Determination of the Newer Quinolones Levofloxacin and Moxifloxacin in Plasma by High-Performance Liquid Chromatography with Fluorescence Detection

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Abstract

A simple, accurate, sensitive, and precise reversed-phase (RP) high-performance liquid chromatographic (HPLC) method with fluorescence detection allowing the sensitive and specific quantitation of the newer fluoroquinolones levofloxacin and moxifloxacin is described. Moxifloxacin is used as the internal standard for the determination of levofloxacin and vice versa. A single-step liquid–liquid extraction from human plasma is sufficient for both quinolones. The method is linear from 0.1 to 15 µg/mL and 0.2 to 7 µg/mL for levofloxacin and moxifloxacin, respectively, covering the clinically relevant plasma concentration range. The limits of quantitation are 0.05 µg/mL (levofloxacin) and 0.2 µg/mL (moxifloxacin). The method is successfully applied to plasma drug level monitoring in a volunteer receiving single therapeutic doses of levofloxacin or moxifloxacin at two different occasions.

Introduction

Nalidixic acid was the first quinolone antibiotic and was introduced in the 1960s. Since that time, quinolone antimicrobial agents have undergone extensive synthetic and clinical development, which resulted in newer substances displaying improved antimicrobial activity and pharmacokinetic features. Levofloxacin and moxifloxacin (Figure 1) are newer fluoroquinolones, both owning a broad spectrum of antibacterial activity (1,2). During the last few years, it has been recognized that fluoroquinolones are among those antibacterials that can be characterized by a concentration-dependent killing over a wide range of concentrations. This is in contrast to another pattern of bactericidal activity, which is largely dependent on the time of exposure (e.g., β-lactam antibiotics, vancomycin, and macrolides) (3). Very recently, it has been clearly demonstrated that clinical response and eradication of pathogenic organisms during treatment with levofloxacin is dependent on the ratio of peak plasma concentration to the organism's minimum inhibitory concentration (4). This feature makes drug level monitoring desirable for the newer quinolone antibiotics. Both levofloxacin and moxifloxacin are increasingly used in the clinical setting. The aim of the present study was the development of a simple high-performance liquid chromatography (HPLC) method that could be used for drug level monitoring in patients. This study focused on the development of a chromatographic method, which allows the determination of both drugs without any analytical modifications. It was intended to develop an assay using moxifloxacin as internal standard for levofloxacin and vice versa.

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Experimental

Apparatus

A Shimadzu (Kyoto, Japan) HPLC system consisting of the following components was used: a Model LC-6A pump and fluorescence HPLC monitor (RF-353, excitation at 295 nm, and emission at 440 nm). Chromatographic separation was carried out on a Nucleosil 100-5C_{18} Nautilus column (125- × 4-mm i.d., 5 µm) (Macherey-Nagel, Düren, Germany). The analytical column was protected by a precolumn (Nucelosil 100-5 C_{18} Nautilus CC8/4, Macherey-Nagel) Chromatographic signals were

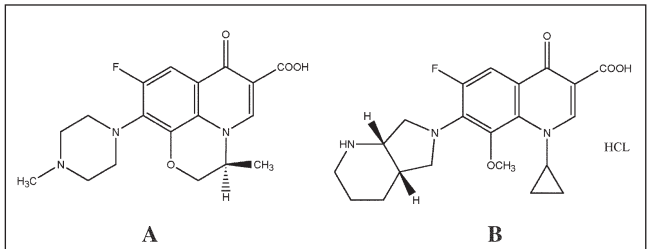

Figure 1. Structures of the newer quinolone antibiotics, levofloxacin (A) and moxifloxacin (B).

processed with an A/D converter (DataApex, Prague, Czech Republic).

Materials and reagents

Levofloxacin was from Aventis (Bad Soden a. Ts., Germany) and moxifloxacin was from Bayer (Wuppertal, Germany). Both pharmaceuticals were obtained as certified reference compounds. The reagents used were of analytical grade. Sodium dihydrogenphosphate and phosphoric acid 85% were obtained from Merck (Darmstadt, Germany). Acetonitrile (HPLC grade) was purchased from J.T. Baker (Gross-Gerau, Germany). Blank plasma was obtained from fasted, healthy volunteers that did not receive any medication.

