The antibiotic efficacy of levofloxacin tablet was determined utilizing a verification method that included computational and experimental techniques. DFT, UV-VIS, and HPLC-20 AD

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Abstract- Levofloxacin is an antibiotic that is sold under the brand names Levaquin and others. It is used to treat a variety of bacterial diseases, including acute bacterial sinusitis, pneumonia, Helicobacter pylori (in conjunction with other drugs), urinary tract infections, chronic prostatitis, and certain types of gastroenteritis. It may be used in conjunction with other antibiotics to treat tuberculosis, meningitis, or pelvic inflammatory illness. Use is normally advised only when no other options are available. It is offered orally, intravenously, and as an eye drop. In this study, we are executing verification analysis to determine the efficacy of levofloxacin at several phases such as linearity, repeatability, precision, accuracy, and robustness to determine how stable our product is in these circumstances.

Index Terms- Levofloxacin, Verification, HPLC, UV-VIS, DFT

I. INTRODUCTION

Levofloxacin is an antibacterial drug in the class of fluoroquinolones. It works against bacteria that cause serious infections in the respiratory, skin, and genitourinary tracts[1, 2]. Levofloxacin is currently used to treat respiratory tract infections, such as acute bacterial exacerbations of chronic bronchitis, acute bacterial sinusitis, and community-acquired and nosocomial pneumonia; complicated and uncomplicated skin and skin structure infections; post-inhalational anthrax; and genitourinary infections, such as uncomplicated and complicated urinary tract infections, acute pyelonephritis[3, 4], 1 and In Europe (Tavanic®) and Asia (Cravit®), levofloxacin has been approved for the same kinds of uses.

As our understanding of pharmacodynamics and the drug's safety and tolerability grew, many different dosing schedules were tested in clinical trials[5]. Initial research mostly looked at regimens with a maximum daily dose of 500 mg. Patients with urinary tract infections were given 250 mg regimens, and patients with respiratory tract infections were given 500 mg regimens[6]. Then, 750 mg dosing regimens were used to study people with skin and skin structure infections2 and patients with nosocomial pneumonia[7, 8]. Recently, 750 mg dose schedules given over 5 days instead of 10 days have been looked at in people with respiratory tract infections. This is because this change could help reduce medication resistance[9, 10].

Some common side effects are feeling sick, having diarrhea, and having trouble sleeping[11, 12]. Some serious side effects include tendon rupture, inflammation of the tendon, seizure, psychosis, and possibly permanent damage to the peripheral nerves[13, 14]. Damage to tendons can show up months after treatment is done. People may also burn more easily in the sun. Muscle weakness and trouble breathing can get worse in people with myasthenia gravis. Even though it's not a good idea to use while pregnant, the risk seems to be low[15]. It seems safe to use other drugs in this class while breastfeeding, but it's not clear if levofloxacin is safe. Levofloxacin is a drug in the class of fluoroquinolones that is an antibiotic with a wide range of effects[16, 17]. Most of the time, it kills the bacteria. It is the drug ofloxacin left-handed isomer[18].

Levofloxacin is used to treat infections in the lungs, the urinary tract, and the stomach. As of 2007, the Infectious Disease Society of America (IDSA) and the American Thoracic Society recommended levofloxacin and other respiratory fluoroquinolines as the first line treatment for community-acquired pneumonia when co-morbidities like heart, lung, or liver disease are present or when in-patient treatment is needed. Levofloxacin is also an important part of the recommended treatment plans for ventilator-associated pneumonia and pneumonia caused by medical care [19, 20].

In this research, we intend to investigate the effect of various parameters on the process of determining the assay for levofloxacin at its various stages of verification analysis. In order to provide an explanation for the absorption spectra while also receiving support from theoretical results and proving it with relation to the absorption value.

