

In-vitro Interaction Study of Levofloxacin with Metformin HCl and Losartan Potassium and Investigation of Antimicrobial Activity

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Abstract

Background: This research work is a drug-drug interaction study between two widely used medications Levofloxacin and Metformin HCl as well as Losartan Potassium and this study is carried out at that time when they are used concomitantly (In- Vitro). Levofloxacin, this medication belongs to class of drugs known as quinolone antibiotics and is used to treat a wide range of gram positive (+) and gram-negative (-) bacterial infections. Metformin HCl is a medicine used to type 2 diabetes and gestational diabetes. Metformin is used with a proper diet and exercise program and possibly with other medications to control high blood sugar. Controlling high blood sugar helps prevent kidney damage, blindness, nerve problems, loss of limbs, and sexual function problems.

Objective: The research work aims to find out the quantitative interaction between Levofloxacin and Metformin HCl as well as Losartan Potassium and to identify how much interaction affects the effectiveness of Levofloxacin that has been done by Antimicrobial Analysis.

Methodology: This research work ensures that there is a possible interaction between the Levofloxacin and Metformin HCl and Losartan Potassium which is confirmed by Job's plot method as well as by the Antimicrobial study. In order to investigate the number of Losartan Potassium and Metformin HCl involved in the complexation with Levofloxacin complexes was demonstrated by exploiting various spectrophotometric methods. The ultraviolet studies of these complexes were carried out and compared.

Findings: The absorbance of Levofloxacin is quite different from the absorbance of Levofloxacin- Metformin HCl and Levofloxacin- Losartan Potassium complexes. Levofloxacin forms a strong 1:1 complex with Metformin and Losartan as 'Λ' shaped curves in the Job's plot. These curves may indicate strong kinetics of complexation between Levofloxacin and Metformin and Losartan.

By antimicrobial investigation, it is observed that the zone of inhibition of the drug Levofloxacin with Metformin and Losartan is reduced from 12 mm, 10 mm and 8 mm respectively.

Keywords: interaction; Levofloxacin; Metformin HCl; losartan potassium; job's plot, zone of inhibition

Introduction

The danger of drug interactions rises disproportionately as the number of drugs taken rises. The increased risk of harmful drug interactions can be caused by over-the-counter medications. Levofloxacin is an antibiotic that belongs to the third generation of fluoroquinolones (1). Levofloxacin (-)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7Hpyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrate is a Japanese originated synthetic broad spectrum antibacterial agent against

Gram-positive and Gram-negative bacteria including Staphylococcus species; Streptococcus pneumonia, Streptococcus pyogenes, Streptococcus hemolyticus, Enterobacter species, Escherichia coli, Salmonella, Klebsiella, Serratia, Enterococcus, Pseudomonas aeruginosa and Proteus species (2). It is a highly effective antibiotic that works against a variety of bacteria. Levofloxacin is used in a variety of bacterial infections, most importantly acute pneumonia (Lung infection), bronchitis (Inflammation of lung

passages), skin infections, as well as urinary tract infection (3). A bacterial infection is a condition in which bacteria grow in the body and cause infection. It can target any body part and multiple very quickly. False-positive urine screening results for opiates have been reported in patients using some quinolones. These false-positives are more likely to occur with levofloxacin, ofloxacin, and pefloxacin when used clinically because they produce urinary concentrations sufficient to interfere with commercially available immunoassay kits (4). Confirmation of positive opiate screens by more specific methods may be necessary. Metformin hydrochloride is an oral anti hyperglycemic agent used to lower blood glucose in patients suffering from diabetes of non-insulin dependent. Metformin hydrochloride (Glucophage), chemically is known as 1,1-Dimethyl biguanide hydrochloride with a molecular formula of $C_4H_{11}N_5.HCl$ (5). It is an oral antidiabetic drug that has been used in the treatment of non-insulin dependent diabetes which improves control of glycemia primarily by inhibiting hepatic

gluconeogenesis and glucogenolysis and seems to ameliorate hyperglycemia by improving peripheral sensitivity to insulin, reducing gastrointestinal glucose absorption and hepatic glucose production (6). Recently, metformin has also become available for the treatment of polycystic ovary syndrome and has been found to improve vascular function, prevent pancreatic cancer and revers fatty liver diseases (7). This study is aimed to evaluate the in-vitro complexation nature and strength of complex which may be formed due to interaction between Levofloxacin and Metformin HCl, Levofloxacin and Losartan Potassium and investigation the bioavailability in terms of antimicrobial study of Levofloxacin with them.

