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## RP-HPLC methods for estimation of Nitazoxanide single and simultaneous estimation of Nitazoxanide with Ofloxacin in pharmaceutical dosage forms

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## ABSTRACT

*Two simple, selective, rapid, precise and economical reverse phase high-pressure liquid chromatography methods have been developed; first method for estimation of nitazoxanide and second method for simultaneous estimation of nitazoxanide & ofloxacin from pharmaceutical dosage forms. The first method carried out on a Hypersil BDS $C_8$ column (5µ particles size) (250 mm X 4.6 mm), with mobile phase consisting of acetonitrile: 0.2M potassium dihydrogen phospate in ratio 70:30 (pH 3.0 adjusted with orthophosphoric acid) at a flow rate of 1.0ml/min. Detection was carried out at 319nm. The retention time of nitazoxanide was 4.51 min. First developed method cannot easily apply for the simultaneous estimation of nitazoxanide and ofloxacin. So second method carried out on same column but different mobile phase which consisting the acetonitrile: 0.2M potassium dihydrogen phospate: methanol in ratio 70:10:20 (pH 3.5 adjusted with orthophosphoric acid) at a flow rate of 1.0ml/min. Detection was carried out at 302nm. The retention time of nitazoxanide and ofloxacin were 6.93 min and 9.32 min, respectively. The results of analysis shows that the amounts of drugs were in good agreement with the labelled claim of the formulations. The method validation parameters checked as per the ICH guidelines. Thus the methods are specific and sensitive.*

**Key words:** Nitazoxanide, Ofloxacin, RP-HPLC.

## INTRODUCTION

Prodrug Nitazoxanide [NTZ] is a 5-nitro heterocyclic containing thiazole ring system; chemically it is N-(5-nitro-2-thiazolyl) salicylamide acetate[1-3]. NTZ is a broad-spectrum drug efficacious in the treatment of infections caused by amitochondriate lumina parasites and

helminthes[4]. Ofloxacin [OFL] is a fluoroquinolones containing oxazine ring system; chemically it is a racemate (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid[5]. It is mainly used as antibacterial for the treatment of urinary tract infection. Extensive literature review revealed that some complicated HPTLC[6], spectrophotometric[7-11], HPLC[12] and stripping voltamatric[13] methods have been reported for the estimation of NTZ in pharmaceutical dosage forms. There are also some complicated spectrophotometric[14] and HPLC[15-16] methods have been reported for the simultaneous estimation of NTZ and OFL.

Present work describes to develop simple, selective, rapid, accurate, precise and economical RP-HPLC methods one is determination of NTZ and second is simultaneous estimation of NTZ & OFL in pharmaceutical dosage forms.

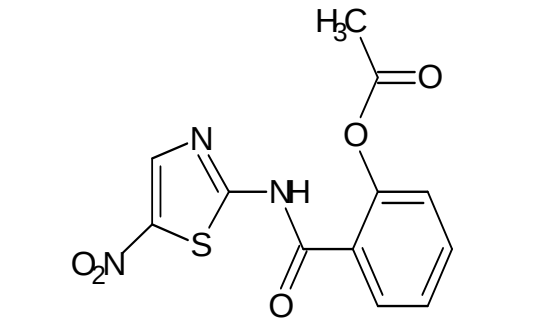
## EXPERIMENTAL SECTION

### Apparatus and chromatographic condition:

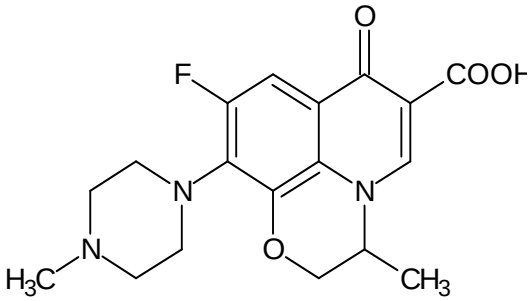
Chromatographic separation was performed on a LC – 2010 AHT HPLC system. Hypersil BDS $C_8$ column (5µ particles size) (250 mm X 4.6 mm), was used for separation for method I and method II Analysis was performed at ambient temperature.

### Materials:

Pharmaceutically pure samples of NTZ and OFL were obtained as generous gifts from Ind-Swift Labs, Chandigarh. All the chemicals used were of HPLC grade.




