*Sci., Tech. and Dev.*, 31 (1): 69-73, 2012

# COMPARATIVE ANALYSIS OF CIPROFLOXACIN IN DIFFERENT PHARMACEUTICAL PRODUCTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPH

**MUHAMMAD NASIMULLAH QURESHI[*1], INAYAT UR RAHMAN[2] AND GUL AKHTAR MARWAT[2]**

*[1]Department of Chemistry, Abdul Wali Khan University Mardan, Pakistan.*

*[2]Medicinal Botanic Centre, PCSIR Laboratories Complex Peshawar, Pakistan.*

## Abstract

Pharmaceutical products with different trade names having ciprofloxacin as an active ingredient were collected from the market. The products were assayed under similar conditions for active ingredient applying HPLC technique. Results obtained from quantification of ciprofloxacin contents of each product were compared with their label claims. Comparative analysis of these products was performed based on the quantity of ciprofloxacin.

**Keywords:** Ciprofloxacin, HPLC, Assay, Comparative analysis.

## 1. Introduction

Fluoroquinolones are broad-spectrum synthetic antibacterial agents used in the treatment of several infectious diseases (Bertino, 2000; Fierens et al., 2000; Hernández-Arteseros et al., 2002; Marzo and Dal Bo, 1998). Ciprofloxacin (CIP) is flouroquinolone with fluorine at position 6 of naphthyridine ring. The chemical structure of ciprofloxacin is shown in Figure 1. It has been shown that fluorine atom broadens their activity spectrum against both gram-negative and gram-positive pathogens (Bertino, 2000; Fierens et al., 2000; Hernández-Arteseros et al., 2002; Lorian, 2005; Samanidou et al., 2003). Fluoroquinolones primarily target bacterial enzyme DNA graze or topoisomerase II (Bertino, 2000; Hernández-Arteseros et al., 2002; Mandell and Petri Junior, 1996; MarzoDal Bo, 1998). They are generally safe and well tolerated. Depending on their physicochemical properties, their adverse effects vary significantly (Bertino, 2000; Mandell and Petri Junior, 1996). Majority of them are well absorbed through oral route. They are widely distributed within body tissues and fluids, therefore, have a long half-life (Marzo and Dal Bo, 1998; Samanidou et al., 2003).

Several analytical approaches have been employed for the determination of fluoroquinolones in pharmaceutical formulations like capillary electrophoresis (Bhowal and Das, 1991; Flurer, 2001; Sun and Chen, 1997), UV spectrophotometry (Fratini and Schapoval, 1996), titrimetry (Belal et al., 1999) and high performance liquid chromatography (HPLC) (Imre et al., 2003; Kamberi et al., 1998; Mazuel, 1991; Pharmacopeia; Pharmacopoeia) amongst others. Amongst them, HPLC is widely employed for the determination of ciprofloxacin in biological fluids, edible animal products, feed and pharmaceutical formulations (Barbosa et al., 1996; Budvari-Barany et al., 1991; Córdoba-Borrego et al., 1999; Haeseker et al., 2011; Horwitz, 2002; Husain et al., 1995; Kassab et al., 2005; Kirkland et al., 1994; Lacroix et al., 1996; Samanidou et al., 2005; Sowinski and Kays, 2004; Thoppil and Amin, 2000; Torniainen et al., 1996; Vybíralová et al., 2005).

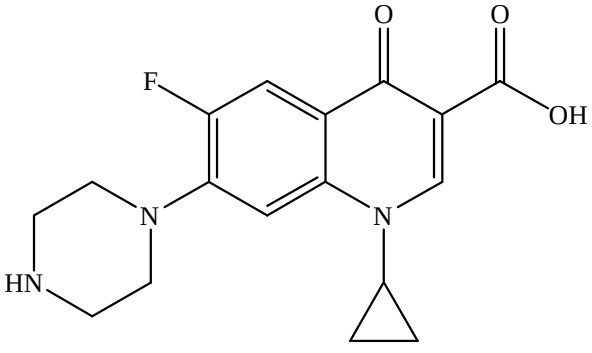


**Figure 1. Chemical structure of ciprofloxacin**

***Author for correspondence E-mail: mnasimuq@yahoo.com**

Ciprofloxacin has been used widely in the country against variety of infections because of its strong antibacterial properties. Many medical doctors all over the country prescribe this medicine for the treatment of a variety of infectious diseases. Because of this reason, a number of ciprofloxacin products with different trade names, manufactured by different pharmaceutical industries are available in the market. Our study aims is to perform a market survey based on the comparative quantification of the amount of ciprofloxacin in each product, to compare these actual amounts of ciprofloxacin obtained with the label claim of the respective products with one another.

