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DERIVATIVE AND DIFFERENCE SPECTROPHOTOMETRIC METHODS FOR THE ANALYSIS OF AMBRISENTAN IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Two new derivative and difference spectrophotometric methods were developed for the determination of ambrisentan in tablets by UV spectrophotometry. Both methods were developed and optimized using Phosphate buffer as solvent system. The difference spectrophotometric method was developed using difference in spectral characteristics of drug was observed in acid buffer pH 2 phosphate buffer solution and basic buffer pH 8 phosphate buffer solution as a solvent system. The derivative spectrophotometric method was developed by differentiating absorbance of a sample with respect to the wavelength of drug observed in 0.2M pH 6.8 phosphate buffer solution as a solvent system. All the determinations were carried out at 263 nm wavelength. The developed methods were validated as per ICH guidelines (ICH Q2 (R1)). Linearity was observed over concentration range of 20-100 mcg/mL for ambrisentan. The coefficient of determination was found to be 0.999 for two methods. The LOD and LOQ were found to be 3.75mcg/mL and 11.37mcg/mL for derivative spectrophotometric method, 2.842mcg/mL and 8.613mcg/mL for difference spectrophotometric method. The methods were found to be precise and accurate. The validated methods were successively applied for the analysis of ambrisentan in tablets. The results demonstrate that the method is precise, linear and accurate. The proposed methods were successfully applied for the determination of ambrisentan in pharmaceutical dosage forms (tablets) with good recovery.

Key words: UV spectrophotometric; derivative; difference; Ambrisentan; LOD; LOQ.

INTRODUCTION

Ambrisentan[1] is an orally active, diphenyl propanoic acid derivative, nonsulfonamide. It is a potent selective type-A endothelin receptor antagonist indicated for the treatment of pulmonary arterial hypertension. It is used in the therapy of pulmonary arterial hypertension (PAH). Ambrisentan has been associated with a low rate of serum enzyme elevations during therapy, but has yet to be implicated in cases of clinically apparent. Ambrisentan is indicated for the treatment of pulmonary arterial hypertension (WHO Group 1) in patients with WHO class II or III symptoms to improve exercise capacity and delay clinical worsening[2-4].

The literature review[5-18] reveals that HPLC, RP-HPLC, LC-MS/MS in biological fluids, spectrophotometric method and HPLC tandem mass spectrometry methods have been reported for the estimation of ambrisentan single drug or in combination with other drug in pharmaceutical dosage forms. There are no derivative and difference spectrophotometric methods for the estimation of ambrisentan in pharmaceutical dosage forms.

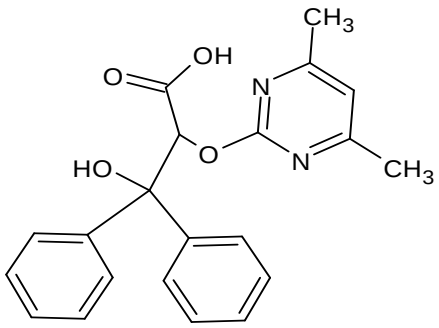

Fig. 1 Structure of ambrisentan

As UV spectrophotometric methods are commonly used methods for quantitative analysis of drug substances and their formulations as they are simple, easy to development and gives accurate and precise results. Present work was planned to develop difference and derivative spectrophotometric methods for the analysis of ambrisentan, pure drug and formulations. The derivative spectrophotometric method provides two general advantages: first, an effective enhancement of resolution, which can be useful to separate two or more components with overlapping spectra; second, discrimination in favor of the sharpest features of a spectrum, used to eliminate interferences by broad band constituents. Difference spectrophotometric method require no empirical input parameters to be computed, do not amplify noise or sharp peaks, and can be quantitated for comparison with other spectra or analytical data. Difference spectra are a useful complement to other methods for analyzing spectroscopic data.

MATERIALS AND METHODS

Chemicals and reagents

Pure drug sample ambrisentan was obtained as gift sample from Ero labs Hyderabad, Pulmonext and Ambrican tablets manufactured by MSN laboratories and Lupin ltd respectively were purchased from local pharmacy. Methanol, Acetonitrile, Potassium dihydrogen phosphate (HPLC grade), Sodium hydroxide, Ammonia, Hydrochloric acid, Acetic acid (AR grade) manufactured by Rankem and SDFCL, Mumbai were used for derivative and difference spectrophotometric methods.

Instrumentation

UV- Vis double beam spectrophotometer (Shimadzu 1800) using UV probe 2.43 software, Analytical balance (Contech instruments Ltd), Ultrasonic bath sonicator (PCI analytics, 6.5 li200H), pH-meter (Analab scientific instruments Pvt. Ltd), Hot air oven (Tempo equipment private limited) used for the analysis.

Selection of solvent system

Solvent selection is the first step involved in the method development. Solvent is selected based on the solubility of the drugs. Four trials were done in derivative spectrophotometric method using different solvents like acetonitrile, methanol, DMSO, pH-6.8 phosphate buffer. For method development by difference spectrophotometric technique acids and bases of different strengths and buffers solution with different pH were selected as solvents and trials were performed for optimization of the solvent system.

