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Method Development and Validation for Assay of Nitazoxanide in Tablet Using RP-HPLC

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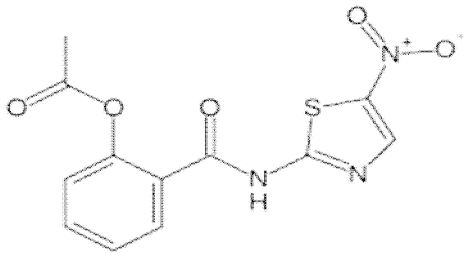
Abstract: A simple, selective, rapid, precise and accurate reverse phase high pressure liquid chromatographic method has been developed for the assay of nitazoxanide (NTZ) in tablets. An isocratic LC separation was performed on a inertsil C_{18} (250mm×4.6mm)
Column with mobile phase consisting of 0.005M tetra butyl ammonium hydrogen sulphate (45): acetonitrile (55): at a flow rate at 1.0 (ml/min). Detection was carried out at 345 nm. The retention time of nitazoxanide was 7.468 min respectively. The developed method was validated for linearity, accuracy, precision, solution stability, robustness, ruggedness. There was no interfearance of excipients on the determination of active pharmaceutical ingredients. It can be applied to the quantitative determination of drug in tablets and powder for oral suspension.
Key words: RP-HPLC, Nitazoxanide.

Introduction

Nitazoxanide (NTZ), 2-acetyloxy-N-(5-nitro-2-thiazolyl) benzamide is a new broad spectrum antiparasitic agent[1, 2]. It is used for the treatment of cryptosporidiosis[3] and giardiasis[4] in children and adults. NTZ inhibits pyruate ferreodoxin oxireductase (PFOR)[5-9] enzyme- dependent electron transfer reactions essential to anaerobic energy metabolism of these organisms. Nitazoxanide is currently available tablets and powder for oral suspension.[10,11,12]

A recent literature survey revealed that few individual methods were available for the determination of Nitazoxanide and metabolites in biological fluids by LC and tandem mass spectrometry (LC-MS-MS)[13,14].Recently spectrophotometer and high performance thin layer chromatographic (HPTLC)methods,[15] RP-HPLC method and UPLC method.[16] Although various analytical methods have been developed for the determination of nitazoxanide. The present work describes the development and validation in compliance with ICH guidelines.



Nitazoxanide

Nitazoxanide used as reference substances (assigned purity) 99.97% was kindly supplied by startech laboratories pvt .Ltd. Hyderabad. The HPLC system consists of with a single pump, a manual injecting device, UV-visible detector, with a chromo Leon version software 6.68. Respectively and tablet purchased from the local market, HPLC grade acetonitrile and Tetra butyl ammonium hydrogen sulphate AR Grade were procured from S.D fine-chemical ltd; Mumbai.

Material and methods

Mobile phase

Filtered and degassed mixture of buffer and acetonitrile was prepared in the ratio of 45:55, where the buffer was (0.005M tetra butyl ammonium hydrogen sulphate).

Chromatographic Conditions:

The liquid chromato graph was equipped with a 345 nm detector and 250 mm of length and 4.6 mm id, octadecyl silane column that contains 5 micron packing chemically bonded to porous silica particle; the flow rate was 1.0 ml/min and at 30°C column temperature.

Standard preparation:

Accurately weighed quantities of nitazoxanide were dissolved in mobile phase to obtain solution having known concentration of about 0.1 mg/ml nitazoxanide respectively.

Assay preparation for commercial formulation:

Twenty tablets were weighed and crushed to fine powder into 100 ml of volumetric flask; mobile phase was added and sonicated until tablets were dissolved. The flask was cooled and made up to volume with mobile phase. The solution was mixed and centrifuged to get clear solution, further diluted quantitatively and stepwise with mobile phase to obtain a solution having concentrations of 0.1 mg/ml respectively.

Procedure:

20 µl of the standard preparation and the assay preparation were separately injected and the chromatographs were recorded (Fig.1)

Linearity and Range:

The result of the method was found to be linear in the range of 20 µg/ml to 160 µg/ml of NTZ. And peak areas recorded for all peaks and plotted peak *vs.* concentration. Coefficient of correlation for NTZ was 0.9997.

