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Application of capillary electrophoresis to the determination of various benzylpenicillin salts

Genowefa Pajchel*, Katarzyna Michalska, Stefan Tyski

Department of Antibiotics and Microbiology, National Institute of Public Health, 30/34 Chełmska St. 00-725 Warsaw, Poland

Abstract

The application of capillary electrophoresis for separation of benzylpenicillin, procaine, benzathine and clemizole was investigated. Phosphate–borate buffer supplemented with sodium dodecyl sulphate 14.4 g/l and electrophoresis voltage 18 kV seem to provide optimal conditions for micellar electrokinetic chromatographic assay of penicillin salts. This method is selective and precise. The results obtained from CE method recovery assay (above 98% for all but procaine—97% substances) and from determination of benzylpenicillin by CE compared with HPLC results, confirmed good accuracy.

Keywords: Pharmaceutical analysis; Benzylpenicillin; Procaine; Benzathine; Clemizole; Antibiotics

1. Introduction

Benzylpenicillin (penicillin G) is a natural penicillin. In pharmaceutical preparations it is available in the form of potassium or sodium (water soluble), procaine, benzathine or clemizole (insoluble in water) salts (Fig. 1). The method of penicillin G analysis includes microbiological assay, iodometric and potentiometric titrations as well as chromatographic assays [1–3]. So far, capillary electrophoresis method was applied by Zhu et al. [4] for separation of benzylpenicillin and its related substances, by Wan et al. [5] to monitoring process in the manufacturing of semisyntetic penicillins, while Chee and Wan [6] used elctrophoresis for separation of basic substances like procaine. Simultaneous determination of penicillin and procaine or benzathine only by the HPLC method is also feasible [7,8].

The aim of this study was to adapt capillary electrophoresis technique for determination of the following compounds: weakly acidic—benzylpenicillin and basic—procaine, benzathine and clemizole, and also to validate the elaborated method.

2. Experimental

2.1. Instruments

Capillary electrophoresis experiments were carried out on Waters Quanta 4000 E CE System, equipped with 30 kV power supply, and UV spectrophotometric detector connected to data collection system suitable to perform both hydrodynamic and voltage injections. The selected detection wavelength was 214 nm. Separations were performed in 60 cm (52 cm effective length) ×75 μm i.d. fused-silica capillary coated with polyimide (AccuSep capillaries, Waters) thermo regulated at 25 °C, with voltage of 18 kV applied (current about 140 μA). Hydrodynamic injection by gravity-driven siphoning 10 s. was used.

The HPLC assays were carried out on Shimadzu LC-10A system with UV detection. A Lichrospher 100, RP-8, 5 μm (250 mm × 4 mm i.d.) column, detection wavelength 220 nm and flow rate 1 ml/min for penicillin clemizole assay, and YMC-Pack ODS-A, 5 μm, 250 mm × 4.6 mm i.d. for benzylpenicillin potassium assay, according to the European Pharmacopoeia were used.

2.2. Standards and reagents

Standards of benzylpenicillin sodium of activity 1645 IU/mg, clemizol hydrochloride 99.6%, and preparation

* Corresponding author. Tel.: +48-22-841-36-83; fax: +48-22-841-06-52.
E-mail address: tyski@il.waw.pl (G. Pajchel).

