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A New RP-UPLC Method Development and Validation for the Bulk Drugs Nitazoxanide and Ofloxacin

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Abstract

A new precise, accurate, rapid method has been developed for the simultaneous estimation of Ofloxacin and Nitazoxanide in pharmaceutical dosage form by RP-UHPLC the optimum wavelength for the determination of Ofloxacin and Nitazoxanide was selected at 230 nm on the basis of isobestic point. Various trials were performed with different mobile phases in different ratios, but finally Sodium Phosphate Buffer pH 3.0: Acetonitrile (80:20%v/v) was selected as good peak symmetry and resolution between the peaks was observed. The Retention time of Ofloxacin and Nitazoxanide were found to be 1.527 and 2.890 min respectively. The Retention times for both the drugs were considerably less compared to the Retention time obtained for the drugs in the other mobile phase. The different analytical performance parameters such as linearity, precision, accuracy and specificity were determined according to International Conference on Harmonization ICH Q2B guidelines. The calibration curve was obtained by plotting peak area versus the concentration over the range of 100-300 µg/ml for Nitazoxanide and250-750 µg/ml for Ofloxacin. From linearity the correlation coefficient R^2 value was found to be 0.999 for Nitazoxanide and 0.999 for Ofloxacin. The proposed HPLC method was also validated for system suitability, system precision and method precision. The %RSD in the peak area of drug was found to be less than 2%. The number of theoretical plates was found to be more than 2000, which indicates efficient performance of the column. The percentage of recovery of Ofloxacin and Nitazoxanide were found to be 99.5 and 99.2 % respectively, shows that the proposed method is highly accurate.

Keywords

Ofloxacin, Nitazoxanide, Acetonitrile, Phosphate Buffer, UPLC, Validation.

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INTRODUCTION

A synthetic fluoroquinolone (fluoroquinolones) antibacterial agent that inhibits the supercoiling activity of bacterial DNA gyrase, halting DNA replication. Ofloxacin acts on DNA gyrase and topoisomerase IV, enzymes which, like human topoisomerase, prevents the excessive supercoiling of DNA during replication or transcription. By inhibiting their function, the drug thereby inhibits normal cell division. Ofloxacin is a quinolone/fluoroquinolone antibiotic. Ofloxacin is bactericidal and its mode of action depends on blocking of bacterial DNA replication by binding itself to an enzyme called DNA gyrase, which allows the untwisting required to replicate one DNA double helix into two. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian. Ofloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. Chemically, it is 8-Fluoro-3-methyl-9-(4-methyl-piperazin-1-yl)-6-oxo-2,3-dihydro-6H-1-oxa-3a-aza-phenalene-5-carboxylic acid. Its molecular formula and molecular weight are $C_{18}H_{20}FN_3O_4$ and 361.367.

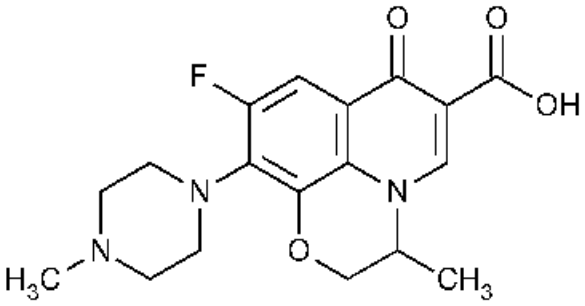


Fig 1: Chemical structure of Ofloxacin

Nitazoxanide belongs to the class of drugs known as thiazolides. Nitazoxanide (NTZ) is a broad-spectrum anti-infective drug that markedly modulates the survival, growth, and proliferation of a range of extracellular and intracellular protozoa, helminths, anaerobic and micro aerophilic bacteria, in addition to viruses. This drug is effective in the treatment of gastrointestinal infections including Cryptosporidium parvum or Giardia lamblia in healthy subjects. It is generally well tolerated. Nitazoxanide is a first-line, standard treatment for illness caused by C. parvum or G. lamblia infection in healthy (not immune suppressed) adults and children and may also be considered in the treatment of illnesses caused by other protozoa or helminths [2]. Recently, this drug has been studied as a broad-spectrum antiviral agent due to its ability to inhibit the replication of several RNA and DNA viruses. Chemically, it is 2-[(5-nitro-1,3-thiazol-2-yl) carbamoyl] phenyl acetate. Its molecular formula is $C_{12}H_9N_3O_5S$ and molecular weight is 307.282.

