DEVELOPMENT OF Rp-HPLC METHOD FOR FEXOFENADINE DETERMINATION IN TABLET FORMULATIONS AND DEVELOPMENT OF DISSOLUTION METHOD

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ABSTRACT:
Fexofenadine hydrochloride is a piperidine derivative. It is indicated to relieve signs and symptoms that are related with seasonal allergic rhinitis, such as rhinorrhea, sneezing, nose, throat and itchy eyes. In the present research work a liquid chromatographic method was developed for the determination of Fexofenadine in tablets and the dissolution method by UV/VIS spectrophotometer was also developed. Method was developed by using Lichrospher 10µm (C18) column. The mobile phase is composed of acetonitrile-5mM ammonium acetate buffer (50:50, v/v) pumped at a flow rate of 1ml/min. The UV detector was operated at 254nm. The method was validated for system suitability, accuracy/recovery, linearity, system precision, method precision, ruggedness, robustness, limit of detection and limit of quantitation. Similarly, for dissolution method different concentrations of standard and sample solutions were prepared i.e. 65µg/ml, 35µg/ml, 17.5µg/ml, 8.75µg/ml and 4.375µg/ml.
The proposed HPLC method was easy, precise and fewer time consuming. The linearity for the dissolution method was found to be acceptable. The correlation coefficient of standard solutions was 0.9979 similarly the correlation coefficient of sample solutions was 0.9963.

Keywords: Fexofenadine, Liquid chromatographic method, Validation, Dissolution.

INTRODUCTION

Fexofenadine α, α-dimethyl-4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidiny] butyl] benzeneacetic acid, is the most important terfenadine metabolite, prevents allergic inflammation (Gelfand et al., 2002). It is nonsedating and does not decrease performance even in extremely high doses (Sussman et al., 1999). There are various reports based on the evaluation of Fexofenadine by HPLC method in different dosage forms (Karakus et al., 2008). Fexofenadine was detected in biological fluids using HPLC method (Hofmann et al., 2002) some spectrophotometric and capillary electrophoresis methods were also developed (Mahgoub et al., 2003; Mikus et al., 2005). Electrospray ionization tandem mass spectrometry methods/High-sensitive liquid chromatography was also used for the evaluation of Fexofenadine in plasma (Yamane et al., 2007).

Dissolution test is very significant for all solid oral dosage forms (Dressman and Reppas, 2000). It ensures that formulated drug product dissolves at a constant rate (Johnson 2007). In order to attain the physiological condition of the gastrointestinal tract the dissolution test conditions have to be cautiously considered (Jantratid and Vertzoni 2010). Various physico-chemical factors like solubility, particle size, ionic strength and

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buffer effects need to be evaluated at the preformulation stage that influences the dissolution test (Skoug et al., 1996).

At the start the selection of the apparatus is very critical, the volume recommended for the dissolution medium is 500-1000ml. For oral dosage forms, the standard temperature for the dissolution medium is 37 ± 0.5°C. Surfactants like (e.g., sodium lauryl sulfate, Tween 80 or CTAB) can be added in different concentrations to enhance the solubility of poorly soluble compounds (Chan, 2004).

Therefore in present study it was aimed to develop and validate HPLC method. Secondly, this study also shows that newly develop dissolution method is suitable to operate in different environment in order to evaluate various parameters.

MATERIAL AND METHODS

Development of assay method

Fexofenadine HCl (Gifted By Aventis Pharma), Natrium Acetate (Merck). HPLC-grade solvents were used for analysis, i.e. Methonal (BDH Hiper Solv for HPLC), Acetonitrile (Riedel-de-haen). Solvents were mixed then filtered through 0.45µm pore size - 47 mm diameter filters (Scheicher & Schuell, Dassel, Germany) by filtration assembly (Sartorius, Gottingen, Germany). All dilutions were performed in standard volumetric flasks (Pyrex England). Double distilled water was used during the whole procedure.

