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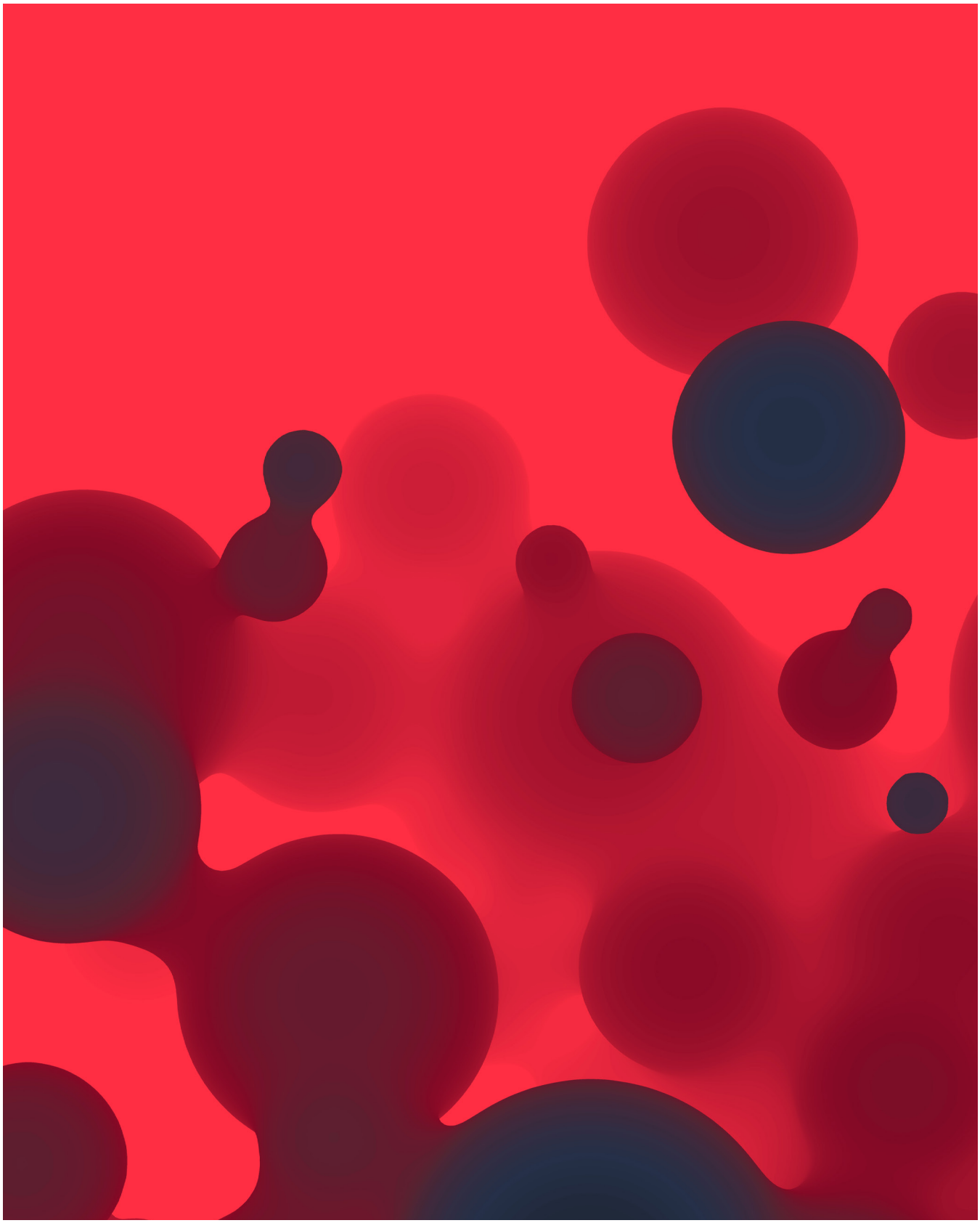

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Publisher
Association of Greek Chemists
Editor-in-Chief
Professor Victoria Samanidou
samanidu@chem.auth.gr
Deputy Editor-in-Chief
Associate Professor Ioannis Katsogiannis
katsogia@chem.auth.gr

Journal Scope and Aims

The Journal of Association of Greek Chemists (JAGC) aims to become an international, peer-reviewed journal, devoted to promoting the latest research and developments in a wide range of fields of chemistry. Starting in 2023 as an initiative of the Association of Greek Chemists, it is aiming to provide a unique platform for original research and scientific articles. Articles will be subjected to peer review by specialists in each field guided by distinguished Section Editors and prominent Greek researchers and academic staff.
During the initial stages, JAGC will cover primarily areas related but not limited to: Analytical Chemistry, Biochemistry- Clinical Chemistry-Biotechnology, Chemical Technology, Computational and Theoretical Chemistry, Environmental Chemistry & Technology, Forensics-Toxicology, Food Chemistry and Technology, Green Chemistry, Inorganic Chemistry, Macromolecular Chemistry, Materials Chemistry, Medicinal Chemistry, Nanochemistry, Natural Products Chemistry, Organic Chemistry, Physical Chemistry, Radiochemistry and Chemical Education.

Representative of Steering committee of AGC to the Editorial Board
Professor Athanasios Papadopoulos
papadnas@ihu.gr
Publishing Manager
Dr Spiros Kitsinelis
eex@eex.gr
Design - Production Edition
Adjust Lane

Steering Committee of AGC

Association of Greek Chemists Headquarters
Kaningos 27, P.C. 10682 Athens,
tel: 210 3821524, 210 3829266, fax: 2103833597
info@eex.gr
www.eex.gr

Celebrating the Second Issue of the Journal of the Association of Greek Chemists

Dear Esteemed Readers and Colleagues,

On behalf of the editorial and publishing team, it is with great pride and enthusiasm that I present to you the second issue of the *Journal of the Association of Greek Chemists (JAGC)*. Following the successful launch of our inaugural issue, we are delighted to continue our mission of disseminating high-quality research, fostering scientific dialogue, and promoting the vital role of chemistry in addressing contemporary and global challenges.

The enthusiastic reception of the first issue reaffirmed our belief in the need for a high-quality, internationally oriented scientific journal that highlights the work of Greek chemists and welcomes contributions from the global scientific community. Our vision remains steadfast: to cultivate a robust platform for original research, comprehensive reviews, and emerging ideas across the full spectrum of chemical science, from traditional disciplines to cutting-edge interdisciplinary fields.

This second issue reflects our unwavering commitment to excellence in chemical research, education, and industrial application. Within these pages, readers will discover a diverse array of peer-reviewed articles covering innovative approaches, novel methodologies, and thought-provoking perspectives. These contributions come from both established researchers and emerging scientists, and represent institutions across Greece.

As chemistry continues to be central in tackling global issues such as climate change, energy sustainability, food safety, and public health, our journal aims to reflect this dynamic role. We encourage submissions that transcend traditional disciplinary boundaries and demonstrate how chemical sciences can contribute meaningfully to society's most pressing needs. Interdisciplinary and collaborative research is not only welcome but necessary and JAGC strives to be a home for such pioneering efforts.

We are also pleased to announce that the *Journal of the Association of Greek Chemists* is now indexed in Google Scholar, which significantly enhances the visibility and citation potential of our published articles. This is an important step in our long-term plan to increase the impact of the journal. As part of this strategy, we are actively pursuing inclusion in additional reputable indexing services and databases, ensuring that the excellent work of our authors reaches a broad international audience.

Our commitment to academic integrity and editorial rigor remains at the core of our operations. Each submission undergoes a thorough peer review process managed by our team of distinguished Section Editors and reviewers. Their dedication ensures the scientific quality and relevance of every article we publish. We owe much to these experts who generously contribute their time, knowledge, and insight. As our journal grows, we are also expanding our editorial board and welcoming new members with diverse expertise to further strengthen our peer-review process and uphold our standards of excellence.

One of our key objectives is to support the next generation of chemists. By offering an inclusive and credible platform for young researchers to publish and engage with the broader scientific community, we hope to foster a culture of mentorship, collaboration, and innovation. We believe that early-career scientists are crucial to the advancement of the field, and we are proud to feature their work prominently in this issue.

Of course, a journal is only as strong as its community. Whether you are an academic, an industrial chemist, a teacher, or a student, your engagement is what fuels our progress. We warmly invite you to submit your research, share the journal within your networks, volunteer as a reviewer, or join our editorial team. This is a journal *for the community, by the community* and its continued growth depends on your contributions and support.

I would like to extend my heartfelt gratitude to our authors, reviewers, and editorial board members, whose dedication and excellence make this journal possible. Finally, I sincerely thank our readers. We hope you find this issue both insightful and inspiring, and we look forward to your continued engagement as we shape the future of chemical research together.

For submission guidelines and access to current and past issues, please visit:
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With best regards,
Dr Spiros Kitsinelis
Publishing Manager
Journal of the Association of Greek Chemists

Green Microextraction Techniques In Therapeutic Drug Monitoring

Kousi Maria and Samanidou Victoria*
Laboratory of Analytical Chemistry, Department of Chemistry,
Aristotle University of Thessaloniki, GR 54124 Thessaloniki, Greece
*samanidu@chem.auth.gr
DOI: 10.62579/JAGC0011

ABSTRACT
Therapeutic drug monitoring (TDM) is crucial for optimizing drug therapy, particularly for medications with narrow therapeutic windows. By measuring drug concentrations in biological fluids, TDM aids in dose individualization, adherence assessment, and the prevention of adverse effects. Recent advancements focus on miniaturized and eco-friendly sample preparation techniques, such as microextraction methods, which are efficient and low-cost. These include liquid- and solid-phase microextraction, offering benefits such as automation and high-throughput performance which have significantly improved TDM efficiency. Additionally, the emergence of microsampling offers a less invasive alternative to traditional blood collection for TDM and pharmacokinetic studies. Coupling these innovations with sensitive analytical techniques, like liquid chromatography-tandem mass spectrometry, enables accurate and reliable drug quantification from minimal sample volumes. These developments collectively enhance the clinical utility of TDM and contribute to improved patient outcomes. This paper provides a comprehensive review of recent advancements in green microextraction techniques as applied to therapeutic drug monitoring.

KEYWORDS
Therapeutic drug monitoring; microextraction techniques; Green Analytical Chemistry; sample preparation; drug analysis

1. INTRODUCTION

Therapeutic drug monitoring (TDM), which involves measuring drug levels or active metabolites and drug antibodies, is a promising strategy that can be used to optimize therapeutic treatments. It is based on the assumption of two facts 1. There is a relationship between drug exposure and outcomes and 2. There is significant inter-individual variability in how patients metabolize a drug (pharmacokinetics) and in the magnitude and duration of the response to treatment (pharmacodynamics). **[1]** TDM is an interdisciplinary clinical specialty aimed at improving patient care through individualizing drug dosing for which clinical experience or clinical trials have shown to improve outcomes in general or specific populations. It can be based on prior pharmacogenetic, demographic, and clinical information or on the subsequent measurement of drug concentrations in the blood (pharmacokinetic monitoring) or biomarkers (pharmacodynamic monitoring). The three main reasons justifying TDM are: i. an experimentally determined and validated relationship between the plasma drug concentration and the pharmacological effect (efficacy & toxicity), ii. The significant variability of pharmacokinetics between and/or within the patient, and iii. The difficulty of monitoring the clinical effect of a specific drug. **[2]**

The criteria involved in a study during therapeutic drug monitoring include: 1. The ratio of the drug concentration in the blood to toxicity. 2. The ratio between the drug concentration in the blood and the drug dose. 3. Clinical indications relating to incompatibility, potential toxicity, and lack of response of the organism to the therapeutic treatment. Therapeutic drug monitoring (TDM) is a valuable tool for optimizing drug therapy by individualizing dosing based on a patient's unique pharmacokinetic and pharmacodynamic characteristics. By measuring drug concentrations in biological fluids, TDM helps to ensure that patients receive the optimal therapeutic dose, minimizing adverse effects and maximizing efficacy. This approach is particularly beneficial for drugs with a narrow therapeutic index, where small changes in drug concentration can have significant clinical consequences. The rationale behind TDM is grounded in the understanding that there is substantial inter-individual variability in drug response due to genetic, environmental, and physiological factors. Consequently, a one-size-fits-all dosing approach may not be suitable for all patients. TDM aims to address this variability by providing a more personalized approach to drug therapy. **[3]**

In recent years, analytical chemistry has witnessed a paradigm shift towards the development of environmentally sustainable methodologies. This transition aligns closely with the twelve principles of green chemistry, which advocate for minimizing sample preparation, re-

ducing sample quantities, conducting in situ analyses, integrating analytical processes, automating methods, avoiding derivatization, minimizing waste generation, employing multi-analyte techniques, conserving energy, utilizing renewable resources, eliminating toxic substances, and ensuring operator safety.**[4]** By adhering to these principles, analytical chemists are actively contributing to a more sustainable future. Modern approaches focus on creating methods that are not only eco-friendly but also maintain high performance standards, including accuracy, repeatability, sensitivity, and selectivity. A key area of innovation lies in the pretreatment of samples. Although the ideal scenario would be to eliminate the need for sample pretreatment, it remains essential due to the presence of interfering substances that complicate the detection of target analytes. Thus, optimizing sample pretreatment is a critical step in chemical analysis, with the goal of adhering to green chemistry principles. Additionally, there is a growing trend towards the development of advanced analytical instruments capable of accurately and sensitively detecting both organic compounds and metal ions. In clinical contexts, measuring drug concentrations in biological fluids is vital for personalizing therapy and monitoring patient adherence, thereby reducing the risk of drug interactions and toxicity. The quantification of these substances, whether for routine toxicological screening or advanced monitoring methodologies, plays a crucial role in public health and patient care. Furthermore, innovative extraction techniques that use minimal sample and solvent volumes are gaining attention for their potential to streamline analyses while minimizing environmental impact. **[5], [6]** This review discusses the application of microextraction techniques in sample preparation for therapeutic drug monitoring. In this review, the papers on microextraction techniques have been selected in such a manner that they cover a time window ranging from 2016 to 2024.

2. AUTOMATION STRATEGIES IN GREEN MICROEXTRACTION TECHNIQUES

The automation of green microextraction techniques marks a transformative development in analytical chemistry, enabling laboratories to achieve greater efficiency, precision, and sustainability in their processes. Traditionally, analytical methods have focused on sensitivity, selectivity, and precision, but recent trends emphasize the importance of minimizing environmental impact and reducing the use of toxic chemicals. Green microextraction techniques align with the principles of Green Analytical Chemistry (GAC) by minimizing the use of hazardous solvents, decreasing energy consumption, and reducing waste. The automation of these techniques further amplifies these benefits by streamlining sample preparation processes, enhancing reproducibility, and minimizing human error. For instance, the integration of robotic systems in sample handling automates labor-intensive tasks like sample transfer, vortexing, centrifugation, and dilution. This automation not only ensures consistency and accuracy across samples but also significantly increases throughput by allowing multiple samples to be processed simultaneously. Additionally, by reducing human intervention, robotic sample handling minimizes the risk of errors such as contamination or incorrect pipetting, which are common in manual processes.

In the realm of automated extraction, devices such as those employed in Solid-Phase Microextraction (SPME) and Liquid-Liquid Microextraction (LLME) have been optimized to function with minimal human input. These devices often feature robotic arms or automated sample injection systems, which allow them to perform complex extraction procedures with high precision and reproducibility. Automated systems can precisely control critical parameters such as temperature, extraction time, and agitation, ensuring that the extraction conditions are optimal for each specific analyte. This level of control leads to more reliable analytical results and reduces variability between experiments. Moreover, automated extraction devices are often integrated with data acquisition and analysis software, enabling real-time monitoring of the extraction process. This integration not only speeds up the analytical workflow but also enhances the accuracy of the data by reducing the likelihood of human error during data interpretation. Consequently, automated extraction systems not only improve the efficiency of the analytical process but also contribute to the development of greener and more sustainable laboratory practices.

The adoption of advanced automation strategies, such as microfluidic devices and lab-on-a-chip (LOC) systems, represents a cutting-edge approach to further refining green microextraction techniques. These miniaturized systems allow multiple analytical steps—such as sample collection, extraction, separation, and detection—to be performed within a single, compact device. This integration not only reduces the need for manual intervention but also significantly lowers the consumption of reagents and solvents, aligning with the goals of Green Analytical Chemistry. Microfluidic devices, for example, utilize very small volumes of solvents and samples, which not only reduces waste but also enhances the efficiency of the extraction process. Lab-on-a-chip systems go even further by incorporating all necessary analytical steps into a single platform, which can be automated to operate continuously or in a stand-alone mode. The potential for coupling these systems with advanced detection methods, such as mass spectrometry,

allows for rapid and highly sensitive analysis with minimal environmental impact.

The integration of advanced data acquisition and analysis tools further streamlines the analytical process and improves the accuracy and reliability of results. These tools allow for real-time data processing, reducing the risk of human error in data interpretation and accelerating the analytical workflow. By automating data analysis, laboratories can obtain timely and reliable results, supporting informed decision-making. As these technologies continue to advance, the automation of green microextraction techniques will likely play an increasingly pivotal role in addressing the growing demand for efficient, sustainable, and accurate analytical methods in a variety of fields, including environmental monitoring, food safety, and pharmaceuticals.**[7-23]**

3. MICROEXTRACTION TECHNIQUES

3.1 Electromembrane Extraction – "EME"

In contrast to traditional liquid-liquid extraction (LLE), where the analyte is separated through diffusion, electro-membrane extraction (EME) utilizes electrokinetic migration to facilitate mass transfer. EME employs a three-phase system that includes two aqueous solutions separated by a supported liquid membrane (SLM). As depicted in figure 1, the system is connected to an external power supply, with electrodes placed in both the donor (sample) and acceptor aqueous solutions. For the extraction of basic analytes, the positively charged electrode (anode) is positioned in the donor solution, while the negatively charged electrode (cathode) is in the acceptor solution. For acidic analytes, the polarity of the electrodes is reversed. EME requires the target analyte to be ionized and carry a charge to be effectively extracted. **[24]**EME is characterized by rapid extraction kinetics, effective matrix removal, environmental friendliness, and superior selectivity attributed to the membrane and applied electric field.**[24,25]**

Tofacitinib (TFB) and cyclosporine A (CsA) are immunosuppressants that can have toxic side effects if not dosed appropriately. Therapeutic drug monitoring (TDM) is crucial to ensure safe and effective treatment with these drugs. Electromembrane extraction (EME) is a promising sample preparation technique for TDM due to its efficiency, selectivity, and low solvent consumption. As presented in table 1, in **[25],** Sætrang investigated the use of EME for the sample preparation of CsA and TFB. For CsA, challenges arose due to its low solubility and detection limit using HPLC-UV. Despite attempts to extract CsA as an anion under alkaline conditions, successful extraction was not achieved. However, for TFB, a suitable EME method was developed. By optimizing conditions such as pH, supported liquid membrane (SLM) composition, voltage, and extraction time, a 100% recovery of TFB was achieved from diluted plasma samples using a specific SLM and extraction conditions. While EME shows promise as a sample preparation technique for TFB, further optimization is necessary to determine its viability for CsA.

Regarding serum samples, Skallvik et al. **[26]** developed a novel conductive vial electromembrane extraction (EME) platform for the analysis of lipophilic basic drugs in serum. This approach innovatively replaces conventional platinum

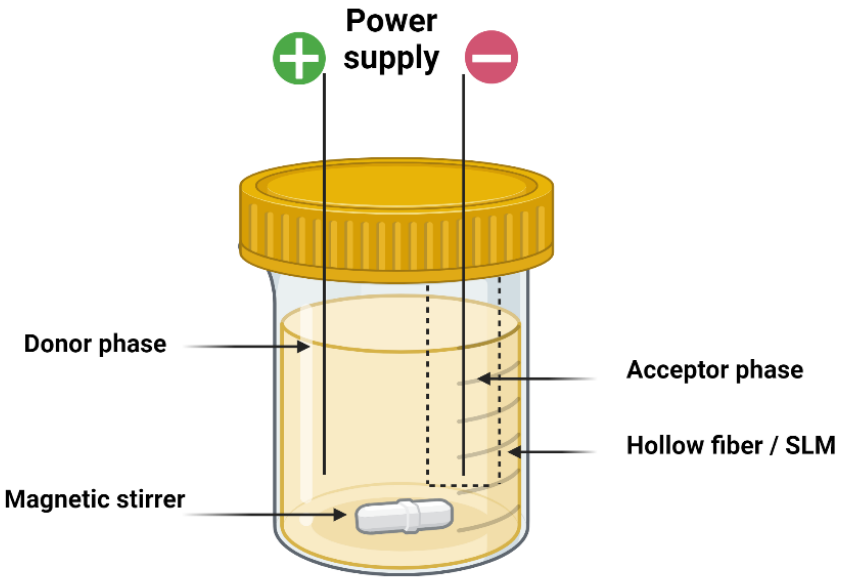

Figure 1 *Electromembrane extraction (created with BioRender.com)*

Table 1 *Bioanalytical procedures using EME for TDM*

Analyte	Sample	Sample Pretreatment and Extraction Procedure	Analytical technique	LOD;LOQ	Linear range	Reference
Tofacitinib (TFB) and Cyclosporin A (CsA)	plasma	• **TFB**: Donor solution 300 µL diluted plasma spiked with 5 µg/mL TFBc (pH 2.1), SLM: 10 µL of 6MethylCoumarin:Thymol, Acceptor solution: 300 µL of 100 mM Formic Acid (pH 2.4),Voltage: 30 V, Agitation: 750 RPM • **CsA**: Donor solution: 250 µL of 20 µg/mL (5 µg) CsA: in 10 mM NaOH (pH 12), SLM: 10 µL 1 octanol, Acceptor solution:250 µL of 10 mM NaOH (pH 12),Agitation: 750 RPM, Extraction time: 15 min	HPLC-UV	TFB:0.05 µg/mL and not mentioned CsA: not mentioned and 2 µg/mL	TFB: 0.05-5 µg/mL CsA: 0.2-50 µg/mL.	**[25]**
Alimemazine, Amitriptyline, Atomoxetine, Clomipramine, Doxepin, Duloxetine, Fluvoxamine, Levomepromazine, Nortriptyline and Trimipramine and metabolites Desmethyl Clomipramine and Desmethyl Doxepin	serum	• 50 V for 15 min from serum samples (100 µL) diluted 1:3 with formic acid (0.1% v/v), using 2-nitrophenyl octyl ether as the supported liquid membrane (SLM), and formic acid (0.1% v/v, 300 µL) as acceptor phase	UHPLC-MS/MS	Estimated LODs ranged from 0.2 to 2 nM. The lowest calibration concentration represented LLOQ and ranged from 5 to 80 nM	not mentioned	**[26]**
Phenytoin (PHT)	plasma	• DBS punch (6 mm) • Internal standard (5 µL) added, nitrogen evaporation (25 °C, 10 min) • Microwave derivatization • Centrifugation (4 °C, 5 min, 13,000 rpm) • Reconstitution (100 µL injection solvent) • GC-MS analysis (1 µL injection)	CE-DAD	0.005 µg/mL and 0.03 µg/mL	0.03–4 µg/mL	**[27]**

electrodes with conductive polymer-based sample and acceptor vials, thereby enhancing the system's portability and disposability. Coupled with UHPLC-MS/MS, the method demonstrated high analytical performance for a panel of psychoactive drugs and their metabolites. Extraction efficiency was optimized using 2-nitrophenyl octyl ether as the supported liquid membrane and formic acid as the acceptor phase. The method exhibited excellent linearity, precision, and accuracy, with near-complete extraction of target analytes. Successful application to clinical samples and comparable results to a reference method validate the potential of conductive vial EME for routine drug monitoring.

Seyfinejad et al. **[27]**, developed and validated a novel electromembrane extraction (EME) method for the quantification of free phenytoin (PHT) concentration in human plasma. Given the clinical significance of therapeutic drug monitoring and the growing demand for accurate free drug levels in drug development, a reliable method for determining unbound drug fractions is essential. This study introduces an EME-based approach that effectively overcomes limitations associated with traditional methods by employing a water-immiscible membrane and leveraging the efficiency of an electric field. The method demonstrated precise protein binding assessment for PHT, enabling accurate determination of the free drug fraction.

