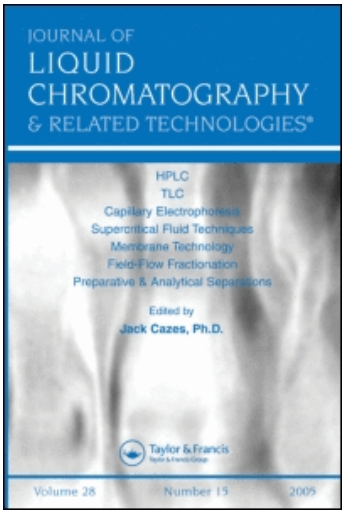

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Abdalla A. Elbashir[a]; Bahruddin Saad[a]; Abdussalam Salhin Mohamed Ali[a]; Khaldun M. M. Al-Azzam[b]; Hassan Y. Aboul-Enein[c]
[a] School of Chemical Sciences, Universiti Sains Malaysia, Penang, Malaysia [b] Hikma Pharmaceuticals, Bayadr Wadi Seer, Amman, Jordan [c] Pharmaceutical and Medicinal Chemistry Department, National Research Center, Dokki, Cairo, Egypt

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Validated Stability Indicating Assay of Gemifloxacin and Lomefloxacin in Tablet Formulations by Capillary Electrophoresis

Abdalla A. Elbashir,[1] Bahruddin Saad,[1] Abdussalam Salhin Mohamed Ali,[1] Khaldun M. M. Al-Azzam,[2] and Hassan Y. Aboul-Enein[3]

[1]School of Chemical Sciences, Universiti Sains Malaysia, Penang, Malaysia
[2]Hikma Pharmaceuticals, Bayadr Wadi Seer, Amman, Jordan
[3]Pharmaceutical and Medicinal Chemistry Department, National Research Center, Dokki, Cairo, Egypt

Abstract: A capillary zone electrophoretic (CZE) method has been developed for the determination of gemifloxacin and lomefloxacin in pharmaceutical tablet formulations. The CZE separation was performed using a 75 μm × 35 cm fused silica capillary under the following conditions: 25°C; applied voltage, 12 kV; 25 mM H_3PO_4-NaOH running buffer (pH 8.5). The detection wavelength was 254 nm. Flumequine was used as internal standard. The method was suitably validated with respect to linearity, limit of detection and quantification, accuracy, precision, specificity, and robustness. The calibration was linear from 5 to 50 μg mL^{-1} for gemifloxacin and 10 to 60 μg mL^{-1} for lomefloxacin, and the limit of detection and quantification were 2.93, 4.91 μg mL^{-1}, and 3.87, 8.93 for gemifloxacin and lomefloxacin, respectively. Recoveries ranging from 94.4–108.6% were obtained for both drugs. The method was successfully applied to the determination of gemifloxacin and lomefloxacin in pharmaceutical tablets. Excipients present in the tablets and degraded products from different stress conditions did not interfere in the assay.

Keywords: Gemifloxacin, Lomefloxacin, Capillary zone electrophoresis, Pharmaeutical analysis

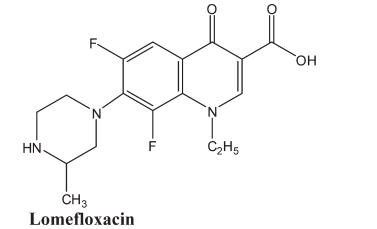
Correspondence: Professor Bahruddin Saad, School of Chemical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia. E-mail: bahrud@usm.my and Professor Hassan Y. Aboul-Enein, Pharmaceutical and Medicinal Chemistry Department, National Research Center, Dokki, Cairo 12311, Egypt. E-mail: enein@gawab.com

INTRODUCTION

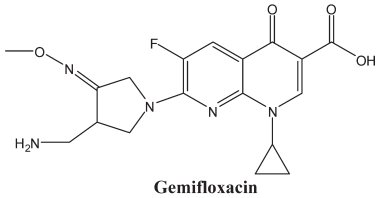
Fluoroquinolones have emerged as one of the most important classes of antibiotics of the past decade.[1] The newest members of this class demonstrate a broad spectrum activity against many pathogenic gram negative and gram positive bacteria, including many of the so called atypical respiratory pathogens.[2]

Gemifloxacin (*R,S*)-7-[(4Z)-3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid and lomefloxacin, (1-ethyl-6,8-difluro-1,4-dihydro-7-(3-methyl-piperazinyl)-4-oxo-3-quinolinecarboxylic acid) (Figure 1) are new fluoroquinolones. Gemifloxacin is recently being approved by the U.S. Food and Drug Administration for the treatment of the upper respiratory tract infections.[3] The determination of gemifloxacin and lomefloxacin are not yet described in any pharmacopoeias. Therefore, a simple, accurate method is required for their determination in pharmaceutical formulations.

