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SIMULTANEOUS ESTIMATION OF TELMISARTAN AND AMLODIPINE BY UV SPECTROSCOPY

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Abstract:

The present work represents a UV spectrophotometric method for the simultaneous estimation of Telmisartan and Amlodipine in bulk and combined tablet dosage form. The method was based on employing the simultaneous equation method for analysis of both drugs, Telmisartan and Amlodipine have shown absorbance maxima at 225nm and 210 nm in methanol. The analytical method was validated for various parameters as per ICH (International Conference on Harmonization) guidelines. The developed simultaneous equation method obeyed Beer-Lambert's law in the concentration range of 4-40 µg/ml for Telmisartan and 0.5-5 µg/ml for Amlodipine. The slope, intercept and correlation coefficient values of telmisartan at 225nm are 0.008, 0.0002 and 0.999. The slope, intercept and correlation coefficient values of Amlodipine at 210nm are 0.0378,0.0137 and 0.999. Accuracy showed acceptable recovery of 90- 110%, as per ICH guidelines. Inter and Intraday precision showed good reproducibility and repeatability with %RSD <2%. Robustness was performed by varying wavelength and the considered method as robust with %RSD <2%. Ruggedness performed by different analysts and considered method as rugged with %RSD <2%. The LOD and LOQ were 0.0107µg/ml and 0.032 µg/ml for Telmisartan and 0.006µg/ml and 0.0183µg/ml for Amlodipine respectively. The developed method was successfully applied for the estimation of drug content in Telmisartan 40mg and Amlodipine 5mg Tablets I.P. Percentage assay of Telma®– AM tablet was found to be 98.3 and 99.94. Hence the proposed method was found to be simple, rapid, accurate and precise and can be applied for the routine quality control studies for assay of Telmisartan and Amlodipine in bulk and tablet dosage forms.

Keywords: *UV spectrophotometry, Telmisartan, Amlodipine, Simultaneous equation method, and validation.*

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INTRODUCTON:

Hypertension is the biggest controllable risk factor for cardiovascular diseases. There are a number of medications (69 drugs in 15 classes) that can reduce the high blood pressure and prevent damage to the heart. If high blood pressure is left untreated, it can cause serious problems like thickening and scarring of the heart muscle (myocardial hypertrophy and fibrosis), ultimately leading to heart failure (HF).

Low adherence and intolerance to medication among hypertensive patients is leading to an increase in hypertension cases by 60 -65 %. Research studies have also shown that around 45.6% of people with high blood pressure were aware of their condition, 36.9% were undergoing treatment, and only 13.8% had a control on high blood pressure levels. A survey has shown that 1.39 billion people had hypertension worldwide in 2010.The number of hypertension cases were higher among low and middle income countries i.e..,1.0 billion when compared to high income countries which was 349 million people.

Factors such as lifestyle changes, food habits (high sodium intake), obesity, alcohol consumption, reduced physical activity are the major causes of hypertension. There is a constant increase in hypertension cases for which new treatment options, new drugs, devices and procedures are being developed.

Telmisartan drug belongs to the class of benzimidazoles which is used in the treatment of hypertension.It plays different roles such as an antihypertensive agent ,an angiotensin receptor antagonist,a peptidyl –dipeptidase inhibitor,a xenobiotic and an environmental contaminant.Telmisartan works by obstructing the binding of angiotensin II to angiotensin II receptors through reverse and selective binding in vascular smooth muscle and in the adrenal gland.The IUPAC name of telmisartan is 2-[4-[[4-methyl-6-(1-methylbenzimidazol-2-yl)-2-propylbenzimidazol-1-yl]methyl]phenyl]benzoic acid.

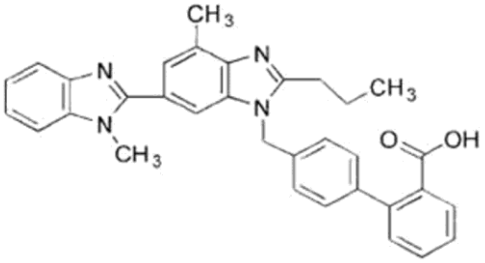

FIGURE 1 : STRUCTURE OF TELMISARTAN

Amlodipine is a completely substituted dialkyl 1,4-dihydropyridne-3,5-dicarboxylate derivative which is used for the treatment of hypertension and angina pectoris.It acts as a calcium channel blocker and vasodilator agent.Amlodipine blocks the entry of calcium ions into the vascular smoth muscle and cardiac muscle.Amlodipine also helps in production of nitric oxide which decreases blood pressure.The IUPAC name of amlodipine is 3-O-ethyl 5-O-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate.

