

In vitro Interaction of Ceftriaxone & Irbesartan and Investigation of Antimicrobial Activity

Tanjin Sharmily, Md. Shahidul Islam

Student, Assistant Professor

Department of Pharmacy

University of Science and Technology Chittagong (USTC) Bangladesh

Corresponding author's email id: -s_i_liton@yahoo.com

Abstract

The research work comprises of interaction studies of Ceftriaxone with irbesartan and investigation of antimicrobial activity of Ceftriaxone. Ceftriaxone is included among the **Cephalosporin** drug class which is active against a wide range of gram positive and negative bacteria. This drug is used to treat many kinds of bacterial infections, including severe or life-threatening forms such as meningitis. Ceftriaxone is also used to prevent infection in people having certain types of surgery. Ceftriaxone interact with antihypertensive drug such as Irbesartan. Irbesartan is used to treat high blood pressure & to protect the kidneys from damage due to diabetes. lowering the blood pressure helps to prevent strokes, heart attack & kidney problems, Irbesartan belongs to a class of drugs called angiotensin receptor blockers (ARBs). It works by relaxing blood vessels so that the blood can flow more easily. The reaction between Ceftriaxone & Irbesartan were simulated to natural environment. Also the antimicrobial activity of the drug and the complexes were determined. There is an effect of p^H on drug-drug complexation. It has observed that Ceftriaxone interacts with Irbesartan on a p^H 7.4, the stability constant of these complexes were determined in order to evaluate their possible in vivo implications. This research work confirms that there was a possible interaction between the Ceftriaxone & Irbesartan which was confirmed by Jobs plot method and by antimicrobial investigation. By antimicrobial investigation it was observed that the zone of inhibition of the drug Ceftriaxone with

Irbesartan reduced from 14mm to 10mm. In order to investigate the interaction of Irbesartan with Levofloxacin were elucidated by exploiting various spectrophotometric methods. The ultraviolet studies of these complexes were carried out and compared.

Keywords: -Ceftriaxone, Irbesartan, Complexation, Interaction, job's Plot, Antimicrobial Study

INTRODUCTION

A drug interaction is defined as the pharmacological activity of one drug is altered by the concomitant use of another drug or by the presence of herbal medicine, food, drink or by some environmental chemical agent. A drug-drug interaction can delay, decrease or enhance absorption of either drug. The outcome can be harmful if the interaction causes an increase in the toxicity of the drug (1). Sometimes when two drugs interact, the overall effect of one or both of the drug may be greater than desired. For example, certain antacid can prevent certain medicines (such as antibiotics) from being absorbed into the blood stream (2). A reduction in efficacy due to an interaction can sometimes be just as harmful as an increase: patients taking warfarin who are given rifampicin need more warfarin to maintain adequate and protective anticoagulation, while patients taking 'tetracycline', or 'quinolones', need to avoid antacids and milky foods (or

separate their ingestion) because the effects of these antibacterial can be reduced or even abolished if admixture occurs in the gut (3). Drug interaction may be the result of various process. The processes may include alterations in the pharmacokinetics of the drug, such as alteration in the Absorption, Distribution, Metabolism, & Excretion (ADME) of a drug. Alternatively, drug interaction may be a result of the pharmacodynamic properties of the drug, e.g. the co-administration of a receptor antagonist and an agonist for the same receptor (4). Ceftriaxone is an antibiotic used for the treatment of a number of bacterial infections. These include middle ear infections, endocarditis, meningitis, pneumonia, bone and joint infections, intra-abdominal infections, skin infections, urinary tract infections, gonorrhea, and pelvic inflammatory disease (5). It is also sometimes used before surgery and following a bite wound to try to prevent infection. Ceftriaxone can be given by

injection into a vein or into a muscle (6). Irbesartan is a medication used to treat high blood pressure, heart failure, and diabetic kidney disease. It is a reasonable initial treatment for high blood pressure. It is taken by mouth. Versions are available as the combination irbesartan/hydrochlorothiazide (7).

Common side effects include dizziness, diarrhea, feeling tired, muscle pain, and heartburn. Serious side effects may include kidney problems, low blood pressure, and angioedema. Use in pregnancy may harm the baby and use when breastfeeding is not recommended(9). It is an angiotensin II receptor antagonist and works by blocking the effects of angiotensin II.

