Research Article

Development and Validation of UV Spectrophotometric Area Under Curve Method for Estimation of Nimorazole in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, precise and economical UV - spectrophotometric method has been developed for the estimation of Nimorazole in pharmaceutical dosage form. Method applied was area under curve (AUC) in which area was integrated in the wavelength range of 292 nm to 302 nm. Calibration curves were plotted for the method by using instrumental response at selected wavelengths and concentrations of analyte in the solution. Linearity for the detector response was observed in the concentration range of 10-60 μg/mL for the method. Tablet formulation was analyzed and the percentage of drug determined in the assays was 99.75 %. The method was validated by the analytical performance parameters suggested by the International Conference on Harmonization. Accuracy and precision studies were carried out and results were satisfactory. Limit of detection and limit of quantitation were determined for the method. All validation parameters were within the acceptable range. The developed method was successfully applied to estimate the amount of Nimorazole in pharmaceutical formulation.

Keywords: Nimorazole, Estimation, UV spectrophotometry, Area under curve, 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.
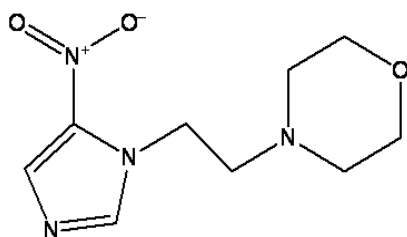

Figure 1: Chemical structure of Nimorazole

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. So far, no area under curve spectrophotometric method has been reported for the estimation of Nimorazole from pharmaceutical dosage form. This paper deals with validation and development of a method by area under curve for the assay of Nimorazole from its bulk drug and in pharmaceutical dosage forms. Thus the objective of present study was to develop a method that can be applied for the routine analysis of Nimorazole in tablet formulation.

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. AR grade methanol used throughout the analysis and were purchased from Merck Chemicals, Mumbai, India

Instrumentation

A JASCO V-530 UV/VIS double beam spectrophotometer was used for measurements of the absorbance spectra with 1cm matched quartz cells was used for all spectral measurements.

Preparation of standard solutions

A standard mixed stock solution of Nimorazole was prepared by accurately weighing Nimorazole 10 mg into a 100 mL volumetric flask. The drug was dissolved in methanol and the solution was diluted to volume. The stock solution was further diluted with methanol to obtain a solution of Nimorazole 10 μg/mL.

Preparation of sample solutions

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 methanol and sonication for 30 min. and filtered through 0.45 µm nylon membrane filter (Pall India Pvt. Ltd). This solution was further diluted to obtain final concentration of 10 µg/mL for Nimorazole. The responses were measured and concentration in the sample was determined by comparing the response of sample with that of the standard.

Area under curve

This method involves calculation of integrated value of absorbance with respect to wavelength in indicated range. Area calculation processing item calculates the area bounded by the curve and horizontal axis. Here horizontal[5] axis represents baseline.

$$\text{Area calculation } (\alpha+\beta) = \int_{\lambda_2}^{\lambda_1} A\, d\,\lambda$$

Whereas, α is area of portion bounded by curve data and a straight line connecting the start and end point, β is area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ1 and λ2 are wavelengths representing start and end point of curve region. In this study area was integrated between wavelength ranges from 292 to 302 nm.

RESULTS AND DISCUSSION

By appropriate dilution of standard stock solution of Nimorazole samples were prepared separately. These solutions were then scanned in the spectrum mode from 400 nm to 200 nm. The area under the curve spectra for Nimorazole was recorded at the wavelength range of 292-302 nm (Fig. 2).
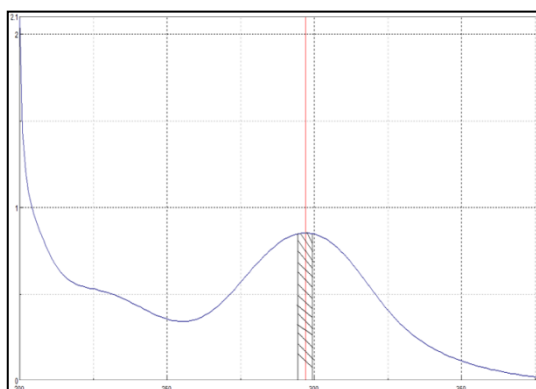

Figure 2: Area under curve spectrum of Nimorazole in methanol

Validation of the Proposed Method

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

Linearity

The calibration curves were plotted over a concentration range of 10-60 µg/mL for Nimorazole. Accurately measured standard solutions of Nimorazole (1, 2, 3, 4, 5, 6 mL) were transferred to a series of 10 mL of volumetric flasks and diluted to the mark with methanol. The areas of the solutions were measured at 292-302 nm against methanol as blank. The calibration curves were constructed by plotting areas versus concentrations and the regression equations were calculated. The linear regression equations were $Y = 0.022X - 0.031$ ($r^2 = 0.999$) for Nimorazole. The calibration curves are given in Fig. 3 for Nimorazole.
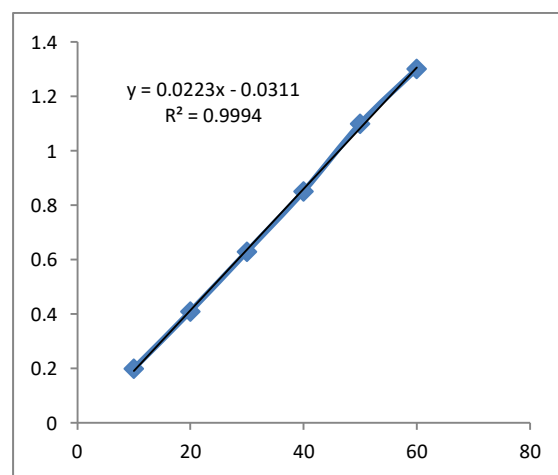

Figure 3: Calibration curve for Nimorazole Limit of detection and limit of quantitation

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve

The results of the LOD and LOQ were found to be 2 µg/mL and 6 µg/mL for Nimorazole.

