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Short communication

Adaptation of capillary electrophoresis to piperacillin drug analysis

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Abstract

A capillary electrophoresis method was developed for the pharmaceutical analysis of piperacillin, a β-lactam antibiotic, in preparations for intramuscular and intravenous injections. Disodium hydrogenphosphate and sodium tetraborate buffer pH 6.2 or 8.7 supplemented with sodium dodecyl sulfate 7 g/l and electrophoresis voltage −18 kV, seem to provide optimal conditions for piperacillin CE assay. The method was validated and good reproducibility, precision, accuracy and assay linearity in antibiotic concentrations 0.08–2.00 mg/ml for both electrolytes were observed. The detection limit for piperacillin was 0.01 mg/ml. Preliminary experiments showed the usefulness of CE for separation and determination of piperacillin and β-lactamase inhibitor co-existing in the drug Tazocin. The results obtained by CE were also compared with those obtained by liquid chromatography. Statistical analysis by Student's *t*-test showed no significant differences between the results obtained by the two methods.

Keywords: Pharmaceutical analysis; Piperacillin; Antibiotics; Lactams

1. Introduction

Capillary electrophoresis (CE) is a relatively new and a very promising analytical technique, which became common and popular in drug analysis for the qualitative and the quantitative determinations of active compounds and their impurities [1,2]. The importance of this method in different fields, has extended in recent years, mainly due to its advantages when compared with more commonly used chromatographic techniques. These advantages include: high efficiency and selectivity, short assay time, reduced usage of organic solvents and low cost of operation.

The aim of this study was to adapt CE for the determination of one of the ureidopenicillins, piperacillin [3], in preparations for intramuscular and intravenous injections. The usual determination of this antibiotic in pharmaceutical and clinical analyses is performed by high-performance liquid chromatography (HPLC) [4–6]. In this paper the abilities of CE and HPLC for assay of piperacillin are also compared.

2. Experimental

2.1. Apparatus

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

HPLC experiments were carried out on a

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Shimadzu LC-10A HPLC system with detection at 220 nm. A μ-Bondapak C_{18} column (30 cm×3.9 mm I.D.; Waters) was used for separation.

2.2. Standards and reagents

Piperacillin monohydrate working standard of activity 97.8% and piperacillin sodium substance of activity 98.6% were obtained from Lederle. Piperacillin 1 g ini. for i.m. and i.v. drug was produced by Tarchominskie Zaklady Farmaceutyczne Polfa.

Disodium hydrogenphosphate, sodium tetraborate, sodium hydroxide, phosphoric acid and monobasic sodium phosphate were of reagent grade. Sodium dodecyl sulphate (SDS) was from Sigma, tetrabutylammonium phosphate from Merck, methanol was HPLC grade from Merck. Water used to prepare standard and sample solutions, running buffers and mobile phase was obtained from a Labconco system.

The CE electrolyte contained constant concentrations of disodium hydrogenphosphate (3.12 g/l) and sodium tetraborate (7.63 g/l) and different contents of SDS (7, 10 and 14 g/l) adjusted with sodium hydroxide or phosphoric acid to pH 5.0, 6.2, 8.7 and 9.0.

The HPLC mobile phase contained methanol–water–0.2 *M* monobasic sodium phosphate–0.4 *M* tetrabutylammonium hydroxide (45:44.7:10:0.3) and was adjusted to pH 5.5.

3. Results and discussion

HPLC is the only chemical method for piperacillin testing. Looking for an easy, quick and cheap method and at the same time selective, specific and repeatable, an attempt was made to assay piperacillin by CE. A micellar system using varying concentrations of SDS in buffer containing disodium hydrogenphosphate and sodium tetraborate was established. Its pH values were chosen according to our earlier experiences from elaboration of Augmentin ini. and Unasyn ini. determination by CE [7,8]. The separation was carried out at four different pH values. Influence of SDS concentrations and buffer pH on piperacillin retention time was investigated. It was observed that reducing the buffer pH below 8.7 and increasing the SDS concentration results in prolongation of piperacillin migration time. This was especially noticed when the SDS concentration was increased from 10 to 14 g/l in pH 5.0. In this case the migration time of piperacillin was almost 50% longer. The change of SDS concentration in the range 7–14 g/l and the buffer pH in the range 8.7–9.0, did not affect the migration time.

