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# Abstract Collection From the Second Conference of Therapeutic Drug Monitoring – TDM 2025

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## EDITORIAL

Dear readers,

With great pleasure, we present you a special issue of the European Pharmaceutical Journal, which is represented by an abstract collection from the Second Conference of Therapeutic Drug Monitoring – TDM 2025. The conference was organised by the Faculty of Pharmacy of the Comenius University Bratislava, in cooperation with the Section of Clinical Pharmacy of the Slovak Pharmaceutical Society. Indeed, from 11 to 12 April 2025, we were pleased to host at the Faculty of Pharmacy in Bratislava more than 100 participants – clinical pharmacists, analytical chemists, pharmaceutical technologists, toxicologists, and physicians, from Slovakia, the Czech Republic, and Poland. The symposium covered three main topics: (a) Preanalytical and analytical stage of therapeutic drug monitoring, (b) Toxicology and antidoping, and (c) Clinical applications and case studies. In addition to the lectures, three practical workshops were a part of the conference.

Two of the practical workshops were dedicated to the analytical methods used in the sample pretreatment and real analysis of biological samples. A special emphasis has been given to the high-resolution tandem mass spectrometry, which is particularly effective in forensics and toxicology due to its unmatched sensitivity, its ability to detect and identify a wide range of substances at low concentrations, and its capacity to handle complex samples with high specificity and precision. With the current progress in both hardware and software, it strongly supports identification of unknowns, quantitation, multi-compound screening, detailed structural information with high throughput, efficiency, and reliable confirmation of results. The last workshop was focused on the practical aspects of the clinical pharmacy in hospital environment. Practical tips, trick, and improvement of clinical skills were trained by the workshop leader Dr. Sharmeen Roy.

The main goal of the Second Conference of Therapeutic Drug Monitoring was to bring together all aspects and procedures accompanied with the therapeutic drug monitoring in clinical, toxicological, forensic, and antidoping environment. We hope that such type of conference is an excellent opportunity to enjoy discussion with specialist and share experience with others from different scientific areas and clinical workplaces.

Yours Sincerely,

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## Oral Presentation

# Personalized medicine system for TDM of cardiological drugs based on LC–MS/MS analysis of samples collected with VAMS

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**Abstract** Cardiac arrhythmia affects approximately 12.6% of people over the age of 65. Ventricular arrhythmias are considered as responsible for 75%–80% of sudden cardiac deaths. Therapeutic drug monitoring in antiarrhythmic drugs (AAD) is essential for patient management because of narrow therapeutic range, association with several serious adverse drug reactions, and long multiphasic elimination or formation of active metabolites with the action distinct from the parent drug. Developed assay named "CardioCarePack" is based on the use of capillary blood collected at home by a patient with volumetric absorptive microsampling (VAMS), quantitative analysis of selected drugs and their metabolites, and a telemedical system that integrates all data between a doctor, patient, and laboratory to support therapy process. The sample preparation procedures for venous and capillary blood collected with 20 µL MITRA® microsampling device, sample transport conditions, and LC–MS/MS method on SCIEX QTRAP spectrometers were developed and validated for 17 compounds. Quantitative analysis covered therapeutic range of the tested compounds: 0.25–25 ng/mL (digoxin and nebivolol) 2.5–250 ng/mL (metoprolol, bisoprolol, propafenone, carvedilol, perindopril, ramipril, spironolactone, and zofenopril), and 25–2500 ng/mL (sotalol, desethylamiodarone, eplerenone, amiodarone, and 5-hydroxypropafenone). A total of 324 patients had been monitored for 2 years during regular pharmacological therapy. Patients were divided into four groups with the main AAD drug: amiodaron, propafenone, sotalol, and digoxin, where additional AAD could also be administered. Every half a year during a visit in medical facility, venous and capillary blood (VAMS) were collected. Between the visits, patients were collecting samples with a MITRA® device themselves at home. Recommended transport conditions for AAD MITRA® samples in string polyethylene bags are 20°C, desiccator, and up to 5 days. Interlaboratory study showed excellent reproducibility of the assay (80%–120%, $p < 0.05$). On the basis of samples collected during visits in medical facility, a correlation between drugs concentration in venous blood (serum) and capillary blood (MITRA®) (S/M) was calculated. S/M ratio was close to 1 (e.g. sotalol – 0.95, %CV – 5.12%, nebivolol – 1.03, %CV – 3.17%) or moderately to more than twofold different (e.g. digoxin – 0.69, %CV – 1.46%, ramipril – 1.18, %CV – 11.1, perindopril – 1.86, %CV – 4.73%, amiodarone – 2.17, %CV – 10.78). The correlation factors are statistically significant ($p < 0.05$) and can be used for accurate concentration estimation in serum. During the clinical study, concentration of the compounds was maintained within the therapeutic range and never exceeded the upper limit of the norm. This resulted in only 11 patients with implantable cardioverter-defibrillator (ICD) or cardiac resynchronization therapy device (CRT-D) interventions and only 31 patients with arrythmia episodes (3.6% and 10.1% of the tested group, respectively). CardioCarePack helps in doctor's supervision over the patient's condition on the basis of data collected within the developed software (history, doses, therapeutic index flagging, cardiograms, and other diagnostic results) and fits in with modern trends of home-based sample collection and personalized medicine.

**Keywords** *cardiac arrhythmia, TDM, VAMS, LC–MS/MS, personalized medicine.*

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# Biobanking: key aspects and benefits for research

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**Abstract** Therapeutic drug monitoring (TDM) and biobanking are relatively new areas that are gaining attention and gradually becoming more established in practice. Their implementation is essential to optimize therapeutic drug levels in order to achieve maximum efficiency while minimising the risk of adverse effects. The success depends on proper implementation in all analytical phases – from the pre-analytical, analytical, and post-analytical phases to the medical and pharmacological interpretation of the results. Biobanks play a key role in providing researchers with access to high-quality biological samples and data, thereby supporting the development of new diagnostics, biomarkers, and pharmaceutical treatments. The collection, processing, and archiving of biological material require well-defined handling procedures, including collection, transport, storage, and quality control of samples. Proper documentation and preservation of data at each stage enable their later use in clinical practice, medical, or pharmacological research. As these fields continue to evolve, their integration into routine practice will play a crucial role in advancing precision medicine and improving patient outcomes. This presentation will focus on the importance of TDM and biobanking, identify key challenges, and suggest strategies to improve sample quality. High-quality samples are crucial for accurate interpretation of results and effective therapeutic decision-making.

**Keywords** *biobanking, pre-analytical phase, analytical phase, post-analytical phase, TDM.*

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# rDUVLAESCI-MS/MSI: a novel approach for direct analysis of biological surfaces

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**Abstract** Ambient mass spectrometry has significantly enhanced the detailed insight into the molecular composition of complex biological surfaces under native conditions, requiring minimal sample preparation (Venter et al., 2008). In the last decade, the laser ablation/desorption followed by the post-ionization of the desorbed neutral aerosol by an ionization source, particularly electrospray ionization, atmospheric pressure chemical ionization, and atmospheric pressure photoionization, has been strongly favored. Advancements in laser-based designs, including UV and deep UV lasers, enable non-destructive, low-fragmentation analysis of various solid samples and dried spots/layers while significantly improving spatial resolution for mass spectrometry imaging (MSI) to just a few micrometers (Papoušková et al., 2025). The presented work introduces a novel, fully automated remote deep-ultraviolet laser ablation coupled with electrospray ionization–atmospheric pressure chemical ionization (rDUVLAESCI) source, which can be easily used for either spot analysis or MSI of a wide range of polarity and molecular weights of organic molecules. The rDUVLAESCI benefits from the coupling of a 193-nm Analyte G2 laser ablation unit (Photon Machines) with a hybrid Q-TOF mass spectrometer Synapt G2S (Waters), enabling the simultaneous acquisition of complementary ESI and APCI mass spectra in a single analytical run. The rDUVLAESCI MS has been utilized in the analysis of dried spots of polar molecules (e.g. caffeine, new psychoactive substances (NPSs), and PEG600) and nonpolar molecules (e.g. squalene, fluorene, anthracene, and wax esters) with excellent limits of detection down to tenths of nmol/mL and attomoles per ablated/desorbed pixel, respectively. The applicability of rDUVLAESCI in molecular MSI was demonstrated on visualization of the NPSs on the latent fingerprints with a variable lateral resolution in the 25–110 µm range. The rDUVLAESCI-MSI provided a detailed insight into the spatial distribution of exogenous naphyrone, butylone, cathinone, flephedrone, tetrahydrocannabinol (THC), hexahydrocannabinol (HHC) as well as human sebum-derived molecules, including free fatty acids, squalene, cholesterol, wax esters, diacylglycerols, and triacylglycerols. The reconstructed 2D maps of sebum constituents were successfully used for personal identification. The unambiguous identification of individuals was achieved even in the case of partially overlapped fingerprints (Papoušková et al., 2025). Furthermore, the rDUVLAESCI-MSI holds great potential for quantitative analysis and imaging of molecules directly in thin tissue sections (12–30 µm thick) due to its ability to optimize laser fluence for complete sample ablation and desorption. The mouse brain and skin melanoma samples were recently subjected to the rDUVLAESCI-MS/MSI. In the brain tissue, the main molecules detected were cholesterol and its derivatives (dehydrocholesterol, oxocholesterol, and desmosterol), free fatty acids (palmitic acid and linolenic acid), sphingosine, diacylglycerols (mainly dipalmitoyl glycerol), phospholipids, and C18 ceramide and adenine. In cancerous tissue, adenine was one of the key analytes, while the free fatty acids were most abundant. Identification was performed based on accurate mass measurements using cholesterol present in the tissue used as the lock mass. To conclude, rDUVLAESCI-MS/MSI opens a new window for the detailed studies of the plethora of molecules with significantly different polarity and molecular weight, which can be adopted in advanced disease diagnosis, environmental protection, and other research fields.