Material and Methods

All the chemicals used here were analytical grade and were sorted under optimum storage conditions.

Table 1: List of chemical reagents and source.

Sl No.	Name	Source
1	Levofloxacin	Albion Laboratories Ltd.
2	Metformin HCl	Albion Laboratories Ltd.
3	Losartan Potassium	Albion Laboratories Ltd.
4	Sodium Di-Hydrogen Phosphate	Department of Pharmacy, USTC
5	Di-Sodium Hydrogen Phosphate	Department of Pharmacy, USTC
6	Demineralized Water	Department of Pharmacy, USTC

Preparations of stock solution (8):

Levofloxacin solution 100 ml of 1×10^{-2} was prepared by dissolving 0.3614 gm. of Levofloxacin in 100 ml of Demineralized water in a 100 ml volumetric flask. The stock solution was diluted to desired strength by buffer solution.

Preparations of Drug-1 (Metformin HCl) solution:

Metformin HCl solution of 100 ml of 1×10^{-2} was prepared by dissolving 0.1656 gm. of Metformin HCl in 100 ml of Demineralized water in a 100 ml volumetric flask. The stock solution was diluted to desired strength by buffer solution.

Preparation of Drug-2 (Losartan Potassium) solution:

For the preparation of 0.01M Drug-2 solution, Losartan Potassium (0.461gm) was weighted accurately and introduced with the help of a funnel

in a 100 ml volumetric flask, dissolved in demineralized water, and makeup to the mark with the same solvent.

Preparation of Standard curve of Levofloxacin (8):

Levofloxacin stock solution at pH 7.4 and concentration of 1×10^{-4} M was added in different concentrations to ten test tubes, to have the following concentrations: 9×10^{-5} M, 8×10^{-5} M, 7×10^{-5} M, 6×10^{-5} M, 5×10^{-5} M, 4×10^{-5} M, 3×10^{-5} M, 2×10^{-5} M, 1×10^{-5} M.

The solutions were then properly mixed. The absorbance values of the solutions were determined at 298 nm by UV-Visible Spectroscopy. As a control of reference sample, phosphate buffer solution of pH 7.4 was used. The standard curve was obtained by plotting the absorbance values against the corresponding concentrations.

Result and Discussion

Spectral studies of interaction of Levofloxacin with Metformin (9):

Table 2: Spectral analysis of Levofloxacin with Metformin

Wavelength (nm)	Absorbance of Levofloxacin	Absorbance of Levofloxacin + Metformin
200	0.0050	0.0053
205	0.0055	0.0060
215	0.0125	0.0146
225	3.4183	1.6547
235	1.1779	0.5893
245	0.8650	0.4441
255	0.8834	0.4020
265	0.6330	0.2795
275	1.0307	0.4003

285	4.0575	0.6551
295	1.3121	0.4262
305	0.4245	0.1202
315	0.4000	0.1119
325	0.5339	0.1939
335	0.6138	0.2530
345	0.4852	0.2007
355	0.2090	0.0738

