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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTANEOUS ESTIMATION OF AMLODIPINE AND TERAZOSIN IN SYNTHETIC MIXTURE BY Q-ABSORBANCE RATIO METHOD

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Abstract

The present work involves simultaneous estimation of Amlodipine and Terazosin in synthetic mixture by UV Spectrophotometric method. Amlodipine has an absorbance maximum at 237 nm and Terazosin has two absorbance maxima at 245 nm and 250 nm in methanol: water (1:1) mixture. For Q absorbance ratio method, Absorbance at isoabsorptive point 230nm and at 250nm was selected. Both the drugs and their mixture obey Beers and Lamberts law at selected wavelength. The linearity was observed in the concentration range 5-25 µg/ml for Amlodipine and 2-10 µg/ml for Terazosin. The result of analysis has been validated as per ICH guideline. The proposed procedures are simple, rapid and economical can be used for the routine analysis of both drugs.

Keywords: Method development, Validation, Q-Absorbance Ratio, Amlodipine, Terazosin.

Introduction

Amlodipine is chemically known as, 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl, 5-methyl ester (±) monobenzenesulfonate. It is a long-acting, calcium channel blocker. It is used in the treatment of hypertension and angina[1-2]., Terazosin hydrochloride dihydrate (THD), 2-[4-(2-tetrahydrofuranyl)carbonyl]-1-piperazinyl-6,7-dimethoxy-4-quinazolinamine monohydrochloride dihydrate , is a highly selective potent α-1 adrenoreceptor antagonist. It is an effective drug for hypertension and benign prostatic hyperplasia.[1].Both the drugs are official in I.P, B.P, and U.S.P. Amlodipine [4] with Terazosin in improving postvoid residual (PVR) in patients with lower urinary tract symptoms (LUTS) and concomitant hypertension. Amlodipine plus

terazosin therapy appears to be a safe and effective combination therapy to control both conditions, especially for those with predominant overactive bladder symptoms. Several UV[6,8,9], HPLC[5,7,10]methods are reported in combination with other drugs for the determination of Amlodipine and Terazosin in the literature for its assay. However, no method is reported for simultaneous estimation of Amlodipine and Terazosin by UV Spectrophotometric method in any literature. In the present investigation, a simple, precise and accurate method is described for the simultaneous estimation of these two drugs.

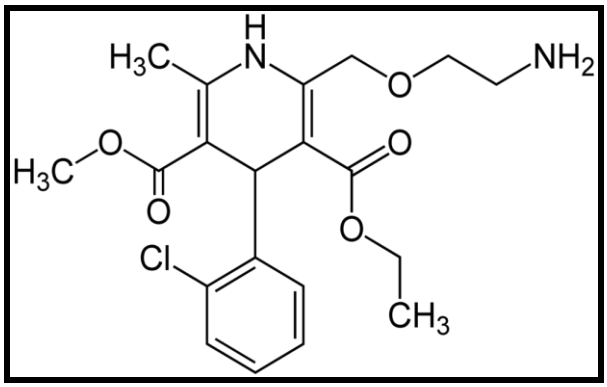

Figure 1: Structure of Amlodipine.

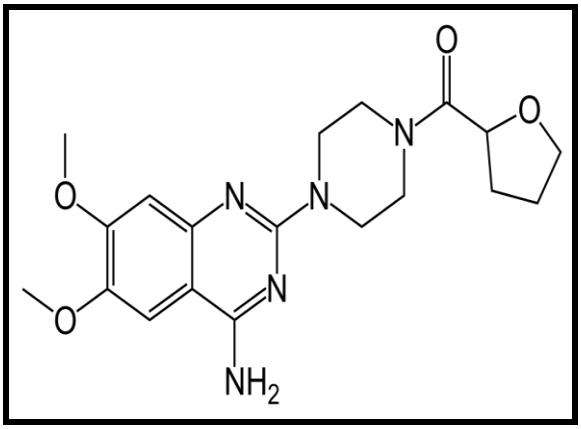

Figure 2: Structure of Terazosin.

