SPECTROPHOTOMETRIC DETERMINATION OF AZILSARTAN MEDOXOMIL IN A PHARMACEUTICAL PREPARATION BY POTASSIUM PERMANGANATE AND SULPHANILIC ACID

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ABSTRACT

For the detection of Azilsartan Medoxomil two simple and sensitive spectrophotometric methods are developed. The first method A is based on the addition of excess $KMnO_4$ of known concentration in the presence of 2M H_2SO_4, reactants are allowed to react and the unreacted $KMnO_4$ is estimated with a fixed amount of Methyl Orange by measuring the absorbance at 510nm. Beer's law was obeyed in the concentration range of 0.5- 5 μg /ml Molar absorptivity was found to be 4.25 X 10^4 L $mol^{-1}cm$. The second Method B is based on the formation of orange color as a result of reaction between drug and diazotised Sulfanilic acid absorbance was measured at 510 nm. All parameters affecting the development of the color were investigated and the conditions were optimized. Under the optimum condition, Beer's law was obeyed in the concentration range 1- 8 μg/ml. Molar absorptivity was found to be 1.348 X 10^4 L mol^{-1} cm^{-1}. The proposed methods are well suited for determination of Azilsartan Medoxomil in pharmaceutical formulations.

KEYWORDS: Azilsartan Medoxomil, Potassium Permanganate, Daizotised Sulphanilic Acid, Spectrophotometry, methylorange, Oxidation.

INTRODUCTION

Azilsartan Medoxomil is a prodrug of azilsartan, It is marketed as "Edarbi" by Takeda. It is used for the treatment of mild to moderate essential hypertension. Azilsartan medoxomil is an angiotensin II receptor antagonist. Angiotensin II is a hormone that contracts blood

vessels and reduces water excretion through the kidneys.[1] Upon hydrolysis, azilsartan selectively and competitively binds to the AT1 subtype angiotensin II receptor and blocks the binding of angiotensin II to the receptor[2,3], 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 $C_{30}H_{24}N_4O_8$ Its IUPAC name is (5-methyl-2-oxo-1,3-dioxol-4-yl)methanol. This ester is more lipophilic than azilsartan itself. Azilsartan medoxomil is a white to nearly white powder with molecular mass: 456.48g/mol. It is practically insoluble in water and freely soluble in methanol.

The U.S. Food and Drug Administration approved azilsartan medoxomil on 25 February 2011, for the treatment of high blood pressure in adults.[5] Health Canada approved the drug on March 8, 2012, for mild to moderate essential hypertension.[6] Azilsartan medoxomil has been currently approved for use in the United States, Japan and Europe.
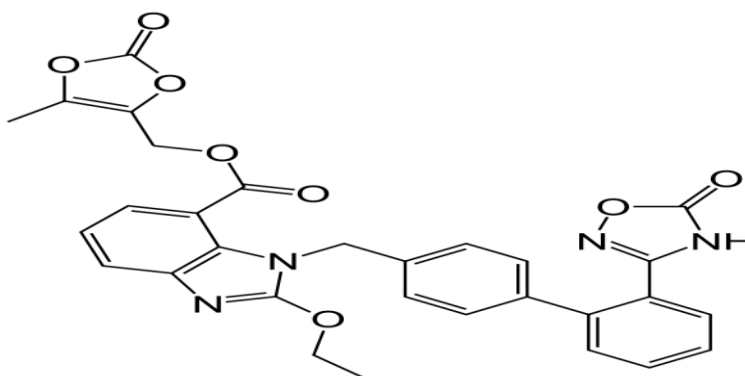

Fig. 1: Structure of azilsartan medoxomil.

The literature survey shows that spectroscopic and chromatographic methods[7,13] for assaying and estimation of azilsartan medoxomil in pharmaeutical dosages.

The present investigation describe two visible Spectrophotometric methods using KMnO₄ as an oxidizing agent and Sulphanilic Acid as a coloring reagent respectively Simplicity, sensitivity, wide linear ranges, mild experimental conditions and above all cost effectiveness characterize the proposed methods. Further the methods were found to possess adequate accuracy and precision.