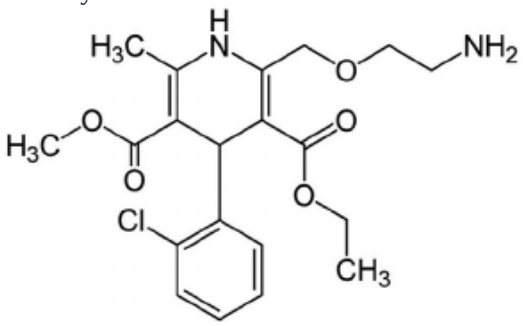

FIGURE 2 : STRUCTURE OF AMLODIPINE

According to recent research studies,it is being prescribed that combination of drugs of two different classes among anti-hypertensive drugs are more effective in reducing blood pressure than a single drug.

To manage hypertension and improve medication compliance, a single-pill combination of ARB and CCB is currently offered. Because of the potent efficacy and lengthy half-lives of each agent, tight BP control is anticipated with the T40/A5 fixed-dose combination (FDC) therapy of telmisartan 40 mg and amlodipine 5 mg.

Telmisartan is a receptor blocker of angiotensin and amlodipine is a blocker of calcium ions.

They both work in different ways to reduce the blood pressure in the blood vessels.

Amlodipine (AML) and Telmisartan (TEL) are one of the commonly combined drugs used in treatment of hypertension. Researchers have developed several methods using ultraviolet (UV) and high-performance liquid chromatography (HPLC) to analyze both drugs simultaneously in these tablets.The present work demonstrates simple, rapid, accurate, reproducible and economical method for the simultaneous determination of AML and TEL.

MATERIALS AND METHODS:

Instruments : The SHIMADZU – double beam-UV-Visible spectrophotometer, was used for the development of new method and validation.The other instruments utilized were digital weighing balance,sonicator.

Chemicals :
The two drugs telmisartan and amlodipine besylate were collected from Dr. Reddy's Laboratories.

Selection of solvent :
To select the appropriate solvent for dissolving the drug ,methanol was selected.As methanol is economical and gave spectra without any noise.

Preparation of Stock solutions (standard) of TEL and AML:
A stock solution of TEL and AML was prepared separately with a concentration of 1mg/ml .

Preparation of working standard solutions :
From the above standard stock solutions, working standard solutions of concentration 100 mg/ml was prepared.

Preparation of test solution:
The average weight of five powdered tablets was taken and the weight equivalent to 40 mg of telmisartan and 5 mg of Amlodipine besylate was taken in 100 ml volumetric flask and dissolved in 50 ml of methanol. After dissolution, the volume was made up to the mark with the same solvent to get a stock solution containing 400 µg/ml telmisartan and 50 µg/ml of amlodipine besylate .The solution was sonicated for about 30 mins and was then filtered through Whatmann filter paper No.41. The solution was suitably diluted with methanol to obtain sample solutions containing TEL and AML in the concentrations ratio of 8:1 µg/ml respectively as in the tablet formulation.

Determination of λ max :
The standard solutions of TEL and AML were prepared and scanned in the UV-Visible Spectrophotometer in the range of 200-400 nm to determine the λ max of each drug. λ max of TEL and AML were found to be 225nm and 210nm respectively.

Method Validaton:
Simultaneous Equation Method:
By applying this method, the absorbance of TEL and AML was measured at 225 nm (λ_1 - λ max of TEL) and 210nm (λ_2 - λ max of AML) respectively. The standard solutions of both drugs and sample solutions were measured at two wavelengths. Absorptivity values calculated for both drugs at two wavelengths λ_1 and λ_2 were used to calculate the concentration of each drug in the sample solutions
Absorptivity can be calculated by the formula,

$$E = A \times 10000 / bc$$

Where, path length (b) = l cm
C= concentration in µg/ml

Concentration of each drug component in the sample solution is calculated using the formula,

$$C_x = A_2 a_{y_1} - A_1 a_{y_2} / a_{x_2} a_{y_1} - a_{x_1} a_{y_2}$$
$$C_y = A_1 a_{x_2} - A_2 a_{x_1} / a_{x_2} a_{y_1} - a_{x_1} a_{y_2}$$

