Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 810 (2004) 77–83
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Simultaneous determination of levofloxacin, gatifloxacin and moxifloxacin in serum by liquid chromatography with column switching

Hoang Anh Nguyen[a], Jean Grellet[a,*], Boubakar B. Ba[a], Claudine Quentin[b], Marie-Claude Saux[a]

[a] *Laboratoire de Pharmacocinétique et de Pharmacie Clinique, Faculté de Pharmacie (EA525), Université Victor Segalen Bordeaux 2, Zone Nord, 146 Rue Léo-Saignat, 33076 Bordeaux Cedex, France*
[b] *Laboratoire de Microbiologie, Faculté de Pharmacie (EA525), Université Victor Segalen Bordeaux 2, Zone Nord, 146 Rue Léo-Saignat, 33076 Bordeaux Cedex, France*

Received 2 March 2004; accepted 16 July 2004
Available online 26 August 2004

Abstract

Liquid chromatography with a column-switching technique was developed for simultaneous direct quantification of levofloxacin, gatifloxacin and moxifloxacin in human serum. Serum samples were injected on a LiChroCART® 4-4 pre-column (PC) filled with a LiChrospher® 100 RP-18, 5 μm where fluoroquinolones (FQs) were purified and concentrated. The FQs were back-flushed from the PC and then separated on a Supelcosil ABZ+ Plus (150 mm × 4.6 mm i.d.) analytical column with a mobile phase containing 10 mM phosphate buffer (pH 2.5), acetonitrile (88:12, v/v) and 2 mM tetrabutyl ammonium bromide. The effects of ion-pair reagents, buffer type, pH and acetonitrile concentrations in the mobile phase on the separation of the three FQs were investigated. Fluorescence detection provided sufficient sensitivity to achieve a quantification limit of 125 ng/ml for levofloxacin and moxifloxacin; 162.5 ng/ml for gatifloxacin with a 5 μl sample size. The on-line process of extraction avoids time-consuming treatment of the samples before injection and run time is shortened. The recovery, selectivity, linearity, precision and accuracy of the method are convenient for pharmacokinetic studies or routine assays.

Keywords: Column switching; Levofloxacin; Moxifloxacin; Gatifloxacin

1. Introduction

A number of new fluoroquinolones (FQs) have become available for use worldwide since the initial introduction of ciprofloxacin in the late 1980s [1]. Their anti-microbial activity results from a selective antagonism between host DNA and bacterial DNA without interfering with eucaryotic topoisomerases. Numerous chemical modifications of the quinolone structure were followed by further observations of related increases of activity, changes in pharmacokinetic characteristics and reduced toxicity. The resulting newer FQs, including gatifloxacin, gemifloxacin, levofloxacin, moxifloxacin, rufloxacin and sparfloxacin have enhanced activity against several important pathogens as well as improved pharmacokinetic parameters in comparison to previous derivatives [2].

Numerous techniques and methods have been developed for the determination of FQs in biological samples. Official, compendial and other methods of analysis of 4-quinolone antibacterials were reviewed briefly by Belai et al. [3], while HPLC analysis of FQs in biological matrix was reviewed recently by Carlucci [4]. Most of utilized methods required relatively time-consuming extraction and/or concentration steps.

In developing an analytical method for pharmacokinetic studies, not only the sensitivity and reproducibility suitability

* Corresponding author. Tel.: +33 5 5679 5503; fax: +33 5 5679 5674.
E-mail address: jean.grellet@chu-bordeaux.fr (J. Grellet).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.07.019

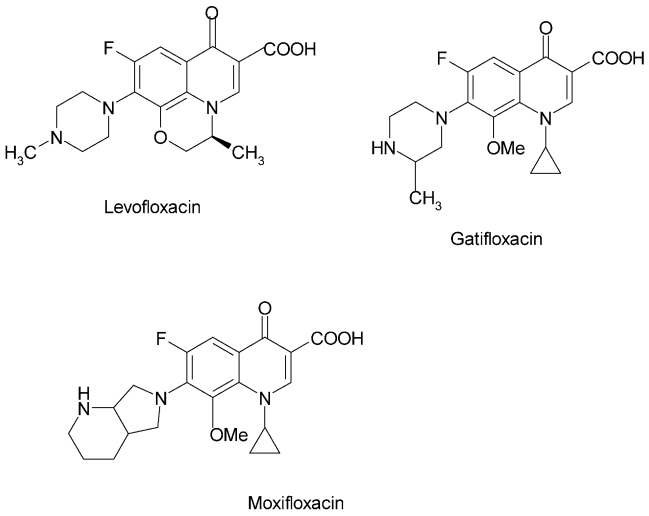

Fig. 1. Chemical structures of levofloxacin (pK_{a1}: 6.27 and pK_{a2}: 6.81); moxifloxacin (pK_{a1}: 6.17 and pK_{a2}: 10.77) and gatifloxacin (pK_{a2}: 8.82) [25].

