IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF AZILSARTAN MEDOXOMIL AND CILNIDIPINE IN MARKETED FORMULATION USING HPLC

Author's Name: Kajal Dwivedi*, Mr. Ankit Diwan, Dr. Kuldeep Ganju

Sagar Institute of Pharmacy and Technology, Bhopal (M.P.)

Corresponding Author: Kajal Dwivedi

ABSTRACT

This study aims to build up the RP-HPLC process for Azilsartan and Cilnidipine and authenticate the RP-HPLC process according to ICH validation code Q2R1. The values of mean percent recoveries were found to shown in table Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curve was linear over the concentration range of 10-50µg/ml and 5-25µg/ml, correlation coefficients were found to be 0.999 for Azilsartan Medoxomil and Cilnidipine respectively. Recovery studies were carried out by applying the method to drug sample to which known amount of Azilsartan Medoxomil and Cilnidipine at three concentration levels of 80, 100 and 120 % were added. 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. The advantage of method was found being simple, economic, rapid and subsequently not required sophisticated technique, instrument and costly solvents. Thus, the proposed method can be successfully applied for determination and dissolution testing of selected drugs in commercial formulation.

Key words: HPLC, Azilsartan Medoxomil and Cilnidipine

1. INTRODUCTION

Drug is broadly defined as "any chemical agent that affects living processes", As drug involves life, its quality becomes most important. So drug should be available in a form in which quality including bioavailability, proper plasma concentration, desired duration of action, rapid onset, accurate dose, safety, efficacy and stability on storage of product, can be assured. The WHO has defined essential drugs as, those that satisfy the healthcare needs of majority of the population; they should be available at all times in accurate amount end in appropriate dosage form. So quality of pharmaceuticals has to be monitored from the very beginning i.e. from raw material to the end, i.e. finished product. Quality control is a concept that strives to produce an ideal product by series of measures, designed to prevent and eliminate errors at different stages in production that is why at various

stages, analysis of product is done. Chemical analysis can be defined as the resolution of a chemical compound into its proximate parts; the determining its elements or the foreign substances it may contain.

The drug products are required by law to confirm a minimum standard of quality. With the rapid development of pharmaceuticals and higher challenges of quality, the volume of analytical work is increasing day by day. This forces the development of analytical methods that are rapid, accurate, precise and reproducible. Analytical chemistry plays vital role in development of science, which involves separation, identification and determination of the relative amounts of components in a sample of matter [1].

Pharmaceutical analysis is a branch of pharmacy and have a very significant role in quality control of pharmaceuticals, through the rigid check on raw materials used in manufacturing of formulation and on finished product .It plays an important role in building up the quality products through in process control. Today in pharmaceutical industry, preparations containing two or more ingredients are more popular than those with single ingredient. As a consequence various problems are being faced by the pharmaceutical analyst who has to develop methods to analyze this mixture [2].

There are various fields of Pharmacy one of them is pharmaceutical analysis which plays a very significant role in quality control of pharmaceuticals through a rigid check of raw materials, in process samples till finished products. Quality is important in every product or service, but it is vital in medicine, as it involves life, so there should not be any compromises to quality of drug products. Secondly to maintain the quality of drugs is a regulatory and mandatory requirement and lastly it is crucial for business in modern and competitive market also. Quality of drugs can only be maintained by analyzing it using analytical method with high degree of accuracy and precision and should satisfy all other validation parameters. The objectives of the research are the development and validation of fast, reproducible, efficient, economic and environmentally viable chromatographic methods for the analyses of some drugs in pharmaceutical dosage form [3].

Azilsartan Medoxomil is an angiotensin II receptor antagonist used in the treatment of hypertension. It inhibits the vasoconstrictive effects of angiotensin II (a peptide) in the body [4]. Also, it is responsible for aldosterone secretion and thereby regulates the fluid balance in the body. This further helps in the control of blood pressure. It is available in doses of 40 mg and 20 mg for the management of hypertension [5, 6].

