IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 18, Issue 6 Ser. 2 (Nov. – Dec. 2023), PP 24-33
www.Iosrjournals.Org

Significance And Approach Of Area Under Curve Method And Amplitude Modulation Of Spectra For Estimation Of Amlodipine And Chlorthalidone In Combined Dosage Form.

Aashish G. Taware, Dnyaneshwar U. Anuse, Prashant S. Suryawanshi, Gurappa K. Dyade
Dept of Post Graduate in Pharmaceutical Quality Assurance, SVPM'S College of Pharmacy, Malegaon (BKII) Tal. Baramati Dist Pune, Maharashtra, India

Abstract
Chemometric based area under curve (AUC) method and amplitude modulation method was developed for the quantitative detection of Amlodipine besylate (ADB) and Chlorthalidone (CTD) by UV-VIS spectrophotometry. Spectroscopic technique, a simple, rapid, precise and accurate process for amount estimation is still in great use for analyst. For estimation of these drugs, solvent alcohol 50% was utilised and 230-220 nm and 242-232 nm were the 2 wavelength regions selected for area under curve method. 237.5 nm And 340 nm were the wavelength of absorbance measurement in derivative method for CTD and ADB respectively. Developed method was validated as per ICH Q 2 R1 regulatory guidelines and linearity of all these drugs was ascertained over the conc range 1-40 µg/ml for ADB and 1-32µg/ml for CTD. The accuracy of assay was found 102.66 % for ADB and 97.16 % for CTD in AUC method and 102.77 % for ADB and 102.41 % for CTD in derivative method; the precision study was shown acceptable data as % RSD data varied from 0.57735 to 1.52752 for ADB, from 0.64292 to 1.96551 for CTD in AUC method. The developed method is rigid, robust and efficient for the estimation of ADB and CTD from the composition of dosage form.

Keywords: *Area under curve method, Amlodipine besylate, Chlorthalidone, derivative method, Chemometry*

Date of Submission: 26-11-2023 Date of Acceptance: 06-12-2023

I. INTRODUCTION

Amlodipine besylate (ADB) is chemically 2-[(2 - amino ethoxy)- methyl]- 4- (2- chloro phenyl)-1, 4-dihydro-6- methyl-3, 5-pyridine di carboxylic acid 3-ethyl-5-methyl ester benzene sulfonate [1], is a potent dihydro calcium channel blocker [2].

Various analytical methods have been reported for the estimation of ADB alone or in combination with other anti - hypertensive agents in pharmaceutical dosage form include UV analytical methods or in combination with CTD [3-5], alone RP-HPLC method [6], chromatographic RP-HPLC methods along with other [7-12], novel approach HPLC method [13], stability indicating HPLC method [14-17] and green analytical method [18] in noted journals.

Chlorthalidone (CTD) chemically (RS)- 2 chloro-5- (1-hydroxy-3-oxo isoindolin-1-yl) benzene sulphonamide [1], is thiazide like diuretic used for the treatment of hypertension and for management of edema caused by conditions such as heart failure or renal impairment. Chlorthalidone improves blood pressure and swelling by preventing water absorption from the kidney through inhibition of the Na+/Cl- symporter in the distal covulated tubule cells in the kidney. However, it is thought that increased diuresis results in the decreased plasma and extracellular fluid volume, decreased cardiac output and therefore overall reduction in blood pressure [2].

Literature survey revealed that for estimation of CTD alone or in combination with other anti - hypertensive agents in pharmaceutical dosage form, UV analytical or chemo metric assisted spectroscopic methods [19-21], chromatographic RP-HPLC methods [22-27], stability indicating HPLC method [28, 29] and response surface methodology [30] have been reported.

Both drugs are official in Indian Pharmacopoeia [31] and British Pharmacopoeia [32]. Chemical structure of both drugs is shown in (Fig No 1).

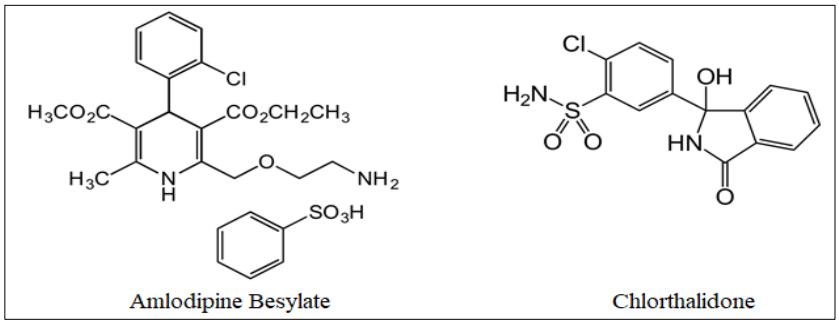

Fig No 1: Chemical structure of Drug molecule

II. 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 solutions Digital Ultrasonic cleaner 1.8 Ltr (Labman scientific Instruments Chennai) was used.

