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Brazilian Journal of Analytical Chemistry
an International Scientific Journal

Quality by Design (QbD)

Key Information Related to Quality by Design (QbD) Applications in Analytical Methods Development

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Scope

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

BrJAC is a quarterly journal that publishes original, unpublished scientific articles, reviews and technical notes that are peer reviewed in the double-blind way.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

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

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EDITORIAL

Multifaces of Modern Analytical Chemistry

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Departamento de Química, Universidade Federal de Santa Maria
Santa Maria, RS, Brazil

The Brazilian Journal of Analytical Chemistry has completed ten years with focus on the integration between academia, research centers, and industry. In this regard, the BrJAC showed rapid growth and great acceptance by the analytical chemistry community and other related science disciplines. There was a rapid increase in its impact factor and visibility, showing that the BrJAC arrived at a good time for analytical chemistry, which is currently one of the most vigorous fields, with researchers cited among the top scientists in the world. Currently, new materials, information technology, advances in instrumentation and the interaction of other areas with analytical chemistry have brought many advances and new developments. In this sense, important advances are observed in areas related to environmental control, the use of chemometrics, quality control of food and pharmaceutical products, among others.

It is possible to observe a clear trend towards the development of faster and less invasive methods, with better detectability and selectivity. Another important aspect that is becoming increasingly more present in analytical chemistry papers refers to the use of methods in agreement to the principles of green chemistry, which presupposes, among other aspects, the use of lower volumes of concentrated reagents and, obviously, the generation of less laboratory waste and a lower need for waste treatment for disposal or eventual reuse.

In this issue of the BrJAC, most of these developments are covered. We can read the excellent **Interview** with a very famous Chilean researcher who has developed plenty of important analytical methods, being the pioneer of a clever system based on a microextraction system using a rotating-disk sorptive extraction technique that has many important applications.

The BrJAC also provides a look at a current challenge related to arsenic speciation. It is well known that arsenic speciation methods are important to understand the toxicity or essentiality of its species for environmental and biological studies. Here, a **Point of View** is presented from an expert in this field, showing the possibilities and recent trends. Sample preparation methods, such as the use of solid phase extraction systems, have been applied to many matrices, even enabling the on-site separation of As species.

The importance of the study of polymorphism and analytical techniques for the structural characterization of food products with a special focus on chocolate is highlighted in the **Letter** section. Solid state characterization is fundamental for the food Industry. It allows the development of food products with many advantages, such as better formulations, processability, stability and functionality.

The **Review** section provides a deep and critical revision of chemometrics applications, with focus on the quality by design approach for the development of new analytical methods. The authors are experts in this field and also demonstrated the effect of the quality by design approach on the recent changes in traditional manufacturing, helping to save both energy and time. It is possible to see that there is a very good expectation for new applications in many areas in addition to the already well-established use for pharmaceutical purposes.

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In this issue, two interesting **Articles** are included. The first describes a comprehensive study related to the effect of ultraviolet radiation (UV-C) on many kinds of paper. The authors performed a full characterization of UV-C irradiated samples with a multitude of techniques (SEM-EDS, DTC-TGA, FTIR, XRD among others). The second article shows the complete evaluation of a validated method using RP-HPLC that provided the accurate determination of pharmaceutical products. A full degradation study was also described, showing the suitability for purity assessment in analytical laboratories.

Finally, the BrJAC also presents a **Technical Note** with a relatively recent improvement in the field of atomic absorption spectrometry (HR-CS FAAS): an important application of HR-CS FAAS for the analysis of B7-diesel oil and multi-element determination of trace elements. The authors used a smart strategy based on the formation of microemulsions that allowed a fast and accurate analysis.

Therefore, this issue brings many exciting articles of researchers from many countries showing that the BrJAC is becoming more important to the field of analytical chemistry and that authors are increasingly more often considering this journal as a suitable channel to spread their research at a high level.

Érico Marlon de Moraes Flores is currently Full Professor of the Department of Chemistry at the Federal University of Santa Maria, Santa Maria, RS, Brazil. He works mainly in research and technological development involving atomic spectrometry and the use of alternative energies, such as ultrasound and microwave for sample preparation with application in several laboratories and also for the intensification of industrial processes. He has also worked in the field of quality control of pharmaceutical products, food, nanomaterials and in the extraction and analytical developments for the determination of rare earth elements.
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INTERVIEW

The inventor of the Rotating-Disk Sorptive Extraction technique kindly spoke to BrJAC

Pablo Richter
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Faculty of Chemical and Pharmaceutical Sciences
University of Chile, Santiago, Chile

Pablo Roberto Richter Duk is Full Professor at the University of Chile and current Academic Director of the Faculty of Chemical and Pharmaceutical Sciences (FCPS) at the same University. He has been the Director of the two Internationalization projects of the FCPS (periods 2016–2018 and 2020–2022).

Prof. Pablo Richter was born in Santiago, Chile and obtained his Bachelor's degree in Chemistry (1984) and his PhD degree in Chemistry (1991) from the University of Chile. Between 1991 and 1992 he carried out postdoctoral studies at the University of Córdoba, Spain, and in 1996 at Oklahoma State University, USA. In the period 1997–2003, Prof. Richter directed the environmental laboratories of the National Center for the Environment (CENMA) at the University of Chile and has visited different environmental institutes in Japan (1998) under the CENMA-JICA international cooperation project. He was also Director of the Center for Studies in the Development of Chemistry (CEPEDEQ) in the period 2003–2015 and was Director of Postgraduate Studies of the FCPS in the period 2010–2018.

His main lines of research and scientific interest are food and environmental sample preparation, microextraction technologies, chromatography, mass spectrometry, and electroanalysis. He is the inventor of the sample preparation technique "Rotating-Disk Sorptive Extraction", RDSE. In the period 2017–2019, he was a member of the Superior Council of Science of the National Fund for Scientific and Technological Development (FONDECYT). He also joined the chemistry study group at FONDECYT (periods 2004–2007 and 2015–2016), becoming its Director in 2016. He has been a responsible researcher and co-researcher in FONDECYT and in international research projects (Spain, Japan, Argentina, Colombia), an evaluator of research projects for FONDECYT, CORFO and universities, and a member and chair of the Organizing Committee and Scientific Committee at different Congresses.

He is the author or co-author of 134 scientific papers in journals of international circulation (ISI), has been cited more than 2700 times and has an "h" index of 31. He is a frequent reviewer of scientific papers published in several WoS journals and is a member of the editorial committee of the *Journal of Analytical Methods in Chemistry* and *Advances in Environmental Chemistry*. He has given lectures by invitation in Chile, Brazil, Argentina, Spain, Ecuador, Peru, Colombia, Uruguay, Mexico and Germany and has been selected by the Chilean Academy of Sciences as "Researcher of the Frontier Science Program 2004–2006". Since 2009 he has been a member of

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the Area Committees of the Advanced Human Capital Program of CONICYT. He has belonged to the following scientific societies: the Chilean Chemistry Society, the Analytical Chemistry Division of the Chilean Chemistry Society, IUPAC, the International Society of Electrochemistry, AOAC and RACAL. He has been president for two periods of the Division of Analytical and Environmental Chemistry of the Chilean Chemical Society, "Observer" in the Division of Analytical Chemistry of the Federation of European Chemical Societies and Professional Institutions (DAC-FECS) and a member of the International Ibero-American Steering Committee of Analytical Chemistry.
Prof. Richter has been a peer evaluator in accreditation processes for the Bachelor of Chemistry programs in Argentina and Ecuador (CONEAU) and in Master's and Doctorate programs in Chile (CNA). He has taught undergraduate and postgraduate courses at the University of Chile and has directed theses for the Bachelor's degree (43), Master's degree (5), Doctorate (17) and two postdoctoral works at the same university.
Currently, Prof. Pablo Richter advises and teaches postgraduate courses in the area of analytical laboratory accreditation, validation of methods, and techniques for sample preparation. He has also carried out accreditation audits as a technical evaluator for the National Institute for Standardization.

How was your childhood? When was your first contact with the scientific area?

I had a very normal childhood, rather far from science. As a child my first interest was sport; I didn't like school very much. My life then turned around soccer and tennis; I was pretty good at both sports. I am the youngest of the family. My father was an industrial technician and had a blanket and yarn factory and my mother was a housewife, buy she had studied Medicine at the University of Chile. I have two siblings, a woman and a man. My first contact with science was through an experiment at school that I never forgot; I think it was one of the first experimental activities I did. The aim was to demonstrate the conductivity of drinking water using a pair of graphite electrodes connected to a light bulb.

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

I remember that my interest in chemistry began three years before finishing high school, but I was mainly interested in teaching. I was passionate about the ways of teaching adopted by my chemistry teacher, Prof. Alejandro Donoso. It was the only course on which I totally concentrated, I could understand everything, and I started to get very good marks in the subject. Although at school I was never very dedicated to study, when I came home after chemistry classes I studied the class on my blackboard and explained it to myself like a teacher, I loved to draw molecules and chemical equations and explain the changes that were happening through those reactions.

When did you decide to go into the field of chemistry? What motivated you? How was the beginning of your career in chemistry?

Linking with the previous answer, at that time, I first decided to study chemistry education. During my last year of high school, I researched more about chemistry careers at the different universities in the country. I realized that in addition to chemistry education I had the possibility to study for a licentiate degree in chemistry (this degree in Chile is an intermediate between Bachelor's and Master's), which prepares university researchers who can also teach chemistry at the university. So, I applied to all universities to follow this career. Fortunately, I was accepted by the University of Chile in 1980.

The first year of my degree was very hard, very difficult, not only regarding the chemistry course but we also had to study mechanics, algebra and calculus together with the undergraduate students of physics. We started with 50 students in the first year and 10 remained in the second year. At that time, that failure rate was totally normal, even more so in a science faculty.

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

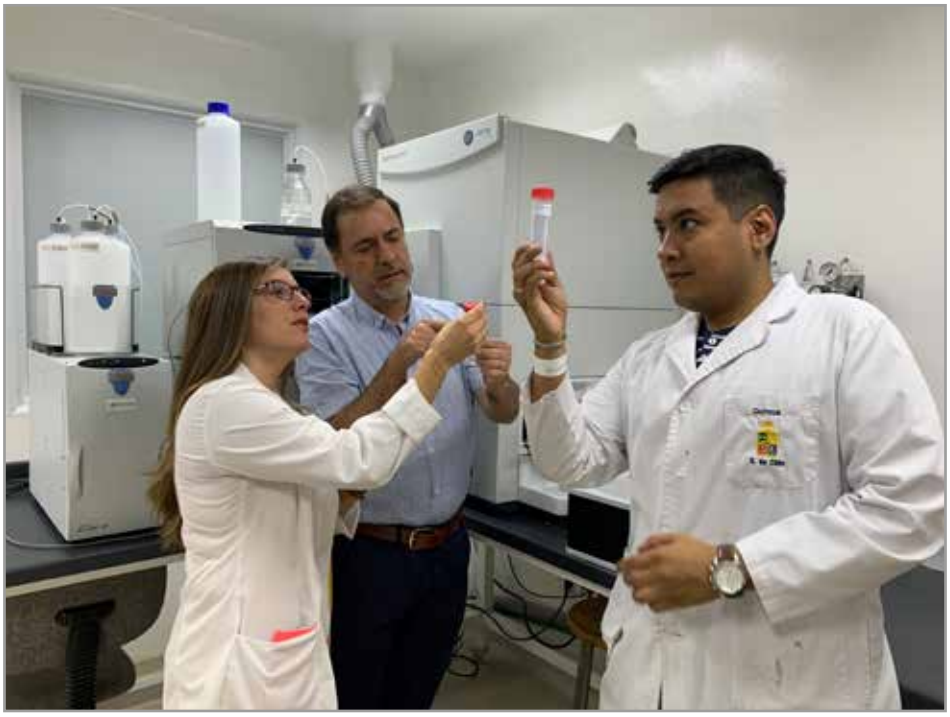
There are considerable differences between the students of the 1980s and those of today. Various aspects and changes have been very positive in this evolution; however, I also see some negative changes.

In general terms, people in different areas of life have rights and duties that should be in a fair balance. In my student days at the university everything turned in the sphere of obligations and duties and we were little concerned about our rights as students. If 80% of the students failed in a subject, the problem was always the fault of the students and nobody thought to question the teaching capacity of the teachers. Today this has changed, driven mainly by the processes of quality assurance and accreditation of careers and universities.

In this context, in my opinion, the balance that in the 80s was displaced towards the obligations or duties of students was reversed in the last decade and displaced towards rights. Although this transition seems totally fair, one realizes that on many occasions it goes to the other extreme. Students themselves must understand that balance is ideal and that duties are also essential in life.

What is it like to be a university professor? What advice would you give to a young scientist who wants to pursue a career in chemistry?

The academic work at the University is very diverse; a professor must do teaching, research, extension and university administration. It is different from working in a research institute, where that unique function is mainly carried out. I really like this variety of tasks and especially the combination of teaching and research that is the basis of the training of new researchers. I am very happy at the University and I consider myself privileged to do what I like. To young people who want to pursue a scientific career, I would say that they should do their work with all seriousness and rigor, and that they should have a holistic vision, since the best way to approach a scientific problem is from an interdisciplinary or transdisciplinary point of view. Furthermore, science today requires solving problems by working as a team.



From left to right: Dr. Valentina Manzo, Prof. Dr. Pablo Richter, Dr. Daniel Arismendi at the laboratory of ICP-MS.

What do you think is the importance of the scientific journals? Tell us a little about your work in this area.

The results of scientific research must be divulged. At this level, scientific journals are essential to show the advances in knowledge, but through rigorous peer-review processes.

In my opinion there are a number of aspects that should be analyzed and improved in the field of publications in scientific journals. Just to mention a couple of aspects: Firstly, the manuscript evaluation procedure, although not in crisis does not work fully satisfactorily at present. I perceive sometimes a lack of commitment to this system by many scientists; many researchers don't even respond to the email invitation to review. It should be a mandatory system; if a researcher submits papers to a journal to be published, they should be willing to evaluate a certain number of manuscripts per year. Some journals demand that commitment. Another relevant aspect related to scientific journals is the full access to manuscripts. I think that knowledge should be universally disclosed and, in this sense, the open access alternative is very attractive, but today its cost is very high. I think that all editorials should tend to open up to this option, understanding that financing is a key aspect that should be solved.

Tell us a little about the importance of the National Fund for Scientific and Technological Development (FONDECYT) and what is it like for you to be a member of it.

I can mention that FONDECYT is the program that distributes most of the resources for science in Chile. The main programs are: postdoctoral positions (3 years), initiation projects (3 years) and regular projects (4 years). A big problem occurring in Chile is that the budget for science, technology and innovation represents only 0.36% of GDP. This value urgently needs to be increased, primarily because there is a group of researchers of a very good scientific level who do not get funding to carry out their research proposals. This proportion has been relatively stable since 2011, which may be due to other areas being prioritized and the lack of clear strategies and policies. Fortunately, since approximately 1 year ago Chile has had a new Ministry of Science, Technology, Knowledge and Innovation. We hope that this new ministry will be endowed with human and material resources so that it can fulfill the expectations that have been generated in the scientific world.

I was for 3 years (2017–2019) a member of the Superior Council of Science of FONDECYT representing the Chemistry area. It was an honor to participate in this independent academic body, which nowadays was replaced in the structure of the new ministry.

How was it for you to be the Director of the environmental laboratories at the National Center for the Environment (CENMA) at the University of Chile?

The National Environment Center (CENMA) was a project agreed between the governments of Chile and Japan to create a center of reference in the environment. This project was delegated to the University of Chile for its execution and Prof. Eduardo Schalscha (a prestigious Chilean researcher in environmental chemistry) invited me to participate. I was in charge of the laboratories of this institution between 1997 and 2003. The project involved setting up a top-tier analytical environmental laboratory with equipment funded by the Japanese government and resources for the operation funded by the Chilean side.

"For me, being the Director of CENMA was a very important challenge because it involved setting up and then running a very complete analytical laboratory in all areas of …"

The Japanese side also provided short- and long-term experts from Japan. The research activities to be carried out in the CENMA project were defined in the document "Record of Discussion, RoD", agreed between both governments when creating the center. For me it was a very important challenge because it involved setting up and then running a very complete analytical laboratory in all areas of the environment and with the best equipment in the country, together with a team of carefully selected young chemists from the country. Undergraduate and postgraduate students also participated of the project. The investment in laboratory instruments was of the order of 6 million dollars. It was a very important period in my life, in which I had the opportunity to learn a lot, not only

about instrumentation and analytical techniques but also about laboratory management. We also had to learn from sampling campaigns and the analysis of air, water and hazardous waste samples. A central objective of the laboratories during the first years of operation (1998–1999) was the implementation of a quality assurance system and its accreditation according to ISO 17025 for more than 80 parameters in environmental matrices.

Could you comment briefly on the recent evolution of Analytical Chemistry, especially in your areas of activity, environment and food, considering your contributions?

Analytical chemistry has seen an important evolution during the last decades, not only in instrumentation but also in new strategies of sample preparation. By means of sample preparation, the analyte is extracted and preconcentrated from a sample to be measured by an instrumental technique practically free from matrix interferences. Since 1990 a sharp increase in the number of publications has been seen covering this area, describing different analytical strategies in different fields of analytical chemistry. Particularly important has been the introduction of solid phase microextraction (SPME) in 1989 by Pawliszyn et al. which produced an inflection point in the evolution of microextraction techniques and the advent of different strategies of sample preparation for organic analytes in different kind of samples such as environment, food and biological samples, making possible the quantification of analytes at concentrations at the level of ppt or ppq. Our contribution in this area has been related to microextraction technology based on Rotating-Disk Sorptive Extraction.

You have published many scientific papers. Would you highlight any?

Several papers have been very important to me during my academic life at the University of Chile. My first paper was in 1984 during the last year of my chemistry degree. Although my supervising professor, Dr. Alfonso Morales, wrote it, I felt that my work was contained there, in an international publication. I read it many times.

- **"Polarographic Behaviour and Determination of Nitrofurantoin Chloramphenicol and Related Compounds". Morales, A., Toral, M.I., and Richter, P., Analyst, 1984, 109, 633.**

Other important works were those carried out during my PhD program and during my postdoctoral positions. Some representative paper in this category are:

- **"Voltammetric Study of 7-Nitro-1,4-Benzodiazepin-2-ones and their acid Hydrolysis Products, 2-Amino-5-Nitrobenzophenones". Richter, P., Morales. A., and Lahsen, J., Analyst, 1990, 115, 409.**
- **"Integrated FIA/HPLC Method for Preconcentration and Determination of Transition Metal Ions". Richter, P., Fernandez, J.M., Luque de Castro, M.D. and Valcárcel, M., Chromatographia, 1992, 34, 445.**
- **"Immobilized Enzyme Reactors: Diffusion/Convection, Kinetics, and a Comparison of Column-Packed and Rotating Bioreactors for Use in Continuous-Flow Systems". Richter, P., López-Ruiz, B., Sánchez-Cabezudo, M., and Mottola, H., Analytical Chemistry, 1996, 68, 1701.**

I would also like to highlight these articles that cover different areas of analytical chemistry:

- **"Screening and Determination of Pesticides in Soil Using Continuous Subcritical Water Extraction and Gas Chromatography-Mass Spectrometry". Richter, P., Sepúlveda, B., Oliva, R., Calderón, K., Seguel, R., J. Chromatogr. A, 2003, 994, 169.**
- **"Total Element Concentration and Chemical Fractionation in Airborne Particulate Matter from Santiago, Chile". Richter, P., Griño, P., Ahumada, I., Giordano, A., Atmospheric Environment, 2007, 41, 6729.**
- **"Flow injection photometric determination of zinc and copper with Zincon based on the variation of the stability of the complexes with pH". Richter, P., Toral, M.I., Fuenzalida, E., Tapia, A.E., Analyst, 1997, 122, 1045.**

And finally, I must highlight the first work in which we introduced the RDSE technique, given that it opened up a very solid research line with international recognition.

- **"Rotating-Disk Sorptive Extraction of Nonylphenol from Water Samples". Richter, P., Leiva, C., Choque, C., Giordano, A., Sepúlveda, B., J. Chromatogr. A, 2009, 1216, 8598.**

How was the process for creating the sample preparation technique "Rotating-Disk Sorptive Extraction" (RDSE)?

Regarding this process there are two important points to highlight. In 1996 I did a postdoc at Oklahoma State University, with Prof. Horacio Mottola. The aim of this work was to demonstrate the greater efficiency of rotating bioreactors compared to microcolumn packed reactors in continuous-flow systems. The rotating-disk reactors afforded a significantly more efficient utilization of immobilized active sites of the enzyme and permitted the effective use of very small amounts of biocatalysts. On the other hand, by studying the Stir Bar Sorptive Extraction (SBSE) technology and focusing on the drawback of the twister device, I realized that a disk-shaped device, where the sorbent phase can be fixed on one of its surfaces, solves many of the deficiencies of SBSE. The extraction device used in RDSE uses an extraction phase with a high surface-area-to-volume ratio and can be stirred at much higher velocities than the twister used in SBSE without damaging the phase because the extraction phase is in contact only with the liquid sample. Thus, higher rotating velocities facilitate analyte mass transfer to the sorptive surface. With this in mind, we got to work with my students until RDSE was a reality. In addition, the costs involved in RDSE are significantly decreased because the device can be reused many times by changing the phase according to the nature of the analytes and samples.

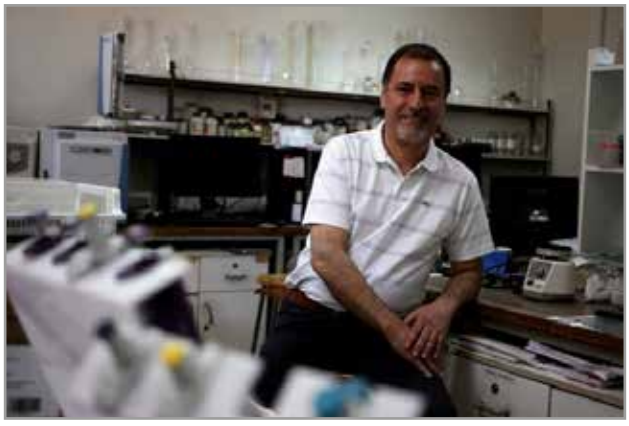
"A scientist of my age has lived through a period of great changes and evolution in analytical chemistry. Among many developments I would highlight two relevant features: ..."

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

A scientist of my age has lived through a period of great changes and evolution in analytical chemistry. Among many developments I would highlight two relevant features: on the one hand, the evolution of mass spectrometry has been very important and has been marked by an ever-increasing number of its applications in science and technology in all areas of analytical chemistry (inorganic, organic, biochemical) and its successful applications to a variety of analytical problems; on the other hand, the development of sensors is another relevant topic in analytical chemistry that is in full development. Although this topic still needs much development ahead of its robust application in real samples, I highlight it because in the future it will allow us to minimize and simplify the sample preparation stages so that the measurements are increasingly direct and fast.

For what would you like to be remembered?

In general, I would like to be remembered for being a good person, whether by those close to me or not. This means that the merits are valued more than the defects. In the case of science and the university sphere, as a scientist who contributed honestly to the formation of many professionals and scientists.



Prof. Dr. Pablo Richter at the laboratory of Environmental Chemistry at the Faculty.

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POINT OF VIEW

Toward Fast and Simple yet Reliable Alternative Arsenic Speciation Methods

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Arsenic (As) can occur in many chemical forms, from harmless to toxic compounds. Its toxicity depends on valence and the chemical environment. Small amounts of toxic forms can even have therapeutic and fortifying effects. The use of As was practiced for hundreds of years, leading to accidental or deliberate poisoning. Waters with high As concentrations (up to 5000 µg/L) adversely affect the drinking water supply of about 200 million people worldwide mainly in Argentina, Chile, Mexico, China, West Bengal (India), Bangladesh, Vietnam and Hungary [1]. In Hungary, the As exposure of the population has been significantly reduced since 2017 by drilling new wells with lower As content and setting up new waterworks in the settlements affected, with financial help from the European Commission. Arsenic possesses a complex water chemistry and occurs in several inorganic and organic species in water depending on pH, salinity, acid dissociation constants of its oxyacids, and the As(V)/As(III) redox potential. The possible technological solution for As removal from water is definitely governed by the species concerned. Chemical oxidation, co-precipitation, adsorption, ion exchange, reverse osmosis and membrane filtration are used to remove As from water. From the technological point of view, As removal processes can be divided into three major groups: i) conventional technologies (coagulation, iron–manganese removal, lime softening); ii) sorption processes (ion exchange, activated aluminum); and iii) membrane technologies (reverse osmosis, nano-, micro- or ultrafiltration). Each of the aforementioned technologies is more efficient for As(V). Therefore, an oxidation step is often needed. Oxidation by simple direct aeration is slow, but there are a number of chemicals that can accelerate the process, such as chlorine gas, sodium hypochlorite, ozone, potassium permanganate, hydrogen peroxide and manganese oxides, and ultraviolet radiation may also be suitable for oxidizing As(III) [2].

Given the lack of a definitive solution for As removal from drinking water, it is important to estimate the exposure of the population in large areas affected by As contamination. For estimation of the As(III)/As(V) ratios, conventional As speciation analysis generally consists of on-line hyphenation of a chromatographic separation technique to an atomic spectrometric detector. Replacement of either the high-performance liquid chromatograph or the atomic spectrometer (e.g. inductively coupled plasma mass spectrometer) – or both – may lead to cost-effective solutions enabling extension of our knowledge with respect to As speciation. Besides its cost-effectiveness, the advantage of the use of solid phase extraction (SPE) microcartridges filled with ion-exchange resins consists of preventing interconversion of As species during sampling and analysis. Recently, significant efforts in this direction can be seen, mainly in the As speciation of water matrixes. Various SPE cartridges enabling on-site separation have been investigated for the speciation of inorganic As(III), As(V) and methylated forms of As(V) [3–5]. In-situ separation of As species in water with a strong anion exchange SPE can easily be accomplished from water matrixes of pH < 9 and/or of higher salinity and temperature (e.g., geothermal water) by applying preconcentration of iAs(V) followed by laboratory analysis of acid-preserved samples [6]. Mono-elemental graphite furnace atomic absorption-based techniques can be recommended for routine measurements. Among multi-elemental techniques,

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total-reflection X-ray spectrometry allows the simultaneous determination of several other trace elements in drinking water [7]. Some new solid-phase sorbents employed for separation and preconcentration of As include nanometer-sized titanium dioxide particles immobilized on silica gel, octadecyl silica, yeast immobilized on controlled-pore glass, PTFE turnings, cetyltrimethylammonium bromide-modified alkyl silica, [3-(2-aminoethylamino)propyl]trimethoxysilane-modified mesoporous silica, macrocyclic materials, hybrid resins based on the activity of hydrated iron oxides and silver chloride, carbon nanofibers and nanotubes, and eggshell membrane [6].

In brief, the pace of development of analytical measurement techniques is extremely rapid, and advances in speciation analysis are also helped by the knowledge acquired in many other scientific fields. In addition to the hyphenation of the chromatographic separation and atomic spectrometric techniques, speciation analysis procedures include a number of sample preparation procedures that can be separate sources of error. These may have already occurred at the time of sampling in the form of unwanted species interconversion, and the interference of the sample matrix constituents may also be significant. Therefore, it would be useful to develop alternative, cost-effective yet reliable analytical methods extensible to as many As species in different matrixes as possible, which could be widely used in routine analysis.

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LETTER

Polymorphic Forms of Chocolate: Application of Solid-State Characterization in the Food Industry

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Background

Polymorphism is very well discussed and consolidated in the pharmaceutical industry related to the impact on formulations and manufacture process; however, it is also an extremely important topic for the field of the chemical food industry [1-5].

Analytical characterization techniques, including X-ray diffraction, differential scanning calorimetry, thermogravimetric analysis, confocal Raman microscopy, laser diffraction, and spectroscopic approaches for structural characterization of food products are powerful tools [1-5].

Chocolate is one of the best-selling products in the food industry worldwide [6-8]**.** The type of chocolate manufacturing process used will interfere with consumers' perception and taste [9]. The perception of chocolate palate is directly related to the crystallographic structure, particle size range (0.01 mm to 0.1 mm), geometry and spatial arrangement [9]. Cocoa butter (CB), a key ingredient in chocolate, has six polymorphs characterized, that is, the same molecule with other crystallographic arrangements, thus influencing the physicochemical properties in chocolate, such as melting, strength, shine, texture and flavor [9]. Several studies are being conducted to elucidate and improve knowledge related to the complexity of CB polymorphism [9]. There are many studies published in the literature on the impact of molecular structure and lipid polymorphism on macroscopic aspects; however, they did not consider the microstructure of the network [10].

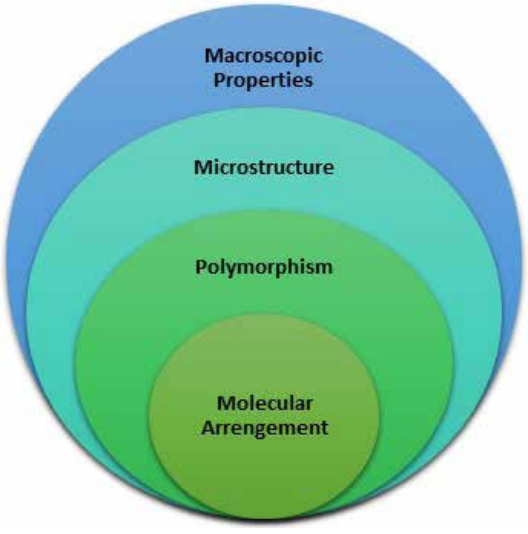

Figure 1. Schematic representation of the influence of the crystallographic profile of fats on macroscopic attributes [10].

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The chemical profile of CB can be affected based on the refinement process and the type of source used, thus being able to influence the crystallization rate and stability. Regarding the microstructural aspect, the polymorphism of cocoa butter affects the shape, size and melting point of the crystal. The alpha (α), beta prime (β′) and beta (β) polymorphs were determined by crystallographic and thermal analysis being classified in increasing order of stability, respectively. These three polymorphs differ in physical properties, but mainly with respect to the melting point [10].

- The β polymorph, which is the most stable, has a triclinic crystal system with a typical short spacing pattern of 4.6 Å. The β′ form is an orthorhombic structure with 3.8 and 4.2 Å [10].
- The α form, which is the less stable, is a hexagonal arrangement with 4.15 Å. In some studies, other metastable forms, called γ and δ, and β′ have been found [10].
- Polymorph γ, like polymorph β′, is an orthorhombic perpendicular system with short spacing characteristics of 3.8 and 4.2 Å [10].

Approaching and understanding the mechanisms of polymorphic transitions of natural fats is not a simple task due to the complexity of the sample matrix. An example would be CB, in which six polymorphic forms were elucidated, however, the nomenclature of these forms is not harmonized [10].

Chocolate companies describe polymorphic forms as I-VI, in increasing order of stability. Margarine and fat companies use the nomenclature γ (or sub-α), α, β′2, β′1, β2 and β1, in increasing order of stability, as well [10].

Regarding the taste, texture and brightness, among the abovementioned polymorphs, it was proven that form V presents greater efficiency and quality in chocolates, in addition to melting in the mouth at the temperature of the human organism [9]. Tables I and II show some of the properties of CB polymorphs.

Table I. Melting point and preparation of cocoa butter polymorphs according to the nomenclature of chocolate companies [11]

Polymorphic Forms	Melting Point	Preparation
Form I	17,3 °C	Melted cocoa butter solidified at 0 °C and lower temperatures.
Form II	23,3 °C	Melted cocoa butter quick-frozen and stored for several minutes to one hour at 0 °C.
Form III	25,5 °C	Solidification of the melt at 5 to 10 °C or by transition of Form II by storage at 5 to 10 °C.
Form IV	27,5 °C	Solidification of the melt at 16 to 21 °C or by transition of a lower melting state by storage at 16 to 21 °C.
Form V	33,8 °C	Direct solidification of the melt, by transition of lower melting products and by crystallization from solvents.
Form VI	36,3 °C	It was never obtained directly, but only by transition phase of Form V.

Table II. Melting point of cocoa butter polymorphs according to the nomenclature of margarine and fat companies [10]

Polymorphic Forms	γ (sub-α)	α	β′2	β′1	β2	β1
Melting Point (°C)	-5 – +5	17 – 22	20 – 27	20 – 27	29 – 34	29 – 34

SUMMARY

Solid state characterization is fundamental for developing food products enabling better knowledge regarding formulation performance, processability, stability and functionality; promoting quality and safety to costumer, to meet regulatory standards.

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REVIEW

Key Information Related to Quality by Design (QbD) Applications in Analytical Methods Development

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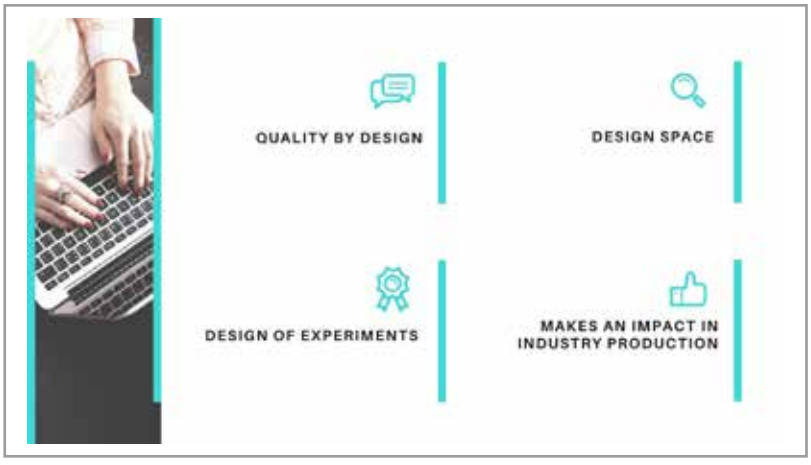

In this review, quality by design (QbD) initiatives are described as applications for many types of processes, such as in the pharmaceutical industry, biotechnology field, and for analytical methods development. Design space (DS) and design of experiments (DoE) provide useful results for analytical methods development and simulation of advanced processes in traditional manufacturing relationships. The three topics, QbD, DS and DoE are the best way to achieve strong and efficient industrial production.

Keywords: chemometrics, quality by design, control strategy, design of experiments, analytical chemistry in industrial applications

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List of Selected Acronyms

Analytical quality by design	AQbD	Plackett-Burman	PB
Box-Behnken design	BBD	Principal component analysis	PCA
Capillary electrophoresis	CE	Process analytical technology	PAT
Central composite design	CCD	Quality by design	QbD
Coefficient of determination	R^2	Quality by testing	QbT
Critical method attributes	CMAs	Quality control	QC
Critical quality attributes	CQAs	Quality target product profiles	QTPPs
Design of experiments	DoE	Response surface methodology	RSM
Design space or Design spaces	DS or DSs	Risk assessment	RA
Fractional factorial design	FFD	United States Food and Drug Administration	US FDA
Method operational design region	MODR	United States Pharmacopeia	USP

INTRODUCTION

Quality by design (QbD) approach has been extensively applied to pharmaceutical products development as can be observed by many applications in industry quality control, and research and development (R&D) laboratories. When compared with quality by testing (QbT), QbD improves the understanding of processes and products with predefined goals based on statistical, mathematical, chemistry and quality risk management. There are four steps related to QbD processes:

1. Analytical target profile (ATP), which includes the purpose of an analytical method and its required performance criteria (critical quality attributes - CQAs);
2. Risk assessment. This step is related to sample preparation for further analytes determination, focuses on data analysis. Then, further potential variables as noise variables can be identified so, they are evaluated by measurement system analysis approaches, and instrumental parameters, which is assessed by design of experiment (DoE) strategies (a critical part);
3. From DoE results, the design space (DS) is obtained. DS shows the analytical chemistry conditions where an analytical method or production procedure can work without compromise the final result. This component is the establishment of reliable methods intended to be used in analytical chemistry laboratories;
4. Control strategy and validation are conducted *via* observation of several analytical figures of merit, such as accuracy and precision expressed as relative standard deviation (RSD) and coefficient of determination (R^2), among others [1-4].

Pharmaceutical laboratories and companies have employed QbD to reach the excellence of their products. Drug efficacy and safety are key criteria to ensure pharmaceutical product quality. Thus, QbD initiatives are able to analyze factors that can compromise the stability of a drug. For example, Michels and coworkers [5] applied the QbD framework to develop and validate a capillary electrophoresis-sodium dodecyl sulfate assay using a laser-induced fluorescence detector for monitoring impurities from the manufacturing process of therapeutic monoclonal antibodies to ensure patient safety and product efficacy.

Since then, QbD was introduced to chemical manufacturing control (CMC), and this approach has gained popularity in the pharmaceutical manufacturing sector [6]. As mentioned before, QbD has been

extensively useful in the pharmaceutical industry to understand drug quality parameters. Excipients, for example, are critical to drug performance, requiring a thorough interpretation of their chemical properties, composition and control of their variability to ensure high product quality.

Direct compression is an important attribute in the manufacturing of new tablets, and thus, it is necessary to know the properties of raw materials, as excipients, to ensure the effectiveness of large batch production. In addition, some properties of these materials are closely correlated, presenting interaction effects, so a multivariate analysis is mandatory to identify and relate these attributes to excipient performance [7].

