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Short communication

The simultaneous separation and determination of five quinolone antibotics using isocratic reversed-phase HPLC: Application to stability studies on an ofloxacin tablet formulation

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Abstract

A rapid and reliable HPLC method was developed for the simultaneously separation and quantitation of five quinolones antibiotics; nalidixic acid, norfloxacin, ofloxacin, ciprofloxacin and lomefloxacin. All five tablet formulations of individual quinolone antibiotics were routinely assayed without interference. The calibration curves were linear ($r^2 \geq 0.999$) over the concentration range of 1.20–4.8 mg/100 ml. Selectivity, precision, sensitivity and accuracy were established and the method is stability indicating with respect to ofloxacin. The limit of detection and quantitation for ofloxacin was 18 and 36 μg/100 ml, respectively. The separation was performed on a Phenomenex ODS C18 column using an isocratic, ion-pairing mobile phase consisting of 35% (v/v) aqueous acetonitrile together with tetrabutylammonium acetate, sodium dodecyl sulphate and citric acid (pH* 3.4). All analyses were conducted at ambient temperature and was monitored using a Diode Array UV/VIS detector set at wavelengths 235, 254, 275 and 300 nm.

Keywords: Antibiotics; Nalidixic acid; Ofloxacin; Norfloxacin; Ciprofloxacin; Lomefloxacin; Isocratic reversed-phase system; Stability indicating

1. Introduction

Since the discovery of nalidixic acid [1], a number of structural modifications to the quinolone nucleus have been made in order to increase the antimicrobial activity and to enhance the pharmacokinetic performance of these drugs. Major findings relating to antibiotic development occurred during the 1980s with the realization that compounds with a fluorine atom at position C-6 and a piperazine or methylpiperazine at position C-7 such as norfloxacin, ciprofloxacin, ofloxacin and lomefloxacin, exhibit a broad spectrum of activity against Gram-positive and Gram-negative bacteria [2,3]. The general structure of fluoroquinolone antibacterial agents consists of a 1-substituted-1,4-dihydro-4-oxypyrine-3-carboxylic moiety combined with an aromatic or heteroaromatic ring (Fig. 1).

A number of analytical methods have been developed for analysing fluoroquinolones in both biological fluids and pharmaceutical formulations however; some of these methods have been found to be cumbersome, possessing poor precision and specificity and are uneconomical [4]. Due to the increased demand for reliability most researchers in the field now utilize HPLC because of its specificity, sensitivity, rapidity and robustness (Fig. 1).

There have been numerous reports describing the analysis of single and various combinations of fluoroquinolones in biological fluids, foods and environmental samples using either UV or fluorescence as the method of detection [5–16]. A HPLC method describing the simultaneous separation of six fluoroquinolones used fluorescence as the method of detection and was validated in order to determine the concentration of levofloxacin in biological fluids using UV as the method of detection [17]. A more recent report describes the simultaneous separation of nine fluoroquinolones from biological and environmental samples using fluorescence as the

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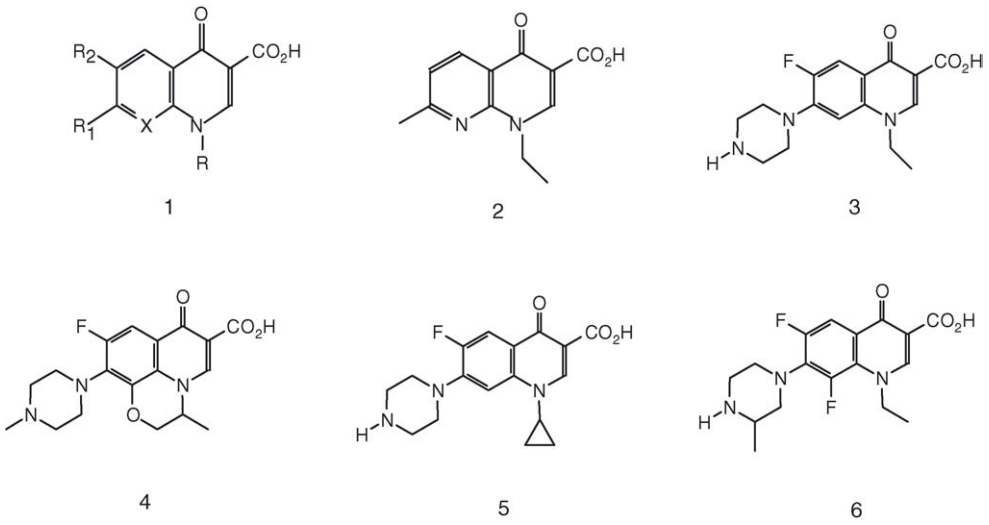

