Journal Of Harmonized Research (JOHR)

Journal Of Harmonized Research in Pharmacy
3(2), 2014, 64-77

ISSN 2321 – 0958

Original Research Article

FORMULATION & ANALYSIS OF LEVOFLOXACIN HEMIHYDRATE BY RP-HPLC IN BULK & TABLET DOSAGE FORM

Dey Bivha, Mahapatra Sujata, Tiwari Prabhakar

School of Pharmacy, Chouksey Engineering College, Lal Khadan,
Masturi Road, Bilaspur (CG)

Abstract

Objective(s)

Developed and validate analytical method for quantitative determination of Levofloxacin tablet formulation. It was prepared by wet granulation method and evaluated by various physicochemical parameters. Analytical method was validating in different parameters like specificity, accuracy, linearity, precision, range, detection limit, robustness. Study in reversed phase high performance liquid chromatographic (RP-HPLC) method for the quantitative determination of Levofloxacin in bulk tablet and finished tablet.

Materials and Methods: A shim-pack CLC-ODS column(25cm X 4.6mm, 5µm) and a mobile phase constituted of Buffer (850 ml of 0.05 (M) Citric acid monohydrate and 10 ml of 1 (M) ammonium acetate) **:**Acetonitirile (150) were used. The flow rate was 1 ml/min and the analyses performed using ultraviolet (UV) detector at a wavelength of 293 nm using levofloxacin Pure drug as an internal standard. Separation was completed within 15 to 17 min.

Results: The developed method showed good resolution between formulated tablet and standard drug. The standard solution of Levofloxacin was prepared to obtain the concentration of about 100 µg /mL, Six (6) replicate injection of homogeneous sample, their % RSD was 0.07 % & test solution was 0.17 %. The assay of Levofloxacin Tablet Obtained by Different Chemist, Date & Column 97.56 %, 98.69 % & 98.24 % respectively. The assay of different flow rate and temperature was 98.07% and 98.12% respectively. The prepared tablet was studied for its stability test for 24 hrs at preselected time interval like initial, 4 hrs, 8 hrs,12 hrs and 24 hours. Their assay % result was obtained 98.62, 98.54, 97.60, 98.50 and 98.41 % respectively.

Conclusion: The method is linear, quantitative, reproducible and could be used as a more convenient, efficient and economical method for the trace analysis of drug in raw material and tablets.

Keywords: Antibiotics, Fluoroquinolone, High Performance Liquid Chromatography, Levofloxacin, Quantitative analysis, Validation studies.

Introduction

Levofloxacin is the L-isomer of the racemate, ofloxacin, a quinolone agent. Chemically, levofloxacin, a chiral fluorinated carboxyquinolone, is the pure (-)-(S)-enantiomer of the racemic drug substance ofloxacin. The chemical name is (-)-(S)-9fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4benzoxazine-6 carboxylic acid hemihydrate. Levofloxacin is a light yellowish-white to yellow-white crystal or crystalline powder. Lefloxacin (Figure 1) is a fluorinated quinolone with an extended antimicrobial spectrum against the majority of Gram negative microbes and staphylococci including methicillin resistant strains (1, 2). Furthermore, pefloxacin possesses some favorable pharmacokinetic properties, including complete absorption after oral administration, long half-life (10-12 hr) permitting infrequent dosage and rapid penetration into the intracellular and extracellular spaces. Clinical studies have shown that Lefloxacin is highly effective in a wide-range of serious infections.

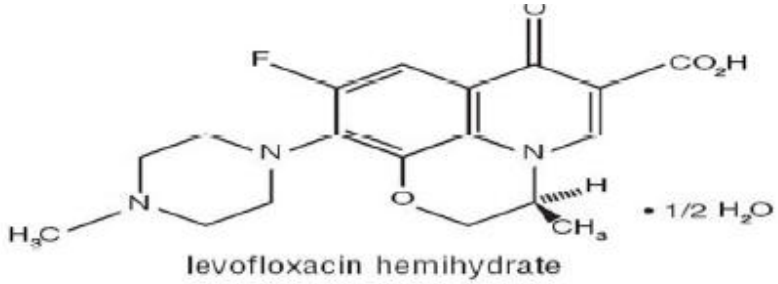


Figure. 1

Developing and validating a simple, efficient, reproducible and economic reversed phase high performance liquid chromatographic (RP-HPLC) method for the quantitative determination of Lefloxacin in bulk material, tablets and in human plasma **S. Gauhar**[{1}]. .Levofloxacin hemihydrate follows linearity in the concentration range 2 – 12 µg/ml with a correlation coefficient of 0.9999. Assay results were in good agreement with label claim. The methods were validated statistically and by recovery studies. The relative standard deviation were found to be less than 2% with excellent precision and accuracy **Surana S. J.** [{3}]**.**This automated method has been validated within the requirements of ICH guidelines Q2A–Q2B. Considerable effort went into developing and validating an automated method. The results obtained in the validation of this automated method were equivalent to the manual method in terms of system precision, linearity, accuracy, robustness and sensitivity (limits of detection, LOD and LOQ**J. F. Dulsat, J. L. Fábregas**[{5}] **.**The objective of the present work is to develop simple, rapid and economical 'UV-spectrophotometry' and 'First Order Derivative' methods for determination of levofloxacin hemihydrates in bulk and finished products**.**

For Correspondence:
bivhadey1707ATgmail.com
Received on: March 2014
Accepted after revision: June 2014

Materials and Methods

The HPLC system consisted of a LC-10AT VP Shimadzu liquid chromatograph (AGILENT, Japan) equipped with SPD-100AVP Shimadzu UVVIS detector. Chromatographic separations were performed on C18 Shim-Pack CLC-ODS column (25cm X 4.6mm, 5µm) attached to a guard column (octadecylsilane guard column), connected to CBM-102 Communications Bus Module Shimadzu (Japan) with PC (P-I I I). In addition, electronic balance, microliter syringe, micropipette and micropore filtration assembly used in this study. Levofloxacin Hemihydrates,

Starch, Aerosil 200, CrossPovidone, Crosscarmellose Sodium, Magnesium Stearate all excipient was available from Alkem PVT. LTD (Sikkim). All solvents were of HPLC grade and reagents of analytical grade. Double distilled water used to prepare mobile phase.

