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"METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF STRONG ANTIBACTERICIDAL ANTIBIOTIC DRUG "

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Abstract

Levofloxacin (DR-3355, Daiichi) is a new fluoroquinolone antibiotic which is the active isomer of ofloxacin (DL-8280, Daiichi). By removing the inactive isomer, the in vitro activity of levofloxacin is 8-128 times higher than that of ofloxacin.

This means that bacterial species, which have borderline susceptibility to ofloxacin and other first generation fluoroquinolones (e.g., pneumococci and organisms causing atypical pneumonia), are considerably more sensitive to levofloxacin.

The pharmacokinetics of levofloxacin, which is available for both oral and i.v. administration, are characterised by a very high bioavailability, low (30-40%) protein binding, high tissue concentrations and elimination via the kidneys with minimal liver metabolism.

As a consequence of the low degree of metabolism, levofloxacin does not interact with other drugs to any major extent. The safety and efficacy of levofloxacin are well documented in lower respiratory tract infections, skin and soft tissue infections, and urinary tract infections.

The safety profile seems advantageous and the risks of phototoxicity, CNS toxicity and cardiac reactions (prolongation of QT-time) are low. Serious liver toxicity, leading to the recent withdrawal of trovafloxacin, has not been a problem in levofloxacin studies. Levofloxacin is a valuable addition to the group of fluoroquinolone antibiotics.

Keywords :-

UV Spectrometry ,validation,levofloxacin,phototoxicity,flouroquinolone.

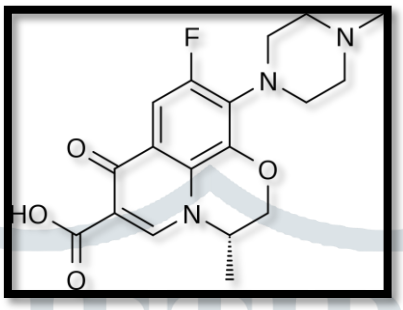
DRUG PROFILE:

LEVOFLOXACIN

GENERAL DATA

Synonym : (-)-Ofloxacin

Chemical Structure :



Drug Bank Code : DB01137

- **IUPAC :** (2S)-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.0^{5},^{13}]trideca-5(13),6,8,11-tetraene-11-carboxylic acid

Molecular Framework : Aromatic heteropolycyclic compounds

Molecular formula : $C_{18}H_{20}FN_3O_4$

Molecular weight : 361.3675

Monoisotopic : 361.143784348

Smiles:
C[C@H]1COC2=C3N1C=C(C(O)=O)C(=O)C3=CC(F)=C2N1CCN(C)CC1

CAS No : 100986-85-4

Physico Chemical Properties

Appearance : White crystals or fine white powder.

Solubility : Practically soluble in water.

Melting Point : 150 °C

Boiling point : 571.5±50.0 °C at 760 mmHg.

pKa : Strongest Acidic - 5.45

Strongest Basic - 6.2

logP : 2.1

Indication : For the treatment of bacterial conjunctivitis caused by susceptible strains of the following organisms: *Corynebacterium* species, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Streptococcus* (Groups C/F/G), Viridans group streptococci, *Acinetobacter lwoffii*, *Haemophilus influenzae*, *Serratia marcescens*.

Pharmacology

Mechanism of action : Levofloxacin inhibits bacterial type II topoisomerases, topoisomerase IV and DNA gyrase. Levofloxacin, like other fluoroquinolones, inhibits the A subunits of DNA gyrase, two subunits encoded by the gyrA gene. This results in strand breakage on a bacterial chromosome, supercoiling, and resealing; DNA replication and transcription is inhibited.

Pharmacodynamics : Levofloxacin, a fluoroquinolone antiinfective, is the optically active L-isomer of ofloxacin. Levofloxacin is used to treat bacterial conjunctivitis, sinusitis, chronic bronchitis, community-acquired pneumonia and pneumonia caused by penicillin-resistant strains of *Streptococcus pneumoniae*, skin and skin structure infections, complicated urinary tract infections and acute pyelonephritis.

Absorption: Absorption of ofloxacin after single or multiple doses of 200 to 400 mg is predictable, and the amount of drug absorbed increases proportionately with the dose.

