Research Article

Biomedical Chromatography

Received: 12 December 2008, Revised: 18 March 2009, Accepted: 19 March 2009 Published online in Wiley Interscience: 1 June 2009

(www.interscience.wiley.com) DOI 10.1002/bmc.1251

Simultaneous determination of ofloxacin and ornidazole in pharmaceutical preparations by capillary zone electrophoresis

Khoo Lay See,[a] Abdalla A. Elbashir,[b] Bahruddin Saad,[a] Abdussalam Salhin Mohamed Ali[a] and Hassan Y. Aboul-Enein[c]*

ABSTRACT: A simple, rapid and validated capillary electrophoretic method has been developed for the separation and determination of ofloxacin and ornidazole in pharmaceutical formulations with detection at 230 nm. Optimal conditions for the quantitative separations were investigated. Analysis times shorter than 4 min were obtained using a background electrolyte solution consisting of 25 mmol/L phosphoric acid adjusted with 1 M Tris buffer to pH 8.5, with hydrodynamic injection of 5 s and 20 kV separation voltage. The validation criteria for accuracy, precision, linearity and limits of detection and quantitation were examined and discussed. An excellent linearity was obtained in concentration range 25–250 μg/mL. The detection limits for ofloxacin and ornidazole were 1.03 ± 0.11 and 1.80 ± 0.06 μg/mL, respectively. The proposed method has been applied for the analysis of ofloxacin and ornidazole both individually and in a combined dosage tablet formulation. The proposed validated method showed recoveries between 96.16 and 105.23% of the nominal contents. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: capillary zone electrophoresis; ofloxacin; ornidazole; pharmaceutical analysis

Introduction

Capillary electrophoresis (CE) is a highly attractive resolving technique used to determine charged components because of the combination of high resolution efficiency, rapid method development with short analysis time and easy automation with small to modest sample requirement and low solvent consumption (Barbosa *et al*., 1999; Maraschiello *et al*., 2001; Panzade and Mahadik, 2001; Zhou *et al*., 2006).

Ofloxacin is chemically kown as (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid [Fig. 1(a)]. It is a broad spectrum antibiotic which belongs to the third generation of fluorinated quinolone (Awadallah *et al*., 2003; Elbashir *et al*., 2007; Zhou *et al*., 2006; Rizk *et al*., 1998; Gandhimathi *et al*., 2006; Shao *et al*., 2008) and is one of the most frequently used fluorinated quinolone antibiotics (Cheng *et al*., 2001).

Ornidazole [Fig. 1(b)] is chemically known as 1-chloro-3-(-2-methyl-5-nitroimidazole-1-yl)propan-2-ol (Chankvetadze *et al*., 1995; Singh *et al*., 2003; Wang *et al*., 2004) or 1-(3-chloro-2-hydroxy)propyl-2-methyl-5-nitroimidazole (Zhang *et al*., 2006). Ornidazole has a single ionization constant with a pK_a of approximately 2.4 (Singh *et al*., 2003).

Several methods have been reported for analysis of ofloxacin and ornidazole individually and in combination with other drugs using HPLC (Hernández *et al*., 2000; Halker and Ankalkope, 2000; Róna and Gachályi, 1987; Lalhriatpuii *et al*., 2006; Paramane *et al*., 2007). Furthermore spectrophotometric (Mruthyunjayaswamy *et al*., 2004; Patel *et al*., 2005; Prasad *et al*., 2004) and electrochemical methods (Patel *et al*., 2006; Özkan *et al*., 1997; Sankar and Reddy, 1989; Yang *et al*., 2008) have been also cited for the analysis of these drugs. However, few citations have been reported for the simultaneous estimation of ofloxacin and ornidazole in tablets. A high-performance thin-layer chromatography (HPTLC) method was reported by Gandhimathi *et al*. (2006).

This paper describes the method development and validation for simultaneous separations and determination of ofloxacin and ornidazole using CE in pharmaceutical formulations. To the best of our knowledge, this method is the first to describe the simultaneous determination of ofloxacin and ornidazole in various pharmaceutical preparation using CE.

Experimental

Materials and Methods

The analysis was carried out on an $HP_{3D}CE$ system (Agilent Technologies, Waldbronn, Germany), model G1600A, which was interfaced to a Agilent Chemstation Software and equipped with a photo diode array detector. Uncoated fused-silica

* Correspondence to: H. Y. Aboul-Enein, Pharmaceutical and Medicinal Chemistry Department, National Research Centre, Dokiki, Cairo 12311, Egypt. E-mail: enein@gawab.com

[a] School of Chemical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

[b] Faculty of Science, Chemistry Department, Khartoum University, PO Box 321, Khartoum, Sudan

[c] Pharmaceutical and Medicinal Chemistry Department, National Research Centre, Dokiki, Cairo 12311, Egypt

Abbreviations used: CE, capillary electrophoresis.

Contract/grant sponsor: Universiti Sains Malaysia Research University.

