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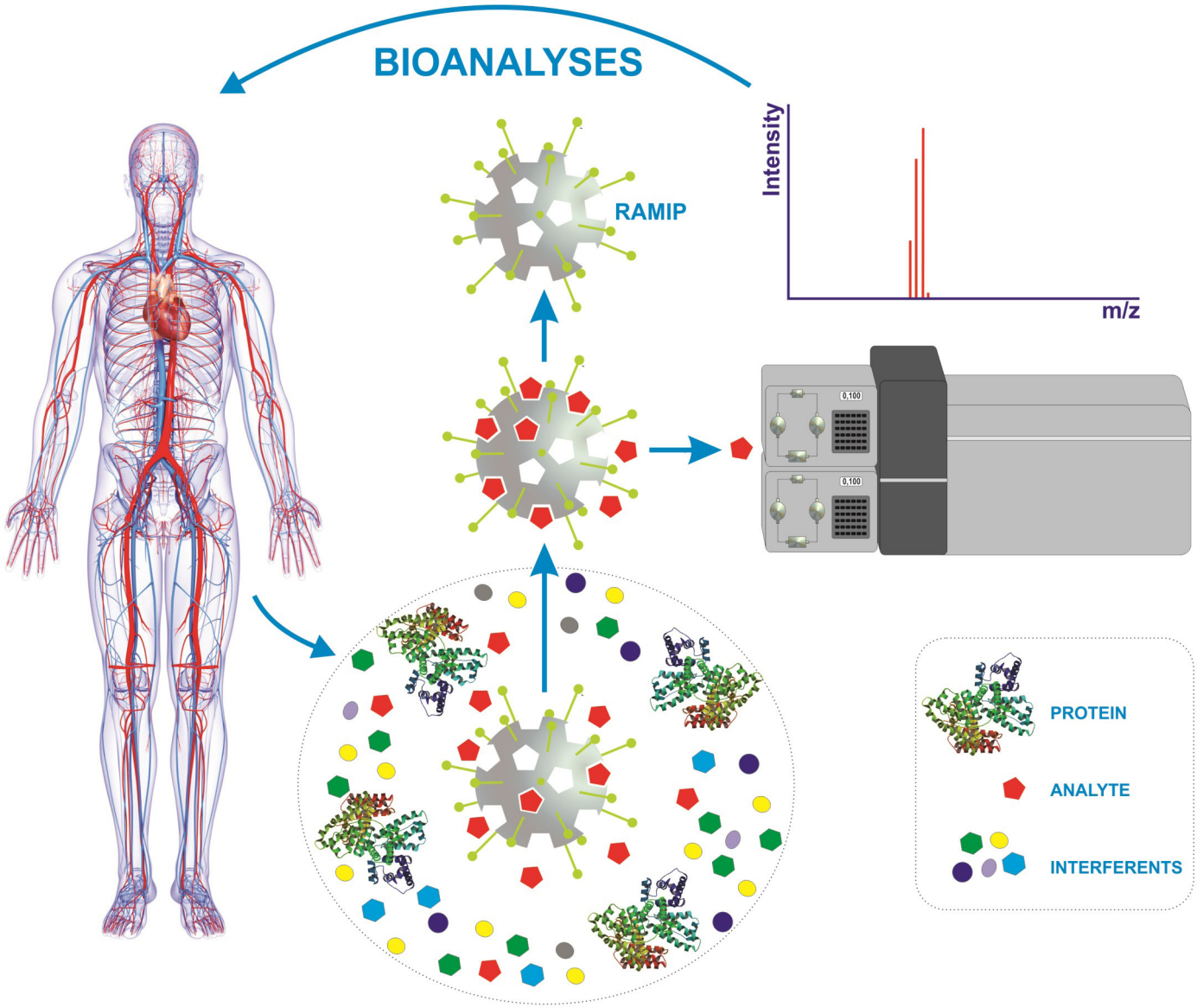

BIOLOGICAL SAMPLE PREPARATION WITH RAMIP

Restricted Access Molecularly Imprinted Polymers for Biological Sample Preparation

Tássia **Venga Mendes, Mariana Azevedo Rosa, Eduardo Costa de Figueiredo**

April – June 2022 Volume 9 Number 35

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BrJAC is dedicated to the diffusion of significant and original knowledge in all branches of Analytical Chemistry and Bioanalytics. BrJAC is addressed to professionals involved in science, technology and innovation projects in Analytical Chemistry at universities, research centers and in industry.

BrJAC is a quarterly journal that publishes original, unpublished scientific articles, reviews and technical notes that are peer reviewed in the double-blind way. In addition, it publishes interviews, points of view, letters, sponsor reports, and features related to analytical chemistry. Once published online, a DOI number is assigned to the paper.

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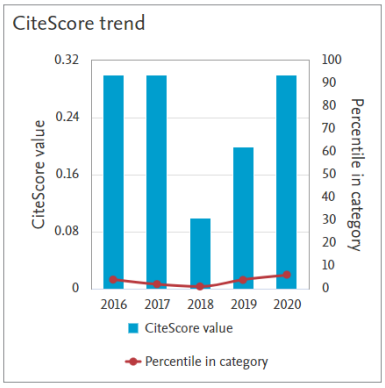

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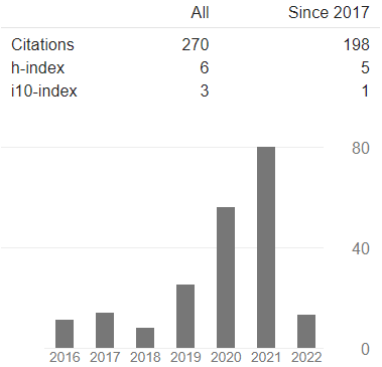

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CONTENTS

CONTENTS

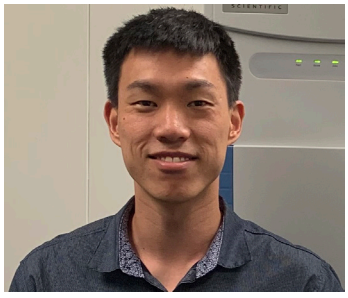
Brazilian Journal of Analytical Chemistry
2022, Volume 9, Issue 35, pp 1-1
doi: 10.30744/brjac.2179-3425.editorial.lwhantao.N35

EDITORIAL

About This Issue

Leandro Wang Hantao
Professor at the Institute of Chemistry, University of Campinas (Unicamp), SP, Brazil
Associate Editor of the Braz. J. Anal. Chem.

Modern analytical chemistry comprises an amazing scope of techniques and methods used for the characterization of analytes in complex matrices. Despite the numerous formats and instrument configurations, the challenges faced by analysts in innovation-driven environments are, in essence, the same. Recent advances in separation science have imposed new requirements for sampling and sample preparation, which has ultimately altered the essence of contemporary sample preparation. Advances in column technology and multidimensional instrumentation has led to the achievement of unprecedented peak capacities. The evolution of hyphenated methods has enabled the generation of information-dense data tensors, which ultimately impact the fundamental role and formats available for data processing and interpretation (see Issue #32). This paradigm shift has created exciting opportunities for analytical chemistry. In this issue #35, we have carefully selected applications covering some of the most important steps of an analytical method, namely, sample preparation and method development using separation and spectroscopy to solve real world tasks. For instance, a review article covers the potential of combining restricted access materials with molecularly imprinted polymers for bioanalytical applications. This article is followed by a report on the extraction and characterization of Tamarind gum from an unusual source of biomass, which is an important step towards the circular economy. Next, applications of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and inductively coupled plasma mass spectrometry (ICP-MS) are also available for the interested reader. In this issue, we have also interviewed Dr. Gisele Tonietto about the dissemination of science, published a point of view with Dr. Emanuel Carrilho, and a letter from Dr. Cesar Tarley. Lastly, I would like to thank all the authors, reviewers, and editorial staff for putting this much effort and dedication into publishing this issue on time. This journal has grown beyond expectations, while accompanying the evolution of research and innovation in Brazil. It is our hope to continue pushing the boundaries of science by inviting interested authors from Latin American and the world. I look forward to publishing manuscripts that will also recognize and promote equality in science, highlighting role models for younger generations of researchers.



Leandro Wang Hantao, PhD, is a Professor of Chemistry at the Institute of Chemistry, University of Campinas (IQ-UNICAMP). His studies aim to sample preparation for analysis of organic compounds, chromatographic techniques, mass spectrometry and data processing. Among the awards, the 2018 Power List “TOP 40 UNDER 40” (Analytical Scientist) and 2019 The “John Phillips Award” stand out.

CV

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Brazilian Journal of Analytical Chemistry
2022, Volume 9, Issue 35, pp 2-4
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INTERVIEW

Gisele Birman Tonietto, a coordinator of development projects and a dynamic researcher also dedicated to the dissemination of science, kindly spoke to BrJAC

Gisele Birman Tonietto
Professor in the Department of Chemistry and Coordinator of Development Projects
at the Pontifical Catholic University of Rio de Janeiro (PUC-Rio)
Rio de Janeiro, RJ, Brazil

Prof. Dr. Gisele Birman Tonietto is a professor in the Department of Chemistry at the Pontifical Catholic University of Rio de Janeiro (PUC-Rio) and Coordinator of Development Projects at the Analytical Center of the same university. Prof. Tonietto holds a degree in Industrial Chemistry from the Fluminense Federal University (1989), a master's degree in Chemistry from PUC-RIO (1995), and a Ph.D. in Chemistry from PUC-RIO (2006). Her research focuses on analytical chemistry, working mainly on ion chromatography, microwaves, arsenic and selenium speciation, and isotopic analysis of carbon, hydrogen and oxygen. Prof. Tonietto is also dedicated to the dissemination of science, developing digital content and promoting interviews, round tables, and training in various areas of chemistry. She received the University Merit Diploma from PUC-Rio in 2021 and is a member of the Technical Chamber of Oil and Gas of the Regional Council of Chemistry of Rio de Janeiro.

What early influences encouraged you to study science? Did you have any influencers, such as a teacher?

Fortunately, I had an excellent basic education. Curiosity and critical thinking were encouraged in me both at home and at school. Regarding science specifically, it was an innate curiosity in me. In elementary and high school, I had excellent teachers in both history and chemistry, and for a long time, I was very unsure about which career to pursue. I took the entrance exam for both university courses.

What motivated you? How was the beginning of your professional career?

I started studying industrial chemistry at the Fluminense Federal University (UFF), as it was, at that time, the only course that was not a Licentiate nor a BSc degree. At that time, I was sure that I would not be a teacher. My dream has always been to be a researcher, no matter the subject or the area of research. I am a very curious person; I like to study and apply scientific methodology towards discoveries. And this has always been my guide.

I took a new entrance exam because my father insisted very much that I study chemical engineering. He believed that chemical engineering would expand my professional possibilities. I ended up attending

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two courses: industrial chemistry and chemical engineering, both at UFF, which was possible at that time.

I was a Scientific Initiation scholarship holder and then an intern at Shell Co. located in "Ilha do Governador", an island in Guanabara Bay, Rio de Janeiro city. For nine months, I sailed through the Guanabara Bay by ferry-boat several times a day. In the morning, I took the ferry-boat from "Praça XV" in downtown Rio de Janeiro, heading to the UFF Chemistry Institute in the "Morro do Valonguinho" in downtown Niterói, a city on the other side of Guanabara Bay. At noon, I took the ferry-boat heading to "Ilha do Governador" for the internship at Shell. In the late afternoon, I returned to Niterói for the chemical engineering classes. I loved being on the ferry-boat and not in a bus!

Later, I did an internship at the Petrobras Research Center – CENPES. As I was studying industrial chemistry and chemical engineering, I was an intern at the Chemistry Department and then at the Refining Division, so I could experience both chemistry and engineering.

After graduating from the university, I worked for the Center of Mineral Technology (CETEM, RJ), a research unit linked to the Ministry of Science, Technology and Innovations. Then, I returned to CENPES, where I worked in the Chemical Department for many years.

What has changed in the student's profile, ambitions and performance since the time you started your career?

My relationship with teaching began at the CENPES research center, where more experienced professionals were required to disseminate their knowledge in different ways, including training less experienced professionals. At CENPES, I gave classes on ion chromatography and on microwave energy and its use in the oil industry. At PUC-Rio, I taught analytical chemistry and general chemistry. Students at a research center are very different from students at a university, obviously. However, on the other hand, a student is always a student, and it is up to us, the teachers, to involve and encourage the students as well as to provide moments where learning can go beyond the classroom. Chemistry offers the possibility of applying theories to practices. I always believed that this is the best way to learn: applying!

Could you comment briefly on the recent evolution of analytical chemistry, considering your contributions?

Working in a research center with super competent and committed colleagues, with a very complete and complex instrumental infrastructure, and with thought-provoking challenges and goals was, without a doubt, a privilege. Working in a university that values professionals and contributes to the viability of their projects is also a privilege. I followed these paths, which, despite being distinct, both made up my professional career. At CENPES, I had the opportunity to work with cutting-edge analytical techniques and to be responsible for their dissemination throughout Brazil. In addition, I was part of international groups of scientists who tested instruments before they were introduced to the market. I gave several courses and trainings in the use of ion chromatography – an analytical technique that was entering the market and would revolutionize the way we detected ions – and courses in the use of microwave energy. In my doctorate, I employed high performance liquid chromatography hyphenated to inductively coupled plasma mass spectrometry (HPLC-ICP-MS) analytical techniques for chemical speciation, which was a great novelty from both a theoretical and practical point of view. Nowadays, hyphenated analytical techniques are being used in different ways, and I am very proud to have been able to contribute to their development.

What are your lines of research?

I am currently the Manager of the Analytical Center at PUC-Rio. In this position, I am responsible for coordinating and making research projects and services possible. I coordinate several teams of specialists dedicated to various lines of research to meet the different demands that come to the Analytical Center. Analytical chemistry provides a very broad view of chemistry, and my professional experience brought me the possibility of management that I really enjoy, in addition to providing me with the best network of friends, colleagues, and professionals! Bringing the best people to work together is my favorite highlight.

There are several conferences on chemistry in Brazil and in the world. To you, how important are these meetings to the scientific community?

Academic meetings are essential for us to meet researchers and update our knowledge.

You also work in the dissemination of science. How do you develop this work?

I promote nice people who do interesting things! I like the backstage, the audience, to put everyone together and be in the organization, in the management, in the support. I do this by being the curator of Platform ECOA (ecoa.puc-rio.br) at PUC-Rio, our scientific dissemination streaming that offers open content in an integrated way, in a browsing experience in the best style of the famous video streaming platforms. Together with other professors, I elaborate digital content on topics of interest to society and participate in scientific podcasts such as SciCast with the purpose of providing access to science.

For you, what is the importance of the national funding agencies for the scientific development of Brazil?

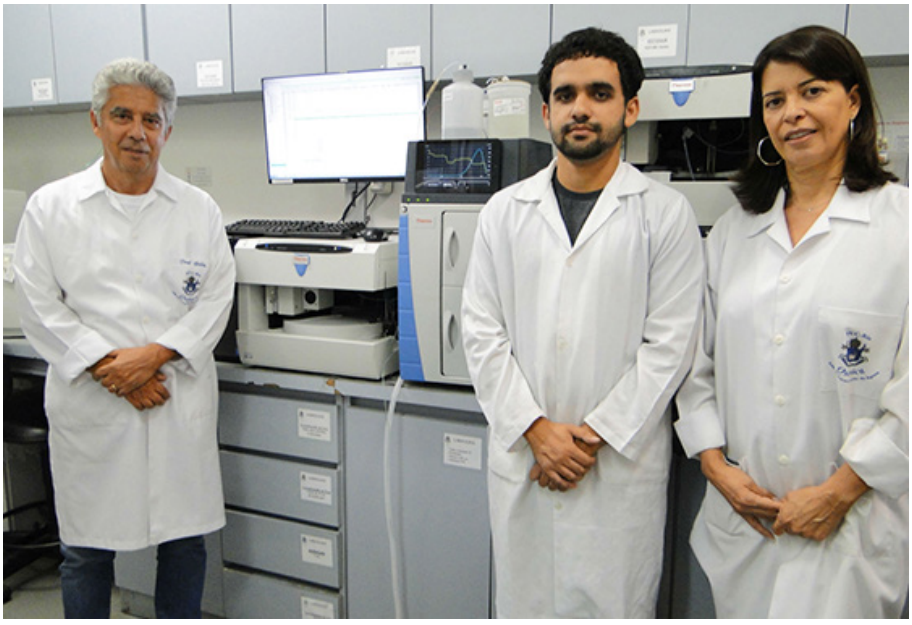
In one word: fundamental! The funding agencies provide autonomy to researchers and national sovereignty.

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?

Although we are living a very serious, worrisome, sad, and even shameful moment, I would say to the young people: Don't give up, because we don't give up on you, and we believe and need you to rebuild this nation. I believe that our role as older researchers is, above all, to encourage the younger ones, always, even in the darkest times.

What advice would you give to a young scientist who wants to pursue a career in analytical chemistry?

Keep your curiosity, your critical spirit. Don't accept obvious answers, formulate your questions, and seek your own answers.



José Marcus Godoy, Diogo Mendes, and Gisele Tonietto are members of the Laboratory of Water Characterization (LabAguas) at the Scientific Technical Center, Pontifical Catholic University of Rio de Janeiro (CTC/PUC-Rio).

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POINT OF VIEW

Analytical and Bioanalytical Chemistry *It is time we innovate*

Emanuel Carrilho

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Instituto Nacional de Ciência e Tecnologia de Bioanalítica, 13083-970, Campinas, SP, Brazil

It is time we innovate from the bottom up! From the analytical chemistry curriculum to the approach we take in teaching it. From the problems we are set to solve to the opportunities we create. Innovation and entrepreneurship are two words that are the "hype of the moment", indeed! But this is for the greater good! Modern societies and highly developed countries invest heavily in education and science and have open economies, with little or no bureaucracy to start a business or create a company. Startups, spin-offs, investors, and profit are words that should be in the minds of young analytical and bioanalytical chemists. Profit, contrary to what our Judaic-Christian religious background has stamped in our subconscious, is not a bad word. It is not a sin!

But let's start from the bottom; let's see how we can improve the way analytical chemistry is taught and what are the changes necessary to bring it to the 21st century. That is right! We are two decades deep into the 21st century, and we still teach our students with the same content and the same way as in the first half of the 20th century, if not earlier!

Is there still a place for the classical analytical march of cations and anions in this scenario?

Of course! Chemical and ionic equilibria in solution, acid-base and complexation reactions are examples of the most fundamental knowledge necessary in analytical and bioanalytical chemistry. It is not a case of not having these formatted as a mandatory course for chemists, but we need to review the methods, the content, and the hours invested in these classics. For example, dozens of cations and anions are separated, identified, and quantified in capillary electrophoresis (CE) experiments, instead of the eight-hour-long class for spot testing, titrations, and precipitations. Let alone running this CE experiment on a microfluidic platform! OK, so is this descriptive analytical chemistry important for the students? Why not present Fritz Feigl's spot tests on the new platform of paper-based microfluidics? These are two simple examples for new and innovative analytical chemistry teaching.

We, analytical and bioanalytical chemists, are passionate and believe it is the most critical discipline in the undergraduate chemistry degree, to the point that Peter Kissinger from Purdue University once said, "three things in life are certain: death, taxes, and the need for analytical chemistry," and that "the importance of analytical chemistry is not arguable!" Unfortunately, this is not the case in some of the most prestigious chemistry departments globally, where analytical chemistry is seen as a service. If this is the case, then we do a pretty good job. Out of 113 Nobel Prizes in Chemistry awarded to date, about a dozen can be directly linked to analytical chemistry fundamentals, instrumentation or methods, and about six others awarded with the Nobel in Physics. Therefore, the contribution of analytical and bioanalytical chemistry to the advancement of chemical sciences is clear. Curiously enough, about half of the chemistry prizes are associated with biochemistry and biomolecules; thus, reinforcing the central role of bioanalytical chemistry

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as the analytical chemistry for life sciences rather than the analyses of small molecules in biofluids.

We now live in a multi- and interdisciplinary time within a complex and integrated world. For example, the SARS-CoV-2 pandemics have shown the values of understanding virology, immunology, epidemiology, physics, statistics, biochemistry, pharmacology, vaccines, and, most importantly, diagnostics! The state-of-the-art Next Generation Sequencing (NGS) is an analytical tool that provides the forefront information on virus sequence and identity, the presence of mutations, and the design of probes for diagnostic tests. The first diagnostics came from quantitative real-time PCR (polymerase chain reaction), the gold standard method. Lateral flow (LF) rapid test diagnostics kicked in afterwards, and became a massive testing tool for antibody search. Later, LF tests against the antigen (the virus) became available. Where did Brazil rank in contributing to fighting the pandemics? Brazil sequenced the virus in just a couple of weeks, started to produce a couple of vaccines, and made alternative molecular diagnostics to PCR. The three major science funding agencies in Brazil, CAPES, CNPq, and FAPESP, quickly invested a fair amount of money and became one of the top producers of papers. So, one might say Brazil is very well-structured? Unfortunately, it is quite the opposite. All the chemicals and instrument supplies are imported. We designed molecular tests, but don't produce the reagents for the synthesis of oligonucleotides; we isolated the virus, but don't produce the disposable material for safely handling it; we made antibodies for rapid tests, but don't produce the membranes needed for LF; we developed biosensors to detect the virus, but we don't have a company producing them. Brazilian science and industry have become entirely dependent on imports.

It is time we innovate and teach modern analytical and bioanalytical chemistry early on in student courses. It is time we innovate and teach the students how to create jobs instead of seeking employment. It is time we innovate and add entrepreneurship in the curriculum of all undergraduate courses, especially in the chemistry degree.

Emanuel Carrilho, FRSC, is a Full Professor at the University of São Paulo (USP) with a Master's degree in Analytical Chemistry from USP (1990) with focus in instrumentation for supercritical fluid chromatography, and a Ph.D. from Northeastern University, Boston, USA (1997) in bioanalytical chemistry, helping in the development of DNA sequencing technologies that led to the sequencing of the Human Genome Project. Later, (2007-2009) he spent a sabbatical leave at Harvard University in the Whitesides Group, working on the emergence of microfluidic paper-based analytical devices (μPAD) and wax-printing. The Carrilho group, or BioMicS – Bioanalytical, Microfabrication, and Separations Group, develops new bioanalytical methods and instrumentation covering the broad aspects of genomics, proteomics, and metabolomics for human health, applied cell and microbiology in microfluidic platforms like organs-on-a-chip, searching for biomarkers for cancer, rare, and neglected diseases. Dr. Carrilho has supervised over 60 graduate students and a dozen of post-docs, has published about 200 papers with 9,000 citations, and an *h* index of 41. CV

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LETTER

Chemical Imprinting Technology Applied to Analytical Chemistry: Current Status and Future Outlook in Brazil

César Ricardo Teixeira Tarley[1,2]

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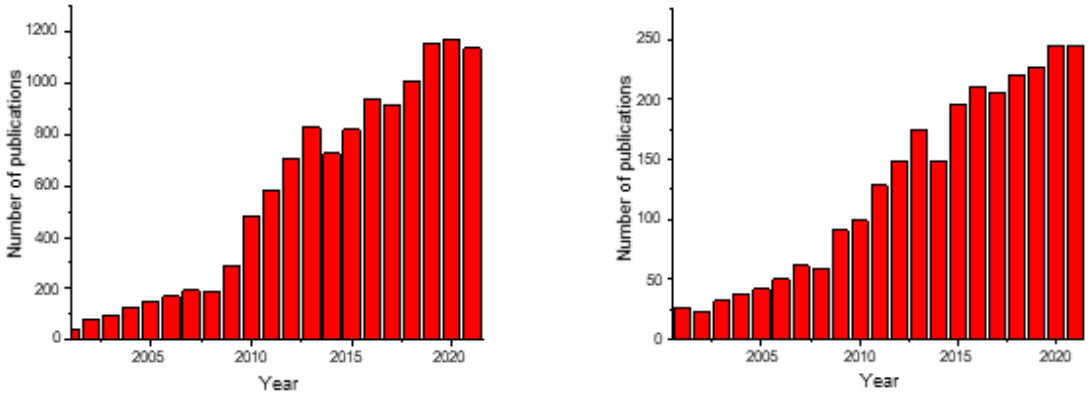
Chemical imprinting technologies based on molecularly imprinted polymers (MIPs) and ion imprinted polymers (IIPs) have been widely applied in different fields of analytical chemistry since their discoveries in 1972[1] and 1976[2], respectively. MIPs and IIPs are considered biomimetic materials with tailor-made synthetic receptors and are, essentially, obtained by co-polymerization of functional and cross-linking monomers in the presence of a target analyte (a molecule for MIPs and an ion for IIPs). The great advantages of these materials over biological recognition systems include their relatively low cost, quick preparation, and, most importantly, their stability in different media. To date, these biomimetic materials have experienced a rapid development with wide applications in electrochemical sensors, luminescence sensors, separation science, sample preparation, and more specific sensor applications such as surface plasmon resonance (SPR) and quartz crystal microbalance (QCM) sensors. In addition, they have been applied for analysis of samples of environmental, food, and forensic interests, as well as for disease diagnostics.[3-8] Figure 1 depicts the increasing number of published papers per year over the past 20 years on the topic of MIPs and IIPs; as expected, most of these papers are devoted to the chemical imprinting of molecules.



(a) (b)

Figure 1. Number of papers published in Web of Science using the keywords (a) "*molecularly imprinted polymer*" and (b) "*ion imprinted polymer*". Retrieved from Web of Science database (2022 March 07).

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A literature survey also reveals that MIPs have been most used for sensor preparation (47%), separation techniques (36%), and sample preparation (17%). On the other hand, IIPs have been mostly prepared for separation techniques, including pre-concentration/extraction (62%), while sensor preparation is the second-most common application (38%). These outcomes were obtained using the Web of Science database platform on March 7th, 2022.

Due to tremendous advances in imprinting technology in the scientific literature, scientists and students now have a facile way to carry out rapid research on imprinted polymers on the free-to-use website MIPdatabase, supported by the Society for Molecular Imprinting, where Professor Mike Whitcombe (University of Leicester, UK) serves as webmaster.[9] This website contains an up-to-date list of papers, books, chapters, patents, reviews, and conference proceedings in the field of imprinting technology.

Success in chemically imprinting polymers in terms of selectivity and stability depends on fully understanding the preparation technologies; therefore, multidisciplinary studies involving scientists with solid knowledge in analytical chemistry, characterization techniques and materials synthesis are indispensable.

Different approaches for MIP and IIP synthesis can be adopted. However, in general, when these materials are applied to separation science, including solid phase extraction-based methods and chromatography, radical polymerization and sol-gel processes have been the most used.[10] Radical polymerization can be carried out in bulk, in which a monolithic morphology material is obtained. However, during the milling process irregular-shaped particles are obtained. To obtain regular-shaped particles, syntheses in heterogeneous media, such as suspensions or emulsions, or homogeneous media, such as precipitation, dispersion or the sol-gel process, have been performed.[10] Aside from these, surface imprinting techniques based on imprinted polymers grafted on the surface of magnetic or carbon-based nanostructured particles (such as carbon nanotubes or graphene oxide) by using the living/controlled “grafting from” polymerization method or exploring free-radical polymerization (FRP) have also been reported.[11] These approaches have given rise to materials with high imprinted sites per volume due to the nanoscale imprinted polymer grafted on the surface of the substrate, thereby increasing the availability of selective binding sites and improving the adsorption kinetics. Another way to improve the adsorption capacity of chemically imprinted polymers is by obtaining a more porous material by using structure-directing agents, such as surfactants or non-ionic block copolymer surfactant templates, exploiting the dual-template docking oriented chemical imprinting strategy.[12] This strategy is well-established in obtaining inorganic polymers synthesized by means of the sol-gel method; however, efforts to understand the influence of structure-directing agents in organic imprinted polymers have only been reported more recently.[13,14]

It is important to emphasize that the use of MIPs and IIPs in complicated matrices, including biological fluids and food samples, is limited due to the adsorption of macromolecules (proteins, carbohydrates, and others) on the surface of the polymers, which hinders the specific recognition of target molecules. For this reason, the synthesis of MIPs and IIPs with restricted access materials (RAM) has been proposed to overcome this drawback. These materials contain hydrophilic functional groups and/or proteins on the external surface with the exclusion properties of macromolecules.[15,16]

Regarding the use of MIPs and IIPs in electrochemical sensor development, their integration onto the surface of the transducer electrode is of paramount importance for the successful performance of the device. Different synthesis/preparation approaches have been reviewed in the literature, such as electropolymerization; carbon/MIP or IIP paste-based electrodes; and nanocomposites based on graphene and carbon nanotubes, grafted on polymeric matrices using drop-casting for film formation.[17] Additionally, there is a trend in the development of electrochemical MIP sensors using metallic nanoparticles (NPs) such as gold or silver for improving sensitivity, and quantum dots (QDs) or carbon dots (CDs) for optical sensing applications.[18]

One important issue that must be considered in the development of chemically imprinted polymers is the presence of selective binding sites. It is known that adsorption capacity and surface area do not have a direct relationship with the presence of selective binding sites, since these features can be influenced by

the porogenic solvent. Therefore, the selectivity should be determined from competitive rebinding studies using the chemically imprinted polymer and the non-imprinted polymer (NIP), followed by the determination of distribution (K_d), selectivity (k), and the relative selectivity (k´) coefficients, as shown in Equations 1–4.[19]

$$K_d = \frac{(C_i - C_f)}{C_f}\frac{V}{M} \quad (1)$$

where C_i and C_f are the initial and final concentration of target analyte (template) and concomitants, *V* is the solution volume, and *M* is the mass of MIP and NIP;

$$k_{MIP} = \frac{K_d(template)}{K_d(structurally\ similar\ concomitant)} \quad (2)$$

$$k_{NIP} = \frac{K_d(template)}{K_d(structurally\ similar\ concomitant)} \quad (3)$$

$$k' = \frac{k_{MIP}}{k_{NIP}} \quad (4)$$

When k´>1, this indicates that successful imprinting has occurred.

The use of imprinting factor (IF) for assessing the selectivity of MIPs and IIPs has also been reported, and is defined by Equation 5. It should be noted that this selectivity parameter can only be used for competitive rebinding studies; however, some studies have wrongly reported the absence of structurally similar concomitants, mainly for sensor applications.[20,21]

$$IF = \frac{Q_{(equilibrium\ binding\ capacity)\ of\ MIP}}{Q_{(equilibrium\ binding\ capacity)\ of\ NIP}} \quad (5)$$

One of the main bottlenecks of chemically imprinted polymers is their commercialization, which has been considered a slow process, most likely due to the possible occurrence of nonspecific binding sites in the material. This, in turn, depends upon the nature of the interaction between the template and binding sites of functional monomers. Despite this, companies have been founded by early proponents of the use of MIPs in separation science. These companies include MIP Technologies, founded in 1999 in Sweden with Sellergren and Mosbach as the principal researchers. Later, this company was acquired by Biotage AB, where SPE cartridges are currently commercially available. Semorex was founded in 2001 in the USA, POLYintell in 2004 in France, NanoMyp in 2011 in Spain, and MIP diagnostics in the UK in 2015; all of these are companies that make use of chemical imprinting technology. These companies offer MIP-based extraction materials, point-of-care devices, drug delivery systems, and MIP particles based on core-shell morphologies.[11]

In Brazil, the first studies published on the topic of chemically imprinted polymers were supervised by Professor Lauro Tatsuo Kubota at UNICAMP in 2005.[5,6] Presently, the number of scientists working with a focus on chemically imprinted polymers has increased and become widely distributed throughout Brazil. Most research involves the use of MIP-based materials for solid phase extraction on columns, magnetic dispersive solid phase extraction, chromatographic purposes, drug delivery systems and

sensor (electrochemical and optical) preparation. These studies have been carried out at several different universities, including UERJ, UFRJ, UFF, Unifal-MG, UNICAMP, USP, UEL, UFSJ, UNESP, UFBA, UESB, UNEB, UFOP, UFMG, UFVJM, UFAM, UnB, UFSE and UFScar.

Considering the number and the quality of published papers by Brazilian scientists, the research on chemical imprinted technology can be considered well-established and mature. However, as also observed worldwide, there are still challenges and opportunities including the large-scale production of particles for a wide range of analytes (ions, small and large molecules); synthesis of chemically imprinted polymers with restricted access and without losses of selectivity; and studies based on chemical speciation and integration of MIPs in microdevices based on microfluidics for rapid diseases diagnostics.

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César Ricardo Teixeira Tarley is currently a Full Professor at the Universidade Estadual de Londrina (UEL), where he teaches undergraduate courses and supervises research projects. He has experience in chemistry, with an emphasis on the development of solid-phase preconcentration systems using chemically imprinted polymers, nanomaterials and mixed oxides, as well as cloud point and dispersive liquid-liquid microextraction coupled to chromatographic, electroanalytical, and spectroanalytical techniques. Tarley has published more than 230 papers, 16 book chapters, and 6 patents, and has supervised more than 40 doctoral dissertations and Master's theses. CV

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IN MEMORIAM

BrJAC mourns the death of Prof. Dr. Miguel Valcárcel Cases and recognizes his great contribution to Analytical Chemistry and Science around the World

Who has not seen or heard about books such as Analytical Chemistry: A Modern Approach to Analytical Science, Principles of Analytical Chemistry: A Textbook, or Foundations of Analytical Chemistry: A Teaching-Learning Approach, or about flow injection analysis-FIA, and sequential injection analysis-SIA? These are, in fact, some contributions from Prof. Miguel Valcárcel Cases, at the University of Córdoba-Spain, who leave us on 9th January 2022 at the age of 75.

Prof. Valcárcel was Dean of the Faculty of Sciences at the University of Córdoba, Vice-Rector for academic guidance and teaching and Vice-Rector for quality, as well as the first Director of the Andalusian Institute of Fine Chemistry and Nanochemistry since 1994.

Born in Barcelona (Spain), Prof. Valcárcel was a graduate of the University of Seville where also obtained his Ph.D., and was an assistant teacher until 1975. He was an associate professor of Analytical Chemistry at the Faculty of Science of Palma de Mallorca in 1975, an institution where he was also Dean and full professor at the University of Cordoba in the year 1976.

He was also President of the Analytical Division of the European Federation of Chemical Societies and was a member for 4 years of the High-Level Expert Group of the European Union's Growth Program.

Valcárcel received the Spanish national Enrique Moles prize for Chemical Science and Technology (2005), the Maimónides prize for scientific-technical research from the Junta de Andalucia (1992), and the Solvay Research Prize in Chemical Sciences from the CEOE Foundation (1997). He has the Robert Boyle Medal from the Royal Society of Chemistry (UK, 2004), the Enrich Planquette Prize from the Austrian Chemical Society (1996), the Gold Medal from the University of Warsaw (2000), and the Medal from the Portuguese Chemical Society (2000). He also received the distinction of Cordoba citizen of the year 2006 in the education/research section, and the Averroes de Oro-Cuidad de Córdoba medal in 2006 for his scientific trajectory. He was also awarded the title of Doctor Honoris Causa by the University of Valencia (2010) and the European DAC-EuChMS (Division of Analytical Chemistry of the European Association for Chemical and Molecular Sciences) award in recognition of his scientific and teaching career (2015).

He was the author of ca. 700 papers, published 9 scientific books, and co-authored 15 chapters of multi-author books.

Owner of a unique vitality and a very accurate vision of Analytical Chemistry, Prof. Valcárcel contributed to the formation of dozens of students, of which he was extremely proud, and some of them are today Full professors spread all over the world.

The Brazilian Journal of Analytical Chemistry mourns his death, and through this simple tribute, recognizes his great contribution to Analytical Chemistry and science around the world.

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IN MEMORIAM

BrJAC pays tribute to Prof. Dr. José Camillo Novello, a pioneer researcher in proteomics in Brazil and a gentle advisor

On January 6, 2022, Dr. José Camillo Novello, Associate Professor at the Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas (IB-Unicamp), SP, Brazil, left us prematurely. Owner of a discreet and subtle humor, Prof. Camillo enjoyed a good chat, always accompanied by a cup of coffee. More recently, he loved to talk about his grandchildren and children who are living abroad.

Prof. Camillo arrived at Unicamp in the 1970s. Initially, he worked at the Laboratory of Protein Chemistry with Professors Benedito de Oliveira and Sergio Marangoni. During this period, he dedicated himself to the studies of biochemical characterization of Arthropod and Snake venoms. The laboratory skills transferred by Prof. Camillo are told in an amusing way by his students, who were always guided with cordiality.

In the late 1990s, Prof. Camillo focused efforts in proteomics, being a pioneer in this field in Brazil. He coordinated the first Brazilian publication in this field[1] based on one of his projects funded by the São Paulo Research Foundation (Fapesp) about *Xylella fastidiosa*'s Functional Genome.

Recently, Prof. Camillo faced many health challenges, which never changed his kindness and good humor when, over a cup of coffee, he would talk about politics, soccer, and the evolution of his former students. He was proud of his "scientific grandchildren", whom he would happily approach in the corridors of IB-Unicamp.

His affectionate figure is missed in the corridors and in the canteen of IB-Unicamp. But his legacy remains and will always be in the world, with his science, and among us, in nostalgia.

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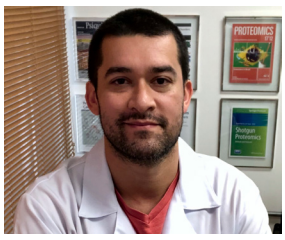

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IN MEMORIAM

José Luís Fontes da Costa Lima

An example to be followed

I am very sad to communicate the death of the Emeritus Professor José Luís Fontes da Costa Lima, a leading chemist who made significant contributions to the development of Analytical Chemistry in Brazil.

His parents, José Coimbra da Costa Lima and Maria de Belém Rodrigues Fontes da Costa Lima, lived in the parish of Cedofeita, Porto (Portugal), where he was born on December 5, 1945. He married Maria Assunção da Costa Lima and both were graced with two children (José Pedro and Rui) and five grandchildren.

He graduated in Chemistry at the Faculty of Sciences of the University of Porto (FF-UP), in 1970. After that, he spent 9 years at the service of Portugal in Africa. In 1985, he concluded the doctoral program in Chemistry, specialty Analytical Chemistry, being approved with distinction and praise.

At the beginning of his scientific career, Prof. Costa Lima experienced important moments when, together with colleagues from Barcelona, proposed a flow-through sandwich PVC matrix membrane electrode for flow injection analysis[1] and, in collaboration with renowned scientists from Cardiff, proposed an ion-selective electrode without inner reference solution[2].

In 1986, he started working at FF-UP and decisively contributed to the formation and consolidation of a research group that today stands out in the context of analytical, bioanalytical and electroanalytical chemistry, especially in the pharmaceutical, biological, and agro-environmental areas.

In 1996, he was appointed Full Professor, and after his compulsory retirement in 2016, he became Emeritus Professor.

Regarding Luso-Brazilian scientific interactions, Prof. Costa Lima was one of the pioneers. In 1989, during an international congress on FIA-HPLC held in Córdoba he was introduced to some researchers from CENA/USP, and invited me to spend a 15-day period in Portugal, where we prepared a research project on multisite detection in flow analysis[3]. This was the initial seed of a fruitful scientific interaction that lasted for a quarter of a century.

This interaction resulted in important scientific contributions involving conceptual, methodological and/or applicative developments in flow analysis, including multi-site detection, multi-commutation, multi-pumping flow analysis, in-line separation/concentration exploiting fluidized beds, single-interface flow analysis, nanomaterial applications, among others[4]. In addition, other Brazilian (e.g., IQ/USP, IQ/Unicamp, Embrapa, UNESP, UFPB, UFPE, UEB), Portuguese (Catholic University of Porto) and Spanish institutions were involved. Apart from the excellent research results, the outstanding number of scholarships, scientific visits and student participations in a different scientific environment was a positive factor towards the improvement of the formation of students and technicians.

In Brazil, Prof. Costa Lima actively participated in the organization of the VII International Conference on Flow Analysis (1997), and collaborated in several Annual Meetings of the Brazilian Chemical Society

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(RA-SBQ) and National Meetings on Analytical Chemistry (ENQA). In 2000, he had a prominent role in the XXIII RA-SBQ, as the theme of the meeting was “The Brazil-Portugal chemical bond”. In 2003, he attended the XII ENQA giving the brilliant conference “Development of flow analysis in the context of the Luso-Brazilian interaction”[5].

He encouraged more than 30 Brazilian collaborators to undertake medium-term internships at the University of Porto, and to participate in scientific events in Portugal, especially the Luso-Galician Congresses, the Euroanalysis XI (Lisbon, 2000), and the X International Conference on Flow Analysis (Porto, 2006). On the other hand, more than 20 Portuguese researchers carried out scientifical missions in Brazil. This resulted in 89 published articles with the participation of both Portuguese and Brazilian researchers.

In 2005, he was sworn in as a member of the Brazilian Academy of Sciences.

The relevance of the scientific work of Prof. Costa Lima can be assessed in terms of its publications (Web of Science: 454 scientific articles, 9293 citations, h = 52), communications at scientific events and training of human resources, especially graduate students, which he affectionately called “scientific children”[5].

He also dedicated himself to academic-scientific management activities, participating in the organization of several congresses, workshops and similar. During the last years as an active scientist, he was the Director of FF-UP, carrying out an outstanding work.

A big party took place on December 5, 2005 to celebrate his retirement. I had the pleasure of being present, offering him an award from the University of São Paulo, which recognized his efforts in favor of Brazil.

As an Emeritus Professor, he continued to work at FF-UP, giving international conferences, strengthening the scientific relations with Brazil, etc.

Throughout his career, he was member of editorial boards of several journals (e.g., Microchim. Acta, Farmaco Sci, Am. J. Enol. Vitic., Port. Electrochimica Acta and Rev. Port. Quim.); member of organizing committees in 26 international meetings; member of 06 scientifical societies; coordinator of the European M.Sc. program in Environmental Analytical Chemistry; integrant of the Chemical Center of the University of Porto - REQUIMTE; collaborator with several research foundations; etc. He was worldwide recognized, as demonstrated by the important awards.

Some personal characteristics of Prof. Costa Lima, such as leadership, creativity, spirit of investigation, tenacity, ease of integration into a teamwork and, above all, friendship and companionship should be highlighted. As for friendship, I received from Prof. Costa Lima an extraordinary example: when serious health problems affected me, he surprised me: I received a visit from this friend who crossed the Atlantic Ocean to comfort me.