Preparation of Solutions

Preparation of standard solutions

10mg of ambrisentan was weighed separately and transferred into a 10mL volumetric flask. 5mL of diluent was added to the volumetric flask and it was sonicated for 2min to dissolve. Further the volume was made up to 10ml with the diluent and mixed well. From this 1ml of the above solution was taken and diluted to 10 ml to yield 100mcg/ml solution which further diluted to 10mcg/ml solution.

Preparation of sample solutions

5 tablets of Ambrisentan (Pulmonext, Ambrican) were weighed and powdered. An accurately weighed portion of the tablet powder equivalent to about 25mg of drug was transferred to a 50 mL volumetric flask, to this 25mL of buffer was added, shaken for 15 min in sonicator. After sonication the volume was adjusted with respective solvent to get the concentration of 500mcg/mL ambrisentan. The solution was the filtered through whatmann filter paper. The above solution was further diluted to 10 mcg/ml solutions.

Preparation of acid buffer pH-2, 0.2M Phosphate buffer solution

0.136g of potassium dihydrogen phosphate was dissolved in 800 mL of water the pH was adjusted to 2.0 with hydrochloric acid and the volume was made up to 1000mL.

Preparation of basic buffer pH-8, 0.2M Phosphate buffer solution

50.0mL of potassium dihydrogen phosphate was dissolved in 200 mL volumetric flask, the pH was adjusted with specified volume of 0.2 M sodium hydroxide, 46.1 mL and the volume was made up to 1000mL with water

Preparation of pH-6.8, 0.2M Phosphate buffer solution

27.2g of potassium dihydrogen phosphate was dissolved in 930 mL of water the pH was adjusted to 6.8 with 0.3%w/v solution of sodium hydroxide and the volume was made up to 1000mL.

Selection of analytical concentration range and verification of beer's law

Derivative spectrophotometric method

Beer's law is obeyed over certain concentration range. This was selected by preparing different calibration standards from standard stock solution of ambrisentan. Concentration range was fixed looking in to the linearity between concentration and absorbance values. From the working standard solution of ambrisentan (100 mcg/mL), appropriate aliquots of 2, 4, 6, 8 and 10mL solutions were pipetted into 10mL graduated tubes separately and the volume was made up to the mark with pH-6.8, 0.2M phosphate buffer.

Difference spectrophotometric method

Beer's law is obeyed over certain concentration range. This was selected by preparing different calibration standards from standard stock solution of ambrisentan. Concentration range was fixed looking in to the linearity between concentration and absorbance values. From the working standard solution of ambrisentan (100 mcg/mL), appropriate aliquots of 2, 4, 6, 8 and 10mL solutions were pipetted into 10mL graduated tubes separately and the volume was made up to the mark with buffer solution pH-12 as reference solvent and buffer solution pH-2 in sample solvent system. The difference absorbance was measured at 263nm.

Method validation

Method validation[19-20] was carried out according to ICH guidelines for the following parameters.

Linearity

The linearity of the analytical method is ability to obtain test results that are directly proportional to the concentration of analyte in the sample. Linearity was checked by preparing calibration standards in the concentration range over which Beer's law was obeyed. Calibration standards were prepared in the concentration range of 20-100mcg/mL for ambrisentan. The absorbance of the solutions was measured at 263 nm for derivative and difference spectrophotometric method. Linearity of the methods was verified by using linear regression analysis and regression equation and correlation coefficient were reported.

Limit of detection and limit of quantification

The sensitivity of the methods for the measurement of ambrisentan was estimated in terms of LOD & LOQ. The limit of detection (LOD) and the limit of quantification (LOQ) were determined using standard deviation method. LOD & LOQ were calculated using the standard deviation (SD) method.

Standard deviation method

The SD of the response was calculated from the calibration curve.

The following formulas were used to calculate LOD & LOQ of ambrisentan.

$LOD = 3.3\sigma S$

$LOQ = 10\sigma/S$

Where σ = the standard deviation of the response

S = slope of the calibration curve

The standard deviation and slope were calculated from the calibration curve constructed from linearity studies data

Precision

The precision of the method was reported in three levels- repeatability, inter and intraday precision. The standard deviation (SD) or %RSD of a series of measurements is used to access the precision of the analytical method.

Repeatability

The repeatability of the analytical method was determined on samples of standard solutions at a single concentration level that is 10mcg/mL of ambrisentan by analyzing six replicates of the same sample as a batch in a single assay run for checking the variation of result on the same day. The absorbance of six determinations was measured and %RSD is calculated.

Intra-day precision and Inter-day precision

Variation of results within the same day (Intra-day) and between days (Inter-day) was reported by calculating Intra-day precision and Inter-day precision.

Intra-day precision

Variations of results within the same day (intraday) were analysed. Repeatability assessment of an analytical method was performed in one laboratory by one analyst using the same equipment on the same day. The intra-day precision of the proposed method was determined on samples of drug at various concentration level that is 48mcg/mL, 60mcg/mL and 72mcg/mL for ambrisentan by analysing 3 replicates of each sample as a batch in a single assay. Each of concentration was analysed for three times and absorbance at 263nm for ambrisentan were recorded. The % relative standard deviation RSD was calculated at each level.

Inter-day precision

The inter-day precision of the proposed method was determined by analysing three replicates of three different samples at various concentration level that is 48mcg/mL, 60mcg/mL and 72mcg/mL for ambrisentan by analysing 3 replicates of each sample for three consecutive days at 263nm. The % relative standard deviation RSD was calculated at each level.

