INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com

Research Article

RESPONSE OF *SALMONELLA TYPHI* AND *SALMONELLA PARATYPHI* TO A NEW EFFERVESCENT CIPROFLOXACIN TABLETS FORMULATION COMPARED TO CONVENTIONAL BRANDS

AHMED M. MASAAD[*1], IBRAHIM A. MAGRABI[4], BADRADDIN M. AL-HADIYA[2], MOHAMMED E. SHAYOUB[3]

[1]Department of Pharmaceutics, College of Pharmacy, Taif University, KSA.
[2]Department of Pharmaceutical Chemistry, College of Pharmacy, Taif University, KSA.
[3]Department of Pharmaceutics, Khartoum University, Sudan.
[4]Department of Clinical Pharmacy, College of Pharmacy, Taif University, KSA.

*Corresponding Author: ahmad.mosaad@hotmail.com

Abstract

Typhoid is an epidemic disease in Sudan and causes morbidty for many people especially in tropical countries. Ciprofloxacin hydrochloride tablets were the drugs of choice for the disease treatment used as alternative to chloramphincol. The present research work aimed to study the response of *Salmonella typhi* and *Salmonella paratyphi* to a newly formulated effervescent ciprofloxacin hydrochloride tablets as compared to five conventional ciprofloxacin marketed brands. Microbiological sensitivity tests were carried out against *Salmonella typhi* and *Salmonella paratyphi* to detect the response of each drug. Comparison was held between the drug formulations. The results showed that the response of *Salmonella typhi* to both drugs is less than that to *Salmonella paratyphi.* This may be due to a genetic factor found in *Salmonella typhi*, in producing more polysaccharide as compared to *Salmonella paratyphi*. Interestingly, the present research study revealed that the inhibition zones of the newly formulated effervescent tablets are greater than those of conventional tablets. This may be an indication for more activity and quicker response or action of the newly formulated drug.

Keywords: *Salmonella typhi*, *Salmonella paratyphi,* effervescent ciprofloxacin HCl tablets, conventional tablet brands, microbiological sensitivity response, treatment activity response.

Introduction

Salmonellae are gram-negative motile bacilli. The genus *Salmonella,* which belongs to the family Enterobacteriaceae, was named after Daniel E. Salmon, an American veterinarian who first isolated *Salmonella choleraesuis* from pigs with hog cholera in 1884[1].

As with the closely related bacterium *Escherichia coli,* salmonellae are potential enteric pathogens and leading cause of bacterial foodborne illnesses. In addition, *Salmonella* species have been implicated in a spectrum of other diseases, including enteric or typhoid fever (primarily *Salmonella typhi* and *Salmonella paratyphi*), bacteremia, endovascular infections, focal infections (e.g. osteomyelitis), and enterocolitis (typically *Salmonella typhimurium, Salmonella enteritidis,* and *Salmonella heidelberg*).

Salmonellae can be isolated in the microbiology laboratory using numerous low-selective media (such as MacConkey agar and deoxycholate agar), intermediate-selective media (*Salmonella-Shigella* [SS] agar, Hektoen [HE] agar), and highly selective media (selenite agar with brilliant green). Salmonellae are oxidase-negative and predominantly lactose-negative species. Fever of more than 1% of nontyphoidal *Salmonella* isolates are lactose-positive (pink on MacConkey agar). Most Salmonellae species produce hydrogen sulfide, which is detectable on HE or SS agar. As facultative anaerobes, they grow well both in bottles of standard automated systems for blood cultures and on culture media routinely used for urine, tissue, and respiratory cultures[(2)]. Individual isolates can then be distinguished with serogrouping, pulsed-field gel electrophoresis, and bacteriophage serotyping techniques.

Nomenclature and Classification of *Salmonella* sp.

The nomenclature and classification of *Salmonella* species have been changed and restructured multiple times. Traditionally, *Salmonella* species were named in accordance with the Kaufmann-White typing system, defined by different combinations of somatic O, surface Vi, and flagellar H antigens[3]. In 2005, *Salmonella enterica* finally gained official approval as one of the *Salmonella species*. The genus *Salmonella* also contains the species *Salmonella bongori* and *Salmonella subterranean,* which was recognized in 2005[3].

