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ORIGINAL ARTICLE **OPEN ACCESS**

Advanced Q-Absorbtion UV Spectroscopic Method for Simultaneous Estimation of Azilsartan Medoximil K and Chlorthalidone in Bulk and Tablet Dosage Form

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ABSTRACT

A specific, sensitive UV spectrophotometric method was developed for the estimation of Azilsartan Medoximil k and Chlorthalidone in bulk and tablet dosage forms. The optimum conditions for the analysis of the drugs were established. The wavelength maxima (λmax) for Azilsartan Medoximil k and Chlorthalidone were found to be 250nm and 284nm respectively. The linearity for this method was found to be in the range of 5-25 µg/ml for AZM and 2-10 µg/ml for CTD. The method showed high sensitivity with reproducibility in their results. The calibration curve was drawn by plotting graph between absorbance and concentration. The linear regression equation was y = 0.0201x + 0.0016 for AZM and y = 0.0152x + 0.0039 for CTD with regression co-efficient of 0.9997 and 0.9953 respectively. This sensitive method was capable to recover accurately and precisely from 80 % level to 120 % level of target concentration. The proposed method suitably applied for the analysis of these drugs in bulk and in tablet pharmaceutical formulation for routine analysis.

Key words: *Azilsartan Medoximil k, Chlorthalidone, UV Spectroscopic Analysis, Bulk and Tablet dosage form, Method validation.*

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INTRODUCTION

High blood pressure, also known as hypertension, is when your blood pressure, the force of your blood pushing against the walls of your blood vessels, is consistently too high.[1] Angiotensin II is a very potent chemical formed in the blood that causes muscles surrounding blood vessels to contract, thereby narrowing the vessels. This narrowing increases the pressure within the vessels and can cause high blood pressure (hypertension). Angiotensin II. receptor blockers (ARBs) are medications that block the action of angiotensin II by preventing angiotensin II from binding to angiotensin II receptors on the muscles surrounding blood vessels. As a result, blood vessels enlarge (dilate) and blood pressure is reduced. Reduced blood pressure makes it easier for the heart to pump blood and can improve heart failure. In addition, the progression of kidney disease caused by the high blood pressure or diabetes is slowed. ARBs have effects that are similar to angiotensin converting enzyme (ACE) inhibitors, but ACE inhibitors act by preventing the formation of angiotensin II rather than by blocking the binding of angiotensin II to muscles on blood vessels. [2]

Azilsartan Medoxomil Potassium 1H-Benzimidazole-7Carboxylic acid, 1-{[2'-(2,5- dihydro-5-oxo-1,2,4-oxadiazol-3-yl)[1,1'-biphenyl]-4-l]methyl}-2-ethoxy-,(5-methyl-2-oxo-1-dioxol-4-yl)methyl ester mono potassium salt. is an orally administered angiotensin II receptor type 1 antagonist (blocker) used in the treatment of adults with essential hypertension. It is a prodrug that undergoes rapid hydrolysis in the gastrointestinal tract after oral administration to the bioactive moiety

azilsartan, before systemic absorption. Azilsartan medoxomil produces antihypertensive effects by selectively blocking the binding of angiotensin II to the angiotensin type 1 (AT1) receptor, thereby antagonizing the pressor response activity of angiotensin II.

Chlorthalidone is chemically described as (RS)-2-chloro-5-(1-hydroxy-3-oxo-2,- dihydro- 1Hisoindol-1-yl) benzene-1-sulfonamide. Is a diuretic drug which is used for treating hypertension. Its method of action is to prevent reabsorption of sodium and chloride by inhibiting the Na+/Cl ¯ symporter in the distal convoluted tubule (DCT). [3]

According to ICH guidelines, an analytical method entails the application of a specific methodology and comprehensive step-by-step instructions for qualitative, quantitative, or structural examination of a sample for one or more analyses. In the assessment and management of equivalence and risk, analytical processes are crucial. It aids in the development of results stability and product-specific acceptability criteria. Validation should show that the analytical method is effective for the desired outcome. [4,5]

MATERIAL AND METHODS

CHEMICALS AND REAGENTS

Azilsartan medoxomil (AZM) and Chlorthalidone (CTD) were obtained as a gift sample from CTX Lifesciences Pvt. Ltd., Sachin, Gujarat. For UV method, All the reagent solutions (Methanol) used in the UV method were prepared. 5-25 and 2-10 µg/ml concentration of AZM and CTD respectively were prepared and used in the UV method. All the absorbances were taken on the 250 nm and 284 nm for AZM and CTD respectively.

