

Q-Absorbance Ratio Spectrophotometric Method for the Simultaneous Estimation of Amlodipine Besylate and Candesartan Cilexetil in Synthetic Mixture

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ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical q-absorbance method for the simultaneous determination of Amlodipine besylate and Candesartan cilexetil in synthetic mixture. Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one which is an Isoabsorptive point at 242 nm in Methanol. The second wavelength is used 255 nm, which is λ_{max} of Candesartan cilexetil in Methanol. The linearity was obtained in the concentration range of 5-25 $\mu\text{g/ml}$ of both drugs. The concentration of drugs was determined by using ratio of absorbance at isoabsorptive point and at the λ -max of Candesartan cilexetil. The method was successfully applied to pharmaceutical dosage form because of no interference. The result of analysis has been validated by recovery studies.

Keywords: Amlodipine besylate, Candesartan cilexetil, Q-Absorbance ratio spectrophotometric method, UV spectroscopy

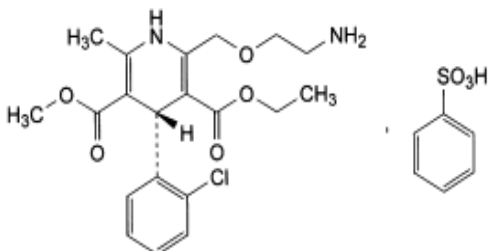
INTRODUCTION: [3, 11, 12, 14, 15, 16, 19]

Amlodipine besylate (AB) (fig. 1a) is the besylate salt of amlodipine, chemically known as, 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid-3-ethyl, 5-methyl ester (\pm) monobenzenesulfonate^[1]. It is a long-acting, calcium channel blocker. It is used in the treatment of hypertension and angina^[12, 16]. Candesartan cilexetil a potent^[4], orally active and selective angiotensin II type 1 receptor blocker, belongs to benzimidazole class with chemical name 1-[[[(cyclohexyloxy) carbonyl]oxy]ethyl-2-ethoxy-1-[[2'-(1H-tetrazol-5-yl) [1, 1'-biphenyl]-4-yl] methyl]-1H-benzimidazole-7-carboxylate-1-3. It is an anti hypertensive drug and is used in treatment of angina. AB is official in USP, BP, EP & IP whereas CC is official in JP^{[14,}

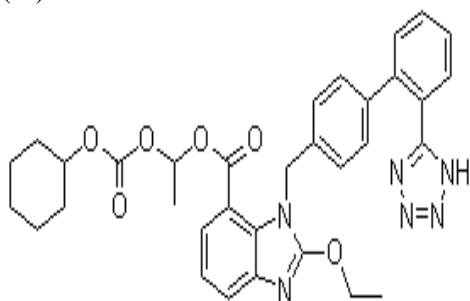
15, and 19]. The chemical structures of AB & CC are shown in Fig. 1. Combination drug products of AB and CC are widely marketed and used in the treatment of hypertension and cardiac disorders^[12, 16]. Several analytical methods like UV spectrophotometry, HPLC, HPTLC, UPLC have been reported for estimation of AB & CC by single drug and also by combining with other drugs^[9, 10, 17, 20, 23, 25, and 26]. However no method has been reported till date for the simultaneous estimation of AB & CC using the UV spectrophotometric method. The present paper describes the development and validation of two analytical methods for simultaneous estimation of AB & CC by UV spectrophotometry in tablet dosage form; the methods include absorbance ratio method. The

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proposed methods are optimized and validated as per the ICH guidelines^[6].



(1a)



(1b)

Fig. 1 Chemical structures of the analytes (1a) AB & (1b) CC^[3, 11]

MATERIALS AND METHODS

SITE OF EXPERIMENTATION: Smt. R. D. Gardi B. Pharmacy College, Nyara, Rajkot

INSTRUMENTATION: UV visible spectrophotometer Helios Alpha, Thermo Scientific, (model UV A 1002E) with 1 cm matched quartz cells were used for all absorbance measurements. Contech, EIE instrument pvt. Ltd. (model CA 34) balance was used for weighing the samples.

