Bioanalytics, lipidomics and metabolomics application of one- and two-dimensional LC-MS techniques

THESIS OF DOCTORAL (PHD) DISSERTATION

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1. Introduction and aims

Bioanalysis is the field of analytical chemistry for the analysis of biological samples. Bioanalysis is not just the analytical measurement itself but it is a complex process that involves problem definition, analytical method design, sample preparation, method development, measurement execution, evaluation of the data, (validation if necessary) and documentation.

In the analysis of biological samples, the compounds may be substances naturally presented in the organism (endogenous) or released from the environment in some way (exogenous), which are involved and transformed in various biochemical processes.

The omics are various disciplines in biology whose names end in the suffix –omics, such as genomics which focuses on the structure, function, evolution, mapping and editing of genomes; transcriptomics used to study a transcriptome of an organism, the sum of all of its RNA transcripts; the large-scale study of proteins is proteomics and metabolomics, which is the scientific study of biochemical processes involving the small molecule substrates, intermediates and products of cell metabolism. Lipidomics ofter referred to as part of metabolomics, is the large-scale study of pathways and networks of cellular lipids in biological systems.

Bioanalytical methods are widely used in drug research, forensic science, food industry, food safety, cosmetics industry, biomedical research (genomics, proteomics, metabolomics, lipidomics, biomarker research), medical diagnostics or environmental protection as well. Therefore, the goal is to develop analytical methods that can analyse a smaller amount and larger number of samples within an appropriate duration in a reliable manner. Widespread liquid chromatography-mass spectrometry (LC-MS) provides an excellent solution for this purpose. One-dimensional liquid chromatography (LC) is one of the most significant widespread separation techniques for the study of complex biological matrices. Offline and online two-dimensional liquid chromatographies (2D-LC) have been shown to be powerful tools to resolve the coelution of the high number of analytes, in particular, in the field of proteomics, metabolomics and lipidomics.

The main goal of this doctoral dissertation was to develop and apply one- and two-dimensional liquid chromatography coupled to mass spectrometry methods to answer biomedical questions in the bioanalytical approach.

Although anxiety disorders are among the most widespread diseases, their pathogenesis is still poorly understood. Lipids can play a key role in neural processes, since lipid composition of the brain significantly influences subjective perception, mood and behaviour. Previous research has shown that the key areas of the brain are the prefrontal cortex, ventral hippocampus and dorsal hippocampus, which functionally interact during innate anxiety tasks. Therefore, our studies focused on these three brain areas. Due to the complexity of the brain, we developed a new online, comprehensive 2D-UHPLC-HRMS method to study the glycerophospholipid and sphingomyelin composition of different brain regions of anxious and non-anxious mice by a combination of hydrophilic interaction liquid chromatography (HILIC) and reversed-phase (RP) separation. The additional aim was to determine significant alterations in the plasma phospholipid composition related to anxiety using hydrophilic interaction liquid chromatography coupled with new, high resolution mass spectrometry (HILIC-HRMS) method.

Dimethyltryptamine (DMT) is an indole alkaloid found naturally in plants and animals. The synthesis of endogenous DMT in mammals begins with the decarboxylation of tryptophan to tryptamine, followed by a methylation step performed by a methyltransferase enzyme, *e.g.*, indolethylamine *N*-methyltransferase (INMT). The second enzymatic metabolism produces DMT from methyltryptamine. INMT is produced in many tissues, and it can be concluded that wherever the INMT enzyme is present, there is also endogenous DMT. INMT is produced in a most abundant manner in the lungs, adrenal glands, thyroid gland and brain. DMT is produced by somatic cells under physiological conditions, and its concentration has been found to increase in response to pathophysiological homeostatic challenges (*e.g.*, hypoxia or oxidative stress). DMT administered at a supraphysiological concentration to experimental rodents reduced the occurrence of cerebral infarction. Thus, the main goals of this study were the development of a fast, targeted heart-cutting 2D-LC-HRMS/MS method and optimisation of sample preparation procedures for the quantitative analysis of DMT in rat brain and plasma using a relatively low amount of biological samples. The method thus developed was applied for the determination of DMT level in rat plasma and brain of experimental model of cerebral ischemia/reperfusion using DMT administration.

