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Application of MEKC for determination of ticarcillin and clavulanic acid in Timentin intravenous preparation☆

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Abstract

A micellar electrokinetic chromatography method for simultaneous assay of ticarcillin and clavulanic acid in Timentin i.v. injection preparation was developed. This method ensures excellent separation of both components of Timentin preparation. The validation of the method was performed, and specificity, reproducibility, precision and accuracy were confirmed. The detection and quantitative limits for Timentin were established in the concentrations 0.04 and 0.08 mg/ml, respectively. The elaborated technique was compared with two methods routinely used-UV and high performance liquid chromatography (HPLC). The obtained results and their statistical analysis proved the same precision of all methods, however, no significant differences were observed between CE and HPLC.

Keywords: Pharmaceutical analysis; Antibiotics; Ticarcillin; Clavulanic acid; β-Lactamase inhibitor; Capillary electrophoresis

1. Introduction

Capillary electrophoresis (CE) becomes an alternative analytical method compared with high performance liquid chromatography (HPLC) in drug research and control [1–3]. We have already elaborated and adapted the CE technique for analysis of some β-lactam antibiotics [4–7]. This method also provided possibilities for amoxicillin and clavulanic acid as well as ampicillin and sulbactam simultaneous assay in pharmaceutical preparations [8]. Our preliminary investigations showed that the CE method offers good resolution of ticarcillin (β-lactam antibiotic/carboxypenicillin) and clavulanic acid (β-lactamase inhibitor) combined in Timentin preparation in proportion 15:1. Actually in our laboratory two techniques are used in routine analysis of both compounds: HPLC and UV spectrophotometric methods, however, UV method does not allow the measurement of both constituents simultaneously.

The aim of this study was to adapt CE in the micellar electrokinetic modification for determination of ticarcillin and clavulanic acid in the Timentin preparation. According to the

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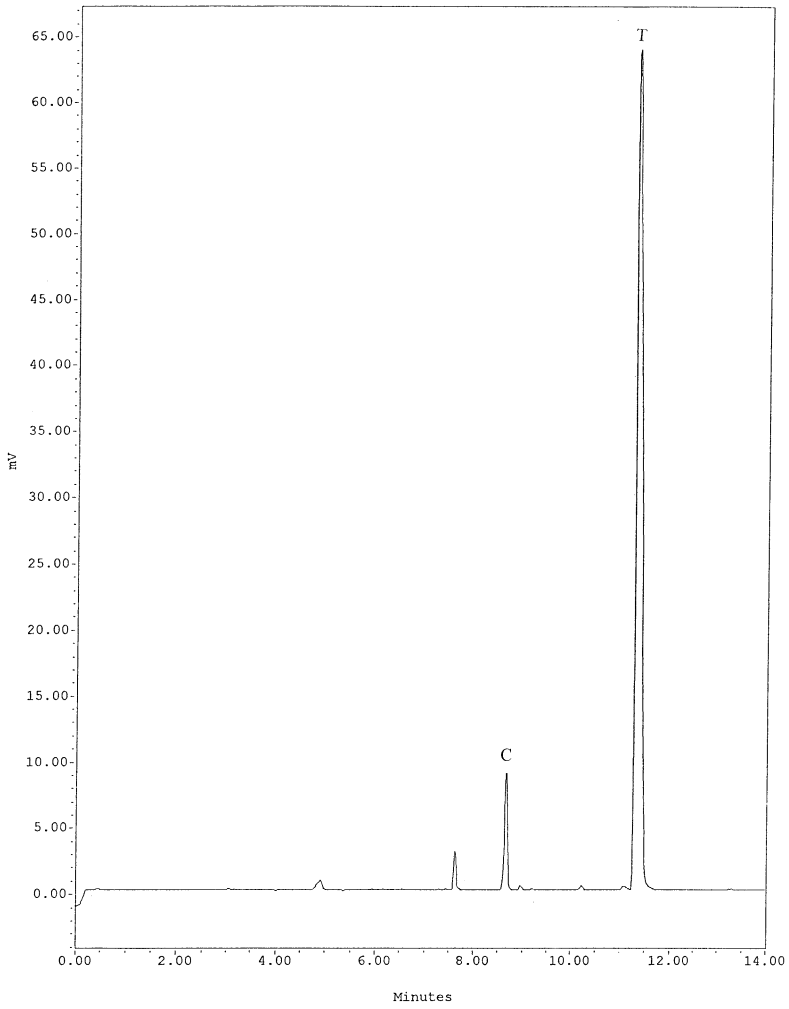

Fig. 1. Typical electropherogram of Timentin preparation. CE conditions—see Section 2. C, clavulanic acid; T, ticarcillin.

pharmacopoeial requirements validation of the new assay should also be performed.

