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Short communication

Separation of seven fluoroquinolones by microemulsion electrokinetic chromatography and application to ciprofloxacin, lomefloxacin determination in urine

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Abstract

A simple, reliable microemulsion electrokinetic chromatography (MEEKC) method is developed for the simultaneous separation of seven fluoroquinolones (FQs). The best separation is achieved in a carrier electrolyte containing 1% (v/v) heptane, 100 mmol/L sodium dodecyl sulfate (SDS), 10% (v/v) 1-butanol, and 8 mmol/L phosphate–sodium tetraborate buffer at pH 7.30. The proposed method was directly applied to the determination of ciprofloxacin (CPF) and lomefloxacin (LMF) in urine samples of subjects administered either with CPF or LMF.

Keywords: Microemulsion electrokinetic chromatography; Fluoroquinolones; Ciprofloxacin; Lomefloxacin

1. Introduction

In generally, following oral administration, fluoroquinolones (FQs) are rapidly and completely absorbed from the gastrointestinal tract and reach the maximal plasma concentration in 1–2 h [1–3]. But how long it takes to reach the maximal urine concentration has not been investigated, and this may be useful to know in clinical pharmacology. Therefore, there is a necessity to develop analytical methods that would allow the directly determination of FQs in urine and make it suitable for routine analysis in clinical laboratories.

Several techniques such as spectrophotometry [4], spectrofluorometry [5], electrochemical detection [6], flow-injection chemiluminescence [7], capillary electrophoresis (CE) [8] and high-performance liquid chromatography (HPLC) [9–11] have been developed for the determination of FQs in urine samples. However, the literature available on determination of FQs in urine is very short and commonly suffers from long pretreatment of the samples and low resolving power.

MEEKC [12] is a relatively new CE technique that often provides higher resolving power. In this study, we develop a selective MEEKC method for the separation of CPF, LMF, norfloxacin (NF), ofloxain (OF), fleroxacin (FL), gatifloxacin (GTF) and sparfloxacin (SPF) and determination of CPF or LMF in urine samples.

2. Experimetal

2.1. Instrumentation

A separation was performed with a Beckman (Fullerton CA, USA) PACE/MDQ capillary electrophoresis system. 32 Karat Software V.7.0 was used for instrument control and data analysis. An untreated fused-silica capillary of 56.9 cm (46.6 cm from inlet to detector) × 75 μm I.D. (Yongnian Optical Fiber Factory, Hebei, China) was used. The detection wavelength was 280 nm. Samples were injected by applying a pressure of 3.4 kPa for 5 s.

2.2. Chemicals and materials

All chemicals were of analytical grade. SDS was obtained from Sigma (St. Louis, MO, USA). CPF, LMF, OF and NF were obtained from the Institute to Pharmaceutical and Biomaterial

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Authentication of China (Beijing, China). GTF, FL and SPF were kindly donated by the Institute to Pharmaceutical and Biomaterial Authentication of Zhaoqing (Guangdong, China). CPF and LMF tablets were purchased from local drugstore.

2.3. Standard solutions

Stock solutions of NF, CPF, OF, LMF, SPF, FL and GTF (1.0×10^{-3} mol/L) were prepared using 0.01 mol/L NaOH and stored under light-protecting condition at 4 °C. Standard working solutions were daily prepared by mixing and diluting the stock solution in water.

2.4. Preparation of the microemulsion

The microemulsion was prepared by adding 1% (v/v) heptane, 10% (v/v) 1-butanol, 2.88 g (100 mmol/L) SDS, 0.125 g (8 mmol/L) sodium phosphate ($NaH_2PO_4 \cdot 2H_2O$) to a 100 mL flask. This mixture was sonicated for 30 min to aid dissolution and an optically transparent microemulsion had formed. Before use, the microemulsion solutions were filtered through a 0.45-μm filter and degassed in an ultrasonic bath for 1 min. The buffer was adjusted to pH 6.0–9.0 with 50 mM sodium tetraborate, if necessary.

