BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **22**: 1288–1295 (2008)
Published online 25 July 2008 in Wiley InterScience
(www.interscience.wiley.com) DOI: 10.1002/bmc.1060

ORIGINAL RESEARCH

Development and validation of a HPLC method for simultaneous quantitation of gatifloxacin, sparfloxacin and moxifloxacin using levofloxacin as internal standard in human plasma: application to a clinical pharmacokinetic study

Nimmagadda Srinivas,[1,2] Lakshmi Narasu,[2] B. Prabha Shankar[3] and Ramesh Mullangi[4]*

[1]Centre for Biotechnology, JNT University, Kukatpally, Hyderabad-500 072, India
[2]Department of Pharmaceutics, S.N. Vanita Pharmacy Maha Vidyalaya, Exhibition Grounds, Hyderabad-500 001, India
[3]Eurodrug Laboratories India, Narayanguda, Hyderabad-500 020, India
[4]Biophramaceutics, Integrated Product Development, Dr Reddy's Laboratories Ltd, Bachupalli, Hyderabad-500 072, India

Received 5 February 2008; revised 11 March 2008; accepted 11 March 2008

ABSTRACT: A highly selective, sensitive and accurate HPLC method has been developed and validated for the estimation of three fluoroquinolones (FQs) viz., gatifloxacin (GFC), sparfloxacin (SFC) and moxifloxacin (MFC) with 500 μL human plasma using levofloxacin (LFC) as an internal standard (IS). The sample preparation involved simple liquid–liquid extraction of GFC, SFC, MFC and IS from human plasma with ethyl acetate. The resolution of peaks was achieved with phosphate buffer (pH 2.5)–acetonitrile (80:20, v/v) at a flow rate of 1 mL/min on a Kromasil C_{18} column. The total chromatographic run time was 18.0 min and the simultaneous elution of GFC, SFC, MFC and IS occurred at approximately 10.8, 12.8, 17.0 and 6.0 min, respectively. The method proved to be accurate and precise at linearity range of 100–10,000 ng/mL with a correlation coefficient (r) of ≥0.999. The limit of quantitation for each of the FQs studied was 100 ng/mL. The intra- and inter-day precision and accuracy values found to be within the assay variability limits as per the FDA guidelines. The developed assay method was applied to a pharmacokinetic study in human volunteers following oral administration of 400 mg GFC tablet.

KEYWORDS: gatifloxacin; sparfloxacin; moxifloxacin; levofloxacin; fluoroquinolones; human plasma; validation; HPLC; pharmacokinetics

INTRODUCTION

Fluoroquinolones (FQs) are extensively used alternatives to β-lactam agents in the treatment of many bacterial infections. FQs act differently compared with β-lactam by having selective antagonism between host DNA and bacterial DNA without interfering with eukaryotic topoisomerases. New generation FQs, viz. gatifloxacin, levofloxacin, sparfloxacin, moxifloxacin, gemifloxacin, trovafloxacin and rufloxacin, have broad spectrum activity against Gram-positive bacteria and anaerobes along with improved pharmacokinetic parameters in comparison to previous derivatives (Andriole, 1998; Bergan, 1998; Aminimanizani *et al.*, 2001).

Only a few HPLC methods have been reported in the literature for simultaneous quantification of three or more FQs, viz. gatifloxacin (GFC), levofloxacin (LFC), moxifloxacin (MFC), ciprofloxacin, grepafloxacin and trovafloxacin (Lubasch *et al.*, 2000); GFC, sparfloxacin (SFC), LFC, ciprofloxacin, trovafloxacin and cinoxacin (Liang *et al.*, 2002); and LFC, GFC, MFC (Nguyen *et al.*, 2004). Lubasch *et al.* (2000) quantified only GFC, MFC and grepafloxacin simultaneously by HPLC using fluorescence detection and the rest of the FQs viz. LFC, ciprofloxacin and trovafloxacin were estimated individually by earlier reported HPLC methods. The method described by Lubasch *et al.* (2000) contains no details on sample processing and validation. The LOQ was reported to be between 0.02 and 0.6 mg/L. Subsequently, Liang *et al.* (2002) published an HPLC method with UV and fluorescence detection for concurrent estimation of GFC, SFC, LFC, ciprofloxacin, trovafloxacin and cinoxacin and showed its application to LFC pharmacokinetic study. The main disadvantages of this method are complex mobile phase with ion-pair agent and involvement of a displacing agent in sample processing followed by ultrafiltration rather than simple liquid–liquid extraction. Of late, Nguyen *et al.* (2004) have described LC with column-switching technique for