## 2. Materials and Methods

### 2.1 Chemicals and reagents

Acetonitrile and phosphoric acid were obtained from Merck KGaA (Darmstadt, Germany). Water purified by double distillation was used through out the experiments. All solvents were filtered through membrane filter (Durapore, 0.2 µm pore size, Ireland) and degassed before use. All solvents were of HPLC grade and reagents were analytical grade. Ciprofloxacin HCl (99.4%) was kindly donated by a local pharmaceutical industry (Z. Jans Pharmaceuticals (Pvt) Ltd, Peshawar) and was used as reference standard without further purification.

### 2.2 Quantification of ciprofloxacin

#### *2.2.1 Standards preparation*

Stock solution of ciprofloxacin HCl was prepared with a concentration of 1mg/ml in DI water. From this stock solution different calibration standards were prepared with concentrations as 5 µg/ml, 10 µg/ml, 20 µg/ml and 30 µg/ml using DI water. All the solutions were filtered through 0.20 µm filter paper before injection into chromatographic column. 20 µl of each calibration standard was injected thrice a time.

#### *2.2.2 Sample preparation*

Ten tablets of each product were ground to powder. Amount of powder equivalent to 10 mg ciprofloxacin, calculated on the basis of product label claim, was transferred to 100 ml volumetric flask. 50 ml water was added into the flask and shaken for 15 minutes. After vigorous shaking make the volume up to the mark with water. The solutions were filtered and 10 ml of filtrate was diluted to 50 ml with DI water. All the solutions were filtered through 0.20 µm filter paper before injection into chromatographic column. 20 µl of each sample was injected thrice a time.

#### *2.2.3 Instrumentation and chromatographic parameters*

HPLC system (LC – 8A) from Shimadzu (Japan) equipped with C-18 (Shimpack-ODS; 250 * 4.6 mm; 5 µm particle size) column was used for the chromatographic separations of analytes. Detection of analytes was performed using UV-Vis detector (Linear). CLASS CR-10 Workstation programme provided by the system suppliers was used to control the system and for data analysis.

HPLC was operated in isocratic mode using mixture of water:acetonitrile:phosphoric acid; 800:200:2 as mobile phase. The system was equilibrated with mobile phase for half an hour at the flow rate of 1.5 ml/min. During standard/sample run column was kept at ambient temperature and a flow rate of 1.5 ml/min was used. Detection was made at the wave length maximum 278 nm.

## 3. Results and Discussion

Different proportions of solvents such as acetonitrile, methanol and water were used in order to optimize the mobile phase. Some ion-pair reagents like phosphoric acid, formic acid and acitic acid were also studied. A flow rate of 1.5 ml/min was selected after preliminary tests. The calibration curve showed linearity over a concentration range from 5 to 30 µg/ml. The correlation coefficient ($R^2$) value obtained with linear regression of curve was 0.9999.

Eleven ciprofloxacin products of different pharmaceutical companies were collected from the market and analyzed for ciprofloxacin contents (active ingredient). The results were calculated, compared and tabulated in Table 1. The table shows that drug no. 8 manufactured by H has the highest quantity of the active ingredient while drug no. 10 manufactured by J has the lowest amount among the others. On the basis of active ingredient the order of contents is: product H (+3.6%) > E (+2.8%) > K (+0.8%) > C (-0.6%) > D (-1.4%) > I (-1.8%) > G (-4%) > B (-8.6%) > A (-9.2%) > F (-10.2%) > J (-12.8%). From the

data it is quite obvious that 10 products out of 11 are within the range of ± 10 % of their claims while one product (J) has lower level of active ingredient. Figure 2 shows the graphical representation of the results obtained from these analyses.

**Table 1. Results from comparative analysis of Ciprofloxacin contents in different Ciprofloxacin based pharmaceutical products.**

| Product codes* | Ciprofloxacin (mg) / tablet (Label claim) | Ciprofloxacin (mg) / tablet (Results obtained) | Results (%) | Content Deviation (%) |
|---|---|---|---|---|
| A | 500 | 454 | 90.8 | - 9.2 |
| B | 500 | 457 | 91.4 | - 8.6 |
| C | 250 | 249 | 99.6 | - 0.6 |
| D | 500 | 493 | 98.6 | - 1.4 |
| E | 250 | 257 | 102.8 | + 2.8 |
| F | 500 | 449 | 89.8 | - 10.2 |
| G | 500 | 480 | 96 | - 4.0 |
| H | 500 | 518 | 103.6 | + 3.6 |
| I | 500 | 491 | 98.2 | - 1.8 |
| J | 500 | 436 | 87.2 | - 12.8 |
| K | 250 | 252 | 100.8 | + 0.8 |

