



SPECTROPHOTOMETRIC DETERMINATION OF AN AZILSARTAN MEDOXOMIL DRUG IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

A new simple, precise, sensitive, accurate and reproducible spectrophotometric method have been developed for determination of azilsartan medoxomil (Azutan) in pure and dosage forms. Method is based on oxidation of drug with 1, 10 phenanthroline producing orange colour chromogen which is measured at 510 nm. Beer's Law is obeyed in the concentration range of 1.0 -7 μ g/ml. for the developed method. The molar absorptivity and sandell's sensivity are found to be $0.59 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $0.0733 \mu\text{g/cm}^2$ respectively. Different experimental parameter affecting the colour development and stability of coloured products are carefully studied and optimized. The developed method could be successfully applied to pharmaceutical formulations. The result obtained are in good agreement with those obtained using standard method.

KEYWORDS: Azilsartan medoxomil, spectrophotometric method 1,10 phenanthroline.

INTRODUCTION

Azilsartan Medoxomil is a prodrug of azilsartan, It is marketed as "Edarbi" by Takeda. It is practically insoluble in water, freely soluble in methanol and dimethylsulfoxide, soluble in acetic acid, slightly soluble in acetone and acetonitrile, and sparingly soluble in tetrahydrofuran and 1-octanol.^[1]

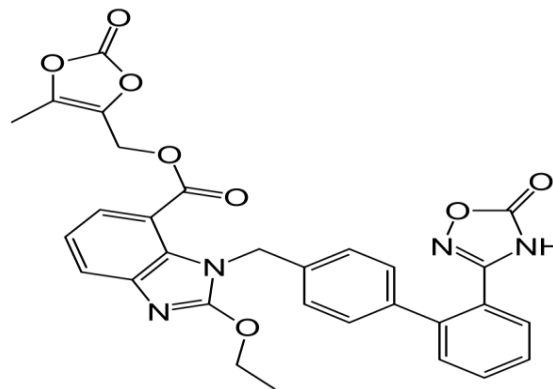
It is used for the treatment of mild to moderate essential hypertension.^[2] Azilsartan medoxomil is an angiotensin II receptor antagonist. Angiotensin II is a hormone that contracts blood vessels and reduces water excretion through the kidneys. Upon hydrolysis, azilsartan selectively and competitively binds to the AT1 subtype angiotensin II receptor and blocks the binding of angiotensin II to the receptor^[3], thus promoting vasodilatation and counteracting the effects of aldosterone. Thus Azilsartan medoxomil lowers blood pressure Azilsartan medoxomil has an ability to remain tightly bound to AT1 receptor for very long period of time. Azilsartan medoxomil was found to be superior to olmesartan and valsartan.

Pharmacokinetics of Azilsartan medoxomil suggests that it is quickly absorbed from the gut. Maximal blood plasma concentrations are reached after one to three hours. The liver enzyme CYP2C9 converts it in the two main metabolites, they are the *O*-deethylation and decarboxylation products of azilsartan. which are pharmacologically inactive. Elimination half life is about 11 hours. 55% are excreted via the faeces, and 42% via

the urine, of which 15% are present as azilsartan and the rest in form of the metabolites.^[4]

The drug formulation contains the potassium salt of azilsartan medoxomil (codenamed TAK-491), an ester of azilsartan's carboxyl group with the alcohol Its molecular formula is C₃₀H₂₄N₄O₈ Its IUPAC name is (5-methyl-2-oxo-1,3-dioxol-4-yl)methanol. This ester is more lipophilic than azilsartan itself. Azilsartan medoxomil (Azilsartan Medoxomil) is a white to nearly white powder with molecular mass: 456.48g/mol.

Structure of azilsartan medoxomil



The literature survey shows that many spectrophotometric and chromatographic methods^[5-9] for assaying and estimation of azilsartan medoxomil in

pharmaceutical dosages which are having some advantages as well as limitations.

The aim of the present work is to develop simple method for the determination of Azilsartan medoxomil in different dosage form. The proposed methods are comparable with reported method with respect to sensitivity moreover the methods neither require extraction nor prior separation of the drug.

MATERIALS AND METHODS

Instrumentation

An systronics UV-VIS Spectrophotometer-118 Model with 1cm length quartz coated optics; Wavelength range 190-1000nm; High stability, linearity, precision instrument is used for all the spectral measurements. All chemicals and reagents used in the analysis are of analytical grade and doubly distilled water is used for the preparation of all the solutions.