3.2 Parallel Artificial Liquid Membrane Extraction – "PALME"

Parallel artificial liquid membrane extraction (PALME) is a microextraction technique characterized by a planar membrane configuration. As depicted in figure 2, a supported liquid membrane (SLM) is immobilized within this membrane, separating the sample and acceptor phases housed in a 96-well plate format. Sample preparation involves pipetting the sample into a well, applying a small volume of organic solvent to the membrane, and subsequently adding the accep-

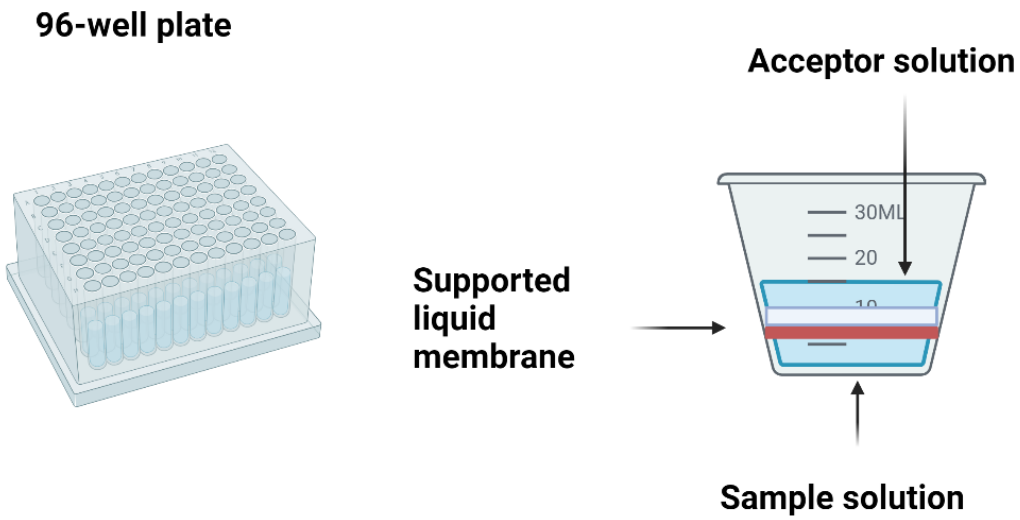

Figure 2 *Parallel Artificial Liquid Membrane Extraction (Created with BioRender.com)*

tor phase to the opposite well before plate assembly. **[28]** PALME offers superior efficiency and enables high-throughput extraction of up to 96 samples simultaneously.**[29]**

According to the summarized data of table 2, in **[30]**, this study introduces a novel approach using parallel artificial liquid membrane extraction (PALME) for the sample preparation of dried blood spots (DBS) prior to ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analysis. The aim was to develop a streamlined and efficient method for analyzing both basic and acidic model analytes in DBS samples. The PALME procedure involved a two-step process: desorption of analytes from the DBS and extraction through a supported liquid membrane (SLM). For basic model analytes, sodium hydroxide solution was used for desorption, while formic acid was used for acidic model analytes. The SLM consisted of either trioctylamine in dodecyl acetate or dihexyl ether, depending on the analyte type. The extracted analytes were then transferred to an acceptor solution for subsequent analysis. The PALME method demonstrated effective desorption and extraction of both basic and acidic model analytes from DBS samples within 60 minutes. High recoveries were achieved for all analytes, ranging from 63 to 85%. The PALME process also effectively removed phospholipids from the DBS samples, ensuring clean acceptor solutions for analysis. The method exhibited excellent linearity, with r^2 values greater than 0.99 for five of the six analytes. Additionally, the accuracy, precision, and matrix effects were found to be in accordance with European Medicines Agency guidelines. The results of this study demonstrate the potential of PALME as a promising sample preparation technique for DBS analysis. The method offers a rapid, efficient, and clean approach for processing DBS samples, enabling the analysis of a wide range of analytes. The ability to process up to 96 DBS samples within 60 minutes highlights the potential for high-throughput analysis using this technique.

It should be noted that in **[31]**, Olsen et al., employed parallel artificial liquid membrane extraction (PALME) for the extraction of serotonin and serotonin-norepinephrine reuptake inhibitors from human plasma samples. This study aimed to develop a semiautomated approach for this extraction technique. The method demonstrated efficient extraction of eight model analytes from 125 µL plasma samples, with recovery rates ranging from 72 to 111% and relative standard deviations (RSDs) below 12.8%. Successful implementation of a semiautomated pipetting system significantly reduced manual labor time. The method was validated using real patient samples, yielding accurate results. These findings highlight the feasibility of semiautomated PALME for the extraction of serotonin and serotonin-norepinephrine reuptake inhibitors from human plasma.

In **[32]**, Ahmed et al., present a novel method for the rapid and efficient therapeutic drug monitoring of repaglinide (RPG) in diabetic patients. The method combines parallel artificial liquid membrane extraction (PALME) with ultraperformance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-ESI-MS/MS). PALME was performed using a 96-well plate containing plasma samples, phosphate buffer, and cetirizine as an internal standard. A polypropylene membrane served as the support for the liquid membrane, which consisted of dodecyl acetate and trioctylamine. The extracted analytes were transferred to an acceptor solution containing DMSO and formic acid. The developed method demonstrated high efficiency and reproducibility, with a linear calibration range, low limit of quantitation, and high recovery. The method allowed for the analysis of 198 samples per hour, highlighting its high throughput capabilities. Additionally, the use of a minimal amount of solvents contributed to

Table 2 *Bioanalytical procedures using PALME for TDM*

Analyte	Sample	Sample Pretreatment and Extraction Procedure	Analytical technique	LOD;LOQ	Linear range	Reference
Amitriptyline,Quetiapine, Ketoprofen, Fenoprofen, Flurbiprofen, Ibuprofen	whole blood	• **Amitriptyline and quetiapine**: DBS were initially subjected to desorption using 250 µL of 10 mM sodium hydroxide solution. The resulting desorbate was transferred to a corresponding well in a sond 96-well plate containing a supported liquid membrane (SLM) composed of 4 µL of 1% trioctylamine in dodecyl acetate. An acceptor phase consisting of 50 µL of 20 mM formic acid was added to the opposite well • **Ketoprofen, fenoprofen, flurbiprofen, ibuprofen**: Analytes were initially desorbed from the DBS matrix using a 20 mM formic acid solution. Subsequently, the desorbed analytes underwent extraction across a supported liquid membrane (SLM) composed of dihexyl ether into an acceptor phase consisting of 25 mM ammonia. This extraction process was conducted for a duration of 60 min, resulting in the efficient transfer of target analytes from the DBS matrix into the acceptor solution, which was subsequently introduced directly into the analytical instrument.	UHPLC-MS/MS	amitriptyline: 0.9 and 2.9 ng/mL ,quetiapine: 0.2 and 0.7 ng/mL , ketoprofen: not evaluated, fenoprofen: not evaluated, flurbiprofen: not evaluated, ibuprofen: not evaluated	not evaluated	**[30]**
O-desmethyl-venlafaxine, Venlafaxine, Citalopram, Paroxetine, Fluvoxamine, Fluoxetine, Norfluoxetine, Sertraline	plasma	• Plasma sample (125 µL) spiked with internal standard (10 µL), pH adjusted to 13 (115 µL 40 mM NaOH) • Donor plate: Plasma sample loaded • Acceptor plate: 1% trioctylamine in dodecyl acetate (5 µL), acceptor phase (50 µL 20 mM formic acid) • Plate assembly, agitation (900 rpm)	UHPLC–MS/MS	LOD was not evaluated for the analytes, O-desmethyl-venlafaxine: 0.23 ng/mL, Venlafaxine: 0.17 ng/mL, Citalopram: 0.23 ng/mL, Paroxetine: 0.16 ng/mL, Fluvoxamine: 0.13 ng/mL, Fluoxetine: 0.28 ng/mL, Norfluoxetine: 1.11 ng/mL, Sertraline: 0.29ng/mL	5-1000 ng/mL for every analyte	**[31]**
Repaglinide (RPG)	plasma	• 96-well donor plate: plasma, 50 mM phosphate buffer (pH 8.0), cetirizine • SLM: PP membrane, dodecyl acetate (1% trioctylamine) • Acceptor phase: DMSO:formic acid (50:50, v/v)	UPLC-ESI-MS/MS	not evaluated and 0.1 ng/mL	0.1–100 ng/mL	**[32]**

the method's eco-friendly profile. The PALME-UPLC-ESI-MS/MS method was successfully applied to monitor RPG levels in diabetic patients following tablet administration, demonstrating its effectiveness for therapeutic drug monitoring.

A novel method for the extraction of polar basic drugs from human plasma was developed by Pilařová et al. **[33]**, utilizing parallel artificial liquid membrane extraction (PALME). Hydralazine, ephedrine, metaraminol, salbutamol, and cimetidine served as model compounds for method development and validation. The extraction process involved the transfer of analytes from alkalinized plasma across a supported liquid membrane into an aqueous acceptor phase. Ion-pair complexation between the hydrophilic analytes and a carrier within the liquid membrane facilitated extraction efficiency. Eliminating the need for solvent evaporation, the direct aqueous-based extraction streamlined sample preparation. Optimized extraction conditions yielded recovery rates ranging from 50 to 89% within a 45-minute extraction period. Comprehensive method validation demonstrated robust performance, aligning with regulatory expectations, and establishing PALME as a promising platform for high-throughput bioanalysis of polar basic drugs.

3.3 Dispersive Liquid-Liquid Microextraction – 'DLLME'

Dispersive liquid-liquid microextraction (DLLME) involves the rapid injection of a mixture of water-immiscible extractant and water-miscible disperser solvent into an aqueous sample. Figure 3 illustrates this action that generates a fine dispersion of the extractant within the sample matrix. Following extraction, the resulting heterogeneous mixture is subjected to centrifugation to facilitate phase separation, with the extractant phase forming a distinct layer at the bottom of the tube. **[34]** DLLME offers several advantages, including operational simplicity, low cost, high preconcentration factors, excellent extraction efficiency, and minimal sample preparation requirements. **[35]**

As shown in table 3,noteworthy is the work carried out by Han et al. **[36]**, who proposed a green and rapid analytical method termed deep eutectic solvent dispersed liquid-liquid microextraction with magnetic particle-assisted retrieval (DES-DLLME-MPAR) for the extraction of antidepressants from biological samples**.** This approach utilized vortex-assisted extraction for efficient analyte transfer into the deep eutectic solvent (DES) and employed magnetic particles for rapid and convenient phase separation. Optimal extraction conditions were established, including the composition of the DES, sample pH, extraction and desorption times, and the amount of magnetic particles. The method demonstrated high extraction recoveries, excellent linearity, low limits of quantification, and satisfactory accuracy and precision. Furthermore, the green nature of the method was confirmed through greenness assessment metrics. The proposed DES-DLLME-MPAR method offers a promising alternative to traditional extraction techniques for the analysis of hydrophobic drugs in biological matrices.

There are some applications of DLLME techniques used for pretreatment of biological samples, mainly plasma. In 2020, Alcantara et al. **[37]**, developed a novel analytical method combining dispersive liquid-liquid microextraction (DLLME) with liquid chromatography-mass spectrometry (LC-MS) for the quantification of the active fraction of antipsychotic drugs in human plasma. Chromatographic separation was achieved using a C_{18} column with a gradient elution of ammonium acetate and acetonitrile. The DLLME procedure involved the use of chlorobenzene as the extractant and acetone as the disperser solvent, with sample pH adjusted to 12.0 and 10% NaCl added for enhanced extraction efficiency. The method underwent full validation according to regulatory guidelines and demonstrated successful application in the therapeutic drug monitoring of schizophrenic patients.

In **[38]**, Turković et al., present the development of a novel dispersive liquid-liquid microextraction (DLLME) method for the simultaneous extraction of six cyclin D-dependent kinase 4/6 (CDK 4/6) inhibitors from human plasma. These inhibitors are used in combination with endocrine therapy for breast cancer treatment, and their monitoring in patient plasma is crucial for effective therapy.

The study explored three different DLLME modes: organic sample DLLME (OrS-DLLME), aqueous sample DLLME (AqS-DLLME), and a modified air-assisted DLLME (AA-DLLME). These modes were optimized to achieve high extraction recoveries while minimizing sample and solvent consumption.The developed DLLME method demonstrated excellent linearity, precision, and accuracy, as validated according to established criteria. The method also achieved high extraction recoveries for all six drugs, with sample volumes as low as 50-100 µL and minimal organic solvent usage. The greenness of the method was confirmed by a high AGREE score, indicating compliance with green analytical chemistry principles. The validated DLLME method was successfully applied to analyze breast cancer patient plasma samples, demonstrating its suitability for the therapeutic drug mon-

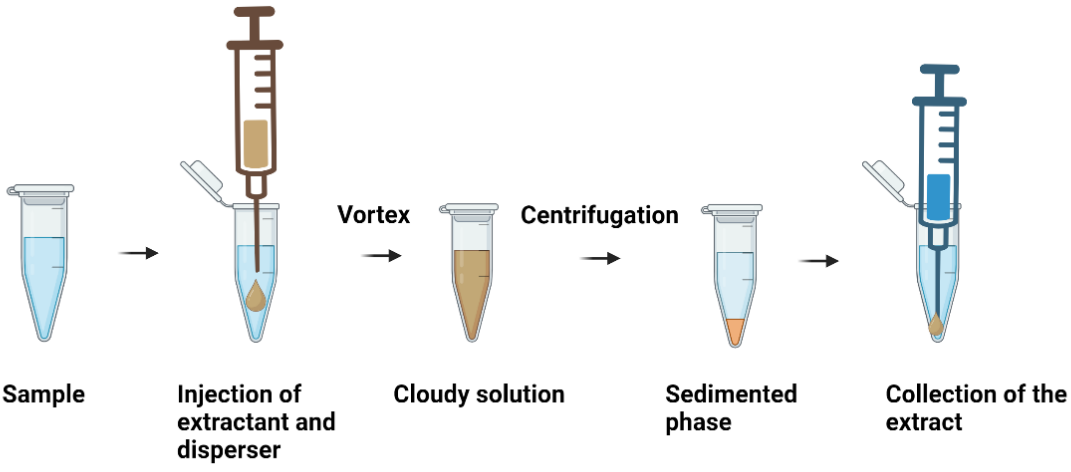

Figure 3 *Dispersive Liquid-Liquid Microextraction (Created with BioRender.com)*

itoring of CDK 4/6 inhibitors. This study highlights the potential of DLLME as a promising sample preparation technique for the analysis of these important anticancer drugs.

3.4 Solid Phase Microextraction- 'SPME'

Solid-phase microextraction (SPME**)** is a solvent-free extraction technique widely employed in various fields, including pharmaceuticals, forensics, and environmental science. SPME integrates sampling, extraction, and sample introduction into a single process. Figure 4 demonstrates a fused silica fiber coated with a stationary phase (liquid or solid) which is exposed to the sample matrix, allowing for analyte partitioning between the sample and the adsorbent. This equilibrium-based extraction can be performed through direct immersion, headspace sampling, or membrane-protected configurations **[39]**. This technique exhibits high speed and sensitivity, enabling simultaneous extraction and preconcentration of target analytes. Additionally, it offers potential for automation **[3]**.

Looby et al. **[40]**, as shown in table 4, employed Solid Phase Microextraction (SPME) methodology to optimize tranexamic acid (TXA) therapeutic drug monitoring in plasma and urine samples from patients with chronic renal dysfunction (CRD). Employing hydrophilic-lipophilic balance (HLB)-coated SPME fibers significantly enhanced sample throughput, enabling the analysis of TXA levels in 96 samples within 25 min. The method demonstrated robust analytical performance with low limits of quantification, excellent accuracy, and precision. Successful implementation in a clinical setting facilitated the adjustment of TXA dosing regimens for CRD patients undergoing cardiac surgery. The proposed SPME approach eliminates

Table 3 *Bioanalytical procedures using DLLME for TDM*

Analyte	Sample	Sample Pretreatment and Extraction Procedure	Analytical technique	LOD;LOQ	Linear range	Reference
Amitriptyline (AMP), Imipramine (IMP), Sertraline (SER), and Fluoxetine (FLX)	blood and urine	• 10 µL DES added to biological sample (1.5 mL tube) • Vortexing, 10 mg magnetic particles added • Magnetic gel formation, separation • Desorption with 100 µL ethanol • 20 µL eluate for LC-UV analysis	LC-UV	IMP: 0.028 µg/mL and 0.092 µg/mL, FLX: 0.020 µg/mL and 0.068 µg/mL, AMP: 0.027 µg/mL and 0.075 µg/mL, SER: 0.025 µg/mL and 0.080 µg/mL	0.1-10 µg/mL for every analyte	**[36]**
Risperidone and its metabolite 9-Hydroxyrisperidone	plasma	• DLLME parameter optimization: solvent types, volumes, ionic strength, vortex time • 3.0 mL aqueous sample, 500 µL acetone (disperser), 80 µL chlorobenzene (extractor) • Centrifugation, evaporation, reconstitution (200 µL acetonitrile-water) • LC-MS/MS analysis	LC-MS/MS	LOD was not evaluated, LOQ values were 5.0 ng/mL for both analytes	5.0-80.0 ng/mL for every analyte	**[37]**
Palbociclib, Ribociclib, Abemaciclib, Anastrozole, Letrozole and Fulvestrant	plasma	• Protein precipitation (PPT): 200 µL ACN added to 50 µL plasma • Supernatant evaporation • Reconstitution in aqueous phase (50-500 µL) • Disperser solvent (0-1000 µL) and extractant solvent (50-500 µL) addition • Mixture vortexed, centrifuged (5 min) • Organic layer collected, evaporated • Residue reconstituted in 40 µL 65% methanol	HPLC-DAD-FLD	LOD and LOQ values were not mentioned	Palbociclib: 0.08-1.92 µg/mL, Ribociclib: 0.25-5.95 µg/mL, Abemaciclib: 0.11-2.61 µg/mL, Anastrozole: 2.51- 60.30 µg/mL, Letrozole: 0.04-1.01 µg/mL and Fulvestrant: 0.50-12.04 µg/mL	**[38]**

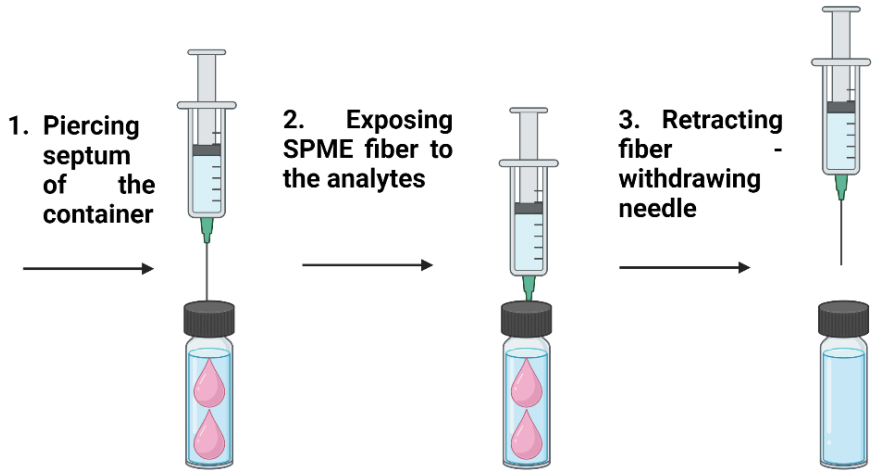

Figure 4 *Solid Phase Microextraction (Created with BioRender.com)*

the need for complex sample pretreatment, rendering it a green and efficient analytical solution for TXA biomonitoring in both plasma and urine.

A novel in vivo lung perfusion (IVLP) technique has been developed, by Bojko et al. **[41]**, for targeted delivery of high-dose doxorubicin (DOX) to residual pulmonary micrometastases. To enable precise monitoring of drug distribution, a biocompatible nitinol wire coated with a sorbent material (Bio-SPME) was employed for in vivo extraction and quantification of DOX and its metabolites. Preclinical and clinical investigations demonstrated the feasibility of real-time DOX level assessment within lung tissue during IVLP. The Bio-SPME approach facilitated the acquisition of pharmacokinetic data and provided insights into the metabolic profile of the lung during drug administration. This innovative methodology offers the potential for personalized treatment strategies by enabling dynamic monitoring of drug exposure and tissue response.

In vivo lung perfusion (IVLP) is a novel technique for delivering high-dose chemotherapy directly to the lungs to treat metastatic lung cancer. The FOLFOX regimen, consisting of folinic acid, 5-fluorouracil, and oxaliplatin, is commonly used for various solid tumors. However, monitoring 5-fluorouracil levels is crucial due to its variability in plasma concentration. Since plasma levels may not accurately reflect tissue drug concentrations, sample preparation methods specifically designed for target organs are essential. In **[42]**, Looby et al., propose in vivo solid-phase microextraction (in vivo SPME) as a valuable tool for quantitative therapeutic drug monitoring of FOLFOX in porcine lungs during pre-clinical IVLP and intravenous (IV) trials. By simultaneously extracting other small molecules from the lung and analyzing them using liquid chromatography-high resolution mass spectrometry (LC-HRMS), this approach allows for an assessment of FOLFOX's impact on the lung's metabolic profile and the identification of metabolic pathways associated with the route of administration. The study demonstrates the importance of immediate instrumental analysis of metabolomic samples, as long-term storage can lead to changes in metabolite content. In vivo SPME provides a valuable tool for monitoring drug levels in the lung and assessing the metabolic impact of FOLFOX therapy.

3.5 Microextraction by Packed Sorbent – 'MEPS'

Microextraction by packed sorbent (MEPS) is a miniaturized solid-phase extraction technique designed to optimize sample throughput and reduce solvent consumption. By significantly decreasing sample volume and eliminating the need for extensive sample preparation, MEPS enables direct injection into chromatographic systems. This method offers enhanced efficiency and compatibility with automation protocols **[27-31]**. Microextraction by packed sorbent (MEPS) is a versatile sample preparation technique that employs a diverse array of sorbent materials, including silica-based phases, ion-exchange resins, polymeric sorbents, and molecularly imprinted polymers. The method encompasses a sequential process involving sorbent preparation, sample loading, washing, and analyte elution, as presented in figure 5. MEPS excels in its ability to efficiently extract and concentrate analytes from complex matrices while minimizing solvent consumption and sample volume. Moreover, the technique's compatibility with automation platforms enhances its throughput and reproducibility. These attributes, coupled with its broad applicability to various analytes and matrices, position MEPS as a valuable tool in analytical chemistry **[43]**.

Table 5 indicates that MEPS has been applied for the extraction of drugs in several biological specimens such as plasma and saliva. In **[44]**, Cruz et al., established a restricted access carbon nanotube (RACNT)-based microextraction by packed sorbent (MEPS) method for the quantification of antipsychotic drugs in untreated plasma samples from schizophrenic patients using ultra-high performance liquid chromatog-

Table 4 *Bioanalytical procedures using SPME for TDM*

Analyte	Sample	Sample Pretreatment and Extraction Procedure	Analytical technique	LOD;LOQ	Linear range	Reference
Tranexamic Acid	plasma and urine	• **Plasma:** The SPME device was initially conditioned by exposure to 1.5 mL of a 50:50 methanol:water (v/v) solution for a duration of 10 minutes. Subsequently, the device was immersed in 1 mL of plasma sample solution, prepared as a 1:3 ratio of sample to phosphate-buffered saline (PBS) containing an internal standard, for a 5-minute extraction period. Following extraction, the device was rinsed with 1 mL of a 90:10 water:methanol (v/v) solution for 10 seconds under static conditions. Finally, the analytes were desorbed from the SPME fiber into 1 mL of a 3:3:4 methanol:acetonitrile:water (v/v/v) solution through a 10-minute desorption step. • **Urine:** The SPME device was initially conditioned in 1.5 mL of a 50:50 methanol/water solution for 10 minutes. Subsequently, the device was immersed in 1 mL of urine sample, diluted 1:3 with 0.5 M phosphate buffer containing an internal standard, for a 5-min extraction period. Following extraction, the device was rinsed in 1 mL of water under agitated conditions for 10 seconds to remove residual salts. Finally, the analytes were desorbed into 1 mL of a 90:10 water/methanol solution through a 10-min desorption step.	LC-MS	LOD values are not mentioned, LOQ: urine: 25 µg/mL and plasma: 10 µg/mL	25 µg/mL-1000 µg/mL in urine, and 10 µg/mL-1000 µg/mL in plasma	**[40]**
Doxorubicin	Lung tissue and perfusate	• 3 Bio-SPME fibers/sampling site • Nitinol wire coated with sorbent particles • Tissue insertion (20 min) • Water rinse, wiping (5 s) • Snap freezing in dry ice	HPLC-MS/MS	Not specified; LOQ: 0.1 µg/mL (perfusate), 5 µg/g (lung)	0.1 - 10 µg/mL (perfusate), 5 - 500 µg/g (lung)	**[41]**
Folinic acid (FOL), 5-Fluorouracil (F), and Oxaliplatin (OX) (FOLFOX)	Lung tissue and perfusate	• SPME fiber sterilization (50:50 methanol:water, 30 min) • Fiber insertion into lung tissue (3 locations, 1-2 cm apart) • Water rinse (5 s), wiping • Storage in 300 µL polypropylene vials, snap-frozen in dry ice (-80 °C)	LC-HRMS	25 µg/g and 50 µg/g were achieved for both F and FOL	50 µg/g - 1000 µg/g for F and FOL	**[42]**

raphy-tandem mass spectrometry (UHPLC-MS/MS). The RACNT sorbent, prepared by coating multi-walled carbon nanotubes with bovine serum albumin, exhibited exceptional protein rejection capabilities, maintaining consistent performance over multiple assays. Method optimization, encompassing sample pH, extraction cycles, desorption conditions, and cleanup steps, resulted in enhanced sensitivity and selectivity. The established MEPS-UHPLC-MS/MS method demonstrated robust analytical performance, characterized by excellent linearity, precision, and accuracy, with minimal matrix effects and carryover. Successful application to therapeutic drug monitoring in schizophrenic patients underscores the clinical utility of this innovative approach.