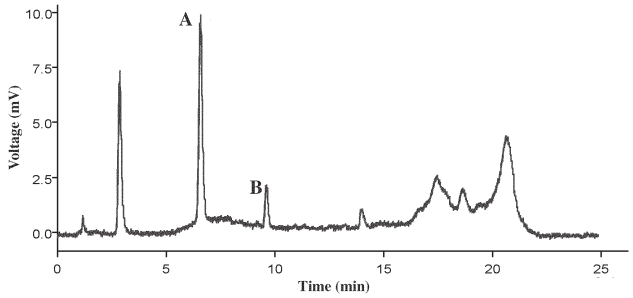

Figure 2. HPLC chromatogram of the extract from a 400-µLhuman plasma sample spiked with levofloxacin (2.0 µg/mL) (A) and moxifloxacin (1.0 µg/mL) (B).

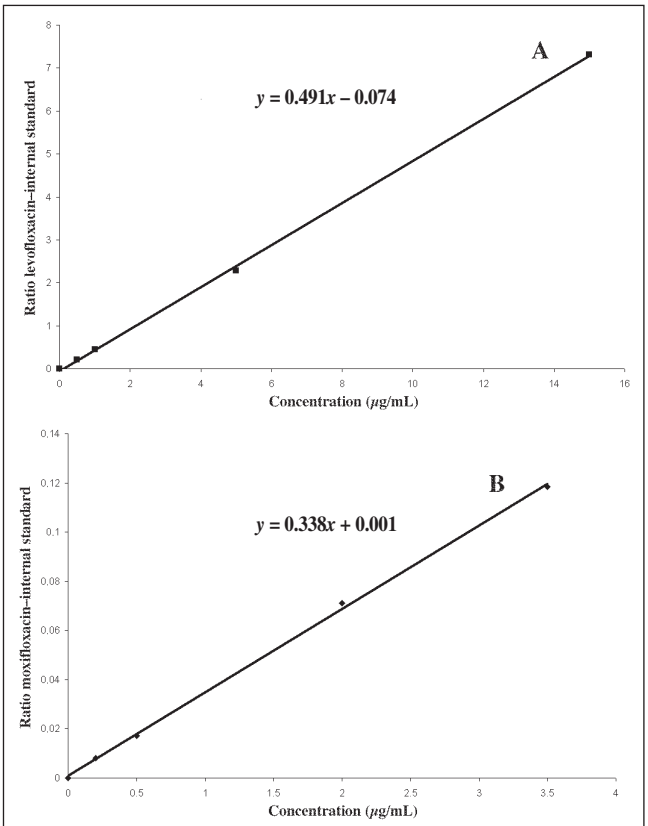

Figure 3. Calibration curve for the HPLC determination of levofloxacin in spiked human plasma samples using moxifloxacin as internal standard (r = 0.9998) (A) and calibration curve for the HPLC determination of moxifloxacin in spiked human plasma samples using levofloxacin as internal standard (r = 0.9995).

Mobile phase

The mobile phase was a mixture of acetonitrile and 0.01 mol/L sodium dihydrogenphosphate at pH 2.7 (3:97, v/v) and of acetonitrile–0.01 mol/L sodium dihydrogenphosphate at pH 2.7 (50:50, v/v). Gradient elution conditions consisted of a percentage of acetonitrile ranging from 9% to 90% in 18 min with a constant flow of 1.5 mL/min.

Sample preparations

A 400-µL aliquot of human plasma was placed in a 10-mL tube and diluted with 400 µL phosphate buffer containing 2 µg of internal standard. For the quantitation of levofloxacin, moxifloxacin was used as internal standard and vice versa. After the addition of 800 µL of acetonitrile, the suspension was vortexed for 30 s on a laboratory vortex followed by centrifugation (10 min, 3500 × g) at room temperature. The supernatant was transferred to a vial and injected via the autosampler onto the column.

Chromatographic separation

Levofloxacin and moxifloxacin were completely separated under the chromatographic conditions chosen (Figure 2).

Results and Discussion

Calibration and linearity

The relationship between the concentration of levofloxacin (or moxifloxacin) and the peak-area ratio of levofloxacin (or moxifloxacin) and the internal standard was analyzed.

