

RESEARCH REGARDING THE PHYSICO-CHEMICAL CHARACTERISTICS OF QUINOLONE DERIVATIVES THROUGH OPTIMIZED ANALYTICAL METHODS

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Antibacterial quinolones (QN) are a class of synthetic broad spectrum chemotherapeutic antibacterial substances widely used due to their enhanced pharmacokinetic properties, particular mechanism of action and extensive and potent activity; being one of the fastest growing antibacterial class in terms of both therapeutic use and global benefit. Their great therapeutic importance is closely linked with the analytical aspects; consequently elaboration of new methods for their analysis is a permanent necessity and also a challenge. The overall objective of this PhD thesis was the physico-chemical characterization of quinolone derivatives using modern optimized analytical methods.

The thesis is structured in two main parts and contains annexes too. The general part includes aspects concerning the evolution of QN class, respectively the current state of knowledge regarding structural characterization, methods of analysis and therapeutical use.

The experimental part contains personal studies presented systematically on specific objectives:

I. Study of specific acid-base equilibrium of quinolone derivatives by NMR experiments.

Since the published studies are controversial regarding the acid-base triprotic equilibria, this study aimed to clarify this issue. In a first step the location of atoms was determined by NMR analysis (^1H -NMR, ^{13}C -NMR, ^{15}N -NMR) for six fluoroquinolones (FQ). Because the literature data are incomplete for moxifloxacin (MOX), the full location of the atoms in the pyrrolidino-piperidine ring for MOX was realized, data not published previously in the literature. The protonation centers for the studied FQ and also for model compounds were researched and identified by preliminary synthesis. The results show that the studied FQ have three protonation centers. Using ^1H -NMR pH titration the protonation macroconstants of the FQs and their corresponding methyl ester were determined accurately by combining pH ^1H -NMR method with a deductive method (using model compounds) the protonation microconstants.

II. The analysis and characterization of quinolone derivatives by UV spectrophotometry.

UV spectra recorded in the quinolone derivatives show three absorption maximum values located around 210-230 nm, 270-290 nm and 315-330 nm. The absorption bands exhibited both hipsocrome and batocrome displacements, by comparison in different solvents (methanol, HCl 0.1M, 0.1M NaOH). We established correlations between the chemical structure of quinolone derivatives and their UV spectra using model compounds. UV spectra were relevant for the identification and purity control of the studied substances and also useful for identifying the analytical signals obtained in the analysis of complex mixtures by capillary electrophoresis (CE).

III. The analysis of the chromatographic behavior of quinolone derivatives by thin layer chromatography (TLC)

TLC methods can be used in order to identify substances and determine their purity degree, being particularly useful in rapid pharmaceutical screenings. We studied the influence of mobile phase composition and elutrope power on the QNs mobility using different types of chromatographic systems. The optimal elutropic power ϵ° is within the range of 0.70 - 0.80. We optimized and selected the following mobile phases as being appropriate for the separation of the studied QNs: ammonia cc. - methanol - acetonitrile - methylene chloride [2: 4: 1: 4], ammonia cc. - methanol - acetonitrile - methylene chloride [1.5: 4: 2: 1], ammonia cc. - methanol - acetonitrile - ethyl acetate [1.25: 1: 1: 1.5], ammonia cc. - methanol - acetonitrile - ethyl acetate [2: 1: 1: 2].

IV. The analysis of quionolone derivatives by capillary electrophoresis (CE)

Due to its speed of analysis, high efficiency, automated analytical equipment, low reagents and sample consumption and rapid development of method, CE has gained momentum in pharmaceutical analysis, being regarded today as an alternative and also a complementary technique to the more frequently used high performance liquid chromatography (HPLC). The electrophoretic behavior of 17 compounds was identified and characterized in different electrophoretic conditions (BGE, pH) using capillary zone electrophoresis (CZE); the correlation between electrophoretic mobility and chemical structure respectively protonation constants of the analyzed compounds was also studied. CZE is applicable for simultaneous separation of QNs with different structural characteristics. A simple and rapid separation method of four compounds from different generations was developed and optimized in less than 10 minutes. This result suggested the possibility of extending the applicability of the CZE method for the analysis of a larger number of derivatives.

The vast majority of CE QN separation use a basic BGE, the separations at an acidic pH being less studied. A separation method for six QN derivatives was developed in less than 7.5 minutes using phosphoric acid like BGE by verifying the analytical performance of the method. QNs have numerous structural similarities that derive to very similar electrophoretic behavior, consequently their simultaneous separation from complex mixtures is challenging. A separation of twelve derivatives was carried out and optimized in less than 10 minutes, using sodium tetraborate as BGE and verifying also the analytical performance of the method. The simultaneous separation of FQ with very similar structural characteristics was solved using micellar electrokinetic capillary chromatography (MEKC), method that extends the applicability of CE to neutral analytes. By adding an anionic surfactant, sodium dodecyl sulfate, to the buffer solution the separation of ciprofloxacin, norfloxacin and ofloxacin from a mixture was achieved in less than approximately 8 minutes.

CE methods (both CZE and MECK) proved to be powerful and adequate analytical tools for the determination and separation of QN derivatives.

In the experimental part of the thesis a comprehensive characterization of the QNs was performed in terms of specific acid-base balance, spectral, chromatographic and electrophoretic behavior. Based on the determined constants (macro-and microconstants) electrophoretic separation methods (CZE, MECK) can be developed and specific properties of microspecies can be interpreted and improved in order to obtain new compounds. The proposed analytical methods can be useful for the qualitative and quantitative determination of QNs from raw materials, pharmaceutical formulations, biological and environmental samples.

Keywords: *quinolone, fluoroquinolone, NMR, microspeciation, protonation constant, UV spectrophotometry, thin layer chromatography, capillary zone electrophoresis; micellar electrokinetic chromatography*