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DEVELOPMENT AND VALIDATION OF ABSORBANCE CORRECTION METHOD FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE BESILATE, OLMESARTAN MEDOXOMIL AND HYDROCHLOROTHIAZIDE

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ABSTRACT

In current study, UV spectroscopy was performed for estimation of Amlodipine Besilate (AMLO), Olmesartan Medoxomil (OLME) and Hydrochlorothiazide (HCTZ). Simultaneous spectrophotometric determination of Amlodipine Besilate, Olmesartan Medoxomil and Hydrochlorothiazide was carried out by absorbance correction method using UV visible double beam spectrophotometer. Validation of simultaneous spectrophotometric method was performed with different parameter like accuracy, precision, recovery, LOD and LOQ. For absorbance correction method, 258 nm, 315 nm and 356 nm were selected as working wavelength because at 315 nm and 258 nm Hydrochlorothiazide, at 258 nm Olmesartan Medoxomil and at 258 nm, 315 nm 356 nm Amlodipine Besilate showed significant absorbance. Linearity of Amlodipine Besilate, Olmesartan Medoxomil and Hydrochlorothiazide was found to be 1-6 µg/mL, 2-24 µg/mL and 2.5-15 µg/mL respectively. The % mean, standard deviation (S.D.), relative standard deviation (% R.S.D.) and standard error (S.E.) were calculated. The % R.S.D. was less than 2% as required by USP and ICH guideline. The experimental work involved is very simple, it requires only measurement of absorbance at selected wavelength. Current study revealed development and validation of

simple, accurate, precise, rapid, cost effective, reproducible and reliable UV spectrophotometric method for estimation of Amlodipine Besilate, Olmesartan Medoxomil and Hydrochlorothiazide in bulk and tablet.

Keywords: UV, validation, analytical method, Amlodipine Besilate, Olmesartan Medoxomil, Hydrochlorothiazide

INTRODUCTION:

Hypertension is a highly prevalent condition, especially in people who are past middle age. It is not a disease in and of itself, but it is a significant contributor to cardiovascular mortality and morbidity. The systolic blood pressure should be 140 mm Hg and the diastolic blood pressure should be 90 mm Hg, while the danger seems to grow as the blood pressure rises above 120/80 mm Hg. Studies shown that the higher the blood pressure (systolic, diastolic, or both), the greater the risk of cardiovascular disease **[1]**. An estimated 1.28 billion adults aged 30-79 years worldwide have hypertension, most (two-thirds) living in low and middle-income countries. Global targets over non communicable diseases reduces the prevalence of hypertension by 33% upto 2030 **[2]**. Olmesartan medoxomil [5-methyl-2-oxo-1,3-dioxol-4-yl) methyl 5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl]methyl]imidazole-4-carboxylate] (OLME) belongs to a group of angiotensin II receptor blockers used as an antihypertensive agent and is currently being used for prevention of hypertension **[3-5]**. Hydrochlorothiazide [6-chloro-1, 1-dioxo-3, 4-dihydro-2H-1λ^6, 2, 4-benzothiadiazine-7-sulfonamide; hydrochloride] [6] (HCTZ) is a thiazide-type diuretic that has been used clinically for more than half a century. The drug has been widely used to treat hypertension globally and is relatively very safe. Hydrochlorothiazide acts on the distal convoluted tubules and inhibits the sodium chloride co-transporter system. Amlodipine Besilate [benzenesulfonic acid; 3-O-ethyl 5-O-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate] **[7]** (AMLO) is a calcium channel blocker used to treat high blood pressure and coronary artery disease. While not typically recommended in heart failure, amlodipine may be used if other medications are not sufficient for treating high blood pressure or heart-related chest pain.

Structure: [8-10]

