proposed method were calculated.

The precision and accuracy of the proposed methods were evaluated by performing five replicate analysis of pure ABS solution at three different concentrations (2,

8 and 14 µg/mL) on the same day and in three consecutive days for intra- and inter-day studies, respectively. The precision is expressed as standard deviation and relative standard deviation while accuracy is expressed as percent recovery and percent error. The accuracy and validity of the proposed methods were further assessed by recovery studies. Recovery studies were performed using the standard addition method. The recovery studies were carried out by measuring percent recovery using powdered tablets spiked with ABS at three different concentration levels (50, 100 and 150% of the labeled claim).

The robustness of the proposed methods was established by the constancy of the absorbance with the deliberated minor changes in the experimental parameters such as change in pH (9.8± 0.1), change in the volume of buffer (2 ± 0.2 mL) and change in the volume of 0.1% MB (1 ± 0.1 mL) for MB method. For SO method these changes include; change in pH (9.8 ± 0.1), change in the volume of the buffer (2 ± 0.2 mL) and change in the volume of 0.05 % SO (1 ± 0.1 mL).

Results and Discussion

An ion-pair complex consists of a positive ion and a negative ion bonded together by the electrostatic force of attraction between them at suitable pH. The ion pair complex is extractable into organic solvents from aqueous phase. In the recent years, ion pair complex extraction has been applied to the estimation of numerous compounds using extractive spectrophotometric method.[17-21] A major advantage of the extractive spectrophotometric method is that they can be applied to the assay of individual substances in a complex mixture with high sensitivity.

Basic dyes such as MB and SO have been used as ion-pair complexing dyes in the development of extractive spectrophotometric method for determination of many pharmaceutical compounds with carboxylic group.[9-15] Since ABS contains a carboxylic group in its structure, it reacts with MB (MB method) and SO (SO method) in ammonia-ammonium chloride buffer (pH 9.8) to give colored chloroform soluble ion-pair complexes [ABS-MB (MB method) and ABS-SO (SO method)], which exhibit absorption maxima at 525 and 515 nm for MB and SO, respectively (Figure 2).

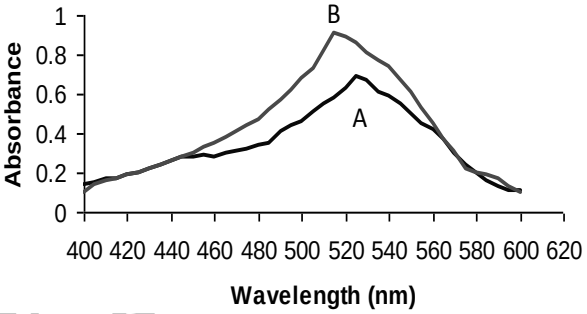

Figure 2. Absorption spectrum: A) ABS-MB ion-pair complex (λ_{max} – 525 nm), B) ABS-SO ion pair complex (λ_{max} – 515 nm).

Under the experimental conditions, the colorless blanks have virtually negligible absorbance. ABS-MB and ABS-SO ion-pair complexes were found to be stable at the room temperature approximately for 1 and 2 hr, respectively. The possible reaction mechanism was based on the reported methods is given in Figures 3 and 4.[9-15]

