Received: 18 February 2018 | Revised: 8 March 2018 | Accepted: 9 March 2018

DOI: 10.1002/chir.22861

REGULAR ARTICLE

WILEY

Structure-retention relationship for enantioseparation of selected fluoroquinolones

Rasha M. Hassan[1] | Ali M. Yehia[2] | Ola A. Saleh[1] | Aida A. El-Azzouny[1] | Hassan Y. Aboul-Enein[1]

[1]Medicinal and Pharmaceutical Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Centre (ID: 60014618), Giza, Egypt

[2]Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt

Correspondence
Hassan Y. Aboul-Enein and Ola A. Saleh, Medicinal and Pharmaceutical Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Centre (ID: 60014618), Dokki, 12622 Giza, Egypt.
Email: haboulenein@yahoo.com; ola.ahmedsaleh@yahoo.com

Funding information
National Research Centre, Grant/Award Number: 11010311

Abstract

Fluoroquinolones are popular class of antibiotics with distinct chemical functionality. Most of them are ampholytes with one chiral center. Stereogeneic center is located either in the side ring of Gatifloxacin (GFLX) or in the quinolone core of Ofloxacin (OFLX). These two amphoteric fluoroquinolones have terminal amino groups in common. The unusual Nadifloxacin (NFLX) is an acidic fluoroquinolone with a core chiral center. Owing to chirality and functionality differences among GFLX, OFLX, and NFLX, we mapped these enantiomers onto structure-retention relationship. Amount of acetic acid modifier was studied in screened mobile phase and cellulose tris(3-chloro-4-methyl phenyl carbamate) (Lux cellulose-2) stationary phase. Experimental design of acetic acid% along with column temperature have been applied. Resolution and enantioselectivity have been related to structural features of the studied enantiomers. High amount of acid (0.4%) was optimum for the separation of either side chirality with a proximate amino group (GFLX) or core chirality without basic functionality (NFLX), while low amount (0.2%) is optimum for core chiral center with distal amino group (OFLX). Temperature has no significant effect on resolution and retention of enantiomers except for OFLX. Enantio-retention explains possible chiral selective and nonselective interactions. The proposed methods have been validated for pharmaceutical analyses.

KEYWORDS

chiral chromatography, experimental design, fluoroquinolones, structure-retention relationship

1 | INTRODUCTION

Fluoroquinolones are immensely popular class of antibiotics. They interfere with bacterial DNA replication leading to rapid bactericidal effect.[1] A common fluoro-substituent in quinolone core is responsible for enhanced bacterial cell penetration and DNA gyrase inhibition.[2] Most of fluoroquinolones are ampholytes with both acidic (carboxylic group attached to quinolone core) and basic (amino) groups in the side chain. For instance, Gatifloxacin (GFLX) and Ofloxacin (OFLX) feature piperazine side ring with secondary and tertiary amino groups, respectively, along with carboxylic group. Unlike usual fluoroquinolones, Nadifloxacin (NFLX) is an acidic antibacterial agent without distal basic functionality.[3] Chemical structures of GFLX, OFLX, and NFLX share 1 stereogenic center (Figure 1). Gatifloxacin possesses chiral center at the side piperazine ring in close proximity to secondary amino group, whereas both OFLX and NFLX feature chiral centers at quinolone core. Their

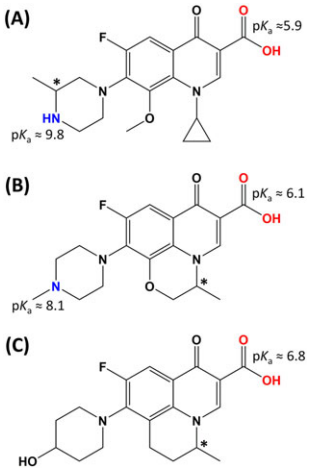

FIGURE 1 Chemical structures of Gatifloxacin A, Ofloxacin B, and Nadifloxacin C, showing their chiral centers (*) and dissociation constants of ionizable groups

enantiomers usually exert different pharmacological effect; for example, the (S)-isomers of OFLX and NFLX have more bactericidal activity than the corresponding (R)-isomers.[3,4]

From the regulatory perspective, guidelines has been issued for marketing racemic drugs.[5] Hazardous side effects are usually encountered for inactive enantiomers, owing to dissimilar mechanism of metabolism, excretion, or clearance.[6,7] To that end, several chromatographic methods have reviewed for enantiomeric separation of fluoroquinolones.[8-11] Most HPLC methods used indirect strategies either by derivatization of GFLX[12] and OFLX[13-19] or by addition of cyclodexrin in the mobile phase for enantiomeric separation of NFLX.[20,21] Some other researches applied direct enantiomeric separations by using chiral stationary phases (CSPs) like amylose-based CSP[22,23] for GFLX. Also cellulose,[24,25] protein,[15] macrocyclic antibiotic,[26] and cyclodextrin-based[27] CSPs were used for OFLX enantioseparation. Usually, efficient chromatographic separation of fluoroquinolone enantiomers requires the optimum use of basic and acidic mobile phase additives. Among the reported methods, few of them consider the effect of basic additive on the separation of GFLX[23] and OFLX.[24,25] Kannappan et al[24] changed some experimental conditions, including diethylamine, in an effective designed protocol to study the separation of S-OFLX on cellulose tris (4-chloro-3-methylphenylcarbamate) stationary phase. Kannappan and Mannemala[24] revealed the significant effect of diethylamine on only the capacity factor of first eluted enantiomer. Probably the fixed higher level of acetic acid (0.4%) in all trials, compared with experimental levels of diethylamine (0.25 ± 0.05%), restricted the authors to resolve other possible effects. Moreover, previous reports[23-25] investigated the effect of basic mobile phase additive without regard to structural features of the cited fluoroquinolones.

