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A reproducible, simple and sensitive high-performance capillary electrophoresis method for simultaneous determination of capreomycin, ofloxacin and pasiniazide in urine

S.S. Zhang [a,b], H.X. Liu [a], Z.B. Yuan [b,*], C.L. Yu [c]

[a] *Center of Instrumental Analysis, Zhengzhou University, Zhengzhou 450052, P.R. China*
[b] *Graduate School, USTC, Academia Sinica, Beijing 100039, P.R. China*
[c] *Department of Clinical Pharmacology, Henan Chest Hospital, Zhengzhou 450003, P.R. China*

Abstract

Separation and determination of capreomycin (Cp), ofloxacin (Oflx) and pasiniazide (Ipa) in urine by high-performance capillary electrophoresis (HPCE) with 280 nm detection have been studied systematically. The calibration lines were linear in the range of $0.5 \sim 50$ mg l^{-1}, and the detection limits ($S/N = 3$) were 0.15, 0.20 and 0.10 mg l^{-1} for Cp, Oflx and Ipa, respectively. The recoveries for these materials from urine were higher than 93.5%. The accuracy and intra- and inter-day reproducibility of Cp, Oflx and Ipa were determined with satisfactory results. This method was successfully used for determining Cp, Oflx and Ipa in human urine.

Keywords: High-performance capillary electrophoresis; Capreomycin; Ofloxacin; Pasiniazide; Simultaneous determination; Urine

1. Introduction

Capreomycin (Cp), a polypeptide antibiotic produced by *Streptomyces capreolus*, is a 'secondary' antituberculous drug and is dosed with other appropriate drugs to patients having strains of tubercle bacilli resistant to the 'primary' antituberculous drugs such as isoniazid, ethambutol, aminosalicylic acid, etc. Cp is produced as a complex of four components amongst which Cp IA and Cp IB (IA and IB; Fig. 1) usually predominate. The study of pharmacokinetics, toxicity, efficacy of liposomal Cp [1], analysis of two Cp-resistance factors [2] and determination of Cp sulfate component IA and IB by TLC-UV spectrometry [3] have been developed.

Ofloxacin (Oflx; Fig. 1) is a fluoroquinolone with high activity against Gram-negative and Gram-positive bacteria in vivo and in vitro [4]. It has shown a large potency against many common bacterial pathogens. Its pharmacokinetics [5], determination in plasma and urine [6], in human hair [7], in plasma and lung tissue [8] by HPLC and in tablet by capillary zone electrophoresis [9] have been reported.

* Corresponding author.

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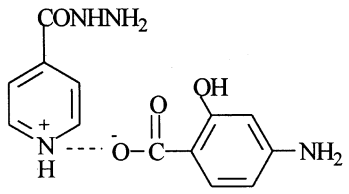
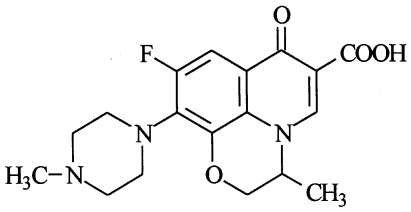
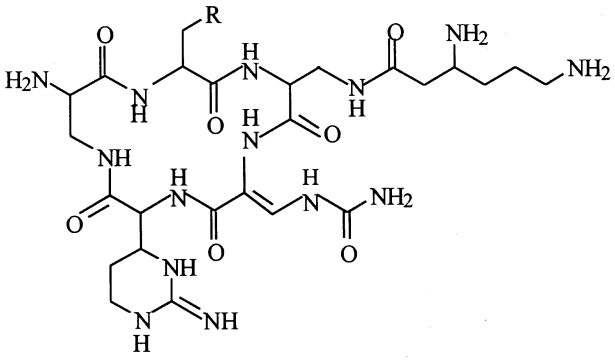


Pasiniazide (Ipa)



Ofloxacin (Oflx)



Capreomycin (Cp)

IA: R=OH; IB: R=H

Fig. 1. The structures of Cp, Oflx and Ipa

Pasiniazide (Ipa; Fig. 1), composed of isoniazid and 4-amino-salicylic acid, has mutual effects coupling isoniazid and 4-amino-salicylic acid to tubercle patients. No report on its pharmacokinetics, efficacy and analytical method has been seen.

In this paper, a reproducible, simple and sensitive high-performance capillary electrophoresis (HPCE) method for determining Cp, Oflx and Ipa in human urine was developed.