Metabolism: Mainly excreted as unchanged drug (87%); undergoes limited metabolism in humans.

Route of elimination :
Mainly excreted as unchanged drug in the urine.

Half life : 6-8 hours.

MATERIALS AND METHODS

Instruments:

- HPLC – WATERS Model NO.2695 series Compact System Consisting of Inertsil-C18 ODS column.
- Electronic balance (SARTORIOUS)
- Digital pH meter(POLOMAN)
- Sonicator(FAST CLEAN)

Chemicals:

- Acetonitrile HPLC Grade

Raw Material:

Levofloxacin Working Standard.

METHOD DEVELOPMENT FOR HPLC:

The objective of this experiment was to optimize the assay method for estimation of Levofloxacin based on the literature survey made. So here the trials mentioned describes how the optimization was done.

Trial: 1

Mobile Phase: 100% pure degaussed methanol.

Preparation of Standard Solution:

10mg of f Levofloxacin drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml was taken from this and diluted to 10 ml with mobile phase

Chromatographic Conditions:

Flow rate : 1.0ml/min

Column : Inertsil - C18 ODS column

Detector wavelength : 250nm

Column temp : Ambient

Injection volume : 20µl

Run time : 6min

Retention time : 2.925

Observation: Peak spliting.The trial 1 chromatogram result was shown in Fig:1

Trail: 2.

Mobile Phase: methanol and Acetonitrile were mixed in the ratio of 90:10V/V and sonicated to degas.

Preparation of Standard Solution:

10mg of Levofloxacin drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phase

Chromatographic Conditions:

Flow rate : 1ml/min

Column : Inertsil -C18 ODS column

Detector wavelength : 250nm

Column temp : Ambient

Injection volume : 20µl

Run time : 5min

Retention time : 2.831

Observation: Got more assymetry. The trial 2 chromatogram result was shown in Fig:

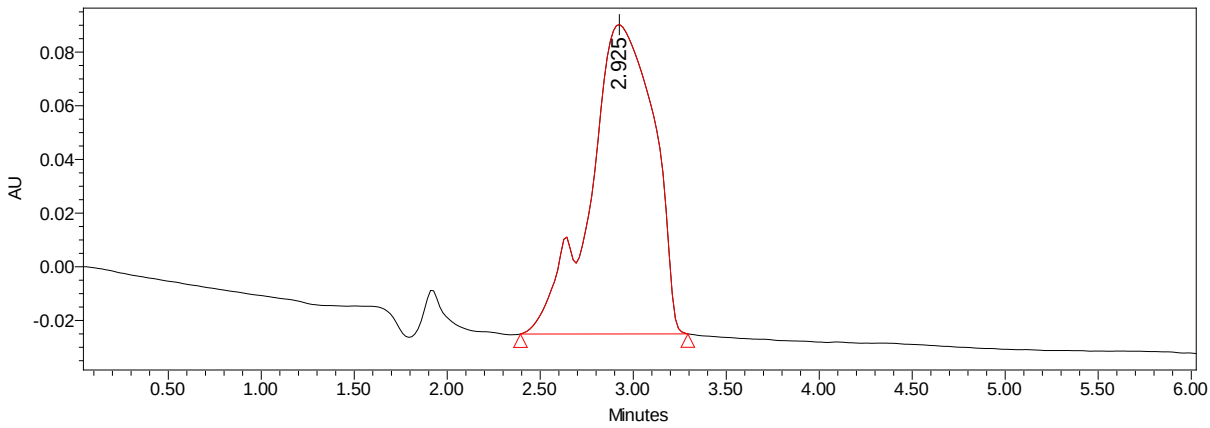
Trail: 3.

Mobile Phase: Methanol and Acetonitrile were mixed in the ratio of 80:20 V/V and sonicated to degas.