MATERIALS AND METHODS

Preparation of Standard solution of drug

An accurately weighed 40 mg of Azilsartan Medoxomil is dissolved in 50 ml of methanol. The final volume is adjusted with 50% methanol to 100ml in standard flask.

Preparation of Reagents

- 0.241% (w/v) Fe (III) solution is prepared by dissolving 241mg of anhydrous ferric ammonium sulphate in 100ml of double distilled water,
- 0.991% (w/v) o-phenanthroline is prepared by dissolving 991mg of the reagent in 100ml of alcohol
- 0.15% (v/v) O-phosphoric acid solution is prepared by diluting 0.15 ml of laboratory reagent (AR Grade) of o-phosphoric acid to 100ml with distilled water.

Experimental Procedure

Different portions (0.5-7.0 ml, 20 µg/ml) of standard Azilsartan medoxomil solution is delivered into a series of 25mL standard flask and then 1.0 mL of 5.0×10^{-3} M of Fe (III) solution, 1.0 mL of 5.0×10^{-2} M o-phenanthroline are added successively. The total volume in each tube is brought to 20 ml with distilled water. The flasks are kept on a boiling water bath for 60 min. The tubes are removed and cooled to room temperature. 1.0 ml of 2.0×10^{-2} M of o-phosphoric acid is added and volume in each flask is made up to the mark with distilled water. The absorbance of the colored complex solution is measured after 5 min against a reagent blank prepared similarly except drug and maximum absorbance is found to be at 510nm (Fig.1). The amount of the Azilsartan drug is computed from the appropriate calibration graph (Fig.2).

Analysis of pharmaceutical sample

Tablets powdered equivalent to 40 mg of the drug is weighed accurately and transferred into 100 ml beaker and shaken with 50 ml methanol. The resulting solution is filtered into 100ml standard flask and volume is

adjusted with 50% methanol. Suitable aliquots of this solution used for the determination of Azilsartan Medoxomil contents by procedure describe earlier.

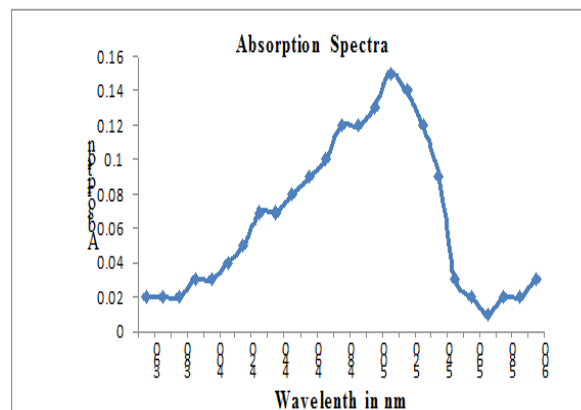


Fig.-1: Absorption spectra Azilsartan Medoxomil with Fe (III)/O-PHEN(50ppm).

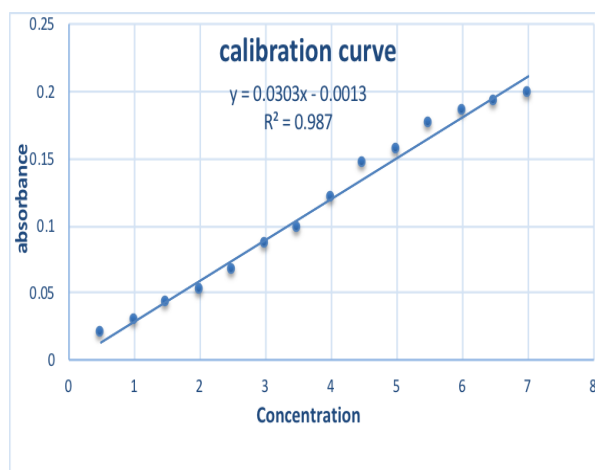


Fig.-2: Linear plot of Azilsartan Medoxomil with Fe (III)/O-PHEN.

The calibration curve is found to be linear over a concentration range of 0.5-7.0 ug/ml of Azilsartan.

