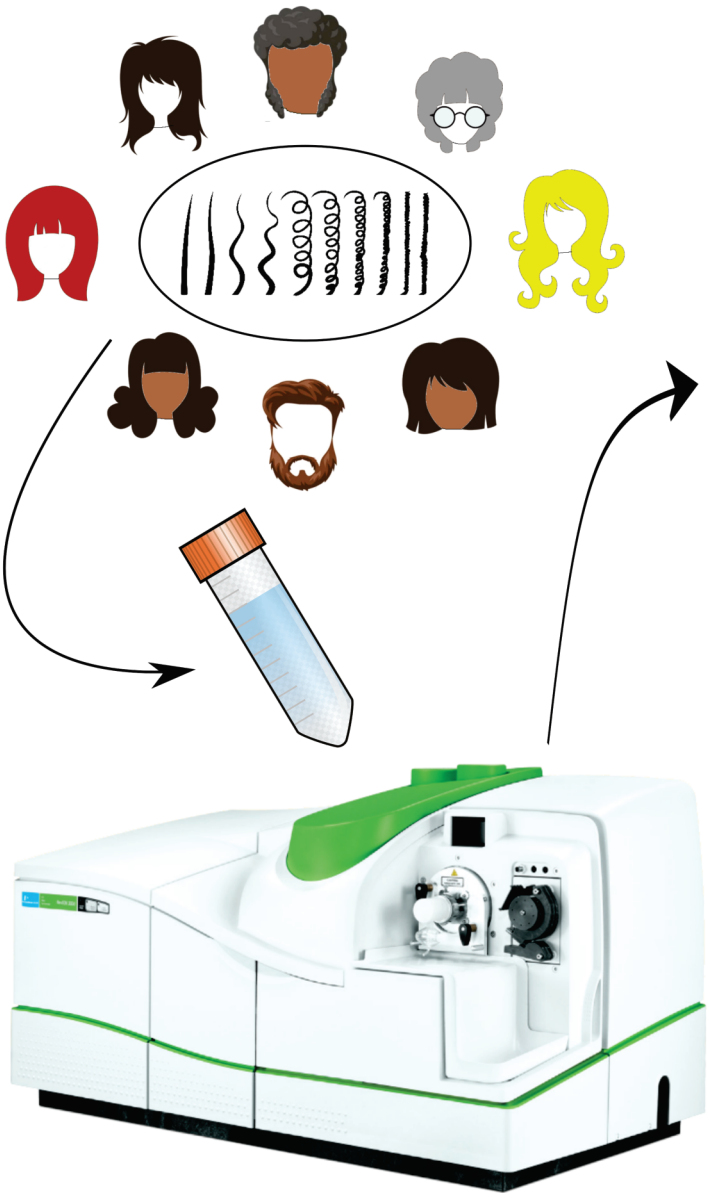
BrJAC
Brazilian Journal of Analytical Chemistry
an International Scientific Journal



Elementos tóxicos				
Elemento	Sigla	Resultado µg/g	Valor máximo	Percentual
Aluminio	(Al)	3.6	14.0	X
Antimônio	(Sb)	0.009	0.03	X
Arsênio	(As)	0.05	0.15	X
Bário	(Ba)	1.09	4	X
Berílio	(Be)	0	0.05	X
Bismuto	(Bi)	0.01	0.03	X
Cádmio	(Cd)	0.000	0.3	X
Chumbo	(Pb)	0.67	9.3	X
Mercúrio	(Hg)	0.61	2.3	X
Tório	(Th)	0.001	0.005	X
Urânio	(U)	0.018	0.02	X
Níquel	(Ni)	0.19	0.6	X
Prata	(Ag)	0.009	0.4	X
Estanho	(Sn)	0.04	0.35	X

Elementos Nutrientes:					
Elemento	Sigla	Resultado µg/g	Intervalo de Referência		Percentual
Cálcio	(Ca)	262	190	684	X
Magnésio	(Mg)	51	13	73	X
Sódio	(Na)	3	20	250	X
Potássio	(K)	0.6	8	75	X
Cobre	(Cu)	11.2	10	32	X
Zinco	(Zn)	181	140	240	X
Manganês	(Mn)	0.01	0.15	1.2	X
Cromo	(Cr)	0.45	0.4	0.65	X
Vanádio	(V)	0.112	0.004	0.03	X
Molibdênio	(Mo)	0.001	0.02	0.05	X
Boro	(B)	1.2	0.25	1.5	X
Iodo	(I)	-0.48	0.05	0.6	X
Lítio	(Li)	0	0.007	0.02	X
Fósforo	(P)	153	160	260	X
Selênio	(Se)	0.84	0.8	1.5	X
Estrôncio	(Sr)	0.73	0.6	4.3	X
Enxofre	(S)	49560	39000	56000	X
Cobalto	(Co)	0.005	0.003	0.03	X
Ferro	(Fe)	8.9	7	18	X

Outros Elementos					
Elemento	Sigla	Resultado µg/g	Referência		Percentual
Ouro	(Au)	0.020	0.002	0.07	X
Paládio	(Pd)	0.016	0.05		X
Germânio	(Ge)	-0.01	0.1		X

Razão				
Elementos	Razão	Intervalo esperado		
Ca/Mg	5	4	30	X
Ca/P	2	0.8	8	X
Na/K	5	0.5	10	X
Zn/Cu	16	4	20	X
Zn/Cd	-	800		

Hair Mineralogram Analysis for Health Assessment: Statistical Bias from Gender and Aesthetic Treatments

Gabrielly Peregrino, Carlos G. Massone, Adriana H. Nudi, Tatiana Dillenburg Saint'Pierre

This work is part of a project to encourage girls to the science, technology, engineering and mathematics (STEM) area

October – December 2021 Volume 8 Number 33

XXIII INTERNATIONAL MASS SPECTROMETRY CONFERENCE

RIO DE JANEIRO 2021

IMSC 2021 will cover all aspects of mass spectrometry, from fundamentals to instrumentation and applications, with the most recent findings and innovations and a very selected group of renowned speakers but with a great addition: our "Brazillian seasoning".

The conference is organized with short courses, oral and poster sessions, workshops, awards, commercial exhibitions, as well as very exciting social programs and companies user meetings. All major MS companies are supporting Rio 2021 and will be in the exposition area presenting their latest products and innovations.

We are working very hard to make Rio 2021 the largest and greatest ever IMSC and for it to be an exceptional occasion for you to enjoy science, to grow your network and make new friends and colleagues, to get new ideas for your research, for establishing new collaborations, starting new projects, and more; all in the unforgettably warm, beautiful and charming city of Rio de Janeiro!

Please come, bring your students, invite friends and colleagues and contribute to make Rio 2021 a very successful, most amazing and unforgettable IMSC ever!

Conference transferred to 2022

Access WEBSITE

Welcome to the

23rd **International Mass Spectrometry Conference** 2021 in Rio de Janeiro

IMSF international mass spectrometry foundation

Sociedade Brasileira de Espectrometria de Massas

BrMASS

Brazilian Journal of Analytical Chemistry

VISÃO FOKKA - COMUNICATION AGENCY

The *Braz. J. Anal. Chem.* (BrJAC) is a peer-reviewed, open access, scientific journal intended for professionals and institutions acting mainly in all branches of analytical chemistry.

ISSN 2179-3425 printed ISSN 2179-3433 eletronic

Scope

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.

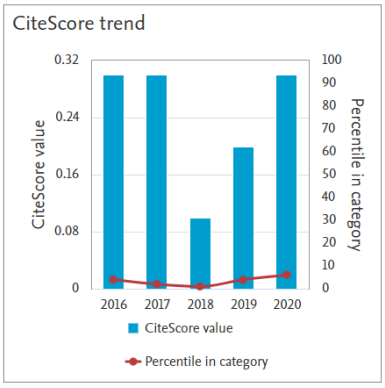
Manuscripts submitted for publication in BrJAC cannot have been previously published or be currently submitted for publication in another journal.

For complete information on ethics and policies on conflicts of interest, copyright, reproduction of already published material and preprints, in addition to the manuscript submission and peer review system, please visit “About us” and Guidelines for Authors at www.brjac.com.br

Indexing Sources

Scopus

CiteScore 2020: 0.3; SJR: 0.131; SNIP: 0.146. CiteScore Tracker 2021: 0.4 Access source details

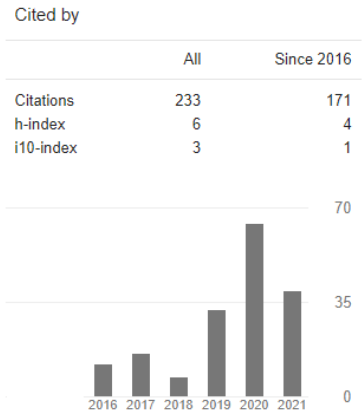


CLARIVATE ANALYTICS
EMERGING SOURCES CITATION INDEX
INDEXED

CAPES
QUALIS

Expectancy: to be classified in the A3 stratum for the Quadrennial Evaluation in 2022

Google scholar



Production Editor
Silvana Odete Pisani

Publisher
Lilian Freitas
MTB: 0076693/ SP
lilian.freitas@visaofokka.com.br

Advertisement
Luciene Campos
luciene.campos@visaofokka.com.br

Art Director: Adriana Garcia

WebMaster: Daniel Letieri

BrJAC’s website: www.brjac.com.br / Contact: brjac@brjac.com.br
Like BrJAC on Facebook: https://www.facebook.com/brjachem

ABEC BRASIL
BrJAC is associated to the Brazilian Association of Scientific Editors

VISÃO FOKKA
BrJAC is published quarterly by:
Visão Fokka Communication Agency
Av. Washington Luiz, 4300 - Bloco G - 43
13042-105 – Campinas, SP, Brazil
contato@visaofokka.com.br
www.visaofokka.com.br

EDITORIAL BOARD

EDITORIAL ADVISORY BOARD

EDITORIAL ADVISORY BOARD (Continuation)

CONTENTS

CONTENTS

Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 33, pp 1-2
doi: 10.30744/brjac.2179-3425.editorial.vgmihucz.N33

BrJAC

EDITORIAL

Quo vadis Spectroscopy?

Victor G. Mihucz

Associate Professor & Associate Editor of Brazilian Journal of Analytical Chemistry (BrJAC)
Institute of Chemistry, ELTE – Eötvös Loránd University, Budapest, Hungary

Since the early days of modern chemistry, analytical chemistry always tried to offer solutions to real-life problems. This is how, in the midst of the Industrial Revolution, **Margueritte** developed the titrimetric determination of iron using the *chameleon solution* (potassium permanganate). In 1860, for the first time, **Bunsen** and **Kirchhoff** used element-specific light emission and absorption of flame-evaporated alkali metal salts for qualitative analysis. Their discovery was groundbreaking because the analytical sensitivity and detection capability of the proposed method was several orders of magnitude lower than that of the contemporary classical analytical ones. Application of this method, leading to the establishment of spectroscopic techniques, also allowed the discovery of about ten chemical elements. Another timeless merit of spectroscopy is the ability to determine the elemental composition of a star in a distant galaxy. In the second half of the 20th century, an era of rapid development of instrumental analysis, atomic spectroscopy also brought several revolutionary results. One such breakthrough was the reduction of the sample volume required for analysis down to microliters with the introduction of graphite furnace atomic absorption spectrometry (GFAAS). The detection limit of GFAAS also decreased by several orders of magnitude. Another important advance was the combination of inductively coupled plasma as a high-temperature ion source with a mass spectrometer (ICP-MS). In the late 1980s, the hyphenation of atomic spectrometric devices to chromatographs, aiming at elemental speciation, also emerged. In Central and Eastern Europe, spectroscopic research was driven by the embargo of the Coordinating Committee for Multilateral Export Controls established by the Western Bloc during the Cold War. In this emerging era, scientific research and applications went hand in hand. Therefore, the science education program and fundamental research were very strong in those countries, contributing considerably to the development of spectrochemistry in Hungary. By the end of the 20th century, development of new equipment and procedures was increasingly carried out by instrument manufacturers, and fundamental research at universities and institutes was relegated to second place. Analytical chemistry has experienced a considerable shift from determination of inorganic compounds and small organic molecules towards that of large (bio)molecules. Expansion of the application of analytical chemistry to forensic, environmental and biochemical questions has been observed and the role of atomic spectrometric techniques seemed to fade away. However, sheer use of these high-performance instruments is not always appropriate or cost-effective; the reliability of the results and elimination of interference must be thoroughly explored. However, when publishing such results, there is a risk that our communications will be rejected due to lack of novelty. Nevertheless, carrying out fundamental research cannot be avoided, as it is not possible to offer appropriate decisions to stakeholders based on questionable results. Presently, GFAAS and ICP-MS allow reliable quantitative determination of virtually any element in any sample. Recently, microwave plasma using nitrogen isolated from the air was launched on the market. This device requires significantly lower operating costs compared to the conventional ICP and reliable simultaneous multi-element analysis has also become possible. Another promising direction is single-particle ICP-MS applied for the characterization of inorganic nanoparticles. Most of the articles of

Cite: Mihucz, V. G. *Quo vadis* Spectroscopy? *Braz. J. Anal. Chem.,* 2021, *8* (33), pp 1-2. doi: http://dx.doi.org/10.30744/brjac.2179-3425.editorial.vgmihucz.N33

the current issue of BrJAC also demonstrate that atomic spectrometry has become indispensable in many areas of our life. Enjoy reading the current issue!

Victor Gábor Mihucz is currently Associate Professor at the Faculty of Science, Eötvös Loránd University, Budapest, Hungary. He works mainly in the field of Atomic Spectrometry, High-Performance Liquid Chromatography–Mass Spectrometry and Elemental Speciation, with emphasis on Environmental Analytical Chemistry. He is Editorial Board member for the *Microchemical Journal* and *Applied Spectroscopy Reviews* and was elected in 2018 as secretary of the Scientific Committee for Analytical and Environmental Chemistry of the Hungarian Academy of Sciences and as a member of the Steering Committee of the Hungarian Chemical Society (HCS) in 2019; he has been president of the Spectrochemical Association of HCS since 2019.

Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 33, pp 3-9
doi: 10.30744/brjac.2179-3425.interview.resantelli

BrJAC

INTERVIEW

Prof. Santelli, a Tireless Researcher and a Gold-Standard Professor in Analytical Chemistry, kindly spoke to BrJAC

Ricardo Erthal Santelli
Full Professor
Federal University of Rio de Janeiro, RJ, Brazil

Prof. Dr. Ricardo Erthal Santelli has a degree in Pharmacy from the Federal Fluminense University, Niterói, RJ, BR (1972), a master's degree in Inorganic Analytical Chemistry from the Pontifical Catholic University, Rio de Janeiro, RJ, BR (1978), a doctorate in Inorganic Analytical Chemistry from the same institution (1985), and a postdoctoral degree from the University of Córdoba, Spain (1988). He was a full professor of Environmental Geochemistry at the Federal Fluminense University from 1994 until 2010 when he retired.
He is currently Full Professor at the Institute of Chemistry at the Federal University of Rio de Janeiro, BR. He works mainly with the development of spectrometric and chromatographic methods, continuous flow injection analysis, and speciation analysis. He develops research mainly on automation in analytical chemistry, environmental geochemistry, and analytical techniques applied to environmental problems.
Prof. Santelli has more than 140 scientific articles published in international journals, with more than 5800 citations and an H-index of 32 in addition to several chapters in international books. He has supervised more than 30 master's and 15 doctoral students. In the editorial field, Prof. Santelli is currently a member of the Editorial Board of the Brazilian Journal of Analytical Chemistry.

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

Since I was a child/teenager, I was very curious about scientific discoveries both in the exact sciences and in the medical field. Also, I liked astronomy (I used to buy the newspaper to read a weekly column about astronomy) and electronics (I used to attend technical courses on electronics by correspondence and also in person). However, my passion at that time was for a children's game called "The Little Chemist". I had a lot of fun performing the experiments described in the game as well as inventing new ones with the available reagents. At the time I attended secondary school, it was not common for schools to have science laboratories, especially in cities in the interior of Rio de Janeiro state, such as Nova Friburgo, where I lived. Only a few high standard schools had laboratories, and I was very eager to visit them, but this was not an easy task.

Cite: Santelli, R. E. Prof. Santelli, a Tireless Researcher and a Gold-Standard Professor in Analytical Chemistry, kindly spoke to BrJAC**.** *Braz. J. Anal. Chem.,* 2021, *8* (33), pp 3-9. doi: http://dx.doi.org/10.30744/brjac.2179-3425.interview.resantelli

Later, already in the last year of high school and preparing to take the university entrance exam, I met Prof. Per Christian Braathen who taught chemistry. In that year (1969), Prof. Per was finishing his Chemistry degree at the State University of Rio de Janeiro (UERJ) and had been hired by the prestigious "Colégio Nova Friburgo", at the time managed by the Getúlio Vargas Foundation, which also taught night classes at the "Colégio Modelo" where I was a student. As I was very interested in Chemistry, I soon became friends with Prof. Per. From this friendship came an invitation to visit the laboratories at "Colégio Nova Friburgo" on a Saturday afternoon. After this first visit, many Saturday afternoons were pleasantly spent in the laboratory, performing the most varied chemistry experiments (and after the practices, we always had a snack carefully prepared by Prof. Per's wife).

Later on, Prof. Per and I were colleagues in the postgraduate course and also professors at the Department of Chemistry at the Pontifical Catholic University, Rio de Janeiro (PUC/Rio) and later professors at the Institute of Chemistry at the Federal Fluminense University (UFF). Therefore, although I already liked Chemistry, Prof. Per proved to be fundamental for my future choices.

When did you decide to study chemistry? What motivated you? How was the beginning of your career?

On the occasion of the university entrance exam, I was thinking of applying for a place in the medical course. I was fascinated by the first heart transplant performed in South Africa by Prof. Christian Barnard. So, I thought about becoming a cardiologist. But, despite loving medicine, I didn't like the hospital environment and much less the sight of blood. Thus, that choice became impossible. Guided by a cousin who was a veterinarian, I decided to take the entrance exam for the Pharmacy course. In a way, there was something about medicine and also about chemistry in the pharmacy course. I passed the entrance exam and started studying Pharmacy at UFF in 1970.

Right in the second semester of the pharmacy course, I was a monitor in organic chemistry. In 1971, UFF created a postgraduate course (master's) in Geochemistry, and I was invited to be the laboratory assistant for this course. This invitation was made by the late Prof. Luiz Fernando Aguiar de Carvalho, who had made his career at the National Department of Mineral Production (currently, Geological Survey of Brazil – CPRM) and had recently been transferred to UFF, where he specialized in emission spectrography with the eminent Prof. Paulo Emídio Ferreira Barbosa.

UFF had received, among other equipment, an emission spectrograph with arc and spark source from the German manufacturer Aus Zena (from the former East Germany). This equipment had been exchanged by the Brazilian government for coffee. I was in charge of operating the emission spectrograph and performing most of the analyses for the first students in the geochemistry course. This was my first real apprenticeship in analytical chemistry. I prepared the samples (usually ores such as cassiterite) either by acid treatment or by fusion with fluxes such as sodium peroxide. Separation and preconcentration techniques such as sorption on silica and solvent extraction were common. The work was hard, but the learning was fantastic. After sample preparation, quantification was done. Those who are more experienced with atomic spectrometry know that at that time, the detector used was a special photographic emulsion (photographic film) (with sensitivity also for the ultraviolet region) that had to be handled in the dark. Before constructing the calibration curve, the photographic emulsion had to be calibrated. It took us days to do this because it involved a lot of calculations, and we had no calculator and even less computers. I ended up becoming a spectroscopist.

I did all this in parallel with the Pharmacy course until my graduation in 1972. In early 1973, I was invited by the then Director of the Institute of Chemistry at UFF, Prof. José Chianelli, to conduct a Chemistry course for students in the geography course, and soon afterwards, I took an internal selection exam (at that time, there were no public tenders as there are today) for assistant professor of experimental analytical chemistry. It was the beginning of my teaching career in analytical chemistry, which has lasted until today.

What has changed in the student profiles, ambitions, and performance since the beginning of your career?

I think everything changes a little over time. Initially, as a student, there is a huge concern to learn and pass the subjects of the university course. Personally, I managed to finish my degree in regular time, and I can say that I was a standard student. In fact, I lived to study.

In professional life, one wishes to have a more solid knowledge of science and particularly of the discipline one has chosen to follow. This requires much more study, effort, dedication, and continuous work when compared to the student. The ambitions are also different. As a student, we want to finish the course and get a good job right away, which is quite difficult nowadays. The student's ambitions are short term. In academic life, we envision success and recognition in the future, but this involves everything I mentioned before. Teaching and scientific research require full dedication, sometimes even hindering one's social life.

Could you briefly comment on recent developments in inorganic analytical chemistry, considering your contributions?

Inorganic analytical chemistry as a whole, worldwide and particularly in Brazil, has evolved a lot in the last decades. This evolution was (and will always be) fundamental for the scientific and technological development of humanity. For the development of new strategic materials and better use of their characteristics, their characterization and production control are necessary. Typically, these materials must have extreme purity, such as the silicon used in the manufacture of semiconductors, or have a well-defined composition, such as catalysts. The characterization and production control of new strategic materials are some of the activities of today's inorganic analytical chemistry, and without the development of these activities, it is impossible to aim for an improvement in our quality of life, in the environmental protection, and improvement of practically everything around us.

... "I believe that the best thing I have done (and still do) in these almost 48 years of teaching is to guide students of analytical chemistry to dedicate themselves to their studies because I believe that this is the only way to form competent professionals."

In the field of human nutrition, the characterization and quantification of inorganic species (such as metals) in human milk and infant formula is fundamental for the nutrition of newborns. In the field of medicine, it is also essential to correctly diagnose several illnesses, such as Parkinson's disease and Wilson's disease.

Nowadays, it is common for an inorganic analytical chemist to handle a "medical" periodic table. Elements that were relatively unknown until recently, such as gadolinium, which is present in contrast agents for magnetic resonance exams, are now being studied in inorganic analytical chemistry laboratories. The interest in gadolinium, in particular, can be explained because its action in the human body, especially in the brain, is still little known and because of its dispersion in water bodies causing an environmental impact.

Regarding my contributions, I believe that the best thing I have done (and still do) in these almost 48 years of teaching is to guide students of analytical chemistry to dedicate themselves to their studies because I believe that this is the only way to form competent professionals. I have also tried to guide students to the correct use of scientific nomenclature in analytical chemistry. Unfortunately, there are some books that insist on not caring about scientific rigor. And so, the students learn wrongly and the mistakes multiply over time.

What are your lines of research? You have published many scientific papers. Would you highlight any?

When I finished my doctorate in inorganic analytical chemistry, I spent some time looking for a research line to start working autonomously. At that time, flow injection analysis (FIA) was under great development in the world, particularly in Brazil, especially with the group of the Center of Nuclear Energy in Agriculture of the University of São Paulo (CENA/USP). I made several contacts with Professors Elias A. G. Zagatto

and Antônio O. Jachinto. I received many teachings from them and started to work with FIA. At that time, everything was still very handmade. It was necessary to build switches to insert the solutions in the continuous system and in the flow cuvettes in addition to improvising a lot. But it worked very well. As I was working in a geochemistry department at UFF, I automated almost all determinations of major elements and some minor elements in rocks by FIA.

Ricardo Santelli, Miguel Valcárcel, Jarda Ruzicka and Gary Christian at *Universidad de Córdoba*, 1992.

Afterwards, I did a post-doctoral period at the *Universidad de Córdoba* in Spain under the supervision of Prof. Miguel Valcárcel (one of the eminent researchers from Spain and the European Community). I learned a lot from him and from Prof. Mercedes Gallego. I could also see how creative we Brazilians are and how productive we can be abroad with a better laboratory structure. There, I published my first article in the Analytical Chemistry journal. Coming back from postdoc, I was able to form a group with many undergraduate students and publish some papers in good journals with several partners from other universities.

As I spent many years in a geochemistry department, whose main interests were environmental issues, I published (and still publish) many works with several colleagues in the area, mainly on the quality of water, soils, and sediments. Also, from that time onwards, I became interested in Atomic Spectrometry, and I have been working a lot with Atomic Absorption Spectroscopy (high resolution), ICP OES, and more recently with ICP-MS. Currently, I am more interested in the application of atomic spectrometry and chemical speciation analysis to understand the action and metabolism of some important inorganic elements in the fields of human nutrition and medicine.

Do you keep yourself informed about the progress of research in chemistry? What is your opinion about the current progress of chemistry research in Brazil? What are the recent advances and challenges in scientific research in Brazil?

I try to keep myself well informed about scientific developments in chemistry and particularly in analytical chemistry. However, I confess that being well informed about recent innovations in analytical chemistry is not an easy task, indeed it is almost impossible. The huge number of papers and information released each day is unbelievable. However, it is necessary to stay alert. Today, there is an industry of scientific production with the appearance of many scientific journals that do not have good quality criteria. I say industry because the publisher of a scientific journal invests little and seeks profit. The authors write the papers, the reviewers comment and suggest changes, the authors adjust their paper, in many cases pay for the publication, and finally the publisher puts it in the final format and sells access to the articles. It is not difficult to see that the profit can be large. One must always pay close attention to scientific quality.

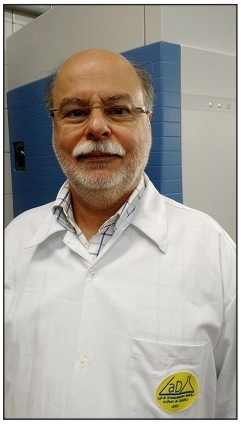
Brazilian chemistry and, in particular, Brazilian analytical chemistry are quite vigorous on the world scene. Over the last few decades, many scientific research groups have been consolidated in Brazil, generally allied to postgraduate courses that were designed to train human resources of great academic and scientific quality. These groups are many, and it is not possible to name them here.

Our challenges are tremendous, and the main one would be to make this unequal country much more egalitarian. I think we need to look for more and more scientific and technological solutions that are genuine and developed by us Brazilians, without leaving aside everything that is good even if imported, in order to advance rapidly in strategic areas, such as engineering, so as to guarantee the full development of our country. However, for some years now, funding for scientific and technological research as well as the training of qualified personnel have been falling sharply in Brazil. Without funding, there is no education, science, and technology, the pillars of a nation. Today, few researchers are able to carry out quality scientific and technological research in Brazil among the many qualified researchers that exist. Our country needs

to immediately reverse this situation of low funding; otherwise, research will become irreversibly unfeasible in the not-too-distant future.

For you, what have been the most important recent achievements in analytical chemistry research? What are the landmarks?

In modern analytical chemistry, we cannot do without equipment. I think the great recent advance in analytical chemistry has been the development, modernization, and improvement of scientific instrumentation. Of course, this is due to the great development of two other related areas, electronics and computer science. In inorganic analytical chemistry, the advent of mass spectrometers that are increasingly sensitive, precise, accurate, and with the most diverse accessories allow the elucidation of questions, mechanisms, total quantification, and speciation of various chemical elements of fundamental importance in the modern world. The possibility of monitoring isotopes, whether in a living organism, in the environment, or in any material, gives us answers to several important questions, such as, for example, in toxicology. Today, we want to live longer and better, and for this, the understanding of the toxicology of a chemical element is fundamental.



Prof. Santelli at the Analytical Development Lab, Federal University of Rio de Janeiro

There are in Brazil, and in the world, several conferences on Chemistry. To you, how important are these meetings to the chemistry scientific community? How do you see the development of national chemistry meetings in Brazil?

Scientific events are fundamental for the dissemination of knowledge and exchange of information with peers. In addition, they are inexhaustible sources of knowledge for new students and researchers. Science is learned in many ways, and one of them is by attending congresses. This activity must be increasingly encouraged, although sometimes, it seems that there are already too many congresses. It is necessary to understand that the scientific community is also growing, hence the need for more events. In Brazil, I have seen this activity growing and with good participation from young students. This has been happening for some decades in our country, and I would dare to say that this activity was very important for the growth of Brazilian analytical chemistry. Unfortunately, the COVID-19 pandemic and more recent financial issues concern the Brazilian scientific community. A remote conference is quite different from a face-to-face one. The collective exchange of information and the conversation with a more experienced researcher are fundamental for a young researcher. I hope that soon the pandemic will slow down and that the financial conditions of our country will allow the return of face-to-face events and the participation of younger people.

Prof. Santelli with his award received at ENQA 2009.

You have already received some awards. What is it like to receive this kind of recognition?

Receiving recognition and being awarded is always good for everyone, but for those who work in science, it is very rewarding. Being recognized brings us joy, pride, and a sense of accomplishment. Of the awards I have received, especially for two of them, I have immense affection and pride. One was the recognition received on the occasion of the 1st National Meeting of Chemical Speciation - EspeQBrasil in São Pedro, SP in 2008, and the other occurred during the 15th National Meeting of Analytical Chemistry - ENQA in Salvador, BA in 2009. However, I believe that the greatest recognition comes from the students at all levels of education, from undergraduate through scientific initiation, master's and doctoral degrees to postdoctoral studies. I have been

fortunate to work with and become friends with countless students to whom I have always tried to pass on lessons of ethics and honesty as well as the encouragement and need for serious work to build a solid career not only in academic but also professional life.

What is the importance of these awards in the development of science and new technologies?

Recognition stimulates our creativity and desire to move forward. In fact, there are awards more focused on the development of new technologies, which are generally supported by Companies, which stimulate science as a whole. But I consider that a scientist should not worry about awards and recognition. This is a natural consequence of their activity and their production carried out in an exemplary manner.

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

I believe that the support of these agencies, whether federal, state, or private, is fundamental for the scientific and technological development of our country. Unfortunately, as mentioned before, our leaders do not have this vision. What we are witnessing is a drastic and gradual reduction in the promotion of research and human resources training (scholarships). This will certainly hinder the country's growth and the construction of a fairer nation in which people can have the education, food, and comfort that every citizen deserves.

Right now, we are facing a radical change in the allocation of master's and doctorate scholarships and in postgraduate courses by the Coordination for the Improvement of Higher Education Personnel (CAPES). I have never been in favor of an excessive distribution of scholarships, but in a continental country like Brazil with countless universities, opportunities must be maintained. However, I assume that the distribution of scholarships should be made considering mainly the merit of the student.

The private sector also has a great responsibility in promoting scientific research in the country. Every company has a social role, and one way to exercise it would be to offer a fraction of its profits for the training of human resources and for scientific and technological research. This is still incipient in Brazil, but I believe that with a good federal government, which is really concerned with the development of our country, such initiatives can be increased and consolidated in the medium term.

"Studying means much more than reading a book or a scientific article. Studying means spending hours on topics of interest and checking all its possibilities, consulting books and related scientific articles."

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?

What I can say to the young researchers is to have hope and perseverance. Take advantage of all the opportunities that are offered and study hard. If you are very well prepared, with broad, strong, and consolidated knowledge, it will be easier to be in a good job and overcome times of crisis like the one we are experiencing at the moment. Knowledge we have to acquire on our own, so it takes a lot of study and dedication. Nowadays, with the quality and quantity of information that we can access, we only depend on ourselves to be prepared and to face the needs and requirements of today's world. Studying means much more than reading a book or a scientific article. Studying means spending hours on topics of interest and checking all its possibilities, consulting books and related scientific articles. It also means listening to the experts, their stories, positions, and interpretations of scientific phenomena.

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

I think this answer has already been given before. Studying is the correct and fundamental action. In the specific case of Inorganic Analytical Chemistry, start studying from "old" books to understand and assimilate the essence of analytical chemistry. The fundamental principles of analytical chemistry do not change and,

therefore, need to be absorbed correctly. Also, nomenclature and scientific rigor must be cultivated. The "Classical Analytical Chemistry", although little used experimentally today, brings us the whole basis and essence of Analytical Chemistry. From this classical knowledge, we then have the possibility to advance in relation to modern analytical techniques, generally instrumental. It is necessary to know the fundamental principles of the techniques, of the instrumentation, and also of how chemical analysis can be carried out reliably. Reliability is fundamental in Analytical Chemistry. A chemical analysis is only valuable if it is performed following a series of analytical quality parameters. I don't think I need to mention that equipment is not magic. Any sample submitted to an instrumental measurement will present a result. But this result must be valid, reliable, and must be validated according to adequate statistical parameters.

I don't know if I will be redundant, but it is worth remembering that it is from the result of a chemical analysis that the decision will be made. Imagine the damage if the result of an analysis is unreliable. Completely wrong decisions will be made.

How would you like to be remembered?

I honestly don't pretend to be remembered. I have always tried to carry out my teaching, research, and management activities with love, dedication, balance, fairness, and honesty. I don't know if I have behaved this way over the years, but that was my intention. I think that for all that I have done in these 48 years of university life, I will leave a small legacy, probably for a few.

In fact, I think it is more important to recognize people's accomplishments while they are active, that is, while they are alive. I would like to think that I have contributed a little and in a possible way to present inorganic analytical chemistry as very important in chemistry courses at all levels.

I want to remember here another professor who inspired me a lot, the late Prof. Dr. Adilson José Curtius, to whom I owe all my master's and doctorate education and my passion for atomic spectrometry.

So, to close this interview, I would really like to be remembered as a professor who helped (or at least tried to help) many students in the field of Chemistry.

Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 33, pp 10-12
doi: 10.30744/brjac.2179-3425.point-of-view-lmgoncalves

BrJAC

POINT OF VIEW

HO^- and OH^-, Reason and Tradition

Luís Moreira Gonçalves

Departamento de Química Fundamental, Instituto de Química
Universidade de São Paulo, São Paulo, SP, Brazil

Writing OH^- is so widespread that one hardly notices that there is no logical reason, apart from being common, not to write HO^- instead. Scientists should be educated to spot irregularities, since often they mean something. Chemistry professors, in particularly at graduate level, when teaching pH should make their students notice such discrepancy.

Albeit pH is not an immensely complex topic, it is intriguing the number of misconceptions, and even plain errors, associated. For example, the limits of the pH scale [1]; it is not uncommon to find students (and not just undergrads) believing pH values cannot be lower than 1 or higher than 14, or that negative pH values do not exist. Herein, it is addressed the odd exception of writing OH^- instead of the most logical form of HO^-. It is fascinating that chemists are so accustomed to see OH^- that they do not longer find it to be an oddity.

First, it is important to highlight why it is a nomenclature exception, i.e. the lack of reason to write OH^-.

For sure, chemists are familiar with these deprotonation equations:

$$H_3PO_4 \rightleftharpoons H^+ + H_2PO_4^-$$

$$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-}$$

$$HPO_4^{2-} \rightleftharpoons H^+ + PO_4^{3-}$$

And this schematic equation:

$$HA \rightleftharpoons H^+ + A^-$$

And even this one:

$$H_3O^+ \rightleftharpoons H^+ + H_2O$$

So, why to write:

$$H_2O \rightleftharpoons H^+ + OH^-$$

Instead of the analogous:

$$H_2O \rightleftharpoons H^+ + HO^-$$

Why are water and the ion hydroxide treated differently? Is it because the negative charge is located on the hydrogen instead of the oxygen? No, it is not, oxygen is far more electronegative. Is it because alphabetically ‘o’ comes before ‘h’? No, it does not. Maybe it is because OH^- looks better than HO^-; or maybe because pOH would be less confused with pH than pHO. Perhaps, but these two explanations are hardly chemically meaningful.

Cite: Gonçalves, L. M. HO^- and OH^-, Reason and Tradition. *Braz. J. Anal. Chem.,* 2021, *8* (33), pp 10-12. doi: http://dx.doi.org/10.30744/brjac.2179-3425.point-of-view-lmgoncalves

In fact, in the red book of the International Union of Pure and Applied Chemistry (IUPAC) [2], rule IR-4.4.3.1, it is clearly stated: *"…the formula for the hydroxide ion should be* HO^- *to be consistent with the above convention."*. However, precisely in the same rule, as example 11, it is stated: "HO^- or OH^-", which the author would find contradictory if 'should' were to be replace by 'must'. By the way, for the naming of the radical $HO^{\bullet}$, only one option is presented (rule IR-4.6.2).

To the author's limited knowledge, there is no other reason apart from historical motif. The author made an effort to better understand the question by seeking the literature, contacting a chemical nomenclature historian and an author of the IUPAC's red book. And the most probable explanation found is that many chemists (if not most of the chemists) used to write OH^- (as can be observed in many older textbooks [3-5]), and the benefits of changing to HO^- would not merit all the effort and nuisance. Thus, the reason for the title of this Point of View: tradition over reason.

Even though there are many scientists that are resilient and make an effort to write HO^- [6-10], and while their effort should be respected, one wonders if a change in paradigm would indeed have positive benefit-cost outcome. Nevertheless, the author of this manuscript advocates that it is important that students are taught that HO^- is a discrepancy. It is something worthy of Chemistry professors' endeavors. It should also be mentioned in lecture books when first referring to OH^-. And not just because it is an indulging curiosity, but because it is a 'vestigial structure' that shows the evolution of Chemistry. Furthermore, quite importantly, scientists should be trained to find irregularities, in many cases in Nature they actually mean something.

Acknowledgements

The author would like to thank Professor Richard Hartshorn (University of Canterbury, New Zealand), Professor Arnaldo Cardoso (São Paulo State University, Brazil), and Professor Rafael Mori (Federal University of ABC, Brazil) for their much-appreciated help. Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP: 2018/14425-7) is also acknowledged.

REFERENCES

1. Lim, K. F. *J. Chem. Educ*., **2006**, *83* (10), p 1465 (https://doi.org/10.1021/ed083p1465).
2. Connelly, N. G.; Damhus, T.; Hartshorn, R. M.; Hutton, A. T. *Nomenclature of Inorganic Chemistry* – International Union of Pure and Applied Chemistry (IUPAC) Recommendation, **2005**.
3. Mori, R. C.; Curvelo, A. A. da S. *Quim. Nova*, **2014**, *37* (5), pp 919-926 (https://doi.org/10.5935/0100-4042.20140148).
4. Teixeira, J. M. *Noções de Chimica Geral* (6th Ed). Francisco Alves, Rio de Janeiro, **1906**.
5. Valeriano, T. P. do A. *Noções Elementares de Chimica Organica: Para Uso dos Alumnos d'esta Disciplina nos Gymnasios e Escolas Superiores da Republica* (5th Ed). Imprensa Nacional, Rio de Janeiro, **1921**.
6. Asthagiri, D.; Pratt, L. R.; Kress, J. D.; Gomez, M. A. *Proc. Natl. Acad. Sci*., **2004**, *101* (19), pp 7229-7233 (https://doi.org/10.1073/pnas.0401696101).
7. Asthagiri, D.; Pratt, L. R.; Kress, J.; Gomez, M. *Chem. Phys. Lett*., **2003**, *380* (5–6), pp 530–535 (https://doi.org/10.1016/j.cplett.2003.09.007).
8. Corrêa, C. *Bol. Soc. Port. Química*, **2002**, *85* (2), 72.
9. Barros, A. A.; Rodrigues, C.; Miguelote, L. *Química 11: Física e Química A, 11º Ano: Ensino Secundário* (1st Ed). Areal, Porto, **2013**.
10. Magalhães, P. J.; Barbosa, A. R.; Gonçalves, L. M.; Pacheco, J. G.; Guido, L. F.; Brányik, T.; Rodrigues, P. G.; Kuncová, G.; Dostálek, P.; Barros, A. A. *J. Inst. Brew*., **2011**, *117* (2) pp 368-376 (https://doi.org/10.1002/j.2050-0416.2011.tb00481.x).

Luís Moreira Gonçalves is currently a chemistry professor in the Institute of Chemistry at the University of São Paulo (IQ-USP), São Paulo, Brazil (Research Group). He holds a BSc and a PhD in chemistry (both from the University of Porto, PT), as well as BSc in medical sciences and MSc in medicine (both from the University of Minho, PT). He previously worked as post-doctoral researcher in the Faculty of Sciences of the University of Porto, with several different research interests including psychiatry, the development of biosensors for medical applications, molecularly imprinted polymers (MIPs), electrochemistry, food quality and research fields combining chemistry and medicine in general. He has ca. 90 published papers (more than 1600 citations, h-index 22) and 4 patents; he has won several prizes including the Young Researcher in Electrochemistry attributed by the Portuguese Electrochemical Society. He is a member of the Portuguese Electrochemical Society (SPE), Portuguese Medical Association (Ordem dos Médicos), and Regional Medical Council of the São Paulo State (CREMESP).

Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 33, pp 13-18
doi: 10.30744/brjac.2179-3425.letter-rmbdezena-N33

BrJAC

LETTER

The applicability of solid state characterization and analytical techniques for reference chemical substance certification: Resolution of the Collegiate Board (RDC) 166/2017 by National Health Surveillance Agency (ANVISA)

Renan Marcel Bonilha Dezena
Preformulation Specialist in the Pharmaceutical Industry

BACKGROUND

Reference chemical substances called primary reference are used which are marketed by official compendia both nationally and internationally in the pharmaceutical industry as a tool for identification and quantification during pre-formulation, development/analytical validation and quality control studies.

The reference or primary standards are almost always imported and resold in the Brazilian market as a consequence, increasing the cost in relation to the sample amount (little amount of mass in mg per bottle).

On July 24, 2017, the National Health Surveillance Agency (ANVISA) published the Resolution of the Collegiate Board (RDC) number 166, which deals with the validation of analytical methods. The Chapter III of this resolution is intended for reference chemical substances and, in Art. 14 § 1, the RDC 166/2017 allows the use of characterized reference chemical substances in the analytical validations.

The characterization study is planned according to the chemical structure of the substance to be characterized and within this new regulation, solid-state characterization and analytical chemistry stands out as an essential ally corroborating with the evolution of the number of publications in the literature regarding this subject in the google scholar database as shown in Figures 1 and 2 [1-15].

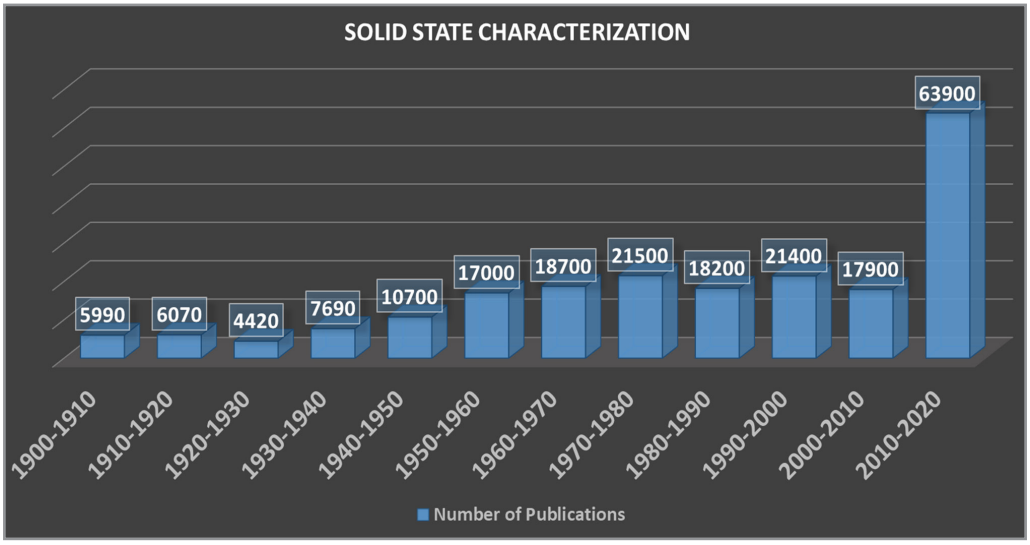


Figure 1. Evolution of the number of references (scientific articles), from 1900 to 2020, containing the concept “solid state characterization” in the google scholar database.

Cite: Dezena, R. M. B. The applicability of solid state characterization and analytical techniques for reference chemical substance certification: Resolution of the Collegiate Board (RDC) 166/2017 by National Health Surveillance Agency (ANVISA). *Braz. J. Anal. Chem.,* 2021, *8* (33), pp 13-18. doi: http://dx.doi.org/10.30744/brjac.2179-3425.letter-rmbdezena-N33

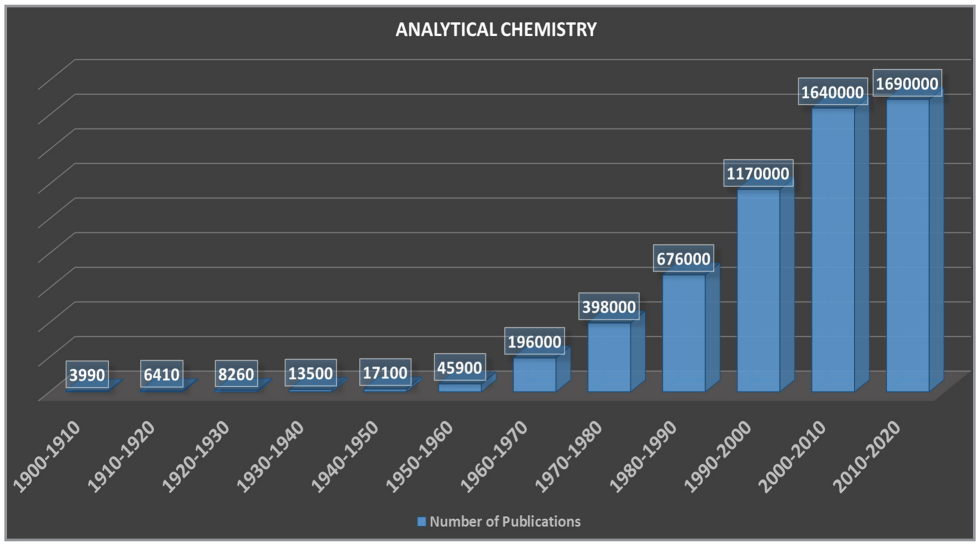


Figure 2. Evolution of the number of references (scientific articles), from 1900 to 2020, containing the concept “analytical chemistry” in the google scholar database.

In Figures 3 and 4, a bibliometric map was performed due to its important role in the evaluation of scientific production worldwide, since its indicators can portray the behavior and progress of the field of knowledge [16].

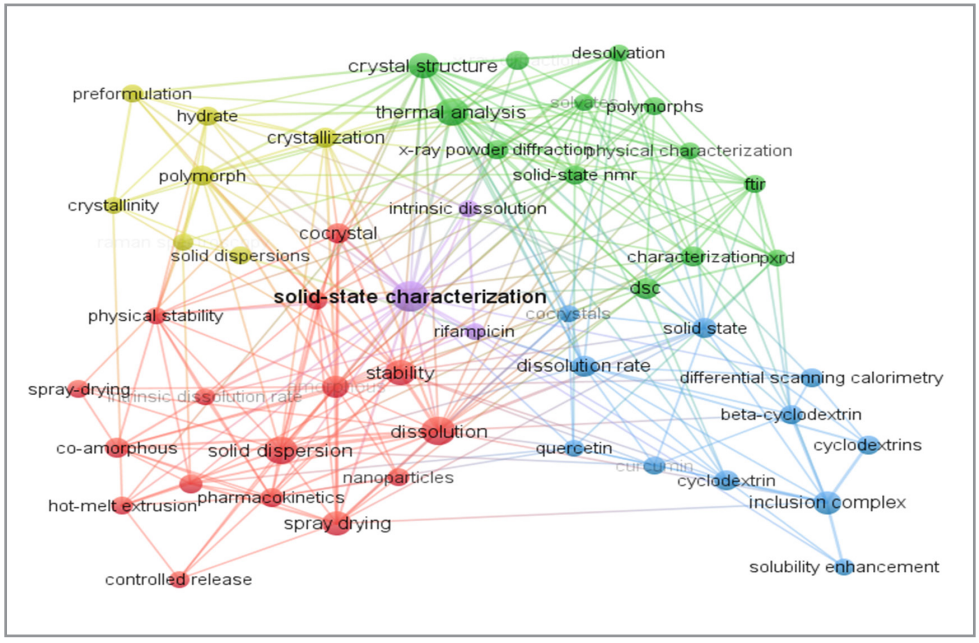


Figure 3. Network visualization map of 50 main items from the 1,029 articles exported from the Web of Science database using the concept “solid state characterization” on 03/29/2021 (VOSviewer software, version 1.6.16).

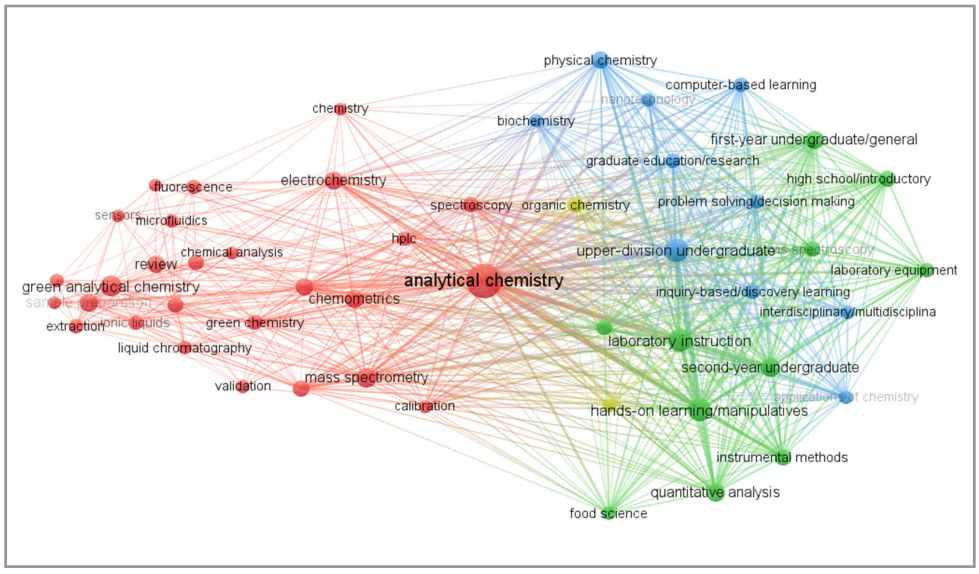


Figure 4: Network visualization map of 50 main items from the 11,712 articles exported from the Web of Science database using the concept "analytical chemistry" on 03/29/2021 (VOSviewer software, version 1.6.16).

High performance liquid chromatography (HPLC)

The HPLC enables the separation, identification and quantification of chemical compounds in complex mixtures and matrices [17-18]. The compounds of a mixture are distributed by the flow of the mobile phase through a column with a stationary phase and the separations are based on differences in migration speed due to the affinity of the components between the mobile phase and the stationary phase [17-18]. Separations are achieved by partition, adsorption, ion exchange, exclusion by size, stereochemical interactions, depending on the type of stationary phase used [17-18].

The studies that are normally conducted are identification; purity, related substances, impurities and assay [17-18].

Mass spectrometry (MS)

The MS allows analyzing the molecules through the mass / charge ratio (m / z) of the ions of these compounds in the gaseous state and consequently enabling the characterization of their chemical structure [19].

Differential scanning calorimetry (DSC)

The DSC performs the measurement of the physical-chemical properties of materials according to difference between the temperature of the reference and the sample, at a constant heat flow in a controlled atmosphere normally nitrogen or oxygen [20]. The technique assesses phenomena, such as melting temperature, recrystallization, polymorphism, desolvation, dehydration, evaporation, decomposition, crystallinity, sublimation, glass transitions, pyrolysis, solid-solid interactions (eutectic mixture) and purity, and such data are extremely relevant in characterization of substances [20].

Thermogravimetric analysis (TGA)

The TGA is used to determine the loss of mass of a sample due to variations in temperature, time and type of atmosphere (nitrogen and oxygen) [21]. Additional data are provided through the changes that heating produces in the mass of substances, making it possible to establish studies regarding the degree of hydration of the molecules, composition, chemical reactions, oxidation induction time, isothermal drying, ash content, filler content, separation of vaporization and decomposition [21].

X-ray powder diffraction (XRPD)

The XRPD is a specific non-destructive crystallographic technique applied for the qualitative or quantitative determination of a sample's crystalline phases, offering information regarding crystal structure, polymorphism, lens size, preferred orientation, and layer thickness [22].

Fourier-transform infrared spectroscopy (FTIR)

The FTIR is responsible for the identification of an organic compound, for the presence of specific bands of functional groups of the molecule through the absorption of infrared radiation (400-4000 cm^{-1}) [23].

Nuclear magnetic resonance (NMR)

The NMR is a spectroscopic technique that makes it possible to determine the properties of a molecule through the correlation of energy absorbed with the frequency in the megahertz (MHz) range [24].

Elemental analysis (C, H, N, S)

It aims to elucidate exactly what are the elements that make up a sample (qualitatively) and in what proportion they appear in the molecular formula (quantitatively), mainly through the levels of carbon, hydrogen, nitrogen and sulfur [25].

Optical rotation

Some molecules after solubilization have the ability to rotate in contact with polarized light, especially those with chiral centers (carbon atoms with four different substituents) [26]. Molecules that rotate clockwise are called dextrogins while those that rotate counterclockwise are called levogiras [26].

SUMMARY

Through RDC 166/2017 approval, the characterization of the solid state has become indispensable within this new scenario providing greater access and autonomy for the pharmaceutical industries regarding the possibility of characterization of raw materials and use during the stages of development and analytical validations as well as in quality control of active pharmaceutical ingredients and finished products.

Acknowledgments

I would like to thank Daniel Fernandes Andrade and Edenir Rodrigues Pereira-Filho from the Group of Applied Instrumental Analysis (GAIA), Department of Chemistry, Federal University of São Carlos (UFSCar), 13565-905 São Carlos - SP, Brazil for the scientific contribution regarding bibliometrics topic and for the files exported from the Web of Science database.

REFERENCES

1. Dezena, R. M. B. *Br. J. Anal. Chem.*, **2017**, *4* (16), pp 8-9.
2. Dezena, R. M. B.; Malta Jr., J. D. S. *Br. J. Anal. Chem.*, **2017**, *4* (15), pp 19-26.
3. Dezena, R. M. B.; Malta Jr., J. D. S.; Meurer, E. C.; Eberlin, M. N. *Br. J. Anal. Chem.*, **2017**, *4* (15), pp 27-34.
4. Dezena, R. M. B. *Braz. J. Anal. Chem.*, **2020**, *7* (26), pp 12-17 (http://dx.doi.org/10.30744/brjac.2179-3425.letter.rmbdezena.N26).
5. Dezena, R. M. B.; Malta Júnior, J. S.; de Godoy, F. P.; Smith, T. *Braz. J. Anal. Chem.*, **2020**, *7* (28), pp 11-19 (http://dx.doi.org/10.30744/brjac.2179-3425.N28-letter).
6. Dezena, R. M. B.; Coelho Silva, R. C.; Luiz, G. F. *Braz. J. Anal. Chem.*, **2019**, *6* (25), pp 10-12 (http://dx.doi.org/10.30744/brjac.2179-3425.letter.rmbdezena.N25).
7. Dezena, R. M. B.; Mauri, R.; Barros, F. A. P.; Rosa, P. C. P. *J. App. Biol. Biotech*., **2019**, *7* (02), pp 64-70 (http://dx.doi.org/10.7324/JABB.2019.70212).
8. Dezena, R. M. B. *Braz. J. Anal. Chem.*, **2021**, *8* (30), pp 11-13 (http://dx.doi.org/10.30744/brjac.2179-3425.letter-rmbdezena-N30).