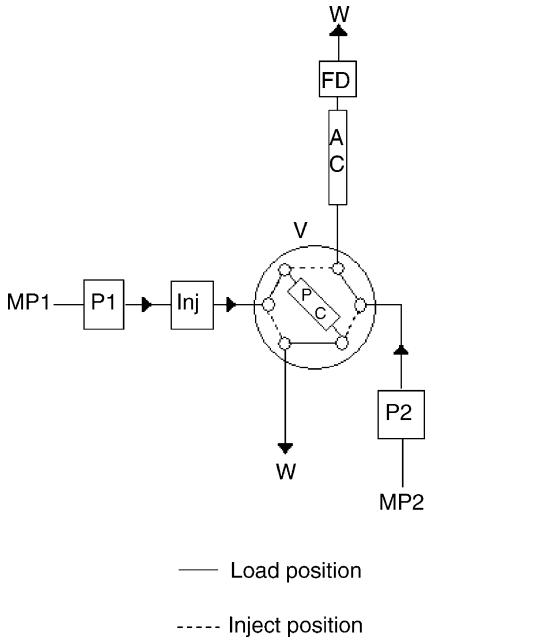

Fig. 2. Schematic diagram of the column-switching system. MP1 and MP2: mobile phases 1 and 2; P1 and P2: pumps 1 and 2; Inj: injector; PC: pre-column; AC: analytical column; V: six-port switching valve; FD: fluorescence detector and W: waste.

for the purpose but also labour-saving factors must be considered because of the need to examine a large number of samples [5]. Facing this problem, Roth et al. [6] developed the fully-automated HPLC using the column-switching technique which allowed on-line drug determination by direct injection of the biological fluids, leading to improvements in the efficiency of analysis for a large number of samples. We, therefore, described here such a method in order to determine simultaneously three FQs: levofloxacin, gatifloxacin and moxifloxacin (Fig. 1) by direct human serum injection without classical sample pre-treatment steps. The first step of this on-line method consists in trapping the analytes in the pre-column and in eluting the biological matrix to waste. In the second step, the analytes were transferred to the analytical column (AC) and the separation occurs.

2. Experimental

2.1. Chemicals

Moxifloxacin hydrochloride (batch 661093 E, purity 87.8%) and gatifloxacin hemihydrate (batch 107, purity 99%) were gifts from Bayer (Puteaux, France) and Grünenthal GmbH Laboratories (Levallois-Perret, France), respectively. Levofloxacin hemihydrate was obtained from infusion solution (Tavanic®, 5 mg/ml, Laboratoire Aventis, France) for initial experiments. Levofloxacin hemihydrate powder (batch W314, purity 99.2%, Aventis Pharma, Romainville, France) was used to confirm initial results and for validation experiments. Dipotassium hydrogen phosphate, potassium dihydrogen phosphate, orthophosphoric acid 85%, tri sodium citrate were provided by Prolabo (Fontenay-sous-Bois, France) (analytical-grade, Normapur). Tetrabutyl ammonium bromide (TBAmBr), hexadecyl-trimethylammonium chloride (HDTAmCl); citric acid were provided by Sigma/Aldrich-Chimie (Saint Quentin Fallavier, France) and tetramethyl ammonium bromide (TMeAmBr) by Merck (Nogent sur Marne, France). Chromatography-grade acetonitrile was also purchased from Prolabo. HPLC-grade water was obtained with a Milli-Q water purification unit, Millipore (Saint Quentin Yvelines, France). Drug-free sera were obtained from blood bank (ETSA, Bordeaux, France).

2.2. HPLC system

The column-switching system (Fig. 2) was composed of an isocratic HP 1050 pump (pump 1), an HP 1050 autosampler (Agilent Technologies, Waldbronn, Germany) and a 420 Kontron pump (pump 2) (Kontron Instruments, Milan, Italy). Pumps 1 and 2 delivered, respectively, pre-analytical (phase 1) and analytical (phase 2) mobile phases. An electric-actuated switching system with a six-port Rheodyne valve was controlled by the external time-events of the HP-1050 pump. Sample injection starts its programmable time-replay.