Fig 1 Chemical structure of Levofloxacin

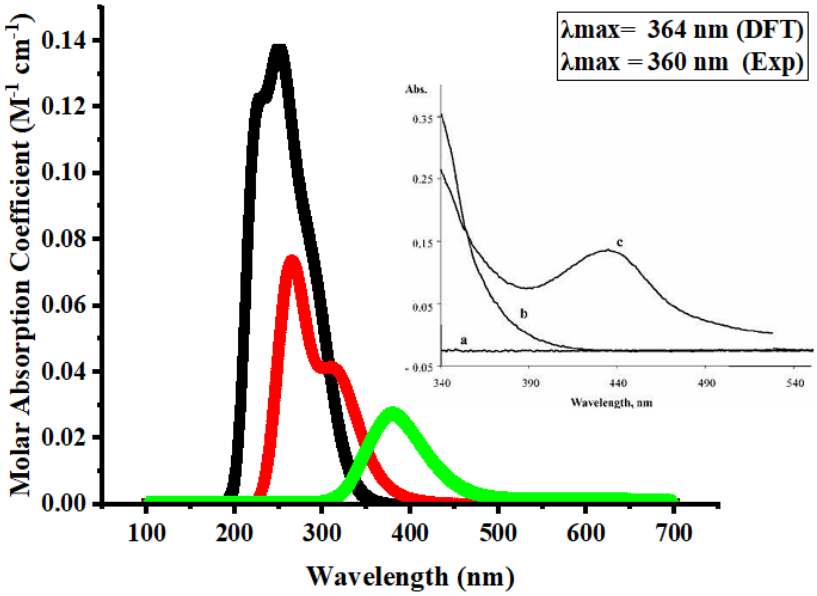

Figure 2 Absorption profile using DFT and UV-1900i with solvent acetonitrile

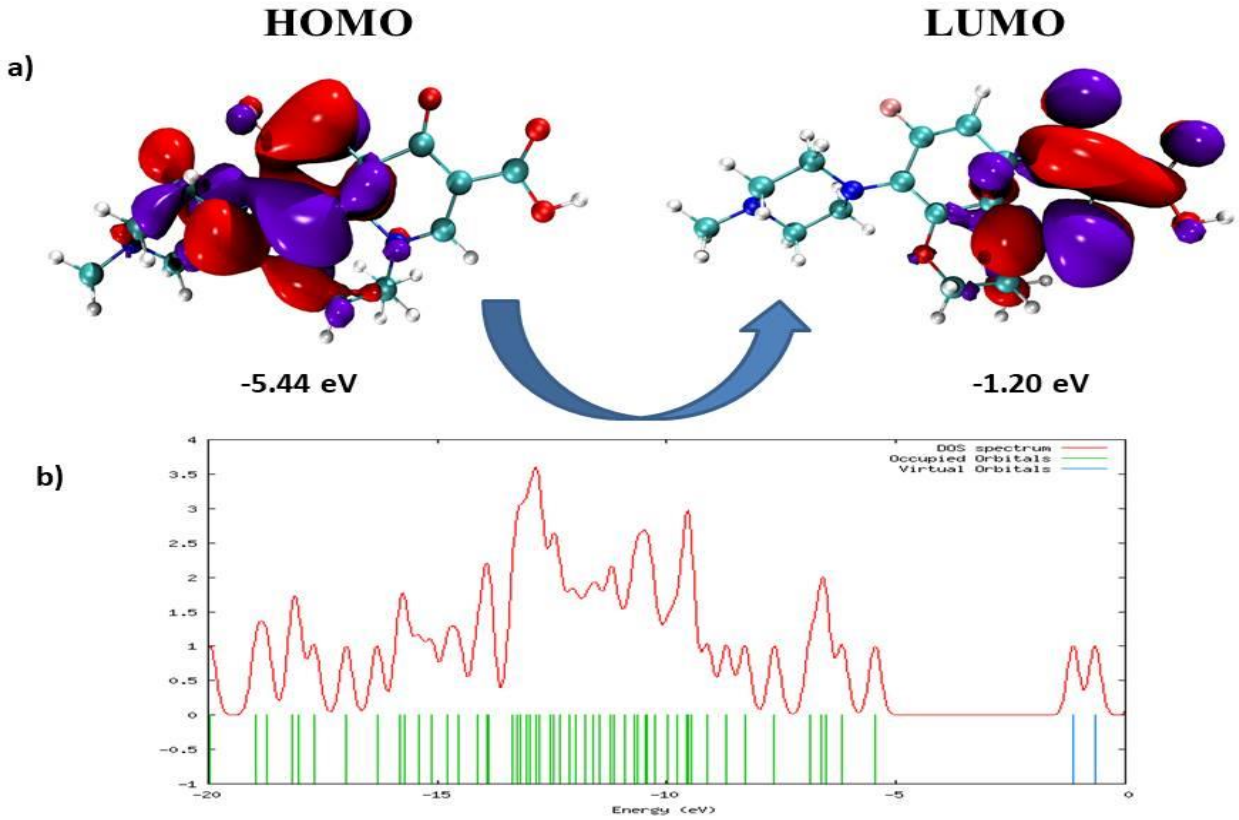

Figure 3 a) Frontiers molecular orbitals for levofloxacin b) Density of state analysis

Analyzing the distribution pattern of the frontier molecular orbitals (FMOs) is a method that is used to characterize the optical as well as the electronic properties of the donor molecules that have been constructed. The distribution pattern of charges in LUMO (lowest unoccupied molecular orbital) and HOMO (highest occupied molecular orbital) can be estimated using frontier molecular orbitals.