The aim of this work is to draw an analogy among the enantiomeric separation of GFLX, OFLX, and NFLX with different molecular skeletons. Position of stereogenic centers and presence of ionizable amino group are main distinguishing features of the selected fluoroquinolones. This comparison has never been proposed in the literature so far. For a primary screened stationary and mobile phases, we applied experimental design for studying the effect of acetic acid and temperature as two main factors affecting our concern. The objectives of design are to maximize enantiomeric resolution within a considerable analysis time. The resolution, selectivity, and capacity factors of the studied enantiomers find a relation to their corresponding structural features. The optimized chromatographic methods were then validated and applied for pharmaceutical analyses of fluoroquinolones under study.

2 | MATERIALS AND METHODS

Pure racemic samples of GFLX (99.79% purity), OFLX (99.90% purity), and NFLX (99.82% purity) were generously obtained from Jamjoom Pharmaceuticals Co, Jeddah, Saudi Arabia, Sanofi-Aventis Egypt sae El Sawah El Amiria, and Quadra pharm, Egypt, respectively, with the corresponding certified purities. Pharmaceutical applications used were Tymer™ sterile ophthalmic solution 0.3% manufactured by Jamjoom Pharmaceuticals Co, Jeddah, Saudi Arabia, B. No. TL0126; Tarivid® 200-mg film-coated tablets, manufactured by Sanofi-aventis Egypt sae El Sawah El Amiria under license of Sanofi-aventis, Germany, B. No. 6EG015 and Nadvulg cream 1%, manufactured by Quadra pharm, Egypt. All solvents used in this work were of HPLC grade: methanol, ethanol, acetonitrile, and hexane (Merck, Darmstadt, Germany) and acetic acid and diethylamine (Sigma Aldrich, Germany).

Agilent HPLC unit; 1100 series apparatus; equipped with a quaternary pump, a vacuum degasser, a column oven and a diode array UV detector. The used chiral column was Lux-Cellulose-2 (cellulose tris(3-chloro-4-methyl phenyl carbamate) column (250 × 4.6 mm, 3-mm particle size) purchased from Phenomenex (Torrance, USA). Chromatographic data acquisition and analysis was performed by Hewlett-Packard Chemstation software for LC 3D systems; Rev. B.03.01 (317) Copyright© Agilent Technologies 2001 to 2007. Design of experiment was carried out by using JMP® Copyright© 2012, SAS Institute Inc, Cary, NC, USA.

Standard stock solutions of racemic GFLX, OFLX, and NFLX (1.0 mg mL^{-1}) were prepared in methanol. Optimization of enantiomeric separation was carried out on

samples having concentration of 500 μg mL^{-1} of GFLX, OFLX, and NFLX. The samples were prepared by suitable dilution of their corresponding stock solutions with methanol. The injection volume was 5 μL, and UV detection was carried out at 290 nm close to absorbance maxima of the studied drugs. Initial separation of enantiomers was studied by using Lux-Cellulose-2 as stationary phase and various mobile phase composition containing ethanol, acetonitrile, and hexane with flow rate of 1.0 mL min^{-1}. Acetic acid (0.2%) and diethylamine (0.1%) were fixed modifiers in this preliminary screening, and column temperature was 25°C. Resolution and retention of enantiomers were examined. Sufficient separation and fast elution were attainable by using acetonitrile-ethanol (90:10 vol/vol) with 2.0-mL min^{-1} flow rate for GFLX and OFLX enantiomers. While the screened mobile phase for NFLX enantiomeric separation was hexane-ethanol (10:90 vol/vol) with 1.5-mL min^{-1} flow rate. Continuous full factorial experimental design was used for optimizing acetic acid percentage and column temperature as crucial factors affecting enantiomeric separation. The designs were built by JMP® by using two levels for each factor and one center point repeated twice. Acetic acid levels were 0.4% (+) and 0.2% (−), whereas for column temperature were 35°C (+) and 25°C (−). A total of six experiments were carried out in random order for each drug. Fixed diethylamine (0.1%) was used in all trials with previously screened mobile phase and appropriate flow rate. Responses used in the design were resolution, selectivity factor, and capacity factors of enantiomers. Statistical comparison between factors' coefficients was applied to evaluate the significant effect of factors on the responses. Desirability function was used to achieve the design targets. Our objectives were to maximize both resolution and selectivity while capacity factors were set to matched target (1-10). Higher weights were provided for both resolution and selectivity. The maximum desirability was achieved when percentages of acetic acid in mobile phase additive were 0.4%, 0.2%, and 0.4% for GFLX, OFLX, and NFLX, respectively. Column temperature was 35°C for all enantiomers.

