Method development and validation for acyclovir in tablet dosage form by RP-HPLC.

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ABSTRACT

Acyclovir is an anti viral drug. A simple, economic, accurate reverse phase isocratic RP-HPLC method was developed for the Acyclovir (200mg) in Tablet dosage form. A Novapak ODS C-18 (150 x 3.9 mm, packed with 4 micron) in an isocratic mode with mobile phase Water:Acetonitrile (78:22) was used with flow rate (0.8ml/min) and monitored at 254 nm. The retention times were 2.453 min for Acyclovir. The linearity range was found to be 1 - 60 µg/ml. The procedure was validated as per ICH rules for Accuracy, Precision, Linearity and Reproducibility. The method has been successfully used to analyze commercial solid dosage containing Acyclovir with good recoveries and proved to be robust.

KEYWORDS: Acyclovir, Tablets, HPLC, Peak area.

INTRODUCTION

Acyclovir is chemically known as 2-amino-9-((2-hydroxyethoxy)methyl)-1H-purin-6-ol. It is a sparingly soluble in water, freely soluble in DMSO, very slightly soluble in alcohol and dissolved in mineral acid. Acyclovir differs from previous nucleoside analogues in that it contains only a partial nucleoside structure: the sugar ring is replaced by an open-chain structure. Acyclovir can be considered as a prodrug, while its administration, it is in an inactive (or less active) form and is metabolized into a more active species after administration[1]. The scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte to be more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation. A survey of literature reveals that good analytical methods are not available for the drugs like Acyclovir. Even though very few methods of estimation of above drugs are available, many of them suffer from one disadvantage or the other, such as low sensitivity, lack of selectivity, simplicity etc. The existing physicochemical methods are inadequate to meet the requirements; hence it is proposed to improve the existing methods and to develop new methods for the assay of Acyclovir in pharmaceutical dosage forms adapting different available analytical techniques like UV spectrophotometry and HPLC. According to the literature survey it was found that few analytical methods such as HPLC and UV-Visible analysis were reported for the estimation of Acyclovir [2-5]. The objective of the proposed method was to develop simple and accurate methods for the determination of Acyclovir by RP-HPLC methods in pharmaceutical dosage forms.

EXPERIMENTAL

Pure Acyclovir was obtained from Biodeal Laboratories Pvt, Ltd. (Himachal Pradesh, India). A commercial sample Prednisone tablet containing Acyclovir (200mg) were procured from Biodeal Laboratories Pvt, Ltd., 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. Quantitative HPLC was performed on Shimadzu HPLC with LC 10AT VP series pumps besides SPD 10AVP UV-Visible detector. Shimadzu Class-VP version 6.14 SPI software was used along with Novapak ODS C-18 (150 x 3.9 mm, packed with 4 micron) column for the chromatographic separation. Automatic injections (20 lì) were used. The detector wavelength was set at 254nm which was ascertained by 10 µg/ml concentration of Acyclovir working standard was scanned between the wavelength ranges of 200 – 400 nm. To develop a suitable and robust HPLC method for the determination of Acyclovir, different mobile phases acetonitrile: water, were used in different compositions of mobile phases (30:70, 40:60, 50:50, 70:30, 80:20) at different flow rates (0.5,0.75,0.8,1.0, 1.2, 1.5, ml/min). The mobile phase Acetonitrile: Water in the ratio of 22:78 at a flow rate of 0.8 ml/min gave peaks good resolution for Acyclovir. The data were collected and analyzed with software in a computer system. Mobile phase used as diluents.

The described method has been validated for the assay of Acyclovir using following parameters [6-8]. Precision was studied to find out variations in the test methods of Acyclovir (10µg/ml) on the same day and on different day by using different make column of same dimensions (Ruggedness). The precision of each method was ascertained separately from the peak area obtained by actual determination of six replicates of a fixed amount of drug. Precision and Ruggedness were done on the same day and the different day respectively and the %RSD was: 

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RESULTS AND DISCUSSION:
A reverse-phase isocratic procedure was proposed as a suitable method for the analysis of Acyclovir in tablets. A mixture of Acetonitrile:Water in the ratio of 17:83 and 27:73 for Acyclovir was varied at 0.7 ml/min to 0.9 ml/min. The Organic composition in the Mobile phase ratio was varied at 0.7 ml/min to 0.9 ml/min. The Organic composition were made to evaluate the impact on the method. The flow rate affected the method significantly. The method was 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 was not robust even by change in the flow rate ±10% and change in the Mobile phase at 17.83 and 27.73 for Acyclovir.

Table 1: Data for Precision

<table>
<thead>
<tr>
<th>SI No.</th>
<th>Concentration (µg/ml)</th>
<th>Peak Area (µm²)</th>
<th>Statistical Analysis</th>
<th>% Recovery of Pure drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>933107</td>
<td>Mean = 933766</td>
<td>98.92 ± 0.0712</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>934258</td>
<td>S.D. = 0.3282</td>
<td>99.09 ± 0.1287</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>933658</td>
<td>% RSD = 0.325</td>
<td>99.27 ± 0.2534</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>934858</td>
<td>Mean = 99.16%</td>
<td>99.39 ± 0.2515</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>934852</td>
<td>S.D. = 0.3255</td>
<td>99.40 ± 0.2534</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>933268</td>
<td>% RSD = 0.3282</td>
<td>99.20 ± 0.127</td>
</tr>
</tbody>
</table>

Table 2: Data for accuracy

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Concentration (µg/ml)</th>
<th>% Recovery of Pure drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>80%</td>
<td>99.11</td>
</tr>
<tr>
<td>S2</td>
<td>80%</td>
<td>98.92</td>
</tr>
<tr>
<td>S3</td>
<td>80%</td>
<td>99.27</td>
</tr>
<tr>
<td>S4</td>
<td>100%</td>
<td>99.39</td>
</tr>
<tr>
<td>S5</td>
<td>120%</td>
<td>99.40</td>
</tr>
<tr>
<td>S6</td>
<td>120%</td>
<td>99.20</td>
</tr>
</tbody>
</table>

CONCLUSION
The presented method was precise, sensitive and accurate. The advantages of proposed method were 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 Acyclovir in pharmaceuticals.

REFERENCES