An important mechanism that take several advantages of QbD is process analytical technology (PAT). Actually, PAT and QbD are aligned. PAT can be defined as a series of analytical control procedures and the manufacturing of new materials that are focused on the quality parameters of a target product. In the chemical industry, PAT concept has been used for some decades, in which process analyzers such as near infrared spectroscopy (NIRS) and nuclear magnetic resonance (NMR) are used to optimize productivity, as well as quality. The data generated by these analytical instrumental techniques are modeled by statistical and mathematical tools, such as principal component analysis (PCA) and partial least squares (PLS). Thus, due to these innovations of analytical instruments and the ability to model the data generated by these detection methods, PAT has been increasingly adopted by the pharmaceutical industry to assist in improving and modernizing the manufacturing of new drugs.

Nowadays, the implementation of PAT in the field of biotechnology has received special attention due to the complexities of the raw materials used in products development [8].

Covering all aspects described in the previous paragraphs, we must mention the Chemometric tools. Chemometrics is a science dedicated to perform a better visualization and interpretation from chemical data. This science has been barely used for optimization in electroanalytical applications when comparing to spectroscopy and chromatography methods [9,10].

This review is intended to present some applications of QbD in pharmaceutical field as also, other promising areas. Chemometrics is “spread” in all text, turning QbD a powerful tool for variables optimization of chromatographic analytical methods allowing that experimental conditions achieve successful separation, identification and quantification of the target species.

DoE can be used for several applications depending on issue to be solved. In a review paper, Hibbert [11] described the use of several DoE processes applied to chromatographic separation and highlighted the most used in optimization and validation studies such as: (1) Factorial design [12-14]; (2) Plackett-Burman (PB) design [15-17]; (3) Central Composite design (CCD) [18-20]; (4) Box–Behnken design (BBD) [21-23]; (5) Doehlert design (DD) [24-26] and (6) Mixture design [27-29]. Other types of DoE have been used and often cited in the literature, as examples Mixed-level fractional factorial design [30-32]; Definitive Screening design (DSD) [33-35] and D-optimal design [36-38].

The interest in QbD by industry and laboratories is increasing. Figure 1 shows the number of publications related to this topic since 1977 using Web of Science database. From 1977 to 2006, only 23 papers were published. It is possible to see the ever-increasing number of publications on this topic which in 2018 and 2019 more than 200 articles were published.

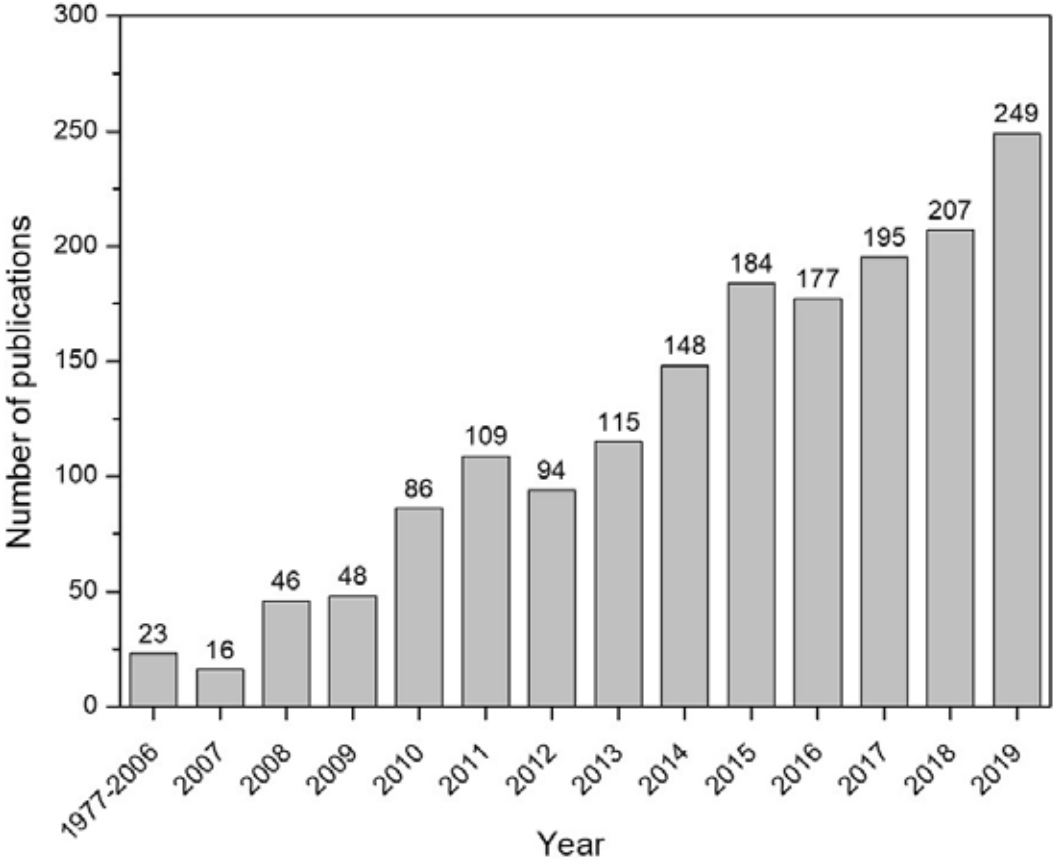

Figure 1. Publications related to quality by design (QbD) from 1977 to 2019.

The dataset used in this research was downloaded from the online database Science Citation Index Expanded (SCI-EXPANDED), and all documents types were considered. These results were exported from the Web of Science using 500 records at a time, file format in full record and cited references as well as tab-delimited (Win) for the file format. A bibliometric map for occurrence analysis of author keywords was obtained using the VOSviewer software (version 1.6.15; www.vosviewer.com). Figure 2 shows the top 50 author keywords with the highest co-occurrence among the 1,607 articles addressed to the keywords “analytical quality by design” OR “quality by design” on July 27, 2020. This bibliometric map shows a set of links connecting different research topics (i.e. clusters indicated in different colors) and how QbD can be associated with other important topics, such as DS, DoE, and PAT. Details explaining how this bibliometric analysis is prepared can be found in the following Youtube Playlist: https://www.youtube.com/playlist?list=PL4CuftF4l_fCc9t1xoQSaYOUq-UBz_2VD

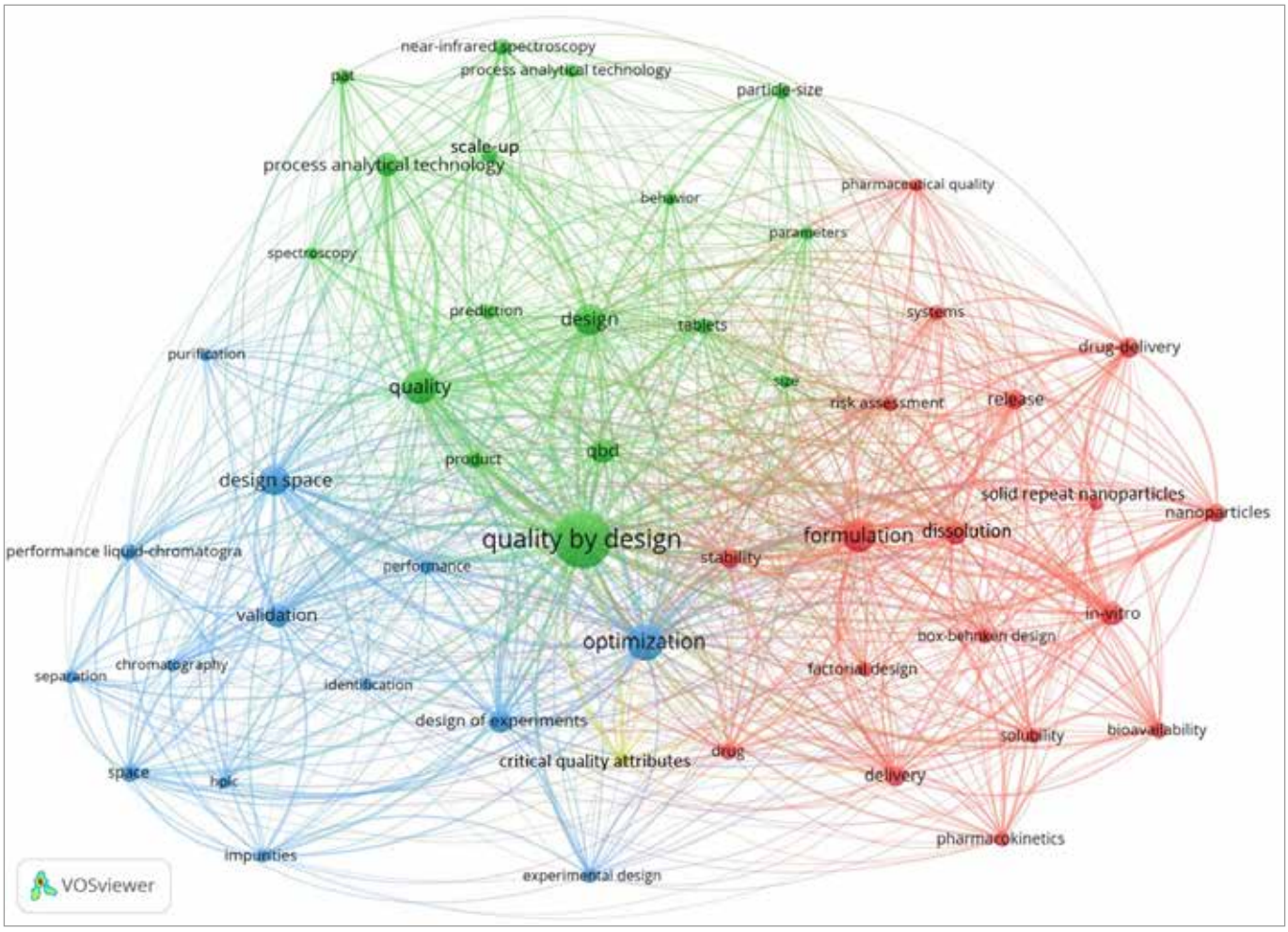

Figure 2. Bibliometric map for quality by design (QbD) relations with main important topics. Keywords used: “analytical quality by design” OR “quality by design”. Number of words: 50 and more details about the bibliometric analysis can be found in the following Youtube Channel: https://www.youtube.com/c/EdenirPereiraFilho

This review aims to introduce the reader to this important topic and mainly, show how the scientific authors applied these concepts. The text addresses three complementary parts: QbD, DS and DoE and, Table I emphasizes recent applications.

Quality by design (QbD), design of space and design of experiments applications

In 2018, Dispas et al. [39] discussed the importance of QbD strategy for analyzing drug impurities. They mentioned that QbD facilitates quality risk management and provides ways to identify and control potential quality issues during both product development and manufacturing. It was also mentioned that one of the key challenges during the implementation of QbD strategies is the development of appropriate analytical methods to achieve the intended goals.

In 2017, a CE-based limit test for levomethorphan was developed. Critical method parameters were determined throughout scouting experiments and screened by FFD to establish the CQAs, which were optimized using Central composite face-centered design to define the method DS. The validation process was performed according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline Q2 (R1) [40].

A rapid colorimetric microplate bioassay for the estimation of bacitracin was proposed using QbD

approach. For the optimization of the method, factorial design and response surface methodologies (RSM) were performed, and DS was established to ensure the method reproducibility [41].

Hashem and El-Sayed [42] proposed a new RP-HPLC method for the simultaneous determination of levetiracetam and pyridoxine HCl in prepared tablets using QbD. Screening of four independent variables: pH of the mobile phase, flow rate, injection volume and percentage of the organic modifier at two levels, and optimization of the chromatographic variables were performed by FFD and CCD, respectively.

Tol et al. [43] developed a selective and robust HPLC method for separation of abacavir, lamivudine and dolutegravir (anti-retroviral formulation) in a drug product. QbD principles combined with a DoE were proposed to establish the relationship between critical response and tested variables.

QbD strategy was used by Mohamed et al. [44] to develop a new method combining vortex-aided salting-out-assisted liquid-liquid microextraction with a core-shell HPLC method establishing an efficient, sensitive and simultaneous determination of dorzolamide hydrochloride (DOR) and timolol maleate (TIM) in rabbit plasma with high analytical frequency. Screening of the knowledge space was performed with a PB design, and a BBD was applied for RSM.

Kozaki et al. [45] also studied the application of QbD to pharmaceutical development for analyzing critical process parameters (CPPs) of poly (lactic-co-glycolic acid) nanoparticle formulations encapsulating triamcinolone acetonide. CPPs were assessed by FFD and CCD, and then the results were visualized using RSM to identify DS.

In 2009, an overview of key developments and a roadmap regarding QbD principles for therapeutic biotechnology products was reported [46].

Terzić et al. [47] developed a specific and robust hydrophilic interaction liquid chromatography (HILIC) method for the determination of bilastine and its degradation impurities with high analytical frequency following analytical QbD (AQbD) strategy. Through the use of BBD, a relationship between the CPPs and the CQAs was established, and further regression models and Monte Carlo simulations were used to identify the DS. Robustness testing was performed using FFD. In another study proposed by Kasagic-Vujanović and Jancic-Stojanovic, the HILIC system was used for the determination of amitriptyline and its impurities as following substances, dibenzosuberone, cyclobenzaprine, nortriptyline and (5EZ,10RS)–5–[3-(dimethylamino)propylidene-10,11-dihydro-5H-dibenzo[a,d][7]annulen–10–ol, and the same statistical treatment was applied [48].

Through QbD, Sylvester et al. [49] developed an HPLC method for the simultaneous determination of curcuminoids and doxorubicin from long-circulating liposomes. The authors established a MODR by means of a DoE and RSM. Within a linear range from 2 to 20 µg/ml, and trueness between 97 to 104% were successfully achieved.

Sankar et al. [50] used QbD for determination of enzalutamide (ENZ), an atypical anticancer drug, in a drug formulation and in spiked plasma samples using UHPLC-electrospray ionization-tandem mass spectrometry (UHPLC-ESI-MS/MS). A DoE and CCD were used to screen and extensively evaluate the critical process attributes (CPAs), which can influence analytical parameters.

Another study using the QbD for method development of the estimation of quercetin dihydrate by HPLC was proposed by Sandhu et al. [51] FFD was used to screen the critical analytical attributes (CAAs), including the organic modifier, injection volume, column temperature and buffer strength. After that, the optimal conditions were determined through BBD and RSM, respectively.

Green analytical chemistry principles and QbD were applied to develop and validate the determination of fenoverine (FEN) in bulk and dosage form. In this study, a two-level FFD (2^{7-3}) was applied to screening and identifying the influential chromatography method variables. Furthermore, the BBD was used to optimize the most important variables that actively affect the CAAs [52].

Mukthinuthalapati et al. [53] simultaneously determined antidiabetic drugs in their combined dosage forms employing HPLC. The effects of the variables, such as mobile phase composition, buffer strength, and flow rate were optimized using a BBD. Thus, the DoE and QbD allowed the separation of five analytes: metformin, pioglitazone, glibenclamide, glimepiride and repaglinide.

In addition, Awotwe-Otoo et al. [54] used QbD to optimize a method based on the separation of hydrolyzed protamine sulfate peptides from a robust RP-HPLC method. Two experimental designs were used: (i) a PB design to evaluate main effect of the different variables, such as mobile phase pH, flow rate of the mobile phase, injection volume, methanol concentration and column temperature, on peak resolution; and (ii) a three-level BBD with three center points to evaluate the principal, quadratic and interaction effects considering the column temperature, flow rate (on peak resolution), USP (United States Pharmacopeia) tailing and mobile phase pH (on the response), reducing the required number of experiments after optimization.

Dobricic et al. [55] used RP-HPLC for the simultaneous determination of telmisartan and impurities using QbD. Variables including the acetonitrile (ACN) content in the first (ACN 1) and second (ACN 2) gradient steps were evaluated. In addition, time (t_2), the second gradient step and their combination affected the separation of telmisartan and its impurities.

QbD was also applied in bioanalytical method development for olmesartan medoxomil (OLM) determination in rat plasma using ultra-performance liquid chromatography (UPLC). For instance, Beg et al. [56] performed a 2^{5-2} FFD as a screening method to identify important variables and, posteriorly, to optimize them (mobile phase ratio and injection volume) using a CCD at three levels. BBD was also used for optimization of the extraction process conditions.

With the help of QbD concept, Sharma et al. [57] used a full factorial DoE 3^3 to optimize the conditions of analyte separation for the determination of bambuterol. In another paper, Schmidt et al. [58] developed a method using DoE for the determination of impurities in the active pharmaceutical ingredient carbamazepine.

QbD was also applied to optimize lipid-polymer nanoparticles from a DoE at three levels of two critical independent variables, the lipidoid 5, L_5 content and the L5:ASO ratio, for customizing luciferase gene transcription of antisense oligonucleotides (Luc-ASO) for delivery in HeLa pLuc/705 cells containing an aberrant luciferase gene [59].

Bade et al. [60] applied QbD using a DoE for optimization of the refolding process for a recombinant, biotech, therapeutic, granulocyte colony-stimulating factor. Risk analysis was performed to identify variables that required more attention. Afterwards, the chosen parameters were evaluated using an FFD.

QbD and PAT were used to investigate particle characterization during in-line, high-shear, wet granulation (HSWG) using focused beam reflectance measurement (FBRM). The optimizations were conducted from a DoE with nine scale-up levels developed in batches and eight clinical sublots using an FBRM probe to assess the variables and improve the process of development of this drug product [61].

A study using the QbD was applied in the production of a pharmaceutical gel. The quality target product profiles (QTPPs) were obtained from reference values of the previously batches. The critical variables of the process (viscosity and pH) were optimized using a DoE that comprised 13 gel with triplicates at the center point. Afterwards, the best synergism values from the optimization of the QTPPs were defined using a desirability function [62].

Pasquini et al. [63] used a DoE to optimize the experimental parameters and variables from an electrokinetic method following QbD. From the optimization, a DS was defined, and the developed method was dedicated to quantify three antimigraine drugs in different pharmaceutical products available on the market.

Design of Experiments (DoE) is a statistical technique for screening of factors, planning, conducting, analyzing, optimizing the conditions of the system to be used and interpreting data from experiments. For QbD, the use of DoE includes advantages as not requiring detailed knowledge of the system, definition of the number of experiments to be performed, and data modelling for empirical functions that are generally linear or quadratic [11,64].

QbD concepts are normally used to analytical method development. Its use is increasing based on predefined requirements for an analytical method stated in the ATP. From this perspective, a DoE is mandatory to visualize the influence of the variables and its interactions on responses. For variable screening studies, the two-level full factorial design is the most widely used. Afterwards, variable screening

and multilevel designs are generally considered for the efficient exploration of response functions from empirical models. The critical conditions of factors as maximum or minimum values are determined by RSM. Among these, the most used are the CCD, BBD, and DD [11,65,66].

Ferreira et al. [10] reported an overview of most common factorial design methods for the optimization of sample preparation procedures and experimental conditions of analytical instrumental techniques dedicated to determination of organic and inorganic analytes in food. According to the authors, CCD is the most commonly used method, although it has a lower efficiency than DD or BBD. They also conclude that there is a high frequent use of chemometric tools for the optimization of analytical methods.

For instance, a DoE and RSM were used to optimize the process of biodiesel production using different types of catalysts (homogeneous and heterogeneous and acid) [67].

Wu et al. [68] proposed the Generalized Multiplicative ANOVA (GEMANOVA) model as an alternative to ANOVA (analysis of variance) for improving the interpretation and understanding of complex data sets obtained from monitoring the physical stability of a solid dispersion with X-ray powder diffraction (XRPD). The results obtained by the GEMANOVA model were easier to interpret and understand than those obtained from the ANOVA model, and a DS was established according to the QbD. Table I shows some selected papers and its remarks about QbD application in Pharmaceutical industry, Industrial purposes and Optimization using DoE.

Table I. Selected papers of applying QbD principles to common scientific fields

Field	Remark	References
Pharmaceutical industry	• Chromatographic methods for separation, identification and determination • Starting materials and reagents evaluation in manufacturing • Identification of unexpected impurities • Granulation and tableting processes • Dosage form of a solid inhaled drug	[69-86]
Industrial purposes	• Development and optimization a chitosan film formulation • Identification of stable and robust microemulsions • Evaluation of the kinetics of a powder mixing process • QbD to assess the subfractions of lipoprotein	[87-95]
Optimization tools using DoE	• Nanoprecipitation (NPR) and nanospray drying (NSD) application • Optimization of cell culture parameters • Determination, development and improvement of analytical methods • Selection of relevant variables in a system	[96-108]

DoEs have been used in these biotechnological processes for some years, as reported by Mandenius et al. [109] in a review paper, in which DoEs are a powerful tool for the optimization of bioprocesses.

QbD has been shown as a relevant scientific tool for developing efficient and safe medicinal products, as well as for reducing costs in the development of these products in health care industries [110].

DoE and RSM are critical tools for QbD implementation. Therefore, to comply with the established guidelines (ICH Q1, Q2 and Q3), studies have reported the importance of these tools to achieve successful results regarding the implementation of QbD. Bezerra et al. [111] outlined a tutorial review about simplex optimization. This particular approach does not need complex mathematical and statistical tools to be performed and, consequently can be easily implemented. Another important study, from Ferreira et al. [112]

is a review about the most known experimental designs, such as the BBD, DD, and the CCD. In addition, a tutorial entitled “Application of free computational program in experimental design: a tutorial” [113] makes DoE, one of the tools discussed in this review, accessible for any analyst or industry professional.

A fast HPLC screening method for the separation of eight antidiabetic compounds: pioglitazone (PZ), rosiglitazone (RZ), glyburide (GB), glimepiride (GM), gliquidone (GQ), gliclazide (GL), glipizide (GP), and repaglinide (RG), was proposed by Mokhtar et al. [114]. DS was developed using an *in silico* simulation of practical robustness testing procedures. The DS was calculated using a full two-level DoE for three different stationary phases varying buffer pH, gradient elution parameters, and ternary solvent ratio. Furthermore, the method offered a fast separation (less than 6 min) of the analytes that achieved the required levels of the CQAs.

Following the QbD principles, Schmidt et al. [115] defined a DS for a chromatographic method for the purity testing of pramipexole. Influential separation parameters were identified and assessed experimentally in a DoE (3^6= 729 experiments were performed *in silico*).

A DS was used by Arai et al. [116] in the granulation process of mefenamic acid tablets. Bootstrap resampling technique was used to evaluate robustness of the procedure. This procedure is used when predictions estimated by the quadratic polynomial model present unsatisfactory results in complex nonlinear problems. Thus, the Bootstrap resampling method is applicable to verify the accuracy of a nonlinear response surface.

DS and QbD concepts were implemented to evaluate the CPPs in ICH, Q8 R2, Q9 and Q10 for analytical method development and optimization of three chiral compounds. These compounds were developed as modulators of small conductance calcium-activated potassium (SK) channels using HPLC [117]. A DoE-DS and CCD were used to investigate the effects of trifluoroacetic acid (TFA) and n-hexane concentration in an acetonitrile mobile phase for enantiomeric separation.

In the pharmaceutical field, the DS and DoE concepts are being increasingly used to optimize analytical methods. For instance, Jambo et al. [118] evaluated the use of mass spectrometric detection supercritical fluid chromatography (SFC-MS) to identify adulteration in medicinal cannabis plants. In order to optimize this method, DoE and DS approaches were applied. Kurmi et al. [119] also used a DoE to optimize the forced degradation conditions of furosemide acid.

In the study by Maeda et al. [120], QbD was used to build a more reliable large-scale D. The experimental model system selected to construct the DS was the lubrication process for theophylline tablet manufacturing.

Mokhtar et al. [121] proposed a method to ensure more confidence in the produced DS regarding the robustness in compliance with ICH Q2 (R1). In this sense, a new DS calculation was developed that was compared to the conventional calculation. For this, a HPLC instrument was used and, with the assistance of a DoE, this DS mapped by the *in silico* robustness simulation provided more compliance to ICH Q2 (R1) than the previous alternatives.

A quantitative structure-retention relationship-design-of-experiment (QSRR-DoE) protocol to propose an optimal DS in a QbD procedure to improve HILIC method development in the separation of pharmaceutical targets was proposed by Taraji et al. [122]. A CCD for three selected chromatographic variables: acetonitrile concentration, mobile-phase pH and salt concentration, was used to model the retention times of a mixture of pharmaceutical analytes in HILIC. A QSRR model was generated and used to predict the retention time and, consequently, the selectivity variable between new target analytes.

Chhatre et al. [123] provided an important discussion about the combination of QbD and biochemical engineering to enable an efficient bioprocess development and manufacturing. The highlights of the main steps of QbD: risk assessment, statistical experimental design, scale-down techniques, rapid assays and graphical representation methods.

A study using QbD and a DS to develop a bioassay for the relative potency of linezolid in pharmaceutical samples was proposed by Saviano et al. [124]. DoE and CCD were applied to evaluate the influence of inoculum concentration and triphenyltetrazolium chloride on microbial growth.

In 2017, Kormány et al. [125] proposed a generic workflow to compare the resolution of a pharmacopeia impurity profiling method in a large DS of 3 measured and 3 calculated variables. A modern LC modeling software (Drylab) was used for comparing critical variables in relation of different parts of the DS. The results showed that it is possible to interchange two columns and map the retention behavior of the compounds of interest in a small number of steps in an experimental study.

Sun et al. [126] combined a computer simulation program and a design of experiments statistical software to develop a multicomponent analytical determination method using HPLC, combining two detectors, a diode-array detector (DAD) and an evaporative light scattering detector (ELSD), connected in series. DoE was applied to in the workflow steps to minimize experimental risk variables and provide an efficient DS method.

Nadella et al. [127] used a DoE by applying QbD to identify a DS for determination of teriflunomide by a UPLC method in the presence of degradation products. A CCD was performed to evaluate the variables: resolution, retention time and peak tailing. The proposed method presented high analytical frequency.

CONCLUSION AND PERSPECTIVE

This review shows that traditional manufacturing relationships have been changing by QbD. These methods are part of analytical quality improvements and mainly, aid to save energy and time. In addition, most methods described here are related to pharmaceutical purposes, but it was possible to verify the first steps in other areas. It is worthy to reinforce the importance of the design tools (QbD, DS and DoE) and that they would be able to enhance other industrial processes. From the practical point of view, there is enough room for other applications in different fields of chemical industry.

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ARTICLE

Effects of UV Radiation on Paper: A Chromatic Study

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A study on Whatman#1, bond, rice, kraft, and amate papers in their original condition and exposed to 2.25, 4.5, 6.75, and 9 W of Ultraviolet-C radiation is presented. The techniques used were scanning electron microscopy + energy dispersive X-ray spectrometry (SEM+EDS), differential scanning calorimetry + thermogravimetric analysis (DTC+TGA), Fourier transform infrared spectrometry (FTIR), X-ray diffraction (XRD), Ultraviolet/Visible (UV/Vis) spectrophotometry, pH measurements, and colorimetry. No changes were found in these papers, length and width of fibers, chemical composition, pH, pyrolysis characteristics, or FTIR and XRD patterns after irradiation. Cellulose (all papers), calcite (bond, rice, and kraft), whewellite (amate), and kaolinite (kraft) were the main components. Non-irradiated bond paper showed a specular effect on the UV spectra, which was progressively reduced by the UV radiation and becoming almost imperceptible at >6 W. Visible spectra of amate paper showed the presence of β carotenoids and chlorophylls, which are degraded by UV radiation. Visible spectra of Kraft paper showed a color reversion at 2.25 W, which progressively diminished as a function of the UV rate energy transfer. UV radiation gradually clarified amate paper. To make a comparison, kraft and amate paper were exposed to gamma radiation at 3, 9, and 15 kGy. The color of Kraft paper was clarified, and amate paper increased in yellowness. Perceptions of chromatic differences due to gamma radiation were smaller than those observed with UV radiation; however, they are still important, especially for kraft paper.

Keywords: UV radiation, bond, amate, rice, kraft, Whatman#1.

INTRODUCTION

Paper has played a prominent role over time, and its permanence and durability are important requirements for librarians, archivists, the electric industry, and conservation of cultural heritage. Permanence of paper was defined [1] as the degree to which it resists chemical action from agents, such as light, air, pollution, high-energy radiation, and microorganisms, over time. Ultraviolet (UV) radiation from both artificial and

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natural light sources are particularly harmful for paper, and that damage is cumulative [2]. Therefore, the studies regarding these effects are useful for cultural heritage preservation and for paper manufacturing techniques, among other areas. Because cellulose is the main component of paper, studies have been devoted to the chemical effects of several agents on this material. Air temperature and relative humidity [2], heat/UV radiation exposure [3], gamma radiation [4], Near-UV and visible pulsed laser [5], thermal oxidation in air at constant temperature and photo-oxidation under Xenon arc lamp [6], and museum environments [7] are among the conditions of these studies. Reduction of the degree of cellulose polymerization and the deterioration of the optical and mechanical properties of papers are among the consequences of aging, and both natural and accelerated processes cause the same effects in cellulose [8]. The processes of aging leads to irreversible changes, usually slowly under environmental conditions [1]; therefore, interest in accelerating those processes, such as by exposition to UV radiation, remains constant.

Wood-derived papers may contain hemicelluloses and lignin in addition to cellulose; on the other hand, some papers also include inorganic and/or organic additives, which may complicate studies on those types of paper [9]. Another aspect to take into consideration among the aging processes is the possibility of color changes in paper [10].

Amate paper is manufactured in a region of Puebla, Mexico, from the bark of trees from the *Moracea* family [11]; today, it is used for handcrafted designs, but pre-Hispanic people used amate for making their codices. Rice paper has been used in China since prehistory for painting and calligraphy [12]; it is usually manufactured out of the Tetrapanax papyrifier tree. Bond paper is commonly used for writing and printing, and it is usually made of cotton, linen, or wood. Kraft paper is a recovered/recycled paper, commonly used for packaging; it is also used in the electrical industry as insulator for winding conductors [13]. As far as we know, amate, rice, and kraft papers have not been studied with regard to the possible effects of UV radiation on them; with respect to cellulose, many studies have been conducted, as mentioned above.

The aim of the present study was to characterize the papers (bond, rice, amate, kraft, and Whatman#1) in their original conditions and after the exposition to UV radiation, in order to evaluate possible changes in their physicochemical characteristics. A study was reported about the physicochemical effects of gamma radiation on several kinds of papers (virtually the same as the present research), not including kraft. A comparison may be made with those data [14].

MATERIALS AND METHODS

The papers were bond, rice, amate, kraft, and Whatman#1 (Sigma-Aldrich), using the last as a reference for the others because it contains virtually only alpha cellulose.

UV irradiations were carried out inside a wooden box 80 cm long, 60 cm wide, and 35 cm high lined inside with aluminum foil. A UVC lamp (Philips TUV 8W G8 T5, 254 nm), a luxometer (UNI-T, UT382), and a thermometer/hygrometer with a probe (Cole-Parmer Traceable) were placed inside the box. Luminescence (Lux), temperature (ºC), and relative humidity (%) were measured multiple times throughout the irradiation. The paper specimens, rectangles of 250 mm x 20 mm in polyethylene bags were placed 25 cm away from the lamp. These experimental conditions are based on a referenced norm [15], in which an accelerated aging of papers by UV radiation is proposed. The samples were analyzed before and after 120, 240, 360, and 480 h of UV radiation exposure.

The experimental conditions during the exposure of paper specimens to UV radiation were the following: lamp intensity of 226±53 Lx, 49±3% relative humidity, and 17.8±0.2 ºC. Luminous intensity (kLx*h) was calculated as the mean value of intensity and the exposure time in hours. This parameter was converted to rates of energy transfer (RET) in Watts (= Joule/s) with Equation 1:

$$\text{RET (W)} = \frac{\text{Luminous intensity} * \text{Dimension of the paper}}{\text{Conversion factor}} \qquad (1)$$

where the dimension of the papers is 0.005 m^2 and the conversion factor for a fluorescent lamp is 60 lm/W. Therefore, the rates of energy transfer were 2.25, 4.5, 6.75, and 9 W for each experimental condition.

2 g of each paper in small pieces (before and after exposure to UV radiation) were gently shaken (wrist stirrer Lab Line Instr., Model 3587) with 0.1 dm^{-3} of distilled water for 6 days. Subsequently, parts of these suspensions were kept at 40 ºC until the de-fibrated papers were completely dried. Samples of de-fibrated paper were placed in an aluminum sample holder, and a gold coating was applied. The specimens were observed with a scanning electron microscope (SEM) (JSM-6610LV with an OXFORD probe, coupled with an EDAX microanalysis system) at 50X and 200X to measure the length and width of fibers, respectively. The chemical compositions of papers were determined by energy dispersive X-ray spectrometry (EDS) analysis. At least five measurements were done for length, width, and chemical composition at the different conditions.

A sample of each paper (before and after exposure to UV radiation) were milled (FRITSCH-Pulverisette Spartan) into fine powder, which was stored in polyethylene bags.

The pH was measured with 0.2 g of each powdered paper and 0.1 dm^{-3} of distilled water in polyethylene flasks. After 15 minutes of shaking the mixture and 10 minutes of centrifugation, the pH of supernatants was measured with a combined electrode coupled with a potentiometer (Orion VersaStar Pro, previously calibrated).

X-ray diffractogram (XRD) of the powdered papers were obtained using a Discover model D8 equipment attached to an X-ray tube with a copper anode. The spectra were compared with data from the Joint Committee on Powder Diffraction Standard Files (JCPDF).

The thermogravimetric spectra of powdered papers were obtained using a differential scanning calorimetry/thermogravimetric analysis (DSC-TGA) equipment (STD Q600 TA Instruments) under the following experimental conditions: temperature between 15 and 800 ºC, rate of 10 ºC/min, and a helium flow of 0.1 dm^{-3}/min.

Fourier transform infrared (FTIR) analysis was conducted with a VARIAN® model 640-IR. For these analyses, a piece of each paper was placed on a quartz sample holder. The absorbance spectra were obtained by rationing the single-beam spectrum against that of the background from 4000 to 400 cm^{-1}, with 40 scans and a resolution of 4 cm^{-1}. The total number of data points was 1869 for each spectrum.

Ultraviolet/visible (UV-Vis) spectra were obtained with integrating sphere equipment (Perkin Elmer Lambda 35). Measurements were made directly with pieces of paper 225 – 325 mm wide and 400 – 800 mm long.

Color was evaluated with the Comission Internationale d'Eclairage System (CIE L*a*b*) using a spectrophotometer PCE-CSM 8 (PCE Instruments) and 3 X 3 cm samples of each paper. The instrument was calibrated using a white standard reflectance plate (L* = 96.37, a* = 0.19, b* = 1.68). The non-irradiated papers were used as control samples to determine changes in color.

Some tests were performed to establish a comparison between UV and gamma radiation. Samples of amate and kraft paper were exposed to gamma radiation at doses of 3, 9, and 15 kGy using a Transelektro LGI-01 irradiator (0.9 kGy/h, Sept. 2016), periodically calibrated with alanine dosimeters. The colors of these samples were evaluated as described above.

RESULTS AND DISCUSSION

Figure 1 shows SEM images of some de-fibrated papers and their EDS spectra. No appreciable changes in the morphology, were observed in the SEM images from the original and UV-irradiated specimens, nor of length, or width of the fibers (Table I). Such changes of the fibers would be an indicator of possible damages, related to the degradation of cellulose [16].

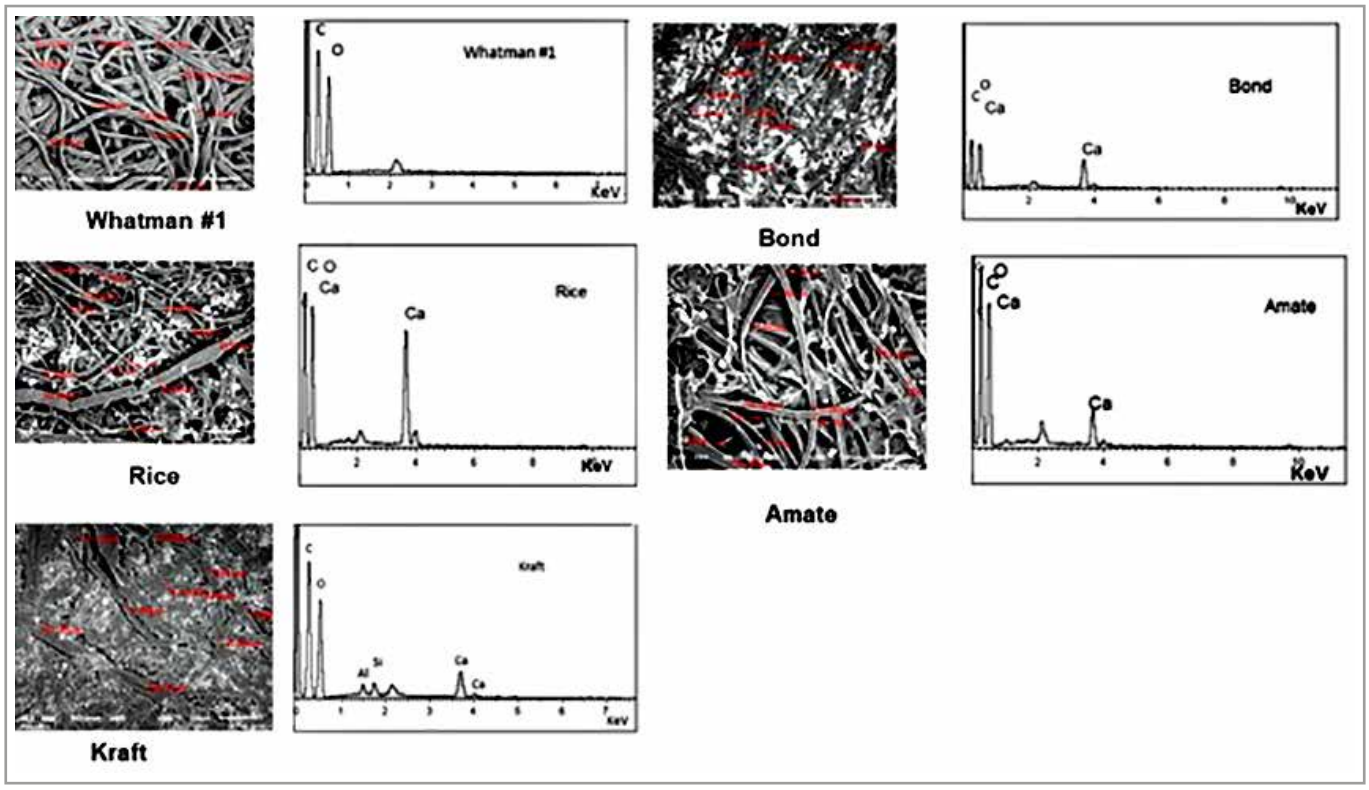

Figure 1. Images of de-fibrated papers (SEM, x200) and EDS spectra.