**Keywords** *mass spectrometry, imaging, new psychoactive substances, fingerprints, laser ablation.*

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# Practical Aspects of Laboratory Accreditation

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**Abstract** The paper provides information on practical aspects in relation to the accreditation of laboratories. Practical aspects relate to the accreditation of laboratories according to the accreditation standards ISO/IEC 17025:2017 and ISO 15189:2022 with a focus on the competence of personnel, verification of equipment and methods, the importance of results from accredited laboratories, and the laboratory management system. The paper also discusses the importance, purpose, and benefits of accreditation for various stakeholders as well as the requirements for maintaining confidentiality and impartiality during the performance of all analytical activities.

**Keywords** *accreditation, laboratory, management system.*

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# Strategies for pretreatment of biological samples for LC–MS analyses in the context of therapeutic drug monitoring

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**Abstract** Liquid chromatography-tandem mass spectrometry (LC–MS/MS) is a well-established technique for identifying and quantifying small molecules across various healthcare fields, including therapeutic drug monitoring. In clinical research, small molecule analysis can be performed on diverse biological samples, such as whole blood, serum, plasma, urine, or cerebrospinal fluid. However, before LC–MS/MS analysis, sample processing is often necessary to remove proteins and other constituents that could precipitate and clog the analytical system, enhance chromatographic performance, improve precision and accuracy, or prolong post-processing stability. The present work compares and describes three methods for processing blood plasma samples applicable to LC–MS analysis: protein precipitation, phospholipid removal, and solid-phase extraction. The protein precipitation technique is presented to determine selected phosphodiesterase inhibitors in guinea pig plasma. Simultaneous phospholipid and protein removal has been done using a commercially available 96-well plate, OSTRO (Waters). This sample preparation approach is illustrated and presented in LC–MS methods for determining selected antidepressive and antipsychotic drugs, direct oral anticoagulants, and first-line antituberculotics. Finally, the solid-phase extraction technique using microelution formate is presented as a method for sample pretreatment of caffeine and its primary metabolites. There is no universal, one-size-fits-all approach to sample preparation in LC–MS/MS analysis. The choice of pretreatment method should consider multiple factors, including the physicochemical properties of the analytes, required concentration ranges or limits of quantification, laboratory workload, and available resources. Today, laboratories performing therapeutic drug monitoring have access to various sample clean-up techniques and should select the most appropriate method to achieve their analytical goals efficiently.

**Keywords** *liquid chromatography, mass spectrometry, sample pretreatment, therapeutic drug monitoring.*

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# The Significance of "Polaromic" Analysis in Biomedical Research

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**Abstract** The development and testing of substances with the potential to influence biochemical and physiological processes in the body, focusing on their possible application in prevention, diagnosis, or therapy, play a crucial role in biomedical research. In our biomedical research, we focus on identifying and quantifying the pharmacological, immunological, and metabolic effects of substances with potential medicinal applications. Our experimental work is based on a combination of several biochemical, enzymatic, cellular, immunological, and analytical methods, among which the newly introduced "polaromic" analysis holds a significant position. This analysis utilizes the analytical capabilities of liquid chromatography (LC) for substance separation and mass spectrometry (MS) for quantification.

"Polaromic" analysis takes advantage of the ability of MS to detect substances based on their relative molecular mass and acquired charge, which have been separated by LC according to their polarity and charge. Since the separation in our chosen LC method does not require derivatization or any other chemical pre-treatment of the sample, apart from dissolving it in an appropriate solvent supplemented with a set of internal standards, the total analysis time – from sample collection to spectrum acquisition – is approximately 40 minutes. The currently established "polaromic" analysis allows the simultaneous quantification of more than 170 polar and ionic metabolites, as well as approximately 30 approved drugs and substances with potential therapeutic effects. We applied this analysis to quantify concentration changes of several drugs, biomarkers, and supplements, both in patient samples and laboratory-prepared samples from various biological models. "Polaromic" analysis also plays a significant role in studying biochemical and metabolomic parameters within neurochemical and oncological research. The introduced "polaromic" analysis method is fast, sensitive, quantitative, reproducible, robust, and cost-effective. Additionally, the obtained spectra can be reanalyzed retrospectively to gather information on substances whose retention times and m/z values can be determined even after the initial analysis. This approach provides the possibility of monitoring an entire group of drugs alongside comprehensive metabolomic analysis.

**Keywords** *metabolomics, LC–MS, biomedical research, drug monitoring, quantitative analysis.*

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# Insights From Our Initial Experience With Therapeutic Drug Monitoring of Linezolid

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**Abstract** Linezolid is a synthetic oxazolidinone with bacteriostatic activity against gram-positive organisms. It inhibits bacterial protein synthesis and is approved for treating nosocomial and community-acquired pneumonia, as well as skin and soft tissue infections. Pharmacokinetic variability in certain patients may lead to therapeutic failure or increased toxicity. Therapeutic drug monitoring (TDM) may help optimize linezolid dosing in these patients. TDM of linezolid was implemented at the Faculty Hospital of Trnava as part of standard care in 2024. Venous blood samples were collected at the end of the dosing interval to assess trough concentrations (Cmin). Samples were centrifuged and pretreated with protein precipitation. Serum concentrations were measured using high-performance liquid chromatography with mass spectrometric detection, with a lower limit of quantification (LLOQ) of 0.5 mg/L. Model-informed precision dosing was performed using MwPharm++ clinical pharmacokinetic software. The target Cmin range was 2–8 mg/L. Data were retrospectively obtained from patient records. In total, 14 patients from the intensive care unit and surgical departments (5 females, 9 males; median age 66 years; weight 94 ± 24 kg; BMI 32 ± 9.36 kg/m$^2$; serum creatinine 161.7 ± 121.3 µmol/L) were included. Pneumonia was diagnosed in nine patients, intra-abdominal infection in three, soft tissue infection in one, and bacteremia of unknown origin in one. The most common etiologic agents were *Staphylococcus aureus* (*n* = 4), *Enterococcus faecium* (*n* = 2), and *Streptococcus pyogenes* (*n* = 2). The standard dose of 600 mg every 12 hours was administered to eight patients, while six patients with severe infection or high body weight received a higher dose of 600 mg every 8 hours. The average duration of treatment before TDM was 4.81 ± 2.46 days. Blood samples were collected repeatedly from 5 patients, resulting in a total of 21 measurements. The mean Cmin was 4.45 ± 4.02 mg/L. The target Cmin was reached in 47% of cases, while 14% were below the target, 19% were below the LLOQ, 14% were above the upper limit (8–9 mg/L), and 5% (one case) had a concentration of 16.2 mg/L. Notably, in four cases, the measured concentrations were below the LLOQ despite the administration of higher doses. One such case involved a 31-year-old man (135 kg, BMI 44 kg/m$^2$, augmented renal clearance of 210 mL/min) with sepsis and pneumonia caused by *S. pyogenes,* whose Cmin was below the LLOQ. Initial experiences with TDM of linezolid suggest that the standard dosing regimen may not be suitable for all patients. Routine implementation of TDM should be considered to optimize linezolid dosing in selected patients.

**Keywords** *linezolid, therapeutic drug monitoring, pharmacokinetic variability, trough concentration.*

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# Therapeutic Monitoring of Vancomycin in Pregnant Patient With Neuroinfection: Case Report

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**Abstract** Vancomycin is a glycopeptide antibacterial indicated for the treatment of serious gram-positive infections, for example, infections caused by methicillin-resistant *Staphylococcus aureus (MRSA)*. Although vancomycin is widely used in hospitals, there is no consensus among clinicians with regard to dosing regimens of vancomycin, and therapeutic drug monitoring (TDM) is suggested due to two reasons. First, under-dosing of vancomycin causes drug resistance and loss of effectiveness, whereas over-dosing causes serious adverse effects, such as nephrotoxicity and ototoxicity. Second, vancomycin is associated with large inter-individual variability in the pharmacokinetic (PK) parameters. Vancomycin is not specifically labeled for use in the pregnant population, and studies published in the literature indicate that vancomycin is not teratogenic at therapeutic concentrations. Hence, prescribers typically use the same dosing regimens that are approved for non-pregnant patients. However, pregnancy is associated with physiological changes and altered drug PK (Goyal et al., 2022; Ingram et al., 2008). For example, for MRSA infection, the recommended trough concentration is 10–20 mg/L, and the recommended concentration for severe infection is 15–20 mg/L. In 2020, the American Society of Health-System Pharmacists, Infectious Diseases Society of America, Pediatric Infectious Diseases Society, and Society of Infectious Diseases Pharmacists issued a new guideline recommending TDM of vancomycin on the basis of the ratio of the area under the curve (AUC) over 24 hours to the minimum inhibitory concentration (AUC/MIC). When the MIC of MRSA is determined to be 1 mg/L using broth microdilution methods, a target AUC between 400 and 600 mg*h/L is suggested for invasive MRSA infections in adults and children according to the clinical efficacy and safety data (Rybak et al., 2020). Our case report describes the case of an overweight pregnant patient who was administered vancomycin due to neuroinfection. The use of the PK program and AUC/MIC dosing regimen calculations was in this patient problematic. We also observed the patient's augmented renal clearance. We regularly monitored the patient's serum vancomycin levels and adjusted the dosage regimen according to the measured concentration.

