FACULTY OF PHARMACY
IN HRADEC KRÁLOVÉ
Charles University

15th Postgraduate conference

28. – 29. January 2025

Abstracts

Section 5 - Pharmaceutical Analysis

NETPHARM

This conference is supported by the project New Technologies for Translational Research in Pharmaceutical Sciences /NETPHARM, project ID CZ.02.01.01/00/22_008/0004607, co-funded by the European Union.

Co-funded by the European Union

MINISTRY OF EDUCATION, YOUTH AND SPORTS

PBA1

NEOPTERIN AND CREATININE ANALYSIS IN DRIED URINE SPOTS USING HPLC

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The dried urine spot (DUS) was developed for analysis of neopterin and creatinine. This microsampling technique requires 10 μL of urine–ten times less than conventional method. It enables home sampling using commercially available volumetric devices, while stability studies confirmed samples remain stable for 5 days at 40 °C and 3 months at ambient temperature. A simple high-performance liquid chromatographic method coupled with fluorescence and diode array detection was adjusted from routine method, incorporating 6,7-dimethylptherin as an internal standard to improve accuracy. This method was successfully applied to 36 DUS samples from healthy volunteers, patients with COVID-19 and cancer, ensuring its capability to use with varied clinical conditions. DUS significantly reduces storage space requirements and supports the regular transportation. It is also environmentally friendly, reflecting modern sustainability in clinical laboratories. By home sampling and integration with telemedicine, the DUS method enhances patient convenience, particularly for those in remote areas. This approach minimizes hospital visits while ensuring timely and reliable clinical results, increase the way for more accessible healthcare solution.

*The study was supported by the Czech Research Health Council (NU21J-02–00021 and NW24-05-00031), Charles University (SVV 260 662), MH CZ—DRO [UHHK, 00179906], Ministry of Education, Youth and Sport (**CZ.02.01.01/00/23_020/0008535**).*

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PBA2

CENTRIFUGATION-ASSISTED EXTRACTION USING C18-COATED GLASS BEADS

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Sample preparation is integral to achieving accurate, reliable, and reproducible analytical results in bioanalysis, addressing challenges posed by complex biological matrices. Various modifications of liquid-liquid extraction and solid-phase extraction are currently being developed to address the shortcomings of these techniques. These drawbacks include the volume of solvents and samples consumed, the multi-step, time-consuming process, or the need for specific apparatus. An innovative microextraction technique has been developed to analyze vitamin A in human serum. The centrifugation-assisted extraction utilizes glass beads (diameter = 30 μm) functionalized with the C18 stationary phase to capture the lipophilic analyte. An inert oil (d = 1.8 g ml^{-1}) is added after binding the analyte from the serum to the sorbent. It serves as a barrier between the solid phase (d = 2.2 g ml^{-1}) and either the aqueous or organic phase (d ~ 1 g ml^{-1}). Due to the different densities of the various layers, removing the residual serum after centrifugation and replacing this layer with the extraction reagent is possible. Subsequently, the extract is taken and prepared for HPLC analysis. The presented method uses small sample and solvent volumes, is fast, and allows the steps of analyte binding, removal of residual matrix, and extraction to be performed in a single tube.

The study was supported by the Charles University (GA UK No. 121124) and the University Hospital in Hradec Králové MH CZ DRO (UHHK, 00179906). The authors also acknowledge the Ministry of Health project NW24-05-00031 and the Ministry of Education, Youth and Sports project CZ.02.01.01/00/23_020/0008535.

PBA3

COMPARING POLYAMIDE NANOFIBERS FOR ON-LINE SOLID PHASE EXTRACTION: OPTIMIZING POLYCYCLIC AROMATIC HYDROCARBONS EXTRACTION FOR UHPLC ANALYSIS