The density of state (DOS) analysis of these molecules was also carried out, and the findings that were obtained from the density of state contribution confirmed the facts that were proven by the FMO results. Additional study of FMOs concerning percentage composition in addition to DOS around HOMOs and LUMOs also indicated that acceptor components with variable electron withdrawing intensities have the potential to affect the distribution of electron density on molecular orbitals (MOs). In a similar manner, the DOS illustrates the energies of all of the intended organic donor molecules' MOs, including those that are occupied and those that are not.

In order to validate the test technique in accordance with the protocol, quantitative determination of levofloxacin, an active component of levofloxacin 250/500 mg tablet, final product, is to be performed by high-performance liquid chromatography (HPLC). The verification procedure was carried out in order to assess the assay's linearity, precision, accuracy, detection limit, quantitation limit, and robustness.

The intended use of this test method is for quantitative determination of Levofloxacin, an active ingredient of Levofloxacin 250/500 mg tablet.

The scope of this study applies to quantitative determination of Levofloxacin, an active ingredient of Levofloxacin 250/500 mg tablet.

HPLC Method:

The use of HPLC as a method for determining the concentration of active pharmaceutical ingredients is a reliable and accurate technique (API). The solubility of the sample in the solvent serves as the foundation for this HPLC procedure. In general, the solvent is comprised of a combination of buffer and methanol solvent (70:30). In this validation study, the following validation parameters, precision, accuracy, linearity, limit of detection, limit of quantitation, and robustness will be assessed and analyzed.

Material and methods:

HPLC (Isocratic Shimadzu SPD-20AD or equivalent), Calibrated Balance, Calibrated PH meter, Purified water, Acetonitrile, filter paper, solution polyamide.

Isocratic USP Method:

Mobile Phase Preparations

Buffer A: In 700 ml water, dissolve 874 mg of cupric sulphate pentahydrate, 918 mg of L-Isoleucine, and 5.94 mg of ammonium acetate using a stirrer.

Mobile phase: Mix the following solvents in a given ratio,

Methanol	:	Buffer A
30%	:	70%

Filter through 0.45µm filter paper and degas the mobile phase in an Ultra sonic bath for 5 minute

Diluents: Acetonitrile: Water (20:80)

Chromatographic Conditions:

The following conditions have been found suitable

Dimensions	:	25cm x 4.6mm
Packing	:	5-µm Packing L1
Temperature	:	45°C
Flow rate	:	0.8 ml/min
Detector	:	UV at 360 nm.
Injection volume	:	25 µl

System suitability requirement: Tailing factor: NMT 1.8; Standard solution RSD: NMT 2.0%, Standard solution.

Sample solution preparation:

Take NLT 20 tablets of sample of Levofloxacin 250 mg tablet and grind to powder and take powder equivalent to 5 tablet and transfer to 250 ml volumetric flask and add about 75% diluents of the final volume, and allow to stand for 15 minutes and sonicate for 2-5 minutes, and dilute to volume with diluents and mix for 10 minutes with stirring.

Filter the above solution with a suitable filter and Take 2 ml of above solution in a 50 ml volumetric flask and dilute to volume with mobile phase for the final concentration 0.2 mg/ml.

Standard preparation:

Weigh accurately equivalent to 100 mg of USP Levofloxacin (……...mg) or working standard, and transfer to 50ml volumetric flask and dissolve it in diluents to the volume and mix for 2-5 minutes.

Take 10 ml of above solution in a 100 ml volumetric flask and dilute to volume with mobile phase for the final concentration 0.2mg/ml.

1. Linearity of Levofloxacin

Linearity experiments were conducted to identify the range over which Levofloxacin exhibit linear response. The stock solution of Levofloxacin was prepared by dissolving equivalent to 100 mg (Levofloxacin) of finely powder homogeneous sample of Levofloxacin 500 mg tablet into 100 ml of diluents for final concentration of 1.0 mg/ ml of Levofloxacin.