MATERIAL AND METHODS

Chemical and reagents:

All the chemicals used here were analytical grade and were sorted under optimum storage conditions. The experimental mixtures and solutions were prepared in standard volumetric flasks about one hour prior to recording the data.

Preparations of stock solution

Ceftriaxone solution 100 ml of 1×10^{-2} was prepared by dissolving 0.554 gm. of Ceftriaxone in 100 ml of buffer solution in a 100 ml volumetric flask. The stock solution was diluted to desired strength by buffer solution.

Table 1: List of chemicals and reagents

Sl. No.	Name	Source
1	Ceftriaxone	Gift samples from ACI laboratories ltd.
2	Irbesartan	Gift samples from ACME laboratories ltd.
3	Sodium di-hydrogen phosphate	USTC, Foy's lake, Chittagong, dept. of pharmacy
4	Disodium hydrogen phosphate	USTC, Foy's lake, Chittagong, dept. of pharmacy
5	Demineralized water	USTC, Foy's lake, Chittagong, dept. of pharmacy

Preparation of drug (Irbesartan) solution

For the preparation of 0.01M drug solution, Irbesartan 0.428gm was weighted accurately and introduced with the help of funnel in 100 ml volumetric flask, dissolved in demineralized water and make up to the mark with the same solvent. This primary solution was further diluted ten folds in the same solvent and the final solution was 0.0001 M concentration.

Preparation of buffer solution

To prepare buffer solution 1.76 gm. of disodium hydrogen phosphate was dissolved in demineralized water with 2.43 gm. of sodium di hydrogen phosphate and p^H was adjusted to 7.4 and the volume was made to 1000 ml with the same solution.

Disk diffusion method:

The disk diffusion test, or agar diffusion test, or kirby-Bauer test is a test of the antibiotics sensitivity of bacteria. It uses antibiotic discs to test the extent to which bacteria are affected by those antibiotics. This method is based on the principle that antibiotic-impregnated disk, placed on agar previously inoculated with the test bacterium, pick-up moisture and the antibiotic diffuse radially outward through the agar medium producing an antibiotic concentration gradient. The concentration of the antibiotic at the edge of the disk is

high and gradually diminishes as the distance from the disk increases to a point where it is no longer inhibitory for the organism, which then grows freely. A clear zone or ring is formed around an antibiotic disk after incubation if the agent inhibits bacterial growth.

RESULTS AND DISCUSSIONS

Preparation of standard curve of Ceftriaxone

Ceftriaxone stock solution at p^H 7.4 and concentration of 1×10^{-4} M was added in different concentrations to nine 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 262 nm by UV spectrophotometer. As a control of reference sample, phosphate buffer solution of p^H 7.4 was used. The standard curve was obtained by plotting the absorbance values against the corresponding concentrations (10).

