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Development and Validation of HPTLC Method for Estimation of Azilsartan Medoxomil in Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

Azilsartan is an angiotensin II receptor antagonist used in the treatment of hypertension. U.S. Food and Drug Administration (FDA) approved azilsartan medoxomil for the treatment of high blood pressure in adults. In present work, a new simple, sensitive, accurate and economical densitometric method has been developed and validated for the estimation of Azilsartan medoxomil (AM) in bulk and pharmaceutical dosage forms. Separation of the drug was carried out using ethylacetate-n-hexane 7:3 (v/v) as mobile phase on precoated silica gel 60 F_{254} plates. The retention factor (R_f) for AM was 0.65 ± 0.05. The detection of band was carried out at 249 nm. The calibration curve was linear in the concentration range 100 to 700 ng per band with correlation coefficient (r^2 =0.999). For AM, the recovery study results ranged from 99.26 to 100.65 % with RSD values ranging from 0.183 to 2.23 %. The assay [%] was 99.786 ± 0.203 in tablet formulation tested. Results of the analysis were validated for accuracy, precision, robustness and were found to be satisfactory. The proposed method is simple, rapid, and suitable for the routine quality control analysis.

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INTRODUCTION

Azilsartan medoxomil (Fig-1) is an angiotensin II receptor antagonist which has the chemical names (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 2-ethoxy-1-{[2'-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]methyl}-1H-benzimidazole-7-carboxylate monopotassium salt and 1H-Benzimidazole-7-carboxylic acid,1-[[2'-(2,5-dihydro-5-oxo-1,2,4-oxadiazol-3-yl)[1,1'-biphenyl]-4-yl]methyl]-2-ethoxy-,(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester [1].

Azilsartan medoxomil is rapidly hydrolysed to the active moiety azilsartan by esterases in the gastrointestinal tract and/or during drug absorption. The enzyme carboxymethylenebutenolidase is a recently discovered hydrolysis mechanism for azilsartan medoxomil in the intestine and liver [2-4]. Azilsartan is an inverse agonist of the AT1 receptor, is a highly potent, selective and competitive antagonist of the angiotensin II type 1 receptor. Molecular basis of Azilsartan medoxomil reveals that it may be responsible for its clinical efficacy [5].

Literature survey reveals that Azilsartan medoxomil can be estimated by RP-HPLC in combination with other drugs and one more reported method in human plasma by solid phase extraction procedure [6, 8]. U.V. Spectrophotometric method reported for the estimation of Azilsartan medoxomil in bulk and pharmaceutical dosage forms [7].

To the date, there have been no published reports for quantitation of Azilsartan medoxomil by HPTLC in bulk and in tablet dosage form [9, 10]. The proposed method is validated as per ICH guidelines [11]. Present work describes a simple, accurate and precise method for the estimation of Azilsartan medoxomil in bulk and tablet formulations.

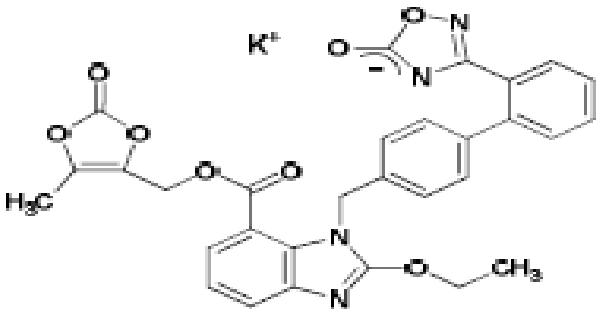

Figure 1. Chemical structure of Azilsartan Medoxomil

MATERIALS AND METHODS

Experimental

Reagents and Chemicals

Pure drug Azilsartan medoxomil and Edarbi (Azilsartan medoxomil-80), a prodrug was provided by our APL Research Centre-II. (A Division of Aurobindo Pharma Ltd). All the reagents and chemicals used were of analytical grade from Merck Chemicals, India.

Standard stock solution (1 mg mL^{-1}) of AM was prepared by dissolving 50 mg of drug in 50 mL acetonitrile. This solution (5 mL) was further diluted to 50 mL to furnish stock solution of 100 ng μL^{-1}.