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

a) Apparatus

An systronics UV-VIS Spectrophotometer-118 Model with 1cm length quartz coated optics; Wavelength range190-1000nm; High stability, linearity, precision instrument is used for all the spectral measurements.

b) Reagents and Materials

For this research project all chemicals used were of analytical grade and double distilled water was used to prepare all solutions.

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

Method A

- **P**otassium Permanganate (0.001) mol/lit was prepared by dissolving about 0.0158gm of chemical (Merck, Mumbai, India) in water and diluting to 100 ml, and standardized[17] using H.A Brights Procedure (A.I. Vogel, 3rd edition, 1961, pg.no280). Stock solution of KMnO4 was further diluted to get the working concentration of $63.2 \mu g\ ml^{-1}(4\ x10^{-4}M)$

- A stock solution of Methyl Orange (160 µg ml^{-1}) was prepared by dissolving the dye (Himedia Laboratories Pvt, Limited, Mumbai) in distilled water. The dye solution was diluted to 80 µg ml^{-1}

- Concentrated H$_2$SO$_4$ (S.D. Fine Chem Limited, Mumbai) diluted appropriately with distilled water to get 2M acid solution.

Method B

Diazotised Sulfanilic Acid: 200 mg of sodium nitrite was taken into a 100 ml volumetric flask and dissolved in 60 ml of distilled water. One ml of hydrochloride acid was added and allowed to stand for one hr. 500 mg of Sulfanilic acid was then added and the final volume was made up to the mark with purified water. This reagent was used after 30 minutes of preparation. This reagent should be freshly prepared for analytical work. This is called colouring agent.

Experimental Procedure

Method A: Aliquots of a drug solution (1 to 5mL, 20µg/ml) were transferred into a series of 10ml calibrated flask. To each flask, 1ml of 2M H$_2$SO$_4$ was added, followed by 1mL of KMnO$_4$ solution (63.2 µg ml^{-1}). The contents were mixed and the flasks were set aside for 15 min under occasional shaking. Finally, 1ml of (80 µg ml^{-1}) Methyl Orange solution was added to each flask, diluted to the mark with water and the absorbance of solution was measured at 510 nm against a reagent blank.

Method B: Into a series of 20 ml calibrated flasks (1 to 8 ml, 20µg/ml) of pure Azilsartan medoxomil was taken with the help of burette and 10 ml of coloring reagent was added. The final volume was made to 20 ml with NaOH (1M). The absorbance was measured at 510 nm after 5.0 minutes after dilution.

Assay Procedure for Tablet

Ten tablets were accurately weighed and powdered. A Portion of tablet powder equivalent to 40 mg of azilsartan medoxomil was accurately weighed and transferred into 100 ml beaker and shaken with 50 ml methanol by following standard method. The standard 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 both the as procedure describe earlier.