(a)




(b)

**Fig. 1. Chemical structure of (a) nitazoxanide and (b) ofloxacin.**

**Pharmaceutical dosage forms**:
Pharmaceutical dosage forms for method I (label claim: 500mg NTZ); nizonide (Batch No.-ND9J009A, Mfg. Dt.-11/09 and Exp. Dt. 10/11) and netazox (Batch No.-NTZ-09015, Mfg. Dt.-07/09 and Exp. Dt. 06/11) and for method II (label claim: 500mg NTZ & 200mg OFL); Nizonide-O (Batch No.-ND9J010D, Mfg. Dt.-10/09 and Exp. Dt. 09/11) and Netazox-OF (Batch No.-9503, Mfg. Dt.-06/09 and Exp. Dt. 05/11) were procured from the local market.

**Mobile phase:**
Optimization of mobile phase was performed based on various parameters such as retention time, theoretical plates, and resolution. The mobile phase consisted acetonitrile: 0.2M potassium dihydrogen phospate in ratio 70:30 (pH 3.0 adjusted with orthophosphoric acid) at a flow rate of 1.0ml/min for method I. For method II mobile phase consisted acetonitrile: 0.2M potassium dihydrogen phospate: methanol in ratio 70:10:20 (pH 3.5 adjusted with orthophosphoric acid) at a flow rate of 1.0ml/min. Mobile phase were premixed and filtered through a 0.2µ membrane filtered and degassed.

**Method I**
**Study of retention time:**
A standard dilution of pure drug containing 10 µg/ml of NTZ was prepared in mobile phase, filtered through 0.2 µg membrane filter and loaded in injection port of instrument fitted with 20 µL fixed volume loop. The solution was injected and chromatogram was recorded. The mean retention time for NTZ was found to be 4.51 min respectively. The representative chromatogram of NTZ is reported in fig. 2.

**Procedure for calibration curve:**
Standard stock drug solutions of NTZ with concentration of 100 µg/ml prepared in mobile phase. For preparation of calibration curve of drug 0.5, 1.0, 1.5, 2.0 and 2.5 ml standard stock solution of NTZ was transferred to series of 10 ml volumetric flasks and volume was made up to the mark with the mobile phase. Each solution was injected after filtration through 0.2 µ membrane filter and chromatogram was recorded. The calibration curve was plotted between concentration of drug and AUC of a peak of NTZ is reported in fig. 3. Linearity was found to be in concentration range of 5-25 µg/ml for NTZ.