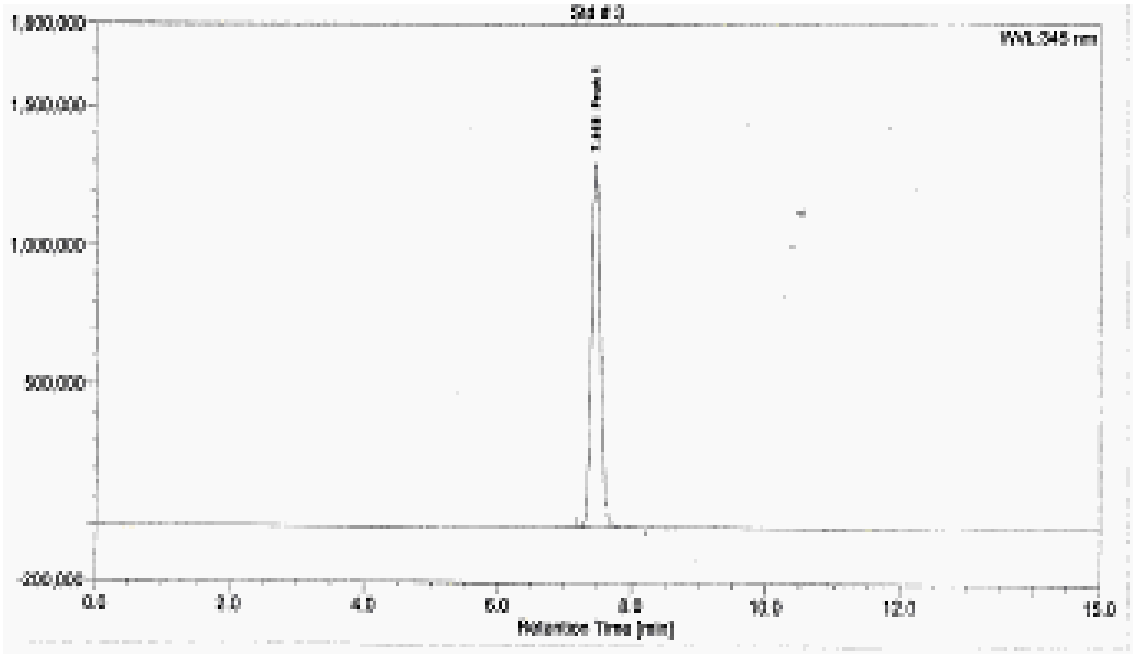


Fig.1 A typical chromatogram of nitazoxanide (7.468min)

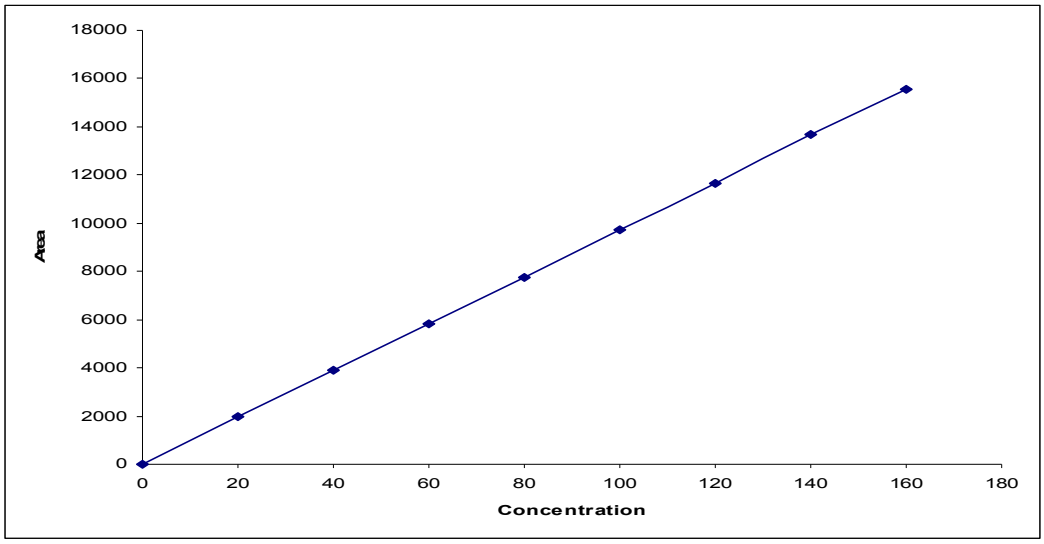


Fig.no.2 Linearity curve for the nitazoxanide

Accuracy:

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three levels of 50, 100, and 150 % and the percentage recovery was calculated and presented in Table no 1. Recovery was within the range of 100 ± 2 % which indicates accuracy of the method.

