DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF AZELNIDIPINE AND TELMISARTAN IN BULK AND PHARMACEUTICAL DOSAGE FORM

Mr. Kolte Durgesh R, Prof. Nemade Mahesh S., Prof. Rane Sachin S., Prof. (Dr.) Chaudhari Rajesh Y., Prof. (Dr.) Patil Vijay R.

Department of Quality Assurance, TVES’s Honorable Lokasevak Madhukarrao Chaudhari College of Pharmacy, Faizpur, Tal- Yawal, Dist- Jalgaon, Maharashtra, PIN-425503

ABSTRACT:

A new simple, rapid, precise, and specific assay method was developed for simultaneous estimation of Azelnidipine and Telmisartan in pure form and tablet form. The analysts were separated by RP HPLC on a RP-Purosphere C18 column (5 µm, 4.6mm, 250 mm). The mobile phase was methanol: acetonitrile: water (40:30:30) at 1.0 mL/min satisfactorily resolved the binary mixture. The UV detector was operated at 225 nm for the determination of both the drugs. Linearity, accuracy and precision were found to be acceptable over the concentration ranges of for Azelnidipine 2-10 µg/ml and Telmisartan 10-50 µg/ml with a R² 0.9988 and 0.9996 values respectively, in binary mixture. The optimized method proved to be specific, robust and accurate for the quality control in bulk drug and pharmaceutical formulations.

KEY WORDS: Azelnidipine, Telmisartan, Validation, RP-HPLC, Simultaneous Estimation

1. INTRODUCTION

Hypertension (HTN) is still the leading cause of death worldwide, and robust randomized trial studies consistently suggest that lowering blood pressure decreases cardiovascular morbidity and mortality. Due to the difficulty of controlling blood pressure (BP) with one anti-hypertensive drug where most patients can only achieve adequate blood pressure control using two or more anti-hypertensive medications, the target has been set to develop an alternative treatment for treating hypertension (HTN) disorder using a combination of the rational drug. The intention for developing the FDC [1] is that by simultaneously administering two comparable drugs in fewer amounts to give lesser side effects, potential benefits attributable to synergistic pharmacological and physiological effects can be obtained. [2] Combining Rennin Angiotensin Aldosterone drugs, including angiotensin converting enzyme (ACE) inhibitors or diuretics, can be a good way to lower blood pressure. [3] The combination of angiotensin II receptor blocker (ARB) telmisartan (TEL), and calcium channel blocker (CCB) Azelnidipine is one such example (AZE) [4] The FDC of Azelnidipine and Telmisartan contains 8 mg of Azelnidipine and 40 mg of Telmisartan.

Azelnidipine is dihydropyridine derivative and chemically 3-[1-(Benzyldrylazetidin-3-yl] 5- isopropyl- 2- amino6methyl-4-(3-nitrophenyl)-1,4- dihydropyridine-3,5dicarboxylate. [5] Azelnidipine category is Dihydropyridine calcium channel blocker. Azelnidipine inhibits trans-membrane Ca2+ influx through the voltage-dependent channels of smooth muscles in vascular walls. [6] It is a vasodilator that induces a gradual decrease in blood pressure in hypertension. When calcium channels are blocked, the vascular smooth muscle does not contract, resulting in relaxation of vascular smooth muscle walls and decreased blood pressure. It is Used in Treatment of Hypertension which lower the Blood Pressure due to Block calcium Channel and Decreases Blood Pressure. and oral dose is 8 – 16 mg once daily. It metabolized in hepatic cytochrome P450 (CYP) 3A4 and has no active metabolite product. [7,8]
Telmisartan is an antihypertensive drug that belongs to the angiotension II receptor blocker class and is marketed under the brand name Micardis. Telmisartan is an angiotensin II receptor blocker. It functions by blocking a material in the body that causes blood vessels to tighten. Telmisartan relaxes the blood vessels as a result. This reduces blood pressure while increasing blood and oxygen supply to the heart. Telmisartan IUPAC name is \( 2-[4-[4-\text{methyl}-6-(1-\text{methylbenzimidazol}-2-\text{yl})-2\text{-propylbenzimidazol}-1-\text{yl}] \text{methyl}] \text{phenyl}] \text{benzoic acid} \). Molecular weight is 514.6 g/mol, Melting point is 261-263°C, pKa value is 3.5. It is freely soluble in formic acid, slightly soluble in methanol, very slightly soluble in ethanol. Log P value is 3.2. A literature survey revealed that several methods have been used for estimation of Telmisartan alone [11,12] and also combined with Amlodipine Ramipril and Hydrochlorthiazide, which includes High Performance Liquid Chromatography (HPLC), Liquid Chromatography, UV spectroscopy [13,14,15].

