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Application of micellar electrokinetic chromatography to the determination of sultamicillin in oral pharmaceutical preparations

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Abstract

A micellar electrokinetic capillary electrophoretic method for determination of sultamicillin in Unasyn oral preparations—tablets and suspension—was evaluated. Phosphate–borate buffer at pH 7.0 containing 1.0% sodium dodecylsulfate was used as a mobile phase. The elaborated method ensures separation of sultamicillin from *p*-toluenesulfonic acid and the impurities, ampicillin, sulbactam and penicillamine. The method was validated for specificity, reproducibility, precision, accuracy and assay linearity (in a concentration range of sultamicillin of 0.05—1.5 mg/ml). Statistical analysis by Student's *t*-test showed no significant differences between the results obtained by micellar electrokinetic chromatography and HPLC, $t_{calculated}$ 0.519 for suspension assays and 0.284 for tablets assays were smaller then $t_{tabulated}$.

Keywords: Pharmaceutical analysis; Antibiotics; Lactams; Sultamicillin

1. Introduction

Capillary electrophoresis (CE) has progressively replaced high-performance liquid chromatography (HPLC) in analytical investigations of drugs. During recent years we have elaborated and optimized CE methodologies to analyze some β-lactam antibiotics: piperacillin, imipenem and cephalosporins (ceftazidime, cefoperazone, cefotaxime, cefuroxime, cephazolin, ceftriaxone) [1–4]. Efforts have also been made to separate the amoxicillin and ampicillin antibiotics from β-lactamase inhibitors in Augmentin and Unasyn preparations for injection [5]. In the case of the oral Unasyn preparations—tablets and suspension, an atypical antibiotic—sultamicillin—is present as the active substance. It is a prodrug of a β-lactam antibiotic—ampicillin and a β-lactamase inhibitor—sulbactam, linked chemically as a double ester (Fig. 1). During absorption in the gastrointestinal tract, sultamicillin is hydrolysed to give ampicillin and sulbactam, in equimolar quantities. Sultamicillin is administered by oral route as tablets (containing sultamicillin tosylate) or suspension (containing sultamicillin base).

The aim of this study was to develop a micellar

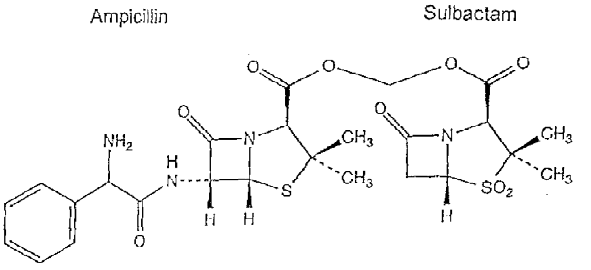

Fig. 1. Sultamicillin formula.

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electrokinetic chromatography (MEKC) method for the determination of sultamicillin antibiotic in pharmaceutical oral preparations. The results were compared with those obtained using HPLC.

2. Experimental

2.1. Apparatus

CE experiments were carried out on Waters Quanta 4000E CE system, equipped with 30-kV power supply, a UV spectrophotometric detector connected to a data collection system. The detection wavelength was 214 nm. Separations were performed using a fused-silica capillary (60 cm×75 μm I.D., Accu-Sep) thermostated at 25 °C, with applied voltage of 18 kV. Hydrodynamic injection was performed.

HPLC experiments were carried out using Shimadzu LC-10A HPLC system with detection at 215 nm. A μBondapak column (30×4.6 mm I.D., 5 μm; Waters) was used for separation.

2.2. Standards and reagents

2.2.1. Standards

Sultamicillin base Pfizer reference standard of activity 98.2%; sultamicillin tosylate Pfizer reference standard containing 72% of sultamicillin and 20.8% *p*-toluenesulfonic acid; *p*-toluenesulfonic acid of BDH reagent; ampicillin trihydrate SKB reference standard of activity 86.0%; sulbactam Pfizer reference standard of activity 90.1%; pencillamine hydrochloride IF working standard.

