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# Analysis of phenoxymethylpenicillin potassium by capillary electrophoresis

Genowefa Pajchel *, Katarzyna Michalska, Stefan Tyski

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

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**Abstract**

The application of capillary electrophoresis for separation of penicillin V and its impurities was investigated. The phosphate–borate buffer supplemented with sodium dodecyl sulfate (SDS) 20.0 g/L (69 mM) and pentanesulfonic acid sodium salt (PS) 2.2 g/L (12.5 mM) adjusted to pH 6.3, and current voltage 15 kV seem to provide optimal conditions for this aim. The resolution between penicillin V and each impurity was very good. The statistical analysis of phenoxymethylpenicillin V assay showed no significant differences between the results obtained by CE and HPLC methods.


*Keywords:* Pharmaceutical analysis; Phenoxymethylpenicillin; Capillary electrophoresis

## 1. Introduction

Capillary electrophoresis is a modern and advantageous tool in the chemical analysis of pharmaceuticals. In such analysis, determination and identification of impurities are very important aspect in order to fulfill the requirements of European Pharmacopoeia (Eur. Ph.). Properly performed analysis is also essential in the quality control of drugs on the market.

Phenoxymethylpenicillin potassium (penicillin V, pen. V) is a natural penicillin produced during the growth of certain strains of *Penicillium notatum* or *Penicillium chrysogenum* in the presence of appropriate side chain precursor (phenoxyacetic acid) or by acylation of 6-aminopenicillanic acid. The biosynthesis products, besides main component—penicillin V, may contain some degradation and by-products [1]. This β-lactam antibiotic, suitable for oral administration, known from many years is still effective and recommended for treatment of bacterial infections caused by susceptible microorganisms. In Eur. Ph. penicillin V monograph, six impurities are mentioned: benzylpenicillin (imp. A, pen. G), phenoxyacetic acid (imp. B), 6-aminopenicillanic acid (imp. C), 4-hydroxyphenoxymethylpenicillin (imp. D), penicilloic (imp. E) and penilloic acids (imp. F) of phenoxymethylpenicillin. According to Eur. Ph. monograph, phenoxymethylpenicillin impurities profile is established by the gradient HPLC method [2].

The aim of this study was to adapt MEKC technique elaborated in our laboratory to assay some β-lactam antibiotics for the separation and determination of phenoxymethylpenicillin and its related substances and compared MEKC with HPLC method described in Eur. Ph. [2].

## 2. Experimental

### 2.1. Apparatus

CE experiments were carried out on Waters Quanta 4000 CE system (Waters, Milford, MA, USA) equipped with 30 kV power supply, UV spectrophotometric detector connected to 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 (60 cm × 75 μm I.D., 52 cm from inlet to detector) AccuSep (Waters) at 25 °C with a voltage of 15 kV applied. Hydrodynamic 10 s injection was performed.

* 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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The HPLC experiments were carried out on chromatograph series LC-10Avp Shimadzu (Kyoto, Japan) with detector set at 254 nm. Separation was achieved on μBondapak HPLC column, 10 μm particle size/300 mm × 3.9 mm (Analysentechnik, Mainz, Germany)

### 2.2. Standards and reagents

Phenoxymethylpenicillin potassium standard (90.2%), and its impurities standards: (F) phenoxymethylpenilloic acid (99.7%), (E) phenoxymethylpenicilloic acid (90%), (B) phenoxyacetic acid (99.3%), (C) 6-aminopenicillanic acid (99.2%) and (D) 4-hydroxyphenoxymethylpenicillin potassium (96.8%) were obtained from Sandoz (Sandoz GmbH, Kundl/Tyrol, Austria). Phenoxymethylpenicillin potassium substances (91.1%) from TZF (Warsaw, Poland) produced in 2002 ("new"), phenoxymethylpenicillin potassium, origin unknown, produced in 1984 ("old"), and benzylpenicillin potassium from TZF (Warsaw, Poland) were used.