At first, the applied voltage values were 18 and 20 kV. Further experiments were performed in 18 kV. The best repeatability of injections (measured by peak area) and piperacillin migration time were obtained using either the buffer pH 6.2 supplemented with 7 g/l SDS or buffer pH 8.7 with the same supplementation. The separation curves looked similar; Fig. 1A presents the assay at pH 8.7, however, obtained piperacillin retention times were about 8 min at pH 6.2 and about 7 min at pH 8.7. Therefore, further investigations were undertaken with both buffers. Comparing piperacillin determination parameters by CE at pH 6.2 and 8.7 (Table 1) linearity was confirmed for antibiotic concentrations of 0.08–2 mg/ml for both electrolytes with correlation coefficients of 0.996 and 0.999, respectively. The detection limit for piperacillin was 0.01 mg/ml in both separation conditions. It was the minimal concentration producing a reproducible peak with a signal-to-noise ratio greater than 3. Repeatability of migration time and peak area were very good in both cases (RSD<2.5%), however, quantitative parameters CE in pH 8.7 were much lower then in pH 6.2. The within-day precision (repeatability) and between-day precision (reproducibility) in both pH were calculated. Statistical analysis of the obtained results for eight samples (in pH 6.2 and 8.7) was performed and confirmed good repeatability, reproducibility and accuracy. At pH 6.2 the mean of 2

Table 1
Results of piperacillin analyses performed by CE in different pH values

	pH 6.2	pH 8.7
Repeatability of migration time	RSD=1.50%	RSD=0.54%
Repeatability of corrected area	RSD=2.37%	RSD=0.68%
Migration time (min)	about 8	about 7
Correlation coefficient	0.996	0.999
Quantification limit (mg/ml)	0.08–2.00	0.08–2.00
Detection limit (mg/ml)	from 0.01	from 0.01

