RESEARCH STUDY REGARDING CEPHALOSPORINS STABILITY,
THEIR DETERMINATION FROM BIOLOGICAL PRODUCTS
REPECTIVELY FROM VETERINARY FORMULATIONS

Author: Corina-Cireșica Mondea
Scientifical guider: Prof.dr.Gyéresi Árpád

Abstract:

Cefuroxime sodium is a second-generation parentally administered cephalosporin, used in both human and veterinary medicine. In veterinary medicine, cefuroxime sodium, active on Streptococcus agalactiae, Streptococcus dysgalactiae, is administered intramammary for the treatment of mastitis in cattle during the lactating period. Cefquinome sulphate is a fifth generation cephalosporin, which is administered only parenterally, being used exclusively in the veterinary medicine. The cefquinome is indicated in affections of the respiratory tract, endometritis, mastitis, urogenital infections, of the pyoderma, polyarthritits, septicemia (caused by Escherichia coli, Salmonella). Pharmacologically active substances, which remain in the organism of the animal or in animal origin products, are harmful for the human body, when exceeding allowed limits. The current legislation establishes maximum limits for antibiotics residues. According to this, the detection of antimicrobian residues in animal products is compulsory, taking into consideration their elimination period from the human body.

HPLC or LC/MS methods are currently widely used in qualitative and quantitative analysis of drugs from biological fluids. There are already some published methods in the literature for quantification of cephalosporins from mixtures, biological matrix, antibiotic residues from biological liquids or stability studies, but these methods usually have the disadvantages of long running-times and laborious sample preparation because of the necessity of liquid-liquid extraction or solid phase extraction. The aim of this work was to
elaborate and validate a simple, rapid, selective and reproducible method that allows the estimation of cephalosporins in cattle plasma, method that subsequently can be used in pharmacokinetic studies and drug residue determinations.

A simple, rapid and sensitive LC/MS/MS method for quantification of cefuroxime sodium and cefquinome sulphate in cattle plasma has been developed and validated. The chromatographic conditions were optimized in order to increase the selectivity of the method for the determination of cephalosporins from biological products.

The analyte was extracted from plasma samples by using protein precipitation with a mixture of 12% perchloric acid in acetonitrile. Separation was achieved using a Zorbax SB-C18 column using a mobile phase containing methanol: ammonium acetate 1mM in water (14:86 v/v). The detection of was realized in MS-MRM mode using an Ion Trap mass spectrometer with electrospray negative ionization. For cefuroxime sodium the linearity domain was established between 63 to 6120 ng/ml; accuracy and precision were less than -4.9% and 9.3 for intra-day assays and -6.9% and 9.3 for inter-day assays, respectively; the recovery ranged between 95 and 105%. For cefquinome sulphate the linearity domain was established between 12,2 to 1464 ng/ml; accuracy and precision were less than 5,5% and 2,2 for intra-day assays and -5,0% and 10,8 for inter-day assays, respectively; the recovery ranged between 86 and 108%.

The proposed method proved to be rapid, accurate and precise for the quantitative determination of cefuroxime and cefquinome in cattle plasma, having the advantage of selectivity due to MS detection, high throughput due to both simple plasma preparation and short analysis time. Without using an internal standard and applying a simple sample preparation by protein precipitation, a specific and efficient analysis of plasma samples could be performed. The method was used for pharmacokinetic studies of cefuroxime and cefquinome and for drug residue analysis in cattle.

The applicability of capillary zone electrophoresis (CZE) for the determination of cephalosporins has also been studied. We choose four cephalosporins with different structural characteristics (cefquinome, cefuroxime sodium, ceftriaxone sodium, ceftazidime).

Our aim was not only to develop a rapid, efficient and simple method for the separation of the studied cephalosporins, but also the optimization of the analytical conditions. The influence of the buffer concentration and pH of the buffer were studied, and the optimum conditions for the separation were chosen.
Using a buffer containing 25 mM NaH₂PO₄ (pH – 6.8), a applied voltage of + 25 kV, at a temperature of 25°C, a 50 cm x 50 μm I.D capillary, and UV detection at 270 nm, we managed the baseline separation for the four studied cephalosporins in less then 10 minutes. The order of migration was the following one: cefuroxime, ceftazidime, cefquinome, ceftriaxone; order which can be explained if we take inconsideration the molecular mass, pKa values and especially the molecular structure and characteristics of the studied cephalosporins (two of them were used as sodium salts and the other two have a zwitterionic structure).

The proposed separation method was evaluated on the basis of precision (migration time and peak area), and linearity (regression equation and correlation coefficient). Also we calculated the electrophoretic mobility of each analyte, taking into consideration the EOF and the individual migration times.

Although the stability of cephalosporins in solid-state are satisfactory, dissolved in water gradually convert to different degradation products by hydrolysis. After dissolution of each cephalosporine in water the samples were reinjected several times over a period of 12 hours. The rate of the concentration decrease was calculated, and major differences were established between the stability of the four compounds. The stability increased in the following order: cefuroxime, ceftriaxone, ceftazidime, cefquinome; the sodium salts proving to be less stable. It was established that the degradation rate of the studied cephalosporins in water was not higher than 20% at room temperature within 4 hours of dissolution. For the cefuroxime sodium we were able to detect on the electrophoregrams two peaks corresponding probably to its degradation products.

The proposed CZE method was applied also for the study of the stability of cephalosporins in water at different temperatures (20°C - 30°C). Cefuroxime exhibited a mild degradation, but cefquinome seemed to be stable over this temperature range.

In conclusion, CE has proven to be a significant and versatile technique for the analysis of the investigated cephalosporins, and can be used probably for the separation of other substances with similar structures. Using the described optimized conditions this technique can be used for the analysis and identification of drugs in formulated products and also for resolving separations from mixtures of drugs; and can be probably applied for the analysis of cephalosporins from biological fluids.

The stability studies showed that the extent of the hydrolysis of cephalosporins in water is highly dependant on the time and also the temperature.