Accuracy

Accuracy is the closeness of test results obtained by the method of true value. The accuracy of analytical method describes the extent to which these results deviate from the expected results and it is a measure of the exactness of an analytical method.

For drug substance

Accuracy for drug substance was determined on samples of drug solutions at varying concentration levels in the range of 80%- 120% (48mcg/mL, 60mcg/mL and 72mcg/mL) of ambrisentan by analysing 3 replicates of each sample as a batch in a single assay.

Recovery studies (drug product)

The accuracy method for drug product was determined by doing recovery studies. Recovery studies were carried out by standard addition method, adding known amount of standard drug 60mcg/mL ambrisentan to different concentration level of drug of product between 80% - 120% (48mcg/mL, 60mcg/mL and 72mcg/mL) prepared from sample solutions.

Analysis of fixed dose tablets

Five fixed dose tablets (Ambrican manufactured by Lupin Pvt Ltd and Pulmonext MSN laboratories Pvt Ltd) containing 5mg of ambrisentan was weighed and powdered. The tablet powder equivalent to 25mg of ambrisentan was weighed and transferred to 50ml volumetric flasks containing methanol and sonicated well for 15 minutes. This solution was filtered through whatmann filter paper and the final volume was made up to the mark to get the concentration of 500 mcg/mL ambrisentan solution. From the filtrate of above sample stock solution (500 mcg/mL ambrisentan), 5ml was transferred into two 25ml graduated tubes separately and the volume was made up to the mark with pH-6.8, 0.2M Phosphate buffer in derivative spectrophotometric method. For difference spectrophotometric method volume was made up to the mark with basic buffer pH-12 as reference solvent and acidic buffer pH-2 in sample solvent system in the UV region to get the final concentration of 100 mcg/mL ambrisentan respectively. The absorbance of the prepared solution was measured at 263nm. The analysis procedure was repeated for three times with tablet formulations. The amount of drug present in the formulation was calculated using the following formula.

$$\text{Amount of drug taken} = \frac{\text{Concentration} \times \text{Dilution factor} \times \text{Average weight of the tablet}}{\text{Weight of tablet powder taken} \times \text{Label claim of the drug}} \times 100$$

RESULTS AND DISCUSSION

DERIVATIVE SPECTROPHOTOMETRIC METHOD

Selection of solvent

Sample solubility was checked in water, methanol, buffer and acetonitrile. Sample is soluble in acetonitrile, pH-6.8 buffer and methanol and is insoluble in water. Suitable solvent was selected based on absorbance observed for the drug without interference shown in fig 2. As the drug shown maximum absorbance in pH-6.8 buffer solution. It was selected as a solvent for method development.

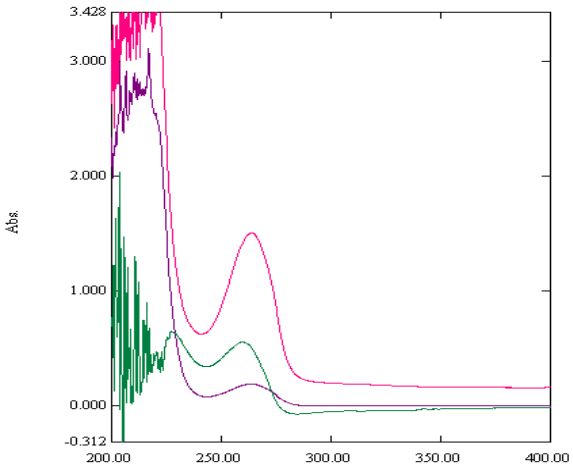

Fig.2. The overlay absorption spectrum of ambrisentan in different solvents

Selection of analytical wavelength

10mcg/mL solution of ambrisentan in 6.8pH buffer was scanned in UV region from 200-400nm. The zero-crossing point 263nm was selected wavelength for derivative spectrophotometric method development.

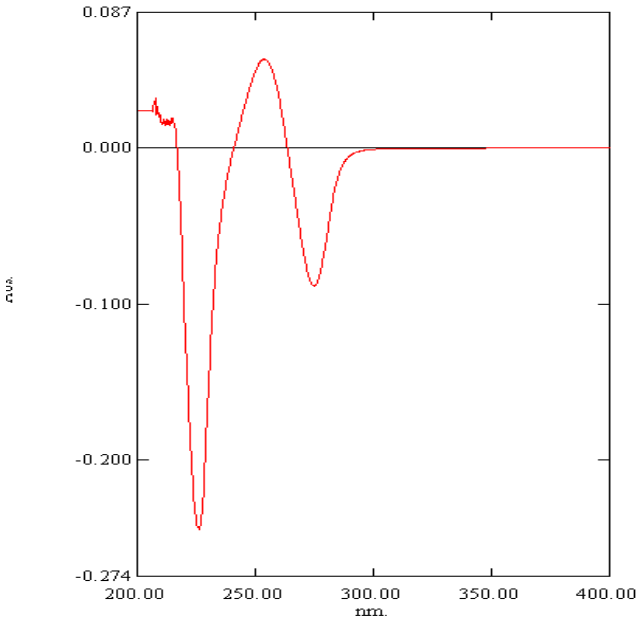

Fig. 3. First derivative spectrum of ambrisentan in pH-6.8 buffer

Selection of analytical concentration range and verification of Beer's law

The drug solutions have shown good proportionality between concentration and absorbance. In the concentration range of 20-100mcg/mL. This range was used for verification of linearity

Analyte solution stability study of ambrisentan

The solution of ambrisentan prepared in buffer, the selected solvent checked for absorbance measurements at different time points for 6 hrs, for checking the stability of drug in pH-6.8 buffer solution. There was not much variation observed in the difference absorbance values indicating stability of the prepared analyte solution.

