International Journal of Research in Pharmacy and Pharmaceutical Sciences
www.pharmacyjournal.in
ISSN: 2455-698X
Received: 13-10-2023, Accepted: 28-10-2023, Published: 14-11-2023
Volume 8, Issue 4, 2023, Page No. 30-36

Chemo metric assisted spectrophotometric method development through QBD approach for the estimation of Azilsartan Medoxomil and Cilnidipine in combined solid dosage form

G K Dyade, Shubham B Babare, Seema S Bansode
Department of Pharmaceutical Quality Assurance, SVPM'S College of Pharmacy, Malegaon (BKII), Baramati, Pune Maharashtra, India

Abstract
Quality by design (QbD) is a systematic process for pharmaceutical development recommended by regulatory agencies like USFDA. Development of various pharmaceutical processes including analytical methods by applying Quality by design aids in ensuring the robustness of the method. QbD approached chemo metric assisted UV-VIS spectrophotometric analytical method was developed for the estimation of Azilsartan medoxomil (ASM) and cilnidipine (CDE) from the combined dosage forms. Simultaneous equation method was selected from the nature of spectra, solvent 90 % alcohol was utilised; and for method 241 nm and 356.6 nm was the wavelength for measurement of absorbance of azilsartan and cilnidipine respectively. Effect of input variables on spectrum characteristics were studied for selection of critical parameters and developed method was validated as per ICH Q 2 R1 regulatory guidelines. Linearity of the drugs was ascertained over the conc range 1-20 mcg/ml (microgram/ml) for CDE and 1-32 mcg/ml for ASM. The percentage purity of assay was found 102.63 % for ASM and 99.65 % for CDE; and the accuracy study data were varied from 0.15759 to 1.26401 for ASM and 0.21761 to 0.49314 for CDE. Precision study was shown acceptable data as % RSD data varied from 0.6369 to 1.3192 for ASM and from 1.08653 to 1.34269 for CDE. The developed method is rigid, robust and efficient for the estimation of ASM and CDM from the composition of dosage form. QbD was applied to build rigid robust method through risk assessment at early stage and defining the design space at the later stage.

Keywords: Cilnidipine, azilsartan medoximil, QbD, ICH, simultaneous equation method

Introduction

Azilsartan (ASM) chemically (5-methyl-2 oxo-1, 3 dioxol-4yl) methyl 2-ethoxy-1- {[2'-(5-oxo-4, 5 dihydro-1, 2, 4-oxadiazol-3-yl)-1, 1'-biphenyl-4-yl] methyl}-1*H*-benzimidazol}-carboxylate [1] is an angiotensin-II receptor antagonist antihypertensive with action similar to those of losartan. The dosage form Azilsartan medoximil is hydrolysed in the GIT to its active metabolite azilsartan [2].

Literature survey revealed that various analytical methods have been reported for estimation of ASM such as UV spectrophotometric method [3], for estimation of ASM alone by RP-HPLC [4], with other drug by RP-HPLC [5], Bio analytical LC [6], LC-MS/MS [7] and Bio analytical LC-MS/MS [8] alone or in combination with other drugs.

Cilnidipine (CDE) chemically 1, 4-Dihydro-2, 6-dimethyl-4-(3-nitrophenyl)-3, 5-pyridine dicarboxylic acid 2-methyloxyethyl (2E)-3-phenyl-2-propenyl ester [1] is a dihydropyridine calcium channel blocker given orally in the management of hypertension [2].

For estimation of cilnidipine UV spectrophotometric method [9-11], chromatographic methods [12], chromatographic methods with other drugs [13], QbD based chromatographic methods [14, 15] alone or in combination with other drugs have been described.

Cilnidipine is official in Indian Pharmacopoeia [16]. Chemical structures of both drugs are shown in (Fig No 1).