Preparation of stock solution in mobile phase:

100 mg of Levofloxacin Hemi hydrate (Standard Drug) was taken in a 100 ml volumetric flask, volume was made up with 0.1 (M) HCl.(1000ug/ml).Further diluted 5 ml to 50 ml with Distilled water(100ug/ml). From that stock solution a series of dilutions were prepared at different concentrations respectively, Area of each solution was measured at 293 nm.Shown in Fig 2.

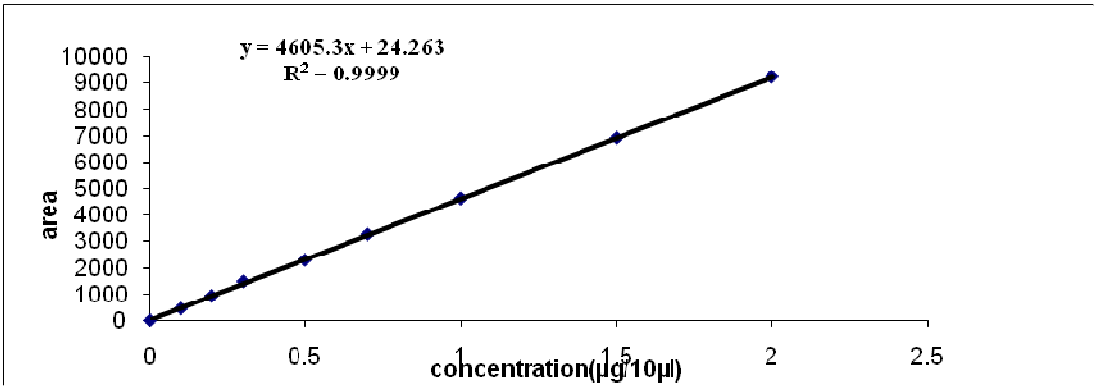


Figure.2 Standard Curve of Levofloxacin by HPLC

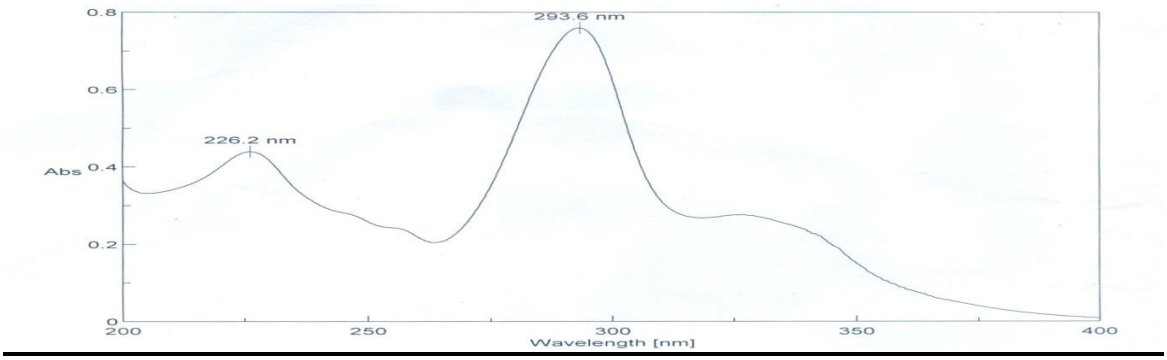


Fig. 3 Estimation of λ_{max} by UV

Preparation of mobile phase: Mobile Phase ratio was Buffer(840 ml of 0.05 (M) Citric acid monohydrate and 10 ml of 1(M) ammonium acetate): Acetonitrile (850:150 v/v) used as mobile phase whereas, thepH of mobile phase was adjusted to 2.9 with 1 N KOH solution. All solvents and solutions

filtered through filtration unit (Millipore, 0.45 µm pore size) and degassed before use. The flow rate maintained at 1 ml/min and volume of injection was 20 µl. Detection performed at a wavelength of 293 nm and analysis was carried out at ambient temperature.

Standard preparation [{1}]:100 mg of Levofloxacin Hemi hydrate WS was weighted, to a 100 ml volumetric flask, dissolve in 0.1(M) Hcl makeup the volume with 0.1 (M) Hcl. Further diluted 5 ml to 50 ml with water.

Test preparation: 20 tablets was randomly selected, crushed and make powdered. Weight accurately a quantity of about 100 mg equivalent of Levofloxacin and into a 100 ml volumetric flask ,add 0.1 (M) Hcl , Filter

through whatman filter paper . Further dilute 5 ml of the filter to 50 ml with Water.

Parameters of Method Validation

The study was conducted to obtain an affordable and convenient method for RP-HPLC determination of Lefloxacin. The experiment carried-out according to the official specifications of United State Pharmacopeias (USP-27), Global Quality Guidelines-2002, International Conference on Harmonization (ICH-1996) and Centre of Drug Evaluation and Research (CDER-1994) . The method validated for the parameters like system suitability, specificity, range and linearity, limit of detection, limit of quantification, accuracy, precision, ruggedness and robustness.

System Precision: The standard solution of Levofloxacin Standard was prepared to obtain the concentration of about 100 µg/mL, Six (6) replicate injection of homogeneous sample ,calculate their % RSD Should be less than 1%. Resulted in % Relative standard Deviation of Levofloxacin Stdwas 0.07 %.