Table IA. Length (mm) of the fibers of the papers before and after the exposition to UV radiation

	Rate of energy transfer, W				
Paper	**0**	**2.25**	**4.5**	**6.5**	**9**
Whatman#1	1±0.7	0.8±0.2	0.9±0.4	0.9±0.4	0.9 ±0.2
Bond	0.4±0.1	0.5±0.1	0.7±0.2	0.9±0.4	0.7±0.1
Rice	1.4±0.7	1.3±0.2	0.9±0.5	1±0.5	0.8±0.2
Amate	1.1±0.5	0.8±0.2	0.8±0.2	1±0.3	0.8±0.3
Kraft	0.8±0.2	0.6±0.2	0.7±0.2	0.7±0.3	0.7±0.2

Table IB. Width* (μm) of the fibers of the papers before and after the exposition to UV radiation

	Rate of energy transfer, W				
Paper	**0**	**2.25**	**4.5**	**6.5**	**9**
Whatman#1	20±6	16±9	16±9	19±6	12±4
Bond	15±2	18±12	16±13	18±14	21±16
Rice	20±18	22±18	21±17	13±9	18±15
Amate	23±10	16±8	21±10	20±8	15±9
Kraft	22±17	22±11	22±20	23±18	20±18

The elemental composition of the papers was analyzed using the EDS spectra (Figure 1), in which the peaks associated with C, O, Ca, Si, and Al are observed, depending on the type of paper. The peaks between 2 and 3 keV correspond the Mα X-ray emission of gold due to the coating applied to the defibrated papers. Table II shows the mean values and standard deviations of elemental concentrations. As no noticeable differences were found between natural and irradiated papers, this table presents the average values obtained under all experimental conditions. The small standard deviations show little change in the chemical compositions attributable to exposure to UV radiation. C and O were quantified in all papers. Calcium is present in bond, rice, amate, and kraft papers, and this last contains in addition small amounts of silicon and aluminum.

Table II. Elemental concentrations of the papers (percentages). Mean values ± s.d. obtained from data before and after the exposition to UV radiation, at 2.25, 4.5, 6.75, and 9 W.

Paper	C	O	Ca	Si	Al
Whatman#1	45 ± 0.3	55 ± 0.3	0	0	0
Bond	37 ± 0.4	54 ± 0.3	9 ± 0.4	0	0
Rice	35 ± 1	54 ± 0.3	10 ± 1	0	0
Amate	42 ± 2	54 ± 0.9	4 ± 1.5	0	0
Kraft	41 ± 2	52 ± 0.8	5 ± 0.9	1 ± 0.2	1 ± 0.2

The pH measurements of each paper, exposed to UV radiation, do not differ of the initial conditions of the papers. Therefore, global results are given in Table III. All papers are alkaline, except for Whatman#1, which is neutral. A slight acidification was observed for kraft paper; however, it is still alkaline after UV exposure. Paper is preferably alkaline to avoid degradation [9].

Table III. Mean values of pH measurements of the papers before and after the exposition to UV radiation, at 2.25, 4.5, 6.75, and 9 W.

Paper	0 W	2.25, 4.5, 6.75, and 9 W
Whatman#1	6.9 ± 0.1	7±0.1
Bond	9.4 ± 0.0	9.4±0.0
Rice	9.6 ± 0.2	9.4±0.3
Amate	8.4 ± 0.4	8.2±0.4
Kraft	8.2 ± 0.1	8±0.1

According to the XRD spectra, the cellulose is semi-crystalline, and the other components are crystalline. No changes occurred due to UV radiation exposure. The diffractogram of Whatman #1 only presented the characteristic peaks of cellulose, which occurred at 14.9°, 16.5°, and 22.6° 2θ; these peaks were present in the spectra of all other papers (Figure 2). Rice, bond, and kraft papers contain calcite ($CaCO_3$), whose main peaks occur at 23.1°, 24.4°, 36°, 39.4°, 43.2°, 47.5°, 48.5°, and 57.4° 2θ. Calcium carbonate gives paper favorable physical and mechanical properties; this compound can be added to the pulp or be formed during the process with calcium hydroxide [9]. The bond paper may contain calcium sulfate (peak at 23.1° 2θ), which is added to improve printing and writing rigidity, whiteness, and retention of paper [17]. This peak is not seen in the spectrum because it overlaps with one of cellulose. The spectra of Kraft paper also showed small peaks due to kaolinite (12.4° and 24.9° 2θ), which sometimes coats this kind of paper [18].

The presence of whewellite (calcium oxalate monohydrate, $CaC_2O_4 \cdot H_2O$) was identified on amate paper spectra (15°, 24.5°,30.2°, 36°, and 38.3° 2θ). This compound is generated by the metabolism of the plant as a defensive measure against external agents, such as insects [19].

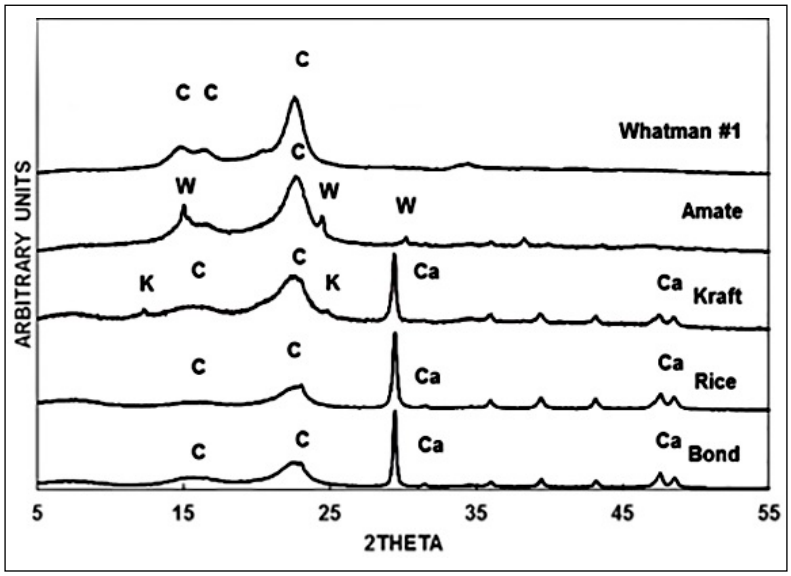

Figure 2. X-ray diffraction patterns of the papers. C: Cellulose, Ca: Calcite ($CaCO_3$), W: Whewellite ($CaC_2O_4\ H_2O$), K: Kaolinite ($Al_2Si_2O_5(OH)_4$).

Few differences were found in the FTIR band intensity and none in their wave numbers due to the UV exposures regarding the original specimens. The bands at 1160, 1107, 1055, and 1031 cm^{-1} corresponding to cellulose [20-22] were present in spectra of all papers. Bands of calcite (C-O stretching and O-C-O bending: 1420, 880, and 712 cm^{-1}) were present in the bond, rice, and kraft spectra, and the stretching vibration of –COOH due to whewellite (1620 and 1350 cm^{-1}) was observed in the amate spectrum. The FTIR spectra of bond, rice, and kraft papers were remarkably similar among them; whereas those of amate paper were like to that reported for soft wood of Southern pine [23].

Some FTIR bands of cellulose, hemicellulose, and lignin occurred in the region between 900 and 1200 cm^{-1}. Other characteristic bands of hemicellulose and lignin are found in the region between 1700 and 1800 cm^{-1}; however, no bands were observed in this region for any paper. The absence of lignin in handmade papers, amate and rice, both of tree barks, is due to the alkaline manufacturing process because lignin is soluble in this medium. Disappearance of spectral bands in the mentioned region after the lignocellulosic fibers were cooked and bleached was reported [24]. On the other hand, a band around 1730 cm^{-1} was observed when Whatman #1 paper was aged under a xenon light source, suggesting a photo-degradation process [6]. This effect was not observed under the conditions of the present study. Common laser-printer paper, newspaper, and thermal fax paper were exposed to UV radiation and analyzed by ATR-FTIR spectroscopy; only the last one was sensitive to UV radiation and scarce effect was observed for the two first [25]. FTIR spectrum of thermally aged Whatman paper was virtually the same that the natural paper spectrum [6]. This scarce effect was observed in the present research for the papers exposed to UV radiation.

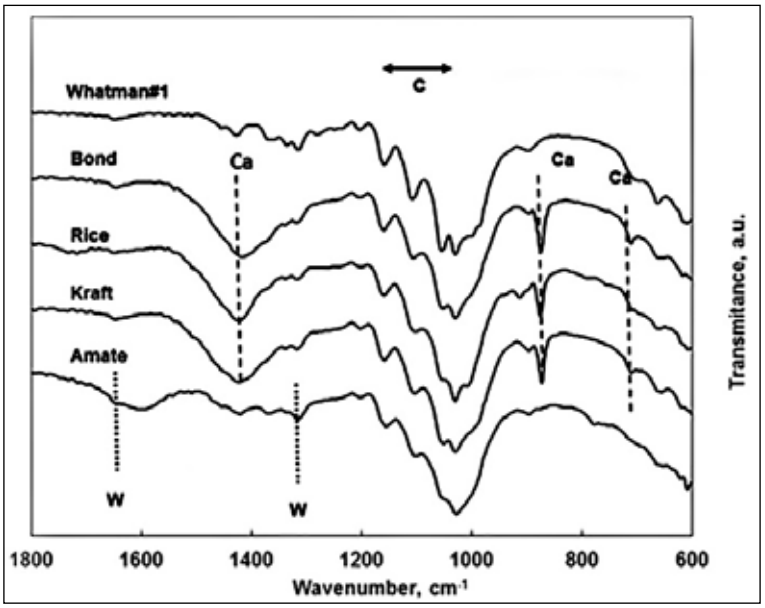

Figure 3. Infrared spectra of the papers exposed at 9 W. C: Cellulose, Ca: Calcite, W: Whewellite.

Deconvolution procedures were applied to the DTC diagrams of the papers to calculate the percentage of each pyrolysis process. The processes observed for all papers were dehydration (20 – 200 °C) and descarboxilation of cellulose (250 – 400 °C). Cellulose decomposes between 277 and 427 °C, with maximum decomposition occurring at 355 °C [20,26,27]. Calcium oxalate monohydrate in amate paper decomposes to calcium carbonate at 400 - 500 °C, creating calcium oxide. Decomposition of this last compound was observed for amate, bond, rice, and kraft papers at 600 - 800 °C. Carbon and calcium oxide, as a residue at >800 °C, represented a large percentage of these four papers, whereas Whatman #1 left a small residue of carbon. Data regarding the exposure (or not) of the papers to UV radiation were virtually identical, as showed by the small standard deviation values of the pyrolysis percentages of the compounds. Therefore, Table IV includes mean values and standard deviations of data obtained in all experimental conditions. Similar results were found with thermally aged Whatman paper, no changes were observed in TGA analysis before and after the thermal treatment [6].

Table IV. Percentages of the compound pyrolysis by TGA/DTA, at different intervals of temperature. Mean values ± s.d. obtained from data before and after the exposition to UV radiation, at 0, 2.25, 4.5, 6.75, and 9 W.

Temperature (°C)	Compound	Whatman#1	Bond	Rice	Amate	Kraft
20 - 200	Humidity	3 ± 1	2.5 ± 1	3 ± 0.4	4 ± 0.3	3 ± 1
250 - 400	Cellulose	92 ± 1	66 ± 1	63 ± 1	64 ± 3	75 ± 1
450 - 500	$Ca(COO)_2$				6 ± 1	
600 - 700	$CaCO_3$		10 ± 0.2	13.5 ± 1	6 ± 1	5.4 ± 0.4
450 - >800	C	2 ± 0.3				
700 - >800	C + CaO		21 ± 1	20 ± 1	21 ± 3	14 ± 4

No peaks were observed in the UV spectra obtained with integrating sphere equipment between 225 and 325 nm for Whatman#1, rice, amate, or kraft paper, and no changes were evident in these papers after exposure to UV radiation between 0 and 9 W (figure not included). Brightness of Whatman paper virtually remained constant when it was exposed to near-UV and visible pulsed laser [5]. On the contrary, due to the brightness of bond paper, a specular effect rather than diffuse reflectance was observed at 276 ± 1 nm (Figure 4A). This effect was particularly noticeable in the original paper and was progressively reduced as a function of the UV rate of energy transfer, being almost imperceptible at >6.75 W (Figure 4B). Then, exposure with UV radiation induced in bond paper the effect known as brightness reversion [8], which can be interpreted as an accelerated aging effect. Absorbance spectral changes below 300 nm were observed using Fabriano paper exposed to ambient solar radiation [2], like was observed for bond paper exposed to UV radiation in the present work. A similar effect was caused by surfactants on photocopy paper; the reflectance of this paper increased but not that of the filter paper [28]. The coatings of the bond, photocopy and Fabriano papers are maybe the responsible of these effects.

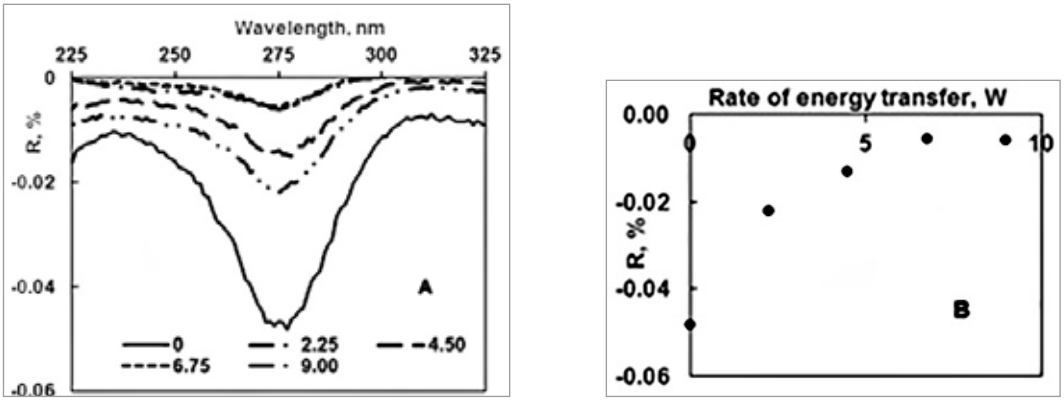

Figure 4. A: UV diffuse reflectance spectra of bond paper, exposed from 0 to 9W.
B: Behavior of R % (at 275 nm) as a function of the rate of energy transfer.

Regarding the diffuse reflectance spectra in the visible region of white papers (Whatman #1, bond, and rice), non-irradiated and irradiated up to 9 W, none presented maximum of diffuse reflectance between 400 and 800 nm (figure not included). Amate paper spectra were very intense, whereas those of kraft paper were less intense.

Figure 5A shows the deconvolution of amate paper visible spectrum of diffuse reflectance. Several maxima were revealed, specifically at 425, 481, 590, and 670 nm, which correspond to the plant's pigments, β carotenoids (400 - 525 nm) and chlorophylls (400 - 500 nm and 575 – 700 nm) [29]. That figure shows the spectrum of non-irradiated amate paper, but deconvolution of all the spectra generated the same results. The visible spectrum of amate was sensitive to the rate of energy transfer (Figure 5B). The natural logarithm of the diffuse reflectance at 481 and 670 nm showed a good fit with the first-order kinetic model (Figure 5C). This effect corresponds to degradation of β carotenoids and chlorophylls due to UV radiation exposition. Thermal degradation of β carotenoids, induced a similar behavior [30].

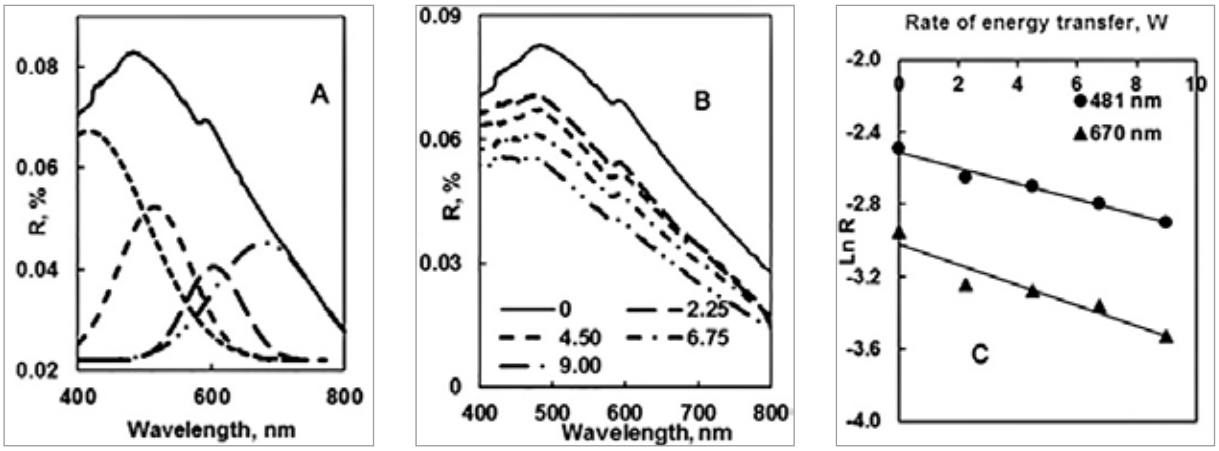

Figure 5. A: Deconvolution of Visible diffuse reflectance spectrum of amate paper. B: Visible diffuse reflectance spectra of amate paper, exposed from 0 to 9 W. C: Behavior of the Ln R as a function the rate of energy transfer at 481 nm (circles), Ln R =-0.005 RET - 2.5 (R^2 = 0.99) and 670 nm (triangles), Ln R =-0.006 RET - 3 (R^2 = 0.96).

The original kraft paper visible spectrum of diffuse reflectance changed when it was exposed to 2.25 W of UV radiation, mainly between 400 and 570 nm (region of green color) and less notably between 700 and 750 nm (region of red color) (Figure 6A). This effect may be explained by a color reversion of cellulose and the resulting increase in yellowness due to accelerated aging [8,31,32] and/or the presence of some additives. Above 2.25 W and up to 9 W, the spectra's intensity decreased (Figure 6A), possibly due to the degradation of dyes present in the kraft paper. These dyes were not identified; however, industrial coloring materials that are used for kraft paper and carton may be suggested [33]. Deconvolution of all the kraft paper visible spectra of diffuse reflectance revealed several maximum regions, specifically at 481, 527, and 718 nm; Figure 6B shows the spectrum corresponding to 2.25 W. The natural logarithm of the diffuse reflectance between 2.25 and 9 W showed a good fit with the first-order kinetic model (Figure 6C).

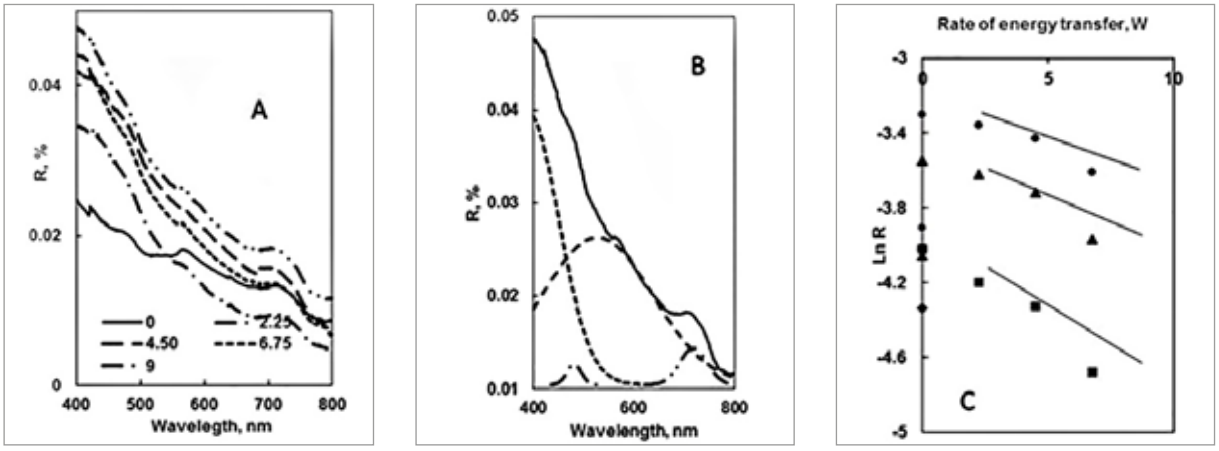

Figure 6. A: Visible diffuse reflectance spectra of kraft paper, exposed from 0 to 9 W. B: Deconvolution of the visible diffuse reflectance spectrum of kraft paper (exposed at 2.25 W). C: Behavior of Ln R as a function rate of energy transfer at 481 nm (circles): Ln R = -0.004 RET - 3.1 (R^2=0.92), 527nm (triangles): Ln R = -0.005 RET - 3.4 (R^2=0.91), and 718 nm (squares): Ln R = -0.008 RET - 3.8 (R^2=0.95).

The results regarding color evaluation are found in Table V. The L* parameter refers to lightness [100 = white, 0 = black); according to this parameter, the order of non-irradiated papers is Whatman #1 = Bond > rice > kraft >> amate. The parameter a* corresponds to the greenness-redness axis (a positive value indicates redness, and a negative one indicates greenness). The values for Whatman #1, rice, and kraft papers are slightly negative, that of bond paper slightly positive, and amate clearly tends toward the red direction (a* = 6). The parameter b* corresponds to the yellowness-blueness axis (negative values for blue and positive values for yellow). The b* values for Whatman #1, bond, and rice paper are small. Kraft paper is yellow (b* = 8), and the value of the brown amate paper is even higher (b* =16). In a study about Whatman paper, this sample was treated by washing, deacidification, immersion in methylcellulose and the CIEL*a*b* color system showed lightness and yellowness due to these treatments [34]. None of these effects was observed for Whatman paper exposed to UV radiation in the present research.

$\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{½}$ expresses the colorimetric difference between the irradiated and original papers. Regarding the effect of UV irradiation, the parameters ΔL*, Δa*, Δb*, and ΔE* of white papers (Whatman #1, bond, and rice) were small and virtually did not change when the luminous intensity was increased to 9 W.

The kraft paper darkened slightly (negative values of ΔL*) throughout the exposure to UV radiation, with a 2.25 W increase in yellowness (Δb* = 9.4), and thereafter gradually decreased when the rate of energy transfer was increased to Δb* = 7.8 with 9 W. The perception of this color difference is appreciated with the values of ΔE* (Table V).

Table V. Results of colorimetric measurements of papers, originals and exposed to UV radiation.

		Rate of energy transfer, W					
Paper	**Parameters**	**0**	**2.25**	**4.5**	**6.75**	**9**	**Mean values or intervals**
Whatman #1	L*	92					
	a*	-0.04					
	b*	1.1					
	ΔL*		0.3	-0.1	0.3	1.1	0.4±0.5
	Δa*		0.004	-0.002	-0.04	-0.02	-0.01±0.02
	Δb*		0.5	0.4	0.7	0.6	0.6±0.2
	ΔE*		0.6	0.4	0.8	1.3	0.8±0.4
Bond	L*	92					
	a*	0.3					
	b*	0.6					
	ΔL*		0.5	0.5	0.2	0.6	0.4±0.2
	Δa*		-0.5	-0.6	-0.6	-0.6	-0.6±0.02
	Δb*		1.7	1.7	1.7	1.5	1.7±0.1
	ΔE*		1.8	1.9	1.8	1.7	1.8±0.1
Rice	L*	87					
	a*	-0.22					
	b*	1.6					
	ΔL*		1.6	1.9	2.1	2.5	2±0.4
	Δa*		-0.1	-0.1	-0.1	-0.1	-0.1
	Δb*		1.3	1.0	0.5	0.8	0.9±0.3
	ΔE*		2.0	2.1	2.2	2.6	2.2±0.2

Table V. Results of colorimetric measurements of papers, originals and exposed to UV radiation. (Continuation)

		Rate of energy transfer, W					
Paper	**Parameters**	**0**	**2.25**	**4.5**	**6.75**	**9**	**Mean values or intervals**
Kraft	L*	82					
	a*	-0.5					
	b*	8					
	ΔL*		-2	-2	-2	-2	-2
	Δa*		-0.07	0.00	0.10	0.10	0.03
	Δb*		9.4	8.6	8.4	7.6	9.4 to 7.6
	ΔE*		9.6	8.8	8.6	7.8	9.6 to 7.8
Amate	L*	61					
	a*	6					
	b*	16					
	ΔL*		7.1	9.3	12.0	16.0	7.1 to 16
	Δa*		-2.3	-1.5	-2.6	-3.7	-3±1
	Δb*		1.4	5.3	2.0	-1.7	2±3
	ΔE*		7.6	10.8	12.4	16.5	7.6 to 16.5

UV radiation gradually increased the amate paper lightness (ΔL*), clarifying its color, and although no significant changes were observed in the values of the greenness-redness (Δa*) and yellowness-blueness (Δb*) axis (between 2.25 and 9 W), the colorimetric (ΔE*) difference is important.

The colorimetric differences (ΔE*) in kraft and amate paper were plotted as a function of the rate of energy transfer. Figure 7 shows that these colorimetric differences behave linearly with this parameter, negatively for the kraft paper, and positively for the amate paper.

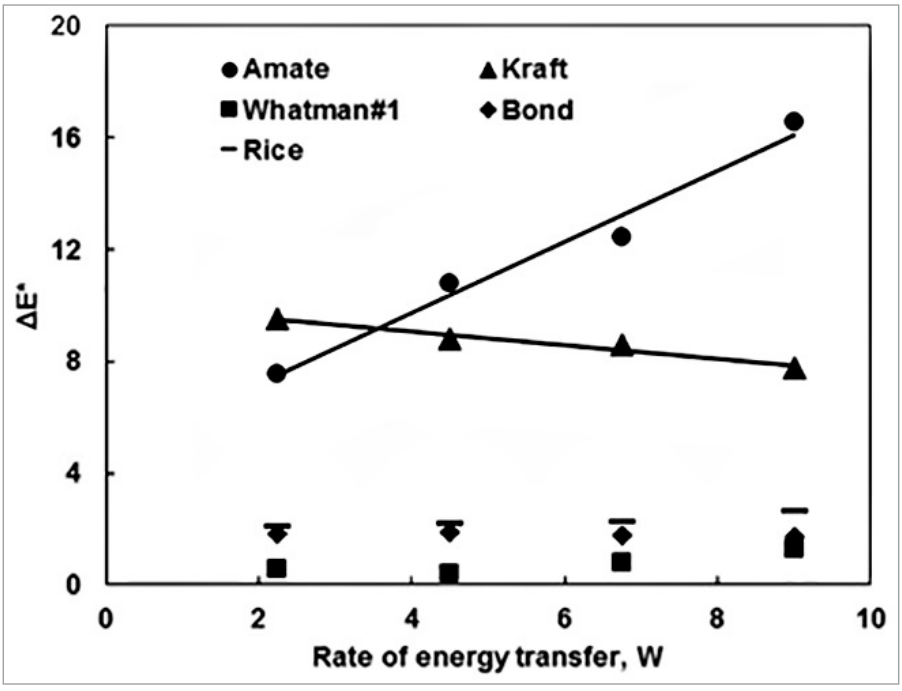

Figure 7. Colorimetric difference (ΔE*) of the papers as a function the rate of energy transfer. For kraft: ΔE* = -0.25 W + 10.1 (R^2 = 0.95) and for amate: ΔE* = 1.3 + 4.7 (R^2 = 0.98).

As mentioned earlier, color was evaluated with the formula in the CIE Lab System, which is similar to NBS standardization. According to that standardization, the perception of chromatic difference is quite important for values of ΔE* > 6, which was the case for amate and kraft paper. These results agree with those obtained by means of spectrometric measurements in the visible light region. The chromatic difference of amate paper emerged due to the degradation of the pigments (β carotenes and α chlorophyll), whereas chromatic differences in kraft paper appeared due to its non-identified dyes or the cellulose itself.

A study was conducted on color and degradation of kraft papers of various compositions and degrees of grinding [10]. When the paper was heated at 200 °C for 40 – 60 minutes, ΔE* values were of the same order of magnitude as those found in the present study with UV radiation. Under these conditions, these authors obtained a ΔE* close to 5 for Whatman#1. The value of this parameter for Whatman #1 in the present research was quite smaller (0.8±0.4), which might mean that cellulose is virtually not affected by UV radiation of up to 9 W.

The colorimetric results of amate and kraft paper exposed to gamma radiation (see Table VI) were the following: A) The color of kraft paper with 3 kGy was clarified (ΔL* = 7.1) and this parameter had virtually the same value for 3, 9, and 15 kGy; on the contrary, changes in coloration (Δa* and Δb*) were virtually not observed. ΔE* was equal to 7.2±0.2 for the interval of gamma doses between 3 and 15 kGy. Even though the values of the perception of chromatic difference (ΔE*) were smaller than those observed with UV radiation, they should be considered especially important. B) The amate paper increased in yellowness (Δb* = 4.9) at 3 kGy, but the values of this parameter remained virtually the same for 3, 9, and 15 kGy, and no changes were observed for ΔL* and Δa*. ΔE* was equal to 5.2±0.5. Even if it is lesser than ΔE* for the UV radiation and < 6, it should be considered of medium importance.

Table VI. Results of colorimetric measurements of papers, non-irradiated and exposed to gamma radiation

		Dose, KGy				
Paper	**Parameters**	**0**	**3**	**9**	**15**	**Mean values**
Kraft	L*	82				
	a*	-0.5				
	b*	8				
	ΔL*		7.1	7.4	7.0	7.1±0.2
	Δa*		-0.2	-0.1	-0.1	-0.1
	Δb*		0.9	1.0	1.1	1±0.1
	ΔE*		7.1	7.4	7.1	7.2±0.2
Amate	L*	61				
	a*	6				
	b*	16				
	ΔL*		1.8	0.4	1.8	1.3±0.8
	Δa*		1.1	1.6	0.9	1.2±0.4
	Δb*		4.9	5.4	4.2	4.8±0.6
	ΔE*		5.3	5.7	4.6	5.2±0.5

CONCLUSIONS

Neither the structure of the fibers, pH, nor the elemental chemical composition seemed to be affected by the exposure of the studied papers to up to 9 W of UV radiation. Cellulose (all papers), calcite (bond, rice, and kraft), whewellite (amate), and kaolinite (kraft) were the main components. These compounds remained unaltered after exposure to UV radiation. The FTIR spectra of the studied papers (irradiated and non-irradiated) did not show peaks corresponding to lignin, so they all lacked it. These results were like those found for papers irradiated with ≤15 KGy of gamma radiation [14].

The UV diffuse reflectance spectra of Whatman #1, rice, amate, and kraft paper (irradiated and non-irradiated) did not show any peaks. Only non-irradiated bond paper showed a specular effect in its UV spectra that was progressively reduced with exposure at > 6.75 W to be almost imperceptible. This effect is knowing as brightness reversion and may be considered as an effect of accelerated aging.

Only colored papers presented visible diffuse reflectance spectra. The spectra of amate paper showed peaks of β carotenoids and chlorophylls, which degraded by UV exposition, according to a first-order kinetic model. The kraft paper spectra changed greatly when the paper was irradiated at 2.25 W, showing a color reversion. This effect progressively diminished as a function of the rate of energy transfer, according to a first-order kinetic model. Spectra deconvolved into several peaks of unknown identity. Degradation of pigments may be interpreted as accelerated aging effects.

Colorimetric differences due to exposure to UV radiation were detectable in Kraft and amate papers. Non-irradiated kraft paper is yellow, it darkened slightly with 2.25 W but this effect gradually decreased at higher rate energy transfer. The non-irradiated amate paper is brown and gradually increased its lightness by UV exposition. Colorimetric differences of kraft and amate paper behaved linearly with the rate energy transfer.

When kraft and amate paper were exposed to gamma radiation between 3 and 15 kGy, the color of the kraft paper was clarified, and amate paper increased in yellowness. The perception of chromatic difference (ΔE*) was smaller than that observed with UV radiation; however, they are still important, especially for kraft paper.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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ARTICLE

Synthesis, Isolation and Characterization of three acid and alkaline hydrolytic products of Nimodipine and Development of a valid RP-HPLC method for simultaneous determination of Nimodipine and Citicoline sodium in the presence of Nimodipine degradation products in Bulk and Tablets

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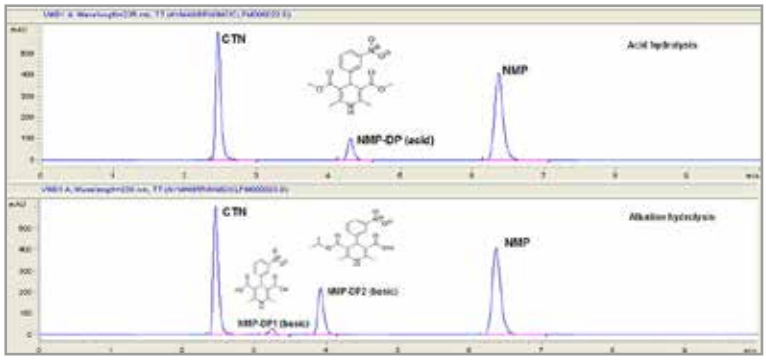

Impurity profiling of active pharmaceutical ingredients is a crucial step in assessing their quality. Moreover, the chemical nature of nimodipine makes it susceptible easily to acidic and alkaline hydrolysis. On the other hand, nimodipine is co-formulated with citicoline sodium pharmaceutically as tablets to treat cerebral ischemia. In this study, three degradation products of nimodipine were synthesized, isolated and characterized with aid of FTIR spectroscopy, ^{1}H-NMR as well as LC-MS/MS after exposing to drastic acidic and alkaline conditions. Subsequently, a simple, selective and valid RP-HPLC method was developed for simultaneous estimation of nimodipine and citicoline in the presence of nimodipine acid and alkaline degradation products in bulk and tablets. Chromatographic separation was achieved using an isocratic mobile phase consisting of acetonitrile: 0.02 M KH_2PO_4 (containing 0.2% v/v, triethylamine and adjusted to pH 3.0 with orthophosphoric acid) (70:30, v/v) at a flow rate 1.0 mL min^{-1} at ambient temperature (25 ºC) on a Eurospher II C18 (250 mm × 4.6 mm, 5 µm) column with UV detection at 270 nm for citicoline and 235 nm for nimodipine and its acidic and alkaline hydrolytic products. Linearity, accuracy and precision were found to be acceptable over a concentration range of (4.5–120 µg mL^{-1}) for nimodipine and (15–400 µg mL^{-1}) for citicoline. The proposed method could be successfully applied for the routine analysis of the studied drugs in their pharmaceutical preparation in the presence of nimodipine common degradation products without any preliminary separation step.

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Keywords: Nimodipine; Citicoline; Acid and alkaline hydrolysis; Structure elucidation; RP-HPLC.

INTRODUCTION

Nimodipine (NMP) (Figure 1.a) is 3,5-pyridine dicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-,2-methoxy ethyl 1-methyl ethyl ester [1]. It is a dihydropyridine calcium channel blocker known for its preferential action on cerebral blood vessels and its potential cytoprotective effects by reducing calcium influx into nerve cells [2]. It is the only available therapy with proven benefits for reducing the impact of ischemic neurological deficits after subarachnoid hemorrhage so far [2,3]. Nimodipine can be of some benefits in patients with various forms of dementia [4].

Citicoline sodium (CTN) (Figure 1.b), is Cytidine 5'-(trihydrogendiphosphate) p'-[2- (trimethylammonio) ethyl] ester inner salt [5]. It is cytidine diphosphate-choline, suggested to increase dopamine receptor densities [6] and it helps prevent memory impairment resulting from poor environmental conditions [7]. It is primarily used in pharmacotherapy of brain insufficiency and other related neurological disorders as stroke, brain trauma and Parkinsonism's disease [8].

Nimodipine and citicoline sodium are co-formulated in pharmaceutical preparations as Nimodilat plus® tablets. This combination was found to have an important role in reducing infarct size, and treating cerebral ischemia [9].

