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Direct liquid chromatographic enantiomer separation of new fluoroquinolones including gemifloxacin ☆

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Abstract

The enantiomers of gemifloxacin mesylate (formerly LB20304a), a new fluoroquinolone compound with potent in vitro and in vivo antibacterial profile were resolved on a commercially available Crownpak CR chiral stationary phase (CSP). All of the fluoroquinolones, including gemifloxacin used in this study, were well enantioseperated on Crownpak CR(+) column. These results are the first reported for the direct separation of the enantiomers of quinolones on chiral crown ether coated Crownpak CR CSP. The behavior of chromatographic parameters by the change of mobile phase additives for the resolution of gemifloxacin was investigated. Also, the effect of structural change of gemifloxacin on chiral recognition was described.

Keywords: Enantiomer separation; Gemifloxacin; Fluoroquinolones

1. Introduction

Since methods that enable simple and accurate assessment of the enantiomeric purity of chiral compounds are frequently required for those in the fields of pharmaceutical chemistry and biochemistry, they have been developed for these tasks [1,2]. Among various techniques, liquid chromatographic separation of the enantiomers on CSPs is considered to be one of the most accurate and convenient means inherent to this technique in determining the enantiomeric purity of chiral compounds.

Recently, racemic gemifloxacin mesylate (formerly LB20304a), an investigational new fluoroquinolone with potent in vitro and in vivo antibacterial profile, has been developed as a chemotherapeutic agent for various infections (Fig. 1). The

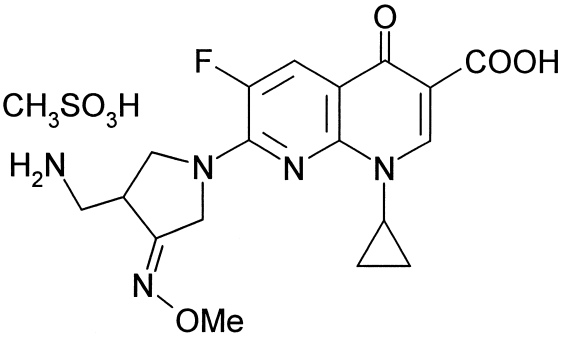

Fig. 1. The structure of gemifloxacin mesylate (formerly LB20304a).

☆Gemifloxacin is the generic name of LB20304a. This work was presented at the 9th International Symposium on Chiral Discrimination, Nagoya, Oct. 27–30, 1997.

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efficacy of gemifloxacin is better than that of ciprofloxacin and ofloxacin in terms of its good in vitro antibacterial activity as well as in vivo pharmacokinetic characteristics [3–7]. Subsequent studies have demonstrated that the antibiotic efficacies of the two enantiomers of gemifloxacin are biologically equivalent to that of racemic gemifloxacin [8]. In order to support the development of the racemate it was necessary to confirm that the two enantiomers were equipotent, and analytical methodology was required in support of this work [9].

For the enantiomer separation of quinolones, however, only a few results have been reported using bovine serum albumin derived CSP and ligand-exchange HPLC [10–13]. The liquid chromatographic method on bovine serum albumin derived CSP has been employed for the determination of only ofloxacin enantiomers and it was not applied for resolution of other quinolone compounds [10,11]. Although the enantioresolution of some ofloxacin-related compounds has been achieved by ligand-exchange chromatography, this method did not provide a good level of enantioselectivity, affording partial separations for three out of six analytes examined [12,13]. Recently, capillary electrophoresis was used to resolve ofloxacin-related quinolone derivatives with poor enantioselectivity (R_s=0.20–0.95) for most of the analytes [14,15].

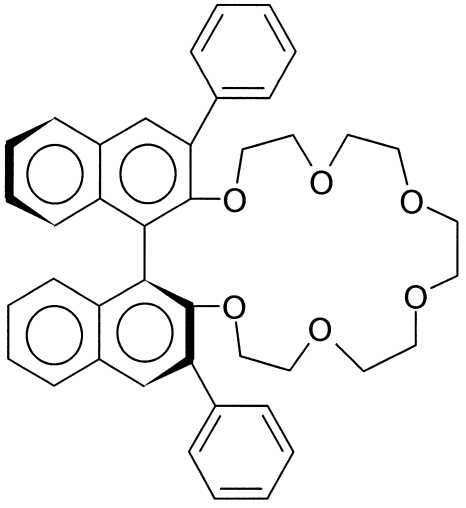


Fig. 2. The structure of chiral crown ether utilized in the Crownpak CR(+).