Currently, *Salmonella* species have the serologically defined names appended as serovars or serotypes. For instance, the current nomenclature of *S typhi* is *S enterica* serovar typhi. *S enterica* is preferred over the confusing name *S choleraesuis*, which is also the name of a commonly isolated serotype [4]. To date, more than 2500 serovars of *S enterica* have been described. Certain serovars are host-restricted, while others have a broad host range [5, 9].

Pathophysiology

The transmission of salmonellae to a susceptible host usually occurs via consumption of contaminated foods. The most common sources of salmonellae include beef, poultry, and eggs. In addition, human-to-human and animal-to-human transmissions can occur. For example, amphibian and reptile exposures are associated with approximately 74,000 *Salmonella* infections annually in the United States. *Salmonellosis* outbreaks have also been associated with handling chicks, ducklings, kittens, and hedgehogs.[10- 15]. Recently, a study of 28 cases of *S typhimurium* identified pet rodents as a previously unrecognized source of human *Salmonella* infection [16].

Ciprofloxacin hydrochloride (HCl)

Quinolone antibacterial drugs have been in use since 1964, when nalidixic acid was released. Oxolinic and cinoxacin were introduced somewhat later. These drugs had fallen into disuse because of their limited antibacterial spectra, and resistance to them rapidly develops [17].

The introduction of 6-fluoro and 7-(1-piperazinyl) group expanded the spectrum, increased potency and appears to have prevented the development of plasmid-mediated resistance in microorganisms (Figure 1 and 2).

The fluoroquinolones are bacteriostatic at low concentrations and bactericidal at high concentrations. They were used as alternative to chloramphincol due to the high risk of chloramphincol (e.g. bone marrow depression). They are now considered as drugs of choice for enteric fever. [17]

Mechanisms of Action

The fluoroquinolone drugs inhibit DNA gyrase (topoisomerase II), which results in abnormal linkage between opened DNA and the gyrase. Negative supercoiling (absent in mammalian nuclei) is impaired, so protein synthesis is prevented. [17]

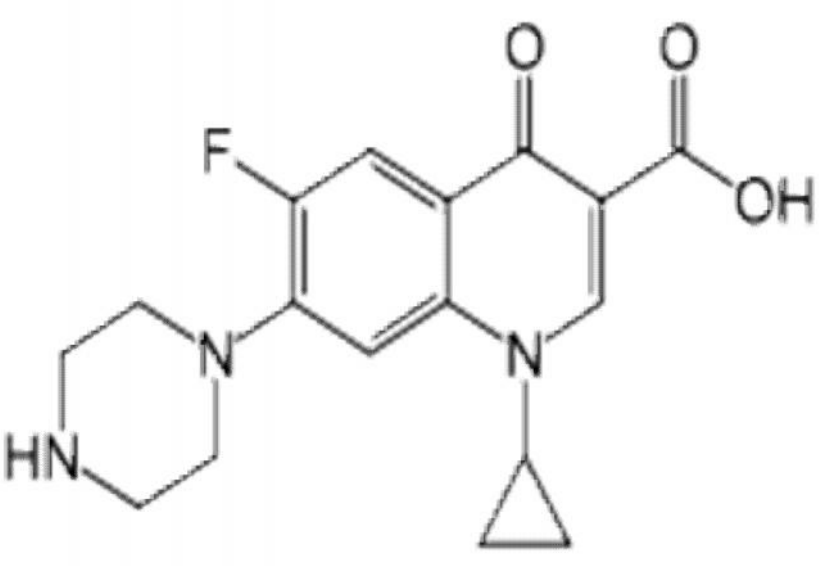

Figure 1. Structure of Ciprofloxacin

Materials and Methods

Microbiological Sensitivity Test

Antimicrobial susceptibility tests measure the ability of an antibiotic or other antimicrobial agents under suitable conditions to inhibit bacterial growth in vitro for evaluating the safety and effectiveness of antibiotic products. Several types of antimicrobial susceptibility (sensitivity) tests were done to detect the response of *Salmonella typhi* and *Salmonella para*typhi to ciprofloxacin.[3]

Ciprofloxacin Sensitivity Test using Disc diffusion Kirby-Bauer

Ciprofloxacin Preparation for the Test

For each test an amount of 1 mg of standard ciprofloxacin authentic powder was dissolved in 10 ml distilled water, then 1ml of this solution was further diluted in 10 ml distilled water to give final concentration of 10 µg/ml[3].