Resulted show in Table.7, Fig.3

Method Precision: Sample preparation was prepared by Levofloxacin tablet as per method precision to obtain the concentration 500 µg/mL of Levofloxacin in each sample preparation .Six (6) replicate injection of the sample. Calculate their % RSD value should be less than 1%. Resulted in % Relative standard Deviation of Levofloxacin test solution was 0.17 %. Resulted show in Table.7, Fig.4

Accuracy: The accuracy means the closeness of test results obtained by the analytical method to the true value. This is sometimes termed trueness. The concentration (in µg/ml) of Levofloxacin was calculated from the linear regression for each level of linearity of 50 % ,75 %, 100 %, 125 %, and 150 % . The calculated values were compared with the actual values injected. The actual and calculated concentration (µg/mL), Accuracy (%). Resulted shown in Table.8, Fig. 5, 6, 7, 8, 9

Calculation Formula:

$$\frac{\text{Avg. ar.(sample)}}{\text{Std Avg. ar.}} \times \frac{\text{Stdwt}}{100} \times \frac{5}{50} \times \frac{100}{\text{test Wt}} \times \frac{50}{5} \times \frac{\text{Potency}}{100} \times \frac{\text{AvgWt}}{\text{Lable claim}} \times 100$$

Sample Preparation 50,75,100,125,150% Conc.:Take 50,75,100,125,150 mg of Levofloxacinstd was taken in 100 ml volumetric flask and added 121,96,71,46,21 mg Placebo respectively, add 0.1 (M) HCl ,Filtered through whatman filter paper.Further diluted 5 to 50 ml volumetric flask with Water. Fig. 5, 6, 7, 8, 9

Linearity & Range: The Linearity of an analytical method was test results that are directly, proportional to the concentration of analyzed in samples. A graph was plotted from 50 % to 150 % of the analyteand their Mathematical transformation was calculated with a correlation coefficient not greater than 0.99.shown in Table 9, Fig. 10

Specificity &Selectivity : Specificity study was designed to prove that Levofloxacin Tablet elutes at specificity retention time, under specified chromatographic conditions, and there is no interference of excipient and Mobile phase. Table 10

Preparation of Placebo solution:355.8 mg of prepared placebo(inactive excipient) was weighted in a 100ml volumetric flask, makeup the volume with 0.1(M) HCl, Sonicate to dissolve and dilute to volume with 0.1(M) HCl. Further diluted 5 ml of the solution to 50 ml with Water.

Preparation of LevofloxacinStandard solution: 100 mg of LevofloxacinWS was weighted, to a 100 ml volumetric flask, add about 50 ml of 0.1(M) HCl, Sonicated and volume made up with methanol. Further diluted 5 ml of the solution to 50 ml with Water.

1. Obtained three (3) replicate chromatograms of Prepared Placebo solution,
2. Three (3) Chromatogram of WS stdsol^{n}(Levofloxacin)

3. Three (3) replicate Chromatogram of prepared Placebo + standard solution as per the experimental condition mentioned.

Limit of detection (LOD): LODwas determined on the basis of signal and noise ratio. Mean of noise peak area and their absolute Standard Deviation value are calculated.**LOD = 3.1 X 10^{-3}**
LOD – S/N > 2 or 3

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

σ =Standard deviation
S = Slope of Standard curve
Limit of Quantification (LOQ): It was expressed as the concentration of analyte in the samples.S/N, ratio not less than 10 with RSD ≤ 3 %.QL = 9.2 X 10^{-3}
The quantitation limit (QL) may be expressed as:

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

Where,
σ,the standard deviation of the response,
S, the slope of the Standard curve,

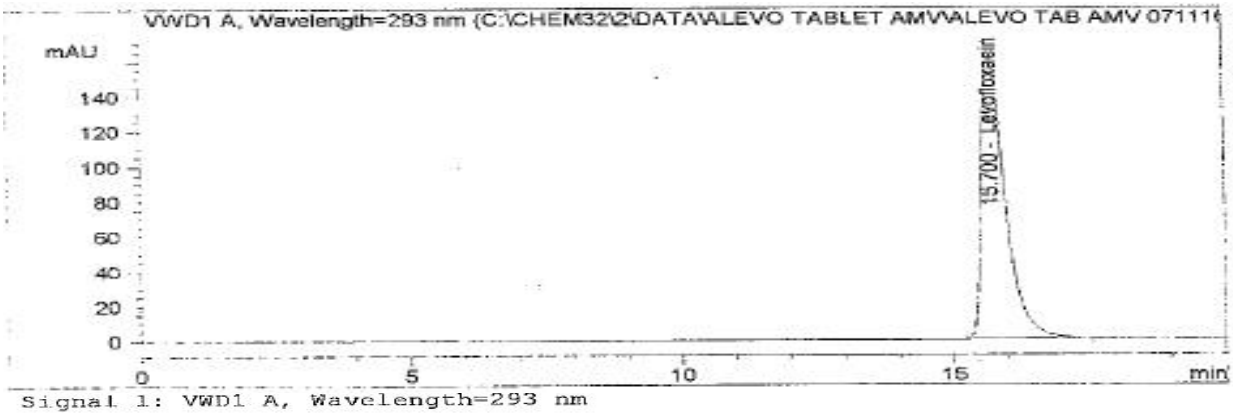
Ruggedness: The ruggedness of analytical method is the degree of reproducibility of test results. Obtained one chromatogram of five (5) replicate chromatograms of the Standard solution, as well as two chromatogram of each sample solution . Recorded the peak area for Levofloxacin in each Injection and calculated percent of Levofloxacin in each assay sample preparation .The results are summarized in Table 11.1, 11.2, 11.3. The average assay of Levofloxacin TabletAssay of Levofloxacin Obtained by Different Chemist, Date & Column 97.56 %, 98.69 % & 98.24 % respectively .Resulted Show in Tablet 11.4
Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in the method parameters. It indicates the reliability of the method during normal usage. Changes to the critical chromatographic conditions such as Flow Rate& Temperature
Stability of solution: Test solution was prepared and studied for its stability up to 24 hrsStored in 27 C temperature appropriately.Assay results was calculated during a preselected time interval, for example, initial, 4 hrs, 8 hrs, 12 hrsand 24 hours, using a single solution. Table.1