Material and Methods

Instrumentation: Spectrophotometric measurements were performed on Shimadzu UV –visible double beam spectrophotometer (Model- 1800). All weighing were done on electronic analytical balance (Wensar Dab220).

Chemicals and Reagents: The bulk drug Amlodipine obtained from West coast Pharmaceuticals, Ahmedabad. And Terazosin obtained from Intas Pharmaceutical Ltd Ahmedabad. Analytical grade methanol was procured from Merck Fine chemicals (Mumbai).

Selection of a Solvent: Methanol: Water (1:1) was selected as solvent for studying spectral characteristic of drugs.

Preparation of Standard Solution

(A) Preparation of Standard Solution of Amlodipine

Preparation of Standard Stock Solution of Amlodipine (100µg/ml): Accurately weighed quantity of AMLO 10 mg was transferred to 100 ml volumetric flask, dissolved in 10 ml of Methanol: Water (1:1) and diluted up to mark with Methanol: Water (1:1) to give a stock solution having strength of 100µg/ml.

Preparation of Working Standard Solution of Amlodipine

From the above stock solution pipette out 0.5 mL, 1.0mL, 1.5mL, 2.0mL, and 2.5mL of solution and transferred to 10 mL volumetric flask and make up the volume up to 10 mL with methanol: Water (1:1) to Produce concentration 5, 10, 15, 20 and 25 µg/mL respectively.

B) Preparation of Standard Solution of Terazosin

Preparation of Standard Stock Solution of Terazosin (100µg/ml)

Accurately weighed quantity of TERA 10 mg was transferred to 100 ml volumetric flask, dissolved in 10 ml of Methanol: Water (1:1) and diluted up to mark with Methanol: Water (1:1) to give a stock solution having strength of 100µg/ml.

Preparation of Working Standard Solution of Terazosin

From the above stock solution pipette out 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, and 1mL of solution and transferred to 10 mL volumetric flask and make up the volume up to 10 mL with methanol: Water (1:1) to Produce concentration 2, 4, 6, 8 and 10 µg/mL respectively.

C) Preparation of synthetic mixture of Amlodipine and Terazosin

The synthetic mixture of Amlodipine and Terazosin was prepared in the ratio of 5:2.Accurately weighed (5mg) Amlodipine and Terazosin (2mg) were transferred in 100 mL volumetric flask and dissolved in methanol: water (1:1) (70 mL). Common excipients, which are used in the tablet formulation, were added in this mixture and sonicated for 20 minutes. This solution was filtered through the Whatmann filter paper No. 41 and the residue was washed thoroughly with methanol: water (1:1). The filtrate and washings were combined and diluted to the mark with methanol: water (1:1) to get solution having Amlodipine (50 µg/mL) and Terazosin (20 µg/mL).

Selection of Analytical Wavelength

To determine wavelength for measurement, standard spectra of TERA and AMLO were scanned between 200-400 nm against Methanol: Water (1:1). Absorbance maxima were obtained at 259 nm and at 302 nm for AMLO and TERA respectively and Iso-absorptive point were obtained at 230 nm.

Preparation of Calibration Curve

(A)Calibration Curve for Amlodipine

Calibration curve for AMLO consists of different concentrations of standard AMLO 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 AMLO (100µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol: Water (1:1). The absorbance of the solutions was measured at 250 nm and 230 nm against Methanol: Water (1:1) as a blank. Calibration curve was plotted at both wavelengths and two equations were formed using the absorptivity.

(B) Calibration Curve for Terazosin

Calibration curve for TERA consists of different concentrations of standard TERA solution ranging from 2 – 10 µg/ml. The solutions were prepared by pipetting out 0.2, 0.4, 0.6, 0.8 and

1 ml of the working standard solution of TERA (100µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol: Water (1:1). The absorbance of the solutions was measured at 250 nm and 230 nm against Methanol: Water (1:1) as a blank. Calibration curve was plotted at both wavelengths and two equations were formed using the absorptivity.