In **[45]**, Magalhães et al., present a novel liquid chromatography (LC) method for the simultaneous quantification of fluoxetine (FLU), norfluoxetine (NFLU), and paroxetine (PAR) in human plasma. The method utilizes microextraction by packed sorbent (MEPS) as a sample preparation technique and fluorescence detection (FLD) for quantification. The LC method employs a reverse-phase C_{18} column

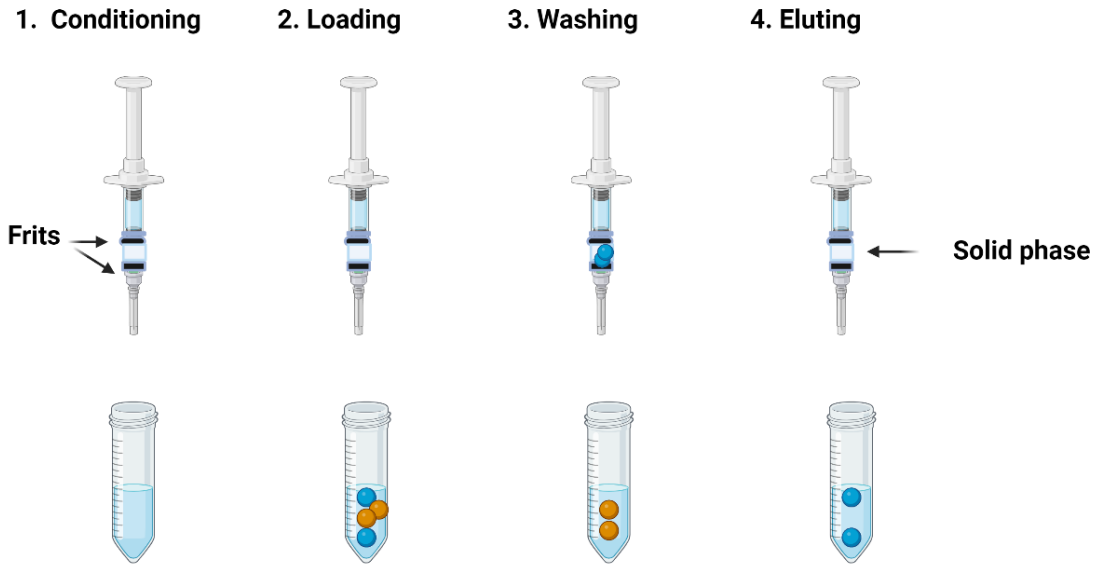

Figure 5 *Microextraction by Packed Sorbent (Created with BioRender.com)*

Table 5 *Bioanalytical procedures using MEPS approaches for TDM*

Analyte	Sample	Sample Pretreatment and Extraction Procedure	Analytical technique	LOD;LOQ	Linear range	Reference
Chlorpromazine (CLOR), Clozapine (CLOZ), Olanzapine (OLA) and Quetiapine (QUET)	plasma	• Plasma sample (150 µL) passed through RACNT (restricted acess carbon nanotube) bed (3 times) • Impurity removal (150 µL ultrapure water) • Analyte elution (2 x 100 µL acetonitrile), drying, reconstitution (50 µL mobile phase) • Sorbent reconditioning (10 x 200 µL acetonitrile, 2 x 200 µL ultrapure water)	UHPLC-MS/MS	LOD values are not mentioned; LOQ: 10 ng/mL for every analyte	200 ng/mL for OLA and 700 ng/mL for CLOR, CLOZ, and QUET	**[44]**
Fluoxetine (FLU), Norfluoxetine (NFLU), Paroxetine (PAR)	plasma	• Plasma (500 µL) spiked; acetonitrile (1.5 mL) added • Residue reconstituted in phosphate buffer (pH 4.0, 500 µL) • MEPS: C_8 cartridge activation, sample loading (3x) • Impurity removal (2 x 200 µL ammonium hydroxide) • Analyte elution (5 x 200 µL methanol-formic acid) • Eluate drying, reconstitution	LC–FLD	LOD: FLU and NFLU: 5 ng/mL, PAR: 1 ng/mL; LOQ: FLU and NFLU: 20 ng/mL, PAR: 5 ng/mL	FLU and NFLU: 20–750 ng/mL, PAR: 5–750 ng/mL	**[45]**
Lamotrigine	plasma and saliva	• Plasma/saliva (100 µL) spiked, acetonitrile (400 µL), centrifugation • Residue reconstituted (0.3% triethylamine-water, 200 µL) • C_{18} sorbent activation (methanol, water, 3 cycles each) • Sample drawn through sorbent (3 times) • Impurity removal (water, 200 µL) • Analyte elution (methanol, 2 x 30 µL), dilution, injection • Sorbent reconditioning (methanol, water)	HPLC-DAD	LOD values are not mentioned; LOQ: 0.1 µg/mL	0.1–20 µg/mL	**[46]**

and an isocratic mobile phase consisting of phosphate buffer and acetonitrile/methanol. The method demonstrates excellent linearity, precision, and accuracy within specific concentration ranges for all three analytes. The developed method was successfully applied to the analysis of authentic plasma samples, demonstrating its suitability for therapeutic drug monitoring and clinical studies involving these antidepressant drugs. The MEPS/LC-FLD assay offers a robust, low-cost, and efficient approach for the simultaneous quantification of FLU, NFLU, and PAR in human plasma.

A novel high-performance liquid chromatography-diode array detection (HPLC-DAD) method incorporating microextraction by packed sorbent (MEPS) was developed, by Ventura et al. **[46]**, for the quantitative determination of lamotrigine (LTG), a therapeutic drug with a narrow therapeutic index, in human plasma and saliva. Method optimization, including meticulous control of sample volume (100 µL) and chromatographic conditions, yielded exceptional selectivity and sensitivity. Isocratic elution on a C_{18} column facilitated rapid analysis of LTG and the internal standard, chloramphenicol, within five min. The method demonstrated robust linearity, precision, and accuracy, with a limit of quantification of 0.1 µg/mL in both biological matrices. Successful application to authentic human plasma and saliva samples from epileptic patients underscores the method's suitability for therapeutic drug monitoring.

3.6 Stir bar sorptive extraction – 'SBSE'

Stir bar sorptive extraction (SBSE) is a sample preparation technique that leverages the principle of analyte partitioning between an aqueous sample and a static phase. In figure 6 it can be clear that in contrast to SPME, SBSE employs a magnetic stir bar coated with a thicker layer of sorbent material, typically polydimethylsiloxane (PDMS), to enhance analyte adsorption capacity. The increased surface area of the stir bar facilitates efficient mass transfer and analyte enrichment during the extraction process. The magnetic core of the stir bar enables its continuous agitation within the sample matrix, promoting analyte diffusion into the PDMS coating. Following extraction, the stir bar is removed from the sample and subjected to thermal or liquid desorption to release the concentrated analytes for subsequent chromatographic analysis. This solvent-free approach, coupled with the high extraction efficiency and compatibility with various analytical platforms, has rendered SBSE a valuable tool for a wide range of analytical applications **[47]**. SBSE capitalizes on an extensive sorbent material, providing a substantial surface area that significantly enhances analyte enrichment and sensitivity. Ongoing research focuses on developing novel coated SBSE media, incorporating advanced materials and nanostructures, to further optimize extraction efficiency **[48]**.

Hypertension constitutes a major risk factor for morbidity and mortality among patients with cardiovascular diseases (CVDs). Angiotensin II receptor antagonists (ARAs) have emerged as effective and well-tolerated alternatives to traditional angiotensin-converting enzyme (ACE) inhibitors in the management of hypertension and heart failure. Losartan and valsartan, as prominent representatives of the ARA class, exhibit superior specificity, selectivity, and prolonged pharmacological effects compared to their peptide-based predecessors. Therefore, Babarahimi et al. **[49]**, as displayed in table 6, developed a novel stir bar coated with a polymeric material for the simultaneous determination of losartan and valsartan in human plasma samples using ultrasound-assisted liquid desorption coupled with high-performance liquid chromatography-ultraviolet detection (HPLC-UV). The proposed stir bar exhibited superior extraction efficiency compared to com-

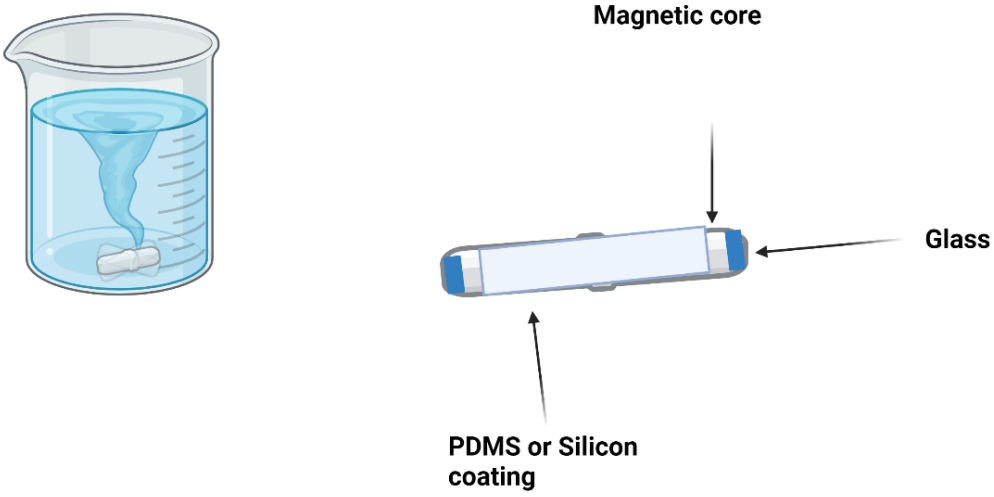

Figure 6 *Stir Bar Sorptive Extraction (Created with BioRender.com)*

Table 6 *Bioanalytical procedures using SBSE approaches for TDM*

Analyte	Sample	Sample Pretreatment and Extraction Procedure	Analytical technique	LOD;LOQ	Linear range	Reference
Losartan (LOS) and Valsartan (VAS)	plasma	• Plasma (1 mL) spiked with LOS, VAS, diluted to 5 mL • Sodium chloride added • Stir bar extraction (1000 rpm, controlled conditions) • Desorption (250 µL solvent, ultrasound) • 20 µL aliquot injected for HPLC analysis	HPLC-UV	LOD; LOS: 7 ng/mL and VAS: 27 ng/mL LOQ; LOS: 24 ng/mL and VAS: 91 ng/mL	24 to 1000 ng/mL for LOS, and 91 to 1200 ng/mL for VAS	[49]
Fluoxetine (FLU)	plasma	• Plasma (240 µL) spiked with FLU standard (10 µL) • Sodium borate buffer (3750 µL, pH 9.00) added • Stirring (840 rpm) • Desorption (methanol-acetonitrile, 4000 µL, 50 °C, 50 min) • Evaporation, reconstitution (250 µL desorption solution) • HPLC-FD analysis	HPLC–FD	9.80 ng/mL and 32.67 ng/mL	25.00–1000.00 ng/mL	[50]
Sertraline, Mirtazapine, Fluoxetine, Citalopram, Paroxetine, Imipramine, Nortriptyline, Amitriptyne, and Desipramine	plasma	• Plasma sample (1 mL) diluted with borate buffer (pH 9.0, 4 mL) • Stir bar extraction (1000 rpm, 50 °C, 45 min) • Desorption (acetonitrile, 15 min, 50 °C) • HPLC analysis	HPLC-UV	LOD values are not mentioned; LOQ: Sertraline: 35 ng/mL, Mirtazapine: 40 ng/mL, Fluoxetine: 25 ng/mL, Citalopram: 10 ng/mL, Paroxetine: 40 ng/mL, Imipramine: 35 ng/mL, Nortriptyline: 15 ng/mL, Amitriptyne: 15 ng/mL, and Desipramine: 35 ng/mL	LOQ-1000.0 ng/mL for every analyte	[51]

mercially available polydimethylsiloxane and polyacrylate stir bars. Method optimization, including extraction and desorption conditions, resulted in excellent linearity, sensitivity, accuracy, and precision. The method demonstrated successful application in the analysis of patient plasma samples, offering a rapid and sensitive alternative to previously reported stir bar sorptive extraction techniques. The use of appropriate sample preparation effectively mitigated the selectivity limitations of UV detection, enabling reliable quantification of target analytes.

Depression is a significant global health issue, affecting millions of people. While antidepressants are effective for many, individual responses can vary. Monitoring plasma levels of antidepressants is crucial for effective treatment, but it presents analytical challenges. In **[50],** Marques et al., aim to develop a robust and efficient method for determining fluoxetine levels in human plasma using stir bar sorptive extraction (SBSE) coupled with high-performance liquid chromatography-fluorescence detection (HPLC-FD). The study optimized the SBSE process, including sorption and desorption steps. Factors such as temperature, time, stirring speed, desorption kinetics, and stirring mode were evaluated to achieve optimal extraction conditions. The developed method was validated and found to be robust, linear, precise, and accurate within a specific concentration range. The method also demonstrated excellent recovery and the ability to quantify fluoxetine without interference from other substances. The validated SBSE-HPLC-FD method was successfully applied to analyze real plasma samples, demonstrating its suitability for therapeutic drug monitoring and clinical studies. SBSE-HPLC-FD represents a promising alternative for the analysis of fluoxetine in low-volume samples. This method offers a cost-effective and efficient approach for therapeutic drug monitoring and can contribute to studies correlating plasma fluoxetine levels with clinical response.

In **[51]**, a sensitive and reproducible stir bar sorptive extraction (SBSE) coupled with liquid chromatography-ultraviolet detection (LC-UV) method was developed for the simultaneous determination of multiple antidepressants in human plasma. The method optimization encompassed extraction time, pH, ionic strength, protein effects, and desorption conditions to achieve optimal extraction efficiency. The SBSE/LC-UV method demonstrated excellent linearity, sensitivity, and precision, with quantification limits ranging from 10.0 to 40.0 ng/mL. Successful application of the method to the analysis of plasma samples from elderly depressed patients highlights its potential for clinical use in therapeutic drug monitoring.

3.7 Microwave Assisted Extraction – 'MAE'

Microwave-assisted extraction (MAE) represents a significant advancement in sample preparation methodologies for extracting analytes from solid matrices. As illustrated in figure 7, this technique leverages the application of microwave energy to create a dynamic extraction environment characterized by rapid and efficient heating, leading to enhanced mass transfer and analyte dissolution. Unlike conventional heating methods, MAE induces direct and homogeneous heating of the sample-solvent mixture, resulting in accelerated extraction kinetics and improved analyte recovery. The simplicity of the MAE process is a key advantage, as it typically involves minimal sample preparation steps. Researchers can readily introduce the target sample and appropriate solvent into a suitable extraction vessel and subject the mixture to microwave irradiation for a specified duration. Following the extraction, the resulting extract is collected and prepared for subsequent analysis. The streamlined workflow, coupled with the potential for automation, makes MAE a highly attractive and versatile technique for a wide range of analytical applications **[52]**. MAE offers significant advantages including accelerated extraction kinetics, reduced solvent consumption, and increased sample throughput. The technique's capacity for simultaneous multi-sample processing further enhances its efficiency **[53]**.

The summarized data from table 7 indicate that Świądro et al. **[54]**, developed an analytical methodology combining dried blood spots (DBS), microwave-assisted extraction (MAE), and capillary electrophoresis-mass spectrometry (CE-MS) for the comprehensive analysis of a broad spectrum of therapeutic drugs in human blood. The method demonstrated the successful quantification of tricyclic antidepressants, selective serotonin reuptake inhibitors, benzodiazepines, and hypnotics within a single analytical workflow. Rigorous optimization of MAE parameters, including extraction solvent, temperature, and time, was conducted to achieve maximal analyte recovery and matrix removal. The resulting method exhibited exceptional analytical performance, characterized by low limits of detection and quantification, excellent linearity, precision, and accuracy. Moreover, the method demonstrated robust stability and minimal matrix effects, ensuring reliable and reproducible quantification of target analytes. Successful application to both post-mortem and clinical samples validated the method's suitability for forensic toxicology and therapeutic drug monitoring, highlighting its potential as a valuable tool for clinical and forensic laboratories.

Another possible application of the MAE extraction procedure is the work of Brahmadhi et al.**[55]**, who developed

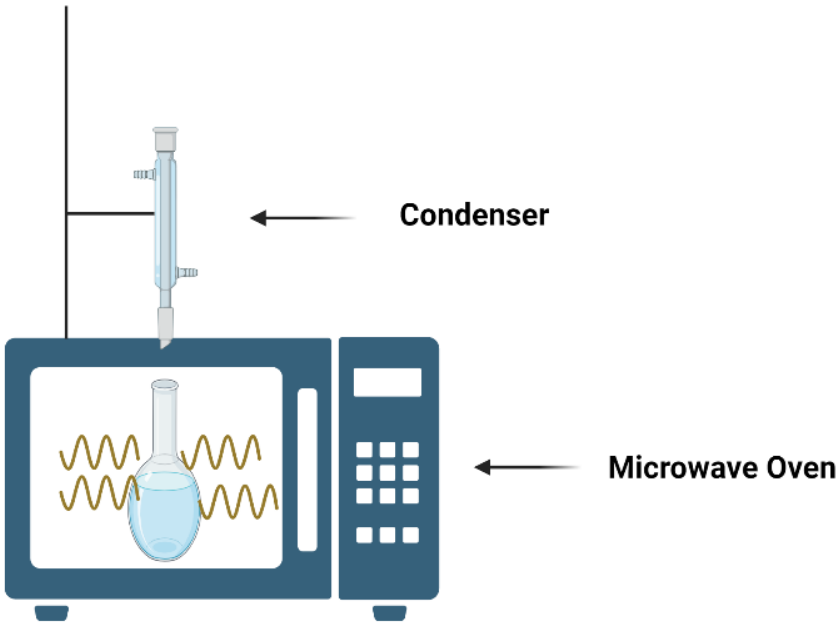

Figure 7 *Microwave Assisted Extraction (Created with BioRender.com)*

Table 7 *Bioanalytical procedures using MAE approaches for TDM*

Analyte	Sample	Sample Pretreatment and Extraction Procedure	Analytical technique	LOD; LOQ	Linear range	Reference
Amitriptyline (AMI), Citalopram (CIT), Imipramine (IMI), Tetrazepam (TET), Zolpidem (ZOL)	whole blood	• Whole blood added, mixture centrifuged • Dried blood spots (DBS) prepared • DBS extraction (MAE, 55°C, 2.5 min) • Solvent extraction, centrifugation • Residue reconstitution, CE-MS analysis • Total time: ~3 h.	CE-MS	LOD: AMI: 1.76 ng/mL, CIT: 9.15 ng/mL, IMI: 2.01 ng/mL, TET: 14.7 ng/mL, ZOL: 6.26 ng/mL, LOQ; AMI: 5.27 ng/mL, CIT: 27.50 ng/mL, IMI: 6.70 ng/mL, TET: 49.0 ng/mL, ZOL: 20.9 ng/mL	LOQ-300 ng/mL	**[54]**
Levofloxacin, Ciprofloxacin, Moxifloxacin	Dried Plasma Spots	• Plasma (15 µL) deposited on paper; air dried (2 h) • 6 mm DPS punched, placed in Eppendorf tube • Extraction solution (90% methanol, IS) added, vortexed • Microwave extraction (400 W, 40 s) • Centrifugation (15,000 x g, 15 min) • LC-MS analysis (4 µL injection)	UHPLC-MS/MS	LOD values are not mentioned; LOQ: 0.2 µg/mL for every analyte	0.2–20 µg/mL	**[55]**
Gamma-hydroxybutyric acid (GHB) and Gabapentin	Dried Blood Spots	• DBS punch (6 mm) • Internal standard (5 µL), nitrogen evaporation (25 °C, 10 min) • Microwave derivatization • Centrifugation (4 °C) • Solvent reconstitution (100 µL) • GC-MS analysis (1 µL injection)	GC–MS	LOD values are not mentioned; LOQ: 10 µg/mL for GHB and 1 µg/mL for Gabapentin	10–100 µg/mL for GHB and 1–30 µg/mL for gabapentin	**[56]**

an analytical method for the quantitative determination of levofloxacin, ciprofloxacin, and moxifloxacin in dried plasma spots (DPS) utilizing microwave-assisted extraction (MAE) coupled with ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). The method involved the deposition of a defined volume of plasma onto a protein saver card to create dried blood spots, followed by microwave-assisted extraction of the analytes from the punched-out spots. The optimized MAE procedure ensured efficient recovery of the target fluoroquinolones. Subsequent UHPLC-MS/MS analysis demonstrated excellent analytical performance, characterized by wide linear ranges, low limits of quantification, and high precision and accuracy. A robust validation study confirmed the method's reliability and suitability for quantitative bioanalysis. Direct comparison of quantification results obtained from DPS with those from conventional plasma samples revealed strong correlation, highlighting the potential of this method for therapeutic drug monitoring of fluoroquinolone antibiotics in tuberculosis patients. The integration of dried blood spot technology with microwave-assisted sample preparation and sensitive mass spectrometric detection offers a promising approach for streamlined and efficient bioanalysis of fluoroquinolones in clinical settings.

Dried blood spot (DBS) sampling is becoming increasingly popular in bioanalysis due to its advantages. However, the limited amount of sample material often requires highly sensitive detection techniques. Derivatization can enhance sensitivity in gas chromatography-mass spectrometry (GC-MS) analysis. In **[56],** Sadones et al., introduce a novel derivatization method, microwave-assisted on-spot derivatization, to minimize sample preparation time for DBS analysis. The study evaluated the applicability of microwave-assisted on-spot derivatization for the determination of gamma-hydroxybutyric acid (GHB) and gabapentin in DBS using GC-MS. The method was validated and found to be robust, with acceptable imprecision and bias values. Calibration lines were linear within specific concentration ranges for both analytes. Stability studies demonstrated the stability of GHB and gabapentin in DBS samples stored at room temperature. The study also

evaluated the impact of DBS-specific parameters, such as hematocrit and volume spotted, on the analytical performance. The method was found to be reliable and applicable for routine toxicology analysis. Microwave-assisted on-spot derivatization represents a promising approach for the analysis of polar low molecular weight compounds in DBS samples. This method offers a rapid, efficient, and reliable alternative for the determination of analytes such as GHB and gabapentin, as well as other compounds of interest in clinical and forensic toxicology.

4. Conclusions and future perspectives

The trajectory of therapeutic drug monitoring (TDM) research should prioritize the development of eco-friendly, high-throughput, and miniaturized extraction techniques that incorporate innovative sorbent materials. A concerted effort to integrate cutting-edge analytical instrumentation, such as high-resolution mass spectrometry, is essential to enhance the sensitivity, selectivity, and overall analytical performance of TDM assays. Along with pursuing entirely novel methodologies, the focus should be also on optimizing existing approaches to address their inherent limitations and expand their applicability. By refining current techniques and disseminating knowledge about these advancements, the field can significantly improve patient care through more precise and individualized therapeutic interventions. Ultimately, the integration of these technological advancements will enable the routine application of advanced TDM strategies in clinical practice, thereby facilitating optimal drug therapy and improved patient outcomes. **[57]**

The integration of microfluidic platforms with advanced sample preparation techniques presents a promising avenue for the development of more efficient and sustainable therapeutic drug monitoring (TDM) workflows. Microfluidic devices, including lab-on-a-chip systems, offer the potential for miniaturization, automation, and increased analytical throughput. Complementary technologies such as dried blood spot sampling and innovative extraction methods, including solvent-assisted microwave extraction, solid-phase microextraction, supercritical fluid extraction, and single-drop microextraction, can significantly enhance sample preparation efficiency and reduce solvent consumption. Moreover, the incorporation of nanostructured materials into these platforms holds promise for the development of novel and environmentally friendly sample preparation approaches. By combining these technologies, researchers can create integrated analytical systems capable of handling complex biological matrices, improving sensitivity and selectivity, and ultimately enabling more precise and personalized patient care.

Abbreviations

Abbreviation	Full Name
LOD	Limit of Detection
LOQ	Limit of Quantification
HPLC-UV	High Performance Liquid Chromatography - Ultraviolet
UHPLC-MS/MS	Ultra-High-Performance Liquid Chromatography - Tandem Mass Spectrometry
CE-DAD	Capillary Electrophoresis - Diode Array Detector
UPLC-ESI-MS/MS	Ultra-Performance Liquid Chromatography - Electrospray Ionization - Tandem Mass Spectrometry
LC-UV	Liquid Chromatography - Ultraviolet
LC-MS/MS	Liquid Chromatography - Tandem Mass Spectrometry
HPLC-DAD-FLD	High Performance Liquid Chromatography - Diode Array Detector - Fluorescence Detector
LC-MS	Liquid Chromatography - Mass Spectrometry
HPLC-MS/MS	High Performance Liquid Chromatography - Tandem Mass Spectrometry
HPLC-HRMS	High Performance Liquid Chromatography - High Resolution Mass Spectrometry
LC-FLD	Liquid Chromatography - Fluorescence Detector
HPLC-DAD	High Performance Liquid Chromatography - Diode Array Detector
HPLC-FD	High Performance Liquid Chromatography - Flame Detector
CE-MS	Capillary Electrophoresis - Mass Spectrometry
GC-MS	Gas Chromatography - Mass Spectrometry

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Perspectives
The Future of Jobs in Chemistry in the Era of Emerging Technologies like AI

Dr Spiros Kitsinelis
Association of Greek Chemists
eex@eex.gr
DOI: 10.62579/JAGC0012

Abstract
The integration of artificial intelligence (AI) and other emerging technologies is rapidly reshaping the field of chemistry, creating unprecedented opportunities and challenges. This short communication explores the potential impact of these technologies on the job market for chemists, highlighting future roles, interdisciplinary applications, and the skills required to thrive in this evolving landscape.

Introduction
Chemistry has always been at the forefront of scientific and technological advancements, driving innovation in pharmaceuticals, materials science, energy, and environmental solutions. However, the advent of artificial intelligence (AI), machine learning, and automation is transforming traditional roles, requiring chemists to adapt to new tools and approaches. This short communication seeks to outline the future job opportunities for chemists, identify the skills they will need, and discuss the implications for education and professional development in the field.

Emerging Roles for Chemists
As AI and other technologies become integrated into chemical research and industry, new roles are expected to emerge. Some of those roles incliude:

1. AI-Assisted Chemical Modeler
Chemists in this role will leverage AI to build predictive models for molecular properties, reaction mechanisms, and process optimization. The integration of computational tools will significantly reduce experimental trial-and-error, enabling faster innovation. By combining chemical intuition with machine learning, chemists will train models on datasets of molecular structures and properties. These models will predict outcomes of chemical reactions, reduce waste, and improve efficiency in chemical synthesis. The potential applications include drug discovery where virtual screening of millions of compounds is enhanced by AI models.

2. Data Scientist in Chemistry
The growing importance of data in chemistry has created a need for professionals skilled in managing, analyzing, and interpreting chemical data. These chemists will use advanced statistical techniques and programming languages (e.g., Python) to process complex datasets from spectroscopy, chromatography, and other analytical tools. The applications include real-time process monitoring in industrial settings, predicting catalyst behavior, and optimizing formulation processes.

3. Sustainable Materials Developer
Chemists will focus on designing eco-friendly alternatives to traditional materials, often using computational tools and AI to guide development. They will collaborate with environmental scientists and engineers to develop biodegradable polymers, energy-efficient coatings, and sustainable packaging materials. The key technologies are AI-driven materials design, life-cycle analysis, and quantum chemistry simulations.