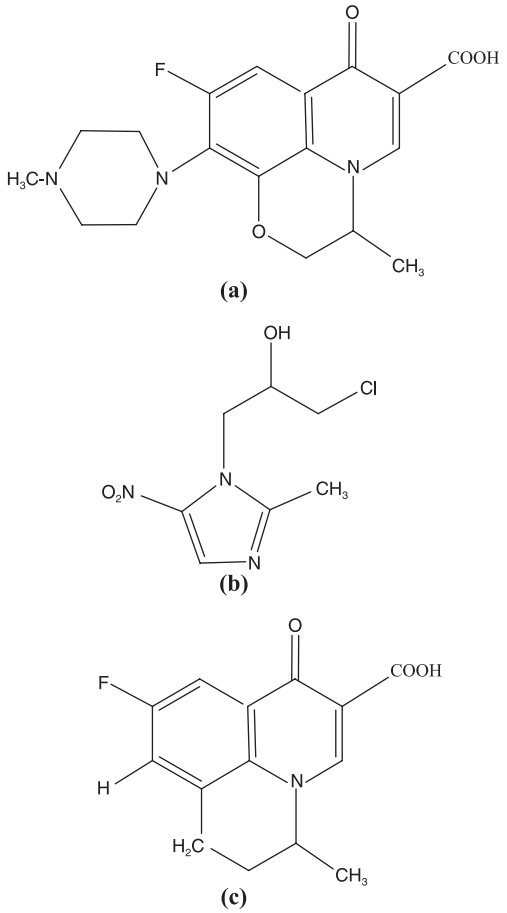

Figure 1. (a) Chemical structure of ofloxacin. (b) Chemical structure of ornidazole. (c) Chemical structure of flumequine, the internal standard.

capillary 50 μm i.d × 56 cm (detection length, 8.5 cm from the outlet end of the capillary) from Agilent Technologies was used.

Data were collected using the software provided connected to PC Workstation. A polyamide coated fused-silica capillary, with a total length of 40 cm and an internal diameter, i.d., of 50 μm, was used for the separation. For pH measurement, a digital pH meter (Orion Research, Expandable Ion Analyzer, EA 940) was used.

Chemicals and Reagents

Racemic ofloxacin, ornidazole and flumequine [Fig. 1(c)] were purchased from Sigma-Aldrich (St Louis, MO, USA).

Commercial tablet pharmaceutical preparations (claimed to contain 500 mg and 200 mg active ingredients; ornidazole and ofloxacin respectively) were obtained from other countries where the combination of the two drugs was available and they were manufactured by different pharmaceutical companies. However single active drug ingredient tablet formulations are available in Malaysia and were obtained from local drug stores. Milli-Q water (Millipore, Bedford, USA) was used for the preparation of all solutions.

Method and Operating Conditions

The separation was carried out at 25 ± 0.1°C with a voltage of +20 kV, in order to keep the total current below 100 μA. Samples were injected hydrostatically at 0.5 psi for 5 s (1 psi = 6894.76 Pa). Detection was achieved at 230 nm. A new coated fused silica capillary was conditioned by flushing with 1 M NaOH for 30 min, then with 0.1 M NaOH for 10 min and water as well as buffer solution, each for 10 and 11 min respectively.

The running buffer consisted of 25 mM phosphoric acid (H_3PO_4) adjusted to the desired pH with 1 M Tris solution. The running buffer solutions were filtered and degassed. All standards, sample solutions, the running buffer and NaOH solution were filtered through a 0.45 μm, 47 mm diameter regenerated cellulose nitrate membrane filter (Whatman International, Maidstone, UK), and degassed by sonication, prior to each use.

Before changing each buffer for different buffer system, the capillary was purged with 1 M sodium hydroxide for 15 min, followed by 20 min Milli-Q water and appropriate buffer electrolyte for 30 min. The final step was the application of a voltage of 20 kV for 20 min with the capillary filled with the buffer solution. Each day, the system was first purged with 0.1 M NaOH for 5 min, followed by water for 15 min and the working buffer solution for 20 min.

Prior to each analysis, the electrophoresis capillary underwent preconditioning and was rinsed with 0.1 M NaOH for 3 min, and then with purified water (Milli-Q water) for 3 min, and finally followed by the background electrolyte, for 4 min between the runs. This was done to equilibrate the capillary and thereby minimize hysteresis effects (Barrón *et al.*, 2000; Barbosa *et al.*, 1999). The capillary was stored overnight filled with working buffer electrolyte.

Stock and Standard Solutions

Ofloxacin standards and ofloxacin tablets were stored at 4°C in the dark to minimize photolytically induced degradation.

Standard stock solution (1000 μg/mL) of ofloxacin was prepared in 0.1 M NaOH and was kept refrigerated. Working standard solutions were prepared daily by diluting the suitable aliquots of the stock solution with water. The standard solutions were stored in brown glass vials for protection from light.

Standard stock solution (1000 μg/mL) of ornidazole was prepared in a minimum amount of HPLC-grade methanol and kept refrigerated. Working standard solutions were prepared daily by diluting the suitable aliquots of the stock solution with water. The standard solutions were stored in brown glass vials for protection from light.

Pharmaceutical Sample Preparation

Ten tablets were weighed, ground and mixed in a mortar. An appropriate amount of the powder was taken and dissolved in 50 mL of 0.1 M sodium hydroxide and a minimum amount of HPLC-grade methanol, and ultrasonicated for three minutes and diluted to 50 mL with Milli-Q water. The sample was filtered through a membrane and the solution was introduced to the CE system for separation.

