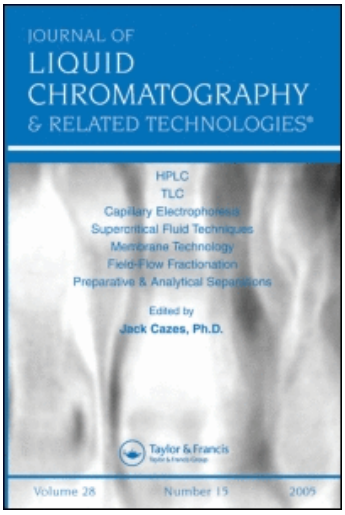

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

DETERMINATION OF FLUOROQUINOLONE ANTIBACTERIALS IN PHARMACEUTICAL FORMULATIONS BY CAPILLARY ELECTROPHORESIS

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Online publication date: 28 January 1999

To cite this Article Sun, Shao-Wen and Wu, An-Cheng(1999) 'DETERMINATION OF FLUOROQUINOLONE ANTIBACTERIALS IN PHARMACEUTICAL FORMULATIONS BY CAPILLARY ELECTROPHORESIS', Journal of Liquid Chromatography & Related Technologies, 22: 2, 281 — 296
To link to this Article: DOI: 10.1081/JLC-100101660
URL: http://dx.doi.org/10.1081/JLC-100101660

J. LIQ. CHROM. & REL. TECHNOL., 22(2), 281–296 (1999)

DETERMINATION OF FLUOROQUINOLONE ANTIBACTERIALS IN PHARMACEUTICAL FORMULATIONS BY CAPILLARY ELECTROPHORESIS

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ABSTRACT

To meet the requirement of quantitative analysis, this work re-investigated the parameters relevant to the separation of seven fluoroquinolone antibacterials, ciprofloxacin, enoxacin, lomefloxacin, norfloxacin, ofloxacin, pefloxacin, and sparfloxacin, based on the method developed previously in our laboratory. With the optimum conditions obtained: electrophoretic media - 65 mM sodium borate, 35 mM sodium dihydrogen phosphate, 60 mM sodium cholate, pH 7.3 / acetonitrile (72 : 28, v/v); fused silica capillary - 50 μm I.D., total length 67 cm, detection length 52 cm; hydrodynamic injection - 60 mbar, 3 sec; voltage - 27 kV; temperature - 25°C and detection wavelength - 275 nm, the fluoroquinolones were well separated within 8.5 min. After being validated the method was used for the assay of the formulation products. The assay results were within 95~105 % of their label claims. The method should be applicable to the analysis of these and other similar formulation products containing these drugs.

INTRODUCTION

Quinolone antibacterial agents are a group of synthetic drugs which exhibit the bactericidal actions by interfering with the bacterial DNA gyrase needed for its DNA synthesis.[1] Fluorinated quinolones, the newer generations of this family, not only have broader antibacterial activities, but also are orally more effective in treating various infectious diseases, compared with the older non-fluororinated congeners.[2]

Following the separation of fourteen quinolone antibacterials by capillary electrophoresis (CE) with the aid of an overlapping resolution mapping scheme in our laboratory,[3] the quantitative work of seven fluoroquinolones, ciprofloxacin, enoxacin, lomefloxacin, norfloxacin, ofloxacin, pefloxacin, and sparfloxacin (Figure 1), was to be continued. Because for quantitative purpose the resolution between peaks plays a key role with regard to the reliability of the results, the previous operating conditions were re-examined. The conditions obtained by combining the optimum value of each relevant factor were tested to see whether a satisfactory and sound separation could be acquired.

EXPERIMENTAL

Apparatus

The CE system consisted of a Lauer Labs' Prince programmable injector including a 30-kV high-voltage supplier (Emmen, the Netherlands), connected with a Dynamax UV-C Absorbance Detector (Rainin, Emeryville, CA, USA) for UV detection. The electropherograms were recorded with a EZChrom chromatographic data system ver. 6 (Scientific Software, San Ramon, CA, USA) on a 486 DX2 66 PC with an appropriate ADC card and interface. A fused-silica capillary of 50 μm I.D. and 375 μm O.D. (Polymicro Technologies, Phoenix, AZ, USA) was used, with total length of 67 cm and detection length of 51 cm. A Mettler delta 320 pH meter with an InLab 410 combination electrode (Essex, England) was used for measurement of pH.