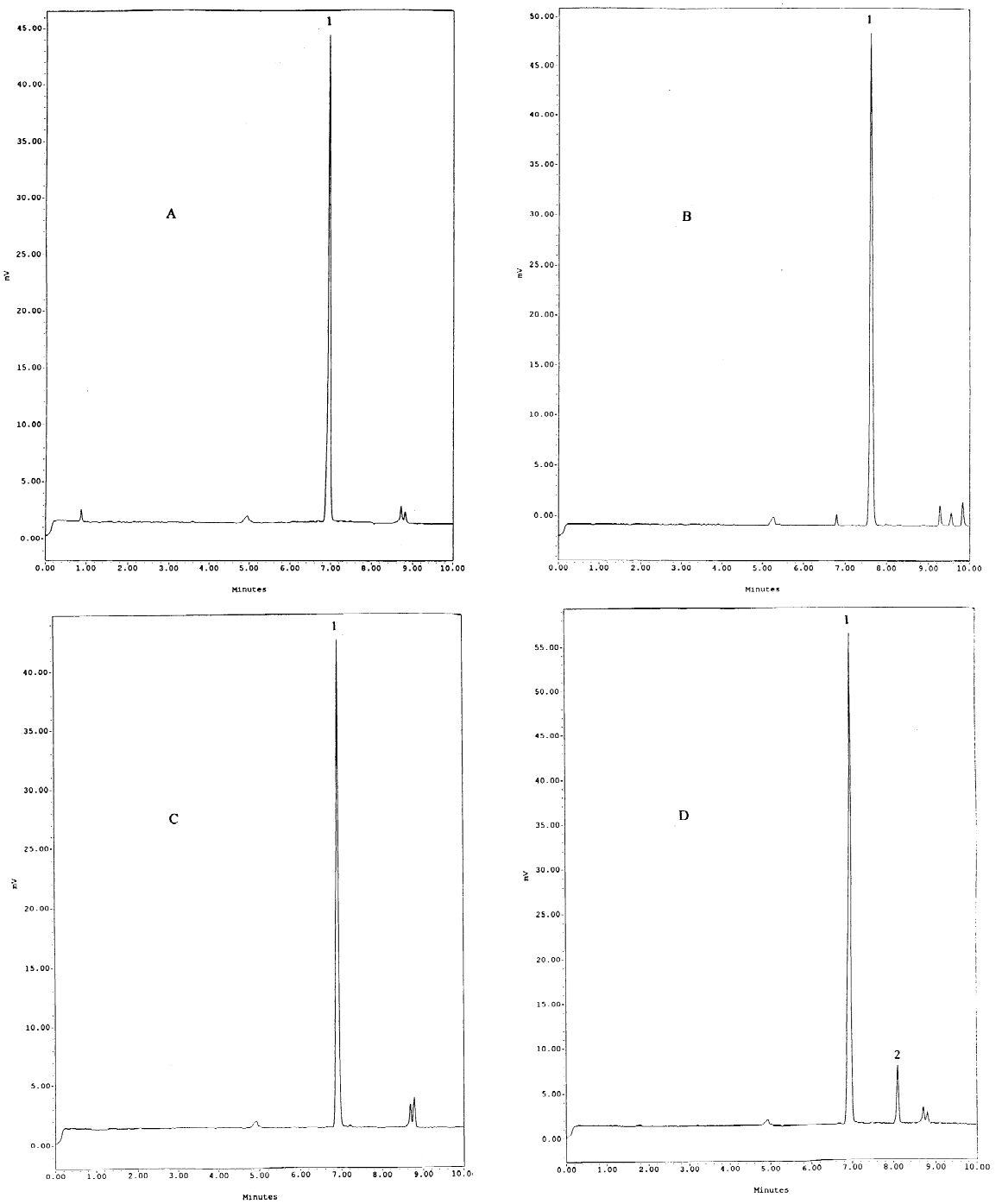

Fig. 1. Electropherograms of piperacillin and Tazocin containing piperacillin and tazobactam. CE conditions are in text. (A) Piperacillin at buffer pH 8.7 containing 7 g/l SDS, (B) piperacillin at buffer pH 6.2 containing 7 g/l SDS, stored at room temperature for 3 days, (C) piperacillin at buffer pH 8.7 containing 7 g/l SDS, stored at room temperature for 3 days, (D) Tazocin at buffer pH 8.7 containing 7 g/l SDS. 1 – Piperacillin, 2 – tazobactam.

Table 2
Comparison of piperacillin analyses results obtained by CE and HPLC

Method	CE		HPLC (method 3)
	pH 6.2 (method 1)	pH 8.7 (method 2)	
Mean (g)	1.02	1.01	1.02
No. of samples	8	8	8
SD	0.022	0.015	0.013
RSD (%)	2.12	1.51	1.25
Student's t value	Method		
for $P=0.05$, $n=14$	1 and 3	2 and 3	
t tabulated=2.145	0.947	1.207	

days determinations was 96.29±2.57% and at pH 8.7 95.4±1.78%. The repeatability and reproducibility were better for the analyses performed in pH 8.7. RSDs of 2.24 and 1.50% for within-two-day determination were obtained. The between-day precision for this assay was 1.88%.

Additionally CE analyses of two piperacillin solutions (the same concentrations, different pH) stored at room temperature for 3 days were performed. It was found that created extra small peaks were more effectively separated in buffer pH 6.2 than in buffer pH 8.7 (Fig. 1B and C).

A sample of piperacillin 1 g for i.m. and i.v. injection, produced by Tarchominskie Zaklady Farmaceutyczne Polfa, was analysed by both CE and HPLC. The values obtained by CE methods and those obtained by the validated HPLC assay were not significantly different $t_{\text{calculated}}<t_{\text{tabulated}}$ for both CE conditions (Table 2).

Using the above mentioned CE conditions, attempts of drug Tazocin ini. (Lederle) assay by CE were undertaken. Tazocin consists of piperacillin and the β-lactamase inhibitor tazobactam in the ratio 8:1. The obtained different migration times and good peak separation allow to conclude that simultaneous determination of both compounds will be possible in the future. In Fig. 1D separation of both active compounds at pH 8.7 is presented. When buffer pH 6.2 was used, migration times of peaks were longer as was observed in the case of piperacillin analysis.

4. Conclusions

CE adapted for determination of piperacillin is precise and accurate. The results obtained by CE and HPLC were not significantly different. Piperacillin CE may be a valuable alternative technique to HPLC. CE may be used for piperacillin/tazobactam analysis in Tazocin preparation.

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