*Alphabets identify the products names

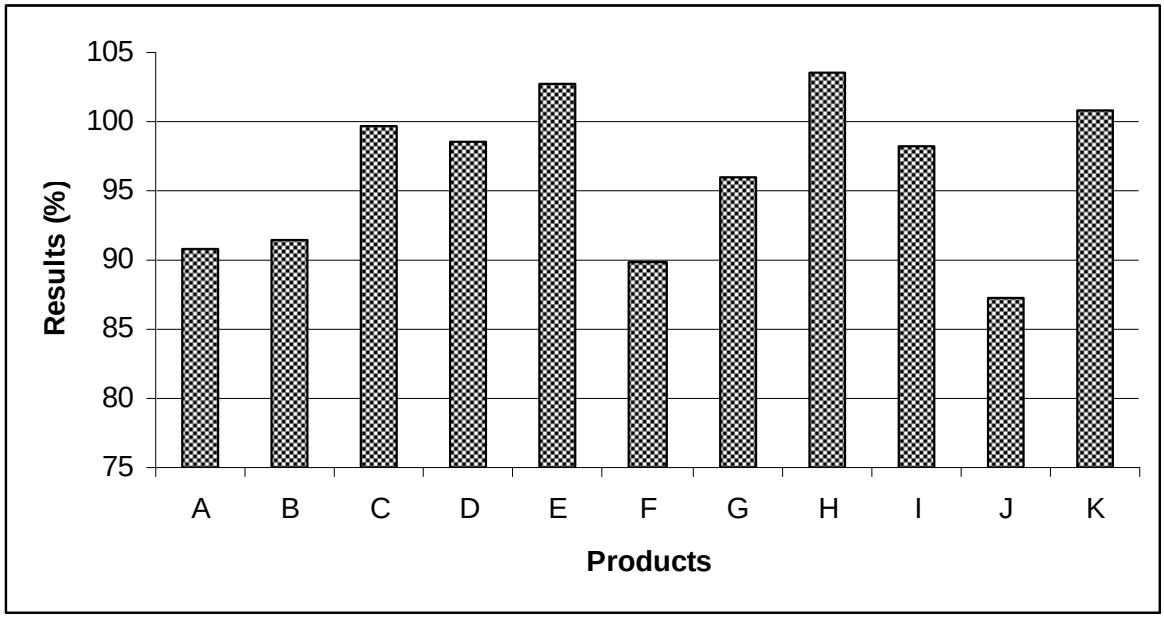


**Figure 2. Comparison of % Ciprofloxacin contents in different Ciprofloxacin based pharmaceutical products**

## References


Barbosa, J., R. Bergés and V. Sanz-Nebot. 1996. Solvatochromic parameter values and pH in aqueous-organic mixtures used in liquid chromatography Prediction of retention of a series of quinolones. *Journal of Chromatography A,* 719(1): 27-36.

Belal, F., A.A. Al-Majed and A.M. Al-Obaid. 1999. Methods of analysis of 4-quinolone antibacterials. *Talanta*, 50(4): 765-786.

Bertino, J. 2000. The safety profile of the fluoroquinolones. *Clinical therapeutics,* 22(7): 798-817.

Bhowal, S.K. and T.K. Das. 1991. Spectrophotometric determination of some recently introduced antibacterial drugs using ferric chloride.*Analytical letters*, 24(1):25-37.

Budvari-Barany, Z., G. Szász, K. Takacs-Novak, I. Hermecz and A. Lore. 1991. The pH influence on the HPLC-retention of chemotherapeutic fluoroquinolone derivatives. *Journal of Liquid Chromatography & Related Technologies*, 14(18): 3411-3424.

Córdoba-Borrego, M., M. Córdoba-Diaz and D. Córdoba-Diaz. 1999. Validation of a high-performance liquid chromatographic method for the determination of norfloxacin and its application to stability studies (photo-stability study of norfloxacin). *Journal of pharmaceutical and biomedical analysis,* 18(6): 919-926.

Fierens, C., S. Hillaert and W. Van den Bossche. 2000. The qualitative and quantitative determination of quinolones of first and second generation by capillary electrophoresis. *Journal of pharmaceutical and biomedical analysis*, 22(5): 763-772.

Flurer, C.L. 2001. Analysis of antibiotics by capillary electrophoresis. *Electrophoresis,* 22(19): 4249-4261.

Fratini, L. and E.E.S. Schapoval. 1996. Ciprofloxacin determination by visible light spectrophotometry using iron (III) nitrate. *International Journal of Pharmaceutics*, 127(2): 279-282.

Haeseker, M., A. Verbon, J. Welzen, C. Neef, C. Bruggeman and L. Stolk. 2011. A Simple and Rapid RP-HPLC Method to Determine Ciprofloxacin Levels In Human Serum. *Asian Journal of Pharmaceutical and Biological Research,* 1(3): 350-354.