The stock solution was gravimetrically diluted in diluents to concentrations of 50 ppm (0.05 mg/ml) ,100 ppm (0.1 mg/ml) ,200 ppm (0.2 mg/ml) ,300 ppm (0.3 mg/ml) , and 500 ppm (0.5 mg/ml) respectively. The calibration graph is shown below, indicates linear relationship observed between the concentration and absorbance of the solutions. The R2 of calibration data point was calculated to 0.9999. This indicates that the test procedure obeys Beer's law.

Table.1 Average peak area of different concentration samples using stock solution to find the linearity curve using calibration curve method

Sr. No.	Concentration (mg/ml)	injections	Retention time	Peak areas	Average peak areas of test solution
1	0.05	1	21.846	993030	993030
2	0.1	1	21.905	2011403	2011403
3	0.2	1	22.047	4037275	4037275

4	0.3	1	22.194	6229148	6229148
5	0.5	1	22.397	10051526	10051526

The calibration graph showing linear relationship between the absorbance and concentration of analyte in the solution is shown below.

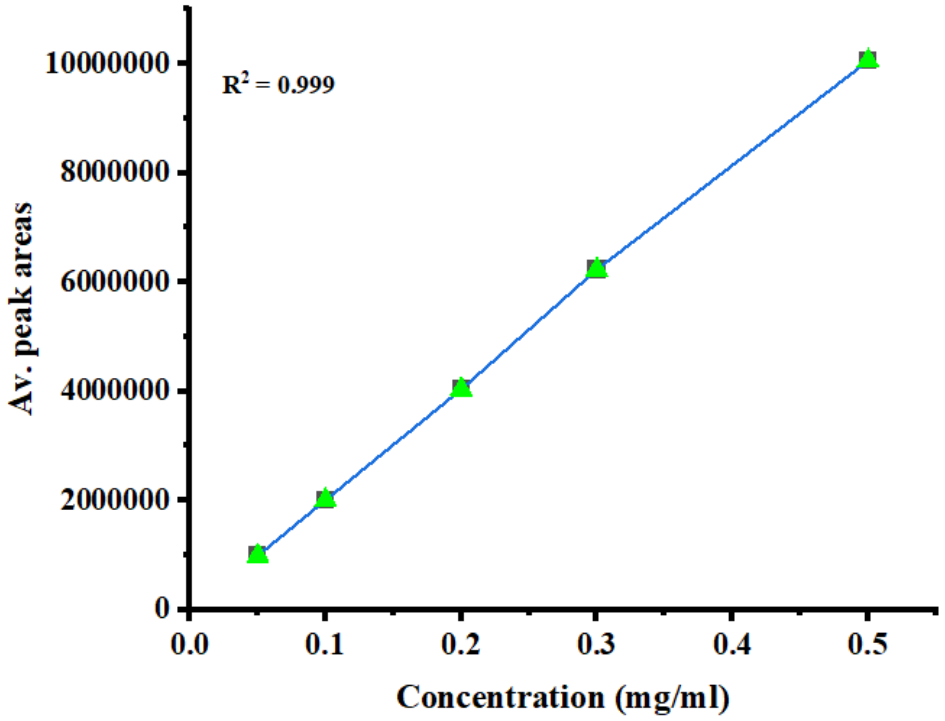

Figure 4 Line graph of all different concentrations (PPM) with peak areas

The connection between the concentration of the solutions and their absorbance may be seen to be linear in the calibration graph. Therefore, this demonstrates that Beer's law is followed by the testing technique.

2. Precision

An inquiry into precision is part of the verification process for tests used for the assay. If the testing procedure was found to be repeatable and reproducible, this will be put through its paces by the repeatability and reproducibility tests.

Repeatability (Levofloxacin)

In other words, repeatability is how close together a series of measurements of the same thing or area are when taken in a row by the same person with the same tool or machine. Repeatability is also called test-retest reliability, which means that when you retest or measure an item or area, you get pretty much the same result. We say that there is variability when the same measurement is not made, which means that a tool or device is not reliable. Variability can be caused by the quality of the tool being used to measure, the fact that the tool isn't calibrated, the person using the tool, or things like the room temperature or the stability of the thing being measured. A measurement that can be done over and over again means that the operator will always get the same measurement value. For a measurement to be truly accurate, it must be repeatable and accurate. Repeatability, on the other hand, is a very important part of accurate measurements in and of itself. We wanted to tell you what repeatability is, why it's so important to us at Higher Precision, and why you should too.

It was worked out by adding up the results of six separate calculations. Six samples were taken and test solutions were made and tested according to the test procedure. This was done to see if the results could be repeated.