9. Jiménez-Reyes, M.; Tenorio, D.; García-Rosales, G.; Jiménez-Becerril, J.; Luna-Castro, G. E. *Braz. J. Anal. Chem.*, **2021**, *8* (30), pp 29-42 (http://dx.doi.org/10.30744/brjac.2179-3425.AR-51-2020).
10. Mohamed, A. A. E. *Braz. J. Anal. Chem.*, **2021**, *8* (30), pp 43-58 (http://dx.doi.org/10.30744/brjac.2179-3425.AR-52-2020).
11. Rajendraprasad, N.; Basavaiah, K. *Braz. J. Anal. Chem*., **2020**, *7* (28), pp 44–69 (http://dx.doi.org/10.30744/ brjac.2179-3425.AR-23-2020).
12. Al-Kharosi, A. S.; Al-Sabari, W. A.; AL-Shidhani, F. H.; Alfarsi, S. A.; Taha, S. M. *Braz. J. Anal. Chem.*, **2020**, *7* (26), pp 18–26 (http://dx.doi.org/10.30744/brjac.2179-3425.AR-23-2019).
13. Lourdes, A. M. F. O.; Cuin, A.; Delbem, I. D.; Campos, N. S.; Sousa, R. A.; Ciminelli, V. S. T.; Silva, J. C. J. *Braz. J. Anal. Chem.*, **2020**, *7* (26), pp 36–50 (http://dx.doi.org/10.30744/brjac.2179-3425.AR-34-2019).
14. Hakme, E.; Herrmann, S. S.; Poulsen, M. E. *Braz. J. Anal. Chem*., **2020**, *7* (26), pp 51–77 (http://dx.doi.org/10.30744/brjac.2179-3425.AR-36-2019).
15. Abe-Matsumoto, L. T.; de Araújo, Y. A. *Braz. J. Anal. Chem*., **2020**, *7* (27), pp 14-19 (http://dx.doi.org/10.30744/brjac.2179-3425.AR-38-2019).
16. Araújo, A. S.; Andrade, D. F.; Babos, D. V.; Castro, J. P.; Garcia, J. A.; Sperança, M. A.; Gamela, R. R.; Machado, R. C.; Costa, V. C.; Guedes, W. N.; et al. *Braz. J. Anal. Chem*., **2021**, *8* (30), pp 14-28 (http://dx.doi.org/10.30744/ brjac.2179-3425.RV-27-2020).
17. Trontelj, J.; Vovk, T.; Bogataj, M.; Mrhar, A. *Pharmacol. Res.*, **2005**, *52* (4), pp 334-339 (https://doi.org/10.1016/j.phrs.2005.05.007).
18. Bueno, L. M.; Manoel, J. W.; Giordani, C. F. A.; Mendez, A. S. L.; Volpato, N. M.; Schapoval, E. E. S.; Sttepe, M.; Garcia, C. V. *Eur. J. Pharm. Sci*, **2017**, *97*, pp 22-29 (https://doi.org/10.1016/j.ejps.2016.11.004).
19. Beccaria, M.; Cabooter, D. *Analyst*, **2020**, *145* (4), pp 1129-1157 (https://doi.org/10.1039/C9AN02145K).
20. Leyva-Porras, C.; Cruz-Alcantar, P.; Espinosa-Solís, V.; Martínez-Guerra, E.; Piñón-Balderrama, C. I.; Compean Martínez, I.; Saavedra-Leos, M. Z. *Polymers*, **2020**, *12* (1), pp 1-21 (https://doi.org/10.3390/polym12010005).
21. Dourado, D. *Biomed. J. Sci. Tech. Res.*, **2019**, *22* (3), pp 16634-16636 (http://dx.doi.org/10.26717/BJSTR.2019.22.003745).
22. Munjal, B.; Suryanarayanan, R. *TrAC Trends Anal. Chem.*, **2021**, *136*, pp 116-181 (https://doi.org/10.1016/j.trac.2021.116181).
23. Amaya-Chantaca, N. J. *Asian Journal of Applied Science and Technology (AJAST),* **2020**, *4* (2), pp 108-114 (Available at SSRN: https://ssrn.com/abstract=3712886).
24. Delaney, S. P.; Nethercott, M. J.; Mays, C. J.; Winquist, N. T.; Arthur, D.; Calahan, J. L.; Sethi, M.; Pardue, D. S.; Kim, J.; Amidon, G.; et al. *Journal of Pharmaceutical Sciences*, **2017**, *106* (1), 338-347 (https://doi.org/10.1016/j.xphs.2016.10.004).
25. Biçer, A.; Taslimi, P.; Yakalı, G.; Gülçin, I.; Gültekin, M. S.; Cin, G. T. *Bioorg. Chem.*, **2019**, *82*, pp 393-404 (https://doi.org/10.1016/j.bioorg.2018.11.001).
26. Farmacopeia Brasileira, Agência Nacional de Vigilância Sanitária (ANVISA), Brasília, **2019**, 6th edition, Volume I. Available at: https://www.gov.br/anvisa/pt-br/assuntos/farmacopeia/farmacopeia-brasileira/arquivos/7985json-file-1 [Accessed on: March 29, 2021].

Renan M. B. Dezena is a Preformulation Specialist with Graduation in Pharmaceutical Sciences from Pontifical Catholic University of Campinas (PUC-Campinas), Brazil (2010), and Specialization in Management of Research and Development of Drugs through the Institute of Sciences, Technology and Quality (ICTQ), Brazil (2014). He has 12 years of experience in the Pharmaceutical Industry with background in the Departments of Production, Quality Control and Research & Development. Perform Preformulation Studies related to the development of pharmaceutical products through the following analytical techniques: Mass spectrometry (LC-MS/MS), Infrared Spectroscopy (MIR and NIR), Ultraviolet Spectroscopy, Liquid Chromatography (HPLC and UPLC), Gas Chromatography (GC-MS), Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), X-Ray Powder Diffraction (XRPD), Optical Microscopy, Particle Size Distribution by Laser Diffraction, Confocal Raman Microscopy, Nuclear Magnetic Resonance Spectroscopy, Zeta Potential and Dynamic Light Scattering.

Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 33, pp 19-41
doi: 10.30744/brjac.2179-3425.RV-58-2021

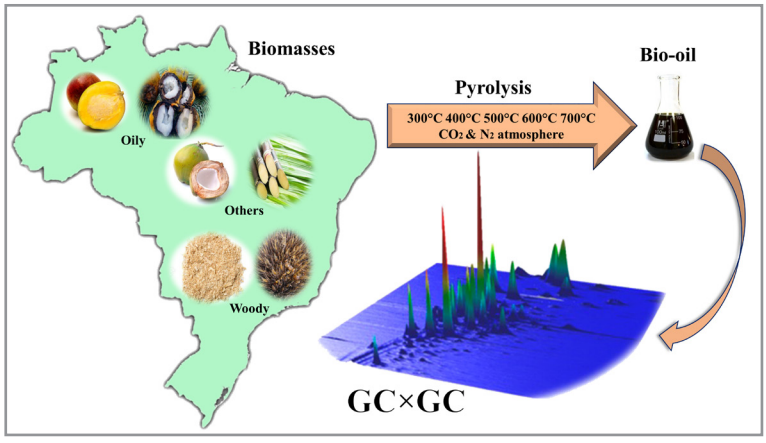
BrJAC

REVIEW

GC×GC in the Characterization of the Bio-Oil from Brazilian Biomass: A Review

Rafael de Oliveira Farrapeira, Yasmine Braga Andrade, Laíza Canielas Krause, Thiago Rodrigues Bjerk, Elina Bastos Caramão and Jaderson Kleveston Schneider*

Instituto de Tecnologia e Pesquisa, ITP, Universidade Tiradentes (UNIT), Av. Murilo Dantas, 300, Aracaju, SE, ZIP 49032-490, Brazil



This study presents and discusses the state of the art of Two-Dimensional Comprehensive Gas Chromatography (GC×GC) developed in Brazil. GC×GC has been the focus of studies in Brazil since 2009, based on successful experiences in cooperation with researchers from Australia and Italy. The result of these researches led to the installation of many laboratories in Brazilian Universities and Research Centers, similar to others in foreign countries and the development of research, mostly involving applications of the technique to Brazilian matrices. In this review we present applications of GC×GC involving the pyrolysis of Brazilian agroindustrial residues, such as cane straw, sawdust, coconut fiber, fruit seeds, rice husks, spent coffee grounds, among others. The most used detection techniques for GC×GC have been mass spectrometry with fast quadrupole analyzer (GC×GC/qMS) and time of flight (GC×GC/TOFMS). These studies showed the possibility of identifying many organic compounds in the bio-oils produced, especially oxygenated ones such as phenols, ketones, acids and esters. Several studies suggest catalytic pyrolysis as a way to generate less oxygen-compounds directing the application of this bio-oil to the area of biofuels. However, the compounds found and their relative concentration, indicates that the best uses should be associated with the processing industry such as pharmaceuticals, chemicals, polymers and food.

Keywords: bio-oil, characterization, Brazilian biomasses, gas chromatography, GC×GC.

INTRODUCTION

Comprehensive two-dimensional gas chromatography (GC×GC) is a technique originally described in 1991 by Professor John B. Phillips and his student Zaiyou Liu [1]. Since then, this technique has been extensively applied to solve complex separation problems such as the large amount of analytes with

Cite: Farrapeira, R. O.; Andrade, Y. B.; Krause, L. C.; Bjerk, T. R.; Caramão, E. B.; Schneider, J. K. GC×GC in the Characterization of the Bio-Oil from Brazilian Biomass: A Review. *Braz. J. Anal. Chem.,* 2021, *8* (33), pp 19-41. doi: http://dx.doi.org/10.30744/brjac.2179-3425.RV-58-2021

Submitted 08 April 2021, Resubmitted 09 June 2021, Accepted 18 June 2021, Available online 09 August 2021.

structural similarities, analytes with large concentration differences between them and also the presence of unresolved complex mixture in 1D. In the last 30 years, GC×GC began to attract attention and their 3D (three-dimensional) chromatograms wake-up the interest of analytical chemists for these new way for analyzing and presenting the results related to complex mixtures [2].

Using GC×GC, the separation power of a gas chromatography system is optimized by coupling two columns with different polarities. Thus, the eluent from 1D (first dimension) column is conducted to the 2D, through a modulator, which segments and focuses the effluent from the first column to the second column [3].

Initially, the main reviews discussed the principles of the technique, the basic theory and the experimental set-up [1,3]. Next, different interfaces among columns became a key topic and the first few applications were reported. Most of these were in the field of petrochemical analysis [4]. In the past few years the main parameter studied were software, new columns and others novelties, indicating that great steps for-ward still have to be made (e.g., in detection, analyte identification and quantification and, specifically, applications). Today, besides petrochemical analysis, areas such as food, air and environmental analysis are detailed studied by GC×GC [2,3,5-7].

Many researches in GC×GC were developed in Brazil, based on successful experiences in scientific exchanges with researchers from Australia and Italy between 2005 and 2007. The first works were related to characterization of oil and derivatives. Then, due to the characteristics of Brazil in terms of environment, energy and biodiversity, this application evolved and the first works involving pesticides, plants and biomass began to be produced. As of 2007, with the installation of some equipment in the Brazilian laboratories of the Universities involved, these researches gained more impact and currently several modern GC×GC systems can be found in the Brazilian Institutions, devoted to a wide variety of researches, from natural products, drugs, pesticides, biomass and biofuels [8-14].

In this review it is covered the literature from 2009 to 2020 on the applications of GC×GC involving the pyrolysis of Brazilian agribusiness residues, such as cane straw, sawdust, coconut fiber, fruit seeds, rice husks, spent coffee grounds, among others.

COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY (GC×GC)

Characteristics

Comprehensive two-dimensional gas chromatography (GC×GC) developed in 1991 by Liu and Phillips has several advantages when compared with one-dimensional gas chromatography. The analyte separation mechanism is performed by two interconnected columns, with a transfer system (modulator) located between them [1], as seen in Figure 1. The modulation process is the main stage of the system, which is responsible for trapping, focusing and introducing fractions of the effluent from the column of the first-dimension (1D) into the second-dimension column (2D). This can be achieved through thermal or flow modulators [2,3,5].

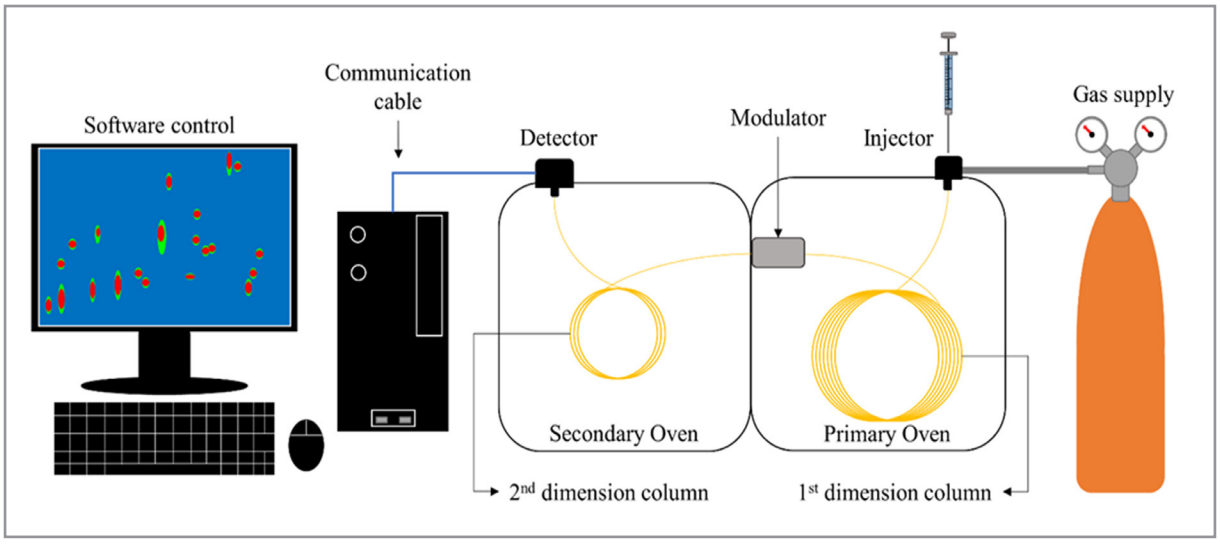


Figure 1. Comprehensive two-dimensional gas chromatography system: injector; 1st dimension column; 2nd dimension column; modulator; detector. (Liu, Zaiyou; Phillips, John B. Comprehensive Two-Dimensional Gas Chromatography using an On-Column Thermal Modulator Interface. *Journal of Chromatographic Science*, 1991, Vol 29, Issue 6, pages 227–231, by permission of Oxford University Press.) [1].

Considering that the peak capacity of a column is the maximum number of peaks separable by it, it can be assumed that the peak capacity of 1D is n_1 peaks, while that of 2D is n_2 peaks. Thus, it can be said that the peak capacity of the GC×GC will be n_1 x n_2 peaks, since the entire sample is subjected to separation in both columns. Comparatively, the peak capacity of the two-dimensional gas chromatography (GC-GC, only a part of the analytes from ^{1}D are also separated in ^{2}D) will be $n_1 + n_2$, since only a fraction of the sample is subjected to separation in 2D [15]. Among these advantages, we can highlight a higher resolution, peak capacity, selectivity and structured elution of sample components in the chromatogram according to their physicochemical properties [6,7].

The first column is usually of low polarity, with analyte separation based on the boiling point. Each modulated fraction is then subjected to a fast GC analysis on the second dimension, generally on a polar column: analytes are resolved on the basis of specific polarity-based characteristics. It is important that the system presents orthogonality, so that the interaction mechanism of 1D is as different as possible from the mechanism used in 2D [6].

The time in which the modulator samples the effluent from 1D and directs it to 2D is a crucial factor for the functioning of the GC×GC, as it directly influences the quality of the chromatogram to be obtained [6]. The sampling sequence followed by injection must be precisely defined and repeated throughout the chromatographic analysis. This time interval is called the modulation period. Ideally, the separation in 2D should occur before the injection of the subsequent chromatographic fraction to minimize the occurrence of peak wraparound [6,7,16].

For bio-oils, the modulation step is important mainly for two reasons: isolating the chromatographic bands coming from the ^{1}D, and re-concentrating these bands to quickly reinject in the ^{2}D. Due to the fact that bio-oils usually present analytes in very different concentration ranges, this re-concentration of the chromatographic band in the modulator facilitates the detection/identification of compounds in low concentrations, which, eventually, could not be detected in 1D.

GC×GC allows a separation of analytes in two-dimensional space according to the chemical classes present in the sample. The orthogonality of the columns used allows this distinction between chemical classes. Some works demonstrate this characteristic, as in the work by von Mühlen et al. [12] shown in Figure 2. The chromatogram in Figure 2 refer to the analysis of *Eucalyptus dunnii* essential oil. The authors managed to divide the chromatograms into distinct regions of: (A) Linear alcohols; (B) aldehydes;

(C) acetates; (D) monoterpenic hydrocarbons; (E) monoterpenic alcohols; (F) monoterpenic acetates; (G) sesquiterpenic hydrocarbons; (H) oxygenated sesquiterpenes.

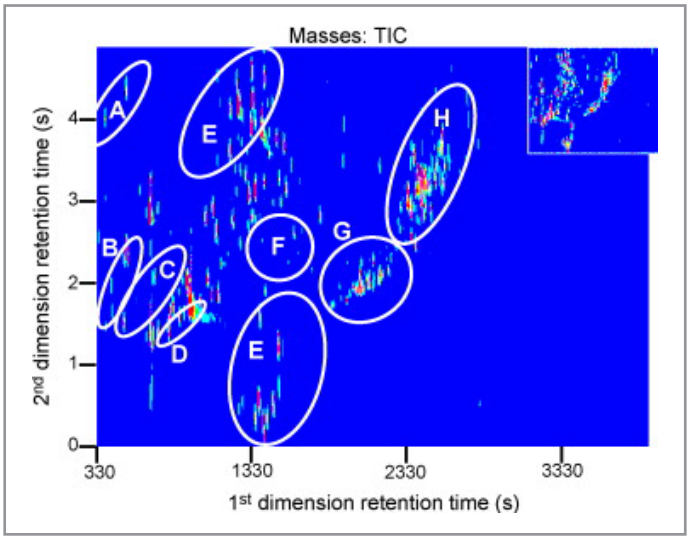


Figure 2. GC×GC/TOFMS total ion current chromatogram (TIC) data colour plot of *E. dunnii* essential oil, showing the distribution of classes of compounds in different regions of the chromatographic space, using a non-polar×polar column set. (A) Linear alcohols; (B) aldehydes; (C) acetates; (D) monoterpenic hydrocarbons; (E) monoterpenic alcohols; (F) monoterpenic acetates; (G) sesquiterpenic hydrocarbons; (H) oxygenated sesquiterpenes. ("Reprinted from *Journal of Chromatography A*, Vol 1200, Issue 1. Authors: Carin von Mühlen, Claudia Alcaraz Zini, Elina Bastos Caramão, Philip J. Marriott. Title: Comparative study of *Eucalyptus dunnii* volatile oil composition using retention indices and comprehensive two-dimensional gas chromatography coupled to time-of-flight and quadrupole masss pectrometry, pages 34–42. Copyright 2008, with permission from Elsevier.) [12]

Several detectors can be coupled to GC×GC, these must have as main characteristics a high acquisition rate, low internal volumes and low time constants [17]. The first detector with high acquisition rates used in GC×GC was the flame ionization detectors (FID) that present acquisition rates from 50 to 200 Hz [15].

Later, mass spectrometry detectors (MS) began to be introduced to this technique; the time-of-flight mass spectrometry detector (TOFMS), allows the collection of up to 500 mass spectra per second. However, the fast-qMS analyzer has consolidated its application in the field of GC×GC due to the development of systems that allow acquisition rates around 50 Hz [12]. The combination of MS with GC×GC provide three analytical dimensions, which stands out as de most important tools for the characterization and identification of complex samples [18].

GC×GC/MS can be used to conduct large scale studies, giving full access to its high-resolution power for targeted and mostly untargeted screening. The current challenges in this area are localized on the data management side, where powerful chemometric tools are required to unlock GC×GC/MS full potential [19].

Comprehensive two-dimensional gas chromatography (GC×GC) has generated a major impact in separation science. In the past 30 years, around 3700 papers were found in the scientific literature through a search on the Scopus Platform using the term "Comprehensive Two-dimensional Gas Chromatography".

History in Brazil

The first research developed in Brazil using GC×GC was in 2009, in which the analysis of organic compounds from water-in-crude oil emulsions separated by microwave heating using comprehensive two-dimensional gas chromatography and time-of-flight mass spectrometry [20] was used. The GC×GC/TOFMS technique proved to be extremely important for the separation and identification of different classes of

compounds. This research represents a milestone in Latin America for separation chemistry, as it was carried in the first GC×GC system in Latin America set up at Universidade Federal do Rio Grande do Sul, Brazil.

Since then, several studies have been developed with different types of matrices. Figure 3 was obtained from a survey of articles in the Scopus database, using the term “Comprehensive two-dimensional gas chromatography” and limiting it to research developed in Brazil. The keywords were processed considering the type of matrices analyzed with at least 3 occurrences, using the VOSviewer 1.6.13 bibliometric software.

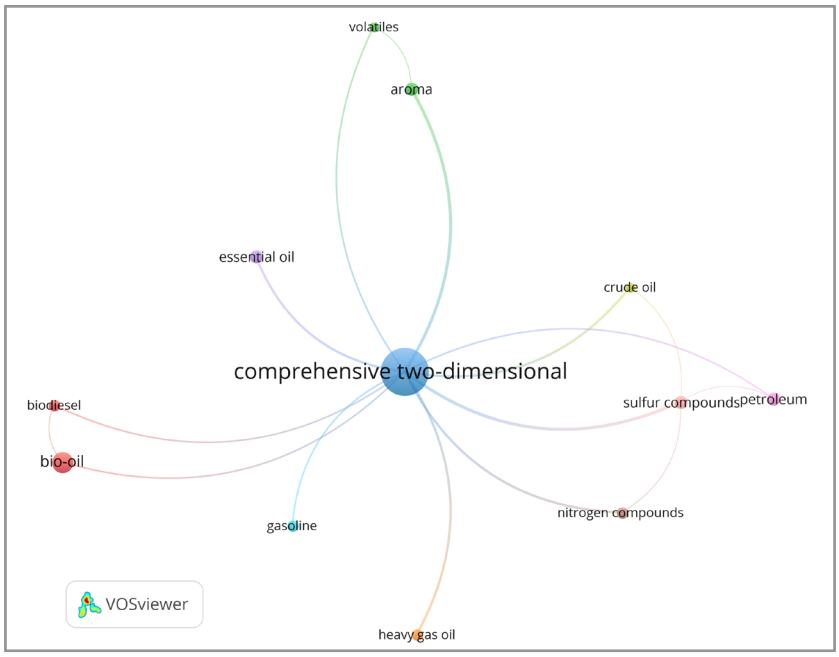


Figure 3. Bibliometric analysis of the most frequently cited keywords related to the type of matrix analyzed by GC×GC in Brazil using the Scopus database.

As shown in Figure 3, several complex matrices have already been analyzed using GC×GC in Brazil, where the largest number of articles found reports their use for bio-oil analysis. This fact can be explained due to the great complexity of this type of matrix, and also by the fact that Brazil presents a large investment in this sector. Brazil has been investing in renewable energy, mainly aiming at the use of agricultural and agro-industrial wastes. For this reason, bio-oil matrices were chosen for the development of this review.

BIO-OIL

Characteristics

The bio-oil, also known as pyrolysis oil, crude bio-oil, pyrolytic tar, wood liquid, wood oil, smoke condensate or wood distillate is the liquid product generated from the pyrolysis of lignocellulosic material. It has a dark brown color, almost black, and a characteristic odor [21,22]. Its composition depends on numerous variables, from the source of the raw material (biomass), through the instrumental conditions of the pyrolytic process (type of reactor, heating rate, final temperature, etc.) to the use of catalysts and the use of upgrading processes (chemical extraction, refining, hydrocracking, hydrodeoxygenation, steam reforming, esterification, emulsification, etc.) [23,24].

For proposing a better use of bio-oil, it is necessary to know its physico-chemical properties and its chemical composition. Several physical and chemical methods for the characterization and analysis of bio-oil have been used. This applied to properties such as ash and water content, elementary analysis, total solid content, heating value, density, viscosity, acidity and solubility in different solvents, in addition, of course, to their chemical composition. Table I lists the main characteristics and physicochemical properties of bio-oil [23,25–30].

Table I. Elementary composition and physicochemical properties of bio-oil

Properties	Characteristics	Reasons
Appearances	Dark brown to black	Bio-oil chemical composition [23]
Smell	Smoke odor	Aldehydes and acids of low molecular weight [23]
Density	1.2 kg L^{-1} (greater than fossil fuels)	High humidity and presence of high molecular weight molecules [23,25]
Viscosity	40 to 1000 centistokes (cSt)	Composition of biomass, water content and light product content [25]
Heating value	Lower than that of fossil fuels; 16-20 MJ kg^{-1}	High oxygen contente [23,25,26]
Miscibility	Miscible in polar solvents, but totally immiscible in oil	Polar nature [23]
Aging	Increased viscosity, decreased volatility, phase separation and resin deposition	Complex structures and acid pH [23]
Water content	15-35%	Residual water from biomass and parallel reactions [23,26,27]
Carbon content	50-64%	Biomass composition and thermal conversion process [23,28]
Hydrogen content	5-7%	Biomass composition and thermal conversion process [23,28]
Oxygen content	15-40%	Biomass composition and thermal conversion process [23,28]
Ash content	0.01-0.6%	Biomass composition [23,29,30]
Acidity	pH bellow 4	Biomass composition and thermal conversion process [23,25]

Chemical characterization

The chemical characterization of bio-oils is important not only to propose the best use of these oils, but also to determine the presence of possible harmful compounds that may be formed during pyrolysis process. Thus, one can evaluate the possibility of bio-oil being a renewable fuel source or used as a starting material for obtaining chemicals. For example, a bio-oil with a higher percentage of phenolic compounds

can be applied as a substitute for fossil phenols in phenolic resins for the production of chemicals. A bio-oil that presents a significant amount of long-chain carboxylic acids and hydrocarbons is more applicable for use as liquid fuel after an upgrading process [30].

The analytical techniques frequently used for bio-oil analysis are chromatographic methods, such as Gas Chromatography (GC) and Liquid Chromatography (LC) (as they allow the separation and identification of organic compounds in complex matrices) and spectroscopy methods such as Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS). These techniques are generally the most used due to their high precision and sensitivity [31]. The GC×GC, on the other hand, has been proved to be more efficient regard about to sensitivity, resolution and peak capacity, when compared to one-dimensional gas chromatography.

A representative list of some compounds present in bio-oils and their respective chemical classes are shown in Figure 4. The compounds and the amount of each analyte present in the bio-oil depend on several factors such as the type and composition of the biomass, pyrolysis process, biomass storage, among others [32].

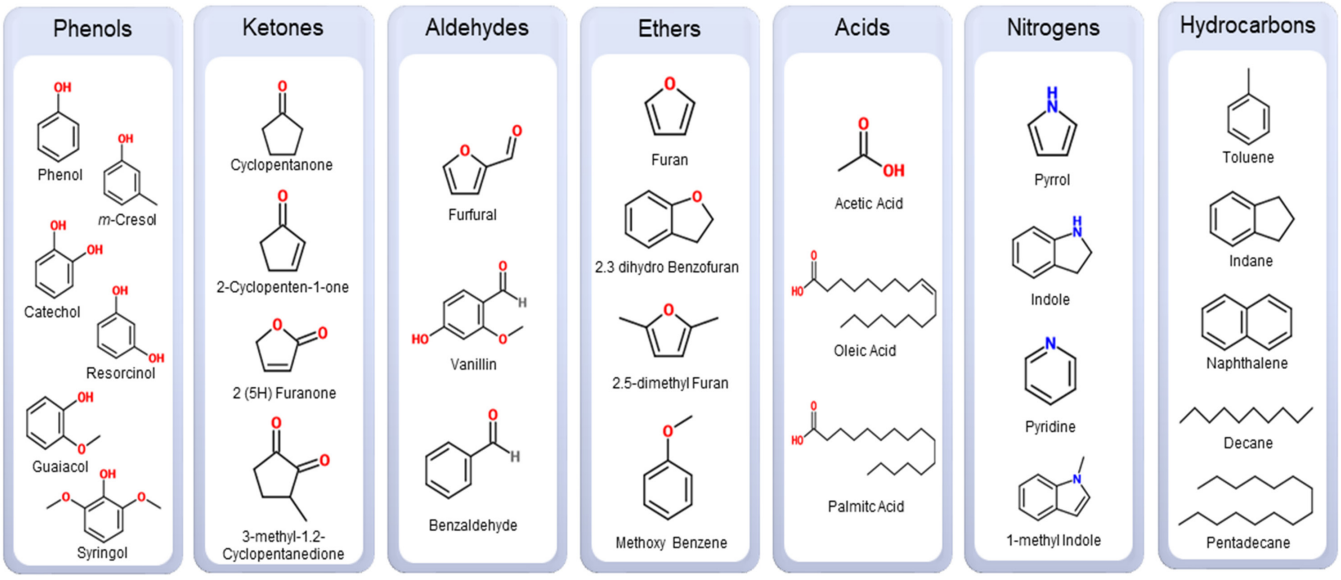


Figure 4. Main compounds found in bio-oils and their respective chemical classes.

GC×GC FOR BIO-OILS OF THE BRAZILIAN BIOMASSES

In Brazil, there are several studies using GC×GC to characterize bio-oils from lignocellulosic biomass, and it is possible to note that the main these bio-oils are produced from biomass from agro-industrial residues, not competing with food production. The research developed for the analysis of bio-oils derived from the pyrolysis of different Brazilian biomasses, were subdivided, in this review, into woody, oily and others biomasses.

In these studies, the injection modes used were split or split/splitless, with 1 µL of sample being used in most cases. Conventional sets of columns were used in most of the analysis: phases with 5% phenyl and 95% poly(dimethylsiloxane) in the first dimension, 30-60 length (m) x 0.18-0.25 i.d. (mm) x 0.10-0.25 thickness (µm), and 50% phenyl and 50% poly(dimethylsiloxane) in the second dimension, 1.10-2.50 length (m) x 0.10-0.25 i.d. (mm) x 0.10-0.25 thickness (µm). Three different types of detectors were coupled to the GC×GC: flame ionization detector (FID), quadrupole mass spectrometry (q/MS) e time-of-flight mass spectrometry (TOFMS). And finally, hydrogen (for GC×GC/FID) and helium (for GC×GC/qMS and TOFMS) were used as carrier gas at 0.70-1.20 mL min^{-1}.

The qualitative characterization of the compounds has been carried out using the chromatographic techniques associated with the Linear Temperature Programming Retention Index (LTPRI), where a standard mixture of linear hydrocarbons is analyzed under the same conditions as the samples [33] and compared with standard compounds and the literature (webbook.nist.gov). The use of retention index identification is tentative identification. For positive identification, it is necessary to use reference standards.

The mass spectra of each analyte are frequently used, facilitating the compounds identification by comparing with the mass spectra of commercial libraries (NIST or Wiley). Some studies also evaluate the compounds semi-quantitatively, using the relative percentage area of the chromatographic peaks and quantitatively, using standards and calibration curves. Table II presents a summary of the studies that have used Brazilian biomass for bio-oil production, and characterization by GC×GC detailing the analysis settings, as well as the main classes of compounds found.

Table II. Researches performed with Brazilian biomass for analysis by GC×GC

Biomass Type	Biomass	Pyrolysis	Chromatographic Parameters						Majority Chemical Classes	Ref.
			Column and Dimensions [length (m) x i.d. (mm) x thickness (μm)]		Oven Temperatures		Carrier gas; Flow (mL min^{-1})	Detector		
			^{1}D	^{2}D	^{1}D	^{2}D				
Woody	*Eucalyptus sawdust*	—	DB-5 (50x0.25x0.25)	DB-17 (2.15x0.18x0.18)	60°C (0.20 min) – 3°C min^{-1} – 330°C (20 min)	10 °C above the ^{1}D	He; 1.20	TOFMS	Phenols and ketones	34
	Sawdust from forest timber	Fast	DB-5 (60x0.25x0.25)	DB-17 (2.15x0.18x0.18)	40°C – 3°C min^{-1} – 120°C – 2°C min^{-1} – 200°C – 10°C min^{-1} – 280°C (5 min)	The same as ^{1}D	He; 0.91	q/MS	Phenols and ketones	35
	Eucalyptus sp and Picea abies	Fast and Catalytic	DB-5 (60x0.25x0.10)	DB-17 (2.15x0.18x0.18)	60°C (3 min) – 3°C min^{-1} – 240°C (10 min)	10 °C above the ^{1}D	-	TOFMS	Phenols and ketones (fast); Polyaromatic hydrocarbons (catalytic)	36
	Pinewood	Catalytic	DB-5 (30x0.25x0.25)	BPX-50 (1.50x0.10x0.10)	35°C (6 min) – 3°C min^{-1} – 330°C	10 °C above the ^{1}D	He	TOFMS	Ketones and hydrocarbons	37
	Pinewood	Fast, Catalytic and hydrodeoxygenation	DB-5 (30x0.25x0.25)	BPX-50 (1.50x 0.10x0.10)	35°C (6 min) – 3°C min^{-1} – 330°C (3 min)	10 °C above the ^{1}D	He; 1.00	TOFMS	Sugars (fast); Aromatic hydrocarbons (catalytic); Alcohols (hydrogeoxygenation)	38
	Palm fruit bunch and pinewood	Flash	DB-5 (30x0.25x0.25)	BPX-50 (1.50x0.10x0.10)	35°C (15 min) – 4°C min^{-1} – 330°C	20 °C above the ^{1}D	He; 1.00	TOFMS	Ketones, cyclopentenones, furanones, furans and phenols	39; 40
	Pinewood	Fast and catalytic	DB-5 (30x0.25x0.25)	BPX-50 (1.50x0.10x 0.10)	35°C (6 min) – 3°C min^{-1} – 330°C	10 °C above the ^{1}D	He; 1.00	TOFMS	Lactones, Ketones and acids (fast); Aromatic hydrocarbons (catalytic)	41

Table II. Researches performed with Brazilian biomass for analysis by GC×GC (continuation)

Biomass Type	Biomass	Pyrolysis	Chromatographic Parameters						Majority Chemical Classes	Ref.
			Column and Dimensions [length (m) x i.d. (mm) x thickness (μm)]		Oven Temperatures		Carrier gas; Flow (mL min^{-1})	Detector		
			^{1}D	^{2}D	^{1}D	^{2}D				
Oily	*Spent coffee grounds*	Fast	DB-5 (60x0.25x0.25)	DB-17MS (2.50x0.18x0.18)	60°C (1 min) – 3°C min^{-1} – 280°C	5 °C above the ^{1}D	He; 1.00	TOFMS	Hydrocarbons, nitrogen compounds and fatty acids	42
	Silverskin	Fast	OV-5 (60x0.25x0.10)	DB-17MS (2.50x 0.18x0.18)	40°C (5 min) – 5°C min^{-1} – 300°C (20 min)	The same as ^{1}D	He; 0.91	q/MS	Phenols and nitrogen compounds	43
	Bark of acuri and endocarp of baru	Fast	DB-5 (60x0.25x0.10)	DB-17MS (2.50x0.18x0.18)	50°C (5 min) – 4°C min^{-1} – 280°C (8 min)	10 °C above the ^{1}D	-	TOFMS	Phenols and ketones (bark of acuri); Hydrocarbons and phenols (endocarp of baru)	44
	Peach pit	Fast	DB-5 (30x0.25x0.25)	DB-17MS (1.84x0.18x0.18)	40°C (5 min) – 5°C min^{-1} – 280°C (10 min)	20 °C above the ^{1}D	-	TOFMS	Phenols and ketones	45
	Castor seed cake	Slow	DB-5 (30x0.25x0.25)	BPX-50 (1.50x 0.10x0.10)	35°C (15min) – 4°C min^{-1} – 330°C	20 °C above the ^{1}D	He; 1.00	TOFMS	Nitrogenous compounds and phenols	32
	Tabacco seeds	Fast	DB-5 (60x0.25x0.10)	DB-17 (2.15x0.18x0.18)	40°C (2 min) – 4°C min^{-1} – 280°C (3 min)	The same as ^{1}D	He; 0.89	q/MS	Phenols and hydrocarbons	46
	Mango seed waste	Fast	DB-5 (60x0.25x0.25)	DB-17MS (1.20x0.18x0.18)	50°C (4 min) – 4°C min^{-1} – 280°C	10 °C above the ^{1}D	He; 1.00	TOFMS	Phenols and ketones	47
	Crambe seeds	Slow	DB-17 e DB-5	BPX-50 e DB-5	35°C (6 min) – 3°C min^{-1} – 330°C	5 and 10 °C above the ^{1}D	He; 1.00	TOFMS	Hydrocarbons (bio-oil); Amides and carboxylic acids (Aqueous phase)	48
	Crambe seeds	Fast	DB-5MS (60x0.25x0.25)	DB-17MS (2.15x0.18x0.18)	50°C (1 min) – 4°C min^{-1} – 280°C (10 min)	5 °C above the ^{1}D	He; 1.00	TOFMS	Fatty acids, hydrocarbons and phenols	49
	Soursop seed cake and bocaiuva seed cake	Slow	DB-5 (30x0.25x0.25)	DB-17 (1.20x0.10x0.10)	35°C (15 min) – 3°C min^{-1} – 330°C	5 °C above the ^{1}D	He; 1.00	TOFMS	Carboxylic acids and amides (soursop seed cake); Hydrocarbons and phenolic derivatives (bocaiuva seed cake)	50

Table II. Researches performed with Brazilian biomass for analysis by GC×GC (continuation)

Biomass Type	Biomass	Pyrolysis	Chromatographic Parameters						Majority Chemical Classes	Ref.
			Column and Dimensions [length (m) x i.d. (mm) x thickness (μm)]		Oven Temperatures		Carrier gas; Flow (mL min^{-1})	Detector		
			^{1}D	^{2}D	^{1}D	^{2}D				
Others	*Sugar cane straw*	Fast	DB-5 (30x0.25x0.25)	DB-17MS (1.20x0.18x0.18)	40°C (5 min) – 3°C min^{-1} – 315°C (10 min)	10 °C above the ^{1}D	He; 1.00	TOFMS	Phenols, ketones and aldehydes	51
	Sugar cane straw	Fast	OV-5 (60x0.25x0.10)	DB-17 (2.15x0.18x0.18)	60°C (1 min) – 3°C min^{-1} – 280°C (25 min)	The same as ^{1}D	He; 0.89	q/MS	Phenols, ketones and aldehydes	52
	Sugar cane straw	Fast	DB-5 (60x0.25x0.25)	DB-17MS (2.15x0.18x0.18)	100°C (0.2 min) – 2°C min^{-1} – 210°C (3 min)	20 °C above the ^{1}D	-	TOFMS	Phenols, aldehydes and ketones	53
	Sugar cane bagasse	Catalytic	DB-5 (30x0.25x0.25)	BPX-50 (1.50x0.10x0.10)	35°C (6 min) – 3°C min^{-1} – 330°C	10 °C above the ^{1}D	He	TOFMS	Ketones and carboxylic acids	37
	Sugar cane bagasse and straw	Fast	DB-5 (60x0.25x0.25)	DB-17 (2.15x0.18x0.18)	40°C – 5°C min^{-1} – 300°C (20 min)	The same as ^{1}D	He; 1.00	q/MS	Phenols and furans	54
	Sugar cane bagasse	Fast and catalytic	DB-5 (30x0.25x0.25)	BPX-50 (1.50x0.10x 0.10)	35°C (6 min) – 3°C min^{-1} – 330°C	10 °C above the ^{1}D	He; 1.00	TOFMS	Lactones, Ketones and acids (fast); Aromatic hydrocarbons (catalytic)	41
	Coconut fiber	Fast	DB-5 (60x0.25x0.25)	DB-17MS (2.15x0.18x0.18)	60°C (1 min) – 3°C min^{-1} – 210°C (3 min)	20 °C above the ^{1}D	He; 1.00	TOFMS	Phenols and aldehydes	55
	Coconut fiber	Fast	DB-5 (30x0.25x0.25) DB-5 (10x0.18x 0.25)	DB-17 (1.25x0.18x0.18) DB-17 (1.10x0.18x0.18)	45°C – 3°C min^{-1} – 260°C 45°C – 10, 15 and 20°C min^{-1} – 260°C	15 °C above the ^{1}D	He; 1.00	TOFMS	Phenols, ketones, aldehydes and acids	56
	Coconut fiber	Fast	DB-5 (60x0.25x0.25)	DB-17 (2.10x0.18x0.18)	45°C – 5°C min^{-1} – 290°C (10 min)	10 °C above the ^{1}D	He; 1.00	TOFMS	Phenols, ketones and aldehydes	57
	Coconut fiber	Fast	DB-5 (10x0.18x0.25)	DB-17 (1.10x0.18x0.18)	45°C (2 min) – 15°C min^{-1} – 260°C (2.5 min)	15 °C above the 1D	He; 1.00	TOFMS	Phenols, ketones and aldehydes	58
	Orange bagasse	Fast	DB-5 (30x0.25x0.25)	DB-17MS (1.30x0.25x0.25)	—	—	H; 1.00 and He; 0.70	FID and TOFMS	Acids, aldehydes, alcohols and ketones	59

Table II. Researches performed with Brazilian biomass for analysis by GC×GC (continuation)

Biomass Type	Biomass	Pyrolysis	Chromatographic Parameters						Majority Chemical Classes	Ref.
			Column and Dimensions [length (m) x i.d. (mm) x thickness (μm)]		Oven Temperatures		Carrier gas; Flow (mL min^{-1})	Detector		
			^{1}D	^{2}D	^{1}D	^{2}D				
Others	*Digester residue and wastewater treatment sludge*	—	DB-5 (50x0.25x0.25)	DB-17 (2.15x0.18x0.18)	60°C (0.20 min) – 3°C min^{-1} – 330°C (20 min)	10 °C above the ^{1}D	He; 1.20	TOFMS	Phenols and Ketones (both biomass); Nitrogen compounds and alcohols (waster treatment sludge)	34
	Rice husk	Intermediate	DB-5 (60x0.25x0.25)	DB-17MS (2.15x0.18x0.18)	40°C (5 min) – 3°C min^{-1} – 280°C	The same as ^{1}D	He; 1.00	q/MS	Phenols and ketones	60
	Rice husk	Fast	DB-5 (30x0.25x0.25)	DB-17MS (1,84x0.18x0.18)	40°C (5 min) – 3°C min^{-1} – 315°C	10 °C above the ^{1}D	—	TOFMS	Phenols and ketones	45
	Rice husk	Intermediate	DB-5 (60x0.25x0.25)	DB-17MS (2.15x0.18x0.18)	40°C (5 min) – 3°C min^{-1} – 280°C	The same as ^{1}D	He; 1.00	q/MS	Phenols and ketones	61
	Endocarp, bark and fiber of bocaiúva	Fast	DB-5 (60x0.25x0.10)	DB-17MS (2.15x0.18x 0.18)	50°C (5 min) – 4°C min^{-1} – 280°C (8 min)	10 °C above the ^{1}D	—	TOFMS	Phenols (endocarp and bark); Hydrocarbons (fiber)	62

Biomass used for bio-oil production and analysis by GC×GC are of the most several types. To facilitate the discussion of literature data, it was classified into 3 types: woody biomass, which corresponds to biomass rich in lignocellulosic materials; oily biomasses that correspond in general to seeds and nuts; and, another biomass, which corresponds to biomass not previously classified.

GC×GC applied to woody biomass bio-oils

Woody biomasses are rich in lignocellulosic materials and the goal of their study was originally for bio-fuels as alternative to fossil fuels. However, the bio-oil produced by pyrolysis of these material, as demonstrated by chromatographic analysis, have many oxygenated compounds which are not indicated for this purpose. Now, woody bio-oils are mainly studied for producing substances with high added value for the chemical industry. Faccini et al. [34], optimized the pyrolysis process (using different temperatures) with residues of Eucalyptus sawdust. The bio-oil from this process has undergone a qualitative and semi-quantitative characterization by GC×GC/TOFMS. The total number of compounds tentatively identified in the bio-oil was 146, which corresponded to a total detected area of 97%. Phenols and ketones were the predominant chemical classes. Despite the great analytical capacity to separate compounds from the chromatographic technique used in this research, some problems of co-elution of compounds could be observed in the analyses. However, mass spectral deconvolution, offered by the GC×GC/TOFMS software (CHROMATOF), played an important role to solve this analytical problem because it provided a separation between two or more compounds through differences in their mass spectra and retention times, once TOFMS assures constant ion ratios along the chromatographic peak.

Schneider et al. [35] studied the most polar fractions of sawdust from forest timber bio-oil produced by fast pyrolysis and characterized by GC×GC/qMS. The use of the fast-quadrupole as a mass analyzer represents a great impulse in the two-dimensional analysis, because this detector is robust, simple, reproductive, sensitive and cheaper if compared to time-of-flight mass detectors, which is the most used for complex samples. From this analysis, 130 compounds were identified (phenols, ethers, ketones, aldehydes, acids, alcohols and aromatic hydrocarbons). From these compounds, 57 were confirmed by LTPRI, corresponding to 43.8% of the total identified. The relative concentration (semi-quantitative) was expressed in relation to the peak volume (volume of the three-dimensional peak of each compound divided by the total peak volume x 100). The results showed phenols as the major class of this bio-oil, with 60% of the total volume, followed by ketones, with 25%. The 4-methyl-1,2-benzenediol (12.1%), 1,2-benzenediol (11.1%), C2-benzenodiol (7.1%) and phenol (4.8%) were the major compounds.

In the research described by Torri et al. [36], bio-oil fractions from fast and catalytic pyrolysis of forest residues (Eucalyptus sp. and Pices abies) were analyzed by GC×GC/TOFMS. The non-catalytic bio-oils presented, mainly, phenols, ketones and aldehydes, while the catalytic ones presented polyaromatic hydrocarbons, phenols and ketones. In this case, the use of the catalyst (ZSM-5, SiO_2/Al_2O_3 ratio of 140) favored the production of polyaromatic hydrocarbons.

Mendes et al. [37], carried out catalytic pyrolysis of pinewood and the bio-oil obtained was characterized by GC×GC/TOFMS. The ketones and hydrocarbons were the predominant chemical classes. The use of the ZSM-5 catalyst was mainly responsible for the significant increase in the content of aromatic hydrocarbons in bio-oil, mainly monoaromatics, such as the toluene and C2-benzene isomers (xylenes and ethylbenzene). These compounds are used as petrochemical intermediates with high added value for the chemical industry. In addition, the ZSM-5 reduced the number of oxygenated compounds such as phenols.

In the research done by Silva et al. [38], the authors performed a characterization (semi-quantitative and quantitative) by GC×GC/TOFMS of pinewood bio-oil from three pyrolytic processes: real thermal(PWT), catalytic process(CP) and hydrodeoxygenation process(HDO). The chromatographic method presented in this article proved to be suitable for the quantification of hydrocarbons and O-containing compounds in real samples of bio-oil with excellent accuracy and precision. The results obtained by the semi-quantitative analysis allowed a preliminary analysis by comparing the distribution of classes, where sugars, aromatic

hydrocarbons and alcohols appeared as the most abundant in PWT, CP and HDO bio-oils from pinewood, respectively. The quantitative evaluation allowed to obtain an individual concentration of target compounds. Among these, the 2(3H)-Furanone, dihydro-3-methyl had a higher concentration in the HDO bio-oil, while in the other samples (PWT and CP) the concentration were more evenly distributed in aromatic compounds.

In the studies developed by Tessarolo et al. [39,40], the authors obtained bio-oils from empty palm fruit bunch and pine wood chips from flash pyrolysis and characterized the fractions by GC/MS and GC×GC/TOFMS. The higher chromatographic resolution and sensitivity of the two-dimensional technique and the use of a detector with a higher data acquisition rate (TOFMS) allowed for better separation and greater identification (four to seven times more) of compounds in both samples, solving problems of co-elution found in the monodimensional analysis. The results of the two-dimensional analyses indicated that a large number of peaks were detected (631 and 857, respectively, for empty palm fruit bunch and pine wood chips bio-oils) and the main classes of compounds in both bio-oil samples were: ketones, cyclopentenones, furanones, furans, phenols and sugars. In addition, esters, aldehydes and pyridines were found for samples obtained from empty palm fruit bunch, while alcohols and cyclopentadiones were found in samples prepared from pine wood chips, indicating different composition profiles due to biomass source.

Tessarolo et al. [41] also submitted pine wood to the thermal and catalytic pyrolysis process using ZSM-5. The bio-oils obtained were characterized by GC×GC/TOFMS. The classes identified were lactones, cyclic ketones, acids, aldehydes, phenols and aromatic hydrocarbons. The use of the ZSM-5 catalyst promoted deoxygenation, reducing the content of oxygenated compounds, such as acids, and increasing the production of aromatic hydrocarbons, with alkyl-benzenes being the main components.

GC×GC applied to oily biomass bio-oils

Brazil is a great agricultural producer and the problem of the destination of agro-industrial waste has been explored in recent decades. Among these discharges stand out oily biomasses, such as seeds from agricultural productions, besides waste of important crops such as crambe, bocaiuva, coffee, soursop, tobacco, among others.

Coffee is an important agricultural product, being one of the most consumed beverages in the world and Brazil leads its worldwide production. Primaz et al. [42] used spent coffee grounds as biomass in the pyrolysis to obtain bio-oil. The characterization (qualitative and semi-quantitative) of the bio-oil was carried out by GC×GC/TOFMS. It was tentatively identified 190 compounds, belonging to the classes of hydrocarbons, nitrogen compounds and oxygen compounds, including ketones and phenols, in addition to oily compounds such as fatty acids and esters. Due to the high level of fats present in this biomass, the esters and fatty acid classes were found significantly, with the major compound being palmitic acid (19%). In this research, dispersion graphics were used to analyze the spatial distribution of the identified components, built from the retention times of the first and second dimensions. This distribution was made according to the molecular weight, and the number of substituents and branches, proving to be an excellent tool on qualitative analysis.

Polidoro et al. [43] submitted silverskin, a derived by-product from the coffee roasting process, to the pyrolysis process. In this research, the pyrolysis process was optimized using the response surface methodology and the bio-oil produced under the optimized conditions was analyzed by GC×GC/qMS. The use of this technique allowed the identification of 228 compounds, where the main chemical class, in terms of percentage of peak volume, was phenols (20.76%), followed by nitrogen compounds (18.51%). In this research, the use of dispersion graphics was also explored, ordering the chemical classes identified in the bio-oil according to molecular weight and polarity. The use of the GC×GC allowed to verify the presence of several groups of analytes with similar retention time in the first dimension, which would imply co-elutions in the use of the conventional GC.

The bio-oil of the acuri bark and baru endocarp residues were studied by Cardoso et al. [44] using GC×GC/TOFMS. In the qualitative analysis, 113 compounds were tentatively identified in the acuri bark

bio-oil, with phenols and ketones as the main chemical classes and guaiacol as the major compound, with 18.67% of the total area. For the baru endocarp residue bio-oil, 71 compounds were identified, with hydrocarbons and phenols being the main chemical classes and toluene as the major compound, contributing with 33.19% of the total area of the chromatogram.

The peach core was used as biomass for studies by Moraes et al. [45]. The pyrolysis parameters were optimized for another biomass (rice rusk) and the best conditions were applied for the production of peach stone bio-oil. This bio-oil was characterized qualitatively and semi-quantitatively using the GC×GC/TOFMS technique and 223 compounds classified as phenols and ketones (majority classes), acids, ethers and aldehydes were identified. The major compound found in this sample was furfural, with 8.82% of area.

Silva et al. [32] submitted the residual castor seed cake (after oil extraction process) to slow pyrolysis, obtaining a bio-oil yield of 22.3%. The chromatographic characterization performed by GC×GC/TOFMS allowed the tentative identification of 995 compounds. The main classes found in crude bio-oil were nitrogenous compounds, with emphasis on pyrroles (13.19%), nitriles (9.04%), pyridine (5.77%) and oxygenated compounds: phenols (10.27%) and carboxylic acids (4.48%). The increase in the separation power enabled the identification and separation into classes by groups of regions of the chromatogram, generating well-ordered 2D maps, which can be used to monitor the transformation process to which the oil may eventually be subjected.

The residual tobacco seeds (after the oil extraction) were pyrolyzed by Onorevoli et al. [46]. An acid-base extraction was performed on the bio-oil in order to obtain extract rich in nitrogenous compounds. The acidic organic phase was extracted using HCl and the nitrogen compounds were recovered using NaOH due to their basic characteristics. The crude bio-oil and the nitrogen-rich extract were subjected to analysis by GC×GC/qMS (qualitatively and semi-quantitatively). In the crude bio-oil, 148 compounds were tentatively identified, among them, phenols, esters, ketones, alcohols, hydrocarbons and nitrogen compounds; while in the nitrogen rich extract, 40 compounds (mostly nitrogen compounds) were identified.

Lazzari et al. [47] submitted mango seed residues (tegument and almond) to pyrolysis. The bio-oils obtained were analyzed by GC×GC/TOFMS, and 108 compounds were identified for the tegument bio-oil sample, with phenols (32.6%) and ketones (22.9%) as major classes. As for the almond bio-oil, 120 compounds were identified, with the major classes being ketones (20.6%) and acids (16.8%).

In another research developed by Silva et al. [48], the liquid products (bio-oil and aqueous phase) obtained through the slow pyrolysis of the crambe seed were analyzed. The organic compounds present in the aqueous phase were lyophilized and diluted in organic solvent for analysis. The elucidation of the chemical composition of the fractions was performed by GC×GC/TOFMS. For the bio-oil sample, an inverse set of columns (DB-17 as the first dimension column and a DB-5 as the second dimension column) was used, which is more suitable for hydrocarbon separation. In this case, using an unconventional set of columns, the separation in the first dimension is done through the volatility and specific interactions of the compounds, while in the second dimension the separation depends only on volatility. The bio-oil analyzed has more apolar characteristics, rich in hydrocarbons, which explains the use of an apolar column in the first dimension for better separation of this class. For the aqueous phase, the conventional system (DB-5 as the first-dimension column and a BPX-50 as the second-dimension column) was adopted, which is more suitable for the separation of organic compounds present in this phase due to the high polarity of their compounds. The identification of the compounds was done by comparing the mass spectra with the NISTTM library associated with the retention index. The semi-quantitative analysis was performed using the relationship between peak areas and the concentration of internal standards. The use of the unconventional set of columns allowed a better separation of the hydrocarbons present in large quantities (67.5%) from the total of identified analytes. Quantitative analysis allowed determining 66% in bio-oil mass. Among the identified classes, the alkyl-benzenes, nitriles and olefins displayed higher concentrations. In total, 137 alkyl-benzenes were identified, with toluene being the major compound (26.4 mg g^{-1}). In the aqueous phase, 136 compounds were identified, with the predominant classes being amides and carboxylic acids, with acetic acid being the major compound (48.7 mg g^{-1}). Semi-quantification by GC×GC/

TOFMS identified only 34.7% by mass, likely due to the highly polar characteristic of this type of sample and, consequently, the strong interaction with the stationary phase used in the chromatographic separation. The characterization of such complex samples was feasible due to the high resolution of the applied technique, which also allowed the use of tools such as spectral deconvolution in the separation of co-elutions of some compounds found.

Onorevoli et al. [49] also used crambe seed to obtain bio-oil by fast pyrolysis. However, before the pyrolytic process, the biomass was extracted with 3 extractive processes to remove vegetable oil: mechanical pressing extraction (MPE), Soxhlet extraction (SE) and compressed propane extraction (CPE). The analysis of bio-oil compounds was performed using GC/qMS and GC×GC/TOFMS techniques, using a conventional column set. The analysis showed similar chromatographic profiles for the three samples and through this it was possible to tentatively identify 195 compounds in the bio-oil obtained after the pressing extraction (MPE), 307 compounds for the bio-oil obtained after the Soxhlet extraction (SE) and, finally, 361 compounds in the bio-oil obtained by the compressed propane extraction (CPE). For the MPE sample, the amounts of acids and phenols were high, showing low efficiency in the extraction of vegetable oil, while the hydrocarbons class appears in higher concentrations for the SE sample. The main differences found between the analysis techniques are related to the classes of alcohols, aldehydes and nitrogen compounds. Alcohols and aldehydes do not appear in the analysis (or appear with small peak areas) by GC×GC/TOFMS, while the amount of nitrogen-compounds is greater when compared to the GC/qMS technique. This is probably due to the co-elutions that occur in GC/qMS, which result in peaks that are not completely separated, preventing the correct identification of the compounds.

In the research developed by Nunes et al. [50], the residual seed-cakes of soursop and bocaiuva (after oil extraction) were used in the slow pyrolysis process. Chromatographic analyses of the obtained bio-oils were performed by GC×GC/TOFMS. Semi-quantification was performed by relating the area of the identified peaks to some internal standards. Thus, 414 compounds were identified in the bio-oil sample of the soursop seed cake, with carboxylic acids (30.7%) and amides (25.4%) as major classes. As for the bio-oil of bocaiuva seed cake, 222 compounds were tentatively identified, with hydrocarbons and phenolic derivatives as major classes, these presenting areas of 32.0% and 29.4%, respectively.