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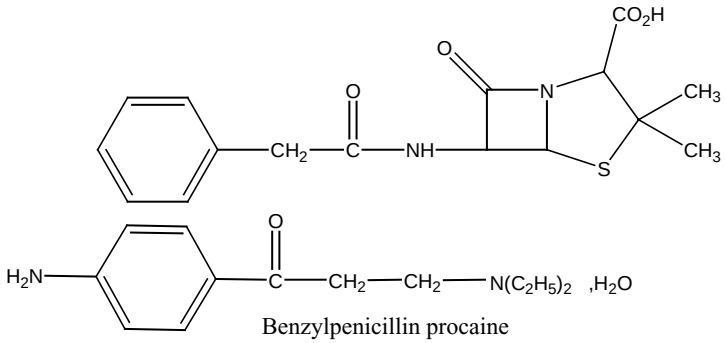
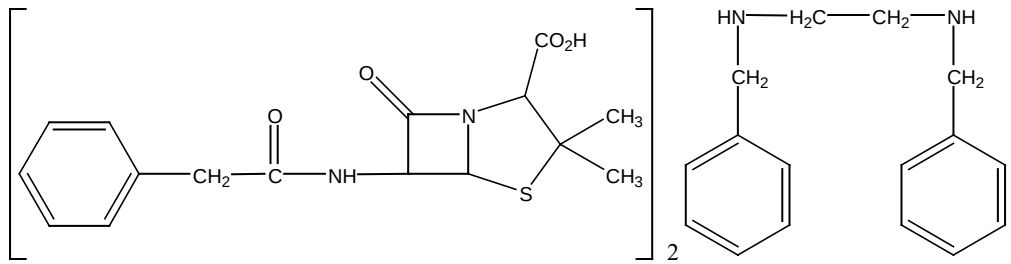
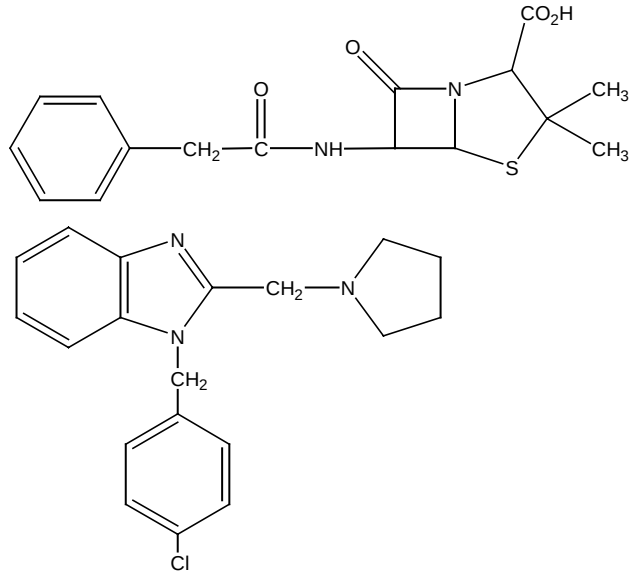


Benzylpenicillin procaine



Benzypenicillin benzathine



Benzylpenicillin clemizole

Fig. 1. Chemical structures of benzylpenicillin salts.

of clemizol–penicillin i.m. 1 000 000 IU/vial were purchased from Grünenthal (Aachen, Germany). Working standards: benzylpenicillin potassium of activity 1570 IU/mg, benzylpenicillin procaine (986.2 IU/mg, containing 39.59% of procaine) and benzylpenicillin benzathine working standard (1183 IU/mg, containing 22.70% of benzathine), as well as raw materials: benzylpenicillin potassium (1581 IU/mg), benzylpenicillin procaine (998 IU/mg, containing 39.47% of procaine) and benzylpenicillin benzathine (1199 IU/mg, containing 24.88% of benzathine) were obtained from Tarchominskie Zakłady Farmaceutyczne Polfa (Warsaw, Poland). Procaine hydrochloride—USP reference standard (100%) (Rockville, MD, USA), benzathine diacetate—Intervet working standard (Boxmeer, The Netherlands), containing 66.1% of benzathine and Biochemie reference substances (Kundl, Austria) of 1-benzylpenilloic acid, 2,6-aminopenicilloic acid, 3-phenylacetic acid, 4-benzylpenillic acid, 5-benzylpenicilloic acid were also used.

Disodium hydrogenphosphate, sodium tetraborate, sodium hydroxide, phosphoric acid were of reagent grade. Sodium dodecyl sulphate (SDS) was from Applichem

(Darmstadt, Germany), methanol HPLC grade from Labscan (Dublin, Ireland). Water used to prepare standard solutions, samples and running buffer was obtained from Labconco System (Kansas City, MO, USA).

The CE electrolyte contained constant concentrations of disodium hydrogenphosphate 3.12 g/l, sodium tetraborate 7.64 g/l and SDS 14.4 g/ml and was adjusted to pH 8.7 with phosphoric acid.

The HPLC mobile phase according to the European Pharmacopoeia for benzylpenicillin potassium assay and 0.067 M phosphate buffer pH 7.0—methanol (6:4) for benzylpenicillin clemizole assay, were used.

