



## A review article on Ciprofloxacin determination with various analytical techniques

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### Abstract

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Ciprofloxacin belongs to the fluoroquinolone family, which exhibits biological activity against both gram-positive and gram-negative bacteria. The previously mentioned drug is of utmost importance due to the high incidence of resistance caused by other antibiotics. Thus, numerous research studies has been conducted to determine Ciprofloxacin as well as its metabolites owing to their contribution in the drug's biological activity. Liquid chromatography, gas chromatography, capillary electrophoresis and other separation techniques were used to develop, adapt and implement analytical techniques for the drug analysis. Other studies has made use of the hyphenation between liquid chromatography and mass spectrometry for Ciprofloxacin assay for quality control, biological fluids, animal tissues and numerous sample types. The recent advances in the ionization methods has led to the use of direct mass spectrometry techniques to analyze various sample types. This mini review aims to focus on the various analytical techniques and recent technologies implemented for Ciprofloxacin analysis.

**Keywords:** Ciprofloxacin; nanospray capillary; ion source; direct nano-electrospray ionization-; targeted detection.

### 1. Introduction

Ciprofloxacin is a broad-spectrum antimicrobial agent, which falls under the fluoroquinolones class. The drug is beneficial as it acts against several infectious diseases where urinary tract infections are the most common ones (Vance-Bryan et al.,1990). Ciprofloxacin is effective against both gram negative and gram-positive bacteria (gram

negative in particular), the mechanism of action targets topoisomerase IV inhibition. Unlike most antibiotics (penicillins, cephalosporins and aminoglycosides), ciprofloxacin is the least resistant antibiotic compared to the ones mentioned above (Navarro et al.,2002).

The drug is partially metabolized in the liver, giving four active metabolites; des-ethylene ciprofloxacin, oxo-ciprofloxacin, sulfo-

ciprofloxacin and N-acetyl ciprofloxacin, where oxo-Ciprofloxacin is the primary urinary metabolite and sulfo-Ciprofloxacin is the fecal metabolite (Vance-Bryan et al.,1990). A large percentage of the drug is excreted unchanged in the urine as well as all four metabolites (Eshar et al.,2018).

Accounting to the importance and the utmost need for such drug, several studies have employed various analytical techniques in owe of Ciprofloxacin analysis along with its metabolites; due to their major biological activity (Vance-Bryan et al.,1990).

Numerous techniques have been carried to analyse ciprofloxacin in various pharmaceutical dosage forms, for the purposes of quality control assays and method optimization. Other techniques have been developed for the drug analysis as well as its metabolites in biological fluids; urine, plasma, serum and saliva. However, when it comes to biological fluids, sample pre-treatment and method optimization are mandatory due to the complexity and endogenous components of such matrices (Eshar et al.,2018, Vance-Bryan et al.,1990).

## 2. Method of analysis

### 2.1. Liquid chromatography

Numerous Liquid chromatographic (LC-MS) techniques have been designed to determine and quantitate Ciprofloxacin in various pharmaceutical dosage forms using ultraviolet (UV) and fluorescence detection systems. The chromatographic system has been the go to tool of analysis when it comes to mixtures, however such golden tool comes with a great deal of parameters to adjust and optimize in order to make use of its sensitivity; for instance the method flow rate, the mobile phase composition and PH, the column, and the detector wavelength.

Several methods were developed to analyze and quantitate Ciprofloxacin in pharmaceutical dosage forms; Kassab et al, developed a technique to determine the drug in question in two pharmaceutical dosage forms using a mobile phase of water: acetonitrile: trimethylamine (80:20:0.3v/v/v) with pH 3.3 at 279nm, UV detection (Kassab et al.,2005). Predrag et al, validated an analytical method for quality control assay of Ciprofloxacin with mobile phase of acetonitrile: water (14:86 v/v), pH 3 at 278nm (Sibinović et al.,2005).