2. Material and Methods

2.1 Chemicals and reagents

AZN and TEL were procured from Torrent Pharmaceutical Ltd. Commercial pharmaceutical preparation UNIAZ T 40, manufactured by Synokem Pharmaceuticals Ltd. containing 40 mg of TEL and 8 mg of AZN was collected from local market. Acetonitrile, methanol and water used were of HPLC grade (Qualigens Fine Chemicals, Mumbai, India). Ortho-phosphoric acid was AR grade (Qualigens Fine Chemicals, Mumbai, India). A 0.2 µm nylon filter (Pall life Sciences, Mumbai, India) was used. All other chemicals and reagents used were analytical grade unless otherwise indicated.

2.2 Apparatus

The chromatographic system (Systronics Corporation, India) consisted of LC-138 at Prominence solvent delivery module, a manual Rheodyne injector with a 20 µL fixed loop and a UV-visible detector. The separation was performed on a Kromstar™ RP-Vertex C18 column (5 µm, 4.6mm* 250 mm) at an ambient temperature. Chromatographic data were recorded and processed using Clarify 2.0 Software. A Fast clean ultrasonicate cleaner (India) was used for degassing the mobile phase. Shimadzu UV 1800 double beam UV visible spectrophotometer and Sansui-vibra DJ-150S-S electronic balance were used for Spectrophotometric and weighing purposes respectively.

2.3 Chromatography Conditions

Chromatographic separations of active (AZN and TEL) substances were obtained by using Kromstar™ RP-Vertex RP-Purosphere Star C18 column (5 µm, 4.6mm* 250 mm). Mobile phase methanol: acetonitrile: water (40:30:30 v/v) PH 6.0 was prepared, filtered through a 0.2 µm nylon filter and degassed for 5 min in an ultrasonicator. The mobile phase was pumped through the column at flow rate of 1.0 mL/min. Analyses were carried out at ambient temperature with detection at 225 nm. The injection volume was 20µL and each analysis required around 14 min.
2.4 Standard Solutions

Stock standard solutions of AZN 1mg/mL and TEL 1mg/mL were prepared by dissolving 5 mg AZN and 10 mg TEL standard in 10 mL methanol. AZN 0.8 ml and TEL 0.4ml dissolve 10ml volumetric flask. Working standard solutions of AZN 1 mg/mL and TEL 1 mg/mL were prepared by diluting suitable aliquots of corresponding stock solutions with mobile phase.

2.5 Sample Solution

Twenty UNIAZ T 40 tablets containing 8 mg of AZN and 40 mg TEL were weighed and ground to fine powder. A quantity of power equivalent to 8 mg of AZN and 40 mg TEL was transferred into 10 mL volumetric flask containing methanol, sonicated for 15 min and the volume was made up to the mark and filtered through 0.45µm nylon membrane filter. This solution was (1 mL) transferred to 10 mL volumetric flaks, dissolved and volume was adjusted to the mark. The response of solution was measured at 225 nm and quantification of AZN and TEL was done by using present HPLC method.

2.6 Selection of Detection wavelength

All the concentration of give samples scanned separately, overly spectra clearly denote optimum wavelength at 277nm with all selected analytes with possible maximum absorbance as shown (fig 1).

3. Method Validation:

3.1 System Suitability:

The standard solution was injected into the Chromatographic system. Percentage relative standard deviations have been found satisfactory. System suitability results are tabulated in table.

3.2 Calibration curve (Linearity):

Linearity of an analytical method is its ability to elicit test results that are directly proportional to concentration of analyte in sample within given range, this was studied by analyzing five different concentrations of drug ranging from 2µgm/ml to 10µgm/ml for Azelnidipine and 10µgm/ml to 50µgm/ml Telmisartan were transferred to series of 10mL volumetric flasks and the contents of the flasks were diluted up to the mark with diluent. A 20µL aliquot of each solution was injected into the chromatographic system. The conditions including the flow rate 1 ml/min and detection wavelength was set to the 225 nm and the run time program was set to the 12 minutes. A calibration curve for each drug was obtained by plotting area under the peak versus concentration. Linearity results tabulated in Table 2. Linearity graphs for Azelnidipine and Telmisartan are presented in Figure 4 and 5 respectively.