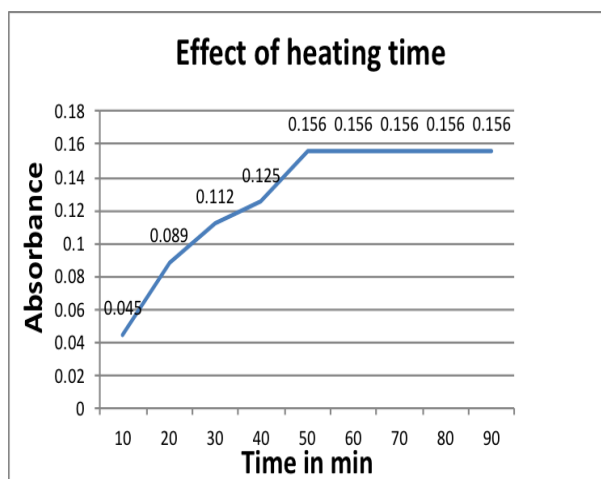


Fig.3: Effect of heating time on absorbance of developed system.

50 minutes are sufficient for full colour development hence 60 minutes time is selected for further studies.

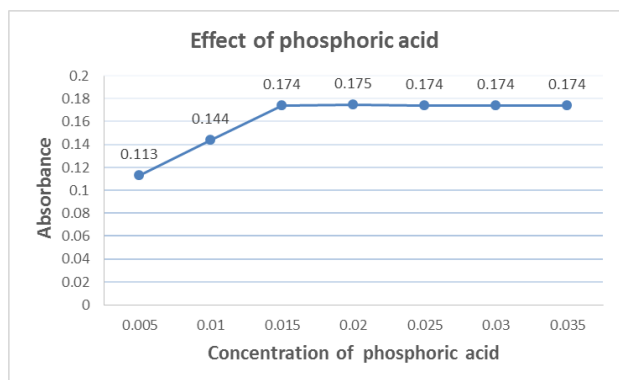


Fig.4: Effect of concentration of H₃PO₄ on colour development.

Absorbance remains constant after 0.015M concentration of H₃PO₄. Hence 0.02M H₃PO₄ is used for colour development and further studies.

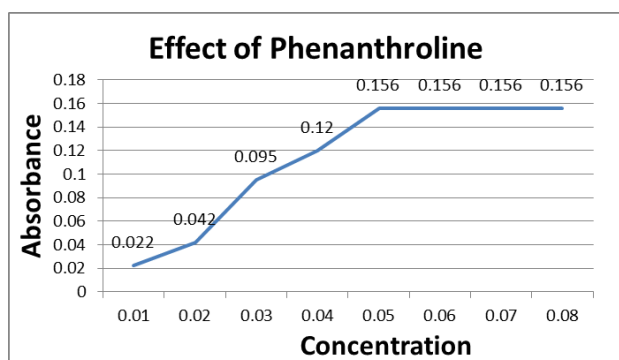


Fig.5: Effect of concentration of 1,10 phenanthroline on absorbance of developed system.

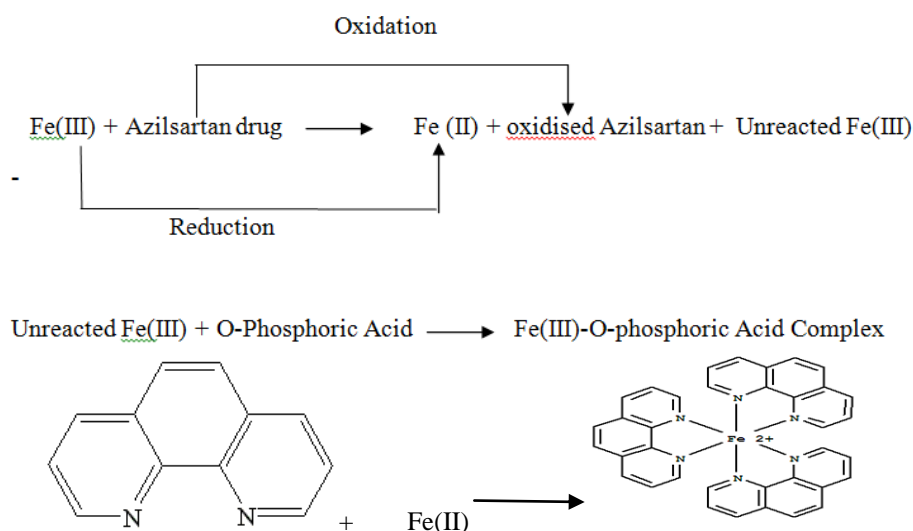
0.05 M Concentration of 1,10 Phenanthroline is sufficient for full colour development. Hence it is used for further study