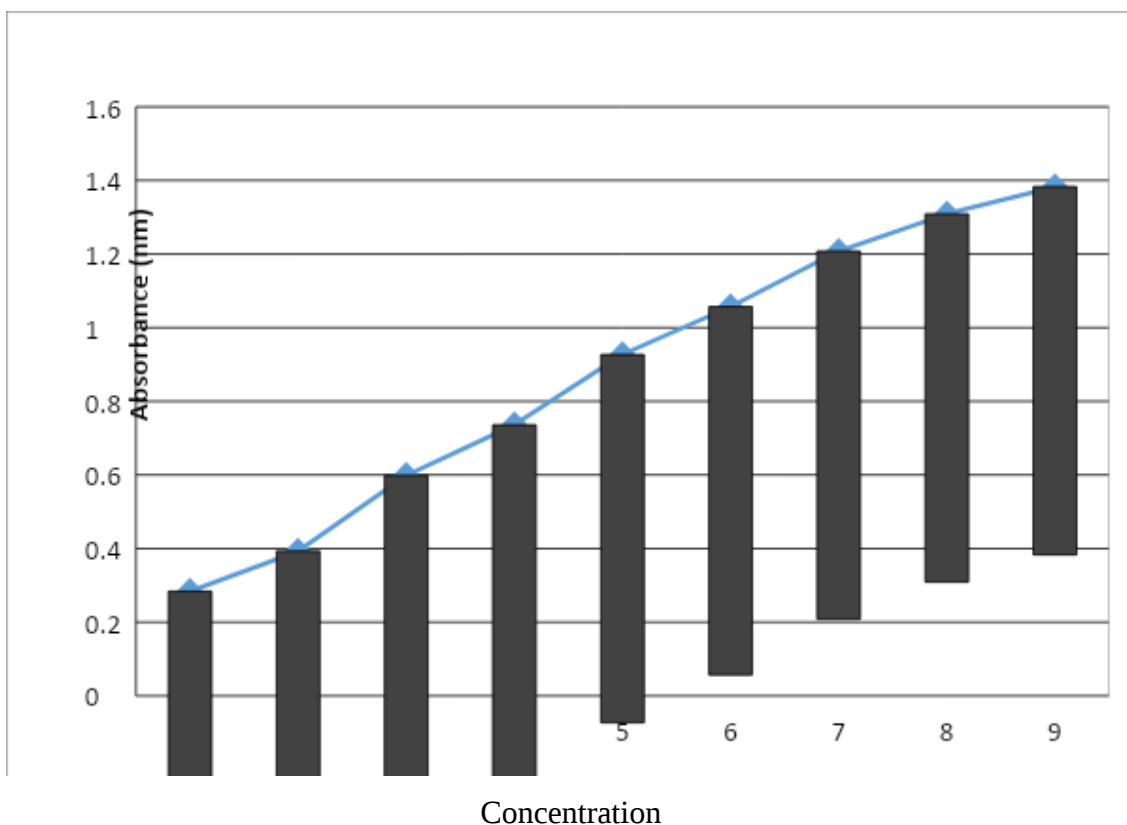


Figure1: Standard curve of Ceftriaxone

From the above figure we can observe that the absorbance of ceftriaxone increases with increasing concentration according to Beer Lambert's law.

Table 2: Spectral studies of interaction of Ceftriaxone & Irbesartan

Wave length (nm)	Absorbance of Ceftriaxone	Absorbance of Ceftriaxone + Irbesartan
200	0.014	0.015
205	0.016	0.012
210	0.018	0.008

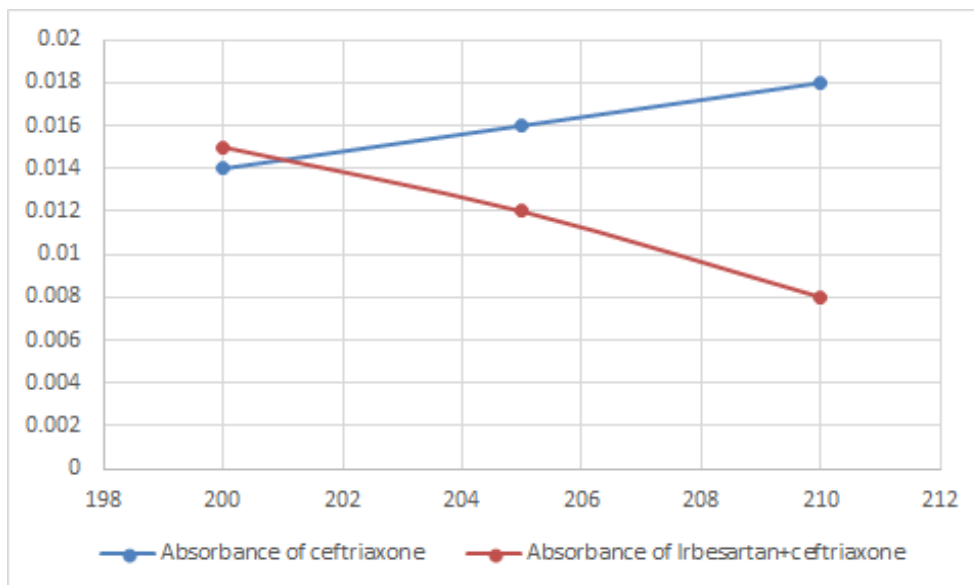


Figure2: Spectral plot of Ceftriaxone & Irbesartan

From the above figure we can observe that, the absorbance of Ceftriaxone is different when it interacts with Irbesartan.