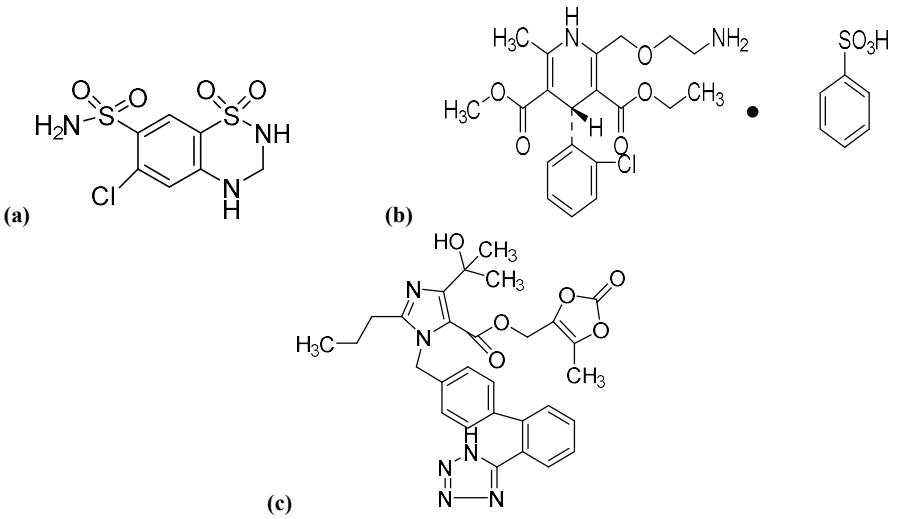

Figure 1: Structure of drugs (a) Hydrochlorothiazide (b) Amlodipine Besilate (c) Olmesartan Medoxomil

MATERIALS AND METHODS:

Preparation of Standard Stock Solution of Amlodipine Besilate, Olmesartan Medoxomil and Hydrochlorothiazide: [11]

10 mg each of Amlodipine Besilate, Olmesartan Medoxomil and Hydrochlorothiazide were weighed separately and transferred in three different 100 mL volumetric flask. All the drugs were dissolved in 100 mL of Acetonitrile with vigorous shaking and then volume was made up to the mark with Acetonitrile to obtain final concentration of 100 µg/mL of each solution of Amlodipine Besilate, Olmesartan Medoxomil and Hydrochlorothiazide.

Selection of analytical Wavelength: [11]

Using appropriate dilution of standard stock solution, the three solutions were scanned separately in order to get good result. By using overlay spectra of three drugs, working wavelength selected were 356 nm, 258 nm and 315 nm **(Figure 2, 3)**. Here, amount of Amlodipine Besilate was determined at 315 nm using standard calibration curve (using equations y = mx + c), where Amlodipine Besilate gives significant absorbance value. At 356 nm, Olmesartan Medoxomil does not show absorbance, but Amlodipine Besilate gives significant absorbance value. So, amount of Hydrochlorothiazide was determined at 356 nm after correcting absorbance of Amlodipine Besilate. The concentration of Olmesartan Medoxomil was determined at 258 nm after correcting absorbance of Amlodipine Besilate and Hydrochlorothiazide.

Selection of Analytical Concentration Range [11, 12]

For each drug appropriate aliquots were pipette out from standard stock solution into series of 10 mL volumetric flask. The volume was made up to the mark with

Acetonitrile to get a set of solution having the concentration of 2.5, 5, 7.5, 10, 12.5 and 15 µg/mL for Hydrochlorothiazide and 4, 8, 12, 16, 20 and 24 µg/mL for Olmesartan Medoxomil and 1, 2, 3, 4, 5 and 6 µg/mL for Amlodipine Besilate. The absorbance of Hydrochlorothiazide was measured at 258 nm and 315 nm. The absorbance of Olmesartan Medoxomil was measured at 258 nm; the absorbance of Amlodipine Besilate was measured at 258 nm, 315 nm and 356 nm. The absorbance was plotted against concentration. The concentration range over which the drugs obeyed beer's law was chosen. The range was found to be 2.5 µg/mL to 15 µg/mL for Hydrochlorothiazide, 2 µg/mL to 24 µg/mL for Olmesartan Medoxomil and 1 µg/mL and 6 µg/mL for Amlodipine Besilate **(Table 2, 3 and 4) (Figure 4, 5, 6, 7, 8 and 9)**.

Determination of Absorptivity at Analytical Wavelength

The absorptivity of all three drugs was calculated at selected wavelength using data of calibration curve **(Table 1)**. The absorptivity was used in forming equations for absorption correction method (equation 1, 2 and 3).