Results

Table.7: System Precision & Method Precision

Levofloxacin Working standard			Levofloxacin sample preparation			
Injection	RT of Levofloxacin Std	Area of Levofloxacin Std.	RT of Levofloxacin test sample	Area of Levofloxacin test sample	Assay (%) (Mean± SEM)	Assay(mg)
1	15.70	5008.09	15.74	4999.88	98.02	490.2
2	15.69	5004.49	15.76	4992.31		
3	15.69	5006.22	15.75	4991.13		
4	15.69	5002.13	15.76	4988.74		
5	15.70	4998.57	15.81	5011.41		
6	15.70	5002.15	15.83	4998.17		
Average	15.70	5003.61	15.78	4996.94		

%RSD	0.03	0.07	0.21	0.17		

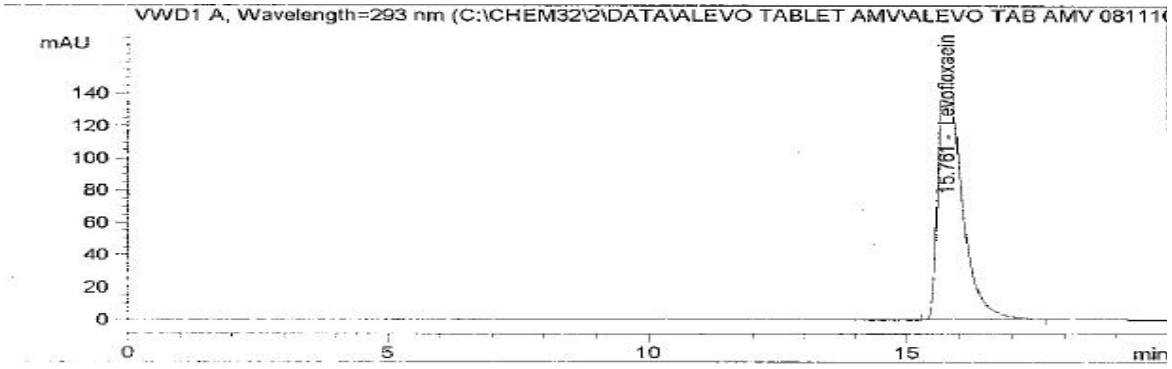


Signal 1: VWD1 A, Wavelength=293 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU	Area *s	Area %	Name
1	15.700	BB	0.4666	5008.09375	100.0000		Levofloxacin

Totals : 5008.09375

Fig.3 Chromatogram of Levofloxacin Std



Signal 1: VWD1 A, Wavelength=293 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU	Area *s	Area %	Name
1	15.761	BB	0.4525	4999.88672	100.0000		Levofloxacin

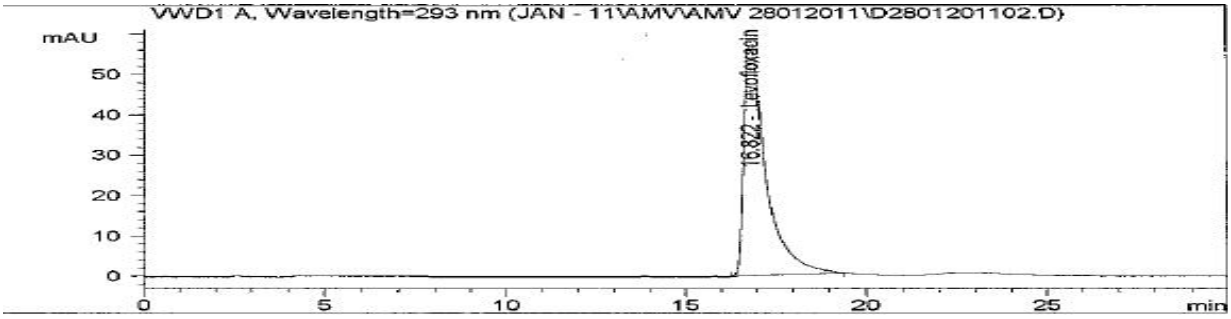
Totals : 4999.88672

Fig.4 Chromatogram of Levofloxacin Sample Solution

Accuracy: The concentration (in µg/ml) of Levofloxacin was calculated from the linear regression for each level of linearity of 50 % ,75 %, 100 %, 125 %, and 150 %.

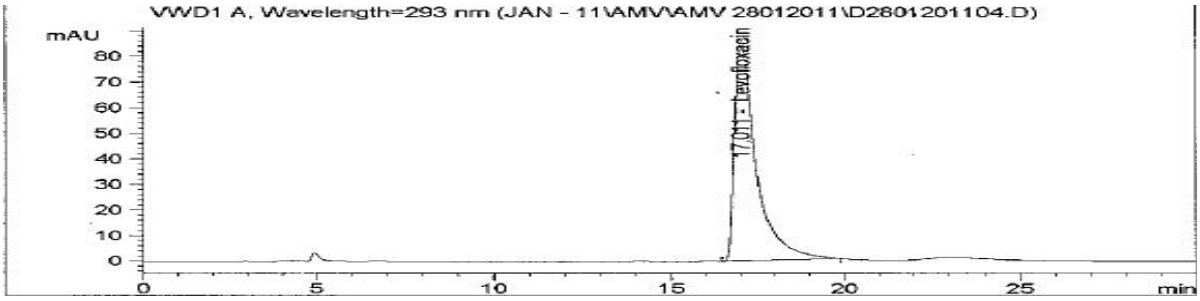
Table.8 Accuracy % data at different Concentration