Chemicals and Reagents

Lomefloxacin, norfloxacin, ofloxacin, and pipemidic acid were purchased from Sigma (St. Louis, MO, USA). Enoxacin and sparfloxacin were provided by Dainippon (Osaka, Japan). Ciprofloxacin and pefloxacin were supplied by Bayer (Leverkeusen, Germany) and Rhône-Poulenc Rorer (Vitry, France), respectively.

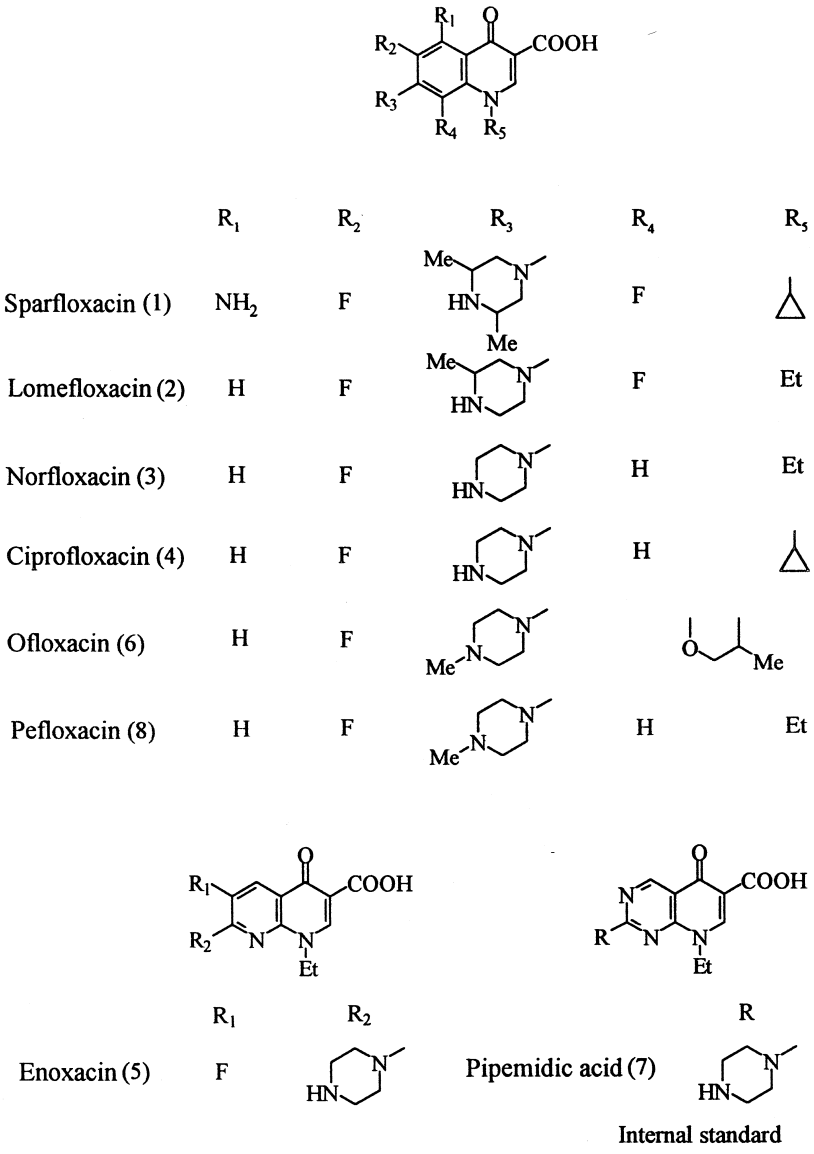

Figure 1. Fluoroquinolone antibacterials analyzed in this work.

