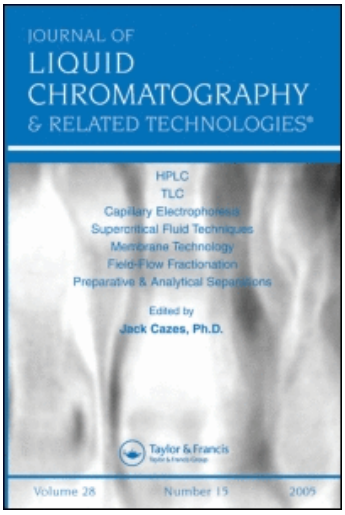

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Elbashir, Abdalla A. , Saad, Bahruddin , Mohamed Ali, Abdussalam Salhin , Saleh, Muhd. Idiris and Aboul-Enein, Hassan Y.(2008) 'Development and Validation of a Capillary Zone Electrophoresis Method for the Determination of Ofloxacin in Tablets', Journal of Liquid Chromatography & Related Technologies, 31: 18, 2771 — 2783
To link to this Article: DOI: 10.1080/10826070802388367
URL: http://dx.doi.org/10.1080/10826070802388367

Journal of Liquid Chromatography & Related Technologies®, 31: 2771–2783, 2008

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070802388367

Development and Validation of a Capillary Zone Electrophoresis Method for the Determination of Ofloxacin in Tablets

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Abstract: A capillary zone electrophoresis (CZE) method has been developed for the determination of the antibiotic ofloxacin in tablets. The CZE separation was performed using 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} and the limit of detection and quantification were 1.24 and 4.01 μg mL^{-1}, respectively. Recoveries ranging from 99.71–102.4% were obtained in the determination of ofloxacin that were spiked to placebos. Excipients in the commercial tablets and degraded products from different stress conditions did not interfere in the assay. The method was successfully applied to the determination of ofloxacin in pharmaceutical tablets.

Keywords: Capillary zone electrophoresis, Ofloxacin, Tablet

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INTRODUCTION

Ofloxacin, (±)-9-fluro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo7H-pyrido[1,2,3-de]-1,4-benzooxazine-6-carboxylic (Figure 1), is a synthetic broad spectrum antibacterial drug that exhibits significant activity against both gram-positive and gram-negative bacteria.[1] The mechanism of the bactericidal effect is based on the inhibition of the DNA gyraze of the bacteria, the enzyme that produces a negative supercoil in DNA, and thus permits transcription and replication.[2] The drug is used for the treatment of gastrointestinal, pulmonary, urinary and other infections, sexually transmitted diseases, as well as for the prevention of infection in the immunocompromised patient.[3]

Several analytical methods for the determination of ofloxacin in pharmaceutical formulations and biological fluids were reported in the scientific literature. These include high performance liquid chromatography (HPLC),[4–7] adsorptive stripping voltammetry,[8] potentiometry,[9] polarography,[10] differential pulse voltammetry,[11] and micellar electrokinetic capillary chromatography (MEKC),[12] amongst others.

The capillary zone electrophoresis (CZE) technique is rapidly gaining popularity in pharmaceutical quality control[13–15] 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 make this technique attractive.[16] Recently, general test chapters involving CZE in the US Pharmacopoeia[17] and European Pharmacopoeia[18] have been added.

CZE methods for the simultaneous separation and determination of fluoroquinolones in pharmaceutical formulation have been reported.[19,20] However, one of these methods does not include ofloxacin, while the other method was not validated for ofloxacin. Sun and Wu[12] proposed a MEKC method for the simultaneous separation of seven fluoroquinolones, including ofloxacin. The best separations were achieved using a mixture of aqueous buffer (containing 65 mM sodium borate, 35 mM sodium dihydrogen phosphate, 60 mM sodium cholate, and acetonitrile

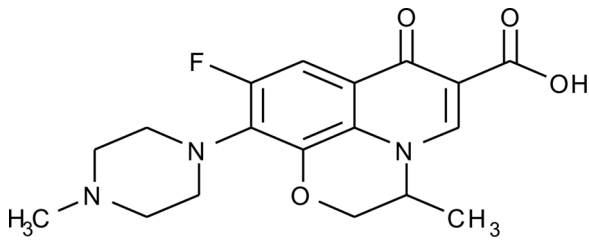

Figure 1. Chemical structure of ofloxacin.

