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Journal of Pharmaceutical and Biomedical Analysis
29 (2002) 75–81

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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CE versus LC for simultaneous determination of amoxicillin/clavulanic acid and ampicillin/sulbactam in pharmaceutical formulations for injections☆

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Received 15 October 2001; received in revised form 19 December 2001; accepted 20 December 2001

Abstract

A rapid, capillary electrophoresis method was evaluated for determination of amoxicillin and clavulanic acid in Augmentin as well as ampicillin and sulbactam in Unasyn preparations for injections. Phosphate–borate buffer at pH 8.66 containing 14.4% sodium dodecyl sulfate was used as a mobile phase. The method was validated. Reproducibility, precision, accuracy and assay linearity in concentration of amoxicillin 0.05–3.03 mg/ml and ampicillin 0.05–3.08 mg/ml, as well as clavulanic acid 0.02–2.02 mg/ml and sulbactam 0.05–2.08 mg/ml were established. This new method is fast, inexpensive and limits consumption of organic solvents when compared with alternative high performance liquid chromatography (HPLC) method, used for drug analysis. Statistical analysis by Student's *t*-test showed no significant differences between the results obtained by the two methods $t_{calculated}$ 0.32 and 1.69 for amoxicillin and clavulanic acid and 0.67 and 1.93 for ampicillin and sulbactam were smaller than $t_{tabulated}$.

Keywords: Pharmaceutical analysis; Antibiotics; Capillary electrophoresis

1. Introduction

Extended spectrum penicillins—ampicillin and amoxicillin have been used in antibacterial therapy for many years. However, more frequent occurrence of β-lactamase producing clinically important bacterial strains resulted in limiting usage of these antibiotics. Co-administration of the labile β-lactam together with an agent capable of inhibiting the β-lactamases was performed to improve the antibacterial therapy and overcome the bacterial resistance. So, oxapenam compound—clavulanic acid accompanied amoxicillin in Augmentin, and penicillanic acid sulphone—sulbactam joined ampicillin in Unasyn preparations [3,4].

Production of composed drugs, always creates a challenge for the pharmaceutical drug control. The modern analytical investigation of antibiotic drugs, content and purity estimation of active

☆ Preliminary results were presented as posters on 2nd European Congress of Chemotherapy, Berlin, 1998 [1,2].

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0731-7085/02/$ - see front matter
PII: S0731-7085(02)00029-8

compounds, very often involves the high performance liquid chromatography (HPLC) [5–12]. This technique has some disadvantages—requires large amount of high purity organic solvents, long system stabilization time, and special sample preparation.

The new analytical separation method—capillary electrophoresis (CE) resolves all above mentioned problems. This fully automated and relatively simple technique is progressively introduced and used alternatively to HPLC. During the last years, we elaborated and adapted CE method to analyze some β-lactam antibiotics: piperacillin, imipenem and cephalosporins (ceftazidime, cefoperazone, cefotaxime, cefuroxime, cephazolin, ceftriaxone) [13–16]. Some preliminary investigations were performed also on amoxicillin and ampicillin combined with β-lactamase inhibitors [1,2].

The drug state control in Poland requires the qualitative and quantitative assay of amoxicillin and clavulanic acid in Augmentin as well as ampicillin and sulbactam in Unasyn pharmaceutical preparations for injections. Two methods: spectrophotometry and HPLC are used routinely to perform analyses of Augmentin and only HPLC method to test Unasyn.

The aim of this study was to adapt CE technique for simultaneous determination of β-lactam antibiotic and β-lactamase inhibitor during one analysis. The elaborated method was validated and compared with routine HPLC method.

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 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 Shimadzu LC-10A HPLC system with detection for both drugs at 230 nm. A μ-Bondapack C_{18} (30 cm × 3.9 mm I.D.; Waters) and Nucleosil 100-5 C_{18} (25 cm × 4 mm I.D.; Machery-Nagel) columns were used for assay of Augmentin and Unasyn preparations, respectively.

