International Journal of Pharmacy Teaching & Practices 2011, Vol.2, Issue 3, 117-119.

Development and Validation of a RP-HPLC Method for Estimation of Nitrendipine in Tablet Dosage Form

Sumeet Dwivedi[1]*and Debadash Panigrahi
Ujjain Institute of Pharmaceutical Sciences, Ujjain, M.P.-India

Research Article

Please cite this paper as: Sumeet Dwivedi[1]*and Debadash Panigrahi. Development and Validation of a RP-HPLC Method for Estimation of Nitrendipine in Tablet Dosage Form. IJPTP, 2011, 2(3),117 - 119.

Corresponding Author:

A/Prof. Sumeet Dwivedi
Ujjain Institute of Pharmaceutical Sciences, Ujjain, M.P.-India
Mob. +91-9893478497
E-mail: herbal0914@rediffmail.com, debmpharm@yahoo.co.in

Abstract

A simple, selective, rapid, precise, sensitive and accurate HPLC method has been developed for the estimation of nitrendipine in tablet dosage form. The method was carried out on a Phenomenox C-18 column (250 mm x 4.6 mm, 5 µm) using acetonitrile, methanol and water (40:30:30 v/v) as mobile phase(pH was adjusted to 3.0 with orthophosphoric acid) at a flow rate of 1ml/min . Detection was performed by UV detector at 232 nm. Total run time was 10 minutes and the drug was eluted at a retention time of 2.619min. The developed method was validated for linearity, precision, accuracy, specificity, sensitivity, limit of detection and limit of quantitation in accordance with ICH. The linearity range was 5 to 30 µg/ml. The recovery was within the range of 99.14-101.21 and the coefficient of variance was < 2%. The inter day and intraday precision were 0.746 and 0.981 respectively. The high percentage recovery and low coefficient of variation confirm the suitability of the method for estimation of nitrendipine in tablet dosage form.

Key words: Nitrendipine, RP-HPLC, Methanol, Acetonitrile, Water.

Introduction

Chemically Nitrendipine is 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5- pyridine di carboxylic acid ethyl methyl ester. It is pharmacologically a calcium ion influx inhibitor (slow-channel blocker or calcium ion antagonist) which selectively inhibits the transmembrane influx of calcium ions into cardiac muscle and vascular smooth muscle. The contractile processes of the muscles depend on the movement of extracellular calcium ions into these cells through specific ion channels. Nitrendipine is used in the treatment of high blood pressure[1].

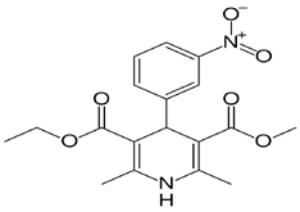

Fig. 1: Chemical Structure of Nitrendipine

Literature survey reveals that chromatographic estimation methods are reported for determination of nitrendipine along with other combinations. HPLC methods have been reported for analysis of nitrendipine in biological material[2–5] and in pharmaceutical formulation by HPTLC[6]. Method reported for separation of nitrendipine from its photodegradable metabolite by gas chromatography[7]. Simultaneous HPLC separation of nitrendipine from its impurities during synthesis was also reported[8]. As no chromatographic method has not yet been reported for quantitative estimation of nitridipine in pharmaceutical formulation hence it is essential to develop a a specific, accurate, precise, and fast HPLC method for determination of nitrendipine in commercial one-component preparations.

Methodology

Reference standard of Nitrendipine was obtained as gift sample by Concept Pharmaceuticals Pvt. Ltd. Aurangabad. Commercial Preparation of Nitrendipine (Cardiff 10) was also gifted from Concept Pharmaceuticals Pvt. Ltd. Aurangabad. HPLC grade acetonitrile, methanol was procured from Rankem (Mumbai, India). othophosphoric acid AR grade was procured from Qualigens fine chemicals, Mumbai. Double distilled water, prepared in the laboratory was used throughout the experiment. Mobile phase was filtered using 0.45µ nylon filters made by millipore (USA).