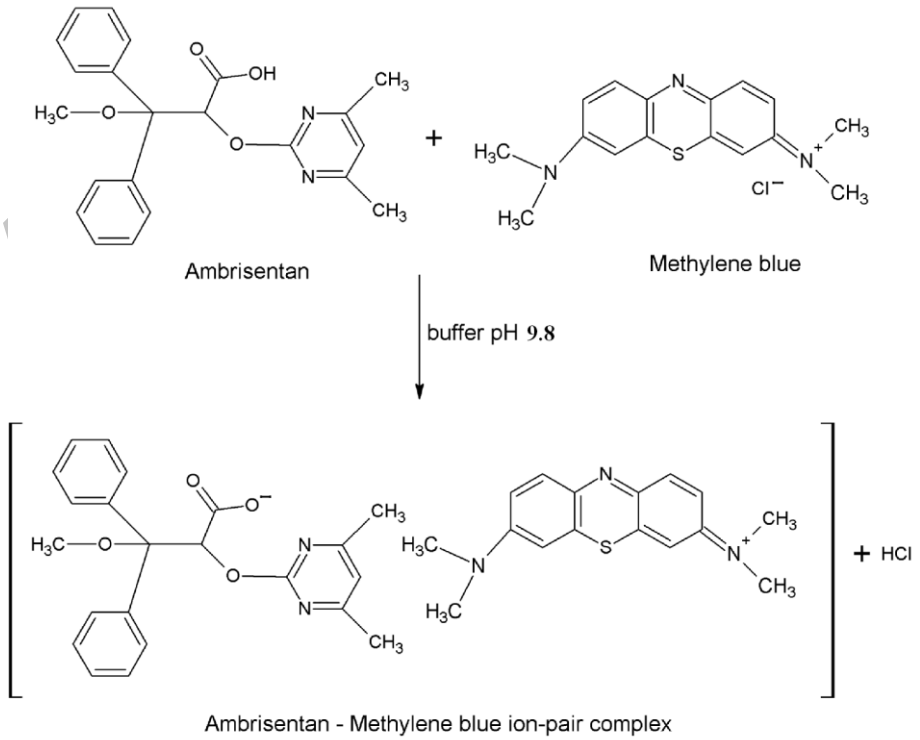

Figure 3. Ion pair complexation of ambrisentan with methylene blue.