Effect of Irbesartan on Ceftriaxone by Job's method of continuous variation

The molar ratio of the complexes of Ceftriaxone with Irbesartan was estimated by job's method of continuous variation. The observed absorbance values were measured in pH 7.4 at various concentrations (1×10^{-5} to 9×10^{-5} M) of Ceftriaxone & Irbesartan at 262 nm. The Job's plots at pH 7.4 were obtained by plotting absorbance difference against the mole fraction of Ceftriaxone which is presented in the following table.

Table 3: Values of job plot of Ceftriaxone & Irbesartan

Concentration of Ceftriaxone $M \times 10^{-5}$	Absorbance of Ceftriaxone A	Concentration of Irbesartan $M \times 10^{-5}$	Absorbance of Irbesartan B	Absorbance of mixture C	Absorbance difference $D=(A+B)-C$
1	0.284	9	0.900	0.430	0.754
2	0.393	8	0.881	0.454	0.82
3	0.599	7	0.829	0.502	0.926

4	0.736	6	0.775	0.617	0.894
5	0.927	5	0.669	0.589	1.007
6	1.057	4	0.569	0.614	1.012
7	1.208	3	0.432	0.678	0.962
8	1.309	2	0.319	0.705	0.923
9	1.383	1	0.216	0.866	0.733

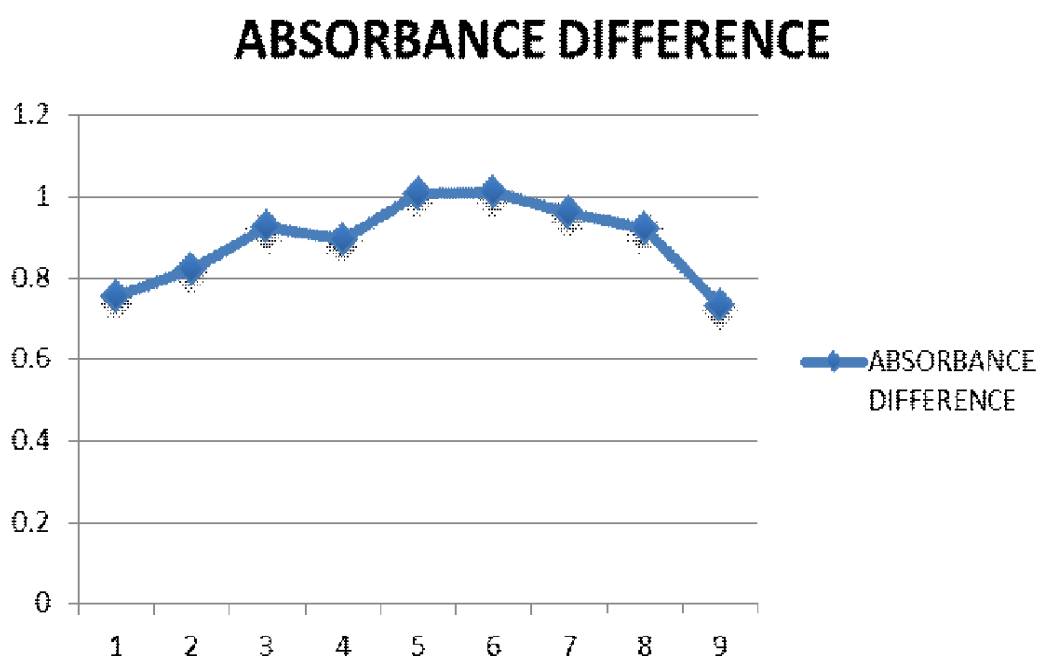


Figure: 3

From the above figure we can observe that Ceftriaxone complexes with Irbesartan which is indicated as 'Λ' shaped curve. This 'Λ' shaped curve confirms the interaction between Ceftriaxone & Irbesartan.