Table 1: Absorptivity Measurement for Absorbance Correction Method

	Amlodipine Besilate	Hydrochlorothiazide	Olmesartan Medoxomil
A_1 (258)	184.125 (ax_1)	405.6 (ay_1)	546.5 (az_1)
A_2 (356)	182.963 (ax_2)	-	-
A_3 (315)	66.7037 (ax_3)	125.8796 (ay_3)	-

The concentration C_{AMLO}, C_{HCTZ} and C_{OLME} can be obtained by solving equation (equation no 1, 2 and 3) **[12, 13]**

For Amlodipine Besilate

$C_{Amlo\ (gm/100\ mL)} = (A_2)/ax_2$

$= (A_2)/182.963$ …… (1)

For Hydrochlorothiazide

$C_{HCTZ\ (gm/100\ mL)} = (A_3 - ax_3 * C_{Amlo})/ay_3$

$= (A_3 - 66.7037 * C_{Amlo})/125.8796$ …… (2)

For Olmesartan Medoxomil

$C_{Olme} = [A_1 - (184.125 * C_{Amlo} + 405.6\ C_{HCTZ})] / az_1$

$= [A_1 - (184.125 * C_{Amlo} + 405.6 * 0.00125)]/ 546.5$……. (3)

Where,

A_1 = Absorbance of sample solution at 258 nm

A_2 = Absorbance of sample solution at 315 nm

A_3 = Absorbance of sample solution at 356 nm

OLME

(az_1) = absorptivity Coefficients of OLME at 258 nm

AMLO

(ax_1)= absorptivity Coefficients of AMLO at 258 nm

(ax_2)=absorptivity Coefficients of AMLO at 356 nm

(ax_3)=absorptivity Coefficients of AMLO at 315 nm

HCTZ

ay_1= absorptivity Coefficients of HCTZ at 258 nm

ay_3= absorptivity Coefficients of HCTZ at 315 nm

C_{OLME}, concentration of OLME in g/100 mL in mixture

C_{AMLO}, concentration of AMLO in g/100 mL in mixture

C_{HCTZ}, concentration of HCTZ in g/100 mL in mixture

Now if a mixture of Amlodipine Besilate, Olmesartan Medoxomil and Hydrochlorothiazide were to be analysed, a solution of suitable dilution should be prepared in solvent. The absorbance of the solution at 258 nm, 315 nm and 356 nm were measured. The values were substituted in equation (1, 2 and 3) to get a concentration of Amlodipine Besilate, Hydrochlorothiazide and Olmesartan Medoxomil.

Procedure for Analysis of Powder Mixture:

Mixed solution of pure drug was prepared by taking suitable volume of standard drug solution. Here, 1.25 mL standard solution of Hydrochlorothiazide (100 µg/mL), 0.5 mL standard solution of Amlodipine Besilate (100 µg/mL) and 2.0 mL standard solution of Olmesartan Medoxomil (100 µg/mL) were transferred into 10 mL volumetric flask to make final concentration of 12.5 µg/mL for Hydrochlorothiazide, 5 µg/mL for Amlodipine Besilate and 20 µg/mL for Olmesartan Medoxomil. Absorbance of this prepared mixed solution was measured at 258, 315 and 356 nm. Concentration of Hydrochlorothiazide, Olmesartan Medoxomil and Amlodipine Besilate was calculated by putting absorbance values into equations (1, 2 and 3). Results for statistical validation for analysis of powder mixture are reported **Table 5**.

Procedure for analysis of Tablet powder

Average weight of tablet powder for unit dosage form was found to be 80 mg. From the tablet powder, 80 mg powder (equivalent to 6.25 mg of Hydrochlorothiazide, 2.5 mg of Amlodipine Besilate and 10 mg of Olmesartan Medoxomil) was weighed and transferred into 100 mL volumetric flask and dissolved in Acetonitrile and the content was kept in ultra sonicator for 20 min. Finally the volume was made upto mark with Acetonitrile. The solution was filtered through whatman filter paper number 41. From this solution, 2 mL was transferred into another 10 ml volumetric flask. Volume was made upto the mark with Acetonitrile to make final concentration of 12.5 µg/mL for Hydrochlorothiazide, 5 µg/mL for Amlodipine Besilate and 20 µg/mL for Olmesartan Medoxomil. Absorbance of this prepared solution was measured at 258 nm, 315 nm and 356 nm. Concentration of

Hydrochlorothiazide, Amlodipine Besilate and Olmesartan Medoxomil in tablet powder was calculated by putting absorbance values into equations (1, 2 and 3). Results of this analysis of synthetic mixture are reported in **Table 6**.

