Disertační práce Summary 133 This dissertation thesis is focused on the analytical evaluation of biologically active compounds with the aid of High Performance Liquid Chromatography, especially with selective and sensitive detection performed by mass and coulometric detector.
In **the theoretical part** of this dissertation thesis, there is attention paid to the importance and status of mass spectrometry as a tool of the modern analytical method of the detection. In this dissertation thesis, there is also a view of electrochemical methods and applications, focusing on coulometric measurements in flow systems.

The results of **the experimental work** have been compiled and presented in three articles published in scientific impact journals.
The list of reached results is following:
The first article deals with **a comparison of four chromatographic columns for their use in bioanalytical studies**. Numerous HPLC columns with different bonded stationary phases are nowadays available, and producers continue to expand the range of columns they offer. Four real mixtures of biologically active compounds were used for the testing. Every mixture contained a parent active compound, its real metabolites and proper internal standard. Measurements were performed on two monolithic and two particle-packed columns with quite new stationary phases. On the basis of chromatographic parameters (retention time, theoretical plate number, height equivalent of theoretical plate (respectively), peak resolution and peak symmetry) the best chromatographic conditions were chosen for each group of compounds. The behaviour of the compounds with a different chemical structure, on sorbents with various bonded chains was also described. The main benefit of our work was that column properties were studied on the mixtures of related compounds - a drug with its real metabolites - which is not usual when properties of columns are presented. Hence, the selectivity of the columns was also tested.

The result of **the second paper** is a fast and sensitive **validated SPE and HPLC method for analytical determination of plasmatic concentrations of eight quinolones used in veterinary practise**. This developed and validated method has been consequently used for the determination of quinolones in real samples.

Quinolones, as well as some other chemotherapeutics or antibiotics, are normally used not only to treat diseases but also in the prophylactic treatment in the veterinary field. The residues of the compounds are accumulated in animal tissue after administration to livestock. Nevertheless, very low concentrations might be dangerous not only for an individual but also for a big population. Individually, these substances may provoke an allergic hypersensitive reaction. The promotion of bacteria resistance is the next important impact for mankind. This work has enabled the determination of ciprofloxacin, the major metabolite of enrofloxacin, which is routinely used in therapy. Commonly used UV techniques were not sensitive enough for its determination. This problem has been solved by the use of mass spectrometry.

The third scientific article shows a sensitive analytical **method for the determination of biotin in pharmaceutical preparations using high performance liquid chromatography coupled with a coulometric detector**. The developed and validated method was then successfully applied for the determination of biotin in chosen pharmaceutical preparations. Biotin is a water-soluble vitamin belonging to the B-complex. It is involved in many biochemical processes and a sufficient intake is necessary for healthy nerve tissue, sweat glands, hair and skin. The defined daily dose of biotin is about 30 μg/day, and thus its content is proportional in pharmaceuticals unlike the amount of other water-soluble vitamins (e.g. vitamin C), in which the content is much higher. This fact together with its nonspecific UV-spectrum causes problems when UV/VIS detection for the determination of biotin is used. Due to the coulometric detection, which was established thanks to electrochemical activity of biotin, much lower detection and quantification limits were reached also in the presence of other vitamins.