Standards and samples for CE and HPLC assays—benzylpenicillin potassium and sodium salts, procaine hydrochloride and clemizol hydrochloride were dissolved with water, while benzylpenicillin procaine, benzylpenicillin benzathine, benzylpenicillin clemizole and benzathine acetate were dissolved in 4 ml of methanol and then diluted with water. The analytes concentration of $a = 0.5$ mg/ml as calculated in relation to penicillin were used in all assays.

3. Results and discussion

3.1. Method development and optimization

Based on our previous studies [9,10], phosphate–borate buffer containing 3.12 g/l disodium hydrogenphosphate and 7.64 g/l sodium tetraborate without SDS and with 14.4 g/l SDS and voltage 18 kV was applied. The first step in the method development process was selection of the optimum pH value and proper choice of capillary electrophoresis mode. Taking into account that some drugs (especially veterinary) contain mixture of penicillin procaine and

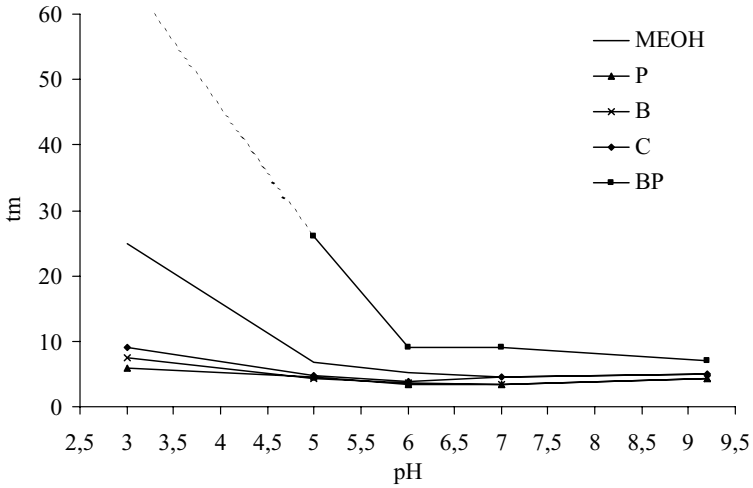


Fig. 2. Dependence of the migration times of basic compounds vs. pH of phosphate–borate buffer. For CE conditions, see Section 2. P, procaine; B, benzathine; C, clemizole; BP, benzylpenicillin; MEOH, methanol.

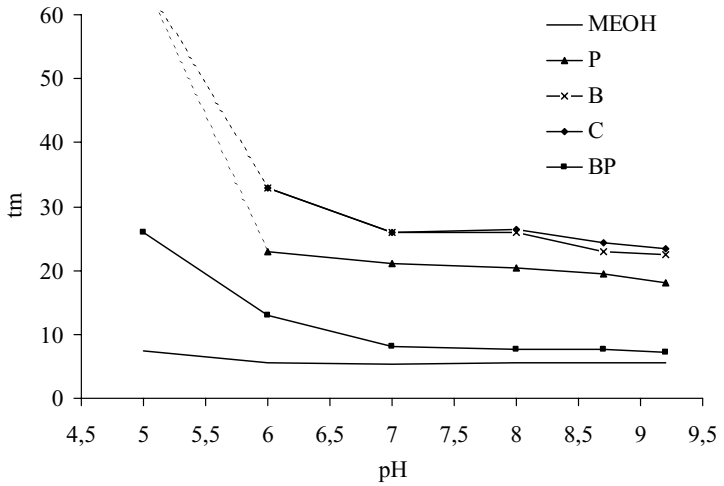


Fig. 3. Dependence of the migration times of basic compounds vs. pH of phosphate–borate buffer supplemented with 14.4 g/l SDS. For CE conditions, see Section 2. P, procaine; B, benzathine; C, clemizole; BP, benzylpenicillin; MEOH, methanol (EOF marker).

benzathine, we considered as very important to achieve good resolution between procaine and benzathine compounds.