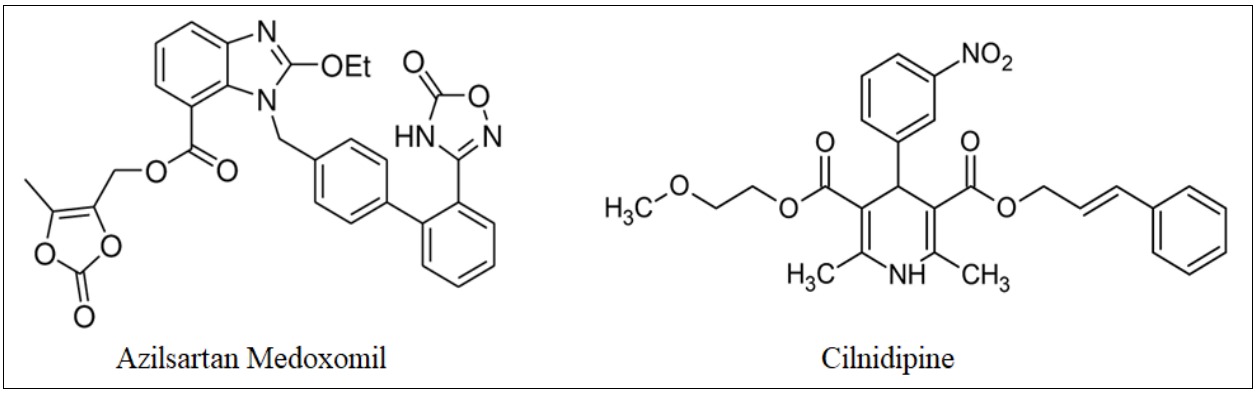

Fig 1: Chemical structure of Drug molecule

Quality by design concept is applied for the development of pharmaceutical processes to assure a predefined product quality. QBD concepts are mentioned in ICH guidelines Q8 (R2) (Pharmaceutical development), Q9 (Quality risk management), and Q10 (Pharmaceutical quality system) [17-19] shown in Fig No 2. ICH guidelines Q8 (R2) defines QBD

as a "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management" [20]. QBD approach in analytical method summarizes a complete understanding of how the analytical technique attributes and operating conditions affect the analytical performance. Factors to study in analytical quality by design (AQbD) approach may include the type of analytical technique chosen, reagents used and instrument parameters.

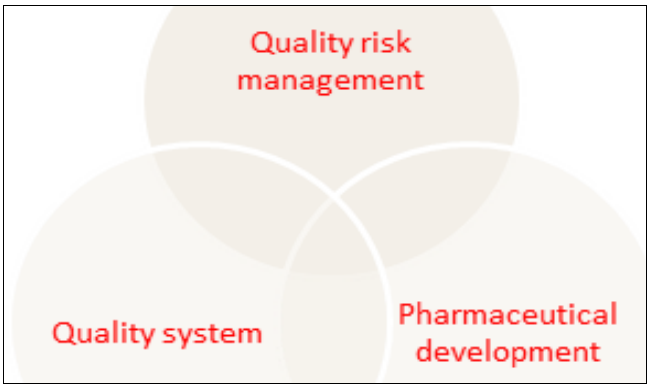

Fig 2: Analytical QbD approach

There are similar advantages of applying QbD principles to analytical methods as to manufacturing processes and product [21]. A QbD approach can be beneficial in the development of suitable, robust, low cost and eco-friendly (eco-friendly solvent, chemicals) method which is applicable at any stage of the lifecycle of the product. Also some regulatory guidelines have mentioned flexibility of changing analytical method without revalidation if the AQbD approach has been implemented during analytical method development. The first stage of AQbD approach is to fix an analytical target profile (ATP) for the method. ATP defines the goal of the analytical method development process and it is the sign of method performance [22, 23]. For analytical method validation ICH Q2 (R1) has given various method performance characteristics for an analytical method. Thus a QbD based UV spectrophotometric was developed, QbD approach was implemented with the study of the effect of method input variables on spectral shape, intensity of absorbance, and absorbance maxima λ_{max} and critical parameters were selected for the proposed method and method was validated as per ICH guidelines Q2 (R1).

Materials and methods

Instrumentation

Analysis was performed with a Shimadzu Double beam UV-Visible spectrophotometer (Shimadzu, Kyoto, Japan) with spectral bandwidth of 2 nm and wavelength accuracy of ± 1 nm with 10 mm matched Quartz cells was used. Electronic balance Afcoset balance (The Bombay Burmah Trading corpo Ltd) with accuracy ±0.1 mg Model No. ER 200A was utilised for weighing and for degassing the solution Digital Ultrasonic cleaner 1.8 Ltr (Labman scientific Instruments Chennai) was used.

Reagents and Chemicals

Pharmaceutically pure samples of ASM and CDE from Swapnroop drugs and pharmaceuticals, Aurangabad, Maharashtra, India were procured as a gift samples and the commercial formulation Myotan CN 40/10 containing azilsartan 40 mg and cilnidipine 10 mg was procured from the local market.

AQbD approach application in method development

AQbD approach was applied to study the influence of input variable parameters on spectrophotometric analytical method performance shown in (Fig No 3).

