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Short communication

Development and validation of an HPLC method for the determination of gatifloxacin stability in human plasma

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Abstract

A simple reversed-phase high performance liquid chromatography (HPLC) method for the determination of gatifloxacin stability in human plasma was developed and validated. Using ciprofloxacin as an internal standard (IS), separation was achieved on X Terra MS C_{18} (3 mm × 50 mm, 5 μm) column. The mobile phase, 0.025 M disodium hydrogen phosphate (pH 3.0) and acetonitrile (80:20 v/v), were delivered at a flow rate of 1.0 ml/min. The eluent was monitored using spectrophotometeric detection at 293 nm. Plasma samples were deproteinized using Amicon Centrifree system. No interference in blank plasma or of commonly used drugs was observed. The relationship between gatifloxacin concentration and peak height ratio of gatifloxacin to the IS was linear over the range of 0.10–6.0 μg/ml. The intra-day and inter-day coefficients of variation were ≤2.77 and ≤4.59%, respectively. The extraction recovery of gatifloxacin and the IS from plasma samples was ≥85%. Gatifloxacin was found to be stable for at least 5 h at RT, 7 weeks at −20 °C, and after 3 freeze-thaw cycles in plasma; 16 h at RT and 48 h at −20 °C in deproteinized plasma; and 24 h at RT and 7 weeks at −20 °C in phosphate buffer.

Keywords: Gatifloxacin; Stability; HPLC; Ciprofloxacin

1. Introduction

Gatifloxacin (1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7-[3-methyl-1-piperazinyl]-4-oxo-3-quinolinecarboxylic acid), a synthetic broad-spectrum antimicrobial fluoroquinolone that is active against both gram-negative and gram-positive bacteria, is used in the treatment of a wide range of infections [1]. Its absolute bioavailability is 96%, with mean peak plasma concentration of 3.1–3.6 μg/ml usually occurring 1–2 h after the ingestion of a 400 mg therapeutic dosage [2,3].A thorough literature search revealed a number of analytical methods for the determination of fluoroquionolones in biological fluids [4–8]. Two reviews of the current status of analytical techniques are also available [9,10]. Nevertheless, only two published papers, one employing electrospray tandem mass spectroscopy [11] and one using fluorescence detection [12] reported complete validation procedures. Published high performance liquid chromatography (HPLC) methods that described the analysis of gatifloxacin levels in biological matrices used ion-pair reagents in mobile phase [13] or were not sufficiently sensitive [14] for therapeutic drug monitoring. The use of ion-pair reagents such as tetrabutylammonium acetate and citric acid is generally avoided not only because of the added complexity of the mobile phase but also because of baseline drift, irregular peak shapes and width, marked sensitivity of separation to temperature, and slow equilibrium of the column [15]. More recently, a method based on the measurement of fluorescence intensity of gatifloxacin at different wavelengths has been reported [16], however, it could not be used for therapeutic drug monitoring because of lack of specificity. There are limited data on the stability of gatifloxacin in human plasma [17,18].We report the validation of a simple and reliable analytical method for the quantitative determination of therapeutic levels of gatifloxacin in human plasma. The method was used to determine gatifloxacin stability under various conditions encountered in the clinical laboratory.

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2. Experimental

2.1. Apparatus

Chromatography was performed on Waters Alliance 2690 Separation Module that included Waters 996 photodiode array detector and an auto injector. A reversed-phase X Terra MS C_{18} (3 mm × 50 mm, 5 μm) column in conjunction with a guard Pak pre-column module and Nova-Pak C_{18} 4 μm insert were used for separation. The data were collected using Pentium III computer with Millennium^{32} Chromatography Manager Software (Waters, Milford, MA, USA). Human plasma samples were deproteinized using an Amicon Centrifree micropartition system (Millipore, Bedford, MA, USA).

2.2. Chemicals and reagents

All reagents were of analytical grade unless stated otherwise. Acetonitrile HPLC grade was supplied by Fisher Scientific, Fair Lawn, NJ, USA. Water for the HPLC analysis was generated by "reverse-osmosis" using Milli-Q-Water (Millipore Co., Bedford, MA, USA). Gatifloxacin sesquihydrate (MRR: 203204, potency: 93.1%, expiry: April 2006) was obtained from Jamjoom Pharma, Jeddah, Saudi Arabia; and ciprofloxacin was purchased from Bayer Chemical, Leverkusen, Germany.