Repeatability can be measured by figuring out the concentration of six samples at 100%, or by making three samples with concentrations of 80%, 100%, and 120%. This part of precision looks at how well the conditions of the method work and estimate how much variation can be expected for a single analyst and HPLC system for a given sample.

Weight of Reference Standard = 51.6 mg Purity 99 %

Peak areas of Reference Standard are 5124273, 5128919, 5122598, 5123760, and 5126915

Average peak area of reference standard = 5125293

Average weight /tablet = 780 mg/tab (Levofloxacin 500 mg tablet)

Table 2 Six readings are taken at the same retention time, concentration to find the difference between the real results and the theoretical ones in percentage.

Sr. No.	Concentration (mg/ml)	Injections	Retention time	Peak areas	Average peak areas of test solution	% age Results of LC	Variation from theoretical Results
1	0.2	1	16.983	5021803	5021509	98.08%	-1.92%
		2	16.983	5022284			
		3	16.982	5020441			
2	0.2	1	16.973	4881324	4877610	99.08%	-0.90%
		2	16.970	4880824			
		3	16.968	4870681			
3	0.2	1	16.797	5022430	5026040	98.55%	-1.45%
		2	16.986	5028481			
		3	16.987	5027209			
4	0.2	1	16.989	4991687	4992245	98.01%	-1.99%
		2	16.980	4993173			
		3	16.981	4991975			
5	0.2	1	16.977	5015322	5019213	98.04%	-1.96%
		2	16.979	5024688			
		3	16.979	5017630			
6	0.2	1	16.977	5032776	5033654	98.32%	-1.68%
		2	16.976	5032442			
		3	16.974	5035723			

After analyzing the six different replicates with the same concentration, peak area, and differences from the theoretical results, they came to the following conclusions. Range: (98.01 --- 99.55%), Mean: 98.34%, Standard Deviation = 0.4475%, Relative Standard Deviation = 0.4551% ± 2.00 %

3. Reproducibility of (Levofloxacin)

The capability of carrying out the test once more is the third and last component of testing for precision. In order to facilitate comparisons between testing locations, samples are created here. This is something that typically occurs when a piece of technology is transferred from one location to another. At both locations, samples are prepared in the same manner, and then they are compared to a predetermined list of acceptability criteria that was previously discussed and agreed upon. According to the ICH, repeatability studies are not required to be included in a submission; nonetheless, conducting them is highly recommended in order to guarantee that all testing facilities are capable of performing the technique in the same manner. It is possible to validate a method using a reproducibility study rather than intermediate precision; however, doing so is not required and will not cause any issues. Either way may be examined.

Analyst 01

Weight of Reference Standard = 51.8 mg Purity 97.01%

Peak areas of Reference Standard = 5136283, 5139459, 5139121, 5138709, 513789

Average peak area of reference standard = 5137893

Average weight / tablet = 390 mg/tab (Levofloxacin 250 mg tablet)

Table 3 Reproducibility 1 at same concentration with some variations from the theoretical results

Sr. No.	Concentration (mg/ml)	Injections	Retention time	Peak areas	Average peak areas of test solution	% age Results of LC	Variation from theoretical Results
1	0.2	1	16.975	5021447	5021496	98.22%	-1.78%
		2	16.975	5020408			
		3	16.976	5022634			
2	0.2	1	16.983	5100753	5101131	99.78%	-0.22%
		2	16.984	5103297			
		3	16.984	5099342			
3	0.2	1	16.984	5035964	5035770	98.50%	-1.50%
		2	16.987	5033202			
		3	16.989	5038143			
4	0.2	1	16.990	4996807	4999053	98.01%	-1.99%
		2	16.993	5002056			
		3	16.994	4998295			
5	0.2	1	16.998	5030130	5033893	98.46%	-1.54%
		2	17.024	5037656			

Range: (98.01 – 99.78%) Mean: 98.59% Standard Deviation = 0.69179 Relative Standard Deviation = 0.7017 % ± 2.00 % **Analyst 02**
Weight of Reference Standard = 51.8 mg Purity 97.01
Peak areas of Reference Standard = 4176143, 4181512, 4170976, 4181644, 418185
Average peak area of reference standard = 417842
Average weight /tablet = 390 mg/tab (Levofloxacin 250 mg tablet)

Table 4 confirmation of analysis by analyst 2 at same concentration with same retention time