(72:28, v/v). In the present study, a simple CZE method for the determination of ofloxacin in pharmaceutical preparation is proposed using H_3PO_4/NaOH as a running buffer.

EXPERIMENTAL

Reagents and Chemicals

Ofloxacin, flumequine (as internal standard), phosphoric acid (85% w/w), sodium hydroxide, hydrochloric acid, and hydrogen peroxide (30%) were purchased from Sigma-Aldrich (St. Louis, USA). Ofloxacin tablets (brands: Olfo-200, Prifloxin, and Tarivid, claimed to contain 200, 200, and 100 mg active ingredient, respectively) manufactured by Unique Pharmaceutical Labs., India; Prime Pharmaceutical Sdn, Penang, Malaysia; and Sanofi-Aventis, Paris, France were purchased from the campus drugstore. Milli-Q water was used for preparing all solutions.

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 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. A glass calomel electrode that was connected to an Orion Research Expandable Ion Analyzer (model EA 940) was used for measurements of pH.

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. Prior to each analysis, 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 ofloxacin and flumequine stock solutions (1000 μg mL^{-1}) were prepared in 0.1 M NaOH and were 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. This solution was stable for two weeks.

Stress Testing

Stress testing of the drug substance can be used to identify the possible degradation products, provide indication of the stability of the analyte, and can also be used to validate the stability and specificity of an analytical method.[21] To investigate the specificity of the proposed method, standard solutions of ofloxacin were prepared and subjected to four different stress conditions. Aliquots of stock solution of ofloxacin (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 completed to 10 mL with water. Each 0.075 mL of these solutions was transferred to a 10 mL volumetric flask containing 0.075 mL of 1 mg mL^{-1} solution of IS, and was topped up to the mark with water. The final solution was injected for analysis.

Pharmaceutical Sample Preparation

Five tablets were weighed, ground, and mixed in a mortar. Of the powder, 30 mg was taken and dissolved in 25 mL of 0.1 M sodium hydroxide by sonication for three minutes and was 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.[22]

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.

The effect of concentration of H_3PO_4 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 3). The optimum concentration of 25 mM was chosen as 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

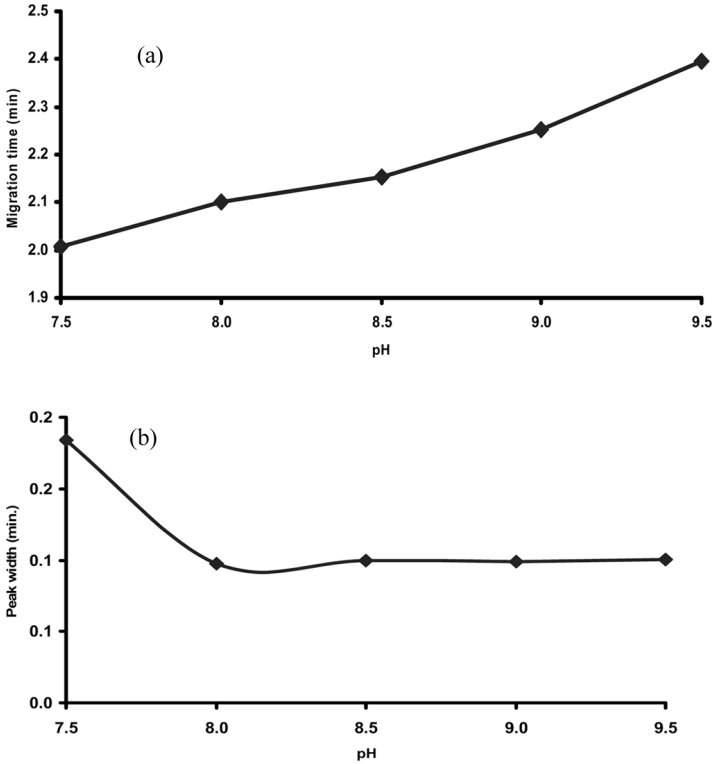

Figure 2. Effect of pH on (a) migration time, and (b) peak width. Running buffer, 25 mM H_3PO_4-NaOH; temperature, 25°C; capillary, 35 (27.5 effective length) cm $\times$ 75 μm ID; applied voltage, 12 kV.