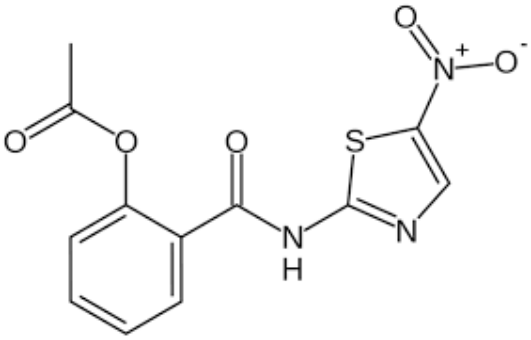


Fig 2: Chemical structure of Nitazoxanide

MATERIALS AND METHODS

Materials and Instruments: The following materials used were either AR/LR grade or the best possible Pharma grade available as supplied by the manufacturer or supplier without further purification or investigation.

Drug Samples

Were obtained from Madras Pharmaceuticals, Chennai.

Chemicals and Solvents used

Water	-	Merck/ HPLC Grade
Acetonitrile	-	Merck/ HPLC Grade
Sodium Phosphate Monobasic	-	Rankem/ AR Grade
Orthophosphoric acid	-	Rankem/ AR Grade
Instruments		
UV-Visible Spectrophotometer	-	Thermo Technology
UPLC	-	Agilent 1290 Infinity
Ultra Sonicator	-	Citizen, Digital Ultrasonic Cleaner
pH meter	-	Thermo
Electronic balance	-	Shimadzu
Syringe	-	Hamilton
HPLC Column	-	Zorbax SB C18(50x2.1mm ID) 1.7 µm

Sample Processing:

Diluents: Based upon the solubility of the drug diluents was selected. Firstly, dissolved in methanol and diluted with Acetonitrile and water.

Preparation of Standard stock solutions: About 10 mg of OFLOXACIN and 10mg of NITAZOXANIDE were weighed into a 50 ml volumetric flask, to this 50 ml of mobile phase was added, sonicated and the volume was made up to mark with the mobile phase.

Preparation of standard working solutions (100% solution):

1ml from each stock solution was pipette out and taken into a 10ml volumetric flask and made up with mobile phase.

Preparation of Sample stock solutions: Crush more than 20tablets then weigh a quantity of powder equivalent to 500 mg of NITAZOXANIDE and 200mg OFLOXACIN in 200 ml volumetric flask and add70mL of mobile phase then sonicated it for 30 min intermittent shacking after 30min make up volume with mobile phase. Pipetted 5 ml of the clear solution in to 25 ml volumetric flask and make up volume with mobile phase. Filter the solution through 0.45µm filter paper.

Preparation of Sample working solutions (100% solution): From the filtered solution 1ml was pipette out into a 10ml volumetric flask and made upto 10ml with diluents.

Preparation of buffer:

Preparation of Phosphate buffer pH 3.0:

2.38gm of Sodium Phosphate Monobasic was weighed and dissolved in 1000 mL of water. Then adjust the pH to3.0±0.02 using diluted orthophosphoric acid. Buffer was filtered through 0.45µm filters to remove all fine particles and gases.