Synthetic cannabinoids (SCs) are the largest group of new psychoactive substances. Their appearance on the black market changes rapidly, so their identification and the establishment of consumption is a particularly important task in the field of forensic

toxicology. Due to the rapid metabolism of synthetic cannabinoids, it is important to analyse not only the parent molecules but also their characteristic metabolites. Methyl 2-({[1-(4-fluorobutyl)-1H-indol-3-yl]carbonyl}amino)-3,3-dimethylbutanoate (4F-MDMB-BICA), as a new dangerous illicit synthetic cannabinoid, was identified in 51 different police seizures, and 11 deaths were attributed to its consumption in Hungary until 11 August 2020. The aim of our work was to identify human liver microsome (*in vitro*) and human urine and blood (*in vivo*) phase I metabolites of 4F-MDMB-BICA. Another goal was to extend our targeted routine analytical LC-MS/MS method with a selected characteristic phase in order to justify the use of 4F-MDMB-BICA even if the level of the parent molecule in both blood and urine falls below the detection limit.

2. Applied substances and experimental methods

Mouse plasma and brain, rat plasma and brain, human liver microsome, human blood and urine biological samples were investigated. Several reversed-phase and HILIC chromatographic columns were tested during method developments, and the related chromatographic and mass spectrometric parameters were optimised. The LC-MS analytical methods were carried out by two LC-MS systems:

- Waters Acquity I-Class UPLC™ (Waters, Manchester, UK) ultra-high performance liquid chromatography system coupled to Thermo Scientific Q Exactive™ Plus hybrid quadrupol-Orbitrap™ (ThermoFisher Scientific, Waltham, USA) mass spectrometer
- Shimadzu Nexera (Kyoto, Japan) UHPLC system coupled to TSQ Fortis triple quadrupole (Thermo Scientific, Waltham, USA) mass spectrometer.

The data were collected using Xcalibur software (ThermoFisher Scientific, Waltham, USA). The non-targeted lipidomic and metabolomic data were processed by Progenesis QI 2.1 (Nonlinear Dynamics, Newcastle, UK). Identification of lipid species was accomplished via database searching of LIPID MAPS and our homemade database built on our own measurements. Multivariate data analysis, including the normalised peak area and the orthogonal partial least square discriminant analysis, was performed by SIMCA software 14.1 (Umetrics, Umeå, Sweden). For additional statistical evaluations, the GraphPad Prism 5 statistical software (GraphPad Software Inc., La Jolla, USA) was used. The semi-targeted UHPLC-HRMS/MS raw files from metabolomics studies were processed manually using

Qual Browser feature of Xcalibur software in order to obtain qualitative information. For quantitative evaluation, the integrated processing application of Xcalibur software was used.

3. Novel scientific results

T1. Significant differences were found in the plasma glycerophospholipids and sphingomyelin concentration of anxious and non-anxious mice by using our new rapid HILIC-HRMS method.

With inbreeding the anxious and non-anxious mouse strains, it is possible to study anxiety disorder at the molecular level. In a mouse model of anxiety disease, we hypothesised that there could be a significant difference in the plasma phospholipid composition of anxious and non-anxious mice. In order to confirm the hypothesis, we developed a rapid comprehensive HILIC-HRMS method and used it for the quantification of 130 phospholipids covering 10 phospholipid classes in mouse plasma. By comparing the plasma phospholipid composition of 8 anxious and 8 non-anxious mice, significant increases in the total concentration of phosphatidylcholine (PC), phosphatidylinositol (PI), lysophosphatidylethanolamine (LPE) and sphingomyelin (SM) classes were found in the anxious group. In the anxious group, 33 phospholipid species were determined to be up-regulated, while down-regulation was found only for PC 38:4 and PC 42:6. The highest observed fold changes (>2) were found compared with the two groups in PI 32:1, PI 36:5, LPE 16:1 and LPE 20:5, respectively.