Method validation

Linearity

The linearity of the method was assessed by performing linear regression analysis for the calibration curve constructed between concentration and absorbance is shown in fig.13. The response of the drug was found to be linear in the investigational concentration range 20-100mcg/mL. The overlay of zero order, first order and second order derivative spectra of ambrisentan standard solution were shown in fig 4-6. The calibration curve was found to be linear with an r^2 value 0.9991 and regression equation was y= 0.0056x+0.0856. For these studies the obtained r^2 value was appropriate to demonstrate the linearity of the method.

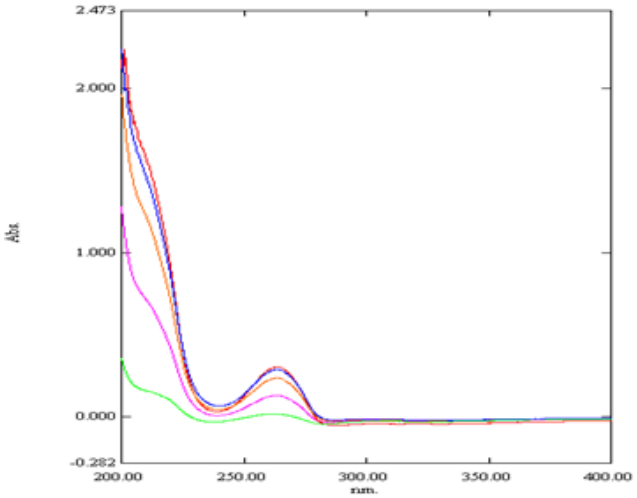

Fig.4. Overlay D^0 spectrum of ambrisentan (20-100mcg/mL)

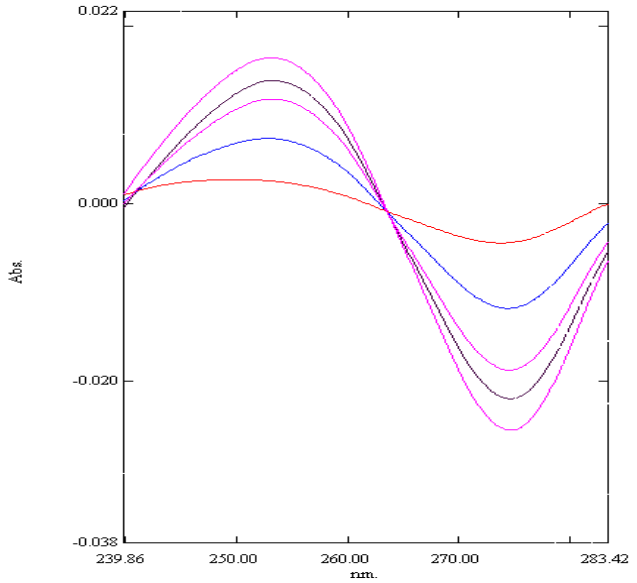

Fig. 5. Overlay D^1 spectrum of ambrisentan (20-100mcg/mL)

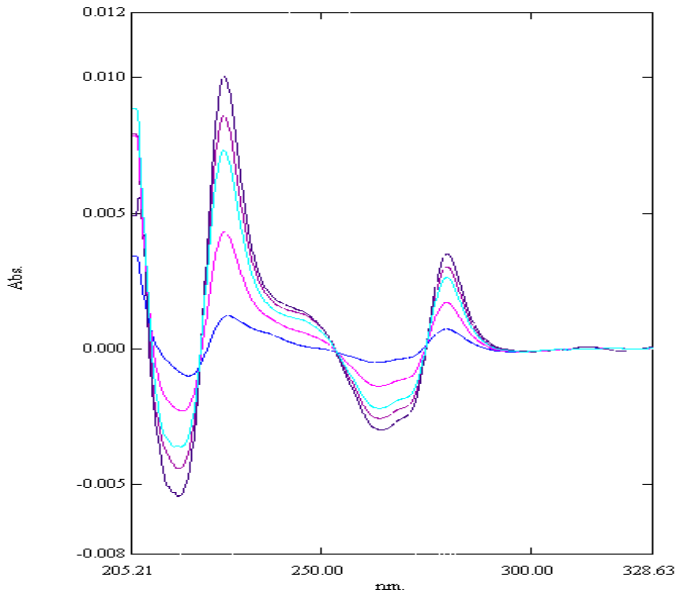

Fig.6. Overlay D^2 spectrum of ambrisentan (20-100mcg/mL)

Table 1. Linearity data of ambrisentan

S.No.	Conc(mcg/mL)	Absorbance*
1	20	0.201
2	40	0.31
3	60	0.415
4	80	0.528
5	100	0.651

