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# Simultaneous Estimation and Validation for Amlodipine & Telmisartan in Tablet Dosage Form by RPHPLC Method

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**Abstract:** A simple, economic, accurate reverse phase isocratic RPHPLC method was developed for the Simultanious estimation of Amlodipine (5mg) & Telmisartan (40mg) in Tablet dosage form. The quantification was carried out using Symmetry C18 (4.6 x 100mm, 5µm, Make:XTerra) with UV detected at 237 nm. The elution was achieved isocratically with a mobile phase comprises of mixture of buffer 650 ml (65%) and 350 ml of Acetonitrile HPLC (35%). The flow rate was 1.0ml/min. The procedure was validated as per ICH rules for Accuracy, Precision, Detection limit, Linearity, Reproducibility and Quantitation limit. The linearity concentration range was 10-50ppm of Amlodipine and 5-25ppm of Telmisartan with the correlation coefficient of 0.9995 and 0.9997 respectively. The percentage recovery for Amlodipine & Telmisartan was found to be 100.5% and 100.21% respectively. Limit of detection values were found to be 0.02mcg/ml and 0.01mcg/ml respectively for Amlodipine & Telmisartan. Limit of quantitation values were found to be 0.08mcg/ml and 0.03mcg/ml respectively for Amlodipine & Telmisartan. The method has been successfully used to analyze commercial solid dosage containing Amlodipine & Telmisartan with good recoveries and proved to be robust.
**Keywords:** Amlodipine, Telmisartan, Tablets, HPLC, Validation.

## Introduction:

Amlodipine besylate (AML) is used as an antihypertensive and antianginal agent chemically 2[(2- aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine carboxylic acid, 3-ethyl, 5-methylester besylate[1,2]. It is official in European Pharmacopoeia[3].Telmisartan (TEL) is a potent antagonis of AT1 receptor, chemically 4[(1,4-Dimethyl-2-propyl-(2,6-bi-1H-benzimidazol]-1-yl)Methyl][1,1-Bipheyl]-2-carboxylic acid[4]. Literature surveys reveal that only a few methods have been reported for these combinations. The paper aims to develop an isocratic RPHPLC method for the estimation of Amlodipine (AML) and Telmisartan (TEL) in tablet dosage forms.

## Materials and methods:

Amlodipine (AML) and Telmisartan (TEL) were obtained as generous gift sample from Dr. Reddy's Laboratory Limited (Hyderabad, India). A commercial sample Telpres-AM (tablet containing Amlodipine (5mg) & Telmisartan (40mg)) were

purchased from local pharmacy store and used within their shelf-life period. The HPLC grade acetonitrile and water from Rankem (New Delhi, India) and all other chemicals used were of pharmaceutical or analytical grade from Rankem. HPLC grade water was prepared using Millipore purification system.

A isocratic Water's HPLC system consisted of a LC-20AT VP (Japan) equipped with diode array detector (SPD-M10 AVP). Manual injections were made using a Rheodyne Injectable valve (20 µl loop). The detector wavelength was set at 237nm. The chromatographic separations were performed at ambient temperature on a Symmetry C18 (4.6 x 100mm, 5µm, Make:XTerra). The mobile phase was a mixture of buffer 650 ml (65%) and 350 ml of Acetonitrile HPLC (35%), filtered and degassed prior to use, and flowing at the rate of 1.0ml/min and run time is 15 minutes. Buffer was prepared by dissolving 7.0g of potassium dihydrogen orthophosphate in 1000ml of water, adjusted the pH to 3.0 with orthophosphoric acid and filtered through 0.45 µ or filter porosity membrane filter. The data were collected and analyzed with software in a computer system. Mobile phase used as diluents.

***Preparations:***

About 100mg of AML & TEL working standard was accurately weighed individually and dissolved in 100ml of mobile phase as diluent in the volumetric flask to get a concentration of 1000mcg/ml. From this stock solution of AML and TEL, suitable dilutions were made to get the concentrations of 100mcg/ml individually. Further 3ml of AML & TEL above solutions were pipette out and place together into a 50ml volumetric flask and dilute up to the mark with diluent and filtered through 0.45 µ or filter porosity membranes filter before use. 20 µl of each of the solution was injected.