Disodium tetraborate decahydrate, sodium dihydrogen phosphate, potassium dihydrogen phosphate, potassium hydroxide were of analytical reagent grade from POCH (Gliwice, Poland). Orthophosphoric acid (85%) was purchased from Fluka (Buchs, Switzerland), pentane-1-sulfonic acid sodium salt from Merck (Darmstadt, Germany), sodium dodecyl sulfate from Applichem (Applichem GmbH, Darmstadt, Germany), methanol HPLC grade from Labscan Analytical Science (Dublin, Ireland). Water used for dissolution mixture, running buffer and mobile phases was obtained from Labconco System (Kansas City, MO, USA).

The MEKC electrolyte contained 3.12 g/L sodium dihydrogen phosphate, 7.64 g/L disodium tetraborate (0.02 M borate–phosphate buffer) with addition of 14.4–28.8 g/L (50–100 mM) of SDS and 2.2 g/L (12.5 mM), 4.4 g/L (25 mM) or 8.8 g/L (50 mM) of PS, adjusted to pH 6.0–8.7 with phosphoric acid or sodium hydroxide. The HPLC mobile phases and dissolution mixture were prepared as described in the phenoxymethylpenicillin potassium Eur. Ph. monograph [2].

All references and sample solutions for CE and HPLC experiments were dissolved in dissolution mixture.

## 3. Results and discussion

For the separation of penicillin V and related substances, MEKC with UV detection as well as CE methods with UV and mass spectrometric detection were developed [1,3]. In MEKC method, buffer containing 40 mM sodium dihydrogen phosphate and 100 mM SDS, adjusted to pH 7.0, were applied [3]. Aqueous and non-aqueous electrolyte systems containing 20 mM ammonium acetate were investigated in CE method [1].

Previously, we elaborated MEKC method using 0.02 M borate–phosphate buffer with addition of 14.4 g/L (50 mM) SDS at pH 8.7, current voltage 18 kV and temperature 25 °C for the determination of some penicillins [4,5]. Therefore, based on above experience, an attempt was made to use the same buffer with SDS for the separation and assay of phenoxymethylpenicillin potassium and its impurities. The specificity of elaborated method was quite good. Peaks of related substances did not comigrate with mother substances, however, the separation of imp. A, imp. F and imp. C was not satisfactory. It was assumed that these peaks were critical for proper separation. The separation factor in MEKC depends on the molecular structure of micelle, the pH of solution, and the addition of organic modifiers [6–8]. Taking above into account, the influence of running buffer pH, concentration of SDS, changes of current voltage and temperature on the resolution of examined substance were investigated.

During preliminary experiments, we ascertained that nature of impurities E and F depending on electrophoretic conditions and injections' repeats, and they were divided into two peaks each. It might be explained that these decomposition products of penicillin V create two isomers (5*R*, 6*R*) and (5*S*, 6*R*) of phenoxymethylpenicilloic acid (imp. E) as well as (5*R*, 5*S*) phenoxymethylpenilloic acid (imp. F) [3].

Two concentrations of SDS in buffer: 20.0 g/L (69 mM) and 28.8 g/L (100 mM) without the change of pH value, were introduced. When 20.0 g/L (69 mM) SDS was added, the migration times of all substances were longer and the resolution between pen. V and pen. G peaks was better. The imp. F presented two well-separated peaks, one of them migrated along with imp. A (pen. G), the other migrated in double peak with imp C. Imp. E created two peaks not completely separated to base line. After enhancing of SDS concentration to 28.8 g/L (100 mM), the resolution between imp. C and second imp. F peak was satisfactory, however, the first imp. F peak migrated with pen. G and also imp. B migrated together with pen. V. These conditions did not improve selectivity, so finally we return to 20.0 g/L (69 mM) SDS. The effect of temperature on the selectivity was investigated additionally at 35 °C. Increase of temperature resulted in decrease of migration time, due to lower electrolyte viscosity, and increase of electroosmotic flow (EOF). The migration time was about 8.7 min for penicillin V and about 7.9 min for penicillin G. Total analysis duration time was 11 min. The resolution between imp. F second peak and imp. C was better, although imp. F first peak co-migrated with penicillin G. The imp. E peaks were well separated. The change of current voltage from 18 to 15 kV at 25 °C led to lengthen of migration times and better separation between pen. V peak, second imp. F peak and imp. C peak, but not between pen. G and first imp. F peaks. In the conditions described above, the peaks order on electropherograms was the same: imp. D, imp. A + one peak of imp. F, imp. C, two peaks of imp. F, pen. V, imp. B and two peaks of imp. E.