Preparation of Sample solution

About 5.0 mg of Synthetic mixture was weighed accurately and transferred into a 50 mL volumetric flask. The content was mixed with Methanol: Water (1:1) (70 ml) and sonicated for 20 min to dissolve the drug as completely as possible. The solution was then filtered through a Whatman filter paper no. 41. The volume was adjusted up to mark with Methanol: Water (1:1). The mixture contain 100µg/ml of Amlodipine and 25µg/ml of Terazosin .An aliquot of this solution (2 ml) was transferred in to a 10 ml volumetric flask and the volume was adjusted up to the mark with Methanol: Water (1:1) to make final concentration of Amlodipine (10 µg/ml) and Terazosin (2.5 µg/ml)

Validation [11]

Linearity and Range

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 5-25 µg/ml and 2-10 µg/ml for AMLO and TERA respectively (n = 5).The calibration curve of absorbance vs. respective concentration was plotted and correlation coefficient and regression line equations for AMLO and TERA were calculated.

Precision

(A) Repeatability

Aliquots of 1.5ml of working standard solution of AMLO (100 µg/ml) were transferred to a 10 ml volumetric flask. Aliquots of 0.6ml of working standard solution of TERA (100 µg/ml) were respectively transferred to a 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 15µg/ml solution of AMLO and 12µg/ml solution of TERA. The absorbance of solution was measured six times and % RSD was calculated.

(B) Intraday precision

Aliquots of 1.0, 1.5, and 2.0 ml of working standard solution of AMLO (100 µg/ml) were transferred to a series of 10 ml volumetric flask. Aliquots of 0.4, 0.6 and 0.8 ml of working standard solution of TERA (100 µg/ml) were respectively transferred to the same series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol: Water (1:1) to get 10, 15 and 20µg/ml solution of AMLO and 4, 6 and 8µg/ml solution of TERA. Solution was analyzed 3 times on the same day and % RSD was calculated.

(C) Interday Precision

Aliquots of 1.0, 1.5, and 2.0 ml of working standard solution of AMLO (100 µg/ml) were transferred to a series of 10 ml volumetric flask. Aliquots of 0.4, 0.6 and 0.8 ml of working standard solution of TERA (100 µg/ml) were respectively transferred to the same series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol: Water (1:1) to get 10, 15 and 20µg/ml solution of AMLO and 4, 6 and 8µg/ml solution of TERA. Solution was analyzed 3 times on the 3 different days and % RSD was calculated.

Limit of Detection (LOD)

The LOD is estimated from the set of 5 calibration curves used to determine method linearity.

The LOD may be calculated as,

$LOD = 3.3 * SD/Slope$

Where, SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

Limit of Quantification (LOQ)

The LOQ is estimated from the set of 5 calibration curves used to determine method linearity.

The LOD may be calculated as,

$LOQ = 10 * SD/Slope$

Where, SD = the standard deviation of Y- intercept of 5 calibration curves.

Slope = the mean slope of the 5 calibration curves.

Accuracy

The accuracy of the method was determined by calculating recovery of AMLO and TERA by the standard addition method. Aliquots of 0.5, 1.0, and 1.5 ml of working standard solution of AMLO(100 µg/ml) were added at 50, 100 and 150 % level to pre-analyzed 1.0 ml sample solutions of AMLO and TERA (100 µg/mL of AMLO and TERA) transferred to a series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 15, 20 and 25µg/ml solution of AMLO. Aliquots of 0.2, 0.4, and 0.6 ml of working standard solution of TERA(100 µg/ml) were added at 50, 100 and 150 % level to pre-analyzed 1 ml sample solutions of AMLO and TERA (100 µg/ mL of AMLO and TERA) transferred to a series of 10 ml volumetric flask. The volume was adjusted up to mark with methanol to get 6, 8 and 10µg/ml solution of TERA. Absorbance of solution was measured at selected wavelengths for AMLO and TERA. The amount of AMLO and TERA was calculated at each level and % recoveries were calculated by measuring the absorbance and fitting the values in equation. Accuracy was assessed using three concentrations and three replicates of each.