Where:
A_1 &; A_2 = the absorbances of sample at λ_1 & λ_2 respectively. (A_1 = 1.119, A_2 = 0.956)
ax_1 & ax_2 = the absorptivities of TEL at λ_1 and λ_2 respectively.
ay_1 & ay_2 = the absorptivities of AML at λ_1 and λ_2 respectively
C_x & C_y = the concentrations of TEL & AML in the sample respectively

Validation Parameters:
Linearity:
A series of solutions in the concentration range of 4 – 40 µg /ml of TEL and 0.5 – 5 µg/ml of AML from the stock solutions were prepared. The prepared solutions were scanned in the range of 200-400mm and the absorbance was recorded. Calibration curve was plotted against absorbance on Y axis and concentration on X-axis. The calibration curve was treated by linear regression analysis. The equation of the calibration curve for TEL and AML was
obtained.

Accuracy:
To determine accuracy, three concentration levels of 50 %,100%,150% were selected.The sample solutions of TEL and AML were spiked at concentration of 8µg/ml along with their different known concentrations of standard TEL (4,8,12µg/ml) and AML (0.5,1,1.5µg/ml) .All the solutions were scanned under the UV range of 200-400mm.
Calculate the % recovery from the absorbances noted.

$$\%\text{Recovery} = \frac{\text{spiked} - \text{unspiked}}{\text{spiked sample}} \times 100$$

Precision:
The precision of the method was confirmed by repeatability and intermediate precision.Intra-day and Inter-day studies were conducted on one particular concentration solution from a linearity range and the absorbance of that particular concentration was measured six times a day at different time intervals and also Inter-day absorbance was measured and the %RSD was calculated.

Robustness:
This procedure was performed by varying wavelengths and checking the absorbance of the

prepared drug concentrations. The standard deviation and %RSD were calculated.

Ruggedness:
The sample solutions were prepared and checked for various analytical parameters like different analysts and different laboratory conditions.

LOD and LOQ:
The limit of detection (LOD) and limit of quantitation (LOQ) parameters were calculated using the following equations;

$$\mathbf{LOD = 3.3\ \sigma / s}$$
$$\mathbf{LOQ = 10\ \sigma / s,}$$

where **σ** is standard deviation of y intercept of calibration curve and
s is slope of regression equation.

RESULTS AND DISCUSSION :

Method:
Simultaneous equation method
Determination of A (1%, 1cm) of Drugs at Selected Wavelengths
For determination of A (1%, 1cm), aliquot portions of TEL and AML were diluted separately by methanol to obtain concentration 8 and 1 µg/ml respectively. The absorbance of each resulting solution was measured at 225 nm and 210 nm. The A (1%, 1cm) values (ax_1, ax_2, ay_1& ay_2) were determined from five different concentrations of 8 and 1 µg/ml of TEL and AML using following equation 1.

$$A = \frac{abs}{Conc\left(\frac{g}{100\ ml}\right)}$$

The absorptivity values of TEL and AML are reported in Table No 1 and 2 respectively.

Table-1 : Absorptivity values of TEL

S.No	Concentration (gm/ml)	Absorbances		Absorptivity	
		210 nm	225 nm	210 nm	225 nm
1	0.008	0.0121	0.04	1.51	5
2	0.008	0.0120	0.041	1.5	5.125
3	0.008	0.0121	0.04	1.51	5
4	0.008	0.0122	0.042	1.525	5.25
5	0.008	0.0120	0.041	1.5	5.125
			Mean	1.51	5.1
			SD	0.0104	0.10458

Table-2 : Absorptivity values of AML

S.No	Concentration (gm/ml)	Absorbances		Absorptivity	
		210 nm	225 nm	210 nm	225 nm
1	0.008	0.0216	0.0152	2.7	1.9
2	0.008	0.0217	0.0153	2.7125	1.9125
3	0.008	0.0215	0.0151	2.6875	1.8875
4	0.008	0.0217	0.0152	2.7125	1.9
5	0.008	0.0215	0.0151	2.6875	1.8875
			Mean	2.7	1.8975
			SD	0.10458	0.10458

Analysis of tablet formulation :
Five tablets were weighed; average weight was determined and triturated to produce fine powder. A quantity equivalent to 40 mg of TEL and 5 mg of AML was weighed and transferred to 100 ml volumetric flask; the volume was made up with methanol. This solution was appropriately diluted with methanol to get concentration of 8 µg/ml of Telmisartan and 1 µg/ml of Amlodipine. The absorbances of sample solutions were measured at 225 nm and 210 nm against blank. The contents of TEL and AML in tablet dosage form were calculated using two framed simultaneous equations and the results of analysis of tablet formulation are reported in Table No 3.