temperature on the separation was investigated at 19, 22, and 25°C (Figure 4). 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 adopted conditions for the assay of ofloxacin were decided: 25 mM H_3PO_4-NaOH, pH 8.5; injection time, 25 s; applied voltage, 12 kV and capillary temperature, 25°C. A typical telectropherogram of ofloxacin 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 components were eluted in less than 3 min. This is considerably faster than the report of Sun and Wu[12] that used a MEKC approach (migration time, 8.5 min.)

Method Validation

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.[23] In this study, flumequine, which belong to the same

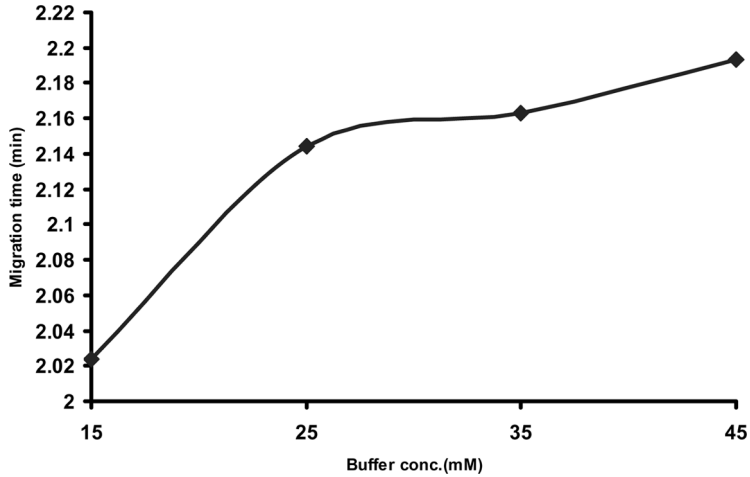

Figure 3. Effect of buffer concentration on migration time. pH of running buffer, 8.5 temperature, 25°C; capillary, 35 (27.5 effective length) cm × 75 μm ID; applied voltage, 12 kV.

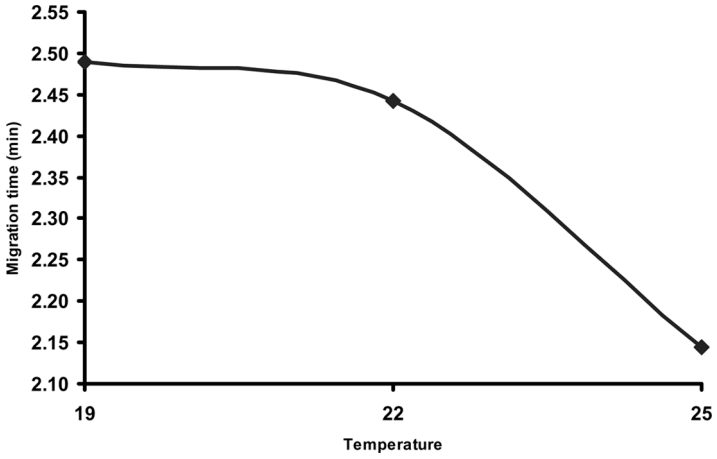

Figure 4. Effect of temperature on migration time. Running buffer, 25 mM H_3PO_4-NaOH; pH 8.5; capillary, 35 (27.5 effective length) cm × 75 μm ID; applied voltage, 12 kV.

group of ofloxacin was selected as the internal standard. In all cases, 7.5 μg mL^{-1} of flumequine was added as internal standard (IS). The assay of ofloxacin was validated with respect to linearity, limit of detection and quantitation, precision, accuracy, robustness, and specificity.[21,24]

Linearity

Using the optimum analysis conditions, linearity was studied in the concentration range of 5 to 50 μg mL^{-1} ofloxacin. The calibration graph was constructed by plotting the ratio of peak area (ofloxacin/IS) (y) as a function of analyte concentration (x) in μg mL^{-1}. The ratio of peak area was chosen rather than the corrected peak area (ratio) as lower RSD (2.21%) was obtained. Each point of the calibration curve corresponded to the mean value obtained from three measurements. The linear regression equation obtained is summarized below:

$$y = 0.0184x + 0.0649$$

Correlation coefficient of 0.9981 was obtained.

