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Area Under Curve Method and First Order Derivative Spectrophotometric Method for Simultaneous Estimation of Candesartan Cilexetil and Amlodipine Besylate in Synthetic Mixture

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ABSTRACT:

A simple, precise and accurate method for simultaneous estimation of Candesartan Cilexetil (CAN) and Amlodipine Besylate (AML) in a synthetic mixture using methanol as solvent. The First method is Area under curve method (Method A) for that wavelength ranges selected were 247.80 - 257.80 nm for CAN and 230 - 240 nm for AML and area were integrated between these selected wavelength ranges for both drugs. The second method is First order derivative method in which CAN shows zero crossing point (ZCP) at 240.40 nm (For estimation of AML) and AML shows ZCP at 224.20 nm (For estimation of CAN). Beer's law is obeyed in the concentration range of 8 – 24 µg/ml for CAN and 5 – 15 µg/ml for AML respectively. The mean % Recoveries was found to be in range of 99.15 – 100.35% for CAN and 98.07 – 101.59% for AML by Area under curve method and 99.21 – 100.66% for CAN and 98.99 – 100.02% for AML by First order derivative method. Both methods were validated as per ICH Q2 (R1) guideline.

KEYWORDS: Candesartan Cilexetil (CAN), Amlodipine Besylate (AML), Area Under Curve method (AUC), First order derivative method, Synthetic mixture.

INTRODUCTION

Candesartan Cilexetil (CAN) is chemically 1-cyclohexyloxycarbonyloxyethyl 2-ethoxy-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]benzimidazole-4-carboxylate (Figure 1) belong to class of Angiotensin (AT1 receptor) blockers, used as Antihypertension [1,2]. It acts by selectively blocking the binding of angiotensin II to angiotensin receptors in many tissues like vascular smooth muscle & adrenal glands. It also inhibits the angiotensin I mediated vasoconstrictive and aldosterone secreting effect of angiotensin II and results in an overall decrease in blood pressure [3,4].

Amlodipine Besylate (AML) is chemically Benzenesulfonic acid;3-O-ethyl 5-O-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (Figure 2) belong to the class of Calcium channel blocker, used as Antihypertension and Angina Pectoris[5,6]. It selectively inhibits the transmembrane calcium channel into the muscle cells, causing relaxation of vascular smooth muscles reducing the blood pressure.

Amlodipine Besylate in the treatment of hypertension combined with the Candesartan Cilexetil has been shown to have a better synergistic therapeutic effect [7].

Literature survey reveals that UV spectrophotometric method (Q- Absorbance Ratio Method) [8] and two HPLC Method [9,10] were reported for the estimation of this combination.

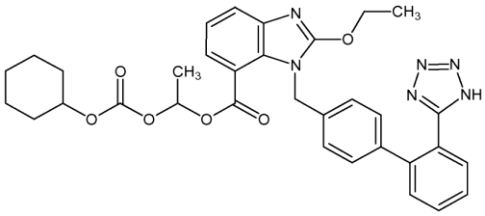

Figure 1 Chemical structure of Candesartan Cilexetil

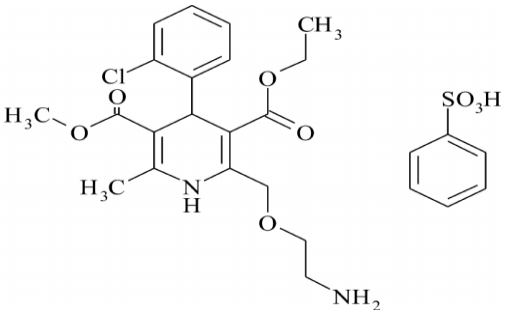

Figure 2 Chemical structure of Amlodipine Besylate

MATERIALS AND METHODS:

Instruments

SHIMADZU UV- 1800 double beam UV- Visible spectrophotometer (UV-Probe software version 2.42 (SHIMADZU) and Electronic analytical balance (REPTECH).