Table 4: Combined absorbance of Ceftriaxone & Irbesartan

Ceftriaxone	Ceftriaxone+Irbesartan
0.284	0.430
0.393	0.454
0.599	0.502
0.739	0.617
0.927	0.589
1.057	0.614
1.208	0.678
1.309	0.705
1.383	0.866

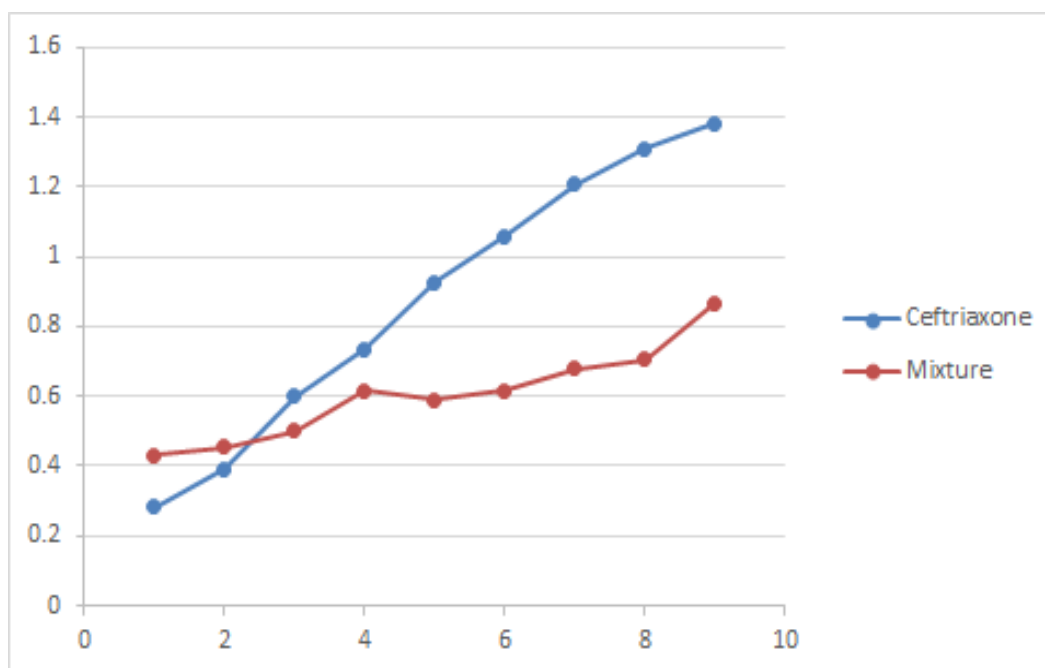


Figure4: Combined absorbance of Ceftriaxone & Irbesartan

The above figure shows that the absorbance of Ceftriaxone differs from the absorbance of Ceftriaxone+Irbesartan due to interaction.

Antimicrobial Study:

The test samples were tested against *Staphylococcus aureus*. The standard Ceftriaxone 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.

Table 5: Results of the antimicrobial activity

Bacteria used	Standard disk (zone of inhibition/mm)	Sample disk (zone of inhibition)
Staphylococcus aureus	16 mm	Ceftriaxone
		14 mm
Staphylococcus aureus	16 mm	Ceftriaxone+Irbesartan
		10 mm