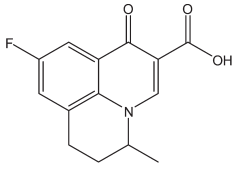
Several analytical methods for quantitative determination of fluoroquinlones in pharmaceutical formulations are reported including, high performance liquid chromatography (HPLC),[4–9] UV spectrophotometry,[10–12] titrimetry,[13] and capillary electrophoresis (CE),[14–19] among others.



Lomefloxacin



Gemifloxacin



Flumequine (internal standard)

Figure 1. Chemical structure of gemifloxacin, lomefloxacin, and internal standard flumequine.

The capillary zone electrophoresis (CZE) technique is rapidly gaining popularity in pharmaceutical quality control[20–22] and has shown great promise in replacing many conventional methods, especially HPLC. The advantages of short analysis times, small injection volumes (a few nanoliters), and low consumption of solvents render this technique attractive.[23] Recently, general test chapters involving CZE has been included in the US Pharmacopoeia[24] and European Pharmacopoeia.[25] Although several capillary electrophoresis methods have been dedicated to determination of fluoroquinolones,[13–19] no method has reported for determination of gemifloxacin in the pharmaceutical formulations.

This study paper describes the optimization, validation, and application of CE for determination of gemifloxacin and lomefloxacin in pharmaceutical tablet formulations.

EXPERIMENTAL

Reagents and Chemicals

Lomefloxacin hydrochloride, flumequine (9-fluoro-6,7-dihydro-5-methyl-1-oxo-1H,5H-benzo[ij]quinolizine-2-carboxylic acid) (Figure 1), phosphoric acid (85% w/w), sodium hydroxide, hydrochloric acid, hydrogen peroxide (30%) were purchased from Sigma-Aldrich (St. Louis, USA). Lomefloxacin tablets (claimed to contain 400 mg active ingredient) manufactured by Gulf Pharmaceutical Industries, Ras Al Khaimah, United Arab Emirates, was purchased from the local market. Gemifloxacin standard and tablets were kindly donated by Hikma Pharmaceuticals PLC (Jordan, Amman). Milli-Q water was used for preparing all solutions used in this study.

Instrumentation

Analytical separations were carried out on a Waters Capillary Ion Analyzer (Milford, USA), which was interfaced to a Waters PC 800 Workstation using uncoated fused silica capillary (total length, 35 cm, effective length 27.5 cm and internal diameter, 75 μm). The separations were conducted at 25°C by applying a voltage of 12 kV. Samples were injected hydrostatically for 25 seconds. Detection was achieved at 254 nm.

Electrophoretic Conditions

A new uncoated fused silica capillary was conditioned by flushing with 1 M NaOH for 30 minutes, then with 0.1 M NaOH for 10 minutes, and finally water and buffer, each for 15 minutes. The running buffer consisted of

25 mM phosphoric acid that had been adjusted to the desired pH with 1 M NaOH solution. The running buffer was passed through 0.2 μm cellulose nitrate membrane filters (Whatman, England) and degassed by sonication prior to use. The capillary was rinsed with 0.1 M NaOH for one minute, and then purified water, followed by the running buffer, each for 2 minutes between the runs.

Stock and Standard Solutions

Standard gemifloxacin and lomefloxacin stock solution (1000 μg mL^{-1}) was prepared in 0.1 M NaOH and was kept refrigerated. Working standard solutions were prepared daily by diluting suitable aliquots of the stock solution with water. The standard solutions were stored in brown glass vials to protect from light.

Stress Testing

Stress testing of the drug substance can be used to identify the possible degradation products, to provide an indication of the stability of the analyte, and also to validate the stability and specificity of an analytical method.[26] To investigate the specificity of the proposed method, standard solutions of gemifloxacin and lomefloxacin were prepared and subjected to four different stress conditions. Aliquots of stock solutions of gemifloxacin and lomefloxacin (2.0 mL of a 2.0 mg mL^{-1}) were transferred into 10.0 mL volumetric flasks. Each flask was then treated in one of the following ways: (i) heated for 15 h at 75°C, (ii) adding 1000 μL of 1 M hydrochloric acid and heated for 15 h at 75°C, (iii) adding 1000 μL of 1 M sodium hydroxide and heated for 15 h at 75°C, (iv) adding 100 μL of 30% hydrogen peroxide and heated for 15 h at 75°C. A corresponding blank solution was also prepared for each condition. After removing from the stress condition, all samples were cooled to room temperature, the acidic and basic samples were neutralized, and all samples were diluted to a final concentration of 30 μg mL^{-1} analytes and were injected for analysis.

