STUDIES ON THE SIMULTANEOUS SEPARATION OF SOME ANTIBACTERIAL COMPOUNDS IN COMPLEX MIXTURES

As a result of the numerous alerts concerning the presence of the antibacterial compounds in environmental and biological samples, the development of new analytical methods for their identification and determination became a permanent necessity and also a challenge. We studied substances from two of the most extensively used antibacterial classes; choosing four penicillin derivatives (amoxicillin, ampicillin, benzylpenicillin, oxacillin) and two fluoroquinolones (ciprofloxacin, norfloxacin), substances with particular importance in both human and veterinary therapy. The aim of our experiments was the development of new chromatographic and electrophoretic analytical methods, which can be used for the identification and separation of the studied compounds in complex mixtures.

We studied the electrophoretic behavior of the analytes in the pH range 2-10, using a phosphate buffer electrolyte solution in the pH range 2-7 and a borate buffer in the pH range 7-10, respectively. In the presence of the acidic phosphate buffer, oxacillin and benzylpenicillin could not be detected, but better results were obtained when using an alkaline buffer solution, as in the presence of this electrolyte solution the analytes are present in ionized forms and the differences between the electrophoretic mobilities generate higher separation resolution. We encountered some difficulties in separating the two aminopenicilins and the two fluoroquinolones, respectively, compounds with homologous chemical structure and almost similar electrophoretic mobilities; we managed to overcome these problems using micellar capillary electrophoresis, by adding an anionic surfactant - sodium dodecyl sulfate (SDS) - to the buffer solution.

The four penicillin derivatives were separated successfully in approximately 5 minutes, using a buffer solution containing 25 mM sodium tetraborate (TB) + 100 mM SDS, at pH = 9.3, the order of separation being: amoxicillin, ampicillin, benzylpenicillin, oxacillin. In the case of the two fluoroquinolones, we used a buffer solution containing 25 mM TB + 100 mM SDS + 100 mM boric acid + 20% acetonitril (ACN), at pH = 8.3. We managed their separation in approximately 9 minutes, the order of separation being: ciprofloxacin, followed by norfloxacin. The optimal electrophoretic conditions in both cases were: applied voltage +25 kV, temperature 25°C; the samples were injected by hydrodynamic injection, at 30 mbar injection pressure for 5 sec. By combining the electrophoretic parameters of the two techniques presented above, we have separated the six compounds in approximately 9 minutes, using a buffer solution containing: 25 mM TB + 100 mM SDS + 100 mM boric acid,

at pH = 8.3, the order of separation being: amoxicillin, ampicillin, benzylpenicillin, oxacillin, ciprofloxacin and norfloxacin.

In order to optimize the separation methods, we studied the effect of the concentration and pH of the electrolyte solution, the effect of the additives, applied voltage and temperature on the separation efficiency. The developed methods were assessed by verifying the analytical performance based on accuracy (migration time and peak area), linearity (calibration curves, regression equations and correlation coefficients) and by calculating the limit of detection and quantification. The correlation coefficient calculated for the separation of the six compounds in the concentration range 6-330 mg/mL, in 9 points of the calibration curve, is above 0.99, indicating a good linearity. The relative standard deviation calculated for migration times and the peak areas registered in the electropherograms obtained by injecting a series of solutions of the same concentration, is below 1%, indicating a good precision of the method. The LOD values were in the range between 1.1 to 1.3 mg / mL; while the LOQ values were in the range between 3.7 to 3.9 mg / mL.

The developed high performance liquid chromatography separation method allows the simultaneous separation of the six antibacterial compounds, at pH = 2.5, using a mobile phase with variable concentration, according to the following elution scheme: isocratic elution with 13% ACN for 3 min, elution with concentration gradient - 13-20% ACN for 3-10 minutes, followed by isocratic elution with 50% ACN for 10-15 minutes and finally an isocratic phase, when the concentration of ACN returns to the value used in the initial phase (13%). The total analysis time is approximately 16 minutes; the elution order being: amoxicillin, ampicillin, norfloxacin, ciprofloxacin, benzylpenicillin and oxacillin.

The stability of the penicillin derivatives was studied by capillary electrophoresis in aqueous, alkaline and acid medium, on two sets of samples, stored at room temperature (20-25°C) and refrigerated (4-8°C). The most emphasized degradation was observed at benzylpenicillin; ampicillin and amoxicillin shows relative stability in acidic medium; while in alkaline medium all compounds decompose rapidly, with formation of specific degradation products. For the preliminary identification of the studied penicillin derivatives we developed a method of thin layer chromatography; using in situ colour reactions and UV detection.

Comparing the separation methods developed, we can say that the use of capillary electrophoresis is advantageous because of the relatively low cost of consumables, low consumption of samples and reagents, shorter analysis time and the rapid development of the analytical method. High performance liquid chromatography offers the advantages of high precision injection and a higher sensitivity (lower detection), which allows identification of

the analytes at much lower levels of concentration at ng / mL. When selecting an analysis method for the determination of a particular sample, aspects regarding the specificity of the sample and the purpose of the analysis of the antibacterial compounds should be taken into consideration.