*Correspondence to: R. Mullangi, Biophramaceutics, Integrated Product Development, Dr Reddy's Laboratories Ltd, Bachupalli, Hyderabad-500 072, India.
E-mail: mullangiramesh@drreddys.com

Abbreviations used: EDTA, ethylene-diaminetetraaceticacid; FQs, fluoroquinolones; GFC, gatifloxacin; LFC, levofloxacin; MFC, moxifloxacin; SFC, sparfloxacin.

simultaneous determination of LFC, GFC and MFC in serum. Column switching is a multi-step process and not feasible in all analytical laboratories. Keeping in mind the drawbacks and disadvantages of the earlier reported HPLC methods for simultaneous estimation of more than three FQs, in this manuscript we are presenting a simple HPLC method with UV detection for simultaneous estimation of GFC, SFC and MFC using LFC as IS with complete validation parameters and application of the newly developed method to derive pharmacokinetic parameters for GFC in humans.

EXPERIMENTAL

Chemicals and reagents

GFC, SFC and MFC (Fig. 1) were procured from Torrent Laboratories (P) Limited, Ahmedabad while LFC (IS) (Fig. 1) was obtained from Aristo Pharmaceuticals (P) Limited, Mumbai. Purity was found to be more than 99% for all the compounds. HPLC-grade acetonitrile and analytical-grade sodium dihydrogen *ortho*-phosphate and ethylenediaminetetraaceticacid (EDTA) dipotassium salt were purchased from Qualigens, Mumbai, India. All aqueous solutions including the buffer for the mobile phase were prepared with Milli Q (Millipore, Milford, MA, USA) grade water. The control human plasma was purchased from Cauvery Diagnostics and Blood Bank, Secunderabad, India.

Instrumentation and chromatographic conditions

The HPLC system was a Shimadzu (Shimadzu Corporation, Japan) SCL-10AVP series LC system equipped with degasser (DGU-14A), quat pump (LC-10ADVP) and thermostat column oven (CTO-10ASVP) along with an auto-sampler (SIL-10ADVP) and UV detector (SPD-10AVP) used to inject 50 µL aliquots of the processed samples onto a Kromasil 100, C_{18} column (4.6 × 250 mm, 5.0 µm, GL Sciences, Tokyo, Japan), maintained at 35 ± 2°C. The isocratic mobile phase system consisting of sodium dihydrogen *ortho*-phosphate–acetonitrile (80:20, v/v) was delivered at a flow-rate of 1.00 mL/min through the column to elute the analytes. The eluate was monitored using the UV detector set at 293 nm. The data were acquired and processed with Class VP software (Version 6.14).

Preparation of stock and standard solutions

Primary stock solutions of GFC, SFC and MFC for preparation of calibration standard (CC) and quality control (QC) samples were prepared from separate weighing. The primary stock solutions (1.00 mg/mL) of these analytes and IS were prepared in diluent, i.e. 60% acetonitrile in water, and stored at –20 ± 2°C for 30 days (data not shown). Appropriate dilutions were made in diluent for GFC, SFC and MFC to produce three-in-one working stock solution of 2.00, 4.00, 10.0, 20.0, 60.0, 120, 160 and 200 µg/mL and on the day of analysis this set of stocks was used to prepare standards for the calibration curve (CC). Another set of working stock solutions of GFC, SFC and MFC were made in diluent (from second primary stock) at 2.00, 6.00, 100 and 150 µg/mL for the preparation of QC samples. Working stock solutions were stored at approximately 5°C and found to be stable for 15 days (data not shown). Individually QC and CC working stock solutions of FQs were made before spiking into QC and CC plasma samples accordingly. A working stock solution of IS (100 µg/mL) was prepared in diluent from primary stock solution of 1.00 mg/mL. Calibration standards were prepared by spiking in 490 µL of control human plasma with the appropriate amount of composite stock of GFC, SFC and MFC