The glycerophospholipid abbreviations (PC, PI, etc.) are used to refer to species with one or two radyl side-chains where the structures of the side chains are indicated within parentheses in the C-atoms:double bonds format. For example, PC 38:4 is a phosphatidylcholine with 38 C-atoms and 4 double bonds in the fatty acid side chains.

T2. By application of the developed comprehensive online 2D-UHPLC/HRMS analytical method, alterations were determined in the phospholipid composition of the three brain areas of anxious and non-anxious mice.

Thanks to the animal model of anxiety, we have been able to investigate the neural pathway that can be associated with anxiety. In this study, we determined the phospholipid composition of three brain areas of anxious and non-anxious mice (dorsal hippocampus, ventral hippocampus and prefrontal cortex) using a novel, comprehensive online 2D-UHPLC-

HRMS coupled technique. The 2D-UHPLC separation was carried out by a combination of HILIC in the first dimension followed by second-dimensional RP chromatography. In the first dimension, lipid classes were distinguished by HILIC, while the second-dimensional separation of individual phospholipid and SM species was achieved by RP chromatography. For the enrichment of diluted HILIC eluent and for the RP separation of trapped lipid species, two RP trapping columns were used separately. The final method provided quantificitaion of 151 phospholipid species in three brain regions within 40-minute run time. With the established method, the differences of phospholipid composition in brain regions of 9 non-anxiety and 8 anxiety mice were compared. Our study revealed that 37 glycerophospholipid and sphingomyelin species had significantly altered concentration in the anxiety group: 20 were found in ventral hippocampus, 6 in dorsal hippocampus and 11 in prefrontal cortex. For PE 40:5, significant changes were observed in all three regions regularly.

T3. Determination of endogenous and exogenous dimethyltryptamine (DMT) concentrations in rat brain and plasma by new, targeted heart-cutting 2D-UHPLC-HRMS/MS method.

DMT produced by somatic cells has been shown to increase in concentration due to oxidative stress or hypoxia. Thus, the putative neuroprotective action of DMT has been studied in models of cerebral and renal ischemia. In our study, we investigated the neuroprotective effect of intravenous infused DMT during cerebral hypoxia and monitored the changes in exogenous DMT concentrations.

By connecting orthogonal HILIC and RP chromatography through the use of RP trap column and HRMS/MS detection, a sensitive and selective analytical method was successfully utilised within a total run time of 10 min for determination of the concentration of DMT in rat plasma and brain of an experimental model of cerebral ischemia/reperfusion. The new liquid–liquid extraction sample preparation procedure and the novel heart-cutting 2D-UHPLC-HRMS/MS method gave an opportunity to improve the limit of detection compared to the literature data, using a smaller amount of biological samples. A further advantage of the new sample preparation method is that the recovery for DMT in plasma is better by approximately 20–30% than data in early studies. The obtained favourable matrix effect values for both compounds from a complex biological matrix clearly indicated the benefit of the application of this 2D-UHPLC-HRMS/MS method. The 10-min total run time is faster than various

published one-dimensional LC-MS/MS methods for DMT analysis in brain and plasma. Another advantage is that α-methyltryptamine as internal standard and DMT as targeted compound eluted with the same retention time, thus the matrix effect was the same for both compounds during the MS detection. The obtained results show that while the concentration of DMT decreases after hypoxia in the plasma then it is increased in the brain compared to the control group. Unforunately, we could not confirm the presence of endogenous DMT in either the brain or the plasma. Based on the obtained results, it can be concluded that the new analytical method was successfully used to confirm the presence of DMT administered to experimental animals with therapeutic purposes.

T4. Characterisation of the latest synthetic cannabinoid metabolites of 4F-MDMB-BICA *in vitro* human liver microsomes, *in vivo* human blood and urine samples with a complex analytical approach consisting of comprehensive LC-HRMS, semi-targeted LC-HRMS/MS and targeted LC-MS/MS analytical methods.

