

Quality Testing and Antimicrobial Activity of Norfloxacin in Pakistan

Nausheen Bibi^a, Irfan Ullah^{b*}, Mazhar Ali^c, Anita Mughal^d, Abdul Jabbar^e,
Muhammad Ihtesham^f, Iqbal Nisa^g, Nusrat Yasin^h, Samina Jehanⁱ, Arshad
Javaid^j, Qurban Ali^k

^{a,e,g,h,i}*Department of Microbiology, Kohat University of Science and Technology, Pakistan.*

^{b,c,j,f}*Programmatic Management of Drug resistant TB Pulmonology, Lady Reading Hospital Peshawar, Pakistan*

^d*Department of Biotechnology and Microbiology, Sarhad University of Science and Information technology
Peshawar, Pakistan*

^k*Ministry of Livestock and Dairy Development National veterinary laboratory Islamabad, Pakistan*

Abstract

There are 400 licensed pharmaceutical companies in Pakistan; one third of Pakistan total consumption of pharmaceuticals is imported. Market potential is good for antibiotics, vaccines, hormones etc. High performance liquid chromatography is an important tool for routine determination of antimicrobial drugs with specific emphasis on fluoroquinolones. The quality testing of norfloxacin was also done by Liquid chromatography coupled with mass spectrometry. Antibacterial activity of norfloxacin is good for aerobic gram negative bacteria, including Enterobacteriaceae and Pseudomonas aeruginosa. It has moderate antibacterial activity against gram positive microorganisms, such as staphylococci; anaerobic bacteria are generally resistant. Our investigation focuses on the quality testing of brands of Norfloxacin available in market with variety of trade names. Reverse phase chromatographic technique RP-HPLC was used for the quality testing of norfloxacin at temperature of 35°C and a flow rate of 0.8ml/ml with Isocratic plution.

Disk diffusion technique–was used to check the antibacterial activity of norfloxacin against Proteus mirabilis, Klebsiella pneumoniae, Escherichia coli and Pseudomonas aeruginosa. Different brands of Norfloxacin (noroxin, uracin, qunor, urid , uroquin, webnor, bactinor, uritac , floxacin, norocin and ecoflaxin) from pharmaceutical companies A, B C, D, F, G, H, I, J & and K were collected from markets of Rawalpindi and Islamabad (Pakistan). The claimed concentration of tablet was 400mg.

* Corresponding author.

The quality testing of norfloxacin with sample and standard showed that brands from companies C and F Qunor, Webnor did not qualify the quality testing, they were of medium standard for the treatment of infections while H Company with a brand Uritac stands highest for quality test. Disk diffusion technique showed that all brands of norfloxacin were sensitive against the mentioned bacteria. This research concluded that special effective measures should be taken by all the pharmaceutical companies in Pakistan to check the quality standards of pharmaceuticals especially antibiotics before manufacturing. If they qualify the quality standards they will be effective to treat infections, if they are of low standard it would be difficult for the clinician to advise the correct amount of dosage for patient.

Key Words: Norfloxacin; HPLC (High Performance Liquid Chromatography); RP (Reverse Phase); UV/Vis (Ultra violet visible).

1. Introduction

Norfloxacin is a broad spectrum fluoroquinolones used for the treatment of urinary tract infections, gonorrhea and prostate infections for many years [1-3]. In Pakistan norfloxacin is available in market with different brand names; few of the pharmaceutical companies are meeting the quality standards while others do not. This may be due to the lack of technical expertise or some other reasons. Special effective measures should be taken by all the pharmaceutical companies in Pakistan to check the quality standards of pharmaceuticals especially antibiotics before manufacturing. If they qualify the quality standards they will be effective to treat infections, if they are of low standard it would be difficult for the clinician to advise the correct amount of dosage for patient. This could be the main reason that most of the pharmaceuticals are imported.

Many methods for quality testing of norfloxacin determination were described, such as UV spectrophotometry, liquid chromatography, high performance thin layer chromatography etc among others [2,4,5]. High performance thin layer chromatography has become the most sophisticated tool for the examination of antimicrobial drugs, with special importance on fluoroquinolones [2,6,7].

Antibacterial activity of norfloxacin is good for aerobic gram negative bacteria, including Enterobacteriaceae and Pseudomonas aeruginosa. It has moderate antibacterial activity against gram positive microorganisms, such as staphylococci; anaerobic bacteria are generally resistant. Our investigation focuses on the quality testing of brands of Norfloxacin available in market with variety of trade names and to check the most effective for treatment of infection.