Nimodipine USP monograph revealed a conventional titrimetric assay method for Nimodipine determination [1]. Nimodipine was determined in different pharmaceutical formulations using HPLC methods in pharmaceutical dosage forms [10,11] and in plasma [12-14]. Besides, nimodipine was estimated in the presence of its process related impurities [15] and in presence of its acidic, alkaline and photolytic degradation products (no degradation was observed under thermal and oxidative stress conditions) [16]. Moreover, nimodipine (NMP) was determined in human plasma and cerebrospinal fluid of patients with subarachnoid haemorrhage using UHPLC-MS/MS [17].

Citicoline sodium was estimated in bulk and dosage form by HPLC methods [18-20]. Moreover, it was estimated in human plasma [21,22]. Citicoline sodium (CTN) was estimated in combination with piracetam using RP-UPLC [23]. Stress degradation studies were estimated for citicoline sodium (CTN) using RP-HPLC [24].

Although, the literature review revealed that stress degradation studies were made on nimodipine (NMP) to indicate its stability under different stress conditions [16]. However, no structure elucidation was reported to any nimodipine (NMP) degradation products. Besides, the literature review revealed that nimodipine (NMP) showed moderate stability under oxidative and high temperature stress conditions [16]. So, in this study the focus was on nimodipine (NMP) acidic and alkaline degradation products preparation, isolation and characterization by LC-MS/MS, ^{1}H-NMR and FTIR spectroscopy (Figures 1.c, 1.d and 1.e). Obviously, due to a lot of overlapping peaks might appear and bad resolutions attained, conducting a forced degradation study on a combination containing both nimodipine (NMP) and citicoline sodium (CTN) was not the target. To date, the combination of nimodipine (NMP) with citicoline sodium (CTN) was not officially reported in any pharmacopeia and no RP-HPLC method was reported for the rapid estimation of both drugs simultaneously. So, in this study, a RP-HPLC method was developed for the quantitation of nimodipine (NMP) and citicoline sodium (CTN) in combination and in the presence of nimodipine (NMP) acidic and alkaline degradation products. The developed method was validated according to ICH Guidelines [25]. The proposed method is suitable for purity assessment of bulk and dosage forms containing nimodipine alone or in combination of citicoline in reasonable time.

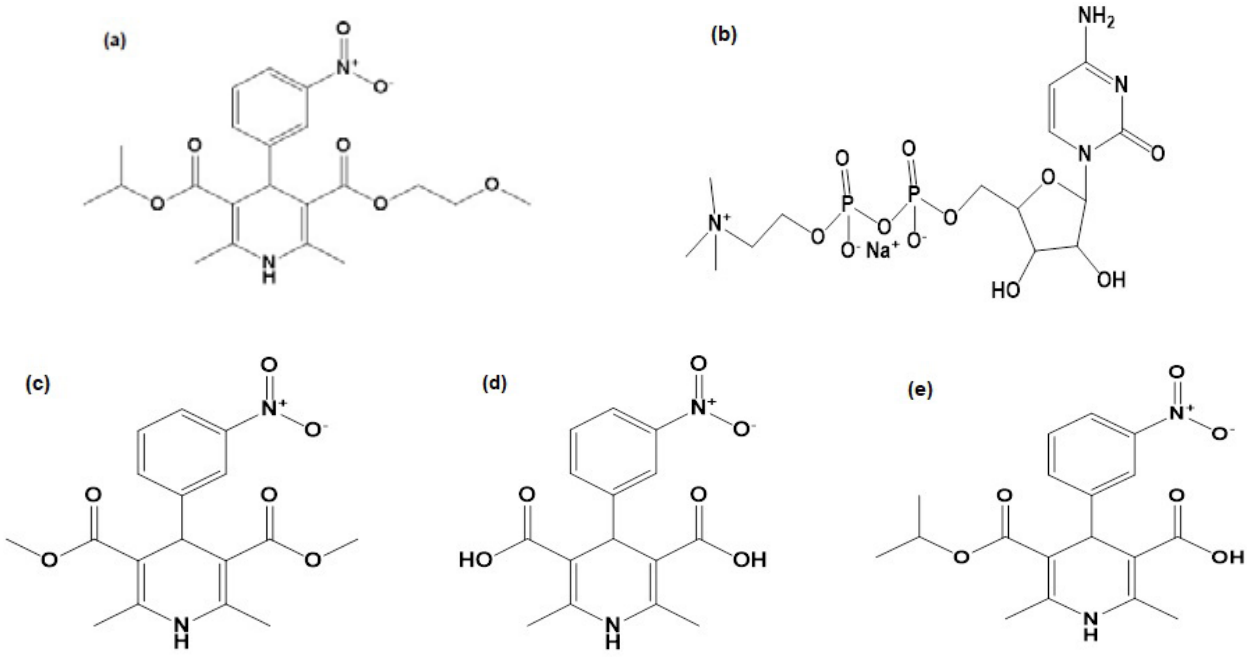

Figure 1. Chemical structures of (a) nimodipine, (b) citicoline sodium, (c) nimodipine acid degradation product (d) nimodipine alkaline degradation product [1] [DP 1], (e) nimodipine alkaline degradation product [2] [DP 2].

MATERIALS AND METHODS

Instrumentation

A chromatographic system consisting of Agilent 1200 series (CA, USA); interface equipped with an Agilent quaternary pump G1311A, Agilent UV-visible detector G1314B, an Agilent manual injector G1328B equipped with (20 µl) injector loop, an Agilent degasser G1322A and an Agilent syringe, LC 50 µL. Separation and quantitation were made on a Eurospher II C18 column (5 µm, 4.6 x 250 mm).

Moreover, tandem mass spectrometer – an Agilent triple quadrupole mass spectrometer with an API source (ESI) coupled with an Agilent pump controlled by an Agilent 1200 controller and equipped with an Agilent 1200 autosampler injector was used for analysis. Agilent Mass Hunter software was used for data acquisition.

Furthermore, NMR instrument – a Bruker NMR instrument (USA), 400 MHz and FTIR spectrophotometer-Shimadzu FTIR spectrophotometer 8400S (Japan), were used.

Materials and Reagents

Pharmaceutical grade nimodipine (NMP) was supplied and certified by GNP Company (6th October -Egypt) to contain 99.87%. Pharmaceutical grade citicoline sodium (CTN) was supplied and certified by October pharma Company (6th October -Egypt) to contain 99.65%. Nimodilat plus® tablets labeled to contain 30 mg nimodipine (NMP) and 100 mg citicoline sodium (CTN) per each tablet, was supplied by Lazar pharmaceutical company (Ecuador). Acetonitrile HPLC grade (Scharlau, Spain) was used. Bi-distilled water was produced in-house (Aquatron Water Still, A4000D, U.K). Membrane filters 0.45 µm from Teknokroma (Barcelona, Spain) were used. All other chemicals and reagents used were of analytical grade unless indicated otherwise.

Chromatographic Conditions

Chromatographic separation was achieved on a Eurospher II C18 column (250 mm×4.6 mm, 5 µm) by applying isocratic elution based on a mobile phase consisting of acetonitrile:0.02 M KH_2PO_4 (containing

0.2% v/v, triethylamine and adjusted to pH 3.0 with orthophosphoric acid) (70:30, v/v). The mobile phase was pumped through the column at a flow rate of 1 mL min^{-1}. Analyses were performed at ambient temperature (25 ºC) and detection was programmed to be at 270 nm from 0-3.49 min for citicoline sodium (CTN) and 235 nm from 3.5-10 min for nimodipine (NMP) and its acidic and alkaline degradation products. The injection volume was 20 µL.

Tandem mass spectrometry (LC-MS/MS) was performed using a mobile phase consisting of acetonitrile: 0.1% formic acid (80:20, v/v), delivered at a flow rate of 0.5 mL min^{-1}. Separation was performed using an analytical Agilent XDB-C18 column (50 × 4.6 mm) with particle size of 1.8 µm.

Standard solution preparation

Standard stock solutions of each of nimodipine (NMP) (1 mg mL^{-1}) and citicoline sodium (CTN) (1 mg mL^{-1}) were prepared by separately dissolving 100 mg of each drug in 100 mL mobile phase. The required concentrations were prepared by serial dilutions with mobile phase.

Sample preparation

Tablet sample preparation

Twenty tablets were accurately weighed and powdered in a mortar. A quantity of the powdered tablets equivalent to (100 mg/30 mg) citicoline sodium (CTN)/ nimodipine (NMP) respectively was sonicated for 15 min with 70 mL of mobile phase, cooled and transferred quantitatively into a 100 mL volumetric flask. The solution was completed to volume with mobile phase and filtered to obtain a concentration equivalent to 1000 µg mL^{-1} for citicoline sodium (CTN) and 300 µg mL^{-1} for nimodipine (NMP).

Nimodipine acid drastic forced degradation product preparation and isolation

An amount equivalent to 125 mg of nimodipine (NMP) was dissolved in 250 mL 5 M methanolic HCl (not aqueous HCl, due to poor solubility of nimodipine in water) and transferred quantitatively to a conical flask. Solution was put on reflux at 80 ºC water bath for 6 hours. Flask content was cooled and neutralized to pH 7 using 5 M NaOH. Neutralized solution was evaporated to dryness. Residue formed was dissolved in dichloromethane and put on sonication for 10 min. Filter and evaporate filtrate obtained to dryness in a beaker of known weight and reweight the beaker. Dissolve residue in acetonitrile to get a solution of 500 µg mL^{-1}. Methanolic degradation products formed are not real time degradation products, but those forming are only induced degradation conditions.

Nimodipine alkaline drastic forced degradation product preparation and isolation

An amount equivalent to 100 mg of nimodipine (NMP) was dissolved in 180 mL dichloromethane in a stoppered conical flask. 20 mL methanol containing 2.1 mg NaOH was added to the conical flask (to make 0.25 M NaOH solution) and flask content was mixed well. Solution was put on reflux at 60 ºC water bath for 2 hours. Flask content was transferred to a beaker and evaporated to dryness. Residue was dissolved in 200 mL water. Make extraction with 45 mL dichloromethane three successive times to remove any traces of unreacted ester and alcohol formed. Aqueous layer was separated and neutralized with 0.25 M HCl to pH 7. The neutralized aqueous solution was re-evaporated to dryness and cooled. 50 mL acetonitrile was added and solution sonicated for 10 min then filtered. Filtrate was evaporated in a beaker of known weight and reweight the beaker. Residue was dissolved in acetonitrile to get a solution of 100 µg mL^{-1}.

Procedure

Linearity and range

Accurately measured aliquots of standard stock solutions of nimodipine (NMP) and citicoline sodium (CTN) equivalent to (45-1200 µg mL^{-1}) and (150-4000 µg mL^{-1}) respectively, were transferred into series of 10 mL volumetric flasks and completed to volume with mobile phase to get concentration ranges equivalent to (4.5-120 µg mL^{-1}) for nimodipine (NMP) and (15-400 µg mL^{-1}) for citicoline sodium (CTN). 20 µL aliquot

of each solution was injected in triplicates onto the chromatograph under the specified chromatographic conditions described in the Chromatographic Conditions item. Calibration curve was constructed by plotting the area under peak (AUP) against the corresponding concentrations (C) of each drug.

Assay of laboratory prepared mixtures and Nimodilat plus® tablet

Laboratory prepared mixtures

The procedures mentioned under the Linearity and Range item were repeated using laboratory prepared mixtures equivalent to 18-110 µg mL^{-1} nimodipine (NMP) (in the presence of 2-30 µg mL^{-1} nimodipine (NMP) acidic and alkaline degradation products (10% to 30% of nimodipine (NMP), w/w)) and 20-370 µg mL^{-1} citicoline sodium (CTN) (Figures 2 and 3).

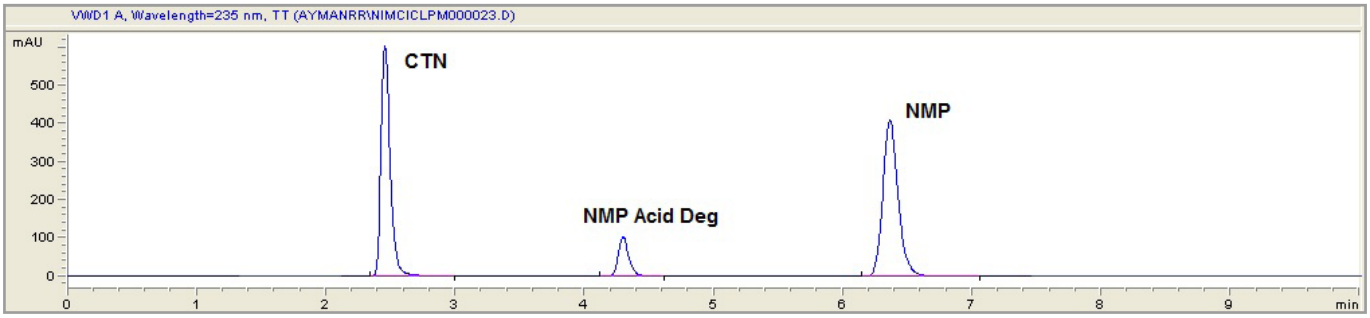

Figure 2. A typical HPLC chromatogram of synthetic ternary mixture of 150 µg mL^{-1} citicoline sodium, 9 µg mL^{-1} nimodipine acid degradate and 45 µg mL^{-1} nimodipine.

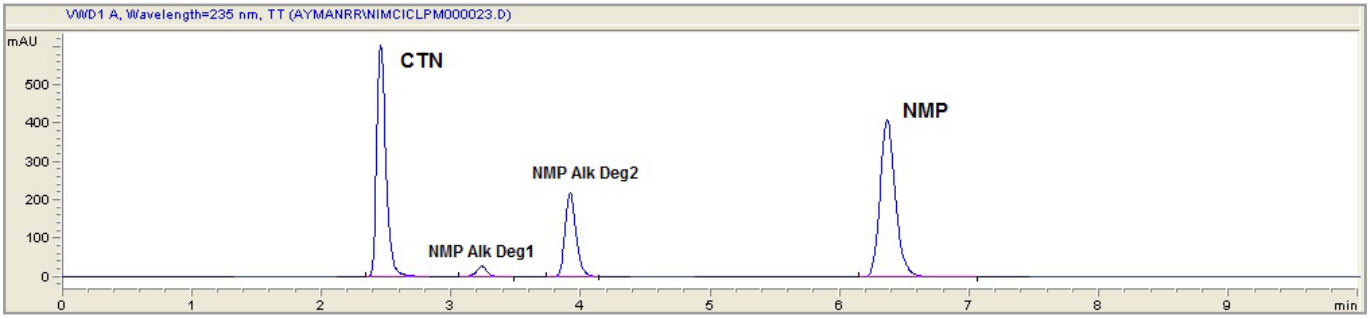

Figure 3. A typical HPLC chromatogram of synthetic ternary mixture of 150 µg mL^{-1} citicoline sodium, 11 µg mL^{-1} nimodipine alkaline degradates and 45 µg mL^{-1} nimodipine

Assay of Nimodilat plus® tablets

Sample solution prepared under the Sample Preparation item was serially diluted with mobile phase to get concentrations equivalent to 9-108 µg mL^{-1} nimodipine (NMP) and 30-360 µg mL^{-1} citicoline sodium (CTN). Samples were injected in triplicates. Concentrations of nimodipine (NMP) and citicoline sodium (CTN) were calculated using calibration equations.

RESULTS AND DISCUSSION

System suitability tests

System suitability tests are important tests of liquid chromatographic methods in order to reach optimized conditions of the proposed USP method [1]. The parameters of these tests include column efficiency (number of theoretical plates) (N), capacity factor (K), tailing of chromatographic peak (T), resolution (R), and repeatability as % RSD of peak area for six injections of a solution of a 200 µg mL^{-1} and 60 µg mL^{-1} for

citicoline sodium (CTN) and nimodipine (NMP), respectively and reproducibility of retention as % R.S.D of retention time. The results of these tests for the proposed method were listed in Table I.

Table I. System suitability results of the proposed RP-HPLC method

	Acid degradation			Alkaline degradation			
Analytical term	**CTN**	**NMP-DP**	**NMP**	**CTN**	**NMP-DP1**	**NMP-DP2**	**NMP**
Number of theoretical plates (N)	3562	14548	15172	3499	7764	10699	14978
Resolution (R)	17.99	11.76		8.38	4.62	14.2	
Capacity factor (K)	1.502	3.352	5.379	1.497	2.231	3.042	5.382
Chromatographic peak tailing (T)	1.348	1.205	1.201	1.321	0.965	1.239	1.187
RSD% of 6 injections							
Peak area	0.160	0.432	0.079	0.183	0.332	0.166	0.067
Retention time	0.184	0.272	0.071	0.166	0.226	0.097	0.082

Validation of analytical method

Linearity and range

In this study, six concentrations were chosen for each drug. Each concentration was analyzed three times. Good linearity of the calibration curve was verified by the high correlation coefficient. The analytical data of the calibration curve including standard deviations for the slope and intercept (S_b, S_a) were summarized in Table II.

Accuracy

Accuracy of the results was calculated by % recovery of laboratory prepared mixtures of 6 different concentrations of citicoline sodium (CTN) and nimodipine (NMP) and also by standard addition technique for Nimodilat plus® tablet. The results obtained including the mean of the recovery and standard deviation were displayed in Table II.

Precision

The intra-day precision of the method was assessed by six determinations for each of the three concentrations (160, 200, 240 µg mL^{-1} for citicoline sodium (CTN) and 48, 60, 72 µg mL^{-1} for nimodipine (NMP)) representing 80%, 100%, 120% for each drug. The repeatability of sample and measurement of peak area for active compound were expressed in terms of percentage relative standard deviation (%R.S.D.) and found to be less than 1% in three concentrations. Besides, inter-day precision (using the same three concentrations in triplicates for three consecutive days) was carried out for both drugs and results were displayed in Table II.

Specificity

Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences. The chromatograms of citicoline sodium (CTN) and nimodipine (NMP) in the sample solutions were found to be identical to the chromatograms obtained by the standard solution. Also, no chromatographic interference from acidic and alkaline degradation products was found by mixing their prepared solution with citicoline sodium (CTN) and nimodipine (NMP). In addition, no chromatographic interference from any of the excipients was found at the retention time of the examined drug after extraction

of the active ingredient. Besides, the chromatograms of the pharmaceutical formulation samples were checked for the appearance of any extra peaks. Good resolution and absence of interference was attained. So, the proposed method could be successfully applied for the routine analysis of the studied drugs in their dosage forms without any preliminary separation step. Results for determination of these drugs by the proposed method in their dosage forms along with standard addition technique were displayed in Table II.

Limit of detection and limit of quantification

Limit of detection (LOD) which represent the concentration of the analyte at S/N ratio of 3 and limit of quantitation (LOQ) representing the concentration of analyte at S/N ratio of 10 was determined experimentally for the proposed method and results were given in Table II.

Table II. Assay and method validation parameters obtained by applying the proposed RP-HPLC method for simultaneous determination of nimodipine and citicoline sodium in bulk and capsule

Analytical term	**CTN**	**NMP**
Retention time	2.51 min	6.40 min
Wavelength of detection	270 nm	235 nm
Linearity		
Range of linearity	15-400 μg mL^{-1}	4.5-120 μg mL^{-1}
Regression equation	y = 18.9246x + 27.3065	y = 70.6529x + 17.1658
Correlation coefficient (R)	0.9999	0.9995
LOD* (μg mL^{-1})	3.487	0.487
LOQ* (μg mL^{-1})	10.567	1.477
S_b*	0.080	0.824
S_a*	19.373	54.638
Confidence limit of the slope	18.9246±0.222	70.6529±2.287
Confidence limit of the intercept	27.3065±53.779	17.1658±151.675
Standard error of the estimation	25.771	74.567
Precision		
Intra day (RSD* %)	0.135-0.175	0.079-0.102
Inter day (RSD* %)	0.432-0.614	0.447-0.887
Drug in dosage form	100.224±0.916	99.994±1.177
Accuracy		
Drug in laboratory mix.	100.405±1.149	99.383±0.898
Drug added	100.158±1.147	100.812±1.027

LOD: limit of detection, LOQ: limit of quantitation, *S_b: slope standard deviation, S_a: intercept standard deviation, RSD: relative standard deviation.

Robustness

Robustness is a measure of the method ability to remain unaffected by small variations in the method conditions and is an indication of the method reliability. Robustness was performed by changes in flow rate (changed from 1.0 mL min^{-1} to 0.8 mL min^{-1} and 1.2 mL min^{-1}), organic strength of mobile phase (acetonitrile percentage changed from 70% to 68% and 72%), molarity of prepared buffer solution (changed from 20 mM to 18 mM and 22 mM) and pH of the buffered solution of mobile phase (changed from pH 3.0 to pH 2.8 and pH 3.2). The most important parameter to be studied was the resolution factor between the two peaks of citicoline sodium (CTN) and nimodipine (NMP). It was found that no significant change in resolution factor between citicoline sodium (CTN) and nimodipine (NMP) peaks indicating good robustness of the proposed method. Results were given in Table III.

Table III. Robustness of the proposed HPLC method

Parameter	Flow rate (mL min^{-1})		pH		Organic composition (%)		Buffer solution molarity (mM)	
	0.8	**1.2**	**2.8**	**3.2**	**68**	**72**	**18**	**22**
CTN/NMP-DP Resolution (acid hydrolysis)	18.21	17.67	18.02	17.89	18.11	17.78	17.88	18.03
NMP-DP/NMP Resolution (acid hydrolysis)	11.83	11.64	11.79	11.68	11.82	11.59	11.69	11.78
CTN/NMP-DP1 Resolution (basic hydrolysis)	8.44	8.32	8.39	8.35	8.45	8.32	8.34	8.40
NMP-DP1/NMP-DP2 Resolution (basic hydrolysis)	4.65	4.59	4.64	4.60	4.67	4.59	4.60	4.64
NMP-DP2/NMP Resolution (basic hydrolysis)	14.24	13.99	14.23	14.18	14.31	14.17	14.16	14.24

Statistical analysis of the results

A statistical analysis of the results obtained by the proposed method and the reference methods for each analyte was carried out by “SPSS statistical package version 11”. The difference between groups was tested by (*t*-test) and (F-value) at p=0.05. The test ascertained that there was no significant difference among the methods. Results were given in Table IV.

Table IV. Statistical analysis of the results obtained by the proposed RP-HPLC and the reference methods

	CTN		NMP	
Statistical Term	**HPLC method**	**Reference method****	**HPLC method**	**Reference method*****
Mean	100.405	100.035	99.383	99.757
SD	1.149	0.938	0.898	0.873
RSD	1.144	0.938	0.903	0.875
n	6	6	6	6

Table IV. Statistical analysis of the results obtained by the proposed RP-HPLC and the reference methods (Cont.)

	CTN		NMP	
Statistical Term	**HPLC method**	**Reference method****	**HPLC method**	**Reference method*****
Variance	1.320	0.880	0.806	0.762
***t*-value**	0.611(2.228)*		0.730(2.228)*	
F-value	0.373(4.965)*		0.533(4.965)*	

* Values in parentheses are the corresponding theoretical *t* -and F-values at P = 0.05.

** Reference method for citicoline sodium using HPLC company method.

*** Reference method for nimodipine using HPLC of USP pharmacopeia.

Method Development Discussion

The development of a simultaneous and sufficiently selective analytical method to determine citicoline sodium (CTN) and nimodipine (NMP) in presence of nimodipine (NMP) acidic and alkaline degradation products, with quantitation sensitivity for the cited two drugs in their binary mixture, was of interest and no analytical method was reported to analyze this mixture simultaneously. I aimed using C18 column which has greater separation ability than C8 columns especially for complex compounds due to chemical nature of packing. Various mobile phase compositions like methanol with water or acetonitrile with water, in different proportions, were attempted in an isocratic mode using Eurosper II C18 column (250 mm x 4.6 mm, 5 µm) but optimized separation of all contents was not obtained. Starting use of a buffer solution of 0.02 M KH_2PO_4 mixed with different proportions of acetonitrile, an improvement in peak shapes and resolutions was noticed. That was expected as buffer solution has the benefit to resist pH change upon the addition of acidic or basic compounds, so maintaining the pH of the solution relatively stable. Using low proportions of acetonitrile gave delayed elution for nimodipine (NMP), because it is strongly attracted to the column particles. But compounds whose polarity is near to that of the mobile phase will be preferentially attracted to it and move faster. Using too high proportions of acetonitrile (more than 82%), salting out of buffer solution was observed due to poor solubility of buffers in organic solvents. Triethylamine was added to buffer solution of mobile phase (0.2%, v/v) to minimize broadening and tailing of nimodipine (NMP) peak. So best resolutions and peak shapes with reasonable retention times for citicoline sodium (CTN), nimodipine (NMP), and nimodipine (NMP) acid and alkaline degradation products were attained using acetonitrile: 0.02 M KH_2PO_4 (containing 0.2% v/v, triethylamine and adjusted to pH 3.0 with orthophosphoric acid) (70:30, *v/v*), Table I. Flow rate was adjusted to 1 mL min^{-1}. Analysis was achieved at ambient temperature (25 ºC) on a Eurospher II C18 (250 mm× 4.6 mm, 5 µm). UV detector was operated at 270 nm for citicoline sodium (CTN) and at 235 nm for nimodipine (NMP) and its acidic and alkaline degradation products, where best sensitivity was attained at these selected wavelengths for each compound.

Acid hydrolysis of Nimodipine

Acid degradation of nimodipine was carried out in 5 M methanolic HCl as mentioned under Section (4.3). Complete degradation was confirmed using TLC plates and its structure was then elucidated by different spectroscopic techniques. Structure elucidation was confirmed using FTIR spectroscopy, ^{1}H-NMR as well as LC-MS/MS spectrometry and supposed to be: 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic methyl ester (Figure 1c).

The spectroscopic data of the intact drug showed a peak at 3550 cm^{-1} in the IR spectrum corresponding to –NH of pyridine ring and a peak at 1500 cm^{-1} corresponding to carbonyl group, presence of septet peak corresponding to –CH of isopropyl group at 4 ppm, presence of triplet peak corresponding to –CH_2 of methoxy ethyl moiety at 3.9 ppm and absence of carboxylic protons at 11-13 ppm in ^{1}H-NMR and the molecular weight of the intact drug at 418.47 *m/z* using LC/MS-MS. Although, the IR spectrum

of nimodipine acid degradation product showed no significant difference from parent drug, its ^{1}H-NMR spectrum showed disappearance of both septet peak of –CH of isopropyl group and triplet peak of $–CH_2$ of methoxy ethyl moiety, and presence of singlet peaks at 3.6 ppm but carboxylic protons still absent. LC-MS/MS confirmed the hydrolysis of two ester groups of nimodipine to its corresponding carboxylic acids followed by their esterification to methyl esters via methanol in solution (Fischer-Speier Esterification) as showing the molecular weight of the obtained acid degradation product at 346.33 *m/z* in the negative ion mode. The suggested scheme for nimodipine (NMP) acid degradation is illustrated in Figure 4.

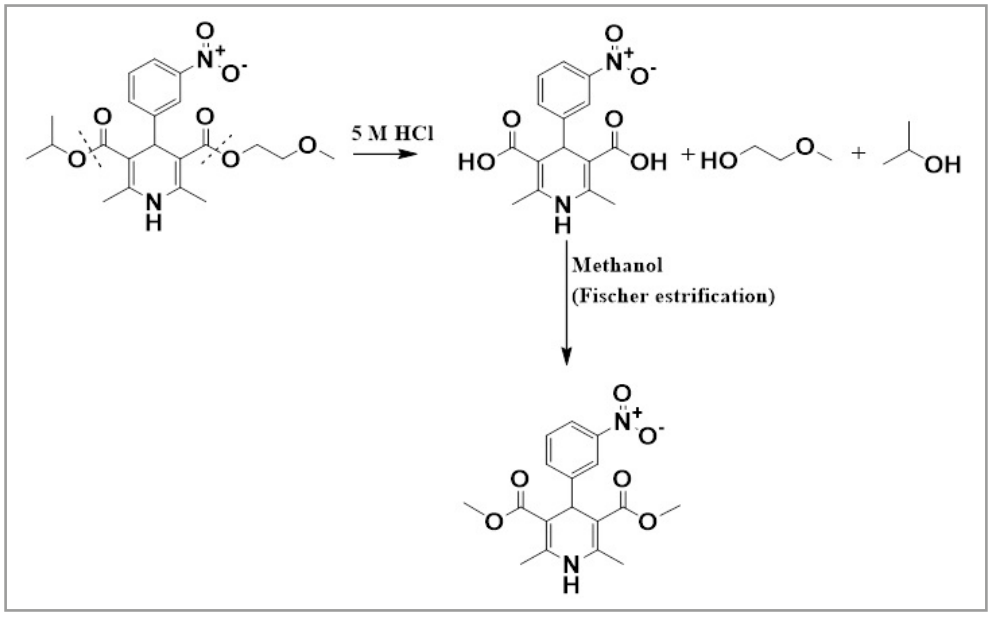

Figure 4. The suggested scheme for nimodipine acid degradation.

Alkaline hydrolysis of Nimodipine

Alkaline degradation of nimodipine was carried out using 0.25 M NaOH as mentioned under Section (4.4). Complete degradation was confirmed using TLC plates and its structure was then elucidated by different spectroscopic techniques. Structure elucidation was confirmed using FTIR spectroscopy and LC/MS-MS spectrometry techniques. Two alkaline degradation products were supposed to be formed and they were: 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylic acid (Figure 1d) and 5-propan-2-yl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (Figure 1e).

Spectroscopic data of intact drug mentioned was utilized. The IR spectrum of nimodipine alkaline degradation products showed appearance of a broad and strong peak at 3630 cm^{-1} corresponding to –OH group. Peak of –NH group was shifted to 3100 cm^{-1}. LC-MS/MS confirmed the hydrolysis of one or two ester groups of nimodipine to its corresponding carboxylic acids as showing the molecular weight of the obtained alkaline degradation products at 360.42 *m/z* and 318.28 *m/z* in the negative ion mode. The suggested scheme for nimodipine (NMP) alkaline degradation is illustrated in Figure 5.

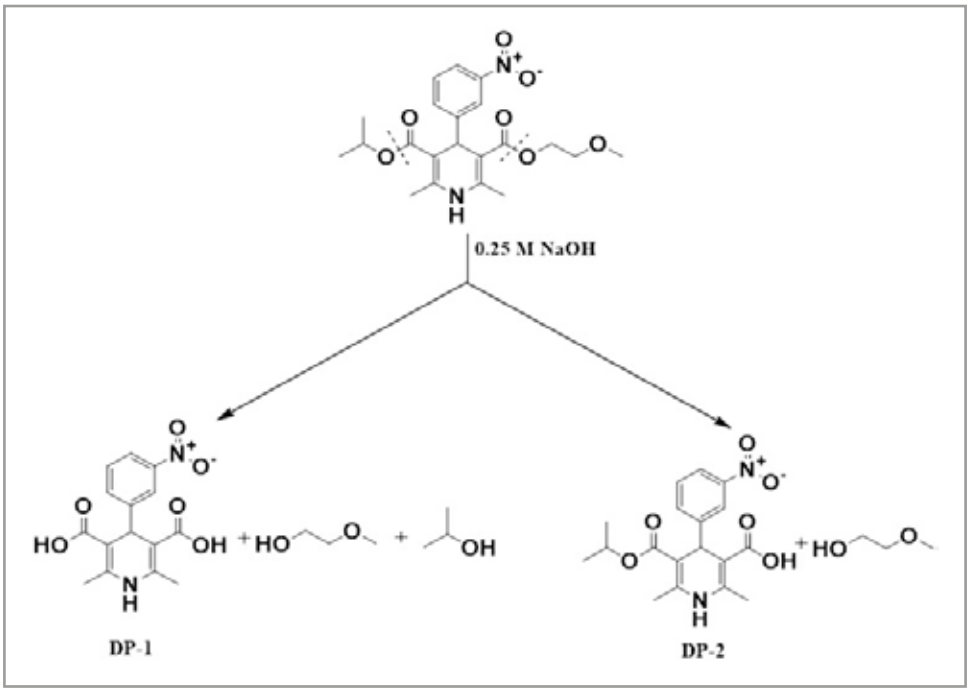

Figure 5. The suggested scheme for nimodipine alkaline degradation.

CONCLUSION

The proposed RP-HPLC provides simple, accurate and reproducible quantitative analysis for the simultaneous determination of citicoline sodium (CTN) and nimodipine (NMP) in bulk and tablets in presence of nimodipine (NMP) acidic and alkaline drastic forced degradation products. This method was validated as per ICH guidelines. The proposed method is suitable for the purity assessment of nimodipine (NMP) alone or in combination with citicoline sodium (CTN) in analytical laboratories.

Acknowledgment

The author thanks National Organization for Drug Control and Research (NODCAR), Giza, Egypt, for carrying out this work.

Conflict of interest

Nothing to declare.