GC×GC applied to bio-oils from other biomasses

Brazil is the world's largest sugarcane producer and it represents a large portion of energy production for industries. For this reason, several studies using residues of this biomass have been standing out in the last decade. Moraes et al. [51] applied the GC×GC/TOFMS technique for identification and semi-quantification of main compounds in bio-oil, derived from intermediate pyrolysis of sugarcane straw, allowing the tentative identification of 123 compounds. Cunha et al. [52] used pressurized solvent fractionation by solvent elution of the bio-oil obtained from the fast pyrolysis of sugarcane straw and analyzed the fractions by GC×GC/qMS. Using LTPRI, 166 compounds were identified. In both studies mentioned above, phenols, ketones, aldehydes and aliphatic hydrocarbons were predominant. Maciel et al. [53] performed extractions of the aqueous phase of the sugarcane straw bio-oil with solvents of different polarities, using SPE (Solid Phase Extraction) and LLE (Liquid-Liquid Extraction) techniques, and the analysis of the aqueous extracts occurred by GC×GC/TOFMS, which allowed the semi-quantitative identification of phenols, aldehydes and ketones mostly. Phenol was the main compound, indicating the potential use of this material as a source of phenolic raw materials for industry.

Mendes et al. [37] carried out a catalytic pyrolysis of sugarcane bagasse and the bio-oil produced was analyzed by GC×GC/TOFMS. Mostly, phenols, ketones, carboxylic acids, aliphatic and aromatic hydrocarbons were identified. Barros et al. [54] characterized the bio-oil of some species of sugarcane (straw and bagasse): Saccharum sp., Saccharum Robustum, Miscanthus sp. and Erianthus sp. using the GC×GC/qMS. The bio-oils presented similar chemical composition and the following compounds were mainly identified: phenols, methoxylated phenols, such as syringol and 4-vinyl guaiacol; furans, such as 2.3-dihydro-benzofuranand and in smaller proportions aldehydes, ketones and ethers.

Tessarolo et al. [41] submitted the sugarcane bagasse to the same pyrolysis process (thermal and catalytic) of pinewood (woody biomass) using ZSM-5 and the bio-oil obtained was also characterized by GC×GC/TOFMS. The samples presented similar profiles with pinewood and the classes identified were lactones, cyclic ketones, acids, aldehydes, phenols and aromatic hydrocarbons (which significantly increased with the use of the ZSM-5 catalyst).

Some studies with coconut fiber biomass have been discussed in recent years, due to the environmental concern that this waste generates by their disposal in open landfills after the coconut-water consumption. Almeida et al. [55] performed fast pyrolysis with green coconut fiber and the characterization of the bio-oil occurred by GC×GC/TOFMS, which allowed the semi-quantification of 94 compounds, mostly oxygenated.

Schena et al. [56] carried out comparative studies between the conventional GC×GC/TOFMS and fast-GC×GC/TOFMS for bio-oils samples from the fast pyrolysis of green coconut fiber. The results showed that in addition to a reduction in analysis time (around 80%) without compromising the separation of compounds, fast-GC×GC/TOFMS allowed a better identification of compounds and narrower peaks, increasing the signal/noise ratio. A total of 327 compounds were tentatively identified by comparing the mass spectrum of the compounds with the NIST™ library. Phenols, ketones, aldehydes and fatty acids were the major chemical classes found in both samples, followed by alkanes and aromatic hydrocarbons, esters and nitrogen compounds. In another study done by Schena et al. [57], two forms of optimization were carried out in the coconut fiber pyrolysis process. One of them was the alkaline extraction of the coconut fiber bio-oil, producing an acidic and neutral fraction. The other optimization process was a pre-treatment of biomass before pyrolysis, using two different extraction techniques (Soxhlet and Sonication). Both processes proved to be efficient and complementary: in the first case, there was a pre-concentration of the phenols in a single fraction and in the second, a large part of the fatty acid derivatives were removed from the bio-oil. These results indicated that the two techniques can be used to improve the quality of the bio-oil produced, removing free fatty acids from the biomass, and isolating compounds with high added value (in the case of phenols). In a third study by Schena et al. [58], the authors evaluated the effect of the TOFMS data acquisition rate on the quality of the analytical information obtained by GC×GC/TOFMS. In the analysis of coconut fiber bio-oil under fast GC×GC/TOFMS conditions, use of high data acquisition rates (200–300 Hz) increased the number of identifiable peaks by more than 50% compared with that achieved at the conventional rate of 100 Hz. The acquisition rate can affect the peak capacity by a factor of 3 or more. For quantitative analyses, it is possible to work with lower acquisition rates, as the number of data points per peak (DPPP) is maintained. This is because the peaks have high intensities, which is an important parameter in this type of analysis. In the case of qualitative analysis, it is important to consider two parameters that directly influence the quantity of identified compounds. The first is S/N ratio. Despite higher signal intensities, lower acquisition rates result in inappropriate S/N ratios for qualitative analysis, mainly owing to the higher noise signal. The same tendency is observed for co-eluted peaks. Higher co-elution degrees were found at 30 Hz, indicating that higher acquisition rates were required. Consequently, in bio-oil qualitative analyses, it is important to work with acquisition rates greater than 200 Hz. This was the first study to demonstrate the importance of optimizing the data acquisition rate, a parameter that has previously been neglected in the literature, in GC×GC/TOFMS development.

Moraes et. al. [59] characterized the bio-oil from the fast pyrolysis of pulp from the industrial processing of oranges by GC×GC/FID e GC×GC/TOFMS. The authors cited the separation in the second dimension and the use of the spectral deconvolution software (to TOFMS) as advantages to characterize the bio-oil and identify a greater number of compounds with co-eluted peaks separation. The GC×GC/FID system was used to optimize the chromatographic process and identify compounds using TOFMS detector. The following parameters were evaluated: temperature setting of the primary furnace, temperature difference between the primary and secondary furnaces (ΔT), modulation period (MP), modulator temperature and duration of the hot jet. With the best conditions defined, 167 compounds were tentatively identified, 26 of which were found with concentrations greater than 1% by GC×GC/TOFMS. The main classes were: acids, aldehydes, alcohols, ketones, phenols, ethers and nitrogen compounds.

Faccini et al. [34] carried out qualitative and semi-quantitative characterization by GC×GC/TOFMS of the bio-oils from two residues originated in the cellulose industry: digester residue (DR) and wastewater treatment sludge (WTS). The total number of compounds tentatively identified in the DR and WTS were 257 and 536, respectively. Phenols and ketones were the major chemical classes in the bio-oil of the DR, while the WTS showed a more complex chemical composition, including a greater variety of chemical classes. In addition to phenols, ketones, nitrogen compounds, alcohols, aliphatic and cyclic hydrocarbons, it was possible to identify the formation of polycyclic aromatic hydrocarbons, which represented 7.6% of the total of tentatively identified compounds. The highest percentage of PAH area corresponded to naphthalenes (4.3%), followed by indenes (2.1%) and indanes (0.5%).

Lazzari et al. [60] studied the intermediate pyrolysis of rice husk and characterized (qualitatively and quantitatively) the organic phase of the bio-oil by GC×GC/qMS. Through the analysis, a total number of 98 compounds was found and 62 were quantified using a developed quantitative method using relative response factors (RRFs). Phenols and ketones (cyclics) were predominant in the organic phase, with 8.21 and 5.9 wt%, respectively; and benzofuran (1.37 wt%) corresponded to the main compounds identified. Rice husk was also the subject of the study by Moraes et al. [45]. For the pyrolysis process, 2^3 factorial planning including the following parameters: granulometry, nitrogen flow and mass of rice husk. The best conditions were applied to the production of bio-oil, which was characterized qualitatively and semi-quantitatively using GC×GC/TOFMS. In total, 106 compounds were identified from the classes of phenols and ketones (major classes), acids, ethers, aldehydes and alcohols. The major compounds found in this sample were guaiacol, contributing to 14.14% of the total area of the identified peaks. In another study by Lazzari et al. [61], for the first time in literature, a systematic approach employing matrix-matched calibration was presented to evaluate the extent of matrix effect in bio-oil analysis and undoubted quantification of its components. A procedure using sequential liquid-liquid extraction (LLE) based on two approaches (organic solvent partitioning and pH-depended reactive extraction) was performed to obtain a blank bio-oil matrix. In order to assess matrix effect, two types of external calibration were employed, namely, matrix-matched calibration and solvent calibration. The procedures proved to be efficient in the extraction of target chemical classes by bio-oil and the evaluation made from the two external calibration methods, allowed to observe the dependence of the matrix effect in the analysis of bio-oil by gas chromatography. This method of matrix-matched calibration was implemented in the quantification of bio-oil by GC×GC/qMS. Were identified 82 compounds in the rice husk bio-oil, 52 of which were quantified using the method of matrix-matched calibration. Ketones (110.1 g kg^{-1}) and phenols (109.2 g kg^{-1}) were majorly present in the bio-oil, together these two classes correspond to 79% of the quantified of the sample. The authors noted that some very important compounds quantified in the rice husk bio-oil showed matrix effect.

The residues of bocaiuva (endocarp, bark and fiber) were submitted to pyrolysis at different temperatures by Cardoso et al. [62]. The bio-oils were subjected to chromatographic analysis using the GC×GC/TOFMS technique. In total 151, 111 and 78 compounds were tentatively identified for the bio-oil of the endocarp (phenol being the major compound with 19.64%), bark (guaiacol being the major compound with 21.98%) and fiber (toluene being the major compound with 50.96%) of bocaiuva, respectively. Some compounds were quantified with commercial standards and their values expressed in g of the compound per g of bio-oil. For the bio-oil of endocarp, bark and fiber of bocaiuva, phenol (0.13 g g^{-1}), furfural (0.11 g g^{-1}) and toluene (0.28 g g^{-1}) were identified in greater concentration for each bio-oil, respectively. Despite the superior performance of the chromatographic technique used, some compounds showed co-elution in both dimensions. In these cases, the use of the spectral deconvolution tool was employed promoting the separation of some compounds by differentiating the mass spectra and retention times.

POSSIBLE APPLICATIONS OF BIO-OILS

The bio-oils analyzed show differences in their composition and concentration of chemical compounds classes. This diversity of chemical compounds makes it possible to suggest its application in the most diverse branches of the fine chemical industry or even in the energy sector. Hydrocarbons, for example,

can be applied as an alternative to fossil fuels, after an adequate up-grade. Oxygenated compounds, such as phenols, are important inputs in the chemical industry for the production of polymeric resins, pesticides, dyes and explosives, as well as nitrogen compounds are widely used for syntheses in the pharmaceutical industry. Compounds derived from aldehydes, such as furfural, can be purified by going through hydrogenation processes and generating high added value products for application in lubricants, plastics, nylon and adhesives. Fatty acids and esters, identified in some samples (mainly oily), can be used in the production of biodiesel. In Figure 5, the main chemical classes found in bio-oil can be observed in all studies with Brazilian biomasses with potential industrial use [63-66].

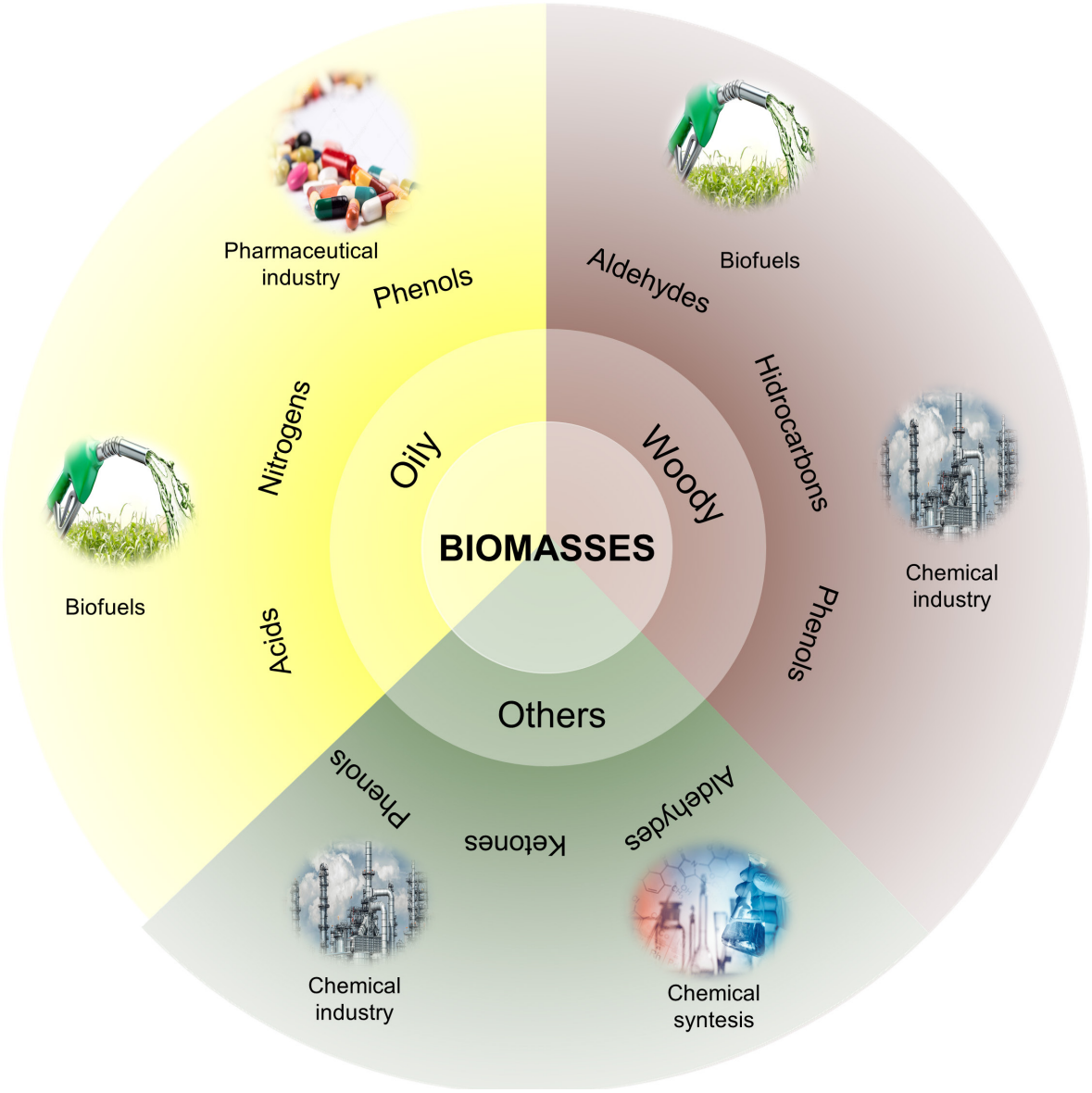


Figure 5. Main classes of compounds found in bio-oils according to the type of biomass and its possible applications.

CONCLUSIONS

This study reveals the state of the art of chromatography developed in Brazil. GC×GC emerges as an extremely important tool in the separation and proper identification of substances, greatly reducing the misidentification with false positives that the one-dimensional technique still allows.

As can be seen, most of the work is related to the application of this technique in the analysis of typical samples of the Brazilian agroindustry, which can assist in the research and use of these important residues that cause environmental impact. The most complete identification, provided by the best separation and by very sensitive and high-resolution detectors, allows the indication of use and consequent application of this bio-oil in the respective industries (food, drugs, chemicals, polymers, ...) to be more effective.

Another important evidence is the high qualification of Brazilian science in chromatographic separation techniques, with laboratories equivalent to the main in the world in the area of GC×GC, including several universities and research centers.

Related to the evolution of GC×GC and its application to biomass derivatives, two paths for the next steps can be proposed: quantitative analysis and rapid analysis. Quantitative analysis requires the use of standards, which is unconventional when talking about more than 200 identified compounds. Furthermore, the type of integration is fundamental, it is possible to treat the “compounds” as peaks, bands or pixels. In this sense, researches have been developed with the objective of recognizing families of compounds and using an approximate response factor for each family. This approach can minimize the use of patterns and optimize analysis time. It is necessary to use peak-to-peak integration, as shown in works throughout the discussion. This procedure still needs further studies to allow its automation, through available software. In fact, the availability of good software is one of the points to be improved in GC×GC, whether in terms of efficiency or acquisition cost.

On the other hand, just as single-dimensional chromatography evolved into fast columns, GC×GC also demonstrated the possibility of using these columns in their first dimension. This makes the characterization of bio-oils by GC×GC more efficient and competitive.

Conflicts of interest

This research has no conflict of interest and no special funding as destined to it. This article does not contain any studies with human participants or animals performed by any of the authors.

Acknowledgements

Author thanks to PETROBRAS, FAPITEC, CAPES and CNPq for the financial support.

REFERENCES

1. Liu, Z.; Phillips, J. B. *J. Chromatogr. Sci.*, **1991**, *29* (6), pp 227–231 (https://doi.org/10.1093/chromsci/29.6.227).
2. Gu, Q.; David, F.; Lynen, F.; Rumpel, K.; Xu, G.; De Vos, P.; Sandra, P. *J. Chromatogr. A.,* **2010**, *1217* (26), pp 4448–4453 (https://doi.org/10.1016/j.chroma.2010.04.057).
3. Phillips, J. B.; Xu, J. *J. Chromatogr. A.*, **1995**, *703* (1-2), pp 327–334 (https://doi.org/10.1016/0021-9673(95)00297-Z).
4. Phillips, J. B.; Beens, J. *J. Chromatogr. A.*, **1999**, 856, pp 331–347 (https://doi.org/10.1016/s0021-9673(99)00815-8).
5. Harynuk, J.; Górecki, T. *J. Chromatogr. A.,* **2006**, *1105* (1-2), pp 159–167 (https://doi.org/10.1016/j.chroma.2005.09.046).
6. Tranchida, P. Q.; Purcaro, G.; Dugo, P.; Mondello, L. *Trends Anal. Chem.,* **2011**, *30* (9), pp 1437–1461 (https://doi.org/10.1016/j.trac.2011.06.010).
7. Bahaghighat, H. D.; Freye, C. E.; Synovec, R. E. *Trends Anal. Chem.,* **2019**, *113*, pp 379–391 (https://doi.org/10.1016/j.trac.2018.04.016).

8. von Mühlen, C.; Zini, C. A.; Caramão E. B.; Marriott, P. J. *J. Chromatogr. A*, **2006**, *1105* (1-2), pp 39-50 (https://doi.org/10.1016/j.chroma.2005.09.036).
9. von Mühlen, C.; Zini, C. A.; Caramão E. B.; Marriott, P. J. *Quím. Nova*, **2006**, 29 (4), pp 765-775 (https://doi.org/10.1590/S0100-40422006000400025).
10. Cardeal, Z.; Marriott, P. J. *Food Chem.*, **2009**, *112* (3), pp 747-755 (https://doi.org/10.1016/j.foodchem.2008.06.057).
11. von Mühlen, C.; de Oliveira, E. C.; Morrison, P. D.; Zini, C. A.; Caramão E. B.; Marriott, P. J. *J. Sep. Sci.*, **2007**, *30*, pp 3223-3232 (https://doi.org/10.1002/jssc.200700172).
12. von Mühlen, C.; Zini, C. A.; Caramão E. B.; Marriott, P. J. *J. Chromatogr. A*, **2008**, *1200*, pp 34-42 (https://doi.org/10.1016/j.chroma.2008.05.070).
13. Dugo, P; Cacciola, F.; Donato, P.; Jacques, R. A.; Caramão, E. B.; Mondello, L. *Chromatogr. A*, **2009**, *1216*, pp 7213-7221 (https://doi.org/10.1016/j.chroma.2009.08.030).
14. Purcaro, G.; Tranchida, P. Q.; Jacques, R. A.; Caramão E. B.; Moret, S.; Conte, L.; Dugo, P.; Dugo, G.; Mondello, L. *J. Sep. Sci.*, **2009**, *32*, pp 3755-3763 (https://doi.org/ 10.1002/jssc.200900343).
15. Dalluge, J.; Beens, J.; Brinkman, U. A. T. *J. Chromatogr. A.,* **2003**, *1000* (1-2), pp 69–108 (https://doi.org/10.1016/S0021-9673(03)00242-5).
16. Edwards, M.; Mostafa, A.; Górecki, T. *Anal. Bioanal. Chem.,* **2011**, *401*, pp 2335–2349 (https://doi.org/10.1007/s00216-011-5100-6).
17. Mostafa, A.; Edwards, M.; Górecki, T. *J. Chromatogr. A.,* **2012**, *1255*, pp 38–55 (https://doi.org/10.1016/j.chroma.2012.02.064).
18. Tranchida, P. Q.; Aloisi, A.; Giocastro, B.; Mondelo, L. *Trends Anal. Chem.*, **2018**, *105*, pp 360-366 (https://doi.org/10.1016/j.trac.2018.05.016).
19. Stefanuto, P.; Smolinska, A.; Focant, J. *Trends Anal. Chem.*, **2021**, *139*, pp 116251-116263 (https://doi.org/10.1016/j.trac.2021.116251).
20. Freitas, L. S.; Von Mühlen, C.; Bortoluzzi, J. H.; Zini, C. A.; Fortuny, M.; Dariva, C.; Coutinho, R. C. C.; Santos, A. F.; Caramão, E. B. *J. Chromatogr. A.,* **2009**, *1216* (14), pp 2860–2865 (https://doi.org/10.1016/j.chroma.2008.09.076).
21. Bridgwater, A. V.; Meier, D.; Radlein, D. *Org. Geochem.,* **1999**, *30* (12), pp 1479–1493 (https://doi.org/10.1016/S0146-6380(99)00120-5).
22. Bridgwater, A. V. *Therm. Sci.,* **2004**, *8* (2), pp 21–50 (https://doi.org/10.2298/TSCI0402021B).
23. Sharifzadeh, M.; Sadeqzadeh, M.; Guo, M.; Borhani, T. N.; Murthy Konda, N. V. S. N.; Garcia, M. C.; Wang, L.; Hallett, J.; Shah, N. *Prog. Energy Combust. Sci.,* **2019**, *71*, pp 1–80 (https://doi.org/10.1016/j.pecs.2018.10.006).
24. Baloch, H. A.; Nizamuddin, S.; Siddiqui, M. T. H.; Riaz, S.; Jatoi, A. S.; Dumbre, D. K.; Mubarak, N. M.; Srinivasan, M. P.; Griffin, G. J. *J. Environ. Chem. Eng.,* **2018**, *6* (4), pp 5101–5118 (https://doi.org/10.1016/j.jece.2018.07.050).
25. Bertero, M.; de la Puente, G.; Sedran, U. *Fuel,* **2012**, *95,* pp 263–271 (https://doi.org/10.1016/j.fuel.2011.08.041).
26. Smets, K.; Adriaensens, P.; Reggers, G.; Schreurs, S.; Carleer, R.; Yperman, J. *J. Anal. Appl. Pyrolysis,* **2011**, *90* (2), pp 118–125 (https://doi.org/10.1016/j.jaap.2010.11.002).
27. Alsbou, E.; Helleur, R. *J. Anal. Appl. Pyrolysis,* **2013**, *101*, pp 222–231 (https://doi.org/10.1016/j.jaap.2013.01.003).
28. Choi, Y. S.; Johnston, P. A.; Brown, R. C.; Shanks, H.; Lee, K. *J. Anal. Appl. Pyrolysis,* **2014**, *110*, pp 147–154 (https://doi.org/10.1016/j.jaap.2014.08.016).
29. Hoekstra, E.; Van Swaaij, W. P. M.; Kersten, S. R. A.; Hogendoorn, K. J. A. *Chem. Eng. J.,* **2012**, *187*, pp 172–184 (https://doi.org/10.1016/j.cej.2012.01.118).
30. Guedes, R. E.; Luna, A. S.; Torres, A. R. *J. Anal. Appl. Pyrolysis,* **2018**, *129*, pp 134–149 (https://doi.org/10.1016/j.jaap.2017.11.019).
31. Acikgoz, C.; Kockar, O. M. *J. Anal. Appl. Pyrolysis,* **2009**, *85* (1-2), pp 151–154 (https://doi.org/10.1016/j.jaap.2008.08.011).

32. Silva, R. V. S.; Casilli, A.; Sampaio, A. L.; Ávila, B. M. F.; Veloso, M. C. C.; Azevedo, D. A.; Romeiro, G. A. *J. Anal. Appl. Pyrolysis,* **2014**, *106*, pp 152–159 (https://doi.org/10.1016/j.jaap.2014.01.013).
33. Van Den Dool, H.; Kratz, P. D. *J. Chromatogr. A.,* **1963**, *11*, pp 463–471 (https://doi.org/10.1016/S0021-9673(01)80947-X).
34. Faccini, C. S.; Vecchia, I. D.; Ribeiro. D.; Zini, C. A.; Caramão, E. B. *J. Braz. Chem. Soc.,* **2013**, *24* (7), pp 1085–1098 (http://dx.doi.org/10.5935/0103-5053.20130143).
35. Schneider, J. K.; da Cunha, M. E.; dos Santos, A. L.; Maciel, G. P. S.; Brasil, M. C.; Pinho A. R.; Mendes, F. L.; Jacques, R. A.; Caramão, E. B. *J. Chromatogr. A.,* **2014**, *1356*, pp 236-240 (https://doi.org/10.1016/j.chroma.2014.06.053).
36. Torri, I. D. V.; Paasikallio, V.; Faccini, C. S.; Huff, R.; Caramão, E. B.; Sacon, V.; Oasmaa, A.; Zini, C. A. *Bioresour. Technol.,* **2016**, *200*, pp 680–690 (https://doi.org/10.1016/j.biortech.2015.10.086).
37. Mendes, F.; Ximenes, V. L.; Almeida, M. B. B.; Azevedo, D. A.; Tessarolo, N. S.; Pinho, A. R. *J. Anal. Appl. Pyrolysis,* **2016**, *122* (http://dx.doi.org/10.1016/j.jaap.2016.08.001).
38. Silva, R. V. S.; Tessarolo, N. S.; Pereira, V. B.; Ximenes, V. L.; Mendes, F. L.; Almeida, M. B. B.; Azevedo, D. A. *Talanta,* **2016**, *164*, pp 626-635 (https://doi.org/10.1016/j.talanta.2016.11.005).
39. Tessarolo, N. S.; dos Santos, L. R. M.; Silva, R. S. F.; Azevedo, D. A. *J. Chromatogr. A,* **2013**, *1279*, pp 68–75 (https://doi.org/10.1016/j.chroma.2012.12.052).
40. Tessarolo, N. S.; Silva, R. C.; Vanini, G.; Pinho, A.; Romão, W.; De Castro, E. V. R.; Azevedo, D. A. *Microchem. J.,* **2014**, *117*, pp 68–76 (https://doi.org/10.1016/j.microc.2014.06.006).
41. Tessarolo, N. S.; Silva, R. V. S.; Vanini, G.; Casilli, A.; Ximenes, V. L.; Mendes, F. L.; de Rezende Pinho, A.; Romão, W.; de Castro, E. V. R.; Kaiser, C. R.; Azevedo, D. A. *J. Anal. Appl. Pyrolysis,* **2016**, *117*, pp 257–267 (https://doi.org/10.1016/j.jaap.2015.11.007).
42. Primaz, C. T.; Schena, T.; Lazzari, E.; Caramão, E. B.; Jacques, R. A. *Fuel*, **2018**, *232*, pp 572–580 (https://doi.org/10.1016/j.fuel.2018.05.097).
43. Polidoro, A. S.; Scapin, E.; Lazzari, E.; Silva, A. N.; dos Santos, A. L.; Caramão, E. B.; Jacques, R. A. *J. Anal. Appl. Pyrolysis,* **2018**, *129*, pp 43–52 (https://doi.org/10.1016/j.jaap.2017.12.005).
44. Cardoso, C. A. L.; Machado, M. E.; Maia, F. S.; Arruda, G. J.; Caramão, E. B. *J. Braz. Chem. Soc.,* **2016**, *27* (11), pp 2149–2159 (https://dx.doi.org/10.5935/0103-5053.20160081).
45. Moraes, M. S. A.; Migliorini, M. V.; Damasceno, F. C.; Georges, F.; Almeida, S.; Zini, C. A.; Jacques, R. A.; Caramão, E. B. *J. Anal. Appl. Pyrolysis,* **2012**, *98*, pp 51–64 (https://doi.org/10.1016/j.jaap.2012.05.007).
46. Onorevoli, B.; Machado, M. E.; Polidoro, A. dos S.; Corbelini, V. A.; Caramão, E. B.; Jacques, R. A. *Energy & Fuels*, **2017**, *31* (9), pp 9402–9407 (https://doi.org/10.1021/acs.energyfuels.7b00405).
47. Lazzari, E.; Schena, T.; Primaz, C. T.; Maciel, G. P. S.; Machado, M. E.; Cardoso, C. A. L.; Jacques, R. A.; Caramão, E. B. *Ind. Crops Prod.,* **2016**, *83*, pp 529–536 (https://doi.org/10.1016/j.indcrop.2015.12.073).
48. Silva, R. V. S.; Pereira, V. B.; Stelzer, K. T.; Almeida, A.; Romeiro, G. A.; Azevedo, D. A. *Biomass and Bioenergy*, **2019**, *123*, pp 78–88 (https://doi.org/10.1016/j.biombioe.2019.02.014).
49. Onorevoli, B.; Machado, M. E.; Dariva, C.; Franceschi, E.; Krause, L. C.; Jacques, R. A.; Caramão, E. B. *Ind. Crops Prod.,* **2014**, *52*, pp 8–16 (https://doi.org/10.1016/j.indcrop.2013.09.034).
50. Nunes, V. O.; Silva, R. V. S.; Romeiro, G. A.; Azevedo, D. A. *Microchem. J.,* **2020**, *153*, 104514 (https://doi.org/10.1016/j.microc.2019.104514).
51. Moraes, M. S. A.; Georges, F.; Almeida, S. R.; Damasceno, F. C.; Maciel, G. P. S.; Zini, C. A.; Jacques, R. A.; Caramão, E. B. *Fuel Process. Technol.,* **2012**, *101*, pp 35–43 (https://doi.org/10.1016/j.fuproc.2012.03.004).
52. Cunha, M. E.; Schneider, J. K.; Brasil, M. C.; Cardoso, C. A.; Monteiro, L. R.; Mendes, F. L.; Pinho, A.; Jacques, R. A.; Machado, M. E.; Freitas, L. S.; Caramão, E. B. *Microchem. J.,* **2013**, *110*, pp 113–119 (https://doi.org/10.1016/j.microc.2013.03.004).
53. Maciel, G. P. S.; Machado, M. E.; Barbará, J. A.; Dal Molin, D.; Caramão, E. B.; Jacques, R. A. *Biomass and Bioenergy,* **2016**, *85*, pp 198–206 (https://doi.org/10.1016/j.biombioe.2015.11.009).

54. Barros, J. A. S.; Krause, M. C.; Lazzari, E.; Bjerk, T. R.; Amaral, A. L.; Caramão, E. B.; Krause, L. C. *Microchem. J.,* **2017**, *137*, pp 30-36 (https://doi.org/10.1016/j.microc.2017.09.015).
55. Almeida, T. M.; Bispo, M. D.; Cardoso, A. R. T.; Migliorini, M. V.; Schena, T.; Campos, M. C. V.; Machado, M. E.; López, J. A.; Krause, L. C.; Caramão, E. B. *Agric. Food Chem.,* **2013**, *61* (28), pp 6812–6821 (https://doi.org/10.1021/jf401379s).
56. Schena, T.; Farrapeira, R.; Bjerk, T. R.; Krause, L. C.; Mühlen, C.; Caramão, E. B. *Sep. Sci. Plus*, **2019**, *2* (3), pp 89–99 (https://doi.org/10.1002/sscp.201800129).
57. Schena, T.; Lazzari, E.; Primaz, C.; Krause, L. C.; Machado, M. E.; Caramão, E. B. *J. Environ. Chem. Eng.,* **2020**, *8*, 103662 (https://doi.org/10.1016/j.jece.2020.103662).
58. Schena, T., Bjerk, T. R., Mühlen, C., & Caramão, E. B. *Talanta*, **2020**, 219, 121186 (https://doi.org/10.1016/j.fuel.2020.119866).
59. Moraes, M. S. A.; Bortoluzzi, J. H.; Migliorini, M. V.; Zini, C. A.; Caramão, E. B. *Sci. Chromatogr.,* **2011**, *3*, pp 301–314 (http://dx.doi.org/10.4322/sc.2011.018).
60. Lazzari, E.; Polidoro, A. dos S.; Onorevoli, B.; Schena, T.; Silva, A. N.; Scapin, E.; Jacques, R. A.; Caramão, E. B. *Renew. Energy,* **2019**, *135*, pp 554–565 (https://doi.org/10.1016/j.renene.2018.12.053).
61. Lazzari, E.; Silva, E. A. S.; Bjerk, T. R.; Schneider, J. K.; Caramão, E. B. *Fuel*, **2021**, 290, 119866 (https://doi.org/10.1016/j.fuel.2020.119866).
62. Cardoso, C. A. L.; Machado, M. E.; Caramão, E. B. *Renew. Energy,* **2016**, *91*, pp 21–31 (https://doi.org/10.1016/j.renene.2015.11.086).
63. Moraes, M. S. A.; Tomasini, D.; da Silva, J. M.; Machado, M. E.; Krause, L. C.; Zini, C. A.; Jacques, R. A.; Caramão, E. B. In: *Frontiers in Bioenergy and Biofuels*. InTech, **2017**, pp 71-116 (http://dx.doi.org/10.5772/66326).
64. Schiller, R.; Tichotová, L.; Pavlík, J.; Buchta, V.; Melichar, B.; Votruba, I.; Kuneš, J.; Špulák, M.; Pour, M. *Bioorg. Med. Chem. Lett.,* **2010**, *20* (24), pp 7358–7360 (https://doi.org/10.1016/j.bmcl.2010.10.052).
65. Rughoonundun, H.; Holtzapple, M. T. *Biomass and Bioenergy*, **2017**, *105*, pp 73–82 (https://doi.org/10.1016/j.biombioe.2017.06.007).
66. Li, Q.; Lam, L. K. M.; Xun, L. *Biodegradation,* **2011**, *22* (6), pp 1215–1225 (https://doi.org/10.1007/s10532-011-9476-y).

Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 33, pp 42-56
doi: 10.30744/brjac.2179-3425.AR-71-2020

BrJAC

ARTICLE

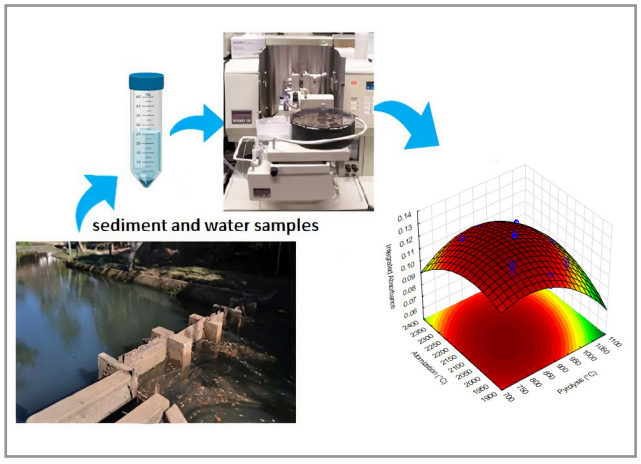
Footprint of Arsenic Contamination in Sediments and Water from Mining Sites

A case study based on multivariate optimization by GF AAS

Glenda Máris Mesquita De Filippis[1], Bruno Elias dos Santos Costa[2], Simone Soares de Oliveira Borges[2], Waldomiro Borges Neto[2], Nivia Maria Melo Coelho[2], Luciana Melo Coelho[1]*

[1]Universidade Federal de Catalão, Av. Dr. Lamartine Pinto de Avelar, 1120, Setor Universitário, 75704-020, Catalão, GO, Brazil

[2]Universidade Federal de Uberlândia, Av. João Naves de Ávila, 2121, Campus Santa Mônica, 38400-902, Uberlândia, MG, Brazil



Arsenic contamination is worrisome in mineral exploration regions. Efficient arsenic monitoring is dependent on detectability at trace level in environmental matrices. This paper presents a procedure to evaluate the occurrence of arsenic in environmental sediment and water samples collected from a mining area in Catalão, Goiás State (GO), Brazil. The water and sediment samples were analyzed by graphite furnace atomic absorption spectrometry (GF AAS) after appropriate chemical treatment. For the arsenic determination, analytical performance was improved employing multivariate tools. The instrumental conditions were optimized using a 2^3 factorial design and the response surface methodology (RSM) was applied with a central composite design (CCD). Iridium was used as a permanent modifier. The results for the sediment samples showed arsenic concentrations below the threshold for adverse effects ranging from 2.06 to 3.82 mg Kg^{-1}. The concentrations in water samples were below LOD. The LOD and LOQ were, respectively, 0.33 and 1.09 µg L^{-1} to water and digested sediment samples. Under the optimal conditions, the dynamic working range was linear of LOQ to 50.0 µg L^{-1}. The method was applied to determine concentrations of arsenic in water and sediments collected from mining sites, which can be used to assess the availability of arsenic in the region.

Keywords: total arsenic, Samambaia stream, mining sites, permanent modifier, graphite furnace

Cite: Filippis, G. M. M.; Costa, B. E. S.; Borges, S. S. O.; Borges Neto, W.; Coelho, N. M. M.; Coelho, L. M. Footprint of Arsenic Contamination in Sediments and Water from Mining Sites: A case study based on multivariate optimization by GF AAS. *Braz. J. Anal. Chem.*, 2021, *8* (33), pp 42–56. doi: http://dx.doi.org/10.30744/brjac.2179-3425.AR-71-2020

Submitted 22 December 2020, Resubmitted 30 March 2021, Accepted 16 April 2021, Available online 14 May 2021.

INTRODUCTION

The presence of arsenic in environmental samples has been extensively reported due to a variety of sources of anthropogenic contamination. This is a matter of great concern since this pollutant can have a subtle but alarming toxic effect.

Arsenic occurs naturally as part of the composition of certain minerals, such as arsenopyrite (FeAsS), realgar (AsS), and orpiment (As_2S_3). It can also be incorporated into the lattice of various other minerals, for instance, during the replacement of Al^{III} and Fe^{III} oxides. Possible host minerals are feldspar, magnetite, galena, blende and apatite. In the form of organic compounds, arsenic is manifested primarily as metabolites derived from marine organisms [1,2].

The toxicity of arsenic compounds is dependent on the nature of the chemical species, and the inorganic forms are around hundred times more toxic than organic compounds [3]. In the case of inorganic species, As^{III} and As^{V} are predominantly found in the aquatic environment, and they are a cause for concern since they can become potentially toxic even in very low concentrations.

In the aquatic environment, the predominant species of inorganic arsenic are strongly dependent on pH and redox potential. Under oxidative conditions (aerobic) and at pH above 2.0, arsenate oxoanions (As^{V}) are the predominant species. In slightly reducing conditions, the neutral specie of As^{III} predominates, manifesting as arsenious acid (H_3AsO_3). This neutral specie converts to anionic species deprotonated with increasing pH. Oxoanions of As^{III} become predominant only at pH above 9.2 [4]. Once released into the water, part of the arsenic is immobilized by adsorption in compounds containing iron, aluminum, manganese and in clay minerals. A small part is complexed to organic compounds. As^{III} is commonly complexed with sulfur amino acids residues like L-cysteine in some peptide metabolites [5].

The presence of arsenic in water has been related to the occurrence of dermatitis, skin cancer, heart problems, cancer and poisoning. The US Environmental Protection Agency (USEPA) revised the regulated limit for arsenic in drinking water from 50 to 10 μg L^{-1} [6,7]. As a result of these revised standard, new technologies have been developed to provide more appropriate monitoring and reduce the levels of this contaminant.

The evaluation of the level of arsenic contamination in a water resource is dependent not only on its determination in water but also in sediment samples. Sediments have a high capacity for the sorption and accumulation of pollutants, especially heavy metals and metalloids such as arsenic. The availability of these contaminants in the aquatic environment can increase by several orders of magnitude, depending on their mobility and environmental changes [8-12].

In Brazil, arsenic has been found in several regions, notably the Iron Quadrangle region in Minas Gerais State, where critical contamination levels have been recorded. It has been reported that total arsenic levels in groundwater samples collected in the cities of Ouro Preto and Mariana, Minas Gerais State, can reach 2980 mg L^{-1} [8].

The occurrence of environmental disasters related to mining activities, such as the rupture of the dam in Brumadinho in January 2019, warns of cases of incorporation and accumulation of arsenic in sediments. Lithogenic compartments are affected by a chain propagation during the course of the mineral waste, spreading out to increasingly distant regions. Parameters such as acidity, oxygen demand and corrosivity of manure can facilitate the availability of arsenic accumulated by leaching over time [13].

Mining is the main economic activity in the city of Catalão, in the state of Goiás, Brazil. Catalão has been the target of investments by large mining companies to explore deposits of phosphate, niobium and rare earths in the region. The water supply in the city occurs through the drainage of the Samambaia Hydrographic Basin. The Samambaia stream constantly suffers from processes of erosion, leaching and silting, which can drag sediments to the riverbed, which are not constantly monitored [14].

Studies on the characterization of sediments point to aluminum, iron, potassium and titanium as the main constituents in addition to silicon [14]. There is still no study aimed at monitoring arsenic in sediments and waters of Samambaia stream. The lack of this information encourages investigations that may reveal history of contamination in recent years.

Due to the above-mentioned factors, it is important to develop a routine methodology for the determination of arsenic in water samples and sludge obtained from mining regions [15], to enable better quality control and reduce the inherent risk of contamination.

In general, the most sensitive analytical techniques for the detection of arsenic are hydride generation atomic absorption spectrometry (HG AAS) and inductively coupled plasma mass spectrometry (ICP-MS). Although the HG AAS technique offers simple and affordable instrumentation, the hydride generation system is restricted to only a few species, and may become unstable under highly reducing conditions. In addition, sample preparation procedures are required, which can lead to losses and contamination [9]. The ICP-MS technique has the advantage of multielement determination, obtaining detection limits of less than 1 μg L^{-1}. However, it has the disadvantage that samples with a high content of dissolved salts are difficult to analyze, and also this method is costly for routine analysis [10]. The graphite furnace atomic absorption spectrometry (GF AAS) technique has proven to be one of the most powerful methods for the determination of trace and ultra-trace levels. This technique has become increasingly attractive in view of the relative simplicity of operation, automation and minimal requirements for processing of the sample.

For the determination of arsenic and heavy metals by GF AAS, the stages of the temperature program (pyrolysis and atomization) are conventionally optimized univariate [16-20]. This study is limited because it does not explore the effects of interaction between the variables in addition to requiring greater amounts of heating cycles for each increase in temperature. These limitations can be critical in determining volatile elements such as arsenic in complex matrix samples. Optimal temperature programs obtained by modeling from data multivariate become efficient tools to ensure analytical performance in GF AAS [21]. In this context, resources such as factorial design and multivariate optimization are applied to achieve more efficient conditions for the determination of arsenic in trace levels.

The aim of this study was to apply a method for the determination of arsenic in environmental sediment and water samples collected from mining sites in Catalão, GO, Brazil. The determination of total arsenic in the water and sediment samples was performed by GF AAS, combining the efficiency of permanent modifiers with an appropriate temperature program, obtained from multivariate optimization.

MATERIALS AND METHODS

Sampling

Sediment and water samples were collected on the banks of the “Samambaia” Stream, in the north and northeast areas of the city of Catalão located in the southeastern region of Goiás State, Brazil.

The samples were collected at 7 points so as to be representative of the influence of the different human activities carried out along the creek, that is, livestock grazing, crop growing, water harvesting and urbanization: *i*) Point 1 (18º05’13”S, 47º59’05”W) near the bridge along the road GO-330, being representative of the entire basin area, *ii*) Point 2 (18º05’13”S, 47º58’41”W), stretch where water is collected by the Catalão City Superintendent of Water and Sewage (SAE) in order to assess the quality of the water for public supply, *iii*) Point 3 (18º05’16”S, 47º58’29” W) along a tributary of the “Samambaia” Stream, *iv*) Point 4 (18º05’08”S and 47º58’23”W) in an area used for pasture, *v*) Point 5 (18º06’34”S, 47º57’05”W) along a tributary of the “Samambaia” Stream in an area where there is the presence of urban activities, this point being the stretch closest to the city, *vi*) Point 6 (18º05’11”S and 47º54’53”W) in an agricultural area and with the presence of livestock in this area and in the vicinity; and *vii*) Point 7 (18º07’08”S, 47º53’47” W) on the edge of the road BR-050. This is the point closest to the head of the “Samambaia” Stream. Figure 1 shows the location of the sampling points along the stream and the study area.

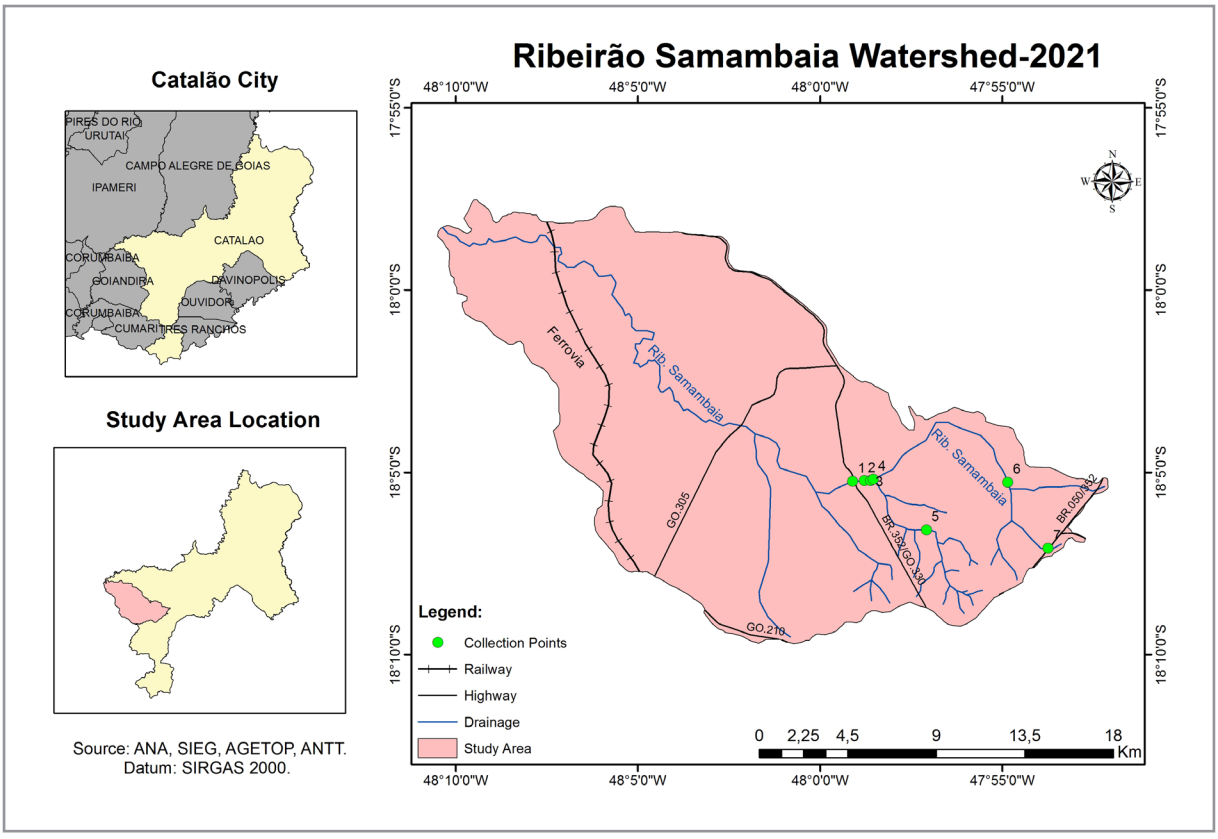


Figure 1. Location of the study area along the Samambaia Stream (Catalão-GO), indicating the points where water and sediment samples were collected.

For each point, one sampling was performed during the collection of sediment and adjacent water. Single sampling was performed, since sediment composition remains stable over an interval of 3 to 5 years [22]. The sampling of water aimed reflecting probable interactions established at the interface, by the diffusion of the analyte in the layer of stagnant water immediately in contact with the collected sediment, in a punctual way.

The water samples were collected in polyethylene bottles previously decontaminated in 10% (v/v) nitric acid for a period of 24 h and washed several times with deionized water. In the sampling procedure, the bottle was washed by placing the mouth of the bottle against the current and filling it about half with water. After this operation, the bottle was completely immersed in the water, leaving only a small gap for sample acidification and homogenization. Then, the samples were preserved by the addition of nitric acid until pH<2.0. In the laboratory, the water samples were cooled to approximately 4 °C and stored for up to 28 days before analysis [22,23].

Sediment samples were collected only from the first 10 cm of the sediment column (about 2 kg per sampling point). When the sediment is sampled at a depth of 10 cm, it is possible to obtain data for up to last 10 years of deposition [24]. This depth was chosen since it is expected to correspond to the relatively recent occupation history of the area. The sediment was collected manually using a plastic grip. The samples were placed in properly labeled plastic bags and transported to the laboratory.

Study of chemical parameters

The chemical parameters pH, conductivity, dissolved oxygen, temperature and salinity were measured at all collection points using a previously-calibrated multiparameter probe (Horiba - Water Quality Checker U-10).

The sediment samples were homogenized and dried at 40 °C to constant weight. They were then pulverized using a porcelain mortar and pestle and sieved using an electromagnetic sieve shaker with a mesh size of 0.062 mm. The samples were packed into plastic bags until the extraction, which was assisted by microwave acid digestion in a Provecto, DGT 100 plus digestor.

Sediment sample preparation

In the sediment preparation procedure, 200 mg of sediment sample and 5 mL of 10% (v/v) HNO_3 were placed in a 100 mL decomposition flask. The flask was placed in a microwave oven and subjected to the digestion program. The program consisted of four stages: *i)* 200 W (8 min); *ii)* 400 W (7 min); *iii)* 600 W (1 min) and *iv)* 0 W (20 min). These conditions were obtained by studies developed from works involving acid digestion by microwave applied to environmental samples [25,26]. Microwave digestion of sediment sample is adequate to promote extraction of elements strongly associated with the crystalline structures of the mineral fraction [12]. After digestion, the solution was filtered using filter paper. The flasks were washed with deionized water and the volume was completed to 20 mL.

Instrumentation

The integrated absorbance signal was obtained using a Varian atomic absorption spectrometer AAnalyst 240Z equipped with an electrothermal graphite furnace atomizer GTA 120 and Zeemann background corrector. A Varian arsenic hollow cathode lamp (Part No.: 5610122200; Serial/Lot No.: 12HO796) was operated at 193.7 nm with a spectral band-pass of 0.5 nm. Argon (99.999%) obtained from White Martins® (Uberlândia - Minas Gerais, Brazil) was used as the purge gas at a flow rate of 300 mL min^{-1}. Pyrolytic graphite coated tubes with a L'Vov platform (Varian Part No: 63-100037-00, Batch: 101816924) were used. In all experiments, the total volume of sample introduced into the graphite tubes was 20 µL. The temperatures applied for drying and cleaning were 85-120 ºC for 55.0 s and 2800 ºC for 2.0 s, respectively.

Reagents and Solutions

All solutions were prepared using water deionized in a Milli-Q system (Gehaka®). Nitric acid (65%; Suprapur®) purchased from Merck (Darmstadt, Germany) was used to prepare the aqueous solutions. Calibration solutions were prepared from a 1000 ± 0.002 mg L^{-1} arsenic stock solution (Merck) in 0.6% v/v HNO_3. Autosampler vials and glassware were cleaned by immersion in 10% (v/v) HNO_3 for at least one day, rinsing several times with Milli-Q water and drying. An autosampler washing solution containing a mixture of 1% (v/v) of nitric acid and 0.1% (w/v) Triton X-100 was used in order to prevent the autosampler sampling capillary tip from clogging due to analyte adsorption and improve the sample solution dispersion on the platform. During the optimization and analysis procedures, water samples and digested sediment were transferred directly into the autosampler vials.

Treatment of graphite tubes with permanent modifiers

Six graphite tubes with integrated platforms were treated by pipetting 25 µL of a specific modifier (palladium, iridium, rhodium, niobium, titanium and tantalum) into each tube. The treatment includes 20 consecutive injections of modifier successively subjected to a specific temperature program [27,28]. The procedure resulted in the deposition of 500 µg of modifier on the inner wall of the tube and the platform.

Optimization the furnace temperature program

A factorial design (2^3) was used to evaluate the pyrolysis variables, atomization temperatures and modifier. A central composite design (CCD) was performed to determine the critical conditions for the pyrolysis and atomization temperature after selecting the best modifier. The experimental data were processed using STATISTICA 6.0 [29].

RESULTS AND DISCUSSION

Study of modifiers

A preliminary study was performed to evaluate the arsenic signal using tubes treated with permanent modifiers. The furnace temperature program recommended by the manufacturer (1500 °C for pyrolysis temperature and 2600 °C for atomization temperature) was used. Arsenic determination by methods involving electrothermal atomization requires greater care. The use of modifiers is crucial to prevent volatilization losses and minimize problematic matrix interference.

The use of chemical modifiers, such as magnesium nitrate or combined with elements of the platinum group such as tungsten are the most traditionally used for arsenic determinations by GF AAS [30]. However serious phosphorus interferences were reported in the sediment analysis, even using this modifier [25,31]. The use of permanent modifiers is more advantageous than those injected in solution [32]. The literature reports that graphite tubes modified with W-Rh were suitable for determination of arsenic in sediments through slurry sampling, however the temperature program was defined by univariate optimization [33].

In this work, palladium, iridium, rhodium, niobium, tantalum and titanium were tested as permanent modifiers. The atomization profile for 50.0 μg L^{-1} arsenic obtained using GF AAS with each permanent modifier are shown in Figure 2.

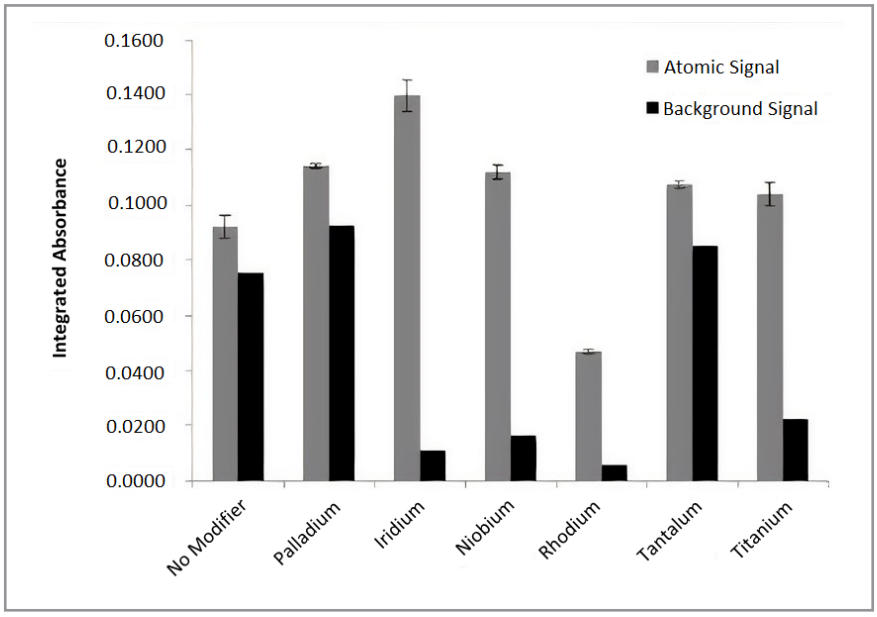


Figure 2. Atomic and background signals for the scan using permanent modifiers for the detection of arsenic. Detail shows the atomization profile for 50.0 μg L^{-1} arsenic obtained using GF AAS with iridium as a permanent modifier.

Elements of the platinum group generally show good performance as permanent modifiers. Intercalation compounds formed with the carbon atoms of the graphite surface could activate the modifier to become an electron donor for the metalloid [34]. This proposed action suggests the thermal stability of arsenic.