Level (%)	Wt. of Std Levofloxacin(mg) Drug Added	Wt. of Placebo (mg) (Drugrecovered)	% Assay (mean± SEM) (n=5)	RSD % (n=5)
50	50.2	121	50.15	0.33
75	75.3	96	75.32	0.32
100	100.2	71	100.76	0.32
125	125.3	46	125.65	0.31
150	150.3	21	149.91	0.32



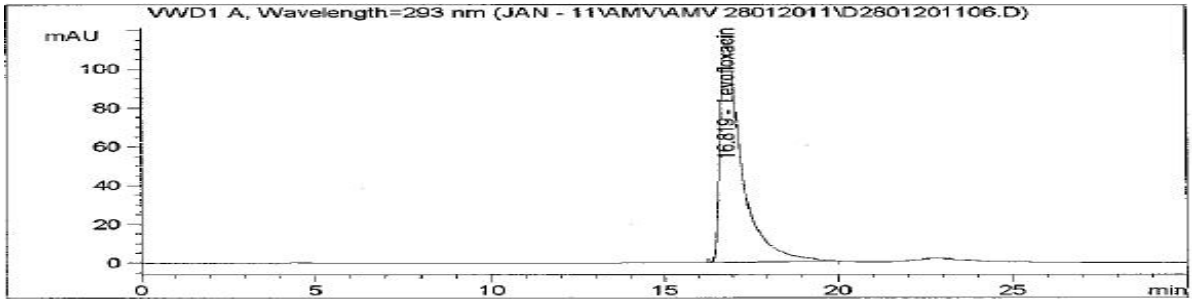
RetTime [min]	k'	Area mAU *s	Height [mAU]	Symm.	Width [min]	Plates	Resol ution	Select ivity
16.822	-	2449.02124	58.04132	0.40	0.5630	4946	-	-

Fig. 5. Chromatogram of 50 % Conc



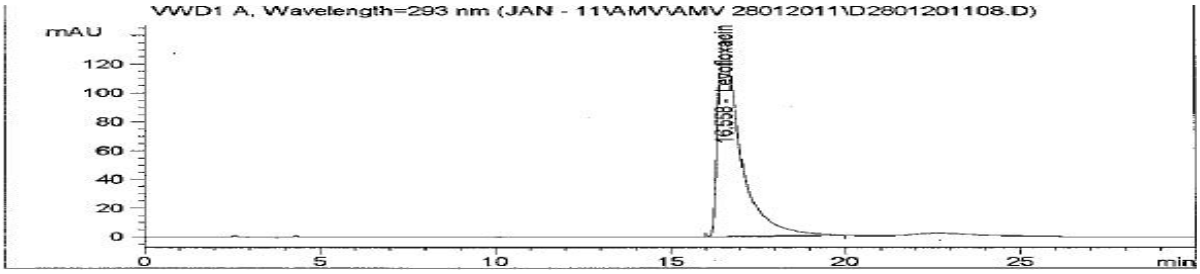
RetTime [min]	k'	Area mAU *s	Height [mAU]	Symm.	Width [min]	Plates	Resol ution	Select ivity
17.011	-	3731.45361	86.73882	0.40	0.5727	4888	-	-

Fig.6 Chromatogram of 75 % Conc.



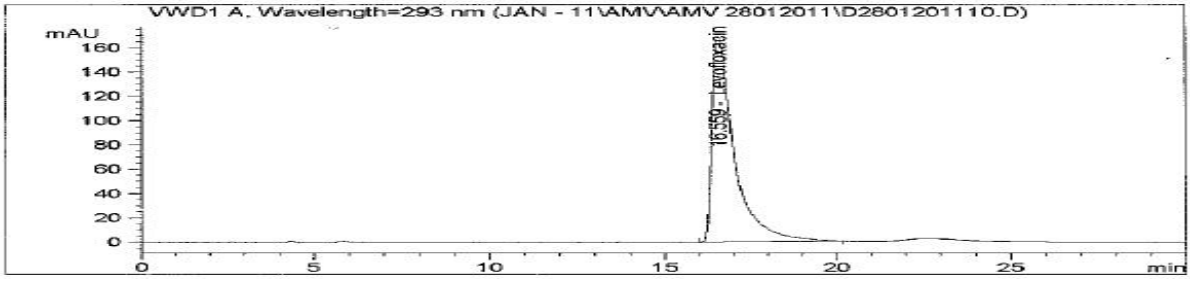
RetTime [min]	k'	Area mAU *s	Height [mAU]	Symm.	Width [min]	Plates	Resol ution	Select ivity
16.819	-	5032.31201	115.20064	0.38	0.5727	4778	-	-

Fig.7 Chromatogram of 100 % Conc.



RetTime [min]	k'	Area mAU *s	Height [mAU]	Symm.	Width [min]	Plates	Resol ution	Select ivity
16.558	-	6279.13135	140.16110	0.38	0.5921	4333	-	-

Fig. 8 Chromatogram of 125 % Conc.



RetTime [min]	k'	Area mAU *s	Height [mAU]	Symm.	Width [min]	Plates	Resol ution	Select ivity
16.559	-	7600.10889	169.09854	0.37	0.5921	4333	-	-

Fig.9

Chromatogram of 150 % Conc.