2. Experimental

2.1. Instrumental parameters

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

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

Spectrophotometer UV/VIS 1700 Shimadzu was used for determinations. Absorption at 313 nm for clavulanic acid assay after incubation in a water-

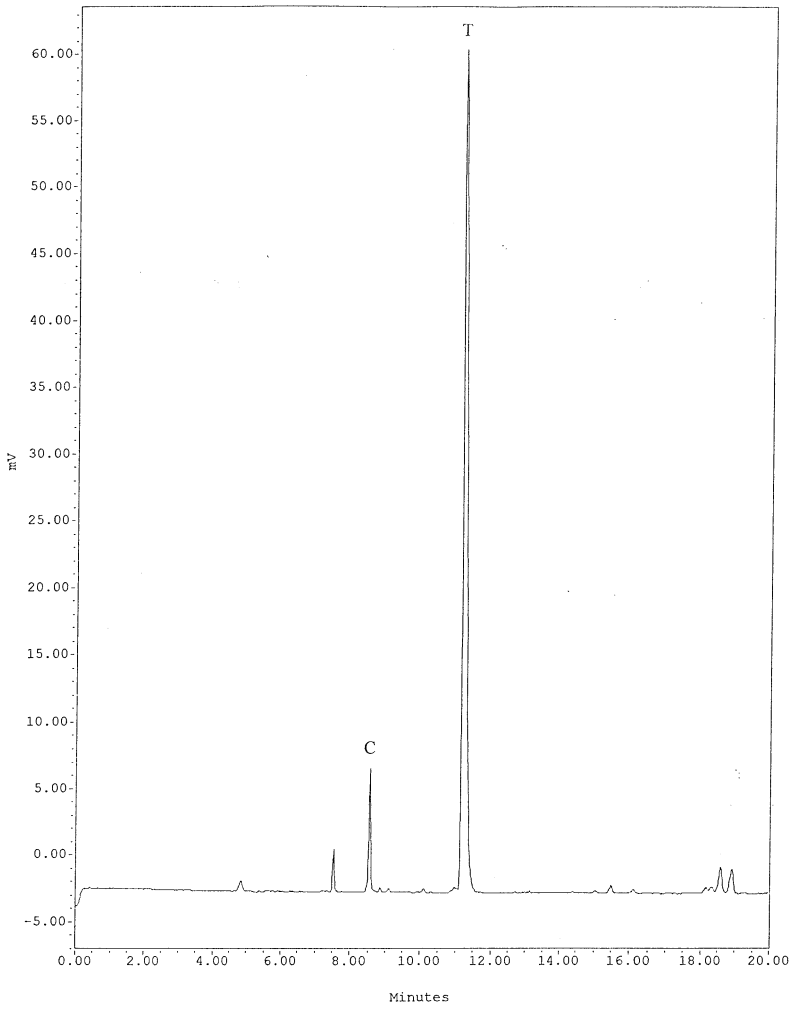

Fig. 2. Typical electropherogram of Timentin preparation stored at room temperature for 24 h. CE conditions—see Section 2. C, clavulanic acid; T, ticarcillin.

bath at 30 °C for 12 min and at 325 nm for ticarcillin after incubation at 60 °C for 25 min, were measured.

2.2. Standards and reagents

2.2.1. Standards

Ticarcillin sodium Smith Kline Beecham (Brentford, UK) reference standard of activity 81.5%; lithium clavulanate Ph.E reference standard of activity 94.4%; potassium clavulanate Smith Kline Beecham (Brentford, UK) reference standard of activity 100.1%

2.2.2. Preparations

SmithKline Beecham (Brentford, UK) intravenous drugs-Timentin 1.6 g, containing 1.5 g ticarcillin and 100 mg of clavulanic acid and Timentin 3.2 g, containing 3.0 g ticarcillin and 200 mg of clavulanic acid.