The permanent modification of graphite tubes was satisfactorily performed and was necessary to improve the detection. The best signal/noise ratio results were obtained using the tubes modified with iridium and niobium. Although the modification with palladium, tantalum and titanium provided a good analytical signal, were not considered for future studies due to the high background signal. Background signals can be easily corrected by the equipment however they can affect the sensitivity. Iridium and niobium were thus selected for the experimental design and the optimization of the temperature program.

Optimization of temperature program

Optimization experiments were performed to establish the optimal thermal conditions in the determination of arsenic by GF AAS. Aliquots (20.0 µL) of a 50.0 µg L^{-1} standard solution of arsenic were injected into the graphite tube using the auto-mix auto-sampler function. The experiments were performed randomly and in triplicate.

A 2^3 factorial design was run for the initial screening of the pyrolysis and atomization temperatures in a joint action with the selected modifiers. The values for each parameter are shown in Table I. The lower and upper levels were normalized to (-1) and (+1), respectively, and the results were expressed as an integrated absorbance.

Table I. Factorial design (2^3) for screening of pyrolysis temperature, atomization temperature and modifier

Experiment	Pyrolysis Temperature / ºC	Atomization Temperature / ºC	Modifier	Integrated Absorbance (*n*=3)		
1	800 (-1)	1400 (-1)	Ir (-1)	0.1130	0.1138	0.1140
2	1400 (+1)	1400 (-1)	Ir (-1)	0.0829	0.0831	0.0852
3	800 (-1)	2400 (+1)	Ir (-1)	0.1165	0.1163	0.1158
4	1400 (+1)	2400 (+1)	Ir (-1)	0.0748	0.0750	0.0752
5	800 (-1)	1400 (-1)	Nb (+1)	0.0822	0.0821	0.0861
6	1400 (+1)	1400 (-1)	Nb (+1)	0.0633	0.0633	0.0639
7	800 (-1)	2400 (+1)	Nb (+1)	0.0937	0.0933	0.0938
8	1400 (+1)	2400 (+1)	Nb (+1)	0.0695	0.0706	0.0708

The effects of each variable were evaluated by analysis of variance (ANOVA) at the 95% confidence level. The main and interaction effects are represented on the Pareto chart shown in Figure 3. The data were treated statistically using Statistica 6.0 software.

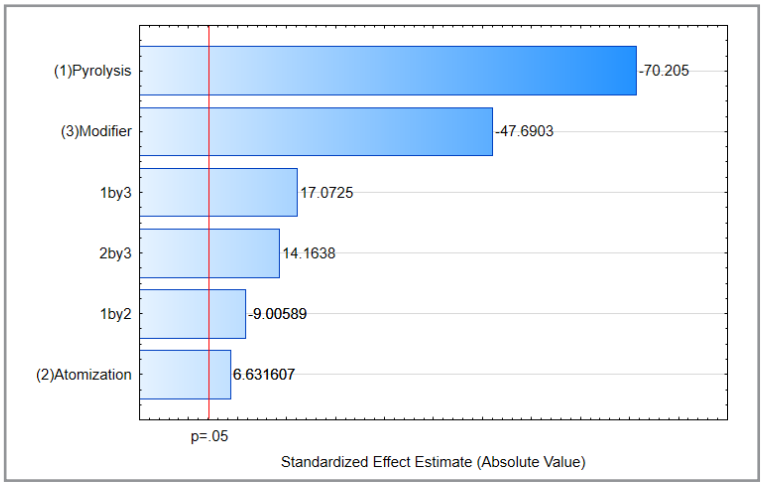


Figure 3. Pareto chart of the effects of different variables and their interaction for the optimization of temperature program.

According to the Pareto chart, all main and interaction effects were significant at the 95% confidence level (p<0.05). The Pareto chart shows the importance of multivariate optimization to determine the occurrence of significant interactions that are neglected in univariate methods. The pyrolysis temperature is the variable with the most significant effect, and the analytical signal is more favorable at the lower temperature level. A high atomization temperature was shown to increase the analytical signal; however, the effect of interaction between the pyrolysis temperature (PT) and atomization temperature (AT) was more significant than the corresponding individual effects.

The modifier also demonstrated a strong contribution, and effective operation occurs at the lower level. When iridium was represented by level (-1) better results were obtained, so this level was fixed in the next steps of the experiment. Figure 2 clearly indicates iridium as the best modifier; however, it is important to check its significance level in relation to the GF AAS temperature program. The inclusion of modifiers as a qualitative variable in multivariate experimental designs showed that the action of iridium does not depend on the interaction with the other variables investigated. This confirms the information obtained from the univariate assay shown in Figure 2. Permanent modification with iridium has been reported as satisfactory for determining arsenic in environmental samples by GF AAS in similar work. It was observed that the graphite tube could be used for at least 200 cycles without any re-treatment [35].

To obtain the optimum values for the pyrolysis and atomization temperatures, experimental modeling of the response surface based on a 2^2 central composite design (CCD) was performed. In this experiment, PT and AT were explored using tubes permanently treated with iridium. The results obtained are shown in Table II.

Table II. Central composite design for optimization of the temperature program using iridium as a permanent modifier

Experiment	Pyrolysis Temperature / ºC	Atomization Temperature / ºC	Integrated Absorbance (n=3)		
1	800 (-1)	2000 (-1)	0.1278	0.1246	0.1310
2	800 (-1)	2300 (+1)	0.1191	0.1193	0.1188
3	1000 (+1)	2000 (-1)	0.1089	0.1129	0.1049
4	1000 (+1)	2300 (+1)	0.1121	0.1114	0.1128
5	759 (-1.41)	2150 (0)	0.1197	0.1197	0.1196
6	1041 (+1.41)	2150 (0)	0.1065	0.1047	0.1083
7	900 (0)	1938 (-1.41)	0.1136	0.1091	0.1181
8	900 (0)	2362 (+1.41)	0.1112	0.1101	0.1122
9	900 (0)	2150 (0)	0.1243	0.1233	0.1252
10	900 (0)	2150 (0)	0.1244	0.1233	0.1254
11	900 (0)	2150 (0)	0.1250	0.1229	0.1232
12	900 (0)	2150 (0)	0.1259	0.1228	0.1289
13	900 (0)	2150 (0)	0.1259	0.1278	0.1281

The response surface was obtained (Figure 4) and the occurrence of the maximum point was verified according to the criterion of Lagrange. The model was tested by Analysis of Variance (ANOVA) with 95% confidence. The quadratic regression model offered a good fit, explaining 93% of the variance explained. The optimum values indicated for the pyrolysis and atomization temperatures were 835 °C and 2106 °C, respectively. The quadratic model was expressed according to Equation 1, where Abs is the integrated absorbance response, PT is the pyrolysis temperature, and AT is the atomization temperature:

$$Abs = -0.9140 + (4.0779\times10^{-4})(PT) - (4.9531\times10^{-7})(PT)^2 + (8.2672\times10^{-4})(AT) - (2.3569\times10^{-7})(AT)^2 + (1.9916\times10^{-7})(PT)(AT) \quad \text{Equation 1}$$

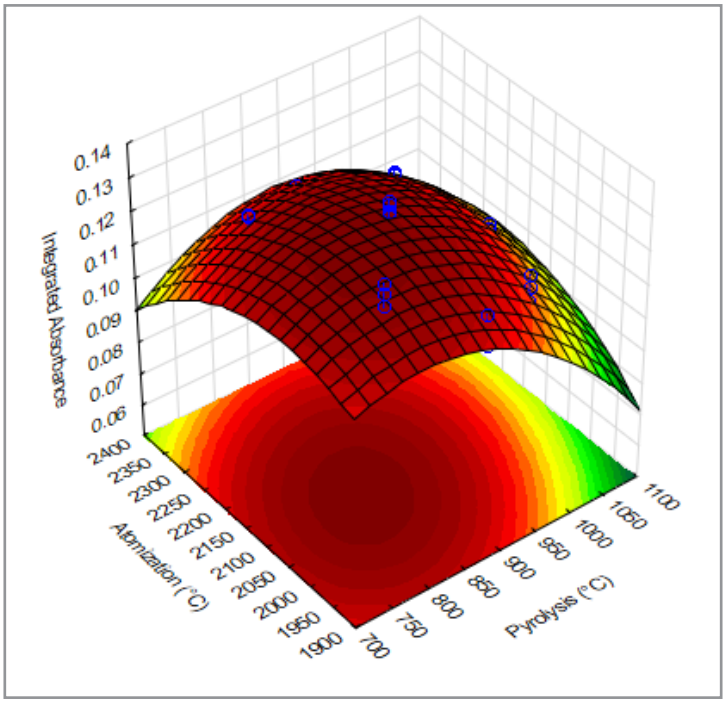


Figure 4. Response surface obtained applying central composite design to optimize the temperature program.

Figures of merit

The optimized method can be assessed by means of the figures of merit shown in Table III. The limits of detection and quantification were calculated, respectively, as three and ten times the standard deviation of the analytic measurement of fifteen blanks divided by the slope of the calibration curve [36]. The limits of detection and quantification were found to be 0.33 µg L^{-1} and 1.09 µg L^{-1}, respectively. Under these conditions, it was possible to achieve a characteristic mass which was less than the recommended value, corresponding to 3.4 pg of arsenic in 20 µL of sample. This confirms the satisfactory sensitivity of the proposed method. The method has a working dynamic range and good linearity ($R^2 > 0.99$) with satisfactory precision with a relative standard deviation of less than 5%.

Table III. Figures of merit obtained for the proposed method

Calibration equation		Abs = $0.0023C_{As}$ + 0.0041
Coefficient of determination / R^2		0.9995
Linear working range / μg L^{-1}		1.10 – 50.0
Limit of detection (LOD)	μg L^{-1}	0.33
	mg Kg^{-1}	0.033
Limit of quantification (LOQ)	μg L^{-1}	1.09
	mg Kg^{-1}	0.109
R.S.D. / % (10.00 μg L^{-1}, *n*=10)		4.1
Characteristic mass / pg (m_o)[a]		3.4

[a]Reference value according to the manufacturer's recommendations = 10.00 pg.

Study area, characterization and analysis of water samples

The "Samambaia" Stream is of great importance since it is the water source which ensures the public water supply of the city of Catalão, Goiás State (population of ca. 90,000). It is also used in other activities including irrigation, ensuring the maintenance of various farming activities practiced in the region. Considering that this municipality is also recognized as the largest mineral region in Goias, the "Samambaia" Stream was selected as the subject of this research, its monitoring being of fundamental importance.

The "Samambaia" Stream is continually subjected to several consequences of human intervention which are of great concern. These include the degradation of riparian forests, the drying up of springs, pressures from urban growth, construction works in the area, the intensive use of pesticides and the disposal of packaging materials, which is performed irregularly.

The economy of Catalão is mainly based on the extraction of niobium and phosphate minerals, which are abundantly distributed, and are also used as raw materials in the manufacture of fertilizers. The investigation of the occurrence of traces of arsenic in this region merits attention given the risk of contamination from the intense extraction of phosphate, due to the chemical similarity between these species.

The physical-chemical parameters of water samples, such as pH, conductivity, dissolved oxygen and temperature, are important since they strongly influence the content of adsorbed contaminants in sediments [37]. The pH values ranged from 4.10 (point 7) to 5.50 (point 6), with the range deliberated by the legislation being 6.0 to 9.0. The standard limits for conductivity, dissolved oxygen, temperature and salinity are respectively: ≤ 0.1 mS cm^{-1}; ≥ 5.0 mg L^{-1}; 0-30 °C and ≤ 0.5%. A variation in conductivity from 0.010 mS cm^{-1} (point 7) to 0.088 mS cm^{-1} (point 2) was observed. Regarding the dissolved oxygen, point 7 showed 4.69 mg L^{-1}, while the other points varied in concentration from 8.23 mg L^{-1} (point 5) to 9.76 mg L^{-1} (point 6). Salinity in all samples was equal to zero. The average water temperature was 19.3 °C between points 1 to 4, and 20.4 °C between points 5 to 7. Based on the results, it can be observed that the pH values are outside the acceptable levels established by Brazilian legislation (CONAMA 357) for all samples analyzed [38,39]. This factor can greatly affect the occurrence of contamination in both water bodies and sediments.

It was found that the concentration of arsenic in all sampling points are below the limit of quantification (<1.09 μg L^{-1}). In the case of dissolved oxygen only one value was outside the acceptable levels established by CONAMA (point 7). In the location of Point 7 there is the presence of a high amount of organic matter and this could result in a high consumption of dissolved oxygen during its degradation.

Analysis of sediment

The sediment fraction analyzed was that composed of particles of less than 0.062 mm (silt and clay). According to the resolution CONAMA 454/12, this fraction is the most suitable for the extraction of metals. The finer the texture of the sediment the higher the metal concentrations found, due to the greater tendency toward adsorption provided by the high surface area/grain size ratio [40].

This resolution defines two levels for the limits of the amount of a metal present in the sediment: threshold effect level (TEL) and probable effect level (PEL). The TEL represents the concentration below which adverse effects on organisms are rarely expected and the PEL represents the concentration above which there is a potential for adverse effects on organisms and the biota. For arsenic, the TEL and PEL values are, respectively, 5.9 and 17 mg Kg^{-1}. In the range between TEL and PEL such effects can occasionally be observed. The results for the sediment samples are shown in Table IV.

Table IV. Quantification of arsenic in the sediment samples (*n*=3)

Sampling point	[As] / mg Kg^{-1}
P1	2.30 ± 0.01
P2	2.24 ± 0.02
P3	2.12 ± 0.01
P4	2.20 ± 0.01
P5	2.45 ± 0.03
P6	2.06 ± 0.01
P7	3.82 ± 0.01

The arsenic concentrations at all points were below the threshold of potential adverse effects (PEL). The sample obtained from point 7 had the highest arsenic concentration. This point is located along the road BR-050 which may have influenced the overall results, since this is the point at which the urban area exerts an influence.

The mining activities associated with visible contamination at some sampling points contribute to the bio-accumulation of arsenic in sediments. These can then act as metal transporters and the partitioning of the arsenic in water bodies can be affected by changes in the physicochemical properties of these environments. A similar study was conducted in the city of Paracatu in Minas Gerais, recognized for its gold mining activities and indiscriminate use of mercury. The study revealed that all of the analyzed sediment samples and 37% of the water samples from the rivers and streams presented arsenic concentrations greater than the quality standards established by CCME and USEPA [41]. The results this work highlight the need for environmental monitoring in order to avoid future adverse effects from this bioavailable fraction.

Comparison with methods and recovery test

The obtained parameters from analytical performance of this procedure demonstrate suitability in relation to similar works found in the literature, with comparable or even better detection limits. For these works, the GF AAS technique was used to determine As in drinking water [42], natural waters [17] and underground mineral waters [43], with detection limits equal to 0.26; 1.4 and 0.42 μg L^{-1} respectively. It is also verified that the characteristic mass achieved here is less than the values reported in Table V, even compared to using high-resolution continuous source solid sampling (HR-CS SS-GF AAS) [18,44]. The present method also showed an advantage even in relation to some electroanalytical techniques. In this case, the study addressed the use of disposable gold screen-printed for voltammetric determination of arsenic in waters, with a reasonably high value for the detection limit (2.5 μg L^{-1}) [45].

Table V. Comparison of analytical parameters involving similar studies for determining As by GF AAS

Sample	PT (°C)	AT (°C)	Optimization Mode	Modifier	LOD[a]	m_o[b] (pg)	Reference
Natural waters	1350	2250	Univariate	$Pd/Mg(NO_3)_2$	1.4 μg L^{-1}	13.4	[17]
Fish oil	1400[c]	2300[c]	Univariate	Ru and Pd	0.03 μg g^{-1}	43	[18]
Groundwater and hemodialysis water	700	2300	Univariate	$Mg(NO_3)_2$	0.13 μg L^{-1}	26,4	[19]
Sediment and soil	1200	2100	Univariate	W-Rh	0.3 μg g^{-1}	39	[33]
Drinking water	1200	2400	Univariate	$Pd/Mg(NO_3)_2$	0.26 μg L^{-1}	4.2	[42]
Mineral groundwaters	1300	2300	Univariate	$Pd/Mg(NO_3)_2$	0.42 μg L^{-1}	N.I.[d]	[43]
Fish and Seafood	1200[c]	2400[c]	Univariate	Pd/Mg/Triton X-100	0.05 μg Kg^{-1}	20	[44]
Hemodialysis water	800[e]	2200[e]	Multivariate	$Pd/Mg(NO_3)_2$	1.0 μg L^{-1}	N.I.[d]	[46]

[a]Limit of Detection; [b]characteristic mass; [c]High-resolution continuum source graphite furnace atomic absorption spectrometry (HR-CS GF AAS); [d]not informed; [e]Simultaneous Graphite furnace AAS spectrometer (SIMAA).

It is important to note that the references cited in Table V address univariate assays in the development of methods for determining As by GF AAS. From this comparison, the suitability of experimental designs for multivariate optimization is highlighted once again, as used here.

During the review, just only a similar method involving simultaneous determination of As, Cd and Pb in water for hemodialysis was developed, resorting to the use of factorial designs to obtain the best pyrolysis and atomization temperatures. Response surface modeling experiments have been carried out to refine the screening results even more since it is a GF AAS equipment that operates in simultaneous mode, which is critique and not well established in most laboratories [46].

In order to verify the analytical applicability of the proposed method, addition and recovery tests were performed on the water and sediment samples. Recovery values were obtained in the range of 82.7 - 108.5% (Table VI).

Table VI. Recovery of spiked samples in the determination of arsenic under GF AAS optimized conditions

Sample	Spiked (μg L^{-1})	Found (μg L^{-1})[a]	Recovery (%)[b]
Water (*mix*)	3.00	2.91 ± 0.0008	97.00
	7.00	6.74 ± 0.0003	96.28
	10.00	10.06 ± 0.0006	100.60
Tap Water	3.00	2.89 ± 0.0016	96.33
	7.00	8.78 ± 0.0021	96.85
	10.00	10.85 ± 0.0012	108.50
Sediment (*mix*)	3.00	2.48 ± 0.0014	82.66
	7.00	7.04 ± 0.0004	100.57
	10.00	8.82 ± 0.0013	88.20

[a]Mean of three experiments ± standard deviation. [b]Acceptable recovery values in the range of 80-120% according to AOAC [36].

Experiments of external calibration and standard addition in the sample, showed that there is no significative difference (p>0.05) in the slopes of the calibration curves. The results did not indicate problematic effects of substances present concomitantly in the samples and confirmed that the matrix does not significantly interfere in the analysis. The results were acceptable, based on a calibration curve automated constructed with a standard aqueous solution of arsenic at 50.0 μg L^{-1} employing the self-dilution GF AAS technique [36].

CONCLUSIONS

The method was successfully applied to determine concentrations of arsenic in samples of water and sediment collected from mining sites and could be used to monitor for arsenic contamination. The use of multivariate optimization tools for the conditions of determination by GF AAS were efficient for the precise determination of arsenic and obtaining limit of detection appropriate to the values tolerated by current legislation.

The results reported herein indicate that human activities carried out in the region of the Samambaia Stream, such as the growing of crops using pesticides, agro-pastoral activities and the advancement of urbanization, have led to an enhancement of arsenic in the environment. The contamination of water resources due to these activities can change the constitution of the surface sediments, increasing the risk of bioavailable arsenic being present in the water body.

The results for the chemical parameters of the water showed that the pH values at all sampling points were outside of the acceptable limits established by CONAMA Resolution 357/05 and this can be considered as a particular characteristic of this location. In the case of dissolved oxygen, one value was below the limit permitted by the legislation, and this result may be due to the presence of a large amount of organic matter at that site. The other parameters were within the limits established by current legislation.

The results for the sediment samples showed arsenic concentrations below the adverse effects threshold. The water samples showed concentrations below the limit of quantification, indicating that this element does not pose a risk at the sampling sites.

Conflicts of interest

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

Acknowledgements

The authors are grateful for financial support from the Brazilian governmental agencies “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq – 477825/2007-9) and “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES – Finance Code 001), the MG state government agency “Fundação de Amparo à Pesquisa do Estado de Minas Gerais” (FAPEMIG), and the GO state government agency “Fundação de Amparo à Pesquisa do Estado de Goiás” (FAPEG). The authors appreciate the contribution of Silas Pereira Trindade (Department of Geography - State University of Goiás) in the elaboration of the location map of the study area of this work.

REFERENCES

1. Henck, K. E. *Arsenic: Environmental Chemistry, Health Threats and Waste Treatment*, 1st ed. Wiley, New York, USA, **2009**.
2. Garcia-Ordiales, E.; Covelli, S.; Rico, J. M.; Roqueñi, N.; Fontolan, G.; Flor-Blanco, G.; Centifuegos, P.; Loredo, J. *Chemosphere*, **2018**, *198*, pp 281-289 (https://doi.org/10.1016/j.chemosphere.2018.01.146).
3. Ma, Z.; Lin, L.; Wu, M.; Yu, H.; Shang, T.; Zhang, T.; Zhao, M. *Aquaculture*, **2018**, *497*, pp 49-55 (https://doi.org/10.1016/j.aquaculture.2018.07.040).
4. Litter, M.; Morgada, M. E. Formas arsenicales en agua y suelos. In: Litter, M. I.; Armienta, M. A.; Farías, S. S. (Eds.). *Metodologías analíticas para la determinación y especiación de arsénico en aguas y suelos.* CYTED, Buenos Aires, AR, **2009**, Chapter 1, pp 19-28.

5. Faita, F.; Cori, L.; Bianchi, F.; Andreassi, M. G. *Int. J. Environ. Res. Public Health*, **2013**, *10* (4), pp 1527-1546 (https://doi.org/10.3390/ijerph10041527).
6. Haider, A. F. M. Y.; Ullah, M. H.; Khan, Z. H.; Kabir, F.; Abedin, K. M. *Opt. Laser Technol.*, **2014**, *56*, pp 299-303 (https://doi.org/10.1016/j.optlastec.2013.09.002).
7. Fagundes, T.; Bachmann, A. W. L.; Tomaz, H. S. O.; Rodrigues, C. A. *Quim. Nova*, **2008**, *31* (6), pp 1305-1309 (https://doi.org/10.1590/S0100-40422008000600006).
8. Borba, R. P.; Figueiredo, B. R.; Cavalcanti, J. A. *Rev. Esc. Minas*, **2004**, *57* (1), pp 45-51 (https://doi.org/10.1590/S0370-44672004000100009).
9. Wang, G.; A, Y.; Jiang, H.; Fu, Q.; Zheng, B. *J. Hydrol.*, **2015**, *520*, pp 37-51 (https://doi.org/10.1016/j.jhydrol.2014.11.034).
10. Ma, Y.; Gong, M.; Zhao, H.; Li, X. *Sci. Total Environ.*, **2018**, *613-614*, pp 1130-1139 (https://doi.org/10.1016/j.scitotenv.2017.09.174).
11. Brady, J. P.; Kinaev, I.; Goonetilleke, A.; Ayoko, G. A. *Mar. Pollut. Bull.*, **2016**, *106* (1-2), pp 329-334 (https://doi.org/10.1016/j.marpolbul.2016.03.029).
12. Silva, L. A.; Coelho, L. M.; Rosolen, V.; Coelho, N. M. M. *J. Braz. Chem. Soc.*, **2011**, *22* (11), pp 2094-2100 (https://dx.doi.org/10.1590/S0103-50532011001100011).
13. Rosolen, V.; De-Campos, A. F.; Govone, J. S.; Rocha, C. *Catena*, **2015**, *128*, pp 203-210 (https://doi.org/10.1016/j.catena.2015.02.007).
14. Purificação, M. M.; Marchi, M. I.; Amado, N. M. P. Ciências Exatas e da Terra: *Exploração e Qualificação de Diferentes Tecnologias*. Atena, Ponta Grossa, **2020**, Chapter 3, p 22.
15. Ono, F. B.; Tappero, R.; Sparks, D.; Guilherme, L. R. G. *Environ. Sci. Pollut. Res.*, **2016**, *23* (10), pp 638-347 (https://doi.org/10.1007/s11356-016-6137-z).
16. Vieira, A. L.; Ferreira, A. C.; Oliveira, S. R.; Barbosa Jr, F.; Gomes Neto, J. A. *Microchem. J.*, **2021**, *160*, 105637 (https://doi.org/10.1016/j.microc.2020.105637).
17. Thermo Scientific Application Note: 40851. *Arsenic in Natural Waters by Graphite Furnace Atomic Absorption using EPA Method* 200.9, **2016**.
18. Pereira, E. R.; Almeida, T. S.; Borges, D. L. G.; Carasek, E.; Welz, B.; Feldmann, J.; Menoyo, J. C. *Talanta*, **2016**, *150*, pp 142-147 (https://doi.org/10.1016/j.talanta.2015.12.036).
19. Santos, L. M. G.; Jacob, S. C. *Ciênc. Tecnol. Aliment.*, **2009**, *29* (1), pp 120-123 (http://dx.doi.org/10.1590/S0101-20612009000100019).
20. Rezende, H. C.; Coelho, N. M. M. *Anal. Methods*, **2014**, *6* (24), pp 9705-9711 (https://doi.org/10.1039/C4AY01443J).
21. Pereira, L. A.; Windmöller, C. C.; Silva, J. B. B.; Borges Neto, W. *Quim. Nova*, **2011**, *34* (7), pp 1167-1172 (https://doi.org/10.1590/S0100-40422011000700012).
22. Nogueira, A. R. A; Souza, G. B. *Manual de laboratórios: solo, água, nutrição vegetal, nutrição animal e alimentos.* Embrapa Pecuária Sudeste, São Carlos-SP, **2005**, p 334.
23. Borba, R.; Coscione, A. R.; Figueiredo, B. R.; Zambello, F. *Quim. Nova*, **2009**, *32* (4), pp 970-975 (https://doi.org/10.1590/S0100-40422009000400027).
24. Costa, R. A.; Rosolen, V. *Hygeia*, **2012**, *8* (15), pp 222-231.
25. Sakan, S.; Popović, A.; Škrivanj, S.; Sakan, N.; Đorđević, D. *Environ. Sci. Pollut. Res.*, **2016**, *23*, pp 21485-21500 (http://doi.org/10.1007/s11356-016-7341-6).
26. Güven, D. E.; Akinci, G. *Gazi University Journal of Science*, **2011**, *24* (1), pp 29-34.
27. Giacomelli, M. B. O.; Lima, M. C.; Stupp, V.; Carvalho Júnior, R. M.; Silva, J. B. B.; Barrera, P. B. *Spectroc. Acta Part B*, **2002**, *57* (12), pp 2151-2157 (https://doi.org/10.1016/S0584-8547(02)00205-7).
28. Costa, B. E.; Coelho, N. M. M.; Coelho, L. M. *Food Chem.*, **2015**, *178*, pp 89-95 (http://dx.doi.org/10.1016/j.foodchem.2015.01.014).
29. Statsoft Inc., *Statistica* 6.0, **2007**.
30. Török, P.; Žemberyová, M. *Spectroc. Acta Part. B*, **2010**, *65* (4), pp 291-296 (https://doi.org/10.1016/j.sab.2010.03.007).

31. Sardans, J.; Montes, F.; Peñuelas, *J. Soil Sediment Contam*., **2011**, *20* (4), pp 447-491 (https://doi.org/10.1080/15320383.2011.571526).
32. Lima, E. C.; Barbosa, R. V.; Brasil, J. L.; Santos, A. H. D. P. *J. Anal. At. Spectrom*., **2002**, *17* (11), pp 1523–1529 (https://doi.org/10.1039/B205905C).
33. Barbosa Jr., F.; Lima, E. C.; Krug, F. J. *Analyst*, **2000**, *125* (11), pp 2079–2083 (https://doi.org/10.1039/B005783P).
34. Ortner, H. M.; Bulska, E.; Rohr, U.; Schlemmer, G.; Weinbruch, S. *Spectroc. Acta Part B*, **2002**, *57* (12), pp 1835-1853 (https://doi.org/10.1016/S0584-8547(02)00140-4).
35. Matusiewicz, H.; Mroczkowska, M. *J. Anal. At. Spectrom*., **2003**, *18* (7), pp 751–761 (https://doi.org/10.1039/B302217J).
36. Association of Official Analytical Chemists (AOAC). Official Methods of Analysis. 16th ed.; Arlington, USA, **1995**.
37. Lemes, M. J. L.; Filho, P. M. F.; Pires, M. A. F. *Quim. Nova*, **2003**, *26* (1), pp 13-20 (https://doi.org/10.1590/S0100-40422003000100004).
38. Conselho Nacional do Meio Ambiente (CONAMA). Resolução Nº 357, 17 de março de **2005**. Available at: http://www.mma.gov.br [Accessed 12 August 2019].
39. Conselho Nacional do Meio Ambiente (CONAMA). Resolução Nº 454, 01 de novembro de **2012**. Available at: http://www.mma.gov.br [Accessed 20 November 2019].
40. Ribeiro, F. A. L.; Ferreira, M. M. C.; Morano, S. C.; Silva, L. R.; Schneider, R. P. *Quim. Nova*, **2008**, *31* (1), pp 164-171 (https://doi.org/10.1590/S0100-40422008000100029).
41. Rezende, P. S.; Costa, L. M.; Windmöller, C. C. *Arch. Environ. Contam. Toxicol*., **2015**, *68*, pp 588-602 (https://doi.org/10.1007/s00244-015-0134-y).
42. Michon, J.; Deluchat, V.; Shukry, R. A.; Dagot, C.; Bollinger, J. *Talanta*, **2007**, *71* (1), pp 479-485 (https://doi.org/10.1016/j.talanta.2006.06.016).
43. Tudorache, A.; Marin, C.; Badea, I. A.; Vladescu, L. *Environ. Monit. Assess*., **2011**, *173*, pp 79–89 (https://doi.org/10.1007/s10661-010-1372-0).
44. Zimozinski, A. V.; Llorente-Mirandes, T.; Damin, I. C. F.; López-Sánchez, J. F.; Vale, M. G. R.; Welz, B.; Silva, M. M. *Talanta*, **2015**, *134*, pp 224-231 (https://doi.org/10.1016/j.talanta.2014.11.009).
45. Laschi, S.; Bagni, G.; Palchetti, I.; Mascini, M. *Anal. Lett*., **2007**, *40* (16), pp 3002–3013 (https://doi.org/10.1080/00032710701645703).
46. Santos, L. M. G.; Gonçalves, J. M.; Jacob, S. C. *Quim. Nova*, **2008**, *31* (5), pp 975-979 (http://dx.doi.org/10.1590/S0100-40422008000500006).

Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 33, pp 57-70
doi: 10.30744/brjac.2179-3425.AR-05-2021

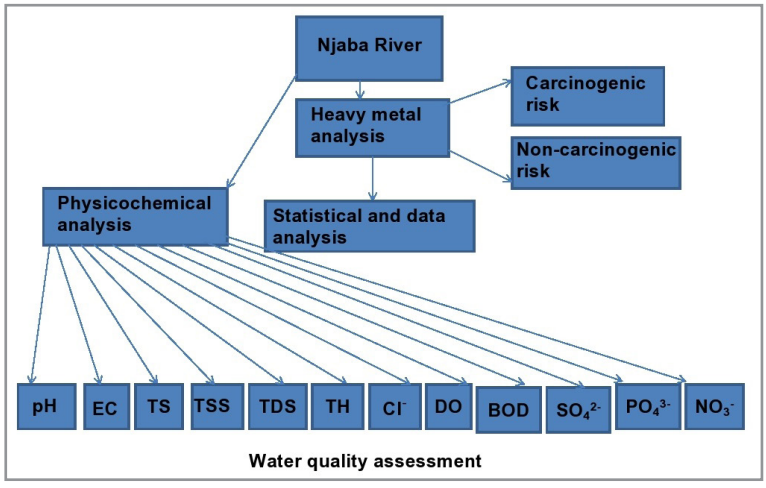
BrJAC

ARTICLE

Carcinogenic and Non-carcinogenic Health Risk Assessment of Heavy Metals in Njaba River, Imo State, Nigeria

Victor Chukwuemeka Eze[1]*, Chidiebere Temple Ndife[2], Miracle Oluebube Muogbo[1]

[1]*Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra State, Nigeria*
[2]*Department of Science Laboratory Technology, Federal Polytechnic Oko, Anambra State, Nigeria*



Daily exposure to potentially toxic elements (heavy metals) through the oral ingestion of water has been a major concern to human health due to its detrimental effects. Studies focusing on health risk assessment of potentially toxic elements in surface and ground waters have been conducted, but none has been reported in Njaba River. Few studies conducted have focused only on the assessment of its water quality. Therefore, this study assessed the carcinogenic and non-carcinogenic effects of the potentially toxic elements (As, Cd, Cr, Cu, Ni, Pb, and Zn) in Njaba River. Overall, a total of 135 water samples was collected for this study and were analyzed using Agilent FS240AA AAS. The potentially toxic elements concentrations were: As (0.015±0.001 to 0.021±0.001 mg L^{-1}), Cd (0.006±0.002 to 0.018±0.002 mg L^{-1}), Cr (0.027±0.001 to 0.074±0.001 mg L^{-1}), Cu (0.016±0.002 to 0.033±0.001 mg L^{-1}), Ni (0.031±0.001 to 0.053±0.002 mg L^{-1}), Pb (0.050±0.002 to 0.092±0.001 mg L^{-1}), and Zn (0.061±0.002 to 0.097±0.002 mg L^{-1}). As, Ni and Pb recorded concentrations above their respective maximum permissible limits. Physicochemical parameters were appraised using the American Public Health Association standard method (APHA). The evaluation of the carcinogenic and non-carcinogenic health risks of the analyzed elements was carried out based on the guidelines of the USEPA. The hazard index values for children via upstream, midstream and downstream sample points were 0.0000128, 0.00000895 and 0.0513 respectively, while the hazard index values for adults via upstream, midstream and downstream sample points were 0.00000551, 0.00000395 and 0.00000581 respectively. The health risk estimation showed that the hazard quotients were within acceptable limits. The total cancer risks of potentially toxic elements were generally within the range of tolerable risk for adults and above the range of tolerable risk for children.

Keywords: Heavy metals, carcinogenic, hazard quotient, physicochemical, Njaba River.

Cite: Eze, V. C.; Ndife, C. T.; Muogbo, M. O. Carcinogenic and Non-carcinogenic Health Risk Assessment of Heavy Metals in Njaba River, Imo State, Nigeria. *Braz. J. Anal. Chem.,* 2021, *8* (33), pp 57-70. doi: http://dx.doi.org/10.30744/brjac.2179-3425.AR-05-2021

Submitted 8 January 2021, Resubmitted 28 February 2021, Accepted 4 March 2021, Available online 26 March 2021.

INTRODUCTION

Availability of uncontaminated water for human use has been a primordial and present issue in developing countries due to the associated health implications [1]. About 2% of the water resources on earth are from rivers, and only about 0.01% of the earth's water resource is fit for human consumption [1,2]. Surface waters such as rivers, streams, creeks, reservoirs, and lakes play an essential role in strengthening the economy of developing countries such as Nigeria [2-4]. Potentially toxic elements, organic and inorganic contaminants often contaminate rivers thereby making them unfit for domestic, agricultural and industrial purposes [3-5]. Due to the unique characteristics of potentially toxic elements (high toxicity at low concentrations, poor biodegradability and bioaccumulation), they pose a serious health threat to humans and aquatic life [5,6]. Similarly, the chronic low-level intake of water contaminated with potentially toxic elements such as chromium, cadmium, arsenic, and lead could seriously threaten human health [7-9]. Also, prolonged intake of potentially toxic elements can lead to accumulation in the kidneys, brain, bones, and livers in the human body [6,7,10]. In most cases, this often results in adverse health effects such as nervous system damage, poor growth and development, and even death, depending on the potentially toxic element and its chemical form [6,10].

Over the years, the discharge of untreated wastewater from municipal, industrial, and agricultural activities into rivers, streams, and lakes have resulted in a high level of potentially toxic elements contamination in them [7,8]. Also, the levels and concentrations of potentially toxic elements in surface and ground waters vary significantly from one geographical area to another [10]. Akubugwo et al. [8] and Ahiarakwem & Onyekuru [11] noted that the level of contamination of Njaba River in Imo State, Nigeria, is prominent. This is because Njaba River receives a large number of contaminants due to frequent rainfalls as well as the enormous superficial runoff due to precipitous water repositories which result in serious flooding within the area [13-15]. During flooding, potentially toxic elements in the soil dissolve in the floodwaters and are transported via seasonal floodplain surfaces into the river where they accumulate, and so, the river becomes enriched with the potentially toxic elements [15]. Numerous studies have revealed that contaminants tend to increase the potentially toxic element concentration in water bodies such as rivers [8,11,12,15,16]. To the best of our knowledge, studies focusing on non-carcinogenic and carcinogenic health risks of heavy metals in Njaba River, Imo State, are lacking and therefore, this study is aimed at filling the knowledge gap. Worthy of note is that potentially toxic elements enter the human body through several pathways, namely: dermal contact, oral ingestion, and inhalation, but in comparison to oral ingestion, all others are trivial [16-18].

In this research, the potential health risks due to exposure to potentially toxic elements were estimated in children and adults. Enyoh & Isiuku [15] opined that health risk assessment is vital as it forms an integral part of safety management and occupational health.

Njaba River also provides water for agricultural purposes to the local population, which is more intense in the dry season. The river water is normally used without any prior assessment, even though many anthropogenic activities are carried out in and around the river [44]. Adults and children often consume water from recreational activities such as swimming in the river. The findings from this research will give an insight into the extent of contamination from potentially toxic elements in Njaba River, and also provide the necessary information that can help the decision-makers to establish comprehensive regulations and policies to protect the health of the populace within and around the area studied.

MATERIALS AND METHODS

Study area

Njaba River is a major tributary of Oguta lake, located in the Niger Delta Basin, Imo State, Southeastern Nigeria. The river originated from Isu-Njaba, flows towards the south-west through Oguta lake and Njaba, passing through the southern parts of Ukworji, Umunnoha, and Oguta [8]. Njaba River lies within Latitudes 5°44' and 5°47' North [8,11] and Longitudes 6°49' and 7°03' East [8,11]. The river has a total stream length of 78.2 km, a mean depth of 4.5 m, a basin area of 145.63 km^2, and an average specific discharge of about

1700 m^3/h [8,11,12]. Geographically, the zone is divided by the lower Niger River into two unequal sections – an eastern section (which is the larger amongst the two) and a western section. Based on geology, the region is divided mainly into the Southeastern scarp lands under the Anambra/Imo river basin, and eastern borderlands under cross-river basins, and the apex of Udi Plateau at 300 m above sea level [43]. The river serves as a source of water for both domestic and agricultural purposes to the poor, and the entire local population. The pressure on the river increases during the dry season and festive periods due to a lack of alternative source of water supply [12]. The map of the study area and sample locations are depicted in Figure 1.

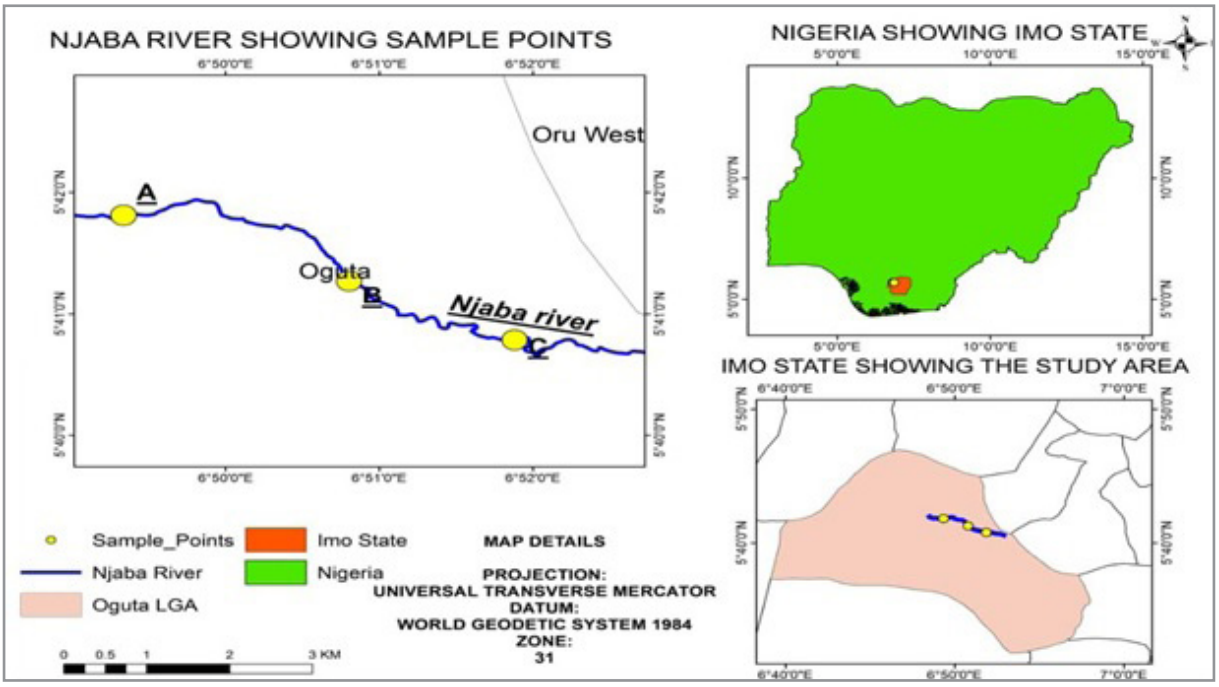


Figure 1. Map of Njaba River showing sample points.

Collection of water samples

The water samples for this study were collected from Njaba River at three sampling points. The sampling points are Upstream (A), Midstream (B) and Downstream (C). The sample points were at least 100 m apart. The water sampling was carried out according to the method prescribed by Akubugwo et al. [8]. Fifteen (15) water samples each were collected from the upstream, midstream and downstream. At each sample point, composite samples were collected and subsequently pooled together as one sample. The samples were kept at room temperature before they were taken to the laboratory for analysis. A total of 135 water samples were obtained for this study. Samples collection was done during the dry season between the months of January and March 2020.

Concentration of potentially toxic elements in water samples

The collected water samples were analyzed for potentially toxic elements using standard methods for the analysis of water samples as described by [13,14]. All chemicals used were analytical grade reagents with >95% purity. De-ionized water was used during samples preparation and dilution of metal solutions. Agilent FS240AA atomic absorption spectrophotometer (AAS) was used to quantify the digested water samples for seven potentially toxic elements: arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn). Following the same protocol as the samples, standard reference material (SRM 2783) filter from the National Institute of Standards and Technology (NIST) was analyzed for

potentially toxic elements concentration, and compared with their certified values to validate the analyses [18] and results obtained were reported to be within ±5% of the certified values [18]. The limit of detection of the measured potentially toxic elements from the water samples are: As = 0.001 mg L^{-1}, Cd = 0.003 mg L^{-1}, Cr = 0.001 mg L^{-1}, Cu = 0.0001 mg L^{-1}, Ni = 0.002 mg L^{-1}, Pb = 0.001 mg L^{-1}, Zn = 0.0003 mg L^{-1}.

Statistical and data analysis

Descriptive statistics (mean ± standard deviation) was reported for the three sampling points (upstream, midstream and downstream). Significant differences were considered at $p < 0.05$. The data obtained were compared with the recommended limits set by the Nigerian Standard for Drinking Water Quality (NSDWQ) [16] and World Health Organization (WHO) [28]. In this study, statistical analyses such as mean and standard deviation were carried out using IBM statistical package for social sciences version 20 (SPSS Inc., Chicago, IL, USA).

Physicochemical parameter assessment

The collected water samples were analyzed according to the method prescribed by the American Public Health Association [13]. The pH and electrical conductivity were measured *in situ* using pH meter and conductivity meter respectively [13]. Total solids (TS), total suspended solids (TSS), and total dissolved solid (TDS) were determined [13,15]. Total hardness (TH) was conducted by EDTA titrimetric method. Chloride (Cl^-) was estimated using the Argentometric method [14]. Dissolved oxygen (DO) was determined using the modified Winkler's method according to [11,12]. Biochemical oxygen demand (BOD) was appraised using the method described by [16]. Sulphate (SO_4^{2-}), phosphate (PO_4^{3-}), and nitrate (NO_3^-) were estimated using the procedures given by [13].

Human health risk assessment

Health risk assessment involves estimating the probable occurrence and impact of a potentially harmful contaminant over a given period [17,18]. The potential health risk of each contaminant is usually based on the estimated risk level; and thus can be classified as non-carcinogenic or carcinogenic health risks [18,19]. In order to ascertain the degree of potentially toxic elements contamination, and possible health risks for children and adults, hazard quotients and incremental lifetime cancer risks were used. In this research, the priority groups were children and adults. The values used for calculating the chronic daily intake through oral ingestion are summarized in Table I.

Table I. Parameters and input assumptions for exposure assessment of potentially toxic elements for children and adults [16,19]

Parameter	Unit	Values			
		Non-cancer		Cancer	
		Adult	Children	Adult	Children
Heavy metal concentration (C_w)	µg L^{-1}				
Daily average intake (DI)	L/day	2.2	1.1	2.2	1.1
Exposure frequency (EF)	Days/year	365	365	365	365
Exposure duration (ED)	Year	70	6	70	6
Bodyweight (BW)	Kg	70	15	70	15
ABS	All	0.001	0.001	0.001	0.001
Average time (AT)	Days	25550	2190	25550	2190

Non-carcinogenic health risk

Equation 1 obtained from [21,44] was applied to determine the chronic daily intake of potentially toxic elements for children and adults via ingestion exposure routes [16,19-21].

$$CDI_{ingestion} = C_W \times DI \times ABS \times EF \times ED/BW \times AT \quad (1)$$

where, C_w (in μg L^{-1}) is the heavy metals concentration in water, *ABS* (no unit) is the dermal absorption factor, *DI* (in L/day) is the daily average intake of water in the area, *EF* (in days/year) represents the annual exposure frequency, *ED* (in years) is exposure duration, *BW* (in kg/person) is bodyweight, and *AT* (in days) is the average time [16,19-21,44].

The *HQ* for individual potentially toxic elements was estimated using the ratio of the calculated mean daily intake (CDI, mg L^{-1}/day) of the metal ingested through contaminated water to the reference dose (RfD). Sum of all the hazard quotients give the total potential health risks or hazard index (*HI*) [16,19]. The calculation of the *HQ* caused by water is presented in Equation 2.

$$HQ = CDI/RfD \quad (2)$$

where *CDI* and *RfD* are expressed in mg L^{-1}/day. The values of the RfD and cancer slope factor for different metals studied are listed in Table II.

Hazard Index (HI)

In order to evaluate the entire non-carcinogenic health impacts caused by exposure to a mixture of potentially toxic elements in River Njaba, the HI for seven potentially toxic elements was computed according to the USEPA guidelines for health risk assessment [16,18,19,21] using Equation 3.

$$HI = HQ_{As} + HQ_{Cd} + HQ_{Cr} + HQ_{Cu} + HQ_{Ni} + HQ_{Pb} + HQ_{Zn} \quad (3)$$

The computed *HI* is compared to standard values: there is the possibility that non-carcinogenic impacts may occur in the residents when *HI* > 1, while the exposed person is unlikely to experience evident harmful health impacts when *HI* < 1 [16,18,24,25].

Table II. Reference dose (RfD) and cancer slope factor (CSF) for different potentially toxic elements [16,21,24]

Potentially toxic elements	$RfD_{oral\ ingestion}$	CSF (mg L^{-1}/day)
As	0.30	1.50
Cd	0.50	6.10
Cr	3.00	41.00
Cu	40.00	NAD
Ni	20.00	0.84
Pb	1.40	8.50
Zn	300.00	NAD

NAD= No Available Data

Carcinogenic health risk

This is usually estimated using the Incremental Lifetime Cancer Risk (ILCR) [16,24,26]. The ILCR is the possibility of a person developing any type of cancer over a lifetime as a result of daily exposure to a given daily amount of a carcinogenic element [16,19,21,26]. Equation 4 was used for the calculation of the lifetime cancer risk.

$$ILCR = CDI \times CSF \quad (4)$$

where CSF is the cancer slope factor. The allowable limits are considered to be 10^{-6}, and less than 10^{-4} for both single and multi-element carcinogens [16,26,27].

RESULTS AND DISCUSSIONS

Concentrations of potentially toxic elements

The concentrations of potentially toxic elements (Table III) in Njaba River revealed the presence of arsenic (0.015±0.001 to 0.021±0.001 mg L^{-1}), cadmium (0.006±0.002 to 0.018±0.002 mg L^{-1}), chromium (0.027±0.001 to 0.074±0.001 mg L^{-1}), copper (0.016±0.002 to 0.033±0.001 mg L^{-1}), nickel (0.031±0.001 to 0.053±0.002 mg L^{-1}), lead (0.050±0.002 to 0.092±0.001 mg L^{-1}) and zinc (0.061±0.002 to 0.097±0.002 mg L^{-1}). Arsenic, nickel, and lead recorded concentrations higher than their respective NSDWQ and WHO [28] maximum permissible limits. The concentrations recorded for cadmium and chromium in midstream were lower than their respective NSDWQ and WHO [28] maximum permissible limits. Also, Cu and Zn concentrations were lower than their respective NSDWQ and WHO [28] maximum permissible limits. These results were slightly in contrast to the data reported by Akubugwo et al. [8] where the authors reported low levels in both upstream and downstream of the river. However, Ahiarakwem et al. [45] reported a high concentration for Cr, Cd, Ni, Zn, and Fe which was attributed to anthropogenic activities around the river. High concentrations of some potentially toxic elements reported in this study are undesirable. Potentially toxic elements such as arsenic cause skin infections, vascular diseases and visceral cancers; cadmium is a human carcinogen that causes renal disorder, and damage of the kidney; chromium causes nausea and vomiting, diarrhea, headache; copper has been associated with Wilson disease, gastrointestinal irritation, insomnia (sleeplessness), and liver damage; nickel, a human carcinogen causes nausea, dermatitis, chronic asthma, and coughing; lead causes damage to the circulatory and nervous system, fetal brain, and diseases of the kidney; zinc causes lethargy, neurological signs, depression, and increased thirst [8,16,29-31]. Generally, human activities such as oil spills from automobiles, bathing, washing vehicles and clothes, causes increased levels of potentially toxic elements in water bodies such as streams, lakes, and rivers [18]. All studied metals showed significant differences ($p<0.05$) in the different sample points of the river. There were no definite trends for the studied potentially toxic elements; some potentially toxic elements (Cd, Cr, Cu, Ni and Zn) showed the highest concentration upstream while some (As and Pb) had the highest concentration downstream of the river.

Table III. Potentially toxic elements concentration levels in Njaba River

Potentially toxic elements	Upstream (mg L^{-1})	Midstream (mg L^{-1})	Downstream (mg L^{-1})	NSDWQ Standards (MPL)* [16]	WHO* Standard [29]
As	0.015±0.001^{a}	0.019±0.002^{ab}	0.021±0.001^{b}	0.01	0.01
Cd	0.018±0.002^{a}	0.006±0.001^{c}	0.012±0.002^{c}	0.03	0.01
Cr	0.074±0.001^{b}	0.027±0.001^{a}	0.050±0.001^{c}	0.05	0.05
Cu	0.033±0.001^{a}	0.016±0.002^{b}	0.025±0.001^{c}	1.00	2.00
Ni	0.053±0.002^{a}	0.031±0.001^{c}	0.044±0.002^{b}	NHB*	0.02
Pb	0.085±0.001^{a}	0.050±0.002^{c}	0.092±0.001^{b}	0.01	0.01
Zn	0.097±0.002^{a}	0.061±0.002^{b}	0.077±0.001^{ab}	3.00	5.00

The results are means and standard deviations of triplicate determinations. Values with similar alphabets along the same row are not statistically significant at $p<0.05$. Values with different alphabets along the same row are statistically significant at $p<0.05$.

*NSDWQ (MPL): Nigerian Standard for Drinking Water Quality; MPL: Maximum Permissible Limits; WHO: World Health Organization; NHB= No Health Baseline.

Physicochemical analysis

The mean concentrations of the physicochemical parameters assessed in Njaba River are represented in Table IV. The physicochemical properties showed significant differences at different points except for some parameters such as BOD, sulphate and phosphate which showed significant differences between downstream and upstream and midstream. From the table, pH values of the sampling points ranged from 5.95±0.23 to 6.43±0.11 and were lower than NSDWQ and WHO [28] maximum permissible limits. Similar pH has been reported previously by [8,45,46] for rivers in Imo state. The pH of the studied water body is acidic. The low pH of water bodies could be attributed to the presence of humic acids generated from decaying aquatic plants and animals [32]. Akubugwo et al. [8] noted that daily ingestion of water with low pH could lead to peptic ulcer. Electrical conductivity values recorded in the studied river ranged from 2847±11.11 to 6838±9.98 µS cm^{-1}. The conductivity values were higher than NSDWQ [16] maximum permissible limit and also higher than the reported values for river Uramiriuka in Owerri, Imo state [46]. A strong relationship between total dissolved solid and electrical conductivity in water has been reported by [33]. Total solid, total suspended solid, and total dissolved solid values ranged from 3050±3.04 to 7800±1.98, 1000±1.03 to 2500±0.99, and 2500±0.5 to 5800±0.53 mg L^{-1} respectively. These reported values are higher than NSDWQ [16] and WHO [28] maximum permissible limit. The reported high values are a clear indication of anthropogenic activities on the studied river. Worthy of note is that people use water from the river for agricultural, industrial, and domestic purposes. Consumption of water with high total solid, total suspended solid, and total dissolved solid are harmful to the body system of humans [8]. The values recorded for total hardness ranged from 55.00±0.77 to 95.50±0.35 mg L^{-1}. These values are lower than the NSDWQ and WHO [28] maximum permissible limits. This implies that Njaba River is moderately soft. Gray [34] stated that water could be classified as soft water when the degree of hardness is 0-50 mg L^{-1}. Also, soft water is known to form lather readily with soap. Chloride ranged from 72±0.08 to 85±0.19 mg L^{-1}. The values reported for chloride were lower than the NSDWQ and WHO [28] maximum permissible limits. However, the presence of chloride in Njaba River is a sign of pollution due to human activities. Dissolved oxygen (DO) and biological oxygen demand (BOD) values ranged from 2.05±0.59 to 3.18±0.65 mg L^{-1} and 5.55±0.66 to 6.00±0.60 mg L^{-1} respectively. The DO and BOD values of Njaba River as revealed in this research are lower than the WHO [28] maximum permissible limit. Garg et al. [35]

noted that dissolved oxygen concentration greater than 5.00 mg L^{-1} supports aquatic life. DO and BOD are essential for the self-purification process in water bodies [36]. Sulphate, phosphate and nitrate values in Njaba River ranged from 15.76±3.33 to 16.23±2.09, 47.45±1.03 to 49.55±0.97 and 0.81±0.13 to 0.99±0.06 mg L^{-1} respectively. The phosphate levels in this research were higher than the WHO [28] maximum permissible limit while the sulphate and nitrate levels were lower than the NSDWQ [16] and WHO [28] maximum permissible limits respectively. The results reported in this study are in contrast to reports of [43], in which lower concentrations were obtained for nitrate and phosphate. High phosphate in the river was recorded due to the continuous use of phosphate-based fertilizers on the surrounding farmlands. High phosphate contents in rivers lead to the existence of blue-green algae on the surfaces [12]. Furthermore, the low sulphate and nitrate concentrations recorded in the river implies that there is a low possibility of the development of methaemoglobinaemia in children (infants) [8,12,37,43].