RESULTS AND DISCUSSION

System suitability:

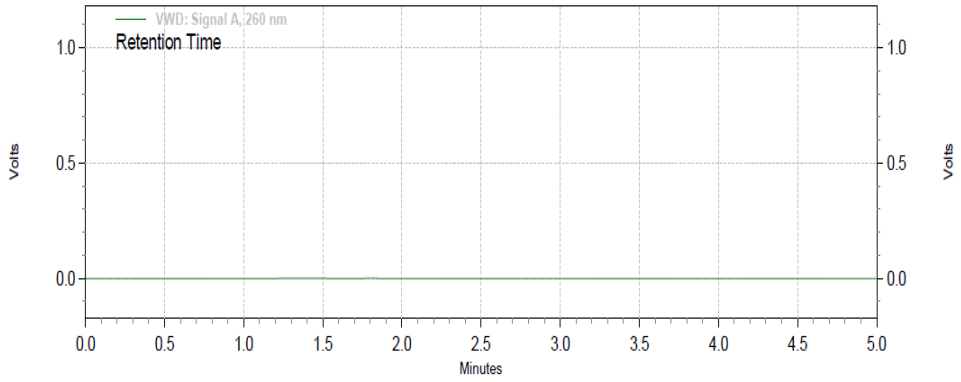
The system suitability parameters were determined by preparing standard solutions of Ofloxacin and Nitazoxanide and the solutions were injected six times and the parameters like peak tailing resolution and USP plate count were determined.

The %RSD for the area of six standard injections results should not be more than 2%.

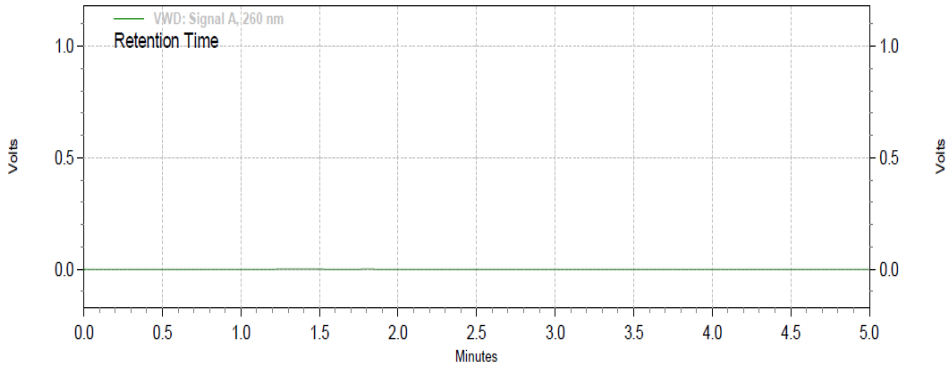
Results of system suitability of Ofloxacin and Nitazoxanide

S.NO	Name	RT	Area	TP	TF	R_S
1	OFLOXACIN	1.527	13689010	3560	1.35	-
2	NITAZOXANIDE	2.890	41583173	6248	1.18	10.9

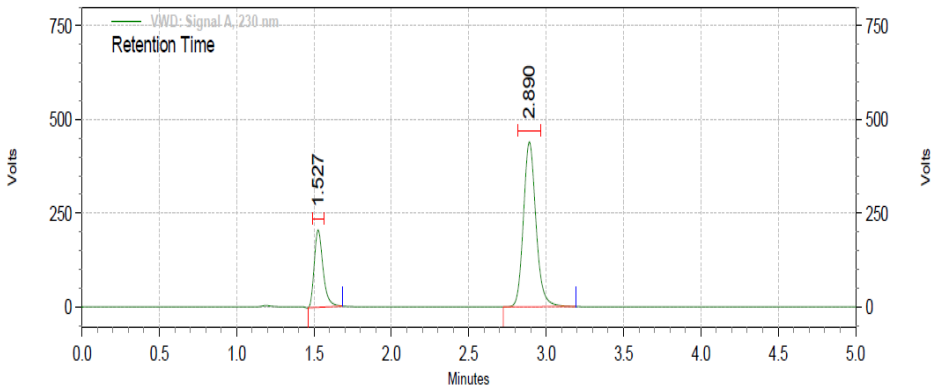
Specificity: Checking of the interference in the optimized method. We should not found interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.



