SESSION I

WEDNESDAY, MAY 24th, 2017

CHAIRPERSONS:

Danica Agbaba

and Anđelija Malenović

1.

Possibilities of instrumental planar chromatography in drug analysis

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Demands to ensure safe and secure medicinal products for protection and treatment of human health and society led to the development and implementation of numerous high performance instrumental techniques for the estimation of their quality. The classical chemical methods of drug analysis have been replaced over the time with the so-called instrumental methods of analysis. The relevant regulatory authorities, EDQM, FDA and the National Drug Agencies following contemporary scientific investigations in drug research and development continuously implemented them as mandatory in the routine drug analysis methodology. In this presentation, different chromatographic methods/systems for the assessment of purity of ziprasidone and moxonidine will be discussed.

2.

Novel insights into the pH-dependent retention behavior of analytes in chaotropic chromatography

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In chaotropic chromatography quite complex mechanisms underlay the solute retention. The chromatographic behavior of analytes in these systems can be affected by type and concentration of chaotropic ions and organic modifiers, mobile phase ionic strength and stationary phase hydrophobicity. Furthermore, we have recently observed an increase in the retention times with increasing pH in the absence of any changes in the ionization state of the solutes. This result was rationalized by the increasing magnitude of surface potential that occurs due to the increasing surface excess of the chaotropic agent. Since the increase in the surface potential can be influenced by ionic strength, in this study we tested whether the observed retention behavior of completely protonated solutes and chaotropic ion adsorption were caused by a mobile phase pH variance or ionic strength effects. To that aim, two sets of experiments were performed and in the first set, the ionic strength (*I*) was varied with the concentration of $NaPF_6$ and additives that adjusted the mobile phase pH, while in the second set, *I* was kept constant by adding the appropriate amount of NaCl. In each set, the retention behavior of 13 analytes was qualitatively examined in 21 chromatographic systems, which were defined by the $NaPF_6$ concentration in their aqueous phases (1–50 mM) and the pH of their mobile phases (2, 3 or 4); the acetonitrile content was fixed at 40%.

The excess of Na^+ ions affected PF_6^- ions adsorption to the stationary phase and the magnitude of the consequential development of the surface potential significantly reducing the differences among retention factors at studied pH. An extended thermodynamic approach was used for a quantitative description of the observed phenomenon. In the set with varying *I* the contribution of ion-pair formation in the stationary phase to the retention of the solutes was confirmed at all the studied pH. On the other hand, in the systems with a constant *I*, the shielding effect of the Na^+ ions on the surface charge lowered the attractive surface potential and diminished the aforementioned interactions and consequently the effect of the mobile phase pH on solute retention.

3.

Applications of NIR Spectroscopy for Qualitative Evaluation of Substances

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Near infrared (NIR) spectroscopy is a fast, non-destructive and cheap method of spectral analysis that gains its importance. Nowadays, the applications of this analytical method are very broad: it is useful as a powerful tool in pharmaceutical industry, food industry as well as in the field of law enforcement, i.e. in identification of counterfeit medicines or quick identification of drugs of abuse, forensic medicine and many more. NIR spectroscopy was recognized as a leading analytical method of process analytical technology (PAT) applied within innovative approaches to the pharmaceutical quality assurance systems. This contribution is focused on qualitative applications of NIR spectroscopy in pharmaceutical analysis, for example, for evaluation of identity, solid forms, particle size, etc., of active pharmaceutical ingredients as well as excipients.

4.

Bioanalytically-Supported Precision Medicine (Personalized Pharmacotherapy)

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Pharmaceutics emerged as a complex of scientific disciplines, dominated by chemistry. Recently: mostly by bioanalytical chemistry. That is due to the down of precision medicine and personalized pharmacotherapy. New approaches to designing single-agent and combination medicine regimens for patient subpopulations and for individual subjects are becoming feasible owing to detailed patient information more and more available from genomic, proteomic and metabolomics platforms, along with molecular imaging and other diagnostic capabilities – all usually subjected to the advanced bioinformatics data processing. Symptomatically enough, President Barrack Obama in 2016 announced the era of precision medicine to prevail in the next decade.

The reason for the change of basic pharmacotherapeutic paradigm from "each drug fits all" to "specific drug for individual patient at optimum dose", has been unsatisfactory progress in the treatment of some diseases, cancer in particular, in spite of the dramatically increasing costs of health care. With the presently available analytical tools one can identify and quantitatively determine any substance present in diverse matrices at minute levels. By means of nanosensors specific disease biomarkers can be determined in various biological material from patients. Genomic analysis forms the basis for subpopulation classifications of patients from the point of view of drug responsiveness or resistance towards them. Large matrices of data on multitude of metabolites, determined by advanced separation techniques, usually combined with mass-spectrometric detection, can be processed chemometrically to detect metabolite profiles of a specific disease-diagnostics potency. There a term emerged: "**Theranostics**", implicating the use of a combination of a given drug (**thera**peutics") with a proper bioanalytical test ("diag**nostics**"): the so-called "companion diagnostics". The sets of drugs, for which the U.S. Food and Drug Administration requires, recommends or just offers biomolecular ("omics") tests before application are systematically increasing..

Modern bioanalytical approach has been expected to determine progress of the Evidence-Based Medicine.