The sample preparation was performed using a LiChroCART® 4–4 pre-column filled with LiChrospher® 100 RP-18, 5 μm (Merck-Clevenot, Nogent-sur-Marne, France). Chromatographic separation was performed at ambient temperature (20 °C) on a Supelcosil ABZ+ Plus analytical reverse phase column (AC) (150 mm × 4.6 mm i.d., 5 μm) (Supelco, Saint Quentin Fallavier, France). An Upchurch 5 μm pre-column filter (Cluzeau Info Labo, Sainte-Foy-La Grande, France) was inserted between the PC and the AC.

Fluoroquinolones were detected using a Bio-Tek spectrofluorimeter (Bio-Tek Instrument, Saint Quentin en Yvelines, France) set at 296 nm excitation and 504 nm emission. Data acquisition was performed with a HP 3396A integrator (Hewlett-Packard) via peak-areas.

2.3. Column-switching procedure

Concentration/clean-up and separation of levofloxacin, gatifloxacin and moxifloxacin in serum samples were done by a column-switching procedure. The flow-rates were adjusted to 1.0 and 1.2 ml/min with pumps 1 and 2, respectively. Switching of concentration/clean-up, elution and separation steps was made by manipulating the six-port valve. The total sequence of automated sample analysis required 17 min and included the following three steps:

- Step I (0–2 min, valve in load position): 5 μl serum sample was injected directly by the auto-sampler onto the pre-column. Then the PC was flushed by the mobile phase 1 for 2 min allowing the three FQs to be retained firmly and many contaminants to be drained.
- Step II (2–15 min, valve in injection position): FQs which were retained near the head of PC, were then transferred into the AC, by back-flushing PC with mobile phase 2 using pump 2, where FQs were separated for quantification.
- Step III (15–17 min, valve in load position): the PC was perfused with mobile phase 1 and conditioned for the next injection.

2.4. Mobile phases

Mobile phase 1 consisted of a mixture of 10 mM K_2HPO_4 buffer adjusted to pH 5.4 with orthophosphoric acid and acetonitrile (98:2, v/v).

Mobile phase 2 was a mixture of 10 mM KH_2PO_4 buffer, pH 2.5 containing 2 mM TBAmBr and acetonitrile (88:12, v/v).

During the optimization of analytical separation, an initial phase containing 10 mM KH_2PO_4 buffer pH 2.5 with 15% acetonitrile was used. Then the effect of ion-pair agents on retention and separation of the three FQs was studied by adding 2 mM TBAmBr, 2 mM TMeAmBr or 2 mM HDTMeCl. For each ion-pairing agent, the influence of organic modifier concentration was tested. Additional experiments were performed to test the influence of TBAmBr concentration. The influence of the buffer was also evaluated using a mobile phase containing 12% acetonitrile and either 10 mM phosphate or 10 mM citrate buffer. Further, the effect of pH variation was tested using 10 mM phosphate buffer containing 2 mM TBAmBr set at pH 2.0, 2.5, 3.0, 3.5 and 4.0 by adding concentrated orthophosphoric acid.

2.5. Calibration standards and quality control samples

Levofloxacin, gatifloxacin and moxifloxacin were made up as 5000, 1600 and 3200 μg/ml individual stock solutions in water, and then used to prepare diluted solutions in water. Six calibrators containing 125, 250, 500, 1000, 2000 and 4000 ng/ml of levofloxacin and moxifloxacin and 156, 312, 625, 1250, 2500 and 5000 ng/ml of gatifloxacin were prepared by adding 10 μl of each individual diluted solution to 970 μl of drug-free human serum. Quality control (QC) samples of low (250 ng/ml of levofloxacin and moxifloxacin; 312 ng/ml of gatifloxacin), middle (1000 ng/ml of levofloxacin and moxifloxacin; 1250 ng/ml of gatifloxacin) and high (4000 ng/ml of levofloxacin and moxifloxacin; 5000 ng/ml of gatifloxacin) concentrations were prepared by the same procedure as the calibration standards but using different stock solutions. Aliquots of 200 μl of all calibrations and QCs were transferred to Eppendorf tubes, stored at −20 °C and protected from light until assayed. Before each run, the blank human serum, calibrators, QC samples were thawed and vortexed for 30 s. A 5 μl-volume was injected into the HPLC system.

2.6. Assay validation

Validation was performed according to the guidelines for development of bioanalytical assay in human biomatrices [7,8].