In further experiments, the pH variation from 8.7 to 6.0 of buffer containing 20.0 g/L SDS (69 mM) was introduced. The electrophoresis was performed at 25 °C and 15 kV. The best peaks resolution in buffer adjusted to pH 6.3 was obtained. In this conditions, migration times ($t_m$) were longer, pen. G $t_m$ = 11.8 min and pen. V $t_m$ = 13.6 min, but better sep-

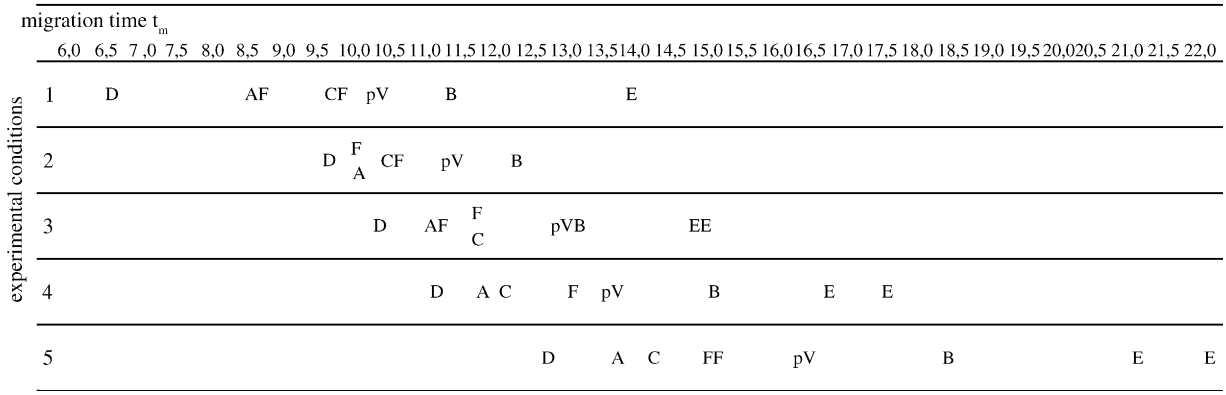


Fig. 1. Migration times dependence on electrophoretic conditions: 0.02 M phosphate–borate buffer; (1) pH 8.7, 69 mM SDS, 18 kV, 25 °C; (2) pH 8.7, 69 mM SDS, 18 kV, 35 °C; (3) pH 8.7, 69 mM SDS, 15 kV, 25 °C; (4) pH 6.3, 69 mM SDS, 15 kV, 25 °C; (5) pH 6.3, 69 mM SDS, 12.5 mM PS, 15 kV, 25 °C; (A) penicillin G; (B) phenoxyacetic acid; (C) 6-aminopenicillanic acid; (D) 4-hydroxyphenoxymethylpenicillin; (E) penicilloic acid of phenoxymethylpenicillin; (F) penilloic acid of phenoxymethylpenicillin.