REAGENTS: Double distilled water and Whatmann filter paper (0.45 μ m) were used for filtration. Active pharmaceutical ingredient (API) working standards of amlodipine besylate (AB), candesartan cilexetil (CC) were obtained as gift sample from Alembic Pharma limited, Baroda, India and test samples (tablets with composition CC-8 mg and AB (equivalent to amlodipine-5 mg) were procured from the local market.

CHEMICAL: Methanol obtained from Chemdyes co. Rajkot.

FOR Q-ABSORPTION RATIO METHOD ^[2, 5, 8, 13]

Mode: Scan

Scan speed: Intelli scan

Wavelength range: 200 - 400 nm

Absorbance scale: 0.00 - 2.00 A

Initial base line correction: Methanol^[27]

PREPARATION OF STANDARD SOLUTION IN METHANOL

(A) Amlodipine besylate standard stock solution: (1000 μ g/ml)

A 100 mg of Amlodipine besylate was weighed and transferred to a 100 ml volumetric flask. Mix with half quantity of Methanol and volume was made up to the mark.

Amlodipine besylate working standard solution: (100 μ g/ml)

Pipette out 10 ml from the standard stock solution in other 100 ml volumetric flask and volume was made up to the mark with Methanol.

(B) Candesartan cilexetil standard stock solution: (1000 μ g/ml)

A 100 mg of Candesartan cilexetil was weighed and transferred to a 100 ml volumetric flask. Mix with half quantity of Methanol and volume was made up to the mark.

Candesartan cilexetil working standard solution: (100 μ g/ml)

Pipette out 10 ml from the standard stock solution in other 100 ml volumetric flask and volume was made up to the mark with Methanol.

PREPARATION OF SYNTHETIC MIXTURE ^[21, 22, 28]

The synthetic mixture of Candesartan cilexetil and Amlodipine besylate was prepared in ratio of 8:5. Accurately weighed 8 mg of Candesartan cilexetil and 5 mg of Amlodipine besylate were

transferred to 100 ml volumetric flask, and 70 ml of Methanol was added. Excipients, such as Mannitol, glycerol, povidone, Mg-stearate and corn starch which were used in tablet formulation, were added in this mixture and mechanically stirred for 20 minutes. This solution was filtered through the Whatmann filter paper No. 41 and filtrate was collected. Residues were washed with Methanol. The volume of filtrate and washing was combined and volume was made up to 100 ml with Methanol.

PROCEDURE FOR DETERMINATION OF WAVELENGTH FOR MEASUREMENT

1.5 ml of working standard stock solution of AB (100 µg/ml) and 1.5 ml of working standard stock solution of CC (100 µg/ml) were pipetted out into two separate 10 ml volumetric flask and volume was adjusted to the mark with Methanol to get 15 µg/ml of AB and 15 µg/ml of CC. Each solution was scanned between 200 - 400 nm against Methanol as a reagent blank. Isoabsorptive point was selected from the overlay spectra of AB and CC.

PREPARATION OF CALIBRATION CURVE

(A) Calibration curve for AB

Calibration curve for AB consists of different concentrations of standard AB solution ranging from 5 - 25 µg/ml. The solutions were prepared by pipetting out 0.5, 1, 1.5, 2.0, and 2.5 ml of the working standard solution of AB (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol. The absorbance of the solutions was measured at 242 nm (λ_1) and 255 nm (λ_2) against Methanol as a reagent blank. Calibration curve was plotted at both wavelengths and two equations were constructed using the absorptivity.