He did several society volunteer activities, promoting social aid, participating in charity parties, etc. An interesting activity is that, when young, he was the table tennis coach of the Portuguese team.

He was always ready to host visiting friends. I was often at his home, and confirmed that he was an exemplary host, and also an excellent father, grandfather, husband and son-in-law. There, I could see that important moral values are passed from generation to generation in his family.

Costa Lima had a special dedication to Brazil and his collaborators lost a very good friend and example to be followed. I am sure that he will be very happy in this new dimension of his life, undoubtedly full of Peace, Joy and Happiness.

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REVIEW

Restricted Access Molecularly Imprinted Polymers for Biological Sample Preparation

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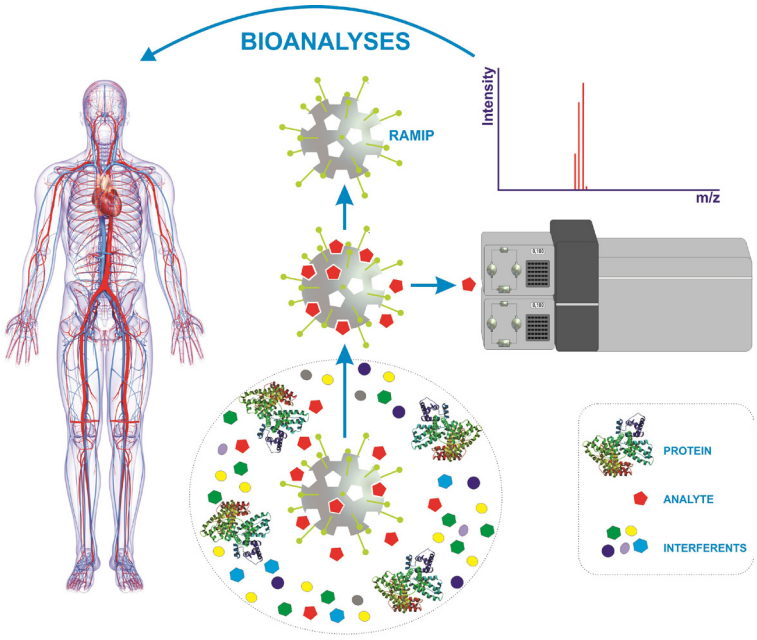

BIOLOGICAL SAMPLE PREPARATION WITH RAMIP

Restricted access molecularly imprinted polymers (RAMIPs) have been efficiently used for the extraction of small organic molecules from untreated biological matrices (e.g. blood, plasma, serum, and milk). These materials have been obtained by modifying the external surface of conventional molecularly imprinted polymers (MIPs) with hydrophilic monomer grafting, crosslinked protein capsule or a combination of both. These sorbents aggregate the selectivity of MIPs with the ability to exclude macromolecules of restricted access materials (RAMs), being widely employed in solid phase extraction techniques, beyond their use in sensors. In this review, we discuss about the design and application of RAMIPs in biological sample preparation, emphasizing the future trends and remaining challenges of this technology for bioanalyses.

Keywords: molecularly imprinted polymers, restricted access materials, restricted access molecularly imprinted polymers, sample treatment, protein exclusion

INTRODUCTION

Molecularly imprinted polymers (MIPs) are selective materials obtained by the copolymerization of functional monomers and crosslinker agents, in the presence of a template molecule [1,2]. The selective recognition ability to the template (in terms of size, shape, and chemical functionality [3]) is the more important characteristic of MIPs. High stability, adsorption capacity, reproducibility and reusability can also

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be highlighted [2]. The first reported application of MIPs in solid phase extraction (SPE) occurred in 1994 [4], and since then, MIPs become well-established materials in sample preparation.

Despite their advantages, the direct used of MIPs to prepare untreated biological samples (e.g. milk, plasma, serum, blood, among others) can be difficult due to the presence of proteins, polypeptides, and lipids, that can be retained on the polymer surface, causing sensitivity and selectivity problems [5]. To circumvent this situation, additional steps of sample preparation have been performed before the molecularly imprinted solid phase extraction (MISPE), as for example, the protein precipitation. On the other hand, sample dilution, drastic pH changes, loss of analytes through co-precipitation, and ionic suppression in mass spectrometry are the main recurrent problems of this strategy [6].

Considering these limitations, in 1999 Haginaka et al. [7] synthesized the first restricted access molecularly imprinted polymer (RAMIP), that was a MIP with the additional capacity to exclude macromolecules. The exclusion mechanisms were based on the concepts of restricted access materials (RAMs), by the presence of physical (size pores) and/or chemical (hydrophilic external layer) diffusion barriers [8,9]. Low molecular target molecules can penetrate through these barriers, being retained by partition, ion exchange and/or adsorption process [10,11], whereas the macromolecules are excluded. The protective barriers can also prevent the influence of water in the selective interactions between target molecule and RAMIP [7,12,13], improving the selectivity.

Some reviews were published in the past years about the use of RAMs or MIPs in sample preparation [1,3,14–18] and/or coupled to analytical techniques [1,19–26]. Future and promising trends for both materials were highlighted [14,17,19,21,27]. However, none of these reviews had RAMIPs as their focus and the RAMIP term hardly was observed (no more than a citation or one topic with a summarized explanation). An exception is the review of De Faria et al. (2017) [3], in which a more complete description of RAMIPs was observed.

The present review is the first totally dedicated to the RAMIP, with an actualization of the types and synthesis methodologies, as well as with recent examples of applications. Different mechanisms of protein exclusion are deeply described. Advances, remaining gaps, advantages, disadvantages, and challenges are also discussed.

RAMIPS OBTAINED BY HYDROPHILIC COMONOMERS GRAFTING

To obtain RAMIPs by hydrophilic comonomers grafting, the comonomers are added in the reactional flask together with the MIP synthesis reagents. Therefore, a dense region of hydroxyl groups is created on the polymer surface during the polymerization step. The hydrophilic comonomers are generally added after a pre-polymerization step (functional monomer + template in porogenic solvent) to avoid possible interferences in the formation of specific imprinted binding sites. Figure 1 shows a general synthesis scheme of these materials.

The mechanism of macromolecules exclusion by the hydrophilic layer is discussed in some papers about the preparation of different RAMs [3,10,28,29]. The authors suggest that the access of macromolecules to the material's inner is avoided by the small pores, and the accumulation of the proteins is averted by a chemical diffusion barrier created by the hydrophilic chains. The primary thermodynamic interaction between protein and support is the hydrophobic effect [30]. Thus, generally, proteins are more adsorbed on hydrophobic surfaces than on hydrophilic ones (releases a large amount of entropy) [30,31]. Therefore, the proteins present in the matrix are eliminated and the binding sites of the RAMIPs are not obstructed.

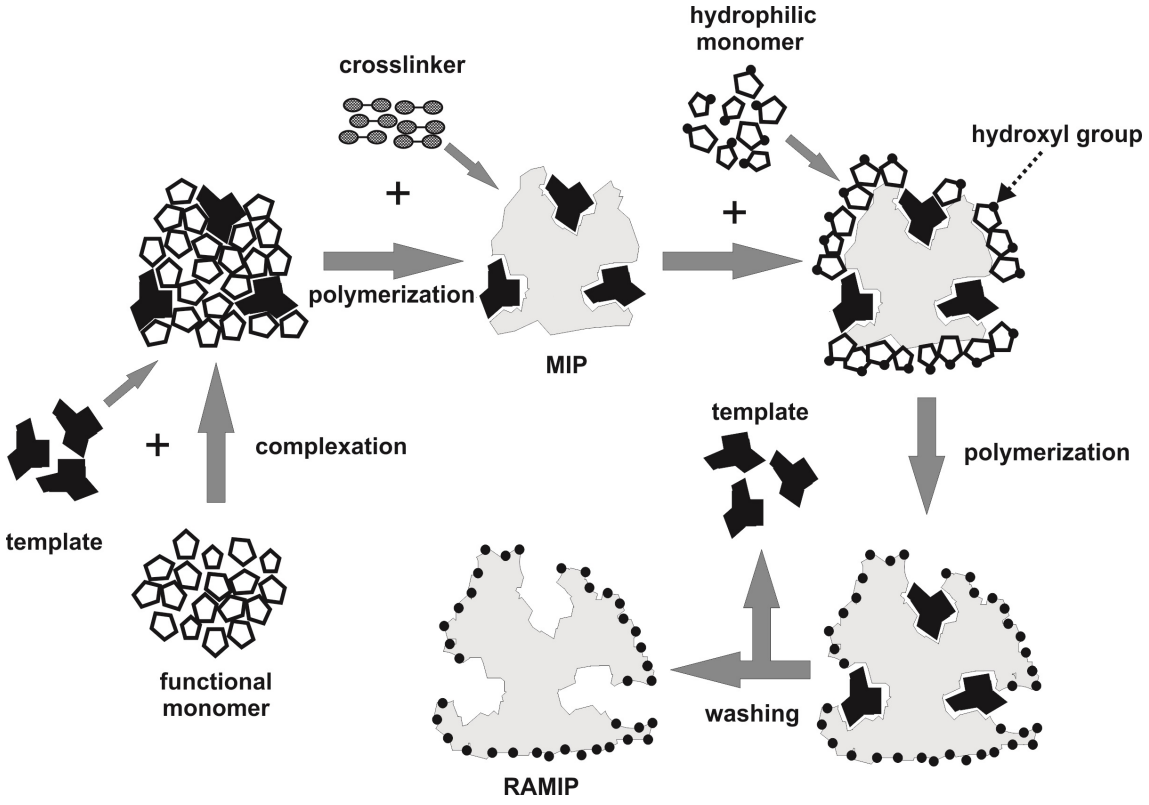

Figure 1. General synthesis scheme of the RAMIP obtained by hydrophilic comonomers grafting. (Reprinted with permission from De Faria, H. D.; Abrão, L. C. de C.; Santos, M. G.; Barbosa, A. F.; Figueiredo, E. C. Anal. Chim. Acta, 2017, 959, pp 43–65 https://doi.org/10.1016/j.aca.2016.12.047 Copyright© (2017), Elsevier.)

Wang et al. (2011) [28] demonstrated that a silica material presented lower total pore area, lower average pore diameter, and higher protein exclusion rate after its functionalization with hydrophilic comonomers: 376.1 and 280.7 $m^2 g^{-1}$, 98.9 and 80.7 Å, and 5.3 and 98.2%, respectively. The pore structures of the materials were analyzed by a nitrogen sorption system, and the protein exclusion test was performed using bovine serum albumin solution. These results showed that the formation of the external layer influenced the macromolecules exclusion by the synthesized material. Xu et al. (2010) [8] observed that the length of the hydrophilic comonomer chains also influenced the protein exclusion. The authors synthesized two different polymers and the length of the chains was calculated by the number of comonomers grafted on each one. While the material with 2.8 comonomers excluded about 4.9% of proteins, the RAMIP with 17.1, excluded approximately 99%.

The RAMIP hydrophilicity can be evaluated by the contact angle (θ) measurements. The means of the contact angle of a drop of water with the surface of the synthesized material is observed, if $\theta > 90°$, the material is hydrophobic, and if $\theta \leq 90°$, the material is hydrophilic [32]. Some authors [33–38] employed this technique to prove that their RAMIPs have hydrophilic properties, being able to exclude proteins. As one example, Liu et al. (2020) [34] measured the contact angle of carbon dots@MIP and carbon dots@RAMIP and the results showed a change in the hydrophilicity of the materials, angles of 102.0° and 73.8° were obtained, respectively. In addition, the Fourier Transform Infrared analysis is commonly used to demonstrate the incorporation of the comonomer in the MIPs.

Haginaka et al. (1999) [7] synthesized the first RAMIP, using a multi swelling polymerization method. The hydrophilic layer was obtained by a mixture of glycerol monomethacrylate and glycerol dimethacrylate, added in the reactional medium, 4 h after the beginning of the polymerization. The authors reported that

the recognition sites remained unchanged even after the surface modification. The material was able to exclude macromolecules from biological samples, allowing the direct injection of the complex matrices in a column switching liquid chromatographic (LC) system. To verify the RAMIP capacity to exclude proteins, a solution of bovine serum albumin (BSA) was injected in the system and the peak area obtained without the column was taking as 100%. Recoveries over 96.8% of BSA was achieved when the same BSA solution was injected in the system with the RAMIP column [7]. RAMIPs for the extraction of ibuprofen [12] and beta-blockers [39] from biological samples were reported by the same group.

RAMIPs grafting with poly(glyceryl monomethacrylate) were used to extract clenbuterol from human serum samples [9]. The macromolecule exclusion rates were higher than 88.9%. In addition, glycerol dimethacrylate and 2-hydroxyethyl methacrylate were used to obtain RAMIPs able to extract beta-blockers from urine samples with good selective and high protein exclusion capacity [40]. In both papers, the samples were direct injected in column switching LC systems. These systems have been efficiently used, main due to the possibility to inject complex matrices without previous treatment (minimizing the sample manipulation and increasing the analytical frequency) [3]. However, some limitations of this procedure can be highlighted, as for example the difficult to uniformly pack the column with the synthesized material (creating preferred pathways), high column pressure or material leakage when particles with small diameters are used, and increment in the pressure along the analyses.

A selective RAMIP to parabens was synthesized via *in situ* polymerization in an open fused silica. The polymerization mixture was introduced into the silanized capillary and poly(glycerol dimethacrylate) was used to obtain a hydrophilic external layer. An *in tube* SPME procedure was performed to recovery the target molecules from breast milk samples. The capacity of the material to excluded matrix interferents was attested from the comparison of chromatograms obtained with sample preparation with MIP and RAMIP [41]. The limits of quantification (3-10 μg L^{-1}) were close to those obtained with different sample preparation techniques: micro solid-phase extraction by packed sorbents (magnesium-aluminum layered double hydroxide coated on graphene oxide nanosheets), 10-15 μg L^{-1} [42], and dispersive liquid-liquid microextraction, 5 μg L^{-1} [43]. Additionally, the nanosheets could be used at least 25 times, while the authors did not report if the RAMIPs were reusable or not.

Zhang and co-works (2013) [44] obtained narrowly dispersed hydrophilic MIP particles with the surface grafted by poly(2-hydroxyethyl methacrylate) brushes. The synthesis was performed using a reversible addition-fragmentation chain transfer precipitation polymerization method, mediated by macromolecular chain-transfer agents. The results suggested that only those polymer brushes with a high enough molecular weight could prevent the accumulation of proteins on the material surface. In addition, the authors related that the hydrophilic RAMIP nanoparticles were compatible with different aqueous matrices, including, diluted and undiluted milk, bovine serum, and river water [44].

Besides the impression of low molecular weight analytes, some authors imprinted MIPs for proteins. However, MIPs for macromolecules can present some problems, such as poor mass transference and low selectivity, due to the high protein complexity, flexible conformation, and large molecular size. To circumvent these limitations, Li et al. (2015) [45] synthesized a magnetic RAMIP to BSA recognition using surface imprinted technique. In this methodology, bindings sites are located near or at the surface of the MIPs, facilitating the mobility of target molecule for into and out the sites. Additionally, 2-methacryloyloxyethyl phosphorylcholine was used as hydrophilic comonomer to prevent the adsorption of non-target proteins. The comonomer decreased the BSA adsorption, when compared with the MIP, but increased the selectivity to BSA, which is an advantage. In relation to the imprinted factors, there was an increment from 1.9 to 8.32 with the addition of 10% of the of 2-methacryloyloxyethyl phosphorylcholine, with a subsequently decreased to 1.44, with the addition of 20% [45]. These results showed that the comonomer was favorable to the BSA extraction until a limit (10%) and, from that, the adsorption was impaired, due to the large amount of 2-methacryloyloxyethyl phosphorylcholine on the RAMIP surface. The target protein selectivity was attested by the selectivity coefficient, which reached up to 1.63, for human albumin and 9.14, for lysozyme [45]. The lowest coefficient value for BSA/human serum albumin is probably because the

high similarity between both molecules. The results demonstrated the suitable application of the surface imprinted technique, using RAMIPs for protein recognition. Additionally, the proposed strategy can be expected to improve the sensitivity of molecularly imprinted sensors for proteins.

A specific type of RAMIPs is obtained by using comonomers with epoxide rings that became hydrophilic after a chemical reaction. This process is performed after the RAMIP synthesis, by treating the polymers with acid solutions. The opening of the comonomer epoxide rings generates a high density of hydroxyl groups, giving rise to the hydrophilic external layer. Figure 2 shows a general synthesis scheme of these RAMIPs.

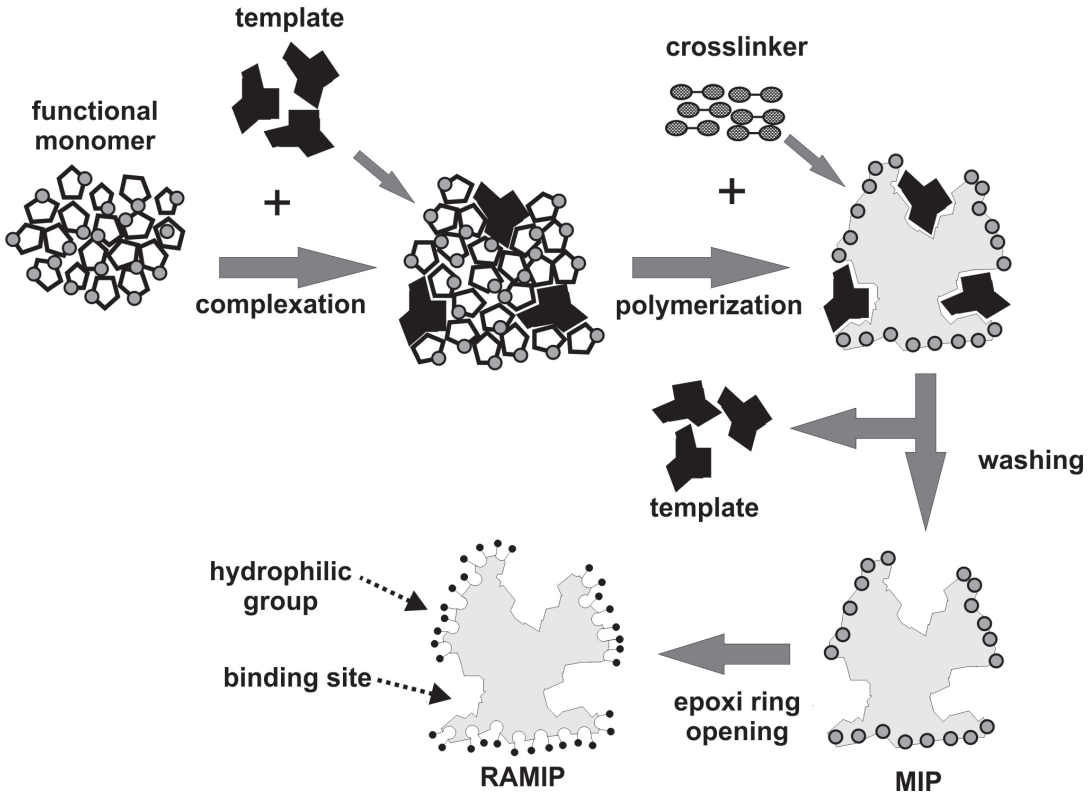

Figure 2. General synthesis scheme of the RAMIPs obtained by the grafting of comonomers that become hydrophilic after a chemical reaction. (Reprinted with permission from De Faria, H. D.; Abrão, L. C. de C.; Santos, M. G.; Barbosa, A. F.; Figueiredo, E. C. Anal. Chim. Acta, 2017, 959, pp 43–65 https://doi.org/10.1016/j.aca.2016.12.047 Copyright© (2017), Elsevier.)

Puoci et al. (2009) [46] developed the first RAMIP using comonomers with epoxide rings. The synthesis occurred by a single step precipitation polymerization method, using glycidyl methacrylate as comonomer. Afterwards, the hydrophilic layer was created by mixing the dried polymers with perchloric acid solution (10% v/v). A protein exclusion rate of 97.5% was obtained after the percolation of a BSA aqueous solution into a solid phase extraction cartridge filled with the synthesized RAMIPs. The material was used for recognition and controlled/sustained release of *p*-acetaminophenol in gastrointestinal simulating fluid.

Other polymers have been obtained by using similar synthesis strategies introduced by Puoci et al. [46], and RAMIPs for ofloxacin [47], 2-methoxyestradiol [48], chloramphenicol [49], organophosphorus [5], and organochlorines [50] were obtained. In all cases, the MISPE technique was employed for the samples preparation, and the authors attested the selectivity and the ability to exclude macromolecules (imprinting factors ranging from 1.5 to 3.1, and BSA exclusion rate ranging from 89.2 to 99.1%).

The modification of MIPs with silicates is also an alternative to obtain RAMIPs. A random free-radical polymerization and sol-gel process was used to synthesize an organic/inorganic hybrid RAMIP. Vinyltrimethoxysilane, tetraethyl-orthosilicate, and 3-glycidyloxypropyltrimethoxysilane were employed as coupling agent, inorganic precursor and comonomer, respectively [51,52]. The hybrid polymer was selective to folic acid and was used in MISPE procedures for the clean-up of milk powder samples. The authors reported that this material presented superior thermal stability, and high hardness when compared to the traditional polymers. However, the capacity to exclude proteins (about 55% of exclusion [52]) was lower when compared to other RAMIPs. Giving the high concentration of proteins in milk samples, 45% of the no-excluded proteins can cause damages in the chromatographic systems, as well as problems in figures of merit. Thus, a pre-treatment was still necessary, in this case. Xu et al. (2010) [8] used silica as support to obtain a RAMIP for the extraction of sulfonamides from milk samples in a column switching LC system. The protein exclusion test was performed using the peak area without column as 100%, and the exclusion rate was over 99%. The material selectivity was confirmed by the imprinted factor of 2.7.

Magnetic materials also improve the dispersive solid phase extraction procedure, turning easier and faster the separation of the material from the sample. In this way, magnetic RAMIPs were synthesized and employed to the extraction of tetracyclines from milk and egg samples. Suitable selectivity for target molecules and protein exclusion capacity above 79.9% were related by the authors [37]. This methodology had a better detectability for tetracycline when compared with a solid phase-extraction using MIPs. Limits of detection of 1.0 and 25.0 μg L^{-1} were respectively obtained. In addition, the process without the RAM employed a previous protein precipitation step using 1 mL of hydrochloric acid and 15 mL of acetonitrile per 5 mL of milk [53].

The RAMIPs can also be used to turn fluorescent probes selective to one specific analyte. Liu et al. (2020) [34] developed a probe based on carbon dots@RAMIP selective to metronidazole. The synthesis was carried out in a single step, using glycidilmethacrylate as comonomer. The probe was used in equine serum samples and the metronidazole was quantified by the variation of fluorescence intensity. The authors reported that coexisting substances in the matrix have no significant influence on the analyte detection.

Both RAMIPs, obtained either by hydrophilic monomers or by monomers that become hydrophilic after a chemical reaction, presented satisfactory selectivity and protein exclusion capacities. A disadvantage can be the need of an additional synthesis step, in the second case. However, the growing use of these comonomers (e.g. glycidilmethacrylate) can be explained due to their lower inference in the polymerization complex, once its oxygen atom has lower capacity to form hydrogen bonds than free hydroxyl groups [46]. Additionally, 3-glycidyloxypropyltrimethoxysilane is consider a cheap silane coupling agent and can be grafted on the surface of silicon dioxide layers through a sol gel-reaction, allowing the obtaining of materials with silicates components [54].

The first RAMIPs synthesis strategies employed a multi swelling procedure, in which various steps previously to the polymerization are needed [7,12,39]. Along the years, the RAMIPs grafted with hydrophilic comonomers are being obtained principally by precipitation polymerization method [5,40,46–50]. This strategy is considered simple and provides particles with uniform size [47], becoming attractive to the researches.

In the case of urine samples [40], which is a matrix with low content of macromolecules, the use of RAMIPs was important to promote selectivity for the target molecules in aqueous media, given that the water interact more with hydrophilic layer than with the selective binding sites [55]. Additionally, the use of hybrid RAMIPs (organic/inorganic) open new possibilities to prepare selective materials, and the *in-situ* polymerization minimizes the difficult of columns uniform packaging. The protein imprinting in RAMIPs [45] turns able their use in sensor to diagnostics and as systems for depletion of abundant proteins. Finally, it is worth to point that most of the described synthesized materials were reusable for a certain period or number of cycles.

The applications of RAMIPs and comparisons in terms of figures of merit, selectivity, and ability to exclude macromolecules are summarized in Table I.

RAMIPS OBTAINED BY HYDROPHILIC COMONOMERS GRAFTING AND COVERING WITH BOVINE SERUM ALBUMIN

In 2013, Figueiredo and co-workers [56] developed the first RAMIP obtained by hydrophilic comonomers (hydroxyethyl methacrylate and glycerol dimethacrylate) grafting, followed by covering with BSA chemically crosslinked. The material was packed in a column and coupled in an online column switching LC system. The procedure was selective to extract chlorpromazine (template, target molecule) from human plasma samples, with a protein exclusion capacity of about 99%. Figure 3 shows a general synthesis scheme of this material.

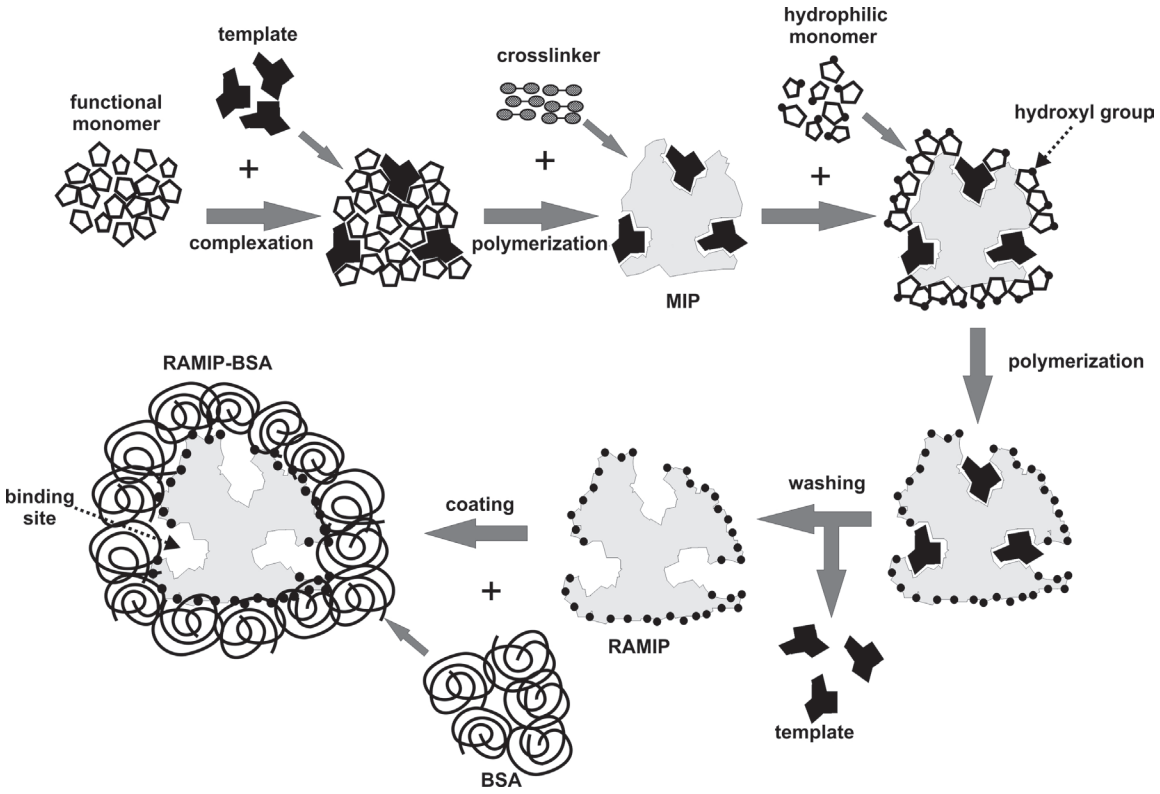

Figure 3. General synthesis scheme of the RAMIP obtained by hydrophilic comonomers grafting and covering with bovine serum albumin. (Reprinted with permission from De Faria, H. D.; Abrão, L. C. de C.; Santos, M. G.; Barbosa, A. F.; Figueiredo, E. C. Anal. Chim. Acta, 2017, 959, pp 43–65 https://doi.org/10.1016/j.aca.2016.12.047 Copyright© (2017), Elsevier.)

The process of the BSA layer incorporation occurs after polymerization and with a clean polymer. Unlike what proceeds with the hydrophilic groups, the BSA layer is not going to build any chemical bond with the polymer surface. In place of this, a cross-linked BSA capsule is formed outside of the MIP particle. Glutaraldehyde has been used as crosslinker reagent, to bond the BSA molecules by reaction with their amino terminal groups (see Figure 4, steps 1 and 2). The high instability of obtained imine groups requests their conversion in amine groups using sodium borohydride as reducing agent (Figure 4, step 3).

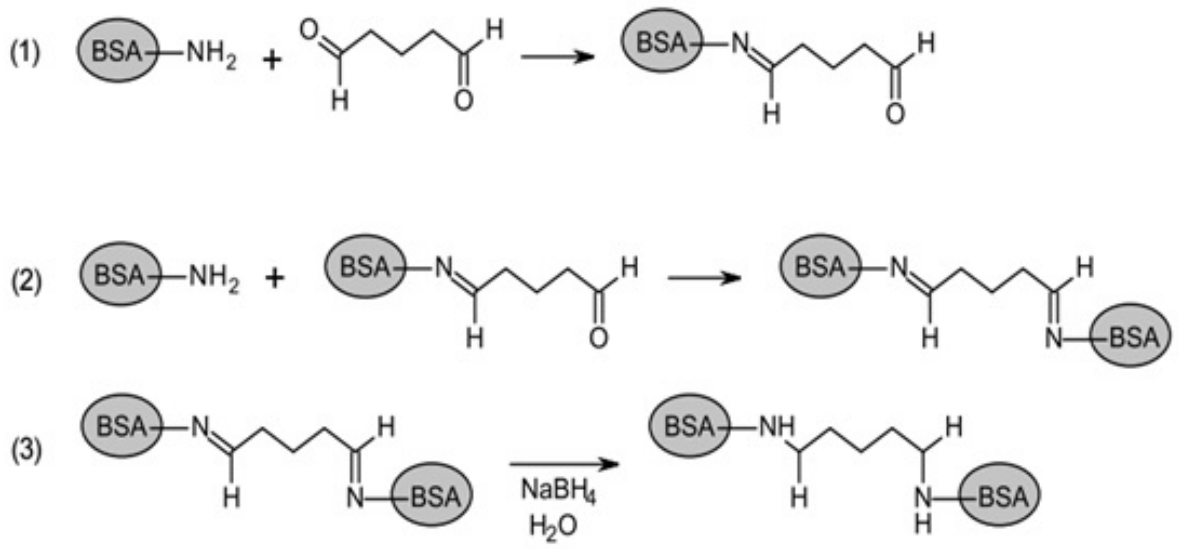

Figure 4. BSA coating reactions steps: (1) interaction between glutaraldehyde (crosslinker) and a BSA molecule; (2) bonding effect from glutaraldehyde on BSA molecules forming the crisscrossed BSA layer; (3) addition of a $NaBH_4$ to reduce imine groups to amine groups.

The physical barrier imposed by the BSA capsule can avoid the direct contact of the macromolecules from the sample with the MIP surface. Additionally, the sample pH needs to be higher or lower than the BSA isoelectric point (4.7) [3,57], in order to maintain both the proteins from the sample and from the BSA layer negatively or positively charged, respectively. In this case, an electrostatic repulsion prevails between the proteins, avoiding their interconnection. This is the principal event in the macromolecule exclusion.

Gomes et al. (2016) [57] investigated the influence of the pH in the protein exclusion capacity in restricted access carbon nanotubes (carbon nanotubes covered with a BSA layer). Despite to be a sorbent different of the MIP, the BSA layer is similar and the behavior in terms of protein exclusion can be compared. A protein exclusion capacity of 85% was obtained for pHs from 2.2 to 3.0 and from 5.6 to 7.0. Meanwhile, this exclusion was about 45% for pHs from 3.5 to 5.2, due to the proximity to the BSA isoelectric point. In other words, in a pH range close to the isoelectric point, the proteins from the BSA layer and the sample are not going to be charged and, consequently, there is no electrostatic repulsion between them. Moreover, this study proved how important is the pH value to promote protein exclusion by electrostatic repulsion using RAMs covered with BSA. Several works about RAMIPs covered with BSA were published, and the most part of them applied pH 7.0 as a better analysis condition [3,56,58,59]. Table I summarizes applications of RAMIPs covered with BSA.

In 2016, da Silva et al. [60] synthetized a RAMIP to catch serotonin reuptake inhibitors from human plasma samples using fluoxetine as a template. Hydroxymethyl methacrylate and glycerol dimethacrylate were added during the MIP synthesis to promote hydrophilic properties to the material and, consequently, decrease the protein retention on the MIP surface. However, the authors also added a BSA cross-linked layer on the MIP surface to reach a higher protein exclusion percentage. The protein exclusion and the adsorption capacity for fluoxetine were >99% and 68.5 mg g^{-1}, respectively. The authors also highlighted the advantages of using the RAMIP with the BSA layer instead of those without the protein covering in a column switching LC system [60]. In the same year, another work applied a similar RAMIP in a column switching LC system, to determine ivermectin in meat samples [61]. Good results in terms of protein exclusion, selectivity, and figures of merit were also obtained.

The RAMIP capacity for macromolecules elimination was also tested by Santos et al. in 2017 [13]. They used a column switching system, coupled to a mass spectrometry to determine tricyclic antidepressants in human plasma. The polymer was synthesized by using glycidyl methacrylate as hydrophilic co-monomer and was covered with BSA layer, differently than the previous works. In order to evaluate the influence of the glycidyl methacrylate and BSA presence, the RAMIPs obtained with the co-monomer and with the co-monomer + BSA layer were compared. The protein exclusion capacities were around 87% and 100%, respectively. This fact indicates that the presence of the BSA external layer was important to improve the RAMIP efficiency [13].

A similar strategy was proposed by Sun et al. (2019) [62] using glycidyl methacrylate and BSA to synthetize a RAMIP and immobilize it on the surface of a mesoporous UiO-66-NH_2 metal-organic framework. The authors called the material UiO-66-NH_2@RAMIP@BSA. Ofloxacin was used as a template and the material was applied to determine fluoroquinolone antibiotics in bovine serum samples. The authors highlighted the use of a hydrophilic bilayer (with hydrophilic groups and the BSA layer) were the better strategy to obtain an excellent protein exclusion efficiency. For that, they compared the protein exclusion capacity of the materials UiO-66-NH_2@MIP (only MIP), UiO-66-NH_2@RAMIP (MIP + hydrophilic groups) and of UiO-66-NH_2@RAMIP@BSA (MIP + hydrophilic groups + BSA) and the percentage obtained were 36.5, 96.0, and 99.4, respectively. Moreover, the adsorption capacity of UiO-66-NH_2@RAMIP@BSA was high as 50.55 mg g^{-1}. Also, the author claimed that the material was better in comparison with others from the literature.

Despite the works involving RAMIPs are mostly applied to protein-rich samples, Oliveira et al. (2019) [38] developed two works about RAMIP to be used in urine samples. In one of their works, they obtained a restricted access mesoporous molecularly imprinted polymer (MMIP) coated with hydrophilic monomers and BSA. This material (called RA-MMIP-HM-BSA) was applied to extract estrone and estriol in pregnant volunteers' urine samples. The analyses were performed by microextraction by packed sorbent followed by a HPLC-UV system. The results to the protein exclusion test were 85% for MMIP, 91% for RA-MMIP-HM, and 99% for RA-MMIP-HM-BSA which means that a material doubly coated can perform better than a material with one hydrophilic layer [38], as already observed in previous described works [59]. Additionally, the obtained quantification limit (10.0 µg L^{-1}) for estriol was lower than the one achieved using a carbon paste electrode modified with ferrimagnetic nanoparticles for voltammetric detection (241.0 µg L^{-1}) [63]; but higher than the one obtained using a multiple monolithic fiber solid-phase microextraction based on a polymeric ionic liquid, for urine preparation and high-performance liquid chromatography with a diode array detector (0.15 µg L^{-1}) [64].

In the second work a magnetic mesoporous molecularly imprinted polymer (MMMIP) coated with a bi-hydrophilic layer (HM+BSA) was synthetized to determine ethinylestradiol and estradiol in urine samples from voluntaries on hormonal contraceptive use. The material called RA-MMMIP-HM-BSA showed a good protein exclusion capacity (99.76%) in comparison with the MMMIP (84.88%) and RA-MMMIP-BSA (96.76%), as well as an excellent efficiency for extracting both studied hormones [36].

RAMIPS OBTAINED EXCLUSIVELY BY COVERING WITH BOVINE SERUM ALBUMIN

RAMIPs can also be obtained by covering of conventional MIPs with BSA layer, without the use of hydrophilic monomers (Figure 5).

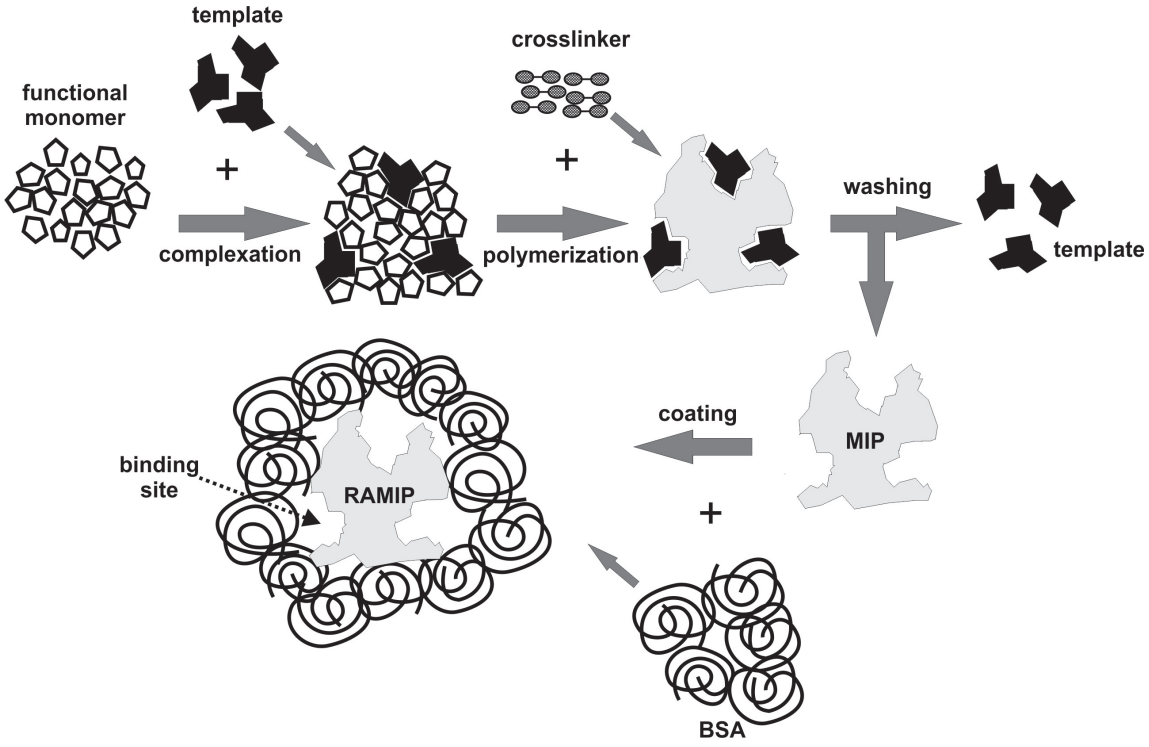

Figure 5. General synthesis scheme of the RAMIP obtained by covering with bovine serum albumin. (Adapted with permission from De Faria, H. D.; Abrão, L. C. de C.; Santos, M. G.; Barbosa, A. F.; Figueiredo, E. C. Anal. Chim. Acta, 2017, 959, pp 43–65 https://doi.org/10.1016/j.aca.2016.12.047 Copyright© (2017), Elsevier.)

This strategy has been presented in some works since 2019 [58,65,66]. Abrão and Figueiredo coated a previous synthesized MIP fiber with BSA, for use in the extraction of benzodiazepines and analogues from plasma samples (Figure 6). According to the authors, this fiber was the first based on RAMIP for SPME. It was synthetized into glass capillary tubes, removed, and treated with BSA according to previous works [56,65]. The solvent volume in the synthesis mixture was an important variable. Low volumes resulted in vitrified and inflexible fibers, whereas high volumes resulted in a powder (non-monolithic polymer). RAMIP fiber remained stable up to 250 °C, being possible it used in gas chromatography with thermal desorption. Fiber selectivity was attested, and its protein exclusion tax was about 98% [65].

