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Development and validation of RP-HPLC method for estimation of process related impurity in nimodipine bulk and formulation

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ABSTRACT

A Simple, linear, precise, accurate, robust and selective RP-HPLC method has been developed for the estimation of Nimodipine impurity in bulk and formulation. The methanol: acetonitrile: water in proportion of (35v:40v:25v) as mobile phase was used. At the flow rate of 0.8ml/min. The HPLC system consisting of LC20AD Prominence Liquid Chromatography SPD 20-A Shimadzu Japan. The UV-VIS detector and C18 column with dimension on 250×4.6 mm was used at wavelength 234 nm. Finally Nimodipine impurity was quantified from bulk Nimodipine and its tablet formulation. It was revealed that amount of impurity present in tablet was found to be 0.0876% and in the bulk 0.0219% respectively. Thus, Nimodipine impurity was found to be within the limit which was given in ICH guidelines. (Not more than 0.1%)

Keywords: Nimodipine, Impurity, HPLC, Validation.

INTRODUCTION

There is an increasing interest in impurities present in API's. Recently, purity profile and impurity profile has become essential as per various regulatory requirements. In the pharmaceutical world, an impurity is considered as any other organic material, besides the drug substance or ingredients; arise out of synthesis or an unwanted chemical that remains with API's. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products.[1,2] The development of HPLC method is a complex procedure that requires simultaneous determination of several factors such as, organic phase, column, temperature, flow rate, type of stationary phase etc.[3] According to ICH guidelines on impurities in new drug products, identification of impurities below the 0.1% level is not considered to be necessary, unless potential impurities are expected to be unusually potent or toxic. According to ICH, the maximum daily dose qualification threshold is considered as follows; ≤2g/day 0.1% or 1mg per day intake (whichever is lower) ≥ 2g/day 0.05% [4]

MATERIALS AND METHODS

Chemicals- Synthesized Nimodipine Impurity, Nimodipine drug, Methanol (HPLC grade), Acetonitrile (HPLC grade), Water (HPLC grade).

HPLC (High-Performance Liquid Chromatography) [5-10]
HPLC method was carried out for 1, 4- Dihydropyridine from bulk and formulation. The LC20AD Prominence Liquid Chromatograph SPD20-A Shimadzu, Japan with UV-Vis detector and C18 column with dimension on

250×4.6 mm was used for the method development with flow rate 0.8 ml/min at wavelength 234 nm. The methanol: acetonitrile: water in proportion of (35v:40v:25v) as a mobile phase, for development of chromatogram. The method was validated for Nimodipine impurity and various parameters according to ICH guidelines (Q2B) were studied.

Stationary Phase
C18 column with dimension on 250×4.6 mm are used.

Preparation of Mobile Phase
The selection of mobile phase was according to polarity and non-polarity of solvents. The methanol: acetonitrile: water was selected as mobile phase in ratio of 35:40:25 and was filtered on membrane filter (0.45µ) to remove degassing and were stirred for 10-15 min.

Preparation of Stock Solution (Standard)
The stock solution of 100µg/ml was prepared by dissolving 10 mg Nimodipine Impurity in 100 ml mobile phase. The dilution was prepared in various concentrations using stock solution and was dissolved in mobile phase.

Preparation of Sample Solution (Formulation)
The sample solution of Nimodipine formulation was prepared as 100µg/ml stock solution for quantification of Nimodipine Impurity in Nimodipine formulation. The dilution was prepared in various concentrations using sample stock and was dissolved in mobile phase for quantification of impurity in Nimodipine formulation.

System Suitability Parameters
The area of respective concentrations, theoretical plates, number of theoretical plates per cm, Tailing factor and the peak symmetry was recorded.

Linearity
Dilution of standard impurity in the range of 2-12µg/ml were prepared by taking suitable aliquots of working standard solution in different 10ml volumetric flasks and diluting up to the mark with mobile phase. 20µl was injected from it each time on column at flow rate of 0.8ml/min. The standard from elute was monitored at 234 nm and corresponding chromatogram were obtained from these chromatograms peak area were calculated. A plot of peak area over concentration was constructed. Regression of the plot was computed by least square regression method.

