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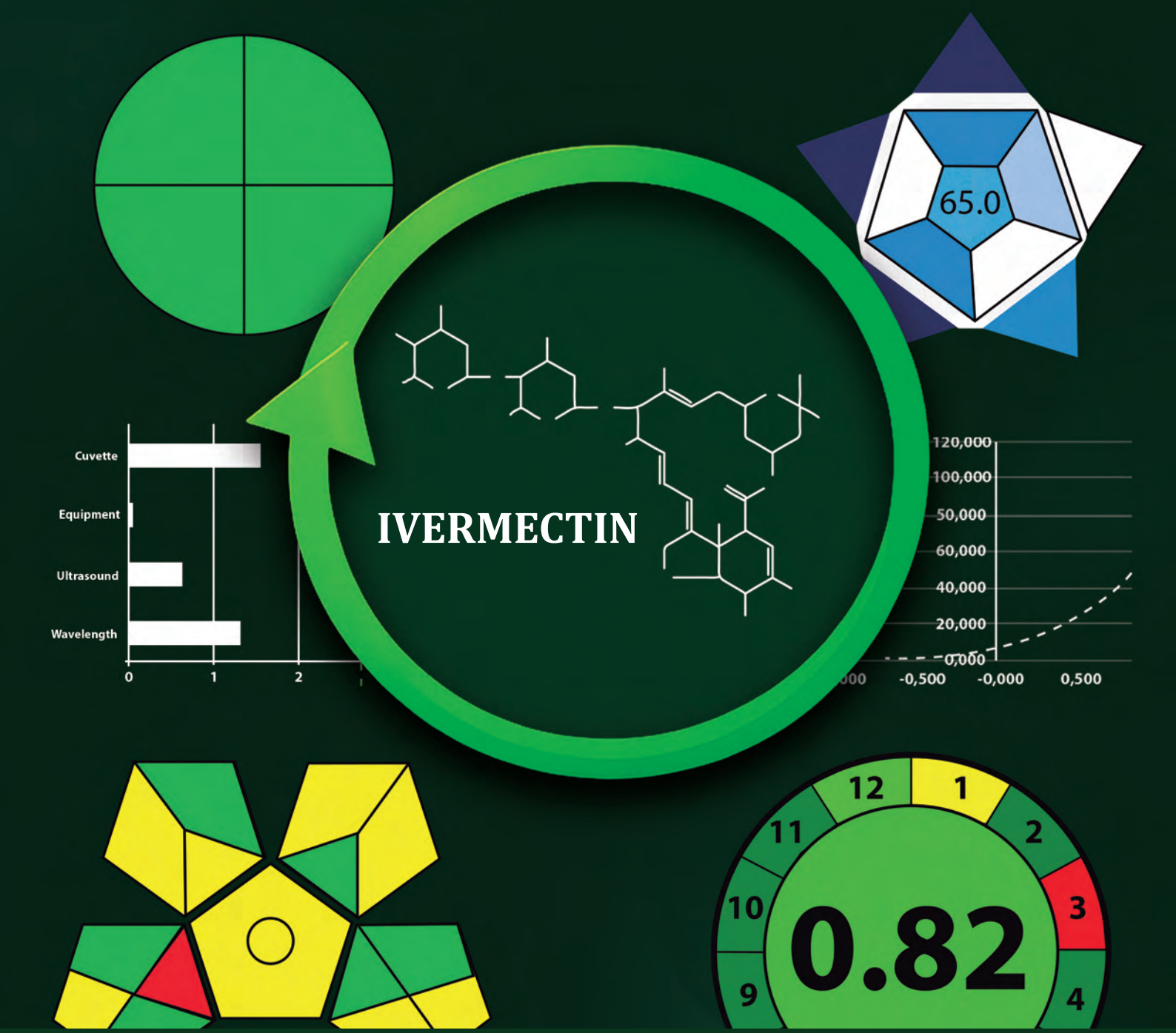

A Green and Lean Method Certified by NEMI, ESA, AGREE, GAPI and BAGI for the Analysis of Ivermectin in Injection Solution for Veterinary Use

Natália Sabina dos Santos Galvão, Ana Carolina Kogawa

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Aims & Scope

BrJAC is a double-blind peer-reviewed research journal, dedicated to the diffusion of significant and original knowledge in all branches of Analytical Chemistry and Bioanalytical Chemistry. It is addressed to professionals involved in science, technology, and innovation projects at universities, research centers and in industry. The **BrJAC welcomes** the submission of research papers reporting studies devoted to new and significant analytical methodologies, putting in evidence the scientific novelty, impact of the research, and demonstrated analytical or bioanalytical applicability. BrJAC **strongly discourages** those simple applications of routine analytical methodologies, or the extension of these methods to new sample matrices, unless the proposal contains substantial novelty and unpublished data, clearly demonstrating advantages over existing ones.

BrJAC is a quarterly journal that publishes original, unpublished scientific articles, reviews and technical notes. In addition, it publishes interviews, points of view, letters, sponsor reports, and features related to analytical chemistry.

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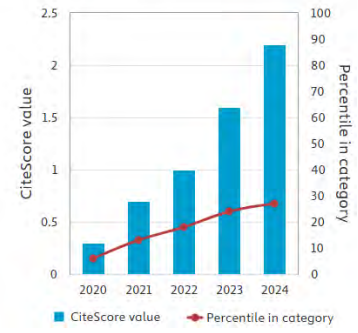

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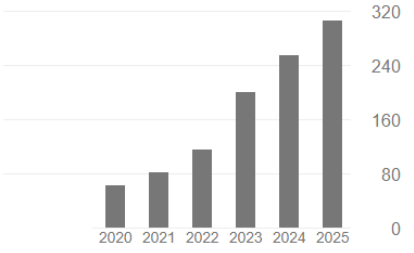

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Brazilian Journal of Analytical Chemistry
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EDITORIAL

Viktor G. Mihucz

Professor at the Institute of Chemistry, ELTE Eötvös Loránd University, Budapest, Hungary

It is with great pleasure that I introduce the 50th issue of the *Brazilian Journal of Analytical Chemistry (BrJAC)*, which once again reflects the vitality, diversity, and innovation of our community. The contributions gathered here span fundamental reflections, methodological advances, and applications that resonate with both academic research and industrial practice.

We begin with an interview with Professor **José Alberto Fracassi da Silva**, whose career and insights exemplify the dedication and creativity that continue to shape analytical chemistry in Brazil and beyond.

The **Point of View** article by **Josué Carinhanha Caldas Santos** addresses the controversial topic of thimerosal, an organic mercury compound historically used as a vaccine preservative.

The Letter by **Wendel Andrade Alves** highlights the promise of polymeric microneedles as analytical interfaces for biosensing and controlled drug release. This concise yet forward-looking piece underscores how analytical chemistry intersects with biomedical innovation, pointing toward exciting future challenges.

Our Review article, authored by **Amanda Mohr and colleagues**, provides a comprehensive overview of the greenness metrics used to evaluate analytical methods. In an era where sustainability is no longer optional but essential, this synthesis offers valuable guidance for researchers and practitioners committed to greener laboratories and more responsible science.

Several original contributions further demonstrate the innovative spirit of our community: updates to quantitative models in validation and routine comparative chemical methods; optimization and validation of ultrasound-assisted extraction for phosphorus analysis in cane syrup; a green and lean method for ivermectin analysis, certified by multiple sustainability metrics; an advanced microwave-assisted digestion method for rare earth element analysis in environmental matrices; and a comparative study of calcination and thermogravimetry techniques for quantifying carbon black in polymeric resins.

Together, these contributions illustrate the breadth of analytical chemistry today: from sustainability metrics to biomedical interfaces, from food chemistry to environmental monitoring, and from methodological refinement to industrial applications. They remind us that analytical chemistry is not only a technical discipline but also a driver of societal progress, sustainability, and innovation.

On behalf of the editorial team, I thank all authors, reviewers, and readers for their commitment to advancing our field. May this issue inspire new ideas, collaborations, and applications that continue to strengthen the role of analytical chemistry in addressing global challenges.

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Viktor G. Mihucz is Professor at the Institute of Chemistry, ELTE Eötvös Loránd University, Budapest, Hungary. His research group specializes in analytical chemistry, with particular emphasis on inorganic trace analysis. Current lines of research include: (i) development and application of ICP methods for elemental analysis, (ii) indoor air quality monitoring with a focus on trace element determination, and (iii) food chemistry studies related to food safety and plant-based diets. His work contributes both to fundamental understanding and to practical applications of analytical chemistry.

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INTERVIEW

Professor José Alberto Fracassi da Silva kindly granted an interview to BrJAC

Professor José Alberto Fracassi da Silva holds a degree in Chemistry from the University of São Paulo (1995), a PhD in Chemistry (Analytical Chemistry) from the University of São Paulo (2001) and completed postdoctoral degree at the Laboratory of Integrable Systems of the Polytechnic School of the University of São Paulo (2003), as well as the Ralph N. Adams Institute for Bioanalytical Chemistry at the University of Kansas, USA (2011).
He has been a professor at the Institute of Chemistry at the State University of Campinas since 2004 and was promoted to Associate Professor in 2019.
His research experience lies in the field of chemistry, with an emphasis on analytical instrumentation. He primarily works on topics such as capillary electrophoresis, electrochemical and fluorescence detection, and microanalysis systems (lab-on-a-chip).

BrJAC: How was your childhood?
Prof. Fracassi: I think that my childhood was fairly normal. My generation was still able to play freely in the streets, and we did not yet have electronic games, so we needed to invent our own toys, which I believe was very beneficial for developing skills. From an early age, I have been curious, and I remember doing simple experiments such as fragrance extraction from flowers, disassembling electronic devices (radios were my favorite), and building fun stuff (e.g., musical instruments—a passion that has stayed with me to this day). Perhaps I was born a scientist, who knows?

I remember getting up early on Saturday mornings to watch the TV series “Cosmos” with Carl Sagan, and by the age of eight, I asked my father to buy me a copy of the book, which I still have.

BrJAC: What early influences encouraged you to study chemistry? Did you have any influencers, such as a teacher?
Prof. Fracassi: I can say that I studied Chemistry by chance. I had a technical degree in Electronics, and when I finished I wanted to have a degree in Mathematics or Physics. But when I was preparing for admission exams at the university, I found that there were many more professional opportunities for Chemists. One day that year I visited the Institute of Chemistry at the University of São Paulo, and realized that Chemistry had many common points with Mathematics and Physics. In particular, I was very impressed by the Raman Spectroscopy laboratory led by Professor Oswaldo Sala. After that day, there were no more doubts about the next steps.

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BrJAC: How was the beginning of your career in chemistry?
Prof. Fracassi: To be honest, I did not expect to build an academic career. When I got my bachelor's degree in Chemistry, I applied for a position at an instrumentation company (Mr. Luiz Bravo may not remember, but I had a job interview with him). But I was also getting interesting results from a research project at the university with Professor Claudimir Lucio do Lago, and one day he said to me "Alberto, this could be used in your doctorate". The research was about the development of a non-contact conductivity detector for use in capillary electrophoresis. So, I decided to take this opportunity and started my graduate studies in 1996.

BrJAC: What has changed in your profile, ambitions, and performance since the time you started your career?
Prof. Fracassi: There is a big shift when you move from 'student' to 'researcher/professor' positions. The researching and teaching role requires the ability to perform many tasks simultaneously. Managing multiple projects and securing funding to support them is challenging. At the same time, you have to teach courses and participate in administrative tasks.

As the career progresses, it is natural to become involved in activities with greater responsibility, larger projects, and broader collaborative networks. I am currently serving as head of the Analytical Chemistry Department at the Institute of Chemistry at UNICAMP, and taking on this role was a significant challenge, since you have to deal with many different interests—both personal and institutional.

Throughout the career journey, we learn how to improve performance. My ambition is to deliver the best I can, day after day.

Prof. Fracassi (left) and Prof. Ivano Gutz (right) at the 29th Annual Meeting of the Brazilian Chemical Society in 2006.

BrJAC: Could you comment briefly on the recent evolution of analytical chemistry, considering your contributions?
Prof. Fracassi: The instrumentation for analytical methodologies has evolved to remarkable levels. Today, it's relatively easy (and inexpensive) to integrate microcontrollers and compact sensors. I thank my former advisor, Professor Claudimir Lucio do Lago, for sharing with me the project of the capacitively coupled contactless conductivity detector (C^4D) – today, one can find commercial products with this type of detection for capillary and microchip formats.

In another direction, protocols involving lab-on-a-chip are well accepted today, and important advances toward organ-on-a-chip, or even body-on-a-chip, have been noted. Our group has contributed to developing

alternative methods for the production of such systems. The use of 3D printing has significantly accelerated the prototyping of microsystems. Our group has contributed to pushing these technologies to the limit to produce fully functional microfluidic devices.

BrJAC: What are your lines of research? You have published many scientific papers. Would you highlight any?
Prof. Fracassi: I have been focusing on the development of methods and instrumentation for capillary electrophoresis and lab-on-a-chip microfluidic platforms. More specifically, we have developed strategies for microfabricating devices and sensors for electrochemical and fluorescence detection, as well as creating novel materials for sensing and device integration.

We have also been interested in bioanalytical applications, with a focus on detecting reactive oxygen and nitrogen species and peptides. More recently, we have adopted additive manufacturing (3D printing) to directly fabricate complex microfluidic systems.

In this regard, I would like to highlight a recent paper published in Lab Chip, one of the most important journals in the field, which describes a very straightforward procedure to enable conventional 3D printers to produce multi-material devices (10.1039/d3lc00356f).

The ability to combine materials during device fabrication is of great interest, as it enables the integration of multiple functions into a single platform.

BrJAC: What is your opinion about the current progress of chemistry research in Brazil? What are the recent advances and challenges in scientific research in Brazil?
Prof. Fracassi: I'm very glad to say that the Analytical Chemistry community in Brazil is very active. I don't think it's an exaggeration to say that each specific area within Analytical Chemistry has Brazilians who are among the leaders in the field. Therefore, I see that scientific research in Analytical Chemistry in Brazil has reached a level of maturity.

Perhaps, the focus for the future would be increasing the impact of the research, which can be achieved by establishing of collaborative networks and increasing investments in scientific research in Brazil.

I can highlight the successful initiative of the Federal Government regarding the implementation of the National Institutes on Science and Technology (INCTs). I hope for more initiatives of this type.

From left to right: Prof. Sue Lunte, Prof. Christian Amatore and Prof. Fracassi at Pittcon 2015.

BrJAC: For you, what have been the most important recent achievements in analytical chemistry research? What are the landmarks?
Prof. Fracassi: This is a difficult question for me because I have the bias of focusing on my actual field. However, if I had to choose one, I would pick the advances in mass spectrometry. I am always impressed with the improvements in spectroscopic resolution, and also the increased sensitivity. Mass spectrometry has taken a leading role among analytical methodologies.

BrJAC: There are, in Brazil and in the world, several conferences on chemistry. To you, how important are these meetings to the chemistry scientific community? How do you see the development of national chemistry meetings in Brazil?
Prof. Fracassi: For me, meetings are important channels for the exchange of experiences in research and the establishment of collaborations, which can significantly accelerate scientific development. Also, meetings offer the opportunity to follow the most recent advances in science.

In particular, for young investigators and students, conferences are very important for strengthening research links (many funding agencies use international engagement as a criterion when evaluating proposals). Personally, I find smaller meetings more productive than large broad conferences.

Prof. Fracassi at Pittcon 2015.

BrJAC: What is the importance of awards for the development of science and new technologies?
Prof. Fracassi: Personally, I do not put too much emphasis on awards and prizes. In general, I view awards as a form of community recognition for an entire body of work, typically granted to individuals at the level of senior researcher. In my humble opinion, the creation of too many awards tends to dilute their overall significance.

BrJAC: For you, what is the importance of the national funding agencies for the scientific development of Brazil?
Prof. Fracassi: Funding agencies are of utmost importance for scientific and technological development. There is a strong link between fundamental research and the high-quality training of human resources. However, I do recognize that other sources, such as partnerships with companies, can complement the budget needed to support the research activities.

BrJAC: At the moment, the situation for scientific research in Brazil is one of decreasing investment. How do you see this situation, and what would you say to young researchers?
Prof. Fracassi: I have to state that conducting research in Brazil is not easy. I am lucky that my research interests do not rely on the acquisition of prohibitively expensive equipment. As a result, my group is able to carry out most of our research using accessible instrumentation.

Conversely, characterization techniques typically involve large-scale equipment, which is often shared within institutions (e.g., scanning electron microscopes). Therefore, it is advisable to participate in collaborative research networks and to establish centralized facilities that consolidate resources and serve the widest range of users.

Another major and growing concern relates to scholarships. There is little value in maintaining a research infrastructure if you do not have people to utilize it. I often say that Brazil needs a long-term development plan of 30, 40, or 50 years, that is not linked to the executive government of the day. Science and technology are strategic assets for the well-being of the nation and cannot be left out of the discussion.

BrJAC: What advice would you give to a young scientist who wants to pursue a career in chemistry?
Prof. Fracassi: Each person is unique, and so is their life path. However, I think that one thing is common: try to follow a career that excites you, which you enjoy at the present moment. If someone is happy in their work, success will come.

Of course, continual learning is also required to achieve excellence in any field, so never stop following the latest advances in the field.

BrJAC: For what would you like to be remembered?
Prof. Fracassi: I would like to be remembered as a person who added positive things wherever I went, and who treated others with respect and kindness.

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POINT OF VIEW

Thimerosal, an organic mercury compound used as a vaccine preservative: A real necessity or regulatory inertia?

Contributions from analytical chemistry

Josué Carinhanha Caldas Santos[1,2]

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Before beginning this text, one point must be made very clear. Vaccination is essential for preventing serious diseases and avoiding outbreaks that threaten public health. Vaccines protect individuals and the community through herd immunity, reducing mortality, hospital costs, and permanent sequelae associated with infections. Vaccination is an act of caring for yourself and others.

Thimerosal (TM) is a mercury-containing organic compound widely used as a preservative in various biological and pharmaceutical products, including many vaccines, to prevent the growth of harmful microbes inadvertently introduced into the vaccine during its use. The documented antimicrobial properties of TM contribute to the safe use of vaccines in multi-dose vials, which are less expensive, easier to store, and help reduce waste. TM, which is approximately 50% mercury by weight, has been one of the most widely used preservatives in vaccines. It is metabolized or degraded to ethylmercury (EtHg) and thiosalicylate. In general, a vaccine containing 0.01% (m/v) TM as a preservative contains 50 µg of TM per 0.5 mL dose, corresponding to approximately 25 µg of mercury per 0.5 mL dose.[1] The use of TM as a preservative in multi-dose vaccines is controversial because this compound has been abolished in the United States and the European Union, either due to its replacement with other preservatives (free mercury) or the adoption of single-dose formulations. In Brazil, it is somewhat surprising that of the use of TM in cosmetics (topical use) has been suspended,[2] partly related to allergic contact dermatitis, but its use in vaccines is still permitted. The World Health Organization (WHO) supports this decision, stating that “ethylmercury is present in thiomersal as a preservative in some vaccines and does not pose a health risk.”[3] However, a growing body of scientific evidence has increasingly challenged this assertion.

Experimental models for assessing the toxicity of a given species are particularly decisive. Regardless of the model (simple or complex) tested with TM, evidence of this compound’s toxicity consistently emerges to varying degrees (Figure 1). TM has demonstrated the ability to form adducts with cysteine, glutathione, and especially with carrier proteins, binding to free thiol groups and thereby being transported throughout the body, reaching other proteins, enzymes, and organs.[4] In this sense, the effect of TM on proteins has been associated with its ability to induce protein fibrillation,[4,5] impair hemoglobin’s capacity to bind oxygen, and increase protein glycation.[6] The use of electrospray ionization–mass spectrometry (ESI-MS) has confirmed TM’s high affinity for proteins containing free thiol groups, leading to metalation and the formation of stable adducts with cytochrome *c*, ribonuclease A, carbonic anhydrase I, and superoxide dismutase, thereby

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compromising the natural activity of these enzymes.[7] When used as a cellular model, erythrocytes exposed to TM show alterations in essential functions, particularly in oxygen transport capacity, along with changes in cellular morphology.[8] Across different cellular models, TM has consistently demonstrated toxicity, indicating that the doses used to achieve antimicrobial activity cannot be considered safe.[9]

Different animal models (flies, fish, and rodents) have consistently demonstrated that TM is a toxic compound, even at sublethal doses.[10–12] In mouse models, TM compromises vaccine potency through thiol modification, affecting the antigenicity and immunogenicity of the formulation by reducing the binding activity between antigens and antibodies.[13] In contrast, a Wistar rat model mimicking TM exposure in infants following childhood vaccination revealed significant damage to bioenergetic pathways within the nervous system, particularly the brain.[14] Moreover, in baby monkeys exposed to TM-containing vaccines, researchers found that the fraction of inorganic mercury in the brain ranges from 21% to 86% of total mercury measured, with an average of ≈ 70%.[15]

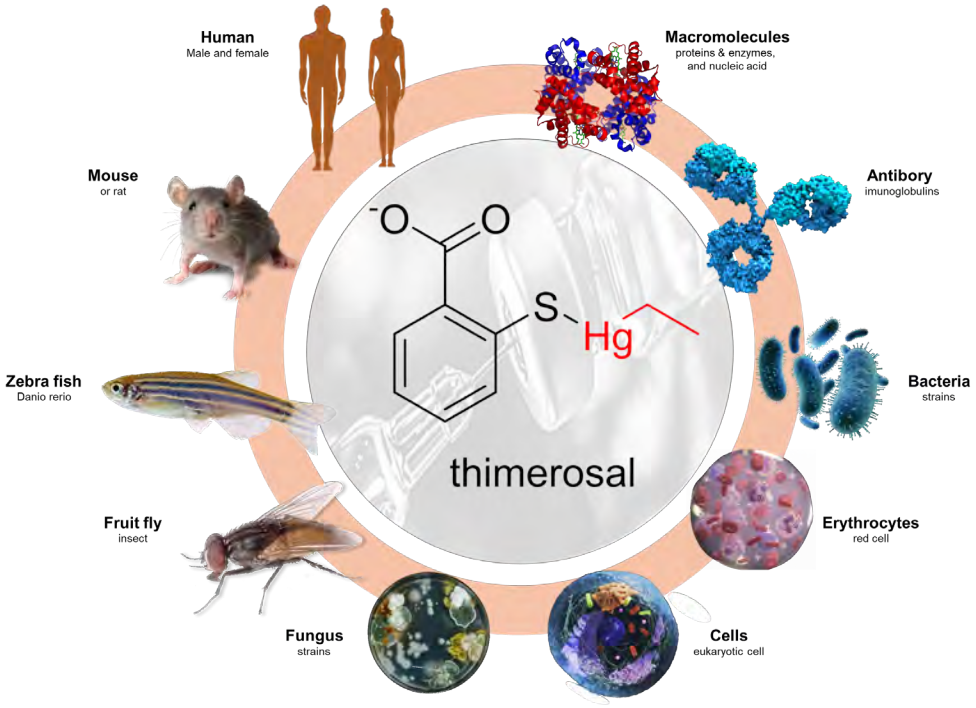

Figure 1. The chemical structure of thimerosal and examples of different experimental models for toxicity assessment.

In vitro studies comparing EtHg with methyl mercury (MeHg) have shown similar outcomes in cardiovascular, neural, and immune cells. However, under *in vivo* conditions, evidence indicates distinct toxicokinetic profiles between MeHg and EtHg, with the latter exhibiting a shorter blood half-life, different compartment distribution, and faster elimination. EtHg's toxicity profile, therefore, differs markedly from that of MeHg, leading to distinct patterns of exposure and associated toxicity risks.[16] From another perspective, studies on the environmental fate and risk of mercury have mostly focused on total mercury and the toxic species MeHg. However, EtHg has long been overlooked, partly due to analytical limitations. The occurrence of EtHg and its possible natural sources in the environment provide essential background information and valuable clues for understanding its natural presence and environmental behavior.[17] Thus, the distribution and toxicological aspects of EtHg are not solely associated with TM use; they are also related to other environmental and chemical pathways. Therefore, expanding research on TM and EtHg is a strategic priority to achieve a more comprehensive understanding of their biological effects and associated impacts.[18]

In this context, the continued use of TM in some vaccines reflects less an unavoidable scientific necessity and more a set of regulatory, logistical, and economic barriers. The proven stability of these formulations, the low rate of serious adverse events, and the reduced cost of multidose vials create a scenario in which regulatory agencies are reluctant to require reformulations that would necessitate new stability, safety, and immunogenicity studies. From an industry perspective, the lack of economic incentives to modify products intended primarily for low-return markets reinforces institutional inertia, even in the face of technically feasible alternatives consistent with global efforts to reduce mercury use.

In this context, analytical chemistry plays a crucial role in providing evidence that extends beyond traditional safety indicators. Sensitive chemical speciation methods, for example, enable the distinction between EtHg, MeHg, and their inorganic forms, thereby revealing metabolic pathways that in the past could not be assessed with conventional toxicological approaches. These advances enable the characterization not only of the kinetics of systemic elimination, but also of the formation and accumulation of inorganic species in target tissues, providing a more accurate basis for reassessing risks in vulnerable subpopulations. In addition, microbiological monitoring techniques and chemical stability analyses provide robust data to validate formulations without TM or with alternative preservatives, demonstrating that microbiological safety can be preserved through optimized packaging systems or the adoption of single-dose presentations.

Based on this evidence, a central conclusion can be drawn: The maintenance of TM today is more a consequence of a regulatory and productive framework that is insufficiently dynamic than it is a result of real scientific limitations. The data generated by analytical chemistry, speciation studies, kinetic analyses, stability evaluations, and post-use surveillance not only allow for a more detailed characterization of the toxicological profile of EtHg, but also provide technical support for transitioning to safer and scientifically sound alternatives. Thus, analytical advances cease to function merely as evaluative tools and become true catalysts for change, providing the scientific basis required for regulators and manufacturers to adopt policies and formulations that progressively reduce dependence on mercury compounds in vaccines.

Finally, it is crucial to emphasize that, regardless of whether TM is present, vaccination remains essential. For adolescents and adults, TM-associated risks are typically minimal; however, for infants and newborns, existing uncertainties deserve more careful consideration. Nevertheless, the choice between a TM-containing vaccine and no vaccination at all is unequivocal: Vaccination unquestionably remains the safer and more responsible option.

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LETTER

Polymeric Microneedles as Analytical Interfaces for Biosensing and Controlled Drug Release: Achievements and Future Challenges

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Microneedle technology has emerged as one of the most dynamic and interdisciplinary frontiers in biomedical science, enabling a unique convergence between analytical chemistry, materials engineering, and clinical diagnostics.[1] Originally developed to overcome the mechanical and diffusional barriers imposed by the stratum corneum, microneedles (MNs) allow precise and minimally invasive access to the epidermal and dermal layers of the skin. This access enables controlled transport of drugs, biomolecules, or diagnostic reagents while avoiding the pain, fear, and infection risks associated with conventional hypodermic needles.[2]

Over the past decade, advances in polymer chemistry, microfabrication, and electrochemical detection have transformed MNs from passive drug delivery tools into multifunctional analytical interfaces capable of both monitoring and treating physiological conditions in real time.

Among the different materials explored, polymeric microneedles have demonstrated remarkable versatility, owing to their biocompatibility, chemical tunability, and mechanical resilience. Natural and synthetic polymers, such as polylactic acid (PLA), polycaprolactone (PCL), hyaluronic acid (HA), and conductive polymers like polypyrrole (PPy) and poly(3,4-ethylenedioxythiophene):polystyrene sulfonate (PEDOT:PSS), have enabled the design of devices that combine flexibility, biodegradability, and electrical conductivity within the same platform.[1]

These features have not only enhanced safety and patient compliance but also expanded the range of applications, particularly in biosensing and controlled drug release.[3,4] Recent approaches have integrated electrochemical transducers and responsive materials into microneedle arrays, enabling continuous monitoring of metabolites or biomarkers in interstitial fluid (ISF) while simultaneously allowing on-demand release of therapeutic agents.[5] In this context, polymeric microneedles have evolved into active analytical–therapeutic systems that function as both sensors and actuators within a single miniaturized interface.

The analytical potential of these devices lies in their ability to access ISF, a complex biological matrix that mirrors systemic biochemical changes. Electrochemical detection of glucose, lactate, uric acid, or inflammatory cytokines using polymeric microneedle electrodes exemplifies a new generation of wearable sensors capable of continuous, non-invasive operation.[6]

The integration of nanostructured conductive polymers and metal nanoparticles has enabled high electroactive surface areas, improved charge-transfer kinetics, and selective immobilization of biorecognition elements, such as enzymes, antibodies, or aptamers.[1,7,8] These characteristics are essential for achieving the sensitivity, reproducibility, and temporal resolution required for real-time clinical analysis.

Although substantial progress has been made, ensuring long-term reliability remains a major challenge. Stable electrical performance in hydrated environments, resistance to biofouling, and mechanical robustness under repeated use are essential prerequisites for successful clinical translation.

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From a therapeutic perspective, polymeric microneedles have demonstrated significant potential as platforms for controlled, localized drug delivery. The intrinsic versatility of polymeric matrices allows modulation of degradation, permeability, and responsiveness, enabling precise control over release kinetics.[9,10]

When combined with electroactive or stimuli-responsive polymers, these systems can respond dynamically to environmental or external factors such as pH, temperature, electric potential, or mechanical pressure. This behavior enables tailoring the temporal and spatial profiles of drug administration, ensuring efficient and patient-friendly delivery.

Additionally, the inherent biocompatibility and degradability of polymers minimizes residual waste and eliminates the need for device removal, an important advantage for chronic or long-term therapeutic applications.

The multifunctional nature of these materials also facilitates the coupling of sensing and release mechanisms within a single structure, laying the foundation for self-regulated systems.[4] In these configurations, microneedles can operate as closed-loop devices, where local biochemical variations detected at the skin interface can trigger or modulate the release of therapeutic agents. This integration of analytical and therapeutic functionalities highlights the transformative role of polymeric microneedles in advancing personalized medicine and point-of-care technologies.

Despite this progress, translating polymeric microneedles into clinical practice requires overcoming critical technological and analytical limitations.[1] Optimizing geometry, tip sharpness, and mechanical robustness is essential to ensure consistent skin penetration without fracture or deformation. Additionally, the surface chemistry of polymer surfaces must be tailored to enable covalent or electrostatic immobilization of biomolecules while preventing nonspecific adsorption that can impair analytical signals.

Advanced fabrication techniques, including micromolding, laser micromachining, and high-resolution 3D printing, have expanded design possibilities, enabling precise control over microneedle dimensions, porosity, and functional gradients. However, large-scale reproducibility and regulatory validation remain major barriers for commercialization. From an analytical standpoint, coupling microneedle sensors with portable potentiostats, flexible electronics, and wireless data transmission modules is essential for transforming laboratory prototypes into reliable wearable devices.

The future of polymeric microneedles lies in the development of integrated analytical and therapeutic platforms that can operate autonomously and safely for prolonged periods. The convergence of conductive polymers, nanocomposites, and biocompatible hydrogels with emerging technologies, such as microfluidics, data analytics, and artificial intelligence, will enable real-time interpretation of biochemical signals and dynamic therapeutic adjustments.

In parallel, the analytical chemistry community plays a central role in ensuring metrological traceability, calibration accuracy, and long-term stability of microneedle-based biosensors, thereby consolidating their reliability in clinical diagnostics.

Ultimately, polymeric microneedles represent a paradigm shift in analytical science applied to health. They embody a fusion of analytical precision, material innovation, and biomedical functionality, transforming the skin into an accessible, information-rich analytical interface. By bridging biosensing and controlled drug release, these systems hold the promise of revolutionizing point-of-care testing, personalized medicine, and minimally invasive therapies. As new challenges emerge—from scalable fabrication to regulatory compliance—the integration of analytical chemistry principles will remain fundamental to unlocking the full potential of this technology, ensuring that microneedles continue to evolve as versatile tools at the intersection of diagnosis, monitoring, and therapy.

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REVIEW

Overview of the Greenness' Metrics used to Evaluate Analytical Methods

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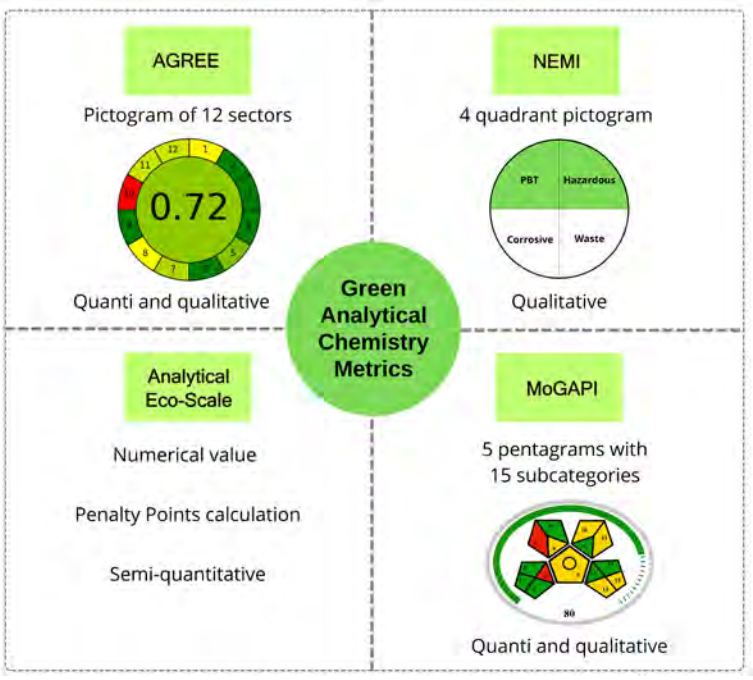

The main goal of Green Analytical Chemistry (GAC) is to reduce the use of hazardous chemicals and waste generation in analytical procedures without compromising method performance. Over the years, several metrics tools were introduced to measure the environmental impact and greenness of analytical procedures. In this context, this paper aims to present an overview of the most used GAC metrics in analytical chemistry, highlighting their criteria, advantages, disadvantages, and comparing their applicability. After extensive research, the metrics selected to be addressed were: National Environmental Method Index (NEMI), Analytical Eco-scale, Modified Green Analytical Procedure Index (MoGAPI), and Analytical GREEnness Metric (AGREE). NEMI is one of the oldest GAC metrics, describing the greenness of the method by a simple pictogram. Analytical Eco-Scale is based on subtracting penalty points from a total score of 100 points. MoGAPI uses a pictogram made up of fifteen categories and a total score to display the greenness of the analytical procedure. AGREE is represented as a circular pictogram divided into 12 parts, where each part corresponds to a principle of GAC. Each discussed metric has its own advantages and disadvantages; however, AGREE stands out as the most widely used and comprehensive GAC metric, applicable to several techniques. Although time-consuming, ideally, the best approach is to apply all metrics in combination to gain as much information as possible.

Keywords: GAC metrics, NEMI, AGREE, MoGAPI, Analytical Eco-Scale

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INTRODUCTION

Over the last few decades, great interest has been raised about the impact of chemicals on the ecosystem.[1] The concept of Green Chemistry emerged in 1990 as the use of chemistry techniques and methodologies that reduce or eliminate the use or generation of hazardous substances.[2] Later, in 1999, following this idea, the term Green Analytical Chemistry (GAC) was proposed, and since then, it has been increasingly applied to minimize health and environmental impact.[3] In 2013, the Twelve Principles of GAC were proposed, with the main goal of reducing the use of hazardous chemicals and waste generation in analytical procedures without compromising method performance.[4-8]

In the field of the chemistry industry, several routine analyses are conducted, from production to quality control of the final product, leading to large amounts of waste generation. Different analytical techniques are employed daily, such as chromatography, spectroscopy, mass spectrometry, and electrochemical analysis, which vary in terms of hazardous chemicals use, chemical consumption, energy consumption, and waste generation.

Therefore, there has been concern about the environmental impact of these analyses and the use of green chemistry. To measure this impact and identify points for improvement in analytical methods, specific metrics have been developed. In this way, besides applying the concepts and principles of GAC, these appropriate evaluation tools are important to conclude whether the analytical procedure can be considered green and its degree of greenness. Over the years, several metrics tools were introduced to measure the greenness of analytical procedures.[9,10] Some of these metrics are: National Environmental Method Index (NEMI),[6] Analytical Method Volume Intensity (AMVI),[11] Analytical Eco-Scale,[12] HPLC-EAT (Environmental Assessment Tool),[13] Green Analytical Procedure Index (GAPI),[14] modified GAPI (MoGAPI),[15] Analytical Method GREEnness Score (AMGS),[16] Analytical GREEnness Metric (AGREE),[17] ChlorTox Scale,[18] and Blue Applicability Grade Index (BAGI).[19]

All the aforementioned metrics combine a score or a coloring pictogram result relating to the degree of greenness of the analytical procedure. Therefore, they can differ in their criteria, content, qualitative or quantitative approach, applicability on sample preparation, and specificity to certain instrumentation.[20,21] Some of them are not widely applied because they focus on particular evaluation parameters, such as the calculation of waste generation (e.g., AMVI), are specific to certain techniques (e.g., HPLC-EAT and AMGS), or are considered complex to use (e.g., ChlorTox Scale). In addition, authors tend to apply the most known metrics to their methods, as they are more established than other metrics and cover more analytical procedures.

In this context, this paper aims to present an overview of the most used GAC metrics in the analytical chemistry field, highlighting their criteria, advantages, disadvantages, and comparing their applicability. For that, extensive research was done about the studies published in the GAC metrics thematic, selecting review and research articles in different databases. As inclusion criteria, it was considered the most widely and generally used metrics once they can be applicable to the majority of analytical procedures. They were NEMI, Analytical Eco-Scale, MoGAPI, and AGREE. Thus, metrics that were less used or more specific were not addressed in this review. Additionally, a case study was conducted applying the four metrics addressed in this paper in an analytical method to compare and discuss the results obtained.

National Environmental Method Index – NEMI

One of the oldest GAC metrics is NEMI, where the greenness of the method is described by a pictogram divided into a four-quadrant circle. The quadrant will be considered and colored green if: (I) none of the reagents are defined as persistent, bioaccumulative, and toxic (PBT) by the Environment Protection Agency's Toxic Release Inventory (EPA-TRI); (II) none of the reagents are considered a hazardous waste by the EPA-TRI (according to the D, F, P, or U lists); (III) the pH of the sample lies in the range of 2 - 12; (IV) the generated waste is less than 50g. Otherwise, if one of these items is not met, the quadrant remains white. Thus representing only a general qualitative tool.[6,9,14,20,22]

The main advantage of NEMI is their simple and easily read representation. Despite their simplicity, no software is available for inputting the data; therefore, it requires a manual process to obtain the pictogram figure. Another disadvantage is the time-consuming process of searching for every compound in the EPA-TRI lists.[10,21,23,24] A while later, a modified-NEMI was proposed, which included a color scale and more assessment details, becoming a semi-quantitative approach.[1] Although the improvements in the modified-NEMI, few studies have reported their application.[25,26] Nowadays, NEMI is usually applied along with other quantitative metrics.[27-31]

Analytical Eco-Scale

Analytical Eco-Scale is a GAC metric based on subtracting penalty points (PPs) from the total score of ideal green analysis of 100 points. The final score allows us to classify the method as excellent (> 75 points), acceptable (75 – 50 points), and non-green (< 50 points). The higher the score, the more environmentally friendly the analytical procedure is.[5,10,12,21,24]

The assignment of the PPs takes into account the hazard and amount of chemicals used, energy consumed by instruments, waste generation, and occupational hazard, as shown in Table I. The metric has no software for calculation, involving a manual process and providing a semi-quantitative result.[1,23,32]

Table I. Analytical Eco-Scale PPs calculation

Parameters	Criteria	PPs*
Hazard	None	0
	Warning	1
	Danger	2
Amount of chemical	< 10 mL (g)	1
	10-100 mL (g)	2
	> 100 mL (g)	3
Energy consumption	≤ 0.1 kWh per sample	0
	0.1 - 1.5 kWh per sample	1
	> 1.5 kWh per sample	2
Waste generation	None	0
	< 1 mL (g)	1
	1 - 10 mL (g)	3
	> 10 mL (g)	5
	Generated waste has a recycling process	0
	Generated waste has a degradation process	1
	Generated waste has a passivation process	2
	Generated waste has no treatment	3
Occupational hazard	Procedure does not release vapors into the environment	0
	Procedure releases vapors into the environment	3

*PPs: Penalty Points

The main advantages of Analytical Eco-Scale are that different aspects of the environmental impacts are evaluated, and it has well-defined criteria for evaluation. The main disadvantage is that the score does not provide information about which were the causes of the PPs, making difficult the improvement and optimization of the process. In fact, from the score, without further information, it is difficult to critically evaluate the procedure and to find the critical points in which to intervene.[12,24]

Furthermore, the PPs are calculated by multiplying the parameter chemical hazard and amount of chemical used, as the influence of hazardous substances depends on their amount.[10,12] However, when assigning a hazard PP to a chemical, the metric simply asks to multiply the number of pictograms with the word symbol of “warning” or “danger”. So, the Analytical Eco-Scale does not consider the type of pictogram used and the severity or hazardous aspect. This can be problematic as some pictograms may indicate more severe hazards than others.[9,21] Table II shows an example of PPs calculation to evaluate an UFLC method for the determination of Omarigliptin in tablets.[33] Table III demonstrates some examples of hazard symbols and their meaning that can be found in some reagents and solvents. Table IV illustrates examples of the amount of energy consumed by some equipment used in the laboratory routine.

Recently, some studies applied the Analytical Eco-Scale to evaluate the greenness of their methods in combination with other metrics.[34-38] Only a few studies were found applying the Analytical Eco-Scale alone and claiming to be “eco-friendly”.[39,40]

Table II. PPs* used to evaluate an UFLC method for determination of Omarigliptin in tablets

			PPs*
Chemicals			
	Hazard	Amount	
Ammonium acetate	0	1	0**
Methanol	6	1	6**
Phosphoric acid	4	1	4**
Instruments			
UFLC			0
Balance			0
Sonicator			0
Total waste (1 - 10 mL)			3
Waste Treatment passivation			2
No vapours released			0
			Σ15
Analytical Eco-Scale Total Score: 85			

*PPs: Penalty Points; **Total Penalty Points = Hazard PP x Amount PP.

Table III. Reagent hazard symbols and their meanings

Meaning	Symbol	Example
Flammable		Acetonitrile
Toxic		Methanol
Health Hazard (eg, sensitisers, carcinogens)		Methanol
Corrosive		Phosphoric acid
Moderate Hazard (eg, harmful if inhaled or in contact with skin, causes eye irritation)		Phosphoric acid

Table IV. Amount of energy consumed by equipment

Equipment	Amount of energy
Raman Optical microscope Titration UV-VIS spectroscopy UPLC	<0.1 kWh per sample
HPLC GC	≤ 1.5 kWh per sample
GC-MS LC-MS	> 1.5 kWh per sample

MoGAPI

Proposed in 2018 by Płotka-Wasylka, GAPI uses a pictogram made up of five pentagrams divided into subsections to display the greenness of the analytical procedure.[14] Recently, in 2024, a modified GAPI tool (MoGAPI) has been developed to address some limitations of the former GAPI metric. The modification implemented a total score to enable comparison between methods and a new software to simplify and expedite its application.[15]

The MoGAPI, as well as the former GAPI, evaluates the environmental hazards of the entire analytical methodology using five colored pentagrams. Each pentagram comprehends a specific step of the procedure: sample handling, type of method, sample preparation, reagents and solvents used, and instrumentation, which are further divided into 15 subsections.[10,15,21]

The subsections are color-coded as green, yellow, and red to indicate the severity of their impact. Green signifies that the subsection is satisfactory and requires no further action. Yellow indicates that there may be minor issues that need to be addressed, while red highlights major problems that demand immediate attention. Additionally, if a circle is placed in the center of the pictogram, it indicates that the method is both qualitative and quantitative.[1,9,23,24] The criteria of MoGAPI and the fifteen subsections are shown in Table V, and the pictogram is illustrated in Figure 1.

Table V. MoGAPI parameters description

			Color (Points)		
Category	**No.**	**Subsection**	**Green (3)**	**Yellow (2)**	**Red (1)**
Sample handling	1	Collection	In-line	On-line or at-line	Off-line
	2	Preservation	None	Chemical or physical	Physicochemical
	3	Transport	None	Required	–
	4	Storage	None	Normal conditions	Special conditions
Method type	5	Direct or indirect	No sample preparation	Simple procedures	Extraction required
Sample preparation	6	Scale of extraction	Nano	Micro	Macro
	7	Solvents/reagents used	None	Green solvents/ reagents	Non-green solvents/reagents
	8	Additional treatments	None	Simple	Advanced
Reagents and solvents	9	Amount	< 10 mL (< 10 g)	10 – 100 mL (10 – 100 g)	> 100 mL (> 100 g)
	10	Health hazard (NFPA health hazard score)	0 or 1	2 or 3	4
	11	Safety hazard (NFPA flammability or instability score)	0 or 1	2 or 3	4
Instrumentation	12	Energy	≤ 0.1 kWh per sample	≤ 1.5 kWh per sample	> 1.5 kWh per sample
	13	Occupational hazard	None (Hermetic sealing)	–	Vapors to the atmosphere
	14	Waste	< 1 mL (< 1 g)	1 – 10 mL (1 - 10 g)	> 10 mL (> 10 g)
	15	Waste treatment	Recycling	Degradation, passivation	No treatment

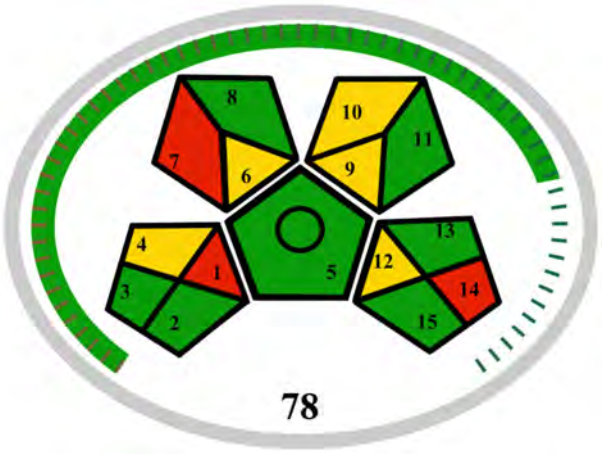


Figure 1. Illustrative MoGAPI pictogram.