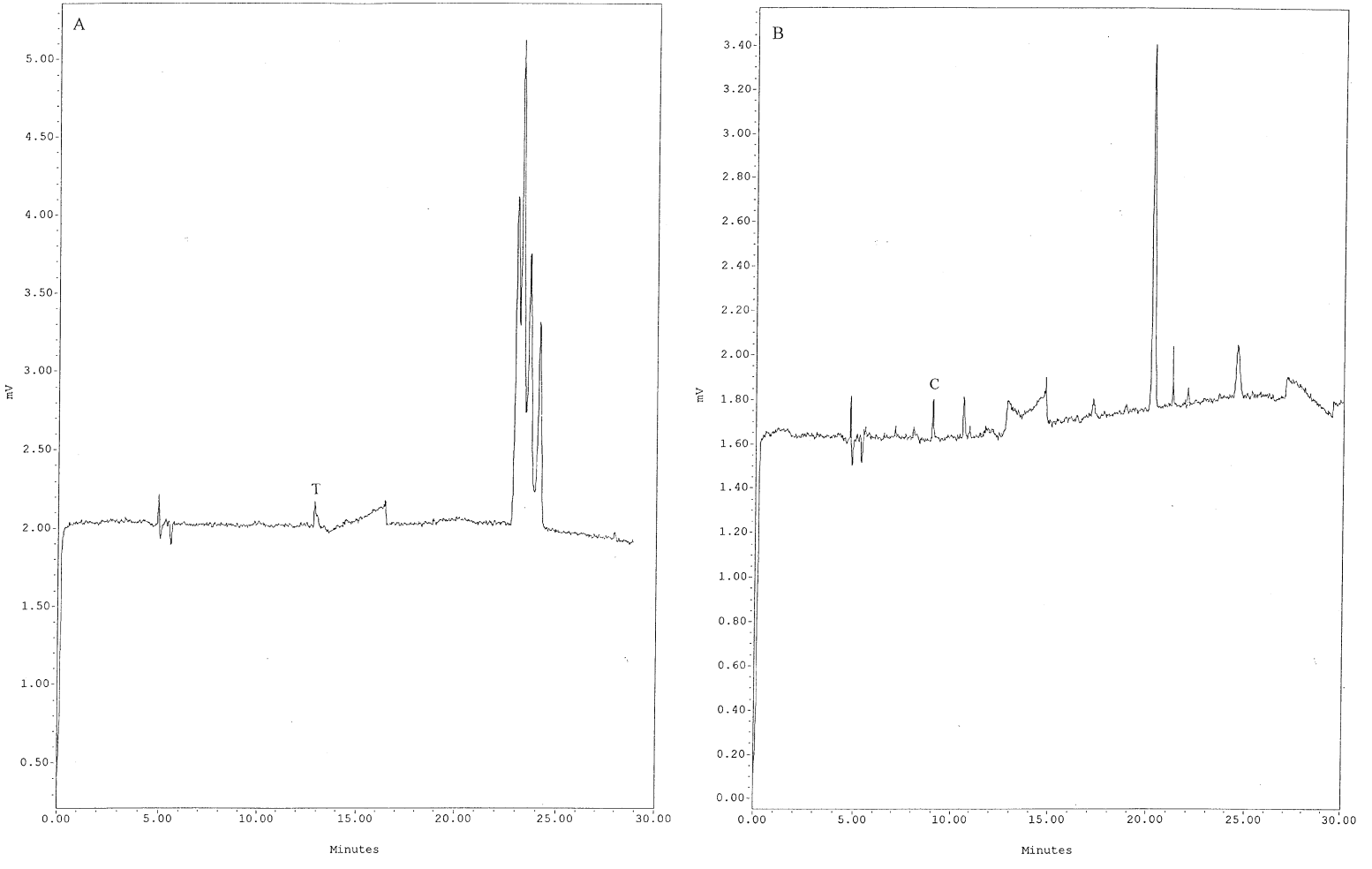

Fig. 3. Electropherogram of solution ticarcillin (A) and clavulanic acid (B) after reaction with 1 M NaOH. CE conditions—see Section 2. C, clavulanic acid; T, ticarcillin.