5.

Analysis of basic drugs in pharmaceutical formulations, biological fluids and tissues by HPLC method

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Sorbents used in chromatographic analysis of active compounds are usually silica-based materials. They ensured high porosity and large surface areas as well as satisfactory pressure stability of materials and possibility to achieving sorbent particles of proper shape. However, the problem is sometimes connected with surface silanols. In chemically modified chromatographic sorbents instead of stationary phase ligands of different origin residual silanols play also role in mechanisms of analytes' separation. It often causes analytical problems because generated incorrect peak shapes and poor system efficiency and influences negatively selectivity of separation.

There are several methods to reduce effect of residual silanols: the use of buffered mobile phases, the use of eluent additives such as amines, acids, ion-pairing reagents and ionic-liquids. Specially synthesized stationary phases with endcaped residual silanols or embedded ligands, are often applied. Recently sorbents possessing moieties enabling π- π interactions are commercially available.

In our investigations various methods were optimized to elaborate procedures for determination of basic drugs and their metabolites in biological fluids and tissues. Often systems with double protection were applied. It means that special stationary phases synthesized for separation of basic analytes and the use of mobile phase additives were necessary for achievement of satisfactory results.

Analysis of group of psychotropic drugs in human serum and saliva were elaborated to the use for control of their level in the serum of psychiatric patients. Optimization of separation conditions was also performed for antiepileptic drugs in mouse brain tissues. Often determination of active metabolites was also necessary. Methods of simultaneous determination of acidic and basic components in pharmaceutical formulations were developed.

6.

Quantitative structure-retention relationship models based on different computational techniques in micellar liquid chromatography of antipsychotic drugs

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In QSRR studies, the retention of a compound in a chromatographic system is modeled as a function of molecular descriptors, the numerical quantities used to characterize certain chemical information. The purpose of this approach is to create a mathematical correlation model, using experimental data, which can be used to predict retention, or some other physicochemical property, of a new compound without need for additional experimentation. QSRR models are commonly built using regression-based approaches like multiple linear regression (MLR) and partial least squares (PLS). In addition to these conventional methods, various machine learning tools, such as artificial neural networks (ANN) and Support Vector Machine (SVM), are recently found to be very useful when underlying mechanisms are unknown and when complex chemical information is bearing a nonlinear relationship with response. Therefore, the aim of the present study was to put on different computational techniques and describe appropriate QSRR models with usable predictive capability for series of structurally related compounds. Presented study could also contribute to understanding of retention mechanisms in micellar liquid chromatography based on the physicochemical meaning of the molecular descriptors used for building QSRR models. The importance of this additional achievement lies in well-known fact that retention behavior of a compound in this system is very complex due to multiple chemical interactions (micelle-compound, micelle-stationary phase and compound-stationary phase).

For all these reasons, a set of compounds consisting of atypical antipsychotic drug aripiprazole and its process-related organic impurities was used. The data table for model building was composed of independent variables represented by molecular descriptors and varied chromatographic conditions. The experimental design methodology based on fractional factorial design, was used in screening the most influential instrumental parameters in the observed chromatographic system. Afterwards, the plan of experiments, according to the Box-Behnken response surface design, was used for exploring the experimental region for statistically significant parameters: the concentration of non-ionic surfactant *Brij L23* and the pH value of the water phase, as well as the percentage of organic modifier acetonitrile in the mobile phase. For every experimental point, structures of investigated compounds in their

dominant ionic and/or non-ionic form were subjected to energy minimization by the semi-empirical MOPAC/AM_1 method and used for calculation of molecular descriptors that encompass all major groups of descriptors (physicochemical, quantum-chemical, topological and spatial structural descriptors). Among large number of descriptors, the selection of ones to be included in model was done taking into consideration the intercorrelation between each pair of descriptors.

Recently developed supervised learning machine techniques were in the focus of the presented study. A computational simulation of biological networks, multi-layer perceptron artificial neural network with back propagation training algorithm, was firstly applied. The input layer in the network architecture was formed by a number of neurons equal to the total number of molecular descriptors and varied chromatographic conditions. Number of nodes in the hidden layer, number of epochs, momentum and the learning rate were optimized through the process of network training. The output layer had one node which corresponded to the retention factor of the compound. Another specific class of learning algorithms that can be used for both classification and regression analysis is represented by Support Vector Machines. They are characterized by usage of kernel functions which operate by constructing hyperplanes in a multidimensional feature space. The adoption of concept known as the structure risk minimization principle which is considered as superior to the traditional empirical risk minimization principle (employed by neural networks) is their main advantage. Additionally, gradient boosted trees and random forests were evaluated. These algorithms are based on ensembles of multiple algorithms and recently showed cutting edge results in many application areas. Finally, traditional linear regression model is evaluated as a benchmark for advanced algorithms.

Data fitting was followed with the model validation in order to ensure good predictability and estimate performance on new data. Internal validation metrics within leave-one out and 10-fold cross-validation methods were applied. Predictive performance of used modeling strategies was evaluated by the statistical significance of the model (squared correlation coefficient for model fitting), root mean square errors, absolute errors and correlation coefficients for the comparison between values predicted by the model and experimentally observed values. Additionally, stability of each model is assessed by analyses of standard deviations over each performance metric.

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