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RP-HPLC Method Development and Validation for Nimorazole

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ABSTRACT

A new reverse phase high performance liquid chromatography (RP-HPLC) method for the quantitative determination of Nimorazole was developed and validated as per ICH guidelines. The analyte was injected into an HIBER C18 column (150 mm × 4.6 mm, 5µm), maintained at ambient temperature and effluent was monitored at 297 nm. The mobile phase consisting of acetonitrile: methanol: buffer (2:3:5 v/v/v). The pH of the mobile phase was adjusted to 4.0 by using *O*-phosphoric acid. The flow rate was maintained at 1.0 mL/min. and retention time was observed 1.76 min. The developed method shows high specificity for Nimorazole. Calibration curve was plotted with a range from 1-5µg/ml (r^2>0.999). The lower limit of quantification (LLOQ) was found to be 0.5µg/ml. The method was validated for parameters like accuracy, precision, recovery, linearity, robustness. This RP-HPLC method is suitable for determining the concentration of Nimorazole and it was applied to routine analysis for determination of the Nimorazole from its formulation during pharmacokinetic study.

Keywords: Nimorazole, RP-HPLC, Validation.

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INTRODUCTION

Nimorazole is a 5-nitroimidazole, which is closely related to Metronidazole in structure and activity. Nimorazole is used as a hypoxic sensitizer concomitantly with radiotherapy for head and neck cancers and could from the similarities with Metronidazole theoretically lead to increased effect of anticoagulant therapy. Nimorazole chemically known as 4-[2-(5-nitro-1*H*-imidazole-1-yl)ethyl] morpholine.[1]

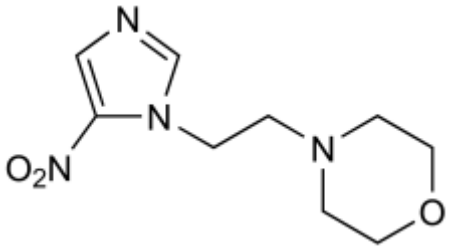

Figure 1: Chemical structure of Nimorazole

Literature survey state that validated RP-HPLC method for the quantification of nimorazole is not reported. The attempt has been made to develop a simple, cost effective, accurate and precise RP-HPLC method for analysis of Nimorazole. The RP-HPLC method developed in this study was validated as per ICH guideline for the parameters like specificity, linearity, range, accuracy and precision.

MATERIALS AND METHODS

Reagents and Materials

Dihydrogen phosphate (AR Grade) was purchased from SISCO Research Lab. Mumbai. Methanol (HPLC Grade) and Acetonitrile (HPLC Grade) were procured from Thomas Baker Chemicals, Mumbai. Nimorazole was obtained as a gift sample from Lupin Ltd, Aurangabad, Maharashtra, India.

Instrument

The HPLC system used was JASCO-2000 series equipped with UV detector. The chromatogram was recorded at 297nm and peaks were quantified by using software.

Preparation of Standard Stock Solutions

Stock solution of Nimorazole having 1mg/ml concentration was prepared. From the prepared stock solution, further dilutions were done by using mobile phase. Resulting solutions were injected into HPLC system with the help of Hamilton syringe.

Chromatographic conditions

Chromatographic separation was performed at ambient temperature on a reverse phase HIBER

(250 x 4.6mm, 5µ) column. Mobile phase was made up of acetonitrile: methanol: buffer in a ratio of 2:3:5(v/v/v). The mobile phase was filtered, degassed before use and the flow rate was maintained at 1ml/min. The 20µl of sample were injected and monitored at 297nm on UV-detector.

Method validation

The validation of the method was carried out as per ICH Guidelines[4,5]. The validation parameters like specificity, linearity, precision, accuracy were validated.

RESULTS AND DISCUSSION

Method development

Separately 20µL of sample of Nimorazole API were injected into the HPLC system and the chromatograms were recorded. The mobile phase was Acetonitrile: Methanol: Buffer in the ratio 2:3:5(v/v/v), pH-4 was adjusted by using *O*-phosphoric acid. The column used was HIBER C18 (150 mm X 4.6 mm i.d. 5µ). The flow rate was maintained at 1mL/min during run and detection was carried out at 297 nm.