Q-Absorbance Ratio Method

• Absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one which is an Iso-absorptive point and other being the λ max of one of the two components.

• From the overlay spectra of two drugs, it is evident that AMLO and TERA show an Iso-absorptive point at 230 nm. The second wavelength used is 250 nm, which is λ max of TERA.

• Five working standard solutions having concentration 5, 10, 15, 20 and 25 µg/mL for AMLO and 2, 4, 6, 8 and 10 µg/mL for TERA were prepared in methanol: Water (1:1) and the absorbance at 230 nm (Iso-absorptive point) and 250 nm (λ max of TERA) were measured and absorptivity coefficients were calculated.

• The absorbance of the sample solution (10 µg/ml of AMLO and 4 µg/ml of TERA) i.e. A_1 and A_2 were recorded at 230 nm (Iso-absorptive point) and 250 nm (λ max of TERA) respectively, and ratios of absorbance were calculated, i.e. A_2/A_1

• Relative concentration of two drugs in the sample was calculated using following equations.

$C_X = [(Q_M-Q_Y)/ (Q_X-Q_Y)] \times A_1/ax_1$…….. (iii)

$C_Y = [(Q_M-Q_X)/ (Q_Y-Q_X)] \times A_1/ay_1$…….. (iv)

The Q-values and absorptivity for both drugs were calculated as follows,

Q_M = Absorbance of Sample solution at 250 nm (A_2)/ Absorbance of Sample solution at 230 nm (A_1)

Q_X = Absorptivity of AMLO at 250 nm (ax_2)/ Absorptivity of AMLO at 230 nm (ax_1)

Q_Y = Absorptivity of TERA at 250 nm (ay_2)/ Absorptivity of TERA at 230 nm (ay_1) Where,

A_1 and A_2 are absorbance of mixture at 230 nm and 250 nm

QX And QY are Q value of AMLO and TERA respectively

ax_1 and ay_1 are absorptivity of AMLO and TERA at 230 nm

ax_2 and ay_2 are absorptivity of AMLO and TERA at 250 nm

• The analysis procedure was repeated 3 times with sample solution.

Results and Discussion

A reliable Q absorption ratio method was developed for simultaneous estimation of Amlodipine and Terazosin in synthetic mixture by UV Spectrophotometry. Beers law was obeyed in concentration range of 5-25 µg/ml for Amlodipine and 2-10 µg/ml for Terazosin at 230 nm and 250 nm wavelengths. The correlation coefficient Amlodipine

and Terazosin was found to be R^2 = 0.988 and 0.992. The mean % recoveries were found to be in the range of 97 - 102% and 90 -110% for Amlodipine and Terazosin respectively. The LOD and LOQ were 0.257µg/ml and 0.716µg/ml of Amlodipin 0.098µg/ml and 0.299µg/ml of Terazosin, respectively. The proposed method was precise, accurate and reproducible and acceptable recovery of the analyte, which can be applied for the analysis of Amlodipin and Terazosin in Synthetic Mixture.

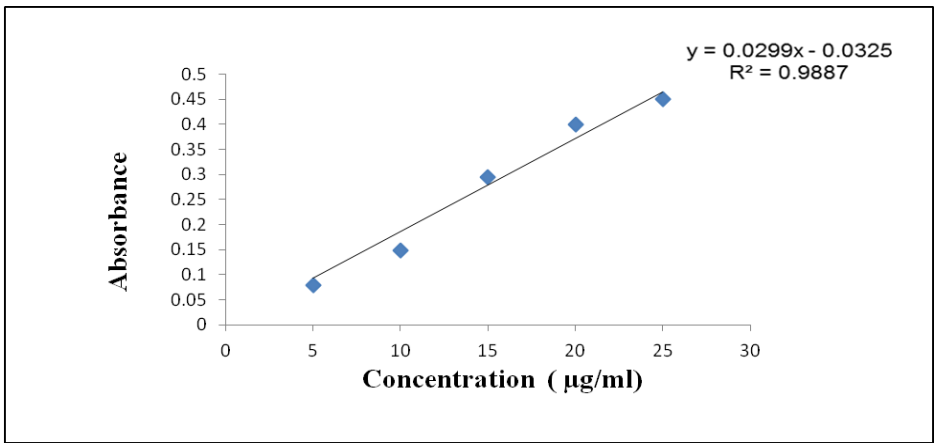

Figure 3: Calibration curve of Amlodipine at 230nm.