(B) Calibration curve for CC

Calibration curve for PAM consisted of different concentrations of standard CC solution ranging

from 5 - 25 µg/ml. The solutions were prepared by pipetting out 0.5, 1.0, 1.5, 2.0, and 2.5 ml of the working standard solution of CC (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol. The absorbance of the solutions was measured at 242 nm (λ_1) and 255 nm (λ_2) against Methanol as a reagent blank. Calibration curve was plotted at both wavelengths and two equations were constructed using the absorptivity.

VALIDATION OF PROPOSED METHOD ^[6]

Parameters to be considered for the validation of method are:

A) Linearity and Range

The linearity response was determined by analyzing 6 independent levels of calibration curve in the range of 5 - 25 µg/ml and 5 - 25 µg/ml for AB and CC respectively (n = 3).

The calibration curve of absorbance vs. respective concentration was plotted and correlation coefficient (r_2) and regression line equations for AB and CC were calculated.

B) Precision

I) Repeatability

Aliquots of 1.5 ml of working standard solution of AB (100 µg/ml) were transferred to a series of 10 ml volumetric flask. Aliquots of 2.0 of working standard solution of CC (100 µg/ml) were respectively transferred to the same above series of 10 ml volumetric flask. The volume was adjusted up to mark with Methanol to get 15 µg/ml solution of AB and 20 µg/ml solution of CC. The absorbance of solutions was measured spectrophotometrically three times and coefficient of variance (% C.V.) was calculated.

II) Intraday precision

Aliquots of 0.5, 1, and 1.5 ml of working standard solution of AB (100 µg/ml) were transferred to a series of 10 ml volumetric flask. Aliquots of 0.5, 1.0, and 1.5 ml of working

standard solution of CC (100 µg/ml) were respectively transferred to the same above series of 10 ml volumetric flask. The volume was adjusted up to mark with Methanol to get 5, 10 and 15 µg/ml solution of AB and 5, 10, and 20 µg/ml solution of CC.

The absorbance of solutions was measured spectrophotometrically three times and coefficient of variance (% C.V.) was calculated.

III) Interday precision

Aliquots of 0.5, 1, and 1.5 ml of working standard solution of AB (100 µg/ml) were

transferred to a series of 10 ml volumetric flask. Aliquots of 0.5, 1.0, and 1.5 ml of working standard solution of CC (100 µg/ml) were respectively transferred to the same above series of 10 ml volumetric flask. The volume was adjusted up to mark with Methanol to get 5, 10 and 15 µg/ml solution of AB and 5, 10, and 15 µg/ml solution of CC.

The absorbance of solutions was measured spectrophotometrically three times on the three different days and coefficient of variance (% C.V.) was calculated.

C) Accuracy

Procedure

I) Preparation of Sample Solution for AB:

Synthetic mixture X: AB (50 µg/ml) + CC (80 µg/ml)

Standard solution Y: AB (100 µg/ml)

Sr. No.	Step 1	Step 2 (diluent as methanol)	Total AB Conc.(µg/ml)
1.	0.5 ml of solution y	Add diluents make up to 10ml	5
2.	0.5 ml sol ⁿ y + 0.8 ml of sol ⁿ x	Add diluents make up to 10ml	9
3.	0.5 ml sol ⁿ y + 1 ml of sol ⁿ x	Add diluents make up to 10ml	10
4.	0.5 ml sol ⁿ y + 1.2 ml of sol ⁿ x	Add diluents make up to 10ml	11

II) Preparation of sample solution for CC:

Synthetic mixture X: AB (50 µg/ml) + CC (80 µg/ml)

Standard solution Y: CC (100 µg/ml)

Sr. No.	Step 1	Step 2	Total CC Con. (µg/ml)
1.	0.8 ml sol ⁿ y	Add diluents make up to 10ml	8
2.	0.8 ml sol ⁿ y + 0.8 ml of sol ⁿ x	Add diluents make up to 10ml	14.4
3.	0.8 ml sol ⁿ y + 1 ml of sol ⁿ x	Add diluents make up to 10ml	16
4.	0.8 ml sol ⁿ y + 1.2 ml of sol ⁿ x	Add diluents make up to 10ml	17.6

Each solution was scanned between 200 to 400 nm against Methanol as a blank.

