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Research Article

RP-HPLC Method for the Estimation of Nitaoxanide in Pharmaceutical Formulation

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ABSTRACT

Objective: A simple and precise RP-HPLC method was developed and validated for the determination of Nitaoxanide in pharmaceutical dosage forms.

Materials and Methods: Chromatography was carried out using waters RP $-C_{18}$ 150×4.6 mm, 3.5 µ, pH 6.8, buffer: acetonitrile (50:50) as the mobile phase at a flow rate 1.2 ml/min. The analyze was monitored using PDA detector at 254 nm. The proposed method was found to have linearity in the concentration range of 25-150µg/ml with correlation co efficient of r^2 =0.9999.

Results: The developed method has been statistically validated and found simple and accurate. The mean recoveries obtained for Nitaoxanide were in the range 100.06-101.9%.

Conclusion: Due to its simplicity, rapidness, high precision and accuracy of the proposed method it may be used for determining Nitaoxanide in bulk and dosage forms.

Keywords: Nitaoxanide, RP-HPLC, ICH guidelines, high precision

INTRODUCTION

Nitaoxanide is a synthetic nitro thiazolyl-salicylamide derivative approved for the treatment of infectious diarrhea [1] caused by Cryptosporidium parvum and Giardia lamblia. This novel agent has a broad spectrum of activity against many other gastrointestinal pathogens, including bacteria, round worms, flat worms and flukes. Nitaoxanide is used in many areas of the world, especially in Central and South America, as broad-spectrum parasiticidal agents in adults and children. In oral administration it is rapidly hydrolyzed to its active metabolite, Nitaoxanide, which is observed 1-4 hours after administration. It is excreted in the urine, bile and faeces. Chemically known as 2-[(5-nitro-1, 3-thiazol-2-yl) carbamoyl] phenyl acetate. A number of methods such as spectrophotometric [2-8], colorimetric [9,10] HPLC [11-13], HPTLC [14], RP HPLC [15-18] for the estimation of Nitaoxanide. The present communication describes 3 UV spectroscopic methods in bulk form and dosage form by using different reagent 4-hydroxy benzaldyhyde and phluroglucinol having maximum absorbance at 460 and 450 nm.

EXPERIMENTAL

Instruments

High performance liquid chromatography (Shimadzu HPLC, Model: SPD M20 A) prominence with high pressure gradient system with photo diode array detector was used.

Chemicals and reagents

Nitaoxanide was obtained as a gift sample from Lupin pharmaceuticals Pondicherry (sample), Water (HPLC grade), acetonitrile (HPLC grade) and methanol (HPLC grade) were used.

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Chromatographic conditions

A chromatographic system (Shimadzu, Japan) consisting of a solvent delivery pump, a degasser, an injector, an RP column, UV detector. An ODS (octadecylsilane) packed C_{18} column was used for separation. The instrumental settings were at the flow rate of 1.2ml/mt. The injection volume was 20µl. The peak purity was checked with the UV detector (SPD 20A). Detection was performed at 254nm. Software used is Spin chrome.

Selection of wavelength

From the UV spectrum of the compound, the λ_{max} of Nitaoxanide was found to be 254nm and that wavelength is suitable for detection. So the appreciable absorbance was found at 254nm Table no 1.

TABLE NO 1

	PARAMETERS	VALUES
1	Wavelength	254nm
2	Flow rate	1.2ml/mt
3	Column	C_{18}
4	Injection volume	20 µl

Preparation of Mobile phase

The mobile phase consisted of di potassium hydrogen phosphate buffer and acetonitrile in the ratio (70:30). The mobile phase was premixed and filtered through a nylon filter and degassed.

Preparation of buffer

Di potassium hydrogen phosphate was prepared as per IP and P^H was adjusted to 6.8.

Preparation of Standard solution [19, 20]

Standard solution was prepared by dissolving 100mg of Nitaoxanide in methanol and it was made up to 100ml with methanol (1000µg/ml).

Preparation of test solution

Tablet powder equivalent to100mg of pure Nitaoxanide was accurately weighed and transferred into a volumetric flask, dissolved in small volumes of diluents and volume was made up with methanol.

Method development

A rapid HPLC method was developed and validated for the estimation of Nitaoxanide. A C_{18} column with mobile phase containing mixture of buffer and acetonitrile was used. Mobile phase was pumped at the flow rate of 1.2ml/mt and the eluents were monitored at 254nm with 20µl loop injector. The selected chromatographic condition was found to effectively separate Nitaoxanide. The method validated in the terms of no: of theoretical plates, tailing factor, linearity, correlation coefficient, limit of detection (LOD) limit of quantization (LOQ) for Nitaoxanide.