Chemicals and reagents

Candesartan Cilexetil and Amlodipine Besylate were obtained as gift sample from Alembic Pharmaceutical Ltd., Vadodara and Ciron Drugs & Pharmaceutical Pvt. Ltd., Maharashtra. Fixed-dose of synthetic mixture of Candesartan Cilexetil 8 mg and Amlodipine Besylate 5 mg were prepared in laboratory-scale as pilot batch. Analytical grade methanol was procured from Thomas Baker, Mumbai.

PREPARATION OF SOLUTION:

Preparation of Standard Stock Solution

Standard solution of Candesartan Cilexetil (1000 µg/ml) and Amlodipine Besylate (1000 µg/ml) was prepared by dissolving 10 mg of drugs in 5 ml methanol and complete to 10 ml with methanol.

Preparation of Working Standard Solution

Aliquot of 2.5 ml from above standard stock solution was pipetted out into 25 ml of volumetric flask and volume was made up to mark with methanol to give a solution containing 100 µg/ml of Candesartan Cilexetil and Amlodipine Besylate.

PROCEDURE AND METHODS:

Area Under Curve Method

The absorption spectra (from 200 to 400 nm) of the solutions were recorded using methanol as a blank. The area under curve values for both drugs were recorded over the wavelength ranges of (247.80 – 257.80) nm (λ1- λ2) and 230 -240 (λ3- λ4) as shown in (Figure 3 & 4) and the calibration graphs were constructed. The area absorptivity values were calculated at each wavelength range for the two components thereafter the concentration of two drugs in mixed standard and the sample solution were calculated using equation

A1 = ax1 C(x) + ay1 C (y) ……..……(1)
A2 = ax2 C(x) + ay2 C(y) ……..(2)
C(x) = [A2 × ax2 – A1 × ay2] / [ax2 × ay1 – ax1 × ay2].....(3)
C(y) = A2 – ax2 × C(x) / ay2……..(4)
Where,
ax1 and ax2 are absorptivities of x at (λ1- λ2) and (λ3- λ4) respectively.
Ay1 and ay2 are absorptivities of y at (λ1- λ2) and (λ3- λ4) respectively.
A1 and A2 are AUC of the mixed standard at (λ1- λ2) and (λ3- λ4) respectively.
C(x) and C(y) are the concentration of x and y, respectively.

Table 1 Formulation of Synthetic Mixture

Sr. No.	Ingredients	Quantity (120 mg)	Amount taken for 1000 mg of synthetic mixture	Role
1.	Candesartan Cilexetil	8	66.66	Antihypertensive
2.	Amlodipine Besylate	5	41.66	Antihypertensive
3.	Mannitol	84.16	701.3	Excipient
4.	Microcrystalline Cellulose	15	125	Compressibility
5.	Hydroxypropyl Cellulose	6	50	Binder

6.	Magnesium Stearate	1.30	10.83	Lubricant
7.	Talc	1.30	10.83	Diluent

First-order Derivative Method

The spectrum obtained was derivatised to obtain first derivative spectrum. The overlain first order spectra of two drugs are shown in figure 11. It appeared that Candesartan Cilexetil showed zero crossing at 240.40 nm while Amlodipine Besylate showed Zero crossing at 224.20 nm. At the zero crossing point of Candesartan Cilexetil (240.40 nm), Amlodipine Besylate showed a substantial dA/dλ, whereas the zero crossing point of Amlodipine Besylate (224.20 nm), Candesartan Cilexetil showed a substantial dA/ dλ. Hence the wavelengths 240.40 nm and 224.20 nm were selected as analytical wavelengths for determination of Amlodipine Besylate and Candesartan Cilexetil, respectively (Figure 9, 10 & 11). Calibration curves were plotted by taking dA/dλ on Y-axis and concentrations on X-axis. Formulation of Synthetic mixture: which is shown in Table 1

Assay of Synthetic Mixture

The quantity of synthetic mixture powder equivalent to 8 mg of Candesartan Cilexetil and 5 mg of Amlodipine Besylate was transferred into 10 ml of volumetric flask and 5 ml methanol was added. After sonication for 2-3 min, the mixture was diluted to volume with methanol and the solution filtered through Whatman filter paper no 42. An aliquot of this solution(1.0 ml) was transferred into 10 ml volumetric flask and volume were made up to mark with methanol to give a solution containing Candesartan Cilexetil (80 µg/ml) + Amlodipine Besylate (50 µg/ml). From the above solution (1.0 ml) was pipette out and transferred to 10 ml volumetric flask. The volume was made up to mark with methanol to give a solution containing Candesartan Cilexetil (8 µg/ml) + Amlodipine Besylate (5 µg/ml) and the absorbance of solution were recorded.