INSTRUMENTATION [6-7]:

- JACSO V-730 UV-Vis Spectrophotometer having wavelength rang 190 to 1100 nm
- Detector(s) - Silicon photodiode
- Light Source - Halogen lamp, Deuterium lamp
- Scanning Speed - 10-8000 nm/min
- Wavelength Accuracy - +/- 0.2 nm (at 656.1 nm)
- Spectral - 1 nm

SAMPLE PREPARATION:

Accurately weighed 10 mg of AZM and CTD were transferred separately into 10 ml volumetric flasks and dissolved in small volume of methanol. Then, the volume was diluted to the mark with methanol to get the final concentration of AZM and CTD (1000 µg/ml). 1 ml of each solution was transferred in 10 ml volumetric flask and volume was adjusted to the mark with methanol to get final concentration of 100 µg/ml of each drug. Further, 1ml pipette out from that solution and make up to 10 ml with methanol to get concentration of 10 µg/ml. Same procedure was followed for mixture solution, too.

III. VALIDATION PARAMETERS

LINEARITY

The linearity was calculated by ordinary linear regression analysis. The constructed calibration curve was linear over the concentration range of 5-25 µg/ml for AZM and 2-10 µg/ml for CTD. The linear regression equation was y = 0.0201x + 0.0016 for AZM and y = 0.0152x + 0.0039 for CTD with regression co-efficient of 0.9997 and 0.9953 respectively. **[8-9]**

LOD AND LOQ

LOD and LOQ were found to be 0.363and 1.10 µg/ml for AZM as well as 1.936and 5.86 µg/ml for CTD respectively indicating high sensitivity of the method. **[8]**

PRECISION

The %RSD values for repeatability, intraday precision and interday precision of AZM and CTD respectively were found to be less than 2 indicating that the proposed method has excellent repeatability and reproducibility. **[10-11]**

ACCURACY

The recovery studies were carried out by adding known amount of standard to samples at 50, 100 and 150% level and analysed by the proposed method, in triplicate. The percentage recovery was found between 99.61 to 100.22 % for AZM while for CTD 99.97 to 100.86 % confirming the accuracy of the proposed method. **[12-14]**

RESULT AND DISCUSSION

Optical Parameter

Table 1. Optical Parameter for AZM and CTD

Parameter	Data for AZM	Data for CTD
λ-max	250 nm	284 nm
Beer's law limit	5-10 µg/mL	2-10 µg/mL
Regression equation	y = 0.0201x + 0.0016	y = 0.0152x + 0.0039
Correlation coefficient	R^2 = 0.9997	R^2 = 0.9953
Slope	0.020	0.015
Intercept	0.2916	1.5564
Limit of detection	0.363 µg/mL	1.936 µg/mL

Estimation of λmax for AZM and CTD:

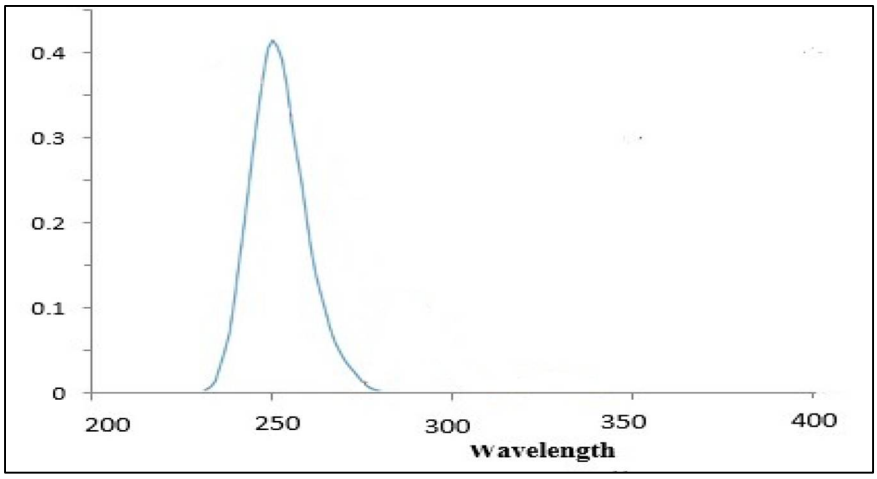

Fig. 1. λ max of Azilsartan Medoxomil

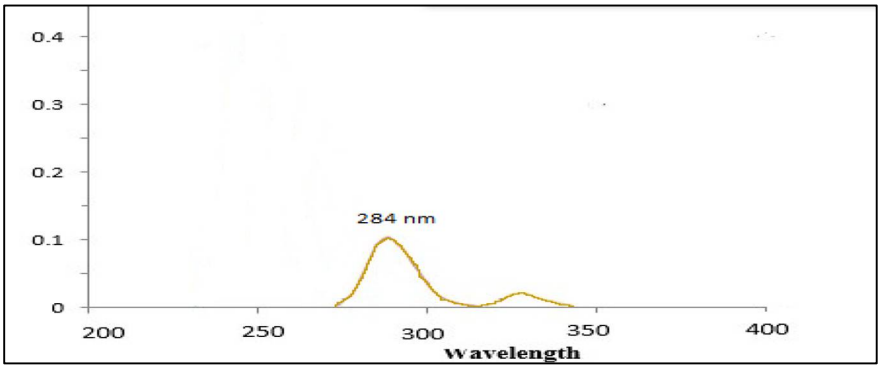

Fig. 2. λ max of Chlorthalidone

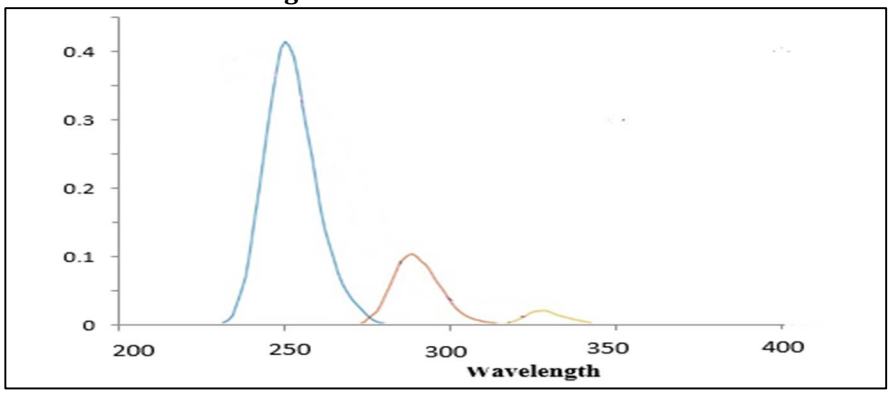

Fig. 3. Iso- Absorptive point

LINEARITY:

Table 2. Linearity data of AZM at 250 nm

Conc. (µg/ml) AZM	Absorbance of AZM	Mean Absorbance AZM	% SD	%RSD at 250nm
5	0.1082	0.1079	0.0003	0.2332
	0.1077			
	0.1079			
10	0.1993	0.1991	0.0004	0.2189
	0.1986			
	0.1994			
15	0.3012	0.3017	0.0006	0.2131
	0.3024			
	0.3014			
20	0.4048	0.4043	0.0005	0.1133
	0.4039			
	0.4042			
25	0.5058	0.5058	0.0005	0.0892
	0.5062			
	0.5053			

Table 3. Linearity data of AZM at 275.2 nm

Conc. (µg/ml)AZM	Absorbance of AZM	Mean Absorbance AZM	% SD	%RSD At 275.2 nm
5	0.1012	0.1022	0.0003	0.2935
	0.0998			
	0.1056			
10	0.2002	0.1995	0.0009	0.4511
	0.1998			
	0.2013			
15	0.3041	0.3017	0.0023	0.7623
	0.3017			
	0.3010			
20	0.4084	0.4016	0.0072	1.7928
	0.3940			
	0.4024			
25	0.5015	0.5033	0.0017	0.3377
	0.5049			
	0.5036			