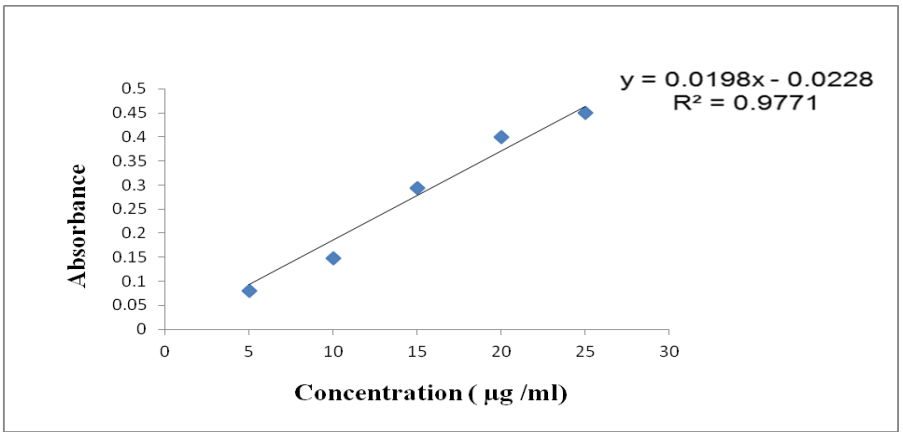

Figure 4: Calibration curve of Amlodipine at 250 nm.

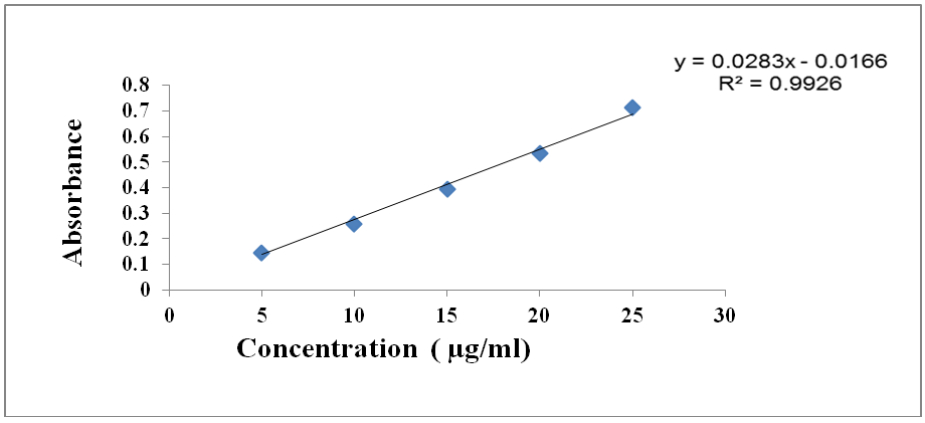

Figure 5: Calibration curve of Terazosin at 230 nm.