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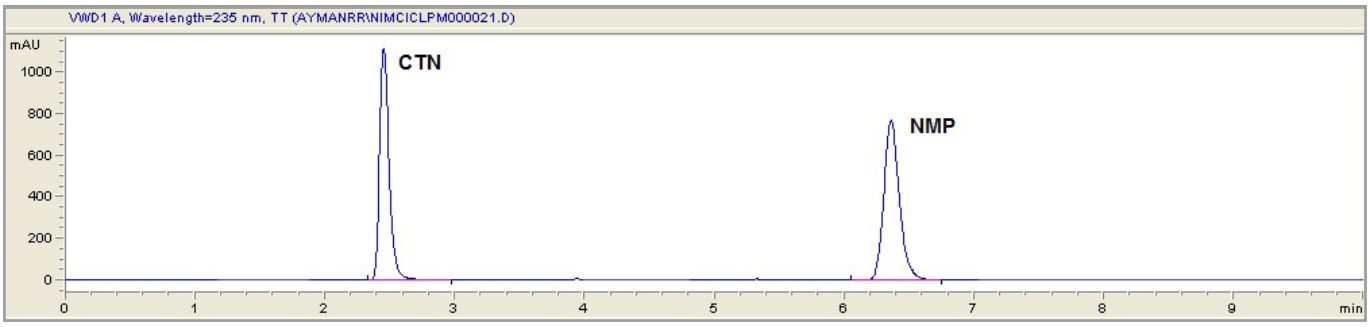
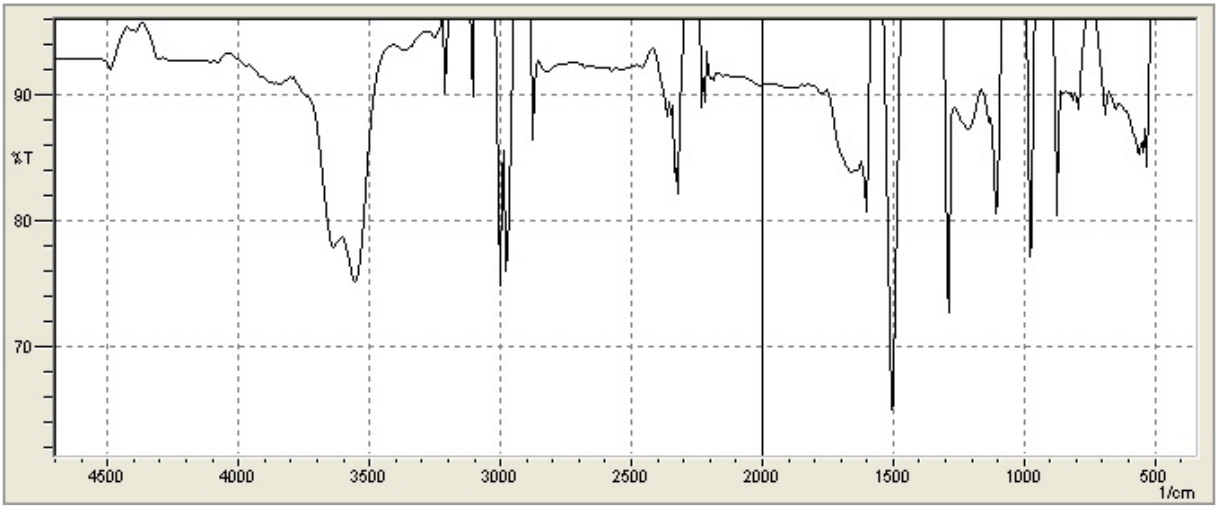
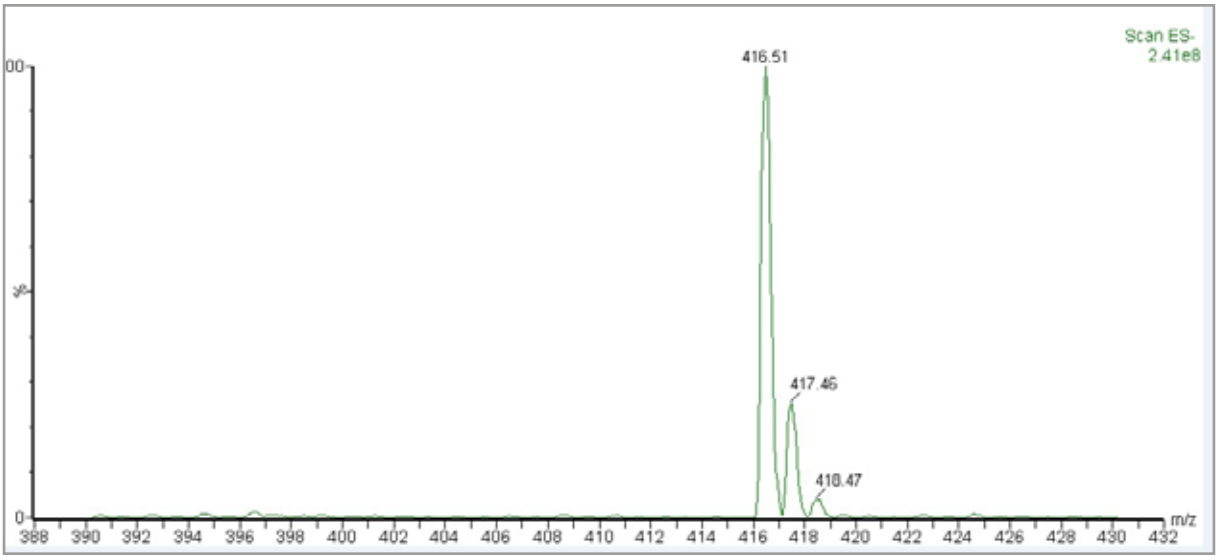
SUPPLEMENTARY MATERIAL



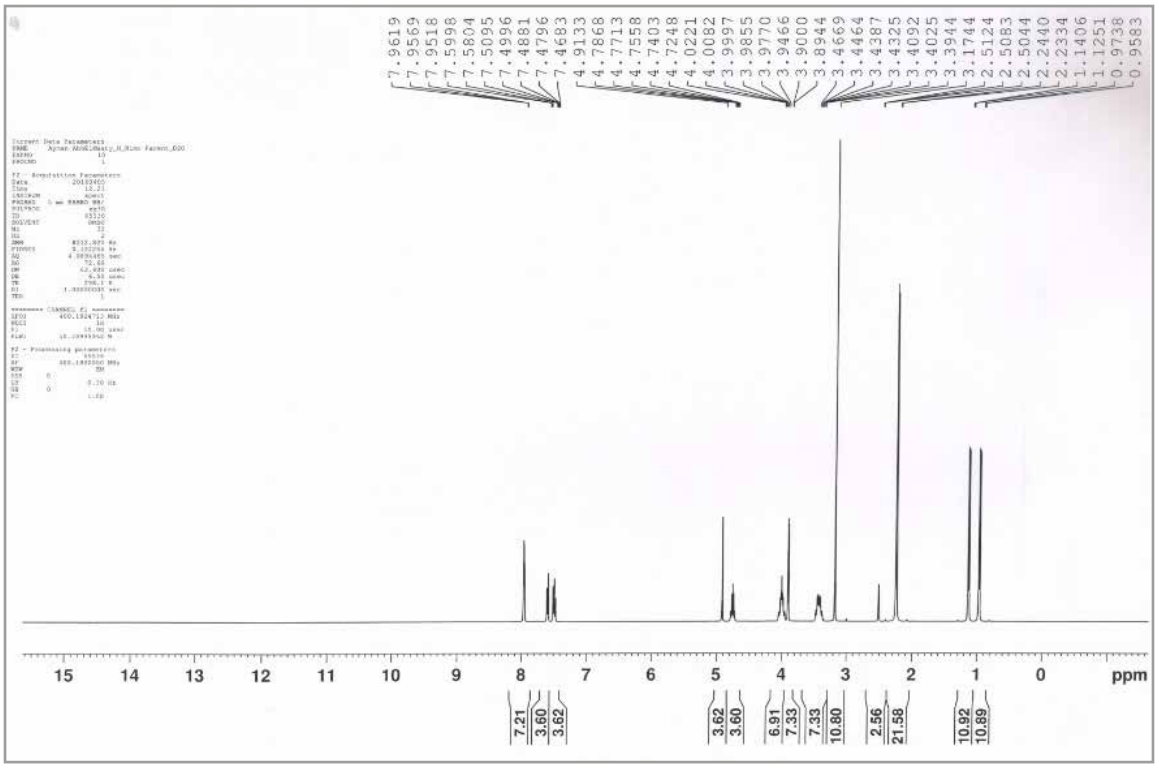
A typical HPLC chromatogram of Nimodilat plus® sample solution (305 μgmL^{-1} CTN and 91.5 μgmL^{-1} NMP)



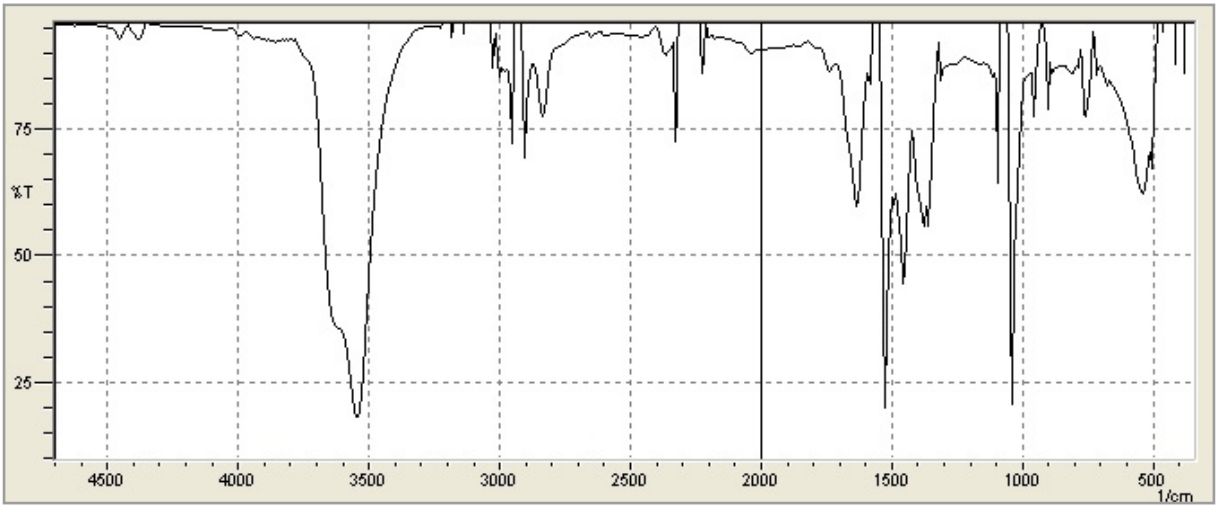
FTIR spectrum of nimodipine



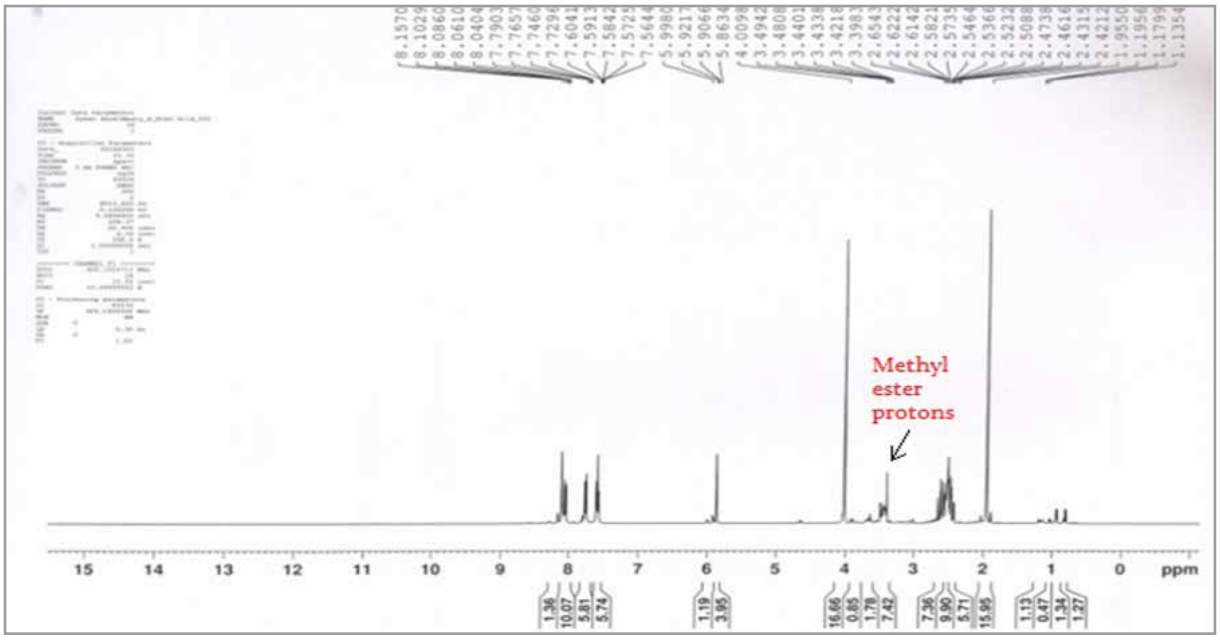
LC-MS/MS spectrometry of nimodipine



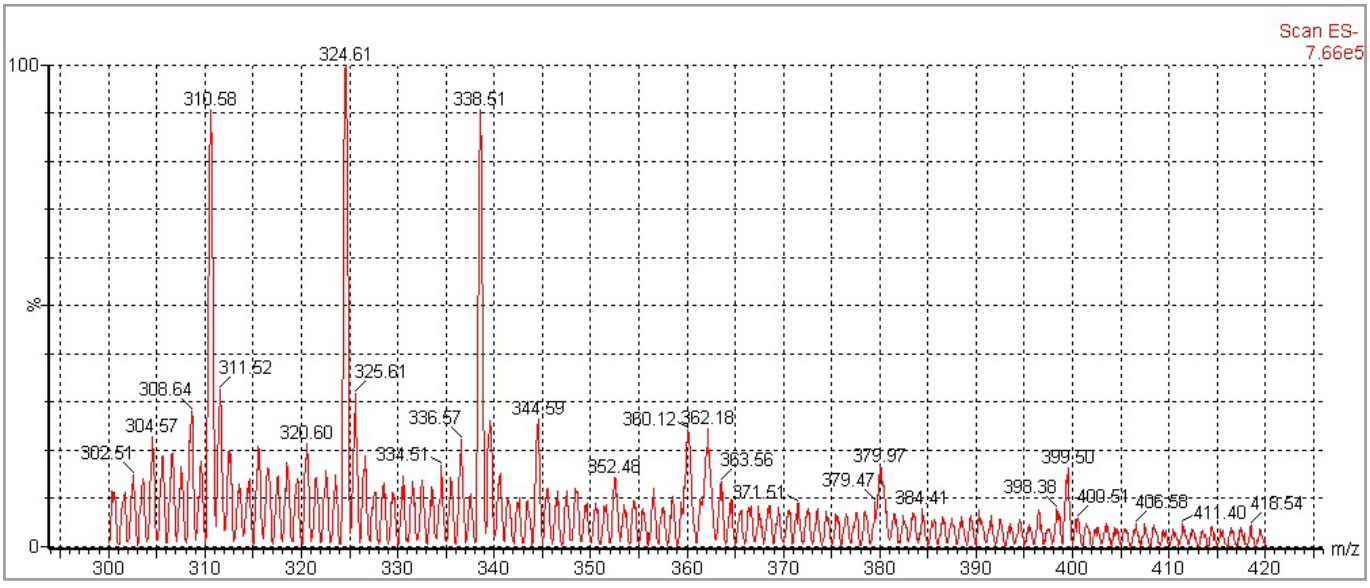
^{1}H-NMR spectrum of nimodipine using deuterium oxide



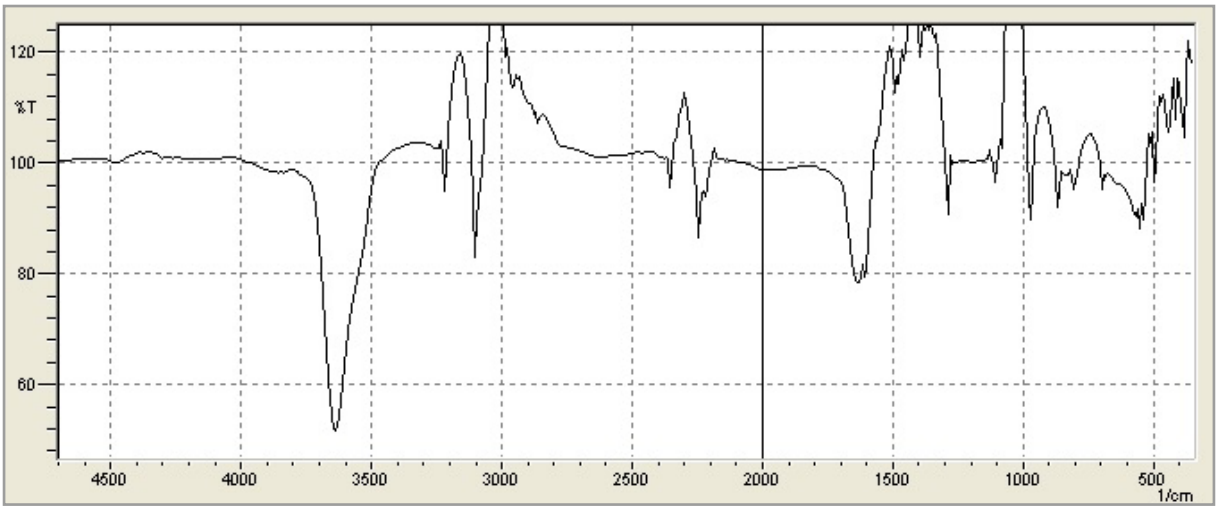
FT-IR spectrum of nimodipine acid forced degradation product



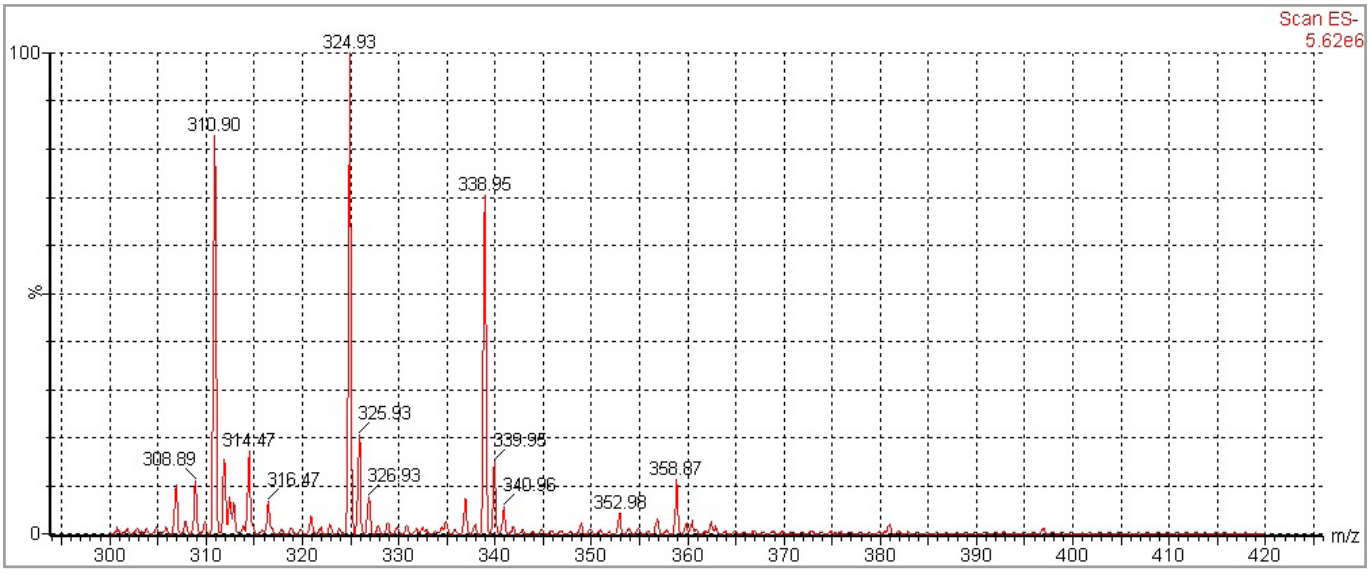
^{1}H-NMR spectrum of nimodipine acid degradation product using deuterium oxide



LC-MS/MS spectrometry of nimodipine acid forced degradation product



FT-IR spectrum of nimodipine alkaline forced degradation products



LC-MS/MS spectrometry of nimodipine alkaline forced degradation products

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TECHNICAL NOTE

Multi-Element Determination of Trace Elements in B7-diesel Oil by High-Resolution Continuum Source Flame Atomic Absorption Spectrometry

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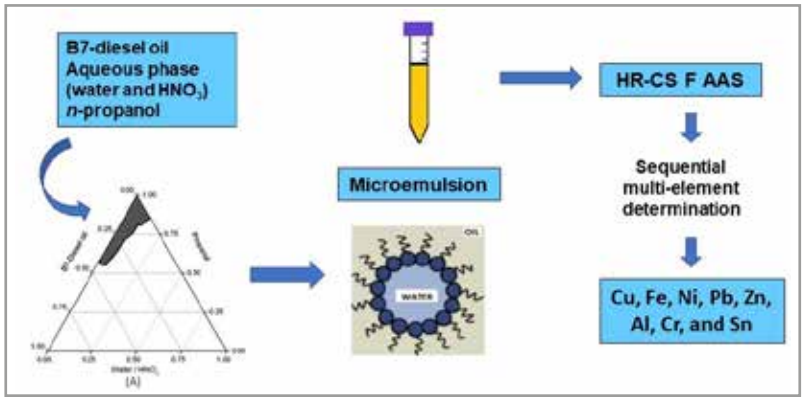

In this work, a method for sequential multi-element determination of Cu, Fe, Ni, Pb, Zn, Al, Cr and Sn in B7-diesel oil samples by high-resolution continuum source flame atomic absorption spectrometry (HR-CS F AAS) was proposed. The sample preparation was based on formation of microemulsion (ME), which was investigated through a three-phase diagram. The ME composition adopted was B7-diesel oil (3.3 g), aqueous phase containing nitric acid (1.4 mol L^{-1}, 300 µL) and *n*-propanol (up to 10 mL). The use of surfactant Triton X-100 in the formation of ME was also evaluated. Limits of detection in the range of 0.01 – 0.4 mg kg^{-1} were obtained. Spike-recovery tests were accomplished and the results varied between 93 and 124%. The accuracy of the proposed method was confirmed by the analysis of certified reference material (CRM) NIST SRM 1084a (Wear-Metals in Lubricating Oil); there was no statistical difference between the obtained results and the certified values (at 95% confidence level). Twelve B7-diesel oil samples were analyzed and Cu, Fe and Cr were found in three samples. The proposed method was simple, fast and accurate. The sequential multi-element determination of trace elements presented advantages as low costs and reduction in analysis time, being appropriate for routine analysis.

Keywords: Diesel oil analysis, F AAS, high-resolution continuum source, microemulsion, sequential multi-element determination

Cite: dos Santos, H. S.; de Jesus, A.; Laroque, D. O.; Piatnicki, C. M. S.; Silva, M. M. Multi-Element Determination of Trace Elements in B7-diesel Oil by High-Resolution Continuum Source Flame Atomic Absorption Spectrometry. *Braz. J. Anal. Chem.*, 2021, *8* (30), pp 59–70. doi: http://dx.doi.org/10.30744/brjac.2179-3425.AR-53-2020

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INTRODUCTION

Diesel oil is one of the most important derivatives from crude oil. It is a mixture of hydrocarbons which contains from 9 to 20 carbon atoms and with boiling point ranging between 170 and 370 °C [1]. Diesel engines present advantages in output power and fuel efficiency; due to this characteristic diesel engine became the choice for several kinds of transport such as passenger vehicles, heavy trucks, buses, trains, boats, ships and others [2]. In Brazil, it is mandatory to add biodiesel to diesel oil commercially available in the country [3]. In 2005, the percentage of biodiesel added was initially 2% (v/v). When the experimental part of this work was started the percentage of biodiesel was 7%, so-called B7-diesel oil. The amount of biodiesel should be increased gradually until a maximum concentration of 15% (v/v), and this percentage should be reached around of 2023, according to Brazilian government projections [3]. Quality control of fuels is important because the presence of some undesirable elements and compounds can lead to fuel degradation and / or instability [4]. Among these elements, metals have a remarkable negative role. The presence of metals (such as Cu, Fe, Ni, Pb, Zn, Al, Cr and Sn) in diesel oil may have several origins: from the raw material, corrosion processes in the storage tanks, leaching during the distillation and refining processes or even contamination from additives [5]. Copper and Ni can promote auto-oxidation reactions, decreasing fuel efficiency due to gum formation [6]. These elements also reduce the effectiveness of vehicles catalysts, increasing the emission of carbon monoxide, sulfur and nitrogen oxides. Iron, Cu, Al and Cr present in storage tank alloys and from the distillation process are known to damage engines and boilers [7]. Moreover, metals as Cr, Ni, Zn, Al and Sn are known by their toxic and / or carcinogenic potential [8,9]. Thus, the control of trace elements such as Cu, Fe, Ni, Pb, Zn, Al, Cr and Sn is essential in order to ensure the fuel quality and the potential harmful effects of releasing these elements to the environment. Even with all negative aspects presented, metals concentration is not controlled in diesel oil, according to American Standard Test Method (ASTM) D396 [10].

Usually, metals are present in fuels in trace levels, requiring sensitive and reliable techniques. Spectrometric techniques are highlighted in relation to accuracy among the techniques used for quantification of trace elements in fuels; some examples are flame atomic absorption spectrometry (F AAS), graphite furnace atomic absorption spectrometry (GF AAS), inductively coupled plasma-optical emission spectrometry (ICP OES) and inductively coupled plasma-mass spectrometry (ICP-MS) [11,12]. The use of a continuum source together with high-resolution monochromator (HR-CS) has brought many advantages for the AAS techniques. The use of high-intensity xenon short-arc lamp as a continuum source, a high-resolution double-echelle monochromator and a CCD array detector provides the possibility to determine many elements in a fast sequential mode when F AAS is used, with high precision, efficient background correction [13], higher tolerance for organic liquids analysis and lower costs than other multi-element techniques.

The direct analysis of fuels without any pre-treatment is limited due to their complex matrices, volatility and flammability. Thus, they must be properly converted in a format compatible with the instrumentation. Moreover, when methods based on nebulization are used, differences of viscosities and in other physical characteristics between samples and calibrations solutions must be carefully evaluated [14,15]. There are several methods for fuel samples preparation. Methods based on dilution, emulsion or microemulsion (ME) preparation and sample decomposition are among the most used [15]. The sample preparation can require excessive manipulation of samples resulting in loss of analytes and/or contamination of the sample with reagents used in the process.

Methods based on formation of ME are flexible and very attractive for complex matrices as petroleum derivatives [12,16], fuels [15,17], and biofuels [14,18,19]. These systems are compatible with external calibration using aqueous standards and the sample preparation time is reduced, when compared with digestion procedures. Moreover, higher stability of the analytes in the ME has been reported [18], especially when acidified solution is used, and no carcinogenic reagents are required, such as xylene (frequently used in the dilution methods) [16,17]. Amorin Filho et al. [20] evaluated different sample preparation methods for the determination of Cu, Cr, Fe, Ni, Pb, Sb and Zn in lubricating oil by HR-CS F AAS. The

compared procedures were microwave-assisted acid decomposition, direct dilution in kerosene, and oil-in-water emulsification. The authors reported that emulsified aqueous medium could be used for calibration and reduction in organic wastes and minimization of time and costs involved in the analysis was achieved.

Microemulsions are optically transparent and thermodynamically stable dispersion systems of at least three components: a polar and a non-polar liquid phase (water and oil respectively), and a suitable surfactant often in combination with a co-surfactant such as an aliphatic alcohol [21,22].The use of microemulsified systems for diesel oil has been reported in the literature for different purposes [23,24]. Quadros et al. [7] reported the use of ME as sample preparation for the determination of trace elements in B5-diesel oil diluted with *n*-propanol and HNO_3 by high-resolution continuum source electrothermal atomic absorption spectrometry (HR-CS ET AAS), using calibrations against aqueous standards for Cu, Fe, Mn and Pb, and standards prepared in *n*-propanol for Al and Cd. The authors highlighted the fast, precise and accurate results. A method based on extraction induced by emulsion breaking (EIEB) was proposed by Cassella et al. [1] for Zn determination in diesel oil by F AAS. This author also determined Al, Cu, Mn, Ni, Sn and V in diesel oil using the same sample preparation (EIEB) but by ICP-MS [25]. In both works, preconcentration factors of five times were obtained improving significantly the limits of detection obtained. In should be pointed out that the sequential multi-element determination of trace elements in diesel oil by HR-CS F AAS has not been reported yet, justifying the investigation of this technique for this application, associated with a very advantageous sample preparation method based on ME.

Thus, the goal of this work is to propose a simple, fast and accurate method for the sequential determination of Cu, Fe, Ni, Pb, Zn, Al, Cr and Sn in microemulsions of B7-diesel oil samples by HR-CS F AAS. The elements selected in this work have negative aspects to the quality of B7-diesel samples or have toxic potential when released into the environment. The ME formation with and without surfactant was investigated through a three-phase diagram. The accuracy of the method was investigated by analyzing a certified reference material (CRM) and by recovery tests.

MATERIALS AND METHODS

Instrumentation

The determinations of Cu, Fe, Ni, Pb, Zn, Al, Cr and Sn in B7-diesel oil were carried out with a ContrAA 300 high resolution continuum source atomic absorption spectrometer (Analytik Jena AG, Germany) equipped with a xenon short-arc lamp (continuum spectrum emitted between 190 and 900 nm, operating in a hot-spot mode), a prism pre-monochromator, an echelle grating monochromator for high resolution (about 1.5 pm per pixel at 200 nm) and a charge-coupled device (CCD) array detector. This arrangement allows a fast sequential multi-element analysis in the flame mode. The determinations were performed with air - acetylene flame (for Cu, Fe, Ni, Pb and Zn) and nitrous oxide - acetylene flame (for Al, Cr and Sn). The aspiration rate of 2.8 mL min^{-1} and a 50 mm burner were used for all elements. Nebulizer chamber with sealing rings resistant to organic solvents were used (Analytik Jena AG, Germany). The optimized instrumental parameters for each analyte are presented in Table I.

High-purity acetylene (99.0%, from White Martins, Brazil) was used as fuel. Compressed air provided by an air compressor (model FIAC CDS 8/50, Araraquara, São Paulo, Brazil) was used as oxidant for Cu, Fe, Ni, Pb and Zn, and nitrous oxide (99.0%, from White Martins, Brazil) was used for Al, Cr and Sn. A sub-boiling distiller (Kürner Analysentechnik, Rosenheim, Germany) was used for nitric acid distillation.

Table I. Instrumental Parameters for Determination of Cu, Fe, Ni, Pb, Zn, Al, Cr and Sn in B7-diesel Oil Samples by HR-CS F AAS

Analyte	Wavelength (nm)	Relative sensitivity (%)	Flame	C_2H_2 flow rate (L h^{-1})	Reading Height (mm)	Pixels evaluated
Cu	324.754	100	C_2H_2 - Air	40	5	CP[a] ± 1
Fe	248.327	100	C_2H_2 - Air	60	6	CP ± 1
Ni	232.003	100	C_2H_2 - Air	45	6	CP ± 1
Pb	217.000	100	C_2H_2 - Air	65	6	CP ± 2
Zn	213.857	100	C_2H_2 - Air	45	6	CP ± 1
Al	396.152	91	C_2H_2 - N_2O	185	5	CP ± 1
Cr	357.868	100	C_2H_2 - N_2O	180	4	CP ± 2
Sn	224.605	100	C_2H_2 - N_2O	235	4	CP ± 2

[a]CP: Center pixel.

Reagents, Solutions and Samples

Analytical grade reagents were used throughout. Solutions and ME were prepared with distilled and deionized water (DDW) from a Milli-Q water purification system (Millipore, Bedford, MA, USA) with 18.2 MΩ cm resistivity. All containers and glassware used were previously decontaminated with a 1.4 mol L^{-1} nitric acid solution for at least one day and rinsed with DDW. A sub-boiling distilled nitric acid (Merck, Germany) was used for ME preparation. Other reagents used, *n*-propanol (Merck, Darmstadt, Germany), base mineral oil (0.87 g cm^{-3} Specsol, Quimlab, São Paulo, Brazil), oleic acid (Vetec, Rio de Janeiro, Brazil) and toluene (Synth, São Paulo, Brazil), Triton X-100 (Union Carbide, Danbury, Connecticut, USA), Cu, Fe, Ni, Pb, Zn, Al, Cr and Sn inorganic and organic standards (1000 mg L^{-1} from Specsol (São Paulo, Brazil)). For accuracy evaluation of the proposed method, the certified reference material SRM 1084a (wear-metals in lubricating oil) from the National Institute of Standards & Technology (NIST Gaithersburg, MD, USA) was used. The B7-diesel oil samples (S10 and S500; this designation denotes the maximum sulfur content allowed in the commercialized fuel) analyzed in this work were supplied by the CECOM (Centro de Combustíveis, Biocombustíveis, Lubrificantes e Óleos, Universidade Federal do Rio Grande do Sul, RS, Brazil) from different regions of Rio Grande do Sul State, Brazil.

Microemulsion Preparation

For the ME preparation, B7-diesel oil, aqueous phase (1.4 mol L^{-1} HNO_3) and *n*-propanol were mixed and shake manually for few seconds. The construction of two ternary diagram phases were accomplished by varying the proportions of B7-diesel oil, diluted nitric acid and *n*-propanol with and without surfactant (Triton X-100), respectively. The ME formation was evidenced through visual transparency. These procedures have been done at 25 °C, maintained by air conditioning of the laboratory. The ME composition adopted for analysis was 3.3 g of B7-diesel oil, 300 µL of water and the final volume of 10 mL filled with *n*-propanol (about 3.7 g). The nitric acid (1.4 mol L^{-1}) was used in ME composition (added within the water component) to increase analytes stability in the medium. The CRM analysis was carried out with 0.1 g of sample followed by the addition of 3.2 g of a previously analyzed B7-diesel oil sample whose concentration was bellow de LOD, i.e., free of analytes, (for the adjust of viscosity) and the final volume of 10 mL filled with *n*-propanol. The final volume was enough for the sequential multi-element determination of all analytes. A 0.74 g of mineral oil was used in the ME standards for calibration (replacing the B7-diesel oil), and metals

inorganic standards added into the aqueous phase. A blank solution was prepared with 0.74 g of mineral oil solution, aqueous phase (1.4 mol L^{-1} HNO_3) and *n*-propanol. Standards were prepared from aqueous stock solutions described above, pipetting the volume into the aqueous phase to give concentrations in the ranges of 0.5 – 2.0 mg L^{-1} for Cu; 1.0 – 3.0 mg L^{-1} for Fe; 0.5 − 2.0 mg L^{-1} for Ni; 1.0 − 4.0 mg L^{-1} for Pb; 1.0 – 3.0 mg L^{-1} for Zn; 5.0 – 20.0 mg L^{-1} for Al; 1.0 − 4.0 mg L^{-1} for Cr and 2.0 – 10.0 mg L^{-1} for Sn in 10 mL volumetric flasks.

Analytical Procedure

The sequential measurements of the eight elements in ME of blank, standards, and B7-diesel oil by HR-CS F AAS were carried out according to type of flame and wavelength. Thus, the sequence of measurement was: Cu, Fe, Ni, Pb, Zn (air-acetylene flame), Al, Cr and Sn (nitrous oxide-acetylene flame). The aspiration rate was manually optimized and a compromise condition based on the maximum absorbance for most of the elements was chosen. Experimental conditions as the acetylene flow rate and burner height were optimized automatically by the software, considering the maximum absorbance as criterion. The most sensitive absorbance lines were used for the measurements, the only exception was for Al, for what the absorbance line with 91% was used, which will be discussed in the results. The multi-element determinations were carried out with optimized conditions which were adjusted for each analyte, being the experimental conditions adjusted by the software before each analyte determination. In order to evaluate matrix effects, recovery experiments were performed with organic and inorganic standards. In the first experiment the sample were spiked with organic standards of each analyte to obtain the following final concentrations: 2.5 mg L^{-1} Cu and Fe; 2.6 mg L^{-1} Ni; 3.0 mg L^{-1} Pb and Cr; 4.0 mg L^{-1} Zn; 15.0 mg L^{-1} Al; and 10.0 mg L^{-1} Sn. A small volume of the organic standards diluted in mineral oil were added directly in the sample and the flask was closed and the mixture was homogenized under vigorous manual agitation for at least 15 min. The spike concentrations values were chosen to be within the linear range of the calibration curves of each analyte. The sample was left to rest and in the next day it was again agitated for 5 min before the ME preparation for analysis. In the spike with inorganic standards, the aqueous standards of each analyte were added to the aqueous phase during the ME preparation to obtain the following final concentrations: 1.5 mg L^{-1} Cu and Ni; 2.0 mg L^{-1} Fe and Zn; 2.6 mg L^{-1} Pb; 3.0 mg L^{-1} Cr; 15.0 mg L^{-1} Al; and 8.0 mg L^{-1} Sn. All measurements of standards, samples and spiked samples were carried out in triplicate (n = 3), according the parameters presented in Table I.

RESULTS AND DISCUSSION

Investigation of microemulsion composition

Based on our previous experience with ME of biodiesel [26-28], the composition of the ME investigated in this work was initially B7-diesel oil, water/nitric acid and *n*-propanol. The use of greater amount of water with nitric acid was attempted in order to facilitate the optimization of instrument after analysis of aqueous solutions. In order to obtain the region where a homogeneous and stable system could be achieved a ternary phase diagram was built (Figure 1-A) as described hereafter. The diagram has three components: B7-diesel oil, water (containing 1.4 mol L^{-1} HNO_3) and *n*-propanol. The points inside the triangle were plotted fixing the quantity of one component and varying the others (the variations were 5% (w/w) for each). Each point represents a mixture and has its proper physical characteristics (emulsified or transparent mixture) visually identified. Several proportions were tested until the emulsion / microemulsion limits could be obtained and a diagram representing the limits was built. Figure 1-A shows two distinct regions: region in gray (where a homogeneous and transparent solution is found (ME)) and region in white (where mixtures form emulsions). As can be seen, with a fraction of water/HNO_3 higher than 0.1 (10% w/w), the stabilization of the hydrophobic phase was not possible (favoring formation of an emulsion). Moreover, the maximum amount of water required a minimum amount of B7-diesel oil, reducing the sensitivity of the method. On the other hand, the minimum of water (about 5% w/w) allowed the use of a fraction of about 0.4 (40% w/w) of B7-diesel oil. In order to investigate if the use of surfactant (Triton X-100) would increase the ME area in

the phase diagram a second ternary phase diagram was built (Figure 1-B) with 13% (w/w) of Triton X-100, and volume completed to 10 mL with *n*-propanol. As can be seen, the use of the surfactant increased the ME region; higher amount of diesel (almost 70% w/w), could be stabilized considering the amount of water not exceeding 5% (w/w). Nevertheless, the amount of water allowed in the system to get the ME has reduced. The use of larger amount of sample increases the concentration of analytes in the in the ME, but also should increase the viscosity, reducing the nebulization efficiency and, thus, not resulting in the expected improvement in the limit of detection (LOD). Therefore, the use of surfactant as a constituent of the system was discarded, favoring the simpler method, avoiding a further preparation step and additional costs with the reagent. It should be pointed out that the homogenization of the ME containing 13% (w/w) Triton X-100 required a few minutes more for sample preparation, reducing the analyses throughput. Thus, the adopted composition was 3.3 g (about 40% w/w) of B7-diesel oil and 0.3 mL of H_2O/HNO_3, completed to 10 mL with *n*-propanol. It is important to emphasize that this composition is not in the border of the ME phase, shown in Figure 1-A. The adopted proportion was chosen because, even with small alterations in the proportion of the components of the system, e.g. the mass of sample or using samples with different characteristics (S10, S500 or different diesel/biodiesel mixtures), the formation of the ME is not affected. The ME developed in this work is water in oil (w/o) type, where the aqueous phase is dispersed in a continuous oil phase.

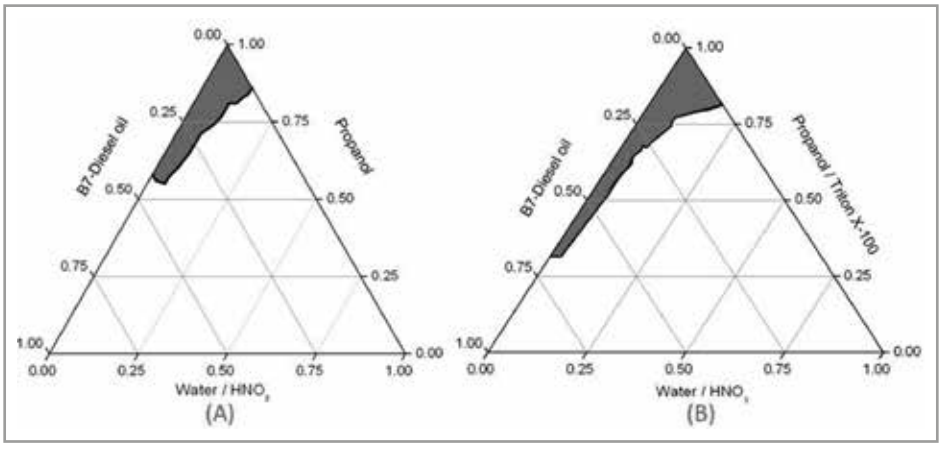

Figure 1. Ternary phase diagrams of B7-diesel oil, water/HNO_3 and *n*-propanol at 25 °C: (A) without Triton X-100 and (B) with 13% (w/w) Triton X-100. The component content in the system is in mass fraction. Microemulsion region is highlighted in gray.