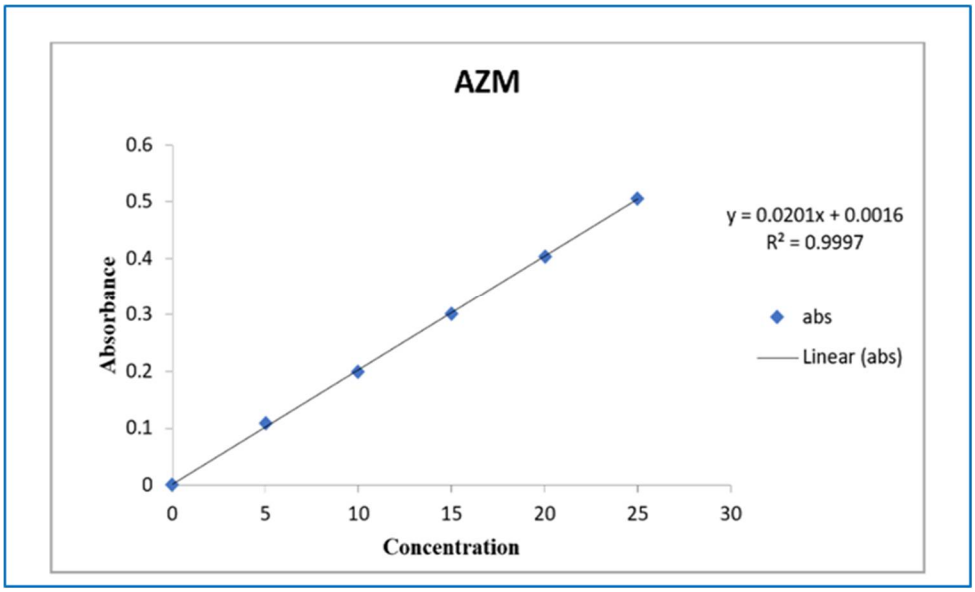

Fig. 4. Calibration Curve for AZM at 250nm

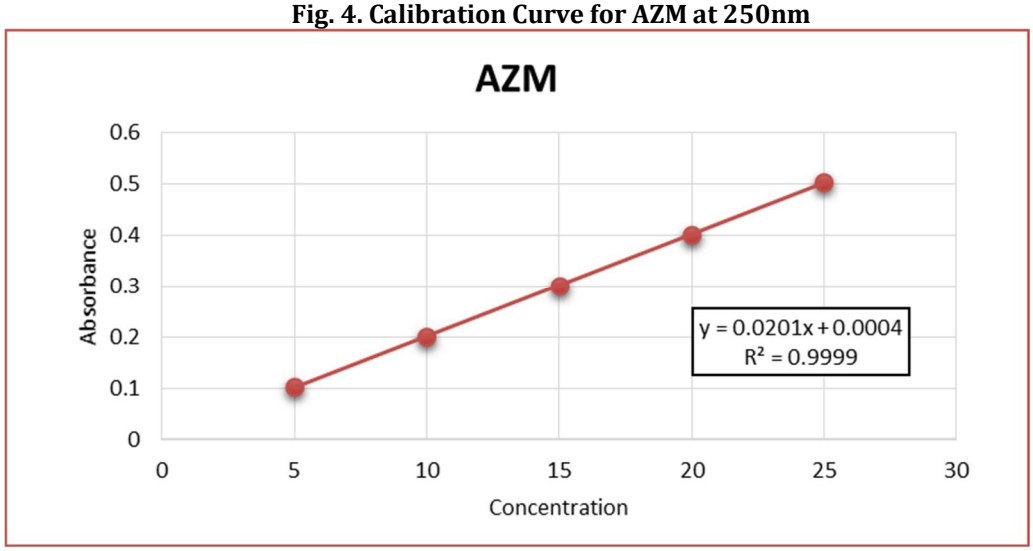

Fig. 5. Calibration Curve for AZM at 275.2nm

Table 4. Linearity data of CTD at 250 nm

Sr. No.	Conc. (µg/ml) CTD	Absorbance of CTD	Mean Absorbance CTD	% SD	%RSD
1	2	0.0336 0.0378 0.0359	0.0373	0.0006	1.4752
2	4	0.0632 0.0674 0.0642	0.0635	0.0005	0.7922
3	6	0.0997 0.1002 0.0992	0.0969	0.0005	0.4893
4	8	0.1244 0.1219 0.1262	0.1282	0.0007	0.2115
5	10	0.1524 0.1533 0.1578	0.1512	0.0003	0.1664

Table 5. Linearity data of CTD at 275.2 nm

Sr. No.	Conc. (µg/ml) CTD	Absorbance of CTD	Mean Absorbance CTD	% SD	%RSD
1	2	0.0379	0.0373	0.0005	1.4765
		0.0368			
		0.0373			
2	4	0.0636	0.0635	0.0005	0.7926
		0.0640			
		0.0630			
3	6	0.1005	0.0998	0.0006	0.6519
		0.0992			
		0.0998			
4	8	0.1283	0.3846	0.0006	0.1705
		0.1288			
		0.1275			
5	10	0.1512	0.1517	0.0006	0.4116
		0.1510			
		0.1515			

