Disertační práce Summary High performance liquid chromatography achieved during its existence enormous boom. Modern, PC controlled and user-friendly chromatographic devices allow performing a number of tens analyses on one apparatus daily. There is possible to analyse wide spectrum of compounds from non-polar to polar and from low molecular to high molecular analytes. Appropriate choice of chromatographic column, mobile phase and detection technique makes the analysis of complicated mixtures possible and allow obtaining both qualitative and quantitative information. HPLC is due to these characteristics utilized not only in the field of pharmacy but also in other areas e.g. biology, medicine, industry, environment monitoring and quality control of various products.

Separation column is the most important part of the chromatographic system. The availability of stable, high performance chromatographic phases is the essential requirement for the development of a rugged and reproducible method. Nowadays, most HPLC separations have been done in the reversed-phase mode on the silica-based columns. This phenomenon is due to many positive properties of silica - mechanical strength, well mastered manufacture of silica particles, easy modification of silica surface, compatibility with water rich mobile phases etc. The insufficient chemical and thermal stability is considered to be the Achilles heel of silica-based stationary phases. Although new technologies have enhanced their stability there is still need for more durable columns. Improvements in stationary phase stability have been and remain a significant driving force for the development of new stationary phases.

The research, that explores non-silica-based supports, has been proceeding for some years. It is focused on, among other things, zirconia-based supports that appear to be suitable alternative to silica supports. Zirconia-based phases differ from silicaneous materials in retention and also selectivity. These differences, based on distinction in chemical properties of both materials, can substantialy influence the separation process. The retention of analytes on zirconia-based stationary phases is influenced by various interactions (reversed-phase, ion-exchange, ligand exchange). Their contribution to the retention depends on the nature of analyte, the mobile phase pH, the type of buffer, its ionic strength and also on the organic component of the mobile phase. The zirconia-based columns offer very high efficiency, which in contrast to polymeric materials is similar to the efficiency of silica. However, zirconia-based phases share the advantage of polymer based supports in that they are vastly superior to silica in terms of their chemical and thermal stability. The rising number of publications and quickly evolving research in this area reflect that the future of zirconia as stationary phases should be rich. The development of monolithic stationary phases, particles which have smaller pores and higher surface areas, the exploration of stationary phases in LC–MS and without doubts extended offer of chiral stationary phases can be expected. The development of coatings that cover

metal oxide surfaces and fully sequester the Lewis acids is the main stream in current research. Such modified zirconia would resemble silica phases more closely without compromising the stability. These phases could be employed in reversed-phase separations of proteins and large peptides for example, because there is not available a zirconia phase useful for these analytes to the date. Additional attention includes development of amino phases, cyano phases and diol phases to emulate the related materials on silica-based bonded phases.

The chiral nature of living systems has implications in biologically active compounds interacting with them. As a consequence, different responses can be often observed for a pair of enantiomers when comparing the chiral drug bioavailability, distribution, and interaction with receptor sites, metabolism and elimination. The differences can manifest themselves as undesirable toxicity, enhanced or different effects or are not significant for activity. Therefore, stereochemistry has to be considered when studying xenobiotics, such as drugs, agrochemicals or food additives. During the last decade chirality has attached increased attention due to scientific and economic reasons, especially in pharmaceutical industry. The liquid chromatographic separation of enantiomers using chiral stationary phases (CSP) has become an indispensable tool in many areas of modern research.

This dissertation touches briefly the current state of HPLC equipment in the beginning of the theoretical introduction and the main part is devoted to the stationary phases that have been used recently. The first chapters are focused on properties of widely used silica-based stationary phases and feasible alternatives. The essential attention is devoted to zirconia-based stationary phases for HPLC. The second part of theoretical introduction deals with HPLC chiral stationary phases. The dissertation had two partial aims 1) to test utilization of zirconia-based stationary phases in drug control and 2) to elaborate a chiral HPLC method enabling analysis of reduced metabolite of the potential antineoplastic agent. The experimental results are summarised in three papers published in the international impacted journals.

The first two papers interrelate and demonstrate the feasibility of using zirconia-based stationary phase on two examples in drug control.

- The zirconia-based stationary phase coated with polystyrene was successfully used for a HPLC separation of ibuprofen from three related compounds and its two decomposition products. Even though the carbon content of PS-ZrO_2 is much lower than that of conventional C18 phase, analytes exhibit sufficient retention and selectivity, but with substantially improved analysis time. The method is applicable for evaluation of studied impurities of ibuprofen in the raw material, for the monitoring of the degradation processes and for the assay of ibuprofen as well. The organic solvent consumption during analysis

has been reduced remarkably, which is not negligible from an economic as well as an environmental viewpoint.

- Two types of zirconia-based stationary phases (PS-ZrO_2; ZrCarbonC18) were tested as a possible alternative to commonly used silica C18 reversed phase in the pharmaceutical analysis. Considerable differences were observed in retention on zirconia stationary phases and on C18 silica stationary phase. The tested zirconia columns differ from each other in selectivity and retention as well. The ZrCarbonC18 column was more appropriate for simultaneous analysis of ibuprofen, parabens and their decomposition product in topical pharmaceuticals due to its higher hydrophobicity. The method can serve also for quantification of the active substance ibuprofen and parabens, using butylparaben as an internal standard.
- Currently, the next paper dealing with the applicability of zirconia-based stationary phases in the pharmaceutical analysis is being prepared.

The goal of the third paper was to develop a chiral HPLC method enabling comparison of carbonyl reduction metabolism of the potential antineoplastic agent dimefluron and its forerunner benfluron.

- Many types of chiral stationary phases were tested for evaluation of biotransformation of both substances in the microsomal fractions of liver homogenates prepared from various species. The best separation of both reduced metabolites were achieved finally on one type of chiral selector - cellulose tris(3,5-dimethylphenylcarbamate) but the mobile phases differ from each other in the organic modifier. Acetonitrile was used for enantio-separation of reduced benfluron and methanol was used for enantio-separation of reduced dimefluron. The intra- and interspecies differences in the biotransformation of both compounds were observed.