# ANTIBACTERIAL QUINOLONES: CIPROFLOXACIN

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***Abstract***

*Quinolones are a class of antibacterial agents with a large number of representatives and enhanced activity against Gram-negative, Gram-positive and anaerobic bacteria frequently used today in the infectious disease treatment.*

*The analysed compound, ciprofloxacin lactate, was obtained from Ciprinol tablets and Ciprinol injectable solution (KRKA) after extraction with dichloromethane and was characterized by appearance, colour, odour and solubility. All these tests were in accordance with the requirements of British Pharmacopoeia IVth edition.*

*Determination of purity of ciprofloxacin was performed by TLC using five mixtures of solvents which allowed us to determine the Rf value for ciprofloxacin. Partial chemical structure studies were performed by UV-VIS spectrophotometry.*

**Key words:** quinolones, infectious diseases, resistant bacterial strains, ciprofloxacin, thin layer chromatography TLC.

## INTRODUCTION

Many human infections are caused by either bacteria or viruses, single-celled organisms thought by some scientists to be among the most successful life forms on the planet. Before the discovery of antibiotics, infectious diseases were the leading cause of mortality in the world. Fortunately, the antibiotics discovery has changed the prognosis of infectious diseases and many bacterial infections are treated successfully today with appropriate antibiotics (http://www.betterhealth; http://www.bt. cdc.gov; Linhares I. et al., 2013).

In the struggle for existence, the germs constantly adapt by selecting resistant strains and thus the study of sensitivity and resistance of germs responsible for the infection is the first step in treatment. Anti-infective chemotherapy is a way to combat the infection diseases with chemicals through their pharmacological effects. Anyway, frequent use of antibiotics and other antimicrobials led to increased incidence of bacterial resistance and the spread of resistant bacterial strains. This is the reason for various classes of antimicrobials have been obtained (Linhares I. et al., 2013; Arjunan M. et al., 2010; Rahman F. et al., 2009; Hryniewicz K. et al., 2001; Schito G.C. et al., 2009).

Quinolones are a class of antibacterial agents entirely obtained by chemical synthesis. In 1962, Lesher and his collaborators introduced the first quinolone derivative, nalidixic acid, a relatively poor antibacterial agent

limited to urinary tract infections against Gram-negative organisms. In the last decades, a large number of antibacterial quinolones with enhanced activity against Gram-negative, Gram-positive and anaerobic bacteria have been synthesized. In the terms of in vitro activity and pharmacokinetic properties, quinolones are categorized into four generations (Chu D.T.W., Fernandes P., 1991; Lesher G.Y. et al., 1962; Limberakis C., 2007; Pintilie L., 2012).

Quinolones are prescribed in treating infections of the upper and lower respiratory tract, urinary tract, gastrointestinal tract, sexually transmitted diseases, soft tissue and skin infections, bacterial meningitis, osteomyelitis, septicemia, endocarditis, surgical infections and on patients with immune deficiencies. Based on global sales, the fluoroquinolone group members Levofloxacin and Ciprofloxacin generated the highest sales in the last decade (Limberakis C., 2007; Pintilie L., 2012; Beale J.M., Block J.H., 2011; IMS, 2005).

**MATERIAL AND METHOD**

*Ciprofloxacin* 1-cyclopropyl-6-fluoro-4-oxo-7(piperazin-1-yl)-quino line-3-carboxylic acid (Fig. 1) sample used in this study comes from Ciprinol (KRKA) tablets 750 mg (series T970131010102015) and Ciprinol injectable solution 100 mg/10 mL (series A49712), respectively by extraction with a suitable solvent. Taking into account that ciprofloxacin is slightly soluble in common solvents such as water, alcohol, acetone, chloroform, ether or ethyl acetate, dichloromethane was chosen as the extraction solvent.

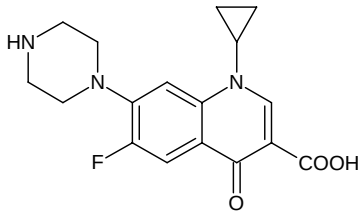


Fig. 1. Ciprofloxacin

Thus, after shredding to a fine powder, the tablets of ciprofloxacin were extracted one time with dichloromethane (partial extraction, 50 ml of dichloromethane). A similar work-up was applied to the extraction of the injectable solution. The organic layer was filtered and then evaporated to dryness under reduced pressure to give pure ciprofloxacin on which the following tests were performed: organoleptic study, solubility, chemical identification, thin layer chromatography, ultraviolet-visible spectral study (Zsurka R., 2012).

**RESULTS AND DISCUSSION**

According to British Pharmacopoeia (BPh), ciprofloxacin is a crystalline powder, white or yellow-white, odorless (British Pharmacopoeia, 2002).

*Organoleptic study* was determined in accordance with experimental techniques provided by British Pharmacopoeia IVth edition and Romanian Pharmacopoeia Xth edition. The results obtained are shown in Table 1.