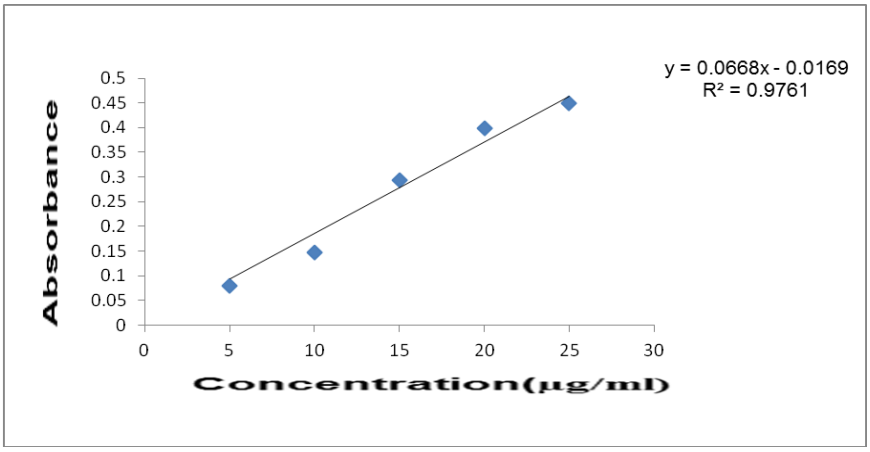

Figure 6: Calibration curve of Terazosin at 250 nm.

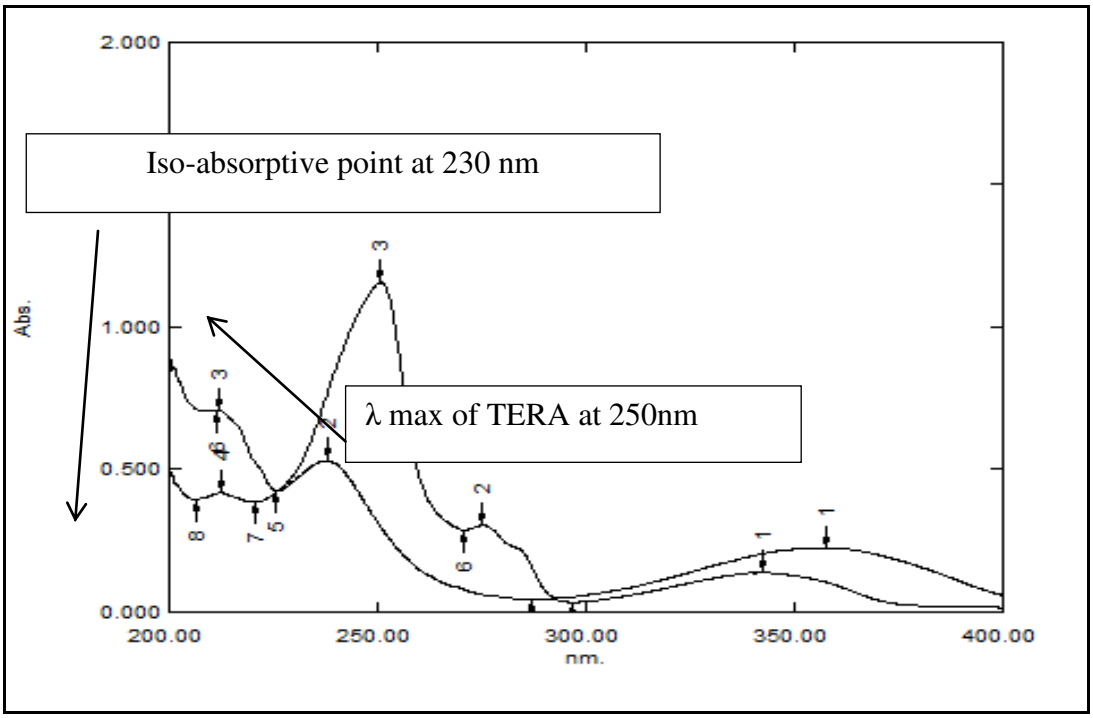

Figure 7: Overlain spectra of Terazosin (6µg/ml) and Amlodipine (15 µg/ml).