Procedure for Recovery Studies [14]

Recovery studies were carried out by applying the method to drug sample present in tablet to which known amount of Amlodipine Besilate, Olmesartan Medoxomil and Hydrochlorothiazide corresponding to 80,100,120% of label claim was added (standard addition method). In 80% recovery study, amount of standard added is 4 mg of Amlodipine Besilate, 16 mg Olmesartan Medoxomil and 10 mg Hydrochlorothiazide (i.e., 80 addition). In 100 % recovery study, amount of standard added is 5 mg of Amlodipine Besilate and 20 mg Olmesartan Medoxomil and 12.5 mg Hydrochlorothiazide. (i.e., 100% addition). In 120% recovery study, amount of standard added is 6 mg of Amlodipine Besilate and 24 mg Olmesartan Medoxomil and 15 mg Hydrochlorothiazide (i.e., 120 addition). After the addition of the standard, the tablet powder were mixed properly. From this tablets and standard mixture, mixed powder (equivalent to 2.5 mg Amlodipine Besilate, 10 mg Olmesartan Medoxomil and 6.25 mg of Hydrochlorothiazide was weighed and transferred into 100 mL volumetric flask and dissolved in Acetonitrile and content was kept in ultrasonicator for 20 min. Finally the volume was made upto the mark with Acetonitrile. The solution was filtered through whatman filter paper No. 41. From the filtrate, 2 ml solution was transferred in 10 ml volumetric flask and volume was made up to the mark with acetonitrile. The concentration of resultant solution was 12.5 µg/mL for Hydrochlorothiazide, 20 µg/mL for Olmesartan Medoxomil and 5 µg/mL for Amlodipine Besilate.

Absorbance of this prepared solution was measured at 258 nm, 315 nm and 356 nm. Concentration of Amlodipine Besilate, Olmesartan Medoxomil and Hydrochlorothiazide was calculated by putting absorbance values into equation (1, 2 and 3)

The statistical evaluation data for recovery studies are shown in **Table 7**.

Procedure for precision [15]

Precision of the method was determined with tablet. Average weight of tablet for unit dosage form was found to be 160 mg. From the tablet powder, 80 mg tablets powder (equivalent to 6.25 mg of Hydrochlorothiazide, 2.5 mg of Amlodipine Besilate and 10 mg of Olmesartan Medoxomil) was weighed and transferred into 100 mL volumetric flask and dissolved in Acetonitrile and the content was kept in ultra sonicator for 20 min. Finally the volume was made up to mark with

Acetonitrile. The solution was filtered through whatman filter paper number 41.

This solution was further diluted as per the analysis of tablet powder to obtain mixed sample solution in Beer Lambert's range for each drug containing 5 µg/mL of Amlodipine Besilate, 20 µg/mL of Olmesartan Medoxomil and 12.5 µg/mL of Hydrochlorothiazide respectively. The mixed sample solutions were analysed to obtain spectra and absorbance value at 258 nm, 315 nm and 356 nm were noted. The concentration of Amlodipine Besilate, Hydrochlorothiazide and Olmesartan Medoxomil were calculated from the equation. In intraday precision, sample having concentration of 5 µg/mL of Amlodipine Besilate, 20 µg/mL of Olmesartan Medoxomil and 12.5 µg/mL of Hydrochlorothiazide was scanned six times at different time interval in the same day **(Table 8)**. Inter day precision was obtained by the assay of six sample sets on different days as per the same procedure **(Table 9)**.

Determination of Limit of Detection and Limit of Quantitation

Limit of Detection and Limit of Quantitation

Calibration curve was repeated six times and the standard deviation of the intercepts was calculated. Then LOD and LOQ were calculated as follow: **[14]**

$$\mathbf{LOD} = \frac{\mathbf{3.3 * D}}{\mathbf{s}} \text{..... (4)}$$

$$\mathbf{LOQ} = \frac{\mathbf{10 * D}}{\mathbf{s}} \text{..... (5)}$$

Where, D = Standard Deviation of y-intercepts of regression line of calibration curves.

S = Slope of the calibration curve

The result for LOD and LOQ are shown in **Table 10**.