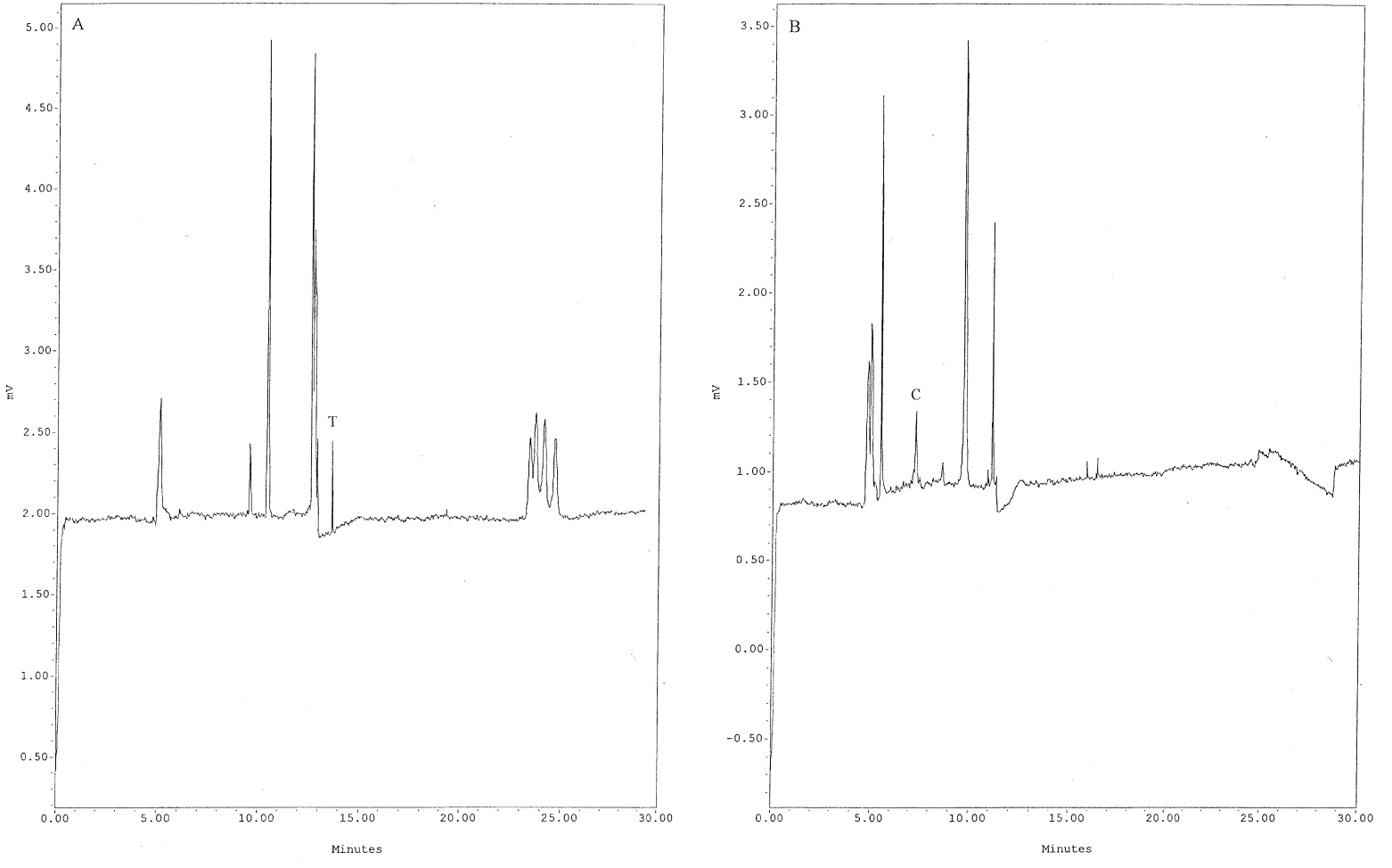

Fig. 4. Electropherogram of solution ticarcillin (A) and clavulanic acid (B) after reaction with 1 M HCl. CE conditions—see Section 2. C, clavulanic acid; T, ticarcillin.