Absorbance of solutions was measured at selected wavelengths for AB and CC.

The amount of AB and CC was calculated at each level (80%, 100%, 120%) and % recoveries were computed.

D) LOD and LOQ ^[5]

The LOD (Limit of Detection) and LOQ (Limit of Quantitation) was estimated from the set of three calibration curves used to determine method linearity. The LOD and LOQ was calculated as,

$$\text{LOD} = 3.3 * \frac{\text{SD}}{\text{Slope}}$$

$$\text{LOQ} = 10 * \frac{\text{SD}}{\text{Slope}}$$

Where,

S.D. = Standard deviation of the Y- intercepts of the 3 calibration curves

Slope = Mean slope of the 3 calibration curves

DRUG – EXCIPIENTS INTERACTION STUDY ^[21, 22, 28]:

Drug – excipients interaction study was performed by accuracy study. First prepare synthetic mixture without excipient was prepared as per dosage form ratio and % recovery was calculated. Then synthetic mixture was prepared by using common excipients present in marketed formulation. In synthetic mixture, mannitol, povidone, glycerol, mg-stearate and corn starch were added as per standard reference. All ingredients were transferred to 100 ml volumetric flask and 70 ml of Methanol was added and mechanically stirred for 20 minutes. This solution was filtered through the whatmann filter paper no. 41 and filtrate was collected. Residues were washed with Methanol. The filtrate and washing were combined and volume of solution was made up to 100 ml with Methanol. Recovery studies were performed at three levels (80%, 100% and 120%). The percentage recovery should lie within the range 98 % - 102%. If it should lie within the range then it indicate that drug does not interact with excipients. If it does not lie within the range then add excipients individually one by one and find out which excipient interact with drug.

ESTIMATION OF CC AND AB IN SYNTHETIC MIXTURE ^[21, 22, 28]

(A) Absorption Ratio Method

The synthetic mixture of Candesartan cilexetil and Amlodipine besylate was prepared in ratio of 8:5. Accurately weighed 8 mg of Candesartan cilexetil and 5 mg of Amlodipine besylate were transferred to 25 ml volumetric flask, and 10 ml of Methanol was added. Excipients, such as mannitol, povidone, glycerol, mg-stearate and corn starch which were used in tablet formulation, were added in this mixture and mechanically stirred for 20 minutes. This solution was filtered through the whatmann filter paper no. 41 and filtrate was collected. Residues were washed with Methanol. The filtrate and washing were combined and volume of solution was made up to 10 ml with Methanol. 2.5 ml of this solution was diluted up to 10 ml with Methanol. Absorbance of the resulting solution was measured at 242 nm and 255 nm against Methanol as a reagent blank. The concentration of AB and CC can be calculated using equation^[2],

$$\text{CX} = \frac{[(\text{QM} - \text{QY}) / (\text{QX} - \text{QY})] \times \text{A1}/\text{ax1}}$$

$$\text{CY} = \frac{[(\text{QM} - \text{Qx}) / (\text{Qy} - \text{Qx})] \times \text{A1}/\text{ay1}}$$

Where,

$$\text{Qm} = \text{A2}/\text{A1}, \text{Qx} = \text{ax2}/\text{ax1}, \text{Qy} = \text{ay2}/\text{ay1};$$

Designates isoabsorptive point at 242 nm (λ_1)

Designates 255 nm λ_{max} of CC;

A1, A2 are absorbance of mixture at 242 nm (λ_1) and 255 nm (λ_2) respectively

ax1 and ax2 are absorptivity of AB at λ_1 and λ_2 respectively,

ay1 and ay2 are absorptivity of CC at λ_1 and λ_2 respectively,

Cx and Cy are concentrations of AB and CC respectively.