Reagents and Chemicals

Pharmaceutically pure samples of Amlodipine besylate, chlorthalidone from IPCA Laboratories Limited, Kandivli, Mumbai was procured as a gift sample. Commercial formulations containing amlodipine besyalte with chlorthalidone was procured from local market.

Solvent selection

ADB is freely soluble in methanol, slightly soluble in water and Isopropyl alcohol, whereas CTD is sparingly soluble in alcohol, dichloromethane, slightly soluble in methanol and practically insoluble in water, ether and chloroform. Although the solubility of the procured drugs was studied in methanol, 0.1 N HCl and 0.1 N NaOH separately; it was found that both drugs were shown appreciable solubility in 90% ethanol. Hence solution with known conc of each analyte was scanned in UV range of 210 nm to 400 nm in the ethanol solvent. The recorded overlain spectra are shown in Fig No 2.

Preparation of stock solutions and standard solutions

10 mg each of drug ADB and CTD were separately and accurately weighed; and transferred into separate 25ml volumetric flask. Dissolved into 90% alcohol and volume was made up to 25 ml with solvent. Subsequent standard solution of each drug with conc 80µg/ml was prepared by diluting aliquot of stock solution to 10 ml capacity volumetric flask with 50% alcohol.

Selection of wavelength and conc range

From UV spectra it was found that ADB has measurable absorbance at 237 nm and 364 nm; and At 364 nm drug CTD was transparent i. e. shows zero absorbance hence this wavelength is suitable for measurement of ADB; CTD has typical devoid of peak spectra where cannot locate the λ_{max}. Chemometric method was applied and which was reasonable remedy to overcome interference of each other. From the nature of spectra working conc range 1to 40 µg/ml for ADB and 1to 32µg/ml for CTD was selected. Also combined drug solution was prepared simulated to marketed formulation and method was validated as per ICH guidelines and by analysing marketed preparations.

Experimental Method for estimation

Multicomponent formulations are beneficial due to greater patient acceptability, increased potency, multiple actions and fewer side effects and so getting significance. Such formulations must meet all the desired standards related to their quality, safety and efficacy and this can be achieved if they are estimated by different methods. Various methods of UV-Visible spectrometry have distinct advantages; and applied by studying the spectra of analytes. Combined formulation of amlodipine and chlorthalidone used in management of hypertension was selected for method development. Overlain spectra of both drugs are shown in Fig No 2 having much interference at each other's wavelength hence decided to apply area under curve and amplitude modulation method.

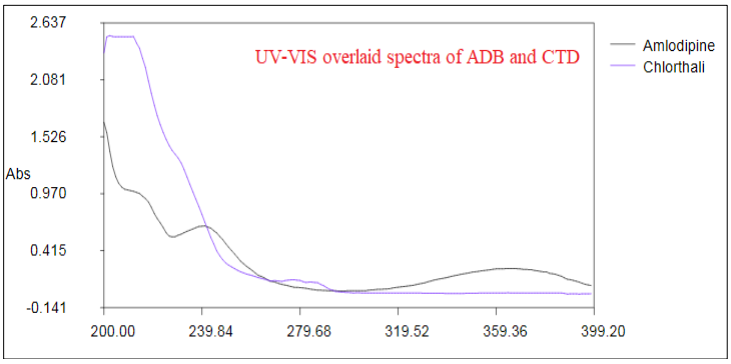

Fig No. 2: Simple overlain spectra of ADB and CTD

Method-I Area under Curve method (AUC)

ADB was shown maximum absorbance at 237.5 nm but CTD has ladder like curve so could not locate λ_{max} for maximum absorbance. So it was decided to apply creamers rule and matrix method to estimate both drugs by area under curve technique. The wavelength region 230-220 as λ_1 and 242 to 232 nm as λ_2 (Fig.No.3 and 4) was considerable to measure area under curve for estimation of combined formulation. The equation A= abc was applied for x (CTD) and y (ADB) determination. By applying Cramer's rule and matrix method two equations derived for these two components are further combined and rearranged to determine the conc of each drug (Equation 1and 2) in the formulation. Working standard solutions of ADB and CTD each of having 12 µg/ml conc were separately prepared and used for the method.