Sodium tetraborate and sodium dihydrogen phosphate were purchased from Merck (Darmstadt, Germany). Sodium cholate was purchased from Sigma (St. Louis, MO, USA). pH of the buffer media was adjusted with 0.1 M hydrochloric acid (Carlo Erba, Milan, Italy) and 0.1 M sodium hydroxide (Fluka). Acetonitrile of chromatographic grade was purchased from Mallinckrodt (Paris, KY, USA). Water was purified on a Barnstead water purification system (Dubuque, IA, USA).

The electrophoretic media were prepared by adding sodium cholate to stock solutions of sodium borate/sodium dihydrogen phosphate (of molar ratio 13:7). After pH adjustment the volume of acetonitrile was added. Water was finally added to complete the volume. The solutions were filtered through a 0.45 μm filter (Millipore, Bedford, MA, USA) before use.

Reference Solution

A test mixture containing 10 mg of each of the the seven fluoroquinolones (ciprofloxacin, enoxacin, lomefloxacin, norfloxacin, ofloxacin, pefloxacin, and sparfloxacin) and pipemidic acid (internal standard) was dissolved in 50 mL methanol to make the reference solution. This solution containing each quinolone at 200 μg/mL was used for resolution studies.

Sample Solutions

For each of the seven fluoroquinolone formulations ten tablets or capsules were weighed and ground to homogeneously fine powders. The powders corresponding to 25 mg of the active ingredients were weighed and then added to 100 mL of methanol. 10 mg of pipemidic acid (internal standard) was added. After sonication the suspensions were filtered through a 0.45 μm PTFE filter (Lida, Kenosha, WI, USA) to obtain clear solutions used as sample solutions.

Electrophoretic Conditions

The quantitative analyses were conducted at 27 kV and 25°C. The detection wavelength was set at 275 nm. Samples were injected hydrodynamically at 60 mbar for 3 sec. The injection volumes are 4.1 nL.

When a new capillary was used, it was flushed with 1.0 M sodium hydroxide for 10 min, and followed by 0.2 M sodium hydroxide for 10 min. Between runs the capillary was flushed with 0.2 M sodium hydroxide for 2 min, and then the run buffer for 3 min.

The resolution (*Rs*) between adjacent peaks was calculated using the following equation:

$$R_S = \frac{1.18 \cdot (t_2 - t_1)}{(W_{1/2})_1 + (W_{1/2})_2} \quad (1)$$

RESULTS AND DISCUSSION

The electrophoretic solution used previously for the separation of fourteen quinolone antibacterials was composed of a mixture of an aqueous buffer and acetonitrile (72:28, v/v), with the aqueous buffer containing 32 mM sodium borate, 18 mM sodium dihydrogen phosphate, 39 mM sodium cholate, and 8 mM sodium heptanesulfonate and having a pH of 7.3.[3] Other electrophoretic conditions were recited as follows: a fused silica capillary (50 μm I.D. and 350 μm O.D.) of 60 cm total length and 44 cm detection length being used; the analytes being injected hydrodynamically at 40 mbar for 3 seconds, and the analysis being performed at 30 kV voltage under room temperature (23 ± 2°C) with the detection at 260 nm.

In the present work, through a series of tests it was found that the addition of sodium heptanesulfonate to the buffer was not necessary to acquire a sound separation. The role played by sodium heptanesulfonate in the solution was regarded as a mediator of ionic strength rather than as an ion-pairing agent. Therefore, its part on the separation could be substituted by the background electrolytes. The optimum detection wavelength was reset to 275 nm based on averaging the absorptions of the seven fluoroquinolones analyzed. The capillary length was increased moderately with a hope to obtain improved separations while still maintaining a short analysis time. Other parameters relevant to the separation were studied and optimized as in the following sections.

Effect of Voltage and Temperature

Resolution can be better enhanced with a more concentrated buffer at a somewhat lowered applied voltage.[4] In this work the effect of varying the voltage from 18 to 30 kV was investigated using 90 mM borate/phosphate buffer (borate-phosphate molar ratio of 13:7) containing 45 mM sodium cholate, which had an ionic strength much greater than that used previously for the separation of fourteen quinolones (see above). A potential of 27 kV yielded the best compromise in terms of resolution, current generated, and run time of separation.