Antibiotic disc preparation

Filter paper was cut into small disks of about 4 mm in diameter then it enclosed in a sealed container and sterilized in oven. Half number of the disks were impregnated with ciprofloxacin test suspension and the others with standard suspension, then the disks were dried in oven at 60ºC for 20 minutes.

Inoculum preparation of *Salmonella typhi* and *Salmonella paratyphi*

Inoculums preparation is the most important step in any susceptibility test. Inocula are prepared directly by inoculating colonies grown overnight on an agar plate, into broth media. Then the numbers of bacteria tested was standardized using McFarland turbidity standards [3]. McFarland turbidity standards: The McFarland 0.5 standard is used, which contains 99.5 ml of 1% sulfuric acid and 0.5 ml of 1.175% barium chloride, this solution was dispensed into tubes comparable to those used for inoculums preparation. The McFarland 0.5 standard provides turbidity comparable to that of a bacterial suspension containing 1.5×10^8colony for unit (CFU/ml) [3].

Inoculation and incubation

After preparation of standard inoculums suspension, a sterile cotton swab was dipped into the suspension, pressed to remove excess liquid, and then swabbed evenly across the surface of a Mueller Hinton agar plate (plates of 9 mm were used). Each inoculum suspension was inoculated into three media labeled test (T), standard (S) and control (C). Within 15 minutes of inoculation, the individual ciprofloxacin disks (one disc per plate) were applied to the agar media with forceps and gently pressed to ensure contact with the agar [3]. The ciprofloxacin Test disks were applied in the plates labeled (T). The ciprofloxacin Standard disks were applied in the plates labeled (S). While other plates labeled (C) without antibiotic disks were used as control. Within 15 minutes of disks placement, plates were inverted and placed in a 37ºC for 18 hours [3]. After incubation the plates were examined, to ensure the satisfactory growth of the test organisms. The diameter of each inhibition zone was measured using ruler or calipers[3]. Once zone measurements were made, the millimeter reading for each brand and effervescent formula were compared with that specified in the interpretive tables of the NCCLS documents.

Results and Discussion

The response of both microorganisms against effervescent ciprofloxacin and conventional five brands compared to that of standard (pure) ciprofloxacin HCl were shown in Figures 3 and 4.

The ciprofloxacin disk was used in concentration 10µg/ml according to Oxoid Labrotaries[3] (Oxoid Ltd, England) to give the required effect. The Accuracy and precision of the results followed the standard of the national committee for clinical laboratory standards (NCCTS)[3].

The diameter of each inhibition zone was measured using a ruler and caliber for each brand as shown in (figure 3 and 4). It was clear that arrangement of inhibition zones from microbiological results were as follows: brand A > brand C > brand D > brand B >E. All brands are active against selected bacteria. This result agreed with study by Mughal et al, (2009) and Asghar et al, (2009)[19]. The inhibition zone of effervescent ciprofloxacin HCl is clear that greater than the five conventional brands. This might solve the problem of microorganisms resistance [3,19].

On comparing tablets formulated as effervescent, wet granulation formulation had inhibition zones were greater than the direct compression ones. This might be due to more distribution of the active ingredient by mixing[5,19].

The inhibition zone response of *Salmonella paratyphi* was more greater than that of *Salmonella typhi* as shown in figure 4.This might be due to variation in genome of *Salmonella paratyphi* compared to that of *Salmonella typhi*. This lead *Salmonella typhi* to produce more polysaccharide and Vi antigen which is responsible for its pathogenic effect and resistance[18].