The influence of pH on migration times of benzylpenicillin, procaine, benzathine, and clemizole in two electrophoretic systems [capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC)], was examined. Dependence of migration times versus pH in range 3.0–9.2 and 5.0–9.2 in phosphate–borate buffer without and with addition of 14.4 g/l SDS, respectively was examined. In case of CZE (Fig. 2) the migration times of benzylpenicillin were increased with decrease (from 6.0 to 3.0) of pH value of the buffer. In pH range 6.0–9.2 the

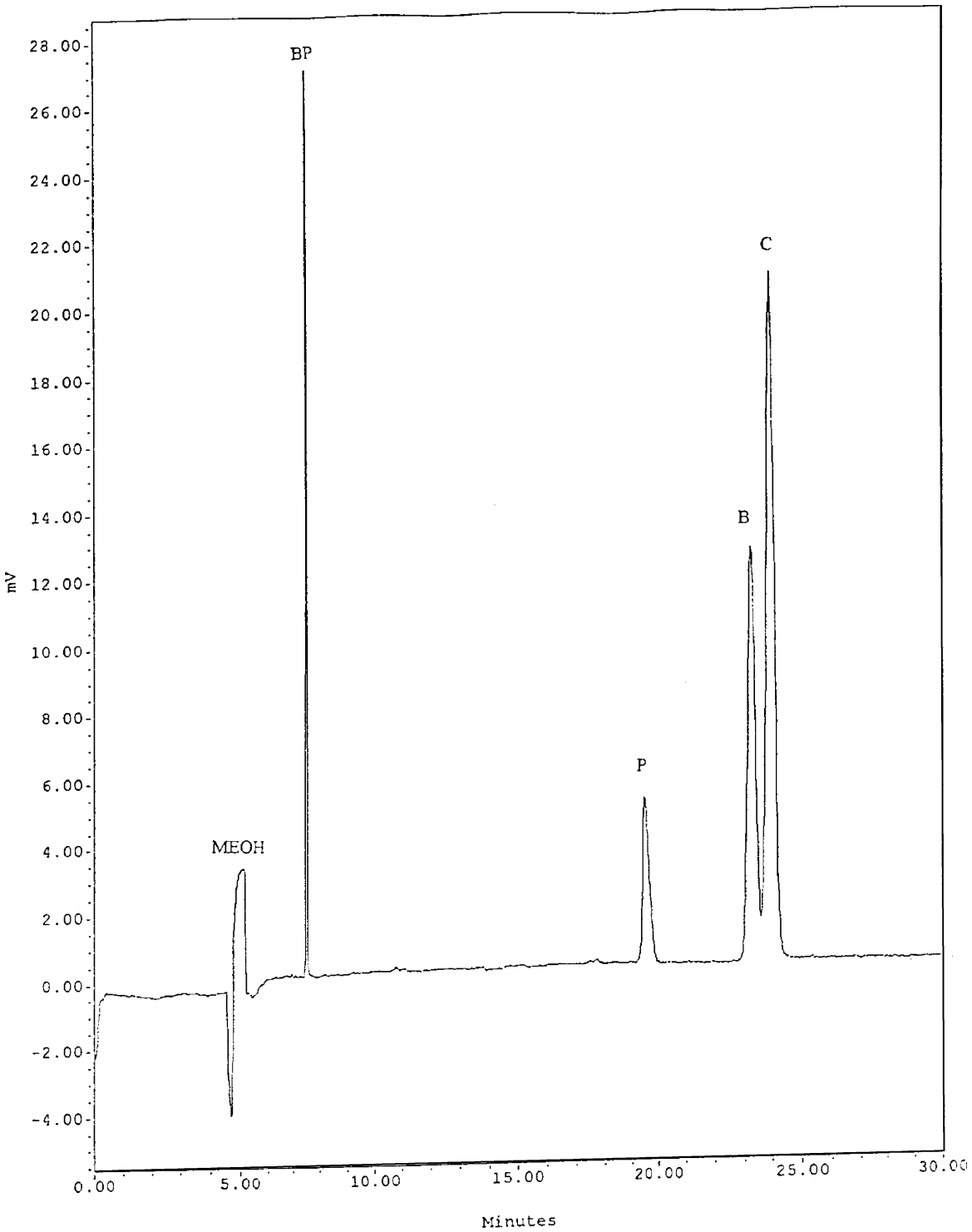


Fig. 4. Electropherogram of benzylpenicillin and procaine, benzathine and clemizole. Buffer phosphate–borate pH 8.7, supplemented with 14.4 g/l SDS. For CE conditions, see Section 2. P, procaine; B, benzathine; C, clemizole; BP, benzylpenicillin; MEOH, methanol (EOF marker).