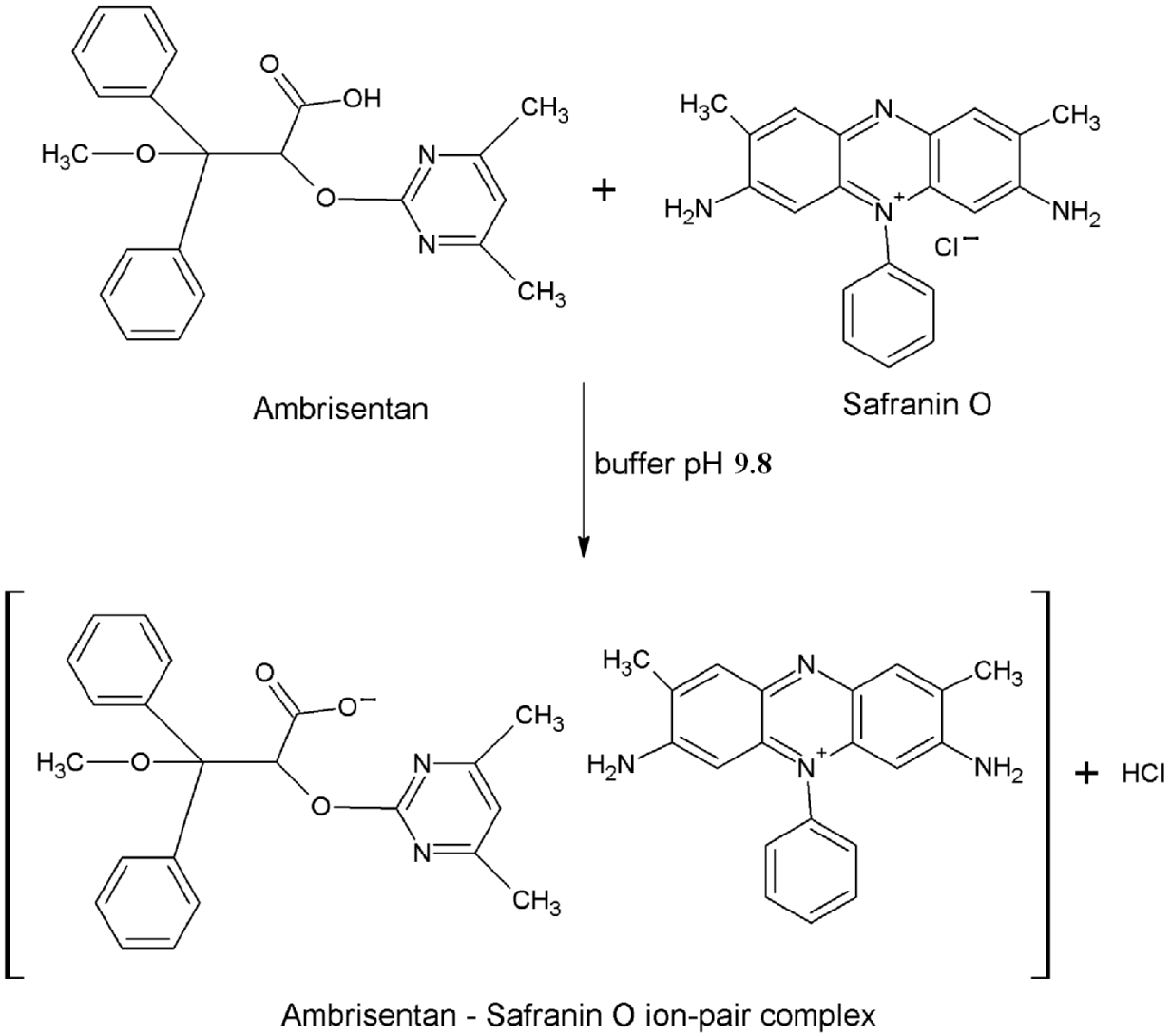

Figure 4. Ion pair complexation of ambrisentan with safranin O.

Optimization of the Methods

In order to optimize the developed MB and SO methods, the effect of experimental parameters such as, dye concentration, pH of the buffer, volume of buffer and extraction solvent, on the formation of ABS-MB and ABS-SO ion-pair complexes has been tested.

Effect of Concentration of Methylene Blue (MB Method) and Safranine O (SO Method)

The influence of the concentration of MB (MB method) or SO (SO method) was studied by treating 10 µg/mL ABS with 2 mL of buffer and varying volumes (0.2–2.0 mL) of 0.1% MB or 0.05% SO. The absorbance of the ABS-MB and ABS-SO ion-pair complexes was increased with increasing volume of 0.2% MB and 0.05% SO, respectively and became constant at 1.0 mL; above this volume, the absorbance remained unchanged (Figure 5). Therefore, 1 mL of 0.2% MB (MB method) and 0.05% SO (SO method) dye solution was chosen as the optimal volume for the quantification process.

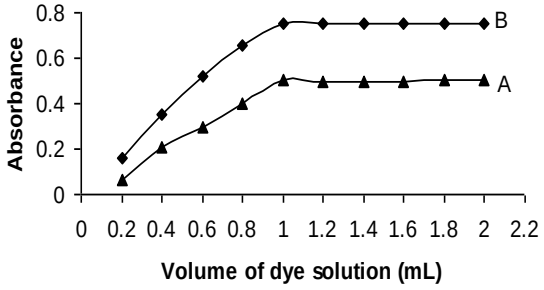

Figure 5. Effect of volume of dye solution: A) 0.1% Mb solution (λ_{max} – 525 nm), B) 0.05% SO solution (λ_{max} – 515 nm).

Effect of PH

At a fixed concentration of ABS (10 µg/mL), the formation of ABS-MB (MB method) and ABS-SO (SO method) ion-pair complexes were investigated over the pH range of 8.0-11.0 using ammonia-ammonium chloride buffer. The absorbance of the ion-pair complexes in both the methods varies only slightly between pH 8-9.8. However the absorbance at pH 9.8 is slightly higher than the absorbance at other pH values.

After pH 9.8, the absorbance decreases (Figure 6). Therefore, pH 9.8 was selected as the optimum pH for the reaction.

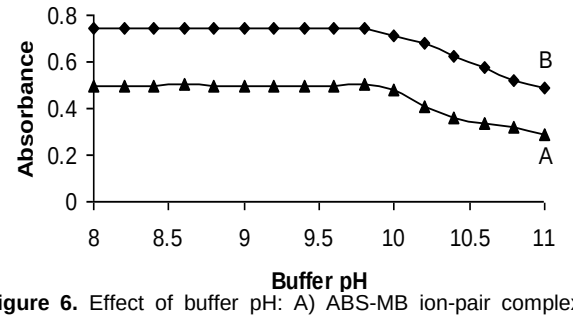

Figure 6. Effect of buffer pH: A) ABS-MB ion-pair complex (λ_{max} – 525 nm), B) ABS-SO ion pair complex (λ_{max} – 515 nm).