Apparatus and chromatographic conditions

Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-10AT-vp solvent delivery system (pump), UV detector, Rheodyne 7725i injector with 20 µl loop volume. A Phenomenox C-18 column (250 mm x 4.6 mm, 5 µm). was used for the separation. The mobile phase was composed of acetonitrile, methanol and water

(40:30:30 v/v) and the pH was adjusted to 3.0 with orthophosphoric acid. It was filtered through a 0.45 µm membrane filter and degassed by sonicating for 10 mins. Detection was carried out at 232 nm.

Preparation of standard solution

A stock solution of Nitrendipine (1mg/ml) was prepared by dissolving 100 mg of working standard of Nitrendipine in 100 ml of mobile phase. Working dilutions were prepared from the above stock solution for further study of linearity.

Preparation of sample solution

Twenty tablets were accurately weighed and their average weight was calculated. The tablets were powdered using mortar pestle to homogenized powder. A quantity of above powder equivalent to 10 mg of Nitrendipine was weighed and transferred into a 100 ml volumetric flask and adjust the volume upto 100 ml by mobile phase and sonicate for 30 mins. The excipeints were separated by filteration through a 0.45µm membrane filter. Further dilution was prepared by diluting 10 ml of filtered solution and make up the volume up to 50ml with mobile phase.

Before injection, both standard and sample solution was filtered through 0.45µm membrane filter. Inject separately 20 µl of the standard and sample solutions in triplicates and their peak area were measured. Chromatogram was obtained for Nitrendipine having a retention time of 2.619 as shown in Fig. 2.

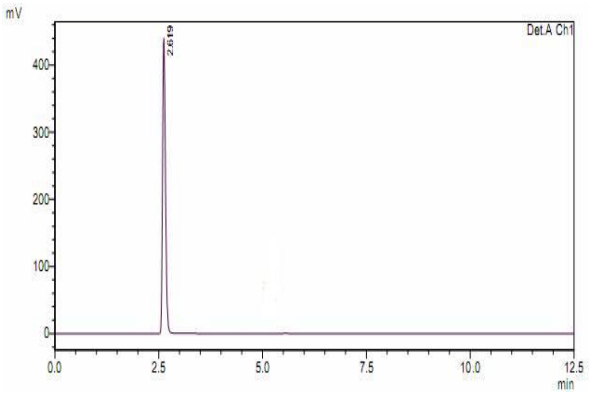

Fig. 2 Chromatogram obtained for Nitrendipine

Method of Validation

The present RP-HPLC method was validated following the ICH guidelines[9] for linearity, precision, accuracy, specificity, sensitivity, limit of detection and limit of quantitation.

Specificity and Selectivity

The interference from endogenous compound was investigated by the analysis of three injection of the system suitability solutions.

Accuracy and Precision

Accuracy of the developed method was studied by performing recovery study at three different levels of 80,100 and 120% of the amount expected from the analysis of the formulation. Precision assessed by performing Intra and Inter day study on the same day and on three different days over a period of one week respectively.**Discussion and Conclusion:**

In the evaluation, the students were overall satisfied with Fall 2009 physical pharmacy Lab organization and quality. Some of the written comments submitted by the students on the evaluation included: "Although it was time consuming I felt the labs were beneficial for pharmacy

Results and Discussion

Linearity

Linearity was assessed by preparing different dilutions of concentrations 5,10,15,20,25,30 µg/ml from the stand stock solution. The above prepared dilutions were injected into the system in hexaplicate manner and their peak areas were note down the data for linearity study was given in Table.1. Calibration graph was plotted between peak area and concentration. From the graph it was found that the drug has a linearity range of 5-30 µg/ml. The graph was found to be linear with a correlation coefficient of 0.999 and the representative linearity regression equation being Y = 20278x + 1292.