Among the advantages of MoGAPI is that the color-system pictogram allows an easy perception of the greenness of each subsection and clearly indicates the weakest points of the procedure. The implementation of the total score provided an overall assessment of the method's greenness, further facilitating visualization and comprehension. This straightforward overview is especially useful for comparing different analytical methods based on their overall scores, especially when the analytical steps differ significantly. Moreover, MoGAPI covers many aspects of the procedure, allowing a more precise assessment of the green profile. Software is also available to directly input the method parameters and result in the pictogram.[9,15]

Although MoGAPI tries to cover the entire analytical process, its functionality can be difficult. Also, some categories can be difficult to fill in correctly into the software, like the concepts of sample preparation in-line, on-line, at-line, and off-line. Another disadvantage is that the subsection amount of reagents and chemicals used and the amount of waste considers the same label for a wide range of volumes.[1,10,21,32]

In 2021, the Complementary Green Analytical Procedure Index (ComplexGAPI) was introduced to assess the sample preparation of the method. It includes an extra hexagonal part that covers the preliminary activities involved in sample preparation and analysis.[41] Since its development, GAPI has been widely used in the literature along with other metrics.[42-47] Despite being very recent, there are already reports of the application of MoGAPI.[47-50] Additionally, a few studies applied the metric alone[51,52] or used the ComplexGAPI.[53-55]

AGREE

Developed in 2020, AGREE is the most widely used metric. It is represented as a circular pictogram divided into 12 parts, where each part corresponds to a principle of GAC. The input of the 12 parts is individually transformed into a score range of 0–1, and a final score is obtained by calculating the average of the parts. Depending on the scores obtained, each part is colored from dark green (score 1) to red (score 0), indicating the impact of each principle.[1,23,24,32]

Additionally, a specific weight is allocated to each part by software default, but that can also be changed by the user. In the pictogram, the length of each part reflects the specific weight assigned.[21] The resulting pictogram is like a clock shape, with a final score colored in the center surrounded by all the 12 parts, also colored,[17] as shown in Figure 2. Therefore, the metric provides both qualitative and quantitative results.[20] Table VI summarizes the criteria for assigning the scores based on the 12 principles of GAC.

Figure 2. Illustrative AGREE pictogram.

The main advantage of AGREE is its comprehensive approach, as it covers all 12 principles of GAC, which makes the assessment more robust. Another advantage is that the assessment can be easily performed with user-friendly software that automatically generates the pictogram. The pictogram has an easy interpretation, with both color and numeric results, allowing the user to determine the overall greenness of the analytical procedure quickly. Moreover, the color scheme varies according to the score range of 0-1 rather than being restricted to the conventional colors of green, yellow, and red.[9,17,21]

As a disadvantage, it can be confusing and quite difficult to allocate and understand the weighting of the 12 parts.[1,10,20] Another difficulty is to correctly input the information on the software, some parts like the sampling procedure step could be difficult to understand, being recommended to read the original article of AGREE by Pena-Pereira, 2020.[17] Furthermore, one related issue is the lack of CAS data for some reagents in the derivatization part; this could be overcome by software updates and alternatively allowing the user to input the missing data manually.

Several authors have used the AGREE metrics.[56-61] A lot of studies applied this metric alone.[62-68] In 2022, the AGREEprep was introduced, designed to evaluate the greenness of the sample preparation process.[1] However, still few studies have applied it.[69,70]

Table VI. 12 criteria of AGREE assessment

No.	Principle/part	Condition	Score
1	Sample pretreatment	Remote sensing without sample damage	1.00
		Remote sensing with little physical damage	0.95
		Non-invasive analysis	0.90
		In-field sampling and direct analysis	0.85
		In-field sampling and on-line analysis	0.78
		On-line analysis	0.70
		At-line analysis	0.60
		Off-line analysis	0.48
		External sample pre-and treatment (reduced number of steps)	0.30
		External sample pre-and treatment (large number of steps)	0.00

(continued on next page)

Table VI. 12 criteria of AGREE assessment (continued)

No.	Principle/part	Condition	Score
2	Amount of sample	Ultra-microanalysis (<1 mL or g)	1.00
		Micro-analysis (1–10 mL or g)	According to equation
		Semi-microanalysis (10–100 mL or g)	
		Macro-analysis (>100 mL or g)	
3	Instrumental position	In-line	1.00
		On-line	0.66
		At-line	0.33
		Off-line	0.00
4	Method's steps	3 or less	1.00
		4	0.80
		5	0.60
		6	0.40
		7	0.20
		8 or more	0.00
5	Level of automation and miniaturization	Automatic, miniaturized	1.00
		Semi-automatic, miniaturized	0.75
		Manual, miniaturized	0.50
		Automatic, not miniaturized	0.50
		Semi-automatic, not miniaturized	0.25
		Manual, not miniaturized	0.00
6	Derivatization	No derivatization applied	1.00
		Derivatization applied	According to equation
7	Amount of waste	≤ 0.1 (mL or g)	1.00
		10 (mL or g)	0.40
		25 (mL or g)	0.25
		100 (mL or g)	0.1
		Any other amount	According to equation
8	Number of analytes/ hour	70	1.00
		50	0.9
		10	0.5
		1	0.0
		Any other number of analytes	According to equation
9	Energy consumption/ sample	<0.1 kWh	1.0
		0.1–1.5 kWh	0.5
		>1.5 kWh	0.0

(continued on next page)

Table VI. 12 criteria of AGREE assessment (continued)

No.	Principle/part	Condition	Score
10	Renewable source reagent	No reagents	1.0
		All reagentes are bio-based	1.0
		Some reagents are bio-based	0.5
		None of the reagents are from bio-based sources	0.0
11	Toxic reagents used	No	1.0
		Yes	According to equation
12	Number of threats to operator	0	1.00
		1	0.80
		2	0.60
		3	0.40
		4	0.20
		5 or more	0.00

DISCUSSION

The increased concern with environmental issues and the incentive to apply GAC principles in procedures emerge the need to create metrics to assess the greenness of methodologies. As a result, various tools such as NEMI, Analytical Eco-Scale, MoGAPI, and AGREE have been proposed. Those metrics are applicable to several methodologies used in analytical chemistry. Their use is very important because it allows us to identify more clearly the specific steps and reagents and solvents of the methodology that have the greatest negative environmental impact. In general, a GAC metric should give easily readable results. Also, the criteria should include several parameters such as waste generation, waste treatment, the hazard of the chemicals, use of renewable source chemicals, the safety of the analyst, energy consumption, and sample preparation.

GAC metrics have been extensively researched and applied since their creation, highlighting their significance in demonstrating the environmental impact of analytical methods. The use of a metric translates into paper publications, and through the amount of papers published applying the metric is possible to measure its utilization. After researching the metrics addressed, it is remarkable the increased number of papers published in recent years (Figure 3). Back in 2019 and 2020, only NEMI and Analytical Eco-Scale were applied, and the concept of GAC metrics was still in its beginning. Later, GAPI and AGREE were created and well-accepted by the researchers. As seen in Figure 3, the paper's publication applying NEMI, Analytical Eco-Scale, GAPI, and AGREE are being used in constant increase, demonstrating their importance. Interestingly, most of the works presented in Figure 3 employed the Liquid Chromatography (LC) technique. Although greener techniques exist, such as ultraviolet-visible spectroscopy and capillary electrophoresis, which consume lower reagents and solvent amounts, LC remains a popular technique. Still, efforts have been made to develop greener LC methodologies and evaluate their environmental impact through the use of the GAC metrics.

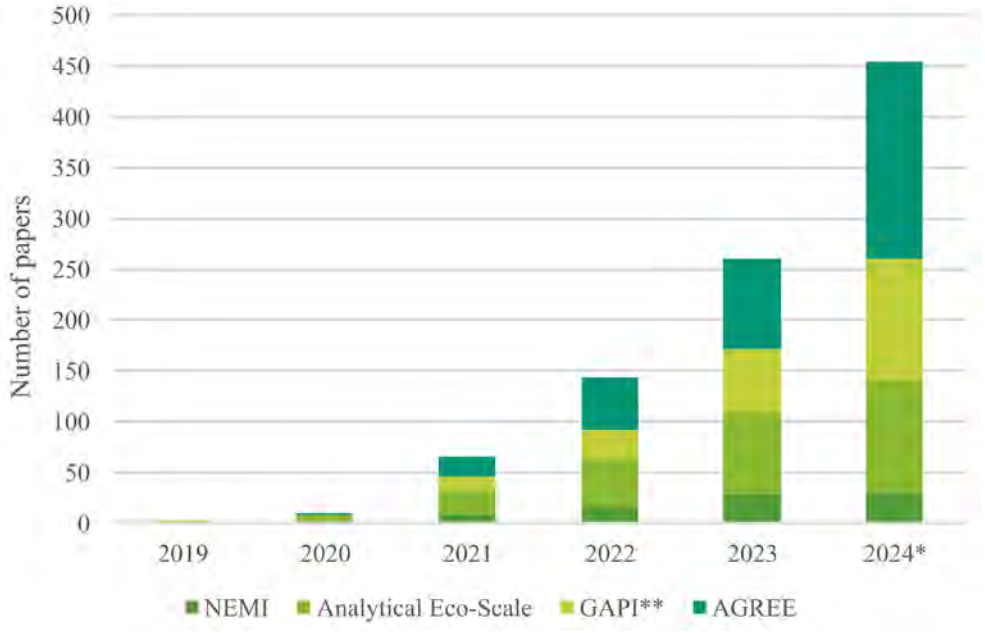


Figure 3. Number of papers using NEMI, Eco-Scale, GAPI and AGREE over the years. Note: *Results up to november 2024. **Results of MoGAPI were also included. Data obtained in Scopus, keywords: “corresponding metric” + green metric.

As a historical timeline, NEMI was the first GAC metric reported, then the Analytical Eco-Scale, GAPI, and AGREE. Two versions of sample preparation tools were also created, the ComplexGAPI and AGREEprep. In 2024, a modification of GAPI was developed, the MoGAPI. Also, a recent metric, namely BAGI, was reported focusing on the White Analytical Chemistry and has been proposed as a complementary to the GAC metrics already established. For this reason, BAGI was not deeply discussed.

To further discuss and compare the GAC metrics, a case study was conducted applying the four metrics addressed in this paper in a previously developed UFLC method for pharmaceutical quantification of the drug Omarigliptin.[33] The results obtained from the four metrics are shown in Figure 4. The application of NEMI resulted in three out of four green quadrants, demonstrating the eco-friendly nature of the method. The hazardous quadrant was not labeled green since the methanol and phosphoric acid used in the mobile phase are considered hazardous waste by the EPA-TRI. The Analytical Eco-Scale total score obtained was 85, classifying the method as excellent greenness. Ten penalty points were assigned to the hazardous and amount of methanol and phosphoric acid used. The total waste per analysis of the method was 1.32 mL, and for that, 3 penalty points were assigned due to the waste being in the range of 1 - 10 mL. Finally, the method received 2 penalty points for the passivation waste treatment.

In the MoGAPI assessment, most categories were considered green, and only two were red in the pictogram. The red categories were due to offline sample preparation and the use of non-green solvents. The yellow categories were received because the sample preparation involves simple procedures and is on a micro-scale, the solvents methanol and phosphoric acid have a health and safety hazard of 3, and similar to the Analytical Eco-Scale, the waste generated has passivation treatment and is on the range of 1 – 10 mL. The method had a total score of 80 and was considered green.

Unlike the other metrics, the AGREE pictogram has a color scheme that varies according to the score received in each category. The case study method only received one absolute red color because none of the solvents used were from bio-based sources. Some of the categories were colored as weak green and weak yellow due to the sample pretreatment being off-line, the amount of waste per analysis of 1.32 mL, only 1 analyte is determined in a single run, the use of approximately 0.43 mL of toxic solvents, and

the chemicals used are flammable and explosive. The method achieved an overall AGREE score of 0.72, indicating its environmental friendliness. In general, the four metrics yield similar results, suggesting that the method can be considered green. However, the complexity level differs among the metrics, and for a more comprehensive and robust evaluation, they should be used combined.

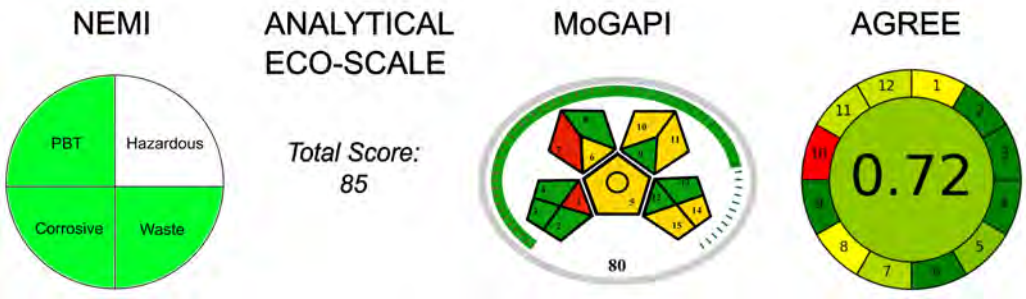


Figure 4. Results of the case study showing the application of the NEMI, Analytical Eco-Scale, MoGAPI and AGREE in an UFLC method for the determination of Omarigliptin.[33]

As illustrated in the case study, NEMI is the only qualitative metric, despite their very easily readable pictogram, it does not show much information and has been replaced by the most new and complete metrics. Analytical Eco-Scale is considered a semi-quantitative approach. It stands out compared to NEMI due to its detailed discussion of the analytical procedure, considering more parameters, and providing an assessment of the greenness as a numerical value. Nevertheless, the main issue of both metrics is the manual and time-consuming process to acquire the necessary information about the chemicals used in the analytical method.

The MoGAPI combines the visual impact of the colored pentagrams with an accurate overall score. The improvement of the total score enabled the metric to give a more accurate and objective comparison between methods instead of just evaluating each step separately. In addition, MoGAPI offers several advantages over Analytical Eco-Scale because it covers a wide range of the analytical procedure aspects, and it gives not only a numerical value but also some colored qualitative information, making MoGAPI more robust. However, none of these metrics consider each one of the 12 principles of GAC.

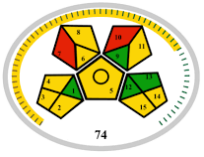
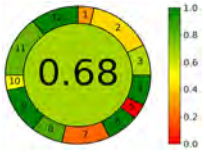
AGREE is the only metric that has the advantage of including all the 12 principles, previously not considered. It also gives both quantitative and qualitative results, similar to the MoGAPI, and has an easy visualization pictogram. This can explain the fact that AGREE is the most GAC metric applied alone without other complementary metrics. An overview of the GAC metrics is represented in Table VII.

Table VII. Overview of the GAC metrics

GAC metric	Outcome data	Representation	Advantages	Disadvantages
NEMI	Qualitative	4 quadrant pictogram 	Simple and easily read representation.	Requires manual process to obtain the pictogram; Time consuming.

(continued on next page)

Table VII. Overview of the GAC metrics (continued)

GAC metric	Outcome data	Representation	Advantages	Disadvantages
Analytical Eco-Scale	Semi-quantitative	Numerical value	Evaluate different aspects of the environmental impacts; Well-defined criteria of evaluation.	Lack of information about which were the causes of the PPs; Difficult to critically evaluate the procedure.
MoGAPI	Quantitative and qualitative	5 pentagram with 15 subcategories 	Color-system allows an easy perception of the greenness; Clearly indicates the weakest points; Total score facilitates comprehension; Covers many aspects of the procedure.	Difficult functionality; Consider the same label for a wide range of volumes.
AGREE	Quantitative and qualitative	Pictogram of 12 sectors 	Easily performed using the software; Automatically generated pictogram; Easy to visualize the weightage; Consider all the 12 principles of GAC.	Confusing to allocate and understand the weighting; Lack of explanation about the terms in Sampling Procedure step.

Nowadays, a change must be made in the evaluation of analytical methodologies. Not only usual parameters are required when assessing the analysis performance and conducting practical studies, but also it must be considered the environmental impact and the sustainability level of analytical techniques. The analytical researchers should know the impact that the process causes on the environment, to limit hazards discharged into the ecosystem. So, the GAC parameters should be evaluated during the construction and planning phase of the analysis. For this, it is worthwhile and important to apply the GAC metrics.

CONCLUSIONS

It was possible to conclude that over the years the GAC metrics have improved and are increasingly being applied. All of the metrics discussed have their own particularities, advantages, and disadvantages. After analysing all the metrics, we observed that AGREE is the most complete and most used GAC metric on its own. In addition, its pictogram is the most encompassing, being the only one that covers all 12 principles. It has an easy comprehension as the color scale allows a better visualization and understanding of the method's greenness profile. The MoGAPI now also provides an easy visual overview of the environmental impact and safety of the method, along with a total score assigned to each method. Moreover, although it is very time-consuming, ideally the best approach is to apply all the metrics in combination to gain as much information as possible, ensuring a comprehensive evaluation of the environmental impact. It is important to note that measuring greenness is not just about determining the quantity of waste but also considering all

factors involved in the methodology. Also, the current GAC metrics need further improvements to enhance their user-friendliness and provide quantifiable reference values. So, it can be expected that more enhanced metrics emerge in the future.

Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

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ARTICLE

Updating of Quantitative Models in Validation and Routine Tests of Comparative Chemical Methods

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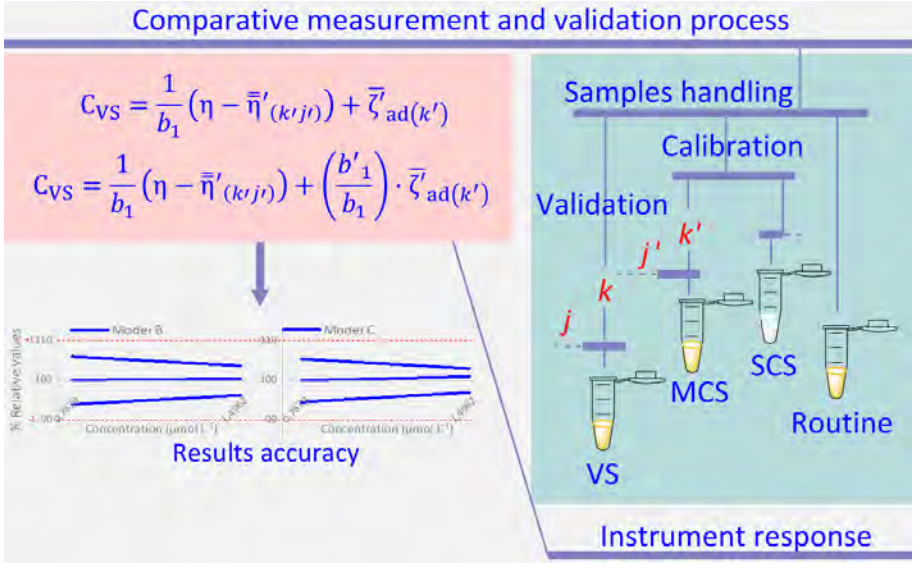

Nowadays, health and safety requirements are becoming more urgent, through normative and regulatory texts, considering the intense demands of customers from different sectors of socio-economic activities. Chemical testing must undoubtedly assume a large part of the tasks related to these concerns, despite the delay observed in their metrological concepts due to the complexity of the chemical and biological samples. Hence, technical and methodological creativity will be well supported, including theoretical revisions and updates of existing methods, in order to overcome the various encountered analytical problems and to fill some frequent lack of metrological tools. In this study, we propose hybrid quantification models, while showing their contributing effects on analytical improvement and decision-making in validation and routine testing of comparative chemical methods. To this end, external calibration plans, with or without a matrix, were established to generate and compare various quantitative models, which make it possible to determine, cleverly, validation and real samples concentrations. The obtained results shed light on the real causes leading to the poor quality that can be found in the obtained validation data. However, highlighted quantitative models show an improvement in both precision and accuracy, which reduce by 5 to 9% the uncertainty measurement. In addition, these estimates show a comparable quality for routinely tests.

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INTRODUCTION

Chemical analyses are infinitely involved in all areas of socio-economic activities. Their related results are intended to be tangible and reliable to meet the daily human needs in product control, pollution evaluation, diagnoses and medical treatments, as well as in international trade and standards establishment, etc. However, this can only be achieved by implementing increasingly creative methodological and metrological practices, although the chemical and biological complexity samples make it difficult to directly fulfill all such requirements.[1,2] Yet, efforts are still maintained by regulatory bodies concerned with chemical measurements, but often individually, with the aim to modernize practices in analytical chemistry by publishing periodically updated recommendations.[3–7] Whose intentions concur with the perspective metrology strategies of the BIPM Consultative Committee for Amount of Substance, across targeted key sectors.[8] Even though, chemical measurements are until nowadays call upon validated and acceptable metrological secondary methods, as the only tools adapted to guaranty comparable results, in order to overcome the various analytical problems.[1]

However, whatever the practice used, we are often opposed to certain unrealizable duties, such as trying to obtain certified reference material (CRM), which is non-existent in some cases, expensive, or even unavailable in time for many laboratories. Added to the unavailability of suitable sample blanks as well as the instability of certain materials whose related errors can enlarge the uncertainty, if they are not wrongly counted, in the contribution of the laboratory staff. In contrast, the replacement of such a material in the validation tests can also make the measurement uncertainty worse, as much if not more, with regard to the above-mentioned drawbacks. This also calls into question the spiking technique, which until now offers a key tool in validation process, provided showing that it is free of any ambiguity accompanying it's use.[4,9-11]

In this sense, a revision has been made in order to update the use of this technique, knowing that, in the absence of certified reference materials, suitable synthetic reference materials can be used in the calibration of comparative methods. Hence, experiments were planned to draw up external calibration plans with or without a matrix, in such a way to derive various equations to determine, mainly and cleverly, the validation standard concentrations and to calculate the validation parameters. Our thinking here is to generate and compare quantitative models, in order to avoid accumulating errors, as when considering ordinary matrices with native content in the designed validation process. In parallel, quality control routine samples were also handled and homologue models quantified their contents. Finally, to highlight results quality arising from those models, measurement uncertainty was estimated according to the single-laboratory validation approach.[12,13]

MATERIALS AND METHODS

Sample handling

Pure Sigma-Aldrich reagents (>95 %) were handled to obtain diluted ethanolic solutions of synthetic and natural retinol, with a constant amount of retinyl acetate as an internal standard (IS). On the other hand, a frozen human transfusion plasma was manipulated in accordance with the ethic committee requirements of the Research Laboratory Spectrochemistry and Structural Pharmacology of the University of Tlemcen, to reach similar ethanolic dilution. The experiments were adapted for a 70/30%, MeOH/MeCN based isocratic reversed phase HPLC analysis. The analytes were eluted at 2.0 mL min^{-1} flow rate and detected at 325 nm wavelength.[14]

Calibration plans

Different solutions were prepared daily to obtain, firstly, five points' synthetic calibration standards type (SCS) within a 0.5 – 2.5 µmol L^{-1} concentrations range. Secondly, the available natural plasma was spiked with the same standard solutions to obtain, after final treatment, on the one hand, validation standards type (VS), and on the other hand, matrix-based calibration standards type (MCS), issued from extra samples (XS) spiked at the concentration range limits.

Preparation of quality control samples

The preparation of the routine quality control (QC) samples was achieved, regarding the difference in physical proprieties of the plasma constituents, mainly, aqueous and no aqueous components. As the lipid matter trend to melt earlier during the plasma thawing process, then we can drop successively different concentrations of the lipophilic compounds until the complete defrosting of the plasma. The selected portions were combined to produce the desired samples, in content and volume, to suit within the validation interval and to allow testing over a period of more than one month.

Theory

Quantitative models

The least squares linear regression based calibration was used to designate the function that links the instrument response to the concentration of the analyte. In the absence of CRM, samples with natural content of analyte of interest are commonly used in spiking practices.[3,4,7,15] In such a situation, the determination of the VS concentration requires measuring, primarily, the endogenous quantity, which will then be subtracted from the whole calculated concentration.[11,15] Unfortunately, without knowing about its influence on the VS quantity measurement, it is even difficult to estimate its contribution to uncertainty in the case of an early standards addition determination.[16,17] While, it should be noted that at this stage the main objective is not yet to determine the native quantity, but rather to eliminate its effect on the validation measurement process. Thus, we intended by establishing the above experimental calibration plans, to address some concerns on the native content estimation of the used matrix, in validation and routine assays, by generating proper quantitation models.

Putting this in mind and assuming an internal standardization, the back calculated concentration (C_{mes}) of the analyte is given as Equation 1:

$$C_{mes} = \frac{1}{b_1}(\eta - b_0) \quad (1)$$

where the instrument response η designate the areas ratio of the analyte of interest over the internal standard and the coefficients (b_1, b_0) represent the slope and the intercept of the calibration curve, respectively.

Alike, the VS concentration (C_{VS}) added to the handled matrix can be quantified as Equation 2:

$$C_{VS} = \frac{1}{b_1}(\eta - b_0) - \zeta_{nat} \quad (2)$$

As we can see, this equation tolerates to acquire two types of data, regarding the natural quantity estimating method, either by the current validated method or from previous tests. Accordingly, we can also deduct, for each validation series, the native content (ζ_{nat}) from the XS quantities samples as Equation 3:

$$\zeta_{nat} = \frac{1}{b_1}\left(\eta'_{(k'j')} - b_0\right) - \zeta'_{ad(k')} \quad (3)$$

where, ($\eta'_{ad(k')}$) and ($\zeta'_{ad(k')}$) represent, respectively, the instrument response and the added content of the (j'th) measurement of the (k'th) XS.

Then, by considering a ($k' \times j'$) plan and combining Equations 2 and 3, we obtain Equation 4:

$$C_{VS} = \frac{1}{b_1}\left(\eta - \bar{\eta}'_{(k'j')}\right) + \bar{\zeta}'_{ad(k')} \quad (4)$$

As can be seen, instead of subtracting an earlier calculated native quantity, which we don't known about its uncertainty, the hybrid Equation 4 allows, in fact, to overcome this inconvenience and highlights only the main actual influential steps of the validation measurement process, such as calibration preparation and instrumental run.

On the other hand, we can express the native response by means of MCS data, as Equation 5:

$$\eta_{nat} = \eta' - b'_1 \zeta'_{ad} \quad (5)$$

where b'₁ designate the slope of the standard addition curve.

Now, assuming we back-calculate the native-content by means of b₁ and b₀, then we can establish, for the same (k' × j') plan, the following Equation 6:

$$\zeta_{nat} = \frac{1}{b_1}\left(\bar{\bar{\eta}}'_{(k'j')} - b_0\right) - \left(\frac{b'_1}{b_1}\right)\bar{\zeta}'_{ad(k')} \quad (6)$$

Therefore, by substituting the Equation 6 into Equation 2, we can rewrite another VS quantitative equation, as Equation 7:

$$C_{VS} = \frac{1}{b_1}\left(\eta - \bar{\bar{\eta}}'_{(k'j')}\right) + \left(\frac{b'_1}{b_1}\right)\bar{\zeta}'_{ad(k')} \quad (7)$$

As it is well noted, this equation appears more complete than the Equation 4, where the slopes ratio factor (b'₁/b₁) proves skillful to redressing the matrix effect if it occurs.

Otherwise, we can also calculate the added concentration of the validation standard by Equation 8:

$$C_{VS} = \frac{\eta - \eta_{nat}}{b'_1} \quad (8)$$

Since η_{nat} is equal to the intercept b'₀ of the standard addition curve, and then we can write, according to Equation 5:

$$\frac{\eta'}{b'_1} - \zeta_{(ad)(k)} = \frac{b'_0}{b'_1} \quad (9)$$

where the ratio (b'₀/ b'₁) recalls the classic standard addition determination of the native content.[18]

When considering the all extra experimental results, the native content can be expressed as Equation 10:

$$\zeta_{nat} = \frac{\bar{\bar{\eta}}'_{(k'j')}}{b'_1} - \bar{\zeta}'_{ad(k')} \quad (10)$$

Another approach to quantify the native content can be established by substituting $\bar{\bar{\eta}}'_{(k'j')}$ by $\left(\bar{\bar{\eta}}'_{std(k'j')} + \eta_{nat}\right)$ into Equation 3 to find Equation 11:

$$\zeta_{nat} = \frac{1}{b_1}\left(\bar{\bar{\eta}}'_{std(k'j')} + b'_0 - b_0\right) - \bar{\zeta}'_{ad(k')} \quad (11)$$

Parameter computation

For a considered (i series × j duplications) validation plan, which agrees an inter-series variability (s^2B) and an intra-series repeatability (s^2r), accuracy profiles were calculated according to the Mee approximations.[19] As for a given average concentration level $\bar{\bar{C}}_{VS\_c(k)}$, the β-expectation tolerance interval (βETI) was expressed by Equation 12:

$$\bar{\bar{C}}_{VS(k)} \pm K \cdot s_{TI} \quad (12)$$

where the factor K represents the Student's β-quantile for a 95% confidence interval, using the degrees of freedom ν of Satterthwaite,[20] as Equation 13:

$$\nu = \frac{\left(\frac{s_B^2}{s_r^2} + 1\right)^2}{\frac{\left(\frac{s_B^2}{s_r^2} + \frac{1}{j}\right)^2}{i-1} + \frac{1-\frac{1}{j}}{ij}} \quad (13)$$

Finally, the standard deviation of the tolerance interval (s_{TI}), itself a function of the intermediate precision standard deviation (s_{TP}), with ($s^2_{TP} = s^2_B + s^2_r$), can be written as Equation 14:

$$s_{TI} = s_{IP} \times \left(\frac{(i+1)js_B^2 + 2s_r^2}{ijs_B^2 + s_r^2}\right)^{\frac{1}{2}} \quad (14)$$

RESULTS AND DISCUSSION

Validation

Experiments were accomplished according to an (i = 3, j = 3 and k = k' = 2) validation plan, to inspect a low level validation standard (LLVS) and a high level validation standard (HLVS). Figure ESI-1 of the electronic supporting information data provides an illustration of the experimental plan and shows the arrangement of these levels relative to the other calibration standards within the desired concentration range. However, validation parameters were calculated using a 95% probability tolerance. The results obtained are summarized in Table I. Models A-1 and A-2 correspond to the Equation 2, in which the natural quantity was estimated daily using the current applied method and when it was estimated from previous tests, respectively. Furthermore, model B agrees with Equations 3 and 4, model C with Equations 6 and 7, model D be in accord with the Equations 8 and 10 and model E links with Equation 11.

As we can see, precision was found to be inferior to 5% for all models, but with rising values for model A-2. On the other hand, the recovery results, which were quantified as the ratio of the mean calculated concentration over the analyte added amount in the plasma samples, give indication on the suitability of the extraction efficiency.[3,21] Nevertheless, they also point out a high bias for the A-2 model.

Table I. Validation and uncertainty parameters estimation results obtained from the different discussed models

	Validation samples										Routine samples				
	LLVS					HLVS									
	Model A-1	Model A-2	Model B	Model C	Model D	Model A-1	Model A-2	Model B	Model C	Model D	Model A	Model B	Model C	Model D	Model E
Sets (I)	3	3	3	3	3	3	3	3	3	3	5	5	5	5	5
Replicates (J)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Concentration (μmol L^{-1})															
Added	0.7639	0.7639	0.7639	0.7639	0.7639	1.4962	1.4962	1.4962	1.4962	1.4962	--	--	--	--	--
Calculated	0.7723	0.7935	0.7635	0.7714	0.7642	1.4916	1.4745	1.5110	1.5075	1.5026	1.1270	1.1157	1.1144	1.1151	1.1161
Precision															
CV intra-series	2.1010	2.0448	2.1251	2.1034	2.0886	1.2413	1.2557	1.1910	1.1938	1.2276	1.0989	0.7849	0.7858	0.7899	0.5354
CV inter-series	0.2862	2.7576	0.5357	1.2020	1.0235	0.8901	2.6225	0.2361	0.3052	0.6927	1.0458	0.7249	0.5456	1.0611	1.1479
CV (IP)	2.1204	3.4330	2.1915	2.4226	2.3259	1.5274	2.9077	1.2142	1.2321	1.4096	1.5170	1.0684	0.9567	1.3228	1.2666
%Recovery	101.10	103.88	99.95	100.98	100.05	99.69	98.55	100.99	100.76	100.43	--	--	--	--	--
S(TI)	2.2392	3.8451	2.3238	2.6158	2.4988	1.6638	3.3049	1.2847	1.3067	1.5213	1.6126	1.1348	1.0079	1.4201	1.3737
Uncertainty															
%RSU	5.212	11.730	5.440	6.394	6.022	4.217	12.032	2.998	3.060	3.714	3.685	2.584	2.234	3.402	3.511
K	2.328	3.051	2.341	2.444	2.410	2.534	3.641	2.334	2.342	2.441	2.285	2.277	2.217	2.395	2.556

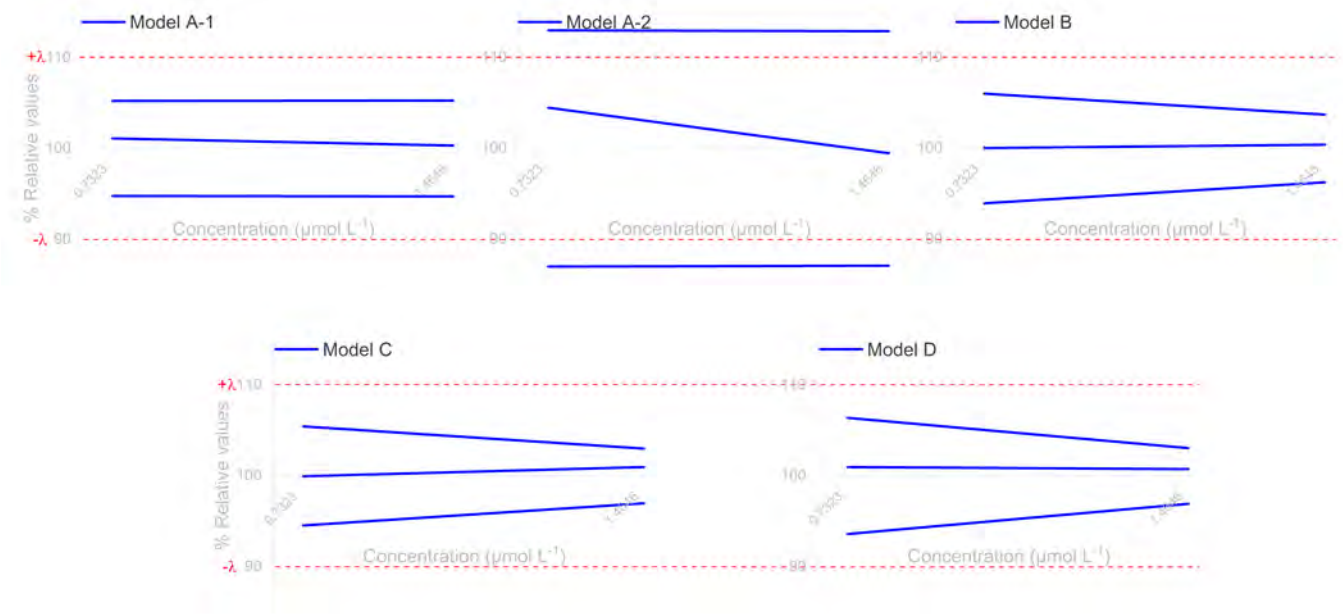

Figure 1. Accuracy profiles relating to the relevant quantitative models obtained by plotting of the relative mean percent values against the introduced concentration.

Figure 1 shows the models-related accuracy profiles obtained by plotting the percent relative values of the interval tolerance limits from either side of the recovery horizontal line. As can be seen, the classic A-2 diagram model shows an excessive variability, which pushes the tolerance interval outside a (λ = ± 10%) predefined acceptance limits and therefore rejects the validity of the results.[22-25] Indeed, this model reveals serious trouble when determining VS concentrations by subtracting the previous defined native content from the actual measured concentration. This fall is also evoked in cases where native quantities are determined by consensus, even if they are provided with an estimate of their uncertainties, moreover, which are considered poor according to this spiking technique.[26] Indeed, such a practice implies a systematic error, which masks wrongly the variability claimed to expose the sample to the random effects of the intermediate precision conditions, while it is requested to eliminate this known error. In this case, the sample must be examined at least for each validation series, as is done when using model A-1. Over and above that, the highlighted hybrid models of Equations 4 and 7 completely exclude this ambiguity, given that the native quantity does not appear in the expression of the VS concentration. This demonstrates that the spiking technique is not responsible for the poor quality that can be found in the investigated validation data. However, the similarity observed of their relating s_{TI}, especially for HLVS indicate a closeness of the calibration curve slopes b_1 and b'_1 and thereby neglected the existence of any matrix effect. Moreover, a two-way ANOVA test was performed using Origin software to support this conclusion. This assessment consists to examine the slope dependency on the curve nature (factor A) and the series (day) variation (factor B). Table II recaps 15 generated values for each slope's type by assuming a min-max calibration points with two replicate each and considering one to two replicates, each time, from one level-point to the other.

Table II. ANOVA-test results on generated slopes data

	Factor B				
Factor A	**day1**	**day2**	**day3**	**Mean**	**SD**
	4.1015	4.0375	4.1137		
	4.1350	4.0449	4.1343		
b_1	4.0680	4.0300	4.0932		
	4.0874	4.0623	4.1261		
	4.1156	4.0126	4.1014	4.07113	0.07065
	4.1046	4.0027	4.1060		
	4.1270	3.9916	4.1394		
b'_1	4.0822	4.0139	4.0727		
	4.0411	4.0825	4.0336		
	4.1682	3.9229	4.1785	4.08424	0.03955
Mean	4.0306	4.02011	4.10988		
SD	0.03613	0.04390	0.03946		
ANOVA	df	SS	MS	F Value	P Value
(factor A)	1	0.00129	0.00129	0.77451	0.38755
(factor B)	2	0.04995	0.02498	15.0152	5.90E-5
Interaction	2	0.00190	9.50E-4	0.57137	0.57225
Model	5	0.05314	0.01063	6.38954	6.62E-4
Error	24	0.03992	0.00166	--	--
Corrected Tot	29	0.09306	--	--	--

The test results show that there is no significant difference between the populations averages for factor A (P-value > 0.05), at 95% confidence level. However, there is a significant difference between the populations of the factor B, which exposes a day effect, but without any influence on factor A and does not cause any interaction. Accordingly, it is confirmed at those concentration levels, intended to cover the validation plan, that no matrix effect will occur and the slopes b_1 and b'_1 are very close to each other.

Routine assays

The results shown in Table I give comparable values for all analogous designated models, which appear consistent with those found for the studied validation concentration range, always with a respected headway for the B and C models. However, model E shows that Equation 11 allows us to detect any possible effects linked to the injected media variation, as it seems to be of the negative drift due to the rapid elution of the polar compounds in the chromatogram of the plasma solution of Figure ESI-2. Indeed, the related results attest that there is no significant difference when it comes to quantify the native concentration using the response of the aqueous solutions of the matrix-based standards or that of the organic standard solutions. Furthermore, which may point out the influencing factor magnitudes, whether qualitatively or quantitatively, during the sample handling process and peaks integration of analytes of interest in the final phase of the chromatographic run.

Uncertainty estimation

The measurement uncertainty was estimated in accordance with the Guide for the Expression of Uncertainty in Measurement (GUM).[12,13] Indeed, the quantitative equations terms normally cover all the potential sources of errors relating to the relevant steps of the measurement process, in particular, the above prospected effects. However, the evaluation was based on the statistical TI's calculations,[26] where the combined uncertainty of the measured sample concentration is defined as the sTI for the I days × J repetitions plan, and whose expanded form leads us back to the ± term of the Equation 12, as Equation 15:

$$U(C_{spl}) = K \times s_{IP} \times \left(\frac{(I+1)Js_B^2 + 2s_r^2}{IJs_B^2 + s_r^2} \right)^{\frac{1}{2}} \qquad (15)$$

As we can see, this estimation seems to be rigorously higher by an effective number times, even if it is close to unity (1.04 – 1.15) in our case, compared to that which can be obtained simply by expanding the s_{IP}. Table I recaps the expanded measurement uncertainty results for all models and indicates that high values always appear at low concentrations, also with clear improvement for highest levels, by decreasing to less than 3% for models B and C. Except, for the model A-2, which shows great variability regardless the examined concentration level, by reaching up to 12% uncertainty.

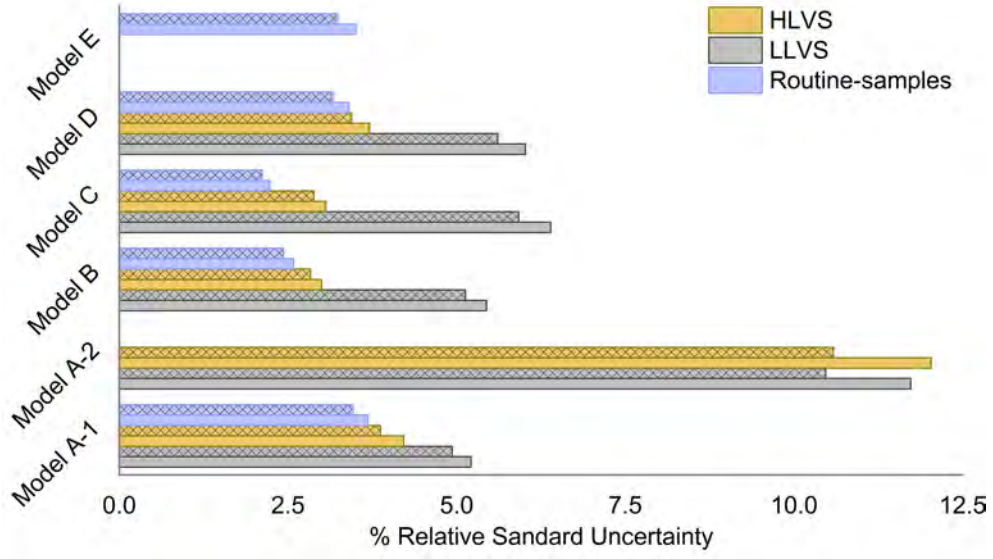

Figure 2. Comparison of the uncertainty estimation results.

Equivalently, these estimates look quite similar for routine samples, not exceeding 4% and with the same advance seen for models B and C. This, when compared to the high validation range results, can be attributed to the high repetitions numbers, which are inherent in the calculating equations of the validation and uncertainty parameters. On the other hand, Figure 2 illustrates these estimations in double, using sTI simple bars and sIP dashed bars. As can be seen, their relative magnitudes are quite similar, whether for HLVS or LLVS, ranging from a deviation of 0.2 to 0.5%, which tolerates a peaceful estimation for somewhat delicate concentration levels. Except in the case of quantification with the A-2 model, which can lead, falsely, to an underestimation of uncertainty of 1.4%. Likewise, these observations are also drawn, by comparing the routine sample results.

CONCLUSIONS

The applied experimental plan has helped to produce different quantitative models that can be used in comparative analysis, such as HPLC-based methods. The valuation of these models was well carried out by determining the validation standard concentrations during the computation of validation parameters, when no certified reference material is available. In addition, this evaluation demonstrates that the spiking technique is not responsible for the poor quality that can be found in the validation data, but rather it is its utilization mode that must be called into question, mostly, when using ordinary matrix with natural content. Indeed, the proposed hybrid models show a clear improvement of this approach, by overcoming errors relating to the native analyte, and by focusing only on the actual influencing factors of the analytical method. Furthermore, these models provide same satisfactions for the determination of the analyte content in routine tests, compared to the results relating to the classical determination model. This supports to open promising perspectives for the validation of comparative chemical methods, which present a shortage for metrological tool and even to promote justified quantitative models for testing and controlling the analytes of interest.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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SUPPLEMENTARY MATERIAL

Electronic Supporting Information Data

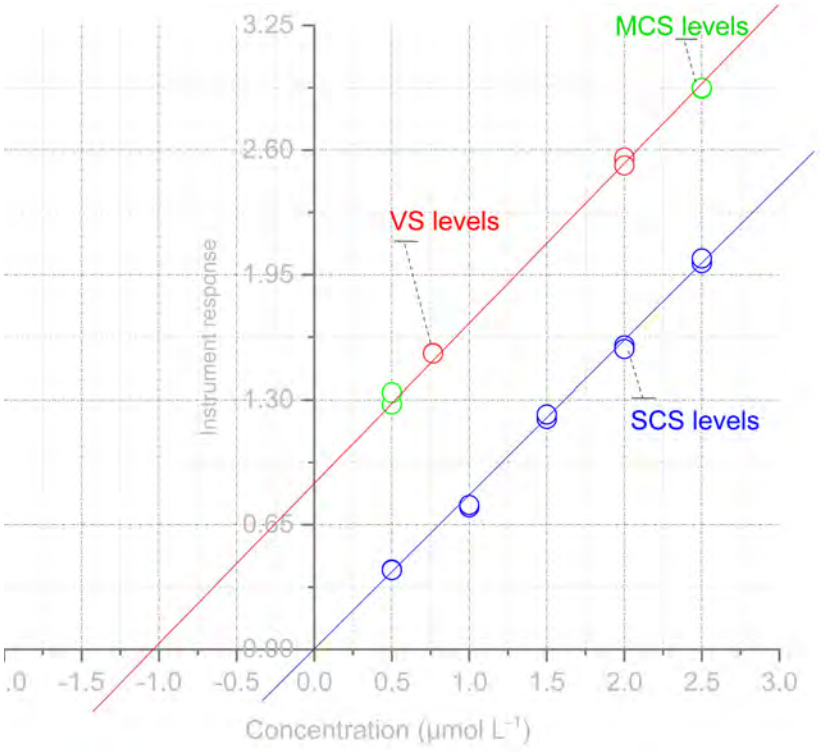

Figure ESI1. Example of a daily constructed experimental plan, showing the arrangement of the different calibration standards in the external standard calibration curve (bleu) and the standard addition calibration curve (red).

