Development and Validation of RP-HPLC Method For Simultaneous Estimation of Indapamide and Telmisartan
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ABSTRACT
A simple, precise, accurate and reproducible Reverse Phase High Performance Liquid Chromatographic method was developed for simultaneous estimation of Telmisartan and Indapamide. The method was carried out on a Hypersil Gold column [250x4.6mm;5µ] with a mobile phase consisting of Acetonitrile:0.1M Potassium dihydrogen phosphate buffer [60:40v/v] at a flow rate 1.0ml/min. Detection was carried out at 237nm. The retention time of telmisartan and indapamide was 7.84, 3.38 min respectively. The developed method was validated for accuracy, precision, linearity, limit of detection, limit of quantification and solution stability as per ICH guidelines Q2 [R1]. The proposed method can be used for simultaneous estimation of these drugs in bulk drugs and formulations.

Keywords: Telmisartan, Indapamide, RP-HPLC, simultaneous estimation., validation.

INTRODUCTION
Telmisartan (TEL), chemically is 4-((2-n-propyl-4-methyl-6-(1-methylbenzimidazol-2-yl)-benzimidazol-1-yl) methyl) biphenyl-2-Carboxylic acid [Figure 1]. It blocks the vasoconstrictor and aldosterone secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscle and the adrenal gland (1) and is effective in lowering blood pressure in hypertensive patients. Indapamide (IND), chemically [3-(aminosulfonyl)-4-chloro-N-(2,3-dihydro-2-methyl-1H-indol-1-yl)-benzamide] [Figure 2] is a thiazide like diuretic. It produces antihypertensive effect by diuresis which causes decrease in plasma extracellular fluid volume, cardiac output and sodium concentration intracellularly in vascular smooth muscle wall and also dampens responsiveness to constrictor stimuli like those of angiotensin II or nor adrenaline. Combination of IND and TEL result in synergistic effect and thus superior blood pressure lowering (2). Many methods have been described in the literature for the determination of IND and TEL individually and in combination with other drugs (3)(4)(5)(6). However, there is no HPLC method reported for the simultaneous estimation of these drugs in combined dosage forms. The aim of this work was to develop Reverse Phase HPLC [RP-HPLC] method for the simultaneous estimation of telmisartan and indapamide in pharmaceutical dosage forms.

Figure 1: Structural formula for telmisartan
Figure 2 : Structural formula for indapamide

EXPERIMENTAL
MATERIALS
Pharmaceutical grade IND was supplied by Unichem Pharmaceuticals, Mumbai and TEL was supplied by Nicholas Piramal, Mumbai. All chemicals and reagents were of HPLC grade and were purchased from Rankem pharmaceuticals.

METHOD
Instrumentation
Analysis was performed on a Perkin Elmer HPLC equipped with Dual piston Pump PU-2080, UV 2075 UV/Visible Detector with manual sampler. Responses of peak area were recorded and integrated using Borwin Chromatographic Software. Stationary phase of the column used was Hypersil Gold column (250x4.6 LD:5µ) operated at room temperature. The λmax of the TEL and IND were obtained by using UV-Visible spectrometer [Jasco V-530].

Mobile phase optimization:
Various mobile phase compositions like methanol : water, methanol : water:acetonitrile, buffer: methanol in different ratios and different columns (e.g. C8, C18) were tried. The best optimized column was found to be C18 and mobile phase was found to be Acetonitrile : 0.1M potassium dihydrogen ortho phosphate buffer [60:40v/v]. The mobile phase was filtered through 0.22µm microfilter prior use and was degassed by sonication. The detector was set at 237 nm. The potassium dihydrogen ortho phosphate buffer solution was prepared by weighing accurately about 3.4gm of potassium dihydrogen orthophosphate in a 500 ml of volumetric flask, and dissolving it in 300 ml of HPLC water with the aid of sonication & making up the volume.

Preparation of Standard and sample Solution
Standard stock solutions (1 mg/ml) of TEL and IND were prepared separately in 0.1N hydrochloric acid. The working standard solution containing mixture of 20µg/ml TEL and 1.5µg/ml IND was prepared.

Assay Sample preparation
Accurately weighed powdered of in house tablet formulation equivalent to 1.5 mg IND and 20 mg TEL was transferred to 100ml volumetric flask, dissolved and volume was made up with 0.1N hydrochloric acid. This solution was ultrasonicated for 1hr and filtered through 0.45µ Whatman filter paper. The solution was suitably diluted and filtered through 0.22µ cellulose acetate membrane filter (Whatman, Japan) and injected into the HPLC system and chromatogram was recorded at 237nm.

Validation
Method validation for parameters like linearity, intra day and inter day precision, limit of detection, limit of quantification, accuracy, specificity was performed as per ICH Q2 (R1) guidelines (7).