2.2. Standards and reagents

Amoxicillin trihydrate working standard of activity 86.7%, ampicillin trihydrate working standard of activity 85.0%, clavulanate lithium working standard of activity 95.5% were obtained from Beecham. Sulbactam sodium reference standard of activity 90.1% was purchased from Pfizer. Ampicillin trihydrate—British Pharmacopoeia Reference Substances of activity 85.0% and amoxicillin trihydrate—European Pharmacopoeia Reference Standard were used. Drugs for injections—Unasyn and Augmentin were obtained from producer—Tarchomińskie Zakłady Farmaceutyczne Polfa.

Monobasic sodium phosphate, sodium tetraborate, sodium hydroxide, phosphoric acid were of reagent grade. Sodium dodecyl sulfate (SDS) was provided by Sigma, tetrabutylammonium hydroxide (TBAH) from Aldrich, methanol and acetonitrile were HPLC grade from Merck. Water used to prepare standard and sample solutions, running buffers and mobile phases was obtained from a Labconco system. The CE electrolyte contained 0.02 M borate–phosphate buffer and 1.44% SDS adjusted to pH 8.66. The HPLC mobile phases contained 0.1 M monobasic sodium phosphate—methanol adjusted to pH 4.0 for Augmentin assay and 0.005 M TBAH pH 5.0—acetonitrile for Unasyn were applied.

3. Results and discussion

The new analytical method, CE has been evaluated and validated for determination of amoxicillin and clavulanic acid in Augmentin as well as ampicillin and sulbactam in Unasyn drugs. Correct separation of both preparations components

during CE, requires usage of proper electrolyte for analysis. The 0.2 M borate–phosphate buffers in range of pH 6.0–10.0, supplemented with different SDS concentrations were used to choose the best separation conditions for components of Augmentin and Unasyn. Finally, above buffer adjusted to pH 8.66 with 1.44% SDS was used for routine electrophoresis. Fig. 1A and B present typical electropherograms of active substances of both drugs. The distinct separations amoxicillin/clavulanic acid and ampicillin/sulbactam as well as a small level of chemical contamination, allow to perform analyses of β-lactam antibiotic and β-lactamase inhibitor parallely during one electrophoresis. Separation conditions for these compounds were established. The migration times of both antibiotics were similar though amoxicillin migrates faster (tm = 7.1 min) than ampicillin (tm = 7.56 min). The migration time for clavulanic acid was 8.6 min and for sulbactam 9.16 min. Comparable HPLC chromatograms of both preparations are presented in Fig. 2A and B.

CE analyses of samples containing different amounts of Augmentin and Unasyn preparations, as well as, analyses of reference substances of β-lactam antibiotics and β-lactamase inhibitors, showed very high correlation between peak areas and analyzed compounds concentrations. Calibration curves were constructed from five different concentrations. Each concentration of sample was injected three times. Depending on the active components ratio: clavulanic acid to amoxicillin (1:5) and sulbactam to ampicillin (1:2), the linearity of preparations were performed in the range: amoxicillin (0.05–3.03 mg/ml), ampicillin (0.05–3.08 mg/ml), clavulanic acid (0.03–2.02 mg/ml) and sulbactam (0.02–2.08 mg/ml). The data concerning calibration curves are summarized in the Table 1. The following, very high correlation coefficients—0.9998 were calculated for all analyzed substances. The detection limit defined as signal to noise ratio of 3:1, was 0.4 μg/ml for amoxicillin and ampicillin as well as 0.3 μg/ml for clavulanic acid and 0.7 μg/ml for sulbactam. The quantitation