Table IV. Physicochemical parameters of Njaba River

Parameters	Upstream	Midstream	Downstream	NSDWQ Standards* [16]	WHO* Standard [28]
pH	5.95±0.23^{a}	6.43±0.11^{a}	6.21±0.34^{a}	6.5-8.5	6.5-8.5
Electrical conductivity (µS cm^{-1})	2847±11.11^{a}	5236±6.05^{b}	6838±9.98^{c}	1000	NHB
Total solids (mg L^{-1})	3050±3.04^{a}	5500±1.01^{b}	7800±1.98^{c}	500 mg L^{-1}	NHB
Total suspended solids (mg L^{-1})	1000±1.03^{a}	2500±0.99^{b}	2000±2.04^{b}	NAD	50.00
Total dissolved solid (mg L^{-1})	2500±0.35^{c}	4000±0.44^{b}	5800±0.53^{a}	NAD	250.00
Total hardness (mg L^{-1})	55.00±0.77^{a}	80.00±0.32^{b}	95.50±0.35^{c}	150	500.00
Chloride (mg L^{-1})	85±0.19^{b}	72±0.08^{a}	78±0.10^{c}	250	250.00
Dissolved oxygen (mg L^{-1})	3.13±0.61^{a}	3.18±0.65^{a}	2.05±0.59^{a}	NAD	10.00
Biochemical oxygen demand (mg L^{-1})	5.55±0.66^{b}	6.00±0.60^{b}	5.58±0.47^{b}	NAD	10.00
Sulphate (mg L^{-1})	15.76±3.33^{a}	15.94±1.01^{c}	16.23±2.09^{b}	100	NHB
Phosphate (mg L^{-1})	49.55±0.97^{a}	48.10±2.55^{b}	47.45±1.03^{b}	NAD	5.00
Nitrate (mg L^{-1})	0.81±0.13^{a}	0.99±0.06^{b}	0.89±0.11^{b}	50.00	50.00

The results are means and standard deviations of triplicate determinations. Values with similar alphabets along the same row are not statistically significant at $p<0.05$. Values with different alphabets along the same row are statistically significant at $p<0.05$.

*NSDWQ (MPL): Nigerian Standard for Drinking Water Quality; MPL: Maximum Permissible Limits; WHO: World Health Organization; NAD= No Available Data; NHB= No Health Baseline.

Non-carcinogenic health risks

In non-carcinogenic risk assessment, the first step is to calculate the chronic daily intake. As shown in Table V, the mean CDI of potentially toxic elements for children and adults through ingestion of contaminated water was reported in the order: upstream>downstream>midstream. As shown in Figure 2, the studied potentially toxic elements recorded hazard quotient values less than 1 for children and adults. Based on the health risk investigation of As, Cd, Cr, Ni, Pb, Zn, and Cu, the mean hazard quotients generally suggests an acceptable level of non-carcinogenic health risk in the water samples collected from Njaba River. After calculation of the individual hazard quotients for children and adults, it can be deduced that the contribution of the potentially toxic elements to the non-carcinogenic health risk for adults was in the order: As > Pb > Cd > Cr > Cu > Ni > Zn, while the contribution of the potentially toxic elements to the non-carcinogenic health risk for children was in the order: Zn > Cd > As > Ni > Cr > Cu > Pb. [24,25,38] stated that when HQ and HI values are less than 1, there is no obvious risk to the residents' health, but if the value exceeds one, there may be a concern for possible non-carcinogenic effects. The non-carcinogenic human health risk of potentially toxic elements for children and adults in Njaba River is presented in Figure 2.

Table V. Chronic daily intake of potentially toxic elements for children and adults in Njaba River

Potentially toxic elements	$CDI_{ingestion}$ Adults			$CDI_{ingestion}$ Children		
	Upstream	Midstream	Downstream	Upstream	Midstream	Downstream
As	4.71E-07	5.97E-07	6.60E-07	1.10E-06	1.39E-06	1.54E-06
Cd	5.66E-07	1.89E-07	3.77E-07	1.32E-06	4.40E-07	8.80E-07
Cr	2.33E-06	8.49E-07	1.57E-06	5.43E-06	1.98E-06	3.67E-06
Ni	1.67E-06	9.74E-07	1.38E-06	3.89E-06	2.27E-06	3.23E-06
Pb	2.67E-06	1.57E-06	2.89E-06	6.23E-06	3.67E-06	6.75E-06
Zn	3.05E-06	1.92E-06	2.42E-06	7.11E-06	4.47E-06	5.65E-06
Cu	1.04E-06	5.03E-07	7.86E-07	2.42E-06	1.17E-06	1.83E-06
Mean CDI	1.18E-05	6.60E-06	1.01E-05	2.75E-05	1.54E-05	2.36E-05

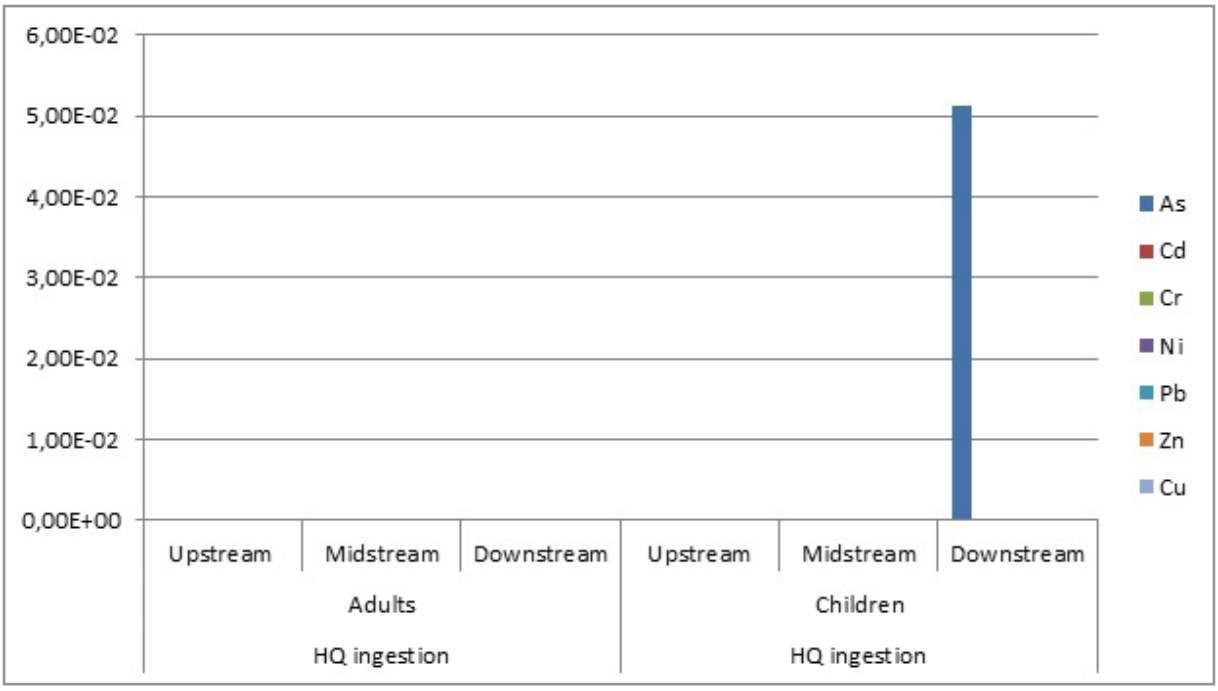


Figure 2. Non-carcinogenic human health risk of potentially toxic elements for children and adults in Njaba River.

Carcinogenic health risks

Potentially toxic elements such as As, Cd, Cr(VI), Ni, and Pb can magnify the risk of cancer in humans [18,19,22,38]. Long term exposure to dangerous metals could result in numerous kinds of cancer [18]. In this research, As, Cd, Cr(VI), Ni, and Pb were investigated as the carcinogens; the total exposure of the population was ascertained using the mean CDI values reported in Table V. Cancer risks of potentially toxic elements in Njaba River is presented in Figure 3, while the cancer slope factor (CSF) values for individual metals are recorded in Table II. According to USEPA [22] and Yang et al. [40], ILCR values less than $1x10^{-6}$ are considered unimportant and can be disregarded, while an ILCR value exceeding $1x10^{-4}$ is considered detrimental.

As shown in Figure 3, the total cancer risks reported for adults are generally within the acceptable limit while the total cancer risk (CR_t) reported for children are above the acceptable limit of potentially toxic elements in the water. Similar results were reported by [7,16,19] in drinking water in Zahedan city and Khorramabad, Iran, and drinking water from shallow groundwater wells in an agricultural area in Ubon Ratchathani province, Thailand, respectively. [7,16,39,42] noted that CR values less than $1x10^{-6}$ are considered unimportant and can be disregarded, while CR values exceeding $1x10^{-4}$ is considered detrimental to health. The carcinogenic risk for children and adults in individual potentially toxic elements are in the order: Cr > Pb > Cd > Ni > As. The findings from this research generally indicate that cancer risks from the studied potentially toxic elements in Njaba River are higher in children than in adults.

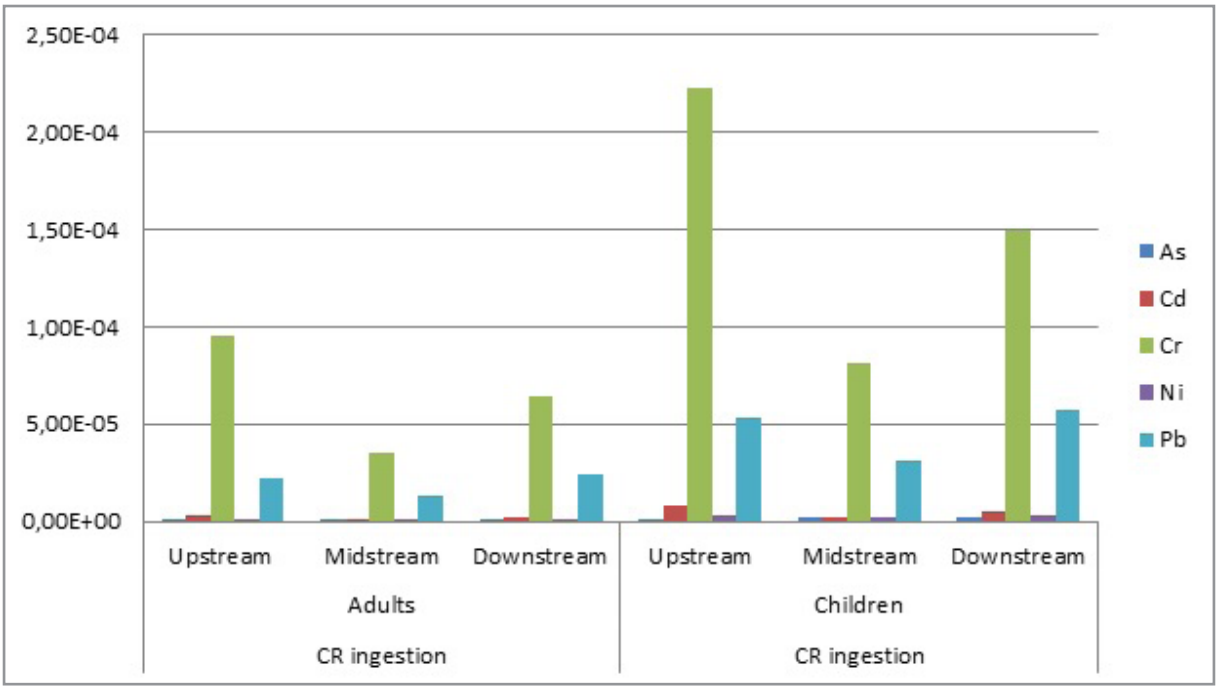


Figure 3. Cancer risks of potentially toxic elements in Njaba River.

CONCLUSION

This research has revealed that Njaba River is acidic (low pH), with a high electrical conductivity, total suspended and dissolved solid, phosphate, and total solid. The high values reported are a clear indication of the influence of human activities within and around the river. It is noteworthy to mention that consumption of water with high total solid, total dissolved and total suspended solid is detrimental to human health. The local populations who consume water from Njaba River are hereby advised to purify the water before usage. This research also investigated the health risk due to potentially toxic elements exposure in the river. Carcinogenic and non-carcinogenic health risks for children and adults were estimated. Mean CDI of potentially toxic elements in mg L^{-1}/day for children and adults through oral intake of contaminated water was reported in the order: upstream > downstream > midstream. Based on the health risk estimation of the potentially toxic elements (As, Cd, Cr, Ni, Pb, Zn, and Cu), the mean hazard quotients generally suggests an acceptable degree of non-carcinogenic health impact on children and adults. Similarly, the total chronic hazard index (THI) which is the addition of the individual potentially toxic element hazard quotients for children and adults recorded a value less than 1. This implies that there is generally no obvious risk to children and adults health. Furthermore, the total cancer risk reported was within the acceptable limit for adults and above the acceptable limit for children. This research has provided the necessary information that can help the decision-makers to establish comprehensive regulations and policies to protect the health of the local population especially the children within and around the study area.

Authors' contribution

All the authors contributed equally to the preparation of this manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors of this research declare that they have no conflict of interest.

REFERENCES

1. Saleh, H. N.; Panahande, M.; Yousefi M. *Biol. Trace Elem. Res*., **2019**, *190* (1), pp 251–261 (https://doi.org/10.1007/s12011-018-1516-6).
2. Pazand, K.; Khosravi, D.; Ghaderi, M. R.; Rezvanianzadeh, R. *Journal of Water & Health*, **2018**, *16* (4), pp 622–634 (https://doi.org/10.2166/wh.2018.034).
3. Kumar, M.; Ramanathan, A. L.; Tripathi, R.; Farswan, S.; Kumar, D.; Bhattacharya, P. *Chemosphere,* **2017**, *166*, pp 135–145 (https://doi.org/10.1016/j.chemosphere.2016.09.086).
4. Barzegar, R.; Asghari, M.; Soltani, E. F.; Tziritis, E.; Kazemian, N. *Exposure and Health,* **2017**, pp 1–15 (https://doi.org/10.1007/s12403-017-0267-5).
5. Li, F.; Qiu, Z.; Zhang, J.; Liu, W.; Liu, C.; Zeng, G. *Int. J. Environ. Res. Public Health,* **2017**, *14*, 768 (https://doi.org/10.3390/ijerph14070768).
6. Lu, S. Y.; Zhang, H. M.; Sojinu, S. O.; Liu, G. H.; Zhang, J. Q.; Ni, H. G. *Environ. Monit. Assess.,* **2015**, *187* (1), article 4220 (https://doi.org/10.1007/s10661-014-4220-9).
7. Mohadeseh, D.; Hossein, K.; Seyed, D. A.; Ayat, H. P.; Amir, H. M.; Davoud, B.; Mohammad, H.; Hossein, A.; Edris, B.; Fatemeh, P. *J. Environ. Health Sci. Eng*., **2019,** *17*, pp 1163-1169 (https://doi.org/10.1007/s40201-019-00430-6).
8. Akubugwo, E. I.; Nwachukwu, M. I.; Odika, P. C.; Duru, M. K. *IOSR J. Environ. Sci., Toxicol. Food Technol.,* **2013**, *4* (6), pp 33-37 (https://doi.org/10.9790/2402-0463337).
9. Xiao, R.; Wang, S.; Li, R.; Wang, J. J.; Zhang, Z. *Ecotoxicol. Environ. Saf*., **2017**, *141*, pp 17–24 (https://doi.org/10.1016/j.ecoenv.2017.03.002).
10. Verla, E. N.; Verla, A. W.; Enyoh, C. E. *Chem. Afr*., **2020**, *3*, pp 427-438 (https://doi.org/10.1007/s42250-020-00124-9).
11. Ahiarakwem, C. A.; Onyekuru, S. O. *J. Water Resour. Prot*., **2011**, *3* (9), pp 686-693 (https://doi.org/10.4236/jwarp.2011.39079).
12. Joseph, A.; Majesty, D.; Friday, U. *J. Ecobiotechnol*., **2019**, *11*, pp 1-5 (https://doi.org/10.25081/jebt.2019.v11.3866).
13. American Public Health, Association (APHA), American Water Works Association (AWWA), and Water Pollution Control Federation (WPCF), **1998**.
14. Standard Methods for the Examination of Water and Wastewater 19th Edition, American Public Health Association (APHA). Washington. DC, **2005**.
15. Enyoh, C. E.; Isiuku, B. O. *Chem. Afr*., **2020**, *3*, pp 1059-1071 (https://doi.org/10.1007/s42250-020-00171-2).
16. Mohammadi, A. A.; Zareib, A.; Majidic, S.; Ghaderpouryd, A.; Hashempoure, Y.; Saghif, M. H.; Mahmood, A. A.; Hosseingholizadehi, N.; Ghaderpoori, M. *MethodsX,* **2019**, *6* (1), pp 1642-1651 (https://doi.org/10.1016/j.mex.2019.07.017).
17. Bempah, C. K.; Ewusi, A. *Environ. Monit. Assess.*, **2016**, *188*, article nº 261 (https://doi.org/10.1007/s10661-016-5241-3).
18. Eze, V. C.; Onwukeme, V. I.; Enyoh, C. E. *Int. J. Environ. Anal. Chem*., **2020** (https://doi.org/10.1080/03067319.2020.1772778).
19. Wongsasuluk, P.; Chotpantarat, S.; Siriwong, W.; Robson, M. *Environ. Geochem. Health,* **2014**, *36*, pp 169–182 (https://doi.org/10.1007/s10653-013-9537-8).
20. Wu, B.; Zhao, D.; Jia, H.; Zhang, Y.; Zhang, X.; Cheng, S. *Bull Environ. Contam. Toxicol.*, **2009**, *82*, pp 405-409 (https://doi.org/10.1007/s00128-008-9497-3).
21. U.S. Environmental Protection Agency. Risk Assessment Guidance for Superfund, Volume 1, Human Health Evaluation Manual (Part A). USEPA Office of Emergency and Remedial Response. Washington (DC), **2010**.
22. Huang, M.; Zhou, S.; Sun, B.; Zhao, Q. *Sci. Total Environ,* **2008**, *405* (1-3), pp 54–61 (https://doi.org/10.1016/j.scitotenv.2008.07.004).

23. Bamuwamye, M.; Ogwok, P.; Tumuhairwe, V. *J. Environ. Pollut. Hum. Health,* **2015**, *3* (2), pp 24–30. Available at: http://pubs.sciepub.com/jephh/3/2/1/ [Accessed 10 March 2020].
24. Kamunda, C.; Mathuthu, M.; Madhuku, M. *Int. J. Environ. Res. Public Health,* **2016**, *13* (7), 663 (https://doi.org/10.3390/ijerph13070663).
25. Guerra, F.; Trevizam, A. R.; Muraoka, T.; Marcante, N. C.; Canniatti-Brazaca, S. G. *Sci. Agric.,* **2012**, *69* (1), pp 54–60 (https://doi.org/10.1590/S0103-90162012000100008).
26. Sultana, M. S.; Rana, S.; Yamazaki, S.; Aono, T.; Yoshida, S. *Cogent Environ. Sci.*, **2017**, *3* (1) (https://doi.org/10.1080/23311843.2017.1291107).
27. Tepanosyan, G.; Maghakyan, N.; Sahakyan, L.; Saghatelyan, A. *Ecotoxicol. Environ. Saf.*, **2017**, *142*, pp 257–265 (https://doi.org/10.1016/j.ecoenv.2017.04.013).
28. World Health Organization, WHO. Environmental Health Criteria 27, *Guidelines on studies on Environmental Epidemiology*. Joint Sponsorship UNEP/ILO/WHO. Geneva, **2003**.
29. Choudhary, S.; Ramteke, S.; Rajhans, K.; Sahu, P.; Chakradhari, S.; Patel, K.; Matini, L. *J. Water Resour. Prot*., **2016**, *8*, pp 12-19 (https://doi.org/10.4236/jwarp.2016.81002).
30. Adamu, G. A.; Sallau, M. S.; Idris, S. O.; Agbaji, E. B. Study of pollution level for drinking water quality assessment of Kafin-Chiri reservoir, Kano State, Nigeria. Proceedings of the 36th Annual International Conference of Chemical Society of Nigeria, Minna, Niger State, Nigeria, **2013**, pp 274-280.
31. Fallahzadeh, R. A.; Ghaneian, M. T.; Miri, M.; Dashti, M. M. *Environ. Sci. Pollut. Res.,* **2017**, *24*, pp 24790–24802 (https://doi.org/10.1007/s11356-017-0102-3).
32. Woke, G. N.; Babatunde, B. B.; Aleleye, W. *Global J. Environ. Sci*., **2013**, *12* (1), pp 41-47 (https://doi.org/10.4314/gjes.v12i1.5).
33. Loucks, D. P.; van Beek, E. Water Resources Planning and Management: An Overview. In: *Water Resource Systems Planning and Management*, Springer, Cham, **2017** (https://doi.org/10.1007/978-3-319-44234-1_1).
34. Gray, N. F. *Drinking Water Quality Problems and Solutions.* John Wiley and Sons, London, UK, **1994**, pp 43-63.
35. Garg, R. K.; Rao, R. J.; Uchchariya, D. U.; Shukla, G.; Saksen, D. N. *Afri. J. Environ. Sci. Technol,* **2010**, *4* (2), pp 61-76. Available at: https://www.ajol.info/index.php/ajest/article/view/56323 [Accessed 10 March 2020].
36. Obire, O.; Tamuno, D. C.; Womedo, S. A. *J. Appl. Sci. Environ. Mgt*., **2003,** *7* (1), pp 43 – 49 (https://doi.org/10.4314/jasem.v7i1.17164).
37. Beka, N. C.; Aga, T.; Eziashi, A. C. *The Pacific Journal of Science and Technology*, **2009**, *10* (2), pp 626-632. Available at: http://www.akamaiuniversity.us/PJST10_2_626.pdf [Accessed 10 March 2020].
38. Enyoh, C. E.; Isiuku, B. O. *Int. J. Environ. Anal. Chem.,* **2020** (https://doi.org/10.1080/03067319.2020.1773455).
39. Zhu, Q.; Wu, J.; Wang, L.; Yang, G.; Zhang, X. *Water, Air, Soil Pollut*., **2015**, *226*, article nº 429 (https://doi.org/10.1007/s11270-015-2680-3).
40. Alidadi, H.; Sany, S. B. T.; Oftadeh, B. Z. G.; Mohamad, T.; Shamszade, H.; Fakhari, M. *Environ. Health Prev. Med*., **2019**, *24*, article nº 59 (https://doi.org/10.1186/s12199-019-0812-x).
41. Abdullah, E. J. *Journal of Natural Sciences Research*, **2013**, *3* (8), pp 63-64 (https://core.ac.uk/download/pdf/234654183.pdf).
42. Eze, V. C.; Enyoh, C. E.; Ndife, C. T. *Chem. Afr*., **2020**, *4*, pp 127-136 (https://doi.org/10.1007/s42250-020-00194-9).
43. Isiuku, B. O.; Enyoh, C. E. *Environmental Advances,* **2020**, *2*, 100018 (https://doi.org/10.1016/j.envadv.2020.100018).
44. Enyoh, C. E.; Ohiagu, F. O.; Verla, A. W.; Verla, E. N. *Int. J. Environ. Anal. Chem.*, **2020** (https://doi.org/10.1080/03067319.2020.1807535).
45. Ahiarakwem, C. A.; Onyekwuru, S. O. *J. Water Res. and Prot*., **2011**, *3* (9), pp 686-693 (https://doi.org/10.4236/jwarp.2011.39079).

46. Wirnkor, V. A.; Ngozi, V. E.; Emeka, A. C.; Ebere, E. C. *International Journal of Advanced Scientific Research*, **2018**, *3*, pp 40-46. Available at: https://www.semanticscholar.org/paper/Water-pollution-scenario-at-river-uramurukwa-owerri-Wirnkor-Ngozi/7efe39a1164c734000544337268b37cd538bef48 [Accessed 10 March 2020].

Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 33, pp 71-88
doi: 10.30744/brjac.2179-3425.AR-20-2021

BrJAC

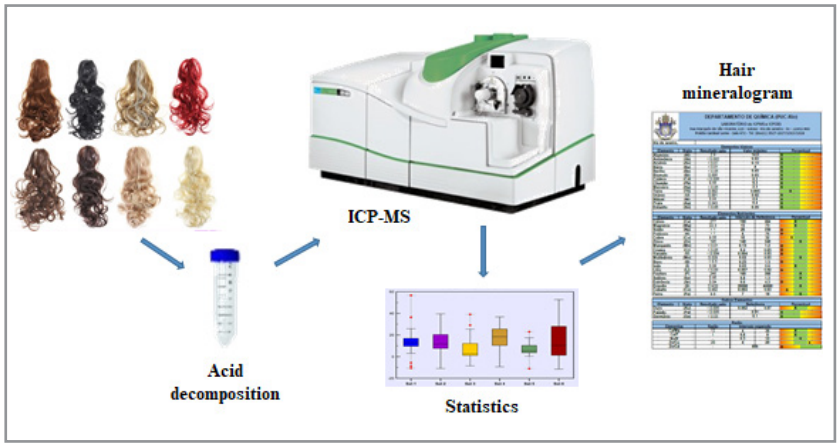
ARTICLE

Hair Mineralogram Analysis for Health Assessment: *Statistical Bias from Gender and Aesthetic Treatments*

Gabrielly Peregrino[1,2], Carlos G. Massone[2], Adriana Haddad Nudi[2], Tatiana Dillenburg Saint'Pierre*[2]

[1]*Instituto Federal do Rio de Janeiro (IFRJ), IFRJ Campus São Gonçalo, São Gonçalo, RJ, Brazil*

[2]*Pontifícia Universidade Católica do Rio de Janeiro (PUC-Rio), Rua Marquês de São Vicente, 225, Gávea, Rio de Janeiro, RJ, CEP 22451-900, Brazil*



The hair mineralogram is a complementary multielement analysis that provides information to aid in the diagnosis of a patient's health status; however, aesthetic treatments can affect the analysis results. This research aimed to identify standard patterns among mineralogram results and some variables, such as gender and the use of aesthetical treatments that can point out differences and causes of variation in elemental concentrations in hair. For this purpose, 151 hair samples were obtained from volunteers and analyzed by inductively coupled plasma mass spectrometry (ICP-MS). This work is pilot research, part of a project to encourage girls to the STEM area, called "Girls in Science", with financial support from the Brazilian Government. Mineralogram results were compared through statistical analysis. The results of natural hair indicate significant differences ($p<0.05$) between genders in the concentrations of Ca, Mg, Sr, and Mo, being higher in women. This behavior was related to the remodeling of minerals in bones, which is different between men and women. The metal concentration in natural hair from women was also compared among different skin colors and no significant differences were observed. Hair treatment, in contrast, has affected significantly the concentrations of many elements. Concentrations increased in hair submitted to dyeing only or with straightening, when compared to natural hair, especially for Ca, Mg, Sr, Ba, and Ni. These results confirm the recommendation of physicians to let the hair grow free of aesthetic treatments for at least 3 months before performing the mineralogram.

Keywords: Mineralogram, hair, ICP-MS, statistic, cosmetic treatment.

Cite: Peregrino, G.; Massone, C. G.; Nudi, A. H.; Saint'Pierre, T. D. Hair Mineralogram Analysis for Health Assessment: Statistical Bias from Gender and Aesthetic Treatments. *Braz. J. Anal. Chem.*, 2021, *8* (33), pp 71–88. doi: http://dx.doi.org/10.30744/brjac.2179-3425.AR-20-2021

Submitted 09 February 2021, Resubmitted 19 April 2021, Accepted 02 May 2021, Available online 08 June 2021.

INTRODUCTION

Nowadays, people are increasingly exposed to chemical substances, many of them being possible harmful to the health, although most people have little information about, or just ignore this fact. As well, the effects on the health of many products are not completely understood, mainly concerning the interactions among different substances. Besides, the lack of supervision by competent authorities and the use of low-quality raw materials have compounded this problem. This is especially true for cosmetic products, which can affect not only the consumers but also the professionals who apply them. Fortunately, it seems that most dyes employed in aesthetical treatment for hair are safe to the health [1].

Exposition to toxic elements can be identified by the analysis of hair, an exam called hair mineralogram, since these elements are excreted also by hair growing, besides other excretion routes. Better than urine analysis, which identifies only acute and recent exposure, hair mineralogram presents advantages also in comparison with blood analysis since elements are concentrated in hair and this is a non-invasive exam. In addition, hair samples are easier to collect and store, and can be employed for temporal monitoring, since hair has a growing rate of about 1 cm $month^{-1}$ [2–9].

The mineralogram is mainly employed by physicians to have an idea of the overall health condition of the patient, by the analysis of nutrient elements, as well as to identify health problems, such as those in thyroid and osteoporosis, that can be indicated by an imbalance in the concentration ratios of nutrient elements, mainly Ca, Mg, Na, K, and Fe. However, the main reason why the mineralogram is so few employed by physicians is that the interpretation of the results is still a challenge. Besides the high amount of data obtained with this exam, it is difficult to establish reference values, by the natural variations among different ethnic groups, genders, ages, food habits, etc [9–14].

Also, although hair has been employed for a long time as biopsy material for the determination of trace elements in the human body, there are also no consensus protocols for hair analysis. Before the 1980s, hair used to be analyzed by inductively coupled plasma optical emission spectrometry (ICP OES), then with the development of the much more sensitive inductively coupled plasma mass spectrometry (ICP-MS), new horizons for hair analysis have emerged. The problem is that, since it has allowed quantifying elements at trace levels, there was overvaluation of hair analysis, which triggered some misdiagnosis [15].

Then, as mentioned, besides the lack of a standardized reference method for hair analysis, the sample preparation procedure is also not a consensus. For example, some authors recommend a previous washing of the sample, while others alert for the possibility of removing endogenous substances by incorrect washing of the sample, which could interfere with the results [16–19]. As well, one requirement seems to be adopted: The analysis should be carried out in natural hair, it means, one should let the hair grow without permanent aesthetical treatments, such as dye or straightening, for at least 3 months before sample collection, since the products employed can incorporate or extract elements from the hair, hampering the interpretation of the results [20].

For these reasons, the objective of this pilot study was to evaluate a dataset of mineralogram results, employing statistical analysis, to identify distribution patterns and significant effects in the analysis results due to permanent aesthetical treatments, such as dyeing and/or straightening. This work was part of a project to encourage young women to sciences, in collaboration with a Rio de Janeiro state high school, and had financial support from the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq).

MATERIALS AND METHODS

Reagents

Water was ultra-purified by deionization (18 MΩ cm minimum resistivity) in a MilliQ system (Millipore, USA). Nitric acid (Vetec, São Paulo, Brazil) was purified by sub boiling bi-distillation in quartz still (Duo-PUR, Milestone, USA).

Analytical solutions were prepared with the multielemental standard solution Merck 23 (B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sr, and Zn at 1000 μg L^{-1} each one) and the monoelemental standard

solutions: As, Hg and Se 1000 µg L^{-1}, P 40 µg mL^{-1}, Be, I, Mo, S, Sb, and V 500 µg L^{-1}, U and Th 200 µg L^{-1}, all from Merck, Darmstadt, Germany. A 40 µg L^{-1} Rh solution (prepared from a 200 µg L^{-1} Rh solution VHG, Manchester, USA) was added online to all other solutions (blank, analytical solutions, and samples), as an internal standard. Nitric acid 10% v/v was added to all blank and analytical solutions to equalize the acid concentration of the sample solutions.

Samples

Samples were collected from 151 volunteers randomly selected, which answered a form with information about ethnic group, gender, age, food and sports habits, health treatments, and kind of hair aesthetical treatment when that was the case. No previous selection of variables was done. The volunteers were advised of the objective of the work before agreeing in giving the hair sample.

Samples were from 116 women and 35 men, from 4 to 88 years old. Table I summarizes the main characteristics of the volunteers.

Table I. Main characteristics of the volunteers

	Women	**Men**
Total	**116**	**35**
Ethnic group:		
White	86	29
Black	9	1
Brown	21	5
Hair treatment:		
Natural	31	34
Dye	45	1
Straightening	15	0
Dye + straightening	25	0

The hair samples were cut from the occipital region, close to the scalp, with a stainless steel scissor cleaned with ethanol. About 3 cm from the scalp were cut and the length was discharged. The samples were stored in identified plastic bags until analysis.

A certified reference sample of hair (NCS DC73347a) from China National Analysis Center for Iron and Steel (2015) was employed for checking the accuracy of the method.

Sample preparation

Sample preparation was adapted from the procedure developed by Miekeley et al. [21]. About 500 mg of the sample were weighted in 50 mL propylene flasks, cut into smaller pieces, and washed alternately with ultra-pure water and acetone, by standing for 10 min in an ultrasonic bath with each solvent. This washing procedure was repeated 2 more times and then, the samples were dried overnight in an oven at 60 ºC. Aliquots of 250 mg were weighted in an analytical balance (Adventurer, Ohaus, USA), added 2.5 mL HNO_3 conc. and stand overnight at room temperature. Then, the flasks were heated on a hot plate at 70 ºC for 1 h. After achieving room temperature, water was added to 25 mL final volume and the resulting solutions were analyzed by ICP-MS.

Analysis by ICP-MS

The ICP-MS spectrometer used for the determination of the elements was a quadrupole, model ELAN DRC II from PerkinElmer Sciex, USA. The operating conditions were optimized according to the

manufacturer's Daily Performance procedure and are presented in Table II. The monitored isotopes were chosen concerning the higher abundance and absence of spectral interferences.

Table II. Operational parameters used in the measurements by ICP-MS

Radio frequency power	1200 W
Plasma Ar flow rate	15.0 L min^{-1}
Auxiliary Ar flow rate	1.0 L min^{-1}
Nebulizer Ar flow rate	0.90 – 1.10 L min^{-1}
% of oxides (CeO^{+})	< 3%
% of bivalent ions (Ba^{2+})	< 3%
Background in *m/z*=220	1 cps
Dwell time	60 ms
Scan per reading	1
Number of replicates	3

Assessment of method accuracy

Figures of Merit

The analytical curves were constructed at different concentration ranges for different groups of analytes, according to the methodology developed in the Labspectro, by Carneiro et al. [22]. The correlation coefficient (R) values, given by the software of the spectrometer, above 0.99 have been accepted. The portion of the total variance explained by the regression was considered significant by ANOVA and F tests [23,24].

The limits of detection and quantification were calculated as 3 or 10 times, respectively, the standard deviation of 10 concentration measurements in the blank solution.

The reference material of hair was used to check for the accuracy of the method, by calculating the percentage of the obtained values in relation to the certified concentrations. For the purpose of this work, recovery values are considered acceptable [25].

Statistical analysis

The concentrations obtained from each element and the information filled out in the form by each volunteer were added to a spreadsheet in Microsoft Excel. The minimum, maximum, mean, median, and standard deviations of the elemental concentrations in hair of women and men measured in this work are presented in Table S1 (Supplementary Material).

The elements determined were divided into two groups, also following the work by Carneiro et al. [22], and the statistical analysis will be presented using the same division: Essential elements and others: B, Ca, Co, Cr, Cu, Fe, I, Mg, Mn, Mo, P, S, Se, Sr, V, and Zn; and toxic elements: As, Ba, Be, Bi, Cd, Hg, Ni, Pb, Sb, Th, and U.

The statistical treatment of the data was started by the Shapiro-Wilk normality test [26]. All statistical analyses were executed in R software and related packages [27–29]. Statistical significant assessments consider a 95% probability ($p < 0.05$). Differences among groups were determined based on the Man Whitney test for single comparisons (e.g. male versus female). In multiple comparisons, as in the different types of hair treatment, the Kruskal-Wallis test (KW) was performed. When Kruskal-Wallis test pointed significant differences among groups the Wilcoxon-Mann-Whitney test (WMW) was performed as *post hoc* method. The KW test indicates the presence or absence of difference among groups but, when the difference is identified, it does not identify in which groups the differences occur. Therefore, *post hoc*

testing (Wilcoxon-Mann-Whitney) is necessary to identify intra-group differences. In the *post hoc* method, the p (probability) values were adjusted according to Holm correction method to avoid type I error, common in multiple comparisons.

RESULTS AND DISCUSSIONS

Assessment of method accuracy

The method employed for the multielemental determination in hair samples was based on previous works of our research group [21,22] and is routinely employed at Labspectro, then figures of merit have already been determined. In this work, for checking the accuracy of the method, instrumental detection limits (LOD, μg L^{-1}) and quantification limits (LOQ, μg g^{-1}) of the method, for each element, were determined for comparison with values obtained previously, as well as the analysis of a certified reference material. The correlation coefficients of the analytical curves, not shown here, were better than 0.997 for all studied elements. The limits obtained in this work were in the same order or better than most of those obtained in previous works [21,22] and are shown in Table III, as well as the results for the certified sample, with the percentage recoveries related to the certified concentrations. The measured concentrations of all studied elements were from 80 to 114% of the certified values, confirming the accuracy of the method.

Table III. Instrumental detection limits (LOD) and quantification limits of the method (LOQ), and certified and measured concentrations obtained for the certified hair sample (average ± standard deviation, n=3). Recoveries are the percentage of obtained concentrations relative to the certified values (Rec).

Monitored isotope	LOD (μg L^{-1})	LOQ (μg g^{-1})	Certified (μg g^{-1})	Measured (μg g^{-1})	Rec (%)
^{75}As	3 x 10^{-2}	9.7 x 10^{-3}	0.28 ± 0.05	0.29 ± 0.01	105.2
^{11}B	2	5.2 x 10^{-1}	2.9 ± 0.5	2.4 ± 0.2	82.1
^{138}Ba	9 x 10^{-2}	3.0 x 10^{-2}	11.4 ± 0.6	10.3 ± 0.3	90.3
^{9}Be	8 x 10^{-2}	2.8 x 10^{-2}	0.11 ± 0.07	0.10 ± 0.01	94.1
^{209}Bi	2 x 10^{-3}	8.0 x 10^{-4}	0.021 ± 0.002	0.020 ± 0.001	96.4
^{44}Ca	4 x 10^{1}	1.5 x 10^{1}	1450 ± 20	1239 ± 19	85.5
^{114}Cd	5 x 10^{-3}	1.5 x 10^{-3}	0.07 ± 0.01	0.07 ± 0.01	97.3
^{59}Co	5 x 10^{-3}	1.6 x 10^{-3}	0.045 ± 0.009	0.041 ± 0.010	90.8
^{53}Cr	2 x 10^{-1}	6.2 x 10^{-2}	0.41 ± 0.12	0.47 ± 0.01	114.0
^{65}Cu	8 x 10^{-2}	2.7 x 10^{-2}	14.3 ± 1.6	13.5 ± 1.2	94.3
^{57}Fe	8	2.7	36 ± 5	31.0 ± 1.2	85.0
^{202}Hg	7 x 10^{-2}	2.3 x 10^{-2}	0.67 ± 0.1	0.64 ± 0.1	96.0
^{127}I	1 x 10^{-1}	3.6 x 10^{-2}	0.8 ± 0.2	0.7 ± 0.1	81.8
^{24}Mg	4 x 10^{-1}	1.3 x 10^{-1}	140*	126 ± 6	90.1
^{55}Mn	3 x 10^{-2}	9.9 x 10^{-3}	2.0 ± 0.3	1.8 ± 0.1	90.0
^{98}Mo	1 x 10^{-2}	3.7 x 10^{-3}	0.17 ± 0.03	0.16 ± 0.01	92.1
^{60}Ni	3 x 10^{-2}	1.0 x 10^{-2}	0.43 ± 0.12	0.40 ± 0.01	91.9

Table III. Instrumental detection limits (LOD) and quantification limits of the method (LOQ), and certified and measured concentrations obtained for the certified hair sample (average ± standard deviation, n=3). Recoveries are the percentage of obtained concentrations relative to the certified values (Rec). (Continuation)

Monitored isotope	LOD (μg L^{-1})	LOQ (μg g^{-1})	Certified (μg g^{-1})	Measured (μg g^{-1})	Rec (%)
^{31}P	2 x 10^{1}	7.4	140 ± 20	139 ± 3	99.6
^{208}Pb	3 x 10^{-2}	9.6 x 10^{-3}	5.7 ± 0.5	5.4 ± 0.3	94.8
^{34}S	1 x 10^{3}	4.1 x 10^{2}	41900 ± 1100	37652 ± 2125	89.9
^{121}Sb	7 x 10^{-3}	2.4 x 10^{-3}	0.065*	0.073 ± 0.002	112.3
^{82}Se	7 x 10^{-1}	2.3 x 10^{-1}	0.58 ± 0.12	0.64 ± 0.03	110.9
^{88}Sr	1 x 10^{-2}	3.6 x 10^{-3}	7.7 ± 0.4	6.2 ± 0.1	80.5
^{232}Th	4 x 10^{-2}	1.3 x 10^{-2}	0.064 ± 0.011	0.061 ± 0.001	95.8
^{238}U	3 x 10^{-3}	1.0 x 10^{-3}	0.099 ± 0.015	0.092 ± 0.001	92.7
^{51}V	2 x 10^{-2}	8.2 x 10^{-3}	0.50 ± 0.18	0.40 ± 0.01	86.3
^{66}Zn	8 x 10^{-1}	2.7 x 10^{-1}	137 ± 9	127 ± 5	92.7

*reference value, no uncertainty is given.

Statistical analysis

Verifying differences

Gender: Evaluating natural hair from men and women

In the first moment, all hair samples would be compared to verify differences promoted by different aesthetic treatments; however, only one male volunteer presented aesthetic hair treatment. Thus, the idea arose to evaluate whether variations due to gender affect the mineralogram results. For this, mineralogram of men and women, all with natural hair, were compared, and the sample from the only man with dyeing treatment was excluded.

As mentioned, one of the objectives of this work is to identify significant distribution patterns among the mineralogram results dataset, and then, initially, only natural hair samples were evaluated. Some differences were observed between women and men for nutrient and toxic elements. Gender differences are exposed in Figure 1.

As observed in Figure 1, the elements Ca, Mg, Sr, and Mo showed significant differences ($p<0.05$) between gender, and the concentrations of all of them were higher in women than in men, all with natural hair. It is expected that Ca, Mg, and Sr, which are in the same group IIA in the periodic table, have similar properties and, in this case, it is also expected that these elements have similar or correlated behavior in hair. Mg and Sr are related to the remodeling of Ca minerals in bones, which process has different rates between men and women, mainly during puberty and old age. The similar behavior of these elements, for both men and women, is confirmed by the strong correlations, presented in Figure 2. Then, the drop in the concentration of Ca and Mg happens concomitantly and the ratio between these elements remains practically steadied. Although, when the Ca/Mg ratio is evaluated, there were no significant differences between men (from 3.87 to 28.00) and women (from 1.69 to 30.54), according to the Man Whitney test ($p=0.39$). This indicates that the ratio should be also checked in addition to the absolute values as a parameter for evaluating the patient health.

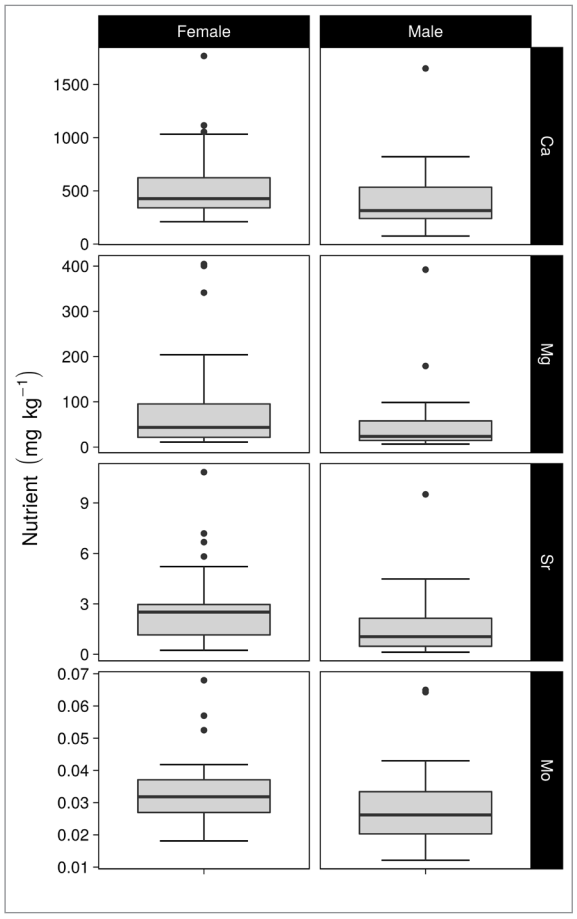


Figure 1. Statistical differences ($p<0.05$) observed between men (n=34) and women (n=31), all with natural hair.

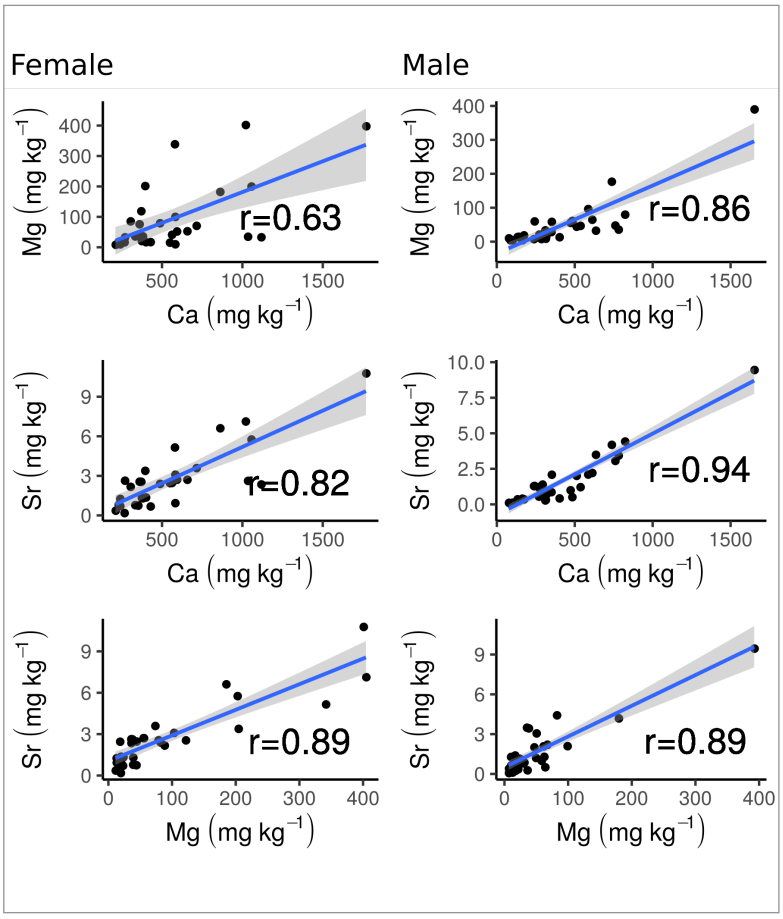


Figure 2. Correlations for Ca, Mg, and Sr in female (n=31) and male (n=34) samples, all with natural hair.

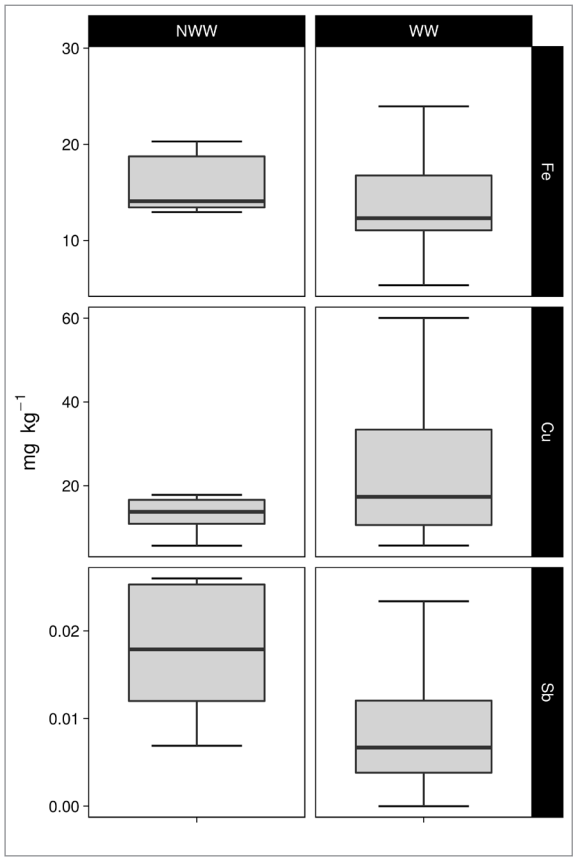
Our findings corroborate most works [6,10,16,30–34]. However, some authors also attribute the concentrations of these elements in hair to environmental, food, and socioeconomic factors. Szynkowska et al. [30] have attributed the differences in Sr concentrations as result of cosmetic treatment, but treated hair samples were excluded in our study, to minimize bias due to it. Also, few studies have found differences in other elements considering gender and each author attributes different causes to it. Luo et al. [35] reported great variability of mean concentrations of elements in hair from different countries and attributed it to environmental exposure, ethnic and geographic origin, and dietary habits, indicating that any country should determine its reference ranges. Higher concentrations of Au or Ni in women have been attributed to the use of jewels, while other metals higher in men were attributed to occupational exposure [31,36–38]

In this research, the reason for higher concentrations of Mo in women than in men could not be elucidated. It is important to mention that the differences were not due to outliers, since the differences are kept by excluding them.

Ethnic group: Evaluating natural hair from white and non-white women

At that moment, we asked ourselves whether differences among ethnic groups, which reflect differences in skin color and hair type, might also promote differences in the mineralogram results. Concerning skin color, we asked the volunteers to declare themselves as white, brown, or black; however, it can be said that, in the Brazilian people, there are no clear limits, but instead, a skin color gradient.

Then, we decided to check whether different ethnic groups present differences in the mineralogram results, by analyzing only samples from women with natural hair. Among these volunteers, most (21) declare themselves as white, 4 as brown, and 3 as black. Because of the skin color issues, already described, and few samples of each group, the differences in the mineralogram results were checked only for 2 groups, white (WW) and non-white women (NWW). Few elements (Cu, Fe, and Sb) presented statistical differences ($p<0.05$) for white and non-white women. However, when we apply the Grubbs test in 3 samples for Cu (285, 153, and 125 mg kg^{-1}) and 1 sample for Fe (182 mg kg^{-1}), all from white women, these values are considered outliers. By excluding them, the concentration ranges of these elements are, respectively, Cu: from 5.7 to 60.0 mg kg^{-1} for WW and from 5.7 to 34.3 mg kg^{-1} for NWW; Fe: from 5.3 to 23.9 mg kg^{-1} for WW and from 12.9 to 35.6 mg kg^{-1} for NWW. In this case, by excluding the outlier bias, there are no differences for Cu and Fe between women from different ethnic groups. The box plots for the concentration distribution of these elements are presented in Figure 3. The outliers were omitted for better range visualization.



Concerning Sb, two outlier values were identified for the group of non-white women (0.052 and 0.030 mg kg^{-1}). Differently from the results found for Cu and Fe, even excluding the outliers, the difference in Sb concentration between WW and NWW is still significant ($p<0.05$), although the concentration ranges are similar (NWW: from 0.007 to 0.026 mg kg^{-1} and WW: from below 0.0024 (LOD) to 0.023 mg kg^{-1}), as evidenced in Figure 3. Antimony is normally associated with pollution due to traffic, since it is a component of alloys employed in vehicle parts, such as breaks and batteries. It has some uses in medicine and other applications; however, we could not find a reason for NWW have higher concentrations of this element than WW in the hair mineralogram. It could be that social-economic differences are responsible for potentially higher exposure to pollution; however, unfortunately, social-economic data were not collected in this research.

Figure 3. Statistical differences ($p<0.05$) observed between white (WW, n=24) and non-white (NWW, n=7) women, all with natural hair. Outliers were excluded.

It is important to mention that, differently than observed in the study concerning differences between genders, in this case, we found outliers in only one of the groups compared and, by excluding them, there are no differences between groups. Maybe these values could be originated from a health problem not recognized in the questionnaires of the volunteers. These samples will be reanalyzed and the volunteers contacted after further analysis.

Aesthetic treatment: Evaluating natural and treated hair from all women

Natural and treated hair mineralogram results were compared only for women, to avoid gender bias observed herein. Also, all women samples were included, except for the 4 mentioned outliers found for Cu, Fe, and Sb. The elements that have presented differences in hair concentrations, when comparing natural

and hair with different aesthetical treatment (dyeing, straightening, or both), for all women, are presented in Figure 4. In this case, the outliers were included in the statistical analysis, since they can be caused by the aesthetical treatments, but omitted in the graphs for better range visualization. It is important to mention that there is a huge amount of different products for hair treatment available on the market. Even products not regulated by health surveillance can be found in Brazilian markets, which can pose risks to the health of consumers. Information about the brand or composition of the hair treatment was not requested for volunteers, since most of them do not know this information, especially when applied in beauty salons.

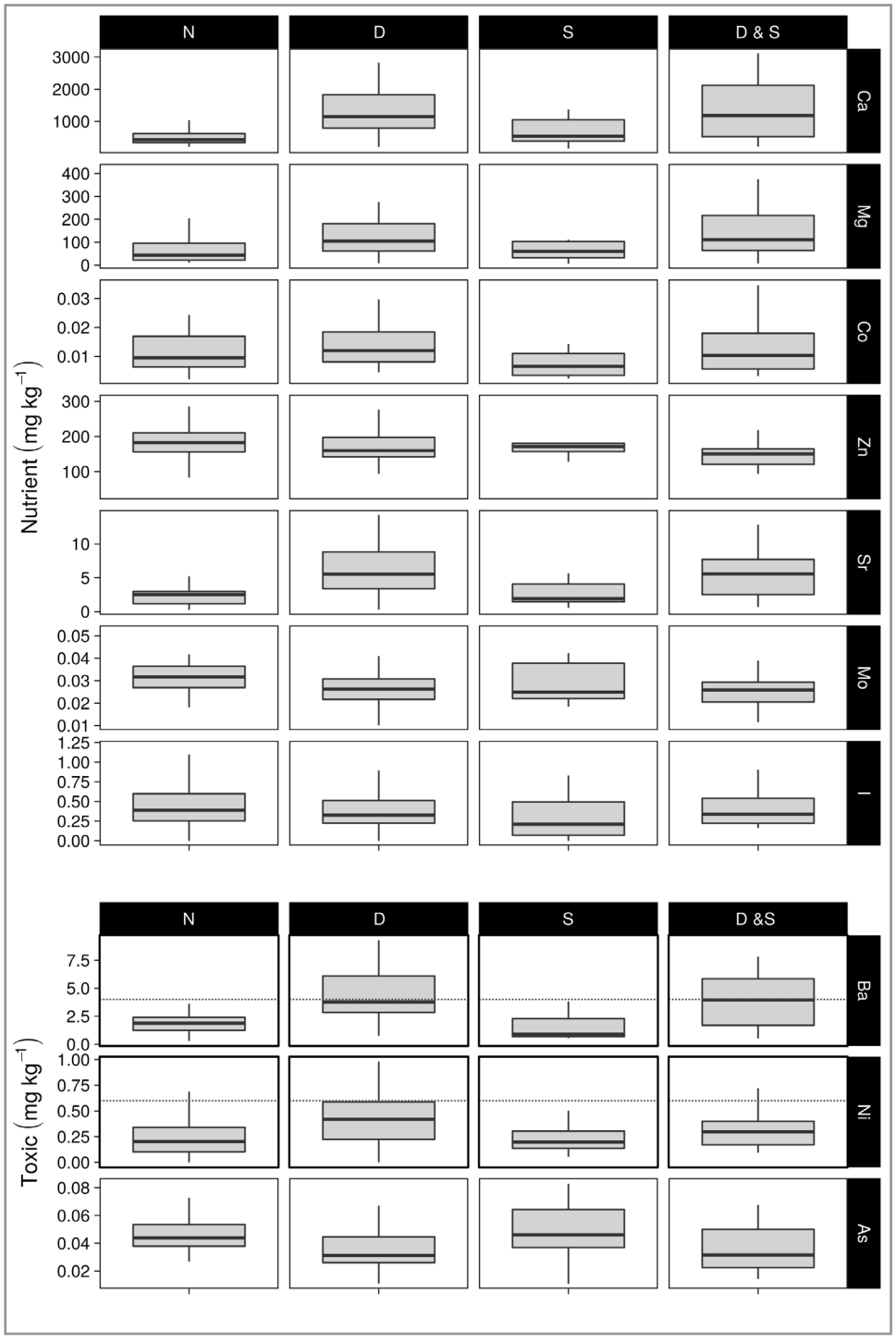


Figure 4. Statistical differences ($p<0.05$) observed among natural hair (N, n=27) and those with aesthetical treatment (D: dyeing n=45, S: straightening n=15, D & S: dyeing + straightening n=25) for all women (n=112) hair samples. Outliers were omitted for better range visualization. The dotted lines indicate the upper limits for toxic elements.