The optimized methods for the separation of fluoroquinolone enantiomers were validated according to International Conference on Harmonization guidelines.[28] Specificity, accuracy, and precision were determined. The linearity of the method was assessed by six concentration levels in the range of 100 to 900 μg mL^{-1} for the three drugs. Calibration samples were prepared by separately transferring aliquots from their respective standard stock solutions (1.0 mg mL^{-1}) into 10-mL volumetric flasks, and the volumes were completed with the methanol. The calibration curves were obtained by plotting peak areas versus the corresponding concentrations, and regression parameters were computed along with detection and quantitation limits. Robustness was also assisted by deliberate small variation in column temperature (35 ± 2°C) and flow rate (2.0 ± 0.2 mL min^{-1}) for GFLX and OFLX or (1.5 ± 0.2 mL min^{-1}) for NFLX. Deviations in peak area and retention were calculated to evaluate the robustness of quantitative and qualitative responses, respectively.

Pharmaceutical applications were applied by accurately transfer of aliquot from Tymer™ sterile ophthalmic solution equivalent to 7.5-mg GFLX, amount of powdered Tarivid® tablets equivalent to 10-mg OFLX, and portion of Nadvulg cream equivalent to 10 mg NFLX, into three separate 50-mL volumetric flasks. Tablet and cream solutions were sonicated for 15 minutes in 30-mL methanol before completing the volumes to the mark with methanol. All pharmaceutical solutions were filtered through membrane filter 0.2 μm then analyzed under the corresponding chromatographic conditions.

3 | RESULTS AND DISCUSSION

Three structurally different fluoroquinolone antibiotics were used in this study. Gatifloxacin is 8-methoxy quinoline derivative (Figure 1A). Its chirality is attributed to the substitution at C3 of the piperazine ring. This side-chain stereogenic center is located in the vicinity of secondary amino group of piperazine ($pk_a \approx 9.8$), whereas both OFLX and NFLX feature chiral substituents at the N-1 position of the quinolone core. In OFLX, the chiral center is distal from the tertiary amine ($pk_a \approx 8.1$) present in the side chain (Figure 1B). On the other hand, NFLX lacks this distal basic functionality in the side chain and has only one acidic pKa (Figure 1C).[3] Owing to this chirality and functionality differences among GFLX, OFLX, and NFLX, we mapped these congeners onto structure-retention relationship.

Polysaccharide stationary phases play a prominent role in chiral separation.[29] Resolution of enantiomers is substantially controlled by mobile phase composition.[30] In the initial assessment of mobile phase on normal mode chromatography, ethanol was mixed with either hexane or acetonitrile in different ratios. The mobile phases were pumped with 1.0 mL min^{-1} on Lux-Cellulose-2 column at 25°C. Fixed amounts of 0.2% acetic acid and 0.1% diethylamine were used as mobile phase additives in this primary trials which provided satisfactory peak shape (tailing factor ≤ 2). It was found that decreasing hexane in mobile phase would decrease retention of enantiomers. When hexane decreased from 30% to 10%, about 20% decrease in retention of GFLX and OFLX was observed, whereas retention of NFLX decreased by about 35%. The

higher impact of changing mobile phase polarity on NFLX is probably due to its low polarity (log$P$ = 1.9), whereas GFLX (log$P$ = −1.1) and OFLX (log$P$ = −2.2) are strongly adsorbed polar fluoroquinolones on normal phase, compared with less polar NFLX with lipophilic tricyclic benzoquinolizine core. On the other hand, resolution of GFLX never exceeds one while OFLX and NFLX show sufficient resolution. A comparable retention can be also achieved by using 90% acetonitrile instead of hexane. Noticeable increase in GFLX and OFLX enantiomer resolution was achieved while decreased for less polar NFLX enantiomers. Based on the above-mentioned results and for achieving suitable resolution and retention, the proposed mobile phases were acetonitrile-ethanol (90:10 vol/vol) for GFLX and OFLX enantiomers and hexane-ethanol (10:90 vol/vol) for NFLX enantiomers. Flow rates of 2.0 and 1.5 mL min^{-1} were used for acetonitrile and hexane containing mobile phases, respectively.