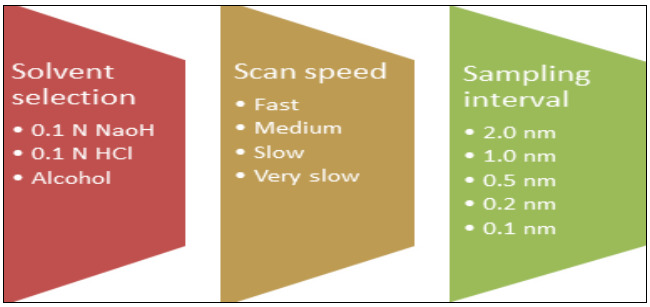

Fig 3: Diagram showing the relationship between input variable parameters and the spectrophotometric method performance characteristics

Solvent selection

ASM is freely soluble in methanol and practically insoluble in water; whereas CDE is very soluble in N, N-Dimethyl acetamide, freely soluble in acetone, soluble in methanol and practically insoluble in water. Although the solubility of the procured drugs were studied in alcohol 90%, 0.1 N HCl and 0.1 N NaOH separately; and found that CDE is insoluble in both these solvents however ASM is soluble in 0.1 N NaOH. Both drugs solubilises in alcohol 90%, hence as a common solvent it was selected. Each solution with known conc of analyte was scanned in UV range of 200 nm to 400 nm. It was found that alcohol 90% is suitable with respect to stable, robust and precise in producing result.

Preparation of stock solutions and standard solutions

10 mg each of drug CDE and ASM were separately and accurately weighed; and transferred into separate 25 ml volumetric flask. Dissolved into alcohol 90% solvent and volume was made to 25 ml with solvent. Subsequent standard solution of each drug with conc 16µg/ml was prepared by diluting aliquot 0.4 ml of stock solution to 10 ml with 50% alcohol into 10 ml capacity volumetric flask.

Selection of wavelength and conc range

From UV spectra it was found that CDE has measurable absorbance at 241 nm (Fig No 4) and less interference was observed by ASM; similarly ASM has maximum absorbance at 246.5 nm and negligible interference by CDE was accounted. Chemometric method using simultaneous equation method was applied and which was reasonable remedy to overcome interference at each other's absorbance. To study linearity, working conc range 1 to 20 µg/ml for CDE and 1 to 32µg/ml for ASM was selected. Also combined drug solution was prepared simulated to marketed formulation. Selected critical parameters based upon above discussion, observations were listed in Table No 1 and by using these; method was validated as per ICH guidelines and by analysing marketed preparations.

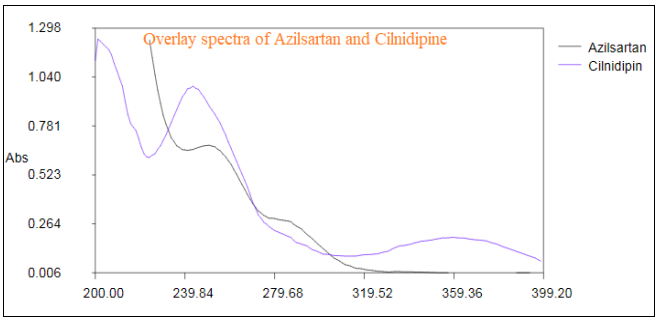

Fig 4: Overlaid spectra of ASM and CDE

Table 1: Selected critical parameter for UV-VIS analytical method of CDE and ASM

Parameter	Selected variables for simultaneous equation method	
	ASM	CDE
Wavelength	241	356.6
Solvent	50% alcohol	50% alcohol
Scan speed	Fast	Fast
Sampling interval	0.2 nm	0.2 nm

Experimental Method for estimation

From the overlain spectra simultaneous equation method was applicable for estimation of both the analytes from the combined dosage form.

Method for estimation of azilsartan by Simultaneous equation method and absorbance correction method

ASM was shown maximum absorbance i.e. λ_{max} at 246.6 nm where moderate interference by CDE found and CDE has maximum absorbance i.e. λ_{max} at 241 nm and 356.6 nm where negligible interference by ASM observed. At 241 nm the λ_{max} of CDE, the ASM was shown consistency in the absorptivity; hence two wavelengths 241 and 356.6 nm were considered as 1 and 2 respectively for the said method to estimate ASM and CDE. The equation A= abc was applied for x (ASM) and y (CDE) determination. Conc of ASM Working standard solutions of ASM and CDE containing 12 mcg/ml conc were separately prepared and used for the method.