Linearity & Range: Table 9: linearity study data of Levofloxacin

Level approx	Conc.(µg/mL)	Retention Time (min)	Observed Peak Area	Average Peak Area	% RSD
50 %	50.2	16.82	2449.02	2460.77	0.68
		17.29	2472.52		
75%	75.3	17.01	3731.45	3762.28	1.16
		17.03	3793.11		
100%	100.2	16.81	5032.31	5032.93	0.02
		16.59	5033.55		
125%	125.3	16.55	6279.13	6275.99	0.07
		16.54	6272.86		
150%	150.3	16.55	7600.10	7551.43	0.09
		16.89	7502.75		

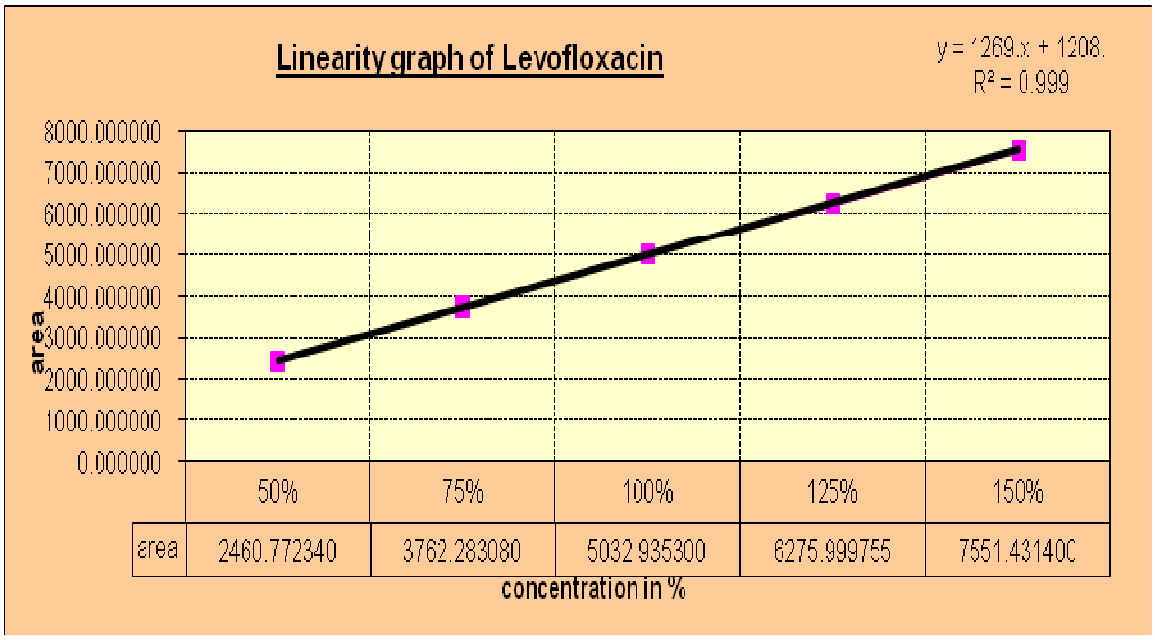


Fig.10 Graph represent data of conc, vs area of Levofloxacin

Table 11.3.1: Ruggedness study data for Levofloxacin Test solution On different Column

	Column ID No: SG021		**Column ID No: SG002**	
Sr.No.	Area of Levofloxacin	Assay Levofloxacin	Levofloxacin	AssayLevofloxacin
1	4609.82	97.95 %	4685.53	98.24 %
2	4643.11		4662.70	
Average	4626.46		4674.12	

Table 11.4 Assay % of Levofloxacin Obtained by Different Chemist, Date & Column

	Working Test	Different Chemist	Different Day	Different Column	% RSD
Levofloxacin % Assay	97.50 %	97.56 %	98.69	98.24	0.58

Robustness:Table 12.1 Avgpeak Area of LevofloxacinStd& test Sample at different flow rates.

Mobile phase flow rate (ml / min)	Avg peak Area of Levofloxacin Std	Avg peak Area of Levofloxacin(test solution)	% Assay Levofloxacin
0.8 ml	6356.05	6408.21	98.55
1.0 ml	5007.32	4986.90	97.70
1.2 ml	4633.51	4626.46	98.91
Avg. RSD (%)			0.44 %

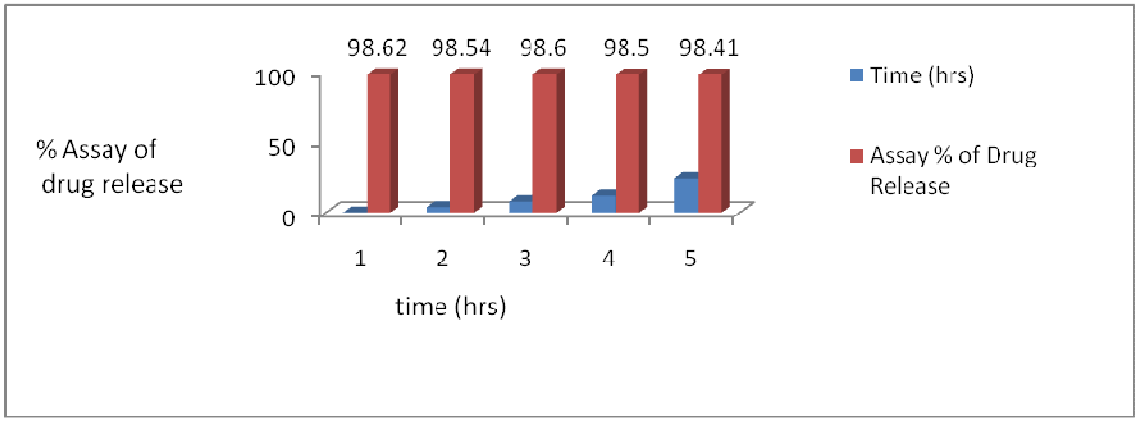
Table 12.2Avg peak Area & % Assay of Levofloxacin drug product at different Column temperature