RESULT AND DISCUSSION

Selection of wavelength for Absorption Ratio Method of AB and CC

To determine wavelength for measurement, standard spectra of AB and CC were scanned between 200-400 nm against Methanol.

Isoabsorptive Point at 242 nm and at 255 nm for λ_{\max} of CC respectively.

Overlay spectra of AB and CC are presented in figure 2.

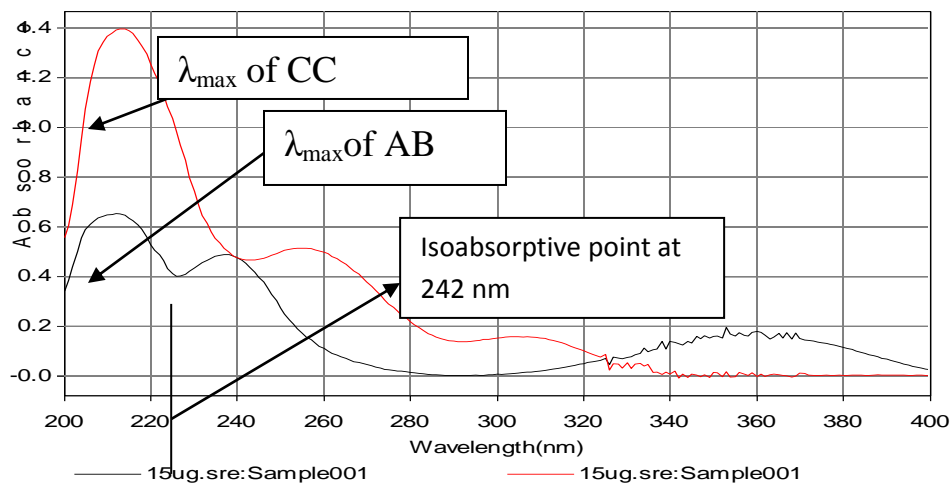


Figure 2: Overlay spectra of AB and CC showing λ_{\max} in Methanol

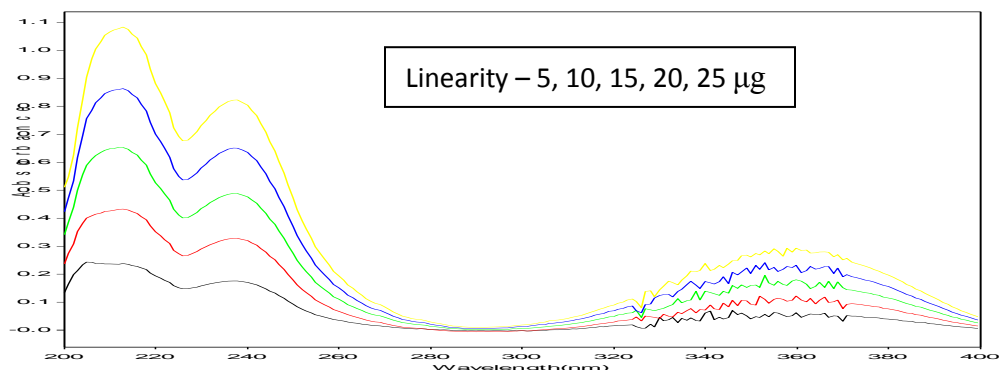


Figure 3: Overlay spectra of AB in Methanol showing Linearity

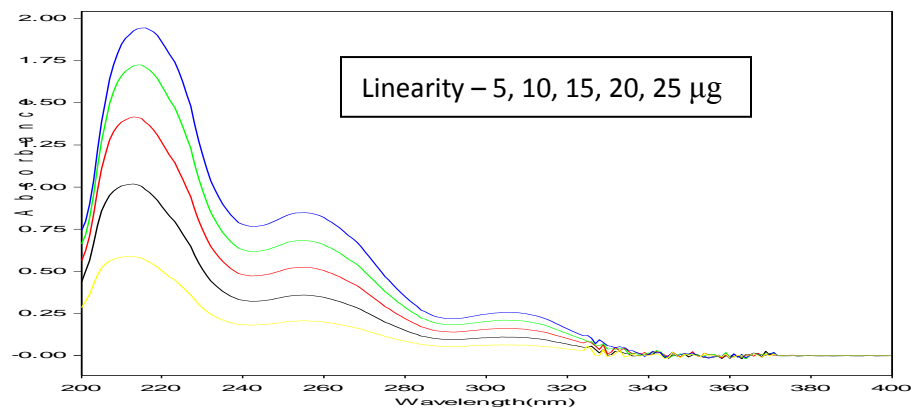


Figure 4: Overlay spectra of CC in Methanol showing Linearity

METHOD VALIDATION

A) Linearity and Range

The linearity range for AB and CC were found to be in the range of 5 - 25 $\mu\text{g/ml}$ and 5 - 25 $\mu\text{g/ml}$ respectively. Linearity data for AB at 242 nm (λ_1) and 255 nm (λ_2) are depicted in table 3. Linearity data for CC at 242 nm (λ_1) and at 255 nm (λ_2) are depicted in table 4.