**Keywords** *vancomycin, TDM, pregnancy, neuroinfection.*

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# Pharmacogenetic Variability of CYP450 Enzymes and Its Influence on Antihypertensive Therapy in Patients With Chronic Heart Failure on Beta Blockers

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**Abstract** Patients with chronic heart failure (CHF) often receive complex pharmacotherapy, including guideline-directed medical therapy (GDMT) and additional co-medications, necessitating careful optimization to balance efficacy and safety. Drug response variability is influenced by interindividual differences in metabolism, particularly genetic polymorphisms in CYP450 enzymes responsible for the biotransformation of many cardiovascular drugs. While pharmacogenetics is gaining attention, its clinical impact on CHF pharmacotherapy, particularly in the context of polypharmacy, remains insufficiently explored. This study aimed to (1) identify GDMTs for CHF that are significantly metabolized by CYP450 enzymes and frequently prescribed, and (2) evaluate the impact of actionable CYP450 polymorphisms on clinical parameters, including blood pressure control, drug tolerability, and the dosage of CHF pharmacotherapy. This observational, retrospective study included patients with CHF (HFrEF, HFpEF) indicated for right-heart catheterization and receiving GDMT. Pharmacogenetic testing was performed to determine CYP450 enzyme polymorphisms (CYP2D6, CYP2C9, CYP2C19, CYP3A4, and CYP1A2) using qPCR. Star alleles with a known metabolic impact were selected based on PharmVar and Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines. Pharmacotherapy data, including beta-blocker prescriptions and total antihypertensive drug burden (expressed in defined daily doses, DDDs), were analyzed. Blood pressure measurements were compared across different CYP450 metabolizer phenotypes. Statistical analyses included Welch's t-test, Mann-Whitney U test, Fisher's exact test, Hardy-Weinberg equilibrium assessment, and multiple comparison correction using the Benjamini-Hochberg procedure. A total of 68 patients (mean age: 56 years, 21% female) were included, 78% classified as NYHA III-IV. The median number of medications was 9. Beta-blockers were prescribed to 87% of patients, with bisoprolol, metoprolol, and nebivolol being the most used. At least one altered CYP450 enzyme was identified in 89% of patients. CYP2D6 intermediate (IM) and poor (PM) metabolizers accounted for 32% and 7% of the cohort, respectively, while CYP2C19 rapid/ultrarapid metabolizers (RM/UM) comprised 32%. No significant differences were observed in beta-blocker dosages across genotype groups. However, CYP2D6 IM/PM patients required lower doses of combination antihypertensive therapy compared to normal metabolizers (4.46 vs. 6.60 DDDs, $p = 0.037$), whereas CYP2C19 RM/UM patients required higher doses (7.59 vs. 4.62 DDDs, $p = 0.020$). Despite these variations in treatment intensity, blood pressure levels remained stable across genotype groups, suggesting dose adjustments based on tolerability rather than predefined pharmacogenetic recommendations. CYP450 polymorphisms are prevalent in CHF patients and influence the overall antihypertensive drug burden rather than individual beta-blocker doses. CYP2D6 IM/PM patients required lower combination therapy doses, whereas CYP2C19 RM/UM patients required higher doses to maintain blood pressure control. These findings highlight the importance of interpreting pharmacogenetic results within the context of polypharmacy and treatment intensity rather than focusing solely on single-drug adjustments. Pharmacogenetic testing may offer valuable insights for optimizing individualized CHF pharmacotherapy by guiding overall treatment intensity and tolerability considerations.

**Keywords** *pharmacogenetics, chronic heart failure, CYP450 polymorphisms, beta-blockers, personalized medicine.*

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# Alternative analytical methods in toxicology: the application of capillary electrophoresis coupled with mass spectrometry for the identification of kratom psychoactive alkaloids in urine

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**Abstract** New psychoactive substances (NPS) are a group of illicit drugs that mimic the effects of illegal drugs, but they are not controlled by international drug conventions, although some may be restricted at the national level. NPS of natural origin, such as kratom (*Mitragyna speciosa*), khat, and *Salvia divinorum*, display addictive properties and have gained significant attention due to their increasing global spread. Kratom contains psychoactive alkaloids, mitragynine, and 7-hydroxymitragynine, which exhibit dose-dependent effects. At lower doses, these compounds act as psychostimulants, while at higher doses, they induce opioid-like effects. The increasing misuse of kratom requires the development of precise and reliable analytical methods for detecting and quantifying these compounds in biological matrices, particularly in urine, where detection is crucial for toxicological assessments. Conventionally, liquid chromatography has been the method of choice for the analysis of kratom alkaloids. However, capillary zone electrophoresis (CZE) coupled with mass spectrometry (MS) offers a promising, environmentally sustainable alternative. CZE provides several advantages, including high separation efficiency, reduced sample and reagent consumption, and lower operating costs. Additionally, a notable benefit of CZE is its ability to employ an online dynamic pH-junction preconcentration strategy, which enhances the method's sensitivity while minimizing the need for complex sample pretreatment, resulting in faster and more efficient analyses. The developed CZE-MS/MS method has been successfully validated according to the Food and Drug Administration (FDA) bioanalytical validation criteria, ensuring both precision and accuracy. The limits of detection for mitragynine and 7-hydroxymitragynine were determined to be 0.5 and 2 ng/mL, respectively, enabling ultra-trace analysis. In conclusion, the CZE-MS/MS approach has proven to be a promising tool for detecting kratom alkaloids in toxicological screening, offering a viable, cost-effective, and environmentally sustainable solution to the growing need to monitor NPS in biological samples.

**Keywords** *kratom, mitragynine, 7-hydroxymitragynine, capillary electrophoresis, tandem mass spectrometry.*

**Acknowledgements** This work was supported by the project VEGA 1/0302/24 and carried out in the Toxicologic and Antidoping Center at the Faculty of Pharmacy Comenius University.

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# Doping Control in 2024: Practices and Insights From Slovakia

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**Abstract** The Slovak Anti-Doping Agency (SADA) aims to protect the health of Slovak athletes and ensure fair competition by testing athletes and preventing the use of prohibited substances and methods. In 2024, SADA conducted 380 doping controls, with 4 athletes refusing to test. A total of 376 urine samples (121 in-competition and 255 out-of-competition) and 62 blood samples (1 in-competition and 61 out-of-competition) were collected. Sample analysis was performed at the World Anti-Doping Agency-accredited laboratories, mainly in Seibersdorf, Austria. Urine samples were primarily analyzed using liquid chromatography-tandem mass spectrometry and gas chromatography-tandem mass spectrometry, while blood samples were analyzed using flow cytometry. Additionally, targeted urine samples underwent additional analysis using gas chromatography/combustion/isotope ratio mass spectrometry or polyacrylamide gel-electrophoretic analytical techniques. Adverse analytical findings (AAFs) were confirmed in four urine samples: dorzolamide ($n$ = 2), a methylphenidate metabolite ($n$ =1), and boldenone with its metabolite ($n$ = 1). However, in all instances, additional non-analytical investigations were required, as analytical data alone were insufficient for case resolution. No AAFs were reported in blood samples, yielding an overall AAF prevalence of 0.91%. These findings emphasize the importance of comprehensive anti-doping efforts and contribute to understanding doping trends in Slovak sports.

**Keywords** *anti-doping, doping, doping control, testing, investigations.*

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# Use of TDM in digoxin intoxication

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**Abstract** Despite being developed many years ago, digoxin is still used in the treatment of heart failure and atrial fibrillation, although it is now less commonly prescribed. Therapeutic drug monitoring (TDM) and measurement of serum digoxin concentrations are valuable tools for managing treatment, preventing toxicity, and addressing cases of digoxin intoxication. The goal is to analyze the use of TDM in the management of digoxin toxicity. Consultations and the measured concentrations of digoxin reported to the National Toxicological Information Center (NTIC) in Slovakia from 2018 to 2024 were analyzed retrospectively. Between 2018 and 2024, a total of 31 cases of digoxin intoxication were consulted with the NTIC. Women (24) outnumbered men (7). Four deaths were reported, all in combination with other medications or in patients with multiple comorbidities. The average age of the consulted patients was 51.8 years, with the youngest being a 13-day-old child and the oldest a 92-year-old woman. Intentional intoxications were the most common cause (30%), followed by accidental intoxications (29%) and iatrogenic cases (16%). In 75% of the consulted patients, serum concentrations of digoxin were measured. The concentrations were reported in various units (nmol/L, ng/mL, and µg/L), and in one case, the units were not provided. The lowest measured concentration was 1.36 ng/mL, while the highest was 25.5 ng/mL. An interesting case involved a 92-year-old woman with an initial serum digoxin concentration of 15.65 ng/mL. After the initiation of digoxin-specific antibodies and symptomatic therapy, the patient gradually improved and was later discharged to ambulatory care. TDM remains a valuable and important tool for assessing digoxin concentrations in both cases of intoxication and during therapy. Measuring digoxin concentration alone is meaningless without proper interpretation. To be useful, the result must be evaluated in the context of the patient's current condition and clinical manifestations. It is also important to consider the units in which digoxin concentrations are measured in different laboratories, as this can lead to errors in the proper interpretation.