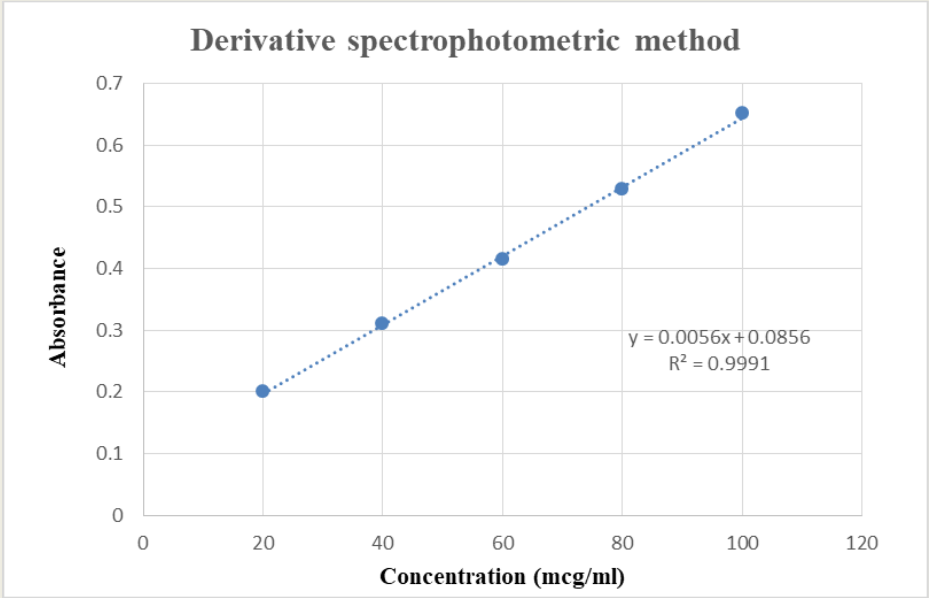

Fig.7. Calibration curve of ambrisentan at 263nm

Sandell's sensitivity

The Sandell's sensitivity is the concentration of the analyte (mcg/mL) which results in an absorbance of 0.001 in a cell of path length 1.0 cm path length. Units: mcg /cm^2 It is calculated using the following equation: Sandell's Sensitivity (л) = Conc. (mcg/100 ml) x 0.001/D1 value = 20 x 0.001/ 0.201 = 0.099 mcg /cm^2.

Limit of Detection (LOD) and Limit of quantitation (LOQ)

The derivative method developed was sensitive and can be used for trace analysis with detection level 3.75mcg/mL and quantitation level 11.37mcg/mL. They are calculated by using standard deviation method.

Repeatability

Repeatability determined with six replicates of 10mcg/mL Ambrisentan solutions (for both drug substance and drug product). The absorbance value was found to be same and calculated %RSD was found to be less than 2.

Intermediate precision

Intermediate precision determined by checking variation of results within the same day (Intra-day) and between days (Inter-day) was reported by calculating relative standard deviation.

Intra-day precision

The intra-day precision of the proposed method was determined on samples of both the drug at various concentration levels (48mcg/mL, 60mcg/mL, 72mcg/mL for ambrisentan) by analyzing three replicates of each sample as a single assay run at 263nm. The % RSD was calculated for each concentration.

Inter-day precision

Inter-day precision of the developed method was determined by analyzing three replicates of three different concentration samples (48mcg/mL, 60mcg/mL and 72mcg/mL for ambrisentan) for three consecutive days at 263nm. The %RSD was calculated and found to be less than 2.

Accuracy

For drug product (Recovery study)

Accuracy of the method for drug product was determined by recovery studies, carried out by adding a known amount of standard drug (60mcg/mL) to sample solution (48mcg/mL, 60mcg/mL, and 72mcg/mL). The % recovery was calculated and reported.

For drug substance

Accuracy for drug substance was determined on samples of drug solutions at varying concentration level in the range of 80%-120% (48mcg/mL, 60mcg/mL and 72mcg/mL) by analyzing three replicates.

The obtained values of recovery studies were in the range of 91 -107% which indicates that the proposed method is accurate.

Assay of fixed dose combination tablets

The validated UV spectrophotometric method was applied for analysis of commercially available Pulmonext (5mg) and Ambrican (5mg) tablets. Five tablets were weighed and powdered. An accurately weighed portion of the tablet powder equivalent to about 25mg of drug was transferred to a 50 ml volumetric flask, to this 25mL of buffer was added, shaken for 15 min in sonicator. After sonication the volume was adjusted with the solvent to get the concentration of 500mcg/mL ambrisentan. The solution was the filtered through whatmann filter paper. From the above stock solution 5ml of sample (500mcg/mL) was transferred into the 25mL volumetric flask and the volume was made up to the mark with buffer to get the desired concentrations of 100mcg/mL. The samples are analysed by

using derivative spectrophotometric method. The results are given in table 3 and the assay spectrum of the sample is shown in fig.9 and 10 for two brands.