Hernández-Arteseros, J.A., J. Barbosa, R. Compañó and M.D. Prat. 2002. Analysis of quinolone residues in edible animal products. *Journal of Chromatography A*, 945(1-2): 1-24.

Horwitz, W. 2002. *Official methods of analysis of AOAC International*. AOAC International Gaithersburg, Maryland, USA.

Husain, S., S. Khalid, V. Nagaraju and R. Nageswara. 1995. High-performance liquid chromatographic separation and determination of small amounts of process impurities of ciprofloxacin in bulk drugs and formulations. *Journal of Chromatography A*, 705(2): 380-384.

Imre, S., M.T. Dogaru, C. Vari, T. Muntean and L. Kelemen. 2003. Validation of an HPLC method for the determination of ciprofloxacin in human plasma. *Journal of pharmaceutical and biomedical analysis,* 33(1): 125-130.

Kamberi, M., K. Tsutsumi, T. Kotegawa, K. Nakamura and S. Nakano. 1998. Determination of ciprofloxacin in plasma and urine by HPLC with ultraviolet detection. *Clinical chemistry*, 44(6): 1251-1255.

Kassab, N.M., A.K. Singh, E.R.M. Kedor-Hackmam, and M.I.R.M. Santoro. 2005. Quantitative determination of ciprofloxacin and norfloxacin in pharmaceutical preparations by high performance liquid chromatography. *Revista Brasileira de Ciências Farmacêuticas,* 41: 507-513.

Kirkland, K.M., D.A. McCombs and J.J. Kirkland. 1994. Rapid, high-resolution high-performance liquid chromatographic analysis of antibiotics. *Journal of Chromatography A*, 660(1-2): 327-337.

Lacroix, P.M., N.M. Curran and R.W. Sears. 1996. High-pressure liquid chromatographic methods for ciprofloxacin hydrochloride and related compounds in raw materials. *Journal of pharmaceutical and biomedical analysis*, 14(5): 641-654.

Lorian, V. 2005. *Antibiotics in laboratory medicine*. Lippincott Williams & Wilkins.

Mandell, G. and W. Petri Junior. 1996. Antimicrobial agents. *In*: Sulfonamides, trimethoprim-sulfamethoxazole, quinolones,

and agents for urinary tract infections. Goodman & Gilmans (Eds.), *The Pharmacological Basis of Therapeutics.* New York: Mcgraw-Hill. 1065-1068.

Marzo, A.and L. Dal Bo. 1998. Chromatography as an analytical tool for selected antibiotic classes: a reappraisal addressed to pharmacokinetic applications. *Journal of Chromatography A,* 812(1-2): 17-34.

Mazuel, C. 1991. Norfloxacin. *Anal. Profiles Drug Subst. Excipients, San Diego* 20: 557-600.

Pharmacopeia, U.S. *27.ed. Rockville: United States Pharmacopeial Convention, 2004. p.454-457, 1342-1343*.

Pharmacopoeia, B. *1999. London: Her Majesty's Stationery Office, 1999. p.369-370, 1034-1035*.

Samanidou, V., C. Demetriou and I. Papadoyannis. 2003. Direct determination of four fluoroquinolones, enoxacin, norfloxacin, ofloxacin, and ciprofloxacin, in pharmaceuticals and blood serum by HPLC. *Analytical and bioanalytical chemistry*, 375(5): 623-629.

Samanidou, V.F., E.A. Christodoulou and I.N. Papadoyannis. 2005. Determination of fluoroquinolones in edible animal tissue samples by high performance liquid chromatography after solid phase extraction. *Journal of Separation Science*, 28(6): 555-565.

Sowinski, K.M. and M.B. Kays. 2004. Determination of ciprofloxacin concentrations in human serum and urine by HPLC with ultraviolet and fluorescence detection. *Journal of Clinical Pharmacy and Therapeutics* , 29(4): 381-387.

Sun, S.W. and L.Y. Chen. 1997. Optimization of capillary electrophoretic separation of quinolone antibacterials using the overlapping resolution mapping scheme. *Journal of Chromatography A,* 766(1-2): 215-224.

Thoppil, S.O. and P.D. Amin. 2000. Stability indicating reversed-phase liquid chromatographic determination of ciprofloxacin as bulk drug and in pharmaceutical formulations. *Journal of pharmaceutical and biomedical analysis*, 22(4): 699-703.

Torniainen, K., S. Tammilehto and V. Ulvi. 1996. The effect of pH, buffer type and drug concentration on the photodegradation of ciprofloxacin. *International Journal of Pharmaceutics* , 132(1-2):53-61.

Vybíralová, Z., M. Nobilis, J. Zoulova, J. Kvetina and P. Petr. 2005. High-performance liquid chromatographic determination of ciprofloxacin in plasma samples. *Journal of pharmaceutical and biomedical analysis*, 37(5): 851-858.