Diff .Colum Temperature	Avg peak Area of Levofloxacin Standard	Avg peak Area of Levofloxacin(Test solution)	% Assay Levofloxacin	(RSD%)
23^0C	4661.72	4647.83	97.80	0.48
25^0C	5007.31	4986.90	97.7	0.15

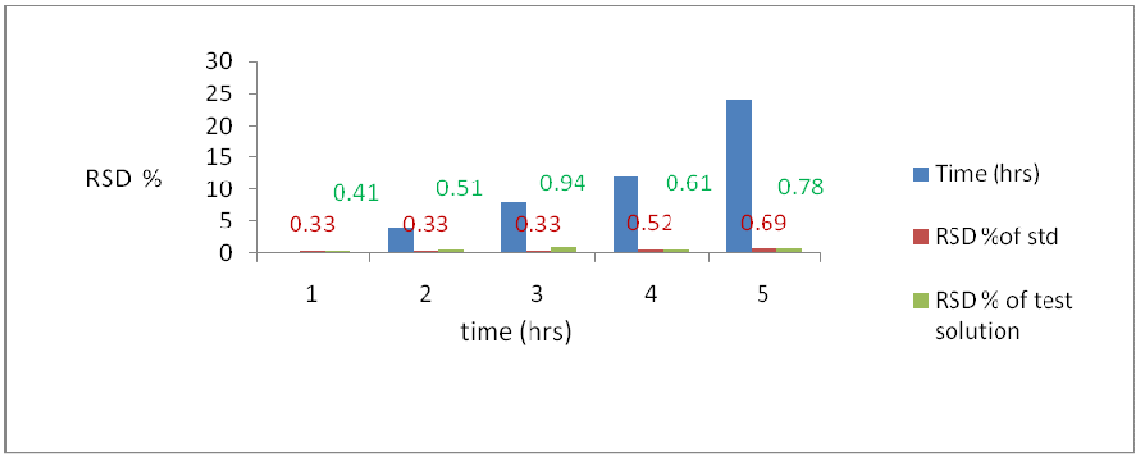
27^0C	4660.42	4693.83	98.82	0.38

STABILITY OF SOLUTION:Table.13 Stability data for Levofloxacin Standard and Sample Solution

TimeInterval (min)	Area of Levofloxacin Std	RSD % of Levofloxacin Std	Area of Levofloxacin Sample	RSD % of Levofloxacin Sample	Assay %
Intial	4505.33	0.33	4533.87	0.41	98.62
4.0 hrs	4633.51	0.33	4626.46	0.51	98.54
8.0 hrs	4687.43	0.33	4665.53	0.94	97.60
12.0 hrs	4819.78	0.52	4844.44	0.61	98.50
24.0 hrs	4663.56	0.69	4695.33	0.78	98.41
Average RSD % : 0.42 %					



Graph. 11 Assay % of drug (test sample) release in different time interval



Graph.12 RSD % of std solution & test sample release in different time interval

IN VITRO DISSOLUTION STUDIES

Table.14.3 % Drug Release of Levofloxacin Core and Coated Tablet

Time (min)	% Drug Release core	% Drug Release Coated
0	0.525	0.525
15	37.25	34.63
30	83.23	81.92
45	100.01	99.82

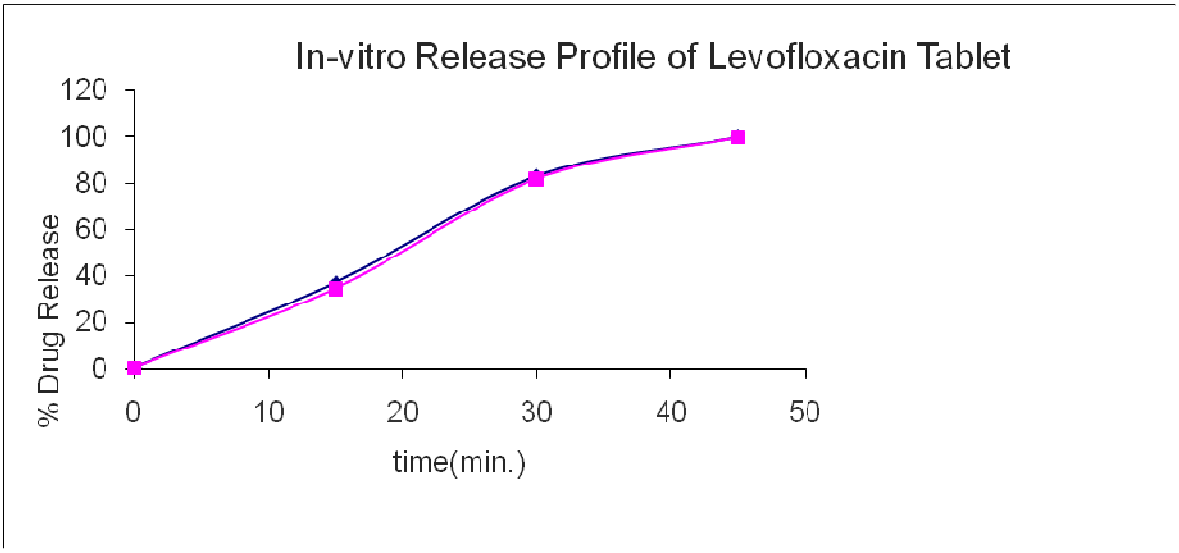


Fig. 10 Graph Represent the % of Drug Release of Levofloxacin Core and Coated Tablet

Discussion

The studied HPLC method has some advantages. First, the extraction procedure is simple and involves only one step. Other advantages are using a commonly used reversed-phase chromatographic column, simple composition of an isocratic mobile phase and UV absorbance measurement for detection. The method has been proved to be linear, reproducible, quantitative and qualitative studies of drug analysis.The invitro dissolution study of core and coasted tablet was determined maximum release was found in 45 min,for core tablet maximum release was 100.01% and coated tablet was 99.82%.To achieve precise component peak with good resolution under isocratic condition, mixture of buffer: Acetonitrile (850:150) mobile phase were tested under different condition on a C_{18} stationary phase (ODS column). Under the above mentioned chromatographic condition the retention time was obtained a 15.72 min. A good linear relationship was observed with R^2 =0.999. The evaluated physicochemical data and analytical datas were found to be within the limit.