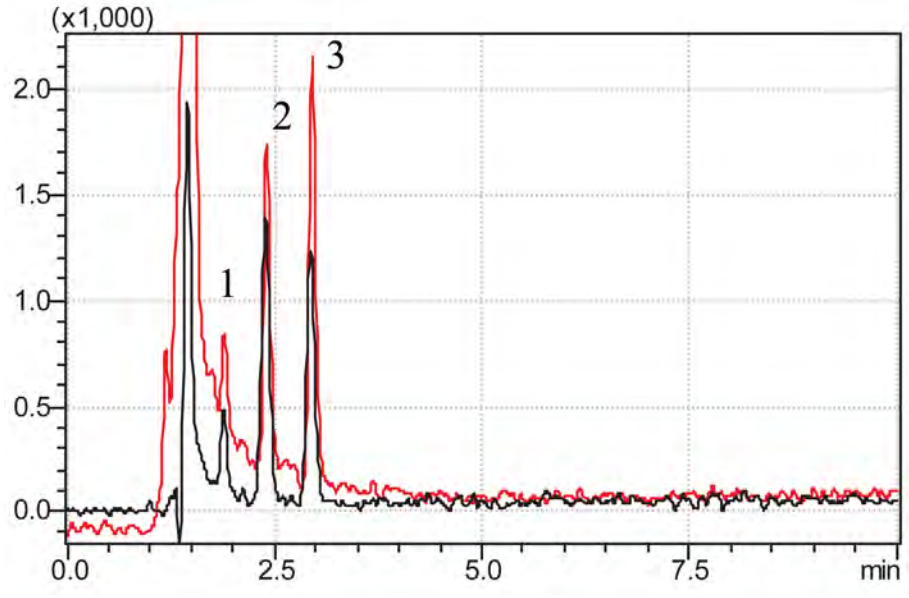

Figure ESI2. HPLC-UV responses of the injected solutions, a synthetic standard solution (black chromatogram) and plasma-sample solution (red chromatogram). Identified peaks: 1) BHT, 2) retinol, 3) retinyl acetate.

Negative drift observed in plasma aqueous medium chromatogram that is due to the rapid elution of the polar compounds, which explains the behavior of the analytical column towards the injected fluid.

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ARTICLE

Optimization and Validation of Ultrasound-Assisted Extraction for Total Phosphorus Analysis in Cane Syrup

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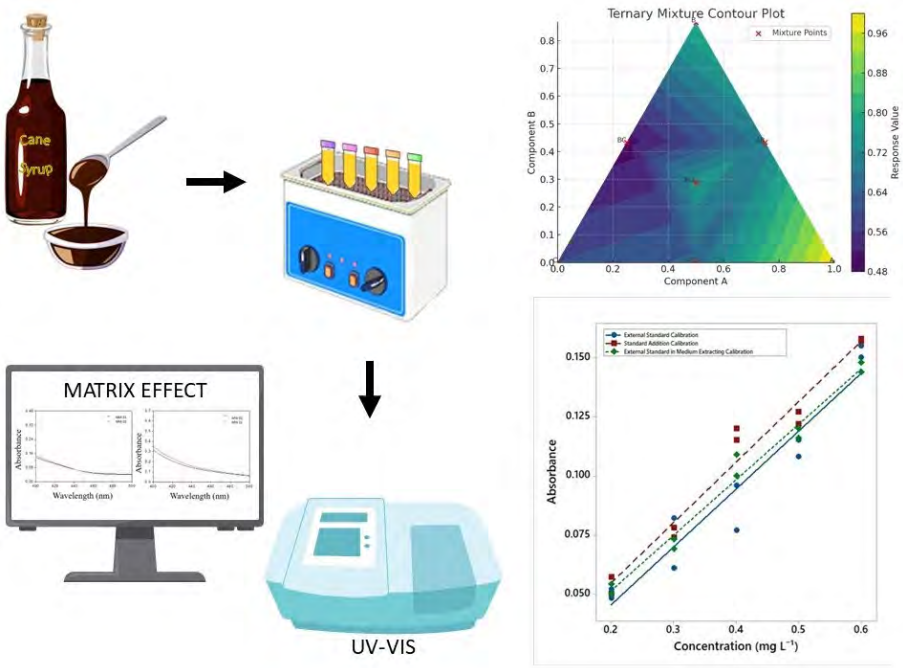

Cane syrup, a nutrient-rich by-product of sugarcane, is valued for its bioactive compounds and mineral content, including phosphorus, a vital macromineral essential for human bone health, enzyme activity, and plant metabolism. Conventional methods for total phosphorus analysis in such viscous matrices face challenges, such as matrix interference, high reagent consumption, and environmental impact. This study optimized and validated an ultrasound-assisted extraction (UAE) method combined with UV-Vis spectrophotometry for determining total phosphorus in cane syrup. UAE parameters were optimized using a simplex centroid mixture design to assess the effects of HNO_3, HCl, and ultrapure water as extraction solvents. UV-Vis spectra revealed that HCl-rich extraction solvents enhanced pigment production via the Maillard reaction, interfering with spectrophotometric detection. In contrast, ternary acid mixtures minimize these effects. The optimal conditions (1.67 mL HNO_3, 2.00 mL HCl, and 1.30 mL H_2O) achieved recovery rates of approximately 100%, without significant matrix interference. The validation of UAE combined with UV-Vis spectrophotometry demonstrated excellent selectivity and linearity ($R^2 > 98.0\%$), low limits of detection and quantification (0.296 μg g^{-1} and 0.898 μg g^{-1}, respectively), and good precision (RSD < 11%). The method's accuracy was confirmed through a paired *t*-test comparison with microwave-assisted digestion (MAD), showing no significant differences ($p > 0.05$). UAE proved to be more environmentally friendly than MAD, with lower energy consumption (4.17 vs. 62.50 Wh/sample) and reduced reagent usage, as indicated

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by the AGREEprep metrics (scores: 0.41 vs. 0.30). The total phosphorus content in cane syrup samples varied significantly (11.48–129.54 mg kg^{-1}), influenced by geographical origin and production processes. The validated UAE method provides a fast, cost-effective, and sustainable alternative for phosphorus analysis in complex food matrices, aligning with the principles of green chemistry.

Keywords: Ultrasound-assisted extraction, total phosphorus analysis, UV-Vis spectrophotometry, simplex-centroid design, method validation

INTRODUCTION

Sugar derived from sugarcane is a natural sweetener primarily composed of sucrose.[1] While sugarcane juice contains bioactive compounds such as phenolic acids, polyphenols, and flavonoids, these are significantly reduced during refining, resulting in a product with low nutritional content.[2] In contrast, less processed sugars, such as cane syrup, have gained consumer preference due to their higher nutrient retention and high energy value (about 300 kcal per 100 g).[3] Cane syrup is widely used in Brazilian cuisine and food industries as a sweetener and flavor enhancer.[4-6]

Cane syrup is a thick, viscous liquid composed of approximately 55% sugars, as well as flavonoid glycosides and phenolic acids.[7,8] Its composition varies according to plant variety, geographic location, and processing conditions.[9] Notably, cane syrup retains minerals naturally present in sugarcane juice, which play essential roles in human health.[10,11]

Phosphorus is a key macromineral, essential for the structure of bones, teeth, and certain muscle proteins.[12] It also participates in digestive enzyme activity and bone formation and interacts with other minerals through complexation reactions.[13] In plants, phosphorus is found in organic and inorganic forms, the latter being more soluble and bioavailable.[14,15] However, insoluble compounds may limit their bioavailability.[16] In sugarcane juice, most phosphorus is in soluble form, and around 10% is organic, with variations depending on cane maturity.[17]

Phosphorus bioavailability in plant-based foods is often reduced by the presence of phytates and phosphates, yet cane syrup shows relatively high bioaccessibility.[15] For laboratory analyses, total phosphorus (P_2O_5) is commonly determined via direct methods, where distinguishing between organic and inorganic forms is less relevant in routine quality control.[18]

Sample preparation for phosphorus determination typically involves techniques like microwave-assisted digestion, wet acid digestion, or dry ashing.[19-22] However, such methods may be inefficient or produce excessive waste when applied to complex matrices like cane syrup.[20,23] Therefore, optimized methods that are both accurate and environmentally sustainable are essential.

Ultrasound-assisted extraction (UAE) has proven effective for solid and semi-solid samples by enhancing dissolution, leaching, and analyte recovery through cavitation-induced disruption of the matrix.[24,25] While simple acid dilution may be sufficient for liquid matrices,[26] UAE offers advantages for viscous, sugar-rich products: (i) breaking macromolecular aggregates; (ii) reducing chromatic interferences; and (iii) minimizing reagent and energy consumption.[27-29]

Previous studies by our research group validated UAE for extracting elements such as iron, manganese, calcium, and magnesium from cane syrup and brown sugar, demonstrating excellent analytical performance.[30,31] However, variables like solvent composition, temperature, pH, ultrasound amplitude, and solid/solvent ratio influence efficiency, especially given cavitation inconsistencies in ultrasonic baths.[32-34]

Although UV-Vis molecular absorption spectrophotometry is widely used in quality control due to its speed and simplicity, no studies have applied it in combination with colorimetric methods and UAE for total phosphorus determination in sugar-rich food matrices, nor have matrix interferences been systematically evaluated.[35,36] This technique also provides structural information and is effective when paired with chemometric tools.[37-39] Additionally, colorimetric methods are widely employed in phosphorus analysis for their low cost and ease of use.[15,40] These methods typically involve the formation of a yellow phosphomolybdate complex, quantified via UV-Vis spectrophotometry.[18,41]

Analytical method development must consider equipment availability, simplicity, and efficiency.[42] However, validation is essential to ensure statistical reliability and regulatory compliance, particularly in food analysis, where it is linked to quality and safety.[43-45]

In this context, this study aimed to optimize and validate an ultrasound-assisted extraction methodology for the determination of total phosphorus in cane syrup, minimizing the influence of potential matrix interferents. Chemometric tools and UV-Vis spectrophotometry were employed for this purpose. The approach prioritized the principles of green chemistry, aiming to minimize reagent consumption, reduce waste generation, and ensure analytical efficiency.

MATERIALS AND METHODS

Cane syrups sampling

A total of 25 cane syrup samples were purchased from supermarkets and other commercial establishments (e-commerce), sourced from different producers and regions across Brazil. The samples were identified with the code "CS" followed by an Arabic number and were collected randomly. They represented the main sugarcane derivative-producing regions of the country: Rio Grande do Sul (RS): CS02, CS08, CS09, CS13, CS14, CS15, CS23, CS24, and CS25; Santa Catarina (SC): CS03, CS04, CS05, CS06, CS12, and CS16; Paraná (PR): CS01, CS17, CS20, and CS22; São Paulo (SP): CS10 and CS19; Minas Gerais (MG), Bahia (BA), and Rio de Janeiro (RJ): CS11, CS18, and CS21, respectively. The samples were stored in their original packaging in a dry, well-ventilated room at room temperature until the tests were performed.

Reagents and solutions

All reagents were of analytical grade. Ultrapure water (18.2 MΩ·cm), obtained from a Milli-Q® system (Millipore Corporation, USA), was used for the preparation of all solutions. For the determination of total phosphorus, a phosphate standard solution was prepared from a stock solution where 1.0 mL was equivalent to 0.2 mg of P_2O_5. Nitric acid (HNO_3, 65% w/v, Sigma-Aldrich, p.a.) and hydrochloric acid (HCl, 37% w/v, Fmaia, Brazil) were used in the ultrasound-assisted extraction and microwave-assisted digestion processes of the samples. To prevent contamination, all glassware was previously immersed in a 5.0% (v/v) nitric acid solution (Sigma-Aldrich, p.a.) for 24 hours and then rinsed with ultrapure water.

Determination of total phosphorus

Microwave-assisted digestion (MAD)

The total phosphorus digestion of the cane syrup matrix was performed using microwave-assisted digestion in a closed system (Anton Paar Multiwave GO Plus, Brazil). For the procedure, 1.0 g of cane syrup (wet basis) was weighed into a high-pressure-rated HVT50 vessel (PTFE-TFM, rated up to 100 bar), with the estimated maximum pressure during digestion ranging from 50 to 80 bar. Then, 5.0 mL of concentrated nitric acid (65%, v/v) and 1.0 mL of ultrapure water were added. The digestion process was conducted in two stages: (1) gradual heating (ramp) for 10 minutes up to 100 °C, followed by a 2-minute hold; (2) gradual heating for 20 minutes up to 200 °C, with an 8-minute hold, and a pressure release rate of 10 $bar \cdot min^{-1}$. The total duration of the procedure was 50 minutes. After cooling, the digested solution was transferred to a volumetric flask and diluted with ultrapure water to a final volume of 25.0 mL. The entire procedure was performed in duplicate. Additionally, for each digestion cycle, a blank was prepared in duplicate, containing only 5.0 mL of concentrated nitric acid.

Ultrasound-assisted extraction (UAE)

Cane syrup samples (0.5 g, wet basis) were directly placed into 15.0 mL Falcon tubes. The extraction solution, containing 1.70 mL of concentrated HNO_3, 2.0 mL of concentrated HCl, and 1.30 mL of ultrapure water, was added to the tube, totaling a volume of 5.0 mL. After manual homogenization, the tubes containing the samples were subjected to sonication in an Elmasonic P-30H ultrasonic bath (Analitica, Brazil) for 10 minutes. The process was carried out using an ultrasonic bath operating at a frequency of 37 kHz, with a

power of 100 W and a controlled temperature of 25.0 ± 2.0 °C. After sonication, the samples were transferred to 10.0 mL volumetric flasks and filled up with ultrapure water. An analytical blank was prepared using the same procedure. All assays were performed in duplicate.

Total phosphorus analysis

The total phosphorus content in cane syrup was determined by its complexation in an acidic medium with molybdate and vanadate ions. This reaction results in the formation of a heteropolyacid, a yellow complex, as described by References No. 46 and No. 47, which absorbs visible radiation at 420 nm. For solution preparation, a 1.0 mL aliquot of the solution resulting from the processes of digestion (UAE and MAD) previously described was taken. This aliquot was transferred to a 10 mL volumetric flask, and 2.5 mL of the vanadomolybdate complexing agent was added. The volume was then completed with ultrapure water. After a 10-minute reaction time, the absorbance was measured using a UV-Vis spectrophotometer (Spectrum model SP-2000 UV) at 420 nm. The analyses were conducted in duplicate. The intensity of the radiation absorbed by the complex was proportional to the amount of phosphorus pentoxide (P_2O_5) present in the sample. Therefore, the total phosphorus concentration was calculated in mg P_2O_5 kg^{-1} and also expressed as the extraction rate, considering microwave-assisted digestion as the reference value, according to Equation 1.

$$Extraction\ (\%) = \frac{C_{UAE}}{C_{MAD}} \times 100 \qquad (1)$$

where:

C_{UAE} is the total phosphorus concentration determined after ultrasound-assisted extraction, expressed in mg kg^{-1}.

C_{MAD} is the total phosphorus concentration after microwave-assisted digestion, also expressed in mg kg^{-1}.

Multivariate optimization using Simplex-Centroid Mixture Design

The instrumental conditions for ultrasound-assisted extraction, such as frequency (37 and 80 kHz), power (100 and 300 W), sonication time (10 and 40 min), temperature (25 °C), tube position in the ultrasonic bath (erosion method with aluminum foil), mass-to-volume ratio (1:5 to 1:50 g mL^{-1}), and HNO_3:H_2O ratio (50:50% v/v), were optimized using a univariate strategy based on the independence of the instrumental factors, as indicated by preliminary tests. The detailed experimental conditions, including the optimized parameters, are described in Alves et al.[30]

To evaluate the total phosphorus extraction efficiency using the solvents HNO_3, HCl, and ultrapure water, a simplex-centroid mixture design was applied (Table I), following the methodology described by Barros Neto, Scarminio, and Bruns.[48] To adjust the mathematical models and assess experimental error, four replicates were performed at the central point, and additional replicates at pure components and binary mixtures to evaluate possible synergistic or antagonistic effects.[49] Additionally, to assess the influence of potential interferents in the total phosphorus analysis by spectrophotometry, arising from the composition of the cane syrup sample or the ultrasound-assisted extraction process, spectra were obtained from the scans of the experiments conducted in the mixture design (Table I) and from the solution resulting from microwave-assisted digestion. These scans were performed in the range of 400 to 500 nm, using a Shimadzu UV-Vis spectrophotometer, model UV-1280.

Subsequently, to determine the optimal extractor solvent conditions, the extraction rate values (Equation 1) for total phosphorus and the analysis of the scan spectrum profiles from the mixture design experiments (Table I) and microwave-assisted digestion were used. An analysis of variance (ANOVA) was applied to generate the adjusted statistical models. The lack of fit and the model significance, at a 95% confidence level, were also verified. Parameters such as the F_{reg} value, F_{lof} value, *p*-values, determination coefficients (R^2 and adjusted R^2), the square root of the residual mean square of the model, residual plots, and contour plots for the mixtures were also evaluated.[50] All analyses were performed using statistical software Minitab for Windows version 16.2.2.[51]

Method validation

The validation study was conducted by regulatory guidelines,[52-54] evaluating the figures of merit: selectivity, linearity, limits of detection (LOD) and quantification (LOQ), precision, and accuracy. Selectivity was investigated by comparing the confidence intervals of the slope, at the 95% confidence level, of the external standard (ES) and standard addition (SA) analytical curves in the concentration range of 0.2 to 1.8 mg L^{-1}, in order to identify possible matrix effects according to the recommendations of the References No. 55 and No. 56. Linearity was evaluated for the analytical curves obtained using three standardization methods: ES (external standard in aqueous solution), ES-ME (external standard in the extraction medium, and SA (standard added to the cane syrup matrix diluted at a 1:10 w/v ratio). All curves were subjected to sonication in an ultrasound bath in optimized UAE conditions.

The adequacy of the analytical curves was verified by linear regression and a lack-of-fit test, both at a 95% confidence level.[43] The limits of detection (LOD) and quantification (LOQ) were determined from the external standard analytical curve in the extraction medium (ES-ME), using the standard deviation (*s*) of ten analytical blanks. The LOD was calculated as 3 *s*/*b* and the LOQ as 10 *s*/*b*, where *b* is the slope of the analytical curve.[52,57] Precision was assessed by repeatability estimates ($n = 5$) and intermediate precision, which was evaluated over five consecutive days, with triplicate measurements performed each day ($n = 3$). The adequacy of the intermediate precision was evaluated by calculating the Horrat value.[54] Accuracy was validated by comparing the total phosphorus content determined by the proposed method (ultrasonic-assisted extraction) with that obtained using the reference method (microwave-assisted digestion) for five cane syrup samples (CS06, CS08, CS15, CS18, and CS19), analyzed in triplicate, using a paired *t*-test at a 95% confidence level.

RESULTS AND DISCUSSION

Optimization of ultrasound-assisted extraction of total phosphorus in cane syrup

The total phosphorus content was determined using a molecular absorption spectrophotometric method in the visible region by forming a yellow-colored complex between phosphorus and vanadate and molybdate ions, which absorb at 420 nm. Patnaik gives the complexation reaction by Equation 2.[58]

$$12\,MoO_3 + H_2PO_4 + HNO_3 \rightarrow [PMo_{12}O_{40}]^{3-} + H_2O \rightarrow [PMo_{12}O_{40}]^{3-} + VO_3^- \rightarrow [PMo_{11}VO_{40}]^{3-} \quad (2)$$

This reaction results in the formation of a heteropolyacid of the Keggin type, responsible for the characteristic yellow color used in the quantification.

To determine the composition of the extraction solvent that would provide the best total phosphorus extraction rates in cane syrup, a simplex-centroid mixture design was applied (Table I). Acidic solvents were chosen as components of these mixtures because their efficiency in the extraction phase is well-documented in the literature, especially when combined, due to their oxidizing or complexing properties.[20,23,59] Additionally, the formation of more reactive products can occur, such as in the case of the mixture of hydrochloric acid and nitric acid (aqua regia), which accelerates the extraction process of elements from the matrix of interest.

Table I. The simplex-centroid mixture design applied to optimize the extraction solvent for total phosphorus analysis in cane syrup

	Extraction solvent composition (mL)			Extraction rate (%)
Experiments	HNO_3	HCl	H_2O	Total Phosphorus
1	5.00	0.00	0.00	77.38
2	0.00	5.00	0.00	131.17
3	0.00	0.00	5.00	121.02
4	2.50	2.50	0.00	78.94

(continued on next page)

Table I. The simplex-centroid mixture design applied to optimize the extraction solvent for total phosphorus analysis in cane syrup (continued)

	Extraction solvent composition (mL)			Extraction rate (%)
Experiments	HNO_3	HCl	H_2O	Total Phosphorus
5	2.50	0.00	2.50	112.47
6	0.00	2.50	2.50	125.45
7	1.67	1.67	1.67	103.40
8	1.67	1.67	1.67	103.30
9	1.67	1.67	1.67	100.47
10	1.67	1.67	1.67	101.73

Color changes were observed in the mixture design experiments (Table I) before and after the ultrasonic-assisted extraction (UAE) (Figure 1). These visual alterations indicate potential matrix interferences in spectrophotometric analysis, as components present in cane syrup or formed during extraction may absorb at the same wavelength (420 nm) as the phosphorus–vanadomolybdate complex. This overlap can lead to overestimated total phosphorus levels, with extraction rates exceeding 100% in some experiments (Table I).

The standard spectrophotometric method employed in this study involves the complete oxidation of organic matter and dissolution of the remaining residue in hydrochloric acid, as in conventional sample preparation techniques (e.g., dry ashing, wet or microwave-assisted digestion).[19] These procedures remove potential interferents. In contrast, the UAE does not eliminate all organic content, depending on the solvent and sample composition, allowing matrix interferences to persist in the final solution.

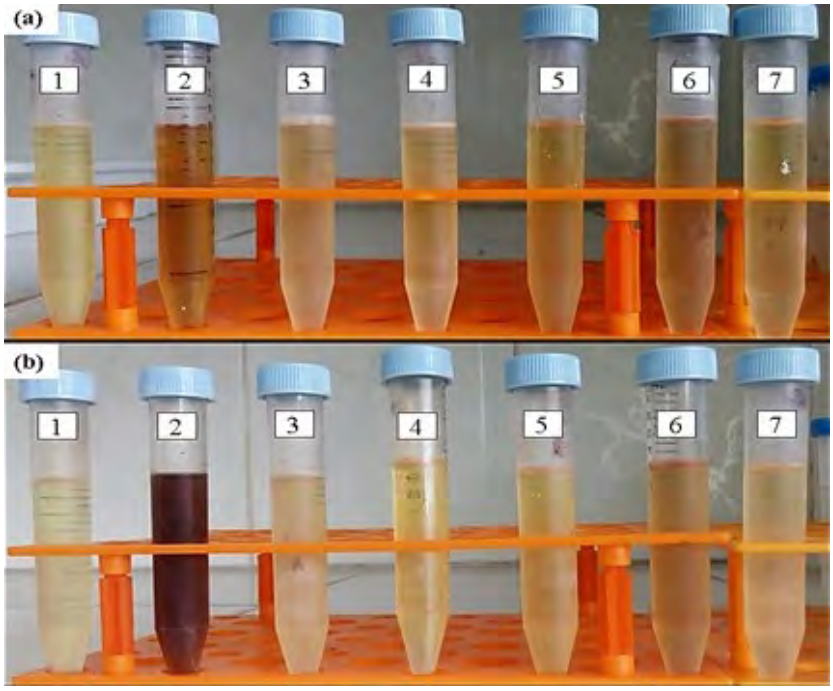

Figure 1. Color of solutions of experiments of simplex-centroid mixture design described in Table I: (a) before the sonication process and (b) after the sonication process.

Before sonication (Figure 1a), cane syrup solutions exhibited a natural yellow hue, attributed to intrinsic pigments such as chlorophylls, carotenoids, and anthocyanins.[58,59] However, processing and storage can lead to the formation of additional pigments, including Maillard reaction products (melanoidins) and oxidized phenolics, which also contribute to browning and may absorb near 420 nm.[27,28,60] After sonication, a reduction in color intensity was observed in experiments 1, 3, 4, 5, and 7, while experiments 2 and 6 showed evident darkening (Figure 1b). These two experiments used concentrated HCl or its binary mixture with water (50:50, v/v), suggesting that solvent composition strongly influences pigment formation and contributes to matrix interference.

To investigate this, visible spectra (400–500 nm) were recorded for the extracts after complexation (Figure S.1, Supplementary Material). Figure 2 presents the spectra for the most affected conditions (Experiments 2, 3, and 6). Extracts obtained using pure HCl showed the most pronounced absorbance at 420 nm and visual darkening, indicating significant matrix interference. This effect arises from the interaction between the solvent composition and ultrasonic cavitation, which enhances solvent–matrix interactions and promotes the release of compounds from the matrix.[61] In HCl-rich systems, cavitation may trigger Maillard-type reactions, leading to the formation of melanoidins that interfere with detection. In contrast, ternary solvent systems (Experiments 7–10) suppressed pigment formation despite being subjected to the same cavitation conditions, highlighting the critical role of solvent composition.

These ternary mixtures (HNO_3:HCl:H_2O) also achieved extraction rates closest to 100% (Table I), with minimal spectral interference (Figure S.1). The mildly acidic environment (pH ≈ 4) promotes solubilization and hydrolysis of organic phosphorus,[62] while cavitation enhances dispersion and fragmentation of macromolecular structures.[25,63,64]

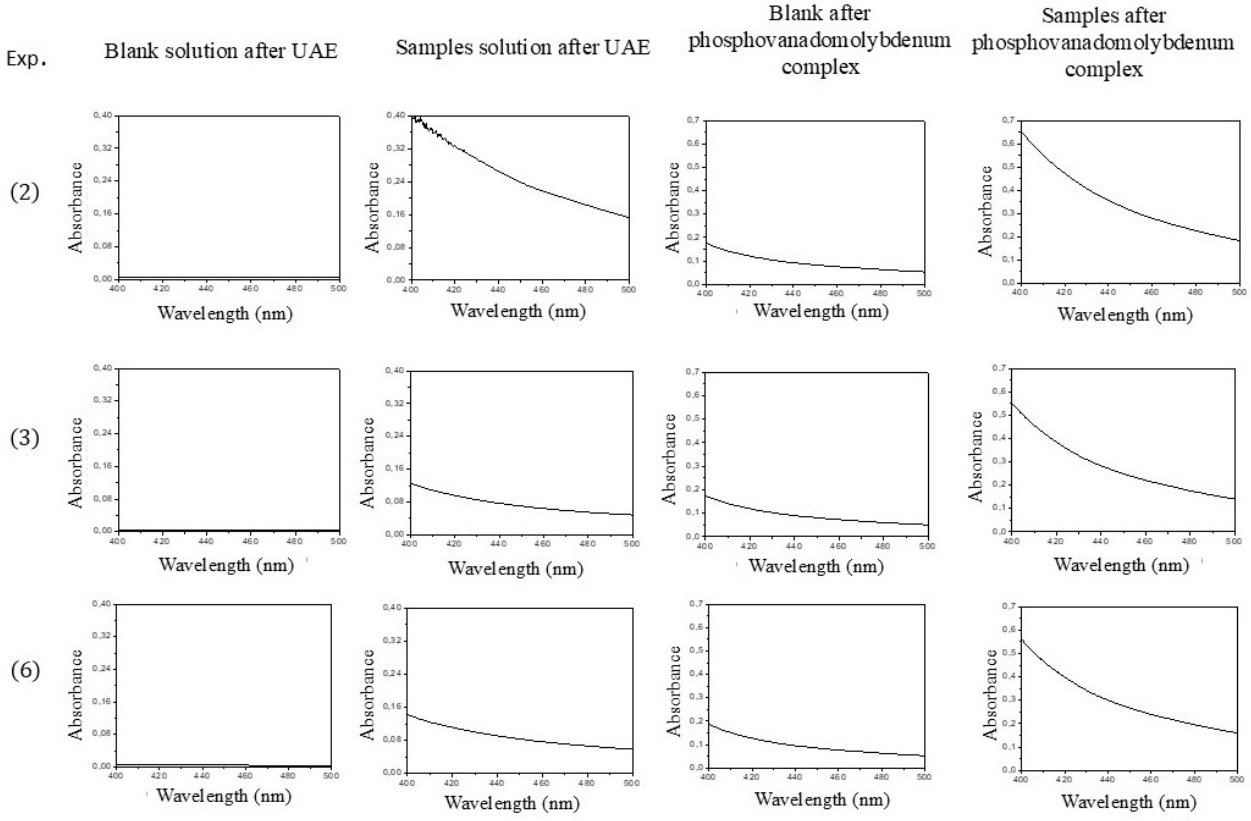

Figure 2. Overlap of UV–Vis Spectra of Experiments 2, 3, and 6 of Table I, considering blank solutions and sample solutions after UAE process and after phosphovanadomolybdenum complex formation.

Nonetheless, phosphorus species in cane syrup (such as phosphates, phytic acid, and phytates) may form insoluble metal complexes depending on pH,[65] which complicates extraction. Phytic acid can also chelate metals, suppress oxidative processes, and hinder phosphorus recovery.[66] These interactions contribute to matrix interference, whether through colored complexes or phosphorus-bound chromophores. Therefore, extraction in a moderately acidic medium is recommended, with conditions optimized to balance recovery efficiency and minimize spectral interference, considering the analytical method and matrix complexity.

To evaluate which mathematical model best fits the experimental data (Table I), an Analysis of Variance (ANOVA) characteristic of the simplex-centroid mixture design was performed. The results of the evaluated models are presented in Table II.

Table II. Analysis of Variance (ANOVA) for the optimization of the total phosphorus ultrasound-assisted extraction from cane syrup at a 95% confidence level

Models	SS[(1)]	df[(2)]	MS_{adj}[(3)]	$F_{regression}$	*p*-values	MS square root	R^2 (%)	R^2_{adj} (%)
Linear	2174.0	2	1087.0	1061[a]	0.008	10.12	75.19	68.10
Quadratic	2881.0	5	576.20	222.8[b]	0.000	1.60	99.64	99.20

Quadratic Model	SS[(1)]	df[(2)]	MS_{adj}[(3)]	F_{lof}	*p*-value
Residual Error	10.34	4	2.585	–	–
Lack of Fit	4.49	1	4.487	2.30[c]	0.227
Pure error	5.85	3	1.951	–	–
Total	2891.35	9	–	–	–

[(1)]SS = Sum of square; [(2)]df = degrees of freedom; [(3)]MS_{adj} = mean square adjusted;
[a]$F_{critical\ (0.05;2;7)}$ = 4.74; [b]$F_{critical\ (0.05;5;4)}$ = 6.26 and [c]$F_{lof\ (0.05;1;3)}$ = 10.1

The evaluated models showed statistical significance ($F_{regression} > F_{critical}$; $p < 0.05$) (Table II). However, the linear model presented a low coefficient of determination (R^2 = 75.19%), indicating a significant influence of the residuals (Figure 3). On the other hand, the quadratic model exhibited high coefficients of determination (> 99%), demonstrating a better fit to the experimental data (Figure 3). Another parameter assessed was the square root of the mean squared residual, which showed a lower value for the quadratic model (1.60) compared to the linear model, again confirming a better fit of the quadratic model to the data of Table I. Additionally, it was verified that there was no lack of fit of this model, at 95% confidence level ($F_{lof} < F_{critical}$; $p > 0.05$). Thus, the quadratic model was considered the most suitable for describing the experimental data and determining the optimal conditions for ultrasound-assisted extraction.

The equation describing the fitted quadratic model was also determined, aiming to identify the optimal extraction conditions for the three components of the mixture. The adjusted model is represented by the polynomial described in Equation 3, where y represents the percentage of total phosphorus extraction, while the coefficients b_1, b_{12}, and b_{123} represent the parameters of the regression function.

$$y = b_1x_1 + b_2\,x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}\,x_1x_3 + b_{23}x_2x_3 \qquad (3)$$

The calculation of the regression equation showed that there are statistically significant interactions between the binary component systems. The generated quadratic model is represented in Equation 4.

$$y = \mathbf{15.52}\ (\pm\mathbf{0.319})x_1 + \mathbf{26.28}\ (\pm\mathbf{0.319})x_2 + \mathbf{24.25}\ (\pm\mathbf{0.319})x_3 - \mathbf{4.253}\ (\pm\mathbf{0.276})x_1x_2 + \mathbf{1.923}\ (\pm\mathbf{0.276})x_1x_3 - 0.304\ (\pm 0.276)x_2x_3 \qquad (4)$$

The values for x_1, x_2, and x_3 represent the individual components of the mixture (HNO_3, HCl, and H_2O), while the values in parentheses indicate the confidence intervals of the coefficients. The significance of the components was evaluated using the *t*-test, with the critical points highlighted in bold. These critical points allow for the calculation of the predicted values for the tested models. The predicted values were then used in the linear regression technique to assess the suitability of the established model, based on the analysis of the plots of the experimental versus the adjusted values from the linear and quadratic models (Figure 3).

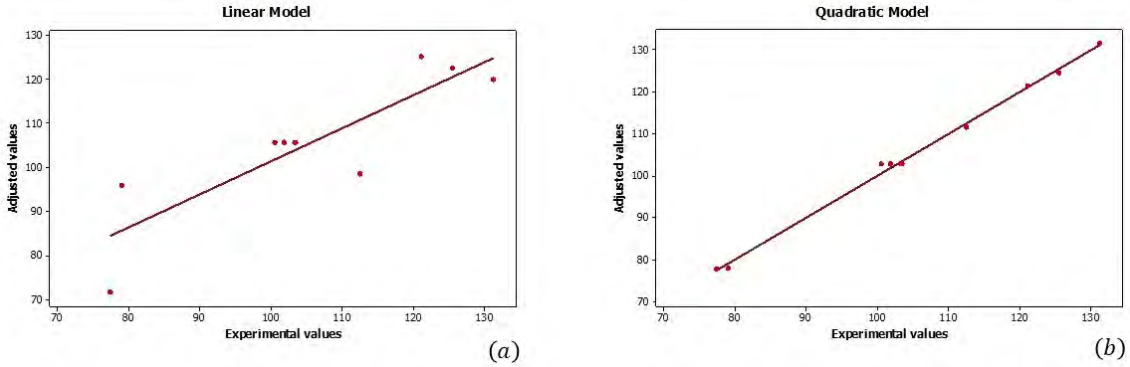

Figure 3. Plots for the correlation between the experimental and adjusted values (a) for the linear model and (b) quadratic model for optimization of the total phosphorus extraction from cane syrup using UAE.

After evaluating the adjusted mathematical model, a contour plot (Figure 4a) was generated to identify the solvent mixture composition with the highest efficiency in total phosphorus extraction rates. Additionally, the application of a *t*-test and the analysis of the Pareto chart (Figure 4b) allowed for the assessment of the individual effects of pure extraction solvents and solvent mixtures on the ultrasound-assisted extraction process.

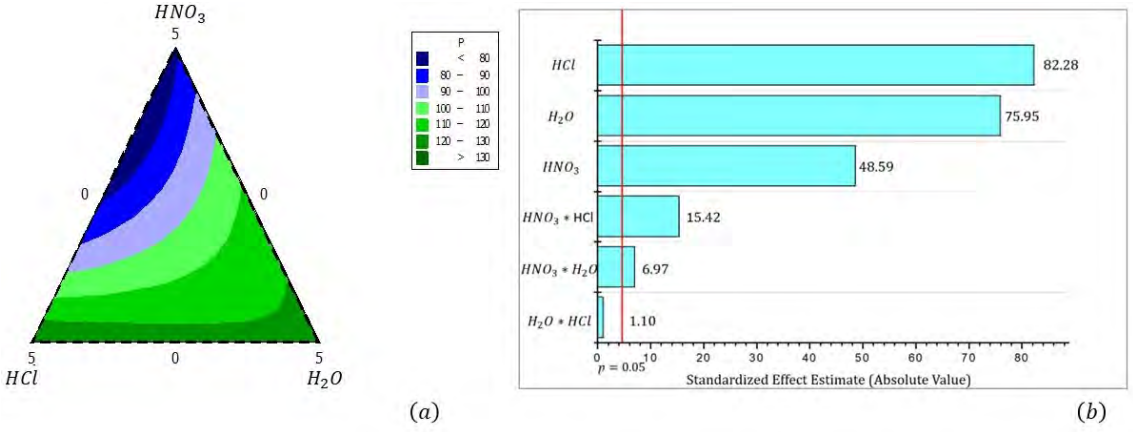

Figure 4. Contour plots (on the left) and Pareto charts (on the right) of the quadratic model adjusted from the simplex-centroid mixture design.

Figure 4a shows a significant interaction among the three extraction components, with total phosphorus extraction rates nearing 100% in the central region of the contour plot, corresponding to a ternary mixture. This indicates that phosphorus is strongly associated with the matrix, as it is a key component of macromolecules like phospholipids, nucleotides, and sugar phosphates.[65] In cane syrup, phosphorus exists mainly as phosphates, phytic acid, and phosphoproteins, which often form insoluble complexes.[66] Therefore, efficient extraction requires reactive solvent systems capable of disrupting these bonds.

The Pareto chart (Figure 4b) confirms that concentrated HCl had a significant positive effect on phosphorus extraction, likely due to its ability to form soluble complexes with phosphorus compounds. However, as previously discussed (Figure 1), pure HCl can also promote the formation of colored byproducts, which may compromise spectrophotometric analysis. Significant interactions were also observed between HNO_3 and H_2O, as well as between HNO_3 and HCl ($p < 0.05$), indicating a synergistic effect when these solvents are combined.

Mixtures of nitric and hydrochloric acid are known to enhance extraction efficiency, benefiting from the oxidative strength of HNO_3 and the complexing capacity of HCl.[23] Additionally, water contributes by lowering the viscosity of the mixture, promoting better cavitation during sonication and enhancing matrix disruption.

An individual desirability profile (Figure S.2, Supplementary Material) was used to identify the optimal solvent proportions. The condition that maximized total phosphorus extraction (desirability = 1.000) consisted of 1.67 mL of HNO_3, 2.00 mL of HCl, and 1.30 mL of ultrapure water.

Method validation of UAE combined with UV-Vis spectrophotometry

Selectivity and linearity

The analysis of total phosphorus in complex matrices, such as cane syrup, poses a significant challenge for conventional analytical methods, mainly due to the matrix effect, which can compromise the accuracy and precision of the results. This issue becomes even more relevant when using ultrasound-assisted extraction, as sonication can amplify matrix-derived interferences, making it essential to assess the selectivity and efficiency of the extraction under different experimental conditions.

In this study, the selectivity of the methodology, combining ultrasound-assisted extraction with spectrophotometric analysis, was evaluated by comparing the confidence intervals of the slope of the external standard (ES) and standard addition (SA) analytical curves. These curves were constructed under the extraction conditions of experiments 2, 3, and 6 from Table I, which, as previously described, exhibited combined effects of matrix and the extraction process, evidenced by changes in the solution color before and after the sonication.

In light of this, the conditions for ultrasound-assisted extraction for total phosphorus quantification in cane syrup were investigated, with particular emphasis on analyzing the matrix effect induced by specific extraction solvents and their combinations. The analytical curves corresponding to experiments 2, 3, and 6 from Table I are illustrated in Figure 5, and the linear regression data, along with the confidence intervals of the slopes, are presented in Table III.

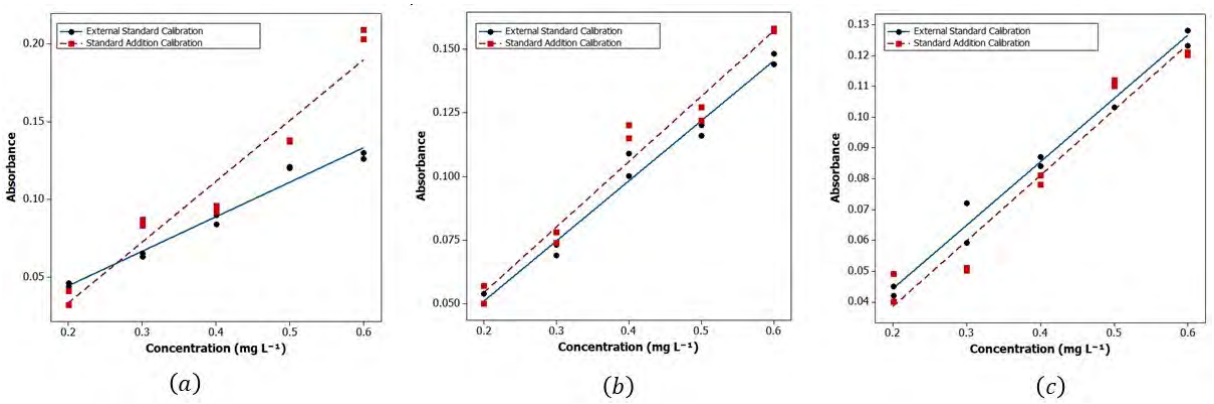

Figure 5. Analytical Curves corresponding to Experiments (a) 2, (b) 3, and (c) 6 of the Simplex-Centroid Mixture Design (Table I): External Standard in Medium Extracting (ES-ME) (●) vs. Standard Addition (■).

Table III. Linear Regression results for total phosphorus analysis using analytical curves of the External Standard in Medium Extracting (ES-ME) and Standard Addition (SA) corresponding to Experiments 2, 3, and 6 of the simplex-centroid mixture design

Extracting solvents[(2)]	Analytical Curves (0.2 – 1.8 mg L^{-1})	Regression[(1)]				
		Slope ± Confidence Interval	r	R^2	*F*	*p*-value
HCl	ES-ME	0.223 (± 0.030)	0.975	96.8%	277.30	0.000
	SA	0.392 (± 0.079)	0.944	93.4%	129.13	0.000
H_2O	ES-ME	0.245 (± 0.054)	0.964	92.2%	107.50	0.000
	SA	0.230 (± 0.068)	0.887	86.9%	60.55	0.000
HCl:H_2O (50:50%, v/v)	ES-ME	0.205 (± 0.021)	0.999	98.1%	468.09	0.000
	SA	0.212 (± 0.039)	0.955	94.5%	154.93	0.000

[(1)] $F_{critical\ (0.05;1;8)}$ = 5.32; [(2)]extracting solvents HCl, H_2O, and mixture of HCl:H_2O (50:50%, v/v) corresponds to the experiments 2, 3, and 6 from Table I.

The results in Figure 5 and the confidence intervals in Table III corroborate the scan spectra from experiments 2, 3, and 6 (Figure 2), confirming matrix interference in the spectrophotometric quantification of total phosphorus under certain extraction conditions. This interference was most pronounced when concentrated HCl or its aqueous mixtures at ≥ 50% v/v were used (Experiments 2 and 3, Table III). According to SANTE guidelines,[55] matrix effects reached 75.9% with pure HCl and 3.40% with the HCl: H_2O mixture, reinforcing the impact of solvent composition on analytical reliability.

These findings highlight the importance of evaluating method selectivity, especially when using concentrated acids that may promote the formation of interfering compounds. Few studies have addressed matrix effects in ultrasound-assisted extraction or microwave-assisted digestion for phosphorus in complex matrices,[12,15] emphasizing the relevance of this investigation.

To assess linearity and method robustness, analytical curves were constructed using five phosphorus standards (0.2–1.8 mg L^{-1}) under three standardization approaches: (i) external standard (ES) in water; (ii) standard addition (SA) in diluted matrix (1:10, v/v); and (iii) external standard in the optimized extraction medium (ES-ME: 1.67 mL HNO_3, 2.00 mL HCl, 1.30 mL H_2O). Despite differences in solvent composition, the slope confidence intervals of the three analytical curves overlapped (Table IV), indicating no significant matrix interference under any condition. All models showed good linearity ($F_{reg} > F_{critical}$, $p < 0.05$) with no lack of fit ($F_{lof} < F_{critical}$, $p > 0.05$), confirming the method's suitability for total phosphorus determination in cane syrup. The ES-ME approach was selected for its analytical throughput advantage.

Detection and quantification limits

The limits of detection (LOD = 0.296 µg g^{-1}) and quantification (LOQ = 0.898 µg g^{-1}) for total phosphorus using UAE combined with UV-Vis spectrophotometry were approximately six times lower than those obtained by microwave-assisted digestion (LOD = 1.772 µg g^{-1}; LOQ = 5.379 µg g^{-1}). These results indicate higher sensitivity of the UAE method for analysing low concentrations of total phosphorus in cane syrup.

Gamela et al.[67] reported LOD and LOQ values of 2 µg g^{-1} and 5 µg g^{-1}, respectively, for UAE in pepper samples, with slightly higher values for microwave-assisted digestion. Similarly, Liu et al.,[68] in a study on honey adulteration, reported a LOQ of 0.5 µg g^{-1} for phosphorus using microwave digestion. Fuentes-Soriano et al.[15] achieved even lower detection limits (LOD = 0.0372 µg g^{-1}; LOQ = 0.1241 µg g^{-1}) in nuts, using optimized microwave-assisted digestion and blue molybdenum spectrophotometry.

Despite variations among matrices and detection systems, the results of this study confirm that UAE offers superior sensitivity for total phosphorus determination in complex matrices such as cane syrup, reinforcing its potential as a promising and efficient alternative to conventional digestion methods.

Precision and trueness

The repeatability estimate showed that the RSD (%) values were below 11%, meeting AOAC criteria,[54] and the Horrat values for intermediate precision were under 1.3, indicating excellent precision of the UAE combined with UV-Vis spectrophotometry (Table IV).

Trueness was evaluated by comparing total phosphorus concentrations in five cane syrup samples obtained using ultrasound-assisted extraction and microwave-assisted digestion, applying a paired *t*-test. The results showed no significant differences between the two methods ($t_{calculated} < t_{critical}$; $p > 0.05$). Previous studies, such as Gamela et al.,[67] demonstrated that UAE yields results consistent with certified reference materials. Similarly, Fuentes-Soriano et al.[15] confirmed the accuracy of a modified spectrophotometric method for phosphorus determination in nuts, with results comparable to ICP OES. These findings support the high accuracy and reliability of the proposed method in this work.

Table IV. Results of the in-house validation study

Analytical curves (0.2 – 1.8 mg L^{-1})	Linear Regression[(1)]				
	Slope ± Confidence Interval	r	R^2	*F*	*p*-value
ES	0.2450 ± (0.0545)	0.964	92.2	107.5	0.000
SA	0.2300 ± (0.0683)	0.886	86.9	60.6	0.000
(ES-ME)	0.2350 ± (0.0263)	0.987	97.9	422.6	0.000

Limits		Precision			Trueness	
LOD	LOQ	Rep (RSD %)	IP	Horrat Value	paired *t*-test	
					$t_{calculated}$[(2)]	*p*-value
0.296[a]	0.898[a]	1.06	0.87	0.07	1.51	0.166
1.772[b]	5.379[b]					

Trueness of Extraction Methods in Cane Syrups		
Samples	Total Phosphorus (mg kg^{-1})	
	UAE	MAD
CS06	58.52±0.008	58.64±0.857
CS08	39.77±0.226	42.81±0.340
CS15	45.17±1.108	47.27±0.422
CS18	75.11±2.964	73.43±0.480
CS19	34.19±0.351	35.65±0.022

[(1)]$F_{critical\ (0.05;1;8)}$ = 5.32; [(2)]$t_{critical\ (0.025;8)}$ = 2.31; [a]Ultrasound assisted extraction and [b]Microwave-assisted digestion; LOD, detection limit (µg g^{-1}); LOQ, quantification limit (µg g^{-1}); Rep = repeatability; IP = intermediate precision. UAE = ultrasound-assisted extraction; MAD = microwave-assisted digestion.

