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RP-HPLC Determination of Nitazoxanide in Bulk and Different Tablet Formulations

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Abstract

A simple, accurate and precise validated RP-HPLC method for determination of Nitazoxanide has been developed. Analysis was carried out on Jasco HPLC system with HiQ-sil C_{18} column (250 x 4.6 mm i.d.) using Acetonitrile: 0.005 mol.L^{-1} Tetrabutyl ammonium hydrogen sulphate in ratio of 55:45 v/v as mobile phase and Satranidazole as an internal standard The detection was carried out using UV detector set at 240 nm. For this method, Beer's law is obeyed in the concentration range of 5.0 to 30.0 µg mL^{-1} of Nitazoxanide. The developed method has been successfully applied for the analysis of drug in bulk and pharmaceutical formulations. The mean percent recoveries were found to be 100.19 ± 0.584 for Brand 1 and 100.26 ± 1.1341 for Brand 2. The method was validated with respect to linearity, precision and accuracy as per the International Conference on Harmonisation (ICH) guidelines.

Keywords: Nitazoxanide, Satranidazole, RP-HPLC.

1. Introduction

Nitazoxanide is used for treatment of diarrhea caused by Giardia lamblia/intestinalis or Cryptosporidium parvum [1]. This novel agent has a broad spectrum of activity against many other gastrointestinal pathogens, including bacteria, roundworms, flatworms, and flukes [2]. Nitazoxanide is used in many areas of the world, especially in Central and South America, as a broad-spectrum parasiticidal agent in adults and children. Chemically Nitazoxanide is [2-[(5-nitro-1, 3-thiazol-2-yl) carbamoyl] phenyl] ethanoate [3, 4].

Extensive literature survey revealed that three colorimetric methods have been reported for determination of Nitazoxanide as single component [5]. Also single UV spectrophotometric method is available for determination of Nitazoxanide in dosage form [6]. Aim of present work was to develop simple, rapid, economical and reproducible HPLC method for determination of drug in bulk and pharmaceutical formulations. This paper describes validated RP-HPLC method for determination of Nitazoxanide in bulk and tablet dosage forms. The proposed method was

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optimized and validated as per the International Conference on Harmonisation (ICH) guidelines [7].

2. Materials and Methods

2.1. Drugs and Chemicals

Acetonitrile (HPLC grade) was purchased from Merck specialties Pvt. Ltd. (Mumbai, India) and Water (HPLC grade) was purchased from Loba Chemie (Mumbai, India). Tetra butyl ammonium hydrogen sulphate salt was purchased from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). All other reagents used were of HPLC grade. Nitazoxanide (99.86 %) and Satranidazole (99.93 %) were kindly supplied by Glenmark pharmaceuticals Pvt. Ltd. (Nasik, India) and Alkem Ltd. (Mumbai, India) respectively used as such without further purification. Commercial tablets of Nitazoxanide, Nitazox-500 (Alembic Ltd., Batch No. ND6J003A, Mfg Date 03/2006, Exp Date 04/2008) and Nitacure-500 (Lupin Ltd., Batch No. 7327997, Mfg Date 12/2006, Exp Date 11/2008) were procured from the local market.

2.2. Instruments

Analysis was carried out using Jasco HPLC system, consisting of Jasco PU-2080 plus HPLC pump on HiQ-SiL C_{18} (250 x 4.6 mm i.d) column at a flow rate of 1 $mL.min^{-1}$. A Rheodyne injector with 20 µL loop was used for injecting the sample. Detection of eluent was carried out using Jasco UV-2075 plus UV/VIS detector set at 240 nm. All weighing were done on electronic balance (Shimadzu AY-120).

2.3. Procedure

Mobile phase selected for this method consists of acetonitrile: 0.005 mol L^{-1} tetrabutyl ammonium hydrogen sulphate (prepared by dissolving 1.6977 gm of TAHS salt in 1000 mL of HPLC grade water) in ratio of 55:45 that was filtered through 0.2 micron membrane filter was used. Method was developed using Satranidazole as internal standard.

2.3.1. Standard Stock Solution

Standard stock solutions of pure drugs were prepared separately in mobile phase containing 100 µg mL^{-1} of Nitazoxanide and 100 µg mL^{-1} of Satranidazole and filtered through a 0.2 micron membrane filter.