2. Experimental

2.1. Reagents

Cp was obtained from Yunan Pharmaceutical Factory (Xinyang, Henan Province, China), Oflx was purchased from Beijing Pharmaceutical Factory (Beijing, China), Ipa was obtained from Guangzhou Baiyun Pharmaceutical Factory (Guangzhou, China). Other analytical-grade reagents were from Beijing Chemical Factory (Beijing, China).

2.2. HPCE

HPCE separations were carried out by using a 1229 HPCE analyser with a fixed wavelength UV detector at 280 nm and PL^{+}-80 integrator (Beijing Institute of New Technology and Application, Beijing, China). Bare fused silica capillaries were from Yongnian Optical Factory (Yongnian, Hebei Province, China). Capillary dimensions were 50 μm i.d., 375 μm o.d. and 55.5 cm length

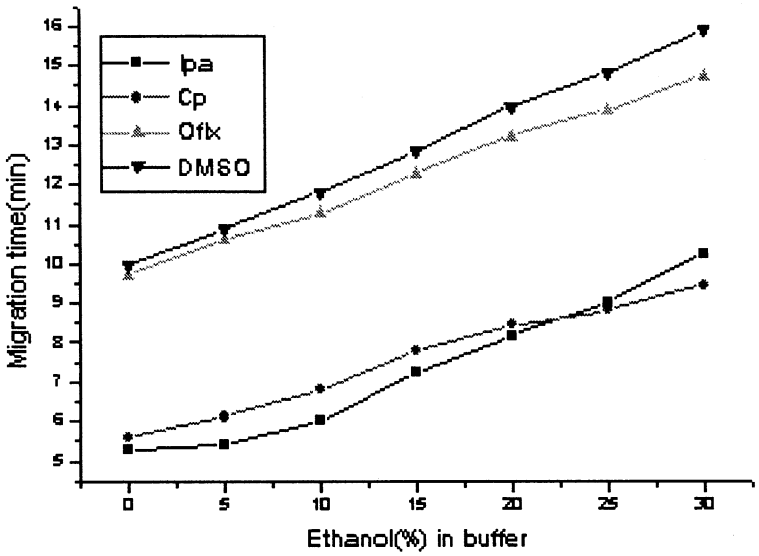


Fig. 2. The effect of ethanol on the migration times of Cp, Oflx and Ipa. The detection wavelength was 280 nm. A 47.5-cm effective length 50 μm i.d. bare fused silica capillary was used. The running buffer was 40 mmol l^{-1} borax-(0 ~ 30%) ethanol-H_3PO_4 (pH 4.0) and the voltage was 25 kV.

(47.5 cm to detector). The pH values of buffers were measured using No. 5944 pH meter (Cole-Parmer Instrument, Chicago, IL). The HPCE system was operated with the anode injecting by applying 20 ± 0.3 kV for 20 s and maintained at 24 ± 0.2°C throughout. The separation voltage was 25 ± 0.3 kV. The capillary was rinsed with 0.1 mol l^{-1} NaOH and double-distilled water for 5 min after each run, then re-equilibrated with running buffer for 10 min. The buffers and sample

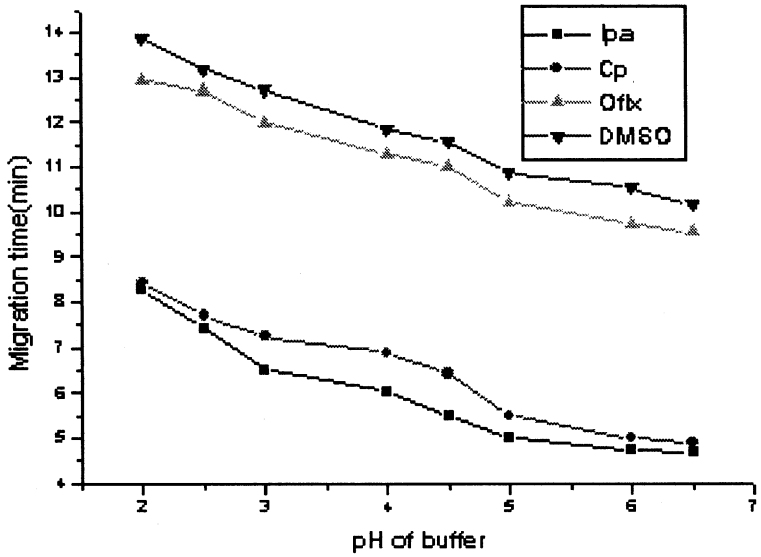


Fig. 3. The effect of pH values on the migration times of Cp, Oflx and Ipa. The running buffer was 40 mmol l^{-1} borax-10% ethanol-H_3PO_4 (2 ~ 6.5) and other conditions as in Fig. 2