Analysis of total phosphorus in cane syrup

The analysis of total phosphorus levels in different cane syrup samples is essential for understanding variations associated with the manufacturer or production region, contributing to ensuring the uniformity and quality of this sweetener. Additionally, this analysis helps identify patterns related to raw materials and production processes, including factors such as soil composition, element mobility in plants, agricultural practices, climatic conditions, cane species, and adopted management, that influence the concentrations of elements absorbed or introduced during processing.[30]

To classify or discriminate samples, the application of statistical tools, such as one-way ANOVA, combined with chemical analysis, is an efficient approach to detect significant differences between manufacturers or production regions.[68-70] Therefore, to evaluate the differences in the mean values of total phosphorus concentration in different cane syrup samples, a one-way ANOVA and a Tukey multiple comparison test were performed at 95% significance level.

The results of the ANOVA, followed by the Tukey post-hoc test, indicated that the cane syrup samples can be grouped based on the variability of total phosphorus content, according to their means, with the variations between region and manufacturer being statistically significant (Figure 6).

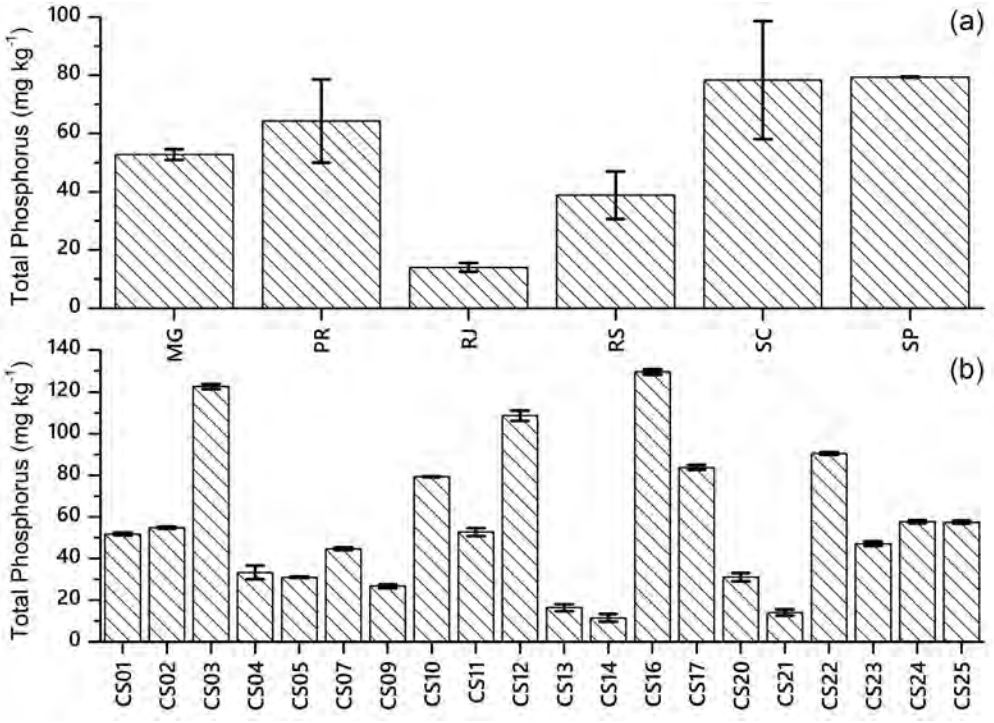

Figure 6. Total phosphorus content in cane syrup: comparison considering the variability between (a) producing regions and (b) among samples. MG = Minas Gerais; PR = Paraná; RJ = Rio de Janeiro; RS = Rio Grande do Sul; SC = Santa Catarina; SP = São Paulo.

Regarding the analysis of total phosphorus content (Figure 6), significant variations were observed between the cane syrup samples, suggesting the influence of several factors such as agricultural practices, soil composition, fertilization, and processing, which can vary both between regions and manufacturers.[30,71] Additionally, the biochemical competition between elements in the soil, such as calcium, magnesium, iron, manganese, and aluminum,[72,73] may affect phosphorus absorption, as exemplified by the differences observed in samples from the same manufacturer, (manufacturer A (CS01 and CS17) and manufacturer I (CS09 and CS13)) (Figure 6b), as well as regions with geographical proximity, such as the States of Santa Catarina (SC) and Rio Grande do Sul (RS) (Figure 6a).

Regarding geographic origin, the samples from Rio Grande do Sul, a Brazilian state recognized for its cane syrup production,[74] generally showed the lowest total phosphorus content. On the other hand, the samples from Santa Catarina (SC) exhibited considerable variability, with concentrations ranging from 30.98 mg kg^{-1} to 129.54 mg kg^{-1}. For instance, the CS16 and CS03 samples from Santa Catarina (SC) stood out with the highest levels (129.54 mg kg^{-1} and 122.65 mg kg^{-1}), while the CS13 and CS14 samples from Rio Grande do Sul (RS) showed the lowest phosphorus content, significantly differing from the other groups. This difference is particularly interesting because, given the geographical proximity, similar production practices would be expected. However, in southern Brazil, sugarcane is grown in a rainfed system, subject to climatic variations that influence the productivity and composition of its derivatives.[75]

Regarding the samples with intermediate concentrations, such as CS12 (108.60 mg kg^{-1}) and CS22 (90.49 mg kg^{-1}), these showed an overlap between groups, indicating a gradual transition between concentration ranges. Notably, the CS10 sample (79.34 mg kg^{-1}) from the State of São Paulo (SP) stood out for its low variability (SD ±0.175), suggesting standardization in its production. On the other hand, CS04 (33.26 mg kg^{-1}), also from Santa Catarina (SC), exhibited high variability (SD ± 2.951), indicating possible inconsistencies in processing.

This suggests that the region of cane syrup production directly influences its manufacturing process. In southern Brazil, the higher variability of total phosphorus content may be due to the involvement of small family-owned farms, which produce artisanal by-products using varied techniques. In contrast, regions with larger-scale production show greater standardization, likely associated with more industrialized processes.[75] Moreover, it is important to note that phosphorus is just one of several elements that can influence the product's composition. Therefore, while total phosphorus content allows for the discrimination of some samples, the inclusion of other chemical parameters or the use of multivariate analyses could generate clearer patterns, enhancing the reliability in identifying and discriminating cane syrup samples.

Evaluating the greenness of sample preparation procedures

The sample preparation stage is crucial in analytical procedures, as it helps minimize matrix interferences and ensures compatibility with instrumental techniques.[76] In total phosphorus analysis, methods such as microwave-assisted digestion and ultrasound-assisted extraction have shown high precision and sensitivity.[12,15,67,68] However, sample preparation can significantly increase the environmental impact of an analytical method due to high reagent consumption, high mass/solvent ratio, long analysis times, waste generation, and energy demand, among others. Therefore, it is essential to optimize this step to strike a balance between analytical efficiency and sustainability. In this study, the AGREEprep metric was applied, the first developed with a focus on sample preparation.[76,77] The pictograms for each of the sample preparation methods (UAE and MAD) are illustrated in Figure 7.

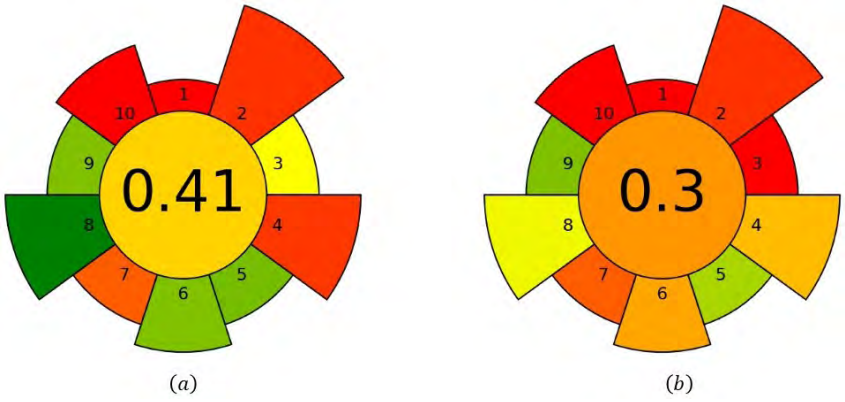

Figure 7. AGREEprep assessment results of sample preparation procedures: (a) UAE and (b) MAD.

The UAE procedure for total phosphorus extraction scores 0.41 on the AGREEprep metric (Figure 7a), making it more environmentally friendly compared to the MAD method, which received a score of 0.3 (Figure 7b). The strengths of the UAE include the reduced use of samples and reagents (criterion 5), the ability to perform simultaneous extractions in the ultrasonic bath, increasing analytical throughput (criterion 6), energy savings (criterion 8), and the use of efficient instrumental techniques after sample treatment (criterion 9). In contrast, the MAD method has lower environmental performance due to the use of concentrated inorganic acid (criterion 3), longer sample preparation times for series samples (criterion 6), and higher energy consumption. While MAD eliminates organics, consumes 62.50 Wh/sample, UAE only requires 4.17 Wh/sample (criterion 8).

CONCLUSIONS

The study highlights that the UAE is an effective method of sample preparation for total phosphorus analysis in cane syrup, particularly when conducted in a moderately acidic medium. The method's efficiency can be optimized while minimizing matrix interferences, as factors like sonication, syrup characteristics, and solvent composition may trigger secondary reactions, such as Maillard reactions, which affect the spectrophotometric analysis.

By combining specific reagents, UAE maximizes analyte extraction while reducing chemical interferences, underscoring the need to refine analytical methods for complex matrices. The study also demonstrates that the UAE offers high sensitivity, precision, and accuracy, with minimal environmental impact compared to MAD.

Additionally, the research indicates that the geographic origin and production practices of cane syrup samples contribute to significant variability in phosphorus levels, emphasizing the method's potential for quality control. The environmental assessment using the AGREEprep metric shows that the UAE is more sustainable than conventional methods, with a much lower energy consumption, reinforcing the importance of adopting greener, more efficient analytical practices.

Overall, the proposed methodology offers a faster, more sustainable approach for food analysis, balancing analytical performance with cost-effectiveness and the principles of green chemistry. It represents a viable alternative for quality control laboratories and the food industry.

Conflicts of interest

The authors confirm there are no financial or personal conflicts of interest related to this work.

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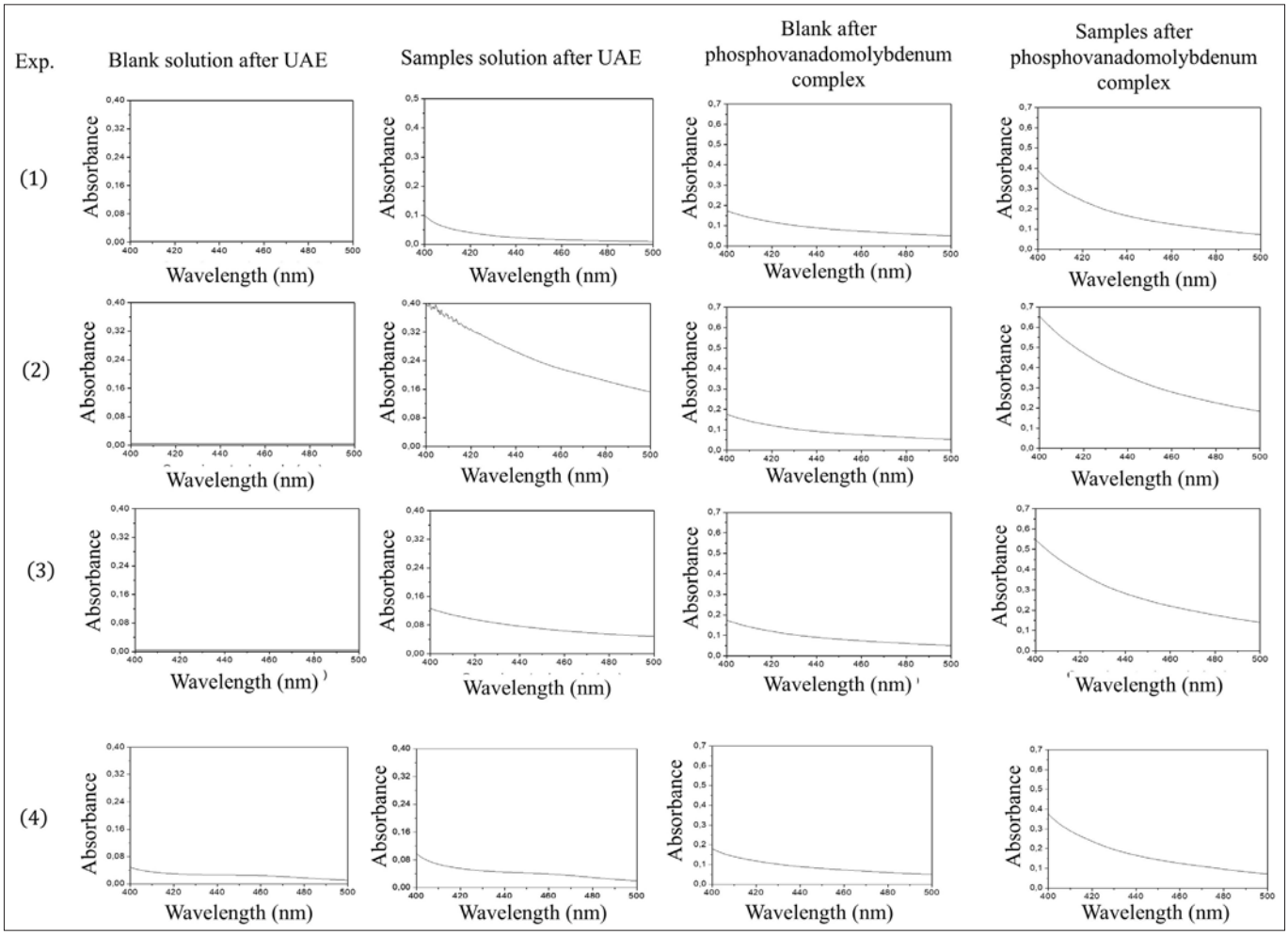
SUPPLEMENTARY MATERIAL



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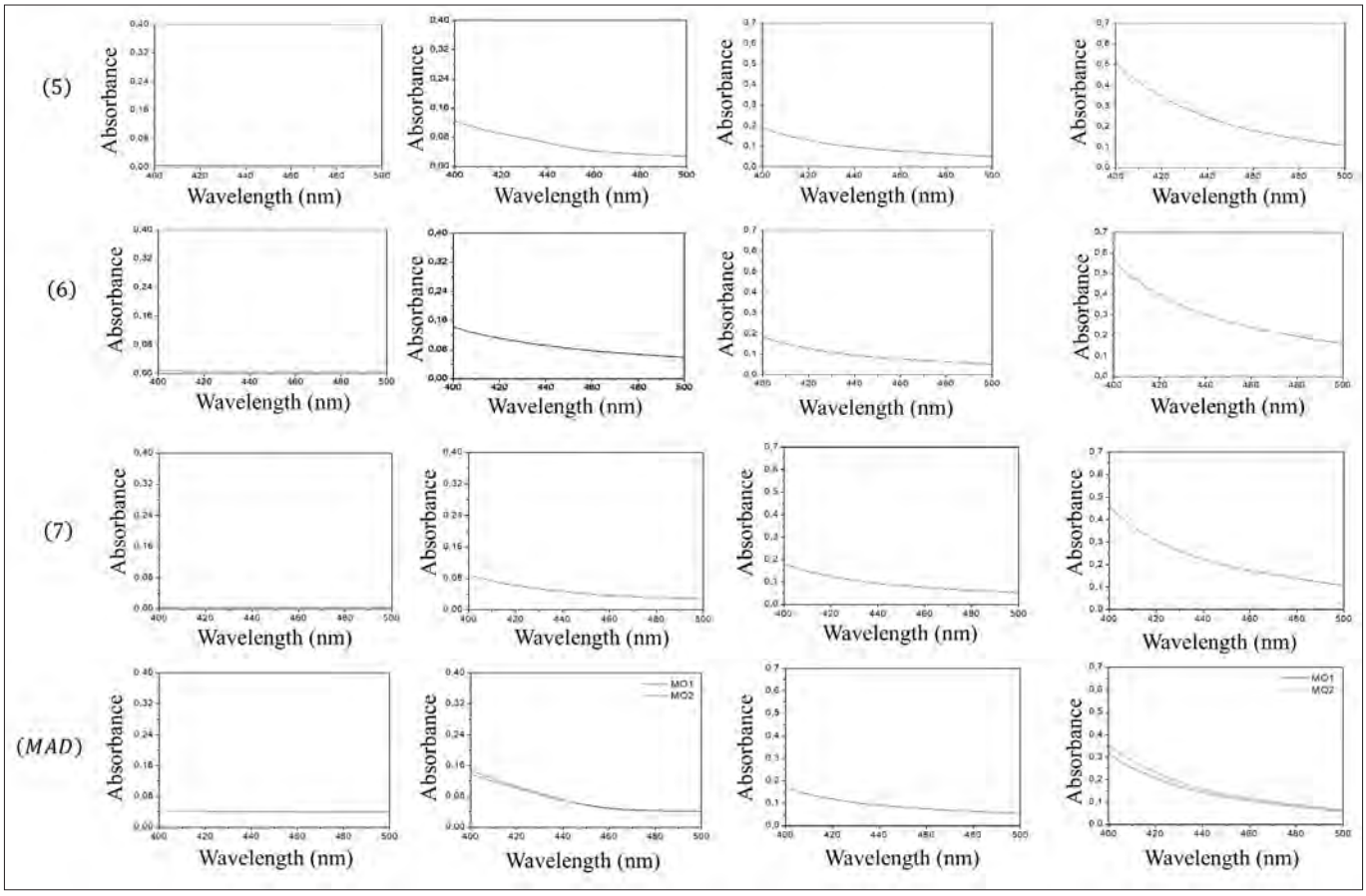

Figure S.1. Overlap of the UV–Vis spectra in the analysis of total phosphorus in the simplex centroid mixture design applied in the extraction solvent optimization.

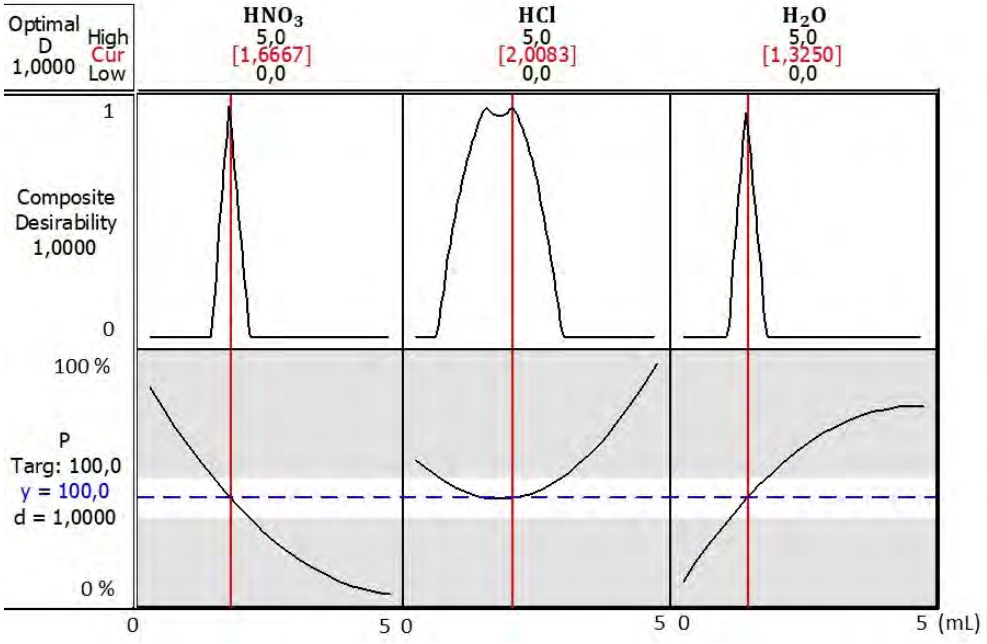

Figure S.2. Profiles for predictive values and individual desirability in optimizing the values of the extraction solvents.

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ARTICLE

A Green and Lean Method Certified by NEMI, ESA, AGREE, GAPI and BAGI for the Analysis of Ivermectin in Injection Solution for Veterinary Use

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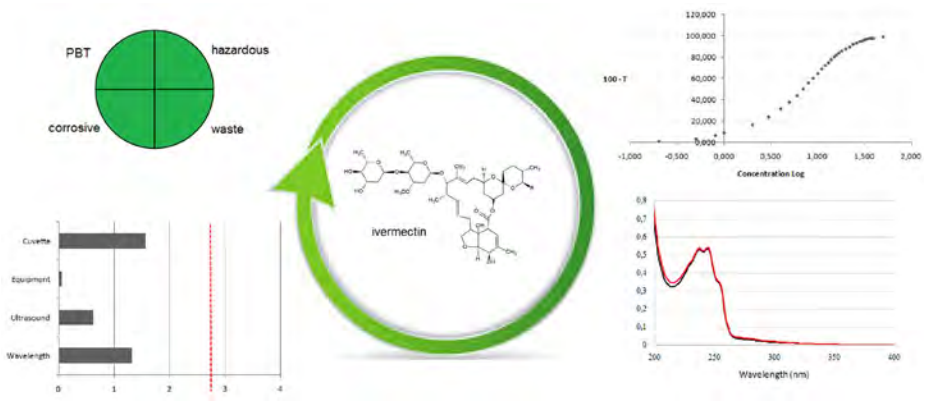

The use of antiparasitics, such as ivermectin (IVE), is extremely important to public health and the economy. Quality control and analytical development are necessary to guarantee the efficacy, safety, and quality of medicines. This work covered the development and validation of a green and lean method by UV to quantify IVE in injection solution for veterinary use. UV methodology using ethanol as a diluent, a quartz cuvette and a spectrophotometer at 245 nm were used. In order to bring objectivity in relation to the greenness of the proposed method, 5 tools were used: National Environmental Method Index (NEMI), Eco-Scale Assessment (ESA), Analytical GREEnness Metric (AGREE), Green Analytical Procedure Index (GAPI), and Blue Applicability Grade Index (BAGI). The proposed method was linear in the range of 6-16 µg mL^{-1}, precise (RSD < 5%), selective and indicative of stability by forced degradation, exact (100.07%) and robust against small and deliberate modifications. NEMI showed the 4 green quadrants, GAPI showed predominantly green and yellow quadrants, ESA, AGREE and BAGI showed scores of 93, 0.82 and 65, respectively. The method is an excellent and lean green option for evaluating final IVE product. It has an environmentally friendly footprint, which can be advantageously employed by pharmaceutical chemical laboratories worldwide.

Keywords: National Environmental Method Index, Eco-Scale Assessment, Analytical GREEnness Metric, Green Analytical Procedure Index, Blue Applicability Grade Index

INTRODUCTION

Ivermectin (IVE) is a macrocyclic lactone used as an anthelmintic in human and animal health. It is a semi-synthetic compound of > 80% IVE B1a ($C_{48}H_{74}O_{14}$) and < 20% IVE B1b ($C_{47}H_{72}O_{14}$) and is sold in pharmaceutical dosage forms such as tablets, pastes, and injectable solutions.[1-3] The fact that parasites cause harm to public health and the economy makes the development of analytical methods fundamental.

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They are indispensable for guaranteeing the effectiveness, safety, and quality of medicines, avoiding cases of resistance, residues in food, allergies, and any negative impacts on human, animal, and ecosystem health.[4-5]

However, just the existence of the analytical method is not enough; currently, the need is for the method to also be clean, green and optimized, based on the fundamentals of Green Analytical Chemistry (GAC).[6-16] This context brings objectivity regarding to the greenness of the methods, tools such as National Environmental Method Index (NEMI), Eco-Scale Assessment (ESA), Analytical GREEnness Metric (AGREE), Green Analytical Procedure Index (GAPI), Blue Applicability Grade Index (BAGI) are used.

NEMI is a graphical representation of a circle divided into four parts, which are categorized. The first corresponds to no products belonging to the list of persistent, bioaccumulative and toxic chemicals (PBT). The second corresponds to no product being on the hazardous waste list. The third corresponds to the pH of the sample being between 2 and 12. The fourth corresponds to the amount of waste generated being less than 50 g. The ESA is a penalizing tool. Thus, penalty points (PP) are reduced from a base of 100. Consequently, the closer the final score is to 100, the more sustainable the analytical method is considered. Scores >75 indicate excellent green analyses, > 50 points mean an acceptable green analysis, and <50 points are considered an inappropriate green analysis. The AGREE tool considers the 12 principles of GAC. Each principle has a score that ranges from orange to green. The result ranges from 0 to 1, and 1 represents the dark green color, i.e. high environmental performance. GAPI takes into account the entire analytical procedure, from sample collection to waste disposal. This tool uses a color scheme, in which there are two or three levels for evaluating the steps. There is an evaluation of reagents, procedures and instrumentation. Five staves are divided into 15 parts, which each part corresponds to a specific parameter. Additionally, a color-coding system, green (low), yellow (medium) and red (high), is used to represent the impact of the analyses. In addition, the central staves indicate the type of method, quantitative (circle present in the center of the pentagon) or qualitative (no circle in the center of the pentagon). BAGI assesses the practicality of the method. It belongs to White Analytical Chemistry (WAC) and is regarded as a complement to sustainable tools. It is represented by an asteroid, which the bluer it is, the more practical and easier the method is. The score can be between 25 and 100, where 25 corresponds to a worse performance of the method in relation to practicality and 100 represents excellent performance. Thus, the method is considered practical when a minimum value of 60 points is assigned.[17-23]

This study aims to develop and validate an ecological and lean method by UV for quantifying IVE in injection solution for veterinary application. Moreover, the NEMI, ESA, AGREE, GAPI, and BAGI tools will be used to evaluate the method's greenness.

MATERIALS AND METHODS

IVE standard with content of 99.8% for H2B1a/(H2B1a+H2B1b) and 96.1% for H2B1a + H2B1b. The sample used was an injection solution (50 mL) with a declared content of 1%. The raw material and sample were donated by Noxon®. Absolute ethyl alcohol (Sciavicco®) and purified water (Gehaka®) were used to prepare the solutions.

Equipment

Spectrophotometer model Genesys 10S UV-Vis (Thermo Scientific®), ultrasound (Unique®), water ultrapurifier (Gehaka®), analytical balance model AUW22OD (Shimadzu®), 10 mm quartz cuvettes with 4 mL capacity (Qualividros®), heating bath and UV light chamber were used.

Stock and work solution preparation

Stock solutions were prepared with the equivalent of 2.5 mg of IVE standard, which was transferred to a 50 mL volumetric flask. Then, a small amount of ethanol was added and taken to ultrasound for 15 minutes and then the volumes were completed with ethanol, obtaining a concentration of 50 µg mL^{-1}.

The working solutions were prepared from the stock solution, so that 6 concentrations, namely 6, 8, 10, 12, 14, 16 µg mL^{-1}, were achieved. The aliquots were transferred to a 5 mL volumetric flask and the volumes were completed with purified water.

Ringbom curve

The Ringbom curve was determined at 245 nm using 33 standard IVE concentrations ranging from 0.2 to 50 µg mL^{-1}, in order to define the linear region and thus proceed with the validation of the method. The analyses were performed in triplicate.

Validation parameters

The international guidelines were followed to validate the proposed analytical method, considering parameters such as linearity, precision, selectivity, accuracy and robustness.[24-31]

Linearity

The linearity of the proposed method was proven through statistical analyzes (straight line equation, least squares, correlation coefficient, analysis of variance – ANOVA and residual graph) of the results from concentrations 6, 8, 10, 12, 14 and 16 µg mL^{-1}.

Precision

Precision was evaluated at a concentration of 12 µg mL^{-1} regarding the proximity of the results at three different levels: intraday, interday, and interanalyst.

Intraday precision evaluated the proximity of results obtained on the same day and with the same analyst. Interday precision evaluated results acquired by the same analyst on different days. Finally, inter-analyst precision evaluated the proximity of results obtained by different analysts on different days.

The precision of the proposed method was established through the dispersion of results, based on the relative standard deviation (DPR %).

Selectivity

The selectivity of the proposed method was proven through comparison of the spectra of the sample and standard solutions at a concentration of 12 µg mL^{-1}, and also by the forced degradation method.

The forced degradation study or stress test for IVE in injection solution occurred at a concentration of 15 µg mL^{-1} under stress conditions: acidic (HCl 0.1 M for 2 hours at 60 °C), basic (NaOH 0.01 M for 1 hour at 60 °C), neutral (diluent for 1 hour at 60 °C) and photolytic (UV light for 24 hours at room temperature).

Accuracy

The accuracy of the proposed method was evaluated by standard recovery test, in which standard solutions are added to the sample solution (6 µg mL^{-1}) and then analyzed. Three levels were used: recovery 1 to 80% (8 µg mL^{-1}), recovery 2 to 100% (10 µg mL^{-1}) and recovery 3 to 120% (12 µg mL^{-1}). Accuracy was assessed in triplicate on three distinct days.

Robustness

The robustness of the proposed method was verified by modifications in the reading wavelength (245 nm - normal x 243 nm - modified), ultrasound time (15 minutes - normal x 10 minutes - modified), equipment (Thermo Scientific®, Genesys 10S UV-Vis - normal x Biospectro® SP-220 - modified) and cuvette capacity (4 mL - normal x 1 mL - modified).

The working solutions were prepared at a concentration of 12 µg mL^{-1} in triplicate. Variations were evaluated using the F test and *t* test.

Content analysis

Standard and sample solutions were prepared at a concentration of 12 µg mL^{-1}. Readings were taken at a wavelength of 245 nm through 6 replicates and over three days. The analysis result must comply with official compendia for IVE in injection solution.

National Environmental Method Index (NEMI)

NEMI issues (a) persistent, bio-accumulative and toxic (PBT); (b) hazardous; (c) corrosive and (d) waste were evaluated. The result was presented through the presence or absence of the color green in 4 quadrants, each representing the parameters mentioned above.

Eco-Scale Assessment (ESA)

The penalty points (PP) were calculated according to the Equation 1.

$$ESA = 100 - \left[\begin{array}{l}(\text{chemical reagents pictogram x quantity of reagents x signal words}) + \\ \text{energy} + \text{ocupacional hazard} + (\text{waste amount x waste characteristic})\end{array}\right] \quad \text{Equation 1}$$

Analytical GREEnness Metric (AGREE)

The analytical conditions of the proposed method were evaluated against the 12 GAC principles and the data was included in the AGREE calculator.

Green Analytical Procedure Index (GAPI)

In addition to the analytical choices, the processes involved and the instrumentation were evaluated and measured by GAPI.

Blue Applicability Grade Index (BAGI)

BAGI evaluated the 10 characteristics of the proposed method, namely: type of analysis, number of analytes evaluated simultaneously, instrumentation and analytical technique, number of samples which can be analyzed simultaneously, sample preparation, number of samples analyzed per hour, type of reagent and materials, preconcentration requirement, degree of automation and sample quantity.

RESULTS AND DISCUSSION

Ringbom curve

The Ringbom curve presents the compatible linear region for the validation stage (Figure 1A). In this case, the points chosen were 6, 8, 10, 12, 14 and 16 μg mL^{-1}.

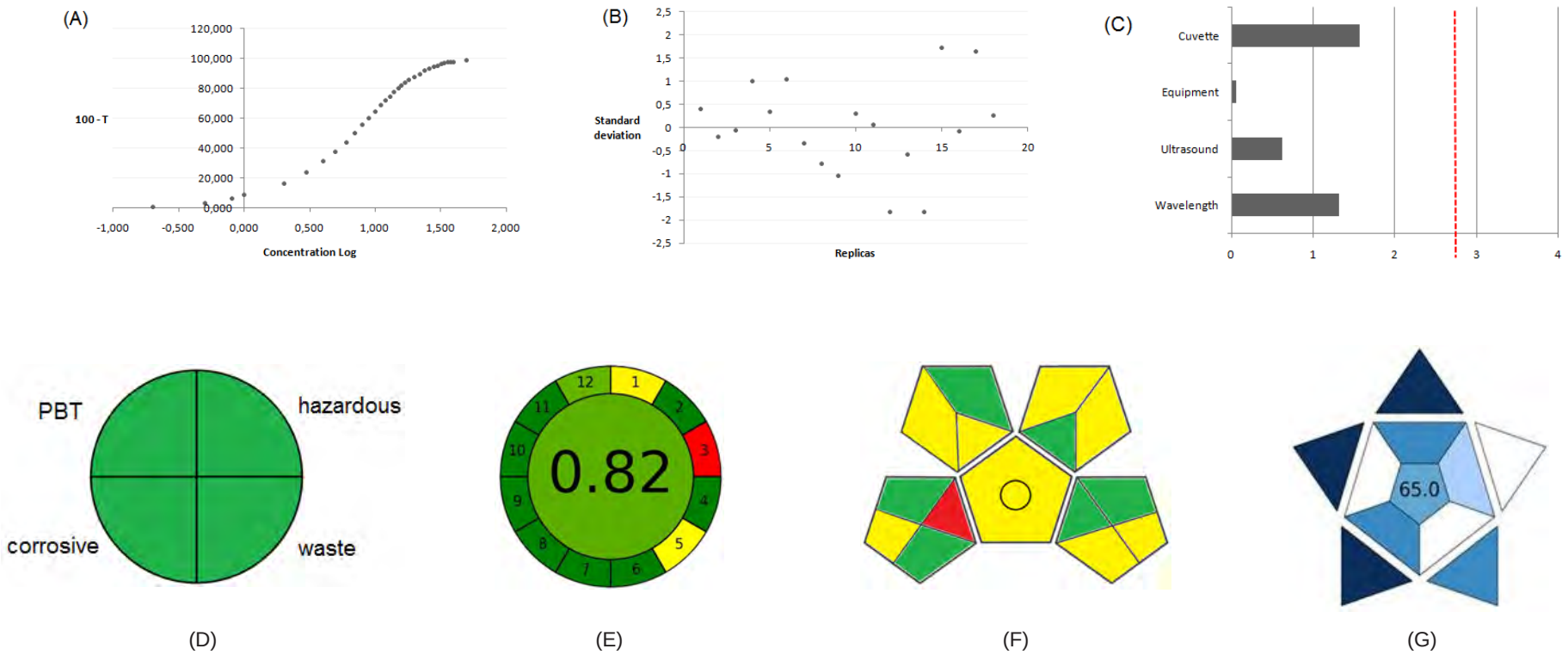
Linearity

The correlation coefficient was 0.9999, therefore, it is a value higher than 0.99 as recommended in the guides.[26,28] The linear regression was significant, while the linearity deviation was not, which corroborates the linearity of the method (Table I). Furthermore, the residual graph shows that the points are distributed randomly without a trend (Figure 1B).

Table I. ANOVA results to evaluate the linearity of the method

Parameters	Value
Wavelength (nm)	245
Linearity range (μg mL^{-1})	6-16
Slope	0.0503
Intercept	0.0557
Correlation coefficient (r)	0.9999
Regression	1994.09* (4.75)
Lack of fit	0.30 (3.26)

*Value p< 0.05



(D) (E) (F) (G)

Figure 1. (A) Ringbom curve, (B) residue graphical, (C) effect of robustness modifications, (D) NEMI, (E) AGREE, (F) GAPI, and (G) BAGI results.

Precision

The RSD (%) of the evaluated precision levels were smaller than 5%, thus demonstrating the proposed method´s precision (Table II).

Table II. Absorbance results to evaluate the precision of the method

Wavelength	Level	Absorbance						RDS (%)
		1	2	3	4	5	6	
245 nm	Intraday	0.529	0.525	0.534	0.536	0.529	0.527	0.79
	Interday	0.545	0.554	0.547	0.538	0.555	0.545	1.34
		0.538	0.537	0.531	0.536	0.542	0.539	
	Interanalyst	0.538	0.537	0.531	0.536	0.542	0.539	0.99
		0.529	0.525	0.534	0.536	0.529	0.527	

Selectivity

The overlap of standard and sample IVE spectra (Figure 2) shows the method's ability to identify IVE in the injection solution, since the presence of adjuvants did not reveal interference.

Furthermore, selectivity was proven through forced degradation. In the development and validation of methods, the stress test is an indication of stability for the method. It is important to highlight that each formulation presents different stress rates and conditions. Therefore, comparison of stress test results should be cautious; the way the pharmaceutical product was exposed (powder or solution), for how long and at what temperature are decisive for the comparison. The conditions chosen were strategically designed so that there was neither excessive nor insufficient degradation (Table III). Exacerbated stress can result in inappropriate conditions and endpoints, which increases the chances of generating degradation products of no interest for study, that is, degradation products that do not correspond to reality. On the other hand, ineffective stress generates insufficient results, providing false methods indicative of stability.[27,29-30] The objective of the present work was to show that the proposed method is indicative of stability, as in all stress conditions tested the method was able to attest to the degradation of IVE due to the change in the absorption profile (Figure 2).

Table III. Degradation values obtained in the stress test for IVE in injection solution

Degradation condition	Absorbance (time 0)	Absorbance (1*, 2**, 24*** hours)	Degradation (%)
Acidic (HCl 0.1 M at 80 °C)	0.615	0.577**	6.18
Neutral (ethanol at 60 °C)	0.860	0.747**	13.14
Basic (0.01 M NaOH at 60 °C)	0.586	0.520*	11.26
Photolytic (UV at 254 nm)	0.783	0.699***	10.73

Accuracy

The average recovery obtained on different days and in triplicate was 100.07% (Table IV). The value obtained in the recovery test is within the specification range for pharmaceutical analysis, which is 98 to 102%, therefore, the proposed method is accurate.

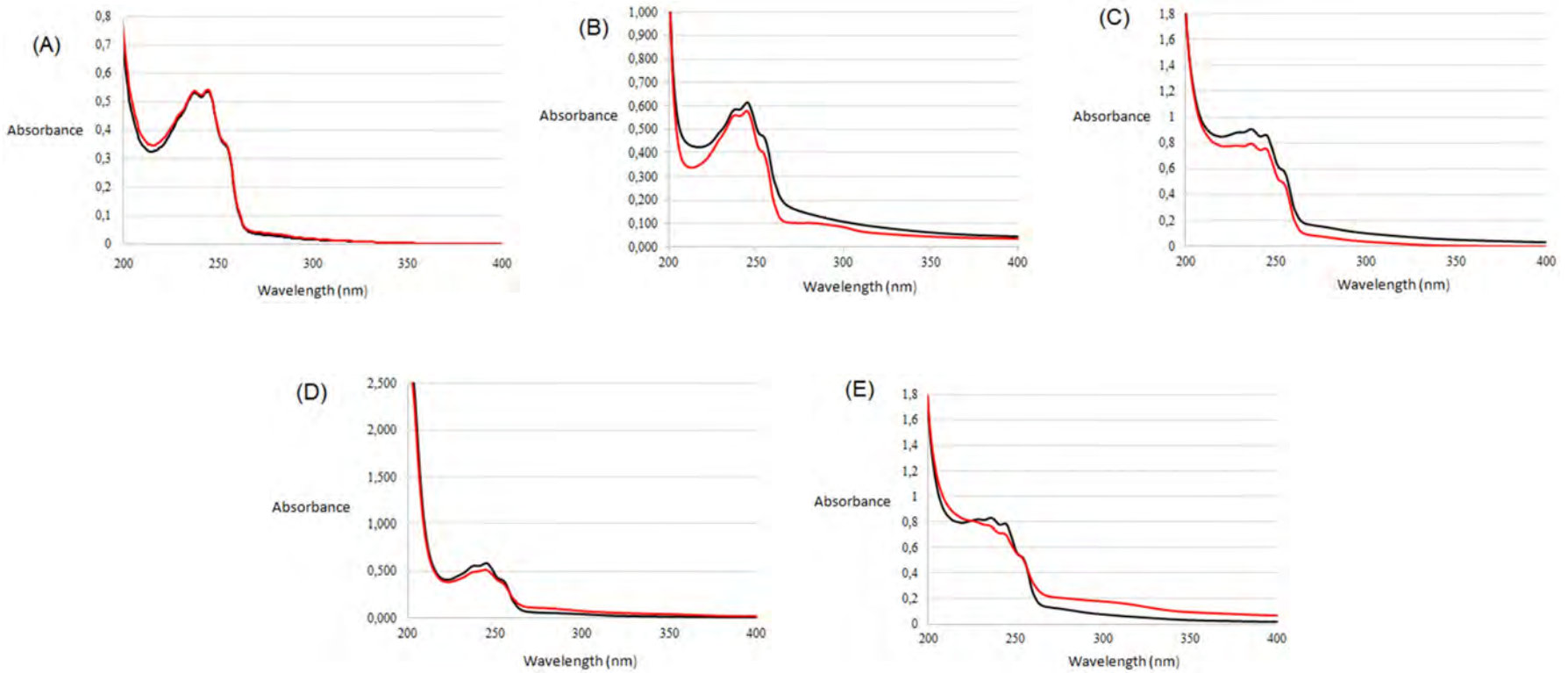


Figure 2. (A) Overlay of the spectra of the standard (black) and sample (red) IVE solutions at 12 μg mL^{-1} and 245 nm. From (B) to (E): absorption spectra profiles from the forced degradation test under the following conditions – (B) acidic, at 0 h (black) and 2 h (red); (C) neutral, at 0 h (black) and 1 h (red); (D) basic, at 0 h (black) and 1 h (red); (E) photolytic, at 0 h (black) and 24 h (red). All (B) to (E) spectra were recorded at 15 μg mL^{-1} and 245 nm.

Table IV. Accuracy results of the proposed method based on the recovery test

	IVE standard added (μg mL^{-1})	IVE standard recovered (μg mL^{-1})	Recovery* (%)	Mean recovery (%)	RSD (%)
R1	2.0	1.98	98.78		
R2	4.0	4.00	100.07	100.07	0.06
R3	6.0	6.08	101.35		

*Average of 3 determinations in triplicate

Robustness

There were no statistically significant differences for changes in wavelength, equipment, ultrasound time and cuvette capacity (Figure 1C). Thus, the proposed method is robust to such deliberate changes, that is, the $t_{calculated}$ was smaller than the $t_{tabulated}$ (2.78), which reveals that such changes do not impact the proposed method.

Content analysis

According to the American Pharmacopoeia,[29] the IVE content in the final product (injection solution) must present a lower value of 95% and an upper value of 105%, thus the value found (101.30%) using the proposed method meets specifications (Table V).

Table V. Results of the content assessment of IVE in injection solution by the proposed method

Day	Average content* (%)	Final content (%)	RSD (%)
1	101.73		
2	100.56	101.30	0.63
3	101.60		

*Average of 3 determinations in triplicate

National Environmental Method Index (NEMI)

NEMI evaluates four different parameters. The proposed method uses only ethanol, which is non-persistent, bioaccumulative, toxic, and dangerous. Therefore, it is not on the TRI and EPA list. In relation to the corrosive quadrant, the pH of the solution was 7, thus, the pH is in the range of 2 to 12. Furthermore, the residue per sample corresponds to 5 mL, being less than 50 mL. Therefore, the proposed method presents the 4 quadrants in green, as shown in Figure 1D.

Eco-Scale Assessment (ESA)

Assessment by ESA is based on PP, as shown in Equation 1. Therefore, in relation to the reagents, the quantity used is less than 10 mL (1 PP), the ethanol label contains two pictograms and the signal word 'Danger' (4 PP). Regarding the instrument, the spectrophotometer consumes energy <0.1 kWh per sample (0 PP). In relation to occupational risk, the analytical process is hermetized. Waste generation was 5 mL per sample (3 PP) and treatment is by degradation (1 PP).

Point count using Equation 1: ESA = 100 - [(1x4) + (0) + (0) + (3x1)] = 100 - 7 = 93

The analysis presented an ESA value of 93, therefore, the proposed method is indicated as an excellent green analysis.

Analytical GREEnness Metric (AGREE)

AGREE showed a score of 0.81 and a green color (Figure 1E), so with a value close to 1, the proposed method is considered green.

Green Analytical Procedure Index (GAPI)

GAPI is a semi-quantitative tool that evaluates processes from start to finish, so the pentagrams for the proposed method were filled mostly in green and yellow, that is, the proposed method has a low-medium environmental impact, as shown in Figure 1F.

Blue Applicability Grade Index (BAGI)

BAGI demonstrated that the proposed method is practical, since the score was 65 with the asteroid predominantly blue, as shown in Figure 1G.

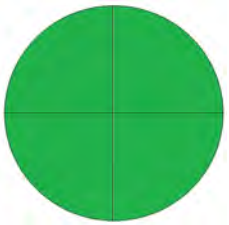
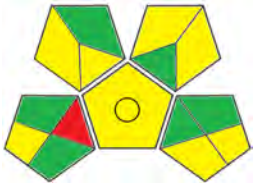
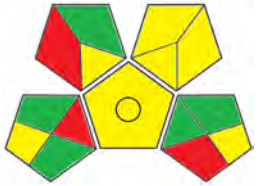
Environmental impact assessment of the proposed method

Considering the objective of routine analysis in the quality control of final IVE products, spectrophotometry is a fast, simple, easy-to-use, and economical technique that requires less solvent and generates less waste than high-performance liquid chromatography, for example. Furthermore, it offers greater sensitivity.[32-33]

Table VI compares the proposed method with a previously reported method for analyzing injectable IVE solutions. The proposed method employs ethanol as a diluent. Ethanol is both less toxic and more economical, not only in terms of purchase cost, but also in disposal. In contrast, methanol is metabolized into formaldehyde and formic acid, which are causing serious poisoning. Similarly, acetonitrile can yield cyanide upon metabolism, which causes respiratory toxicity.[10,35] The traditional method for evaluating IVE in injectable solution, as described in the USP, is HPLC. The mobile phase consists of acetonitrile, methanol, and purified water (106:55:39, v/v/v), using a 250 x 4.6 mm column with a flow rate of 1.5 mL min^{-1} and injection volume of 20 µL.[29]

Therefore, the proposed method offers advantages over both the literature method and the conventional method, the solvent used is considered green, it generates less waste, and it requires a lower concentration of the stock solution. Furthermore, compared to the traditional method, it is faster and economical. Overall, based on the tools employed, the method can be considered more eco-efficient than existing methods and innovative for evaluating IVE in injectable solution.