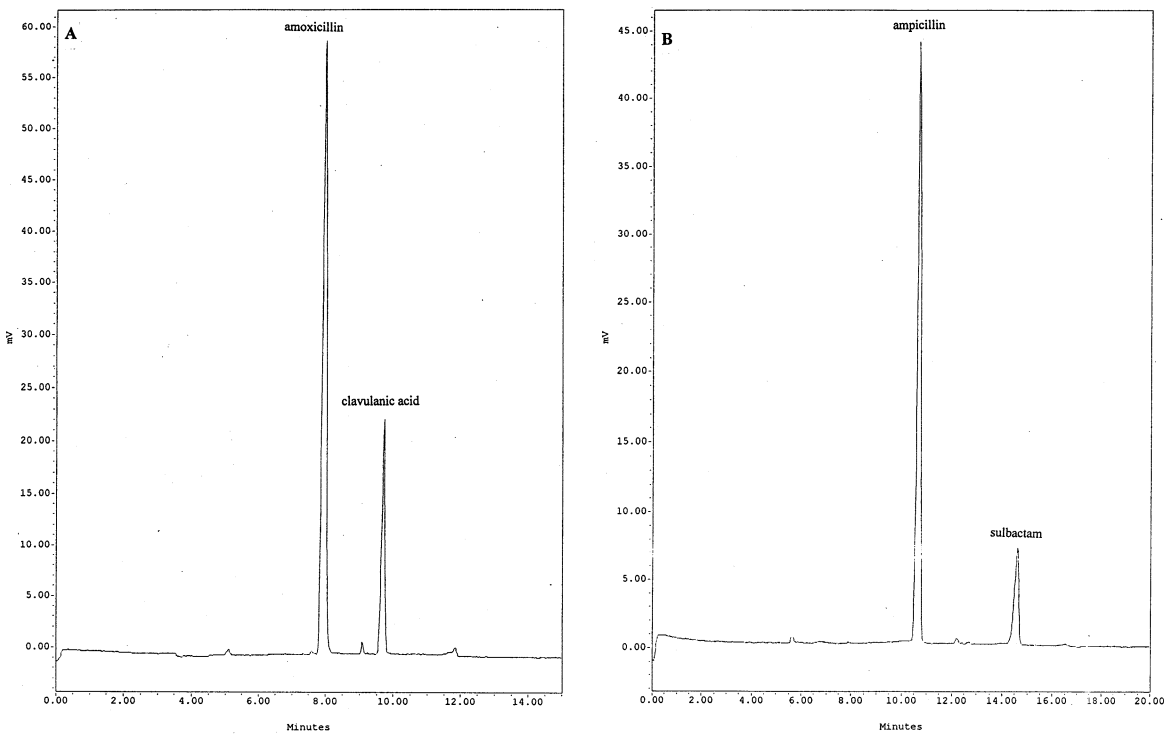

Fig. 1. Typical CE electropherograms of the Augmentin (A) and Unasyn (B) preparations. Conditions—see Section 2.

