

Development of a Separation Method of Four Penicillin Derivatives by Capillary Electrophoresis

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Introduction: The objective of this paper is the development and optimization of a capillary electrophoresis method, which allows the separation of four frequently used penicillin derivatives (amoxicillin, ampicillin, benzilpenicillin and oxacillin), with possible application in the analysis of environmental samples.

Material and method: In our experiments we worked on water solutions of the studied penicillins. The analysis was performed on an Agilent Capillary Electrophoresis System with a diode array detector. The data were recorded and processed by Chemstation software.

Results: Different buffer solutions were tried out in order to reach the most efficient separation of the studied compounds. The influence of different analytical parameters was evaluated by varying the buffer concentration, buffer pH, voltage, temperature, injection time and pressure. The analytical performance of the method was verified, in order to estimate reproducibility and sensitivity.

Conclusions: A micellar electrokinetic capillary chromatography method has been developed for the separation of the four penicillins. We obtained the best results with a buffer solution containing 25 mM sodium tetraborate and 100 mM sodium dodecyl sulfate (pH = 9.3), the separation being achieved in approximately 5 minutes.

Keywords: amoxicillin, ampicillin, benzilpenicillin, oxacillin, capillary electrophoresis

Introduction

In the last decade, the environmental chemistry studies focused on the identification and separation of the polluting pharmaceutical substances, especially antibiotics, compounds with important biological effects. These substances reach different compartments of the environment (water, soil) as metabolites, conjugated and/or unmetabolized forms. The main pollution source is represented by hospitals (about 40% of the pollutants). As their metabolic transformation has a rate of about 50–60%, these compounds reach the environment in a relatively high quantity [1].

Taking into account that beta-lactams are still the most frequently used antibiotics in the human and veterinary therapy, the aim of the paper was to elaborate a separation technique suitable for the identification and separation of four extensively prescribed penicillin derivatives: amoxicillin, ampicillin, benzylpenicillin and oxacillin, with possible

further application in environmental analysis. The chemical structure of the studied penicillin are presented in figure 1.

For this purpose we chose capillary electrophoresis, a modern method of analysis which allows a fast and sensitive separation and doesn't require high quantity of samples [2,3]. This analytical method is based on a relatively simple principle: the different migration of the electrically charged particles in the running buffer solution, induced by an electric field. The separation of the compounds is due to the difference between the electrophoretic mobilities of the analytes. Since penicillins have similar electrophoretic mobilities and structural characteristics, an efficient separation by the conventional capillary zone electrophoresis (CZE) is difficult to achieve. Micellar electrokinetic chromatography (MEKC) proved to be the suitable option for the separation of the analyzed penicillins, providing a secondary separation through the addition of a surfactant that forms into micelles. MEKC is based on a micellar "pseudostationary" phase added above its critical micellar concentration (CMC) to the buffer solution, which interacts with the analytes according to partitioning mechanism, in a chromatography-like mode, the electroosmotic flow (EOF) acting as the chromatographic "mobile phase" [4,5,6,7,8].

Material and method

The penicillin derivatives (amoxicillin trihydrate, ampicillin trihydrate, benzilpenicillin sodium, oxacillin sodium monohydrate) were purchased from SC Antibiotice SA (Iaşi, Romania). During the experimental work the following reagents were used: boric acid, sodium tetraborate, sodium dodecyl sulfate (Merck, Germany), sodium hydroxide solution 0.1 N (Agilent). All reagents used