2.6.1. Linearity of the standard curves

The concentration of each FQ was determined using the calibration curves from 125 to 4000 ng/ml for levofloxacin and moxifloxacin and from 162.5 to 5000 ng/ml for gatifloxacin. The curves were obtained daily by computing linear non-weighted least-square regressions of the peak areas (y) versus FQ's concentrations (x) and used to determine FQs concentrations in unknown samples. Ten standard curves were prepared on 10 separate days. The mean slope value and standard deviation were determined from these 10 experiments. Moreover, mean intercept was calculated and statistically compared to zero by Student t-test (statistical significance as $P < 0.05$). The back-calculated concentrations were also determined for all calibration standards and accuracy and precision calculated from these data.

2.6.2. Precision and accuracy

For the within-day assay precision and accuracy, 10 replicates of each low, middle and high quality control samples were analyzed for the same day. For the between-day assay precision and accuracy, triplicates of each low, middle and high QC samples were analyzed daily for 10 days. These QCs replicates were analyzed together with serum blank and calibrators in the same analytical sequence. The mean concentrations and standard deviations were calculated from within-day and between-day experiments; the precision

and the inaccuracy were computed using standard methods [7,8].

2.6.3. Recovery

The extraction efficiency (recovery) was determined by comparing peak areas of aqueous standards directly injected into the AC and those from serum standards containing similar FQ concentrations (n = 10), submitted to the "on-line" extraction by the column-switching technique.

2.6.4. Limit of detection and limit of quantification

The limit of detection (LOD) in serum was defined as the concentration providing a signal-to-noise ratio of 3. The limit of quantification (LOQ) is the minimum injected amount that demonstrated precise measurements (inaccuracy and coefficient of variation (CV) less than 15%).

2.6.5. Stability

The short term stability of FQs in serum were studied under two experimental conditions: during three freeze–thaw cycles and after storage at room temperature for 24 h. Long term stability in serum was assessed after 1 month storage at −20 °C. Stability was evaluated by comparing measured concentrations before and after storage.

3. Results and discussion

3.1. Method development

3.1.1. Optimisation of chromatographic separation

In our laboratory, different columns have been used successfully to separate FQs: Kromasil® C_{18} [9], Ashahipack® ODP50 [10] and Supelcosil ABZ+ Plus [11]. Among these columns, silica-based deactivated Supelcosil ABZ+ Plus provided the best peak shapes and efficiencies, so it was chosen for our experiments.

Acidic pH of the mobile phase was chosen for the method development to reverse quinolone carboxylic function ionisation and to increase chromatographic retention of FQs. Nevertheless, when initial mobile phase (10 mM phosphate buffer; pH 2.5 acetonitrile; 85:15, v/v) was used, levofloxacin and gatifloxacin were little retained (t_R were 2.1 and 4 min, respectively) and their peaks overlapped.

The low retention was due to the eluting strength of the mobile phase and led us to reduce organic modifier concentration. However, the peak tailing was extremely serious probably due to the secondary interactions at the working pH, between silanol groups on the support and the fully protonated basic groups of the FQ molecules despite the use of a deactivated column packing (Supelcosil ABZ+ Plus). To reduce the peak tailing, different methods are available: adding a ion-pairing reagent in the mobile phase which competes for the available adsorption sites or modifying mobile phase pH to reduce interactions with the silanol groups. The influence of ion-pairing agents was studied by adding amines such as

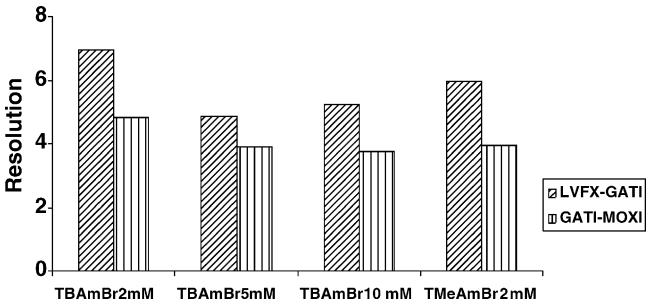

Fig. 3. Effect of ion-pairing agents on separation of the tested quinolones. Chromatographic column: Supelcosil ABZ+ Plus (150 mm × 4.6 mm i.d.; 5 μm particle size). Mobile phase: 10 mM KH_2PO_4 pH 2.5 and 15% acetonitrile with 2, 5 and 10 mM TBAmBr or 2 mM TMeAmB; flow-rate: 1.2 ml/min. Injected volume: 5 μl. All experiments were carried out at ambient temperature: 20 °C. LVFX: levofloxacin; GATI: gatifloxacin and MOXI: moxifloxacin.