Precision
Precision of analytical method was studied by multiple injections of homogeneous samples. 6 replicate of 4 ppm solution were prepared and injected for precision at the same flow rate of 0.8ml/min. The inter-day and intermediate precisions were used to study the variability of the method S.D. and %R.S.D. was calculated for both.

Robustness
Robustness was studied by changing parameters like change in flow rate. The S.D. and %R.S.D. between the change parameter were calculated.

Ruggedness
Ruggedness studied was carried out by using different analysts. The S.D. and %R.S.D. were calculated.

Recovery
Recovery of the method was studied using the method of standard addition. Standard impurity solutions were added to the unknown bulk and tablet formulation of Nimodipine. The percent recovery was determined at three different levels (50%, 100%, and 150%). Impurity content was determined and the percent recovery was calculated.

LOD and LOQ
Limit of detection and limit of Quantitation of the method was calculated by formula given below,
LOD= 3.3×S.D/Slope
LOQ=10×S.D./Slope

Quantitation of Impurity
The total amount of impurity present in Nimodipine bulk and formulations was calculated for synthesized compound and the result was compared to ICH limit for impurities in new drug substance is 0.1%.

RESULTS AND DISCUSSION

The synthesis of diethyl 2, 6-dimethyl-4-(2-nitrophenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate one of the process related impurity in Nimodipine bulk and formulation. The RP-HPLC method was developed for detection and determination of process related impurity in Nimodipine bulk and formulation. The optimized chromatographic conditions are stated in, Table 1.

Table No.1 Optimized Chromatographic Condition for RP- HPLC

Chromatographic Conditions	SHIMADZU HPLC System
Mobile phase	Methanol: Acetonitrile: Water (35:40:25)
Column	ARP-C18 (250mm×4.6 mm), 5µ column
Flow rate	0.8 ml/min
Wavelength detection	234nm
Injection volume	20µl
Temperature	Ambient
Retention time	8.5
Run time	15Min

Table No.2 Summary of Method Validation Parameters of HPLC

Sr. No.	Parameter	Observation
1.	Linearity range	2-12µg/ml
2.	Slope	14.97
3.	Intercept	48.96
4.	Correlation coefficient	0.980
5.	LOD	0.2177µg/ml
6.	LOQ	0.6597µg/ml

Table No.3 Summary of Repeatability Studies

Sr. No.	Parameter	SD	%RSD
1.	Precision	0.987	0.824
2.	Intraday precision	0.901	0.755
3.	Interday precision	1.279	1.046
4.	Robustness	1.970	0.467
5.	Ruggedness	0.831	0.690

The summary of the repeatability is given in the above table and %RSD was found to be ≤2

Table No.4 Result of Recovery Study by HPLC

Sr. No.	Drug/ Formulation	Percentage Recovery			Mean	SD	%RSD
		50%	100%	150%			
1.	Bulk	97	98.62	99.2	98.27	1.140	1.160
2.	Tablet	97.83	98.87	99.5	98.73	0.843	0.854

Table No.5 System Suitability Parameter

Sr. No.	Property	Values	Official limits
1.	Retention time (t_R)	8.530	-
2.	Theoretical plate (N)	4614	N≥ 2000
3.	Resolution (R)	4.310	R≥ 2
4.	Tailing factor (T)	0.96	T≤ 2

Table No.6 Result of Quantitation of Process Related Impurity of Nimodipine Bulk and Formulation.

Sr. No.	Bulk/ Formulation	Quantitation of Impurity
1.	Bulk Nimodipine	0.0219%
2.	Nimodipine Tablet	0.0876%

CONCLUSION

The proposed method was simple, rapid and selective. The percentage relative standard deviation was found to be below 2.0% which indicate method was highly precise and accurate. The retention time of Nimodipine impurity by HPLC method was found to be 8.530 the amount of impurity present in bulk was found to be 0.0219% and in tablet was found to be 0.0876% the above method was used for routine analysis.

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