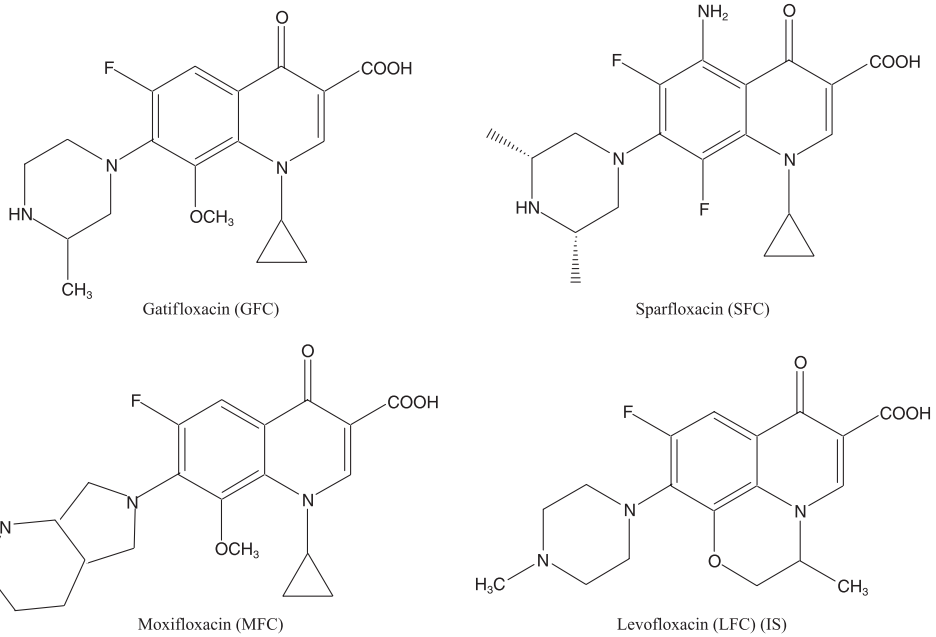

Figure 1. Structural representation of GFC, SFC, MFC and LFC (IS).

DOI: 10.1002/bmc

(10 µL) and IS (50 µL) on the day of analysis. Samples for the determination of recovery, precision and accuracy were prepared by spiking control human plasma in bulk at appropriate concentrations [100 ng/mL (lower limit of quantitation, LLOQ), 300 ng/mL (quality control, QC, low), 5000 ng/mL (QC medium) and 7500 ng/mL (QC high)] and 500 µL volumes were aliquoted into different tubes and were stored at –80 ± 10°C until analysis.

Recovery

The recovery of all three analytes, viz. GFC, SFC and MFC along with IS, through liquid–liquid extraction procedure, was determined by comparing the responses of the analytes extracted from replicate QC samples (n = 6) with the response of analytes from aqueous standard samples at equivalent concentrations. Recoveries of GFC, SFC and MFC were determined at QC low, medium and high concentrations, viz. 300, 5000 and 7500 ng/mL, whereas the recovery of the IS was determined at a single concentration of 10 µg/mL.

Sample preparation

A simple liquid–liquid extraction method was followed for extraction of GFC, SFC and MFC from human plasma. To 500 µL of plasma aliquot, IS solution (50 µL of working stock) equivalent to 5000 ng was added and mixed for 30 s on a cyclomixer (Remi Instruments, Mumbai, India), followed by extraction with 3.00 mL of acetonitrile. The mixture was vortexed for 5 min, followed by centrifugation for 5 min at 4500 rpm on Biofuge at 4°C (Heraus, Germany). The organic layer (2.5 mL) was separated and evaporated to dryness at 40°C using a gentle stream of nitrogen (Turbovap® LV evaporator, Zymark® Kopkinton, MA, USA). The residue was reconstituted in 500 µL of the mobile phase and 50 µL was injected onto analytical column.

Validation procedures

A full validation according to the FDA guidelines (US DHHS, FDA, CDER, 2001) was performed for the assay in human plasma.

Specificity and selectivity. The specificity of the method was evaluated by analyzing human plasma samples from at least six different sources to investigate the potential interferences at the LC peak region for analyte and IS.