2.5. Sample preparation

2.5.1. Drug administration

Eight healthy males and females adult volunteers were participated in the study. All subjects were healthy on the basis of their medical history, clinical and laboratory examination. After the overnight fast, one 500 mg CPF tablet or LMF tablet of the test was administered orally. The urine samples were respectively collected at 0, 0.75 (0.5), 1.75 (1.5), 3.75 (2.75), 4.25(3.75), 5.25 (4.5), 5.75 (5.5), 6.25 (6.0), 6.75 (7.0), 8.5 (8.0), 12.0 (12.0) h and stored at −20 °C until analysis.

2.5.2. Urine

The frozen urine samples were thawed at room temperature and centrifuged at 3500 × *g* for 15 min. The supernatants were transferred to clean glass tube and filtrated through a 0.45 μm filter, and directly injected into the electrophoresis system.

3. Results and discussion

3.1. MEEKC profiles

The standard microemulsion conditions [0.81% (w/w) heptane, 6.61% (w/w) 1-butanol, 3.31% (w/w) SDS, and 89.27% (w/w) 10 mmol/L sodium tetraborate buffer] were taken as the starting point and performed a series of optimizations. The pH was a critical factor in a series of optimizations. Thus, a pH interval of 7.0–9.5 was investigated. The best separation was achieved in a carrier electrolyte containing 1% (v/v) heptane, 100 mmol/L SDS, 10% (v/v) 1-butanol, and 8 mmol/L phosphate–sodium tetraborate buffer at pH 7.30. Figs. 1 and 2 show the optimized MEEKC separation of a standard solution of all seven FQs and independent sources of drug-free urine. No interfering peaks in the retention time of CPF, LMF were observed in blank drug-free urine.

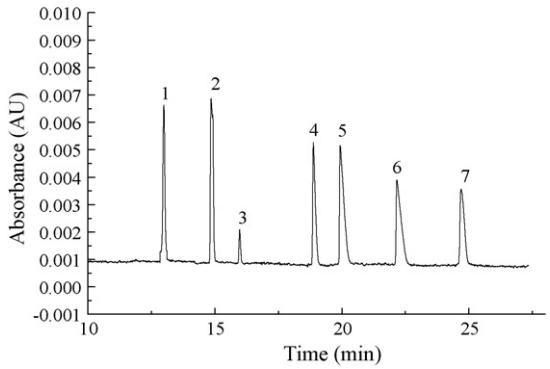

Fig. 1. MEEKC electropherogram of FQs standard mixture each at 5.0×10^{-5} mol/L (except for OF: 1.0×10^{-5} mol/L). Separation conditions: 1% (v/v) heptane + 100 mmol/L SDS + 10% (v/v) 1-butanol + 8 mmol/L phosphate–sodium tetraborate buffer at pH 7.30. The applied voltage was 22 kV, temperature was 20 °C. Peaks: 1 = FL; 2 = LMF; 3 = OF; 4 = GTF; 5 = CPF; 6 = NF; 7 = SPF.

3.2. Linearity of the method and limits of detection and quantitation

The calibration curves were constructed using response of peak areas (*y*) versus the urine sample concentration (*x*, mol/L). The calibration curves for LMF and CPF in the range of $1.2 \times 10^{-6} - 5.0 \times 10^{-4}$ mol/L were: $y = 1.93 \times 10^9 x + 8.55 \times 10^3$, and $y = 9.82 \times 10^8 x + 3.58 \times 10^3$, with $r = 0.9987$ and $r = 0.9972$, respectively. The detection limits (LODs, S/N = 3) were 0.95 and 0.97 μmol/L for LMF and CPF, respectively. The quantification limits (LOQs, S/N = 9) were 2.85 and 2.91 μmol/L for LMF and CPF, respectively.