Table VI. Comparison of the proposed method with another method from the literature

Method	Diluent	Stock solution (concentration used)	Work solution (volume used)	Greeneess profile				
				NEMI	ESA	AGREE	GAPI	BAGI
UV*	Ethanol	50 µg mL^{-1}	5 mL		93			
UV[34]	Methanol or acetonitrile	100 mg L^{-1}	10 mL		79			

*Proposed method

CONCLUSIONS

Current scientific literature and chemical-pharmaceutical laboratories advance with the proposed green and lean method. It is linear (6-16 μg mL^{-1}), selective, precise (RSD < 5%), exact (100.07%) and robust. In addition to being indicative of stability and an excellent green option by ESA, NEMI, AGREE, GAPI and BAGI to evaluate IVE in injection solution for veterinary use.

Conflicts of interest

The authors declare that they have no conflict of interest.

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ARTICLE

Enhanced Microwave-Assisted Digestion Method for Accurate Trace-Level Analysis of Rare Earth Elements in Environmental Matrices

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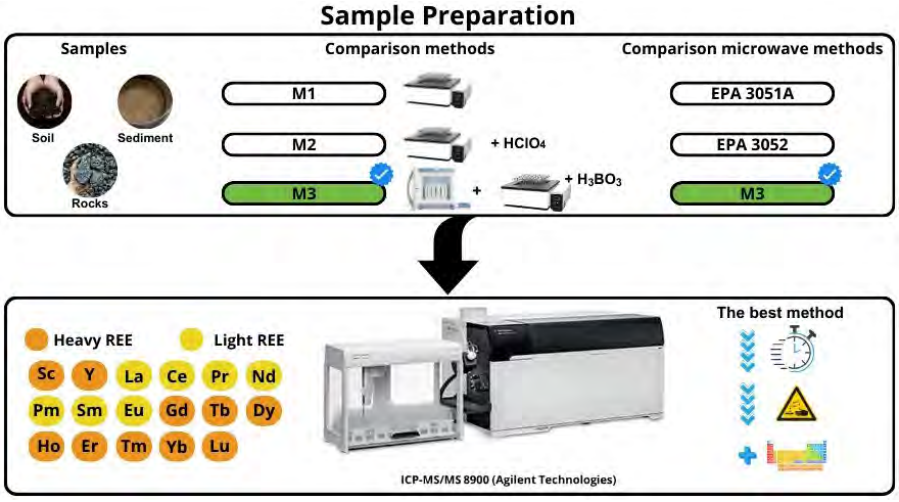

Rare Earth Elements (REEs) play an essential role in modern technologies, with soils and sediments often acting as the final repository for REE-containing products. This study evaluates sample preparation methodologies using digestion methods and triple quadrupole inductively coupled plasma mass spectrometry (ICP-QQQ) for accurate REE quantification in soils, sediments, and rocks. Five digestion methods—differing in hydrofluoric acid (HF) usage and fluoride interference mitigation (Methods 1, 2, 3, USEPA 3052, and 3051)—were compared, identifying Method 3 as the optimal approach. Method 3, which includes 3.0 mL of HF, 3.5 mL of HCl, 1.5 mL of HNO_3, and boric acid (H_3BO_3) to neutralize fluorides, achieved REE recovery rates exceeding 84% across all certified reference materials (CRMs), including soil (TILL-3, NIST SRM 2709a), sediment (NIST SRM 8704), and rock (ITA-1 Friable Itabirite) samples. This method significantly reduces digestion time from 12 hours to 3 hours, minimizes acid consumption, and enhances sample throughput, offering a highly efficient workflow. In addition, Method 3 demonstrated high precision within a 95% confidence interval, excellent linearity, and minimal matrix interference for all REEs (except scandium). Detection limits (LOD: 0.0025–0.0610 μg g^{-1}) and quantification limits (LOQ: 0.0072–0.1448 μg g^{-1}) were markedly lower than previously reported values, enabling sensitive, trace-level REE analysis in complex environmental matrices. Overall, Method 3 stands

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out as an efficient, precise, and environmentally sustainable method for multi-element analysis, providing a rapid and reliable solution for REE quantification in soils, sediments, and rocks using ICP-MS/MS.

Keywords: rare earth elements, microwave digestion, soil, rocks, CRM, ICP-MS

INTRODUCTION

In recent decades, the global mining landscape has increasingly focused on a specific group of elements, the Rare Earth Elements (REEs), driven by a transition in the global energy matrix toward cleaner, renewable energy sources.[1] REEs are now central to raw material policies and critical for advanced industrial applications.[2]

According to the International Union of Pure and Applied Chemistry's (IUPAC) Nomenclature of Inorganic Chemistry, IUPAC Recommendations 2005, REEs include 17 metals from the periodic table: all 15 lanthanides (La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu) alongside scandium (Sc) and yttrium (Y). Among these, promethium (Pm) is a naturally radioactive and exceptionally rare element in the Earth's crust, predominantly produced as a byproduct of nuclear fission in reactors; thus, it is generally excluded from REE analysis. Sc and Y, while not lanthanides, are often categorized with REEs due to their frequent co-occurrence in mineral deposits and shared chemical properties.[3]

Quantifying REEs in soil, sediment, and rock samples is essential for understanding their environmental impacts and informing sustainable management strategies.[2] Highly sensitive and precise analytical methods are necessary to measure REE concentrations accurately in these complex matrices. Inductively coupled plasma mass spectrometry (ICP-MS), particularly the Triple Quadrupole Inductively Coupled Plasma Mass Spectrometry (ICP-MS/MS), has become the standard for REE quantification, offering high sensitivity and selectivity.[2] This method enables the detection and quantification of trace REE concentrations, overcoming the previous analytical challenges and costs associated with pre-separation techniques such as solvent extraction, ion exchange, and precipitation required for X-ray fluorescence or ICP optical emission spectrometry.[4,5]

Sample preparation remains a critical determinant of analysis quality, as ICP-MS typically requires samples in liquid form, a necessary condition for sample introduction, which is normally performed via pneumatic nebulization.[2,4] As found in the literature, the two main methods routinely used for REEs determinations are preconcentration in resins[6] and microwave-assisted digestion.[7] Additional approaches, including acid leaching[8] and alkaline fusion,[9] have also been explored. Some methods involve pre-concentration steps, such as solvent extraction, co-precipitation, or ion-exchange separation, to enhance detection limits.[6] However, these pre-concentration techniques are labor-intensive and time-consuming.

Microwave-assisted digestion typically utilizes concentrated acids like hydrofluoric acid (HF), nitric acid (HNO_3), and hydrochloric acid (HCl) to decompose soil matrices, releasing REEs for analysis. However, HF can lead to the formation of poorly soluble fluorides, including those of Al (III), Ca (II), Fe (III), Mg (II), and REEs, which can impair analytical accuracy. To address this, methods such as evaporate samples to dryness, boric acid (H_3BO_3) addition, or post-digestion treatment with perchloric acid ($HClO_4$) have been introduced to minimize fluoride formation, thereby enhancing REE measurement accuracy.[7,10-12]

The strategy of evaporating samples to dryness, while energy- and time-intensive, has shown high recovery rates. For example, Kasar et al. (2020) reported recovery rates exceeding 90% for 18 elements (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Th, Sr, Cs, and U) using microwave digestion followed by evaporate samples to dryness.[12] Similarly, Ivanova et al. (2001) and other authors, demonstrated that REEs determination in soils via ICP-MS, using HF and HNO_3 with overnight digestion followed by HF and H_3BO_3 addition, resulted in recoveries exceeding 80%.[6,10,13–15] Other studies have applied HF, HCl, and HNO_3 for microwave digestion, recommending $HClO_4$ post-digestion to remove residual fluoride and achieve recoveries above 80%.[7,11]

A review of current literature highlights the lack of standardized methods for rare earth element (REE) analysis in geological samples, as well as the limited data on REE concentrations in soils, sediments, and rocks represents a key gap in supporting the development of international standards and geochemical baselines. To address this gap, it is essential to develop and implement standardized analytical methods

and to establish comprehensive databases that consolidate data from various studies and regions. Additionally, there is a significant lack of REE data in waste materials, an emerging area of interest due to the environmental contamination risks associated with dam failures and electronic waste. To mitigate these risks, systematic studies should be conducted to quantify and monitor REE concentrations in industrial and electronic waste, promoting a proactive approach to waste management and enabling the development of more effective environmental regulations.

This work presents a systematic comparison of five microwave-assisted digestion methods to identify the most effective method for comprehensive REEs quantification. By optimizing triple quadrupole ICP-MS/MS detection conditions, we enhance measurement precision and selectivity across complex soil, sediment, and rock matrices. Using certified reference materials, this study aims to establish a reliable analytical framework for accurate REE analysis, contributing to standardized methods in environmental and geological sample assessment.

MATERIALS AND METHODS

Chemicals and reagents

All chemical reagents used were of analytical grade, ensuring minimal contamination and high reagent purity. Ultrapure deionized water (resistivity: 18 MΩ·cm) was obtained using a Millipore Nanopure system (Millipore, Bedford, MA, USA). Hydrochloric acid (37% HCl), nitric acid (65% HNO_3), and hydrofluoric acid (40% HF) were purified by sub-boiling using a Milestone DuoPur Quartz Acid Purification system (Milestone, Sorisole BG, Italy). Boric acid (99.6% H_3BO_3, Merck) and ACS-grade perchloric acid ($HClO_4$, Merck) were utilized for neutralizing residual HF and preventing the formation of REE fluorides.

Certified Reference Materials (CRM) were selected based on their REE content and representativeness of natural environmental matrices. These included multi-element REE standards (Sigma Aldrich, Buchs, Switzerland) and a 1000 mg L^{-1} Rhodium CRM (Inorganic Ventures, Christiansburg, USA) as an internal standard. The analytical curve was prepared with concentrations ranging from 2 to 65 ug L^{-1} in 2% HNO_3, with Rh added as an internal standard to control for instrumental drift.

Certified reference materials and quality control samples

This study employed two certified soil reference materials: TILL-3 (Canadian Soil CRM) and NIST SRM 2709a (San Joaquin Soil), as well as one sediment reference material, NIST SRM 8704 (Buffalo River Sediment). For quality control, a friable itabirite sample (ITA-1) sourced from Iron Quadrangle, Brazil, provided by the Federal University of Ouro Preto, served as a geological control to simulate rock matrix behaviour in REEs recovery assessment. The accuracy of each method was evaluated by calculating recovery rates based on certified values, while each CRM was measured in triplicate to ensure robustness in statistical assessments.

Digestion methods

To determine the most effective method for REE extraction, 3 microwave-assisted digestion methods were evaluated, each designed to optimize REEs solubilization in complex matrices. Each method used approximately 250 mg of sample and followed unique procedural steps. For the three initial methods focused on fluoride removal from digests to avoid poorly soluble fluoride formation, was used a standardized acid mix: 3.5 mL of 37% HCl, 1.5 mL of 65% HNO_3, and 3.0 mL of 40% HF, with a 3-hour of contact time.

1. **Method 1 (Evaporation to dryness):** Post 3 hours of contact time digestion, samples were evaporated to dryness at 90 °C, in a hot block (DigiBlock, Italy), followed by reconstitution with HCl and HNO_3 for two cycles. Total time: 48 hours.
2. **Method 2 ($HClO_4$ addition):** After the 3 hours of contact time digestion, 1 mL of $HClO_4$ was added and the samples were heated to 200 °C in a hot block (DigiBlock, Italy), dried, and reconstituted to eliminate HF interference. Total time: 24 hours.

3. **Method 3 (H_3BO_3 addition):** This method was processed in an ETHOS UP Microwave Digestion System with SR15 pressure vessels (Milestone, Belgium), using a ramp to 230 °C, followed by a 15-minute hold and cooling phase. To address HF complexation, 1.1 g of H_3BO_3 was added post-digestion, avoiding sample drying. This method aimed to neutralize HF and prevent fluoride precipitation without requiring evaporation. Total time: 6 hours.

The optimized method with superior accuracy and precision was then compared against two standardized microwave-assisted digestion methods by the U.S. Environmental Protection Agency (USEPA): USEPA 3051a and USEPA 3052.[23,24]

4. **USEPA 3051a (Pseudo-total digestion):** A standard method using 9.0 mL of HNO_3 and 3.0 mL of HCl at 175 °C for 4.5 minutes, designed for metals analysis in environmental samples.
5. **USEPA 3052 (Total digestion with HF and H_3BO_3):** This total digestion method included 9.0 mL of HNO_3, 3.0 mL of HF, with post-digestion addition of H_3BO_3, targeting complete REE recovery from silicate matrices.

For these two standardized methods, the digestion was processed in the same equipment ETHOS UP Microwave Digestion System with SR15 pressure vessels (Milestone, Belgium), using descripting ramp of both standardized methods.

Each sample batch was assigned a unique identifier to ensure traceability, and each digestion method was applied to all CRM samples in triplicate.

Instrumental analysis and ICP-MS/MS optimization

Sample analysis was performed using an Agilent 8900 ICP-MS/MS system (Agilent Technologies, Japan). The instrumental parameters are described in Table I.

Table I. Instrumental parameters for the determination of REE by ICP-MS/MS

Instrumental parameters	Operating conditions
Nebulizer	Mira Mist (peek)
Nebulization chamber	Scott double-pass (Quartz)
Torch	Quartz torch, 2.5 mm diameter
Sampling and Skimmer cones	Ni
Tygon® tubes	1.02 mm
Radiofrequency power (W)	1550
Sample flow (mL min^{-1})	0.4
Nebulization gas flow (L min^{-1})	1.07
Plasma gas flow (L min^{-1})	15.0
Auxiliary gas flow (L min^{-1})	0.90
Rinse time (s)	10
Peristaltic pump speed (rps)	0.5
Sample aspiration time (s)	30
Stabilization time (s)	20
Rising time (s)	10
Total time analysis (s)	120

Isotope selection was critical in mitigating isobaric interferences. By selecting non-interfering isotopes, we minimized the impact of overlapping signals from polyatomic ions, a technique that is consistently recommended in the literature to improve analytical specificity.[16] This approach allowed for more precise quantification of REEs, especially when analysing samples with complex matrix backgrounds that could otherwise lead to signal distortion. The isotopes selected for determination included ^{139}La, ^{140}Ce, ^{141}Pr, ^{146}Nd, ^{147}Sm, ^{153}Eu, ^{158}Gd, ^{159}Tb, ^{163}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{172}Yb, and ^{175}Lu. This method of interference reduction aligns with practices highlighted by Anders and Grevesse (1989) and Pradhan et al. (2015), who demonstrated that careful isotope selection enhances both sensitivity and selectivity in ICP-MS applications.[14,17] These tailored adjustments led to improved sensitivity and reproducibility in REE analysis, establishing a solid foundation for accurate and reproducible measurements in analytical applications.

The reaction cell was optimized, and a comparison was made between NO GAS and Helium (He) modes, with the He mode operated at a flow rate of 4.5 mL/min.

The Shapiro-Wilk test was applied to assess data normality, and ANOVA followed by Tukey's post-hoc test was used for normally distributed data at 0.05 significance level. For non-normal data, the Kruskal-Wallis test and Dunn's post-test were applied to compare group means. Method accuracy was determined through recovery percentage comparisons to CRM values using *t*-tests at 0.05 significance level.

For method validation, selectivity, linearity, repeatability, intermediate precision, accuracy/recovery, limit of detection (LOD), and limit of quantification (LOQ) were verified. Statistical tests employed included the Jackknife outlier test, Cochran's test for residue variance homogeneity, coefficient of determination, F-test, *t*-test, and coefficient of variation to evaluate these figures of merit.

Calibration curves were prepared in triplicate using seven multi-element standards with REE concentrations from 2 to 65 µg L^{-1}. To monitor signal consistency, an internal standard mix (^{103}Rh, ^{204}Tl) was added to all solutions, and the signal was correct using the ratio "signal analyte/signal internal standard" for all samples. Each batch analysis began and ended with calibration standards, with blank solutions interspersed every 5 to 7 samples to check system stability. After completion, trace element data were processed in MassHunter and corrected for instrumental drift. Quality control was verified by evaluating recoveries from multi-element standards and Certified Reference Materials (CRM).

Precision was measured through repeatability and intermediate precision tests. Repeatability, reflecting agreement in results under identical conditions, and intermediate precision, which measures consistency under variable conditions (e.g., across different days), were assessed. Both repeatability and intermediate precision were expressed as relative standard deviation (RSD).

LOD and LOQ were calculated based on ten replicate blanks (9 degrees of freedom), each subjected to the complete digestion process, to provide robust estimates for sensitivity.

RESULTS AND DISCUSSION

ICP-MS/MS optimization and minimization of interference

The analytical performance of the method was evaluated for a range of REEs under no-gas and helium (He) collision cell conditions. The coefficients of determination (R^2) in no-gas mode ranged from 0.9539 (Gd) to 0.9698 (Pr), while in He mode, all R^2 values were markedly improved, ranging from 0.9993 (Ce) to 0.9999 (Gd), indicating excellent linearity. In terms of precision, expressed as relative standard deviation (RSD%), the no-gas mode showed a wider variation, with values ranging from 1.0–38.4% depending on the element, such as 3.0–38.4% for Gd and 1.0–17.2% for Nd. In contrast, the He mode provided significantly better precision, with RSDs ranging from just 0.1% (La) to 3.4% (Lu). These results demonstrate that the use of He as a collision gas greatly enhances both signal stability and analytical accuracy for the determination of REEs by reducing interferences and improving repeatability. The implementation of the He mode, which utilizes helium as a collision gas, was essential in reducing these interferences. Literature supports the use of kinetic energy discrimination in the He mode to minimize oxide formation, which is essential for obtaining accurate results, particularly for elements such as Ce and Nd.[18]

Comparison of digestion Methods 1, 2 and 3 for SRM's soils

Figure 1 presents a comprehensive comparison of the mean concentrations (μg g^{-1}), standard deviations (μg g^{-1}) and recovery rates (%) for three analytical methods applied to both CRMs. Mean values (n = 3) followed by the same letter (A, B, or C) are not significantly different, whereas values followed by different letters indicate statistically significant differences. Statistical analysis was performed using one-way ANOVA, followed by Tukey's post-hoc test for parametric data, or using Kruskal-Wallis analysis of variance, followed by Dunn's test for non-parametric data at 0.05 significance level.

Table S1 (Supplementary Material section) presents the results of mean, standard deviation and recovery for the 3 methods and the two standardized methods USEPA 3051a and 3052, for the TILL-3 CRM of Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu obtained after digestion (n = 3). The efficacy of each digestion method was analyzed with respect to REE recovery rates across diverse environmental matrices. This analysis allows for a deeper understanding of the complex factors influencing REE extraction and offers insights into the operational advantages and limitations of each method. The criteria used to determine acceptable recovery rates for the methods was a range of 80% to 120%.

Method 1 (Evaporation to dryness) showed limitations, especially with elements prone to forming stable, insoluble compounds, such as La, Ce, and Pr. Recovery rates for these elements, Figure 1, were below 50%, which is consistent with reports from Fedyunina et al. (2012) and Zimmermann et al. (2020), who observed that complete drying can lead to volatilization losses or incomplete solubilization.[6,15]

These findings suggest that the use of evaporation steps may not be suitable for matrices containing REEs, particularly those with a tendency to form volatile or refractory compounds. Additionally, the high standard deviations observed, Figure 1, indicate that the method's reproducibility is compromised, likely due to the partial crystallization of REE compounds, which can limit their redissolution during subsequent reconstitution steps.

Method 2 ($HClO_4$ addition) exhibited moderate recovery rates for specific REEs, such as Gd and Dy, achieving values above 80%, Figure 1. However, the recovery of La, Er, and other light REEs was particularly low, with values as low as 20% in some cases, Figure 1.

This trend may be attributed to the inability of $HClO_4$ alone to completely dissolve fluoride-bound REEs, especially those in highly resistant silicate phases, as documented by Balaram (2019).[2] $HClO_4$'s oxidative potential helps remove organic contaminants and some matrix interferences, but its lack of complexation ability with fluorides highlights a major limitation in recovering the full spectrum of REEs without additional treatments, as noted by Cotta and Enzweiler (2010).[4]

In fact, incomplete dissolution of fluoride-bound REEs is a recurring challenge in environmental samples, especially in matrices containing complex silicate structures.[16]

In contrast, Method 3 (H_3BO_3 addition) demonstrated a consistent and high recovery rate across all reference materials and REEs analyzed, Figure 1. This method achieved recovery rates exceeding 84% for most REEs, closely matching certified values in TILL-3, NIST SRM 2709a, NIST RM 8704 and ITA-1, Figure 2. The addition of boric acid post-digestion effectively neutralizes excess HF, preventing the formation of insoluble fluoride precipitates.[7] This approach aligns with the findings of Ebihara et al. (2020)[7] and Zimmermann et al. (2020),[15] who showed that boric acid acts as a fluoride scavenger, forming stable BF_4^- complexes that help maintain REE solubility. The efficiency of boric acid in eliminating fluoride interference is particularly notable with heavy REEs (HREEs), which are more prone to forming stable fluorides, thereby enhancing method consistency and reproducibility.

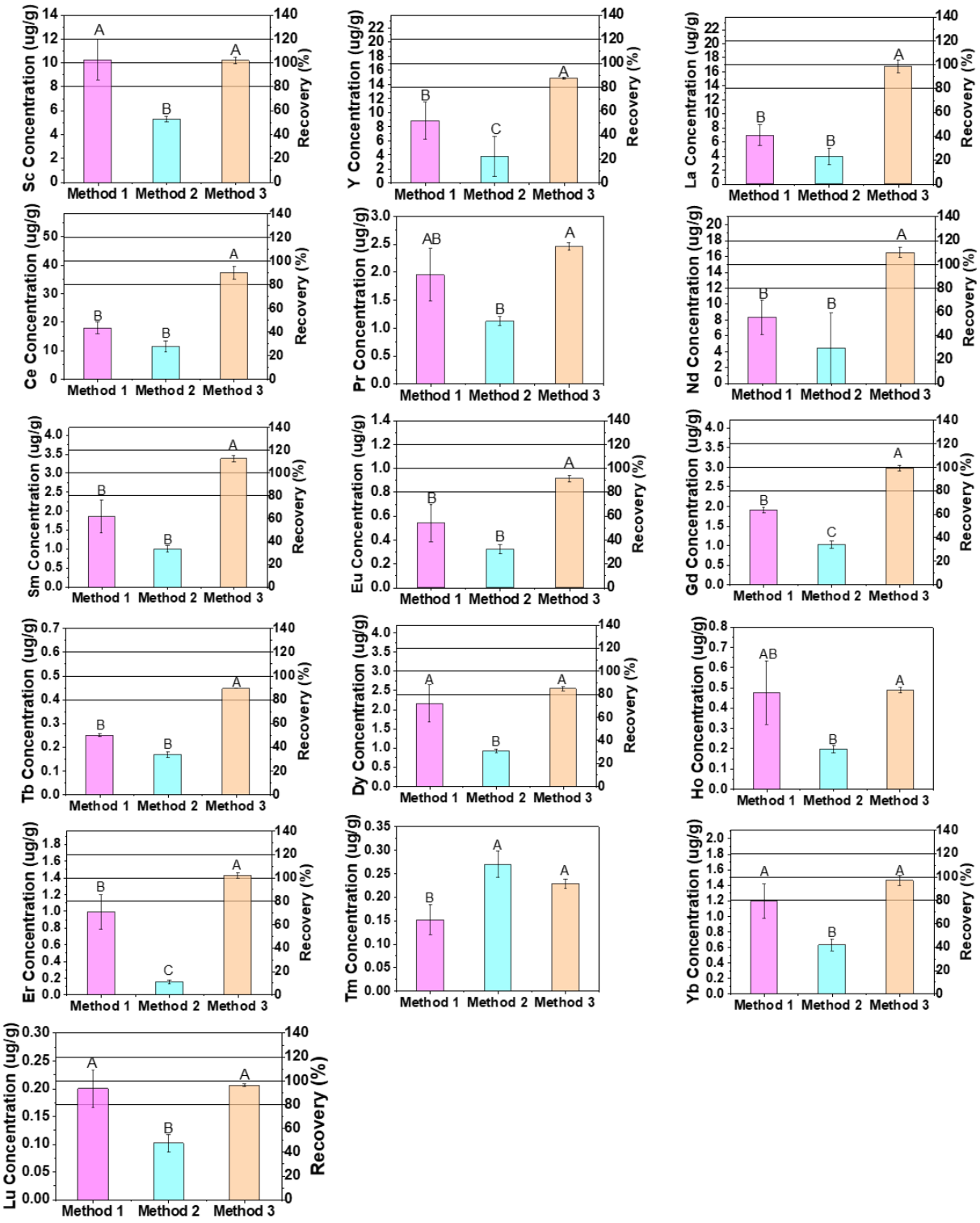

Figure 1. Comparison between microwave digestion of the 3 digestion methods, for concentration and recovery REEs in TILL-3 (Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Tb, Ho, Er, Tm, Yb, and Lu), and NIST SRM 2709a (Gd and Dy), obtained after digestion (continuous lines correspond to 80, 100, and 120% of the certified value of the respective metal). Mean values (n = 3) followed by the same letter (A, B, or C) are not significantly different, whereas values followed by different letters indicate statistically significant differences. Statistical analysis was performed using one-way ANOVA, followed by Tukey's post-hoc test for parametric data, or using Kruskal-Wallis analysis of variance, followed by Dunn's test for non-parametric data at 0.05 significance level.

This study compared two open-vial digestion methods (Methods 1 and 2) with a closed-vial microwave-assisted digestion method (Method 3). The latter obtained significantly higher recoveries and a higher analytical frequency. It is worth noting that open-vial digestion methods have been shown to be more efficient than closed-vial methods, such as for the analysis of mercury in soils.[19]

Comparing these results with previous methods, Method 3's recovery rates for HREEs such as Lu and Yb were significantly higher, indicating that boric acid addition may be an optimal solution for complex matrices Figure 1. Studies by J. Ivanova et al. (2001), support these observations, as their work with boric acid-enhanced methods also reported improvements in REE recoveries, particularly for HREEs in geological matrices.[10] Comparing Method 3 with the proposed method by J. Ivanova et al. (2001),[10] it has the advantage of less time consumable and consequently higher analysis frequency, because of the reducing the sample contact time from 12 to 3 hours and no use of a second step of adding HF after microwave digestion. This finding highlights boric acid's dual role as both a stabilizing agent for REEs and a means of mitigating HF-related matrix interferences, thus promoting efficient solubilization and facilitating accurate quantification in ICP-MS/MS.

Comparison of microwave-assisted digestion Method 3 and standardized microwave-assisted digestion methods USEPA 3051a and USEPA 3052 for SRM's soils, sediments and QCM ITA-1

Figure 2 presents a comparison between Method 3 and two standardized methods, USEPA 3051a and 3052, across four reference materials: two soil standards (TILL-3 and NIST SRM 2709a), sediment reference material NIST RM 8704, and the Itabirito Rock Quality Control Material (QCM ITA-1). Similar to Figure 1, mean values (n = 3) followed by the same letter (A, B, or C) are not significantly different, whereas values followed by different letters indicate statistically significant differences. Statistical analysis was performed using one-way ANOVA, followed by Tukey's post-hoc test for parametric data, or using the Kruskal-Wallis analysis of variance, followed by Dunn's test for (non-parametric data) at 0.05 significance level. Tables S1, S2, S3 and S4 of the Supplementary Material section present the results of mean, standard deviation and recovery for the methods and the two standardized methods USEPA 3051a and 3052, for the TILL-3, NIST SRM 2709a, NIST RM9704 and ITA-1 samples CRMs; of Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu obtained after digestion (n = 3).

The standardized USEPA methods (3051a and 3052), widely recognized for metals analysis, displayed partial efficacy in REEs recovery, Figure 2. USEPA 3052, which incorporates HF and post-digestion boric acid, yielded better results than 3051a, especially for silicate-rich matrices where REEs are more tightly bound.

However, neither method achieved the recovery consistency or levels observed with Method 3, Figure 2. These results are in line with studies by Sucharová and Suchara (2006), who observed that USEPA 3052 provided satisfactory recovery for some metals but was less effective for REEs due to fluoride complexation issues.[13] This limitation is particularly pronounced in matrices like soils and sediments, where REEs are often bound to mineral phases that resist complete dissolution in HF without additional complexing agents, such as boric acid, which are not explicitly recommended in the USEPA methods.[20]

Figure 2 also presents the results for the sediment CRM NIST SRM 8704. For this CRM as well, Method 3 outperformed Methods 3052 and 3051a in the digestion of the sediments, resulting in significantly higher mean concentrations and recovery rates across all rare earth elements (REEs). For example, scandium (Sc) recovery was 86.13% with Method 3, compared to 82.96% and 41.77% for Methods 3052 and 3051a, respectively.

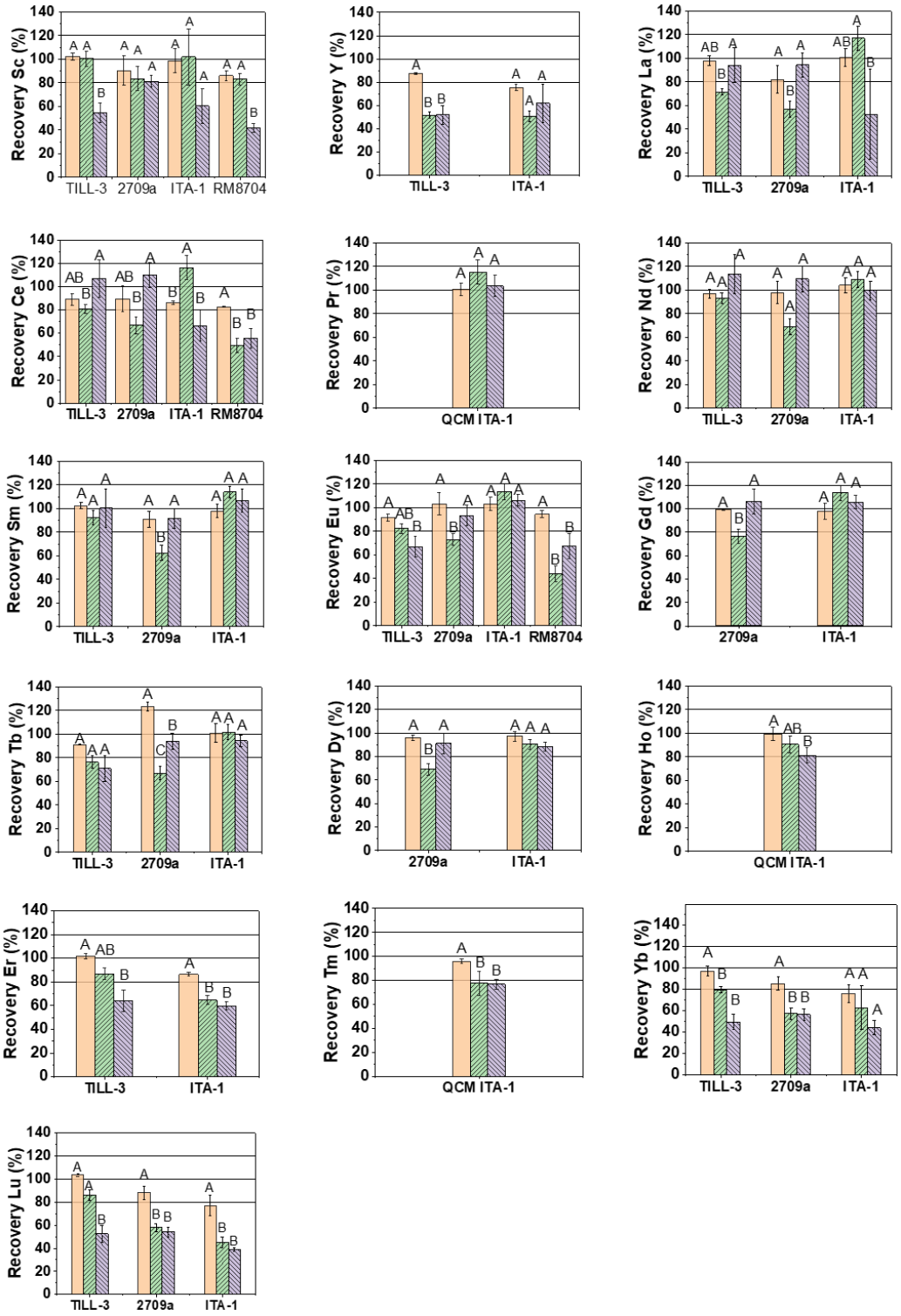

Figure 2. Comparison between microwave digestion of Method 3 (▭) and the two standardized methods USEPA 3052 (▨) and 3051a (▨), for concentration and recovery REEs certified in TILL-3, NIST SRM 2709a, RM8704 and QCM ITA-1 obtained after digestion. Continuous lines correspond to 80, 100, and 120% of the certified value of the respective metal. Mean values (n = 3) followed by the same letter (A, B, or C) are not significantly different, whereas values followed by different letters indicate statistically significant differences. Statistical analysis was performed using one-way ANOVA, followed by Tukey's post-hoc test for parametric data, or using Kruskal-Wallis analysis of variance, followed by Dunn's test for (non-parametric data) at 0.05 significance level.

Method 3 also delivered higher concentrations for elements like yttrium (Y), lanthanum (La), and cerium (Ce), suggesting more effective matrix digestion and reduced matrix interference. Furthermore, Method 3 provided particularly strong results for europium (Eu) with a recovery of 94.33%, surpassing the other methods by over 20%, Figure 2. These consistent improvements highlight Method 3's efficiency in quantifying REEs in sediment matrices, benefiting from lower acid consumption and higher analytical frequency.

Comparative performance in soil, sediment, and rock matrices

The efficacy of Method 3 was further confirmed by comparing its performance across different matrix types. In soil CRMs (TILL-3 and NIST SRM 2709a), Method 3 consistently achieved recovery rates close to certified values, with an average recovery range of 84–108% for TILL-3 and 82–105% for NIST SRM 2709a. In sediment CRM (NIST RM 8704) achieving recovery rates 83-84%. In rock samples (ITA-1), Method 3 achieved recovery rates within 76–111%, demonstrating its robustness even in silicate-rich matrices. The stability of recovery rates across these diverse matrices suggests that Method 3 offers matrix compatibility and adaptability, essential qualities for analytical methods in environmental geochemistry.

The matrix resilience observed with Method 3 is especially relevant given the diverse composition of environmental samples, which may contain a wide range of silicates, organic matter, and metal oxides. These complex matrices present significant challenges in REE quantification, as traditional methods often struggle to achieve high recovery rates across the REE spectrum.[20] The ability of Method 3 to maintain high recovery rates in both.

The higher temperature and extended digestion time of Method 3 likely contributed to its superior performance compared to USEPA 3052.[21] Method 3 also uses a smaller volume of HNO_3 but incorporates HCl, similar to aqua regia, which, combined with the higher temperature, improves efficiency, given that HF quantities are the same in both methods.[21,22]

Given these findings and the study's objective to achieve accurate and precise REE measurements in soil, sediments and rock samples, Method 3 met all required criteria. Figure 2 demonstrates that Method 3 was the only approach yielding over 84% recovery for all 13 REEs in soil CRMs and 13 of 16 REEs in QCM ITA-1. For the remaining three REEs (Y, Yb, and Lu), average recoveries with Method 3 were close to 80%, higher than those obtained with USEPA methods. Additionally, Method 3, compared to the USEPA methods, requires a smaller volume of acid than 3051a and 3052 and enables a higher analytical throughput.

Statistical validation and method consistency

Statistical comparisons with CRM certified values (using a *t*-test) are provided in Tables S1, S2, S3 and S4 (Supplementary Material). The statistical analysis of recovery rates provided robust validation of the digestion methods. Tukey's post-hoc test revealed that recovery rates obtained with Method 3 did not significantly differ from the certified values at 0.05 significance level for the majority of REEs across the TILL-3, NIST SRM 2709a, NIST RM9704 and ITA-1 samples. This result underscores Method 3's high accuracy, further supporting its adoption as a standardized approach for REE analysis.

The reproducibility of recovery rates across different CRMs demonstrates Method 3's reliability, consistent with studies by Fedyunina et al. (2012) that advocate for the use of boric acid as a stabilizing agent to maintain REE solubility.[6]

Table II reports the results for the selectivity, linearity, repeatability, intermediate precision, accuracy, LOD and LOQ for REEs in TILL-3 (Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Tb, Ho, Er, Tm, Yb, and Lu), and NIST SRM 2709a (Gd and Dy), using Method 3 and comparison with other works in literature.

Table II. Results for the selectivity, linearity, repeatability, intermediate precision, accuracy, LOD and LOQ for REEs in TILL-3 (Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Tb, Ho, Er, Tm, Yb, and Lu), and NIST SRM 2709a (Gd and Dy), using Method 3 and comparison with other works in literature

REEs	Linearity		Selectivity	Repeatability RSD (%)	Intermediate precision RSD (%)	Recovery (%)	This study LOD (μg g^{-1})	LOD (μg g^{-1})[25]	This study LOQ (μg g^{-1})	LOQ (μg g^{-1})[26]
	Solvent curve	Matrix Curve								
	Coefficient of Determination (R2)	Coefficient of Determination (R2)	F-test (residual variances) *t*-test (combined variances)							
Sc**	0.9996	0.9998	Homoscedastic - matrix effect	5.42	5.96	100	0.0701	-	0.1448	-
Y**	0.9998	0.9991	Homoscedastic - no matrix effect	4.27	4.91	87	0.0147	-	0.0424	-
La*	0.9997	0.9993	Homoscedastic - no matrix effect	6.83	6.84	84	0.0145	0.017	0.0299	0.010
Ce*	0.9993	0.9990	Homoscedastic - no matrix effect	7.02	7.78	90	0.0163	0.017	0.0210	0.029
Pr**	0.9996	0.9997	Homoscedastic - no matrix effect	5.66	7.01	-***	0.0055	0.006	0.0129	0.021
Nd**	0.9997	0.9997	Homoscedastic - no matrix effect	5.78	7.73	103	0.0128	0.013	0.0313	0.040
Sm**	0.9997	0.9997	Homoscedastic - no matrix effect	4.22	5.55	98	0.0070	0.023	0.0188	0.012
Eu**	0.9996	0.9997	Homoscedastic - no matrix effect	2.73	3.79	92	0.0055	0.007	0.0167	0.020
Gd**	0.9999	0.9995	Homoscedastic - no matrix effect	3.06	5.99	101	0.0610	0.023	0.0149	0.033
Tb**	0.9997	0.9997	Homoscedastic - no matrix effect	2.94	3.58	91	0.0042	0.005	0.0122	0.013
Dy**	0.9997	0.9997	Homoscedastic - no matrix effect	4.38	4.51	90	0.0066	0.022	0.0158	0.018
Ho**	0.9995	0.9997	Homoscedastic - no matrix effect	2.93	3.67	-***	0.0040	0.006	0.0121	0.018
Er**	0.9996	0.9997	Homoscedastic - no matrix effect	2.98	4.89	99	0.0033	0.007	0.0080	0.008
Tm**	0.9994	0.9995	Homoscedastic - no matrix effect	3.74	6.29	-***	0.0039	0.006	0.012	0.020
Yb**	0.9996	0.9997	Homoscedastic - no matrix effect	2.98	4.89	99	0.0025	0.008	0.0072	0.010
Lu**	0.9998	0.9997	Homoscedastic - no matrix effect	4.20	5.00	108	0.0027	0.013	0.0079	0.008

n=10 for all measurements; *No internal Standard; **Internal Standard Rh.

As observed in Table II, the selectivity of the Method 3 was evaluated using statistical comparison between the calibration curves and the certified reference materials. Among the 17 rare earth elements analyzed, only scandium (Sc) presented *t*-values exceeding the critical threshold, suggesting that its quantification may require matrix-matched calibration, especially in complex soil matrices (Table II). Regarding linearity, the method demonstrated excellent performance across all analytes. The data exhibited homoscedasticity, with no significant differences in residual variances, fulfilling this criterion for all calibration curves. Additionally, all coefficients of determination (R^2) exceeded 0.9990, confirming the method's strong and consistent linear response.

Method 3 showed consistently low relative standard deviations (RSDs) across all certified reference materials (CRMs), reinforcing the method's precision and repeatability. For the TILL-3 material, RSDs ranged from 2.79% to 7.12%; for NIST SRM 2709a, from 5.62% to 14.0%; and for ITA-1, from 1.75% to 11.5% (Table II). These values fall within acceptable limits for environmental analytical methods. Notably, the HorRat values remained below 2 for all analyses, in accordance with international guidelines, further supporting the method's precision. This level of reproducibility aligns with the recommendations of Zimmermann et al. (2020),[15] who emphasize that robust precision is a critical parameter for reliable analytical methods.

The method also demonstrated high sensitivity, with limits of detection (LOD) ranging from 0.0025 to 0.070 μg g^{-1} and limits of quantification (LOQ) from 0.0072 to 0.145 μg g^{-1} (Table II). These results indicate that Method 3 is suitable for trace-level detection of rare earth elements in soil matrices, ensuring its applicability in environmental monitoring and geochemical studies. These values are in agreement with studies by Coedo et al. (1998) and Fedyunina et al. (2012).[25,26] The LOD and LOQ values achieved were lower than or comparable to reported values in chondritic data, supporting the applicability of Method 3 in quantifying REE concentrations in TILL-3 and NIST SRM 2709a, as concentrations were below certification levels. The LOD and LOQ values achieved are suitable for REE quantification in environmental monitoring programs, where trace-level detection is essential for regulatory compliance and ecological assessments.

CONCLUSIONS

This study provides a comprehensive evaluation of five digestion methods for the analysis of Rare Earth Elements (REEs) in environmental matrices. The results demonstrate that Method 3, characterized by the addition of boric acid and optimization of time reaction, provides significant advantages over other methods in terms of recovery rates, precision, and applicability across diverse matrix types.

Recovery rates with Method 3 consistently exceeded 84% across all certified reference materials, showing minimal deviation from certified values. This high level of consistency confirms the method's reliability for accurate REE determination in soils, sediments, and geological materials. The effective use of boric acid played a key role in mitigating fluoride precipitation, ensuring complete digestion of refractory minerals and improving matrix compatibility, an important factor in environmental and geochemical studies.

In addition to recovery performance, Method 3 exhibited excellent sensitivity, with low limits of detection and quantification, and robust repeatability, as indicated by HORRAT values below 2. These results confirm its suitability for trace-level analyses, particularly in regulatory and ecological monitoring contexts where analytical reliability is essential.

Furthermore, the method's efficiency, reflected in reduced acid consumption, shorter reaction times, and compatibility with ICP-MS/MS instrumentation, supports its scalability for routine laboratory applications. Its environmentally conscious design also contributes to safer and more sustainable analytical workflows.

In conclusion, Method 3 provides a robust and reliable approach for comprehensive REE quantification in environmental matrices. Its combination of high recovery rates, matrix compatibility, and sensitivity aligns well with the needs of environmental monitoring and industrial applications, supporting its recommendation as a standardized method for REE analysis in complex environmental samples.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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SUPPLEMENTARY MATERIAL

This supplementary material presents comparisons of rare earth element (REE) concentration, recovery, and relative standard deviation values obtained using the digestion methods M1, M2, and M3, and the standardized methods EPA 3052 and 3051a, applied to TILL-3, NIST SRM 2709, QCM ITA-1, and NIST RM 8704 matrices.

Table S1. Results of mean concentration, standard deviation, recovery and relative standard deviation (RSD) obtained using the digestion methods M1, M2, M3, and the standardized methods USEPA 3051a and 3052 applied to the TILL-3 CRM (n = 3 for all REEs).