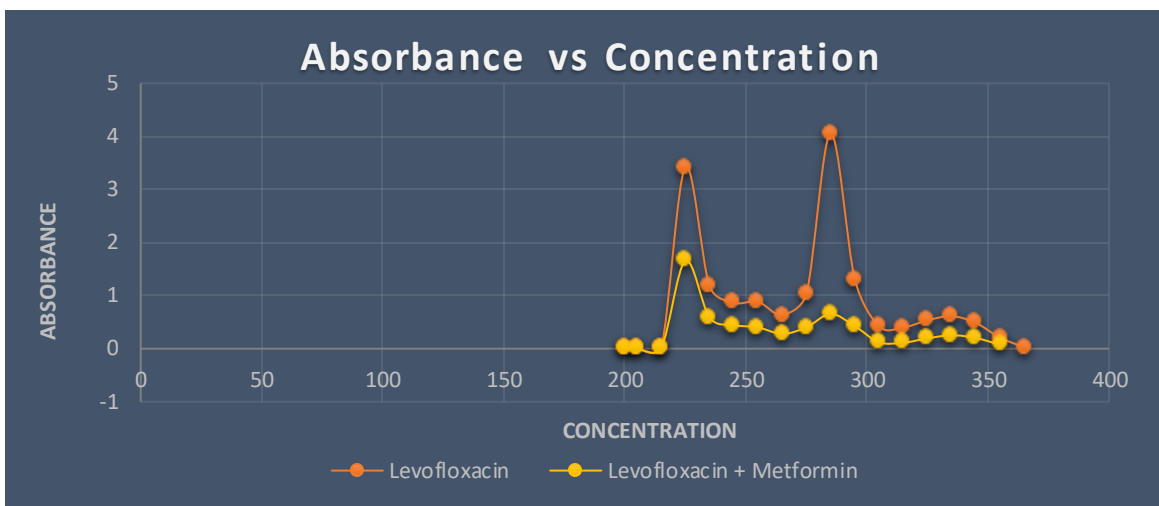


Figure 1: Spectral plot of Levofloxacin and Levofloxacin with Metformin

In x-axis: wavelength

In y-axis: absorbance

From the figure we can observe that the absorbance of Levofloxacin is different when it interacts with Metformin.

Spectral studies of interaction of Levofloxacin with Losartan:

Table 3: Spectral analysis of Levofloxacin with Losartan

Wavelength (nm)	Absorbance of Levofloxacin	Absorbance of Levofloxacin + Losartan
200	0.0050	0.0022
205	0.0055	0.0137
215	0.0125	0.0171
225	3.4183	3.4310
235	1.1779	1.4367
245	0.8650	0.8121
255	0.8834	0.7404
265	0.6330	0.4790
275	1.0307	0.4816
285	4.0575	0.6405
295	1.3121	0.3827
305	0.4245	0.0870
315	0.4000	0.0805
325	0.5339	0.1632
335	0.6138	0.2228
345	0.4852	0.1748
355	0.2090	0.0538