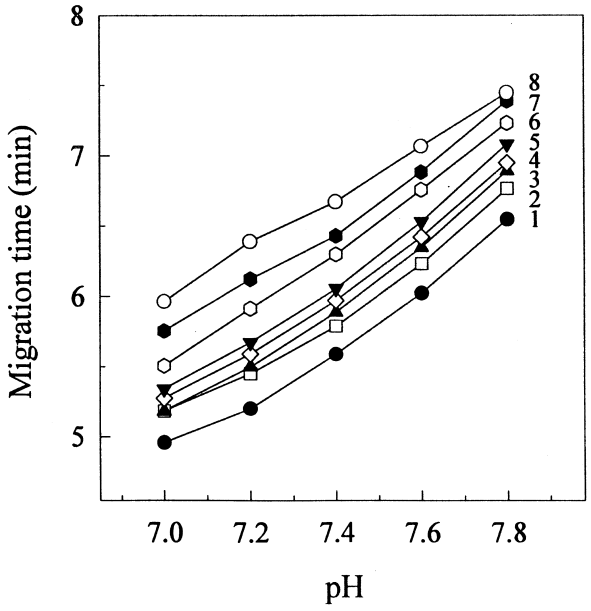

Figure 2. Effect of pH on migration time. Electrophoretic condition: 58.5 mM Na borate, 31.5 mM NaH_2PO_4, 45 mM Na cholate, pH 7.0~7.8/ACN (72:28, v/v); voltage, 27 kV; temperature, 25°C; fused silica capillary, 50 μm I.D., total length 67 cm, detection length 52 cm; injection, 60 mbar, 3 sec; detection at 275 nm. Compound numbers are shown in Figure 1.

Increasing the temperature will lower the viscosity of buffer and shortens the analysis time. However, the column efficiency fell off at high temperatures.[5] The effect of the temperature on separation was investigated between 15 and 55°C. When the temperature was raised from 15 to 25°C, the gain of analysis time was dramatic. Over 35°C the overall resolutions gradually decayed. The effect of temperature is quite similar to that of the applied voltage. The voltage 27 kV and the temperature 25°C were thus used for the subsequent optimization process.

Effect of pH

The pH of the buffer is of pivotal importance to the separation of the fluoroquinolines. Its effect on migration time was studied in the range 7.0~7.8 (Figure 2) which encompassed the pI (isoelectric point) values of the fluoroquinolines.[6,7] The migration times of the fluoroquinolones increased with

the solution pH because the higher the pH was, the more the solutes were negatively charged, and the faster they would move in the opposite direction of the electroosmotic flow (EOF). At pH 7.0 lomefloxacin was completely overlapped with norfloxacin and at pH 7.8 the resolution between norfloxacin and ciprofloxacin dropped to 1.1, and so did the resolution between pefloxacin and pipemidic acid.

The optimum separation was found at pH 7.4, at which the resolution between adjacent peaks were all greater than 1.8. The coincidence of this optimum pH with that used previously showed that the pH for an optimum separation could generally be found at or near the overall pI value of the tested compounds, independent of the changes in the compositions of the buffer media.

Effect of Acetonitrile

The addition of acetonitrile to the buffer electrolyte altered the polarity and the viscosity of the mobile phase.[8] This caused changes of both the EOF and the electrophoretic mobilities of the analytes. The effect of acetonitrile on migration time was investigated from 24% to 32% of acetonitrile, using 100 mM borate/phosphate buffer containing 45 mM sodium cholate. The migration times increased with the acetonitrile percentage in a manner very similar to the situation of sodium cholate described below. Because the addition of acetonitrile caused only slight changes in EOF, the large increase of migration times of the solutes could be attributed to the more enhanced interaction between the bile salt micelles and the solutes, as a consequence of decreasing solubilities of the ionic solutes (as zwitterions in pH 7.4) in the buffer media (becoming less polar due to addition of acetonitrile).

At 24% of acetonitrile the resolution between norfloxacin and ciprofloxacin and that between ciprofloxacin and ofloxacin were both below 1.2. At 32% of acetonitrile lomefloxacin was barely resolved with norfloxacin. At 28% of acetonitrile the lowest resolution between adjacent peaks reached 1.8. This optimum volume percentage corresponds to that used previously.