Nowadays, the black market for designer drugs is changing dynamically. Consequently, to establish consumption, it is essential to identify and quantify newly released synthetic cannabinoids as quickly as possible. Due to their rapid metabolism, in many cases, despite the recognition of their consumption, the mother compound could not be identified in biological fluids. In our work, we aimed to identify the phase I metabolites of 4F-MDMB-BICA synthetic cannabinoid released in Hungary in 2020. In our research, we used a new analytical approach to determine metabolites consisting of comprehensive LC-HRMS, semi-targeted LC-HRMS/MS and targeted routine LC-MS/MS analytical methods. The obtained HRMS data from *in vitro* comprehensive LC-HRMS measurements were statistically deconvoluted by Progenesis QI software. Molecular ions selected were further studied by semi-targeted LC–HRMS/MS measurements in order to obtain structural information of interests. The possible substructures or structures of unknown molecular ions were determined by manually comparing the related HRMS/MS spectra with their hypothetical fragmentation, which was based on the fragmentation of the parent compound and literature data of other SCs with similar core, linker and linked group. This study provides the first characterisation of the *in vitro* and *in vivo* metabolites of 4F-MDMB-BICA with a new analytical approach. We identified several biotransformations affecting the structure of 4F-MDMB-BICA (core, tail, linker and linked group), such as ester hydrolysis, dealkylation, oxidative defluorination,

dehydrogenation, mono- and di-hydroxylation, amide hydrolysis, carboxylation and their combinations. In general, the selection of at least two main metabolites is recommended in routine toxicological analysis to obtain reliable qualitative data to prove synthetic cannabinoid consumption. Thus, based on the results of the semi-targeted LC-HRMS/MS measurement of human urine and blood samples, we selected the ester hydrolysis metabolite as the primary biomarker of both body fluids. The urinary mono-hydroxylation metabolite and the ester hydrolysis and dehydrogenation substances in blood were selected as secondary confirmatory targets for the screening of 4F-MDMB-BICA.

T5. For verification of consumption of 4F-MDMB-BICA in human blood and urine, the identified characteristic metabolites were adapted to our routine targeted LC-MS/MS method.

Our targeted LC-MS/MS forensic analytical method was further developed to be suitable for the quantitative determination of the 4F-MDMB-BICA synthetic cannabinoid and the qualitative determination of selected characteristic metabolites in urine and blood samples. In the 6-min analysis, the transitions (quantifier and qualifier ions) of 4F-MDMB-BICA parent compound and the three selected metabolites were aligned to the 32 synthetic cannabinoids (parent molecule and metabolites). For the quantitative analysis of 4F-MDMB-BICA, the main validation parameters, such as limit of detection, limit of determination, linearity, accuracy, precision, recovery, matrix effect, process efficiency and carryover were determined. Overall, the obtained main validation parameters verify that our targeted LC–MS/MS method with related sample preparation procedures is suitable to screen 4F-MDMB-BICA in both urine and whole blood samples. As a next step, we determined the concentration of the parent molecule 4F-MDMB-BICA in two blood and five urine samples. By the application of the targeted method, the parent compound was detectable only in one urine sample (0.970 ng/mL), while it was quantifiable (0.920 ng/mL and > 10 ng/mL) in both blood samples. However, the selected characteristic metabolites could be detected in all blood and urine samples. This underlines the importance of the metabolomic approach in the analysis of synthetic cannabinoids in forensic and toxicological practice.

