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ORIGINAL ARTICLE

Comparative Study of *In Vitro* Evaluation of Two Quinolone Derivatives by HPLC

Tanweer Alam[1], Asra Hameed[1,*], Farman Ali[2], Kashif Maseed[2], Neelam Sharif[1], Ghulam Sarwer[1], Khan Usmanghani[1]

[1]Department of Pharmacy, Jinnah University for Women, 5C, Nazimabad, Karachi 74600, Pakistan
[2]Central Drug Laboratory, Drug Regulatory Authority of Pakistan, 4-B, SMCHS, Karachi 74400, Pakistan

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Address of Correspondence Author:
asra_hameed1@hotmail.com

ABSTRACT

Background: Quinolones are the antibacterial agents which vigorously inhibit the DNA synthesis. Pharmacodynamically they are DNA Gyrase inhibitor.

Objective: The aim of this study is to compare two quinolone derivatives using HPLC.

Result: The drug content is found to be as 96.25% in ofloxacin sample tablets and 99.64% in ciprofloxacin sample tablets when compared with standards.

Conclusion: The given method has found to be speedy, exact, defined, trustworthy and minimum time taking for the concurrent process of estimating of ofloxacin as well as ciprofloxacin drugs in the system of loose drug mockups, its preparations exhausting the supreme frequently engaged C-18 column through UV indicator.

INTRODUCTION

Quinolones briskly inhibit the synthesis of DNA by endorsing breakdown of bacterial DNA on DNA-enzyme complexes at DNA gyrase like wise type IV topoisomerase, substantial on hasty bacterial death [1-3]. As a universal tenet, gram-negative bacterial action associates through embarrassment of DNA gyrase, as well as gram-positive infective actiontallies through embarrassment of DNA type IV topoisomerase [1]. The quinolones are categorized into four generations on the basis of their antimicrobial [4]. Drugs or agents included in First generation, are used less frequently nowadays, due to their adequate gram-negative activity with slight systemic distribution. Drugs of agents included in Second-generation of quinolones have shown extended gram-negative action likewise atypical infectious agent devotion, however at the same time, limited action against gram-positive. These representatives are utmost energeticin contradiction of the aerobic gram-negative bacilli. Ciprofloxacin vestiges the best quinolone that is alloutvigorous against Pseudomonas aeruginosa [5,6]. Third-generation quinolones preserve the stretched gram-negative in addition atypical intracellular action on the other hand have amended gram-positive handling. Lastly, drugs in the fourth-generation expand gram-positive attention, preserve gram-negative handling, also achievement anaerobic handling [6]. Ofloxacin defeats gram negative besides gram positive microorganism in addition to accommodates each gram positive also as gram negative microorganism taints [7,8]. It has an intensive sort of claims as antibacterial drug, anti-infective agents, urinary; nucleic acid synthesis inhibitors

[9]. Ofloxacin is considerably persuasive in contradiction of Escherichia coli that could be a dare for additional medication corresponding chloramphenicol also rifampicin [10] chemically Ofloxacin (OFL) may be a fluorinated carboxyaquinolone, with chemicals may be a racemate (+) – 9 fluro - 2,3 - dihydro – 3 - methyl - 10 - (4 - methyl 1 piperazinyl) – 7 – oxo - 7H - pyrido [1,2,3 - de] 1,4 benzoxazine - 6 - carboxylicacid [11]. bacterial DNA gyrase, for instance paralleled toward different enzymes is vulnerable to 4-quinolone antimicrobials in conjunction with ofloxacin that retain a further assassination tool [12]. Ofloxacin stumble upon anaerobic bacteria, chlamydiae, also as sure connected organisms, like mycoplasmas instead mycobacteria [13]. Ciprofloxacin is time-honored broad-spectrum fluoroquinolone antibiotic exhibiting action in contradiction of mutually Gram-positive as well as Gram-negative bacteria pathogens [14-15]. It acts principally by hindering DNA gyrase besides topoisomerase IV existent in bacterial cells which remain accountable meant for imitation of DNA of bacteria [16]. It is principally effective in contradiction of Pseudomonas aeruginosa. Among patients having cystic fibrosis in addition to bronchiectasis, P. aeruginosa causes thrilling deterioration in lung function, that one oral in addition to parenteral prescription designated in place for management of exacerbations of respiratory tract infection [17-20]. Ciprofloxacin is prescribed in numerous clinical diseases for instance infectious enteritis other than inflammatory bowel disease, induction of nitric oxide, clampdown of pro-inflammatory cytokines besides equally it is clever to persuade apoptosis in an assortment of human cancer cell lines together with the human colonic cancer cells [21].

EXPERIMENTAL

Arrangement

A Shimadzu HPLC system fitted out through LC-10 AT VP pump as well as SPD-10 A VP ultraviolet–VIS sensor was used. Chromatographic system stayed cohesive via Shimadzu model CBM-102 to P-IV computer encumbered through Shimadzu CLASS-VP software system (Version 5.03) for information acquirement besides mathematical calculations. Rheodyne manual injector fixed using a 20 µL loop, Hypersil, ODS, C18 (150×4.6mm, 5micron) and Purospher® STAR RP-18 column as well as DGU-14 AM on-line degasser. Furthermore, Mattler Toledo electronic balance, micro liter syringe plus micro pore filtration assemblage was employed in this study.

METHODOLOGY

All chemicals used in this method were HPLC grade. Reference standard for both the quinolone derivatives were taken from Central Drug Laboratory (CDL), Drug Regulatory Authority of Pakistan (DRAP), Karachi. While dosage formulation for both quinolone derivatives were acquired from the market. Entirely these drugs had an expiry of not below one year while studying. Water used in procedure was suitably purified for HPLC (double distilled and de-ionized through “GFL Water STILL” purification system and Elgacan B114 deionizer. Fresh employed solutions were arranged day-to-day. Entirely solutions were clarified through 0.45 µmbesides degassed by means of sonicator.