Preparation of Standard Solution:

10mg of Levofloxacin drug was weighed and dissolved in 10ml of Mobile phase and taken in 10ml of volumetric flask and sonicated for 20 minutes to get 1000ppm and 1 ml. was taken from this and diluted to 10ml.with mobile phase

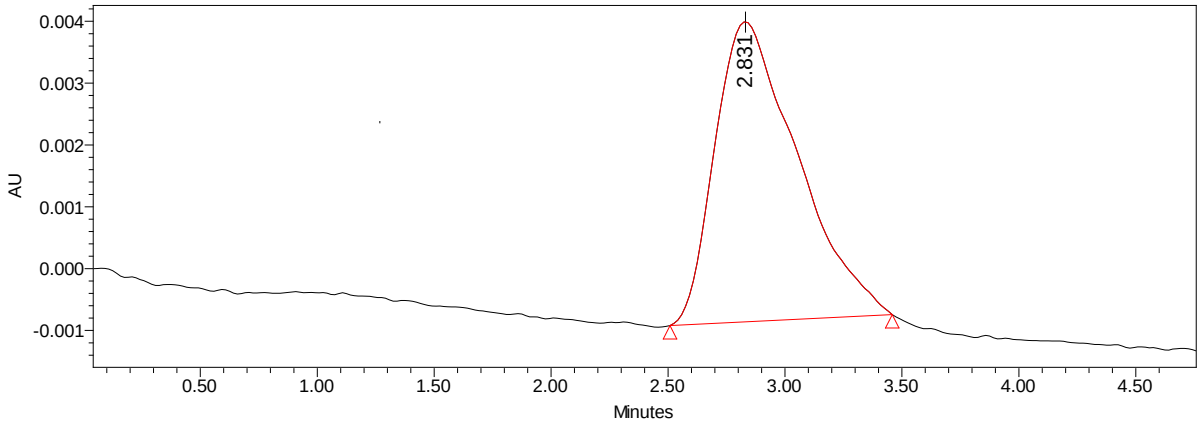
Fig1: Chromatogram of Trial 1



Inference : Peak spliting.

S.NO	Name of the peak	Retention time(min)
1.	LEVOFLOXACIN	2.925

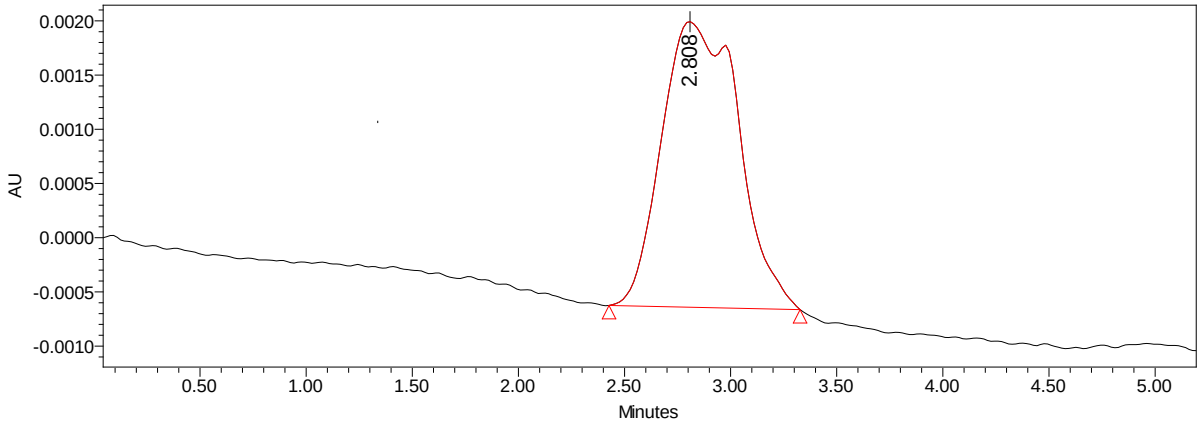
Fig 2: Chromatogram of Trial 2:



Inference : Got more assymetry

S.NO	Name of the peak	Retention time(min)
1	**LEVOFLOXACIN**	2.831

Fig 3: Chromatogram of Trial3

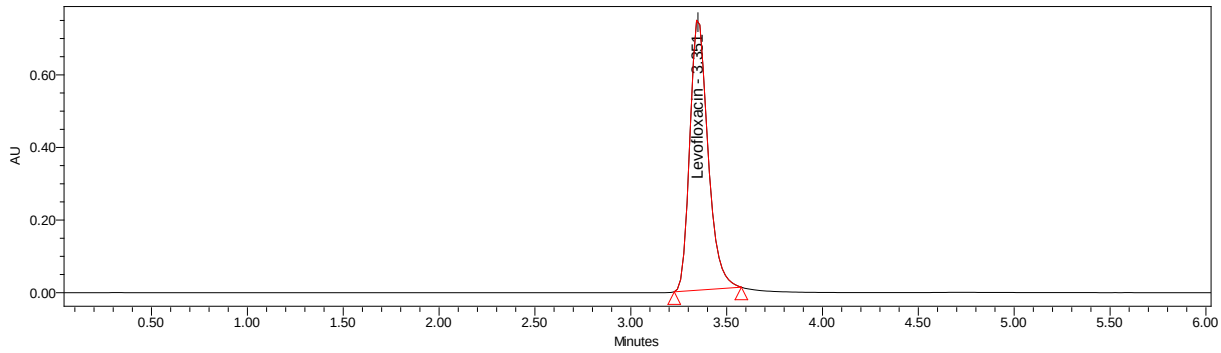


Inference : Got Bad peak.

S.NO	Name of the peak	Retention time(min)
1.	**LEVOFLOXACIN**	2.808

OPTIMIZED METHOD:

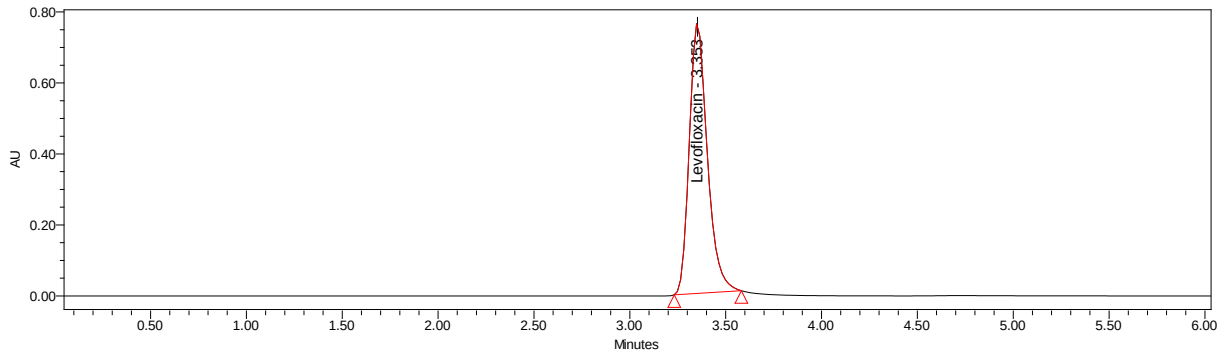
Fig 4: Chromatogram of standard



Inference : Got chromatogram at an Rt of 3.351 for standard

S.NO	Name of the peak	Retention time(min)
1	**LEVOFLOXACIN**	3.351

Fig5:Chromatogram of sample



Inference : Got same peak with same Rt 3.353 as of standard.

S.NO	Name of the peak	Retention time(min)
1.	**LEVOFLOXACIN**	3.353

VALIDATION DATA

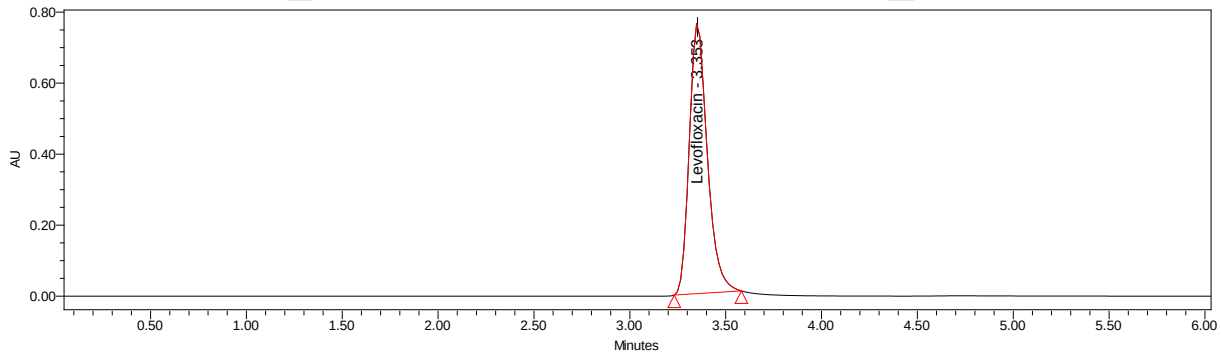
1 SYSTEM SUITABILITY:

TABLE-1: Data of System Suitability

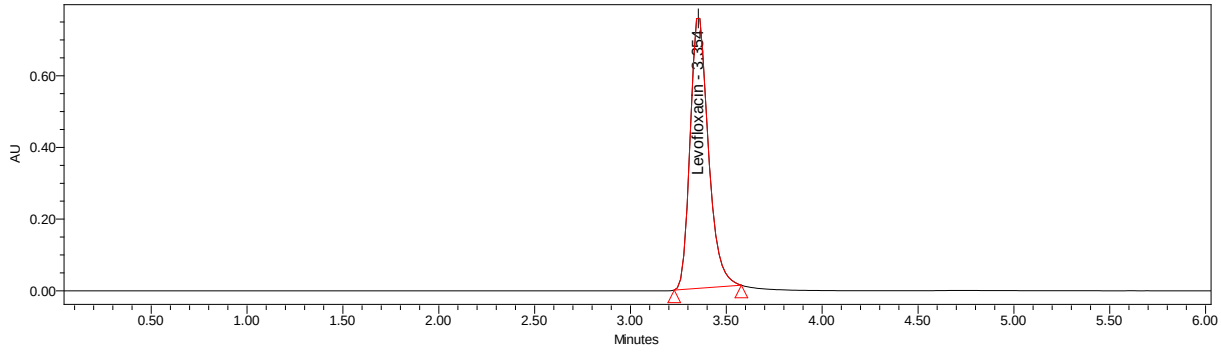
Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.353	645478.48	10621	1.101
2	3.354	645449.32	10630	1.103
3	3.352	645455.29	10632	1.101
4	3.352	645423.23	10645	1.103

5	3.353	645480.63	10650	1.102
Mean	3.3528	645457.39	10635	1.102
SD	0.000837	23.5654	-------	-------
% RSD	0.024954	0.00365	-------	-------

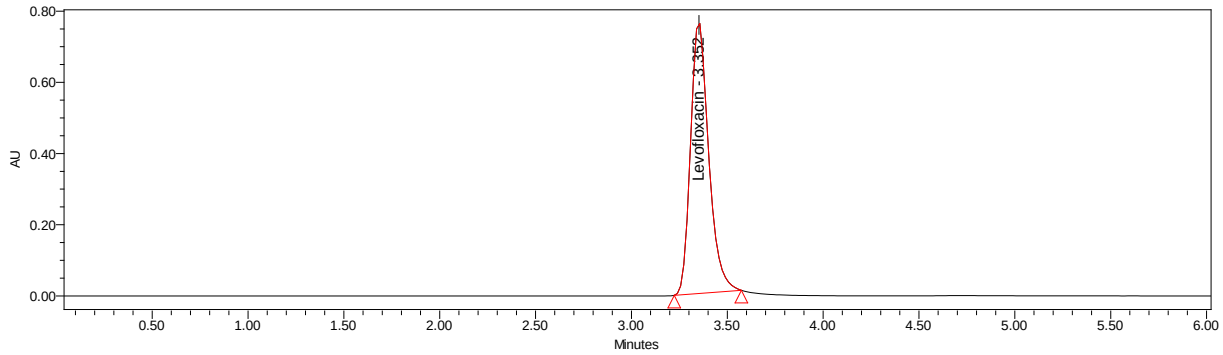
Fig6 : Chromatograms of system suitability(standards 1-5)



