ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF NATAMYCIN IN EYE DROP BY RP-HPLC

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ABSTRACT

A simple, rapid, sensitive, specific, accurate, HPLC method was developed and validated for the determination of natamycin in eye drop. Agilent Eclipse XBD C18, 250 x 4.6 mm, 5 μm column with a mobile phase consisting of phosphate buffer pH 5.5 and acetonitrile are taken in the ratio 70:30 % v/v with a flow rate of 1.0 mL/min was used. Detection was carried out at 304 nm using UV detector. Validation parameters were performed to demonstrate specificity, precision, linearity, accuracy, system suitability, LOD and LOQ. The method was linear over the concentration range of 10 – 150 μg/mL. The proposed method was found to be precise, accurate, specific and rapid for the determination of natamycin in quality control estimation.

Keywords: Natamycin, eye drop, RP-HPLC, estimation.

INTRODUCTION

Natamycin, is a product of naturally occurring fungi Streptomyces natalensis. Natamycin is an effective macroide polyene antifungal agent, active against both yeast and molds without interfering with bacterial fermentation processes. It usually occurs as a white, virtually tasteless and odorless crystalline powder with low water solubility. The drug can be estimated in rabbit tears by LC-MS/MS method for the analysis of natamycin in Wine3 also reported. Present work deals about the RP-HPLC estimation of natamycin in pure form and in eye drop formulation.

EXPERIMENTAL

Instrumentation
Waters 2695 HPLC system equipped with Agilent Eclipse XBD C18, 250 x 4.6 mm, 5 μm column, Rheodyne injector with 50 μL loop, 2996 PDA detector and Empower-2 software was used.

Chemicals and reagents
Potassium dihydrogen orthophosphate, sodium hydroxide were analytical grade. HPLC grade acetonitrile and water were from Merck, India. Pure drug of Natamycin was obtained from Cipla Ltd. (Mumbai, India) as gift sample. The formulation of natamycin received from local pharmacy.

Chromatographic conditions
Chromatographic separation was carried out on Agilent Eclipse XBD C18, 250 x 4.6 mm, 5 μm column. Mobile phase was a mixture of phosphate buffer pH 5.5 and acetonitrile are taken in the ratio 70:30 % v/v, flow rate was 1.0 mL/min., temperature 25°C. Injection volume was 50 μL and detector wavelength was at 304 nm.

Preparation standard solution
The stock solution (1 mg/ml) of natamycin was prepared in Acetoneitrile by dissolving accurately weighed 100 mg of natamycin in a 100 ml clean calibrated volumetric flask and protected from light.

<table>
<thead>
<tr>
<th>Drug</th>
<th>USP Tailing</th>
<th>USP Plate Count</th>
<th>Retention time (min), (n=6)</th>
<th>Peak area, (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natamycin 50 µg/mL</td>
<td>1.08</td>
<td>5696</td>
<td>Mean ± S.D 6.29±0.0863</td>
<td>%RSD 1.371</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean ± S.D 547268±3448</td>
<td>%RSD 0.6300</td>
</tr>
</tbody>
</table>

Linarity

Standard solution was diluted further using mobile phase to the various concentrations ranging from 10 to 150 μg/ml of natamycin. The various concentration solutions were injected into the HPLC system and chromatograms were recorded. A typical chromatogram of natamycin is given in figure 1. The linearity graph was plotted by comparing concentration of natamycin (µg/ml) and area of chromatographic peak.

RESULT AND DISCUSSION

The chromatographic parameters were fixed and HPLC system was studied for the suitability of drug analysis. The system suitability parameters were given in Table 1. The developed method was validated to make suitable it for drug analysis. Validation of the HPLC method was performed by linearity, precision, accuracy, specificity, LOD and LOQ.
Validation of HPLC method

Specificity

The components of the eye drop (benzalkonium chloride 0.02%, sodium hydroxide) did not show any interference at 304 nm and no detector signal was produced during the analysis.

Linearity

Calibration curve was prepared for natamycin in the concentration range of 10 µg/ml to 150 µg/ml. The regression analysis was performed, shows the equation: \( y = 10111x + 10611 \). Correlation coefficient was 0.999. This shows good linearity of the method (Table 2).

Limit of detection (LOD)

It is the lowest concentration of natamycin in a sample that can be detected. LOD value was calculated from the calibration curve using equation LOD = 3.3 SD / b. (Where, SD = Standard Deviation of intercepts of calibration curves and b = Slop of corresponding calibration curve). The LOD was found to be 0.2031 µg/ml.

Limit of quantitation (LOQ)

It is the lowest concentration of analyte in the sample that can be determined with the acceptable precision and accuracy under stated experimental condition. LOQ value was calculated from the calibration curve using equation LOQ = 10 SD / b (Where, SD = Standard Deviation of intercepts of calibration and b = Slop of corresponding calibration curve). The LOQ was found to be 0.6157 µg/ml.

Precision

Precision of the assay was determined by intra-day and intermediate assay of the developed method. Intra-day analysis refers to the use of the analytical procedure within a laboratory over a short period of time that was evaluated by assaying six sample solutions, at the final concentration corresponding to 50 µg/ml of natamycin during the same day. Intermediate assay was did by two different analyst in two different days (Table 3).

Accuracy

The accuracy of the developed method was carried out by adding the known amount of natamycin pure drug to the pre analyzed eye drop sample and subjected to the proposed method. Results of recovery study are shown in Table 3. The study was done at 50, 100 and 150 % of test concentration levels. All the results indicate that the method is highly accurate.

Analysis of eye drop

The developed method was used to determine the amount of natamycin available in eye drops. Six replicate determinations were carried out for sample solution in the concentration of 50 µg/ml and the results are summarized in Table 3.

CONCLUSION

The validation study shows that the developed method is accurate, rapid, precise, reproducible and inexpensive with acceptable correlation coefficient, RSD (%) and standard deviation which make it useful for determination of natamycin in its pharmaceutical preparation. The proposed method is simple and do not involve time-consuming sample preparation. So this RP-HPLC method can be used in the quality control estimation of the drug in its formulation.

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REFERENCES