Blank Chromatogram



Placebo Chromatogram



Optimized Chromatogram

Accuracy:

Preparation of Standard stock solutions: About 10 mg of OFLOXACIN and 10mg of NITAZOXANIDE were weighed into a 50 ml volumetric flask, to this 50 ml of mobile phase was added, sonicated and the volume was made up to mark with the mobile phase. **Preparation of 50% spiked solution:** 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipette out, and make up to the mark with diluents. **Preparation of 100% spiked solution:** 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

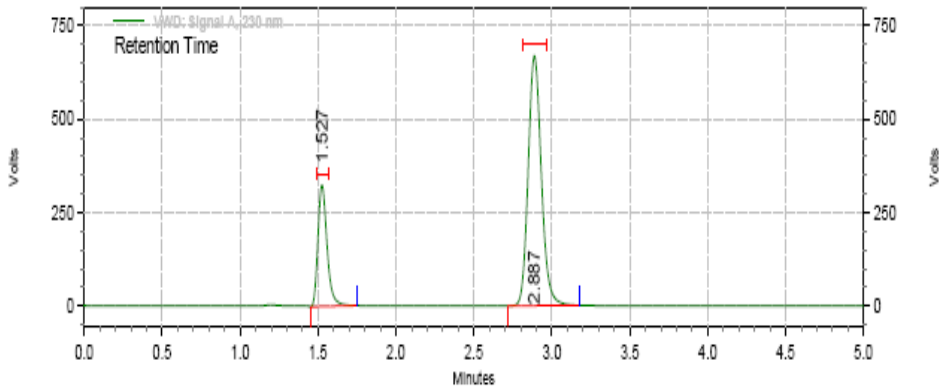
Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluents.

Accuracy table of Ofloxacin

%Recovery	Amount present (µg/ml)	Amount found (µg/ml)*	Percent Recovery *	% Mean Recovery
50%	250	252.35	100.9	
100%	500	498.21	99.6	100.5
150%	750	756.76	100.9	

Accuracy table of Nitazoxanide

%Recovery	Amount present (µg/ml)	Amount found (µg/ml) *	Percent Recovery *	% Mean Recovery
50%	250	252.35	100.9	
100%	500	498.21	99.6	100.5
150%	750	756.76	100.9	



Accuracy chromatogram

Precision:

Preparation of Standard stock solutions: About 10 mg of OFLOXACIN and 10mg of NITAZOXANIDE were weighed into a 50 ml volumetric flask, to this 50 ml of mobile phase was added, sonicated and the volume was made up to mark with the mobile phase.