Calibration curve. The calibration curve was acquired by plotting the ratio of sum of peak area of each FQ to that of IS against the nominal concentration of calibration standards. The final concentrations of calibration standards obtained for plotting the calibration curve were 100, 200, 500, 1000, 3000, 6000, 8000 and 10,000 ng/mL. The results were fitted to linear regression analysis using $1/X^2$ as weighting factor. The calibration curve had to have a correlation coefficient (r) of 0.999 or better. The acceptance criteria for each back-calculated standard concentration were ±15% deviation from the nominal value except at LLOQ, which was set at ±20% (US DHHS *et al.*, 2001).

Precision and accuracy. The intra-day assay precision and accuracy were estimated by analyzing six replicates at four different QC levels, i.e. 100, 300, 5000 and 7500 ng/mL. The inter-assay precision was determined by analyzing the four levels QC samples on three different runs. The criteria for acceptability of the data included accuracy within ±15% standard deviation (SD) from the nominal values and a precision of within ±15% relative standard deviation (RSD), except for LLOQ, where it should not exceed ±20% of accuracy as well as precision (US DHHS *et al.*, 2001).

Stability experiments. The stability of GFC, SFC and MFC and IS in the injection solvent was determined periodically by injecting replicate preparations of processed samples up to 36 h (auto-sampler) after the initial injection. The peak-areas of the each analyte and IS obtained at 0 h on day 1 were used as the reference to determine the relative stability of the analyte at subsequent points. In all stability studies two QC concentrations were used, viz. QC low and QC high. Stability of three analytes, viz. GFC, SFC and MFC, in the biomatrix during 12 h exposure at room temperature in human plasma (bench-top) was determined at ambient temperature (25 ± 1°C) in six replicates at each concentration. Freezer stability of each FQs in human plasma was assessed by analyzing the QC samples stored at –80 ± 10°C for at least 30 days. The stability of GFC, SFC and MFC in human plasma following repeated freeze–thaw cycles was assessed using QC samples spiked with GFC, SFC and MFC. The samples were stored at –80 ± 10°C between freeze–thaw cycles. The stability of GFC, SFC and MFC was assessed after third freeze–thaw cycle. Samples were considered to be stable if assay values were within the acceptable limits of accuracy (i.e. ±15% SD) and precision (i.e. ±15% RSD).

Pharmacokinetic study in humans

A pharmacokinetic study was performed in healthy male subjects. The ethics committee approved the protocol and the volunteers provided written informed consent. Blood samples were obtained following oral administration of 400 mg GFC tablet into polypropylene tubes containing EDTA solution as an anti-coagulant at pre-dose, 0.25, 0.50, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 6, 8, 12, 24, 36 and 48 h. Plasma was harvested by centrifuging the blood using Biofuge (Hereaus, Germany) at 1760***g*** for 5 min and stored frozen at –80 ± 10°C until analysis.

An aliquot of 500 µL of thawed plasma samples was spiked with IS and processed as mentioned in sample preparation section. Along with study samples, QC samples at low, medium and high concentration were assayed in duplicate and were distributed among unknown samples in the analytical run. The criteria for acceptance of the analytical runs encompassed the following: (i) not more than 33% of the QC samples were greater than ±15% of the nominal concentration; and (ii) not less than 50% at each QC concentration level must meet the acceptance criteria. Plasma concentration–time data for GFC was analyzed by a non-compartmental method using WinNonlin Version 5.1 (Pharsight Corporation, Mountain View, CA, USA).

DOI: 10.1002/bmc

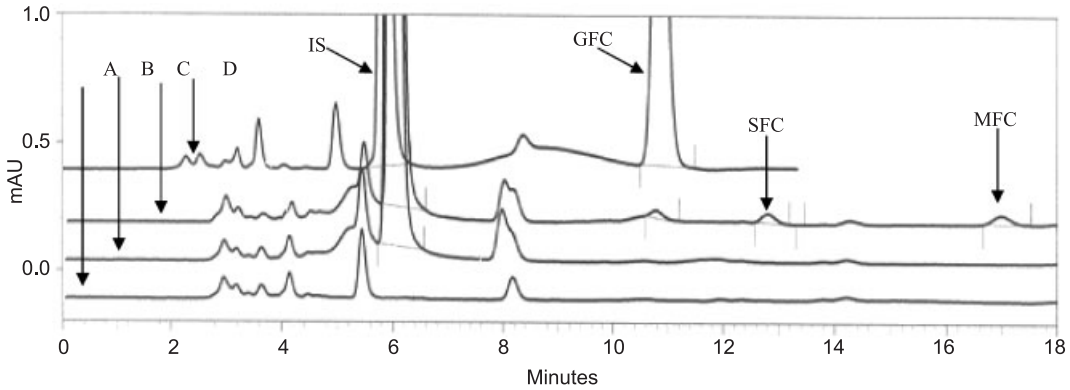

Figure 2. HPLC chromatograms of a 50 µL injection of an extract from (a) human blank plasma; (b) human blank plasma spiked with IS; (c) human plasma spiked GFC, SFC and MFC at LLOQ with IS; and (d) a 1.5 h *in vivo* plasma sample obtained following oral dose of GFC 400 mg tablet to a subject.