2.3.2. Preparation of Calibration Curve

For preparation of the calibration curve, aliquots 0.5, 1,..., 3 mL of the standard stock solution of Nitazoxanide (100 µg mL^{-1}) were transferred in a series of 10 ml volumetric flasks. In each flask, 1 mL stock solution of Satranidazole (100 µg mL^{-1}) was added and the volume was made up to the mark with the mobile phase. Each solution was injected and a chromatogram was recorded. Mean retention time and standard deviation for Nitazoxanide and Satranidazole was found to be 6.578 ± 0.0065 and 3.663 ± 0.0049 min respectively. The peak areas were recorded for Nitazoxanide and Satranidazole using Borwin software and calibration curve was plotted of peak area ratio of drug to internal standard against concentration of drug.

2.3.3. Procedure for Analysis of Tablet Formulation

Twenty tablets were weighed accurately; the average weight was determined and then ground to a fine powder. Powder equivalent to 10 mg of Nitazoxanide was dissolved in the 50 mL of mobile phase with the aid of ultrasonication for 10 min; volume was filtered to a 100 mL volumetric flask and made up to the mark with mobile phase. One mL of this solution was transferred to 10 mL volumetric flask, to this 1 mL of standard stock solution of Satranidazole was added and the volume was made up to the mark with the mobile phase to get final concentration 10 µg mL^{-1} of Nitazoxanide (10 µg mL^{-1} of Satranidazole)

The tablet sample solution was injected and a chromatogram was obtained. The injections were repeated six times and the peak areas were recorded. A representative chromatogram has been given in Fig.1. The peak area ratios of the drug to the internal standard were calculated and the amount of drug present per tablet was determined from the calibration curve. Same procedure was followed for both the tablets.

2.3.4. Robustness Studies

The robustness of a method is its ability to remain unaffected by small deliberate variations in the method parameters. The following changes in the optimum parameter values were examined: the flow rate of the mobile-phase (adjusted by ± 0.02 $mL.min^{-1}$) and the detection wavelength (adjusted by ± 1 nm).

2.3.5. Recovery Studies

To study the accuracy, reproducibility and precision of the above method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample (containing 10 µg mL^{-1} of Nitazoxanide and 10 µg mL^{-1} of Satranidazole as internal standard) at three different levels 50 %, 100 % and 150 %.

