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Brazilian Journal of Analytical Chemistry
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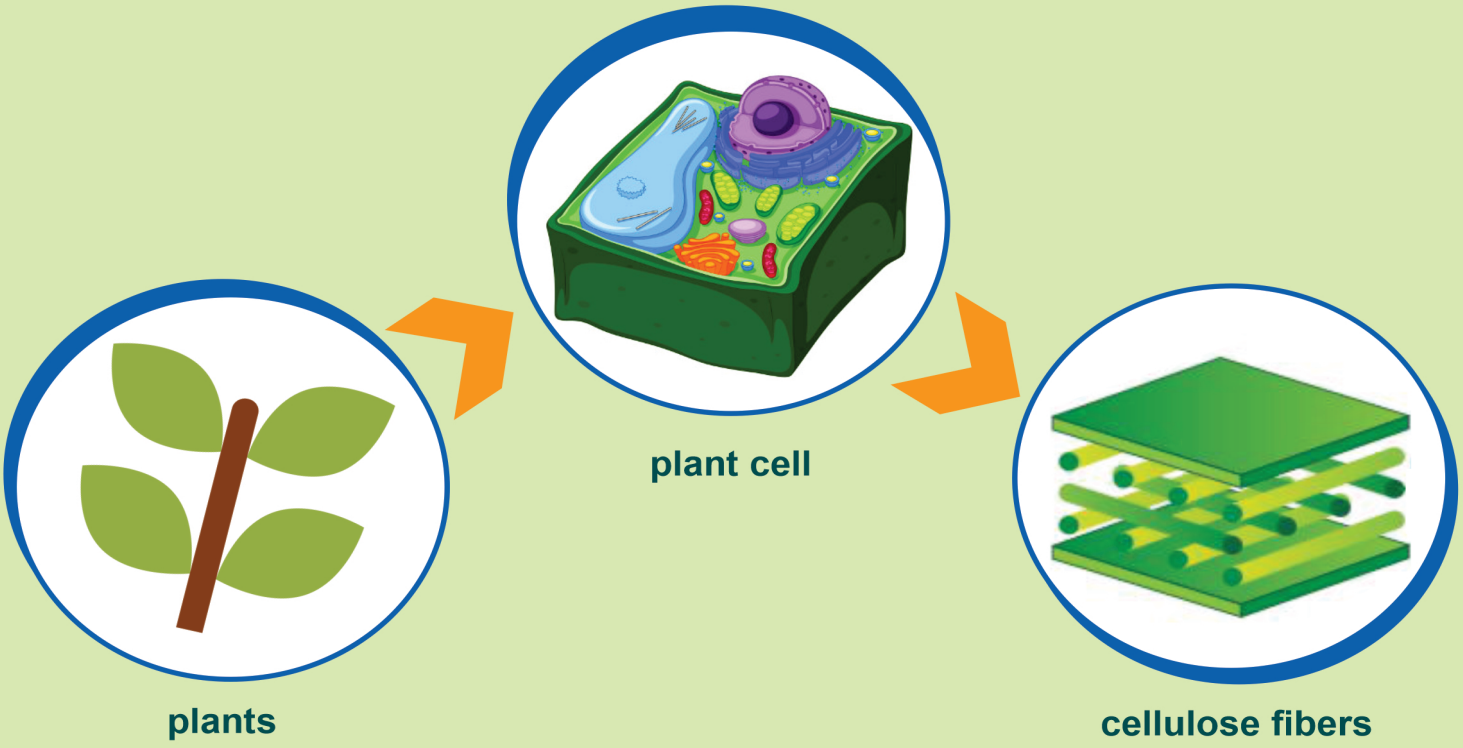

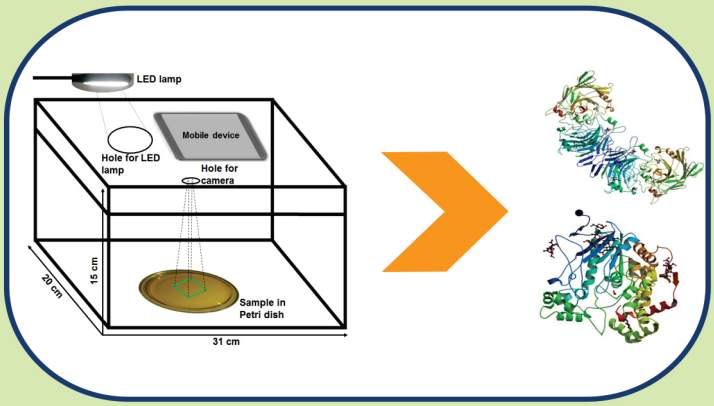

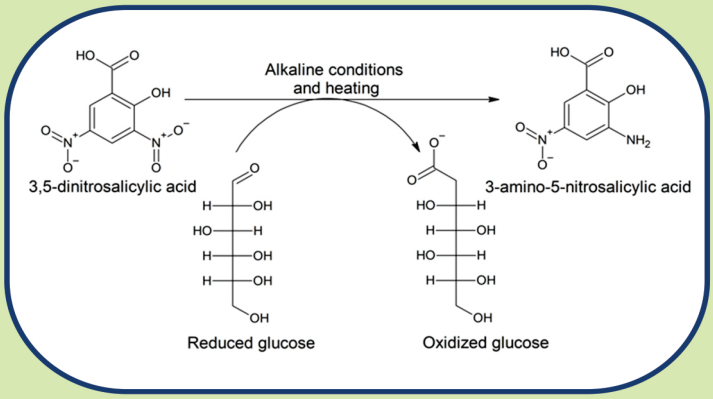

Easy Estimation of Endoglucanase Activity Using a Free Software App for Mobile Devices

Wesley Nascimento Guedes, Guilherme Nunes Lucena, Ariela Veloso de Paula, Rodrigo Fernando Costa Marques, Fabíola Manhas Verbi Pereira

January – March 2020 Volume 7 Number 26

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Scope

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

BrJAC publishes original, unpublished scientific articles and technical notes that are peer reviewed in the double-blind way. In addition, it publishes reviews, 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 manuscript preparation and submission, please see the Guidelines for the Authors section at the end of this edition.

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BrJAC

EDITORIAL

Reproducibility in Science

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Analytical chemists are particularly interested in measuring things, whether it is the amount of a substance or its concentration. When we develop a new method or technique to obtain such quantitative information, it must substantially improve the measurement conditions, perhaps by providing significant decreases in analysis times or by an enhancement in the analytical figures of merit. Like any other scientist, we produce results, and they have to be reproducible and reliable. Hence, an experiment should be described in such a way that other researchers with sufficient skills and resources can follow the steps and obtain identical results, within the margins of experimental error.

The reproducibility in an experiment is the foundation of the scientific method, and we have to validate the results at every stage of discovery and development, i.e., we have to be able to reproduce the work with precisely the same output. Reproducibility testing offers us the ability to deal with different parameters that can influence measurement results and estimated uncertainty. This is crucial because if we know the parameters that significantly impact measurements, we can take action to control the measurement process and reduce uncertainty in the results.

When it comes to reproducibility testing, there are plenty of different conditions that can be evaluated, and a distinction regarding the concept of “repeatability” should be introduced. Measurement repeatability states the closeness of the results obtained with the same sample using the same measurement procedure, same operators, same equipment, same operating conditions, and the same laboratory over a short period. The short period is typically one day or one analytical run. On the other hand, “reproducibility” expresses the precision between measurement results obtained at different laboratories (also named between-lab reproducibility) or the precision obtained in the same laboratory over a more extended time. Reproducibility considers more changes than repeatability, such as different analysts, operators, or instruments, and these parameters act randomly in the context of precision. Hence, because more variables are accounted for in the assessment of reproducibility, its value (expressed as the standard deviation) is larger than the repeatability. Reproducibility can be best explained as the standard deviation of multiple repeatability tests carried out under different experimental conditions.

Communicating a scientific result requires enumerating, recording, and reporting all experimental conditions to attempt repetition straightforwardly. However, several reports have suggested severe issues with the reproducibility of scientific research. Results that are not reproducible are unsound or derived from flawed experimental methods, or there is dishonesty in reporting the measurements. In a scenario in which science is questioned, such lack of rigor may be used against scientists, and, not surprisingly, concerns have been expressed in both scientific and popular media. To overcome such a problem, especially in the field of Analytical Chemistry, a possibility we might want to take into account is based on increased attention to statistics and data analysis, as well as using larger sample sizes. In a broader context, researchers and academic institutions should employ all efforts to improve reproducibility in science.

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Mauro Bertotti graduated from the Institute of Chemistry at the University of São Paulo (IQ-USP), Brazil, with a degree in chemistry (1983), and holds a master's degree (1986) and a doctoral degree (1992) from the same IQ-USP and a postdoctoral degree from the University of Southampton (UK) (1995-1996).

He is currently a Full Professor at the IQ-USP and works mainly on electroanalytical chemistry, with emphasis on microelectrodes, modified electrodes, scanning electrochemical microscopy, and miniaturized and integrated chemical sensors as new manufacturing platforms for biological, clinical and environmental applications.

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INTERVIEW

Professor Ronei Jesus Poppi, a chemist with a clear vocation for research, recently gave an interview to BrJAC

Ronei Jesus Poppi
Full Professor
Institute of Chemistry, University of Campinas – Unicamp
Campinas, SP, Brazil

Prof. Dr. Ronei Jesus Poppi graduated from the Institute of Chemistry at the University of Campinas (IQ-Unicamp) in 1986 with a degree in chemistry, and holds a master's degree (1989) and a doctoral (1993) degree from the same IQ-Unicamp and a postdoctoral degree from the Free University of Brussels, Belgium (1996).

He is currently a full professor in the Analytical Chemistry Department at IQ-Unicamp. He works mainly with chemometrics and spectroscopic methods of analysis. In chemometrics, he has worked in multivariate calibration, neural networks, support vector machines, curve resolution and methods for processing multimode data. In spectroscopy, his studies have emphasized near- and medium-infrared, Raman, molecular fluorescence and image spectroscopy.

Could you tell us a little about your childhood?

I was born in the city of Campinas, SP, Brazil. My father had a barber shop in the 'Bonfim' neighborhood, my mother always worked at home taking care of the family, and I have an older sister. I had no luxury, but I never lacked anything, and my childhood was like that of any other boy growing up in the 1970s in the neighborhood, playing ball on the street all day and dreaming of being a football player.

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

In the elementary school, I was always one of the best students in my class at the state public school named 'Dom João Nery'. When I started taking science classes, I was delighted to be able to understand how things worked. I was very curious to know about the cells in the body, how the light bulb lit or the car battery could generate electricity. At this point, I had a science teacher (Dona Terezinha) who, seeing my interest and how well I was doing in the exams, one day called me aside and said: "You have a lot of potential. You must take a technical course and then go to Unicamp". No one had ever spoken to me like that, and it opened my horizon. I ended up taking her advice, and today I am here.

Cite: Poppi, R. J. Professor Ronei Jesus Poppi, a chemist with a clear vocation for research, recently gave an interview to BrJAC. *Braz. J. Anal. Chem.,* 2020, *7* (26), pp 4-8. doi: http://dx.doi.org/10.30744/brjac.2179-3425.interview.rjpoppi

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

When I finished elementary school, it was already clear to me that I wanted to pursue a career related to science. I intended to attend a technical school, and at the time there were two free schools in Campinas: the Technical School of Unicamp (COTUCA) and the State Technical School 'Conselheiro Antônio Prado' (Etecap), the latter with technical courses in the area of chemistry. To study at these schools, it was necessary to take entrance exams. I enrolled at both schools, and when I went to enroll at Etecap, I visited the school and was very impressed with the chemistry labs and school structure. As I wanted to take a course that involved laboratories and experiments, I opted for the chemical technician course at Etecap. I really liked the area, and at the end of the technical course, I decided to take a course of chemistry at the university. When I finished my bachelor's degree in chemistry at Unicamp, and after having done an internship at a private company in the region, I decided to take a master's degree and follow the research area. My profile is much more suitable for the research area, because I always want to do something new, different and nonstandard. That's what motivates me. I can't work for a long time on the same topic; I'm always changing and looking for new things.

As soon as I defended my doctoral thesis, I had the privilege of being hired at IQ-Unicamp to work in the Analytical Chemistry Department. It's always like that; you need to be in the right place at the right time. I was going to another institution, but at that time Unicamp was interested in the area in which I had specialized. I don't know if today I would be in the same position in another institution, without the facilities that I had at Unicamp at the beginning of my career. I had to learn to teach classes, something I had never done before. This was perhaps the biggest challenge at the beginning of my career. As for research, the beginning was surprising, because I was able to quickly form a group of students and publish in great scientific journals in the area.

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

"Classes need to be more dynamic and with greater student participation. We need to teach students how to think chemically (or analytically) and not simply show how to push buttons on a device…"

I have been a professor for over 25 years, and I was able to follow all the changes that the Internet and the ease of access to information caused in the world, in education and in students. While at the beginning of my career all information could be absorbed and passed on more slowly, today students have immediate access to content and are no longer interested in disciplines in which it is necessary to retain information in memory. Classes need to be more dynamic and with greater student participation. We need to teach students how to think chemically (or analytically) and not simply show how to push buttons on a device, because the robots will push the buttons in the near future. I can mention an interesting fact in a laboratory class, where it was necessary to know the molar mass of a given compound to prepare a solution. A student took out his smartphone and simply asked the molar mass, which was promptly provided. This exemplifies the fact that much information is readily available and may no longer need to be memorized. The worst is that even today there are professors who prohibit the use of smart phones in class.

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

The analytical chemistry area, more than other areas of chemistry, is strongly influenced by technological advances. Some tools, which could not have been thought of a few years ago, today are easily available. I believe that today there are two important trends in analytical chemistry. The first is the sensors, which can be spectroscopic, electrochemical and others, which together with the development of microfluidic systems, portable equipment and data processing, enable fast, inexpensive analytical methodologies that can be used for measurements outside the laboratory, as in the field, in process or even in body. For example, to mention my area, interferometers are now manufactured on a chip for ultraportable infrared

systems. The other trend is the technological development of the systems for separation and detection by mass spectrometry, that allow more reliable determinations of low concentrations in complex matrices. I believe that my contribution is in the area of information processing in spectroscopic sensors.

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

My line of research is mainly related to the use of chemometrics together with spectroscopic techniques for the development of new analytical methodologies that are simpler, more practical and faster. Chemometrics, which emerged in the late 1970s, is based on the use of multivariate statistical methods for processing chemical data and has provided the development of methods of analysis that were previously impractical.

I always had a high scientific productivity, having today 280 published articles, about 6,000 citations, and an H index of 39. Regarding highlighting an article, I think it is important to mention a work done by my research group at Unicamp, with the participation of students, and of which I am the corresponding author: "Least-squares support vector machines and near infrared spectroscopy for quantification of common adulterants in powdered milk", published in Analytica Chimica Acta. This article has 220 citations and summarizes the main areas of activity that mark my work, such as near-infrared spectroscopy, determination of adulterants in food and use of machine learning.

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 always try to keep myself informed about the progress of research in chemistry, and in Brazil the research in chemistry has had great evolution and has achieved a high degree of excellence. Recent advances in the areas of sensors and analytical methodologies for "omic" sciences are of relevant importance. The main challenge in Brazil is, undoubtedly, obtaining financial resources, which are increasingly scarce, and not obtaining human resources, because we have always had human talent.

"… intelligent sensors will undoubtedly dominate a large part of chemical analysis, especially now with the advance of the 'industry 4.0'…"

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

Among the advances in the analytical chemistry research, the miniaturization of equipment and the new mass spectrometry analyzers can be highlighted. As I said before, intelligent sensors will undoubtedly dominate a large part of chemical analysis, especially now with the advance of the 'industry 4.0' or the fourth industrial revolution. We must also consider the artificial intelligence that will revolutionize many of the analytical methodologies that exist today.

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

Scientific meetings are always important for the dissemination of research that is being carried out, and more than that, for personal contacts to be made. It is in conversations that new ideas, new collaborations and new research emerge. Personal contact is essential to leverage these interactions that often become very impersonal in contacts by email or phone. There are some scientific meetings in Brazil in the area of analytical chemistry which are well consolidated and need to be maintained and expanded so that, in this country of continental dimensions, personal contacts can be made.

You have already received some awards. What is the importance of these awards in the development of science and new technologies?

I have already received awards, and the most important thing is that I have already been invited to hold the opening conference at meetings on chemometrics in Brazil. The importance is in being recognized by the academic community as a reference for those who are starting or wanting to enter the research career. It is always important to have references in the country.

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

Funding agencies are fundamental to the scientific development in Brazil. Although there is much discussion today about private research funding, which is also fundamental and necessary, I do not know any country in the world with high scientific development that does not have strong public funding for research. We have to do what we can to inform Brazilian society in general about the importance of public funding, that is fundamental to our independence as a free country.

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?

I see this reduction in investment with great concern. An interruption now in the studies that are in progress means a break in many lines of research that have been consolidated over many years and that have reached a high degree of excellence. Most often, it is not possible to proceed from the point at which a study was interrupted, as advances are dynamic and make restarting work from the beginning mandatory. I know that the current moment is difficult, especially for young researchers, but it is necessary to continue with the dream because, despite everything, there are many moments of personal fulfillment that, in other fields of work, it is not possible to achieve.

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

My advice would be: “Chase after your dreams”. I did this 30 years ago, when the research situation in Brazil was not good. When I was a postgraduate student, I often received my scholarship payment late or was informed on the day of payment that my payment deposit had been forgotten. This is not good, and it is not happening anymore in Brazil, which is an improvement. However, this did not discourage me, because I always wanted to work in research, despite knowing that I would not receive the highest salaries or benefits that other careers could offer.

“I would like to be remembered as that researcher who always tried to escape from the conventional in the field of analytical chemistry and was important in establishing chemometrics in the country.”

How would you like to be remembered?

I was privileged to be able to develop a line of research that today is important, but that was not very well regarded by the most classic analytical chemists at the time of the beginning of my career. This was most likely due to the lack of knowledge of the topic and lack of vision, but time has shown the importance of my line of research. I also had the privilege of mentoring many masters and doctors, and I believe that this shows my contribution to the development of chemometrics in Brazil. Thus, I would like to be remembered as that researcher who always tried to escape from the conventional in the field of analytical chemistry and was important in establishing chemometrics in the country.

Current members of the Laboratory of Chemometrics in Analytical Chemistry at IQ-Unicamp. From left to right: Carlos Alberto Teixeira, Victor Kelis Cardoso, Victor Hugo Cavalcante Ferreira, Rássius Alexandre Medeiros Lima, Marina De Géa Neves, Ronei Jesus Poppi, Carlos Alberto Rios, Sandro Keiichi Otani, Aline Guadalupe Coelho, Felipe Bachion de Santana.

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POINT OF VIEW

3D Printing in Separation Science: Hype or Reality?

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Three-dimensional (3D) printing is an emerging and enabling technology that is paving its way in different fields of research, including analytical science, for the fabrication of custom devices and portable sensing platforms based on additive manufacturing of objects from Computer-Aided Design (CAD) models. In fact, the last five years have witnessed tremendous advances in novel materials and composites with improved chemical properties (e.g., noble metals, carbon nanomaterials, and chemically resistant polymers). Printing platforms for fabrication of low-cost devices have capitalized on stereolithography (SLA) or dynamic light processing (DLP), inkjet printing, fused deposition modelling (FDM), and selective laser sintering (SLS) that enable decentralized (in situ) measurements. The main advantage of 3D printing is the capability of rapid and single-step prototyping of holders, scaffolds, and integrated complex systems with geometries that cannot easily be manufactured by conventional means, such as computer numerical controlled milling and soft-lithographic approaches. Furthermore, the outreach of this technique has been expanded by the lowering costs of the machinery, the user-friendliness of the CAD software and especially the commercial strategies addressed to the nontechnical and nonscientific collective, currently called the 'maker community'.

In the scientific sphere, a variety of 3D printing technologies, including multimaterial printing, is now available in the market for the fabrication of cost-effective fluidic platforms for microscale sample preparation and column separation systems. Efforts toward designing unrivalled 3D printing structures have triggered the development of printable sorbent materials and membranes using either pristine or chemically modified polymers for on-line sorbent extraction and concentration, and platforms for chromatographic and even electrophoretic separation by using conductive printable materials. Also, 3D printing fostered a new dawn for flow injection analysis (FIA) with the fabrication of versatile millifluidic platforms within the concept of "3D-printed μFIA". Recent literature in the field of 3D printing in separation science aiming at so-called "Office Chromatography" focused on the (i) exploration of chromatographic features of tailorable 3D-printed HPLC column geometry with complex designs, (ii) feasibility of in situ fabrication of covalently-attached porous organic monoliths into 3D-printed polymeric or metal scaffolds, and (iii) cost-effective fabrication of printed thin-layer chromatographic plates and entire gel electrophoresis setups. Current trends are also focused on the development of 3D-printed microchips with features of size less than 50 μm for on-line electrophoretic separation of peptides and proteins as biomarkers and the exploitation of multimaterial polyjet-type printers for integration of membrane separation and electrokinetic separation-on-chip for clean-up and separation of pharmaceuticals.

However, 3D-printing in analytical science is still in its infancy because most of the applications reported in the literature are overly simplistic. In fact, the three main 3D-printing technologies (FDM, SLA/DLP and SLS) have a series of problems or disadvantages that must be solved before their acceptance and full

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integration in the analytical chemistry lab. For example, the use of FDM prints at relatively high pressures (>15 bar) is troublesome and can only be achieved by expensive 3D printers. In the case of SLA, most of the photopolymerizable resins are proprietary, and custom-grade printers only accept resins provided by the manufacturer. In both cases, resolution of the 3D-printers does not suffice for truly microfluidic separations, and tailor-made printer configurations with optimized laser sources and optical disposition are needed in SLA applications. Last but not least, post-printing curing is of high importance in 3D-printed SLA objects to prevent leaching of monomers or oligomers that might otherwise jeopardize the analytical detection step, in particular when coupled to mass spectrometric detection. In the case of SLS, the available instruments are still too expensive to be incorporated as a common instrument in analytical research laboratories, and the resolution and features are still at similar levels to those of SLA/DLP machines. Similarly to SLA, post-printing steps are necessary in SLS for removal of the nonsintered metal from the channels, which is not a straightforward task. In this context, the high printing precision achieved by the two-photon polymerization technique, where submicron features can be fabricated, is worth mentioning. However, the high cost, tedious workflow, and small printer volume are still the main challenges of this cutting-edge technique.

In brief, 3D printing in analytical science has been mostly used in proof-of-concept studies or disposable platforms, and no application to complex matrices (wastewaters, whole blood or industrial samples) has been demonstrated as of yet. At this juncture, we can conclude that 3D printing is still a hype in analytical science, yet this technique is proven to outperform conventional techniques in terms of flexibility in fabrication of unique and singular designs. We anticipate that 3D printing will become a standard technique for prototyping in most R&D laboratories when inert materials will be printable at low cost. Apart from the mainstream printers targeted to a wider market, other technically oriented approaches will become key players for further development of 3D printing, such as the usage of electronic components or chemically active materials inside of the prints or the elimination of post-printing procedures for a truly single-step fabrication.

Manuel Miró received his M.Sc. (1998) and Ph.D. (2002) in Chemistry at the University of the Balearic Islands, Spain. He has conducted post-doctoral research in several universities including the Technical University of Berlin, Technical University of Denmark and University of Natural Resources and Applied Life Sciences in Austria. He is currently Full Professor in Analytical Chemistry (from September 2017) at the University of the Balearic Islands; Visiting Professor at Charles University (Czech Republic); and member of the IUPAC Chemistry and Environment Division (Subcommittee on Chemical and Biophysical Processes in the Environment).

He is the Reviews Editor of the journal Analytica Chimica Acta (Elsevier, IF:5.23, second highest IF across general journals in AC) and Associate Editor of the Enclyclopedia of Analytical Science, 3rd Edition, Elsevier, since 2007 and 2016, respectively. His publication record shows 190 refereed publications including 11 book chapters, and a foreword, with an h-index of 39 and over 4500 citations. He has published 23 articles in the prestigious journal 'Analytical Chemistry' from ACS and is corresponding author of 95 articles.

Enrique Javier Carrasco-Correa received his Ph.D. degree from the University of Valencia (Spain) in 2015. He worked as a Ph.D student in Prof. G. Ramis-Ramos' group at the Department of Analytical Chemistry in the University of Valencia. Currently, he is working as a researcher at the Department of Analytical Chemistry of the UV (CLECEM Group) and the Department of Chemistry at the University of the Balearic Islands (FI-TRACE group). His current research interests focus on the assessment of novel hybrid materials (e.g., monoliths, nanomaterials, 3D-printed devices) in separation and microextraction techniques. He participates in the training of novice researchers and in the organization of workshops for young researchers. He also collaborates with industry for the development of novel products and methodologies.

David J. Cocovi-Solberg received his PhD in Chemical Science and Technology from the University of the Balearic Islands (UIB) in 2017. He is currently a postdoc researcher at the University of Natural Resources and Life Sciences of Vienna (BOKU), a scientific collaborator of the UIB and a visiting professor at the Gdansk University of Technology (GUT). His research is focused in the development of analytical instrumentation for exposome evaluation, for which he is the principal investigator of 2 projects with public and private funding. This research has a strong component in sample preparation, microfluidics, chromatography, elemental and molecular mass spectrometry, 3D printing, software programming and statistics. Most recent contributions resort to microfluidics for single-step, fast and universal sample preparation as a front end to LC-MS.

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LETTER

Ritonavir Polymorphism: Analytical Chemistry Approach to Problem Solving in the Pharmaceutical Industry

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Although multinational/innovative companies invest resources and time in studying the impact of different polymorphic forms on medicines, in Brazil the polymorphism issue is very recent. Control of the crystalline form or structure of solids should be carefully evaluated in the pharmaceutical industry because, despite the same chemical composition, polymorphs have distinct physicochemical properties, such as solubility, dissolution, chemical stability, appearance of the raw material, melting point and bioavailability. In addition, polymorphs can undergo phase conversion during a sequence of manufacturing steps, such as milling, micronization, drying, wet granulation, spray-drying and compaction.

The most widely known and well-known example in the history of the pharmaceutical industry regarding the influence of polymorphism leading to deviations in quality control and problems with the pharmacological effect in patients is the case of ritonavir.

The ritonavir molecule was developed by Abbott Laboratories in 1992. A new drug application was submitted in December 1995, marketing began in January 1996 and FDA approval was granted in March 1996. Ritonavir, sold under the tradename Novir®, was introduced to the market as a gelatin capsule and as a liquid formulation [1]. Two years after its launch (early 1998), some batches began to fail the dissolution specifications and there were reports of clinical ineffectiveness, causing a market crisis for Abbott Laboratories [1]. Further investigation revealed the existence of a previously unknown new crystalline form that was thermodynamically more stable and much less soluble. This new polymorph was referred to as ritonavir Form II, with the originally known crystalline form being called ritonavir Form I [1]. The contents of the capsules were examined by microscopy. Crystals were detected on capsule filling that had a distinctly different crystal habit from bulk ritonavir [1]. The Form I morphology is generally observed as lath-shaped crystals or rods, whereas Form II crystals appear as thin needles (see Figure 1) [1].

The structures of the polymorphs were determined from x-ray powder diffraction (XRPD) and are shown in Figures 2 and 3. By analysing the diffractograms of the two polymorphic forms it was possible to verify that they present different XRPD profiles from each other: Form I has a characteristic peak at 6.75 (2θ) whereas Form II has characteristic peaks at 9.51 (2θ), 9.88 (2θ) and 22.2 (2θ) [1]. The crystal structures of Forms I and II were obtained from the Cambridge Crystallographic Data Center (CCDC), deposition numbers 710528 and 710529, respectively, using the Mercury 4.3 program.

Hydrogen bond evaluation

In both polymorphic forms the total number of hydrogen bonds is the same, however the crystallographic network of hydrogen bonds is different [4].

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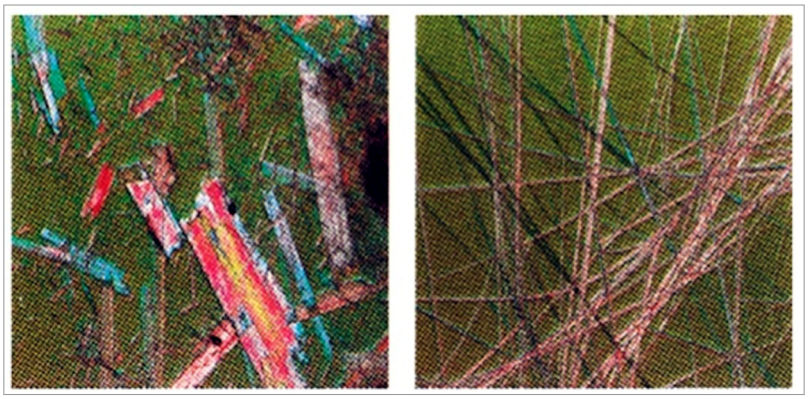


Figure 1. Micrograph of ritonavir crystals: (left) Form I; (right) Form II [1].

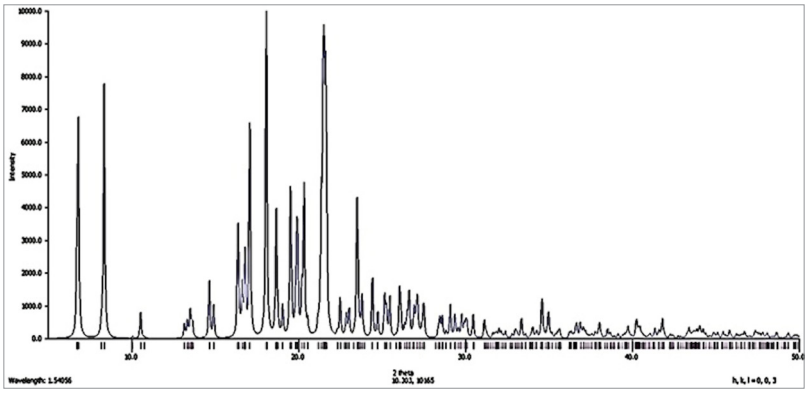


Figure 2. X-ray powder diffractogram of ritanovir Form I [2].

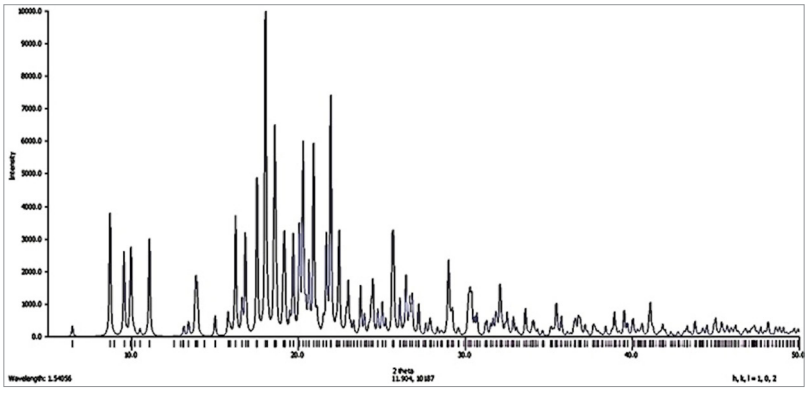


Figure 3. X-ray powder diffractogram of ritanovir Form II [3].

Form I

Figures 4 and 5 show the hydrogen bond arrangements for Form I: a continuous beta-like stack parallel to the short crystallographic axis due to alignment between the amide bonds of one molecule with the same functional group in the next molecule [4]; and pairing of these stacks due to hydrogen bonds between the alcohol of one molecule and the thiazole ring of a second related molecule [4].

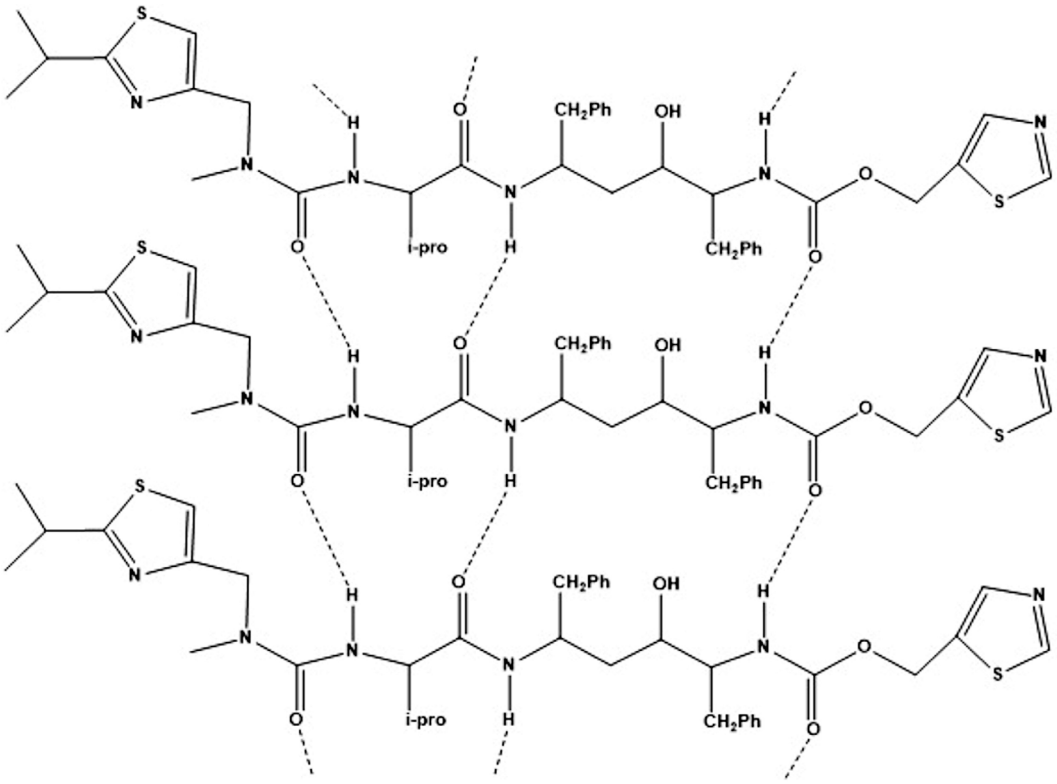


Figure 4. Crystallographic network for ritonavir Form I: beta-like stack [4].

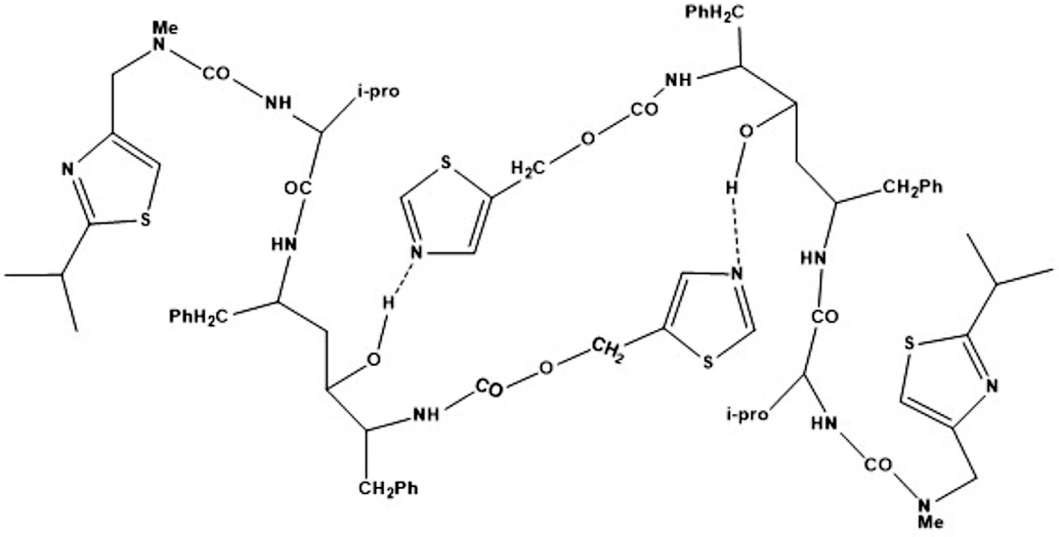


Figure 5. Crystallographic network for ritonavir Form I: twofold screw axis [4].

Form II

Figure 6 shows the hydrogen bond arrangement for Form II, from which it is possible to verify that the hydrogen bonds are strong and that both the hydrogen bond donors and acceptors are fully bonded [4].

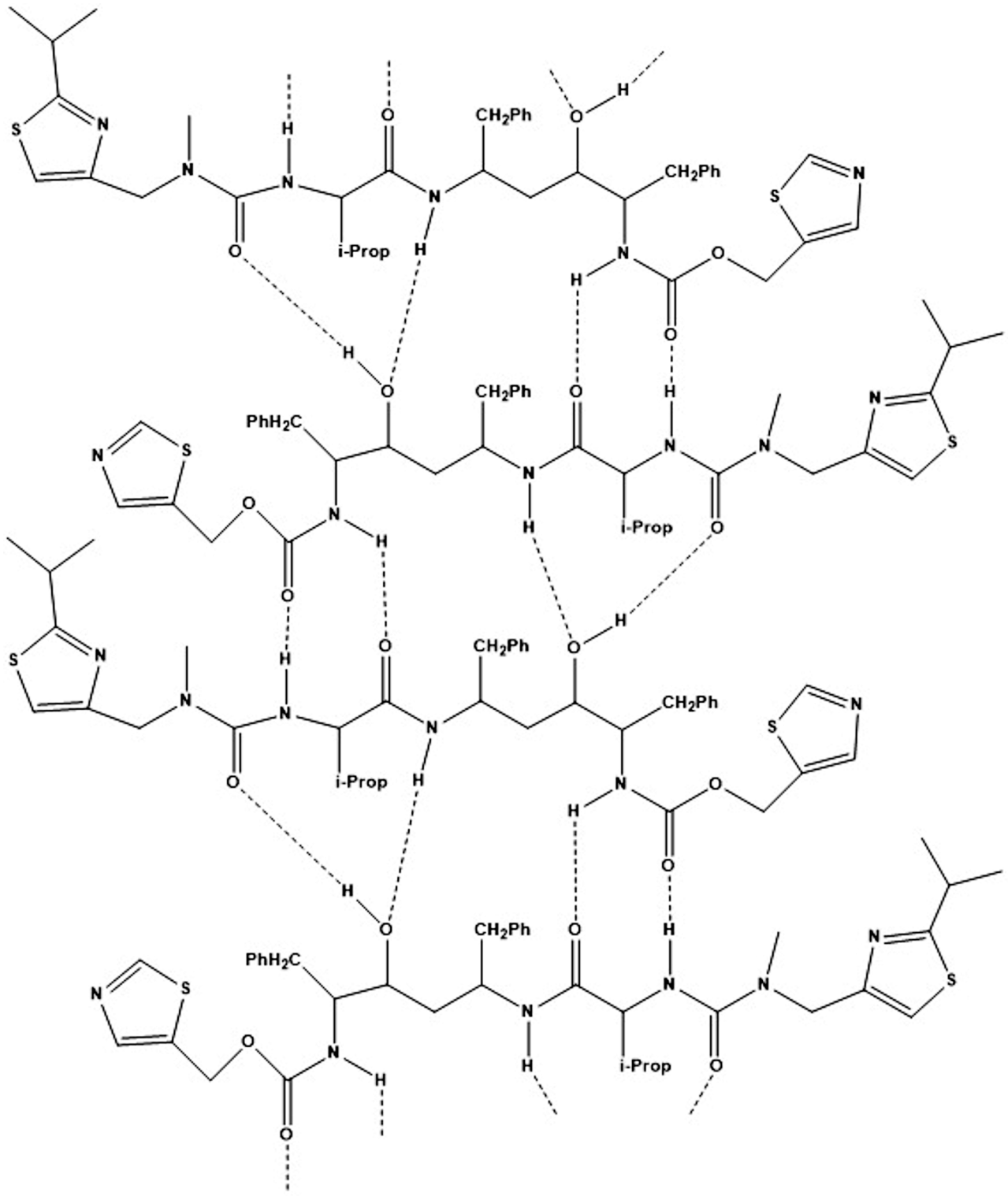


Figure 6. Crystallographic network for ritonavir Form II: needle growth occurs due to orientation promoted through hydrogen bond alignment [4].