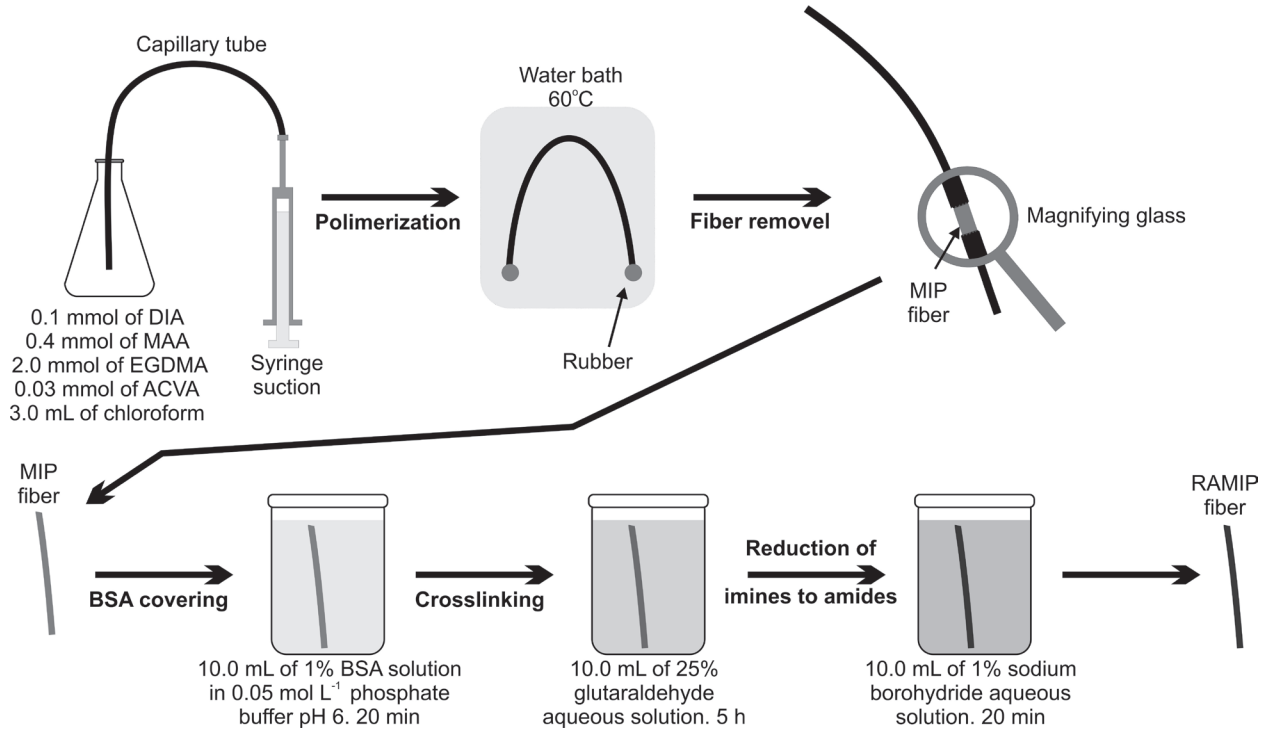

Figure 6. Schematic figure from Abrão and co-workers work [30] with an explanation about their RAMIP fibers synthesis. (The authors are thankful to Royal Society of Chemistry for permission to reproduce this figure from L.C.D.C. Abrão and E.C. Figueiredo, Analyst, 2019, 144, 4320, https://doi.org/10.1039/C9AN00444K)

At the same line, Wang et al. (2019) [65] modified stainless steel fibers to coat them with a RAMIP based on BSA layer to select hesperitin and its metabolites [66]. 3-(tri-methoxysilyl) propyl methacrylate and dopamine were used to promote a bridge between the stainless fiber and the MIP, that was coated with BSA following the Moraes et al. protocol [56,66]. RAMIP fiber was used for an *in vivo* assay. The rats used were kept alive during all the procedure, and the fibers were exposed inside their livers. Because of that, one of the authors' concern was the fiber performance to exclude BSA and bovine hemoglobin as well. The RAMIP could eliminate about 98.96% of BSA and 94.80% of bovine hemoglobin while the MIP fibers eliminated only 59.99% and 47.08%, respectively [66].

Mendes et al. (2020) [58] used a similar strategy to attach a RAMIP on Fe_3O_4 nanoparticles surface [66]. Tetraethyl orthosilicate provides silanol groups which can be attached to a nanoparticle surface and also prepares the metal to receive the 3-(trimethoxysilyl) propyl methacrylate, the second reagent. It is important to highlight that coating nanoparticle surfaces is a strategy to decrease the dipolar attraction among them. This improves their dispersion capacity, protects them from the oxidation process and reduces the nanoparticles aggregation [36,58]. The 3-(trimethoxysilyl) propyl methacrylate offers the methacrylic groups to bind the iron nanoparticle (inorganic material) with the MIP (organic material) during the polymerization. The BSA layer was added after the polymerization step. The magnetic susceptibility of the RAMIP allowed its use in magnetic dispersive SPE of nicotine from biological fluids [58]. The protein exclusion taxes were 79 and 99% for MIP and RAMIP, respectively. RAMIP captured more nicotine (template) than cotinine, lidocaine, and cocaine, attesting the presence of the selective binding sites. However, the selectivity of RAMIP was lower in comparison with MIP (without the BSA layer). This result was explained due to the possible obstruction of some selective binding sites by the BSA layer as well as due to possible unspecific retention of molecules in the BSA structure [58].

To clarify the unspecific retention of analytes in the BSA, Rosa et al. (2019) [67] tested if restricted-access nanoparticles from BSA (obtained by coacervation) would be able to adsorb drugs that have high and low affinity for proteins. The particles presented capacity to adsorb the analytes as well as to exclude proteins, being possible its use in biological sample preparation.

According to all the papers described, covering a MIP with BSA is a good strategy to exclude proteins from non-treated samples such as plasma, serum, blood, and milk, for instance, reaching about 100% of protein exclusion. Moreover, in comparison with the hydrophilic monomers' strategy, the BSA layer can be added any time after the material is synthetized.

Another advantage is that the BSA layer can promote a biocompatibility and stability to be applied on *in vivo* experiments with no damage for the individual. However, in some cases, the BSA layer can prejudice the MIP selectivity and the bidding sites adsorption. So, it is up to the researcher to decide which RAM strategy is the best to apply on the MIP to reach satisfactory results.

RAMIPS OBTAINED BY OTHER STRATEGIES

Hua et al. (2011) [68] used biocompatible carbohydrates to obtain a hydrophilic layer in a RAMIP selective to phenobarbital. The alcoholysis process was performed afterward the synthesis to deprotected the blocking groups of the carbohydrates, introducing the sugar moiety in the external layer of MIP particles. The authors related that uniform sized polymers were obtained and that the recognition sites of phenobarbital were unchanged even after the surface modification. In addition, more than 85% of BSA could be recovery from the surface modified material, attesting its capacity to exclude macromolecules [68]. However, it is important to point out that other RAMIPs have presented better protein exclusion capacities [37,39,48,58,60,62,65].

RAMIPS COMPARISION: APPLICATIONS AND CHARACTERISTICS

As demonstrated by the examples, RAMIPs obtained by hydrophilic comonomers grafting, protein covering, or both strategies were suitable for the biological matrices sample preparation. The materials were able to selectively extract the analyte(s) and exclude macromolecules concomitantly. In addition, the authors did not report difficulties in the synthesis repeatability or problems with clogging when column switching systems were used. The comparison between the applications of RAMIPs and the characteristics from each type of them can be found in Tables I and II, respectively.

Table I. Applications of RAMIPs

RAMIPs obtained by hydrophilic comonomers grafting										
Monomer(s)	**Material**	**Analyte(s)**	**Method**	**Sample(s)**	**LOD**	**LOQ**	**Protein exclusion rate**	**IF**	**Reusability**	**Ref.**
GMA*	RAM-MIM	Organophosphorus	MISPE-GC-FPD	Honey	0.5-1.9 μg L^{-1}	-	-	3.1	-	[5]
GMMA GDMA	RAMIP	(S)-naproxen	CS -LC-UV	Serum	-	-	≥ 96.8	-	-	[7]
GMA*	RAMIP	Sulfonamides	CS -LC-UV	Bovine milk	0.2-0.8 μg L^{-1}	0.7-2.7 μg L^{-1}	≥99.0	2.7	-	[8]
GMMA	RAMIP monolithic column	Clenbuterol	CS-LC-UV	Human serum	0.7 μg L^{-1}	2.0 μg L^{-1}	≥ 90.0	7.2	15 months	[9]
GMMA GDMA	RAMIP	Ibuprofen	CS -LC-UV	Rat plasma	50 μg L^{-1}	200 μg L^{-1}	~100	-	~500 injections	[12]
GMA*	CDs@ RAMIPs probe	Metronidazole	Fluorescence intensity	Equine serum	17.4 μg kg^{-1}	-	95.5	-	-	[34]
GMA*	M- RAMIP	Tetracyclines	MSPE-LC-UV	Milk and egg	1.03-2.67 μg kg^{-1}	3.46-8.97 μg kg^{-1}	99.4	~2.3	At least 6 times	[37]
GMMA GDMA	RAMIP	Beta blocker drugs	CS LC-UV	Rat plasma	-	12.5 μg L^{-1}	≥ 97.0	-	-	[39]
HEMA GDMA	RAMIP	Beta blocker drugs	CS -LC-MS/MS	Urine	0.1-1.0 μg L^{-1}	1.0-3.0 μg L^{-1}	-	~1.2	~150 cycles	[40]
GDMA	RAMIP	Methyl, ethyl, propyl, butylparaben	in-tube SPME-LC-MS/MS	Breast milk samples	-	3-10 μg L^{-1}	≥ 67 (exclusion of endogenous compounds)	8.0-14.0	-	[41]
PHEMA	RAMIP	2,4-dichlorophenoxyacetic acid (1 study) and propranolol (2 study)	-	River water, milk, and bovine serum	-	-		-	-	[44][a]
MPC	M-RAMIP	BSA	-	-	-	-	-	8.32	-	[45][b]
GMA*	RAMIP	*p*-acetaminophenol	-	-	-	-	95.7	-	-	[46][c]
GMA*	RAMIP	Ofloxacin	MISPE-LC-UV	Milk	-	-	92.4	~1.8	10 cycles	[47][d]
GMA*	RAMIP	2-methoxyestradiol	MISPE-LC-UV	Rat plasma	20 μg L^{-1}	60 μg L^{-1}	93.5	~1.5	9 cycles	[48]

Table I. Applications of RAMIPs (Continuation)

RAMIPs obtained by hydrophilic comonomers grafting										
Monomer(s)	**Material**	**Analyte(s)**	**Method**	**Sample(s)**	**LOD**	**LOQ**	**Protein exclusion rate**	**IF**	**Reusability**	**Ref.**
GMA*	RAMIP	Chloramphenicol	MISPE-LC-UV	Bovine serum	1.2 μg L^{-1}	-	≥92.6	~3.0	At least 8 times	[49]
GMA*	RAMIP	Organochlorides	MISPE-GC-EDC	Pork	1-2 μg kg^{-1}	5. μg kg^{-1}	97.3	~2.5	At least 4 cycles	[50]
GPTMS*	RAMIP/ SiO_2 hybrid material	Folic acid	MISPE-LC-PDA	-	-	-	-	~1.2	At least 120 times	[51]
GPTMS*	RAMIP/ SiO_2 hybrid material	Folic acid	MISPE-LC-DAD	Milk powder	1.45 μg L^{-1}	4.83 μg L^{-1}	53.3	-	At least 100 times	[52]
HEMA GDMA	RAMIP	Oxprenolol	-	Human plasma	-	-	87.8	~1.2	-	[59]
GMA*	RAMIP	Caffeine	-	-	-	-	≥73.9	-	At least 5 times	[69][e]
RAMIPs obtained by comonomers and BSA covering										
Monomer(s)	**Material (named according to the authors)**	**Analyte(s)**	**Method**	**Sample(s)**	**LOD**	**LOQ**	**Protein exclusion rate**	**IF**	**Reusability**	**Ref.**
GMA BSA	RAMIP	Tricyclic antidepressants	CS-LC-MS/MS	Human plasma	-	15 μg L^{-1}	~100	-	-	[13]
HEMA GDMA BSA	M-RAMIP	Ethinylestradiol and estradiol	MSPE dispersive-LC-UV	Human urine	-	80 μg L^{-1}	99.76	-	At least 5 times	[36]
HEMA GDMA BSA	M-RAMIP	Estrone and estriol	MEPS-LC-UV	Human urine	-	100 μg L^{-1}	99.0	-	Only 1 use	[38]
HEMA GDMA BSA	RAMIP	Chlorpromazine	CS-LC-UV	Human plasma	-	30 μg L^{-1}	99.0	3.8	90 cycles	[56]
HEMA GDMA BSA	RAMIP	Oxprenolol	-	Human plasma	-	-	98.7	~1.2	-	[59]

Table I. Applications of RAMIPs (Continuation)

RAMIPs obtained by comonomers and BSA covering										
Monomer(s)	**Material**	**Analyte(s)**	**Method**	**Sample(s)**	**LOD**	**LOQ**	**Protein exclusion rate**	**IF**	**Reusability**	**Ref.**
HEMA GDMA BSA	RAMIP	Serotonin reuptake inhibitors	CS-LC-UV	human plasma	-	20 μg L^{-1}	~100	~1.5	-	[60]
HEMA GDMA BSA	RAMIP	Ivermectin	CS-LC-UV	Meat	30 μg kg^{-1}	50 μg kg^{-1}	~100	-	-	[61]
GMA BSA	UiO-66-NH_2@ RAMIP@ BSA	Ofloxacin and enrofloxacin	MISPE-LC-UV	Bovine serum	15.6 μg L^{-1}	-	99.4	~3.1	-	[62]
RAMIPs obtained by BSA covering										
Protein	**Material**	**Analyte(s)**	**Method**	**Sample(s)**	**LOD**	**LOQ**	**Protein exclusion rate**	**IF**	**Reusability**	**Ref.**
BSA	M-RAMIP	Nicotine	MSPE dispersive - LC-UV and GC-MS	Human plasma	-	-	99.0	~1.4	50 extractions cycles	[58]
BSA	RAMIP	Oxprenolol	-	Human plasma	-	-	87.3	~1.2	-	[59]
BSA	RAMIP fiber	Benzodiazepines	SPME-LC-DAD	Human plasma	5-30 μg L^{-1}	15-100 μg L^{-1}	98.0	-	-	[65]
BSA	RAMIP fiber	Hesperetin and its metabolites	SPME - UPLC-MS/MS	Rat livers in vivo	20 μg L^{-1}	50 μg L^{-1}	≥94.8	~3.1	-	[66]
RAMIPs obtained by other strategies										
Monomer(s)	**Material (named according to the authors)**	**Analyte(s)**	**Method**	**Sample(s)**	**LOD**	**LOQ**	**Protein exclusion rate**	**IF**	**Reusability**	**Ref.**
Glycomonomer	glyco-MIP	Phenobarbital	MISPE-LC-UV	Calf serum	-	-	≥ 85.0	-	-	[68]

*The comonomer has epoxide rings that become hydrophilic after a chemical reaction.

Continuation of Table I footer:
BSA: bovine serum albumin; CD: carbon dot; CS: column switching; DAD: diode array detector; EDC: eletron capture detector; FPD: flame photometric detector; GC: gas chromatography; GDMA: glycerol dimethacrylate; GMA: glycidilmethacrylate; GMMA: glycetol monomethacrylate; GPTMS: 3-glycidyloxypropyltrimethoxysilane; HEMA: hydroxymethylmethacrylate; IF: imprinting factor (adsorption capacity MIP/adsorption capacity NIP); LC: liquid chromatography; LOD: limit of detection, LOQ: limit of quantification; MEPS: microextraction by packed sorbent; MIP: molecularly imprinting polymer; MISP: molecularly imprinted solid phase extraction; MPC: 2-methacryloyloxyethyl phosphorylcholine; M-RAMIP: restricted access media-magnetic molecularly imprinted polymer; MS: mass spectrometry; MSPE: magnetic solid phase extraction; NIP: non imprinted polymer; PDA: photodiode array; PHEMA: poly(2-hydroxyethyl methacrylate); RAM-MIMM: restricted access materials–molecularly imprinted magnetic micro- spheres; RAMIP: restricted access molecularly imprinting polymer; SPE: solid phase extraction; SPME: solid phase microextraction; UV: ultraviolet-visible detector.

[a]The paper focus in the material compatibility with the different complex matrices.
[b]The paper aimed to study the adsorption capacity and selectivity of the synthesized microspheres.
[c]The paper focus in the recognition and controlled/sustained release. Initial investigations to drug delivery systems.
[d]The paper evaluated the best synthesis conditions to obtain the final material.
[e]The paper compares different synthesis conditions and does not focus on the material application.

Table II. Comparison of the characteristics of each type of RAMIP

Characteristics	**RAMIPs obtained by**		
	Hydrophilic comonomers	**Protein covering**	**Both strategies**
Advantages	> The hydrophilic comonomers contributed to better molecular recognition in aqueous media [59]. > The comonomers can be included in the polymerization flask, Thus, some RAMIPs are obtained with a one-step synthesis.	> The external BSA layer confers more biocompatibility to the material. > It was already employed in in vivo studies [66]. > Any synthesized MIP can be functionalized with the BSA layer. Thus, MIPs can be transformed into RAMIPs even after their synthesis.	> A higher protein exclusion rate can be obtained due to the combination of the protein exclusion mechanisms (physical barriers, hydrophilic layer, and electrostatic repulsion).
Disadvantages	> The presence of the comonomers can interfere in the formation of the pre-polymerization complex [46]. > The opening ring reaction, when necessary, demands a synthesis step of 24h.	> Limited pH range for protein exclusion. > The BSA can interact non-specifically with some drugs present in the samples [67].	> Limited pH range for electrostatic repulsion mechanism. > Demand more synthesis steps to the functionalization.

Table II. Comparison of the characteristics of each type of RAMIP (Continuation)

Characteristics	RAMIPs obtained by		
	Hydrophilic comonomers	**Protein covering**	**Both strategies**
Exclusion pH range	> Most of the authors did not report that the protein exclusion rates were impaired by the sample pH. One exception in the work of Sanbe and Haginaka (2003) [39].	> Do not have a suitable protein exclusion rate in the pH range of 3.5 to 5.2, due to the proximity of the BSA isoelectric point [57].	> Do not have the electrostatic repulsion mechanism in the pH range of 3.5 to 5.2.
Non-specific interactions	> Some authors suggested that the hydroxyl groups can be bound non-specifically with some compounds beyond the analyte(s) [3].	> It was demonstrated that the BSA can extract some drugs from untreated biological samples [67]. Therefore, non-specific interactions can occur.	> Non-specific interactions can occur in both functionalization layers.
Reusability*	> Eleven of the exemplified papers reported the reusability of this type of RAMIP. The number of extraction cycles using the same material without losses in efficiency, ranged from 4 to 500.	> Just one of discussed papers reported the reusability of this type of RAMIP. The material was efficiently used in 50 extraction cycles.	> Three works brought information about the reusability of this type of RAMIP. Two papers related that the material could be used in various extraction cycles (5 and 90), and the other one reported that the RAMIP was not reusable, being used just one time.

* Not all the papers used in this review reported if the material was reusable or not as well as already presented in Table I.
BSA: bovine serum albumin.

CONCLUSIONS

RAMIPs are a suitable alternative to complex sample preparation procedures, because of their high selective, ability to exclude proteins and resistant to high temperatures, pHs, and solvents. According to the papers presented in this review, the average of proteins exclusion percentages for each strategy were 89.60% (53.3-100%), 94.75% (87.3-99%) and 99.48% (98.7-100%) to MIPs surface modified with hydrophilic comonomers, bovine serum albumin and both, respectively. These results corroborate with the study of Santos et al [14], in which the MIPs covering with double layer (hydrophilic monomers + BSA) are better in terms of protein exclusion. Additionally, the BSA-based RAMIPs are promising as materials for biomedical applications, due to their biocompatibility and low inability to generate immune response. However, the presence of BSA layer can result in loss of selectivity, probably due to the obstruction of selective binding sites, as well as by unselective binding of molecules in the BSA structure.

Owing to these results, all the demonstrated strategies to convert MIPs into RAMIPs result in selectively materials able to exclude macromolecules from untreaded complex samples. Moreover, RAMIPs are promising materials to be used in different sample preparation techniques, sensors, proteins depletion systems, and routine analyses, and commercial materials such as columns and fibers can be available in the future.

Conflicts of interest

Authors declare that there is no conflict of interest.

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ARTICLE

Methodology for Preconcentration and Determination of Silver in Aqueous Samples using Cloud Point Extraction

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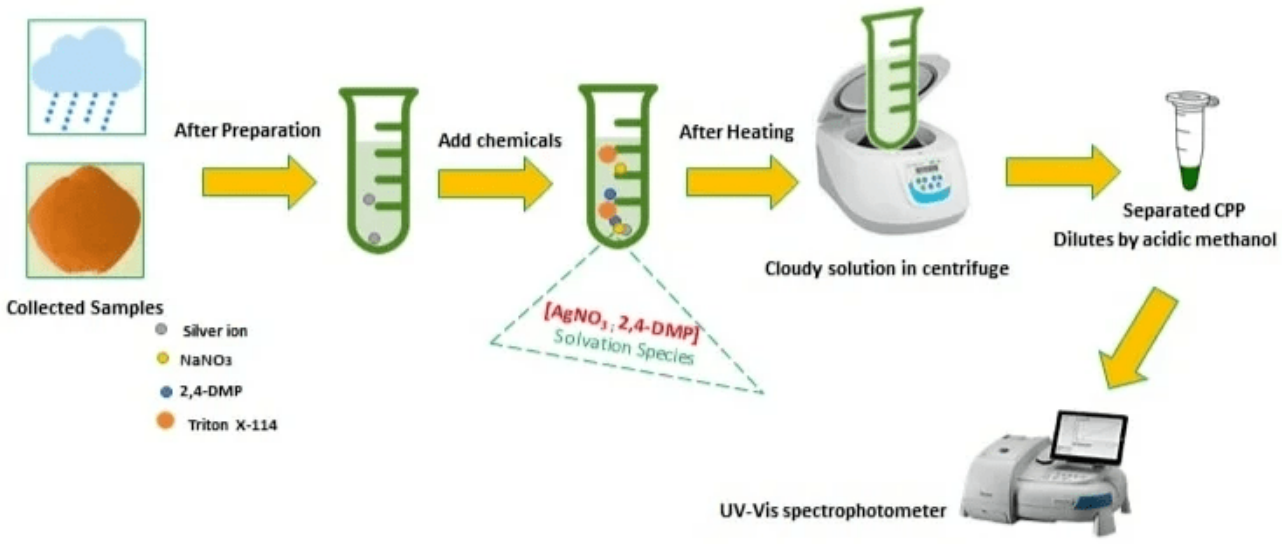

For the selective extraction of silver, a cloud point extraction (CPE) procedure was developed. After synthesizing the solvation species through the reaction of silver ions with 2, 4-dimethyl pentane-3-one (2,4 DMP), the salting-out agent (0.4 mol L^{-1} $NaNO_3$) was added at 35 °C and, after 10 min, Triton X-114 was used to separate silver ions from aqueous solution. The type and quantity of salting-out agent, silver ion, temperature, heating time, and surfactant volume were all examined as important factors determining the CPE. The analytical curve in the 0.1-100 µg L^{-1} Ag range was straight at optimal conditions. The detection limit (LOD), quantification limit (LOQ), and enrichment factor (E) were 0.05 µg L^{-1}, 0.15 µg L^{-1}, and 200, respectively. The relative standard deviation (RSD) was estimated as 0.2-3.9% (n = 5) in relation to 1, 40,

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and 80 µg L^{-1} Ag. Flame atomic absorption spectrometry and spectrophotometry exploiting dithizone were used to assess the CPE accuracy. The proposed approach was then applied to river water, rain water and sand samples.

Keywords: cloud point extraction, salting out effect, silver, solvation system, solvent extraction.

INTRODUCTION

Non-polluting and accurate methods for trace element determination are desirable [1,2]. To remove and extract tiny quantities of elements, a variety of approaches have been utilized [3-5], including solid-phase extraction [6,7], liquid-phase extraction [8-12], and cloud point extraction (CPE) [13-16]. Researchers are interested in CPE because it follows green chemistry principles. After all, the chemicals utilized are tiny and non-toxic. CPE also low-cost, accurate, efficient, and quick [17-22]. Achieving a critical micellar concentration (CMC) requires a cloud point temperature that is just right. Cloud point phase (CPP) (non-hydrophilic, with large micelle quantity and tiny volume) and an aqueous phase (large volume, hydrophilic nature) are produced as a result. To create hydrophobic complexes, target components are combined with an organic chelating reagent, which is further transported towards CPP [23-25]. Solvation molecules have hydrophobic properties and can be equally distributed in the micelle phase [13]. In the condition of salting-out processes, nitrate and hydrophobic reagents [M $(NO_3)_x$; b (organic reagent)] are combined. Many agents, including aldehydes, ketones, asters, etc. Generally, the salting-out process is caused by ions desorbing from the hydrophilic portions of micelles, enhancing inter-micelle attraction and, as a result, causing surfactant molecules to precipitate. The salting-out effect was absence and presence of $NaNO_3$, KNO_3, and other chemicals. The solution ionic strength is mostly influenced by nitrate salts, which reduce the hydration shell surrounding metallic ions and aid in the transport of ionic species to CPP [26-28].

Silver is antibiotic, thermally and electrically conductive. Silver compounds are commonly utilized in water purification filters, medical materials, food, and advanced materials. Because the amount of silver in the crustal ground is low, water naturally includes trace amounts of it. As a result, a particularly sensitive methodology should be utilized for silver detection in water for evaluating the toxicity effect in bio-organisms [30,31]. In this instance, an ultrasound-assisted cloud point extraction procedure was implemented. Silver was extracted from samples using copolymer and Fe_3O_4 nanoparticles at pH 5.0, and spectrophotometrically detected. The detection limit (LOD) was 0.85 µg L^{-1} [32].

The primary goal of this study was to extract and determine silver in water and sand samples using CPE. The surfactant, chelating, and salting-out reagents were Triton X-114, 2, 4-DMP and $NaNO_3$, respectively. The factors that influence CPE were examined.

MATERIALS AND METHODS

Reagents and solutions

All the substances (with purity ranging from 99.0 to 99.2%) used in this study were from Merck, Darmstadt, Germany. Deionized water was used in the preparation of all samples and solutions. A standard solution 1.00 g L^{-1} Ag was prepared by dissolving $AgNO_3$ with water in the presence of 1 mL of HNO_3. Then, the solution was transferred to a suitable volumetric flask and the volume was completed to the mark using water. The 2,4-DMP solution (1×10^{-2} mol L^{-1}) was prepared in water which contains drops of surfactant. The $NaNO_3$ solution (1.00 mol L^{-1}), was prepared in water. The dithizone solution (1×10^{-2} mol L^{-1}) was prepared in CCl_4.

Instrumentation

A water bath model Cambridge, England, (WNB7-45), (±0.0001 g precision) A and D company, Japan, a UV-Vis Biochrom spectrophotometer Libra, S60 (Cambridge, England), and an AA 6800 Shimadzu flame atomic absorption spectrophotometer (Kyoto, Japan) with a silver hollow cathode lamp were employed.

Test methodology

An aliquot comprising 25 mL of an aqueous solution, 100 µg L^{-1} silver ions, 0.4 mol L^{-1} $NaNO_3$, $1x10^{-4}$ mol L^{-1} 2,4-DMP solution, and 0.5 mL of Triton X-114 were mixed and deionized water was diluted to the line of the 50 mL centrifuge tube. This was done in a water bath to accelerate the formation of the micelle at 35 °C for 10 min. The mixture was centrifuged for 12 minutes at 4000 rpm and then refrigerated for 4.0 minutes in the freezer. Then, the hydrophobic phase was separated and 5 mL of an acidic methanol solution containing nitric acid (1.0 mol L^{-1}) was added. Afterward, the absorbance was measured at λ_{max} = 325 nm. A blank solution was prepared and analyzed in the same way.

To extract silver ions from aqueous solutions, the dithizone (H_2Dz) methodology [41] was employed. The 0.001% H_2Dz in CCl_4 was added to a silver solution containing HNO_3 until the concentration reached 1-2 mol L^{-1}, then shacked for 5 minutes. The step was fivefold repeated. The color of an aqueous solution after the last amount of H_2Dz has been added should not change. Separation of the silver-containing aqueous layer by lowering the pH to 4-5 with ammonia, then removing the silver in CCl_4 with 0.001% H_2Dz. To exclude any residual H_2Dz from the sample, a dilute ammonia solution (2 drops of conc. NH_3 solution in 25 mL of water) was utilized [42]. The analytical curve was used to determine the amount of silver ions. Equation 1 gives the distribution ratio (D). Log D data is placed in all of the graphics to provide more structured and wide data [33].

$$D = \frac{[Ag(I)]\ CPP}{[Ag(I)]\ aq} \qquad (1)$$

Sample Preparation

Water samples

All water samples were filtered through a 0.45 µm Millipore filter and stored in a polyethylene container for future application after being acidified to pH 2.0 with HNO_3 to reduce silver ions adsorption on the container surface.

Sand samples

The sand samples were gathered from several sites throughout the Najaf desert in Iraq. The samples were dehydrated and mashed at 90 °C for 15 hours before being put through a 130 grit filter. In a Teflon bottle, 5 mL of each HNO_3, H_2O_2, and H_2O of the measuring sand were added to 1.5 g and destroyed using a microwave digestion oven with an application cooking program [25]. The extra H_2O_2 was then neutralized using 20 mL of H_3BO_3 (5% w/v). After that, the mixture was filtered, and the volume was filled up to 50 mL. Masking reagents (KCN and $K_2S_2O_3$) were applied to samples at a concentration of 0.1 mol L^{-1} [25].

RESULTS AND DISCUSSION

The spectrophotometric investigation involved 50 mL of aqueous solutions containing 100 µg L^{-1} Ag(I), 0.4 mol L^{-1} $NaNO_3$, 1.0% Triton X-114 and $1×10^{-4}$ mol L^{-1} 2,4- DMP. The heating temperature was maintained as 35 °C for 10 minutes. The extracted components were spectrophotometrically evaluated (λ_{max} = 325 nm). Effects of the main involved factors were investigated for dimensioning the proposed procedure.

Influence of the sodium nitrate concentration

Aqueous solutions (50 mL) including 100 µg L^{-1} silver ions were handled with different quantities of salting-out ($NaNO_3$) in the range of (0.1-1 mol L^{-1}) according to the test methodology at 40 °C for 15 minutes. After treating the Ag(I) aqueous solutions as above described, it was realized that 0.4 mol L^{-1} was the most effective concentration of the salting-out agent ($NaNO_3$). This concentration corresponded to the fastest formation equilibrium with the development of solvation species. This was not attained with a concentration just under or higher than the optimal value. Equation 2 depicts the structure of solvation species. Figures 1 and 2 show the influence of $NaNO_3$ concentrations on salting-out.

$$Ag^{+} + NO_3^{-} + 2,4\text{-DMP} \rightleftharpoons [AgNO_3, 2,4\text{-DMP}] \quad (2)$$

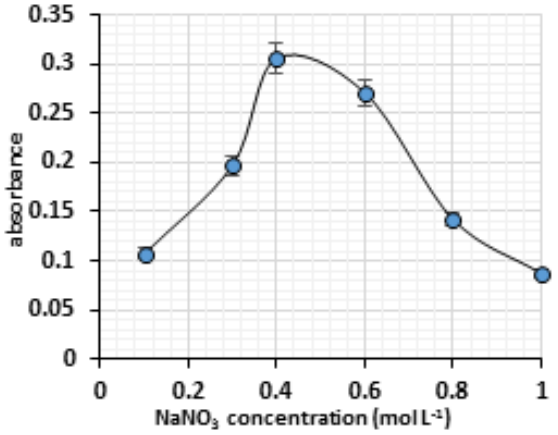

Figure 1. Influence of the $NaNO_3$ concentration on the extraction efficiency.

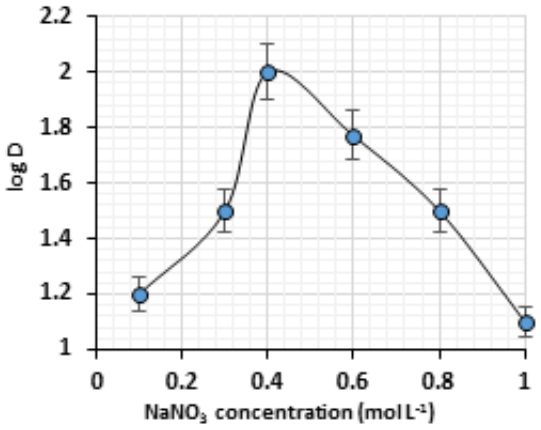

Figure 2. Influence of the $NaNO_3$ concentration on the D.

Influence of the concentration of the silver ions

Figures 3 and 4 display the effectiveness of silver ion solutions at different concentrations (0.1-100 µg L^{-1}) including 0.4 mol L^{-1} $NaNO_3$, and completing the task according to the applied methodology. The findings show that silver ions act a parameter that influences the thermodynamic equilibrium of the synthesis solvation species, as well as the transport to the micelle. The optimal concentration amount of silver was then found as 100 µg L^{-1} and this result is in agreement with references [33,41].

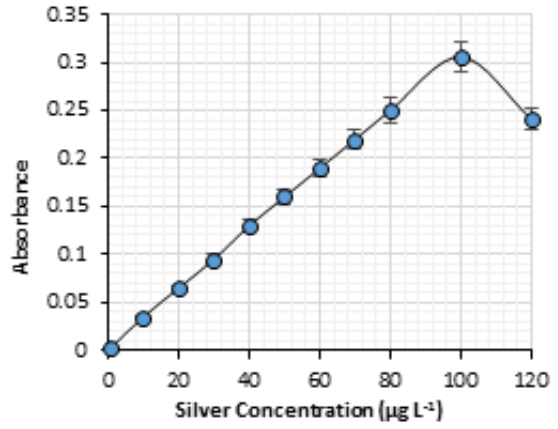

Figure 3. Influence of the silver ion concentration on the absorption of solvation species.

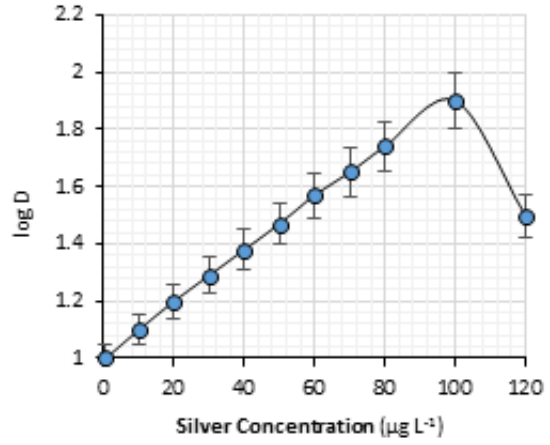

Figure 4. Influence of the silver ion concentration on extraction efficiency.

Influence of the ketone nuture

Five different ketones were tested as organic agents (1×10^{-4} mol L^{-1}) for the formation of solvation species with silver nitrate separation according to the test methodology. The results in Table I show a different extraction efficiency using different ketones. The 2,4-DMP appears to be the best value for D and absorbance depends on chemical structure [34]. It increases the lipophilic properties of solvation species, which allows transfer to the CCP for easy separation. It permits the preconcentration at a specific

temperature with the presence of a salting-out impact. 2,4-DMP was then selected to be used in the following studies.

Table I. Effects of various ketones on the extraction efficiency and distribution ratio (D)

Ketones	λ_{max} nm	Absorbance	D
2,4-DMP	325	0.342	95.460
Acetone	256	0.105	43.461
Acetophenone	291	0.201	54.621
Methyl Iso Butyl Ketone	292	0.247	80.752
Propanone	297	0.233	75.500

Influence of the temperature and heating time

In CPE, temperature and heating time are important parameters. Figure 5 refers to a thorough process for extracting silver ions at various temperatures. The ideal temperature was selected as 35 °C. Thus, it was suitable for forming a hydrophobic CPP and provided enhanced extraction efficiency for silver ion removal [33,34]. Enthalpy, entropy and Gibbs free energy were then determined as ΔH_{ex} = 0.0959 kJ/mol, ΔS_{ex} = 200.668 J/mol and ΔG_{ex} = -77.655 kJ/mol.

Then, under selected conditions for all chemical concentrations and temperatures, the CPE procedure was used to determine various heating times ranging from 5 to 30 minutes (Figure 6). The selected heating time was 10 minutes. Heating time offers the required energy to receive the right rate of thermodynamic equilibrium for the synthesis of solvation species, and higher extraction efficiency [14,15].

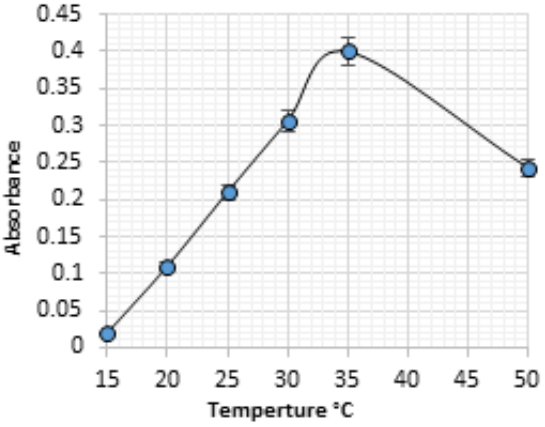

Figure 5. Influence of the temperature on extraction efficiency.

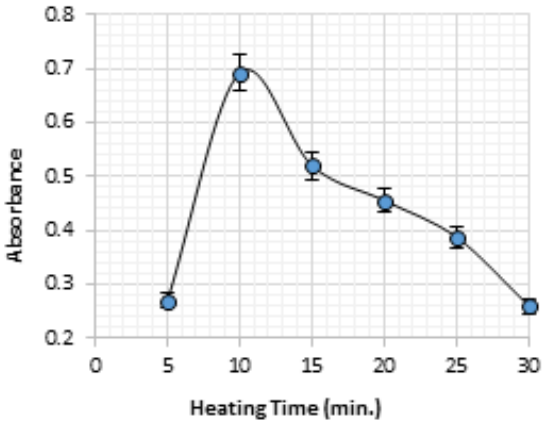

Figure 6. Influence of the heating time on extraction efficiency.

Influence of the Triton X-114 volume

Some features of the non-ionic surfactant Triton X-114, such as lipophilicity and ability to endure the electrolytes caused by corresponding silver ions, enabled the CPE and analytical selectivity for silver ions [33,34]. Figures 7 and 8 show the influence of Triton X-114 (1.0% v/v) at various volumes (0.1-1.0 mL). Better results, such as a high extraction efficiency, were obtained with 0.5 mL of Triton X-114, as the critical micellar concentration (CMC) was surpassed.

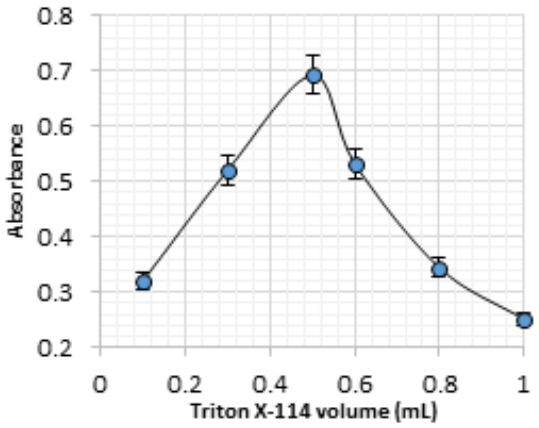

Figure 7. Influence of the Triton X-114 volume on the absorbance of extracted species.

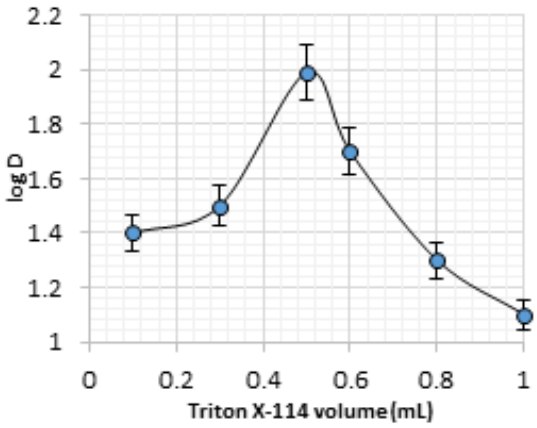

Figure 8. Influence of the Triton X-114 volume effect on D.

Comparison with other procedures

Some parameters of the proposed CPE, such as organic ligands, LOD and E were compared with those related to other strategies for preconcentration of silver ions using Triton X-114 as a surfactant [25, 33-44]. Table II summarizes the results. It can be seen that the LOD of the present method is lower, in relation to other procedures [35,37,39,45], whereas the best LOD was obtained by the CPE method [36]. The large and excellent E is probably due to the higher extraction efficiency of silver ions.

Table II. Comparison of Procedures for Silver Determination using CPE and Triton X-114

Organic ligands	LOD	E	References
2,4-DMP	0.05 μg L^{-1}	200	this work
6-(4-BrPAA)	0.0054 μg mL^{-1}	12.4	[44]
AgNPs	1.50 ng L^{-1}	-	[38]
APDC	0.42 μg L^{-1}	20	[35]
BIES	1.40 ng mL^{-1}	42	[43]
BIMPI	10.00 μg L^{-1}	35	[37]
BMAA	0.43 ng mL^{-1}	-	[34]
DDTC	1.00 μg L^{-1}	24	[45]
DDTC	0.30 ng mL^{-1}	33	[36]
H_2Dz	0.56 ng mL^{-1}	43	[40]
HPCTS	0.08 μg L^{-1}	46	[25]
MBT	2.20 ng mL^{-1}	-	[33]
PAR	6.00 μg L^{-1}	-	[39]

6-(4-BrPAA) 6-(4-bromo-phenylazo)m-anisidine, AgNPs silver nanoparticles, APDC ammonium pyrrolidine dithiocarbamate, BIES bis((1H-benzo [d] imidazol-2yl)ethyl) sulfane, BIMPI 2-((2-((1H-benzo[d]imidazole-2-yl)methoxy)phenoxy)methyl)-1H-benzo[d]imidazol, BMAA bis(2-mercaptoanil) acetylacetone, DDTC diethyl dithiocarbamate, H_2Dz Dithiazone, HPCTS 4-(p-chlorophenyl)-1-(pyridin-2-yl)thiosemicarbazide, MBT 2-mercaptobenzothiazole, PAR 4,2 pyridylazo resorsinol.

Analytical Figures of Merit

Limit of detection, limit of quantification, enrichment factor, precision, and linearity of the analytical curve were also examined, and the results are summarized in Table III. Analysis of this table reveals that silver recoveries were quantitative (> 98.7%), so the analytical accuracy was good. For C = 30 μg L^{-1}, RSD was 1.5%, based on five-fold assaying times of a 50 mL of solution. Under ideal conditions, the linearity was notes within 0.1 and 100 μg L^{-1}. The standard error of detection limit detection limit for the blank solution (3.3 S/b) was determined as 0.05 μg L^{-1}. Instead, the E was estimated as 200, by using the ratio of the slopes of the analytical curve after and before CPE.