Linearity was determined by building two calibration curves. For each calibration curve six standard solutions were prepared at concentrations ranging from 5-30...
The intraday precision and interday precision were expressed in terms of relative standard deviation (RSD). For intraday precision %RSD for TEL and IND was estimated to be 0.01, 0.01 µg/ml respectively.

Table 2: Result of Intra day and Inter-day precision using HPLC method for simultaneous estimation of telmisartan and indapamide (n=3)

<table>
<thead>
<tr>
<th>Analytical parameters</th>
<th>Telsmisartan</th>
<th>Indapamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>7.84±0.01</td>
<td>3.38±0.01</td>
</tr>
<tr>
<td>Range µg/ml</td>
<td>5-30</td>
<td>0.6-2.1</td>
</tr>
<tr>
<td>Regression coefficient ($r^2$)</td>
<td>0.9991</td>
<td>0.9994</td>
</tr>
<tr>
<td>Slope ± %RSD</td>
<td>162948.7±0.36</td>
<td>72115±0.32</td>
</tr>
<tr>
<td>95% confidence limits of slope</td>
<td>162327,163571</td>
<td>72002,72499</td>
</tr>
<tr>
<td>Intercept (± S.D.)</td>
<td>101.9±1.67</td>
<td>3680±0.924</td>
</tr>
<tr>
<td>95% confidence limits of intercept</td>
<td>1010,1010</td>
<td>36300,36303</td>
</tr>
</tbody>
</table>

a) RSD: Relative standard deviation

Table 3: Result of accuracy studies using HPLC method for simultaneous determination of telmisartan and indapamide (n=3)

<table>
<thead>
<tr>
<th>Amount taken (µg)</th>
<th>Amount detected (µg)</th>
<th>% Recovery</th>
<th>Mean % recovery</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telsmisartan</td>
<td>16</td>
<td>15.9</td>
<td>99.2</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>121</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100%</td>
<td>100</td>
</tr>
<tr>
<td>Level 1</td>
<td>20</td>
<td>20</td>
<td>100</td>
<td>1.5</td>
</tr>
<tr>
<td>Level 2</td>
<td>24</td>
<td>24.26</td>
<td>108.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Level 3</td>
<td>24</td>
<td>24.26</td>
<td>108.6</td>
<td>1.79</td>
</tr>
<tr>
<td>Indapamide</td>
<td>16</td>
<td>15.9</td>
<td>99.2</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>121</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>100%</td>
<td>100</td>
</tr>
<tr>
<td>Mean % recovery</td>
<td>99.93</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%RSD</td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Accuracy of the method was determined by performing the recovery experiment. Three replicate samples at each concentration level were prepared and % recovery of added standard was calculated.

Specificity was studied for interference peaks of excipients, at retention times of TEL and IND. Lack of interfering peaks of excipients in the tablet formulation at the retention times of two drugs was taken as the indication of specificity/selectivity of the method.

RESULTS AND DISCUSSION

The solutions of 10 µg/ml of IND and TEL were scanned in the UV range of 200-400 nm and their λmax were found to be 241 and 296 respectively. The concentration of IND in formulation was lower as compared to TEL. Both drugs showed reliable sensitivity at 237 nm, therefore, the wavelength used in the study was found to be suitable for assay of both compounds in combination. The mobile phase acetonitrile: 0.1 M dihydrogen potassium phosphate buffer (60:40 v/v) was selected as optimized mobile phase to achieve good resolution between two peaks. The retention time (Rt) of TEL and IND was 7.84, 3.38 min respectively [Figure. 5].

The linear regression analysis of calibration curves of TEL and IND in combination is tabulated in table 1. The calibration curves were found to be linear in the range of 5-30 µg/ml for telmisartan (R²=0.9991) & 0.6-2.1 µg/ml for indapamide (R²=0.9994). The % assay or average amount of TEL and IND was found to be 99.04% ± 0.02 and 99.87% ± 0.03 respectively. For TEL and IND limits of quantitation (LQO) were 0.03, 0.03 µg/ml and limits of detection (LOD) were estimated to be 0.01, 0.01 µg/ml respectively.

The intraday precision and interday precision were expressed in terms of relative standard deviation (RSD). For intraday precision %RSD for TEL and IND was found to be 1.2 and 1.5 respectively. The interday precision at three concentration levels (n=3) on three different days was also evident with a low % RSD providing ruggedness of the method [Table 2].

Accuracy was evaluated by performing recovery studies by the standard addition method. The recovery of the added standard was studied at three different levels viz 120%, 100% and 80% of the estimated amount of the drug. Each set of recovery of added standard was calculated.

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CONCLUSION

It can be concluded that HPLC method was successfully developed for simultaneous estimation of telmisartan and indapamide in the prepared tablet formulations.

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REFERENCES


7. ICH Q2 R1, Text on validation of analytical procedures, International Conference on Harmonization tripartite guidelines, adapted 27 June 1995.

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