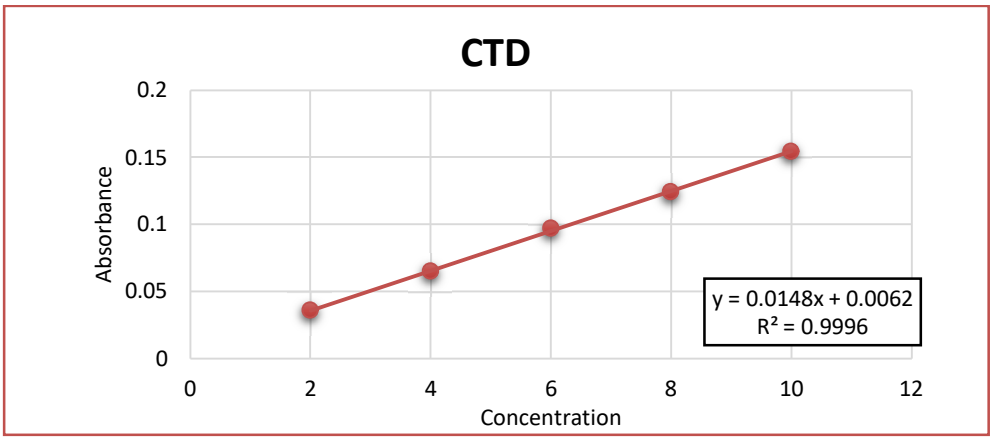

Fig. 6. Calibration Curve for CTD at 250nm

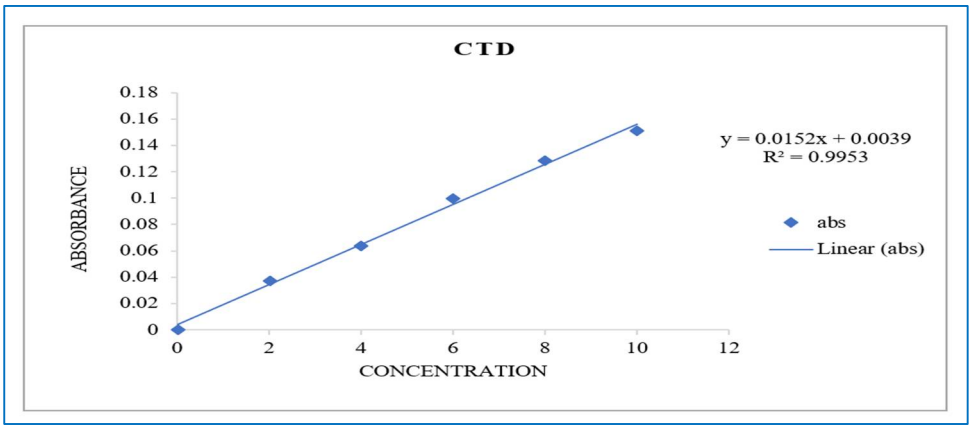

Fig. 7. Calibration Curve for CTD at 275.2nm

L.O.D. (µg/ml):

The limit of detections were found to be: ***3.3 * SD / Slope***

LOD of AZM = (3.3 ∗ 0.0022)/0.020
= 0.363

LOD of CTD= (3.3 ∗ 0.0088)/0.015
= 1.936

L.O.Q. (µg/ml):

The limit of detections were found to be: ***10 * SD / Slope***

LOD of AZM = (10 ∗ 0.0022)/0.020
= 1.10

LOD of CTD= (10 ∗ 0.0088)/0.015
= 5.86

Table 6. LOD and LOQ

Parameters	AZM	CTD
LOD	**0.363**	1.936
LOQ	**1.10**	5.86

PRECISION

Table 7. Intraday precision of AZM at 250 nm & 275.2 nm

Validation Parameters	AZM						
	Conc. (µg/ml)	Mean abs	% SD	%RSD at 250 nm	Mean abs	% SD	%RSD at 275.2 nm
Intraday Precision	5	10.0068	0.0057	0.0578	10.0249	0.0226	0.2259
	10	20.0072	0.0089	0.0447	20.0066	0.0069	0.0349
	15	30.0461	0.0546	0.1819	30.2575	0.4280	1.4146