RESULTS AND DISCUSSION:

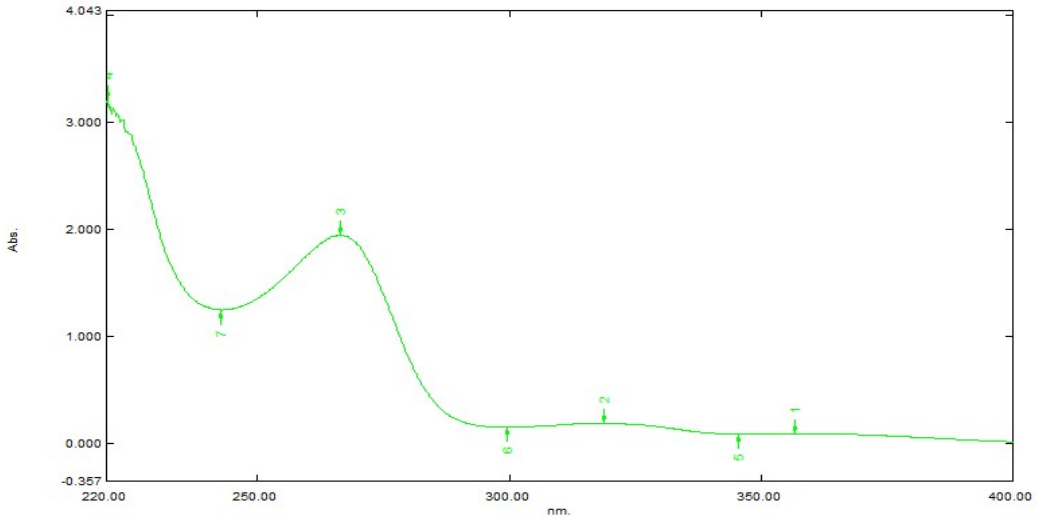

Figure 1: UV Spectra of mixture of Olmesartan Medoxomil – 20 µg/mL, Hydrochlorothiazide – 12.5 µg/mL and Amlodipine Besilate – 5 µg/mL in Acetonitrile

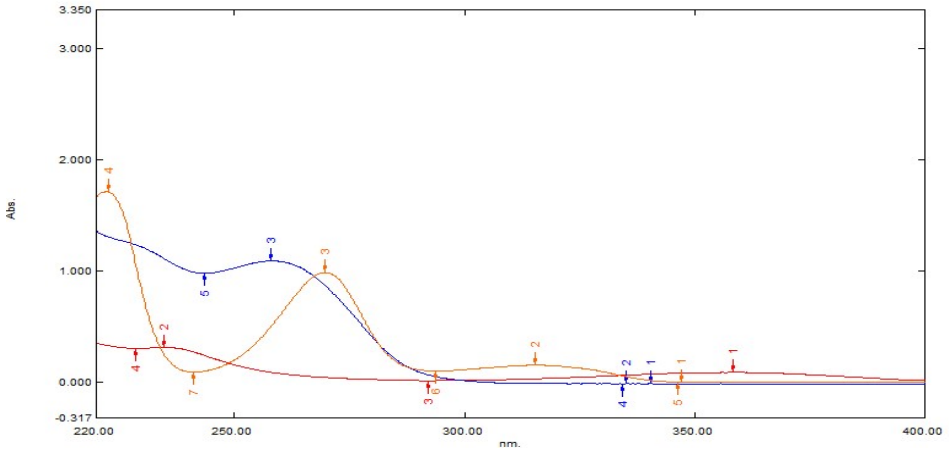

Figure 2: Overlay Spectra of Olmesartan Medoxomil – 20 µg/mL, Hydrochlorothiazide – 12.5 µg/mL and Amlodipine Besilate – 5 µg/mL in Acetonitrile

From examination of overlay spectra of drugs, the three wavelength chosen were 258 nm, 315 nm and 356 nm. Here, Hydrochlorothiazide showed absorbance at 258 nm and 356 nm, Amlodipine Besilate showed absorbance at 258 nm, 315 nm and 356 nm, whereas Olmesartan Medoxomil showed absorbance at 258 nm.

Selection of analytical Concentration Range:

Analytical concentration ranges for which drug obeys Beer Lambert's law were determined for all three drugs at selected wavelengths.