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A novel on-line solid phase extraction method coupled with ultra-high performance liquid chromatography with fluorescence detection was demonstrated for the analysis of nine PAHs in river water. We investigated various polyamide nanofibers differing in the alkyl chain length and heteropolyamides as modern sorbent materials. PA4/6 nanofibers demonstrated the highest retention of tested analytes and comparable performance with commercial C18 sorbent in terms of peak areas and shapes. The on-line SPE-HPLC-FD method showed excellent analytical performance, with linearity ranging from 0.01 – 0.5 μg L^{-1} for acenaphthene, anthracene, pyrene, chrysene, and 0.05 – 0.3 μg L^{-1} for benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, indeno(c,d)pyrene and benzo(g,h,i)perylene, achieving determination coefficients (R^2) between 0.9989 – 0.9999. Limits of quantification were in range 0.01 – 0.05 μg L^{-1}. Recoveries were between 92% and 105%, with RSD 1.5 – 6.7 %. The total time for the extraction and analysis of nine analytes was 14 minutes. Furthermore, the polyamide-based sorbent requires no modifications and can be cost-effectively manufactured through electrospinning as homogeneous mats. New sorbent can be cut and manually packed into the column, allowing for easy and affordable column replacement, when necessary, with repeatability between columns yielding RSD < 13%.

The study was supported by the Czech Science Foundation through the project no. 23-05586S and Charles University project GAUK no. 110424 and SVV 260 662.

PBA4

AUTOMATED CENTRIFUGATION-LESS MILK DEPROTEINIZATION AND SALTING-OUT HOMOGENOUS LIQUID-LIQUID EXTRACTION COUPLED ONLINE TO HPLC FOR THE DETERMINATION OF WATER-SOLUBLE VITAMINS IN PLANT-BASED MILK

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An overview is given on the development of an automated methodology for determination of selected water-soluble vitamins, namely B1 (Thiamine), B2 (Riboflavin), B3 (Niacinamide), B3 (Nicotinic acid), B6 (Pyridoxine), B9 (Folic acid), B12 (Cyanocobalamin) and internal standard (Theophylline) in plant-based milk samples. The chosen milk samples were oat, soy, almond, hazelnut, and coconut, with fat contents ranging from 0.8 to 3.5 %. The method is based on centrifugation-less milk deproteinization and salting out homogenous liquid-liquid extraction was automated via the flow-batch technique Lab-In-Syringe (LIS) [1] and coupled online to high-performance liquid chromatography (HPLC) with photodiode array detection.

The extracts obtained were evaporated to increase sensitivity and transferred to online coupled liquid chromatography for analyte separation. The separation process was carried out in parallel to the subsequent extraction, evaporation, and system cleaning. Parameters influencing the method performance including salt solution type and volume, solvent-to-sample ratio, extraction pH, stirring rate, stirring time, and evaporation conditions were optimized as well as the HPLC method for the separation of the analytes. A preliminary evaluation of the applicability of the developed procedure will be presented.

This study was supported by Charles University SVV 260 662 and GAUK project no. 206623.
This study was co-funded by the European Union under the ATEBIO project (Advanced Techniques for Biomedical Diagnostics, Project ID CZ.02.01.01/00/23_020/0008535)

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PBA5

BIOANALYTICAL STUDY OF A NOVEL POTENTIAL CARDIOPROTECTIVE DRUG

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JM-437 is a promising novel cardioprotective drug that demonstrates exceptional potential in mitigating anthracycline-induced cardiotoxicity. With its unique mechanism of action, this compound has attracted significant attention in both *in vitro* and *in vivo* studies. Consequently, establishing a reliable bioanalytical method to study its behavior in the organism is essential. The primary objectives of this study were: (a) to identify the *in vitro* metabolites of JM-437 and to confirm their presence in *in vivo* samples, and (b) to develop a robust UHPLC-MS/MS method for the quantification of JM-437 and its main metabolite in plasma and whole blood microsamples collected by volumetric absorptive microsampling (VAMS) devices.

We used UHPLC coupled with MS/MS or HRMS (ESI+ ion source) and a ZORBAX Eclipse Plus C18 column (150 × 2.1 mm, 1.8 μm) with a mobile phase of 0.1% formic acid and acetonitrile. Six phase I metabolites of JM-437 were identified *in vitro* using human and mouse microsomes and *in vivo* in rabbit and mice samples. Seven phase II metabolites were detected only *in vivo*. Subsequently, a method for the determination of JM-437 and its demethylated metabolite in plasma and blood microsamples was developed, validated, and utilized to quantify the analytes in rabbit's *in vivo* samples, providing a detailed pharmacokinetic profile.

The study was supported by Charles University (SVV 260 666) and INTER-EXCELLENCE II programme of The Ministry of Education, Youth and Sports of the Czech Republic. (Project No. LUAUS24335).

