

SUMMARY

Development and validation of methods for analysis of several drugs or their metabolites are described in this thesis. The document is presented as a commentary to the original papers, which were published in peer reviewed journals. Discussion on the optimization of each method is presented and covers also method development and influence of preanalytical aspects. Additionally, examples of the application of the developed methods in clinical pharmacology and toxicology are shown. This dissertation consists of three parts: enantiomeric determination of tramadol and its metabolite, determination of some antihypertensive drugs, and qualitative analysis of benzodiazepines.

Development of a method for chiral analysis of tramadol and its desmethylated metabolite *O*-desmethyltramadol (ODT) in human urine and plasma is described in the first part of the thesis. Tramadol is a centrally acting analgetic drug, which is used as racemate in clinical practise. Each enantiomer displays different binding properties for various receptors: (+)-tramadol preferentially inhibits serotonin reuptake while (-)-tramadol mainly inhibits noradrenalin reuptake. (+)-tramadol is considered 10-times more potent than (-)-tramadol. Major active metabolite (ODT), which is considered to be the main agent responsible for the drug-induced opioid analgesia, is formed in the liver predominantly via CYP2D6 enzyme. (-)-ODT possesses potent monoamine reuptake inhibitory activity while (+)-ODT has approximately 200-times the affinity of the parent drug. The metabolic fate of tramadol is also stereoselective. From this reason it was necessary to determine concentrations of both enantiomers for parent drug and ODT for phenotypization of polymorphic cytochrome P450 2D6. Phenotype results were compared with the results of genotypization. Metabolic ratios ODT/T correlated well with genotypes: extensive metabolizers had higher metabolite concentrations while poor metabolizers had metabolic ratios shifted to tramadol. The developed methods were also used for measurement of enantiomers in tramadol treated patients, who were hospitalised in a paediatrics intensive care unit.

LC-MS/MS methods for determination of several antihypertensive drugs are described in second part of the thesis. These analyses were performed to document non-compliance of patients, who were treated with one or more antihypertensive drugs. Eleven methods were developed for determination of antihypertensive drugs including verapamil and doxazosin. To apply the methods blood samples were analysed from 215 (104 female and 111 male) patients aged from 16 to 90 years. Interpretation of the obtained results was based on published therapeutic ranges for each drug that served as discriminating values for low, therapeutic or high levels. The results were compared with age, sex, number of prescribed drugs, and institutionalized or out-patient care. Generally better compliance was observed in men, whilst large non-compliance was observed especially in young and middle aged women. Poor compliance was also noted in patients using more than two pills. The therapeutic group of the prescribed drug did not influence compliance.

The last part of this thesis describes development of analytical method for qualitative screening for benzodiazepines in urine. Benzodiazepines belong among the most widely analysed drugs in toxicological praxis. They undergo extensive metabolic conversion that makes difficult to detect parent compounds in urine. It was therefore necessary to develop analytical method suitable for detection of parent compounds, metabolites and glucuronides. Final method consists of LC-MS/MS detection of analytes after acid hydrolysis and consecutive double liquid-liquid extraction. This method was applied for a screening of all common benzodiazepines and it was also implemented in routine laboratory praxis. The developed method is conveniently applicable for the routine use since it is accurate and time non-consumable.