World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: http://www.wjpsonline.org/
Original Article

Antimicrobial Screening Tests of Boric acid with Fluoroquinolones or Meropenem in some ophthalmic preparations

Bassam M. Al-Abachi[1], Adeeba Y. Shreef [2] and Faris T. Abachi[3]

[1]Department of Ophthalmology, Al-Mosul General Hospital, Nineveh Health Directorates, Ministry of Health, Mosul, Iraq
[2]Department of Biology, College of Science, [3]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Mosul, Mosul, Iraq

Received: 21-06-2019 / Revised Accepted: 03-08-2019 / Published: 31-08-2019

ABSTRACT

The aim of this study is to prepare boric acid ophthalmic solution with antibiotic to treat the mild infections. In this study four ophthalmic formulas were prepared containing boric acid alone or combination with fluoroquinolone (moxifloxacin) or meropenem and screening some properties as well as screening tests for their antibacterial activities *in vitro* against Gram positive and Gram-negative bacteria using gatifloxacin formula as a reference. The purpose of using boric acid is common to treat the mild infections caused by a bacterial or fungal infection of the external eye local therapy. The use of combination therapy with moxifloxacin as potent antibiotic in the treatment of bacterial conjunctivitis. Docking of boric acid with two different types of bacteria, compare some physical and chemical properties and stability study of boric acid at four different temperatures (formula 1), as well as the screening tests for their antimicrobial activities *in vitro* against Gram positive and Gram-negative bacteria using gatifloxacin and glycerin as references.

Keywords: Moxifloxacin HCl, mild infections, Conjunctivitis, Gram positive.

Address for Correspondence: Faris T. Abachi, Department of Pharmaceutical Chemistry, College of Pharmacy, University of Mosul, Mosul, Iraq. E-mail: farisabachi@yahoo.com

How to Cite this Article: Bassam M. Al-Abache, Adeeba Y. Shreef and Faris T. Abachi. Antimicrobial Screening Tests of Boric acid with Fluoroquinolones or Meropenem in some ophthalmic preparations. World J Pharm Sci 2019; 7(9): 101-106.

INTRODUCTION

Boric acid $B(OH)_3$ is a weak inorganic acid, has been externally used and known safer and cheaper product that found in many pharmaceutical preparations [1] such as solutions, ointments, and dust powders. It is widely used in many ophthalmic solutions as eye wash or antiseptic alone or with other agents, due to therapeutic aspects involving the bacteriostatic antimicrobial action.

Fluoroquinolones, are antibacterial antibiotic derivatives with four generations [2]. They inhibit one or more of a group of topoisomerase enzymes. Moxifloxacin hydrochloride and gatifloxacin hydrocholride are fourth generation of fluoroquinolones with a broad-spectrum bactericidal activity against Gram positive and Gram-negative bacteria [3]. Many fluoroquinolone derivatives are formulated as ophthalmic preparations in different concentrations due to their broad antibacterial activities, safer agents.[4]

Meropenem is a carbapenem antibiotic, a non classical beta – lactam derivative, as a broad spectrum antibiotic for several bacterial infection with highest activity against Gram negative aerobic bacteria[5]. The goal of this paper was designated to prepare four opthalamic solutions and determine their antimicrobial activity using boric acid alone or combination with fourth generation fluoroquinolone or meropenem in some ophthalomic solutions.

METHODOLOGY

A. Docking Studies [6]

Molecular docking of boric acid with two types of substrate (*E.coli* and *staphylococcus aureus*) using Mmcule docking online program. This type of study was used to determine the interaction of boric acid and proteins of microorganisms (*E.coli* and *staphylococcus aureus*) would form a complex with overall minimum energy Kcal/mole theoretically. The inorganic acid (boric acid) small organic molecule (Moxifloxacin or Gatifloxacin) as ligands usually fits within protein's cavity which is predicted by the search.

B. Pharmaceutical Formula as eye drops

General Procedure [7]

All chemicals and drugs are used in this study are of highly purity.

Formula 1: Boric acid with allantoin

Dissolve 1.008g of boric acid in water for injection, add 0.21g of borax and 0.08g of allantoin mixed well in electric mixer, then 0.72g chlorbutanol was added as preservative and the volume was completed to 100 ml with distill water. The pH of the solution was adjusted between 6-7.5.

Formula 2: Boric acid with Naphthazoline

A 0.01g of Naphthazoline nitrate was dissolved in water for injection, add 1.0g of boric acid and complete volume with water for injection to 100ml at pH 5-7.

Formula 3: Boric acid with combination Naphthazoline and Meropenem

Mix the contents of the formula 2 with beta-lactam antibiotic 0.3g of meropenem and adjust at the same pH.