In this paper, we present the direct analytical HPLC separation of the enantiomers of several fluoroquinolones including gemifloxacin on a chiral crown ether coated Crownpak CR(+) column (Fig. 2).

2. Experimental

2.1. Apparatus

Chromatography was performed at room temperature using an HPLC consisting of a Waters model 510 pump, a Rheodyne model 7725 injector with a 20 μl loop, a variable wavelength UV detector Waters 490 operated at 272 nm and a Waters 746 data module integrating recorder. All separations of quinolone compounds were carried out at ambient temperature (approximately 25°C) with the use of a flow-rate of 1.2 ml/min. The injected volume of the sample was 1–3 μl of 2 mg/ml, unless specified otherwise. Crownpak CR(+) (150×4 mm I.D., 5 μm packing) was purchased from Daicel Chemical Company (Tokyo, Japan). The Crownpak CR chiral column was found to be equally effective for chiral separation after the use of more than 1 year under our experimental conditions.

2.2. Chemicals

HPLC-grade methanol was obtained from J.T. Baker. Water was purified using a milli-Q water purification system (Bedford, MA, USA). All acid chemicals were of analytical-reagent grade. Sulfuric acid, hydrochloric acid and perchloric acid were obtained from Fluka company (Switzerland). Trifluoroacetic acid was obtained from Aldrich (Milwaukee, WI). Methanesulfonic acid was obtained from Janssen Chimica (Belgium). All analytes used in this study were available from Biotec Research Institute in LG Chemical Ltd. [3,4].

3. Results and discussion

Chiral crown ether coated Crownpak CR column has been developed for the direct resolution of the enantiomers of amino acids [16,17]. Since it has

been found to be applicable in the resolution of racemic primary amino compounds [18], we attempted to separate the enantiomers of 3-aminomethyl-(*Z*)-4-methoxyimino pyrrolidine dihydrochloride, a racemic primary amine HCl salt as a precursor of gemifloxacin. And it was found that they are readily resolved on a Crownpak CR(+) column (separation factor 2.65). The chromatogram of its direct enantiomeric separation using pH 1.0 $HClO_4$ as a mobile phase is shown in Fig. 3. Since the enantiomers of a precursor of gemifloxacin were separated on Crownpak CR, we investigated to resolve racemic gemifloxacin on the same CSP. Because the Crownpak CR chiral column is prepared by dynamic coating of chiral crown ether on reversed-phase packing, it is recommended that a mobile phase that contains more than 15% methanol is not used for this column. Since the retention times are decreased with an increase in methanol concentration, 15% methanol in water containing acid additive was used as a mobile phase in this experiment. Although some fluoroquinolones used in this study have tremendous hydrophobicity compared to amino acids, all injected analytes with zwitterion structure eluted well, not causing any problem in spite of the long retention times on Crownpak CR CSP. For reduced retention times of some analytes in our study, 20% methanol in water containing acid additive has been used, but this harsh operation condition has been avoided for column safety.

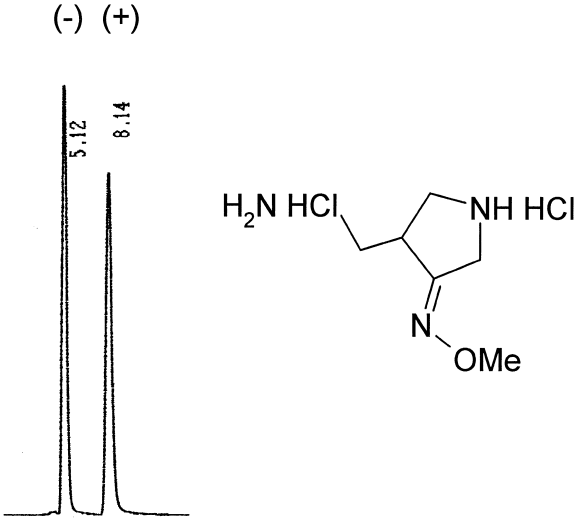


Fig. 3. Resolution of the racemic amine precursor of gemifloxacin on a Crownpak CR(+) CSP; mobile phase=pH 1.0 $HClO_4$; flow-rate=0.5 ml/min; UV 210 nm; injection amount 7.5 μg.