$$Cx = \frac{A2\ .ay1 - \ A1.ay2}{ax2.ay1 - \ ax1.ay2}$$

$$Cy = \frac{As}{ay2}$$

Where C_X = Conc of ASM in sample solution

A_1 and A_2 = absorbance of sample solution at 1 and 2 wavelength

ay_1 and ay_2 = absorptivity of CDE at 1 and 2 wavelength of standard solution

ax_1 and ax_2 = absorptivity of ASM at 1 and 2 wavelength of standard solution

C_y = Conc of CDE in sample solution

As = Absorbance of Sample solution at 2 wavelength

Validation of the Method

Selected critical parameters should meet the performance characteristics of the analytical method so as to attain analytical target profile of the method. An ICH guideline Q2 R1 was applied to study methods performance with critical parameters in order to implement AQbD approach. The method was validated as per ICH guidelines

System suitability

System suitability is studied to demonstrate the suitability of the developed procedure under consideration for the analytical method. Six replicates of working standard solutions with conc12mcg/ml and 20mcg/ml of ASM and CDE respectively were prepared separately and absorbance was recorded, calculated SD and % RSD of the response.

Linearity

The linearity of an analytical method is its ability to obtain response i.e. absorbance which is directly proportional to the conc of analyte. Series of working standard solutions were prepared in conc. range of 1-32 µg/ml for ASM and 1-20 µg/ml for CDE and scanned in 400 to 200 nm range in spectrum mode of the spectrophotometer, absorbance of the standard solutions were recorded at their respective wavelength; i.e. 246.6 for ASM and 356.6 nm for CDE in spectrum order. Microsoft office excel software tool was used to obtain the standard regression curve and its analysis as slope, intercept, and correlation coefficient.

Assay of formulation

Assay was carried out by proposed methods and assay was validated by statistical parameters.

Estimation of formulations by simultaneous equation and absorbance correction method

Tablet powder equivalent to 10 mg ASM and 2.5 mg CDE was weighed and transferred into 25 ml volumetric flask. Dissolved into 90% alcohol, mixed well for 10 mins and volume was made to 25 ml with the solvent. Solution was filtered through what man filter paper and aliquots of solution were further diluted with the 50% alcohol to obtain tablet sample solution. Solution was scanned in the range of 400 to 200 nm to obtain absorbance of tablet solution at 241 nm and 356.6 nm in spectrum order. Obtained absorbance were utilised to estimate unknown conc of formulation; and results were statistically validated to obtain % of nominal conc, standard deviation and % of RSD.

Accuracy and Precision

The accuracy of an analytical method expresses the closeness of an agreement between test result and true result. Accuracy study was performed by recovery study i.e. standard addition method; diluted standard solutions of ASM and CDE were prepared and standard solutions added in 80,100 and 120% proportionate to the tablet solution. Three replicates at each of these three levels were prepared, measured and % of conc, SD and RSD were calculated.

The precision study was carried out by performing assay of tablet six times; also the reproducibility in result was studied by inter day and intraday precision.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of ASM and CDE by the proposed method were determined using calibration graph method and calculated as 3.3σ/s and 10 σ/s for LOD and LOQ respectively; σ is the standard deviation of calibration curve and s is the slope of regression line.

Robustness and Ruggedness

It is measure of capacity of analytical procedure to remain unaffected by small but deliberate variations in method parameter.

Results and Discussion

Method development comprises numerous steps, and of which solvent selection, selection of method for measurement are significant one. Uses of aqueous solvents, eco-friendly solvents like hydrotropic have got remarkable weightage due to low cost, readily available and environmentally sound. Drugs underlying analysis must have appreciable solubility in the selected solvent. Chemical structure of the drug and physico-chemical properties available in the literature guides about use of appropriate solvent in the method.

From UV spectra two wavelengths were selected as 241 nm (λ_{max} of ASM) and 356.6 nm (λ_{max} of CDE) for calculation of both drugs in combined solution shown in Fig No 4.

System Suitability

The absorbances of six replicates of standard solutions of tabulated respective conc are reported in Table No 2. The SD and % RSD was found for ASM and CDE and meets the system suitability requirements indicate method was suitable for analysis.

Table 2: System suitability study of ASM and CDE

Sr No	Conc in mcg/ml	Absorbance of ASM	Conc in mcg/ml	Absorbance of CDE
1	20 mcg/ml	0.8263	12 mcg/ml	0.9934
2	20 mcg/ml	0.8241	12 mcg/ml	1.0867
3	20 mcg/ml	0.8173	12 mcg/ml	1.0452
4	20 mcg/ml	0.8185	12 mcg/ml	0.9923
5	20 mcg/ml	0.8323	12 mcg/ml	1.0762
6	20 mcg/ml	0.8185	12 mcg/ml	0.9890
	SD RSD	0.58551 0.71134	SD RSD	0.44768 0.43928

Linearity

The calibration curve of both drugs was found to be linear shown in (Fig No 5) in the conc range of 1-32 µg/ml for ASM and 1-20 µg/ml for CDE as shown in Fig No 6. The regression equation of line and parameters slope, r^2 value and intercept are tabulated in Table No 3, which proved the linear relationship between conc and obtained response.