Further, crystallographic elucidation of the polymorphic forms identifies the three torsion angles (A, B, C) represented in Figure 7, showing areas with significant differences between the different polymorphs [4].

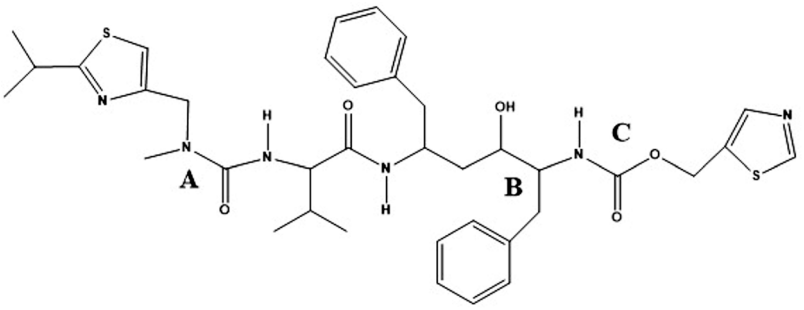
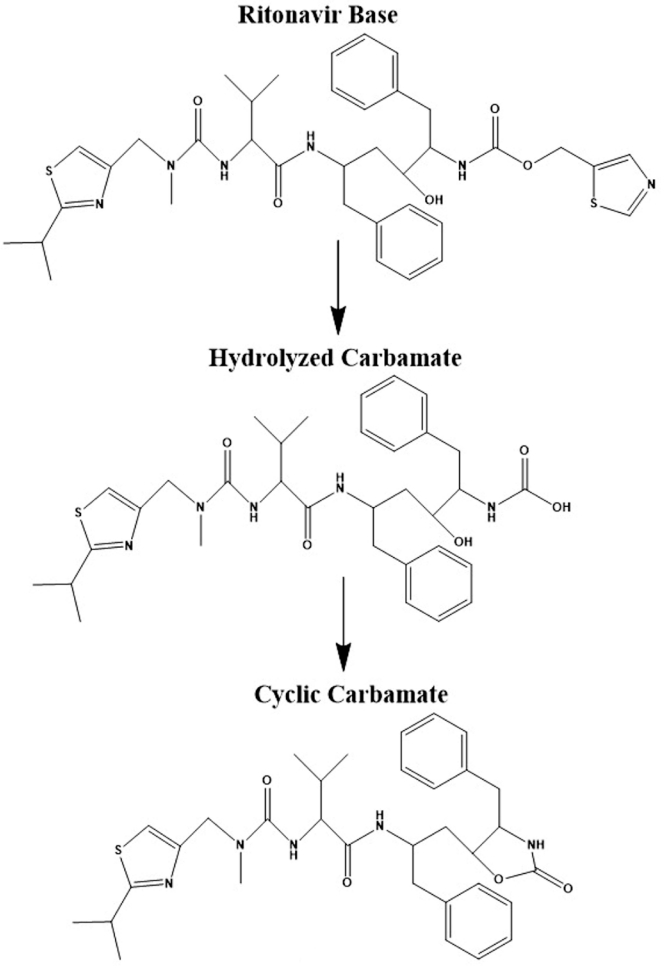


Figure 7. Three torsion angles (A, B, C) identified in ritonavir polymorphism [4].

The main question that still remained was how Form II emerged initially, given its resistance to nucleation [4]. Crystallization of Form II in supersaturated solutions can only occur if Form II seeds are present, so it has been hypothesized that a possible source for the formation of these original seeds would involve heterogeneous nucleation of an impurity of similar chemical structure capable of acting as a mold for Form II. Possible degradation steps for ritonavir are shown in Scheme 1 [4].



Scheme 1. Possible degradation steps for ritonavir.

Cyclic carbamate bonding of ritonavir yields a *cis*-like conformation ring and may serve as a template or seed for promoting the nucleation of Form II [4]. Using ritonavir characterization studies, the existence of conformational polymorphism ("*cis*" and "*trans*") around the carbamate bond was confirmed, leading to two unique crystallographic networks with significantly different solubility properties [4].

Nucleation of Form II requires the generation of a less stable conformation in solution, with subsequent organization for crystal growth [4]. Owing to the very low probability of nucleation, this new polymorphic form could remain hidden from scientific knowledge if it were not for the combination of a solution that was highly supersaturated (around 400%) with respect to Form II and an unknown nucleation enhancer that was able to seed the solution heterogeneously [4].

Summary

This case study has demonstrated the need to invest financial resources in analytical chemistry to conduct research focused on identifying all possible polymorphs and determining the effects related to bioavailability and stability in pharmaceutical products.

Background

Dealing with polymorphs seems imperceptible and mysterious but the initial investment in preformulation studies regarding identification, characterization and quality control of active pharmaceutical ingredient (API) polymorphism provides strategic insights to ensure drug quality and patent issues.

In this extremely competitive market between generic and innovative products, the API cannot be considered based solely from a molecular point of view; changes in solid-state properties also must be considered to ensure the interchangeability, efficacy and safety between these products.

Analytical chemistry has played a critical role in understanding the characterization and identification of different polymorphic forms through the use of analytical techniques such as microscopy, mid-infrared spectroscopy, differential scanning calorimetry, thermal gravimetric analysis, x-ray powder diffraction and nuclear magnetic resonance, thus becoming a well-established and well-known tool with essential practical application for the pharmaceutical industry [5-9].

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ARTICLE

A Simple Method for Simultaneous Determination of Commonly used Artificial Food Colors and Preservatives in Soda, Jam, and Yogurt by HPLC-PDA

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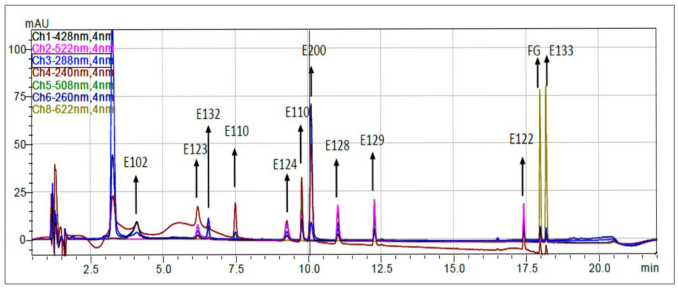

In this study, a simple and sensitive analytical method for the analysis of an expanded scope of food additives in three different matrices using HPLC-PDA was developed. This method was validated for the simultaneous analysis of the commonly used ten food colors and two preservatives of different polarities and chemical structures. The studied matrices included: soda juice, jam, and yogurt. The mixture of H_2O:MeOH (1:3, v:v) with 0.01 mol L^{-1} NaOH allowed low limits of quantifications (LOQ) for most of the studied food additives. Acceptable recoveries within the range 60–100% with an associated precision (RSD) <20% were obtained for all the studied food additives at a low concentration of 1.00 mg kg^{-1} in jam and yogurt except tartrazine and benzoic acid which could be determined at concentrations of 5.00 mg kg^{-1} in jam and equal to 5.00 and 10.00 mg kg^{-1} in yogurt, respectively. The studied food additives could also be determined at lower concentrations in soda juice samples, as analyzed with a lower dilution factor. A complete separation for the tested additives was achieved at run time of 22 min using a mobile phase consisting of solvent A of 0.10 mol L^{-1} ammonium acetate buffer (pH 4.60) in a mixture of H_2O:MeOH (95:5, v:v) and solvent B of ACN:MeOH (7:3, v:v). This method was successfully applied for the analysis of eighteen real samples. The eight collected soda juice samples were found to contain at least one food preservative with one or more food color.

Keywords; Artificial colors, Food preservatives, Soda Juice, Jam, Yogurt, HPLC-PDA

INTRODUCTION

Artificial food colors are usually used to add more purchasing values to the foodstuff and juices especially that of children's interest. The main reasons for the increment usage of such synthetic colors

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are attributed to its high stability and being of a lower price when compared to natural colors [1]. However, overuse concentrations from these artificial colors may lead to frequent headaches for adults and increase children's hyperactivity [2]. Azo dyes may be transformed under specific conditions to form genotoxic and carcinogenic products [3,4]. Benzoic acid and sorbic acid are the most commonly used food preservatives that prevent deterioration of foodstuffs caused by microbial contamination [5-7]. Therefore, there is a high probability of finding one or more colors together with benzoic and/or sorbic acid in the same sample. Although liquid chromatography-tandem mass spectrometry technique enables the simultaneous analysis of not well chromatographically separated compounds, only a few papers were published on LC-MS/MS analysis of a low number of artificial colors in limited matrices [1,8-10]. This is mainly attributed to inefficient extraction methods that enable such multi food additives (FA) analysis in various food matrices. In addition, most of the current extraction methods for the analysis of FA require intensive sample preparation techniques, especially for the determination of artificial colors in high protein content sample, applying Solid Phase Extraction (SPE) clean up [11-15] or many sample preparation steps [16].

In the current paper, a simple and cheap method for the simultaneous analysis of ten food colors and two preservatives in different matrix categories was established using HPLC-PDA. This method was fully validated for soda juice, jam, and yogurt samples according to SANTE/11813/2017 [17].

MATERIAL AND METHODS

Chemicals and reagents

Methanol and a ready prepared NaOH solution (1 mol L^{-1}) were purchased from PROLAB chemicals. Ammonium acetate was obtained from Fischer Scientific. The used deionized water (DIW) of 17 Ω cm was obtained using Veolia water solutions and Technologies. All the studied food colors were purchased from Sigma Aldrich Inc. Benzoic and sorbic acids were obtained from LRSD Fine-Chem Ltd. and Sisco Research Laboratories, respectively.

Preparation of standard working solutions

A stock standard solution for each FA standard (Table I) was prepared at a concentration of 1000 μg mL^{-1} in 10 mL of H_2O:MeOH (1:3, v:v) for most of the studied FA. A stock standard solution for tartrazine was prepared in H_2O:MeOH (3:1, v:v), and for Amaranth, Indigo, and Ponceau in H_2O:MeOH (1:1, v:v). A working standard solution mixture of 25 mg kg^{-1} for all the studied FA and of 50 mg kg^{-1} for tartrazine and benzoic acid was prepared by diluting a suitable aliquot of the stock solutions in H_2O:MeOH (1:3, v:v). This mixture was then stored at 4 ºC and was used for the fortification of the blank samples and the analytical curve preparation.

Table I. Analyzed food additives, respective retention times and wavelengths used for their detection

N	Food Additive	Molecular formula	MWt	E number[a]	t_R (min)	Wavelength (nm)
1	Tartrazine	$C_{16}H_9N_4Na_3O_9S_2$	534.36	E 102	4.06	428
2	Indigo	$C_{16}H_8Na_2N_2O_8S_2$	466.36	E 132	6.57	289
3	Benzoic A	$C_7H_6O_2$	122.1	E 110-113	7.49	240
4	Sorbic A	$C_6H_8O_2$	112.12	E 200, 202, 203	10.11	240
5	Amaranth	$C_{20}H_{11}N_2Na_3O_{10}S_3$	604.49	E 123	6.20	518
6	Ponceau	$C_{20}H_{11}N_2Na_3O_{10}S_3$	604.48	E 124	9.27	518
7	Sunset Y	$C_{16}H_{10}N_2Na_2O_7S_2$	452.37	E 110	9.78	518
8	Acid R 33	$C_{18}H_{14}N_2Na_2O_8S_2$	496.41	E 128	11.02	518
9	Allura R	$C_{18}H_{14}N_2Na_2O_8S_2$	496.43	E 129	12.28	518

Table I. Analyzed food additives, respective retention times and wavelengths used for their detection (Cont.)

N	Food Additive	Molecular formula	MWt	E number[a]	t_R (min)	Wavelength (nm)
10	Carmoisine	$C_{20}H_{12}N_2Na_2O_7S_2$	502.44	E 122	17.42	518
11	Fast G	$C_{37}H_{34}N_2Na_2O_{10}S_3$	808.84	-	17.98	622
12	Brilliant B	$C_{37}H_{34}N_2Na_2O_9S_3$	792.85	E 133	18.19	622

[a]Codes for substances used as food additives within the European Union and European Free Trade Association.

Final extraction method procedure

One gram of yogurt & jam and 2 mL of soda juice (previously sonicated for 2 h) samples were weighed into a 10 mL measuring separating flask. For the recovery experiments, the samples were fortified by adding appropriate volumes of the FA working mixture, which were then left for 15 min with applying vortex per 5 minutes interval times. Ten milliliters of H_2O:MeOH (1:3, v:v) containing 0.01 mol L^{-1} NaOH was added to the mark of the used measuring flask. Then, this solution was vortexed, transferred into 15 mL centrifugation tubes, and shaken by hand for 1 min. Then after, a soda juice extract solution was collected in a vial using an acrodisc syringe filter of 0.45 µm. While the tubes of jam and yogurt extract solution were centrifuged (3700 rpm) for 5 min and subsequently collecting the supernatants using acrodisc syringe filter of 0.45 µm.

HPLC-PDA conditions

A liquid chromatograph (Nexera LC 2040, Shimadzu, Kyoto, Japan) coupled to UV–Vis detector with a photodiode unit was used for the chromatographic analyses in this study. Chromatographic separation was carried out using a C18 column of small internal diameter and particle size (Restek column, 3.00 mm × 100 mm × 2.7 µm) at 25 ºC and a sample injection volume of 2 µl. A fast HPLC runtime of 22 min was developed for the analysis of 12 different FA (10 food colors and 2 food preservatives). The mobile phase components consisted of solvent (A): a mixture of H_2O:MeOH (95:5, v:v) with pH 4.60 adjusted using ammonium acetate buffer (0.10 mol L^{-1}); and solvent (B): a mixture of ACN:MeOH (7:3, v:v). The mobile phase was pumped at a flow rate of 0.30 mL min^{-1} with an optimized solvent gradient program, as follows: 0–2 min, 3% solvent B; 2–4 min, 3-10% solvent B; 4–14 min 10-25% solvent B; 14–18 min 25-60% solvent B; then, the program was held constant at 3% to the end of the run time 22 min.

Method Validation

The developed method was fully validated for the analysis of the studied food additives, according to SANTE/11813/2017, in yogurt, jam, and soda juice. For linearity evaluations, six calibration levels were prepared in soda, jam, and yogurt extracts at 0.05, 0.10, 0.25, 0.50 and 2.50 mg L^{-1} for all the studied FA except for tartrazine and benzoic acid which were prepared at 0.10, 0.20, 0.50, 1.00, and 5.00 mg L^{-1}.

The intra-day accuracy and precision of the developed method were evaluated at three different concentration levels of 1.00, 2.50, and 5.00 mg kg^{-1} for all the studied FA, except for tartrazine and benzoic acid whose concentration levels were 2.00, 5.00, and 10.00 mg kg^{-1}, in jam and yogurt. These three concentration levels are corresponding to lower levels of 0.5, 1.25, and 2.5 mg kg^{-1} for all the studied FA, except for tartrazine and benzoic acid at 1.00, 2.50, and 5.00 mg kg^{-1}, in soda juice, as it has a lower dilution factor.

The inter-day evaluation was carried out within five repeated days, at a concentration level of 5.00 mg kg^{-1} for most of the studied FA, except for tartrazine and benzoic acid at 10.00 mg kg^{-1} in jam and yogurt. These experiments were also carried out for soda juice sample at a concentration level of 2.50 mg kg^{-1} for all of the studied FA, except for tartrazine and benzoic acid at 5.00 mg kg^{-1}.

RESULTS AND DISCUSSION

Development of the food additive analysis method

The two main goals of this study were to achieve satisfactory peak separations corresponding to the extracted FA, and to develop a unified extraction method for these different FA from the highly different studied matrices.

Optimizing the chromatographic separation

Different mobile phases and gradient elution were tested to obtain an efficient separation for all the studied FA (Table I). A mixture of H_2O:MeOH (95:5, v:v) was selected for sample loading on the used RP-column. The presence of organic solvent in the used loading solution is important to prevent algae formation and enhance the evaporation of the mobile phase in case applying this study for ESI-LC-MS/MS analysis. Ammonium acetate of 0.10 mol L^{-1} was added to the studied loading solution (pH 4.60) to enable the simultaneous analysis of both food colors and preservatives. Ammonium acetate buffer was selected as it has lower aggressive characters than using phosphate buffer. ACN, MeOH, and their mixtures at different ratios were tested for the proper elution of the retained FA. A deep focusing was paid on increasing the retaining of the highly polar studied FA, tartrazine, amaranth, and indigo, with obtaining a satisfactory separation between sorbic acid and the moderately polar FA (ponceau, sunset Y, acid R, and Allura R) and also proper separation for the late eluted FA (carmoisine, fast G, and brilliant B) without further increasing the overall run time. Therefore, different temperatures (25-40 ºC) were also optimized on the used RP-Phase C18 column, of a smaller particle size of 2.7 µm. The best optimized chromatographic separation conditions were obtained using a loading solvent of H_2O:MeOH (95:5, v:v), elution solvent of ACN:MeOH (7:3, v:v), and applying a temperature of 25 ºC for the used column. Details of the elution program are mentioned in Material and Methods item. Figure 1 shows the obtained chromatogram for the analysis of the studied FA in yogurt (dilution factor equal 10), at a concentration of 5 µg kg^{-1} for all of the studied FA, except for tartrazine and benzoic acid at 10.00 mg kg^{-1}.

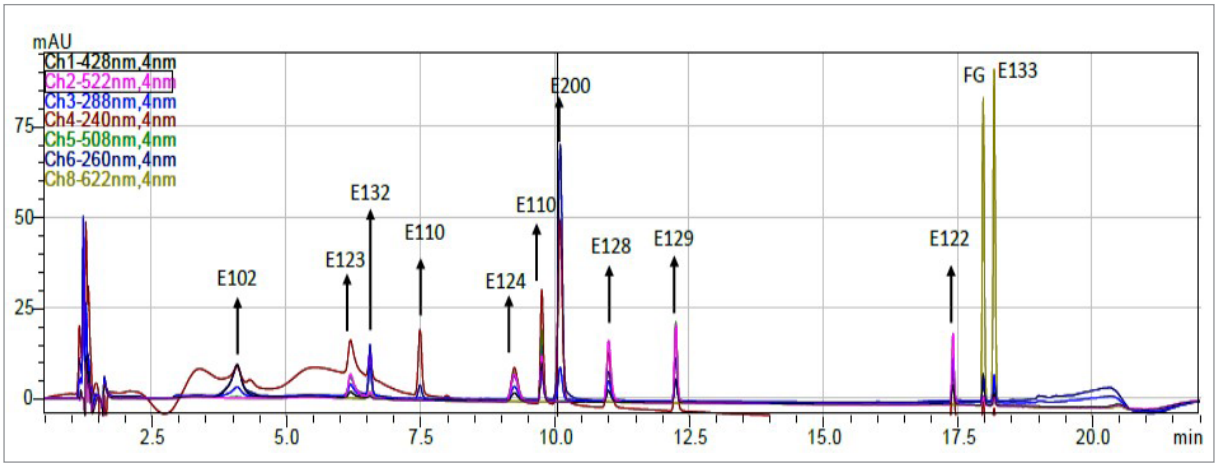

Figure1. HPLC chromatogram of the analyzed food additives mixture solution: Tartrazine (E 102, 10 mg kg^{-1}); Amaranth (E 123, 5 mg kg^{-1}); Indigo (E 132, 5 mg kg^{-1}); Benzoic A (E 110, 10 mg kg^{-1}); Ponceau (E 124, 5 mg kg^{-1}); Sunset Y (E 110, 5 mg kg^{-1}); Sorbic A (E 200, 5 mg kg^{-1}); Acid R 33 (E 128, 5 mg kg^{-1}); Allura R (E 129, 5 mg kg^{-1}); Carmoisine (E 122, 5 mg kg^{-1}); Fast G (FG, 5 mg kg^{-1}); Brilliant B (E 133, 5 mg kg^{-1}) in yogurt extract using the developed method.

Optimizing the extraction method

To obtain a simple and efficient multi-detection method for analysis of the twelve studied FA with shortening the sample preparation steps, a mixture of H_2O:MeOH (1:3) was firstly tested for jam and yogurt analyses both with a dilution factor (DF) of 10 and for soda sample of DF 5. Satisfactory recoveries were

obtained for soda juice samples for nearly all the studied FA except for Ponceau. On the other hand, lower recoveries were obtained for all the studied artificial colors in jam and yogurt, only acceptable recoveries were obtained for benzoic and sorbic acid.

Based on these results, it was suggested that there is an adsorption interaction between the studied artificial colors and the membrane of the used acrodisc syringe filter, as these colors have high molecular weights (Table I) with planar structures. Such adsorption may be increased in the presence of matrices which may bind with these colors and results in an increment in its adsorption on the membrane of the acrodisc syringe filter used. This adsorption was confirmed by comparing the obtained recoveries when applying this method with and without using acrodisc syringe filter. As shown in Table II, better results were obtained for jam sample in the absence of acrodisc syringe filter. Also, Ponceau's recovery in soda sample was largely improved in the absence of acrodisc syringe filter. In addition, lower recoveries were obtained for all the studied food colors in the soda sample when applying acrodisc syringe filter of 0.20 µm. On the other side, only slightly enhancement was obtained for the recoveries of the studied food colors in the yogurt sample. This is maybe attributed to the presence of an additional factor concerning the co-precipitation between the studied colors and the precipitated protein.

Table II. Comparison of percentage recoveries (RSD in parentheses) obtained for the analysis of the studied food additives in soda juice, jam, and yogurt using the developed method with and without acrodisc syringe filter of different pore sizes

Food Additive	Soda Juice			Jam		Yogurt	
	Acrodisc SZ of		Without filtering	Acrodisc SZ of	Without filtering	Acrodisc SZ of	Without filtering
	0.4 µm	0.2 µm		0.4 µm		0.4 µm	
Tartrazine	66 (17)	0	81 (6)	0	71 (14)	0	0
Indigo	87 (2)	27 (18)	80 (0)	0	70 (5)	15 (15)	46 (1)
Benzoic A	128 (5)	128 (9)	108 (14)	84 (9)	113 (0)	129 (4)	137 (19)
Sorbic A	92 (3)	83 (7)	88 (2)	93 (4)	91 (2)	81 (8)	93 (3)
Amaranth	61 (6)	0	87 (3)	0	62 (8)	0	0
Ponceau	38 (10)	0	77 (3)	0	75 (6)	0	4 (15)
Sunset Y	79 (4)	28 (23)	78 (3)	0	133 (17)	28 (15)	54 (1)
Acid R 33	70 (6)	0	79 (2)	0	79 (7)	0	35 (1)
Allura R	78 (4)	20 (19)	80 (0)	0	81 (6)	25 (13)	57 (1)
Carmoisine	61 (7)	0	79 (0)	0	81 (5)	0	39 (3)
Fast G	82 (4)	47 (15)	79 (0)	0	83 (6)	41 (7)	54 (2)
Brilliant B	84 (4)	57 (11)	80 (0)	0	82 (6)	48 (6)	59 (1)

Therefore, the mixture of H_2O:MeOH (1:3, v:v) was further tested with the addition of NaOH solution in order to enable the formation of the corresponding soda salts of the studied food additives. The formation of sodium salts of the studied FA (especially food colors) may results in an increment in its solubility with subsequently lowering its adsorption on the membrane of the acrodisc syringe filter (0.45 µm). As expected, better recoveries were obtained for all the studied FA when using a mixture of H_2O:MeOH (1:3, v:v) containing 0.01 and 0.03 mol L^{-1} NaOH. Higher concentrations of 0.06 and 0.12 mol L^{-1} of NaOH have also been tested but it led to degradation of indigo. Finally, the mixture of H_2O:MeOH (1:3, v:v) containing

0.01 mol L^{-1} NaOH was selected for all the tested samples as it doesn't lead to degradation for indigo even after 24 h. Therefore, this extraction solution was further full validated in all the studied matrices and used for the analysis of different real samples.

Method validation

As presented in Table III, most of the studied FA has acceptable recoveries at range 60–100% with RSDs ≤ 20% at all the studied concentration levels, which is in agreement with the European guidance document on quality control and method validation for pesticides residues analysis in food and feed (SANTE/11813/2017). Also, acceptable results were obtained for the inter-day measurements, listed in Table IV. The coefficient of determination (r^2) for each of the studied FA was calculated by plotting signal responses against its expected concentration. As presented in Tables IV, r^2 for each studied FA, in the three studied matrices, was ≥ 0.9960, indicating excellent linearity for the analyzed FA using the developed method.

Table III. Percentage recoveries (Rec) and relative standard deviation (RSD) at three concentration levels of 0.50, 1.25, and 2.50 mg kg^{-1} for all the studied FA except for tartrazine and benzoic acid at 1.00, 2.50, and 5.00 mg kg^{-1} in soda juice (dilution factor of 5), with Rec and RSD at twofold values in Jam and yogurt (dilution factor of 10), n= 5 in the same day

Food Additivies	Soda Juice			Jam			Yogurt		
	L1	L2	L3	L1	L2	L3	L1	L2	L3
	Rec/ RSD	Rec/ RSD	Rec/ RSD	Rec/ RSD	Rec/ RSD	Rec/ RSD	Rec/ RSD	Rec/ RSD	Rec/ RSD
Tartrazine	<LOQ	68 (4)	80 (3)	<LOQ	121 (3)	81 (2)	<LOQ	82 (10)	82 (9)
Indigo	85 (27)	100 (6)	90 (6)	81 (9)	82 (21)	102 (9)	56 (9)	83 (5)	85 (2)
Benzoic A	<LOQ	105 (1)	96 (2)	<LOQ	133 (5)	98 (1)	<LOQ	<LOQ	106 (18)
Sorbic A	80 (9)	101 (4)	97 (3)	99 (11)	97 (11)	98 (2)	82 (4)	86 (15)	88 (2)
Amaranth	69 (25)	81 (10)	79 (4)	98 (16)	86 (5)	87 (3)	87 (7)	92 (2)	78 (11)
Ponceau	68 (17)	87 (5)	89 (4)	93 (8)	92 (5)	88 (2)	78 (3)	79 (3)	86 (2)
Sunset Y	75 (18)	96 (4)	95 (1)	78 (17)	81 (24)	92 (8)	78 (16)	80 (2)	87 (2)
Acid R 33	74 (10)	96 (4)	93 (1)	84 (10)	88 (7)	93 (2)	78 (7)	86 (4)	88 (1)
Allura R	76(15)	99 (3)	96 (2)	93 (8)	89 (6)	96 (1)	91 (9)	87 (3)	87 (2)
Carmoisine	67 (17)	90 (7)	90 (3)	90 (10)	90 (6)	89 (1)	75 (7)	88 (3)	89 (1)
Fast G	83 (14)	102 (2)	97 (1)	90 (6)	90 (5)	95 (0)	87 (4)	87 (3)	89 (1)
Brilliant B	84 (11)	100 (3)	93 (2)	94 (5)	93 (5)	100 (1)	89 (2)	87 (3)	89 (1)

LOQ: Limit of quantification

Table IV. Recoveries (Rec) and relative standard deviation (RSD) at concentration levels of 2.50 mg kg^{-1} for all the studied FA except for tartrazine and benzoic acid at 5.00 mg kg^{-1} in soda juice (dilution factor of 5), with Rec and RSD at twofold values in jam and yogurt (dilution factor of 10), n= 5 in five replicate days, with the calculated coefficient of determination (r^2)

Food Additivies	Soda juice		Jam		Yogurt	
	Rec/RSD	r^2	Rec/RSD	r^2	Rec/RSD	r^2
Tartrazine	100 (19)	0.99961	81 (5)	0.996036	103 (8)	0.99869
Indigo	99 (14)	0.99947	94 (7)	0.999936	96 (4)	0.996481
Benzoic A	98 (22)	0.99740	97 (9)	0.99878	114 (20)	0.999424
Sorbic A	101 (5)	0.99993	98 (9)	0.999884	88 (5)	0.999957
Amaranth	84 (16)	0.99964	87 (6)	0.998702	75 (9)	0.999859
Ponceau	93 (9)	0.99989	88 (3)	0.999756	76 (22)	0.999917
Sunset Y	96 (4)	0.99986	92 (8)	0.999745	89 (3)	0.999986
Acid R 33	98 (5)	0.99984	94 (3)	0.999835	87 (6)	0.999983
Allura R	99 (4)	0.99982	96 (2)	0.999728	92 (5)	0.999981
Carmoisine	93 (6)	0.99974	89 (3)	0.999816	82 (18)	0.999975
Fast G	99 (3)	0.99981	95 (2)	0.999779	92 (4)	0.99999
Brilliant B	99 (3)	0.99985	100 (2)	0.999771	96 (4)	0.999963

The lowest validation concentration levels studied (Table III) were considered as the limit of quantification of the developed method for most of the studied FA, according to the SANTE/11813/2017. Only tartrazine and benzoic acid have a higher limit of quantification in all the studied commodities. The LOQ for tartrazine analysis in jam and yogurt equals to 5.00 mg kg^{-1} and in soda juice equals 2.50 mg kg^{-1}. While, LOQ for benzoic acid analysis using the developed method in soda, jam, and yogurt equals 2.50, 5.00, and 10.00 mg Kg^{-1}. However, these LOQs are lower than previously reported LOQs in a method applied using LC-MS/MS for food color analysis in species samples [9]. Although this study reported an extraction method using a complex mixture of solvents (H_2O/MeOH/ACN/THF, 9:1:5:5, v/v/v/v) and carried out longer stirring extraction time of 30 min, the reported LOQs for tartrazine, amaranth, and ponceau analysis are 10, 3.00, 2.00, mg kg^{-1}. These LOQs higher than that obtained in the current study (in all the studied matrices) may be attributed to the acrodisc syringe filter of 0.20 µm used without the NaOH additive in the extract solution. However, Waleska et al. [16] have reported lower LOQs for the analysis of 10 artificial colors in yogurt. This study reported repeated extraction steps using methanol: ammonium hydroxide (2 mol L^{-1}) (80:20) after protein precipitation using $K_4Fe(CN)_6 3H_2O$ (0.35 mol L^{-1}) and 0.5 mL $ZnSO_4 7H_2O$ (1 mol L^{-1}). This study confirms our suggestion about the importance of adding basic additives to the extract solution to enhance the recoveries of the tested food colors. However, this study didn't discuss the importance of such basic additives in minimizing adsorption of tested colors on the membrane of the used acrodisc syringe filter. Besides, it involves many preparation steps. A previously reported method [18] also used NaOH just to neutralize the soda solution (as mentioned by the authors) for the analysis of allura red and ponceau with LOQ of 0.30 mg L^{-1} without discussing its rule in lowering the adsorption of tested food colors in the membrane of the acrodisc syringe filter as investigated in the current study, especially for ponceau in soda sample. While nearly all the studied food colors cannot be analyzed in jam and yogurt without adding NaOH to the extract solution.

Analysis of real samples

The validated method was applied for the analysis of eighteen real samples, including eight soda juices, five jams, and five yogurt samples, all of which were purchased from local markets in Oman. The obtained results for these analyses are presented in Table V. As can be seen, soda samples were the most susceptible samples containing one or more food additives. Most of the soda samples were containing benzoic and or sorbic acid together with at least one food color. These results confirm the importance of our developed method that simultaneously enables the analysis of FA with food colors. There is one jam sample containing sunset yellow and another one containing a high concentration of benzoic acid of 767 mg kg^{-1}, exceeding its EU maximum permissible limits. On the other hand, no food colors were found in the collected yogurt samples, with the presence of only low concentrations of benzoic acid.

Table V. Levels of the detected food additives and its concentrations in the analyzed real samples

Sample	Nº of sample	Food Additive	Nº of detection	Range min-max (mg kg^{-1})
Soda Juice	8	Tartrazine	2	5.4 — 8.7
		Benzoic Acid	6	83.25 — 181
		Sorbic Acid	6	0.50 — 173
Jam	5	Benzoic	1	767
		Sorbic Acid	1	2
		Sunset Y	1	4.3
Yogurt	5	Benzoic Acid	3	1.5 — 4.65

CONCLUSION

In the current study, a complete liquid chromatography separation for eluted peaks of 10 food colors together with 2 food additives was achieved at a run time of 22 min. The addition of NaOH to the used extraction solution is a highly advantageous approach for the determination of different food colors simultaneously with food preservatives. A mixture of H_2O:MeOH containing 0.01 mol L^{-1} NaOH largely decreases the adsorption of the analyzed food colors in the membrane of the acrodisc syringe filter of 0.45 µm used. In addition, it enables proper extraction of the tested food additives in the high protein contents samples, like yogurt. The developed method was fully validated in three sample matrices include soda, jam, and yogurt. Analysis of real samples using the developed method indicates the presence of more than one food color together with one or two food preservatives at elevated concentrations, especially in the analyzed soda samples.

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ARTICLE

Easy Estimation of Endoglucanase Activity Using a Free Software App for Mobile Devices

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This study describes the estimation of the enzymatic activity of endoglucanase based on the 3,5-dinitrosalicylic acid (DNS) method and carboxymethylcellulose (CMC) substrate using glucose as the analyte. As an alternative to measuring the colored product obtained by the reaction between glucose and DNS, a free software app for mobile devices was used for estimating the color intensity of solutions, replacing the need of a UV-Vis spectrophotometer (reference method). The app is able to convert images in color histograms to red (R), green (G) and blue (B) scales and to provide univariate and multivariate calculations of the image data. The chemometric technique partial least squares (PLS) was employed with the app and presented accurate results that were comparable to those of the reference method. A correlation coefficient of 0.983 was obtained for the linear range between 0.60 and 2.60 mg mL^{-1} of glucose, and the standard error of cross-validation (SECV) was 0.219 mg mL^{-1}. The proposed method (imaging – PLS) was applied for samples of cellulase enzymes from *Aspergillus niger* and *Trichoderma reesei*, revealing an interesting alternative to estimate the enzymatic activity of endoglucanase.

Keywords: Endoglucanase activity, digital imaging, PLS, free software app.

INTRODUCTION

Lignocellulosic biomass has been shown to be an important natural raw material due its abundance and versatility in terms of applications in areas such as energy, materials, pharmaceuticals and food science [1,2]. Therefore, lignocellulosic biomass has grown as an important alternative to raw fossil-based materials, not only from an industrial perspective but also from an economic and environmental perspective. Currently,

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the biomass conversion process involves three main steps: delignification pretreatment, saccharification and fermentation. The challenge in the use of lignocellulosic biomass as a renewable raw material has been the obtention of reducing sugars, which can be converted into several value-added chemicals by several chemical and biologic routes, in the saccharification step [3,4].

Cellulase enzyme complexes hydrolyze β-1,4 linkages in cellulose chains and play an important role in cellulose saccharification. Endoglucanase hydrolyzes glycosidic bonds at amorphous regions of the cellulose, generating long chain oligomers, which are hydrolyzed into short chains, especially cellobiose, by the action of the exoglucanase. Finally, cellobiose is broken down to glucose by β-glucosidase enzymes [5]. In addition to being the first to act in cellulose hydrolysis, endoglucanase enzymes are outstanding for their importance in different industrial applications, such as in textiles, detergent and paper [6].

Several methods have been employed to determine endoglucanase activity in cellulase complexes from fungi, such as *Trichoderma reesei* and *Aspergillus niger*, which have been extensively studied due to their high cellulolytic activity during biomass conversion processes [7]. Basically, these methods have been carried out by colorimetric techniques, such as 3,5-dinitrosalicylic acid (DNS) and mainly spectrophotometric measurements [8,9], which requires a large consumption of energy and expensive instruments, hampering, for example, field analyses.

On the other hand, for this application, it is possible to use methods that minimize energy consumption and the use of expensive instruments and decrease waste generation but that provide results comparable to the reference method with high analytical frequency, simplicity and low cost [10,11], as an example, analyses using digital images from software apps for mobile devices.

Software apps for mobile devices that use digital images enable qualitative and even quantitative analyses [12], as shown in the study of Masawat et al. [13] in which a software app for mobile devices (iPhone) named “ColorConc” was developed that utilizes an image matching algorithm to determine the concentration of tetracycline in bovine milk. The proposed app was able to determine concentrations in a range from 0.5 to 10 μg mL^{-1} and provided a limit of detection (LOD) of 0.5 μg mL^{-1}. Moonrungsee et al. [14] developed a software app for mobile devices named “Phosphorus Analysis” that recorded digital images and used information from the Red-Green-Blue (RGB) color system to determine the content of phosphorus in soil. The calibration was performed by measuring the blue color intensity of standard phosphorus solutions (0.0 to 1.0 mg L^{-1}). The results obtained agreed well with the spectrophotometric reference method, with a detection limit of 0.01 mg L^{-1}, analytical frequency of 40 samples per hour, accuracy in terms of relative error smaller than 5% and precision in terms of relative standard deviation smaller than 2%, in addition to providing fast and low-cost method, which are convenient for in-loco analysis.