TBAmBr, TMeAmBr and HDTACl in the mobile phase to saturate the free silanol groups. HDTACl was rejected due to unreliable retention times.

The best peak shapes and resolutions of quinolones were obtained with TBAmBr (Fig. 3). Increasing concentrations of TBAmBr from 2 to 10 mM did not improve FQ's separation (Fig. 3) and thus, 2 mM TBAmBr was chosen for the final mobile phase.

The effect of pH mobile phase was also studied in the range of 2.0–4.0. The mobile phase pH had a little impact on resolution of FQs and the best separations were observed between 2.5 and 3.5 (Fig. 4).

Further, we studied the effect of buffer nature on the separation of quinolones. The resolution was similar when using citric acid instead of phosphate buffer (data not shown) and the latter was chosen for the final mobile phase.

Finally, we investigated the effect of acetonitrile concentration in the mobile phase. As expected, the retention times and resolutions of the quinolones increased with decreasing acetonitrile concentration from 15 to 11% (Fig. 5). A concentration of 12% organic modifier provided the best compromise between resolution and run duration.

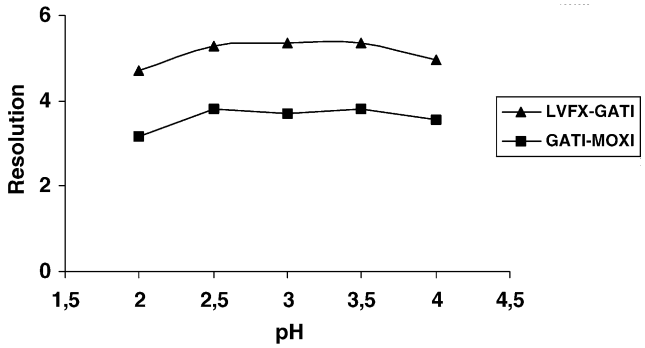

Fig. 4. Effect of mobile phase pH on resolution of the tested quinolones. Mobile phase: 10 mM K_2HPO_4, 2 mM TBAmBr with 15% acetonitrile, at pH ranging from 2.0 to 4.0. Other experimental conditions as in Fig. 3. LVFX: levofloxacin; GATI: gatifloxacin and MOXI: moxifloxacin.

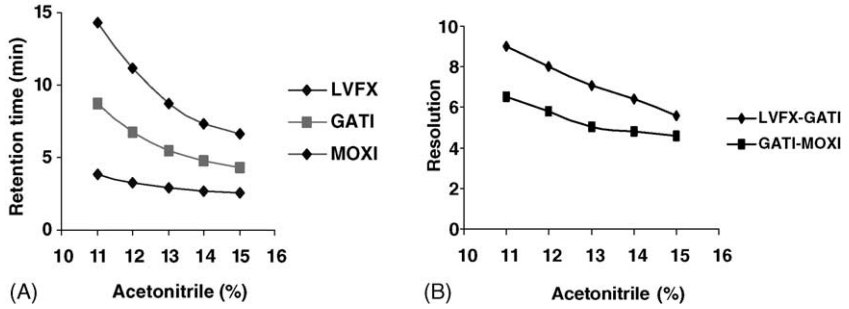

Fig. 5. Effect of mobile phase acetonitrile concentration on retention (A) and resolution (B) of the tested quinolones. Mobile phase: 10 mM K_2HPO_4 pH 2.5, 2 mM TBAmBr with 11–15% acetonitrile. Other experimental conditions as in Fig. 3. LVFX: levofloxacin; GATI: gatifloxacin and MOXI: moxifloxacin.

The final optimized analytical mobile phase consisted of 2 mM TBAmBr, 10 mM KH_2PO_4 buffer pH 2.5 and 12% acetonitrile.

3.1.2. Extraction of FQs from human serum

We adopted on-line extraction of FQs from the biological matrix by a column-switching technique to reduce the classical labor-intensive and time consuming preparation processes [9,11,12]. The composition of the mobile phase was adjusted to allow the retention of the FQs on the pre-column and to elute the serum contaminants. The parameters to consider were the proportion of organic modifier, the pH value and buffer capacity of the final mixture [12]. An acidic pH and a low elution strength (2% of acetonitrile) were used for the mobile phase to increase lipophilicity of FQs and to improve their retention on the pre-column. Among different acidic pH, pH 5.4 provided the best sample clean-up while obtaining recoveries greater than 85% (Table 1). With respect of a small volume of injection (5 μl), a low capacity of the phosphate buffer (10 mM) was chosen to avoid precipitation during this clean-up step. Extraction and sample cleaning remained unchanged after 100 on-line extractions. Nevertheless, the extraction pre-column and the 5 μm filter were replaced after about 100 injections to avoid AC damage.