Significant differences ($p<0.05$) were observed for many elements, most of them presented increased concentrations in hair submitted to dyeing only (D) or with straightening (D+S) when compared to natural hair (N). This behavior was especially observed for the earth alkaline elements, Ca, Mg, Sr and Ba, and also for Ni. Surprisingly, the concentrations of Mo and As have decreased in hair submitted to treatments including dyeing, when compared to natural hair. For As, although not significant, a slight increase in concentration was observed in straightened hair. In fact, straightening only (S) seems not to affect significantly the mineralogram results, when compared to natural hair, only for I and Co, whose concentrations have decreased with this aesthetical treatment. Zn concentration has also decreased with straightening, but only when associated with dyeing. It can be that straightening treatments employ chemical substances to break the hair protein bounds, while dyeing treatments require extracting substances from the hair and adding colorful others, to allow changing the natural color, which can be more aggressive, concerning the hair composition, than straightening.

Still, concerning the essential elements, the lower and upper limits considered in this work were not indicated in the figure, since it would result in too much information, difficult to visualize. The common ranges of essential elements in the hair mineralogram of the Brazilian people were already established in the previous research of our group [22] and are present in Table IV. It is important to mention that, for the earth alkaline elements, about 22% for Ca, 39% for Mg, and 16% for Sr, of the samples with natural hair (only for women) presented concentrations above the upper limits, which are 684, 73 and 4.3 mg g^{-1}, respectively. The percentage of samples with concentrations above the upper limit increases to more than 71%, for Ca and Mg, and more than 64% for Sr, of the treated hair including dyeing (D or D+S). For Ca and Sr, the straightening also increased their concentration (not for Mg), but only about 10% more than those observed for natural hair. Among these elements, just a few samples presented concentrations below the lower limits (about 10%) and no tendency was observed for the different treatments. The other essential elements that presented statistical differences ($p<0.05$) among the hair treated or not, are Co, I, Zn, and Mo. For Co, about 6% of the natural hair samples presented values above the upper limit, which are increased to about 16% when D or D+S were applied. No difference was observed for S compared to N hair and less than 10% of the total female samples were below the lower limit. For I and Zn, the opposite effect was observed, about 42% and 32%, respectively, of the women with N hair, presented I and Zn concentration above the upper limits, and these numbers decrease to less than 30% and 25% respectively, above the limit for treated hair. Indeed, the Zn concentrations were below the lower limit for 10% of N and up to 40% for D+S hair. Decreasing in concentration was observed also for Mo, being about 15% of the N samples, 24% of D, 27% of S, and 32% of D+S samples, with concentrations below the lower limit. For Mo, less than 10% of the samples presented concentrations above the upper limits.

Table IV. Reference ranges for nutrient elements and upper limits for toxic elements employed in this work. The values were established in previous work by our research group [22].

Element	Lower limit (μg g^{-1})	Upper limit (μg g^{-1})	Element	Upper limit (μg g^{-1})
	Nutrient		**Toxic**	
B	NE	0.3	**As**	0.15
Ca	190	684	**Ba**	4.0
Co	0.003	0.03	**Be**	NE
Cu	10	32	**Bi**	0.03
Fe	7	18	**Cd**	0.3
I	0.05	0.6	**Cr**	0.3

Table IV. Reference ranges for nutrient elements and upper limits for toxic elements employed in this work. The values were established in previous work by our research group [22]. (Continuation)

Element	Lower limit (μg g^{-1})	Upper limit (μg g^{-1})	Element	Upper limit (μg g^{-1})
	Nutrient		Toxic	
Mg	13	73	**Hg**	2.3
Mn	0.15	1.2	**Ni**	0.06
Mo	0.02	0.05	**Pb**	9.3
P	161	257	**Sb**	0.03
S	39965	46000	**Th**	0.005
Se	0.8	1.5	**U**	0.02
Sr	0.6	4.3		
V	0.004	0.03		
Zn	140	239		

NE: not established

Still evaluating the limits for essential elements, it was interesting to observe that, among those that did not present statistical differences between natural and treated hair, some presented concentrations above the upper limits, such as Fe (from 27 to 50% of the samples) and S (from 24 to 40%), while others presented concentrations below the lower limits, such as B (from 56 to 73% of the samples), P (from 73 to 88%) and Se (from 28 to 72%). Men with natural hair presented more or less similar tendencies than women with natural hair, except for those elements with statistical differences between genders, as presented above.

Concerning toxic elements, from the 11 elements quantified, only As, Ba and Ni presented significant differences ($p<0.05$) in treated hair when compared to natural hair. A worrisome observation was the number of treated hair samples whose Ba and Ni concentration exceeded the upper limits. The dotted lines in Figure 4 indicate the upper limits for Ba (4 mg g^{-1}) and for Ni (0.6 mg g^{-1}). For As, only 2 samples presented values above the upper limit (0.15 mg g^{-1}) and were considered outliers. All the others had As concentrations below the reference value employed in this work. For this reason, the upper limit was not highlighted in the figure. However, when analyzing the values obtained for Ba and Ni, it was interesting to observe that about 45% and 30%, respectively, of the hair samples with dyeing, exceeded the upper limits. It corresponds to 20 samples for Ba and 14 for Ni among the 45 hair samples with dyeing. Also, among the hair with dyeing and straightening (n=25), 56% (n=14) of the samples presented Ba concentration above the upper limit, while only 12% (n=3) for Ni. Among the other toxic elements, which did not present statistical differences among treated and non-treated hair, only a few samples have surpassed the upper limits for some elements and were considered outliers.

Few works are reporting the mineral composition of hair treated with aesthetical products. Chojnacka et al. [39] also compared naturally colored hair with those that were artificially colored. They have found that colored hair contained more Sr, Ba, Ca, Mg, W, Mo, Ag, and Mn than in natural one. Also, the same authors reported lower V, Zr, Sb, Pb, As, Si, K, and Hg concentrations in colored hair. Moreover, they have also compared and found differences in the elemental composition of hair samples with different natural colors. However, they analyzed a total of 83 people, male and female, smokers and non-smokers, from children to elderly, with natural (5 different colors) and colored hair. Besides having too many variables, it is supposed that they analyzed few individuals of each hair color.

Massadeh et al. [40] also pointed out differences in the composition of natural and dyed hair, besides other variables, such as age and smoking habit. They have found higher concentrations for some elements, mainly heavy metals, such as Cd and Pb, and also Cu, Fe, and Zn, in dyed hair when compared to non-dyed ones. Differently from our results, they observed lower Ca concentrations in dyed than in non-dyed hair, but they also mention that the differences can be attributed to other variables, such as environment, food habits, and use of medicine. It reinforces the idea that the patient should let the hair grow up for, at least 3 months, before collecting the sample for the mineralogram exam, to exclude one more variable to the results, making more precise the diagnosis.

Verifying grouping

Cluster analyses based on Euclidean Distance and in Ward's method were applied to all women data, including the kind of aesthetic treatment, ethnic group, and age. This statistical evaluation allowed to segregate the samples (n=116) into 3 groups (Figure 5). It was interesting the relation of the 3 groups with age, shown in the density distribution graph in the upper left corner of Figure 5. Although the age is not normal and homogeneously distributed among the groups, by comparing the median ± sd of the age, G1, G2, and G3 could be related to younger (24 ± 13, n=28), middle-age (34 ± 16, n=9), and older (52 ± 16, n=79) women, respectively.

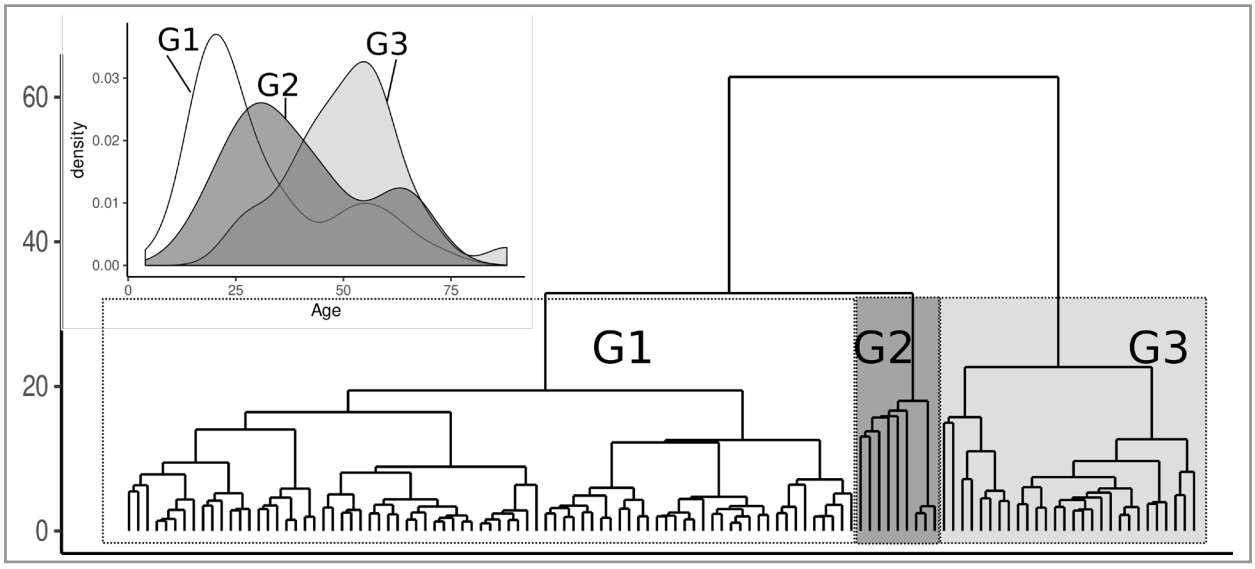


Figure 5. Cluster-based in Euclidean distance and Ward's Method applied to all women group. The density distribution graph is in detail.

Corroborating with the discussion in the "*Ethnic group"* item, the skin color was not relevant for clustering the data, since non-white women (NWW) were more or less equally distributed among groups, about 25% (n=20) in G1, 22% (n=2) in G2, and 28% (n=8) in G3. On the other hand, the dyeing hair treatment was not homogeneously distributed among groups, as discussed below.

The elemental concentrations determined in the 3 groups are presented in the box plot graphs in Figure 6. Only the elements that presented significant differences ($p<0.05$) in concentration among groups are presented, besides the distribution of hair treatment for each group in the lower-left corner of Figure 6.

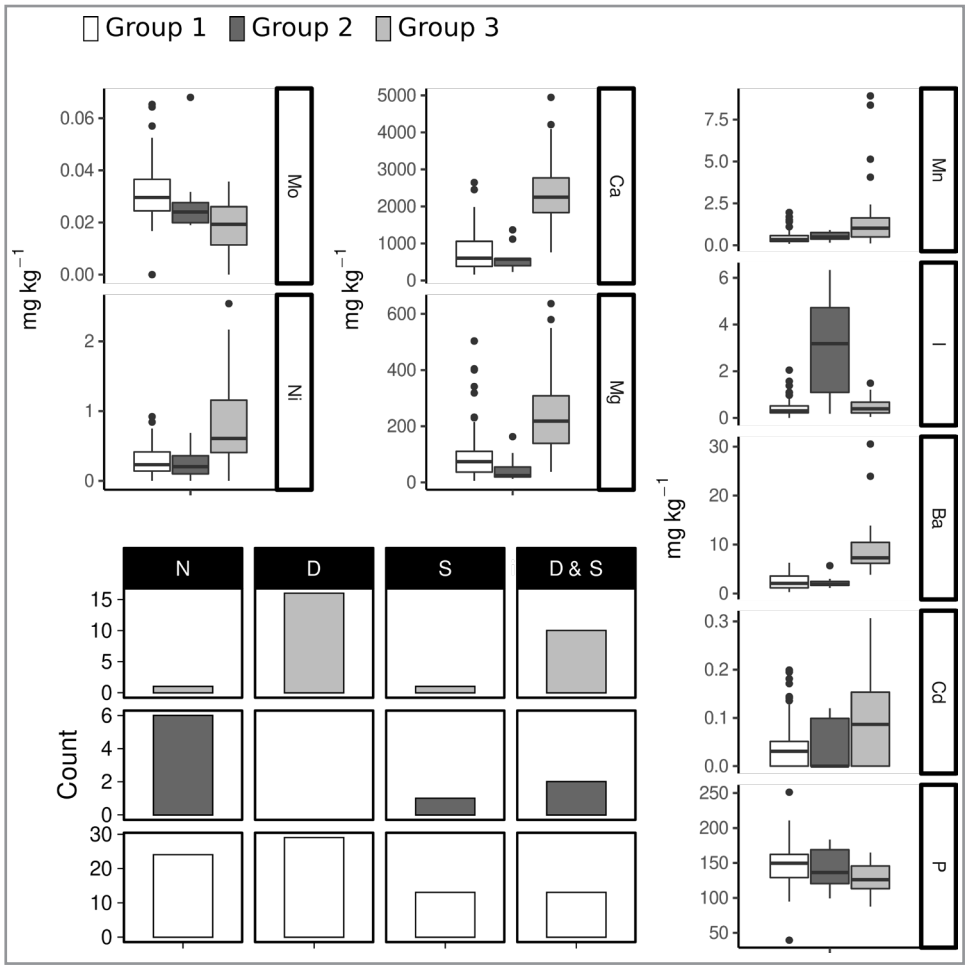


Figure 6. Boxplot of elemental concentrations determined in the 3 groups segregated in the dendrogram. The number of samples with each aesthetical treatment is in detail. D: dyeing, S: straightening, D & S: dyeing + straightening.

By analyzing Figures 5 and 6, it can be said that G3 is characterized by older age women with dyed hair (93%) and higher elemental concentrations, many of them essential. G1 and G2 are younger and middle-age women, with lower concentrations of most elements, when compared to G3 (except Mo and P). These two groups differ, one from each other, mainly by iodine concentration, being those for G2 much higher than for the other groups. It is important to mention that, although the clustering analysis indicates a relationship between the higher concentrations of elements in hair and older age, this correlation is not linear, at least for essential elements, since they are rather related to changes due to life cycles, mostly ruled by hormones. However, concerning toxic elements, it can be related to cumulative effects, since most of them are known for accumulating in body tissues over long periods of exposure. Also, as already mentioned, the hair treatments affect the mineralogram results, and then, a study concerning age should be repeated only for natural hair and with a larger number of samples.

Concerning the aesthetical treatment, almost all women in G3, those older and with the highest metal concentration, have their hair dyed, as expected, while in G2, whose median age is 34 years old, women with natural hair predominate. This group is more related to natural hair than to age, since G2 is distributed over a wide age range, combining the characteristics, in terms of metals concentration, of young and older women. In G1, the group of younger women and lower concentrations of most elements, many women have dyed hair, but many also have natural hair. Differently from G3, the high percentage of dyed hair in G1 (53%) is rather attributed to fashion than to white hair, but even that, present a lower concentration of metals, indicating that it is not possible to attribute a direct relationship between dyeing hair and any tendency in metals concentration.

CONCLUSION

This study fulfilled the main objective of involving high school students of a public school from the metropolitan region of Rio de Janeiro in scientific research conducted by a group of mostly female researchers/professors. The study evaluated the factors that affect the results of the capillary mineralogram, such as gender and use of aesthetic treatment. Although this study was carried out on a pilot scale, the variation between men and women, for Ca, Sr, and Mg, was observed, as well as that these elements must be evaluated by the concentration and by the relationship between them, concomitantly.

The main difference between natural hair and hair with aesthetical treatments was observed to the earth alkaline elements, Ca, Mg, Sr, and Ba, with increased concentrations in hair submitted to dyeing. The toxic elements As, Ba, and Ni also presented significant differences in treated hair when compared to natural hair. However, it was interesting to note that the treatments involving only straightening have significantly affected just a few elements (I and Co). It is important to reinforce that, even knowing these findings, it is difficult to preview the effects of any specific aesthetic treatment in any individual. Then, this work corroborates the doctors' recommendation, that the patient should let the hair grow up for, at least 3 months, before collecting the sample for the mineralogram exam.

It is also noteworthy that the sampling complexity must be better evaluated in future studies. Throughout this research, to eliminate possible interference in statistical evaluations, many samples were removed, such as those for women undergoing aesthetic treatment when comparing whites and non-whites. Despite this procedure, the presence of outliers remains and prejudiced statistical evaluation.

Cluster analysis performed herein was able to indicate a relationship between the higher concentrations of elements in hair and older age, not achieved from univariate analysis. This relationship is related to changes due to life cycles, mostly ruled by hormones, and is not linearly related to age values.

The limitations presented herein are intrinsic to pilot research but guide future studies and other researches to the ideal minimum number of samples or to limit a certain class during sampling. As the samples obtained were donated, the distribution of samples between the different classes is discovered after sampling and, perhaps, several sampling efforts by different classes is a more advantageous strategy than a generalist sampling.

Acknowledgements

The authors would like to thank to Gabrielle da Silva Ribeiro, Maria Eduarda Sousa Amaral, Isabelle Cunha da Silva and Pedro de Andrade Vasconcelos for the samples collection and to Rafael C. C. Rocha for the technical support. This study was financed in part by the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES) – Brazil (finance code 001) and to "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq), "Edital Meninas nas Ciências" [Project 20242/2013-9]. T.D.S thanks to CNPq and FAPERJ for the scholarships.

Compliance with Ethical Standards

The volunteers were advised of the objective of the work and the ethical procedures, before agreeing in giving the hair sample.

Conflicts of interest

The authors do not have any conflict of interest concerning the involved institutions or the Brazilian financial agencies.

REFERENCES

1. Nohynek, G. J.; Fautz, R.; Benech-Kieffer, F.; Toutain, H. *Food Chem. Toxicol*., **2004**, *42*, pp 517–543 (https://doi.org/10.1016/j.fct.2003.11.003).
2. Dongarrà, G.; Varrica, D.; Tamburo, E.; D'Andrea, D. *Environ. Toxicol. Pharmacol.*, **2012**, *34*, pp 160–169 (https://doi.org/10.1016/j.etap.2012.03.005).

3. Martorell, I.; Nadal, M.; Vilavert, L.; Garcia, F.; Schuhmacher, M.; Domingo, J. L. *Trace Elem. Electrolytes*, **2015**, *32*, pp 43–51 (https://doi.org/10.5414/TEX01346).
4. Sahoo, S. K.; Žunić, Z. S.; Kritsananuwat, R.; Zagrodzki, P.; Bossew, P.; Veselinovic, N.; Mishra, S.; Yonehara, H.; Tokonami, S. *J. Environ. Radioact.*, **2015**, *145*, pp 66–77 (https://doi.org/10.1016/j.jenvrad.2015.03.020).
5. Samanta, G.; Sharma, R.; Roychowdhury, T.; Chakraborti, D. *Sci. Total Environ*., **2004**, *326*, pp 33–47 (https://doi.org/10.1016/j.scitotenv.2003.12.006).
6. Senofonte, O.; Violante, N.; Caroli, S. *J. Trace Elem. Med. Biol*., **2000**, *14*, pp 6–13 (https://doi.org/10.1016/S0946-672X(00)80017-6).
7. Kempson, I. M.; Lombi, E. *Chem. Soc. Rev.*, **2011**, *40*, pp 3915-3940 (https://doi.org/10.1039/c1cs15021a).
8. Menezes, M. Â. B. C.; Maia, E. C. P.; Albinati, C. C. B.; Sabino, C. V. S.; Batista, J. R. *J. Radioanal. Nucl. Chem*., **2004**, *259*, pp 81–86 (https://doi.org/10.1023/B:JRNC.0000015810.22775.72).
9. Wołowiec, P.; Michalak, I.; Chojnacka, K.; Mikulewicz, M. *Clin. Chim. Acta*, **2013**, *419*, pp 139–171 (https://doi.org/10.1016/j.cca.2013.02.001).
10. Chojnacka, K.; Zielińska, A.; Michalak, I.; Górecki, H. *Environ. Toxicol. Pharmacol.*, **2010**, *30*, pp 188–194 (https://doi.org/10.1016/j.etap.2010.06.002).
11. Michalak, I.; Chojnacka, K.; Saeid, A. *Chinese Sci. Bull.,* **2012**, *57*, pp 3460–3465 (https://doi.org/10.1007/s11434-012-5385-7).
12. Chojnacka, K.; Michalak, I.; Zielińska, A.; Górecki, H. *Arch. Environ. Contam. Toxicol.*, **2011**, *61*, pp 512–520 (https://doi.org/10.1007/s00244-011-9647-1).
13. Shiobara, Y.; Yoshida, T.; Suzuki, K. T. *Toxicol. Appl. Pharmacol*., **1998**, *152*, pp 309–314 (https://doi.org/10.1006/taap.1998.8537).
14. Skalny, A. V.; Skalnaya, M. G.; Tinkov, A. A.; Serebryansky, E. P.; Demidov, V. A.; Lobanova, Y. N.; Grabeklis, A. R.; Berezkina, E. S.; Gryazeva, I. V.; Skalny, A. A.; et al. *Environ. Monit. Assess.*, **2015**, *187*, pp 1–8 (https://doi.org/10.1007/s10661-015-4903-x).
15. Pozebon, D.; Dressler, V. L.; Curtius, A. J. *Quim. Nova*, **1999**, *22*, pp 838–846 (https://doi.org/10.1590/s0100-40421999000600011).
16. Raposo, J. C.; Navarro, P.; Sarmiento, A.; Arribas, E.; Irazola, M.; Alonso, R. M. *Microchem. J.,* **2014**, *116*, pp 125–134 (https://doi.org/10.1016/j.microc.2014.04.012).
17. Morton, J.; Carolan, V. A.; Gardiner, P. H. E. *Anal. Chim. Acta,* **2002**, *455*, pp 23–34 (https://doi.org/10.1016/S0003-2670(01)01578-1).
18. Mikulewicz, M.; Chojnacka, K.; Gedrange, T.; Górecki, H. *Environ. Toxicol. Pharmacol.,* **2013**, *36*, pp 1077–1086 (https://doi.org/10.1016/j.etap.2013.09.012).
19. Chojnacka, K. W.; Saeid, A.; Michalak, I.; Mikulewicz, M. *Pol. J. Environ. Stud.*, **2012,** *21*, pp 1563-1570.
20. Kosanovic, M.; Jokanovic, M. *Environ. Monit. Assess.*, **2011**, *174*, pp 635–543 (https://doi.org/10.1007/s10661-010-1484-6).
21. Miekeley, N.; Carneiro, M. T. W. D.; da Silveira, C. L. P. *Sci. Total Environ.*, **1998**, *218*, pp 9–17 (https://doi.org/10.1016/S0048-9697(98)00185-5).
22. Carneiro, M. T. W. D.; da Silveira, C. L. P.; Miekeley, N.; Fortes, L. M. C. *Quim. Nova*, **2002**, *25*, pp 37–45 (https://doi.org/10.1590/s0100-40422002000100008).
23. Instituto Nacional de Metrologia, Qualidade e Tecnologia (Inmetro). *Orientações sobre Validação de Métodos de Ensaios Químicos*, **2003** (in Portuguese). Available at: http://www.inmetro.gov.br/Sidoq/Arquivos/CGCRE/DOQ/DOQ-CGCRE-8_01.pdf [Accessed November 5, 2020].
24. Agência Nacional de Vigilância Sanitária (ANVISA). *Guia para Validação de Métodos Analíticos e Bioanalíticos*, **2003** (in Portuguese). Available at: https://www.gov.br/anvisa/pt-br [Accessed November 5, 2020].

25. Official Methods of Analysis (AOAC). Appendix D: *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis*, **2002**. Available at: https://members.aoac.org/AOAC_Docs/StandardsDevelopment/Collaborative_Study_Validation_Guidelines.pdf [Accessed November 5, 2020].
26. Shapiro, S. S.; Wilk, M. B. *Biometrika,* **1965**, *52*, pp 591–611 (https://doi.org/10.1093/biomet/52.3-4.591).
27. Wickham, H. *ggplot2 Elegant Graphics for Data Analysis*. Springer International Publishing, **2016**. (https://doi.org/10.1007/978-3-319-24277-4).
28. Wickham, H. *J. Stat. Softw.,* **2007**, *21*, pp 1–20 (https://doi.org/10.18637/jss.v021.i12).
29. Wei, T.; Simko, V.; Levy, M.; Xie, Y.; Jin, Y.; Zemla, J. *Statistician,* **2017**, *56*, pp 316–324.
30. Szynkowska, M. I.; Marcinek, M.; Pawlaczyk, A.; Albińska, J. *Environ. Toxicol. Pharmacol*., **2015**, *40*, pp 402–408 (https://doi.org/10.1016/j.etap.2015.07.005).
31. Tamburo, E.; Varrica, D.; Dongarrá, G. *Sci. Total Environ*., **2016**, *573*, pp 996–1002 (https://doi.org/10.1016/j.scitotenv.2016.08.178).
32. Rodushkin, I.; Axelsson, M. D. *Sci. Total Environ.,* **2000**, *262*, pp 21–36 (https://doi.org/10.1016/S0048-9697(00)00531-3).
33. Zaichick, S.; Zaichick, V. *Biol. Trace Elem. Res.,* **2010**, *134*, pp 41–54 (https://doi.org/10.1007/s12011-009-8456-0).
34. Zaichick, S., Zaichick, V. *Int. J. Environ. Heal.,* **2011**, *5*, pp 106–124 (https://doi.org/10.1504/IJENVH.2011.039860).
35. Luo, R.; Zhuo, X.; Ma, D. *Ecotoxicol. Environ. Saf.,* **2014**, *104*, pp 215–219 (https://doi.org/10.1016/j.ecoenv.2014.03.006).
36. Rao, K. S.; Balajib, T.; Rao, T. P.; Babu Y.; Naidu, G. R. K. *Spectrochim. Acta - Part B*, **2002**, *57*, pp 1333–1338 (https://doi.org/10.1016/S0584-8547(02)00045-9).
37. Dongarrà, G.; Lombardo, M.; Tamburo, E.; Varrica, D.; Cibella, F.; Cuttitta, G. *Environ. Toxicol. Pharmacol*., **2011**, *32*, pp 27–34 (https://doi.org/10.1016/j.etap.2011.03.003).
38. Michalak, I.; Mikulewicz, M.; Chojnacka, K.; Wołowiec, P.; Saeid, A.; Górecki, H. *Environ. Toxicol. Pharmacol*., **2012**, *34*, pp 727–734 (https://doi.org/10.1016/j.etap.2012.09.015).
39. Chojnacka, K.; Górecka, H.; Górecki, H. *Environ. Toxicol. Pharmacol*., **2006**, *22*, pp 52–57 (https://doi.org/10.1016/j.etap.2005.11.006).
40. Massadeh, A.; El-Rjoob, A. W.; Smadi, H. *Toxicol Environ Chem*., **2011**, *93*, pp 494–503 (https://doi.org/10.1080/02772248.2010.532797).

SUPPLEMENTARY MATERIAL

Table S1. The minimum, maximum, mean, median and standard deviations of the elemental concentrations (mg g^{-1}) measured in hair of women and men

	Women					Men				
	Nutrient									
Element	**Min**	**Max**	**Mean**	**Median**	**Std Dev**	**Min**	**Max**	**Mean**	**Median**	**Std Dev**
B	< LOQ	79.0	2.56	< LOQ	8.99	< LOQ	9.21	1.51	< LOQ	2.63
Be	< LOQ	0.03	0.00	< LOQ	0.01	< LOQ	0.03	0.01	< LOQ	0.01
Ca	158	4946	1174	882	969	75.6	1651	411	313	302
Co	0.00	0.34	0.02	0.01	0.05	< LOQ	0.02	0.01	0.01	0.01
Cr	0.33	1.14	0.60	0.56	0.18	0.31	1.61	0.59	0.57	0.22
Cu	5.68	5662	86.7	15.7	532	4.61	412	37.6	13.6	76.0
Fe	3.26	182	20.39	15.66	19.28	5.43	33.75	14.73	13.51	7.53
I	< LOQ	6.34	0.66	0.37	0.95	0.04	2.03	0.49	0.39	0.41
Mg	5.52	637	136	91.8	134	6.56	392	47.5	26.5	70.0
Mn	0.08	8.91	0.76	0.43	1.27	0.05	0.82	0.32	0.26	0.21
Mo	< LOQ	0.07	0.03	0.03	0.01	0.01	0.07	0.03	0.03	0.01
P	39.5	251	141	143	27.8	90.0	210	144	143	29.0
S	25459	82748	53047	54209	8151	41592	72756	52575	52337	6572
Se	0.25	262	3.18	0.82	24.56	0.56	1.85	0.99	0.93	0.29
Sr	0.24	22.8	5.37	4.08	4.58	0.13	9.51	1.61	1.01	1.83
V	0.01	0.18	0.08	0.07	0.03	0.03	0.20	0.08	0.08	0.04
Zn	34.6	2315	222	172	234	114	995	234	185	171

Table S1. The minimum, maximum, mean, median and standard deviations of the elemental concentrations (mg g^{-1}) measured in hair of women and men (Continuation)

	Women					Men				
	Toxic									
Element	**Min**	**Max**	**Mean**	**Median**	**Std Dev**	**Min**	**Max**	**Mean**	**Median**	**Std Dev**
As	0.01	0.40	0.04	0.04	0.04	0.02	0.14	0.06	0.06	0.02
Ba	0.30	30.5	4.00	2.84	4.23	< LOQ	5.43	1.61	1.54	1.22
Bi	< LOQ	0.34	0.03	< LOQ	0.07	< LOQ	0.15	0.02	< LOQ	0.04
Cd	< LOQ	0.31	0.05	0.03	0.07	< LOQ	0.41	0.04	< LOQ	0.08
Hg	< LOQ	5.77	0.62	0.38	0.73	0.03	8.56	0.96	0.38	1.67
Ni	< LOQ	2.54	0.43	0.32	0.45	< LOQ	0.69	0.20	0.17	0.17
Pb	0.05	11.5	0.97	0.52	1.34	0.09	19.5	2.02	0.90	3.46
Sb	< LOQ	0.05	0.011	0.01	0.009	< LOQ	0.19	0.02	0.01	0.03
Th	< LOQ	0.024	0.002	< LOQ	0.004	< LOQ	0.012	0.002	< LOQ	0.003
U	< LOQ	0.13	0.01	0.01	0.02	< LOQ	0.16	0.02	0.01	0.03

Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 33, pp 89–100
doi: 10.30744/brjac.2179-3425.AR-67-2021

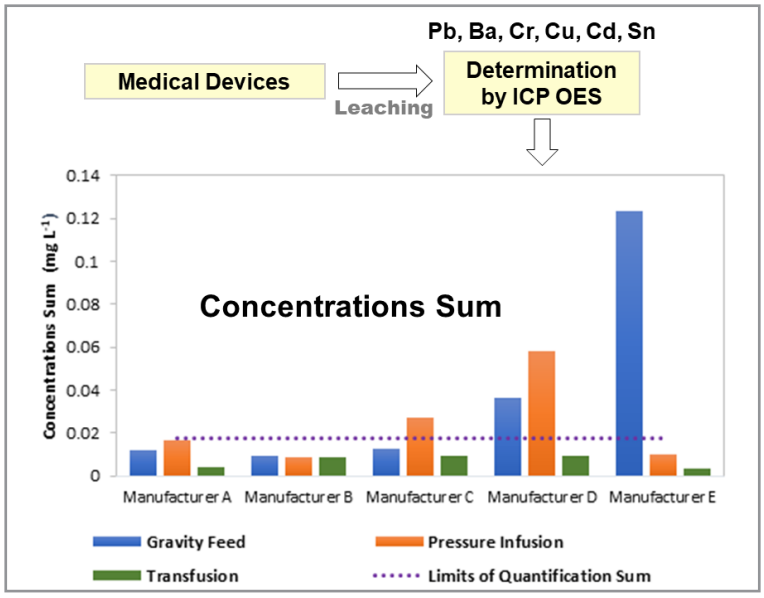
BrJAC

ARTICLE

Method Validation and Determination of Leachable Metals from Infusion and Transfusion Medical Devices

Alexandra Janine Schuh[1], **Dirce Pozebon**[2]*

[1]*Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Avenida Ipiranga, 681, 90619-900, Porto Alegre, RS, Brazil*
[2]*Instituto de Química, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, 91501-970, Porto Alegre, RS, Brazil*



This work deals with method validation for regulated metals (Cd, Pb, Ba, Sn, Cr and Cu) determination in infusion and transfusion medical devices. The metals were extracted with water at (37 ± 1) °C followed by their determination in the extract by using inductively coupled plasma optical emission spectrometry (ICP OES). The validated method was applied in the analysis of infusion and transfusion devices commercialized in Brazil to verify compliance with current legislation, which establishes that the sum of Pb, Ba, Sn, Cr and Cu in the extract must not exceed 1 mg L^{-1} and that of Cd must not 0.1 mg L^{-1}. Samples from five manufacturers of infusion and transfusion devices, produced in Brazil or imported, were analysed. The results of the analysis showed that all devices complied with the legislation, whereas the sum of Pb, Ba, Sn, Cr and Cu concentrations and that of Cd in the extract were lower than the maximum permissible; Cd was not detected in any sample extract and the sum of the other elements was < 0.14 mg L^{-1} in all extracts of the analysed samples.

Keywords: method validation, regulated metals, infusion and transfusion medical devices

INTRODUCTION

A multitude of disposable medical devices (DMDs) such as infusion and transfusion devices, catheters and syringes are widely used in clinical practice [1,2]. The materials composing DMDs must be biocompatible and allow the intended function without causing undesirable side effects such as necrosis to patients and allergic reactions to patients and medical staff [3,4]. Moreover, such materials must be quite

Cite: Schuh, A. J.; Pozebon, D. Method Validation and Determination of Leachable Metals from Infusion and Transfusion Medical Devices. *Braz. J. Anal. Chem.*, 2021, *8* (33), pp 89-100. doi: http://dx.doi.org/10.30744/brjac.2179-3425.AR-67-2021

Submitted 03 May 2021, Resubmitted 05 June 2021, Accepted 07 June 2021, Available online 25 August 2021.

pure because impurities present can directly enter the bloodstream of patients and cause intoxication of them. The DMDs are composed of polymers that are considered safe, but they may be harmful to patients and medical staffs [1,2,5,6]. Therefore, the quality of DMDs must be controlled. The DMS are mainly constituted of silicone, polyvinyl chloride (PVC), latex rubber [5,6] and additives. Different processes are involved in DMDs manufacturing, which are usually not disclosed by the manufacturers and potential contaminants not informed.

Besides insufficient sterility and structural defects, DMDs can release microparticles, toxic elements, or compounds that are then transferred to patients under treatment. As such, the DMDs toxicity is apparently related to multiple leachable compounds that are released from the DMDs during their clinical use [2]. Infusion devices are usually in prolonged contact with patients and can induce local inflammation, allergic reactions, systemic toxicity, and infections [1,2,4-6]. Furthermore, whatever is released by the infusion devices goes directly into the patient's bloodstream [1,4].

Infusion and transfusion devices are currently evaluated with respect to cytotoxicity, sensitization, intracutaneous irritation or reaction, acute systemic toxicity, and hemocompatibility. Recommendations for this type of devices are described in the ISO 8536 series for infusion devices and in the ISO 1135 series for transfusion devices [7-10]. Research about infusion and transfusion devices side effects has dealt with biological aspects [1-3,5,6] and any research regarding the presence of toxic elements that could be leached from these devices has not been published so far.

Surveillance of production and commercialization of transfusion and infusion devices in Europe and The United States are conducted according to the Directive (EU) 2017/745 [11] and FDA (Food and Drug Administration) [12], respectively. The National Health Surveillance Agency (ANVISA) is responsible for such surveillance in Brazil [13], whereas the certification of DMDs (produced in Brazil or imported) is conducted by the National Metrology Institute, Quality and Technology (INMETRO) [14]. The laboratories that carry out the analysis for certification of DMDs must be accredited to the Brazilian Network of Testing Laboratories [14,15]. One of the requirements for certification of diffusion and infusion devices is the determination of leachable Ba, Cr, Cu, Pb, Sn and Cd; the sum of Ba, Cr, Cu, Pb, Sn concentrations in the leached must not exceed 1 mg L^{-1} and that of Cd must not 0.1 mg L^{-1}. Such determination shall be carried out following a validated method.

Atomic spectrometry techniques such as atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP OES) can be employed for Ba, Cr, Cu, Pb, Sn and Cd determination in the leachate of infusion and transfusion devices. The laboratory that performs the analysis must validate non-standard methods, that is, methods that are outside the intended scope (methods planned/developed in the laboratory or modified standard methods) [14-17]. An analytical method can be validated through interlaboratory analysis or not (single laboratory approach), depending on the interests involved [17,18]. Selectivity, linearity (including the working range), sensitivity, limit of detection (LOD), limit of quantification (LOQ), bias, accuracy, overall uncertainty, precision, and robustness [17,18] are parameters evaluated in a method validation. Robustness assessment is optional and generally not required for well-established methods [19]. The purpose of validation is to guarantee that the candidate method provides results that are equivalent to those provided by the standard method [18].

A method of DMDs (infusion and transfusion devices in the present case) analysis was validated in the present work using ICP OES for determination of regulated metals; leachable Cd, Pb, Ba, Sn, Cr and Cu were determined. The validated method was applied in the analysis of infusion and transfusion devices used in Brazil to verify the compliance with current legislation.

MATERIALS AND METHODS

Instrumental

For elemental analysis, an ICP OES spectrometer (model ICAP 6200, dual view, Thermo Scientific) was employed. Argon with purity of 99.9992% (White Martins/Praxair, Brazil) was used as principal, auxiliary and nebulizer gas. Instrumental parameters and the operating conditions of ICP OES are summarized in Table I.

Table I. Instrumental and operating conditions of ICP OES and spectral lines monitored

Parameter	Settings
Radio frequency power	1150 W
Plasma gas flow rate (L min^{-1})	15.0
Auxiliary gas flow rate (L min^{-1})	0.5
Nebulizer gas flow rate (L min^{-1})	0.5
Peristaltic pump speed	45 rpm
Plasma view	Axial
Nebulizer	Concentric
Spray chamber	Cyclonic
Replicates	7
Wavelength (nm)	Pb II (220.3 nm); Sn I (189.9 nm); Ba II (233.5 nm); Cr II (283.5 nm); Cd II (214.4 nm); Cu I (324.7 nm)

Standards, reagents, and solutions

The following certified standard solutions from Accustandard were used for preparation of calibration solutions: ICP-29N-1 containing (1.014 ± 0.024) mg kg^{-1} of Pb; ICP-63N-1 containing (0.9881 ± 0.0024) mg kg^{-1} of Sn; ICP-4N-1 containing (0.986 ± 0.002) mg kg^{-1} of Ba; ICP-13N-1 containing (1.015 ± 0.002) mg kg^{-1} of Cr; ICP-08N-1 containing (1.013 ± 0.002) mg kg^{-1} of Cd; and ICP-15N-1 containing (0.987 ± 0.024) mg kg^{-1} of Cu. To evaluate the accuracy of the method, aliquots of samples were spiked with the following certified reference materials from NIST (National Institute of Standards and Technology): SRM 3128 – (9.995 ± 0.014) mg kg^{-1} Pb; SRM 3161a – (10.011 ± 0.025) mg kg^{-1} Sn; SRM 3104a – (6.994 ± 0.017) mg kg^{-1} Ba; SRM 3112a – (10.009 ± 0.020) mg kg^{-1} Cr; SRM 3108 – (10.007 ± 0.027) mg kg^{-1} Cd; and SRM 3114 – (10.005 ± 0.024) mg kg^{-1} Cu.

Nitric acid (HNO_3 65% from Merck) was used in calibration solutions. All solutions and samples were prepared using ultrapure water (minimum resistivity of 18.2 MΩ cm (purified in a Millipore Direct-Q Ultrapure system), type 1 according to ISO 3696 [20].

Volumetric flasks (Brand, Germany), digital micropipettes (Transpette, Brand, Germany) and analytical balance (AUX320, Shimadzu) calibrated to the Brazilian calibration network (RBC) were used.

Samples and procedure

Samples of infusion and transfusion devices produced by five different manufacturers (two national and three international) and commercialized in Brazil were analysed: 45 gravimetric infusion devices, 45 infusion devices for connection to pumps, and 45 transfusion devices. The manufacturers were identified by letters A, B, C, D and E.

Figure 1 shows a scheme of an infusion device for use in pumps. Infusion devices and transfusion devices are similar; the main differences are in the drip chamber (6) and fluid filter (7), being this filter more porous and larger in transfusion devices.

Infusion and transfusion devices were leached in a closed way encompassing three devices of the same model connected in series (through silicone tubes) to a 300 mL-borosilicate glass flask containing 250 mL of purified water (see Figure 2). This water was heated at (37 ± 1) °C, passed through the three

devices and then returned to the flask under heating. A peristaltic pump was employed to transport the water at a flow rate of 1 L h^{-1} for 2 h continuously. This procedure was carried out following reference [7]. The extract was transferred to a 250 mL-volumetric flask, to which 12.5 mL of HNO_3 were added and the volume adjusted to the mark by adding water. Then, Cd, Pb, Ba, Sn, Cr, and Cu were determined in this solution according to the validated method.

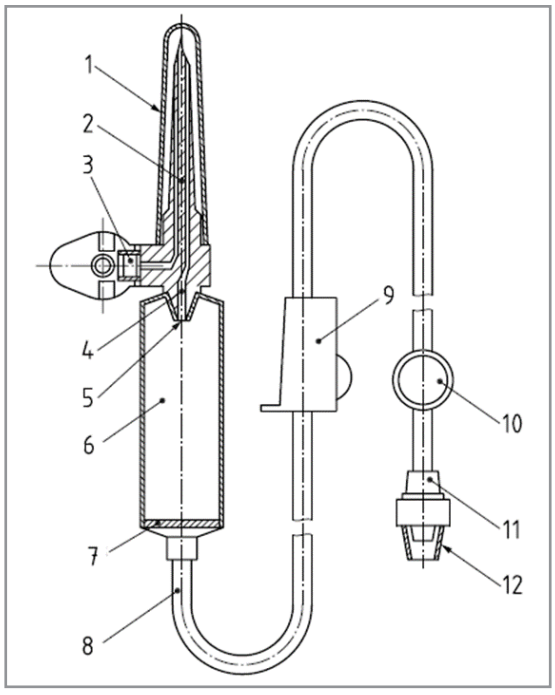


Figure 1. Scheme of an infusion medical device. 1: protector; 2: perforating tip; 3: air filter; 4: fluid channel; 5: dripper; 6: drip chamber; 7: fluid filter; 8: transfer tube; 9: flow regulator; 10: lateral injector; 11: luer lock connector; 12: protector. (Reprinted with permission from *Associação Brasileira de Normas Técnicas. ABNT NBR ISO 8536-4. Equipamento de infusão para uso médico. Parte 4: Equipos de infusão para uso único, alimentação por gravidade*, 2011. ISO 8536-4, 2019 [7].)

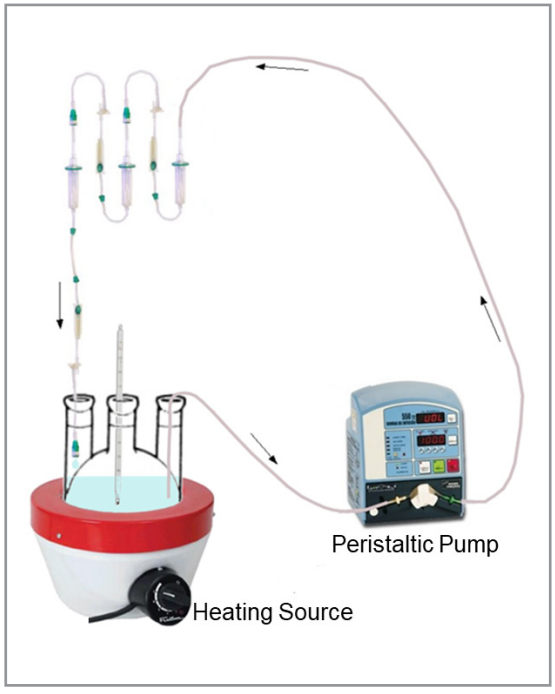


Figure 2. Scheme illustrating the leaching procedure of infusion and transfusion devices; arrows indicate the circulating water direction.

Method Validation

Selectivity, linearity, bias, sensitivity, LOD, LOQ, accuracy, uncertainty, and precision were evaluated for method validation, following Eurachem guidelines [18].

Two calibration curves were prepared for each analyte; solution calibrations were prepared in 5% (v/v) HNO_3 for one of the calibration curves and in the sample leached medium + 5% (v/v) HNO_3 for the other.

Each calibration curve was composed of seven points corresponding to 7 solutions with different concentrations of the analytes: 100, 200, 300, 400, 500, 600 and 700 μg L^{-1} of Pb, Sn, Ba, Cr and Cu; and 10, 25, 50, 75, 100, 125 and 150 μg L^{-1} of Cd. The analytes concentrations in the prepared calibration solutions were equidistant to avoid leverage effect in calibration curves.

The Snedecor F-test was applied to assess the variance homogeneity and the analysis of variance (ANOVA) was applied to assess the similarity of the calibration curves [17,21]. The slopes of calibration curves were compared to evaluate the selectivity of the method. In the linearity evaluation, outlier values were detected and excluded using the Grubbs test [19,22]. The linear regression coefficient (r) was then calculated, and it was acceptable if r ≥ 0.9950.

The LOD and LOQ were calculated using Equations (1) and (2), respectively:

$$LOD = C + 3s \qquad \text{Equation (1)}$$

$$LOQ = C + 10s \qquad \text{Equation (2)}$$

where C is the mean concentration of ten consecutive analyte determinations in the sample blank (leached from a sample where none of the analytes were detected) and *s* is the standard deviation of them.

Aliquots of samples leachate were fortified with known amounts of the analytes at two concentration levels and within the concentration range of the respective calibration curves for accuracy evaluation. Analyte recovery in the range of 80 to 110% was accepted [17].

The method precision was evaluated through repeatability, intermediate precision, and reproducibility [17,21]. As such, the coefficient of variation (CV) and ANOVA of data obtained in analyses conducted by different analysts in different days and participation in an Interlaboratory program were considered. The interlaboratory program was coordinated by the Metrological Network of Rio Grande do Sul, Brazil. This interlaboratory program was part of a project of the Brazilian Technology System (SIBRATEC) that assists the development of technological services in metrology, standardization and conformity assessment approved by the Ministry of Science, Technology and Innovations (MCTIC) of Brazil.

The semi-empirical model was followed in the evaluation of the expanded uncertainty (*U*) of the method. The uncertainty was obtained by multiplying the combined standard uncertainty (μC_a) by a coverage factor (k = 2), for a confidence interval of 95.45% [23,24]. *U* was calculated using the linear squares fit, assuming that the uncertainties of the values on the abscissa are considerably smaller than the uncertainty of the values in the ordinate. The contribution of calibration standards to the overall uncertainty was considered negligible; μC_a was calculated by Equation (3) and the standard deviation of residues by Equations (4) and (5).

$$\mu C_a = \frac{S}{b_1}\sqrt{\frac{1}{n_a} + \frac{1}{n} + \frac{(C_a - \bar{C})^2}{S_{xx}}} \qquad \text{Equation (3)}$$

$$S = \frac{\sum_{j=1}^{n}\left[Y_j - \left(b_0 + b_1.C_j\right)\right]^2}{n-2} \qquad \text{Equation (4)}$$

$$S_{xx} = \sum_{j=i}^{n} \left(C_j - \bar{C}\right)^2 \qquad \text{Equation (5)}$$

where:
b_1 : angular coefficient of the linear equation of calibration curve (slope)
b_0 : intercept point on the ordinate axis (linear coefficient)
Y_j : j^{th} measurement of the intensity of the i^{th} calibration solution
S : residual standard deviation
n : number of measurements for calibration
n_a: number of measurements performed for the sample under analysis
C_a: concentration of the analyte in the sample solution
$\bar{C}$: avarege value for each calibration solution (for *n* measurements)
i : index for the number of calibration solution
j : index for the number of measurements to obtain the calibration curve

RESULTS AND DISCUSSION

Method Validation

Selectivity

In method validation it is necessary to prove that the method is selective even if any matrix effect is not expected. According to normative documents [17-20], selectivity is evaluated by calibration curves comparison. This makes evident that there is matrix effect or not. Calibration curves were obtained from calibration solutions prepared in 5% (v/v) HNO_3 in presence or absence of sample leached medium. The angular coefficient (slope) values of the linear regression equation of the calibration curves obtained were compared by ANOVA and they did not differ significantly (see Table II). Besides, parallelism of lines was observed for the two calibration curves. Therefore, the calibration solutions could be prepared in 5% (v/v) HNO_3 solely because the sample matrix does not interfere. The homogeneity of variance for each concentration level also demonstrated the absence of matrix effect. Additionally, the homogeneity of variance by concentration level (Table III) demonstrated that the sample matrix does not interfere regardless of the concentration. Thus, the method was considered selective, that is, the analyte determination does not depend on other substances present in the sample leached.

Linearity

The linear dynamic range of ICP OES is about five orders of magnitude. Even so, linearity must be evaluated because this is required in a method validation, according to normative documents [17-20]. As previously mentioned, calibration curves were composed of 7 equidistant points to avoid leverage. For each point 7 solutions were prepared, and the mean intensity value plotted as a function of concentration. The correlation coefficient (*r*) values were ≥ 0.9950 (Table II), which was acceptable. The residues (difference among the obtained *y* value and the predicted y value) were randomly distributed and close to zero, denoting the linearity of the established working range.

Table II. Calibration curve parameters and results of ANOVA; $F_{critical}$ = 4.60; confidence level of 95.45%

Parameters	Pb	Sn	Ba	Cr	Cu	Cd
Slope - 5% (v/v) HNO_3	1519	1902	4813	15354	18216	11062
Slope - sample leached + 5% (v/v) HNO_3	1570	1983	4946	17130	19931	11249
r - 5% (v/v) HNO_3	0.9998	0.9980	0.9984	0.9984	0.9982	0.9959

Table II. Calibration curve parameters and results of ANOVA; $F_{critical}$ = 4.60; confidence level of 95.45% (Continuation)

Parameters	Pb	Sn	Ba	Cr	Cu	Cd
r - sample leached + 5% (v/v) HNO_3	0.9989	0.9999	0.9998	0.9983	0.9979	0.9960
$F_{calculated}$	0.018	0.04	0.53	0.53	0.86	0.05
P_{value}	0.90	0.84	0.48	0.48	0.37	0.99

Table III. Values of $F_{calculated}$ for each concentration level of calibration curves; 12 degrees of freedom (n-2) and confidence level of 95.45%; $F_{critical}$ = 4.28

Calibration Solutions	Pb	Sn	Ba	Cr	Cu	Cd
Blank	1.08	0.02	0.23	2.47	0.32	0.39
1	0.18	0.18	0.15	3.17	2.58	2.45
2	1.36	0.29	0.11	0.99	0.61	2.96
3	0.45	0.19	0.01	0.13	0.17	0.97
4	0.75	0.19	0.18	0.12	0.37	3.96
5	1.26	0.09	0.20	0.39	0.67	3.51
6	0.22	2.70	0.22	1.42	1.39	3.16
7	2.98	0.13	0.38	3.99	3.72	0.93

Accuracy

The accuracy was assessed through the analyte recovery in spiked sample and normalized error. The obtained results are presented in Table IV where one can observe the analyte recovery and the normalized error were 80-110% and less than ≤ 1, respectively. These are established values for the accuracy acceptance [17,21,25] and, therefore, the method can be considered accurate.

Table IV. Normalized error and analyte recovery; samples were spiked with aliquots of NIST SRMs

Analyte	Fortification Level (mg L^{-1})	Recovery (%)	Normalized Error
Pb	0.200	104	0.169
	0.500	100	0.047
Sn	0.200	101	0.043
	0.500	100	0.048
Ba	0.200	100	0.043
	0.500	102	0.382

Table IV. Normalized error and analyte recovery. Samples were spiked with aliquots of NIST SRMs (Continuation)

Analyte	Fortification Level (mg L^{-1})	Recovery (%)	Normalized Error
Cr	0.200	103	0.280
	0.500	104	0.888
Cu	0.200	102	0.127
	0.500	101	0.189
Cd	0.050	104	0.306
	0.100	106	0.952

Precision

Precision was assessed through repeatability, intermediate precision, and reproducibility. To access repeatability, two solutions (A and B) named “blind samples” were analysed. The analytes concentrations in these solutions were close to those in the calibration solutions corresponding to the intermediate points of the calibration curves; the analytes concentrations in the “blind samples” were unknown by the analysts. The CV of the obtained data (Table V) met the repeatability acceptance criteria, considering the concentration range involved [17,21,25,26]. To assess the intermediate precision (which represents the variability of the results in a laboratory) two analysts conducted the samples analyses in different days. To this end, they prepared calibration curves and spiked aliquots of samples to obtain 0.050 or 0.100 mg L^{-1} of Cd, and 0.200 or 0.500 mg L^{-1} of the other elements. Results obtained by the two analysts were submitted to ANOVA that demonstrated they were not different ($F_{calculated} < F_{critical}$ and $p > 0.05$).

Table V. Coefficient of variation (CV) in “blind samples” analysis, accepted values [17-21] and results obtained in the analyses conducted by two analysts in different days; n = 5 for each element and sample

Analyte	Blind Samples	Obtained CV (%)	Accepted CV (%)	Concentration Found (mg L^{-1})	
				Analyst 1	Analyst 2
Pb	A	0.19	≤ 11	0.207	0.192
	B	0.98	≤ 11	0.502	0.491
Sn	A	0.84	≤ 11	0.202	0.213
	B	1.01	≤ 11	0.503	0.509
Ba	A	0.25	≤ 11	0.201	0.203
	B	0.18	≤ 11	0.508	0.494
Cr	A	1.45	≤ 11	0.206	0.204
	B	0.63	≤ 11	0.519	0.501
Cu	A	1.65	≤ 11	0.205	0.202
	B	0.06	≤ 11	0.507	0.491
Cd	A	0.19	≤ 15	0.052	0.051
	B	0.38	≤ 15	0.105	0.100

The method reproducibility was confirmed through participation in an interlaboratory program involving three laboratories. Results for the laboratory where the present method has been validated are summarized in Table VI for one sample of transfusion device whose leached has been spiked and distributed to participating laboratories of the interlaboratory program. A value of z-score lower than 2, in module, means that the results found are satisfactory; between 2 and 3 they are "questionable"; and higher than 3 they are "unsatisfactory". As can be seen in Table VI, the results were satisfactory.

Table VI. Laboratory performance in the interlaboratory program

Analyte	Found (mg L^{-1})	CV (%)	Accuracy and Precision	Z-score	Laboratory Performance
Pb	1.041	0.7	Satisfactory	1.14	Satisfactory
Sn	0.177	4.2		0.71	
Ba	1.848	0.6		-0.71	
Cr	0.507	0.6		-0.10	
Cu	1.589	0.5		-0.73	
Cd	0.214	1.0		0.19	

Limit of detection and quantification

The LOD and LOQ of the method were calculated by Equations (1) and (2). As can be observed in Table VII, the sum of Pb, Sn, Ba, Cu and Cr concentrations is ≤ 1 mg L^{-1}, and the Cd concentration is ≤ 0.1 mg L^{-1}. Therefore, the calculated LOQs met the maximum limits allowed for these elements in infusion and transfusion devices. The overall uncertainty was < 4% for all analytes, which is also satisfactory.

Table VII. Limits of detection (LOD) and quantification (LOQ) of the method

Analyte	LOD (mg L^{-1})	LOQ (mg L^{-1})	Relative Uncertainty (%)
Pb	0.0031	0.0102	2.5
Sn	0.0010	0.0034	2.0
Ba	0.0019	0.0061	1.4
Cr	0.0012	0.0036	3.4
Cu	0.0005	0.0016	2.2
Cd	0.0001	0.0008	1.4

Sample Analysis

The results of the analysis of infusion and transfusion devices are depicted in Figure 3. As shown in this figure, the sum of the metals concentrations was above the LOQs sum for pressure infusion devices from manufacturers C and D and for gravity infusion devices from manufacturers D and E. However, the sum of Pb, Ba, Cr, Cu and Sn concentrations in the leached was lower than 1 mg L^{-1} (the maximum accepted concentration) for all samples and manufacturers. The noteworthy highest value for manufacturer E was

due to Sn whose concentration was higher in the leachate of samples from this manufacturer than in the others. Cadmium concentration (not shown in Figure 3) was lower than the LOQ (0.008 mg L^{-1}) in all analysed samples. Thus, Cd concentration followed the legislation which recommends the concentration of this element in the leached must be lower than 0.1 mg L^{-1}.

