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High performance liquid chromatography determination of prulifloxacin and five related impurities in pharmaceutical formulations

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ABSTRACT

A novel HPLC method for the simultaneous determination of Prulifloxacin, Ulifloxacin, its process impurities in a tablets formulation and Enrofloxacin, used as Internal Standard, is developed. The separation was successfully carried out with a new stationary phase, HILIC, under isocratic elution mode using ammonium acetate buffer (5 mM, pH 5.8) and acetonitrile (12:88, v/v) at a flow rate of 1.0 mL/min. Column was thermostated at 25 °C (±1 °C) and 20 μL were injected for the analysis.

Calibration curves were linear in the investigated range with correlation coefficient better than 0.9880, while the limit of quantifications ranged from 0.25 to 5 μg/mL, depending from the analyte.

The within and between batch precision (RSD%) values ranged from 0.11% to 13.9% while within and between batch trueness (bias%) values ranged from 14.0% to −11.3%.

This method for the direct determination and quantification of process impurities in pharmaceutical formulations is suitable for routine analyses in quality control laboratories and was applied to evaluate for the first time, the presence and the quantities of cited analytes in commercially available formulation.

1. Introduction

According to IUPAC nomenclature, Prulifloxacin is a thiazetoquinolone 6-fluoro-1-methyl-7-[4-[(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl]-1-piperazinyl]-4-oxo-1H,4H-[1,3]thiazeto[3,2-a] quinoline-3-carboxylic acid.

It is anti-bacterial agent pro-drug of the quinolone carboxylic acid Ulifloxacin (Fig. 1), characterised by a potent and broad-spectrum anti-bacterial activity.

Prulifloxacin contains a quinolone skeleton with a four-member ring in the 1, 2 position including a sulphur atom that increase anti-bacterial activity and an oxodioxolenylmethyl group in the 7-piperazine ring that improve oral absorption.

In addition, as recently reviewed [1], Prulifloxacin was generally well tolerated in clinical trials, with a comparable tolerability profile than Ciprofloxacin and showed remarkable activity at the dose of 600 mg once daily for 10 days in patients with acute exacerbation of chronic bronchitis or other complicated lower urinary tract infections.

The *in vitro* anti-microbial activity studies were performed using Ulifloxacin that is generally more active than other commercially available fluoroquinolones against a large variety of clinical gram-negative bacteria including community and nosocomial isolates (*Escherichia coli*, *Klebsiella* spp., *Proteus*, *Providencia*, *Morganella* spp., *Moraxella catarrhalis* and *Haemophilus* spp.) and gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus* spp., and Italian community isolates of *Streptococcus pneumonie*) bacteria [2,3].

Activity against *Pseudomonas aeruginosa* varies between countries, especially related to the fact that the relative distribution of resistance in organisms arising from different clinical conditions may be of some relevance, with multiple resistances being more common in isolates from cystic fibrosis [4].

Prulifloxacin pharmaceutical behaviour (pharmacokinetic and pharmacodynamic) and its use on daily uptake (key factor in successfully infection treatment [5]) account for considering this pro-drug as an interesting antibacterial option to treat acute exacerbations of chronic bronchitis [6].

Prulifloxacin shows also an important effect against a worldwide collection of gastroenteritis producing pathogens, including those causing traveller's diarrhoea [7,8].

After oral administration, Prulifloxacin is absorbed in the upper small intestine and then metabolised to Ulifloxacin by esterases, mainly paraoxonase, partially by the intestinal membrane and mostly by the portal blood and the liver (first pass or pre-systemic metabolism) [2,3,9].

Abbreviations: CV, coefficient of variation; LOD, limit of detection; LOQ, limit of quantification; QC, quality control; ULOQ, upper limit of quantification.