4. Application of the results

The developed new bioanalytical methods presented in my dissertation have been successfully applied to answer the biomedical questions arising during the research. The developed one- and two-dimensional analytical methods were effectively used to determine brain and plasma phospholipid levels. On the other hand, the obtained results clearly confirmed the role of phospholipids in the mouse model of anxiety disorders. We developed a rapid and reliable targeted two-dimensional bioanalytical method that was successfully used to determine the brain and plasma concentrations of DMT administered to reduce the cytotoxic effect during ischemia. Additional biological experiments (not part of my doctoral dissertation) demonstrated that DMT administered intravenously during the experiment prevented ischemic cell damage. Phase I metabolites of 4F-MDMB-BICA, both *in vitro* and *in vivo*, were identified using the comprehensive and semi-targeted analytical approach. The selected characteristic metabolites of 4F-MDMB-BICA were detectable in blood and urine, regardless of the detectability of the parent molecule. With the parent compound and selected metabolites, our routine targeted analytical method was improved and it has been used successfully in forensic practice since then.

5. Publications

ID of Hungarian Collection of Scientific Publications (MTMT): 10062186

5.1. Papers related of the Theses published in refereed journals

1. R. Berkecz, **T. Körmöczi**, F. Tömösi, V. Szegedi, J. Horváth, N. Kovács, T. Janáky
   Plasma phospholipid profiling of a mouse model of anxiety disorder by hydrophilic interaction liquid chromatography coupled to high-resolution mass spectrometry
   Biomedical Chromatography, 32 (**2018**) e4202.
   DOI: 10.1002/bmc.4202 **IF: 1.760** (SJR Indicator: Q2)

2. R. Berkecz, F. Tömösi, **T. Körmöczi**, V. Szegedi, J. Horváth, T. Janáky
   Comprehensive phospholipid and sphingomyelin profiling of different brain regions in mouse model of anxiety disorder using online two-dimensional (HILIC/RP)-LC/MS method
   Journal of Pharmaceutical and Biomedical Analysis, 149 (**2018**) 308-317.
   DOI: 10.1016/j.jpba.2017.10.043 **IF: 3.077** (SJR Indicator: Q1)

3. **T. Körmöczi**, Í. Szabó, E. Farkas, B. Penke, T. Janáky, I. Ilisz, R. Berkecz
   Heart-cutting two-dimensional liquid chromatography coupled to quadrupole-orbitrap high resolution mass spectrometry for determination of N,N-dimethyltryptamine in rat plasma and brain; Method development and application
   Journal of Pharmaceutical and Biomedical Analysis, 191 (**2020**) 113615
   DOI: 10.1016/j.jpba.2020.113615 **IF: 3.935** (SJR Indicator: Q1)

4. **T. Körmöczi**, É. Sija, L. Institóris, É. Kereszty, I. Ilisz, R. Berkecz
   Analytical Methodologies for the Characterisation and Analysis of the Parent Compound and Phase I Metabolites of 4F-MDMB-BICA in Human Microsome, Urine and Blood Samples
   Journal of Analytical Toxicology, (**2021**) bkab004.
   DOI: 10.1093/jat/bkab004 **IF(2020): 3.367** (SJR Indicator: D1)
   Summarized IF: 12.139

5.2. Additional journal publications also related to the topic of thesis

1. K. Kovács, É. Kereszty, R. Berkecz, L. Tiszlavicz, É. Sija, **T. Körmöczi**, N. Jenei, H. Révész-Schmehl, L. Institóris
   Fatal intoxication of a regular drug user following N-ethyl-hexedrone and ADB-FUBINACA consumption
   Journal of Forensic and Legal Medicine, 65 (**2019**) 92-100.
   DOI: 10.1016/j.jflm.2019.04.012 **IF:1.302** (SJR Indicator: Q1)

2. Í. Szabó, V.É. Varga, S. Dvorácskó, A.E. Farkas, **T. Körmöczi**, R. Berkecz, S. Kecskés, Á. Menyhárt, R. Frank, D. Hantosi, N.V. Cozzi, E. Frecska, C. Tömböly, I.A. Krizbai, F. Bari, E. Farkas
   N,N-Dimethyltryptamine attenuates spreading depolarisation and restrains neurodegeneration by sigma-1 receptor activation in the ischemic rat brain
   Neuropharmacology, 192 (**2021**) 108612
   DOI: 10.1016/j.neuropharm.2021.108612 **IF(2020): 5.250** (SJR Indicator: Q1)