2. Materials & Methods

The present research was carried out at drug residue and testing section National Veterinary Labs, Ministry of Livestock and Dairy Development Chak Shahzad Islamabad (Pakistan).

2.1 Chemicals and Reagents

Products of norfloxacin (Floxacin, Noroxin, Urid, Uracin, Uritac, Ecofloxacin, Norocin, Bactinor, Qunor,

Webnor and Uroquin) eighteen batches were collected from markets of Rawalpindi and Islamabad (Pakistan). The claimed concentration of norfloxacin was 400mg/ tablet. All chemicals and Reagents used were of HPLC grade obtained from Merck.

2.2 Instrument

The samples were analyzed with D-700 series HPLC system (Hitachi, Japan) consisting of column (C8) oven (L-7300), UV detector (L-7400), Autosampler (L-7200), pump (L-7100) and degasser (L-7610).

2.3 Experimental Conditions

The parameters used for quality testing of norfloxacin samples and standards were taken from United States pharmacopoeia 2004. Reverse phase chromatographic technique was used in HPLC for detection of norfloxacin using RPC8 HPLC column at a temperature of 35°C and a flow rate of 0.8ml/min with Isocratic plution. The absorption pattern of mobile phase and norfloxacin were studied by scanning with UV/Vis spectrophotometer to ensure that mobile phase do not interfere with peak absorption of norfloxacin.

2.4 Preparation of mobile phase and standard stock solution

Mobile phase was prepared by mixing 0.5ml phosphoric acid into 500ml volumetric flask and filling the flask upto the mark with deionized water. Then 100ml of HPLC grade acetonitrile was measured with the help of graduated cylinder and mixed with the phosphoric acid solution. Mobile phase was filtered with the help of vacuum filtration assembly using a 47mm (046µm) FTFE membrane filter (Sartorius, Germany). A mobile phase was degassed for 15 minutes in ultrasonic water bath at ambient temperature. Norfloxacin reference standard 99.978% supelco was used for standards preparation. Norfloxacin (50mg) was weighed and added upto 50ml volumetric flask. It was dilated to volume with mobile phase. 10ppm standard was prepared by taking 0.5ml (1000ppm) standard solution of norfloxacin in 50 ml volumetric flask. It was dilated to volume with mobile phase. Scanning of mobile phase and standards was done with UV/Vis spectrophotometer within the range of 200-500nm to find out the absorption pattern of mobile phase and norfloxacin. 10ppm norfloxacin solution was prepared for each sample. Before HPLC analysis, the sample solution was filtered through a 0.45µ syringe filter PTFE (Sartorius, Germany).

2.5 Antibiotic sensitivity test

10µg disk of norfloxacin was prepared by dissolving 20mg of powdered tablet in 1ml of 0.1 N NaOH, which was further diluted with 9ml of distilled water. 5µl solution of each tablet was poured on each disk. The process was performed in laminar flow hood. The test microorganisms (*P.mirabilis*, *K.pneumoniae*, *E.coli*, *P.aeruginosa*) isolated from urine were mixed in 0.85% normal saline and their turbidity was matched with 0.05% Mc Farlands standard solution. Disk diffusion technique was used for this test (NCCLS document M-7A3, 13(25) NCCLS Villanova, PA).

3. Results

3.1 *Uv /Vis scanning of mobile phase and norfloxacin*

UV/Vis scanning showed that norfloxacin had maximum absorbance at 278nm while the mobile phase at 220nm. Norfloxacin in sample solution was identified on the basis of retention time which was same for standard and sample (5.2min) as shown in figure 1 and 2.

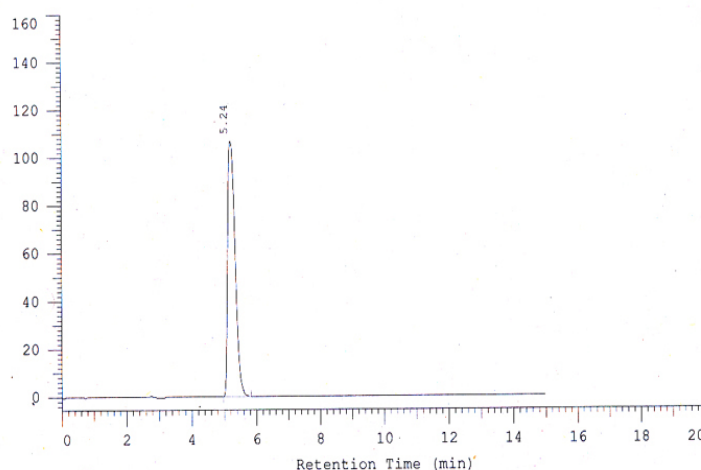


Figure 1: HPLC chromatogram of Norfloxacin standard solution (10 ppm)