Cilnidipine is a novel analogue in the category of calcium channel antagonist [7]. Chemically it is named as 1,4-dihydro-2,6-dimethyl- 4-(3-nitrophenyl)-3,5-pyridine carboxylic acid 2-methoxyethyl(2E)- 3-phenyl-propenyl ester (fig. 1) [8]. It acts on long-acting Ca+2 channels, thereby restricting calcium ions' entry inside the small blood vessels. Blockade of entry of Ca+2 leads to inhibition of vasoconstriction cascade, eventually resulting in vasodilatation. It helps to decrease peripheral resistance and, therefore, blood pressure [9, 10]. It also acts on N-type calcium channels present at the neuronal terminals [11]. It reduces the out flow of norepinephrine from the neuronal terminal and aids in reducing stress and hence blood pressure [12].

The objective of present work is to develop RP-HPLC for Azilsartan medoxomil and Cilnidipine in combined pharmaceutical dosage form and to validate the developed methods according to ICHQ2R1 & ICHQ2R2 guidelines to ensure their precision, accuracy, repeatability, reproducibility and other analytical method validation parameters.

2. MATERIAL AND METHODS

Selection of Mobile Phase

Initially to estimate Azilsartan medoxomil and Cilnidipine in fix dosage form number of mobile phase in different ratio were tried. A result was shown in Table.

Taking into consideration the system suitability parameter like RT, Tailing factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was Acetonitrile: Methanol in the ratio of 50:50v/v. The mobile phase was filtered through 0.45µ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed for analysis was 1.0 ml/min.

Selection of Diluent

Diluent used for preparation of sample were compatible with mobile phase and no any significant affect retention and resolution of analyte. After various trials methanol was used as diluents.

Preparation of Stock Solution

Accurately weighed 10 mg API of AZM and CLD was transferred into 10 ml volumetric flask separately and added 5ml of methanol as diluents, sonicated for 20 minutes and volume was made up to 10ml with methanol to get concentration of solution 1000µg/ml (Stock-A)

Preparation of Sub Stock Solution

5 ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50 ml with diluent (methanol) to give concentration of 100µg/ml of AZM and CLD respectively (Stock-B).

Preparation of Different Solution

1ml, 2ml, 3ml, 4ml and 5ml of stock-B were taken separately in 10 ml volumetric flask and volume was made up to 10ml with (methanol). This gives the solutions of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml, for AZM. In same manner 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml of CLD also prepared.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 10-50 µg/ml for AZM and 5-25µg/ml for CLD were prepared. All the solution were filtered through 0.45µm membrane filter and injected, chromatograms were recorded and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, six replicates of working standard of AZM 1µg/ml for AZM and 10µg/ml CLD was injected separately. Peak report and column performance report were recorded for all chromatogram.

Validation of developed Method

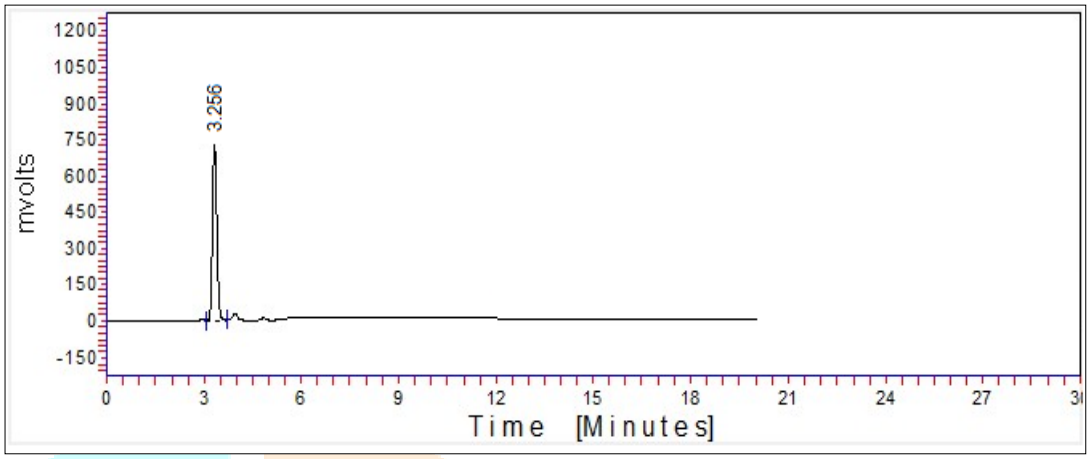
Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different concentrations (from 5 to 25 µg/ ml for AZM) and (5 to 25µg/ ml for (CLD) and areas for each concentration