		TILL-3					
REE	**Certified values (ug g^{-1})**		**M1**	**M2**	**M3**	**3052**	**3051**
Sc	10	Mean (ug g^{-1})	10.3±1.7 (NS)	5.303±0.022	10.21±0.30 (NS)	10.05±0.64	5.45±0.86 (NS)
		Recovery (%)	103	53.0	102.1	103.7	54.5
		RSD (%)	16.74	0.22	2.95	6.40	8.59
Y	17	Mean (ug g^{-1})	8.8±2.6	3.78±0.48	14.85±0.15	8.76±0.45	8.8±1.4
		Recovery (%)	51.9	22.2	87.4	51.5	51.6
		RSD (%)	15.46	2.84	0.89	2.67	8.08
La	21	Mean (ug g^{-1})	6.93±1.5	3.92±0.25	16.67±0.89 (NS)	15.02±0.62 (NS)	19.8±3.1
		Recovery (%)	33.0	18.7	98.1	71.7	94.2
		RSD (%)	7.04	1.19	4.24	2.97	14.73
Ce	42	Mean (ug g^{-1})	18.1±2.1	11.55±0.63	37.4±2.2 (NS)	33.86±1.5 (NS)	44.8±6.7
		Recovery (%)	43.0	27.5	89.0	82.4	106.7
		RSD (%)	8.45	1.49	5.17	3.65	15.98
Pr	-	Mean (ug g^{-1})	2.12±0.26	1.12±0.079	2.451±0.070	3.83±0.20	3.31±0.75
		Recovery (%)	-	-	-	-	-
		RSD (%)	23.97	7.09	2.86	5.04	22.74
Nd	16	Mean (ug g^{-1})	8.3±2.2	4.45±0.31	16.47±0.66 (NS)	14.92±0.71 (NS)	18.2±2.7 (NS)
		Recovery (%)	52.0	27.8	96.9	95.8	113.8
		RSD (%)	13.54	1.92	4.10	4.17	16.61
Sm	3.3	Mean (ug g^{-1})	1.85±0.44	1.003±0.087	3.374±0.086 (NS)	3.05±0.20 (NS)	3.31±0.54 (NS)
		Recovery (%)	56.1	30.4	102.2	93.8	100.4
		RSD (%)	13.18	2.65	2.62	5.90	16.32
Eu	<1.0	Mean (ug g^{-1})	0.54±0.16	0.32±0.039	0.913±0.029	0.822±0.040	0.669±0.084
		Recovery (%)	54.5	32.0	91.3	83	66.9
		RSD (%)	15.79	3.87	2.91	4.03	8.40
Gd	-	Mean (ug g^{-1})	2.00±0.73	1.024±0.09	2.758±0.072	2.67±0.23	2.49±0.41
		Recovery (%)	-	-	-	-	-
		RSD (%)	36.43	8.83	2.62	8.73	16.51
Tb	<0.5	Mean (ug g^{-1})	0.245±0.068	0.168±0.012	0.4466±0.0010	0.382±0.026	0.355±0.055
		Recovery (%)	51.0	33.6	91.1	80.7	71.1
		RSD (%)	13.65	2.36	0.19	5.27	11.05
Dy	-	Mean (ug g^{-1})	2.41±0.79	0.933±0.045	2.537±0.050	2.27±0.17	1.84±0.31
		Recovery (%)	-	-	-	-	-
		RSD (%)	32.72	4.87	1.98	7.58	16.53

(continued on next page)

Table S1. Results of mean concentration, standard deviation, recovery and relative standard deviation (RSD) obtained using the digestion methods M1, M2, M3, and the standardized methods USEPA 3051a and 3052 applied to the TILL-3 CRM (n = 3 for all REEs). (continued)

TILL-3							
REE	**Certified values (ug g^{-1})**		**M1**	**M2**	**M3**	**3052**	**3051**
Ho	-	Mean (ug g^{-1})	0.47±0.16	0.196±0.018	0.486±0.015	0.440±0.026	0.342±0.050
		Recovery (%)	-	-	-	-	-
		RSD (%)	33.11	9.38	3.11	5.83	14.71
Er	1.4	Mean (ug g^{-1})	0.99±0.20	0.162±0.021	1.427±0.033 (NS)	1.217±0.066	0.90±0.12
		Recovery (%)	70.7	11.6	101.9	89.1	64.3
		RSD (%)	14.60	1.49	2.33	4.74	8.88
Tm	-	Mean (ug g^{-1})	0.152±0.032	0.27±0.029	0.2290±0.0093	0.1912±0.0092	0.131±0.017
		Recovery (%)	-	-	-	-	-
		RSD (%)	20.98	10.65	4.07	4.82	12.75
Yb	1.5	Mean (ug g^{-1})	1.19±0.22	0.628±0.077	1.454±0.067 (NS)	1.188±0.046	0.74±0.10
		Recovery (%)	79.3	41.9	96.9	80.6	49.5
		RSD (%)	14.70	5.11	4.44	3.09	6.85
Lu	0.2	Mean (ug g^{-1})	0.200±0.033	0.102±0.016	0.2064±0.0023	0.1720±0.0093	0.105±0.015
		Recovery (%)	100.0	53.0	103.2	86.8	52.3
		RSD (%)	16.42	7.77	1.13	23.18	7.46

(NS) = Do not differ statistically ($p<0.05$) according to the *t*-test (n=3), n=3 for all measurements.

Table S2. Results of mean concentration, standard deviation, recovery and relative standard deviation (RSD) obtained using the digestion method M3 and the standardized methods USEPA 3051a and 3052 applied to the NIST SRM 2709 (n = 3 for all REEs).

NIST SRM 2709					
REE	**Certified values (ug g^{-1})**		**M3**	**3052**	**3051a**
Sc	11.1±0.1	Mean (ug g^{-1})	10.0±1.4 (NS)	9.3±1.2	9.03±0.58 (NS)
		Recovery (%)	90.3	79.8	81.3
		RSD (%)	9.7	10.5	5.3
Y	-	Mean (ug g^{-1})	13.74±0.56	8.2±0.81	12.9±1.10
		Recovery (%)	-	-	-
		RSD (%)	4.04	9.84	8.23
La	21.7±0,4	Mean (ug g^{-1})	17.8±2.5 (NS)	12.3±1.5	20.5±2.2 (NS)
		Recovery (%)	82.1	60.6	94.4
		RSD (%)	6.9	6.9	10.3
Ce	42±1	Mean (ug g^{-1})	37.5±4.7 (NS)	28.0±3.1 (NS)	46.1±4.6
		Recovery (%)	89.3	70.1	109.9
		RSD (%)	7.3	7.4	10.9
Pr	-	Mean (ug g^{-1})	2.01±0.17	3.01±0.36	3.37±0.52
		Recovery (%)	-	-	-
		RSD (%)	8.46	11.66	15.37

(continued on next page)

Table S2. Results of mean concentration, standard deviation, recovery and relative standard deviation (RSD) obtained using the digestion method M3 and the standardized methods USEPA 3051a and 3052 applied to the NIST SRM 2709 (n = 3 for all REEs). (continued)

NIST SRM 2709					
REE	**Certified values (ug g^{-1})**		**M3**	**3052**	**3051a**
Nd	17	Mean (ug g^{-1})	16.6±1.6 (NS)	11.7±1.2 (NS)	18.6±1.9
		Recovery (%)	97.9	70.8	109.3
		RSD (%)	9.3	7.5	11.1
Sm	4	Mean (ug g^{-1})	3.67±0.27 (NS)	2.49±0.26 (NS)	3.66±0.34
		Recovery (%)	90.96	62.27	91.56
		RSD (%)	6.69	6.54	8.38
Eu	0.83±0.02	Mean (ug g^{-1})	0.855±0.077 (NS)	0.604±0.040 (NS)	0.77±0.07
		Recovery (%)	103	73.2	93.1
		RSD (%)	9.26	4.86	8.64
Gd	3,0±0.1	Mean (ug g^{-1})	2.974±0.025 (NS)	2.28±0.19 (NS)	3.17±0.33
		Recovery (%)	99.1	76.2	105.6
		RSD (%)	0.82	6.21	10.84
Tb	0.5	Mean (ug g^{-1})	0.616±0.018	0.335±0.027	0.470±0.033 (NS)
		Recovery (%)	123.3	66.7	94.0
		RSD (%)	3.5	5.4	6.7
Dy	3	Mean (ug g^{-1})	2.871±0.070 (NS)	2.07±0.15 (NS)	2.73±0.27
		Recovery (%)	95.7	69.3	90.9
		RSD (%)	2.32	4.85	8.87
Ho	-	Mean (ug g^{-1})	0.602±0.050	0.412±0.033	0.511±0.040
		Recovery (%)	-	-	-
		RSD (%)	8.26	8.02	7.82
Er	-	Mean (ug g^{-1})	1.511±0.085	1.18±0.11	1.36±0.11
		Recovery (%)	-	-	-
		RSD (%)	5.62	9.34	8.01
Tm	-	Mean (ug g^{-1})	0.263±0.014	0.180±0.014	0.194±0.019
		Recovery (%)	-	-	-
		RSD (%)	5.47	7.54	9.55
Yb	2	Mean (ug g^{-1})	1.70±0.12	1.14±0.11	1.13±0.11
		Recovery (%)	85.1	57.7	56.3
		RSD (%)	5.99	5.53	5.23
Lu	0.3	Mean (ug g^{-1})	0.264±0.018 (NS)	0.174±0.010 (NS)	0.163±0.013 (NS)
		Recovery (%)	88.0	58.0	54.2
		RSD (%)	6.04	36.85	4.24

(NS) = Do not differ statistically ($p<0.05$) according to the *t*-test (n=3), n=3 for all measurements.

Table S3. Results of mean concentration, standard deviation, recovery and relative standard deviation (RSD) obtained using the digestion method M3 and the standardized methods USEPA 3051a and 3052 applied to the QCM ITA-1 (n = 3 for all REEs).

QCM ITA-1					
REE	**Certified values (ug g^{-1})**		**M3**	**3052**	**3051a**
Sc	0.44±0.05	Mean (ug g^{-1})	0.50±0.13 (NS)	0.45±0.10 (NS)	0.265±0.064 (NS)
		Recovery (%)	113.9	91.9	60.2
		RSD (%)	3.6	23.6	14.5
Y	4.5±0.2	Mean (ug g^{-1})	3.38±0.12	2.27±0.21 (NS)	2.77±0.74
		Recovery (%)	75.1	49.3	61.6
		RSD (%)	2.6	4.7	16.5
La	1.9±0.04	Mean (ug g^{-1})	1.92±0.15 (NS)	2.22±0.20 (NS)	1.00±0.73 (NS)
		Recovery (%)	100.8	114.3	52.7
		RSD (%)	7.7	10.5	38.2
Ce	3.8±0.1	Mean (ug g^{-1})	3.266±0.057	4.42±0.40 (NS)	2.52±0.51
		Recovery (%)	85.9	113.5	66.3
		RSD (%)	1.5	10.6	13.4
Pr	0.47±0.01	Mean (ug g^{-1})	0.471±0.024 (NS)	0.540±0.048 (NS)	0.484±0.043 (NS)
		Recovery (%)	100.2	114.8	102.9
		RSD (%)	5.1	10.3	9.1
Nd	2.2±0.1	Mean (ug g^{-1})	2.30±0.14 (NS)	2.40±0.15 (NS)	2.19±0.18 (NS)
		Recovery (%)	104.1	108.4	99.6
		RSD (%)	6.1	8.3	8.2
Sm	0.58±0.02	Mean (ug g^{-1})	0.567±0.034 (NS)	0.663±0.029 (NS)	0.618±0.057
		Recovery (%)	97.8	116.4	106.5
		RSD (%)	5.80	4.9	9.9
Eu	0.200±0.004	Mean (ug g^{-1})	0.226±0.011 (NS)	0.227±0.013 (NS)	0.212±0.010 (NS)
		Recovery (%)	102.9	111.4	105.9
		RSD (%)	5.7	6.7	4.8
Gd	0.80±0.02	Mean (ug g^{-1})	0.781±0.055 (NS)	0.905±0.053 (NS)	0.843±0.044 (NS)
		Recovery (%)	97.7	109.5	105.3
		RSD (%)	6.9	6.6	5.5
Tb	0.120±0.002	Mean (ug g^{-1})	0.121±0.010 (NS)	0.1221±0.0078 (NS)	0.1135±0.0059 (NS)
		Recovery (%)	100.7	98.4	94.6
		RSD (%)	8.0	6.5	4.9
Dy	0.70±0.02	Mean (ug g^{-1})	0.679±0.028 (NS)	0.631±0.029	0.617±0.026 (NS)
		Recovery (%)	96.9	90.1	88.2
		RSD (%)	4.0	4.1	3.7
Ho	0.130±0.005	Mean (ug g^{-1})	0.1290±0.0073 (NS)	0.1173±0.0092	0.1053±0.0081 (NS)
		Recovery (%)	99.2	94.3	81
		RSD (%)	5.6	7.0	6.3
Er	0.39±0.02	Mean (ug g^{-1})	0.3376±0.0073	0.253±0.015	0.234±0.014
		Recovery (%)	86.6	65.1	59.9
		RSD (%)	1.7	3.9	3.5

(continued on next page)

Table S3. Results of mean concentration, standard deviation, recovery and relative standard deviation (RSD) obtained using the digestion method M3 and the standardized methods USEPA 3051a and 3052 applied to the QCM ITA-1 (n = 3 for all REEs). (continued)

QCM ITA-1					
REE	**Certified values (ug g^{-1})**		**M3**	**3052**	**3051a**
Tm	0.052±0.003	Mean (ug g^{-1})	0.0499±0.0011 (NS)	0.0404±0.0053	0.0399±0.0021 (NS)
		Recovery (%)	96	72.8	76.8
		RSD (%)	2.1	10.2	4.1
Yb	0.36±0.02	Mean (ug g^{-1})	0.272±0.029	0.225±0.073	0.158±0.023 (NS)
		Recovery (%)	75.7	51.7	44.0
		RSD (%)	8.0	20.4	6.4
Lu	0.058±0.003	Mean (ug g^{-1})	0.0446±0.0051	0.0260±0.0026	0.02±0.00093
		Recovery (%)	76.8	43.4	40.0
		RSD (%)	8.8	126.4	1.6

(NS) = Do not differ statistically ($p<0.05$) according to the *t*-test (n=3), n=3 for all measurements

Table S4. Results of mean concentration, standard deviation, recovery and relative standard deviation (RSD) obtained using the digestion method M3 and the standardized methods USEPA 3051a and 3052 applied to the NIST RM 8704 (n = 3 for all REEs).

NIST RM 8704					
REE	**Certified values (ug g^{-1})**		**M3**	**3052**	**3051a**
Sc	11.26±0.19	Mean (ug g^{-1})	9.7±0.46	9.34±0.55	4.7±0.39
		Recovery (%)	86.13	82.96	41.77
		RSD (%)	4.10	4.88	3.43
Y	-	Mean (ug g^{-1})	25.33±0.95	12±1.6	13.7±2.3
		Recovery (%)	-	-	-
		RSD (%)	3.75	13.13	16.79
La	-	Mean (ug g^{-1})	24.91±0.72	14±2	14.8±2.3
		Recovery (%)	-	-	-
		RSD (%)	2.88	14.49	15.53
Ce	66.5±2	Mean (ug g^{-1})	54.96±0.22	32.8±4.2	37.1±5.6
		Recovery (%)	82.65	49.3	67.2
		RSD (%)	0.32	6.37	8.36
Pr	-	Mean (ug g^{-1})	5.52±0.18	3.99±0.54	2.88±0.8
		Recovery (%)	-	-	-
		RSD (%)	3.33	13.56	27.89
Nd	-	Mean (ug g^{-1})	26.59±0.58	16.2±2.2	18.8±3
		Recovery (%)	-	-	-
		RSD (%)	2.17	13.89	16.18
Sm	-	Mean (ug g^{-1})	5.88±0.23	3.73±0.38	4.37±0.72
		Recovery (%)	-	-	-
		RSD (%)	3.91	10.30	16.52

(continued on next page)

Table S4. Results of mean concentration, standard deviation, recovery and relative standard deviation (RSD) obtained using the digestion method M3 and the standardized methods USEPA 3051a and 3052 applied to the NIST RM 8704 (n = 3 for all REEs). (continued)

NIST RM 8704					
REE	**Certified values (ug g^{-1})**		**M3**	**3052**	**3051a**
Eu	1.31±0.038	Mean (ug g^{-1})	1.236±0.039	0.818±0.075	0.88±0.14
		Recovery (%)	94.333	62.44	67.22
		RSD (%)	2.97	5.72	10.54
Gd	-	Mean (ug g^{-1})	5.29±0.16	3.43±0.4	4.07±0.75
		Recovery (%)	-	-	-
		RSD (%)	2.94	11.55	18.43
Tb	-	Mean (ug g^{-1})	0.781±0.024	0.522±0.046	0.586±0.096
		Recovery (%)	-	-	-
		RSD (%)	3.02	8.78	16.43
Dy	-	Mean (ug g^{-1})	4.98±0.16	3.81±0.65	3.16±0.54
		Recovery (%)	-	-	-
		RSD (%)	3.31	17.14	17.25
Ho	-	Mean (ug g^{-1})	0.944±0.034	0.602±0.065	0.58±0.1
		Recovery (%)	-	-	-
		RSD (%)	3.58	10.86	17.46
Er	-	Mean (ug g^{-1})	3.07±0.13	1.69±0.14	1.43±0.25
		Recovery (%)	-	-	-
		RSD (%)	4.19	8.25	17.73
Tm	-	Mean (ug g^{-1})	0.4157±0.0059	0.262±0.028	0.198±0.03
		Recovery (%)	-	-	-
		RSD (%)	1.41	10.71	15.14
Yb	-	Mean (ug g^{-1})	2.88±0.11	1.75±0.19	1.12±0.18
		Recovery (%)	-	-	-
		RSD (%)	3.73	10.75	15.73
Lu	-	Mean (ug g^{-1})	0.409±0.02	0.27±0.19	0.157±0.023
		Recovery (%)	-	-	-
		RSD (%)	4.81	69.42	14.54

(NS) = Do not differ statistically ($p<0.05$) according to the *t*-test (n=3), n=3 for all measurements.

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ARTICLE

Comparative Study Between Calcination and Thermogravimetry Techniques in the Quantification of Carbon Black Content in Polymeric Resins

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In the characterization laboratory involving polymeric resins from the petrochemical industry, the carbon black content in polymeric resins is analyzed using two techniques: calcination and thermogravimetric analysis (TGA). The objective of this study is to verify whether there is a significant difference between the results obtained by these two methods, with the aim of reducing response times for customers while maintaining the same level of quality and accuracy and improving Health and Safety Environment (HSE) matters, such as reducing analysts' exposure to the high temperatures used in microwave oven for calcination. In the experimental conditions, the calcination technique obtained a higher uncertainty value compared to TGA, but the results showed precision and accuracy in both techniques. Furthermore, the method developed by TGA provided a 175% increase in productivity and advancement for the analysts safety involved in carrying out the activities as it has low risks when compared to calcination.

Keywords: calcination, TGA, carbon black, HSE, HDPE

INTRODUCTION

Carbon black is a commercial product manufactured by thermal decomposition (detonation or by incomplete combustion of carbon hydrogen compounds – oil or natural gas). According to IUPAC, it is an

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industrially manufactured colloidal carbon material in the form of spheres and of their fused aggregates, with sizes below 1000 nm.[1] Different categories of carbon black are available including furnace black (the most representative one), channel black, thermal black, acetylene black and lamp black.[2] From the technological point of view, carbon black is an additive incorporated into the polymer with the purpose of protecting against photooxidation, increasing the life cycle of the polymer exposed to the sun, acting as a radical inhibitor and as a bridge between molecules. Empirically it was found that an amount of 2 to 3% of carbon black prevents degradation and makes the polymer more resistant.[3]

The quantification of carbon black in polymeric materials has been investigated using calcination techniques, thermogravimetric analysis (TGA) and dispersion measurements. Examples of carbon black quantification by other techniques involve, for example, transmittance electron microscopy (TEM) through the quantification of structural parameters (on a nanometric scale),[4] physical quantification of interfacial interactions with polymer matrices[5] or laser welding.[6] In the petrochemical industry, the determination of carbon black content in polymeric resins is performed using gravimetric and TGA methods based on ASTM and ISO standards. The determinations aim to quantify and demonstrate the real concentration of carbon black present in polymeric resins, ensuring the specification, quality and life cycle of the polymer exposed to the sun.[7-9] The experimental conditions vary between the two methodologies: in the case of calcination analysis, 2 g of sample are used per duplicate, with a limitation of 2 analyses/day with manual steps due to the weighing, heating and cooling time of the microwave oven, while the TGA analysis uses 20 mg of sample per duplicate and has an average of 11 analyses/day that were performed automatically. The sample masses were defined through previous internal studies based on standards.[9] Thus, evaluating the process as a whole, the increase in productivity is evident, in addition to the simplification of the process and agility in terms of response time.

Therefore, the present study aimed to evaluate the potential differences between carbon black in polymeric matrix quantification by calcination and TGA measurements, seeking to reduce the response time of results for customers with the same quality, accuracy and improvements in terms of Health, Safety and Environment (HSE).[3] It is worth remarking that the fundamental responsibility of each company and employee is to minimize the environmental impact where one operates, focusing on the HSE and well-being in the workplace.

MATERIALS AND METHODS

Samples

A more common Braskem high-density polyethylene (HDPE) resin was selected and collected with a specification of 2.0 to 2.5% carbon black and, to carry out this study, the composition of the master used to additive the resins contains 48% carbon black.

Calcination

The technique is based on mass difference, where a sample is subjected to microwaves (CEM-PHOENIX, 2450 MHz and 122 mm band) in a compressed air atmosphere typical of an industrial plant and the energy is absorbed by the sample molecules, increasing the kinetic energy of the sample and causing internal heating and differentiated polarization, which expands, agitates and heats the material.[10] In this case, the carbon black content is determined from the degradation that occurs with the formation of volatiles, causing mass loss in the sample subjected to temperatures of 600 °C and 800 °C, with an average of 2 analyses/day.[8-10]

Thermogravimetric analysis (TGA)

This technique was performed on a TA TGA Q500 thermal analyzer (TA Instruments, New Castle, US). HDPE samples weighing 10.0 ± 2.0 mg were heated from room temperature to 500 °C at 10 °C min^{-1} under N_2. The inert gas N_2 ensures samples stability whithout the possibility of samples degradation (carbon black releasing) between the range of 500 and 600 °C. The percentage of carbon black is determined from the mass loss curve after changing gases from N_2 to O_2, with an average of 11 analyses/day.[8-11]

Method validation

Both methods were evaluated in terms of the following parameters:

Precision

It is the proximity among several readings carried out on the same sample, and is usually expressed by the standard deviation, variance or coefficient of variation (CV) of replicates, with ≤ 5% being considered appropriate, determined via Excel for the calculations.[12-14]

Repeatability (r)

It is the degree of agreement between the results of successive measurements of the same sample carried out under the same measurement conditions.[12-14]

Reproducibility (R)

It is the degree of agreement between the results of successive measurements of the same sample carried out under different measurement conditions, in this case, by a different analyst.[12-14]

Gage Repeatability and Reproducibility (Gage R&R)

It is the statistical tool that measures the amount of variation in the measurement system resulting from the measuring device and the people making the measurement, with ≤ 5% being considered adequate, determined by Excel for the calculations.[12-14]

Uncertainty

It is the expression of statistical dispersion of the values assigned to a measured quantity. All measurements are subject to uncertainty and a measurement result is only complete when it is accompanied by a statement of the associated uncertainty, such as the standard deviation, determined via Excel.[12-14]

Statistical tests

Complementary *t* and Grubbs' tests have been employed. The latter has been employed aiming at checking the presence of extreme values in sample observations. Extreme values can be considered as manifests of the random variability inherent in the data, or just an error in the calculation during data collection and even a hasty note by the operator, determined by minitab software for the calculations.[12-14] Expanded coefficient of variation (CVE) is the acceptance criterion for a quantitative analysis carried out with more than one route. It is the repeatability limit, i.e. the maximum value admitted for a given analysis, with acceptance criteria in the laboratory of ≤ 5%, determined via Excel calculations.[12-14] Statistical analyses were also performed using analysis of variance (ANOVA) using SPSS (IBM). A value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS

Analytical results

The sample was evaluated on five different days in triplicate by two analysts, totaling 15 repetitions per analyst, with the aim of testing homogeneity and amplitude. The results can be found in Table I.[12-14]

It is observed through the reference values present in Table I that the results obtained are within the sample specification range, therefore, it is considered that the HDPE sample is homogeneous and stable. It can also be seen that, using the TGA technique, the standard deviation found is smaller than that using calcination.[10-14]

Table I. Quantification results by calcination and TGA

Repetition	Calcination Results		TGA Results	
	Analyst 1 (%)	Analyst 2 (%)	Analyst 1 (%)	Analyst 2 (%)
1	2.3	2.1	2.2	2.1
2	2.2	2.1	2.2	2.2
3	2.2	2.0	2.2	2.2
4	2.1	2.1	2.2	2.2
5	2.1	2.2	2.2	2.2
6	2.1	2.2	2.1	2.2
7	2.1	2.2	2.1	2.2
8	2.1	2.2	2.2	2.2
9	2.1	2.1	2.1	2.2
10	2.2	2.2	2.1	2.2
11	2.3	2.2	2.1	2.2
12	2.2	2.2	2.2	2.2
13	2.1	2.0	2.1	2.2
14	2.1	2.0	2.2	2.2
15	2.2	2.0	2.0	2.2
Average	2.2	2.1	2.1	2.2
Reference value (% Carbon Black)	2.1 ± 0.10		2.2 ± 0.04	

A typical thermogram is shown in Figure 1a and presents two stages of mass loss. In an inert atmosphere (N_2), the first stage, close to 500 °C, corresponds to the mass loss (TG) related to the decomposition of the polymer (HDPE). Subsequently, the atmosphere is changed from inert (N_2) to oxidizing (oxygen) close to 500 °C and the second stage of mass loss refers to the carbon black, which is oxidized and released in the form of carbon dioxide (CO_2). The maximum temperatures of polymer decomposition and carbon black oxidation can be obtained from the DTG curve. In the case of HDPE (Figure 1b), we observed the absence of the signal related to the degradation of carbon black between 500 and 600 °C at the decomposition of the polymer (HDPE) close to 450 °C. By comparing Figure 1a with Figure 1b, it is possible to observe the difference in initial temperature of the polymer decomposition (HDPE) and relate it to the thermal stability characteristic of carbon black.[2,8,11]

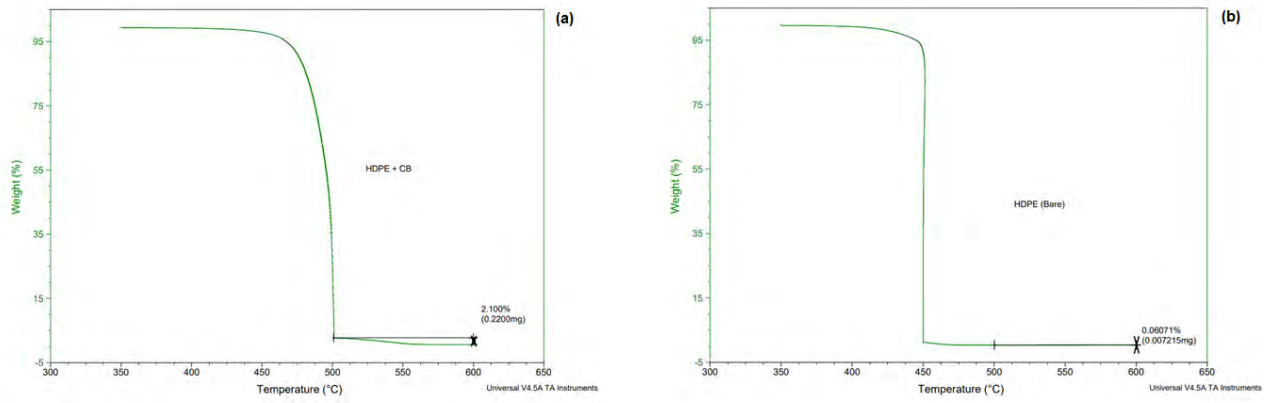

Figure 1. Typical TGA thermogram of (a) HDPE containing CB; (b) HDPE (bare).

Precision / Repeatability and Reproducibility

Repeatability and reproducibility were calculated according to laboratory results acceptance criteria standards (it is worth noting that internal control was performed and verified by Braskem's quality management through control charts and is based on standards cited in references),[12-13] using the data available in Table I. From these values, repeatability and reproducibility calculations were performed, as shown in Table II.[12-14]

Table II. Calcination and TGA results from the Repeatability and Reproducibility assessment

Assessment	Calcination Results (%)	TGA Results (%)	Conclusion
CV R&R	2.1	1.2	Adequate
CV REPE	1.63	1.07	Adequate
CV REPRO	1.25	0.59	Adequate
Max CV Analyst 1	2.8	2.7	Adequate
Max CV Analyst 2	2.7	4.8	Adequate

Where, %CV ≤ 5 is adequate; 5 < %CV ≤ 15 may be appropriate depending on the importance of the application, the cost of the instrument, the maintenance cost, etc.; %CV > 15 % is inadequate, measurement system needs improvements.

Through the values obtained by the Repeatability and Reproducibility calculations, the results of the calcination and TGA techniques are considered adequate, i.e., the coefficient of variation values are equal to or less than 5%, in accordance with specific laboratory standards.[12-14]

Uncertainty calculation

The data present in Table I were used to calculate and generate uncertainties, with the calcination method presenting an uncertainty of 4.3% and the TGA method 2.6%. Combined uncertainty was used, where the square root of the squared deviation results is taken according to results acceptance criteria standards.[12-14]

Expanded coefficient of variation (CVE)

The CVE for the TGA analysis is 3% and for the calcination method 4.7%, determined based on the repeatability limit over the analysts' average multiplying by 100, in accordance with results acceptance criteria standards.[12-14]

Statistical tests

Two-Way ANOVA was conducted to determine to what extent analytical technique and technician have an effect on income. The statistical analysis revealed that there was not a statistically significant interaction between both effects ($F(2, 57) = 1.4172$, $p = 0.251$).

The results obtained in Table I were also subjected to the Grubbs Test to verify whether there is significant variation between the two techniques and the presence of outliers (anomalous results). (Figure 2)

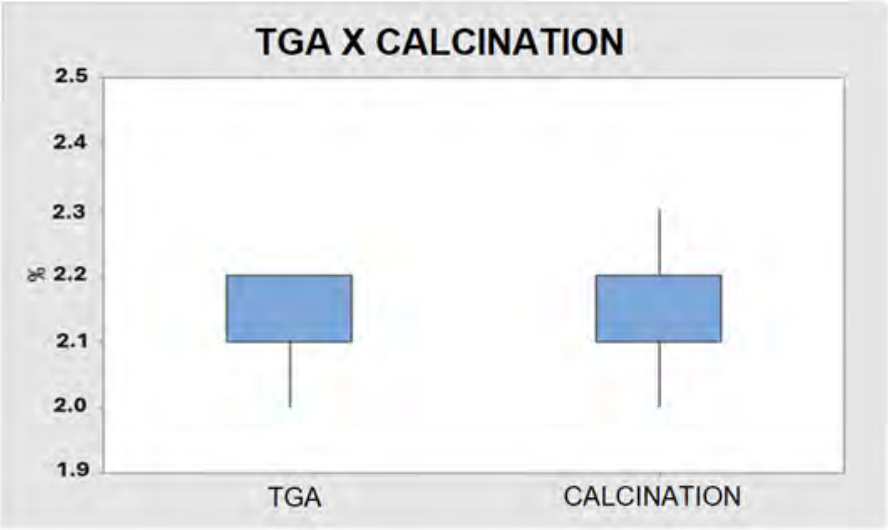

Figure 2. Grubbs test results between TGA x calcination techniques.

Through the Grubbs test it was possible to verify that the TGA results presented precision and accuracy, as well as the calcination results. Furthermore, it can be observed that there is no presence of outliers.

FINAL REMARKS

It was possible to observe that the calcination technique achieved a higher uncertainty value compared to TGA, which was already expected as it has more steps during the execution of the analysis (use of analytical balance, microwave oven, desiccators and handling) that may influence. The TGA technique is automated, having an internal scale system, which results in a reduction in analytical errors. Thus, with the help of statistical tools, after determining the analytical uncertainty of the techniques and correlating the data obtained in this study, it is concluded that the TGA technique presents precision and accuracy as much as the absolute calcination technique, being able to correlate the proximity to the real value of the samples with their dispersion in a series of measurements.

Furthermore, considering the number of daily analyses possible through calcination (2 analyses) compared to TGA (11 analyses), a 175% increase in productivity was obtained, as well as an improvement in the safety of the analysts involved in the execution of the activities, since the calcination technique required exposure to high temperatures, around 800 °C, and for each sample, this exposure was repeated 4 times. While using the TGA technique, the existing risk is considered low, where there is also heating to high temperatures, but the analyst does not have contact with the heated region.

Thus, through this study, it was possible to understand the process improvements resulting in increased safety, health and environment (HSE), as well as the importance of using statistical calculations as problem solutions, as each tool has its own importance, however the use of them in combination associated with correct interpretation is fundamental, thus allowing opportunities for growth and development.

Conflicts of interest

The authors declare to have no conflicts of interest.

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49th Annual Meeting of the Brazilian Chemical Society (RASBQ)

Following the success of the 48th RASBQ and numerous requests for the event's return, the 49th Annual Meeting of the Brazilian Chemical Society (49th RASBQ) will once again take place in Campinas from June 15 to 18, 2026.

The theme of the 49th RASBQ is "Chemistry for National Sovereignty," an urgent issue in the international context. In Brazil, this theme calls for a broad discussion regarding the country's natural resources and their processing. New technologies are needed for renewable energy generation, sustainable agricultural production, addressing the climate emergency, and much more. Chemistry is one of the most strategic fields for a sovereign nation facing these challenging circumstances. In Brazil, chemistry related to natural resources is a strong and reliable field, both in research and innovation, as well as in industry. Chemistry is a cornerstone of national development that must be recognized and increasingly strengthened.

PROGRAM **REGISTRATION**

ABSTRACTS **VENUE**

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FEATURE

The 10th Analitica Latin America Congress Consolidates Brazil as a Scientific Hub

Lecture given during the 10th Analitica Latin America Congress.
Photo: Analitica Latin America

From September 23 to 25, 2025, the 10th Analitica Latin America (ALA) Congress was held in São Paulo, bringing together around 90 participants and reaffirming its position as the leading scientific meeting on analytical chemistry in Latin America. Held alongside the ALA Fair—the largest event in the analytical chemistry sector in Latin America—the ALA Congress fostered dialogue among academia, industry, and research institutes in an environment focused on innovation, sustainability, and technological development.

The 2025 edition of the ALA Congress introduced an unprecedented change to the event's scientific structure: Each day's agenda was curated by a different institution, reinforcing both the scientific and technical depth and the diversity of topics and specializations.

- Day 1 (September 23) was curated by the Organizing Committee of the ALA Congress.
- Day 2 (September 24) was curated by the Brazilian Chemical Society (SBQ).
- Day 3 (September 25) was jointly curated by the Organizing Committee of the National Meeting of Analytical Chemistry (ENQA) and the Ibero-American Congress of Analytical Chemistry (CIAQA)

This new configuration reinforced the ALA Congress's role as a strategic space for collaboration across diverse fields of contemporary analytical chemistry.

Theme of the First Day: Instrumental Advances and Critical Applications

The first day, led by the Organizing Committee of the ALA Congress, highlighted emerging research in electrochemistry, detection methods, and quality control. Davi Marques de Farias (USP) presented the opening lecture, "*Tunable Mass Transport and Enhanced Electrochemical Performance of CO_2 Laser-*

Engraved Electrodes," showcasing advances in high-performance electrode engineering. Next, Alexandre Cunha, from ELGA LabWater – Veolia, discussed the role of ultrapure water in analytical reliability and the execution of critical methods—a central theme for research laboratories, quality control, and industrial development. The program continued with discussions on ion chromatography, flow cytometry automation, macromolecule separation, and advanced international detection methods. The Federal Council of Chemistry (CFQ) also held a roundtable discussion on cannabis inputs.

Theme of the Second Day: The SBQ Emphasizes Basic Science, Metrics, and Sustainability

The program for September 24 was organized by the SBQ, an entity recognized for its historical role in consolidating chemistry in Brazil. It presented topics of significant contemporary importance:

- Green analytical chemistry applied to food
- Sample preparation for halogens and complex chemical species
- Metrics and validation of analytical methods
- Environmental geochemistry applied to monitoring
- Ionomic effects and nanoparticles in plants
- Rare earth fractionation in natural samples

The lectures spanned from theoretical foundations to emerging environmental applications, reinforcing the relevance of analytical chemistry in understanding natural processes, supporting public policies, and overcoming regulatory challenges.

Attentive participants listening to another lecture held during the 10th Analitica Latin America Congress. Photo: Analitica Latin America

Theme of the Last Day: The ENQA and CIAQA Project the Frontiers of Analytical Chemistry

The ENQA and CIAQA jointly curated the final day, expanding the international scope of the ALA Congress. The strategic topics included:

- Emerging contaminants and microplastics in aquatic environments
- Ionic adsorption for the petroleum industry
- Functional microneedles for diagnosis and controlled release
- Multimodal platforms for non-target chemical speciation
- Laser-induced breakdown spectroscopy (LIBS) applied to the recovery of metals from electronic waste

This Ibero-American integration highlights the growing internationalization of the field of analytical chemistry and Brazil's active participation in global scientific agendas related to energy, the environment, diagnostics, and the circular economy.

Scientific Highlights and Integration

The 10th ALA Congress emphasized three key pillars of scientific advancement:

- Institutional integration, marked by shared curatorship among the ALA Congress, the SBQ, the ENQA, and the CIAQA. This collaboration united specialists from various research fields, creating a comprehensive and diverse scientific landscape.
- Technical innovation and interdisciplinarity, expressed through discussions on electrochemistry, spectrometry, LIBS, separations, green chemistry, automation, and microtechnologies—demonstrating the continuous and multifaceted expansion of the field.
- The connection between research, industry, and public policy, reinforced through the participation of companies, researchers, and regulatory bodies such as the CFQ, underscoring analytical chemistry's potential to address strategic societal challenges.

In this context, the 10th ALA Congress reaffirmed Brazil's position as a scientific hub, supported by established research groups, robust infrastructure, and strong links with industry—consolidating analytical chemistry as a driving force for innovation, sustainability, and scientific development in Latin America.

Awards

The three winners of the awards given to the best scientific posters presented at the 10th Analitica Latin America Congress. Photo: Analitica Latin America

A committee of experts evaluated the best scientific papers presented at the ALA Congress. Three winners received prizes of up to R$2,500 and gained visibility at one of the largest analytical chemistry events in Latin America.

- **First place** went to Davi Farias for "*Tunable Mass Transport and Enhanced Electrochemical Performance of CO_2 Laser-Engraved Electrodes*." This study presents advanced electrochemical platforms for sensing molecules of interest, with potential applications in environmental monitoring and public health.
- **Second place** was awarded to Higor B. de Oliveira, for research on X-ray fluorescence as a characterization technique in producing a domestic sludge reference material—a contribution to standardization and rigorous analytical control.
- **Third place** went to "*Catalytic Exploring Digital Videos for the Sequential Determination of Copper and Sucrose in Cachaça*." This project proposes an accessible analytical method for small producers, enabling monitoring of copper contaminants and sucrose levels to improve artisanal quality control.

Tribute to Professor Lauro Tatsuo Kubota Moves Participants

Ceremony for the “Lauro Kubota Award for Young Talent in (Bio) Analytical Chemistry”. Photo: Analitica Latin America

A moving tribute marked the morning of September 24. During a special coffee break hosted by the *Brazilian Journal of Analytical Chemistry* (*BrJAC*), with support from Nova Analítica, Metrohm, and NürnbergMesse, family members, colleagues, and researchers gathered to celebrate the distinguished career of Professor Lauro Tatsuo Kubota, one of the most influential figures in analytical chemistry in Brazil.

The ceremony highlighted Professor Kubota’s importance as a scientist, educator, and academic leader. Recognized for his pioneering contributions to electrochemistry, sensor development, and scientific innovation, he left a lasting legacy that has shaped generations of researchers and strengthened Brazil’s presence on the international stage of analytical chemistry.

In addition to the fond memories shared, the ceremony featured the presentation of the *Lauro Kubota Award for Young Talent in (Bio)Analytical Chemistry*, an award created to inspire young researchers and to perpetuate Professor Kubota’s lifelong commitment to scientific excellence. The moment was marked by emotion, respect, and recognition, reaffirming the profound impact of his life and work on the national scientific community.

The award will be presented annually. This year’s recipient was Prof. Dr. Vagner Bezerra dos Santos, Adjunct Professor at the Federal University of Pernambuco. The award was presented by Prof. Marco Aurélio Zezzi Arruda and Luciene Campos, Editor-in-Chief and Sales Manager of *BrJAC*, respectively.

Analitica Latin America Expo 2025: Technological Innovation, Applied Science, and Market Expansion Mark the Biggest Edition of the Decade

The ALA Expo 2025, also held from September 23 to 25 at the São Paulo Expo Center, established itself as the largest and most representative trade fair in the analytical chemistry sector in the past decade. This edition of the ALA Expo registered 14,330 visitors—a 50% increase compared to the previous year—and welcomed 450 international professionals, reflecting a 30% growth in foreign participation. This broad and highly qualified audience included technical and university students, young researchers, laboratory managers, industry professionals, and specialists in analytical chemistry. With 350 exhibiting brands, the event served as a showcase for innovations in analytical instrumentation, laboratory technologies, and advanced solutions for sample preparation, automation, and quality control.

Aerial view of the Analitica Latin America Expo 2025. Photo: Analitica Latin America

The booths featured technologies ranging from high-performance spectrometry and chromatography systems to sample-preparation robots, drone-mounted sensors, artificial intelligence (AI)-driven platforms, and portable systems for environmental and industrial analyses. The growing presence of sustainable technologies—such as green solvents, eco-efficient materials, and low-energy methodologies—confirmed the sector's alignment with global Environmental, Social, and Governance (ESG) standards. Within the first two hours of the opening day, the exposition had already generated R$13.5 million in business, underscoring the commercial dynamism and strategic importance of Brazil's analytical sector.

The ALA Expo also served as a technological advancement platform for educational institutions. A notable example is the group of students from the National Service for Industrial Training (SENAI), who encountered equipment more sophisticated than that available in their own educational units—highlighting the event's crucial role in training future professionals.

The Analitica Latin America Expo exhibition area brought together established Brazilian companies as well as global leaders in analytical instrumentation. Photo: Analitica Latin America

The exhibition pavilion brought together established Brazilian companies and global leaders in analytical instrumentation, including Bio Scie, Metrohm Brasil, Agilent Technologies, Nova Analitica, Shimadzu, Thermo Fisher Scientific, Waters, PerkinElmer, Bruker, Sciex, Eppendorf, Merck, and Bio-Rad, among others. This diversity of exhibitors underscores the consolidation of the ALA Fair as the main continental meeting point for manufacturers, distributors, researchers, and end users.

The ALA Expo demonstrated a mature integration among academic research, industry, and technological development. In addition to the technical exhibition, the program featured seminars, lectures, and networking events that brought together university researchers, application engineers, regulatory specialists, representatives of public and private laboratories, and suppliers of equipment and automation solutions. This collaboration reinforces the ALA Expo as a strategic environment for identifying trends, establishing partnerships, prospecting technologies, and discussing solutions to contemporary analytical challenges.

The Analitica Latin America Expo has reaffirmed itself as the main barometer of the future of analytical instrumentation in Latin America. Photo: Analitica Latin America

The ALA Expo 2025 leaves a significant legacy: It strengthens Brazil's position as a hub for analytical science, boosts the laboratory innovation ecosystem, and expands international connections.

Future dates for the event have already been confirmed:

- 2026: The Analitica Road Show in Santiago, Chile
- 2027: The 19th Edition of the Latin America Analytical Expo in São Paulo (September 28–30)

With record numbers, a wide variety of exhibitors, and a strong emphasis on technological innovation, the ALA Expo has reaffirmed itself as the main barometer of the future of analytical instrumentation in Latin America.

Text: Lilian Freitas – BrJAC Publisher
Source: Analitica Latin America

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FEATURE

COLACRO XX Brings Together 450 Participants and Becomes One of the Most Significant Events in Latin American Chromatography

Opening ceremony. Photo: Gabriele Maciel

The 20th Latin American Congress on Chromatography and Related Techniques (COLACRO XX), held from October 28 to 31, 2025, in Campos do Jordão (SP, Brazil), marked one of the most relevant editions in its history. With 450 participants from various countries across Latin America, Europe, and North America, the event has reaffirmed its strategic role in advancing separation techniques and sample preparation.

The organizer of the event—Professor Fernando Lanças, from the São Carlos Institute of Chemistry at the University of São Paulo (IQSC-USP)—emphasized that the congress has fulfilled its mission of promoting scientific excellence and integration among academic communities: "COLACRO achieved its audience goal and exceeded expectations in terms of scientific quality. This demonstrates the strength and relevance of the congress, even in the face of the challenges of resuming in-person events after the COVID-19 pandemic and the economic difficulties that many Latin American countries are facing."

Considered one of the five most important international events in the field, COLACRO XX provided a space for scientific convergence, promoting in-depth discussions on current challenges and emerging technologies that are shaping the future of analytical chemistry.

The scientific program was one of the highlights of this edition. The plenary lectures, delivered by world-renowned experts, addressed the most advanced topics in separation science.

Lecture by Professor Luigi Mondello. Photo: Gabriele Maciel

The professors who presented plenary lectures and their respective titles were:

- Luigi Mondello (Italy)—*Cryogenic Zone Compression after Gas Chromatography Separation to Enhance Sensitivity*
- Elia Psillakis (Greece)—*The Journey from Green Sample Preparation to Sustainable Analytical Chemistry*
- Valérie Pichon (France)—*Environmentally Friendly and Selective Sample Preparation Methods for Target Analytes in Complex Samples*
- Paola Dugo (Italy)—*Comprehensive Two-Dimensional Liquid Chromatography for the Characterization of Food and Natural Products*
- Elena Stashenko (Colombia)—*Listening to Insect–Soil–Plant Chemical Conversations with Chromatographic Techniques*
- José Manuel Nogueira (Portugal)—*Emerging Sustainable Trends in Passive Microextraction Techniques*

Hence, COLACRO XX provided a platform for researchers from throughout the world to discuss cutting-edge science that integrates sample preparation, chromatographic multidimensionality, advanced spectrometry, and new materials applied to instrumentation.

In addition to the plenary sessions, there were several satellite workshops. The traditional Workshop on Recent Advances in Sample Preparation (WARPA)—a regional reference in this field—brought together experts who discussed microextraction, new green solvents, portable instrumentation, and the integration of artificial intelligence in sample preparation. In parallel, the Brazilian Symposium on Chromatography and Related Techniques (SIMCRO) and other specialized symposia addressed topics such as GC×GC, LC×LC, electrophoresis, new materials, microfabrication, and chromatography applied to food, pharmaceuticals, the environment, and biotechnology, expanding the technical depth of the congress.

COLACRO XX also reinforced its role in training generations of researchers by awarding several traditional and highly prestigious honors. The medal ceremony was a symbolic moment of the event.

The COLACRO Medal

The COLACRO Medal is one of the most prestigious honors awarded to researchers in the field of Separation and Related Techniques worldwide. It recognizes individuals who have made outstanding international contributions to the development, promotion, and application of separation techniques. The medal is traditionally awarded during the Opening Ceremony. This year's recipients were:

- Prof. José Manuel Nogueira (Portugal)
- Prof. Leandro W. Hantao (Brazil)

The CIOLA Medal

The CIOLA Medal honors the memory of one of the pioneers of instrumental chromatography in Brazil, Professor Dr. Remolo Ciola. It recognizes either a senior researcher who has made significant contributions to the development of the field in Brazil or a *young researcher* (under 35 years of age at the time of nomination) with proven creativity and productivity in the area. The CIOLA Medal is sponsored by Nova Analítica. This year's recipient was:

- Prof. Luiz Antônio D'Ávila (Brazil)

The WARPA–Janusz Pawliszyn Medal

The WARPA–Janusz Pawliszyn Medal is awarded during the WARPA to a researcher who has made a significant contribution to the development and application of contemporary sample preparation techniques. The award is named after Prof. Dr. Janusz Pawliszyn, a pioneer in micro-techniques for sample preparation, particularly solid-phase microextraction (SPME). This year's recipient was:

- Prof. Ednei Gilberto Primel (Brazil)

Awards for Posters and Oral Presentations

The five best posters presented at the event were recognized and awarded by a group of researchers appointed by the Scientific Committee. They made their selections based on established evaluation criteria. The young researchers were recognized for innovative studies in:

- Miniaturized techniques
- GC-MS and LC-MS
- Green sample preparation
- Food and beverage analysis
- Environmental chemistry
- Toxicology and forensics

This recognition of undergraduate and graduate students reinforces COLACRO's role in training new Latin American scientific leaders.