In this context, we used a free software app called PhotoMetrix [15] to estimate the enzymatic activity of endoglucanase enzymes. PhotoMetrix has been used for efficiently helping to determine iron in vitamin supplement tablets [16], iodine in biodiesel [17], ethanol in sugarcane spirit samples [18], and for monitoring of acid-base titrations on paper platforms [19], estimation of pH in milk samples [20] and chromium speciation in leather samples [21].

Thus, the goal of this study was to estimate the enzymatic activity of endoglucanase enzymes using color information data from digital images and reference values from the standard 3,5-dinitrosalicylic acid (DNS) method, by performing analyses with a free software app for mobile devices.

MATERIALS AND METHODS

Chemicals

Cellulase enzymes from *Aspergillus niger* (AN) and *Trichoderma reesei* (TR), and 3,5-dinitrosalicilic acid (DNS) were purchased from Sigma-Aldrich (St Louis, MO, USA). Carboxymethylcellulose (CMC) sodium salt substrate was obtained from Neon (Suzano, SP, Brazil). No additional purification was required for the chemicals.

Instrumentation

A smartphone ASUS ZE550KL (Beitou, TPE, Taiwan) equipped with a camera with 13-megapixel resolution and an Android 6.0 operating system Marshmallow were used. The software app for mobile devices PhotoMetrix Pro that includes the chemometric technique PLS computed with the NIPALS algorithm was downloaded for free from a website [15]. For spectrophotometric measurements, a Perkin Elmer Lambda 465 spectrophotometer (Waltham, MA, USA) equipped with a UV-Vis photodiode array detector was used.

Endoglucanase activity assay

The enzymatic activity assay was determined by measuring reducing sugar production after the reaction of the endoglucanase with the CMC, based on the DNS method and using glucose as a standard [8], as shown in Figure 1. The cellulase enzymes AN and TR were solubilized in citrate buffer, pH 5, with an initial concentration of 5.0 mg mL^{-1} and previously diluted before the reaction began. The reaction mixture contained 0.9 mL of 0.44% CMC solution in citrate buffer, pH 5, and 0.1 mL of enzymatic solution. The CMC hydrolysis reaction was carried out for 1 h at 50 °C, after which 1.5 mL of DNS reagent was added to stop the reaction.

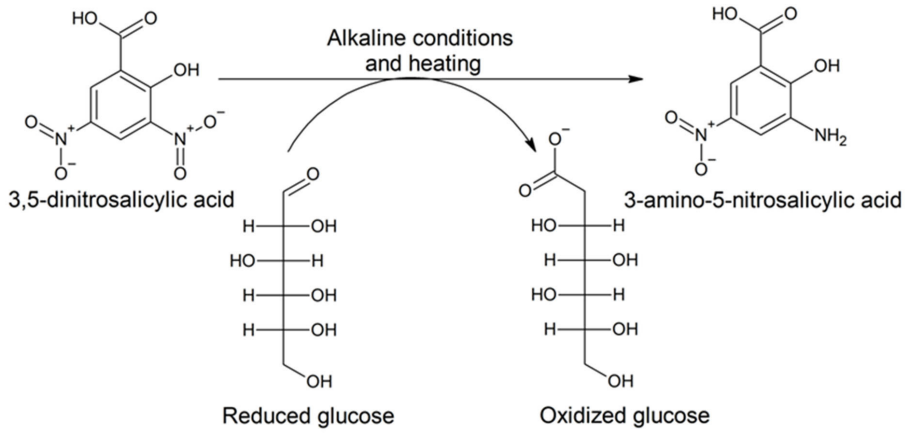

Figure 1. Chemical reaction of the 3,5-dinitrosalicylic acid (DNS) method for glucose determination.

The cellulase enzymes were heated in boiling water for 5 minutes to allow color formation. After cooling to room temperature, the concentration of reducing sugar was measured at 540 nm on a spectrophotometer. One unit of enzyme activity is defined as the amount of reducing sugar produced per mL of enzyme used over time and was stated as U mL^{-1} (μmol mL^{-1} min^{-1}). The units were calculated according to Equation (1):

$$\text{Endoglucanase activity } \left(\text{U mL}^{-1}\right) = \frac{\text{Df x reducing sugar } \left(\mu\text{mol mL}^{-1}\right) \text{ x } V_T\text{(mL)}}{\text{Time (min) x } V_E\text{(mL)}} \qquad (1)$$

where Df is the dilution factor of cellulase enzymes: 200 for TR and 25 for AN; V_T is the total volume of the reaction mixture (1 mL) and V_E is the enzyme volume (0.1 mL).

Reference method using UV–Vis data

The glucose standard curve for the DNS method was prepared by dissolving glucose in citrate buffer, pH 5, for concentrations in the range between 0.60 and 2.60 mg mL^{-1}. The reaction solutions of the standard curve were prepared according to the endoglucanase activity assay. Briefly, 0.1 mL of glucose solution was mixed with 0.9 mL of citrate buffer and incubated for 1 h at 50 °C. Subsequently, 1.5 mL of DNS reagent was added, and the samples were heated in boiling water for 5 minutes to allow color formation. After

cooling to room temperature, the solutions of glucose were measured at 540 nm on a spectrophotometer and submitted to image acquisition analysis. In the blank samples, glucose was substituted for citrate buffer, and the procedure was carried out in the same manner.

Proposed method using image - PLS

The acquisition of images of the solutions of various glucose concentrations was performed under controlled lighting provided by a light emitting diode (LED) lamp (6 W, 12 V), with a white paper underneath the Petri dishes to minimize the background reflectance and the influence of external light [22]. The LED lamp was used to illuminate the environment without interference in image quality [17].

The mobile device was placed on top of the laboratory-made imaging system adapted from Helfer et al. [20], which included a box (20 width x 31 length x 15 height cm) with a hole for the mobile camera device (15 cm above the samples) and another hole for the LED lamp, as shown in Figure 2. Approximately 5 mL of solutions was placed in a Petri dish (5.5 cm diameter x 1.0 cm height), and images were acquired using parallel alignment of mobile device on sample surface in a region of interest (ROI) of 64 × 64 pixels.

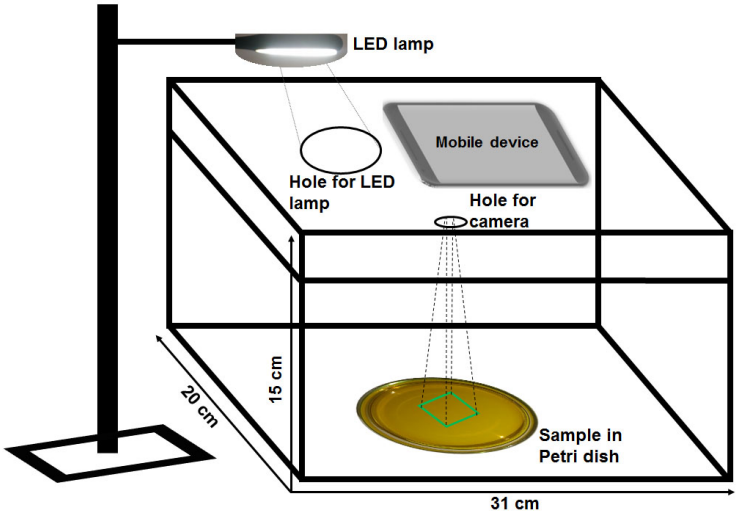

Figure 2. Schematic diagram for acquisition of the digital images.

RESULTS AND DISCUSSION

Figure 3 shows the univariate analytical curve obtained with the UV-Vis spectrophotometer from six glucose concentration values, obtained in triplicate and ranging between 0.60 and 2.60 mg mL^{-1}. As shown in Figure 1, the reducing sugars, such as glucose, have the capability to reduce DNS to its analogue 3-amino-5-nitrosalicylic acid. This aromatic compound absorbs strongly visible light at 540 nm and, therefore, can be used to establish a direct relationship between the amount of the reducing sugar and the colorimetric measurement from a UV-Vis spectrophotometer (reference method) [23]. The mean absorbances ±standard deviation (SD), (n = 3) were (1) 0.116 ±0.008; (2) 0.238 ±0.01; (3) 0.357 ±0.001; (4) 0.484 ±0.004; (5) 0.613 ±0.01 and (6) 0.730 ±0.02. These data are used according to the reference method based on the color formation during the reaction between DNS and the reducing sugars from the CMC hydrolysis to estimate the enzymatic activity of endoglucanase enzymes.

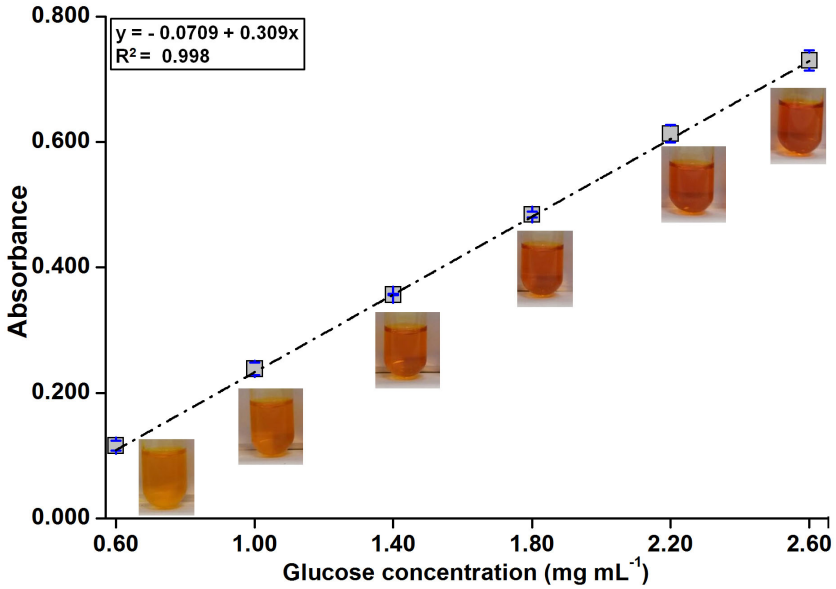

Figure 3. Analytical curve for glucose obtained using the reference method (UV-Vis spectrophotometry) for reducing sugar determination.

The robustness of the analytical curve of the UV-Vis was evaluated using analysis of variance (ANOVA), as shown in Table I. ANOVA revealed that the curve was well adjusted. The calculated F-value for the regression with 1 and 16 degrees of freedom (D.F.) was equal to 8954 at the 95% confidence level, being 1993 times higher than the tabulated F-value of 4.49. The calculated F-value for the lack of fit with 4 and 12 D.F. presented a value 9.42-fold smaller than the tabulated F-value at the 95% confidence level, confirming that the regression did not show a lack of fit. Moreover, neither of the regression coefficients assumed the value of zero, and these coefficients were significant at same level. The residues between the empirical and predicted values showed no heteroscedastic tendency in the data, implying a normal distribution.

Table I. Description of sum of squares from analysis of variance (ANOVA) for the analytical curve for UV-Vis spectrophotometry data

Source	Sum of squares	Degrees of freedom (D.F.)	Mean of squares	F-test	F-tabulated (95%)
Regression	0.801	1	0.801	8954	4.49 (1 and 16 D.F.)
Residual	0.00143	16	0.0000894		
Total	0.802	17	0.0472		
Pure error	0.00128	12	0.000107	0.346	3.26 (4 and 12 D.F.)
Lack of fit	0.000148	4	0.0000370		
R^2	0.998	R	0.999		
R^2 maximum	0.998	R maximum	0.999		

After this test, the UV-Vis data were considered appropriate as a reference for estimating enzymatic activities. The glucose concentration (mean ±SD, n=3) values were equal to 1.90 ±0.002 mg mL^{-1} and 2.02 ±0.03 mg mL^{-1} for cellulase enzymes from AN and TR, respectively. The relative standard deviations (RSD%) were 0.0853% and 1.27% for AN and TR, respectively.

The endoglucanase enzymatic activity calculated from the reference method according to Equation 1 was 44.0 ±0.04 and 375 ±5 U mL^{-1} for cellulase enzymes from AN and TR, respectively. Generally, *Trichoderma* spp. show almost four times more endoglucanase activity than *Aspergillus* spp. [24]. The higher endoglucanase activity found for TR can be attributed to its high endoglucanase production. Almost 20-36% of the cellulase production of TR was attributed to endoglucanase enzymes, which makes TR an excellent fungus for cellulose hydrolysis [25].

Afterwards, pictures were taken from the same solutions used in the reference method (UV-Vis spectrophotometry). The Photometrix Pro app automatically converted the images into color information data by representing the frequency of the pixels (histograms) of the RGB color system, generating 768 variables per image, with 256 variables for each individual color. According to ANOVA shown in Table II, there is no significant difference at the 95% confidence level, since that the F-value for the regression with 1 and 16 D.F. was equal to 467, being 104 times higher than the tabulated F-value of 4.49.

Table II. Description of sum of squares from analysis of variance (ANOVA) for imaging-PLS method

Source	Sum of squares	Degrees of freedom (D.F.)	Mean of squares	F-test	F-tabulated (95%)
Regression	7.85	1	7.85	467	4.49 (1 and 16 D.F.)
Residual	0.269	16	0.0168		
Total	8.11	17	0.477		
Pure error	0.0283	12	0.00236	25.5	3.26 (4 and 12 D.F.)
Lack of fit	0.241	4	0.0602		
R^2	0.967	R	0.983		
R^2 maximum	0.997	R maximum	0.998		

At the same app, it is possible to predict the glucose concentration using the chemometric technique PLS as an in-app tool. The histograms from the RGB colors, represented by color counts, were the independent variables and the glucose concentrations obtained by the reference method UV-Vis spectrophotometry were the dependent variables. The preprocessing applied to the independent variables included mean-centering for the counts of each color. The PLS model was built with only 3 latent variables (LVs) with leave-one-out cross-validation that explained 97% of the data variance. The glucose concentrations (mean ±SD, n=3) in mg mL^{-1} predicted by the PLS model were (1) 0.760 ±0.03; (2) 0.927 ±0.01; (3) 1.35 ±0.1; (4) 1.83 ±0.01; (5) 2.01 ±0.006 and (6) 2.72 ±0.03. The correlation between predicted (image – PLS) and reference values (UV-Vis) was equal to 0.983 and the value of coefficient of determination was 0.967, as shown in Figure 4. The SECV was used to evaluate the predictive ability of the PLS model and presented a low value equal to 0.219 mg mL^{-1}. The absolute errors ranged between -0.190 and 0.200, with an estimated bias of -0.000556.

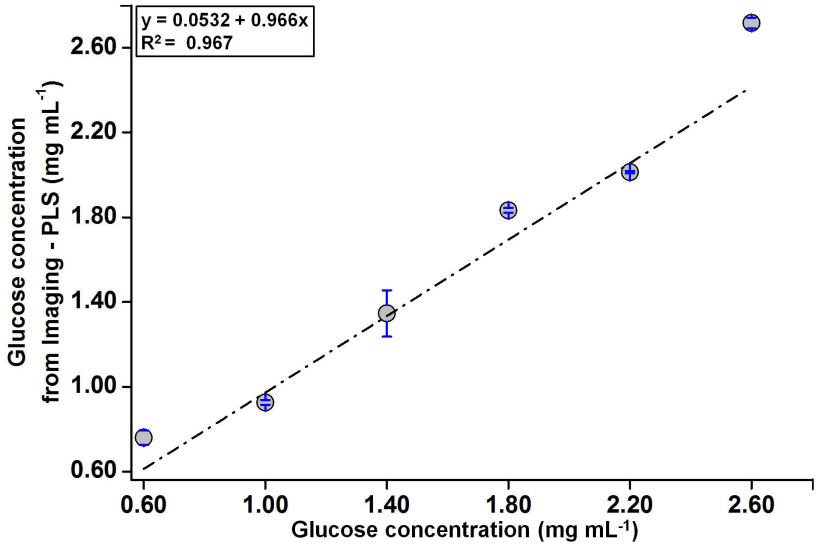

Figure 4. Correlation between values of glucose concentrations (mg mL^{-1}) of the standard samples *versus* predict by imaging-PLS model using Photometrix®.

Samples of cellulase enzymes from AN and TR were used in triplicate to evaluate the predictability of the PLS model. The glucose concentrations values (mean ±SD, n=3) for AN and TR predicted by the PLS model were 1.74 ±0.05 mg mL^{-1} and 2.08 ±0.1 mg mL^{-1}, respectively. The results of the SEP and the correlation coefficient of prediction were 0.164 mg mL^{-1} and 0.867, respectively. The range of absolute errors was between -0.208 and 0.171 with -0.0547 of bias. Using Equation 1, the cellulase enzymes from TR showed higher endoglucanase activity of 385 ±27 U mL^{-1} than those of AN at 40.2 ±1 U mL^{-1}.

The variances between the methods, UV-Vis spectrophotometry and imaging – PLS, were calculated. For the AN enzyme, the variance between the methods was not comparable, with a calculated F-value of 775 and a tabulated F-value of 19.0 with 2 D.F. (3 replicates measured per method equal to 2 D.F.), which suggests a 40.8-fold increase. Comparison of means was performed using a paired Student´s *t*-test for same samples with different variances since the tabulated *t*-value was 4.30 and the calculated *t*-value was 6.25 (2 D.F.).

For cellulase enzymes from TR, the endoglucanase activities obtained were 375 ±5 U mL^{-1} for UV-Vis and 385 ±27 U mL^{-1} for the image-PLS model. In this case, the variance was 31.8 for calculated F-value and 19.0 for tabulated F-value with 2 D.F. In this case, the variances between methods were still not comparable. Afterwards, a paired Student's *t*-test was calculated for the same samples with different variances, showing that there was no significant difference at the 95% confidence level between the two mean concentration values because the calculated *t*-value of 0.630 is smaller than the tabulated *t*-value of 4.30, and thus the mean was comparable. The advantages and limitations from both methods, UV-Vis and imaging - PLS were summarized in Table III.

Table III. Advantages and limitations between reference and proposed method

Parameter	Reference method UV – Vis Spectrophotometer	Proposed Method Imaging – PLS Mobile device
Cost	high	low
Robustness	high	high
Analysis *in loco*	no	yes

Table III. Advantages and limitations between reference and proposed method (Cont.)

Parameter	Reference method UV – Vis Spectrophotometer	Proposed Method Imaging – PLS Mobile device
Maintenance cost	high	low
Portability	no	yes
Analytical frequency	medium	high
User friendly interface	no	yes
Energy consumption	medium	low

CONCLUSIONS

The proposed method developed by using digital images processed by a cellphone app provides potential for evaluating enzymatic activity in cases where the enzymatic activity is higher than 385 U mL^{-1}, taking advantage of the calibration curve that can be quickly assessed using only a cellphone. The imaging – PLS method showed good agreement with the results of the colorimetric method using the DNS reaction at the 95% confidence level.

The traditional method using a spectrophotometer could be replaced by quick screening of enzymatic activity for *in loco* analysis in industries or processes that require this estimation, mainly those dedicated to biomass processing.

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Conflicts of interest

All authors declare that they have no conflict of interest.

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ARTICLE

Chemical Characterization and Human Health Risk Assessment Analysis of Cd, Cr, Fe, Mn and Zn Contents in Street Dust from Different Urban Sites in Minas Gerais State, Southeastern Brazil

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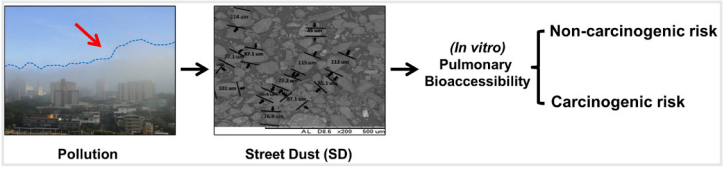

Health risk assessment of different metals (Cd, Cr, Fe, Mn and Zn) was evaluated in 12 street dust samples (SD) collected in Juiz de Fora and Congonhas cities, Southeast Brazil, from May to July 2017. Quartz, hematite, magnesium and aluminum silicate were found in Congonhas' samples, while quartz, hematite, andaluzite, diopside, dolomite and calcite phases have been found in Juiz de Fora's samples. The main elements observed in the samples were Al, Fe, O and Si, elements such as Ca, K, Mg, Na and Ti were found in minor concentrations. The major mineral phases observed in SD samples by MLA were iron oxides and hydroxides, quartz, clay compounds and many phases containing the Al element, which are in accordance with the XRD analysis characterization. Analytes were detected in the following order: Fe> Zn> Mn> Cr> Cd, except for Mn> Zn in the Congonhas samples. The Zn contents found in some samples were higher than prevention value for industrial areas (2000 mg kg^{-1}). The metals Cd (56 to 90%) and Zn (37 to 59%) showed high gastrointestinal bioaccessibility. The Cr and Fe analytes presented low solubility ranging from 10 to 18% and from 8 to 10%, respectively. It was verified that Cd and Zn presented non-carcinogenic risk through dermal, ingestion and inhalation contact for the most samples studied. However, non-carcinogenic risk factor (HQ) obtained for Zn in all samples collected were greater than the limit set by USEPA. No carcinogenic risk was observed for Cd and Cr in the investigated samples.

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Keywords: Trace elements, Street dust, Human health risk, Bioaccessibility.

INTRODUCTION

Street dust can be used as an indicator of environmental pollution. It is one of the main pathways of the plant, animal, and human exposure to toxic elements [1]. Street dust is defined as a complex matrix containing particles of different sources such as vehicles exhaust, organic materials, soil, deposited atmospheric particulate matter, etc., which can be a source of metallic elements [1-3].

Street dust also offers risk to human health due to the easy mobility of its particles, which may lead to human exposure through digestive, dermal, and inhalation [4]. Among the adverse effects, it can be mentioned the irritation of the upper respiratory tract, respiratory diseases such as lung cancer, acute respiratory infections, and also cardiac diseases [5].

Some studies have shown that trace elements, such as, As, Cd, Cr, Cu, Mn, Ni, Pb, and Zn can be found in street dust samples (SD), which can be a point of concern since exposure to high levels of some of these elements can cause chronic poisoning [2,6]. In summer periods, the atmospheric concentrations are usually lower due to the leaching caused by the rains, and in the winter there is a higher concentration of pollutants due to the intense dry season [2,3]. In this sense, it is important the development of studies regarding the chemical characterization, including both mineral and trace elements composition, of this kind of samples from urban areas around the world.

Several techniques can be used to characterize atmospheric pollutants, such as X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), X-ray fluorescence (XRF), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) and Atomic Absorption Spectrometry (AAS). These techniques provide relevant information regarding the mineral phases, morphology and the elemental chemical composition of the investigated samples. Recently, the technique of mineral liberation analysis (MLA) has been used to measure the composition and texture of the minerals [7-10]. Although there are a large number of papers using these techniques [11-15], their using together in order to obtaining SD information may be new, especially when applied to bioaccessibility studies.

Bioaccessibility may be defined as the quantity of a compound that is available for absorption when dissolved in body fluids, such as gastrointestinal and pulmonary. Then, bioaccessibility assays with street dust samples can be used to estimate urban pollution and its possible effects on human health [4]. The metallic bioaccessible fractions are also used to estimate these effects through health-risk models [16,17]. These calculations involve the determination of the exposed amount of pollutants as well as estimating factors for carcinogenic and non-carcinogenic risks, for example [2].

In Brazil, there are few works regarding the characterization of street dust sample [7,8,18]. Furthermore, there are no studies available concerning the chemical characterization of street dust from Brazil's southeastern cities associated with bioaccessibility assays. Thus, the goals of this work were the chemical characterization of urban dust samples from Juiz de Fora and Congonhas cities, in the Minas Gerais State, Brazil, and the evaluation of the potential health risks associated with its exposure.

MATERIALS AND METHODS

Studied Area

The studies were carried out with four samples collected in two different cities of Minas Gerais State, Brazil. Juiz de Fora is located in Zona da Mata, Minas Gerais State, at 43°20'40" west longitude and 21°41'20" south latitude. It has a total area of 1,435.749 km^2 with a population of approximately 560,000 inhabitants and approximately 242,000 vehicles. The city is located in a mountainous region, with altitudes ranging from 467 m to 1104 m and the climate is tropical high-altitude [19,20]. According to Environmental Company of the State of São Paulo [21] the 75% of air pollution in urban centers is due to the vehicles. Thus, an increase of vehicle traffic can affect air quality due to higher pollutants emission in the atmosphere. Mining-related activities emit atmospheric particulate matter (APM) for nearby areas, and air quality

impacts are a concern, as local soil, mineral dust, heavy equipment emissions and combustion sources and resuspension of particulate matter (PM) may all contribute to PM in surrounding areas [22]. High metal content in APM was observed by Mimura et al. [18] near a zinc mining industry, established in Juiz de Fora since 1980. The second city, Congonhas, is located in the macro region of Planning I in Minas Gerais state (20°29'59" south latitude and 43°51'59" west longitude). The city presents a total area of 306.45 km^2 with a terrain elevation varying from 800-1000 m. The annual mean temperature is 20.9 °C and the climate is humid subtropical. The estimated population of Congonhas in 2018 was about 54,200 inhabitants [20, 23]. United Nations Educational, Scientific and Cultural Organization (UNESCO) [25] recognize Congonhas as a World Cultural Heritage. The famous Sanctuary of "Bom Jesus de Matozinhos" was built in the 19th century on Baroque style. The atmospheric pollution resulting from fossil fuels burning and intense mining activities has been caused progressive corrosion of the statues over the years. The mining-metallurgical industry is the base of Congonhas' economy, and some researchers estimate that an amount of 120 tons of iron ore dust/mon is deposited on the city's public roads. As a result, this deposition in addition to the health problems, affect the integrity of humanity's historical heritages located in Congonhas [23].

Sample Collection

Street dust samples (SD) were collected in the following sampling sites: Juiz de Fora site 1-JF: Igrejinha district (latitude: 21°42'30.67"S, longitude: 43°29'8.90" W, altitude: 713 m) that is a region close to an industrial area; Juiz de Fora site 2-JF: (latitude: 21°46'10.34" S, longitude: 43°21'50.82" W, altitude: 935 m) at the meteorological station of the Federal University of Juiz de Fora, a region with mild traffic and high altitude. Congonhas site 1-CON: São Vicente village (latitude: 20°51'0792" S, longitude: 43.847277"), in the historical center; Congonhas site 2-CON: Federal Institute of Minas Gerais (latitude: 20.485256" S, longitude: 43.843066" W) near to the BR 040 federal highway that connects Brasília, the capital of Brazil, to Rio de Janeiro and shows an intense vehicle traffic. Samples were collected by sweeping 1 m^2 of a paved surface using plastic brooms and shovels at each of the collection site from May to July 2017, totalizing 12 samples. The contents were stored in plastic bags and transferred to the laboratory. Materials such as stones, branches and leaves were removed and the samples were sieved to a particle size less than 63 µm using a stainless steel sieve [26-28]. Areas with distinct characteristics were chosen in order to clearly demonstrate their influence on the samples investigated.

Instrumentation

A Thermo Scientific atomic absorption spectrometer with double atomization (flame and graphite furnace), model SOLAAR M5 (Waltham, MA, USA), equipped with a deuterium lamp as background corrector was employed for all element determinations. Flame Atomic Absorption Spectrometry (F AAS) was used to determine the contents of Cr, Fe, Mn and Zn while Cd and Cr pulmonary fractions were determined by graphite furnace atomic spectrometry (GF AAS). Hollow cathode lamps of the analytes were employed as radiation sources (Cd: 228.8 nm; Cr: 357.8 nm; Fe: 248.3 nm; Mn: 279.5 nm; Zn: 213.9 nm) and resolution of 0.5 nm for all analytes. For the F AAS, the gas mixture N_2O/C_2H_2 (Cr) and air/C_2H_2 (Cr, Fe, Mn, and Zn) have been used. The fuel flow rate were 4.2 L min^{-1} (Cr), 1.1 L min^{-1} (Fe) and 1.1 L min^{-1} (Mn and Zn). For GF AAS determinations, 99.99% purity argon was used as purge gas (0.2 L min^{-1}). The gas flow was stopped during atomization. The optimized pyrolysis temperatures were 300 and 1600 ºC while atomization temperatures were 1000 and 2600 ºC, for Cd and Cr, respectively. All measurements were performed in triplicate.

An ultrasound bath Unique Ultrasonic Cleaner, model USC 2800A, with a frequency of 40 kHz, power of 150 W and with time and temperature control was employed to extraction procedures (total and gastrointestinal contents). The samples were accurately weighted in an analytical balance (ME 204, Mettler Toledo, Columbus, OH, USA) and a metabolic bath (SOLAB, model Dubnoff SL-157/22) were used to bioaccessible essays.

The mineral phases of the samples were identified by X-Ray Diffraction (XRD) using a diffractometer

(D8 Advance - Vinci, Bruker). Morphological information was obtained by Scanning Electron Microscopy (SEM) and microanalysis of the samples were done using an Energy Dispersive X-Ray Spectrometer, EDX (Hitachi TM 3000) spectrophotometer. For the Mineral Liberation Analyzes (MLA), a FEV Quanta 650 model SEM-FEG equipped with two Bruker Quanta X-Flash 5010 EDS analyzers and the MLA software package version 3.1.1.283 was used. These analyzes were done at the Microscopy Center of the Federal University of Minas Gerais (CM-UFMG). For each of the particles detected in the images, EDS spectra were collected considering a range of pixels pre-defined by the system operator. The particles were classified by comparing the spectra acquired during measurements with those belonging to a mineral reference list. In this study, the ford analysis or grain-based X-ray mapping (GXMAP) measurement method was applied to identify the phases (minerals or not) present in the sample. In this type of measurement, a series of images is collected. The X-ray mapping is employed within a range of grayness intensities stipulated by the operator [24]. The SEM and MLA instrumental operating parameters are showed in the Table I.

Table I. Related parameters to SEM and to MLA

SEM Parameters	Values	MLA Parameters	Values
Voltage (kV)	25	Scan Speed	8
Working distance (mm)	11	Resolution	500 x 500
Spot size	5	Pixel size (μm/px)	0.60
Brightness	79.6	Acquisition time (ms)	10
Contrast	22.9	GXMAP BSE trigger	18-255
BSE calibration	Cu 245	Minimum grain size (px)	4
		GXMAP X-ray step (px)	6

Reagents and Solutions

All reagents used were analytical grade chemical reagents. The standards used for the construction of the analytical curves were prepared from stock solutions of Fe and Mn (Merck), Cd, Cr and Zn (Qhemis) with concentration of 1000 mg L^{-1}. The standards were prepared in acid medium (1.0% v/v HNO_3). The concentrations of the standards used in the construction of the analytical curves ranged from 0.5 to 3.0 mg L^{-1} and 1.0 to 8.0 μg L^{-1} (Cr), 0.5 to 16.0 mg L^{-1} (Fe), 0.1 to 4.0 mg L^{-1} (Mn), 0.25 to 2.0 mg L^{-1} (Zn), and 0.5 to 4.0 μg L^{-1} (Cd). The reagents used in the extraction procedures were HCl 37% v/v (Vetec), HNO_3 65% w/v (Vetec, Cosmos, Rio de Janeiro - Brazil) and H_2O_2 30% v/v (Isofar, Duque de Caxias, Brazil), $NaHCO_3$ (Isofar, Duque de Caxias, Brazil), Pepsin (Sigma Aldrich, St. Louis, MO, USA), Pancreatin (Sigma Aldrich, St. Louis, MO, USA), Bile (Sigma Aldrich, St. Louis, MO, USA), $MgCl_2$, NaCl, Na_2HPO_4, $CaCl_2.2H_2O$, $C_6H_5Na_3O_7.2H_2O$ (Sigma-Aldrich, St. Louis, MO, USA), $C_2H_3O_2Na$ (Isofar, Duque de Caxias, Brazil), KCl, $NaHCO_3$, Na_2SO_4 (Isofar, Duque de Caxias, Brazil). Used glassware were decontaminated in acid bath (10% v/v HNO_3) for 24 hours and rinsed at least three times with deionized water before use. All solutions were prepared with purified water at 18.2 MΩ in Elga Purelab Option-Q system.

Sample Preparation for AAS determination

Method 1: Extraction using ultrasonic bath (Total contents)

Total metallic contents were determined in SD samples after an ultrasound extraction according to the method developed by Mimura et al. [18]. For this, 100 mg of SD samples were mixed with 5 mL of aqua regia in 50 mL flasks. This mixture was placed in the ultrasonic bath for 10 min at room temperature (± 25 °C). The obtained extracts were filtered, centrifuged and transferred quantitatively into volumetric flasks. The volume was completed with deionized water to 50 mL.

Method 2: Procedure for in vitro gastrointestinal digestion

The bioaccessible fractions of the analytes were determined based on the method from Campos et al. [29]. For this, 100 mg of SD samples were mixed with 3.50 mL of 0.01 mol L^{-1} HCl solution. The pH was adjusted to 2.0 with a 1 mol L^{-1} HCl solution and 400 µL of pepsin solution (6% w/v) was added. The mixtures were incubated in a metabolic bath with mild agitation (level 5) for 2 hours at 37 ºC. Next, the pH of the solutions were adjusted to 7.0 by drop wise additions of $NaHCO_3$ 1 mol L^{-1} solution and 700 µL of pancreatin (0.4 w/v) and bile salts (2.5% w/v) were added. The mixtures were incubated again for more 2 hours under the same heating and conditions described above. After this procedure, the obtained extracts were cooled to room temperature to inhibit the enzymatic activity and filtered on common paper filter. The filtered solutions (called chyme) were then centrifuged at 2000 rpm for 15 minutes and then filtered through 0.45 µm Milipore® membrane filters.

To the determination of the bioaccessible fractions, the chyme solutions were submitted to an ultrasound-assisted extraction. For this, 5.00 mL of the extracts were mixed with 3.00 mL of HNO_3 and 1 mL of H_2O_2. Then, the mixture was placed in the ultrasonic bath for 1 h at 65°C. After that, the volume was brought to 20 mL with deionized water. The bioaccessibility was calculated by dividing the gastrointestinal soluble contents of the analytes by total content determined by Method 1 and expressed in percentage.

Method 3: Procedure for in vitro pulmonary bioaccessibility

The Gamble method [30] was employed to determine pulmonary bioaccessible fractions. Details about the composition of the synthetic pulmonary fluid used can be reached in Table II. Approximately 1.0 g of SD samples were mixed with 20 mL of this solution at pH = 7.4. Next, the mixtures were incubated in a metabolic bath with mild agitation (level 5) for 24 hours at 37 ºC. After this, the solutions were centrifuged for 15 minutes at 2,500 rpm and the soluble part was filtered through a 0.45 µm Milipore® membrane filter and stored in plastic flasks (acidified with 2% nitric acid) at refrigeration (10 °C) until the analysis. In this case, the analytes contents were determined by direct analysis and no sample preparation steps were necessary. Only sample dilution for analyte adjustment in the analytical curves used.

Table II. Composition of Gamble solution employed to pulmonary bioaccessibility experiments

Salt	Concentration (g L^{-1})
$MgCl_2$	0.095
NaCl	6.019
KCl	0.298
Na_2HPO_4	0.126
Na_2SO_4	0.063
$CaCl_2.2H_2O$	0.368
$C_2H_3O_2Na$	0.574
$NaHCO_3$	2.604
$C_6H_5Na_3O_7.2H_2O$	0.097

Analysis by MLA, SEM and MEV

For the other techniques used, it was not necessary samples pretreatment, so the direct analysis was done.

Health-risk assessment

Health-risk models, including carcinogenic (CR) and non-carcinogenic risk ratio (HQ) ones, from USEPA [16,17] were used to investigate the levels of the elements determined in the samples collected in this study. These estimating factors were calculated according to equations 1 to 4, as follow:

$$CDI_{ingestion} = \frac{C \times IngR}{BW} \times \frac{EF \times ED}{AT} \times CF \quad \text{Equation 1}$$

$$EC_{inalation} = C \times \left(\frac{ET \times EF \times ED}{ATn}\right) \quad \text{Equation 2}$$

$$DAD_{dhermal} = \frac{C \times SA \times AF \times ABS}{BW} \times \frac{EF \times ED}{AT} \times CF \quad \text{Equation 3}$$

$$HQ = \frac{CDI_{ingestion}}{RfDo} = \frac{EC}{RfCi \times 1000 \mu g mg^{-1}} = \frac{DAD}{RfDo \times GIABS} \quad \text{Equation 4}$$

$$CR = CDI_{ingestion} \times SFo = IUR \times EC = DAD \times \frac{SFo}{GIABS} \quad \text{Equation 5}$$

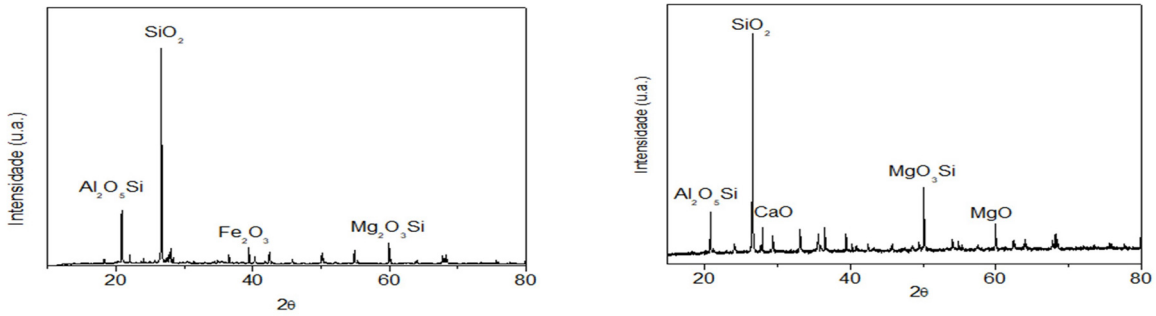
Where C is the element concentration (mg kg^{-1}) in SD samples. The concentrations obtained after the total extraction of the gastric fraction were used for determination of the ingestion parameter (CDI). The inhalation parameter (EC) values were obtained after the extraction of the pulmonary fraction. IngR is the ingestion rate (the default values for the ingestion exposure of soil/dust used were 200 mg day^{-1} for children and 100 mg day^{-1} for adults); EF: exposure frequency (180 days $year^{-1}$ in this study); ED: exposure period (6 years for children and 24 years for adults); BW: the average body weight (15 kg for children and 70 kg for adults); AT: average in time (for non-carcinogens, AT = EDx365 days; for carcinogens, AT = 70x365 = 25,550 days); CF: conversion factor (10^{-6} kg mg^{-1}); SA: surface area of the skin in contacts with the airborne particulates (3300 cm^2 for adults and 2800 cm^2 for children); AF: skin adherence factor for the airborne particulates (0.2 mg cm^{-2} for both adults and children); ABS: dermal absorption factor (0.001 for Cd; no values were available for other elements; therefore, 1.0% was used in the present study); ET: exposure time (hours/day); ATn: average time (for non-carcinogens, AT = ED x 365 days x 24 hours/day; for carcinogens, ATn = 70 year x 365 days/year x 24 h). All parameters used in the calculation of CDI, DAD and EC were found in reports published by the USEPA during different periods [16]. For the calculations of HQ and CR it was used the parameter RfDo that is the oral reference dose (mg $kg^{-1}day^{-1}$); RfCi as inhalation reference concentrations (mg m^{-3}); SFo as oral slope factor ((mg $kg^{-1}day^{-1})^{-1}$); GIABS as gastrointestinal absorption factor; IUR as inhalation unit risk ((mg $m^{-3})^{-1}$).