Limit of Detection and Quantitation

The limit of detection and the limit of quantitation of the method was estimated to be 1.24 μg mL^{-1} and 4.01 μg mL^{-1}, respectively. This

was obtained by following the recommendations as stipulated in the literature.[21]

Precision

The repeatability of the method was examined by injecting ten consecutive injections of 30 μg mL^{-1} of ofloxacin (keeping the other operating conditions identical). The results were evaluated by considering the migration time, peak area, corrected peak area, ratio of corrected peak, ratio of peak area values of ofloxacin and IS. The precision values with their RSD are summarized in Table 1.

The precision of a method is defined as the closeness of agreement between independent test results obtained under optimum conditions. Three different concentrations of ofloxacin (10.0, 25.0, and 50.0 μg 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 three times. The %RSD values of intra-day and inter-day studies varied from 0.81 to 3.69%, suggesting that the intermediate precision of the method was satisfactory (Table 2).

Recovery

The accuracy of the proposed method was performed by spiking a synthetic mixture with a known amount of ofloxacin. The synthetic mixture contained lactose, microcrystalline cellulose, and magnesium stearate in the ratio 60.0:32.0:0.5.0 (w/w %) and was prepared in a 10 mL volumetric flask. Recoveries ranging from 97.71 to 102.4% were found (Table 3).

Table 1. Repeatability of various parameters expressed as % RSD

Parameter	Ofloxacin IS
Migration time	0.29
0.95	
Peak area	0.50
0.63	
Corrected peak area	0.67
1.85	
Ratio of corrected peak area	2.21
Ratio of peak area	2.27

Table 2. Precision and accuracy of the method for the analysis of ofloxacin (n = 6)

	Intra-day			Inter-day		
Ofloxacin Conc. (μg/mL)	Found[a]	Precision, RSD (%)	Accuracy bias[b] (%)	Found[a]	Precision RSD (%)	Accuracy bias[b] (%)
10	10.53 ± 0.085	0.81	5.35	10.29 ± 0.38	3.69	2.91
25	24.92 ± 0.29	1.17	−0.32	24.99 ± 0.28	1.11	−.007
50	49.94 ± 0.54	1.08	−0.116	49.85 ± 0.62	1.24	−0.30

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

Robustness

Robustness tests were performed to investigate the reliability of results when the experimental parameters were changed. Only one parameter was changed at a time. The determination of 25 μg mL^{-1} ofloxacin standard solution under slight changes in pH and buffer concentration was studied. No significant difference was found between the results, indicating the robustness of the method (Table 4).

Stress Testing and Specificity

The specificity of the method was evaluated by forcibly degrading ofloxacin standards and blanks. The drug was found to be relatively stable under acidic (Figure 3), basic, and elevated temperature conditions since good recoveries were found (Table 5). However, slight degradation when stressed under basic condition was noted, but serious degradation was found when stressed using H_2O_2. No evidence of interference from

Table 3. Recoveries obtained from the determination of ofloxacin in placebos that contained different levels of spiked standards

Ofloxacin spiked, (μg mL^{-1})	Amount found (μg mL^{-1})	Recovery (%) (mean ± S.D) (μg mL^{-1})
5	4.98 ± .08	99.71 ± 1.52
30	30.72 ± 41	102.4 ± 1.38
50	49.91 ± 1.2	99.82 ± 2.54

Mean ± S.D. (n = 6).