Table III. Analytical Figures of Merit for CPE

Parameters	CPE
Enrichment factor	200
Limit of Detection	0.050 μg L^{-1}
Limit of Quantification	0.15 μg L^{-1}
Linearity	0.1-100 μg L^{-1}
Recovery	99.2-107.1%
Relative Standard Deviation (n = 5)	0.2-3.9%

CPE conditions: 50 mL sample, 1% (v/v) Triton X-114 (0.5 mL), 0.4 mol L^{-1} $NaNO_3$, 1×10^{-4} mol L^{-1} 2,4-DMP, temperature 35 ºC and time 10 min.

Applications

The proposed innovation was applied to silver determination in natural waters and sand tests from Al-Najaf city. Accuracy was assessed by analyzing samples already run by other methods. CPE was used to allow the estimation of recoveries after addition of known amounts of silver ions to the samples. The results (Table IV) demonstrate the CPE ability to extract and determine silver ions in water tests with various materials in the appropriate conditions, as well its ability to isolate silver ions. Results of sliver of determination by two classical methods (FAAS and UV-Vis spectrophotometry) are also presented. Table V summarizes the obtained results.

Table IV. Silver determination in river and rain water samples three times

Tests	Added (μg L^{-1})	CPE			FAAS (μg L^{-1})	Dithizone (μg L^{-1})
		Found (μg L^{-1})	RSD (%)	Recovery (%)		
Shatt Al-Kufa water	-	N.D.	-	-		
	2.00	1.63	2.10	99.22	2.10	1.90
	4.00	4.11	0.23	107.10	4.02	3.85
	6.00	5.88	1.47	99.30	6. 01	6.10
Rainwater[b]	-	N.D.	-	-	-	-
	2.00	2.14	0.12	98.50	2.00	1.78
	4.00	4.40	1.23	102.50	4.80	4.82
	6.00	6.06	1.50	100.60	6.39	6.31

[b]Collected at Al-Najaf city, Iraq (December 2020). N.D. not detected.

Table V. Silver determination in sand samples by the proposed innovation

Samples*	mean ± RSD (n = 5) μg L^{-1} Ag
1	3.02 ± 3.40
2	5.88 ± 1.60
3	3.38 ± 1.40
4	4.25 ± 2.90
5	5.01 ± 0.70
6	3.33 ± 2.00

*These sand samples were collected from various locations in the Al-Najaf desert.

CONCLUSIONS

An environmentally friendly, low-cost, and selective procedure exploiting CPE was proposed for separating and determining silver ions in water and sand samples. All reagents and solvents were safe for the environment. Outstanding limits of detection and quantification, and analytical recovery were attained. The procedure involves a short separation and preconcentration time (10 minutes). For the analysis of several samples with different concentrations, the advantages of combining CPE (easy, safe, rapid, and efficient) and the solvation process utilizing a lipophilic agent (selectivity and sensitivity) for silver ions were demonstrated. For the fast extraction of silver in biological samples, the developed strategy produced excellent results in terms of selectivity, environmental safety, simplicity, and accuracy.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

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ARTICLE

Determination of the Trace Element Contents of Fruit Juice Samples by ICP OES and ICP-MS

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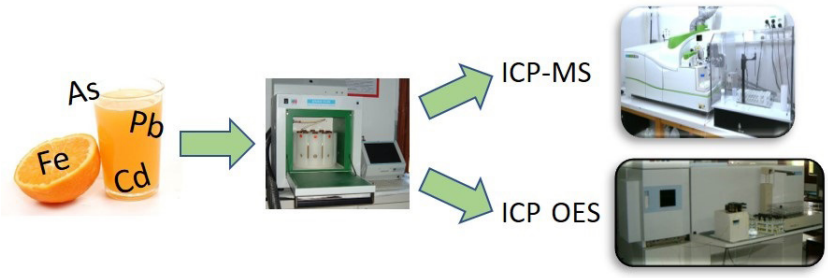

Fruit juices were analysed for their contents in a series of essential and toxic elements (Al, As, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Na, Ni, Pb, V, Sb, Mo and Zn). Samples were subjected to microwave-assisted acid digestion (HNO_3/H_2O_2), and analytes were determined by inductively coupled plasma optical emission spectrometry (ICP OES), in axial mode, or by inductively coupled plasma mass spectrometry (ICP-MS), with kinetic energy discrimination, using ^{103}Rh and ^{89}Y as internal standards. The methods were validated, showing good precision (relative standard deviations < 10%), linearity and mean analytical recovery values (89 to 103%). The ICP OES instrumental limits of detection (LODs) were between 0.3 µg L^{-1} (for Mg) and 1.5 µg L^{-1} (for Ca), whereas ICP-MS instrumental LODs varied from 2 ng L^{-1} (for Co, Mo and V) to 5.7 µg L^{-1} (for Na). Some toxic elements (As and Sb) were not detected in the fruit juices analyzed, Cd concentrations were below the maximum permitted level established by Brazilian and European regulations and only in one sample the Pb concentration (61.7 µg L^{-1}) exceeded current legal limits. Besides, essential elements such as Ca, Mg and Na were in high concentrations in the samples analyzed.

Keywords: fruit juice, toxic and essential trace elements, ICP OES, ICP-MS.

INTRODUCTION

The World Health Organization [1], in its report on diet, nutrition and chronic diseases prevention, considers that a diet with abundant ingestion of fruits and vegetables is essential for chronic diseases prevention. This current call for a healthy life and nutrition care also produces a demand of fruit juices, nectars and concentrates that are foods with nutritional and sensory characteristics that resemble foodstuff *in natura*.

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Fruits and juices contain mineral elements, carbohydrates, proteins, are cholesterol free and also have vitamins, carotenoids, essential micronutrients, folate and natural antioxidants. Among the trace elements, Ca, Fe, Mg, Zn, K and Na are essential nutrients for the organism, because they participate in the constitution of bones, teeth, muscles, blood and nerve cells among others [2].

Moreover, fruits and juices may contain potentially toxic elements such as As, Cd, Pb or Hg. These elements may be present due to absorption processes of plants from soil, water, atmospheric air, manufacturing processes of juices and migration processes from packaging. Therefore, regulatory organizations responsible of food safety pay special attention to these elements. Their contents in fruit juices are included in regulations such as the Codex Alimentarius [3] and European Commission Regulations [4,5], which set 0.03 mg kg^{-1} as the Maximum Permitted Level (MPL) for Pb in fruit juices and 0.05 mg kg^{-1} in fruit juices exclusively from berries and other small fruits and 0.02 mg kg^{-1} of Cd in drinks (including fruit juices) for infants and young children. The Brazilian Health Regulatory Agency (ANVISA) [6] established a maximum allowed limit of 0.05 mg kg^{-1} for Pb in fruit juices, 0.05 mg kg^{-1} for Cd and 0.10 mg kg^{-1} for As in fruit juices and nectars.

The most used techniques to determine the total concentration of metals in fruit juices are flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS) [7]**,** inductively coupled plasma optical emission spectrometry (ICP OES) [8-10], inductively coupled plasma mass spectrometry (ICP-MS) [9-12], and for the speciation analysis, high performance liquid chromatography (HPLC) coupled to ICP-MS [10,13] and ICP-MS/MS [14]. Hafez et al. [15] combined a supramolecular solvent-based liquid-phase microextraction method with spectrophotometric measurements at 550 nm for Al determination in water and fruit juices. Jedryczko et al. [16] determined the labile species fraction of Cd, Pb and Zn in apple beverages, including apple juices, by anodic stripping chronopotentiometry. FAAS is usually, restricted to the determination of major elements due to its low sensitivity. Although, GFAAS, ICP OES and ICP-MS are the most recommended techniques for trace elements analysis, ICP OES and ICP-MS are the techniques of choice due to their well-known advantages of sensitivity, selectivity and multi-elemental analysis capacities.

Sample preparation procedures for fruits or fruit juices analysis include calcination [17,18] and wet digestion in open or closed systems such as hot plate [19] or digestion block [7]. The use of microwave heating [14,20-23], which reduces drastically the time required for the pretreatment of the sample is today the most common technique. The reagents used in digestions are often acids with strong oxidizing power for the sample organic matter destruction, such as HNO_3, which has been used alone [23-25], or combined with H_2SO_4 [26], $HClO_4$ [19] or H_2O_2 [7,20,21,27].

In previous studies reported in the literature, high levels of trace elements were found in samples of commercial fruit juices. Savić et al. [8] obtained concentration levels of 1.19 mg L^{-1} Pb in clear orange juices and Fathabada et al. [22] found Pb values up to 66.1 µg kg^{-1} in fruit juices and Kowalska et al. [12] found up to 0.067 mg kg^{-1} of Pb in black currant nectars, and As values up to 0.149 mg kg^{-1} in apple juices and 0.144 mg kg^{-1} in orange juices. Other authors, such as Demir et al. [23], reported Pb concentrations of 0.121 mg L^{-1} in apricot nectar and 0.135 mg L^{-1} in peach nectar and Mohamed et al. [28] reported a value of 8.07 mg L^{-1} of Pb in mango juice purchased in a Yemeni market.

The main objective of this study was to determinate trace elements in fruit juices. To achieve this objective, the sample was prepared using a microwave-assisted acid digestion, followed by ICP OES or ICP-MS determination.

MATERIALS AND METHODS

Instrumentation

Major elements determination in acid-digested samples was performed using an Optima 3300 DV inductively coupled plasma atomic emission spectrometer equipped with an AS 91 autosampler and a Gem-Cone crossflow nebulizer (Perkin Elmer, Waltham, MA, USA). Trace elements in digested samples were determined using a Perkin Elmer NexIon 300X ICP-MS instrument equipped with a SeaFast SC2 DX

autosampler (Elemental Scientific, Omaha, NB, USA). Sample digestion was carried out using a microwave oven Ethos Plus MW Labstation with 100 mL closed Teflon vessels and Teflon covers, HTC adapter plate and HTC safety springs (Milestone, Sorisole, Italy).

Reagents

Nitric acid 69% Hiperpur (Applichem Panreac – ITW Companies, Barcelona, Spain); H_2O_2 33% ACS, ISO (Applichem Panreac) and ultrapure water, resistivity 18 MΩ cm (Millipore, Bedford, Maryland, USA). ICP OES calibration solutions were prepared with standard monoelemental solutions of 1.000 µg mL^{-1} of Zn (Scharlau, Barcelona, Spain), Ca, Mg, (CertiPUR® - MERCK, Darmstadt, Germany). ICP-MS calibration solutions were prepared from a multielemental standard solution containing Al, As, Cd, Co, Cr, Cu, Fe, Mn, Na, Ni, Pb and V, each element at 10 µg mL^{-1} (PerkinElmer Pure Plus) and individual standard solutions of 1,000 µg mL^{-1} of Sb and Mo (CertiPUR® - MERCK). Y and Rh were used as internal standards at concentrations of 10 µg L^{-1}. This solution was prepared from a 10 mg L^{-1} standard solution of Y (Perkin Elmer Pure Plus) and a 1000 mg L^{-1} standard solution of Rh (Fluka TraceCERT™).

Samples

The 21 samples analysed in this study include 16 fruit juices (orange, tangerine, red berries, peach, pineapple, apple and grape, mango and orange) and 2 nectars (passion fruit and purple pear nectar) of different brands bought in supermarkets in Spain and Portugal, and 2 orange juices and one lemon juice squeezed in the laboratory (denoted, respectively, as “Orange 4”,“Orange 5” and “Lemon”). These samples were treated in a manual domestic squeezer, where most of the solid fraction was retained in the filter and therefore an homogeneous sample was obtained.

Microwave-assisted acid digestion procedure

Just before being sampled, each commercial juice bottle was manually shaken for homogenization. Aliquots of 2.5 mL of the homogenized juice samples were transferred into a 100 mL high pressure Teflon vessel with 3.0 mL of 69% HNO_3, 1.0 mL of 33% H_2O_2 and 2.0 mL of ultrapure water. Closed vessels were introduced in the microwave oven and subjected to a four steps temperature program for digestion. Firstly, the temperature was linearly increased from room temperature to 90 ºC with increasing the power up to 1000 W for 2.5 min. The temperature was then elevated to 140 ºC in 6 min (power 1000 W). Finally, the temperature was increased to 180 ºC in five minutes (800 W), maintaining these conditions for 15 min. The digestion procedure was carried out in triplicate for each sample. Besides, a sample blank digestion was prepared for each series of digestions using ultrapure water instead of sample.

Both digested samples and blanks were diluted to 25 mL with ultrapure water and analysed by ICP OES or ICP-MS after appropriate dilution.

Trace element determination by ICP OES and ICP-MS

Trace element determination in the acid-digested samples were carried out by ICP OES and ICP-MS. Instrumental conditions for ICP OES and ICP-MS, as well as the concentration ranges used for calibration, are shown in Tables I and II, respectively. Samples were analysed in quintuplicate with an integration time of 5 s by ICP OES and in triplicate with an integration time of 50 ms by ICP-MS. ICP-MS polyatomic interferences were minimized with a helium collision cell using helium flows of 1 mL min^{-1} (Kinetic Energy Discrimination, KED 1) or 4 mL min^{-1} (KED 4).

Table I. Instrumental operational conditions for ICP OES

Component/Parameter	Type/Value/Mode
RF Power	1300 W
Nebulizer	Cross Flow GemTip
Plasma gas flow	16.0 L min^{-1}
Auxiliary gas flow	0.5 L min^{-1}
Nebulizer gas flow	0.8 L min^{-1}
Sample uptake rate	1.5 mL min^{-1}
Integration time	5 s
Replicates per sample	5
Mode of operation	Axial
Wavelength	393.366 nm (Ca); 279.553 nm (Mg); 206.205 nm (Zn)
Calibration interval	0.050-50 mg L^{-1} (Ca, Mg); 0.050-1.5 mg L^{-1}(Zn)

Table II. Instrumental operational conditions for ICP-MS

Component/Parameter	Type/Value/Mode
Nebulizer	PFA-ST Microflow Nebulizer
Spray chamber	PC^3 Peltier Cooler - Quartz cyclonic
Triple cone interface material	Nickel
Plasma gas flow	16.0 L min^{-1}
Auxiliary gas flow	1.2 L min^{-1}
Nebulizer gas flow	0.94 L min^{-1}
Sample uptake rate	400 µL min^{-1}
RF power	1600 W
Integration time	50 ms
Replicates per sample	3
Mode of operation	KED (Kinetic Energy Discrimination) 1 mL min^{-1} He (^{27}Al, ^{111}Cd, ^{63}Cu, ^{55}Mn, ^{98}Mo, ^{208}Pb) 4 mL min^{-1} He (^{75}As,^{59}Co, ^{52}Cr, ^{54}Fe, ^{23}Na, ^{60}Ni, ^{121}Sb, ^{51}V)
Isotopes	^{27}Al, ^{75}As, ^{111}Cd, ^{59}Co, ^{52}Cr, ^{63}Cu, ^{54}Fe, ^{55}Mn, ^{23}Na, ^{60}Ni, ^{208}Pb, ^{98}Mo, ^{121}Sb, ^{51}V
Calibration interval	0.5-100.0 µg L^{-1}
Internal standard	10 µg L^{-1} (^{103}Rh, ^{89}Y)

Material cleaning and waste treatment

All glassware and polypropylene bottles were immersed in a 10%(v/v) HNO_3 solution for 48 h followed by a minimum of three rinses with ultrapure water, dried and finally stored ready for use.

The waste generated throughout the work was collected in suitable containers and subsequently treated by the Waste Management Service of the University of Santiago de Compostela.

RESULTS AND DISCUSSION

Analytical performance

The procedure for the determination of major and trace elements in digested juice samples by ICP OES and ICP-MS was evaluated for its linearity, accuracy, precision and detection and quantification limits (respectively, LOD and LOQ).

Calibration

To study the matrix effect, external calibration and standard addition methods were compared for element determination in digested fruit juices. Calibration standards and standard addition solutions were prepared by volume dilution with concentrations ranging from 0,050 to 15 mg L^{-1} for Zn and from 0,50 to 50 mg L^{-1} for Ca and Mg for analysis by ICP OES. Calibration solutions were prepared in 1%(v/v) HNO_3. A digested juice sample was used to prepare the solutions for the standard addition method. Each calibration solution was analyzed by quintuplicate using the measurement conditions shown in Table I.

In the determinations by ICP-MS, external calibration and standard addition were in the range 0.5-100 µg L^{-1} for Al, As, Cd, Co, Cr, Cu, Fe, Mn, Na, Ni, Pb, Mo, Sb and V. ^{89}Y and ^{103}Rh were used as internal standards at a concentration of 10 µg L^{-1} and prepared in 1%(v/v) HNO_3. These solutions were analyzed using the instrumental conditions included in Table II.

The slopes obtained for all the elements studied as well as the correlation coefficients are shown in Table III. Slopes of external and standard addition calibration were compared using a t-test (5% significance level) [29] to evaluate the matrix effect. According to the results obtained, As, Co, Na, Ni and V were measured using external calibration, and Ca, Cr, Mg Mn, Mo and Zn using the standard addition method. Al and Sb were analyzed using external calibration and ^{103}Rh as internal standard. For Cd, Cu and Pb it was necessary to use the standard addition method with ^{103}Rh as internal standard, whereas Fe was determined using the standard addition method with ^{89}Y as internal standard.

Table III. Slopes and correlation coefficients of external calibrations and standard addition lines for ICP OES or ICP-MS determinations. Each internal standard used is indicated in brackets.

Element	External calibration		Standard addition	
	Slope	Correlation coefficient (r)	Slope	Correlation coefficient (r)
Al (^{103}Rh)	$4 \cdot 10^{-4}$	0.9998	$4 \cdot 10^{-4}$	0.9998
As	260.3	0.9998	268.9	0.9978
Ca[a]	1145	0.9983	1126	0.9964
Cd (^{103}Rh)	$1.02 \cdot 10^{-2}$	1	$1.0 \cdot 10^{-2}$	0.9979
Co	3842	0.9999	3986	0.9978
Cr	1467	0.9999	1581	0.9979
Cu (^{103}Rh)	$2.76 \cdot 10^{-2}$	0.9997	$3.1 \cdot 10^{-2}$	0.9979
Fe (^{89}Y)	$2 \cdot 10^{-3}$	0.9998	$2.2 \cdot 10^{-3}$	0.9975
Mg[a]	2643.5	1	2675.8	0.9999
Mn (^{89}Y)	$4.9 \cdot 10^{-2}$	0.9997	$5.6 \cdot 10^{-2}$	0.9978
Mo	12075	0.9950	20128	0.9983
Na	309.1	0.9961	314.2	0.9996

Table III. Slopes and correlation coefficients of external calibrations and standard addition lines for ICP OES or ICP-MS determinations. Each internal standard used is indicated in brackets. (Continuation)

Element	External calibration		Standard addition	
	Slope	Correlation coefficient (r)	Slope	Correlation coefficient (r)
Ni	1130	0.9999	1160	0.9977
Pb (^{103}Rh)	0.1303	0.9998	0.1366	0.9979
Sb	$1.04 \cdot 10^{-2}$	0.9996	$1.07 \cdot 10^{-2}$	0.9974
V	1057.8	0.9999	1154.2	0.9976
Zn[a]	13.1	1	13.77	1

[a] determined by ICP OES.

Sensitivity

LODs were calculated as (3 SD/m) and the of LOQs were calculated as (10 SD/m), where SD is the standard deviation of the blank signal (n=11) and m is the slope of the calibration line. Table IV shows the instrumental LODs and LOQs, in μg L^{-1}, as well as such limits referred to sample, considering the whole sample preparation process and analysis. Digested samples were analysed without dilution by ICP OES and were diluted ten times before the analysis by ICP-MS. The instrumental LODs by ICP OES were between 0.3 μg L^{-1} (for Mg) and 1.5 μg L^{-1} (for Ca). Instrumental LODs from 2 ng L^{-1} (for Co, Mo and V) to 5.7 μg L^{-1} (for Na) were obtained for ICP-MS. These limits can be improved using a higher volume of sample to carry out the digestion process. Moreover, the LOQs referred to the sample were lower than the MPLs established in the different regulations for Pb and Cd [3-6].

Table IV. Limits of detection (LODs) and quantification (LOQs) and analytical recovery (expressed as mean ± standard deviation) for the elements determined by ICP OES or ICP-MS

Element	LOD_i[b] (ng L^{-1})	LOQ_i[b] (ng L^{-1})	LOD_s[c] (μg L^{-1})	LOQ_s[c] (μg L^{-1})	Analytical recovery (%)
Al	1.0[d]	3.3[d]	98.7	329.0	89 ± 1
As	21.3	71.1	2.1	7.1	97 ± 1
Ca[a]	1.9[d]	6.2[d]	18.5	61.7	93 ± 11
Cd	5.2	17.2	0.5	1.7	103 ± 1
Co	2.3	7.6	0.2	0.8	102 ± 1
Cr	12.3	40.9	1.2	4.1	102 ± 1
Cu	0.2[d]	0.7[d]	21.6	72.2	99 ± 1
Fe	0.3	0.9[d]	27.4	91.4	99 ± 1
Mg[a]	2.3[d]	7.6[d]	22.7	75.6	108 ± 5
Mn	3.7	12.2	0.4	1.2	97 ± 2
Mo	2.0	68.0	0.2	0.7	97 ± 1
Na	5.7[d]	19.1[d]	574.2	1913.9	89 ± 1

Table IV. Limits of detection (LODs) and quantification (LOQs) and analytical recovery (expressed as mean ± standard deviation) for the elements determined by ICP OES or ICP-MS (Continuation)

Element	LOD_i[b] (ng L^{-1})	LOQ_i[b] (ng L^{-1})	LOD_s[c] (µg L^{-1})	LOQ_s[c] (µg L^{-1})	Analytical recovery (%)
Ni	27.5	91.6	2.7	9.2	98 ± 1
Pb	33.4	111.4	3.3	11.1	100 ± 1
Sb	16.1	53.8	1.6	5.4	93 ± 1
V	2.3	7.5	0.2	0.8	102 ± 1
Zn[a]	1.3[d]	4.3[d]	15.2	50.7	96 ± 7

[a] determined by ICP OES; [b] subscript "i" means "instrumental", i.e., instrumental LODs or instrumental LOQs; [c] subscript "s" means "sample", i.e., LODs and LOQs referred to sample; [d] expressed as µg L^{-1}.

The instrumental LODs obtained in this study were lower than those reported in previous studies [7,10,28]. Therefore, Pereira et al. [7] obtained LODs of 0.24, 0.03, 0.008, and 0.056 mg L^{-1} for Cr, Cu, Mg and Zn determination by FAAS, 0.026 mg L^{-1} for Na by flame emission spectrometry and 0.99 µg L^{-1} for Pb determination by ETAAS. Coelho et al. [10], for element determination in juices samples by ICP OES and ICP-MS, achieved instrumental LODs of 0.07, 0.02, 0.08, 0,2, 0.1, 0.07, 0.1 and 1 µg L^{-1} for As, Cd, Co, Cr, Mn, Mo, Pb and Zn, respectively; and 0.01 and 0.12 mg L^{-1}, for Fe and Mn, respectively, whereas for Cu they obtained a similar LOD as the reported here. The method proposed in this work also shows better sensitivity for As, Ca, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb and Sb than those obtained by Savić et al. [8] for the analysis in orange juice samples. Hong et al. [9] reported LODs of 0.009, 0.017, 0.008, 0.941, 0.120, 0.051, 0.019, 0.081 and 0.176 µg L^{-1} for As, Cd, Co, Cr, Cu, Mn, Pb, V and Zn, respectively by ICP-MS, and 0.115, 0.124, 0.057 and 0.058 µg L^{-1} for Al, Ca, Fe and Mg by ICP OES for element determination in citrus fruits samples.

Precision and accuracy

The precision of the method was studied in terms of repeatability and reproducibility. The repeatability was studied using a digested juice sample spiked with 10 µg L^{-1} and 10 mg L^{-1} for elements determined by ICP-MS and ICP OES, respectively. The relative standard deviation (RSD) was below 2% for all the elements studied. For reproducibility studies, samples were digested in triplicate and analysed in triplicate by ICP-MS and ICP OES. The RSDs obtained were lower than 10% for all the samples studied. Taking into account these results, it can be concluded that the method is precise.

The accuracy of the method was estimated using analytical recovery. Recovery percentages for Ca, Mg and Zn were calculated using a digested juice sample spiked with element concentrations of 0.1, 1 and 10 mg L^{-1}. For the elements determined by ICP-MS the digested juice sample was spiked with concentrations of 0.5, 10 and 100 µg L^{-1}. Analysis was carried out in quintuplicate for each concentration. The mean analytical recoveries (Table IV) were in a range from 89 to 108%, Therefore, it can be concluded that the method presents a good recovery.

Analysis of trace elements in juice samples

The proposed method was applied to determine trace elements in 21 juice samples (previously described in "Samples" section). Aliquots of samples were subjected to microwave assisted acid digestion and analysis by ICP OES and ICP-MS. The digestion of the sample and also the analysis were carried out in triplicate.

The results obtained for minor trace elements are shown in Table V. According to those results, in the group of toxic elements, the levels of As and Sb were below the LODs (2.1 and 1.6 µg L^{-1}, respectively, see

Table V) in all analysed samples and Pb was only detected in “Apple 1” juice sample, which contains 61.7 ± 7.2 μg L^{-1} Pb. This Pb concentration is above current regulations, in which the maximum permitted limit is 0.03 mg kg^{-1} - 0.05 mg kg^{-1} for fruit juices [3-6]. Cadmium was only quantified in “Apple 1” and “Pineapple 1” juice samples with concentration of 2.6 ± 0.1 and 2.8 ± 0.1 μg L^{-1}, respectively. These Cd levels are far below the maximum permitted level established by the ANVISA [6] of 0.05 mg kg^{-1} Cd for fruit juices and by European regulation [5], which establishes 0.02 mg kg^{-1} of Cd in drinks (including fruit juices) for infants and young children.

Co was detected in 13 of the 21 samples analysed. Co concentrations varied between 1.2 μg L^{-1} (“Orange 3”) and 37.8 μg L^{-1} (“Passion fruit nectar”). Kiliç et al. [30] obtained Co concentrations of 1.6-17 μg L^{-1} for orange and apricot juices, respectively.

Cr was detected in all the samples analysed except the orange juices “4” and “5” (juices squeezed in the laboratory). The Cr concentration varied between 3.1 and 18.9 μg L^{-1}. Anastácio et al. [21] found Cr concentrations from 5.58-26.52 μg L^{-1} in juices.

Cu was detected in 19 of the juices analysed with concentrations between 99.2 μg L^{-1} (“Apple juice 3”) and 501.4 μg L^{-1} (“Pineapple”). These results are comparable to those obtained by Coelho et al. [10], who reported Cu concentrations of 69.8-79.1 μg L^{-1} (for apple juice), and 359 μg L^{-1} (for peach, apple and grape juice).

Mo was quantified in 20 samples in a concentration range from 0.8 (“Red berries” juice) to 5.9 μg L^{-1} (“Pineapple” juice). Savić et al. [8], found Mo concentrations of 0.34-0,48 mg L^{-1} in clear orange juice and, in a recent study, Demir et al. [23] reported Mo concentrations of 0.007 mg L^{-1} in apple and peach juice samples.

Ni was detected in 14 of the samples analysed and the concentrations obtained varied from of 16.6 to 113.9 μg L^{-1}. The highest value was obtained for the “Passion fruit (nectar)” sample. Demir et al. [23] reported Ni concentrations of 0.018 and 0.003 mg L^{-1} in apricot and peach samples, respectively and concentrations below 0.018 mg L^{-1} for apple, orange, grape and cherry juice samples. Bora et al. [31] in the study of trace elements and heavy metals in fruit juices in the Romanian market, found Ni concentrations in a range between 13.85 μg L^{-1} (pear juice) and 72.83 μg L^{-1} (kiwi juice).

V concentrations varied in a range between 11.7 μg L^{-1} (“Orange juice 3”) and 91.4 μg L^{-1} (“Orange 1”), that are smaller values than those obtained by Gaiad et al. [32], who analysed 74 lemon juice samples and found mean V levels of 0.15 ± 0.02 μg g^{-1}.

The concentrations obtained for major trace elements are included in Table VI.

Table V. Concentration of minor trace elements in fruit juice and nectar samples (expressed as mean ± standard deviation; n = 6)

Sample	Concentration (µg L^{-1})								
	As	Cd	Co	Cr	Cu	Mo	Ni	Pb	Sb
Orange 1	< 2.1	< 0.5	1.5 ± 0.1	4.7 ± 0.1	< 21.6	2.70 ± 0.03	< 2.7	< 3.3	< 1.6
Orange 2	< 2.1	< 0.5	1.2 ± 0.1	6.0 ± 0.4	213 ± 17	2.96 ± 0.04	38.2 ± 0.3	< 3.3	< 1.6
Orange 3	< 2.1	< 0.5	1.2 ± 0.1	5.7 ± 0.3	218 ± 12	5.0 ± 0.1	< 2.7	< 3.3	< 1.6
Orange 4	< 2.1	< 0.5	< 0.2	< 1.2	293 ± 12	0.95 ± 0.02	48 ± 3	< 3.3	< 1.6
Orange 5	<2.1	< 0.5	< 0.2	< 1.2	265 ± 3	1.7 ± 0.1	16.6 ± 0.2	< 3.3	< 1.6
Tangerine	< 2.1	< 0.5	2.6 ± 0.3	4.9 ± 0.4	323 ± 22	2.9 ± 0.2	25 ± 4	< 3.3	< 1.6
Apple 1	< 2.1	2.6 ± 0.1	1.5 ± 0.1	9 ± 1	< 21.6	1.19 ± 0.03	< 2.7	62 ± 7	< 1.6
Apple 2	< 2.1	< 0.5	< 0.2	5.8 ± 0.5	122 ± 10	1.2 ± 0.1	< 2.7	< 3.3	< 1.6
Apple 3	< 2.1	< 0.5	1.2 ± 0.1	4.7 ± 0.1	99 ± 3	1.1 ± 0.1	< 2.7	< 3.3	< 1.6
Peach 1	< 2.1	< 0.5	< 0.2	10.3 ± 0.7	323 ± 11	4.5 ± 0.1	36 ± 3	< 3.3	< 1.6
Peach 2	< 2.1	< 0.5	< 0.2	4.4 ± 0.2	303 ± 19	3.8 ± 0.1	23.8 ± 0.5	< 3.3	< 1.6
Lemon	< 2.1	< 0.5	< 0.2	3.1 ± 0.3	194 ± 5	0.9 ± 0.1	< 2.7	< 3.3	< 1.6
Pineapple 1	< 2.1	2.8 ± 0.1	7.4 ± 0.4	15.3 ± 0.2	172 ± 9	2.81 ± 0.02	84 ± 5	< 3.3	< 1.6
Pineapple 2	< 2.1	< 0.5	5.8 ± 0.2	19 ± 1	501 ± 6	5.9 ± 0.1	31 ± 3	< 3.3	< 1.6
Red berries	< 2.1	< 0.5	1.5 ± 0.2	16.3 ± 0.8	130 ± 7	0.8 ± 0.2	< 2.7	< 3.3	< 1.6
Peach, apple and grape	< 2.1	< 0.5	< 0.2	9 ± 1	376 ± 17	4.9 ± 0.3	29 ± 1	< 3.3	< 1.6
Mango and orange	< 2.1	< 0.5	< 0.2	4.6 ± 0.2	149 ± 5	0.78 ± 0.02	27 ± 1	< 3.3	< 1.6
Pineapple, apple and grape 1	< 2.1	< 0.5	2.0 ± 0.1	19 ± 2	209 ± 2	4.3 ± 0.1	27 ± 4	< 3.3	< 1.6
Pineapple, apple and grape 2	< 2.1	< 0.5	5.8 ± 0.3	14 ± 1	189 ± 5	2.4 ± 0.2	66 ± 4	< 3.3	< 1.6
Passion fruit (nectar)	< 2.1	< 0.5	38 ± 2	6.4 ± 0.2	140 ± 9	< 0.2	114 ± 9	< 3.3	< 1.6
Purple Pear (nectar)	< 2.1	< 0.5	2.7 ± 0.4	4.9 ± 0.3	281 ± 27	2.079 ± 0.001	24 ± 2	< 3.3	< 1.6

Table VI. Concentration of major trace elements in fruit juice and nectar samples (expressed as mean ± standard deviation; n = 6)

Sample	Concentration (mg L^{-1})						
	Al	Ca	Fe	Mg	Mn	Na	Zn
Orange 1	1.50 ± 0.03	97 ± 1	0.53 ± 0.03	46.2 ± 0.4	0.179 ± 0.003	28.5 ± 0.4	< 0.015
Orange 2	< 0.098	67 ± 3	0.44 ± 0.07	85 ± 1	0.16 ± 0.02	23.7 ± 0.9	0.139 ± 0.001
Orange 3	< 0.098	73.4 ± 0.4	0.57 ± 0.01	93 ± 2	0.16 ± 0.01	39.9 ± 0.4	0.22 ± 0.02
Orange 4	< 0.098	40 ± 1	0.39 ± 0.02	93.9 ± 0.4	0.139 ± 0.003	3.1 ± 0.3	0.4 ± 0.1
Orange 5	< 0.098	20.4 ± 0.9	0.35 ± 0.01	100.3 ± 0.2	0.114 ± 0.001	< 0.6	0.5 ± 0.1
Tangerine	< 0.098	79 ± 3	0.44 ± 0.01	80 ± 1	0.201 ± 0.005	13 ± 1	0.18 ± 0.01
Apple 1	0.42 ± 0.02	54 ± 2	0.47 ± 0.01	48.3 ± 0.6	0.201 ± 0.003	15.2 ± 0.2	< 0.015
Apple 2	< 0.098	28 ± 1	< 0.027	34.5 ± 0.3	0.170 ± 0.001	21.8 ± 0.4	0.16 ± 0.02
Apple 3	< 0.098	35 ± 3	0.20 ± 0.02	38 ± 1	0.210 ± 0.004	3.3 ± 0.2	0.15 ± 0.01
Peach 1	< 0.098	25.2 ± 0.4	0.59 ± 0.03	33.9 ± 0.9	0.202 ± 0.005	7.2 ± 0.5	0.5 ± 0.1
Peach 2	< 0.098	22 ± 2	0.68 ± 0.02	37 ± 2	0.24 ± 0.01	14.6 ± 0.5	0.50 ± 0.05
Lemon	< 0.098	10.9 ± 0.5	0.16 ± 0.01	53.3 ± 0.2	0.121± 0.001	9.4 ± 0.6	0.62 ± 0.07
Pineapple 1	< 0.098	64.0 ± 0.2	0.61 ± 0.01	72.4 ± 0.7	12.1 ± 0.2	12.5 ± 0.1	0.41 ± 0.01
Pineapple 2	0.60 ± 0.02	91 ± 1	1.81 ± 0.03	105 ± 1	11.90 ± 0.04	16.5 ± 0.2	0.98 ± 0.01
Red berries	0.338 ± 0.006	30.9 ± 0.7	0.61 ± 0.02	17.5 ± 0.2	0.209 ± 0.002	17.9 ± 0.5	0.08 ± 0.01
Peach, apple and grape	0.44 ± 0.06	41 ± 1	0.88 ± 0.05	48 ± 2	0.26 ± 0.01	8.5 ± 0.2	0.76 ± 0.04
Mango and orange	< 0.098	31 ± 2	0.27 ± 0.02	33.0 ± 0.5	0.377 ± 0.007	27 ± 1	< 0.015
Pineapple, apple and grape 1	0.40 ± 0.05	68 ± 2	3.7 ± 0.1	84 ± 2	2.14 ± 0.04	24.7 ± 0.4	0.9 ± 0.1
Pineapple, apple and grape 2	0.40 ± 0.01	95.8 ± 0.8	1.07 ± 0.04	76 ± 2	7.1 ± 0.2	22 ± 2	0.4 ± 0.1
Passion fruit (nectar)	< 0.098	10.2 ± 0.6	0.712 ± 0.004	26.7 ± 0.6	0.252 ± 0.005	21.7 ± 0.4	0.59 ± 0.02
Purple Pear (nectar)	< 0.098	32.6 ± 0.9	0.28 ± 0.01	25.4 ± 0.4	0.156 ± 0.003	11.8 ± 0.6	0.39 ± 0.01

Among the major trace elements, Ca and Mg are the ones found at the highest concentrations, followed by Na. The concentrations of these elements vary from 10.2 to 96.5 mg L^{-1} for Ca, 17.5 to 104.7 mg L^{-1} for Mg and from 1.9 to 28.5 mg L^{-1} for Na. The highest Mg concentrations were found in pineapple juices, orange juices, tangerine juice and juices prepared by mixing pineapple, apple, and grape juices. The lowest concentration was found in red berries juice. Similar behavior was observed for Ca, but in this case the lowest concentration was found in the "Passion fruit (nectar)" sample (10.2 mg L^{-1}); moreover, there was lower concentration in the orange juices squeezed in the laboratory (20.4 and 39.7 mg L^{-1} for "Orange 5" and "Orange 4", respectively) than in the ones acquired in the supermarket and commercialized in tetra pack. The lowest Na concentration was also found in the orange juices squeezed in the laboratory. The values are smaller than the values obtained by Musa and Lal [19]: 33.8-638.7 mg kg^{-1} for Ca and 17.0-1281.8 mg kg^{-1} for Na in several juices (apple, orange, mango, multifruit, pulpy orange and aam panna) and the results obtained by Ekşi and Kirtiş [33] who reported concentration ranges from 75 to 160 mg L^{-1} Mg in 103 different sour cherry juice samples.

Al was only quantified in seven of the 21 samples analyzed, with concentrations of 0.4-1.5 mg L^{-1}. The lowest Al content was measured in "Pineapple, apple and grape 1" juice (0.4 mg L^{-1}) and the highest in "Orange 1" (1,5 mg L^{-1}). Kowalska et al. [12] found concentrations of 0.462-6.646 mg kg^{-1} in orange juice and black currant nectars, respectively. These authors suggest that the packaging of food products can have an impact on the level of trace elements in juices, since fruit juices and nectars stored in tetra pack packaging were characterised for a high concentration of Al.

Fe was quantified in 20 of the 21 samples analysed and its concentration varied from 0.16 to 3.7 mg L^{-1}. The highest Fe concentration was also found in the multifruit juice containing pineapple ("Pineapple, apple and grape 1") and in "Pineapple 2" juice sample. These concentrations are higher than those obtained by Demir et al. [23], who observed Fe levels between 0.066 mg L^{-1} (orange nectar) and 0.894 mg L^{-1} (apricot nectar) for juices samples purchased in Istanbul. Mohamed et al. [28] found Fe concentrations fruit juices purchased in a Yemeni market of 0.04-11.5 mg L^{-1}.

Mn was quantified in all the samples analyzed, with concentrations from 0.114 to 12 mg L^{-1}. The highest concentrations were found in pineapples and multifruit juices containing pineapple. These values are in accordance with those obtained by Coelho et al. [10] (190-9220 µg L^{-1}) in fruit juices and by Rocha et al. [34] (116-3296 µg L^{-1}) in orange and grape juices and nectars (orange, mango, passion fruit, peach and grape).

Finally, Zn was quantified in 18 of 21 of samples analyzed and the concentrations varied from 0.08 to 0.9 mg L^{-1}. These results agree with those found by Coelho et al. [10].

The differences observed among the concentrations of these elements in the samples analysed, could be attributed to different factors such as metal cation exchange capacity and soil pH, fungi presence, different species of the same plant, geographical and climatic variability conditions as well the industrial production process applied and packaging material. To investigate these differences further, data were subjected to exploratory multivariate analysis techniques, namely, PCA and CA (Principal Component Analysis and Cluster Analysis, respectively). The results –not included here– were inconclusive, since no groups of samples were revealed on the basis of their elemental contents, in spite of the different raw materials (i.e., fruits) used for their manufacture. Maybe that the manufacture process of the juices itself covers up such differences in the final products.

CONCLUSIONS

According to the results obtained, the determination of trace elements in fruit juices by ICP OES and ICP-MS provides good sensitivity, precision, analytical recovery, and it is easy to implement in laboratories for routine analysis. The proposed method provides certain advantages, such as reduction of volume of reagents (nitric acid) and time require for sample preparation, larger number on analytes determined and higher sensitivity for most of the elements.

In the juices analyzed, toxic trace elements like As and Sb were not detected and only in one apple juice was found a content of 61.7 μg L^{-1} of Pb, which is above of the current regulations. Cd was only quantified in two of the samples analyzed with values below the maximum permitted level established by the ANVISA and European regulations. In addition, essential elements such as Ca, Mg and Na were in high concentrations in the samples analyzed. Considering the results obtained in this study, we can conclude that fruit juices are a good source of essential elements for humans, and the presence of toxic elements do not pose a risk for human health. However, further studies will be necessary to evaluate the bioavailability of these trace elements.

Conflicts of interest

The authors declare that the conclusions achieved are supported by the data included in this paper. Of course, raw data are available from the corresponding author on request.

The authors declare that there are no conflicts of interest between them, affiliations, funding, financial or management relationships.

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ARTICLE

Extraction, Characterization and Rheological Behavior of Tamarind Gum Under High Salinity

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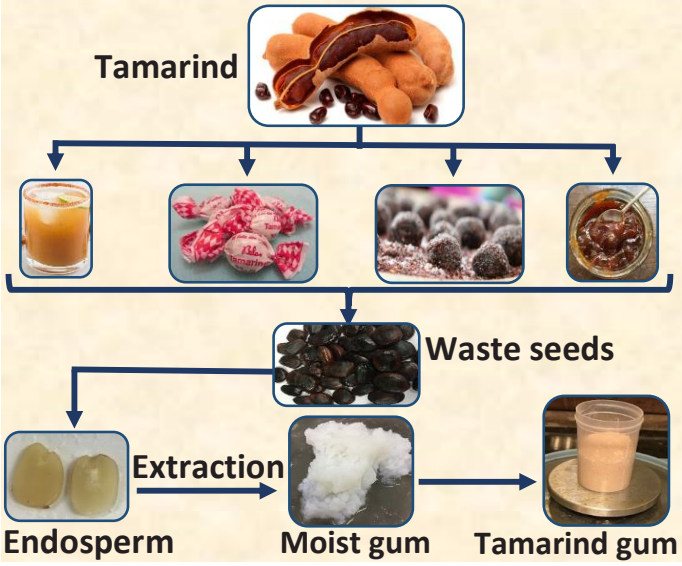

The objective of this work was to obtain tamarind gum from *Tamarindus indica* L. seeds, which are waste from the food industry. Tamarind gum was extracted by two methods and the highest yield achieved was 32.6% w/w, containing 69.25% w/w of organic matter, which was composed mostly of the nonionic polysaccharide xyloglucan. The greatest molar mass of the tamarind gum was Mw = 7.16 x 10^5 g mol^{-1} with polydispersity index (PI) of 1.7. Evaluation of the rheological behavior of tamarind gum samples were carried out in two brines (total dissolved solids values of 29,711 mg L^{-1} and 68,317 mg L^{-1}, containing divalent ions) that simulated petroleum reservoir salinity levels, with different temperatures (25, 60 and 80 °C). The rheological curves indicated high salt resistance of the gum samples. Under a shear rate of 7.3 s^{-1} the highest viscosity values found were approximately 86, 41 and 50 cP with a concentration of 5,000 ppm and temperatures of 25, 60 and 80 ºC, respectively.