RESULTS

Optimization of the experimental conditions

Preliminary experiments were carried out to optimize the experimental parameters affecting the chromatographic separation of GFC, SFC and MFC along with IS in the preselected LC-column selected and their detection by UV. In order to detect GFC, SFC and MFC simultaneously with good sensitivity, 293 nm was selected as UV_{max}. The feasibility of different mixtures of solvents such as acetonitrile and methanol using different buffers such as phosphate, formic acid and ammonium acetate, along with variable pH range (3–5) and different flow-rates (in the range of 0.5–1.0 mL/min) were tested for complete chromatographic resolution of GFC, SFC and MFC along with IS. It was found that the resolution of GFC, SFC, MFC and IS was satisfactory on Kromasil 100 C_{18} column maintained at 35°C using an isocratic mixture of sodium dihydrogen *ortho*-phosphate (pH 2.5):acetonitrile (80:20, v/v) with 1 mL/min flow rate. The total run time was 18 min.

Recovery

The results of the comparison of pre-extracted standards vs post-extracted plasma standards were estimated for GFC, SFC and MFC at LQC, MQC and HQC, i.e. 300, 5000 and 7500 ng/mL, respectively. The absolute mean recovery at LQC, MQC and HQC was 56.39 ± 8.56, 57.75 ± 8.25, 57.33 ± 9.56; 74.27 ± 7.1, 79.79 ± 5.39 77.82 ± 5.26; 67.85 ± 7.08, 65.13 ± 7.11, 62.50 ± 6.53 for GFC, SFC and MFC, respectively. The absolute recovery of IS at 10 µg/mL was 63.47%.

Validation procedures

Specificity and chromatography. In the chosen completely optimized chromatographic conditions, specificity was indicated by the absence of any endogenous interference at retention times of peaks of interest as evaluated by chromatograms of blank human plasma and plasma spiked with GFC, SFC, MFC and IS. When single FQs were injected at the highest concentration in the chromatographic system, at the retention times of all FQs, no interference was observed (data not shown). GFC, SFC, MFC and IS were well separated with retention times of 9.8, 12.2, 16.0 and 5.8 min, respectively. Figure 2 shows a typical overlaid chromatogram for the control human plasma (free of analytes and IS), control human plasma with IS, human plasma spiked with GFC, SFC and MFC at their respective LLOQ and IS at 100 ng/mL concentration and 1.25 h *in vivo* sample collected from human subject following oral administration of GFC tablet.

Calibration curve. The plasma calibration curve was constructed using eight calibration standards (viz. 100–10,000 ng/mL). The calibration standard curve had a reliable reproducibility over the standard concentrations across the calibration range. The calibration curve was prepared by determining the best fit of peak-area ratios (peak area analyte/peak area IS) vs concentration, and fitted to the $y = mx + c$ using weighing factor $(1/X^2)$. The average regression ($n = 3$) was found to be ≥0.999. The lowest concentration with the RSD <20% was taken as LLOQ and was found to be 100 ng/mL. The percentage accuracy observed for the mean of back-calculated concentrations for three calibration curves was within 95.6–103. Figure 3 shows the representative calibration curve for GFC, SFC and MFC, in human plasma.

Accuracy and precision. Accuracy and precision data for intra- and inter-day plasma samples are presented in Table 1. The assay values on both occasions (intra- and inter-day) were found to be within the accepted variable limits.