Formula 4: Boric acid with Moxifloxacin

Dissolve 0.545g moxifloxacin HCl (equivalent to 0.5g moxifloxacin free base) in normal saline (0.9% sodium chloride), add 1.0g boric acid and complete to 100ml with sodium chloride adjust at pH 4-6.

Formula 5: Gatifloxacin as reference.

Dissolve 3.22g Gatifloxacin Sesquihydrate (equivalent to 0.3 g of Gatifloxacin free base) in water for injection, add 0.05g of benzalkonium chloride and complete to 100 ml at pH 4-6. All formulas were filtered using 0.45µ sterilized millipore membrane filter for certain medications as the preparations are not sufficient it stable to heat.

Clarity test [8]

All solutions are clear and colorless except formulas 2&3 are fait yellow color as compare with 10ml water for injection.

Assay of boric acid

Mix a volume equal to 0.1g of boric acid with 0.8g of mannitol dissolve in 20ml distill water and titrate with 0.1N sodium hydroxide using phenolphthalein solution as indicator. Each one of sodium hydroxide is equivalent to 0.06183g of boric acid.

Spectroscopic determination the content of boric acid [10]

Transferred aliquots of standard stock solution into 25 ml volumetric flask and diluted with distill water. Ultraviolet scanning was done for the sample solutions in the range 200- 400 nm (CARY 100 concdouble beam spectrometer) using 1 cm quartz cells, UV spectrophotometer and absorption maxima of moxifloxacin and gatifloxacin were determined.

Stability studies [11]

The method of shelf-life predication based on 3 months accelerated stability data was utilized. The

graphic techniques have been employed to predict the degradation that may occur over prolonged storage at 25°C. In the present work samples were incubated for 3 months at 40 °C, 60 °C and 75 °C degrees, and other set of samples were preserved at 25 °C for stability studies for prolonged period to determine the actual shelf-life. The degradation of boric acid and its combination with antibiotics behaves according to first – order kinetic. The time for the loss lines at 40 °C & 60 °C to reach 90% of the initial concentration is labelled by arrows on the curve, only formula 1 was studied.

Table 1: Some physical and chemical properties

Test	Formula -1	Formula -2	Formula-3	Formula-4	Formula- 5 Reference (Gatifloxacin)
Description	Colorless clear solution	Yellow, clear solution	Yellow clear solution	Colorless clear solution	Colorless clear solution
Content of Boric acid UV, λ_{max} (nm)	100.2% Assay -	96% Assay -	96% Assay -	- 98.2% At 367.2(nm)	- 101.3% At 384.3(nm)
Particulate	No	No	No	No	No
pH	7.0	5.5	5.2	4.1	4.3
Stability Below 25°C	2 Years*	3 Years	One month*	3 Years	3 Years
Sterility	Sterile	Sterile	Sterile	Sterile	Sterile

* Four different temperatures.

C- Antimicrobial tests[12,13]

The microorganisms are characterized and stocked in the Department of Biology, College of Science, University of Mosul. The microorganisms were Gram positive and Gram negative bacteria. These microorganisms were grown in nutrient broth; the number of bacteria was adjusted at 1.5 x 10^8 cells/mL by comparison with Macfarland tube No. 0.5.

The test ophthalmic solutions were obtained from different formulas dissolved in water for injection (aqueous solutions) of dimethylsulfoxide (DMSO). The activity of each formula was determined by inhibition zone measurement of bacteria growth caused by this test. Nutrient agar plates were seeded with 0.1mL of the broth culture of each bacteria, filter paper disc impregnated with 10µL of each solution were distributed on the surface of the seeded agar plates, incubated for 24-48h at 37°C. Finally, the inhibition zones were measured in mm.

RESULTS & DISCUSSION

Four different ophthalmic formulas were prepared containing boric acid alone or combination with different drugs, naphthazolin, decongestive agent, beta-lactam carbapenem derivative, moxifloxacin antibacterial antibiotic agents. All four ophthalmic are clear solution, no particulate and with a weak pH rang, and they stable at least for two years (Table 1).

The work presented herein is primarily focused on comparing boric acid with other combination their antibacterial activities using gatifloxacin & glycerin as references. [14]. Firstly, the theoretical docking study of boric acid with two types of substrate *E.coli* and *staphylococcus aureus* was study (figures 1 & 2) using Mmcule docking online program, and was showed a -6.1 Kcal/mole & - 5.4 Kcal/mole respectively[15]. This means that a good binding between boric acid with two bacterial substrates. According to the theoretical study, four different formulas were prepared and testing their antibacterial activities.

The contents of the inorganic agent (boric acid) can be calculated using direct titration. While, UV spectrophotometric method was used for determination the concentrations of moxifloxacin and gatifloxacin (Table 1). Both methods are analytical techniques demonstrate good sensitivity, accuracy and highly selective for determination of above mentioned ophthalmic solutions [16&17].