**Keywords** *digoxin, intoxication, therapeutic drug monitoring, concentration.*

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# Therapeutic drug monitoring of voriconazole – our first experience

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**Abstract** Voriconazole is a broad-spectrum triazole antifungal agent commonly used in the treatment of aspergillosis and other invasive fungal infections. The pharmacokinetics of voriconazole are non-linear due to the saturation of its metabolism. It is metabolized hepatically to inactive metabolites. Cytochrome P450 CYP2C19 is the enzyme primarily responsible for its metabolism, with a smaller contribution of isoenzymes CYP3A4 and CYP2C9. It has been recognized that the metabolic activity of CYP2C19 can vary due to multiple factors, which may lead to variability in voriconazole plasma concentrations. Additionally, voriconazole plasma levels are affected by age, drug-drug interactions, liver disease, bioavailability if orally administered, and some other circumstances. All these factors result in great plasma concentration variability – not only in different patients but also for the same patient in various clinical situations. Having a narrow therapeutic range, therapeutic drug monitoring (TDM) of voriconazole is widely recommended to ensure both safety and efficacy. While underdosing was associated with treatment failure, overdosing increases the risk of toxicity. At University Hospital in Pilsen, we introduced TDM of voriconazole in September 2023. The main role of clinical pharmacists in this process is to interpret measured plasma concentration in the context of all available patient data and to provide an optimal dosing strategy for an individual patient. Our experience is in concordance with the published data about the unpredictability and variability of voriconazole plasma concentrations in treated patients. TDM, therefore, proved useful in treatment optimization. Our presentation shows various case studies demonstrating this variability as well as chosen approaches.

**Keywords** *voriconazole, therapeutic drug monitoring, TDM, clinical pharmacist, treatment optimizing.*

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# Therapeutic drug monitoring in AGEL SK

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**Abstract** In many countries, the principles of personalized therapy are applied as part of the basic pharmacokinetic service, of which therapeutic monitoring of drug levels is an integral part. We first discussed the idea of TDM at AGEL Hospital Komárno in 2015, and it took a long time before we were able to implement it in real practice. We introduced the TDM service at our hospital in September 2022 with the aim of making the healthcare provided more efficient and shortening the hospitalization of our patients. Pharmacists always interpret the results of drug-level determinations provided by laboratory diagnostics by subsequently creating a TDM report and entering it into the hospital information system. The TDM report is created using the MWPharm+ software. To follow the correct procedure, we have created a standard that defines all the steps required for TDM and is approved by the accreditation process. Currently, we focus on monitoring TDM, especially antimicrobial drugs, such as vancomycin, gentamicin, and amikacin, but we also determine valproic acid and digoxin. Since June 2024, we have also started monitoring the levels of these drugs in other AGEL healthcare facilities. In other AGEL hospitals, we have successfully consulted a total of 174 patients in the last 6 months. We prepared a total of 390 reports for these patients, representing the analysis of 351 blood samples. In 2024, at the AGEL Hospital in Komárno, we determined the level of gentamicin in 94 patients, of whom up to 81% required dosage adjustment. In retrospect, we found that up to 55% of patients are underdosed, and therefore, their therapy is ineffective. Our recommendations were accepted by 68%. For vancomycin, we measured concentrations in 39 patients, of whom up to 90% had an incorrectly adjusted dose and required dosage adjustment. We found that even in the case of vancomycin, up to 57% of patients are underdosed, and their therapy is also ineffective. The success rate of our recommendations and their subsequent acceptance is 66%. The lecture will present four case studies that demonstrate the positive impact of TDM on the health status of at-risk patients. We can safely say that TDM monitoring is becoming an integral part of our hospital and is increasingly sought after. While when this service was introduced to clinical pharmacists, the acceptance rate was around 20%; after 2 years of successful work and practice, we were able to increase not only the number of patients for whom TDM is indicated but also the acceptance rate to 70%. In the future, we would like to achieve acceptance of around 90% and for the TDM service to be provided to all patients for whom the drug we can determine is indicated.

**Keywords** *therapeutic drug monitoring, gentamicin, vancomycin, acceptation, clinical pharmacy service.*

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# The impact of clinical pharmacists to optimizing vancomycin therapy using a pharmacokinetic software

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**Abstract** Vancomycin is the most commonly used bactericidal antibiotic for treating serious infections caused by methicillin-resistant strains of bacteria. Its pharmacokinetics exhibit high variability, and the efficacy and safety of treatment depend on achieving optimal drug levels through therapeutic drug monitoring (TDM). The aim of this study was to investigate the relationship between vancomycin area under the curve in 24 hours ($AUC_{0-24}$) and variables, such as dose, trough serum concentration ($C_{min}$), patient age, body mass index (BMI), and kidney function, and to evaluate the benefit of clinical pharmacist interventions in optimizing therapy. A retrospective study was conducted in Teaching Hospital Žilina with 70 hospitalized patients set on intravenous vancomycin treatment. Patients were divided into groups according to age, BMI, renal function, co-medication with another nephrotoxic antibiotic, and clinical pharmacist intervention. A strong correlation was observed between vancomycin $C_{min}$ and $AUC_{0-24}$ values in our study group ($r = 0,809$, $p \leq 0.0001$). We also found a strong correlation between $AUC_{0-24}$ value and patient's age, with elderly patients exhibiting the highest $AUC_{0-24}$, which frequently exceeded the optimal range ($p = 0.0128$) compared to the middle-aged patients. Although no significant correlation was found between $AUC_{0-24}$ and BMI, the greatest variability in $AUC_{0-24}$ was observed in obese patients. A statistically significant difference between $AUC_{0-24}$ was noted in patients with preserved glomerular filtration rate (GFR) and those with reduced GFR ($p = 0.0026$), with a negative correlation between $AUC_{0-24}$ and GFR values. TDM, combined with clinical pharmacist interpretation using pharmacokinetic software, is crucial for optimizing vancomycin therapy, particularly in high-risk patient groups. These groups include elderly patients, those with obesity, individuals with altered kidney function, and patients receiving concomitant nephrotoxic antibiotics.

**Keywords** *$AUC_{0-24}$, pharmacokinetic software, serum concentration, therapeutic drug monitoring, vancomycin.*

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# Therapeutic drug monitoring of meropenem – experience from the pilot project

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**Abstract** Meropenem is a β-lactam antibiotic with a broad spectrum of activity. It is widely used, particularly in patients with severe infections in situations where the causative infectious agent is unknown, and rapid initiation of antibiotic therapy is required. However, patients could exhibit significant pharmacokinetic variability due to altered renal filtration function, which undergoes dynamic changes over time. Additionally, the presence of artificial access devices and potential obesity further modify the drug's volume of distribution. As a result, achieving adequate therapeutic concentrations of meropenem is often challenging, with subtherapeutic levels posing the most critical clinical concern. This may lead to therapeutic failure and increased mortality at an individual level, and in general, it contributes to the spread of antimicrobial resistance. One of the solutions is therapeutic drug monitoring (TDM) of meropenem, followed by the interpretation by a clinical pharmacist and/or clinical pharmacologist, leading to individualized dose optimization. The aim of this study was the pilot implementation of an innovative and simplified meropenem quantification method for TDM purposes, followed by interpretation in relation to clinical and laboratory outcomes in patients. Using a simple online LC-MS method with a protein precipitation-based sample pretreatment, meropenem concentrations were determined in patients hospitalized at the University Hospital Bratislava – Hospital Ružinov. The primary inclusion criterion was intravenous administration of meropenem. Based on this criterion, a total of 25 patients and 26 blood samples were enrolled in the pilot study between January and May 2024. The results were analyzed using descriptive statistics to determine the proportion of patients achieving therapeutic, subtherapeutic, or supratherapeutic meropenem levels. These findings were then interpreted in relation to clinical characteristics and laboratory results. The pilot study showed that out of 26 blood samples, 17 (65%) had subtherapeutic, and 3 (11%) had supratherapeutic meropenem concentrations at the time of TDM, respectively. Only four patients (15%) achieved therapeutic levels, while in one patient, concentration assessment was not possible due to incorrect sampling time. Among the four patients with therapeutic levels, three had reduced glomerular filtration rates. Additionally, our study will present a case report of a patient exhibiting persistently low meropenem levels across two consecutive hospitalizations. The findings indicate that nearly two-thirds of patients had subtherapeutic meropenem levels. Under-dosing of meropenem significantly increases the risk of mortality. This pilot study highlights the clinical importance of TDM for β-lactam antibiotics and provides strong justification for its routine implementation in clinical practice.