migration times were almost constant. In the low buffer pH (3.0) the electroosmotic flow (EOF) was fairly slow [migration time of EOF marker—MeOH (methanol) was about 25 min]. All measured migration times of penicillin under these conditions were above 50 min while migration times for basic compounds were shorter then 10 min. In the pH range 6.0–9.2 the electrophoretic mobility of basic compounds was similar to EOF, and tested compounds migrated together. The separation of procaine, benzathine and clemizole was possible only at pH 3. In MEKC (Fig. 3) the migration times of benzylpenicillin also increased with decreasing pH value from 6.0

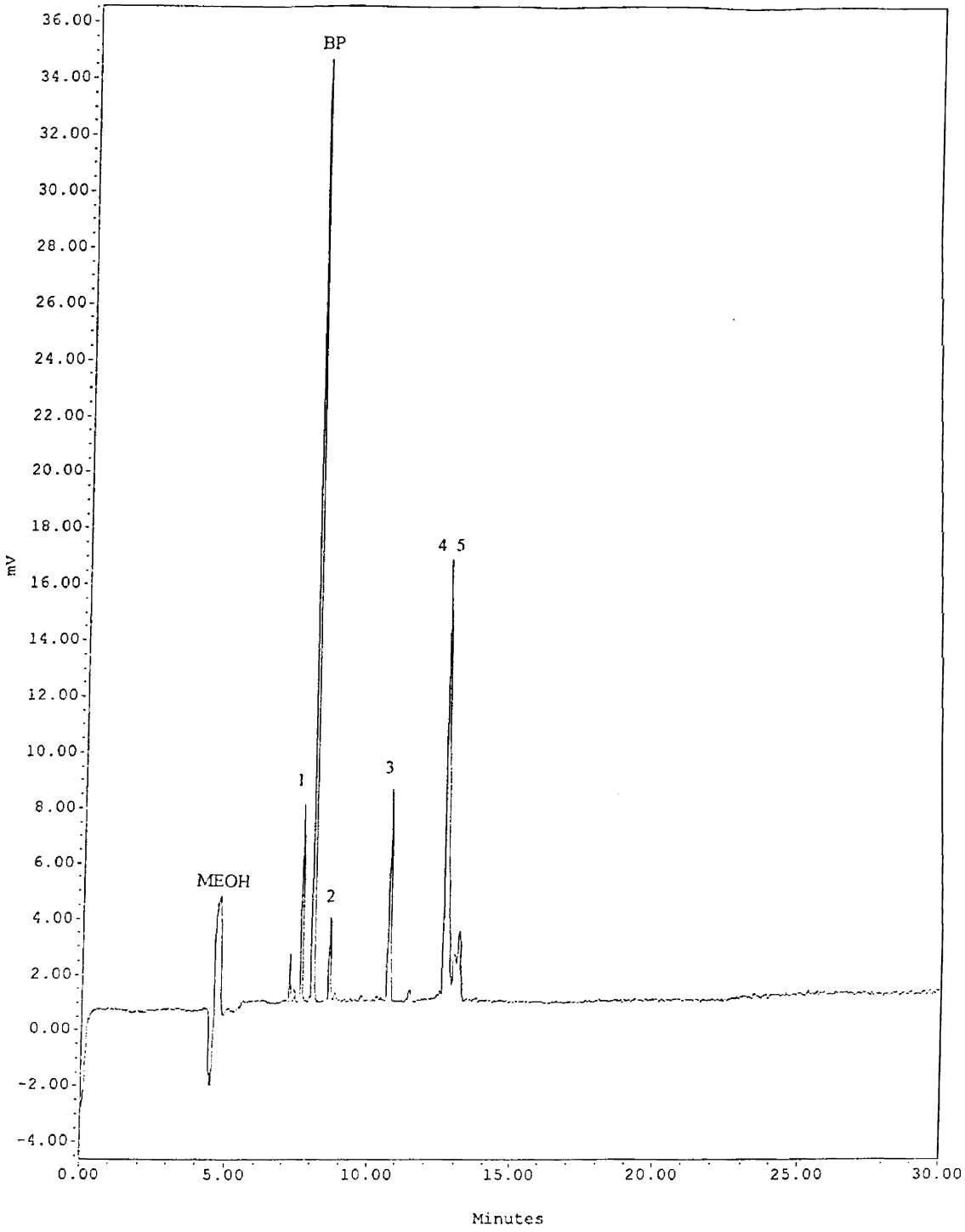


Fig. 5. Electropherogram of benzylpenicillin and related substances. Buffer phosphate–borate pH 8.7, supplemented with 14.4 g/l SDS. For CE conditions, see Section 2: (1) benzylpenilloic acid, (2) 6-aminopenicilloic acid, (3) phenylacetic acid, (4) benzylpenillic acid, (5) benzylpenicilloic acid, (BP) benzylpenicillin, (MEOH) methanol.

Table 1
Quantitative performance for CE

Parameter	Benzylpenicillin	Procaine	Benzathine	Clemizole
Migration time (min)	7.5	18.5	23.5	24.5
Repeatability of migration times (R.S.D., %)	0.79	0.88	0.72	0.77
Repeatability of peaks area (R.S.D., %)	1.60	2.00	1.92	1.06
Linearity				
Correlation coefficient	0.999	0.993	0.994	0.998
Range (mg/ml)	0.01–1.50	0.1–0.45	0.1–0.45	0.005–1.0
LOD (mg/ml)	0.001	0.02	0.02	0.0004
LOQ (mg/ml)	0.0035	0.05	0.05	0.001

Table 2
Intermediate precision, accuracy and results of recovery tests for benzylpenicillin procaine assayed by CE method

	Benzylpenicillin (%)			Procaine (%)		
	Day 1	Day 2	Average recovery (%)	Day 1	Day 2	Average recovery (%)
Sample concentration						
