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FORCED SIMULTANEOUS ESTIMATION OF NITAZOXANIDE AND OFLOXACIN BY UPLC AND THEIR DEGRADATION STUDIES

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Abstract: A simple, Accurate, precise method was developed for the simultaneous estimation of the Nitazoxanide and Ofloxacin in Tablet dosage form. Chromatogram was run through Zorbax SB C18 (50x2.1mm ID) 1.7µm Mobile phase containing Sodium Phosphate Buffer (pH 3.0): Acetonitrile (80:20) %v/v was pumped through column at a flow rate of 0.5ml/min. Temperature was maintained at 15°C. Optimized wavelength selected was 241.0 nm. Retention time of Nitazoxanide and Ofloxacin were found to be 3.373min and 1.953min. % RSD of the Nitazoxanide and Ofloxacin were found to be 0.3and 0.6respectively. %Recovery was obtained as 99% and 99.6% for Nitazoxanide and Ofloxacin respectively. LOD, LOQ values obtained from regression equations of Nitazoxanide and Ofloxacin were 2.67µg/ml, 3.18µg/ml and 8.10µg/ml, 9.66µg/ml respectively. Regression equation of Nitazoxanide is y = 2468.x + 811365 and y = 206946x -2E+06 of Ofloxacin. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Keywords: Nitazoxanide, Ofloxacin, UPLC.

INTRODUCTION:

OFLOXACIN:[1-4]IUPAC Name:8-Fluoro-3-methyl-9-(4-4 methyl-piperazin-1-yl)-6-oxo-2,3-dihydro-6H-1-oxa-3a -aza-phenalene-5-carboxyl acid.

Molecular Formula: C18H20FN3O4

Molecular Weight: 361.368

Pharmacological Category: Anti-Bacterial Agent.

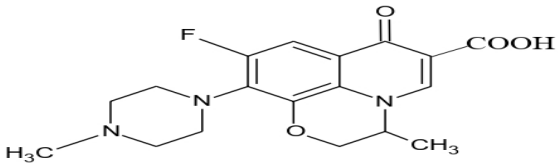

Fig 1. structure of Ofloxacin

NITAZOXANIDE: [5-11] IUPACName:2-[(5-nitro-1,3-thiazol-2-yl)carbonyl]phenyl acetate
Chemical Formula: C12H9N3O5-S

Molecular Weight:307.281

Pharmacological Category: Anti-Infective Agents

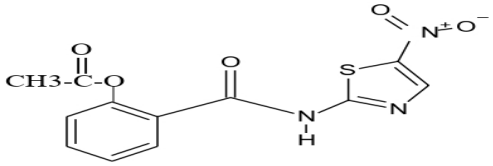

Fig 2.structure of Nitazoxanide

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Materials and Methods
Materials
Equipment's used in present study.

UV-Visible Spectrophotometer	Thermo Technology Ltd
UPLC	Agilent model no. 1290
Ultra Sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Thermo Pvt
Electronic balance	Shimadzu 124
Syringe	Hamilton glass type
HPLC Column	Zorbax SB C18 column (50x2) 1.7µm

Chemicals: Chemicals and Solvents for present study: Sodium Phosphate Monobasic, Acetonitrile, Water, HPLC Grade Orthophosphoric acid, Nitazoxanide drug and Ofloxacin bulk drugs, Nitazoxanide drug500mg and Ofloxacin200mg Tablets.

Methods Procedure to prepare 3.0 phosphate buffer: Weigh and dissolve 2.38gm of Sodium Phosphate Monobasic1000 mL of water by stirring slowly. Adjust the pH of solution to 3.0 by using diluted orthophosphoric acid. Prepared buffer was filtered to remove fine particles through 0.45µm filters.