Others were concerned with the analysis of the targeted drug in biological fluids; analysis of human plasma to determine ciprofloxacin with an HPLC-UV technique, prior to protein precipitation using acetonitrile at 277nm (Vella et al.,2015). Plasma and urine samples assay using an isocratic RP-HPLC technique with UV detection at 280 nm; where the plasma samples were pretreated using acetonitrile and centrifuged to get rid of the proteins, while the urine samples were diluted with distilled water (Kamberi et al.,1998). Another HPLC-UV assay for Ciprofloxacin determination in human plasma following protein precipitation at 280nm was carried (Maya et al.,2001).

While several articles focused on Ciprofloxacin analysis using UV detection, others have utilized the fluorescent properties of the drug in question for analysis; an HPLC technique has been developed and validated for Ciprofloxacin assay in human plasma post excitation at 278nm, where protein precipitation was carried prior to analysis (Imre et al.,2003). Another study was carried by employing an isocratic HPLC assay of ciprofloxacin in-addition to three of the drug's metabolites in serum and diluted urine samples (Krol et al.,1995). Furthermore, an assay was done to analyze rabbit and human serum to quantitate Ciprofloxacin after excitation at  $\lambda_{exc}$  274 nm and emission at  $\lambda_{emi}$  418 nm (Pei et al.,1994).

### 2.2. Capillary electrophoresis (CE)

While most of the separation techniques used to analyze Ciprofloxacin are dependent on Liquid chromatography, some studies implemented Capillary electrophoresis (CE) as an alternative; due to the lesser amounts of organic solvents needed, the feasibility, safety and shorter run times. Michalska et al. developed and validated a CE technique to determine Ciprofloxacin and its impurities simultaneously (Michalska et al.,2004). Other studies applied CE to analyze drugs in biological matrices; Hernandez et al. carried an isotachopheresis-capillary zone electrophoretic technique to determine three quinolones in pig plasma samples, where a solid phase extraction step prior to analysis, was done as sample pretreatment (Hernández et al.,2002). Another biological study to determine two fluoroquinolones was proposed; Barron et al. implemented a solid-phase extraction procedure for chicken muscles, followed by a capillary electrophoretic method to determine Enrofloxacin and Ciprofloxacin (Barrón et al.,2001).

### 2.3. Spectrophotometry detection

Numerous studies have utilized the spectrophotometry technique to analyze ciprofloxacin in mixtures along with other drugs; determination of Ciprofloxacin using a solid phase extraction (SPE) procedure followed by spectrophotometric analysis using UV detection (Pascual-Reguera et al.,2004). A quality control assay using a zero order spectrophotometry to analyze Ciprofloxacin in a mixture with Metronidazole was carried (Attia et al.,2016). Another quality control application was done to determine Ciprofloxacin in a mixture with Tinidazole using UV spectrophotometry (Bhalerao and Rote,2012).

### 2.4. Spectrofluorometric detection

Ciprofloxacin is characterized by its high native fluorescence, which led to the development of several studies in this area. Navalon et al. developed a solid-phase spectrofluorometric technique to analyze residual amounts of Ciprofloxacin in human urine specimen (Navalón et al.,2000). Tatar proposed a fast, specific and sensitive spectrofluorometric method to analyze Ciprofloxacin along with three other fluoroquinolones in pharmaceutical dosage forms (Ulu,2009).

### 2.5. Mass spectrometry detection

Mass spectrometry (MS) is one of the most recent powerful tools in the analytical field; such tool has given many privileges in terms of sensitivity and selectivity. Where the mass spectrometer has allowed determining the exact mass of an atom or a molecule, ionization of almost any compound being analyzed and consequently, ion fragmentation that allowed the interpretation of the resulting fragments. Thus, structural information regarding the targeted compound could be obtained. The many ionization techniques discovered have widened the scope of efficiency of the MS, which allowed the ionization of non-volatile complex compounds (e.g. proteins and polysaccharides) (De Hoffmann,2000) .