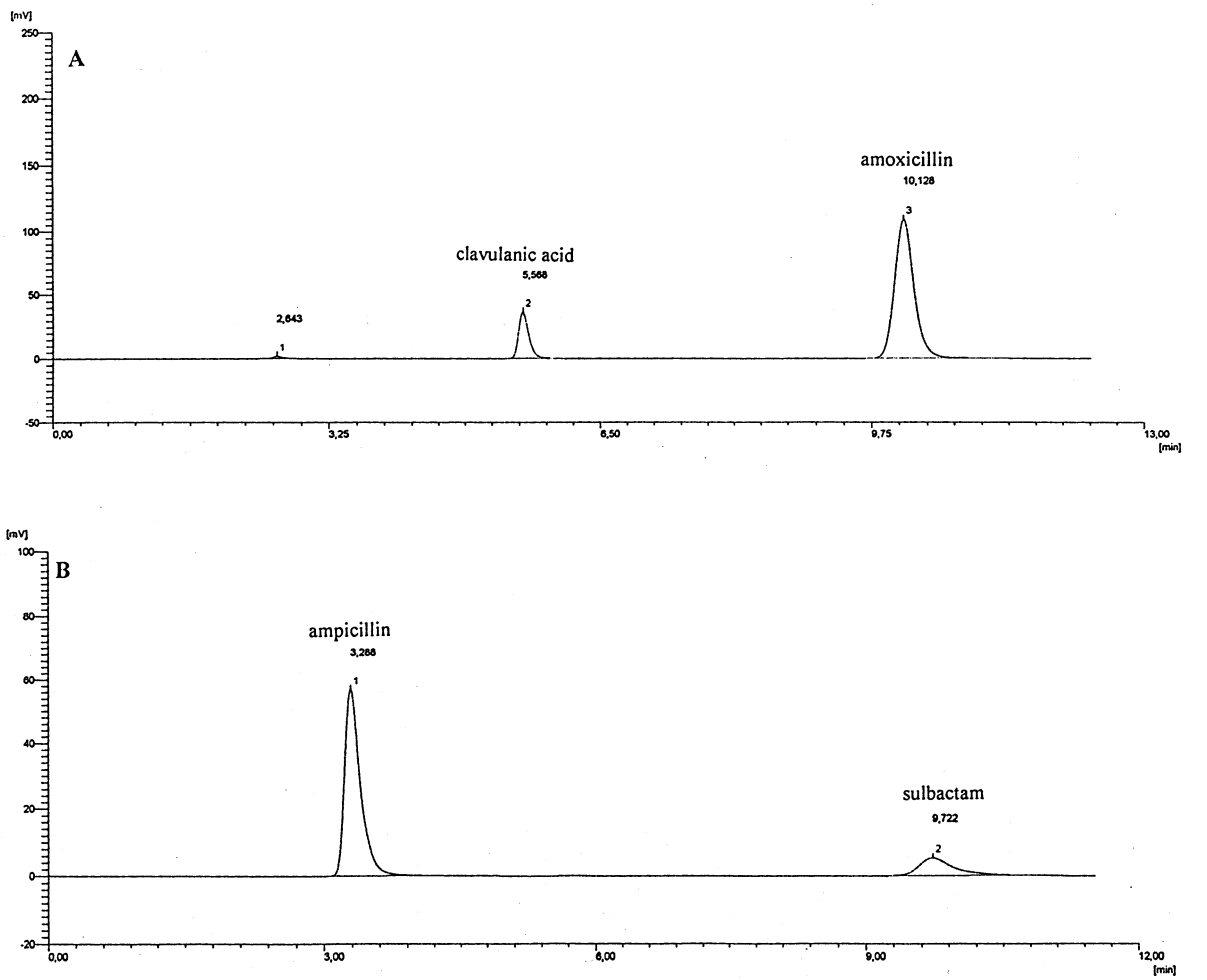

Fig. 2. Typical HPLC chromatograms of the Augmentin (A) and Unasyn (B) preparations. Conditions—see Section 2.

limit defined as signal to noise ratio of 10:1, was 0.8 μg/ml for amoxicillin and ampicillin as well as 0.5 μg/ml for clavulanic acid and 2 μg/ml for sulbactam.

Instrumental precision was calculated from 10 consecutive Augmentin solution injections and 9 consecutive Unasyn injections performed during 2 days.

Augmentin solution analysis showed very good by-day and day-to-day repeatability for each of analyzed substance. Particular retention times of amoxicillin and clavulanic acid were stable (RSD = 0.51, 0.47, 0.58% and 0.71, 0.62, 0.65%, respectively). High precisions of repeatability of peak areas of active substances in Augmentin (RSD = 1.57, 1.95, 1.73% and 1.92, 1.62, 1.76% for amoxicillin and clavulanic acid, respectively) were also noticed.

CE by-day and day-to-day analyses of Unasyn for injection samples, showed better repeatability for ampicillin than for sulbactam. Stable retention times of ampicillin and sulbactam (RSD = 0.52, 1.00, 1.79% and 0.59, 1.56, 1.61%, respectively) and good precision of repeatability of peaks area of active substances in Unasyn (RSD = 1.68, 1.75, 1.79% and 2.92, 2.19, 2.55% for ampicillin and sulbactam) were found. The estimation of repeatability was performed during 3 h. Solutions

were stable and showed no significant difference in the peaks area after this time. That was sufficient period to perform such assays.