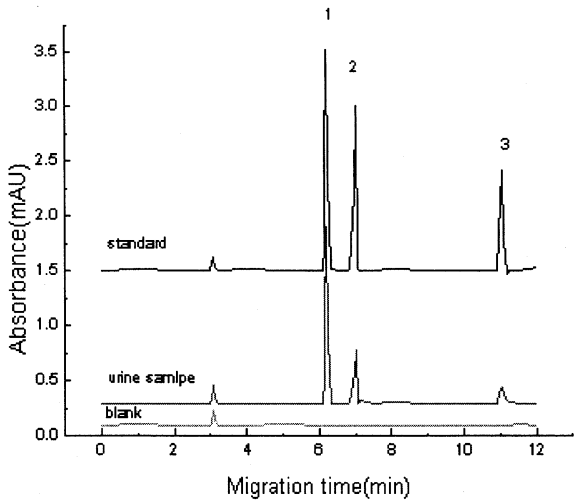


Fig. 4. The electropherograms of standard, blank and urine samples. The running buffer was 40 mmol l^{-1} borax-10% ethanol-H_3PO_4 (pH 4.0), other conditions as in Fig. 2. Peaks: 1, Ipa (10 mg l^{-1}); 2, Cp (5 mg l^{-1}); 3, Oflx (5 mg l^{-1}).

solutions were filtered through a 0.45-μm filter. The peak area of each standard was plotted against its concentration to form a calibration line. In the present work, t_{mark} was the migration time of a neutral marker, dimethyl sulfoxide (DMSO).

2.3. Stock and standard solutions

Stock solutions of Cp, Oflx and Ipa, 200 mg l^{-1}, were prepared by dissolving 20 ± 0.10 mg of each compound into a separate 100-ml volumetric flask. Then, 40 mmol l^{-1} borax-H_3PO_4 (pH 4) for Cp and Oflx, and 40 mmol l^{-1} borax for Ipa were added to the vial. The stock solutions (200 mg l^{-1}) were diluted 4-fold with 40 mmol l^{-1} borax-H_3PO_4 (pH 4) for final stock solutions at 50 mg l^{-1}.

Table 2
The recoveries of Cp, Oflx and Ipa

Added (mg l^{-1})	Found (mg l^{-1})	Recovery (%)
Cp ($n = 5$)		
0	16.84 ± 0.05	—
2	18.72 ± 0.04	94.0 ± 2.1
10	27.13 ± 0.13	102.9 ± 1.2
Oflx ($n = 6$)		
0	4.25 ± 0.03	—
2	6.34 ± 0.04	104.5 ± 1.9
10	14.47 ± 0.11	102.2 ± 1.1
Ipa ($n = 5$)		
0	2.38 ± 0.02	—
2	4.25 ± 0.03	93.5 ± 1.6
10	12.51 ± 0.08	101.3 ± 0.8

Standard solutions were prepared by putting the final stock solutions to 10-ml flask, respectively. Then, blank urine was added to the vial for the standard solution concentrations of 50, 20, 10, 5, 2, 1, 0.75 and 0.50 mg l^{-1}, respectively.

2.4. Recovery test for Cp, Oflx and Ipa

The test samples were prepared by adding 2 and 10 mg l^{-1} Cp, Oflx and Ipa to urine samples from one healthy volunteer dosed with Cp, Oflx and Ipa (200 mg, orally), respectively. The technique was carried out as described.

3. Results and discussion

3.1. The optimal HPCE buffer

Analyses were carried out in acidic buffer. Acidic buffer ensures higher positive density on

Table 1
The calibration lines, detection limits of Cp, Oflx and Ipa

Compound	Concentration range	Calibration Line ($A = a + bC$)		r	n	Detection limit
	(mg l^{-1})	Intercept (a)	Slope (b) (mg^{-1} l)			(mg l^{-1})
Cp	0.5 ~ 50	-13.8 ± 0.12	57.5 ± 1.06	0.9989	8	0.15
Oflx	0.5 ~ 50	-8.50 ± 0.09	41.2 ± 0.93	0.9972	8	0.20
Ipa	0.5 ~ 50	-9.3 ± 0.07	81.2 ± 1.98	0.9995	8	0.10

Table 3
Accuracy and intra-day[a] and inter-day[b] reproducibility of Cp, Oflx and Ipa