Table 2. Assay data of formulations

Brand name	Drug name	Label claim (mg)	Amount found(mg)	Assay (%)
Ambrican	Ambrisentan	5	89	96
Pulmonext	Ambrisentan	5	92	101

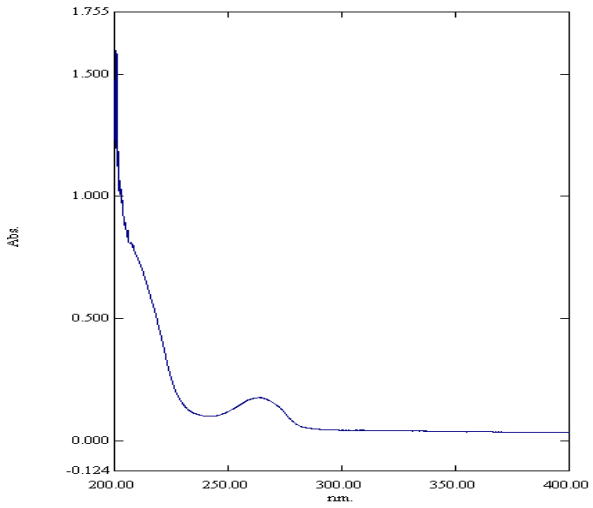

Fig.8. Assay spectrum of Ambrican tablets

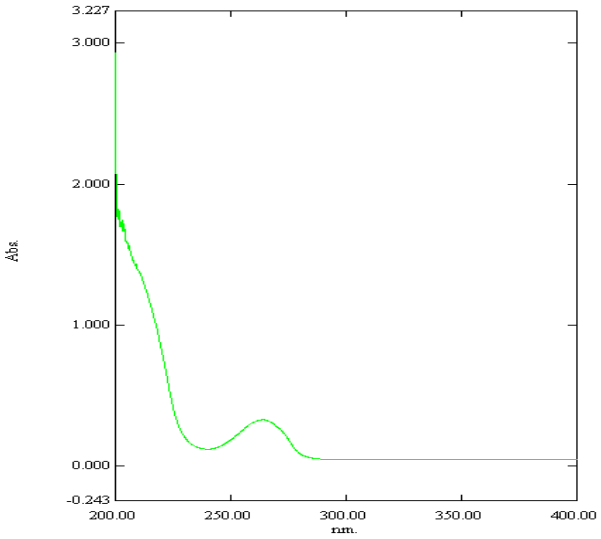

Fig.9. Assay spectrum of Pulmonext tablets

Ambrisentan in tablets was found to be 96% and 101%. The results of assay revealed that the drug content of the commercially available product was within the acceptable limits described in the Indian Pharmacopoeia (90-110%)

DIFFERENCE SPECTROPHOTOMETRIC METHOD

Selection of analytical wavelength

Difference absorption spectrum of drug was scanned in UV region from 200 to 400nm. Wavelength was selected.

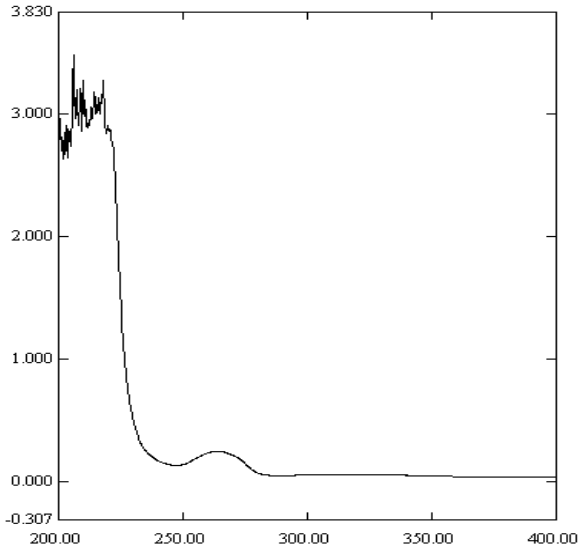

Fig. 10.Difference absorption spectrum of ambrisentan in buffer solution pH-8 as reference solvent and buffer solution pH-2 in sample solvent system

Selection of analytical concentration range and verification of Beer's law

The concentration range was proved for ambrisentan in the concentration range of 20 – 100mcg/mL which obeys beer lamberts law, with absorption maximum at 263nm.

Analyte solution stability study of ambrisentan

The solution of ambrisentan prepared in buffer, the selected solvent checked for absorbance measurements at different time points for 6 hrs, for checking the stability of drug in buffer solution pH-8 as reference solvent and acidic buffer pH-2 in sample solvent system. There was not much variation observed in the difference absorbance values indicating stability of the prepared analyte solution.

Method validation

Linearity

The linearity of the method was assessed by performing linear regression analysis for the calibration curve constructed between concentration and absorbance shown in fig 11. The response of the drug was found to be linear in the investigational concentration range 20-100mcg/mL. The overlay difference spectra of ambrisentan standard solution were shown in fig. 12. The calibration curve was found to be linear an r value 0.9994 and regression

equation was y= 0.0101x+1.0236. For these studies the obtained r^2 value was appropriate to demonstrate the linearity of the method.