Acidic and basic modifiers are acknowledged to change the retention behavior of enantiomers[31] to study the activity of amino groups and their position with respect to chiral centers. Acidic modifier has a priority over the basic one in our design. All the studied fluoroquinolones have carboxylic moiety; therefore, basic modifier is secondary to be considered. We fixed diethylamine% at low level (0.1%) to subtly estimate the effect of changing acetic acid%. Level of acetic acid was changed from 0.2% to 0.4%. It is worth noting that total amount of additives should not exceed 0.5% as per column user manual. The second factor in this study was column temperature. Temperature plays a principal role in thermodynamics of retention process, affecting retention factors, and selectivity of ionizable compounds.[32] Temperature levels were raised from 25 to 35°C to facilitate the fast elution of peaks and hence fast analysis. Least squares fit model was applied to estimate the factors effects on resolution, selectivity, and capacity factors. Quadratic terms were too trivial to be included in prediction formulae, and only main effects were included for optimization purpose. All regression models had an excellent fit to the experimental data with an adjusted R value above 0.992 in all cases.

Relative importance of the studied two factors on each response were quantified and statistically compared. Calculated coefficients along with their corresponding P values are summarized in Table 1.

The table shows that acetic acid has significant effects on all GFLX responses which possesses primary amino group close to its side chain chiral center. The positive coefficients of this factor on GFLX indicate improved enantiomeric resolution and selectivity at high levels of acetic acid% (Figure 2A and B). Positive significant effects of acetic acid% on GFLX enantiomer capacity factors were also registered and depicted in Figure 2C. The figure shows that GFLX enantiomers have completely different migration rates by increasing acetic acid% which reveals the favorable effect of adjacent amino ionization on chiral recognition. Another explanation is that acetic acid masks some nonenantioselective retention sites on stationary phase; thus, enantioselective recognition sites are dominating.[33] On contrary, increasing percentage of acetic acid significantly decreases both resolution and enantionselectivity of OFLX with a core chiral center away from the secondary amino group. These negative coefficients on OFLX can be also observed in the sharp decline of responses in (Figure 2A and B). Acetic acid% has opposite effect on OFLX enantiomer capacity factors as concluded from positive coefficients in Table 1. This effect is significant on the first eluted OFLX enantiomer where its migration rate becomes close to the more retained enantiomer at high level of acetic acid% (Figure 2D). Increased retention with lower resolution and selectivity at high levels of acetic acid% is likely attributed to the ionization of distal amino group which augments the nonenantioselectivity hydrogen bond interaction with residual silanol active sites. Nadifloxacin lacks this ionizable amino group, and hence, no significant effect was registered for acetic acid% (Table 1). Acetic acid% has

TABLE 1 Parameter estimates of the effect of factors on responses for the studied fluoroquinolones enantiomers

Fluoroquinolone		Gatifloxacin		Ofloxacin		Nadifloxacin	
Factor	**Response**	**Estimate**	***P* Value**	**Estimate**	***P* Value**	**Estimate**	***P* Value**
Acetic acid %	Resolution (R_s)	5.3	.034[a]	−7.3	.030[a]	−0.375	.702
	Selectivity (α)	0.349	.042[a]	−1.242	.031[a]	0.192	.352
	Capacity factor ($k'1$)	10.375	.033[a]	6.025	.031[a]	−3.370	.324
	Capacity factor ($k'2$)	15.435	.017[a]	2.613	.364	−3.444	.341
Temperature	Resolution (R_s)	−0.046	.147	0.187	.019[a]	−0.038	.158
	Selectivity (α)	−0.005	.078	0.011	.126	−0.008	.144
	Capacity factor ($k'1$)	−0.239	.025[a]	−0.111	.036[a]	−0.182	.073
	Capacity factor ($k'2$)	−0.323	.016[a]	−0.112	.129	−0.343	.025[a]

[a]Significant estimates.

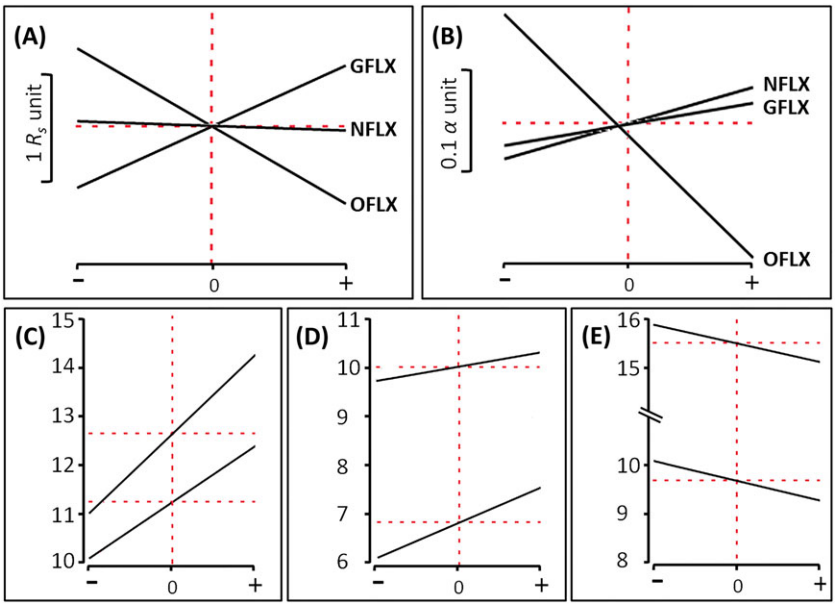

FIGURE 2 Prediction profiles of the effect of acetic acid % in mobile phase on resolution A, selectivity B, capacity factors of Gatifloxacin C, Ofloxacin D, and Nadifloxacin E