Table 4. Determination of ofloxacin standard solution (25 μg mL^{-1}) under different conditions using the CZE method (n = 6)

CZE running conditions	Mean ± SD	RSD (%)
Standard	25.1 ± 0.15	0.61
pH 8.4	25.68 ± 0.58	2.25
pH 8.6	25.44 ± 0.62	2.45
24 mM H_3PO_4 buffer	25.08 ± 0.55	2.19
26 mM H_3PO_4 buffer	25.29 ± 0.51	2.01

Table 5. Results for the determination of ofloxacin (30 μg mL^{-1}) when subjected to different stressed conditions[a]

Stress condition	Recovery (%)
Temperature 75°C	97.94±2.48
1 M HCl	94.65±1.31
1 M NaOH	91.34±0.47
30% H_2 O_2	59.87±1.17

[a]All sample were stressed at 75°C for 15 h.

any excipients in the formulation was found (Figure 3), indicating the specificity of the method.

Application

The developed method was used to quantify ofloxacin in three 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).

Table 6. Results for the determination of ofloxacin in commercial tablets

Sample	Manufacturer's claim, mg	Obtained, mg	Agreement ± SD (%)
Oflo 200	200	199.9	99.99 ± 2.13
Prifloxin	200	202.1	101.0 ± 1.21
Traivid	100	100.8	100.8 ± 2.24

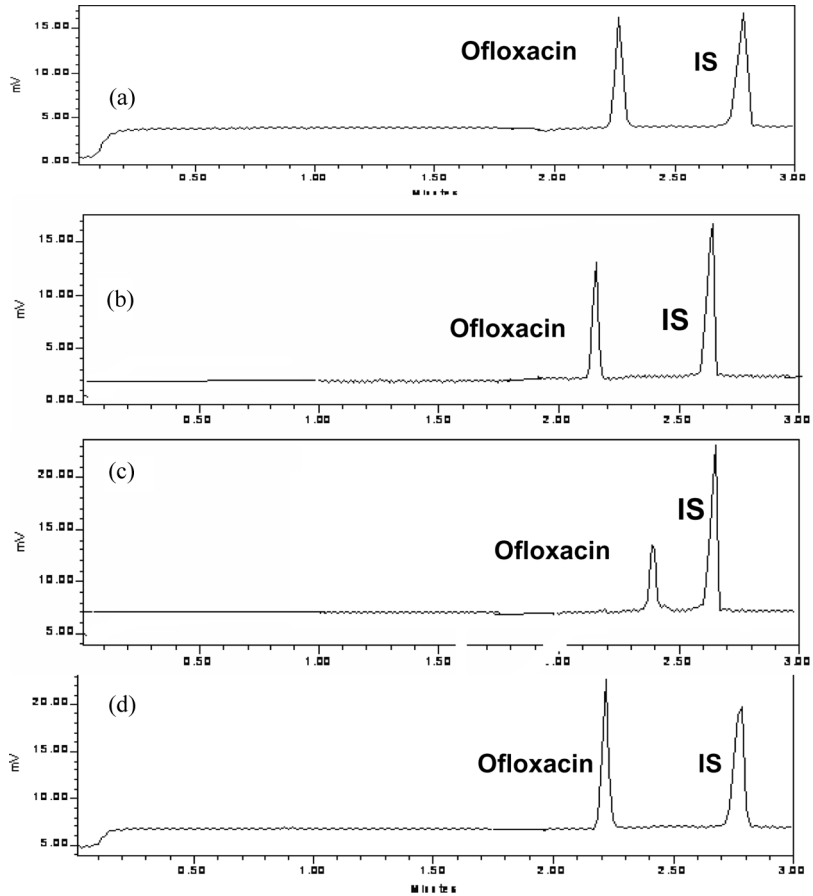

Figure 5. Electropherogram of (a) ofloxacin standard, (b) ofloxacin standard containing HCl, heated to 75°C for 15 h, (c) ofloxacin standard containing H_2O_2, heated to 75°C for 15 h, (d) pharmaceutical tablet. Concentration of all ofloxacin standard, 30 μg mL^{-1}. Refer to text for CE conditions.