2.3. Chromatographic conditions

The mobile phase consisted of 0.025 M disodium hydrogen phosphate buffer (pH 3.0, adjusted with phosphoric acid) and acetonitrile (80:20 v/v). Before delivering into the system, the mobile phase was filtered through 0.45 μm polytetrafuloroethylene (PTFE) filter and sonicated for 5 min. The analysis was carried out under isocratic conditions using a flow rate of 1.0 ml/min at room temperature (26 °C). Chromatograms were recorded at 293 nm using a photodiode array detector.

2.4. Stock solutions

Stock solution of gatifloxacin was prepared by dissolving 10.741 mg sesquihydrate gatifloxacin (equivalent to 10 mg anhydrous gatifloxacin) in 10 ml of 0.025 M disodium hydrogen phosphate buffer (pH 3.0) to enhance solubility. The solution was shaken completely by hand and sonicated for 10 min in a Model 425E Bransonic ultrasonic cleaner (Branson, Danbury, CT, USA). Stock solution of the internal standard, ciprofloxacin, was similarly prepared. The concentration of both stock solutions was equivalent to 1000 μg/ml.

2.5. Working solutions

One thousand microliters of gatifloxacin stock solution was added to 10 ml of blank plasma to produce a working solution of 100 μg/ml. Five hundred microliters of stock solution of ciprofloxacin was added to 100 ml of 0.025 M disodium hydrogen phosphate buffer (pH 3.0) to produce a working solution of 5 μg/ml. Only freshly prepared working solutions were used.

2.6. Calibration standards/quality control samples

Calibration standards were prepared by mixing nine volumes of gatifloxacin working solution in the range 10–600 μl with up to 10 ml blank human plasma for final concentrations of 0.10–6.0 μg/ml. Four quality control samples were prepared by mixing 10–550 μl gatifloxacin working solution with up to 10 ml blank human plasma. The yielded concentrations were 0.10, 0.35, 3.50, and 5.50 μg/ml. The solutions were vortexed for 1 min then 0.5 ml aliquots were transferred into 1.5 ml eppendrof microcentrifuge tubes and stored at −20 °C until used.

2.7. Sample preparation

Five hundred microliters of plasma samples of volunteers, calibration standards, or quality control samples were placed in a 1.5 ml microcentrifuge tubes. Two hundred microliters of IS working solution (containing 1.0 μg of IS) were added to each tube. The solutions were vortexed for 30 s, sonicated for 1 min, transferred to an Amicon Centrifree micropartition system (Millipore Corporation, Bedford, MA), and centrifuged at 3000 rpm for 30 min. One hundred microliters of the ultrafiltrate was injected into the chromatographic system using an autosampler.

2.8. Stability studies

QC samples: Five aliquots of each of the two QC samples: 0.35 and 5.50 μg/ml were deproteinized and immediately analyzed at zero time (baseline). Five aliquots of each QC sample were allowed to stand on the bench-top for 5 h at room temperature (counter stability, 5 h at room temperature) or were stored at −20 °C for 7 weeks before being deproteinized and analyzed (long term freezer storage stability). Fifteen aliquots of each QC sample were stored at −20 °C for 24 h, they were then left to completely thaw at room temperature. Five aliquots of each QC sample were deproteinized and analyzed, and the rest returned to −20 °C for another 24 h. The cycle was repeated three times (freeze-thaw stability). Finally, five aliquots of each QC sample were deproteinized and the ultrafiltrate stored at room temperature for 16 h or at −20 °C for 48 h before analysis (autosampler stability).

Stock solutions: Five aliquots of gatifloxacin stock solution were diluted to 10 μg/ml in 0.025 M disodium hydrogen phosphate buffer (pH 3.0) and analyzed at baseline, after storage for 24 h at room temperature, or after storage at −20 °C for 7 weeks. Similarly, five aliquots of IS stock solution were diluted to 10 μg/ml in deionized water and analyzed at baseline, after storage for 24 h at room temperature, or after storage for seven weeks at −20 °C.

3. Results and discussion

Fig. 1 depicts the chemical structures of the compounds used in the present study. In order to improve specificity and minimize interferences from plasma or solvent system that may occur at lower wavelengths, we performed the analysis at 293 nm.