3.3 Accuracy (% Recovery):

Accuracy refers to the closeness of a measured value to a standard value. Accuracy studies were carried out by adding a known amount of pure drugs of AZN and TEL to the pre-analyzed sample solution. The percentage recovery studies carried out by spiking 80%, 100% and 120% of respective drug, each level was injected 3 times, shown in Table 5. According to the results the method is capable to estimate both drug components accurately in the tablet dosage form at a time and the results were within acceptable limits, i.e, above 99 % and below 101 %.

3.4 Precision (Repeatability):

The precision of the method has been evaluated by injecting the six replicate sample preparations. The percentage assay for both Azelnidipine and Telmisartan were calculated and tabulated in Table 3. The % R.S.D. values of the results corresponding to the peak area and retention time were expressed for intra-day precision and on 3 days for inter-day precision. Percentage RSD results show that the method is precise and can be used to estimate the drug components in the tablet dosage form.

3.5 Intermediate Precision (Reproducibility):

The intraday and interday precisions of the proposed method were determined by estimating the corresponding responses 5 times on the same day and on 5 different days for present method. The results are reported in terms of relative standard deviation (RSD).

3.6 Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD and LOQ of the drug were calculated using the equations according to International Conference on Harmonization (ICH) guidelines.

3.7 Robustness:

Robustness of the method was determined by making slight changes in chromatographic conditions. Effect of percentage of methanol in mobile phase on the retention time and slight changes in flow rate were applied as variable parameters. Flow rate varied
at three levels (-1, 0, 1). One factor at the time was changed to estimate the effect. Thus standard solution at varied pH (pH 4.0, 5.0 and 6.0) three pH levels was performed.

3.8 Specificity:

Specificity of an analytical method is its ability to measure accurately, and specifically, the concentration of analyte without interference from other API, diluents, mobile phase. Specificity was checked by determining AZN, TEL in laboratory prepared binary mixture and in binary mixture containing different degradation products.

3.9 System Suitability Test:

In the system suitability test, the binary solution of 8 µg/ml of AZN, 40 µg/ml of TEL (n=6) was prepared and injected. Then the system suitability parameters like retention time, theoretical plates, tailing factor and resolution were calculated from the chromatogram.

4. Results and Discussion:

The absorption spectra of AZN and TEL greatly overlap; so conventional determination of these compounds in mixture is not possible. To optimize the LC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for AZN, and TEL were obtained with a mobile phase consisting of methanol:acetonitrile:water (40:30:30 v/v), pH 6.0. Quantification of the drugs was performed at 225 nm. Resolution of the components with clear baseline separation was obtained.

4.1 Linearity:

Linear correlation was obtained between peak areas and concentrations of AZN, and TEL in range of 8 µgm/ml to 40 µgm/ml, for both drug compounds. The linearity of calibration curves was found to be acceptable over the concentration ranges of 8 µgm/ml 40 µgm/ml for Azelnidipine and Telmisartan, with a R² values 0.9988 and 0.9996. The results show that good correlation existed between the peak area and concentration of the analysts.

4.2 Accuracy:

The recovery experiments were performed by the standard addition method. The recoveries obtained were 99.25 and 99.92% for AZN and TEL, respectively. The high values indicate that the method was accurate.

4.3 Precision:

Precision study was carried out using parameter like method repeatability study which showed that results were within acceptable limit 0.5894 and 0.1419 i.e. % RSD below 2.0 indicating that the method is reproducible. The results are shown in (Table No.2)

4.4 Intermediate Precision:

The intraday % RSD values for AZN and TEL were 0.5894, 0.1419 and respectively. The interday % RSD values for AZN, and TEL were 0.7135, 0.9479 respectively. The % RSD (< 2%) values indicate that the method was sufficiently precise (Table 2).

4.5 LOD and LOQ

LOD values for AZN and TEL were found to be 0.127036 µg/mL, 0.256725 µg /mL, respectively. LOQ values for AZN and TEL were found to be 0.42345 µg /mL, 0.85575 µg /mL, respectively (Table 2). These data showed that the method was sensitive enough for the determination of AZN and TEL.

4.6 Robustness:

The method was found to be robust with no significant changes on test result upon change of analytical conditions like different flow rate, % methanol in mobile phase and pH of mobile phase with the standard deviation was found to be bellow 1 and % RSD is less than 2 for all results. It was found that under small deliberate changes of chromatographic factors, there was no considerable change in under study parameters.