PBA6

TARGETED UHPSFC-MS/MS STEROID ANALYSIS: EVALUATION OF UNISPRAY OVER ELECTROSPRAY IONIZATION

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Over the years, steroid hormones, originating from a single precursor of cholesterol, have represented several indisputable roles, among which their clinical relevance, e.g., in stress research, and their frequent adoption as model compounds must be raised. Yet, despite the vast number of published studies using traditional GC-MS and LC-MS techniques, accurate steroid quantification represents an ongoing analytical challenge. These hurdles are associated with high structural similarity, resulting in overlapping retention times, steroid crosstalk between stereoisomers and positional isomers, and similar fragmentation patterns. Another hurdle is then linked with the lack of ionizable functional groups and the neutral character of steroids, differing among steroid classes, e.g., progestogens, corticosteroids, androgens, and crucially affecting method sensitivity.

In the present study, we aimed to (i) investigate the separation selectivity of the UHPSFC-MS/MS method for 36 steroid hormones, covering 15 stereoisomers and 17 positional isomers with overlapping fragmentation patterns, by optimizating chromatographic separation on 19 analytical columns; (ii) explore the effect of UniSpray on ionizability of steroids in UHPSFC by thorough optimization of source setup, ionization source conditions using design of experiment approach, and makeup solvent composition and flow rate; (iii) compare its performance with ESI source by estimation of signal intensity increase, signal-to-noise ratio, limits of detection and quantification, linear dynamic range, and matrix effects, and (iv) validate the final UHPSFC-UniSpray-MS/MS in terms of matrix-matched calibration curve range, its linearity and %-errors, and parameters of recovery, matrix effects, accuracy and precision evaluated on 6 concentration levels.

The study was supported by the GA UK project No. 348 221, the project of specific research SVV 260 662, and the project New Technologies for Translational Research in Pharmaceutical Sciences (NETPHARM CZ.02.01.01/00/22_008/0004607) co-funded by the European Union.

PBA7

ALTERNATIVE WEAK ACIDIFIERS FOR IMPROVED DETECTION SENSITIVITY IN BOTTOM-UP PROTEOMICS

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Although formic acid is widely accepted as the additive of choice for peptide reversed-phase LC-MS/MS applications, other weak acidifiers may present a better alternative. In this study, we evaluated the applicability of alternate weak acids as mobile phase additives for bottom-up proteomics using columns packed with a C_{18} stationary phase with a positively charged surface. Using 0.5% acetic and propionic as mobile phase additives, maintained excellent chromatographic performance and increased MS signal response compared to the standard 0.1% formic acid. Relative to formic acid we found an overall MS intensity increase of 2.5x for acetic acid and 3.5x for propionic acid. The increase in the MS intensity led to improved peptide identification. The method can be easily implemented on any LC-MS/MS system without modification and at no additional cost.

The study was supported by the Czech Science Foundation (GAČR No. 22-21620S), Charles University Grant Agency (GAUK No. 370522), and specific research project No. SVV 260662

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PBA8

PHYSICOCHEMICAL CHARACTERIZATION AND IDENTIFICATION OF DEGRADATION PRODUCTS OF LENVATINIB MESYLATE WITH EXCIPIENTS BY LC-MS/MS

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The aim of this work was to evaluate the characterization and identification of compatibility study of Lenvatinib Mesylate (LEN) with the commonly used excipients for solid and liquid dosage form using thermogravimetric analysis, differential scanning calorimetry, isothermal stress testing (IST) by HPLC, LC-MS/MS, and Fourier transform infrared spectroscopy to contribute to the interpretation of the obtained results.[1] The selected excipients were magnesium stearate (MS), sodium starch glycolate (SSG), croscarmellose sodium (CCS), pregelatinized starch (PS), microcrystalline cellulose (MCC), pluronic acid (PA), stearic acid (SA), and sodium taurocholate (ST). During IST studies less than 10 % change in LEN content was observed in all stressed binary mixtures stored at 50 °C except of LEN–PA, LEN–SA and LEN–ST which showed incompatibility with LEN. On the basis of the LC-MS/MS results, two degradation products (DP1 and DP2) were identified with all the excipients mixtures. The *m/z* of the identified DP1 and DP2 were 410.0821 and 465.0642 simultaneously. The obtained information was utilized for the outlining the comprehensive degradation pathway of the LEN.

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