RESULTS AND DISCUSSION

X-Ray Diffraction (XRD)

The XRD technique was used to map the main minerals present in the investigated samples. The obtained diffractograms (Figure 1) were compared with the Crystallography Open Database standard (COD) files. In the samples from Congonhas sampling sites, the identified minerals were quartz (SiO_2), hematite (Fe_2O_3), magnesium (MgO_3Si) and aluminum silicate (Al_2O_5Si). Thus, the main elements found in

this type of sample were Si, Fe, O, Al and Mg. These mineralogical data show the same profile found by Queiroz et al. [7] for samples of atmospheric particulate material from Belo Horizonte (Minas Gerais State). For Juiz de Fora samples, the identified phases were quartz (SiO_2), hematite (Fe_2O_3), andaluzite (Al_2O_5Si), magnesium silicate (MgO_3Si), periclase (MgO) and lime (CaO). It can be seen that both the SD and APM have common majority constituents indicating common origin.



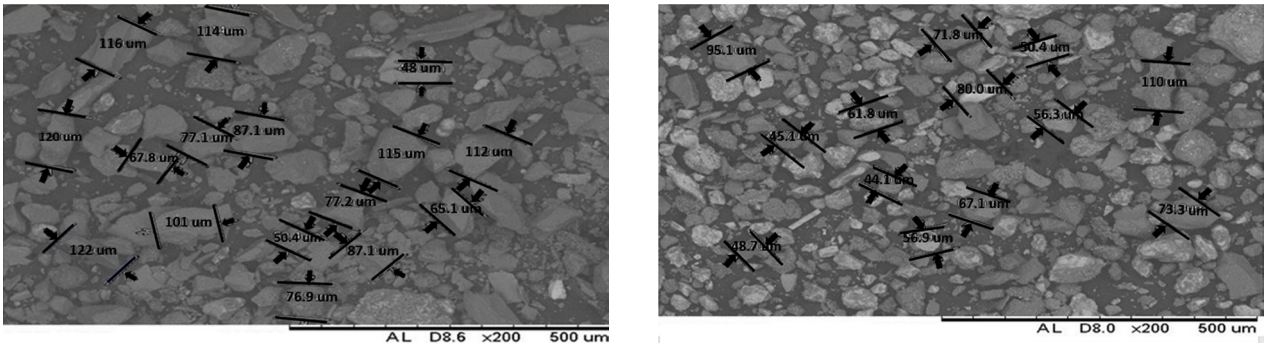
(a) (b)

Figure 1. X- ray diffraction of SD samples collected in May 2017 (a) Congonhas-Site 1 (b) Juiz de Fora-Site.

Scanning Electron Microscopy (SEM) and Energy Scattering X-ray Spectroscopy (EDS)

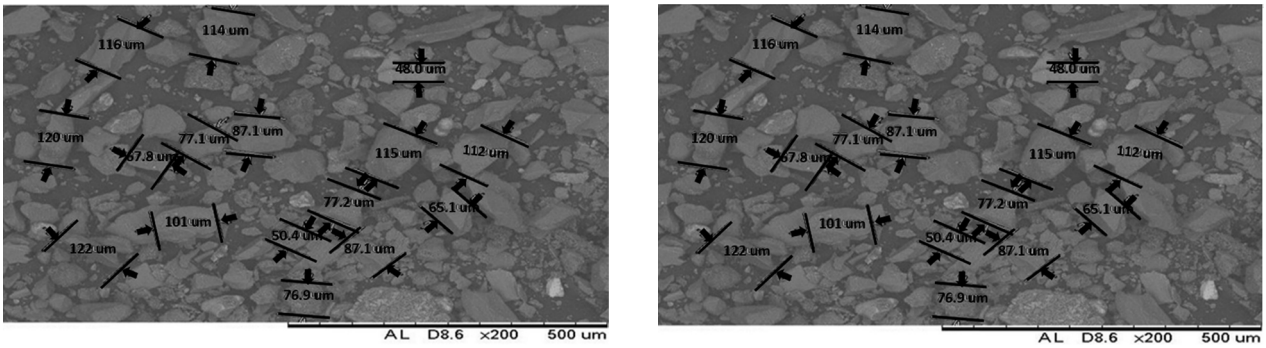
The SD samples from Congonhas presented particle size distribution from 36 to 125 μm while SD samples from Juiz de Fora showed particle size varying from 32 to 156 μm (Figure 2). In general, the particle size distribution for both cities did not show significant differences, probably due to the origins of the particles emissions, such as constructions, industrial activities and burning of fossil fuels, which is similar for both regions.

The investigated samples presented particles (rounded and cylindrical) with characteristic of biological origin, which include pollen, spores, bacteria and viruses [29]. Rough and schistose particles are irregularly shaped groups and were observed as individual particles or as small particles nucleated onto larger particles forming agglomerates. The observed characteristics reflect the minerals found in the soil, and may be associated with natural source [32]. Additionally, in the samples from Juiz de Fora it was observed crystalline-form particles that are characteristic of natural and marine origin [33-34]. According to the literature [35] particles smaller than 100 μm can penetrate the respiratory tract while particles smaller than 10 μm (PM_{10}) can penetrate in the nasal and oral cavities and penetrate into the larynx, while particles smaller than 2.5 μm ($PM_{2.5}$) can penetrate even deeper into the respiratory system reaching the lungs (bronchi and alveoli), these particles are also known as thoracic particles. In the present study particles with those characteristics were found in al investigated samples.



(a) (b)

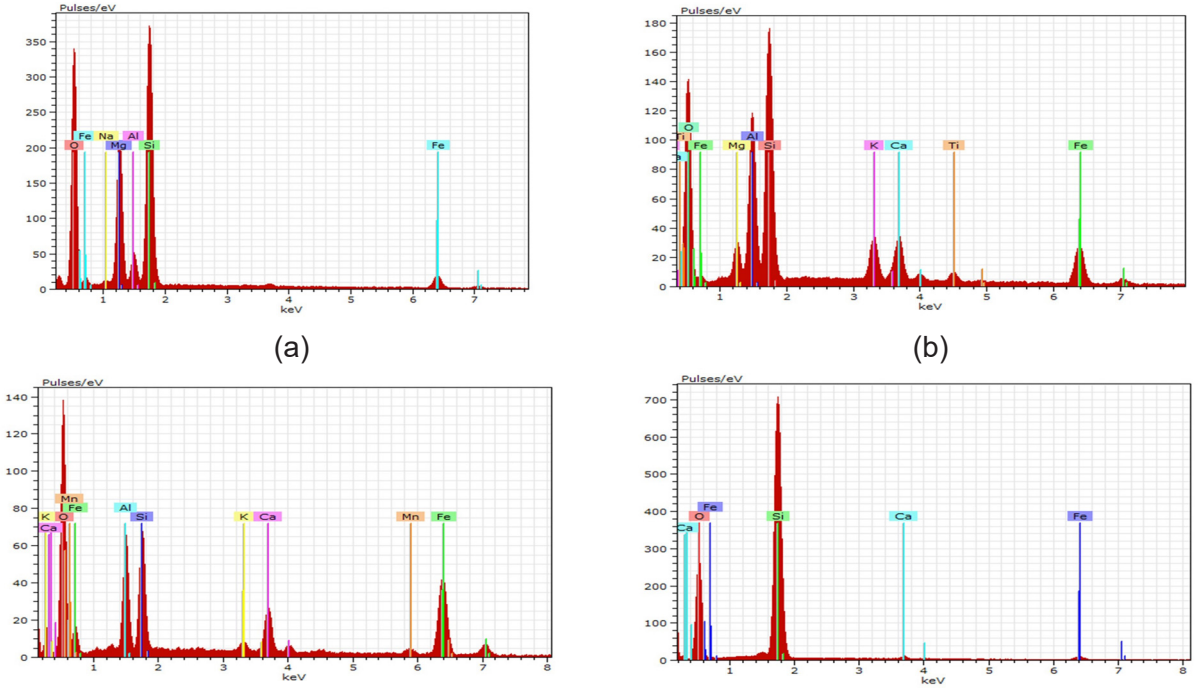
Figure 2. Image obtained by SEM for SD samples collected in Juiz de Fora and Congonhas in May 2017. Magnification: x 200 (a) Site 1-JF; (b) Site 2-JF; (c) Site 1-Con; (d) Site 2-Con.



(c) (d)

Figure 2. Image obtained by SEM for SD samples collected in Juiz de Fora and Congonhas in May 2017. Magnification: x 200 (a) Site 1-JF; (b) Site 2-JF; (c) Site 1-Con; (d) Site 2-Con. (Continuation)

Regarding EDS results (Figure 3 and Table III), the SD samples from the two cities presented Al, Fe, O and Si in their main constitution. Elements such as Ca, K, Mg, Na and Ti were also found in the samples, but their contents were less than 3% (w/w). The presence of Na in the samples collected in Juiz de Fora may be an indicative of the marine contribution, which is explained by the relative proximity of the studied area with the sea, as observed by Mimura et al. [18]. The EDS data are in agreement with those observed in XRD, and the presence of Fe and Al oxides are in agreement with data reported in literature [36–37].



(a) (b)

(c) (d)

Figure 3. EDS spectra obtained for SD samples collected in May 2017 in Juiz de Fora (a) Site 1; (b) Site 2; and Congonhas (c) Site 1; (d) Site 2.

Table III. Composition of SD samples collected in May 2017 obtained by EDS (% w/w)

Site	Analytes (% w/w)			
	Al	Fe	O	Si
P1-Juiz de Fora	2.3	2.2	64.4	18.4
P2-Juiz de Fora	7.3	0.3	60.9	14.3
P1-Congonhas	8.2	13.4	64.7	8.5
P2-Congonhas	5.9	21.3	66.4	5.0

Mineral Liberation Analysis (MLA)

The major mineral phases observed in SD samples by MLA (defined as > 1% wt) are Fe oxides and hydroxides, quartz, clay compounds and many phases containing the Al element, which are in accordance with the XRD analysis characterization. According to these results, Fe is mainly bonded to phases with oxides while Mn was observed in association with the group of pyroxenes found in many igneous and metamorphic rocks (Table IV).

Table IV. Mineral phases (% by weight) in SD samples

Phases	Samples			
	P1 – Con.	P2 – Con.	P1 – JF	P2 – JF
Fe Oxides/Hydroxides	78.05	72.25	4.71	10.99
Quartz	13.55	17.27	22.10	46.20
Andesine	<1%	1.26	15.18	10.78
Andratite	<1%	<1%	<1%	1.25
Calcite with Fe	1.34	1.10	<1%	<1%
Albite	<1%	<1%	<1%	1.68
Ortoclase	<1%	0.85	2.75	6.10
Siderite	<1%	<1%	<1%	1.18
Hornblende	<1%	<1%	2.35	2.92
Ilmenite	<1%	<1%	3.07	2.15
Pyroxenes	[a]ND	[a]ND	2.27	1.32
Vesuvianite	<1%	<1%	<1%	1.22
Biotite	<1%	<1%	1.63	<1%
Clay Minerals	<1%	<1%	33.45	5.77
[b]Mixture	<1%	<1%	<1%	<1%
Total	100.0	100.0	100	100.0
Total Particles	6457	7157	8140	16132

[a]Not determined; [b]Al, Si, P, S, Cl, Ca, Ti, Mn, Fe, Zn and O.

The pyroxenes are silicon and aluminum oxides with Ca, Na, Fe, Li, Mg, Mn, Zn substituting Si and Al. The general formula is $XY(Si, Al)_2O_6$, where X represents Ca, Na, Fe, Mg, Mn, Zn and Li; and Y represents smaller ions, such as Al, Cr, Co, Fe^{3+}, Mg, Mn, Sn, Ti and V. Chromium appeared mainly in FeOOH

0.12 (P1) and 0.59 (P2) for samples from Juiz de Fora, and 0.54 (P1) and 0.43 (P2) for Congonhas samples. Zn appeared mainly connected to mixtures with other compounds. The Cd probably due to its low concentration was not observed in the MLA analysis. The MLA tool allows the quantitative analysis of single particles of large number of grains. The total number of particles varied from 6,457 to 16,132, which provides good statistics in both cases. The difference observed was due to the larger number of particles that will be analyzed in the samples with a finer particle size distribution.

Health-risk assessment

Regarding total contents (Table V), Fe was the most abundant trace element found in SD samples from Juiz de Fora followed by Zn > Mn > Cr > Cd. For Congonhas SD samples the observed order was Fe > Mn > Zn > Cr > Cd. According to the Brazilian legislation [38] Site 1 (Juiz de Fora) showed both Cd and Cr contents above VP (prevention value – limit value of a substance in soil that cause no changes in its characteristics) for these elements (Cd: 1.3 mg kg^{-1} and Cr: 75 mg kg^{-1}) which needs attention. Values for Cr were also found above allowed in locations 2 and 3. On the other hand, the Zn contents found in the samples from Sites 1-JF and 1-CON were higher than VP values for industrial areas (2000 mg kg^{-1}). These results are probably due to the influence of industrial activities, such as mining and metallurgy, on the study area. Mimura et al. observed similar behavior in rainwater [40] and atmospheric particulate matter [18].

Table V. Metal contents obtained by F AAS and GF AAS for a resuspended dust sample by ultrasonic bath extraction (mg kg^{-1}) (mean ± standard deviation, n = 3)

Month	Site	Analyte				
		[a]Cd	[b]Cr	[b]Fe	[b]Mn	[b]Zn
May	P1-JF	1.84 ± 0.07	65.6 ± 3.6	54164 ± 217	1148 ± 60	10638 ± 57
	P2-JF	0.0587 ± 0.0023	28.2 ± 0.8	32313 ± 174	767 ± 59	545 ± 3.09
	P1-Con	0.0948 ± 0.0087	141 ± 7.3	53286 ± 181	2087 ± 85	2450 ± 17
	P2-Con	0.0468 ± 0.0024	48.8 ± 4.1	35424 ± 33	855 ± 13	153 ± 13
June	P1-JF	1.31 ± 0.03	92.4 ± 5.0	60281 ± 842	1490 ± 130	13091 ± 13
	P2-JF	0.0380 ± 0.0009	58.4 ± 2.48	39227 ± 59	880 ± 13	365 ± 17
	P1-Con	0.0229 ± 0.0006	74.8 ± 6.95	52094 ± 529	2974 ± 67	1365 ± 44
	P2-Con	0.0465 ± 0.0017	50.39 ± 3.59	23276 ± 117	3128 ± 15	278 ± 12
July	P1-JF	2.21 ± 0.34	98.2 ± 1.1	54149 ± 90	817 ± 4	10948 ± 60
	P2-JF	0.0569 ± 0.0002	82.2 ± 1.9	42955 ± 111	607 ± 5	640 ± 12
	P1-Con	0.0994 ± 0.001	36.7 ± 3.1	58940 ± 54	1097 ± 21	873 ± 63
	P2-Con	0.195 ± 0.001	46.3 ± 1.3	48055 ± 907	2800 ± 24	449 ± 60

[a]μg kg^{-1} / [b]mg kg^{-1}

The extracted contents of the metallic elements after *in vitro* gastrointestinal digestion are showed in Figure 4. The bioaccessible fraction levels were smaller than total contents showing that investigated trace elements were partially soluble in gastrointestinal tract. The metals Cd (56 to 90%) and Zn (37 to 59%) showed high gastrointestinal bioaccessibility while others, such as, Mn (19 to 35%), presented moderate solubility. The Cr and Fe analytes presented low solubility ranged from 10 to 18% and from 8 to 10%, respectively. The same behavior was observed by Xi Hun et al. [4] in APM from Nanjing (China).

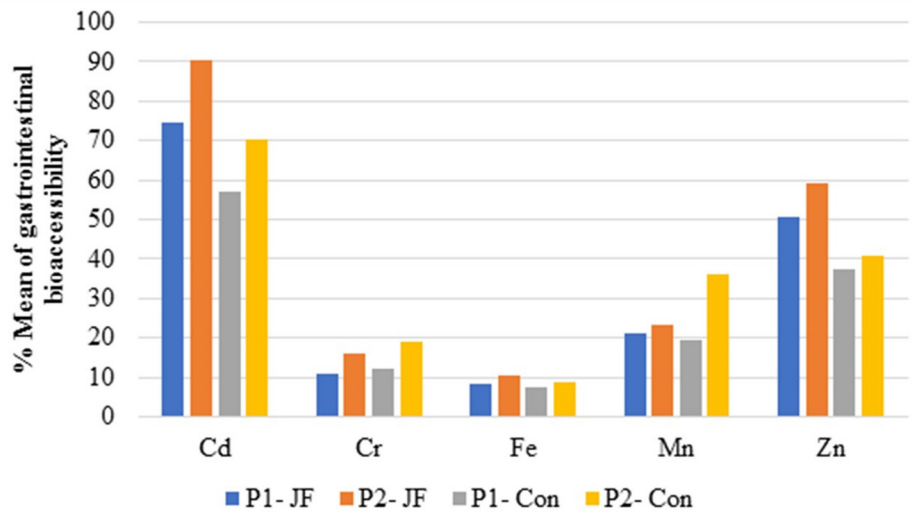


Figure 4. Gastrointestinal bioaccessible contents of Cd, Cr, Fe, Mn and Zn in SD samples from Juiz de Fora (JF) and Congonhas (Con) (mean of 12 samples expressed in %, RSD < 5%, n = 3 readings).

The results obtained after *in vitro* pulmonary bioaccessibility are shown in Figure 5. A lower solubility in synthetic pulmonary fluids was observed for all samples collected, with extractions minor above 50%. The Zn concentration in the pulmonary bioaccessible fractions varied in the range of 5 to 35% while the Cd concentration ranged from 8 to 26%. For Cr, Mn and Fe the concentration values ranged from 0.33 to 0.60, 0.34 to 1.1 and 0.21 to 2.0% respectively.

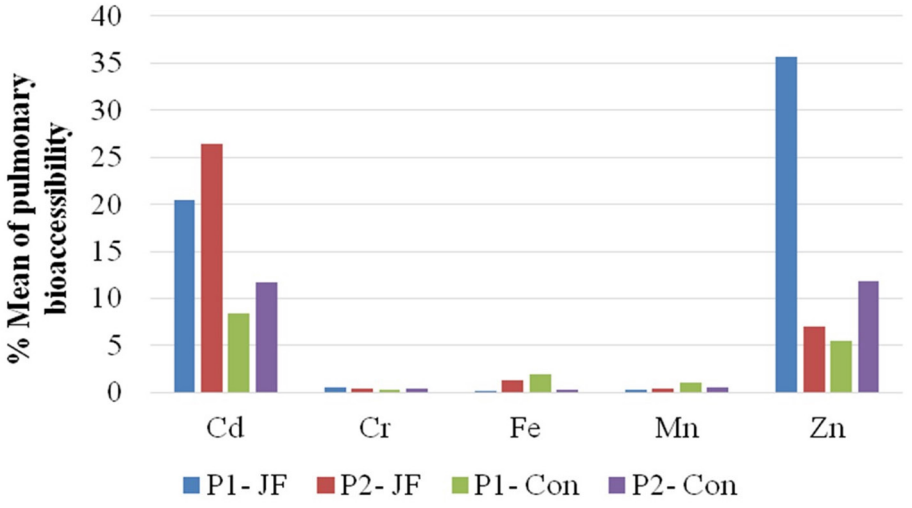


Figure 5. Pulmonary bioaccessible contents of Cd, Cr, Fe, Mn and Zn in SD samples from Juiz de Fora (JF) and Congonhas (Con) (mean of 12 samples expressed in %, RSD < 5%, n = 3 readings).

Carcinogenic (CR) and non-carcinogenic risk ratio (HQ) were calculated and the results are presented in Tables VI – VIII. For dermal contact risk, (Table VI) HQ values for Cd, Cr, Fe and Mn were lower than reference value (1) for adults and children in all samples collected (USEPA 2013). These results indicate no health-risk associated to dermal contact with the SD samples analyzed in this study. For Zn, HQ values for Site P1-JF were higher than one indicating health risk. Dermal contact with zinc compounds can cause skin irritation which may be an absorption pathway for those compounds into the bloodstream [5]. CR values were determined by Cd and Cr due to their carcinogenic classification according the International Agency

for Research on Cancer (IARC). For children the highest CR values found were 4.14 x 10^{-6} (Cd) and 5.0 x 10^{-4} (Cr) while for adults the CR values were 4.1 x 10^{-6} (Cd) and 7.7 x 10^{-5} (Cr). These results are considered acceptable since they are below 10^{-4} [16].

Table VII shows HQ and CR values for ingestion through gastrointestinal tract for Cd, Cr, Fe and Mn. All HQ values are below the set limit for adults and children. However, HQ values calculated for Zn in Sites P1 (32 for children and 14 for adults) and P2 (1.7 for children) from Juiz de Fora were higher than 1 indicating health risk. Ingestion of zinc compounds can cause nausea, diarrhea and intestinal irritation [5]. The maximum CR obtained for Cd and Cr were classified as acceptable for all points since its values were 1.4 x 10^{-5} (Cd) and 4.1 x 10^{-6} (Cr) for children, and 6.1 x 10^{-6} (Cd) and 1.8 x 10^{-5} (Cr) for adults.

Finally, the health risk assessment through inhalation was estimated by CR and HQ calculations which results are shown in Table VIII. Regarding CR calculations for Cd and Cr the highest values were 5.28 x 10^{-5} (Cd) and 9.2 x 10^{-8} (Cr) for adults and 1.3 x 10^{-5} (Cd) and 2.3 x 10^{-8} (Cr) for children, these values can be considered acceptable showing no carcinogenic risk. For Site 1-JF the values of HQ obtained for Fe were equal to 2.93 also indicating non-carcinogenic health risk. Additionally, for Cd and Zn, all HQ values for samples from both cities were higher than set limit indicating great health human risk. It demonstrates that remediation studies are needed for the studied regions because Cd is not desirable at any level for humans, as it plays no role in our body, and Zn can be harmful in large quantities.

Table VI. Values of non-carcinogenic risk (HQ) and carcinogenic risk (CR) dermal contact of analytes

Classification	Analyte	Samples							
		P1-JF		P2-JF		P1-Con.		P2-Con.	
		CR	HQ	CR	HQ	CR	HQ	CR	HQ
Children	Cd	1.0 x 10^{-6}	1.7 x 10^{-4}	3.0 x10^{-8}	4.8 x 10^{-6}	5.5 x 10^{-8}	8.7 x 10^{-6}	2.0 x10^{-7}	3.1 x 10^{-5}
	Cr	5.0 x 10^{-4}	2.0 x 10^{-1}	1.7 x 10^{-4}	1.1 x 10^{-1}	3.0 x 10^{-4}	2.0 x 10^{-1}	1.3 x 10^{-4}	8.4 x 10^{-2}
	Fe[a]	-	1.1 x 10^{-5}	-	6.5 x 10^{-6}	-	1.1 x 10^{-5}	-	4.7 x 10^{-6}
	Mn[a]	-	7.5 x 10^{-4}	-	4.0 x 10^{-4}	-	1.6 x 10^{-3}	-	1.5 x 10^{-3}
	Zn[a]	-	3.5	-	1.6 x 10^{-1}	-	4.8 x 10^{-1}	-	2.6 x 10^{-1}
Adults	Cd	4.1 x 10^{-6}	6.58 x 10^{-5}	1.2 x 10^{-7}	1.9 x 10^{-5}	5.5 x 10^{-8}	1.30 x 10^{-4}	2.0 x 10^{-7}	1.81 x 10^{-4}
	Cr	7.6 x 10^{-5}	5.9 x 10^{-2}	4.4 x 10^{-6}	2.9 x 10^{-2}	7.7 x 10^{-5}	5.1 x 10^{-2}	3.2 x 10^{-5}	2.1 x 10^{-2}
	Fe[a]	-	2.8 x 10^{-2}	-	1.6 x 10^{-6}	-	2.8 x 10^{-6}	-	1.2 x 10^{-6}
	Mn[a]	-	1.9 x 10^{-4}	-	1.2 x 10^{-4}	-	3.9 x 10^{-4}	-	3.7 x 10^{-4}
	Zn[a]	-	8.9 x 10^{-1}	-	4.0 x 10^{-2}	-	1.2 x 10^{-1}	-	6.6 x 10^{-2}

[a]There is no reference value

Table VII. Non-carcinogenic risk (HQ) and Carcinogenic risk (CR) values for the gastric fraction of the analytes

Type	Analytes	Samples			
		P1-JF	P2-JF	P1-Con.	P2-Con.
Children	Cd [a]	2.25 x 10^{-3}	7.5 x 10^{-5}	3.3 x 10^{-4}	3.72 x 10^{-4}
	Cd[b]	1.4 x 10^{-5}	4.7 x10^{-7}	2.0 x 10^{-6}	2.3 x10^{-6}
	Cr[a]	2.54 x 10^{-3}	6.5 x 10^{-6}	3.3 x 10^{-4}	3.7 x 10^{-3}
	Cr[b]	1.6 x 10^{-6}	4.1 x 10^{-6}	2.1 x 10^{-6}	2.3 x 10^{-6}
	Fe[a]	4.7 x 10^{-3}	4.0 x 10^{-3}	8.2 x 10^{-7}	2.6 x 10^{-7}
	Mn[a]	5.7 x 10^{-4}	4.1 x 10^{-4}	4.7 x 10^{-7}	5.3 x 10^{-7}
	Zn[a]	32	1.7	1.9 x 10^{-3}	1.2 x 10^{-3}

Table VII. Non-carcinogenic risk (HQ) and Carcinogenic risk (CR) values for the gastric fraction of the analytes (Cont.)

Type	Analytes	Samples			
		P1-JF	P2-JF	P1-Con.	P2-Con.
Adults	Cd [a]	9.6×10^{-4}	3.2×10^{-5}	1.30×10^{-4}	1.81×10^{-4}
	Cd[b]	6.1×10^{-6}	2.0×10^{-7}	8.22×10^{-7}	$1.14x\ 10^{-6}$
	Cr[a]	1.1×10^{-3}	2.8×10^{-3}	1.3×10^{-4}	1.8×10^{-4}
	Cr[b]	1.6×10^{-6}	1.8×10^{-5}	8.2×10^{-7}	1.1×10^{-6}
	Fe[a]	1.1×10^{-2}	9.4×10^{-3}	4.7×10^{-7}	5.3×10^{-7}
	Mn[a]	3.1×10^{-2}	2.8×10^{-2}	2.2×10^{-1}	1.3×10^{-1}
	Zn[a]	14	7.2×10^{-1}	4.3×10^{-4}	6.0×10^{-4}

[a]HQ and [b]CR

Table VIII. Non-carcinogenic risk (HQ) and Carcinogenic risk (CR) values for the lung fraction of the analytes

Type	Analytes	Samples			
		P1-JF	P2-JF	P1-Con.	P2-Con.
HQ (Adults/ Children)	Cd	2.0	7.7	4.5	4.2
	Cr	3.6×10^{-2}	2.0×10^{-1}	2.0×10^{-1}	$2.5x\ 10^{-1}$
	Fe	2.93	$1.1x\ 10^{-1}$	6.5×10^{-2}	7.0×10^{-2}
	Mn	3.14×10^{-2}	2.8×10^{-2}	2.2×10^{-1}	1.33×10^{-1}
	Zn	8320	786	1170	757
CR (Children)	Cd	1.3×10^{-5}	$4.98x\ 10^{-7}$	2.9×10^{-7}	2.7×10^{-7}
	Cr	$2.3x\ 10^{-8}$	1.5×10^{-8}	1.3×10^{-8}	$1.6x\ 10^{-8}$
CR (Adults)	Cd	5.28×10^{-5}	$2.0x\ 10^{-6}$	1.2×10^{-6}	1.1×10^{-6}
	Cr	$9.2x\ 10^{-8}$	5.8×10^{-8}	5.2×10^{-8}	$6.5x\ 10^{-8}$

CONCLUSION

This work describes the characterization of street dust samples from two southeastern Brazilian cities (Juiz de Fora and Congonhas) using different instrumental techniques such as F AAS, GF AAS, XRD, SEM-EDX and MLA. According to the results the street dust samples contained different minerals such as quartz hematite and clay. Additionally, the analyzed samples showed particles size below 100 μm, which can penetrate respiratory tract. Regarding health risk assessment, it was verified that Cd and Zn presented non-carcinogenic risk through dermal, ingestion or inhalation for the most samples studied. This behavior was also observed for Fe in samples from Site 1 of Juiz de Fora. Additionally, non- carcinogenic risk factor (HQ) obtained for Zn in all samples collected are greater than the set limit of USEPA, which suggests attention of Brazilian authorities as well. On the other hand, carcinogenic risk factor (CR) obtained for Cd and Cr in all collected samples are considered acceptable showing no carcinogenic risk through contact with street dust samples.

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ARTICLE

Data Processing Approach for the Screening and Quantification of Pesticide Residues in Food Matrices for Early-Generation Gas Chromatography Time-of-Flight Mass Spectrometry

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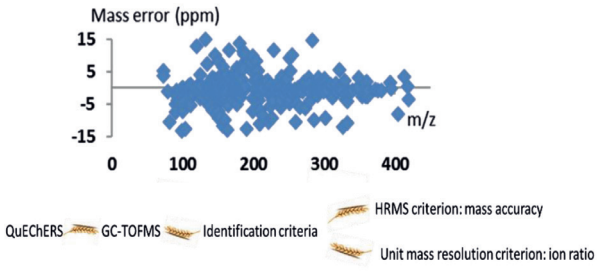

The application of high-resolution mass spectrometry (HRMS) in pesticide residue analysis is gaining popularity worldwide. Upgrading from early-generation gas chromatography time-of-flight mass spectrometry (GC-TOFMS) to higher resolution analysers is quite challenging, economically wise, for third countries' laboratories. Given the insufficient resolution of 12000 full width at half maximum (FWHM) or less offered by GC-TOFMS, meeting the HRMS identification requirements in some *Analytical Quality Control* guidelines may be challenging. This paper presents a useful approach for GC-TOFMS data processing for the screening and quantification of pesticide residues in cereals for laboratories disposing of that same equipment. The data obtained from spiking four types of cereals (wheat, rye, rice, and barley) at three different concentrations, 0.01, 0.02, and 0.1 mg kg^{-1} were evaluated with an "in-house" accurate-mass database of 102 pesticides, on the basis of two processing approaches. The data were first evaluated by considering the identification criteria in HRMS, which consists of the detection of two fragment ions of mass ≤5 ppm. The screening detection limits in that case were above 0.1 mg kg^{-1} for 25% of the compounds, owing to the high mass error (> 5ppm) obtained for some ions at low levels. The unsatisfactory results obtained were examined, and the data were re-evaluated by comparison with injected standards for identification (ion ratio). With this validated approach, the screening detection limit achieved for 85% of the compounds was 0.01 mg kg^{-1}. Therefore, given the insufficient resolving power of the instrument for some pesticide/commodity pairs, the HRMS requirement of 12000 FWHM in TOFMS was demonstrated to be inapplicable. Consequently, we recommend applying the requirements for identification of unit mass resolution for these specific mass spectrometers, to ensure accurate screening and quantification.

Keywords: Cereals, pesticide residues, mass accuracy, resolving power, screening detection limit, data processing.

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INTRODUCTION

The last true paradigm shift in the field of screening of pesticide residues in food was the shift from low-resolution mass spectrometry to high-resolution mass spectrometry (HRMS). HRMS enables broad-spectrum analysis and the collection of full scan spectra with excellent mass accuracy and mass resolution. Because of the variety and complexity of food matrices and the different matrix/pesticide combinations that may be present, accurate mass information by HRMS is required for good selectivity and identification capabilities. High-resolution analysers can resolve the ions of interest from most possible interferences originating from both matrix ions and the chemical background in complex samples. However, Balogh [1] has noted that the sensitivity decreases as the mass resolving power is increased in ToF analyzers, and the argument for higher mass resolution does not become persuasive until the molecular weights being measured become significant. A higher resolution is theoretically a better option in the presence of co-eluting isobaric compounds.

Numerous applications of HRMS in the literature indicate that this technique is highly suitable for screening pesticide residues in various food matrices [2,3]. Time-of-flight mass spectrometry (TOFMS) is a valuable approach that operates in full scan mode and can be combined with the development of an accurate-mass database for screening purposes; it has led to substantial improvements in food monitoring. GC-TOFMS is a powerful tool for screening pesticide residues in fruits and vegetables at a resolving power between 12500 and 18000 FWHM [4-7].

With the availability of GC-Orbitrap-MS, Mol *et al.* [8] have evaluated and demonstrated the efficiency of the full scan at a resolving power of 60000 FWHM in fruits and vegetables. The efficiency of a full scan with GC-Orbitrap-MS operated at a resolving power of 17500 FWHM for the analysis of pesticide residues in complex matrices, such as spices, has been described [9]. GC-Orbitrap-MS (resolving power: 100000 FWHM) is also effective in the analysis of pesticide residues in matrices that are difficult to analyse, such as wheat, maize and animal feed [10]. Thus, in the literature, a resolution ranging between 17500 and 100000 FWHM has been found to allow screening of pesticide residues in various matrices. However, generally, the more complex a sample extract is to analyse, the higher the resolution power is needed [11] to avoid false positive detection. If resolving power is a limitation, the sample preparation may be optimized for complex samples to decrease the complexity.

Cereals are known as difficult/dirty dry matrices with a fat content varying from 2% (e.g., wheat) to 6.5% (e.g., oats) [12]. Sample preparation aims to decrease potential chemical interference and consequently the resolution requirements. However, even with the latest extraction methods, because of the development of multiresidue methods aiming to cover as many compounds as possible, a certain resolving power is necessary to distinguish isobaric compounds in difficult matrices such as cereals. Maximum residue limits (MRLs) for cereals have been established by the European Union (EU) in Directive 32/ EC for 353 pesticides, at values between 0.01 and 0.1 mg kg^{-1} for 94% of the compounds [13]. Therefore, low screening detection limits (SDLs) and limits of quantification (LOQs) are essential to fulfil the MRL requirements.

In this paper, the use of high-resolution full scan GC-TOFMS as a screening and quantification platform for pesticide residue analysis in cereals is described and evaluated. Compound identification was performed with an in-house updated high-resolution accurate mass database covering 102 pesticides. The 102 pesticides selected are not among the most frequently found pesticides (only 15 compounds are included in the European Union multi-annual monitoring program), hence the relevance of their use in testing the screening method. The European Union monitoring program and the European Union member states focus on the control of pesticides and the commodities that contribute most to the dietary intake of pesticides. Though new pesticides are authorised and illegal uses occur and it is therefore relevant to supplement the control programmes with a wide scope screening programme. GC-TOFMS operating in full scan mode at a resolving power of 12000 FWHM was evaluated with respect to mass resolution, mass accuracy, ion ratio, precision, and sensitivity. The main research question was whether a resolution of 12000 FWHM might be sufficient for accurate qualitative and quantitative screening of pesticide residues in cereals, in accordance with the EU screening requirements. Therefore, with spiking of four types of cereals at 0.01, 0.02, and 0.1 mg kg^{-1} and a QuEChERS extraction method, the SDLs were assessed

according to SANTE guidelines, on the basis of accurate mass measurement of at least two representative ions [14]. The results were then re-evaluated by using additional requirements (standard and ion ratio). The validated method based on the second evaluation approach was finally applied in the screening of pesticide residues in 38 real samples of cereals and feeding stuffs.

MATERIALS AND METHODS

Chemical and reagents

Pesticide standards (purity > 96%) were purchased from Sigma-Aldrich or LGC Standards. Pesticide standard stock solutions of 1.000 mg mL^{-1} were prepared in toluene and stored at -18 ºC in ampoules under an argon atmosphere. A standard mixture of 10 mg mL^{-1} was prepared from these stock solutions. Working standard solutions were prepared with standard-matched calibrations with cereal blank extract. The blank extracts were obtained from the extraction procedure described in the 'Extraction method' section. Acetonitrile (HPLC Grade 5) was purchased from Rathburn Chemicals (Walkerburn, UK). The buffer salt mixture was purchased from Thermo Scientific, and the clean-up sorbent Supel™QuE (EN) tubes were purchased from Supelco (Belleffonte, PA, USA).

Extraction method

The extraction procedure was performed with an acetate-buffered version of the QuEChERS method. The adopted method was previously validated by Herrmann et al. [15] with 25 mg Primary Secondary Amine (PSA) per mL extract in the clean-up step for wheat, in accordance with EN 15662 [16]. Five grams of sample was weighed into a 50 mL centrifuge tube; 10 mL of cold deionized water and ceramic homogenizers were added; and the tubes were shaken vigorously so that the sample was soaked thoroughly. Afterward, 10 mL of acetonitrile was added, and the tubes were shaken vigorously by hand for 1 min. A buffer-salt mixture from Thermo Scientific™, consisting of 4 g magnesium sulfate ($MgSO_4$), 1 g sodium chloride (NaCl), 1 g trisodium citrate dehydrate, and 0.5 g disodium hydrogencitrate sesquihydrate was added. The tubes were shaken with an automatic shaker (SPEX SamplePrep 2010 Geno/Grinder®) for 1 min at 750 rpm and then centrifuged for 10 min at 4500 rpm with a Heraeus™ Multifuge™ X3 Centrifuge. Aliquots comprising 8 ml of the acetonitrile extracts were transferred to 15 mL centrifuge tubes and stored in a freezer for a minimum of 1 hour at -80 ºC. The still-cold extracts were centrifuged in a cool centrifuge (at 5 ºC) for 5 min to precipitate the low-soluble matrix co-extractives. Then 6 mL of acetonitrile extract was transferred to Supel™QuE tubes containing 150 mg of PSA and 900 mg of $MgSO_4$. PSA allows the removal of fatty acids from the extract. The tubes were shaken in an automatic shaker for 1 min at 750 rpm and then centrifuged for 5 min at room temperature (20 ºC) at 4500 rpm. Subsequently, 4 mL of the cleaned-up extracts was transferred into 15 ml centrifuge tubes, and 40 µl of 5% formic acid solution in acetonitrile was added to each extract to adjust the pH for storage stability. The extracts were later diluted by a factor of 2 with acetonitrile (0.25 g of sample/mL) to obtain the same matrix concentrations as those in the calibration standards, according to the in-house routine procedure for the quantitative methods.