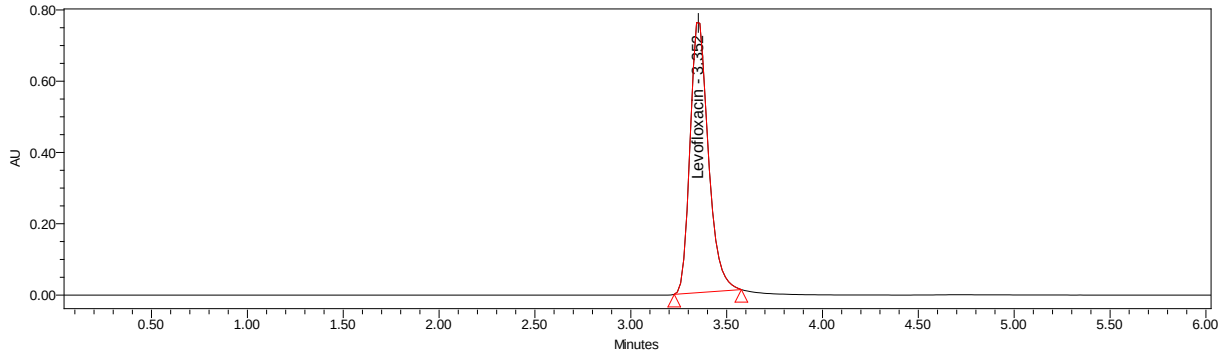
Inference : System sutabilty Chromatogram for standard – 1



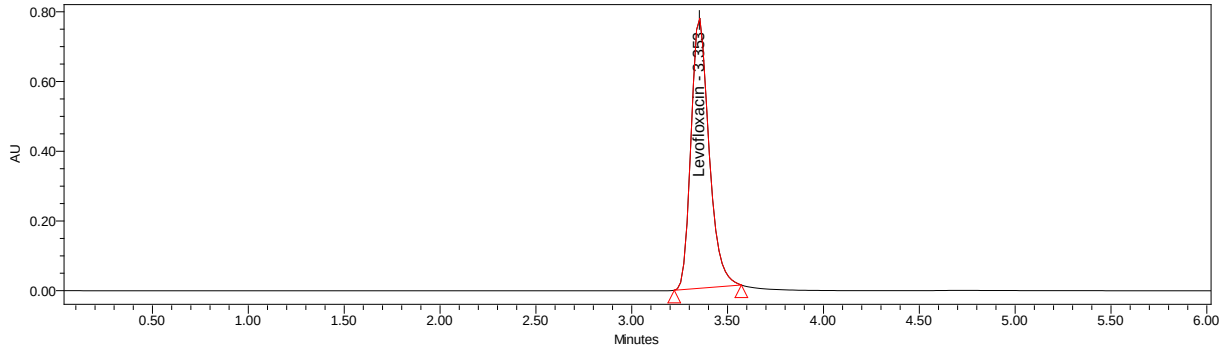
Inference : System sutabilty Chromatogram for standard – 2



Inference : System sutabilty Chromatogram for standard - 3



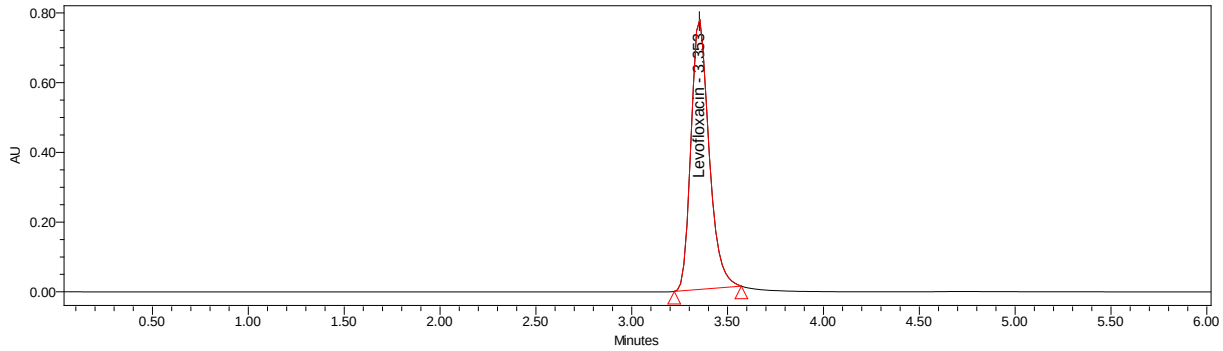
Inference : System sutabilty Chromatogram for standard - 4



