A Simple Rp- HPLC Method for Simultaneous Estimation of Six Cardiovascular Drugs in Bulk and Dosage Form

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Abstract: A simple, convenient Rp-HPLC method has been developed and validated for the simultaneous estimation of Metolazone, Indapamide, Nebivolol, Rosuvastatin, Olmesartan and Spironolactone. The column used was an Inertsil ODS 3 V column of 250 mm length × 4.6 mm ID, with 3 micron particle size of adsorbent. Separation was achieved using isocratic elution in a buffer-acetonitrile-methanol mobile phase at a flow rate of 1.2 ml/min. The detection was performed at wavelength of 225 nm using a UV detector. The column temperature was 45°C and injection volume was 20µl. The method was validated for precision, linearity and accuracy. The % RSD for all the drugs was found to be less than 2%. The correlation coefficient (r2) was not less than 0.999 for all drugs. The mean percent recovery of the drugs from tablet placebo at 50%, 100% and 150% were within limits. The marketed formulations of the drugs were analyzed and the mean assay results were found to be within limits. The developed method can thus be employed for routine simultaneous analysis of Metolazone, Indapamide, Nebivolol, Rosuvastatin, Olmesartan and Spironolactone in bulk and in their marketed formulations.

Keywords: Rp-HPLC method, Indapamide, Metolazone, Spironolactone, Olmesartan, Nebivolol, Rosuvastatin.

I. Introduction

As per World Health Organization, Cardiovascular Diseases (CVDs) are the number one cause of death globally. Although many CVDs can be treated or prevented, an estimated 17.1 million people die of CVDs each year1. CVDs are caused by disorders of the heart and blood vessels, and include coronary artery disease (heart attacks), cerebrovascular disease (stroke), raised blood pressure (hypertension), peripheral artery disease, rheumatic heart disease and congenital heart disease. The major causes of CVDs are tobacco use, stress, inadequate or lack of sleep, physical inactivity, an unhealthy diet and harmful use of alcohol.

Congestive Cardiac Failure (CCF) is one of the complications of Coronary Artery Disease. It is a chronic and usually progressive illness which occurs when, cardiac output is insufficient to meet the demands of tissue perfusion, resulting in a clinical syndrome of decreased exercise tolerance with pulmonary and systemic venous congestion2. Combination of drugs are needed to control the risk factors associated with CCF and these include diuretics, angiotensin II receptor antagonists, β1 receptor blockers and statins.

Metolazone [3-7] is an oral diuretic agent commonly classified with the thiazide diuretics. It is useful to treat Congestive Heart Failure and Hypertension. Indapamide [3-7] is a non-thiazide, sulphonamide diuretic drug which reduces blood pressure at doses causing little diuresis. It is generally used in the treatment of hypertension, as well as decompensated cardiac failure. Nebivolol hydrochloride [5-8] is a β1-blocker (anti-hypertensive) which reduces peripheral vascular resistance and significantly increases stroke volume, with preservation of cardiac output. Olmesartan medoxomil [5-7, 9], a recent member of angiotensin receptor blocker (ARB) class of drugs, is indicated in the treatment of hypertension and prevention of diabetic nephropathy and congestive cardiac failure. Rosuvastatin [5, 7, 10] reduces levels of low-density lipoprotein, apolipoprotein B and triglycerides in the blood, while increasing levels of high-density lipoprotein in the management of hyperlipidaemia. Spironolactone [3-7, 11] is a potassium sparing diuretic agent.

Literature revealed that different analytical methods like UV spectroscopy, Rp-HPLC, High Performance Thin Layer Chromatography (HPTLC) [12-33] have been reported for the analysis of the above drugs individually and in combination with other drugs. However, there has been no study involving simultaneous estimation by HPLC of above six drugs. Hence, in the present study a simple Rp-HPLC method, for the simultaneous analysis of above mentioned cardiovascular drugs in bulk and tablet dosage form has been developed and validated.
II. Materials And Methods

2.1 Chemicals and reagents
All chemicals and reagents used were of analytical grade. Olmesartan medoxomil was obtained as a gift sample from Unichem laboratories Ltd., Pilerne, Goa; Rosuvastatin from VerGo Pharma Research Laboratories Pvt. Ltd., Verna, Goa; Nebivolol HCl from Glenmark Generics, Goa; Metolazone and Spironolactone from Centaur pharmaceuticals Pvt. Ltd., Tivim, Goa and Indapamide from Adcock Ingram Ltd., Bangalore. Tablet formulations were procured from the local market.