2.2.2. Preparations

Pfizer pharmaceuticals: Unasyn tablets containing sultamicillin tosylate equivalent to 375 mg sultamicillin base and Unasyn powder for oral suspen-

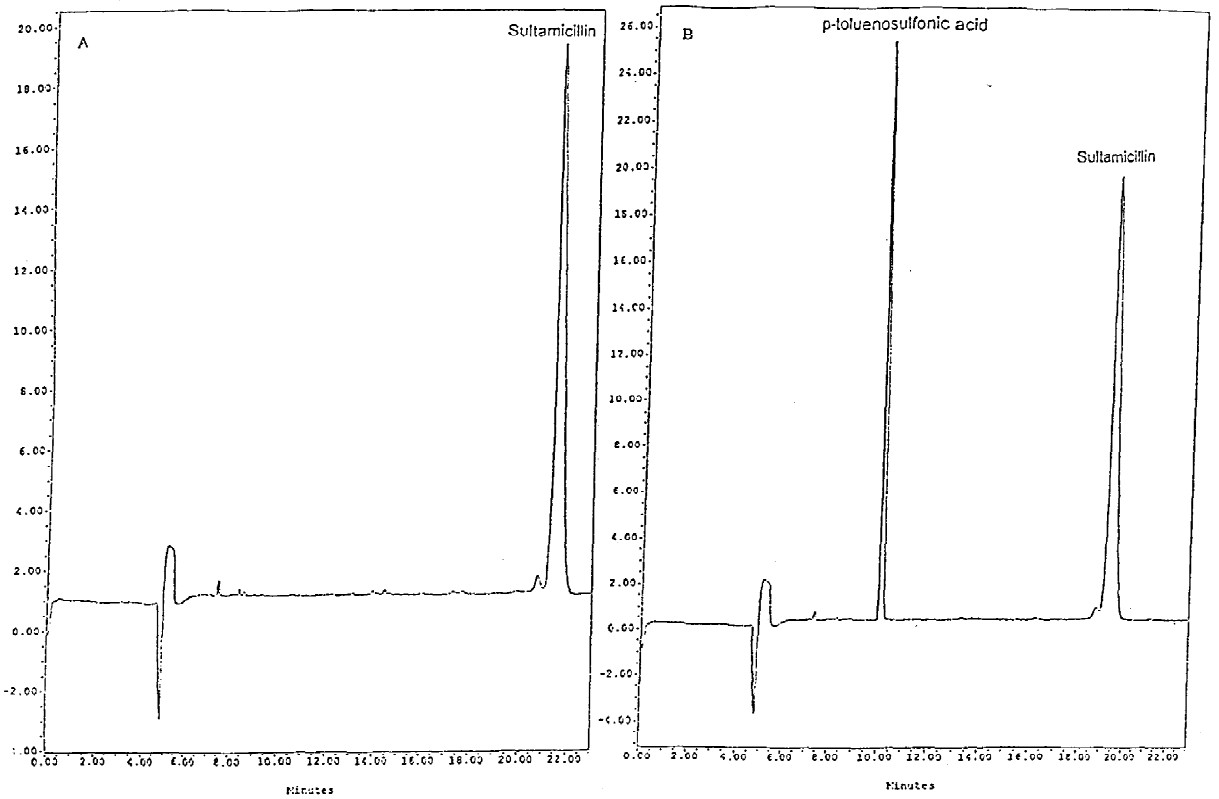

Fig. 2. Typical electropherograms of Unasyn oral preparations: powder (A) and tablets (B). CE conditions as in the text.

sion containing 250 mg sultamicillin base in a 5-ml dose.

Standard solutions were prepared by dissolving the sultamicillin base in the mobile phase at a concentration of about 0.8 mg/ml for CE and 0.1 mg/ml for HPLC. In the case of sultamicillin tosylate, the standard solutions were prepared at a concentration of about 1 mg/ml for CE and 0.1 mg/ml for HPLC.

