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SIMULTANEOUS DETERMINATION OF AZILSARTAN MEDOXOMIL AND CHLORTHALIDONE BY UV SPECTROPHOTOMETRIC METHOD

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ABSTRACT

Simple, precise and accurate UV-Spectrophotometric simultaneous equation method for estimation of Azilsartan medoxomil (AZM) and Chlorthalidone (CTD) were developed and validated as per ICH guidelines. This Method involves solving of simultaneous equations based on measurement of absorbance at two wavelengths 244 nm and 272 nm (λ_{max} of AZM and CTD) in methanol: water (80:20v/v). Both the drugs obey the Beer's law in the concentration ranges 5-25 and 10-50µg/ml for AZM and CTD respectively. % Recovery for both the drugs was in the range of 99.82±0.308 % indicating excellent accuracy. The methods were precise, with a relative standard deviation of less than 2% for both drugs. The developed methods were validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. Thus, method can be used for routine monitoring of drugs in industry for the assay of bulk drugs and commercial formulation.

Keywords: Azilsartan medoxomil, Chlorthalidone, Spectrophotometric analysis, Simultaneous equation method

INTRODUCTION

Azilsartan medoxomil (AZM) [(5-methyl-2-oxo-2*H*-1,3- dioxol-4-yl)methyl 2-ethoxy-1-({4-[2-(5-oxo-4,5-dihydro- 1,2,4-oxadiazol-3-yl)phenyl]phenyl}methyl)-1*H*-1,3-benzodiazole- 7-carboxylate] is an angiotensin (AT)-II-receptor blocker which

selectively blocks the binding of angiotensin II to the AT1 receptors found in the vascular smooth muscle and the adrenal gland, thereby promoting vasodilatation and a decrease in the effects of aldosterone. Chlorthalidone (CTD) [2-chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1*H*-isoindol- 1-yl)benzene-1-sulfon amide] is a thiazide-like diuretic which produces diuresis with increased excretion of sodium and chloride at the cortical diluting segment of the ascending limb of Henle's loop of the nephron by inhibiting Na^+ and Cl^- ions re-absorption by blocking the Na^+/ Cl^- symporter. The combination of AZM/CTD has demonstrated safety and efficacy in lowering blood pressure in hypertensive patients, thus it is used in the treatment of mild to moderate hypertension **[1]**. CTD is official in the Indian Pharmacopoeia (IP) 2018, British Pharmacopoeia (BP) 2018, European Pharmacopoeia (EP) 2018 and United States Pharmacopeia (USP) 2018. AZM is not official in any pharmacopoeia **[2-4]**. The chemical structures of AZM and CTD are shown in **Figure 1**.

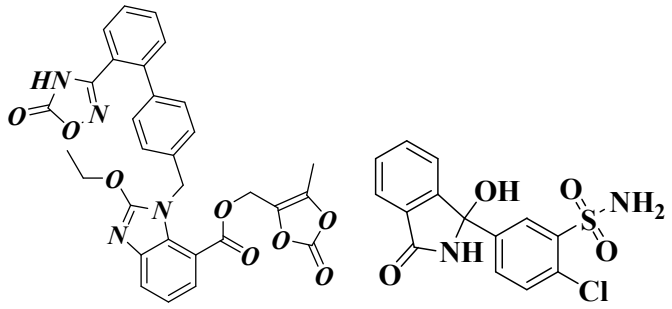

Figure 1: Chemical structure of (A) Azilsartan medoxomil and (B) Chlorthalidone

CTD has been determined either alone or in combination with other medications in different matrices using LC **[5, 6]**, LC–MS-MS **[7]**, HPTLC **[8, 9]**, chemiluminometry **[10]**, spectrophotometry **[11]** and capillary electrophoresis (CE) techniques **[12]**. Combination therapy of AZM and CTD proved to induce reductions in systolic and diastolic BP **[13]**. Accordingly, it was very important to develop a rapid and accurate method for the analysis of this combination. Few HPLC methods develop for the determination of both drugs **[14-16]**. However, no UV-spectrophotometric simultaneous equation method is available for simultaneous determination of the AZM and CTD in combined pharmaceutical dosage form. In the present study, an attempt was made to develop a simple, precise and accurate method for the simultaneous estimation of these drugs in combined pharmaceutical dosage form and validate as per International Conference on Harmonization (ICH) guidelines.

MATERIALS AND METHODS

Reagents and chemicals

AZM and CTD standard were obtained from Alembic Pharmaceutical (Baddi). Methanol, acetonitrile were procured from Rankem, RFCL Limited, New Delhi, India. The 0.45- mm pump nylon filter was obtained from Advanced Micro devices (Ambala Cantt, India). HPLC grade water was used throughout the study. Other chemicals used were of analytical or HPLC grade.

Instrument

In UV-spectrophotometric method, Labindia model-3000+ series were used, which is a wavelength accuracy ±1 nm, with 1cm quartz cells.

Method development

Preparation of Standard Stock Solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80ml methanol: water (80:20v/v) in 100 ml volumetric flask. The flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark 100ml with methanol: water (80:20 v/v) to get a concentration of 1000 µg/ml (Stock-A) for both drugs.