Effect of Sodium Cholate

The effect of concentration of sodium cholate on the migration times of the fluoroquinolones was investigated between 30 mM and 70 mM (Figure 3). The migration times of the solutes increased apparently in even proportion with the concentration of sodium cholate; nevertheless, the resolution window (not shown) yet provide an optimum for separation (with minimum resolution between adjacent peaks larger than 2.0) at 60 mM.

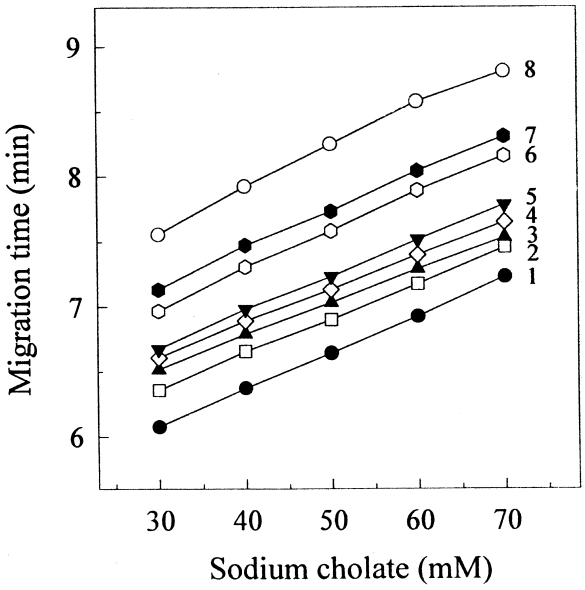

Figure 3. Effect of Na cholate concentration on migration time. Electrophoretic condition: 65 mM Na borate, 35 mM NaH_2PO_4, 30~70 mM Na cholate, pH 7.4/ACN (72:28, v/v), other conditions as shown in Figure 2. Compound numbers are shown in Figure 1.

Effect of Buffer Concentration

Because both the electroosmotic and the solute electrophoretic mobilities are varied by changing the buffer concentration, the buffer concentration can be manipulated to improve the resolution.[4,9,10] As mentioned above, the buffer concentration was elevated to trade off the action of sodium heptanesulfonate. The total borate /phosphate buffer concentrations were varied from 60 mM to 140 mM at pH 7.4 (Figure 4). At 60 mM ofloxacin was completely overlapped with the internal standard (pipemidic acid).

The migration times of all the eight quinolones increased linearly with the increase of the buffer concentration in the range of 60 mM to 100 mM. However, above 100 mM the migration times of the large part of the quinolones fell off, with sparfloxacin and lomefloxacin being entirely overlapped at 140 mM. This is due to the Joule heating caused by the high ionic strength of the buffer solution. Therefore the 100 mM was chosen as the optimum buffer molarity.

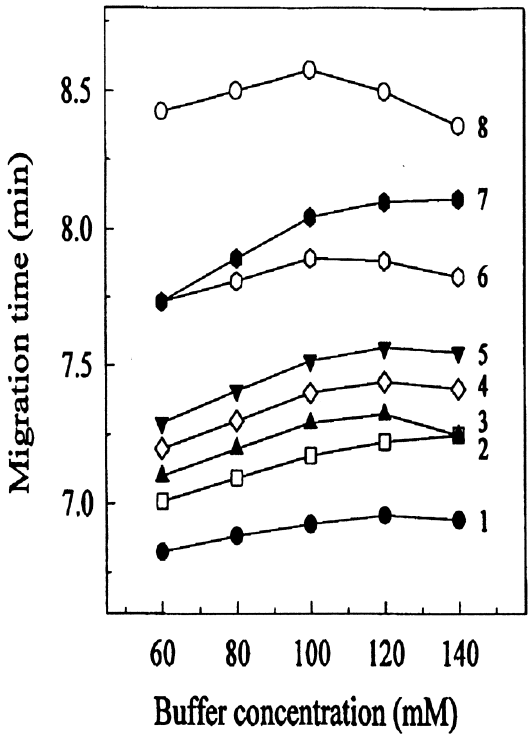

Figure 4. Effect of buffer concentration on migration time. Electrophoretic condition: 39~91 mM Na borate, 21~49 mM NaH_2PO_4, 60 mM Na cholate, pH 7.4/ACN (72:28, v/v), other conditions as shown in Figure 2. Compound numbers are shown in Figure 1.

