SUMMARY

“Problems of Stability Testing of the Active Substances and Pharmaceutical Preparations Using HPLC”

The presented thesis deals with using of high performance liquid chromatography for the analysis of pharmaceutical active substance, its degradation products, and impurities in pharmaceutical preparations.

The theoretical part describes in a more detailed way the topic stability and impurity testing in pharmaceutical preparations. The HPLC theory, method optimization, isolation procedures, method validation, HPLC/MS, and UPLC are characterized briefly in separate chapters.

The practical part of the thesis focuses mainly on the development and validation of new chromatographic methods for simultaneous determination of active substances, impurities, and preservatives in topical pharmaceutical preparations. Six original methods, which are used for the quality control and stability testing of pharmaceuticals, were developed and validated.

The active substance indomethacin and two degradation products (5-methoxy-2-methylindoleacetic acid and 4-chlorobenzoic acid) were monitored in antiflogistic topical preparation Indomethacin gel (see attachment 5.2). For antimycotically active topical preparation Terbinafin cream, method for determination of the active substance terbinafine hydrochloride, degradation products β-terbinafine, 4-methylterbinafine, Z-terbinafine, and impurity 1-methylaminomethylnaphtalene was developed (attachments 5.3). In the Calcium pantothenate ointment the active substance calcium pantothenate and two preservatives were determined (attachment 5.4). An HPLC method for the determination of the active substance estradiol and its seven known impurities ($\Delta^{9(11)}$–estradiol, 17α estradiol, estrone, $\Delta^{9(11)}$–estrone, ethynylestradiol, estradiol 3-methyl ether, and estradiol 17-acetate) in hormonally active preparation Estrogel gel (attachment 5.5) was developed. The active substance chlorhexidine gluconate and its degradation product p-chloraniline were monitored in Amastol neo, a topical preparation for veterinary use (attachment 5.6). Two preservatives, methyl- and propylparaben, were quantified in the topical preparation Heparin gel.

Modern stationary phases were used for the chromatography because of the improvement of the separation parameters. A cyanomodified stationary phase enabled the separation of degradation products of terbinafin and estradiol. The phenyl modified stationary phase was

used for Indomethacin gel and Amastol neo ointment. Conventional ODS stationary phases were used during the method development for Heparin gel and Calcium pantothenate ointment.

All of these presented methods are developed and optimized in order to be used in drug-control laboratory. The methods were developed by using appropriate internal standard. The isolation procedures for Indomethacin gel, Estrogel gel, and Heparin gel were not difficult to be developed. The isolation of analytes from Calcium Pantotenát ointment, Terbinafin cream, and Amastol neo was more complicated. Binary extraction mixtures (Calcium Pantotenát, Amastol neo), pH modification (Terbinafin cream) or increasing temperature (Calcium Pantotenát, Amastol neo) were necessary for the quantitative isolation. All presented methods were completely validated; all tested validation and SST parameters meet the requirements of authorities.

A new UPLC method for the simultaneous determination of vitamine A and E was developed. UPLC enables fast analysis with high separation efficiency. The UPLC method was compared with a classical HPLC method performed on the ODS column (cooperation with the Department of Metabolic Care and Gerontology, Teaching Hospital, L.Urbánek, D.Solichová, B.Melichar, J.Dvořák, I.Svobodová, P.Solich, Anal. Chim. Acta, 573 (2006), 267). The UPLC analysis is three times faster and the peak shape is better than in the HPLC method. The comparison with the HPLC method performed on the monolithic column is more interesting. While UPLC works under high pressure (15.000 psi) and the flow-rate is low, low working pressure and high flow-rate are typical for the monolithic columns. Both of these methods enable fast analyses (less then two minutes); the values of the validation and SST parameters do not differ a lot. The biggest advantage of using the UPLC method is the decrease of the consumption of the mobile phase (UPLC flow-rate 0.48ml min^{-1} in contrast to HPLC flow-rate 2.5 ml min^{-1}). A statistically significant difference was not demonstrated.

The last part of the thesis deals with the analysis of esters of erythromycin in pharmaceuticals using HPLC and HPLC/MS. The great advantage of using the HPLC/MS method is the possibility of identifying impurities and degradation products resulting from the active substance.