Equipment

The samples were analysed with an Agilent 7200 Accurate-Mass Q-ToF-GC/MS, 7890A GC coupled to a PAL-GC automated Sampler 80.

For gas chromatographic separation, a 7890A gas chromatograph (Agilent Technologies) was used. The samples were injected in programmed temperature vaporizer (PTV) mode. The PTV enables the injection of solvents with high vapor expansion, in contrast to the split/splitless injection mode, thereby enabling the use of acetonitrile as injection solvent. Thus, solvent exchange involving exchanging the acetonitrile from the final QuEChERS extracts with, e.g., ethyl acetate was unnecessary. The injection volume was 5 µL, and the inlet temperature was 60 ºC. The analytes were separated in two fused silica HP-5MSUI capillary columns with 15 m x 250 µm inner diameter and a film thickness of 0.25 µm (Agilent). Helium (99.999% purity) was used as a carrier gas with a flow of 1.2 mL min^{-1} in the first column

and 1.4 mL min^{-1} in the second. The oven temperature program was as follows: 60 °C hold for 3 min, increase to 180 °C at 30 °C min^{-1}, and then increase to 300 °C at a rate of 5 °C min^{-1}. The total run time was 31.8 min, with four additional minutes for backflushing at 310 °C. The benefits of backflushing in capillary gas chromatography include better quality data and lower operating costs; it reduces the carryover of high boiling point compounds and it also helps to keep the electron ionization (EI) source clean resulting in less chemical background.

For the mass spectrometric analysis, a 7200 Accurate-Mass Q-ToF-GC/MS quadrupole time of flight (Q-ToF) mass spectrometer Agilent 7200 (Agilent Technologies) was used. The ion source operates in EI mode and spectra were collected at 70 eV. The EI emission value was set at 3.8 μA. The ion source temperature was set at 230 °C, and the transfer line temperature was set at 300 °C. The high-resolution mode of 4 GHz (12000 FWHM) at which the TOFMS operated in full scan enabled higher confidence in analyte identification. Internal mass calibration with perfluorotributylamine was performed before each injection for improved accurate mass operation. An automatic stop of the sequence occurred when the mass error exceeded 5 ppm. Data acquisition was performed with GC-Q-ToF MassHunter Data Analysis at a mass range of 69–500 Da with an acquisition rate of 5 spectra/s. MS data were collected in centroid mode.

Database

An exact mass database was created and optimized for 102 compounds (Supplementary Material). For each pesticide, the chemical formula and molecular weight, as well as the formulas and exact masses of at least three selective/sensitive fragment ions were added.

When a new compound was to be included in the database, the ion fragmentation was first predicted with ChemDraw (up to five fragments). The percentage of fragment probability (mass accuracy) and the assigned formula were verified in Agilent Qualitative Software (*Formula Calculator* Tool) and ChemCalc software (*Molecular Formula Finder* tool). The exact mass of the fragment ions was calculated in Agilent Qualitative MassHunter software with the *Mass calculator* tool and on the Scientific Instrument Services, INC (SIS) website. The mass-to-charge ratio (*m/z*) corresponding to the molecular ion (M^+) was obtained by subtraction of the electron´s mass from the neutral mass (M). Even though the electron mass was very low (0.00054858 Da), not subtracting it would have resulted in an initial theoretical mass error of 5 ppm for ion masses of $m/z \leq 100$.

Later, a standard mix of the 102 pesticides was injected in the GC-TOFMS at different concentrations to create the database. The retention times were collected, and the three most selective/sensitive fragment ions in the total ion chromatogram were selected.

The database also included isotope clusters. Isotopes are variations in chemical elements with different numbers of neutrons and thus different masses: they have the same number of protons and electrons but a different number of neutrons. Their occurrence increases with increasing molecular weight. Chlorine, e.g., exists as a pair of isotopes, ^{35}Cl and ^{37}Cl, in a near 3:1 ratio. M+2 (^{37}Cl) are elements with an isotope mass of 1.997050 Da above that of the most abundant isotope (^{35}Cl). In some cases, and in accordance with selecting the most abundant ions for optimizing the method sensitivity, isotope clusters were included in the database to provide the most intense peaks. Bixafen (formula: $C_{18}H_{12}Cl_2F_3N_3O$, exact mass: 413.030951) is one such example. One of the three selective and sensitive ions of bixafen is an isotope cluster (formula: $C_{18}H_{12}Cl(37Cl)F_3N_3O$; exact mass: 415.028001).

For sample screening, an automatic library search using the “Find compounds by formula option” in Agilent Qualitative Software is useful. The search can be filtered, selecting at least three fragment ions, a mass accuracy threshold of ± 5 ppm, a minimum peak intensity and a score of formula matching including isotopes (above 70%). However, with this approach, the number of false positive findings is high. To decrease the number of incorrect hits, the results must be assessed manually, thus making data review time consuming [17]. This approach is not recommended for sample screening on GC-QTOF. Therefore, the optimized database that was first maintained as a Microsoft Excel spreadsheet was imported into Agilent MassHunter Quantitative software for rapid data review and sample screening.

Spiking procedure

Four different types of cereals including blank samples of rice, rye, and barley, and two blanks of wheat were spiked at three concentrations of 0.01, 0.02, and 0.1 mg kg^{-1}. The spiking experiment was performed with five replicates for each matrix at each concentration level. A total of 75 samples were injected for the validation of the screening and quantification method. Another 75 non-diluted extracts were injected to study the effect of the increased sensitivity on mass accuracy.

Identification criteria

Mass accuracy is generally reported as a ppm error and is calculated by taking the difference between the theoretical mass and the measured experimental mass, dividing by the theoretical mass, and then multiplying by 10^6. According to SANTE guidelines [14], the requirements for identification with HRMS (>10000) include a mass accuracy ≤ 5 ppm for m/z > 200, whereas for m/z < 200, the mass accuracy must be < 1 mDa. For example, a fragment ion of m/z 141.06988 must have a mass error less than ± 7.1 ppm ($0.001/141.06988 \times 10^6$). Consequently, the mass error threshold is higher for lower m/z. However, for low mass range molecules and fragments, the probability of possible elemental composition is lower because there are fewer elements to combine. Consequently, a higher mass error can be accepted [18]. Moreover, regarding identification requirements, the signal to noise ratio must be ≥ 3, and the analyte peaks of the fragment ions in the extracted ion chromatogram (EIC) must fully overlap.

Method validation for screening

The qualitative screening method was validated according to SANTE guidelines. The validation involved at least 20 samples. The SDL was set at the lower concentration for which a certain analyte could be identified in at least 95% of the samples.

Method validation for quantification

The quantification method was validated according to SANTE guidelines [14]. The analytical performance of the method was determined by evaluation of the linearity, the recoveries and repeatability, the LOQ, the inter-day and intra-day precision, and estimation of the expanded uncertainty of the entire method. The matrix effect was also assessed.

Recovery and repeatability in terms of relative standard deviation (RSD) were calculated with each matrix at the three concentrations in the five replicates. The inter-day precision of the method was evaluated at the concentrations of 0.01, 0.02, and 0.1 mg kg^{-1} and was obtained by performing the same spiking process for five consecutive days. The intra-day precision was obtained by measuring the analytes five times on the same day and was also estimated at the three concentrations. The uncertainty (u') was estimated by the sum of the bias component results (u'bias) and the recovery's uncertainty u'(Rw) as follow:

$$u' = u'(bias) + u'(Rw) \quad \text{where } u' \text{ bias} = \sqrt{RMS'(bias)}$$

The u'(bias) was estimated by calculation of the root mean square of the bias (RMS'bias) derived from the inter-day reproducibility. The u'(Cref) contributions could be included in the bias component. The u'(Rw) was obtained from the standard deviation of recoveries obtained on the same day. The expanded uncertainty U' was expressed as 2u' and was required to be less than 50%. The uncertainty was also estimated at the three concentrations. The assessment of the matrix effect was performed by comparison of the slopes obtained with the calibration curves of wheat, rice, and barley to the slope of the calibration curve obtained with rye, the supposed easiest matrix, by calculating the slopes' relative standard deviation.

Real samples

The validated GC-TOFMS method was applied to 38 real samples of cereals and feeding stuffs sampled from the Danish market as part of the annual control program and corresponding to samples of wheat, barley, rye, basmati rice, jasmine rice, parboiled rice, white rice, red rice, pudding rice, rapeseed, linseed, sunflower seed, and hemp seed. Feeding stuffs have similar matrix profile than cereal samples, therefore it was relevant to analyse feeding stuff samples using the validated method of cereals.

RESULTS AND DISCUSSION

Evaluation of screening detection limits

First approach

First, the examination of the average mass accuracy of the 306 ions was conducted, after screening of 75 samples spiked at different concentrations. The average mass accuracy obtained from the 25 injections at the concentration of 0.1 mg kg^{-1} was below ± 5 ppm for 88% of the fragment ions. In addition, 78% of the ions showed an average mass accuracy below ± 5 ppm at 0.02 mg kg^{-1}, and 72% of the ions showed an average mass accuracy below ± 5 ppm at 0.01 mg kg^{-1}. Overall, the average mass accuracy obtained at different concentrations for all the compounds indicated that the probability of mass error was higher at low concentrations (0.01 mg kg^{-1}) and low *m/z* (<200).

However, according to SANTE guidelines, to calculate the SDL for accurate screening, the results must be evaluated for each compound, as described in the 'Identification criteria' section. Figure 1 shows the percentage of compounds that achieved an SDL of 0.01, 0.02, 0.1, and above 0.1 mg kg^{-1}. The SDL was 0.01 mg kg^{-1} for only 16% of the compounds, 0.02 mg kg^{-1} for 27% of the compounds, and 0.1 mg kg^{-1} for 37% of the compounds. No SDL could be established for 25% of the compounds. These compounds were not validated, but an SDL above 0.1 mg kg^{-1} was associated with those compounds.

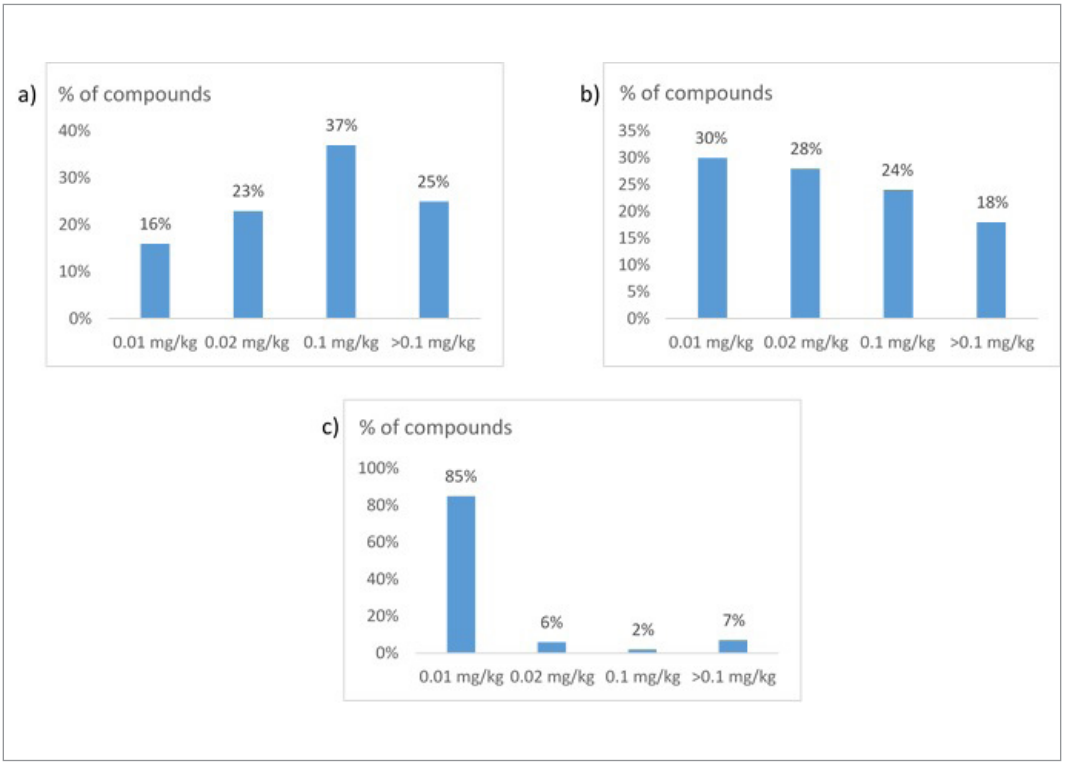

Figure 1. SDLs in mg kg^{-1} of the 102 compounds obtained with a) diluted extracts, b) non-diluted extracts, c) non-diluted extracts and considering the standards.

Some of the compounds were not well extracted with acetonitrile and required a single residue method to obtain satisfactory results; these compounds included cycloxidim (log P: 1.36), chloridazon (log P: 1.19), 8-hydroxyquinoline (log P: 1.915), and cyromazine (log P: 0.069). Dicrotofos (log P: -0.5) and amidosulfuron (log P: -1.56) were also not well extracted with acetonitrile. Other compounds showed poor extraction efficiency, such as dimoxystrobin (log P: 2.2), flumioxazin (log P: 2.55), and tralkoxydim (log P: 2.1). Ametoctradin (pKa: 2.78) is a strong acid and may be retrieved in its ionized form, and therefore is better extracted with water or other water-miscible solvents. The other compounds exhibited low sensitivity. The small molecules carvone ($C_{10}H_{14}O$, exact mass: 150.104465) and fuberidazole ($C_{11}H_8N_2O$, exact mass: 184.063663), having small fragments, showed very low sensitivity, in agreement with findings from Ramanathan *et al.* [19] who have shown that a mass resolution of 12000 FWHM is insufficient for detecting small molecules at low concentrations, owing to interference from endogenous compounds. The high SDL obtained for some compounds was associated with the four different matrices examined in this study. A major matrix peak was observed in the total ion chromatogram between 15 and 21 min with rice matrix. The matrix peak was generated by the high amounts of fatty acids in rice. In the 15–21 min time segment, a high number of pesticides also eluted.

In light of the data obtained, and given that low sensitivity was associated with the diluted samples, we assessed the mass accuracy without sample dilution. The spiked samples were injected without final dilution in the vial (0.5 g sample/mL). The SDLs obtained were 0.01 mg kg^{-1} for 30% of the compounds, 0.02 mg kg^{-1} for 28% of the compounds, and 0.1 mg kg^{-1} for 24% of the compounds. Moreover, 18% of the compounds showed an SDL above 0.1 mg kg^{-1} (Figure 1). Lower SDLs were obtained without the final dilution; however, using 0.5 g of sample/mL rather than 0.25 g of sample/mL would also increase the amount of matrix introduced into the instrument (liner, column, and ion source) and more maintenance would be required. Another approach to increase sensitivity is increasing the injection volume. The PTV injector allows use of high injection volumes [20]. However, an increase in sensitivity will not necessarily be accompanied by an increase in mass accuracy.

The SDLs were re-evaluated by considering a mass accuracy threshold of 10 ppm, in accordance with the US-FDA guidelines [21]. More satisfactory results in this case were obtained: 50% of the compounds showed an SDL of 0.01 mg kg^{-1}, 22% of the compounds showed an SDL of 0.02 mg kg^{-1}, and 16% of the compounds showed an SDL of 0.1 mg kg^{-1}.

Those results prompt the question of whether a resolving power of 4 GHz, 12000 FWHM, might be sufficient for screening pesticides at low concentrations in difficult matrices as cereals, without the use of any standard.

Second approach

HRMS instruments were expected to allow the identification of compounds without the use of standards, by relying only on the mass accuracy of two fragment ions. However, given the poor results obtained with the first approach, even though three ions were assessed, the data were re-evaluated with identification criteria commonly applied to unit mass data [14]. For unit mass spectrometry, using certified standards and comparing the ion ratios obtained for a sample with that of a standard is a key requirement for identification [14]. Thus, identification is inadequate without the use of standards. Fortunately, in simultaneous screening and quantification studies, standards were also injected, thus enabling the ion ratios to be used for identification. When this second approach was applied, 85% of the compounds achieved an SDL of 0.01 mg kg^{-1}, 6% of the compounds had an SDL of 0.02 mg kg^{-1}. Only 2% of the compounds showed an SDL of 0.1 mg kg^{-1}, and 7% of the compounds had an SDL above 0.1 mg kg^{-1} (Figure 1).

To improve understanding the differing results obtained with the two approaches, the correlation between the two identification criteria used was studied: the mass accuracy (first approach) and the ion ratio (second approach). Figure 2 provides a summary of the percentage of compounds that met the mass accuracy requirement (± 5 ppm or < 1 mDa compared with the database) and/or the requirement to ion ratio (± 30% of standard) for identification.

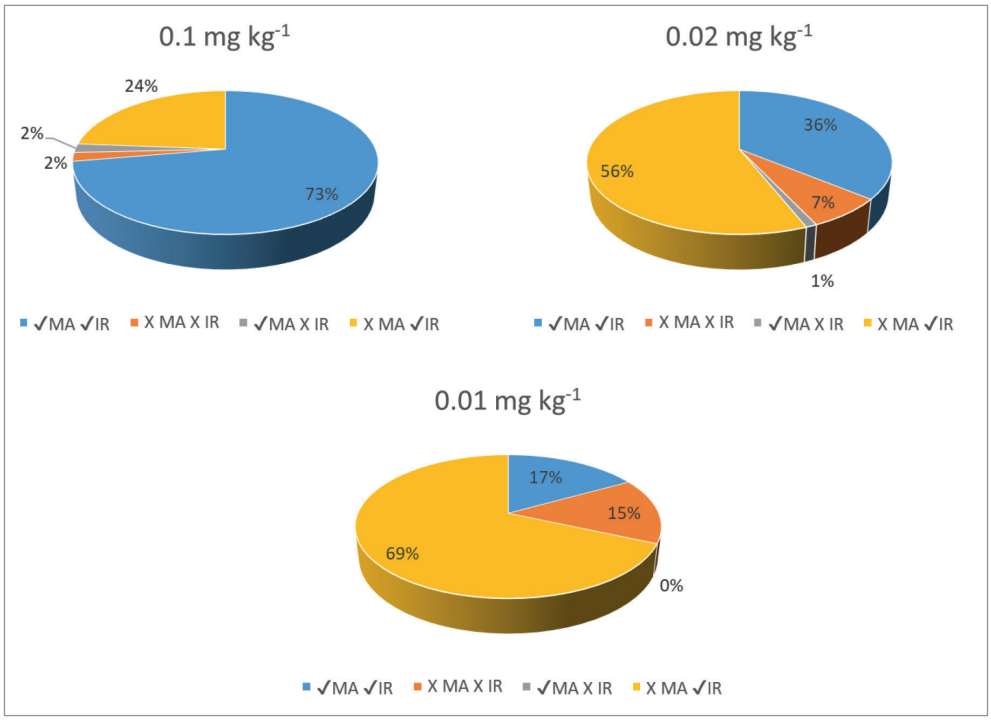

Figure 2. Correlation between mass accuracy and ion ratio at 0.1, 0.02 and 0.01 mg kg^{-1} (blue: percentage of compounds for which fragment ions have fulfilled both mass accuracy and ion ratio criteria. Orange: percentage of compounds that did not fulfill both ion ratio and mass accuracy criteria. Grey: percentage of compounds that have fulfilled the mass accuracy criterion but failed the ion ratio criterion. Yellow: percentage of compounds that have failed the mass accuracy criterion but have met the ion ratio criterion).

At 0.1 mg kg^{-1}, 75% of the compounds showed a positive correlation between the ion ratio and mass accuracy; when the mass accuracy criterion is met, so did the ion ratio results, and when the mass accuracy did not meet the criterion for peak identification, so did the ion ratio results. Therefore, peak identification based on mass accuracy was completely acceptable in that case, without the use of the ion ratio criterion (use of standards), because the two criteria gave the same indication. At 0.02 mg kg^{-1}, 43% of the compounds showed a good correlation between mass accuracy and ion ratio. At 0.01 mg kg^{-1}, the percentage of compounds was even lower: only 32% of the compounds showed a good correlation between mass accuracy and ion ratio. Therefore, the criterion of mass accuracy at low concentrations was insufficient in the last cases for compound identification.

At all the concentrations studied, a mass accuracy less than 5 ppm was accompanied by an ion ratio less than 30%, but not vice versa. At 0.1 mg kg^{-1}, 24% of the compounds showed high mass error (>5 ppm) but acceptable ion ratio (< 30%). At 0.02 and 0.01 mg kg^{-1}, approximately 56% and 69% of the compounds, respectively, showed good ion ratio but low mass accuracy (above 5 ppm or 1 mDa). These compounds met not only the ion ratio criterion but also the signal to noise ratio criterion, and the fragment ions were fully overlapping in the EIC. In the EIC of carfentrazone-ethyl and fenoxaprop-p-ethyl (Figure 3), carfentrazone-ethyl showed a good ion ratio and good mass accuracy for both qualifiers. Fenoxaprop-ethyl showed a good ion ratio, but a high mass error was observed with both qualifiers. Ignoring these compounds because of their low mass accuracy might have resulted in false positive detection.

Therefore, at high concentrations, the first approach relying solely on mass accuracy was applicable. However, at low concentrations, the use of standards and the evaluation of ion ratio was found to be an applicable identification criterion. Relying only on mass accuracy resulted in many false positives.

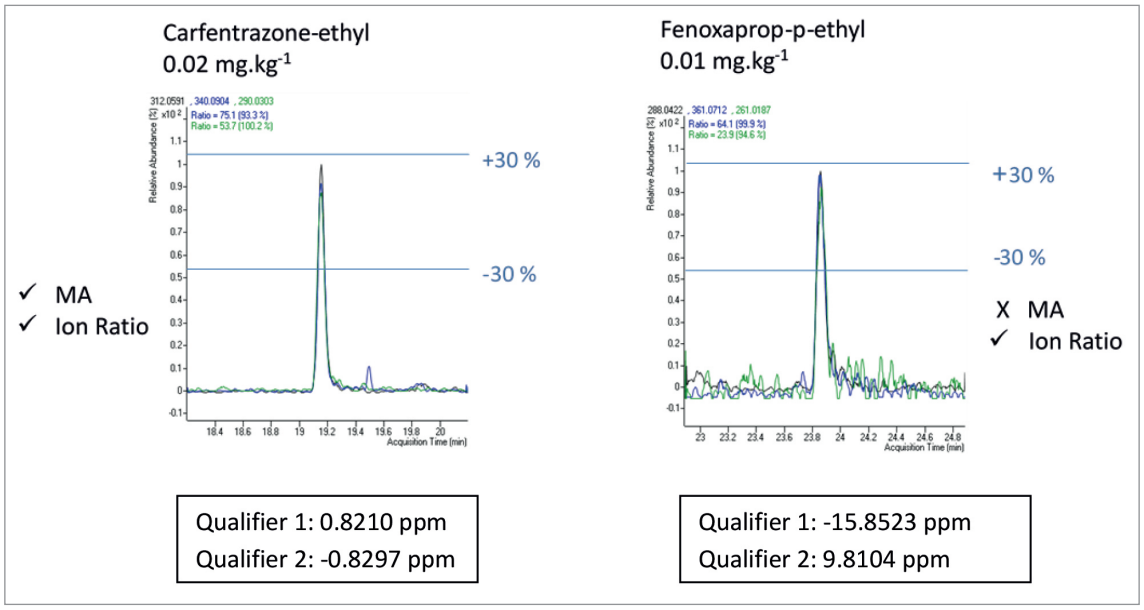

Figure 3. The extracted ion chromatograms of the two qualifiers of carfentrazone-ethyl and fenoxaprop-p-ethyl; ion ratio and mass accuracy.

The question was addressed again about how much resolution is needed to reach low detection limits with high accuracy. Regarding the identification criteria for screening pesticide residues in cereals at a resolving power of 12000 FWHM, the use of standards remains the most important tool for identification. With the availability of different HRMS with different resolving power, the identification requirements cannot be generalised. The limitations of equipment offering a resolution of 12000 FWHH or less must be considered when analysing pesticide residues in difficult matrices.

The EU MRLs in force for the studied compounds in cereals vary from 0.01 up to 0.1 mg kg^{-1}. Among the 102 compounds, 60 compounds had established EU MRLs. Using the extraction procedure described above and applying the first approach, SDLs equal to or below the corresponding EU MRLs were achieved for only 35% of the compounds. When the dilution step was omitted, 47% of the compounds fulfilled the MRL requirements. If the ion ratio was considered along with the mass accuracy, *i.e.,* the second approach was applied, 90% of the compounds were suitable for MRL compliance check. Table I lists the SDLs obtained by the application of both the first and the second approach along with the MRLs in force for each compound.

Table I. The SDLs achieved with 0.25 g sample/mL of extract and with 0.5 sample/mL of extract considering the identification requirements of the SANTE guidelines (Mass accuracy (MA), S/N, peaks overlapping), and the SDLs obtained with 0.25 g of sample/mL by considering the ion ratio (IR) criteria, and the EU MRLs established for cereals.

			SDL (mg kg^{-1})		
	Compounds	**MRLs (mg kg^{-1})**	**SDL (0.25 g sample/mL)+MA**	**SDL (0.5 g sample/mL)+MA**	**SDL (0.25 g sample/mL)+MA+IR**
1	1-Naphthylacetic acid	0.06	0.02	0.02	0.01
2	1-Naphtylacetamide	0.06	0.1	0.02	0.01
3	8-Hydroxyquinoline	0.01	>0.1	>0.1	>0.1
4	Acetochlor	0.01	0.02	0.02	0.01
5	Aclonifen	0.01	0.1	0.1	0.01
6	Ametoctradin	0.05	>0.1	>0.1	0.02

Table I. The SDLs achieved with 0.25 g sample/mL of extract and with 0.5 sample/mL of extract considering the identification requirements of the SANTE guidelines (Mass accuracy (MA), S/N, peaks overlapping), and the SDLs obtained with 0.25 g of sample/mL by considering the ion ratio (IR) criteria, and the EU MRLs established for cereals. (Cont.)

			SDL (mg kg^{-1})		
	Compounds	**MRLs (mg kg^{-1})**	**SDL (0.25 g sample/mL)+MA**	**SDL (0.5 g sample/mL)+MA**	**SDL (0.25 g sample/mL)+MA+IR**
7	Amidosulfuron	0.01	>0.1	>0.1	0.01
8	Amisulbrom	0.01	>0.1	>0.1	0.01
9	Anthraquinone	0.01	>0.1	>0.1	0.01
10	Benalaxyl	0.05	0.02	0.02	0.01
11	Benfluralin	0.02	0.02	0.01	0.01
12	Biphenyl	0.01	0.01	0.01	0.01
13	Bixafen		0.1	0.1	0.01
14	Butralin	0.01	0.1	0.02	0.01
15	Carbophenothion		0.1	0.02	0.01
16	Carfentrazone-ethyl	0.05	0.1	0.02	0.01
17	Carvone		>0.1	>0.1	>0.1
18	Chlorantraniliprole		>0.1	>0.1	0.01
19	Chloridazon	0.1	>0.1	>0.1	>0.1
20	Chloropropylate		0.02	0.01	0.01
21	Chlorthal-dimethyl	0.01	0.1	0.02	0.01
22	Cinidon-ethyl	0.05	>0.1	>0.1	0.01
23	Clodinafop-propargyl	0.02	>0.1	0.1	0.01
24	Cycloxydim		>0.1	>0.1	>0.1
25	Cyflufenamid		0.1	0.01	0.01
26	Cyromazine	0.05	>0.1	>0.1	>0.1
27	Dialifos		>0.1	>0.1	0.1
28	Dichlobenil	0.01	0.01	0.01	0.01
29	Dichlofenthion		0.02	0.01	0.01
30	Dicrotofos		>0.1	>0.1	0.01
31	Diflufenican		0.02	0.01	0.01
32	Dimetachlor	0.02	0.02	0.02	0.01
33	Dimethenamid	0.01	0.01	0.01	0.01
34	Dimoxystrobin		>0.1	>0.1	0.02
35	Diniconazole	0.01	0.02	0.01	0.01
36	Dioxathion	0.01	>0.1	>0.1	0.01
37	Ethalfluralin	0.01	0.1	0.02	0.01
38	Ethofumesate	0.03	0.01	0.01	0.01
39	Etoxazole	0.01	0.1	0.1	0.01
40	Etridiazole	0.05	0.1	0.02	0.01
41	Etrimfos		0.1	0.1	>0.1
42	Famoxadone		0.1	0.1	0.01
43	Fenchlorphos	0.01	0.01	0.01	0.01

Table I. The SDLs achieved with 0.25 g sample/mL of extract and with 0.5 sample/mL of extract considering the identification requirements of the SANTE guidelines (Mass accuracy (MA), S/N, peaks overlapping), and the SDLs obtained with 0.25 g of sample/mL by considering the ion ratio (IR) criteria, and the EU MRLs established for cereals. (Cont.)

			SDL (mg kg^{-1})		
	Compounds	**MRLs (mg kg^{-1})**	**SDL (0.25 g sample/mL)+MA**	**SDL (0.5 g sample/mL)+MA**	**SDL (0.25 g sample/mL)+MA+IR**
44	Fenoxaprop-p-ethyl	0.1	0.1	0.02	0.01
45	Flonicamid		0.02	0.02	0.01
46	Fluazinam	0.02	>0.1	>0.1	0.01
47	Flucythrinate I and II	0.01	0.1	0.1	0.01
48	Flufenacet		0.1	0.02	0.01
49	Flumetralin	0.01	0.01	0.02	0.01
50	Flumioxazin	0.02	>0.1	0.1	0.01
51	Fluopicolide	0.01	0.01	0.01	0.01
52	Fluopyram		0.01	0.01	0.01
53	Flurochloridone	0.1	0.02	0.02	0.01
54	Flurprimidol	0.02	0.01	0.01	0.01
55	Flurtamone	0.01	>0.1	0.1	0.02
56	Flutolanil		0.01	0.01	0.01
57	Fluxapyroxad		0.02	0.01	0.01
58	Fonofos		0.01	0.01	0.01
59	Fuberidazole		>0.1	>0.1	0.02
60	Furathiocarb	0.01	0.01	0.01	0.01
61	Heptachlor	0.01	0.02	0.01	0.01
62	Isocarbofos		0.1	0.1	0.01
63	Isofenfos		0.01	0.01	0.01
64	Isoprocarb		0.01	0.01	0.01
65	Isopyrazam		0.1	0.02	0.01
66	Isoxaflutole	0.02	0.1	0.02	0.01
67	Isoxathion		0.1	>0.1	0.01
68	Metazachlor	0.02	0.1	0.02	0.01
69	Metobromuron		0.1	0.1	0.01
70	Metolachlor	0.05	0.02	0.1	0.01
71	Metrafenone		>0.1	0.1	0.01
72	Molinate	0.01	0.1	0.1	0.01
73	Napropamide	0.05	>0.1	0.1	0.02
74	Novaluron	0.01	0.1	0.1	0.01
75	Oxadiargyl	0.01	0.1	0.1	0.02
76	Oxasulfuron	0.01	0.1	0.02	0.01
77	Oxyfluorfen	0.05	0.1	0.02	0.01
78	Penflufen		0.02	0.01	0.01
79	Pentachloroaniline	0.02	0.01	0.01	0.01
80	Penthiopyrad		0.02	0.01	0.01

Table I. The SDLs achieved with 0.25 g sample/mL of extract and with 0.5 sample/mL of extract considering the identification requirements of the SANTE guidelines (Mass accuracy (MA), S/N, peaks overlapping), and the SDLs obtained with 0.25 g of sample/mL by considering the ion ratio (IR) criteria, and the EU MRLs established for cereals. (Cont.)

			SDL (mg kg^{-1})		
	Compounds	**MRLs (mg kg^{-1})**	**SDL (0.25 g sample/mL)+MA**	**SDL (0.5 g sample/mL)+MA**	**SDL (0.25 g sample/mL)+MA+IR**
81	Picolinafen		0.02	0.01	0.01
82	Picoxystrobin		0.02	0.01	0.01
83	Piperonylbutoxide		0.1	0.01	0.01
84	Pirimiphos-ethyl		0.02	0.02	0.01
85	Propachlor	0.02	0.1	0.02	0.01
86	Propanil	0.01	0.1	0.02	0.01
87	Proquinazid	0.02	0.02	0.02	0.01
88	Pyraclofos		0.1	0.1	0.01
89	Pyridalyl	0.01	0.1	0.02	0.01
90	Quinalphos	0.01	0.1	0.1	0.01
91	Quintozene	0.02	0.1	0.1	0.01
92	Siafluofen		0.02	0.01	0.01
93	Spiromesifen	0.02	0.02	0.01	0.01
94	Sulfotep		0.1	0.1	0.01
95	Terbuthylazine		>0.1	0.1	0.01
96	Tetrachlorvinphos		0.1	0.02	0.01
97	Tetrasul		0.1	0.02	0.01
98	Thiobencarb	0.01	0.01	0.01	0.01
99	Tralkoxydim	0.01	>0.1	>0.1	0.1
100	Tralomethrin		>0.1	>0.1	0.01
101	Trichloronate		0.02	0.01	>0.1
102	Triflumizole	0.1	0.1	0.02	0.01

Method validation for quantification

Recoveries and repeatability

At 0.01 mg kg^{-1}, the percentages of compounds exhibiting good recovery values between 70 and 120% were 82% in rye, 76% in wheat, 75% in barley, and 33% in rice. Among the cereals injected, and as mentioned before, rice is the most difficult matrix because of the relatively high amount of fat. At low concentrations, the pesticides may be discriminated by the high signal to noise of the co-extractive components of rice. Some non-polar compounds may also remain in the fat precipitate, such as tralomethrin (log P: 5). At 0.02 mg kg^{-1}, good recoveries were obtained with rye, wheat, barley, and rice for 90%, 81%, 92%, and 90% of the compounds, respectively. At 0.1 mg kg^{-1}, 94% of the compounds showed good recoveries in rye, 89% showed good recoveries in wheat, 94% showed good recoveries in barley, and 95% showed good recoveries in rice. Some compounds exhibited poor recoveries for all four matrices at all three concentrations studied, *e.g.,* carvone, cycloxidim, chloridazon, cyromazine, and 8-hydroxyquinoline.

RSDs obtained for almost all the compounds were below 20% except for some compounds analysed in barley and rice at the lowest concentration of 0.01 mg kg^{-1}.

Intra-day precision and inter-day precision

The intra-day precision is influenced by many factors, such as the efficiency of the extraction procedure and potential errors during the extraction. It is also influenced by the instrument calibration and stability during the sequence; an internal mass calibration with perfluorotributylamine was performed before each injection on the TOFMS, to improve the resolution and accuracy of mass operation. Another parameter that may influence the results is the different types of cereals used for this study, which exhibited different responses depending on the matrix effect. The poor recoveries obtained for rice and barley at 0.01 mg kg^{-1} were the main reason why only 40% of the compounds showed an RSD for intra-day precision below 20%. The results were quite different at 0.02 and 0.1 mg kg^{-1}, at which almost all compounds showed an intra-day precision below 20%

The inter-day precision indicates the reproducibility of the method applied on different days. In our study, the different matrices used were also considered. Only 33% of the compounds showed good inter-day precision at 0.01 mg kg^{-1}, whereas 84% of the compounds showed good reproducibility below 20% at 0.02 mg kg^{-1}, and 98% of the compounds showed good inter-day precision standard deviation at 0.1 mg kg^{-1}.

Uncertainty

The results of uncertainty were consistent with the intra-day and inter-day precision results. Figure 4 shows the estimated uncertainty obtained at the three concentrations. Only 58% of the compounds showed low uncertainty below 50% at 0.01 mg kg^{-1}. At 0.02 mg kg^{-1} and 0.1 mg kg^{-1}, 90% and 98% of the compounds showed low uncertainty, respectively. On the basis of the recovery and uncertainty results, the LOQs of the method ranged between 0.01 and 0.02 mg kg^{-1}.

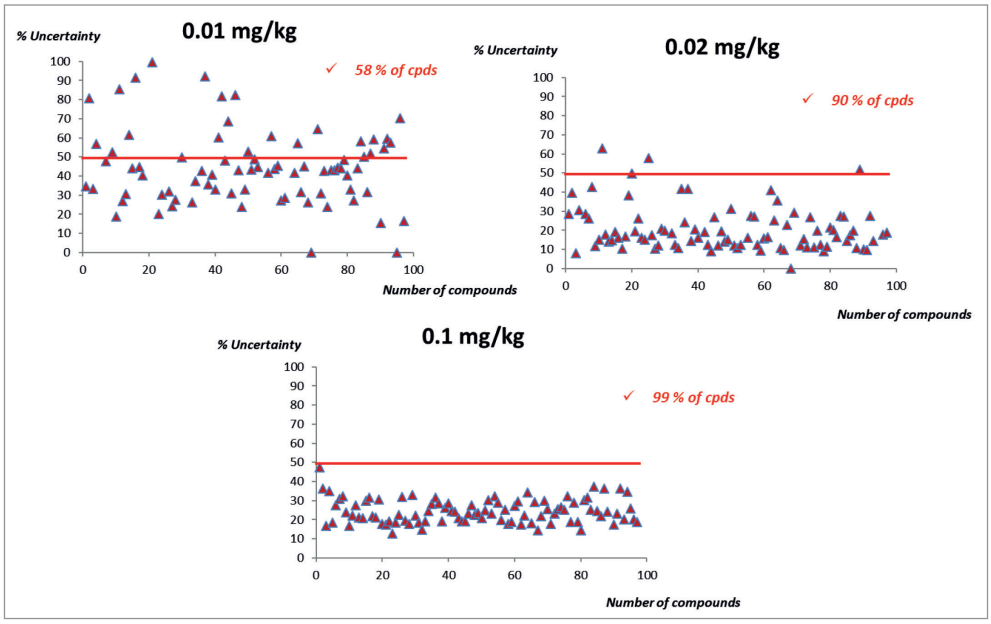

Figure 4. Uncertainty estimation in percentage obtained for the 102 compounds at 0.01, 0.02 and 0.1 mg kg^{-1}.