Optimum Separation Conditions

An electropherogram obtained by using the optimum values of the parameters described above, namely, 65 mM sodium borate, 35 mM sodium dihydrogen phosphate, 60 mM sodium cholate, buffer pH 7.4, 28% acetonitrile, 27 kV, and 25°C, and other selected conditions including capillary total length of 67 cm, detection length of 52 cm, hydrodynamic injection at 60 mbar for 3 sec, and detection at 275 nm, is shown in Figure 5.

It demonstrates that the method developed permits a complete separation of all the seven compounds and the internal standard within 8.5 min. This method was validated before being used for the determination of the fluoroquinolones in pharmaceutical dosage formulations.

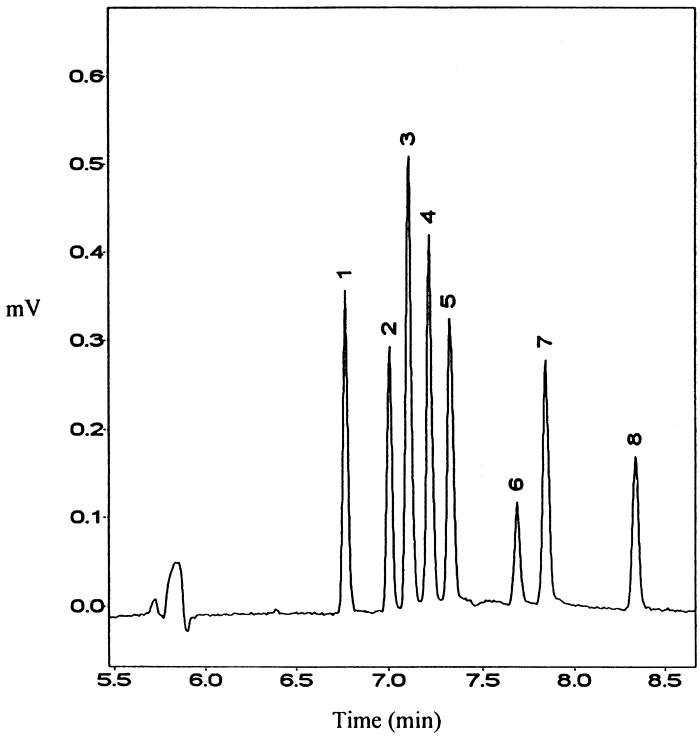

Figure 5. Electropherogram of the fluoroquinolones. Electrophoretic condition: 65 mM Na borate, 35 mM NaH_2PO_4, 60 mM Na cholate, pH 7.4/ACN (72:28, v/v), other conditions as shown in Figure 2. Compound numbers are shown in Figure 1.

Method Validation

Specificity

The resolutions of the compound pairs, calculated with equation (1), are listed in Table 1. The separation obtained was better than that in the previous report. The minimum resolution value of 2.1 was sufficient for the quantitative purpose.

Precision

The precision of the method was assessed by measuring the run-to-run and day-to-day reproducibilities of migration times and peak area ratios (with respect to internal standard) for each fluoroquinolone. As can be seen from

Table 1

Resolutions (*Rs*) of the Compound Pairs

Compound Pair	1-2	2-3	3-4	4-5	5-6	6-7	7-8
Rs	4.96	2.23	2.11	2.15	6.59	2.83	8.28

Table 2

Reproducibilities of Migration Times and Peak Area Ratios

(a) Run-to-Run (n=7)

Analyte	Migration Times (RSD %)	Peak Area Ratios[a] (RSD %) (50 μg/mL)	(200 μg/mL)
Sparfloxacin	0.55	1.04	1.13
Lomefloxacin	0.54	1.26	1.23
Norfloxacin	0.57	0.98	0.98
Ciprofloxacin	0.58	2.40	0.87
Enoxacin	0.55	1.61	1.04
Ofloxacin	0.57	2.31	1.27
Pipemidic	0.60		
Pefloxacin	0.64	2.15	1.16