counteracting effects on resolution and selectivity. Working at low level of acetic would improve resolution but worsen the selectivity. Also, nonsignificant negative effects of acetic acid% on NFLX enantiomer capacity factors are registered in Table 1. Similar effect of acetic acid on migration rates of NFLX enantiomers can be recognized from their close numerical coefficients. Two parallel prediction profiles of this response in Figure 2E indicate a balance between enantioselective and nonselective interactions. The higher capacity factors of NFLX enantiomers at low level of acetic may be attributed to relatively higher ratio of diethylamine at this investigated level. Diethylamine could possibly endow NFLX enantiomers with some polar character through ionization of carboxylic acid ($pk_a \approx 6.8$), increasing their retention.

Equilibrium mass transfer of enantiomers from mobile phase to stationary phase is usually an exothermic process. For this reason, negative coefficients were registered for most of responses in Table 1. Negative effects of temperature were registered for resolution, selectivity, and retention for GFLX and NFLX enantiomers. These effects are significant on capacity factor for GFLX and most retained NFLX enantiomers. Therefore, working at high level of column temperature would significantly decrease retention and insignificantly decrease resolution and selectivity of GFLX and NFLX. Quite uncommon finding is the positive effect of temperature on OFLX enantiomeric resolution and selectivity. Unique temperature-induced effect of some other enantiomers was discussed earlier for acidic drugs on polysaccharide-based CSPs[34,35] and amino acids on *Cinchona* alkaloid-based zwitterionic CSPs.[36,37]

Coefficients in Table 1 show that the retention of OFLX enantiomers decreased while resolution and selectivity improved with increasing temperature. This unusual OFLX enantiomer behavior could be ascribed to the attenuated chiral nonselective interaction of amino group at high temperature, and separation is mostly based on chiral recognition mechanisms.

Moreover, similar extent of temperature effect on the retentions of OFLX enantiomers, suggesting that adsorption–desorption kinetics is probably related to the distal basic functionality.

Based on desirability function,[38] multiple responses have been compromised for optimum resolution of drugs. Using 0.4% acetic acid as an additive in mobile phase is optimum for the separation of GFLX and NFLX enantiomers, while 0.2% acetic acid in mobile phase is optimum for OFLX enantiomers. Column temperature is optimum at 35°C for drugs under study. The optimized chromatographic conditions achieved the design targets of separating GFLX, OFLX, and NFLX enantiomers with high resolution and selectivity along with moderate retentions (Figure 3).

System suitability parameters were calculated in Table 2 indicating satisfactory results for the chromatographic parameter. The methods were finally validated according to the International Conference on Harmonization guidelines for validation of analytical procedures.[28] Linear regression parameters obtained from the calibration curves for each enantiomer in the specified concentration range were provided in Table 3.

Repeatability and intermediate precision were checked and expressed in %RSD values. The calculated LOD and LOQ were also illustrated in Table 3. Robustness of the method against minor changes was examined and almost the obtained %RSD results upon changing column temperature (35 ± 2°C) were less than 8% in both

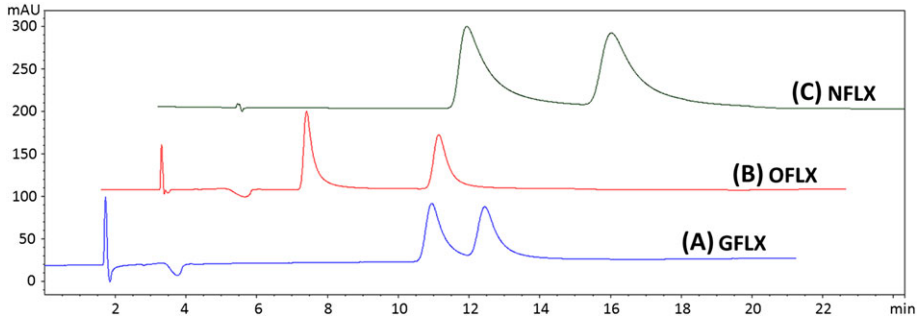

FIGURE 3 Separation of the studied fluoroquinolones on Lux-cellulose-2 thermostated at 35°C by using acetonitrile-ethanol (90:10 vol/vol) as mobile phase either with acetic acid (0.4%) and diethylamine (0.1%) for Gatifloxacin A, or with acetic acid (0.2%) and diethylamine (0.1%) for Ofloxacin B, at 2-mL min^{-1} flow rate while using hexane-ethanol (10:90 vol/vol) as mobile phase with acetic acid (0.4%) and diethylamine (0.1%) for NFLX at 1.5-mL min^{-1} flow rate (C)

TABLE 2 System suitability parameters for the proposed chromatographic separation of the studied fluoroquinolones