METHOD VALIDATION:

The method was validated according to ICH guideline Q2 (R1).

1. **Linearity and construction of calibration curves**
   Different aliquots of Candesartan Cilexetil and Amlodipine Besylate were prepared from working standard solution (100 µg/ml) ranging from 8-24 µg/ml for Candesartan Cilexetil and 5-15 µg/ml for Amlodipine Besylate were transferred to 10 ml volumetric flasks and completed to volume with methanol. The absorption spectra from 200 to 400 nm of these solutions were recorded using methanol as a blank.
2. **Precision**
   - **Repeatability**
     Repeatability of the method was assessed by analysing samples from the same batch 6 times with a standard solution containing concentration 16 µg/ml for Candesartan Cilexetil and 10 µg/ml for Amlodipine Besylate and % RSD was calculated.
   - **Intraday and Interday**
     It was assessed by analysing samples from the same batch with three standard solutions containing concentrations 12, 16, 20 µg/ml for Candesartan Cilexetil and 7.5, 10, 12.5 µg/ml for Amlodipine Besylate. Solutions were analyzed thrice on the same day within a short interval of time for intraday and on a different day for interday assay and %RSD was calculated.
3. **Recovery study**
   The accuracy of the proposed methods was checked by recovery study, by addition of standard drug solution to pre analysed sample solution at three different concentration level (80%, 100% and 120%) within the range of linearity for both the drugs. The basic concentration of the drugs standard solution was 8 µg/ml of Candesartan Cilexetil and 5 µg/ml of Amlodipine Besylate for all two methods.
4. **LOD and LOQ**
   Limit of Detection (LOD)
   The LOD (Limit of Detection) was estimated from the set of 5 calibration curves that were used to determine the linearity of the method. The LOD was calculated by using the formula: **LOD = 3.3 × S.D./Slope**
   The LOQ (Limit of Quantitation) was estimated from the set of 5 calibration curves that were used to determine the linearity of the method. The LOQ was calculated by using the formula: **LOQ = 10 × S.D./Slope**
   Where, S.D. = Standard deviation of the Y - intercepts of 5 calibration curves
   Slope = Mean slope of 5 calibration curves

RESULT AND DISCUSSION:

The spectrophotometric methods have the advantages of being the most simple, fast and applicable in all laboratories, as most of the active compounds show absorbance in the UV region. Since only one method is reported for the simultaneous analysis of the two drugs earlier by Absorbance Ratio Method, the developed methods can be used for routine analysis in their synthetic mixture. The linearity range for Candesartan Cilexetil 8-24 µg/ml and for Amlodipine Besylate 5-15 µg/ml. The proposed method was validated as per ICH guideline. The accuracy of method was determined by calculating mean percentage recovery at three level 80,100 and 120%. Both drugs showed good regression value at their respective wavelengths.

Method A: Area Under Curve (AUC)

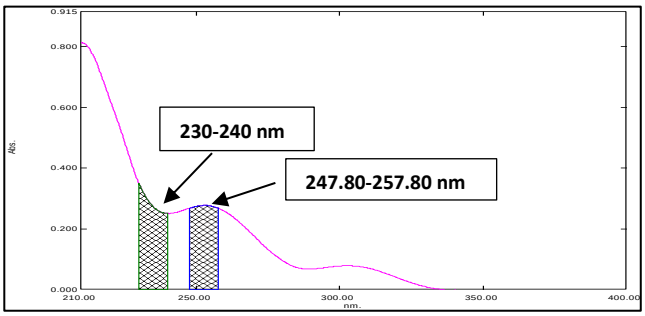

Figure 3 AUC Spectra of CAN 8 µg/ml in wavelength 247.80 to 257.80 nm and 230 to 240 nm