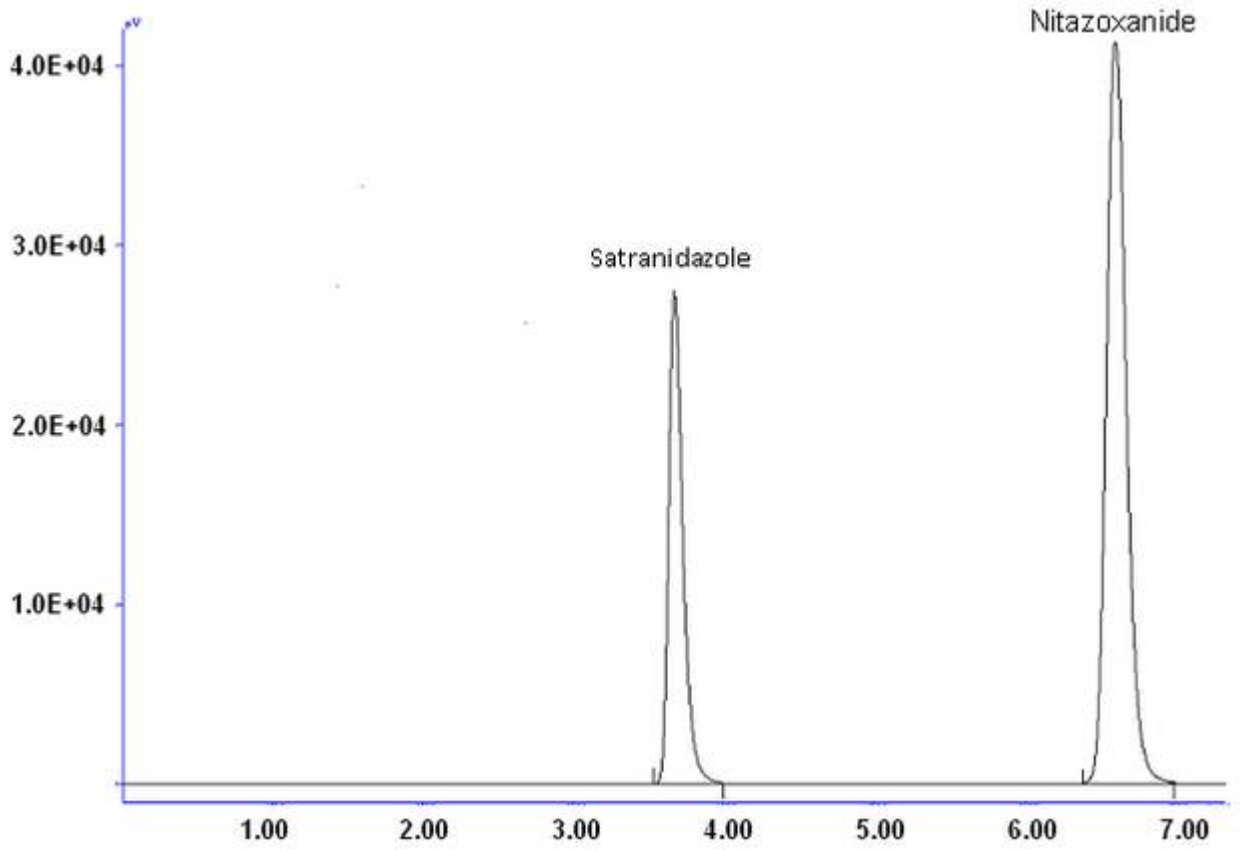

Fig.1. Chromatogram of Nitazoxanide (10 µg mL^{-1}, 6.575 min), Satranidazole (10 µg mL^{-1}, 3.667 min).

3. Results and Discussion

Under experimental conditions described, calibration curve, assay of tablets and recovery studies were performed. A critical evaluation of proposed method was performed by statistical analysis of data where slopes, intercepts, correlation coefficients are shown in Table 1. System suitability parameters are given in Table 2. The proposed methods were also evaluated by the assay of commercially available tablets containing Nitazoxanide. Six replicate determinations were performed on the accurately weighed amounts of tablets. The % recovery was found to be 100.19 ± 0.5848 and 100.26 ± 1.1312 for Brand 1 and Brand 2 respectively. The result of analysis of commercial formulations is presented in Table 3. % R.S.D. values were found to be less than 2, indicating reproducibility of the method. For recovery study volume of standard solution of different concentration were added to the fixed volume of sample solution. The recovery study results ranged from 100.06 % to 100.26 % and 99.33 % to 100.60 %, with % RSD values ranging from 0.488 to 1.96 % and 0.6124 to 1.1219 % for Brand 1 and Brand 2 respectively. Results of recovery studies were found to be satisfactory and are reported in Table 4. The robustness studies (data not shown) showed that results are unaffected by small but deliberate changes in method parameters (RSD < 2).

Table 1: Regression analysis of calibration curves.

Parameters	Values
Detection wavelength (nm)	240
Beer's law limits (µg mL^{-1})	5 - 30
Regression equation (Y = a + bx)[a]	
Slope (b)	0.2212
Intercept (a)	0.1137
Correlation coefficient	0.9998
Limit of Detection (µg mL^{-1})	1.49
Limit of Quantitation (µg mL^{-1})	4.51

[a]Y = a + bx, where x is the concentration in µg mL^{-1} and Y is absorbance units.

Table 2: System Suitability Parameters for RP-HPLC Method.

Parameters	Values
Theoretical plates	13408
Resolution	3.53
Asymmetry Factor	1.209
Tailing factor	1.0678
HETP (cm)	0.00187
Capacity Factor (K')	3.109

Table 3. Results of Analysis of Commercial Formulation

Brand Name	Label Claim (mg/tablet)	% of Label Claim Estimated*	Standard Deviation	R.S.D., %
Nitazox-500	500	100.19	0.5848	0.5837
Nizonide-500	500	100.26	1.1341	1.1312

*Average of six Determinations

Table 4: Recovery Studies of Nitazoxanide from Both Tablets:

Level of % Recovery	% Mean Recovery*		Standard Deviation		% R.S.D.†		Standard Error	
	Nitazox 500 (Brand-1)	Nizonide 500 (Brand-2)	Brand-1	Brand-2	Brand-1	Brand-2	Brand-1	Brand-2
50	100.23	100.37	1.9779	0.9544	1.9603	0.9495	1.3986	0.6749
100	100.06	100.60	0.5848	1.1341	0.5792	1.1219	0.4135	0.4096
150	100.26	99.33	0.4822	0.6150	0.4880	0.6124	0.3410	0.4349

*avg. of three determinations.
†is the relative standard deviation.

4. Conclusion

The validated RP-HPLC method employed here proved to be simple, fast, accurate, precise and sensitive enough. This developed method can be used for routine analysis of drug in bulk and tablet dosage forms.

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