Fig. 1. The general structure of quinolone antibiotics (labelled 1). X, C; R, cyclopropyl, ethyl, fluoroethyl, methylamino, fluorophenyl group and thiazine or oxazine ring. R_1, piperazin-1-yl, 4-methylpiperazin-1-yl, 3-methylpiperazin-1-yl; R_2, fluorine. The quinolone antibiotics labelled 2–6 are nalidixic acid, norfloxacin, ofloxacin, ciprofloxacin and lomefloxacin, respectively.

method of detection, however, the separation was achieved using capillary electrophoresis [18].

Our investigation involved the modification of the method described above involving the separation of six fluoroquinolones in order to simultaneously separate and quantitate nalidixic acid, norfloxacin, ofloxacin, ciprofloxacin and lomefloxacin (Fig. 1) raw materials, and to carryout routine analysis of tablet formulations of these antibiotics. In addition, the method was further validated as a reliable stability indicating method for the analysis of ofloxacin tablets.

Although a combination of these actives would not normally be present in the same tablet formulation, it could provide a useful method for laboratories involved in the routine analysis of these antibiotics.

2. Materials and methods

2.1. HPLC instrumentation

The two HPLC systems used in the study consisted of: a Perkin-Elmer Series 410 Bio LC pump coupled to a Diode array UVD 340S Dionex detector and a Dell Optiplex GX1 computer with a Chomeleon Version 6.10 software package. The printer was a Hewlett Packard DeskJet 710C; a Varian Vista 5000 Liquid Chromatograph pump, coupled to a Waters 486 Tunable Absorbance UV detector and a Hewlett-Packard HP3395 integrator.

2.2. Chemicals

HPLC grade water and acetonitrile were purchased from Riedel-dettaen, dodecyl sulphate, sodium salt 98% (SDS), citric acid 99.5% and tetrabutylammonium acetate 97% (TBAA) were purchased from Aldrich. Nalidixic acid was purchased from Sigma. Ciprofloxacin, norfloxacin and lomefloxacin and excipients (microcrystalline cellulose, methylcellulose, magnesium stearate, hydroxypropylcellulose, lactose, maize starch, hypromellose croscarmellose, sodium starch glycollate, titanium dioxide, carnauba wax, propylene glycol, iron oxide, povidone and talc), were a generous gift from the Jordanian Pharmaceutical Manufacturing Company (Jordan). The ofloxacin was kindly provided by the United Pharmaceutical Manufacturing Company (Jordan). All the actives were of USP grade. Nalidixic acid tablets (500 mg) were manufactured by Sanofi Synthelabo (UK), norfloxacin tablets (400 mg) were manufactured by Merck Sharp and Dohme (UK), ofloxacin tablets (200 mg) were manufactured by Aventis Pharma Ltd. (UK), ciprofloxacin tablets (250 mg) were manufactured by Bioglan Generics (Ireland) and lomefloxacin tablets (400 mg) were manufactured by Pharmacia (Sweden). All the five tablets were purchased from The Royal Hospital Pharmacy, Preston (UK).

2.3. Chromatography

The chromatography was performed in the reversed-phase mode using a Phenomenex ODS C18(2), 150 mm × 4.6 mm i.d. column, linked to a Phenomenex ODS C18(2), 30 mm × 4.6 mm guard column. Both columns consisted of particle sizes equivalent to 5 μm. Manual injections were carried out using a Rheodyne model 9125 injector with a 20 μl loop. The mobile phase was 35% (v/v) aqueous acetonitrile consisting of 10 mM tetrabutylammonium acetate (TBAA), 10 mM sodium dodecyl sulphate and 25 mM citric acid. The pH* of the mobile phase was 3.4. Analysis was performed only after the column had reached equilibrium (approximately 1.5 h at a flow rate of 1 ml/min). The final flow