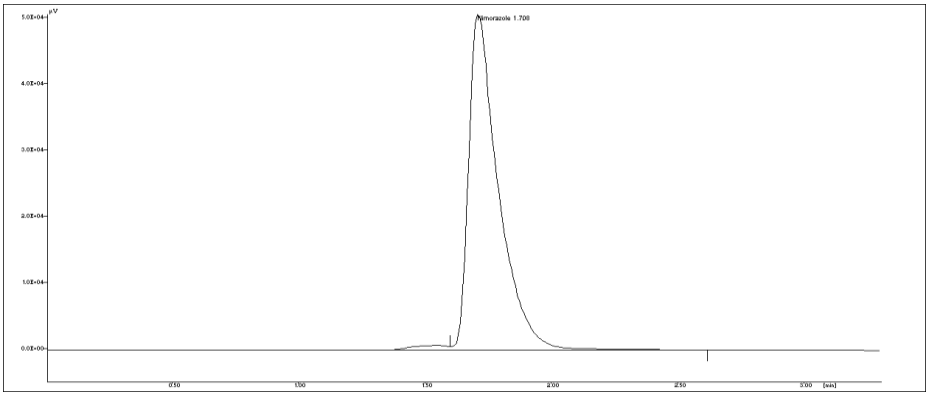

Figure: 2 Typical Chromatogram of Nimorazole

Specificity

The method was found to be precise and specific to the analyte. There is no interference found in the retention of drug.

Linearity

Linearity of the method was determined by mean of calibration graph using an increasing amount of each analyte (Table.1). Linearity was evaluated by visual inspection of a calibration plot (Figure. 3). At least three concentration levels were tested in agreement to ICH. The slope, intercept was reported for developed method. LOD and LOQ were estimated from the standard deviation of the response and the slope of the calibration curve. The standard deviation can be determined either from the standard deviation of multiple blank samples or from the standard

deviation of the intercepts of the regression lines done in the range of the detection limit.

Accuracy

The accuracy of the method was studied by recovery studies. The recovery was determined at three levels, viz. 80%, 100%, and 120% of the selected concentrations. Three samples were prepared for each recovery level (Table.2).

Table 1: Data of calibration curve

Concentration	Peak area	R^2 Value
1 µg/ml	69385.03	
2 µg/ml	137093.5	
3 µg/ml	199274.5	**0.999**
4 µg/ml	269970.3	
5 µg/ml	336246	

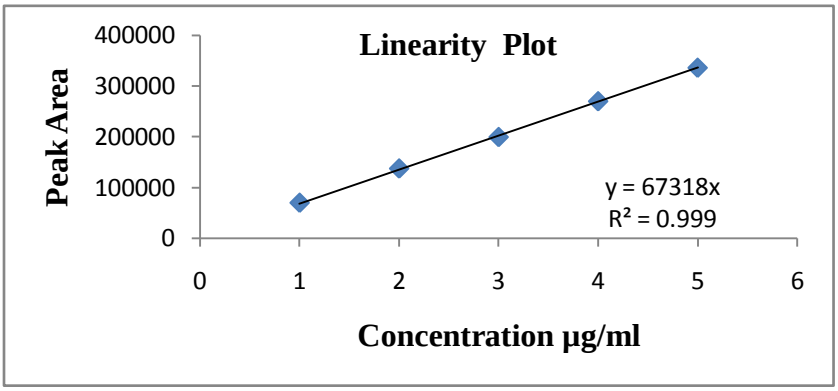

Figure 3: Linearity Plot of Nimorazole

Table 2: Data for accuracy studies

Accuracy level	Area of sample	% Recovery	% Average recovery	SD	% RSD
80%	880438	99.11	99.10	2.51	0.1
	880443	99.11			
	880441	99.10			
100%	1116750	99.45	99.40	18.56	1.7
	1115046	100.29			
	1083781	98.97			
120%	1326182	98.49	98.92	99.15	0.7
	1310825	99.42			
	1309065	98.86			

Precision

Precision was investigated at three levels, intra-day, inter-day, and reproducibility. The intra- and inter- day variability were assessed by using standard drug solution at three different concentration. Intra-day precision was carried out by analyzing the drug solutions within same

day. The inter-day precision was measured using standard solution over three consecutive days (Table.3).

Table 3: Data for precision study

A) Interday precision

Conc. Of Drug (µg/ml)	Nimorazole	
	R_t	Peak area
3	1.76	380500.75
	1.75	373868.11
	1.76	388115.99
SD	**0.006**	**7129.58**
% RSD	**0.32**	**1.87**

B) Intraday precision

Conc. of Drug (µg/ml)	Nimorazole	
	R_t	Peak area
3	1.75	401512.57
	1.76	395121.12
	1.76	402153.80
SD	**0.006**	**3888.46**
% RSD	**0.32**	**0.97**

CONCLUSION

A simple RP-HPLC method was developed and validated to quantify Nimorazole. The validated method covers the wide range of linearity over 1-5 µg/ml. The mobile phase used is acetonitrile: methanol: buffer (2:3:5 v/v/v). The % mean recovery was found to be in the range of 98.92% to 99.40%. The developed method was simple, selective, precise and accurate. The developed method can be applied to monitor plasma concentrations of Nimorazole in pharmacokinetic studies. It can also be used for therapeutic drug monitoring in order to optimize drug dosage on an individual basis.

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