Chromatography

The samples were spotted in the form of bands of width 6 mm with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel 60 F_{254} aluminum HPTLC plates (20 cm x 10 cm) with 250 µm thickness (E. Merck, Darmstadt, Germany) using a CAMAG Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110°C for 5 min, prior to chromatography.

Linear ascending development was carried out in a 20 cm x 10 cm twin-trough glass chamber (CAMAG) using the mobile phase ethyl acetate - n-hexane 7:3 (*v/v*). The chamber saturation time was 15 min. The development distance was 9 cm and the development time 20 min. The plates were dried in air with the help of a hair dryer. Densitometric scanning was performed with a CAMAG TLC Scanner 3 at 249 nm operated by Wincats software version 4.06. The source of radiation was a deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. The slit dimension was 5 mm x 0.45 mm and the scanning speed of 20 mm s^{-1}.

For preparation of the calibration plot aliquots (1-7 µL) of the fresh standard stock solution (100 ng μL^{-1}) of AM were applied by over spotting on a TLC plate and the plate was developed and scanned as described above. Each standard was analyzed in five replicates and peak areas were recorded. Calibration plot for AM was constructed separately by plotting peak area against respective concentration of AM.

Table 1. Summary of validation data for the proposed method

	Azilsartan Medoxomil [AM]
Detection wavelength [nm]	249
Beer's law limit [ng per band]	100-700
Correlation coefficient (r)	0.9996
Linear regression equation[a)] ($y = mx + c$)	
Intercept (c)	-144.29
Slope(m)	9.145
Detection limit [ng per band]	22
Quantitation limit [ng per band]	68

[a)]Where y is peak area and x is concentration (ng per band) $n = 5$

Validation

In accordance with the ICH guidelines, the method was validated for specificity, linearity, range, accuracy, precision, limits of detection, quantitation, and robustness. The specificity of the method was ascertained by analyzing standard drug and samples of equivalent concentration (200 ng per band). The band for azilsartan medoxomil in the sample was confirmed by comparing the R_f values and spectra of the band from the sample with those from the standard. The peak purity of azilsartan medoxomil was assessed by comparing the spectra at three different levels, i.e., peak start, peak apex and peak end. The linearity of the response (peak area) was assessed in the concentration range 100 to 700 ng per band. To check accuracy, recovery was studied by addition of standard drug solution at three different levels of 50, 100, and 150 % to pre-analyzed sample solution. Densitograms were obtained and the peak areas were noted. The concentrations of drug and thus mean percentage recovery was calculated from respective calibration curves. To study intra-day variation, six standard solution containing AM (200 ng per band) was analysed on the same day. To study inter-day variation, analysis of three replicates of standard solution of the same concentration was performed on three different days. The specificity of the method was ascertained by analyzing standard drug and sample. The spots for drug were confirmed by comparing the R_f values and spectra of the sample spots with those of

standard drug. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$ respectively; where σ is the standard deviation of the response (*y*-intercept) and *S* is the slope of the calibration plot. The robustness of the method was studied, by small but deliberate variation of chamber saturation period (±10 %), mobile phase composition (± 2%), and time from development to scanning (0, 30, 60, 90 min). One factor at a time was changed to study the effect. The robustness of the method was checked at concentration of 200 ng per band to study the effect on the peak area of the drug. Results of Robustness studies are reported in Table 3.

Analysis of Tablet Formulation

Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to (50 mg of AM) was weighed and transferred to a 50 mL volumetric flask containing approximately 25 mL acetonitrile, sonicated for 5 min, and the volume was made up to the mark with the acetonitrile. The solution was filtered using Whatman no. 41 paper. From the filtrate 5 mL was further diluted to 50 mL to furnish sample stock solution of AM (100 ng μL^{-1}). From sample stock solution, 2 µL was applied to an HPTLC plate to furnish final amount of 200 ng per band for AM. After chromatographic development peak areas of the bands were measured at 249 nm and amount of drug present per tablet was estimated from the respective calibration plots. The procedure was repeated six times for analysis of homogenous sample.

Table 2. Results from study of recovery of AM

Drug	Amount taken [ng/band]	Amount added [ng/band]	Total amount found [ng/band]	Mean recovery[%]	RSD[%][a]
AM	200	100	301.962	100.654	2.230
	200	200	397.031	99.258	1.043
	200	300	498.988	99.797	0.183

[a]Average from three determinations

Table 3. Results from testing of robustness, as RSD [%] of peak area.