(b) Day to Day (n=3)

Analyte	Migration Times (RSD %)	Peak Area Ratios[a] (RSD %) (50 μg/mL)	(200 μg/mL)
Sparfloxacin	0.77	3.26	1.72
Lomefloxacin	0.84	2.33	1.78
Norfloxacin	0.92	2.50	1.63
Ciprofloxacin	0.82	4.36	0.92
Enoxacin	0.80	2.05	1.50
Ofloxacin	0.85	2.88	1.38
Pipemidic	0.91		
Pefloxacin	0.99	3.91	1.82

[a] With respect to pipemidic acid as internal standard at 100 Mg/mL.

Table 3

Linearities Between Peak Area Ratios (y) and Concentratons Injected (x)

Compound	Slope	Intercept	r[a]
Sparfloxacin	0.0065	-0.0743	0.998
Lomefloxacin	0.0076	-0.0147	0.998
Norfloxacin	0.0133	-0.1167	0.998
Ciprofloxacin	0.0106	-0.0247	0.997
Enoxacin	0.0097	-0.0221	0.999
Ofloxacin	0.0037	-0.0073	0.999
Pefloxacin	0.0074	-0.0323	0.999

[a] r, correlation coefficient.

Table 2, the RSD values of run-to-run reproducibilities are 0.6% for migration times, 1.0-2.4% and 0.9-1.3% for peak area ratios at 50 and 200 μg/mL, respectively. The RSD values of day-to-day reproducibilities are 0.9% for migration times, 2.0-4.4% and 1.4-1.8% for peak area ratios at 50 and 200 μg/mL, respectively. The values are somewhat higher at lower concentration levels for day-to-day reproducibilities of peak area ratios.

Linearity

The linear relationship between peak area ratios and the concentrations injected was assessed (Table 3) by running five standard solutions for the seven fluoroquinolones over the range 25-300 μg/mL, with pipemidic acid as internal standard (100 μg/mL). These regression lines were used, thereby, as the calibration curves.

Accuracy

The accuracy of the method was tested by analyzing the solutions prepared from commercial formulations (tablets or capsules) of the fluoroquinolones to which two different known amounts of standards were added.

The concentration of the solutions being spiked and the two spiking concentrations were 150, 50, and 100 μg/mL, respectively. The results listed in the Table 4 show that except for the low level spikings of lomefloxacin and norfloxacin all the other recoveries are within 100 ± 5%.

Table 4

Recoveries

Concentration Added (μg/mL)	Concentration Found (μg/mL)[a]	Recovery (%)
Sparfloxacin		
---	149.0 ± 5.4	---
50	199.3 ± 2.2	100.6
100	253.6 ± 1.0	104.5
Lomefloxacin		
---	153.0 ± 5.1	---
50	200.0 ± 5.4	93.6
100	249.6 ± 1.5	96.6
Norfloxacin		
---	143.0 ± 3.0	---
50	195.8 ± 2.4	105.6
100	239.0 ± 0.9	96.0
Ciprofloxacin		
---	150.3 ± 3.6	---
50	198.6 ± 3.8	96.7
100	247.6 ± 1.4	97.4
Enoxacin		
---	152.1 ± 2.7	---
50	202.6 ± 3.5	101.0
100	256.1 ± 0.3	104.1
Ofloxacin		
---	155.1 ± 1.0	---
50	205.0 ± 3.2	99.9
100	256.0 ± 2.3	101.0
Pefloxacin		
---	144.7 ± 3.9	---
50	196.8 ± 4.2	104.3
100	249.3 ± 2.0	104.6

[a] Mean ± s.d. (n=3).