Table 3. Linearity data for ambrisentan

S.No.	Concentration (mcg/mL)	Absorbance
1	20	1.234
2	40	1.415
3	60	1.623
4	80	1.829
5	100	2.032

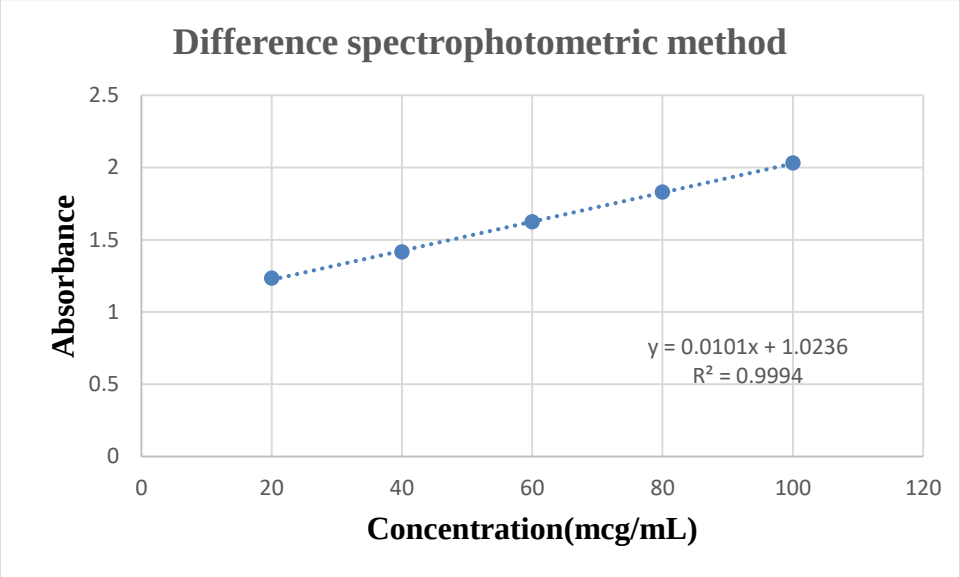

Fig.11. Calibration curve of ambrisentan at 263nm

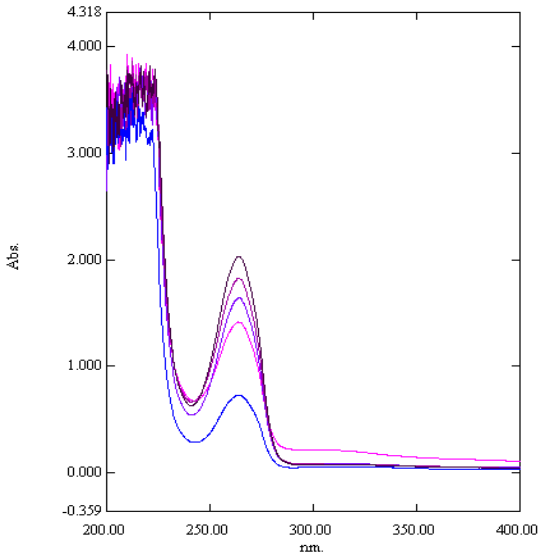

Fig. 12. Overlay absorption of ambrisentan (20-100mcg/mL)

Sandell's sensitivity

The Sandell's sensitivity is the concentration of the analyte (mcg/mL) which results in an absorbance of 0.001 in a cell of path length 1.0 cm path length. Units: mcg /cm^2

It was calculated using to the following equation:

Sandell's sensitivity (л) = Conc. (mcg/100 ml) x 0.001/D1 value

= 20 x 0.001/ 1.236

= 0.016mcg/ cm^2

Limit of Detection (LOD) and Limit of quantitation (LOQ)

The difference spectrophotometric method developed was sensitive and can be used for trace analysis with detection level 2.842mcg/mL and quantitation level 8.613mcg/mL. They are calculated by using standard deviation method.

Precision

Precision of the method was determined and reports in terms of repeatability and intermediate precision.

Repeatability

Repeatability determined with six replicates of 10mcg/mL Ambrisentan solutions (for both drug substance and drug product). The absorbance values was found to be same and calculated %RSD was found to be less than 2.

Intermediate precision

Intermediate precision determined by checking variation of results within the same day (Intra-day) and between days (Inter-day) was reported by calculating relative standard deviation.

Intra-day precision

The intra-day precision of the proposed method was determined on samples of both the drug at various concentration levels (48mcg/mL, 60mcg/mL, 72mcg/mL for ambrisentan) by analyzing three replicates of each sample as a single assay run at 263nm. The % RSD was calculated for each concentration.

Inter-day precision

Inter-day precision of the developed method was determined by analyzing three replicates of three different concentration samples (48mcg/mL, 60mcg/mL, and 72mcg/mL for ambrisentan) for three consecutive days at 263nm. The %RSD was calculated and found to be within limits.

The results revealed that percentage relative standard deviation (%RSD) values are within the limits hence the method is repeatable. There is no much difference in absorbance values measured in sessions of the day and low % RSD values indicate that the method was precise. Low RSD values indicate high precision of the method for the determination of ambrisentan in pure form and in dosage forms.

Accuracy

For drug substance

Accuracy is the closeness of test results to the true value. It describes the systematic error of the measurement results. Accuracy for drug substance was determined on samples of drug solutions at varying concentration levels in the range of 80%-120% (48mcg/mL, 60mcg/mL, and 72mcg/mL) by analyzing three replicates.

For drug product (Recovery study)

Accuracy of the method for drug product was determined by recovery studies, carried out by adding a known amount of standard drug (60mcg/mL) to sample solution (48mcg/mL, 60mcg/mL, and 72mcg/mL). The % recovery was calculated and reported. The obtained values of recovery studies were in the range of 90 -109% which indicates that the proposed method is accurate.