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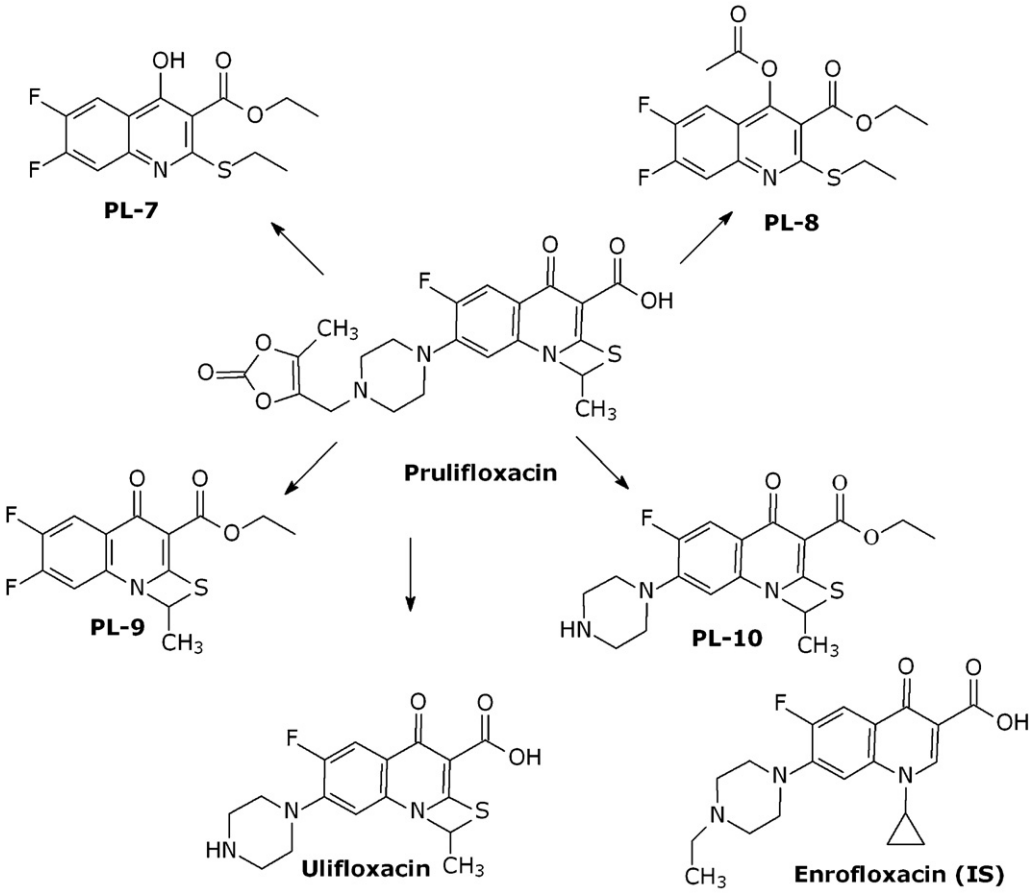

Fig. 1. Analytes and Internal Standard (IS) chemical structures.

In previously reported methods, analysis of Ulifloxacin was prevalently performed by HPLC with several type of detector, in order to improve the overall analytical performance, especially related to sensibility and selectivity [10].

Tougou and co-workers [9] used ethylchloroformate for the derivatization of Ulifloxacin into ethoxycarbonyl ulifloxacin to give a reasonable retention time on the Mono Q anion exchange column. Nakashima and co-workers [11] used liquid–liquid extraction for sample preparation, while Picollo and co-workers [12] used multi-step extraction and solid phase extraction followed by liquid–liquid extraction and derivatization.

Several papers report high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) method for the determination of Ulifloxacin in plasma [13,14].

There are some works that report an HPLC method with fluorescence detection [15,16]. The assays employed solid phase extraction (SPE) involving multi-step purification and nitrogen evaporation and 0.5 mL plasma was needed.

In literature are also present some works that involved in the determination of Prulifloxacin active metabolite by the use of spectroscopic analysis [17,18].

Other papers report the determination of Prulifloxacin active metabolite with different detection methods, such as traditional UV–vis [19] coupled also with a very powerful separation technique [20] such as capillary zone electrophoresis [21], fluorescence [22,23], and chemiluminescence [24,25].

Most of the drugs tend to undergo physicochemical degradation upon storage and it is essential to characterise the degradation products and/or impurities from its synthetic process in drug discovery and development process [26].

Studies of the drug stability under various different handling conditions and report the presence of impurities into their final formulation as per the guidelines of International Conference on Harmonization (ICH) and other international agencies [27–31] are required.

In particular the impurities generally present are often derived from synthetic process and are defined as “by-product” or “intermediates”.

In the International Conference on Harmonization (ICH) guidelines, was also reported that all impurities and/or degradation products that are present over a defined thresholds must be reported, identified, and quantified.

Until now, at least in our knowledge, a study on synthetic process impurities profile on tablet pharmaceutical formulation was not carried out.

In literature are present several Patent regarding different synthetic Prulifloxacin pathways [32–34] and a possible process intermediates can be identified in ethyl 6,7-difluoro-2-ethylmercapto-4-hydroxyquinoline-3-carboxylate (CAS 154330-67-3, here namely PL7), ethyl 4-acetoxy-6,7-difluoro-2-(ethylthio)quinoline-3-carboxylate (CAS 154330-68-4, here namely PL8), ethyl 6,7-difluoro-1-methyl-4-oxo-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylate (CAS 113046-72-3, here namely PL9), and ethyl 6-fluoro-1-methyl-4-oxo-7-(1-piprazinyl)-4H-[1,3]thiazeto[3,2-a]quinoline-3-carboxylate (CAS 113028-17-4, here namely PL10) (Fig. 1).

The main objective of the present study was to develop a simple, rapid and sensitive HPLC method suitable for routine quality control analysis of Prulifloxacin, its metabolite Ulifloxacin (active principle) and related synthetic process impurities in drug tablets.