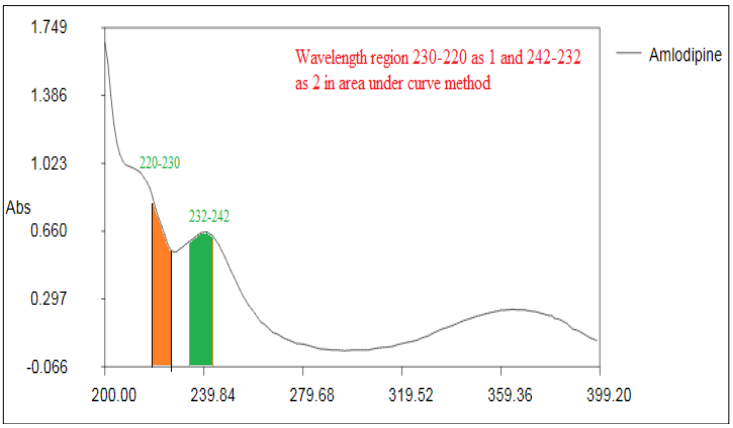

Fig No 3: Wavelength region Y1 (230-220) and Y2 (242-232) for ADB in AUC method

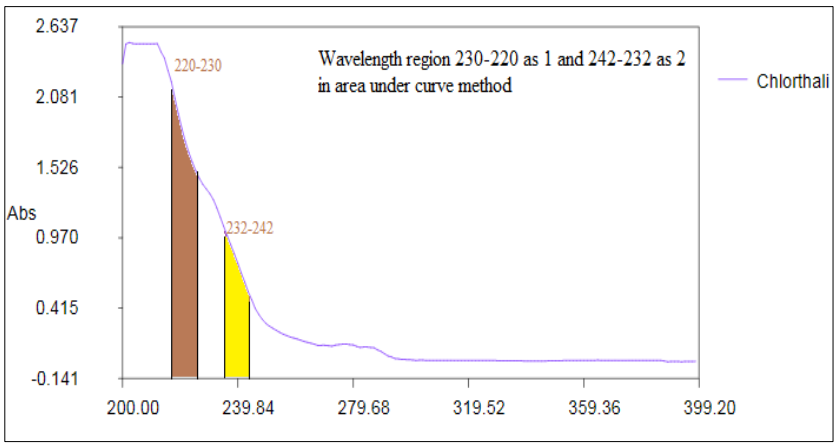

Fig No 4: Wavelength region X1 (230-220) and X2 (242-232) for CTD in AUC method

Equation – 1 and 2

$$Cx = AUC_2 . Y_1 - AUC_1 . Y_2 / Y_1 . X_2 - Y_2 . X_1 \quad \text{——} (1)$$

$$C_Y = AUC_2 . X_1 - AUC_1 . X_2 / Y_2 . X_1 - Y_1 . X_2 \quad \text{——} (2)$$

Where Cx = Conc of CTD in sample solution
AUC_1 and AUC_2 = Area under curve of sample solution at 1 and 2 wavelength
Y_1 and Y_2 = area under curve of ADB at 1and 2 wavelength range of standard solution /conc in g/L
X_1 and X_2 = absorptivity of CTD at 1 and 2 wavelength range of standard solution /conc in g/L
C_y= Conc of ADB in sample solution

Method II: Amplitude Modulation Method

This method comprises the conversion of zero order/normal spectra to its first, second or higher derivative spectrum. The amplitude is directly proportional to the conc of solution provided Beer's law is obeyed by spectrum. In derivative method zero crossing wave length for both drugs is found such that at the zero crossing of one drug the other drug should show substantial absorbance.

Here standard solutions 16 µg/ml each of amlodipine and chlorthalidone were prepared in 10 ml volumetric flask and scanned from 400 to 200 nm wavelength range against ethanol 50% as blank. Absorption spectra of both drugs were recorded and the λ_{max} of ADB was found at 237, but CTD spectra doesn't show appreciable peak.

From overlain spectra both drug has absorption at 237 nm wavelengths Fig No. 2 hence to overcome the interference it was decided to apply derivative and area under curve method.
Zero order spectra of both drug was modulated and converted to first order derivative spectra Fig No.5.

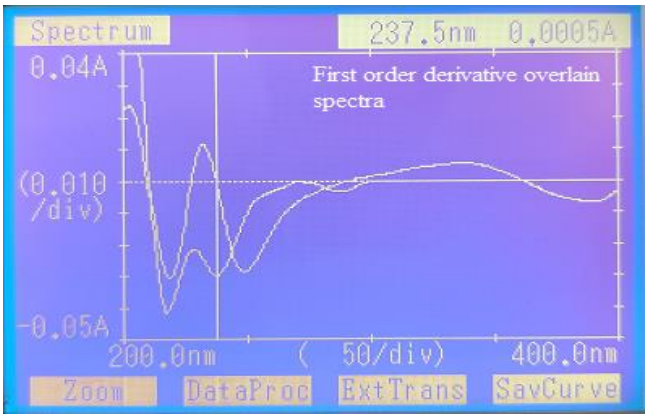

Fig No.5: First order derivative overlain spectra of ADB and CTD

From overlain spectra it clearly shows that no interference or zero absorbance of CTD was found at 340 nm hence it was selected λ_{max} of ADB and 237.5 nm was zero crossing of amlodipine besyalte, so it was selected as λ_{max} of CTD. Both drugs were obeying Beer's law in first order derivative mode at the respective wavelength.