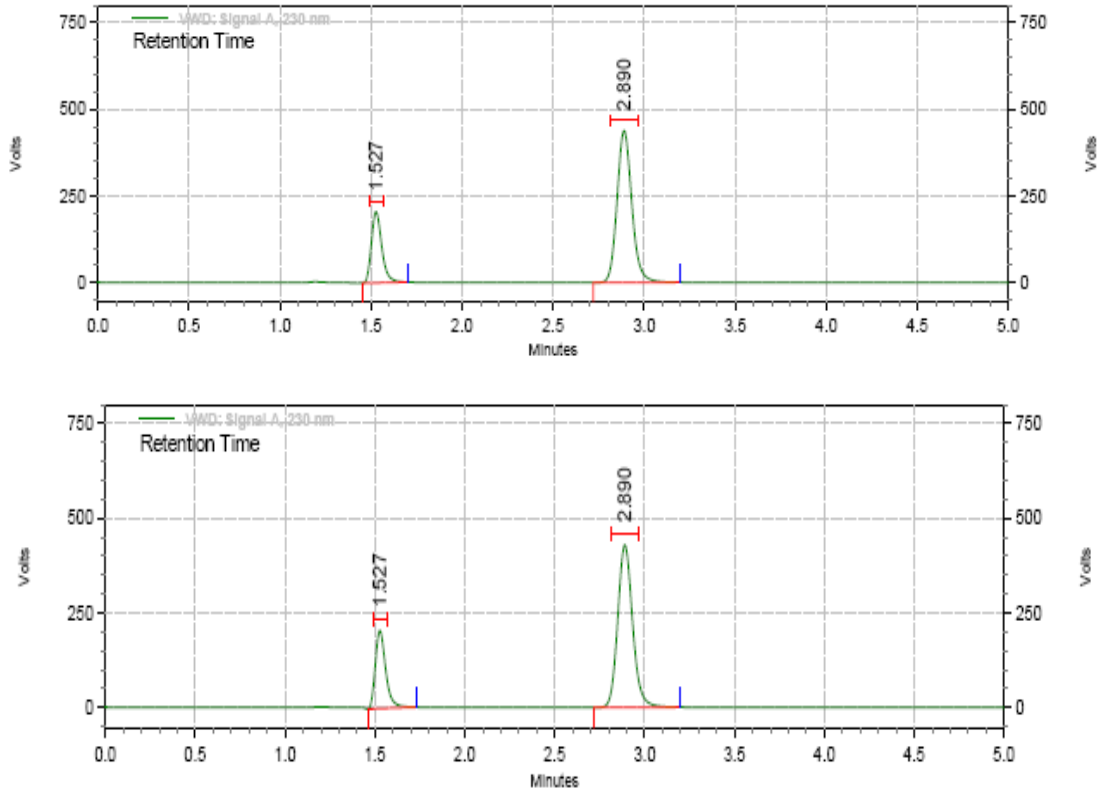
Preparation of Sample stock solutions: Crush more than 20tablets then weigh a quantity of powder equivalent to 500 mg of NITAZOXANIDE and 200mg OFLOXACIN in 200 ml volumetric flask and add70mL of mobile phase then sonicated it for 30 min intermittent shacking after 30min make up volume with mobile phase. Pipetted 5 ml of the clear solution in to 25 ml volumetric flask and make up volume with mobile phase. Filter the solution through 0.45µm filter paper.

Preparation of Sample working solutions (100% solution): From the filtered solution 1ml was pipette out into a 10ml volumetric flask and made up to 10ml with diluents.

System Precision:

Injection	OFLOXACIN		NITAZOXANIDE	
	Area	%Assay	Area	%Assay
1	13970043	100.2	41588622	99.5
2	14027516	100.6	41633302	99.6
3	14034587	100.6	41460630	99.2
4	13942258	100.0	41238796	98.7
5	13788168	98.9	41582369	99.5
6	13986421	100.3	41519805	99.3
Average	-	100.1	-	99.3
SD	-	0.6	-	0.3
%RSD	-	0.6	-	0.3

System precision table of Ofloxacin and Nitazoxanide



System precision chromatogram

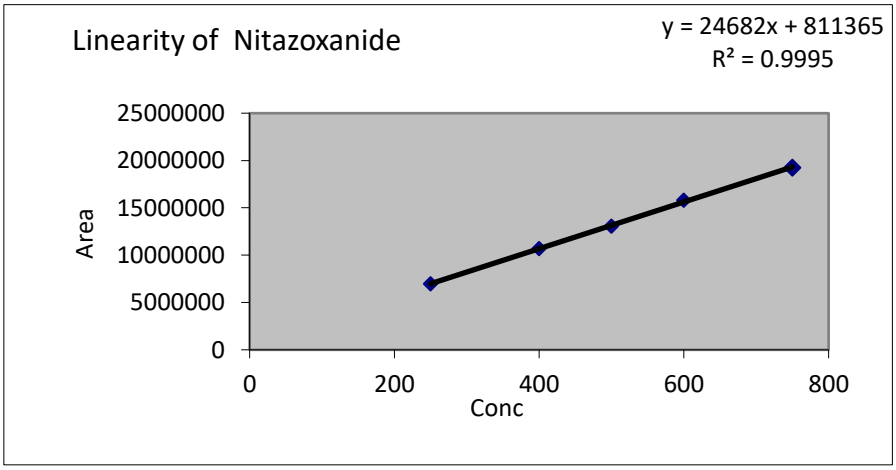
Linearity and Range:

Preparation of standard stock solution

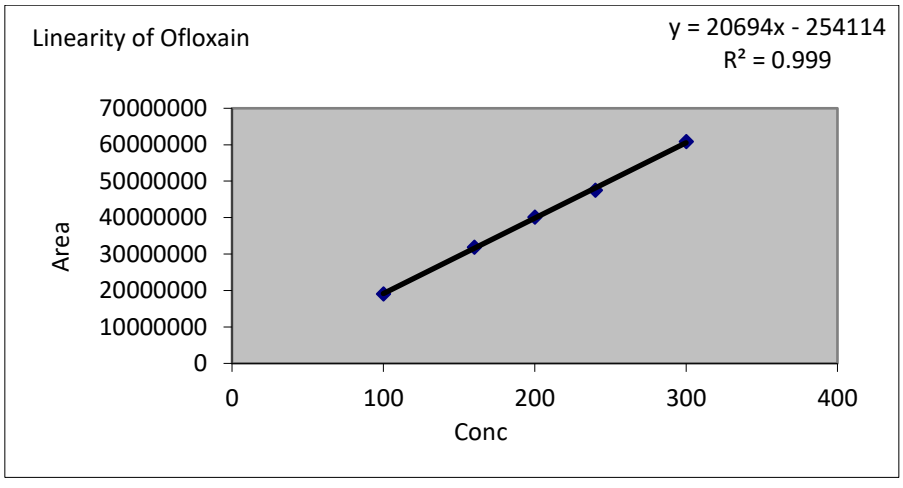
Standard stock solutions of OFLOXACIN (2000µg/ml) and NITAZOXANIDE (5000mg/ml) were prepared by dissolving 200 mg of OFLOXACIN and 500 mg of NITAZOXANIDE in 100 ml of mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min.

Linearity Preparations.

Preparations	Volume from standard stock transferred in ml	Volume made up in ml (with mobile phase)	Conc. obtained (µg/ml)	
			NITAZOXANIDE	OFLOXACIN
Preparation 1	1.0	20	250	100
Preparation2	1.6	20	400	160
Preparation 3	2.0	20	500	200
Preparation 4	2.4	20	600	240
Preparation 5	3.0	20	750	300



Calibration curve of Nitazoxanide

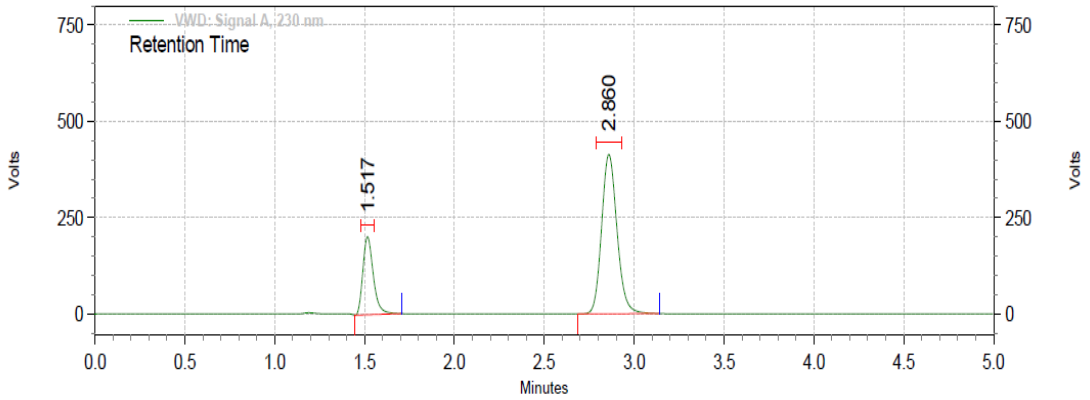


Calibration curve of Ofloxacin

Robustness:

The Robustness of the method was determined. The results obtained by deliberate variation in method parameters are summarized below in Table