Results and Discussion

Optimization of Separation Conditions

Wavelength selection. The UV spectra of ofloxacin in aqueous solution showed two peaks, a strong peak at 287 nm and a weak peak at 332 nm. The observed strong peak corresponds to the chromophore involving the *N*-1 position to the carboxylic group

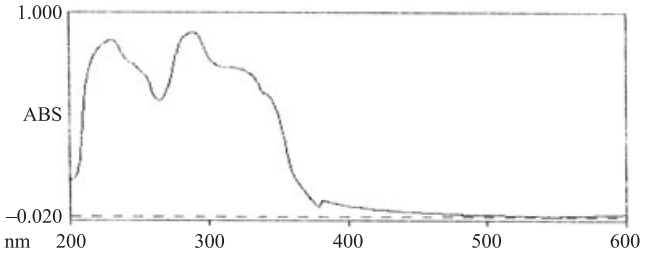

Figure 2. UV spectrum of ofloxacin and ornidazole mixture. Experimental result obtained with a mixture of 100 μg/mL solution of ornidazole and ofloxacin, dissolved in a minimal amount of methanol and 2 mL 0.1 M sodium hydroxide.

while the weak absorption peak corresponds to the chromophore involving the nitrogen of the piperazinyl group to carbonyl group. This spectrum is affected when ofloxacin form complexes with metallic cations, especially divalent cations, leading to a red shift of the strong absorption peak to 285 nm and then blue shift of the weak absorption peak to 330 nm (Lin *et al*., 2004). On the other hand, Singh *et al*. (2003) reported that ornidazole exhibited maximum peaks at 230 and 311 nm when a 20 μg/mL solution of ornidazole was dissolved in isopropanol. Hence, the wavelength of 230 nm was chosen in this study. This was complementary for the ornidazole because at wavelength 287 nm, the peak sensitivity was too low and the height obtained from the electropherogram was too low. Moreover, wavelength 332 nm was not chosen as it was close to the weak peak for ofloxacin (Fig. 2).

Effect of pH

A better understanding of the influence of various experimental parameters is essential in predicting the migration behavior of individual solutes in a mixture and, consequently, in optimizing their separation. In electrophoretic separation of ionizable compounds, pH plays an important role as it determines the extent of ionization of each individual solutes (Zhou *et al*., 2006). Buffer acidity may affect mobility and electroosmosis flow (EOF) by changing the dissociation constant of analyte and Si–OH groups on the capillary. Previous work on ofloxacin resolution indicated that electrophoretic separation of the analytes should be possible at nearly all pH values except at their isoelectric points, which are between pH 6.5 and 7.5. The optimum pH is always smaller than the pK_a by 0.30 regardless of the ratio K_{a1}/K_{a2} (Elbashir *et al*., 2007; Zhou *et al*., 2006).

The effect of pH on the resolution (R_s) and migration time was investigated over the pH range 5.0–9.0, using 25 mmol/L buffer solutions prepared at different pH. At pH 6.5, the mixture of ofloxacin and ornidazole standards was separated. However, with increasing pH the two active ingredients separated more efficiently. At pH 8.5, the compounds migrated faster due to increasing EOF. Based on the result obtained (Fig. 3), pH 8.5 was chosen as the optimal pH for separation of the two analytes**.**

Effect of Buffer Concentration

The chemical composition and the concentration of the buffer can affect the baseline stability, peak shape and separation selectivity. In this study, a new set of experiments was proposed taking into account a variable made from the Tris–phosphoric acid (pH 8.5) buffer. The experimental levels were established

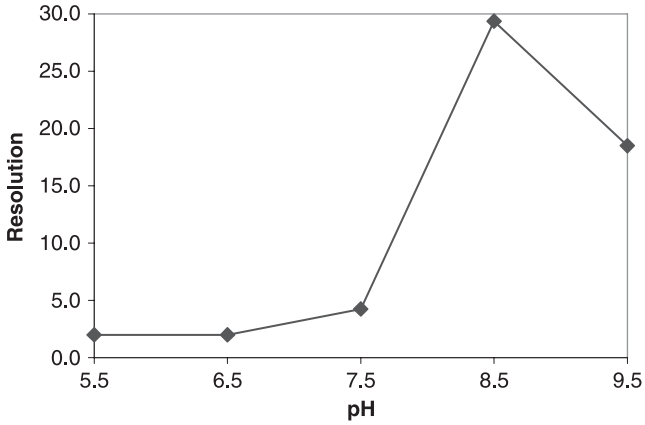

Figure 3. Effect of buffer pH on resolution. Conditions: 25 mM Tris–H_3PO_4 buffer; voltage, 20 kV; temperature, 25°C; and injection time, 5 s.