Precision

The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of 20, 40, 60 µg/mL for Nimorazole. The result was reported in terms of relative standard deviation (% RSD). The values less than 2.0 indicate that there were no significant variations in the analysis of Nimorazole at the given concentration levels (Table 1).

Table 1: Precision studies

Conc. (µg/mL)	Intra-day precision (n=3)		Inter-day precision (n=3)	
	Measured Conc. ± SD	(%) RSD	Measured Conc. ± SD	(%) RSD
Nimorazole				
20	19.8 ± 0.208	1.05	19.7 ± 0.219	1.11
40	39.7 ± 0.444	1.12	39.6 ± 0.416	1.05
60	59.5 ± 0.601	1.01	59.3 ± 0.670	1.13

Instrumental Precision

The precision of the instrument was checked by repeated scanning and measurement of area of solutions six times without changing the parameter of the proposed spectrophotometric method. The % RSD for measurement of peak area for the drug was calculated to determine the instrumental precision. The % RSD for 20 µg/mL of Nimorazole was found to be 1.11.

Accuracy

The accuracy of the method was carried out by adding known amounts of drug corresponding to three concentration levels; (80, 100, and 120% of the label claim) along with the excipients. The mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate. The percentage recoveries of Nimorazole at each level and each replicate were determined. The mean percentage recovery and the relative standard deviation were calculated (Table 2).

Table 2: Recovery studies

Label claim (mg/tablet)	Amount Added (%)	Total amount (mg)	Amount recovered (mg)	(%) Recovery	Mean (%) Recovery (± SD)
Nimorazole 500	80	900	895.10	99.46	99.41 ± 0.235
	100	1000	996.12	99.61	
	120	1100	1090.60	99.15	

Robustness

For the evaluation the robustness of the developed method, small deliberate variation in the optimized method parameter was done (Change in Wavelength ±1 nm.). The effect of change in Wavelength was studied. In term of % RSD which was less than 2% for Nimorazole.

Analysis of marketed formulation

The present method is applied to the estimation of drug in the commercially available tablet dosage form. There was no interference from the excipients commonly present in the conventional formulation. The % drug content found for Nimorazole was 99.72 %.

CONCLUSION

The proposed area under curve method was found to be simple, sensitive, accurate and precise for estimation of Nimorazole. This method utilizes commonly and easily available and economic solvent, so this method is cost effective method for estimation of Nimorazole. The method was validated as per ICH guidelines in terms of linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ) and robustness. The common excipients and other additives are usually present in the tablet do not interfere in the analysis of Nimorazole. Hence the proposed method can be used for routine analysis and quality control assay of Nimorazole in tablet dosage form.

REFERENCES

1. Timothy A R, Overgaard J, Overgaard M. A phase I clinical study of Nimorazole as a hypoxic radiosensitizer. Int J Radiat Oncol Biol Phys, 10 (9), 1984, 765-8.

2. Overgaard J, Overgaard M, Timothy A R. Studies of the pharmacokinetic properties of Nimorazole. British Journal of Cancer, 48 (1), 1983, 27-34.

3. Bjarnason N H, Chritiansen M, Specht L. The nimorazole regimen in patients with head and neck cancer can increase the effect of vitamin K antagonists. Acta Oncologica, 47(1), 2008, 150-151.

4. Sreevatsav A S K, Mamatha N, Fiaz M D, Karthikayan W A, Latha G, Method development and validation of nimorazole tablet dosage form by UV spectrophotometry, World Journal of Pharmacy and Pharmaceutical Sciences, 3(8), 2014, 440-445.

5. Giriraj P, Sivakkumar T, Spectrophotometric simultaneous estimation of ofloxacin and nimorazole in pure and pharmaceutical dosage form by vierordt's method. Int. J. ChemTech *Res*. 6 (7), 2014, 3799-3806.

6. Umamaheswari D, Jayakar B, Analytical method development and validation for the simultaneous estimation of nimorazole and ofloxacin in pure and its pharmaceutical dosage form by RP-HPLC, Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry. 2(4), 2014, 268- 275.

7. Kashid A M, Dawra N S, Dhange A A, Mulani A I, Ghorpade D A, Dhawale S C. RP-HPLC Method Development and Validation for Nimorazole, American Journal of pharmtech research, 2(6), 2012, 818-823.

8. Giriraj P, Sivakkumar T A rapid - chemo metrics assisted RP-HPLC method with PDA detection for the simultaneous estimation of ofloxacin and nimorazole in pure and pharmaceutical formulation, European Journal of Pharmaceutical and Medical Research, 1 (1), 2014, 58-74.

9. ICH Harmonized-Tripartite Guidelines. Validation of Analytical Procedure: Text and Methodology Q2 (R1), November, 2005.

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