Table 1 and Fig. 4 show the effect of acid additives on direct separation of the enantiomers of gemifloxacin on Crownpak CR. These data show that chromatographic parameters such as resolutions and retention times are greatly influenced by the nature of acid additive in the mobile phase. Among several acids used, the best separation is obtained using sulfuric acid (entry 1). The use of perchloric acid, the most commonly used acid provides increased retention times and worse resolution (entry 4). It has been observed that an increase in the lipophilicity of the acid anion leads to significantly enhanced retention times due to relatively poor solvation of lipophilic anion by solvent molecules [16]. Here, the use of perchloric acid as an acid additive in the mobile phase provides a great increase of retention times than that of chloric acid, since the perchloric anion is more lipophilic than the chlorine anion (entries 3 and 4) [16]. Also, an increase in the acid concentration (or a lowering of the pH) in the mobile phase increases the retention times with better resolution (entries 1 and 6, entries 4 and 7) [19]. The pH of the mobile phase affects more significantly the chromatographic parameter than the lipophilicity of the acid anion [16]. As an example, although methanesulfonic acid has a more lipophilic acid

Table 1
Effect of acid additive on direct separation of the enantiomers of gemifloxacin on Crownpak CR[a]

Entry	Acid additive	α[c]	k_1'[d]	R_s[e]
1	10 m*M* H_2SO_4	1.53	34.03	3.75
2	10 m*M* CH_3SO_3H	1.59	31.24	1.85
3	10 m*M* HCl	1.61	31.65	1.87
4	10 m*M* $HClO_4$	1.78	96.08	3.60
5	10 m*M* CF_3COOH	1.60	61.23	3.63
6	5 m*M* H_2SO_4	1.53	28.96	2.78
7	114 m*M* (pH 1) $HClO_4$	2.14	193.59	3.72
8	14 m*M* CF_3COOH	1.51	127.24[b]	4.09

[a] Mobile phase: acid additive in water–methanol (85:15, v/v); see Experimental for chromatographic conditions.
[b] Data using 14 m*M* trifluoroacetic acid in water–methanol (90:10, v/v) as a mobile phase.
[c] Separation factor.
[d] Capacity factor for the first eluted enantiomer.
[e] Resolution factor.