2.2.3. Sample solutions

Unasyn powder: About 700 mg of powder was dissolved in 50 ml mobile phase; the solution was filtered and then used for CE assay. For HPLC a portion of the same solution was further diluted (ratio 1:5) with mobile phase.

Unasyn tablets: About 110 mg of blended tablets were dissolved in 50 ml mobile phase, the solution was filtered and used for CE assay. For HPLC the filtrate was diluted 10 times with mobile phase.

2.2.4. Reagents

Monobasic sodium phosphate, sodium tetraborate, sodium hydroxide, phosphoric acid were of reagent grade. Sodium dodecylsulfate (SDS) was provided

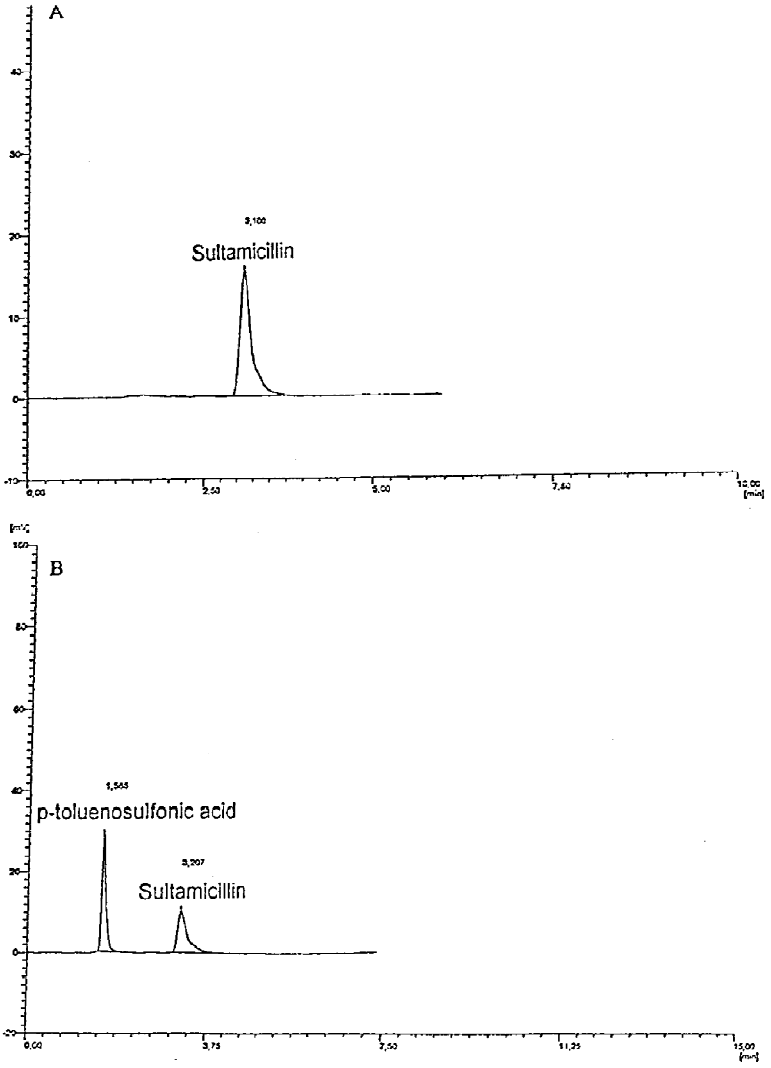

Fig. 3. Typical chromatograms of Unasyn oral preparations: powder (A) and tablets (B). HPLC conditions are in text.

by Sigma, acetonitrile was HPLC grade supplied by Merck. Water used to prepare running buffer and mobile phase was obtained from a Labconco System. The background electrolytes were prepared by adding 1.4% of SDS to 0.02 *M* borate–phosphate buffer at pH 8.7 or adding 1.0% of SDS to 0.02 *M* borate–phosphate buffer at pH 7.0. The mobile phase consisting of 0.02 *M* monobasic sodium phosphate adjusted to pH 3.0 (with 40% phosphoric acid) and acetonitrile in the ratio of 40:60 (v/v) was used for HPLC.

