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Determination of Nimorazole in Pharmaceutical Dosage Form by HPLC

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ABSTRACT

RP-HPLC method has been developed and validated for estimation of Nimorazole from pharmaceutical dosage form. A chromatographic separation was accomplished on ODS C18 column (100 mm X 4.6mm, 5 µm)with mobile phase consisting of acetonitrile: water (70: 30% v/v)and ultraviolet detection at 297 nm are used for the determination. Under these conditions, the studied Nimorazole elute at 1.60 ± 0.02 min. at a 1 mL/min flow rate. Linearity of Nimorazole was found in the concentration range of 5-30 µg/mL. The LOD and LOQ were found to be 0.2 µg/mL and 0.6µg /mL, respectively. The mean percent recovery of Nimorazole was found to be 99.78 %. All validation parameters were within the acceptable range. Hence, the proposed method can be useful for routine analysis of Nimorazole in pharmaceutical dosage form.

Key words: Nimorazole, HPLC, Validation.

INTRODUCTION

Nimorazole (NIM) is a 5-nitroimidazole derivative. It has antimicrobial actions and uses similar to those of metronidazole. 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-1H-imidazole-1-yl)ethyl] morpholine [1-3]. The chemical structure of Nimorazole shown in Figure 1.

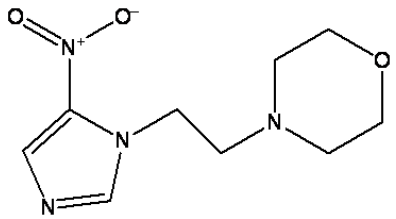

Figure 1: Chemical structure of Nimorazole

Literature survey revealed that there are several methods available to determine Nimorazole either alone or in combination with other drugs in pharmaceutical formulations and biological fluids using various analytical techniques such as spectrophotometric techniques [4-5], several methods based on separation techniques, such as HPLC [6-8] have been also reported. The attempt has been made to develop a simple, economic, accurate and

precise HPLC method for analysis of Nimorazole. The developed method is simple, precise, selective, and rapid and can be used for routine analysis.

MATERIALS AND METHODS

Chemicals and reagents

Nimorazole was kindly supplied by Lupin's Pharmaceuticals Inc. Aurangabad, India. Nimorazol tablet containing 500 mg Nimorazole was procured from local market within their shelf life. All the chemicals used were of HPLC grade and were purchased from Merck Chemicals, Mumbai, India

Instrumentation

The HPLC system consisted of Intelligent HPLC pump model (Jasco PU 2080 Plus) with sampler programmed at 20 µL capacity per injection was used. The detector consisted of a UV/ VIS (Jasco UV 2075 Plus). Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. ODS C18 (100 mmX 4.6 mm id, 5 µm particle size) column was used as the stationary phase. **Preparation of standard and sample solutions**

A standard mixed stock solution of NIM was prepared by accurately weighing NIM (5 mg) into a 10 mL volumetric flask. The drugs were dissolved in methanol and the solution was diluted to volume. The stock solution was further diluted with mobile phase to obtain a solution of NIM (5 µg/mL), respectively.

Twenty tablets of the pharmaceutical formulation Nimorazol (containing 500 mg Nimorazole) were assayed. They were crushed to a fine powder and an amount of the powder corresponding to approximately 500 mg Nimorazole was weighed in a 100 mL volumetric flask. The powder obtained was dissolved in methanol. After that, an adequate volume of aliquot was taken and diluted with mobile phase and sonication for 30 min. and filtered through 0.45 µm nylon membrane filter (Pall India Pvt. Ltd). Finally, an aliquot of the clean solution was injected into the chromatograph.

System suitability

From the filtered sample solution 5 µg/mL for NIM was injected into the chromatograph. The analysis was repeated six times to test the system suitability for their retention time, theoretical plates number (N) and tailing factors (T).

Method Validation

The developed HPLC method was validated as per International Conference on Harmonization (ICH) [9] guidelines for the parameters like specificity, linearity and range, LOD and LOQ, precision (intraday and interday precision), accuracy and robustness.

RESULTS AND DISCUSION

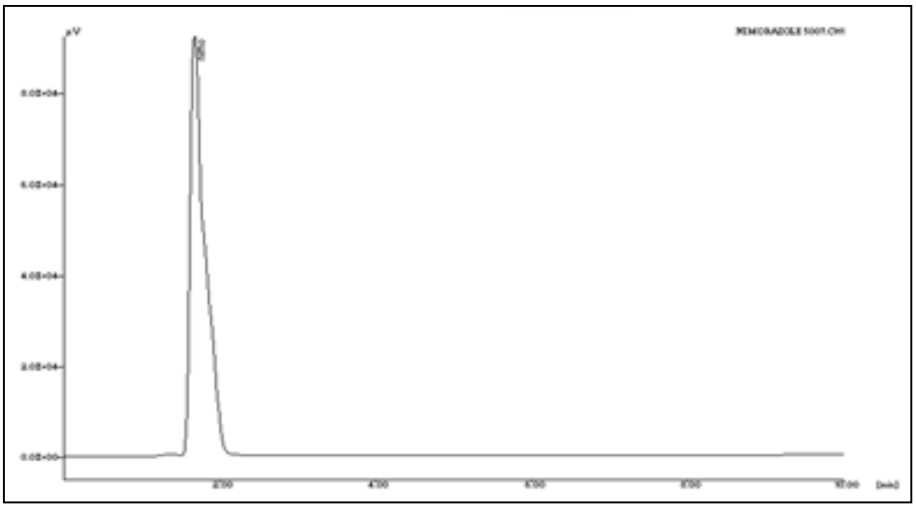

Figure 2: Chromatogram of NIM

Method development

The HPLC method was optimized for determination of NIM. Good resolution for NIM was obtained with acetonitrile: water (70: 30% v/v). The flow rate of 1 mL/min was optimum. UV detection was made at 297 nm. The average retention time for NIM was found to be 1.60 min. (Figure 2).