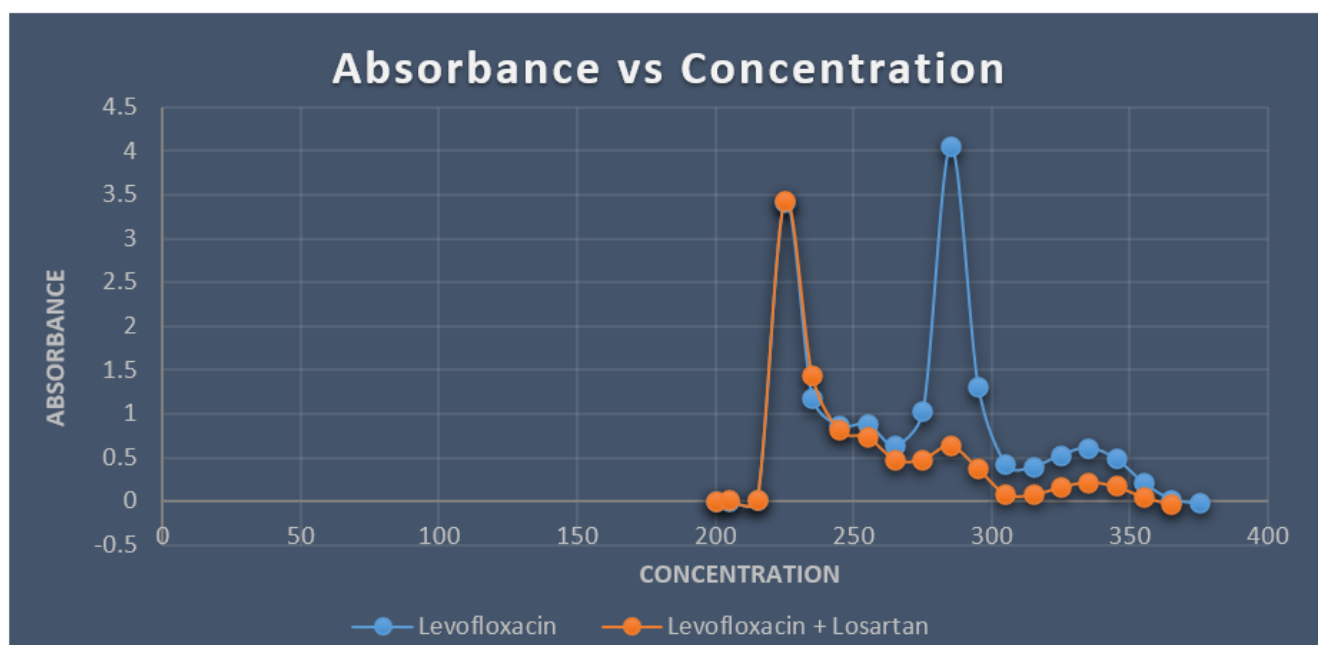


Figure 2: Spectral plot of Levofloxacin and Levofloxacin with Losartan

In x-axis: wavelength

In y-axis: absorbance

From the figure we can observe that the absorbance of Levofloxacin is different when it interacts with Losartan.

Table 3: Combined Spectral Curve of Levofloxacin and Levofloxacin with Metformin and Levofloxacin with Losartan (11):

Wave length (nm)	Absorbance of Levofloxacin	Absorbance of Levofloxacin + Metformin	Absorbance of Levofloxacin + Losartan
200	0.0050	0.0053	0.0022
205	0.0055	0.0060	0.0137
215	0.0125	0.0146	0.0171
225	3.4183	1.6547	3.4310
235	1.1779	0.5893	1.4367
245	0.8650	0.4441	0.8121
255	0.8834	0.4020	0.7404
265	0.6330	0.2795	0.4790
275	1.0307	0.4003	0.4816
285	4.0575	0.6551	0.6405
295	1.3121	0.4262	0.3827
305	0.4245	0.1202	0.0870
315	0.4000	0.1119	0.0805
325	0.5339	0.1939	0.1632
335	0.6138	0.2530	0.2228
345	0.4852	0.2007	0.1748
355	0.2090	0.0738	0.0538

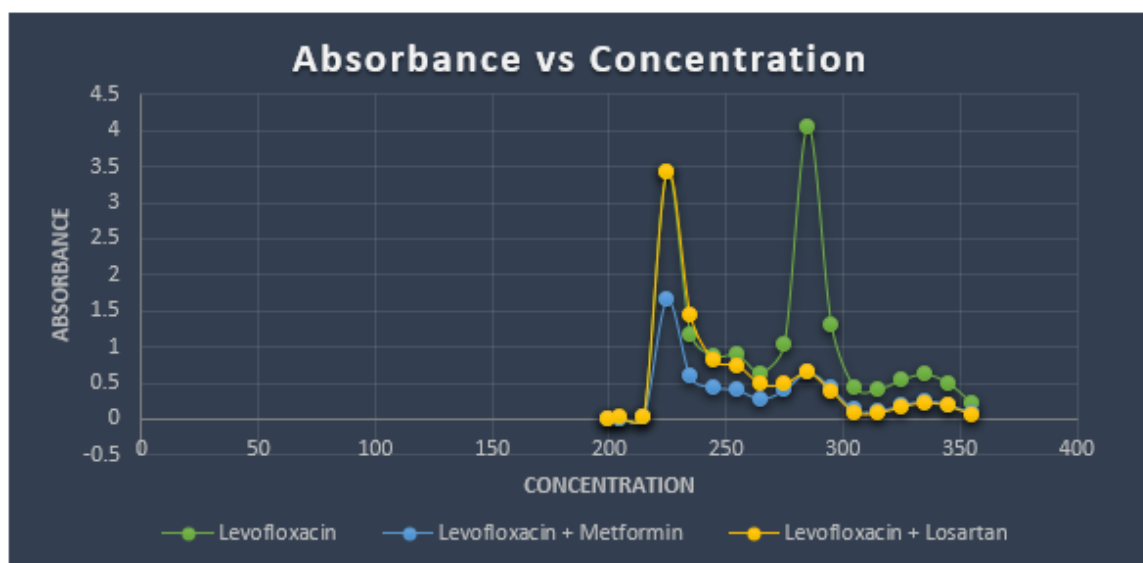


Figure 3: Combined Spectral Curve of Levofloxacin and Levofloxacin with Metformin and Levofloxacin with Losartan. This figure shows that the absorbance of Levofloxacin is quite different from the absorbance of Levofloxacin and Metformin and Losartan.