Results and Discussions Solubility data: These solubility studies were conducted at 25 0C Solubility study data as follows:

Solvent Name	OFLOXACIN	NITAZOXANIDE
water	Soluble	Soluble
Methanol	Soluble	Soluble
Acetonitrile	Sparingly Soluble	Sparingly Soluble

Assurance of Working Wavelength (λmax): In concurrent estimation of two medications isosbestic wavelength was utilized. Isosbestic point was where the molar absorptivity was the equivalent for two substances that were interconvertible. So, this wavelength was utilized in concurrent estimation to evaluate the two medicationsprecisely.

Planning of Standard arrangement: Around 10 mg of OFLOXACIN and 10mg of NITAZOXANIDE DRUG were weighed into a 50 ml volumetric cup, to this 50 ml of portable stage was included, sonicated and the volume was made up to stamp with the versatile stage.

Assurance of ISOBESTIC POINT (λmax): The ingestion bend indicates trademark assimilation maxima at 210 nm for OFLOXACIN, 237 nm for NITAZOXANIDE DRUG and at 241 nm same absorbance for both the medications, i.e., isosbestic point. Consequently, 241nm was chosen as identifier wavelength for the UHPLC chromatographic strategy.

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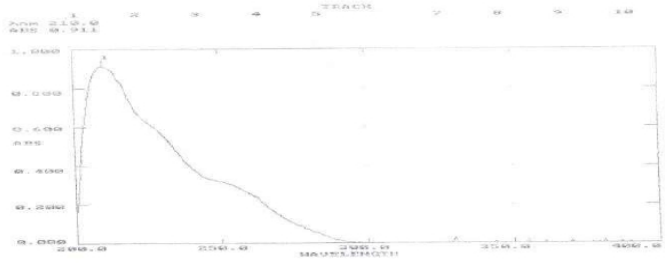

Fig-1 Scanning of drug OFLOXACIN (210 nm) under UV-VIS

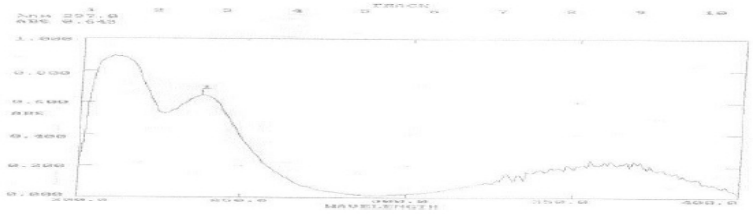

Fig-2 UV-VIS Spectrum scanning for NITAZOXANIDE DRUG (237 nm)

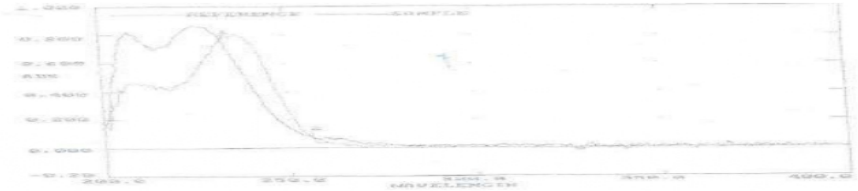

Fig-3 Over lay of scanning graphs of both drugs (241 nm)

Optimized Chromatographic Conditions for Nitazoxanide Drug and Ofloxacin Drug
Optimised conditions to run chromatogram.

Mobile phase	SodiumphosphateBufferpH3.0: Acetonitrile (80:20) %v/v
Column	Zorbax SB C18 (50x2.1mm ID) 1.7µm
Flow rate	0.5ml/min
Column temperature	30°C
Sample temperature	15°C
Wavelength of Detection	241 nm
Injection volume	10µl
Run time	5 min
Rt maintenance time	OFLOXACINDRUGANDNITAZOXANIDE DRUG 1.953and 3.733min individually.

ASSAY

200mg of drug Ofloxacin and 500mg of drug Nitazoxanide were weighed accurately and taken into 200ml volumetric flask, To this add 70ml of mobile phase and sonicate this to eradicate bubbles, make up volume with mobile phase. From above solution pipette out 5ml of solution and make up this to 25 ml in a separate volumetric flask.