Pharmaceutical Sample Preparation

Five tablets were weighed, ground, and mixed in a mortar. An amount of 30 mg of the powder was taken and dissolved in 25 mL of 0.1 M sodium hydroxide by ultrasonic sonication for three minutes and diluted to 100 mL with water. The sample was filtered through a membrane (0.22 μm) and 1 mL of the filtrate was diluted with water to 10 mL.

RESULTS AND DISCUSSION

Optimization of Electrophoretic Conditions

Buffer pH is an important parameter in CZE optimization as it affects the ionization of analytes and also their electrophoretic mobility.[27] For the pH interval 2.5–7.0, the fluoroquinlones have been found to interact with the internal capillary wall. Therefore, the pH in ranges from 7.5–10.0 was investigated. The effect of pH from 7.5–10 of the running buffer (25 mM H_3PO_4-NaOH buffer) on the migration time and peak width were investigated (Figure 2). When the pH was increased, the migration time slightly increased and peak tailing was observed at pH 7.5. The peak widths were virtually unaffected at pH $\geq$8.0. Therefore, pH 8.5 was selected as the optimum value of the running buffer due to the short analysis time and good peak shapes that were obtained.

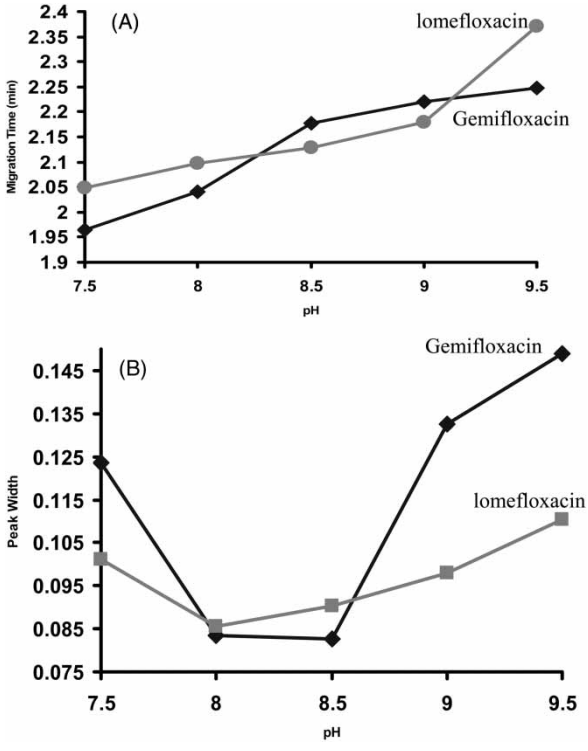


Figure 2. Effect of pH on (A) migration time, and (B) peak width. Running buffer, 25 mM H_3PO_4-NaOH; temperature, 25°C; capillary, 35 cm (27.5 cm effective length) cm × 75 μm ID; applied voltage, 12 kV.

The effect of concentration of phosphate running buffer was examined by varying its concentration from 15–45 mM. A slight increase in the migration time was observed with increasing buffer concentration (Figure not shown). Twenty-five mM was chosen as the optimum concentration of the running buffer.

The effect of voltage (8–14 kV) on the migration time of the analyte was also studied. As expected, higher voltage resulted in shorter migration time. At 12 kV, the analysis time was the shortest and the current was not excessive, so this voltage was selected. The influence of temperature on the separation was investigated at 19, 22, and 25°C. Because of its shorter analysis time, 25°C was chosen.

Injection times are known to affect the peak width and peak height. Studies were carried out by varying the injection times from 15–35 s. When the injection times were more than 25 s, peak width increased and the peak shape was distorted. Therefore 25 s was chosen for further studies.

From the above experiments, the optimized conditions used for the assay were: 25 mM hosphate buffer (pH 8.0); injection time, 25 s; applied voltage, 12 kV; and capillary temperature, 25°C. Typical electropherograms of gemifloxacin and lomefloxacin standard is shown in Figure 3A. The suitability of flumequine as internal standard is evident as it is well resolved from the ofloxacin peak. Under these conditions, both analytes were eluted in less than 3 min.