(1/2)*a*	55.21	55.03		37.79	37.80	
(1/2)*a*	55.01	55.11	98.35	38.29	38.01	96.21
a	56.13	55.62		39.10	38.79	
a	55.70	55.87	99.67	39.68	38.40	98.79
2*a*	54.69	55.27	98.16	37.56	38.03	95.76
Mean	55.35	55.38	98.73	38.48	38.21	96.92
R.S.D. (%)	1.031	0.643		2.318	1.024	
CE assay on days 1 and 2						
Mean		55.36			38.35	
R.S.D. (%)		0.810			1.736	
Confidence range		55.36 ± 0.32			38.35 ± 0.48	

Claim: benzylpenicillin 998 IU; Procaine 39.47%.

to 5.0, but much faster then in CZE (the migration time above 50 min was at pH 5). The procaine, benzathine and clemizole migration times were longer when pH value of buffer with 14.4 g/l SDS was decreased and moreover the lack of benzathine and clemizole separation was observed at pH 7.0 and below. Using MEKC, the electrophoretic mobility of these substances was slower then of penicillin. The satisfactory separation of all studied compounds was achived at pH 8.7. For further studies, taking into account resolution and migration times of all compounds, buffer pH 8.7 containing 14.4 g/l SDS was chosen (Fig. 4).

Table 3
Intermediate precision, accuracy and results of recovery tests for benzylpenicillin benzathine assayed by CE method

	Benzylpenicillin (%)			Benzathine (%)		
	Day 1	Day 2	Average recovery (%)	Day 1	Day 2	Average recovery (%)
Sample concentration						
(1/2)*a*	65.85	65.91		24.58	24.68	
(1/2)*a*	65.44	66.16	97.85	24.45	24.64	98.82
a	67.22	67.24		24.86	25.15	
a	67.21	67.20	99.87	25.09	24.84	100.42
2*a*	66.16	65.99	98.19	24.96	25.09	100.58
Mean	66.38	66.50	98.74	24.79	24.88	99.94
R.S.D. (%)	1.216	0.998		1.074	0.934	
CE assay on days 1 and 2						
Mean		66.44			24.83	
R.S.D. (%)		1.053			0.969	
Confidence range		66.44 ± 0.50			24.08 ± 0.17	

Claim: benzylpenicillin 1199 IU; benzathine 24.88%.