Microemulsion for Calibration Curves

As pointed out by Sánchez et al. [14,15] the organic solvents can affect the nebulization process in many ways. Considering that the pneumatic aerosol is generated by interaction between the solution with the gas stream at high speed, the mean droplet size distribution in the aerosol depends on the physical properties of the solution, mainly the surface tension and viscosity. For organic solvents, surface tension is generally lower than for aqueous samples, while the viscosity shows a more variable behavior. The viscosity, e.g., affects both aspiration rate and nebulization, while density and volatility, plays important role in the transport of the aerosol [14,15]. All these events influence the amount of sample that reaches the atomizer. Thus, it is necessary that standards and samples have similar physical characteristics to prevent nebulization and transport interferences in F AAS analysis. In this work, oleic acid, toluene and mineral oil (in different proportions) were investigated as a substitute for the sample in the calibration standards. The results obtained by recovery tests with ME prepared with B7-diesel sample (free of analytes) were compared with those obtained with ME prepared with oleic acid, toluene or mineral oil (in different proportions). The best results were obtained when the sample was replaced by 0.74 g of mineral oil (about 9% w/w) as

shown by the results obtained for Cu (Figure 2). This study was carried out only for Cu considering that the physical interferences affect the transport of the solution, thus affecting all analytes in the same way. Consequently, the standards for calibration were prepared in the same way, replacing the sample by 0.74 g of mineral oil to simulate the diesel oil matrix in the ME and adding the inorganic standards into the aqueous phase. After the addition of all components, the system was manually shaken for a few seconds and the formation of a clear and stable solution was achieved. It should be emphasized that using DDW, sub-boiling distilled nitric acid and other analytical grade reagents, analytical signals measured in the ME blank were below de LOD for all analytes.

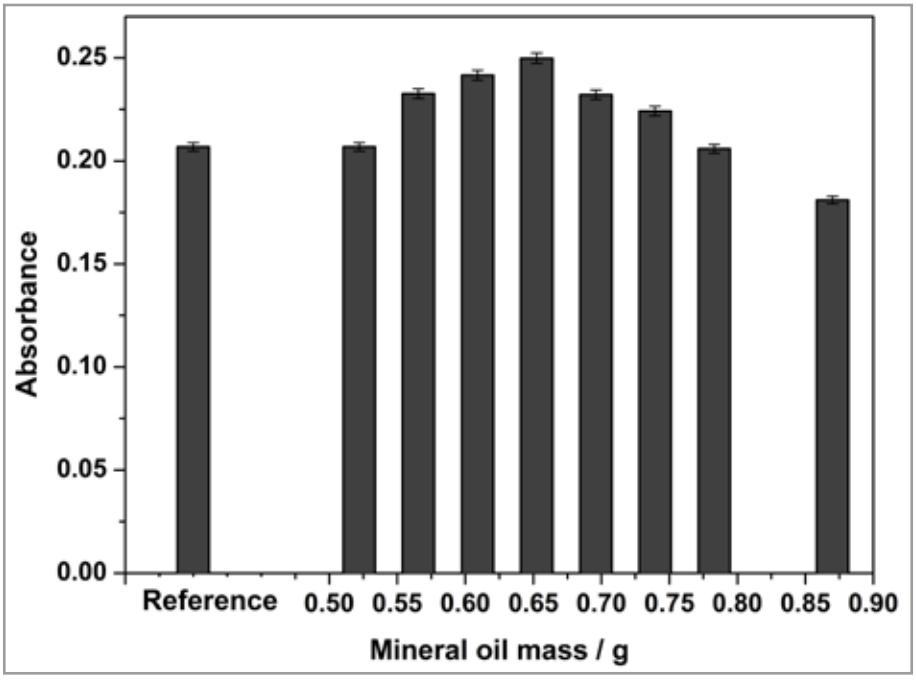

Figure 2. Evaluation of mineral oil mass added to the microemulsion for calibration. All systems contain 2 mg L^{-1} Cu, 300 µL of aqueous phase (1.4 mol L^{-1} HNO_3) and *n*-propanol (up to 10 mL). Reference: instead of mineral oil, this system contains 3.3 g of B7-diesel oil.

Optimization of the Spectral and Instrumental Parameters

The most sensitive analytical lines were used for all analytes, except for Al, for which the secondary line at 396.152 nm (91% relative sensitivity), was used. The main line for Al at 309.271 nm is within the range of strong OH absorption bands, which might cause some deterioration of the signal-to-noise ratio if not properly corrected [29]. The number of pixels used for measurements of the analytical signal was evaluated; for Pb, Cr and Sn an increase the sensitivity was obtained using 5 pixels for measurements (center and two lateral pixels; CP $\pm$ 2). For the other elements, no significant increase in the signal was obtained, thus only 3 pixels (center and one lateral pixel; CP $\pm$ 1) were used. These different behaviors are related with the peak profile of each element [29].

The optimization studies were performed for the following instrumental parameters: acetylene flow rate, burner height and aspiration rate. The adjustment of the aspiration rate was performed manually, observing the intensity of the analytical signal of each element, considering the maximum value of the analytical signal. With the aspiration rate near 2.8 mL min^{-1}, higher values of analytical signals were obtained for most of the analytes. Thus, the aspiration rate of 2.8 mL min^{-1} was adopted. The optimization of the other equipment conditions for analysis (burner height and acetylene flow rate) was automatically

carried out by the spectrometer software, taking the highest absorbance signal as parameter. Thus, the aspiration rate was the unique compromise condition, while the atomization and measurement conditions chose were the optimum for each element. Table II shows the selected conditions.

Figures of Merit

After the optimization procedures, calibration curves with inorganic standards added to ME were carried out for each analyte. The calibration parameters as linear regression equations, correlation coefficients as well as the figures of merit obtained for the method are presented in Table II. The characteristic concentration, (C_0), is defined as the analyte concentration that produces an absorbance signal of 0.0044 (1% absorption) with flame atomization [29]. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to International Union of Pure and Applied Chemistry (IUPAC) recommendations, as 3 times and 10 times, respectively, the standard deviation of 10 measurements of a blank solution, divided by the sensitivity of calibration curve. The characteristic concentrations obtained by the proposed method, presented values slightly higher than those reported in the literature [29]. Correlation coefficients greater than 0.99 (except for Zn) were obtained for all investigated analytes.

Table II. Figures of Merit Obtained for the Determination of Trace Elements in B7-diesel Oil Microemulsion by HR-CS F AAS and Comparison with Data from the Literature

	Proposed method					Other methods		
Analyte	Sensitivity (L mg^{-1})	R^2	C_0 [a] (mg L^{-1})	LOD[b] (mg kg^{-1})	LOQ[b] (mg kg^{-1})	LOD[c] (mg kg^{-1})	LOD[d] (mg kg^{-1})	LOD[e] (mg kg^{-1})
Cu	0.0960	0.9983	0.04	0.01	0.02	0.09	n.d	0.003
Fe	0.0403	0.9983	0.10	0.01	0.04	0.14	n.d	0.001
Ni	0.0380	0.9990	0.11	0.01	0.04	0.05	0.01	n.d
Pb	0.0247	0.9995	0.18	0.01	0.05	n.d[f]	0.01	0.0006
Zn	0.1316	0.9867	0.03	0.02	0.06	0.11	n.d	n.d
Al	0.0093	0.9995	0.47	0.07	0.3	0.12	n.d	0.003
Cr	0.0431	0.9943	0.08	0.4	1.4	n.d	n.d	n.d
Sn	0.0040	0.9998	1.00	0.3	1.1	n.d	n.d	n.d

[a] C_0: Characteristic concentration.
[b] The LOD and LOQ were calculated considering the mass of sample and final volume used in sample preparation (3.3 g and 10 mL, respectively).
[c] LOD obtained by ICP OES.
[d] LOD obtained by GF AAS.
[e] LOD obtained by HR-CS ET AAS.
[f] n.d: not determined.

Comparing the LODs obtained in this work with the values reported in the literature for fuel analysis such as diesel-oil, B5-diesel oil and gasoline, our results were similar to those obtained by other more sensitive techniques, such as ICP OES for Al, Cu, Fe, Ni and Zn [4] and GF AAS for Ni and Pb [30], and higher than the values obtained by F AAS for Zn with preconcentration (2.0 µg kg^{-1}) [1], and by HR-CS ET AAS for Al, Cu, Fe and Pb [7].

Analytical Results

To assure the accuracy of the proposed method the CRM NIST SRM 1084a (Wear-metals in lubricant oil) was analyzed. For this, the ME was prepared according to the procedure described in the Experimental

section (ME preparation). The results obtained (Table III) were not significantly different of the certified values at the 95% confidence level (Student's *t* test; the calculated *t*-values: Pb (1.083), Ni (3.897), Fe (2.853), Cu (2.665), Sn (1.915) and Cr (3.343) were lower than the *t*-critical = 4.303). Unfortunately, there are not certified values for Al and Zn in this material.

Table III. Analytical Results Obtained for NIST SRM 1084a by the Proposed Method (mean ± sd; n=3)

Analyte	Certified value (mg kg^{-1})	Obtained value (mg kg^{-1})
Cu	100.0 ± 1.9	102.0 ± 1.3
Fe	98.9 ± 1.4	104.5 ± 3.4
Ni	99.7 ± 1.6	101.5 ± 0.8
Pb	101.1 ± 1.3	103.6 ± 4.0
Cr	98.3 ± 0.8	106.6 ± 4.3
Sn	97.2 ± 2.6	91.2 ± 5.4

In order to evaluate the matrix effect, recovery experiments were performed by spiking some selected samples of B7-diesel oil with inorganic aqueous standard and organic oily standard of all analytes as described in the Experimental (Analytical Procedure). The recovery values obtained were very satisfactory for most of elements, ranging from 93% to 110%, confirming the absence of the matrix effects; the exceptions were the results obtained with organic standard for Al (124%) and Sn (119%), as shown in Table IV. These relatively worse recovery values may have occurred because the mixture of samples and organic standards was not sufficiently homogeneous. However, the good results presented for most elements show also the similar behavior of organic and inorganic standard, corroborating the possibility of using inorganic aqueous standards for calibration.

Table IV. Recovery Tests Carried out with Inorganic and Organic Standards Added to B7-diesel Oil Prepared as Microemulsions (mean ± sd; n=3)

	Inorganic standards		Organic standards	
Analyte	Spike (mg L^{-1})	Recovery (%)	Spike (mg L^{-1})	Recovery (%)
Cu	1.5	101.7 ± 2.1	2.5	94.7 ± 0.1
Fe	2.0	99.7 ± 5.3	2.5	96.7 ± 0.2
Ni	1.5	96.4 ± 3.8	2.6	109.6 ± 0.03
Pb	2.6	93.2 ± 0.5	3.0	96.5 ± 0.1
Zn	2.0	104.7 ± 2.9	4.0	95.2 ± 0.05
Al	15.0	99.8 ± 4.5	15.0	124.2 ± 0.3
Cr	3.0	103.7 ± 2.1	3.0	102.9 ± 0.1
Sn	8.0	96.0 ± 1.8	10.0	118.8 ± 0.4

Twelve B7-diesel oil samples were analyzed in this work (ten S10 and two S500). Low concentrations of Cu (0.18 ± 0.02 mg kg^{-1}**)** and of Fe (0.05 ± 0.02 mg kg^{-1}**)** in one S10 diesel oil sample and of Cr (0.9 ± 0.2 and 0.8 ± 0.1 mg kg^{-1}**)** in two S500 diesel oil samples were found. The other samples did not present concentrations of the analytes above the LOD of the method, evidencing the good quality of the diesel oil samples. The results obtained in this work for Cu and Fe were similar to the concentrations range found in the literature using different sample preparations and techniques [4,7,31,32]. Some papers reported concentrations above the LOD for Ni, Pb, Zn and Al in diesel oil using more sensitive techniques [4,7,25,31,32] or preconcentration procedures [1,33,34]. It should be mentioned that in Brazil there is no legislation establishing maximum values for concentrations of any trace metal in diesel oil.

CONCLUSION

The method proposed in this work proved to be fast, simple and accurate for the multi-element sequential determination of Cu, Fe, Ni, Pb, Zn, Al, Cr, and Sn in B7-diesel oil samples by HR-CS F AAS. The sample preparation based on simple formation of ME of samples with *n*-propanol and water/nitric acid showed to be a fast approach for trace elements determination, when compared with digestion procedures. The construction of a phase diagram has provided the variations possibilities for sample preparation (composition, amount of sample, etc.). Moreover, the good results obtained in this work suggest that this procedure might be used with minor modification for analysis of other biodiesel/diesel mixtures or determinations of other elements in B7-diesel oil. It should be stressed that a minimum sample preparation, the use of aqueous standard in calibration and the sequential multi-element determination of analytes allowed a fast and low-cost analysis. The total time to determine eight analytes was around 5 min, due to the adjustment of wavelengths, flame parameters and composition. These features, together with LODs comparable to those of multi-elemental or more sensitive techniques, added to the robustness of flame for organic liquids analysis and the lower cost of implementation and operation, are the clear advantages of the proposed method. Moreover, it can be considered appropriate to the principles of Green Analytical Chemistry, as a small amount of a nontoxic solvent is used (5.7 mL of *n*-propanol/sample). Thus, the proposed method can be suggested as an alternative for routine analysis.

Conflict of interest

The authors declare no competing financial interest.

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BrJAC

FEATURE

PDF

Analytical Chemistry in the Fight for Life

According to a survey released on 26/01/2021 by Johns Hopkins University, the new coronavirus has already infected more than 100 million people and killed 2 million around the world. In Brazil, there have been almost 8 million confirmed cases of COVID-19 and more than 200,000 deaths from the disease. The United Nations (UN) has declared that the current pandemic is humanity's greatest challenge since World War II.

Faced with this situation, short-, medium-, and long-term actions, such as understanding how the virus causing COVID-19 acts in the human body, developing inputs for diagnostic tests, researching new uses of established drugs, using technology to produce and repair mechanical ventilation equipment, and producing 70% alcohol on a large scale for distribution in public health units, are being developed by chemistry professionals in association with specialists in other scientific areas to confront the coronavirus pandemic throughout Brazil.

An example of these actions is the test for COVID-19 that employs artificial intelligence algorithms and generates results in just 20 minutes, which was developed by a group of pharmaceutical, biological, medical and computer scientists from the University of Campinas (Unicamp), University of São Paulo (USP), São Paulo state regional hospitals, together with professionals from institutions in the State of Amazonas, Brazil.

This test developed by Brazilian scientists for COVID-19, and coordinated by Prof. Dr. Rodrigo Ramos Catharino, coordinator of the Innovare Biomarkers Laboratory at the Faculty of Medical Sciences at Unicamp, uses artificial intelligence algorithms to analyze data obtained by high-resolution mass spectrometry using blood plasma samples and metabolomics concepts to identify a characteristic pattern of molecules in patients with the disease. According to the article published in the scientific journal *Analytical Chemistry* [1], in addition to replacing the reagents with artificial intelligence algorithms, this test is able to predict which infected people will be at higher risk of suffering complications due to infection by the SARS-CoV-2 virus.

"The software captures the signals measured on the mass spectrometer and learns which molecules in the body are responsible for the positive and negative diagnosis. In addition, it also learns which molecules are responsible for severe and mild cases of infection with SARS-CoV-2. This test is an important tool for the medical team to make decisions for the treatment because it makes a very accurate screening of those who need more care and hospitalization. In this way, the test helps to save lives at the front lines of the fight against COVID-19," said the research coordinator, Dr. Catharino.

"Initially, the project had the participation of 728 patients, of which 369 were diagnosed with COVID-19 clinically and by RT-PCR. Samples from uninfected individuals were used for comparison, as a kind of control group. In the case of some infected patients who developed complications and needed to be hospitalized, a second blood sample was collected. In general, among patients with confirmed COVID-19, there were individuals with mild and severe symptoms," explained one of the research coordinators, the PhD student Jeany Delafiori.

The use of the machine learning technique to search for markers capable of assisting in the diagnosis is very important because this technique is capable of accumulating knowledge and improving its performance as more samples are analyzed. "If today this test has a 90% accuracy rate, it is likely that this rate will increase even more when we reach thousands of patients analyzed," says Prof. Dr. Anderson Rocha of the Institute of Computing at Unicamp. In addition to COVID-19, other diseases may be diagnosed by the platform through databases on other diseases.

Prof. Dr. Rodrigo R. Catharino
Innovare Biomarkers Lab
Coordinator

Jeany Delafiori, PhD student
Faculty of Medical Sciences
Unicamp

Prof. Dr. Anderson Rocha
Institute of Computing
Unicamp

Other advantages of this test are the speed of diagnosis, practicality of use, and low price in relation to the COVID-19 tests that are currently available on the market. This new test provides a COVID-19 diagnosis in about 20 minutes, while other tests can take days. In addition, the cost of the RT-PCR test in Brazil is between R$ 80 and R$ 100, while the cost of the test developed by Brazilian scientists should be R$ 40.

This diagnostic method seeks to minimize one of the greatest difficulties in halting the progress of the new coronavirus pandemic in Brazil, which is the low testing done on the population. The joint effort of universities aims to provide testing, case locations, and tracking of the spread of the virus at low cost and high speed. “As it is about developing a new diagnostic concept, we need financial support for the implementation of this test. Cooperation agreements can be established through the Innovation Agency Inova Unicamp,” said Dr. Catharino.

The test has not yet been approved by the National Health Surveillance Agency (Anvisa); however, the expectation is to submit for approval as soon as possible.

Another important example of action in Brazil to face the COVID-19 pandemic came from Fleury Group, a private diagnostic center, which also developed a new test for the diagnosis of COVID-19. The project led by the biochemists Dr. Valdemir Melechco Carvalho and Dr. Karina Helena Morais Cardozo resulted in a pioneer proteomics assay. The test is based on the analysis of specific peptides from the new coronavirus proteins directly from respiratory tract clinical samples. This method is considered as reliable as the gold standard RT-PCR test, which detects the RNA of the virus.

Dr. Valdemir Melechco Carvalho
Scientific Advisor to Fleury Group

Dr. Karina Helena Morais Cardozo
Scientific Advisor to Fleury Group

The Fleury test was initially developed with micro chromatography and high-resolution mass spectrometry and subsequently implemented in multidimensional turbulent flow chromatography coupled to tandem mass spectrometry, which enables robustness and speed for routine work. It is fully automated, which reduces the analysis time and risks in handling the samples. With the potential to analyze more than 1,500 samples per day, this new test is a high-throughput targeted proteomics assay that generates results in up to 2 days. According to the Fleury Group, the new test may be an alternative in case of a shortage of consumables for the RT-PCR test. Also, it provides a testing option for the most remote areas of Brazil since the proteins are more stable than RNA simplifying the logistics for specimen transportation.

This proteomics test is recommended for use in the first three to seven days of COVID-19 symptoms and is performed directly from nasopharyngeal and oropharyngeal swabs, similar to RT-PCR. It was validated in a study with more than 1000 samples previously analyzed by RT-PCR, and it was able to detect more than 83% of positive cases [2]. This proteomics test is currently available for hospitals, laboratories, and clinics in regions far from major centers in Brazil.

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FEATURE

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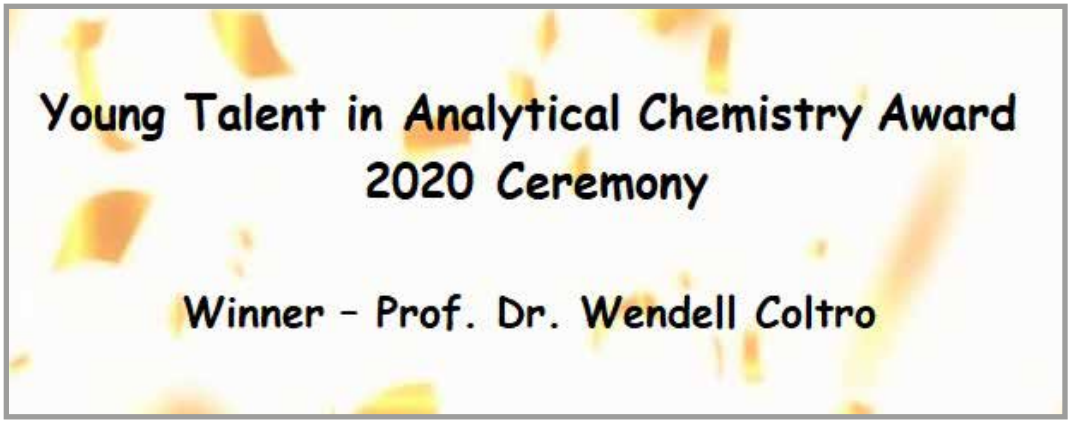
BrJAC Young Talent in Analytical Chemistry Award 2020

BrJAC created the annual Young Talent in Analytical Chemistry award to recognize outstanding young researchers in Analytical Chemistry. The winner of the 2020 award was Prof. Dr. Wendell Karlos Tomazelli Coltro, Associate Professor and Director of the Institute of Chemistry at the Federal University of Goiás (IQ-UFG), Brazil.



“I was extremely happy and honored with the recognition given to me by BrJAC. This award crowns almost 20 years of total dedication to science, including almost 7 years of post-graduation studies involving master’s, doctorate, and post-doctorate education and 12 years leading a dedicated and committed research group at the Federal University of Goiás. Therefore, there are no words to express my feeling for the recognition that BrJAC gave me by awarding me the Young Talent in Analytical Chemistry Award. Without the dedication of all members of our research group and without family support from my wife and two daughters, this certainly would not have happened. For this reason, I dedicate this award to my research group and my family” thanked Coltro when receiving the award.

The award ceremony was held online on November 26, 2020 with the participation of the special guest Prof. Dr. Boniek Gontijo Vaz (IQ-UFG) who presented the scientific curriculum of the awardee. After the award ceremony, Prof. Wendell Coltro presented a live webinar entitled “Portable Analytical Tools for Bioanalytical and Forensic Applications”.

Access a video of the award ceremony and the webinar **here**

In a brief interview for BrJAC, Prof. Dr. Wendell Coltro described how his career started and advised those who are starting their scientific careers. Check it out below:

BrJAC: How did you start your professional career?
Coltro: My career began in 1997. I was finishing high school and liked chemistry and mathematics subjects. At first, I thought about taking a course in Chemical Engineering at the State University of Maringá in Paraná, where I passed the third call in a winter entrance exam. However, at the end of the same year, after clarifying my doubts about a career in chemical engineering, I decided to take a new entrance exam, now for the Chemistry course, and I was approved at the same institution. I concluded the Chemistry course in 2002, and in the middle of the same year I decided to do postgraduate studies. I participated in the selection processes of the post-graduate programs in Chemistry at the University of Campinas and

at the University of São Paulo in the city of São Carlos. I chose to attend the post-graduation course in São Carlos under the guidance of Prof. Dr. Emanuel Carrilho, who proposed a research project involving microfabrication, electrophoresis, and electrochemical detection. It was very challenging, and I had many instrumental and operational difficulties because the area I chose was very innovative. Today, seeing everything we have built scientifically, I would do it all over again in the same way and with the same advisor. I feel very proud to have been guided by a professional like Prof. Carrilho, who always showed me the paths to follow, with great humility and sincerity.

BrJAC: What advice would you like to offer to those at the beginning of a career in science?
Coltro: We are experiencing a moment of extreme difficulty, where the uncertainties are increasing. The best advice I would give the youngest is to have perseverance, dedication and commitment. This tripod is essential to achieve our goals. If it does not happen as planned, it will happen through new discoveries! This is what I learned from my advisor and what I pass on to all the students that I guide. The result is always positive.

BrJAC: What are your plans for the future?
Coltro: In the face of all the adversities we are going through, the future is very uncertain. However, I hope to continue contributing to scientific and technological progress with the great goal of seeing society benefit from new technologies that favor improved quality of life. For this to happen, we must narrow the divide between academia and the productive sector.

BrJAC: What you are currently working on?
Coltro: Currently, I coordinate the Microfluidics and Electrophoresis Group, located at the Institute of Chemistry of the Federal University of Goiás, Brazil. Our group focuses on the development of portable devices for applications in diverse areas, such as environmental, bioanalytical, biomedical, forensic, and clinical diagnostics areas. Among the devices, it is worth highlighting the effort in search of simple, accessible, inexpensive materials that, at the same time, offer reliability for quick analysis directly at the point of need. In addition, our group has been active in the manufacture of microfluidic devices, electrochemical devices, and wearable sensors via 3D printing.

Prof. Dr. Wendell K. T. Coltro with the Young Talent in Analytical Chemistry Award certificate.

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FEATURE

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Live BrJAC Webinars
Check out the recent BrJAC initiative

BrJAC, like all of us, also had to adapt to the new reality of the year 2020 with the new coronavirus pandemic. To remain connected to its readers, in addition to maintaining its regular publications, BrJAC held live webinars.

The first live webinar was held on August 6, 2020, with the presentation entitled “Development and Application of Methods for the Determination of Residues and Contaminants in Food and Environmental Samples” by Prof. Dr. Renato Zanella, Full Professor of the Department of Chemistry at the Federal University of Santa Maria, Brazil.

At the end of this webinar, the book titled *Preparo de Amostras para Análise de Compostos Orgânicos*, by Keyller Bastos Borges, Eduardo Costa de Figueiredo, and Maria Eugenia Costa Queiroz (authors), was drawn among the participants. The winner of this book was Dr. Carla Toledo Neira from the Universidad de Santiago de Chile, Chile.

Access a video of this webinar **here**

The second live webinar was held on September 25, 2020, with the presentation entitled “Analysis of Clinical Biomarkers by ICP-MS: From Speciation to Single-Cell Approaches” by Prof. Dr. Maria Montes-Bayón, Full Professor at the Dept. of Physical and Analytical Chemistry, University of Oviedo, Spain.

The book drawn was *Metallomics: The Science of Biometals* by Marco Aurélio Z. Arruda (editor). The winner of this book was Prof. Dr. Márcia M.A.S. Veiga, Associate Professor in the Dept. of Chemistry at the Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo, Brazil.

Access a video of this webinar **here**

The third live webinar was held on October 30, 2020, with the presentation entitled “Analytical Strategies for Environmental and Ecotoxicological Studies” by Prof. Dr. Marco Tadeu Grassi, Associate Professor in the Dept. of Chemistry, Federal University of Paraná, Brazil.

The book drawn was *Principles of Environmental Chemistry* by James E. Girard (author), and the winner was Prof. Dr. Gilberto Abate from the Federal University of Paraná, Brazil.

Access a video of this webinar **here**

The fourth and last live webinar in 2020 was held on November 26th and was entitled “Portable Analytical Tools for Bioanalytical and Forensic Applications” by Prof. Dr. Wendell Karlos Tomazelli Coltro, Associate Professor and Director of the Institute of Chemistry at the Federal University of Goiás, Brazil.

In addition to presenting this webinar, Prof. Wendell Coltro was also honored with the Young Talent in Analytical Chemistry Award 2020, which is an award offered by BrJAC to recognize outstanding young researchers in Analytical Chemistry.

The book drawn was *Forensic Analytical Methods* by Thiago R. L. C. Paixão, Wendell K. T. Coltro and Maiara Oliveira Salles (editors), and the winner was Dr. Marcos Aurélio Gomes da Silva, Dept. of Chemistry, Federal University of Juiz de Fora, Brazil.

Access a video of this Webinar **here**

In 2021, BrJAC will offer a New Program of live webinars, so stay tuned to our social networks so as not to miss the next editions of the BrJAC Live Webinars.

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SPONSOR REPORT

PDF

This section is dedicated for sponsor responsibility articles.

Total Fat Determination in any Matrix by Simultaneous, Robust and Comprehensive Hydrolysis and Extraction Microwave Approach

This report was extracted from the Milestone Application Report: Total Fat Determination in Food and Feed samples

The determination of total fat content in food and feed samples is a common task for food industry quality control labs and for third party contract labs. The classical procedures use different methods based on the matrix, antique technology and approaches. Analytical laboratories have to manage several methods in order to cover the demand while increasing the overall analysis costs. The Milestone ETHOS X used for total fat determination enables a simultaneous hydrolysis and extraction and can be applied on all food matrices, as it is not matrix dependent. With the ETHOS X, the fat analysis costs and turnaround time are strongly reduced. The ETHOS X enables to perform the total fat determination in just a few hours.

INTRODUCTION

The Nutrition Facts Label is nowadays fundamental for packaged foods and drinks. The U.S. Food and Drug Administration (FDA) has recently updated the information required on nutrition facts labels in order to better inform consumers on quality and nutritional parameters of products [1].

Fat content is by far one of the most important parameters. The determination of total fat, saturated fat, monounsaturated fat and *trans* fat content in food samples is necessary to comply with the food labeling requirements. In particular, total fat content plays a pivotal role for several reasons. The evaluation of total fat content allows to properly dose other expensive ingredients, to comply with nutritional labeling regulations, to produce healthy and quality food with a low fat content, and to select the right process conditions according to the lipid content. All these considerations elevate total fat value to an important parameter for Economic, Legal, Health, Quality and Process evaluations. Fat determination is of interest also for the feed industry since its value allows to set the quality and price of feed products.

Several determination protocols are available for the analysis of total fat content in food and feed samples; most of them are selective for specific food classes and cannot be applied to others. This leads to the application of several determination protocols depending on the food stuff that has to be analyzed.

Moreover, most of the conventional protocols involve an elevated use of organic solvents and long processing times since they are often based on hydrolysis with inorganic acids, followed by the Soxhlet extraction with organic solvents. This leads to considerable costs due to a high solvent consumption and a long turnaround time.

Microwave energy sources have been widely applied both in elemental and molecular sample preparation and have been tested in this application report for the total fat determination. In particular, an innovative total fat determination protocol was developed enabling the simultaneous hydrolysis and extraction process within a sole step. This approach aims to provide a unique method for the total fat determination of all food and feed samples by dramatically reducing both the overall analysis time and the solvent consumption. This method allows analytical laboratories to deliver total fat analysis in a few hours reducing costs and solvent wastes.

EXPERIMENTAL

Equipment

- Milestone ETHOS X
- SR-15 eT extraction rotor
- RAR-15 evaporation rotor
- Aluminum caps
- Vacuum system with condensation module
- Analytical balance (with direct interface to ETHOS X terminal)

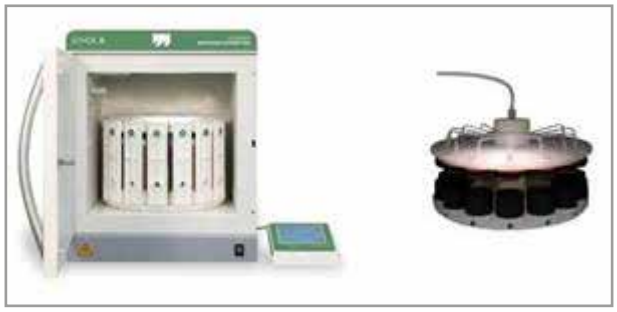

Figure 1. Milestone's ETHOS X with SR-15 extraction rotor (left) and RAR-15 evaporation rotor (right).

Solvents and Reagents

Solvents and reagents were purchased by Sigma Aldrich. Sulphuric acid (25%) and Cyclohexane ACS reagent grade were used.

Samples

For this study, certified reference materials (BCR and ERM), quality control samples and labeled food stuff (purchased at the grocery store) were used (Tables 2-4). Regarding commercial food stuff, the samples should be homogenized before the weighing step in order to get a representative aliquot of sample. The sample has been used as it is, avoiding any drying step.

Procedure

Approximately from 1 to 3 g of sample was directly weighed into the SR-15eT extraction vessels; 10 mL of sulphuric acid (25%) and 25 mL of cyclohexane were subsequently added, recording its final mass. Magnetic stirring bars were added to each vessel. The SR-15eT was properly assembled. The microwave program is reported in Table 1.

Table 1. ETHOS X microwave hydrolysis and extraction program

STEP	TIME	T	POWER	Stirrer
1	00:03:00	90 °C	1400 W	80%
2	00:04:00	135 °C	1400 W	80%
3	00:40:00	135 °C	1400 W	80%
		Cooling		

At the end of the program, the SR-15 eT vessels were opened and the aliquots of the organic phase were transferred into aluminum caps and then weighed. After a fast solvent evaporation, using the RAR-15 evaporation rotor, the aluminum caps were newly weighed. The ETHOS X-easyCONTROL software tracks and records all the steps and weights necessary for the calculation. Total fat values are delivered at the end of the run thanks to the capabilities of the easyCONTROL software and the direct interface with the analytical balance. The data can be saved with the possibility to generate customized reports.

RESULTS AND DISCUSSION

In this study, the total fat content of several food samples was analyzed by applying the ETHOS X simultaneous hydrolysis and extraction method.

Despite the availability of several sequential total fat determination methods, the ETHOS X method enhances total fat determination by simultaneously performing hydrolysis and extraction during the same heating run with up to 15 matrices. Moreover, thanks to the RAR-15 evaporation rotor, the ETHOS X microwave extraction platform also extends its capacity to the evaporation step. For this purpose, a mix of certified reference materials (CRM), quality control samples and foodstuff locally purchased at grocery stores were used.

Several food samples were tested, ranging from cookies, dairy, meat, sausages and even feed samples, among others. Tables 2 to 4 report all the samples tested with the total fat results and the relative standard deviations. The samples were selected to explore a wide range of total fat content, from condensed milk (0.33%) to butter (81.37%) samples.

Table 2. ETHOS X total fat method – Data on CRM materials (n=12)

		Reference values		ETHOS X results	
Sample	**ID**	**Total Fat (%)**	**Uncertainty (%)**	**Total Fat (%)**	**RSD (%)**
Whole Milk Powder	BCR-380R	26.95	± 0.16	26.3	0.23
Wheat Flour	ERM-BC382	1.39	± 0.17	1.41	0.2
Lyophilized Pork Muscle	ERM-BB384	8.99	± 0.2	8.63	0.21
Condensed Milk	TET036RM	0.33	± 0.07	0.29	0.06
Dairy Feed	BCR-708	6.5	± 0.8	6.32	0.28

Table 3. ETHOS X total fat method – Data on quality control samples (n=12)

		Reference values		ETHOS X results	
Sample	**ID**	**Total Fat (%)**	**Acceptability [Range for |z|≤2] (%)**	**Total Fat (%)**	**RSD (%)**
Porridge Oats	T2477QC	7.82	7.36 –8.28	8.03	0.187
Butter	T25160QC	81.37	80.83 –81.91	81.38	1.28
Fish Paste	T25163QC	4.43	3.77 –5.10	4.4	0.07
Chocolate	T25166QC	34.85	33.52 –36.17	35.74	0.67
Fat Spread	T14190QC	66.47	64.8 -68.1	68.0	0.48

Table 4. ETHOS X total fat method – Data on samples purchased at grocery store (n=12)

	Reference values	ETHOS X results	
Sample	**Total Fat (%)**	**Total Fat (%)**	**RSD (%)**
Taralli	19.1	18.95	0.19
Cookies	19	18.42	0.1
Wurstel	26	25.2	0.28
Skimmed Milk	1.6	1.35	0.01
Pudding	1.9	1.55	0.073
Semolina	1.9	2.17	0.05
Cooked Ham	12	12.14	0.37
Raw Sausage	24	23.94	1.4
Condensed Milk	8	7.82	0.06
Cream	21.5	20.94	0.1

For all the ranges tested, the measured total fat content was always in the acceptance range of the certified materials, with very high reproducibility proven by very low relative standard deviations even when working on 12 repetitions. Figure 2 summarizes the accuracy of the ETHOS X method in all the tested ranges in relation to the reference values of each sample.

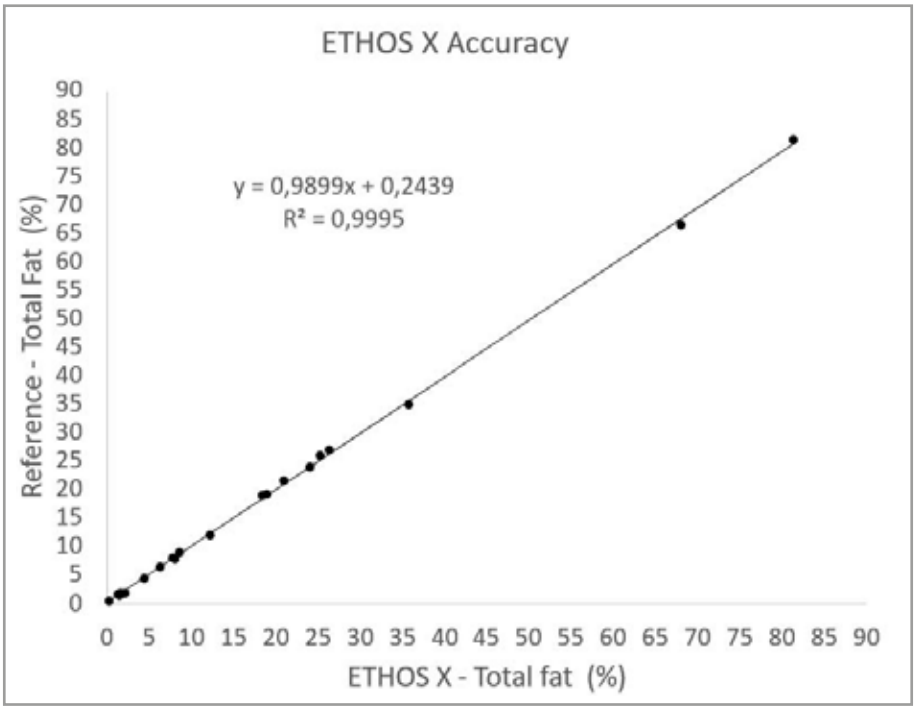

Figure 2. Milestone's ETHOS X accuracy evaluation (data source: Tables 2-4).