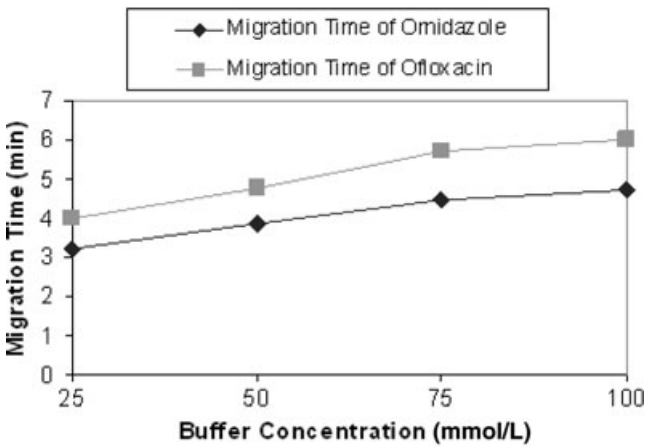

Figure 4. Effect of buffer concentration on migration time. Conditions: Tris–H_3PO_4 buffer, pH 8.5; voltage, 20 kV; temperature, 25°C; and injection time, 5 s.

through a series of concentration variations of buffer from 25 to 100 mmol/L. The experiments were carried out with a standard mixture containing the two active ingredients under study.

Figure 4 indicates that, as the buffer concentration increased, the resolution slightly increased but at the same time the migration time and the peak width also increased. Symmetrical peaks were observed at 25 mmol/L but were broadened when the buffer concentration was 50 mmol/L. However as expected peak tailing was observed when the buffer concentration changed from 75 to 100 mmol/L. There was an increase in analyte migration times due to a decrease in electroosmosis flow (EOF) with increasing buffer concentration. Hence, a 25 mmol/L buffer was selected to achieve good resolution without sacrificing the migration time.

Effect of Separation Voltage

It is well known that increasing the voltage gives rise to shorter migration times. However, the generation of Joule heat may limit the theoretical gain in the resolution and efficiency when the voltage is increased (Elbashir *et al*., 2007). In this study, voltages between 15 and 30 kV were investigated. A decrease

Table 1. Optimum CE operating conditions

Parameters	Conditions
Electrolyte	25 mmol/L acid phosphoric (H_3PO_4) adjusted to pH 8.5 with 1 M Tris solution
Applied voltage	20 kV
Sample injection	5 s hydrodynamically
Capillary temperature	25°C
Fused silica capillary	40 cm effective length × 50 μm i.d.
Detection wavelength	230 nm

in migration times was observed from 15 to 30 kV. The voltage of 20 kV was chosen because there was adequate resolution of the two drugs and the signal-to-noise ratio was satisfactory.

Effect of Injection Time and Capillary Temperature

In order to reduce the detection limits, the injection time was varied from 3 to 15 s. Using hydrostatic injection, a 5 s injection time was selected as the optimal value. At the same time, capillary temperatures ranging from 17 to 29°C were investigated. In this study, the best operating CE conditions were obtained at 25°C.

Details of optimized electrophoretic separation conditions are summarized in Table 1, while Fig. 5 shows the electropherogram of the compounds separated under these optimized conditions.

Method Validation

Internal standard selection. In order to significantly reduce the injection-related impression and to ensure better reproducibility and greater control over the sample amount injected, the use of an internal standard (IS) in quantitative analysis is generally preferred (Taverniers *et al*., 2004). In this method, an internal standard was selected to be added to the solution of mixture ofloxacin and ornidazole.

Lomefloxacin, sparfloxacin, flumequine and phenobarbital were added to the mixture. While limited resolution was obtained for lomefloxacin and phenobarbital, there was an unexpected overlapping of peaks of sparfloxacin with ofloxacin. A complete recognition of resolution was obtained with flumequine, the structure of which is shown in Fig. 1(c) with a satisfactory analysis time.

Linearity. The linearity of the analytical procedure was evaluated by plotting the detector response (peak area) vs the nominal concentration of ornidazole and ofloxacin present in the mixture of standard solutions. Each calibration curve was constructed using seven different standard solutions of 5 to 250 μg/mL (5, 10, 25, 50, 100, 150 and 250 μg/mL) and spiked with 20/μg mL of internal standard, performed in triplicate. The correlation coefficient R^2 for the two analytes was 0.999 over a relatively wide concentration range (5–250 μg/mL).

The linear regression equations obtained were as follows:

$$\text{ofloxacin: } y = (0.0465 \pm 4.18)x - 0.1641$$

$$\text{ornidazole: } y = (0.0166 \pm 1.50)x + 0.9125$$

Precision. Intra-day precision was assessed by injecting a standard mixture of ofloxacin and ornidazole at three different concentrations within the linear range of the calibration curve, at 50, 150 and 250 μg/mL. The relative standard deviation (RSD) for migration times and corrected peak areas were less than 2.5 and 5.0%, respectively, as shown in Table 2.

The inter-day precision was assessed with standard mixture containing both drugs at 50, 150 and 250 μg/mL. The validation of the analytical procedure was performed over a period of 6 days. The relative standard deviations were less than 4.0 and 6.0% for migration time and corrected peak areas, respectively.