4. Healthcare and Pharmaceuticals
Traditional drug discovery takes years, but AI algorithms can predict drug efficacy and toxicity in silico. Chemists working in this field of drug design with AI will need to bridge medicinal chemistry and computational sciences. They will also play a key role in the design of personalized treatments (precision medicine), including tailored drug-delivery systems.

5. Energy and Environment
Chemists will be at the forefront of developing sustainable processes that minimize environmental impact and promote ''green chemistry''. Another field will be in energy storage solutions with the development of advanced batteries and fuel cells that will require expertise in materials science and electrochemistry.

6. Materials Science
Chemists will develop smart materials with tunable properties for applications in sensors, robotics, and wearable technology as well as nanostructured materials with unique optical, electronic, or catalytic properties.

Skills for the Chemist of the Future

The skills that will be required from the future chemists include

Technical Skills
- **Programming and Data Analysis**: Knowledge of Python and software like MATLAB will be essential.
- **AI and Machine Learning**: Familiarity with algorithms and frameworks (e.g., TensorFlow, PyTorch) will be highly advantageous.
- **Automation Tools**: Understanding the operation and programming of robotic platforms for automated synthesis and analysis.

Soft Skills
- **Interdisciplinary Collaboration**: Working effectively with professionals in computer science, engineering, and biology.
- **Lifelong Learning**: The rapid pace of technological advancement requires chemists to continuously update their skills.

All the above mean that educational institutions will have to adopt, adjust and develop curricula that include and integrate computational chemistry, AI, and data science. More over professional organizations should offer training in the form of **workshops and certifications** in all the emerging tools and techniques. And finally academia-industry partnerships will be crucial for aligning education with real-world needs.

Conclusion
The integration of AI and other technologies into chemistry is not a distant reality but an ongoing transformation. Chemists must embrace interdisciplinary approaches and acquire new skills to remain relevant in the job market. By adapting to these changes, they can lead innovation in healthcare, energy, and sustainability, shaping a better future.

Conformational characteristics decisive for their biological action of organotin metallotherapeutics using a combination of NMR methods and *in silico* calculations

N. Zoupanou[a], Christina N. Banti [b], Sotiris K. Hadjikakou [b], M.E Stathopoulou[b], S. Kiriakidi[a], Carlos Silva Lopez [c], Uroš Javornik[d] , T.Mavromoustakos[a]

[a]Department of Chemistry, National and Kapodistrian University of Greece Zografou, 15771, Athens, Greece
[b]Section of Inorganic and Analytical Chemistry, Department of Chemistry, University of Ioannina, 45110 Ioannina, Greece
[c]Department of Organic Chemistry, University of Vigo, Lagoas-Marcosende Campus, Vigo, Spain
[d]Slovenian NMR Centre, National Institute of Chemistry of Ljubljana, Hajdrihova 19, Slovenia

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Abstract

This study involves the structure elucidation and conformational analysis of three synthetic metallotherapeutics organotin (IV) derivatives of cholic acid (CAH) with the formulate $R_3Sn(CA)$ (R= Ph- (**1**), *n*-Bu- (**2**)) and $R_2Sn(CA)_2$ (R= Me- (**3**)**.** The structures of compounds (**1-3**) were assigned using a combination of homonuclear and heteronuclear 2D NMR experiments. Subsequently, ^{119}Sn NMR experiments and semi-empirical quantum mechanic computations (method PM6) were applied to determine the geometry surrounding the tin atom. Results showed that their optimized geometry is distorted tetrahedral and thus their biological activity of these compounds may be related with the conformation of atoms which endue the metal of Sn. *In silico* molecular docking studies were performed to the ER-α (estrogen receptor alpha ligand) justifying the compounds' biological activity.

Key Words : metallotherapeutics, organotin, NMR spectroscopy, semi-empirical quantum mechanic computations.

INTRODUCTION

Metallotherapeutic compounds, in particular metal complexes, present an increasing interest in the field of medicinal chemistry. Their unique characteristics, such as redox activity, variable mode of coordination, antineoplastic properties and reactivity [1][2][3] to the organic substrate, make them attractive molecules in designing metallotherapeutics. Studies have shown that metallotherapeutic compounds could indicate significant action as anti-cancer agents, antibiotics and antibacterials[4]. Equally important is their role as medical and diagnostic factors too. Their obvious utility leads to the necessity for a further synthesis and thorough structure and conformational analysis, aiming to an optimized version of them with reduced serious side effects. According to similar studies Organotin (IV) complexes are associated with anti-proliferative activity, they have the ability to show better excretion from the body, cells do not develop resistance to them, and they also exhibit low toxicity levels. These factors make them valuable leads in the field of medicine [5][6][7][8][9].

According to many studies available in the literature, the biological activity and the role of the anti-cancer tin (IV) complexes, depend on the number and the type of group R, which bind to Sn [3]. Organotin (IV) compounds, which bound to carboxyl groups, provide antiproliferative properties too. Despite the cytotoxicity studies of organotin compounds (IV) against cancer cells, there is not much data on in vitro toxicity against healthy cells. In this context, it has been found that certain compounds of di- and tri-organotin (IV) showed remarkably selective activity against cancer cell lines (HCT-116 and MCF-7) from normal cells. The results obtained from the studies and tests carried out in the field of organotin (IV) compounds seemed extremely interesting, starting a new era of research on the antiproliferative and anti-cancer activity of tin compounds. Small tri-organotin (IV) molecules have been shown to have better anti-cancer activity than their respective di-organotin (IV) derivatives, while among tri-organotin (IV) derivatives, triphenyl derivatives show improved activity [10][11][12].

In this study synthetic organotin (IV) compounds include the tin atom complexed with a different group R (R = Me, Bu, Phe) (**Figure 1**). In particular, the compounds that were analyzed were organotin (IV) derivatives of cholic acid (CAH) with the formulae $R_3Sn(CA)$ (R= Ph- (**1**),

n-Bu- (**2**)) and $R_2Sn(CA)_2$ (R= Me- (**3**)). The combination of a strong antitumor agent, such as organotins, with the molecule of cholic acid, which exhibits hormone conformation, could contribute to a new formulation with selective activity against hormone depended tumour cells [13][14]. Compounds **1-3** were tested for their *in vitro* bioactivity against MCF-7 (positive to hormone receptors) and MDA-MB-231 (negative to hormone receptors) cells. The *in vitro* toxicity of **1-3** was evaluated towards MRC-5, while their *in vitro* genotoxicity was tested by the MN assay. The *in vivo* toxicity of **1-3** was tested by *Artemia salina* assay, while the *in vivo* genotoxicity by *Allium cepa* test. Moreover, the molecular mechanism of **1-3** against essential intracellular components was examined by their (i) DNA binding affinity, (ii) LOX inhibitory activity and (iii) catalytic activity on the peroxidation of linoleic [15][16].

1-3 were structurally elucidated using 1D and 2D homonuclear and heteronuclear NMR experiments. Subsequently, the conformational analysis and recognition of complexes was completed through experiments ^{119}Sn NMR and semi-empirical quantum mechanic computations. Molecular docking and quantum mechanics and molecular mechanic computations (QM/MM) are methods utilized to study both the binding of molecules to potential receptors that they may act, as well as to find their optimal geometry. MD calculations could confirm the stability of the complexes and justified their biological activities as binders at the ER-α.

2. Material and Methods*NMR Analysis*

All of the Organotin (IV) derivatives were synthesized in the lab of Inorganic Chemistry at University of Ioannina and their synthesis was published elsewhere [1]. The structural elucidation of protons and carbons of the compounds **(1-3**) was achieved using 700 MHz Bruker Avance III HD magnet equipped with an ultra-sensitive triple-resonance TCI cryoprobe, installed at Pharmaceutical Department of University of Patras. 5 mg of each compound were dissolved in 550ml of DMSO-d6. The experiments, performed using Bruker library pulse sequences, included homonuclear ^{1}H ,^{13}C, ^{13}C APT NMR, 2D COSY, 2D ROESY and heteronuclear 2D HSQC and 2D HMBC experiments. All measurements are carried out at 25°C, and the data are acquired and processed with the MestreNova [17] and TopSpin 3.5 [18] software. Final-

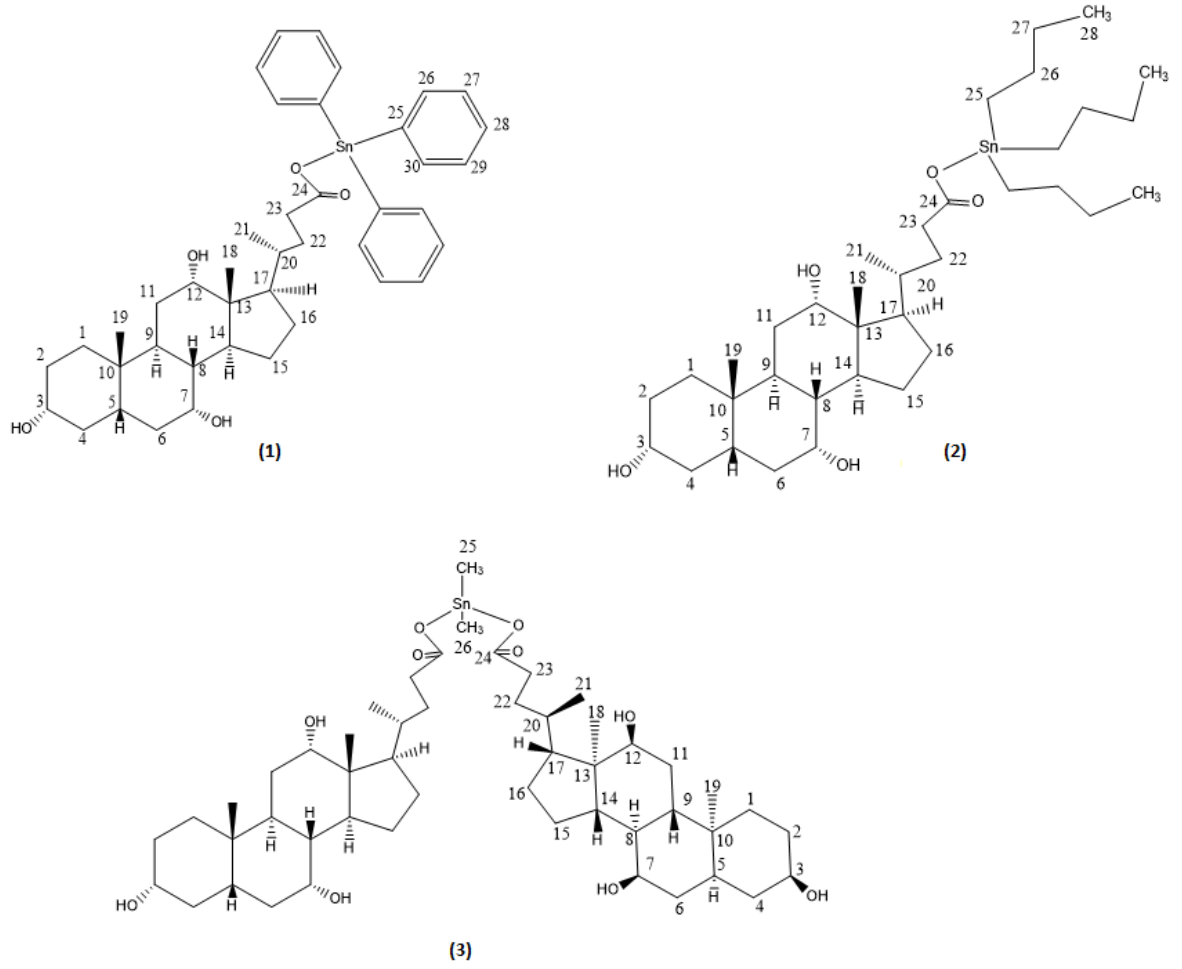

Figure 1. *Structure of the compounds (1-3).*

ly, ^{119}Sn NMR experiments were performed at NMR Lab of Slovene using spectrometer of 300MHz (Unity Inova NMR) and DMSO-d6 solvent.

Semi-empirical Quantum Mechanic Computations

Semi-empirical quantum mechanics computation was an effective tool for the analysis of the geometry of molecules. The Neglect of Diatomic Differential Overlap (NDDO)-based semi-empirical method PM6 [19] was used in order to calculate the geometries of the coordination complexes under study. The choice of this method was based upon its suitability for computing metal coordination complexes since it takes into account both the adoption of Viotyuk's core-core diatomic interaction term [20] and Thiel's d-orbital approximation [21][22][23]. The starting geometry for each complex was guessed based upon the results of the performed ^{1}H-NMR and Mössbauer experiments [24], which have already performed. Initial structures were designed in Molden and were used as input for geometry optimization in the PM6 level of theory with the Gaussian 09 software [25]. Frequency calculations were performed in the optimized geometries in order to assure that the resulting structures correspond to global minima and not any transition states.

In silico Molecular Docking studies

I*n silico* studies, have been performed on the estrogen receptor alpha (ER-a). The structures of the organotin (IV) derivatives derived by PM6 calculations were used as input structures for the docking studies. The crystal structure of the macromolecule/receptor was downloaded from Protein Data Bank (PDB: 1A52).

AutoDock Tools (ADT) 4.5 was utilized for preparing the ligands and protein, as well as for analyzing the results. Molecular docking calculations were conducted by AutoDock4. Regarding the protein preparation, parameters were adjusted and polar hydrogen's were added to the receptor. Gasteiger charges were assigned to the protein's structure. Grid was generated based on coordinates the entire protein (blind docking) while, the grid box size was set to 126 Å in all directions. The center of the grid box was set to the active site, which was acquired by the cocrystallised ligand (estradiol), included in the crystal structure with the cod 1A52.

Docking software AutoDock4 and Autogrid4 algorithms were implemented in order to generate the grid maps. Also, the parameters for Sn (IV) were extracted from the literature and were manually incorporated in AutoDock's parameter files. Finally, docking calculations were performed using the Genetic algorithm, determining the conformation of each complex with the most suitable binding mode to the receptor.

3.Strategy and results

The structure of molecules under study, contain one or two cholic acids and one different group R for each compound. The structures of complexes are shown below, and the strategy used for the structural identification of protons and carbons of the compounds (**1-3**) follows at **Figure 1**.

3.1 NMR analysis of compound 1

As a starting point for the structure elucidation, analyzing the NMR spectra of compound **1,** was taken the double peak of H21 (0.82ppm), which corresponds to CH_3. Through the combination of 2D HSQC and 2D COSY experiments, C21, H20 and C20, have been determined and they are resonated at 41.52ppm, 1.16ppm and 31.07ppm correspondingly. The second starting step is the singlet at 0.49 ppm, which corresponds to CH_3 H18. This was confirmed by the 2D HMBC spectrum which showed correlation of H18 with C12 resonated at 71.02ppm. Through 2D HSQC and 2D COSY confirmed the chemical shifts of H12 (3.74ppm) and OH12 (4.04ppm) were confirmed. The other singlet peak of CH_3 at 0.80ppm corresponds to H19. Through 2D HSQC it was found the C19 resonating at 22.64ppm.

Then next step in the strategy was the identification of H20. In particular, H20 at 2D COSY spectrum had correlation with protons which resonated at 1.70ppm and at 1.09ppm. According to the structure of molecule, these protons must be H17 and H22. Using the 2D HMBC spectrum, the already identified C18 correlated with proton at 1.70ppm, confirming its identity as H17. Therefore, H22 resonated at 1.09ppm. Through 2D HSQC C17 and C22 were identified. As H22 included two protons, the second H22 was identified using the 2D HSQC spectrum resonating at 1.60ppm. Apparently, through the 2D COSY experiment, the H23 protons were identified to resonate at 1.94ppm and 2.04ppm. C23 was identified through 2D HSQC.

A following step was to utilize identified C17 which through the 2D HMBC, correlated with proton H14 (1.93ppm). Using the 2D HSQC, C14 was determined. Through 2D COSY H14 correlated with H8 and this with H7 which subsequently correlated with OH7. Through 2D HSQC experiments C8 and C7 have been determined to resonate at 39.95ppm, 66.26ppm correspondingly. Identified H14 correlated through bond with H15 which resonated at 0.88ppm, and H15 with H16 which resonated at 1.67ppm. The second protons of H15 and H16 were revealed by 2D HSQC experiment.

Through the identified H7 and using 2D COSY experiment H6-H1 were identified and subsequently through 2D HSQC C6-C1. Then, through 2D HMBC experiment, identified C7 correlated with H9, which resonated at 2.09ppm. 2D COSY

allowed through identified H9 to assign H11 (1.31ppm) and 2D HSQC the identification of C11.

It followed the identification of the triphenyl group. H28, is expected to show triplet, at the range of 7.38-7.42 ppm. The rest of the protons, H26/30 as a doublet and H27/29 as a doublet of doublets have been observed at 7,77ppm and 7,38-7,42ppm correspondingly. The carbons bearing protons have been identified through 2D HSQC and the tertiary C25 resonated at 143,38ppm according to 2D HMBC. The results of the analysis, the correlations of protons with carbons and the proton and carbon chemical shifts, are shown analytically in the following **Table 1**. At **Figure 2 and 3** are presented the ^{1}H NMR spectrum and the ^{13}C NMR spectrum analytically.

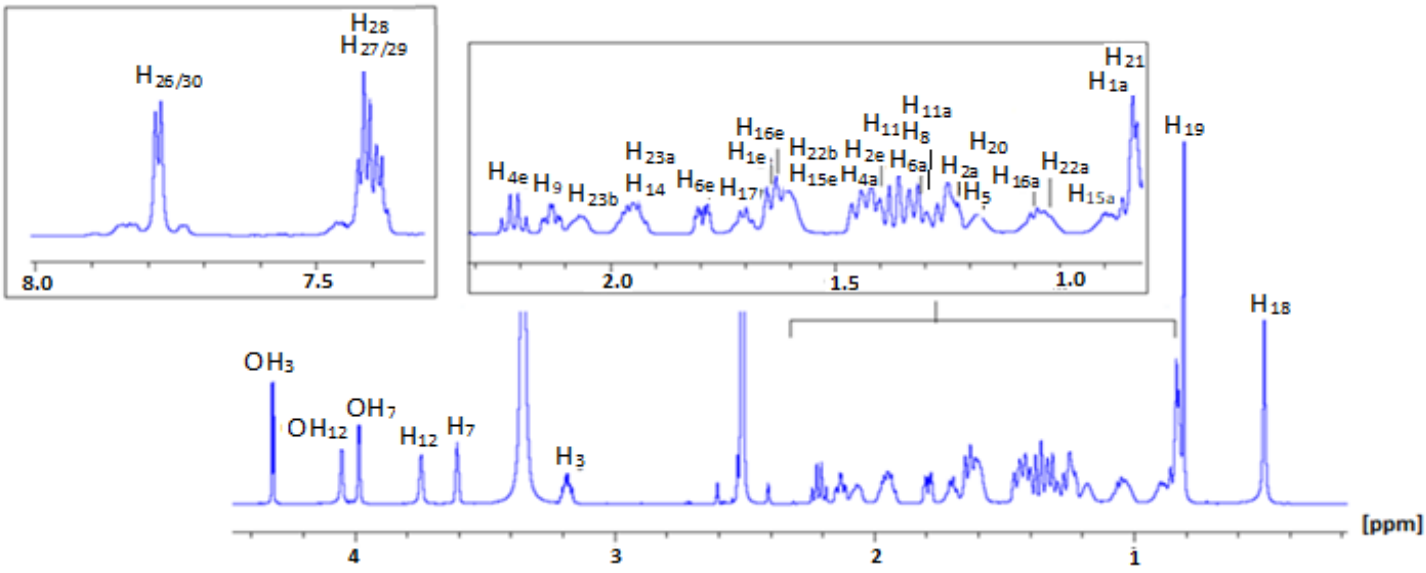

Figure 2: *^{1}H NMR spectrum of compound 1 obtained at ambient temperature in DMSO-d6 using 700 MHz Bruker NMR spectrometer.*

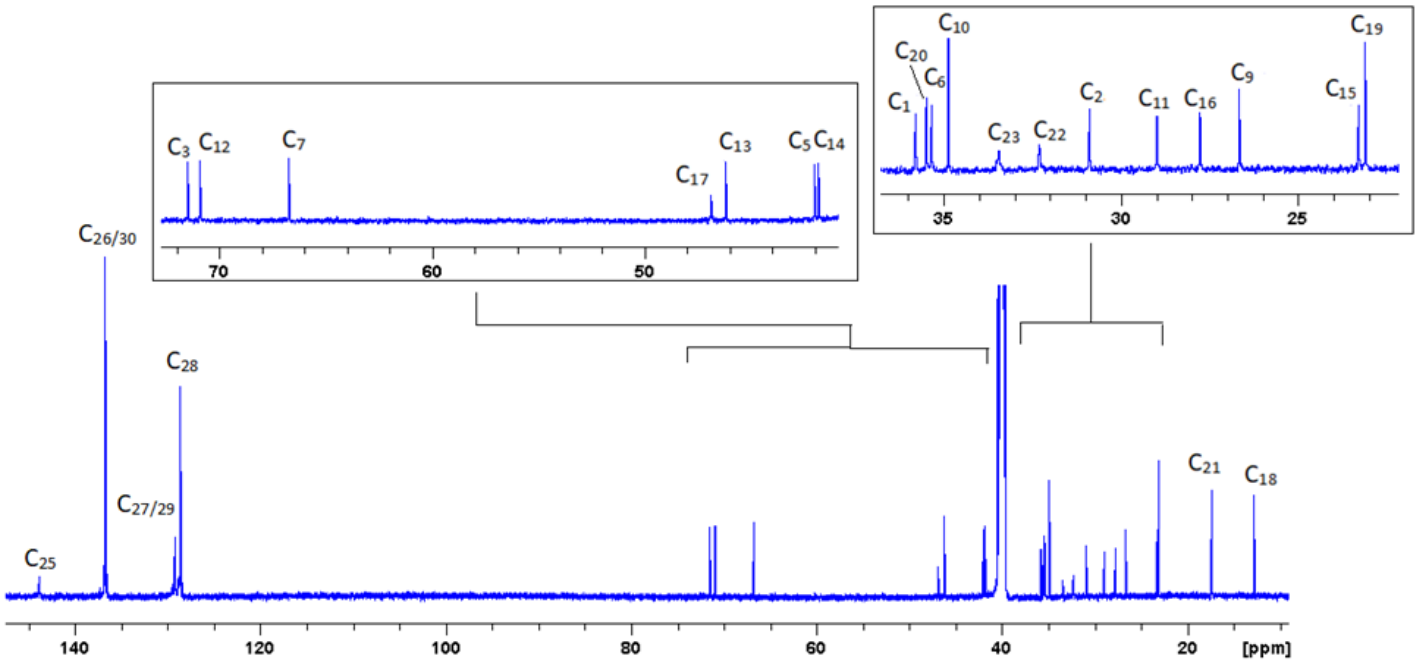

Figure 3: *^{13}C NMR spectrum of compound 1 obtained at ambient temperature in DMSO-d6 using 700 MHz Bruker NMR spectrometer.*

Table 1. *Chemicals Shifts of protons and carbons of compound (1) expressed in ppm.*

Position	^{1}H (ppm)	^{13}C (ppm)	Position	^{1}H (ppm)	^{13}C (ppm)
1a	0.83	35.31	16a	1.04	27.35
1e	1.63	35.31	16e	1.62	27.35
2a	1.25	30.41	17	1.70	46.46
2e	1.41	30.41	18	0.49	12.36
3	3.17	70.45	19	0.80	22.70
4a	1.44	-	20	1.16	35.07
4e	2.19	-	21	0.82	16.95
5	1.22	41.53	22b	1.00	31.89
6a	1.36	34.86	22a	1.59	31.89
6e	1.78	34.86	23b	1.94	33.05
7	3.60	66.26	23a	2.07	33.05
8	1.30	-	24	-	-
9	2.11	26.19	25	-	143.38
10	-	34.39	26	7.77	136.70
11a	1.31	28.50	27	7.38-7.42	128.76
11e	1.40	28.50	28	7.38-7.42	128.17
12	3.74	71.02	29	7.38-7.4	128.76
13	-	-	30	7.77	136.70
14	1.93	41.40	OH-3	4.31	-
15a	0.88	22.88	OH-7	3.98	-
15e	1.58	22.88	OH-12	4.05	-

3.2 NMR analysis of compound 2

The strategy of compound **2** involved the assignment of the triplet resonated at 0.85 ppm as H28, while the rest protons and carbons have been assigned through 2D COSY and 2D HSQC. Especially, through 2D COSY was recognized that H27, H26 and H25 resonated at 1.54ppm, 1.27ppm and 1.02ppm. Proton and carbon chemical shifts are shown analytically at **Table 2** and the ^{1}H NMR and ^{13}C NMR spectra at **Figure 4 and 5**.