High reproducibility and data traceability are ensured thanks to the easyTEMP sensor, which controls the temperature and, therefore, the reaction conditions in all the positions of the SR-15 rotor.

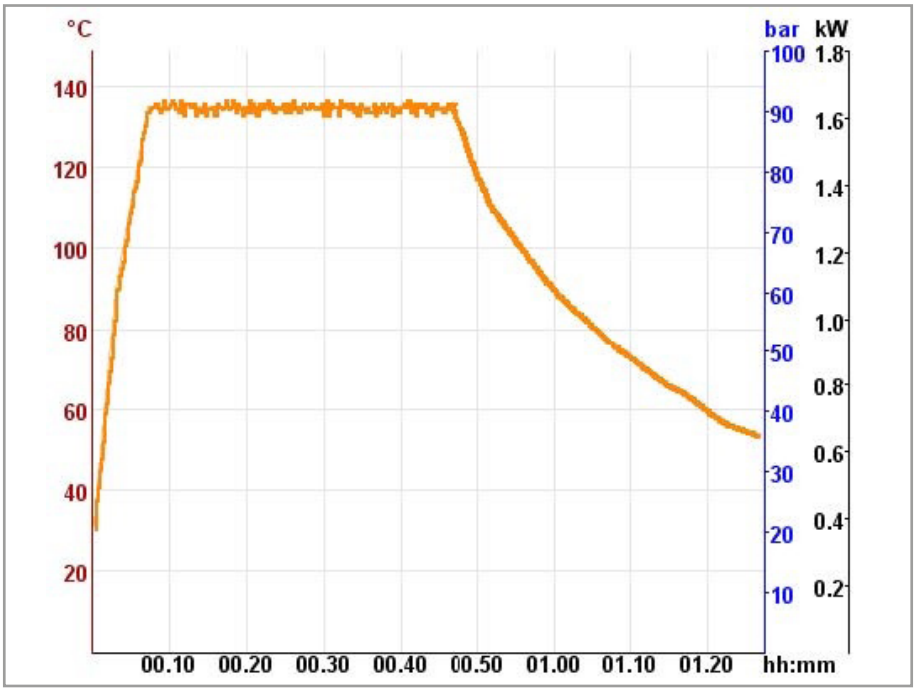

Figure 3. Milestone's ETHOS X run profile.

Working Range

The working range of the ETHOS X during the total fat determination method depends on the sample mass and on the balance capability, being thus calculated from the minimum mass able to be weighed by the analytical balance. Furthermore, the higher the sample mass, the lower the value of the detectable fat content. A typical working range, using an analytical balance, varies from 0.1 to 100% of total fat content, depending on the sample mass.

CONCLUSION

The ETHOS X method for total fat determination proved to be precise and accurate on a wide working range from 0.1 to 100% of total fat content.

Thanks to the performance of the ETHOS X, and to the innovative simultaneous hydrolysis and extraction capability during the same run, this method allows an unmatchable turnaround time, a low solvent consumption and a streamlined workflow. Moreover, the 15 positions of both the SR and RAR rotors ensure the highest throughput available in the market. The total fat residue may be further exploited, after gravimetric evaluation, for the fatty acid methyl ester (FAME) analysis. See dedicated application reports.

The ETHOS X with its unique features fully addresses the needs of food laboratories in terms of productivity, ease of use, running costs, and turnaround time.

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About Milestone

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

This Sponsor Report is the responsibility of Milestone.

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SPONSOR REPORT

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This section is dedicated for sponsor responsibility articles.

Rapid Qualitative and Quantitative Analysis of Residual Solvents in Food Packaging by Static Headspace coupled to GC-FID/MS

Giulia Riccardino and Cristian Cojocariu / *Thermo Fisher Scientific, Runcorn, UK*

This report was extracted from the Thermo Scientific Application Note 10689

The aim of this report is to demonstrate the qualitative and quantitative performance of the Thermo Scientific™ TriPlus™ 500 Gas Chromatography Headspace Autosampler coupled to a dual-detector GC-FID/MS for the determination of residual solvents in food packaging according to the European Standard EN 13628-1 method [1] and to highlight a highly efficient workflow through extended automation from sampling to data reporting.

Keywords: Residual solvents, flexible food packaging, food safety, valve and loop, headspace-gas chromatography, HS-GC, multiple headspace extraction, MHE, flame ionization detector, FID, mass spectrometer detector, MS, single quadrupole GC-MS, ISQ 7000, TriPlus 500 HS.

INTRODUCTION

Packaging materials are essential for maintaining food integrity and to ensure safe handling, transportation, and storage. Common food packaging materials are polymer-based thin films or paper-based coatings often layered or imprinted on the outside with inks, dyes, and paints intended to address the consumer appeal and convenience. The chemical components of such food packaging (especially from polymers, dyes, and inks) can migrate into the food products, modifying the organoleptic properties and the composition of the food and posing health risks to the consumer. As a consequence, regulatory measures are in place to make sure that food contact materials do not transfer any components to the packed foodstuff in quantities that could affect human health, change the composition, or modify the organoleptic properties of the product [2]. In the United States a migration limit of 50 ppm is applicable for residual solvents and non-volatile food additives [3]. In addition, precise quantification of residual solvents in flexible packaging is also regulated through set methods such as EN 13628-1:2002.

Analysis of volatile impurities in solid polymers is challenging, especially with regard to sampling and extraction techniques. Liquid injections of such samples require dissolution of packaging polymers into a suitable solvent prior to gas chromatography (GC) injection. This can result in high viscosity solutions containing non-volatile, long chain polymers that can potentially contaminate the GC injector ports. This, in turn, will require frequent inlet liner replacement and system maintenance that will increase the cost of analysis.

An alternative to liquid injections is headspace sampling: a fast and simple technique that enables the extraction of volatile and semi-volatile compounds from food packaging samples without the need for time-consuming sample preparation. In particular, static headspace with multiple headspace extraction (MHE) [4] can be used for absolute quantitative analysis of volatiles in solid matrices. This technique is particularly useful when matrix-matched calibration reference materials are not available.

In this study, the quantitative results for residual solvent analysis in food packaging materials, obtained with the TriPlus 500 Headspace (HS) autosampler, are reported. A dual detector FID/MS configuration allowed for the detection, identification (flame ionization detection), and confirmation (mass spectrometry

detection) of unknown impurities. The experiments also focused on assessing method linearity [1] according to EN 13628:1:2002 and precision, as well as the overall quantitative performance of the analytical setup for routine analysis of residual solvents in food packaging.

EXPERIMENTAL

In all experiments, a TriPlus 500 HS autosampler was coupled to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph equipped with a Thermo Scientific™ Instant Connect Split/Splitless SSL Injector. A Thermo Scientific™ Dual Detector Microfluidics device (P/N 19071030) was used to split 1:1 the carrier gas flow from the analytical column between a Thermo Scientific™ Instant Connect Flame Ionization Detector (FID) and a Thermo Scientific™ ISQ™ 7000 Single Quadrupole GC-MS system.

Chromatographic separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-1MS GC capillary column, 30 m × 0.32 mm × 3.0 µm (P/N 26099-4840). Additional HS-GC-FID/MS conditions are given in Table 1. The GC oven temperature program was optimized to decrease the analysis time and improve sample throughput; all peaks of interest are eluting in <7 minutes with adequate peak chromatographic resolution (Rs > 1). An incubation time of 40 minutes per MHE step was optimized to cover the majority of food packaging material types. According to the EN 13628-1:2002 method, linearity was assessed on n = 4 headspace extraction cycles.

Table 1 (part 1). HS-GC-FID and ISQ 7000 mass spectrometer operating conditions for residual solvent determination

TRACE 1310 GC	
Inlet Module and Mode:	SSL, split
Split Ratio:	20:1
Septum Purge Mode, Flow (mL/min):	Constant, 5
Carrier Gas, Carrier Mode, Pressure (kPa):	He, constant pressure, 110
Oven Temperature Program	
Temperature 1 (°C):	50
Hold Time (min):	1
Temperature 2 (°C):	110
Rate (°C/min):	30
Temperature 2 (°C):	250
Rate 2 (°C/min):	20
FID	
Temperature (°C):	250
Air Flow (mL/min):	350
H2 Flow (mL/min):	35
N2 Flow (mL/min):	40
Acquisition Rate (Hz):	25
ISQ 7000 Single Quadrupole GC-MS system	
Ion Source:	ExtractaBrite
Transfer Line Temp. (°C):	250
Source Temperature (°C):	250
Ionization Mode:	EI
Electron Energy (eV):	70
Acquisition Mode:	Full-scan (*m/z* 25-350)

Table 1 (part 2). HS-GC-FID and ISQ 7000 mass spectrometer operating conditions used for residual solvent determination

TriPlus 500 HS Autosampler Parameters (MHE)	
Incubation Temp. (°C):	120
Incubation Time (min):	40
Vial Shaking:	Medium
Vial Pressurization Mode:	Pressure
Vial Pressure (kPa) (Auxiliary Gas Nitrogen):	55
Vial Pressure Equilibration Time (min):	1
Loop Size (mL):	1
Loop/Sample Path Temp. (°C):	120
Loop Filling Pressure (kPa):	34
Loop Equilibration Time (min):	1
Extraction Cycles:	4
Needle Purge Flow Level:	4
Injection Mode:	MHE
Injection Time (min):	1

Table 1 (part 3). HS-GC-FID and ISQ 7000 mass spectrometer operating conditions for residual solvent determination

TriPlus 500 HS Autosampler Parameters (total vaporization)	
Incubation Temp. (°C):	120
Incubation Time (min):	40
Vial Shaking:	Medium
Vial Pressurization Mode:	Pressure
Vial Pressure (kPa) (Auxiliary Gas Nitrogen):	55
Vial Pressure Equilibration Time (min):	1
Loop Size (mL):	1
Loop/Sample Path Temp. (°C):	120
Loop Filling Pressure (kPa):	34
Loop Equilibration Time (min):	1
Needle Purge Flow Level:	4
Injection Mode:	Standard
Injection Time (min):	1

Data acquisition, processing and reporting

The data was acquired, processed, and reported using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2. Integrated instrument control ensures full automation from instrument set-up to raw data processing, reporting, and storage. Simplified e-workflows deliver effective data management ensuring ease of use, sample integrity, and traceability. Chromeleon CDS also offers the option to scale up the data handling process in the laboratory from a single workstation to an enterprise environment to further improve productivity [5].

Standard and sample preparation

Two standard mixtures, each containing different residual solvents that can be found in packaging materials (mixture 1 and mixture 2 at 7.14% v/v and 9.09% v/v, respectively), were purchased from Sigma Aldrich® (P/N 48994-U and 48995-U). A volume (1 µL) of each standard solution (corresponding to 71.4 µg and 90.9 µg of mixture 1 and 2, respectively) was spiked into the same 10 mL empty sealed headspace glass vial and used as retention time reference for compound identification as well as for MHE linearity assessment with total vaporization. A complete list of analyzed compounds is reported in Table 2.

Samples of packaged foods (pizza, cookies, bread, salad, and salami) were purchased locally and the packaging (cling film, wraps, and trays) was separated from the food and analyzed following the EN 13628-1:2002 method. A sample surface of 40 cm^2 (2 × 20 cm) was cut, coiled, and sealed into a 10 mL crimp cap headspace vial (vials P/N 10CV, caps P/N 20-MCBC-ST3). As specified in the EN 13628-1:2002 method, the ratio between the sample area (in cm^2) and the vial volume (in mL) was maintained between 3 and 5.

Table 2. Correlation coefficients (R^2) calculated using the full-scan EI traces. *For all compounds in the reference standard $R^2 \geq 0.997$. Correlation coefficients for FID data were 1.000 for all components, hence data are not shown*

MHE Linearity		
Component Name	**RT (min)**	**Correlation Coefficient (R^2)**
Methanol	1.72	0.997
Ethanol	2.11	0.997
Acetone	2.37	0.998
2-Propanol	2.44	0.999
Methyl acetate	2.73	0.999
1-Propanol	2.98	0.998
2-Butanone	3.33	0.999
2-Butanol	3.42	1.000
Ethyl acetate	3.53	0.999
2-Methyl-1-propanol	3.68	0.999
2-Methoxyethanol	3.74	0.997
Tetrahydrofuran	3.80	0.999
Isopropyl acetate	4.04	0.998
1-Methoxy-2-propanol	4.20	0.997
Cyclohexane	4.34	0.998
Propylacetate	4.57	0.999
4-Methyl-2-pentanone	4.89	0.998
Isobutyl acetate	5.22	0.999
Toluene	5.38	0.997
Butyl acetate	5.63	0.999
2-Methoxyethyl acetate	5.74	0.997
2-Etoxyethyl acetate	6.47	0.998
Cyclohexanone	6.66	0.999

RESULTS AND DISCUSSION

MHE linearity assessment according to EN 13628-1:2002 method

A reference solvent standard mix was prepared as described in the standard and sample preparation section and analyzed using the total vaporization technique [4] applying the MHE conditions reported in Table 1. MHE allows the extrapolation of the total content of analytes in a liquid or solid matrix through multiple headspace cycles. The amount of analyte present in the sample is calculated by direct comparison of the peak area responses to external standards previously analyzed in a similar way but without matrix.

MHE linearity was assessed by plotting the natural logarithm of the peak areas in the standard solution versus the number of headspace cycles (n = 4). Chromeleon CDS interactive charts and reprocessing features allowed for fast MHE calibration plots and correlation coefficient calculations without the need of external calculation tools, as shown in Figure 1. For all the investigated compounds, the calculated correlation coefficients (R^2) were 1.000 for FID data and ≥0.997 for EI full-scan MS traces (Table 2). In both cases calculated correlation coefficients met the method requirement ($R^2 \geq 0.98$) confirming an excellent linearity.

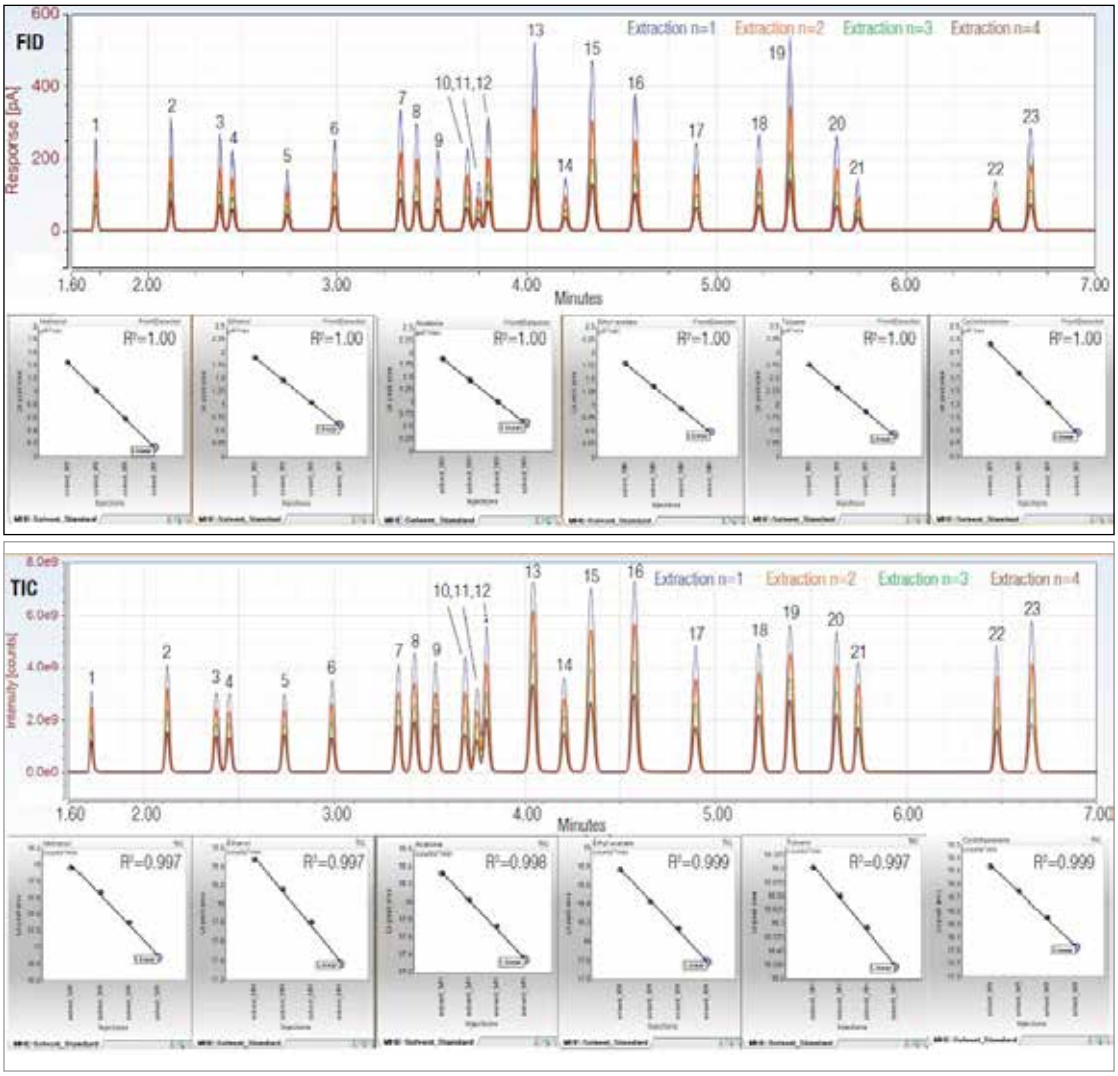

Figure 1. FID and TIC (full-scan, EI at 70 eV) traces for reference standard and corresponding MHE calibration curves for selected compounds (left to right: methanol, ethanol, acetone, ethyl acetate, toluene, and cyclohexanone) as examples. *Calibration curves were obtained by plotting the natural logarithm of peak area responses (total vaporization MHE) versus the corresponding MHE extraction step.*

Quantification of residual solvent in food packaging materials using MHE

The packaging materials were prepared as described and analyzed using the MHE conditions reported in Table 1. The microfluidic device allowed for splitting the gas flow 1:1 to the FID and the ISQ single quadrupole mass spectrometer, ensuring a minimal effect on the retention times (max RT shifts 0.04 min) by choosing either the FID or MS chromatogram as reference. The sample and the standard solution FID chromatograms were compared to verify the presence of known residual solvents. Several residual solvents such methanol (RT = 1.72 min) and ethylacetate (RT = 3.53 min) were detected in the sliced salami lid (D) and plastic tray (E), whereas ethanol (RT = 2.11 min) and acetone (RT = 2.37 min) were present in salad wrap (C) (Figure 3).

Full-scan data were used to putatively confirm the identity of detected solvent impurities, increasing the confidence in compound identification. When searching the mass spectrum of the peak eluting at RT = 1.72 min against NIST17 library, the best library match was acetaldehyde (not included in the standard mixtures) with a SI score of 953 (sliced salami tray:E) and 729 (sliced salami lid:D) (Figure 3). Acetaldehyde is usually present in meat and meat products [6]. Using the same approach, ethanol and acetone in salad wrap (C) and ethyl acetate in sliced salami (lid:D and tray:E) were also putatively confirmed with a SI score of 929, 913, 874, and 950, respectively. These chemicals are actually released by the packaging since they are typically used in solvent-based inks imprinted on the external layer of flexible packages [7]. Additional unknown compounds (*) detected in the samples were confirmed using spectral library comparison against NIST17 library (Figure 2).

Obtaining good ($R^2 \geq 0.98$) MHE linearity is fundamental to achieve accurate quantitation of residual solvents in solid food packaging materials. MHE linearity in the samples was assessed as previously described. The correlation coefficients (R^2) were 0.998 and 0.995 for ethyl acetate in sliced salami (lid and tray, respectively). R^2 for ethanol and acetone in salad wrap were 0.996 and 0.998, respectively (Figure 4).

The concentration (in mg/m^2) of residual solvents detected in the samples was calculated using the FID data applying the formula reported in paragraph 9.2.10.1 of the EN method. No residual solvents were found in the majority of samples. Traces of ethyl acetate were found in the sliced salami wrap (lid: 0.76 mg/m^2, tray: 29 mg/m^2). Ethanol (0.97 mg/m^2) and acetone (1.9 mg/m^2) were also present in salad wrap. All levels were within the safety limits reported for residual solvent and non-volatile food additives [3].

Peaks (A):

1. Methanol
2. Ethanol
3. Acetone
4. 2-Propanol
5. Methyl acetate
6. 1-Propanol
7. 2-Butanone
8. 2-Butanol
9. Ethyl acetate
10. 2-Methyl-1-propanol
11. 2-Methoxyethanol
12. Tetrahydrofuran
13. Isopropyl acetate
14. 1-Methoxy-2-propanol
15. Cyclohexane
16. Propyl acetate
17. 4-Methyl-2-pentanone
18. Isobutyl acetate
19. Toluene
20. Butyl acetate
21. 2-Methoxyethyl-acetate
22. 2-Ethoxyethyl acetate
23. Cyclohexanone

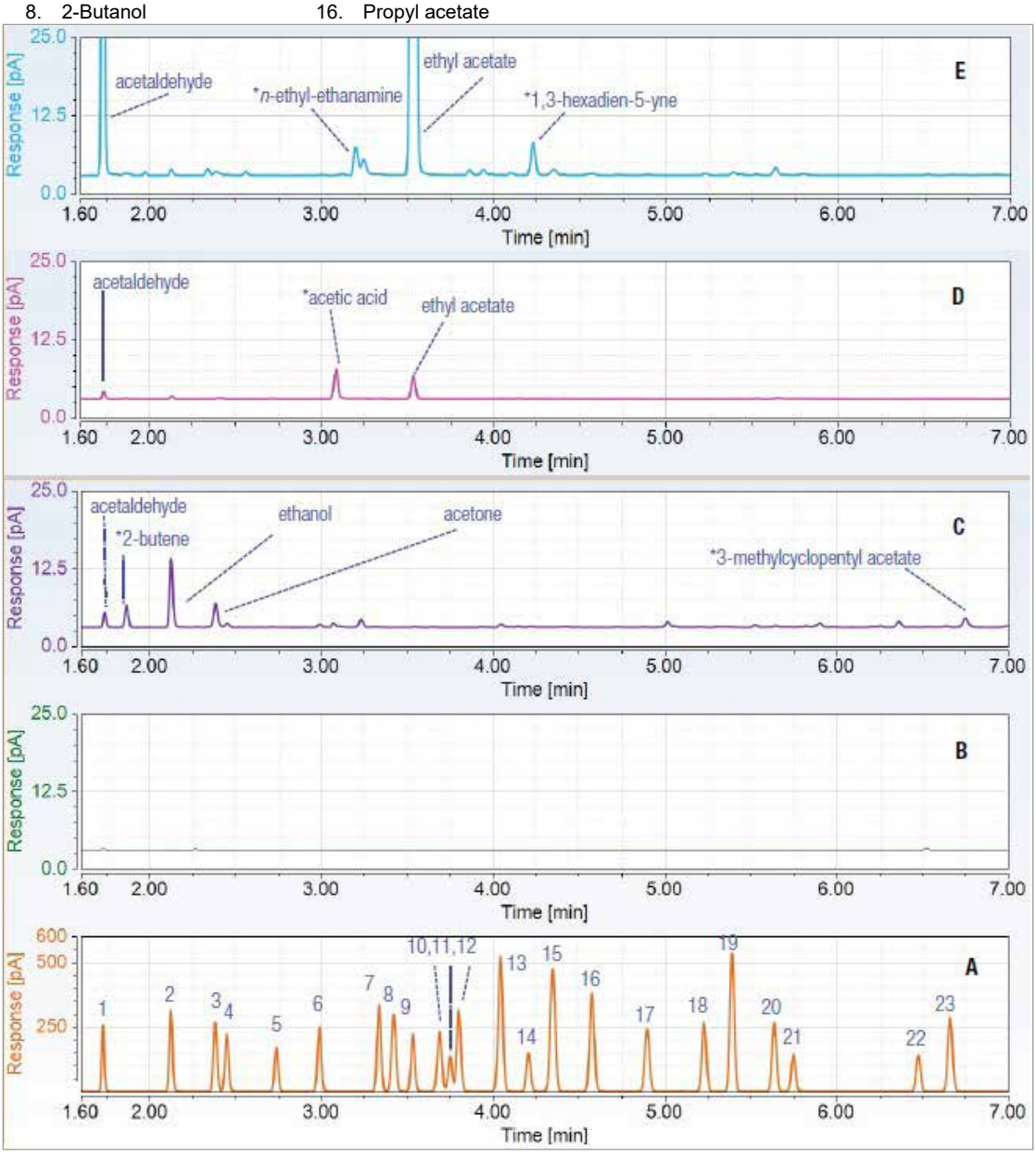

Figure 2. FID chromatograms showing a comparison between the residual solvents in the reference standard solution (A), empty blank vial (B), salad wrap (C), sliced salami wrap: lid (D) and tray (E). *Based solely on retention time comparison, methanol and ethyl acetate were detected in both sliced salami samples (lid:D, tray:E). Ethanol and acetone were found in salad wraps (C). FID signal responses (y-axis) are normalized for the empty vial (B) and samples (C,D,E). Unknown peaks (*) in the samples were confirmed comparing their mass spectra (full-scan, EI traces) against the NIST17 library and are reported as an example. Peaks not annotated were below the integration threshold of 0.04 pA * min.*

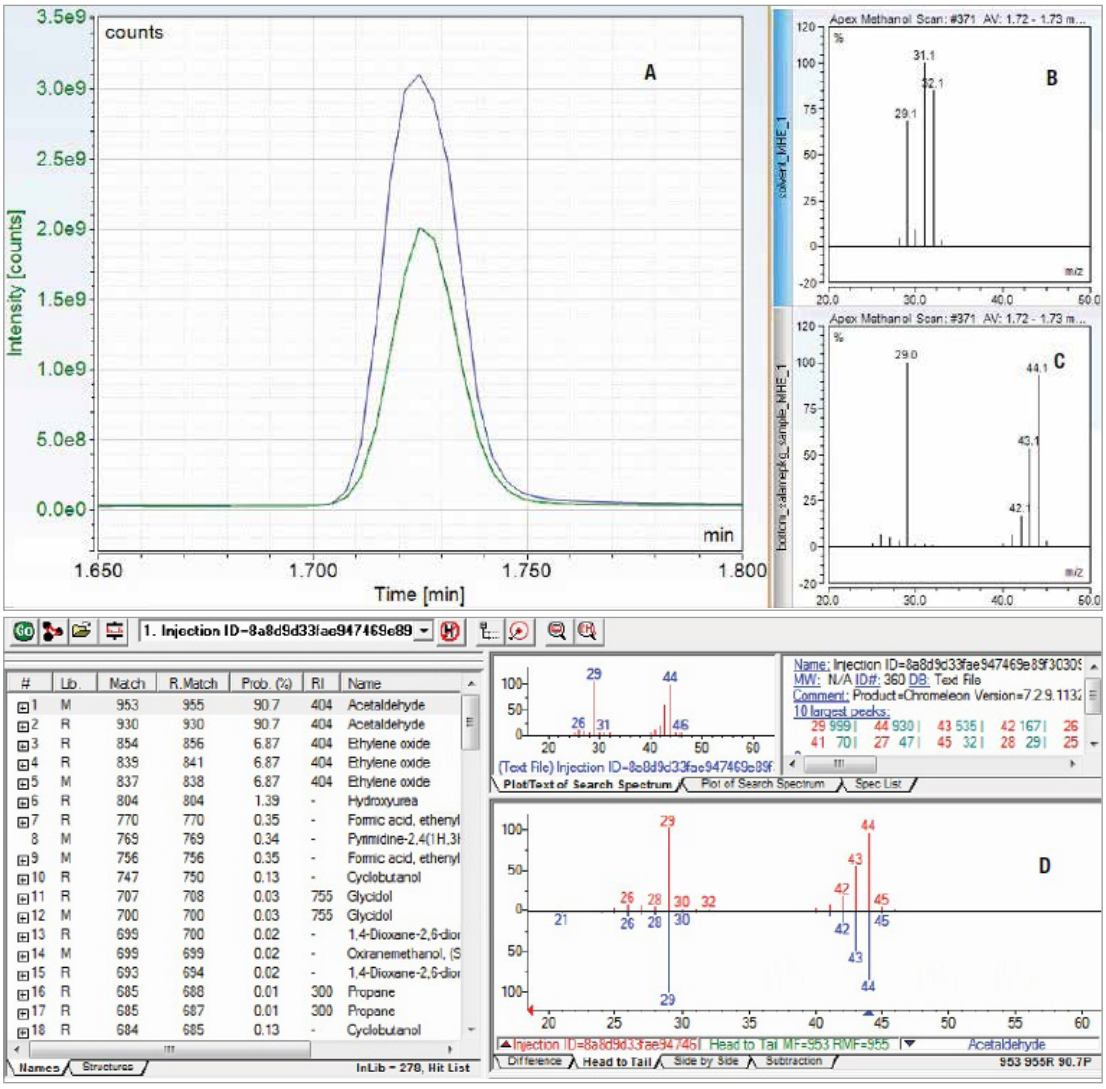

Figure 3. Identification of residual solvent peak eluting at RT=1.72 min in salami tray sample. *Comparison of TIC chromatograms (full-scan, EI at 70 eV) showing retention time comparison of peak eluting at RT=1.72 min in solvent standard (blue) and salami tray (green) (A). Background subtracted EI mass spectra for this peak in solvent standard (B) and in the sliced salami tray (C) did not confirm methanol. NIST library result (D) putatively identified this compound as acetaldehyde with a SI score of 953 and a probability of 91%.*

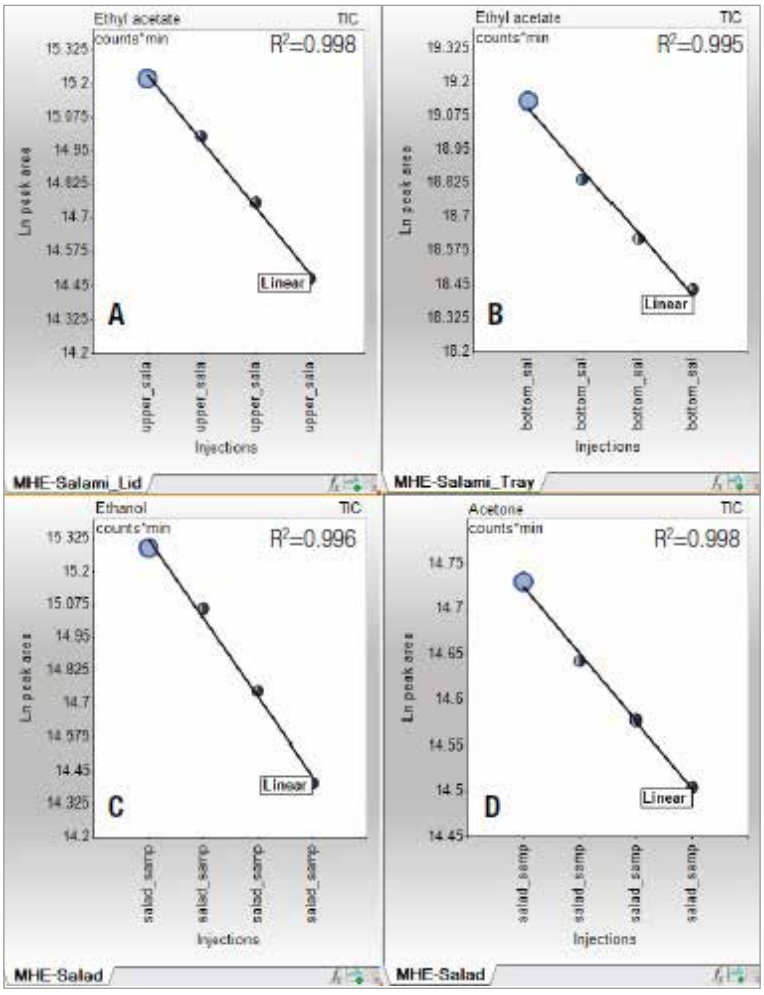

Figure 4. MHE linearity for ethyl acetate in sliced salami lid (A) and sliced salami tray (B), ethanol (C), and acetone (D) in salad wrap. *The resulting correlation coefficients (R^2) were 0.998 and 0.995 for sliced salami (lid and tray, respectively) and 0.996 and 0.998 for ethanol and acetone, respectively, in salad wrap.*

CONCLUSIONS

The results obtained with the TriPlus 500 HS autosampler are compliant with the EN 13628-1:2002 standard method requirements.

- The MHE capability allows for absolute quantitative analysis of residual solvent impurities in solid samples, overcoming the matrix effect and eliminating the need of sample preparation. Using the MHE mode, excellent linearity with correlation coefficient $R^2 \geq 0.995$ was obtained for all analytes in both solvent standard and samples, meeting the minimum required value of $R^2 \geq 0.98$, thus confirming excellent instrument performance for MHE quantitative analysis.

- Traces of residual solvents were found in three of the six analyzed food packaging samples. Acetone and ethanol were detected at 1.9 and 0.97 mg/m^2 in salad wrap samples, respectively, and ethyl acetate was found in sliced salami tray at 29 mg/m^2 and lid at 0.76 mg/m^2. No residual solvents were present in pizza cling film, cookies, and bread wraps.

- The dual detector configuration FID/MS increases the confidence in compound identification, allowing for the detection of possible analyte co-elution, otherwise difficult to assess in the absence of MS data. Moreover, several unknown peaks in the samples have been putatively confirmed (using spectral library match score thresholds of >950 SI) through comparison with NIST17 spectral library.

- The low bleed and superior inertness of the TraceGOLD column allowed for highly reliable results. The high analytical column efficiency allowed for fast GC oven ramp with adequate chromatographic separation (Rs ≥ 1.0) for all the analyzed compounds, reducing analysis time. Moreover, up to 240 sample vials can be accommodated into the trays for unattended 24-hour operation. The automated cycle time optimization allows for continuous sample processing ensuring the overlapping between the MHE cycles of the same sample. The overlapping capability is maintained between the final injection of one sample and the incubation of the next one increasing the sample throughput.

- Chromeleon CDS software ensures data integrity, traceability, and effective data management from instrument control to the final report. The integrated charts and the advanced report capability allowed for easy and integrated MHE data reprocessing, thus eliminating the need for external calculation tools.

Overall, the results obtained show that the TriPlus 500 HS autosampler coupled to the TRACE 1310 GC and the ISQ 7000 single quadrupole GC-MS system represents a robust analytical configuration for routine laboratories delivering outstanding reliability for MHE quantitative analysis of residual solvents in food packaging.

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Speciation of Bromine Compounds in Ozonated Drinking Water using Ion Chromatography and Inductively Coupled Plasma Mass Spectrometry

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This report was extracted from the Thermo Scientific Application Note 43227

The goal of this study is to speciate and quantify bromine (Br) species in drinking water by means of ion chromatography (IC) coupled with Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Applying the Environmental Protection Agency (EPA) method 321.8 for bromate detection in drinking water.

Keywords: Bromate, Drinking Water, EPA 321.8, Inductively Coupled Plasma Mass Spectrometry, Ion Chromatography, Speciation

INTRODUCTION

Bromine speciation in drinking water is required worldwide by major regulatory bodies. A maximum contaminant level (MCL) of 10 µg L^{-1} in the US for bottled drinking water and in the EU of 3 µg L^{-1} for natural mineral water and spring water treated with ozonation are stipulated for the bromate anion. As a result of the ozonation process, a common water disinfection method, bromate can be formed via the oxidation of the naturally occurring bromide. Whereas bromide is non-toxic, bromate is toxic and carcinogenic.

Differentiating bromate from bromide is therefore important due to the toxicity differences between the two species.

Bromine analysis with ICP-MS is challenging because the ionization potential of bromine is relatively high and it lowers sensitivity in ICP based techniques. Additionally, there are a number of isobaric interferences on *m/z* 81 and 79 (the two Br stable isotopes) as shown in Table 2.

The United States Environmental Protection Agency (EPA) and the International Organization for Standardization have developed several methods for bromate determination in drinking water (EPA 300, 300.1, 302.0, 317, 326: ISO 10304-4 and 15061) all of which have been validated in Thermo Scientific™ Dionex™ application notes (AN167, AN168, AN171, AN184, AN187).

The EPA Method 321.8 provides an analytical procedure for bromate determination in drinking water using IC-ICP-MS. This method significantly reduces the risk for contamination and has the advantage of a stable and quantitative bromate recovery (Table 1, Figure 6) through the IC column and it tolerates high salt conditions. The method also provides guidance for reducing polyatomic interferences that overlap with the two most abundant Br isotopes.

For this method, a Thermo Scientific™ Dionex™ ICS-5000 IC can be coupled to a Thermo Scientific™ iCAP™ RQ ICP-MS (data presented was acquired with a Thermo Scientific™ iCAP™ Q ICP-MS.). The iCAP RQ ICP-MS is equipped with a collision/reaction cell, the Thermo Scientific™ QCell™, which enables a single interference reduction approach using He-KED (Kinetic Energy Discrimination). This measurement mode filters out polyatomic interferences on bromine and allows for the accurate quantification of bromide and bromate species, all without the need for additional interference correction steps. Additionally, the

QCell design maintains high transmission and high sensitivity so that quantification at the sub-ppb levels required by the EPA method can be easily achieved.