The HPLC system consisting of a pump (LC-5A, Shimadzu Corporation, Tokyo, Japan) with a spectrophotometric detector (SPD -2A, Shimadzu Corporation, Tokyo, Japan) put at a working wavelength of 254nm, a manual injector fitted with a 20µl loop. Data collection was done with software for data handling (LC-10A, Shimadzu Corporation, Tokyo, Japan). Validation procedures were achieved on a Lichrospher 10µm (C18) column. Mixture of 5mM of ammonium acetate buffer and acetonitrile (50:50, v/v) was used as a mobile phase, pumped at a flow rate of 1ml/min.

Preparations of solutions

Reference solution

Reference solution of 500µg/ml Fexofenadine was prepared in mobile phase and its peak was observed on HPLC at 254nm.

Sample preparation

Twenty tablets (prepared at home) were accurately weighed and crushed, 46.33mg was taken in order to make a solution in mobile phase of strength equivalent to 500µg/ml, solution was then sonicated for 15 min, filtered, injected and peak was observed at 254nm.

Table-1

<table>
<thead>
<tr>
<th>No of Injections</th>
<th>Sample Amount (µg/ml)</th>
<th>% Recovery</th>
<th>% Mean Recovery</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>250</td>
<td>100.95</td>
<td></td>
</tr>
<tr>
<td>2</td>
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<td>99.86</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>101.59</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>500</td>
<td>99.52</td>
<td>100.213</td>
</tr>
<tr>
<td>2</td>
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</tr>
<tr>
<td>MEAN</td>
<td></td>
<td></td>
<td>101.165</td>
</tr>
</tbody>
</table>
Validation Procedure

System suitability
System suitability test was carried out by injecting five consecutive injections of 500µg/ml of the standard solution.

Accuracy/Recovery
Method accuracy was evaluated by injecting three consecutive injections of solutions of 250µg/ml, 500µg/ml and 750µg/ml as given in table 1.

Linearity
Linearity was evaluated by the analysis of five diverse concentrations of 500µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml and 31.25µg/ml; three consecutive samples of each solution were injected as presented in fig. 1.

System precision and Method precision
System precision/Repeatability were studied by injecting five consecutive injections of the standard solution of 500µg/ml and method precision was determined by observing the reproducibility of the method i.e. by applying five injections of standard and sample solution of 500µg/ml.

Ruggedness and Robustness
Ruggedness was performed by other analyst by following the similar procedure described in the preparation of standard

Fig. 1. A chromatogram of 500µg/ml Fexofenadine standard solution.

Fig. 2: Presented the linearity for Fexofenadine response.
solution and in the preparation of test solution on another day by using another HPLC system, system consisting of a pump (LC-10A, Shimadzu Corporation, Tokyo, Japan) with a spectrophotometric detector (SPD -10A, Shimadzu Corporation, Tokyo, Japan), Shimpack CLC-ODS (C18) column, data collection was done with software (GC-10A, Shimadzu Corporation, Tokyo, Japan).

Robustness was tested after intentional alterations of mobile phase composition i.e. Acetonitrile and 5mM Ammonium acetate buffer (52:48) and flow rate i.e. 1.1 ml/min in order to examine the necessary changes of the performance of the chromatographic system, for that purpose three consecutive injections of Fexofenadine standard solution were applied.

Limit of detection and limit of quantitation:
Limit of detection and limit of quantitation were examined by injecting three consecutive injections of 15.625µg/ml, 7.8125µg/ml, 3.90625µg/ml, 1.95312µg/ml, and 0.97656 µg/ml solutions.

Development of dissolution method
Dissolution method was determined by using dissolution apparatus II (Erweka DT 700, Heuesnstand, Germany). Sodium
dodecyl sulphate (Merck, Darmstadt, Germany) was used for analysis. All dilutions were performed in standard volumetric flasks (Pyrex England). Double distilled water was used during the entire practice.