In the next experiments, robustness-influence of deliberate small changes in the pH buffer on the results was tested. It varied from pH 8.6 to 8.8 (Table 2). Despite small changes in the migration times and peaks area, stability towards the pH change (8.6–8.8) was proved. During these investigations, some deviations of migration times for all substances were noticed. Migration times were shorter than obtained during earlier experiments. We have realized, that usage of new capillary, effects the migration times. During electrophoresis run in the new capillary, analyzed substances migrated faster.

Specificity of the CE method was confirmed by addition of ampicillin and sulbactam reference substances to Unasyn sample solution as well as amoxicillin and clavulanic acid reference powders to Augmentin sample solution. Only two properly separated peaks on Unasyn and Augmentin electropherogram were obtained. Augmentin and Unasyn preparations consist of antibiotics and β-lactamase inhibitors and do not have any other interfering matrix.

Good repeatability and intermediate precision were shown in independent assays performed by two analysts in different days (Table 3).

Table 1
Quantitative performance test for CE

Parameter	Amoxicillin	Clavulanic acid	Ampicillin	Sulbactam
By-day repeatability	$n = 10$		$n = 9$	
Migration time (RSD%)	0.51, 0.47	0.71, 0.62	0.52, 1.00	0.59, 1.56
Corrected area (RSD%)	1.57, 1.95	1.92, 1.62	1.68, 1.75	2.92, 2.19
Day-to-day repeatability	$n = 20$		$n = 18$	
Migration time (RSD%)	0.58	0.65	0.99	1.61
Corrected area (RSD%)	1.73	1.76	1.79	2.55
Linearity	$r = 0.9998$	$r = 0.9998$	$r = 0.9998$	$r = 0.9998$
y = corrected area	$y = 149670x + 605$	$y = 355656x + 251$	$y = 223790x + 4054$	$y = 97912x + 947$
x = concentration (mg/ml)	$Sxy = 4561$	$Sxy = 5303$	$Sxy = 6470$	$Sxy = 1664$
Range (mg/ml)	0.05–3.08	0.02–2.02	0.05–3.03	0.05–2.08
LOD (μg/ml)	0.4	0.3	0.4	0.7
LOQ (μg/ml)	0.8	0.5	0.8	2.0

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

Preparations	Buffer pH 8.6		Buffer pH 8.7		Buffer pH 8.8	
	tm	Peak area	tm	Peak area	tm	Peak area
Augmentin						
Amoxicillin	6.86 (0.49%)	152 713 (1.09%)	6.94 (0.29%)	151 799 (1.19%)	6.99 (0.92%)	151 265 (0.85%)
Clavulanic acid	8.24 (0.29%)	44 583 (1.19%)	8.29 (0.31%)	43 650 (0.51%)	8.27 (0.36%)	43 588 (0.91%)
Unasyn						
Ampicillin	7.35 (0.31%)	144 378 (1.13%)	7.36 (0.34%)	144 436 (1.57%)	7.27 (0.89%)	143 475 (0.33%)
Sulbactam	8.75 (0.32%)	27 041 (1.04%)	8.75 (0.32%)	26 874 (1.01%)	8.74 (0.26%)	26 474 (1.45%)

Each sample was injected five times RSDs are listed in brackets. Peaks areas were calculated on 1 mg/ml preparations of Augmentin and Unasyn.