aration of all peaks—impurities and penicillin V, was obtained. However, good repeatability of migration times and peaks area could not be achieved. In our previous experiments on cephalosporins separation [9], better selectivity and good repeatability using pentane-1-sulfonic acid were received. Then, the influence of PS supplementation: 50, 25 and 12.5 mM, of the buffer containing 20.0 g/L (69 mM) SDS, adjusted to pH 6.3 at 25 °C and 15 kV, were examined. Generally, PS addition extended migration times of pen. V and its impurities and electrophoresis performed in the buffer with higher amount of this reagent led to worse separation between pen. G and imp. C peaks. They migrated together in buffer containing 50 mM of PS while were partly or completely separated in buffer supplemented with 25 or 12.5 mM of PS, respectively. The imp. F was partly separated into two peaks and had higher $t_m$ than imp. C. The imp. E presented two peaks. The migration order was as follows: imp D, imp. A, imp. C, imp. F, pen. V, imp. B, and imp. E. In Fig. 1, the influence of different electrophoresis conditions on migration times is presented. The electrophoretic conditions: 0.02 M borate–phosphate buffer supplemented with 20.0 g/L (69 mM) SDS and 12.5 mM PS, pH 6.3, applied current voltage 15 kV at 25 °C were selected as the best compromise between selectivity and stability of migration times and peaks area. The electropherogram of mixture: pen V (4 mg/ml) and impurities A–C, E and F (0.04 mg/ml) and impurity D (0.16 mg/ml) are presented in Fig. 2. The analysis duration time in chosen condition was 20–25 min.

The quantitative aspects of this method were examined. The linearity was estimated by analysing penicillin V and impurity standards. In this study, five concentrations were chosen ranging from 0.02 to 4.5 mg/ml for penicillin V and from 0.02 to 0.2 mg/ml for each impurity. Each sample concentrations were injected twice. The correlation coefficients for all analysed substances were calculated. The detection limit (LOD) and quantification limit (LOQ) were defined as signal to noise ratio of 3:1 and 10:1, respectively. They were calculated according to the formulas: $3c/(h/2H)$ and $10c/(h/2H)$, where $c$ is a concentration (mg/ml), $h$ is an analyte peak height and $H$ is noise signal height. Instrumental precision was calculated from five consecutive injections of mixture: pen. V (4 mg/ml) and impurities A–C, E and F (0.04 mg/ml) and im-

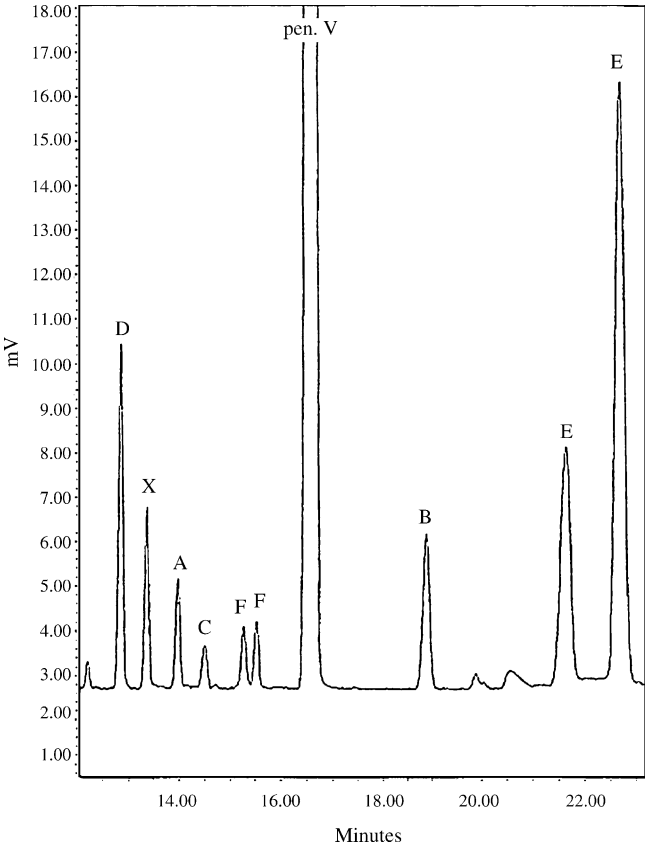


Fig. 2. Electropherogram of penicillin V and its impurities mixture: 0.02 M phosphate–borate buffer, pH 6.3, supplemented with 69 mM SDS and 12.5 mM PS, 15 kV, 25 °C; (A) penicillin G; (B) phenoxyacetic acid; (C) 6-aminopenicillanic acid; (D) 4-hydroxyphenoxymethylpenicillin; (E) penicilloic acid of phenoxymethylpenicillin; (F) penilloic acid of phenoxymethylpenicillin.

