Introduced dissertation thesis deals with the development and validation of the chromatographic methods for analytical evaluation of selected biologically active substances. HPLC coupled with UV and MS detection was chosen for determination of analytes, because of the dominant role of the HPLC in pharmaceutical analysis.

The theoretical part is focused on the theory of chromatographic methods and topics of the experimental work. At first, summary of all the chromatographic methods is briefly introduced and subsequently, the most common analytical method HPLC is described in details. The next part deals with the mass spectrometry. Thank to its high sensitivity and ability to provide structural information about the analytes, MS became an indispensable tool not only in modern pharmaceutical analysis. Besides MS theory and instrumentation, its applications and new trends are also mentioned. Last chapters deal with the transdermal application of drugs, specifications of antiretroviral therapy and especially they provide basic information about the physical - chemical and biological properties of analysed substances.

The experimental part is consisted of the original research papers with appropriate comments divided into two thematic sections. The first one is composed of three papers focused on analytical determination of the novel accelerant of transdermal penetration – transkarbam 12. The second section deals with the analytical determination of antiretroviral efavirenz in biological material.

The first thematic section deals with the development and validation of the chromatographic methods suitable for analytical determination of Transkarbam 12 (T 12). This new accelerant of transdermal penetration was developed at the Faculty of Pharmacy in Hradec Králové in the nineties and it was patented in 2001. The limited availability of highly effective accelerants in clinical practise was the main reason which initiated this development. In fact, Azon® has been the only, clinically widely used accelerant since 1976. Introduction of a new and highly effective accelerant can significantly contributes into the widespread of transdermal drug delivery which is an advantageous alternative to another, commonly used drug delivery.

Because of the absence of any analytical method for determination of T 12, it was necessary for its introduction to pharmaceutical praxis, to develop and validate chromatographic methods, which allow impurity and stability determination of T 12. At first, fast, easy and inexpensive TLC method was developed for the evaluation of T 12 and its two hydrolytic degradation products 6-aminohexanoic acid (AH) and dodecanol (D). Derivatization of the

sample using benzoyl chloride was needed before each analysis, because of absence of the chromophore in the structure of T 12, as well as in degradation products. Beside TLC, in this pilot study HPLC/UV method was also developed to obtain sufficient separation of T 12 from the derevatization reagent and/or side products of the derivatization reaction. Further experimental work was focused on the development of a new HPLC/UV method, which enabled simultaneous determination of T 12 and its degradation products - AH a D. Due to the low sensitivity of the previously used derivatization, the new derivatization reagent (3,5-dinitrobenzoyl chloride) was chosen in these experiments and whole derivatization process was newly optimized. However, it was obvious that time-consuming derivatization is not suitable for routine use in pharmaceutical manufacturing. Therefore, further effort was focused on searching for an alternative for elimination of this time-consuming step. Thus, mass spectrometry was chosen as a proper method, which allows to determine the analytes according to their *m/z* ratio and therefore it requires no chromophore in the structure of the analytes. **Novel HPLC/MS method developed and validated in this study, allowed sensitive determination of purity and stability of the T 12 substance. Following impurities can be evaluated: AH – hydrolytic degradation product, CA – initial substance for T 12 synthesis and DAH – side product of the synthesis. DAH was discovered and identified as a novel T 12 impurity during HPLC/MS method development. Currently, this HPLC/MS method is used by T 12 manufacturer to determine impurity and stability of T 12 bulk substance.**

Considering the need to quickly introduce novel method for analytical evaluation of T 12 to pharmaceutical practice, the time available for the method development was limited. Therefore ideal optimization of all method parameters was not done. Total analysis time and gradient profile of the method are the main drawbacks. Hence, further work was concentrated to overcome these disadvantages. Currently, a new scientific paper describing the development and validation of the novel isocratic HPLC/MS method with 12 min of the total analysis time is prepared.

The second thematic section is focused on analytical determination of efavirenz in biological material. This work was a part of a wide clinical study proceeded in cooperation with other research laboratories wordwide at Ruprecht-Karls-Universität Heidelberg, Germany. Efavirenz is recommended by WHO for medication of HIV infection in African countries. As well as most of other antiretrovirotics, efavirenz (EFV) is characterized by high inter-individual variability of plasmatic concentrations. Hence, antiretrovirotics belong to the drugs recommended for therapeutic drug monitoring in order to adjust dose for particular patient

individually. CYP-450 is extensively involved in EFV biotransformation. The high risk of drug-drug interaction can be expected in HIV/TBC co infection, where rifampicin, other potential CYP-450 inducer, is recommended as a first line drug. In this case the analytical method capable to determine the concentration profile of EFV in biological material is of particular importance. Presented part of the project was focused on the development and validation of chromatographic methods suitable to determine concentration of EFV in three biological materials plasma, ultrafiltrate and intracellular concentration in PBMC. **A new, highly sensitive method HPLC/MS/MS was developed for determination of EFV in these biological materials. Isolation processes of EFV from each biological material were developed as well. Finally this method was used for the determination of EFV in samples taken from 14 healthy volunteers receiving EFV**.