	Gatifloxacin Enantiomers		Ofloxacin Enantiomers		Nadifloxacin Enantiomers	
Parameter	**1**	**2**	**1**	**2**	**1**	**2**
Retention time (min)	10.8	12.3	5.8	9.6	8.7	12.8
Capacity factor (k')	5.5	6.4	2.45	4.68	2.9	4.8
Symmetry factor	1.3	1.5	1.2	1.3	1.9	1.5
Resolution (R_s)	1.8		6.7		2.9	
Selectivity (α)	1.2		1.9		1.7	
Theoretical plates (N)	2981	3408	2947	3074	699	1133

TABLE 3 Regression and validation parameters of the proposed chromatographic methods for the determination of studied fluoroquinolone enantiomers in bulk powder

	Gatifloxacin Enantiomer		Ofloxacin Enantiomer		Nadifloxacin Enantiomer	
Parameter						
Linearity	**1**	**2**	**1**	**2**	**1**	**2**
Concentration range ($\mu g\ mL^{-1}$)	100-900		100-900		100-900	
Slope	5.32	4.88	5.04	5.21	4.96	4.91
Intercept	−218.93	−113.76	−68.49	−88.10	−196.09	−165.44
Correlation coefficient	0.9998	0.9997	0.9997	0.9993	0.9997	0.9999
Accuracy (mean ± SD)	100.54 ± 1.436	99.98 ± 0.384	98.80 ± 1.295	99.58 ± 1.503	99.44 ± 1.912	99.88 ± 1.158
Precision (%RSD)						
Repeatability	1.227	1.976	1.161	1.043	1.121	1.269
Intermediate precision	1.656	2.098	1.507	1.771	1.895	1.711
LOD ($\mu g\ mL^{-1}$)	24.97	25.18	18.92	22.88	29.58	27.43
LOQ ($\mu g\ mL^{-1}$)	75.67	76.32	57.35	69.32	89.64	83.11

peak area and retention time of all enantiomers. Quantitative and qualitative GFLX responses were barely affected (%RSD < 4%) by changing flow rate (2.0 ± 0.2 mL min^{-1}). Also little effect of flow rate was encountered for OFLX peak area (%RSD < 9%) and retention (%RSD < 12%). However, changing flow rate (1.5 ± 0.2 mL min^{-1}) has apparent effect on NFLX peak area (%RSD < 12%) and retention (%RSD < 16%). Wide flow rate variation in robustness study for NFLX (about 13% of nominal value) explains its larger deviation values.

TABLE 4 Determination of studied fluoroquinolone enantiomers in their pharmaceutical formulations by the proposed and reported methods

Parameter	Gatifloxacin			Ofloxacin			Nadifloxacin		
	Proposed Method		Reported Method[39 a]	Proposed Method		Official Method[40 b]	Proposed Method		Reported Method[39 c]
	Enantiomer 1	Enantiomer 2		Enantiomer 1	Enantiomer 2		Enantiomer 1	Enantiomer 2	
Mean	100.88	100.94	100.25	100.64	100.32	99.80	99.63	100.08	98.70
SD	1.013	0.879	0.433	1.240	0.841	1.095	0.947	0.881	0.837
N	6	6	6	6	6	6	6	6	6
Variance	2.027	1.758	0.866	2.480	1.682	2.191	1.894	1.762	1.673
t (2.306)[d]	0.907	1.043	—	0.952	0.647	—	1.206	1.829	—
F (5.05)[d]	2.34	2.03	—	1.13	1.30	—	1.13	1.05	—

[a]HPLC method using C18 column as stationary phase and acetonitrile-phosphate buffer pH 3.3 (25:75, vol/vol) as a mobile phase with 1-mL min^{-1} flow rate and UV detection at 293 nm.

[b]HPLC method using C18 column as stationary phase and acetonitrile-phosphate buffer pH 3.3 (3:22, vol/vol) as a mobile phase with 1 mL min^{-1} flow rate and UV detection at 294 nm.

[c]HPTLC using methanol-ethyl acetate-toluene-acetonitrile-3 M ammonium formate in water (1:2.5:6.0:0.3:0.2, by volume) as mobile phase and UV detection at 224 nm.

[d]The values in parentheses are the corresponding theoretical values for t and F at $P = .05$.

The proposed methods have been successfully used for the determination of GFLX in opthalmic solution, OFLX in tablets, and NFLX in cream formulation with good accuracy and precision (Table 4). Statistical comparison between results of pharmaceutical analyses obtained by the proposed and reported or official method for GFLX,[40] OFLX,[41] and NFLX[39] showed no significant difference at .05 probability.

4 | CONCLUSION

Fluoroquinolones with distinctive structural features exhibit different retention activities on Lux-Cellulose-2. The most suitable organic modifier in ethanol as a mobile phase is either acetonitrile for GFLX and OFLX or hexane for the less polar NFLX. Experimentally designed optimization of acidic modifier and column temperature revealed the extent by which resolution and retention of these enantiomers are affected. The proximity of basic functionality to chiral center is decidedly improving GFLX enantiomeric recognition at high acidic modifier%, while increased acidity may attenuate the chiral recognition of OFLX which possess distal basic group and core chiral center. Remarkable similar retention of acidic fluoroquinolone enantiomers with core chiral center was registered. Nadifloxacin enantiomers showed balanced chiral selective and nonselective interactions. Results emphasized the important complementary effect of chemical functionality on enantiomeric discrimination. Temperature effect is typically an exothermic process; however, increased resolution and selectivity at high column temperature were registered for OFLX. The proposed chromatographic conditions meet the design targets of optimum resolution, selectivity, and retention. The analytical procedures were validated and applied successfully for pharmaceutical analyses.