Table 4. Values of Jobs Plot of Levofloxacin and Metformin (12):

Conc. of Levofloxacin ($M \times 10^{-5}$)	Abs. of Levofloxacin A	Conc. of Metformin ($M \times 10^{-5}$)	Abs. of Metformin B	Abs. of mixture C	Abs. Difference $D=(A+B)-C$
1	0.0211	9	0.4903	0.3944	0.1170
2	0.1148	8	0.4397	0.0613	0.4932
3	0.2357	7	0.3999	0.5739	0.0617
4	0.3322	6	0.3223	0.5553	0.0992
5	0.5025	5	0.2774	0.1716	0.6083
6	0.7408	4	0.2134	0.7589	0.1953
7	0.8568	3	0.1595	0.8914	0.1249
8	0.9222	2	0.1071	0.9294	0.0999
9	0.9819	1	0.0476	1.0053	0.0242

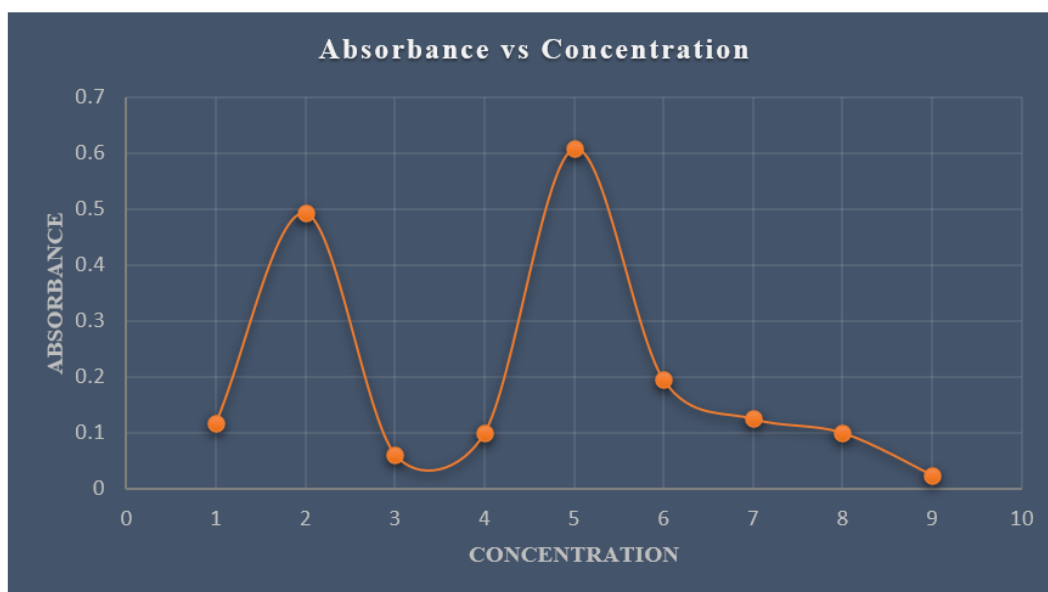


Figure 4: Jobs Plot of Levofloxacin and Metformin

From the above figure we can observe that Levofloxacin forms strong 1:1 complex with Metformin which is indicated as '∧' shaped curve. This '∧' shaped curve confirms the interaction between Levofloxacin and Metformin.

Table 5. Values of Jobs Plot of Levofloxacin and Losartan:

Conc. of Levofloxacin (M $\times 10^{-5}$)	Abs. of Levofloxacin A	Conc. of Losartan (M $\times 10^{-5}$)	Abs. of Losartan	Abs. of mixture C	Abs. Difference D=(A+B)-C
1	0.0211	9	0.9559	0.4601	0.5169
2	0.1148	8	0.8833	0.3802	0.6179
3	0.2357	7	0.7596	0.2685	0.7268
4	0.3322	6	0.5919	0.5980	0.3261
5	0.5025	5	0.4126	0.1250	0.7901
6	0.7408	4	0.2672	0.7842	0.2238
7	0.8568	3	0.1356	0.8188	0.1736
8	0.9222	2	0.0657	0.8648	0.1231
9	0.9819	1	0.0124	0.9261	0.0682

