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ORIGINAL ARTICLE

Ion-pairing and reversed phase liquid chromatography for the determination of three different quinolones: Enrofloxacin, lomefloxacin and ofloxacin

Alaa S. Amin [a,*], Hassan A. Dessouki [a], Ibrahim A. Agwa [b]

[a] *Chemistry Department, Faculty of Science, Benha University, Benha, Egypt*
[b] *Quality Control, Spimaco Addwaeih, Saudi Arabia*

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Abstract Two simple and sensitive high performance liquid chromatographic (HPLC) methods have been developed for the simultaneous determination of three different quinolones: enrofloxacin, lomefloxacin and ofloxacin in their pure and dosage forms, one with reversed phase HPLC and the other with ion-pair HPLC. In reversed phase HPLC, method (A), the mobile phase consists of 2.18% aqueous solution of KH_2PO_4 with pH adjusted to 2.4 ± 0.2 with acetonitrile (80:20; v/v), the mobile phase pumped at flow rate of 1.2 ml min^{-1}. A Neucleosil C_{18} column (10 μm, 100 Å), 250 mm length × 4.6 mm diameter was utilized as stationary phase. Detection was affected spectrophotometrically at 294 nm. While in ion-pair HPLC, method (B), the mobile phase was aqueous solution of 0.65% sodium perchlorate and 0.31% ammonium acetate adjusted to pH 2.2 ± 0.2 with orthophosphoric acid: acetonitrile (81:19; v/v), the mobile phase pumped at flow rate of 1.5 ml min^{-1}. A μ bondapack C_{18} column (10 μm, 100 Å), 250 mm length × 4.6 mm diameter was utilized as stationary phase. Detection was affected spectrophotometrically at 294 nm. Linearity ranges for enrofloxacin, lomefloxacin and ofloxacin were 4.0–108, 7.0–112 and 8.0–113 μg ml^{-1}, respectively using method A and 8.0–112, 7.0–112 and 5.0–105 μg ml^{-1}, respectively applying method B. Minimum detection limits obtained were 0.013, 0.023 and 0.035 μg ml^{-1} for enrofloxacin, lomefloxacin and ofloxacin, respectively using method A, and 0.028, 0.023 and 0.011 μg ml^{-1} using method B.

* Corresponding author.
E-mail address: asamin2005@hotmail.com (A.S. Amin).

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The proposed methods were further applied to the analysis of enrofloxacin in injection and tablets containing the ofloxacin and lomefloxacin drugs, and the results were satisfied.

1. Introduction

Enrofloxacin (Enro), lomefloxacin (Lome) and ofloxacin (Oflo) are fluorinated 4-quinolone (Fig. 1) and have a wide spectrum of antibacterial activity (Monk and Campoli-Richards, 1987). Ofloxacin is among the fluoroquinolones considered promising for the treatment of ocular infections (Borrmann et al., 1988). An ophthalmic solution of ofloxacin was introduced for the topical treatment of ocular infections caused by susceptible gram-negative and gram-positive bacteria (Gwon, 1992). Analysis of fluoroquinolones in pharmacokinetic studies has rlied mainly on a variety of microbiological method (Wise et al., 1986) which are non-selective and imprecise compared with more recent approaches using high performance liquid chromatography (HPLC) (Basci et al., 1996; Nemutlu et al., 2007; Suna et al., 2007). Selective determination of flouroquinolone derivatives from tablets by reverse-phase was investigated (Shinde et al., 1998; Marilyn et al., 2007; Espinosa-Mansilla et al., 2005). Quantitative determination of Enro, Lome and Oflo in pharmaceutical dosage, bulk drugs and process monitoring of enrofloxacin by RP-HPLC was studied (Argekar et al., 1996). A simple, rapid and sensitive HPLC method was developed for the assay of Enro in raw material and injection (Souza et al., 2002; Salehzadeh et al., 2007). The fate of Enro present in raw sewage at a swine-breeding facility was investigated by liquid–liquid extraction and reversed phase liquid chromatography with photodiode array detection (Pierini et al., 2004). Determination of a series of quinolone antibiotics using liquid chromatography–mass spectrometry was studied (Ballesteros et al., 2004; Santoro et al., 2006). A rapid and simple procedure for determination of enrofloxacin and ciprofloxacin in bovine milk and plasma is described (Idowu and Peggins, 2004). The aim of the present work is to develop a simple, rapid, sensitive and reliable HPLC assay procedures to quantify ofloxacin, lomefloxacin and enrofloxacin in their pharmaceutical dosage forms.

2. Experimental

2.1. Apparatus

Chromatographic separation and detection was performed on high performance liquid chromatography (HPLC) system which consisted of pump (WATERS Model 515), an autosampler (WATERS Model 717) and a Dual λ absorbance detector (WATERS model 2489) with 10 mm path length cell. The data were recorded on a personal computer, using the manufacturer software package (Millennium 32, Version 3.02, WATERS). A Jenway Instruments (Germany) pH meter was used for pH control; the instrument has previously been calibrated against standard buffer solutions of pH 2.0, 4.0 and 7.0.

2.2. Drugs

Enrofloxacin (99.92%), lomefloxacin hydrochloride (99.95%) and ofloxacin (99.52%) were kindly supplied by Egyptian International Pharmaceutical Industries Company (EIPICO), Egypt, Pharmaceutical dosage forms were bought from local market.

2.3. Reagents

- All reagents were obtained from VWR Chemicals (Pool, England).
- Methanol (HiPerSolv for HPLC).
- Acetonitrile (HiPerSolv).
- Potassium dihydrogen phosphate (AnalaR).
- Orthophosphoric acid (about 85%, AnalaR), were obtained from VWR Chemicals (Pool, England).
- Millipore 0.45 μm nylon membrane filter (USA).
- Sodium perchlorate (GPR) and ammonium acetate crystals (AnalaR).
- The high purity water was prepared using WATERS Ultra pure water system (WATERS, USA).

2.4. Solution preparations

2.4.1. Stock and working standards solutions

– Enro, Lome and Oflo stock solutions containing 1.0 mg ml^{-1} of each in methanol were prepared separately by weighing 100 mg each of Enro, Lome and Oflo in 100 ml volumetric flask and diluted to the mark with the same solvent (standards stock solutions).
– Working standards solutions of Enro, Lome and Oflo were prepared separately by diluting 5.0 ml from each standard stock solution, to 100 ml with mobile phase A or B for

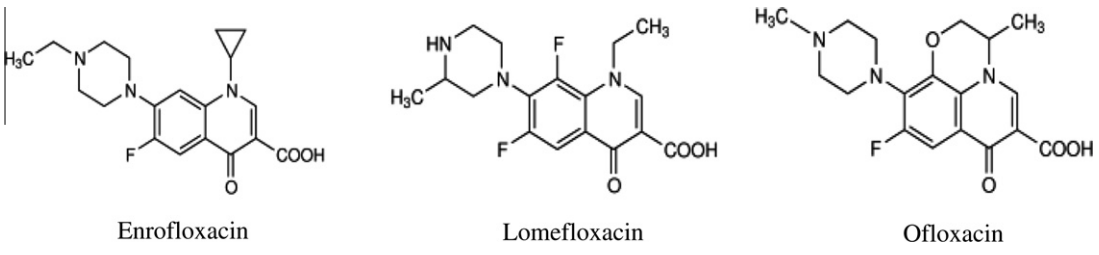

Figure 1 The chemical structure of three pure drug materials.