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Preparation of Sample solution

Samples: Nitazoxanide drug-500mg and Ofloxacin-200mgTablets

Take 20 tablets and weigh them and crush all these 20 tablets and from this powder collect powder equivalent to 500 mg of Nitazoxanide and 200 mg of Ofloxacin. Above collected powder mixed with 70ml mobile phase in a 200ml volumetric flask, sonicate this for 30 min with intermittent shaking. From these sonicated solutions pipetted out 5ml of clear solution in to a 25 ml flask and make up the volume with same mobile phase. Filter this solution using 0.45 micro filterpaper.

The quantity of OFLOXACIN and NITAZOXANIDE present in the pharmaceutical dosage form by using the formula shown here, results were noted in table: %

Assay/purity=AT/AS×P/100×AW/LC×WS/DS×DT/WT×100 Were,
AS: Average pinnacle region because of standard arrangement AT: Peak zone because of measure readiness

WS: Standard Weight of OFLOXACIN/NITAZOXANIDE DRUG in mg WT: Weight of test in test readiness

DT: Dilution of test readiness

DS: Dilution of standard arrangement

Table: 1Assay results table

	OFLOXACIN DRUG		NITAZOXANIDE DRUG	
	Standard Area	Sample Area	Standard Area	Sample Area
Trial -1	13970043	13862751	41588622	43574129
Trial -2	13965702	13862458	41585689	43574258
Trial - 3	13875680	13862515	41585781	43574215
Trial - 4	13878674	13862720	41598964	41575025
Trial -5	13970251	13862781	41598964	38576027
Average Area	13932070	13862645	41591502	38574731
Assay (%)	99.50		101.40	

Table-2 Results of assay:

Drug	Label claim(mg)	Amount found(mg) %	% Assay(purity)
NITAZOXANIDE	500	497.87	500.39
OFLOXACIN	200	200	198.57

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Observations from above results: So, the both drugs % assay (purity) was found to be within the range. The percentage purity of both OFLOXACIN and NITAZOXANIDE was found within the limits I.e., 98-102%.

Method Validation

System Suitability& Precision

Results for System Suitability of Drug Ofloxacin And Nitazoxanide Drug.Table-3

OFLOXACIN					NITAZOXANIDE DRUG.			
Injection	RT	Peak area	Theoretical plates	Tailing factor	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	1.527	13689010	3506	1.39	2.890	41583173	1.14	6.58
2	1.525	13947497	3782	1.52	2.890	41423589	1.19	6.47
3	1.526	13804506	3846	1.24	2.893	41621024	1.24	6.55
4	1.521	13961832	3975	1.10	2.890	41575805	1.44	5.98
5	1.525	14036560	3544	1.12	2.890	41528013	1.27	6.75
6	1.528	13887923	3877	1.25	2.893	41518183	1.41	-
Mean	1.525	13887888	-	-	2.891	41541631	-	-
SD	0.002	124603	-	-	0.002	69088	-	-
RSD (%)	0.015	0.9	-	-	0.1	0.2	-	-

Method Precision Results for Ofloxacin And Nitazoxanide Drug

Method precision was determined by injecting 6 different sol'n of sample solutions OFLOXACIN (200µg/ml) & NITAZOXANIDE (500µg/ml) for 6 times are prepared separately.

Table-4 The chromatograms were recorded and the results were summarized

INJECTION	OFLOXACIN		NITAZOXANIDE DRUG	
	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

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Linearity and Range

Standard stock solutions of OFLOXACIN (2000µg/ml) and NITAZOXANIDE DRUG (5000mg/ml) was prepared by dissolving 200 mg of OFLOXACIN and 500 mg of NITAZOXANIDE in 100 ml of mobile phase. Filter the solution using 0.45-micron syringe. Sonicate for 5 min furtherdilutions.