CONCLUSION

An alternative analytical method for the determination of ofloxacin in tablets using CZE is proposed. When compared with the reported HLPC method,[6] the proposed method offers distinct advantages such as not requiring expensive analytical columns, large amounts of organic solvents not required as mobile phase, and shorter analysis time (3 min compared to 8 min by HPLC). The proposed method is also superior to the reported MEKC[12] technique in terms of shorter analysis time (3 min compared to 8.5 min) and can be operated using a simple running buffer. Ofloxacin

was found to be stable at an elevated temperature (75°C) for 15 h, under acidic or basic conditions, but was degraded in the presence of the strong oxidizing agent, H_2O_2. However, the degraded products do not interfere in the determination. The method is specific, precise, accurate, and robust and is, therefore, recommended for the routine analysis of ofloxacin in pharmaceuticals.

ACKNOWLEDGMENTS

Financial support of the work by the Universiti Sains Malaysia Short Term grant scheme is gratefully acknowledged.

REFERENCES

1. Cheng, F.C.; Tsai, T.R.; Chen, Y.F.; Hung, L.C.; Tsai, T.H. J. Chromatogr. A. **2002**, *961*, 131–136.
2. Rang, H.P.; Dale, M.M.; Ritter, J.M.; Moore, P.K.; *Pharmacology*, 5th Ed.; Churchill Livingstone: London, 2003; 648.
3. *Therapeutic Drugs*, 2nd Ed.; Dollery, C., Boobis, A., Rawlins, M., Thomas, S., Wilkins, M., Eds.; Churchill Livingstone: London, 1999; 9.
4. Macek, J; Ptacek, P. J. Chromatogr. B. 1995, *673*, 316–319.
5. Ohkubo, T.; Kudo, M.; Sugawara, K. J. Chromatogr. B. **1992**, *573*, 289–293.
6. Shervington, L.A.; Abba, M.; Hussain, B.; Donnelly, J. J. Pharm. Biomed. Anal. **2005**, *39*, 769–775.
7. Zivanovic, L.; Zigic, G.; Zecevic, M. J. Chromatogr. A. **2006**, *1119*, 224–230.
8. Tamer, A. Anal. Chim. Acta. **1990**, *231*, 129–131.
9. Tuncel, M.; Atkosar, Z. Pharmazie **1992**, *47*, 642–643.
10. Zhou, G.; Pan, J.; Wang, Anal. Chim. Acta. **1995**, *307*, 49–53.
11. Rizk, M.; Belal, F.; Aly, F.A.; El-Enany, N.M. Talanta. **1998**, *46*, 83–89.
12. Sun, S.W.; Wu, A.C. J. Liq. Chromatogr. & Rel. Technol. **1999**, *22*, 281–295.
13. Altria, K.D. J. Chromatogr. A. **1996**, *735*, 43–56.
14. Altria, K.D. J. Chromatogr. **1993**, *646*, 245–257.
15. Altria, K.D.; Chanter, Y.L. J. Chromatogr. A. **1993**, *652*, 459–463.
16. Dawson, L.A. J. Chromatogr. B. **1997**, *697*, 89–99.
17. *United States Pharmacopoeia*, 27-NF23; Pharmacopoeial Convention: Rockville, MD, 2005; Ch. *727*, 2425–2429.
18. *European Pharmacopoeia*, 5th Ed.; Method of analysis 2.2.47, Council of Europe: Strasbourg, France, 2005; 74–79.
19. Faria, A.F.; De Souza, V.N.; De Almeida, M.V.; De Oliveira, M.A.L. Anal. Chim. Acta. 2006, *579*, 185–192.
20. Fierens, C.; Hillaert, S.; Van den Bossche, W. J. Pharm. Bimed. Anal. **2000**, *22*, 763–772.
21. International Conference Harmonization (ICH) Validation of Analytical Procedures: Methodology, Q2B (CPMP/ICH/281/95), 1995.

22. Rabanal, B.; Paz, E.D.; Walser, N.; Negro, A. J. Liq. Chromatogr. & Rel. Technol. 2001, *24*, 29–45.
23. Mayer, B.X. J. Chromatogr. A. **2001**, *907*, 21–37.
24. Toasaksiri, B.X.; Massart, D.L.; Heyden, Y.V. Anal. Chim. Acta. **2000**, *419*, 29–42.

Received April 6, 2008
Accepted May 3, 2008
Manuscript 6327