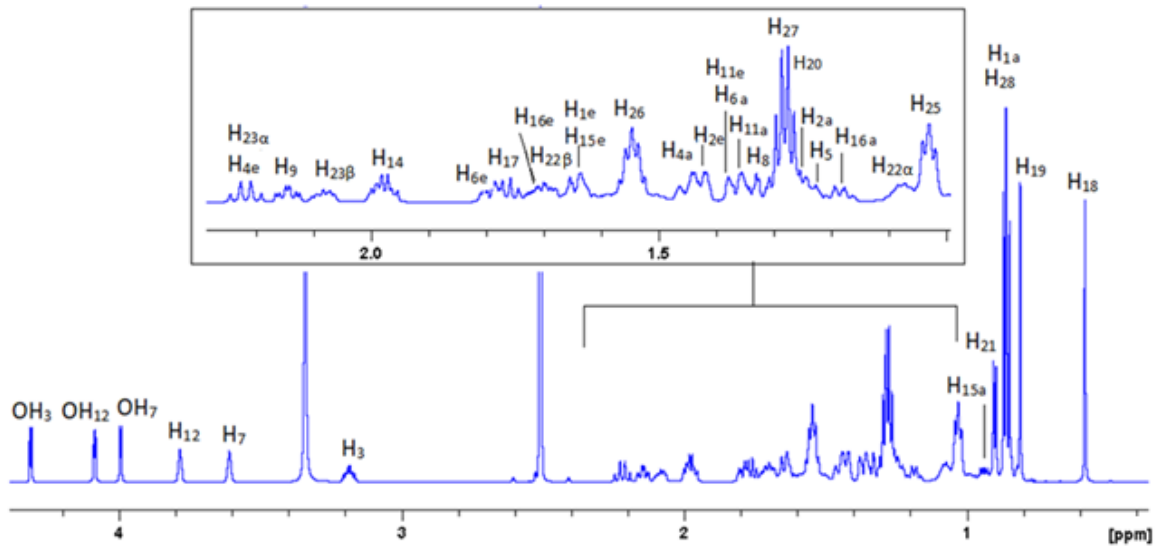

Figure 4: *^{1}H NMR spectrum of compound (2) obtained at ambient temperature in dmso-d6 and using 700 MHz Bruker NMR spectrometer.*

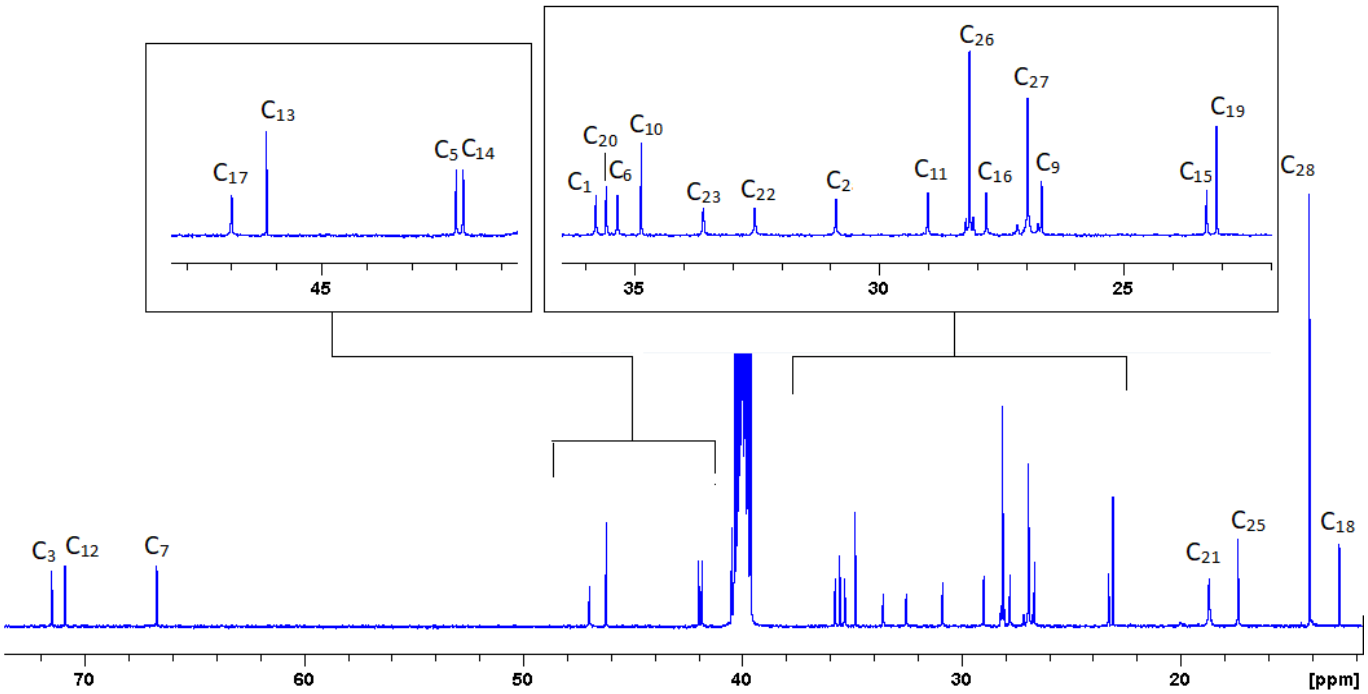

Figure 5: *^{13}C NMR spectrum of compound (2) obtained at ambient temperature in DMSO-d6 and using 700 MHz Bruker NMR spectrometer.*

Table 2. *Chemicals Shifts of protons and carbons of compound (2) expressed in ppm.*

Position	^{1}H	^{13}C	Position	^{1}H	^{13}C
1e	1.63	35.38	15e	1.63	22.90
1a	0.83	35.38	16e	1.72	27.40
2e	1.42	30.46	16a	1.17	27.40
2a	1.25	30.46	17	1.76	46.59
3	3.18	70.53	18	0.58	12.37
4e	2.21	-	19	0.80	22.70
4a	1.44	-	20	1.28	35.16
5	1.22	41.60	21	0.90	17.00
6e	1.79	34.94	22a	1.67	32.14
6a	1.36	34.94	22b	1.07	32.14
7	3.60	66.33	23b	2.07	33.18
8	1.32	-	23a	2.20	33.18
9	2.14	26.28	24	-	177.76
10	-	34.45	25	1.02	18.31
11e	1.40	28.60	26	1.53	27.74
11b	1.34	28.60	27	1.27	26.56
12	3.79	71.12	28	0.85	13.73
13	-	45.81	OH-3	4.30	-
14	1.97	41.45	OH-7	3.99	H-7
15a	0.94	22.90	OH-12	4.09	H-12

3.3 NMR analysis of compound 3

Finally, chemical shifts of proton and carbon are shown at **Table 3** and the ^{1}H NMR and ^{13}C NMR spectra at **Figure 6 and Figure 7**. Finally, compound **3** involved an extra step, which involved the assignment of the singlet resonance at 0.66 ppm and which is assigned as H26, in particular at CH_3 (**Table 3**, **Figure 6 and 7).**

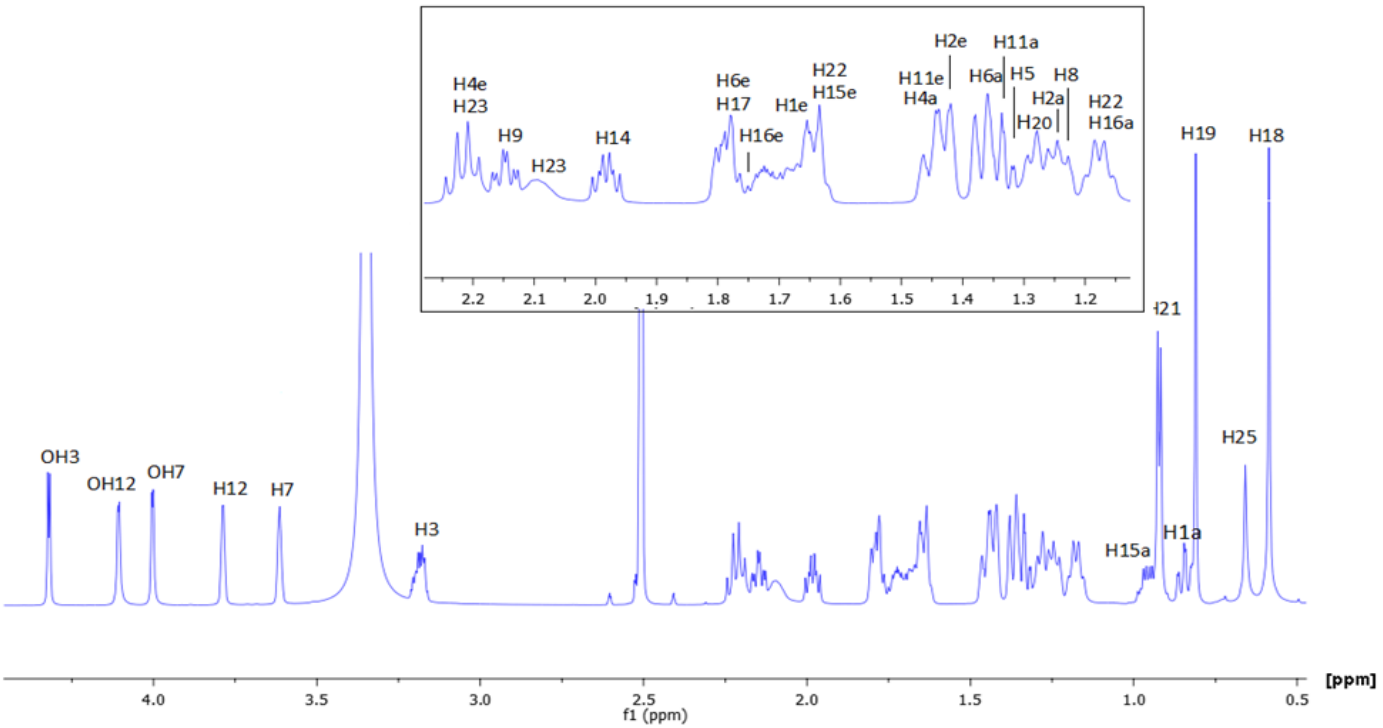

Figure 6: *^{1}H NMR spectrum of compound (3) obtained at ambient temperature in DMSO-d6 using 700 MHz Bruker NMR spectrometer.*

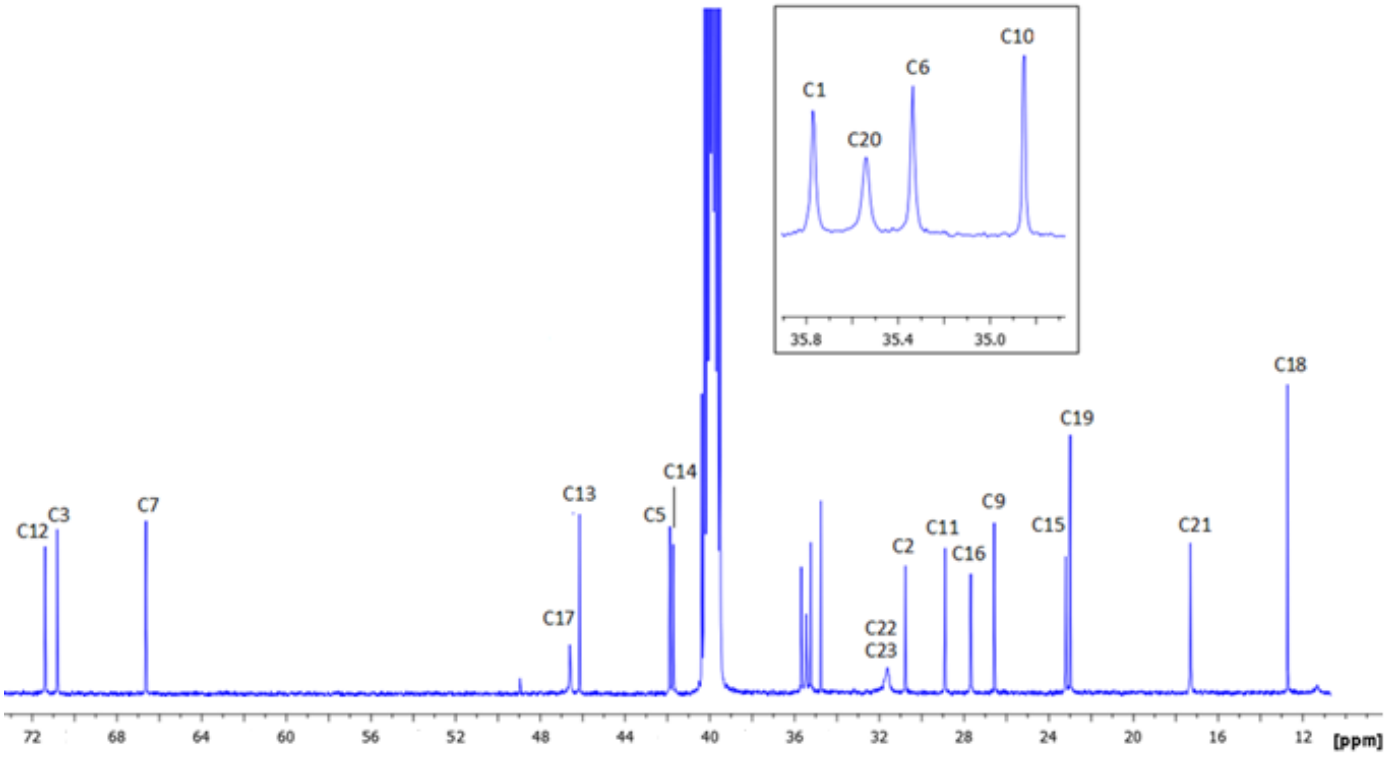

Figure 7: *^{13}C NMR spectrum of compound (3) obtained at ambient temperature in DMSO-d6 using 700 MHz Bruker NMR spectrometer.*

Table 3. *Chemicals Shifts of protons and carbons of compound (3) expressed in ppm.*

Position	^{1}H	^{13}C	Position	^{1}H	^{13}C
1a	0.86	35.31	15e	1.63	2283
1e	1.63	35.31	16e	1.75	27.31
2e	1.42	30.57	16a	1.17	27.31
2a	1.25	30.57	17	1.77	46.24
3	3.18	70.44	18	0.59	12.35
4e	2.22	-	19	0.81	22.62
4a	1.44	-	20	1.28	35.08
5	1.22	41.52	21	0.92	16.94
6a	1.36	34.87	22b	1.17	34.39
6e	1.78	34.87	22a	1.64	34.39
7	3.61	66.26	23b	2.09	31.24
8	1.32	-	23a	2.20	31.24
9	2.14	26.20	24		
10	-	45.78	25	0.66	11.10
11a	1.34	28.95	26	0.66	-
11e	1.44	28.95	27		
12	379	71.01	OH-3	4.32	-
13	-	48.60	OH-7	4.00	-
14	1.98	41.36	OH-12	4.10	-
15a	0.94	22.83			

3.4 ^{119}Sn NMR analysis of compounds

^{119}Sn NMR experiments were performed to study its geometry with the surrounding substituents. The nature of the substituents determines the observed chemical shifts. Subsequently, the chemical shifts are correlated with certain geometries. The use of known spectroscopic data with determined geometries are in aid of this effort. ^{119}Sn NMR spectra were obtained in DMSO-d6 solvent. $SnCl_4$ was used as reference compound with known chemical shift (-150.41) (**Figure 8**).

According to the literature a four coordinated Sn provides a ^{119}Sn NMR spectrum shows resonances that range between -60 - 200 ppm [2-3] with a possible tetrahedral geometry [5]. The five-coordinated Sn gives peaks that resonate between -330ppm-(-) 90ppm, with a possible triangular dipyramid or tetrahedral dipyramid geometry [5]. Finally, a six-coordinated Sn gives peaks that resonated between -210ppm until -515ppm [2-3], with a possible octahedral geometry [5]. Based on these literature data the observed chemical shifts for the three complexes designate geometries shown in **Table 4**. [26][27][28][29]

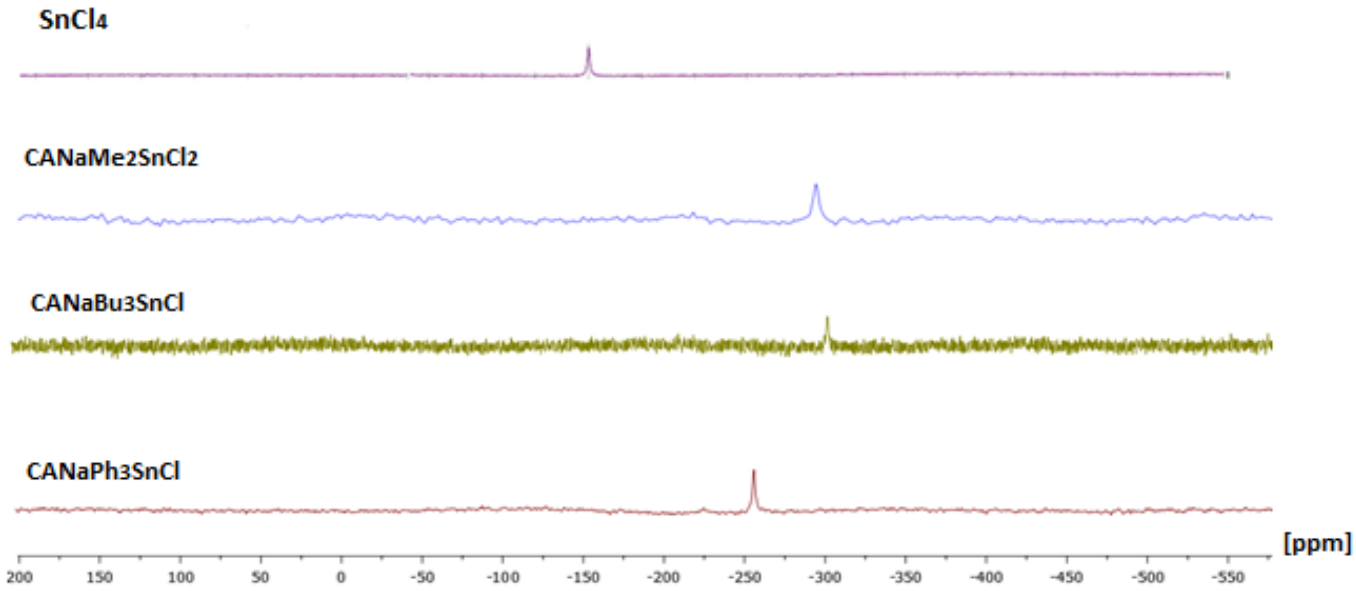

Figure 8: *Spectra of 119Sn NMR of compounds (1-3) and the reference compound SnCl4.*

Table 4. *Chemical shifts observed with 19Sn NMR spectroscopy for compounds (1-3) and their possible geometry according to literature data.*

a/a	Compound	Chemical shift	Δδ to reference compound $SnCl_4$	Geometry of Compound
1	$SnCl_4$	-150.41	-	-
2	$CANaPh_3SnCl$	-257.44	107.04	triangular dipyramid or tetrahedral dipyramid or octahedral geometry -
3	$CANaBu_3SnCl$	-305.82	155.50	triangular dipyramid or tetrahedral dipyramid or octahedral geometry
4	$CANaMe_2SnCl_2$	-294.61	144.21	triangular dipyramid or tetrahedral dipyramid or octahedral geometry

4. Semi-empirical quantum mechanic computations

Semi-empirical method PM6 was used in order to calculate the geometries of the coordination complexes under study. The starting geometry for each complex was guessed based upon the results of the performed ^{1}H-NMR and Mossbauer experiments. Initial structures were designed in Molden and were used as input for geometry optimizations in the PM6 level of theory with the Gaussian 09 software. Frequency calculations were performed in the optimized geometries in order to assure that the resulting structures correspond to global minima and not any transition states.

The resulting geometries of the coordination complexes under study are presented in **Table 5** and **Table 6**.

Table 5. *Bond lengths for both organometallic complexes. The numbers 1,2 & 3 correspond either to butyl or phenyl groups depending on the structure. The complexes exhibit a distorted tetrahedral geometry with angles very close to the 109 ° of the perfect tetrahedron.*

Structure	Bond(Å)				
	Sn-CANa (1)	Sn-CANa (2)	Sn-C1	Sn-C2	Sn-C3
$CANa(Bu)_3Sn$	2.13	-	2.15	2.15	2.15
$CANa(Ph)_3Sn$	2.12	-	2.12	2.12	2.12
$CANa(Me)_2Sn$	2.06	2.10	2.11	2.11	-

Table 6. *Relative angles for both organometallic complexes. The numbers 1,2 & 3 correspond either to butyl or phenyl groups depending on the structure. The complexes exhibit a distorted tetrahedral geometry with angles very close to the 109 ° of the perfect tetrahedron.*

Structure	(degrees)								
	C1-Sn-C2	C2-Sn-C3	C3-Sn-C1	C1-Sn-CANa (1)	C2-Sn-CANa (1)	C3-Sn-CANa (1)	C1-Sn-CANa (2)	C2-Sn-CANa (2)	CANa(1)-Sn-CANa(2)
$CANa(Bu)_3Sn$	113.79	114.83	116.02	105.78	98.39	105.52	-	-	-
$CANa(Ph)_3Sn$	114.39	116.10	114.07	106.02	107.03	96.63	-	-	-
$CANa(Me)_2Sn$	120.82	-	-	108.61	105.97	-	102.27	110.89	107.72

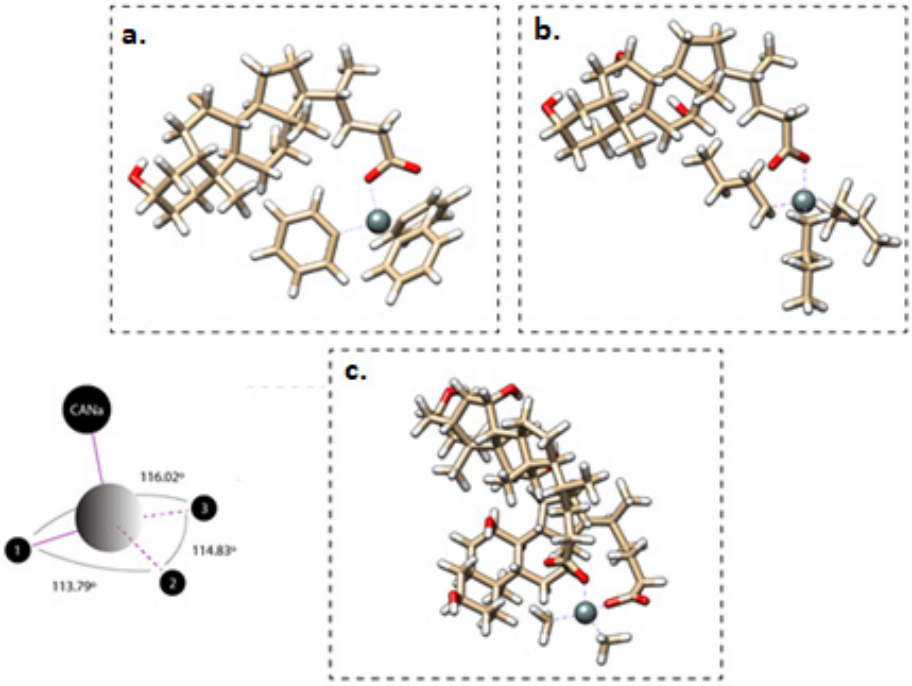

Figure 9. *Schematic Representation of the PM6 geometry of CANa(Ph)3Sn(a), CANa(Bu)3Sn(b) and CANa(Met)2Sn(c). The geometry is distorted tetrahedral and the organometallic bonds are illustrated as purple dashed lines. A simplified version with some angle degrees are presented in the bottom left.*

Table 7: *Binding Energy, binding constant (K_D) values and H-bonds of molecular docking calculations.*

Receptor 1a52 -Compounds	Binding Energy Kcal/mol	Constant K_D	H Bonds
Receptor 1a52/ complex 1	-9.94 ±0.5	0.003 nM	OH(3) with His 501 H(24) with Leu 509
Receptor 1a52/ complex 2	-6.52±0.5	0.010 μM	OH(3) with Glu 441 OH(3) with Asn 449 OH(7) with Glu 397
Receptor 1a52/ complex 3	-7.37±0.5	0.061 nM	OH(3) with Glu 330 OH(3) with Arg 352 OH(12) HOH 560

As it can be observed in **Figure 9**, all complexes exhibit a distorted tetrahedral geometry with angles very close to the 109 ° of the perfect tetrahedron.
Interestingly, there is no agreement with the experimental data. A possible reason is that the solvent was not taken into account in the theoretical calculations.

5. Results of Molecular Docking Studies

The *in silico* experiments of molecular docking showed the binding of compounds **(1-3**) with the estrogen receptor (1a52). The molecular docking calculations were conducted three -times, in order to obtain reliable results. The average of the values of binding energy and binding constant (K_D), are presented at **Table 7**.
In all the "enzyme-ligand" complexes that were formed, the receptor (PDB:1A52) was interacting with the ligands through electrostatics and H-bond formation. The binding energy of all the complexes demonstrates a significantly strong energy. In particular, It was observed that the cholic acid binds in the active side of the receptor, creating H-bonds, between the OH(3) and H(24) of cholic acid to the amino acids His 501 and Leu 509 correspondingly **(Figure 10a).** The corresponding interactions for complex 2 with the receptor were H-bonds of OH(3) to amino acids Glu 441 and Asn 449 and OH(7) to amino acid Glu 397 as it is presented at **Figure 10b**. Complex 3 had interactions through H-bonds. In particular, the one cholic acid presented H-bond between OH(3)/OH(12) with Asp 351 and OH(7) with Lys 529. The other cholic acid had H-bonds between OH(3') and the amino acids Glu 330 and Arg 352 **(Figure 10c)**. The visualization of docking results were performed by Pymol program for all complexes.

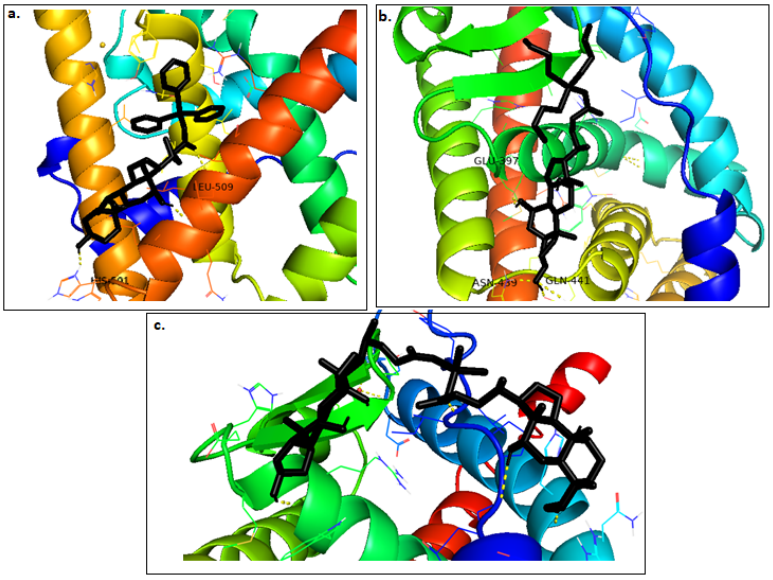

Figure 10. *Interaction of complex (1-3) with the receptor (PDB:1a52).*

6. Conclusions

In this study, we carried out the structural elucidation and conformational analysis of three synthetic organotin(IV) derivatives of cholic acid (CAH) with the general formulas $R_3Sn(CA)$ (R = Ph (1), n-Bu (2)) and $R_2Sn(CA)_2$ (R = Me (3)). To achieve this, we employed a combination of 1D and 2D homonuclear and heteronuclear NMR experiments along with ^{119}Sn NMR spectroscopy. Additionally, semi-empirical quantum mechanical calculations (PM6 method) were applied to investigate the geometry surrounding the tin atom. Their consistent results pointed out that their optimized geometry is distorted tetrahedral. This conformation may be responsible for their biological activity. To justify this, *in silico* molecular docking studies were performed to the ER-α (estrogen receptor alpha ligand). Their high binding affinity illustrates the possible relation between the putative bioactive conformation and possible bioactivity.