Effect of Volume of Buffer

The influence of the volume of ammonia-ammonium chloride buffer (pH 9.8) on the absorbance value of the ion-pair complexes was studied by treating 10 µg/mL ABS with varying volumes (0.5–4.0 mL) of buffer and 1 mL of 0.1% MB (MB method) or 0.05% SO (SO method). As shown in Figure 7, 2 mL of ammonia-ammonium chloride buffer (pH 9.8) was sufficient to get the optimum pH value (pH 9.8). Therefore, 2 mL was chosen as the optimum buffer volume.

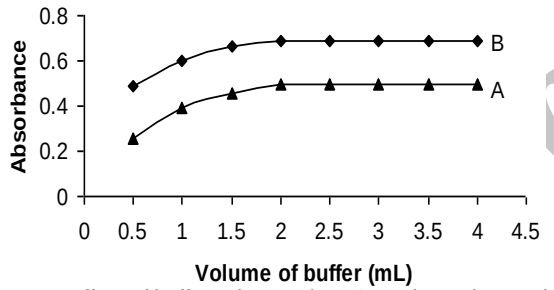

Figure 7. Effect of buffer volume: A) ABS-MB ion-pair complex (λ_{max} – 525 nm), B) ABS-SO ion pair complex (λ_{max} – 515 nm).

Effect of Extraction Solvent

The effect of extraction solvent was tested using different solvents such as chloroform, benzene, dichloromethane and butanol. Using chloroform as extraction solvent, the ion-pair complexes showed the highest absorbance value and reproducibility. Therefore, chloroform was chosen as the best extraction solvent for extraction of ABS-MB and ABS-SO ion-pair complexes.

Method Validation

Calibration curve was constructed by plotting the absorbance *vs* concentration of ABS. Linearity was found to be 1-15 µg/mL ABS for MB and SO methods. The linear regression equations for MB and SO methods were:

MB method: $y = 0.0458x + 0.0095$ ($R^2 = 0.9991$)

SO method: $y = 0.0602x + 0.0140$ ($R^2 = 0.9999$)

Where y is absorbance and x is the concentration of ABS (µg/mL)

Sensitivity of the proposed methods was evaluated by molar absorptivity, Sandell's sensitivity, LOD and LOQ. The results, presented in Table 1, indicated the high sensitivity of the proposed methods.

Table 1. Sensitivity parameters of the propose methods.

Parameters	Method	
	MB	SO
Molar Absorbtivity(L mole^{-1} cm^{-1})	1.7911 x 10^5	2.3272 x 10^5
Sandell's sensitivity(µg cm^{-2}/0.001 Absorbance unit)	0.0215	0.0162
LOD (µg mL^{-1})	0.182	0.175
LOQ (µg mL^{-1})	0.551	0.531

The intra day and inter day assays were performed by conducting five replicate analyses of ABS at the 2, 8 and 14 µg/mL concentration levels using the proposed methods on one day and on three consecutive days. The results are summarized in Table 2. The standard deviation, relative standard deviation, percent recovery and percent error values can be considered to be very reasonable. Thus the proposed methods are precise and accurate.

Table 2. Precision and accuracy of the proposed methods.