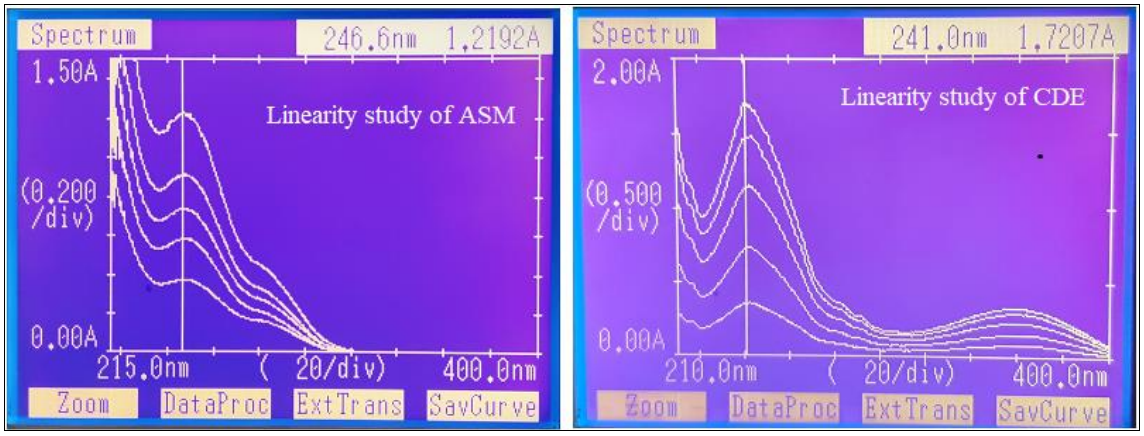

Fig 5: Overlaid spectra of both drugs obtained in linearity study

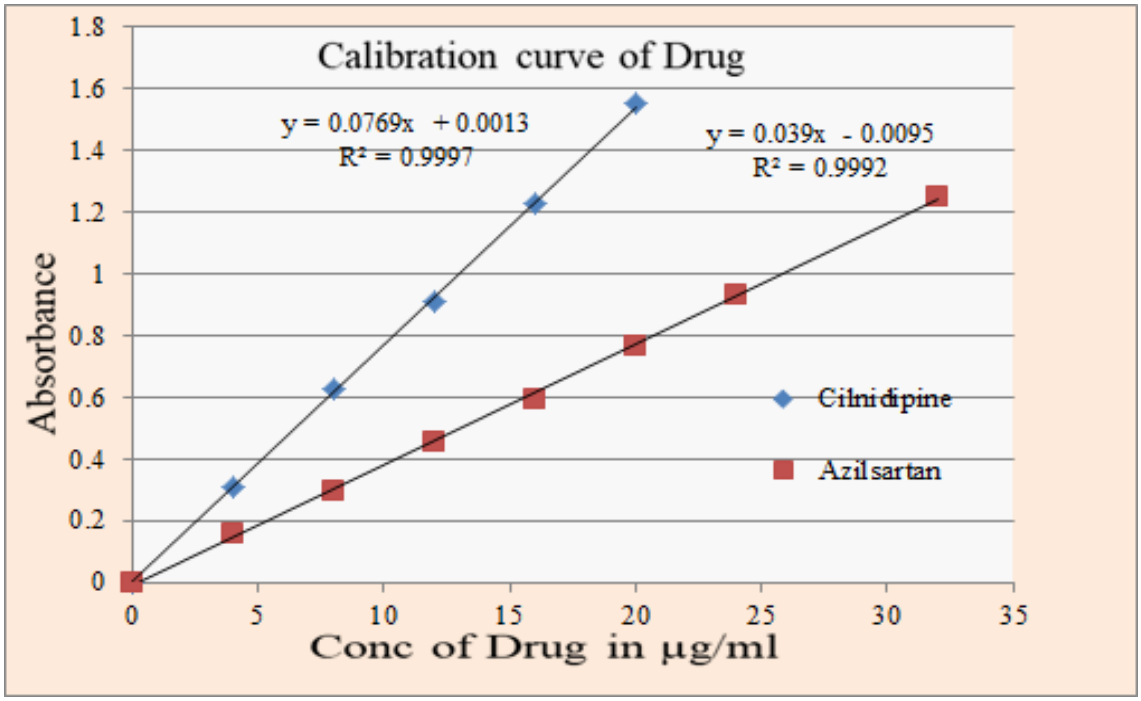

Fig 6: Calibration curve of ASM and CDE

Table 3: Parameters of regression equation obtained in Microsoft excel

Parameters	ASM	CDE
Detection wavelength	246.6	241
Beer's law limit (µg/ml)	1–32 mcg/ml	1–20 mcg/ml
Correlation coefficient (r^2)	0.9992	0.9997
Regression equation (y = mx + c)	Y = 0.039X - 0.0095	Y = 0.0769X + 0.0013

