Capillary Zone Electrophoretic Determination of Tosufloxacin and Trovafloxacin in Urine

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Key Words

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Summary

A simple and rapid capillary zone electrophoretic method with UV detection has been developed for determination of tosufloxacin and trovafloxacin. The separation was performed in fused-silica capillaries (57 cm length × 75 µm i.d.); the running buffer was 35 mM borate + 35 mM phosphate buffer solution, pH 8.6, containing 6% (v/v) acetonitrile. The applied potential was 15 kV, the temperature 30 °C, and detection was at 262 nm. Piromidic acid was used as the internal standard. Response was linearly dependent on concentration in the range 1.0-120.0 µg mL⁻¹ and the detection limit was 0.2 µg mL⁻¹ for both compounds. The analysis was highly reproducible (RSD between 3.41 and 1.25%). The method was applied to the determination of tosufloxacin and trovafloxacin in human and rat urine. The method was validated by using HPLC as a reference method. Recovery was between 96.8 and 102%.

Introduction

In the last decade the fluoroquinolones have emerged as one of the most important classes of antibiotic [1]. These synthetic antimicrobial agents have broad-spectrum activity against many pathogenic gram-negative and gram-positive bacteria both in vitro and in vivo [2]. They act by inhibiting the DNA gyrase, resulting in bacterial death [3]. Tosufloxacin, 7-(3-amino-l-pyrrolidinyl)-1-(2,4-difluorophenyl)-6-fluoro-1,4-dihydroxy-4-oxo-1,8-naphthyridine-3-carboxylic acid (Figure 1), is a new antibacterial fluoroquinolone agent used in the treatment of urinary and respiratory tract infections and soft tissue and enteric infections [4, 5]. Trovafloxacin, (1x,5x,6z)7-(6-amino-3-azabicyclo[3.1.0] hex-3-yl)-1-(2,4-difluorophenyl)-6-fluoro-1,4-dihydroxy-4-oxo-1,8-naphthyridine-3-carboxylic acid (Figure 1), is another new fluoroquinolone, used to treat skin complaints, respiratory-tract infections, sexually transmitted diseases, and meningitis [6]. Recommended oral doses of trovafloxacin are 200-300 mg day⁻¹ for 7-14 days once daily [6]; for tosufloxacin oral medication is approximately 150 mg three times a day for 14 days [7].

The widespread use of these compounds and the need for clinical and pharmacological study require fast and sensitive analytical techniques for determination of the presence of the drugs in biological fluids. As far as we are aware only high-performance liquid chromatographic (HPLC) methods have been reported for determination of the presence of tosufloxacin and trovafloxacin in biological fluids [8-11]. Capillary electrophoresis (CE), which is highly efficient and uses less solvent than HPLC, separates sample components according to size and charge. The resolution of CE procedures is greater than that of HPLC and the precision is of the same order. CE is less costly than HPLC and requires only modest quantities of sample. The concentration sensitivitv of UV-visible absorbance detection in CE is, however, relatively poor compared with that in HPLC. CE has become an advanced analytical methods for drug analysis in pharmaceutical, therapeutic, diagnostic, and forensic applications [12]. Reliable and automated CE instruments are commercially available and have promoted the exploration of an increasing number of CE methods for drug analysis in several matrices [13-17].

Although many CE methods have been used for analysis of antibiotics [18], little attention has been devoted to the separation of quinolone antibiotics. CE has been used to determine ciprofloxacin in pharmaceutical formulations [19] and for the enantioselective separation of ofloxacin and DU-6859 [20]. Sun and Wu [21] determined seven quinolone antibiotics by CE in pharmaceutical formulations. Although
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**Experimental**

**Apparatus and Software**

Electrophoresis was performed with a Beckman (Fullerton, CA, USA) P/ACE 5500 capillary electrophoresis system equipped with a diode-array detector, a thermostatted column cartridge, a high-voltage built-in power supply, and an autosampler. The System Gold (version 810) software package (Beckman) was used for acquisition and subsequent processing of electropherograms. Separations were conducted in uncoated fused-silica capillaries (Beckman; 57 cm × 75 μm i.d. × 375 μm o.d.) with an capillary inlet-to-detector distance of 50 cm.

A Hewlett Packard (Norwalk, CT, USA) 8453 UV-visible spectrophotometer was used to record absorbance spectra of tosufloxacin, trovafloxacin, and piromidic acid.

HPLC was performed with a Hewlett-Packard series 1050 chromatograph with UV detector set at 274 nm. The output from the detector was monitored by means of a Hewlett-Packard model 3395A integrator. Separation was achieved on a 125 mm × 4 mm i.d., 5-μm particle, LiChrospher 100RP18 column (Merck, Darmstadt, Germany). The column was protected with a 10 mm × 4 mm i.d. LiChrocart 4–4 pre-column (Merck).

Statgraphics [25] and Alamin [26] software packages were used for statistical analysis of the data and for regression analysis (linear model).

**Reagents**

All reagents were analytical grade, unless stated otherwise, and all solvents were HPLC grade (Panreac, Barcelona, Spain). Water was purified by means of a Milli-Q plus system (Millipore Bedford, MA, USA).

Tosufloxacin and trovafloxacin were kindly provided by Abbott S.A. (Spain) and Pfizer S.A. (Spain), respectively.

several reports have shown that CE is suitable for pharmaceutical analysis, few methods have been applied to biological samples [22, 23]. Pérez-Ruiz et al. [24] determined nalidixic acid and two metabolites in serum and urine samples by micellar electrokinetic capillary chromatography (MEKC).

Figure 1. The chemical structures of (A) tosufloxacin and (B) trovafloxacin.

Figure 2. Effect of pH on the migration time (○, ●) and plate number (□, ○) of tosufloxacin (○, ○) and trovafloxacin (●, ○). The buffer solution was 50 mM borate + 50 mM phosphate-acetonitrile, 95:5 (v/v), the potential 13 kV, and the temperature 25°C.

Figure 3. Effect of buffer solution (pH 8.6) concentration on the migration time (○, ●) and plate number (□, ○) of tosufloxacin (○, ○) and trovafloxacin (●, ○). The potential was 13 kV and the temperature 25°C.

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