*Table 1*

Organoleptic characteristics of ciprofloxacin

| COMPOUND | APPEARANCE | COLOUR | ODOUR |
|---|---|---|---|
| **Ciprofloxacin** (BPh) | crystalline powder | white or yellow-white | odorless |
| **Ciprofloxacin lactate** (KRKA) | crystalline powder | white | odorless |

*Solubility* in different solvents was determined in accordance with British Pharmacopoeia IVth edition. The solubility of ciprofloxacin is presented in Table 2.

*Table 2*

Solubility of ciprofloxacin

| SOLVENTS | CIPROFLOXACIN (BPH) | CIPROFLOXACIN LACTATE (KRKA) |
|---|---|---|
| **water** | practically insoluble | sparingly soluble |
| **ethanol** | very sparingly soluble | very sparingly soluble |
| **dichloromethane** | very sparingly soluble | sparingly soluble |
| **acetic acid** | soluble in diluted acetic acid | soluble in 30% acetic acid<br>sparingly soluble in 10% acetic acid |

As the British Pharmacopoeia does not mention any chemical reaction for ciprofloxacin, we used the *chemical identification reactions* indicated by Romanian Pharmacopoeia Xth edition to identify the lactate ion (oxydation by kalium permanganat, iodine reaction and feric chloride colloration) and all of them were in agreement with RPhX techniques.

*Thin layer chromatography* (TLC) used solutions of $10^{-4}$-$10^{-5}$ M of ciporfloxacin in dichloromethane and methanol (1:1). Merck chromato graphic plates with dimensions of 20x20 cm and silica gel 60 F254 applied on poliester were used. The samples were spotted at a distance of 1 cm from the edge of TLC plates using microcapillary. Migration was performed in a tightly closed flask, saturated by the vapors of solvents. The development

over a distance of 12 cm led to the separation of a single area. The following mixtures of solvents were used (Table 3).

*Table 3*

Mixtures of solvents for TLC

| MIXTURES OF SOLVENTS | COMPONENTS | RATE |
|---|---|---|
| **$S_1$** | hexane-ethyl acetate-acetic acid glacial | 15:5:1 |
| **$S_2$** | hexane-ethyl acetate-10% acetic acid | 15:5:1 |
| **$S_3$** | ethyl acetate-ethanol | 1:1 |
| **$S_4$** | acetone-ethanol | 1:1 |
| **$S_5$** | dichloromethane-methanol | 1:1 |
| **$S_6$** | dichloromethane-methanol | 1:3 |
| **$S_7$** | ethanol-water | 2:1 |
| **$S_8$** | acetonitrile-ammonia-methanol-dichloromethane | 1:2:4:4 |
| **$S_9$** | acetonitrile-ammonia-chloroform | 1:2:2 |

*Table 4*

$R_f$ values for ciprofloxacin

| MIXTURES OF SOLVENTS | $R_f$ | |
|---|---|---|
| | **Ciprofloxacin lactate** | **Impurities** |
| **$S_5$** | 0.11 | - |
| **$S_6$** | 0.76 | - |
| **$S_7$** | 0.076 | - |
| **$S_8$** | 0.29 (ciprofloxacin tablets)<br>0.34 (ciprofloxacin injectable sol) | 0.61 (impurity in tablets) |
| **$S_9$** | 0.47 | - |

Identification of ciprofloxacin lactate was based on $R_f$ values after highlighting the spots in UV light at $\lambda = 254$ nm (Table 4). Ultraviolet light has been selected as visualizing agent because the quinoline ring system shows, due to chromophore C=C-C=C-C=C-C=O, an intense absorption in UV. Ciprofloxacin lactate shows blue-purple fluorescence in UV light.

Among the developing mixtures, $S_8$ and $S_9$ systems with $R_f$ values between 0.29-0.47 are most recommended for the ciprofloxacin lactate study. The spots are well separated and with oval-rounded shapes. The ideal separation system has proven to be $S_9$ (acetonitrile-ammonia-chloroform 1:2:2) as can be seen from the Rf values ($R_f$=0.47), $S_8$ system (acetonitrile-ammonia-methanol-dichloromethane in a ratio of 1:2:4:4, officinal in British Pharmacopoeia) being the next in term of efficiency ($R_f$=0.29 for ciprofloxacin extracted from tablets and $R_f$=0.34 for ciprofloxacin extracted from injectable solution). Systems $S_1$-$S_4$ proved to be ineffective ($R_f$=0). According to system $S_8$, thin-layer chromatography revealed an impurity

visible in UV light as blue-purple spot with lower intensity compared to ciprofloxacin. According to British Pharmacopoeia, the structurally related impurities in the sample can be the compounds in the Figure 2:

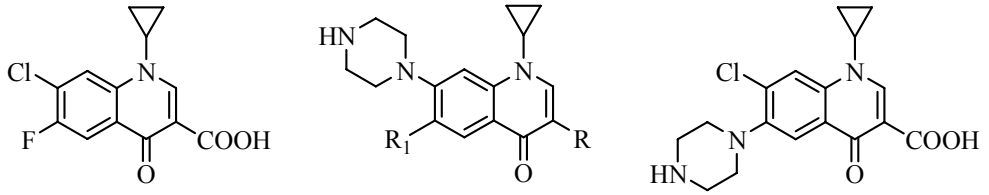


Fig. 2. Structurally related impurities of Ciprofloxacin

*Ultraviolet-visible spectral study* of ciprofloxacin lactate was performed with a Beckman DU-64 spectrometer using a solution 0.001% m/v of ciprofloxacin lactate in dichloromethane. The spectrum was recorded for 200-400 nm area and shows three absorption bands with the maximum values at 273.5 nm, 316.5 nm and 330 nm which caractherize the quinoline-4-one heterocycle (Table 5).