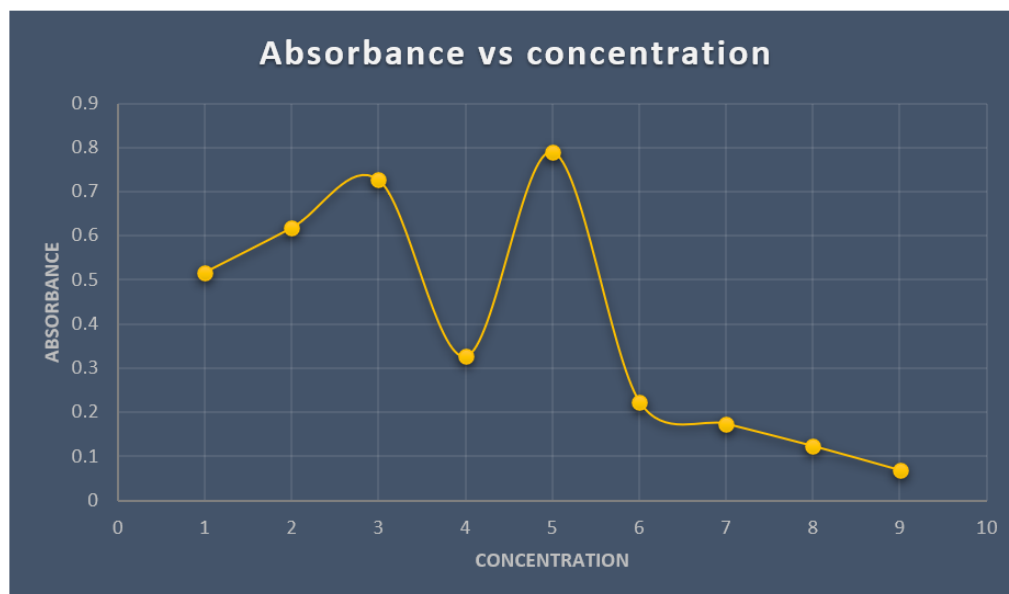


Figure 5: Job's Plot of Levofloxacin and Losartan

From the above figure we can observe that Levofloxacin forms strong 1:1 complex with Losartan which is indicated as '^' shaped curve. This '^' shaped curve confirms the interaction between Levofloxacin and Losartan.

Table 6. Combined absorbance of Levofloxacin and Levofloxacin with Metformin and Levofloxacin with Losartan at different concentration:

Levofloxacin	Levofloxacin + Metformin	Levofloxacin + Losartan
0.0211	0.0613	0.1250
0.1148	0.1716	0.2685
0.2357	0.3944	0.3802
0.3322	0.5553	0.4601
0.5025	0.5739	0.5980
0.7408	0.7589	0.7842
0.8568	0.8914	0.8188
0.9222	0.9294	0.8648
0.9819	1.0053	0.9261