Table-3 : Analysis of tablet formulation

Drug	Brand Name	Available form	Label Claim	Amount found	%Assay
TEL	Telma®– AM	Tablet	40mg	0.8734	98.3
AML	Telma®– AM	Tablet	5mg	0.111	99.94

Method Validation :

The method was validated for linearity, accuracy (recovery), precision, robustness, ruggedness and sensitivity. The method validation was performed as per ICH guidelines.

Linearity:

Absorbances of all the dilutions were taken at λ_{max} of TEM and AML i.e., 225 nm and 210nm respectively. The absorbances were plotted against the respective concentrations to obtain the calibration curves (Figure 4 and 5). The r^2 value obtained was 0.999 for TEM and AML respectively.

Table-4 : Linearity of TEL and AML

Telmisartan		Amlodipine	
Concentration (µg/ml)	Absorbances	Concentration (µg/ml)	Absorbances
4	0.035	0.5	0.005
8	0.064	1	0.024
12	0.094	1.5	0.044
16	0.127	2	0.064
20	0.163	2.5	0.08
24	0.188	3	0.099
28	0.219	3.5	0.118
32	0.254	4	0.134
36	0.289	4.5	0.157
40	0.324	5	0.178

Table-5 : Values obtained from Calibration curve

Parameters	**Telmisartan**	**Amlodipine**
Linearity range	4-40 µg/ml	0.5-5 µg/ml
Correlation coefficient	0.999	0.999
Slope	0.008	0.0378
Intercept	0.0002	0.0137

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The recovery studies were performed by standard additional methods. The results of recovery studies were found to be satisfactory and as reported in Table No. 6 and 7.

Table-6: Accuracy of Telmisartan

Level of recovery	**Concentration (µg/ml)**		**Absorbance**	**%recovery**	**% mean recovery**	**Mean**	**SD**	**%RSD**
	Sample	SD						
50	8	4	0.102	98.52941	99.01961	0.102333	0.000577	0.564186
	8	4	0.103	100				

	8	4	0.102	98.52941				
100	8	8	0.133	101.4706	100.4902	0.132333	0.001155	0.87257
	8	8	0.131	98.52941				
	8	8	0.133	101.4706				
150	8	12	0.163	101.4706	100.9804	0.162667	0.000577	0.354928
	8	12	0.162	100				
	8	12	0.163	101.4706				

Table-7: Accuracy of Amlodipine

level of recovery	Concentration (µg/ml)		absorbance	%recovery	% mean recovery	mean	SD	%RSD
	Sample	SD						
50%	8	0.5	0.073	100	99.5098	0.072667	0.000577	0.794519
	8	0.5	0.073	100				
	8	0.5	0.072	98.52941				
100%	8	1	0.093	101.4706	100.9804	0.092667	0.000577	0.62304
	8	1	0.092	100				
	8	1	0.093	101.4706				
150%	8	1.5	0.113	101.4706	101.9608	0.113333	0.000577	0.509427
	8	1.5	0.113	101.4706				
	8	1.5	0.114	102.9412				

The percentage recovery varied from 99.019 to 100.9608 for TEL and 99.5.98 to 101.9608 for AML, indicating good accuracy of the method.

Precision:

Precision of the method was verified by using tablet stock solution. Intraday precision was determined by repeating assay six times in the same day and on different day for Interday precision studies. The results of this analysis are shown in Table No 8 and 9.

Table-8: Precision values of Telmisartan

Intraday Precision		**Interday Precision**	
Concentration (µg/ml)	**Absorbance**	**Day**	**Absorbance**
8	0.641	1	0.642
8	0.637	2	0.639
8	0.639	3	0.641
8	0.643	4	0.643
8	0.647	5	0.648
8	0.645	6	0.647
Mean	0.642	**Mean**	0.643333333
SD	0.00341565	**SD**	0.003197221
% RSD	0.53203275	**% RSD**	0.49697736

Table-9: Precision values of Amlodipine

Intraday Precision		Interday Precision	
Concentration (µg/ml)	**Absorbance**	**Day**	**Absorbance**
1	0.024	1	0.023
1	0.024	2	0.023
1	0.025	3	0.024
1	0.024	4	0.023
1	0.024	5	0.023
1	0.024	6	0.023
Mean	0.024167	**Mean**	0.023167
SD	0.000373	**SD**	0.000373
% RSD	1.542116	**% RSD**	1.608682

Robustness:

Robustness of the method was determined by changing the wavelength ±1 nm from 225nm (TEL) to 210nm (AML) and results were offered in Table No.10 and 11.