Assay

The assay was carried out by the proposed method. The spectrum of formulation by method was shown in Fig No 7. The assay of formulation was carried out by proposed method and calculated % of nominal conc and RSD was found within acceptable limits are summarized in Table No 4. The results indicated applicability of the method for estimation of formulation.

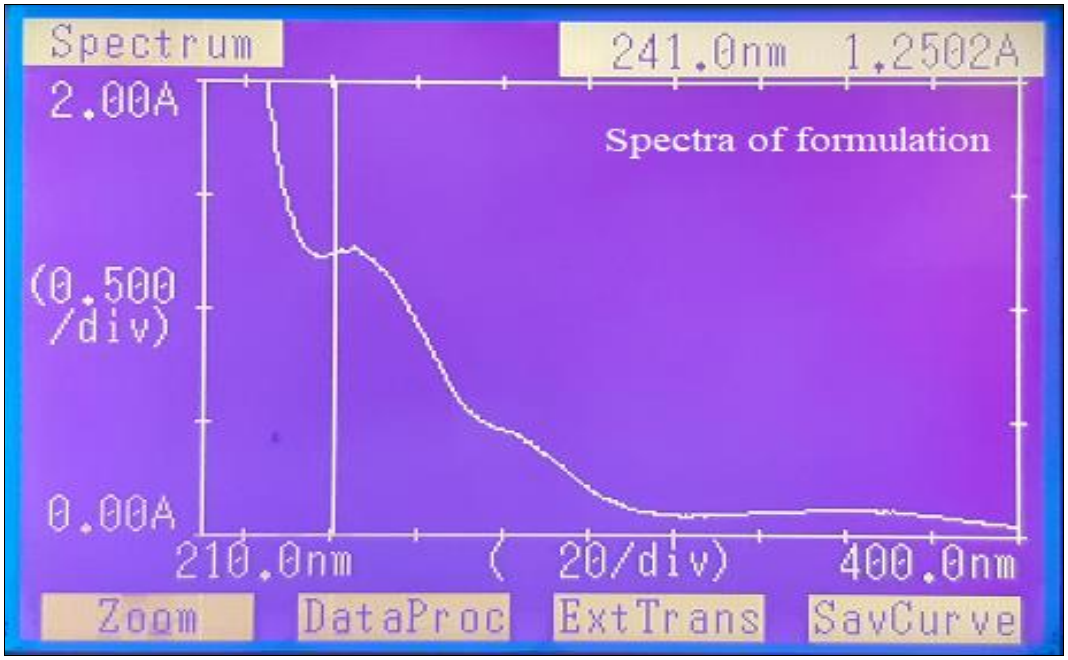

Fig 7: Spectra of formulation obtained in the assay

Table 4: Results of assay of formulation by proposed method

Formulation	Drug	Label Claim (mg/Tablet; n=6)	Amount found/mg	Drug Content %	Std Deviation	% RSD
Method	ASM	40	41.06	102.65	0.68091	0.69895
	CDE	10	9.96	99.63	0.42182	0.42023

Accuracy and Precision

The accuracy study carried out at 3 levels was shown in Fig No 8. The results of accuracy are summarised in Table No 5, the obtained results were within acceptable limit; and methods accuracy was justified by calculating % drug content. The precision study was carried out by performing assay of solutions; further the reproducibility in result was studied by interday and intraday precision. The values obtained SD and % RSD was shown methods precision and are summarised in Table No 5.