The biological activity was designated to study the inhibition zones of antimicrobial agents of four different ophthalmic solution formulas, the formula 1 (boric acid) had a weak to moderate activity towards six types of bacteria were listed in (Table 2). This formula was containing boric acid in borax and allantoin, and shows a mild antibacterial activity against three types of bacteria.

The formula 2 containing boric acid and naphthazoline (decongestive agent). It had been suggested that addition such antibiotic to the formula will increase the total activity. The results

were indicated to significant effect towards. *S. aureusn* Strain 1, while a negative activity towards S. *aureus* Strain 2. In formula 3 the contents is similar to the formula 2 except the addition of meropenem (beta-lactam) leads to significant effect in the inhibition zone of *pseudomonas aeruginosa* 32 mm, but the solution was showed unstable formula due to the change in pH, color during storage at 25 °C. Further studies to enhance its stability studies against storage conditions[18].

The combination between boric acid and moxifloxacin (formula four) showed highest inhibition zone by *p.aerugnosa* 30 mm, *S. aureus* 40 mm[5].The fourth generation fluoroquinolones are known to be of broad spectrum of activity by inhibiting both Gram positive and Gram negative bacteria. Our conclusion, the boric acid may play an important role as a buffer (excipient) and mild antibacterial agent, and leads to synergistic effect of moxifloxacin.

Table 2: Preminlary screening antimicrobial tests.

Formula No.	*P.aeruginosa* Strain 1	*S. aureus* Strain 1	*E.coli*	*S. aereus* Strain 2	*P.aeruginosa* Strain 2	*Klebsiella Spp.*
1	16	18	20	21	16	23
2	-	32	14	12	-	25
3	32	39	13	20	-	29
4	29	39	38	33	18	35
5 (Reference) (Gatifloxacin)	26	36	29	35	15	32
Glycerin (Reference)	-	28	20	18	-	20

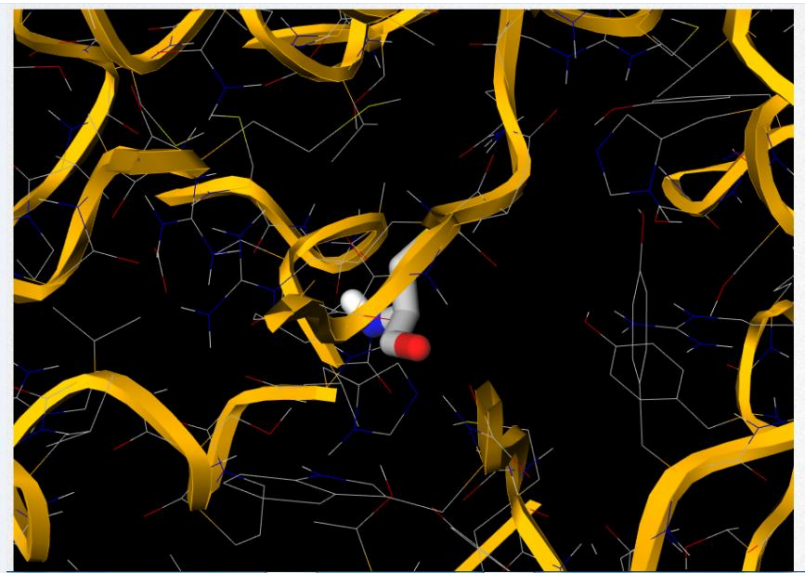

Figure 1: Docking of Boric acid with *E. coli*

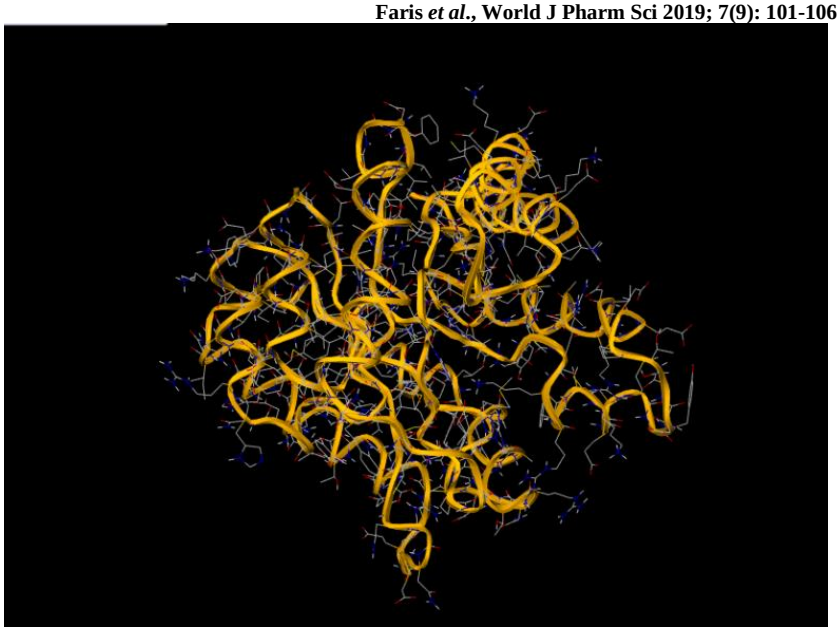

Figure 2: Docking of Boric acid with *S. aerue*

Acknowledgments
We would like to thank Department of Pharmaceutical Chemistry, College of Pharmacy, and Department of Biology, College of Science, University of Mosul, for their financial support. Also, our thanks to Mmcule for docking online.