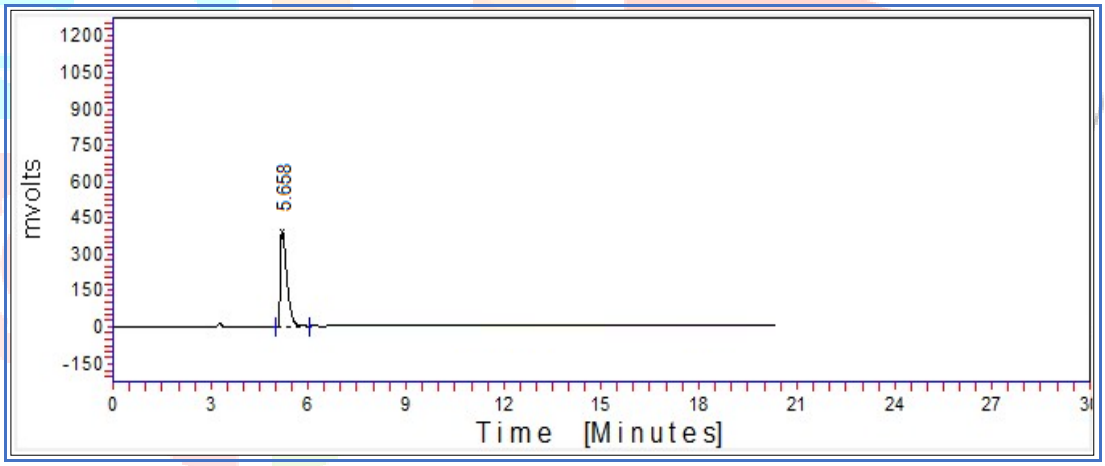
were recorded three times and mean area was calculated. The response ratio (response factor) was found by dividing the AUC with respective concentration.

Specificity

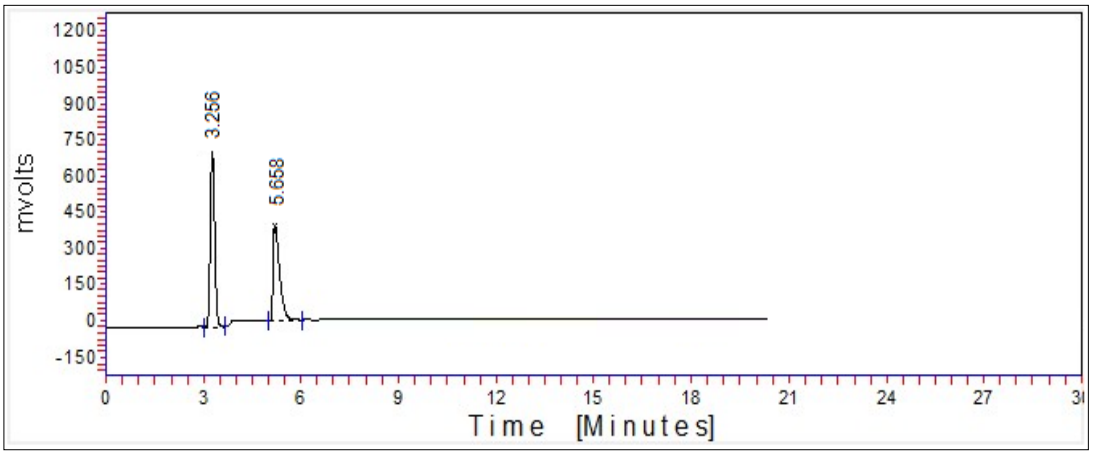
Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present such as impurities, degradation products and matrix components Figure 1.