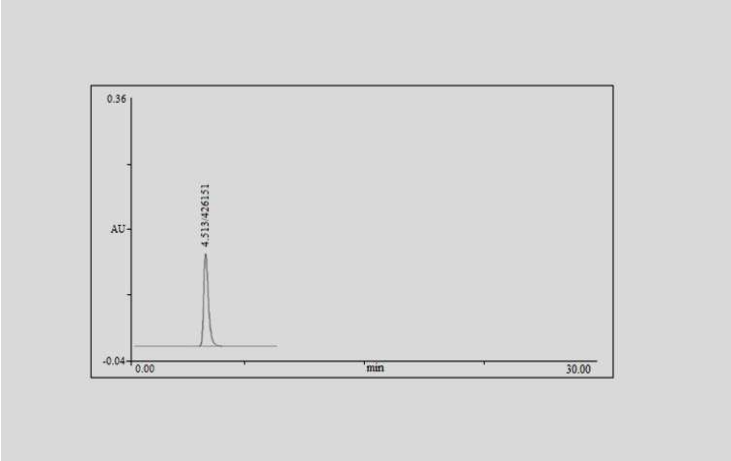



**Fig. 2. Chromatogram of nitazoxanide**

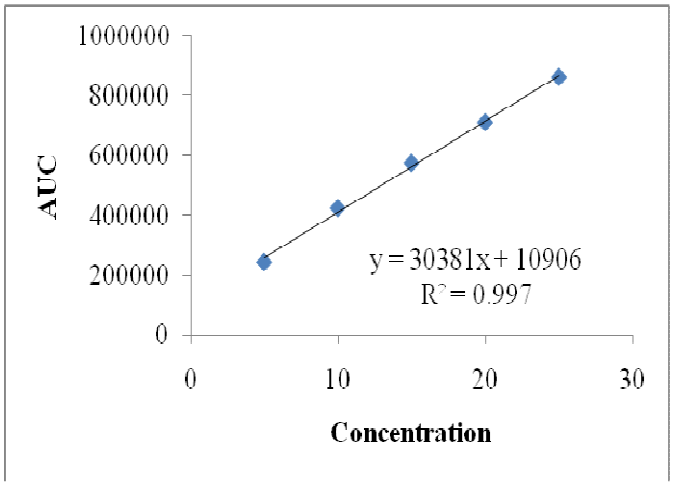



**Fig. 3. Calibration curve of nitazoxanide**

## Assay procedure:

Twenty tablets (each tablet containing 500mg NTZ) of NTZ were weighed accurately. The tablets were finely powdered and powder equivalent to 10.00 mg of NTZ was taken in a 100 ml volumetric flask containing 75 ml of mobile phase. The powder mixture was dissolved in the mobile phase with aid of ultrasonication for 10 min. The resultant was filtered through Whatman filter paper no. 41 into another 100 ml volumetric flask. The filter paper was washed several times with mobile phase. The washings were added to the filtrate and final volume was made up to the mark with mobile phase. From this filtrate 1.5 ml was further diluted to 10 ml with mobile phase. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady base line, the final dilution of tablet sample solution was loaded in sample loop of the injection port of the instrument. The solution was injected and chromatogram was recorded. The procedure of analysis for tablet formulation was repeated five times with two different tablet formulation and results are reported in table 2.

**Table 1 : System suitability parameters for method I**

| Parameters | Values |
|---|---|
| **Detection wavelength** | 319nm |
| **Linerity range** | 5-25 µg/ml |
| **Slope** | 30381 |
| **Intercept** | 10906 |
| **Correlation coefficient** | 0.997 |
| **Retention time** | 4.51min |

## Method II

## Study of retention time:

Standard stock solutions of pure drugs were made separately in mobile phase containing 100µg/ml of NTZ and OFL, filtered through a 0.2 µ membrane filter. In a 10 ml volumetric flask, 2.5 ml standard stock solution of NTZ with 1.0 ml standard stock solution of OFL was taken and volume made to the mark with mobile phase. This mixed standard solution was loaded in the injector port of the instrument. The solution was injected and a chromatogram was

recorded. This was done to check the resolution of two drugs. The two drugs were found to be perfectly resolved. The representative chromatogram is reported in fig. 4.

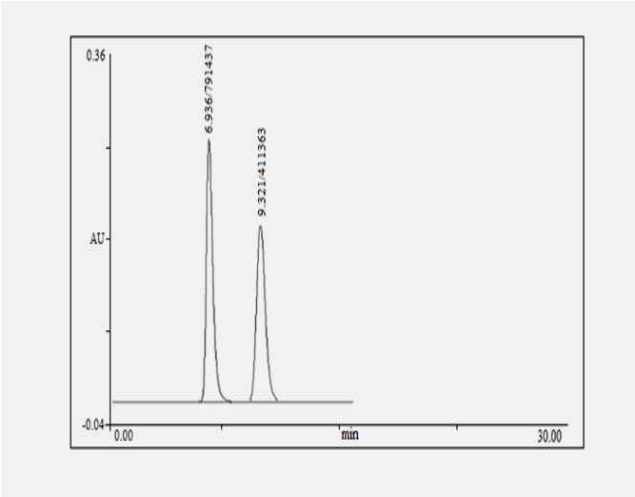
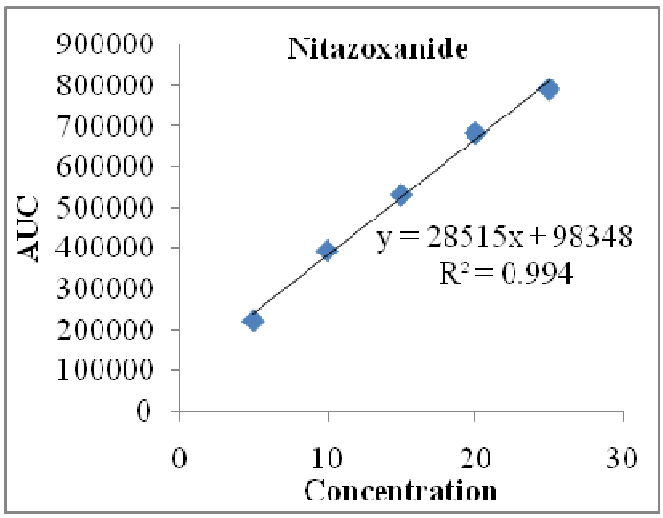