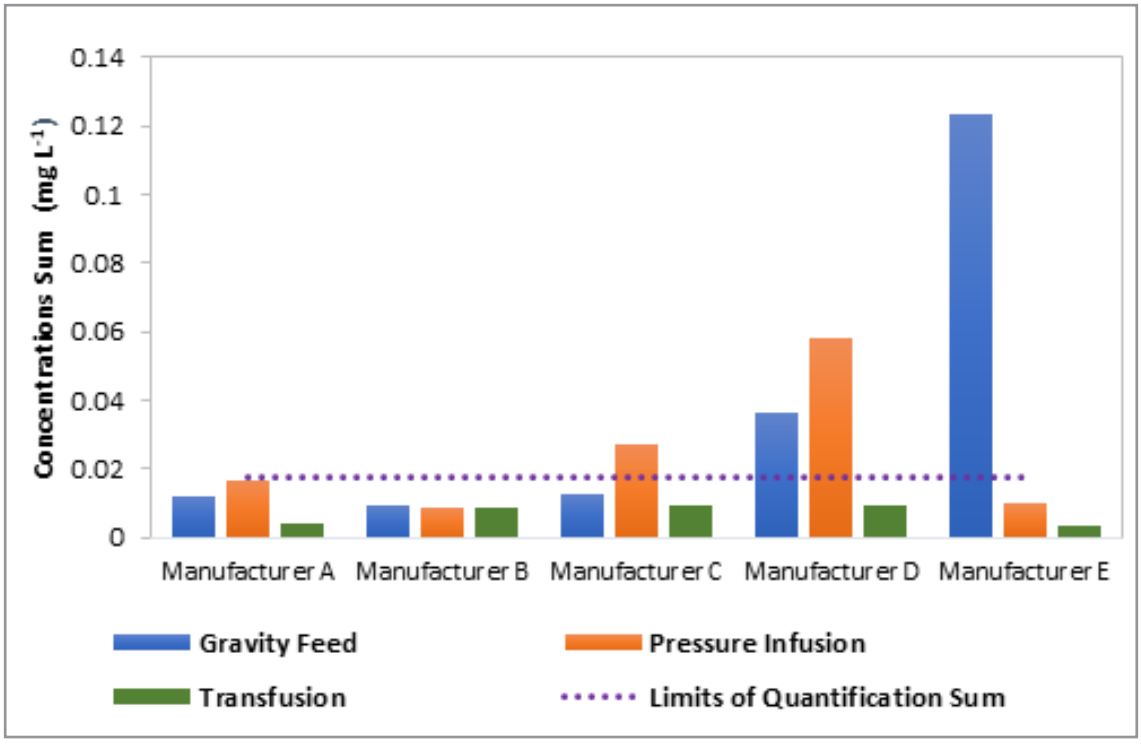


Figure 3. Sum of Pb, Ba, Cr, Cu and Sn concentrations in the leachate of infusion and transfusion devices.

CONCLUSIONS

The validated method is suitable for infusion and transfusion devices analysis with respect to regulated metals determination. The LOQs are adequate and ICP OES can be employed for Pb, Ba, Cr, Cu, Cd and Sn determination in the leachate from these devices, and the validated method meets the requirements of the current legislation.

The infusion and transfusion devices commercialized in Brazil, produced in the country or imported, are in accordance with the current legislation, having the expected quality with respect to leachable Pb, Ba, Cr, Cu, Cd and Sn.

Currently, DMDs like syringes, needles, and transfusion and diffusion devices are regulated and must be certified. However, the regulation should be extended to other DMDs used in Brazil, to ensure the minimum quality necessary regarding leachable toxic elements.

Conflicts of interest

The authors declare that there is not any conflict of interest.

Acknowledgements

The authors are grateful to LABELO of PUCRS by providing the necessary infrastructure to carry out the work.

REFERENCES

1. Kozlovskaya, L.; Stepensky, D. *Int. J. Pharm.,* **2015**, *179* (2), pp 693-701 (https://doi.org/10.1016/j.ijpharm.2014.12.020).
2. Kozlovskaya, L.; Popilski, H.; Gorenbein, P.; Stepensky, D. *Int. J. Pharm.,* **2015**, *489* (1-2), pp 285-293 (https://doi.org/10.1016/j.ijpharm.2015.04.065).
3. Litman, R. S.; Smith, V. I.; Mainland, P. *Pediatr. Anesth*., **2017,** *28* (1) (https://doi.org/10.1111/pan.13279).
4. International Organization for Standardization. ISO 10993-1:2018. *Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process*. Available at: https://scc.isolutions.iso.org/obp/ui#iso:std:iso:10993:-1:ed-5:v2:en [Accessed April 2021].
5. Dakwar, G. R.; Kaplun, V.; Kojukarov, L.; Gorenbein, P.; Schumacher, I.; Kontorovich, D.; Förster, C.; Beit-Yannai, E.; Stepensky, D. *Int. J. Pharm.,* **2012**, *434* (1-2), pp 20-27 (https://doi.org/10.1016/j.ijpharm.2012.05.019).
6. Schumacher, I.; Tsiperman, E.; Tarnopolsky, A.; Raskin, A.; Sigler, E.; Domb, A.J. *Polym. Adv. Technol*., **2007**, *18*, pp 207-212 (https://doi.org/10.1002/pat.856).
7. Associação Brasileira de Normas Técnicas (ABNT). ABNT NBR ISO 8536-4. *Equipamento de infusão para uso médico. Parte 4: Equipos de infusão para uso único, alimentação por gravidade*, 2011. ISO 8536-4, **2019**.
8. International Organization for Standardization. ISO 8536-8. *Infusion equipment for medical use – Part 8: Infusion sets for single use with pressure infusion apparatus*, **2015**.
9. International Organization for Standardization. ISO 1135-4:2015. *Transfusion equipment for medical use - Part 4: Transfusion sets for single use, gravity feed*, **2015**.
10. International Organization for Standardization. ISO 1135-5:2015. *Transfusion equipment for medical use - Part 5: Transfusion sets for single use with pressure infusion apparatus*, **2015**.
11. European Union. Directive 2017/45/EEC. Regulation (EU) 2017/745 of the European Parliament and of the Council, amending Directive 2001/83/EC, Regulation (EC) No 178/2002 and Regulation (EC) No 1223/2009 and repealing Council Directives 90/385/EEC and 93/42/EEC. Available at: https://op.europa.eu/en/publication-detail/-/publication/83bdc18f-315d-11e7-9412-01aa75ed71a1/language-en/format-PDF/source-58036705 [Accessed: December 2020].
12. Food and Drug Administration (FDA). Biocompatibility Testing of Medical Devices – Standards Specific Information for the Accreditation Scheme for Conformity Assessment (ASCA) Pilot Program. Available at: https://www.fda.gov/media/142388/download [Accessed April 2021].
13. Agência Nacional de Vigilância Sanitária (ANVISA). Resolução RDC nº 342 de 06 de março de **2020**. *Estabelece os requisitos mínimos de identidade e qualidade para os equipos de uso único de transfusão, de infusão gravitacional e de infusão para uso com bomba de infusão*. Available at: http://www.so.com.br/legislacao_anvisa/2020/RDC_342_2020_.pdf [Accessed: September 2020].
14. Instituto Nacional de Metrologia, Qualidade e Tecnologia (Inmetro). Portaria nº 289 de 04 de setembro de **2020**. *Requisitos de Avaliação da Conformidade para Agulhas Hipodérmicas Estéreis para Uso Único e Agulhas Gengivais Estéreis para Uso Único, para Equipos de Uso Único de Transfusão, de Infusão Gravitacional e de Infusão para Uso com Bomba de Infusão e para Seringas Hipodérmicas Estéreis de Uso Único, respectivamente*. Available at: http://inmetro.gov.br/legislacao/rtac/pdf/RTAC002666.pdf [Accessed: September 2020].
15. Associação Brasileira de Normas Técnicas (ABNT). ISO/IEC 17025. *Requisitos gerais para a competência de laboratório de ensaios e calibrações*. Available at: https://pt.scribd.com/document/414735599/Abnt-Nbr-Iso-17025 [Accessed: September 2020].
16. Instituto Nacional de Metrologia, Qualidade e Tecnologia (Inmetro). DOQ-CGCRE 0087. *Orientações gerias sobre os requisitos da ABNT NBR ISO/IEC 17025:2017*. Available at: http://www.inmetro.gov.br/credenciamento/eventos-cgcre/13-14-15Workshop/00-DOQ-CGCRE-087_rev_00_-_Orientacoes_gerais_sobre_os_requisitos_da_ABNT_NBR_ISO_IEC_17025_2017.pdf [Accessed: September 2020].

17. Instituto Nacional de Metrologia, Qualidade e Tecnologia (Inmetro). DOQ-CGCRE 0008. Orientação sobre validação de métodos analíticos. Available at: http://www.inmetro.gov.br/Sidoq/Arquivos/CGCRE/DOQ/DOQ-CGCRE-8_01.pdf [Accessed: September 2020].
18. Magnusson, B; Örnemark, U. Eurachem Guide: *The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics*, **2014**, 2nd Ed. Available at: https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf [Accessed: November 2020].
19. Barwick, V. *Planning and Reporting Method Validation Studies*. Supplement to Eurachem Guide on the Fitness for Purpose of Analytical Methods, **2019**, 1st Ed. Available at: https://eurachem.org/images/stories/Guides/pdf/MV_Guide_planning_supplement_EN.pdf [Accessed: December 2020].
20. International Organization for Standardization. ISO 3696: *Water for analytical laboratory use, Specification and test methods*, **1987**. Available at: https://www.iso.org/standard/9169.html [Accessed: May 2021].
21. Association of Official Agricultural Chemists (AOAC). Appendix D: *Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis*, **2002**. Available at: https://members.aoac.org/AOAC_Docs/StandardsDevelopment/Collaborative_Study_Validation_Guidelines.pdf [Accessed: September 2020].
22. Miller, J. N.; Miller, J. C. *Statistics and Chemometrics for Analytical Chemistry*, 6th Ed. Pearson, **2010**, chapter 3, pp 49-52.
23. International Organization for Standardization (ISO). *Uncertainty of Measurement. Guide to the Expression of Uncertainty in Measurement* (GUM:1995), **2008**. Available at: http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf [Accessed: May 2021].
24. Ellison, S. L. R; Wiliams, A. Eurachem/CITAC Guide CG4: *Quantifying Uncertainty in Analytical Measurement*. Available at: https://www.eurachem.org/images/stories/Guides/pdf/QUAM2012_P1.pdf [Accessed: May 2021].
25. Horwitz, W. *Pure & Appl. Chem.,* **1995**, 67, pp 331-343. Available at: http://list.iupac.org/publications/pac/1995/pdf/6702x0331.pdf [Accessed: May 2021].
26. Larrea-Marín, M. T.; Pomares Alfonso, M. S.; Gómez-Juaristi, M.; Sánchez-Muniz, F. J.; Ródenas de la Rocha, S. *J. Food. Comp. Anal.,* **2010**, *84*, pp 814-820 (https://doi.org/10.1016/j.jfca.2010.03.015).

Brazilian Journal of Analytical Chemistry
2021, Volume 8, Issue 33, pp 101-104

BrJAC

FEATURE

PDF

Agronomic Institute of Campinas – One of the Most Renowned Research Centers in Brazil

The Agronomic Institute of Campinas (IAC) was founded on June 27, 1887 by the Emperor of Brazil D. Pedro II as the Imperial Agronomical Station of Campinas. It is the oldest institution dedicated to agronomy in Latin America. In 1892, it was transferred to the São Paulo State government, and today, it is part of the São Paulo's Agency for Agribusiness Technology, linked to the São Paulo State Secretariat of Agriculture and Supplies.

The Agronomic Institute of Campinas headquarters is located in the city of Campinas, SP, Brazil

Currently, the IAC staff includes the Director-General Dr. Marcos Guimarães de Andrade Landell, 161 scientific researchers, and 319 support employees. The IAC physical area of 1,279 hectares houses the Headquarters, the Campinas Experimental Center, and 12 Research Centers distributed among the cities of Campinas, Cordeirópolis, Jundiaí, Ribeirão Preto, and Votuporanga, which are occupied by laboratories, vegetation houses (structures built with various materials, such as wood, concrete, iron, aluminum, etc., covered with transparent materials that allow passage of sunlight for growth and development of plants), and other infrastructure suitable for the work carried out.

IAC's mission is to generate and transfer science and technology for the agricultural business, aiming to optimize plant production systems and contributing to socioeconomic development with environmental quality. Its objectives are: to develop research actions in tune with the demands of the agricultural sector; predict and propose demands; develop and transfer products and processes; produce technical and scientific bibliographic material; guide the formulation of public policies; form scientific and critical competencies; contribute to food security; propose innovative products and processes.

The Agronomic Institute is a source of pride for Campinas, which for over 100 years has been home to one of the most renowned centers of intelligence and research in the field of agriculture, a rich scientific heritage to contribute to the quality of life and wealth generation from the countryside.

Research developed by the IAC

The Agronomic Institute conducts research on production systems of more than 100 types of plants. Due to this research, the State of São Paulo is able to cultivate plants from the most diverse regions and climates in the world with economic success.

Agronomic Institute conducts research on the production systems of more than 100 types of plants.

The questions about what, when, and where to plant, how to improve the soil and protect it, or how to produce agricultural products economically without causing damage to the environment find answers in the technologies generated by the IAC.

The State of São Paulo could not produce the enormous variety of typical fruits of the most diverse climates if it were not for the extensive research at the Agronomic Institute. The introduction and adaptation of new varieties and genetic improvement allowed for the diversification of cultures, creating new options for producers and meeting the growing demand of consumers.

Keeping up with the demands of the industries, IAC has been offering state-of-the-art technology for the production of high-quality raw materials. In addition, the Agronomic Institute maintains one of the most complete collections of native and exotic plants in Latin America to recover degraded areas and form riparian forests.

The partnership between the IAC and the Brazilian cosmetics company Natura helped to develop and launch on the market a line of perfumes whose fragrance is unprecedented in the world.

The perfumes brands “Química do Humor” and “Urbano Noite” began to be researched in 2006 when a group of IAC researchers, of which Dr. Marcia Ortiz was a part, entered the Atlantic Forest biome in São Paulo State to search for plants with aromatic potential able to innovate the national perfumery. According to Dr. Ortiz, 120 species were selected by their smell. From then on, the process was long: obtaining the

essential oils, analyzing their chemical compositions, olfactory evaluations, etc. From the extraction of the plant to the flask, it took 12 years of work.

Researcher Dr. Marcia Ortiz is one of the authorities in Brazil when it comes to the development of new essential oils from Brazilian biodiversity for use in the perfumery industry.

The perfumes developed contain essential oil from Piper, which is a pepper from the Atlantic Forest, and have been a sales success up to today not only for the fragrance, unknown to the human sense of smell (among those catalogued in perfume bottles), but also for the quality of the products, as attested by experts in the field.

“Some people question the need for the Brazilian State to invest in research institutes. There are those who say that research does not give profit or financial return to society. Although generating profit is not the function of public institutions, it is not true that there is no financial return from research. In this specific case of the partnership with the Natura company, the IAC obtained a return in the form of royalties. In other words, the IAC has a share in product sales”, explained Dr. Ortiz.

Dr. Ortiz makes a point of stressing that research on the Brazilian biodiversity carried out directly in the Atlantic Forest has always been associated with environmental preservation. For the manufacture of perfumes, for example, plants were not removed from the natural habitat of the Atlantic Forest. Instead, the IAC team developed planting technologies for Natura so that the company could cultivate and extract Piper pepper from its own greenhouses.

Dr. Marcia Ortiz is currently one of the most experienced researchers in Brazil when it comes to the development of new essential oils from Brazilian biodiversity for use in the perfumery industry.

IAC Award

Since 1994, the Agronomic Institute has offered the IAC Award in recognition of scientific merit in two categories: Support (technical or administrative) and Scientific Researcher.

Since 2001, this award has also been offered in recognition of scientific merit and institutional performance to professionals and institutions outside the IAC who are outstanding in agriculture in the State of São Paulo and in Brazil. The IAC aims to offer this award to honor external individuals or legal entities that, in the agricultural area, have distinguished themselves by their contribution in the scientific and technological spheres or in practical activities that promote the development of sustainable agriculture and the improvement of the farmer's income and of the agribusiness of São Paulo State.

This award is given to up to three individuals or institutions chosen from the following categories: Research Funding Agency, Special Highlight, Personality of Agribusiness, Personality of Research or Teaching or Extension, Parliamentarian linked to Agribusiness and/or to Science and Technology, and Rural Producer. Candidates are nominated by individuals or institutions linked to the Brazilian agribusiness, class entities, associations, unions, companies, cooperatives, universities, and research and rural extension institutions. The nominated names are evaluated by the commission in charge of the IAC Award, which is composed of employees of the Agronomic Institute and endorsed by its Board of Directors.

The IAC Award consists of a miniature of the D. Pedro II Building in bronze on black granite created by the artist Giuseppe Botica and executed by FUNDIART – Fundição Artística LTDA. The D. Pedro II Building, the first site to house the IAC headquarters, was built in 1888 in an art nouveau style and is protected by the Defense Council of the Cultural Heritage of Campinas (CONDEPACC) and by the Defense Council of the Historical, Archaeological, Artistic and Tourist Heritage (CONDEPHAAT).

The IAC Award ceremony is held during the formal sitting that closes the festivities to celebrate the Agronomic Institute's anniversary on June 27.

Medal Franz Wilhelm Daffert

In 2009, the "Franz Wilhelm Daffert Merit Medal" was instituted to honor personalities and institutions for their personal values and relevant services rendered to Brazilian agriculture.

Franz Wilhelm Daffert was the founder and first director of the Agronomic Institute. A young Austrian scientist with a doctorate in agricultural chemistry, he was hired by the Brazilian government to organize and direct the agronomic research institute. His management, considered admirable, was a period in which important transformations occurred in the institution, making it possible to better meet demands and improve services.

Under his direction, the Agronomic Institute was the first institution to perform soil and plant analysis in Brazil, receiving the Silver Medal for Soil Analysis at the 1904 Universal Exposition in Saint Louis, USA.

BrJAC

SPONSOR REPORT

PDF

This section is dedicated for sponsor responsibility articles.

Fully Automated, Intelligent, High-Throughput Elemental Analysis of Drinking Waters using SQ-ICP-MS

Marcus Manecki, Daniel Kutscher, Christoph Wehe, Robert Henry, Julian Wills and Shona McSheehy Ducos

Thermo Fisher Scientific, Bremen, Germany

This report was extracted from the Thermo Scientific Application Note 43323

The goal of this work is to demonstrate robust high-throughput analysis of environmental samples using SQ-ICP-MS in He-KED mode, in accordance with the requirements of U.S. EPA method 200.8 Revision 5.5 and to demonstrate the performance of the Thermo Scientific™ iCAP™ RQ ICP-MS coupled to the ESI prep*FAST* Autodilution system.

Keywords: Autodilution, Drinking water, EPA method 200.8 revision 5.5, He KED, ICP-MS, Sample preparation, SQ-ICP-MS.

INTRODUCTION

EPA Method 200.8 analyses for the quantification of trace metals in drinking and waste waters are performed routinely in many laboratories. Thousands of analyses are performed per week to support the monitoring and control of drinking water contaminants and water quality. Due to the complexity of the standard operating procedure (SOP), skilled technicians are required to setup and prepare the daily analysis, as well as actively monitor the results and perform further sample manipulation as required throughout the analytical run. The need for technical staff is a factor that keeps the overall expense of routinely running the 200.8 method relatively high.

Recent advances in autodilution offer the potential to automate much of the sample preparation and data review with automated re-runs of any samples that do not meet predefined limits. By automatically creating a calibration set of standards from one stock standard and then diluting each sample to a predefined dilution level, an autodilution system can save valuable analysts' time and reduce costs overall through the lowered consumption of utilities and lab supplies.

Fast sample throughput is another driving factor when implementing routine SOPs. Throughput in the method described herein is improved by the discrete sampling of the autodilution system, dramatically reducing uptake and washout time, as well as the use of a single measurement mode for the analysis of all the analytes in the method.

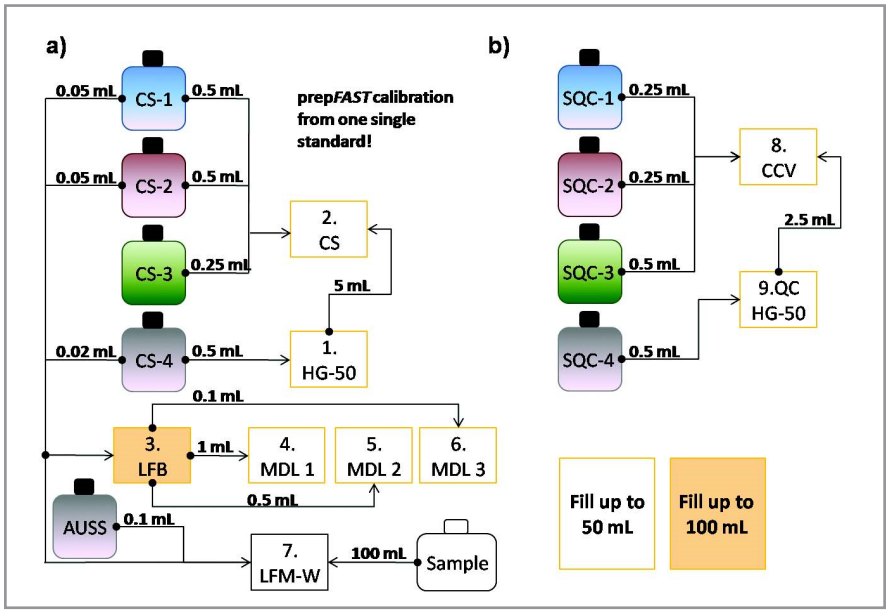
The use of kinetic energy discrimination with helium as a reaction cell gas (He KED) ensures comprehensive interference removal and confidence in the accuracy of the analytical results. Whereas other single quadrupole (SQ) ICP-MS systems require multiple methods for the analysis of drinking water, the iCAP RQ ICP-MS collision/reaction cell (QCell) has a high ion transmission across the mass range so that all of the analytes in the method, including low mass analytes such as Li and Be, can be measured in He KED mode. This eliminates the extra overheads of switching times between different modes and simplifies method development.

This report describes the fully automated, intelligent, high throughput EPA 200.8 analysis of environmental samples using a prep*FAST* Autodilution system (Elemental Scientific Inc., Omaha, NE, USA) integrated with the iCAP RQ ICP-MS.

MATERIALS AND METHODS

Sample Preparation for U.S. EPA 200.8 Rev 5.5

All samples were prepared according to the EPA 200.8 method. For the determination of dissolved analytes in drinking water, tap water was collected in an HDPE tank and acidified to 1% v/v HNO_3 (Optima™ grade acid, Fisher Chemicals). Aliquots (20 mL) from the tank were filled into 50 mL polypropylene centrifuge tubes for analysis. The standards and quality control (QC) solutions were prepared according to the protocol outlined in Figure 1.



AUSS: Gold Standard Solution, CCV: Continuous Calibration Verification, CS-1 to 4: Calibration Standards, HG-50: Mercury Standard (50 ppb), LFB: Laboratory Fortified Blank, LFM-W: Laboratory Fortified Matrix, MDL-1 to 3: Solutions to determine Method Detection Limit, SQC-1 to 4: Standards for Quality control.

Figure 1. Scheme of (a) standard and (b) QC solutions required for EPA 200.8.

Mass Spectrometry

The iCAP RQ ICP-MS coupled to the prep*FAST* Autodilution system with an SC-2DX Autosampler (Figure 2) was used for acquisition of all data. The iCAP RQ ICP-MS was operated in He KED mode for all analytes. Instrumental parameters are listed in Table I.

Table I. Instrument conditions

Parameter	Value
iCAP RQ ICP-MS	
Nebulizer	PFA-ST
Nebulizer Gas Flow	1.02 L min^{-1}
Interface Setup	Ni Cones, High Matrix Skimmer insert
Cell Gas Flow	4.8 mL min^{-1} He
KED Voltage	3 V
prep*FAST*	
Sample Loop	1.5 mL
Time per Analysis	66 s

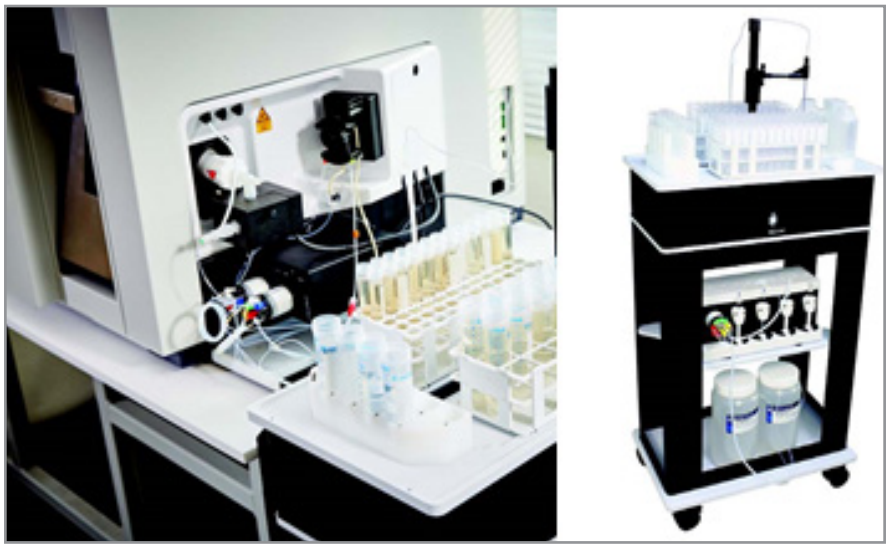


Figure 2. prep*FAST* Autodilution system connected to the iCAP RQ ICP-MS (left). ESI SC-2DX Autosampler (right).

Data Analysis

Thermo Scientific Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software was used for quantitative assessment of the data. Working from a predefined EPA 200.8 template, the only user action needed is to enter the number of samples to be analyzed in the analytical batch. All parameters that must be monitored and achieve certain criteria to comply with EPA 200.8 are automatically checked by the Quality Control feature set included in the default installation of the Qtegra ISDS Software. Samples that do not meet all criteria e.g. Internal Standard (ISTD) recovery rates or over-range analyte concentrations, are automatically diluted to an appropriate level as calculated or defined within the software and the measurement automatically repeated.

Intelligent Autodilution with prepFAST

Dilution factors of up to 400-fold are performed reliably and accurately, with all flows controlled by high precision syringe pumps. With the intelligent dilution feature, Qtegra ISDS Software registers every analyte that falls outside of the defined quality control requirements.

If an analyte exceeds the calibration range (Figure 3) the intelligent autodilution dilutes the sample and re-measures only the affected analytes without manual interaction. The applied dilution factor is recorded in the software for full traceability of all dilution steps executed during data acquisition.

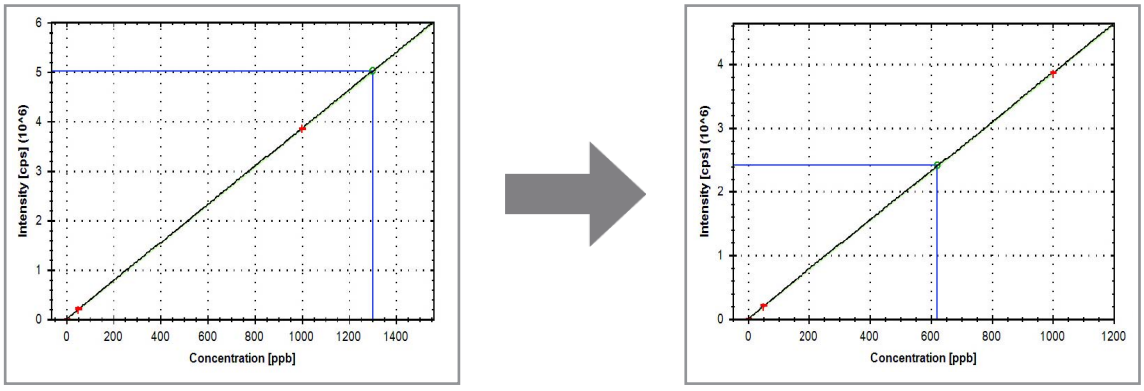


Figure 3. Analyte concentration re-analyzed by intelligent auto-dilution.Original sample (left), reanalyzed analyte with dilution factor 2.165 (right).

RESULTS AND DISCUSSION

Routine Performance of the iCAP RQ ICP-MS

Over 320 tap water samples were analyzed according to method EPA 200.8. The analysis time was, on average, 66 s per sample for the analysis of 21 elements listed in EPA method 200.8 plus 6 different internal standards, leading to a total number of 48 individual isotopes being read out per sample. The concentration of all analytes and their ISTD recovery was monitored throughout the whole analysis time. In total, 508 analyses were run in less than 10 h. Internal standard recovery was well within the EPA 200.8 method requirements of 60 to 125% (Figure 4).

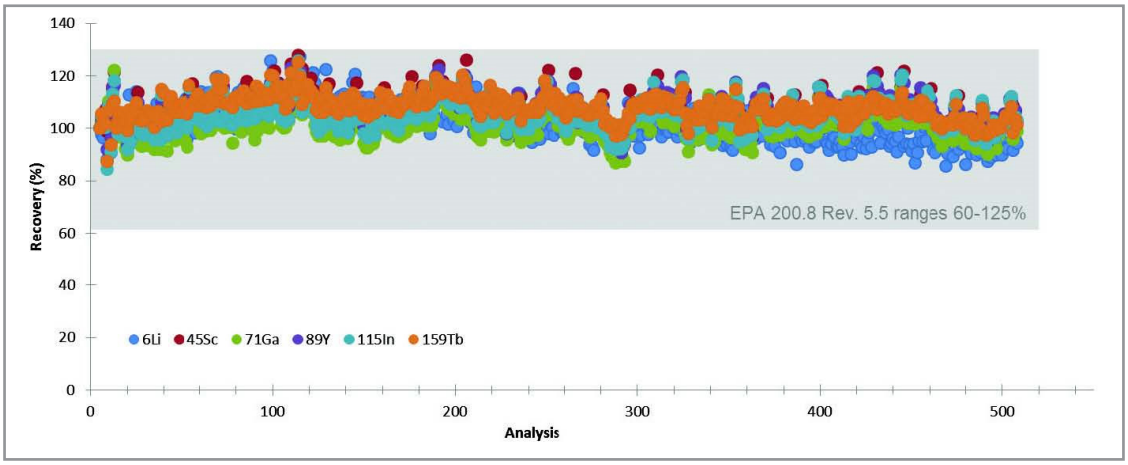


Figure 4. Internal standard response of running tap water samples and QCs showing recoveries well within the 60 – 125% range specified in EPA Method 200.8.

Quality Control (QC) Samples

During the analysis run, a Continuing Calibration Verification (CCV) QC sample was analyzed every 10 samples to assess the accuracy of the calibration throughout the entire batch.

The EPA 200.8 method requires that the recovery of this QC must be within ± 10%, or within the acceptance limits of the method (EPA 200.8, rev 5.5, Table 8). All elements were found to be accurate to within ± 10% of the known concentration, as well as the acceptance criteria, and were stable over all repeated analyses (Figure 5).

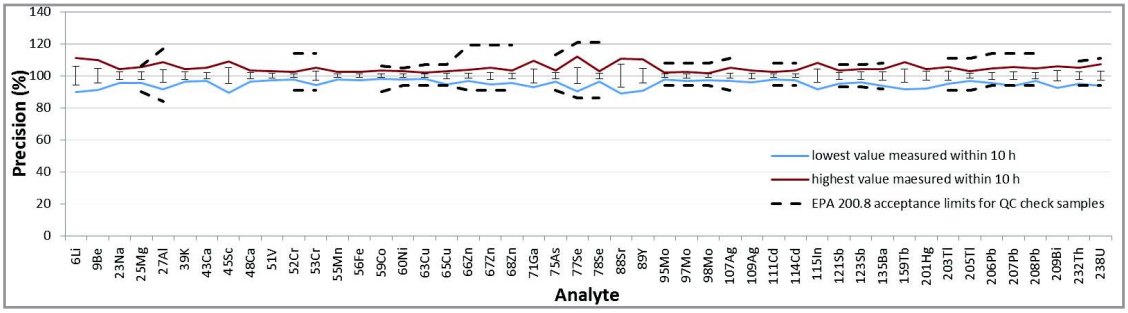


Figure 5. QC recovery and stability of the continuous calibration samples over the entire batch.

Laboratory Fortified Blank and Laboratory Fortified Matrix Recoveries

The recovery of a Laboratory Fortified Blank (LFB) with known added amounts of analytes (Figure 1a, solution 3) must be measured at least once per batch of samples. During this assessment, the LFB was analyzed 32 times and the calculated recovery rates are shown in Figure 6. All analytes show recoveries

within the limits (85–115%) of EPA 200.8. Similar to the LFB recovery for every batch, one sample must also be spiked with a known amount of analytes, (Laboratory Fortified Matrix sample; LFM). All 32 LFM (Figure 1a, solution 7) samples were within the EPA 200.8 recovery limits (75-130%).

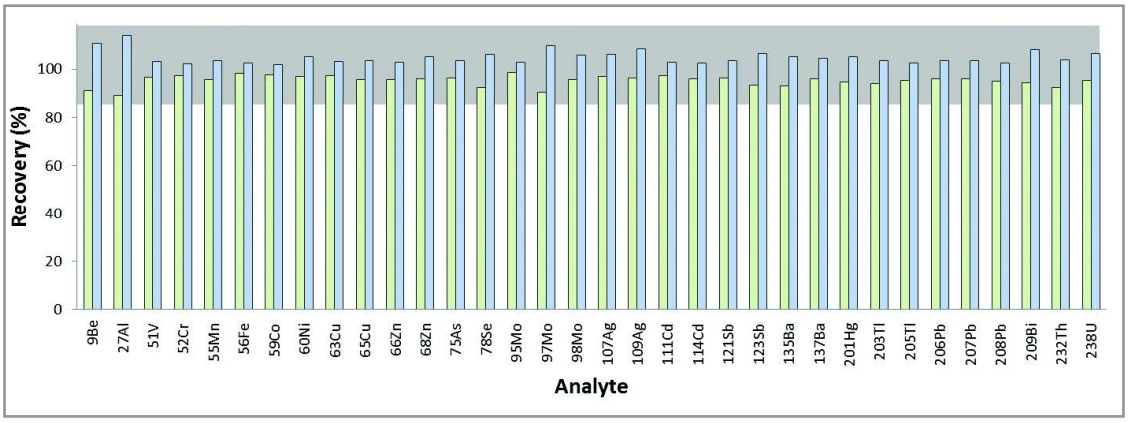


Figure 6. Laboratory Fortified Blank (LFB) recoveries from measurements. Blue bars show the highest (green lowest) recovery of the analyte measured during the 10 h run. The grey area represents the EPA 200.8 acceptance range (85-115%) for LFB recoveries.

Driven by Qtegra ISDS Software

Fully Integrated

The Qtegra ISDS Software provides all required features needed for the high throughput analysis of environmental samples. Together with the fully integrated prep*FAST* Autodilution system, Qtegra ISDS Software offers:

- Prescriptive dilution of samples and calibration standards.
- Continuous monitoring of all quality controls (LFB and LFM recoveries or duplicate sample verification)
- LabBook feature that starts an intelligent sequence, with full QA/QC protocols, and subsequently processes and reports results.
- Comprehensive, user definable reports enabling flexible export to external LIMS software packages.

Intelligent autodilution for samples exceeding the calibration range is fully integrated. Samples re-measured by the Qtegra ISDS Software are added automatically to the sample list and clearly identified by a plus sign (Figure 7).

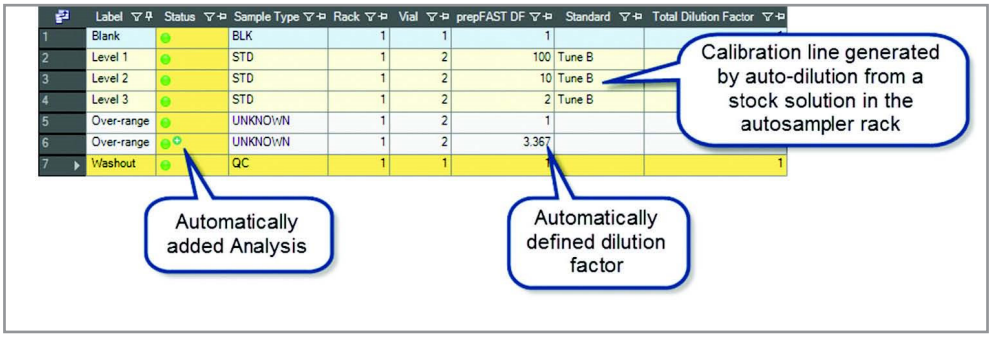


Figure 7. Screenshot of the intelligent auto-dilution process in Qtegra ISDS Software.

CONCLUSION

The Thermo Scientific iCAP RQ ICP-MS equipped with an ESI Autosampler and prep*FAST* Autodilution System was successfully validated for use with US EPA Method 200.8. With the robust iCAP RQ ICP-MS paired with an ESI prep*FAST* Autodilution system, it is possible to run the entire analysis (encompassing sample dilution, calibration and measurement) with minimal manual intervention. After optimizing the uptake and washout parameters, the high sensitivity and stability of the iCAP RQ ICP-MS readily achieved the goal of 52 EPA Method 200.8 analyses per hour.

Robustness

The iCAP RQ ICP-MS delivers reliable analysis of drinking water with minimal drift when equipped with the high matrix insert. For extra robust operation in the face of higher matrix samples, the system can be equipped with the robust plasma interface.

Productivity

The iCAP RQ ICP-MS in combination with the ESI prepFAST Autodilution System is the ideal system to measure environmental samples in a high throughput laboratory.

Simplicity

With the prescriptive and intelligent dilution capabilities provided by the system, manual sample preparation and data post-processing is minimized.

No Impact on Bench Space

The integrated dual valve assembly is mounted directly beneath the sample introduction system, minimizing sample pathways.

This sponsor report is the responsibility of Thermo Fisher Scientific.

SPONSOR REPORT

PDF

This section is dedicated for sponsor responsibility articles.

US EPA Method 200.7 using the Thermo Scientific iCAP PRO XPS Duo ICP-OES

Sanja Asendorf

Thermo Fisher Scientific, Bremen, Germany

This report was extracted from the Thermo Scientific Application Note 44422

Keywords: Drinking water, Environmental, analysis, Method 200.7, US EPA

Goal

This note describes the use of the Thermo Scientific iCAP PRO XPS Duo ICP-OES for the analysis of water samples using the US EPA Method 200.7.

INTRODUCTION

The analysis and monitoring of natural, produced and drinking waters is essential to ensure both human and environmental health. Levels of permissible contamination are controlled by local, national and international regulations. In the United States of America the Environmental Protection Agency (EPA) is the body responsible to set and regulate national standards for the quality of supplied drinking water and drinking water resources, such as ground waters. The EPA Office of Ground Water and Drinking Water (OGWDW) administers control under the Federal Regulation 40 CFR part 141 & 143. This regulation states that all supplied waters must comply with the Maximum Contaminant Levels (MCL) for the contaminants specified in the National Primary Drinking Water Regulations (NPDWR). Table 1 lists the MCL and Maximum Contaminant Level Goals (MCLG) that the EPA defines as the maximum level of an element in drinking water at which no known or anticipated adverse effect on the health of persons would occur.

Table 1. MCLs and MCLG for the national drinking water regulations

National primary drinking water regulations		
Contaminant	**MCL (mg L^{-1})**	**MCLG (mg L^{-1})**
Antimony	0.006	0.006
Arsenic	0.01	0
Barium	2.0	2.0
Beryllium	0.004	0.004
Cadmium	0.005	0.005
Chromium (total)	0.1	0.1
Copper	1.3	1.3
Lead	0.015	0
Mercury	0.002	0.002
Selenium	0.05	0.05
Thallium	0.002	0.002
Uranium	0.03	0

Further contaminants are given suggested maximum values in the National Secondary Drinking Water Regulations (NSDWR) as these elements will affect water properties such as taste and color (Table 2). The Unregulated Contaminant Monitoring Rule 3 (UCMR-3) requires that measurements are taken and recorded for two areas at every water treatment plant; the metals to be tested and their Maximum Reporting Limits (MRL) are shown in Table 3.

Table 2. MCLs for the national secondary drinking water regulations

National secondary drinking water regulations	
Contaminant	**MCL (mg L^{-1})**
Aluminium	0.05 – 0.2
Copper	1
Iron	0.3
Manganese	0.05
Silver	0.1
Sulphate	250
Zinc	5

Table 3. MRLs for Unregulated Contaminant Monitoring Rule 3

Unregulated Contaminant Monitoring Rule 3 (UCMR-3)	
Contaminant	**MRL (mg L^{-1})**
Chromium (total)	0.0002
Cobalt	0.001
Molybdenum	0.001
Strontium	0.0003
Vanadium	0.0002

The EPA Method 200.7 "Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry" is used extensively for the analysis and monitoring of a range of waters including, ground, river, drinking and waste water. The results of the analysis are used for a variety of purposes, in the case of drinking water the results are used to ensure consumer safety and in the case of waste waters the results are to determine compliance with the permits issued within the National Pollutant Discharge Elimination System (NPDES) under the Clean Water Act (CWA) (40 CFR part 136).

Large numbers of water samples are analyzed using this method, including supplied waters, natural waters and waste waters. The method is commonly used in US States that require well water on private property to be analyzed prior to the purchase of real estate. Method 200.7 is used globally as the basis of water analysis methods by ICP-OES, particularly in regions where environmental monitoring developed later than in the US.

METHOD 200.7 SUMMARY

Method 200.7 describes the determination of 31 elements in water samples and suggests preferred wavelengths, calibration and quality control procedures in addition to specifying procedures for determining method performance characteristics, such as detection limits and linear ranges. A brief overview of the method procedures follows below.

Method detection limit

The method provides a protocol for determining the method detection limit (MDL). The instrument hardware and method are set up as intended for the analysis. A reagent blank solution spiked at 2-3 times the estimated instrument detection limit is subjected to seven replicate analyses. The standard deviation (SD) of the measured concentrations is determined and multiplied by 3.14 (the Student's *t* value for a 99% confidence interval for 6 degrees of freedom) to calculate the MDL. It is important that contamination is kept under control, especially for environmentally abundant elements such as Al and Zn, since any contamination will degrade the MDL. Interference corrections also affect the MDL, since they employ the monitoring of additional wavelengths and propagate the measurement errors accordingly.

Linear dynamic range

The upper linear range limit of a calibration is termed the linear dynamic range (LDR). Method 200.7 defines the upper LDR to be the highest concentration at which an observed signal deviates by less than 10% from that extrapolated from lower standards. Sample dilution can facilitate the measurement of high concentrations, but with additional effort, cost and error. Therefore, a wide LDR is desirable.

Quality control

Method 200.7 specifies a variety of quality control (QC) standards. These are summarized in Table 4.

Table 4. Summary of Method 200.7 QC requirements

Check name	Check code	Purpose	Frequency	Limits
QCS	Quality Control Standard	Checks the accuracy of the calibration with a second source standard	Post calibration	95-105% recovery
SIC	Spectral Interference Check Solution(s)	Checks for the presence of spectral interference and the effectiveness of inter-element corrections	Periodically	No specific requirements
IPC	Instrument Performance Check	A continuing check of accuracy and drift normally done by re-measuring a standard as a sample	Every 10 analyses and at the end of the run	95-105% recovery immediately following calibration; 90-110% recovery thereafter
Blank	Check Blank	A continuing check of the blank level by re-measuring the calibration blank as a sample	Every 10 analyses and at the end of the run	< IDL
LRB	Laboratory Reagent Blank	Checks the laboratory reagents and sample preparation process for contamination	1 per batch of 20 or fewer samples	< 2.2 x MDL
LFB	Laboratory Fortified Blank	Checks the recovery of analytes by spiking a known quantity into a blank	1 per batch of samples	85-115% recovery or within ±3 standard deviations of the mean recovery

Table 4 continuation. Summary of Method 200.7 QC requirements

Check name	Check code	Purpose	Frequency	Limits
LFM	Laboratory Fortified Matrix	Checks the recovery of analytes in a matrix by spiking a known quantity into a batch sample	1 in 10 samples	85-115% recovery or within ±3 standard deviations of the mean recovery

*<IDL: below instrument detection limit.

Instrumentation

A Thermo Scientific™ iCAP™ PRO XPS Duo ICP-OES was used for this analysis. The duo view plasma allows for elements expected at trace levels to be analyzed axially, for best sensitivity and for elements expected at high concentrations to be measured radially, for best dynamic range. In conjunction with this instrument, a Teledyne CETAC ASX-560 Autosampler was used. An internal standard mixing kit was also used to introduce a 5 mg L^{-1} yttrium internal standard solution online. Sample introduction details and instrument parameters are given in Table 5.

Method

A LabBook was set up using the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software for all 31 elements covered by Method 200.7. Sulfur, which is not part of Method 200.7 but is often required in this type of analysis, was also added to the method. Additionally, yttrium wavelengths were added, to be used as an internal standard. The acquisition parameters used are shown in Table 5.

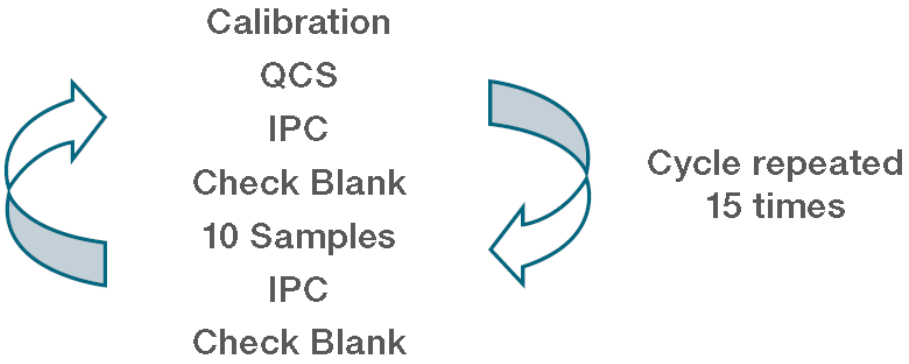
Table 5. Instrument parameters

Parameter	Setting	
Pump tubing	Sample Tygon® orange/white Drain Tygon® white/white	
Pump speed	45 rpm	
Spray chamber	Glass cyclonic	
Nebulizer	Glass concentric	
Nebulizer gas flow	0.5 L min^{-1}	
Auxiliary gas flow	0.5 L min^{-1}	
Coolant gas flow	12 L min^{-1}	
Center tube	2 mm	
RR power	1150 W	
Repeats	3	
Radial viewing height	10 mm	
Exposure time	Axial 10 sec	Radial 10 sec

All samples were preserved in 1.5% TraceMetal™ grade nitric acid (Fisher Chemicals, Loughborough, UK). Calibration standards and QC solutions were prepared using 1000 mg·L^{-1} standard solutions (Fisher Chemicals, Loughborough, UK); acid matched to the samples and made up to volume with ultra-pure deionized water (≥18.2 mΩ).

Analytical procedure

A linear dynamic range (LDR) and method detection limit (MDL) study was performed as described in Method 200.7. The MDL study was performed with a reagent blank spiked with low concentrations of each element. An interference study was performed using single element SIC solutions as described in Method 200.7. To demonstrate the performance of the iCAP PRO XPS Duo ICP-OES for typical routine analysis of a variety of water samples with Method 200.7, a sequence was set up as follows:



The 10 samples analyzed between each IPC and blank pair consisted of a variety of aqueous matrices. Three sample types were analyzed, a drinking water, a trench water and a well water; each was spiked for analysis as a laboratory fortified matrix (LFM). The samples were analyzed multiple times throughout the process, replicating a run consisting of a total number of 114 samples (152 samples, including QC and calibration solutions).

Interference study

A comprehensive interference evaluation was performed using single element SIC solutions of the following concentrations: 300 mg L^{-1} Fe, 200 mg L^{-1} Al and 50 mg L^{-1} of each, Ba, Be, Cd, Ce, Co, Cr, Cu, Mn, Mo, Ni, Sn, Si, Ti, Tl, and V. If the apparent concentration of an interferent was above the quantification limit of the method, an inter-element correction (IEC) factor was established and applied. During this study, a few minor and only 9 significant (contribution of up to 1 mg L^{-1}) interferences were identified in accordance with Table 2 of the annex of method 200.7, showing that the selected wavelengths are relatively interference free. The interferences observed (shown in Table 6) can easily be corrected for by applying the automatically calculated interference correction factors when necessary.

RESULTS

Table 6. Comprehensive interference evaluation results

Element and wavelength (nm)	SIC solution	Contribution (mg L^{-1})	Element and wavelength (nm)	SIC solution	Contribution (mg L^{-1})
Al 308.215	Ce	0.212	S 182.034	Mo	0.085
Al 308.215	Mo	1.314	S 182.034	Sn	0.074
Al 308.215	V	0.539	Sb 206.833	Ce	-0.111
As 193.759	Al	0.123	Sb 206.833	Cr	0.905
B 249.678	Co	0.066	Se 196.090	Fe	-0.031
Ba 455.403	Mo	-0.014	Se 196.090	Mn	0.021
Ca 315.887	Mo	0.14	Si 251.611	Mo	0.434
Co 228.616	Ti	0.109	Sn 189.989	Ce	0.009

Table 6 continuation. Comprehensive interference evaluation results

Element and wavelength (nm)	SIC solution	Contribution (mg L^{-1})	Element and wavelength (nm)	SIC solution	Contribution (mg L^{-1})
Cu 224.700	Mo	0.058	Ti 334.941	Cr	0.01
Cu 224.700	Ti	0.015	Tl 190.856	Ce	0.025
Hg 194.227	V	0.027	Tl 190.856	Co	0.055
Hg 194.227	Mn	0.022	Tl 190.856	V	0.040
P 177.495	Cu	0.131	V 292.402	Mo	-0.02
P 177.495	Ni	0.044	V 292.402	Ti	0.027
S 182.034	Mn	0.201	Zn 213.856	Ni	0.041

LDR

The high standards analyzed for the linear dynamic range check showed little deviation from their expected values, indicating linearity up to at least the levels indicated in Table 7. These levels are normally more than sufficient for the analysis of typical water samples.

Table 7. Analytical wavelengths, plasma views used, LDR and MDL achieved

Analyte	Wavelength (nm)	Plasma view	LDR (mg L^{-1})	MDL (µg L^{-1})	Level of interest (µg L^{-1})
Ag	328.608	Axial	>10	0.84	100
Al	308.215	Radial	>1000	21	50-200
As	193.759	Axial	>100	2.1	10
B	249.678	Axial	>100	1.2	N/A
Ba	455.403	Axial	>2	0.47	2.000
Be	234.861	Axial	>10	0.08	4
Ca	315.887	Radial	>100	6.0	N/A
Cd	226.502	Axial	>10	0.25	5
Co	228.616	Axial	>10	0.75	1*
Cr	284.325	Axial	>10	0.29	100/0.2*
Cu	224.700	Axial	>10	0.51	1.300
Fe	258.940	Radial	>1000	3.7	300
Hg	194.227	Axial	>100	1.0	2
K	766.490	Radial	>1000	42	N/A
Li	670.784	Radial	>100	3.3	N/A
Mg	279.079	Radial	>1000	21	N/A
Mn	257.610	Axial	>10	0.06	50
Mo	203.844	Axial	>10	0.90	1*
Na	589.592	Radial	>100	20	N/A
Ni	231.604	Axial	>10	0.85	N/A
P	177.495	Axial	>10	2.5	N/A
Pb	220.353	Axial	>100	3.2	15

Table 7 continuation. Analytical wavelengths, plasma views used, LDR and MDL achieved

Analyte	Wavelength (nm)	Plasma view	LDR (mg L^{-1})	MDL (µg L^{-1})	Level of interest (µg L^{-1})
SO_4	182.034	Axial	>300	16.5	250000
Sb	206.833	Axial	>100	3.3	5
Se	196.090	Axial	>10	4.8	50
SiO_2	251.611	Radial	>2000	11.8	N/A
Sn	189.989	Axial	>10	0.73	N/A
Sr	421.552	Axial	>1	0.02	0.3*
Ti	334.941	Axial	>10	0.61	N/A
Tl	190.856	Axial	>10	1.8	2
V	292.402	Axial	>10	0.50	0.2*
Zn	213.856	Axial	>2	0.02	5000

*Maximum report limit required for UMCR-3

N/A: value not available

MDL

The method detection limits calculated from analysis of the MDL solution were generally in the low and sub µg L^{-1} range for the majority of elements. All MDLs were sufficiently below the typical levels of interest for drinking water analysis according to National Primary and Secondary Drinking Water Regulations, with the exception of aluminium, antimony, mercury and thallium. The MDLs for these elements were of the same magnitude as the level of interest. For this reason ICP-MS, such as delivered by the Thermo Scientific™ iCAP™ RQ ICP-MS may be a more appropriate alternative for the regulatory drinking water measurements for these elements.

Accuracy, precision and stability

The iCAP PRO XPS Duo ICP-OES produced consistently accurate results with minimal intensity drift, as shown by the results for the QCS and IPC solutions (see Table 8). The ongoing IPC results were consistently within the allowed range of 90-110% of the known value, as shown in Figure 1. The precision of the 9 IPC measurements across the 150 sample run were also shown to be very good. Table 8 indicates that the relative standard deviations (RSDs) of these measurements were within 5% across the duration of the run.

Table 8. QCS and IPC results

	QCS			IPC (n=9)			
Analyte	Measured (mg L^{-1})	Known (mg L^{-1})	Recovery (%)	Measured (mg L^{-1})	Known (mg L^{-1})	Recovery (%)	RSD (%)
Ag	0.207	0.2	103.5	0.201	0.2	100.5	1.7
Al	0.998	1	99.8	1.989	2	99.5	1.1
As	9.828	10	98.3	1.917	2	95.9	1.8
B	9.869	10	98.7	2.009	2	100.5	0.7
Ba	0.980	1	98.0	2.009	2	100.5	1.5
Be	1.473	1.5	98.2	1.964	2	98.2	0.7
Ca	97.964	100	98.0	1.914	2	95.7	0.9

Table 8 continuation. QCS and IPC results

	QCS			IPC (n=9)			
Analyte	**Measured (mg L^{-1})**	**Known (mg L^{-1})**	**Recovery (%)**	**Measured (mg L^{-1})**	**Known (mg L^{-1})**	**Recovery (%)**	**RSD (%)**
Cd	0.957	1	95.7	2.011	2	100.6	1.3
Co	0.967	1	96.7	2.014	2	100.7	1.0
Cr	1.012	1	101.2	2.039	2	102.0	0.8
Cu	0.998	1	99.8	1.981	2	99.1	0.5
Fe	9.803	10	98.0	2.001	2	100.1	0.5
Hg	0.976	1	97.6	2.052	2	102.6	1.8
K	5.029	5	100.6	9.598	10	96.0	2.3
Li	2.088	2	104.4	2.004	2	100.2	2.7
Mg	0.971	1	97.1	2.019	2	101.0	1.2
Mn	0.985	1	98.5	1.996	2	99.8	0.8
Mo	0.989	1	98.9	1.963	2	98.2	1.6
Na	1.001	1	100.1	2.085	2	104.3	3.4
Ni	0.980	1	98.0	2.035	2	101.8	1.1
P	5.071	5	101.4	2.106	2	105.3	2.7
Pb	0.976	1	97.6	2.044	2	102.2	1.3
SO_4	61.84	60	103.1	5.78	6	96.3	2.2
Sb	0.986	1	98.6	1.984	2	99.2	1.1
Se	9.664	10	96.6	1.923	2	96.2	1.8
SiO_2	2.14	2.14	100.0	21.68	21.4	101.3	0.5
Sn	0.950	1	95.0	2.099	2	105.0	1.6
Sr	1.983	2	99.2	1.953	2	97.7	0.7
Ti	0.983	1	98.3	2.008	2	100.4	0.7
Tl	1.009	1	100.9	2.023	2	101.2	1.6
V	0.983	1	98.3	1.935	2	96.8	0.6
Zn	1.022	1	102.2	1.898	2	94.9	1.5

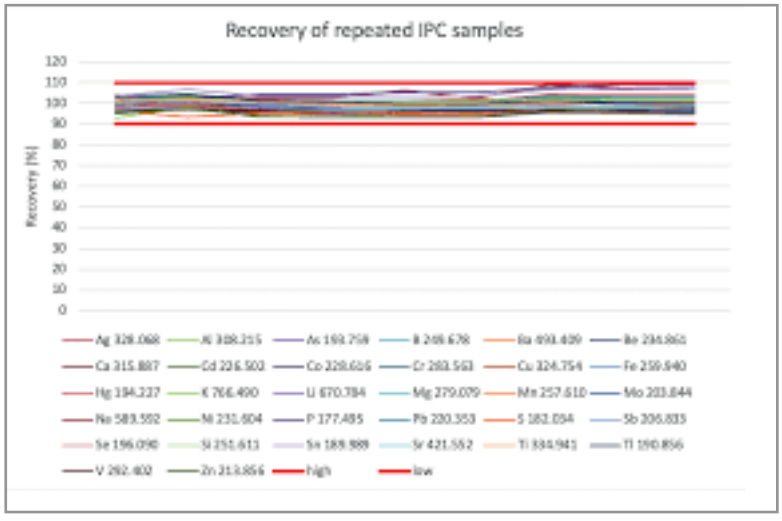


Figure 1. Recovery graph of successive IPC measurements for all analyzed elements during the 114 sample analyses with the accuracy interval of 90-110% indicated as high and low.