A linear relationship was obtained between the concentration of levofloxacin (or moxifloxacin) and the peak height ratio of levofloxacin (or moxifloxacin) and the internal standard (Figure 3). The same was true for the peak area ratio. The calibration curves were linear in the clinically relevant range of 0–15 µg/mL (levofloxacin, Figure 3A) and 0–3.5 µg/mL (moxifloxacin, Figure 3B).

Sensitivity

The limit of detection was 0.01 and 0.1 µg/mL for levofloaxcain and moxifloxacin, respectively. At these concentrations, the signal-to-noise ratio was 5:1 and 10:1. The limits of quantitation were 0.05 µg/mL and 0.2 µg/mL for levofloxacin and moxifloxacin, respectively.

Extraction efficiency

The extraction efficiency of both quinolones was calculated by comparison of peak-area ratios obtained after extraction of spiked plasma samples and in 1.2 mL phosphate buffer–acetonitrile aliquots spiked immediately prior to injection. The extraction efficiency of levofloxacin was assessed at two different concentration levels (2.0 and 3.0 µg/mL, n = 12). The arithmetic mean value was 79% [standard deviation (SD) 1.5% and range 76–88%]. The extraction efficiency of moxifloxacin was assessed at 1.0 and 1.5 µg/mL. The arithmetic mean value was 81% (SD 1.6% and range 71–83%).

Recovery

Recovery was determined by splitting a 0.8-mL serum probe containing 10 µg/mL moxifloxacin or levofloxacin into two 0.4-mL samples (A and B). Prior to extraction, 2 µg moxifloxacin or levofloxacin was added to sample B. After extraction and HPLC analysis, the concentrations in samples A and B were compared with each other. Recovery was calculated from the measured difference between probe A and B and compared with the given difference. The recovery for levofloxacin was 106% (SD 1.4%, range 104% to 107%, and n = 8). The corresponding value for moxifloxacin was 97% (SD 1.7%, range 95% to 100%, and n = 6).

Table I. Inter- and Intraday Precision and Accuracy of the Determination of Levofloxacin in Human Plasma

	Nominal concentration of levofloxacin (µg/mL)			
	0.50	**1.00**	**5.00**	**15.00**
Concentration found (µg/mL, arithmetic mean value)				
Day 1 (n = 6)	0.47	1.03	5.02	15.42
Day 2 (n = 6)	0.57	1.05	4.81	15.06
Day 3 (n = 6)	0.53	1.04	4.91	15.07
Interday (n = 18)	0.53	1.04	4.91	15.18
Accuracy (%, arithmetic mean value)				
Day 1 (n = 6)	94	103	100	103
Day 2 (n = 6)	114	105	96	100
Day 3 (n = 6)	106	104	98	101
Interday (n = 18)	105	104	98	101
Precision (%, arithmetic mean value)				
Day 1 (n = 6)	2.1	3.9	2.4	2.5
Day 2 (n = 6)	3.5	3.8	4.2	0.9
Day 3 (n = 6)	3.8	1.0	1.8	0.9
Interday (n = 18)	9.4	3.3	3.1	1.9

Table II. Inter- and Intraday Precision and Accuracy of the Determination of Moxifloxacin in Human Plasma

	Nominal concentration of moxifloxacin (µg/mL)			
	0.20	**0.50**	**2.00**	**3.50**
Concentration found (µg/mL, arithmetic mean value)				
Day 1 (n = 6)	0.19	0.49	2.03	3.47
Day 2 (n = 6)	0.22	0.50	1.95	3.53
Day 3 (n = 6)	0.19	0.47	2.07	3.46
Interday (n = 18)	0.20	0.49	2.02	3.48
Accuracy (%, arithmetic mean value)				
Day 1 (n = 6)	95	98	102	99
Day 2 (n = 6)	110	100	98	101
Day 3 (n = 6)	95	94	104	99
Interday (n = 18)	100	97	101	100
Precision (%, arithmetic mean value)				
Day 1 (n = 6)	5.3	4.1	2.5	1.4
Day 2 (n = 6)	4.5	2.0	1.5	1.1
Day 3 (n = 6)	5.3	2.1	2.4	1.2
Interday (n = 18)	1.0	4.1	3.5	1.4

Precision (reproducibility) and accuracy

Assay precision and accuracy were assessed by analyzing spiked plasma samples on the same day (within-day, n = 6) and on three different days (between-day, n = 6).