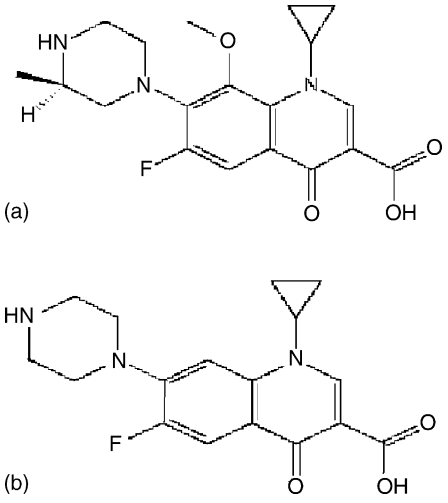

Fig. 1. Chemical structure of (a) gatifloxacin and (b) ciprofloxacin (internal standard).

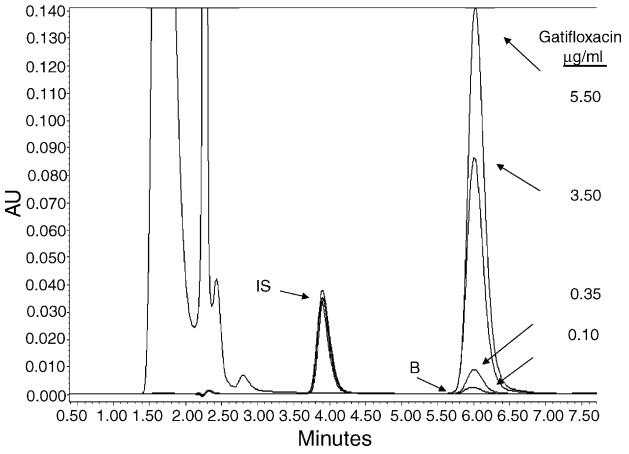

Fig. 2. Overlay of chromatograms of extracts of 0.5 ml human plasma spiked with the internal standard and one of five concentrations of gatifloxacin: 0.00; 0.10; 0.35; 3.50; 5.50 μg/ml.

Under the described conditions, gatifloxacin and the IS were well resolved with a resolution factor greater than 3.2 with a run time of 7.50 min. To evaluate the assay specificity, we screened eight frequently used medications for potential interference, namely, acetaminophen, aspirin, ibuprofen, ranitidine, nicotinic acid, ascorbic acid, caffeine, and omeprazole. None was found to co-elute with gatifloxacin or the IS. Only ranitidine, acetaminophen, caffeine, and aspirin eluted during the run time of the assay; their relative retention times with respect to the IS were 0.52, 0.71, 0.78, and 1.76, respectively. The plasma calibration curves were constructed using peak heights ratios of gatifloxacin to the IS and gatifloxacin concentrations. Linear regression analysis was used to calculate the slope, intercept, and correlation coefficient (r^2). The linearity over the range of 0.10–6.0 μg/ml was found to be quite satisfactory and reproducible over time. The r^2 ranged from 0.9994 to 1.0000 ($n = 10$). The limit of quantification was determined as 0.10 μg/ml with 0.5 ml of plasma. Table 1 summarizes the intra- and inter-day precision and accuracy of the assay determined at gatifloxacin concentration of 0.10, 0.35, 3.50, and 5.50 μg/ml over three different days. The intra-day precision ($n = 10$) was ≤2.77%. The inter-day precision ($n = 20$) was ≤4.59%. The intra-day and inter-day accuracy were in the range of 99–107%. The extraction recovery for gatifloxacin ranged from 85 to 90% for the concentrations 0.10–5.50 μg/ml. The extraction recovery of the internal standard at a concentration of 1.0 μg/ml was 85%. In regard to assay robustness and ruggedness, no significant effect was observed using two different HPLC instruments. Further, the method was found to be reproducible from one analyst to another. The chromatographic resolution and peak responses were stable after the injection of at least 1000 deproteinized plasma samples.

Fig. 2 shows an overlay of chromatograms of four gatifloxacin standards: 0.10, 0.35, 3.50, and 5.50 μg/ml. Fig. 3 shows an overlay of chromatograms of plasma samples collected over 36 h from a healthy volunteer after the oral administration of a single gatifloxacin 400 mg tablet. The maximum concentration was around 3.77 μg/ml. Reported gatifloxacin levels after the oral administration of a therapeutic dosage of 400 mg are 0.4 to 4.6 μg/ml [19], indicating that the described method is potentially suitable for therapeutic drug monitoring.