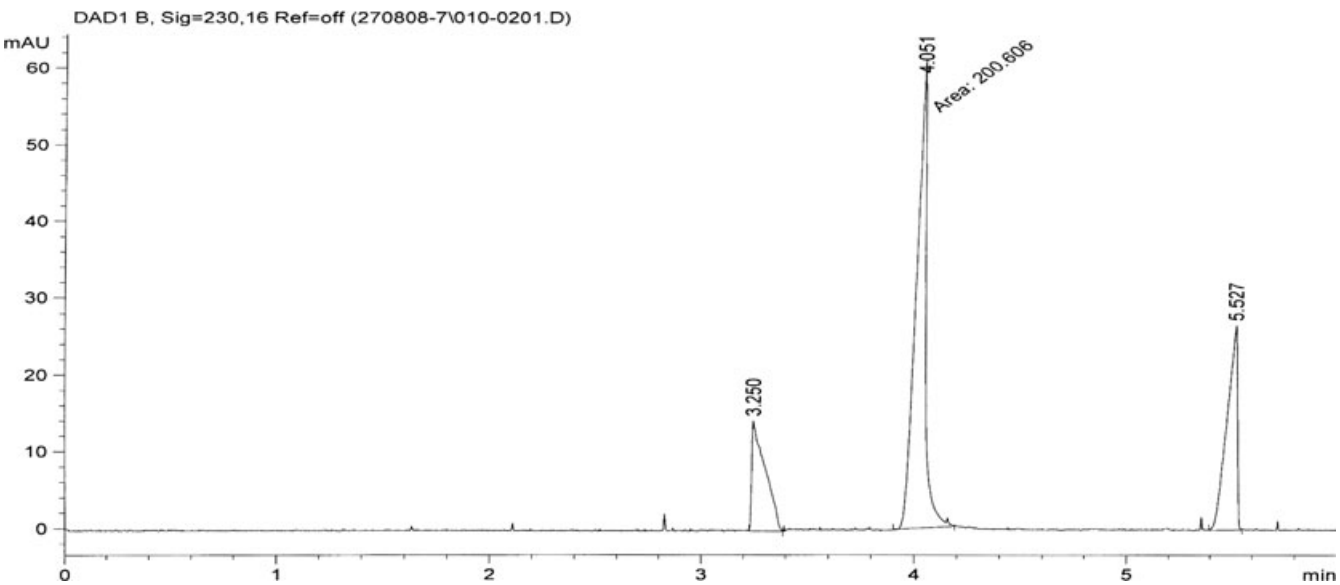

Figure 5. Typical electropherogram of the separated compounds under the optimized conditions, where peak 1 is ornidazole; peak 2 is ofloxacin and peak 3 is IS flumequine. Conditions: 25 mM Tris–H_3PO_4, pH 8.5, voltage, 20 kV; temperature, 25°C; and injection time, 5 s.

Table 2. Intra- and inter-day reproducibility for the repeated injection of different concentrations of both ofloxacin and ornidazole

Concentration (μg mL^{-1})	RSD% (migration time)		RSD% (corrected peak areas)	
	Ornidazole	Ofloxacin	Ornidazole	Ofloxacin
Intra-day precision (n = 9)				
50	0.88	1.00	4.07	4.91
150	0.58	1.44	3.92	2.38
250	1.77	2.29	3.92	5.07
Inter-day precision (n = 54)				
50	2.28	1.28	5.60	5.26
150	2.99	3.64	6.22	6.01
250	2.57	2.90	5.20	5.96

Table 3. Recoveries obtained from the determination of ofloxacin and ornidazole in commercial formulations that contain different levels of spiked standards

Sample no.	Standard mixture spiked (μg/mL)	Recovery (%, mean ± SD)	
		Ornidazole	Ofloxacin
1	50	110.59 ± 3.65	98.37 ± 3.33
2	100	102.07 ± 2.30	92.51 ± 1.81
3	150	103.03 ± 1.79	97.59 ± 3.45

Accuracy/recovery. The proposed method was validated in two commercial tablet formulations. Several aliquots of the ofloxacin and ornidazole mixture at three different concentrations, namely, 50, 100 and 150 μg/mL, were added to a weighted, ground powder of ofloxacin and ornidazole combination tablet. Table 3 shows that the recovery results for ofloxacin and ornidazole were 92.51–98.37 and 102.07–110.59%, respectively.

Limit of detection. In this study, the limit of detection (LOD) was determined based on the standard deviation of the response, σ, and the slope of the calibration curve, *S*. The LOD can be expressed from the following equation:

$$\mathrm{LOD} = \frac{3.3\sigma}{S} \quad (1)$$

Based on the standard deviation of the blank, measurement of the magnitude of analytical background response was performed by analyzing an appropriate number of blank samples, i.e. 10 (Taverniers *et al*., 2004). Calculation of standard deviation, σ, was performed from the responses. From the result, the LODs for ofloxacin and ornidazole were 1.03 ± 0.11 and 1.80 ± 0.06 μg/mL, respectively.

Limit of quantification. In this study, the LOQ was determined based on the standard deviation of the response, σ, and the slope of the calibration curve, *S*. The LOQ can be expressed from the following equation:

$$\mathrm{LOQ} = \frac{10.0\sigma}{S} \quad (2)$$

Based on the standard deviation of the blank, measurement of the magnitude of analytical background response was performed by analyzing an appropriate number of blank samples, i.e. 10. Calculation of the standard deviation, σ, was performed from the responses. From the result, the LOQs calculated were 3.19 ± 0.11 and 5.46 ± 0.06 μg/mL, for ofloxacin and ornidazole, respectively.