Table 3: Linearity data for AB at 242 nm and 255 nm

Conc. $\mu\text{g/ml}$	Absorbance at $\lambda_1 = 242 \text{ nm}$ \pm S.D. (n=3)	% RSD	Absorbance at $\lambda_2 = 255 \text{ nm}$ \pm S.D. (n=3)	% RSD
5	0.171 \pm 0.00124	0.72	0.086 \pm 0.00081	0.94
10	0.321 \pm 0.00124	0.38	0.140 \pm 0.00047	0.33
15	0.475 \pm 0.0004	0.099	0.198 \pm 0.00163	0.82
20	0.633 \pm 0.0009	0.14	0.261 \pm 0.00244	0.93
25	0.803 \pm 0.00163	0.203	0.318 \pm 0.0021	0.66

*n= 3

Table 4: Linearity data for CC at 242 nm and 255 nm

Conc. $\mu\text{g/ml}$	Absorbance at $\lambda_1 = 242 \text{ nm}$ \pm S.D. (n=3)	% RSD	Absorbance at $\lambda_2 = 255 \text{ nm}$ \pm S.D. (n=3)	% RSD
5	0.183 \pm 0.001247	0.726	0.204 \pm 0.001247	0.6
10	0.335 \pm 0.0029	0.387	0.364 \pm 0.00094	0.24
15	0.476 \pm 0.0028	0.099	0.518 \pm 0.0029	0.559
20	0.629 \pm 0.0047	0.148	0.682 \pm 0.00163	0.239
25	0.793 \pm 0.002357	0.2033	0.853 \pm 0.002828	0.331