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The Role of ISO 14001 in Food Waste Management: Supporting Compliance and Sustainable Practices

Eftychia G. Karageorgou
Chemist, M.Sc., PhD
Manager, North Certification Division
NBIS P.C. Cargo Inspections and ISO Certification, Thessaloniki, Greece
email: ekarageorgou@nbis.gr
DOI: 10.62579/JAGC0014

Perspective Article

Abstract
The food industry places considerable pressure on the environment due to its high levels of resource consumption and waste generation. In response to growing demands for sustainability and regulatory compliance, ISO 14001 has emerged as a key standard for the implementation of Environmental Management Systems. This article explores its role in improving waste management in the food sector, particularly in relation to European legislation such as the Waste Framework Directive, Regulation (EC) No 1069/2009, and cross-border waste shipment regulations. Drawing on international case studies, the article highlights measurable improvements in resource efficiency, cost control, and employee engagement. It also examines how ISO 14001 complements food safety standards such as ISO 22000, supports regenerative practices like composting and food redistribution, and leverages digital innovations for waste forecasting and optimization. Despite financial and institutional challenges in some regions, the integration of ISO 14001 with legal frameworks and a sustainability-oriented organizational culture contributes to advancing circular economy objectives in the global food industry. This perspective provides practical insights for regulators, industry leaders, and sustainability professionals seeking to align food waste strategies with international standards and EU legislation.

Keywords
ISO 14001 implementation; Food waste management; Environmental compliance; Regulatory compliance; ISO 22000; Sustainability

1. Introduction

The food industry contributes significantly to global environmental degradation. It generates large amounts of waste and consumes high levels of water and energy. Growing concerns over sustainability and increasing regulatory pressures have prompted organizations to adopt structured environmental management practices. ISO 14001, the leading international standard for Environmental Management Systems, provides a systematic approach to identifying environmental aspects, improving compliance, and reducing negative impacts (1,2,3). Developed by the International Organization for Standardization – ISO, it offers a systematic approach that helps organizations address their environmental responsibilities effectively and sustainably. The standard focuses on minimizing environmental impact, complying with regulatory requirements, and continually improving environmental performance. ISO 14001 follows the Plan-Do-Check-Act cycle and is applicable to organizations of all types and sizes across various sectors. By promoting lifecycle thinking, pollution prevention, and continuous improvement, ISO 14001 enables food companies to align their operations with both sustainability goals and regulatory obligations.

This perspective article investigates the role of ISO 14001 in enhancing waste management within the food sector. It explores how the standard complements European Union legislation – such as the Waste Framework Directive and animal by-product regulations – and how it fosters synergy with ISO 22000, regenerative practices, and digital innovation. The analysis draws from diverse international case studies to demonstrate ISO 14001's practical and strategic value in advancing circular economy principles.

2. Optimizing Food Industry Waste Management Through ISO 14001

Nearly one-third of global food production (about 1.3 billion tons annually) is lost or wasted (4). This has severe environmental, economic, and ethical implications. It leads to more greenhouse gas emissions, overuse of natural resources, and greater food insecurity.

ISO 14001 offers a structured framework that helps organizations respond to these challenges by fostering pollution prevention, encouraging efficient use of resources, and embedding lifecycle thinking into environmental practices (2,3,5).

Within the food processing sector, ISO 14001 enables companies to systematically identify the environmental aspects and impacts of their operations, establish measurable environmental objectives and performance targets, and implement mechanisms for continuous monitoring and improvement. Moreover, it supports firms in maintaining full compliance with relevant environmental regulations, thus aligning their operational practices with both sustainability principles and legal obligations (3,6,7).

3. EU Regulatory Framework on Food Waste Management

The EU has established a detailed legislative framework aimed at reducing food waste throughout the supply chain. Fundamental to this approach is the EU Waste Framework Directive (2008/98/EC), revised by Directive (EU) 2018/851, which establishes a legally binding waste hierarchy. This hierarchy prioritizes prevention, followed by preparation for reuse, recycling, other recovery methods, and finally, disposal (8). The directive obliges Member States to adopt national food waste prevention programs, promote food donation and redistribution for human consumption, and regularly monitor and report progress. These policy objectives are closely aligned with the core principles of ISO 14001, such as lifecycle thinking, pollution prevention, and the pursuit of continual environmental improvement.

In addition, Regulation (EC) No 1013/2006 governs the transboundary movement of waste and ensures that such transfers – whether within the EU or involving third countries – are managed in an environmentally sound manner (9). This is especially relevant for food companies operating internationally, as ISO 14001 emphasizes transparency, traceability, and legal conformity in environmental performance.

Furthermore, waste derived from animal by-products is regulated by Regulation (EC) No 1069/2009 and its implementing act, Commission Regulation (EU) No 142/2011. This legislation establishes strict health standards for collecting, treating, and disposing of materials not intended for human consumption (10,11). They also ensure that waste minimization strategies do not compromise food safety or public health protection.

These EU regulations provide a strong legal foundation for food companies to apply ISO 14001 in ways that fulfill both environmental goals and regulatory requirements. This alignment also contributes to the EU's efforts to achieve Sustainable Development Goal 12.3, which aims to halve per capita food waste by 2030. The flowchart depicted in Figure 1 illustrates how ISO 14001 and the EU legislative framework operate in synergy, directing environmental actions that lead to concrete outcomes such as waste reduction, improved efficiency, and full regulatory compliance.

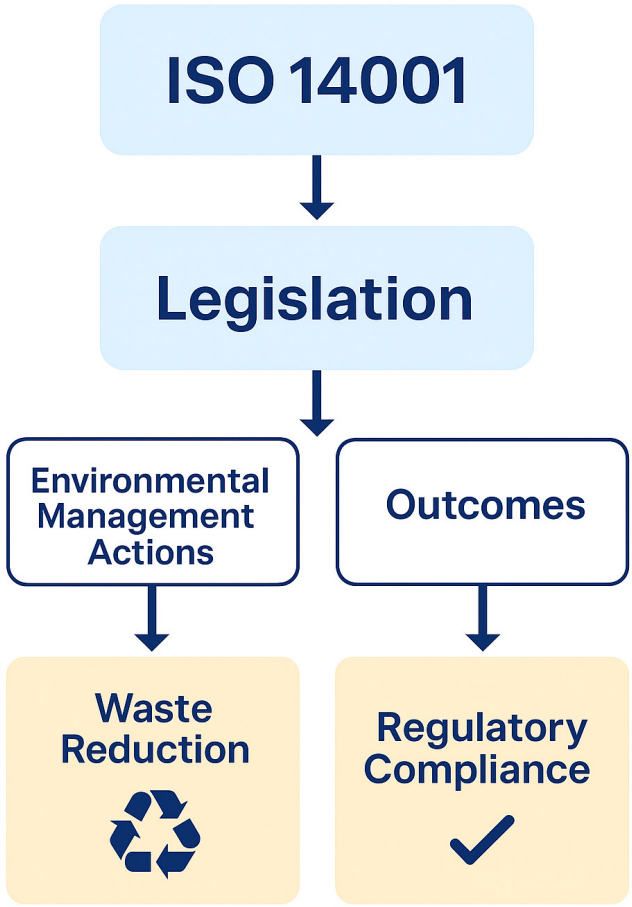

Figure 1. *Flowchart showing the interaction between ISO 14001, EU legislation, environmental actions, and outcomes in the food industry.*

4. ISO 14001 Outcomes in the Food Sector

Studies from Europe and South Africa show that ISO 14001 adoption in the food industry leads to a broad range of measurable benefits. A frequently observed outcome is the increased awareness and involvement of employees in environmental initiatives, which helps cultivate a stronger culture of sustainability within organizations (5). In parallel, many companies have achieved notable reductions in operational costs, primarily through waste minimization and water reuse practices (12). Additionally, the implementation of reuse, recycling, and remanufacturing activities has generated new revenue opportunities, strengthening the economic rationale for adopting ISO 14001 (5,12). These environmental improvements also contribute to an enhanced brand image and facilitate access to international markets particularly in export-oriented sectors where environmental certification offers a competitive advantage (2,13).

These benefits are clearly demonstrated in real-world

cases. For example, a food-processing plant in the United Kingdom that introduced wastewater reuse strategies managed to cut its use of caustic cleaning agents by 40% and reduce daily wastewater discharge by up to 500 cubic meters (12). In Brazil, food establishments managed by dietitians showed higher ISO 14001 compliance, highlighting the role of staff expertise in achieving environmental performance targets (13). Similarly, ISO 14001-certified manufacturers in South Africa reported financial gains, improved environmental data handling, and additional income from material reuse and recycling especially in sectors with strict environmental oversight, such as the food and automotive industries (5). Although the standard's implementation can be costly and technically demanding, especially for small businesses, ISO 14001 remains a critical instrument for meeting regulatory requirements and advancing sustainability. In South Africa, its alignment with national frameworks such as the National Environmental Management Act (NEMA) and the Companies Act further reinforces its practical impact and institutional relevance.

5. ISO 22000 as a Supporting Standard in Food Waste Reduction

ISO 14001 offers a structured framework for managing environmental aspects. Complementing this, ISO 22000 integrates HACCP principles with the structure of ISO management systems to form a proactive approach to food safety that also contributes significantly to waste reduction. It applies to all organizations in the food chain, from primary production to distribution, creating a consistent and well-organized approach throughout the food industry. By promoting the systematic identification, evaluation, and control of food safety hazards, it supports traceability, regulatory compliance, and continuous improvement. These elements not only safeguard public health but also enhance operational efficiency by minimizing production losses, rejected batches, and avoidable waste, while building trust with consumers and business partners.

In complex facilities operating multiple production lines, ISO 22000:2018 certification has been associated with a 44% reduction in non-compliance events, along with notable decreases in operational downtime and unnecessary waste generation (4,14). These performance gains are directly linked to the standard's requirements for systematic risk assessment, real-time monitoring, corrective actions, and a commitment to continual improvement principles that align closely with those of ISO 14001.

The integration of ISO 22000 with ISO 14001 creates a robust and synergistic management architecture that supports both food safety and environmental sustainability. Together, these standards enable food companies to ensure regulatory compliance, enhance resource efficiency, reduce waste at its source, and strengthen both consumer trust and market competitiveness. In this role, ISO 22000 not only reinforces food safety standards but also acts as a regulatory compliance tool that supports the fulfillment of broader sustainability objectives within national and international policy frameworks.

6. Regenerative Food Waste Management Driven by QCC Practices

Including Quality Control Circles (QCCs) in Environmental Management Systems can enhance organizational performance by fostering innovation, teamwork, and employee-driven problem solving (15). In the context of waste management, QCCs empower frontline staff to detect inefficiencies in operational processes and develop targeted, context-specific solutions that directly contribute to waste reduction. By promoting active participation across all organizational levels, this approach cultivates a culture of continuous improvement and shared environmental responsibility.

Beyond structured process enhancements, ISO 14001 is also well aligned with the principles of regenerative sustainability. Practices such as food redistribution, composting of organic waste, and prioritization of local sourcing extend the impact of Environmental Management Systems beyond mere regulatory compliance, supporting circular economy objectives (16). These regenerative strategies reframe waste not as a burden but as a potential input for new value-generating cycles. This shift from basic compliance to system-level innovation illustrates how ISO 14001 is evolving into a strategic tool that helps the food industry reduce waste and reintegrate it into useful production cycles.

7. Discussion

The alignment of ISO 14001 with both national and European legislative frameworks demonstrates the added value of integrating voluntary standards with binding environmental regulations. Legal instruments such as the EU Waste Framework Directive and Regulation (EC) No 1069/2009 provide the structural basis for food waste governance, while ISO 14001 offers the practical tools, such as planning, performance tracking, and continual improvement, that enable organizations to meet and often surpass these regulatory requirements.

Yet, the effectiveness of Environmental Management Systems does not rely solely on technical procedures. Their success is closely tied to leadership engagement and organizational culture. Evidence from countries including Brazil and South Africa underscores the critical role of staff education, managerial commitment, and a unified sustainability vision in driving meaningful change. Environ-

mental practices yield lasting results only when they are embedded in everyday operations, rather than treated as administrative obligations.

The impact of ISO 14001 is amplified when it operates in tandem with related standards like ISO 22000. While ISO 22000 focuses primarily on food safety, its structured emphasis on risk prevention, process optimization, and traceability also contributes to operational efficiency and reduced waste generation. Together, these standards establish an integrated management approach that addresses both environmental performance and public health protection.

On a global scale, ISO standards facilitate valuable knowledge exchange between industrialized and developing regions. Countries in the Global North can provide technical guidance and regulatory experience, while the Global South contributes adaptive, cost-effective innovations and regenerative solutions rooted in local contexts. Supporting this mutual exchange is essential for advancing sustainable and resilient food systems worldwide.

Table 1 summarizes the key environmental, economic, and organizational benefits associated with ISO 14001 implementation in the food sector, drawing on practical case studies and alignment with relevant legislation.

8. Conclusions

ISO 14001 offers the food industry a structured and scalable approach to improving environmental performance, particularly in the management of waste and resource use. It provides organizations with the tools to systematically identify environmental impacts, define objectives, and maintain legal compliance—supporting the transition toward more sustainable operations.

This impact becomes significantly greater when ISO 14001 is integrated with ISO 22000, the global standard for food safety management systems. Although ISO 22000 is pri-

Table 1. *Summary of environmental, economic, and organizational benefits resulting from ISO 14001 implementation in the food industry.*

Benefit category	**Description**	**Examples/sources**
Environmental Benefits	Significant reductions in waste, water, and energy use, improved pollution control, and overall enhancement of environmental performance.	40% decrease in caustic use and 500 m^3/day reduction in wastewater at a UK food-processing facility (12).
Economic Benefits	Decreased operational costs and additional income through resource efficiency, material reuse, and recycling.	Cost savings from reuse and recycling practices in South Africa (5).
Legal Compliance	Enhanced conformity with EU and national legislation, such as Directive 2018/851 and Regulations 1013/2006 and 1069/2009.	Compliance with EU Waste Directive 2018/851 (8); management of animal by-products per Regulation 1069/2009 (10).
Human Capital and Culture	Strengthened employee awareness and engagement, leadership involvement, and promotion of a sustainability-driven organizational culture.	Use of Quality Control Circles (QCCs) to encourage innovation and staff participation (15).
Market and Reputation	Broader access to export markets and improved brand reputation and stakeholder confidence as a result of ISO 14001 certification.	Increased reputation and market opportunities linked to ISO 14001 adoption (2).
Innovation and Digital Tools	Integration of artificial intelligence technologies for forecasting demand, optimizing inventory, predicting shelf life, and managing food donation logistics.	Use of artificial intelligence in food waste reduction to minimize overproduction and enhance distribution efficiency (14).

marily focused on identifying and controlling food safety hazards, its emphasis on preventive action, traceability, and process optimization directly supports environmental goals. By minimizing operational inefficiencies and reducing product loss, ISO 22000 indirectly contributes to waste reduction across the supply chain.

Together, ISO 14001 and ISO 22000 form a complementary and synergistic system that enables food companies to address two critical priorities simultaneously: ensuring food safety and improving environmental sustainability. This dual-framework approach strengthens organizational capacity not only to meet regulatory expectations, but also to operate responsibly and competitively in a globalized food system.

When combined with regenerative practices and digital tools, this integrated approach helps organizations move beyond simply meeting regulations, supporting circular economy solutions and long-term sustainability.

Figure and Table

Figure 1. Flowchart showing the interaction between ISO 14001, EU legislation, environmental actions, and outcomes in the food industry.

Table 1. Summary of environmental, economic, and organizational benefits resulting from ISO 14001 implementation in the food industry.

Conflict of Interest

The author declares no conflict of interest.

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Regulatory Requirements as Control Measures: A HACCP-Based Model for Food Safety Compliance in the EU

Eftychia G. Karageorgou*
Chemist, M.Sc., PhD
Manager, North Certification Division
NBIS P.C. Cargo Inspections and ISO Certification, Thessaloniki, Greece
Email: ekarageorgou@nbis.gr
*Corresponding author
Zissis Tzikas
Department of Agriculture, School of Geosciences
International Hellenic University, 57400
Thessaloniki, Greece
Email: ztzikas@ihu.gr

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Perspective Article

Abstract

Ensuring food safety in the European Union requires food business operators (FBOs) to apply structured, risk-based systems that respond to a wide range of regulatory requirements. Operational Prerequisite Programs (OPRPs), as part of Hazard Analysis and Critical Control Point (HACCP) plans, offer a practical means of managing significant food safety hazards that are not addressed at Critical Control Points (CCPs). This perspective article discusses how regulatory expectations related to hygiene, traceability, allergens, chemical contaminants, and food contact materials can be translated into effective OPRPs. Emphasis is placed on how these measures can be implemented by small and medium-sized enterprises (SMEs), which often face challenges related to technical capacity, documentation, and auditing. The article highlights examples of OPRPs linked to sanitation, supplier control, production procedures monitoring, packaging safety, and water quality, and considers their role in supporting both compliance and system improvement. By aligning operational controls with compliance obligations through OPRPs, FBOs can enhance transparency, audit readiness, and consumer confidence. This perspective provides a structured approach for integrating regulatory compliance into food safety management systems in a way that is practical, scalable, and adaptable to ongoing changes in the regulatory environment.

Keywords:

European food safety legislation; HACCP; OPRP; food safety management; risk assessment

1. Introduction

Food safety in the European Union is managed through a common regulatory system that applies across all stages of food production and distribution. The core of this system is the General Food Legislation, which defines basic principles such as risk assessment, traceability, taking preventive action in case of potential risks even without full scientific certainty, and the obligation of food business operators to ensure the safety of the products they place on the market [1]. The foundation for many of today's regulatory requirements was laid by the European White Paper on Food Safety, which emphasized prevention, transparency, and scientific risk assessment [2]. These principles are applied through a set of EU food hygiene Regulations, which include specific hygiene rules, sector-based requirements, and official control procedures conducted by national authorities [3].

FBOs must implement food safety systems based on the Hazard Analysis and Critical Control Point approach. However, applying detailed regulatory requirements within these systems can be challenging, especially for small and medium-sized enterprises [4,5,6]. European food legislation covers many technical areas, such as chemical contaminants, labelling, packaging, and food contact materials, which must be translated into practical controls [7–9].

In the context of food safety management systems, as structured by ISO 22000, the implementation of Prerequisite Programs (PRPs) is essential to establish and maintain a hygienic environment throughout the food chain. PRPs comprise the basic conditions and activities that are necessary to maintain food safety, such as cleaning and sanitation, pest control, maintenance, supplier approval, and personal hygiene, and are gen-

erally applied across all processing steps. In addition to PRPs, ISO 22000 introduces the concept of Operational Prerequisite Programs – preventive control measures – that are determined by hazard analysis as necessary to control significant hazards, but which do not meet the criteria to be managed as Critical Control Points. OPRPs require specific monitoring and verification procedures, and their management is more stringent than that of general PRPs due to their direct role in hazard control. The clear differentiation between PRPs, OPRPs, and CCPs is a fundamental aspect of the ISO 22000 methodology, enabling food business operators to ensure effective hazard control and compliance with regulatory and customer requirements. When linked to compliance obligations, OPRPs also provide structured and verifiable evidence of compliance [4,10].

This article presents a practical approach for integrating food safety legislation into OPRPs. The aim is to help FBOs, particularly SMEs, develop systems that comply with EU food safety legislation, audit-ready, and adaptable to risk.

2. How European Food Legislation Supports HACCP Implementation

Food safety in the European Union is based on strict Regulations that apply across all Member States. The main reference to applicable legislation is Regulation (EC) No 178/2002, which introduces key principles such as the precautionary principle, traceability, risk analysis, and the statutory responsibility of FBOs to ensure the safety of food placed on the market [1]. It also establishes the European Food Safety Authority (EFSA) as the scientific body responsible for risk assessments.

To apply these principles in practice, the EU adopted comprehensive food hygiene Regulations, commonly referred to as the Hygiene Package, which includes Regulations (EC) No 852/2004 [11] and 853/2004 [12], as well as Regulation (EU) 2017/625 on official controls. Regulation (EC) No 852/2004 requires all FBOs to follow hygiene rules based on HACCP principles. It also defines key supporting measures, such as sanitation, water quality monitoring, and pest control, as PRPs [3,11]. According to ISO 22000:2018, some PRPs that control specific hazards may be classified as OPRPs [4].

Regulation (EU) 2017/625 ensures that official inspections are applied consistently across Member States. It requires competent authorities to use a risk-based approach and to assess the effectiveness of HACCP systems, including OPRPs. It also establishes rules for transparency and enforcement [3].

These Regulations provide the regulatory basis for turning food safety requirements into practical, documented control systems that can be monitored and audited [13].

3. Critical Regulatory Requirements for Integration into OPRPs

Operational Prerequisite Programs help food business operators implement food legislation in a practical way. They are used to control significant food safety hazards that are not addressed at Critical Control Points. Instead of relying on a single control step, OPRPs are integrated into daily activities and preventive measures. Several areas of EU food legislation align closely with the structure of OPRPs and can be incorporated into HACCP-based systems.

3.1. Food Hygiene and Microbiological Criteria

Regulation (EC) No 2073/2005 establishes safety and hygiene limits for microorganisms in food, including *Listeria monocytogenes* in ready-to-eat products and *E. coli* in meat [5]. It defines microbiological criteria for different categories of food and specifies sampling plans, testing methods, and actions to be taken when limits are exceeded. These legislative requirements are typically addressed through OPRPs involving environmental monitoring, cleaning procedures, and cold chain temperature controls [4]. In most cases, the presence of *Listeria monocytogenes* or *Salmonella* is not controlled by a single step, but through a combination of hygiene measures, proper handling, and refrigeration, making them typical examples of hazards managed by OPRPs [5]. Actually, all specific microorganisms for which microbiological criteria are laid down by Regulation (EC) No 2073/2005 may be identified as food safety hazards related to the safety of each officially defined food category.

3.2. Food Additives, Flavourings, and Smoke Substances

The use of additives and flavourings is controlled under Regulations (EC) 1333/2008, 1334/2008, and 2065/2003. Additionally, Regulation (EU) 2024/2067 removed ten smoke flavourings from the Union list [14,15,16,17]. To comply, food operators must ensure that only approved substances are used. OPRPs can include procedures such as checking ingredient lists, requesting supplier specifications, verifying that discontinued flavourings are not used, and ensuring that weighing equipment used for additives is properly calibrated. Detailed guidance on the application of Regulation (EC) No 1333/2008 is also provided by the European Commission to help food businesses understand how to apply additive and processing aid rules in practice [18].

These controls are often integrated into purchasing, receiving, and production control procedures.

3.3. Labelling, Allergens, and Consumer Information

Regulation (EU) 1169/2011 requires clear labelling and the correct declaration of allergens [7]. Failure to declare allergens properly can result in regulatory non-compliance and potential harm to consumers. In addition to allergens, the regulation also covers ingredients that may cause food intolerances, such as lactose and gluten, which must also be clearly declared on the label. OPRPs in this category can include cleaning of production lines between allergen and non-allergen products, verifying label accuracy before packaging, personnel training on allergen management, and maintaining records for traceability and recall [19].

3.4. Contaminants and Residues

Regulation (EC) 396/2005, Regulation (EC) 37/2010 and Regulation (EU) 915/2023 define maximum allowable levels for chemical hazards such as pesticide residues, veterinary drug residues and food contaminants (e.g. heavy metals and mycotoxins), respectively [8,20,21]. Chemical hazard identification and determination of acceptable levels, as part of a hazard analysis, may be based primarily on the above regulatory requirements. To control these risks, OPRPs may focus on supplier approval, raw material testing, and reviewing certificates of analysis. These procedures are especially important in sensitive product categories such as baby food, cereal products, and seafood. In addition to supplier controls, food business operators should carry out internal verification, such as periodic in-house testing, to confirm compliance with established limits.

3.5. Food Contact Materials and Packaging

Regulation (EC) 1935/2004, as well as Regulations (EU) 10/2011 and 2025/40 establish rules for the safety and sustainability of packaging and food contact materials [9,22,23]. These Regulations aim to prevent harmful substances from migrating into food and promote the use of recyclable and safe materials. OPRPs in this area may include collecting supplier declarations, testing for compliance with migration limits, avoiding materials containing substances such as per- and polyfluoroalkyl substances (PFAs) or bisphenol A (BPA), and auditing packaging suppliers.