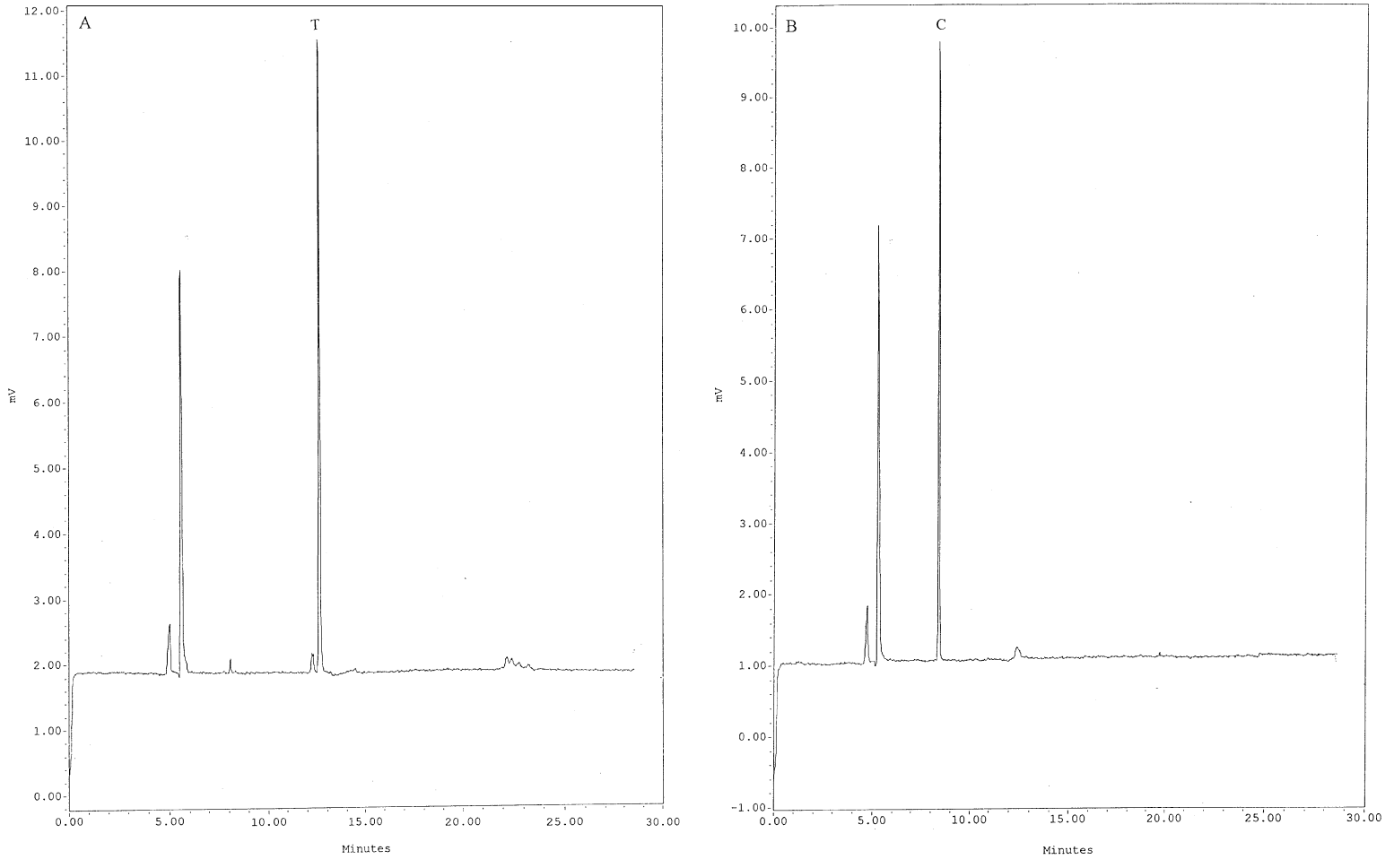

Fig. 5. Electropherogram of solution ticarcillin (A) and clavulanic acid (B) after reaction with 30% H_2O_2. CE conditions—see Section 2. C, clavulanic acid; T, ticarcillin.

Table 1
Parameters of Timentin determination by CE method

	Clavulanic acid	Ticarcillin
Repeatability of migration time	RSD ≤ 0.420%	RSD ≤ 0.344%
Repeatability of corrected area	RSD ≤ 0.761%	RSD ≤ 0.661%
Migration time	8.1–8.9 min	10.5–11.8 min
Correlation coefficient	0.9997	0.9999
Quantification limit	0.005–2 mg/ml Timentin	0.005–3 mg/ml 0.08–3.2 mg/ml
Detection limit	From 0.003 mg/ml Timentin	From 0.0025 mg/ml From 0.05 mg/ml

2.2.3. Standard solutions

Standard solutions were prepared in water for CE at concentration about 1.4 mg/ml ticarcillin and 0.1 mg/ml of clavulanic acid as well as 1 mg/ml of ticarcillin and 0.05 mg/ml of clavulanic acid for HPLC. For UV assay one water solution containing 0.05 mg/ml of ticarcillin and second water solution containing 0.02 mg/ml of clavulanate acid. Solutions for specificity experiments: 2 ml of solution containing 1 mg/ml reference standard was mixed with 1 ml 1 M NaOH or 1 ml 1 M HCl or 1 ml 3% H_2O_2, stored 1 h in the cases of NaOH and HCl and 15 min in the case of H_2O_2 followed by dilution to 10 ml.

2.2.4. Sample solutions

Water solutions of Timentin preparation at concentrations of about 0.5, 1 (this concentration was used for quantitative determinations) and 1.5 mg/ml for CE, about 1 mg/ml for HPLC, and 0.4 mg/ml for UV assay of clavulanic acid and 0.7 mg/ml for UV assay of ticarcillin.