(a) Chromatogram of AZM



(b) Chromatogram of CLD



(c) Chromatogram of Both the drug

Figure 1: (a) Chromatogram of AZM (b) Chromatogram of CLD (c) Chromatogram of Both the drug

Accuracy

Recovery studies were performed to calculate the accuracy of developed method to preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

The stock solution was prepared. The precision are established in three differences:

1. Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 1, 2, 3, 4 and 5µg/ml for AZM and 5, 10, 15, 20 and 25µg/ml for CLD indicates the precision under the same operating condition over short interval time.

Intermediate Precision

a) Day To Day Precision

Intermediate precision was also performed within laboratory variation on different days and different analyst in five replicate at five concentrations. Results of day to day intermediate precision for AZM and CLD reported in table.

Robustness

As per ICH norms, small but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, Acetonitrile: Methanol (50:50 % v/v) to (45:55 % v/v). Results of robustness are reported in table.

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of both the drug in Tablet Sample

Twenty tablets were accurately weighed and their mean weight was determined. The tablets were grinded to fine powder, an accurately weighed quantity of powder equivalent to 20 mg of AZM was transferred to 10 ml volumetric flask containing methanol. The solution was sonicated for 25 min and the final volume was made with mobile phase. The mixture was then filtered through a 0.45 µm filter. The stock solution was further diluted sufficiently with methanol to get sample solution of drug concentration of 20µg/mL AZM and 5µg/mL CLD respectively. The amounts of AZM and CLD in tablets formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with formulation. Results of tablet analysis are reported in table.

3. RESULTS AND DISCUSSION

The developed methods were found to be linear table 1. The values of mean percent recoveries were found to shown in table 2, and results of validation were shown in Table 3. The mean percent label claims of tablets by the proposed methods were close to 100, indicating the accuracy of the proposed method and low values of standard deviation, percent coefficient of variation and standard error further validated the proposed method as shown in Table .

Table 1: Results of Linearity of Azilsartan Medoxomil (AZM) and Cilnidipine (CLD)

S. No.	Parameter	AZM	CLD
1	Linearity	10-50µg/ml	5-25µg/ml
2	Correlation Coefficient (r^2)*	0.999	0.999
3	Slope (m)*	25.24	36.49
4	Intercept (c)*	10.10	1.668

*Average of five determination

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curve was linear over the concentration range of 10-50µg/ml and 5-25µg/ml, correlation coefficients were found to be 0.999 for Azilsartan Medoxomil and Cilnidipine respectively.

Table 2: Results of Recovery Studies on Marketed Formulations

Recovery Level %	% Recovery (Mean±SD)*	
	AZM	CLD
80	98.76±0.251	97.66±1.338
100	99.38±0.154	98.50±0.726
120	99.06±0.294	98.42±0.869

Recovery studies were carried out by applying the method to drug sample to which known amount of Azilsartan Medoxomil and Cilnidipine at three concentration levels of 80, 100 and 120 % were added. The results are given in Table 3.

Table 3: Results of validation (%R.S.D.)

Parameter		(Mean±SD)	
		AZM	CLD
Precision (% R.S.D.)*	Repeatability	99.312±0.110	98.341±0.111
	Day to Day	99.033±0.117	98.405±0.079
	Analyst to Analyst	99.201±0.083	99.523±0.047
	Robustness	99.093±0.101	90.376±1.047

*Average of five determination

The precision of the analytical method was studied by multiple sampling of the homogenous sample. The precision was done by measuring the absorbance for five times. The results are given in table 4.