3.6. Water Quality and Process Inputs

Directive (EU) 2020/2184 on the quality of water intended for human consumption requires that water used in

***Table 1**: Indicative EU legislation and their corresponding OPRPs within HACCP systems.*

EU Regulation	Hazard Controlled	Example OPRP Measure
Reg. 852/2004 (Hygiene)	Microbiological contamination	Check of the effectiveness of cleaning and sanitation schedules
Reg. 853/2004 (Animal-origin foods)	Animal-origin contamination	Check of Food Chain Information, temperature controls
Reg. 2073/2005 (Microbiological Criteria)	Listeria, Salmonella	Cold chain monitoring, environmental testing
Reg. 1169/2011 (Labelling)	Allergen cross-contact	Check of product labelling and equipment sanitation
Reg. 1333/2008, 2024/2067 (Additives, Smoke)	Unauthorized additives	Ingredient verification, supplier specifications
Reg. 1935/2004, 10/2011, 40/2025 (Packaging)	Chemical migration from packaging	Supplier declarations, migration testing
Reg. 396/2005, 915/2023 (Pesticides, Contaminants)	Pesticide residues, heavy metals	Review of certificates of analysis, raw material testing
Dir. 2020/2184 (Water)	Unsafe water use	Water analysis, filter maintenance
Reg. 37/2010 (Pharmacologically active substances)	Veterinary drug residues	Supplier validation, residue monitoring

food production is clean and suitable for human consumption [24]. Monitoring system and verification activities for OPRPs related to water safety often include regular microbial and chemical testing, checking the function of filters or disinfectants, and keeping records of results and corrective actions [25].

The application of European food safety legislation within HACCP systems relies on the ability to convert compliance obligations into structured and verifiable control measures. Table 1 outlines indicative EU regulatory requirements and demonstrates how these can be systematically addressed through corresponding OPRPs, ensuring both compliance and operational consistency.

By integrating legislative requirements, such as those on hygiene, additives, labelling, and contaminants, into clear and structured OPRPs, FBOs can create food safety systems that are practical to apply, easy to verify, and compliant with EU Regulations. This also helps prepare for inspections, improves operational reliability, and supports continuous improvement across the food chain.

4. Applying Regulatory Requirements through OPRPs in HACCP Systems

To effectively apply European food safety legislation within HACCP-based systems, the food industry must convert regulatory requirements, such as those concerning hygiene, labelling, and contaminants, into practical control measures. Operational Prerequisite Programs, as defined in ISO 22000:2018, are designed to manage significant hazards that are not controlled at Critical Control Points but still require documented, preventive action. Compared to general Prerequisite Programs, which provide basic conditions for food safety, OPRPs focus on targeted hazards and require regular monitoring, and verification [4].

4.1 Determining OPRPs Using HACCP and ISO 22000 Criteria

The ISO 22000:2018 standard provides a structured method for categorising control measures to be managed as OPRPs, or at CCPs. Controls that are essential to food safety and do not require critical limits' establishment for their monitoring, but measurable or observable specifications instead, are typically designated as OPRPs [4]. Meeting or achieving these specifications, defined as "action criteria" in ISO 22000:2018, indicates that the OPRPs are functioning as intended. Examples of OPRPs include monitoring cold chain temperatures, validating sanitation between allergen runs, verifying supplier documentation, and controlling physical hazards through visual inspection. These measures are part of preventive systems and typically involve regular checks and documented procedures to demonstrate consistent performance and compliance with food safety regulations.

4.2 Integration of Regulatory Requirements into OPRPs

Several parts of EU food legislation can be applied in practice through well-designed OPRPs. For example, to meet the requirements of Directive (EU) 2020/2184 on water used in food processing, an OPRP may include regular monitoring to ensure that filtration and disinfection systems are maintained in good working condition [24]. In the case of allergen control, Regulation (EU) 1169/2011 requires clear labelling and the prevention of allergen cross-contact. An OPRP can support these goals by including proper cleaning between product batches, visual checks, ATP testing, label verification, and personnel training [7,19]. Similarly, to comply with Regulation (EC) 396/2005, Regulation (EC) 37/2010 and Regulation (EU) 915/2023 regarding chemical hazards, as well as with Regulation (EC) 2073/2005 regarding microbiological hazards, the food industry may establish OPRPs involving monitoring of incoming materials and supplier approval, through

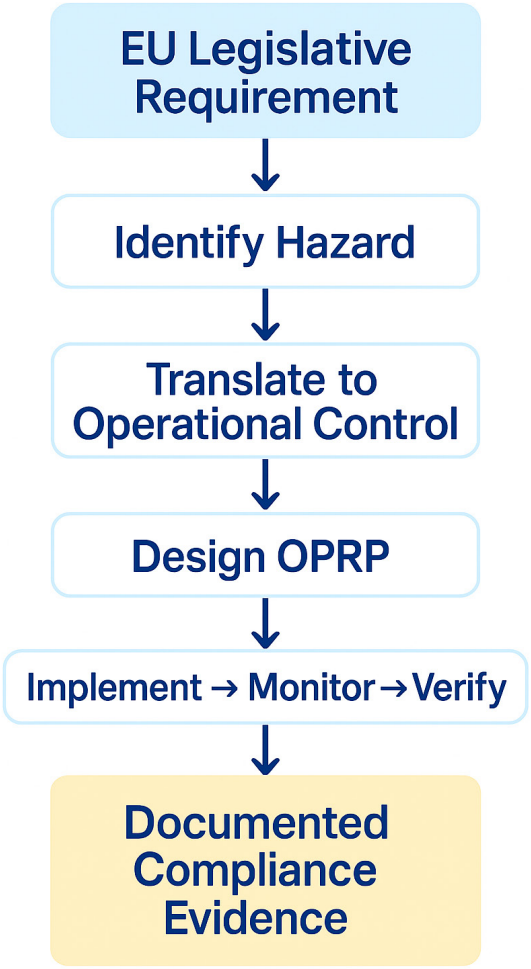

Figure 1. *OPRP development process based on EU regulatory requirements.*

reviewing certificates of analysis and microbiological examination results.

Regulation (EU) 2024/2067 bans the use of certain smoke flavourings, requiring the food industry to update ingredient lists, obtain confirmation from suppliers, and carry out internal checks to ensure these substances are not used [17]. For food contact materials and packaging, Regulation (EC) 1935/2004 and Regulation (EU) 2025/40 establish rules on safety and recycling. OPRPs can be used to ensure that packaging materials are approved by suppliers, tested for compliance with chemical migration limits, and free from harmful substances such as per- and polyfluoroalkyl substances or bisphenol A.

These examples show how the food industry can turn regulatory requirements into everyday control procedures that are clear, effective, and easy to monitor.

Figure 1 illustrates the sequential process of establishing Operational Prerequisite Programs (OPRPs) based on EU regulatory requirements, following the hazard analysis and control measure categorization approach defined in ISO 22000.

4.3 Documentation, Monitoring, and Verification

Each OPRP must be clearly documented and include evidence that it functions effectively in practice. The documentation should describe the control measure in detail, define who is responsible for its implementation, and explain how it should be carried out. Monitoring records are essential and may include cleaning and temperature logs, or supplier documentation. To confirm that the OPRP is working as intended, verification activities, such as internal audits, third-party laboratory testing of end product samples, regular microbial and chemical testing of water quality, or cross-checks like label inspections and traceability exercises, are often used [13,26]. When a problem occurs, it is necessary to investigate the root cause, implement corrective actions, and apply preventive measures to reduce the likelihood of recurrence.

This structured approach supports consistency, traceability, and readiness for both internal reviews and official inspections.

4.4 Linking OPRPs to Regulatory Compliance

When an OPRP is clearly derived from a regulatory requirement, it functions not only as a food safety control but also as documented evidence of compliance with the legislation. For instance, temperature monitoring in ready-to-eat foods – although not classified as a CCP – helps demonstrate that the microbiological limits established by Regulation (EC) 2073/2005 are being met [5]. Similarly, OPRPs related to hygiene support the requirements set out in Regulation (EC) 852/2004 [11]. During official inspections, authorities operating under Regulation (EU) 2017/625 may use OPRP records to confirm that a business is fulfilling its compliance obligations and managing food safety risks effectively [3].

By integrating regulatory requirements into clear and structured OPRPs, the food industry enhances transparency, reduce the risk of non-compliance, and strengthen their systems for long-term improvement.

5. Implementing Legislation in HACCP Systems: Challenges for SMEs

Although OPRPs provide a practical approach for applying EU food safety legislation, small and SMEs often face significant challenges in their implementation. These difficulties are both technical and structural in nature, and they may affect the consistency and reliability of HACCP-based food safety systems.

5.1. Lack of Internal Expertise

Many SMEs do not employ personnel with sufficient knowledge of food legislation or HACCP system design. Extensive and strict legislation, such as Regulation (EC) 396/2005 on pesticide residues and Regulation (EU) 2025/40 on packaging and sustainability requirements, can be difficult to interpret without technical guidance [9,20]. For this reason, many businesses rely on external consultants. While this may be helpful in the short term, it often reduces system ownership and limits continuity over time [4,6].

5.2. Complexity of Documentation

SMEs frequently have difficulty managing the volume and overlap of regulatory obligations. Regulatory requirements related to traceability under Regulation (EC) No 178/2002, hazard control as defined in ISO 22000, microbiological safety under Regulation (EC) 2073/2005, food additives usage under Regulation (EC) 1333/2008, and contaminant limits under Regulation (EU) 915/2023 all require structured documentation [1,4,5,8,14]. Without clear guidance, businesses may produce either too many or too few records, increasing the risk of non-conformities during audits and reducing operational efficiency.

5.3. Inconsistent Auditing Practices

Despite the intent of Regulation (EU) 2017/625 to harmonize official controls, differences remain in how national authorities evaluate OPRPs [3]. Some inspections focus heavily on documentation, while others prioritize on-site performance. This inconsistency creates confusion and may lead SMEs to adapt their systems to indi-

vidual inspector expectations rather than follow a risk-based approach [13].

5.4. Weak Supplier Verification

Supply chain compliance is essential, especially when dealing with restricted substances such as banned smoke flavourings under Regulation (EU) 2024/2067 or materials regulated under food contact legislation, such as Regulation (EC) 1935/2004 [17,22]. However, SMEs may lack the leverage or technical capacity to demand full transparency from their suppliers. This exposes them to a higher risk of supplier non-compliance and makes it harder to show that proper controls have been followed.

5.5. Resource Limitations

Many SMEs operate with limited resources, which makes it difficult for them to invest in tools such as final product testing, digital traceability systems, external laboratory analyses, or structured employee training on food legislation [4,19,27]. This limits their ability to keep food safety systems up to date, especially as EU legislation continues to evolve.

Despite these challenges, support measures such as simplified OPRP templates, targeted training, and risk-based audit approaches can help SMEs strengthen regulatory compliance and improve the overall effectiveness of their food safety systems [25].

6. Discussion: From Regulatory Compliance to Food Safety Culture

The role of OPRPs in food safety goes beyond meeting compliance obligations. When regulatory requirements are integrated directly into daily processes, they support a broader shift in food safety thinking from basic compliance to a culture of responsibility, transparency, and continuous improvement.

6.1. Regulatory Requirements as a Basis for Improvement

EU legislation, such as Regulation (EU) 2025/40 on packaging and Regulation (EU) 1169/2011 on food information, can support more than just minimum standards. These regulatory requirements provide a structured approach that encourages companies to strengthen traceability, improve supplier control, and promote sustainable packaging design [7,9,13]. When applied in this way, legislation becomes a tool for advancing operational maturity and supporting better decision-making, even in complex or high-risk situations such as product recalls.

Integrating regulatory requirements into structured OPRPs also contributes to broader environmental, social, and governance (ESG) goals. Clear documentation, traceable sourcing, and safe packaging design not only ensure compliance, but also align with corporate sustainability strategies. In this context, food safety becomes part of a company's social responsibility and long-term value creation.

6.2. Increasing Trust Through Transparency

Clear alignment between Operational Prerequisite Programs and regulatory requirements, such as hygiene practices under Regulation (EC) 852/2004 or allergen management under Regulation (EU) 1169/2011, demonstrate that a business is complying with the legislation and acting responsibly [7,11,28]. This level of transparency helps build trust with regulators, customers, and certification bodies. It also makes food safety efforts more visible, consistent, and measurable.

6.3. Empowering Food Handlers

When personnel understand that their actions, such as cleaning, final product testing, or label verification, are not only part of daily routines but also stem from specific legislative requirements aimed at protecting public health, they become more committed to performing them correctly [19]. This strengthens awareness, personal ownership, and teamwork. With proper training and clear communication, frontline personnel can help transform food safety from a checklist activity into a shared responsibility [26].

6.4. Turning Compliance into Competitive Advantage

Businesses that align OPRPs with legislative requirements can differentiate themselves in the marketplace. Well-documented systems support certifications, export approvals, and participation in supply chains that prioritize sustainability and traceability [27]. For these businesses, food safety becomes not just a legislative obligation, but a strategic advantage.

By treating legislation as part of daily operations and not just as an external demand, the food industry can improve their systems, build trust, and support a stronger food safety culture at all levels.

7. Conclusions and Policy Recommendations

Integrating European food safety legislation into HACCP-based Operational Prerequisite Programs is not merely a regulatory obligation, it is a strategic approach to building stronger, more adaptable food safety systems. By incorporating official requirements into routine operational controls, the food industry can establish systems that are not only compliant with EU legislation, but also clear, auditable, and responsive to emerging risks. OPRPs provide

a practical means of translating strict rules on hygiene, contaminants, labelling, packaging, and sustainability into daily activities, aligning food operations with regulatory expectations while supporting long-term performance.

However, many small and SMEs continue to face significant challenges when attempting to translate regulatory requirements into effective procedures. Frequent difficulties include limited access to regulatory expertise, lack of technical capacity, and inconsistent inspection practices, all of which can delay or disrupt implementation efforts. These factors make it difficult for SMEs to establish HACCP-based systems that are fully aligned with current EU regulatory expectations.

To support practical implementation, policy efforts should focus on making food safety legislation more accessible at the operational level. This includes developing sector-specific guidance that translates regulatory requirements into applicable OPRPs, expanding training for both front-line personnel and SMEs managers, and promoting digital tools—such as templates and simplified verification systems—to reduce complexity. Risk-based audits should also recognize well-documented OPRPs as valid evidence of compliance, especially in low-risk settings.

Innovation also plays a key role in advancing food safety. Tools such as smart packaging and environmental performance indicators can help integrate regulatory compliance with sustainability. Ongoing collaboration between authorities, the food industry, and certification bodies is crucial to ensure that HACCP systems adapt to evolving regulatory and societal demands.

When OPRPs are used as practical tools for applying food legislation, they enable FBOs to move beyond basic compliance. This approach enhances transparency, strengthens stakeholder trust, and supports the EU's goals for a safe and sustainable food system.

Figure and Table

Table 1: Indicative EU legislation and their corresponding OPRPs within HACCP systems.

Figure 1. OPRP development process based on EU regulatory requirements.

Conflict of Interest

The authors declare no conflict of interest.

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Sorption of thorium from aqueous solutions onto natural and modified zeolites

Filippos Karantoumanis, Fotini Noli
Radiochemical Laboratory of the General & Inorganic Chemistry Department,
School of Chemistry, Aristotle University, Thessaloniki, 54124 Greece
Corresponding author: noli@chem.auth.gr

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Abstract
A natural HEU-type zeolite from Greece was embedded with iron oxyhydroxides via a simple and fast precipitation method following acid pretreatment. The raw and modified materials were subsequently evaluated as sorbents for thorium removal from acidic aqueous solutions. Surface techniques (BET, pHpzc) and structural characterization methods such as Fourier Transform Infrared Spectroscopy (FTIR) and X-Ray Diffraction (XRD) were implemented for both materials before and after metal sorption. The experimental results were used for the construction of sorption isotherms while the data were also fitted using mathematical models for a brief insight into the sorption mechanism accompanied by kinetic and thermodynamic studies. Results showed that the modification process caused changes in the characteristics of the precursor material and that thorium uptake was greatly affected by the presence of iron oxyhydroxides.

Keywords Zeolites; Fe-oxyhydroxides; thorium; sorption; isotherms

INTRODUCTION

Thorium is one of the most commonly occurring actinides in the Earth's crust, forming a variety of compounds with many elements. Among others such as thorianite (ThO_2), thorite, and uranothorite, monazite is the most important commercially exploitable mineral, and often a common by-product of ore mining. However, thorium does not exhibit the complex aqueous chemistry of other actinides like uranium. It is present in nature only in the 4+ oxidation state, Th(IV), and most of its complexes with common ligands have very low solubility. As a result, its concentration in natural waters is significantly lower [1, 2].

Nonetheless, thorium is an important waste produced by ore mining and milling operations that can contaminate ground- and surface water deposits, posing a threat to the ecosystem and humans. Exposure to high concentrations of thorium, although rare, can lead to serious health issues due to its chemical toxicity, including organ failure, respiratory problems, or even cancer [3, 4].

Among the procedures employed for thorium removal, sorption by natural sorbents like zeolites and bentonites exhibits a series of advantages including high sorption capacities, high active surface, low cost, and widespread availability. Many examples of environmental remediation utilizing aluminosilicate sorbents entail the treatment of highly acidic wastewater. Most notably in the cases of ore mining and milling, high volumes of acidic liquid waste (tailings) are produced. When in contact with such media, zeolites can undergo structural changes, like dealumination or alteration of surface characteristics, which then affect the materials' sorption properties [5–8].

Moreover, the iron oxyhydroxides are a group of porous Fe(III) and Fe(II) compounds with oxide and/or hydroxide groups in their crystal lattice. In this category some of the most common iron minerals in soil substrates like ferrihydrite, goethite, magnetite, etc. are included. A multitude of applications of these compounds have been reported due to their useful chemical, structural, and physical properties, including sorption of toxic and radioactive elements [9, 10].

To the best of our knowledge, the studies related to thorium sorption using aluminosilicate materials are quite limited, especially where acid pretreatment or modification by iron compounds is concerned [3–6]. The scope of the present work was to evaluate the effect that the iron oxyhydroxide introduction in the crystal matrix and acid pretreatment caused the sorption properties of the precursor material regarding thorium removal. Natural and modified sorbents were characterized by FTIR, XRD before and after thorium loading, their pHpzc and BET surface area were calculated, and the nature of the oxyhydroxides produced was investigated. Isotherm, kinetic, and thermodynamic studies were carried out, accompanied by data fitting to theoretical models.

MATERIALS AND METHODS

Natural zeolite from Petrota region in Thrace, Greece was acquired in its powder form, which was then sieved and separated into fractions of different grain size. In all experiments de-

scribed in the following sections, the fraction of particle size smaller than 50 μm was only used. All chemical reagents were of analytical grade and a water purification system (Millipore) with Elix and Milli-Q was used to provide ultra-pure water.

Modified zeolite preparation

The modified zeolite (FeZ-HCl) was prepared by acid pretreatment followed by precipitation in alkaline environment as described in the literature [11–13]. 1.85 g of precursor zeolite was suspended in a large beaker with 100 mL distilled water at room temperature and stirred for 15 min. A 50 mL solution of 0.1 mol L^{-1} Fe(III) was prepared by dissolution of iron trichloride hexahydrate $FeCl_3 \cdot 6H_2O$ (Riedel-de Haën) and was mixed with 50 mL of 1.25 mol L^{-1} HCl. The final solution was added to the beaker and further stirred for 40 min. Slow addition of 100 mL 1.25 mol L^{-1} NaOH solution to create alkaline environment (pH 10-11) resulted in the immediate formation of reddish-brown precipitate, which was filtered, washed with distilled water until a neutral pH, and dried for 24 hours at 60 °C. The final iron-modified zeolite was grinded and sieved to a particle size less than 50 μm. For comparison, the iron-modified zeolite omitting the acid pretreatment step (FeZ), as well as the pure oxyhydroxides were also prepared, according to the procedure reported in our previous study [14].

Materials characterization

The mineralogical composition of the sorbents was determined through Powder X-Ray Diffraction (pXRD) using a Rigaku MiniFlex diffractometer with Ni-filtered Cu*Ka* radiation. Each pulverized material was scanned with a step size of 0.01° 2θ in the 2θ interval of 3-90°. Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) was carried out in the range of 4000-400 cm^{-1} using a Thermo Scientific Nicolet iS20 spectrometer. The characterized materials were in contact with a diamond reflectance crystal.

The point of zero charge (pHpzc) of the sorbents was found by the pH-drift method. Solutions of 0.1 mol L^{-1} KNO_3 were prepared and fixed at initial pH values from 1 to 9. The sorbents were shaken for 18 hours at room temperature and the pHpzc was determined by plotting the ΔpH (difference between the initial and final pH) against the initial pH. BET surface area and volume were measured with an Autosorb-1MP, Quantachrome porosimeter by recording the adsorption/desorption of nitrogen at –196 °C in the partial pressure range of 0.01 to 0.995 and after heating the samples under vacuum at 150 °C.

Sorption experiments

Stock solution of 250 mg L^{-1} thorium was prepared by dissolving $Th(NO_3)_4 \cdot 5H_2O$ (Merck) in bi-distilled water and was diluted to achieve the desired initial metal concentrations (5-100 mg L^{-1}). Sorption experiments were conducted at room temperature (ca. 298 K) using 10 mg of the sorbents contacted with 10 mL of the solutions (ratio of 1.0 g L^{-1}) in centrifuge tubes for 24 hours, enough time for the establishment of equilibrium. The liquid phase was

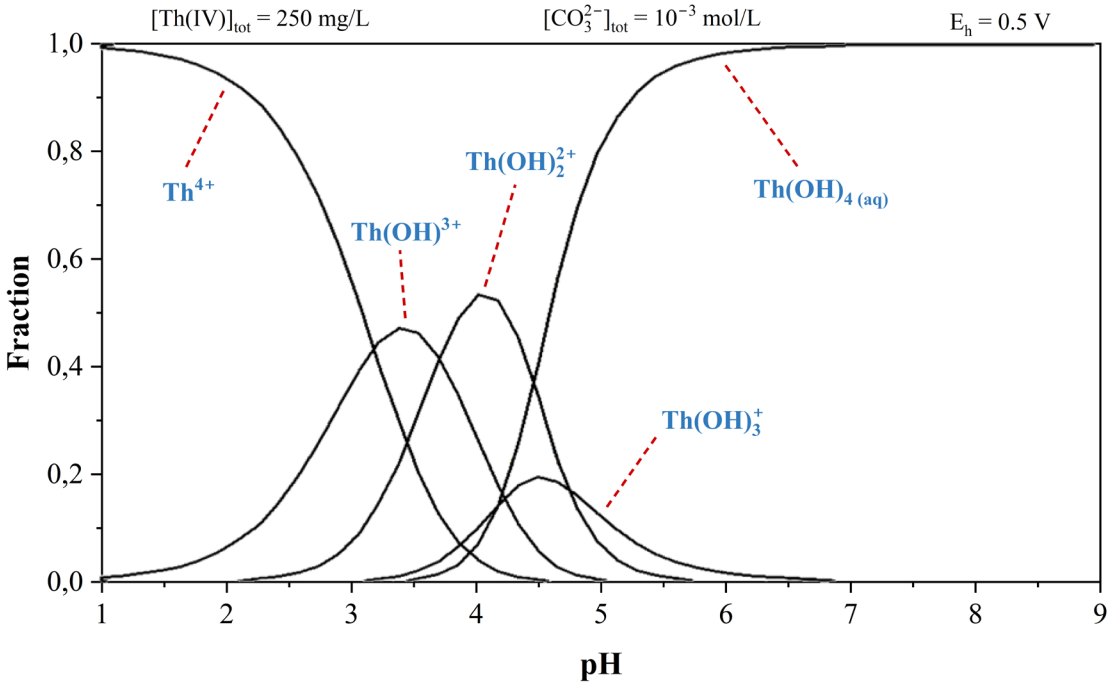

Fig. 1 *Speciation diagram of ionic thorium species in aqueous solutions as calculated by the code MEDUSA.*

afterwards separated by centrifugation (4000 rpm for 10 min) and filtration, and the equilibrium pH was measured. Thorium concentrations in the supernatant solutions were determined photometrically with the Arsenazo III method at 660 nm working wavelength. The initial solution pH was adjusted to 3 by slow, dropwise addition of HNO_3 or NaOH, so as to avoid possible precipitation of thorium hydroxides occurring at higher pH values (Fig.1) [5, 15].

The experimental data were then used for uptake calculations and for modelling with the Langmuir (Eq.1), which considers monolayer coverage of specific adsorption sites, and Freundlich (Eq. 2) linear isotherm, which assumes heterogeneity in the solid/liquid interface [16]. Evaluation of the linear correlation coefficients, R^2, revealed the best-adjusted model.

Langmuir (L)
$$\frac{C_e}{q_e} = \frac{1}{K_L q_{max}} + \frac{1}{q_{max}} C_e \quad (1)$$

Freundlich (F)
$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e \quad (2)$$

In the above equations q_e and C_e are the equilibrium uptake (in mg g^{-1}) and liquid metal concentrations (in mg L^{-1}), respectively, q_{max} the maximum sorption capacity (in mg g^{-1}), K_L and K_F the Langmuir and Freundlich equilibrium constants and n, in the case of Freundlich equation, a parameter associated with the heterogeneity.

Kinetic and thermodynamic studies

The effect of contact time and temperature was investigated using stock thorium solutions of 100 mg L^{-1} concentration (at pH 3) at three different temperatures, 298, 308, and 318 K. Known amounts of the raw zeolite and each solution (with 1.0 g L^{-1} ratio) were contacted, and at predetermined time intervals between 2 and 60 min, 10 mL aliquots were drawn with a syringe, filtered, and transferred to polypropylene tubes for photometric thorium determination.

The results were afterwards fitted to the pseudo-first (PFO) and pseudo-second order (PSO) kinetic models, using the linear forms of the equations (Eq. 3 and Eq. 4). According to the PFO model, sorption is due to physical interactions and mass transfer by diffusion, while according to the PSO model, the critical step of the process may involve chemical interactions between the adsorbent and the adsorbate [17].

PFO
$$\ln(q_e - q_t) = \ln(q_e) - k_1 t \quad (3)$$

PSO
$$\frac{t}{q_t} = \frac{1}{q_e^2 k_2} + \frac{1}{q_e} t \quad (4)$$

In both previous cases, q_t (in mg g^{-1}) is the thorium uptake at time t (in min), and q_e (in mg g^{-1}) is the thorium uptake at equilibrium. The pseudo-first and pseudo-second order reaction rate constants are represented by k_1 (in min^{-1}) and k_2 (in g mg^{-1} min^{-1}) respectively.