Table 3
Determination of active compounds of Augmentin and Unasyn preparations by CE, performed independently by two analysts in two assays

Sample	Augmentin				Unasyn			
	Amoxicillin (%)		Clavulanic acid (%)		Ampicillin (%)		Sulbactam (%)	
	Assay 1	Assay 2	Assay 1	Assay 2	Assay 1	Assay 2	Assay 1	Assay 2
1	75.66	76.05	13.69	14.21	62.50	62.73	30.06	31.07
2	72.65	74.22	13.58	14.00	61.03	64.42	29.52	29.68
3	73.21	75.45	14.08	14.36	62.58	65.50	31.08	30.53
4	74.03	77.39	13.70	14.33	63.65	63.58	31.71	30.00
5	72.91	73.22	13.27	14.48	64.52	65.99	31.14	30.45
6	74.37	73.57	13.52	13.60	64.41	63.98	30.39	29.79
7					63.74	64.81	30.79	30.08
Mean	73.81	74.98	13.26	14.20	63.20	64.43	30.67	30.23
SD	1.12	1.60	0.27	0.31	1.24	1.12	0.74	0.49
RSD	1.52	2.14	1.95	2.18	1.97	1.74	2.40	1.61
Mean from two assays		74.39		13.90		63.82		30.45
SD		1.46		0.39		1.30		0.64
RSD		1.96		2.82		2.04		2.11

SD, standard deviation; RSD, relative standard deviation.

Table 4
Comparison of CE and HPLC for determination of active compounds of Augmentin and Unasyn preparations

Method	Augmentin				Unasyn			
	Amoxicillin		Clavulanic acid		Ampicillin		Sulbactam	
	CE	HPLC	CE	HPLC	CE	HPLC	CE	HPLC
Mean	73.81	73.97	13.12	13.00	1043.42	1046.67	513.13	518.70
SD	1.12	0.73	0.17	0.08	10.45	6.98	6.52	6.47
RSD	1.52	0.99	1.301	0.62	1.00	0.67	1.27	1.25
Student's t-value for $P = 0.05$	0.32		1.69		0.67		1.93	
n	12				14			
t-tabulated	2.179				2.145			

CE, capillary electrophoresis; HPLC, high performance liquid chromatography.

In the following step, comparable assays of examined drugs by CE and HPLC methods were performed (Table 4). Both amoxicillin and clavulanic acid contents in Augmentin as well as ampicillin and sulbactam contents in Unasyn, determined by CE and HPLC have not differed significantly. The Student's t for four compounds, determined by the t-test, were lower than theoretical (tabular) value. HPLC method used routinely for Unasyn assay is also pharmacopeial method [12], but for analysis of Augmentin ini. preparation, only manufacturer HPLC method exists. Parameters of CE and HPLC methods used to assay Augmentin and Unasyn injection drugs, are summarized and compared on Table 5.

Table 5
The comparison of CE and HPLC methods

Method	CE	HPLC	
Parameters		Unasyn	Augmentin
The mobile phase volume	50 ml	500 ml	500 ml
The mobile phase composition	Buffer	Buffer–acetonitrile	Buffer–methanol
The system stabilize time	30 min	2 h	2 h
The migration time	10 min	10 min	10 min
The analysis total time	2.5 h	4 h	4 h
The sample concentration	3 mg/ml	1 mg/ml	1.5 mg/ml

Presented results showed that CE method is a useful alternative to HPLC method to assay ampicillin and amoxicillin together with β-lactamase inhibitor during one analysis. Performed validation also proved that new analytical method is as good as the compendial HPLC technique for pharmaceutical analysis of active compounds in Augmentin and Unasyn preparations for injections.

4. Conclusions

Different retention times of amoxicillin and clavulanic acid peaks, as well as ampicillin and sulbactam peaks during evaluated CE of Augmentin and Unasyn injection preparations ensure good separation and precise, parallel determination of estimated compounds.

Results obtained during this study, and performed validation, enable to use the CE alternatively to HPLC for pharmaceutical analysis of Augmentin and Unasyn preparations for injection.

Taking into consideration the necessity of nature and environmental preservation, the amount of organic solvents waste from LC should be limited. This can be achieved, replacing HPLC method by CE.

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