Exhibitor area. Photo: Luciene Campos

Although primarily a scientific congress, COLACRO XX also saw strong participation from analytical instrumentation companies, which sponsored the event and presented technological solutions aligned with the topics discussed. Among the companies sponsoring, supporting, or traditionally involved with COLACRO were Nova Analítica, Agilent Technologies, Waters, Shimadzu, Merck, Sciex, and others. The presence of these companies reinforced the essential connection between scientific research and technological innovation, allowing researchers to engage directly with state-of-the-art instruments, consumables, and software.

COLACRO XX was more than just a commemorative edition—it was a confirmation of Latin America's scientific strength in separation techniques and sample preparation. With 450 participants, dozens of international lectures, specialized workshops, and an extensive network of collaborations, the congress has reaffirmed its status as one of the world's leading scientific forums in the field.

Text: Lilian Freitas – BrJAC Publisher
Source: COLACRO XX

SPONSOR REPORT

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Keywords: Pharmaceutical products, Leachables, Extractables, GC-MS, Deconvolution, Unknown screening, ISQ 7000

Introduction

From manufacturing to administration, pharmaceutical products come in contact with multiple packaging systems made of different materials. Detailed compatibility studies on these materials may be required to ensure that product quality remains acceptable and that no safety concern is raised due to product/material incompatibility, especially when the administration method associated with a particular dosage or form of the product might maximize the risk of exposure and interaction.[1,2]

Among these studies, extractables and leachables represent a huge portion of the work the analyst does to characterize the substances potentially leaching into the product. The terms “extractables” and “leachables” are well defined by industrial working groups,[3,4] mixed working groups[5] (such as the Product Quality Research Institute, the Leachables and Extractables Working Group, including pharmaceutical development scientists representing industry, health agencies, and academia) and subject experts.[6]

Often extended to process materials, these definitions are consistent with the one proposed by the United States Pharmacopeia, which has added specific chapters of extractables and leachables studies:[7,8]

- Extractables are substances such as “organic or inorganic chemical entities that can be released from a pharmaceutical packaging/delivery system, packaging component, or packaging construction material under laboratory, conditions. Depending on the specific purpose of the extraction study […] these laboratory conditions (e.g., solvent, temperature, stoichiometry, etc.) may accelerate or exaggerate the normal storage/ use conditions for a packaged form. Extractables themselves, or substances derived from extractables, have the potential to leach into a drug product under normal conditions of storage and use.”
- Leachables are “organic or inorganic chemical entitiesthat migrate from a packaging/delivery system, packaging component, or packaging construction material into an associated drug product under normal conditions of storage and use or during drug product stability studies. Leachables are typically a subset of extractables or are derived from extractables.”

The identification of potential leachables through a preliminary extractable study and the attribution to the contact component from which they originate are important. Such species may react with the drug product or formulation ingredients, compromise the efficacy of the drug product or interfere with dosage consistency, and finally, may cause a negative health effect.

Studies for the determination of extractables and leachables are typically carried out using different analytical approaches e.g. by inductively coupled plasma (ICP) for elemental composition, or by liquid chromatography mass spectrometry (LC-MS) for nonvolatiles.

Gas chromatography mass spectrometry (GC-MS) is mostly applied for volatile components using direct headspace (HS) analysis, or liquid injection after a solvent extraction step for semi-volatile compounds.
This application note describes a part of an extractable analysis of an elastomeric plunger considered for potential use in a dental injectable cartridge using different extraction techniques, derivatization and HS analysis by single quadrupole GC-MS. A parallel classical flame ionization detection (FID) channel was configured for use in a future routine method, if required. While the composition of the plunger is known from the manufacturer, the drug product manufacturer has very little information about its composition and consequently about the substances that might migrate into the applied medicine.

Experimental

Sample preparation

The elastomeric plunger material was examined in several ways. The volatiles were determined via direct HS analysis. For the headspace injection, 10 plungers were placed in a 20 mL HS vial.
The extractables of the sample were studied by preparing three different liquid extracts for injection using three extraction procedures:

1. Aqueous extraction of the plunger material followed by a dichloromethane (DCM) extraction of the aqueous phase, no derivatization
2. The above DCM extract was derivatized using BSTFA (10 × 1 mL ampule pack, P/N TS-38830)
3. Isopropanol (IPA) extraction

These extractions were selected for the purpose of this application note and were a part of a global extraction study including additional methods and extraction techniques.

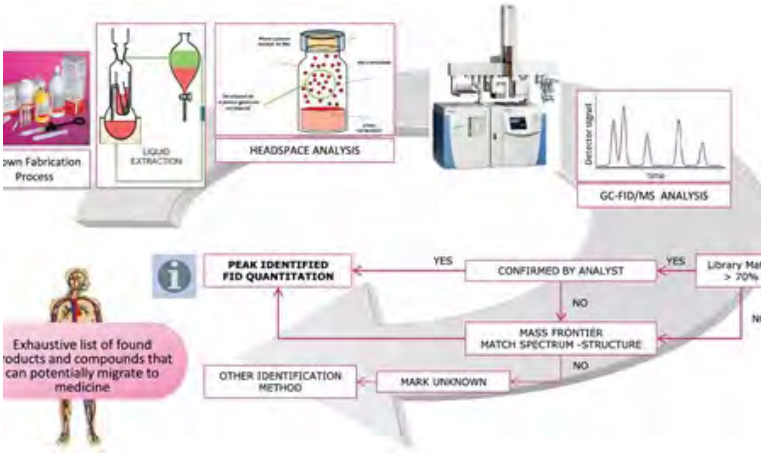

Figure 1. Workflow for extractable analysis.

Conditions

Figure 2. ISQ GC-MS system with TriPlus RSH autosampler.

The analyses were performed using a Thermo Scientific™ TRACE™ 1310 GC system with parallel FID and MS detection with the Thermo Scientific™ ISQ™ single quadrupole MS sytem* as shown in Figure 1. The parallel FID detection was accomplished by using a Silflow® connection, which also allowed a no-vent option for easy column change without the time-consuming venting of the mass spectrometer.

The TRACE 1310 GC system was equipped with a Thermo Scientific™ TriPlus™ RSH™ autosampler for both liquid and HS injections.

*Equivalent or better performances with the Thermo Scientific™ ISQ™ 7000 single quadrupole GC-MS system.

Table 1. GC system conditions.

TRACE GC 1310	Headspace
Injector:	Split/splitless, 320 °C Split 20 mL/min
Injection volume:	1 mL headspace
Inlet liner:	Splitless liner with glass wool, 4 mm ID (P/N 453A1925)
Oven program:	30 °C, 3 min, 8 °C/min to 280 °C, 280 °C, 10 min

Table 1 (cont'd). GC system conditions.

TRACE GC 1310	Liquid injection
Injector:	Split/splitless, 320 °C 1 min splitless time for liquid extracts
Injection volume:	1 µL of liquid extract
Inlet liner:	Splitless liner with glass wool, 4 mm ID (P/N 453A1925)
Oven program:	40 °C, 1 min, 8 °C/min to 325 °C, 325 °C, 10 min
TRACE GC 1310	Liquid and headspace injection
Carrier gas:	He, constant pressure 125 kPa
Column type:	Thermo Scientific™ TraceGOLD™ column 5MS, 30 m × 0.25 mm ID × 0.25 µm film thickness (P/N 26098-1420)
Transfer capillaries:	0.2 m × 0.2 µm to FID and 2 m × 0.15 mm to MS
FID:	300 °C, Air 350 mL/min, Hydrogen 35 mL/min, Nitrogen 40 mL/min
Transfer line:	300 °C

Table 2. MS system conditions.

ISQ Mass Spectrometer	
Ion source type:	Thermo Scientific™ ExtractaBrite™
Ion source temp.:	220 °C
Ionization mode:	EI, 70 eV
Emission current:	50 µA
Full-scan:	25–700 Da, 4 scans/s (250 ms/scan)

Data processing

The ISQ mass spectrometer in full-scan mode was used for identification of the unknown compounds with a parallel FID detection. The chromatograms of the HS analysis are shown in Figures 3 and 4. The retention times for compound identification match perfectly between the MS and the FID detection.

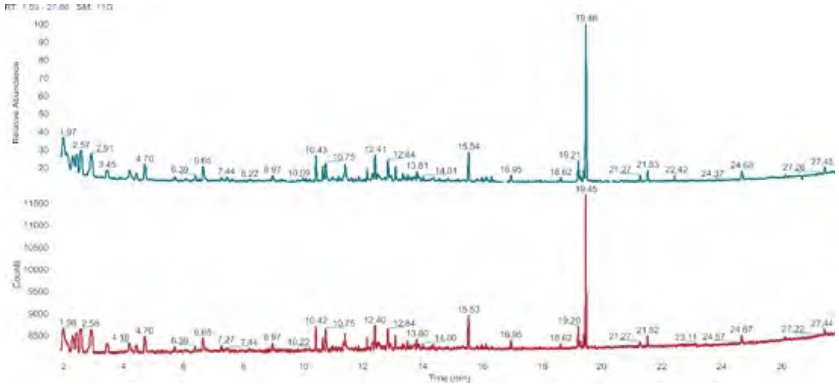

Figure 3. Headspace chromatograms of the elastomeric plunger (top MS TIC, bottom FID).

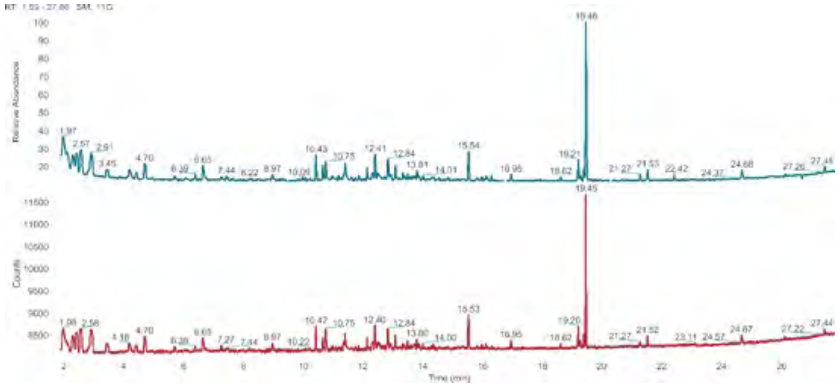

Figure 4. Zoom display—MS and FID retention times match perfectly (top MS TIC, bottom FID).

The analyses of the different liquid extraction and derivatization procedures follow in Figures 5–7, all of them with the total ion chromatogram (TIC) and FID traces. All chromatograms demonstrate that the parallel FID plus MS detection is in very good agreement of the eluted compound pattern.

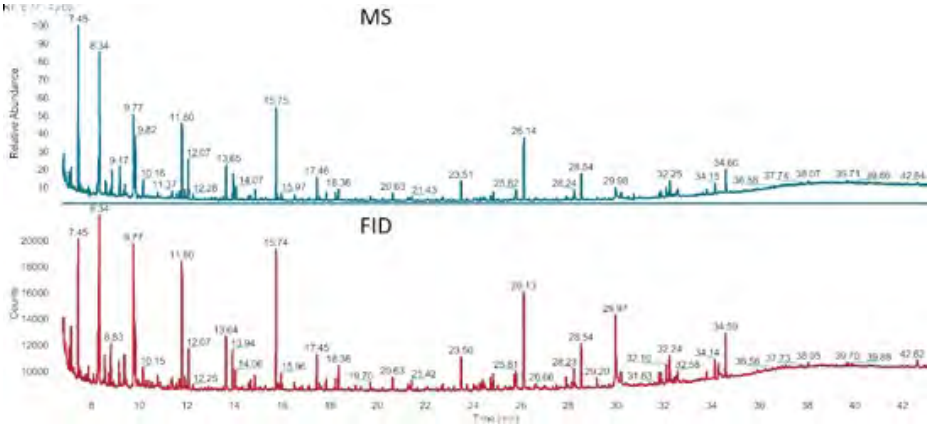

Figure 5. Chromatogram of the aqueous extract concentrated in DCM (top MS TIC, bottom FID).

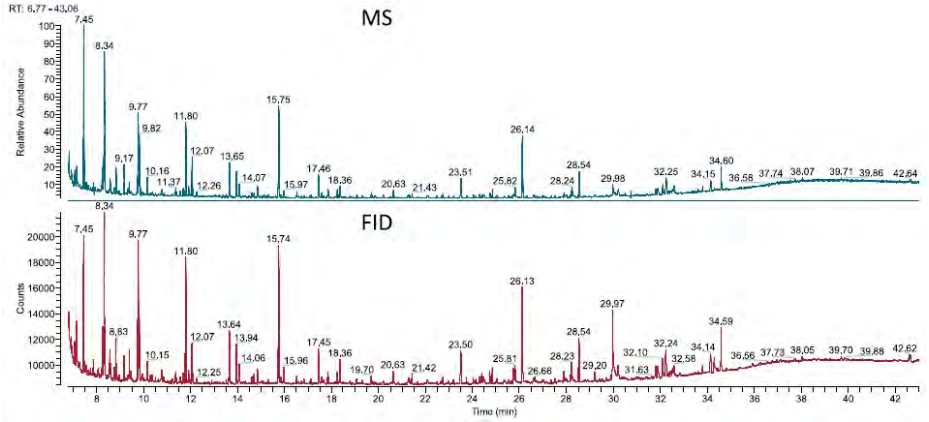

Figure 6. Chromatogram of the liquid DCM extract, derivatized with BSTFA (top MS TIC, bottom FID).

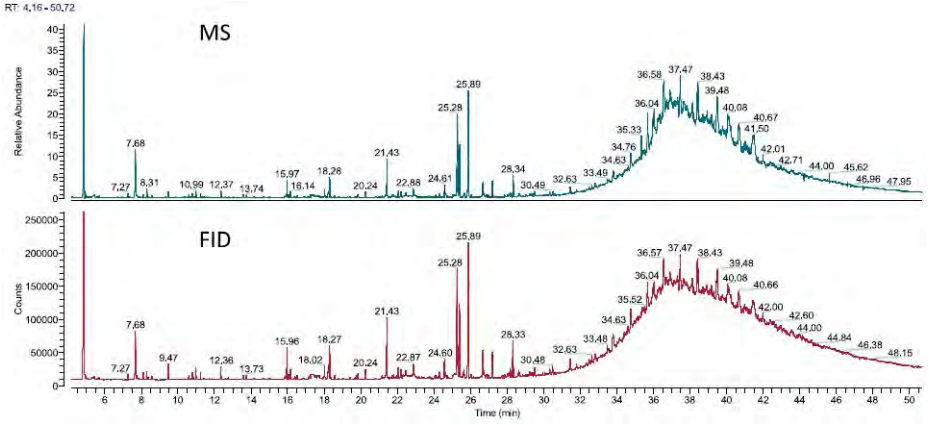

Figure 7. Chromatogram of the liquid IPA extract (top MS TIC, bottom FID).

AMDIS, the Automated Mass spectral Deconvolution and Identification System was used for a deconvolution of the complex chromatograms extracting the “clean” single compound mass spectra. For search and spectral comparison, the National Institute of Standards and Technology (NIST) library was used. AMDIS associates the found retention time with the mass spectrum for an improved identification. All results can optionally be transferred to Microsoft® Excel® for further investigation.

Mass spectra that could not be identified by library search were analyzed in the fragmentation pattern using Thermo Scientific™ Mass Frontier™ spectral interpretation software resulting in realistic structural proposals.

AMDIS chromatogram deconvolution

The AMDIS deconvolution program works in three steps:[9]

- *Step 1*: AMDIS analyzes the chromatogram. It counts the number of eluted compounds based on a minimum number of ions that show at the apex a common retention time (maximizing masses peak finder). The corresponding mass spectrum is extracted and cleared from a potential contribution to baseline and coeluting compound mass intensities.
- *Step 2*: AMDIS checks from a user library if target compounds are present by simultaneously matching retention time (or retention index, if available) and mass spectrum.
- *Step 3*: All detected compound spectra are compared with the reference spectra of the linked libraries allowing a filter with different criteria:
  - Only match those that are better than a chosen value
  - Only the top 'T' most abundant compounds in terms of their peak area
  - Only those 'S' compounds with a minimum % area over a given value
  - All compounds

Results and discussion

Tables 3 to 6 show the results from the same sample material using different extraction methods and filter candidates that reach at least a 75% match.

The list of identified compounds from the HS analysis (Figure 1) is presented in Table 3 with compound name, as well as peak information, retention time, and the reverse library matching factor. The 'Reverse' fit column tells the match quality in % of the proposed library entry with the unknown spectrum.

Table 3. Compounds identified by HS analysis with a library match >75% (of a total of 53 peaks detected).

Name	RT	Reverse	Area	Area	Height	Height
	[min]	Fit	[cts]	%	[cts]	%
Pentane, 2-methyl-	2.28	85	1917	4.68	368	2.36
Pentane, 3-methyl-	2.40	87	2308	5.63	442	2.84
Cyclopentane, methyl-	2.93	79	2847	6.95	515	3.31
Cyclohexane, methyl-	4.72	82	1333	3.25	346	2.22
2,3-Hexanedione	6.40	83	238	0.58	106	0.68
Hexanal	6.65	82	825	2.01	300	1.92
Propanal, 2,2-dimethyl-	10.43	88	950	2.32	538	3.46
Octane, 2,2,6-trimethyl-	10.66	88	445	1.09	259	1.67
Benzaldehyde	10.75	92	1031	2.52	461	2.96
Octane, 2,6,6-trimethyl-	13.34	79	164	0.40	115	0.74
2-Propenoic acid, […] Ageflex/Sipomer IBOA	19.46	95	6465	15.77	3438	22.08
Ethanone, 2,2-dimetho5 [...] DMPA, Photocure 51	27.44	82	217	0.53	125	0.8

Table 4. Compounds identified in the IPA extract, library match >75% (of a total of 118 peaks detected) (* see Mass Frontier Figure 6).**

Name	RT	Reverse	Area	Area	Height	Height
	[min]	Fit	[cts]	%	[cts]	%
Isopropanol P117	4.85	78	1737928	31.0	626333	28.9
Tricyclo[3.1.0.0(2,4)]hex-3-ene-3-carbonitrile	8.52	78	11506	0.2	5183	0.2
Benzyl alcohol	9.48	94	43713	0.8	23090	1.1
Benzene, (bromomethyl)-	10.80	90	23050	0.4	10571	0.5
Benzyl isopentyl ether	10.99	89	31562	0.6	18724	0.9
Benzyl isocyanate	11.25	94	21095	0.4	10942	0.5
Heptanoic acid, propyl ester	11.41	86	3680	0.1	2310	0.1
Decanone-2	12.60	79	3716	0.1	2230	0.1

Table 4 (cont'd). Compounds identified in the IPA extract, library match >75% (of a total of 118 peaks detected) (* see Mass Frontier Figure 6).**

Name	RT	Reverse	Area	Area	Height	Height
	[min]	Fit	[cts]	%	[cts]	%
Dodecane	12.77	92	5686	0.1	3521	0.2
Butane, 1,2,2-tribromo-	13.12	79	1611	0.0	1096	0.1
Tridecane	14.69	81	3736	0.1	2055	0.1
Benzene, (isothiocyanatomethyl)-	15.97	91	114760	2.1	47845	2.2
2-Dodecanone	16.40	84	7773	0.1	3757	0.2
Tetradecane	16.52	94	11436	0.2	5989	0.3
1-Bromo-3-(2-bromoethyl)heptane	18.02	65	57416	1.0	19643	0.9
1-Bromo-3-(2-bromoethyl)heptane ***	18.31	66	143599	2.6	50332	2.3
Pentadecane, 3-methyl-	19.39	88	5468	0.1	2763	0.1
2-Tetradecanone	19.82	89	16697	0.3	7514	0.4
Hexadecane	19.87	90	18647	0.3	8612	0.4
Impurity P116	21.43	93	245631	4.4	91711	4.2
N-Benzylidenebenzylamine	22.32	94	17660	0.3	5632	0.3
Tetradecanenitrile	24.29	81	17783	0.3	8962	0.4
Hexadecanoic acid, methyl ester	24.61	97	61160	1.1	28869	1.3
Phthalic acid, butyl cyclobutyl ester	25.06	88	10308	0.2	4055	0.2
Decane, 5,6-bis(2,2-dimethylpropylidene)-, (E,Z)-	25.29	72	403320	7.2	163977	7.6
Isopropyl palmitate	25.89	76	572407	10.2	204258	9.4
Oleanitrile	26.72	80	90096	1.6	41750	1.9
Heptadecanenitrile	26.97	76	8891	0.2	4527	0.2
Octadecanoic acid, methyl ester	27.19	92	73043	1.3	38750	1.8
Isopropyl stearate	28.34	90	99362	1.8	53712	2.5
Diisooctylphthalate @ P1828	31.81	82	22826	0.4	6778	0.3

Table 5. Compounds identified in the DCM extract, library match >75% (of a total of 88 peaks detected).

Name	RT	Reverse	Area	Area	Height	Height
	[min]	Fit	[cts]	%	[cts]	%
Toluene	4.17	96	17526	3.7	2914	3.2
Benzene, 1-fluoro-4-methyl-	4.30	88	6223	1.3	2039	2.2
Benzene, 1-fluoro-2-methyl-	4.37	93	244339	51.0	27516	29.7
Benzaldehyde	7.89	90	800	0.2	307	0.3
Benzylalcohol	9.38	88	3632	0.8	1081	1.2
Cyclopropyl carbinol	10.63	77	55086	11.5	9086	9.8
Decanal	12.79	88	4070	0.9	2030	2.2
Diethylphthalate	19.72	92	1195	0.3	682	0.7
Dibutyl phthalate	25.05	90	1253	0.3	559	0.6
Ethyl hexyl phtalate	31.81	93	6673	1.4	3399	3.7

Table 6. Compounds identified in the BSTFA derivatized DCM extract, library match >75% (of a total of 88 peaks detected).

Name	RT	Reverse	Area	Area	Height	Height
	[min]	Fit	[cts]	%	[cts]	%
Disiloxane, hexamethyl-	6.83	91	1426	0.5	1588	1.0
Dimethyl sulfone	7.14	92	5078	1.7	3467	2.1
Trifluoromethyl-bis-(trimethylsilyl)methyl ketone	7.45	94	14274	4.8	10694	6.6
Octane, 4-ethyl-	7.85	94	1932	0.6	1158	0.7
1,2-Bis(trimethylsiloxy)ethane	8.34	93	20903	7.0	12757	7.8
Cyclopropane, 1-heptyl-2-methyl-	8.58	87	3614	1.2	2127	1.3
Silane, (cyclohexyloxy)trimethyl-	8.83	89	4655	1.6	3102	1.9
Tetrasiloxane, decamethyl-	9.34	86	3844	1.3	2285	1.4
Silane, (1-cyclohexen-1-yloxy)trimethyl-	9.77	90	18016	6.0	10881	6.7
Propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	9.82	95	8888	3.0	4868	3.0
Glycolic acid	10.17	88	3744	1.3	1516	0.9
Silane, trimethyl(phenylmethoxy)-	11.80	94	16044	5.3	9758	6.0
3,6,9-Trioxa-2-silaundecane, 2,2-dimethyl-	11.93	89	1501	0.5	974	0.6
Benzoic acid trimethylsilyl ester	13.65	94	7195	2.4	4126	2.5
Octanoic acid, trimethylsilyl ester	13.95	87	5175	1.7	3037	1.9
Octane, 2,4,6-trimethyl-	14.70	85	1338	0.5	790	0.5
Butanedioic acid, bis(trimethylsilyl) ester	14.86	89	1936	0.6	1089	0.7
Nonanoic acid, trimethylsilyl ester	15.75	91	19185	6.4	10747	6.6
Benzene, (isothiocyanatomethyl)-	15.97	86	2663	0.9	1295	0.8
Decanoic acid	17.46	90	5016	1.7	2737	1.7
Lauric acid TMS	20.63	91	1867	0.6	1006	0.6
Tetradecanoic acid, trimethylsilyl ester	23.51	86	4824	1.6	2576	1.6
Phthalic acid, butyl cyclobutyl ester	25.05	89	655	0.2	348	0.2
Hexadecanoic acid, trimethylsilyl ester	26.14	89	14483	4.8	7421	4.6
Octadecanoic acid, trimethylsilyl ester	28.55	86	6429	2.1	3375	2.1
Ethyl-hexyl-phthalate	31.81	89	1724	0.6	869	0.5
4-Methyl-2,4-bis(4'-trimethylsilyloxyphenyl) pentene-1	33.43	91	652	0.2	380	0.2

Mass Frontier spectrum interpretation software

While some of the acquired spectra are not included in commercial libraries, some matches show structural similarities. The Mass Frontier software analyzes the unknown mass spectrum and associates fragmentation pathways and ion structures to the unknown spectral pattern calculated from the included knowledge base of known fragmentation rules.[10] Two examples of spectrum interpretation of unknown compounds are given in Figure 8 for the DCM extract and in Figure 9 for the IPA extract. The expert Mass Frontier software system generated plausible proposals to explain the mass spectrum pattern. Conclusions Parallel detection using full-scan MS and FID show very good compliance in the detected compound pattern.

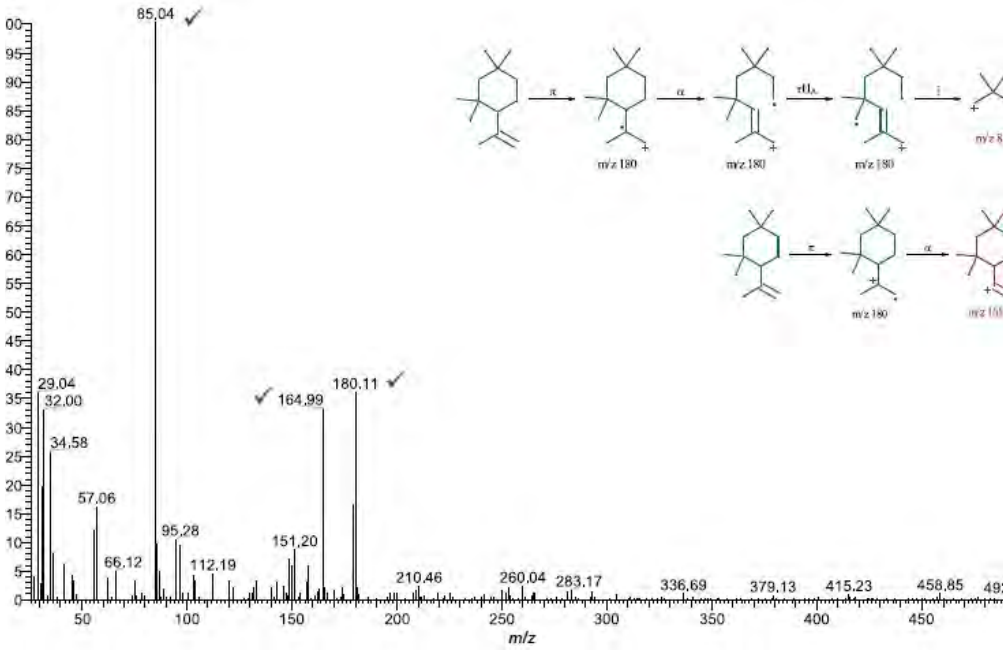

Figure 8. DCM extract, unknown peak at RT 12.68 min.

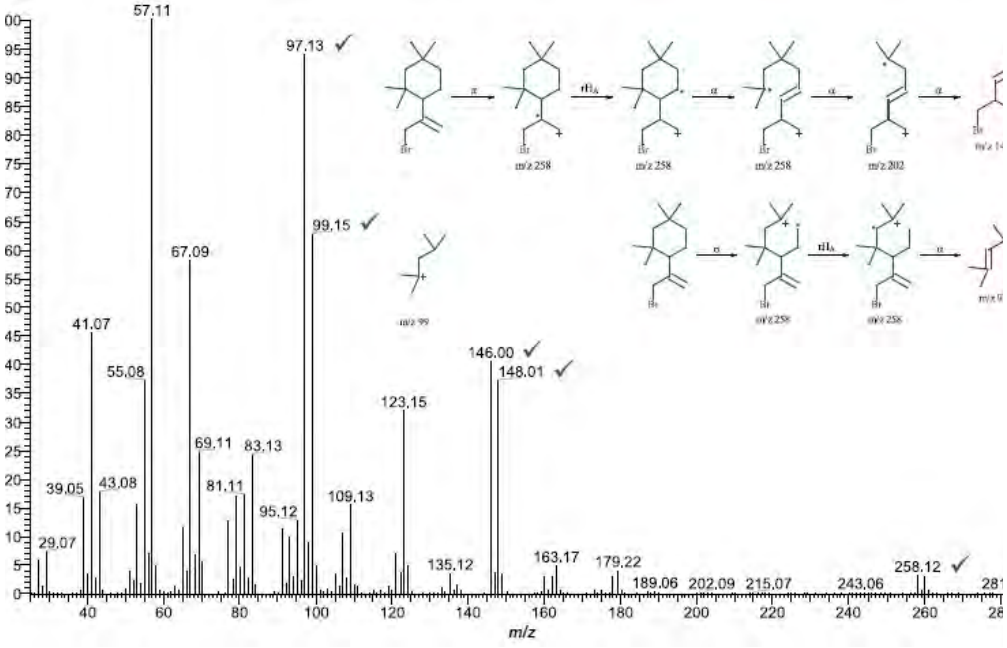

Figure 9. IPA extract, unknown peak at RT 18.31 min.

Conclusions

Parallel detection using full-scan MS and FID shows very good compliance in the detected compound pattern. After identification of typical major components using the mass spectrometer, routine analysis for such compounds can be run reliably using FID.

Deconvolution using AMDIS software provides a precise isolation of the mass spectra even from co-eluting compounds. The possibility of using an individual library of target compounds and combining retention time with the mass spectrum makes it a powerful tool for analytical control. Moreover, the Thermo Scientific™ TraceFinder™ mass spectrometry software allows transfer of mass spectral libraries already available in the laboratory.

For unknown mass spectra, Mass Frontier software is a unique tool for interpretation. Structure proposals and fragmentation pathways are provided for mass spectra allowing a deeper sample and unknown elucidation.

The complete analytical system using the ISQ as a single quadrupole MS with the parallel FID on the TRACE 1310 GC system, associated with acquisition and processing software, is a powerful and easy-to-use solution for the identification of unknowns, routine screening, and if required, also compound quantitation for product safety control and similar quality control applications.

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APPLICATION NOTE 44479

Integration of rare earth elements (REE) into a novel ICP-MS method for environmental analysis

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Thermo Fisher Scientific

Keywords: Environmental analysis, rare earth element (REE), emerging contaminants, sensitivity and robustness, selectivity, surface waters, TQ-ICP-MS

Goal

To demonstrate the suitability of the Thermo Scientific™ iCAP™ TQe ICP-MS using a single measurement mode for rare earth elements in a variety of environmental and geological samples.

Introduction

Rare earth elements (REEs) are a group of 14 elements, (all metals) that tend to be found together in geological deposits. REEs represent useful chemical tracers and are often used as geochemical fingerprints in hydro geochemical processes to study ocean circulation, rock-water interactions, water physical mixing, etc.[1]

In addition to this, REEs are valuable for modern industries and widely used in advanced technologies, such as medical diagnostics (magnetic resonance imaging, MRI), permanent magnets, rechargeable batteries, electric cars, and electronic products.[2,3] However, despite their utility, REEs pose significant risks to the environment if handled inappropriately as electronic or medical waste, etc. For example, increased concentrations of gadolinium (Gd) were reported recently in tap[4] and river water collected close to medical facilities where it is used as a contrast agent in MRI or computerized tomography (CT).[5,6] Other elements could accidentally leach out into the environment from consumer electronics or residues from industrial production of batteries disposed of incorrectly. Consequently, it is important to monitor REE levels in ground and surface waters, and therefore, most of the elements are mentioned in regulated methods for the analysis of drinking and surface waters, such as ISO method 17294, governing water analysis in the European Union.

Inductively coupled plasma mass spectrometry (ICP-MS) is the most widely employed technique for the analysis of trace elements in environmental samples. Although the most common analytes, such as chromium, arsenic, selenium, cadmium, mercury, or lead, are well established in methods used by analytical testing laboratories, quantifying REEs in such samples still comes with challenges. These include the ultra-low concentrations of these elements in water samples (typically ng·L^{-1}), variable chemical composition of samples, and spectral interferences. Besides their potential to cause interferences on key analytes by formation of doubly charged interferences (e.g., $^{150}Nd^{++}$ on $^{75}As^{+}$),[7] lighter members of this group of elements can contribute to and therefore create false positives on the resulting signal for the heavier homologs (e.g., formation of $^{156}Gd^{16}O^{+}$ on $^{172}Yb^{+}$).

This application note describes how interference free, low level analysis of rare earth elements can be integrated into a fast, sensitive, and robust ICP-MS method for the analysis of different water samples (e.g., drinking and surface waters). This analytical method was tested using water samples collected locally as well as applicable certified reference materials (CRMs).

Experimental

Experimental optimization of instrument parameters An iCAP TQe ICP-MS was used for all measurements. The sample introduction system consisted of a Peltier cooled, baffled cyclonic spraychamber, PFA nebulizer, and quartz torch with a 2.5 mm i.d. removable quartz injector. To avoid unwanted matrix effects, the High Matrix skimmer cone insert was selected for this application. Table 1 gives an overview of the full configuration of the system. For automation of the sample introduction process, a Teledyne CETAC™ ASX-560 autosampler (Omaha, NE, USA) was used.

To remove potential interferences, the ICP-MS was operated in single mode (TQ-O2) using the parameters presented in Table 1. Although kinetic energy discrimination (KED) using helium as an inert collision gas is often used to remove abundantly occurring polyatomic interferences, the use of a triple quadrupole mass analyzer in conjunction with oxygen as a reactive gas provides significant improvements:

- Polyatomic interferences are removed with equivalent or even higher efficiency, especially in the higher mass range (e.g., WO^+ interferences on mercury).
- Other types of interferences, such as doubly charged ions, are removed effectively in comparison to He-KED mode.
- In comparison to a method using different settings for some analytes, time savings can be realized at no expense of achievable detection limits.

In short, the TQ-O_2 mode removes spectral interferences in the following way: the collision reaction cell (CRC) is pressurized with oxygen as a reaction gas. For all analytes, Q1 is set to analyte mass (M^+), whereas Q3 is set to either the analyte mass as well (for elements unreactive or with low reactivity towards oxygen), or to MO^+ or even MO_2^+ (for analytes reactive to oxygen). Based on the mass filtration in the first quadrupole, potential side reactions with other ionic species are suppressed, and other elements, potentially occupying the intended product ion mass of MO^+, are removed. This mode allows for complete interference removal and improved sensitivity.
Rare earth elements are well known to form doubly charged ions (M^{++}) due to their moderate 2nd ionization potential, leading to interferences in the mass range between *m/z* 70 and 88, but they can also create interferences among themselves through the formation of oxides (MO^+). This is highlighted in Figure 1, showing how potential interferences on erbium (Er) caused by the presence of neodymium (Nd) and samarium (Sm) can be avoided.

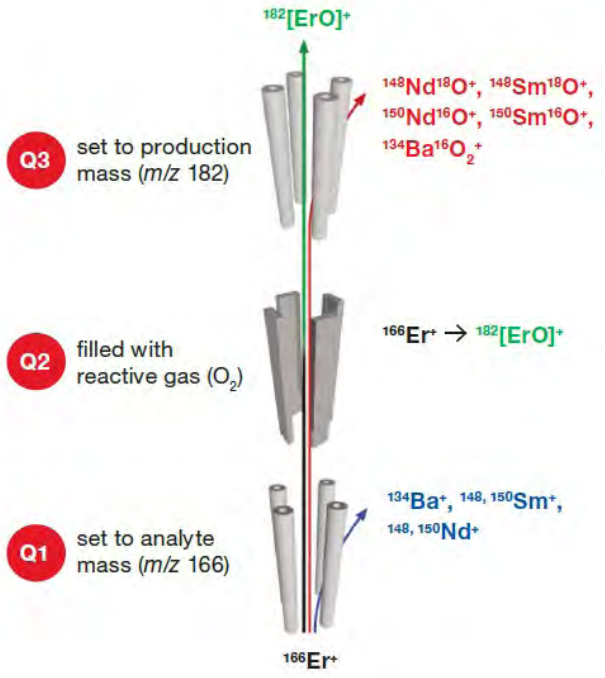

Figure 1. Schematic showing the use of TQ-O2 mode and a mass shift reaction for interference free detection of erbium (Er).

Table 1. Instrument configuration and operating parameters

Parameter	Value
Nebulizer	Borosilicate glass micromist, 400 µL·min^{-1}, pumped at 40 rpm
Pump tubing	Orange – green, 0.38 mm i.d.
Spraychamber	Quartz cyclonic, cooled at 2.7 ˚C
Injector	2.5 mm i.d., quartz

Table 1 (cont'd). Instrument configuration and operating parameters

Parameter	Value
Interface	Nickel sampler and nickel skimmer cone with High Matrix insert
Plasma power	1,550 W
Nebulizer gas	1.04 $L \cdot min^{-1}$
QCell setting	TQ-O_2
Gas flow	100% O_2, 0.34 $mL \cdot min^{-1}$
CR bias	-6.3 V
Q3 bias	-12 V
Scan setting	0.1 s dwell time, 5 sweeps, 3 main runs
Lens setting	Optimized using autotune
Sample uptake	55 s
Wash time	55 s
Total analysis time	2 min 50 s

Data acquisition and data processing

All parameters in the measurement mode were defined automatically using the autotune procedures provided in the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software. The autosampler was controlled using the Qtegra ISDS Software as well using a dedicated software plug-in.

Quality control is critical in analysis, especially when running long batches containing different sample matrices. To ensure quality control, the internal standards were monitored, and continuing calibration checks (CCVs) were performed periodically throughout the analytical run. A full suite of quality control tests is included in the Qtegra ISDS Software and can be configured (with respect to applicable % limits, repetition rate, and actions on warning/failure) as required.

Sample preparation

Precleaned polypropylene bottles were used for the preparation of all blanks, calibration standards, and samples. The bottles were rinsed with ultrapure water (18.2 MΩ·cm) and left to dry in a laminar flow clean hood before use. Two CRMs were used: SLRS-5 (River water, National Institute of Standards and Technology) and BCR-2 (Basalt, Columbia River, United States Geological Survey). In addition, a total of eight individual water samples were collected from various locations in and around Bremen, Germany (see Table 2 for details) and analyzed for 35 elements. All water samples were acidified with 2% v/v HNO_3 (OPTIMA™ grade, Fisher Scientific) after collection. In addition, the samples were filtered through a 0.45 µm membrane to remove particles.

The BCR-2 CRM required autoclave digestion using a combination of HNO_3, $HClO_4$ and HF prior to analysis. The total dilution factor incurred throughout the digestion process was 2,500

All blanks, calibration standards, and quality control standards (QC) were prepared using 2% v/v HNO_3 and single element standards (SPEX CertiPrep, Metuchen, NJ, USA) to result in the concentration ranges listed in Table 3. In addition to major elements (typical concentration ranges in the $mg \cdot L^{-1}$ range) and common contaminants (expected concentrations in the $\mu g \cdot L^{-1}$ range). This allowed to establish instrumental detection limits for these analytes.

An internal standard solution, containing Ga, In, and Bi, all at 5 $\mu g \cdot L^{-1}$ in 2% v/v HNO_3, was added on-line to all samples via a T-piece (mixing rate between internal standard and samples 1:1) before entering the nebulizer. The internal standards were selected to cover the entire mass range of the analytes selected to get the best possible correction for potentially occurring matrix effects or instrumental drift. The allocation of the different internal standards to the individual elements is highlighted in Table 4.

Further details of the measurement modes, acquisition parameters, and internal standards used for each element are summarized in Table 4. To analyze all elements using a single mode, the default settings of the Reaction Finder Method Development Assistant were modified accordingly.

Table 2. Overview of the samples analyzed, including location

	Item	Place	Category	Note
1	SLRS-5	Ottawa	River	CRM
2	Drinking water	Bremen	Tap water	–
3	Achterdieksee	Bremen (north)	Lake	Sampling location is close to a major highway
4	Creek (no name)	Weyhe	Stream	Sampling area is rural
5	Weser River	Bremen (middle)	River	Main river, sampling location close to a harbor

Table 2 (cont'd). Overview of the samples analyzed, including location

Item		Place	Category	Note
6	Creek (no name)	Bremen (south)	Stream	Industrial area
7	Sodenmattsee	Bremen (west)	Lake	Sampling location is close to an area with heavy traffic
8	Sebaldsbrück	Bremen (east)	Lake	Sampling location is close to a major highway
9	Tweelbäkersee	Oldenburg	Lake	Sampling location is close to a major highway
10	BCR-2	Portland, OR	Basalt sediment	CRM

Table 3. R^2 and IDL data for 35 elements in 2% HNO_3

Analyte and mass	Concentration range in calibration solutions [µg·L^{-1}]	Coefficient of determination (R^2)	Instrumental detection limit (IDL) [µg·L^{-1}]
^{9}Be	1–20	0.997	0.006
^{23}Na	5,000–100,000	0.999	13.3
^{24}Mg	5,000–100,000	0.999	3
^{27}Al	1–20	0.999	0.3
^{39}K	5,000–100,000	0.999	2.1
^{44}Ca as $^{44}Ca.^{16}O$ at *m/z* 60	5,000–100,000	>0.999	12.9
^{51}V as $^{51}V.^{16}O$ at *m/z* 67	1–20	0.999	0.002
^{52}Cr as $^{52}Cr.^{16}O$ at *m/z* 68	1–20	0.999	0.012
^{55}Mn	1–20	0.999	0.005
^{57}Fe	5,000–100,000	0.999	0.57
^{60}Ni	1–20	0.999	0.024
^{63}Cu	1–100	>0.999	0.3
^{66}Zn	1–20	0.999	0.048
^{75}As as $^{75}As.^{16}O$ at *m/z* 91	1–20	0.999	0.0038
^{80}Se as $^{80}Se.^{16}O$ at *m/z* 96	1–20	>0.999	0.0041
^{89}Y as $^{89}Y.^{16}O$ at *m/z* 105	0.01–1	>0.999	0.0009
^{98}Mo as $^{98}Mo.^{16}O$ at *m/z* 114	1–20	>0.999	0.0082
^{107}Ag	1–20	0.999	0.002
^{111}Cd	1–20	0.999	0.0016
^{121}Sb	1–20	>0.999	0.0016
^{139}La as $^{139}La.^{16}O$ at *m/z* 155	0.01–1	0.999	0.0002
^{140}Ce as $^{140}Ce.^{16}O$ at *m/z* 156	0.01–1	>0.999	0.0004
^{141}Pr as $^{141}Pr.^{16}O$ at *m/z* 157	0.01–1	>0.999	0.0002
^{146}Nd as $^{146}Nd.^{16}O$ at *m/z* 162	0.01–1	0.999	0.0006
^{149}Sm as $^{149}Sm.^{16}O$ at *m/z* 165	0.01–1	>0.999	0.0005
^{153}Eu	0.01–1	>0.999	0.0001
^{157}Gd as $^{157}Gd.^{16}O$ at *m/z* 173	0.01–1	>0.999	0.0005
^{159}Tb as $^{159}Tb.^{16}O$ at *m/z* 175	0.01–1	0.999	0.0002
^{163}Dy as $^{163}Dy.^{16}O$ at *m/z* 179	0.01–1	>0.999	0.0002
^{165}Ho as $^{165}Ho.^{16}O$ at *m/z* 181	0.01–1	>0.999	0.0001
^{166}Er as $^{166}Er.^{16}O$ at *m/z* 182	0.01–1	0.999	0.0001
^{169}Tm as $^{169}Tm.^{16}O$ at *m/z* 185	0.01–1	>0.999	0.0001
^{172}Yb	0.01–1	>0.999	0.0003

Table 3 (cont'd). R^2 **and IDL data for 35 elements in 2% HNO_3**

Analyte and mass	Concentration range in calibration solutions [µg·L^{-1}]	Coefficient of determination (R^2)	Instrumental detection limit (IDL) [µg·L^{-1}]
^{175}Lu as $^{175}Lu.^{16}O$ at *m/z* 191	0.01–1	>0.999	0.0001
^{238}U as $^{238}U.^{16}O_2$ at *m/z* 270	0.01–1	0.999	0.0003

Result and discussion

Sensitivity, linearity, and limit of detection

Although for some elements, other modes such as kinetic energy discrimination might be able to provide at least equivalent interference removal and, in some cases, also slightly improved detection limits, the use of a single mode for all elements was preferred to reduce the analysis time per sample by omitting a gas switching cycle in the CRC. Especially when using valve-based systems for discrete sampling, a time saving of 10 seconds (corresponding to a typical flush/fill cycle in a CRC) makes up for a significant amount of the turnover time per sample.

Achieving high sensitivity is important especially when analyzing REEs in aqueous samples, where these elements are often present in ultra-trace amounts. The absolute sensitivity is significantly enhanced when using TQ-O_2 mode although both TQ-O_2 and He-KED mode have the capability of achieving detection limits in the sub ng·L^{-1} range. However, in comparison, the TQ-O_2 mode performed significantly better (Figure 2).