*n= 3

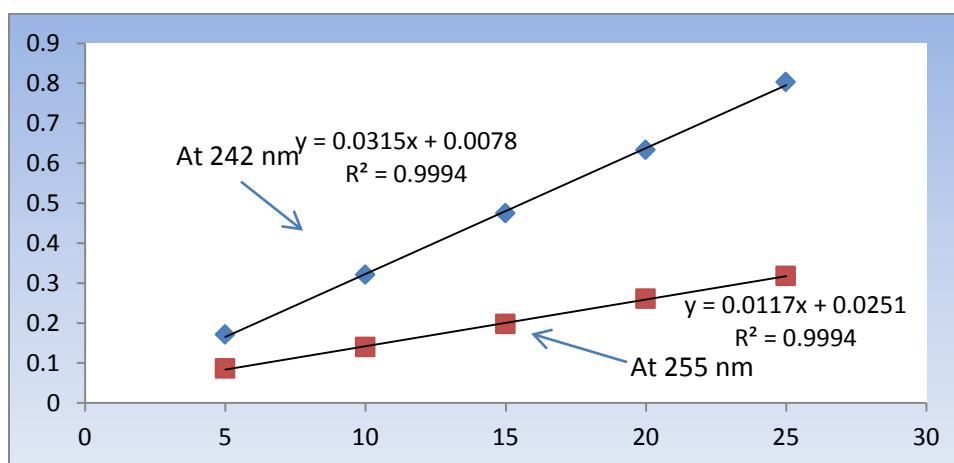


Figure 5: Calibration curve for AB at 242 nm and 255 nm

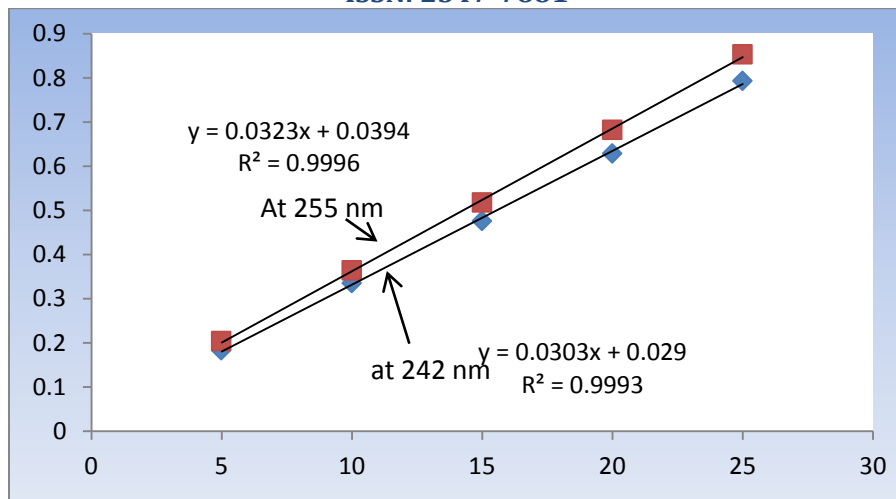


Figure 6: Calibration curve for CC at 242 nm and 255 nm

Table 5: Statistical data of AB and CC

CHARACTERISTICS	AMLODIPINE BESYLATE		CANDESARTAN CILEXETIL	
	λ_1	λ_2	λ_1	λ_2
Wavelength (nm)	242	255	242	255
Linearity ($\mu\text{g/ml}$)	5-25	5-25	5-25	5-25
Regression Equation	$y = 0.031x + 0.007$	$y = 0.011x + 0.025$	$y = 0.030x - 0.029$	$y = 0.032x + 0.039$
Slope	0.031	0.011	0.030	0.032
r^2	0.9990	0.9996	0.9994	0.9990
Intercept	0.007	0.025	0.029	0.039
LOD ($\mu\text{g/ml}$)	0.11	0.444	0.2651	0.292
LOQ ($\mu\text{g/ml}$)	0.357	1.35	0.8034	0.599

B) Precision

i) Repeatability

The data for repeatability for AB and CC at 242 nm and 255 nm are shown in Table.

Table 6: Repeatability data of AB at 242 nm and 255 nm

Sr. No.	Conc. ($\mu\text{g/ml}$)	Absorbance at 242 nm	%RSD At 242 nm	Absorbance at 255 nm	%RSD at 255 nm
1	15	0.478	0.335	0.171	0.81
2		0.476		0.172	
3		0.475		0.174	
4		0.480		0.175	
5		0.478		0.173	
6		0.479		0.171	

Table 7: Repeatability data of CC at 242 nm and 255 nm

Sr. No.	Conc. (µg/ml)	Absorbance at 242 nm	%RSD At 242 nm	Absorbance at 255 nm	%RSD at 255 nm
1	20	0.629	0.664	0.655	0.682
2		0.625		0.653	
3		0.637		0.655	
4		0.635		0.664	
5		0.630		0.660	
6		0.634		0.662	