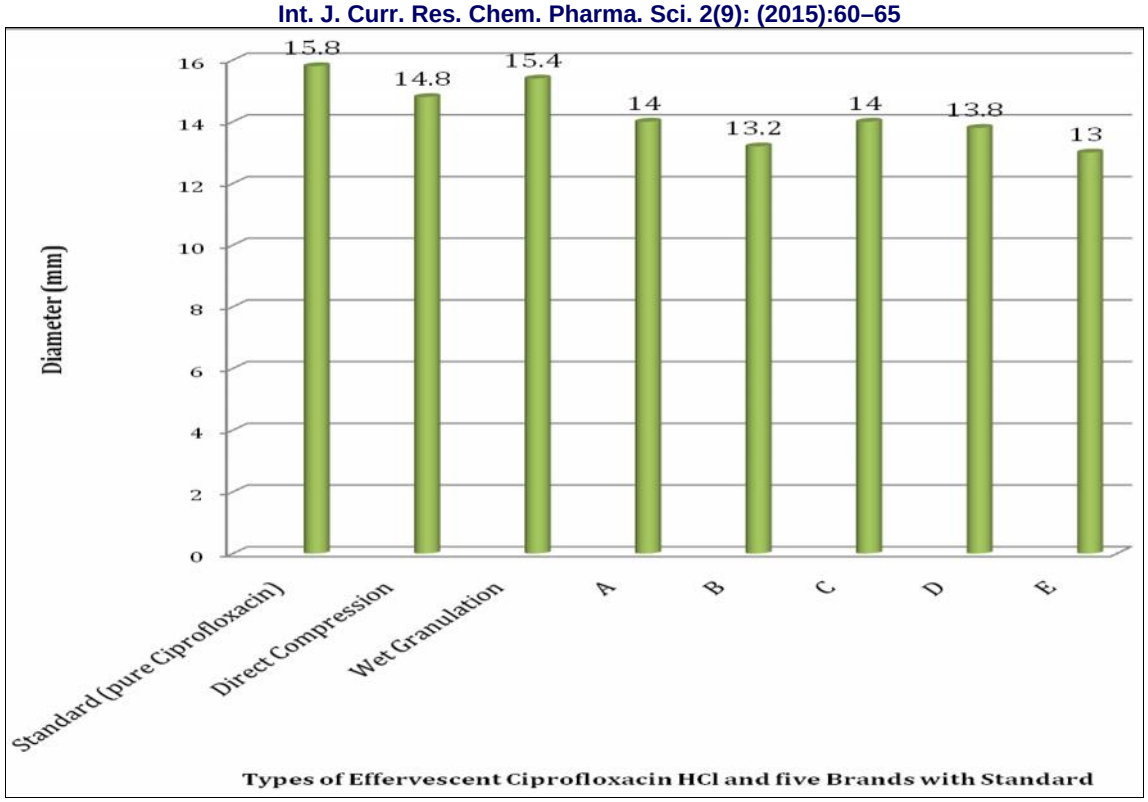

Figure 3. Response of *Salmonella typhi* to Effervescent Ciprofloxacin and Conventional Five Brands compared to that of Standard Ciprofloxacin HCl.