The %RSD value calculated from the robustness study was found to be less than 2% for TEL and AML, indicating the method is robust.

Table-10: Robustness of Telmisartan

Concentration (µg/ml)	224	225	226
8	0.64	0.643	0.645
8	0.643	0.647	0.648
8	0.637	0.641	0.642
8	0.642	0.639	0.641
8	0.643	0.645	0.647
8	0.635	0.637	0.64
Mean	0.64	0.642	0.643833
SD	0.003055	0.003416	0.003023
%RSD	0.477352	0.532033	0.469541

Table-11: Robustness of Amlodipine

Concentration (µg/ml)	209	210	211
1	0.023	0.024	0.024
1	0.023	0.024	0.024
1	0.023	0.023	0.024
1	0.023	0.024	0.023
1	0.024	0.024	0.024
1	0.023	0.024	0.024
Mean	0.023167	0.023833	0.023833
SD	0.000373	0.000373	0.000373
%RSD	1.608682	1.563684	1.563684

Ruggedness:

Ruggedness was carried out by analyzing the sample of Telmisartan and Amlodipine utilizing two distinct instruments and analysts. The results were determined in in terms of %RSD. The
results was offered in TableNo-12 and 13.

The %RSD value calculated from the ruggedness study was found to be less than 2% for TEL and AML, indicating the method is rugged.

Table-12 : Ruggedness of Telmisartan

Concentration (µg/ml)	Day-1		Day-2	
	analyst-1	analyst-2	analyst-1	analyst-2
	absorbance	absorbance	absorbance	absorbance
8	0.62	0.643	0.643	0.647
8	0.635	0.645	0.645	0.649
8	0.637	0.642	0.649	0.648
8	0.635	0.652	0.645	0.653
8	0.623	0.655	0.647	0.652
8	0.631	0.657	0.652	0.66
Mean	0.630167	0.649	0.646833	0.6515
SD	0.007055	0.006481	0.003251	0.004764
%RSD	1.119474	0.998573	0.502547	0.731305

Table-13: Ruggedness of Amlodipine

Concentration (µg/ml)	DAY-1		DAY-2	
	analyst-1	analyst-2	analyst-1	analyst-2
	absorbance	absorbance	absorbance	absorbance
1	0.023	0.025	0.022	0.024
1	0.023	0.024	0.022	0.023
1	0.023	0.024	0.022	0.023
1	0.023	0.025	0.021	0.023
1	0.023	0.025	0.022	0.023
1	0.022	0.025	0.022	0.023
Mean	0.022833	0.024667	0.021833	0.023167

Limit of Detection and Limit of Quantification

The limit of detection and limit of quantification i.e. LOD & LOQ were calculated by use of the equations **LOD = 3.3 × σ/s** and **LOQ = 10 × σ/s,** where **σ** is the standard deviation of the response areas of the drugs, taken as a measure of noise, and **s** is the slope of the corresponding calibration plot. The LOD and LOQ were recorded in Table-14.

The LOD and LOQ were 0.0107µg/ml and 0.032µg/ml for Telmisartan and 0.006µg/ml and 0.0183µg/ml for Amlodipine respectively.

Table-14 : LOD&LOQ values of Telmisartan and Amlodipine

Name of the Drug	LOD (ppm)	LOQ (ppm)
Telmisartan	**0.0107**	**0.032**
Amlodipine	**0.006**	**0.0183**

CONCLUSION:

We developed and validated U.V Spectrophotometric method for the combination of Telmisartan and Amlodipine in both Bulk and Dosage formulation. The developed method was successfully applied for the estimation of drug content in Telmisartan 40mg and Amlodipine 5mg Tablets I.P. Percentage assay of Telma ® – AM tablet was found to be 98.3 and 99.94.

After observing the validated parameters: accuracy, precision, LOD& LOQ were within limits. We are able to justify that by taking the available label claim, we have improved the linearity range of the drugs. Lower LOQ value indicated that the proposed method would be suitable for analyzing the samples containing even small quantities of drugs. The proposed method was found to be robust and rugged in nature and was successfully used

for estimation of Telmisartan and Amlodipine.

The results indicates that the method was simple, accurate and precise. Hence it can be used for the analysis, which would involve detection of absorbance of sample and standard solution at two wavelength and single calculation.

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