Chromatographic changes		Theoretical Plates		Tailing factor		Resolution
		NITAZOXANIDE	OFLOXACIN	NITAZOXANIDE	OFLOXACIN	Between OFLOXACIN&NITAZOXANIDE
Flow rate (ml/min)	0.4	4236	7185	1.18	1.48	11.9
	0.6	3708	5930	1.42	1.18	10.9
Temperature(°C)	25	3358	5345	1.32	1.14	10.3
	35	3330	5630	1.28	1.17	10.4



Robustness chromatogram

Limit of Detection:

$$\mathrm{LOD} = \frac{3.3\sigma}{S}$$

= (3.3) *(0.002)/24682
= 2.67µg/ml (Nitazoxanide)
= (3.3) * (0.002)/20694
= 3.18µg/ml (Ofloxacin)

Where, σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

Limit of Quantification:

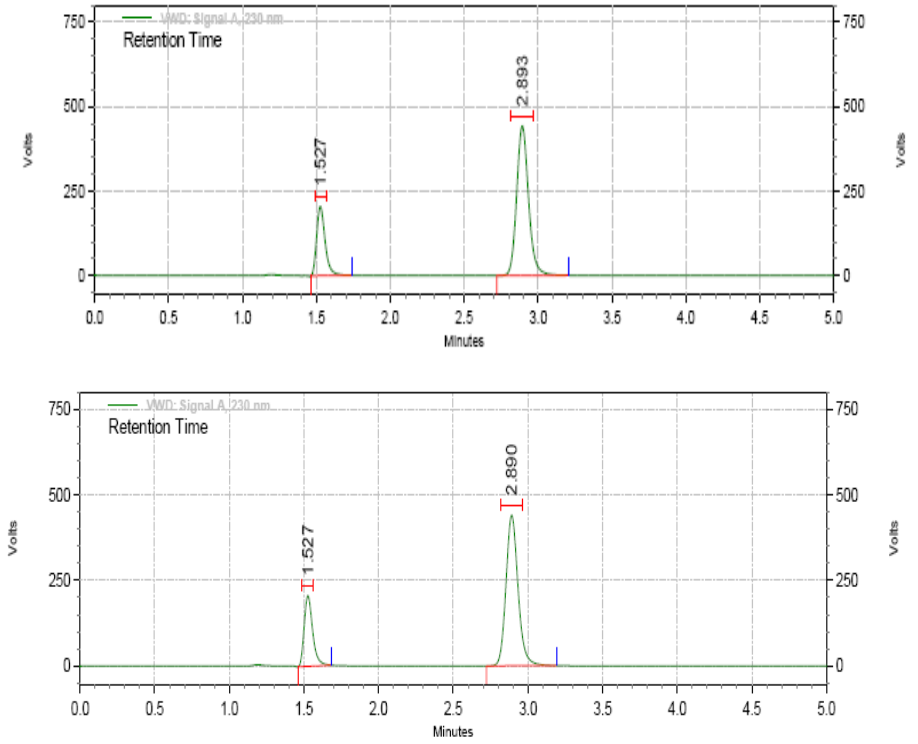
$$\mathrm{LOQ} = \frac{10\sigma}{S}$$

= (10) *(0.002)/24682
= 8.10µg/ml (Nitazoxanide)
= (10) * (0.002)/206946
= 9.66µg/ml (Ofloxacin)