Mobile phase

Acetonitrile: Solution A (3:22) was used as mobile phase for ofloxacin. 1000ml Solution A was prepared with 2.72g Potassium dihydrogen phosphate (KH_2PO_4), adjusting pH at 3.3 by phosphoric acid. The rate of flowduring the course of the analysis of ofloxacin was 1 mL/min in addition to UV recognition was performed on 294 nm. Acetonitrile: Buffer (13:87) was used as mobile phase for ciprofloxacin. 1000ml Buffer was prepared by 2.8ml of orthophosphoric acid, adjusting pH at 3 by triethanolamine. The flow rate throughout the analysis of ciprofloxacin was 1.5 mL/min and UV recognition were

accomplished on 278 nm. Mobile phase was clarified over and done with 0.45µm Millipore filter, mixed thoroughly and degassed by sonication for 20 minutes.

Preparation of solutions

Ofloxacin: 100mcg Standard solution and sample solution of drug was prepared with 100mg of drug. Dilution are made twice for both sample and standard in solution i and solution ii. Solution i was prepared with methanol: glacial acetic acid (3:1) while solution ii was prepared with Acetonitrile: water (1:9).

Ciprofloxacin: 250mcg Standard solution of drug was prepared using 25mg active while 200mcg sample solution was prepared 100mg of sample drug. Dilution was made with HPLC grade water. Lot of these solutions were clarified by the usage of a disposable 0.45µm strainer formerly injected.

For the evaluation of sample drug, the material of 20 tablets were pulverized weigh up lot of the powder correspondent to the appropriate quantity of drug (rendering to the labeled appealed) was shifted into a volumetric flask. The drug was entirely liquefied and so diluted through solvent up to the spot. Portion of these solution were clarified with the usage of a disposable 0.45µm strainer and formerly injected.

Chromatographic conditions

The chromatographic scrutiny remained accomplished at ambient temperature using isocratic elution. The thrust remained set at a pour rate of 1.0 mL min-1 for ofloxacin as well as 1.5 mL min-1 for ciprofloxacin, tester measurements of 20µL was injected in triplicate onto the HPLC column then elute was censored at 294nm and 278nm, respectively. Optimal retention times for both ofloxacin and ciprofloxacin were found to be 13 min.

RESULTS

Assay of two fluoroquinolone derivatives was performed and compared. The drug content is found to be as 96.25% in ofloxacin sample tablets and 99.64% in ciprofloxacin sample tablets when compared with standards.

DISCUSSION

The improvement in HPLC technique to conclude the medication has acknowledged extensive courtesy in contemporary centuries attributable to their significance in repetitive quality control scrutiny. HPLC strategies usually need endowment to be used and dumping of solvents, effortful sample preparation procedure besides personal skilled in chromatographical practices. The penalty area of this concrete study was to check two quinolone derivatives with developed speedy, a lot of correct, defined consistent tiniest interval overwhelming HPLC strategies intended for the coincidental regulate of ofloxacin as well as ciprofloxacin medication within the kind of loose drug tasters, its preparations consuming the foremost ordinarily utilized C-18 column by way of ultraviolet sensor.

The average area for standard of ofloxacin is found to be as 3717638.3 with the standard deviation of 102133.6468 and relative standard deviation as 2.7473. The average area for sample of ofloxacin is found to be as 3578442.5 with the standard deviation of 33847.0803 and relative standard deviation as 0.9459. The stated amount of ofloxacin is 50mg and determined amount is 48.128, so the determined percentage is 96.256. The average area for standard of ciprofloxacin is found to be as 31214326.0 with the standard deviation of 506825.9225 and relative standard deviation as 1.6237. The average area for sample of ciprofloxacin is found to be as 24880370.0 with the standard deviation of 704793.1278 and relative standard deviation as 2.8327. The stated amount of ofloxacin is 100mg and determined amount is 99.6352, so the determined percentage is 99.64 (Table **1-7**).

Table 1. System Suitability Test (Standard Observation for Ofloxacin).

CT#	D01	D02	D03
R. Time	13.598	13.666	13.550
Standard	2753124	3797296	3602495
Average	3717638.3		
Std. dev	102133.6468		
RSD	2.7473		

Table 2. Sample Observation for Ofloxacin.

CT#	D01	D02
R. Time	13.511	13.535
Standard	3554509	3602376
Average	3578442.5	
Std. dev	33847.0803	
RSD	0.9459	

Table 3. Assay Percentage for Ofloxacin.

Determine Amount	48.128
Stated Amount	50
Determine %	96.256

Table 4. System Suitability Test (Standard Observation for Ciprofloxacin).

CT#	D01	D02	D03
R. Time	13.802	13.758	13.689
Standard	31315541	30664530	31662907
Average	31214326.0		
Std. dev	506825.9225		
RSD	1.6237		

Table 5. Sample Observation for Ciprofloxacin.

CT#	D01	D02
R. Time	13.725	13.671
Standard	25378734	24382006
Average	24880370.0	
Std. dev	704793.1278	
RSD	2.8327	

Table 6. Assay Percentage for Ciprofloxacin.

Determine Amount	99.6352
Stated Amount	100
Determine %	99.64

Table 7. Comparison of Two Quinolone Derivatives.

Quinolone derivatives	Drug analyzed
Ofloxacin	96.256
Ciprofloxacin	99.64

CONCLUSION

Both the derivatives of quinolones are equivalent and their available formulations in the market are also equivalent and within the official limits of assay.

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