Assay of fixed dose combination tablets

The validated UV spectrophotometric method was applied for analysis of in commercially available Pulmonext (5mg) and Ambrican (5mg) tablets. Five tablets were weighed and powdered. An accurately weighed portion of the tablet powder equivalent to about 25mg of drug was transferred to a 50 mL volumetric flask, to this 25mL of buffer was added, shaken for 15 min in sonicator. After sonication the volume was adjusted with the solvent to get the concentration of 500mcg/mL ambrisentan. The solution was the filtered through whatmann filter paper. From the

above stock solution 5mL of sample (500 mcg/mL) was transferred into the 25mL volumetric flask and the volume was made up to the mark with buffer to get the desired concentrations of 100 mcg/mL. The samples are analysed by using difference spectrophotometric method. The results are given in table. 4 and the assay spectrum of the sample is shown in fig 13 and 14 for two brands.

Table 4.Assay data of formulations

Brand name	Drug name	Label claim(mg)	Amount found(mg)	Assay (%)
Ambrican	Ambrisentan	5	89	98
Pulmonext	Ambrisentan	5	92	109

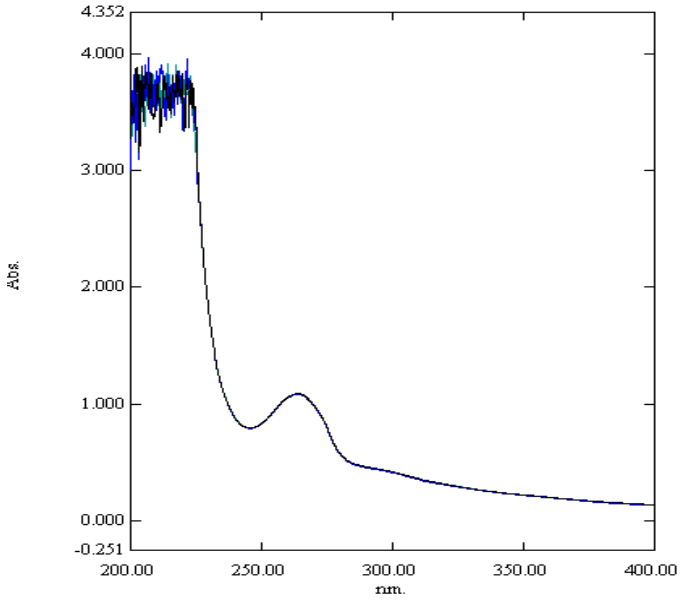

Fig 13.Assay Spectrum of Ambrican tablets

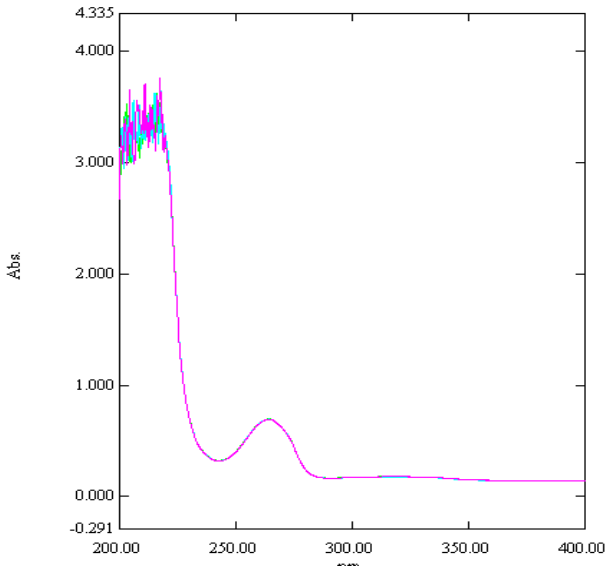

Fig 14. Assay Spectrum of Pulmonext tablets

Ambrisentan in tablets was found to be 98% and 113%. The results of assay revealed that the drug content of the commercially available product was within the acceptable limits described in the Indian Pharmacopoeia (90-110%)

CONCLUSION

Two new derivative and difference spectrophotometric methods were developed for the determination of ambrisentan in tablets by UV spectrophotometry. Both methods were developed and optimized using phosphate buffer as solvent system. These methods were developed and optimized with instrumental conditions; for each methods absorbance was measured at 263nm respectively. The methods were found to be precise and accurate. The validated methods were successively applied for the analysis of ambrisentan in pharmaceutical dosage forms. These methods were validated according to ICH guidelines and the results of the parameters were indicated within the limits. Due to high sensitivity and simple sample preparation, the methods described can be used for quality control studies. Moreover, simple spectrophotometric methods have obvious advantages over sophisticated instrumental analysis such as HPLC. This method was validated for precision, linearity and accuracy as per ICH guidelines. All the above parameters lead to the conclusion that the proposed method is linear, accurate, precise, reliable, simple, sensitive and cost effective and can be applied successfully for the routine estimation of ambrisentan in bulk and pharmaceutical formulation. From the results it can be concluded that the proposed first derivative and difference spectrophotometric method is effective for the analysis of ambrisentan in pharmaceutical dosage form and without any interference of other constitute in the formulation. The first and second order derivative spectroscopy can be converted in to higher derivative spectroscopy like third, fourth and fifth derivative technique for combination of two to three drugs. The difference spectrophotometric method can increase sensitivity by changing the pH of the solvent system. This method compensates spectral interferences of other absorbing components.

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