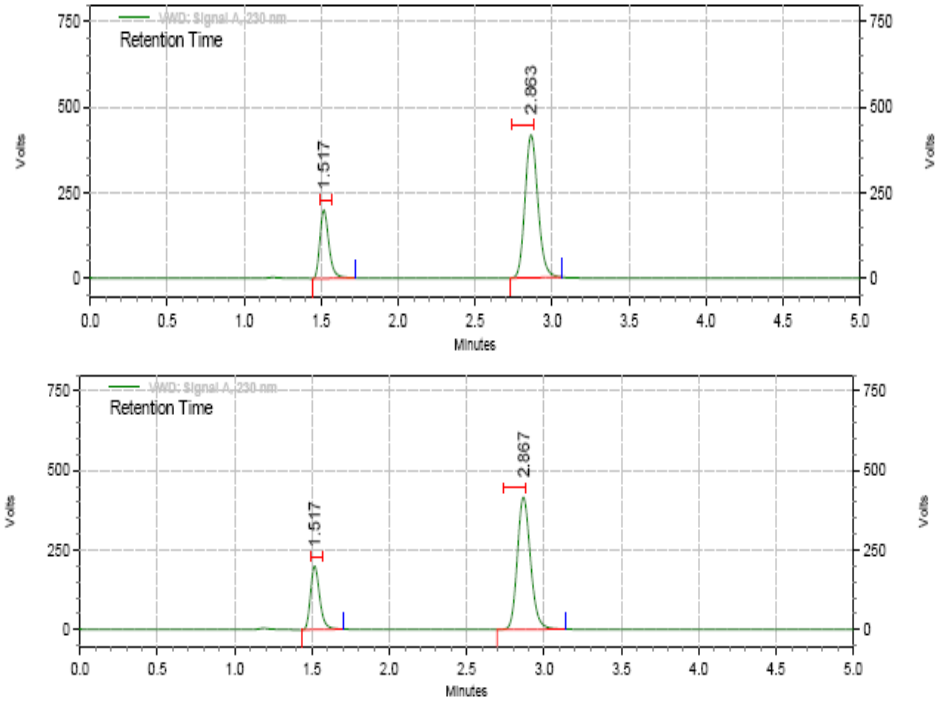
Where
σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

Assay:

About 500 mg of NITAZOXANIDE and 200mg OFLOXACIN of were weighed into a 200 mL volumetric flask, to this 70mL of mobile phase was added, sonicated and the volume was made up with the mobile phase. Pipetted 5 mL of the clear solution in to 25 mL volumetric flask and make up volume with mobile phase.



Chromatogram of standard solution



Chromatogram of sample solution

OFLOXACIN			NITAZOXANIDE	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	13970043	13862751	41588622	43574129
Injection-2	13965702	13862458	41585689	43574258

Injection-3	13875680	13862515	41585781	43574215
Injection-4	13878674	13862720	41598452	41575025
Injection-5	13970251	13862781	41598964	38576027
Average Area	13932070	13862645	41591502	38574731
Assay(%purity)	99.50		101.40	

CONCLUSION

A simple precise, precise rapid method has been developed for the simultaneous estimation of OFLOXACIN and NITAZOXANIDE in pharmaceutical dosage form by RP-UHPLC.

The optimum wavelength for the determination of OFLOXACIN and NITAZOXANIDE was selected at 230 nm on the basis of isobestic point. Several trials were performed with dissimilar mobile phases in dissimilar ratios, but finally Sodium Phosphate Buffer pH 3.0: Acetonitrile (80:20) %v/v) was selected as good peak symmetry and resolution between the peaks was observed. The Retention time of OFLOXACIN and NITAZOXANIDE were found to be 1.953 and 3.733 min respectively. The Retention times for both the drugs were considerably less compared to the Retention time obtained for the drugs in the other mobile phase.

The calibration curve was obtained by plotting peak area versus the concentration over the range of 100-300 µg/ml for NITAZOXANIDE and250-750 µg/ml for OFLOXACIN. From linearity the correlation coefficient R^2 value was found to be 0.999 for NITAZOXANIDE and 0.999 for OFLOXACIN. The proposed UHPLC method was also validated for method precision, system precision and system suitability. The percentage of recovery of OFLOXACIN and NITAZOXANIDE were found to be 99.5 and 99.2respectivelyshows that the proposed method is highly accurate.

Hence the proposed method is highly accurate, sensitive and precise and it successfully applied for the quantification of API content in the commercial formulations of OFLOXACIN and NITAZOXANIDE in Educational institutions and Quality control laboratories.

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