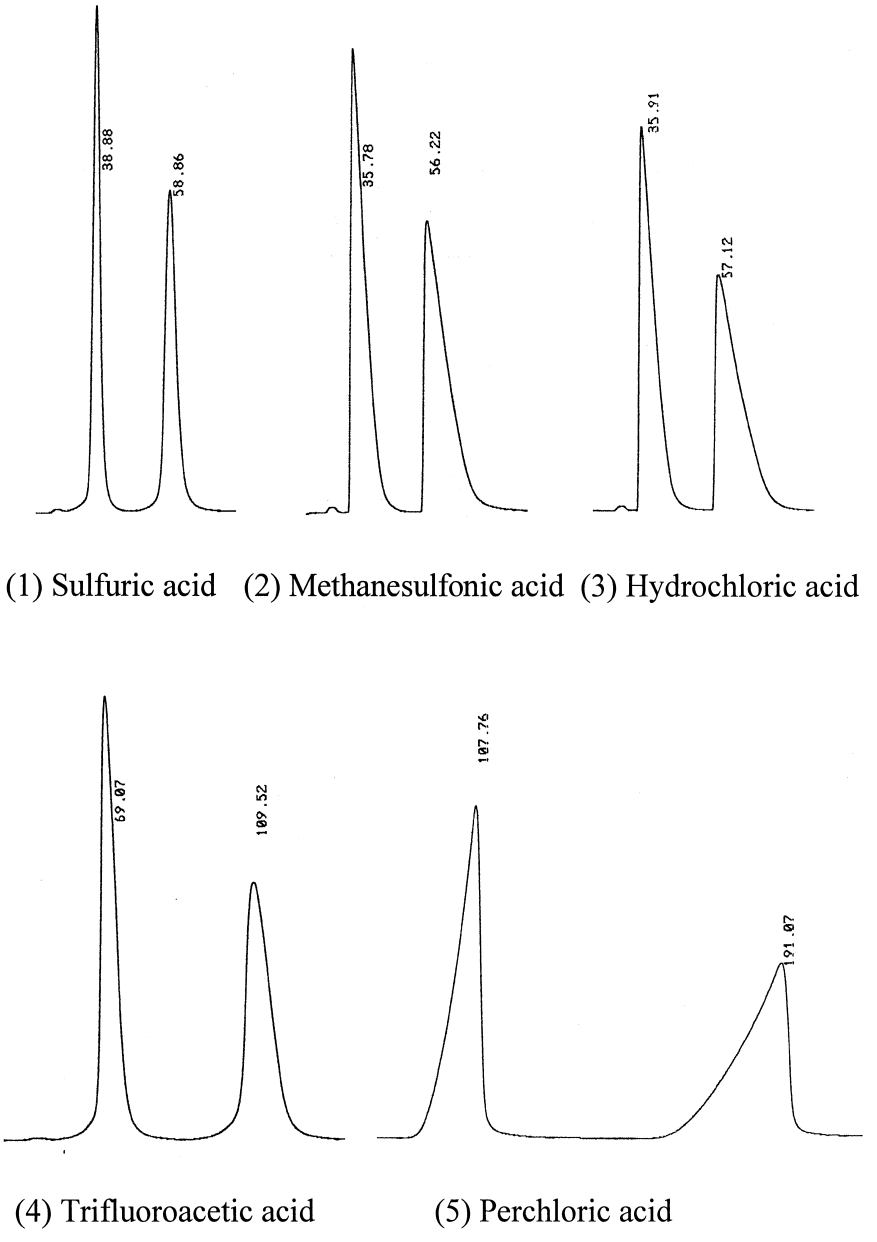


Fig. 4. Resolution of gemifloxacin on Crownpak CR CSP; mobile phase: 10 m*M* acid additive in water–methanol (85:15, v/v); flow-rate=1.2 ml/min; UV 272 nm; injection amount 60 μg.

anion than sulfuric acid, the use of the former acid gives slightly shorter retention times than that of the latter acid, because 10 m*M* sulfuric acid solution (pH 2.16) is more acidic than 10 m*M* methanesulfonic acid solution (pH 2.36) (entries 1 and 2). For the same reason, it can be explained that the use of trifluoroacetic acid, the most lipophilic acid in our study gives much shorter retention times than that of perchloric acid (entries 4 and 5).

Fig. 5 shows chromatograms of the direct enantiomer separation of various fluoroquinolone compounds including gemifloxacin. All of the fluoro-