ACKNOWLEDGMENT

This work was supported by the National Research Centre, Dokki, Giza, Egypt (project no. 11010311).

ORCID

Hassan Y. Aboul-Enein http://orcid.org/0000-0003-0249-7009

REFERENCES

1. Drusano G, Labro M-T, Cars O, et al. Pharmacokinetics and pharmacodynamics of fluoroquinolones. *Clin Microbiol Infect*. 1998;4:2S27-22S41.

2. Domagala JM, Hanna LD, Heifetz CL, et al. New structure-activity relationships of the quinolone antibacterials using the target enzyme. The development and application of a DNA gyrase assay. *J Med Chem*. 1986;29(3):394-404.
3. de Souza NJ, Gupte SV, Deshpande PK, et al. A chiral benzoquinolizine-2-carboxylic acid arginine salt active against vancomycin-resistant Staphylococcus aureus. *J Med Chem*. 2005;48(16):5232-5242.
4. Morrissey I, Hoshino K, Sato K, et al. Mechanism of differential activities of ofloxacin enantiomers. *Antimicrob Agents Chemother*. 1996;40(8):1775-1784.
5. FDA policy statement for the development of new stereomeric drugs. *Chirality*. 1992;4:338-340.
6. Ali I, Aboul-Enein HY, Ghanem A. Enantioselective toxicity and carcinogenesis. *Curr Pharm Anal*. 2005;1(1):109-125.
7. Basheer A. Chemical chiral pollution: impact on the society and science and need of the regulations in the 21st century. *Chirality*. 2017;1-5. https://doi.org/10.1002/chir.22808
8. Sousa J, Alves G, Fortuna A, Falcão A. Analytical methods for determination of new fluoroquinolones in biological matrices and pharmaceutical formulations by liquid chromatography: a review. *Anal Bioanal Chem*. 2012;403(1):93-129.
9. Grellet J, Ba B, Saux MC. High-performance liquid chromatographic separation of fluoroquinolone enantiomers: a review. *J Biochem Biophys Methods*. 2002;54(1-3):221-233.
10. Ali I, Suhail M, Asnin L. Chiral separation of quinolones by liquid chromatography and capillary electrophoresis. *J Sep Sci*. 2017;40(14):2863-2882.
11. Asnin L, Ali I. Chiral chromatography of quinolones: trends and application in the analysis of fluoroquinolone antibiotics. *Bull Perm State Pharmaceut Acad No*. 2016;18:43-44.
12. Machida M, Izawa S, Hori W, Ishida R, Uchida H. Pharmacokinetics of gatifloxacin, a new quinolone, and its enantiomers: II. Enantioselective method for the determination of gatifloxacin and its application to pharmacokinetic studies in animals. *Jpn J Chemo*. 1999;47(Suppl 2):124-130.
13. Liu Y, Wang X. Enantioseparation of ofloxacin and its four related substances with ligand exchange-micellar electrokinetic chromatography using copper(II)-L-isoleucine complex as chiral selector. *Chirality*. 2017;29(8):422-429.
14. Liang X, Zhao L, Deng M, Liu L, Ma Y, Guo X. Separation of ofloxacin and its six related substances enantiomers by chiral ligand-exchange chromatography. *Chirality*. 2015; 27(11):843-849.
15. Bi W, Tian M, Row KH. Chiral separation and determination of ofloxacin enantiomers by ionic liquid-assisted ligand-exchange chromatography. *Analyst*. 2011;36:379-387.
16. Shao B, Sun X, Zhang J, Hu J, Dong H, Yang Y. Determination of ofloxacin enantiomers in sewage using two-step solid-phase extraction and liquid chromatography with fluorescence detection. *J Chromatogr A*. 2008;1182(1):77-84.
17. Yan H, Row KH. Rapid chiral separation and impurity determination of levofloxacin by ligand-exchange chromatography. *Anal Chim Acta*. 2007;584(1):160-165.
18. Garcia MA, Solans C, Calvo A, et al. HPLC separation and quantification of ofloxacin enantiomers in rabbit plasma. Application to pharmacokinetic studies. *Chromatographia*. 2002;56(1-2):39-42.
19. Wong FA, Juzwin SJ, Flor SC. Rapid stereospecific high-performance liquid chromatographic determination of levofloxacin in human plasma and urine. *J Pharm Biomed Anal*. 1997;15(6):765-771.
20. Dutt Sharma S, Singh G. Enantioseparation of nadifloxacin by high performance liquid chromatography. *Adv Anal Chem*. 2012;2:25-31.
21. Yeole RD, Jadhav AS, Patil KR, et al. Validated chiral high-performance liquid chromatography method for a novel anti-methicillin-resistant staphylococcus aureus fluoroquinolone WCK 771. *J Chromatogr A*. 2006;1108(1):38-42.
22. Bhavyasri K, Rambabu D, Prasad PSS, Balaram VM. Separation of the two enantiomers of gatifloxacin by SFC on amylose based stationary phase. *J Chem Pharm Res*. 2012;4:4915-4920.
23. Nirogi R, Kota S, Vennila S, et al. High-performance liquid chromatographic method for the separation of enantiomeric gatifloxacin. *J Chromatogr Sci*. 2010;48(2):100-103.
24. Kannappan V, Mannemala SS. Multiple response optimization of a HPLC method for the determination of enantiomeric purity of S-ofloxacin. *Chromatographia*. 2014;77(17-18):1203-1211.
25. Sun X, Wu D, Shao B, Zhang J. High-performance liquid-chromatographic separation of ofloxacin using a chiral stationary phase. *Anal Sci*. 2009;25(7):931-933.
26. Maia AS, Castro PML, Tiritan ME. Integrated liquid chromatography method in enantioselective studies: biodegradation of ofloxacin by an activated sludge consortium. *J Chromatogr B*. 2016;1029:174-183.
27. Fang Z, Guo Z, Qin Q, Fan J, Yin Y, Zhang W. Semi-preparative enantiomeric separation of ofloxacin by HPLC. *J Chromatogr Sci*. 2012;51:133-137.
28. Guideline ICH. Validation of analytical procedures: text and methodology 2005; Q2 (R1).
29. Ali I, Aboul-Enein HY. Impact of immobilized polysaccharide chiral stationary phases on enantiomeric separations. *J Sep Sci*. 2006;29(6):762-769.
30. Aboul-Enein HY, Ali I. Optimization strategies for HPLC enantioseparation of racemic drugs using polysaccharides and macrocyclic glycopeptide antibiotic chiral stationary phases. *Il Farmaco*. 2002;57(7):513-529.
31. Mosiashvili L, Chankvetadze L, Farkas T, Chankvetadze B. On the effect of basic and acidic additives on the separation of the enantiomers of some basic drugs with polysaccharide-based chiral selectors and polar organic mobile phases. *J Chromatogr A*. 2013;1317:167-174.
32. Gagliardi LG, Tascon M, Castells CB. Effect of temperature on acid–base equilibria in separation techniques. A review. *Anal Chim Acta*. 2015;889:35-57.
33. Ye YK, Stringham RW. Effect of mobile phase acidic additives on enantioselectivity for phenylalanine analogs. *J Chromatogr A*. 2001;927(1-2):47-52.