**Keywords** *TDM, meropenem, pilot study, PK/PD, interpretation.*

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# Use of gentamicin as part of metaphylaxis in patients undergoing free flap reconstruction surgeries at the department of maxillofacial surgery and interpretation of its levels

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**Abstract** Gentamicin is a broad-spectrum aminoglycoside antibiotic with rapid bactericidal activity against most gram-negative microorganisms. Due to the poor oral absorption of aminoglycosides, intravenous administration is preferred. Peak concentrations are reached within 30–120 minutes after intramuscular or intravenous administration, with therapeutically effective levels maintained for 6–8 hours. A key characteristic of aminoglycosides is their clinically significant post-antibiotic effect, for which gentamicin ranges from 0.5 to 8 hours. Gentamicin is administered either as a slow intravenous bolus over 2–3 minutes or via intravenous infusion over 30 minutes. Since it is primarily excreted by the kidneys, renal function must be assessed before administration. The main adverse effects of gentamicin include ototoxicity and nephrotoxicity, both of which are dose- and duration-dependent. Therefore, therapeutic drug monitoring is essential. A once-daily dosing regimen is commonly used to treat infections, as it helps reduce toxicity risks. This regimen is also suitable for surgical metaphylaxis in free-flap oral reconstructive surgery, a preventive approach to reduce infection risk in procedures with a high likelihood of bacterial contamination. For surgical metaphylaxis, the standard gentamicin dose is 5 mg/kg body weight over 24 hours. To minimize toxicity, gentamicin blood levels should be below 1 mg/L before administering the next dose. Depending on creatinine levels and renal function, the dosing interval may need to be adjusted to every 36 or 48 hours to maintain optimal drug levels while reducing toxicity risks

**Keywords** *gentamicin, surgical metaphylaxis, infection risk, TDM.*

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# RECIPE – Your Recipe for TDM

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**Abstract** Therapeutic drug monitoring (TDM) plays a key role in evaluating the therapeutic response of administered drugs. Based on the information obtained by TDM, treatment can be optimized, thereby increasing the effectiveness and safety of treatment for the patient. However, a fast, efficient, and selective analytical method is necessary for these evaluations. Liquid chromatography (LC) has been increasingly used, especially with tandem mass spectrometry (LC–MS/MS). Developing LC and LC–MS/MS analysis methods in TDM can be time-consuming and personnel-intensive. To accelerate the implementation of new strategies, it is possible to use commercially available solutions in the form of LC or LC–MS/MS kits, for example, from the manufacturer RECIPE. Commercial kits are packages of consumables necessary for TDM analyses and are certified methods suitable for determining a wide range of analytes. TDM kits from the manufacturer RECIPE include LC–MS/MS methods for determining more than 160 different analytes from 9 groups, such as antibiotics, antidepressants, and antiepileptics.

**Keywords** *kit, liquid chromatography, tandem mass spectrometry.*

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# Phenyl selectivity

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**Abstract** Aliphatic hydrocarbon-bonded phase columns, such as C18 and C8, continue to be the most popular reversed-phase HPLC columns on the market today. These phases are primarily designed for and excel at hydrophobic-based liquid chromatography selectivity and retention. However, for complex mixtures, additional selectivities may be needed to achieve the desired chromatographic separation. Phenyl phases are often the first choice in orthogonal selectivity for aromatic and polar compounds due to the π–π interaction offered by the double bonds within the phenyl ligand. The increase in selectivity correlates to the amount of π electrons in the analyte and can also be affected by the choice of organic modifier used in the mobile phase. For the highest π–π interaction, methanol is the preferred solvent over acetonitrile as the π electrons associated with the nitrile (C≡N) bond in acetonitrile compete for the π–π interaction between the phenyl phase and the analyte. However, because of the higher viscosity of methanol/water mobile phases, the addition of acetonitrile with methanol when using phenyl phases is often recommended to reduce the mobile-phase viscosity, thus reducing the backpressure associated with methanol/water mixtures. In this study, we show that the addition of acetonitrile to methanol does not disrupt the π–π interaction of a phenyl phase but rather suppresses the interaction as a function of the relative amount versus methanol. Depending on the analyte characteristics, overall selectivity may also be improved.

**Keywords** *phenyl selectivity, HPLC.*

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## Poster Presentation

# Optimization of chromatographic conditions for the analysis of 5-bromo-2′-deoxyuridine

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**Abstract** The widely used analog of thymidine, 5-bromo-2′-deoxyuridine (BrdU) is a helpful tool for the identification of S-phase cells undergoing DNA replication during the cell cycle. Therefore, BrdU integration into DNA could be used for the detection and evaluation of cell proliferation. Reversed-phase high-performance liquid chromatographic RP-HPLC on a C18 column, with a binary mobile phase containing acidified water and a polar organic solvent with diode-array detection (DAD), is usually preferred for the routine analysis of bioactive compounds. This work is focused on the optimization of a simple HPLC method for the determination of 5-BrdU. The analysis was carried out using a Dionex UltiMate 3000 RS system equipped with a DAD detector, and the analysis was carried out by Chromeleon 7.0 version software. Different HPLC columns were examined to achieve the best separations with the shortest possible time. The best separation was carried out using a Polaris C18-A column (250 × 4.6 mm i.d. and 5 µm particle size). The separation was performed under isocratic conditions using a mobile phase consisting of $CH_3CN$ and 0.1% HCOOH in a ratio of 10:90 (v/v). A flow rate of 0.8 mL/min produces a run time of less than 10 minutes. Detection is accomplished using UV absorbance at 282 nm. All samples were prepared in triplicate and injected three times in HPLC with a 10 µL injection volume. The calibration plot was linear over the concentration range of 5–70 µg/mL for 5-BrDU. The correlation coefficient $R^2 = 0.9998$ indicates a linear relationship between the concentration of the analyte and the peak area.

**Keywords** *5-bromo-2′-deoxyuridine, RP-HPLC, cell proliferation.*

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# Enhancing Solubility of Ivermectin Through Encapsulation Into Magnetic Mesoporous Silica Nanoparticles: Detection Using HPLC

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**Abstract** Ivermectin, a widely employed antiparasitic drug, faces significant formulation and therapeutic challenges due to its poor water solubility and limited bioavailability. To overcome these limitations, encapsulation into porous carriers represents a promising strategy, enhancing solubility by maintaining the drug in an amorphous state and improving bioavailability through controlled release. Among potential carriers, magnetic mesoporous silica nanoparticles (MSNs) have recently emerged as innovative nanoplatforms for biomedical applications, distinguished by their excellent physicochemical properties, including tunable particle size, customizable pore structure, large surface area, dual-functional surfaces (internal and external), and facile surface modification. In our study, ivermectin was encapsulated into magnetic MSNs, exploiting the magnetic core for enhanced targeting capabilities. This approach could potentially enable precise delivery of antiparasitic medication directly to parasite-infected tissues, optimizing therapeutic outcomes and decreasing the side effects of the therapy (Comanescu, 2022). For monitoring drug performance, specifically the quantification of ivermectin release from MSNs in a simulated body fluid (pH 7.4) at selected time intervals, we utilized reverse-phase ultrahigh-performance liquid chromatography (RP UHPLC). Based on the obtained chromatographic records, the desorption profile of the ivermectin was evaluated. The simple and rapid RP UHPLC method for the quantitative determination of ivermectin released from MSNs was established on ASTRA® C18-HE column (150 × 4.6 mm, 5 µm) eluted with mobile phase consisted of methanol:water in a volume ratio of 97:3 (v/v). Separation was achieved by isocratic elution. The flow rate was kept at 1 mL/min, the injection volume was set at 20 µL, and the column oven temperature was maintained at 25°C. The effluent was monitored at 248 nm. The linearity of the chromatographic methods was evaluated by injecting nine standards of ivermectin solutions into the HPLC system in the concentration range of 0.5–350 µg/mL.

**Keywords** *ivermectin, silica, release kinetics, UHPLC.*

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# Therapeutic drug monitoring of thiopurine nucleotides for Crohn's disease therapy optimization using an innovative porous graphitic carbon-based HPLC-QTOF MS method

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**Abstract** Thiopurines (TPs) are immunosuppressive drugs commonly administered in the treatment of inflammatory bowel diseases (IBD) such as Crohn's disease or ulcerative colitis. TPs are biotransformed by a complex set of metabolic pathways before the active metabolites, that is, thiopurine nucleotides (TPN), are obtained. Therapeutic drug monitoring of TPN is a useful tool for the optimization of TP therapy. One of the most effective analytical approaches for such monitoring is based on ion exchange chromatography hyphenated with mass spectrometry (IEC-MS). In this work, a new approach based on the HPLC–MS (MS = QTOF) method employing a porous graphitic carbon column (PGC-MS) was developed. The new PGC–MS approach brought a significant enhancement of key performance parameters, for example, selectivity (at least partial chromatographic separation of all 12 TPN plus MS/MS resolution), sensitivity (up to 9 times higher peak heights), and sample throughput (reduction of analysis time by 25%). Hence, PGC-MS currently represents the most effective solution for the therapeutic drug monitoring of TPN, suitable to replace IEC-MS. The developed method was fully validated according to the requirements of the FDA guidelines and applied to 10 IBD patient samples (red blood cells). The developed PGC-MS approach offers a unique ability of the fast, sensitive, accurate, and precise monitoring of the full TP metabolome, urgently demanded by routine gastroenterological laboratories. PGC-MS is currently being used to profile TPNs in ongoing research studies aimed at elucidating their mechanism of action in IBD therapy.