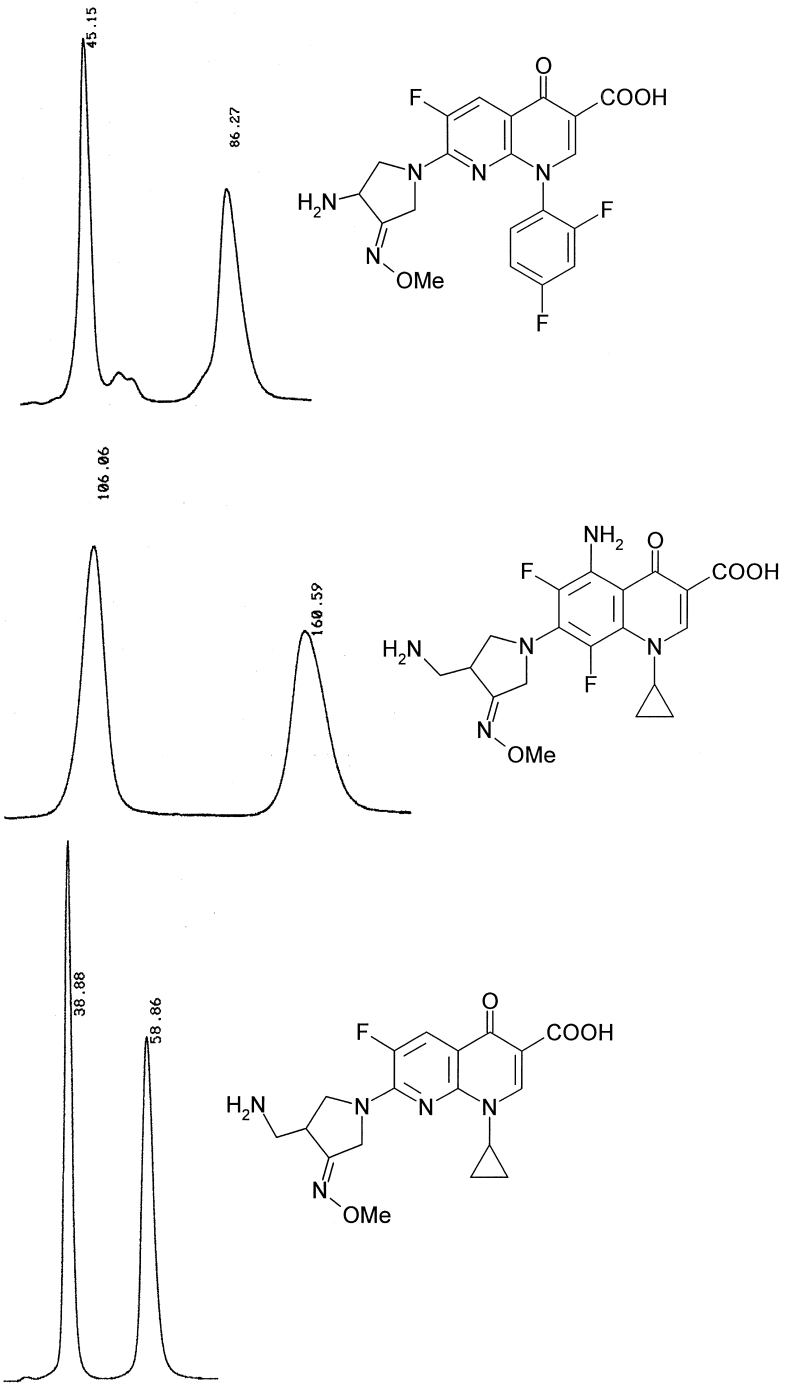


Fig. 5. Chromatograms of the direct enantiomer separation of fluoroquinolone compounds including gemifloxacin. Mobile phase: 10 m*M* H_2SO_4 in water–methanol (85:15, v/v). See Experimental for chromatographic conditions.

quinolones used in this study are well resolved on the Crownpak CR(+) column. Figs. 6 and 7 show examples of determination of the enantiomeric purity of two enantiomerically enriched samples. Elution orders were determined only for two configurationally known quinolones, the (*S*)-enantiomers being preferentially retained on Crownpak CR(+).

Table 2 shows direct separation of the enantiomers of gemifloxacin and its analogs on Crownpak CR. For the enantioresolution of (*E*)-LB20304 instead of gemifloxacin with (*Z*)-methoxyimine moiety, it affords greatly decreased elution time and marginal enantioselectivity (entry 2). When the methoxyimine group of pyrrolidone on gemifloxacin is replaced with the ketone moiety, the separation factor for its enantiomers is diminished (entry 3). The absence of the methoxyimine group from gemifloxacin provides significantly lowered enantioselectivity (entry 4). It is well known that a primary interaction between the ammonium ion of the analyte and the oxygens of the crown ether of the chiral selector leads to the formation of the transient diastereomeric complex [20]. From the study of CPK molecular models, all of the data in Table 2 suggests that when the aminomethyl group on gemifloxacin interacts with the chiral selector, the (*Z*)-methoxyimine moiety of gemifloxacin that provides stereoselective steric interaction for the diastereomeric complex is essential for chiral recognition. Therefore, it is considered that the increase of alkyl group of the (*Z*)-alkoxyimine moiety from methyl (gemifloxacin) to ethyl (LB20326) increases enantioselectivity (entry 5).

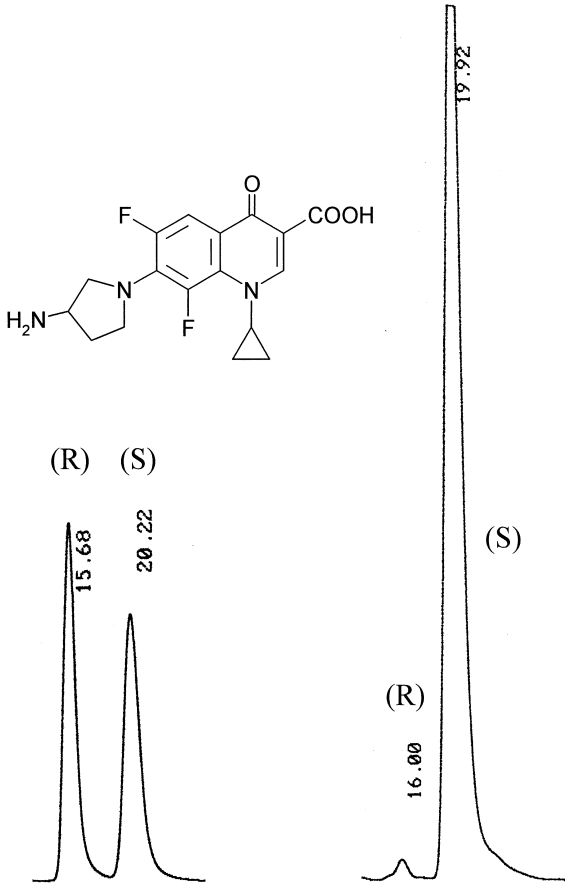


Fig. 6. Chromatograms of a racemic quinolone compound and its enantiomerically enriched sample (*R*:*S*=1.5:98.5) on Crownpak CR(+); mobile phase: 10 m*M* sulfuric acid in water–methanol (85:15, v/v); amount injected 5–12 μg.