34. Matarashvili I, Chankvetadze L, Tsintsadze T, Farkas T, Chankvetadze B. HPLC separation of enantiomers of some chiral carboxylic acid derivatives using polysaccharide-based chiral columns and polar organic mobile phases. *Chromatographia*. 2015;78(7-8):473-479.

35. Matarashvili I, Chankvetadze L, Fanali S, Farkas T, Chankvetadze B. HPLC separation of enantiomers of chiral arylpropionic acid derivatives using polysaccharide-based chiral columns and normal-phase eluents with emphasis on elution order. *J Sep Sci*. 2013;36(1):140-147.

36. Ilisz I, Gecse Z, Pataj Z, et al. Direct high-performance liquid chromatographic enantioseparation of secondary amino acids on Cinchona alkaloid-based chiral zwitterionic stationary phases. Unusual temperature behavior. *J Chromatogr A*. 2014;1363:169-177.

37. Ilisz I, Pataj Z, Gecse Z, et al. Unusual temperature-induced retention behavior of constrained β-amino acid enantiomers on the zwitterionic chiral stationary phases ZWIX(+) and ZWIX(−). *Chirality*. 2014;26(8):385-393.

38. Derringer G, Suich R. Simultaneous optimization of several response variables. *J Qual Technol*. 1980;12(4):214-219.

39. Aljuffali IA, Kalam MA, Sultana Y, Imran A, Alshamsan A. Development and validation of stability-indicating high performance liquid chromatography method to analyze gatifloxacin in bulk drug and pharmaceutical preparations. *Saudi Pharm J*. 2015;23(1):85-94.

40. United States Pharmacopoeia. United States Pharmacopeial Convention, Rockville, Maryland, USA; 2017. 5413–5415.

41. Patel KG, Shah PM, Shah PA, Gandhi TR. Validated high-performance thin-layer chromatographic (HPTLC) method for simultaneous determination of nadifloxacin, mometasone furoate, and miconazole nitrate cream using fractional factorial design. *J Food Drug Anal*. 2016;24(3):610-619.

How to cite this article: Hassan RM, Yehia AM, Saleh OA, El-Azzouny AA, Aboul-Enein HY. Structure-retention relationship for enantioseparation of selected fluoroquinolones. *Chirality*. 2018;1–9. https://doi.org/10.1002/chir.22861