3. V. Kovács, G. Remzső, **T. Körmöczi**, R. Berkecz, V. Tóth-Szűki, A. Pénzes, L. Vécsei, F. Domoki
   The Kynurenic Acid Analog SZR72 Enhances Neuronal Activity after Asphyxia but Is Not Neuroprotective in a Translational Model of Neonatal Hypoxic Ischemic Encephalopathy
   International Journal of Molecular Sciences, 22.9 (**2021**) 4822
   DOI: 10.3390/ijms22094822 **IF(2020): 5.923** (SJR Indicator: D1)

4. L. Institóris, K. Kovács, É. Sija, R. Berkecz, **T. Körmöczi**, I. Németh, I. Elek, Á. Bakos, I. Urbán, C. Pap, É. Kereszty
   Clinical symptoms and blood concentration of new psychoactive substances (NPS) in intoxicated and hospitalised patients in the Budapest region of Hungary (2018-19)
   Clinical Toxicology, (**2021**) 1-7.
   DOI: 10.1080/15563650.2021.1928162 **IF(2020) 4.467** (SJR Indicator: Q2)

5. S. Hornok, R. Berkecz, E. Sós, A. D. Sándor, T. Körmöczi, N. Solymosi, J. Kontschán, A. Hunyadi
   Arthropod moulting hormones (ecdysteroids) are present in the blood of insectivorous bats
   Mammal Review, (**2022**)
   DOI: 10.1111/mam.12283 **IF(2021) 4.927** (SJR Indicator: D1)

Summarized IF: 21.869
ΣΣ IF: 34.008

9.3. Oral presentations related to the topic of thesis

1. **T. Körmöczi**, É. Sija, R. Berkecz
Dizájner drogok fogyasztásának igazolása a metabolomika segítségével
Tavaszi Szél Konferencia 2018: Nemzetközi Multidiszciplináris Konferencia, 4-6th May **2018**, Győr, Hungary

2. É. Sija, R. Berkecz, L. Institóris, **T. Körmöczi**, T. Janáky, É. Kereszty
Detections and Metabolism of Three Synthetic Cannabinoids
26th International Meeting on Forensic Medicine, Alpe-Adria-Pannonia, 30th May-2nd June **2018**. Pula, Croatie

3. É. Sija, R. Berkecz, L. Institóris, **T. Körmöczi**, É.M. Kereszty
Adb-Fubinaca okozta intoxikáció büntetés-végrehajtási intézetben, Esetbemutatás
A Magyar Igazságügyi Orvosok Társasága XVI. Nemzetközi Konferenciája, 30th August-1st September **2018**, Pécs, Hungary

4. B. Barna, **T. Körmöczi**, É. Sija, R. Berkecz
Laboratory challenges of detecting synthetic cannabinoids in urine samples – a new sample preparation method
24th International Symposium on Analytical and Environmental Problems, 8-9th October **2018**, Szeged, Hungary

5. É. Sija, R. Berkecz, L. Institóris, **T. Körmöczi**, É. Kereszty
Szintetikus kannabinoidok in vitro és in vivo képződő metabolitjainak vizsgálata
TOX'2018 Tudományos Konferencia, 17-19th October **2018**, Lillafüred, Hungary

6. **T. Körmöczi**, O. Kovács, É. Sija, Á. Hunya, R. Samavati, R. Gáspár, L. Institóris, I. Ilisz, R. Berkecz
Analysis of Designer Drugs and Their Metabolites in Blood and Urine Samples
25th International Symposium on Analytical and Environmental Problems, 7-8th October **2019**, Szeged, Hungary

7. É. Sija, L. Institóris, R. Berkecz, **T. Körmöczi**, K. Kovács, É.M. Kereszty
5F-MDMB-PICA és 5F-MDMB-PINACA metabolitjainak in vitro és in vivo vizsgálata
TOX'2019 Tudományos Konferencia, 9-11th October **2019**, Szeged, Hungary