Preparation of Sub Stock Solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of AZM and CTD and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with methanol: water (80: 20v/v) that gave concentration of 100 µg/ml (Stock-B).

Preparation of Working Standard Solution

1) 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with distilled water. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for AZM.

2) Aliquots of 1 ml, 2 ml, 3 ml, 4 ml and 5 ml withdrawn with help of pipette from standard stock solution (Stock-B) separately in 10 ml volumetric flask and volume was made up to 10ml with distilled water. This gave the solutions of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml respectively for CTD.

Selection of wavelength for linearity

Solutions of 10 µg/ml of AZM and 10 µg/ml CTD were prepared separately. Both the solutions were scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of AZM and CTD was observed at 244.0 nm and 272.0 nm, respectively. AZM and CTD showed linearity in the concentration range of 5-25µg/ml and 10-50µg/ml at their respective maxima. Calibration curve was plotted,

absorbance versus concentration.

Study of Overlay Spectra

Working standard solution from the standard stock solution prepared in concentration 10µg/ml of AZM and 10µg/ml of CTD were scanned in the spectrum mode over the range of 200-400 nm against methanol: water (80:20 v/v) as blank and the overlain spectra of the two were recorded. AZM showed an absorbance peak at 244.0 nm, whereas CTD at 272.0 nm. The overlain spectra also showed isoabsorptive points at 260.0 nm **Figure 2-4**.

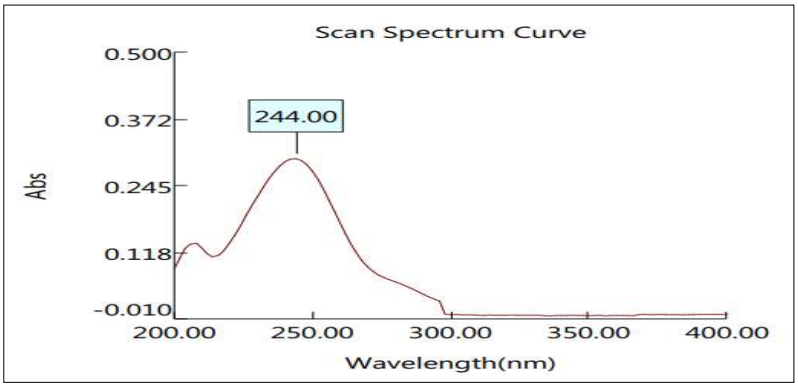

Figure 2: Determination of λ_{max} of Azilsartan medoxomil at 244 nm

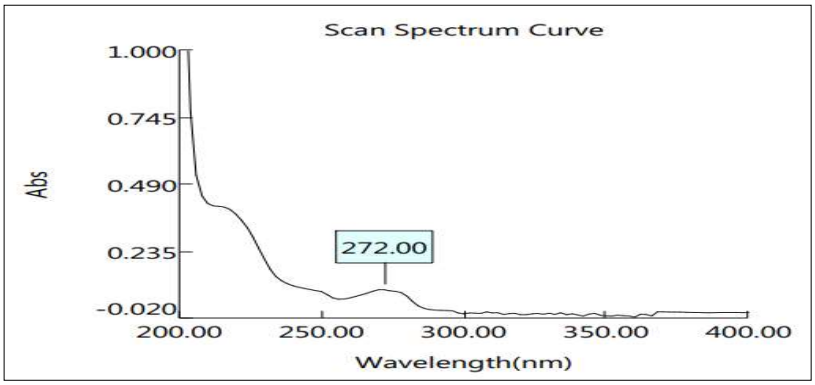

Figure 3: Determination of λ_{max} of Chlorthalidone at 272 nm

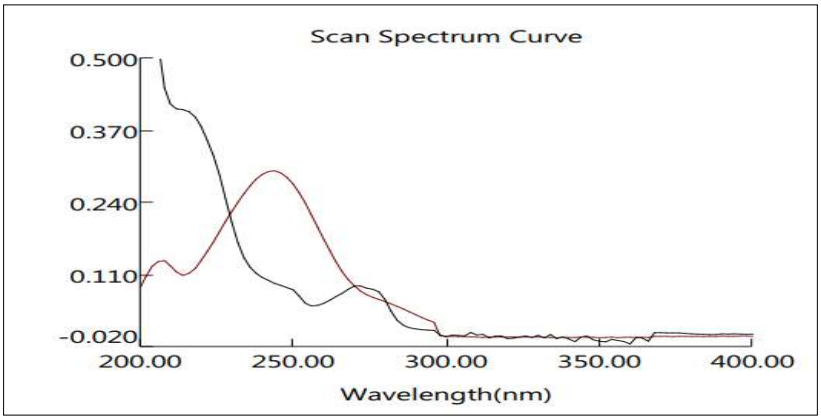

Figure 4: Overlain spectra of drugs (isoabsorptive points)

Simultaneous equation method (Vierordt's)

Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method. Simultaneous equation method is based on the absorption of drugs (X and Y) at the wavelength maximum of the other. Two wavelengths selected for the method are 244.0 nm and 272.0 nm that are λ_{max} of AZM and CTD respectively. The absorbances were measured at the selected wavelengths and absorptivities ($A^{1\%, 1cm}$) for both the drugs at both wavelengths were determined as mean of five independent determinations. Concentrations in the sample were obtained by using following equations.

$$C_x = \frac{A_1 a_{y_2} - A_2 a_{y_2}}{a_{x_1} a_{y_2} - a_{x_2} a_{y_1}} \quad \ldots\ldots\ldots\ldots\ldots \text{Eq. (1)}$$

$$C_y = \frac{A_1 a_{x_2} - A_2 a_{x_1}}{a_{x_1} a_{y_2} - a_{x_2} a_{y_1}} \quad \ldots\ldots\ldots\ldots\ldots \text{Eq. (2)}$$

Where, A_1 and A_2 are absorbances of mixture at 244.0 nm and 272.0 nm respectively, ax_1 and ax_2 are absorptivities of AZM at λ_1 (244.0 i.e. λ_{max} of AZM) and λ_2 (272.0 i.e. λ_{max} of CTD) respectively and ay_1 and ay_2 are absorptivities of CTD at λ_1 and λ_2 respectively. C_{CTD} and C_{AZM} are concentrations of AZM and CTD respectively.

Methods validation

Validation of the method was carried out in accordance with the International Conference on Harmonization Q2B guidelines 2005 **[17]**.

Linearity

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and estimate into the UV and the results was recorded. The results of linearity are reported in **Table 1**.

Accuracy

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study shown in **Table 2**.

Precision

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD are

less than 2 indicate the precision of method. Result of precision shown in **Table 3**.

Analysis of tablet sample

Twenty marketed tablets of AZM and CTD were weighed and ground to a fine powder; amount equal to 20 mg of AZM was taken in 10 ml volumetric flask and sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with hydrotropic solution. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with methanol: water (80:20 v/v) to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from Simultaneous Equation Method. The procedure was repeated for five times.

Table 1: Results of Linearity of Azilsartan medoxomil (AZM) and Chlorthalidone (CTD)

	Method	
Parameter	**AZM**	**CTD**
Working λ	**244 nm**	**272 nm**
Beer's law limit (µg/ml)	**5-25**	**10-50**
Correlation Coefficient (r^2)*	**0.999**	**0.999**
Slope (m)*	**0.030**	**0.012**
Intercept (c)*	**0.004**	**0.003**

Table 2: Results of Recovery Studies on Marketed Formulations

Recovery Level %	% Recovery (Mean±SD)*	
	AZM	CTD
80	99.03±0.501	99.04±0.287
100	99.31±0.394	99.05±0.400
120	99.19±0.560	99.82±0.308

*Average of three determination

Table 3: Results of validation (Mean±SD)*

Parameter		Method	
		AZM	CTD
Precision*	Repeatability	99.715±0.043	99.211±0.033
	Day-to-Day	99.530±0.038	99.592±0.080
	Analyst-to-Analyst	99.450±0.150	99.364±0.131
	Reproducibility	99.666±0.039	99.504±0.114

*Average of five determination

Table 4: Analysis of Tablet Formulation of AZM and CTD

Drug	Label claim (mg)	Amount found (mg)	Label claim (%)	S.D.	% RSD
AZM	40	24.838	99.72	0.290	0.291
CTD	25	39.886	99.352	0.297	0.299

RESULTS AND DISCUSSION

Method development by UV-Spectrophotometer is cost effective and time saving as compared to HPLC method of analysis **[18]**. Thus, for estimation of routine sample of drugs simple, rapid, sensitive and accurate analytical UV methods were utilized which reduces unnecessary tedious sample preparations and use of costly materials. To develop

suitable methods of analysis, various solvents were studied. Based on sensitivity of the method and non-toxic behaviour methanol: water (80:20v/v) was selected as a solvent for the methods. Overlain spectra **(Figure 4)** shows that at λ_{max} of AZM (244 nm) interference of CTD and at λ_{max} of CTD (272nm) interference of AZM occurs which suggested development of simultaneous equation method. The optimized methods showed good reproducibility and mean recovery with 99.666±0.039 (AZM), 99.504±0.114 (CTD) and 99.31±0.394 (AZM), 99.82±0.308 (CTD) respectively. The standard deviation, coefficient of variance and standard error were obtained for AZM and CTD were satisfactorily low. Result of precision at different levels was found to be within acceptable limits (RSD < 2). Thus, the method provides a simple, convenient, rapid and accurate way to determine AZM and CTD simultaneously.

CONCLUSION

A new, simple, sensitive and economical UV spectrophotometric method was developed for the simultaneous estimation of AZM and CTD in their tablet formulation. Validation of developed methods was performed according to ICH guidelines. The standard deviation, % RSD for the methods are low, reflecting a high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods. Vierordt's method has the advantage of being simple, economic, rapid and subsequently not required sophisticated technique, instrument and costly solvents. Thus, the proposed methods can be successfully applied for determination and dissolution testing of AZM and CTD in commercial formulation.

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