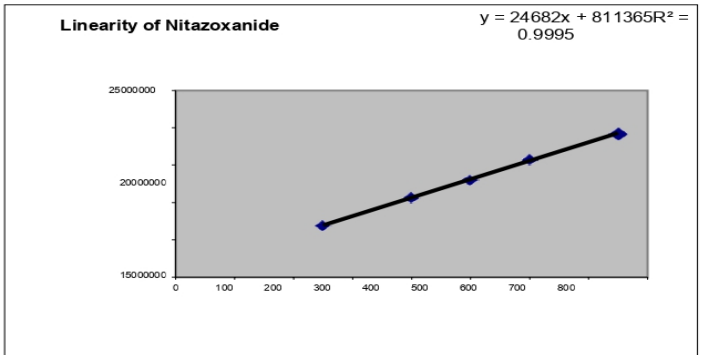
Linearity Preparations.Table-5

Preparations	Volume from Standard stock transferred in ml	Volume made upin ml(with mobile phase)	Con.obtained((µg/ml)	
			NITAZOXANIDE DRUG	DRUG 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

LINEARITY DATA OF NITAZOXANIDE DRUG:Table-6

S.No	Concentration (µg/ml)	Area
1	250	6967182
2	400	10692464
3	500	13063312
4	600	15797447
5	750	19240546

Fig-4 Graph for Linearity data of NITAZOXANIDE drug



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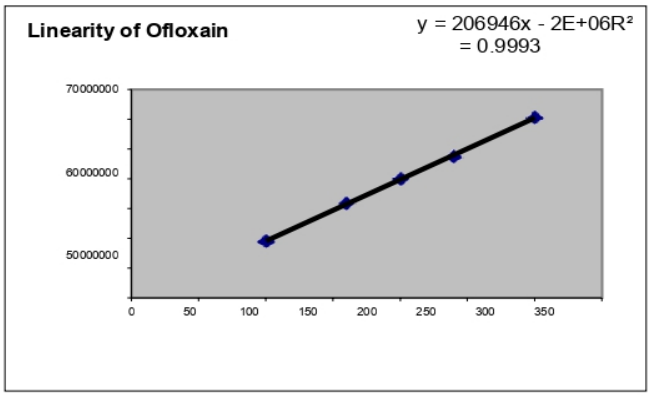
LINEARITY DATA OF DRUG OFLOXACIN.Table-7

S.NO	Concentration (µg/ml)	Area
1	100	19050341
2	160	31852575
3	200	40083328
4	240	47461995
5	300	60817935

Fig-5 Graph for Linearity data of drug OFLOXACIN



Observation for linearity.Tabe-8

Parameter	NITAZOXANIDE DRUG	DRUG OFLOXACIN
Correlation coefficient	0.9995	0.9993
Slope	24682	206946
Intercept	811365	254114

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SPECIFICITY:

Blank solution was injected and the chromatogram was recorded.
Placebo solution was prepared and it was injected and the chromatogram was recorded.

