7. SUMMARY

DEVELOPMENT AND VALIDATION OF HPLC METHODS

The doctoral thesis concerns with development of HPLC methods for analysis of pharmaceutical formulations, which meet all requirements for suitability, precision and reliability. Documentation of these parameters is called validation.

At first theoretical principles of chromatography are described in this work, with the emphasis to liquid chromatography, included HPLC. The instrumentation in HPLC is described, mentioning the new trends in stationary phase's development – for example monolithic columns, zirkonia stationary phase, HILIC and sub-2-microns phases. In the other part UPLC system, one of the new trends in liquid chromatography development is discussed. Next part deals with the development of HPLC methods for analysis of pharmaceutics and is followed by chapter, in which analysis of pharmaceutical formulations is described, included preparation of the samples before analysis, stability monitoring and further the analysis of impurities.

The last, relatively large chapter is dealing with the validation of analytical methods. Several institutions are concerned with validation requirements. They issue recommendation and/or guidelines, which are cogent for this process. In the Czech Republic there exists State Institute for Drug Control (SUKL – Bulletin of SUKL, Czech Pharmacopoeia). The validations are mentioned in European Pharmacopoeia (Ph.Eur.5) too. The validation chapter is described in the US Pharmacopoeia (USP 29); FDA (Food and Drug Control) and ICH (International Conference on Harmonization) are dealing with validation too. The ICH associates validation requirements for the European Union, USA and Japan. The validation process is controlled by several institutions and it was the reason for transparent and clear description of this problem. In this chapter the validation process is threshed relatively closely. The described parameters of validation procedure are precision, accuracy, linearity, selectivity, robustness, limit of detection, limit of quantitation. One part is dealing with the system suitability test, very important part of validation process (mainly in instrumental methods for analysis of pharmaceutical formulations). Each parameter is described with comments. The parameters are classified in term of two authorities – SUKL and the ICH.

In term of this work seven new methods for analysis of topical formulations (ointments, gels, and pastes) were developed; the papers were accepted in international journals: Journal of Chromatography A and B, Talanta, Analytical and Bioanalytical

Chemistry and Journal of Pharmaceutical and Biomedical Analysis. Other three methods, which were published as posters on several analytical conferences, were developed.

The pharmaceutical formulations are analyzed using the above mentioned methods during their stability testing and quality and homogeneity control. The methods solved analysis of active substances, preservatives and degradation products in one analysis.

The new methods were applied to pharmaceutical formulations, in most cases to gels and creams. In *Triamcinolon cream* triamcinolone acetonide as an active substance, methylparaben and propylparaben as preservatives and triamcinolone as degradation product are determined. In *Ketoprofen gel* ketoprofen parabens and two degradation products are similarly determined. Two methods for analysis of *Estrogel gel* were developed, because SUKL increased its requirements for monitoring impurities and producer changed composition of this preparation. Thus the latest method monitors assay of estradiol and its six impurities. In terms of analysis of *Indometacin gel* indomethacin and its two degradation products – 4-chlorbenzoic acid and 5-methoxy-2-methylindolacetic acid - are determined. *Terbinafin cream* is next formulation, in which more impurities are tested. In this formulation terbinafine as an active substance, methylaminomethylnaphtalene as impurity erased in a production process and three degradation products of terbinafine – Z-terbinafine, β-terbinafine and 4-methylterbinafine - are determined. The last pharmaceutical is *Calcium pantothenát mast*. Calcium pantothenate as an active substance and methylparaben and propylparaben as preservatives are determined. During analysis of these formulations internal standard method, which increases precision and accuracy, was used. This method is very suitable with regard to isolation from ointment matrices.

All of the methods were completely validated with respect to guidelines of all mentioned institutes. Methods were found sufficiently suitable, precise and reliable for analysis of the mentioned formulations and they are applied for their routine analysis (for example during stability testing or quality control).

The developed methods were transferred to the new types of stationary phases. They were represented by monolithic columns; on those Ketoprofen gel and Estrogel gel were analyzed. An advantage of these phases is very low back pressure, so they can be used with high flow rates of mobile phases. It leads to shorter analyses. High consumption is debatable considering environmental and financial aspects (for details see attachment VIII).

A comparison of developed methods using conventional ODS columns and acquity columns in the UPLC system was described in attachment IX. Five formulations were tested – Diclofenac emulgel, Triamcinolon cream, Estrogel gel, Indomethacin gel and Hydrocortison

cream, in those contents of active substances, preservatives and degradation products were monitored. The UPLC method was advantageous from the point of view of separation quality, resolution, time of analysis and consumption of solvents. By virtue of minor band broadening more precise data evaluation and hence better analysis precision were reached. This method would be debatable considering determination of undefined impurities. The shorter recording of analysis and shorter distance between peaks on chromatogram makes monitoring of these impurities practically impossible.