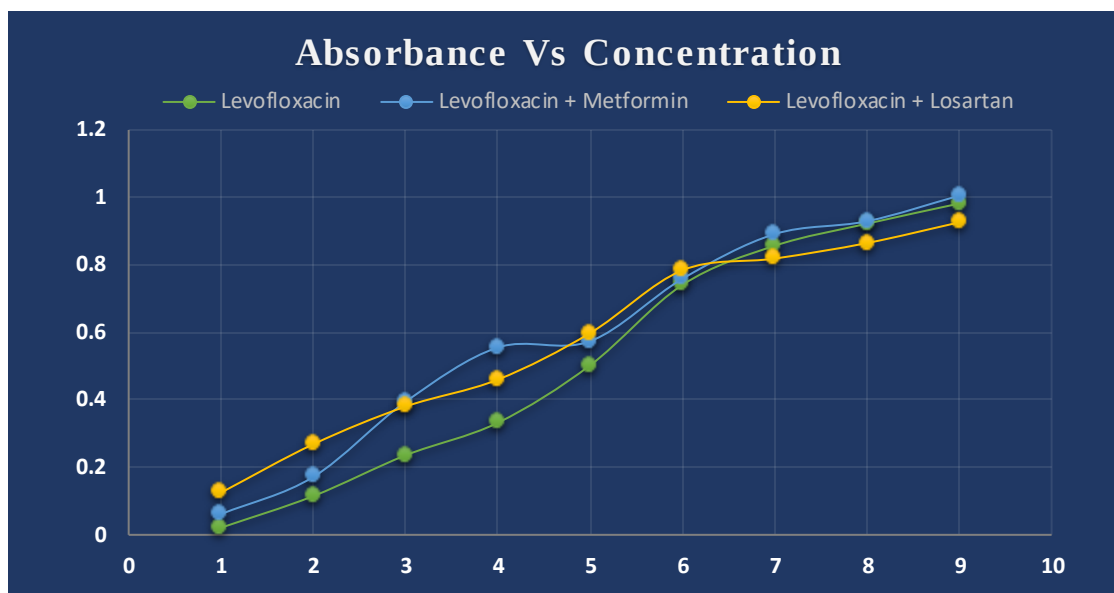


Figure 6: Combined absorbance of Levofloxacin and Levofloxacin with Metformin and Levofloxacin with Losartan

The above figure shows that the absorbance of Levofloxacin differs from the absorbance of Levofloxacin + Metformin and Levofloxacin + Losartan due to interaction.

Activity against gram-positive bacteria (13)

The test samples were tested against *Staphylococcus aureus*. The standard Levofloxacin disk also tested against *Staphylococcus aureus*. The results of the antimicrobial activity, measured in terms of diameter of zone of inhibition in mm are showed in table.

Bacteria used	Standard disk (zone of inhibition/mm)	Sample disk (zone of inhibition)
<i>Staphylococcus aureus</i>	16 mm	Levofloxacin (L)
		12 mm
<i>Staphylococcus aureus</i>	16 mm	Levofloxacin + Metformin (L+M)
		10 mm
<i>Staphylococcus aureus</i>	16mm	Levofloxacin + Losartan (L+L)
		8 mm

Figure 7: Evaluation of antimicrobial study of Levofloxacin with Metformin and Losartan.



In the figure-

L: Levofloxacin Solution

L+M: Levofloxacin + Metformin Solution

L+L: Levofloxacin + Losartan Solution

Conclusion

This research work ensures that there is a possible interaction between Levofloxacin with Metformin and Levofloxacin with Losartan. This research work has been done by two methods one by UV-Visible Spectroscopic method and another one by antimicrobial study.

For the UV-Visible Spectroscopic method, the standard curve for Levofloxacin, Metformin as well as Losartan is prepared and their absorbance vs concentration curve is almost linear means the absorbance is increased with increasing their concentration. After that the spectrum curve of Levofloxacin is measured at a different concentration from 200-355 nm then this spectrum of Levofloxacin compared with the spectrum of Levofloxacin + Metformin mixture and Levofloxacin + Losartan mixture, Levofloxacin spectrum curve is quite different from each other. From these spectrum differences, we would primarily guess there is a possible interaction between Levofloxacin with Metformin and Levofloxacin with Losartan, which will be proved via job's plot in the next stage. In the job's method, a vital method for this research work, it has been seen that Levofloxacin forms strong complexes with Metformin as well as Losartan when they are mixed at a 1:1 ratio (pH 7.4) and provides a “^” shaped curve that confirms the interaction has occurred between Levofloxacin with Metformin as well as Losartan which ultimately affects the antimicrobial activity of Levofloxacin. For the Antimicrobial study, Levofloxacin have been tested against *Staphylococcus aureus* and *Escherichia coli* bacteria and the test have been done via disk diffusion method. Antimicrobial activity confirmed that the zone of inhibition of Levofloxacin, Levofloxacin with Metformin and Losartan against *Staphylococcus aureus* (gram positive bacteria) is lowered from standard disk 16mm to 12 mm, 10 mm and 8 mm respectively. This represents interaction between one another. But the zone of inhibition of Levofloxacin with Metformin and Losartan against *Escherichia coli* (gram negative bacteria) don't show any notable antimicrobial effect. It is known to us; the availability of the drug represents the amount (quantity or concentration) of the drug. It helps in the study of the selection of the best dosage form for treatment. And very important in adjusting the effective dose and dose ranges.

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