Table 8: Intraday precision data of AB at 242 nm and at 255 nm

Sr. No.	Conc. (µg/ml)	Absorbance at 242 nm ± S.D	%RSD At 242 nm	Absorbance at 255 nm ± S.D	%RSD at 255 nm
1	5	0.176±0.001247	0.7059	0.067±0.00047	0.7
2	10	0.325±0.001633	0.5024	0.122±0.00129	0.81
3	15	0.477±0.001247	0.2611	0.183±0.00163	0.89

Table 9: Intraday precision data of CC at 242 nm and at 255 nm

Sr. No.	Conc. (µg/ml)	Absorbance at 242 nm ± S.D	%RSD At 242 nm	Absorbance at 255 nm ± S.D	%RSD at 255 nm
1	5	0.176±0.001247	0.7059	0.202±0.0017	0.83
2	10	0.331±0.0017	0.5124	0.361±0.00094	0.26
3	15	0.477±0.00216	0.4528	0.519±0.000816	0.15

Table 10: Interday precision data of AB at 242 nm and at 255 nm

Sr. No.	Conc. (µg/ml)	Absorbance at 242 nm ± S.D	%RSD At 242 nm	Absorbance at 255 nm ± S.D	%RSD at 255 nm
1	5	0.179±0.001247	0.69	0.063±0.00326	0.52
2	10	0.328±0.001247	0.37	0.122±0.000816	0.66
3	15	0.478±0.00471	0.098	0.178±0.00124	0.696

Table 11: Interday precision data of CC at 242 nm and at 255 nm

Sr. No.	Conc. (µg/ml)	Absorbance at 242 nm ± S.D	%RSD At 242 nm	Absorbance at 255 nm ± S.D	%RSD at 255 nm
1	5	0.198±0.000471	0.23	0.216±0.000471	0.21
2	10	0.343±0.001414	0.41	0.371±0.00216	0.58
3	15	0.502±0.002357	0.46	0.542±0.00216	0.39

Table 12: Accuracy data of AB

Amount of Drug in sample (µg/ml)	Amount of Std. Drug added (µg/ml)	Total amount of Drug (µg/ml)	Mean Total amount of Drug recovered (µg/ml) Mean ± S.D.*	%Recovery	% R.S.D.
5	0	5	5.05 ± 0.017	101	0.80
5	4	9	9.00 ± 0.0012	100.38	0.38
5	5	10	9.89 ± 0.0016	99.8	0.45
5	6	11	10.95 ± 0.0021	99.56	0.50

Table 13: Accuracy data of CC

Amount of Drug in sample (µg/ml)	Amount of Std. Drug added (µg/ml)	Total amount of Drug (µg/ml)	Mean Total amount of Drug recovered (µg/ml) Mean ± S.D.*	%Recovery	%R.S.D.
8	0	8	8.1 ± 0.0040	102.56	0.19
8	6.4	14.4	13.74 ± 0.0015	95.5	0.48
8	8	16	15.67 ± 0.0025	98.00	0.25
8	9.6	17.6	17.42 ± 0.0009	99.05	0.09

Applicability of the Method

Analysis using synthetic mixture

Applicability of the proposed method was tested by analyzing synthetic mixture prepared in laboratory.

Table 14: Analysis of synthetic mixture

Synthetic mixture	Label claim		Assay (% of label claim) Mean* ± S. D.	
	AB	CC	% AB	% CC
	5 mg	8 mg	101.02±0.37	99.95±0.452

The assay results were comparable to labeled value of each drug in synthetic mixture. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

CONCLUSION

Spectrophotometric methods Q – absorption Ratio method was developed for estimation of AB and CC in bulk drugs and synthetic mixture without prior separation. Methods were found to be precise and accurate as can be reflected from validation data. Developed methods were successfully applied for estimation of AB and CC in Synthetic mixture.

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27. Drug Bank Open drug and open target database :

i. drugbank.ca/drugs/DB00381 (Amlodipine)

ii. drugbank.ca/drugs/DB00796 (Candesartan)

28. For Marketed preparation : MIMS.com/mimsphilippines