Condition varied	Azilsartan Medoxomil
Time from chromatography to scanning [min]	0.355
Chamber saturation period [min]	0.425
Mobile phase composition [%]	0.652

RESULTS AND DISCUSSION

Method Development

Initially ethyl acetate and n-hexane both pure and mixed in different ratios were tried. Finally the mobile phase ethylacetate - n-hexane 7:3 (*v/v*) was selected as optimum for obtaining well defined peak. The retention factor for AM was 0.65 ± 0.05. The wavelength 249 nm was selected for detection because drug showed considerable absorbance at this wavelength (Figure 2). A densitogram obtained from a standard solution of AM is shown in Figure 3.

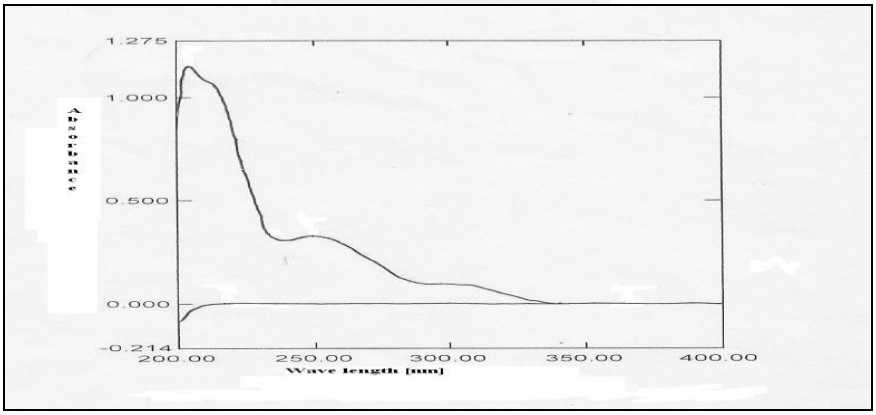

Figure 2. Spectra of AM measured from 200 to 400 nm

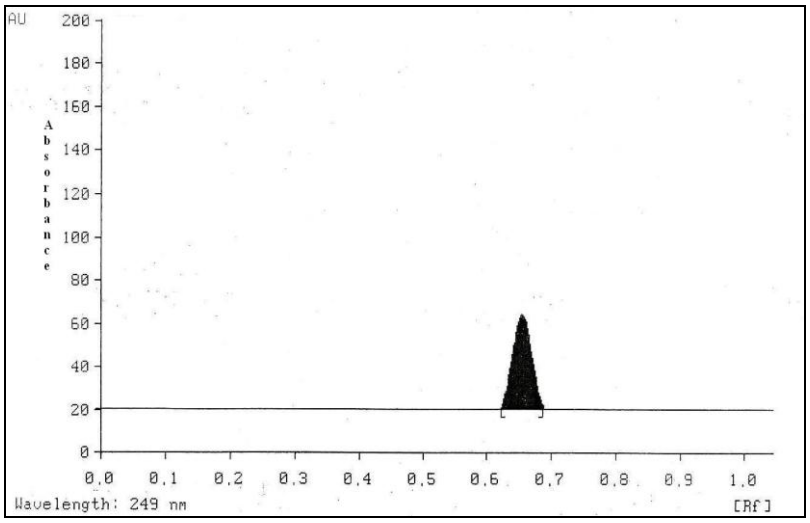

Figure 3. Standard chromatogram of AM (200 ng per band, R_F 0.65 ± 0.05)

Validation

The standard calibration plots were linear over a range 100-700 ng per band with correlation coefficient of 0.9996 ± 0.006. The validation data is summarized in Table 1.

Which gives Beer's law limit, linear regression equation, correlation coefficient, and LOD and LOQ for drug. Recovery study results ranged from 99.26 to 100.65 % with RSD values ranging from 0.183 to 2.23 %. Results of recovery studies are reported in Table 2. Intra-day variation, as RSD, was 0.325 % and inter-day variation, as RSD, was 0.395 %. The spectra acquired for AM extracted from the tablet was also compared with those acquired for AM standard, good correlation between the corresponding spectra indicated that the method is specific for the drug and no other tablet components interfere with the drug.

CONCLUSION

The validated densitometric method used in present study is accurate, precise, and robust, thus can be used for routine analysis of AM in tablet dosage form.

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