Inference : System sutabilty Chromatogram for standard - 5

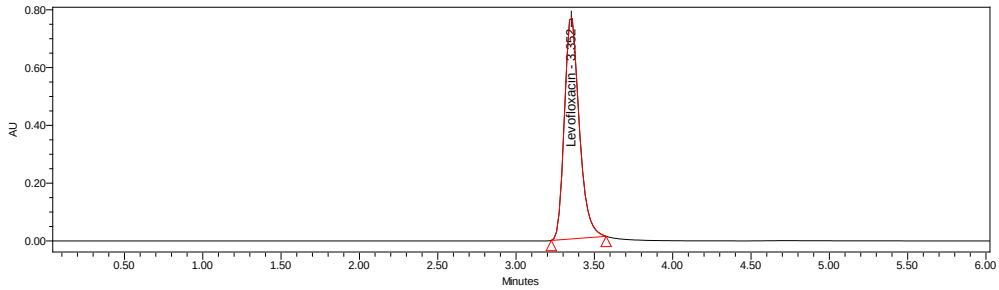
2: SPECIFICITY:

Fig 8: Chromatogram of standard



Inference : Got a peak for standard at an Rt of 3.353

Fig 9: Chroatogram of sample



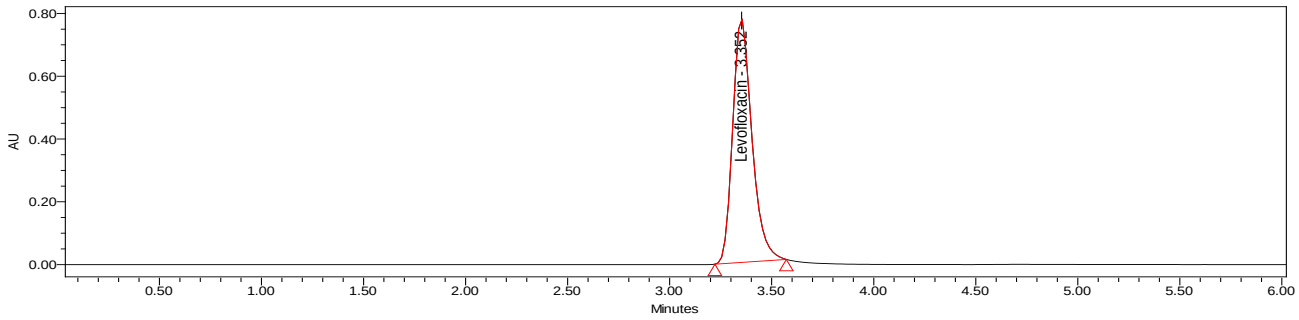
Inference : Got a peak for sample at an Rt of 3.352

3.Method precision:

TABLE-: Data of Repeatability (Method precision)

	Injection	**Peak Areas of Levofloxacin**	**%Assay**
Concentration 40ppm	1	645522.29	100.23
	2	645499.64	100.23

	3	645531.85	100.23
	4	645481.56	100.22
	5	645539.93	100.23
	6	645510.45	100.23
Statistical Analysis	**Mean**	645514.28	100.23
	SD	21.5888	0.00336
	% RSD	0.00334	0.00335



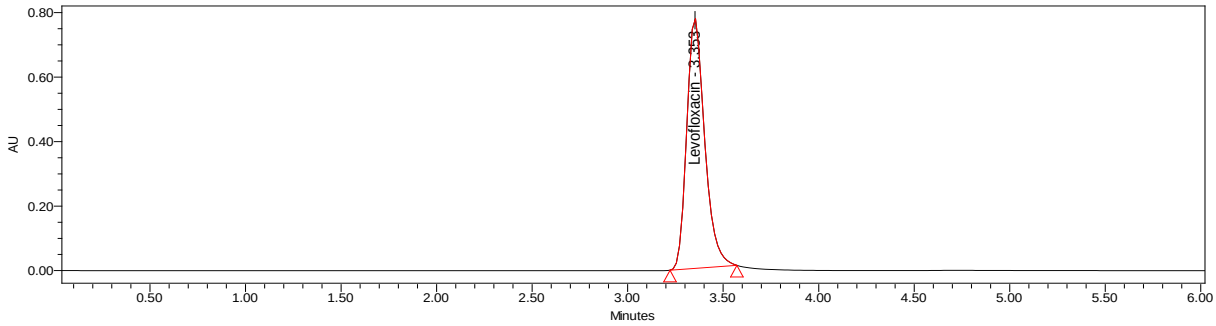
Chromatograph of Retability graph

4.Intermediate precision:

Table4: Data of Intermediate precision (Analyst)

	Injection	**Peak Areas of Levofloxacin**	**%Assay**
Concentration 40ppm	1	645413.13	100.21
	2	645440.85	100.22
	3	645482.67	100.22

	4	645429.99	100.22
	5	645461.16	100.22
	6	645466.66	100.22
Statistical Analysis	**Mean**	645449.07	100.22
	SD	25.7209	0.00400
	% RSD	0.00398	0.00399



Summary and conclusion

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 250nm and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Inertsil C_{18} chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Different pH and ratios of mobile phase were studied, mobile phase with ratio of Methanol and Water (90:10)was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Methanol was selected because of maximum extraction sonocation time was fixed to be 10min at which all the drug particles were completely soluble and showed good recovery. Run time was selected to be 6min because analyze gave peak around 3.351 and also to reduce the total run time.

The present recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 0.0048.

Linearity study was, correlation coefficient and curve fitting was found to be. The analytical method was found linearity over the range of 20-70ppm of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

REFERENCES:

1. http://www.rxlist.com/levaquin-drug.htm (accessed Nov25 , 2010).
2. Wong a FA, Juzwin SJ, Flor SC. Rapid stereospecific high-performance liquid chromatographic determination of levofloxacin in human plasma and urine. J. P harm. Biomed. Anal 1997;15:765-771
3. Djabarouti S, Boselli E, Allaouchiche , Baa B, Nguyena AT. Determination of levofloxacin in plasma, bronchoalveolar lavage and bone tissues by high-perfor mance liquid chromatography with ultraviolet detection using a fully automated extraction method. J . Chromatogr. 2004; 799: 165– 172.
4. Watabe S, Yokoyama Y, Nakazawa K, Shinozaki K, Hiraoka R, Takeshita K, Suzuki Y. Simultaneous m easurement of pazufloxacin, ciprofloxacin, and levofloxacin in human serum by high performance liqu id chromatography with fluorescence detection. J. Chromatogr B. 2010; 878: 1555-1561.
5. Fanga PF, LinCaia H, DeLi H, HuaZhua R, YouTana Q, WeiGaoa, Xua P, PingLiua Y, YuanZhanga W, ChangCh ena Y, FengZhanga. Simultaneous determination of isoniazid, rifampicin, levofloxacin in mouse tissues a nd plasma by high performance liquid chromatography–tandem mass spectrometry. J. Chromatogr B. 2010; 878: 2286-2291.
6. Nguyena HA, Grelleta J, Boubakar BB, Quentinb C, Sauxa MC. Simultaneous determination of levofl oxacin, gatifloxacin and moxifloxacin in serum by liquid chromatography with column switching. J. Chro matogr B. 2004; 810: 77-83.
7. Siewert S. Validation of a levofloxacin HPLC assay in plasma and dialysate for pharmacokinetic stud ies. Journal of Pharmaceutical and Biomedical Analysis. 2006; 41: 1360– 1362.
8. http://www.fda.gov/cder/guidance/index.html. (accessed on Aug15, 2010.)
9. Indian Pharmacopoeia. The India Pharmacopoeia commission. Vol 2 Ghaziabad, 2007, 678.
10. Rang & Dale's, Pharmacology, 6th Edn., 2007, 672.
11. Harkishan Singh, Medicinal and Pharmaceutical Chemistry, 3rd Edn., 2012, 572.
12. Satoskar, Pharmacology. 20th Edn., 2012, 677.
13. Tamilarasi G, Vetrichelvan T, Venakappayya D, Tharabai R and Yuvarajan K, Int J Pharm Sci., 2014, 6, 646-648.
14. Rajeshree C K, Savaliya B M, Lakkad A J, Soriya S V, Kapuriya K G and Faldu S D, Int Bulletin Drug Res., 2012, 2(3), 22-30.
15. Shivangi D P and Farhatjahan Shaikh, World J Pharm & Pharmaceutical Sci., 2014, 6, 921-940.