III. METHOD VALIDATION

The objective of method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, assay, precision (repeatability and intermediate precision), accuracy, LoD and LoQ; and robustness ruggedness. 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.

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 known conc each of ADB and CTD were prepared separately and area under curve for method I (12µg/ml), absorbance in derivative order for method II (8 and 12 µg/ml) was recorded, and SD and % RSD of the response was calculated.

Linearity

Method -I

The linearity of an analytical method is its ability to obtain response i.e. area under curve 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 ADB and 1-32 µg/ml for CTD and scanned in 400 to 200 nm range in spectrum mode of the spectrophotometer, area under curve of the each standard solutions were recorded at 1 and 2 selected wavelength region for ADB and CTD. Microsoft office excel software tool was used to obtain the standard regression curve and its analysis as slope, intercept, and correlation coefficient.

Method -II

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 1-40 µg/ml for ADB and 1-32 µg/ml for CTD and scanned in 400 to 200 nm range in spectrum mode of the spectrophotometer, absorbance of the standard solutions were recorded at 237.5 for CTD and 340 nm for ADB 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 obtained data was validated by applying statistical parameters.

Weighed 20 tablets and crushed to fine powder, tablet powder equivalent to 2.5 mg ADB and 6.25 mg CTD was weighed and transferred into 25ml volumetric flask. Dissolved into alcohol 90% mixed well for 15 mins and volume was made to 25 ml with solvent. Resulting solution was filtered through whatman filter paper and aliquots of solution were further diluted with 50% alcohol to obtain six replicates tablet solution.

Method-I-Solution was scanned in the range of 400 to 200 nm to measure AUC of tablet solution at selected 1 and 2 wavelength regions. Measured AUC was utilised to estimate unknown conc of formulation; and results are statistically validated to obtain % of nominal conc, standard deviation and % of RSD.

Method-II- All the solutions were scanned; and spectra's were modulated to first order to measure absorption of solution at 237.5 and 340 nm shown in Fig No.5 Amount of each drug in solution was calculated obtained results are validated statistically.

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 ADB and CTD 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 and measured and % of conc, SD and RSD of replicates were calculated.

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

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

The LOD and LOQ of ADB and CTD by the proposed method were determined using calibration graph method and calculated as $3.3\sigma/s$ and $10\ \sigma/s$ 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.

IV. RESULTS AND DISCUSSION

Method development comprises numerous steps of which solvent selection, selection of method for measurement and validation, essential calculation are significant one. Use of common, low noise, interference free and eco-friendly solvent 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.

Area Under Curve Method

Linearity

The calibration curve of AUC against conc of both drugs was found to be linear in the conc range of 1-40 µg/ml for ADB and CTD as shown in Fig No 6. The regression equation of line and its parameters slope, r^2 value and intercept are tabulated in Table No 1, which proved the linear relationship between conc and obtained response.

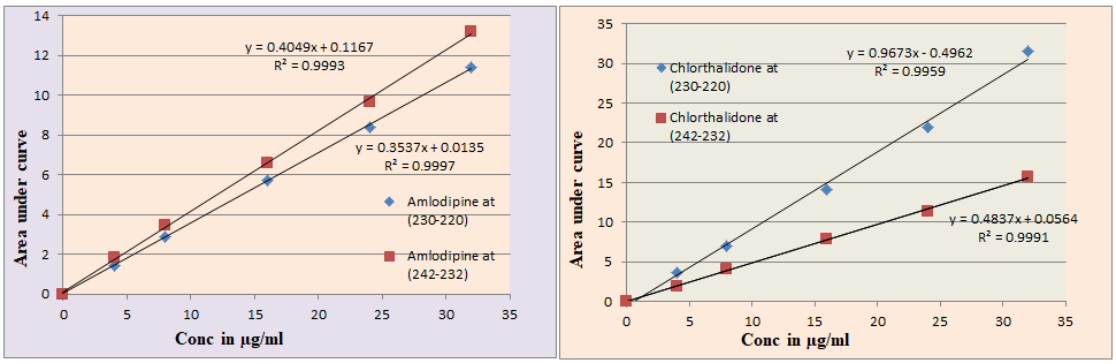

Fig No 6: Calibration curve of ADB and CTD obtained in AUC method

Table No 1: Parameters of regression equation obtained in Microsoft excel

Parameters	**ADB**		**CTD**	
Selected wavelength range to measure AUC	230-220	242-232	230-220	242-232
Conc range (µg/ml)	1-32 µg/ml		1-32 µg/ml	
Correlation coefficient (r^2)	0.9997, 0.9993		0.9959, 0.9991	
Regression equation (y = mx + c)	Y=0.3537 X + 0.0135 Y=0.4049 X+0.1167		Y=0.9673 X – 0.4962 Y=0.4837 X+0.0564	

Assay

The assay was carried out by described method. The obtained results by proposed method and calculated % of nominal conc and RSD was found within acceptable limits are summarized in Table No 2. The results indicated applicability of the method for estimation of formulation.