*Table 5*

UV spectral parameters for ciprofloxacin lactate

| COMPOUND | $\lambda_{max}$ (nm) | ABSORBANCE (A) |
|---|---|---|
| **Ciprofloxacin lactate** | 273,5 | 2,97 |
| | 316,5 | 1,02 |
| | 330 | 0,97 |

The intensity of the molecular extinction coefficient (ε) for a determined absorption is proportional to the probability of electronic transition. Taking into account the structure of ciprofloxacin, the absorption bands observed in the UV-VIS spectrum is corresponding to $n \rightarrow \pi^*$ electronic transition in heterocycle and $\pi \rightarrow \pi^*$ electronic transition in the aromatic ring.

**CONCLUSIONS**

The analysed compound, cyprofloxacin lactate, was obtained from Ciprinol tablets and Ciprinol injectable solution after extraction with dichloromethane and was characterized by appearance, colour, odour and solubility. All these tests were in accordance with the requirements of British Pharmacopoeia IVth edition.

Determination of purity of ciprofloxacin was performed by TLC using five mixtures of solvents which allowed us to determine not only the $R_f$ value for ciprofloxacin but also some impurity in the analysed sample.

Partial chemical structure studies were performed by UV-VIS spectrophotometry. UV-VIS spectrum recorded for 200-400 nm area showed three absorption bands which caractherize the heterocycle ring.

**REFERENCES**


1. Arjunan M., Al-Salamah A.A., Amuthan M., 2010, *Prevalence and antibiotics susceptibility of uropathogens in patients from a rural environment. Tamilnadu*, Am. J. Infect Dis., 6, pp. 29-33.
2. Beale J.M., Block J.H., 2011, *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*, 12th edition, Lippincott Williams and Wilkins, pp. 179-241.
3. Chu D.T.W., Fernandes P., 1991, *Recent developments in the field of quinolone antibacterial agents*, in: Advances in drug research, Academic Press, London-San Diego-New York, 21, pp. 39-144.
4. Hryniewicz K., Szczypa K., Sulikowska A., Jankowski K., Betltjewska K., Hryniewicz W., 2001, *Antibiotic susceptibility of bacterial strains isolated from urinary tract*, J. Antimicrob. Chemother., 47, pp. 773-780.
5. Lesher G.Y., Foelich E.J., Garnett M.D., Bayley J.H., Brundage P.R., 1962, *1,8-Naphtyridine derivatives, a new class of chemotherapeutic agents*, Journal of Medicinal Chemistry, 5(5), pp. 259-279.
6. Limberakis C., 2007, *Quinolone antibiotics: Levofloxacin, Moxifloxacin, Gemifloxacin and Garenoxacin*, in: The Art of Drug Synthesis, edited by Johnson D.S. and Li J.J., John Wiley and Sons, Inc., pp. 39-69.
7. Linhares I., Raposo T., Rodrigues A., Almeida A., 2013, *Frequency and antimicrobial resistence patterns of bacteria implicated in community urinary tract infections: a ten-year surveillance study (2000-2009)*, BMC Infectious Diseases, 13:19.
8. Pintilie L., 2012, *Quinolones: Synthesis and Antibacterial Activity*, in: Antimicrobial Agents, edited by Bobbarala V., InTech, pp. 255-272.
9. Rahman F., Chowdhury S., Rahman M.M., Ahmed D., Hossain A., 2009, *Antimicrobial resistance pattern of gram-negative bacteria causing urinary tract infection*, S. J. Phar. Sci., 2, pp. 44-55.
10. Schito G.C., Naber K.G., Botto H., Palou J., Mazzei T., Gualco L., Marchese A., 2009, *The ARESC study: an international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections*, Int. J. Antimicrob. Agents, 34, pp. 407-413.
11. Zsurka R., 2012, *Chinolone antibacteriene. Ciprofloxacina*, Lucrare de licenţă, Universitatea din Oradea, Facultatea de Medicină şi Farmacie.
12. * * *, 1993, *Farmacopeea Română Ediţia a X-a*, Ed. Medicală, Bucureşti.
13. * * *, 2002, *British Pharmacopoeia*, 4th edition.
14. * * *, 2005, IMS Health World Review, www.imshealth.com
15. http://www.betterhealth.vic.gov.au/bhcv2/bhcarticles.nsf/pages/infections_bacterial_and_viral?open
16. http://www.bt.cdc.gov/training/smallpoxvaccine/reactions/bac_inf_frequency.html