The accurate results for the LFM samples (shown in Table 9) show that quantitative recovery can be achieved in a variety of real environmental matrices. All spike recoveries were well within the allowable range of 85-115%.

Table 9. Laboratory fortified matrix results

Analyte	Drinking water			Trench water			Well water			Laboratory fortified blank		
	Unspiked (mg L^{-1})	Spiked (mg L^{-1})	Recovery (%)	Unspiked (mg L^{-1})	Spiked (mg L^{-1})	Recovery (%)	Unspiked (mg L^{-1})	Spiked (mg L^{-1})	Recovery (%)	Unspiked (mg L^{-1})	Spiked (mg L^{-1})	Recovery (%)
Ag	<MQL	0.097	97.0	<MQL	0.087	87.0	<MQL	0.107	107.0	<MQL	0.104	104.0
Al	<MQL	1.999	100.0	<MQL	1.999	100.0	<MQL	1.958	97.9	<MQL	2.085	104.3
As	<MQL	0.200	100.0	<MQL	0.203	101.5	<MQL	0.203	101.5	<MQL	0.195	97.5
B	<MQL	0.224	112.0	0.167	0.364	98.5	0.116	0.314	99.0	<MQL	0.198	99.0
Ba	0.017	0.215	99.0	0.066	0.255	94.5	0.246	0.440	97.0	<MQL	0.209	104.5
Be	<MQL	0.206	103.0	<MQL	0.206	103.0	<MQL	0.215	107.5	<MQL	0.206	103.0
Ca	40.52	42.43	95.5	49.92	57.39	99.6	46.20	53.66	99.5	<MQL	1.950	97.5
Cd	<MQL	0.199	99.5	<MQL	0.196	98.0	0.001	0.199	99.0	<MQL	0.203	101.5
Co	<MQL	0.195	97.5	<MQL	0.191	95.5	<MQL	0.193	96.5	<MQL	0.199	99.5
Cr	<MQL	0.202	101.0	<MQL	0.200	100.0	<MQL	0.199	99.5	<MQL	0.207	103.5
Cu	0.024	0.319	98.3	<MQL	0.291	97.0	0.007	0.296	96.3	<MQL	0.301	100.3
Fe	0.045	0.239	97.0	1.360	8.701	97.9	27.40	34.82	98.9	<MQL	0.202	101.0
Hg	<MQL	0.196	98.0	<MQL	0.196	98.1	<MQL	0.197	98.5	<MQL	0.198	99.0
K	2.747	7.795	101.0	12.56	15.31	110.0	1.401	4.116	108.6	<MQL	4.311	86.2
Li	<MQL	0.231	115.5	0.018	0.225	103.5	0.013	0.226	106.5	<MQL	0.207	103.5
Mg	4.271	11.60	97.7	7.863	14.95	94.5	6.953	14.02	94.2	<MQL	7.777	103.7
Mn	0.003	0.201	99.0	0.065	0.256	95.5	2.583	2.790	103.5	<MQL	0.204	102.0
Mo	<MQL	0.194	97.0	<MQL	0.194	97.0	<MQL	0.194	97.0	<MQL	0.193	96.5
Na	14.24	19.67	108.6	145.31	170.8	102.0	92.85	118.3	101.8	<MQL	1.535	102.3
Ni	<MQL	0.197	98.5	<MQL	0.194	97.0	<MQL	0.195	97.5	<MQL	0.203	101.5
P	0.015	1.644	108.6	0.102	1.730	108.5	1.185	2.742	103.8	<MQL	1.696	113.1
Pb	<MQL	0.197	98.5	<MQL	0.192	96.0	0.077	0.266	94.5	<MQL	0.204	102.0
SO_4	40.43	43.82	113.2	77.69	93.13	103.1	1.295	16.80	103.5	<MQL	2.864	95.6
Sb	<MQL	0.200	100.0	<MQL	0.195	97.5	<MQL	0.197	98.5	<MQL	0.194	97.0
Se	<MQL	0.193	96.5	<MQL	0.193	96.5	<MQL	0.194	97.0	<MQL	0.189	94.5
SiO_2	20.05	22.39	109.4	15.82	21.88	113.3	26.14	32.07	110.9	<MQL	0.412	100.1
Sn	<MQL	0.201	100.5	<MQL	0.196	98.0	<MQL	0.200	100.0	<MQL	0.206	103.0
Sr	0.129	0.324	97.5	0.362	0.539	88.5	0.466	0.645	89.5	<MQL	0.211	105.5
Ti	<MQL	0.193	96.5	<MQL	0.193	96.5	<MQL	0.193	96.5	<MQL	0.205	102.5
Tl	<MQL	0.294	98.0	<MQL	0.281	93.7	<MQL	0.283	94.3	<MQL	0.301	100.3
V	<MQL	0.198	99.0	<MQL	0.198	99.0	<MQL	0.197	98.5	<MQL	0.201	100.5
Zn	0.0009	0.22	109.6	0.0013	0.22	109.4	0.282	0.48	99.0	<MQL	0.218	109.0

*<MQL: measured concentration below method quantification limit (MQL = 3 x MDL).

CONCLUSION

The Thermo Scientific iCAP PRO XPS Duo ICP-OES demonstrated compliance with the requirements of EPA Method 200.7 for a wide range of water sample types. The instrument was successfully used to follow stringent analytical quality control requirements of the method, these were easily implemented in the LabBook by the built-in QC checking capability of the Qtegra ISDS Software which is designed to meet the requirements of EPA methods.

In this study, the full wavelength range was covered for both, axial and radial view, keeping analysis time to a minimum. For trace elements, ideal detection limits are established in the axial view while matrix elements are analyzed in radial view so that the full potential of the linear dynamic range is used. This reduces the need for sample reruns and dilution and improves overall productivity of high throughput laboratories. Detection limits may even be further improved utilizing eUV (enhanced UV) capabilities of the iCAP PRO XPS ICP-OES, which improves detection limits in the UV region by up to 20%.

The compact high transmission optical design and nonblooming CID detector produce optimum performance, as indicated by the excellent method detection limits obtained. The optimized vertical torch interface combined with the high resolution optics minimizing physical and spectral interferences as demonstrated by the interference study, making the iCAP PRO Series ICP-OES ideal for analyzing waters and other environmental sample types.

The productivity tools of Qtegra ISDS Software combined with the speed of the iCAP PRO XPS ICP-OES drive rapid analysis times. Samples in this study were processed at a speed of 1 sample every 1 minutes and 58 seconds, or 30 samples per hour. In addition, an external discrete sampling valve could be used to speed up the sample uptake time even further and therefore increase sample throughput. The system also incorporates fast start up ensuring the system is purged and stable within minutes to allow for maximum instrument utilization during a working day. These combined features make the iCAP PRO XPS ICP-OES the ultimate instrument for cost-effective elemental analysis.

Find out more at thermofisher.com/ICP-OES

This sponsor report is the responsibility of Thermo Fisher Scientific.

SPONSOR REPORT

PDF

This section is dedicated for sponsor responsibility articles.

Sample Preparation of Environmental Samples for Trace Metal Analysis

Ensuring high-quality and productivity in elemental analysis of environmental samples using the Milestone ETHOS UP

This report was extracted from a Milestone Industry Report on Ethos UP/Environmental

INTRODUCTION

Demand for trace metals analysis in environmental laboratories is growing strongly due to stricter environmental regulations. ICP has been the standard for metals analysis, but as demand for lower levels of detection grows, the laboratories are experiencing a significant transition to ICP-MS. This transition is placing increased emphasis on the sample preparation method. Traditional sample preparation techniques for environmental matrices include hot block digestion, closed vessel microwave digestion and ashing; all of which include 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.

Closed vessel microwave digestion is now included in the US EPA official sample preparation methods for most environmental samples.

ETHOS technology is perfectly designed for the three US EPA methods:
- EPA 3015A: Microwave assisted acid digestion of aqueous samples and extracts.
- EPA 3051A: Microwave assisted acid digestion of sediments, sludges, soils and oils.
- EPA 3052: Microwave assisted acid digestion of siliceous and organically based matrices.

The Milestone ETHOS UP, microwave digestion system, incorporates all of the benefits of closed vessel microwave digestion while making sample preparation fast, easy, effective, and the highest quality.

EXPERIMENTAL

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

Instrument

The ETHOS UP meets the requirements of modern analytical labs. It offers several unique benefits including:
- Increased ease of use and productivity
- Enhanced control in all vessels
- Fast, accurate and traceable
- Superior safety and digestion quality

The ETHOS UP is a flexible and high performing platform used for elemental analysis and routine determination in many applications. Its construction of stainless steel coated with five PTFE layers accommodates both high-pressure and high-throughput rotors.

Figure 1. Milestone's ETHOS UP.

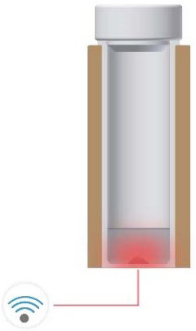


Figure 2. easyTEMP contactless direct temperature sensor

easyTEMP

Milestone's easyTEMP contactless sensor directly controls the temperature of all samples and solutions, providing accurate temperature feedback to ensure complete digestion in all vessels and high safety.

The superior temperature measurement of easyTEMP allows the processing of different samples of similar reactivity, thus reducing labor time and increasing overall throughput.

This technology combines the fast and accurate reading of an in-situ temperature sensor with the flexibility of an infrared sensor. The ETHOS UP software provides digestion history traceability and temperature measurement for every sample. The temperature diagram and profiles are displayed real time, and can be subsequently saved on the ETHOS UP terminal.

SK-15 HIGH PRESSURE ROTOR

The SK-15 rotor perfectly matches the needs of a modern analytical lab to determine trace elements, thanks to its ability to digest large sample amounts at high temperature (up to 300 °C) and pressure (up to 100 bar).

The 15-position rotor is controlled by a contactless direct temperature sensor that controls the internal temperature of all vessels throughout the digestion cycle. This ensures complete and reproducible digestions of even the most difficult and reactive samples. The SK-15 also features Milestone's patented "vent-and-reseal" technology for controlling the internal pressure of each vessel.

MAXI-44 HIGH THROUGHPUT ROTOR

The MAXI-44 is a high throughput rotor capable of digesting a large variety of environmental samples, improving throughput in the lab.

The MAXI-44 is fully controlled by contactless temperature/pressure sensors that directly control each vessel. This assures maximum safety and digestion quality.

Figure 3. SK-15 easyTEMP High Pressure Rotor.

Figure 4. MAXI-44 easyTEMP High Throughput Rotor.

USER INTERFACE

The ETHOS UP comes with a dedicated touch screen terminal and easyCONTROL software which incorporates our expertise and know-how in microwave sample preparation. The ETHOS UP user interface provides full control of all digestion parameters, provides complete documentation and expedites the overall digestion procedure. The terminal is equipped with multiple USB and ethernet ports for interfacing the instrument to external devices and the laboratory network. The ETHOS UP controller is user-friendly, icon-driven, Multilanguage and 21 CFR Part 11 compliant. To find the method which best suits your application, simply select from the vast library of pre-stored methods.

Included with the ETHOS UP is a unique web-based application: Milestone Connect. This app allows you to become a part of the Milestone community and gain exclusive access to a robust library of information: lists of parts, technical notes, user manuals, video tutorials, continuously updated application notes and all relevant scientific articles.

Figure 5. easyCONTROL built-in library.

ANALYTICAL PROCEDURE

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

ETHOS UP

Rotor	Sample	Sample amount	Acid mixture	Reference official method
SK-15 easyTEMP	Sandy Loam soil (CRM 027)	0.5 g	9 mL of HNO_3 65%, 3 mL HCl 37%	EPA 3051A
	Lake sediment (BCR 280R)	0.5 g	9 mL of HNO_3 65%, 3 mL HCl 37%	EPA 3051A
	Fly ash (BCR 176R)	0.5 g	8 mL of HNO_3 65%, 1 mL HCl 37%, 1 mL HF 48%	UNI EN 14385
MAXI-44 easyTEMP	Sandy Loam soil (CRM 027)	0.5 g	9 mL of HNO_3 65%, 3 mL HCl 37%	EPA 3051A
	Lake sediment (BCR 280R)	0.5 g	9 mL of HNO_3 65%, 3 mL HCl 37%	EPA 3051A

SK-15 eT method and microwave run report:

Table 2. SK 15 microwave program used for digestion of samples

STEP	TIME	T2	POWER
1	00:20:00	210 °C	1800 W
2	00:15:00	210 °C	1800 W

Final dilution: 50 mL with deionized water

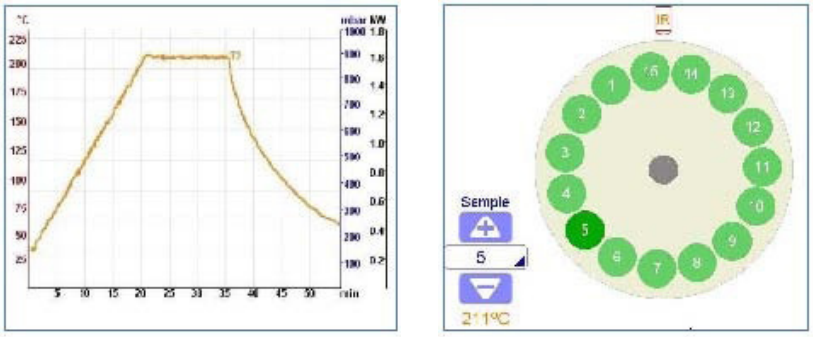


Figure 6. SK-15 Microwave Run Report and Multiple temperature traceability.

MAXI-44 eT method and microwave run report:

Table 3. MAXI-44 microwave program used for digestion of Samples

STEP	TIME	T2	POWER
1	00:10:00	150 °C	1800 W
2	00:10:00	180 °C	1800 W
3	00:10:00	180 °C	1800 W

Final dilution: 50 mL with deionized water

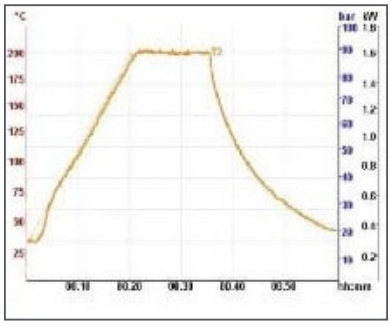


Figure 7. MAXI-44 Microwave Run Report and Multiple temperature traceability.

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 SK-15 easyTEMP rotor was evaluated through a recovery study on sandy loam soil (CRM027), lake sediment (BCR280R), fly ash (BCR 176R). The samples were digested with Milestone's ETHOS UP and subsequently analyzed via ICP-OES.

Table 4. Data of the recovery study on sandy loam soil CRM027

		SK-15 eT		MAXI-44 eT	
	Certified value (mg Kg^{-1})	**Recovery % (n=3)**	**RSD (%)**	**Recovery % (n=3)**	**RSD (%)**
As	59.0 ± 0.939	93.1	1.9	90.5	1.8
Ba	233 ± 4.27	107.3	1.5	97.9	1.9
Be	59.5 ± 1.06	88.9	0.8	87.9	2.0
B	79.6 ± 3.18	102.6	2.6	99.0	2.1
Cd	98.7 ± 1.64	93.6	0.5	93.0	1.7
Co	153 ± 2.67	94.8	2.4	98.0	1.4
Cr	240 ± 3.82	103.8	2.9	99.6	1.9
Hg[a]	16.0 ± 0.327	81.9	1.1	83.1	2.3
Mo	56.4 ± 1.36	101.1	1.1	99.5	2.1
Ni	298 ± 5.20	94.3	1.0	93.0	1.4
Pb	276 ± 4.59	91.3	2.5	87.0	2.6
Cu	89.6 ± 1.66	111.6	2.4	106.4	2.7
Se	100.00 ± 1.59	99.1	1.1	97.6	1.6
Sn	90.7 ± 2.63	88.	1.6	88.6	1.2
Tl	128 ± 2.96	93.0	1.6	92.2	1.1
V	201 ± 2.70	103.0	2.8	99.0	2.1
Zn	590 ± 9.75	94.7	1.0	96.1	1.8

[a]Analyzed with ICP cold vapor generator module.

Table 5. Data of the recovery study on lake sediment BCR280R

		SK-15 eT		MAXI-44 eT	
	Certified value (mg Kg^{-1})	**Recovery % (n=3)**	**RSD (%)**	**Recovery % (n=3)**	**RSD (%)**
As	33.4 ± 2.9	102.7	1.7	98.4	2.1
Co	16.8 ± 0.9	94.4	2.2	91.3	2.4
Cr	126 ± 7	98.5	2.9	95.2	2.0
Cu	53 ± 6	89.9	2.2	90.3	1.7
Ni	69 ± 5	75.9	1.8	71.6	1.8
Zn	224 ± 25	91.8	1.9	93.4	2.1

Table 6. Data of the recovery study on fly ash BCR 176R

	SK-15 easyTEMP[a]		
	Certified value (mg Kg^{-1})	**Recovery % (n=3)**	**RSD (%)**
As	54 ± 5	103.1	1.0
Cd	226 ± 19	91.2	2.8
Co	26.7 ± 1.6	97.0	0.7
Cr	810 ± 70	101.2	1.5
Cu	1050 ± 70	97.1	2.7
Fe	13100 ± 500	100.0	2.3
Ni	117 ± 6	98.3	1.4
Pb	5000 ± 500	98.8	1.4
Se	18.3 ± 1.9	105.7	1.2
Zn	16800 ± 400	107.1	3.0

[a]SK-15 easyTEMP is the recommended rotor for the sample preparation of fly ash samples

The analytical results are shown in tables 4, 5 and 6 with good recoveries of all elements and RSDs below 3%. This demonstrates the robustness and reproducibility of microwave digestion using the ETHOS UP equipped with SK-15 easyTEMP technology.

CONCLUSION

The data shown in this technical note demonstrates full recovery of the elements reported in the certificates of the reference materials.

Milestone's ETHOS UP with SK-15 and MAXI-44 easyTEMP rotors demonstrates full compatibility with official EPA environmental methods with accurate and full control of the digestion process. The easyTEMP sensor ensures superior digestion quality and reliable results even for large amounts of different samples

with similar reactivities. In addition to full analyte recovery, microwave digestion using the Milestone ETHOS UP provides the highest level of reproducibility, great ease of use and high productivity.

ABOUT MILESTONE

At Milestone we help chemists by providing the most innovative technology for metals analysis, direct mercury analysis and the application of microwave technology to extraction, ashing and synthesis. Since 1988 Milestone has helped chemists in their work to enhance food, pharmaceutical and consumer product safety, and to improve our world by controlling pollutants in the environment.

This Sponsor Report is the responsibility of Milestone.

20ºENQA | 8ºCIAQA

Encontro Nacional de Química Analítica
20th Brazilian Meeting on Analytical Chemistry

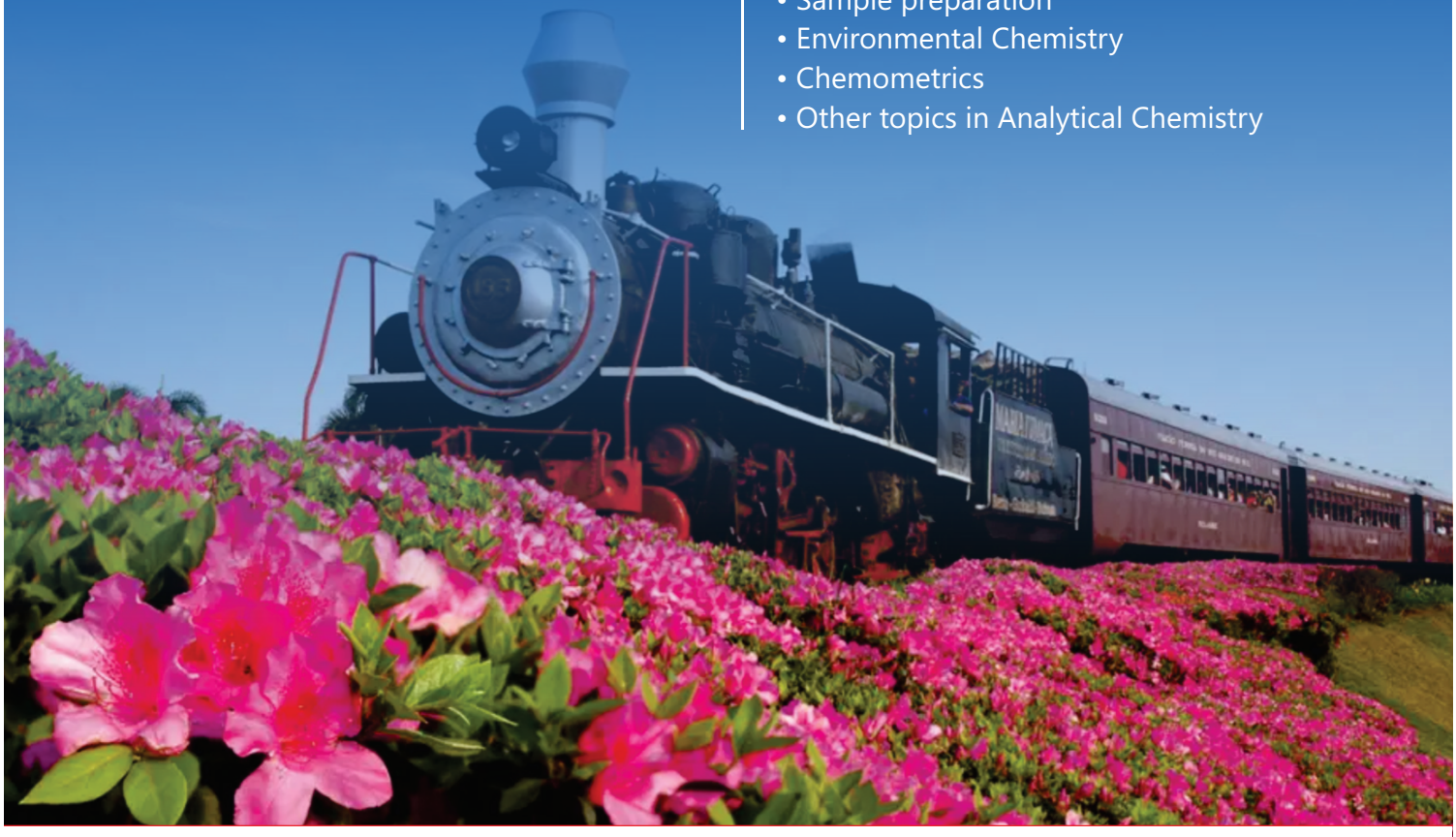
Congresso Ibero-Americano de Química Analítica

September • 25 to 28, 2022 • Dall'Onder Grande Hotel • Bento Gonçalves • RS • Brazil

These events are organized with short courses, conferences, oral and poster sessions, workshops, awards and commercial exhibitions.

Event topics

- Electrochemistry and Electroanalysis
- Atomic Spectrometry
- Mass Spectrometry
- Analytical Instrumentation
- Separation methods
- Sample preparation
- Environmental Chemistry
- Chemometrics
- Other topics in Analytical Chemistry



For more information: www.enqa.com.br

Held by: Sociedade Brasileira de Química, UFSM

Sponsorship – Diamond: Anton Paar, ThermoFisher Scientific

Sponsorship - Gold: Agilent, Astro 34, Metrohm, analitica

Sponsorship – Silver: HORIBA, Waters

Sponsorship - Bronze: ALLCROM, SHIMADZU

Organized by acontece

RELEASE

Thermo Scientific iCAP RQ ICP-MS

Simplicity, productivity and robustness for routine labs in agribusiness

This innovative single quadrupole (SQ) ICP-MS is the ideal trace elemental analyzer for a wide range of several agricultural samples.

The Thermo Scientific™ iCAP™ RQ ICP-MS is particularly suited to the analysis of food, simultaneously determining trace level contaminants and macro level nutrients. This system provides a simple, robust, multielemental analysis method for major and minor concentrations of elements in food.

The simultaneously determination of all elements of interest in a wide range of food samples can be done efficiently and quickly by the Thermo Scientific™ iCAP™ RQ ICP-MS.

DESCRIPTION

Simplicity guarantees user-friendly operation

- Quick connect sample introduction system
- Easy access cones via bench-height drop-down door
- Open geometry architecture for easy peripheral connection
- Intuitive platform software for seamless workflows

Productivity delivers more analysis in less time

- Less training for software and hardware
- Compatibility with automation and sample handling systems
- Comprehensive interference removal in single measurement mode
- Reduced drift and operator intervention

Robustness ensures high up-time and low maintenance

- Improved matrix tolerance interface
- All new state-of-the-art electronics
- New sturdy design RF generator
- Reliable hot and cold plasma operation

Comprehensive interference removal assures data accuracy, while our innovative helium Kinetic Energy Discrimination (He KED) technology enables measurement of all analytes in a single mode.

Our highly effective QCell collision/reaction cell, combined with unique flatapole design reduces BECs even further than He KED alone, through the clever, dynamic application of low mass cut off (LMCO).

Intuitive Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) software delivers all the support features essential to any lab, while containing all the flexibility needed to achieve the most challenging applications.

ThermoFisher SCIENTIFIC

Complete trace elemental analysis

Innovation inspired by real laboratory needs lies at the core of our trace elemental analysis instruments. The iCAP RQ ICP-MS part of the new Thermo Scientific™ iCAP™ Qnova™ Series ICP-MS, delivers the accuracy, robustness and ease of use essential to every high-throughput lab. Driven by our user-friendly Qtegra™ Intelligent Scientific Data Solution™ (ISDS) software, this powerful new system is ideal for labs up-grading to ICP-MS for the first time. The new Element Finder™ plug-in for Qtegra ISDS software, automates wavelength selection, simplifying method development for interference free analysis with the iCAP 7000 Plus Series ICP-OES.

maximum productivity

VIDEO **WEBSITE**

• powerful solutions at thermofisher.com/TEA

Thermo Scientific™ iCE™ 3000 Series AAS

Thermo Scientific™ iCAP™ 7000 Plus Series ICP-OES

Thermo Scientific™ iCAP™ RQ ICP-MS

Thermo Scientific™ ELEMENT 2™ & XR™ HR-ICP-MS
ELEMENT GD™ PLUS GD-MS

RELEASE

Perform like a PRO

Thermo Scientific™ iCAP™ PRO X ICP-OES system delivers Simplicity, Speed and Robustness

Deliver robust, uncomplicated trace elemental analysis for your laboratory with the Thermo Scientific™ iCAP™ PRO X ICP-OES system. This system combines powerful multi-element capability with flexibility, so your lab is ready for any challenge. Produce consistent, reliable data quickly and easily. Experience enhanced sample throughput, matrix tolerance, and flexibility to produce results you can trust.

The iCAP™ PRO X ICP-OES offers fast start-up, easy-to-use software and incredible speed with pre-optimized method conditions, providing multi-element detection technology far superior to that of single-element AAS and multi element microwave plasma techniques. This system is ideal for laboratories with low sample throughput requirements. For ease of use a number of optimized settings are defined as standard, making them ideal for users new to the technique or those who require a simple solution for multi elemental analysis.

Experience more simplicity without compromising on detail

- Flexibility to fulfil demanding projects
- Long-term stability through gas MFCs and temperature control
- Full frame view immediately after measurement
- Intelligent monitoring of analytes with Qtegra ISDS Software
- Plasma optimization tool with tune sets and auto-tune for automated method development

Fast, powerful performance

- Advanced, high-speed charge injection device detection technology produces results in the fastest possible time
- A small optical tank ensures fast start up time and reduced purge gas requirements. With start up times of just 30 minutes from power off and 5 minutes from standby (model dependent)
- Detect from % range to sub ppb detection limits with a high dynamic range detector

Optimized vertical torch for ultimate robustness

- Both duo and radial view configurations of the instruments feature vertical torch orientation. When combined with the unique plasma interface, a new level of robustness is achieved
- Adjustable radial viewing height on both duo and radial view instruments, enabled by the vertical plasma interface
- Increase robustness further with dedicated accessories and analyze the most challenging samples, such as saturated brine solutions.

Accurately quantify the elemental composition of a wide range of samples in: Agricultural screening, Food production and safety, Environmental analysis, Pharmaceutical and nutraceutical compliance, Chemical QA and QC, Petrochemical, Metals and materials

Find out more at thermofisher.com/icp-oes

thermo scientific

iCAP PRO Series ICP-OES

Perform like a PRO
Simplicity, robustness and speed

Analyze even the most challenging sample matrices
The Thermo Scientific™ iCAP™ PRO Series ICP-OES combines powerful multi-element capability with flexibility so your lab is ready for any challenge.

Produce consistent, reliable data quickly and easily. Experience enhanced sample throughput, matrix tolerance and flexibility to produce results you can trust.

Achieve precise results first time, every time with the innovative Get Ready feature. This automated technology sets up the instrument for you and checks performance. Manage instrument processes using a logical dashboard interface. Trust in ICP-OES technology driven by Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software.

VIDEO

WEBSITE

ThermoFisher SCIENTIFIC

RELEASE

Milestone ETHOS UP

High Performance Microwave Digestion System

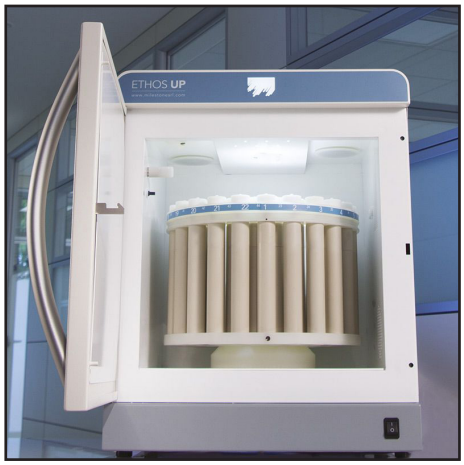
The new ETHOS™ UP is simply the most advanced yet easy to use system for microwave sample preparation ever manufactured. It offers a perfect integration between microwave hardware, user interface, digestion sensors and pressure vessels.

Productivity UP / Simplicity UP / Safety UP

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.

Featuring a comprehensive choice of accessories, ETHOS UP microwave digestion system encompasses Milestone's visionary concept of "Total Microwave Sample Preparation" offering a complete first-class solution for microwave solvent extraction, high-temperature fusion, vacuum evaporation and even organic and inorganic synthesis or protein hydrolysis.

- **Safety and reliability** *Best microwave hardware, temperature and pressure sensors*
- **Performance and throughput** *easyTEMP temperature control and advanced rotor technology*
- **Ease of use and control** *EasyCONTROL operating software*
- **Expertise and know-how** *30-years experience at your lab with the Milestone Connect*
- **Flexibility** *Rotor-based digestion system for hundreds of applications*



- **The Largest Stainless-Steel Microwave Cavity**
- **Pressure-Responsive Door for Max Safety**
- **The Most Powerful Microwave Digester**
- **Live Monitoring with Milestone SafeVIEW**

Find out more at milestonesrl.com

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

HIGHEST THROUGHPUT ROTORS

NO METHOD DEVELOPMENT

REMOTE SYSTEM CONTROL

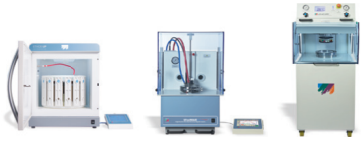
ENHANCED SAFETY FEATURES

JUST WHEN YOU THINK METALS PREP TECHNOLOGY CANT GET ANY BETTER, IT DOES.

The Milestone Ethos UP isnt just intelligent, its the most powerful microwave digestion system on the market today.

Featuring the highest throughput rotors, stainless steel construction and patented vent-and-reseal technology, the Ethos UP ensures market-leading safety and productivity.

Plus, new Milestone Connect offers remote system control, 24/7 technical support and direct access to a comprehensive library of content developed especially for lab professionals.



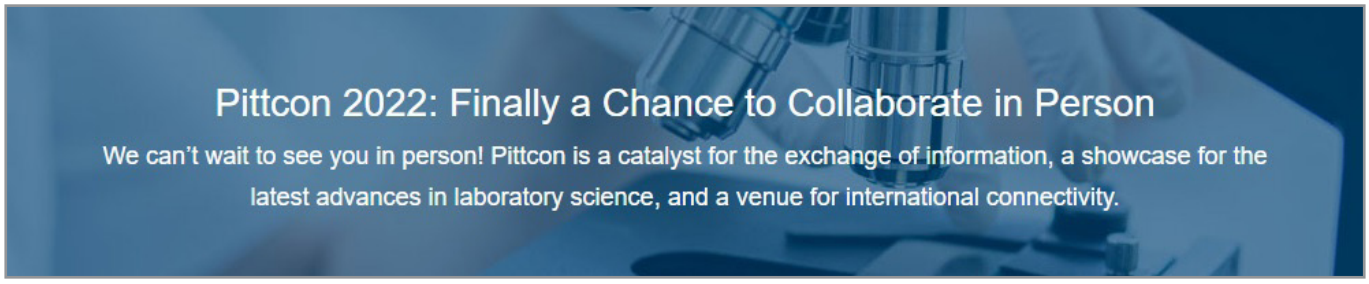
ETHOS Line UltraWAVE UltraCLAVE

See how Ethos UP can help you work smarter.
Go to www.milestonesrl.com/ethosup

VIDEO WEBSITE

MILESTONE

RELEASE



Reconnect with colleagues, feel the instruments, and experience the sights. Celebrate bringing together a world of knowledge to impact, enrich, and inspire the future of science.

Pittcon is a friendly, welcoming environment where analytical chemists at all professional levels meet. It is a platform for sharing ideas and cooperating to form new ones. Here, you will find that spark that drives your research, your career, and above all, your scientific perspective forward.

What to Expect
Our goal is to advance scientific endeavor through collaboration, bringing together a world of knowledge to impact, enrich, and inspire the future of science. Pittcon is a catalyst for the exchange of information, a showcase of the latest advances in laboratory science, and a venue for international connectivity.

Technical Program
One of the foremost analytical chemistry conferences, Pittcon's technical program provides participants with direct access to the latest research and developments from top scientists and innovators found throughout the world.

Short Courses
With over 60 from which to choose, these courses cover significant topics in bioanalytics, cannabis, energy, enviroscience, food science, forensics, industry, life sciences, nanotechnology, materials science, pharmaceuticals, and many more.

Exposition
Pittcon's transnational exposition gives you the opportunity to see the latest laboratory instrumentation, participate in demonstrations and product seminars, talk directly with technical experts, and find solutions to all your laboratory challenges.

WHEN
Technical Program Dates: Sunday, March 6 – Wednesday, March 9, 2022
Short Courses Dates: Saturday, March 5 – Wednesday, March 9, 2022
Exposition Dates: Monday, March 7 – Wednesday, March 9, 2022

VENUE: Georgia World Congress Center, Atlanta, GA, USA

For more information visit https://pittcon.org/conferees/

PITTCON®
CONFERENCE & EXPO

March 5-9, 2022
Georgia World Congress Center
Atlanta, GA USA

Pittcon: Catalyzing a World of Collaborative Science

Pittcon is a dynamic, transnational exposition and comprehensive technical conference, a venue for presenting the latest advances in research and scientific instrumentation, and a platform for continuing education and career-enhancing opportunity

Exposition

Technical Program

Short Courses

Networking Sessions

Employment Bureau

Learn More

RELEASE

SelectScience® Pioneers online Communication and Promotes Scientific Success

SelectScience® promotes scientists and their work, accelerating the communication of successful science. SelectScience® informs scientists about the best products and applications through online peer-to-peer information and product reviews. Scientists can make better decisions using independent, expert information and gain easy access to manufacturers.

SelectScience® informs the global community through Editorial, Q&A and Application Articles, Featured Topics, Event Coverage, Video and Webinar programs.

Some recent contributions from SelecScience® to the scientific community

Video: ***Water quality: A non-target screening approach for characterizing micropollutants***

Dr. Christian Zwiener, Professor of Environmental and Analytical Chemistry at the University of Tuebingen, explains how, using HRMS followed by statistical modeling, his team is able to overcome the challenges posed by the identification and characterization of a large number of poorly described or unknown chemical compounds, and address critical questions ranging from the effect of disinfection byproducts in drinking water to the pharmaceutical impact on surface water and groundwater quality. Watch this video here

Webinar: ***Ion-exchange chromatography in metabolomics: Exploring analytical performance and metabolome coverage***

James McCullagh, Professor of Biological Chemistry and Director of the Mass Spectrometry Research Facility at the University of Oxford, presents the technical capabilities of IC-MS in the context of untargeted metabolomics applications, explores metabolome coverage and reports insights into altered central carbon metabolism in disease models. Access this webinar here

Editorial Article: ***'Inspiring others and rebuilding trust in science'***

Ronald Kittle, Ph.D. student at the University of Louisiana, shares the inspiration behind his lifelong curiosity for learning and his hopes for the future of science communication. In this article, he tells us about the nature of his research and the people who have inspired his unshakable curiosity with science. Ronald also stresses the need for building trust in science and shares his hopes for the future of science communication. Read this article here

SelectScience® is the leading independent online publisher connecting scientists to the best laboratory products and applications.

Access 2 Million+ Decision Makers

- Working with Scientists to Make the Future Healthier.
- Informing scientists about the best products and applications.
- Connecting manufacturers with their customers to develop, promote and sell technologies.

SelectScience®
The Fastest Way to Expert Opinion

RELEASE

CHROMacademy is the leading provider of eLearning for analytical science

CHROMacademy *helps scientific organizations acquire and maintain excellence in their laboratories*.

For over 10 years, CHROMacademy has increased knowledge, efficiency and productivity across all applications of chromatography. With a comprehensive library of learning resources, members can improve their skills and knowledge at a pace that suits them.

CHROMacademy covers all chromatographic applications – HPLC, GC, mass spec, sample preparation, basic lab skills, and bio chromatography. Each paradigm contains dozens of modules across theory, application, method development, troubleshooting, and more. Invest in analytical eLearning and supercharge your lab.

For more information, please visit www.chromacademy.com/

LC GC's CHROMacademy
powered by crawford scientific

CHROMacademy Lite members have access to less than 5% of our content. Premier members get so much more !

Video Training courses

Fundamental HPLC
Fundamental GC
Fundamental LCMS
Fundamental GCMS
HPLC Method Development
GC Method Development

Ask the Expert

We are always on hand to help fix your instrument and chromatographic problems, offer advice on method development, help select a column for your application and more.

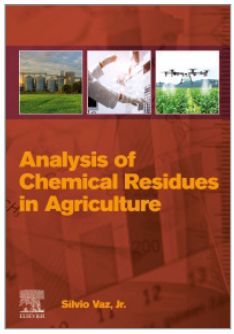
To find out more about Premier Membership contact:

Glen Murry: +1 732.346.3056 | Glen.Murry@ubm.com
Peter Romillo: +1 732.346.3074 | Peter.Romillo@ubm.com

www.chromacademy.com

The worlds largest e-Learning website for analytical scientists

NOTICES OF BOOKS

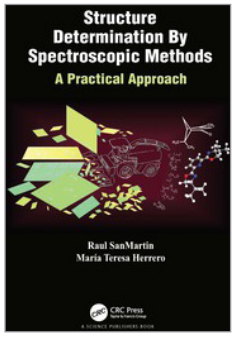


Analysis of Chemical Residues in Agriculture

Silvio Vaz Jr., Author

August 2021. Publisher: Elsevier

This book presents a focused, yet comprehensive guide on how to identify, evaluate and analyze the wide range of chemicals that impact our food production system. Presents also a variety of analytical technologies and methods in order to help professionals, researchers, and graduate and undergraduate students understand chemical residues in agriculture and apply them to applications for the detection and quantification of chemical residues – both organic and inorganic – in several agricultural matrices, including crops, fruits, meat, food, feed, soil and water. Read more

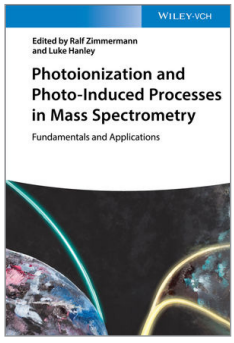


Structure Determination by Spectroscopic Methods – A Practical Approach

Raul SanMartin, Maria Teresa Herrero, Authors

November, 2020. Publisher: CRC Press

The authors travel with the reader through the challenging maze of structure determination, showing how to distinguish between valuable and deceiving data from IR, NMR and MS spectra, extracting structural conclusions and putting all the pieces together to solve the structure elucidation puzzle. In addition to the spectra themselves, each chapter is supplemented with figures and tables that decipher the data and serve as maps for the journey. Read more

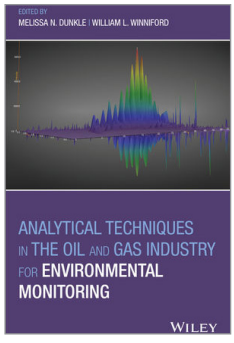


Photoionization and Photo-Induced Processes in Mass Spectrometry: Fundamentals and Applications

Ralf Zimmermann, Luke Hanley, Editors

October 2020. Publisher: Wiley

Provides comprehensive coverage of laser-induced ionization processes for mass spectrometry analysis. Covers both the theory and current applications of photo-induced ionization processes. It places widely used techniques such as MALDI side by side with more specialist approaches such as REMPI and RIMS, and discusses leading edge developments in ultrashort laser pulse desorption, to give readers a complete picture of the state of the technology. Read more



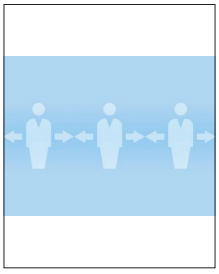
Analytical Techniques in the Oil and Gas Industry for Environmental Monitoring

Melissa N. Dunkle, William L. Winniford, Editors

July 2020. Publisher: Wiley

The book discusses the conventional analyses for oil and natural gas feeds, along with their limitations. It offers detailed descriptions of advanced analytical techniques that are commercially available, plus explanations of gas and oil industry equipment and instrumentation. This book can also serve as your comprehensive resource on key techniques in the characterization of oil and gas samples, within both refinery and environmental contexts. Read more

PERIODICALS & WEBSITES



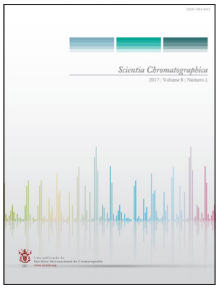
American Laboratory

American Laboratory® is a platform that addresses basic research, clinical diagnostics, drug discovery, environmental, food and beverage, forensics and other markets, and combines in-depth articles, news, and video to deliver the latest advances in their fields. ***Driving Productivity in the Socially Distanced Lab*** To facilitate social distancing and schedule staff access for the use of lab equipment, digitalized solutions may include scheduling functionality software that can reserve specific resources and spaces, control access to sensitive or validated equipment, and track utilization. Read more

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, thereby making laboratories more productive and businesses around the world more successful.

Article: ***Analyzing Per- and Polyfluoroalkyl Substances in Drinking Water Using EPA Methods 533 and 537.1 with Semi-Automated SPE*** A semi-automated system that combines elements of fully manual and automated procedures was developed and tested. Read more



Scientia Chromatographica

Scientia Chromatographica is the first and to date the only Latin American scientific journal dedicated exclusively to Chromatographic and Related Techniques (Mass Spectrometry, Sample Preparation, Electrophoresis, etc.). With a highly qualified and internationally recognized Editorial Board, it covers all chromatography topics (HPLC, GC, SFC) 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

Select Science

SelectScience® promotes scientists and their work, accelerating the communication of successful science. SelectScience® informs scientists about the best products and applications through online peer-to-peer information and product reviews. Scientists can make better decisions using independent, expert information and gain easy access to manufacturers. SelectScience® informs the global community through Editorial, Features, Video and Webinar programs. Read more

Spectroscopy

With the Spectroscopy journal, scientists, technicians, and lab managers gain proficiency through unbiased, peer-reviewed technical articles, trusted troubleshooting advice, and best-practice application solutions.

Article: ***Calibration: Effects on Accuracy and Detection Limits in Atomic Spectroscopy*** A thorough discussion of statistics in analytical chemistry is available in a series of 52 articles, with six articles being the most relevant to this discussion. Read more

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

October 4 – 8, 2021
20th IUPAB Congress, 45th Annual SBBf Meeting, and 49th Annual SBBq Meeting – Virtual
http://iupab2020.sbbq.org.br/interna-278/home

October 11 – 15, 2021
34th Latin American Congress of Chemistry – CLAQ 2020; 18th Latin American Congress of Chromatography – COLACRO; 10th Colombian Congress of Chromatography – COCOCRO; 4th Colombian Congress of Biochemistry and Molecular Biology - C2B2
Convention Center, Cartagena de Indias, Colombia
https://claq2020.com/en/bienvenida/

October 18 – 21, 2021
Metrology 2021 – Online
https://metrologia2021.org.br/

November 16 – 19, 2021
60th Brazilian Chemistry Congress – Virtual
http://www.abq.org.br/cbq/

November 24 – 27, 2021
Analitica Congress – Virtual
https://www.analiticanet.com.br/

November 30 – December 2, 2021
FCE Pharma
São Paulo, SP, Brazil
https://www.fcepharma.com.br/o-evento

XXIII International Mass Spectrometry Conference (IMSC 2021) / Conference transferred to 2022
Sheraton Grand Rio Hotel & Resort, Rio de Janeiro, RJ, Brazil
https://www.imsc2020.com/

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

June 4 – 8, 2022
18th International Conference on Electroanalysis (ESEAC 2022)
Vilnius, Lithuania
http://www.eseac2020.com/

June 21 – 23, 2022
Analitica Latin America Expo
São Paulo, SP, Brazil
https://www.analiticanet.com.br/

September 25 – 28, 2022
20th ENQA & 8th CIAQA
Bento Gonçalves, RS, Brazil
https://enqa.com.br/

GUIDELINES FOR AUTHORS

Scope

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.

The BrJAC does not consider submissions of manuscripts that have been posted to preprint servers prior to submission, and will withdraw from consideration any papers posted to those servers prior to publication.

The submitted manuscripts are the full responsibility of the authors. Manipulation/invention/omission of data, duplication of publications, the publication of papers under contract and confidentiality agreements, company data, material obtained from non-ethical experiments, publications without consent, the omission of authors, plagiarism, the publication of confidential data and undeclared conflicts of interests are considered serious ethical faults.

The BrJAC discourages and restricts the practice of excessive self-citation by the authors.

The BrJAC does not practice coercive citation, that is, it does not require authors to include references from BrJAC as a condition for achieving acceptance, purely to increase the number of citations to articles from BrJAC without any scientific justification.

Misconduct will be treated according to the COPE's recommendations (https://publicationethics.org/) and the Council of Science Editors White Paper on Promoting Integrity in Scientific Journal Publications (https://www.councilscienceeditors.org/).

For more detailed information on the BrJAC's ethics and integrity policy, please see the "About us" menu at www.brjac.com.br

Manuscripts that can be submitted to BrJAC

- **Articles:** Full descriptions of an original research finding in Analytical Chemistry. Articles undergo double-blind full peer review.
- **Reviews:** Articles on well-established subjects, including critical analyses of the bibliographic references and conclusions. Manuscripts submitted for publication as Reviews must be original and unpublished. Reviews undergo double-blind full peer review.
- **Technical Notes:** Concise descriptions of a development in analytical methods, new techniques, procedures or equipment falling within the scope of the BrJAC. Technical notes also undergo double-blind full peer review.
- **Letters:** Discussions, comments, suggestions on issues related to Analytical Chemistry, and consultations to authors. Letters are welcome and will be published at the discretion of the BrJAC editor-in-chief.

Documents Preparation

It is highly recommended that authors download and use the templates to create their four mandatory documents to avoid the suspension of a submission that does not meet the BrJAC guidelines.

Download templates here

Cover Letter

In addition to the usual content of a Cover Letter to the Editor-in-Chief of the journal, the submitting author must declare any conflicts of interest on behalf of all co-authors and their agreement with the copyright policy of the BrJAC. Please read about conflicts of interest and copyright in the About us menu at www.brjac.com.br

It is the duty of the submitting author to inform their collaborators about the manuscript content and obtain their permission for submission and eventual publication.

The Cover Letter must be signed by the submitting author.

Title Page

The Title Page must contain information for each author: full name, affiliation, full postal address, e-mail, phone number, ORCID and information on the contribution of each author to the work. Acknowledgments must be entered on the Title Page. The submitting author must sign the Title Page.

Novelty Statement

The Novelty Statement must contain clear and succinct information about what is new and innovative in the study in relation to previously related works, including the works of the authors themselves.

Manuscript

It is highly recommended that authors download the Manuscript template and create their manuscript in this template, keeping the layout of this file.

- **Language**: **English** is the language adopted by BrJAC. The correct use of English is of utmost importance. In case the Editors and Reviewers consider the manuscript to require an English revision, the authors will be required to send an English proofreading certificate before the final approval of the manuscript by BrJAC.
- **Required items**: the manuscript must include a title, abstract, keywords, and the following sections: Introduction, Materials and Methods, Results and Discussion, Conclusion, and References.
- **Identification of authors**: as the BrJAC adopts a double-blind review, the manuscript file must **NOT** contain the authors' names, affiliations nor acknowledgments. Full details of the authors and their acknowledgements should be on the Title Page.
- **Layout**: the lines in the manuscript must be numbered consecutively and double-spaced.
- **Graphics and Tables**: must appear close to the discussion about them in the manuscript. For **figures** use **Arabic** numbers, and for **tables** use **Roman** numbers.
- **Permission to use content already published**: to use figures, graphs, diagrams, tables, etc. identical to others previously published in the literature, even if these materials have been published by the same submitting authors, a publication permission from the publisher or scientific society holding the copyrights must be requested by the submitting authors and included among the documents uploaded in the manuscript management system at the time of manuscript submission.
- **Chemical nomenclature, units and symbols**: should conform to the rules of the International Union of Pure and Applied Chemistry (IUPAC) and Chemical Abstracts Service. It is recommended that, whenever possible, the authors follow the International System of Units, the International Vocabulary of Metrology (VIM) and the NIST General Table of Units of Measurement. Abbreviations are not recommended except for those recognized by the International Bureau of Weights and Measures or those recorded and established in scientific publications. Use L for liters. Always use superscripts rather than /. For instance: use mg mL^{-1} and NOT mg/mL. Leave a space between numeric values and their units.
- **References throughout the manuscript:** the references must be cited as **numbers in square brackets [ ]**. It is recommended that references older than 5 (five) years be avoided, except in relevant cases. Include references that are accessible to readers.
- **References item:** include a DOI (as a hyperlink) whenever it is available. **References should be thoroughly checked for errors by the authors before submission.**

See how to format the References item below:

Journals

1. Orlando, R. M.; Nascentes, C. C.; Botelho, B. G.; Moreira, J. S.; Costa, K. A.; Boratto, V. H. M. *Anal. Chem*., **2019**, *91* (10), pp 6471-6478 (https://doi.org/10.1021/acs.analchem.8b04943).
   - *Publications with more than 10 authors, list the first 10 authors followed by a semicolon and et al.*
   - *Titles of journals must be abbreviated as defined by the Chemical Abstracts Service Source Index (http://cassi.cas.org/search.jsp).*

Books

2. Burgot, J.-L. *Ionic Equilibria in Analytical Chemistry*. Springer Science & Business Media, New York, **2012**, Chapter 11, p 181.
3. Griffiths, W. J.; Ogundare, M.; Meljon, A.; Wang, Y. Mass Spectrometry for Steroid Analysis. In: Mike, S. L. (Ed.). *Mass Spectrometry Handbook*, v. 7 of Wiley Series on Pharmaceutical Science and Biotechnology: Practices, Applications and Methods. John Wiley & Sons, Hoboken, N.J., **2012**, pp 297-338.

Standard methods

4. International Organization for Standardization. ISO 26603. Plastics — *Aromatic isocyanates for use in the production of polyurethanes — Determination of total chlorine*. Geneva, CH: ISO, **2017**.

Master's and doctoral theses or other academic literature

5. Dantas, W. F. C. *Application of multivariate curve resolution methods and optical spectroscopy in forensic and photochemical analysis*. Doctoral thesis, **2019**, Institute of Chemistry, University of Campinas, Campinas, SP, Brazil.

Patents

6. Trygve, R.; Perelman, G. US 9053915 B2, June 9, **2015**, Agilent Technologies Inc., Santa Clara, CA, US.

Web pages

7. http://www.chromedia.org/chromedia [Accessed 10 January 2019].

Unpublished source

8. Viner, R.; Horn, D. M.; Damoc, E.; Konijnenberg, A. *Integrative Structural Proteomics Analysis of the 20S Proteasome Complex* (WP-25). Poster presented at the XXII International Mass Spectrometry Conference (IMSC 2018) / August 26-31, **2018**, Florence, IT.
9. Author, A. A. *J. Braz. Chem. Soc*., in press.
10. Author, B. B., **2019**, submitted for publication.
11. Author, C. C., **2019**, unpublished manuscript.

Note: *Unpublished results may be mentioned only with the express authorization of the author(s). Personal communications can be accepted in exceptional circumstances.*

Manuscript Submission

The BrJAC uses an online manuscript manager system for the submission of manuscripts. This system guides authors stepwise through the entire submission process.

Submit manuscripts at www.brjac.com.br

The submitting author must add all co-authors to the Authors section of the manuscript manager system. After submission, all co-authors will receive an alert and will have the opportunity to confirm whether or not they are co-authors.

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

The four documents mentioned above must be uploaded into the manuscript manager system as Word files. The manuscript Word file will be converted by the system to a PDF file which will be used in the double-blind peer review process.

All correspondence, including notification of the Editor's decision and requests for revision, is sent by e-mail to the submitting author through the manuscript manager system.

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.

Manuscripts that are in accordance with the journal's guidelines are submitted for the analysis of similarities by the iThenticate software.

The manuscript is then forwarded to the Editor-in-Chief who will check whether the manuscript is in accordance with the journal's scope and will analyze the similarity report issued by iThenticate.

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.

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

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.

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

Copyright

When submitting their manuscript for publication, the authors agree that the copyright will become the property of the Brazilian Journal of Analytical Chemistry, if and when accepted for publication. The submitting author declares in the Cover Letter, on behalf of all other co-authors, to consent to this transfer.

The copyright comprises exclusive rights of reproduction and distribution of the articles, including reprints, photographic reproductions, microfilms or any other reproductions similar in nature, including translations.

Final Considerations

Whatever the nature of the submitted manuscript, it must be original in terms of methodology, information, interpretation or criticism.

With regard to the contents of published articles and advertisements, the sole responsibility belongs to the respective authors and advertisers; the BrJAC, its editors, editorial board, editorial office and collaborators are fully exempt from any responsibility for the data, opinions or unfounded statements.