THE RESULT AND DISCUSSIONS

Method Development

Method A: $KMnO_4$ plays a prominent role in spectrophotometric determination of many pharmaceutical drug substances acting as an oxidant[14] The proposed spectrophotometric methods are indirect after allowing the reaction between $KMnO_4$ and drug the excess amount of $KMnO_4$ is to be determined. The excess of $KMnO_4$ was made to react it with a fixed amount of Methyl Orange dye. $KMnO_4$ brings oxidative destruction of the (bleaches) dye. Drug when added in increasing concentrations to a fixed concentration of $KMnO_4$, consumes the latter proportionally and there occurs a fall in the concentration of $KMnO_4$. When a fixed concentration of dye is added to decreasing concentrations of $KMnO_4$ a increase in the concentration of dye is obtained. Consequently, a proportional increase in the absorbance at the respective λmax is observed with increasing concentration of drug.
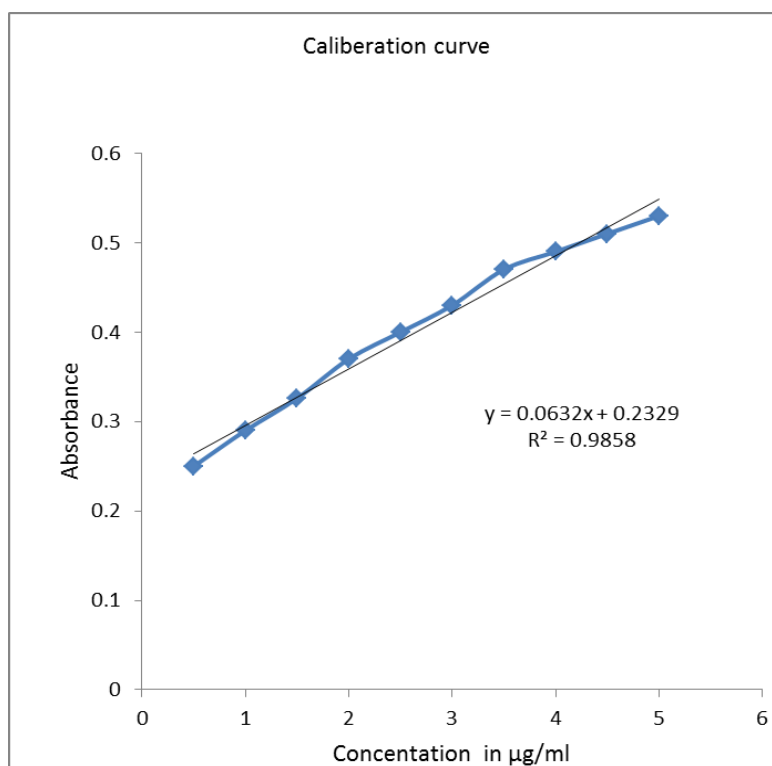

Fig. 2: Calibration Curve for Method A.

Optimisation of Parameters for Method A

In order to determine the maximum concentrations of Methyl Orange spectrophotometrically preliminary experiments were conducted by measuring the absorbance of their acidic solutions at their respective λmax and 8 µg ml^{-1} of Methyl Orange was found to be the upper limits. $KMnO_4$ concentration of 6.32µg ml^{-1} was found to be sufficient to bleach the color

due to 8µg ml^{-1} Methyl Orange. Hence different amounts of drug was made to react with 6.32 µg ml^{-1} KMnO$_4$ in this method before determining the residual KMnO$_4$ as described under the respective procedure.

Sulphuric acid was found to be a convenient medium for these methods.

For a quantitative reaction between drug and KMnO$_4$, a contact time of 15min was found sufficient. It is evident from the fig given below.
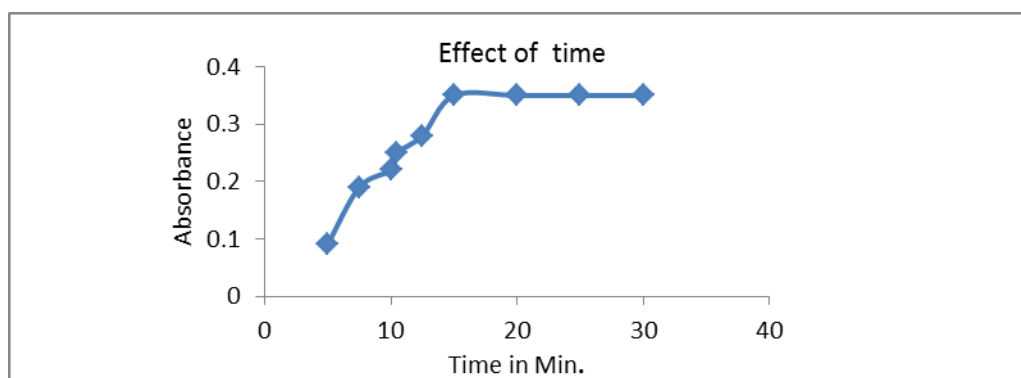

Fig. 3: (Effect of contact time for the formation of color product in method A).

Method B: Coloring reagent (diazotized sulphanilic acid) plays an important role in spectrophotometric determination of many pharmaceutical drug substances.[15,16] Coloring reagent was prepared by diazotization reaction with Sulphanilic Acid. In second method reaction proceeds with increasing absorbance This reagent react with Azutan to form Orange colored complex.