Keywords: Xyloglucan, Biopolymer, Tamarind seeds, Rheology, Harsh reservoir conditions.

INTRODUCTION

Biopolymers can be derived from plant, animal or microbial biomass. These may be polysaccharides, lipopolysaccharides, glycolipids, proteins or polyhydroxyalkanoates [1]. Many complex polysaccharides are found in nature, among which xyloglucans are potentially useful for various purposes [2].

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Xyloglucans are hemicelluloses that occur in the primary cell walls of all vascular plants, as well as in different seeds, such as *Tamarindus indica* L., *Impatiens balsamina*, among others. Its chemical structure is formed by a β-(1→4)-glucan backbone substituted with α-(1→6)-xylosyl residues in a regular pattern, as well as occasional galactosyl or fucosyl residues [3,4].

Xyloglucans can be extracted from tamarind seeds or from waste pulp after fruit processing, containing as high as 72% wt of biopolymers [5]. Tamarind xyloglucan (also called tamarind gum) is used as a thickening agent in foods, pharmaceuticals and cosmetics [6]. Besides these traditional industrial applications, new functionalities have been proposed for native and modified xyloglucans [7], such as textile production, tissue engineering, wastewater treatment, food processing (emulsifiers, stabilizers, gelling agents), paste materials and freshness-maintaining agents, among others [8,9].

In addition to the applications mentioned above, biopolymers in general are being studied for application in polymer flooding that is a broadly applied enhanced oil recovery (EOR) method. Where synthetic polymers, which are generally based on partially hydrolyzed polyacrylamide (HPAM), have presented low salinity resistance, mainly in the presence of divalent cations (Ca^{2+} and Mg^{2+}), which in some systems can cause premature polymer precipitation [10,11].

The polymer function in the EOR injection fluid is to increasing its viscosity, resulting in mobility control, enabling a larger sweep area, increasing the oil recovery efficiency.

Biopolymers are less susceptible to high salinity, so they have been considered promising for this application [12].

In this study, a new potential application of tamarind gum extracted from *Tamarindus indica* seeds, an untapped waste material of tamarind pulp processing, was evaluated. Tamarind is an abundant resource in Brazil, so additional applications of it can promote economic development of production areas. After extraction, biomolecules were characterized and their rheological behavior under harsh reservoir conditions never related before (high salinity and temperature) was evaluated to assess their potential as viscosifying agents for EOR application.

MATERIALS AND METHODS

Materials

The *Tamarindus indica* seeds were obtained from the company Arbocenter Comércio Sementes (São Paulo, Brazil). Sodium chloride, calcium chloride anhydrous, magnesium chloride hexahydrate, potassium chloride, sodium sulfate anhydrous, strontium chloride hexahydrate all P.A/A.C.S from Synth, Brazil. Dimethyl sulfoxide-d6 (99.9 atom%D) from Sigma-Aldrich (São Paulo, Brazil); polyethylene oxide standard 99% from PolyAnalytik (São Paulo, Brazil); and ethanol P.A (99.8%) from Isofar (Rio de Janeiro, Brazil).

Experimental methods

Determination of tamarind seed kernel

Storage xyloglucans is the most abundant polysaccharide from the group of hemicelluloses and they are found in the cotyledons of some leguminous seeds such as *Tamarindus indica* [13,14]. Tamarind seeds were weighed and then placed in the oven at 80 °C until reaching constant weight (dry seed weight). Subsequently, the seed hulls were removed to isolate all seed kernel, which were dried under the same condition described above (dry seed kernel weight). All experiments were performed in duplicate and the seed kernel (cotyledons) was calculated by Equation 1

$$Content\ of\ tamarind\ seed\ kernel\ (\%) = \frac{mass\ of\ gry\ seed\ kernel}{mass\ of\ dry\ seed} x100 \quad \text{(Equation 1)}$$

Tamarind gum extraction from Tamarindus indica seeds

The tamarind gum was extracted by two methods. Method A described by Arruda et al. [15] with some modifications. The tamarind seeds (100 g) were washed and boiled in distilled water at 100 °C for 4 hours for enzyme inactivation and hull softening. The seeds coat were removed, seeds kernel was ground with 0.1 mol L^{-1} NaCl [15% (w/v)] at 25 °C in a blender and the crude extract obtained was then centrifuged twice for 20 min at 4,000 rpm.

The supernatant was precipitated with 95% ethanol [1:3 (v/v)] for 15 minutes and the solid material was separated with a standard sieve (45 µm), washed with ethanol [1:3 (w/v)] for 30 min and tamarind gum was isolated again with the same standard sieve. Lastly, it was lyophilized and powdered.

Method B was performed as described by Sousa et al. [16] with a few adjustments. The seeds were weighed (40 g) and soaked for swelling in deionized water at 40 g L^{-1} for 12 hours at 8 °C, to extract the xyloglucan. Then the seeds were separated by filtration through a standard sieve (1000 µm) and the liquid phase was centrifuged at 4,000 rpm for 20 minutes. Next, the seeds were boiled in deionized water at 100 °C for 2 hours for enzyme inactivation, hull softening and first hot aqueous extraction. After that, this blend (crushed seeds and deionized water) was ground, boiled for another hour and ground again. This was followed by two centrifugations at 4,000 rpm for 10 minutes each to separate the particulate matter. The extract obtained was subsequently precipitated in ethanol (1:3 v/v) under slow stirring, washed with 95% ethanol, filtered through a standard sieve (45 µm) and then lyophilized.

Proton magnetic resonance spectroscopy (^{1}H-NMR)

The tamarind gums were characterized by ^{1}H-NMR using a Varian Mercury VX 300 spectrophotometer, equipped with a 5 mm universal probe, at spectral width of 4800 Hz, acquisition time of 2.5 s, pulse width calibration of 90 degrees, pulse intervals of 10 s, transient number 80 and temperature of 60 °C. The samples were solubilized in deuterated dimethyl sulfoxide (C_2D_6SO) at concentration of 12.5 mg mL^{-1}.

Fourier-transform infrared spectroscopy (FTIR)

The samples for FTIR analysis were analyzed in pellet form, prepared with 5 mg of tamarind gum and 15 mg of KBr. A PerkinElmer Frontier FT-IR/FIR spectrometer with triglycine sulfate (TGS) detector was employed with operating parameters of 20 scans, 4 cm^{-1} resolutions, and wavelength from 4000 to 400 cm^{-1}.

Thermogravimetric analysis (TGA) and derivative thermogravimetry (DTG)

Thermogravimetric analyses were carried out to correlate the tamarind gum mass loss temperatures with the composition and purity of the samples. The equipment used was a TA Instruments TGA Q500 analyzer and the conditions used were nitrogen atmosphere (≥ 99.99), heating rate of 10 °C/min and maximum temperature of 700 °C. Initial mass of Method A was 20.6680 mg and Method B was 9.3190 mg.

Elemental analysis (CHN)

An elemental analysis (CHN) was performed to verify the presence of nitrogen in the tamarind gum samples and thus calculate the protein concentration in the final product, using Equation 2. For this purpose, gum samples weighing approximately 3 mg were analyzed with a PerkinElmer 2400 series II CHNS/O elemental analyzer [17].

$$\% \text{ Protein} = N\% \times 6.25 \qquad \text{(Equation 2)}$$

Size-exclusion chromatography (SEC)

First of all, the specific refractive index increment (dn/dc) of tamarind gum was calculated. For this, a stock solution was prepared in the mobile phase (0.1 M $NaNO_3$ + 0.0025% sodium azide, in deionized water) and filtered through a 0.45 µm filter unit (Millipore). The dilutions were obtained in the range

between 0.05 to 2.5 mg mL^{-1}, according to the detection limit of the detection system composed of a Wyatt Technology DAWN8+ multi-angle light scattering detector and Wyatt Technology Optilab T-rEX refractive index detector. Subsequently, for SEC analysis the stock solutions were passed through 0.45 µm and 0.22 µm filters (Millipore) and injected into an Agilent Technologies 1260 Infinity gel permeation chromatograph with a Shodex LG-G 6B pre-column and two Shodex LB- 806M columns and the detectors described before.

Low molecular weight poly(ethylene oxide) was used as standard and the analysis temperature was 40 °C with flow rate of 0.5 mL/min. The data were processed with the Astra 1.7.3 software to obtain Mw, Mn and the polydispersity values relative to the tamarind gum.

X-Ray diffraction analysis (XRD)

This technique was used to identify the tamarind gum morphology. After being ground the samples were passed through a 170 mesh sieve to standardize the mesh. Subsequently, they were compacted in a specific sample holder and evaluated with a Proto AXRD benchtop diffractometer with wavelength λ = 0.1542 nm, corresponding to CuKα radiation. The samples were analyzed in stepwise mode at angular amplitude from 7° to 90° (2θ), with resolution of 0.04°, speed of 0.05º sec^{-1}, 30 kV and 20 mA.

Rheological behavior

The rheological behavior of tamarind gum was carried out with a TA Instruments DHR3 rotational rheometer with titanium cone/plate accessory (40 mm 2°) and TA Instruments TRIOS software. Flow curves were obtained with shear rates ranging from 0.1 to 100 s^{-1} with two different synthetic brines: water I (WI), which simulated seawater used as injection with total dissolved solids (TDS) equal to 29,711 mg L^{-1}; water II (WII), with TDS = 68,317 mg L^{-1}, which simulated a mixture of injection water and formation water with high salinity. The composition of WI was NaCl 27,936 mg L^{-1}, anhydrous $CaCl_2$ 372 mg L^{-1}, $MgCl_2.6H_2O$ 1,275 mg L^{-1}, KCl 748 mg L^{-1}, and Na_2SO_4 58 mg L^{-1}, while that of WII was NaCl 55,306 mg L^{-1}, anhydrous $CaCl_2$ 7971 mg L^{-1}, KCl 1,569 mg L^{-1}, Na_2SO_4 72 mg L^{-1}, and $SrCl_2.6H_2O$ 1703 mg L^{-1}.

The tamarind gum concentrations used were 2,000, 3,000 and 5,000 ppm and the analyses were carried out at three temperatures, ambient temperature (25 ºC) and 60 °C [18] and 80 °C [19] to simulate typical injection and reservoir temperatures.

RESULTS AND DISCUSSION

Seed kernel content

The evaluated tamarind seeds showed 67% w/w of its weight being the seed kernel, which is the part of the seed with highest biopolymer concentration. How higher is seed kernel percentage better will be the biopolymer source because xyloglucan is obtained from tamarind kernel. Therefore, tamarind seeds presented a high potential for biopolymer production.

Extraction yield (% w/w)

The yield of each extraction was calculated by dividing the final product weight by the initial dry weight of seeds, expressed as % w/w. The yields were 7.7% (method A) and 32.6% (method B).

Method B produced the highest yield (32.6% w/w), possibly due to the performance of a sequential aqueous extraction process, one cold and two hots. This yield was higher than found by Alpizar-Reyes et al. [5], of 29.83% w/w, and by Singh et al. [20], of 18.39% w/w, but it was less than found by Sousa et al. [16], of 40.63% w/w. These yield variations are frequent and depend on factors such as geographical origin, variety and growing conditions, besides the conditions and stages of the extraction process, as mentioned by Shao et al. [21].

Proton nuclear magnetic resonance spectroscopy (^{1}H-NMR)

As mentioned before, xyloglucan is a polysaccharide with a complex structure consisting of a main chain formed by D-glucopyranose units, linked by β-type glycosidic bonds (1 → 4), with O-6 substituted side units in O-6 per unit of α-D-xylopyranose, which may also be substituted at O-2 by β-D-galactopyranose units [7]. Figure 1 shows the ^{1}H-NMR spectrum of gum extracted from *Tamarindus indica* seeds, indicating its composition mostly of xyloglucan. All the ^{1}H-NMR spectra showed typical characteristic polysaccharides signals, in a narrow region between 3 and 5 ppm, indicating the presence of sugar residues [22].

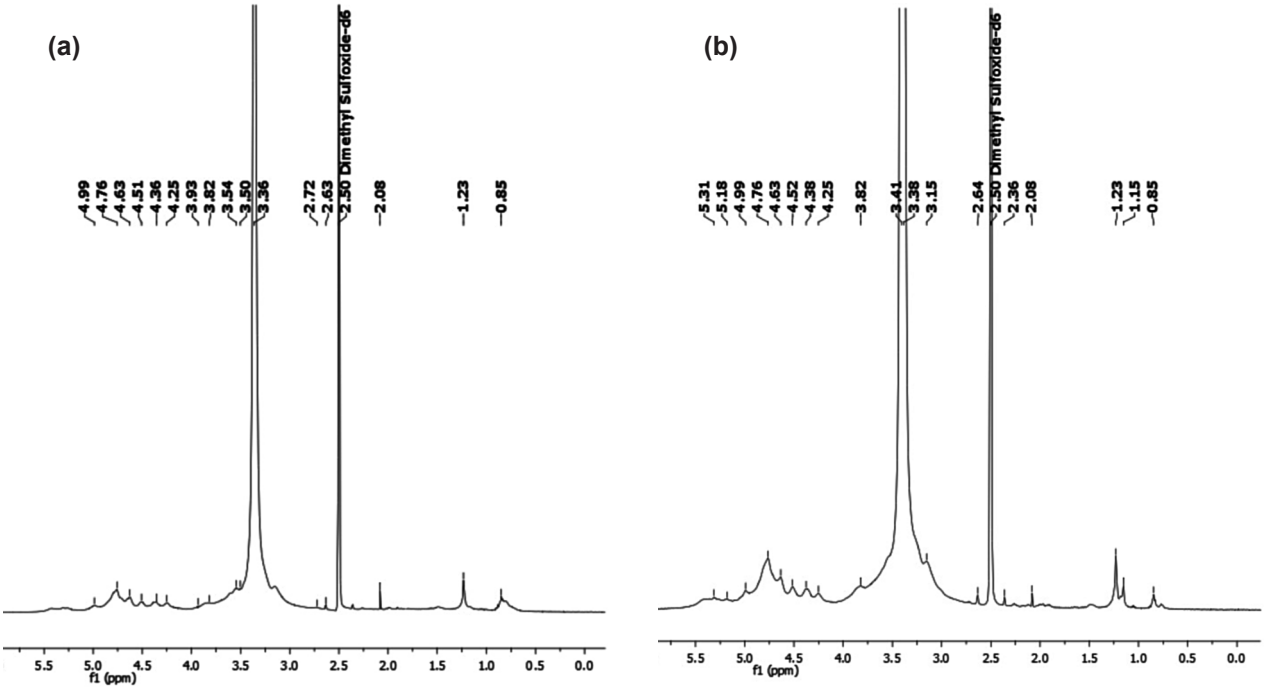

Figure 1. ^{1}H NMR spectrum of tamarind gum from (a) method A and (b) method B.

Figure 1 also indicates the presence of peaks between 4.0 and 5.5 ppm, referring to the anomeric hydrogens related to the polysaccharides [23]. Within this region, one can observe the anomeric α and β hydrogens, but unfortunately the signals of carbohydrates in NMR spectra are frequently crowded in a narrow region, especially in the case of complex carbohydrates. As a result, the interpretation of ^{1}H-NMR spectra is difficult if a polysaccharide contains many similar sugar residues [24]. The other peaks located in the region of low chemical displacement (0.8 to 2.0 ppm) are related to the methyl and methylene groups of remaining impurities in the extraction process, such as ethanol [25].

Fourier-transform infrared spectroscopy (FTIR)

The FTIR analysis was carried out to corroborate the structure of the xyloglucan extracted from the *Tamarindus indica* seeds (Figure 2). The chemical structure identified for method A and B was very similar but not the same because minor differences can be observed in the peak intensities. The main peak assignments were: O–H stretching vibration for broad peak between 3000-3700 cm^{-1} (could be related to water and/or the xyloglucan), CH_2 or CH_3 stretching (2900 - 3100 cm^{-1}), C–H symmetric stretching (2926 cm^{-1}), adsorbed water (1656 cm^{-1}); amide I (1600-1700 cm^{-1}) and amide II (1500-1560 cm^{-1}) are prominent features of a typical protein spectrum, the first is due to C=O stretching vibration and the second to the N-H bending and C-N stretching vibrations of peptide backbone; CH_2 angular deformation 1470 – 1430 cm^{-1}, CH_3 angular deformation 1390 – 1370 cm-^{1}, C-N axial distortion (1441 cm^{-1}), C–O and C–C stretching vibrations of the hexopyranosyl skeleton (1040 cm^{-1}, 943 cm^{-1}, 896 cm^{-1}) [26-32].

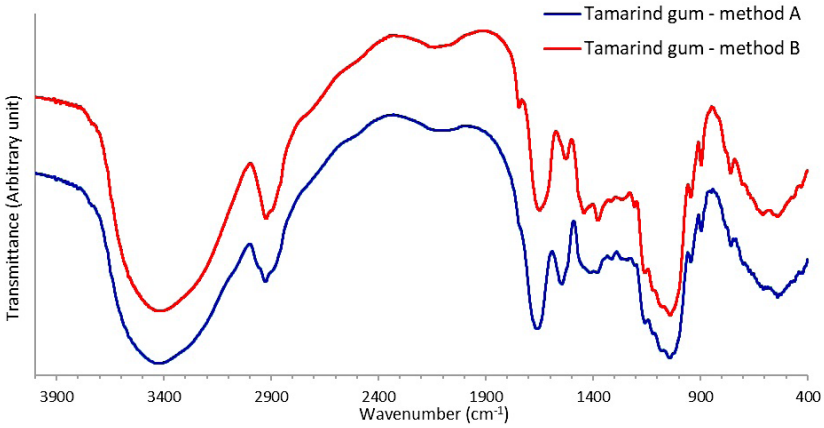

Figure 2. FTIR spectra of tamarind gum produced by method A and B.

The FTIR spectra in Figure 2 are typical for xyloglucan and peaks between 400 and 1000 cm^{-1} consists of fingerprint region it also has other vibrations such as CH_2, OH, CO deformations, etc. These were disregarded due to the high complexity in this region [33]. Among recorded peaks, changes in intensities were observed and can be attributed to protein, nucleic acids bases and fatty acids, suggesting the presence of contaminants, a possibility later ascertained by elemental analysis (CHN).

Thermogravimetric analysis (TGA)

Figure 3 shows TGA and DTG curves of tamarind gum extracted using methods A and B where two steps can be observed. In both TGA thermograms, the initial most pronounced mass loss up to 150 ºC can be associated with the loss of adhered water or other volatile solvents, such as ethanol, which was used in the precipitation stage. The second event (approximately 200–500 ºC) refers to the degradation of the organic fraction biopolymer chain cleavage with a high weight loss and the extrapolated onset temperatures of the DTG curve were 257 ºC for method A and 299 ºC for method B. These temperatures are close to those found for xyloglucan by Bergströma et al. [34] and Marais et al. [35].

The residual mass obtained at the end of analysis, at temperature of 700 ºC, can be correlated with the inorganic compounds, usually salts, contained in the raw material and/or from the extraction process.

According to the TGA/DTG thermograms (Figure 3), the mass losses were: for method A – 11.24% w/w of volatile solvents (water and alcohol), 61.77% w/w of organic matter and 26.99% w/w of inorganic matter; and for method B – 11.22% w/w of volatile solvents (water and alcohol), 69.25% w/w of organic matter, and 19.53% w/w of inorganic matter. Greater total mass lost, indicated by a red circle in the graphs in Figure 3, is theoretically associated with purer tamarind seed polysaccharide.

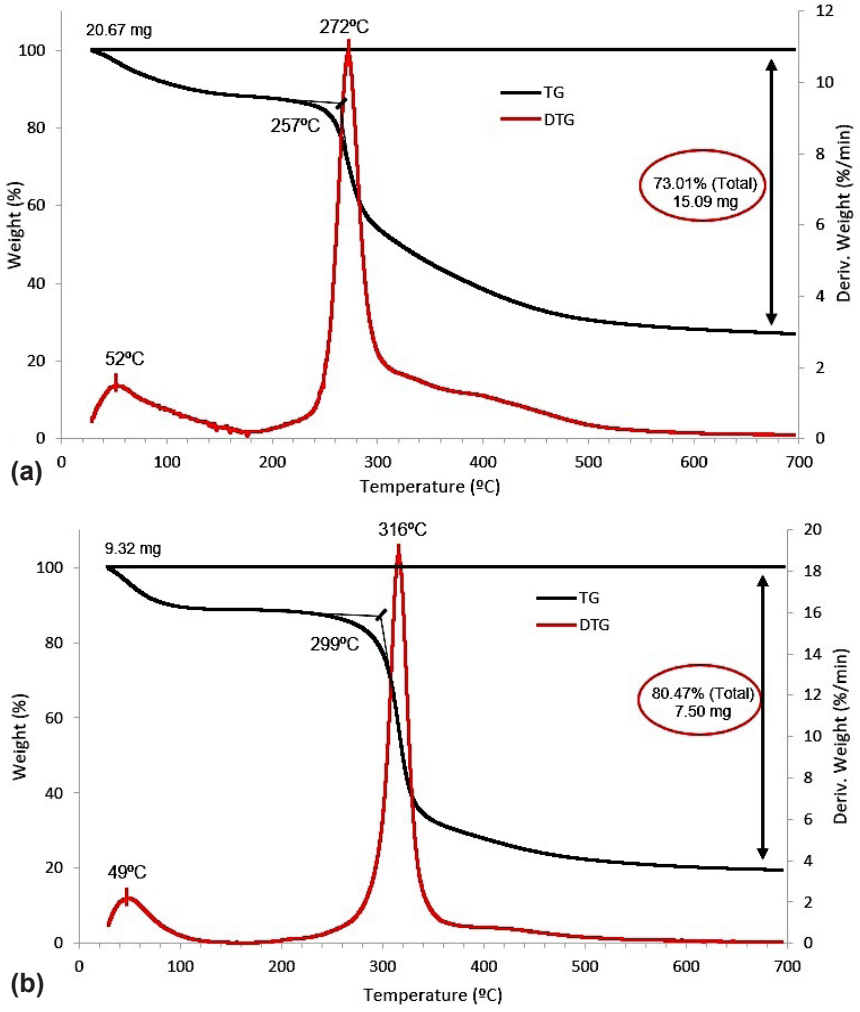

Figure 3. Thermogravimetric (black) and differential thermogravimetric curves (red) of tamarind gum extracted by (a) method A and (b) method B.

Figure 3 (b) represents the best method (B), which presented the highest yield (32.2% w/w) and the highest organic matter concentration. The total mass loss value is similar to that reported by Alpizar-Reyes et al. [36] and Khounvilay and Sittikijyothin [37], of 80.25% and 80.66% of total carbohydrates.

It should be mentioned that higher organic matter concentration is generally associated with greater efficiency in increasing the saline water viscosity.

Based on the results obtained from TGA, new yields were calculated, considering only the organic matter of interest: 4.8% (method A) and 22.6% w/w (method B). Compared with other published studies [25,38], the final extraction yield of polysaccharide was lower, but this determination procedure followed our methodology described in previous work [39].

CHN analysis

Through this analysis, the presence or absence of proteins adhered to the tamarind gum was determined. Since the primary structure contains no nitrogen, the analysis allows verifying the purification process efficiency. According to the manufacturer, the equipment Limit of Detection (LOD) is 0.3%, meaning that concentrations equal or below this value are considered to indicate absence of a certain chemical element. The analyses were carried out in duplicate and the results are shown in Table I.

Table I. CHN analysis results for extracted biopolymers

Sample	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Total (%)	Protein (%)
Tamarind gum method A	37.65	7.25	3.64	48.50	20.88
Tamarind gum method A	37.67	6.61	3.61	47.90	20.69
Tamarind gum method B	39.71	6.92	0.89	47.50	3.69
Tamarind gum method B	40.00	7.20	0.90	48.10	3.75

Method A produced gum with the highest protein concentration, making it less efficient in terms of protein-free gum production. Method B, on the other hand, produced gum with protein content of 3.7%, lower than the values reported by Alpizar-Reyes et al. [36], 14.24%, Khounvilay and Sittikijyothin [37], 13.51% and Jones and Jordan [40], 17-19%.

Lower protein content means greater content of other substances, such as high molar mass carbohydrates. This increases aqueous solutions viscosity, meaning greater thickening efficiency of the gum.

Size-exclusion chromatography (SEC)

The dn/dc found was 0.1453 mL g^{-1} for method B, which is in the range of values calculated by Sawadaa et al. [41] and Patel et al. [42], of 0.141 mL g^{-1} and 0.153 mL g^{-1}, respectively. The dn/dc of the tamarind gum obtained from method B was used to determine de number average molar weight (Mn), weight average molar weight (Mw) and polydispersity index (Mw/Mn) of the tamarind gum, obtained through methods A and B, were:

Method A: Mn = 2.03 x 10^5, Mw = 3.66 x 10^5 and Mw/Mn = 1.8

Method B: Mn = 4.161 x 10^5, Mw = 7.16 x 10^5 and Mw/Mn = 1.7

These Mw values are in agreement with those obtained by Freitas et al. [6] of 8.7x10^5 g/mol, and Newton, Indana and Kumar [43], between 1.15x10^5 g/mol and 6.5x10^5 g/mol.

Method B produced the highest concentration of organic matter and also the highest Mw value (7.16 x 10^5 g mol^{-1}), indicating that the sequential extraction method used favored obtaining molecules with greater molar mass.

The two methods to determine polydispersity studied are similar to those described by Freitas et al. [44] with values between 1.4 and 1.7 for xyloglucans extracted from different seeds, including tamarind seeds.

X-ray powder diffraction (XRD)

X-ray diffractometry was used to investigate the samples composition. Figure 4 shows the XRD pattern of tamarind gum obtained by methods A and B. The observed profile is characteristic of amorphous materials, indicated by the absence of sharp peaks in the entire 2θ limit range. These are in accordance with the literature for xyloglucans (Arruda et al. [15], Kaur et al. [45], Madgulkar et al. [46], Mahajan et al. [47]).

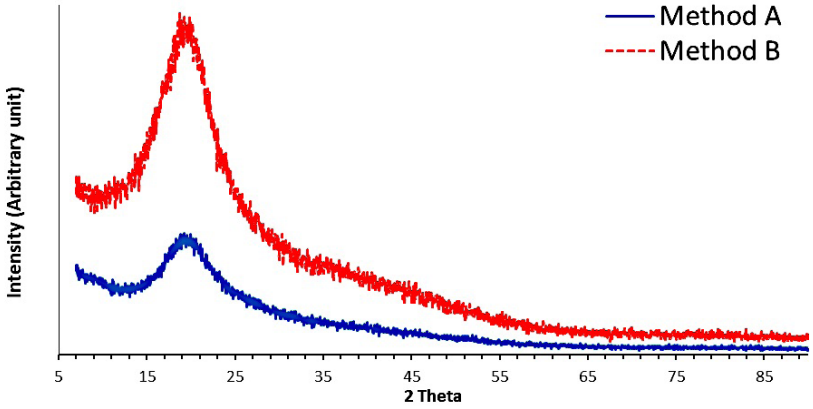

Figure 4. X-ray diffraction pattern of tamarind gum samples obtained by different methods.

Rheological and solution characteristics

Shear behavior, effect of concentration and salinity on viscosity

The curves in Figure 5 show a pseudoplastic fluid behavior, which is characterized by a reduction of viscosity with rising shear rate. This behavior is typical of high molar mass molecules, such as polymers and biopolymers. This reduction in viscosity is observed, because when the sample is shear-free, the macromolecule chains are tangled, causing high flow resistance and hence high viscosity. When shear is applied, the molecules disentangle and align in the same flow direction. This (re)organization of the biopolymer chains causes pseudoplastic behavior (Xu et al. [48]).

Figures 5 (a) and (b) show that the biopolymers prepared with water I had smaller viscosities than those prepared in water II. Therefore, in these cases higher water salinity was associated with greater viscosities. Other authors have also reported the same behavior of some biopolymers and explained this by high ionic strength of the water and changes in the conformation of macromolecules (Al-Saleh et al. [49], Diaz, Vendruscolo and Vendruscolo [50], Rodrigues et al. [14])

Between the two extraction methods, the sample obtained by method B (Figure 5.b) had the highest viscosity at the dosage of 5,000 ppm in the water with highest salinity. This behavior is in accordance with the results shown in the thermogravimetric and molar mass determination analyses, because method B presented the highest percentage of organic matter (69.25% w/w) and molecules with higher molar masses (Mn = 4,161 x 10^5 g mol^{-1} and Mw = 7.16 x 10^5 g mol^{-1}).

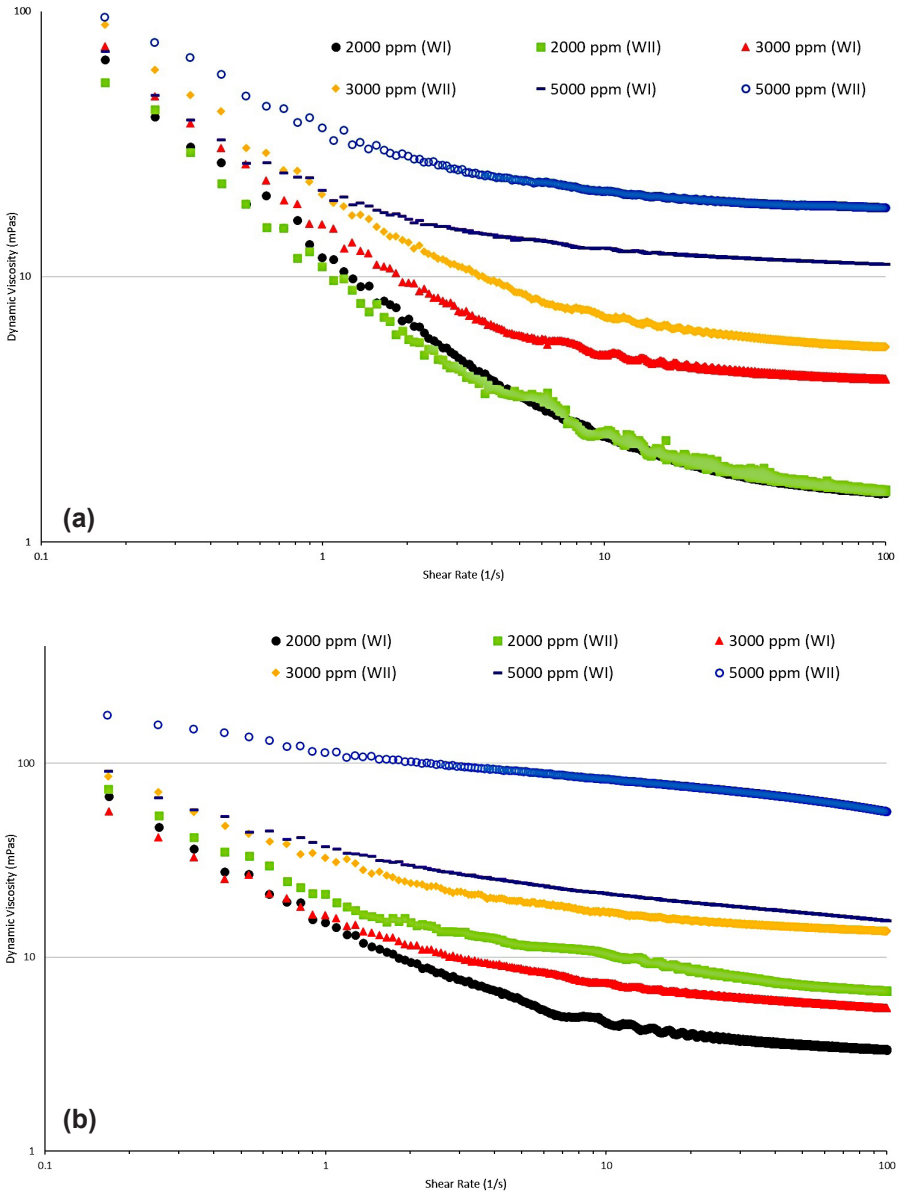

Figure 5. Flow curves of tamarind gum extracted by (a) method A and (b) method B at different concentrations (2000, 3000 and 5000 ppm), two synthetic brines (WI and WII) at the same temperature (25 °C).

Effect of temperature on the biopolymer viscosity (temperature tolerance)

In order to study the temperature influence on the solutions prepared with the extracted tamarind gum, rheological flow curves were plotted at three different temperatures, 25, 60 and 80 ºC. However, for better discussion of results, viscosities were compared at a shear rate of 7.37 s^{-1}, which simulates the flow inside a typical reservoir (Kamal et al. [51], El-hodhoudy et al. [52]). Figure 6 represents the viscosities of the solutions prepared with tamarind gum extracted by methods A and B with different concentrations (2,000, 3,000 and 5,000 ppm), salinity (TDS 29,711 mg L^{-1} for water I and 68,317 mg L^{-1} for water II) and temperature (25, 60 and 80 ºC).

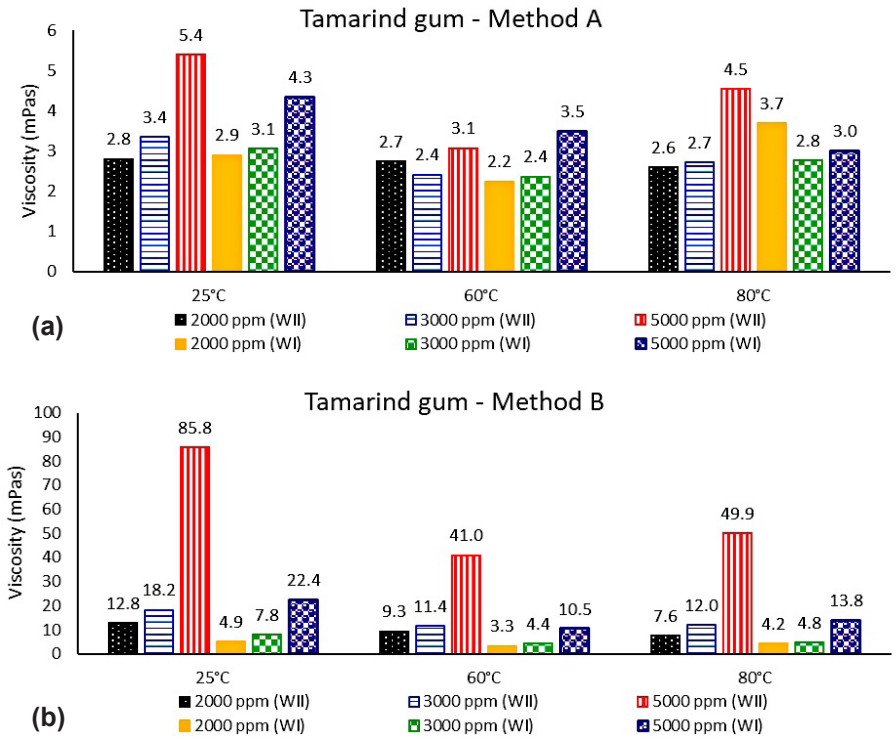

Figure 6. Viscosities of Tamarind gum solutions at shear rate of 7.3 s^{-1} extracted by methods (a) A and (b) B with different concentrations (2000, 3000 and 5000 ppm), salinity (TDS 29,711 mg L^{-1} for WI and 68,317 mg L^{-1} for WII) and temperatures (25, 60 and 80 ºC).

Most of the systems illustrated in Figure 6 showed a reduction in viscosity with rising temperature, which was expected, since dynamic viscosity is directly proportional to the attraction force between molecules suggesting that they have a temperature-dependent viscosity property (Zaim et al. [53]). As the temperature increases, this attraction force decreases, also decreasing the dynamic viscosity (Canciam [54]). In the Figure 6, it is possible to observe the viscosity increase for tamarind gum when evaluated at 80 ºC, but the expected behavior was a viscosity reduction with increasing temperature. It is suggesting this behavior was due to the high analysis temperature (80 ºC), which favored solvent evaporation, resulting in higher viscosities due to higher concentration of biopolymer.

According to Figures 6 (a) and (b), the increase in salt concentration did not result in a proportional increase in viscosity. In all of these systems, the increase in concentrations had virtually no influence on rheology, confirming the theory that these systems were in the dilute solution state.

Method B (Figure 6. B) presented the highest rheological values. The highest values with concentration of 5,000 ppm in water 80:20 was 85.8, 41.0 and 49.9 cP at temperatures of 25, 60 and 80 ºC, respectively. This tamarind gum proved to be advantageous compared to other polymers generally applied in EOR fluids. For example, Akbari et al. [55] evaluated the rheological properties of four polyacrylamide-based co-polymers: AN132VHM (medium M_W [molecular weight], high anionicity, high sulfonation degree); FLOCOMB C7035 (high M_W, medium anionicity, low sulfonation degree); SUPERPUSHER SAV55 (low M_W, high anionicity, high sulfonation degree); and THERMOASSOCIATIF (medium M_W, medium anionicity, medium sulfonation degree). All were prepared at a concentration of 4500 ppm in saline water composed of $CaCl_2$-NaCl, at 25 ºC and the viscosities at a rate of 7.37 s^{-1} were 60, 26, 36 and 35 cp. These synthetic polymers showed significant reductions in viscosities in the presence of divalent ions, a behavior that was not observed for tamarind gum, making this biopolymer even more promising for application as a viscosifying agent in EOR fluids. It is important to highlight that there is no optimum range of polymer

viscosity for polymer flooding and the polymer viscosity is not the same as that of oil to increase oil recovery. The water-oil mobility is essential control that depend on several parameters not only of viscosities (Guo et al. [56], Wang and Dong [57]).

CONCLUSIONS

In this work it was possible to extract tamarind gum from *Tamarindus indica* seeds in order to investigate its applicability as a viscosifying agent for enhanced oil recovery, and thus add economic value to these seeds, typically discarded as waste. The extracted tamarind guns were composed mainly of xyloglucan-type polysaccharides, and according to the structural characterization methods performed (^{1}H-NMR and FTIR), was noted that regardless of the extraction method, the gums had similar chemical composition.

Extraction method B produced the highest yield, and thermogravimetric analysis was used to determine the final extraction yield based on the organic matter content, which was 22.6% w/w. Furthermore, this method generated molecules with higher molar masses with Mw = 7.16 x 10^5 g mol^{-1}. It was also verified that this method produced gum with the lowest protein content in the final product, indicating that the purification process was efficient.

Tamarind gum extracted by method B proved to be advantageous from the standpoint of rheology for reservoir conditions when compared to other synthetic polymers generally applied in EOR fluids, due to its good resistance to high salinity with the presence of divalent salts.

Conflicts of interest

The authors declare no conflict of interest.

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TECHNICAL NOTE

Development of Paraquat Pesticide Determination Methodology in Urine Samples by UHPLC-MS/MS

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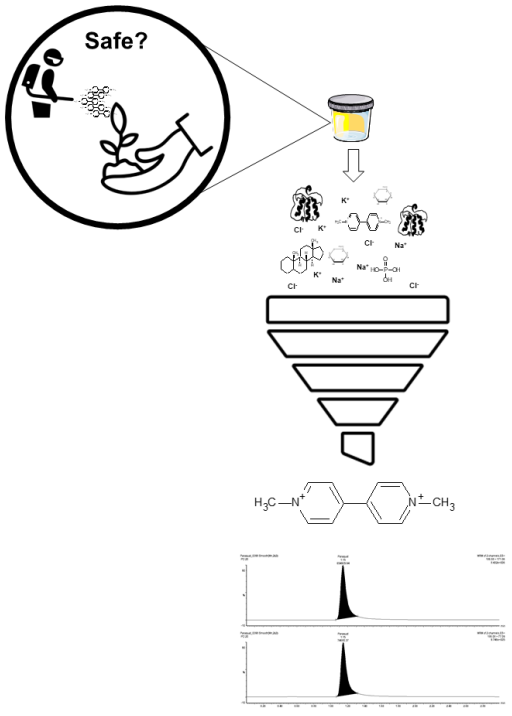

In technical-scientific development, mankind has developed different ways of managing pests, diseases and weeds, increasing agricultural production impacting social, economic and environmental-aspects. As major grains producer (such as corn, soybeans and beans), the Brazilian State, through the Brazilian agencies as Institute for the Environment and Renewable Natural Resources (IBAMA), the National Health Surveillance Agency (ANVISA), and the Ministry of Agriculture, Livestock and Supply (MAPA) authorize and regulate the use of different pesticides, including Paraquat, to improve and guarantee the production. However, the direct contact and long-term exposure to these substances offer environmental and occupational risks, impacting negatively on the workers, population of neighbor cultures, and environmental health. Considering the potential damages, it is of utmost importance to develop effective analytical methodologies for the biomonitoring and assessment of the level of exposure of farmers and ranchers in direct contact with pesticides. Thus, this work proposed to develop a sensitive and selective analytical method, using the ultra-high efficiency liquid chromatography technique, coupled to mass spectrometry

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seeking a fast and simple sample preparation for the Paraquat quantification in urine samples. To achieve this, two sample preparations were compared in terms of speed and practicality. The first method (A) used C18 silica as adsorbent to remove the non-polar interferences. The second preparation (B) consisted in direct sample dilution in acetonitrile. Both methodologies used centrifugation under refrigeration to precipitate suspended artifacts. The linearity of the Paraquat detection by the analytical methodology, developed in a HILIC column, was evaluated between 10 to 70 μg L^{-1} (r^2=0.9911; y=19427x+479868), enabling the matrix evaluation after applying the best sample preparation. The method developed was simple, fast, which makes it useful and efficient for toxicology laboratories routine to monitor the exposure levels of farmers dealing with Paraquat daily.

Keywords: Paraquat, farmers, biomonitoring, urine, UPLC-MS/MS.