Table No 2: Results of assay of formulation by Area under Curve method

Tablet Formulation	Label claim(mg/Tab)		% of Label claim estimated*		Standard deviation		Coefficient of variance	
Sample I	ADB	CTD	ADB	CTD	ADB	CTD	ADB	CTD
	5	12.5	102.66 %	97.16 %	1.9248	1.7389	1.8749	1.7897

*Mean of six determinations

Accuracy and Precision

The results of accuracy are summarized in Table No 3, 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 3.

Table No 3: Results of accuracy and precision obtained in AUC

Sr. No.	**Parameter**	**Level of study**	**Drug Name**	**S.D.**	**% RSD**
1	Precision	Intraday Precision	ADB	0.57735	0.55278
			CTD	1.96551	2.10163
		Interday precision	ADB	1.52752	1.94431
			CTD	0.64292	0.63684
2	Accuracy study of ADB and CTD	80%	ADB	0.19025	0.21142
		100%	ADB	0.19622	0.24372
		120%	ADB	0.29458	0.35214
		80%	CTD	0.19857	0.21417
		100%	CTD	0.12152	0.10055
		120%	CTD	0.24006	0.24616

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

The LOD and LOQ of ADB and CTD was calculated from slope of regression line and standard deviation of the calibration curve with the help of Microsoft office excel word and found -- for ADB and --- for CTD.

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; similarity the result was produced by performing the analysis by different analyst.

Amplitude Modulation Method

The method was validated as per ICH guidelines

System Suitability

The absorbances of six replicates of standard solution (8 and 12 µg/ml for ADB and CTD) at selected derivative order wavelength are reported in Table No 4. The SD and % RSD was found for ADB and CTD and meets the system suitability requirements indicates suitability of the method for analysis.

Table No 4: System suitability study of ADB and CTD

Sr No	Conc in µg/ml	Absorbance of ADB 340 nm	Conc in µg/ml	Absorbance of CTD 237.5 nm
1	8 µg/ml	0.0024	12 µg/ml	-0.0419
2	8 µg/ml	0.0023	12 µg/ml	-0.0427
3	8 µg/ml	0.0023	12 µg/ml	-0.0431
4	8 µg/ml	0.0025	12 µg/ml	-0.0429
5	8 µg/ml	0.0024	12 µg/ml	-0.0424
6	8 µg/ml	0.0025	12 µg/ml	-0.0428
	SD	0.89442	SD	0.42739
	RSD	0.96895	RSD	0.44109

Linearity

The linearity was ascertained for both drugs shown in (Fig No 7 and 8). The calibration curve of both drugs derivative spectra was found to be linear in the conc range of 4-40 µg/ml for AMD and 4-32 µg/ml for CTD as shown in Fig No 9. The regression equation of line and its parameters slope, r^2 value and intercept are tabulated in Table No 5, which proved the linear relationship between conc and obtained response.