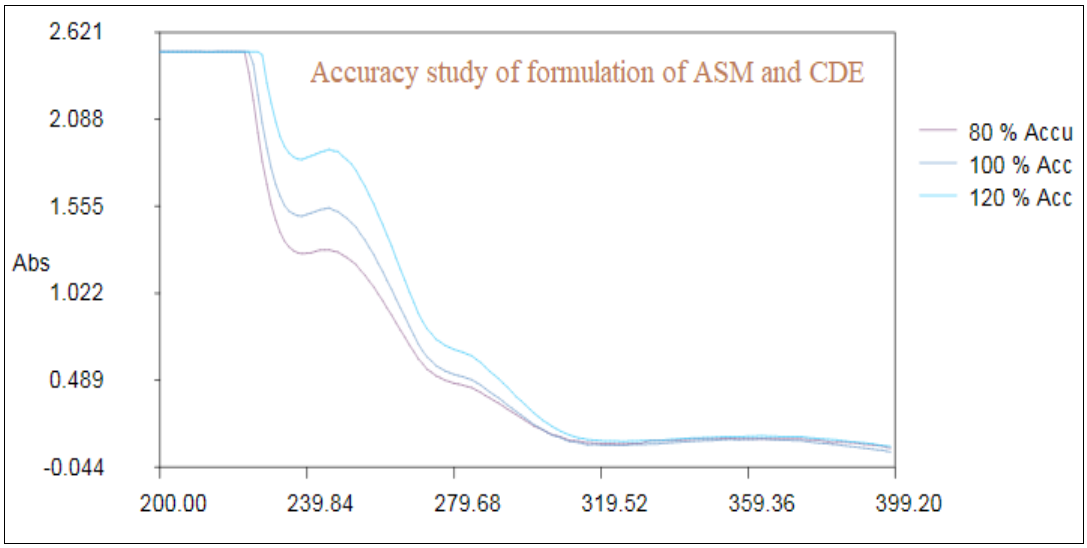

Fig 8: Spectra of formulation obtained in the accuracy study

Table 5: Results of accuracy and precision

Sr. No.	Parameter	Level of study	Drug Name	S.D.	% RSD
1	Precision	Intraday Precision	ASM	1.31513	1.31658
			CDE	1.33631	1.08653
		Inter day precision	ASM	0.51152	0.56804
			CDE	1.42473	1.34269
2	Accuracy study of ASM and CDE	80%	ASM	1.26401	1.29936
		100%		0.15759	0.17738
		120%		0.64834	0.65454
		80%	CDE	0.21761	0.23123
		100%		0.22428	0.22746
		120%		0.49314	0.52905

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of ASM and CDE by the proposed method were shown in Table No 6.

Robustness and Ruggedness

Robustness was studied and capacity of analytical procedure to measure analyte was remain unaffected by small but deliberate variations in method parameter. The analytical method was found rugged during development; similarly the result was produced by performing the analysis by different analyst.

Table 6: Results of LOD and LOQ, robustness

Parameters	ASM	CDE
LOD mcg/ml	1.00717	0.32961
LOQ mcg/ml	1.52031	0.99881

Conclusion

Both the drugs were estimated from the combined formulation by simultaneous equation method. Results were found within acceptable limits, statistical data obtained were shown rigidity of the method. The validated method was employed 50% alcohol as solvent thus become economical. The proposed method is precise, accurate, robust and reproducible hence can be routinely used for simultaneous estimation of azilsartan and cilnidipine from combined dosage form.

Conflict of Interest

All Authors declared that there is no conflict of interest

Acknowledgement

Authors are thankful to Management and Principal of SVPM'S college of Pharmacy Malegaon (BKII), Baramati Dist. Pune for providing necessary facilities chemicals, instruments etc. for research.