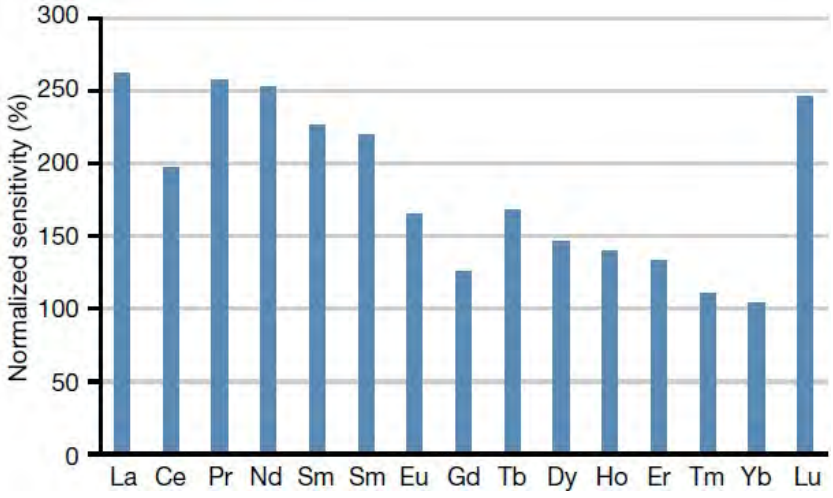

Figure 2. Comparison of the sensitivity in TQ-O_2 mode and He-KED mode for all REEs. The sensitivity in TQ-O_2 mode is normalized relative to the sensitivity observed in He-KED mode.

Table 3 summarizes the obtained instrumental detection limits together with the coefficient of determination (R^2) for all elements analyzed in this study. The IDLs were calculated using three times the standard deviation of ten replicate measurements of the calibration blank. Although there are no regulatory limits specified yet for REEs in environmental samples, the IDLs obtained were significantly below the measured concentrations in the unknown samples collected for this study.

Table 4. Internal standards used for each element with corresponding target isotopes, Q1, and Q3

Analyte and mass	Q1 resolution	Q3 resolution	Internal standard
^{9}Be	High	Normal	^{71}Ga
^{23}Na	High	High	^{71}Ga
^{24}Mg	High	High	^{71}Ga
^{27}Al	High	Normal	^{71}Ga
^{39}K	High	High	^{71}Ga
^{44}Ca as $^{44}Ca.^{16}O$ at *m/z* 60	High	High	^{71}Ga
^{51}V as $^{51}V.^{16}O$ at *m/z* 67	iMS	Normal	^{71}Ga
^{52}Cr as $^{52}Cr.^{16}O$ at *m/z* 68	iMS	Normal	^{71}Ga

Table 4 (cont'd). Internal standards used for each element with corresponding target isotopes, Q1, and Q3

Analyte and mass	Q1 resolution	Q3 resolution	Internal standard
^{55}Mn	iMS	Normal	^{71}Ga
^{57}Fe	High	High	^{71}Ga
^{60}Ni	iMS	Normal	^{71}Ga
^{63}Cu	iMS	Normal	^{71}Ga
^{66}Zn	iMS	Normal	^{71}Ga
^{75}As as ^{75}As.^{16}O at *m/z* 91	iMS	Normal	^{115}In
^{80}Se as ^{80}Se.^{16}O at *m/z* 96	iMS	Normal	^{115}In
^{89}Y as ^{89}Y.^{16}O at *m/z* 105	iMS	Normal	^{115}In
^{98}Mo as ^{98}Mo.^{16}O at *m/z* 114	iMS	Normal	^{115}In
^{107}Ag	iMS	Normal	^{115}In
^{111}Cd	iMS	Normal	^{115}In
^{121}Sb	iMS	Normal	^{115}In
^{139}La as ^{139}La.^{16}O at *m/z* 155	iMS	Normal	^{115}In
^{140}Ce as ^{140}Ce.^{16}O at *m/z* 156	iMS	Normal	^{115}In
^{141}Pr as ^{141}Pr.^{16}O at *m/z* 157	iMS	Normal	^{115}In
^{146}Nd as ^{146}Nd.^{16}O at *m/z* 162	iMS	Normal	^{115}In
^{149}Sm as ^{149}Sm.^{16}O at *m/z* 165	iMS	Normal	^{115}In
^{153}Eu	iMS	Normal	^{115}In
^{157}Gd as ^{157}Gd.^{16}O at *m/z* 173	iMS	Normal	^{115}In
^{159}Tb as ^{159}Tb.^{16}O at *m/z* 175	iMS	Normal	^{115}In
^{163}Dy as ^{163}Dy.^{16}O at *m/z* 179	iMS	Normal	^{115}In
^{165}Ho as ^{165}Ho.^{16}O at *m/z* 181	iMS	Normal	^{209}Bi
^{166}Er as ^{166}Er.^{16}O at *m/z* 182	iMS	Normal	^{209}Bi
^{169}Tm as ^{169}Tm.^{16}O at *m/z* 185	iMS	Normal	^{209}Bi
^{172}Yb	iMS	Normal	^{209}Bi
^{175}Lu as ^{175}Lu.^{16}O at *m/z* 191	iMS	Normal	^{209}Bi
^{238}U as ^{238}U.^{16}O$_2$ at *m/z* 270	iMS	Normal	^{209}Bi

Interference removal

As mentioned previously, the different REEs may not only create interferences on key analytes under regulation, such as arsenic or selenium, but also interferences on other REEs can be expected and need to be resolved to avoid false positive results. False positive results can arise for the analysis of erbium in the presence of different concentrations of samarium, which can interfere if, for example, $^{150}Sm^{16}O^{+}$ is not resolved from the common isotope for erbium analysis, ^{166}Er. TQ-O_2 mode showed excellent interference removal with no false positive being returned. A potential bias of up to 2.5 µg·L^{-1} was observed for ^{166}Er in He-KED mode for concentrations of samarium between 10 µg·L^{-1} and 1,000 µg·L^{-1}.

To highlight the ability of the iCAP TQe ICP-MS to remove all potential interferences caused in the presence of different rare earth elements, a river sediment CRM (BCR-2, United States Geological Survey) was analyzed. Although not a water sample, it is one of the few materials available certified for its content of REEs and contains between 0.5 µg·g^{-1} (Tm, Lu) and >25 µg·g^{-1} (e.g. La, Nd). Additionally, method detection limits (MDLs) for the REEs of choice were determined and results are summarized in Table 5. MDLs were calculated from the IDLs values determined experimentally (Table 4) but considering the dilution factor of 2,500 because of the digestion procedure. As can be seen from Table 5, good agreement between the experimental results and the certified/informative concentrations was obtained.

Table 5. Quantitative results obtained for the CRM BCR-2 sample analyzed in TQ-O_2 mode. All REEs concentrations are reported as µg·g^{-1}.

Analyte and mass	MDL	Measured (n=4)	CRM consensus values
^{139}La as $^{139}La.^{16}O$ at *m/z* 155	0.001	26 ± 0.5	25 ± 1
^{140}Ce as $^{140}Ce.^{16}O$ at *m/z* 156	0.001	55 ± 1	53 ± 2
^{141}Pr as $^{141}Pr.^{16}O$ at *m/z* 157	0.001	7.0 ± 0.2	6.8 ± 0.3
^{146}Nd as $^{146}Nd.^{16}O$ at *m/z* 162	0.002	30 ± 1	28 ± 2
^{149}Sm as $^{149}Sm.^{16}O$ at *m/z* 165	0.001	6.9 ± 0.5	6.7 ± 0.3
^{153}Eu	0.0003	2.1 ± 0.1	2.0 ± 0.1
^{157}Gd as $^{157}Gd.^{16}O$ at *m/z* 173	0.001	7.1 ± 0.1	6.8 ± 0.3
^{159}Tb as $^{159}Tb.^{16}O$ at *m/z* 175	0.001	1.10 ± 0.03	1.07 ± 0.04
^{163}Dy as $^{163}Dy.^{16}O$ at *m/z* 179	0.001	7.1 ± 0.2	–
^{165}Ho as $^{165}Ho.^{16}O$ at *m/z* 181	0.0003	1.45 ± 0.05	1.33 ± 0.06
^{166}Er as $^{166}Er.^{16}O$ at *m/z* 182	0.0003	4.2 ± 0.3	–
^{169}Tm as $^{169}Tm.^{16}O$ at *m/z* 185	0.0003	0.6 ± 0.1	0.54
^{172}Yb	0.001	3.7 ± 0.2	3.5 ± 0.2
^{175}Lu as $^{175}Lu.^{16}O$ at *m/z* 191	0.0003	0.55 ± 0.02	0.51 ± 0.02

Analysis of REEs in environmental samples

As part of this study, a river water reference material (SLRS-5) and eight different water samples were analyzed as technical replicates to assess the method performance. The results for the river water CRM were also found to be in excellent agreement with the reference values (Table 6). As the water samples were aspirated directly without any dilution, the MDL is effectively the same as the IDL. As can be seen, the different samples analyzed were significantly variable in their overall matrix content or composition, with total concentrations of the most common alkaline and alkaline earth elements (Na, K, Mg, and Ca) between less than 20 mg·L^{-1} (SLRS-5 CRM) to over 200 mg·L^{-1} (Weser River). This again may cause a difference in the response of the plasma, so that internal standardization is key to avoid bias caused by potentially occurring matrix effects. No correlation of the combined concentration of the REEs with the concentration of other elements (such as alkaline/ alkaline earth elements) could be found.

Table 6. Quantification results for different water samples. All concentrations are reported as µg·L^{-1}. Values annotated with * are known reference values (expected values).

Analyte and mass	MLD for water samples	SLRS-5 Measured (n=8)	SLRS-5 CRM value	Concentration range in 8 water samples
^{9}Be	0.006	0.004 ± 0.005	0.005*	0.003–0.07
^{23}Na	13.3	5,374 ± 98	5,380 ± 100	10,972–110,328
^{24}Mg	3	2,443 ± 110	2,540 ± 160	3,332–35,128
^{27}Al	0.3	50.1 ± 6.0	49.5 ± 5.0	0.002–0.2
^{39}K	2.1	822 ± 60	839 ± 36	2,216–19,681
^{44}Ca as $^{44}Ca.^{16}O$ at *m/z* 60	12.9	10,060 ± 380	10,500 ± 400	18,100–48,082
^{51}V as $^{51}V.^{16}O$ at *m/z* 67	0.002	0.291 ± 0.020	0.317 ± 0.033	0.36–0.92
^{52}Cr as $^{52}Cr.^{16}O$ at *m/z* 68	0.012	0.199 ± 0.021	0.208 ± 0.023	0.09–0.46
^{55}Mn	0.005	4.21 ± 0.28	4.33 ± 0.18	0.16–519.8
^{57}Fe	0.57	93.5 ± 2.8	91.2 ± 5.8	50.1–1,051
^{60}Ni	0.024	0.495 ± 0.038	0.476 ± 0.064	0.82–1.93
^{63}Cu	0.3	18.7 ± 1.8	17.4 ± 1.3	0.77–127.02
^{66}Zn	0.048	0.89 ± 0.018	0.845 ± 0.095	3.8–163.3
^{75}As as $^{75}As.^{16}O$ at *m/z* 91	0.0038	0.389 ± 0.03	0.413 ± 0.039	0.06–1.08
^{80}Se as $^{80}Se.^{16}O$ at *m/z* 96	0.0041	0.09 ± 0.02	-	0.04–0.12
^{89}Y as $^{89}Y.^{16}O$ at *m/z* 105	0.0009	0.11 ± 0.006	-	0.01–0.77
^{98}Mo as $^{98}Mo.^{16}O$ at *m/z* 114	0.0082	0.5 ± 0.1	0.5*	0.1–1.2
^{107}Ag	0.002	0.005 ± 0.001	-	0.004–0.019
^{111}Cd	0.0016	0.0069 ± 0.0012	0.0060 ± 0.0014	0.001–0.031
^{121}Sb	0.0016	0.29 ± 0.02	0.3*	0.029–0.31
^{139}La as $^{139}La.^{16}O$ at *m/z* 155	0.0002	0.21 ± 0.01	-	0.003–0.575
^{140}Ce as $^{140}Ce.^{16}O$ at *m/z* 156	0.0004	0.26 ± 0.01	-	0.002–1.288
^{141}Pr as $^{141}Pr.^{16}O$ at *m/z* 157	0.0002	0.05 ± 0.003	-	0.001–0.176
^{146}Nd as $^{146}Nd.^{16}O$ at *m/z* 162	0.0006	0.18 ± 0.01	-	0.003–0.768
^{149}Sm as $^{149}Sm.^{16}O$ at *m/z* 165	0.0005	0.039 ± 0.004	-	0.007–0.171
^{153}Eu	0.0001	0.008 ± 0.001	-	0.002–0.042
^{157}Gd as $^{157}Gd.^{16}O$ at *m/z* 173	0.0005	0.033 ± 0.004	-	0.008–0.162
^{159}Tb as $^{159}Tb.^{16}O$ at *m/z* 175	0.0002	0.003 ± 0.0002	-	0.001–0.02
^{163}Dy as $^{163}Dy.^{16}O$ at *m/z* 179	0.0002	0.018 ± 0.001	-	0.001–0.112
^{165}Ho as $^{165}Ho.^{16}O$ at *m/z* 181	0.0001	0.0038 ± 0.0002	-	0.0004–0.025
^{166}Er as $^{166}Er.^{16}O$ at *m/z* 182	0.0001	0.011 ± 0.001	-	0.001–0.074
^{169}Tm as $^{169}Tm.^{16}O$ at *m/z* 185	0.0001	0.0016 ± 0.0001	-	0.0002–0.011
^{172}Yb	0.0003	0.010 ± 0.001	-	0.001–0.074
^{175}Lu as $^{175}Lu.^{16}O$ at *m/z* 191	0.0001	0.0017 ± 0.0009	-	0.0004–0.012
^{238}U as $^{238}U.^{16}O_2$ at *m/z* 270	0.0003	0.100 ± 0.003	0.1*	0.014–0.596

To fully confirm the absence of any drift or matrix effect as an influencing factor to the results, a spike recovery test for all REEs was performed in all water samples analyzed, including the river water CRM. To reflect the typically observed concentrations in natural waters, a concentration of 0.05 µg·L^{-1} was added to each sample. The overall spike recovery observed across all samples was excellent with an average recovery between 90% and 112%.

Robustness

For reliable analysis in an essential testing laboratory, it is important that the results obtained are accurate and precise also in longer batches comprising different sample types. Commonly, quality control (QC) standards containing a known concentration of all analytes are analyzed periodically during a batch to monitor method performance.

To simulate a high-volume sample analysis, a larger sample batch was scheduled for analysis containing all water samples previously analyzed. Each sequence in the batch (consisting of 23 individual samples) was concluded with a quality control standard (continuing calibration verification, CCV, containing 0.05 µg·L^{-1} of REEs) before restarting the next sequence. In summary, eight CCVs were analyzed in a batch containing 197 samples in approximately 10 hours. The relative standard deviation of all CCVs (n=8) in the batch did not exceed 3%. The response of the internal standards are shown in Figure 3. All internal standards showed excellent recovery (within approximately 70% to 110%) over the entire runtime of the batch, demonstrating robust analytical performance

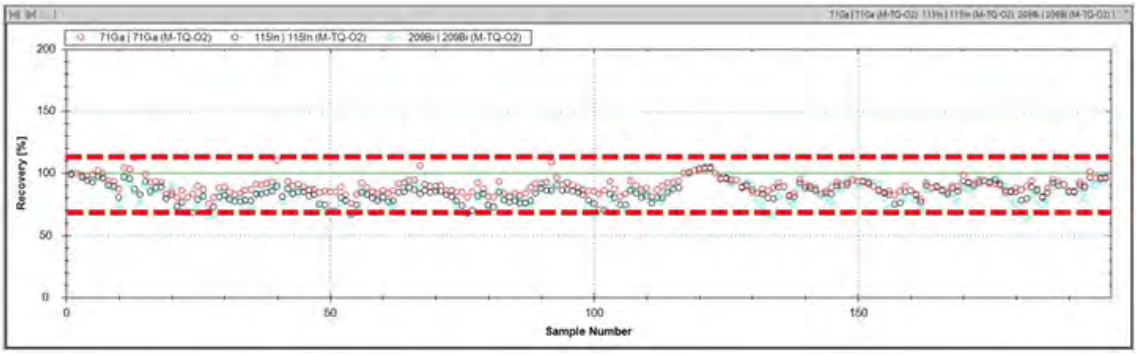

Figure 3. Response of the internal standards assessed over a period of ~10 hours of uninterrupted acquisition of 197 samples

Conclusion

The iCAP TQe ICP-MS was successfully employed to analyze 35 elements in different environmental samples (water samples and a previously digested sediment sample) following a simple sample preparation. This analytical method was rigorously tested, and the results obtained clearly demonstrated the following analytical advantages:

- The combination of a triple quadrupole mass analyzer with O_2 as the cell gas is effective for the removal of spectral interferences such as complicated isobaric and/ or polyatomic interferences during the analysis of REEs.
- TQ-O_2 mode allows for high sensitivity analysis required for the accurate determination of the entire mass range (beryllium to uranium) with outstanding IDLs and linear response.
- The TQ-O_2 single measurement reduced the total analysis time to <3 min/sample (including uptake and wash time) for 35 elements (at both major and ultra-trace level). This sample turnover time can be reduced to <90 s by using a discrete sampling valve and will positively impact high sample throughput laboratories.
- The large linear dynamic range of up to 10 orders of magnitude allows for precise determination of multi elements at low and high concentrations without further sample concentration or dilution.
- Robust and stable analytical performance was demonstrated over 10 hours of continuous acquisition of 200 samples.
- In summary, the iCAP-TQe ICP-MS system together with Qtegra ISDS Software allows for fast, sensitive, and robust determination of ultra-trace REEs in environment and geological samples, making it ideal for laboratories analyzing a high volume of samples per day.

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APPLICATION REPORT

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MILESTONE
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Microwave-assisted acid digestion of geological samples for elemental quantification on ICP-MS: Exotic rocks with rare and resistant minerals.

SUMMARY

Historically, the preferred analytical technique for mineral analysis was arc emission spectroscopy, but this is no longer suitable in the isotope ratios determination. This is one of the reasons why, in contemporary laboratories, more advanced techniques such as Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) have become standard. These techniques, particularly for trace element analysis, necessitate complete sample dissolution—a critical step that is typically achieved through strong acid digestion. It is important to make an analytical distinction when dealing with samples containing refractory minerals that are not readily dissolved in open-vessel HF-HNO_3 mixtures (e.g., $ZrSiO_4$, $FeCr_2O_4$, $BaSO_4$). For such materials, closed-vessel digestion is required to reach significantly higher temperatures and pressures and effectively digest the sample. This is where Milestone's Single Reaction Chamber (SRC) technology becomes essential. The SRC system utilizes microwave-assisted digestion in a fully stainless-steel, 1-liter pressurized reactor. With the UltraWAVE 3 platform, temperatures up to 300 °C and pressures up to 199 bar can be achieved. In collaboration with Petrology Professor Kamber Balz (*Queensland University of Technology*) and with contributions from geological laboratories, Milestone has developed a comprehensive sample preparation method applicable to a wide range of geological materials. These have been classified into five categories based on their chemical behavior and composition. This document presents a four-step protocol designed for the complete dissolution of exotic rocks samples that contain rare and refractory minerals (Pegmatites, ores, hydrothermally altered rocks, carbonatites,), significantly reducing the time required for elemental analysis sample preparation.

| EXPERIMENTAL

INSTRUMENTATION

- UW3 system
- 20-position rack
- PTFE 15 mL vials with caps
- Chiller 1 kW
- Nitrogen gas line (40 bar / or min. 10 bar using gas booster)
- Hotblock with fumehood connection
- ICP-MS

REAGENTS

- HF 48%
- HNO_3 67%
- HNO_3 50%
- Distilled water

| REAGENT HANDLING

Reagent addition, a common task during the sample preparation process, poses safety concerns for the operator, is time-consuming, and can lead to contamination if not performed correctly. The use of an automatic dosing station, such as Milestone easyFILL, mitigates and limits these risks, especially when the operator is exposed to toxic acid as HF, very common on geochemical methods. Specifically designed to precisely add reagents to digestion vessels and vials, easyFILL helps chemists optimize their procedures by reducing the risk of human error in trace analysis. Capable of dispensing various types of reagents into digestion vessels and vials, easyFILL minimizes manual handling of the digestion mixture, thereby reducing the risk of contamination. EasyFILL is fully compatible with all UW3 racks, including the 20-position rack.

Figure 1: Milestone's easyFILL

SINGLE REACTION CHAMBER (SRC) TECHNOLOGY: UW3 OVERVIEW

SRC technology represents the latest revolution in microwave closed-vessel digestion. Unlike traditional microwave ovens, SRC utilizes a pressurized one-liter stainless steel microwave reactor which is also the digestion vessel. The process is straightforward: samples and reagents are loaded into vials and placed inside the reactor cavity together with a suitable baseload that allows heat homogeneity around the samples. Once loaded, the reactor is securely closed and sealed. Automatic introduction of nitrogen gas (40 bar) follows for internal pressurization, which also serves to raise the boiling points of the solutions and not lose the volatile elements. At the end of the process, the reactor is cooled using a powerful water-cooling system.

Figure 2: UW3 microwave digestion system with focus on the SRC chamber

METHOD DESCRIPTION

The acid digestion protocol developed for the preparation of geological samples, for exotic rocks containing rare and refractory minerals. Due to the wide range of potential minerals present, no generalized method can be provided. However, for pegmatite- associated silicates and oxides, this four- step method is a good starting point. The method was successfully deployed to analyze REE-rich samples that also contain Ti-Nb-Ta minerals.

The **first stage** involves a high-temperature digestion aimed at the decomposition of silicate structures. This is achieved through the addition of concentrated hydrofluoric acid to the samples placed within a 20- position rack. Digestion is performed using the SRC technology, which maintains a stable temperature of 250 °C for an extended duration. Under these conditions, silicon is effectively complexed with fluoride ions, as hydrofluoric acid is capable of cleaving strong metal–Si–O bonds. The second step consists of an evaporation phase carried out on a hotblock, in which the samples are brought to dryness; This step does not affect the recovery of elements, not even volatile ones. This step promotes only the volatilization and removal of silicon in the form of silicon tetrafluoride (SiF_4), effectively reducing the sample matrix and facilitating the digestion of the remaining elements. The third step involves the addition of 4.5 mL of hydrochloric acid to each sample, followed by a second high-temperature digestion (280°C) on the Ultrawave 3 system. This advanced SRC-based microwave digestion unit is capable of fully dissolve compounds such as aluminum fluoride (AlF_3) and other fluoride species residues that may have formed during earlier steps. The **fourth and final step** consists of an evaporation phase

carried out on a hotblock for the drydown of all samples. Then, it involves the addition of 2 mL of nitric acid (50%) to each sample, followed by gentle heating on the hotblock. This step serves to reconvert any residual fluoride species into soluble complexes and ensures the breakdown of intermediate fluoride salts, thereby preparing the sample for the final digestion.

OPERATING CONDITIONS ON UW3

For the first digestion, a mixture of 3.2 mL of hydrofluoric acid and 1.3 mL of nitric acid is added to 0.100 g of sample in each 15mL test tube. The digestion program followed by the instrument is as follows:

First digestion step, operating conditions:

Step	Time	Power (W)	Temp T1 (°C)	Temp T2 (°C)	Pressure (bar)
1	00:35:00	1500	250	70	120
2	00:20:00	1500	250	70	140

The second digestion step, performed using SRC technology, involves the addition of 4.5 mL of hydrochloric acid, setting the following program:

Last digestion step, operating conditions:

Step	Time	Power (W)	Temp T1 (°C)	Temp T2 (°C)	Pressure (bar)
1	00:40:00	1500	280	70	160
2	00:20:00	1500	280	70	160

| RESULTS AND **DISCUSSION**

Table 1: Borate/peroxide fusion ICP-MS data from supplier datapack original, supplemented with 4-acid digest data.

Element	**Reference value (µg/g)**	Uncertainty **(µg/g)**	Silicate method **digestion (n=2) (µg/g)**	**RSD (µg/g)**	Accuracy **(fraction)**
Li	19562	852	18379	54	0.939
Sc	27883	1260	29888	5	1.072
Ti	12003118	277788	12097595	30053	1.008
Cr	393185	20848	389028	2250	0.989
Ni	61699	3952	61301	43	0.994
Cu	41735	1997	41138	301	0.986
Zn	120560	23277	92089	13	0.764
Sr	304797	11828	299360	359	0.982
Zr	472360	21450	432441	4543	0.915
Nb	697566	38980	698343	5120	1.001
Nd	781295	46653	78081	3864	1.010
Sb	3708	391	3383	9	0.912
Ba	807945	52913	807493	2704	0.999
La	1369293	75075	1351814	9484	0.987
Eu	22740	963	22184	26	0.976
Pb	67209	5351	59953	247	0.892

Table 1 lists the main elements analyzed by ICP-MS following acid digestion according to the method presented in this document. For a complete list of analyzed elements, please refer to the eBook (*Efficient rock digest preparation for geochemists: a practical handbook*). All recoveries were accurate, exceeding 75%, with an average of 97%.

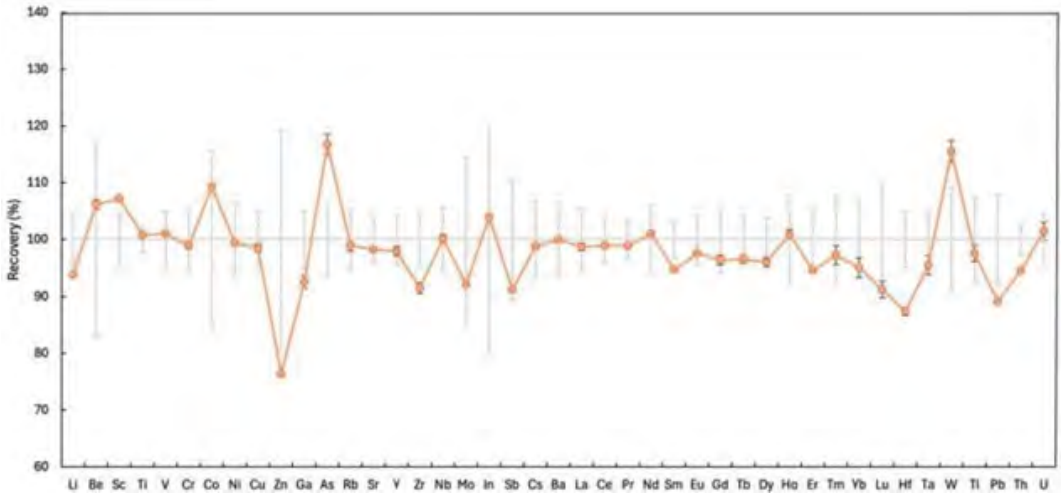

Figure 3: Graph BCR-2, elements sorted by atomic number and relative recovery.

Figure 3 graphically displays the recoveries of each element, ordered by atomic number. Notably, even for volatile elements (Cd, Pb), recovery rates fall within the optimal range, yielding reproducible results.

| CONCLUSION

SRC technology successfully achieved complete digestion of exotic rock samples, obtaining excellent recoveries of all analyzed elements and optimizing sample preparation times without losing any performance quality. Using Milestone easyFILL system, reagent handling has been improved, significantly reducing the risk of operator exposure to hazardous acids (such as HF) and automating their addition in all 20 positions.

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To stay ahead, analytical testing laboratories need the ultimate confidence of a GC-MS system that easily and reliably produces trusted results, day after day. For this, you can count on the Thermo Scientific™ ISQ™ 7610 Single Quadrupole GC-MS System. Simplified operation, automated workflows, and extended dynamic range deliver consistent results from system to system in every laboratory. Thermo Scientific™ NeverVent™ technology, extended-life detector, and intelligent software eliminate unnecessary downtime to maximize sample throughput. To ensure you are ready for any analytical challenge, the system is upgradeable from entry-level to advanced configurations. Now you can take the lead with rapid return on investment (ROI) for your regulated GC-MS analyses

Increase instrument uptime

Eliminate unnecessary, unplanned instrument downtime and maximize productivity to achieve unprecedented efficiency, day after day. The ISQ 7610 Single Quadrupole GC-MS System combines robustness with the ability to change the GC column and clean the ion source without interrupting your analytical workflows.

Monitor key aspects of your GC-MS system

With Thermo Scientific™ SmartStatus™ instrument tracking within Chromeleon CDS, quickly see whether instruments are ready to run samples, if maintenance is required or if consumables should be replaced. The fully customizable software enables you to make data-driven decisions on when to perform maintenance and avoid unnecessary downtime.

Maximize sample throughput

When high sample throughput is essential, the system delivers results on time and with ease. Automated workflows and simplified instrument operation ensure every user produces consistent results, sample after sample. Extended linear range enables method consolidation so you can analyze more compounds at varying concentrations in a single run.

Realize rapid return on investment

Ensuring your system delivers results as soon as it's installed is necessary to achieve rapid ROI. With built-in intelligence that simplifies instrument setup, analytical methods, and everyday operation, the ISQ 7610 Single Quadrupole GC-MS System is designed for accelerated deployment. Reduced needs for operator training and faster time to full productivity together with maximum sample throughput provide fast return on your instrument investment.

Thermo Fisher Scientific is working to provide more information about our products' environmental impact by stepping up to participate in the ACT Label program with selected products.

Find out more at **thermofisher.com/ISQ7610**

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VIDEO **WEBSITE**

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Triple quadrupole ICP-MS with advanced interference removal

The Thermo Scientific iCAP MTX Triple Quadrupole ICP-MS system is a high-performance instrument that can be integrated into the most challenging analysis workflows. The system gives you confidence in a streamlined sample pathway that consistently provides highly accurate results.

Excellent performance

The iCAP MTX ICP-MS leverages the advantages of triple quadrupole technology, incorporating field-proven QCell technology and IntelliLens, to provide exceptional performance across the entire mass range for advanced levels of accuracy in elemental characterization.

Simple sample introduction

With an optimized sample introduction system, including a built-in solution for diluting high-matrix-containing samples using argon gas dilution (AGD), the iCAP MTX ICP-MS system helps to ensure consistent and stable analytical performance no matter the sample type.

Minimal analytical drift

A unique implementation of AGD combined with a new, innovative cone design ensures exceptional long-term stability. The instrument's matrix minimization mode also supports long-term performance, avoiding interruptions to your productivity from unnecessary cone cleaning.

Minimize downtime

The built-in Thermo Scientific Hawk Consumables and Maintenance Assistant and Instrument Performance Monitor provides visibility to consumables and instrument performance and facilitates active monitoring to minimize user intervention and enabling maintenance to be scheduling.

Flexible interference removal

The iCAP MTX ICP-MS enables quick and dependable results. Users can easily switch between different analysis modes, such as Kinetic Energy Discrimination (KED) mode and triple quadrupole (TQ), depending on the complexity of analysis required for a particular sample.

Simple method development

The Reaction Finder Method Development Assistant is a powerful tool that automates mode selection for complex analyses. It identifies the best isotope, reaction gas, and product ion to remove interferences and achieve optimal results. With this tool, users do not need advanced knowledge of complex reaction chemistry, making the process much simpler and more streamlined.

Find out more at iCAP MTX Triple Quadrupole ICP-MS

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Ignite your confidence

iCAP MTX triple quadrupole ICP-MS

The Thermo Scientific iCAP MTX Triple Quadrupole ICP-MS system is a high-performance instrument that can be integrated into the most challenging analysis workflows. The system gives you confidence in a streamlined sample pathway that consistently provides highly accurate results.

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ultraWAVE 3

Taking Productivity and Performance to New Heights

The new ultraWAVE 3 is the latest generation of SRC technology that further elevates the value of this technology for elemental analysis in terms of performance, time, workflow, and cost of ownership.

Single Reaction Chamber Technology
Updated construction that includes several technology advances further enhances the well-proven benefits of the SRC technology.
The new features of ultraWAVE 3 merge with those already intrinsic in the technology, so that labs will experience higher performance, greater productivity, and more streamlined workflow, providing them with improved competitiveness and a lower cost of ownership.
Thanks to its superior digestion capabilities that result from its higher temperature and pressure features, ultraWAVE's unique SRC technology provides greater digestion efficiency.
Several aspects of the system, such as reduced handling and cleaning and the ability to process any samples simultaneously, reduce turnaround time and increase lab efficiency.

BENEFITS
Rugged construction
Designed with all wetted components made of PTFE-TFM, fully compatible with any acid mixture and ensuring minimal maintenance to lower the cost of ownership.

High-pressure lines
Made of acid-resistant stainless steel, the two pressure lines, one for inlet and one for outlet, ensure high safety and lower blanks.

Racks
Available with racks of 7, 20, 27 and 40 vials to provide even higher throughput than previous generations.

easyTEMP Temperature control
True contactless temperature sensor to directly control the digestion of the samples from the inside out, without reading delays.

Advanced heating technology
The noiseless water-cooled magnetron ensures higher heating efficiency along with superior working conditions.

User interface
Equipped with the most up-to-date features to bring all digestion information within easy reach of the operator.

Find out more at milestonesrl.com

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ultraWAVE 3

Single Reaction Chamber Technology

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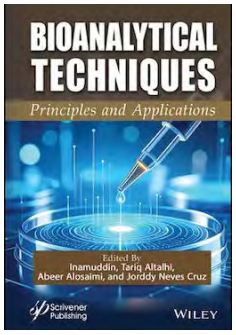
VIDEO WEBSITE

www.milestonesrl.com/products/microwave-digestion/ultrawave-3

New Books & Upcoming Events & Trusted Sources for Analytical Chemistry

Notices of Books on Analytical Chemistry



Bioanalytical Techniques: Principles and Applications

Inamuddin, Tariq Altalhi, Abeer Alosaimi, and Jorddy Neves Cruz, Editors

August 2025, Wiley

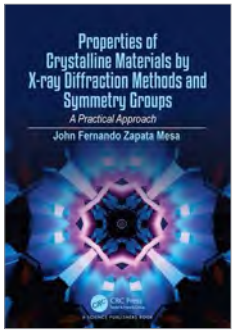
This is a comprehensive and authoritative book that explores the principles, methodologies, and applications of bioanalytical techniques in the field of life sciences. It covers a wide range of analytical techniques used for the characterization, quantification, and analysis of biological samples. It provides a solid foundation in the fundamental principles of spectroscopy, chromatography, electrophoresis, immunoassays, mass spectrometry, and biosensors. The book incorporates case studies, examples, and practical tips to illustrate how these techniques are used to solve biological problems. It also discusses emerging trends and technologies in bioanalytical techniques, such as microfluidics, nanotechnology, and omics approaches.

Analytical and Chemometric Tools

Alessandra Biancolillo, Editor

May 2026, Elsevier

This book provides a one-stop tutorial in understanding the tools required for applying chemometrics to food and forensic chemistry. With a generous compilation of practical examples, the book covers a theoretical discussion of chemometric methods, summarizes up-to-date targeted and untargeted analytical methods in the field of forensic science, and presents real-life case studies applied to methods of chemometrics for food and forensic chemistry. This book is a valuable resource for chemists, forensic scientists, food scientists, students, and all those who wish to broaden their knowledge in the allied field. Read more

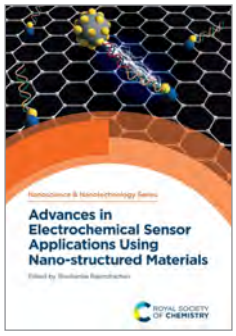


Properties of Crystalline Materials by X-ray Diffraction Methods and Symmetry Groups

John Fernando Zapata Mesa

September 2025, CRC Press

This book is fundamental for those seeking to understand the structure and properties of crystalline materials from a rigorous and systematic approach. Its coverage, which ranges from the physical principles of X-rays to structural refinement using the Rietveld method, provides a solid theoretical and practical foundation. The inclusion of symmetry group analysis and the study of elasticity reinforce its value in areas such as electronics and engineering. With an educational and precise approach, this book becomes an indispensable reference for materials characterization. Read more



Advances in Electrochemical Sensor Applications Using Nano-structured Materials

Shashanka Rajendrachari, Editor

Jun 2025, The Royal Society of Chemistry

Various nanomaterials can be used as possible electrocatalysts for the determination of huge amounts of bioactive compounds, surfactants, dyes, toxic chemicals, food additives, fertilizers, heavy metals, etc. The detection of such compounds in the human body, the environment, food or water is very important for our safety and well-being. There are many methods available to detect these compounds and determine their concentration, but electrochemical methods are proved to be: highly responsive, comparatively inexpensive, sensitive, simple. This state-of-the-art book focuses on recent electrochemical and nanomaterials research, taking the reader from basic principles to recent advances, before discussing different techniques and tools for determining the presence of a variety of compounds.

Events on Analytical Chemistry in 2026

March 7 to 11
PITTCON Conference & Expo
Henry B. González Convention Center
San Antonio, Texas, USA
https://pittcon.org/

April 06 to 07
XX International Conference on Chemometrics in Analytical Chemistry
Rome, Italy
https://waset.org/chemometrics-in-analytical-chemistry-conference

May 17 to 22
44th International Symposium on Capillary Chromatography and 21st GCXGC Symposium
Congress Centre, Riva del Garda, Italy
https://iscc44.chromaleont.it/

June 3 to 6
XXIV Brazilian Congress of Toxicology (CBTOX) 2026
WTC Events Center, São Paulo, SP, Brazil
www.cbtox.com.br

June 8 to 9
MassSpecMeet 2026
Lisbon, Portugal
https://scisynopsisconferences.com/mass-spectrometry/

June 15 to 18
49th Annual Meeting of the Brazilian Chemical Society (RASBQ)
Expo Dom Pedro
Campinas, SP, Brazil
https://www.sbq.org.br/49ra/

July 23 to 24
ANALYTICA ACTA 2026
Paris, France
https://analytical-bioanalytical.pharmaceuticalconferences.com/

August 22 to 28
26th International Mass Spectrometry Conference (IMSC)
Lyon, France
https://imsc26.com/

September 15 to 18
22nd National Meeting on Analytical Chemistry (ENQA) & 10th Ibero-American Congress on Analytical Chemistry (CIAQA)
Ruth Cardoso Cultural and Exhibition Center, Maceió, AL, Brazil
#enqa2026

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Pittcon is a catalyst for the exchange of information, a showcase for the latest advances in laboratory science, and a venue for international connectivity.

Pittcon is a dynamic, transnational conference and exposition on laboratory science, a venue for presenting the latest advances in analytical research and scientific instrumentation, and a platform for continuing education and science-enhancing opportunity. Pittcon is for anyone who develops, buys, or sells laboratory equipment, performs physical or chemical analyses, develops analysis methods, or manages these scientists.

Pittcon Awards

Honoring scientists who have made outstanding contributions to Analytical Chemistry

Each year, Pittcon provides a venue where scientists who have made outstanding contributions to laboratory science, analytical chemistry, and applied spectroscopy are honored.

Among these awards is the **Pittcon Heritage Award** which honors those visionaries whose entrepreneurial careers shaped the instrumentation and laboratory supplies community and by doing so have transformed the scientific community at large.

The award has been presented jointly with Pittcon since 2002 and is given out each year at a special ceremony during the Pittcon Conference and Expo. The recipient's name and achievements are added to the Pittcon Hall of Fame, which conference attendees can visit at the show each year.

Pittcon 2026 – Conference on Analytical Chemistry and Applied Spectroscopy
March 7-11, 2026
Henry B. González Convention Center
San Antonio, Texas, USA

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Venue
Henry B. González Convention Center

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What is Pittcon?

Pittcon is a dynamic, international conference and exposition on laboratory science, a venue for presenting the latest advances in analytical research and scientific instrumentation, and a platform for continuing education and science-enhancing opportunity.

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Pittcon is a dynamic, international conference and exposition on laboratory science, a venue for presenting the latest advances in analytical research and scientific instrumentation, and a platform for continuing education and science-enhancing opportunity.

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Some recent contributions from SelecScience® to the scientific community

Editorial article

How to adopt automation in food and beverage testing: A checklist for your lab

Higher throughput, fewer manual errors, more walk-away time – the rewards of automation are well recognized. Achieving them in practice, however, is often another story. Even the most 'plug-and-play' instruments can present new challenges for staff, infrastructure, and day-to-day operations. Explore a checklist designed to help you plan, implement, and review your transition to automation with clarity and confidence. Access **here**

Webinar

Why biphenyl is replacing C18 in HPLC

While C18 remains a widely trusted and globally adopted stationary phase, this webinar introduces a fresh perspective on what's often considered a 'generic' choice. Join Application Scientist, Samantha Herbick as she explores the retention profiles of various C18 columns, highlighting that not all C18 phases are created equal, and examines the structural modifications permitted within the USP L1 classification. She will also introduce biphenyl phase and compare it to C18, showcasing how its unique ligand chemistry offers alternative retention mechanisms that can reshape your approach to method development. Access **here**

Video

Tackling the challenge of forever chemicals with advanced mass spectrometry

Dr. Gauthier Eppe from the University of Liège discusses the challenges of monitoring PFAS (per- and polyfluoroalkyl substances) when less than 50 compounds are currently regulated, despite their forever chemical status. Hear about the importance of advanced analytical methods to detect trace-level contamination in water and food matrices, and the critical need for international discussion to improve regulation and understanding. Access **here**

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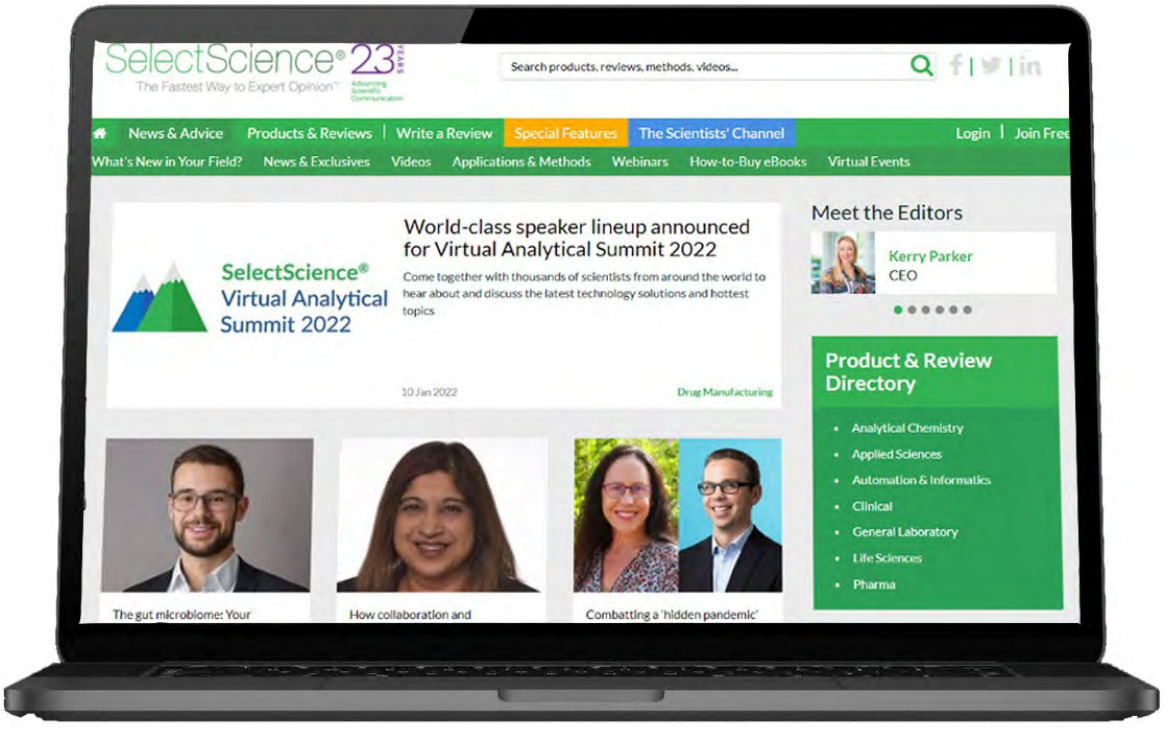

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LCGC

Chromatographyonline delivers practical, nuts-and-bolts information to help scientists and lab managers become more proficient in the use of chromatographic techniques and instrumentation. **Article:** ***A Practical Excel Tool to Support the Development of Comprehensive 2D-LC Separations.*** By Davy Guillarme, Megane Aebischer, Mathias Buff, and Sabine Heinisch. This article explores advanced oligonucleotide analysis techniques, including chromatography methods for therapeutic RNA, siRNA, and mRNA, enhancing nucleic acid research. **Read more**

Scientia Chromatographica

Scientia Chromatographica is the first and to date the only Latin American scientific journal dedicated exclusively to Chromatographic and Related Techniques. With a highly qualified and internationally recognized Editorial Board, it covers all chromatography topics in all their formats, in addition to discussing related topics such as “The Pillars of Chromatography”, Quality Management, Troubleshooting, Hyphenation (GC-MS, LC-MS, SPE-LC-MS/MS) and others. It also provides columns containing general information, such as: calendar, meeting report, bookstore, etc. **Read more**

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Spectroscopy

With the *Spectroscopy* journal, scientists, technicians, and lab managers gain proficiency through unbiased, peer-reviewed technical articles, trusted troubleshooting advice, and best-practice application solutions. **Article:** ***Recent Research in Chemometrics and AI for Spectroscopy, Part I: Foundations, Definitions, and the Integration of Artificial Intelligence in Chemometric Analysis.*** By Jerome Workman, Jr. **Read more**

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BrJAC editors are grateful to all those who have reviewed papers in 2025 using significant time and effort to provide constructive inputs.

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Aims & Scope

Brazilian Journal of Analytical Chemistry is a double-blind peer-reviewed research journal dedicated to the diffusion of significant and original knowledge in all branches of Analytical and Bioanalytical Chemistry. It is addressed to professionals involved in science, technology, and innovation projects at universities, research centers and in industry. **BrJAC welcomes** the submission of research papers reporting studies devoted to new and significant analytical methodologies, putting in evidence the scientific novelty, the impact of the research and demonstrating the analytical or bioanalytical applicability. BrJAC **strongly discourages** those simple applications of routine analytical methodologies, or the extension of these methods to new sample matrices, unless the proposal contains substantial novelty and unpublished data, clearly demonstrating advantages over existing ones.

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See the next items for more information on the journal, the documents preparation, manuscript types, and how to prepare the submission.

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Note: In case the Editors and Reviewers consider the manuscript to require an English revision, the authors will be required to send an English proofreading certificate, by the ProofReading Service or equivalent service, before the final approval of the manuscript by the BrJAC.

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The manuscripts accepted for publication are forwarded to the BrJAC production department. Minor changes to the manuscripts may be made, when necessary, to adapt them to BrJAC guidelines or to make them clearer in style, respecting the original content. The articles are sent to the authors for approval before publication. Once published online, a DOI number is assigned to the article.

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