RESULTS AND DISCUSSION

Scheme of coloured product

Ferric salts play a prominent role in spectrophotometric determination of many pharmaceutical drug substances acting as an oxidant^[12,13,14]. The Fe⁺³/O-phenanthroline (Fe⁺³/Phen) system is valuable reagent for any analytes with reducing properties, because the final product is intensely colored and extractable chelate [Fe(phen)₃]²⁺. Drug Azilsartan undergo oxidation by FPL reagent in a weakly acidic medium, forming an orange red colored complex with absorption maximum at 510 nm. The optimum reaction parameters were established via a number of preliminary experiments.

Ferric salt oxidises the drug and is itself converted into a ferrous salt and can be easily detected by the usual reagent O-Phenanthroline. The reaction product is tris complex of Fe (III), well known as ferroin. The colored product of the reaction is given below.



In order to test whether the coloured product formed in this method adhere to Beer's Law, the absorbance at maximum wavelength of series of eight concentrations are plotted against concentration of drug in $\mu\text{g}/\text{cm}^3$. Beer's Law is obeyed within the limits 0.5 to 7 $\mu\text{g}/\text{ml}$ of cetirizine, Molar absorptivity and sandell sensitivity are

found to be $0.59 \times 10^4 \text{ Lmol}^{-1}\text{cm}^{-1}$ and $0.0733 \mu\text{g}/\text{cm}^2$ respectively. Regression analysis of Beer's Law plots at λ_{max} reveals a good correlation. The graph show negligible intercept and described by regression equation $y = 0.0303X + 0.0013$. Where Y is the absorbance of 1 cm layer, b is the slope, a is the intercept and C is the

concentration of the measured solution in $\mu\text{g/mL}$) The high molar absorptivity of resulting coloured complex indicates the high sensitivity of method.

Limit of detection (LOD)

LOD was calculated based on standard deviation of response and the slope of calibration curve. The limit of detection was expressed as. $\text{LOD} = 3.3 \times \sigma/S$

Where σ is the standard deviation of intercept, S is the slope of calibration curve.

Limit of Quantitation (LOQ)

LOQ was calculated based on standard deviation of intercept and slope of calibration curve.

In this method the limit of quantitation is expressed as.

$$\text{LOQ} = 10 \times \sigma/S$$

Where σ is the standard deviation of intercept, S is the slope of calibration curve.

The results were summarized in table above indicating good sensitivity of proposed method. According to USP validation guidelines (TUSP, 2002).

Table-1: Optical characteristics, Regression parameters, Precision and Accuracy of the proposed method.

Parameters	Method
Maximum Wavelength λ_{max}	510 nm
Beer's Law Limits $\mu\text{g/mL}$	0.5- 7.0
Sandell's Sensitivity ($\mu\text{g/cm}^2 / 0.0001$ Absorbance)	0.0733
Molar Absorptivity L/mole/cm	0.59×10^4
Slope(b) ^a	0.0303
Standard Deviation (S) on slope	0.001004
Intercept(a) ^a	0.0013
Standard Deviation (S) on Intercept(a) ^a	0.004276
LOD	0.4657
LOQ	1.41122

^aRegression equation $Y = a + b C$, Where Y stands for absorbance and C is concentration in $\mu\text{g/mL}$ %Relative standard deviation is calculated for ten determination

The proposed method has been used for the analysis of Azilsartan. The result obtained are comparable with standard method^[6] (Table- 2).

Table 2: Analysis of Pharmaceutical Formulations of Azilsartan.

Drug	Manufacturing company	Labelled amount(mg)	*Amount found by Proposed MethodA (mg)	*Amount found by Proposed Method B(mg)	*Amount found by HPLC Method(mg)
Azilsartan Medoxomil	Synokem	40	39.76	39.79	39.87
ABEL	Lupin	40	39.86	39.75	39.88
AZILCAD	Cadila	40	39.65	39.86	39.96

* Average of three determinations

CONCLUSIONS

The developed method is simple, sensitive, accurate and reproducible. This method can be successfully applied for the analysis of pharmaceutical formulations in any laboratory.

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