Abstract

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Title of Doctoral Thesis: Quantitative measurement of drugs in biological samples using chromatography methods.

The thesis deals with the application of instrumental analytical methods to the quantitative measurement of drugs and their metabolites in biological samples using liquid and gas chromatography.

Various procedures of sample preparation were evaluated during optimization of analytical assays. Precipitation of proteins with acetonitrile was employed (analysis of cefuroxime), liquid-liquid extraction (analysis of topiramate, 8-methoxypsoralen) and samples were also purified using a mixed-mode solid-phase extraction (reversed-phase and ion-exchange principle) during the analysis of galantamine and its metabolites. The unbound cephuroxime fraction in the plasma and protein binding were determined using an ultrafiltration method.

Chromatography conditions – gas or liquid chromatography- depended on physico-chemical characteristics of a drug. Due to the thermal decomposition of topiramate, derivatization was used to form a more stable methyl derivative for GC.

During method development in case of liquid chromatogramy, optimum separation was studied using a wide spectrum of columns, most often based on silica gel bonded structural functions with unique selectivity and polarity. Ascentis (Supelco) column with amide groups was suitable for the analysis of polar cephuroxime.

In terms of resolution and peak symmetry, Discovery HS F5 column with pentafluorophenylpropyl bonded to silica gel was selected for the separation of galantamine and its metabolites epigalantamine, *O*-desmethyl-galantamine and *N*-desmethyl-galantamine. Separation of galantamine and its stereoisomer epigalantamine under reverse-phase conditions was especially remarkable.

Special section deals with the chiral separation of (*R)*-warfarin and (*S)*-warfarin. Polar organic mode and chiral cyclodextrin-based column were replaced by reverse-phase column containing pure spheric silica gel with amphotheric glycopeptide vancomycin.

HPLC detection of analytes involved UV photodiode-array detector. For fluorophore-containing structures a more sensitive and selective fluorescence detector was used (eg. analysis of galantamine and metabolites or warfarin enantiomers). Galantamine and its metabolites were identified in the liver extracts using ESI mass spectra obtained with the ion trap analyzer.

The HPLC method with fluorescence detection for determination of 8-methoxypsoralen (8-MOP) was abandoned and the capillary GC/MS method was used to improve sensitivity and selectivity. A capillary column based on the Crossbond® silarene phase was employed for the separation of 8-MOP or methyl topiramate on the GC/MS analyzer. Switching from nitrogen phosphorus detector to quadrupole mass analyzer increased sensitivity of the GC method for the determination of antiepileptic topiramate by one order of magnitude.

Following the optimization of the analytical methods, the validation characteristics were evaluated. The methods met the required limits of sensitivity, precision and accuracy, and were therefore applied to the analysis of samples from experimental studies or used for therapeutic drug monitoring.

For example, the HPLC method with fluorescence detection was employed for the quantitative determination of galantamine in the study focused on the intestinal absorption of galantamine and its modulation by transmembrane enhancer L-carnitine in rat using an in situ perfusion method.

The HPLC method using the UV photodiode-array detector allowed the determination of the bound and unbound cephuroxime fraction in the plasma and the concentration of cephuroxime in the dialysates from interstitial microdialysis of the skeletal muscle during a cardiac surgery. Pharmacokinetics of cephuroxime including plasma binding was influenced by physiological changes during cardiopulmonary bypass. Cephuroxime concentrations in the plasma and in the dialysates (in the skeletal muscle) were higher than the minimum inhibitory concentrations of potential pathogens. The dosage of cephuroxime tested in such way provided effective antibacterial concentrations in the peripheral tissue during a cardiac surgery using cardiopulmonary bypass.

Chiral separation of (*R*)- and (*S*)-warfarin on a vancomycin-based column with the fluorescence detection confirmed the same accumulation of both isomers in hepatoma HepG2 cell line.

The GC/MS methods were used for the determination of topiramate in epileptic patients and for optimization of 8-MOP dosage in patients with a bone marrow transplantation undergoing photopheresis.