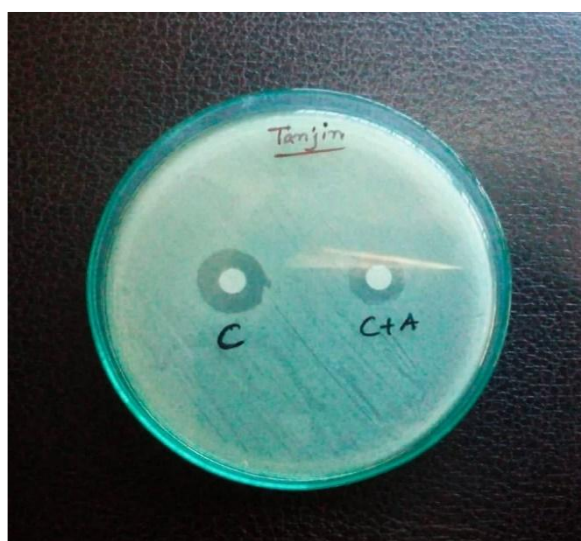


Figure 5: Evaluation of antimicrobial study of Ceftriaxone & Irbesartan

CONCLUSION

In the present work, the interaction of an important antimicrobial drug, Ceftriaxone with Irbesartan at p^H 7.4 by a variety of physical method like inspection of spectral behavior, Job's method of continuous variation. From spectral study, it has been seen that Ceftriaxone gives a peak at 262 nm. When Irbesartan mixed with Ceftriaxone at 1:1 ratio, the intensity of the peak changes remarkably (absorbance decreases) i.e. absorption characteristics are altered due to interaction but the position of the compound do not shift. The curve may indicate strong kinetics of complexation between Ceftriaxone & Irbesartan. Job's plot has given the molar ratio of complexes of Ceftriaxone and Irbesartan. At p^H 7.4 Ceftriaxon forms strong 1:1 complexes with Irbesartan indicated as almost '^' shaped curves. These curves may indicate strong kinetics of complexation between Ceftriaxone & Irbesartan. It is known to us; the availability of the drug represents the amount (quantity or concentration) of drug. It helps in the study of selection the best dosage form for treatment. And obviously very important in adjusting the effective dose and dose ranges. The antimicrobial screening of an agent is essential to ascertain its spectrum against various types of pathogenic organisms. the

susceptibility of organism (bacteria in this case) to antimicrobial agents can be measured *in vitro* by a number of techniques among which the disk diffusion method using different concentration of the agents absorbed on material filter paper disks, is widely acceptable for the preliminary evaluation of antimicrobial activity. From my work, the antimicrobial activity of Ceftriaxone was 14mm, when Ceftriaxone interact with Irbesartan the zone of inhibition was decreased in 10mm.

REFERENCES

1. Dr Per HartvigHonore, Drug interactions, Volume 21, Department of Drug Design & Pharmacology, Faculty of Health & Medical Sciences, University of Copenhagen, Available from: <http://dx.doi.org/10.1136/ejpharm-2013-000388>.
2. James M Ritter, Lionel D Lewis, Timothy GK Mant and Albert Ferro, *A Textbook of Clinical Pharmacology and Therapeutics*, Fifth edition, Hodder Arnold, an imprint of Hodden Education, part of Hachette Livre UK, Available from: <http://www.pharmaresearchlibrary.com/wp-content/uploads/2013/03/A->

- Textbook-of-Clinical-Pharmacology-and-Therapeutics-5th-edition.pdf.
3. Roger Walker, Cate Whittlesea. *Clinical Pharmacy and Therapeutics*, Fifth edition, Churchill Livingstone Elsevier, 2012. Available from: <http://www.prip.edu.in/img/ebooks/Roger-Walker-Clinical-Pharmacy-and-Therapeutics-5th-Ed.pdf>.
 4. Singh Y. Martin's physical pharmacy and pharmaceutical sciences. Department of Pharmaceutics Ernest Mario School of Pharmacy Rutgers, The State University of New Jersey. 2006.
 5. DeFord DD, Hume DN. The determination of consecutive formation constants of complex ions from polarographic data. *Journal of the American Chemical Society*. 1951 Nov;73(11):5321-2.
 6. Sultana NA, Arayne MS, Sharif SA. Ceftriaxone interactions with essential and trace elements. *Pakistan journal of pharmaceutical sciences*. 2004 Jul;17(2):67-76.
 7. Ref.Emily Steadman,Dennis W.Raisch& Marc H.Scheetz.Evaluation of a potential clinical interaction between Ceftriaxone & Calcium.January 19,2010.Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2849391/>
 8. Angehrn P, Probst PJ, Reiner R, Then RL. Ro 13-9904, a long-acting broad-spectrum cephalosporin: in vitro and in vivo studies. *Antimicrob Agents Chemother*. 1980 Dec;18(6):913-921. Available from: <https://ncib.com>
 9. Pool JL, Guthrie RM, Littlejohn TWIII et al.: Dose-related antihypertensive effects of irbesartan in patients with mild-to-moderate hypertension. 2010.
 10. Olson EJ , Bühlmann P . Getting more out of a Job plot: determination of reactant to product stoichiometry in cases of displacement reactions and n: n complex formation. *The Journal of organic chemistry*. 2011 Sep 26;76(20):8406-12.