DOI: 10.1002/bmc

Table 1. Intra- and inter-day precision and accuracy data of GFC, SFC and MFC quality controls in human plasma

		Measured concentration (ng/mL)											
		GFC				SFC				MFC			
QC ID (ng/mL)	Run	Mean	SD	RSD (%)	Accuracy (%)	Mean	SD	RSD (%)	Accuracy (%)	Mean	SD	RSD (%)	Accuracy (%)
Intra-day variation (six replicates at each concentration)													
100	1	108	5.83	5.36	108	85.15	5.48	6.44	85.15	98.7	4.66	4.71	98.7
	2	101	4.22	4.14	101	104	13.9	13.29	104	93.1	9.80	10.52	93.1
	3	111	3.75	3.35	111	109	11.6	10.66	109	92.3	9.90	10.71	92.3
300	1	339	1.32	0.38	113	274	16.9	6.19	91.4	326	6.08	1.86	108
	2	331	7.37	2.22	110	285	28.4	9.95	95.2	322	2.97	0.92	107
	3	334	15.70	4.69	111	289	5.27	1.81	96.6	315	3.13	0.99	105
5000	1	5419	11.44	0.21	108	5144	69.9	1.35	102	5355	11.2	0.20	107
	2	5391	18.59	0.34	107	5147	79.1	1.53	102	5320	33.1	0.62	106
	3	5292	187	3.53	105	5042	52.9	1.04	100	5165	43.6	0.84	103
7500	1	7734	8.26	0.10	103	7171	92.6	1.29	95.6	7446	4.06	0.05	99.2
	2	7698	47.7	0.62	102	7258	69.86	0.96	96.7	7367	76.9	1.04	98.2
	3	7541	263	3.49	100	7111	65.5	0.92	94.81	7130	81.0	1.13	95.0
Inter-day variation (Eighteen replicates at each concentration)													
100		107	6.19	5.76	107	99.7	14.8	14.9	99.7	94.7	8.49	8.96	94.7
300		334	10.0	3.00	111	283	19.4	6.86	94.44	321	6.23	1.94	107
5000		5367	116	2.17	107	5111	81.3	1.59	102	5280	90.0	1.70	105
7500		7657	169	2.21	102	7180	95.5	1.33	95.7	7314	151	2.07	97.5

RSD, relative standard deviation (SD × 100/mean).

Stability. Over a period of 36 h injection time in the auto-sampler at ambient temperature, over the bench-top for a period of 12 h, following three freeze–thaw cycles –80 ± 10°C and when stored at –80 ± 10°C for 30 days, the predicted concentrations for GFC, SFC and MFC at 300 and 7500 ng/mL samples deviated within the nominal concentrations. The results were found to be within the assay variability limits (Table 2).

Pharmacokinetic study in humans

The sensitivity and specificity of the assay were found to be sufficient for accurately characterizing the pharmacokinetics of GFC in humans. GFC concentrations in plasma were seen up to 36 h. The profile of the mean plasma concentration vs time of one subject is shown in Fig. 4. The maximum concentration in plasma (C_{max}: 5.55 μg/mL) was achieved at 1.25 h (t_{max}). The half-life ($t_{1/2}$) of GFC was 6.14 h, while the $AUC_{(0-\infty)}$ was 45.07 μg h/mL.

DISCUSSION

Only a few HPLC methods have been reported in literature for simultaneous quantification of three FQs in literature (Lubasch *et al.*, 2000; Liang *et al.*, 2002; Nguyen *et al.*, 2004). The main disadvantages of the reported methods are usage of complex mobile phase with ion-pair agent, involvement of displacing agent in sample processing followed by ultra filtration, multi-step column switching process, etc. Considering these disadvantages of the earlier reported HPLC methods, we have developed a simple HPLC method for simultaneous estimation of more than three FQs with UV detection in human plasma with complete validation parameters. To the best of our knowledge this is first HPLC-UV method utilizing liquid–liquid extraction technique showing the baseline separation of four FQs considering the IS, which is also an FQ. The applicability of the method in clinical pharmacokinetic studies has been demonstrated in healthy humans.

CONCLUSIONS

In conclusion we have developed and validated a simple, specific and reproducible HPLC-UV assay to quantify three FQs viz. GFC, SFC and MFC using another FQ, i.e. LFC as IS in human plasma and demonstrated its utility to GFC pharmacokinetic study in humans.