**Keywords** *thiopurines, inflammatory bowel diseases, HPLC, QTOF, porous graphitic carbon column.*

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# Epitachophoresis – ways to stabilize LE/TE border

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**Abstract** The successful analyses of complex biological samples depend on sensitive and selective separation and detection methods as well as sample quality. The quality of the sample is strongly affected by its collection and pretreatment. The sample pretreatment includes purification, concentration, and simplification of complex, primarily biological samples. The complexity of biological matrices impacts the method's sensitivity and reproducibility. Epitachophoresis is a newly developed method for the separation, purification, and concentration of ionic compounds from large volumes up to several milliliters of complex sample matrices (Foret, 2019). The analytes are separated by the electric field in a discontinuous electrolyte system according to their electrophoretic mobilities. The circular design of the epitachophoretic device allows the injection of a large volume of samples and their concentration and collection in the center of the device. Similarly, to isotachophoresis, only anionic (Foret, 2019) or cationic (Hrušková, 2022) analytes can be concentrated and separated. The circular design raises the necessity of stabilization of the leading (LE) and trailing (TE) electrolytes border. When no stabilizing medium is added, the mixing of LE and TE, caused by the introduction of electrolytes and diffusion, destroys the separation process. There are several requirements for stabilization media, such as preventing the mixing of LE and TE, large pores for free movement of analytes through its structure, no analyte adsorption, time stability, and, also, effortless introduction of LE into the stabilizing medium. Several media, such as agarose gels, foamed polymers, polymer frits, 3D printed (Voráčová, 2021), and PDMS structure, for LE/TE border stabilization were tested. Anionic dyes Patent Blue and SPADNS and DNA were selected as model analytes. Recovery of DNA was used as the main criteria for media selection. The best results were obtained for NEEO agarose gel, agarose gel for pulsed-field electrophoresis, ultra-high molecular weight polyethene frit, and PDMS structure with DNA recovery from 75% to 100%. In the case of agarose gel, it was necessary to select the LE and TE composition carefully to prevent the shrinkage of the gel. In the case of the PDMS structure, the LE/TE border was not perfectly stabilized because its open structure cannot completely prevent the mixing of electrolytes. On the other hand, it should perfectly fit the concentration of very large analytes such as whole bacteria or viruses because there is no sieving effect. Gels and polymeric frits are perfect for small-size analytes due to better stabilization of the LE/TE border. From the above, it can be seen that media selection strongly depends on the physicochemical properties of the analyte to be concentrated

**Keywords** *epitachophoresis, sample preparation, border stabilization.*

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# Development of LC-MS/MS Method for Simultaneous Quantification of Seven Antibiotics and Two β-Lactamase Inhibitors in Human Serum

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**Abstract** Therapeutic drug monitoring (TDM) is an essential tool for optimizing antibiotic therapy, ensuring efficacy while minimizing toxicity (Lu et al., 2022). Given the variability in drug metabolism, renal function, and the increasing challenge of antimicrobial resistance, precise and rapid quantification of antibiotic concentrations is necessary (Rehm & Rentsch, 2020). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as a key technique for TDM due to its high sensitivity, specificity, and ability to quantify multiple antibiotics simultaneously (Radovanovic et al., 2022). This study presents the development of an LC-MS/MS method for simultaneous quantification of seven antibiotics (meropenem, linezolid, cefepime, ceftazidime, cefotaxime, piperacillin, and ampicillin) with two beta-lactamase inhibitors (tazobactam and sulbactam) commonly used in clinical practice. During method development, various chromatographic parameters, including mobile-phase composition (water with 0.1% formic acid/acetonitrile with 0.1% formic acid, water with 0.1% formic acid/methanol, 10 mM ammonium formate/acetonitrile), mobile-phase flow rate (0.2–0.5 mL/min), sample injection volume (1–10 µL), column temperature (35°C–45°C), and gradient elution were evaluated. Additionally, mass spectrometric conditions, such as gas flow rates (Sheath Gas, Aux Gas, and Sweep Gas), vaporizer temperature, and collision energies, were optimized. Full-scan Q1 and product ion scans were performed for each analyte. The analyses were conducted using a Vanquish Horizon LC system coupled with a TSQ Quantis mass spectrometer (Thermo Scientific). Chromatographic separation was achieved in 7.5 min on Hypersil Gold™ column (2.1 × 100 mm, 1.9 µm; Thermo Scientific) at an optimal temperature of 35°C. The mobile phase consisted of water and acetonitrile with 0.1% formic acid, with a flow rate of 0.5 mL/min. A 1 µL injection volume was used. Electrospray ionization was performed in both positive and negative modes, with selected reaction monitoring for targeted detection. Optimal MS conditions included positive ion spray voltage of 3500 V, negative ion spray voltage of 2500 V, sheath gas at 63 Arb, aux gas at 5 Arb, sweep gas at 0 Arb, ion transfer tube temperature of 325°C, and a vaporizer temperature of 350°C. Acetonitrile was used for protein precipitation and antibiotic extraction from human serum samples. Preliminary analysis of model serum samples demonstrated the method's capability to detect and quantify antibiotics within expected concentration ranges. The results indicate its potential for clinical application in TDM, providing a valuable tool for personalized antibiotic therapy and improved patient outcomes. Future work will focus on full method validation to ensure accuracy, precision, and long-term reliability. The validated method will be applied in clinical settings for routine TDM.

**Keywords** *therapeutic drug monitoring, antibiotics, liquid chromatography, mass spectrometry*

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# LC–MS/MS analysis of the GLP-1 analog semaglutide in plasma and brain tissue

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**Abstract** Semaglutide, a highly potent glucagon-like peptide-1 analog, is widely used for the treatment of type II diabetes mellitus and obesity. Recent studies indicate that semaglutide also exerts effects on the brain, highlighting its potential therapeutic applications for neurodegenerative diseases, such as Parkinson's and Alzheimer's. This study aimed to develop a novel liquid chromatography-tandem mass spectrometry (LC–MS/MS) method for the quantification of semaglutide in both plasma and brain tissue. Semaglutide was extracted using protein precipitation with acetonitrile/methanol, followed by solid-phase extraction from plasma and brain samples. Liraglutide served as the internal standard. Chromatographic separation was achieved using gradient elution with mobile phases consisting of 5% formic acid in water and acetonitrile. LC-MS/MS analysis was conducted using an ACQUITY™ UPLC™ Peptide C18 Column, an ACQUITY UPLC I-Class Plus System, and a Waters Xevo TQ Absolute Mass Spectrometer. This method enabled fast, sensitive, and highly reproducible quantification of semaglutide in both plasma and brain tissue. Our findings demonstrate a selective, sensitive, and robust LC–MS bioanalytical approach for the accurate measurement of semaglutide in biological samples.

**Keywords** *therapeutic drug monitoring, antibiotics, liquid chromatography, mass spectrometry.*

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# Therapeutic drug monitoring supported by lipidomics

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**Abstract** Colistin is a naturally occurring cyclic lipopeptide with an antibacterial spectrum against gram-negative pathogens. Typically, it is used as a last-resort antibiotic in the treatment of infections caused by *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, and *Klebsiella pneumoniae*. The use of colistin in clinical practice should be monitored due to its high toxicity and serious adverse events. Therefore, it is necessary to perform therapeutic drug monitoring (TDM) of this antibiotic during pharmacotherapy management. Typically, TDM is performed with the use of chromatographic analytical methods, which represent the 'gold standard' in clinical laboratories. However, recent analytical procedures have been pushed to methods characterized by high separation efficiency, sensitivity, selectivity (specificity), sustainability, eco-friendliness, and minimal requirements on sample amount. Capillary electrophoresis fulfills the aforementioned requirements and represents a very promising alternative to the convenient chromatographic methods. Moreover, its combination with mass spectrometry overcomes the issue of low sensitivity. Recent trends in clinical practice are oriented towards personalized medicine. Such an approach typically demands the fundamental understanding of the disease, identification of drug targets for therapy, and also discovery of relevant biomarkers of diseases or for monitoring the effectiveness of drug treatment leading to clinical follow-up in medical therapy. These goals can be fulfilled with the use of 'omics' disciplines, for example, lipidomics. Here, a comprehensive therapy optimizing strategy based on a combination of an alternative TDM approach (combination of capillary zone electrophoresis, CZE, with MS/MS) with an innovative lipidomics strategy is presented. The obtained results showed a correlation of the therapy with the plasma levels of some lipids accompanied by bacterial infections and/or inflammation.

**Keywords** *collistin, lipidomics, capillary electrophoresis, liquid chromatography, mass spectrometry*

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# Monitoring of direct oral anticoagulants – is it needed?