Table 1
Extraction recovery of levofloxacin, gatifloxacin and moxifloxacin from serum

Theoretical concentration (ng/ml)	Recovery (%) (mean ± S.D.) (n = 10)
Levofloxacin	
250	98.8 ± 4.6
1000	83.9 ± 1.76
4000	94.7 ± 1.23
Gatifloxacin	
312.5	90.7 ± 4.23
1250	87.6 ± 2.13
5000	94.2 ± 1.26
Moxifloxacin	
250	91.5 ± 2.14
1000	92.2 ± 1.84
4000	94.7 ± 1.11

3.2. Method validation

3.2.1. Selectivity

"Trap and flush" back-flushing column-switching methods may suffer from decrease selectivity compared to extraction methods. Using the common packing materials such as C_8 or C_{18}, only proteins, salts and other highly polar serum components are flushed to waste, while most of endogenous compounds are retained on the PC and transferred to the AC, together with the substances of interest [13]. This drawback was compensated in the proposed method by taking advantage of fluorescence detection technique selectivity. Typical chromatograms of a blank and a spiked serum with levofloxacin, gatifloxacin and moxifloxacin are shown in Fig. 6. The retention times of levofloxacin, gatifloxacin and moxifloxacin were 5.1, 8.9 and 13.4 min, respectively.

3.2.2. Linearity

Linear least-square regression analysis of the calibration graphs in 10 different days demonstrated linearity between the response and corresponding concentrations of FQs over the range of 125–4000 ng/ml for levofloxacin and moxifloxacin and 162.25–5000 ng/ml for gatifloxacin. The mean slopes (±S.D.) were 0.52 (±0.02), 1.06 (±0.06), 0.75 (±0.04) for levofloxacin, gatifloxacin and moxifloxacin, respectively, and the corresponding mean intercepts (respectively: −20.3 (±27.27), −11.32 (±15.32), −44.4 (±41.9)) were not significantly different from zero ($P > 0.05$). The results of linear regression analysis show that the correlation coefficients of all standard curves were ≥0.999. In addition, the accuracies (given as inaccuracies) and precisions of all back-calculated standard concentrations demonstrated ruggedness of the calibrations. The accuracy ranged from −0.1 to 12.6% and precision (CV) from −0.2 to 13% (Table 2).

3.2.3. Precision and accuracy

The between-day assay and within-day assay precision (CV) and accuracy (given as inaccuracy, i.e. the difference between measured and theoretical concentrations) were checked using quality control samples. For all tested concentrations, the CV and the inaccuracies were lesser than 6% (Table 3). These results indicate that the method is reliable,

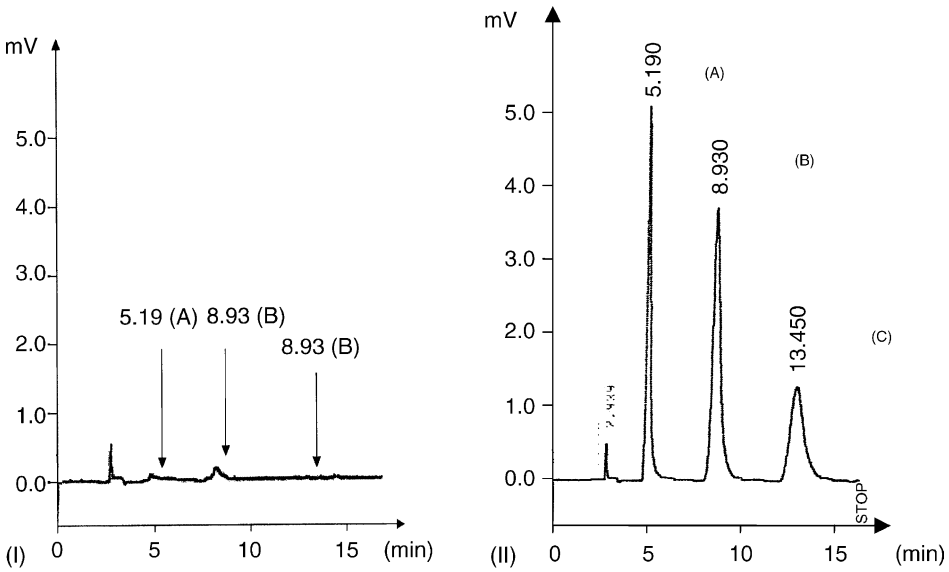

Fig. 6. Chromatograms of a blank (I) and a serum sample spiked (II) with levofloxacin (250 ng/ml), gatifloxacin (312.5 ng/ml) and moxifloxacin (250 ng/ml) processed by "on-line" extraction. Mobile phase: 12% acetonitrile at pH 2.5. Other experimental conditions as in Fig. 3. (A) Levofloxacin, (B) gatifloxacin and (C) moxifloxacin.

reproducible and accurate and so, the use of an internal standard is not necessary.