3.3. Precision and accuracy

Precision and accuracy were assessed for LMF and CPF at three concentrations covering the linearity range (six replicate analyses at each level during 5 consecutive days) by spiked urine. The intra-day and inter-day precision were <5.0% and <7.0%,

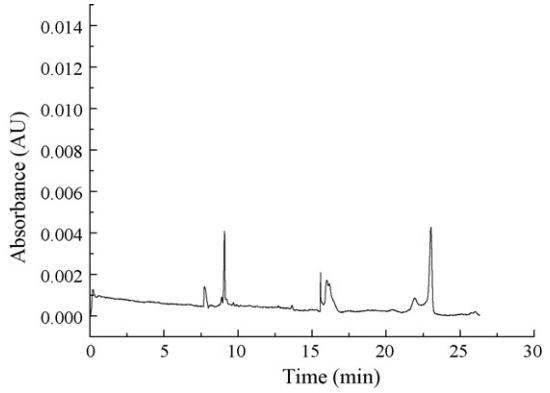

Fig. 2. Electropherograms of a blank drug-free pooled urine; Analytical conditions were as for Fig. 1.

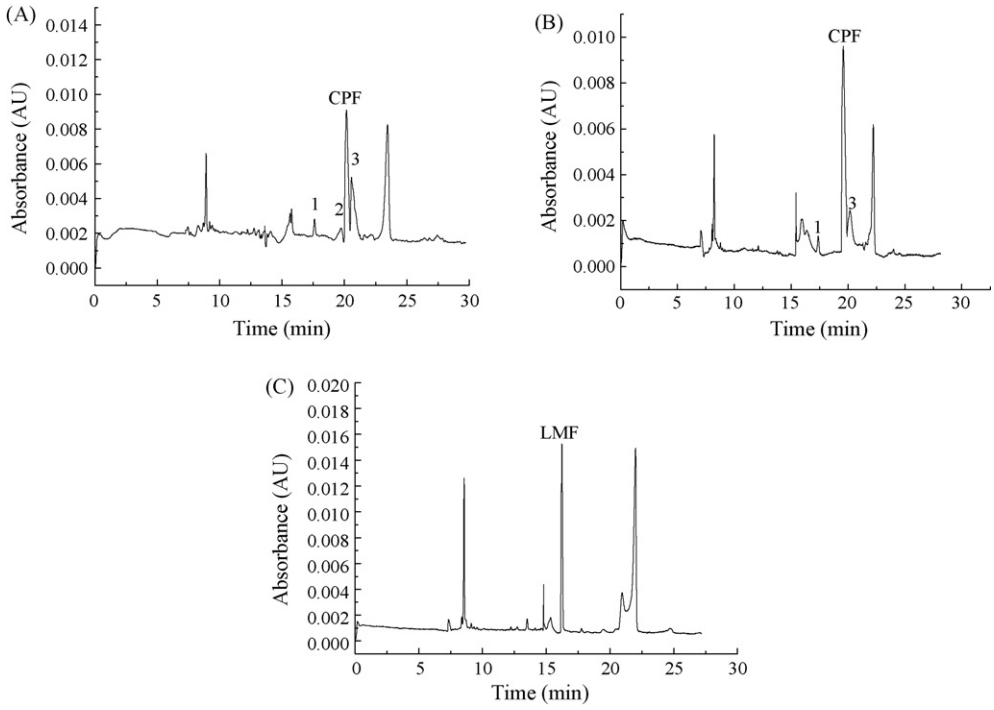

Fig. 3. Electropherograms of: (A) urine 0.75 h and (B) 3.75 h after oral administration of one tablet with 500 mg CPF; (C) urine 4.0 h after oral administration of one tablet with 500 mg LMF. Analytical conditions were as for Fig. 1. Peaks 1, 2 and 3 were probably CPF metabolites.

respectively, for LMF and CPF. The intra-day and inter-day accuracy were in the range of 99.0–104.0% and 100.0–105.5%, respectively, for LMF and CPF. The above results indicated that the method was reliable, reproducible and accurate.