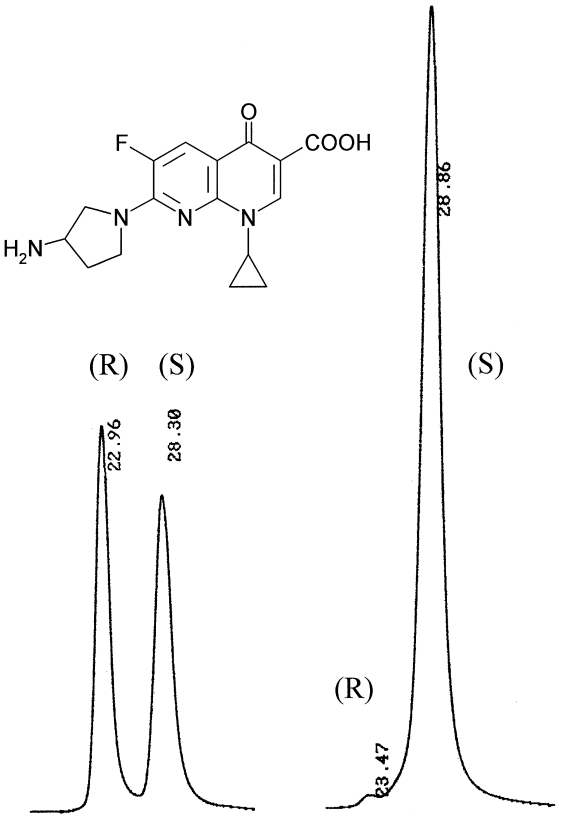
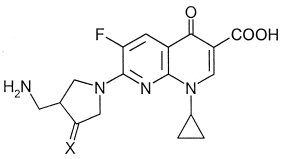


Fig. 7. Chromatograms of a racemic quinolone compound and its enantiomerically enriched sample (*R*:*S*=0.9:99.1) on Crownpak CR(+); mobile phase: 10 m*M* trifluoroacetic acid in water–methanol (85:15, v/v); amount injected 3–5 μg.

Table 2
Direct separation of the enantiomers of gemifloxacin and its analogs on Crownpak CR[a]



Entry	Analyte	X	α[b]	k_1'[c]	R_s[d]
1	Gemifloxacin (LB 20304)	(*Z*)-Methoxyimine	1.60	61.23	3.63
2	(*E*)-LB 20304	(*E*)-Methoxyimine	1.03	42.90	0.13
3	keto-LB 20304	oxygen	1.23	12.24	1.11
4	LB 20393		1.12	31.22	1.09
5	LB 20326	(*Z*)-Ethoxyimine	1.72	141.27	5.72

[a] Mobile phase: 10 m*M* CF_3COOH in water–methanol (85:15, v/v); see Experimental for chromatographic conditions.
[b] Separation factor.
[c] Capacity factor for the first eluted enantiomer.
[d] Resolution factor.

The presence of (*E*)-methoxyimine on the pyrrolidine ring gives rise to significant steric hindrance to the approach towards the chiral selector. Consequently, this steric prevention of the primary ammonium group of (*E*)-LB20304 from forming diastereomeric complexation with the chiral selector results in decreased elution time and poor enantioseparation (entry 2). On the other hand, either the substitution of the ketone moiety for the methoxyimine group (entry 3) or its absence on the pyrrolidine ring (entry 4) provides relatively a freedom of rotation of the aminomethyl group, which affords lowered enantioselectivity [21]. It is also observed that the increased hydrophobicity of LB20326 results in a dramatic increase in retention behavior (entry 5).

In conclusion, liquid chromatographic separation of the enantiomers of several fluoroquinolones including gemifloxacin was achieved on Crownpak CR(+) CSP. This is the first report on the direct separation of the enantiomers of quinolones on a chiral crown ether coated Crownpak CR column. The enantiomers of all the fluoroquinolones investigated were readily separated on chiral Crownpak CR CSP. Although these fluoroquinolone analytes frequently provide long retention times, this method using the commercially available Crownpak CR column has an advantage of convenience and versatility over other techniques reported for the resolution of quinolone compounds [10–15]. Furthermore, this method is expected to be useful for the resolution of other racemic quinolones related to the examined analytes with a primary amino group.

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