Table 1. Linearity study report of Nitridepine

Replicate	Concentration µg/ml	Mean Peak area	RSD%
1	5	51589	0.321
2	10	112911	0.309
3	15	163479	0.089
4	20	221936	0.068
5	25	283089	0.088
6	30	333988	0.029
Slope	20278		
Y-intercept	1292		
Coorelation coefficient	0.999		

Table2. Recovery study result

S/No.	Amount added	% Recovery	% RSD
1.	8	99.14	0.3480
2.	10	100.25	0.4700
3.	12	101.21	0.5750

Precision

The precision was assessed by performing inter day and intraday changes in peak area of drug solution. The intra-day and inter-day variation was calculated in terms of percentage relative standard deviation and the result was found to be 0.981 and 0.746 for intraday and intra-day respectively.

Table 3: System Suitability Data

Parameters	Obtained Value
Tailing Factor	1.207
Retention Time	2.619
No. of Theoretical Plates	5448.462

Accuracy

Accuracy was performed by the method of standard addition at three different levels, by multiple level recovery studies. Three levels of solution were made which correspond to 80, 100 and 120% the amount expected from the analysis of the formulation. Each level was made in triplicate. These solutions were then analyzed for recovery studies. The percentage of recovery was found to be within the range of 99.14-101.21, % of recovery and % RSD was listed in Table.2, which indicates that the proposed method is accurate.

Limit of Detection and Quantification

Limit of Detection (LOD) and Limit of Quantification (LOQ) were estimated from signal to noise ratio. LOD and LOQ were found to be 0.384 µg/ml and 1.146µg/ml respectively.

Specificity

Specificity was tested against standard compounds and against potential interferences in the presence of placebo. The comparison of the chromatograms of the synthetic placebo mixture and the spiked drug solution revealed that there was no interference of placebo with the peaks of Nitrendipine in sample solution. No interference from placebo was observed at the retention time of the drugs. Therefore, it was concluded that the method is specific.

System suitability

System suitability tests are an integral part of chromatographic method. They were used to verify that the reproducibility of the chromatographic system is adequate for the analysis. The application of the method was checked by analyzing the Nitrendipine in commercial tablets. The results are given in Table. 3.

Conclusion

The developed RP-HPLC method for the estimation of Nitrendipine in pharmaceutical dosage forms, using the UV detector is simple, precise, specific and highly accurate and less time consumption for analysis. So, it can be employed for the routine analysis and quality control analysis of Nitrendipine in tablet formulation.

Acknowledgments

The authors are grateful to Concept Pharmaceuticals Pvt. Ltd. Aurangabad for providing gift sample of raw drug and formulation.

References

1. Goodman and Gillman (1996). The Pharmacological Basis of Therapeutics. NewYork: MacMillan, 767–774.
2. D. Marszałek, A. Goldnik, R. Dabrowski , and M. Gajewska (1999). Chem. Anal. 44 705–709.
3. E. Georgaraki and F. Zougrou , J. Liq. Chromatogr. Related Technol. 22 (1999) 1381–1390.
4. X. Chen, D. Schong, H. Yang, Y. Yuan , and H. Xu , Biomed. Chromatogr. 15 (2001) 518–524.
5. W.E. Lambert, E. Meyer , and A.P. De Leenheer , J. Anal. Toxicol. 19 (1995) 73–78.
6. Y. Li, Q. Zheng, Z. Huang , and Y.Z. Yiyuan , Zhongguo Yiyuan Yaoxue Zazhi 20 (2000) 602–603; Chem. Abstr. 134 (2001) 9454.
7. G. Ragno and M. Veronico , Int. J. Pharm. 99 (1993) 351–355.
8. V. Marinkovic, D. Agbaba, S. Vladimirov , and S. Stankovic , J. Pharm. Biomed. Anal. 24 (2001) 993–998.
9. ICH, Validation of Analytical Procedure: Methodology (Q2B), International Conference on Harmonization, IFPMA, Geneva 1996.

AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed

CONFLICTS OF INTEREST

The authors declare that they have no competing interests