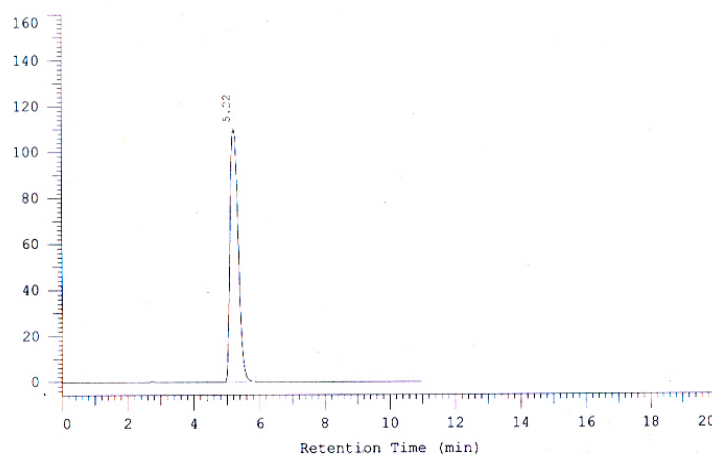


Figure 2: HPLC chromatogram of Norfloxacin sample solution (10 ppm)

3.2 *HPLC analysis*

Three batches of norfloxacin noroxin were tested; the average concentration per tablet was 385mg. So the percent Norfloxacin concentration was 96.14% and this brand had 3.86% less concentration of Norfloxacin.

Two batches of uracin were tested; the average concentration of norfloxacin was 408.3mg per tablet. The

percent concentration was 102.07% and this brand had 2.75% more concentration of norfloxacin. Three batches of uritac were tested and the concentration of norfloxacin was 423.84 mg per tablet. The percentage of norfloxacin was 105.96 and this product had 5.96% more concentration than claimed. One batch of quonor was tested and norfloxacin concentration was 321.63mg per tablet. The percentage of norfloxacin was 80.41% and this product had 19.56% less concentration than claimed.

One batch of urid was tested and norfloxacin concentration was 378.08mg per tablet. The percentage of norfloxacin was 94.52 and this product had 5.48% less concentration of norfloxacin than claimed. Two batches of bactinor were tested and the norfloxacin concentration was 391.42mg. The percentage of norfloxacin was 97.85 and this product had 2.15% less concentration than claimed.

One batch of uroquin was tested and norfloxacin concentration was 360.06mg per tablet. The percentage of norfloxacin was 90.01% and this product had 9.99% less concentration of norfloxacin than claimed. One batch of webnor was tested and the concentration of norfloxacin was 310.65mg per tablet. The percentage of norfloxacin was 77.66% and this product had 22.44% less concentration of norfloxacin than claimed.

Two batches of floxin were tested and the concentration of norfloxacin was 395.44mg per tablet. The percentage of norfloxacin was 98.86 and this product had 1.14% less concentration of norfloxacin than claimed. One batch of Ecoflaxin was 388.69mg. The percentage of norfloxacin was 97.17 and this product had 2.83% less concentration than claimed. One batch of noroxin was tested and the concentration of norfloxacin was 363.74mg. The percentage was 90.93 and this product had 9.07% less concentration of norfloxacin than claimed.

Results showed that products of norfloxacin quonor and webnor did not show the deviation of percentage % deviation within the range ($\pm 10\%$). So they do not qualify the quality testing.

3.3 Antimicrobial susceptibility testing

Antimicrobial sensitivity test showed that *Proteus mirabilis*, *Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa* were sensitive against all products of norfloxacin.

3.4 Discussion

Human health, more specifically patient's life depends on the quality of marketed drugs. Quality is never an accident; it is always the result of intelligent efforts. The quality of drugs means the quality of treatment that ensures the well being of patients. Certified companies that are producing safe quality products have good marketed value. There are almost 400 pharmaceutical companies in Pakistan, 30 of them are multinational having 53% of market share. One third 1/3 of Pakistan's total consumption of pharmaceuticals is imported. Market potential is good for antibiotics, vaccines, hormones etc. [8].