Table 8. Intraday precision of CTD at 250 nm & 275.2 nm

Validation Parameters	CTD						
	Conc. (µg/ml)	Mean abs	% SD	%RSD at 250 nm	Mean abs	% SD	%RSD at 275.2 nm
Intraday Precision	2	3.096	0.015	0.0578	3.019	0.013	0.2259
	4	6.0554	0.053	0.0447	6.027	0.034	0.0349
	6	9.9826	0.027	0.1819	9.977	0.023	1.4146

Table 9. Interday precision of AZM at 250 nm & 275.2 nm

Validation Parameters	AZM						
	Conc. (µg/ml)	Mean abs	% SD	%RSD at 250 nm	Mean abs	% SD	%RSD at 275.2 nm
Interday Precision	5	10.0258	0.020	0.2071	10.0405	0.043	0.4293
	10	20.0022	0.003	0.0153	20.0066	0.007	0.0366
	15	30.3349	0.479	1.5821	29.9904	0.090	0.3001

Table 10. Interday precision of CTD at 250 nm & 275.2 nm

Validation Parameters	CTD						
	Conc. (µg/ml)	Mean abs	% SD	%RSD at 250 nm	Mean abs	% SD	%RSD at 275.2 nm
Interday Precision	2	3.0113	0.013	0.4529	3.0209	0.027	0.9078
	4	6.0554	0.053	0.8762	6.0235	0.025	0.4165
	6	9.0730	0.067	0.7478	9.0189	0.024	0.2726

ACCURACY

Table 11. Accuracy data of AZM and CTD

Drug	Recovery Level	Mean Conc.	% Recovery	SD	%RSD
AZM	80%	18.028	100.15	0.0661	0.3666
	100%	19.994	99.97	0.0748	0.3741
	120%	22.191	100.86	0.1602	0.7219
CTD	80%	10.042	100.42	0.052	0.516
	100%	11.994	99.95	0.074	0.624
	120%	13.191	101.46	0.160	1.21

Analysis of formulation

Table 12. Analysis of formulation

Sr. No.	Tablet Formulation	Drug	Label claim (mg/tablet)	Amount Found (mg)	Mean Amount Found	% Assay ±%SD
1	MYOTAN CT	AZM	40	39.96	40.05	100.12%±0.09
2				40.05		
3				40.14		
1		CTD	12.5	12.48	12.48	99.86%±0.21
2				12.46		
3				12.50		

DISCUSSION

Methanol was used as the solvent for the measurement of AZM and CTD on the Jasco V730 spectrophotometer. On each day of the estimating trial, fresh sample solutions containing AZM and CTD were made. [15-16] When utilising regression analysis, the linear equations for AZM and CTD are y = 0.0201x + 0.0016 and y = 0.0152x + 0.0039, respectively, with regression co-efficient of 0.9997 and 0.9953. Beer's law is seen over the concentration range of 5-25 g/ml for AZM and 2-10 g/ml for CTD. From 80% level to 120% level of target concentration, this sensitive approach could recover precisely and accurately. The limit of detection was found to be 0.363 µg/mL for AZM and 1.936 µg/mL for CTD. The quantification limits were found to be 1.10 g/ml and 5.86 g/ml, respectively. A recovery study was done on the formulations, and it revealed % RSD. Table 1 lists the estimated optical parameters, such as the Beer's law limit, slope, and intercept values. [17-18] The newly developed method can be used for routine analysis as method for the simultaneous estimation of Azilsartan Medoxomil k and Chlorthalidone in tablet dosage forms.