Six spiked plasma samples at concentrations of 0.5, 1, 5, and 15 µg/mL (levofloxacin) or 0.2, 0.5, 2, and 3.5 µg/mL (moxifloxacin) were analyzed within 1 day (intraday variability) or on three consecutive days (interday variability). The results are shown in Tables I and II.

Analysis of biological samples

A practical application of the described HPLC method was tested by determination of the plasma concentration time curves of levofloxacin and moxifloxacin after application of a single therapeutic dose to a healthy volunteer at two different occasions (500 mg levofloxacin and 400 mg moxifloxacin, respectively). The results are shown in Figure 4.

HPLC determination of levofloxacin has been described by other authors (5,6). The method described by Wright et al. focused on other quinolones, but levofloxacin determination was not validated in the publication (5). Wong et al. described a stereospecific HPLC determination (6). Our sample preparation was somewhat easier as compared with the method of Wong, which requires evaporation and reconstitution. With respect to precision, accuracy, and recovery, both methods were comparable. The retention time of levofloxacin in the HPLC assay presented here was similar (7.1 vs. 8.0 min). Wong et al. report that ciprofloxacin (internal standard) was well resolved from levofloxacin metabolites (desmethyl- and *N*-oxide-levofloxacin). Under the conditions used in our assay, interference of the desmethyl metabolite of levofloxacin was observed (retention time: 7.7 min) with ciprofloxacin (retention time: 7.7 min). Moxifloxacin (retention time: 9.7 min), however, was separated by more than 2 min from the desmethyl metabolite, making this quinolone an excellent internal standard. In accordance with Wong et al., levofloxacin was found to be stable in plasma stored frozen at –20°C for at least 6 months and at ambient temperature in the autosampler for at least 48 h after extraction.

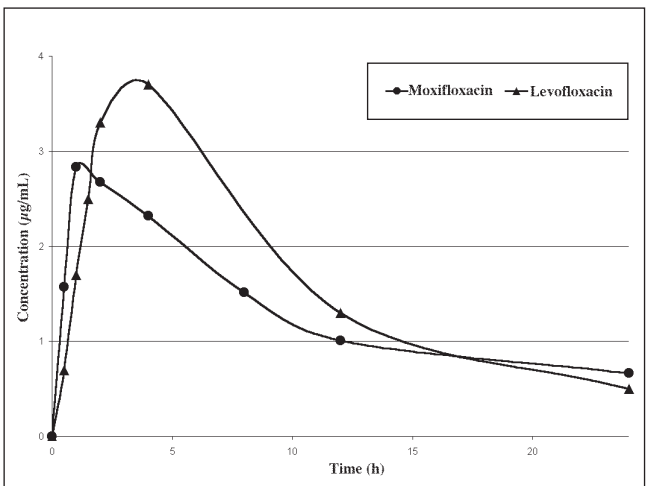

Figure 4. Plasma concentration-time profiles from a healthy volunteer receiving (at two different occasions) a 400-mg oral dose of moxifloxacin or 500-mg oral dose of levofloxacin.

Very sensitive HPLC assays with fluorescence detection for the determination of moxifloxacin have been described by Stass and Dalhoff (7), Lemoine et al. (8), Fuhrmann et al. (9), and Nguyen et al. (10). These methods had a lower limit of detection compared with the limit of detection presented in the present paper (0.1 µg/mL). The very low limits of detection in the literature were all measured with a signal-to-noise ratio of 3:1. However, the signal-to-noise ratio of the present study was 10:1. This method proved to be adequately sensitive to determine the plasma level time course of moxifloxacin in a healthy volunteer after application of a single therapeutic dose. With respect to sample preparation and assay validation, all methods were comparable.

Conclusion

The HPLC assay described here allows the determination of the newer quinolones, levofloxacin and moxifloxacin. It is simple enough to be used in a routine clinical setting. Currently, levofloxacin plasma levels are determined in critically ill patients undergoing renal replacement therapy in our institution. This method can be used without any modification for plasma level monitoring in patients receiving either moxifloxacin or levofloxacin.

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