SUMMARY

For at least ten years electrophoretic techniques were indicated as those which may, in the future, displace costly and less environmentally friendly various types of chromatography. Nevertheless, CE techniques have some restrictions included primarily low sensitivity, resulting from the detection process, carried out directly in the capillary. Another limitation is significant salinity of biological samples, which has negative affect on the reproducibility of CE analyzes, or in some cases can completely prevent them. Good solutions for this restrictions are stacking techniques used directly in the measurement system and additional appropriate preparation techniques of test materials.

Researches carried out in the context of this PhD thesis comprise the development of four new electrophoretic methods for the determination of selected biologically active compounds, in four different matrices. The choice of compounds wasn't accidental and had been designed to test wide variety of samples in terms of the matrix complexity and concentration of ingredients usually disturbing in the analysis.

In this work was described new unconventional method enabling determination of pyridoxine (PN) in selected pharmaceuticals. Method allowed to approx. 20-fold reduction of the quantification limit in comparison to a conventional zone electrophoresis. Electrokinetic introduction of the sample and presence of SDS micelles in base electrolyte allowed to reach the low limit of quantification (LOQ) and the limit of detection (LOD) of the method about 0.25 μmol/L and 0.5 μmol/L, respectively. A buffer solution consisting of 25 mmol/L sodium borate (pH 8.0), 17.5% acetonitrile (MeCN), 7.5 mmol/L trichloroacetic acid (TCA) and 12.5 mmol/L sodium dodecyl sulfate (SDS) was found to be the most suitable BGE for the separation. The procedure relies on electrokinetic sample introduction, the use for the separation maximum voltage of 30 kV and UV detection at 320 nm. Linearity in detector response was observed over the range of 0.5–30 μmol/L with the correlation coefficient 0.9952. The intra- and interday precision and recovery were 1.84–9.12%, 2.55-9.12% and 98.15–109.53%, respectively. Resulting LOQ suggests that in the future, after a slight modifications, the method can be used for analysis of biological samples for PN content.

A MEKC-UV based method was developed for the determination of total apigenin (API) in selected herbs. Application of pseudostationary phase in the form of SDS micelles resulted in great repeatability of retention times and peak areas. A buffer solution consisting of 30 mmol/L sodium borate (pH 10.2), 10% MeCN, and 10 mmol/L SDS was found to be the most suitable for the separation. The method was validated and calibrated for total API

in the range of 1.0–100.0 μmol/L (R^2=0.9994). The LOQ and LOD values were 0.48 μmol/L and 0.92 μmol/L, respectively. This precise and robust method was successfully applied to the analysis of plant samples for total API content.

Sensitive electrophoretic method for the determination of total sodium 2-mercaptoethanesulfonate (MESNA) in human plasma, based on the stacking with high salt concentration in MEKC and in-capillary derivatization with 2-chloro-1-methyllepidinium tetrafluoroborate followed by UV detection was developed. In the method 0.03 mol/L pH 7 phosphate buffer with the addition of 0.01 mol/L SDS, and 10% MeCN was used as a BGE. LOQ of the method was 0.5 μmol/L. Linearity in detector response was observed over the range of 0.5–10 μmol/L with the correlation coefficient 0.9971. The intra- and interday accuracy (three concentration levels, 5 days, n=3) of the method ranged from 97.2 to 110.0% and from 94.0 to 101.2%, respectively. The novel MEKC method with UV detection proved to be suitable for determination of total MESNA in human plasma.

A new assay for the determination of homocysteine thiolactone (HTL) in human urine based on a field amplified sample injection and sweeping MEKC with UV detection has been developed. The two steps procedure relies on sample liquid–liquid extraction followed by CE separation and UV detection at 240 nm. HTL standard added to the urine before the extraction step shows that the response of the detector is linear within the range studied, from 0.1 to 1.0 μmol/L urine. The intra- and interday precision and recovery were 3.2–14.4% (average 5.1% and 9.3%) and 92.5–112.6% (average 99.8% and 99.1%), respectively. The LOQ was 0.09 nmol HTL in 1 mL of urine. The proposed method was applied for the analysis of 15 urine samples donated by apparently healthy volunteers. The average concentration of the analyte was 0.170 ± 0.029 μmol/L.

It has been shown that all developed methods described in the present dissertation can be used to the real samples. Conducted experiments showed, among others, that regardless of the matrix complexity, the stacking technique of the analyte in the measuring system is a great tool to lowering the limit of quantification. Consequently, the developed analytical procedures could be an alternative for the similar, based on chromatography.

In conclusion, one should give again an attention on the fact, that the sample preparation is a key step of the whole analytical procedure. On the other hand, carried out experiments seem to confirm the thesis concerning possibility of replacing at least some of the chromatographic methods, with much cheaper and simpler CE based procedures.