Table 1
The method validation results

| Parameter | Imp. D | Imp. A | Imp. C | Imp. F | Pen. V | Imp. B | Imp. E |
|---|---|---|---|---|---|---|---|
| Migration time (min) | 12.7 | 13.7 | 14.3 | 15.0, 15.2 | 16.3 | 18.5 | 21.1, 22.1 |
| Repeatability of migration time (RSD%) | 1.96 | 2.11 | 1.60 | 1.86, 2.48 | 1.68 | 2.84 | 3.21, 3.44 |
| Repeatability of corrected area (RSD%) | 2.30 | 2.49 | 2.96 | 2.58 | 1.61 | 2.62 | 6.11 |
| Correlation coefficient | 0.9998 | 0.9999 | 0.9998 | 0.9999 | 0.9999 | 0.9999 | 0.9998 |
| LOQ (μg/ml) | 5 | 4 | 7 | 7 | 5 | 3 | 7 |
| LOD (μg/ml) | 1 | 1 | 2 | 2 | 2 | 1 | 2 |
| RRF | 0.64 | 0.79 | 0.46 | 0.86 | 1 | 1.64 | 0.97 |
| 1/RRF | 1.56 | 1.27 | 2.17 | 1.16 | 1 | 0.61 | 1.03 |

purity D (0.16 mg/m). The data concerning method validation are summarized in Table 1.

According to the current Eur. Ph. requirements, each impurity excluding imp. D is calculated in reference to penicillin V diluted solution. The content of impurity D is compared with its reference substance. In the following steps of the study, the reciprocal response factor (1/RRF) was calculated, to find the dependence response of impurity peak area on penicillin V peak area. Relative response factor (RRF) was determined by calculation of ratio between the average re-

Table 2
Determination of penicillin V by CE and HPLC methods

| Sample | Penicillin V "old" (%) | | | | Penicillin V "new" (%) | |
|---|---|---|---|---|---|---|
| | CE | | HPLC | | CE | HPLC |
| | 1 Day | 2 Days | 1 Day | 2 Days | 1 Day | 1 Day |
| 1 | 92.52 | 90.39 | 91.74 | 91.76 | 89.17 | 87.43 |
| 2 | 90.66 | 92.28 | 91.56 | 91.24 | 89.48 | 91.83 |
| 3 | 91.33 | 93.73 | 90.72 | 91.91 | 89.17 | 90.54 |
| 4 | 90.26 | 90.41 | 91.49 | 91.97 | 88.64 | 88.74 |
| 5 | 90.84 | 90.87 | 91.23 | 91.26 | 89.4 | 90.73 |
| 6 | 91.63 | 89.01 | 91.35 | 91.37 | 89.15 | 88.94 |
| Mean | 91.21 | 91.12 | 0.254 | 91.59 | 89.17 | 89.70 |
| SD | 0.806 | 1.656 | 0.354 | 0.333 | 0.293 | 1.610 |
| RSD% | 0.884 | 1.817 | 0.388 | 0.364 | 0.329 | 1.809 |
| Mean from two assays | | 91.16 | | 91.47 | | |
| SD | | 1.242 | | 0.350 | | |
| RSD% | | 1.363 | | 0.383 | | |
| Comparison between CE and HPLC methods | | | | | | |
| $P = 0.05$; $n - 2 = 10$; $t_{tabulated} = 2.228$ | | | 0.390 | 0.681 | | 0.752 |