8. **T. Körmöczi**, O. Kovács, É. Sija, Á. Hunya, R. Samavati, R. Gáspár, L. Institóris, I. Ilisz, R. Berkecz
Dizájner drogok és metabolitjaik az igazságügyi gyakorlatban
XLII. Kémiai Előadói Napok, 28-30th October **2019**, Szeged, Hungary

9. **T. Körmöczi**, É. Sija, R. Berkecz
The Most Common Synthetic Cannabinoids in the Last Year; Focusing on Their Metabolites in Biofluids
26th International Symposium on Analytical and Environmental Problems, 23-24th November **2020**, Szeged, Hungary, online

5.4. Poster presentations related to the topic of thesis

1. R. Berkecz, F. Tömösi, **T. Körmöczi**, T. Janáky
Development and Application of a Novel Comprehensive Online Two-Dimensional Liqud Chromatograpy Coupled with High-Resolution Mass Spectrometry Method in Lipidomics
11th Balaton Symposium on High-Performance Separation Methods in memoriam of Ernö Tyihák, 6-8th September **2017**, Siófok, Hungary

2. **T. Körmöczi**, É. Sija, R. Berkecz
Analysis of new synthetic cannabinoid in human urine by LC-MS/MS
23rd International Symposium on Analytical and Environmental Problems, 9-10th October **2017**, Szeged, Hungary

3. É. Sija, **T. Körmöczi**, F. Tömösi, T. Janáky, L. Institóris, É.M. Kereszty, R. Berkecz
ADB-FUBINACA és CUMYL-PEGACLONE metabolitjainak azonosítása human májmikroszómából
TOX'2017 Tudományos Konferencia, 11-13th October **2017**, Bükfürdő, Hungary

4. **T. Körmöczi**, É. Sija, R. Berkecz
Challenges in Detecting Synthetic Cannabinoids in Human Urine Samples (Focus on their Metabolism)
36th Informal Meeting on Mass Spectrometry, 6-9th May **2018**, Kőszeg, Hungary

5. **T. Körmöczi**, É. Sija, O. Nagy, Zs. Ruppert, Á. Hunya, R. Berkecz
Challenges in Detection of the Recetly Emerged Syntethic Cannabinoids in Human Urine Samples
26th International Meeting on Forensic Medicine, Alpe-Adria-Pannonia, 30th May-2nd June **2018**, Pula, Croatie

6. A. Dweny, **T. Körmöczi**, O. Kovács, R. Samavati, R. Gáspár, R. Berkecz
Development of targeted LC-MS/MS method for analysis of diclofenac and its main metabolites in rat liver perfusion solution obtained by new type of ex vivo perfusion system
24th International Symposium on Analytical and Environmental Problems, 8-9th October **2018**, Szeged, Hungary

7. **T. Körmöczi**, É. Sija, R. Berkecz
Identification of the main metabolites of three synthetic cannabinoids using LC-MS/MS technique
24th International Symposium on Analytical and Environmental Problems, 8-9th October **2018**, Szeged, Hungary

8. **T. Körmöczi**, O. Kovács, R. Samavati, É. Sija, R. Gáspár, R. Berkecz
Májperfúziós ex vivo vizsgálat lehetőségei a dizájner drog metabolit kutatásban
TOX'2018 Tudományos Konferencia, 17-19 October **2018**, Lillafüred, Hungary

9. **T. Körmöczi**, O. Kovács, A. Dweny, R. Samavati, R. Gáspár, R. Berkecz
Újtípusú májperdúziós módszer kísérleti beállítása diklofenák hatóanyag segítségével
Elválasztástudományi Vándorgyűlés, 8-9th November **2018**, Tapolca, Hungary
*poster first prize

10. O. Kovács, R. Samavati, **T. Körmöczi**, R. Berkecz, R. Róbert
Development of vapor chamber rat liver perfusion system for metabolite research
RECOOP 14th Bridges in Life Sciences Conference, 10-12th April **2019**, Bratislava, Slovakia