The sorption activation energy, E_a, is the amount of energy required for the reaction to occur, and can be easily calculated using the appropriate k constant, as determined by previous analysis of the kinetic data, with the Arrhenius equation (Eq. 5), where A (same units as k) is the Arrhenius factor, R (=8.314 J mol^{-1} K^{-1}) is the universal gas constant, and T (in K) the absolute temperature.

$$\ln k = \ln A - \frac{E_a}{RT} \quad (5)$$

Finally, thermodynamic parameters were calculated. The Gibbs free energy change, $\Delta G°$ (in J mol^{-1}), is related to the spontaneity of the sorption and is given by Eq. 6, in which $K_D = q_e/C_e$ (L g^{-1}) is the distribution coefficient at equilibrium [18].

$$\Delta G° = -RT \ln K_D \quad (6)$$

The enthalpy change, $\Delta H°$ (in J mol^{-1}), which determines the exothermic or endothermic character of the reaction, and the entropy change, $\Delta S°$ (in J mol^{-1} T^{-1}), which is a measure of the degree of disorder in the system, can be calculated using the van't Hoff equation (Eq. 7).

$$\ln K_D = \frac{\Delta S°}{R} - \frac{\Delta H°}{R} \frac{1}{T} \quad (7)$$

RESULTS AND DISCUSSION

Materials characterization

The FTIR spectra of the raw and loaded sorbents are presented in Fig. 2. The pattern observed for the natural zeolite is characteristic for HEU-type zeolites [19–21]. The broad band at approximately 3390 cm^{-1} is attributed to stretching O–H vibrations of water molecules and hydroxyl groups while the band at 3616 cm^{-1} is due to surface Si–OH and Al–OH groups of the lattice. The respective bending O–H vibrations can be seen at 1630 cm^{-1}. Typical aluminosilicate bands are detected at 1005, 792, and 585 cm^{-1} corresponding to the asymmetric stretching vibrations of both the zeolitic tetrahedra (T–O–T) and similar vibration modes of possible mineral impurities (quartz, feldspars), the symmetrical internal stretching vibrations of O–T–O bonds, and finally vibrations related to the pore structure of HEU-type zeolites.

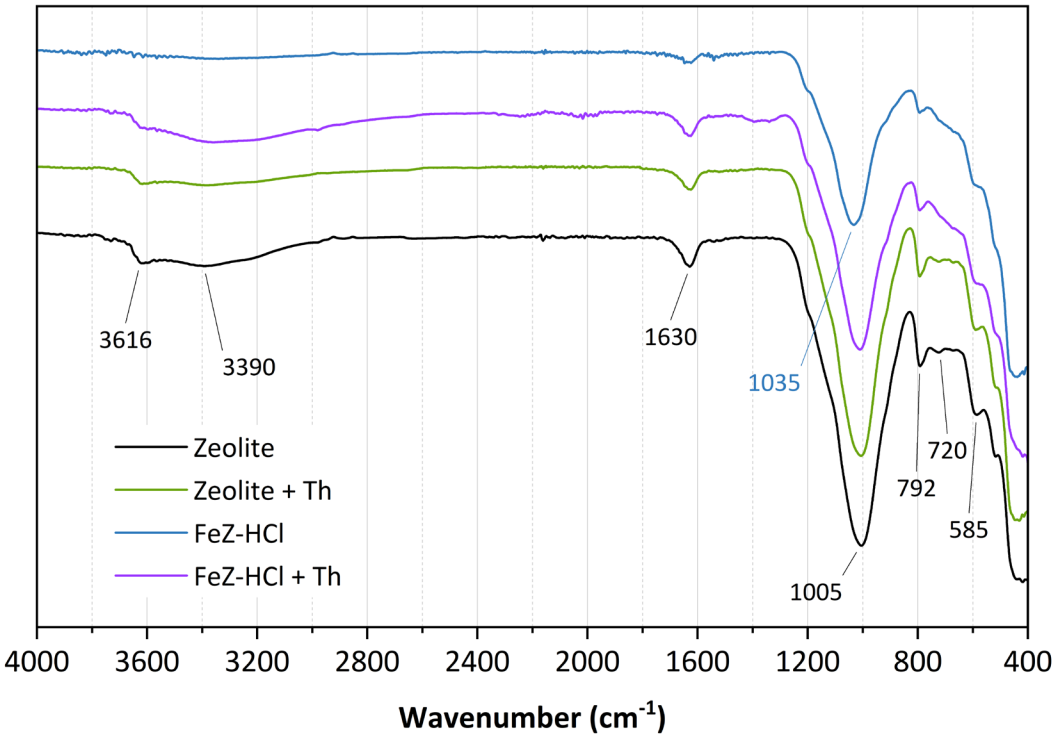

Fig. 2 *FTIR spectra of the raw (Zeolite) and modified zeolites (FeZ-HCl) before and after thorium loading.*

Acid treatment and the introduction of iron oxyhydroxides did not alter the peak pattern of the precursor material pointing to retention of the basic zeolite structure after modification [13, 21, 22]. However, minor changes in the intensity and position of the main band can be observed. Specifically, the shift towards higher wavenumbers (1035 cm^{-1}) could be explained by the interaction between newly-introduced Fe–O groups of the oxyhydroxides and surface tetrahedra, as well as the acid pretreatment step. A high concentration of H^+ ions can attack and break Si–O–Al bonds, leading to dealumination and removal of Al^{3+} from the lattice. The stronger Si–O bonds are detected in higher wavenumbers [23]. Thorium sorption did not affect band position and number for both materials.

The powder XRD pattern of the zeolite (Fig. 3) reveals the characteristic clinoptilolite diffraction peaks at 2θ=10.01, 11.33, 22.55, 30.16° which correspond to the (020), (200), (400), (151) planes. The natural zeolite consisted of up to 89% HEU-type zeolite, but small quantities of impurities like quartz, feldspars, clay, and mica were also present, as indicated by the diffraction peaks at 2θ=26.77 and 28.27° [22, 23]. In the modified material (FeZ-HCl) the clinoptilolite peaks remained unchanged in number and position, confirming the zeolite structure without any new mineral phases. However, the material has a more amorphous character, induced by acid treatment and partial dissolution of the lattice [23]. Thorium sorption did not alter the basic peak pattern for both materials, meaning that no new mineral phases were created. Minor shifts in the range of 0.2° to lower 2θ values indicate lattice expansion, related to thorium binding.

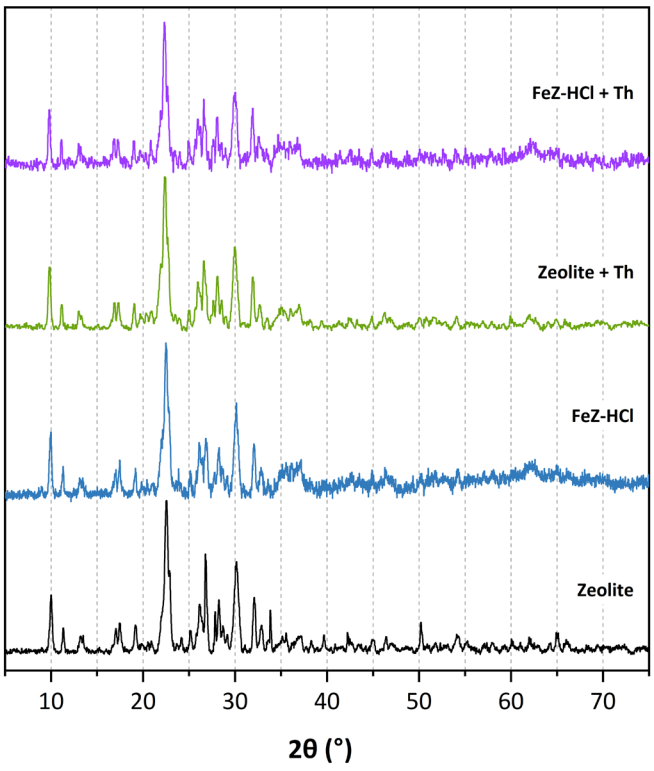

Fig. 3 *pXRD patterns of raw zeolite and iron-modified zeolite, before and after uranium sorption.*

Table 1 *Surface areas and pore volumes calculated with the BET method, and the point of zero charge of the evaluated materials.*

Material	S_{BET} (m^2/g)	S_{micro} (m^2/g)	V_{BET} (cm^3/g)	V_{micro} (cm^3/g)	pHpzc
Zeolite	32	9	0.106	0.004	7.00
FeZ-HCl	90	13	0.133	0.006	4.60

Table 1 shows the results of the BET and pHpzc methods. The point of zero charge has decreased by almost 2.5 units in the modified sorbent. As revealed by the pHpzc curves (Fig. 4) surface charge characteristics are different for FeZ-HCl. Similar results have been observed by other researchers and were attributed to a possible positively charged film of Al^{3+} ions or otherwise by the more intense H^+ adsorption due to defects by dealumination and partial amorphization [23, 26].

Concerning BET measurements, it is obvious that both surface area as well as volume have been significantly enhanced post modification. The amorphous nature of the surface-deposited iron oxyhydroxide layer results in the creation of vacant space among the randomly precipitated particles, thus enhancing the surface area [21, 26, 27]. This is further confirmed by the pore size distribution (Fig. 5) showing a large increase in the pores with diameters in the region of 1-4 nm. The increase in total volume can be attributed to the structural changes caused by acid treatment.

A detailed presentation of the characterization results for FeZ and the pure iron oxyhydroxides is given in our previous study [14], where it was observed that even without acid pretreatment, iron modification had in general similar effects as discussed above. Concerning the nature of iron oxyhydroxides produced, it was concluded that they were a mixture consisting mostly of amorphous ferrihydrite co-existing with small amounts of low-crystallinity goethite (α-FeOOH).

Thorium sorption

Fig. 6 displays the thorium sorption isotherms on natural and modified zeolites, which demonstrate the change of uptake values as a function of the equilibrium metal concentration. These data can afterwards give information on the phenomenon and help elucidate the sorption mechanism. A significant decrease in the amount of thorium sorbed can be observed concerning the modified materials. At initial concentration equal to 25 mg L^{-1}, almost 70% of the metal has been adsorbed onto the natural zeolite, compared to the 55% and 32% removed by the FeZ and FeZ-HCl modified zeolites respectively.

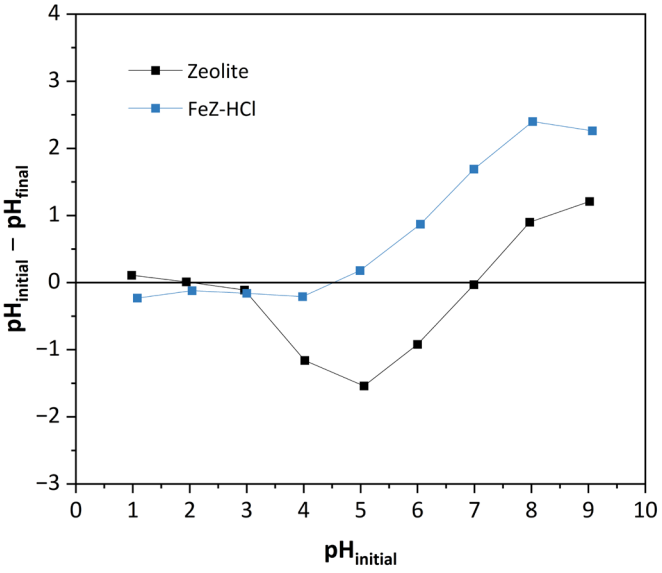

Fig. 4 *N_2 adsorption-desorption isotherms and pore size distribution calculated according to the NLDFT model for the raw and iron-modified zeolites.*

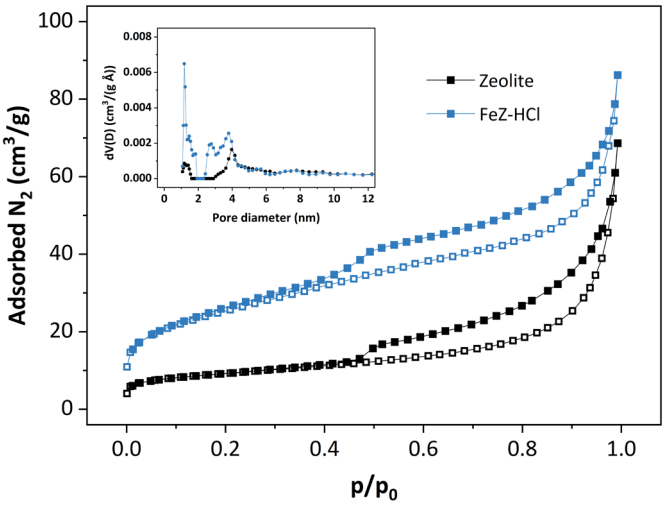

Fig. 5 *N_2 adsorption-desorption isotherms and pore size distribution calculated according to the NLDFT model for the raw and iron-modified zeolites.*

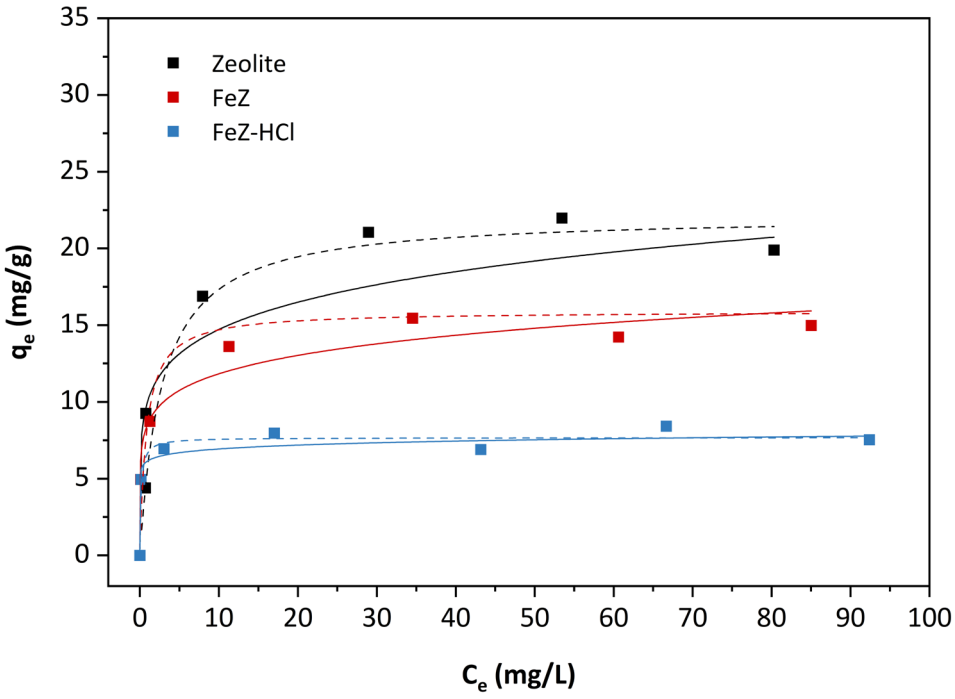

Fig. 6 *Thorium sorption isotherms onto the raw and modified zeolites (dosage: 1 g L^{-1}, T: 295 K). Solid lines depict the Freundlich fitting and dashed lines the Langmuir fitting.*

As it is known, the zeolitic framework hosts mainly negative charge owing to the $[AlO_4]^{5-}$ tetrahedra, which is then compensated by exchangeable cations like Na^+, Ca^{2+}, K^+, Mg^{2+} [20, 24]. As a result, cations like Th^{4+}, $[Th(OH)]^{3+}$, $[Th(OH)_2]^{2+}$ which dominate in acidic media (Fig. 1) readily sorb on the material's surface. However, since the initial solution pH was quite acidic, the competition for active site binding between the cationic thorium species and H^+ is high.

The further decrease in uptake in the case of the modified materials can be explained by considering the following two points. Firstly, researchers have observed a decrease in porosity in iron oxyhydroxide-treated sorbents [28] which in turn deters the large size thorium ions from reaching the active binding sites. This is supported by BET surface area results, showing a sharp increase in small diameter micropores. Secondly, concerning specifically FeZ-HCl the dealumination process described before has affected surface charge characteristics. As a result, the electrostatic repulsion is higher and inhibits the approach of thorium ionic species to the surface.

Table 2 contains fitting data of the experimental results for the Langmuir and Freundlich models. As can be seen for all investigated materials, the Langmuir isotherm shows a higher correlation factor, meaning that the sorption proceeds via the monolayer coverage described by the model.

There are few literature-works concerning thorium sorption on natural aluminosilicate sorbents under different conditions (e.g. pH, concentration, dosage) [3–6, 29, 30]. Even more limited is the number of works examining sorption on oxyhydroxide-modified zeolites or bentonites [31–33]. In most cases the authors have concluded that the sorption mechanism includes mainly the formation of

Table 2 *Parameters of the Langmuir and Freundlich models for thorium adsorption.*

	Langmuir			Freundlich		
Material	**K_L** ($L\ mg^{-1}$)	**q_{max}** ($mg\ g^{-1}$)	**R^2**	**K_F**	**1/n**	**R^2**
Zeolite	0.9355	20.88	*0.9948*	7.4940	0.2722	0.8052
FeZ	1.7846	14.90	*0.9983*	7.9526	0.1649	0.9466
FeZ-HCl	2.7845	7.74	*0.9911*	5.9787	0.0670	0.7765

Table 3 *Literature data for thorium sorption on natural and iron-modified aluminosilicates, as well as on iron oxyhydroxides.*

Material	q_{max} (mg g^{-1})	Concentration range (mg L^{-1})	pH	Dosage (g L^{-1})	Reference
Clinoptilolite	**19.89**	**5-100**	**3**	**1.0**	**This work**
ZSM-5	2.55	0-2.78	3.6	2.4	[3]
NKF-6	64.97	10-33	3	0.45	[4]
Activated bentonite	26.22	1.76-23.2	2.5	0.3	[5]
Na-clinoptilolite	175	154-3870	4	10.0	[29]
Phillipsite/chabazite tuff	21.9	5-40	3	1.0	[30]
Na-bentonite	41.24	1.2-235	6.2	2.5	[31]
Clinoptilolite/FeO(OH)s	**14.99**	**5-100**	**3**	**1.0**	**This work**
Clinoptilolite/FeO(OH)s/HCl	**7.54**	**5-100**	**3**	**1.0**	**This work**
Na-bentonite/Fe_3O_4	31.34	1.2-235	6.2	2.5	[31]
Ferrihydrite/Fe_3O_4	0.016/0.024	0.255	2.9	10	[32]
Fe_3O_4	4.64	0.25-29.5	2.6	0.6	[33]

surface complexes, especially when iron oxyhydroxides are present, while ion exchange can also take place. The results of the current study are compared to the literature works in Table 3.

Effect of contact time and temperature

The effect of contact time and temperature on thorium sorption on the natural zeolite is depicted in Fig. 7. Equilibrium conditions are achieved very fast, within the first 20 min of contact time, which is generally observed if ion exchange takes place [34]. By increasing the solution temperature, adsorption is strengthened and thus, the reaction is endothermic. In the literature, thorium sorption on aluminosilicate materials has mostly been found to be an endothermic phenomenon [3–6], attributed to the increased degree of hydrolysis and easier elimination of the hydration spheres at higher temperatures [33], but it has also been reported as exothermic [30, 31].

Kinetic results from the three different temperatures are best fitted to the pseudo-second order (PSO) model (Table 4), which signifies that the reaction-controlling step of adsorption may be of chemical nature. The activation energy calculated by the Arrhenius equation (Fig. 8a) is also indicative of a complex mechanism. Complex processes consist of separate reaction steps each having a certain activation energy. This way, the negative value of the overall experimentally observed activation energy can be explained if it is considered that an increase in temperature has a different effect on each of these individual steps. Negative activation energies of metal sorption of comparative magnitude have also been reported in other works [35, 36].

Finally, the van't Hoff diagram (Fig. 8b) was used to determine the thermodynamic parameters ΔG°, ΔH°, and ΔS° after calculating the distribution coefficients K_D at equilibrium (Table 5). As can be seen, the phenomenon is spontaneous (due to the negative values of the Gibbs free energy), is confirmed to be endothermic (due to the positive enthalpy), and leads to increased disorder in the solid/liquid interface (due to the positive entropy).

CONCLUSIONS

- A natural zeolite was successfully modified with hydrochloric acid and/or iron oxyhydroxides using a simple and fast precipitation by base method. The oxyhydroxides were identified as mostly amorphous ferrihydrite coexisting with poorly crystalline α-FeOOH.
- Consideration of FTIR, XRD, N_2-BET, and pHpzc revealed that after modification, the fundamental zeolite structure was preserved, but charge and surface characteristics were greatly affected.

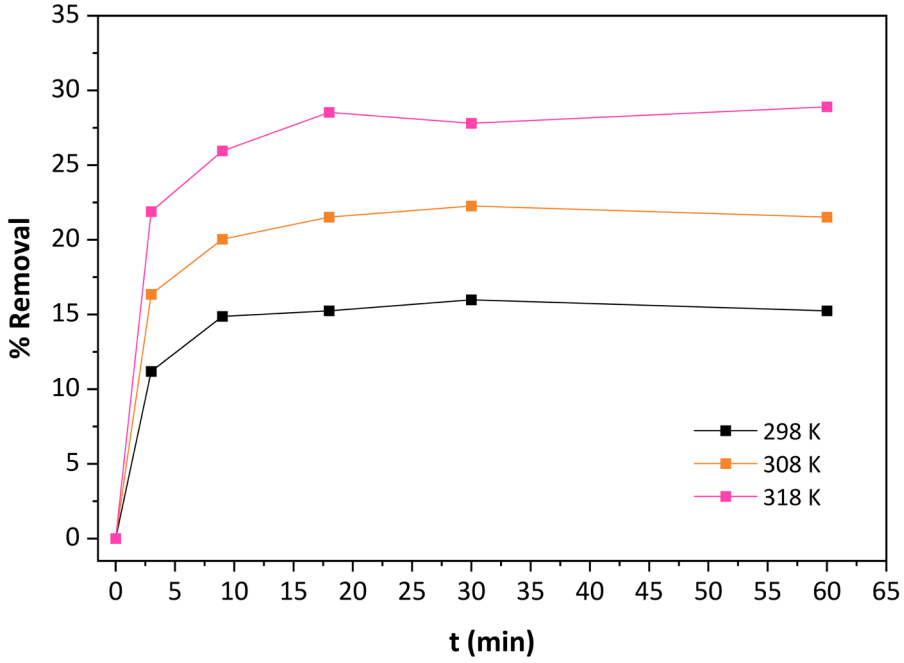

Fig. 7 *Effect of contact time and temperature on thorium sorption onto the natural zeolite (dosage: 1 g L^{-1}, C_0: 100 mg L^{-1})*

Table 4 *Kinetic parameters for thorium sorption at different temperatures and activation energy as calculated by modeling of experimental data.*

T (K)	Pseudo-First Order			Pseudo-Second Order			E_a (kJ mol^{-1})
	k_1 (min^{-1})	q_e (mg g^{-1})	R^2	k_2 (g mg^{-1} min^{-1})	q_e (mg g^{-1})	R^2	
298	0.4258	15.43	0.9707	0.1613	15.51	*0.9991*	−62.99
308	0.4657	21.46	0.9164	0.0819	22.27	*0.9992*	
318	0.4969	27.93	0.8744	0.0325	29.33	*0.9997*	

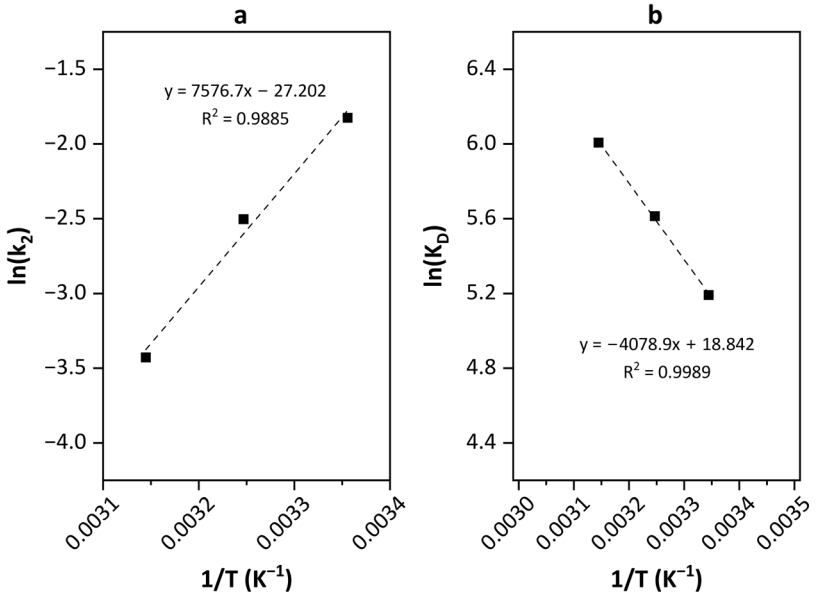

Fig. 8 *Arrhenius plot (a) and van't Hoff diagram (b) for the adsorption of thorium on zeolite*

Table 5 *Distribution coefficients and thermodynamic parameters of thorium sorption on zeolite.*

T	K_D	ΔG°	ΔH°	ΔS°
(K)	(mL g^{-1})	(kJ mol^{-1})	(kJ mol^{-1})	(kJ mol^{-1} K^{-1})
298	179.9	–12.9	33.91	0.157
308	274.2	–14.4		
318	406.5	–15.9		

- Thorium sorption was lower on the modified zeolites possibly due to greater electrostatic repulsion and limited access of Th(IV) ions to the active binding sites.
- Isotherm fitting data showed better correlation with the Langmuir theoretical model, indicating monolayer coverage.
- Evaluation of kinetic and thermodynamic parameters revealed a complex phenomenon, fitted to the pseudo-second order reaction model with a spontaneous, endothermic character.

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