Optimisation of Parameters for Method B
The optimum concentration of Sulphanilic acid was 500 mg and of Sodium Nitrite was 200 mg, optimum volume of coloring reagent was 10 ml. The absorbance was measured between first 10 minutes.
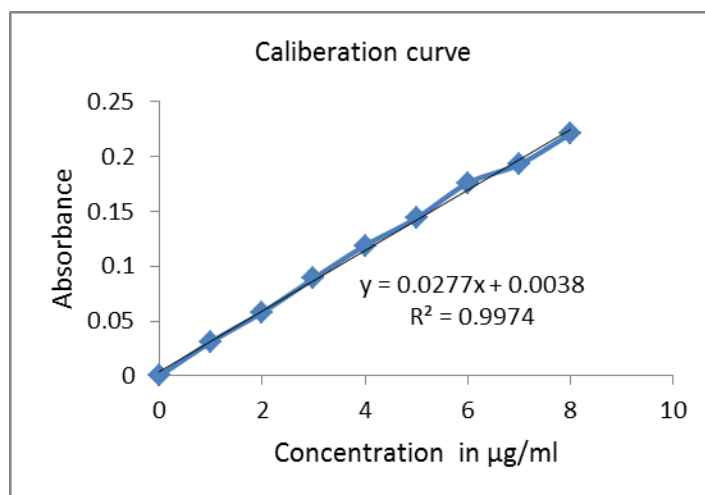
Fig. 4: Calibration Curve for Method B.

Method Validation

The developed method were validated for it's accuracy, precision, reproducibility and selectivity. Also the experiment was repeated three times in a day to determine intra-day precision and on three different days to determine inter-day precision. The percent relative standard deviation was calculated at each concentration level and the results are tabulated. The Reproducibility was confirmed by repeating the three different analyst and the % RSD was calculated

Table 1: Evaluation of Precision of the proposed spectrophotometric methods for Azilsartan medoxomil.

Sr.No	Amount taken (µg/ml)	Amount found (µg /ml) Method A(KMnO4 & Methylorange)	Amount found (µg/ml) Method B(sulphanilic acid)
1	2	1.96666 ± 0.15055 variation with 95% confidence limit=0.120463	1.983333 ± 0.11690 variation with 95% confidence limit=0.093542
2	5	4.983333 ± 0.14719 variation with 95% confidence limit=0.117779	5.10667 ± 0.075277 variation with 95% confidence limit=0.060227

Accuracy and Precision of the proposed methods

Accuracy and precision was checked according to USP validation guidelines (TUSP, 2002) at three concentration levels within the specified range, six replicates measurements were recorded at concentration levels. The results are summarized in (table-1) below.

Table 2: Optical characteristics, Regression parameters, Precision and Accuracy of the proposed method.

Parameters	Method A	Method B
Maximum Wavelength λ_{max}	510	510
Beer's Law Limits µg/mL (Linearity Range)	0.5- 5 µg/mL	1-8 µg/mL
Sandell's Sensitivity (µg/cm^2 /0.0001 Absorbance)	0.0108	0.0338
Molar Absorptivity Lt/mole/cm	4.25×10^4	1.34×10^4
Slope(b)[a]	0.0632	0,0277
Standard Deviation on slope	0.003723	0.000535
Intercept(a)[a]	0.2329	0.0038
Standard Deviation on y intercept	0.011551	0.002547
LOD (µg/ml)	0.6048	0.303
LOQ (µg/ml)	1.833	0.9194

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.

LOD= $3.3 \times \sigma/S$

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).

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.

LOQ= $10 \times \sigma/S$

Application to Formulation

The proposed methods are applied to the determination of drugs in tablets. The results in **Table 3** showed that the methods are successful for the determination of drugs and that the excipients in the dosage forms do not interfere. The results are compared to the available validated reported methods on each drug and the results agree well.

Table 3: 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[10] Method(mg)
AZUTAN	Synokem	40	39.76	39.79	39.80
ABEL	Lupin	40	39.86	39.75	39.79
AZILCAD	Cadila	40	39.79	39.85	39.84

ACKNOWLEDGEMENTS

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