3. Results and discussion

HPLC was previously reported for testing the quality of Unasyn preparations [6,7]. In our previous paper [5], the CE method for ampicillin and sulbactam simultaneous assay in Unasyn injection was developed. The CE separation is based on MEKC application using 1.4% SDS in phosphate–borate buffer at pH 8.7. Now, an attempt is made to adapt this method for the assay of the prodrug (sultamicillin) in oral Unasyn preparations. Sultamicillin is insoluble and unstable in water, for that reason our investigations started with the selection of a proper solvent for assuring good solubility and stability during assays. Methanol–water and acetonitrile–water mixtures were tested, but phosphate buffer (pH 3.0)–acetonitrile (40:60, v/v) (mobile phase for the HPLC assay) was chosen. The CE separations were carried out at two different pH values, 8.7 and 7.0, in buffers containing, respectively, 1.4 and 1.0% of SDS. During CE separations under both conditions, a single peak from the solution of sultamicillin and two well-separated peaks from the sultamicillin tosylate solution were detected. Independently of the buffer pH value and SDS concentration, the retention time was about 20 min for sultamicillin and 10 min for the second peak, identified as *p*-toluenesulphonic acid. For further determination the buffer containing 1.0% SDS at pH 7.0 was used. Under the above conditions sultamicillin is more stable in solution. Elution profiles of Unasyn powder sample and Unasyn tablets containing sultamicillin tosylate obtained by CE and HPLC are presented in Figs. 2 and 3, respectively. The total run time using the CE method is longer than HPLC on which faster retention times (*p*-toluenesulfonic acid 1.6 min and sultamicillin 3.2 min) are obtained. In such condition good repeatability of migration times as well as repeatability of peak area for sultamicillin and *p*-toluenesulphonic acid were obtained (Table 1). CE analyses of samples containing different amounts of sultamicillin and sultamicillin tosylate showed a very high correlation between peak areas and the analysed compounds concentrations. The linearity of method was achieved in the concentration range 0.05–1.5 mg/ml of sultamicillin base and of sultamicillin tosylate calculated for sultamicillin, with correlation coefficient of 0.999. Calibration curves are based on five concentration points; for each concentration the sample was injected three times. The detection limit, defined as signal-to-noise ratio of 3:1, was 0.01 mg/ml for sultamicillin. The quantitation limit, defined as signal-to-noise ratio of 10:1, was 0.05 mg/ml for sultamicillin [8]. Table 2 presents results of sultamicillin base analysis by CE. The specificity of the method was confirmed by analysis of solutions obtained by spiking the pure compound with a placebo of powder and tablet preparations. The absence of interfering peaks in the electropherograms confirms the specificity of the assay. The specificity was additionally confirmed by the separation analysis

Table 1
Repeatability of migration time (t_m) and peak area of sultamicillin tosylate obtained applying the CE method

Injection	*p*-Toluenesulfonic acid		Sultamicillin	
	t_m (min)	Peak area	t_m (min)	Peak area
1	10.27	114 574	19.90	285 021
2	10.33	115 767	20.30	296 964
3	10.20	116 020	19.83	292 416
4	10.20	115 938	19.92	285 669
5	10.25	115 575	19.90	290 018
Mean	10.25	115 575	19.98	290 018
SD	0.054	584.95	0.192	4946.74
RSD (%)	0.53	0.506	0.963	1.706