In Pakistan norfloxacin is available in market with different brand names. Few of the pharmaceutical companies are meeting the quality standards while others do not. This may be due to technical expertise or some other

reasons. Norfloxacin is the 1st generation antibacterial drug used to treat various bacterial infections such as urinary tract infections, gonorrhoea and prostate infections. High performance liquid chromatography has become an important tool for routine quality testing of antimicrobial drugs, with specific emphasis on fluoroquinolones.

So the present study focuses on the quality testing of norfloxacin; its correct dosage and efficacy by antimicrobial sensitivity testing. Different brands of norfloxacin from pharmaceutical companies A, B, C, D, E, F, G, H, I, J, K with specific names noroxin, uracin, qunor, URID, uroquin, webnor, bactinor, uritac, floxacin, norocin and ecoflaxin were collected from markets of Rawalpindi and Islamabad (Pakistan). The claimed concentration of tablet was 400mg per tablet. UV/Vis scanning showed that norfloxacin showed maximum absorbance at 278nm while the mobile phase at 220nm. Norfloxacin in sample solution was identified on the basis of retention time. The quality testing of norfloxacin with sample and standard showed that brands of norfloxacin from pharmaceutical companies C, F, qunor and webnor did not qualify the quality testing. They were of medium standards for the quality treatment of urinary tract, gonorrhoea and prostate infections.

The antimicrobial sensitivity test of norfloxacin against *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *E.coli* showed that all brands were sensitive against the test microorganism. Wide spread antibiotic resistance amongst these organisms necessitates sensitivity testing to determine the appropriate antibiotic treatment. Often a combination of two bactericidal antibiotics (antipseudomonal and fluoroquinolone) is required [9].

4. Conclusion

One third of Pakistan total consumption of pharmaceuticals is imported. Market potential is good for antibiotics, vaccines, hormones etc. Human health, more specifically patient's life depends on the quality of marketed drugs. Quality is never an accident; it is always the result of intelligent efforts. The quality of drugs means the quality of treatment that ensures the well being of patients. Certified companies that are producing safe quality products have good marketed value. There are almost 400 pharmaceutical companies in Pakistan, 30 of them are multinational having 53% of market share. Effective measures should be taken by the authority to ensure the quality of all certified medicines. If the antibiotic is of low quality standard, it would be difficult for the clinician to administer the correct amount of dosage to patients. This study can be done on other group of fluoroquinolones such as ciprofloxacin.

Acknowledgment

Dr Qurban Ali, Director General National Veterinary Labs, Ministry of Livestock and Dairy Development Islamabad Pakistan for providing research facility.

References

- [1]. Indian pharmacopoeia vol.3", Ghaziabad (2010): The Indian Pharmacopoeia commission, C 2007, pp-1793-1795.
- [2]. Sing, NR, Sahoo S, Mishra U, Garnaik B, Sahoo K, Sudhir HD (2013). Stability indicating RP-HPLC

method development and validation of Norfloxacin.voll(5) ;pp 743-758.

- [3]. Rang HP, Dale MM, Ritter JM, and Flower RJ (2007). "Rang and Dale's Pharmacology", 6th ed., Churchill Livingstone, Philadelphia ,pp.672-674.
- [4]. Xiao Y, Chang H, Hu J, Jia A, (2008). Journal of Chromatography A, 1214, 1-2,100-108.
- [5]. Rahman N, Ahmed Y (2004). European Journal of Pharmaceutics and Biopharmaceutics, 57, 2, , 359-367.
- [6]. Gigoso PG, Revesado PR (2000). Journal of Chromatography A, 871, 1-2, 31-36.
- [7]. Galarini R, Fioroni L and Angelucci F (2009). European Pharmacopoeie, British Pharmacopoeie, Stationary Office Limited (UK). Journal of Chromatography A, 1216, 46, 8158-8164.
- [8]. Christodoulou EA, Samanidou VF and Papadoyannis IN (2007). Validation of an HPLC-UV method according to the European Union Decision 2002/657/EC for the simultaneous determination of 10 quinolones in chicken muscle and egg yolk, Journal of Chromatography B, 859,2, 246-255.
- [9]. William AS, Harriet Rouse, Bruce D. Fisher. Lippincott, William and Wilkins, Baltimore, MD. (2001). Lippincott's illustrated Reviews: Microbiology.