Fig-6 Chromatogram of DRUG OFLOXACIN and NITAZOXANIDE DRUGBlank

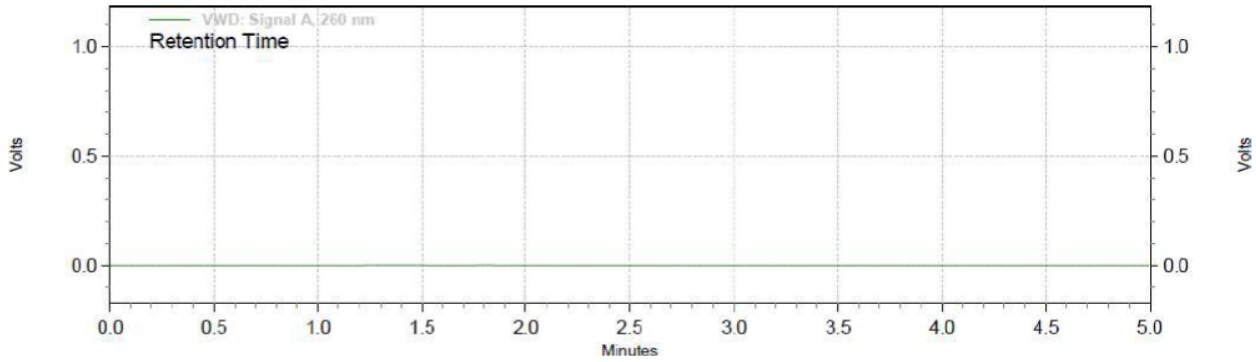
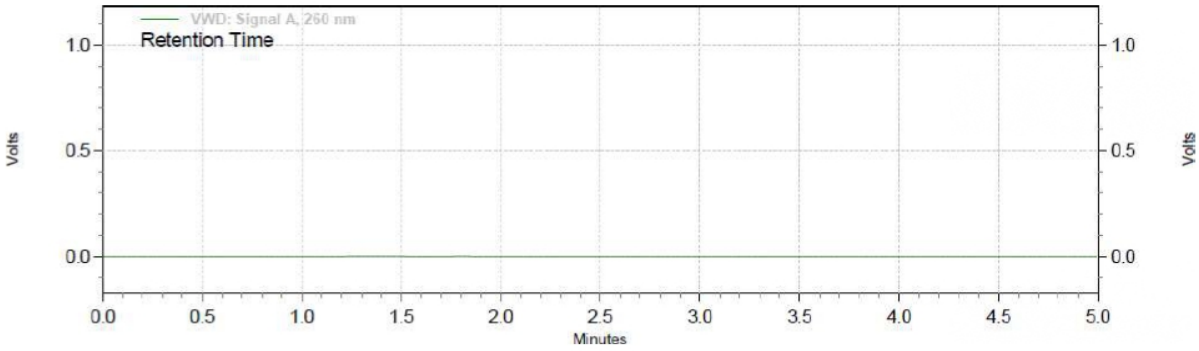


Fig-7 Chromatogram of Placebo



ACCURACY:

Table-9 Results for Recovery OFLOXACIN.

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

* Mean of three observations

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Table -10Results for Recovery of NITAZOXANIDE.

%Recovery	Amount present (µg/ml)	Amount found (µg/ml)*	Percent Recovery *	% Mean Recovery
50%	100	101.2	101.2	100.3
100%	200	198.03	99.0	
150%	300	301.66	100.6	

* Mean of three observations

Acceptance criteria

The % recovery of OFLOXACIN and NITAZOXANIDE should lie with in the range of 98% - 102%.

Result

The % mean recovery of OFLOXACIN and NITAZOXANIDE were founded in between the range from98.0to102%.

LIMIT OF DETECTION (LOD)

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

= (3.3)*(0.002)/24682

= 2.67µg/ml (Nitazoxanide drug)

=(3.3)* (0.002)/20694

=3.18µg/ml (Drug Ofloxacin)

Where, σ = the standard deviation of the response

S = the slope of the linearity curve

The slope S may be estimated from the linearity graph of the analyte.

Observation:

The LOD for this method was found to be 2.67µg/ml (Nitazoxanide drug) and 3.18µg/ml (Drug Ofloxacin)

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LIMIT OF QUANTIFICATION (LOQ)

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

$$= (10)*(0.002)/24682$$

$$= 8.10\mu g/ml \text{ (Nitazoxanide drug)}$$

$$=(10)* (0.002)/206946$$

$$=9.66\mu g/ml \text{ (Drug Ofloxacin)}$$

Where

σ = the standard deviation of the response

S = the slope of the linearity graph

. **OBSERVATION :**

The LOQ was found to be 8.10µg/ml (NTZ) and 9.66µg/ml (OFL).