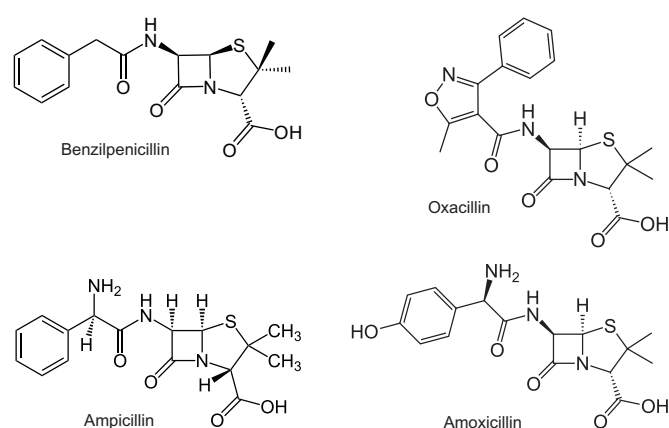


Fig. 1. Penicillin structures

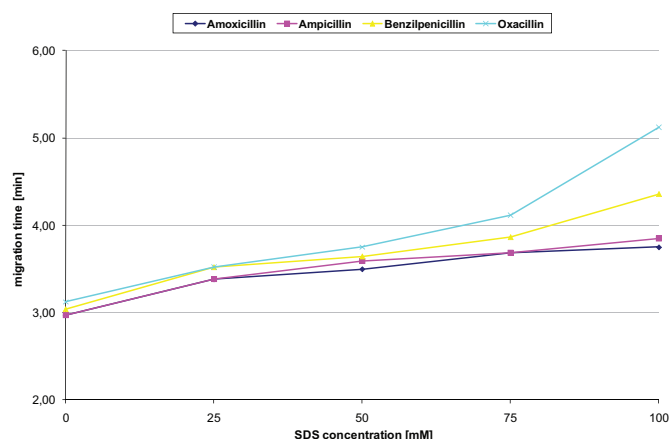


Fig. 2. SDS concentration – migration time

were of analytical grade. The deionized water was prepared with a Milli-Q system (Millipore). Penicillin stock solutions were prepared in water at concentrations of 1 mg/ml and later diluted to the appropriate concentrations. The antibiotic solutions were stored at 2–8°C between measurements.

The CE experiments were conducted using an Agilent 6100 capillary electrophoresis system and data were recorded and processed by use of Chemstation software version 7.01 (Agilent). In all measurements hydrodynamic sample injection was used, by applying a pressure of 30 mbar for 5 seconds, the sample solutions being introduced at the anodic end of the capillary. Separations were performed using a fused-silica capillary of 56 cm × 50 µm I.D. (effective length: 48 cm) (Agilent). The applied voltage was +25 kV; the current was kept below 200 µA. The temperature of the capillary was kept at 25°C. Detection of the analytes was performed using a photodiode array detection system set to 210 nm and 220 nm. The pH of the buffer solutions was determined with the Terminal 740 pH-meter (Inolab). At the beginning of each day the capillary was conditioned with NaOH 0.1 N for 15 minutes and buffer solution for another 5 minutes.

Results

Different buffers solutions were tried out in order to establish the proper buffer solution. Using different con-

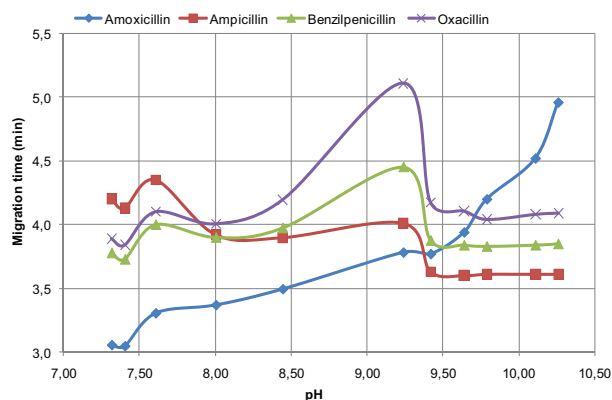


Fig. 4. pH influence

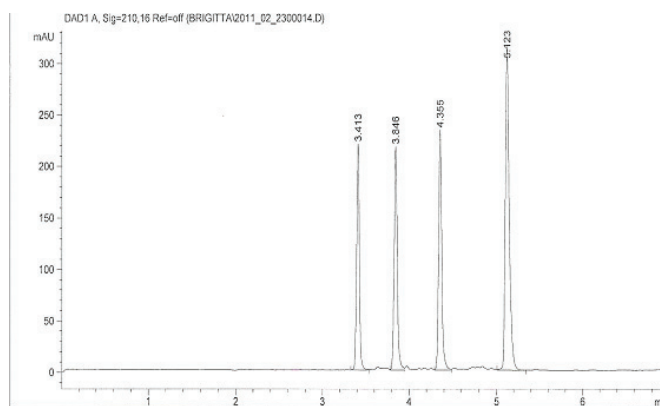


Fig. 3. Electropherogram

centrations (between 25 mM and 100 mM) of sodium tetraborate as running buffer, the separation of the four compounds was inefficient. The migration times of the two aminopenicillin derivatives (amoxicillin and ampicillin) were very close, so these compounds couldn't be separated. Growing the content of sodium tetraborate in the buffer solution doesn't improve the efficiency of the separation but increases the migration times.

Subsequent experiments were performed using a 25 mM sodium tetraborate buffer and adding sodium dodecyl sulfate to the solution (between 25 mM and 100 mM). In the presence of the surfactant, the separation of the penicillins was significantly improved (Figure 2). The best results were obtained with a buffer solution containing 25mM sodium tetraborate and 100 mM sodium dodecyl sulfate.

The electropherogram recorded using the selected buffer solution is presented in Figure 3, the order of migration being: amoxicillin, ampicillin, benzilpenicillin, oxacillin. The separation has been achieved in approximately 5 minutes.