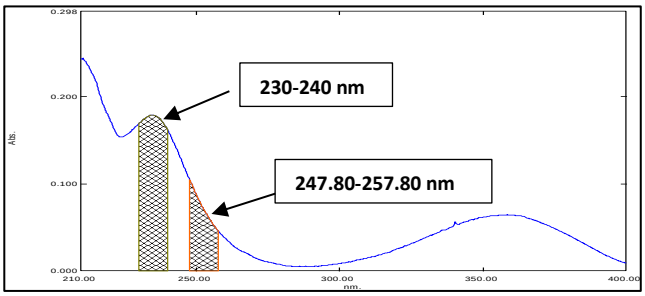

Figure 4 AUC Spectra of AML 5 µg/ml in wavelength 230 to 240 nm and 247.80 to 257.80 nm

Method Validation:

Table 2 Linearity data for CAN at 247.80 – 257.80 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1	8	2.7288 ± 0.0111	0.4075
2	12	4.0498 ± 0.0175	0.4338
3	16	5.4484 ± 0.0326	0.5984
4	20	6.7304 ± 0.07132	1.0597
5	24	8.2114 ± 0.07088	0.8633

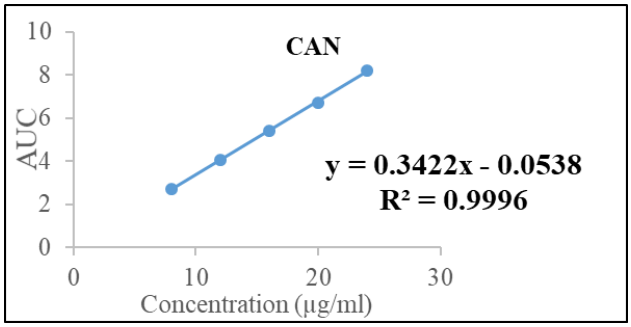

Figure 5 Calibration curve for CAN at 247.80 – 257.80 nm

Table 3 Linearity data for AML at 230 - 240 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1	5	1.7368 ± 0.0055	0.3216
2	7.5	2.7274 ± 0.0072	0.2615
3	10	3.5730 ± 0.0103	0.2901
4	12.5	4.4830 ± 0.0151	0.3382
5	15	5.3772 ± 0.0440	0.8198

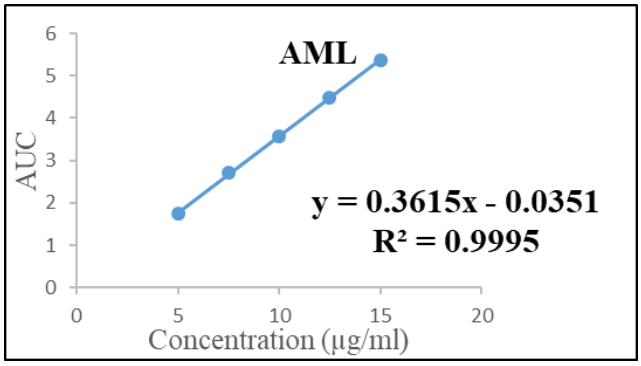

Figure 6 Calibration curve for AML at 230 – 240 nm

Table 4 Linearity data for CAN at 230 – 240 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1	8	2.8210 ± 0.0087	0.3090
2	12	4.2252 ± 0.0061	0.1453
3	16	5.678 ± 0.0498	0.8782
4	20	7.1888 ± 0.0896	1.2471
5	24	8.740 ± 0.0519	0.5948

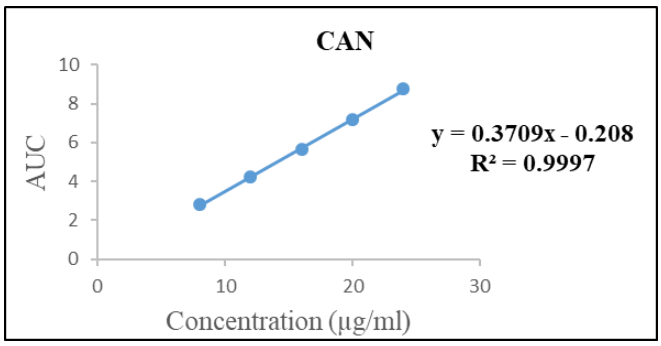

Figure 7 Calibration curve for CAN at 230 – 240 nm

Table 5 Linearity data for AML at 247.80 – 257.80 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1	5	0.7204 ± 0.0024	0.3343
2	7.5	1.1166 ± 0.0064	0.5755
3	10	1.4624 ± 0.0133	0.9156
4	12.5	1.8638 ± 0.0079	0.4248
5	15	2.2446 ± 0.0074	0.3313

