Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 6, Suppl 1, 2013 ISSN - 0974-2441

Research Article

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

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Received:1 December 2012, Revised and Accepted:1 January 2013

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, 5µm column with a mobile phase consisting of phosphate buffer pH 5.5 and acetonotrile 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 macrolide polyene antifungal agent, active against both yeast and molds without interfering with bacterial fermentation processes. It usually occurs as a white, virtually tasteless and odourless crystalline powder with low water solubility. The drug can be estimated in rabbit tears by LC-MS/MS[1], in doogh by LC with uv detection[2]. LC-MS/MS method for the analysis of natamycin in Wine[3] 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, 5µm column, Rheodyne injector with 50 µL loop, 2996 PDA detector and Empower-2 software was used.

Chemicals and reagents

Potasium 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, 5µm column. Mobile phase was a mixture of phosphate buffer pH 5.5 and acetonotrile are taken in the ratio 70:30 % v/v, flow rate was 1.0 mL/min., temperature 25^{0}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 Acetonitrile by dissolving accurately weighed 100 mg of natamycin in a 100 ml clean calibrated volumetric flask and protected from light.

Linearity

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.

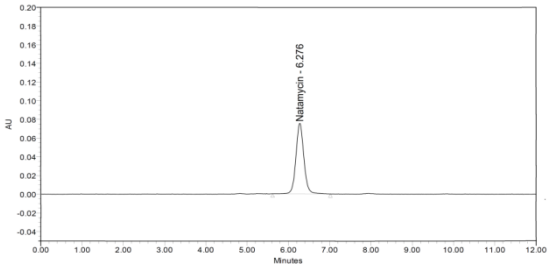

Figure 1:A typical chromatogram of Natamycin.

Preparation of samples for HPLC analysis

2 ml of the test sample was transfer to a 100 ml volumetric flask containing mobile phase and mixed to dissolve and make up to the volume with mobile phase and filtered through 0.22 µm filtration membrane. 1 ml of the above filtered solution was further diluted using the mobile phase to get the required concentration of natamycin. The sample solution was injected in to the HPLC system and chromatograms were compared with area produced by standard and estimation was carried out.

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.

Table 1:System suitability study

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

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).

Table 2:Linear regression data for calibration curve

Parameters	Values
Concentration range, µg/mL	10- 150
Slope	10111
Intercept	10611
Correlation coefficient	0.999

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.

Table 3:Assay results, precision and recovery studies

Formulation	Labeled amount (mg/5ml)	(%) label claim* ± S.D	% Recovery	Precision (% RSD)	
				Intermediate (n=24)	Repeatability (n=6)
Natamycin Eye drop	250	99.96 ± 0.1484	99.41 % to 100.46 %	0.4937	0.1485

*** Average of six determinations.**

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.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to Spectrum Pharma Research Solutions, Hyderabad and the management, SIMS College of pharmacy, Guntur, for providing the necessary facilities to carry out the research work.

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