**Standard preparation**

Prepare 130µg/ml Fexofenadine standard solution from this serial dilutions of 65µg/ml, 35µg/ml, 17.5µg/ml, 8.75µg/ml and 4.375µg/ml were made their absorbance were determined spectrophotometrically (Heliosa UV-VIS spectrophotometer 150, England) at 220nm.

**Sample preparation**

Add 900ml of 1% sodium dodecyl sulphate solution in the flask of dissolution apparatus II (Erweka DT 700, Husenstamm, Germany) at 37°C, at a paddle speed of 100 r.p.m, Fexofenadine tablets were placed inside the vessel, filtered the sample at the end, then serial dilutions were prepared to make 65µg/ml, 35µg/ml, 17.5µg/ml, 8.75µg/ml and 4.375µg/ml solutions their absorbance were determined spectrophotometrically (Heliosa UV- VIS spectrophotometer 150, England) at 220nm.

**RESULTS AND DISCUSSION**

By means of well-documented experimental studies validation procedures are established. The results are important for determining the efficacy of pharmaceuticals (Ermer, 2005). In the present study a rugged method has been developed and validated by different tests from which information was obtained.

System suitability test was performed the % CV was 1.024%. A chromatogram obtained from standard solution (500µg/ml) is shown in fig. 1. In the present study mean percent recovery was in the range from 100.213 – 102.48% at all levels. The overall mean percent recovery was 101.165 ± 1.178 % with a % CV of 1.164 as given in table 1. Linearity is the capability of the analytical method to extract test outcome that is proportionally related to the analyte concentration in the sample (Lister, 2005). Fig. 2 present the correlation coefficient that was 0.9984, slope and intercept values were 300000 and – 927.16 respectively. Precision is the evaluation of the method to create results which are reproducible (Lister, 2005). System precision was conducted having % CV of 1.096. Similarly, method precision was performed its mean recovery is 99.508 ± 0.932 % with a % CV of 0.937. Ruggedness is the determination of the reproducibility of test results carried out by the examination of similar samples under variable environment (Chan, 2004). Ruggedness studies have the mean recovery of 98.66 ± 0.37 % with a % CV of 0.37. Robustness test was performed having % CV of 0.77. In the present research work, the LOD and LOQ were calculated and the response for limit of detection was found at the concentration of 0.97656 µg/ml and the response for limit of quantitation was found at the concentration of 3.90625 µg/ml.

In order to challenge the drug release performance of the dosage form, the dissolution test should be considered during the premature design phase and should afterwards be modified to get the excellent control tool (Storey, 1996). For the evaluation of the release of lasofoxifene tartrate low dose tablets a new HPLC method has been developed and validated (Space et al., 2007). Dissolution methods should be validated because it is essential for good manufacturing practices (Guo et al., 2000), in order to calculate the linearity various statistical methods should be used and the results should consist of correlation coefficient, slope of the regression line, y-intercept and plot of the data (Brown et al., 2005).

In the present work the linearity of the dissolution method was examined by analyzing standard solutions of Fexofenadine and sample solutions of Fexofenadine tablet. 65-4.375µg/ml standard solutions were prepared. The results showed that the absorbance was linear within the concentration range of the analysis. The correlation
Development of Rp-HPLC Method

The coefficient of standard solutions was 0.9979, its slope and intercept were 385.88 and –0.0695 respectively as shown in fig. 3. Similarly the sample solutions were prepared in the range of 65-4.375 µg/ml solution. The results showed that the absorbance was linear within the concentration range of the analysis. The correlation coefficient of sample solutions was 0.9963, its slope and intercepts were 337.92 and –0.0524 respectively as shown in fig. 4. The purpose of this study is to develop a fast and specific dissolution method by UV/VIS spectrophotometer for the evaluation of Fexofenadine in tablet.

CONCLUSION

For the investigation of drug compounds and detecting compounds in biological fluids, liquid chromatographic method is the most generally used method. Validation data confirmed that method is cost effective and robust. The data also showed that the dissolution methodology was simple for the Fexofenadine determination in tablet formulations.

REFERENCES