Limit of Quantization (LOQ)

It is a characteristic of quantitative assays for low level of compounds in sample matrices, such as impurities in bulk drug and degradation products in finished pharmaceuticals. It is the lowest level of analyze in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. The quantization limits is expressed as concentration of analyze (e.g.: percentage, parts per billion) in sample.

Determination: For instrumental and non-instrumental methods, LOQ is determined by analysis of sample with known concentration of analyze and by establishing the minimum level at which the analyze can be determined with acceptable accuracy and precision.

$$LOQ = \frac{3.3 \times \text{Standard deviation}}{\text{Slope}}$$

Limit of Detection (LOD)

It is a characteristic of limit tests. It is the lowest amount of the analyze in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. The

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detection limit is expressed as concentration of analyze (e.g.: percentage, parts per billion).
Determination: It is determined by assaying a sufficient no: of aliquots of homogenous sample to be able to calculate statistically valid estimates of standard deviation or % RSD.

$$LOD = 10 \times \frac{\text{Standard deviation}}{\text{Slope}}$$

Linearity
It is the ability to elicit test results that are directly, or by a well defined mathematical transformation, proportional to concentration of analyze in samples which in a given range. If linearity is not attainable, a non-linear model may be used, however, the goal is to have a model, whether linear/non-linear that describes closely the concentration-response relationship.

System suitability
Tests are based on the concept that the equipment, electronics, analytical operation and samples constitute an integral system that can be evaluated as such.

Preparation of Calibration curve
For the preparation of calibration curve, aliquots of 1ml, 2ml, 3ml, 4ml, 5ml were pippeted out and the volume was made up to 100ml with methanol to produce concentrations in the range of 10-50µg/ml. Each solution was injected and a chromatogram was recorded. The peaks were recorded. Calibration curve was constructed by plotting concentration vs. peak area and was recorded in table no: 2 .

TABLE NO 2

Concentration	Peak area
10	1231.309
20	2456.657
30	3730.062
40	4906.374
50	6163.697

Assay
10 tablets were accurately weighed and ground to fine powder. Powder equivalent to 50mg of Nitaoxanide was accurately weighed, dissolved and made up with methanol. From this 5ml was pippeted and transferred to 100ml standard flask and the volume was made up with mobile phase. 20µl of the solution was injected and the chromatogram was recorded. In the similar manner chromatogram of pure drug as same concentration was also recorded in table no 3,4 .

$$\frac{\text{Test absorbance}}{\text{Std absorbance}} \times \frac{\text{Wt of std drug(mg)}}{50} \times \frac{5}{100} \times \frac{50}{\text{wt of tablet powder (mg)}} \times \text{Avg wt oftablet (mg)}$$

DATA FOR ASSAY OF TABLET

(Label claim: 500mg)

TABLE NO 3

Sl no:	Brand name	Avgwt of tablet (mg)	Wt of std drug (mg)	Avg peak area for test	Wt of tab powder (mg)	Avg peak area for std	Avg content (mg)
1	NITAOXANIDE - 500mg	1285.1	50.4	3220.388	68.2	6160.406	496.58

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System suitability parameters

TABLE NO 4

Sl no:	Parameters	Observed values	Acceptance Criteria
1	No: of theoretical plates (N)	1917.22	Not less than 2000
2	Tailing factor (T)	1	≤ 2.0
3	Linearity	--	--
4	Correlation coefficient	0.9999507	0.9999
5	Limit of Detection	0.0747	--
6	Limit of Quantitation	0.0246	--
7	% RSD	0.185	--

RESULTS AND DISCUSSION

HPLC method was developed and validated for various parameters as per ICH guidelines. The system suitability parameters proved that the proposed method was suitable for the estimation of Nitaoxanide in bulk drug and pharmaceutical formulation. The observation and results obtained for each of the parameters like system suitability, linearity, correlation coefficient, LOD and LOQ lies within the acceptance criteria. So the given method was simple, specific, linear, precise and accurate. Therefore this method can be used for the routine estimation of the drug. The chromatogram of Nitaoxanide was run in mobile phase, buffer: acetonitrile using C_{18} column. The calibration graph was found to be linear at the concentration range 10-50µg/ml. It was observed that the concentration range showed a good relation. The LOD and LOQ were found to be 0.0747 µg/ml and 0.0246 µg/ml respectively. It proved the sensitivity of the method. The average amount of Nitaoxanide in formulation was found to be 496.58mg. The low values of standard deviation and coefficient of variation indicates high precision of the method.

CONCLUSION

The proposed RP-HPLC method was sensitive and reproducible for the analysis of Nitaoxanide in tablet dosage forms. It found to be accurate, precise, simple, and rapid. Hence the present RP-HPLC method may be used for routine analysis of the raw materials.

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