Twenty tablets were weighed and crushed into fine powder. The powder equivalent to 100mg of AML & TEL was taken in a 100ml volumetric flask containing 50ml of mobile phase used as diluent and kept for sonication for 20min with intermittent shaking. The volume made upto the mark with diluent and centrifuged the solution at 5000RPM for 5 minutes. From this stock solution of AML and TEL, suitable dilutions were made to get the concentrations of 100mcg/ml individually. Further 3ml of AML & TEL above solution were pipette out and place together into a 50ml volumetric flask and dilute up to the mark with diluent and filtered through 0.45 µ or filter porosity membranes filter before use. 20 µl of each of the solution was injected. The experiments were performed six times under the chromatographic conditions described above. The peak areas were measured at 237nm and concentrations in the sample were determined by comparing the peak areas of sample with that of the standards.

***Validation:***

The described method has been validated for the assay of AML & TEL using following parameters.[5-7]

Precision was studied to find out variations in the test methods of AML & TEL on the same day and on different day by using different make column of same dimensions (Ruggedness). The standard solution was injected for five times and measured the area for all five injections in HPLC. Precision and Ruggedness were done on the same day and the different day respectively and the %RSD was calculated for each.

The accuracy of the method was shown by analyzing model mixtures which were obtained by adding known amount of AML & TEL to pharmaceutical preparation. The model mixtures contained 50, 100 and 150% of AML & TEL compared to the labeled drug amount. After injected the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions, the Amount found, Amount added for AML & TEL, individual recovery and mean recovery values were calculated.

The linearity of the method was shown by analyzing model mixtures of concentration range from 10 to 50ppm for AML and 5 to 25ppm for TEL. After Injection of each level into the chromatographic system, peak area was measured. A graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) was plotted.

As part of the Robustness, deliberate change in the Flow rate and Mobile Phase composition were made to evaluate the impact on the method. The flow rate was varied at 0.8 ml/min to 1.2 ml/min. The Organic composition in the Mobile phase was varied from 25% to 45%.

Limit of detection and limit of quantitation were calculated by the method which was a common approach which is to compare measured signals from samples with known low concentrations of analyte with those of blank samples, the minimum concentration at which the analyte can be reliably detected is established. Ratio of Signal Obtained from LOD or LOQ solution (S) and Average Baseline Noise obtained from Blank (N) was calculated for both the drugs.