Table 2. Stability data GFC, SFC and MFC quality controls in human plasma

Nominal concentration (ng/mL)	Stability	GFC			SFC			MFC		
		Mean ± SD,[a] $n = 6$ (ng/mL)	Accuracy (%)[b]	Precision (% CV)	Mean ± SD,[a] $n = 6$ (ng/mL)	Accuracy (%)[b]	Precision (% CV)	Mean ± SD,[a] $n = 6$ (ng/mL)	Accuracy (%)[b]	Precision (% CV)
300	0 h (for all)	339 ± 1.33	113.09	0.39	274 ± 16.9	91.41	6.2	326 ± 6.08	108.82	1.86
	3rd freeze–thaw	324 ± 20.0	108.30	6.18	280 ± 4.60	93.53	1.64	294 ± 12.8	98.02	4.38
	12 h (bench-top)	335 ± 4.85	111.69	1.45	297 ± 2.58	99.08	0.87	300 ± 15.2	100.15	5.08
	36 h (in-injector)	321 ± 18.2	107.23	5.68	276 ± 8.71	92.33	3.15	294 ± 30.3	98.10	10.3
	30 day at –80°C	296 ± 12.4	98.78	4.20	266 ± 17.10	88.67	6.43	265 ± 7.34	88.62	2.76
7500	0 h (for all)	7734 ± 8.26	103.12	0.11	7171 ± 92.6	95.61	1.29	7446 ± 4.06	99.29	0.05
	3rd freeze–thaw	7608 ± 139	101.44	1.84	6936 ± 88.7	92.49	1.28	7179 ± 83.4	95.73	1.16
	12 h (bench-top)	7687 ± 23.7	102.49	0.31	7251 ± 35.6	96.69	0.49	7451 ± 23.1	99.34	0.31
	36 h (in-injector)	7645 ± 121	101.93	1.59	6923 ± 97.2	92.31	1.41	7201 ± 76.9	96.02	1.07
	30 day at –80°C	7765 ± 259	103.53	3.34	7654 ± 235	102.02	3.08	7667 ± 315	102.24	4.11

[a] Back-calculated plasma concentrations; [b] (mean assayed concentration/mean assayed concentration at 0 h) × 100.

DOI: 10.1002/bmc

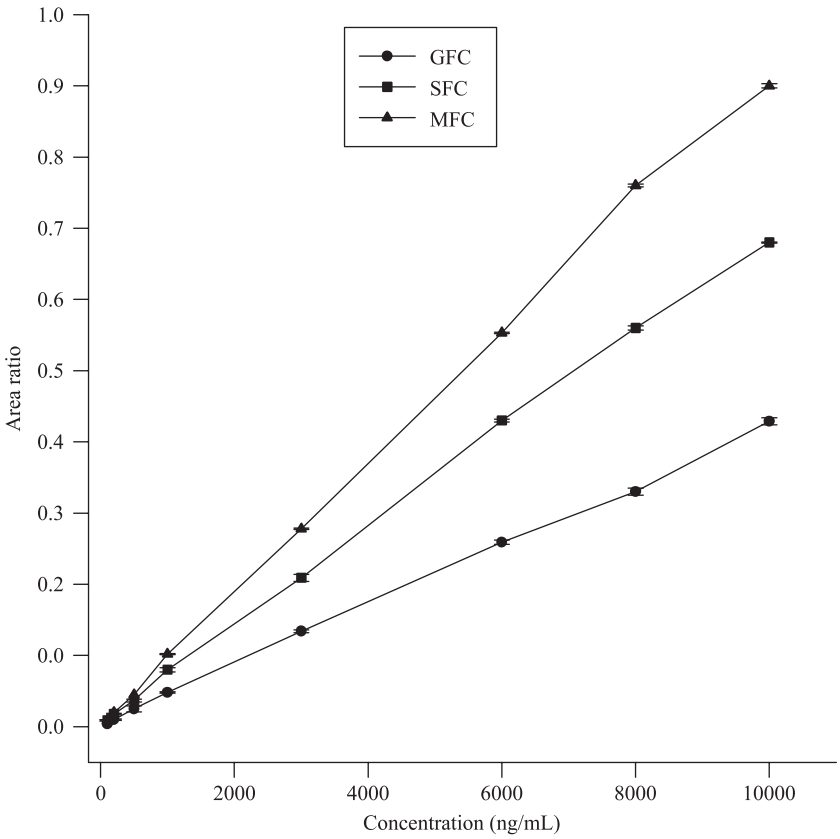

Figure 3. Representative calibration curve for GFC, SFC and MFC in human plasma.