**Fig. 4. Chromatogram of nitazoxanide and ofloxacin**

**Table 2: Results of analysis of commercial formulations method I**

| Brand name | Labelled claim (mg/tablet) | % labelled claim estimated* | ± standard deviation | % relative standard deviation |
|---|---|---|---|---|
| **Nizonide** | 500 | 99.26 | 0.1407 | 0.1417 |
| **Netazox** | 500 | 99.38 | 0.2955 | 0.2973 |

***** Each value is an average of five determinations



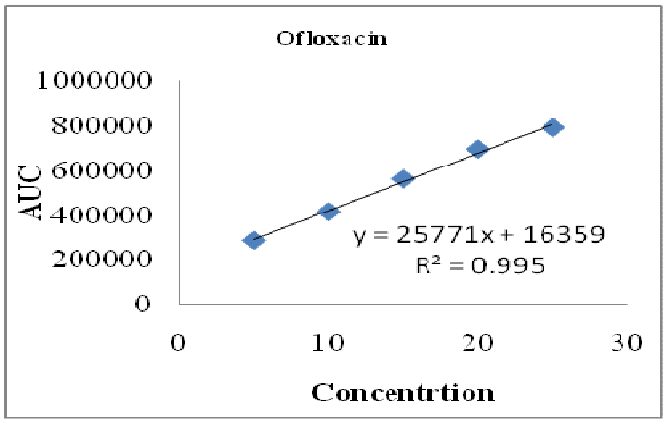



**Fig. 5: Calibration curve of nitazoxanide and ofloxacin for HPLC method**

**Procedure for calibration curve:**

For preparation of drug solutions for the calibration curves, in a series of 10 ml volumetric flask, several dilutions of NTZ and OFL were prepared in the mobile phase. Each solution was injected and a chromatogram was recorded. The peak area of drug was calculated for each concentration level of two drugs and a graph was plotted between drug concentrations against the AUC of the peak. The linearity was observed in the concentration range of 5-25µg/ml for NTZ and 5-25µg/ml for OFL.

**Assay procedure:** Twenty tablets [NTZ - 500 mg and OFL – 200 mg] were weighed accurately and. the tablets was finely powdered and powder equivalent to 10 mg of NTZ and 4 mg OFL was weighed and extracted with 75 ml of mobile phase. The powder mixture was dissolved in the mobile phase with aid of ultrasonication for 10 min. The resultant was filtered through Whatman filter paper no. 41 into 100 ml volumetric flask. The filter paper was washed several times with mobile phase. The washings were added to the filtrate and final volume was made up to the mark with the mobile phase. Filtrate (1.5 ml) of the sample solution was diluted to 10 ml with solvent. This solution was filtered through a 0.2 µ membrane filter. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was loaded in the 20 µL sample loop of the injection port of the instrument, and the peak areas were recorded The peak areas of each of the drug were recorded and amount of each drug present per tablet was estimated from the respective calibration curves. The procedure of analysis was repeated five times with two different tablet formulations. The results of analysis are presented in table 4.

**Tab. 3: System suitability parameters for method II**

| Parameters | Values | |
|---|---|---|
| | **NTZ** | **OFL** |
| **Linerity range (µg/ml)** | 2-25 | 2-25 |
| **Slope** | 28515 | 25771 |
| **Intercept** | 98348 | 16359 |
| **Correlation coefficient** | 0.994 | 0.995 |
| **Retention time (min.)** | 6.93 | 9.31 |

**Recovery studies:**
Recovery studies were carried out for both the methods by addition of known amount of standard drugs solutions to pre-analyzed tablet sample solutions at three different concentration levels. The resulting solutions were analyzed by proposed methods. The results of recovery studies were found to be satisfactory and are results are reported in table 5 & 6.

**Tab. 5: Results of recovery studies of method I**

| Brand name | % amount added | % amount recovered | % recovery |
|---|---|---|---|
| **Nizonide** | 80 | 79.48 | 99.35 |
| | 100 | 99.43 | 99.43 |
| | 120 | 119.03 | 99.19 |
| **Netazox** | 80 | 79.39 | 99.24 |
| | 100 | 99.48 | 99.48 |
| | 120 | 119.77 | 99.81 |

**Tab. 4: Results of analysis of commercial formulations method II**

| Brand name | Labelled claim (mg/tablet) | | % labelled claim estimated* | | ± standard deviation | | % relative standard deviation | |
|---|---|---|---|---|---|---|---|---|
| | NTZ | OFL | NTZ | OFL | NTZ | OFL | NTZ | OFL |
| **Nizonide-O** | 500 | 200 | 99.56 | 99.42 | 0.1232 | 0.2815 | 0.1237 | 0.2831 |
| **Netazox-OF** | 500 | 200 | 99.38 | 99.60 | 0.1714 | 0.2092 | 0.1724 | 0.2101 |