CONCLUSION

The chosen combination of drug is a day to day usage medication which lacked an analytical part in its development of method and validation by Q Absorbance ratio method. The presented work attempts for the development and to statistically validate spectrophotometric method on AZM and CTD in their bulk and tablet dosage forms. The spectrophotometric method which was employed was done by using Q-Absorbance ratio method and it was also validated on the basis of guidelines outlined by ICH. The method was examined to be rapid, precise, accurate and reproducible. The process was well employed for determining the drugs in their pharmaceutical dosage forms.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Carey RM, Whelton PK, (2017). ACC/AHA Hypertension Guideline Writing Committee*. Prevention, detection, evaluation, and management of high blood pressure in adults: synopsis of the 2017 American College of Cardiology/American Heart Association Hypertension Guideline. Annals of internal medicine. 2018 Mar 6;168(5):351-8.
2. Omudhome O, Jay W. Marks, (2020). "Angiotensin II Receptor Blockers", December, 2019, https://www.medicinenet.com/angiotensin_ii_receptor_blockers/article.html.
3. Touyz RM. (2005). Molecular and cellular mechanisms in vascular injury in hypertension: role of angiotensin II–editorial review. Current opinion in nephrology and hypertension. 1;14(2):125-31.
4. Perry CM. Azilsartan medoxomil: a review of its use in hypertension. Clinical drug investigation. 2012 Sep;32:621-39.
5. Breaux J, Jones K, Boulas P. Analytical methods development and validation. Pharm. Technol. 2003;1:6-13.
6. Clark BJ, Frost T, Russell MA, editors. UV Spectroscopy: Techniques, instrumentation and data handling. Springer Science & Business Media; 1993 Apr 30.
7. Guideline IH. Validation of analytical procedures Q2 (R2). ICH: Geneva, Switzerland. 2022.
8. Gawai MN, Surwade KS, Phadatare DG. UV Spectrophotometric Method for the estimation of Azilsartan Medoxomil in Bulk Form. Asian Journal of Research in Chemistry. 2018;11(5):791-3.
9. Singh G, Kumar D, Sharma D, Singh M, Kaur S. Q-Absorbance ratio spectrophotometric method for the simultaneous estimation of prednisolone and 5-Amino salicylic acid in tablet dosage form. Journal of applied pharmaceutical science. 2012 Jul 30;2(7):222-6.
10. Shah PB, Patel PU. Q-absorbance ratio spectrophotometric method for the simultaneous estimation of cinnarizine and dimenhydrinate in their combined dosage form. Journal of Pharmaceutical Science and Bio-Scientific Research. 2012;2(2):83-7.
11. Patil SM,Galatage ST,Choudhari AU.Evelopment Of Uv Spectrophotometric Method For Estimation Of Letrozole In Pure And Pharmaceutical Dosage Form. Indo American Journal of Pharmaceutical Research.2018 May;8(04):1080-1085.
12. Galatage ST, Trivedi R, Bhagwat DA. Characterization of camptothecin by analytical methods and determination of anticancer potential against prostate cancer. Future Journal of Pharmaceutical science,202;7(104):1-9. doi.org/10.1186/s 43094- 021- 00236-0.
13. Galatage, S. T., Trivedi, R., & Bhagwat, D. A., Oral self-emulsifying nanoemulsion systems for enhancing dissolution, bioavailability and anticancer effects of camptothecin. Journal of Drug Delivery Science and Technology, 78 (2022) 103929.
14. Galatage ST, Manjappa AS, Bhagwat DA, Trivedi R, Salawi A, Sabei FY, Alsalhi A. Oral self- nanoemulsifying drug delivery systems for enhancing bioavailability and anticancer potential of fosfestrol: In vitro and In vivo characterization. Eur J Pharm Biopharm. 2023;17:S0939-6411(23)00273-4. doi: 10.1016/j.ejpb.2023.10.013.
15. Galatage, S.T., Hebalkar, A.S., Gote, R.V. Design and characterization of camptothecin gel for treatment of epidermoid carcinoma. Futur J Pharm Sci,2020; 6, 50. https://doi.org/10.1186/s43094-020-00066-6.
16. Galatage ST, Manjappa AS, Kumbhar PS, Salawi A, Sabei FY, Siddiqui AM, Patil RV, Akole VS, Powar RD, Kagale MN. Synthesis of silver nanoparticles using Emilia sonchifolia plant for treatment of bloodstream diseases caused by Escherichia coli. Ann Pharm Fr. 2022 Dec 15:S0003-4509(22)00179-1. doi: 10.1016/j.pharma.2022.12.007.
17. Kodoli, R.S., Galatage, S.T., Killedar, S.G. et al. Hepatoprotective activity of Phyllanthus niruri Linn. endophytes. Futur J Pharm Sci 7, 97 (2021). https://doi.org/10.1186/s43094-021-00243-1.
18. Kumbhar PS, Diwate SK, Mali UG, Shinde TU, Disouza JI, Manjappa AS. (2020). Development and validation of RP-HPLC method for simultaneous estimation of docetaxel and ritonavir in PLGA nanoparticles. InAnnales Pharmaceutiques Françaises.1 (Vol. 78, No. 5, pp. 398-407). Elsevier Masson.

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