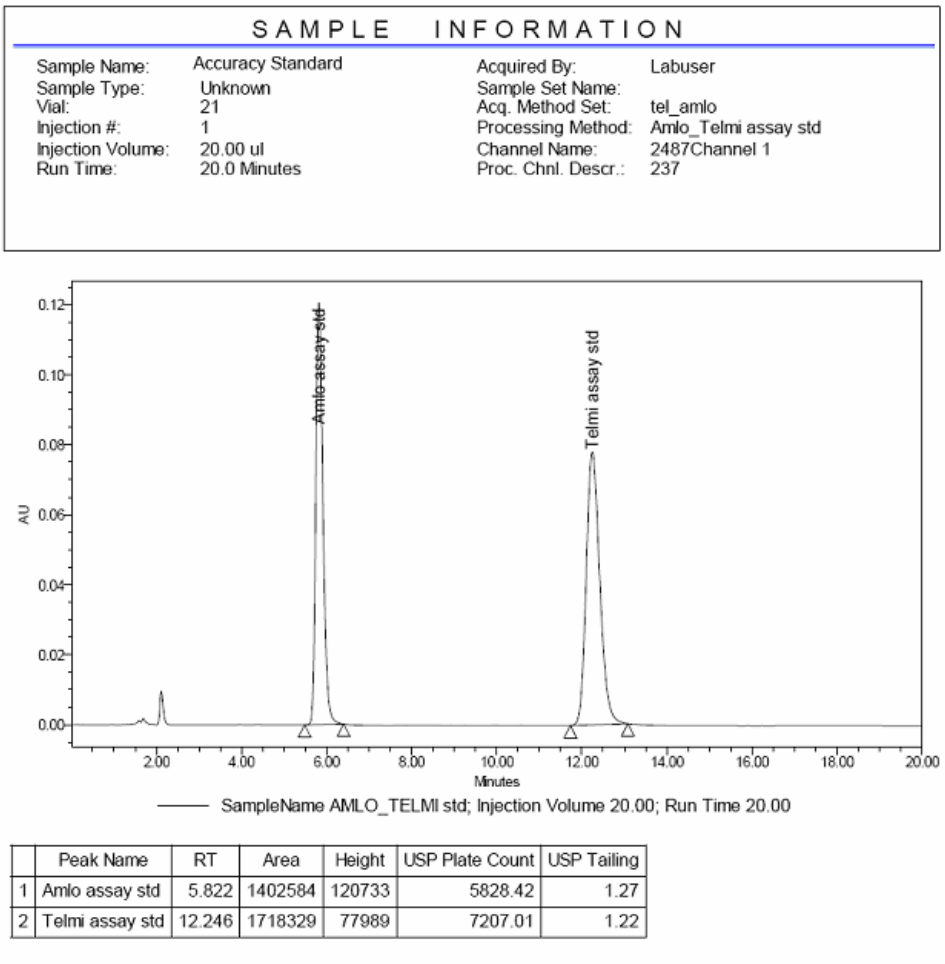
**Table 1: Data For Precision**

| Precision | Precision (Intra) | | Intermediate Precision (Ruggedness) | |
|---|---|---|---|---|
| | AML | TEL | AML | TEL |
| Peak area | 1188915 | 1388580 | 596535 | 696627 |
| | 1190442 | 1389028 | 604589 | 703540 |
| | 1191217 | 1374326 | 602442 | 706905 |
| | 1190077 | 1370785 | 606791 | 717522 |
| | 1193069 | 1355986 | 611921 | 725613 |
| **Mean** | 1190744 | 1375741 | 604456 | 710041 |
| **Standard deviation** | 1542.2 | 13767.8 | 5656.6 | 11516.3 |
| **%RSD** | 0.13 | 1.00 | 0.94 | 1.62 |

**Table 2: data for accuracy**

| Drug | %Concentration (at specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|---|
| Amlodipine | 50% | 789610 | 6.58 | 6.59 | 99.8% | 100.5% |
| | 100% | 1194288 | 10.0 | 9.97 | 100.3% | |
| | 150% | 1593413 | 13.5 | 13.3 | 101.5% | |
| Telmisartan | 50% | 920716 | 6.45 | 6.56 | 98.3% | 100.21% |
| | 100% | 1398893 | 10.01 | 9.97 | 100.4% | |
| | 150% | 1864298 | 13.52 | 13.29 | 101.7% | |

**Figure 1: Chromatogram for a mixture of Amlodipine & Telmisartan**




| | Peak Name | RT | Area | Height | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Amlo assay std | 5.822 | 1402584 | 120733 | 5828.42 | 1.27 |
| 2 | Telmi assay std | 12.246 | 1718329 | 77989 | 7207.01 | 1.22 |