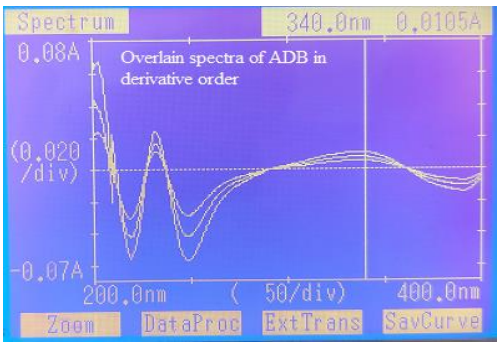

Fig No 7: Overlaid spectra of ADB in First order derivative method

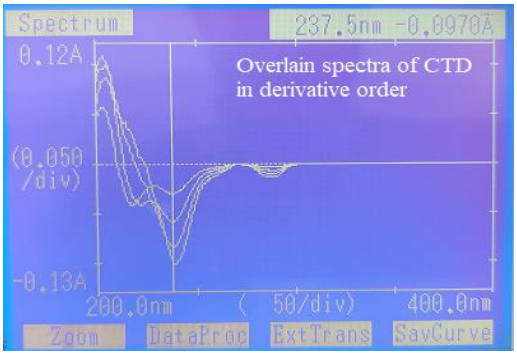

Fig No 8: Overlaid spectra of CTD in First order derivative method

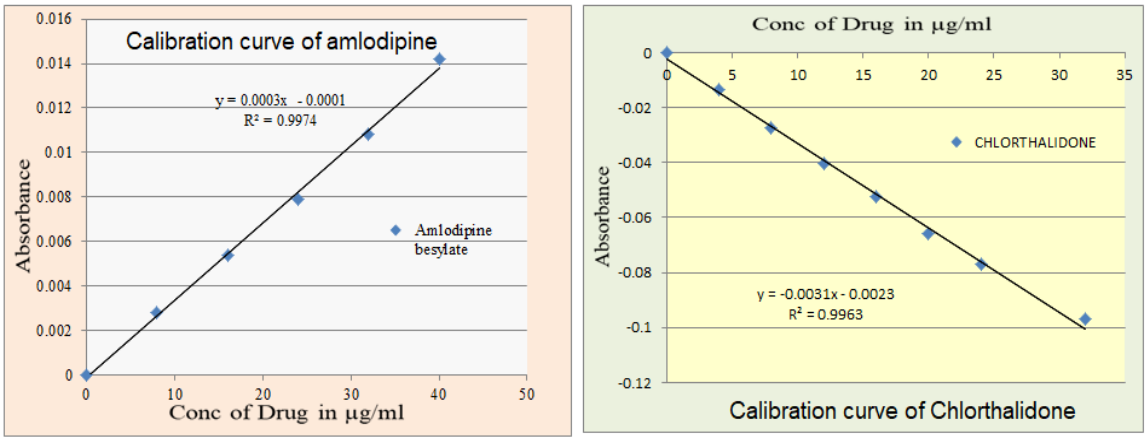

Fig No 9: Calibration curve of ADB and CTD obtained in derivative method

Table No 5: Parameters of regression equation obtained from Microsoft excel

Parameters	ADB	CTD
Detection wavelength	340	237.5
Beer's law limit (µg/ml)	4-40 µg/ml	4-32 µg/ml
Correlation coefficient (r^2)	0.9974	0.9963
Regression equation (y = mx + c)	Y = 0.0003 X - 0.0001	Y = 0.0031 X-0.0023

Assay

The assay was carried out and results of formulation were validated by proposed method; calculated % of nominal conc, SD and RSD was found within acceptable limits are summarized in Table No 6. The results were proved the effectiveness of derivative method for rapid estimation of dosage forms.

Table No 6: Results of assay of formulation by derivative method

Formulation	Drug	Label Claim (mg/Tablet; n=6)	Amount found/mg*	Drug Content %	Std Deviation	% RSD
Formulation Method -I	ADB	12.5	102.77	12.846	0.19248	0.17859
	CTD	80	102.41	81.928	0.73891	0.90197

*Mean of six determinations

Accuracy and Precision

The results of parameter studied accuracy are given in Table No 7, the obtained results were within acceptable limit; and methods accuracy was justified by calculating standard deviation.

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 tabulated in Table No 7.

Table No 7: Results of accuracy and precision obtained in derivative method

S. No.	Parameter	Level of study	Drug Name	S.D.	% RSD
1	Precision study	Intraday Precision	ADB	0.57735	0.55278
			CTD	1.96553	2.10163
		Inter day precision	ADB	1.52752	1.94431
			CTD	0.64291	0.63682
2	Accuracy study of ADB and CTD	80%	ADB	0.19052	0.21142
		100%	ADB	0.19621	0.24372
		120%	ADB	0.50846	0.48181
		80%	CTD	0.19857	0.21417
		100%	CTD	0.10001	0.10055
		120%	CTD	0.24001	0.24616

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

The LOD and LOQ of ADB and CTD by the proposed method were found in acceptable limits. 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; similarity the result was produced by performing the analysis by different analyst.

V. CONCLUSION

All these drugs were quantitatively estimated from the combined formulation by area under curve and derivative method. Results were found within acceptable limits, statistical data obtained were shown rigidity of the method. Derivative method is rather simple, precise as compare to area under curve method and free from arithmetic calculations hence would be accepted. However both these proposed methods are precise, accurate, robust and reproducible hence can be routinely used for simultaneous estimation of amlodipine and chlorthalidone.

Conflict of Interest

All Authors declared that there is no conflict of interest

Acknowledgement

Authors are thankful to IPCA Laboratories Limited, Kandivli, Mumbai Maharashtra, for providing amlodipine besylate and chlorthalidone as a gift sample. Authors are thankful to Principal and Management SVPM'S college of Pharmacy Malegaon (BKII) Tal. Baramati Dist. Pune, Maharashtra for providing necessary facilities for research.