Sr. No.	Concentration (mg/ml)	injections	Retention time	Peak areas	Average peak areas of test solution	% age Results of LC	Variation from theoretical Results
1	0.2	1	16.914	4079625	4076144	98.04%	-1.96%
		2	16.912	4072068			
		3	16.909	4076738			
2	0.2	1	16.907	4057708	4057333	98.09%	-1.91%
		2	16.905	4055955			
		3	16.905	4058337			

3	0.2	1	16.987	4024729	4026130	98.01%	-1.99%
		2	16.897	4026565			
		3	16.895	4027096			
4	0.2	1	16.898	4060827	4062918	98.22%	-1.78%
		2	16.894	4063591			
		3	16.897	4064336			
5	0.2	1	16.918	4189404	4189795	99.75%	0.25%
		2	16.917	4190183			
		3	16.917	4189798			

Range: (98.01 – 99.75%), Mean : 98.42%, Standard Deviation = 0.7467%, Relative Standard Deviation = 0.7587% ± 2.00 %

4.0 Accuracy (Levofloxacin)

In the laboratory, three samples with a total weight of 250 grams were prepared in accordance with the steps involved in the production of the product. Quantities of analyte that were comparable to 80%, 100%, and 120% of the quantity that was stated on the label were added to each placebo. In order to test the product, test solutions of each concentration (namely 80%, 100%, and 120%) were produced, analyzed, and tested in duplicate in accordance with the product's testing process. The following are the tabulated results:
Weight of Reference Standard = 51.8 mg, Purity 97.01%
Peak areas of Reference Standard = 4201337, 4199890, 4200504, 4199613, 4200406
Average peak area of reference standard = 4200350

Table 5 at different percentages of active material find the assay of each sample with respect to label claim

Contents of Active Added in Placebo (% of Label Claim)	**80 % (200mg/tab)**		**100 % (250mg/tab)**		**120 % (300 mg/tablet)**	
Weight of Placebo for each tablet			122 mg/tablet			
Standard Compression weights for each sample	328 mg/Tab	328 mg/Tab	380 mg/Tab	380 mg/Tab	431 mg/Tab	431 mg/Tab
Weights of each concentration Samples taken for analysis eq. to 1250 mg Levofloxacin	2040 mg	2040 mg	1900 mg	1900 mg	1800 mg	1800 mg
Peak area of test solution	4095425	4081430	3973972	4084779	4221416	4111303
Recovery for each concentration	200.8 mg/tab	200.19 mg/tab	245.46 mg/tab	248.26 mg/tab	298.90 mg/tab	294.43 mg/tab
% age of label claim	100.44%	100.09%	98.18%	99.30%	99.66%	98.14%
Variation from Theoretical Results or Difference	0.44%	0.09%	-1.816%	-0.70%	-0.34	-1.86%
Average results of each concentration	100.26%		98.74%		98.90%	
Standard Deviation	0.26%		-1.26%		-1.1%	

5.0 Robustness (Levofloxacin)

It is the measure how stable the test procedure is under slight variation in test procedure. The following changes were made deliberately in testing procedure. The test solution prepared according to the test procedure and kept at 15°C and 35°C for 24 hours and assayed according to the test procedure. The results are compound with initial results and tabulated below.

Weight of Reference Standard = 51 mg, Purity 97.01%

Peak areas of Reference Standard = 4065095, 4073895, 4071783, 4069561, 4069561

Average peak area of reference standard = 4070083

Average weight /tablet = 780 mg/tab (Levofloxacin 500 mg tablet)

Table 6 to validate the stability of product at various temperatures to find its efficiency

Storage Condition	**15°C**		**25°C**		**35°C**	
	Sample I	Sample II	Sample I	Sample II	Sample I	Sample II
Weight of Samples	780 mg	780 mg	780 mg	780 mg	780 mg	780 mg
Peak area of test solution	4078236	3962988	4235366	4092135	4099778	4025494
% of Label Claim	99.14%	98.88%	99.89%	99.48%	99.67%	98.36%
Average	99.89%		99.68%		99.01%	

The test samples are stable for up to twenty-four hours at temperatures ranging between 15°C and 35°C.

II. Conclusion

The purpose of this study is to reflect the efficacy of levofloxacin under different conditions in order to meet the USP requirements by assessing it using the verification method. Our results show that the product levofloxacin is stable at the following stages: linearity curve using different ppm solutions to find the calibration curve, repeatability to find the replicates peak areas at the same concentration, reproducibility to determine whether or not our results are reproducible, accuracy to make active material mixing with inactive at different percentages 80%, 100%, and 120% to compare the obtained results from HPLC chromatogram with theoretical values.

Conflict of interest

The writers have no financial or other competing interests.

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