11. R. Berkecz, **T. Körmöczi**, Í. Szabó, E. Farkas, E. Frecska, T. Janáky
Development and application of heart-cutting 2D-LC-MS/MS method for analysis of N,N-dimethyltryptamine in brain samples
12th Balaton Symposium on High-Performance Separation Methods, 11-13th September **2019**, Siófok, Hungary

12. **T. Körmöczi**, O. Kovács, R. Samavati, R. Gáspár, R. Berkecz
Ex vivo Pharmmacokinetic Profiles of CUMYL-PeGaCLONE Synthetic Cannabinoid and Its Metabolites
12th Balaton Symposium on High-Performance Separation Methods, 11-13th September **2019**, Siófok, Hungary

13. N. Kmetykó, **T. Körmöczi**, Í. Szabó, E. Farkas, T. Janáky, I. Ilisz, R. Berkecz
Determination of Dimethyltryptamine in Rat Plasma Using 2D-LC-MS/MS Method
25th International Symposium on Analytical and Environmental Problems, 7-8th October **2019**, Szeged, Hungary

14. L. Institóris, É. Sija, I. Elek, R. Berkecz, **T. Körmöczi**, É. Kereszty
Designer drogok gyakorisága és jellemzése intoxikált droghasználók vérmintáinak idősoros analízise alapján
TOX'2019 Tudományos Konferencia, 9-11th October **2019**, Szeged, Hungary

15. Í. Szabó, **T. Körmöczi**, S. Dvorácskó, D. Hantosi, A. Menyhárt, F. Bari, R. Berkecz, C. Tömböly, B. Penke, E. Farkas
Dimethyltriptamine attenuates spreading depolarisation int he ischemic rat brain
IBRO Workshop, 29-30th January **2020**, Szeged, Hungary

16. Í. Szabó, V.E. Varga, **T. Körmöczi**, S. Dvorácskó, D. Hantosi, A. Menyhárt, F. Bari, R. Berkecz, B. Penke, E. Farkas
Dimethyltriptamine Attenuates Spreading Depolarization and Apoptotic Cell Death in the Ischemic Rat Brain
4th Hungarian Neuroscience Meeting for Undergraduate Students, Graduate Students, and Junior Post-Docs, HUNDOC 2020, 28th January **2020**, Szeged, Hungary

17. **T. Körmöczi**
Quantitative analysis of synthetic cannabinoids ant their metabolites in human urine and blood samples
EUGLOH Annual Student Research Conference, 28-30th September **2020**, online

5.5. Supervisors

1. Olivér Nagy, BSc in Biology
Legújabb dizájner drogok metabolitjainak LC-MS/MS azonosítása (**2018**)
(LC-MS/MS-based identification of metabolites of newest designer drugs)
Supervisors: Róbert Berkecz senior lecturer, **Tímea Körmöczi** PhD student
Internal advisor: András Szekeres senior research fellow

2. Dweny Mohamed Ayaallah Mohamed, MSc in Chemistry
   Development and application of liquid chromatography tandem mass spectrometry (LC-MS/MS) method for identification of designer drug metabolites (**2018**)
   Supervisors: Róbert Berkecz senior lecturer, **Tímea Körmöczi** PhD student
   Internal advisor: István Pálinkó professor

3. Noémi Kmetykó, BSc in Chemistry
   Célzott UHPLC-MS/MS analitikai módszer kifejlesztése és alkalmazása kinurénsav, xanturénsav és legújabb származékaiknak mennyiségi meghatározására (**2020**)
   (Development and application of a targeted UHPLC-MS/MS method for the quantitative analysis of kynurenic acid, xanthurenic acid and their newest derivatives)
   Supervisors: Róbert Berkecz senior lecturer, **Tímea Körmöczi** assistant research fellow
   Internal advisor: Tünde Alapi senior lecturer

4. Petra Kovács, Pharmacist (**2022**)
   Supervisors: Róbert Berkecz senior lecturer, **Tímea Körmöczi** assistant research fellow, PhD student