2.2.5. Reagents

Monobasic sodium phosphate, sodium tetraborate, sodium hydroxide, phosphoric acid, hydro-

Table 2
Robustness of migration times and peaks area upon change buffer pH in CE

	Buffer pH 8.6		Buffer pH 8.7		Buffer pH 8.8	
	tm	Peak area	tm	Peak area	tm	Peak area
Clavulanic acid	8.29 (0.324%)	37 277 (0.494%)	8.11 (0.136%)	37 715 (0.827%)	8.23 (0.01%)	37 442 (2.065%)
Ticarcillin	10.62 (0.574%)	545 430 (0.637%)	10.39 (0.105%)	543 368 (0.689%)	10.65 (0.084%)	528 769 (2.068%)

Each sample was injected five times, RSDs are listed in brackets. Timentin concentration was 2 mg/ml.

Table 3
Repeatability and intermediate precision of CE assay

	Timentin 1.6 g				Timentin 3.2 g			
	Day 1		Day 2		Day 1		Day 2	
	Clavulanic acid	Ticarcillin	Clavulanic acid	Ticarcillin	Clavulanic acid	Ticarcillin	Clavulanic acid	Ticarcillin
No. samples	6	6	6	6	5	5	5	5
Mean/vial	99.96 mg	1.503 g	98.61 mg	1.513 g	209.26 mg	3.137 g	208.46 mg	3.144 g
S.D.	2.004	0.024	1.232	0.026	1.166	0.016	2.171	0.032
RSD%	2.005	1.613	1.240	1.687	0.557	0.494	1.041	1.024
Mean from assays on day 1 and day 2			99.29 mg	1.508 g			208.86 mg	3.139 g
S.D.			1.735	0.024			1.697	0.023
RSD%			1.748	1.610			0.812	0.735
Accuracy			99.29 ± 1.10	1.508 ± 0.02			208.86 ± 1.21	3.139 ± 0.02

Assay requirements for both substances: 95–105% of the declaration.