3.2.4. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD were 60 ng/ml for levofloxacin, 120 ng/ml for gatifloxacin and 35 ng/ml, for moxifloxacin. At concentrations of 125, 162.5 and 125 ng/ml for levofloxacin, gatifloxacin and moxifloxacin, the percent deviation from the nominal concentrations and the CV were lesser than 15%. Thus, 125 ng/ml for levofloxacin and moxifloxacin and 162.5 ng/ml for gatifloxacin were defined as the LOQs.

3.2.5. Stability

Since many serum samples are expected to be analyzed daily, the stability of spiked serum samples was examined at room temperature (20 °C) over 24 h. No significant difference in the FQs measured concentrations was observed during

Table 2
Ten-day standard curve validation for the three tested FQs

Added concentrations (ng/ml)	Back-calculated concentrations (ng/ml) (mean ± S.D.) (n = 10)	CV (%)	Inaccuracy (%)
Levofloxacin			
125	138.6 ± 14.5	13.3	12.6
250	247.8 ± 10.9	4.4	−0.9
500	499.1 ± 10.3	2.1	−0.2
1000	993.6 ± 30.2	3.3	−0.6
2000	1984.1 ± 29.6	1.5	−0.8
4000	4008.2 ± 18.0	0.4	0.2
Gatifloxacin			
156.25	180.0 ± 13.6	7.5	13.2
312.5	314.2 ± 17.7	4.5	0.5
625	627.2 ± 21.1	3.3	0.35
1250	1224.1 ± 52.1	4.1	−2.8
2500	2503.3 ± 29.8	1.1	0.13
5000	5004.9 ± 9.38	0.1	0.09
Moxifloxacin			
125	142.9 ± 18.5	12.9	14.3
250	250.1 ± 15.2	6.0	0.4
500	508.5 ± 21.3	4.19	1.7
1000	990.4 ± 47.0	4.75	−0.9
2000	1968.2 ± 56.4	2.86	−1.58
4000	4014.6 ± 17.1	0.42	0.36

Table 3
Within- and between-day precisions (CV) and inaccuracies of levofloxacin, gatifloxacin and moxifloxacin assays in serum

Added concentrations (ng/ml)	Within-day assays (n = 10)			Between-day assays (n = 10)		
	Measured (mean ± S.D.) (ng/ml)	CV (%)	Inaccuracy (%)	Measured (mean ± S.D.) (ng/ml)	CV (%)	Inaccuracy (%)
Levofloxacin						
250	246.5 ± 10.4	3.9	5.8	240.9 ± 13.3	5.5	−3.6
1000	1044.2 ± 22.4	2.1	4.4	962.1 ± 13.0	1.3	−3.7
4000	3959.6 ± 92.0	2.3	−1.0	3825.6 ± 79.4	2.0	−4.3
Gatifloxacin						
312.5	304.1 ± 10.4	3.4	−2.6	294.7 ± 10.4	3.5	−5.6
1250	1284.2 ± 22.3	1.8	2.7	1186.11 ± 37.2	3.1	−5.1
5000	4955.2 ± 66.0	1.3	−0.8	4798.1 ± 71.3	1.4	−4.0
Moxifloxacin						
250	260.1 ± 12.5	4.8	4.0	239.4 ± 12.9	5.3	−4.2
1000	1034.0 ± 37.7	3.6	3.4	968.5 ± 29.3	3.0	−3.1
4000	4170.1 ± 58.7	1.4	4.2	3870.8 ± 63.7	1.6	−3.2

the 24 h-period of assay (for middle QC concentrations (ng/ml): 1021.5 ± 18.4 after 24 h versus 1032.4 ± 21.6 for levofloxacin; 1254.3 ± 26.4 after 24 h versus 1271.4 ± 31.2 for gatifloxacin and 1008.4 ± 47.2 after 24 h versus 1026.8 ± 38.1 for moxifloxacin, $P > 0.05$). No significant decrease of FQs concentrations was noted after three freeze–thaw cycles and after 1 month storage at −20 °C (data not shown).