ROBUSTNESS:

RESULTS FOR ROBUSTNESS OF DRUG OFLOXACIN AND NITAZOXANIDE DRUG.Table-11

Chromatographi cchanges		Theoretical Plates		Tailing factor		Resolution
		NITAZ OXAN IDE DRUG	DRU G OFL OXA CIN	NITA ZOX ANI DE DRU G	DRU G OFL OXA CIN	Between DRUG OFLOXA CIN&NIT AZOXANIDE DRUG
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

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RUGGEDNESS AND ACCEPTANCE CRITERIA:

The % Relative std deviation of Assay values between two analysts should be not more than2.0%.

Results for Ruggedness

DRUG OFLOXACIN	%Assay	NITAZOXANIDE DRUG	%Assay
Analyst 01	99.74	**Analyst 01**	101.24
Analyst 02	99.84	**Analyst 02**	101.30
RSD(%)	0.15	RSD (%)	0.31

Conclusion

Another exact, exact fast technique has been produced for the synchronous estimation of DRUG OFLOXACIN and NITAZOXANIDE DRUG in pharmaceutical measurement frame by RP- UHPLC.

The ideal wavelength for the assurance of DRUG OFLOXACIN and NITAZOXANIDE DRUG was chosen at 230 nm based on isosbestic point. A few preliminaries were performed with divergent versatile stages in unique proportions, however at last Sodium Phosphate Buffer pH 3.0: Acetonitrile (80:20) %v/v) was chosen as great pinnacle symmetry and goals between the pinnacles was watched. The Rt maintenance time of OFLOXACIN DRUG and NITAZOXANIDE DRUG were observed to be 1.953and 3.733 min individually. The Rt maintenance times for both the medications were impressively less contrasted with the Rt maintenance time acquired for the medications inthe other portablestage.

The linearity diagram was gotten by plotting top zone versus the focus over the scope of 100-300µg/ml For NITAZOXANIDE and 250-750 µg/ml for OFLOXACIN. From linearity the relationship coefficient R^2 esteem was observed to be 0.999 for NITAZOXANIDE and 0.999 for OFLOXACIN. The proposed UHPLC strategy was additionally approved for technique exactness, framework accuracy and framework reasonableness. The level of recuperation of OFLOXACIN and NITAZOXANIDE were observed to be 99.5 and 99.2respectivelyshows that the proposed technique was very precise.

Subsequently the proposed strategy was exceptionally exact, touchy and exact and it effectively connected for the measurement of API content in the business details of DRUG OFLOXACIN and NITAZOXANIDE DRUG in Educational establishments and Quality control labs.

References

1. Chatwal, R. G.; Anand, K. S. Elite fluid chromatography. Instrumental techniques for substance investigation, 5thed.; Himalaya distributers: Mumbai, 2010; 2.570-2.629.
2. Sharma, B. K. Elite fluid chromatography. Instrumental techniquesfor substance investigation, 24th ed.; Goelpublishers: Meerut, 2005; 295 -300.
3. Dong, W. M. HPLC instrumentation and patterns. Current HPLCfor rehearsing researchers, USA, 2006; 5-10,78-110.
4. Swartz,M. E.; Ira Krull, S, Analytical strategy improvement. Systematic technique advancement and approval, first ed.; Marcel Dekker, Inc: New York, 2009; 17-80.
5. Satinder, A.; Dong, M. W. Strategy improvement and approval. Pharmaceutical examination by HPLC, fifteenth ed.; New York, 2005;16-70.
6. Snyder, R. L.; Kirkland, J. J.; Glajch, L. J. Beginning. Down to earth HPLC Method Development, second ed.; New York, 1997; 30-100.
7. ICH, Text on Validation of Analytical Procedures, ICH – Q2A,International Conference on Harmonization, IFPMA, Geneva, 1995, 2-3, A– 1 to A–3.
8. ICH, Validation of Analytical Procedures: Methodology, ICH –Q2B, International Conference on Harmonization, 1996,1-3.
9. ICH Guidelines, Q2 (R1) - Validation of Analytical Procedures: Textand Methodology,2005,1-6.
10. Drug Ofloxacin tranquilize profile -https://www.drugbank.ca/drugs/DB01165