4.7 System Suitability Test:

A binary solution of 8µg/mL of AZN and 40µg/mL TEL (n=5) was prepared and same was injected, then the system suitability parameters were calculated from the chromatogram. The parameters, retention times, resolution factor, tailing factor and theoretical plates were evaluated. The results (Table 5) obtained from system suitability tests are in agreement with the official requirements.
5. Conclusion:

The proposed LC method presented in this paper has advantages of simplicity, accuracy, precision and convenience for separation and quantitation of AZN, and TEL in combination and can be used for the assay of their respective dosage form. Moreover, the proposed HPLC method is a stability indicating assay method that can determine AZN and TEL in presence of their degradation products. Thus, the proposed HPLC method can be used for the quality control of AZN and TEL in typical laboratories.

6. Acknowledgements:

The authors wish to thank to Synokem Pharmaceuticals Ltd., for supplying generous gift samples of Azelnidipine and Telmisartan.

Table No. 1 Regression Analysis of the calibration curves for Azelnidipine and Telmisartan in the proposed HPLC method.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Azelnidipine</th>
<th>Telmisartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity Range(µg/ml)</td>
<td>2 – 10 µg/ml</td>
<td>10 – 50 µg/ml</td>
</tr>
<tr>
<td>Detection Wavelength</td>
<td>225 nm</td>
<td></td>
</tr>
<tr>
<td>Slope ± S.D.</td>
<td>73.104</td>
<td>148.98</td>
</tr>
<tr>
<td>Intercept ± S.D.</td>
<td>6.641</td>
<td>12.87</td>
</tr>
<tr>
<td>Correlation Coefficient($R^2$)</td>
<td>0.9988</td>
<td>0.9996</td>
</tr>
</tbody>
</table>

Table No. 2 Summary of the validation parameters for the proposed HPLC Method:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Azelnidipine</th>
<th>Telmisartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOD</td>
<td>0.127036</td>
<td>0.256725</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.42345</td>
<td>0.85575</td>
</tr>
<tr>
<td>Accuracy %</td>
<td>99.25</td>
<td>99.92</td>
</tr>
<tr>
<td>Repeatability (% RSD, n=5)</td>
<td>0.05806</td>
<td>0.11077</td>
</tr>
<tr>
<td>Precision (RSD %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interday, n=3</td>
<td>0.7135</td>
<td>0.9479</td>
</tr>
<tr>
<td>Intraday, n=3</td>
<td>0.5894</td>
<td>0.1419</td>
</tr>
</tbody>
</table>

LOD = Limit of Detection

LOQ = Limit of Quantification

RSD = Relative Standard Deviation

Table No. 3 Assay results for the combination dosage form using the proposed HPLC method.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Azelnidipine</th>
<th>Telmisartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNIAZ T 40</td>
<td>99.50 ± 0.4246</td>
<td>99.74 ± 0.1796</td>
</tr>
</tbody>
</table>
Table No. 4 System suitability test parameters for AZN and TEL for the proposed HPLC method.

<table>
<thead>
<tr>
<th>System Suitability Parameters</th>
<th>AZD</th>
<th>TEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention Time ($t_R$)</td>
<td>4.003</td>
<td>2.87</td>
</tr>
<tr>
<td>Area</td>
<td>603.98</td>
<td>6049.1</td>
</tr>
<tr>
<td>Area (%)</td>
<td>7.847</td>
<td>92.153</td>
</tr>
<tr>
<td>Number of Theoretical Plates</td>
<td>4636</td>
<td>13305</td>
</tr>
<tr>
<td>Asymmetry</td>
<td>1.150</td>
<td>1.324</td>
</tr>
<tr>
<td>Capacity Factor ($R$)</td>
<td>3.003</td>
<td>1.874</td>
</tr>
<tr>
<td>Resolution</td>
<td></td>
<td>8.628</td>
</tr>
</tbody>
</table>

Figure No. 1 Overlay Spectra of AZD and TEL

Figure No. 2 Typical liquid chromatogram obtained for a 20 µL injection of a binary mixture of AZN and TEL
Figure No. 3 Typical liquid chromatogram obtained for a 20 µL injection of a binary Formulation of AZN and TEL

Figure No. 4 Calibration curve of Azelnidipine

\[ y = 73.104x + 6.641 \]
\[ R^2 = 0.9988 \]

Figure No. 5 Calibration curve of Telmisartan

\[ y = 148.98x + 12.87 \]
\[ R^2 = 0.9996 \]
7. References