Table 4 presents the obtained results for linear coefficient, R^2, regression equation, precision, recovery, LOD and LOQ.

Table 4. Validation parameters for the analysis of ofloxacin and ornidazole

Validated results	Ofloxacin	Ornidazole
Linear coefficient, R^2	0.9996	0.9992
Regression equation	y = 0.0465 x – 0.1641	y = 0.0166 x + 0.9125
Linear range (concentration)	25–250 μg/mL	25–250 μg/mL
Precision (corrected peak area)		
Inter-day	5.26–6.01%	5.20–6.22%
Intra-day	2.38–5.07%	3.92–4.07%
Precision (migration time)		
Inter-day	1.28–3.64%	2.28–2.99%
Intra-day	1.00–2.29%	0.58–1.77%
Recovery	96.16% (92.51–98.37%)	105.23% (102.07–110.59%)
LOD	1.03 ± 0.11 μg/mL	1.80 ± 0.06 μg/mL
LOQ	3.19 ± 0.11 μg/mL	5.46 ± 0.06 μg/mL
Concentration/MDL	3.94	2.61
Signal-to-noise	6.17	9.57

Table 5. Determination of pharmaceutical formulations containing ofloxacin and ornidazole (figures in bracket denote the manufacturer's claimed value)

Sample no.	Commercial formulation[a]	Ofloxacin (mg)	Ornidazole (mg)	Total active ingredients (mg)
1	Oflotas-OZ; INTAS Pharmaceuticals, India (ofloxacin 200 mg and ornidazole 500 mg)	204.90 ± 2.59	451.75 ± 11.29	656.65 ± 13.88
2	JAKA Pharmaceuticals, Russia (ofloxacin 200 mg and ornidazole 500 mg)	195.71 ± 3.92	517.33 ± 9.71	713.04 ± 13.63
3	OXWALOZ, Wallace Pharmaceuticals Pvt, Ltd, India (ofloxacin 200 mg and ornidazole 500 mg)	210.38 ± 2.92	502.43 ± 11.13	712.81 ± 14.05
4	Tarivid Tablet; Thialand (ofloxacin 200 mg)	186.68 ± 1.26	—	186.68 ± 1.26
5	Oflicin; Penang General Hospital, Malaysia (ofloxacin 100 mg)	94.8 ± 0.84	—	94.8 ± 0.84
6	OFLO; Unique Pharmaceutical, Malaysia (ofloxacin 200 mg)	182.73 ± 2.43	—	182.73 ± 2.43
7	Tiberal; Sun Pharmaceutical, Russia (ornidazole 500 mg)	—	462.58 ± 7.30	462.58 ± 7.30
8	Roche, Russia (ornidazole 500 mg)	—	485.03 ± 3.68	485.03 ± 3.68

Analysis of Pharmaceutical Formulations

The developed method was applied for the analysis of combination ofloxacin and ornidazole tablets in various commercial pharmaceutical formulations. Triplicate determinations were carried out. The results obtained are summarized as in Table 5. Three different pharmaceuticals formulations were analyzed in this study for the simultaneous analysis of ofloxacin and ornidazole in a tablet dosage form (samples 1–3). The remaining five samples (samples 4–8) were individual tablets for ofloxacin and ornidazole.

All the pharmaceutical formulations analyzed showed good agreement with the claimed amounts of the active ingredient(s), as shown in the electropherograms in Fig. 6.

Conclusion

A validated capillary electrophoretic method is described for the simultaneous determination of of ofloxacin and ornidazole in pharmaceutical tablet formulations. The proposed method is fast, accurate, precise, reproducible and inexpensive. Several variables were studied and the optimized CE conditions were as follows: 25 mmol/L phosphoric acid adjusted to pH 8.5 with 1 M Tris solution; applied voltage 20 kV; capillary temperature 25°C; and injection time 5 s. The analytes were determined quantitatively at 230 nm. The proposed method could be adopted in quality control laboratories for the analysis of these two drugs individually or in combination.

Acknowledgements

Financial support of the project by the Universiti Sains Malaysia Research University grant scheme is acknowledged.

References

Awadallah B, Schmidt PC and Wahl MA. Quantitation of the enantiomers of ofloxacin by capillary electrophoresis in the parts per billion concentration range for *in vitro* drug absorption studies. *Journal of Chromatography A* 2003; **988**: 135–143.

Barbosa J, Barrón D and Jiméne-Lozano E. Electrophorestic behaviour of quinolones in capillary electrophoresis: effect of pH and evaluation of ionization constants. *Journal of Chromatography A* 1999; **839**: 183–192.

Barrón D, Jiménez-Lozano E and Barbosa J. Electrophoretic behaviour of zwitterionic compounds in capillary electrophoresis: Prediction of mobility of several quinolones. *Analytica Chimica Acta* 2000; **415**: 83–93.

Chankvetadze B, Endresz G and Blaschke G. Enantiomeric resolution of chiral ingredients in composite pseudoephedrine hydrochloride with cyclodextrin-type buffer modifiers. *Journal of Chromatography A* 1995; **700**: 43–49.