Table 2
Quantitative performance test of sultamicillin

	Sultamicillin
Migration time (min)	~20
Repeatability of migration time	RSD = 1.131%
Repeatability of peak area	RSD = 1.920%
Correlation coefficient	0.999
Quantification limit	0.05–1.50 mg/ml
Detection limit	From 0.01 mg/ml

of the degradation compounds of sultamicillin: ampicillin, sulbactam and penicillamine. Under the established conditions, sultamicillin is well separated from impurities. Fig. 4 shows the separation of a mixture of sultamicillin base, ampicillin, sulbactam and penicillamine, while Fig. 5 presents the separation of sultamicillin tosylate, ampicillin and sulbactam. Penicillamine (Fig. 4) co-emigrated with the peak of the buffer. A sufficient within-day precision (repeatability) and between-day precision (reproducibility) for the determination of the two preparations were confirmed. Statistical analysis of the results (Tables 3 and 4) showed good repeatability, reproducibility and accuracy (RSD=0.539% for tablets and RSD=1.470% for powder). The analysis of both preparations by MEKC and HPLC were also compared. The values obtained for Unasyn powder and Unasyn tablets were not significantly different. The Student's $t_{calculated}$ (0.519 for powder and 0.284 for tablets) determined by the *t*-test, is much lower then $t_{tabulated}$ values (Tables 3 and 4).

4. Conclusions

The elaborated MEKC method ensures good separation of sultamicillin from *p*-toluenesulfonic acid

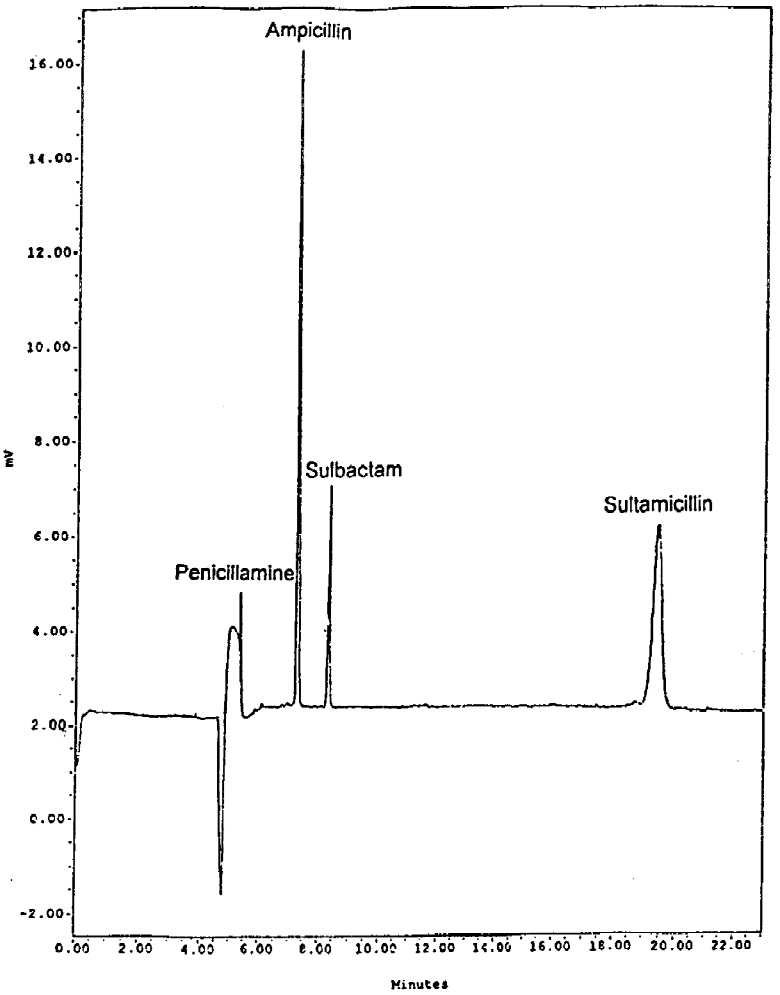

Fig. 4. Electropherogram of mixture of sultamicillin base, ampicillin, sulbactam and penicillamine. CE conditions as in the text.