Matrix effect

The matrix effect is a well-known problem in GC-EI [22] that can influence the accuracy of the results, producing signal suppression or enhancement depending on the compound. Matrix effects can be decreased through efficient sample preparation methods resulting in very clean extracts and therefore less interference. However, this approach may also lead to loss of analytes. Matrix effects are also decreased with dilution of the sample extract. Another way to diminish the matrix effect is to use HRMS; the accurate

mass measurements yielded by HRMS decrease the chance of isobar detection, thus decreasing the matrix effect. According to our laboratory experience, rye was chosen as a representative matrix among the cereals included in the present study. Rye matrix provides good protection of the analytes and has a moderate matrix effect compared with those in other types of cereal matrices. Thus, matrix-matched calibration standards are commonly prepared with rye. The matrix effects presented in this study for barley, wheat, and rice were therefore calculated as percentage increases or decreases in response, normalized to that for rye. Figure 5 shows the matrix effects obtained with the comparison of slopes of the calibration curves prepared with each matrix. The results indicated that 94% of the compounds in wheat showed a weak matrix effect compared with rye (≤ ±20% signal suppression or enhancement). Only 6% of the compounds showed a moderate matrix effect (|20–50|% signal enhancement or suppression). In barley, 83% of the compounds showed a weak or non-significant matrix effect, 14% showed a moderate matrix effect, and 3% showed a strong matrix effect (≥ ±50% signal suppression or enhancement). In rice, 77% of the compounds showed a weak matrix effect, 21% of the compounds showed a moderate matrix effect, and only 2% of the compounds showed a strong matrix effect. Most of the compounds that showed any matrix effect at all exhibited a signal enhancement (approximately 60% of the compounds in wheat and rice, and 88% of the compounds in barley). The combination of an effective extraction method and a relatively high accurate mass measurement decreases the matrix effect. Therefore, the quantification of any cereal sample could be performed with a matrix-matched calibration prepared in rye.

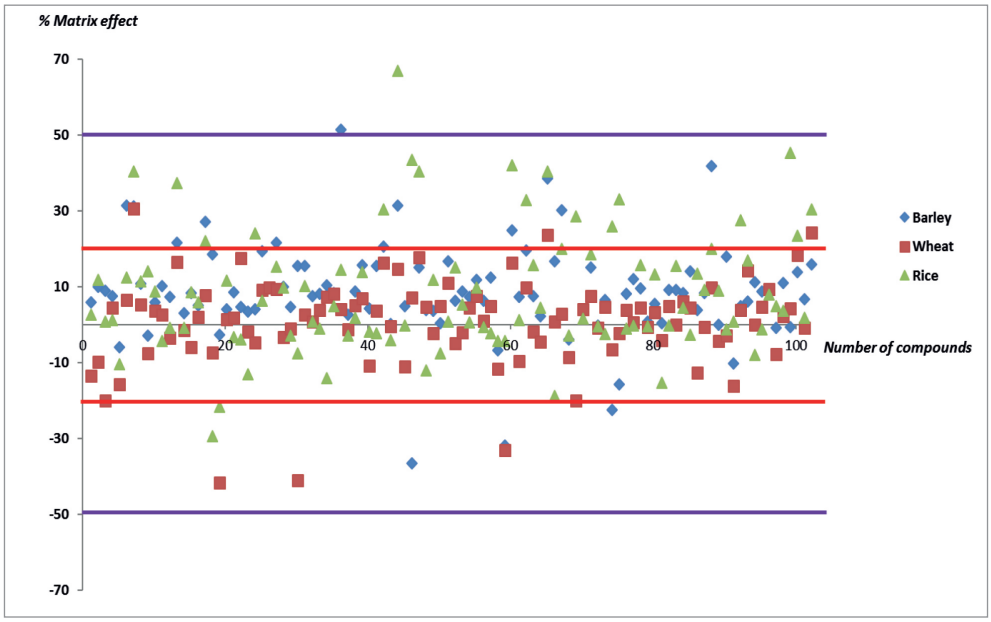

Figure 5. Percentage of matrix effect obtained from the comparison of slopes of matrix-matched calibrations of wheat, barley and rice to the slope of matrix-matched calibration prepared with rye.

Real samples

Only one pesticide was detected in real cereal samples, piperonyl butoxide, which is not included in the EU monitoring program for cereals [23]. Piperonyl butoxide is used as an insecticide synergist. It was detected in two parboiled rice samples, one jasmine rice sample, and one basmati rice sample at concentrations of 0.020, 0.021, 0.055 and 0.113 mg kg^{-1}, respectively. Piperonyl butoxide was detected at a high concentration exceeding the MRL of 0.060 mg kg^{-1}, only in jasmine rice. The presented analytical method and evaluation approach was also applied to the green beans sample of the European Union Proficiency Test for screening methods (EUPT-SM10). All the spiked compounds were identified, among them, etoxazole, isopyrazam, metrafenone, penflufen, pentachloroaniline, penthiopyrad, and proquinazid,

which demonstrate the applicability of the screening method described in this paper not only for cereal samples, but also for fruits and vegetables.

CONCLUSION

In recent years, the application of GC-TOFMS has been demonstrated to be a valuable and highly effective analytical tool in the analysis of pesticide residues in food. High mass accuracy TOF instruments can produce spectra with narrow mass peaks enabling high mass resolution. The benefit of high resolution is the elimination of background interference by using narrow mass window settings for extracting target ions, thus providing high selectivity. The resolution required for pesticide screening at lower concentrations depends on the complexity of the matrix analysed. High sensitivity is considered necessary for each application in food and pesticide food control. Cereals have relatively high lipid content, and the amount of interfering matrix retrieved in the extracts may affect the results. Accurate results are obtained through a combination of highly effective extraction procedures and instrumentation with adequate resolving power. The SDLs for cereals assessed without the use of any standards, through the citrate buffered QuEChERS method followed by GC-TOFMS analysis at 12000 FWHM, were in the range of 0.01 to 0.1 mg kg^{-1} or higher. Considering the injection of standards for identification, 85% of the compounds would show an SDL of 0.01 mg kg^{-1}. Therefore, the recommendation for third countries laboratories disposing of mass spectrometry of insufficient resolution to use standards for the identification of compounds if they are economically unable to switch to instruments offering higher mass resolving power and higher mass accuracy. Simultaneous screening and quantification are possible with HRMS. On the basis of recovery studies and uncertainty estimation, almost all the compounds achieved an LOQ between 0.01 and 0.02 mg kg^{-1}.

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Supplementary Material

The database of 102 compounds including: CAS number, retention times, molecular formula and exact mass of the compounds, and the formula, neutral mass and exact ion mass of fragment ions.

Compounds		CAS number	Rt (min)	Exact molecular mass	Molecular formula	Ion formula	M^+ , M+2	Neutral mass
1	1-Naphthylacetic acid	86-87-3	13.3	186.068080	C12H10O2	C11H9	141.069876	141.070425
						C9H7	115.054226	115.054775
						C11H7	139.054226	139.054775
2	1-Naphtylacetamide	86-86-2	13.2	185.084064	C12H11NO	C12H11NO	185.083515	185.084064
						C9H7	115.054226	115.054775
						C11H10	142.077701	142.078250
3	8-Hydroxyquinoline	148-24-3	7.7	145.052764	C9H7NO	C9H7NO	145.052215	145.052764
						C8H7N	117.057300	117.057849
						C7H6	90.046401	90.046950
4	Acetochlor	34256-81-1	12.4	269.118256	C14H20ClNO2	C10H12N	146.096425	146.096974
						C9H10N	132.080775	132.081324
						C11H12NO	174.091340	174.091889
5	Aclonifen	74070-46-5	18.2	264.030171	C12H9ClN2O3	C12H9ClN2O3	264.029622	264.030171
						C12H8N2O2	212.058029	212.058578
						C12H9NO	183.067865	183.068414
6	Ametoctradin	865318-97-4	23.7	275.210995	C15H25N5	C8H10N5	176.093071	176.093620
						C9H12N5	190.108721	190.109270
						C8H11N5	177.100896	177.101445
7	Amidosulfuron	120923-37-7	27.7	369.041294	C9H15N5O7S2	C4H5N4O4S2	236.974676	236.975225
						C5H11N4S	159.069894	159.070443
						C4H5N4O4(34S)S	238.971086	238.971635
8	Amisulbrom	348635-87-0	23.8	464.957638	C13H13BrFN5O4S2	C5H2N5O2S2	227.963656	227.964205
						C8H4FNO2S2	228.966202	228.966751
						C9H6BrFN	225.966214	225.966763
9	Anthraquinone	84-65-1	13.9	208.052430	C14H8O2	C14H8O2	208.051880	208.052429
						C13H8O	180.056966	180.057515
						C12H7	151.054226	151.054775

The database of 102 compounds including: CAS number, retention times, molecular formula and exact mass of the compounds, and the formula, neutral mass and exact ion mass of fragment ions. (Cont.)

	Compounds	CAS number	Rt (min)	Exact molecular mass	Molecular formula	Ion formula	M^+ , M+2	Neutral mass
10	Benalaxyl	71626-11-4	19.1	325.167794	C20H23NO3	C11H14NO	176.106990	176.107539
						C12H16NO2	206.117555	206.118104
						C13H16NO3	234.112470	234.113019
11	Benfluralin	1861-40-1	10.1	335.109291	C13H16F3N3O4	C10H9F3N3O4	292.053967	292.054516
						C8H5F3N3O4	264.022667	264.023216
						C7H5F3N2O2	206.029763	206.030312
12	Biphenyl	92-52-4	7.8	154.078250	C12H10	C12H10	154.077701	154.078250
						C12H9	153.069876	153.070425
						C12H8	152.062051	152.062600
13	Bixafen	581809-46-3	27.0	413.030951	C18H12Cl2F3N3O	C18H12Cl(37Cl)F3N3O	415.027453	415.028002
						C18H12Cl2F3N3O	413.030403	413.030952
						C6H5F2N2O	159.036445	159.036994
14	Butralin	33629-47-9	14.5	295.153207	C14H21N3O4	C12H16N3O4	266.113533	266.114082
						C11H14N3O2	220.108053	220.108602
						C9H10N3O4	224.066583	224.067132
15	Carbophenothion	786-19-6	19.0	341.973862	C11H16ClO2PS3	C7(35Cl)H6S	156.987326	156.987875
						C7(37Cl)H6S	143.979501	158.984925
						C3H6ClOS	124.982241	124.982790
16	Carfentrazone-ethyl	128639-02-1	19.2	411.036432	C15H14Cl2F3N3O3	C13H9F3N3O3	312.059052	312.059601
						C15H13F3N3O3	340.090352	340.090901
						C11H8ClF3N3O	290.030250	290.030799
17	Carvone	99-49--0	8.9	150.104465	C10H14O	C7H7	91.054226	91.054775
						C7H9	93.069876	93.070425
						C6H7	79.054226	79.054775
18	Chlorantraniliprole	500008-45-7	21.5	480.970792	C18H14BrCl2N5O2	C13H8Cl2N2O	278.000820	278.001369
						C9H14BrClN2O	279.997253	279.997802
						C13H8ClN2O	243.031967	243.032516
19	Chloridazon	2698-60-8	19.4	221.035590	C10H8ClN3O	C10H8ClN3O	221.035041	221.035590
						C10H8(37Cl)N3O	223.032091	223.032640
						C6H5	77.038576	77.039125

The database of 102 compounds including: CAS number, retention times, molecular formula and exact mass of the compounds, and the formula, neutral mass and exact ion mass of fragment ions. (Cont.)

	Compounds	CAS number	Rt (min)	Exact molecular mass	Molecular formula	Ion formula	M^+ , M+2	Neutral mass
20	Chloropropylate	5836-10-2	17.9	338.047651	C17H16Cl2O3	C7H4ClO	138.994519	138.995068
						C13H9Cl2O	251.002500	251.003046
						C6H4Cl	110.999604	111.000153
21	Chlorthal-dimethyl	1861-32-1	14.4	329.902022	C10H6Cl4O4	C9H3Cl4O3	298.883083	298.883632
						C10H6Cl4O4	329.901473	329.902022
						C7Cl3O2	220.895840	220.896389
22	Cinidon-ethyl	142891-20-1	31.2	393.053465	C19H17Cl2NO4	C17H13ClNO4	330.052763	330.053312
						C19H17ClNO4	358.084063	358.084612
						C15H9ClNO4	302.021463	302.022012
23	Clodinafop-propargyl	105512-06-9	19.5	349.051715	C17H13ClFNO4	C11H6ClFNO2	238.006560	238.007110
						C13H10ClFNO2	266.037860	266.038410
						C17H13ClFNO4	349.051170	349.051715
24	Cycloxydim	101205-02-1	24.0	325.171165	C17H27NO3S	C10H12NO2	178.086255	178.086804
						C6H6NO	108.044390	108.044939
						C5H9S	101.041948	101.042497
25	Cyflufenamid	180409-60-3	17.7	412.121018	C20H17F5N2O2	C7H6	90.046401	90.046950
						C8H2F4N	188.011787	188.012336
						C7H7	91.054226	91.054775
26	Cyromazine	66215-27-8	11.2	166.096694	C6H10N6	C5H7N6	151.072670	151.073219
						C6H9N6	165.088320	165.088869
						C4H5N4	109.050872	109.051421
27	Dialifos	10311-84-9	23.7	393.002510	C14H17ClNO4PS2	C10H7ClNO2	208.015983	208.016532
						C7H4O	104.025666	104.026215
						C10H7(37Cl)NO2	210.013033	210.013582
28	Dichlobenil	1194-65-6	7.6	170.964255	C7H3Cl2N	C7H3Cl2N	170.963706	170.964255
						C7H3(37Cl)ClN	172.960756	172.961305
						C7H2N	100.018174	100.018723
29	Dichlofenthion	97-17-6	12.4	313.970011	C10H13Cl2O3PS	C6H5ClO3PS	222.938009	222.938558
						C10H13ClO3PS	279.000609	279.001158
						C8H9ClO3PS	250.969309	250.969858

The database of 102 compounds including: CAS number, retention times, molecular formula and exact mass of the compounds, and the formula, neutral mass and exact ion mass of fragment ions. (Cont.)

	Compounds	CAS number	Rt (min)	Exact molecular mass	Molecular formula	Ion formula	M^+ , M+2	Neutral mass
30	Dicrotofos	141-66-2	10.0	237.076612	C8H16NO5P	C2H6O3P	109.004909	109.005458
						C3H6NO	72.044390	72.044939
						C6H10O5P	193.026039	193.026588
31	Diflufenican	83164-33-4	20.1	394.074068	C19H11F5N2O2	C13H7F3NO2	266.042339	266.042888
						C19H11F5N2O2	394.073519	394.074068
						C13H6F2N2O	246.036110	246.036659
32	Dimetachlor	50563-36-5	12.5	255.102607	C13H18ClNO2	C9H12N	134.096425	134.096974
						C10H12ClNO	197.060193	197.060742
						C9H10NO	148.075690	148.076239
33	Dimethenamid	87674-68-8	12.5	275.074679	C12H18ClNO2S	C8H12NS	154.068497	154.069046
						C10H13ClNOS	230.040090	230.040639
						C8H10ClNOS	203.016615	203.017164
34	Dimoxystrobin	149961-52-4	21.1	326.163043	C19H22N2O3	C8H6N	116.049475	116.050024
						C7H5	89.038576	89.039125
						C11H13N2O2	205.097154	205.097703
35	Diniconazole	83657-24-3	18.1	325.074868	C15H17Cl2N3O	C11H8Cl2N3O	268.003894	268.004443
						C11H7ClN3O	232.027216	232.027765
						C8H5Cl	136.007429	136.007978
36	Dioxathion	78-34-2	25.1	456.008753	C12H26O6P2S4	C8H16O4PS2	271.022218	271.022767
						C4H10O2PS2	184.985438	184.985987
						C4H10O2PS	153.013366	153.013915
37	Ethalfluralin	55283-68-6	9.9	333.093641	C13H14F3N3O4	C10H9F3N3O3	276.059052	276.059601
						C13H13F3N3O3	316.090352	316.090901
						C10H9F3N3O4	292.053967	292.054516
38	Ethofumesate	26225-79-6	13.5	286.087497	C13H18O5S	C10H9O2	161.059705	161.060254
						C12H15O3	207.101571	207.102120
						C8H9O2	137.059705	137.060254
39	Etoxazole	153233-91-1	21.5	359.169685	C21H23F2NO2	C7H3F2O	141.014647	141.015196
						C18H16F2NO	300.119446	300.119995
						C13H18NO	204.138290	204.138839

The database of 102 compounds including: CAS number, retention times, molecular formula and exact mass of the compounds, and the formula, neutral mass and exact ion mass of fragment ions. (Cont.)

	Compounds	CAS number	Rt (min)	Exact molecular mass	Molecular formula	Ion formula	M^+ , M+2	Neutral mass
40	Etridiazole	2593-15-9	8.3	245.918819	C5H5Cl3N2OS	C3HCl2N2OS	182.918117	182.918666
						C5H5Cl2N2OS	210.949417	210.949966
						C5H5(35Cl)ClN2OS	212.946467	212.947016
41	Etrimfos	38260-54-7	11.8	292.064668	C10H17N2O4PS	C9H13N2O2	181.097154	181.097703
						C10H17N2O4PS	292.064119	292.064668
						C7H9N2O2	153.065853	153.066402
42	Famoxadone	131807-57-3	29.8	374.126658	C22H18N2O4	C21H18N2O2	330.136279	330.136828
						C15H12O2	224.083181	224.083730
						C14H12O	196.088266	196.088815
43	Fenchlorphos	299-84-3	13.1	319.899739	C8H8Cl3O3PS	C8H8Cl2O3PS	284.930337	284.930886
						C8H8(37Cl)ClO3PS	286.927387	286.927936
						C2H6O2PS	124.982066	124.982615
44	Fenoxaprop-p-ethyl	71283-80-2	23.9	361.071702	C18H16ClNO5	C15H11ClNO3	288.042198	288.042747
						C18H16ClNO5	361.071152	361.071701
						C13H8ClNO3	261.018723	261.019272
45	Flonicamid	158062-67-0	9.4	229.046295	C9H6F3N3O	C7H3F3NO	174.016124	174.016673
						C6H3F3N	146.021209	146.021758
						C6H4F3N	147.029034	147.029583
46	Fluazinam	79622-59-6	15.1	463.951380	C13H4Cl2F6N4O4	C13H4Cl2F6N3O2	417.957927	417.958476
						C13H4Cl2F6N2	371.965023	371.965572
						C13H4(37Cl)ClF6N3O2	419.954977	419.955526
47	Flucythrinate I and II	70124-77-5	26.6	451.159515	C26H23F2NO4	C8H7F2O	157.045947	157.046496
			27.0			C11H13F2O	199.092897	199.093446
						C14H11NO2	225.078430	225.078979
48	Flufenacet	142459-58-3	14.1	363.066461	C14H13F4N3O2S	C9H10FN	151.079178	151.079727
						C5H2F3N2O2S	210.978360	210.978909
						C8H7FN	136.055703	136.056252
49	Flumetralin	62924-70-3	16.2	421.045247	C16H12ClF4N3O4	C7H5ClF	143.005832	143.006381
						C7H5(37Cl)F	145.002882	145.003431
						C16H11ClF4N3O3	404.041958	404.042507

The database of 102 compounds including: CAS number, retention times, molecular formula and exact mass of the compounds, and the formula, neutral mass and exact ion mass of fragment ions. (Cont.)

	Compounds	CAS number	Rt (min)	Exact molecular mass	Molecular formula	Ion formula	M^+ , M+2	Neutral mass
50	Flumioxazin	103361-09-7	27.9	354.101586	C19H15FN2O4	C19H15FN2O4	354.101037	354.101586
						C13H8FN2O3	259.051347	259.051896
						C18H15FN2O3	326.106122	326.106671
51	Fluopicolide	239110-15-7	19.7	381.965431	C14H8Cl3F3N2O	C7H3Cl2O	172.955547	172.956096
						C14H8Cl2F3N2O	346.996029	346.996578
						C6H3Cl2	144.960632	144.961181
52	Fluopyram	658066-35-4	15.3	396.046409	C16H11ClF6N2O	C8H4F3O	173.020875	173.021424
						C7H4F3	145.025960	145.026509
						C8H7ClF3N2	223.024436	223.024985
53	Flurochloridone	61213-25-0	14.3	311.009153	C12H10Cl2F3NO	C8H4F3NO	187.023949	187.024498
						C8H7F3N	174.052509	174.053058
						C12H10Cl2F3NO	311.008605	311.009154
54	Flurprimidol	56425-91-3	12.5	312.108562	C15H15F3N2O2	C12H8F3N2O2	269.053238	269.053787
						C5H3N2O	107.023989	107.024538
						C8H4F3O2	189.015790	189.016339
55	Flurtamone	96525-23-4	22.2	333.097663	C18H14F3NO2	C8H10N	120.080775	120.081324
						C18H14F3NO2	333.097114	333.097663
						C10H8F3N	199.060334	199.060883
56	Flutolanil	66332-96-5	16.6	323.113313	C17H16F3NO2	C8H4F3O	173.020875	173.021424
						C7H4F3	145.025960	145.026509
						C17H16F3NO2	323.112764	323.113313
57	Fluxapyroxad	907204-31-3	21.3	381.090052	C18H12F5N3O	C6H5F2N2O	159.036445	159.036994
						C18H12F5N3O	381.089503	381.090052
						C12H6F3N	221.044684	221.045233
58	Fonofos	944-22-9	11.3	246.030197	C10H15OPS2	C2H6OPS	108.987151	108.987700
						C4H10OPS	137.018451	137.019000
						C10H15OPS2	246.029648	246.030197
59	Fuberidazole	3878-19-1	12.7	184.063663	C11H8N2O	C11H8N2O	184.063114	184.063663
						C10H8N2	156.068199	156.068748
						C10H7N2	155.060374	155.060923

The database of 102 compounds including: CAS number, retention times, molecular formula and exact mass of the compounds, and the formula, neutral mass and exact ion mass of fragment ions. (Cont.)

	Compounds	CAS number	Rt (min)	Exact molecular mass	Molecular formula	Ion formula	M^+ , M+2	Neutral mass
60	Furathiocarb	65907-30-4	22.2	382.156245	C18H26N2O5S	C10H11O2	163.075356	163.075905
						C10H10O2S	194.039603	194.040152
						C7H7O	107.049140	107.049690
61	Heptachlor	76-44-8	12.9	369.821096	C10H5Cl7	C5H2Cl6	271.809619	271.810168
						C5H5Cl	100.007429	100.007978
						C5H2(37Cl)Cl5	273.806669	273.807218
62	Isocarbofos	24353-61-5	14.2	289.053769	C11H16NO4PS	C7H4OS	135.997738	135.998287
						C7H4O2	120.020581	120.021130
						C6H4O	92.025666	92.026215
63	Isofenfos	25311-71-1	15.2	345.116369	C15H24NO4PS	C7H5O2	121.028406	121.028955
						C9H10O4P	213.031124	213.031673
						C7H6O4P	184.999824	185.000373
64	Isoprocarb	2631-40-5	8.9	193.110279	C11H15NO2	C8H9O	121.064790	121.065339
						C9H12O	136.088266	136.088815
						C8H7	103.054226	103.054775
65	Isopyrazam	881685-58-1	23.9	359.180918	C20H23F2N3O	C6H5F2N2O	159.036445	159.036994
						C20H23F2N3O	359.180369	359.180918
						C12H10NO	184.075690	184.076239
66	Isoxaflutole	141112-90-0	14.4	359.043914	C15H12F3NO4S	C14H8F3NO2	279.050550	279.050713
						C7H3F3O	160.013500	160.013599
						C13H7F3O2	252.039990	252.039814
67	Isoxathion	18854-01-8	17.5	313.053769	C13H16NO4PS	CH5OS	105.033491	105.034040
						C9H7NOS	177.024287	177.024836
						C11H12NO4PS	285.021920	285.022469
68	Metazachlor	67129-08-2	14.9	277.098190	C14H16ClN3O	C9H10N	132.080775	132.081324
						C11H12ClNO	209.060193	209.060742
						C4H5N2	81.044724	81.045273
69	Metobromuron	3060-89-7	7.0	258.000389	C9H11BrN2O2	C7H4BrNO	196.947076	196.947625
						C6H4BrN	168.952161	168.952710
						C6H4N	90.033825	90.034374

The database of 102 compounds including: CAS number, retention times, molecular formula and exact mass of the compounds, and the formula, neutral mass and exact ion mass of fragment ions. (Cont.)

Compounds		CAS number	Rt (min)	Exact molecular mass	Molecular formula	Ion formula	M^+ , M+2	Neutral mass
70	Metolachlor	51218-42-2	13.9	283.133907	C15H22ClNO2	C11H16N	162.127725	162.128274
						C13H17ClNO	238.099318	238.099867
						C13H17(37Cl)NO	240.096368	240.096917
71	Metrafenone	220899-03-6	23.8	408.057236	C19H21BrO5	C18H18BrO5	393.033212	393.033761
						C18H18BrO4	377.038297	377.038846
						C11H13O4	209.080836	209.081385
72	Molinate	2212-67-1	8.9	187.103086	C9H17NOS	C7H12NO	126.091340	126.091889
						C6H12N	98.096425	98.096974
						C9H17NOS	187.102537	187.103086
73	Napropamide	15299-99-7	16.5	271.157229	C17H21NO2	C4H10N	72.080775	72.081324
						C7H14NO	128.106990	128.107539
						C9H7	115.054226	115.054775
74	Novaluron	116714-46-6	7.8	492.012310	C17H9ClF8N2O4	C6H5ClNO	142.005418	142.005967
						C6H5(37Cl)NO	144.002468	142.005967
						C9H6ClF6NO2	308.998576	308.999125
75	Oxadiargyl	39807-15-3	18.2	340.038149	C15H14Cl2N2O3	C8H5ClN	150.010503	150.011052
						C9H5Cl2NO	212.974271	212.974820
						C8H5Cl2N	184.979356	184.979905
76	Oxasulfuron	144651-06-9	12.5	406.094707	C17H18N4O6S	C8H12NS	154.068497	154.069046
						C5H5N3O6	203.017288	203.017837
						C6H7S	111.026298	111.026847
77	Oxyfluorfen	42874-03-3	17.2	361.032871	C15H11ClF3NO4	C13H7F3O2	252.039265	252.039814
						C13H6ClF3NO2	300.003367	300.003916
						C15H11ClF3NO4	361.032322	361.032871
78	Penflufen	494793-67-8	18.7	317.190340	C18H24FN3O	C6H6FN2O	141.045867	141.046416
						C15H17FN3O	274.135016	274.135565
						C18H24FN3O	317.189791	317.190340
79	Pentachloroaniline	527-20-8	12.3	262.862988	C6H2Cl5N	C6H2Cl5N	262.862440	262.862988
						C6H4Cl5N	264.878090	264.878639
						C6H6Cl5N	266.893740	266.894289

The database of 102 compounds including: CAS number, retention times, molecular formula and exact mass of the compounds, and the formula, neutral mass and exact ion mass of fragment ions. (Cont.)

	Compounds	CAS number	Rt (min)	Exact molecular mass	Molecular formula	Ion formula	M^+ , M+2	Neutral mass
80	Penthiopyrad	183675-82-3	18.4	359.127918	C16H20F3N3OS	C12H11F3N3OS	302.056944	302.057493
						C16H20F3N3OS	359.127369	359.127918
						C6H4F3N2O	177.027023	177.027572
81	Picolinafen	137641-05-5	21.2	376.083490	C19H12F4N2O2	C12H7F3NO	238.047424	238.047973
						C19H12F4N2O2	376.082941	376.083490
						C12H8F3NO	239.055249	239.055798
82	Picoxystrobin	117428-22-5	16.5	367.103143	C18H16F3NO4	C10H9O	145.064791	145.065340
						C17H12F3NO3	335.076379	335.076928
						C9H7	115.054226	115.054775
83	Piperonylbutoxide	51-03-6	20.2	338.209325	C19H30O5	C11H12O2	176.083181	176.083730
						C9H9O2	149.059705	149.060254
						C9H11	119.085526	119.086075
84	Pirimiphos-ethyl	23505-41-1	14.7	333.127602	C13H24N3O3PS	C7H10N3S	168.058995	168.059544
						C12H21N3O3PS	318.103578	318.104127
						C11H19N3O3PS	304.087928	304.088477
85	Propachlor	1918-16-7	9.5	211.076392	C11H14ClNO	C8H10N	120.080775	120.081324
						C11H14NO	176.106990	176.107539
						C8H8ClNO	169.028893	169.029442
86	Propanil	709-98-8	12.4	217.006120	C9H9Cl2NO	C6H5Cl2N	160.979356	160.979905
						C6H5(37Cl)ClN	162.976406	162.976955
						C9H9Cl2NO	217.005571	217.006120
87	Proquinazid	189278-12-4	20.0	381.965431	C14H8Cl3F3N2O	C8H5IN2O2	287.939031	287.939580
						C7H4INO	244.933217	244.933766
						C8H3INO2	271.920307	271.920856
88	Pyraclofos	89784-60-1	23.7	360.046431	C14H18ClN2O3PS	C9H7ClN2O	194.024142	194.024691
						H2O2PS	96.950766	96.951315
						C14H18ClN2O3PS	360.045882	360.046431
89	Pyridalyl	179101-81-6	26.8	488.967990	C18H14Cl4F3NO3	C9H9F3NO	204.063074	204.063623
						C3H3Cl2	108.960632	108.961181
						C6H5F3NO	164.031774	164.032323

The database of 102 compounds including: CAS number, retention times, molecular formula and exact mass of the compounds, and the formula, neutral mass and exact ion mass of fragment ions. (Cont.)

	Compounds	CAS number	Rt (min)	Exact molecular mass	Molecular formula	Ion formula	M^+ , M+2	Neutral mass
90	Quinalphos	13593-03-8	15.3	298.054103	C12H15N2O3PS	C8H6N2O	146.047464	146.048013
						C10H8N2	156.068199	156.068748
						C10H9N2	157.076024	157.076573
91	Quintozene	82-68-8	11.3	292.837169	C6Cl5NO2	C5Cl5	234.843716	234.844265
						C6Cl4	211.874851	211.875400
						C6Cl5	246.843716	246.844265
92	Siafluofen	105024-66-6	27.0	408.192086	C25H29FO2Si	C10H15OSi	179.088669	179.089218
						C15H15FOSi	258.087072	258.087621
						C17H19FOSi	286.118372	286.118921
93	Spiromesifen	283594-90-1	20.7	370.214410	C23H30O4	C17H20O3	272.140700	272.141245
						C17H18O2	254.130130	254.130679
						C17H21O3	273.148520	273.149070
94	Sulfotep	3689-24-5	10.2	322.022740	C8H20O5P2S2	C8H20O5P2S2	322.022196	322.022745
						C4H11O3PS2	201.988178	201.988727
						C2H8O5P2S2	237.928296	237.928845
95	Terbuthylazine	5915-41-3	11.2	229.109423	C9H16ClN5	C8H13ClN5	214.085399	214.085948
						C5H8ClN5	173.046274	173.046823
						C5H8N5	138.077421	138.077970
96	Tetrachlorvinphos	22248-79-9	16.1	363.899260	C10H9Cl4O4P	C10H9Cl3O4P	328.929858	328.930407
						C2H6O3P	109.004909	109.005458
						C10H9Cl4O4	332.924948	332.925497
97	Tetrasul	2227-13-6	18.5	321.894434	C12H6Cl4S	C12H6Cl2S	251.956179	251.956728
						C12H6(37Cl)ClS	253.953229	253.953778
						C12H6(37Cl)Cl3S	323.890935	323.891484
98	Thiobencarb	28249-77-6	13.7	257.064114	C12H16ClNOS	C5H10NO	100.075690	100.076239
						C3H6NO	72.044390	72.044939
						C7H6Cl	125.015254	125.015803
99	Tralkoxydim	87820-88-0	22.8	329.199094	C20H27NO3	C7H7NO2	137.047130	137.047679
						C6H7NO	109.052215	109.052764
						C18H21NO2	283.156680	283.157229

The database of 102 compounds including: CAS number, retention times, molecular formula and exact mass of the compounds, and the formula, neutral mass and exact ion mass of fragment ions. (Cont.)

Compounds		CAS number	Rt (min)	Exact molecular mass	Molecular formula	Ion formula	M^+ , M+2	Neutral mass
100	Tralomethrin	66841-25-6	29.0	660.809838	C22H19Br4NO3	C13H9O	181.064791	181.065340
						C7H9Br2	250.906548	250.907097
						C6H9Br2O	254.901462	254.902011
101	Trichloronate	327-98-0	14.4	331.936124	C10H12Cl3O2PS	C2H6OPS	108.987151	108.987700
						C8H8Cl2O2PS	268.935422	268.935971
						C10H12Cl2O2PS	296.966722	296.967271
102	Triflumizole	68694-11-1	15.6	345.085574	C15H15ClF3N3O	C8H4ClF3N	205.997887	205.998436
						C7H3ClF3	178.986988	178.987537
						C12H12ClF3NO	278.055402	278.055951

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FEATURE

PDF

Institute of Chemistry of the University of Campinas Renews International Quality Certificate

In recognition of the excellence of its chemistry courses, infrastructure, program, and academic staff, the Institute of Chemistry at the University of Campinas (IQ-Unicamp), Brazil, which has been accredited since 2014, obtained renewal of the Accreditation of the Royal Society of Chemistry (RSC). In 2019, a team from the RSC came to IQ-Unicamp to revalidate the accreditation, which lasts for five years.

Facade of the Institute of Chemistry building, Unicamp.

In South America, only Unicamp and the Federal University of Rio Grande do Sul, both in Brazil, have this certificate. The coordinator of the undergraduate course at IQ-Unicamp, Prof. Dr. Italo Odone Mazali, emphasized the importance of this certificate. “More than a seal, certification by RSC shows IQ’s willingness to establish partnerships with high-level academic partners. Accreditation facilitates students’ participation in RSC programs, such as exchanges at UK universities, through short term scholarships. We will implement a new model that may be unprecedented in the country; all of our undergraduate exams will be evaluated by our peer professors before being applied to students. The purpose of this innovation is to ensure uniform teaching. For the students, this measure is good because it means that the institution is concerned with the evaluation being applied to them. This aims to transform the evaluation into an institutional one and not an evaluation of the individual”.

Italo Odone, graduation coordinator of IQ-Unicamp, highlights that the institute easily surpassed all requirements for certification by RSC.

The director of IQ-Unicamp, Prof. Dr. Marco Aurélio Zezzi Arruda, explained that the accreditation offers a certificate with the diplomas issued by the Institute. “The importance is fundamental for both students and the academic community. Students have two very important things: the quality of education, as we have reached an international level, and the opening of job possibilities not only in Brazil but also abroad”, said Prof. Arruda. According to the director, for the academic community, this certification marks an important recognition of the professors for their work developed in teaching and, for Unicamp itself, the excellence of its courses is recognized.

For Marco Aurélio Z. Arruda, director of IQ-Unicamp, the certification expresses recognition of the courses’ excellence and opens up greater opportunities for students.

Master lecture celebrates the partnership

In order to celebrate the renewal of accreditation and the partnership between the Institute of Chemistry and the Royal Society of Chemistry, on March 10, 2020, a master class opened to the public was given by Dr. Roberto Rinaldi, a professor at Imperial College London, UK. The researcher, graduated and post-graduated by IQ-Unicamp, shared his academic trajectory.

The ceremony, held at the Unicamp Convention Center, was also attended by Elizabeth Magalhães, a representative of RSC in Brazil; Marco Antonio Zago, the president of the São Paulo Research Foundation (FAPESP); Eliane Amaral, Unicamp's pro-rector of graduation; and representatives of other academic units at Unicamp.

Prof. Arruda and Prof. Mazali emphasized that, more than celebrating the re-accreditation, the event aimed to give visibility to the partnership and the importance of internationalization.

About the Royal Society of Chemistry

The Royal Society of Chemistry is a non-profit international association of researchers in the field of chemistry. It promotes several scientific activities in the field, in addition to being a publisher that is currently responsible for 50 scientific journals. Born in the United Kingdom in 1841, the RSC has offices in the United States, China, Japan, Germany, India, and Brazil.

For the representative of RSC in Brazil, Dr. Elizabeth Magalhães, the accreditation process offers a differential recognition in the professional and academic trajectory of the students. "Students from universities accredited by RSC are considered to have better quality training than others. When it is known that a student has undergone such scrutiny, there is certainty that the student has a good base and is able to meet the needs of research. Elizabeth Magalhães points out that "it is a form of recognition".

"In addition to promoting RSC in Brazil, I am a former student at Unicamp, and it is with great pride that I see this university being raised to this position. It shows that the quality of research carried out in Brazil is as good as that done in any other part of the world", added Magalhães.

By Luciene Campos - With information from "Jornal da Unicamp"

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FEATURE

PDF

Institute of Chemistry of the University of São Paulo Celebrates 50 years

The Institute of Chemistry of the University of São Paulo (IQ-USP), Brazil, celebrated its 50th anniversary in a ceremony that brought together its directors, professors, employees, and students. The commemorative ceremony was held at USP's Camargo Guarnieri Amphitheater and was also attended by important members of the university, such as USP rector Vahan Agopyan, IQ-USP director Paolo Di Mascio and deputy director Pedro Vitoriano Oliveira, and Senior Professor Shirley Schreier, who was a student in the 1958 chemistry class.

IQ-USP director Paolo Di Mascio (left) and vice director Pedro Vitoriano Oliveira (right).

"Today we celebrate 50 years of the Institute of Chemistry and also 85 years of the chemistry program at USP. Reminders have a more individual nature; each has its own reminders. Memory, which is more material and corresponds to a set of information, is important to build and maintain reminders, avoiding situations that happened in the past", stated the director of IQ, Paolo Di Mascio, at the opening of the event.