Cheng G-W, Wu H-L and Huang Y-L. Simultaneous determination of malondialdehyde and ofloxacin in plasma using isocratic high-performance liquid chromatography. *Analytica Chemica Acta* 2001; **616**: 230–234.

Elbashir AA, Saad B, Abdussalam Ali ASM, Saleh MI and Aboul-Enein HY. Determination of ofloxacin enantiomers in pharmaceutical formulations by capillary electrophoresis. *Journal of Liquid Chromatography and Related Technologies* 2007; **31**: 348–360.

Gandhimathi M, Ravi TK and Shukla N. Validated high performance thin layer chromatography method for simultaneous estimation of ofloxacin and ornidazole in tablet dosage form. *Indian Journal of Pharmaceutical Sciences* 2006; **68**: 838–840.

Halkar UP and Ankalkope PB. Reverse-phase high-performance liquid chromatographic determination of ofloxacin and tinidazole in tablets. *Indian Drugs* 2000; **37**: 585–588.

Hernández M, Borrull F and Caluli M. Determination of quinolones in plasma samples by capillary electrophoresis using solid-phase extraction. *Journal of Chromatography B: Biomedical Sciences and Applications* 2000; **742**: 255–265.

Lalhriatpuii TC, Jatav RK, Goel PK and Kawathekar N. Simultaneous determination of metronidazole and ofloxacin in suspension dosage form using UV–visible spectrophotometer. *Asian Journal of Chemistry* 2006; **18**: 559–565.

Lin C-E, Deng Y-J, Liao W-S, Sun S-W, Lin W-Y and Chen C-C. Electrophoretic behaviour and pKa determination of quinolones with a piperazinyl substituent by capillary electrophoresis. *Journal of Chromatography A* 2004; **1051**: 283–290.

Maraschiello C, Cusido E, Abellán M and Villageliu J. Validation of an analytical procedure for the determination of the fluoroquinolone ofloxacin in chicken tissues. *Journal of Chromatography B: Biomedical Sciences and Applications* 2001; **754**: 311–318.

Mruthyunjayaswamy BHM, Mali Ptil SM and Apalla Raju S. Spectrophotometric determination of ornidazole. *Journal of the Indian Chemical* Society 2004; **81**: 346–348.

Özkan SA, Senturk Z and Biryol I. Determination of ornidazole in pharmaceutical dosage form based on reduction at an activated glassy carbon electrode. *International Journal of Pharmaceutics* 1997; **157**: 137–144.

Panzade PD and Mahadik KR. Simultaneous estimation of ofloxacin and tinidazole in table dosage form. *Indian Drugs* 2001; **38**: 368–370.

Paramane S, Kothapalli L, Thomas A and Deshpande AD. Simultaneous RP-HPLC estimation of gatifloxacin and ornidazole in tablet dosage form. *Indian Journal of Pharmaceutical Sciences* 2007; **67**: 525–528.