Precision:

The accuracy of the method was demonstrated by inter day and intra day variation studies. The intra day studies 3 repeated injections of standard and sample solutions were made in a day and the response of the factor of drug peaks and percentage RSD were calculated and found to not more than 0.18 % of NTZ. In the inter day variation studies 3 repeated injections standard and sample solutions were made on 3 consecutive days and the response of the factor of drug peaks and percentage RSD were calculated and found to not more than 0.13 % of NTZ. The data obtained indicates that the developed RP-HPLC method is precise.

Table -1 Accuracy for Nitazoxanide

Conc. add	Found conc.	Mean % RE	Standard deviation	Relative standard deviation
50	100.18	100.36	0.822	0.82
50	100.08			
50	100.32			
100	99.84	99.98	0.30	0.30
100	99.96			
100	100.15			
150	99.49	99.68	0.21	0.21
150	100.18			
150	99.38			

Solution stability:

The standard solution prepared by using nitazoxanide as working standard as per the test method and injected initial and after 25 hours into the HPLC system. The solution stability parameters were evaluated and found to be with in the limits. The deviations of the both initial and after 25 hrs 0. 00 and 0.40 of nitazoxanide.

Robustness:

Robustness was checked by making slight deliberate change in the experimental procedures. In the present method a deliberate change of flow rate, temprature and wavelength was made and the effects were noted. The method was found to be robust with respect to change in flow rate, temperature and wavelength.

Ruggedness:

Ruggedness was checked by using different instrument, different laboratory and different analyst. In the present method six different concentration of sample solution were prepared by one different analyst and each solution is injected six times respectively.

Results and Discussion

Above method of the development and validation of the nitazoxanide in tablet dosage form with RP-HPLC method was evaluated and was found to be the accurate in the range given above. All the parameters of the validation were passed as per the compliance of the ICH guidelines, the results showed that accuracy was between 99.68% to 100.36%.

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References

1. www. rxlist. com/ nitazoxanide.htm.
2. www. wikepedia.org .
3. Marcelo Donadel Malesuik1, Simons Goncalves Cardoso and Martin .include Int. J. Rapid Communication in Chromatography, Electrophoresis and Associated Techniques 0454-9(2007).
4. www.fda.gov/cder/foi/label/2002/21498_Alinia_lbl.
5. ZhaoZ, Zhang L,Xue F,Wang X, Zheng W,Zhang T, FieC,Zhang K,Qiu M, Xing R, Yang F. J. Chromatogram B Analyt Technol Biomed Life Sci. 875(2):427-36(2008) Sep.
6. P.D,Sethi.,Quantitative Analysis of Drugs in Ph armaceuticalFormulations,3rdEdn.,BS Publisher and Distributor, New Delhi, 51(1997).
7. D. Saravolatz, Louis Chicago press Journals, Clinical Infection Diseases, 15th April 2005, Nitazoxanide.
8. Ashok S. Jadhav1, Dnyandeo B. pathare and Murlidhar.S.Shingare., Sprinkerlink Journal, Vieweg Verlag 66, October, ,17(2007).
9. ICH Harmonized Tripartite guidelines, validation of analytical procedure, methodology, NOV 6th, 3(1996).
10. ICH Harmonized Tripartite guidelines, validation of analytical procedure, methodology, OCT, 59(1996).
11. USP validation of compendia Method,28thEdn., 2440(2005)
12. BP validation procedure A-456(2005).
13. The Merck Index,: Merck research lab, Division of Merck & Co. INC, White house station, N.J.13th Edn, 1177.
14. C. L .GOPU., Thomas Shibu., .A.R. Paradkar., .K.R. Mahadik., In., Journal of scientific & industrial research, , 66, . 141-145(2007).
15. GK Kapse., Indian. J. sci., 68 403-406(2006).
16. B.P, London, Vol.II 1225 (2003).

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