Professor Shirley Schreier spoke about the history of IQ-USP since its origin as the Department of Chemistry of the former Faculty of Philosophy, Sciences and Letters located in downtown São Paulo city, its change of address to the campus of USP "Armando Salles de Oliveira" on the west side of São Paulo in 1966, and the creation of the IQ-USP to meet the norms of the Brazilian reform of higher education in 1969.

Rector Vahan Agopyan pointed out that this celebration consolidates an institute that is already a source of pride for USP and for the whole of São Paulo society. "Institutions don't come out of nowhere. There is always a story behind them, and there is the intense work of several people who build a successful institution. In 1934, the founders of USP had the view that quality education is essential for building a society. We have to maintain that vision. Even if the current Brazilian conditions are not the most favorable, they motivate us to work even harder, produce more, and make a difference in our São Paulo State" he said.

Vahan Agopyan, USP Rector, speaking at the ceremony commemorating the 50th anniversary of IQ-USP.

Golden Jubilee

In 1934, together with USP, the Faculty of Philosophy, Sciences and Letters (FFCL) was created with the vision of developing a culture of teaching in a research environment. Over the course of three decades, the FFCL developed, and the need arose for its dismemberment into various institutes. Thus, the IQ-USP was born. It was created on December 16, 1969, by Decree No. 52,326 of the Brazilian reform of higher education.

IQ-USP currently has 126 professors, 190 employees, 672 undergraduate students, about 400 postgraduate students, 96 post-doctors, and five collaborating researchers.

“IQ-USP follows its path with the mission of promoting the training of highly qualified human resources to be citizens and leaders in the areas of chemistry and biochemistry, meeting the needs of Brazilian society, and contributing to the development of our State and our Country”, said the vice director Pedro Vitoriano Oliveira.

Homages

During the ceremony, the pioneers of the chemistry course at the former Faculty of Philosophy, Sciences and Letters and from the IQ-USP were honored. A souvenir of the celebration was given personally to those who are alive and to the relatives of those who have passed away.

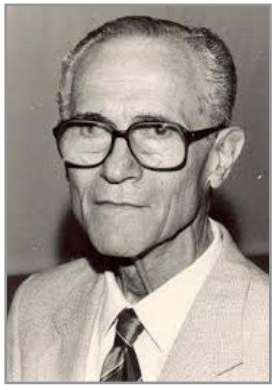
More about the honorees

Heinrich Rheinboldt (Karlsruhe, Germany, Aug. 11, 1891 – São Paulo, Brazil, May 12, 1955) was a professor at the University of Bonn, Germany. In 1934, he was hired to be responsible for the Chemical Sciences sector of the newly founded FFCL, which he was the director of until his death. He organized the teaching of chemistry according to the German tradition, which is based on lectures accompanied by demonstrative experiments and on the individual work of students in the laboratory. He was a scientist with an eclectic background, an educator, and a profound cultivator of the history of Chemistry. He was President of the Brazilian Society for the Advancement of Science (Sociedade Brasileira para o Progresso da Ciência − SBPC) in the 1953-1955 biennium.

Heinrich Hauptmann (Breslau, Germany, April 10, 1905 – São Paulo, July 21, 1960) was German by birth and a naturalized Brazilian. He graduated and obtained a PhD degree in Chemistry in his hometown, and worked with great researchers in the European centers at a high academic level. He arrived in São Paulo in February 1935 and was hired as an assistant to Heinrich Rheinboldt. He became responsible for the Organic and Biological Chemistry sector in 1939 and obtained the chair in 1946. He was the director of the Department of Chemistry at FFCL from December 1955 until his death. He was the mentor and coordinator of the construction project for the set of buildings called by the responsible architects the “Conjunto das Químicas”, which included the Institute of Chemistry, the Faculty of Pharmaceutical Sciences, and the Faculty of Chemical Engineering.

Isaias Raw (São Paulo, Brazil, March 26, 1927) was a physician, professor of Physiological Chemistry at the Faculty of Medicine, and a cultivator of advanced ideas about the university. He was the first to settle with his team in the still unfinished “Conjunto das Químicas” in late 1965. Isaias Raw was compulsorily retired in 1969 by Institutional Act number 5 (AI-5) of the Brazilian military government. However, from 1970 onwards, the select team of researchers created by him became part of IQ-USP as the most numerous group in the Department of Biochemistry. Its members have achieved notoriety in the international scientific community.

Blanka Wladislaw (Warsaw, Poland, June 3, 1917 – São Paulo, Brazil, Jan. 26, 2012) was a Polish naturalized Brazilian. She graduated from FFCL-USP in 1941. As a doctoral student and later collaborator of Heinrich Hauptmann, she became responsible for the Organic Chemistry sector after the death of Dr. Hauptmann in 1960. She defended his associate professorship thesis in 1958 and became an associate professor in 1965, rising to the position of full professor in 1971. She developed research in the fields of organic sulfur compounds, organic electrosynthesis, and organic physical-chemistry. She was head of the Department of Fundamental Chemistry at IQ-USP from 1976 to 1980.



Otto Richard Gottlieb (Brno, Czechoslovakia, current Czech Republic, Aug. 31, 1920 – Rio de Janeiro, Brazil, June 19, 2011), whose mother was Brazilian, emigrated in 1939 to Brazil, where he became a Brazilian citizen. With a degree in industrial chemistry from the University of Brazil, Rio de Janeiro in 1945, he was a pioneer in the study of plant composition and became a world reference in the area of Phytochemistry. He taught courses and created research groups at universities throughout Brazil. He was the founder of the Natural Products Chemistry Laboratory created by FAPESP in 1967 and incorporated into IQ-USP in 1970. In 1999, his name was proposed for the Nobel Prize in Chemistry in recognition of his vision of science and for his proposal for classification of plants based on their chemical characteristics.

Simão Mathias (São Paulo, Brazil, Aug. 26, 1908 – São Paulo, Brazil, Aug. 25, 1991), graduated in the first class of Chemistry at USP. He remained at the institution as an assistant and was the first student to obtain a PhD degree in Chemistry from USP in 1942. In 1946, he began to develop research in Physics-Chemistry at the Department of Chemistry at FFCL, of which he was the director from 1960 to 1969. In this period, the Department of Chemistry was transferred to USP's campus in the São Paulo University City. He was the pro tempore director of IQ-USP from January to March 1970 and headed the Department of Fundamental Chemistry from 1970 to 1972. He strove to attract researchers to IQ-USP and to open new fields of research. He retired in 1972 and started to dedicate himself to the History and Philosophy of Science, in particular to the History of Chemistry.

Paschoal Ernesto Américo Senise (São Paulo, Brazil, Aug. 19, 1917 – São Paulo, Brazil, July 21, 2011), graduated in the first class of the Chemistry course at USP in 1937. He remained at the institution as an assistant to Heinrich Rheinboldt and obtained a PhD degree in Chemistry in 1942. In the 1950s, he introduced instrumental methods of chemical analysis in the Department of Chemistry at FFCL and became a full professor in 1965. He was a member of the USP University Council (1968-1987), coordinated the Post-Graduate Chamber for 17 years, and implemented the current USP post-graduate system. He was director of IQ-USP from 1970 to 1974 and from 1978 to 1982. He received the titles of Professor Emeritus at USP (1987) and Emeritus Researcher at the National Council for Scientific and Technological Development (CNPq) (2006).

By Lilian Freitas – With information from "Jornal da USP"

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SPONSOR REPORT

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Determination of Chromium Species using Ion Chromatography coupled to Inductively Coupled Plasma Mass Spectrometry

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The goal of this report is to highlight a simple method to separate and quantify both major Cr species, Cr (III) and Cr (VI) in drinking water.

Keywords: Chromium speciation, Drinking water, Hexavalent Cr, IC-ICP-MS, IonPac AG7 column.

INTRODUCTION

Chromium is found naturally in rocks, soil, plants and animals, but can also be introduced into the environment as a result of human activity. Like many elements, chromium is found in multiple oxidation states, which can vary significantly in their toxicity, nutritional value, bioactivity, and environmental mobility. In trace amounts, trivalent chromium (Cr (III)) is considered an essential nutrient that promotes insulin, sugar, and lipid metabolism. In contrast, hexavalent chromium (Cr (VI)) is toxic and can lead to respiratory tract, stomach, and intestinal irritation, anemia, and is known to be a human carcinogen [1]. Cr (VI) can leach into drinking water sources naturally, but drinking water can also be contaminated by industrial processes such as wood treatment with copper dichromate, leather tanning with chromic sulfate, and stainless steel cookware. Because of the varying toxicity attributable to the different oxidation states of chromium, simply knowing the total chromium concentration in a solution is not sufficient to determine its true toxicity following exposure, and therefore speciation analysis is required. While inductively coupled plasma mass spectrometry (ICP-MS) can readily determine the total amount of an element present, chromatographic separation prior to the ICP-MS system is required to separate the different elemental species. Because Cr (III) and Cr (VI) have different charges, ion chromatography (IC) using anion exchange is the ideal separation method for analysis of these species.

One of the challenges with chromium speciation is that Cr (VI) can be degraded to Cr (III) and Cr (III) can be converted to a precipitate ($Cr(OH)_3$), depending on the solution pH [2]. An additional difficulty in the accurate speciation analysis of Cr by ICP-MS are the numerous spectral interferences (e.g. $^{35}Cl^{16}O^{1}H^{+}$ or $^{40}Ar^{12}C^{+}$) on the most abundant chromium isotope, ^{52}Cr [3].

In this application note, the Thermo Scientific™ Dionex™ Aquion™ Ion Chromatography system was coupled with the Thermo Scientific™ iCAP™ RQ ICP-MS to determine the concentration of Cr (III) and Cr (VI) in drinking water.

MATERIALS AND METHODS

Sample preparation

The tap water was acidified with 10 µL of concentrated nitric acid per 10 mL aliquot to yield a pH of around 4. A fortified sample was spiked with 0.1 mL of a standard solution containing 10 µg L^{-1} of both Cr species to 10 mL of the sample to give a final concentration of 0.1 µg L^{-1}.

Instrument configuration

The ion chromatography system used for this work consisted of a Dionex Aquion IC system and a Thermo Scientific™ Dionex™ AS-AP autosampler. All components of the IC system were controlled using the ChromControl plug-in for Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ Software. The system was purged and equilibrated prior to the start of sample analysis on each day. Data evaluation was accomplished using the tQuant virtual evaluation module of Qtegra Software. The chromatographic method was developed and published elsewhere [4], in brief, an isocratic separation using 0.3 mol L^{-1} nitric acid was used to separate both Cr species using a Thermo Scientific™ Dionex™ IonPac™ AG7 anion exchange guard column. Using a guard column alone (length of only 5 cm) effectively reduces the analysis time per sample and therefore increases sample throughput. At the same time, the chromatographic resolution and sample capacity are sufficient for the analysis.

The iCAP RQ ICP-MS was operated using the conditions summarized in Table 1. After optimization of the instrument using the autotune routines delivered with the Qtegra ISDS Software, the outlet of the column was directly connected to the PFA-LC nebulizer using a zero dead volume connector. The instrument was operated using kinetic energy discrimination (KED) with He as a collision gas to effectively eliminate all potential polyatomic interferences on Cr.

Table 1. Instrument configuration

Ion Chromatography	
Column	Dionex IonPac AG7, 2 x 50 mm
Flow rate	0.4 mL min^{-1}
Eluent	0.3 mol L^{-1} Nitric Acid
Injection volume	25 µL
ICP-MS	
Spray chamber	Quartz cyclonic, chilled at 2.7 ºC
Nebulizer	PFA-LC
Injector	2.5 mm I.D., quartz
Interface	Nickel sampler and skimmer cone High matrix skimmer cone insert
Forward power	1550 w
Nebulizer gas	1.12 L min^{-1}
Collision cell gas	He at 4.5 mL min^{-1}
KED voltage	3 V
Dwell times	0.1 s
Total acquisition time	3 min 20 sec

RESULTS AND DISCUSSION

The second chromatogram (from top to bottom) in Figure 1 shows the separation of a solution containing Cr (III) and (VI) at a concentration of 0.1 µg L^{-1}. Both species are completely separated and complete elution is achieved within 120 s. To assure complete elution if a much higher concentration of Cr (III) is present in a sample, the total runtime of the method was extended to 200 s. For calibration of the system,

a three-point calibration curve was generated using standard solutions containing both Cr species at concentrations between 0.1 and 10 µg L^{-1}. The analytical figures of merit obtained are shown in Table 2. The stability of retention times was verified using 10 injections of tap water spiked with 0.1 µg L^{-1} of both species. The attainable detection limits were calculated based on the standard deviation of the peak area observed in the peak area of repeated injections (N = 15) of unspiked tap water. This allows for a rather conservative and realistic assessment of this parameter.

Table 2. Analytical figures of merit

	Cr (VI)	Cr (III)
Retention time (s)	36 ± 0.2	101 ± 1.2
Sensitivity (kcps/µg L^{-1})$^{-1}$	114	123
Detection Limit (ng L^{-1})	4.0	9.0

Next, the performance of the guard column was evaluated with a locally sourced drinking water. Drinking water typically contains a high amount of both different cationic (for example, alkaline and alkaline earth elements) as well as anionic species (for example carbonate, sulfate and chloride), leading to an increased column load and potentially compromising the separation efficiency for the species under investigation. As can be seen from the chromatograms in Figure 1, no difference in the elution profile is observed between the injection of a standard solution (in ultrapure water) or a spiked drinking water sample.

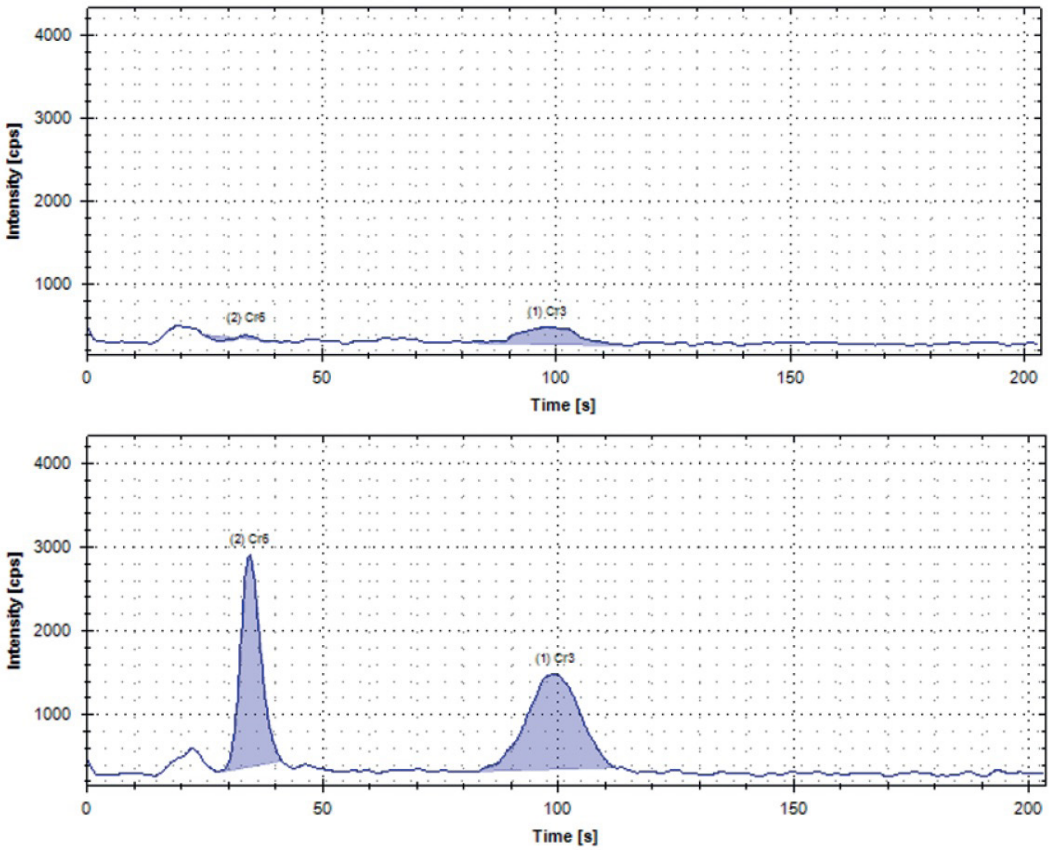

Figure 1. Chromatograms showing the injection of (from top to bottom) a blank, a standard solution containing 0.1 µg L^{-1} of both species (top), tap water, and spiked tap water (0.1 µg L^{-1}). For better comparability, all are scaled identically.

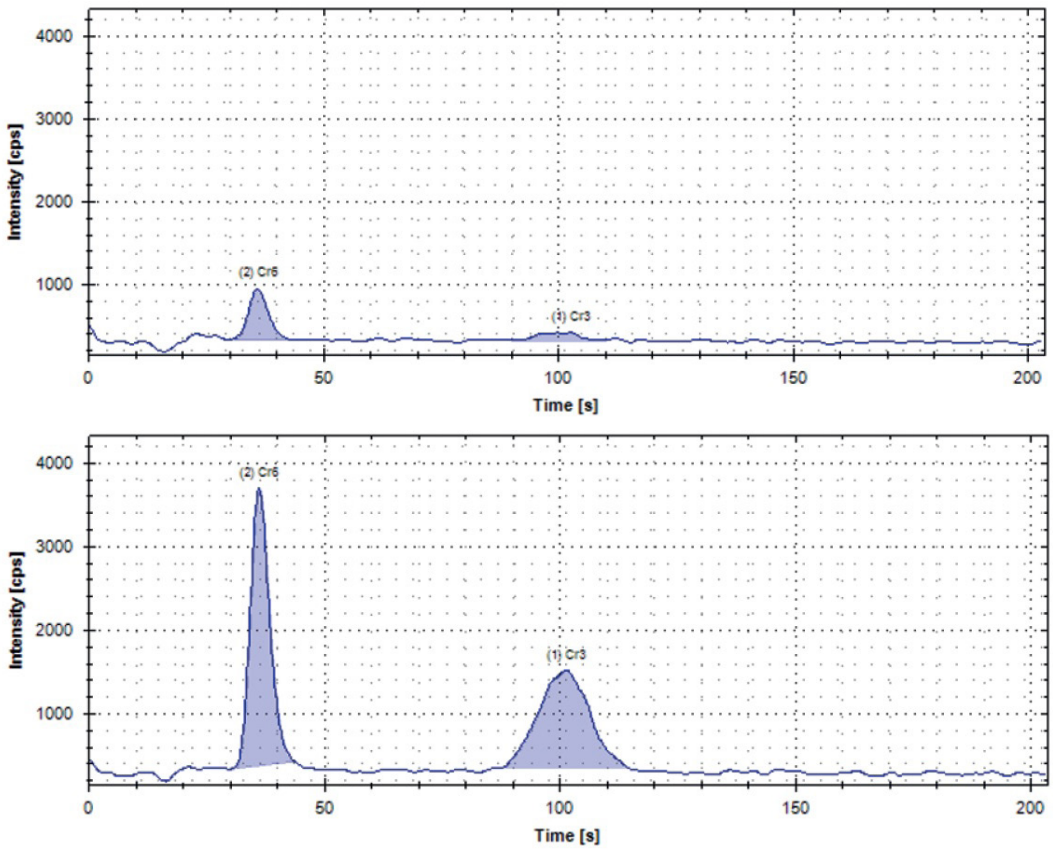

Figure 1. Chromatograms showing the injection of (from top to bottom) a blank, a standard solution containing 0.1 µg L^{-1} of both species (top), tap water, and spiked tap water (0.1 µg L^{-1}). For better comparability, all are scaled identically. (Continuation)

As can be seen, the blank did contain very low amounts of Cr (III) (approximately 20 ng L^{-1}) and the tap water sample contained a very low amount of Cr (VI), which was quantified to be approximately 25 ± 1 ng L^{-1}. To address the accuracy of the method, a sample was spiked with both Cr species at a concentration of 0.1 µg L^{-1} and the spike recovery was determined. In all cases, the spiked amount was recovered accurately with a recovery of 93 ± 1% for Cr (III) and 113 ± 5% for Cr (VI). The lower deviation for Cr (III) can be explained by the absence of naturally occurring Cr (III) in the samples, so that the recovery is based on the spiked amount only and excludes any variation from the actual content of water sourced from different taps.

CONCLUSION

In this report, a method was outlined that coupled the Dionex Aquion IC system with the iCAP RQ ICP-MS system, which demonstrated linear calibrations over three orders of magnitude, good stability (based on multiple injections), and suitable accuracy and Limits of Detection (LOD). The use of a guard column alone is sufficient for this application and allows reduction of runtimes to around 3 min and therefore improves sample throughput. With an eluent ideally suited to ICP-MS, superior column chemistry specifically designed to provide both anion and cation exchange sites, and dedicated hardware for ion chromatography, that completely eliminates trace metal contamination, IC-ICP-MS is the optimal combination for chromium speciation.

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U.S. EPA Method 557 Quantitation of Haloacetic Acids, Bromate and Dalapon in Drinking Water Using Ion Chromatography and Tandem Mass Spectrometry

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The purpose of this report is to demonstrate a simple and sensitive IC-MS/MS method for analyzing haloacetic acids, the pesticide dalapon, and bromate in water using EPA method 557, by direct injection of drinking water samples using IC-MS/MS. Sub-ppb levels of nine haloacetic acids, bromate, and dalapon are achieved in the quantitative analysis.

INTRODUCTION

Haloacetic acids (HAAs) are formed as disinfection byproducts when water is chlorinated to remove microbial content. Chlorine reacts with naturally occurring organic and inorganic matter in the water, such as decaying vegetation, to produce disinfection byproducts (DBPs) that include HAAs. Of the nine species of HAAs, five are currently regulated by the U.S. Environmental Protection Agency (EPA) (HAA5): monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), monobromoacetic acid (MBAA), and dibromoacetic acid (DBAA). The remaining four HAAs are currently unregulated: bromochloroacetic acid (BCAA), bromodichloroacetic acid (BDCAA), dibromochloroacetic acid (DBCAA), and tribromoacetic acid (TBAA). However, they are also of health concern, and are often analyzed along with the HAA5. This method allows for the analysis of all nine HAAs, plus bromate and the pesticide dalapon in the same IC-MS/MS run without sample preparation.

According to the U.S. EPA, there is an increased risk of cancer associated with long-term consumption of water containing levels of HAAs that exceed 0.06 mg/L [1]. U.S. EPA Methods 552.1, 552.2, and 552.3, are used to determine the level of all nine HAAs in drinking water [2-4]. These methods require derivatization and multiple extraction steps followed by gas chromatography (GC) with electron capture detection (ECD).

By comparison to the conventional U.S. EPA methods using GC with ECD, the combination of ion chromatography and mass spectrometry (IC-MS and IC-MS/MS) offers sensitive and rapid detection without the need for sample pre-treatment. In order to develop a simple, easy-to-use direct injection method, the U.S. EPA promulgated method 557 [5] for the analysis of haloacetic acids, bromate, and dalapon in drinking water by IC-MS/MS.

MATERIALS AND METHODS

Sample Preparation

Drinking water samples were collected from municipal tap water sources. NH_4Cl was added as a preservative at 100 mg/L to all water samples. No further sample preparation was performed prior to injection.

Ion Chromatography

IC analysis was performed on a Thermo Scientific™ Dionex™ ICS 5000 system. Samples were directly injected and no sample pre-treatment was required. The IC conditions used are shown in Table 1.

Table 1. Ion chromatography system conditions

Column	Dionex IonPac AG24 (2x50 mm), IonPac AS24 (2x250 mm)
Suppressor	ASRS 300 2 mm
Column Temperature	15 °C
Injection Volume	100 µL
Flow Rate	0.3 mL/min KOH gradient, electrolytically generated

The sample is injected without cleanup or concentration onto a Thermo Scientific™ Dionex™ IonPac™ AS24 column specifically designed to separate method analytes from the following common anions (matrix components) in drinking water: chloride, carbonate, sulfate, and nitrate.

Hydroxide eluent is generated using an electrolytic eluent generation which provides smoother gradients then conventional pump proportioning valves, and a continuously regenerated trap column continuously removes contaminants to provide pure eluent throughout the run. A Thermo Scientific™ Dionex™ ASRS 300 suppressor is placed in line after the column and electrolytically converts hydroxide eluent into water and simultaneously removes cations present in the drinking water and eluent. The gradient profile used is shown in Table 2. An overall schematic diagram of the system is shown in Figure 1.

Table 2. Electrolytically formed hydroxide gradient

Retention Time (min)	[KOH] mM
0.0	7.0
15.1	7.0
30.8	18.0
31	60
46	60
47	7
58	7

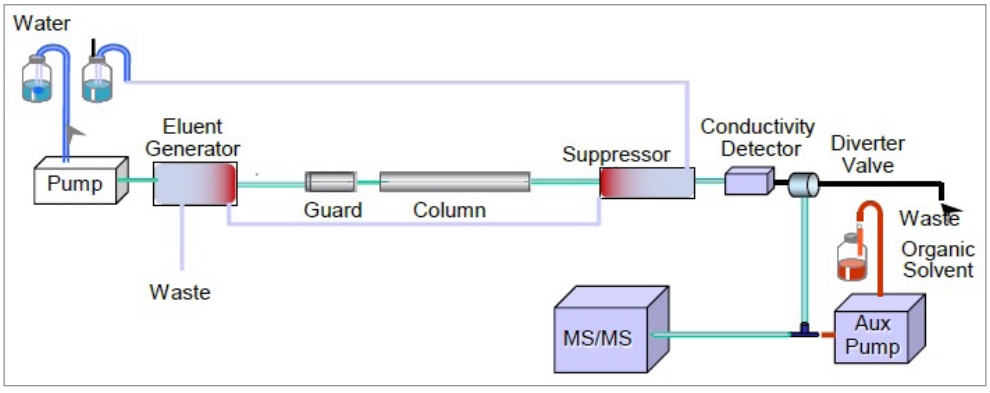

Figure 1. Schematic diagram of the flow path of the IC-MS/MS system.

A matrix diversion valve was placed in line prior to the mass spectrometer to divert the high sample matrix anions from the MS source that normally cause signal suppression in the MS. Thus, the use of hydroxide eluent and suppression in the Reagent Free™ IC system is more powerful for the separation and detection of organic acids than reversed phase separations that require acidic addition (to protonate the compounds to acetic acids) or addition of stabilizing salts, both of which undermine analysis. Isopropyl alcohol was added into the eluent stream via a mixing T immediately after the matrix diversion valve. The isopropyl alcohol was added at a flow rate of 0.2 mL/min. The isopropyl alcohol had two main purposes: to assist in the desolvation of the mobile phase and to act as a makeup flow when the IC eluent was diverted to waste. Acetonitrile can also be used instead of isopropyl alcohol, however the lower cost of isopropyl alcohol is an advantage to the chemist.

Mass Spectrometry

MS analysis was carried out on a Thermo Scientific™ TSQ Endura™ triple stage quadrupole mass spectrometer with a heated electrospray ionization (H-ESI-II) probe. The MS conditions used are shown in Table 3.

Table 3. Mass Spectrometer Source Conditions

Parameter	Value
Ion Source Polarity	Negative Ion Mode
Spray Voltage	3200 V
Vaporizer Gas Pressure	45 units N_2
Auxiliary Gas Pressure	10 units N_2
Capillary Temperature	200 °C
Vaporizer Temperature	200 °C
Collision Gas Pressure	1.5 mTorr Argon
Ion Cycle Time	0.5 seconds

Individual standards were infused into the mass spectrometer to determine optimum RF lens settings and collision energies for the product ions. Table 4 describes the MS conditions for specific HAAs, dalapon, bromate, and internal standards.

Table 4. Optimized MS transitions for each compound analyzed in this experiment. As per the U.S. EPA method, only one product ion was monitored for each precursor ion.

Analyte	Q1 (m/z)	Q3 (m/z)	RF lens (V)	CE (V)
MCAA	92.9	35.0	67	10
MBAA	136.9	79.0	60	13
DCAA	126.9	82.9	70	10
DBAA	216.8	172.8	72	12
BCAA	172.9	128.9	70	11
TCAA	160.9	116.9	45	8
BDCAA	162.9	81.0	60	10
DBCAA	206.9	81.0	90	16
TBAA	252.8	81.0	70	17

Table 4. Optimized MS transitions for each compound analyzed in this experiment. As per the U.S. EPA method, only one product ion was monitored for each precursor ion. (Cont.)

Analyte	Q1 (m/z)	Q3 (m/z)	RF lens (V)	CE (V)
Dalapon	140.9	96.8	56	7
Bromate	126.9	110.9	90	22
MCAA-ISTD	94.0	35.0	67	10
MBAA-ISTD	138.0	79.0	60	13
DCAA-ISTD	128.0	84.0	70	10
TCAA-ISTD	162.0	118	45	8

Data Analysis

Data acquisition and processing were carried out using Thermo Scientific™ TraceFinder™ software version 3.2.

RESULTS AND DISCUSSION

Calibrators and Simulated Sample Matrix

The separation of the nine HAAs and two other analytes is shown in Figure 2. This chromatogram is from the laboratory synthetic sample matrix (LSSM) fortified at 20 ppb. The LSSM is a prepared matrix of 250 mg/L of each of chloride and sulfate, 150 mg/L of bicarbonate, 20 mg/L of nitrate, and 100 mg/L ammonium chloride preservative, for a total chloride concentration of 316 mg/L. All 11 compounds are shown in Figure 2. The selectivity of the IC-MS/MS system allows separation of the HAAs from common inorganic matrix ions. This allows matrix peaks of chloride, sulfate, nitrate, and bicarbonate to be diverted to waste during the analytical run and avoids premature fouling of the ESI-MS/MS instrument source.

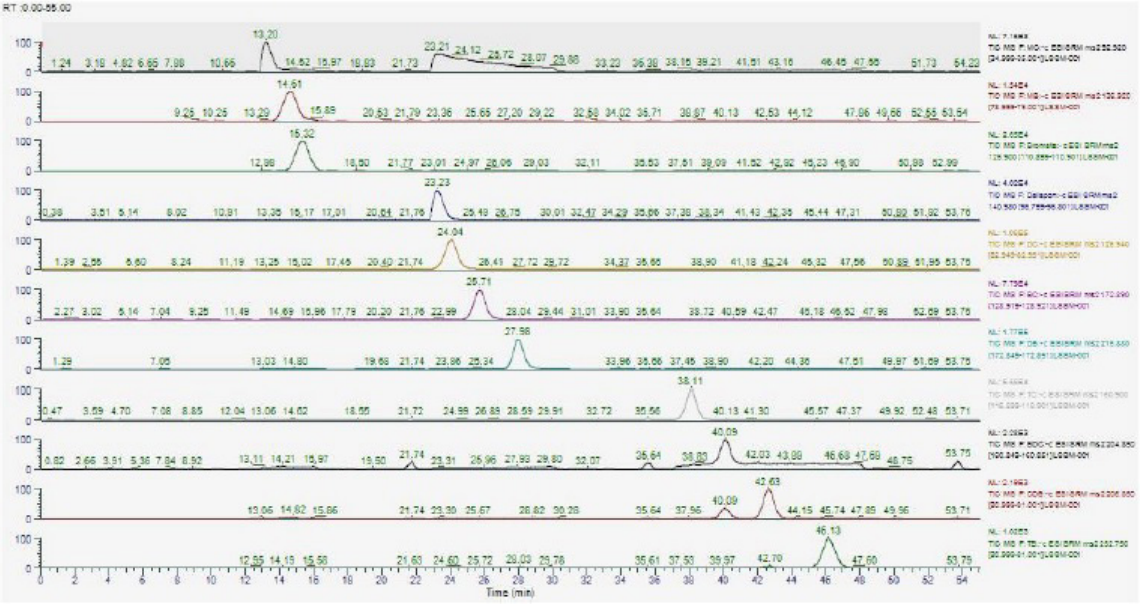

Figure 2. Laboratory Synthetic Sample Matrix (LSSM) spiked with 20 ppb haloacetic acids, bromate and dalapon. The internal standard peaks are not shown. From top to bottom, MCAA, MBAA, bromate, dalapon, DCAA, BCAA, DBAA, TCAA, BDCAA, DBCAA, and TBAA

Figure 3 shows the conductivity detector response chromatogram. The response from the Cl-, SO_4- and NO_3- can be seen in the trace. These ions do not coelute with the HAAs and are diverted to waste using the method controlled six-port valve on the mass spectrometer. The IC stream is diverted to waste from 0–12 minutes, 16–22.75 minutes, 30–37 minutes, and from 48 minutes until the end of the run.

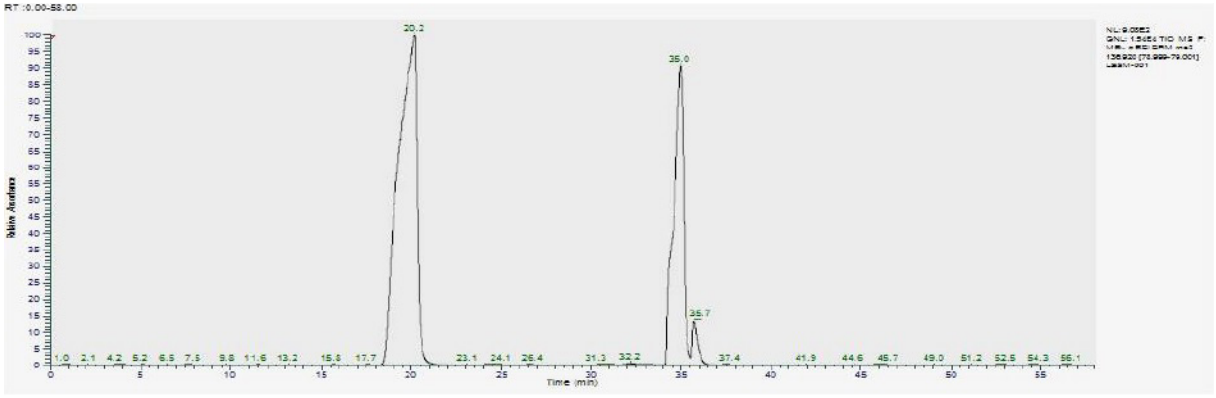

Figure 3. Conductivity detector response showing signals from LSSM salts.

An internal standard mixture of ^{13}C labeled MCAA, MBAA, DCAA, and TCAA was spiked into each sample at 4 ppb. All calibration standards were prepared in deionized water containing 100 mg/L NH_4Cl as a preservative. The calibration curves were generated using internal standard calibrations for all of the HAA compounds in water. Excellent linearity results were observed for all compounds. Analytes were run at levels of 250 ppt to 20 ppb in a seven-point calibration curve. All of the HAAs were detected at all concentration levels. It should be noted that TCAA sensitivity is very strongly correlated with the source temperature of the mass spectrometer as well as the column temperature of the IC column. For this reason, the column temperature was maintained at 15 °C as specified in the U.S. EPA method. Additionally, to improve the TCAA detection, the effect of temperature of the MS source on TCAA's response was tested. Temperatures of 200 °C for both the ion transfer tube and vaporizer were found to be optimal for TCCA detection without impacting the detection of the other eight analytes. This phenomenon of TCAA temperature sensitivity has been reported in studies with other MS instrumentation configurations [6].

Method detection limits were calculated by seven replicate injections of 0.5 ppb of each analyte and the equation MDL=*t*99%xS(n-7), where: *t* is Student's *t* at 99% confidence intervals (*t*99%, n=7 = 3.143) and S is the standard deviation. These MDLs are listed in Table 5.

Table 5. Method Detection Limits for each compound

Analyte	MDL (ppb)
MCAA	0.105
MBAA	0.104
DCAA	0.044
DBAA	0.021
BCAA	0.059
TCAA	0.033
BDCAA	0.141
DBCAA	0.214
TBAA	0.159

Table 5. Method Detection Limits for each compound (Cont.)

Analyte	MDL (ppb)
Dalapon	0.050
Bromate	0.059

Tap Water Sample Analysis

Additionally, tap water from San Jose, CA was analyzed for the presence of any of the analytes contained in the method. Tap water samples were collected in accordance with EPA method 557's procedure, with NH_4Cl added as a preservative as it reacts with residual chlorine preventing further formation of haloacetic acids. Internal standards were added and the samples were quantified. The total amount of haloacetic acids for all nine HAAs was 35.62 ppb. For the regulated HAA5, the total was 30.21 ppb. The MCL set by the US EPA for the HAA5 is 0.060 mg/L. This sample was below that limit, at 0.03021 mg/L.

CONCLUSION

- Reagent-Free IC systems coupled with an MS/MS detector is a powerful tool used in the quantitation of haloacetic acid samples.
- When compared to the conventional U.S. EPA methods using GC with electron capture, using the combination of the Dionex ICS 5000 Ion Chromatography system and the TSQ Endura triple quadrupole mass spectrometer to analyze for haloacetic acids saves analysts several hours of sample preparation.
- The resolution between the matrix peaks and haloacetic acids is excellent, which allows for minimum interference in detection, as well as ensuring a cleaner ion source of the mass spectrometer.
- Excellent reproducibility and quantitation of HAAs was achieved when samples were spiked into a simulated matrix.

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6. Slingsby, R.; Saini, C.; Pohl, C.; Jack, R. The Measurement of Haloacetic Acids in Drinking Water Using IC-MS/MS–Method Performance, Presented at the Pittsburgh Conference, New Orleans, LA, March 2008.

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SPONSOR REPORT

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Determination of Total Mercury in Environmental Samples Utilizing Direct Analysis

As more emphasis is placed on the monitoring of mercury emissions, both private and public institutions are looking at characterizing soil, sediments and waste water samples. Contaminated soil has to be excavated and remediated, or depending on the level of contamination, disposed of as hazardous waste. Several methods are available for mercury analysis in environmental samples like waste water and soil. Most of these methods however, require elaborate preparation procedures that are labor intensive and subsequently expensive. Direct mercury analysis, as described in EPA Method 7473, is an alternative to these methods and has been used successfully to determine total mercury in environmental samples. This technique requires no sample preparation and delivers results in as little as six (6) minutes per sample making it significantly faster than traditional wet chemistry.

INTRODUCTION

Mercury is naturally present in the earth and enters the air and water streams through the burning of fossil fuels, discharge of industrial waste and use of pesticides. Companies have also discharged mercury onto their property via production by-products. Now, with more emphasis being placed on the monitoring of this neurotoxin, both private and public institutions are looking at characterizing the soil, sediment and waste water, on their property.