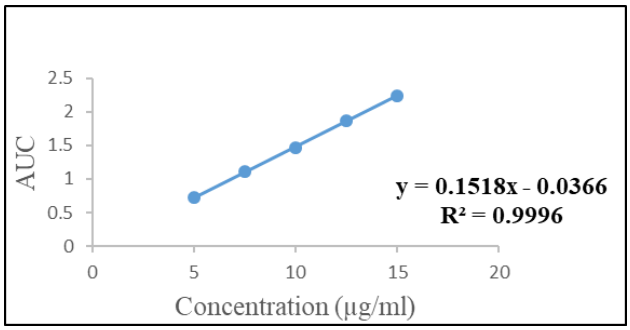

Figure 8 Calibration curve for AML at 247.80 – 257.80 nm

Table 6 Repeatability data of CAN and AML

Drugs	Concentration (µg/ml)	Mean AUC ± S.D. (n=6)	%R.S.D.
CAN (247.80-257.80 nm)	16	5.4438 ± 0.0312	0.5738
AML (230-240 nm)	10	3.5701 ± 0.0115	0.3244

Table 7 Intraday Precision data of CAN at 247.80 – 257.80 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=3)	%R.S.D.
1	12	4.0896 ± 0.0161	0.3952
2	16	5.4480 ± 0.0345	0.6350
3	20	6.7573 ± 0.0421	0.6244

Table 7. Intraday Precision data of AML at 230 – 240 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=3)	%R.S.D.
1	7.5	2.7270 ± 0.0072	0.2644
2	10	3.5700 ± 0.0147	0.4145
3	12.5	4.4803 ± 0.0197	0.4409

Table 8. Interday precision data of CAN at 247.80 – 257.80 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=3)	%R.S.D.
1	12	4.0373 ± 0.0421	1.0436
2	16	5.4070 ± 0.0697	1.2902
3	20	6.7173 ± 0.0798	1.1891

Table 9. Interday Precision data of AML at 230 – 240 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=3)	%R.S.D.
1	7.5	2.7060 ± 0.0268	0.9922
2	10	3.5250 ± 0.0476	1.3513
3	12.5	4.4266 ± 0.0617	1.3957

Table 10. Determination of Accuracy of CAN and AML

Drugs	Level	Amount of sample (µg/ml)	Amount of std. spiked (µg/ml)	Total Amount (µg/ml)	% Recovery
CAN	0%	8	0	8	100.16
	80%	8	6.4	14.4	100.35
	100%	8	8	16	98.83
	120%	8	9.6	17.6	99.15
AML	0%	5	0	5	99.43
	80%	5	4	9	99.04
	100%	5	5	10	98.07
	120%	5	6	11	101.59

Table 11. Determination of Assay of CAN and AML

Synthetic Mixture	Actual Concentration (µg/ml)		Amount obtained Mean ± S.D. (µg/ml)		%CAN ± S.D. (n=3)	%AML ± S.D. (n=3)
	CAN	**AML**	**CAN**	**AML**		
	8	5	8.01 ± 0.0010	4.97 ± 0.0001	100.16 ± 0.0125	99.43 ± 0.0010