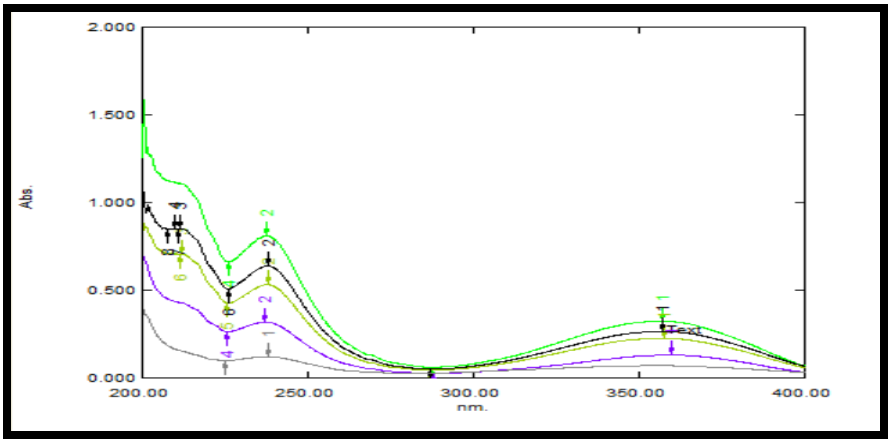

Figure 8: Overlain spectra of Amlodipine (5-25 µg/ml)

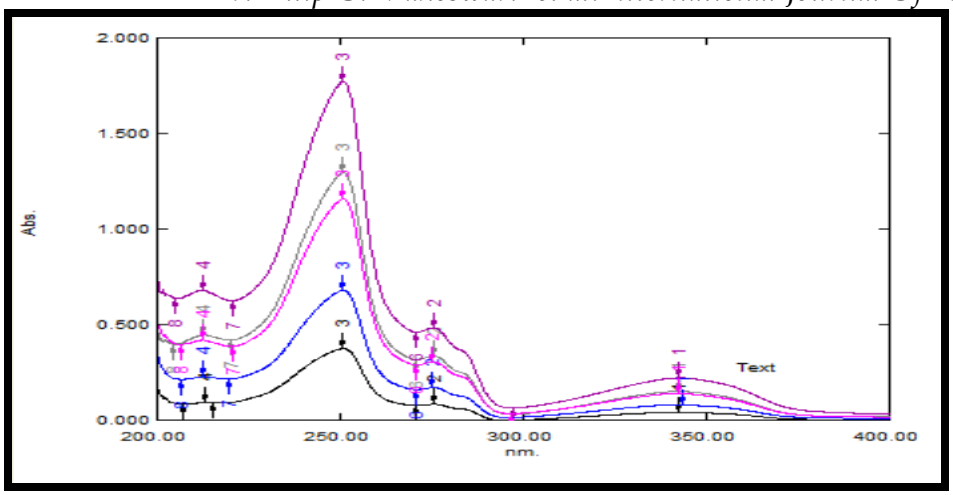

Figure 9: Overlain spectra of Terazosin (2-10 µg/ml).

Table-1: Linearity data of Amlodipine.

AT 230nm			At 250 nm		
Con (µg/ml)	**Mean absorbance ±SD(n=5)**	**%RSD**	**Con (µg/ml)**	**Mean absorbance ±SD (n=5)**	**%RSD**
5	0.115 ± 0.0005	0.477	5	0.080 ±0.0008	1.043
10	0.245 ± 0.0017	0.709	10	0.148 ±0.0013	0.879
15	0.444 ± 0.0008	0.201	15	0.294 ±0.0013	0.443
20	0.591 ± 0.0013	0.220	20	0.399 ±0.0007	0.177
25	0.689 ± 0.0011	0.165	25	0.450 ±0.0015	0.351

Table-2: Linearity data of Terazosin.

AT 230 nm			At 250 nm		
Con (µg/ml)	**Mean absorbance ±SD (n=5)**	**%RSD**	**Con (µg/ml)**	**Mean absorbance ±SD (n=5)**	**%RSD**
2	0.143 ± 0.0011	0.78850	2	0.376 ± 0.0012	0.3273
4	0.257 ± 0.0015	0.61522	4	0.667 ± 0.0017	0.2682
6	0.392 ± 0.0019	0.49094	6	0.852 ± 0.0013	0.1528
8	0.535 ± 0.0020	0.38782	8	1.301 ± 0.0011	0.0876
10	0.714 ± 0.0008	0.18256	10	1.729 ± 0.0008	0.0483

Table 3: Repeatability data of Amlodipine.