System suitability

To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solution. The parameters obtained are shown in Table 1.

Table 1: System suitability parameters (n=6)

Parameters	NIM
Retention Time in min	1.60
Theoretical plates number (N)	3865.75
Tailing Factor	1.21

Specificity

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

Linearity

Linearity of the method was evaluated by mean of calibration graph using an increasing amount of each concentration. The points distributed equally above and below the trend line showed linearity. The linearity was established over the concentration range of 5-30 µg/mL for NIM. The linear regression equations was found to beY = 45964X + 16892 (r^2 = 0.9995) for NIM. The plots obtained from linear regression given in Figures 3.

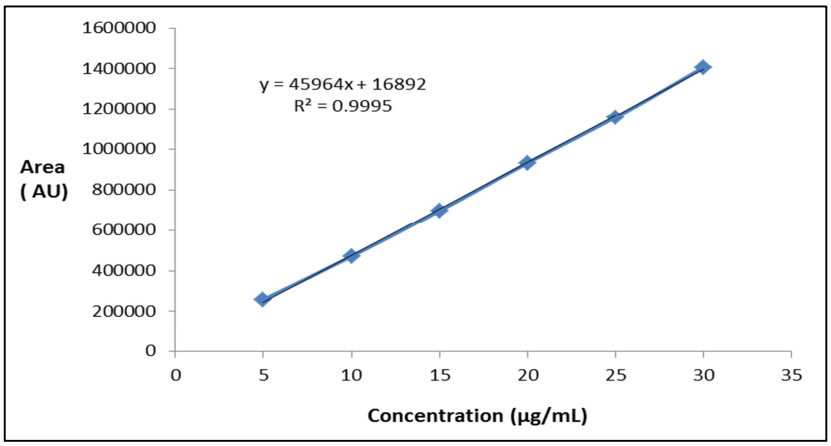

Figure 3: Calibration curve for NIM

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

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 from the standard deviation of the intercepts of the regression lines done in the range of the detection limit. The limits of detection and quantitation were found to be 0.2 µg/mL and 0.6 µg/mL for NIM, respectively. This indicates the method is sufficiently sensitive.

Precision

Precision was carried out to establish the intraday and interday precision of proposed method. 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 over a period of a week. The precision of the method was expressed as relative standard deviation (RSD, %). Results calculated as % RSD values for intraday and interday precision studies are shown in Table 2 and found to be satisfactory.

Table 2: Precision studies

Conc. (µg/mL)	Intra-day precision (n=3)			Inter-day precision (n=3)		
	Measured Conc.	(%) RSD	Recovery (%)	Measured Conc.	(%) RSD	Recovery (%)
Nimorazole						
10	9.901	1.07	99.01	9.887	1.04	98.87
20	19.89	1.15	99.45	19.82	1.11	99.10
30	29.78	1.10	99.27	29.72	1.21	99.07

Accuracy

The accuracy of the method was studied by recovery studies. Accuracy was determined at three levels 80%, 100% and 120% of the target concentration in triplicate and the percentage recovery for the amount added was calculated. The results are presented in Table 3.

Table 3: Recovery studies

Label claim (mg/tablet)	Amount Added (%)	Total amount (mg)	Amount recovered (mg)	(%) Recovery	Mean (%) Recovery (± SD)
NIM 500	80	900	898.50	99.83	99.78 ± 0.047
	100	1000	997.60	99.76	
	120	1100	1097.14	99.74	

Robustness

Robustness of the method was determined by small deliberate changes were made in the method parameters such as wavelength (±2nm), flow rate(±0.1ml), mobile phase ratio (±2%) and pH (± 0.05).But these changes, not affected the method results indicated that the method was robust. There were no significant changes in the retention times of NIM, when the various parameters were changed. The low values of the % RSD indicate the robustness of the method, as shown in Table 4.

Table 4: Robustness evaluation

Conditions	NIM	
	Rt (min.)	% RSD
A: Flow rate mL/min.		
0.9	1.62	1.05
1.0	1.60	1.10
1.1	1.58	1.14
B: % composition of the mobile phase (± 1mL)		
Acetonitrile: water (69: 31 % v/v)	1.63	1.07
Acetonitrile: water (70: 30 % v/v)	1.60	1.04
Acetonitrile: water (71: 29 % v/v)	1.59	1.10

Analysis of marketed formulation:

The chromatogram of the sample extracted from conventional tablets showed peak of NIM (Rt 1.60 min.) well resolved from other tablet excipients. The percent content of NIM per tablet by proposed method was found to be 99.87 %.

CONCLUSION

An accurate, sensitive and precise high performance liquid chromatographic method has been developed and fully validated for quality control analysis of Nimorazole in bulk and Pharmaceutical dosage form. The developed method was found to be simple and have certain advantages associated with this method such as high selectivity and sensitivity. Moreover, the lower solvent consumption along with the short analytical run time leads to aneconomic and eco-friendly chromatographic procedure. Hence the present RP-HPLC method can be used in the pharmaceutical industry for the routine analysis of Nimorazole in pharmaceutical dosage form.

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