Method B: First Order Derivative Method

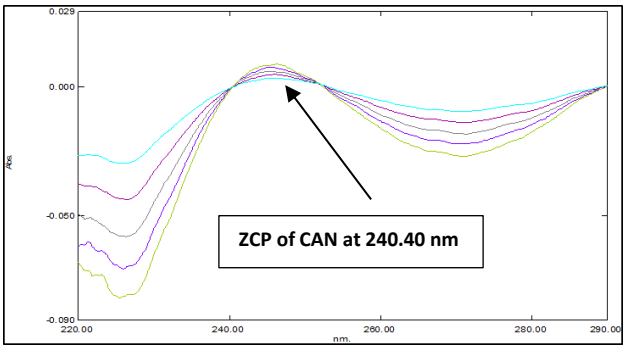

Figure 9: Overlain first order spectra of CAN

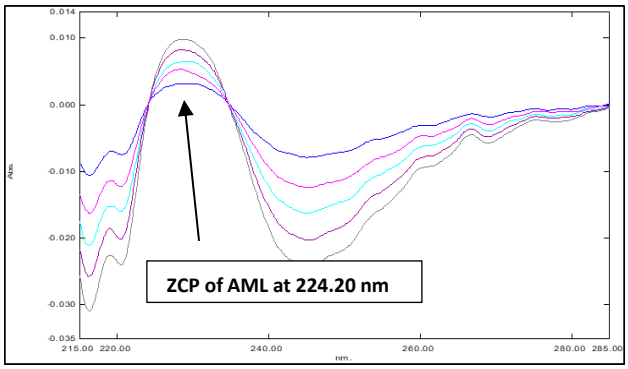

Figure 10: Overlain first order spectra of AML

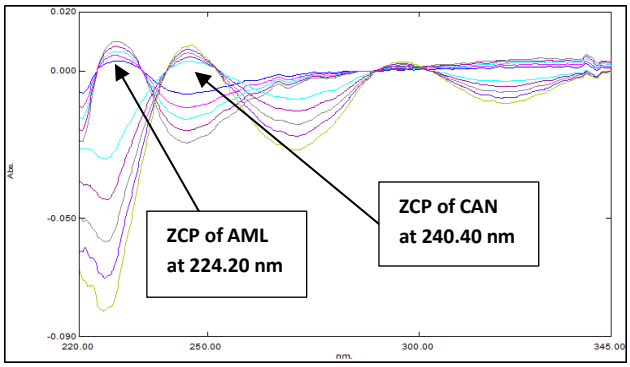

Figure 11: Overlain first order spectra of CAN and AML

Table 12. Linearity data for CAN at 224.20 nm (ZCP of AML)

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=5)	%R.S.D.
1	8	0.0287 ± 0.00020	0.7215
2	12	0.0416 ± 0.00037	0.9041
3	16	0.0551 ± 0.00057	1.0504
4	20	0.0664 ± 0.00062	0.9357
5	24	0.0778 ± 0.00093	1.2000

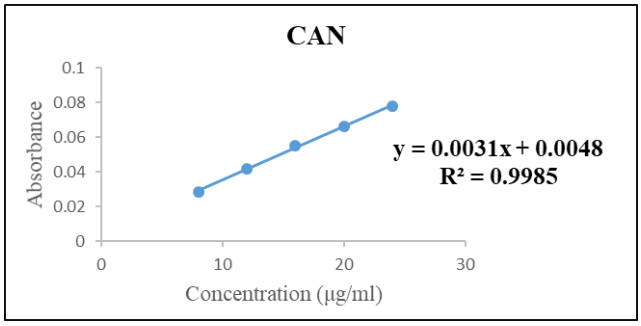

Figure 12: Calibration curve for CAN at 224.20 nm (ZCP of AML)

Table 13. Linearity data for AML at 240.40 nm (ZCP of CAN)

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=5)	%R.S.D.
1	5	0.0060 ± 0.00003	0.5325
2	7.5	0.0099 ± 0.00006	0.6141
3	10	0.0128 ± 0.00011	0.8900
4	12.5	0.0157 ± 0.00015	0.9622
5	15	0.0188 ± 0.00015	0.8041

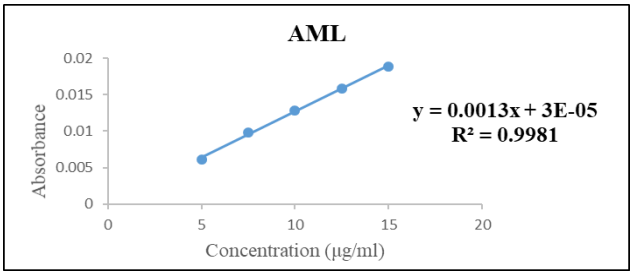

Figure 13: Calibration curve for AML at 240.40 nm (ZCP of CAN)