Method Validation

Various studies have shown that the use of internal standards is crucial to obtain good reproducibility in CZE and chromatographic techniques in order to compensate for injection errors and minor fluctuations of the migration time.[28] In this study flumequine, which belong to the same group of gemifloxacin and lomefloxacin, was selected as the internal standard. In all cases, 15 $\mu g\ mL^{-1}$ of flumequine was added as internal standard (IS). The assay of gemifloxacin and lomefloxacin were validated with respect to linearity, limit of detection, and quantitation, precision, accuracy, robustness, and specificity.[26,29]

Linearity

Using the optimum analytical conditions, linearity was studied in the concentration range of 5 to 50 for gemifloxacin and 10 to 60 $\mu g\ mL^{-1}$ for lomefloxacin. The calibration graph was constructed by plotting the ratio of peak area (analyte/IS) (y) as a function of analyte concentration (x) in $\mu g\ mL^{-1}$. The ratio of peak area was chosen rather than the corrected peak area ratio, as lower RSDs were obtained for both gemifloxacin and lomefloxacin. Each point of the calibration curve corresponded to the mean value obtained from three measurements. The statistical linear regression data obtained are summarized in Table 1.

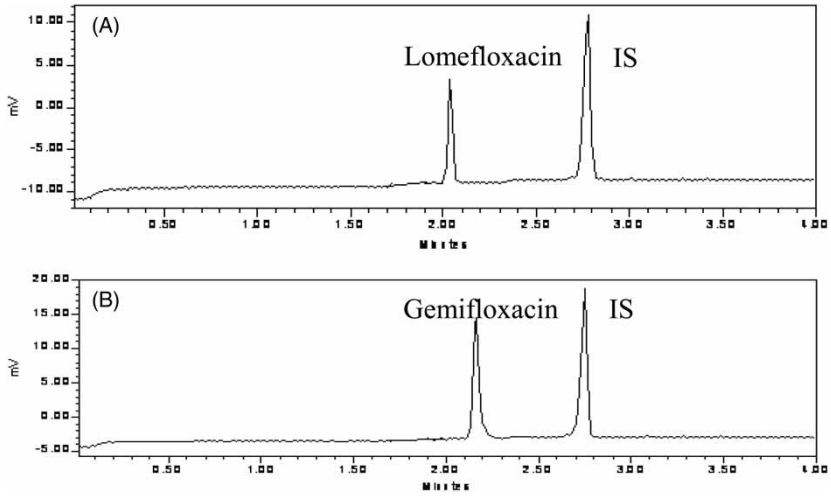


Figure 3. Electropherogram of (A) lomefloxacin standard, (B) gemifloxacin concentration of lomefloxacin and gemifloxacin 30 μg $mL^{-1}$and 15 μg mL^{-1} for internal standard. Refer to Experimental section for CZE conditions.

Limit of Detection and Quantitation

The limit of detection of the method was estimated to be 2.93 for gemifloxacin and 3.87 μg mL^{-1} for lomefloxacin. This was obtained by multiplying the standard deviation by 3.[21] The limit of quantitation, estimated by multiplying the standard deviation by 10, was found to be 4.91 and 8.93 μg mL^{-1} for gemifloxacin and lomefloxacin, respectively.

Precision

The repeatability of the method was examined by injecting ten consecutive injections of 30 μg mL^{-1} of gemifloxacin and lomefloxacin (keeping the other operating conditions identical). The results were evaluated by

Table 1. Analytical parameters

	Gemifloxacin	Lomefloxacin
Intercept	0.3023	0.2288
Slope	0.0205	0.0177
Correlation coefficient	0.9954	0.9985
linearity range (μg/mL)	5–50	10–60
Detection limit (μg/mL)	2.93	3.87
Quantification limit (μg/mL)	4.91	8.93

considering the migration time, peak area, corrected peak area, ratio of corrected peak, ratio of peak area values of two analytes under study, and the internal standard (IS). The precision values with their RSD are summarized in Table 2.

Three different concentrations of gemifloxacin and lomefloxacin (10.0, 30.0, and 50.0 $\mu g\ mL^{-1}$) were analyzed over six independent series in the same day (intra-day precision) and six consecutive days (inter-day precision). Within each series, every sample was injected six times. The %RSD values of intra-day and inter-day studies varied from 1.38 to 2.21%, and from 1.7 to 4.43 for gemifloxacin and lomefloxacin, respectively, suggesting that the intermediate precision of the method was satisfactory (Table 3).

Recovery

The accuracy of the proposed method was performed by spiking the synthetic mixture with known amounts of lomefloxacin and gemifloxacin. Recoveries ranged from 94.4–108.6% for both drugs (Table 4).