INTRODUCTION

It is known that the use of pesticides of different chemical classes, such as herbicides, insecticides and fungicides, aims to increase in productivity and to deal with the life forms that directly affect agricultural production [1,2].

Brazil is recognized by the large-scale agricultural production also in the use of pesticides, and this using is increasing year by year. If the large-scale use of pesticides favors the development of agriculture, on the other hand, its indiscriminate or irregular use, can lead to problems: in the environment, soil, water, food, human and animal health. In addition, rural workers who have direct and chronic contact with the paraquat pesticide, may suffer serious damage to health, this kind of substance can influence the human body functioning, since this substance has a wide distribution in the tissues [3–5].

The Paraquat (PQ) is an herbicide, widely used in crops as a desiccator in soybean plantations, and the poisoning is a very serious situation that unfortunately almost all physicians in the countryside have already encountered. The poor prognosis, with mortality of almost 100% for ingestion above 40/45 mg kg^{-1}, is associated with the lack of reliable therapeutic protocols [6].

Paraquat, 1,1'-dimethyl-4,4'-dipyridyl cation (Figure 1), has low, but rapid gastrointestinal absorption (5-10%), plasma peak concentrations appear in less than 2 h after ingestion. It is transported to all major organs, especially to the lung, where it is reduced to form highly reactive free radicals, and is slowly excreted unchanged in urine and faeces [6–8].

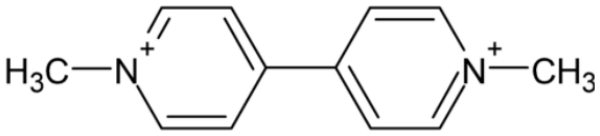


Figure 1. Structural formula of paraquat ion pesticide.

Studies indicate that chronic exposure to the PQ promotes an imbalance in the antioxidant system and behavioral changes. This exposure is also considered a risk factor to neurodegenerative diseases development, such as Parkinson's disease. Up until now, it was observed that PQ acts on dopaminergic neurons inducing the state of oxidative stress through its redox cycle on an adenine nicotinamide dinucleotide (NADPH) and also on mitochondria, inducing mitochondrial dysfunction and death of neurons [6,9–11].

Determining the level of PQ in the urine is a useful way to measure the degree of absorption for evaluating the exposure. It is suspected that direct and daily contact with this pesticide may compromise the farmer's health by affecting the kidneys, lungs and liver (due to the reactive oxygen species). Given the severity of the chronic exposure consequences, biomonitoring is necessary to evaluate the exposure levels of rural workers, who have greater chances of poisoning by these agents due to chronic and/or acute risk [12,13].

This exposure follow-up can be made by evaluating levels of PQ in urine, and different analytical methodologies are used: radioimmunoassay, colorimetric methods, gas chromatography coupled with mass spectrometry detection (CG-MS), capillary electrophoresis coupled with mass spectrometry detection (CE-MS), and high efficiency liquid chromatography coupled with mass spectrometry (HPLC-MS) [7,12–14].

For the urine preparation to chromatographic analysis, different strategies can be adopted: the use of acid or base, followed or not by centrifugation, use of an ion exchange or C18 cartridge, direct dilution, and other methodologies that make possible the matrix effect dealing. As Paraquat is insoluble in organic solvents, and hydrophilic, the most common extraction method is SPE with weak cation exchange SPE cartridges. In these methodologies ion-pairing reagents are used, such as heptafluorobutyric acids (HFBA) and trifluoroacetic acids (TFA), used to promote the separation, which can retain analyte on LC columns used in some HPLC–MS methods. But the presence of these ions can suppress the PQ signal decreasing the MS detection [15–19].

These limitations associated with high costs or many steps always reinforce the need for methods of sample preparation for investigation. Thus, to enrich the sample preparation alternatives and to help in the diagnosis of rural workers exposed to Paraquat, this paper proposed to develop a sensitive and rapid methodology for the PQ quantification in human urine samples by ultra-high efficiency liquid chromatography technique coupled with mass spectrometry (UHPLC-MS/MS), with fast and simple sample preparation.

MATERIALS AND METHODS

Materials

In this project development the ultra-performance liquid chromatography (UPLC) equipment used was in the following configuration: automatic sampler, coupled to a triple quadrupole detector (MS/MS) (Waters® Acquity Class I). The data were analyzed and processed using Mass Lynx software version 4.2. Chromatographic columns tested: ACQUITY UPLC BEH C18 12.1 x 30 mm, 1.7 µm; Agilent Poroshell UPLC BEH C8 12.1 x 30 mm, 1.7 µm; the Acquity type UPLC BEH HILIC 2.1 x 50 mm, 1.7 µm.

For the sample preparation a centrifuge (NT 805, Nova Técnica, Brazil), sample concentrator (LV Concentration TurboVap® Workstation, Biotage), water purification system (EPOD IQ 7003 - Merck Millipore®) were used. Sample solutions and 99.9% HPLC acetonitrile mobile phase (Sigma Aldrich®); Ammonium acetate (Merck®), Formic Acid (Merck®), Paraquat standard 99.9% (Sigma Aldrich®), Modified C18 silica adsorbent (Merck®).

Standard solution

The standard paraquat mother solution, as well as other dilutions for the linearity test was performed in methanol, with ultrasound bath homogenization.

Urine sample preparation

The urine standard sample was prepared with a known paraquat concentration. To avoid analytical signal suppression and loss of sensitivity of detection of the analyte, two methods of sample preparation were evaluated A and B, Figure 2.

The A method consisted of adding 100 µL of sample and 500 µL of methanol, followed by the addition of a modified C18 silica spatula tip. The system was shaken for 1 minute in a vortex mixer, and centrifuged at 9000 rpm for 10 minutes under refrigeration, then the supernatant was transferred to the injection vial.

The B preparation consisted of diluting 100 µL of urine in 900 µL of methanol. The flask was homogenized for 1 minute in a vortex mixer and centrifuged at 9000 rpm for 10 minutes under refrigeration, then the supernatant was analyzed.

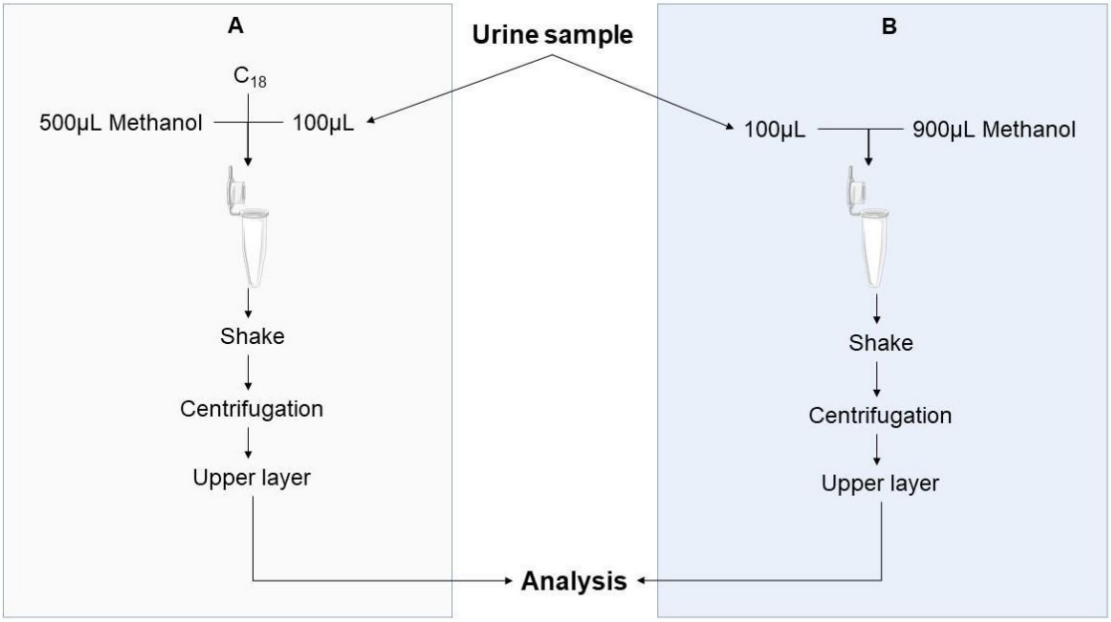


Figure 2. Sample preparation strategies: A with C18 adsorbent addition, and B only the sample and solvent.

Chromatographic parameters evaluated by UPLC-MS / MS

To develop the chromatographic system, the following parameters were evaluated: signal intensity, peak symmetry, retention time, peak resolution, and sensitivity. In this study C-8, C-18, and HILIC stationary phases were tested. In the mobile phase selection, as organic phase methanol and acetonitrile were tested; and as aqueous phase, buffer solutions containing formic acid, acetic acid and the ammonium acetate, ammonium phosphate and ammonium formate salts were tested. After comparing the developed methods, the one that best favored the sensitivity (improving the Paraquat ionization), symmetry and the analyte detection was chosen.

Method evaluation

The analytical methodology evaluation was based on some criteria of the Brazilian National Health Surveillance Agency (ANVISA by Resolution No. 27, of May 17, 2012, for bioanalytical methods validation, and National Institute of Metrology, Standardization and Instrumental Quality (INMETRO). The evaluated parameters were selectivity, linearity and matrix effect.

Specificity / Selectivity

Selectivity is the property of a procedure of providing measured values for one or more substances, so that the values of each measure are independent of each other. For this, each solution was injected independently, with visual assessment.

Linearity

Linearity is the ability of an analytical method to produce results that are directly proportional to the concentration of the analyte, in a given concentration range. The linearity was evaluated in tree replicates at concentrations of 10, 20, 30, 40, 50, 60, and 70 µg L^{-1}. One of the mathematical models used to assess linearity is to describe this dependency and the adjustment of this equation by the method of ordinary least squares. The equation of the line that relates the regression parameters a and b is given by:

$$y = ax + b$$

where y = Peak area; x = Concentration; a = Slope of the calibration curve (sensitivity) and b = Intersection with the y axis, when x = 0.

The determination coefficient (r^2) is often used to indicate how adequate the straight line can be considered as the model obtained. The working range of a method is the interval between the lower and upper levels of analyte concentration where it was possible to be demonstrated the required precision, accuracy and linearity, under the conditions specified in rehearsal [20,21].

Matrix Effect

The matrix effect is a selectivity study that aims to ascertain possible interferences caused by the substances that make up the sample matrix, basically generating phenomena of decrease or enlargement of the instrumental signal. This parameter was evaluated comparing two standard curves with and without urine. Then, the two curves were plotted and compared, also variance values were compared by F test with 0.05 significance.

RESULTS AND DISCUSSION

After a sequence of tests, the stationary phase that best met the requirements was the column with separation based on hydrophilic interaction liquid chromatography (HILIC), since the paraquat molecule (1,1'-dimethyl-4,4'-bipyridinium) is an ionized bicyclic system with two protonated nitrogen with a high-water solubility. Hilic separation showed a good efficiency in different kinds of polar molecules, showing adequate retention that provides the analyte separation from the matrix. The results evaluation leads to the analytical method adopted in this work: 5 mM ammonium formate, in 0.3% formic acid (mobile phase A), and acetonitrile (mobile phase B), to facilitate system stabilization the isocratic elution mode at 60% of FM B was chosen, at a flow rate of 0.3 mL min^{-1}, column oven at 35 ºC and samples kept at 10 ºC [14,22,23].

The mass spectrometer condition that best favored the detection of the analyte and the diagnostic ions were: capillary 0.5 kV, 13 V cone, dissolution, temperature 550 ºC, at a gas flow of 100 L h^{-1}. The ions of *m/z* 186, and their fragments *m/z* 171 and *m/z* 77.9 were monitored in the MS^n mode.

A fragile point of analytical quantification is the sample preparation: a crucial step, and more susceptible to errors. Pre-analytical errors compromise the reliability of the results, so the simpler and more efficient this step, the better. In the sample preparation, an extraction was tested with the modified silica C-18, added directly to the microcentrifuge. After chromatographic analysis (Figure 3), it was not possible to observe the presence of the analyte.

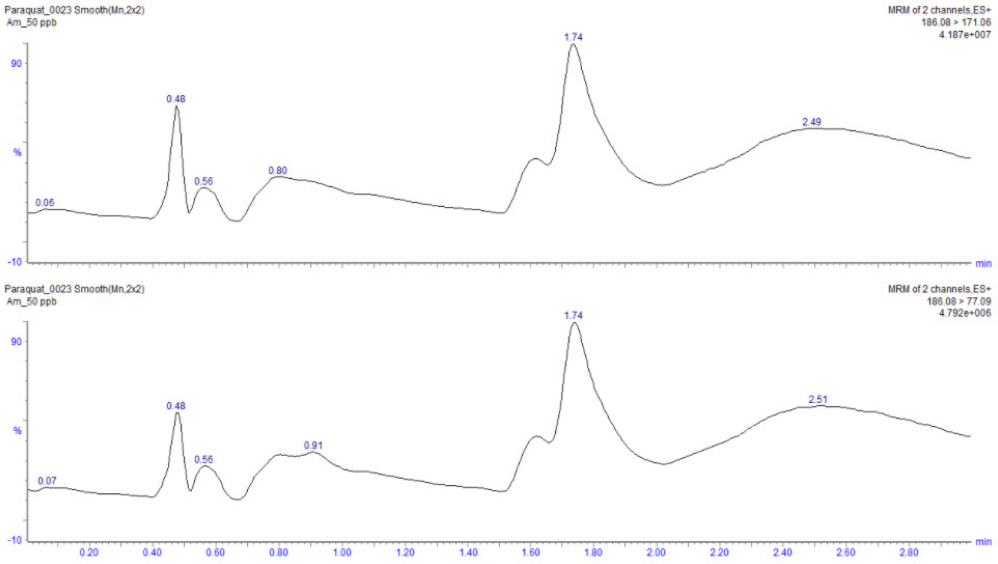


Figure 3. Chromatogram resulting from sample preparation with C-18.

The choice of sorbent C18 in sample preparation was based on reviews in the literature. In environmental analysis, particularly in the isolation and pre-concentration of pesticides in water, non-selective hydrophobic sorbents, such as C18, are the most used. These phases can differentiate and isolate the analyte of interest from the interfering compounds present in the sample, and it is common to remove non-polar interfering compounds in the matrix before chromatographic analysis. According to Carvalho et al. (2008) the most used sorbents in SPE for the extraction of pesticides in aqueous matrices are: C18, used in extraction of organochlorine insecticidal herbicides and in methods with different particle classes. Many studies used for fungicide and pesticide determinations already dissipate between 70 to 110% demonstrating the suitability of the C18 sorbent for these compounds, which makes it a good option in the extraction of interferers, but it was not applicable to this work [17,24–26].

In view of the obtained results, and still envisioning simplicity in this preparation step, it was decided to test the direct dilution of the sample and later centrifugation. The methanol has the capacity of precipitating urine protein content, and the chromatogram represented by Figure 4 shows the presence of the interested analyte. For evaluation of this preparation method, the recovery was calculated revealing almost 100% [18].

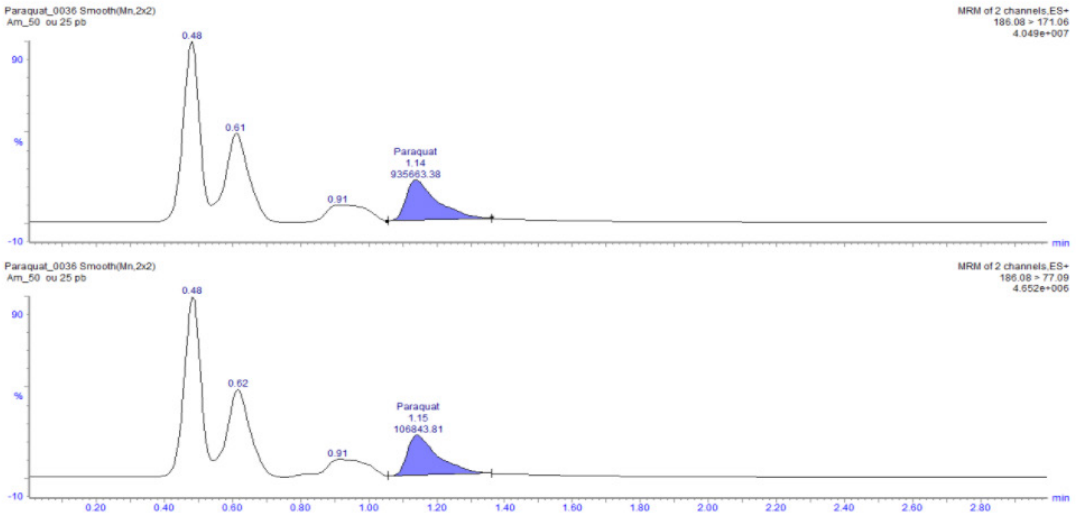
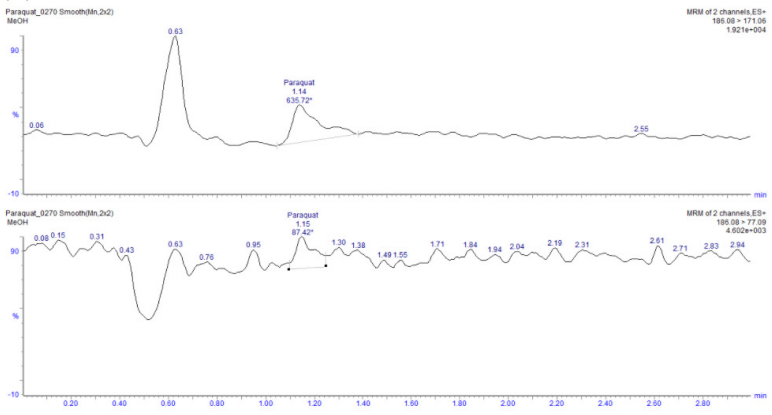


Figure 4. Chromatogram of the sample preparation by centrifugation.

Selectivity was determined by comparing the chromatogram, after the injection of the diluent (methanol), mobile phases A and B, urine without the analyte, and urine with the analyte (illustrated by Figure 5). Note the absence of signs of the pesticide ions studied in the retention time of 1.14 minutes.



(A)

Figure 5. (A) Chromatograms of the diluent; (B) mobile phase; (C) Urine without the analyte (D) urine with the standard (E) analyte in diluent.

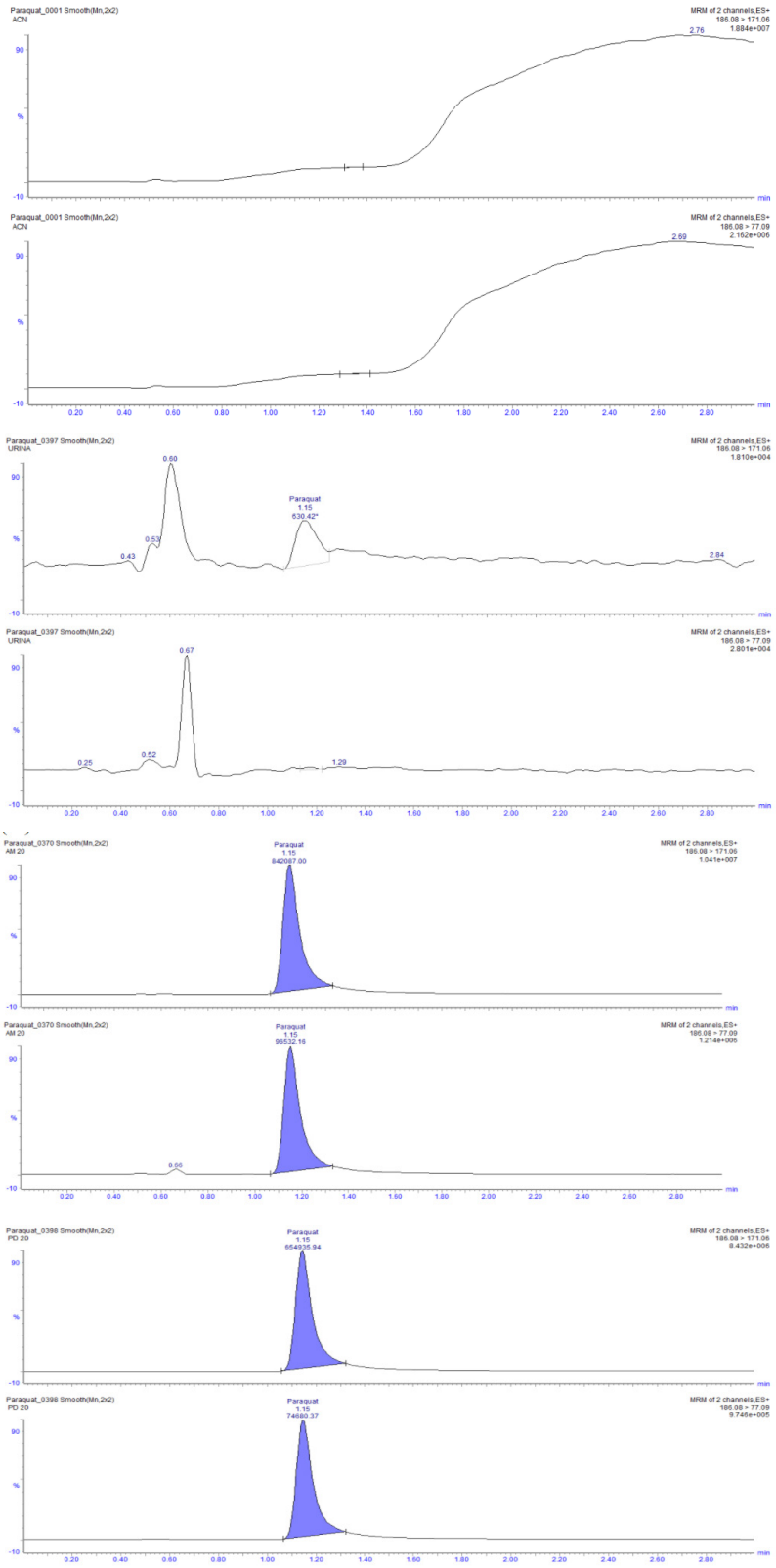


Figure 5 continuation. (A) Chromatograms of the diluent; (B) mobile phase; (C) Urine without the analyte (D) urine with the standard (E) analyte in diluent.

The Figure 6 (A) shows the calibration curve of the analyte studied in the diluent (methanol). It is observed that the method developed was linear with a determination coefficient of 0.99, thus confirming the linearity of the method. In the standardized residuals analysis (Figure 6 B), it reveals that the data obtained shows independence and randomness.

The matrix effect is an important analysis to be performed, specially to attest the sample preparation, the possible sources of potential errors in the chromatographic response need to be verified. In addition, the matrix effect allows it to generate recoveries above 100% of the studied analyte, leading to mistakes and still determine possible changes during the ionization process such as ion suppression, low response of the studied analyte, and other interferences [10].

The analysis of this effect through the mass spectrometer showed that there were no interferences that influenced the analysis of paraquat in urine samples. For this, an analytical curve was performed on the matrix (urine) (Figure 6A). The analysis interval was linear, obtaining a coefficient of determination of 0.99. Furthermore, in the standardized residual analysis (Figure 6C), it also showed that the data obtained were independence and randomness. Comparing the curves obtained in the matrix and in the solvent, it was possible to perform the F test (α = 0.05), revealing that the sample preparation minimized the effect of the matrix in the studied analytical range.

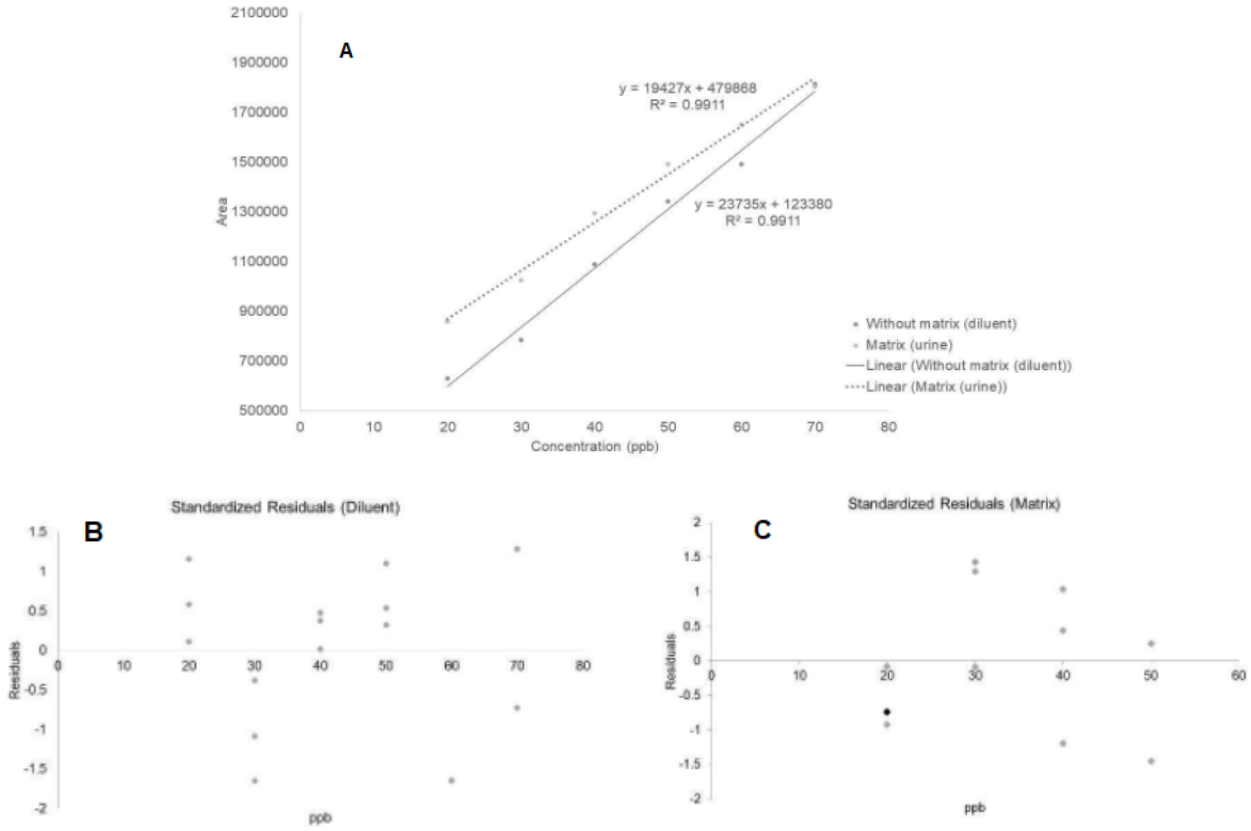


Figure 6. (A) Paraquat analytical curve in urine (matrix), and in the diluent (without matrix). (B) Standardized Residual analysis of paraquat samples on diluent. (C) Residue analysis of paraquat samples in urine (matrix).

CONCLUSIONS

Based on the results obtained, it can be concluded that the direct dilution and centrifugation to prepare the sample makes the paraquat detection possible. This strategy minimized the matrix influence. The chromatographic elution using HILIC column was possible, and it demonstrated to be able of quantifying paraquat in urine samples using UHPLC-MS/MS.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank INPES, which supplied the standards and main reagents, and the CAEPETOX laboratory of the Synvia group for having provided part of the laboratory, as well as reagents and equipment for carrying out the experiments. CAPES for Karolyne's and Gabriela's scholarship; FAEPEX-UNICAMP for the financial support to develop this project.

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FEATURE

PDF

SIRIUS – The Brazilian Particle Accelerator

Sirius is a 68,000 m^2 particle accelerator inaugurated in 2018 at the Brazilian Synchrotron Light Laboratory (LNLS), in the city of Campinas, SP, Brazil. The name is a reference to Sirius, the brightest star in the night sky. Budgeted at R$ 1.8 billion (about US$ 350 million), Sirius is the largest and most complex scientific infrastructure ever built in Brazil and one of the most advanced synchrotron light sources in the world. It was designed and developed by researchers and engineers from the Brazilian Center for Research in Energy and Materials (CNPEM), a private and non-profit organization, under the supervision of the Brazilian Ministry of Science, Technology, and Innovations (MCTI) – in partnership with Brazilian industry. It is financed with resources from the MCTI.

Brazilian Synchrotron Light Laboratory (LNLS). (Photo: Divulgação/CNPEM)

ACCELERATORS

The great scientific equipment Sirius has at its core state-of-the-art particle accelerators capable of producing and controlling the movement of electrons at speeds close to the speed of light, which generate synchrotron light, a special type of light capable of revealing the microscopic structure of organic and inorganic materials.

A synchrotron light source is composed of two main sets of particle accelerators: an Injector System and a Storage Ring. The conditioning of the electron beam in the accelerators requires an ultra-high vacuum chamber that delimits the region traversed by the electrons, radiofrequency cavities used to replace the energy lost by the electrons in the form of radiation, and a set of auxiliary systems that allow the particle accelerator to function as a whole.

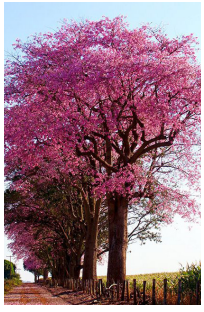
Sirius – the Brazilian Synchrotron Light Source. (Photo: Divulgação/CNPEM)

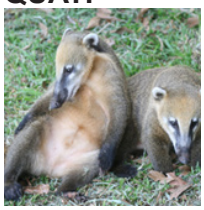
BEAMLINES

The beamlines are the experimental stations where the materials are analyzed. They are like complex microscopes that focus the synchrotron radiation so that it illuminates the samples being studied and allowing the observation of their microscopic properties. The Sirius beamlines, named after Brazilian fauna and flora, are designed to house advanced scientific instrumentation, suitable for solving Brazilian development's strategic areas. The light source can house up to 38 beamlines (from bending magnets and insertion devices), six of which are between 100 and 150 meters long, extending outside the main building experimental hall. Initially, a set of 14 beamlines is planned to cover a wide variety of scientific programs. The first six beamlines are in the installation and commissioning stage and are part of a first delivery package. A second beamline package will deliver another eight beamlines, with construction expected by 2022.

Technical Information Regarding the Beamlines

BEAMLINES Photo of what originated the BL name	**Main Technique / Description / Application**
CARNAÚBA 	**X-Ray Nanoscopy** CARNAÚBA (Coherent X-rAy NAnoprobe BeAmline) is the longest beamline of the Sirius with approximately 145 meters between the light source and the sample environment, which allows a high optical demagnification and to reach nanometric spatial resolutions.
CATERETÊ 	**Coherent and Time-resolved X-ray Scattering** The CATERETÊ (Coherent And TimE REsolved ScatTEring) beamline will provide unique capabilities in biological and soft materials imaging and dynamics experiments with particular focus on the application of coherent X-ray scattering and diffraction techniques.
CEDRO 	**Circular Dichroism** CeDRO (Circular DichROism Beamline) will be a beamline dedicated to Circular Dichroism (CD) spectroscopy in the ultraviolet region. This spectroscopy is applied to the structural analysis of chiral molecules, including biomolecules such as proteins, nucleic acids, and carbohydrates.
EMA 	**X-ray Spectroscopy and Diffraction in Extreme Condition** The EMA (Extreme condition Methods of Analysis) beamline is planned to make a difference where a high brilliance (high flux of up to 1×10^{14} photons/sec with beamsize down to 0.1×0.1 μm^2) is essential, as is the case with the study of materials under extreme thermodynamic (pressure, temperature and magnetic field) conditions.
IMBUIA 	**Infrared Micro and Nanospectroscopy** IMBUIA (Infrared Multiscale Beamline for Ultra-resolved Imaging Applications) is a beamline dedicated to experiments in micro and nano-infrared spectroscopy in the medium IR range. These experiments allow for compositional analysis of virtually any material and are essential for the research in new materials, with emphasis on biological and synthetic materials.

BEAMLINES Photo of what originated the BL name	**Main Technique / Description / Application**
IPÊ 	**Resonant Inelastic X-ray scattering and Photoelectron spectroscopy** IPÊ (Inelastic scattering and PhotoElectron spectroscopy) is a beamline optimized for high resolution Resonant Inelastic X-Ray Scattering (RIXS) and X-ray Photoelectron Spectroscopy (XPS) applied to the study of the chemical composition, electronic structure and elementary excitations of solids, liquids and gases.
JATOBÁ 	**Full X-ray Scattering and PDF Analysis** The JATOBÁ beamline is being built to produce a high-energy, high-photon flux beam focused on micrometric dimensions and will be dedicated to the study of a wide range of materials using the full X-ray scattering technique.
MANACÁ 	**Macromolecular Micro and Nanocrystallography** MANACÁ (MAcromolecular Micro and NAno CrystAllography) will be the first macromolecular crystallography beamline of Sirius and will be optimized for micrometric and sub-micrometric focus. The project includes two experimental stations, also including beams with dimensions of 0.5×0.5 micrometers (nano station) and 10×7 to 100×80 micrometers (micro station), dedicated to the study of three-dimensional structures of macromolecules, particularly complex arrangements such as viruses, membrane proteins and protein complexes and ligands.
MOGNO 	**X-ray Micro- and Nanotomography** The MOGNO beamline will be dedicated to obtaining three-dimensional images of different materials, in multiscale, in a fast, non-invasive way.
PAINEIRA 	**Powder X-ray Diffraction** The PAINEIRA will be a beamline optimized for the X-ray diffraction of polycrystalline materials in Debye-Scherrer geometry. It is aimed primarily at the structural characterization of materials in the powder form, such as ceramics, pharmaceuticals, minerals, catalysts, amongst others. The beamline will operate in high-throughput mode to rapidly characterize numerous samples. In addition, it will offer reaction cells and accessories for conducting experiments under in situ conditions as well as perform studies on functional materials and devices for energy storage and catalysis, for example.

BEAMLINES Photo of what originated the BL name	**Main Technique / Description / Application**
QUATI 	**X-ray Spectroscopy with Temporal Resolution** The QUATI (Quick X-Ray Absorption Spectroscopy for Time and space resolved experiments) beamline will be dedicated to high-quality X-ray absorption spectroscopy experiments, with temporal and spatial resolution on a millisecond scale and in situ conditions.
SABIÁ 	**Soft X-ray Absorption Spectroscopy and Imaging** The SABIÁ (Soft x-ray ABsorption spectroscopy and ImAging) beamline operates in the soft X-rays region using undulators to provide polarization control and plane gratings for monochromatization. The main techniques available are X-rays absorption spectroscopy (XAS) and photoemission electron microscopy (PEEM).
SAPÊ 	**Angle-Resolved PhotoEmission Spectroscopy** SAPÊ (Angle-resolved PhotoEmission) is a beamline dedicated to angle-resolved photoemission spectroscopy (ARPES) experiments, with high energy and momentum resolution, in the vacuum ultraviolet (VUV) spectrum range. Such experiments allow the analysis of the electronic structure of crystalline materials and are an essential tool for the study of the frontier of new materials, with special emphasis on topological materials and 2D materials.
SAPUCAIA 	**Small Angle X-ray Scattering** SAPUCAIA is a beamline dedicated to small-angle X-ray scattering for solid, liquid and gel-like samples. This technique allows to comprehend fundamental biological mechanisms through the determination of complex structures of proteins without the need for their crystallization. Moreover, this technique also is capable to give information of many different nanometric structures and agglomerates.

Although the project is well advanced, there is still a lot of work to be done, as highlighted by Harry Westfahl Jr., Director of the Brazilian Synchrotron Light Laboratory (LNLS/CNPEM). The next beamlines to be opened to researchers will support frontier scientific research with the potential to benefit areas such as agriculture, the environment, energy and new materials, as well as health.

About CNPEM

The Brazilian Center for Research in Energy and Materials (CNPEM) houses four laboratories mentioned below, which are considered world references and are open to the scientific and business community:

- The Brazilian Synchrotron Light Laboratory (LNLS) is a state-of-the-art lab responsible for operating Sirius. This lab houses multi-user facilities open to the Brazilian and international scientific community

and allows the investigation of the composition and structure of matter in its most varied forms, opening new perspectives for research in areas such as materials science, nanotechnology, biotechnology, environmental sciences, and many others.

- The Brazilian Biosciences National Laboratory (LNBio) is dedicated to cutting-edge research and innovation focused on biotechnology and drugs development.
- The Brazilian Biorenewables National Laboratory (LNBR) employs biotechnology to address scientific and technological challenges of sustainable economic development. It aims to accelerate the transition from an industrial production based on fossil resources to a bio-based and renewable industry, which promotes Brazil's technological independence and reduces environmental impacts.
- The Brazilian Nanotechnology National Laboratory (LNNano) conducts research with advanced materials, with great economic potential for the country

In addition, the four laboratories mentioned have their own research projects and participate in the transverse research agenda coordinated by CNPEM, which articulates scientific facilities and competences around strategic themes.

Aerial view of the Brazilian Center for Research in Energy and Materials. (Photo: Divulgação/CNPEM)

Source: With information from the Brazilian Synchrotron Light Laboratory (LNLS) and the Brazilian Center for Research in Energy and Materials (CNPEM).

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SPONSOR REPORT

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Total Elemental Analysis of Food Samples for Routine and Research Laboratories using the Thermo Scientific iCAP RQ ICP-MS

Tomoko Vincent[1], Simon Lofthouse[2], Daniel Kutscher[1] and Shona McSheehy Ducos[1]
[1]Thermo Fisher Scientific, Bremen, Germany [2]Thermo Fisher Scientific, UK

This report was extracted from the Thermo Scientific Application Note 43326

Keywords: Arsenic, Automation, Food safety, He KED, High matrix, High-throughput, iCAP RQ ICP-MS, Multielement, Quality control, Rice, Speciation

INTRODUCTION

The measurement of toxic, essential and nutritional elements in food has become a major topic of public interest in recent years. Intergovernmental bodies sponsored by the Food and Agricultural Organization and the World Health Organization are responsible for developing standard test methods for the analysis of food samples.

Alongside this regulatory compliance, it is important to monitor toxic contaminants that could potentially enter the food chain via a series of pathways such as industrial pollution or environmental contamination. Once toxic elements are in the food chain, they can pose significant health risks.

For these reasons, it is essential to have a simple, robust, multielemental analysis method for major and minor concentrations of elements in food. The elemental and dynamic range of single quadrupole (SQ) ICP-MS makes it particularly suited to the analysis of food, simultaneously determining trace level contaminants and macro level nutrients. In some cases, a sample may contain matrix that leads to specific interferences that can only be effectively resolved using triple quadrupole (TQ) ICP-MS.

The goal of this work is to demonstrate how simultaneous determination of all elements of interest in a wide range of food samples can be efficiently and rapidly performed using the Thermo Scientific™ iCAP™ RQ ICP-MS.

MATERIALS AND METHODS

Sample preparation

Certified Reference Materials (Rice Flour IRMM-804 and Chicken NCS ZC73016) were prepared to evaluate the proposed SQ-ICP-MS method. Approximately 0.5 g of each sample were acid digested using a mixture of HNO_3 and HCl in a closed vessel microwave digestion system.

After digestion, the samples were made up to volume (50 mL) using ultrapure water. The standard calibration solutions, blank and rinse solution were all prepared in 1% (v/v) HNO_3. The major elements (Na, Mg, P, S, K and Ca) were prepared at calibration concentration levels of 25, 50 and 100 mg L^{-1}, while the minor elements (balance of analytes) were prepared at concentrations of 25, 50 and 100 µg·L^{-1}. Internal standard correction was applied with Ga, Rh, and Ir at 20, 10 and 10 µg L^{-1} respectively.

Instrument configuration

A Thermo Scientific™ iCAP™ RQ ICP-MS was used for all measurements. The sample introduction system used consisted of a Peltier cooled (3 ºC), baffled cyclonic spraychamber, PFA nebulizer and quartz torch with a 2.5 mm i.d. removable quartz injector. The instrument was operated using kinetic energy discrimination (KED) using pure He as the collision gas in the collision/reaction cell (CRC). To automate the sampling process, an Elemental Scientific SC-4 DX Autosampler (Omaha, NE, USA) was used.

General analytical conditions

The iCAP RQ ICP-MS was operated in a single He KED mode using the parameters presented in Table I.

Table I. Instrument Operating Parameters

Parameter	Value
Forward Power	1500 W
Nebulizer Gas	0.9 L min^{-1}
Auxiliary Gas	0.8 L min^{-1}
Cool Gas Flow	14.0 L min^{-1}
CRC Conditions	4.5 mL min^{-1} at He, 3V KED
Sample Uptake/Wash Time	45 s each
Dwell Times	Optimized per analyte
Total Acquisition Time	3 min

RESULTS

The use of a single, comprehensive He KED mode is made possible through the use of unique Thermo Scientific QCell™ flatapole technology. Sample throughput is significantly improved with the single analysis mode – a key advantage for the analysis of food, since large numbers of samples may have to be screened rapidly. High transmission of the iCAP RQ ICP-MS QCell provides sufficient low mass sensitivity for accurate analysis of low mass analytes such as Li, so that all analytes can be reliably measured in one single measurement mode.

Table II shows the typical detection limits achievable for a range of analytes measured by this method. Taking into account the 1:100 dilution factor required for this analysis, the data shows that µg kg^{-1} range method detection limits are achieved with ease for all analytes. Detection limits for all the major constituent elements are well below the target levels required for food analysis.

Figures 1 and 2 show typical external calibration curves for the low concentration (Li, 0-100 µg L^{-1}) and high concentration (Na, 0-100 mg L^{-1}) analytes determined in the same analytical run with the iCAP RQ ICP-MS in single He KED mode. The results of the rice flour and chicken reference material measurements are presented in Table II. Excellent agreement was observed between the measured and reference values for all target analytes in the two reference materials analyzed.

As part of this study, the reference materials were repeatedly analyzed during the analysis. Five independent measurements were made of separate aliquots of each reference material to assess the repeatability of the method.

The results in Table II demonstrate that excellent reproducibility was achieved for the five repeat analyses of rice flour and chicken reference materials over 8 hours, with RSD's of <2 % obtained for all of the elements determined.

Table II. Dilution corrected MDLs and results for two certified reference materials: relative standard deviation is calculated for 5 independent analyses. All concentrations reported in µg L^{-1} except where stated.