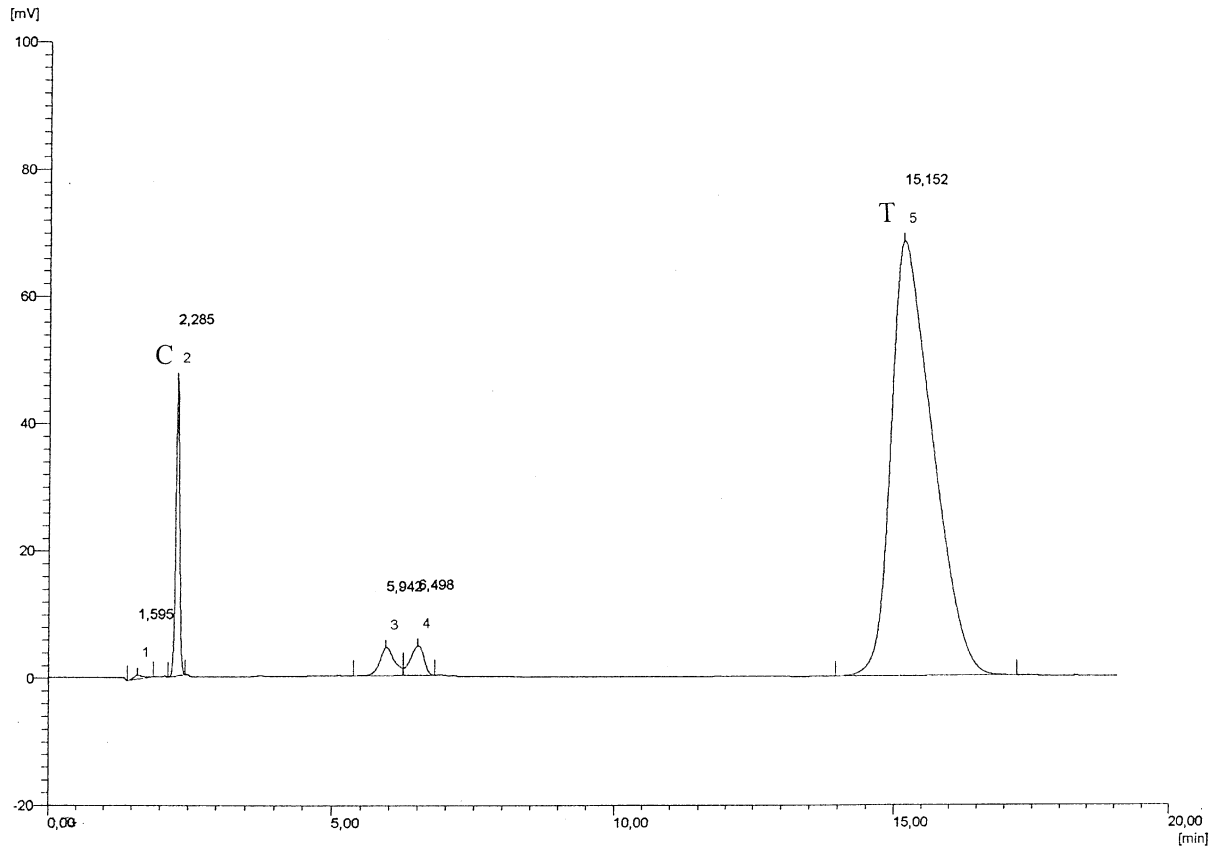

Fig. 6. Chromatogram of Timentin preparation. HPLC conditions—see Section 2. C, clavulanic acid; T, ticarcillin.

gen peroxide and mercuric chloride were of reagent grade. Sodium dodecyl sulphate (SDS) was provided by Sigma (St. Louis, MO), imidazole extra pure grade and acetonitryle HPLC grade were received from Merck (Germany, Darmstad). Water used to prepare running buffer and mobile phase was obtained from a Labconco System (Kansas City, KS). The CE electrolyte contained 0.02 M borate, 0.02 M of phosphate solution and 1.44% SDS, pH of this electrolyte is 8.66 (only if it is necessarily adjusted to pH 8.66 with sodium hydroxide). The mixture (1:20) of acetonitryle and 0.1 M monobasic sodium phosphate solution adjusted to pH 4.3 (add about 1 ml of 1 M phosphoric acid to 1000 ml of mixture to adjust to pH 4.3) was used as HPLC mobile phase. 1.2 M imidazole solution adjusted to pH 6.8 and solution containing 8.25% imidazole and 0.027% mercuric chloride adjusted to pH 6.8 were used for UV determinations.

3. Results and discussion

The elaborated MEKC method using borate-phosphate buffer at pH 8.66 supplemented with 14.4 g/l SDS, allows the separation of semisynthetic penicillins—amoxicillin and ampicillin from β-lactamase inhibitors—clavulanic acid and sulbactam [8]. Under these conditions migration times of ampicillin and amoxicillin are similar: 7.1 and 7.6 min, and are shorter than migration time of clavulanic acid: 8.6 min or sulbactam 9.2 min. In the case of Timentin preparation, migration time of ticarcillin was longer than of clavulanic acid and varied from 10.5 to 11.8 min,

Table 4
Results of comparative CE and UV assays of Timentin preparation 3.2 g

Method	Clavulanic acid		Ticarcillin	
	CE	UV	CE	UV
Mean/vial	205.41 mg	211.20 mg	3.109 g	3.138 g
No. samples	5	5	5	5
S.D.	2.553	1.982	0.0102	0.0156
RSD%	1.243	0.938	0.329	0.498
Student's value from CE and UV assays				
t_{tab} (0.05;8) = 2.306	4.028		3.005	

Assay requirements for both substances: 95–105% of the declaration.

depending on the exploitation of the capillary. The resolution between ticarcillin and clavulanic acid peaks was about 8 [9]. It means that separation of both peaks is essentially complete (Fig. 1). The solutions of ticarcillin and clavulanic acid under these conditions were stable and showed no significant difference in the peaks area after a 1.5-h period of fifth injections. That time was sufficient to perform the assay. There were also, in the assay range, no significant degradation differences in solutions after 24 h storage at room temperature (Fig. 2). Specificity of the CE method was confirmed by addition of ticarcillin and clavulanic acid reference substances to Timentin sample and only two peaks were obtained on the electropherogram. Timentin preparation consists of only antibiotic and β-lactamase inhibitor and does not have other interfering components.

For the confirmation of the method specificity electrophoresis of ticarcillin and clavulanic acid solution after hydrolysis with 1 M sodium hydroxide and 1 M hydrochloride acid as well as oxidation with 3% hydrogen peroxide were performed (Figs. 3–5). New degradation peaks obtained from mother substances did not comigrate with these compounds with the exception of acid degradation product of clavulanic acid with the same migration time as ticarcillin. Taking into account that the concentration of ticarcillin in Timentin was 15 times higher than concentration of clavulanic acid, and the peak after degradation was very small, we assumed no degradation product influence on ticarcillin assay.