**Figure 2: Linearity curve for Amlodipine**

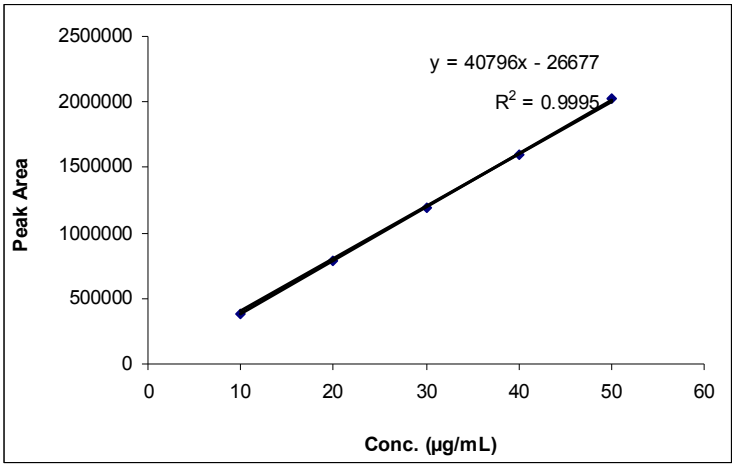
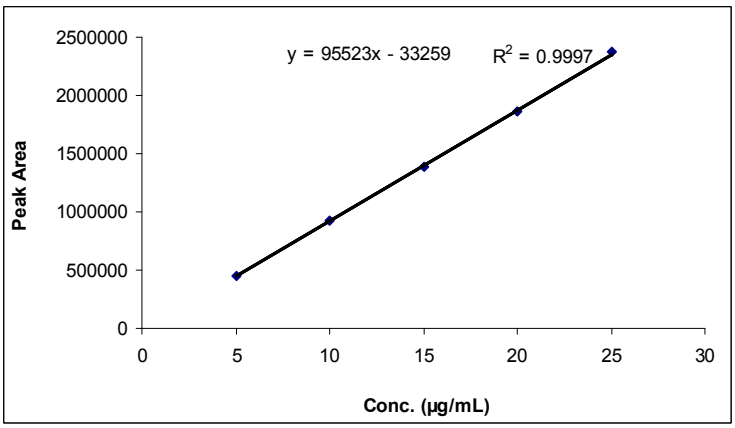



**Figure 3: Linearity curve for Telmisartan**




## Results and Discussion:

A reverse – phase isocratic procedure is proposed as a suitable method for the analysis of AML & TEL in tablets. A mixture of mixture of buffer 650 ml (65%) and 350 ml of Acetonitrile HPLC (35%) at a flow rate of 1.0ml/min was found to be an appropriate mobile phase allowing adequate and rapid separation of analyte. The retention time was found to be 5.8 and 12.2 for AML & TEL respectively. The percentage of purity of AML & TEL in tablet dosage form is 99.8 and 101.3%. System suitability for the AML, Tailing factor Obtained from the standard injection was 1.22 and Theoretical Plates Obtained from the standard injection was 6481. System suitability for the TEL, Tailing factor Obtained from the standard injection was 1.22 and Theoretical Plates Obtained from the standard injection was 6832. As shown in the Fig. 1 the substances were eluted forming well shaped, symmetrical single peaks, well removed from the solvent front.

The precision of the HPLC system was determined using the %RSD of the peak areas for five injections of the standard solution of AML & TEL. Precision data were present in Table 1. The %RSD was less than 2.

In order to verify the accuracy of the described method, recovery studies were carried out by analyzing model mixtures of AML & TEL. The recovery of AML & TEL was evaluated from 50 to 150% of the labeled tablet. The mean percentage recoveries were found to be 100.5% and 100.21% for AML & TEL respectively. Accuracy data were present in Table 2.

For quantitative application a linear calibration curve was obtained over the concentration range from 10 to 50ppm for AML and 5 to 25ppm for TEL. The parameters of the calibration graph for AML & TEL were y = 40796x - 26677 and y = 95523x - 33259 respectively where x is concentration and y is peak area; correlation coefficient for AML & TEL were 0.9995 and 0.9997. Percentage curve fitting for AML & TEL was found to be 99.95% and 99.97% respectively. Calibration curve was present in figure 2 and 3.

The results of robustness indicate that the variation in flow rate affected the method significantly. The method is robust only in less flow condition. Even variation in organic composition in the mobile phase affected the method significantly. Hence it indicates

that the method is not robust even by change in the flow rate ±10% and change in the Mobile phase ±10% for AML & TEL.

Limit of detection values were found to be 0.02mcg/ml and 0.01mcg/ml respectively for AML & TEL. S/N ratio values for LOD were found to be 3.1 and 3.2 for AML & TEL respectively. Limit of quantitation values were found to be 0.08mcg/ml and 0.03mcg/ml respectively for AML & TEL. S/N ratio values for LOQ were found to be 10.7 and 10.25 for AML & TEL respectively.

## Conclusion:

The presented method is precise, sensitive and accurate. The advantages of proposed method are its short analysis time and a simple procedure for sample preparation. The satisfying recoveries and low coefficient of variation confirmed the suitability of proposed method for the routine analysis of mixtures of AML & TEL in pharmaceuticals.

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