Table 2: Selection of analytical Concentration Range for Hydrochlorothiazide (Linearity)

S. No.	Concentration (µg/mL)	Absorbance	
		258 nm	315 nm
1	2.5	0.1031±0.001941	0.0324±0.000875
2	5	0.2058±0.001835	0.0608±0.004708
3	7.5	0.2950±0.005404	0.0892±0.001169
4	10	0.4080±0.010863	0.1295±0.002074
5	12.5	0.5037±0.004676	0.1575±0.001517
6	15	0.6075±0.106720	0.1943±0.001366

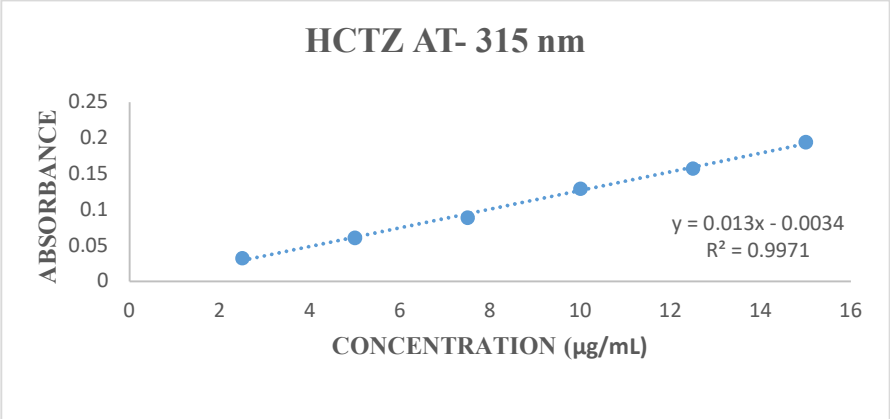

Figure 3: Calibration curve of Hydrochlorothiazide at 315 nm

From the calibration curve of Hydrochlorothiazide at 315 nm, the linearity was observed for 2.5 µg/mL to 15 µg/mL with correlation coefficient (r^2) value of 0.9971.

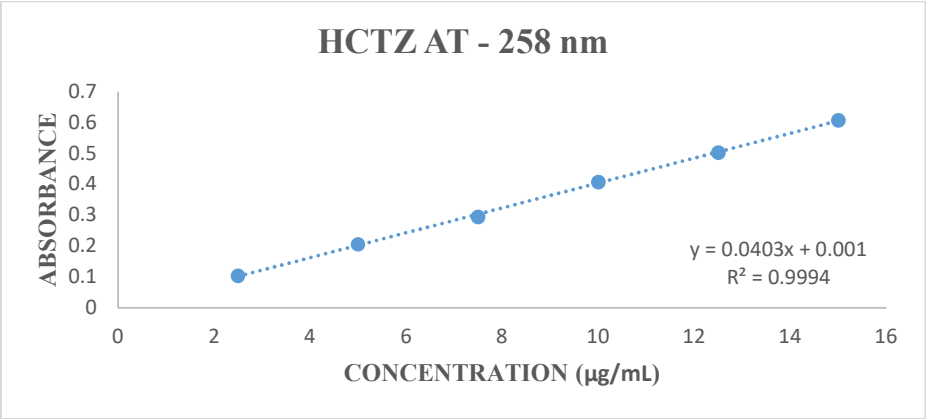

Figure 4: Calibration curve of Hydrochlorothiazide at 258 nm

From the calibration curve of Hydrochlorothiazide at 258 nm, the linearity was observed for 2.5 µg/mL to 15 µg/mL with correlation coefficient (r^2) value of 0.9994.

Table 3: Selection of analytical Concentration Range for Olmesartan Medoxomil (Linearity)

S. No.	Concentration (µg/mL)	Absorbance 258 nm
1	4	0.2157±0.007916
2	8	0.4430±0.011900
3	12	0.6436±0.025230
4	16	0.8927±0.026560
5	20	1.0997±0.075280
6	24	1.3007±0.010558

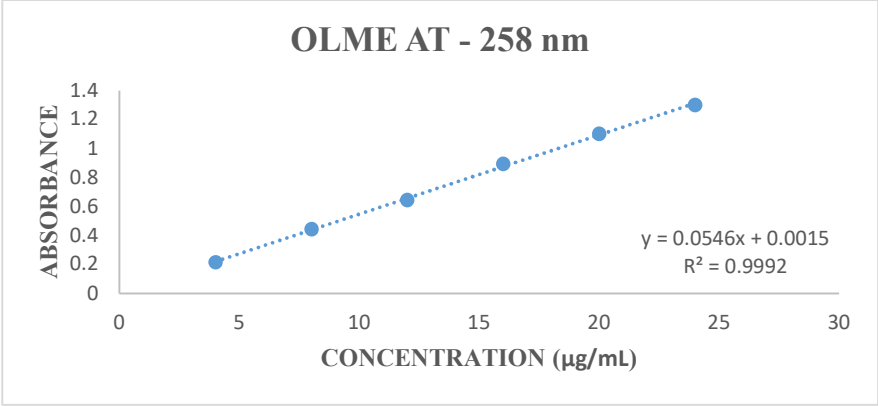

Figure 5: Calibration curve of Olmesartan Medoxomil at 258 nm

From the calibration curve of Olmesartan Medoxomil at 258 nm, the linearity was observed for 4 µg/mL to 24 µg/mL with correlation coefficient (r^2) value of 0.9992.