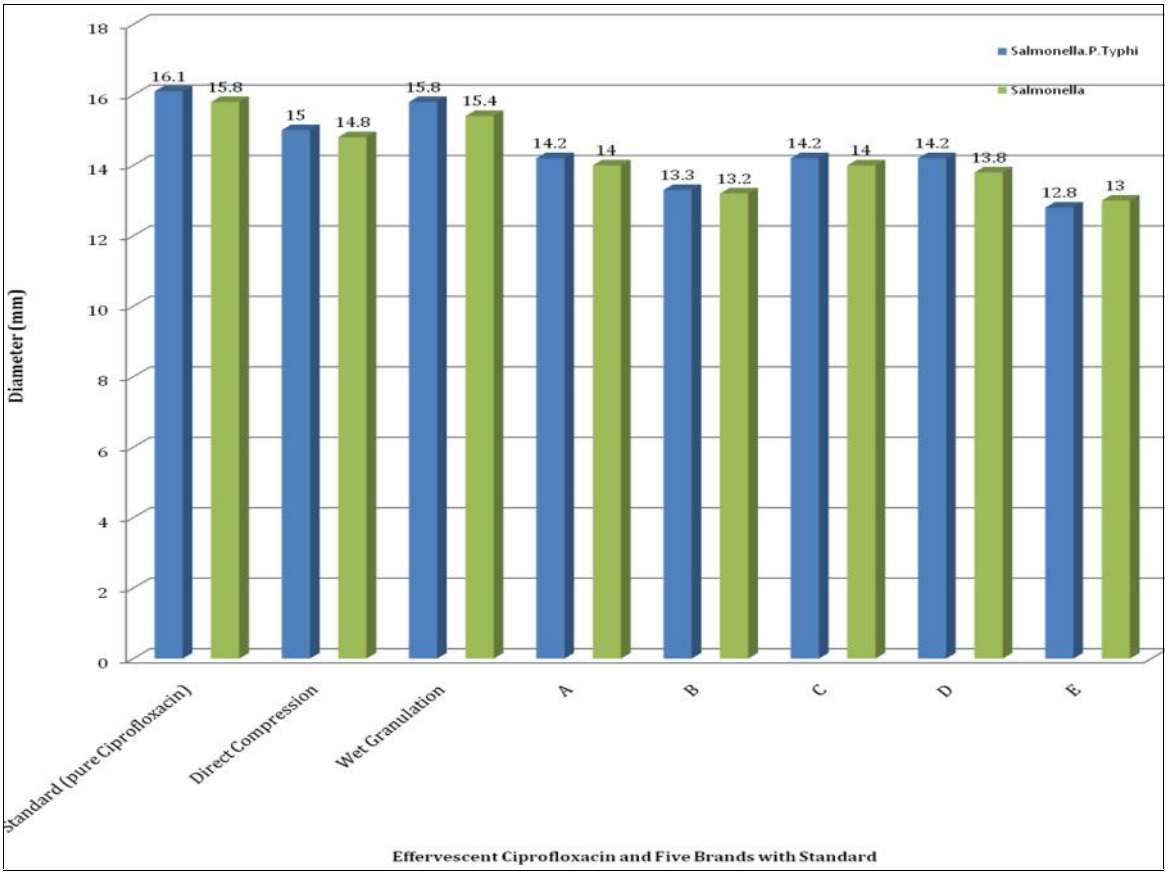

Figure 4. Comparison of the Effect of all tablets and standard on *Salmonella typhi* and *Salmonella paratyphi* by Inhibition Zone Diameter [19]

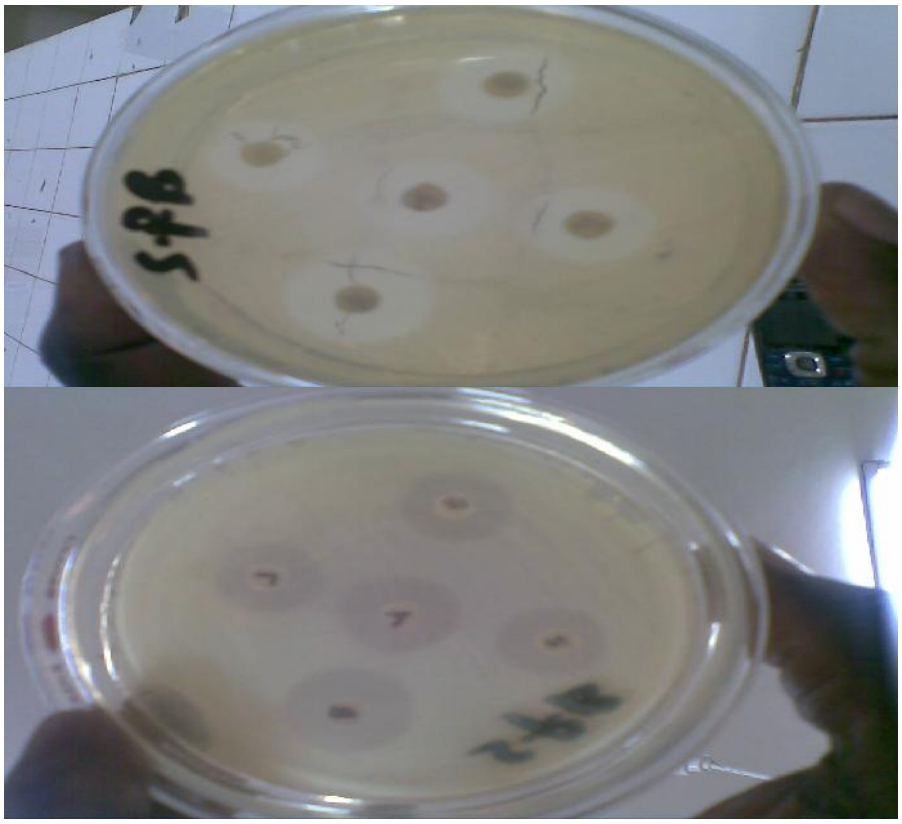

Figure 5. Inhibition Zones of *Salmonella typhi* and *Salmonella paratyphi*

Conclusion

- The higher sensitivity of *Salmonella paraty*phi compared to *Salmonella typhi* may be due to the genetic factor that makes *Salmonella typhi* produces more polysaccharide which might be responsible for its resistance to antibiotics[19].
- Effervescent ciprofloxacin tablets showed higher inhibition zones compared to conventional ciprofloxacin HCl brands, this lead to quicker response.
- The effervescent ciprofloxacin tablets should be restricted for the treatment of the enteric fever, and not to be used irrationally.
- Effervescent tablets were selected due to their convenience, easy to use, more effective and the increased patient acceptability and palatability.