Contaminated soil has to be excavated and remediated, or depending on the level of contamination, disposed of as hazardous waste. Several methods exist for the determination of mercury in environmental samples. Traditional analytical methods such as Cold Vapor Atomic Absorption (CVAA) and ICP MS both require sample preparation prior to analysis. This results in both techniques being costly, labor-intensive and subsequently, having a long turnaround time. Direct mercury analysis, as described in EPA Method 7473, is a cost-effective, proven alternative to these labor-intensive, wet chemistry techniques.

Direct analysis affords the laboratory many benefits including:

- Reduced Sample Turnaround (6 Minutes)
- No Sample Preparation
- Reduced Hazardous Waste Generation
- Reduction of Analytical Errors
- General Cost Savings (70% versus CVAA)
- The goal of this study was to evaluate the effectiveness of the DMA-80 to analyse both soil and ground water samples – two vastly different matrices.

EXPERIMENTAL

Instrument

The DMA-80, Direct Mercury Analyzer, as evidenced in EPA Method 7473, from Milestone Srl (www.milestonesrl.com) was used in this study. The DMA-80 features a circular, stainless steel, interchangeable 40 position autosampler for virtually limitless throughput and can accommodate both nickel (500 mg) and quartz boats (1500 µL) depending on the requirements of the application. It operates from a single phase 110/220V, 50/60 Hz power supply and requires regular grade oxygen as a carrier gas.

As the process does not require the conversion of mercury to mercuric ions, both solid and liquid matrices can be analyzed without the need for acid digestion or other sample preparation. The fact that zero sample preparation is required also eliminates all hazardous waste generation. All results, instrument

parameters including furnace temperatures, are controlled and saved with easy export capabilities to Excel or LIMS.

Figure 1. Milestone's DMA-80 evo.

Principles of Operation

Direct mercury analysis incorporates the following sequence: Thermal Decomposition, Catalytic Conversion, Amalgamation, and Atomic Absorption Spectrophotometry. Controlled heating stages are implemented to first dry and then thermally decompose sample introduced into a quartz tube. A continuous flow of oxygen carries the decomposition products through a hot catalyst bed where halogens, nitrogen, and sulphur oxides are trapped. All mercury species are reduced to Hg(0) and are then carried along with reaction gases to a gold amalgamator where the mercury is selectively trapped. All non-mercury vapors and decomposition products are flushed from the system by the continuous flow of gas. The amalgamator is subsequently heated and releases all trapped mercury to the single beam, fixed wavelength atomic absorption spectrophotometer. Absorbance is measured at 253.7 nm as a function of mercury content.

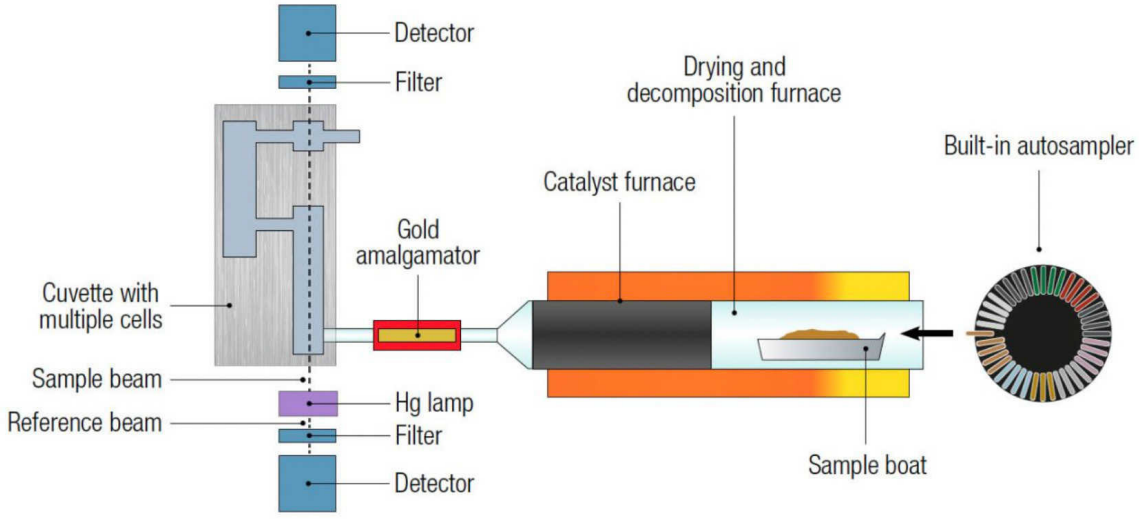

Figure 2. An Internal Schematic of Milestone's DMA-80 evo.

Samples

All soil and water samples were obtained from an independent contract laboratory that had already analysed the samples via CVAA. For this study, the soil and water samples were analysed at various weights in the nickel and quartz sample boats respectively.

Calibration

Calibration standards were prepared using a NIST traceable stock solution of 1000 ppm Hg preserved in 5% HNO_3. Working standards of 100 ppb and 1 ppm were prepared and preserved in 37% HCl and stored in amber glass vials. By injecting increasing sample volumes of standard into the quartz sample boats, calibration graphs of 0 – 20 ng (Figure 3) and 20 – 500 ng (Figure 4) of mercury were created using the 100 ppb and 1 ppm standards respectively.

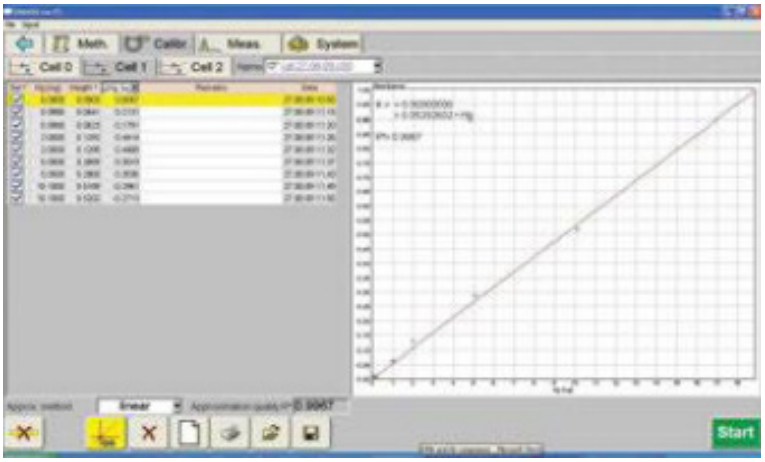

Figure 3. 0 ng – 20 ng Calibration Graph for ultra-level.

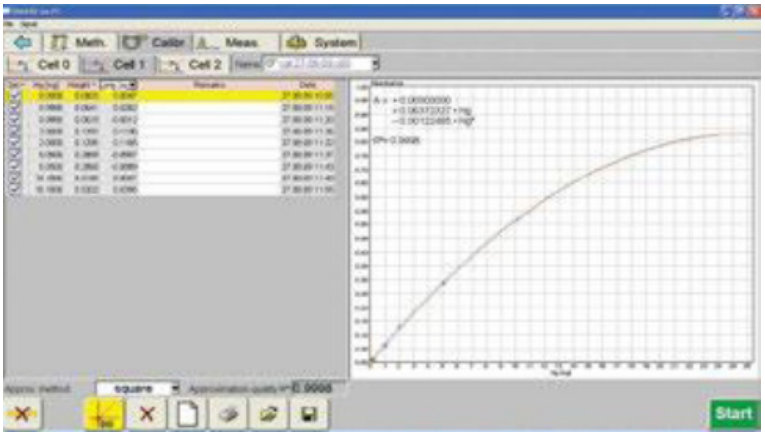

Figure 4. 20 ng – 1000 ng Calibration Graph for low to mid-level analysis (ppb, ppm).

Operating Conditions

The DMA-80's operating conditions for all analyses are shown in Table 1.

Table 1. Analysis Operating Parameters

Parameter	Setting
Drying Temp/Time	90 seconds to 200 °C
Decomposition Ramp	120 seconds to 650 °C
Decomposition Hold	90 seconds at 650 °C
Catalyst Temp	565 °C

Table 1. Analysis Operating Parameters (Cont.)

Parameter	Setting
Purge Time	60 seconds
Amalgamation Time	12 seconds at 900 °C
Recording Time	30 seconds
Oxygen Flow	120 mL/min

RESULTS

Table 2 shows the results for all samples analysed on the DMA-80. The far column indicates the results previously obtained via CVAA. All sample results were within satisfactory range of previous analyses. The slight discrepancy in the soil sample results can be attributed to using smaller sample sizes which leads to samples being inhomogeneous.

Table 2. Unknown Environmental Samples on DMA-80 vs Contract Laboratory

Sample	Concentration (µg/Kg)	Contract Laboratory (µg/Kg)
0,2 (µg/Kg)	0,211	0,205
1 (µg/Kg)	0,915	1,05
5 (µg/Kg)	4,72	5,04
10 (µg/Kg)	9,717	10,3
ICV (5 µg/Kg)	5,446	5,2
blank	0,01	0,03
LCS (5 µg/Kg)	5,773	5,1
soil	0,663	0,9
soil	5,092	5
soil	0,535	0,63
CCV (5 µg/Kg)	5,765	5,5

In addition to the unknown environmental samples, NIST 2709 San Joaquin Soil, a matrix matched Standard Reference Material (SRM) was periodically analysed.

Table 2. Unknown Environmental Samples on DMA-80 vs Contract Laboratory (Part II)

Sample	Concentration (µg/Kg)	Certifield (µg/Kg)
LCS (5 µg/Kg)	5,541	5,9
Soil	398,013	520
Soil	7684,281	6590
Soil	8408,259	7630

Table 3. Summary of results of QA/QC analysis

Sample	Concentration (mg/Kg)	Certified (mg/Kg)
NIST 2709	1,378	1,40 ± 0,08
NIST 2709	1,404	1,40 ± 0,08
NIST 2709	1,406	1,40 ± 0,08
NIST 2709	1,404	1,40 ± 0,08
NIST 2709	1,424	1,40 ± 0,08

CONCLUSION

All results obtained on the DMA-80 were in good agreement with the previous CVAA results. The DMA-80, direct mercury analyser, successfully analysed five replicates of the NIST 2709 SRM. Similar success can be expected when analysing other soil and sludge-type samples. As previously mentioned, a possible reason for the discrepancy in Table 2 can be sample homogeneity. All the liquid standard results were within their expected range. The DMA-80 is a fast, reliable alternative to wet chemistry techniques. No sample preparation enables quick sample turnaround which enable higher throughput for environmental contract laboratories.

Further Reading

Please visit our Hg info center for complete access to application notes, technical papers, as well as links to valuable resources for mercury testing.
Go to http://www.milestonesrl.com

To learn more about mercury and other related topics, feel free to visit these websites.

EPA Method 7473
http://www.epa.gov/waste/hazard/testmethods/sw846/pdfs/7473.pdf

ASTM Method D6722-01
http://www.astm.org/Standards/D6722.htm

Methyl Mercury
http://en.wikipedia.org/wiki/Methylmercury

Mercury in Fish
http://www.epa.gov/waterscience/fish/advice/mercupd.pdf

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RELEASE

IC-ICP-MS Analyzer for Speciation Analysis

While total elemental content can be analyzed by ICP-MS, speciation analysis of trace elements is ideally performed by a Thermo Scientific IC-ICP-MS system. The speciation analyzer includes a metal-free IC system with high resolution ion exchange columns and simple online connectivity, together with high sensitivity ICP-MS and integrated software.

Dionex™ Aquion™ Ion Chromatography (IC) System

Perform basic ion analysis reliably. Simple, compact platform with straightforward operation for budget-limited labs. This basic IC system features dual-piston pumping, electrolytic suppression, high sensitivity and set-and-forget convenience. Contamination-free, trouble-free IC with durable PEEK flow paths, which are compatible with the full range of IC eluents. Enjoy ease of use, fast start up times and reliable, stable performance for demanding applications in environmental, food safety, and academic laboratories. The system comes with Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software for automation and data handling.

Single Quadrupole Inductively Coupled Plasma Mass Spectrometry (SQ-ICP-MS)

Simplicity, productivity and robustness for routine labs.

With comprehensive interference removal for assured data accuracy and intuitive workflows to boost productivity, this single quadrupole (SQ) ICP-MS will expand your analytical capabilities.

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.

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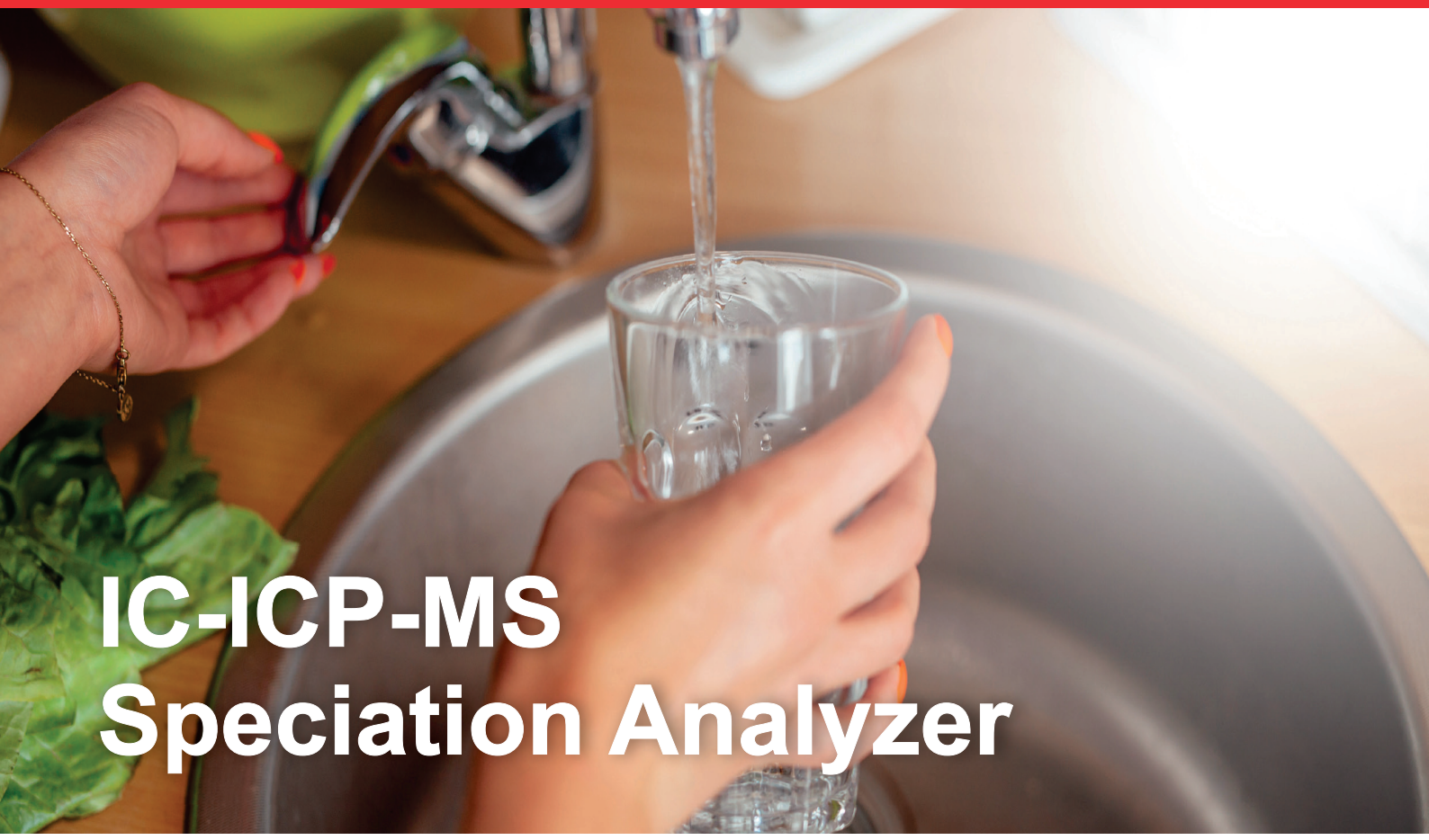

IC-ICP-MS Speciation Analyzer

Speciation Analysis

Distinguishing between chemical forms of an element is critical for multiple industries, including the food, environmental, and pharmaceutical sectors. In the past, measuring the total amount of an element was suffcient. Unfortunately, the effects of an element extend far beyond its absolute amount. Different forms of an element can exhibit very different physicochemical properties, including varying toxicities. The process of separation and quantification of different chemical forms of an element, more specifically termed speciation analysis, delivers a better understanding of the environmental or health-related impact associated with a particular sample. Speciation analysis can be split into two components: separation of individual ionic species by ion chromatography (IC), and trace elemental detection and quantification using inductively coupled plasma mass spectrometry (ICP-MS). This combined method is termed ion chromatography inductively coupled plasma mass spectrometry (IC-ICP-MS).

WEBINAR WEBSITE

Benefts of IC-ICP-MS

- Metal-free IC separates the individual species without contributing trace metal contamination.
- IC-ICP-MS acts as a highly sensitive and element-specifc detection system.

ThermoFisher SCIENTIFIC

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TSQ Altis™ Triple Quadrupole Mass Spectrometer

Sensitivity with Robustness: No Compromise

Address the most stringent analytical challenges for targeted quantitation workflows with the Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer. With its improved Active Ion Management (AIM™) technology, segmented quadrupoles, novel electron multipliers, and enhanced ion transmission tubes, the TSQ Altis MS helps you achieve unprecedented experimental sensitivity for all molecular species in complex matrices without sacrificing robustness.

The TSQ Altis Triple Quadrupole Mass Spectrometer, together with its industry-leading quantitation software, UHPLC system, and superior sample preparation kits, helps researchers address critical analytical challenges using robust, reliable, and sensitive targeted quantitation.

The main benefits of the TSQ Altis MS include

- Ultimate sensitivity for all molecular types in matrices ranging from simple to complex.
- Outstanding instrument robustness enables increased confidence in generated data with no loss of instrument uptime.
- Ultrafast selected reaction monitoring (SRM) increases the amount of molecular quantifications in less time.
- Close integration with application-specific software results in increased productivity across all application markets.
- Simplicity and ease-of-use allow users of all expertise levels to acquire high quality results with increased confidence.

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THE POWER OF MASS SPECTROMETRY FOR IC ANALYTICAL CHEMISTS

Increase the power of IC with MS detection

Utilize mass detection when you need to ensure analytical confidence and dramatically improve the detection capability of your Thermo Scientific Dionex Integrion or ICS-6000 HPIC system. Ion chromatography with mass spectrometry (IC-MS) maximizes the ability to detect and quantify unexpected co-elutions of components and to confirm trace components.

An application example is the analysis of haloacetic acids, the pesticide dalapon, and bromate in water using EPA method 557. Haloacetic acids (HAAs) are formed as disinfection byproducts when water is chlorinated to remove microbial content. Chlorine reacts with naturally occurring organic and inorganic matter in the water, such as decaying vegetation, to produce disinfection byproducts (DBPs) that include HAAs. Of the nine species of HAAs, five are currently regulated by the U.S. Environmental Protection Agency (EPA) (HAA5). The method allows for the analysis of all nine HAAs, plus bromate and the pesticide dalapon in the same IC-MS/MS run without sample preparation.

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WEBSITE

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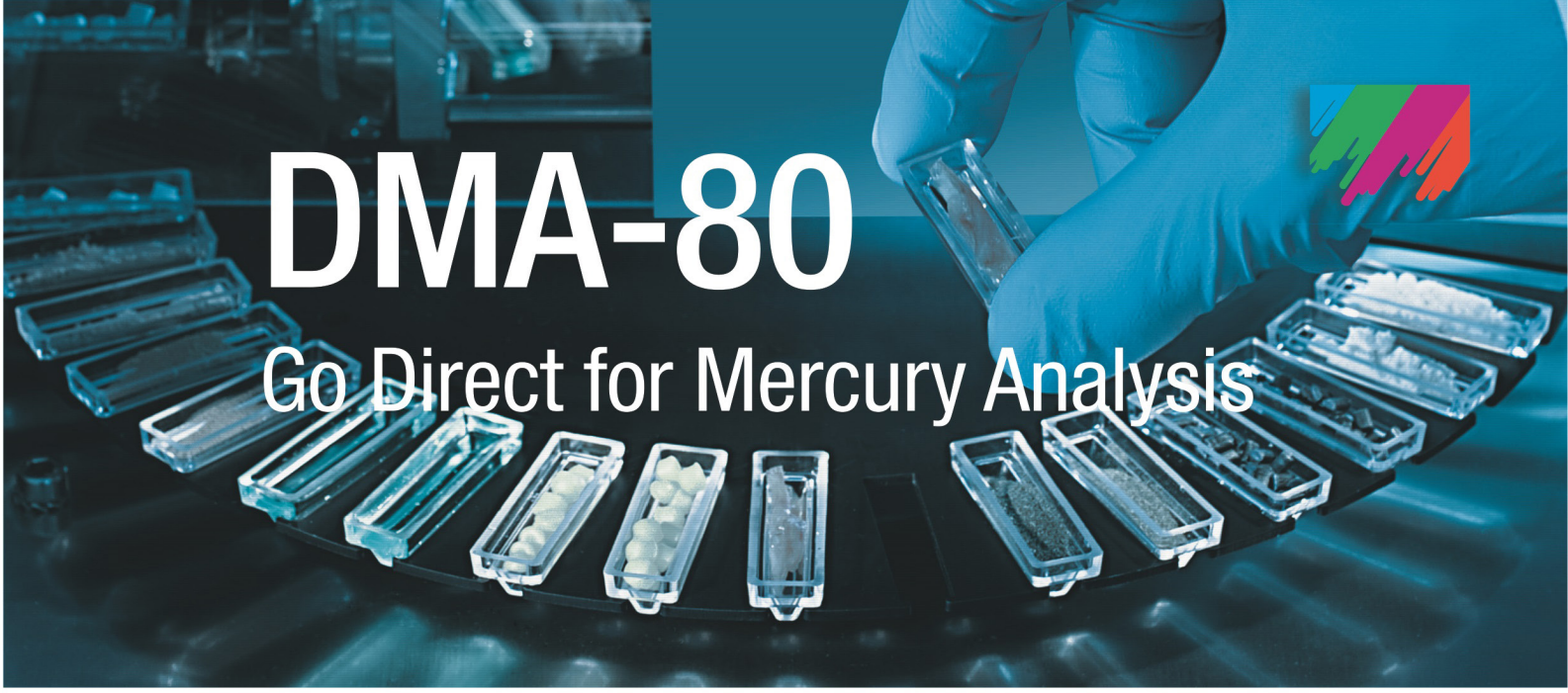
Save Time — Go Direct for Mercury Analysis

The Milestone DMA-80 has been at the forefront of direct total mercury analysis for almost two decades

The challenges in mercury determination are well known to analysts who often face a number of issues connected with tedious sample preparation process or the mercury analysis steps causing memory effect even after long cleaning cycles performed using latest generation of ICP-MS or cold vapor systems.

The DMA-80 mercury determination system can analyze any matrix (solid, liquid or gas) without any pre-treatment or chemical additions in as few as 6 minutes in full compliance with EPA method 7473. With thousands of units installed, Milestone defines the benchmark for direct mercury determination in a wide range of industries: environmental, food energy, cement, cosmetics, agriculture, mining and petrochemical.

- **Improved design** from the outside out for superior performance event at the ppt level.
- **Easy-to-use technology** with a single long-lasting calibration for all matrices.
- **No memory effect** thanks to the revolutionary autoblank feature.
- **Milestone Connect** for 30-years of experience at your fingertips
- **Fully compliant** with US EPA Method 7473, ASTM method D-6722-01 and D-7623-10.
- **Maximize your ROI** with an easy-to-maintain system and reliable components.



DMA-80

Go Direct for Mercury Analysis

SIX MINUTE, PREP-FREE MERCURY ANALYSIS

If youre looking for unsurpassed processing speed, efficiency and precision for mercury samples, its time for the Milestone DMA-80offering more than double the productivity of traditional cold vapor techniques at a fraction of the cost.

NO SAMPLE PREP REQUIRED

Eliminating the need for any wet chemistry sample treatment prior to analysis.

WIDEST DYNAMIC RANGE

Capable of achieving a detection limit of 0.001 to 30,000 ng of mercury

RESULTS IN JUST 6 MINUTES

Dramatically improves your laboratorys turnaround time and productivity.

UP TO 70% COST REDUCTION

Compared to traditional mercury analysis techniques such as CV-AAS.

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DMA-80

DMA-1

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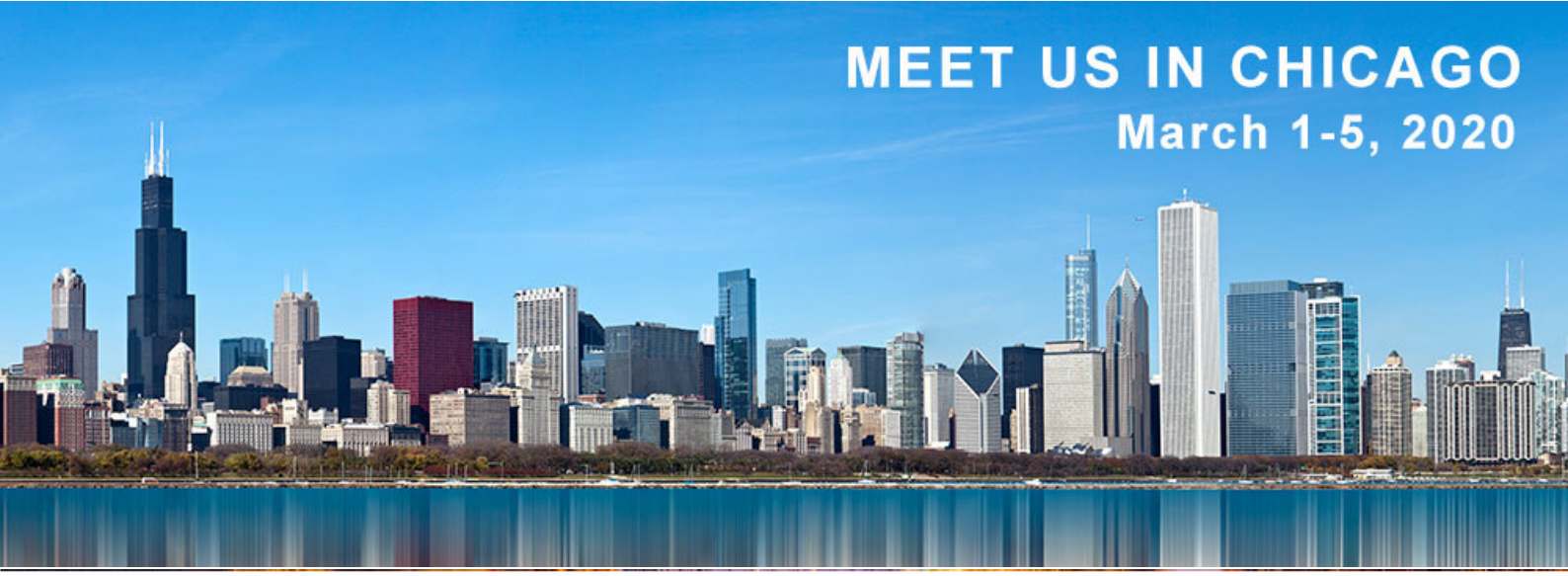

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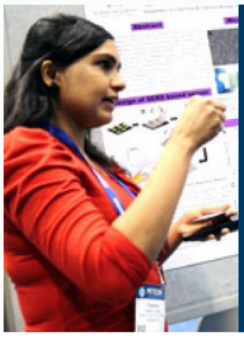

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Some recent contributions from SelecScience® to the scientific community

Editorial Article

Striving for product quality assurance in the 'wild west' of medical cannabis

Ever since the medicinal properties of cannabis and its components were first recognized in pain relief for a wide range of ailments, debates about its safety have loomed large. Not least of these concerns has been the quality of the cannabis product administered and how its components transform during use, for example under heating. Learn how a diagnostic testing company in the U.S. is meeting the increasing demand for patient safety in medical cannabis products here

Video

Cannabis testing: Overcoming challenges in edibles analysis

Dr. Rob O'Brien
CEO and CSO of Supra Research and Development, Kelowna, Canada

Following the legalization of edible cannabis products in Canada in October 2019, effective testing and quality control are vital. In this video, Dr. Rob O'Brien explores the analytical processes, testing protocols and challenges involved in meeting new regulations and reveals a breakthrough method for cannabis testing in chocolate for the Canadian market. Watch this video here here

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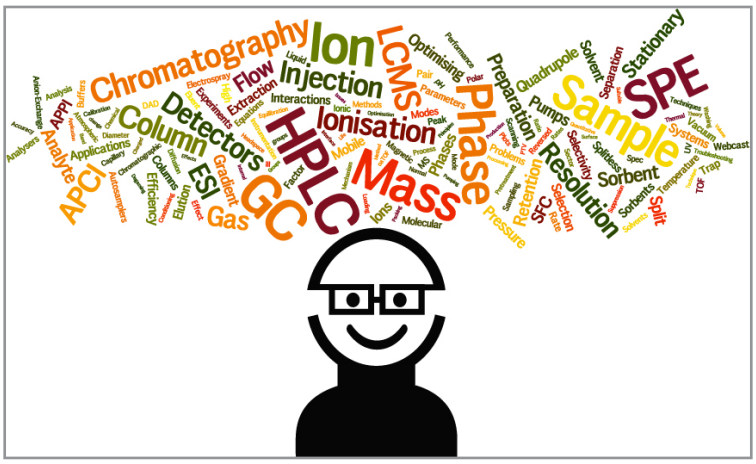

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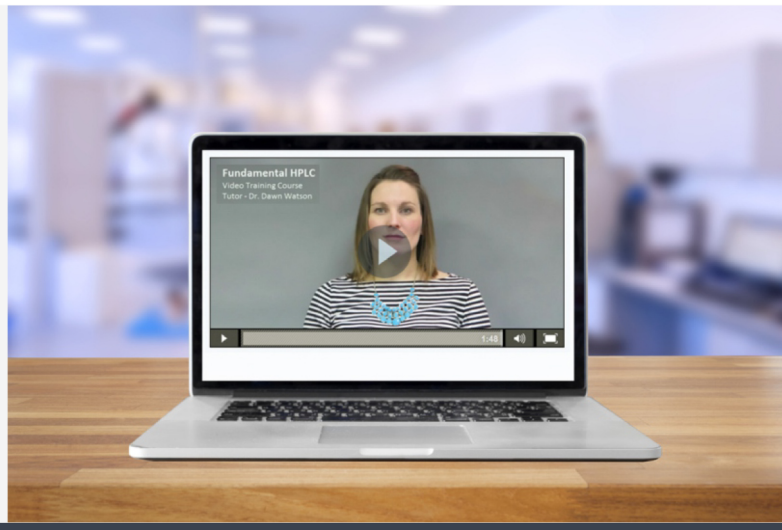

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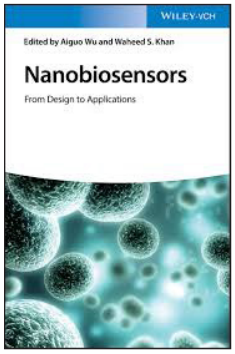
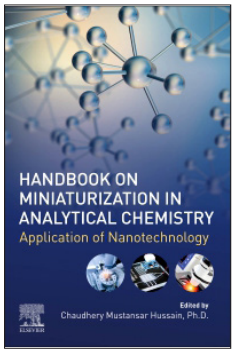
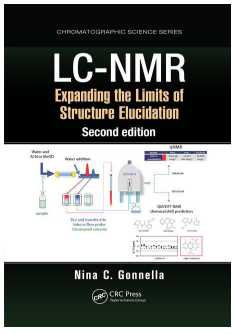


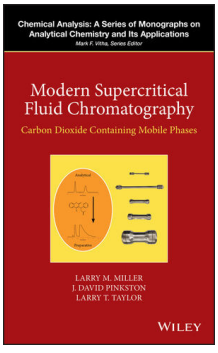
Nanobiosensors: From Design to Applications
Aiguo Wu, Waheed S. Khan (Editors)
February 2020. Publisher: John Wiley & Sons
This book covers biosensors related material in broad spectrum such as basic concepts, biosensors&their classification, biomarkers&their role in biosensors, nanostructures-based biosensors, applications of biosensors in human diseases, drug detection, toxins, and smart phone based biosensors. Discusses the latest technology and advances in the field of nanobiosensors and their applications in human diseases, drug detection, toxins. Read more …



Handbook on Miniaturization in Analytical Chemistry
Chaudhery Mustansar Hussain, Editor
August 2020. Publisher: Elsevier
Provides fundamentals, interdisciplinary knowledge and primary literature for researchers who want to fully understand how nano-technologies work. Covering all stages of analysis, from sample preparation to separation and detection, the book discusses the design and manufacturing technology of miniaturization and includes safety risks, ethical, legal and social issues, the economics of nanotechnologies, and a discussion on sustainability with respect to nano- and lab-on-chip technologies. Read more …



LC-NMR: Expanding the Limits of Structure Elucidation, 2nd Edition
Nina C. Gonnella, Author
January 2020. CRC Press Taylor & Francis Group
Comprehensive overview of key concepts in HPLC and NMR required to achieve definitive structure elucidation with very low levels of analytes. Provides introductory background to facilitate readers' proficiency in both areas, including an entire chapter on NMR theory. Provides guidance in setting up LC-NMR systems, discussion of LC methods that are compatible with NMR, and an update on recent hardware and software advances for system performance. Read more …



Modern Supercritical Fluid Chromatography: Carbon Dioxide Containing Mobile Phases
Larry M. Miller, J. David Pinkston, Larry T. Taylor, Authors
November 2019. Publisher: John Wiley & Son
Explains why modern SFC is the leading "green" analytical and purification separations technology. Covers current SFC instrumentation as it relates to greater robustness, better reproducibility, and increased analytical sensitivity. Topics covered include: Milestones of SFC; Physical Properties of SF; Instrumentation for SFC; Detection in SFC; Achiral SFC Method Development; Chiral SFC... Read more …

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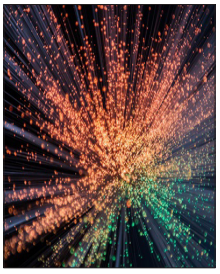

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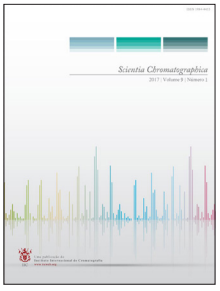

Scientia Chromatographica

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

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EVENTS 2020

March 1 – 5
PITTCON Conference and Expo 2020
McCormick Place, Chicago, IL, USA
https://pittcon.org/pittcon-2020/

August 10 – 13
Braz. Meeting on Forensic Chemistry (7th EnqFor) & 4th Meeting of the Braz. Society of Forensic Sciences (SBCF)
Ribeirão Preto, SP, Brazil
https://www.en.enqfor2020.sbcf.org.br/

August 29 – September 4
XXIII International Mass Spectrometry Conference (IMSC 2020)
Windsor Oceânico Hotel, Rio de Janeiro, RJ, Brazil
https://www.imsc2020.com/

August 30 – September 4
71st Annual Meeting of the International Society of Electrochemistry — "Electrochemistry towards Excellence"
Belgrade, Serbia
https://annual71.ise-online.org/

September 21 – 24
Rio Oil & Gas 2020
Riocentro, Rio de Janeiro, RJ, Brazil
https://www.riooilgas.com.br/

October 7 – 9
FCE Pharma, International Exhibition of Technology for the Pharmaceutical Industry
São Paulo Expo, São Paulo, SP, Brazil
https://www.fcepharma.com.br/

October 20 – 21
18th Congress on Quality in Metrology (ENQUALAB 2020)
São Paulo, SP
https://www.enqualab.net/

November 8 – 11
XVI Latin American Congress on Organic Geochemistry (ALAGO)
Niterói, RJ, Brazil
https://alago2020.com/

December 3 – 6
43rd Annual Meeting of the Brazilian Chemical Society (43rd RASBQ)
Centro de Convenções, Maceió, AL, Brazil
http://www.sbq.org.br/43ra/

December 10 – 11
International Conference on Metrology, Measurement and Inspection (ICMMI 2020)
New York City, USA
https://waset.org/metrology-measurement-and-inspection-conference-in-december-2020-in-new-york

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. BrJAC is addressed to professionals involved in science, technology and innovation projects in Analytical Chemistry, at universities, research centers and in industry.

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Manuscripts submitted for publication in BrJAC cannot have been previously published or be currently submitted for publication in another journal. BrJAC publishes original, unpublished scientific articles and technical notes that are peer reviewed in the double-blind way.

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BrJAC's review process begins with an initial screening of the manuscripts by the editor-in-chief, who evaluates the adequacy of the study to the journal scope, and an analysis of similarities. Manuscripts accepted in this screening are then forwarded to at least two reviewers who are experts in the related field. 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 to the BrJAC's guidelines, and the clarity of writing and presentation.

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

Brief description of the documents that can be submitted by the authors

- **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 a critical analysis 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 method, new technique, procedure or equipment falling within the scope of 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.

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The mandatory files to be uploaded are: **Cover letter**, **Manuscript** and **Title Page** (double-blind review). **Templates** for these files are available at http://brjac.com.br/guidelines-download.php

Manuscript preparation

- **Language**: **English** is the language adopted by BrJAC.
- **Required items**: the manuscript must include a title, an abstract, keywords, and the following sections: Introduction, Materials and Methods, Results and Discussion, Conclusion, and References.

- **Identification of authors**: the manuscript file must **NOT** contain the authors' names nor affiliations. This information must be in the **Title Page**. This rule is necessary because the manuscript is subjected to double-blind review.
- **Layout**: the lines in the manuscript must be numbered consecutively and double-spaced.
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- **References**: must be cited by **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 should be thoroughly checked for errors by the authors before submission.** See how to format the references in the following item.

Examples of reference formatting

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).*

Electronic journals

2. Sapozhnikova, Y.; Hoh, E. *LCGC North Am*., **2019**, *37* (1), pp 52-65. Available from: http://www.chromatographyonline.com/suspect-screening-chemicals-food-packaging-plastic-film-comprehensive-two-dimensional-gas-chromatogr [Accessed 20 January 2019].

Books

3. Burgot, J.-L. *Ionic Equilibria in Analytical Chemistry*. Springer Science & Business Media, New York, **2012**, Chapter 11, p 181.
4. 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

5. 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

6. 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

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

Web pages

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Unpublished source

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