***** Each value is an average of five determinations.

**Tab. 6: Results of recovery studies of method II**

| Brand name | % amount added | | % recovery | |
|---|---|---|---|---|
| | NTZ | OFL | NTZ | OFL |
| **Nizonide-O** | 80 | 80 | 98.36 | 98.41 |
| | 100 | 100 | 99.62 | 98.55 |
| | 120 | 120 | 99.22 | 99.31 |
| **Netazox-OF** | 80 | 80 | 99.25 | 99.25 |
| | 100 | 100 | 99.50 | 99.59 |
| | 120 | 120 | 98.59 | 99.45 |

## DISCUSSION

Method for the individual determination of the NTZ cannot be easily applied for the simultaneous estimation of NTZ and OFL drugs because combined formulation owing to their large differences in physical and chemical properties such as polarity and solubility. If developed method applied for the analysis of combined formulation the method would not be rapid.

**Method I-** The proposed method was found to be simple and linear in the concentration range 5-25µg/ml for NTZ. The method was found to be accurate and precise as indicated by the recovery studies and %RSD not more than 2. Thus the method is specific and sensitive.

**Method II-** The proposed method was found to be simple and linear in the concentration range 5-25µg/ml for NTZ and OFL. The method was found to be accurate and precise as indicated by the recovery studies and %RSD not more than 2. Thus the method is specific and sensitive.

## CONCLUSION

The proposed RP-HPLC methods for estimation of nitazoxanide and simultaneous estimation of nitazoxanide & ofloxacin from pharmaceutical dosage forms were found to be sensitive, accurate, precise, simple and rapid. Hence the present RP-HPLC method may be used for routine analysis of the pharmaceutical dosage forms.

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## REFERENCES

[1] S.C. Sweetman, Eds., In: Martindale; The Complete Drug Reference, 33rd Edn., The Pharmaceutical Press, London, 598 (**2002**).
[2] L. Debreuil, Antimicrob. *Agents Chemother*., 40, 2261 (**1996**).
[3] R. Cravier, *Eur. J. Med. Chem*., 13, 539 (**1978**).
[4] J.R. Murphy and J.C. Fried Mann, *J. Appl. Toxicol*., 5, 491 (**1985**).
[5] S. Budavari, Eds., In: The Merck Index, 13th Eds, Merck & Co. Inc., White House Station, NJ, 1213, 1229 (**2001**).
[6] C.L. Gopu, S. Thomas, A.R. Paradkar and K.R. Mahadik, *J. Sci. Ind. Res*., 66, 141-145 (**2007**).
[7] Nandini R. Pai, Deepnandan S. Dubhashi, *Der Pharmacia Lettre*, 2, 4, 1-10 (**2010**).
[8] Sagar B.Wankhede, Somnath S. Zambare, Nitin R.Dixit and Sohan S. Chitlange, *Der Pharmacia Lettre*, 2(3), 315-320 (**2010**).
[9] G.K. Kapse, G. Prabhakar and S.A Raju, *Indian J. Pharm. Sci.*, 68, 403-406 (**2006**).
[10] K.V. Lakshminarayana, Y.N. Manohara and B.M. Gurupadayya, *Indian J. Pharm. Sci*., 69, 147-149 (**2007**).
[11] A.L Santos, R.M. Takeuchi and N.R. Stradiotto, *Current Pharmaceutical Analysis*, 5, 69-88 (**2009**).
[12] V.Y. Jadhav, S.V. Gandhi, N.D. Dhavale and S.S. Sabnis, *Eurasian Journal of Analytical Chemistry*, 3, 318-323 (**2008**).
[13] H.S. El-Desoky, M.M. Ghoneim and M.M. Abdel-Galeil, *J. Braz. Chem. Soc*., 1-11 (**2009**).
[14] V.P Rane, and D.B. Shinde, *Eurasian J. Anal. Chem*., 4, 304-310 (**2009**).
[15] Neha Yadav and B. P Srinivasan, *Der Pharma Chemica*, 2(4), 103-112 (**2010**).

[16] S. Jayaseelan, M. Rajasekar, S. Ganesh, V. Sekar, P. Perumal, *Der Pharma Chemica*, 2(3), 31-36 (**2010**).