Determination of Pharmaceutical Formulations

The contents of the fluoroquinolones in pharmaceutical dosage formulations were determined using the developed and validated method

Table 5

Contents of Fluoroquinolones in Pharmaceutical Formulations

Formulation[a]	Label Claim (mg)	Amount Found[b] (mg)	% of Label Claim
Sparfloxacin			
Tablets 1	100	104.6 ± 2.3	104.6
Tablets 2	100	102.2 ± 2.5	102.2
Tablets 3	100	104.6 ± 1.7	104.6
Lomefloxacin			
Capsules 1	100	99.2 ± 1.2	99.2
Capsules 2	100	98.0 ± 1.5	98.0
Capsules 3	100	98.2 ± 0.3	98.2
Norfloxacin			
Tablets 1	100	96.5 ± 3.1	96.5
Tablets 2	100	94.7 ± 0.9	94.7
Tablets 3	100	95.0 ± 1.5	95.0
Ciprofloxacin			
Tablets 1	250	250.8 ± 4.7	100.3
Tablets 2	250	245.9 ± 1.5	98.4
Tablets 3	250	246.1 ± 5.0	98.4
Enoxacin			
Tablets 1	100	103.5 ± 1.2	103.5
Tablets 2	100	102.4 ± 2.8	102.4
Tablets 3	100	102.0 ± 1.2	102.0
Ofloxacin			
Tablets 1	100	103.3 ± 1.6	103.3
Tablets 2	100	104.5 ± 2.4	104.5
Tablets 3	100	102.4 ± 2.2	102.4
Perfloxacin			
Tablets 1	400	406.5 ± 9.5	101.6
Tablets 2	400	416.2 ± 8.9	104.1
Tablets 3	400	402.1 ± 6.2	100.5

[a] Ten tablets or capsules were taken each time, as described in the section, EXPERIMENTAL.

[b] Mean ± s.d. (n=3).

described above. The assay results shown in Table 5 indicate that all the pharmaceutical formulations contain the active ingredients within 100 ± 5 % of the label claim. The USP 23 specifies that the ciprofloxacin and norfloxacin tablets should contain not less than 90 % and not more than 110 % of the labeled amount of the active ingredient, respectively.[11]

CONCLUSIONS

The proposed method is simple, rapid, and specific. It permits a simultaneous determination of seven fluoroquinolone antibacterials in pharmaceutical tablet and capsule formulations by CE. The method can be used for routine analysis of these drugs and also as an alternative method for the assay of these drugs by HPLC in bulk materials and in formulations products.[11,12]

REFERENCES

1. J. P. Monk, D. M. Campoli-Richards, Drugs, **33**, 346-391 (1987).

2. G. L. Mandell, W. A. Petri Jr., "Antimicrobial Agents," in **The Pharmacological Basis of Therapeutics**, ed. J. G. Hardman, A. G. Gilman, L. E. Limbird, 9th ed., McGraw-Hill press, New York, 1996, p. 1065.

3. S. W. Sun, L. Y. Chen, J. Chromatogr. A, **766**, 215-224 (1997).

4. H. J. Issaq, I. Z. Atamna, G. M. Muschik, G. M. Janini, Chromatographia, **32**, 155-161 (1991).

5. J. H. Knox, K. A. McCormack, Chromatographia, **38**, 207-214 (1994).

6. K. Takács-Novák, B. Noszál, G. Keresztúri, B. Podányi, G. Szász, J. Pharm. Sci., **79**, 1023-1028 (1990).

7. Z. Budvári-Bárány, G. Szász, K. Takács-Novák, I. Hermecz, A. Lóre, J. Liq. Chromatogr., **14**, 3411-3424 (1991).

8. R. Kuhn, S. Hoffstetter-Kuhn, **Capillary Electrophoresis: Principles and Practice**, Springer-Verlag, Berlin Heidelberg, 1993, p. 99.

9. S. C. Smith, M. G. Khaledi, Anal. Chem., **65**, 193-198 (1993).

10. G. M. Mc Laughlin, J. A. Nolan, J. L. Lindahl, R. H. Palmieri, K. W. Anderson, S. C. Morris, J. A. Morrison, T. J. Bronzert, J. Liq. Chromatogr., **15**, 961-1021 (1992).

11. **United States Pharmacopeia**, 23 ed., United States Pharmacopeial Convention, Rockville, MD, 1995, pp. 379, 1104.

12. ibid, pp. 376, 379, 1104.

Received January 2, 1998
Accepted March 16, 1998
Manuscript 4702

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