In order to optimize the analytical conditions, we studied the influence of the applied voltage and temperature on the separation. The increase of the voltage results in the decrease of the migration times. The increase of the temperature has a similar effect. The modification in the injection parameters (pressure and injection time) has little influence on the migration times. The following analytical

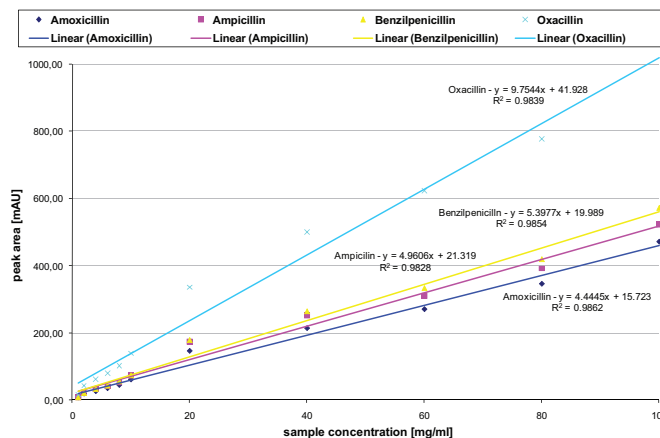


Fig. 5. Calibration graph

parameters were chosen: applied voltage +25 kV, temperature 25°C, injection pressure 30 mbar for 5 seconds.

The buffer pH influence on the separation was studied on the interval 7–10.5. The changes in the pH of the buffer solutions were induced by adding increasing quantities of 1M boric acid and 0.1M NaOH, respectively. The modification of the migration times at different pH values can be observed in Figure 4.

In order to evaluate the reproducibility of the method, we performed a repeated measurement of 9 samples and observed the variation of the migration times, peak height and the peak area. We calculated the average of the values and the standard deviation for each parameter. Very similar migration times, peak heights and areas were obtained, the RSD values were smaller than 1% indicating that the precision of the method is good.

We also calculated the individual linear regression equation and the correlation coefficient for each compound, injecting fourteen solutions with different concentrations in a specific range (0.4–100 mg/100 ml) and three replicates per concentration (Figure 5).

Discussions

Capillary electrophoresis is an alternative and complementary method to the more frequently used high performance liquid chromatography, which is prescribed in the European Pharmacopoeia for the dosage and purity control of the penicillin derivatives [9]. CE has important advantages: small quantity of reagents required, short running time and the possibility to analyze even unprocessed biological samples.

Perez and coworkers [10] and Nozal and coworkers [11] developed capillary electrophoresis methods for the separation of the penicillin derivatives using sodium dodecyl sulfate as buffer additive, but our method proves its effectiveness especially regarding the short analysis time.

In the proposed method we used a similar buffer – a 25 mM sodium tetraborate solution enriched with the same surfactant – 100 mM sodium dodecyl sulfate, at pH = 9.3. The applied voltage was 25 kV at a temperature of 25°C, while the injection pressure was 30 mBar for 5 seconds, respectively. The higher voltage and temperature applied during analysis significantly shortens the running time of the analysis and improves the resolution of the separation. The antibiotics average migration time was 3.30 for amoxicillin, 3.74 for ampicillin, 4.22 for benzilpenicillin and 4.96 for oxacillin.

Conclusions

We obtained the most efficient separation of the four penicillin derivatives with a buffer solution containing 25 mM

sodium tetraborate and 100 mM sodium dodecylsulfate. The migration order of the four compounds studied was the following: amoxicillin, ampicillin, benzilpenicillin and oxacillin. The influence of the instrumental and chemical variables was evaluated, as well. With the increase of the voltage and of the temperature, respectively, we observed the decrease of the migration times. A voltage of 25 kV and a temperature of 25°C were selected.

The effect of the pH of the buffer solution was studied in order to evaluate the efficiency of the separation. Our measurements were performed in the pH interval 7–10.5. The variation of the pH affected the migration order of the compounds. At pH <8 the compounds studied migrate in the following order: amoxicillin, benzilpenicillin, oxacillin and ampicillin; while at pH >9.5 amoxicillin exhibited the longest migration time. The change in the migration time of the amoxicillin at higher pH values can be explained with the phenolic hidroxil group present in this molecule, which can participate at the formation of a double sodium salt of the compound.

The analytical performance of the optimized method was evaluated on the basis of precision (by calculating regional standard deviation – RSD for the migration time and peak area), linearity (individual regression equation and correlation coefficient)

MEKC proved to be a useful and suitable tool for the identification and separation of penicillin. We suggest that the method can find practical application in the separation of penicillin from environmental samples.

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