Table 4: Selection of Analytical Concentration Range for Amlodipine Besilate (Linearity)

S. No.	Concentration (µg/mL)	Absorbance		
		258 nm	315 nm	356 nm
1	1	0.0197±0.001630	0.0069±0.000117	0.0192±0.000186
2	2	0.0380±0.001673	0.0139±0.001029	0.0373±0.001506
3	3	0.0548±0.003430	0.0199±0.001114	0.0545±0.002258
4	4	0.0728±0.002787	0.0260±0.001414	0.0723±0.004320
5	5	0.0885±0.002950	0.0335±0.001470	0.0900±0.001793
6	6	0.1053±0.001363	0.0387±0.000749	0.1062±0.001169

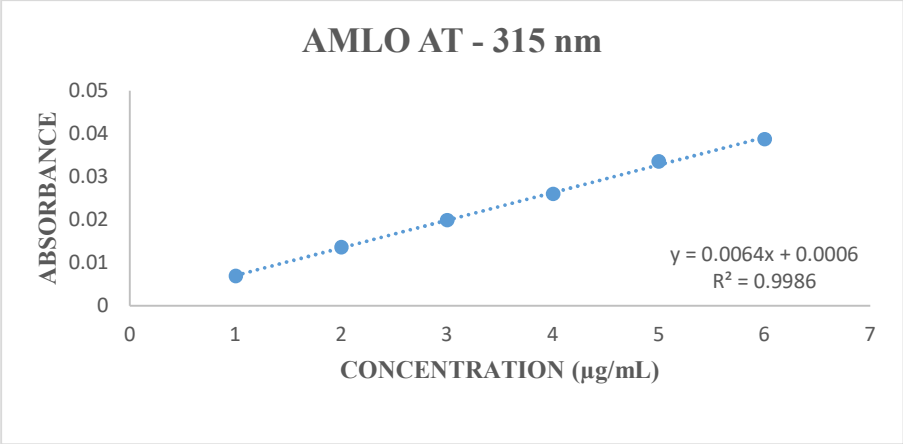

Figure 6: Calibration curve of Amlodipine Besilate at 315 nm

From the calibration curve of Amlodipine Besilate at 315 nm, the linearity was observed for 1 µg/mL to 6 µg/mL with correlation coefficient (r^2) value of 0.9986.

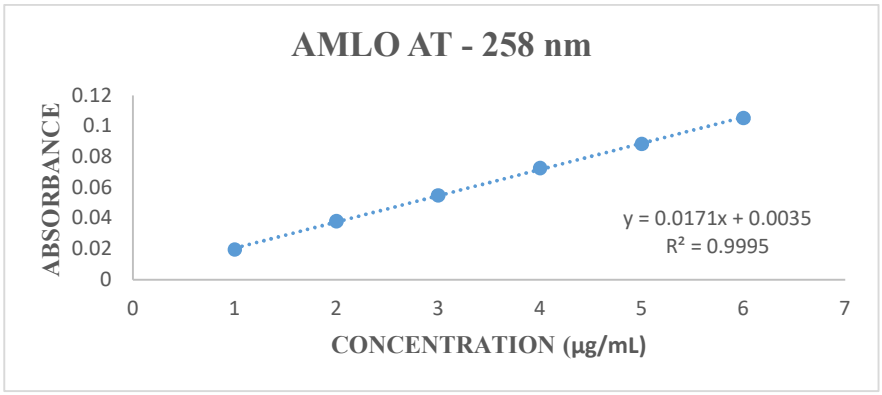

Figure 7: Calibration curve of Amlodipine Besilate at 258 nm

From the calibration curve of Amlodipine Besilate at 258 nm, the linearity was observed for 1 µg/mL to 6 µg/mL with correlation coefficient (r^2) value of 0.9995.