AT 230 nm			At 250nm		
Con (µg/ml)	**Mean absorbance ±SD (n=6)**	**%RSD**	**Con (µg/ml)**	**Mean absorbance ±SD (n=6)**	**%RSD**
15	0.445± 0.00098	0.496	15	0.293± 0.00098	0.1706

Table-4: Repeatability data of Terazosin.

AT 230 nm			At 250 nm		
Con (µg/ml)	Mean absorbance ±SD (n=6)	%RSD	Con (µg/ml)	Mean absorbance ±SD (n=6)	%RSD
6	0.392 ± 0.0012	0.2203	6	0.850 ± 0.0011	0.1840

Table-5: Intraday Precision data of Amlodipine.

AT 230 nm			At 250 nm		
Con (µg/ml)	Mean absorbance ±SD (n=3)	%RSD	Con (µg/ml)	Mean absorbance ±SD (n=3)	%RSD
10	0.450 ± 0.004	0.897	10	0.304 ± 0.0049	1.062
15	0.628 ± 0.004	0.619	15	0.474 ± 0.0056	0.851
20	0.834 ±0.007	0.915	20	0.658 ± 0.0078	1.076

Table-6: Intraday Precision data of Terazosin.

AT 230nm			At 250 nm		
Con (µg/ml)	Mean absorbance ±SD (n=3)	%RSD	Con (µg/ml)	Mean absorbance ±SD (n=3)	%RSD
4	0.396 ± 0.002	0.6344	4	0.441 ± 0.002	0.4713
6	0.575 ± 0.002	0.3478	6	0.638 ± 0.002	0.3134
8	0.735 ± 0.002	0.2721	8	0.816 ± 0.002	0.3082

Table-7: Interday Precision data of Amlodipine.

AT 230 nm			At 250 nm		
Con (µg/ml)	Mean absorbance ±SD	%RSD	Con (µg/ml)	Mean absorbance ±SD	%RSD
10	0.317 ± 0.0045	1.4231	10	0.302 ± 0.0054	1.1615
15	0.482 ± 0.0061	1.2503	15	0.484 ± 0.0067	1.0515
20	0.636 ± 0.0075	1.1745	20	0.662 ± 0.0079	1.2971

Table-8: Interday Precision data of Terazosin.

AT 230 nm			At 250 nm		
Con (µg/ml)	Mean absorbance ±SD	%RSD	Con (µg/ml)	Mean absorbance ±SD	%RSD
4	0.399 ± 0.005	1.2531	4	0.444 ±0.004	1.014074
6	0.575 ± 0.005	0.8743	6	0.637 ±0.006	1.044171
8	0.741 ± 0.008	1.0819	8	0.814 ±0.008	0.982801

Table-9: Accuracy data of Amlodipine and Terazosin.

Name of sample	Level	Amount taken (µg/mL)	Amount added (µg/mL)	Recovered Concentration (µg/mL)	%Recovery ± SD (n=3)
AMLO	50	10	5	15.23	101.53 ± 0.19
	100	10	10	19.66	99.15 ± 1.01
	150	10	15	24.89	99.59 ± 1.10
TERA	50	4	2	6.06	101.06 ± 0.18
	100	4	4	7.9	99.64 ± 1.02
	150	4	6	9.98	99.85 ± 1.30

Table 10: Assay Study Parameter.

Amlodipine			Terazosin		
Label claim (mg)	Amount found (mg)	% Assay ± SD (n=3)	Label claim (mg)	Amount found (mg)	% Assay ± SD (n=3)
10	9.98	99.8 ± 0.657	5	4.92	99.4 ± 1.3

Conclusion

The proposed Spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of AMLO and TERA in synthetic mixture. The method utilizes easily available and solvent for analysis of AMLO and TERA hence, the method is economic for estimation of AMLO and TERA in synthetic mixture. The common excipients and additives are present in the synthetic mixture form do not interfere in the analysis of AMLO and TERA in method, Hence it can be conveniently adopted for routine quality control analysis of the drugs in mixture.

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