Compound	Known conc. (mg l^{-1})	Dedected conc. (mg l^{-1})		Accuracy (%)		RSD (%)	
		Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
Cp	5	4.95 ± 0.03	5.09 ± 0.04	99.0	101.8	0.61	0.78
	15	15.12 ± 0.10	14.87 ± 0.13	100.8	99.1	0.66	0.87
	30	29.54 ± 0.21	29.39 ± 0.18	98.5	97.9	0.71	0.61
Oflx	5	5.10 ± 0.05	4.89 ± 0.06	102.0	97.8	0.98	1.22
	10	9.89 ± 0.12	9.92 ± 0.09	98.9	99.2	1.21	0.91
	25	25.54 ± 0.24	24.75 ± 0.32	102.2	99.0	0.94	1.29
Ipa	2	2.05 ± 0.01	1.97 ± 0.02	102.5	98.5	0.49	1.01
	10	9.86 ± 0.06	9.73 ± 0.09	98.6	97.3	0.61	0.92
	20	20.45 ± 0.21	19.54 ± 0.24	102.3	97.7	1.03	1.23

[a] $n = 5$; [b] $n = 10$, 5 separate days run in duplicate.

Ipa and Cp than Oflx. The effects of concentration of borax, pH values and the proportions of ethanol (EtOH) in buffer on the HPCE behaviours of Cp, Oflx and Ipa were investigated.

When the borax concentration was increased from 20 to 60 mmol l^{-1}, the capillary current was increased from 18 to 70 μA, and the peak shape became asymmetric owing to the temperature gradient caused by joule heat. Fig. 2 showed the effect of EtOH on the migration times. It was observed from Fig. 2 that the migration time increased with the increasing concentration of EtOH in the range of $5 \sim 30\%$. The viscosity increase and electroosmosis mobility decrease resulted in a migration time increase. Peak shape of Oflx was improved owing to reducing adsorption and increasing analyte solubility. The effect of pH values on the migration times is shown in Fig. 3. When the pH value was decreased from 6.5 to 2.0, the negative charge on the capillary inner wall was suppressed (the density of SiO^- on capillary inner wall was reduced). The migration times were increased resulting from electroosmosis mobility decrease, even though electrophoretic mobilities of analytes increase.

Taking into consideration of the migration time, peak symmetry and capillary current, 40 mmol l^{-1} borax-10 mmol l^{-1}-10% EtOH-H_3PO_4 (pH 4.0) was selected as the running buffer for the HPCE separation of Cp, Oflx and Ipa with capillary current of 45 μA. An asymmetry factor of near 1.0 and resolution of over 2.0 were achieved. Fig. 4 shows the typical electropherogram of standards within 13 min.

3.2. Quantitative data

By HPCE, the equations of calibration lines, detection limits ($S/N = 3$) and recoveries were listed in Tables 1 and 2. The accuracy and intra- and inter-day reproducibility of Cp, Oflx and Ipa were displayed in Table 3.

Tables 1 and 2 indicated that the proposed method had low detection limit and high recovery. The values in Table 3 indicated that the method was satisfactory with respect to accuracy and reproducibility.

3.3. Determination of Cp, Oflx and Ipa in urine

One healthy volunteer was dosed with Cp of 100 mg, Oflx of 150 mg and Ipa of 100 mg altogether. The urine samples were obtained within $0 \sim 3$ and $3 \sim 6$ h, and determined in triplicate ($n = 3$) by HPCE. The concentrations in urine were 7.3 ± 0.07 mg l^{-1} ($0 \sim 3$ h, 500 ml urine) and 9.2 ± 0.10 mg l^{-1} ($3 \sim 6$ h, 650 ml urine) of Cp, 2.3 ± 0.06 mg l^{-1} and 3.5 ± 0.09 mg l^{-1} of Oflx, and 1.4 ± 0.02 mg l^{-1} and 1.8 ± 0.04 mg l^{-1} of Ipa. The electropherograms of blank and urine samples ($3 \sim 6$ h) were shown in Fig. 4. The peak in the blank urine did not interfere with the determination of Cp, Oflx and Ipa.

4. Conclusion

A reproducible and simple HPCE method has been developed to determine Cp, Oflx and Ipa in urine simultaneously. It is useful for pharmacokinetic studies with determination of Cp, Oflx and Ipa in plasma of patients. The further clinical applications to pulmonary tuberculosis therapy by using different dosage of Cp, Oflx and Ipa are being conducted in Henan Chest Hospital.

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