Table 3
Determination of penicillin V "old" purity by CE and HPLC methods

| | Impurity D (%) | | | Impurity F (%) | | | Impurity B (%) | | | Impurity E (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 Day | 2 Days | 3 Days | 1 Day | 2 Days | 3 Days | 1 Day | 2 Days | 3 Days | 1 Day | 2 Days | 3 Days |
| CE | | | | | | | | | | | | |
| 1 | 1.89 | 1.79 | 1.82 | 0.7 | 0.56 | 0.58 | 0.39 | 0.36 | 0.35 | 0.84 | 0.75 | 0.92 |
| 2 | 1.81 | 1.83 | 1.9 | 0.58 | 0.55 | 0.62 | 0.35 | 0.36 | 0.36 | 0.82 | 0.76 | 0.92 |
| 3 | 1.86 | 1.81 | 189 | 0.59 | 0.57 | 0.26 | 0.36 | 0.36 | 0.36 | 0.88 | 0.73 | 0.85 |
| Mean ($n = 9$) | | | 1.84 | | | 0.59 | | | 0.36 | | | 0.83 |
| SD | | | 0.041 | | | 0.046 | | | 0.012 | | | 0.071 |
| RSD% | | | 2.243 | | | 7.81 | | | 3.24 | | | 8.58 |
| HPLC | | | | | | | | | | | | |
| 1 | 1.77 | 1.68 | 1.85 | 0.63 | 0.63 | 0.71 | 0.18 | 0.18 | 0.24 | 0.8 | 0.78 | 0.9 |
| 2 | 1.8 | 1.71 | 1.91 | 0.68 | 0.67 | 0.7 | 0.24 | 0.23 | 0.22 | 0.89 | 0.81 | 0.93 |
| 3 | 1.83 | 1.69 | 1.87 | 0.71 | 0.7 | 0.69 | 0.22 | 0.22 | 0.22 | 0.83 | 0.86 | 0.96 |
| Mean ($n = 9$) | | | 1.79 | | | 0.68 | | | 0.22 | | | 0.86 |
| SD | | | 0.083 | | | 0.0312 | | | 0.022 | | | 0.062 |
| RSD% | | | 4.632 | | | 4.592 | | | 10.16 | | | 7.21 |

sponse of each impurities and average peak of penicillin V in regard to concentrations (Table 1). The calculated value of 1/RRF for impurities D, B and C (about 1.56, 0.61 and 2.17, respectively) considerably differed from value 1.0 accepted for penicillin V. For these impurities, 1/RRF estimated from HPLC assay were 2.14, 0.41 and 2.0 and also differed from 1.0.

During next experiments, comparable analysis of two penicillin V raw materials, which were produced 2 and 20 years ago, by both elaborated MEKC and HPLC, according to Eur. Ph. were performed. For determination of penicillin V content, solutions of samples and reference standard containing 1 mg/ml were used. The results obtained by CE and by HPLC were not significantly different; $t_{calculated}$ was lower than $t_{tabulated}$ for the two substances analysed by both methods. Also the within-day precision (repeatability) and between-days precision (reproducibility) were satisfactory, in case of the "old" penicillin during independent assays performed by two analysts in different days (Table 2). Then, the purity of "old" penicillin V by MEKC and by HPLC method was estimated. Electropherogram and chromatogram from above assays are presented in Figs. 3 and 4. Penicillin V "old" sample was chosen for the purity assay, because the screening examination showed high purity