3.4. Urine samples

The proposed method was applied to determination of CPF and LMF in urine samples. Fig. 3A and B shows an electropherogram of one healthy volunteer after oral administration of 500 mg CPF tablet, collected at 0.75 h and 3.75 h, respectively. Comparing the electropherogram obtained in Fig. 2, three new peaks respectively at t_R = 17.60 min, 19.72 min and 20.53 min can be observed in Fig. 3A and B. Their areas changed with the collection time and their spectra concordance with CPF. Therefore, Peaks 1, 2 and 3 were probably CPF metabolites. Fig. 3C shows an electropherogram of another healthy volunteer after oral administration of 500 mg LMF tablet, collected at 3.75 h. Comparing the electropherogram obtained in Fig. 1 and Fig. 2, few differences in migration times can be observed. These are due to the time taken to remove the sample matrix from the capillary, since the sample matrix is different.

The urine concentration–-time profile of two healthy human volunteer after respectively receiving one table with 500 mg CPF and LMF were shown in Fig. 4. The concentration of CPF and LMF in the urine respectively reached a maximum value of 39 mg/l and 49 mg/L after about 5 h oral administration. Twelve hours after oral administration, the concentration of CPF and LMF in the urine was, respectively, 2.5 mg/L and 3.9 mg/L, which corresponded to a rapid elimination. The above

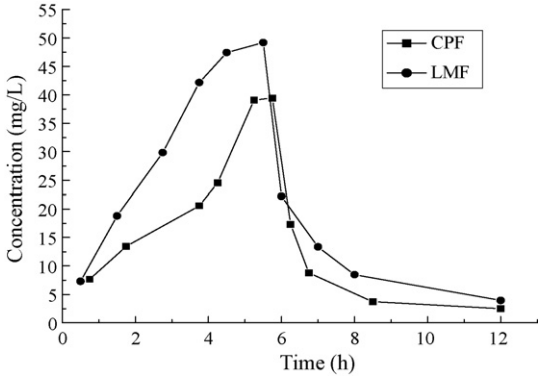

Fig. 4. Urine concentration–time profiles after oral administration of one tablet with (A) 500 mg CPF, (B) 500 mg LMF.

results may be useful in clinical pharmacology for detecting the efficacy and side effects of LMF and CPF.

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References

[1] T. Nekvindová, J. Suchopár, Remedia 4 (1993) 206.
[2] K.G. Nabe, Chemotherapy 42 (1996) 1.
[3] A.E. Struck, D.K. Kim, F.J. Frey, Clin. Pharmacokinet. 22 (1992) 116.

[4] M. Jelikic-Stankov, J. Odovic, D. Stankov, P. Djurdjevic, J. Pharm. Biomed. Anal. 18 (1998) 145.
[5] L.M. Du, Y.Q. Yang, Q.M. Wang, Anal. Chim. Acta 516 (2004) 237.
[6] M. Rizk, F. Belal, F.A. Aly, N.M. El-Enany, Talanta 46 (1998) 83.
[7] Y.D. Liang, J.F. Song, X.F. Yang, Anal. Chim. Acta 510 (2004) 21.
[8] C. Fierens, S. Hillaert, W.V.D. Bossche, J. Pharm. Biomed. Anal. 22 (2000) 763.
[9] S. Husain, S. Khalid, V. Nagaraju, R.N. Rao, J. Chromatogr. A 705 (1995) 380.
[10] G.J. Krol, G.W. Beck, T. Benham, J. Pharm. Biomed. Anal. 14 (1995) 181.
[11] A. Zotou, N. Miltiadou, J. Pharm. Biomed. Anal. 28 (2002) 559.
[12] K.D. Altria, P.-E. Mahuzier, B.J. Clark, Electrophoresis 24 (2003) 315.