Isotope	MDL*	IRMM-804 Rice			NCS ZC73016 Chicken		
		Measured	Certified	%RSD	Measured	Certified	%RSD
^{7}Li	3	-	-	-	28 ± 1	34 ± 7	1.9
^{11}B	10	-	-	-	730 ± 23	760 ± 130	1.9
^{23}Na	0.3 (mg L^{-1})	-	-	-	1310 ± 25	1440 ± 90	1.3
^{25}Mg	0.01 (mg L^{-1})	-	-	-	1200 ± 22	1280 ± 100	1.1
^{31}P	0.6 (mg L^{-1})	-	-	-	8950 ± 220	9600 ± 800	1.7
^{34}S	9 (mg L^{-1})	-	-	-	8310 ± 220	8600 ± 500	1.9
^{39}K	0.5 (mg L^{-1})	-	-	-	14000 ± 480	14600 ± 700	1.8
^{44}Ca	0.2 (mg L^{-1})	-	-	-	200 ± 4	220 ± 20	1.7
^{52}Cr	0.2	-	-	-	450 ± 10	590 ± 110	0.9
^{55}Mn	1	35800 ± 470	34200 ± 2300	0.5	1640 ± 20	1650 ± 70	0.8
^{56}Fe	4	-	-	-	32700 ± 260	31300 ± 3000	0.7
^{60}Ni	2	-	-	-	153 ± 2	150 ± 30	0.8
^{65}Cu	0.8	2650 ± 30	2740 ± 240	0.4	1350 ± 11	1460 ± 120	0.7
^{66}Zn	2	23100 ± 270	23100 ± 1900	0.7	25300 ± 220	26000 ± 1000	0.6
^{75}As	0.2	52.3 ± 0.8	49 ± 4	1.4	115 ± 1	109 ± 13	0.9
^{78}Se	1	35.1 ± 1.0	38 (Reference value)	1.3	549 ± 11	490 ± 60	1.6
^{88}Sr	0.1	-	-	-	611 ± 11	640 ± 80	1.6
^{98}Mo	1	-	-	-	112 ± 1	110 ± 10	1.9
^{111}Cd	0.3	1620 ± 9	1610 ± 70	0.7	-	-	-
^{138}Ba	0.3	-	-	-	1610 ± 16	1500 ± 400	1.4
^{141}Pr	0.02	-	-	-	2.6 ± 0.1	2.8 ± 0.6	1.6
^{208}Pb	0.1	460 ± 8	420 ± 70	0.8	90.7 ± 2.0	110 ± 20	1.0

*Method Detection Limit

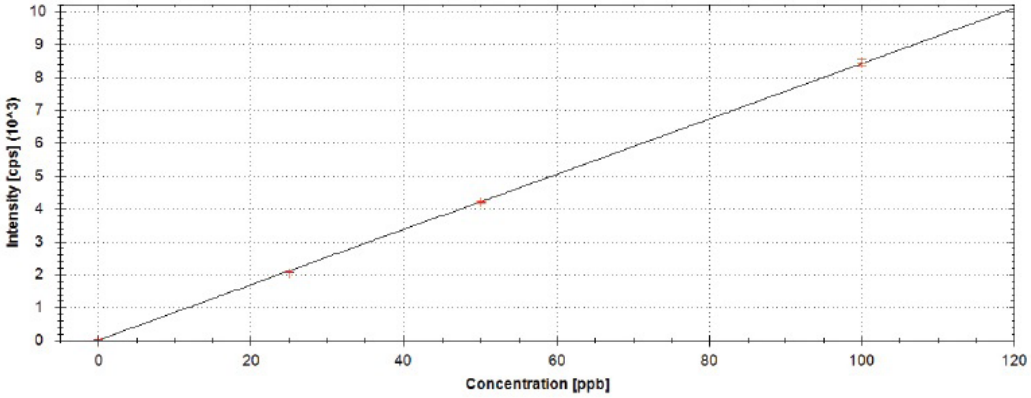

Figure 1. Calibration curve for ^{7}Li in He KED mode.

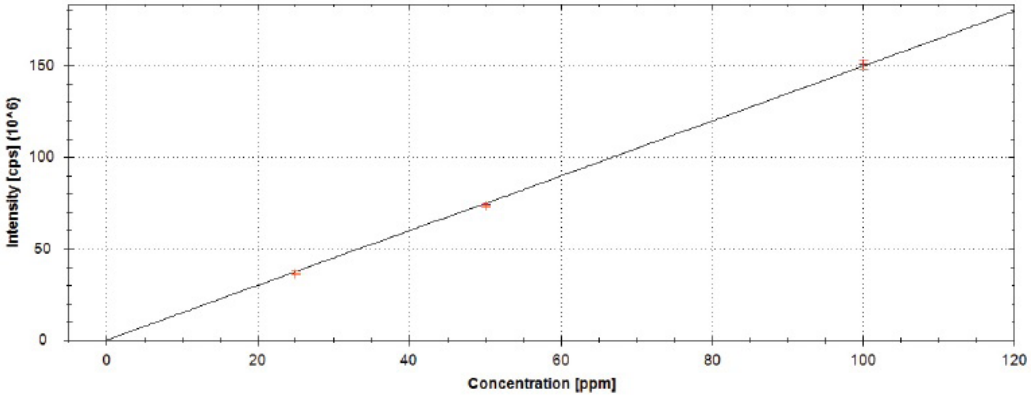

Figure 2. Calibration curve for ^{23}Na in He KED mode.

Quality control with Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software

Quality control is critical in routine analysis. To ensure quality control with the high matrix samples described in this method, the internal standards were monitored and continuing calibration checks (CCVs) were performed periodically throughout the analytical run.

The absolute suppression and relative drift of the internal standards was evaluated throughout the analysis, further demonstrating the stability and robustness of the iCAP RQ ICP-MS for prolonged measurement of high matrix samples. The variation in the internal standard signals during the run is shown in Figure 3.

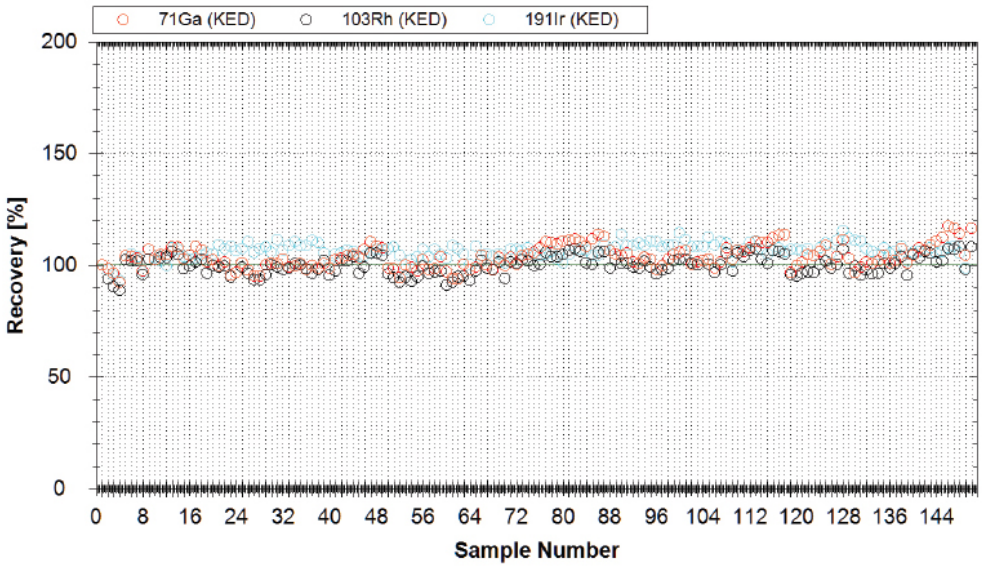

Figure 3. Variation of the internal standard intensities throughout the 8 hours analysis.

The analysis was evaluated for 8 hours, allowing the analysis of more than 150 samples. The minimal variation in the internal standard signals highlights the excellent robustness of the iCAP RQ ICP-MS in terms of both matrix resistance and interference removal for food samples analysis. Powerful, comprehensive He KED mode effectively removed complicated interferences and delivered accurate measurement results.

Continuing calibration checks (CCVs) and the reference materials were periodically analyzed throughout the analytical run with good agreement to expected levels illustrating the robustness of the method.

Six CCV checks were analyzed at intervals during the 8 hours analysis. Figure 4 shows the average concentration of the CCV standard and the in-run relative error for a range of high and low level analytes. The results from the CCV checks throughout the analysis show that there was minimal drift between the batches of food samples, eliminating the need for any sensitivity re-calibration within the 8 hours analysis period.

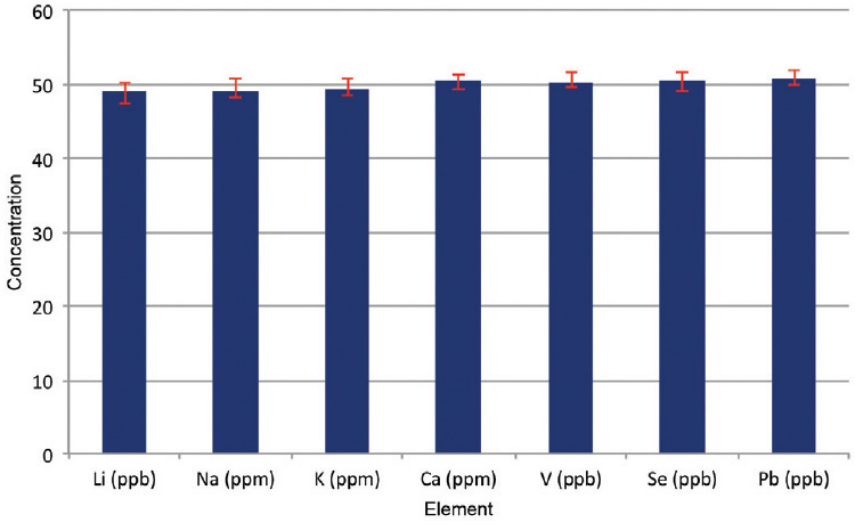

Figure 4. Calibration checks verification standards measured.

Dynamic range control with user defined mass resolution

Normal resolution or high resolution modes can be selected easily within the Qtegra ISDS Software (Figure 5). This function is particularly useful to extend dynamic range, in food, environmental and clinical research samples.

	Identifier	Dwell time (s)	Channels	Spacing (u)	Measurement mode	
	23Na (KED)	0.01	1	0.1	KED	High
	24Mg (KED)	0.01	1	0.1	KED	Normal
	39K (KED)	0.01	1	0.1	KED	Normal

Figure 5. Screen shot measurement mode from Qtegra ISDS Software.

The normal resolution mode has 0.75 u peak width at 10% of the peak height and high resolution mode has a narrow 0.25 u peak width. Using this user selectable, high resolution mode, sensitivity is reduced in order to generate a linear calibration curve with a wide concentration range.

This feature can be used for analytes such as sodium, where due to low ionization potential energy (5.1 eV) and high sensitivity in hot plasma, a calibration up to 1000 mg L^{-1} can be outside the performance capabilities of the SQ-ICP-MS detector's dynamic range.

Figure 6 shows a full calibration of ^{23}Na at 0, 5, 250, 500 and 1000 mg L^{-1} with R^2=1.000 linearity and background equivalent concentration (BEC) of only 6 µg L^{-1} using high resolution and He KED mode.

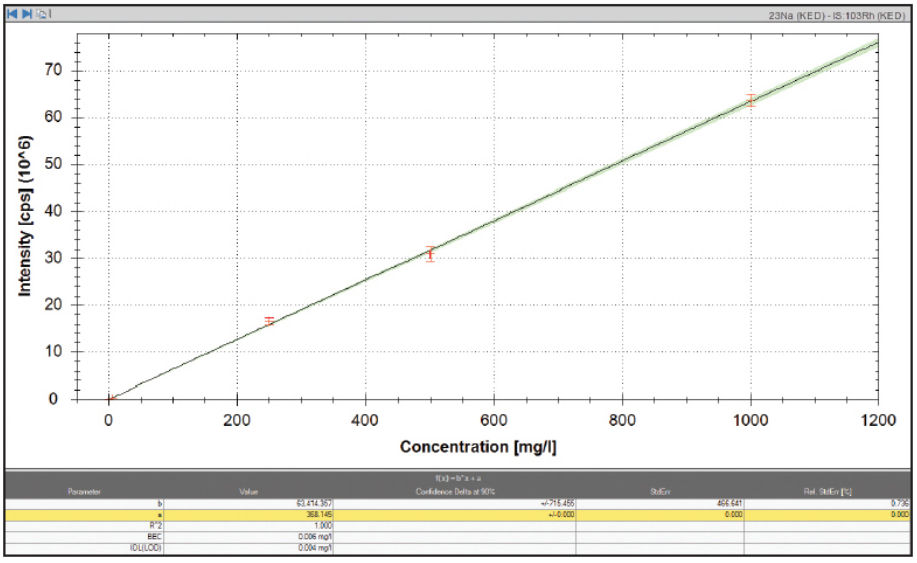

Figure 6. Calibration curve for ^{23}Na in He KED mode at 5, 250, 500 and 1000 mg L^{-1}.

IC-ICP-MS speciation analysis in organic brown rice syrup with the iCAP RQ ICP-MS

For some types of food, the concentration of a given element may not be sufficient to judge potential hazards. For example, As may be present in both inorganic forms, such as As (III) and As (V), as well as organic forms (e.g. arsenobetaine and methylated forms), which exhibit different toxicological properties. Elevated concentrations of As in foodstuffs such as rice or rice derived products are occasionally reported in the media, and speciation analysis is required to determine whether the As found is either toxic inorganic As or rather harmless organic As. Currently, regulatory authorities strive for maximum concentration levels for As in a variety of foodstuffs.

Speciation analysis comprises the separation of different compounds containing a given element using, for example, Ion Chromatography (IC) followed by selective and sensitive detection of the element using ICP-MS. Based on previous work undertaken by Thermo Fisher Scientific Application Specialists, speciation analysis of As was accomplished using the Thermo Scientific Dionex™ ICS-5000+ ion chromatography system coupled to the iCAP RQ ICP-MS. The hyphenated system can be integrated into the Qtegra ISDS Software used for operation of the iCAP RQ ICP-MS using the Thermo Scientific ChromControl Plug-in.

As for conventional As analysis, He KED mode was used to efficiently reduce polyatomic interferences affecting the detection of monoisotopic As at *m/z* 75. The method allows the determination of six As species often encountered in food analysis: The two toxic inorganic As species, and four organic species which are considered harmless.

Whereas some samples, for example water or beverages may be simply diluted, for rice and rice derived products such as organic brown rice syrup (OBRS, often used as an organic sweetener for example in cereals and cereal bars), a mild extraction is required.

Preparation of OBRS samples for As speciation was achieved by taking 1.5 g of sample, adding 15 mL of 0.28 M HNO_3 and refluxing for 90 minutes. This procedure is also suitable for As species extraction from rice grains.

Chromatographic separation of the As species under investigation is shown in Figure 7. As can be seen, OBRS contains mostly As (III) and As (V), so one of the toxic forms of As, but also methylated DMA can be observed. Each species of As was identified using comparative retention times of a standard, and automatic peak area integration for quantification was accomplished using the tQuant data evaluation plugin included in the Qtegra ISDS Software.

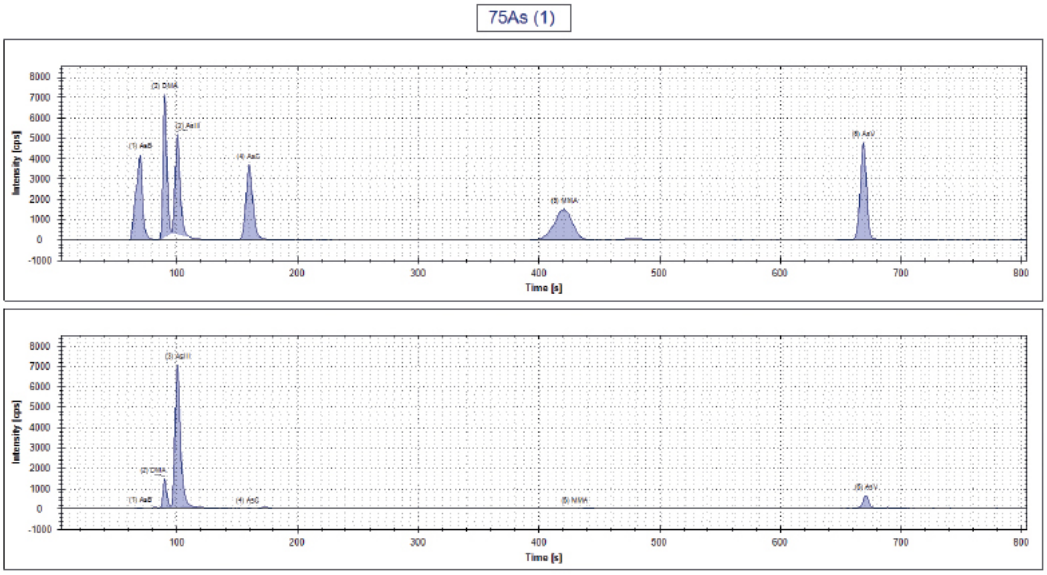

Figure 7. IC-ICP-MS chromatogram of (top) arsenic standards and (bottom) Arsenic species found in a OBRS sample. As(III) was the most abundant species detected.

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Polar pesticides in honey
Optimized chromatographic workflow

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This report was extracted from the Thermo Scientific Application Note 73853

Keywords: AMPA, glufosinate, glyphosate, IC-MS, ion chromatography, mass spectrometry, pesticide residues, TSQ Altis Triple Quadrupole MS

Goal

To develop and validate an integrated sample-to-result analytical workflow with integrated sample preparation and based on ion chromatography (IC) coupled with triple quadrupole mass spectrometry (MS/MS), for the multi-residue determination of polar anionic pesticides in representative honey samples.

INTRODUCTION

The Codex Alimentarius defines honey as the natural sweet substance produced by honeybees from plant nectar, from secretions of living plant parts, or from excretions of plant-sucking insects.[1] Since ancient times honey has been used for sweetening, but also in medicine to treat burns, gastrointestinal diseases, asthma, infected wounds, and skin ulcers.[2] The main components of honey are sugar (70–80%), water (15–20%), organic acids, enzymes, amino acids, pollen, minerals, and solid particles. Honey composition is influenced by the plant species, climatic and ecological conditions, and the beekeeper's contribution.[3]

The global production of honey has increased in the last 20 years. According to the Food and Agriculture Organization of the United Nations (FAO), 1.85 million tons of honey were produced in 2018, with China accounting for 24% of world production, followed by Turkey, Argentina, Iran, and Ukraine.[4] In addition to the main components mentioned above, trace contaminants must also be determined to assess the quality of honey. These include, for example, pesticides whose maximum contents are regulated by the EU.[5]

There are two main contamination pathways in honey:

- Cross-contamination through the collection of contaminated pollen and nectar by bees.
- Contamination through the treatment of hives with insecticides, fungicides, and acaricides to protect against parasites such as *Varroa destructor*, *Acarapis woodi*, and *Paenibacillus larvae*.

Tolerance levels for glyphosate in cereal crops up to four hundred times higher than for honey[5] suggest the possibility of cross-contamination. The development of glyphosate- and glufosinate-tolerant, genetically modified crops encouraged the use of these broad-spectrum herbicides, which are still used in horticulture. As a result, these polar components occur as environmental contaminants and thus in food such as honey. The EU set the maximum residue level (MRL) for glyphosate and glufosinate in honey to 0.05 mg/kg as the lower limit of the analytical determination procedure.[5,6] In 2017 and 2018, two German consumer organizations reported glyphosate-contaminated honey, referencing LC-MS/MS as the analytical method.[7,8] The State Office for Consumer Protection and Food Safety of Lower Saxony (Germany) tested domestic honey samples in 2016. Out of the 193 samples, 94% did not contain glyphosate and 3% of the samples

contained glyphosate below the permitted limit. The remaining 3% of samples were found to be above the maximum level.[9] A more recent local study reported several pesticide residues but no glyphosate.[10] The controversial debate on glyphosate in honey and the relevance of glyphosate for human health[11] suggests the need for an optimized method for the accurate determination of polar pesticides and their metabolites in honey.

The chromatographic separation of polar and ionic pesticides is one of the more challenging tasks in food evaluation. Due to their high polarity, classical reversed-phase chromatography (RPLC) of glyphosate, aminomethylphosphonic acid (AMPA), glufosinate, and other polar pesticides requires their derivatization[12] or the use of unique separation columns.[13] More recent approaches are based on hydrophilic interaction liquid chromatography (HILIC) without derivatization of the polar pesticides. Frequently reported experimental limitations in the routine use of HILIC or of special RPLC applications refer to the robustness of the columns used. Their occasional rapid aging has drastic effects, e.g., on retention time, peak efficiency, and resolution, and thus on evaluation and quantification.[14,15]

In contrast to classical RPLC and HILIC, ion chromatography (IC) is the method of choice for the separation of polar and ionic compounds. Initially designed for the analysis of inorganic ions, today IC is successfully used for the separation of, e.g., organic anions and cations, sugars, amino acids, peptides, proteins, and nucleotides.[16]

At high pH values, glyphosate, AMPA, and glufosinate are anionic, suggesting the use of anion exchange chromatography. Derivatization is not necessary, and modern analytical ion exchangers are optimized for the separation of small polar ionic compounds. Until recently, coupling IC with mass spectrometry (MS) has been considered rather unusual, due to the eluents consisting of aqueous corrosive alkalis or acids.[16] With the introduction of electrolytically regenerated membrane suppressors, however, the robust continuous desalting of the eluents, or more precisely their chemical conversion into water, is now possible before the eluent enters the mass spectrometer.[17]

Mass spectrometry has become an accepted technique for the detection of pesticides. Triple quadrupole MS/MS systems are currently in widespread use in food analysis. These systems meet the current requirements for sensitivity and selectivity in the selected reaction monitoring (SRM) mode.[18-22] Additional improvements in detection specificity and selectivity result from the use of high-resolution accurate mass spectrometry (HRAM).[23-29]

A matrix-specific challenge arises in the application of MS due to the high sugar content of honey, which can lead to contamination of the mass spectrometer inlet cone, resulting in instrument downtime.

This paper describes an IC-MS/MS method for the direct analysis of glyphosate, AMPA, and glufosinate in honey. Our evaluation is supplemented by an automated inline elimination of sugars before the mass spectrometer.

EXPERIMENTAL

A metal-free ion chromatograph (Thermo Scientific™ Dionex™ ICS-6000) with a Thermo Scientific™ Dionex™ AS-AP autosampler was coupled to a Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer (Figure 1). A Thermo Scientific™ Dionex™ IonPac AS19-4µm polymeric based separation column and guard column were used. The KOH gradient was generated in-situ with an eluent generator without the use of external chemicals (RFIC™). After separation, eluent and eluites passed through the Thermo Scientific™ Dionex™ ADRS 600 Suppressor being electrolytically regenerated in external water mode. For matrix elimination, a second valve was integrated, diverting the effluent from the MS for a selected time segment. In this state, the effluent is first collected in a loop (750 µL), the contents of which are fed separately to waste after switching back. To improve the evaporation of the effluent (desolvation), 2-propanol was added post-column before the mass spectrometer interface. The Thermo Scientific™ Chromeleon™ Chromatography Data System software was used for data acquisition and analysis. All chemicals used in these investigations were of analytical grade quality or better; the deionized water used was freshly taken from the ultrapure water system.

Equipment

- Dionex ICS-6000 HPIC™ system*, including:
  - SP Pump, Isocratic with Degas (P/N 22181-60003)
  - DC Microbore Compartment with Dual Temperature Zone, Two Injection Valves (P/N 22181-60049)
  - EG Module (P/N 22181-60019)
  - EG Degas Unit (SB/MB) (P/N 075522)
  - CD Detector (with Cell) (P/N 079829)
  - EO Eluent Organizer Tray with two 2 L bottles (P/N 072057)
  - IC PEEK Viper Fitting Kit for Dionex ICS-6000 with Conductivity Detector (Microbore 2 mm) (P/N 302965)
  - Dionex Suppressor External Regenerant Installation Kit (P/N 038018)

  * or a Thermo Scientific™ Dionex™ Integrion™ HPIC™ system (RFIC model) with two injection valves, and CD Detector with cell.

- Dionex AS-AP Autosampler, with Tray Temperature Control Option (P/N 074926) with three vial trays (P/N 074936)
- Thermo Scientific™ Dionex™ AXP Auxiliary Pump (P/N 063973)
- Thermo Scientific™ Dionex™ AXP-MS Auxiliary Pump (P/N 060684)
- TSQ Altis Triple Quadrupole Mass Spectrometer (P/N TSQ02-10002)
- Chromeleon Chromatography Data System software, version 7.2.9 or higher (P/N 7200.0201-ICSP) with Spectral License—3D/MS Data Acquisition (P/N 7000.0020-ICSP)
- Thermo Scientific™ Barnstead™ Pacific™ GenPure™ ultrapure water system with UV-photo-oxidation, ultrafiltration membrane, and TOC monitor (P/N 50131256) with Pacific TII 40 (UV) (P/N 50132133) and double cartridge pretreatment system (P/N 09.4000)

Reagents and supplies

- AMPA, (Aminomethyl) phosphonic acid (P/N 05164-50MG) Sigma-Aldrich
- Deionized (DI) water, (18.2 MΩ·cm, TOC < 5 ppb, 0.2 µm inline filter), Thermo Scientific (see Equipment)
- Glufosinate-ammonium, Pestanal™ (P/N 45520-100MG) Sigma-Aldrich
- Glyphosate, Pestanal™ (P/N 45521-250MG) Sigma-Aldrich
- Isopropanol, Optima™ LC/MS Grade, Fisher Chemical™ (P/N 10091304) Fisher Scientific
- Fisherbrand™ Non-sterile Nylon Syringe Filter, 25 mm, 0.2 µm (P/N 15121499) Fisher Scientific
- Vial Kit, 1.5 mL Polypropylene with Caps and Septa, 100 each (P/N 079812) Thermo Scientific

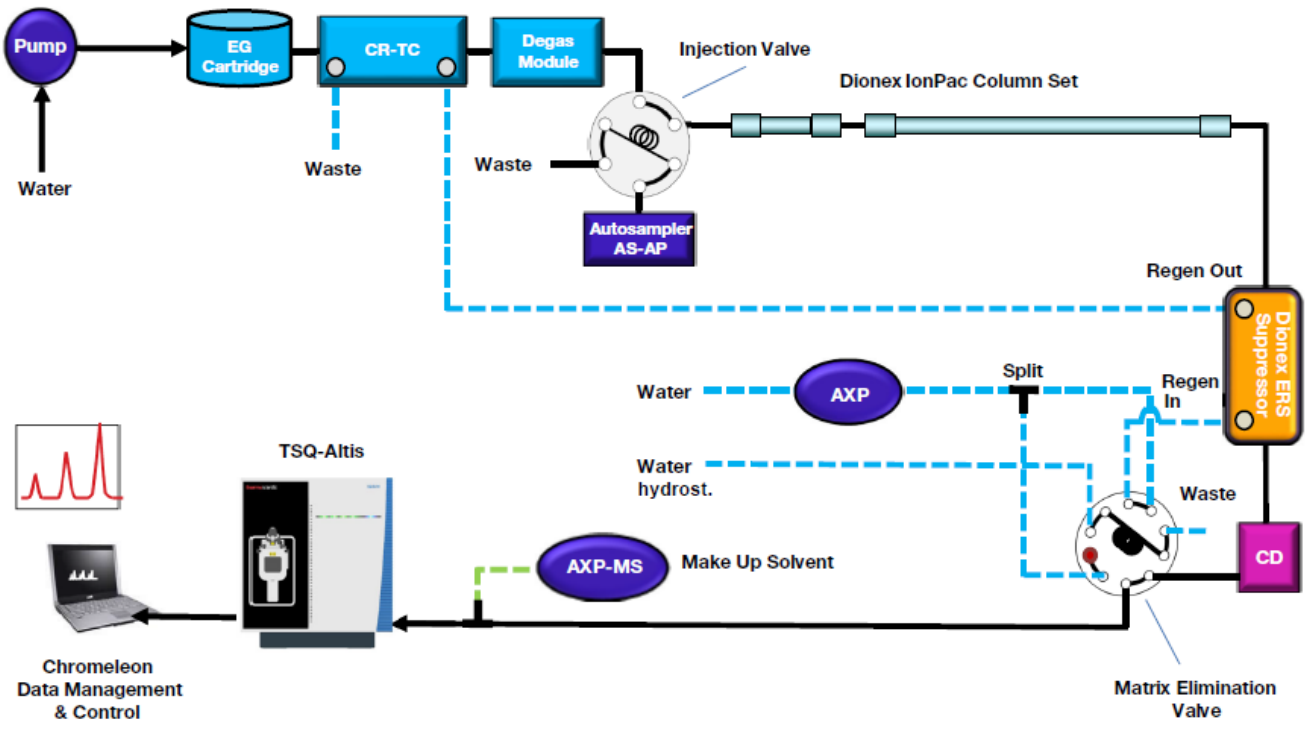

Figure 1. Schematic IC-MS/MS-configuration; Position of matrix elimination: "Off".

Table 1 (part 1). Conditions for ion chromatography

IC system:	Dionex ICS-6000 HPIC system		
Columns:	Dionex IonPac AG19-4µm Guard, 2 × 50 mm (P/N 083225) Dionex IonPac AS19-4µm Analytical, 2 × 250 mm (P/N 083223)		
Eluent source:	Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge (P/N 075778) with Thermo Scientific™ Dionex™ CR-ATC 600 (P/N 088662)		
KOH gradient:	Time (min)	KOH (mM)	Matrix elimination
	0.0	Start	Off
	0.0	20	
	2.5		On*
	4.0	20	
	5.5		Off*
	16.0	60	
	18.0	60	
	18.1	80	
	19.0	80	
	19.1	20	On*
	24.0	End of Run	

*On: Effluent to waste Off: Effluent to MS

Table 1 (part 1). Conditions for ion chromatography (Continuation)

Flow rate:	0.25 mL/min
Injection volume:	10 µL (push full mode)
Temperature:	25 °C (column compartment) 20 °C (detector compartment) 35 °C (conductivity detector cell)
System backpressure:	<3300 psi (100 psi = 0.6895 MPa)
Suppressor:	Suppressed Conductivity, Dionex ADRS 600 Suppressor (2 mm) used in the dynamic regeneration mode (3.8 V), AutoSuppression, external water mode via a Dionex AXP pump, external water flow rate (0.5 mL/min)
Background conductance:	<0.5 µS/cm
Run time:	24 min
IC-MS interface:	Tee union (PEEK, P/N 00101-18204) to combine the effluent from the conductivity detector via Thermo Scientific™ Viper™ tubing with the makeup solution. Use Viper connections between grounding union and H-ESI spray insert
Post-suppressor makeup solution:	2-propanol at 0.15 mL/min via a DionexAXP-MS pump

Table 1 (part 2). Conditions for mass spectrometric detection

Ion source settings	
Ion source type:	H-ESI
Spray voltage:	Static
Negative ion:	3,500 V
Sheath gas:	30 Arbitrary units (Arb)
Aux gas:	10 Arb
Sweep gas:	0 Arb
Ion transfer tube temp.:	250 °C
Vaporizer temp.:	350 °C
Probe setting:	Vertical: L/M Horizontal: 1.1 Side-to-side: Center
MS global settings	
Start time:	0 min
End time:	24 min

Master scan	
Scan mode:	SRM
Polarity:	Negative
Use cycle time:	True
Cycle time:	0.6 s
Q1 resolution (FWHM):	0.7
Q3 resolution (FWHM):	1.2
CID gas:	2.0 mTorr
Source fragmentation:	0 V
Chromatographic peak width:	6 s
Transition conditions:	Optimized for each compound using the automated compound optimization tool (Tab 2)

Table 2. IC-MS/MS parameters for selected SRM transitions for glyphosate, AMPA, and glufosinate

Compound	t_{ms} (min)*	Transition	Precursor *(m/z)*	Product *(m/z)*	Collision Energy (V)	RF Lens (V)
Glufosinate	10.3	Quantifier	180	63	40	60
		Qualifier 1	180	95	20	
		Qualifier 2	180	136	18	
AMPA	10.5	Quantifier	110	79	28	49
		Qualifier 1	110	63	25	
		Qualifier 2	110	81	14	
Glyphosate	20.6	Quantifier	168	63	24	50
		Qualifier 1	168	79	28	
		Qualifier 2	168	124	12	
		Qualifier 3	168	150	10	

MS conditions

All precursors, quantifiers, and qualifiers were individually determined using standards. Typical conditions are summarized in Table 1 and Table 2. Because the target analytes are small molecules with low mass-to-charge (*m/z*) product-ions, the mass spectrometer was calibrated using the Thermo Scientific™ Pierce™ Triple Quadrupole Extended Mass Range Calibration Solution (P/N 88340), which contains 14 components (mass range from 69 *m/z* to 2800 *m/z*) for calibration in both positive and negative ionization modes. This solution improves mass accuracy and transmission compared to conventional polytyrosine mass calibration solution, especially in the low *m/z* range.[18]

Samples and sample preparation

The honey samples were sourced from regional commercial and private production. The samples (~2.8 g) were diluted with DI water to a volume of 25 mL, thoroughly mixed, and filtered through a nylon filter (0.2 µm pore size). The ready to inject solutions (original and spiked) were adjusted to hold 100 ± 0.5 g/L honey. Aliquots were transferred to polymeric sample vials, which prevent analyte loss, avoiding wall adsorption effects known for glass vials.[18]

RESULTS AND DISCUSSION

Direct analysis of the honey samples

For direct examination of the diluted honey samples, the setup shown in Figure 1 was chosen, and the second valve was left in the "Off"-position shown. Thus, the sugar matrix and the anions and target components reached the MS.

Based on the findings of the pilot study by Pareja et al., we decided not to use isotopically labeled internal standards (ILIS) and evaluate the honey samples using the standard addition method.[25] To determine the matrix effects (ME), the target components were calibrated externally with aqueous, matrix-free standards in the range of 0.1 µg/L to 5 µg/L. Analytical characteristics are listed in Table 3.

Due to the high sensitivity of the TSQ Altis MS, 10 µL of the diluted honey solution (100 g/L) was injected. The amount of sugar injected, and thus the load on the inlet cone, increases at the same time. The absolute value of ME (Equation 1) for glyphosate was less than 30% in our experiments and, therefore, comparable to the reported literature values.[25]

$$ME = \frac{Slope\ of\ standard\ addition - Slope\ of\ external\ calib.}{Slope\ of\ external\ calibration} X\ 100$$

Equation 1. Calculation of the ME30

Our tests showed excellent instrument stability. Despite the reduced injection volume, deposits on the inlet cone could still form with continued analysis.

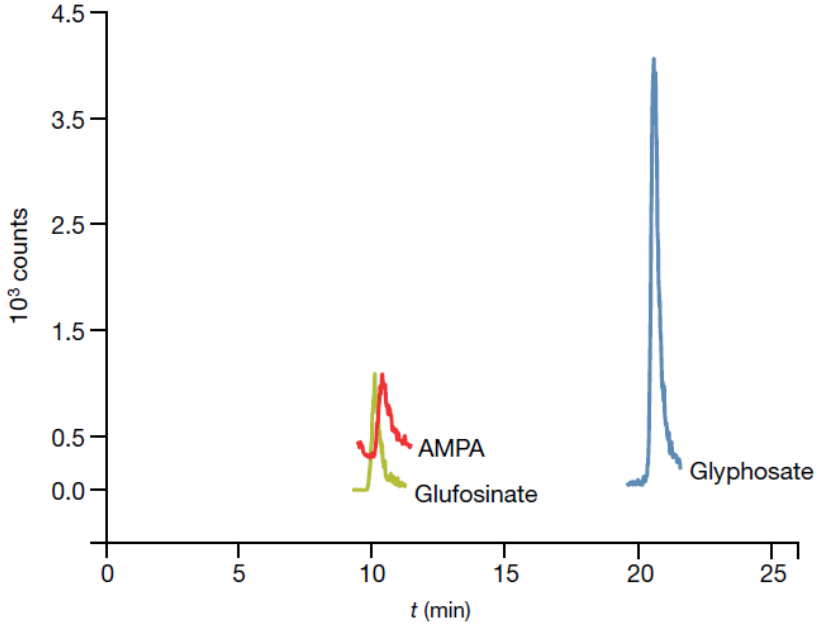

Figure 2. SRM chromatograms for glyphosate, AMPA, and glufosinate from a standard solution (ρ = 0.4 µg/L for each target component). Conditions: see Experimental section.

Table 3. Characteristics of the external calibration and retention times; calibration range for the target components: 0.1–5 µg/L

Component	Correlation coefficient (r^2)	Evaluation	Limit of detection LOD (µg/L)	Limit of quantitation LOQ (µg/L)	Standard deviation t_{ms} (min)*
Glufosinate[a]	>0.9999	Peak Area	0.06	0.2	<0.1
AMPA[b]	>0.9998	Peak Area	0.20	0.5	<0.1
Glyphosate[b]	>0.9997	Peak Area	0.20	0.7	<0.1

[a] Calculation of the limit of detection and limit of quantification according to ISO 8466-2.31; [b] Calculation of limit of detection and limit of quantification according to DIN 32645.32; n = 5, confidence level = 99.5%, tolerated error at the limit of quantification 33.3%; * n = 42, including real samples and matrix-free solutions.

Figure 3 and Figure 4 show representative chromatograms of wild honey and blossom honey. Both figures combine the chromatogram of the conductivity detector and the SRM traces of the analytes. The method is suited for the simultaneous determination of anionic honey constituents (e.g., organic acids, inorganic anions) after appropriate peak assignment and calibration.[33,34] The glyphosate content in wild honey was below the required detection limit of 50 µg/kg. The investigated blossom honey, however, showed a glyphosate content of more than three times the permitted value (Figure 4).

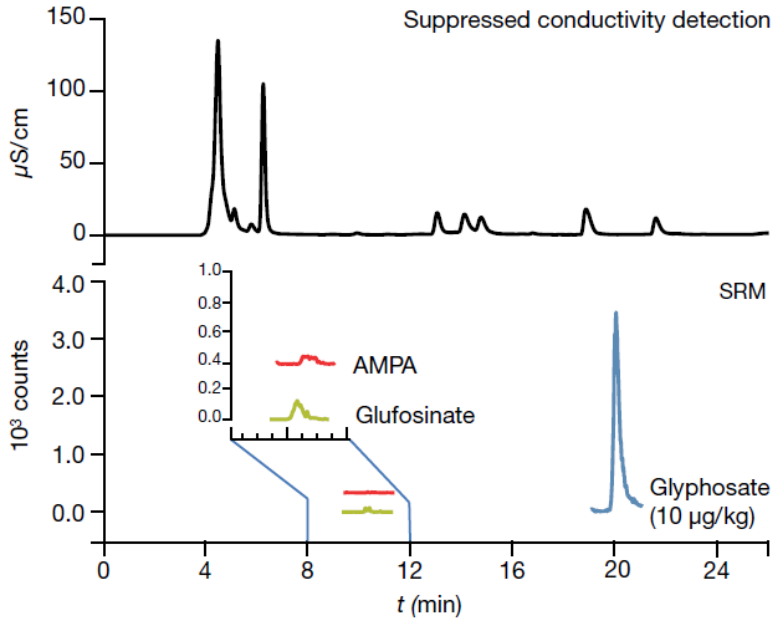

Figure 3. Representative chromatogram of diluted wild honey. Conditions: see Experimental section. Detection: conductivity after suppression (black) and SRM chromatograms for glufosinate (green), AMPA (red), and glyphosate (blue). Contents: Glufosinate (<3 µg/kg), AMPA (<2 µg/kg), glyphosate (10 µg/kg). The concentrations are those calculated for the original honey sample.

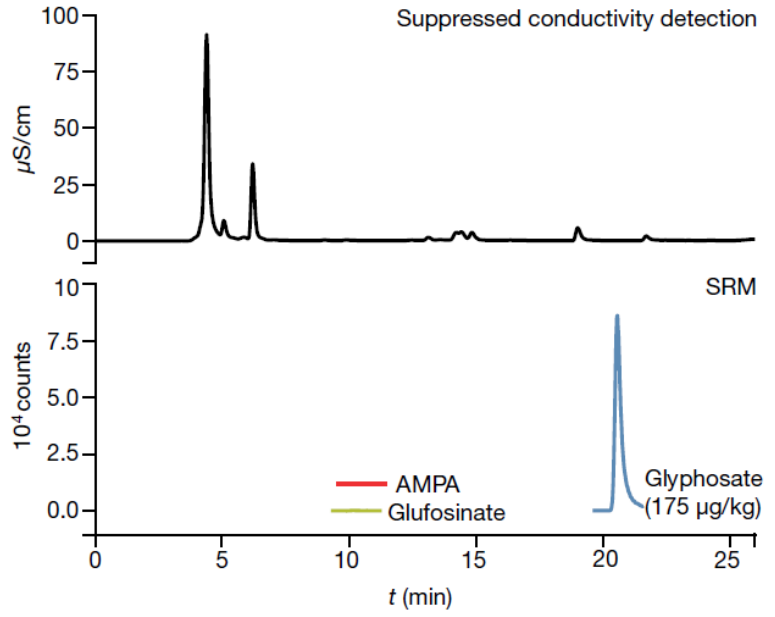

Figure 4. Representative chromatogram of diluted blossom honey showing high glyphosate content. Conditions: see Experimental section. Detection: conductivity after suppression (black) and SRM chromatograms for glufosinate (green), AMPA (red), and glyphosate (blue). Contents: Glufosinate (<1 µg/kg), AMPA (not detectable (n.n.), glyphosate (175 µg/kg). The concentrations are those calculated for the original honey sample.

Inline matrix elimination

Although only 10 µL of the diluted honey solution were injected, the high sugar load (70–80 g/L) was sufficient to lead to discoloration of the MS inlet cone. To minimize the effect, automated matrix elimination was set up. It uses a timed second switching valve, which directs the effluent to the waste instead of the mass spectrometer (Figure 1).

Determination of the switching times

Guyong et al.[35] reported mono- and disaccharides to elute from a classical Dionex IonPac anion exchange column at the beginning of the chromatogram. The appropriate switching times were determined using an amperometric detector instead of suppressed conductivity detection, allowing the carbohydrate detection at high pH.[36-39] The elution of the sugar matrix (glucose, fructose, sucrose) starts at 2.5 min, and the main part of the sugar matrix has eluted at 5.5 min (Figure 5). Through timed actuation of the matrix elimination valve (Figure 1, Table 1) the column effluent does not reach the MS, and the sugar matrix is diverted to waste.