Robustness

Robustness tests were performed to investigate the reliability of results when the experimental parameters were slightly changed deliberately. Only one parameter was changed at a time. The determination of 30 $\mu g\ mL^{-1}$ gemifloxacin and lomefloxacin standard solution under slight changes in pH and buffer concentration was studied. No significant

Table 2. Repeatability of various parameters expressed as % RSD

Parameter	Gemifloxacin		IS
Migration time	0.268		0.27
Peak area	3.67		1.28
Corrected peak area	3.67		1.61
Ratio of corrected peak area		2.77	
Ratio of peak area		3.03	
	Lomefloxacin		IS
Migration time	0.34		0.616
Peak area	1.72		1.58
Corrected peak area	1.58		1.51
Ratio of corrected peak area		1.61	
Ratio of peak area		1.96	

Table 3. Precision and accuracy of the CZE method (n = 6)

	Intra-day precision			Inter-day precision		
	Found[a]	RSD (%)	Accuracy bias[b] (%)	Found[a]	Precision RSD (%)	Accuracy bias (%)
Gemifloxacin conc. (μg/mL)						
10	10.13 ± 0.29	2.82	1.3	10.18 ± 0.14	1.38	1.8
30	29.41 ± 0.59	1.99	−1.97	30.06 ± 0.66	2.21	3.34
50	49.68 ± 0.83	1.67	−0.64	50.0 ± 0.09	1.80	0.18
Lomefloxacin conc. (μg/mL)						
10	9.99 ± 0.46	4.58	−0.1	9.74 ± 0.43	4.43	−2.6
30	30.89 ± 0.53	1.71	2.96	30.75 ± 0.75	2.44	2.5
50	50.1 ± 1.32	2.63	0.2	50.34 ± 0.86	1.70	0.66

[a]Mean ± standard error.
[b]Accuracy: [(found-added/added)] × 100.

Table 4. Recoveries obtained from the determination of gemifloxacin and lomefloxacin in placebos that contained different levels of spiked standards

Compound	Spiked ($\mu g\ mL^{-1}$)	Amount found ($\mu g\ mL^{-1}$)	% Recovery (mean ± S.D.) ($\mu g\ mL^{-1}$)
Gemifloxacin	10	10.21	102.1 ± 2.16
	30	31.11	103.72 ± 1.07
	50	50.68	101.37 ± 0.66
Lomefloxacin	10	10.86	108.6 ± 4.03
	30	30.46	101.54 ± 1.6
	50	46.38	094.44 ± 2.78

Mean ± S.D. (n = 6).

difference was found between the results, indicating the robustness of the method (Table 5).

Stress Testing and Specificity

The specificity of the method was evaluated by forcibly degrading gemifloxacin and lomefloxacin standards and blanks. Both drugs were found to be relatively stable under acidic (Figure 3), basic, and elevated temperature conditions since good recoveries were obtained (Table 5). However, both drugs suffered serious degradation when stressed using H_2O_2. There was no evidence of interference from the tablet excipients used in the formulation (Figure 3), indicating the specificity of the method.

Table 5. Determination of gemifloxacin and lomefloxacin standard solution (30 $\mu g\ mL^{-1}$) under different conditions using the CZE method (n = 6)

Compound	CEZ running conditions	Mean ± SD	RSD (%)
Gemifloxacin	Standard	30.63 ± 0.48	1.56
	pH 8.4	30.22 ± 0.25	0.82
	pH 8.6	29.95 ± 0.19	0.60
	24 mM H_3PO_4 buffer	29.05 ± 0.78	2.69
	26 mM H_3PO_4 buffer	29.24 ± 0.15	0.51
Lomefloxacin	Standard	30.63 ± 0.48	1.56
	pH 8.4	30.22 ± 0.25	0.82
	pH 8.6	29.95 ± 0.19	0.60
	24 mM H_3PO_4 buffer	29.05 ± 0.78	2.69
	26 mM H_3PO_4 buffer	29.24 ± 0.15	0.51

Table 6. Analysis results of gemifloxacin and lomefloxacin in tablets

Compound	Manufacturer's claim, mg	Found mg ± RSD (%)	Purity (%)
Gemifloxacin	325	327.8 ± 0.44	100.86
Lomefloxacin	400	395.04 ± 2.14	98.76

Application

The developed method was used to quantify gemifloxacin and lomefloxacin in commercial pharmaceutical tablets. The preparation of the sample was as described in the experimental section. Good agreement between the proposed method and the manufacture's claimed values were found for all samples (Table 6).

CONCLUSION

The present study describes a rapid, robust, and precise method for determination of gemifloxacin and lomefloxacin in tablet formulations. The stress tests indicate that these drugs are stable at elevated temperature (75°C) and under acidic and basic conditions; however, they are seriously degraded in the presence of oxidizing agents such as H_2O_2.

The method is specific, precise, accurate, and robust and is recommended for the routine analysis of lomefloxacin in pharmaceuticals.

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