The stability of gatifloxacin in human plasma was determined using two concentrations: 0.35 and 5.50 μg/ml. Table 2 shows the stability of gatifloxacin in undeproteinized or deproteinized

Table 1
Precision and accuracy of gatifloxacin assay

Nominal concentration (μg/ml)	Concentration found (μg/ml)	Precision[a] (CV, %)	Accuracy[b] (%)
Intra-day ($n = 10$)			
0.10	0.103 ± 0.002	1.49	103
0.35	0.354 ± 0.009	2.44	101
3.50	3.589 ± 0.099	2.77	103
5.50	5.721 ± 0.091	1.58	104
Inter-day ($n = 20$)			
0.10	0.107 ± 0.005	4.59	107
0.35	0.347 ± 0.011	3.23	99
3.50	3.548 ± 0.106	2.99	101
5.50	5.666 ± 0.130	2.29	103

[a] Precision as coefficient of variation (CV, %) = standard deviation divided by mean measured concentration × 100.
[b] Accuracy = mean measured concentration/nominal concentration × 100.

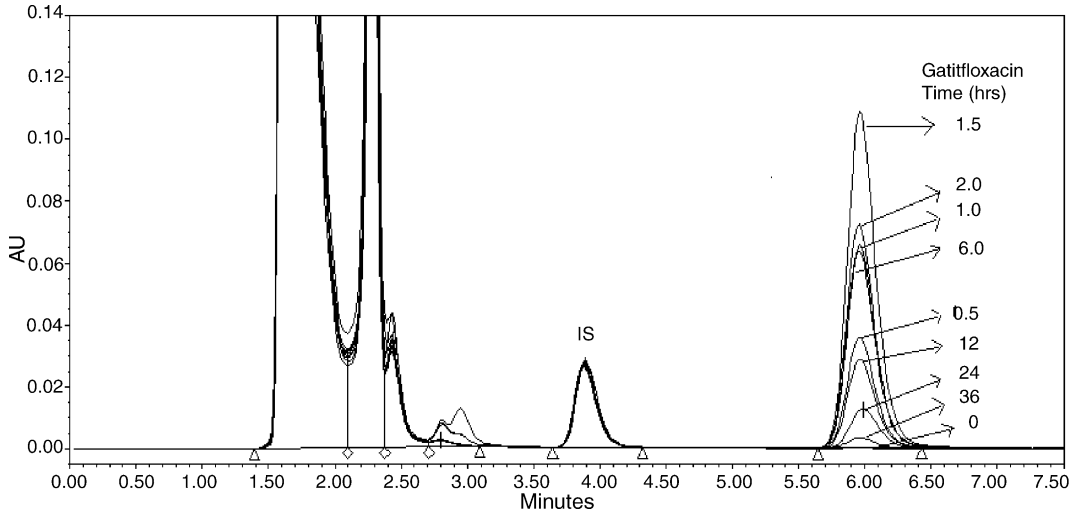

Fig. 3. Overlay of chromatograms of extracts of 0.5 ml human plasma samples obtained from a healthy volunteer before and 0.5, 1, 1.5, 2, 6, 12, 24, and 36 h, after the administration of a single 400 mg gatifloxacin tablet.

Table 2
Stability of gatifloxacin under various conditions

Nominal concentration (μg/ml)	Stability (%)								
	Plasma samples[a]							Stock solution[b]	
	Undeproteinized		Deproteinized		Freeze-thaw			24 h at RT	7 weeks at −20 °C
	5 h at RT	7 weeks at −20 °C	16 h at RT	48 h at −20 °C	Cycle				
					1	2	3		
0.35	99	99	100	104	95	98	103	108	108
5.50	99	102	100	104	97	99	96	108	108

Stability (%) = mean measured concentration ($n = 5$) at the indicated time divided by mean measured concentration ($n = 5$) at baseline × 100.

[a] Spiked plasma samples were filtered through Amicon Centrifree micropartition system and analyzed immediately (baseline, data not shown), after 5 h at room temperature (5 h RT), after freezing at −20 °C for 7 weeks (7 weeks −20 °C), or after 1–3 cycles of freezing at −20 °C and thawing at room temperature (freeze-thaw); or filtered (deproteinized) and analyzed after leaving the filtrate 16 h at room temperature (16 h RT) or 48 h at −20 °C (48 h −20 °C).

[b] Gatifloxacin stock solution, 1 mg/ml in phosphate buffer.

human plasma kept under various conditions. Gatifloxacin stability in phosphate buffer (1 mg/ml) after 24 h at room temperature or 7 weeks at −20 °C is also shown in Table 2. The data indicate that gatifloxacin is stable under various clinical laboratory conditions.

4. Conclusions

In summary, the described method is rapid, sensitive, specific, accurate, and reproducible. It was successfully used to study gatifloxacin stability under various clinical laboratory conditions. It is of potential value for the determination of therapeutic gatifloxacin levels.

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