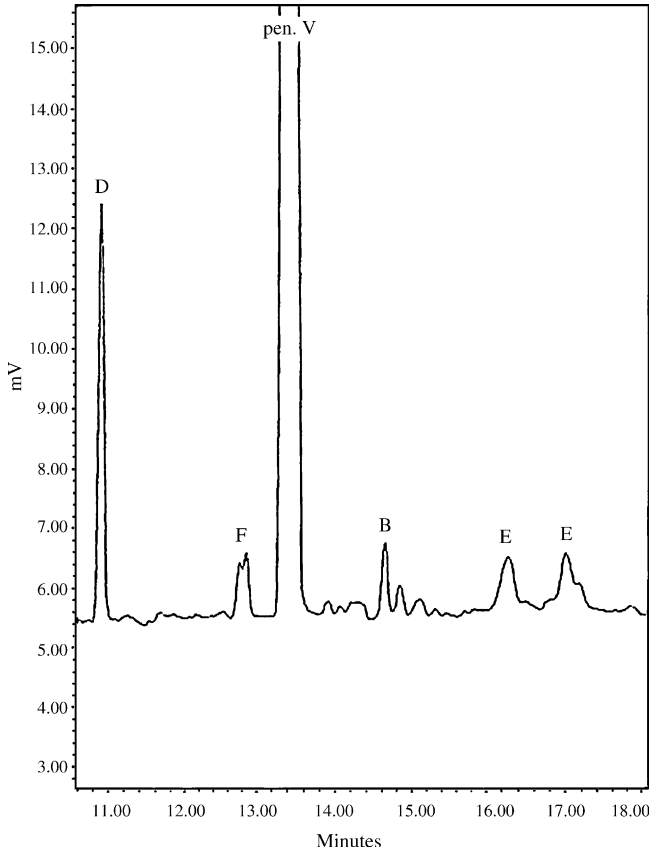


Fig. 3. Electropherogram of penicillin V "old": 0.02 M phosphate–borate buffer, pH 6.3, supplemented with 69 mM SDS and 12.5 mM PS, 15 kV, 25 °C; (D) 4-hydroxyphenoxypenicillin; (E) penicilloic acid of phenoxymethylpenicillin; (F) penilloic acid of phenoxymethylpenicillin.

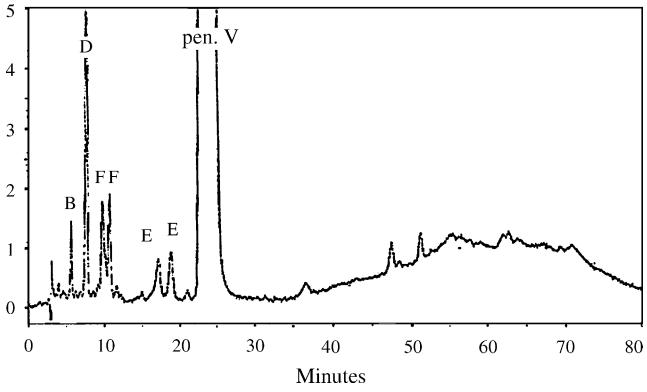


Fig. 4. Chromatogram of penicillin V "old": conditions according to Eur. Ph. [2]; (D) 4-hydroxyphenoxymethylpenicillin; (E) penicilloic acid of phenoxymethylpenicillin; (F) penilloic acid of phenoxymethylpenicillin.

Table 4
Determination of imp. D by CE and HPLC methods as against 4-hydroxyphenoxymethylpenicillin standard

| Sample | Imp. D (%) | |
|---|---|---|
| | CE | HPLC |
| 1 | 2.77 | 2.97 |
| 2 | 2.85 | 2.84 |
| 3 | 2.81 | 2.73 |
| 4 | 2.87 | 2.81 |
| 5 | 2.87 | 2.88 |
| 6 | 2.82 | 2.91 |
| Mean | 2.83 | 2.85 |
| SD | 0.039 | 0.083 |
| RSD% | 1.39 | 2.92 |
| Comparison between CE and HPLC methods $P = 0.05$; $n - 2 = 10$; $t_{tabulated} = 2.228$ | | 0.534 |

of the "new" one. For this assay, concentrations of sample (4 mg/ml), phenoxymethylpenicillin potassium standard (0.04 mg/ml) and 4-hydroxyphenoxymethylpenicillin standard. (0.02 mg/m) were prepared. The purity analysis was performed during 3 days with the usage of three samples every day. The results obtained in both methods are comparable (Table 3). From these data, we could conclude that MEKC method is suitable for evaluation of impurities content in the same manner like described in Eur. Ph. monograph against the diluted standard penicillin V. The results of imp. D determination were also satisfactory. Since $t_{calculated} < t_{tabulated}$, there is no significant difference (at confidence limits) between the results obtained with the two methods (Table 4).

## 4. Conclusions

The elaborated MEKC method is suitable not only for the penicillin V assay but also for the estimation the impurities level in the same manner like in Eur. Ph. MEKC may be a valuable alternative technique to HPLC in the analysis of phenoxymethylpenicillin.

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