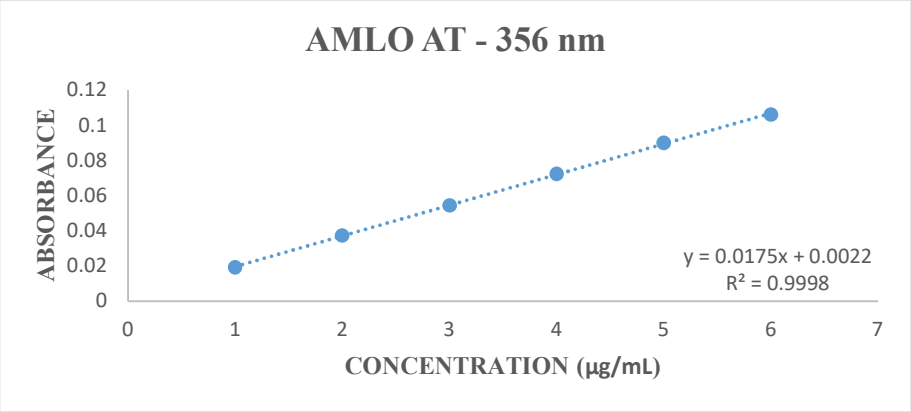

Figure 8: Calibration curve of Amlodipine Besilate at 356 nm

From the calibration curve of Amlodipine Besilate at 356 nm, the linearity was observed for 1 µg/mL to 6 µg/mL with correlation coefficient (r^2) value of 0.9998.

Table 5: Statistical Validation for Powder Mixture

Drug	Mean* %	Standard Deviation	Co–efficient of Variation* (% R.S.D)	Standard Error*
HCTZ	99.50	0.5764	0.5793	0.2353
OLME	99.21	0.3596	0.3624	0.1468
AMLO	100.75	0.6645	0.6596	0.2713

The % R.S.D. was found to be less than 2 % as required by USP and ICH guideline.

Analysis of Tablet

Table 6: Statistical Validation for Tablet

Drug	Mean* %	Standard Deviation*	Co–efficient of Variation* (% R.S.D)	Standard Error*
HCTZ	99.70	1.0560	1.0586	0.4309
OLME	99.23	0.3771	0.3800	0.1540
AMLO	99.74	1.0870	1.0902	0.4439

Table 7: Statistical Validation for Recovery Studies

Level of % Recovery	% Mean Recovery*			Standard Deviation*			Co–efficient of Variation* (% R.S.D.)			Standard Error*		
	HCTZ	OLME	AMLO	HCTZ	OLME	AMLO	HCTZ	OLME	AMLO	HCTZ	OLME	AMLO
80	100.72	100.14	99.07	0.9153	0.4735	0.1680	0.9087	0.4728	0.1696	0.5284	0.2734	0.0970
100	100.34	100.72	100.01	1.4640	0.1706	1.0320	0.4624	0.1694	1.0319	0.8454	0.0984	0.5958
120	100.10	100.24	99.87	0.4277	0.4124	1.4470	0.4273	0.4114	1.4489	0.2469	0.2381	0.8354

Table 8: Statistical Validation for Intra–day Precision

Drug	Mean* (%)	Standard Deviation*	Co–efficient of Variation* (%R.S.D.)	Standard Error*
HCTZ	99.73	0.9230	0.9255	0.3768
OLME	99.46	0.6881	0.6918	0.2809
AMLO	101.14	0.9919	0.9905	0.4049

Table 9: Statistical Validation for Intra–day Precision

Drug	Mean* (%)	Standard Deviation*	Co–efficient of Variation* (%R.S.D.)	Standard Error*
HCTZ	100.28	0.8668	0.8644	0.3539
OLME	99.57	0.4748	0.4768	0.1938
AMLO	100.03	1.0138	1.0135	0.4139

Table 10: LOD and LOQ Data for Amlodipine Besilate, Olmesartan Medoxomil and Hydrochlorothiazide

Drug	LOD (µg/mL)	LOQ (µg/mL)
Amlodipine Besilate (at 356 nm)	0.2368	0.7177
Hydrochlorothiazide (at 315 nm)	0.6676	2.0230
Olmesartan Medoxomil (at 258 nm)	0.5812	1.7613

CONCLUSION:

Present study revealed that absorbance correction method was found to be simple, accurate and precise. With the help of developed UV method three drugs Amlodipine Besilate, Hydrochlorothiazide and Olmesartan Medoxomil were determined simultaneously with precision of 99.46 % to 101.14 %.

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