References

1. The Merck Index. An Encyclopaedia of chemicals, drugs and Biological. 15th ed. the Royal Society of Chemistry Cambridge UK,2013:161:406.
2. Alison Brayfield. Martindale (The complete drug reference). 39th ed. Pharmaceutical press London UK,2017(A):P. A1341, A1365.
3. Gawai MN, Surwade KS, Phadatare DG. UV Spectrophotometric Method for the estimation of Azilsartan Medoxomil in Bulk Form. Asian J. Research Chem,2018:11(5):791-793.
4. Masthanamma SK. Pradeepthi, Jahnavi. Stability Indicating RP-HPLC Method for Determination of Azilsartan Medoxomil in Pharmaceutical Dosage Form. Research J. Pharm. and Tech,2014:7(2):168-172.
5. Kavita wagh, Sandeep Sonawane, Santosh Chhaajed, Sanjay Kshirsagar. Development of RP-HPLC method for separation of atorvastatin calcium, amlodipine besylate and azilsartan medoxomil and its application to analyze their tablet dosage forms. Asian J. Pharm. Res,2017:7(3):148-154.
6. Mukta D. Naykode, Durgacharan A. Bhagwat, Swapnil D. Jadhav, Harinath N. More. Analytical and Bioanalytical Method for Quantification of Pure Azilsartan, Not its Salts by RP-HPLC. Research J. Pharm. and Tech,2017:10(3):708-714.
7. Dhiraj Kaushik, Jasmeen Kaur, Vaneet Paul Kaur, Balraj Saini, Yogita Bansal, Gulshan Bansal. Forced degradation, LC–UV, MSn and LC–MS–TOF studies on azilsartan: Identification of a known and three new degradation impurities. Journal of Pharmaceutical and Biomedical Analysis,2016:120:202-211. Doi.org/10.1016/j.jpba.2015.12.027
8. Rachumallu Ramakrishna, Santosh kumar Puttrevu, Manisha Bhateria, Veenu Bala, Vishnu L. Sharma, Rabi Sankar Bhatta. Simultaneous determination of azilsartan and chlorthalidone in rat and human plasma by liquid chromatography-electrospray tandem mass spectrometry. Journal of Chromatography B,2015:990:185-197. Doi.org/10.1016/j.jchromb.2015.03.018
9. Farhana V Buchiya, Ashif I Bhim, Hasumati A Raj, Vineet C Jain. Simultaneous Determination of Cilnidipine and Valsartan in Synthetic Mixture using Spectrophotometric Technique (Simultaneous Equation Method). Asian J. Pharm. Ana,2015:5(1):21-25.
10. Sushmita Sawant, Celina Nazareth, Saumya Vernekar. Development and Validation of a Novel, Cost Effective UV Spectrophotometric Method for Simultaneous Estimation of Cilnidipine and Olmesartan. Research Journal of Pharmacy and Technology,2022:15(2):863-9.
11. Samantha Fernandes, Celinana Zareth, Bhakti Naik. Simultaneous Analysis of Cilnidipine and Nebivolol by Absorbance Correction Method and Q Absorption Ratio Method. Research Journal of Pharmacy and Technology,2023:16(7):3213-8.
12. Mohammed M Safhi, Manohara Yagaina Nagaraj. Development and validation of a Rapid Stability Indicating chromatographic determination of Cilnidipine in Bulk and Dosage form. Research J. Pharm. and Tech,2013:6(3):296-299.
13. Vijaykumar T Pawar, Shubhangi V Pawar, Harinath N More, Anita S Kulkarni, Dinanath T Gaikwad. RP-

HPLC Method for Simultaneous Estimation of Cilnidipine and Chlorthalidone. Research J. Pharm. and Tech,2017:10(11):3990-3996.
14. Shirleen Miriam Marques, Rupesh K Shirodkar, Lalit Kumar Analytical. Quality-by-Design' paradigm in development of a RP-HPLC method for the estimation of cilnidipine in nano formulations: Forced degradation studies and mathematical modelling of *In-vitro* release studies. Microchemical Journal,2023:193:109124. Doi.org/10.1016/j.microc.2023.109124.
15. Kanaka Parvathi Kannaiah, Hemanth Kumar Chanduluru, Reem H. Obaydo, Hayam M. Lotfy, Nevin Erk,
16. Manikandan Krishnan, Mohamed A. El Hamd. Application of advanced environmentally benign assessment tools in determining ternary cardiovascular drug combination by RP-HPLC with analytical quality by design: Application to stability indicating method evaluation. Sustainable Chemistry and Pharmacy,2023:35:101197. Doi.org/10.1016/j.scp.2023.101197.
17. Indian Pharmacopoeia, Govt. of India, ministry of Health and family welfare, The Indian pharmacopoeia commission Ghaziabad. 8th ed (II, III). 2018; pp.1616.
18. ICH Expert working group. ICH Harmonized tripartite Guideline-Pharmaceutical development Q 8 R2. In current step 4 version, 2009, 1-28.
18. ICH Expert working group. ICH harmonized tripartite Guideline-Quality Risk Management Q 9. In current step 4 version, 2005, 1-23.
19. ICH Expert working group. ICH Harmonized tripartite Guideline-Pharmaceutical Quality system Q 10. In current step 4 version, 2008, 1-21.
20. ICH Expert working group. ICH Harmonized tripartite Guideline-Validation of analytical procedures: Text and methodology Q 2 R1. In current step 4 version, 2005, 1-17
21. Schweitzer M, Pohl M, Hanna-Brown M, Nethercote P, Bormanare P, Hansen G, *et al*. Implications and opportunities of applying QbD principles to analytical measurements. Pharm Technology,2010:34:12-29.
22. Vogt FG, Kord AS. Development of quality-by-design analytical methods. J of Pharm science,2011:100:797-812.
23. Bhatt DA, Rane SI. QbD approach to analytical RP-HPLC method development and its validation, Int J of Pharma Science,2011:(3):179-187.