High performance liquid chromatography (HPLC) is one of the most frequently used analytical techniques for the analysis of drugs. HPLC methods are widely employed in all fields of the modern pharmaceutical analysis - new drugs development, quality control and assurance, the analysis of drugs and metabolites in a biological material (e.g. therapeutic drug monitoring, bioequivalence). The theoretical part of this thesis deals with the main specifics and aspects associated with HPLC analysis of drugs. The experimental part is consisted of six original research papers with appropriate comments divided in to two sections (The analysis of the drug candidates from the group of aroylhydrazone iron chelators and The stability study on selected drugs).

The first section concerns with development, validation and application of new HPLC methods in the analysis of drug candidates from the group of iron chelators – pyridoxal isonicotinoyl hydrazone (PIH), salicylaldehyde isonicotinoyl hydrazone (SIH) and pyridoxal 2-chlorobenzoyl hydrazone (o-108).

Aroylhydrazone iron chelators are under the investigation **as promising drug candidates**. Despite these chelators have demonstrated number of interesting pharmacological effects, in fact there were no modern analytical techniques suitable for the analysis of these drug candidates. The lack of the analytical methodology has not allowed to determine the basic pharmacokinetic parameters of these drug candidates. At the beginning of the experiments, **the chromatographic methods** suitable for **purity** and **stability evaluation** of these chelators were developed and validated. The stability-indicating method was thereafter employed for the **evaluation** of the **selected aspects of the stability** of a water soluble salt - **PIH·2HCl** (The stability of the chelator in water media of different pH and two common pharmaceutical excipients was investigated, photostability and thermal stability studies on the solid substance were performed as well). These experiments have demonstrated that PIH is relatively sensitive to hydrolytic splitting of the hydrazone bound in aqueous solutions; however the solid substance is stable under the tested conditions (photo and thermal stability). These experiments provided the practical information which could be useful in further investigations of this chelator as a drug candidate (e.g. for the selection of storage conditions, an application media and pharmaceutical excipients).

The development and validation of HPLC methods appropriate **for the analysis of SIH and o-108 in rabbit plasma** was described **in the second part** of

this section. Precipitation using acetonitrile with an addition of small amount of acid was employed for the isolation SIH. In the case of o-108, the method of solid phase extraction employing C8 columns was used. The desirable separations were achieved on C18 columns using mobile phases of different compositions. These methods were employed for the **determination of plasma concentration-time profile and the basic pharmacokinetic parameters** followed i.v. application of these chelators to rabbits. Rather short half-lives of elimination were observed these studies. The results of these experiments could be important for further investigation in this field.

The **second part** of the dissertation thesis concerns **with the development of stability indicating analytical methods** and **the applications** of these procedures in **the stability evaluation** of two modern drugs.

Investigation of the stability of drugs is one of a specific part of the pharmaceutical analysis. The stress testing can help to identify the likely degradation products which can in turn help to establish the degradation pathways and intrinsic stability of the drug molecule and to validate the stability indication power of the analytical methodology. The modern hypoglycemic agent - **glimepiride** was chosen for the stability investigation. **The stability** of glimepiride **in the different aqueous media** (0.1 M HCl, 0.1 M NaOH, water, 2 % H_2O_2) under the selected stress conditions was tested and expressed as a fall of the concentration of drug in the time course of the experiments. It was observed, that the susceptibility of glimepiride to degradation is decreasing in following manner: 2 % H_2O_2 > acid condition > alkaline condition > water. The five main degradation products were detected in the exposed samples.

The last topic of this dissertation thesis was to determine the photochemical stability of modern anti-inflammatory drug – **nimesulide**. The chromatographic methods (HPLC and TLC) suitable for the separation of nimesulide and its two potential degradation products have been developed and employed in the **photostability evaluation** on this drug. These experiments indicated that nimesulide is relatively resistant against the influence of UV light. The sample solution had to be exposed to UV light for relatively long time period to detect the degradation products.

This part of the thesis provides new analytical methods suitable for the stability evaluation of glimpiride and nimesulide. Information presented herein could be useful for quality control and assurance of these two clinically used drugs.

Summary