2.2 HPLC Instrument and Chromatographic conditions
The instrument used for analysis was of Agilent Technologies “1120 Compact LC” with UV detector and Inertsil ODS 3 V column of 250 mm length × 4.6 mm ID, with 3 micron particle size of stationary phase. The mobile phase used was buffer-methanol-acetonitrile in the proportion of 45:33:22, v/v/v. The column temperature was 45°C, the flow rate was 1.2 ml/min and injection volume was 20µL.

2.3 Preparation of standard and sample solutions
A mixed standard solution of the drugs was prepared by accurately weighing the quantity of drugs as in Table 1, into a 100 ml volumetric flask. About 75ml of diluent (ACN & MilliQ water, 1:1, v/v) was added and the solution was sonicated for 10 minutes. The volume was made to 100ml with diluent and mixed. The solution was centrifuged at 4000 rpm for 10 mins. A volume of 4 ml diluted to100ml using diluent was injected 5 times and peak areas were determined.

2.4 Method validation
The developed method was validated for linear range, accuracy, precision and specificity [33].

2.4.1 Linear range
The linearity of the method for each drug was studied by preparing 5 different concentrations of the drugs as in Table 2. The solutions were injected in the HPLC system and peak areas were recorded. Calibration curves were constructed by plotting peak areas versus concentration of each drug and the linear range was determined. The linear regression equation and correlation coefficient for each drug was determined.
Table 2 Concentration of drugs in working standard solutions

<table>
<thead>
<tr>
<th>Vol (ml) of std stock in 100ml</th>
<th>Metolazone in ppm</th>
<th>Indapamide HCl in ppm</th>
<th>Nebivolol in ppm</th>
<th>Olmesartan medoxomil in ppm</th>
<th>Rosuvastatin in ppm</th>
<th>Spironolactone in ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>6</td>
<td>12</td>
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<td>3</td>
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<td>6</td>
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<td>18</td>
<td>36</td>
<td>18</td>
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<td>18</td>
</tr>
</tbody>
</table>

2.4.2. Precision

Precision of the method for each drug was determined by performing repeatability studies by the successive analysis of six injections of above solution, corresponding to 100% of drug concentration. The percentage RSD was determined.

2.4.3. Accuracy

Accuracy of the developed HPLC method was determined by carrying out recovery studies for each drug at spike level 50% (L1), spike level 100% (L2) and spike level 150% (L3) concentration by replicate analysis (n=3). A volume of 2ml, 4ml and 6ml of standard drug solution (corresponding to 50%, 100% and 150% concentration of each drug) was added to 50 mg of placebo powder taken in 3 different 50ml volumetric flask. Around 35ml of diluent was added and solution was sonicated for 10 mins. The volume was made up with diluent and mixed. Aliquot of the solution was centrifuged at 4000 rpm for 10 mins. The clear centrifugate was diluted appropriately and injected. The percentage of total drug content recovered was calculated.

2.4.4. Specificity

The specificity of the method was determined by injecting the diluent and placebo solution in the chromatographic system and observing the chromatograms.

2.5 System suitability testing

System suitability of the system was determined by six replicate injections. The acceptance criteria adopted was less than 2 % RSD for peak areas, greater than 2000 (USP) theoretical plates and asymmetry factor between 0.85 and 2.0.

III. Results And Discussion

3.1 Method development

The solubility of the drugs was tested in acetonitrile (ACN), methanol, ACN & MilliQ water mixture (1:1, v/v), methanol & MilliQ water mixture (1:1, v/v), 0.1N HCl and phosphate buffer pH 6.8. Based upon the free solubility of the drugs, ACN & MilliQ water (1:1, v/v) mixture was selected as diluent for method development and validation. The drug concentrations were optimized so as to obtain absorbance values in the range of 0.3 to 0.9. UV Spectra of the drugs as studied from Fig. 2 revealed that a wavelength of 225 nm could be used as a common wavelength for analysis.

Chromatographic separation of the drugs was tried on YMC Pack Pro C18 RS column having a length of 250mm with 4.6 mm ID and particle size of stationary phase being 5 micron. The mobile phase used was buffer-methanol-acetonitrile in the proportion of 45:33:22, v/v/v. The column temperature was maintained at 25°C and flow rate of mobile phase chosen was 1 ml/min. However, results were not satisfactory as Nebivolol and Rosuvastatin were not resolved and Retention time (Rt) of Spironolactone was more than 20 mins. Several parameters were verified including, alteration of column temperature (30°C, 40°C, 45°C) and flow rate of mobile phase (1.2 ml/min), with no improvement in the resolution. Hence, change of column, so as to increase the surface area of adsorbent and increased carbon loading, was tried. An Inertsil ODS 3 V column of 250 mm length × 4.6 mm ID, with 3 micron particle size was used. Optimum separation of the drugs was finally achieved on the column as seen in Fig. 3, with column temperature of 45°C and 1.2 ml/min flow rate of mobile phase. The injection volume was 20µL. There were no interferences from the diluent and placebo, as seen in Fig. 4.