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**Abstract** Direct oral anticoagulants (DOACs) provide a safe and effective alternative to previous anticoagulant therapies for the treatment of thromboembolic conditions, including stroke prevention in atrial fibrillation and the prevention and treatment of venous thromboembolism. DOACs are classified based on their mechanism of action into two groups: direct factor Xa inhibitors (apixaban, edoxaban, and rivaroxaban) and direct thrombin inhibitors (dabigatran). Compared to vitamin K antagonists (warfarin), DOACs offer several advantages, including more straightforward dosing, faster onset of action, and the absence of routine monitoring requirements. However, in specific clinical situations, such as severe bleeding, acute stroke, or in particular patient populations (e.g. obese patients), monitoring may be helpful. Routine coagulation tests, such as activated partial thromboplastin or thrombin time, are unreliable for assessing DOAC efficacy. More specific assays, including dilute thrombin time, ecarin clotting time, and anti-Xa activity assays, can be used to measure DOAC levels, but liquid chromatography-mass spectrometry (LC-MS) remains the gold standard for accurate quantification. In this study, we used our in-house developed and validated LC-MS method to simultaneously quantify all four DOACs in human plasma. We assessed plasma levels in 71 patients (37 men and 34 women, median age 80 years), receiving DOAC therapy primarily for atrial fibrillation. The dosing regimen was individualized based on clinical characteristics. Specifically, 36 patients were taking apixaban (2.5–10 mg twice daily), 12 dabigatran (110–150 mg twice daily), three edoxaban (30–60 mg once daily), and 20 rivaroxaban (15–20 mg once daily). Blood samples were withdrawn at two time points – right before drug administration (trough level) and 2 hours post-dose (peak level). The measured trough plasma levels ranged from 34.5 to 492.6 ng/mL for apixaban, 35.9 to 164.3 ng/mL for dabigatran, 32.4 to 49.1 ng/mL for edoxaban, and 10.4 to 265.4 ng/mL for rivaroxaban. The peak plasma levels ranged as follows: 106.8–922 ng/mL for apixaban, 71.5–214.2 ng/mL for dabigatran, 198–379.5 ng/mL for edoxaban, and 118.3–670.8 ng/mL for rivaroxaban. Compared with the expected trough and peak levels recommended by the International Council for Standardization in Haematology in 2021, we observed that 11 apixaban patients had higher trough levels, and 13 had higher peak concentrations, possibly due to initial higher dosing. Among dabigatran users, one patient had lower and two had higher trough levels, while seven had lower peak levels. Of the three patients using edoxaban, two had higher peak levels. Rivaroxaban patients exhibited inconsistent results: one had a trough level above and another below the recommended range, while five patients had lower and two had higher peak levels. These findings suggest that therapeutic monitoring of DOAC therapy may be necessary for certain patients, as plasma concentrations vary significantly and may sometimes fall outside the recommended therapeutic range.

**Keywords** *direct oral anticoagulants, liquid chromatography, mass spectrometry, therapeutic drug monitoring.*

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# Application of "polaromics" to simultaneous monitoring of drugs and their effects on cellular metabolism

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**Abstract** Experimental research plays an important role in the development and testing of new substances that could find their application not only in biomedical research but also in clinical practice. As part of our biomedical research in the field of cancer cell metabolism, we use several methodological approaches aimed at identifying and quantifying the impact of potential as well as already approved drugs on selected cellular and metabolic features of cancer cells. In addition to methods aimed at testing the cytotoxicity of substances and changes in the activity of selected key enzymes, we managed to put into practice a new protocol for quantitative liquid chromatography–tandem mass spectrometry (LC–MS) analysis of polar and ionic compounds in various biological matrices. Temozolomide (TMZ), 2-deoxyglucose (2-DG), and metformin (MF) are among the substances that have the ability to influence the survival of cancer cells. While TMZ is an alkylating cytostatic used to treat specific forms of brain tumors, 2-DG is considered a substance with antitumor properties as a probable consequence of glycolysis inhibition, which is also currently the subject of clinical testing. MF is a substance used in the treatment of type 2 diabetes mellitus, with a not fully understood molecular mechanism of action. Using the newly introduced LC–MS analysis of polar and ionic compounds – "polaromics" – we monitored changes in the concentration of TMZ, 2-DG and MF in media from glioblastoma cells, as well as their influence on the chemical composition of the media, as a manifestation of cellular metabolism. "Polaromic" analysis was also applied to determine the stability or thermal or cellular degradation of TMZ. "Polaromic" analysis of media obtained from glioblastoma cell cultures allowed the quantification of the specific decrease in the concentration of TMZ, 2-DG, and MF. In the case of TMZ, the formation of aminoimidazole carboxamide (AIC) was observed in the culture medium, which is also one of the products of the thermal decomposition of TMZ. At the same time, we quantified the effect of the studied drugs on the capacity of cells to change the composition of the culture media. In parallel with the "polaromic" analysis based on LC–MS, we tested the effect of the drugs on the increase in biomass or cell viability but also the effect on anaerobic metabolism. The decrease in the amount of TMZ and the increase in its degradation product AIC, as well as the decrease in 2-DG in the media, indicate that the given substances are taken up into the cells, or their chemical transformation occurs. At the same time, changes in the composition of the culture medium compared to the control indicate that they affect cell metabolism. In the case of MF, a decrease in its amount in the medium was not observed, but changes in the composition of the medium after incubation indicate its effect on cell metabolism. The newly introduced "polaromic" analysis allows for the simultaneous quantification of the tested active substances – TMZ, 2-DG, and MF, together with the metabolomic analysis of the media. The simplicity and speed of sample preparation, as well as the sensitivity and complexity of the established "polaromic" analysis, make it a tool suitable for both drug monitoring and biomedical research in a broader context.

**Keywords** *temozolomide, 2-deoxyglucose, metformin, cellular metabolism, LC–MS.*

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# Therapeutic drug monitoring of ethanol in ethylene glycol intoxication: biochemical insights

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**Abstract** Ethylene glycol (EG) is a colorless, relatively non-toxic alcohol with a characteristic sweet taste (Achappa, 2019). However, even small ingested amounts can lead to severe toxicity due to its metabolism by alcohol dehydrogenase (ADH) into toxic metabolites, including oxalate, glycolate, and glyoxylate. The management of EG intoxication includes hemodialysis, sodium bicarbonate administration, and the use of antidotes, such as fomepizole (an ADH inhibitor) or ethanol (EtOH), which acts as a competitive substrate for ADH (Mégarbane, 2010; Sasanami, 2020). Regular monitoring of serum ethanol concentrations and dose adjustments are required to maintain the therapeutic range (22–30 mmol/L) due to the variability in ethanol elimination rates. The objective of our study was to monitor the biochemical changes during EtOH therapy in a patient with EG intoxication. In addition to EtOH, we monitored parameters such as pH, base excess (BE), bicarbonate ($HCO_3^-$), creatinine, and lactate. EG intoxication is characterized by severe metabolic acidosis in its early stages, which was in our case confirmed by initial pH, BE, and $HCO_3^-$ values. The patient also exhibited an elevated lactate concentration (34.48 mmol/L); however, this could have been affected by glycolate (a metabolite of EG). Glycolate has structural similarities with lactate and may interfere in lactate determination. Correlation analysis of lactate with EG was not possible, as only two EG values were available: 178 mg/dL (on the second day of treatment) and 15.0 mg/dL (on the fourth day). Significant correlations were identified, primarily between BE and pH ($R = 0.954$), BE and $HCO_3^-$ ($R = 0.988$), BE and lactate ($R = -0.861$), and urea and creatinine ($R = 0.922$). No other statistically significant correlations were observed. Due to the small molecular size of EtOH, its elimination was rapidly accelerated in the presence of hemodialysis. As a result, serum EtOH concentrations fell below the therapeutic range, and an increase in the EtOH dose from 20 to 60 mL/hour through a nasogastric tube was required. Throughout and after ethanol therapy, both urea and creatinine levels increased. The serum urea-to-creatinine ratio was relatively low (<40 mmol/L), suggesting the possibility of acute tubular injury after EG intoxication. Biochemical parameter analysis revealed statistically significant correlations that may provide insights into the metabolic compensatory mechanisms and the progression of intoxication. Our findings indicate that monitoring these biochemical markers could facilitate early assessment of metabolic disturbances and treatment efficacy.

**Keywords** *clinical biochemistry, ethanol, ethylene glycol, toxicology.*

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# Medication Errors by Nurses in the Provision of Nursing Care

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**Abstract** Medication errors represent a significant issue in nursing care, encompassing mistakes in the preparation, administration, and monitoring of medications. These errors can occur at any stage of the treatment process and may lead to serious consequences for patients, including adverse effects, health deterioration, or even death. Medication errors are defined as preventable events that result in the inappropriate use of medications.

**Objective**: The aim of the study was to analyze the adherence to the principles of proper medication administration by nurses and the verification of patient identity before medication application. We focused on identifying the most common procedures and methods used by nurses in medication administration and the potential shortcomings in these processes. The research sample consisted of 245 nurses working in healthcare facilities, with the largest group being nurses from intensive care units (32%). The majority of nurses had more than 15 years of experience (43%), and regarding education, the largest group consisted of nurses with a Bachelor's degree (52%). As a research tool, we used a self-constructed questionnaire focused on medication administration principles and patient identity verification. The collected data were analyzed using descriptive and analytical statistics with a significance level of 5% ($\alpha = 0.05$). The research showed that nurses in different workplaces adhere to medication administration principles to a similar extent, with no statistically significant differences between them ($p = 0.138$). In contrast, statistically significant differences were found between the length of nurses' experience and adherence to medication administration principles ($p = 0.001$), with nurses having more than 15 years of experience showing the highest adherence to proper medication administration. In the area of patient identity verification, statistically significant differences were found between the workplaces ($p = 0.001$), with nurses from intensive care units achieving the best results. Differences in patient identity verification among nurses with varying lengths of experience were found, but they were not statistically significant ($p = 0.152$).