Table 14. Repeatability data for CAN and AML

Sr. No.	Drugs	Concentration (µg/ml)	Mean Abs. ± S.D. (n=6)	%R.S.D.
1	CAN	16	0.0549 ± 0.00063	1.1552
2	AML	10	0.0128 ± 0.00011	0.8959

Table 15. Intraday precision data of CAN at 224.20 nm

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=6)	%R.S.D.
1	12	0.0415 ± 0.00045	1.0856
2	16	0.0558 ± 0.00070	1.2699
3	20	0.0663 ± 0.00065	0.9890

Table 16. Interday precision data of CAN at 224.20 nm

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=6)	%R.S.D.
1	12	0.0415 ± 0.00049	1.1990
2	16	0.0557 ± 0.00073	1.3225
3	20	0.0661 ± 0.00078	1.1815

Table 17. Interday precision data of AML at 240.40 nm

Sr. No.	Concentration (µg/ml)	Mean Abs. ± S.D. (n=6)	%R.S.D.
1	7.5	0.0101 ± 0.00011	1.0877
2	10	0.0128 ± 0.00015	1.2249
3	12.5	0.0157 ± 0.00017	1.1383

Table 18. Determination of Accuracy of CAN and AML

Drugs	Level	Amount of sample (µg/ml)	Amount of std. spiked (µg/ml)	Total Amount (µg/ml)	Amount of sample found (µg/ml)	% Recovery
CAN	0%	8	0	8	8.05	100.66
	80%	8	6.4	14.4	14.34	99.60
	100%	8	8	16	16.09	100.59
	120%	8	9.6	17.6	17.46	99.21
AML	0%	5	0	5	4.95	99.01
	80%	5	4	9	9.00	100.02
	100%	5	5	10	9.89	98.99
	120%	5	6	11	10.97	99.78

Table 19. Determination of Assay of CAN and AML

Synthetic Mixture	Actual Concentration (µg/ml)		Amount obtained Mean ± S.D. (µg/ml)		%CAN ± S.D. (n=3)	%AML ± S.D. (n=3)
	CAN	AML	CAN	AML		
	8	5	8.05 ± 0.0488	4.95 ± 0.0438	100.66 ± 0.6110	99.01 ± 0.8775

Table 20. Summary of validation parameters

Parameters	Area Under Curve		First order Derivative Method	
	CAN	AML	CAN	AML
Wavelength (nm)	247.80 - 257.80	230 - 240	ZCP = 240.40	ZCP = 224.20
Linearity (µg/ml) (n=5)	8 - 24	5 - 15	8 - 24	5 - 15
Regression equation	y = 0.3422x – 0.0538	y = 0.3615x – 0.0351	y = 0.0031x + 0.0048	y = 0.0013x + 0.00003
Repeatability (%R.S.D) (n=6)	0.5738	0.3244	1.1552	0.8959
Intraday precision (%R.S.D) (n=3)	0.3952 - 0.6350	0.2644 - 0.4409	0.9890 - 1.2699	0.9209 - 1.0553
Interday precision (%R.S.D) (n=3)	1.0436 - 1.2902	0.9922 - 1.3957	1.1815 - 1.3225	1.0877 - 1.2249
%Recovery	98.02 - 100.35	98.07 - 101.59	99.21 - 100.66	98.99 - 100.02
LOD (µg/ml) (n=5)	0.3332	0.1396	0.6452	0.7667
LOQ (µg/ml) (n=5)	1.0097	0.4231	1.9553	2.3233
% Assay (n=3)	100.16	99.43	100.66	99.01

CONCLUSION:

The developed Area under curve and First-order derivative method was found to be simple, precise and accurate & it was validated as per ICH Q2 (R1) guideline. Thus developed UV spectroscopic method was found to be suitable for deterimation of Candesartan cilexetil and Amlodipine Besylate in synthetic mixture without interference from the excipient.

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