Method	Assay type	Concentration of ABS (µg mL^{-1})		RSD (%)	Recovery (%)	Error (%)
		Taken	Found ± SD[a]			
MB	Intra-day	2	1.98 ± 0.013	0.656	99.00	1.00
		8	7.94 ± 0.041	0.516	99.25	0.75
		14	13.93 ± 0.044	0.315	99.50	0.50
	Inter-day	2	1.98 ± 0.017	0.858	99.00	1.00
		8	7.95 ± 0.053	0.666	99.37	0.63
		14	13.92 ± 0.092	0.660	99.42	0.58
SO	Intra-day	2	2.03 ± 0.027	1.310	101.50	1.50
		8	7.97 ± 0.054	0.654	99.62	0.38
		14	14.03 ± 0.029	0.206	101.21	1.21
	Inter-day	2	1.98 ± 0.021	1.076	99.00	1.00
		8	8.04 ± 0.048	0.592	100.50	0.50
		14	13.94 ± 0.098	0.703	99.57	0.43

a-Average of five determinations

The accuracy and validity of the proposed methods were further assessed by performing recovery studies using standard addition technique. The results are presented in the Table 3. The recovery of pure ABS added indicates that common excipients did not interfere in the assay procedures. The results obtained were reproducible with low relative standard deviation.

Table 3. Recovery results of ABS by the proposed methods.

Method	Concentration of ABS (mg)			RSD (%)	Recovery (%)
	Tablet	Pure drug added	Total found ± SD[a]		
MB	10	5	15.08 ± 0.121	0.802	100.53
	10	10	19.86 ± 0.175	0.881	99.30
	10	15	25.15 ± 0.260	1.033	100.60
SO	10	5	14.96 ± 0.118	0.788	99.73
	10	10	20.10 ± 0.096	0.477	100.50
	10	15	24.97 ± 0.184	0.736	99.88

a-Average of three determinations

The robustness of the proposed methods was investigated by making small intentional changes in the experimental parameters at two different concentration levels (2 and 14 µg/mL). The results (Table 4) revealed that the slight changes expected to take place did not adversely influence the absorbance intensity.

Table 4. Robustness of the proposed methods.

Method	Experimental Parameter		ABS Taken (2 µg mL^{-1})		ABS Taken (14 µg mL^{-1})	
			Absorbance	RSD (%)	Absorbance	RSD (%)
MB	0.1% MB (mL)	0.9	0.101	1.666	0.634	0.470
		1.0	0.104		0.638	
		1.1	0.103		0.640	
	Buffer pH	9.7	0.100	1.960	0.628	0.270
		9.8	0.104		0.631	
		9.9	0.102		0.630	
	Buffer volume (mL)	1.8	0.099	1.700	0.632	0.315
		2.0	0.101		0.635	
		2.2	0.102		0.636	
SO	0.05% SO (mL)	0.9	0.132	1.278	0.838	0.238
		1.0	0.134		0.842	
		1.1	0.135		0.841	
	Buffer pH	9.7	0.134	1.111	0.836	0.309
		9.8	0.137		0.840	
		9.9	0.135		0.841	
	Buffer volume (mL)	1.8	0.128	0.781	0.842	0.355
		2.0	0.129		0.848	
		2.2	0.129		0.846	

Application to Letairis Tablets

Applicability of the proposed methods was evaluated by determination of ABS in its dosage form, Letairis tablets. The results are presented in Table 5. Excellent recoveries with low SD and RSD values were obtained. Common excipients present in the tablet dosage form did not interfere with the assay in the two applied methods.

Conclusion

Two simple, rapid, accurate, precise and robust extractive spectrophotometric methods were developed for the estimation of ABS, using MB and SO as ion-pair complexing dyes, in bulk drug and in tablet. The reagents used in the proposed methods are cheaper and readily available. The developed methods have the advantages over the reported spectrophotometric methods in being more sensitive, stable colored complex, robust, precise and accurate. Furthermore, the developed methods are inexpensive and do not require sophisticated instrumentation & elaborate treatments allied with chromatographic methods. Therefore, the proposed methods can be used for the routine analysis of ABS in quality control laboratories.

Table 5. Analysis of tablet dosage form by the proposed methods.

Formulation	Labelled claim (mg)	Method	Found ± SD[a]	RSD (%)	Recovery (%)
Letairis	10	MB	9.96 ± 0.098	0.968	99.60
		SO	10.04 ± 0.071	0.707	100.40
a: Average of five determinations					

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Conflict of Interest

The authors report no conflicts of interest in this work.

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