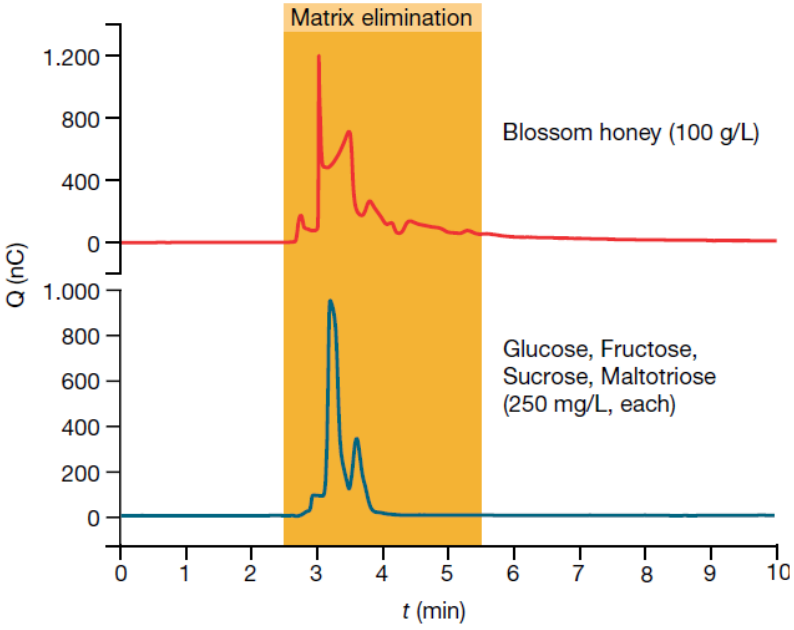

Figure 5. Chromatograms of diluted blossom honey and a sugar reference solution to determine the time segment of the matrix elimination. Conditions: see Experimental section. Detection: Pulsed amperometry on Au (four-potential pulse sequence against Ag/AgCl).[37] The first ten minutes of chromatograms are shown.

Figure 6 shows the chromatographic analysis of a local beekeeper honey, where the sugar matrix was eliminated before MS.

In addition to the LOD and LOQ calculations based on the calibration function, we determined the method detection limit (MDL). The sample used for the repetitive injections contained 0.8 µg/L of glufosinate, AMPA, and glyphosate.

$$MDL = t_{(n-1,\ 1-\alpha=0.99)} \cdot S$$

Equation 2. Calculation of MDL[40]

MDL = the method detection limit based on samples; t(n-1, 1-α=0.99) = the Student's t-value, single-tailed 99th percentile t statistic, n-1 degrees of freedom; S = sample standard deviation of the replicate sample analyses.

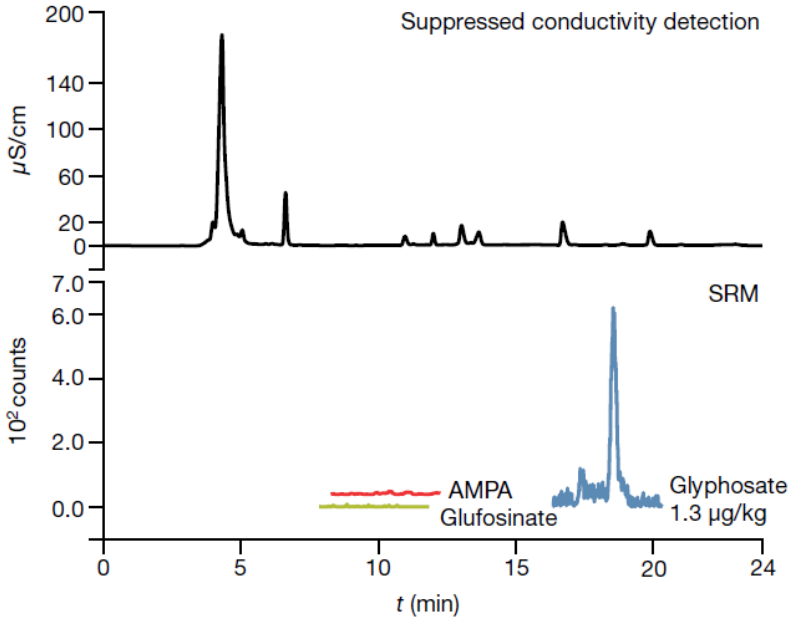

Figure 6. Representative chromatogram of diluted honey from a local beekeeper using matrix elimination. Conditions: see Experimental sections. Detection: conductivity after suppression (black) and SRM chromatograms for glufosinate (green), AMPA (red), and glyphosate (blue). Contents: Glufosinate (n.n.), AMPA (n.n.), glyphosate (1.3 µg/kg). The concentrations are those calculated for the original honey sample.

Table 4. Determination of MDL. Sample target concentration 0.8 µg/L, each; n = 9

	Amount (µg/L) MS quantitation peak		
Replicate	**Glufosinate**	**AMPA**	**Glyphosate**
1	0.80	0.63	0.83
2	0.79	0.63	0.80
3	0.85	0.77	0.76
4	0.77	0.63	0.78
5	0.80	0.80	0.75
6	0.78	0.66	0.79
7	0.79	0.71	0.83
8	0.77	0.63	0.78
9	0.75	0.57	0.83
Sample standard deviation (*S*)	0.03	0.07	0.03
MDL	0.09	0.21	0.09

$t_{(8,\ 0.99)}$ = 2.896

Our results show that the original analytical characteristics of the glufosinate, AMPA, and glyphosate determination remain unchanged. The most prominent advantage of matrix elimination is, therefore, the prevention of undesirable matrix effects and matrix buildup on the MS inlet cone (Figure 7).

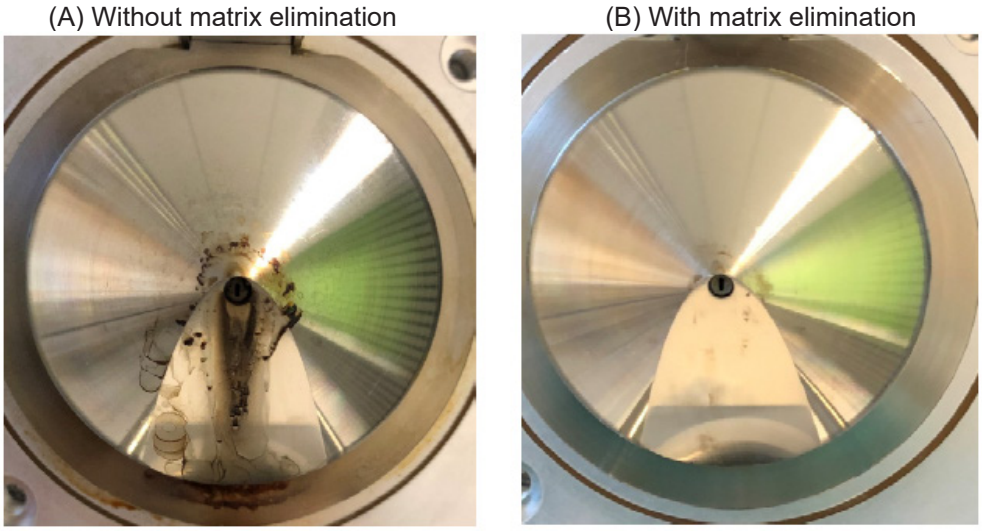

Figure 7. Comparison of inlet cone (~40 test injections): (A) Without matrix elimination, (B) With matrix elimination. Conditions: see Experimental section.

SUMMARY

Trace levels of glyphosate, AMPA, and glufosinate can reliably be determined using IC-MS/MS in diluted honey. In the combination of IC with MS, the continuously electrolytically regenerated membrane suppressor acts as a desalter through which the alkaline eluent is converted into water. The resulting effluent is directed to the MS interface. The applied chromatographic conditions allow the automated, inline elimination of the sugar matrix. It reduces the matrix effect on the MS hardware, and the uninterrupted operating time of the analysis system increases. The LODs and LOQs are well below the values required by the EU. The method presents itself as a reliable and cost-effective analytical tool for routine analysis of glyphosate, AMPA, and glufosinate in honey.

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New Performance for High Volume Agriculture Laboratories

Enabling high throughput in elemental analysis of several agriculture matrices using Milestone's ETHOS UP with MAXI 24 HP

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INTRODUCTION

Fertilizer is a fundamental component for the growing of plants. However, too much of the wrong nutrient can have adverse effects such as burning the roots. Characterizing the fertilizer content will indicate the formula's macronutrient content, as well as other nutrients such as calcium, magnesium, potassium, etc. Analysis on a fertilizer product gives the ratio of each macronutrient, which must be correct to ensure optimal efficacy. ICP analysis can provide a lot of information on fertilizer composition but the choice of the correct sample preparation technique is fundamental. Traditional sample preparation techniques include hot block digestion, closed vessel microwave digestion and ashing; each of them posing different challenges. Hot block digestions suffer from long run times, airborne contamination, poor digestion quality, and poor recovery of volatile compounds. Closed vessel microwave digestion has proven to be an effective technique with fast, complete digestions, a clean environment, and full recovery of volatile compounds. Milestone's ETHOS UP equipped with the MAXI 24 High Performance (HP) rotor incorporates all of the benefits of closed vessel microwave digestion while making sample preparation fast, easy, effective, and of the highest quality. This innovative solution perfectly integrates with the powerful ETHOS UP, matching both performance and throughput requirements of agricultural elemental analysis.

EXPERIMENTAL

In this industry report, a recovery study on certified reference materials has been performed to prove the efficacy of the ETHOS UP in sample preparation for metal analysis.

Instrument

The ETHOS UP is the most advanced microwave sample preparation equipment. It meets the requirements of modern analytical labs.

Figure 1. Milestone's ETHOS UP

Figure 2. MAXI-24 HP Rotor

The ETHOS UP used in this study was equipped with MAXI-24 HP rotor controlled via Milestone's easyTEMP contactless temperature. The superior temperature measurement of easyTEMP allows the processing of different samples of similar reactivities, thus reducing labor time and increasing the overall throughput.

MAXI-24 HP Rotor

The latest Milestone's development is the MAXI-24 HP, which combines performance and throughput within a single rotor-based platform. It completely innovates the rotor-base solutions providing high throughput without sacrificing the performance. Thanks to its 24 positions, it is the first high pressure and throughput rotor available in the market. The completely new design of its vessels allows to achieve conditions never seen for high throughput rotors. Thicker high purity PTFE-TFM vessels and caps, along with rugged PEEK shields are key ingredients to handle the conditions required to completely digest these samples.

Procedure

Table 1 reports the conditions used to prepare the sample.

Table 1. Sample amount and acid mixture used for the microwave digestion run

SAMPLE	SAMPLE AMOUNT	ACID MIXTURE
Multi-nutrient	0.2 g	8 mL of HNO_3 (65%), 0.5 mL of H_2SO_4 (96%), 2 mL of HF (48%)
Marine sediment (IAEA-457)*	0.5 g	9 mL of HNO_3 (65%), 3 mL of HCl (37%)
San Joaquin soil (NIST 2709a)*	0.5 g	9 mL of HNO_3 (65%), 3 mL of HCl (37%)
Tomato leaves (NIST 1573a)	0.5 g	5 mL of HNO_3 (65%) + 1 mL of H_2O_2 (30%)
Cabbage (IAEA-359)	0.5 g	5 mL of HNO_3 (65%) + 1 mL of H_2O_2 (30%)

* EPA 3051A was applied

All samples were weighted into the MAXI–24 HP vessel, approximately 0.5 g (as reported in Table 1). The acid mixture (trace metal grade) was added according to the data reported in Table 1 and the proper microwave method has been used as reported in Table 2.

Table 2. Microwave program

STEP	TIME	T2	POWER
1	00:10:00	160 °C	1500 W
2	00:15:00	210 °C	1800 W
3	00:10:00	210 °C	1800 W

After microwave digestion, the samples were diluted to 50 mL with deionized water and analyzed by ICP-OES.

Quantification

ICP-OES Instrumental Parameters: RF power (W): 1300; Plasma flow (L/min): 15.0; Auxiliary Flow (L/min): 1.5; Nebulizer Flow (L/min): 0.75; Replicate read time (s): 10; Instrument stabilization delay (s): 15; Sample Uptake Delay (s): 30; Pump Rate (rpm): 15; Rinse Time (s): 10; Replicates: 3.

RESULTS AND DISCUSSION

The performance of the Milestone ETHOS UP equipped with MAXI-24 HP rotor and easyTEMP technology was evaluated through a recovery study on multi-nutrient fertilizer (NIST SRM695), marine sediment (IAEA 457), San Joaquin soil (NIST 2709a), tomato leaves (NIST 1573a) and cabbage (IAEA 359) samples. The samples were digested with Milestone's ETHOS UP and subsequently analyzed via ICP-OES.

Table 3. Data of the recovery study on multi-nutrient fertilizer (NIST SRM695) sample

	Certified value	Recovery % (n=3)	RSD (%)
Al	0.61 ± 0.03%	89.3	2.3
As	200 ± 5 mg/Kg	96.7	2.6
Ca	2.26 ± 0.04%	103.5	2.8
Cd	16.9 ± 0.2 mg/Kg	93.5	1.7
Co	65.3 ± 2.4 mg/Kg	94.1	2.3
Cr	244 ± 6 mg/Kg	88.9	2.6
Cu	1225 ± 9 mg/Kg	91.7	1.7
Fe	3.99 ± 0.08%	89.7	2.3
Hg	1955 ± 0.036 mg/Kg	95.6	2.6
K	11.65 ± 0.13%	92.6	1.9
Mg	1.79 ± 0.05%	105.9	2.7
Mn	0.305 ± 0.005%	101.5	2.4
Mo	20.0 ± 0.3 mg/Kg	93.3	1.4
Na	0.405 ± 0.007%	95.6	2.2
Ni	135 ± 2 mg/Kg	90.0	2.3
Pb	273 ± 17 mg/Kg	102.3	2.6
V	122 ± 3 mg/Kg	99.6	1.0
Zn	0.325 ± 0.005 mg/Kg	101.3	2.1

Table 4. Data of the recovery study on marine sediment (IAEA-457) sample

	Certified value	Recovery % (n=3)	RSD (%)
Ag	1.93 ± 0.38 mg/Kg	94.1	1.4
Al	82660 ± 3430 mg/Kg	96.3	1.3
As	10.2 ± 1.0 mg/Kg	109.4	2.5
Cd	1.09 ± 0.08 mg/Kg	102.9	1.9
Co	14.7 ± 1.0 mg/Kg	90.0	2.1

Table 4. Data of the recovery study on marine sediment (IAEA-457) sample (Continuation)

	Certified value	Recovery % (n=3)	RSD (%)
Cr	144 ± 8 mg/Kg	89.9	2.0
Cu	365 ± 19 mg/Kg	91.3	1.4
Fe	41450 ± 2240 mg/Kg	91.6	0.9
Hg	0.143 ± 0.012 mg/Kg	93.2	1.3
Li	64.2 ± 5.5 mg/Kg	94.7	1.8
Mn	427 ± 30 mg/Kg	93.1	2.8
Ni	53.1 ± 2.7 mg/Kg	93.0	1.4
Pb	105 ± 7 mg/kg	91.4	1.2
Sn	27.40 ± 0.75 mg/Kg	93.7	2.1
Sr	137 ± 10 mg/Kg	94.1	1.1
V	87.4 ± 8.1 mg/Kg	101.3	2.0
Zn	425 ± 25.8 mg/Kg	96.8	2.5

Table 5. Data of the recovery study on San Joaquin soil (NIST 2709a) sample

	Certified value	Recovery % (n=3)	RSD (%)
Al	7.37 ± 0.16%	93.4	2.3
Ba	979 ± 28 mg/Kg	91.6	2.6
Ca	1.91 ± 0.09%	89.9	2.8
Cd	0.371 ± 0.002 mg/Kg	<LOQ	—
Co	12.8 ± 0.2 mg/Kg	94.2	2.7
Cr	130 ± 9 mg/Kg	90.7	2.4
Fe	3.36 ± 0.07%	83.3	1.4
K	2.11 ± 0.06%	92.8	2.2
Mg	1.46 ± 0.02%	102.8	2.3
Mn	529 ± 18 mg/Kg	98.1	2.6
Na	1.22 ± 0.03 %	93.6	2.8
P	0.0688 ± 0.0013%	113.1	1.7
Pb	17.3 ± 0.1 mg/kg	92.4	1.4
Sb	1.55 ± 0.06 mg/Kg	<LOQ	1.9
Si	30.3 ± 0.4%	96.3	1.2
Sr	239 ± 6 mg/Kg	95.7	2.3

Table 5. Data of the recovery study on San Joaquin soil (NIST 2709a) sample (Continuation)

	Certified value	Recovery % (n=3)	RSD (%)
Ti	0.336 ± 0.007%	91.3	2.6
V	110 ± 11 mg/Kg	102.0	2.8
Zr	195 ± 46 mg/Kg	94.4	1.7

Table 6. Data of the recovery study on tomato leaves (NIST 1573A) sample

	Certified value	Recovery % (n=3)	RSD (%)
Al	598.4 ± 7.1 mg/Kg	94.1	2.3
As	0.1126 ± 0.0032 mg/Kg	<LOQ	—
Ca	50450 ± 550 mg/Kg	96.7	1.0
Cd	1.517 ± 0.027 mg/Kg	92.1	2.1
Co	0.5773 ± 0.0071 mg/Kg	<LOQ	—
Cr	1.988 ± 0.034 mg/Kg	90.9	2.6
Cu	4.70 ± 0.14 mg/Kg	96.0	2.8
Fe	367.5 ± 4.3 mg/Kg	96.8	1.7
Hg	0.0341 ± 0.0015 mg/Kg	92.3	2.3
K	26760 ± 480 mg/kg	99.7	2.6
Mn	246.3 ± 7.1 mg/Kg	101.0	1.7
Na	136.1 ± 3.7 mg/Kg	97.1	2.3
Ni	1.582 ± 0.041 mg/Kg	93.5	2.6
P	2161 ± 28 mg/Kg	90.6	2.2
Rb	14.83 ± 0.31 mg/K.g	90.2	1.4
Sb	0.0619 ± 0.0032 mg/kg	<LOQ	—
Se	0.0543 ± 0.0020 mg/Kg	<LOQ	—
V	0.835 ± 0.034 mg/Kg	<LOQ	—
Zn	30.94 ± 0.55 mg/Kg	94.5	2.4

Table 7. Data of the recovery study on cabbage (IAEA-359) sample

	Certified value	Recovery % (n=3)	RSD (%)
As	0.10 ± 0.004 mg/Kg	<LOQ	—
Ba	11.0 ± 0.5 mg/Kg	91.6	1.2
Ca	18500 ± 510 mg/Kg	92.5	2.6

Table 7. Data of the recovery study on cabbage (IAEA-359) sample (Continuation)

	Certified value	Recovery % (n=3)	RSD (%)
Cd	0.12 ± 0.005 mg/Kg	<LOQ	—
Cr	1.30 ± 0.06 mg/Kg	90.3	2.0
Cu	5.67 ± 0.18 mg/Kg	94.8	2.1
Fe	148 ± 3.9 mg/Kg	95.2	1.6
Hg	0.013 ± 0.002 mg/Kg	94.1	0.7
K	32500 ± 690 mg/Kg	96.3	1.6
Mg	2160 ± 50 mg/Kg	91.4	1.7
Mn	31.9 ± 0.6 mg/Kg	98.9	2.9
Na	580 ± 21 mg/Kg	103.4	1.1
Ni	1.05 ± 0.05 mg/kg	95.5	1.4
Se	0.12 ± 0.011 mg/Kg	<LOQ	—
Sr	49.2 ± 1.4 mg/Kg	93.1	1.9
Zn	38.6 ± 0.7 mg/Kg	91.9	2.1

The analytical results were shown in Tables 3-7 with good recoveries of all elements and RSDs below 3%. This demonstrates the robustness and reproducibility of the digestion process with the ETHOS UP – MAXI-24 HP.

Figure 3 shows the temperature profile of the digestion as well as the multiple temperature visualization and recording for all the samples digest in the run.

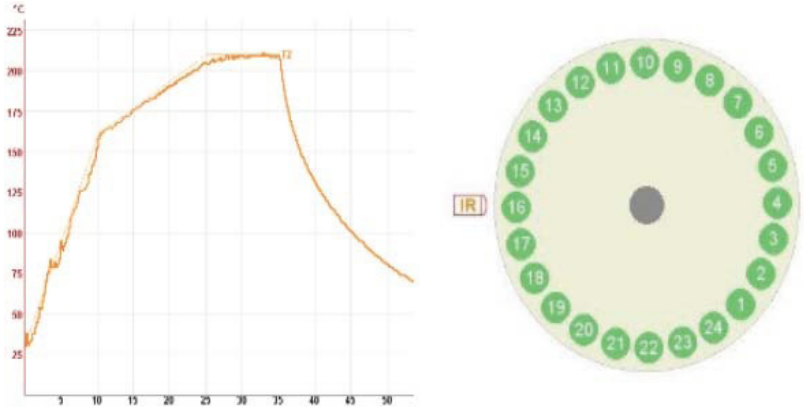

Figure 3. MAXI-24 HP Microwave Run Report and Multiple temperature traceability.

CONCLUSION

The data shown in this industry report demonstrates full recovery of the elements reported in the certificates of the reference material. Highly reactive samples such as fertilizer has been completely digested even in large sample amounts. The digestion process has been accurately controlled by the

easyTEMP sensor, ensuring same digestion quality and reliable results. In addition, microwave digestion using the Milestone ETHOS UP with easyTEMP control, provides the highest level of reproducibility and great ease of use, ensuring high quality digestion run after run.

Further reading here

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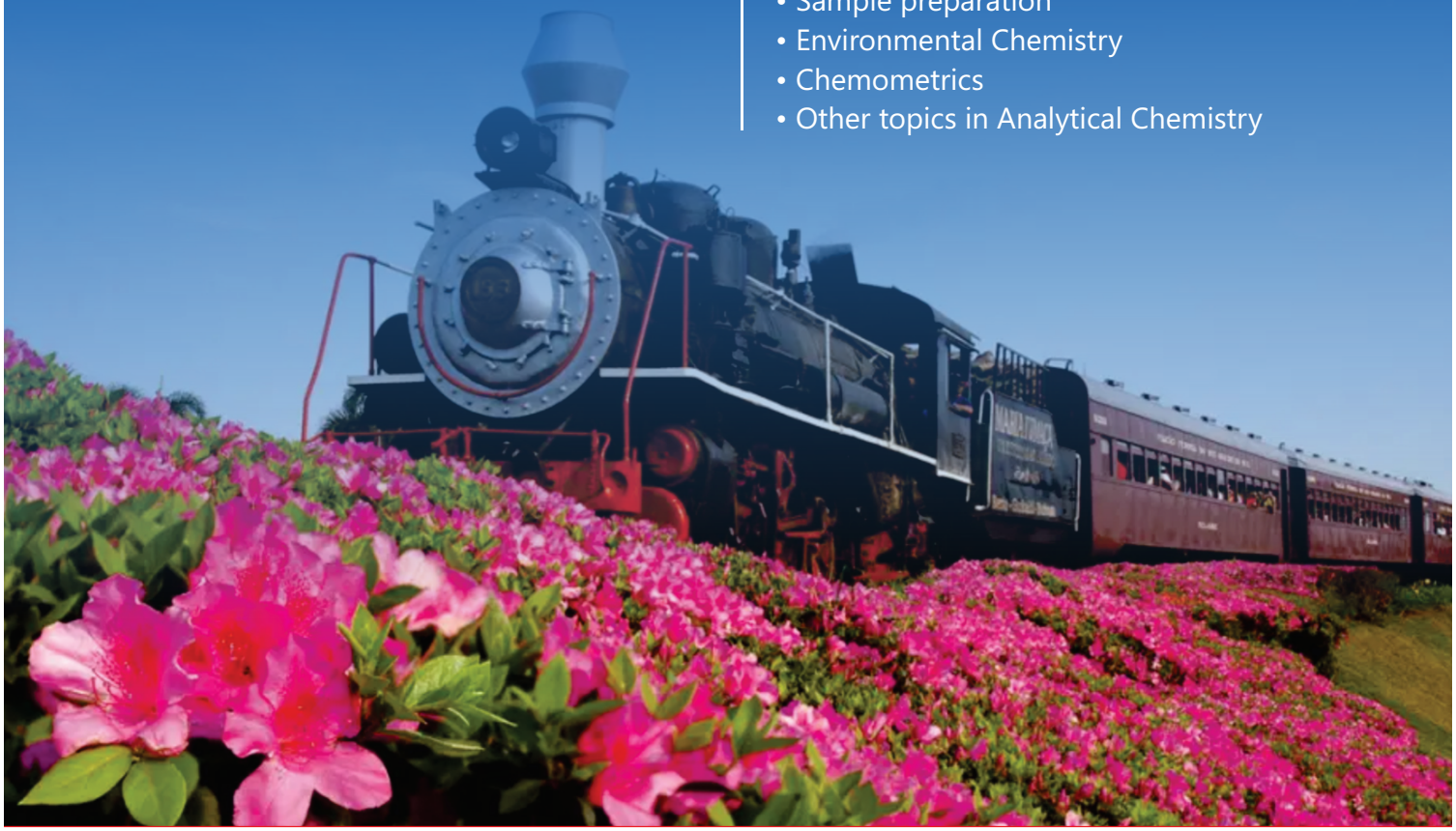

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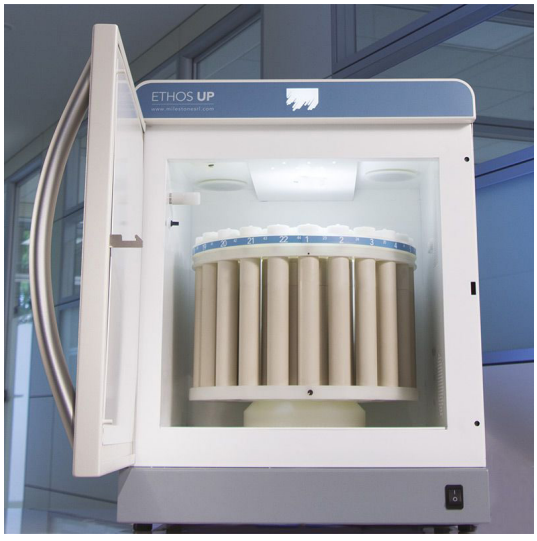
ETHOS UP High Performance Microwave Digestion System



Quality Analisis Starts with Great Sample Prep

The ETHOS UP™ fully embodies Milestone's philosophy in microwave sample preparation. Specifically designed for closed vessel microwave acid digestion, it offers productivity, safety, ease of use, connectivity, expertise and application flexibility.

Safety and reliability

Best microwave hardware, temperature and pressure sensors. The cavity has a volume in excess of 70 L, and is constructed entirely of stainless steel to ensure longevity and structural integrity. Its unique pressure responsive door, also made of stainless steel, ensures superior safety even with the most reactive samples. The safeVIEW high definition digital camera, allows to safely look into the microwave cavity.

Performance and throughput

The MAXI-24 High Performance (HP) and high throughput rotor was designed with double pressure limits and greater capacity than other high throughput rotors. It processes increased volumes of sample and a wide variety of matrices within a single rotor-based platform. Quick-assembly vessels are closed with a simple hand-tightening, improving your daily productivity.

EasyCONTROL operating software

Milestone pioneered the use of infrared sensors combined with an in-situ temperature sensor to more accurately control the digestion cycle.

Today, Milestone innovates again by combining all the benefits of in-situ and infrared sensors into a single solution: **the Milestone easyTEMP, a direct contactless temperature sensor.** The MAXI-24 HP, along with the built-in easyTEMP, contactless temperature control in all vessels, automatically controls the temperature even in case of exothermic reactions.

Flexibility

Rotors and pressure vessels are crucial components to ensure high digestion quality and safety. Milestone's rotors are build with high-purity PTFE-TFM, PEEK safety shields and a unique safety release mechanism.

Smart software to save your time

The ETHOS UP comes with a dedicated touch screen terminal with easyCONTROL software which incorporates our expertise and knowledge in microwave sample preparation. The ETHOS UP user-interface provides full control of all digestion parameters, complete documentation and improves the overall procedure.

Read more

Productivity UP.
Simplicity UP.
Safety UP.
Ethos UP.

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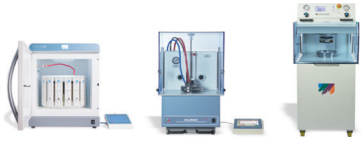

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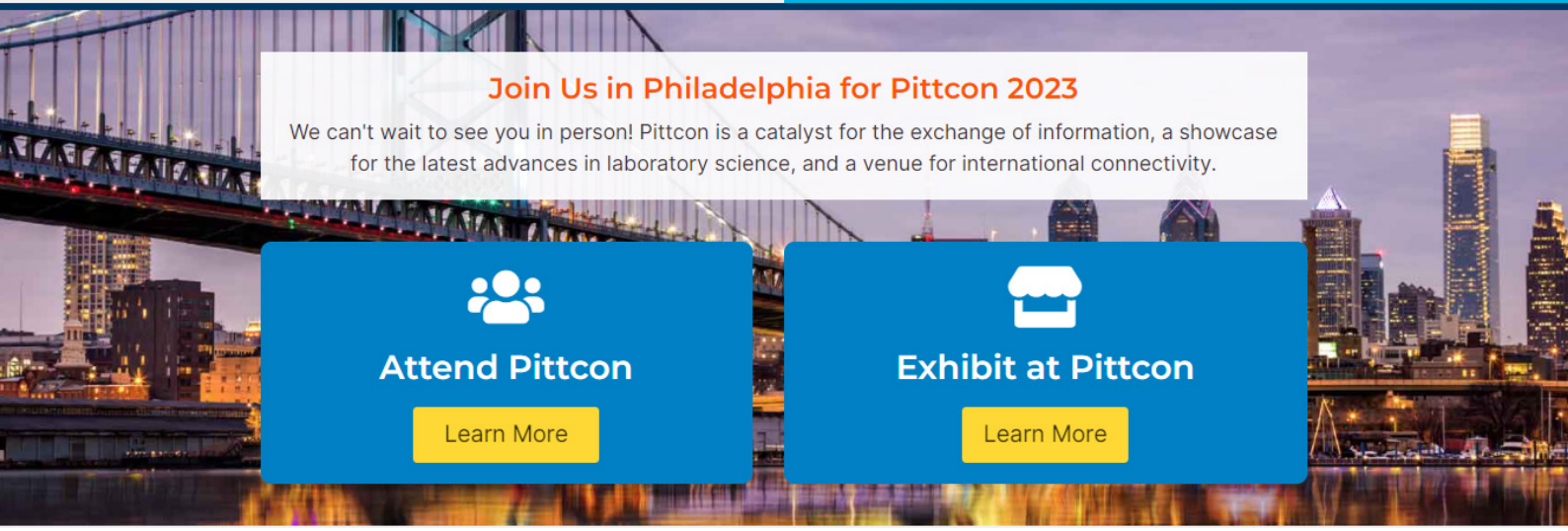

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By Dr. Michael Witting, Metabolomics & Proteomics Core, Helmholtz Centre Munich. Access **here**

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By Prof. Ian Wilson, Imperial College London. Access **here**

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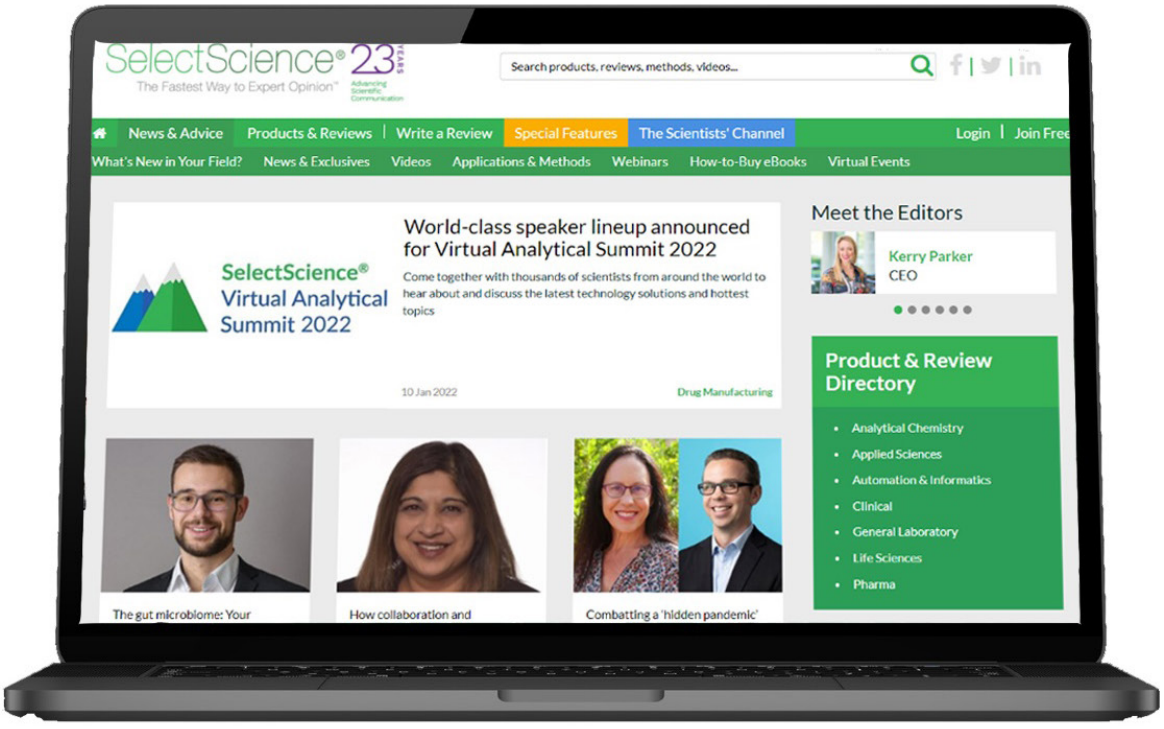

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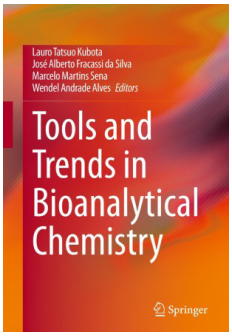
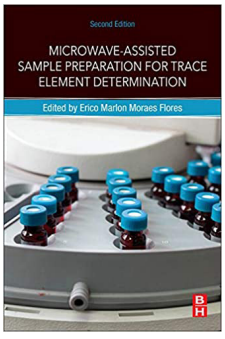


Tools and Trends in Bioanalytical Chemistry

Lauro T. Kubota, José Alberto F. da Silva, Marcelo M. Sena, Wendel A. Alves, Editors

2022. Publisher: Springer, Cham

This textbook covers the main tools and techniques used in bioanalysis, provides an overview of their principles, and offers several examples of their application and future trends in diagnosis. Explores the role of bioanalysis in different areas such as biochemistry, physiology, forensics, and clinical diagnosis, including topics from sampling/sample preparation, chemometrics in bioanalysis to the latest techniques used in the field. Read more

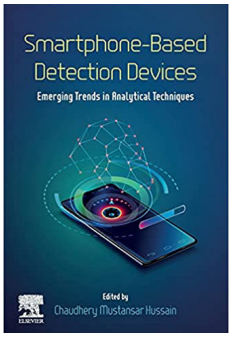


Microwave-Assisted Sample Preparation for Trace Element Determination 2nd Ed.

Érico M. M. Flores, Editor

December 2021. Publisher: Elsevier

This book covers all the new devices and more powerful systems that have emerged in the last several years, such as Ultrawave and Ultraclave systems. It offers a summary of the state-of-the-art ways to meet the challenges in the fields of geology, environmental and biological studies – as the need for further determination of rare earth elements and halogens. Read more

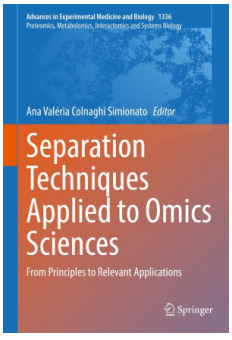


Smartphone-Based Detection Devices / Emerging Trends in Analytical Techniques

Chaudhery Mustansar Hussain, Editor

August, 2021. Publisher: Elsevier

This book gathers the modern developments in smartphone analytical methods into one comprehensive source, covering recent advancements in analytical tools while paying special attention to the most accurate, highly efficient approaches. It is an important source for researchers who require accurate analysis of their on- and off-site samples. Read more



Separation Techniques Applied to Omics Sciences / From Principles to Relevant Applications

Ana Valéria Colnaghi Simionato, Editor

October, 2021. Publisher: Springer International

First book to show relevant applications in genomics, proteomics, metabolomics and foodomics, concomitantly. It demonstrates that a multiplatform methodology is required for a comprehensive omics analyses, and separation techniques is of outcome importance. The main characteristics of each separation technique suitable to each omic approach is presented. Read more

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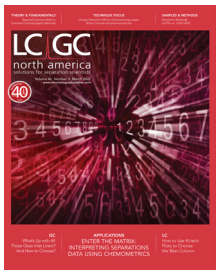
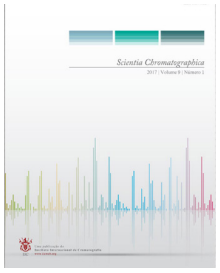


LCGC

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:** ***Enter the Matrix: Improving the Interpretation of Separations Data Using Chemometrics in Analytical Investigations*** Paiva, A.C.; Teixeira, C.A.; Cardoso, V.G.K.; Ferreira, V.H.C.; Sabin, G.P.; Hantao, L.W. This article describes a compendium of chemometrics applications in separation science to demonstrate the importance and synergy of data handling. Read more



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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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EVENTS in 2022 – It is suggested to consult the event's official website for updates.

May 25 – 28
XXII Brazilian Congress of Toxicology (CBTox 2022)
Balneário Camboriú, SC, Brazil
www.cbtox2021.com.br

May 29 – June 2
19th International GCxGC Symposium – VIRTUAL
https://www.gcxgc-symposium.com

May 29 – June 2
241st Meeting of the Electrochemical Society (ECS)
Vancouver, BC, Canada
https://www.electrochem.org/241

May 30 – June 3
Colloquium Spectroscopicum Internationale (CSI XLII 2022)
Gijon, Asturias, Spain
https://www.csi2022spain.com/en/

May 31 – June 3
45th Annual Meeting of the Brazilian Chemical Society (RASBQ)
Maceió, AL, Brazil
http://www.sbq.org.br/reunioes-anuais

June 5 – 9
18th International Conference on Electroanalysis (ESEAC 2022)
Vilnius, Lithuania
http://www.eseac2020.com/

June 21 – 23
Analitica Latin America Expo & Conference
São Paulo, SP, Brazil
https://www.analiticanet.com.br/

July 18 – 21
8th International Caparica Conference on Analytical Proteomics
Caparica, Portugal
https://www.icap2022.net/

August 24 – 26
Journées de Chimie Analytique, 11th Edition (JCA2022)
Yaounde, Cameroun
https://jca-2021.sciencesconf.org

August 29 – September 2
XVIII Chemometrics in Analytical Chemistry (CAC2022)
University of Rome La Sapienza, Rome, Italy
http://cac2022.sciencesconf.org

EVENTS in 2022 – It is suggested to consult the event's official website for updates.

September 5 – 8
National Meeting of Forensic Chemistry (ENQFor) & Meeting of the Brazilian Society of Forensic Sciences (SBCF)
Ribeirão Preto, SP, Brazil
https://www.enqfor.org.br/

September 5 – 8
51th Annual Meeting of the Brazilian Society of Biochemistry and Molecular Biology (SBBq) & 46th Congress of the Brazilian Society of Biophysics (SBBf)
Convention Center of the Majestic Hotel, Águas de Lindóia, SP, Brazil
https://www2.sbbq.org.br/reuniao/2022/

September 25 – 28
20th National Meeting of Analytical Chemistry (ENQA) & 8th Ibero-American Congress of Analytical Chemistry (CIAQA)
Bento Gonçalves, RS, Brazil
https://enqa.com.br/

September 25 – 29
XX Brazilian Materials Research Society Meeting (SBPMat)
Foz do Iguaçu, PR, Brazil
https://www.sbpmat.org.br/pt/

December 10 – 15
III Ibero American Conference on Mass Spectrometry (IBERO 2022)
Rio de Janeiro, RJ, Brazil
https://www.ibero2022.com/

GUIDELINES FOR AUTHORS

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The *Brazilian Journal of Analytical Chemistry* (BrJAC) is dedicated to the diffusion of significant and original knowledge in all branches of Analytical Chemistry and Bioanalytics. The BrJAC is addressed to professionals involved in science, technology and innovation projects at universities, research centers and in industry.

Professional Ethics

Manuscripts submitted for publication in BrJAC cannot have been previously published or be currently submitted for publication in another journal.

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- **Articles:** Full descriptions of an original research finding in Analytical Chemistry. Articles undergo double-blind full peer review.
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The Cover Letter must be signed by the submitting author.

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Four documents are mandatorily uploaded by the submitting author: Cover letter, Title Page, Novelty Statement and the Manuscript. Templates for these documents are available at www.brjac.com.br

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Review process

Manuscripts submitted to the BrJAC undergo an initial check for compliance with all of the journal's guidelines. Submissions that do not meet the journal's guidelines will be suspended and an alert sent to the corresponding author. The authors will be able to resend the submission within 30 days. If the submission according to the journal's guidelines is not made within 30 days, the submission will be withdrawn on the first subsequent day and an alert will be sent to the corresponding author.

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If the manuscript passes the screening described above, it will be forwarded to an Associate Editor who will also analyze the iThenticate similarity report and invite reviewers.

Manuscripts are reviewed in double-blind mode by at least 2 reviewers. A larger number of reviewers may be used at the discretion of the Editor. As evaluation criteria, the reviewers employ originality, scientific quality, contribution to knowledge in the field of Analytical Chemistry, the theoretical foundation and bibliography, the presentation of relevant and consistent results, compliance with the BrJAC's guidelines, clarity of writing and presentation, and the use of grammatically correct English.

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The 1st-round review process usually takes around 5-6 weeks. If the manuscript is not rejected but requires corrections, the authors will have one month to submit a corrected version of the manuscript. In another 3-4 weeks, a new decision on the manuscript may be presented to the corresponding author.

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