Table 4: LOD and LOQ of AZM and CLD

Name	LOD (µg/ml)	LOQ (µg/ml)
AZM	0.50	1.50
CLD	0.35	0.98

Table 5: Result of assay of tablet formulation

	AZM*	CLD*
Label Claim (mg)	**40mg**	**10mg**
% Found (mg)	39.85	9.85
% Assay	99.62	98.5
% RSD	0.115	0.145

*Average of three determination

CONCLUSION

The developed methods were found simple, sensitive and economical for the simultaneous estimation of selected active pharmaceutical ingredients 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. The advantage of method was found being simple, economic, rapid and subsequently not required sophisticated technique, instrument and costly solvents. Thus, the proposed method can be successfully applied for determination and dissolution testing of selected drugs in commercial formulation.

REFERENCES

1. Skoog DA, West DM, Holler JF. Eds., In; Analytical Chemistry- An Introduction, 6 th Edn., Saunders college Publishing. 1994; 1:724-726.
2. Ewing GW. Eds., Liquid Chromatography;In; Instrumental Methods of Chemical Analysis, 2nd Edn., McGraw Hill Book company, Inc, New York. 1960; 2-3:377-380.
3. Corners KA. Eds., In; A Text Book of Pharmaceutical Analysis, 3rd Edn., JohnWiley and Sons. NJ. 2002; 581:620-622.
4. Baker WL, White WB. Azilsartan medoxomil: a new angiotensin II receptor antagonist for the treatment of hypertension. Ann Pharmacother. 2011;45(12):1506-15.
5. White WB, Weber MA, Sica D, Bakris GL, Perez A, Cao C, Kupfer S. Effects of the angiotensin-receptor blocker azilsartan medoxomil versus olmesartan and valsartan on ambulatory and clinic blood pressure in patients with stages 1 and 2 hypertension. Hypertension. 2011;57(3):413-20.
6. Perry CM. Azilsartan medoxomil: a review of its use in hypertension. Clin Drug Investig. 2012;32(9):621-39.
7. Datar PA, Aher SB, Washimkar MH, Auti PB. 2DQSAR of novel 1, 4-dihydropyridine derivative blocking n-type-calcium channels. J Chem Pharm Res. 2012;4:1117-22.
8. Rupareliya RH, Joshi HS. Stability indicating simultaneous validation of telmisartan and cilnidipine with forced degradation behavior study by RP-HPLC in tablet dosage form; 2013
9. Iyer RP, Lindsey ML, Chilton RJ. A two-for-one bargain: using cilnidipine to treat hypertension and its comorbidities. J Clin Hypertens (Greenwich). 2013;15(7):455-7.

10. Takahara A, Nakamura Y, Wagatsuma H, Aritomi S, Nakayama A, Satoh Y, Akie Y, Sugiyama A. Long-term blockade of L/N-type Ca(2+) channels by cilnidipine ameliorates repolarization abnormality of the canine hypertrophied heart. Br J Pharmacol. 2009;158(5):1366-74.
11. Fujii S, Kameyama K, Hosono M, Hayashi Y, Kitamura K. Effect of cilnidipine, a novel dihydropyridine Ca ++ -channel antagonist, on N-type Ca++ channel in rat dorsal root ganglion neurons. J Pharmacol Exp Ther. 1997;280(3):1184-91.
12. Hosono M, Fujii S, Hiruma T, Watanabe K, Hayashi Y, Ohnishi H, Takata Y, Kato H. Inhibitory effect of cilnidipine on vascular sympathetic neurotransmission and subsequent vasoconstriction in spontaneously hypertensive rats. Japan J Pharmacol.1995;69(2):127-34.
13. Shrinivasan R, Kamal Chandra J, Kumar R, Dushyant Kumar N. Stability indicating RP-HPLC method for determination of azilsartan medoxomil in bulk and its dosage form. IJPAR. 2014;3(4):445-52.
14. Masthanamma SK, Jahnavi P. Stability indicating RP-HPLC method for determination of azilsartan medoxomil in pharmaceutical dosage form. Res J Pharm Technol. M2014;7(2):168-72.