REFERENCES

[1]. The Merck Index. An Encyclopaedia Of Chemicals, Drugs And Biological. 15th Ed. The Royal Society Of Chemistry Cambridge UK. 2013. P. 87,390.
[2]. Alison Brayfield. Martindale (The Complete Drug Reference). 39th Ed. Pharmaceutical Press London UK. 2017(A). P. A1341, A1365
[3]. Sanap R M, Wavhale S R, Kunjir V V, Shete R V. Analytical Method Development And Validation For Telmisartan, Chlorthalidone And Amlodipine By UV-Spectroscopic Method. Research Journal Of Pharmacy And Technology. 2021; 14(11):6049-6054.
[4]. Usharani N, Divya K, Ashrtiha V V. Development And Validation Of UV-Derivative Spectroscopic And RP-HPLC Methods For The Determination Of Amlodipine Besylate And Valsartan In Tablet Dosage Form And Comparison Of The Developed Methods By Student's T-Test. Indian J. Pharmaceu. Edu. Res. 2017; 51(4s): S776-782.
[5]. Kavathia A, Misra M. Development And Validation Of RP-HPLC And UV-Spectrophotometric Methods For Rapid Simultaneous Estimation Of Amlodipine And Benazepril In Pure And Fixed Dose Combination. Arabian Journal Of Chemistry. 2017; 10(2s):S3021-3028.
[6]. Burugu R. RP-HPLC Determination Of Amlodipine From Tablet Dosage Form. Asian Journal Of Research In Chemistry. 2010; 3(3): 656-658.
[7]. Mandale T R, Kondawar M S, Kadam S D. Development And Validation Of Analytical Method For Simultaneous Estimation Of Amlodipine Besylate And Celecoxib In Pure And Combined Dosage Form. Research Journal Of Pharmacy And Technology. 2020; 13(9):4280-4284.
[8]. Priyadharisini J, Saraswathi D, Aruna A, Suresh AJ. RP-HPLC Determination Of Olmesartan Medoxomil And Amlodipine Besylate In Tablets. Research Journal Of Pharmacy And Technology. 2011; 4(6): 903-904.
[9]. Çelebier M, Kaynak MS, Altınöz S, Sahin S. HPLC Method Development For The Simultaneous Analysis Of Amlodipine And Valsartan In Combined Dosage Forms And In Vitro Dissolution Studies. Brazilian Journal Of Pharmaceutical Sciences. 2010; 46: 761-768.
[10]. Jagadeeswaran M, Gopal N, Sivakumar T. Simultaneous Estimation And Validation Of Amlodipine Besylate And Nebivolol Hydrochloride In Tablet Formulation By RP-HPLC Method. Asian Journal Of Research In Chemistry. 2010; 3(3): 640-642.
[11]. Vora DN, Kadav AA. Development And Validation Of A Simultaneous HPLC Method For Estimation Of Bisoprolol Fumarate And Amlodipine Besylate From Tablets. Indian Journal Of Pharmaceutical Sciences. 2008; 70(4): 542-546.
[12]. Shah DA, Bhatt KK, Shankar MB, Mehta RS, Baldania SL. RP-HPLC Determination Of Atorvastatin Calcium And Amlodipine Besylate Combination In Tablets. Indian Journal Of Pharmaceutical Sciences. 2006; 68(6): 796-799.
[13]. Dyade G K, Sawant R L. Validated RP-HPLC Method And Unique Mobile Phase For The Simultaneous Estimation Of Amlodipine Besylate And Valsartan From Solid Dosage Form And Rosuvastatin And Valsartan From Bulk. Asian Journal Of Pharmaceutical And Clinical Research. 2019; 12(4): 156-162.
[14]. Chitlange S S, Bagri K, Sakarkar D M. Stability Indicating RP-HPLC Method For Simultaneous Estimation Of Valsartan And Amlodipine In Capsule Formulation. Asian Journal Of Research In Chemistry. 2008; 1(1): 15-18.
[15]. Jain PS, Patel MK, Gorle AP, Chaudhari AJ, Surana SJ. Stability-Indicating Method For Simultaneous Estimation Of Olmesartan Medoxomile, Amlodipine Besylate And Hydrochlorothiazide By RP-HPLC In Tablet Dosage Form. Journal Of Chromatographic Science. 2012; 50(8): 680-687.
[16]. Naidu K R, Kale U N, Shingare M S. Stability Indicating RP-HPLC Method For Simultaneous Determination Of Amlodipine And Benazepril Hydrochloride From Their Combination Drug Product. Journal Of Pharmaceutical And Biomedical Analysis. 2005; 39(1-2): 147-155.
[17]. Bodapati K, Vaidya JR, Siddiraju S, Gowrisankar D. Stability Indicating RP-HPLC Studies For The Estimation Of Irbesartan And Amlodipine Besylate In Pharmaceutical Formulation And Indentification And Characterization Of Degradants Using LC-MS. Journal Of Liquid Chromatography And Related Technologies. 2015; 38(2): 259-270.
[18]. El Jamal M K, Gazy A A. Analysis Of Three Cardiovascular Drugs In Their Ternary Mixture Using Green Analytical Methodology