REFERENCES

1. Sarbjeet SG. UV – Visible Spectral Analysis of Boric Acid in Different Solvents: A Case Study. International Journal of Pharmaceutical Science and Research 2015; 6(2): 830-834.
2. Bax, B.D et.al., Type IIA topoisomerase inhibition by a new class of antibacterial agents. Nature, 2010; 466(7309): 935-940.
3. Scoper SV. Review of third-and fourth-generation fluoroquinolones in ophthalmology: in-vitro and in-vivo efficacy. Adv Ther. 2008; 25(10):979-94. doi: 10.1007/s12325-008-0107-x.
4. McDonald MB, et al. Efficacy and safety of besifloxacin ophthalmic suspension 0.6% compared with moxifloxacin ophthalmic solution 0.5% for treating bacterial conjunctivitis. Ophthalmology. 2009;116(9):1615-1623.e1.
5. Noor AW, Hashim JA, Faris TA. Combinations of Carbapenem with Fluoroquinolones or 1,3,4 - Oxadiazole-2 -Thione Derivatives as New Broad Spectrum Bactericidal Antibiotics for Ophthalmic Preparation. Journal of Drug Design and Medicinal Chemistry.2016; 2(5): 47-50.
6. Digvijay S. et.al. Antimicrobial Activity and Molecular Docking Studies of a Sesquiterpenoid Alcohol from Leaf Solvent Extracts of *Juniperus communis L.* International Conference on Commercialization of Medicinal Plant Products - Lab techniques to Trade. 2018.
7. Qusay FA, et.al. Formulation and Characterization of Dextran-70 0.1% and Hypermellose 0.3% Artificial Tears Eye Drop. Journal of Iraqi Industrial Research. 2016 ; 3(2) : 8-11.
8. Md. SU.et.al.,. Quality Control Tests for Ophthalmic Pharmaceuticals: Pharmacopoeial Standards and Specifications. Journal of Advances in Medical and Pharmaceutical Sciences. 2017; 14(2): 1-17.
9. Rajesh S. Handbook of Practical Pharmaceutical Chemistrry [A Systematic Approach to Titrimetric Analysis]. Ahivalik College of Pharmacy, Nagal, Ropar, Punjab. 2019.
10. Ni, Y., Du S. , and Kokot S., Molecular Spectroscopy and Chemometrics: An Analytical Study of Synergistic Effects of Drugs--Interaction Between Fluoroquinolones and DNA. The Analyst, 2009; 134(9): 1840-7.
11. Sanjay B., Dinesh S., Neha S., Stability Testing of Pharmaceutical Products. Journal of Applied Pharmaceutical Science. 2012; 2(3):129 DOI: 10.7324/JAPS.2012.2322.
12. Bauer, AW., Kirby, WM., Sherris, JS. and Turk, M. Antibiotic susceptibility testing by standarized single disk method. Am.J.Clin.Pathol.1966; ,45:493-496.
13. Brook, I. et.al, In vitro resistance of Bacillus anthracis Sterne to doxycycline, macrolides and quinolones. International Journal of Antimicrobial Agents, 2001;18(6): p. 559-562.

14. Abdel-Meguid AAE. et.al, Comparative Study Between Topical Gatifloxacin 0.5% and Moxifloxacin 0.5% as a Prophylactic Measure Before Intraocular Surgery. J Ocul Pharmacol Ther. 2019; 35(5):315-318. doi: 10.1089/jop.2018.0088**.**
15. Elavarasan T., Bhakiarajand D., Gopalakrishnan M. Antimicrobial screening and molecular docking studies of some novel triazoloquinazolinone derivatives. Der Pharma Chemica, 2014; 6(2):391-400.
16. Andréa Martins et.al. Determination of eye Irritation Potential of Low-irritantProducts: Comparison of in vitro Results with the in vivoDraize Rabbit Test. Chives. Brazilian Archives of Biology and Technology. 2012; 55(3}: 381-388.
17. Ayaka T. et.al. Spectrophotometric Measurement of Boric Acid in a Case of Accidental Ingestion. AJMHS, 2017; Vol. 48(1): 49-53.
18. Silver, LL., Challenges of Antibacterial Discovery. Clinical Microbiology Reviews, 2011; 24(1): 71-109.