Acknowledgment

Deep appreciation for the Department of Pharmaceutics, Faculty of Pharmacy, S'O'A University and School of Pharmacy, for giving us moral and generous help during this research. The authors declare that they have no conflict of interests.

References

1. ShahnazGauhar, et.al, Development and Validation of a HPLC Method for Determination of Lefloxacin in Tablet and Human Plasma, Iranian Journal of Basic Medical Sciences Vol. 12, No. 1, Spring Jan 20 ,2009, Pages 33-42.
2. K. M. Kothekar,Amit P. Khandhar,et.al, Quantitative Determination of Levofloxacin and Ambroxol hydrochloride in Pharmaceutical Dosage Form by Reversed-Phase High Performance Liquid Chromatography, Eurasian Journal of Analytical Chemistry Volume 2, Number 1, 2007 ISSN: 1306-3057.
3. J. S .Surana et.al, Quantitative Determination of Levofloxacin hemihydrate in bulk and tablets by uv-spectrophotometry and first order derivative methods, RC Patel College of Pharmacy, Karwand Naka, Shirpur, Dist: Dhule (MS), 425 405, India.
4. S. Siewert et.al ,Validation of a levofloxacin HPLC assay in plasma and dialysate for pharmacokinetic studies, Department of Pharmacology and Toxicology, Faculty of Medicine, University of Rostock, Germany
5. J. F. Dulsat, J. L. Fábregas ,et.al, Development and validation of a fully automated method for the chromatographic determination of content uniformity of drug tablets,AlmirallProdesfarma S.A., Analysis R&D Department, C/Cardener 68–74, Spain.
6. R. Bano1, S. Gauhar et.al, Pharmaceutical Evaluation Of Different Brands Of Levofloxacin Tablets (250mg) Available In Local Market Of Karachi (Pakistan), Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi, Department of Pharmaceutics, Faculty of Pharmacy,University of Karachi
7. M. Feinberg et.al, Validation of analytical method based on accuracy profiles ,Institut National de la RechercheAgronomique, (INRA), 16 rue Claude Bernard, 75231 Paris Cedex 05, France.
8. M. E. Gil-Alegre, J. A. Bernabeu, et.al,, Statistical evaluation for stability studies under stress storage conditions .Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, Complutense University of Madrid, Volume 56, Issue 11, Pages 877-883 .
9. J. Chaet.ol, T. Gilmol, P. Lane et.al, Stability Studies, Johnson & Johnson Pharmaceutical Research and Development, Raritan, New Jersey, USA.
10. K. C. Waterman et.al ,Accelerated aging: Prediction of chemical stability of pharmaceuticals, . International Journal of Pharmaceutics ,Volume 293, Issues 1-2, Pages 101-125,
11. G. Altiokka et.al , The determination of levofloxacin by flow injection analysis using UV detection, potentiometry, and conductometry in pharmaceutical preparations ,Journal of Pharmaceutical and Biomedical AnalysisVolume 30, Issue 3, Pages 881-885.
12. A. Lambert et.al. Structure of levofloxacin in hydrophilic and hydrophobic media: Relationship to its antibacterial properties, SRSMC, Nancy-University, CNRS, BP 239, 54506 Vandoeuvre-lès-Nancy Cedex, France,Volume 442, Issues 4-6, Pages 281-284 .
13. M. Walton et.al ,Validation of laboratory tests and methods, Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA. USA, Volume 10, Issue 2, Pages 59-65 .
14. H. Liang, M. B. Kays et.al,Separation of levofloxacin, ciprofloxacin, gatifloxacin, moxifloxacin, trovafloxacin and cinoxacin by high-performance liquid chromatography: application to Levofloxacindetermination in human plasma ,Journal of Chromatography B:

Analytical Technologies in the Biomedical and Life Sciences Volume 772, Issue 1, Pages 53-63.

15. M. L. Devia et.al, A validated stability-indicating RP-HPLC method for levofloxacin in the presence of degradation products, its process related impurities and identification of oxidative degradant , Journal of Pharmaceutical and Biomedical AnalysisVolume 50, Issue 5 , Pages 710-717.
16. D. Baoa et.al, Simultaneous determination of rifampicin and levofloxacin concentrations in catheter segments from a mouse model of a device-related infection by liquid chromatography/electrospray ionization tandem mass spectrometry , Journal of Pharmaceutical and Biomedical AnalysisVolume 46, Issue 4, Pages 723-727
17. S. Tatar et.al Rapid and sensitive spectrofluorimetric determination of enrofloxacin, levofloxacin and ofloxacin with 2,3,5,6-tetrachloro-*p*-benzoquinone , SpectrochimicaActa Part A: Molecular and Biomolecular SpectroscopyVolume 72, Issue 5, Pages 1038-1042 .
18. In the Proceedings of International Conference on Harmonization Guidance, ICH: Q2A: Guideline for Industry, Text on validation of analytical procedures, March 1995.
19. 19. In the Proceedings of International Conference on Harmonization Guidance, ICH: Q2A: Guideline for Industry, Text on validation of analytical procedures, May 1997.
20. M . G Bures, L.L. Shen, (August 1996). "Mechanism of differential activities of ofloxacin enantiomers." (PDF). Antimicrob Agents Chemother 40.