Of Smart Spectrophotometric Methods And RP-HPLC Method. International Journal Of Pharmacy And Pharmaceutical Sciences. 2016; 8(8): 243-250.
[19]. Patel SN, Hinge MA, Bhanushali VM. Development And Validation Of An UV Spectrophotometric Method For Simultaneous Determination Of Cilnidipine And Chlorthalidone. Journal Of Pharmacy Research. 2015; 9(1):41-45.
[20]. Bhanushali VM, Hinge MA, Patel SN. Development And Validation Of UV Spectrophotometric Methods For Simultaneous Determination Of Chlorthalidone And Losartan Potassium. Journal Of Pharmacy Research. 2015; 9(1):54-59.
[21]. Charde M, Welankiwar A, Chakole R. Simultaneous Estimation Of Atenolol And Chlorthalidone In Combine Tablet Dosage Form By Absorption Ratio Method Using UV-Vis Spectrophotometry. International Journal Of Advanced Pharmaceutics. 2014; 3(1)20-27.
[22]. Kalaiselvi P, Lalitha K G. Development And Validation Of RP-HPLC Method For The Simultaneous Estimation Of Chlorthalidone And Irbesartan In Pharmaceutical Dosage Form. Pharmacophore. 2014; 5(2): 278-286.
[23]. Sravani P, Rubesh Kumar S, Duganath N, Devanna N. Method Development And Validation For The Simultaneous Estimation Of Azilsartan And Chlorthalidone By RP-HPLC In Pharmaceutical Dosage Form. International Journal Of Pharma Sciences. 2014; 4 (5): 725-729.
[24]. Charde MS, Welankiwar AS, Chakole RD. Development Of Validated RP-HPLC Method For The Simultaneous Estimation Of Atenolol And Chlorthalidone In Combine Tablet Dosage Form. International Journal Of Advanced Pharmaceutical Sciences. 2014; 3(1): 6-18.
[25]. Mhaske RA, Sahasrabudhe S, Mhaske AA. RP-HPLC Method For Simultataneous Determination Of Irbesartan, Losartan, Hydrohlorothiazide And Chlorthalidone-Application To Commercially Available Drug Products. International Journal Of Pharmaceutical Sciences And Research. 2012; 3(4): 1116-1123.
[26]. Pawar V T, Pawar S V, More H N, Kulkarni A S, Gaikwad D T. RP-HPLC Method For Simultaneous Estimation Of Cilnidipine And Chlorthalidone. Research Journal Of Pharmacy And Technology. 2017; 10(11): 3990-3996.
[27]. Sawale V, Dangre P, Dhabarde DI. Development And Validation Of RP-HPLC Method For The Simulteneous Estimation Of Olmesartan Medoxomil And Chlorthalidone In Tablet Dosage Form. International Journal Of Pharmacy And Pharmaceutical Sciences. 2015; 7(5): 266-269.
[28]. Elkady E F, Fouad M A, Faquih A A. A Versatile Stability-Indicating Liquid Chromatographic Method For The Simultaneous Determination Of Atenolol, Hydrochlorothiazide And Chlorthalidone. Current Pharmaceutical Analysis. 2020; 16(8): 1037-1051.
[29]. Youssef RM, Maher HM, El-Kimary EI, Hassan EM, Barary MH. Validated Stability-Indicating Methods For The Simultaneous Determination Of Amiloride Hydrochloride, Atenolol, And Chlorthalidone Using HPTLC And HPLC With Photodiode Array Detector. Journal Of AOAC International. 2019; 96(2): 313-323.
[30]. Kayesh R, Jahan MS, Sultan MZ. Development Using Response Surface Metho Dology And Validation Of A Stability-Indicating RP-HPLC Method For Simultaneous Estimation Of Azilsartan Medoxomil And Chlorthalidone In Solid Dosage Form. Chromatographia. 2017; 80: 593-603.
[31]. Indian Pharmacopoeia, Govt. Of India, Ministry Of Health And Family Welfare, The Indian Pharmacopoeia Commission Ghaziabad. 8th Ed (II). 2018; Pp.1219, 1603.
[32]. British Pharmacopoeia. Medicines And Healthcare Products Regulatory Agency London; 2019. Pp. I-156, 550.