The calibration curve of the drugs as in Fig. 5 gave linear lines. The results of accuracy and precision studies as depicted in Table 3 proved that the results were satisfactory. The proposed Rp-HPLC method was applied to marketed formulations of the drugs. The results, as tabulated in Table 4, were within acceptable limits.

The results of system suitability testing as depicted in Table 5 proved that the parameters were within the acceptable limits.
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<table>
<thead>
<tr>
<th>Metolazone</th>
<th>Indapamide</th>
<th>Nebivolol</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="UV Spectra" /></td>
<td><img src="image2.png" alt="UV Spectra" /></td>
<td><img src="image3.png" alt="UV Spectra" /></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Olmesartan</th>
<th>Rosuvastatin</th>
<th>Spironolactone</th>
</tr>
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<tbody>
<tr>
<td><img src="image4.png" alt="UV Spectra" /></td>
<td><img src="image5.png" alt="UV Spectra" /></td>
<td><img src="image6.png" alt="UV Spectra" /></td>
</tr>
</tbody>
</table>

Fig. 2 UV Spectra for standard drugs

Fig. 3 Chromatogram showing separation of drugs

Fig. 4 Chromatogram for (a) diluent and (b) placebo
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Fig. 5 Calibration curves for drugs

Table 3 Results of Accuracy and Precision analysis

<table>
<thead>
<tr>
<th></th>
<th>Met</th>
<th>Ind</th>
<th>Neb</th>
<th>Ros</th>
<th>Olm</th>
<th>Spi</th>
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</thead>
<tbody>
<tr>
<td>% Recovery L1</td>
<td>100.95</td>
<td>100.97</td>
<td>100.75</td>
<td>100.71</td>
<td>99.83</td>
<td>100.47</td>
</tr>
<tr>
<td>% Recovery L2</td>
<td>100.45</td>
<td>100.11</td>
<td>100.57</td>
<td>100.39</td>
<td>99.70</td>
<td>100.46</td>
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<tr>
<td>Precision % RSD</td>
<td>0.07750</td>
<td>0.06111</td>
<td>0.18673</td>
<td>0.13247</td>
<td>0.20606</td>
<td>0.16094</td>
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</table>

Table 4 Results for assay of marketed formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Metolazone</th>
<th>Indapamide</th>
<th>Nebivolol</th>
<th>Rosuvastatin</th>
<th>Olmesartan</th>
<th>Spironolactone</th>
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<tr>
<td>Mfg. By</td>
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<td>Serdia</td>
<td>Otsira Genetica</td>
<td>USV</td>
<td>USV</td>
<td>RPG Life Sciences</td>
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<td></td>
<td>Metoz 2.5</td>
<td>Natriix</td>
<td>Nebi 5</td>
<td>Roseday</td>
<td>Olmetrack 20</td>
<td>Aldactone</td>
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<tr>
<td>% Purity</td>
<td>98.38</td>
<td>104.33</td>
<td>97.51</td>
<td>104.72</td>
<td>102.15</td>
<td>98.38</td>
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</table>

Table 5 Results for system suitability testing

<table>
<thead>
<tr>
<th>Drug</th>
<th>Metolazone</th>
<th>Indapamide</th>
<th>Nebivolol</th>
<th>Rosuvastatin</th>
<th>Olmesartan</th>
<th>Spironolactone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Area (%RSD)</td>
<td>0.3</td>
<td>0.269</td>
<td>0.5</td>
<td>0.293</td>
<td>0.170</td>
<td>0.225</td>
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<tr>
<td>Theoretical plates</td>
<td>8580</td>
<td>9291</td>
<td>5947</td>
<td>9466</td>
<td>10780</td>
<td>10690</td>
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<td>Asymmetry factor</td>
<td>1.112</td>
<td>1.076</td>
<td>1.064</td>
<td>1.040</td>
<td>1.046</td>
<td>0.955</td>
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</table>

IV. Conclusion

A simple, isocratic LC method has been developed, optimized and validated for the simultaneous estimation of Metolazone, Indapamide, Nebivolol, Rosuvastatin, Olmesartan and Spironolactone. The method is simple, rapid, accurate, precise and specific. It can be used for the routine analysis of the six cardiovascular drugs without the need for separation, in bulk and in their dosage form.

Acknowledgements

Authors are thankful to VerGo Pharma Research Laboratories Pvt. Ltd., Verna, Goa, India, for providing the facilities to conduct this research work and also to Unichem laboratories Ltd., Pilerne, Goa, Glenmark Generics, Goa, Centaur pharmaceuticals Pvt. Ltd., Tivim, Goa, and Adcock Ingram Ltd., Bangalore for gift samples of the drugs.

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