References

1. Kim AY, Goldberg MB, Rubin RH. Salmonella infections. In: Gorbach SL, Bartlett JG, Blacklow NR, eds.. Infectious Diseases, 3rd ed. Lippincott Williams and Wilkins. (2004): 68.
2. Mohrle, R. Effervescent Tablets, In: Pharmaceutical Dosage Forms: Tablets, vol. 1, Chapter 6, 2nd ed. Lieberman, HA, Lachman, L and Schwartz, JB (Eds.). Marcel Dekker Inc. New York. (1989).
3. Hohmann EL. Nontyphoidal salmonellosis. Clin Infect Dis. 2001; 32(2): 263-269.
4. Su LH, Chiu CH. Salmonella: clinical importance and evolution of nomenclature. Chang Gung Med J. 2007; 30 (3): 210-219.
5. World Health Organization (2004). WHO Medicine Strategy. Countries at the Core 2004-2007. P. 68. Available at http://libdoc.who.int/hg/2004/WHO_EDM_2004.5.
6. Rang, H. P.; Dale, M. M.; Ritter, J. M. and Moore, P. K. (2003). Drugs Used In the Treatment of Infections and Cancer Pharmacology, 5th ed. Churchill Livingston.
7. Koletar, S. L. (2000). Concepts in Antimicrobial Therapy. In: Textbook of Diagnostic Microbiology 2nd (ed) by W. B. Saunders Company. Philadelphia London Toronto Montreal Sydney Tokyo. Pp.1 53-04.
8. Brenner FW, Villar RG, Angulo FJ, Tauxe R, Swaminathan B. Salmonella nomenclature. *J Clin Microbiol*. 2000; 38(7): 2465-2467.
9. Grassl GA, Finlay BB. Pathogenesis of enteric Salmonella infections. *Curr Opin Gastroenterol*. 2008; 24(1): 22-26.
10. Centers for Disease Control and Prevention. From the Centers for Disease Control and Prevention. African pygmy hedgehog-associated salmonellosis--Washington, 1994. *JAMA*. 1995; 274(4): 294.

11. Mermin J, Hutwagner L, Vugia D, et al. Reptiles, amphibians, and human Salmonella infection: a population-based, case-control study. *Clin Infect Dis*. 2004; 38 Suppl 3: S253-261.
12. Outbreaks of multidrug-resistant *Salmonella typhimurium* associated with veterinary facilities--Idaho, Minnesota, and Washington, 1999. *MMWR Morb Mortal Wkly Rep*. Aug 24 2001; 50(33): 701-704.
13. Reptile-associated salmonellosis--selected states, 1998-2002. *MMWR Morb Mortal Wkly Rep*. Dec 12 2003; 52(49): 1206-1209.
14. Salmonellosis associated with chicks and ducklings--Michigan and Missouri, spring 1999. *MMWR Morb Mortal Wkly Rep*. Apr 14 2000; 49(14): 297- 299.
15. Woodward DL, Khakhria R, Johnson WM. Human salmonellosis associated with exotic pets. *J Clin. Microbiol*. 1997; 35(11): 2786 - 2790.
16. Swanson SJ, Snider C, Braden CR, Boxrud D, Wunschmann A, Rudroff JA, et al. Multidrug resistant *Salmonella enterica* serotype *typhimurium* associated with pet rodents. *N Engl J Med*. 2007; 356: 21- 28.
17. Nichols, W. K. (2000). Oral Solid Dosage Form. In: Remington: The Science and Practice of Pharmacy. 20th ed. Alfonso, R.G. Philadelphia College of Pharmacy and Science. Pp. 1507-89.
18. httpgenome.cshlp.orgcontent17161.full. (April 2011).
19. Massaad et al, (2013). Comparison of physicochemical properties of formulated effervescent ciprofloxacin tablets to conventional tablets brands. M. Pharm. University of Khartoum, Sudan.