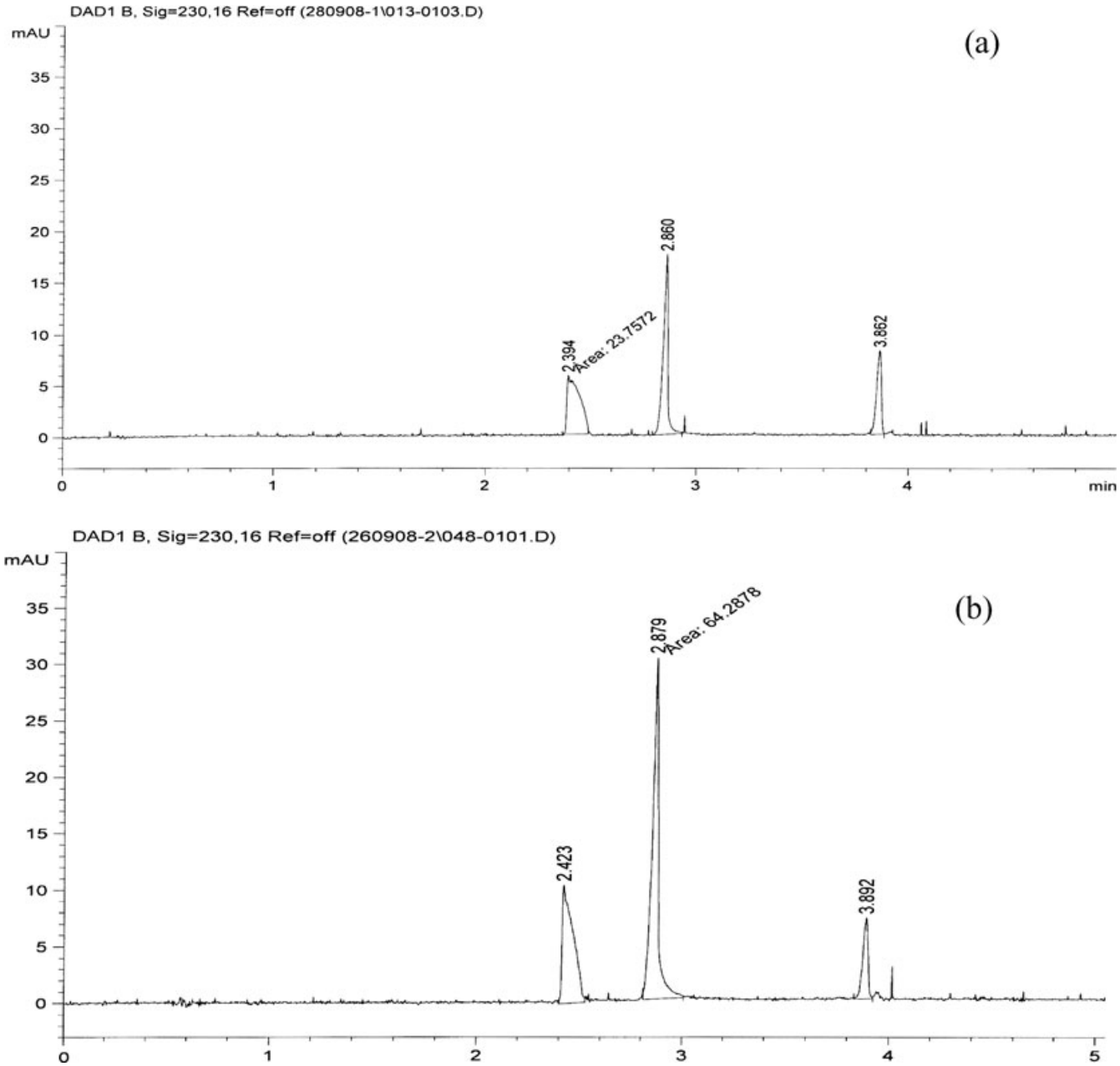

Figure 6. Typical electropherograms of some of the pharmaceutical formulations: (a) INTAS Pharmaceuticals; (b) JAKA Pharmaceuticals, where peak 1 is ornidazole; peak 2 is ofloxacin and peak 3 is IS flumequine. Conditions: 25 mM Tris–H_3PO_4, pH 8.5; voltage, 20 kV; temperature, 25°C; and injection time, 5 s.

Patel PU, Suhagia BN, Patel CN, Patel MN, Patel GC and Patel GM. Simultaneous spectrophotometric estimation of gatifloxacin and ornidazole in mixture. *Indian Journal of Pharmaceutical Sciences* 2005; **67**: 356–357.

Patel SA, Patel NM and Patel MM. Simultaneous spectrophotometric estimation of ciprofloxacin and ornidazole in mixture. *Indian Journal of Pharmaceutical Sciences* 2006; **68**: 665–667.

Prasad AB, Reddy N, Reddy YVB and Chakravathy IE. Simultaneous spectrophotometric determination of ofloxacin and tinidazole from combined dosage forms from pharmaceutical formulations. *Asian Journal of Chemistry* 2004; **16**: 1905–1910.

Rizk M, Belal F, Aly FA and El-Enany NM. Differential pulse polarographic determination of ofloxacin in pharmaceuticals and biological fluids. *Talanta* 1998; **46**: 83–89.

Róna K and Gachályi B. Simple liquid chromatographic method for the determination of ornidazole and metronidazole in human serum. *Journal of Chromatography: Biomedical Applications* 1987; **420**: 228–230.

Sankar PS and Reddy SJ. Electrochemical study and polarographic determination of ornidazole. *Indian Journal Environment Protection* 1989; **9**: 589–591.

Shao B, Sun X, Zhang J, Hu J, Huiru Dong and Yang Y. Determination of ofloxacin enantiomers in sewage using two-step solid-phase extraction and liquid chromatography with fluorescence detection. *Journal of Chromatography A* 2008; **1182**: 77–84.

Singh P, Mittal R, Sharma GC, Singh S and Singh A. Ornidazole: comprehensive profile. *Profiles of Drug Substances, Excipients and Related Methodology* 2003; **30**: 123–184.

Taverniers I, De Loose M and Van Bockstacle EI. Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. *Trends in Analytical Chemistry* 2004; **23**: 535–552.

Wang M-H, Tan Z-C, Sun X-H, Liu Y-F, Sun L-X and Tao Zhang. Heat

capacity and thermodynamic properties of crystalline ornidazole ($C_7H_{10}ClN_3O_3$). *Thermochimica Acta* 2004; **414**: 25–30.
Yang C, Zhang S, Liu Y and Huang W. Electrochemical behaviours of ofloxacin and its voltammetric determination at carbon nanotubes film modified electrode. *Frontiers Chemistry in China* 2008; **3**: 353–358.
Zhang L, Zhang Z and Wu K. *In vivo* and real time determination of ornidazole and tinidazole and pharmacokinetic study by capillary electrophoresis with microdialysis. *Journal of Pharmaceutical and Biomedical Analysis* 2006; **41**: 1453–1457.
Zhou S, Ouyang J, Baeyens WRG, Zhao H and Yang Y. Chiral separation of four fluoroquinolone compounds using capillary electrophoresis with hydroxypropyl-β-cyclodextrin as chiral selector. *Journal of Chromatography A* 2006; **1130**: 296–301.