#### 2.5.1. Liquid chromatography mass spectrometry (LC-MS)

The hyphenation between the resolving power of liquid chromatography (LC) and the sensitivity of MS has led to the LC-MS technique, which evidently became the tool of choice when it comes to chromatography. Where the liquid chromatography separates the liquid phase and converts it to gas and the MS ionizes the gas phase

resulting into ions, which are fed into the MS detector for subsequent separation and analysis. LC-MS has proven superior to numerous techniques such as Gas chromatography mass spectrometry (GC-MS) because it is suitable for the analysis of non-volatile, thermally unstable and large ionic compounds (Packard and Primer,2017). Several studies adapted the LC-MS technique to determine Ciprofloxacin in various matrices and dosage forms; Wastewater samples were analyzed using LC-MS procedure with fluorescence detection to determine several fluoroquinolones including Ciprofloxacin at  $\lambda_{exc}$  278nm and  $\lambda_{emi}$  445nm (Nakata et al.,2005). Another LC-MS technique was developed to determine the drug in question, where a double step SPE procedure was done prior to the assay (Caro et al.,2006). The use of such combined technique has given the analytical process an edge; where tandem mass spectrometry (LC-MS/MS) was used to elucidate the structure of the targeted compound in a mixture with Enrofloxacin in chicken tissues following a SPE step (Ferrari et al.,2015). Other numerous LC-MS/MS techniques have been developed and adapted to analyze Ciprofloxacin in mixtures with other quinolones in food samples and meat products (Hatano,2004, Lee et al.,2013).

#### 2.5.2. Direct Electrospray ionization mass spectrometry (ESI-MS)

The typical mass spectrometer consists of an ion source, a mass analyzer, a detector and a computer device for data display, and while all components are of great importance, the ionization technique has a huge impact on the process in terms of sensitivity and ionization efficiency. The recent advances in ionization technologies has allowed direct analysis of intact samples and subsequently shortcutting the separation step (chromatography), which minimized sample pretreatment, carryover effect accompanied by the chromatography system and most importantly less run times (Klampfl and Himmelsbach,2015). Ester caro etal, adapted the direct ESI-MS technique to determine Ciprofloxacin after a two-step SPE pretreatment. Which greatly reduced the matrix effect threatened by the numerous endogenous compounds present in the urine samples analyzed, and subsequently allowed direct analysis and evidently less run time (Caro et al.,2006)

#### 2.5.3. Direct Nano-electrospray ionization Mass spectrometry (nano-ESI-MS)

Further advances in the mass spectrometry ionization techniques has led to the evolution of the

standard ESI source into nano-ESI technology. While the typical ESI source has proven efficient and sensitive, Nano-ESI technology has a few extra remarkable features. Minute sample volumes are needed for analysis which makes it very suitable when it comes to limited volume samples, much lower flow rates and higher ionization efficiency, more stable spraying, smaller sizes of the initial droplets and subsequently less analyte amount lost (Karas et al.,2000).

Demeiry et al, adapted the direct nano-esi tandem mass spectrometry technique to identify and quantify Ciprofloxacin in pharmaceutical dosage form as well as human urine samples (El Demeiry et al.,2020).

### 3. Conclusion

Fluoroquinolones are an important class of antibiotics that is active against numerous bacterial infections. Ciprofloxacin belongs to previously mentioned class; it is a broad-spectrum quinolone-carboxylic acid derivative, which is effective against gram-negative and positive bacteria (especially those resistant to other antibiotic classes like penicillins and cephalosporins). The drug is used to treat various respiratory tract infections as well as gastrointestinal disorders. The primary metabolism of the drug in question takes place in the liver resulting in four metabolites that contribute to the drug's biological activity. Thus, numerous studies and articles have developed and adapted several analytical techniques to determine and analyze Ciprofloxacin as well as its metabolites. A lot of research was carried to analyze dosage forms and pharmaceutical preparations. Other research was for the purpose of biological fluids and matrices assay of the drug in question, while some work was done as an adaptation and optimization of complex analytical techniques using Ciprofloxacin as the target analyte.

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