The precision of the method was calculated from five consecutive injections of three different concentrations (50, 100 and 150% of assay concentration) of Timentin preparation. Timentin solution analysis showed good repeatability for each of the analysed substances independently on drug concentration. Particular migration times of clavulanic acid and ticarcillin were stable (RSD = 0.123, 0.420, 0.100% and 0.244, 0.344, 0.112% for aforementioned concentrations, respectively). High repeatability of peak areas for both substances (RSD = 0.404, 0.761, 0.392% and 0.661, 0.562, 0.493% for clavulanic acid and ticarcillin, respectively) were also noticed. CE analyses of sample containing different amounts of Timentin preparation showed very high correlation between peak areas and analysed compounds concentrations. The linearity for ticarcillin disodium reference substance was established in the range from 0.005 to 3.0 mg/ml ($r = 0.9992$) and for lithium clavulanate reference substance in the range from 0.005 to 2 mg/ml ($r = 0.9993$). The detection limit defined as signal to noise ratio of 3:1, was 0.003 mg/ml for clavulanic acid, and 0.0025 mg/ml for ticarcillin. The quantitation limit defined as signal to noise ratio of 10:1, was 0.005 mg/ml for both compounds. The detection and quantification limits for Timentin preparation depending on concentration of clavulanic acid concentrations were 0.05 and 0.08 mg/ml, respectively. The

Table 5
Results of comparative CE and HPLC assays of Timentin preparations

Method	Timentin 1.6 g				Timentin 3.2 g			
	CE		HPLC		CE		HPLC	
	Clavulanic acid	Ticarcillin	Clavulanic acid	Ticarcillin	Clavulanic acid	Ticarcillin	Clavulanic acid	Ticarcillin
No. samples	5	5	5	5	5	5	5	5
Mean/vial	100.13 mg	1.536 g	100.33 mg	1.529 g	209.26 mg	3.137 g	208.90 mg	3.107 g
S.D.	0.702	0.005	1.026	0.009	1.166	0.016	4.284	0.042
RSD%	0.701	0.341	1.023	0.560	0.557	0.494	2.051	1.345
Student's value from CE and HPLC assays t_{tab} (0.05;8) = 2.306			0.361	1.477			0.181	1.498

Assay requirements for both substances: 95–105% of the declaration.

following, very high correlation coefficients were calculated from clavulanic acid peak—0.9997 and from ticarcillin peak—0.9999. Calibration curves were constructed from five different concentrations of ticarcillin substance and Timentin preparation. Each concentration of sample was injected three times. In the case of clavulanic acid we used our earlier calibration results [8]. Timentin determination parameters are assumed in Table 1. In further experiments, robustness-influence of deliberate small changes in the pH buffer on results was tested. It varied from pH 8.6 to pH 8.8 (Table 2). Despite small changes in the migration times and peaks area, stability towards pH changing (8.6–8.8) was proved. In a buffer of pH 8.8 the worst reproducibility of peaks area both clavulanic acid and ticarcillin were observed (RSD = 2.065% for clavulanic acid and 2.068 for ticarcillin). Good repeatability and intermediate precision were shown in independent assays of Timentin 1.6 and 3.2 g on different days (Table 3).

Further comparable assays of examined drug by CE and UV methods, as well as CE and HPLC methods were performed. Older UV methods were applied to assay other penicillins, for example ampicillin and amoxicillin as well as cephalosporins and clavulanic acid [10–13]. In the UV method ticarcillin reacts with imidazole in the presence of mercuric chloride to form a mercuric mercaptide with absorption at 325 nm, under optimum reaction conditions of temperature and time. Clavulanic acid and its salts and imidazole, under optimum temperature and reaction time, form ultraviolet absorbing complex with maximum at 313 nm. The statistical analysis of results obtained from CE and UV methods showed the same accuracy but did not confirm that methods can be used alternatively. Under HPLC method conditions, migration time of clavulanic acid was about 2.5 min and ticarcillin about 15 min (Fig. 6). Parameters of CE and HPLC methods used to assay Timentin preparation, are summarised and compared in Table 4. The content of both clavulanic acid and ticarcillin in Timentin preparations 1.6 g as well as 3.2 g determined by CE and HPLC did not differ significantly. The Student's t value determined by the t-test was lower than the theoretical value (Table 5).

4. Conclusions

The elaborated MEKC method ensures good separation of components of Timentin preparation and sufficient stability during analysis.

The results obtained in this study and performed validation, enables to use CE alternatively to HPLC for pharmaceutical analysis combination of ticarcillin and clavulanic acid.

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