Table 4
Assay of benzylpenicillin clemizole by CE and HPLC methods

	CE Benzylpenicillin (IU/vial)		HPLC	CE Clemizole (%)	
	Day 1	Day 2	Day 2	Day 1	Day 2
Sample					
1	1024398	1009018	1004090	53.7	50.6
2	1002288	1030650	1002288	51.8	51.9
3	1028956	1024303	1028956	52.2	52.2
4	1042475	1032643	1042475	52.2	52.5
5	1037917	1024398	1037917	52.5	51.9
Mean	1027207	1024202	1023145	52.5	51.8
R.S.D. (%)	1.524	0.905	1.844	1.383	1.401
CE assay day 1 and 2					
Mean		1025705			52.2
R.S.D. (%)		1.192			1.471
Confidence range		1025705 ± 8747			52.2 ± 0.55
Student's *t*-test for CE and HPLC assays on day 2		0.112			
ttab (0.05, 8)			2.306		

Declared benzylpenicillin 1 000 000 IU. Teoretical content of clemizole 49.35%.

3.2. Performance of the method

To confirm selectivity and specificity of the method, the electrophoresis of related penicillin substances: benzylpenilloic acid, benzylpenillic acid, benzylpenicilloic acid, 6-aminopenicillanic acid and phenylacetic acid was performed. The satisfactory resolution of related substances and benzylpenicillin (Fig. 5) was observed. They were well separated from procaine, benzathine and clemizole which have migration time above 15 min. Specificity of the method appeared to be the poorest for two degradation products—benzylpenillic and benzylpenicilloic acids, with identical migration time. The repeatability of migration times and corrected peak areas for benzylpenicillin, procaine, banzathine and clemizole was additionally determined. Using concentrations higher than 0.5 mg/ml for procaine and benzathine, peaks were too wide and their repeatability was found to be not satisfactory. The R.S.D. for six replicated analyses of examined substances in concentration 0.5 mg/ml calculated in relation to benzylpenicillin is presented in Table 1. The limit of detection (LOD) defined as the lowest concentration of analyte that can be detected was estimated as three times signal-to-noise ratio. The limit of quantification (LOQ) was found as ten times signal-to-noise ratio. The sensitiveness of the method was the best for clemizole, what may be explained by the presence of multiple chromophor groups in the molecule.

The linearity for four compounds was established and presented in Table 1. Calibration curves were constructed based on five different concentrations for each compound. Furthermore, each concentration was injected three times. Very good linearity was observed over range with correlations coefficient 0.999 and 0.998 for benzylpenicillin and clemizole, and was slightly worse ($r = 0.993$ and 0.994) for procaine and benzathine.

Precision of the method was evaluated by repeated analysis of benzylpenicillin and benzylpenicillin salts during two days. The content of both components for each salt was calculated and statistical analysis was performed (Tables 2–4). The accuracy of CE method by recovery characteristic (Tables 2 and 3) or HPLC comparable assay (Tables 4 and 5) was then established. Recovery for all compounds estimated in MEKC system was higher then 98%, except for procaine for which it was 96.92%. Determination of benzylpenicillin by CE and HPLC methods showed no significant differences between both methods. In case of clemizole precision only at the level of R.S.D. = 1.524% and 0.905% was es-

Table 5
Assay of benzylpenicillin potassium by CE and HPLC method

	Benzylpenicillin (IU/mg)		
	CE assay		HPLC assay
	Day 1	Day 2	
Sample concentration			
(1/2)*a*	1564	1553	1564
(1/2)*a*	1554	1551	1568
a	1558	1583	1581
a	1563	1576	1580
2*a*	1581	1558	1561
2*a*	1575	1539	1542
Mean	1566	1560	1566
R.S.D. (%)	0.655	1.057	0.917
CE assay on days 1 and 2			
Mean		1563	
R.S.D. (%)		0.896	
Confidence range		1563 ± 8.96	
Student's test for CE and HPLC assays on day 2		0.672	
ttab (0.05, 10)			2.228

Claim: 1581 IU.

tablished, but accuracy was not calculated because of lack of a comparative method to assay this component.

4. Conclusion

The MEKC technique appeared to be suitable for the separation of benzylpenicillin from procaine, benzathine and clemizole. The results obtained proved, that this method is valuable to assay benzylpenicillin and its salts.

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