4. Conclusion

A column-switching HPLC technique was established for simultaneous determination of levofloxacin, gatifloxacin and moxifloxacin in human serum. It allows a direct, efficient and reproducible on-line extraction of three FQs. In addition, the method is selective, sensitive and reliable. The precisions, accuracies and limits of detection and quantification are comparable with those of previously reported methods for levofloxacin [14–16], gatifloxacin [17] and moxifloxacin [18–20]. Analysis of a serum sample required only 17 min. The on-line processes of extraction avoid time-consuming treatment of the samples before injection [21–24]. As a consequence, analysis was shortened, allowing to perform consecutively a large number of assays. This method appears convenient for pharmacokinetic studies of these newer FQ agents.

Acknowledgment

This study was supported by a grant from the Ministère de l'Education Nationale et de la Recherche (EA 525), Université de Bordeaux 2, Bordeaux, France.

References

[1] P.C. Appelbaum, P.A. Hunter, Int. J. Antimicrob. Agents 16 (2000) 5.
[2] A. Aminimanizami, P. Beringer, R. Jelliffe, Clin. Pharmacokinet. 40 (2001) 169.
[3] F. Belai, A.A. Al-Majed, A.M. Al-Obaid, Talanta 50 (1999) 765.
[4] G. Carlucci, J. Chromatogr. A 812 (1998) 343.
[5] E. Matsui, M. Hoshino, A. Matsui, A. Okahira, J. Chromatogr. B 668 (1995) 299.
[6] W. Roth, K. Beschke, R. Jauch, A. Zimmer, F.M. Koss, J. Chromatogr. 222 (1981) 13.
[7] S. Braggio, R.J. Barbany, P. Grossi, M. Cugola, J. Pharm. Biomed. Anal. 14 (1996) 375.
[8] H.T. Karnes, C. March, Pharm. Res. 10 (1993) 1420.
[9] B.B. Ba, D. Ducint, M. Fourtillan, M.C. Saux, J. Chromatogr. B 714 (1998) 317.
[10] P. Rispal, J. Grellet, C. Celerier, D. Breilh, M. Dorian, J.L. Pellegrin, M.C. Saux, B. Leng, Arzneim. Forsch/Drug Res. 46 (1996) 316.
[11] B.B. Ba, R. Etienne, D. Ducint, C. Quentin, M.C. Saux, J. Chromatogr. B 754 (2001) 107.
[12] P. Camp-Falco, R. Herraez-Hernandez, A. Sevillano-Cabeza, J. Chromatogr. 619 (1993) 177.
[13] U. Timm, R. Zumbrunnen, R. Erdin, J. Chromatogr. B 691 (1997) 397.
[14] C.M. Tobin, J. Sunderland, L.O. White, A.P. MacGowan, J. Antimicrob. Chemother. 43 (1999) 434.
[15] H. Liang, M.B. Kayes, K.M. Sowinski, J. Chromatogr. B 772 (2002) 53.
[16] U. Neckel, C. Joukhadar, M. Frossard, W. Jager, M. Muller, B.X. Mayer, Anal. Chim. Acta 463 (2002) 199.
[17] M. Nokashima, T. Uematsu, K. Kosuge, H. Kusajima, Antimicrob. Agents Chemother. 39 (1995) 2635.
[18] H. Stass, A. Dalhoff, J. Chromatogr. B 702 (1997) 163.
[19] C.M. Tobin, J. Sunderland, L.O. White, A.P. MacGowan, D.S. Reves, J. Antimicrob. Chemother. 42 (1998) 278.
[20] T. Lemoine, D. Breilh, D. Ducint, J. Dubrez, J. Jougon, J.F. Velley, M.C. Saux, J. Chromatogr. B 742 (2000) 247.
[21] K. Friedand, I.W. Wainer, J. Chromatogr. B 689 (1997) 91.
[22] F.K. Chow, Column switching techniques in pharmaceutical analysis, in: G.W. Fong, S.K. Lam (Eds.), HPLC in the Pharmaceutical Industry, Marcel Dekker, New York, USA, 1991, pp. 41–61.
[23] W.S. Letter, LC/GC Int. 10 (1997) 798.
[24] T. Ohkudo, M. Kudo, K. Sugawara, J. Chromatogr. 573 (1992) 289.
[25] Chemical Abstracts Service, American Chemical Society, Chemical Abstracts, 2003.