**Conclusion**: The results of the research highlight the importance of experience in adhering to medication administration principles, as nurses with more experience demonstrated a higher level of proper execution of these tasks. On the other hand, patient identity verification was most consistently performed in intensive care units, indicating the significance of organizational settings and the work environment. Failure to adhere to proper medication administration principles and failure to verify patient identity pose a significant risk of medication errors, which can lead to administering the wrong medication, wrong dosage, or to the wrong patient. These errors can have serious consequences, including adverse effects, deterioration in the patient's health, or, in extreme cases, endangering the patient's life. In the case of therapeutic drug monitoring, medication errors by nurses can result in inadequately determined drug levels. The research emphasizes the need for continuous education of nurses in medication safety, regular educational activities, and the implementation of effective control mechanisms to minimize the risk of medication errors. Key measures to improve safety include standardized protocols, interprofessional collaboration, and technological tools, such as electronic patient identification systems and automated drug dispensing systems.

**Keywords** *medication errors, medication administration, patient identity verification, nurse, nursing care.*

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# Management of open-angle primary glaucoma therapy

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**Abstract** The aim of this study is to compare selected case reports from clinical practice with the results of clinical studies published by foreign scientists. We compared risk factors and current therapeutic options for the treatment of primary open-angle glaucoma. In the first part of the study, we examined the age factor as one of the potential risk factors for glaucoma. The results of the study published by Hashemi et al. (2018) point out that older age is one of the significant risk factors for the development of this disease. In our study, the first patient was 71 years old, which confirms the risk of developing the disease at an older age, but the second patient was only 27 years old. The results of the study published by Bak et al. (2020) confirm that although older age is a risk factor for the development of glaucoma, primary open-angle glaucoma can also be diagnosed in younger individuals. In the second part of our study, we investigated treatment options and the occurrence of side effects. The first patient experienced adverse effects after using eye drops containing the alpha-adrenergic agonist, brimonidine. After discontinuation of brimonidine, the treatment was modified and changed to the carboanhydrase inhibitor brinzolamide. However, the intraocular pressure value remained unchanged, and adverse effects reappeared. Despite the addition of artificial tears, the condition did not improve. For this reason, the treatment was adjusted to the last option – prostaglandin analogues (tafluprost). Due to the lack of efficacy of monotherapy, escalation of treatment to a fixed double combination (timolol and bimatoprost) was indicated. Despite this modification, glaucoma was not sufficiently stabilized, and therefore, it was necessary to proceed to surgical treatment (trabeculectomy), which subsequently led to a long-term stabilization of the patient's health condition. According to a study by Distelhorst (2003), surgical trabeculectomy is indicated when the target intraocular pressure cannot be achieved medically, if there is a worsening of optic nerve damage despite maximum treatment, or when the patient cannot adhere to or tolerate drug treatment. The second patient was indicated for monotherapy with the prostaglandin analogue tafluprost based on diagnosis. The chosen therapy led to an effective reduction of intraocular pressure without the need for modification or drug change. No adverse effects were observed during the therapy and were well-tolerated by the patient until the current stabilization of the clinical condition. The results of our study point to various treatments, the success of which depends not only on the age of the patient but also on his response to drug or surgical treatment. Even though the patients we monitored were diagnosed with the same type of glaucoma, they tolerated the treatment differently. In the first case, stabilization of the condition was achieved only after several treatment adjustments due to the occurrence of adverse effects that required surgery. In the second case, pharmacological treatment was sufficiently effective and without the occurrence of allergic reactions. We also pointed out that although the older age of the patient is a risk factor for glaucoma, the disease can also be diagnosed in younger individuals. The presented study confirms that the treatment of glaucoma requires a multidisciplinary approach involving not only ophthalmologists but also pharmacists. Research and development of new therapeutic strategies and other clinical trials may help improve treatment outcomes and improve the quality of life of glaucoma patients in the future.

**Keywords** *risk factors, glaucoma, antiglaucoma preparations and miotics, pharmacotherapy, intraocular pressure.*

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# The effect of selective serotonin re-uptake inhibitors use on patient's body weight

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**Abstract** The aim of this study is to review the effect of selective serotonin reuptake inhibitors use on a patient's body weight. In the past, the effect of selective serotonin reuptake inhibitors on body weight was considered neutral or, even in some cases, resulting in weight reduction. The newest research, based on several studies, shows that long-term use of SSRI drugs can lead to weight gain. Based on inclusive and exclusive criteria, a set of 101 patients from the same psychiatric outpatient clinic has been selected. The monitored group of patients consisted of 89 women (88.12%) and 12 men (11.88%). The patient's BMI value was calculated and recorded after each weight measurement. Weight measurements were carried out in a psychiatric outpatient clinic, and the weight at the beginning of treatment (m1), after 3 months of treatment (m2) and after 6 months (m3), was recorded. These records were used in the calculation of BMI (BMI1, BMI2, BMI3). The dosage of the prescribed medication was consistent for all patients during the period of this study. Specifically, citalopram 20 mg once daily, escitalopram 10 mg once daily, paroxetine 20 mg once daily, sertraline 50 mg once daily, fluoxetine 20 mg once daily, and fluvoxamine 100 mg once daily. At the start of the treatment, there was a gradual dose titration period, lasting for up to a maximum of 14 days. During the 6-month follow-up period, only a few clinically relevant BMI elevations were observed. In most cases, there was a slight increase in BMI values. We also recorded a case of stagnation and a decrease in BMI values. In many cases, we saw a trend of an initial decline in BMI, but this was followed by an increase. The result of the study proves that the largest weight gain was recorded with fluvoxamine (0.67 kg/observed period), followed by escitalopram (0.58 kg/observed period), fluoxetine (0.5 kg/observed period), citalopram (0.4 kg/observed period), and sertraline (0.3 kg/observed period). A decrease in weight within our patient group was recorded with paroxetine (-0.17 kg/observed period). Based on the results of our study, we assume that the use of selective serotonin reuptake inhibitors in our group had a minimal, although not completely negligible, effect on weight gain, as clinically significant changes in BMI occurred in several cases. We did not notice anorexic effects in any patient.

**Keywords** *compliance, adverse reactions, selective serotonin reuptake inhibitors, metabolism, BMI, body weight.*

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title

# Optimizing vancomycin therapy: the importance of therapeutic drug monitoring and individualized dosing

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**Abstract** Vancomycin is a glycopeptide antibiotic with potent activity against a broad range of Gram-positive bacteria. It is primarily administered intravenously for the treatment of various systemic infections. The pharmacokinetic profile of intravenous vancomycin is highly complex and exhibits significant interpatient variability across different patient populations. Therapeutic drug monitoring (TDM) of vancomycin is recommended to facilitate individualized dosing and optimize therapeutic outcomes. Subtherapeutic concentrations (<10 mg/L) have been associated with an increased risk of bacterial resistance, whereas concentrations exceeding 20 mg/L may contribute to toxicity (Mauliņa, 2022).

In this retrospective study, we evaluated vancomycin plasma levels in hospitalized patients between 2023 and 2024, with a particular focus on individual concentration ranges. Vancomycin levels were measured using a Roche biochemical analyzer in conjunction with the Vancomycin diagnostic kit, ensuring compliance with all preanalytical and analytical quality control requirements.

A total of 259 samples were analyzed, comprising 84 samples (32.43%) from female patients and 175 samples (67.57%) from male patients. Among the female patients, 6 samples (3.43%) had subtherapeutic vancomycin concentrations below 10 mg/L, while 43 samples (24.57%) were within the therapeutic range of 10–20 mg/L. Elevated vancomycin levels between 20 and 30 mg/L were observed in 25 samples (14.29%), and concentrations exceeding 30 mg/L were found in 10 samples (5.71%).

Similarly, among male patients, 22 samples (12.57%) had subtherapeutic vancomycin levels below 10 mg/L, whereas 83 samples (47.30%) were within the therapeutic range of 10–20 mg/L. Elevated concentrations between 20 and 30 mg/L were observed in 50 samples (28.57%), and levels exceeding 30 mg/L were identified in 20 samples (11.43%). Overall, vancomycin concentrations above 20 mg/L were observed in 35 samples (41.7%) from female patients and in 70 samples (51.4%) from male patients.

Our findings highlight the importance of accurate vancomycin monitoring to optimize patient management. Maintaining plasma concentrations within the therapeutic range (10–20 mg/L) is crucial to minimizing the risk of antimicrobial resistance associated with subtherapeutic levels (<10 mg/L) while simultaneously reducing the potential for toxicity at concentrations exceeding 20 mg/L. These results underscore the necessity of individualized dosing strategies and routine therapeutic drug monitoring to ensure both efficacy and safety in vancomycin therapy.

**Keywords** *antibiotics; therapeutic drug monitoring; vancomycin*

**References**

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