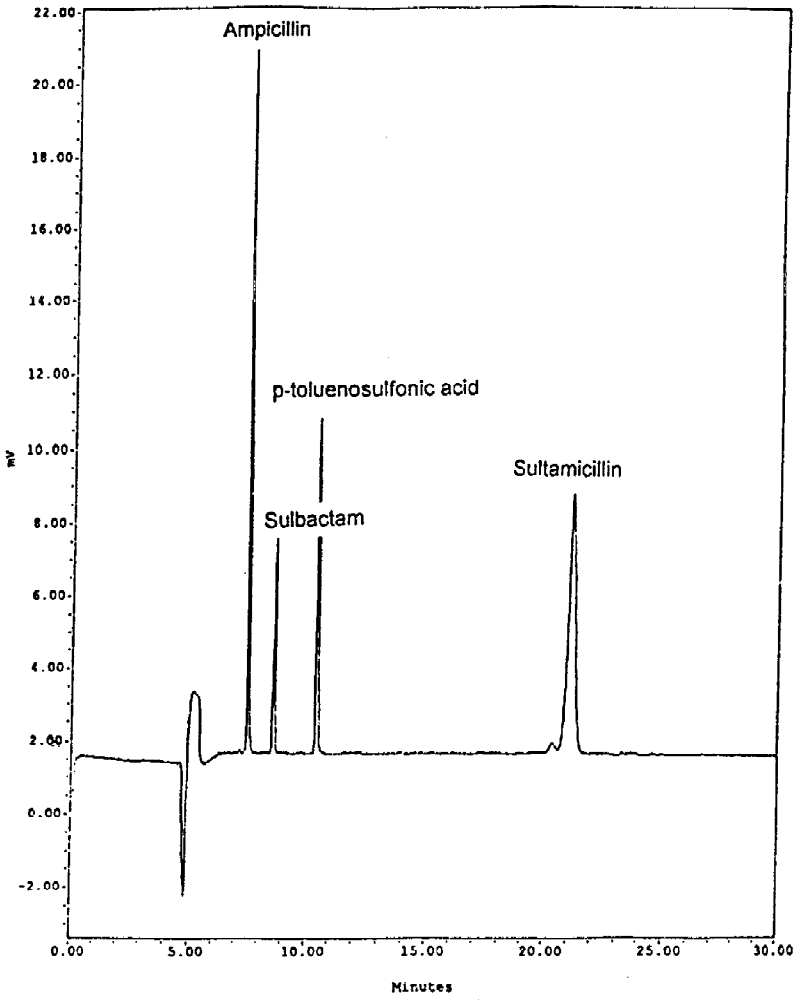

Fig. 5. Electropherogram of a mixture of sultamicillin tosylate, ampicillin and sulbactam. CE conditions as in the text.

Table 3
Results of assay of sultamycillin in the Unasyn tablets preparation

	CE		HPLC
	Day 1	Day 2	Day 2
Mean (mg/tablet) (quota 356–394 mg)	369.68	368.67	368.99
Number of samples	5	6	6
SD	2.400	1.680	2.196
RSD (%)	0.649	0.456	0.595
CE assays on days 1 and 2			Student's t value from CE and HPLC assays on day 2
Mean (mg/tablet)	369.12		
RSD (%)	0.539		$t_{tab.}(0.05,10)=2.228>t_{calc.}=0.284$

Table 4
Results of assay of sultamicillin in Unasyn powder preparation

	CE		HPLC
	Day 1	Day 2	Day 2
Mean (mg/5 ml)	240.58	238.37	237.17
Number of samples	6	6	5
SD	3.552	3.421	3.794
RSD (%)	1.477	1.435	1.600
CE assays on days 1 and 2			Student's t value from CE
Mean (mg/5 ml)	239.47		and HPLC assays on day 2
RSD (%)	1.470		$t_{tab.}(0.05,9)=2.262>t_{calc.}=0.519$

and the drug related impurities such as ampicillin, sulbactam and pencillamine.

The results obtained in this study as well as the validation performed, show that MEKC is a suitable alternative to HPLC for the pharmaceutical analysis of Unasyn oral preparations.

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