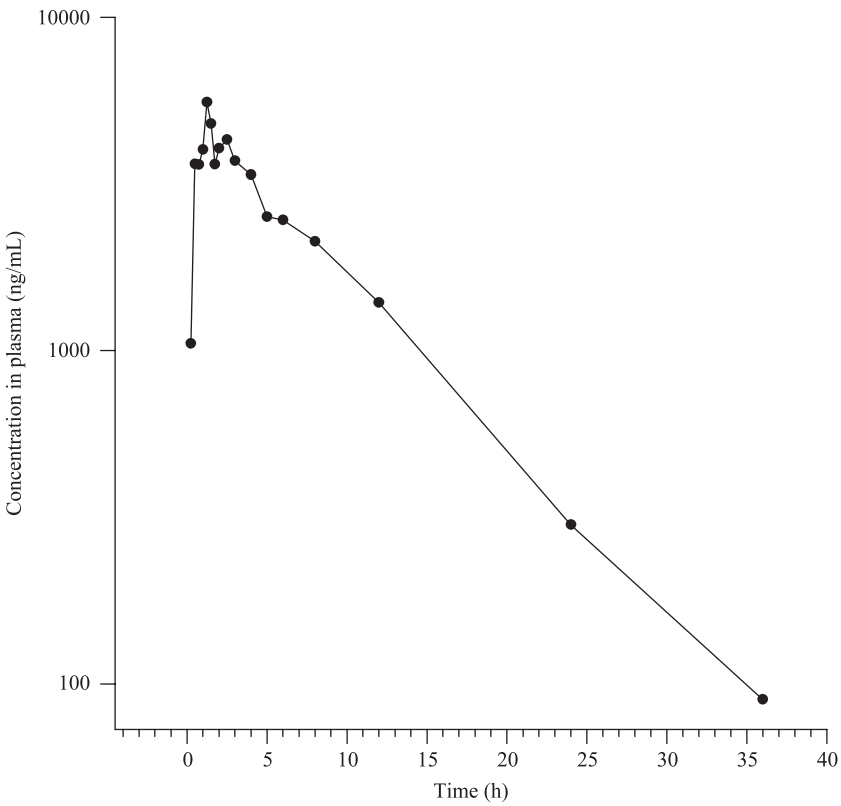

Figure 4. Plasma concentration–time profile of GFC in human plasma following oral dosing of 400 mg of GFC tablet to a subject.

DOI: 10.1002/bmc

REFERENCES

Aminimanizani A, Beringer P and Jelliffee R. Comparative pharmacokinetics and pharmacodynamics of the newer fluoroquinolone antibacterials. *Clinical Pharmacokinetics* 2001; **40**: 169–187.

Andriole VT. The quinolones: prospects. In Andriole T (ed.). *The Quinolones*, 2nd edn. Academic Press: San Diego, CA, 1998; 417–429.

Bergan T. Pharmacokinetics of the fluoroquinolones. In Andriole T (ed.). *The Quinolones*, 2nd edn. Academic Press: San Diego, CA, 1998; 143–182.

Liang H, Kays MB and Sowinski KM. Separation of levofloxacin, ciprofloxacin, moxifloxacin, trovafloxacin and cinoxacin by high-performance liquid chromatography: application to levofloxacin determination in human plasma. *Journal of Chromatography B* 2002; **772**: 53–63.

Lubasch A, Keller I, Borner K, Koeppe P and Lode H. Comparative pharmacokinetics of ciprofloxacin, gatifloxacin, grpafloxacin, levofloxacin, trovafloxacin and moxifoloxacin after single oral administration in healthy volunteers. *Antimicrobial Agents and Chemotherapy* 2000; **44**: 2600–2603.

Nguyen HA, Grellet J, Ba BB, Quentin C and Saux M. Simultaneous determintation of levofloxacin, gatifloxacin and moxifloxacin in serum by liquid chromatography with column switching. *Journal of Chromatography B* 2004; **810**: 77–83.

US DHHS, FDA and CDER. *Guidance for Industry: Bioanalytical Method Validation*. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research and Center for Veterinary Medicine, 2001. Available at: http://www/fda.gov/cder/guidance/index.htm

DOI: 10.1002/bmc