MATERIAL AND METHODS

General Analytical Conditions

All data collection was performed according to the protocols outlined in EPA 321.8 for "Determination of bromate in drinking waters by Ion Chromatography – Inductively Coupled Plasma – Mass Spectrometry". The QCell, operated in He-KED mode, provides simple and accurate quantification of Br at both *m/z* 79 and 81. However, in accordance with EPA 321.8, ^{81}Br was only measured to screen for potential interferences. All bromate results were quantified based upon the isotope at mass 79. The KED measurements were conducted with a mixture of 7% H_2/He as collision gas.

Table 1. Acquisition parameters for the IC-ICP-MS method

Parameter	**IC**
Columns	Thermo Scientific™ Dionex™ IonPac™ AS19 Analytical, 2 × 250 mm (P/N 062886) Dionex IonPac AG19 Guard, 2 × 50 mm (P/N 062888)
Eluent	10 mM KOH from 0–25 min, 45 mM from 25–30 min, 10 mM from 35 min*
Flow Rate	300 µL min^{-1}
Sample Loop	100 µL
System Back Pressure	2300 psi
Run Time	35 min
Conditions	**ICP-MS**
Measurement Mode	KED, STD
Isotopes Measured (Dwell Time)	^{79}Br (200 ms), ^{81}Br (200 ms)
Gas Flow for QCell (He/H_2)	4.5 mL min^{-1}
Cool Gas	14 L min^{-1}
Auxiliary Flow	0.8 L min^{-1}
Nebulizer	1.13 L min^{-1}
Analysis Mode	tQuant
Spray Chamber	2.6 °C
Forward Power	1550 W
Injector	2 mm i.d.
KED Voltage	2.5 V

*Method returns to 10 mM KOH for 5 min re-equilibration prior to injection.

Equipment

- Thermo Scientific™ Dionex™ ICS-5000 (or Thermo Scientific Dionex ICS-2100) HPIC System consisting of:
  - DP Dual Pump or SP single Pump
  - EG Eluent Generator module
  - Injection Loop, 100 µL
- Thermo Scientific™ Dionex™ Eluent Generator KOH Cartridge
- Polystyrene Autoselect vials with caps and septa 10 mL
- Thermo Scientific™ iCAP™ Q ICP-MS

Autosampler and Software

- Thermo Scientific™ Dionex™ AS-AP Autosampler
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System
- Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution (ISDS)

Preparing Standards and Samples

Six samples of bottled water and tap water (numbered 1 through 6) were analyzed for this application. The samples came from various sources in the US, where the ozonation approach for water disinfection is still commonly applied.

Bromide and bromate stock standards were diluted with deionized water acidified with 2% HNO_3 to produce calibration standards containing 1, 5, 10 and 25 µg L^{-1} of each Br species. The blank was prepared by dissolving NaOH in deionized water in order to increase the pH of the solution to 10. Standards and water samples were also brought to a pH of 10 and measured without further sample preparation. The samples and standards were analyzed using the Dionex ICS-5000 IC system for chromatographic analysis (although the Dionex ICS-2100 IC system with KOH cartridge would be a suitable alternative). The method could be followed using KOH as per EPA recommendation with no ICP-MS compatibility issues. A solution containing 100 ppm of PO_4^{3-} and SO_3H^- was prepared and analyzed to check for interferences at *m/z* 79 and 81. Several potential plasma- and solution-based interferences are listed in Table 2.

Table 2. Possible sources of isobaric interference

Interferente Source	MASS 79	MASS 81
Plasma	$^{40}Ar^{38}Ar^{1}H^{+}$	$^{40}Ar^{40}Ar^{1}H^{+}$
Sulfate		SO_3H^+
Phosphate	$^{31}P^{16}O_3^+$	$^{31}P^{16}O_3{}^{1}H_2^+$
Potassium	$^{40}Ar^{39}K^+$	

RESULTS AND DISCUSSION

Data sets were collected over three consecutive days. On each day of analysis the blanks and standards were analyzed. Samples were quantified against the external calibrations. On each day of analysis the correlation coefficient was always better than 0.999 (Figure 1).

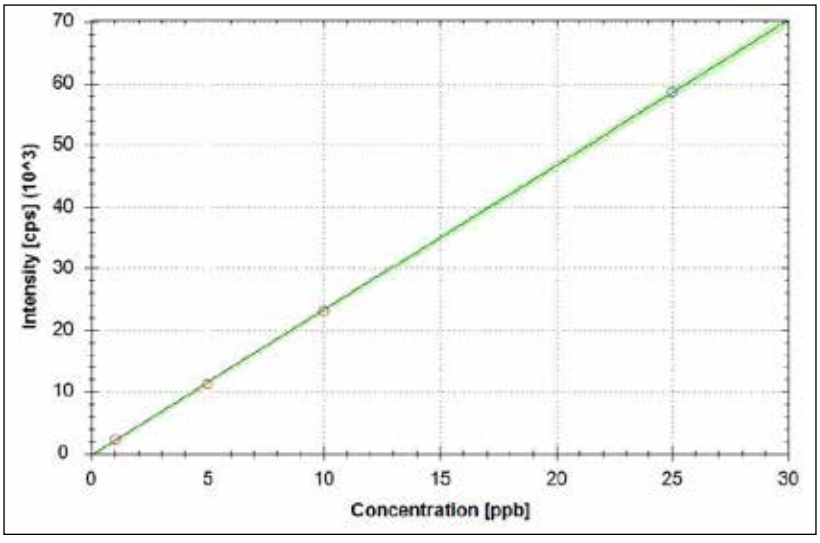


Figure 1. Calibration curve for bromate standards measured at ^{79}Br in He-KED mode.

As per EPA 321.8, each sample was prepared in quadruplicate. One of the replicate preparations was spiked with 1 µg L^{-1} of bromate and 5 µg L^{-1} of bromide for determining spike recoveries. The remaining three preparations were analyzed without further modification. Spike recoveries were calculated according to the following equation:

$$\%R = \frac{(\text{spiked sample result} - \text{unspiked sample result})}{(\text{known spike added concentration})} \times 100$$

For purposes of comparison, Br was measured at *m/z* 79 and 81 in both He KED and STD modes (STD mode data not shown). Due to increased accuracy using KED mode, quantitative results for mass 79 in KED mode only are shown (Table 3).

Table 3. Results for bromate in KED mode of the six water samples (spiked and unspiked)

Water	Average Unspiked Sample Result (µg L^{-1})	Samples Spiked with 1 µg L^{-1} Bromate (µg L^{-1})	Recovery %
1	N/A	0.999	99.9
2	1.218	2.221	100.3
3	1.732	2.729	99.7
4	N/A	1.000	100.0
5	N/A	0.999	99.9
6	1.127	2.124	99.7

Results in Table 3 indicate that recovery for bromate is quantitative and reproducible. The average recovery is 99.8% with a SD of 0.4%.

A Method Detection Limit (MDL) was calculated for bromate according to the EPA method instructions (3.14 × s.d. of 7 replicates of 5 µg L^{-1} target species). Figure 2 shows the chromatographic overlay of the 7 replicates. Based on those results, a bromate MDL of 0.014 µg L^{-1} was achieved. This is significantly below the maximum contamination level (MCL) for bromate in water (3 µg L^{-1}) and twenty times lower than the required EPA MDL of 0.3 µg L^{-1}. Figure 3 shows the chromatographic elution of bromate at the EPA MDL concentration of 0.3 µg L^{-1}. To demonstrate the necessity of He KED mode for the accurate analysis of bromine, a 25 ppb standard of both bromate and bromide were analyzed in standard mode (STD). The baseline offset for mass 81 is due to the isobaric interference from $^{40}Ar^{40}ArH^{+}$ detected in STD mode. This interference is completely eliminated using He KED, as shown in Figure 5 where the baseline signals for ^{79}Br and ^{81}Br overlap perfectly.

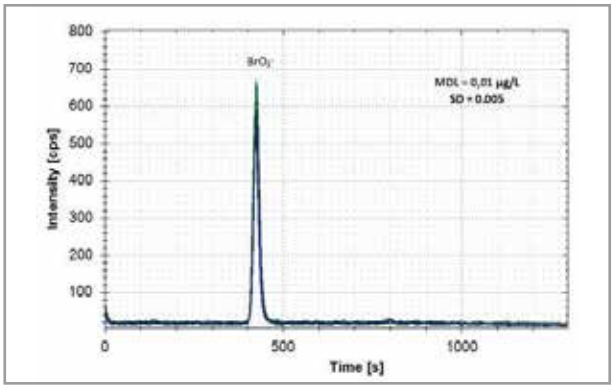


Figure 2. Overlay of chromatograms from 7 repeated injections of 5 ppb standard are shown in He- KED mode.

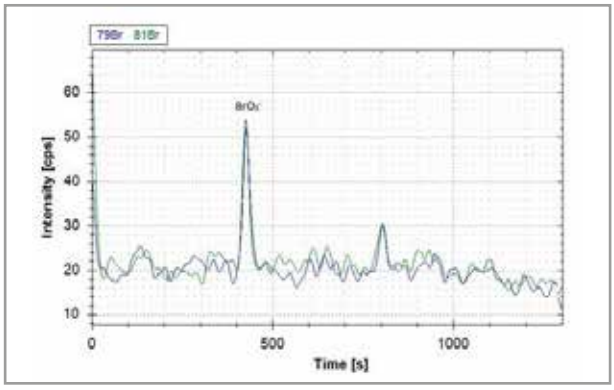


Figure 3. Test for EPA recommended MDL of bromate at 0.3 µg L^{-1}.

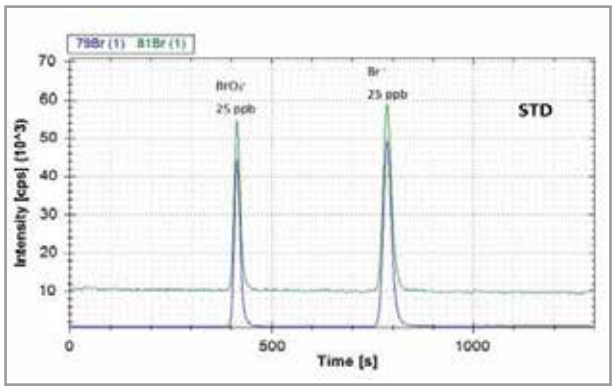


Figure 4. Signal for 25 ppb bromate and 25 ppb bromide in STD mode. Green upper trace is ^{81}Br, blue lower trace is ^{79}Br.

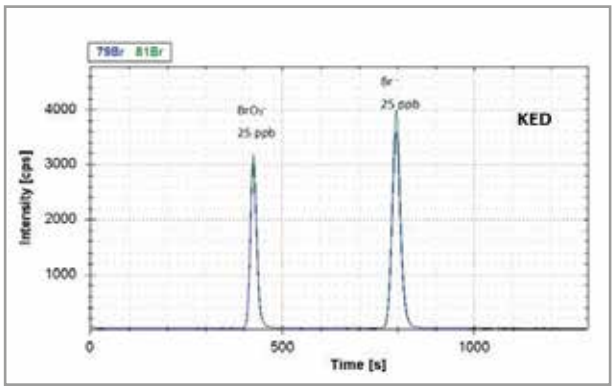
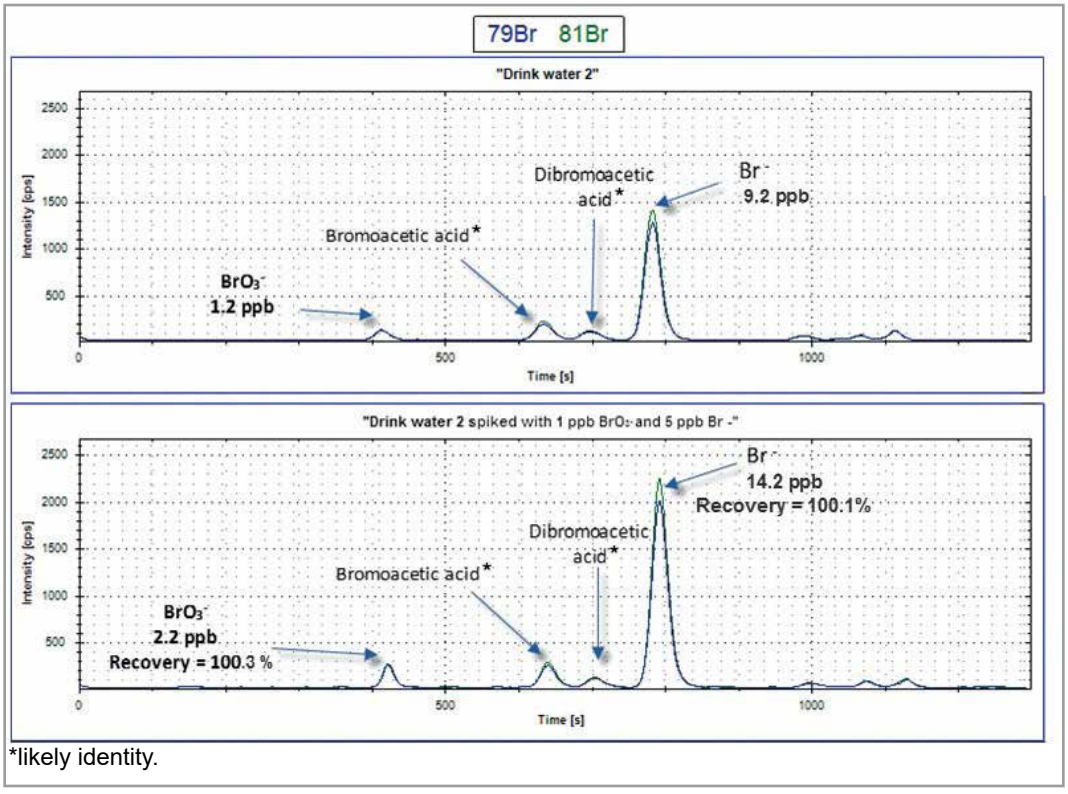


Figure 5. Signal for 25 ppb bromate and 25 ppb bromide in He-KED mode. Green trace is ^{81}Br, blue trace is ^{79}Br.



*likely identity.

Figure 6. Separation for Br species in water sample 2 (upper trace) and the same sample with a 1 µg L^{-1} bromate and 5 µg L^{-1} bromide spike (lower trace). *Note the consistency in elution times for all the species detected, the excellent separation and quantitative recovery.*

The chromatographic separation (Figure 6) demonstrates that the measured analytes are effectively eluted from the column with baseline resolution, even in the presence of other brominated species and the sample matrix. This chromatographic separation coupled with the effective KED interference removal ensures that the detected peaks correspond only to the target analytes giving highly precise and accurate results. Results for bromate in samples 2 (Figure 6), and 3 (not shown) corroborate that both samples were collected from a municipality which ozonates its drinking water as part of the disinfection treatment process. Bromate concentrations are reasonably close to each other, as they come from the same supply although different locations within the same city.

Based on the results of the interference check with sulfate and phosphate and from the isotope ratios, the two peaks at 630 and 700 s were identified as bromine-containing species. Following the order of elution outlined in the EPA methods, it is likely that these two species are bromoacetic acid and dibromoacetic acid respectively, although this was not unequivocally confirmed (via spiking experiments or other approaches).

Sample 6 (not shown) is a bottled water from a company that does not state the purification method adopted, but according to the bromate content it is reasonable to assume that it is ozonation.

Samples 1, 4 and 5 are all bottled waters, but any bromate present was below the MDL.

CONCLUSION

Combining the resolving capability of IC with the detection power of ICP-MS allows fast, easy and reliable analysis of bromine species in drinking waters. Low background and He-KED interference removal permit robust conditions so that sample preparation is virtually eliminated and analysis of the water can be performed from the tap to the vial after simple alkalinization. This method enables robust and reliable speciation of bromine containing species in drinking water, with no salinity-related recovery issues. Thanks to the high sensitivity and completely metal free sample and mobile phase flow paths, the application complies to EPA 321.8 quality control requirements and provides MDLs well below the values necessary for accurate monitoring of water from various sources.

REFERENCES

1. ISO method 11206.
2. EPA Method 326.0 and 317.0 "Determination of inorganic oxhalide disinfection by-products in drinking water using ion chromatography incorporating the addition of a suppressor acidified post column reagent for trace bromate analysis"
3. EPA Method 300.0 and 300.1 "Determination of inorganic anions by ion chromatography."
4. EPA Method 321.8 "Determination of bromate in drinking waters by Ion Chromatography – Inductively Coupled Plasma – Mass Spectrometry".

Find out more at thermofisher.com/ICP-MS

This Sponsor Report is the responsibility of Thermo Fisher Scientific.

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.

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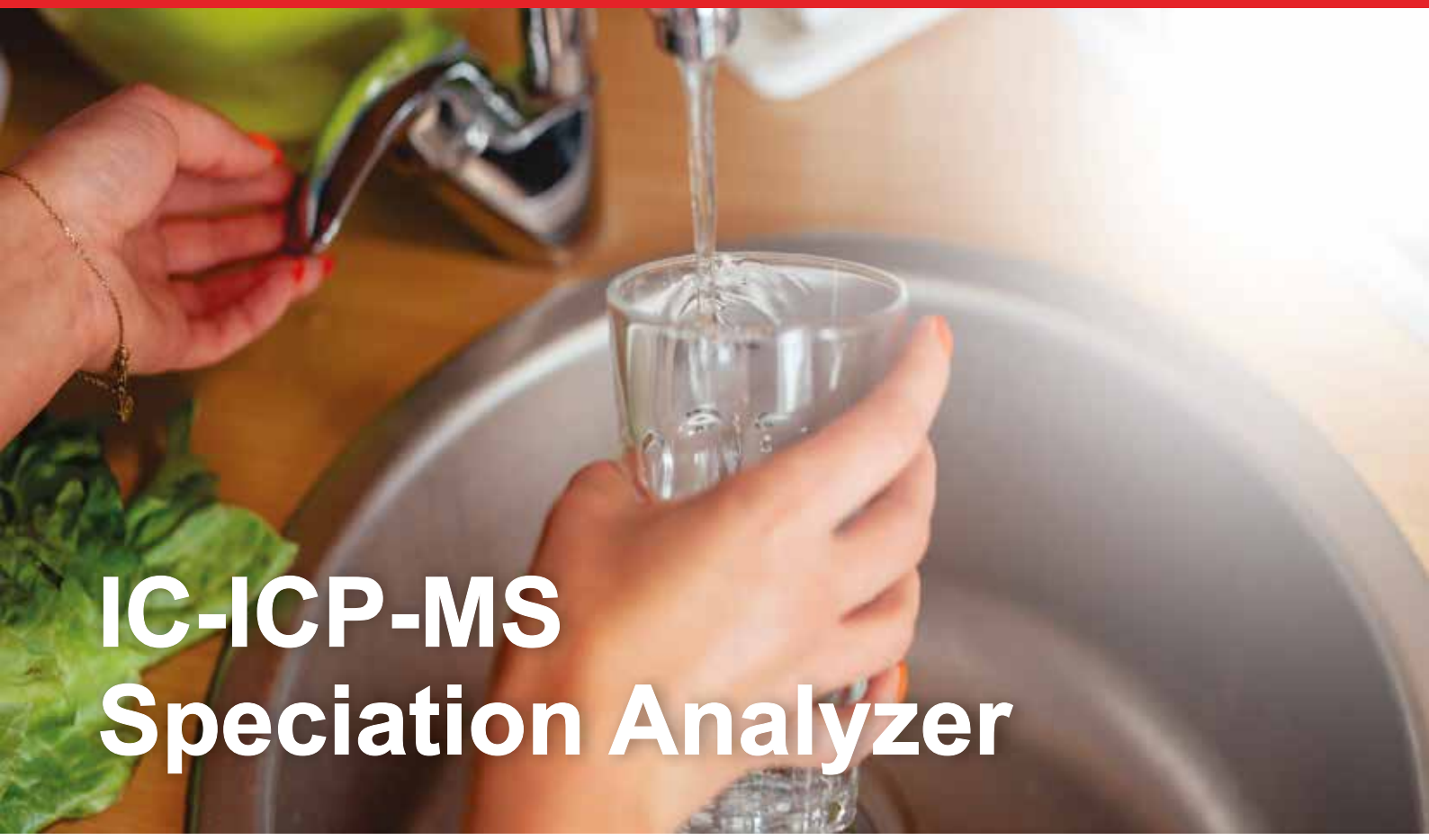

IC-ICP-MS Speciation Analyzer

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.

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High data quality.
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TriPlus 500 Headspace Autosampler coupled to the TRACE 1310 Gas Chromatograph

VIDEO WEBSITE

ThermoFisher
SCIENTIFIC

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ETHOS™ X Microwave – Gain Productivity in Fat Determination on any Matrix

Fat determination in food and feed matrices is a routine analysis for several testing and food processor labs. Nowadays, the process still relies on outdated and matrix-dependent methods which use obsolete and time-consuming technologies.

The ETHOS X offers a new approach to gravimetric fat determination, moving toward a more modern, greener and more efficient approach to total fat, Fatty Acid Methyl Ester (FAME) and free fat determinations in food and feed matrices.

The ETHOS X applies the well-known selectivity of microwave heating and the advantages of closed vessel technology to fat determination. Its configuration and methods have been specifically built to address lab requirements in terms of throughput, ease of use and universal method approach.

In the ETHOS X hydrolysis and extraction are done in a single step and in less time, ensuring higher productivity and better workflow.

Applicable to any matrix
The ETHOS X is not matrix-dependent, so it uses a single method and setup for virtually any food and feed sample. This approach leads also to a lighter accreditation procedure, as it is carried out on several samples using a single technique.

Improved Turnaround Time & Productivity
The ETHOS X processes 15 samples simultaneously, and as many as 45 samples in a single working day, using one rotor and 75 samples with two rotors. Its specific method combines the hydrolysis and extraction into a single step, thus having a direct impact on the lab's turnaround time and throughput.

Lower cost per sample
The ETHOS X uses closed vessels technology to increase the temperature and improve extraction efficiency, reducing the reagents volume too. The unique setup offers a reagent and waste reduction of up to 60%. In combination with the higher productivity and compelling initial investment, it provides a lower cost per sample.

Complete fat determination
The flexibility of the ETHOS X configuration and its dedicated procedures enables to perform FAME and free fat determinations of food samples, performimg the complete fatty acids profile within a single platform.

Safe and compact
The ETHOS X setup allows to carry out all the steps within a single platform while improving productivity, it is not installed in the fume hood and saves over 50% of the lab space. Its dedicated exhaust system combined with up-to-date features, ensures the highest operator safety level throughout the entire process.

Find out more at milestonesrl.com

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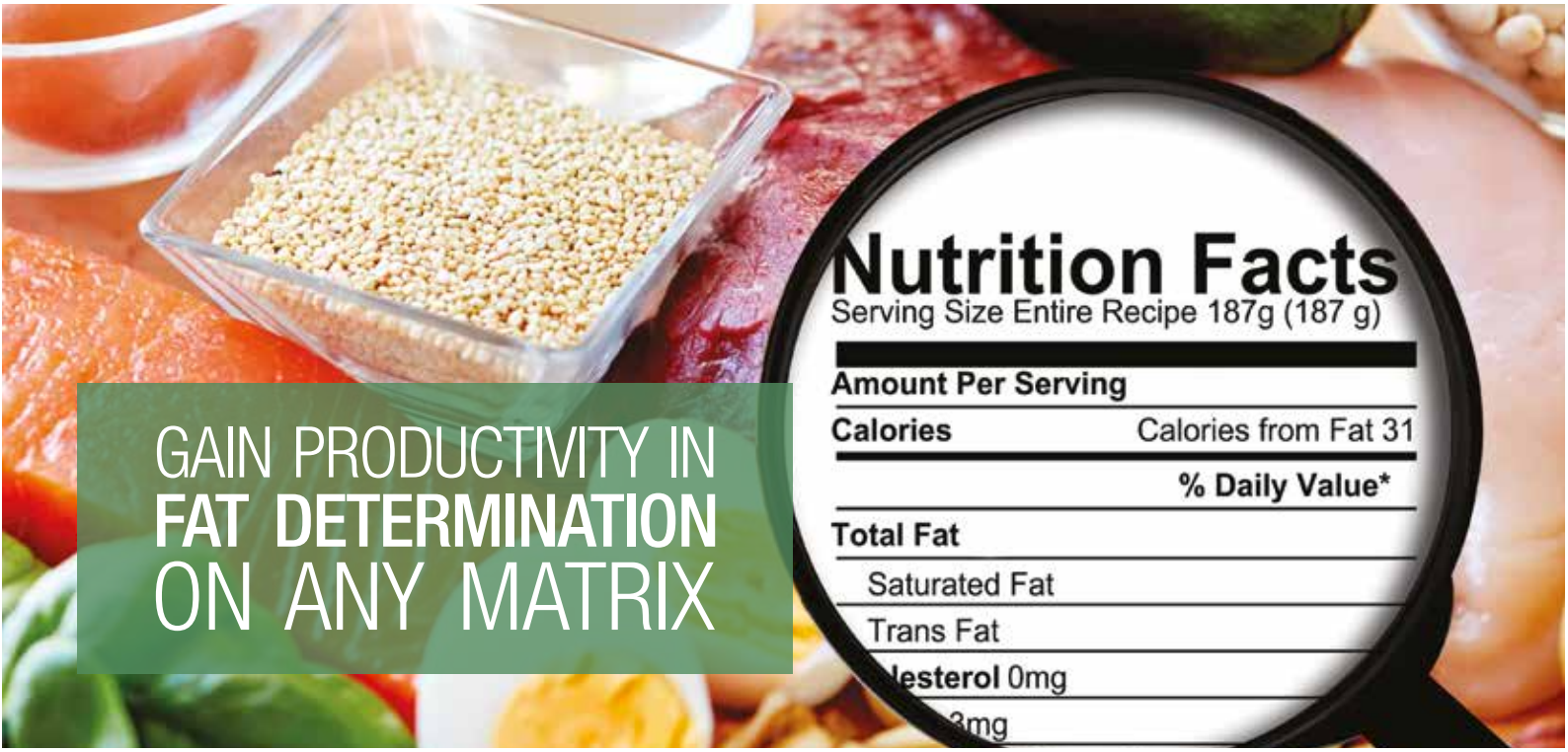


GAIN PRODUCTIVITY IN **FAT DETERMINATION** ON ANY MATRIX

ETHOS™ X

Microwave Fat Determination in Food and Feed

The ETHOS X system matches modern lab requirements offering an efficient approach to total fat determination by gravimetric analysis. Applicable to virtually any food and feed sample, this universal method combines the acid hydrolysis & solvent extraction into a single step, enhancing the overall process and improving the lab workflow.

ONE METHOD FITS ALL
A single platform and method for any samples

LOWER REAGENTS CONSUMPTION
Both acid and solvent volumes are reduced by 2/3

SIMULTANOUS HYDROLYSIS AND EXTRACTION
Fats are hydrolyzed and extracted within a single step

HIGH PRODUCTIVTY
Up to 45 samples/day with one system

VIDEO

Watch Videos, Download Brochures & Application Notes at **www.milestonesrl.com/fatdetermination**

RELEASE

Pittcon 2021 Conference & Expo is going Virtual!

Pittcon 2021 attendees and exhibitors – the very people who comprise the international science community – will have access to the same dynamic exposition and technical programming made available at the 71 in-person Pittcons prior. This installment of Pittcon may look different, and it will be because it is our sole mission to offer all the benefits associated with a face-to-face Pittcon, tailored to a virtual environment.

The platform that will be used is LabRoots, which has been heralded as the best of the best virtual event platforms on the market. **Take a tour of LabRoots Virtual Platform** to see why they're the leading producer of scientific virtual events.

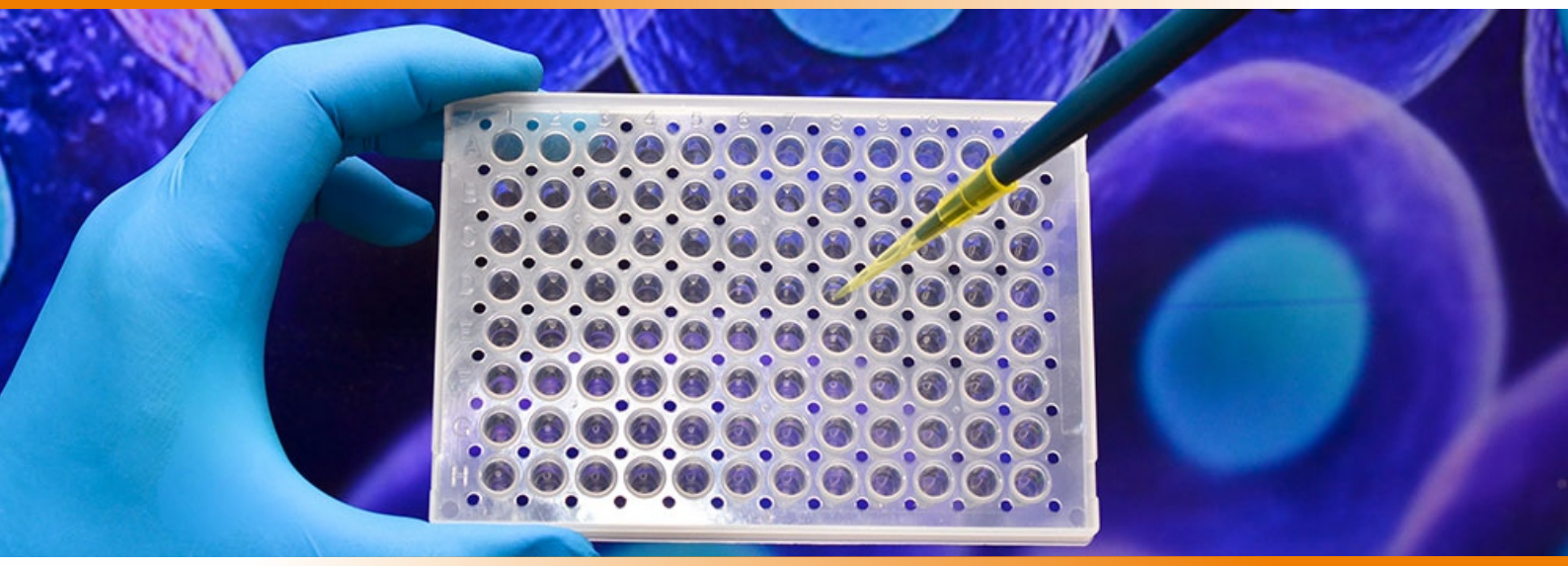
Abstract submissions for (contributed) Oral and Poster presentations
Deadline: January 18, 2021
Technical presentations in all areas of analytic chemistry and applied spectroscopy are being accepted.

Virtual Undergraduate Poster Session
Undergraduate students who are attending Pittcon 2021 are invited to present a poster describing their research in a special session dedicated to such presentations by students who have not yet completed their B.S. or B.A. degree. The purpose of this session is to provide a forum in which undergraduate students can share research presentations with their peers and to encourage networking amongst undergraduate students. An advisor must be listed on the abstract in order to be considered an undergraduate abstract.

WHEN

Short Courses	Monday (March 1) – Friday (March 19)
Technical Program	Monday (March 8) – Friday (March 12)
Exposition	Monday (March 8) – Wednesday (March 10)
Employment Bureau	Monday (March 8) – Friday (March 2)

For more information visit https://pittcon.org



The results are in…Pittcon 2021 is going virtual!

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.

Science is a dynamic and constantly evolving discipline. Every day, all over the world, scientists are making new discoveries; formulating new medicines to enhance the quality of life and solving global environmental, energy and food related challenges.

March 6 – 12, 2021
Virtual Event

Pittcon, a vital resource for knowledge, happens yearly to help keep you informed of, connected to and up-to-date on these significant ongoing findings and new instrumentation.

PITTCON™
CONFERENCE & EXPO

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Interview: ***The secret to Salmonella detection: Quick, accurate and low-cost screening in one day***

In this exclusive interview, we hear from Dr. Daniele Sohier, Senior Manager, Global Scientific Affairs - Food Protection at Thermo Fisher Scientific, about how her team helps combat foodborne pathogens by developing state-of-the-art workflows for easy and fast *Salmonella spp*. detection, with same-day results for fresh food products. Read this Interview here

Webinar: ***Pesticide residue analysis in cannabis: Method fundamentals and confirmation strategies***

In this on-demand webinar, cannabis and hemp consultant Julie Kowalski explains why confirmation testing is a must when screening for residual pesticides in consumer products. Access this webinar here

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Impact of STEC on the global food supply chain: Your questions answered

Shiga toxin-producing *Escherichia coli* (STEC) is a common pathogen found in ruminant animals that can lead to contamination of meat and milk during harvesting and processing. It can also contaminate other foods such as fresh produce if the water is contaminated. To learn about the impact of STEC on our global food supply, read the highlights from the question and answer discussion or register to watch the full webinar on demand. Access here

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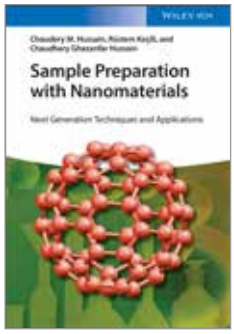
NOTICES OF BOOKS

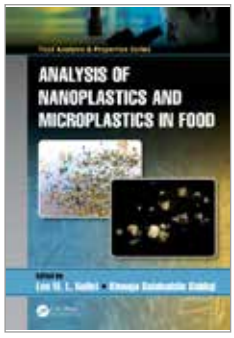


Sample Preparation with Nanomaterials: Next Generation Techniques for Sample Preparation

Chaudhery Mustansar Hussain, Authors

June 2021. Publisher: Wiley

The use of nanomaterials opened new perspectives also in analytical chemistry. Despite significant advances in instrumentation, sample preparation remained a bottleneck. This book gives a comprehensive overview of this issue: from special properties of nanomaterials over physico-chemical background up to most recent and representative applications (e.g., membranes, lab-on-a-chip echnology, and SERS), all important aspects are covered. Additional chapters on toxicity, legal and environmental aspects make this book a unique reference. Read more

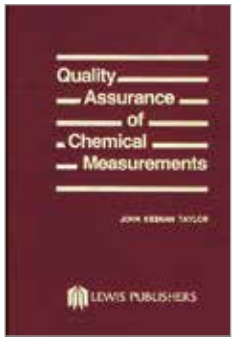


Analysis of Nanoplastics and Microplastics in Food

Leo M.L. Nollet, Khwaja Salahuddin Siddiqi, Editors

December 2020. Publisher: CRC Press

This book discusses sampling and analysis of nano- and microplastics; details the impacts of plastic residues in diverse compartments of the environment; includes a discussion of microplastics in freshwater; and discusses interactions of microplastics and POPs. Read more



Quality Assurance of Chemical Measurements

John K. Taylor, Author

December 2020. Publisher: Routledge

This definitive new book provides guidance for the development and implementation of a credible quality assurance program, plus it also provides chemists and clinical chemists, medical and chemical researchers, and all scientists and managers the ideal means to ensure accurate and reliable work. Each chapter has a degree of independence so that it may be consulted separately from the others. Read more

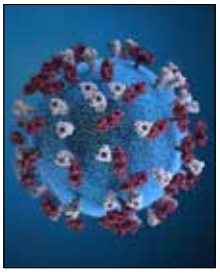
Advanced Materials and Techniques for Biosensors and Bioanalytical Applications

Pranab Goswami, Editor

November 2020. Publisher: CRC Press

This book provides a comprehensive review of the subject, including a knowledge platform for both academics and researchers. Considering biosensors as a central theme to this book, an outline on this subject with background principles has been included, with a scope of extending the utility of the book to coursework in graduate and postgraduate schools. Read more

PERIODICALS & WEBSITES

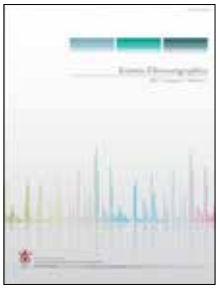


American Laboratory

American Laboratory® is a platform that addresses basic research, clinical diagnostics, drug discovery, environmental, food and beverage, forensics and other markets, and combines in-depth articles, news, and video to deliver the latest advances in their fields.
Featured Article: ***The Pandemic's Lasting Impact: Key Takeaways for Laboratory Professionals***. Posted: January 11, 2021, by Arvind Kothandaraman, General Manager, Specialty Diagnostics. Learn about the key takeaways from COVID-19 and its impact on the modern laboratory. Read more

LCGC

Chromatographyonline delivers practical, nuts-and-bolts information to help scientists and lab managers become more proficient in the use of chromatographic techniques and instrumentation, thereby making laboratories more productive and businesses around the world more successful.
Article: ***Tips for LC Coupled with Charged Aerosol Detection***. Author: Imad A. Haidar Ahmad. LC users that are new to CAD will find behaviours that are different from UV detectors. This article discusses some of the most important of these behaviours and provides tips to shorten the path to usable data. Read more



Scientia Chromatographica

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

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Spectroscopy

With the Spectroscopy journal, scientists, technicians, and lab managers gain proficiency through unbiased, peer-reviewed technical articles, trusted troubleshooting advice, and best-practice application solutions.
Article: ***ICP-MS Analysis of Nanomaterials for Use in Nuclear and Material Applications*** Authors: Mohamud, H.; Russell, B.; Pollard, A.; Schettino, G.; Liu, R.; Ge, G. This article outlines the progress made at UK's NPL in using ICP-MS for nanomaterial research using three case studies. Read more

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

March 3 – April 4, 2021
National Chemistry Congress (III Condequi) – Online
https://congresse.me/eventos/condequi

May 30 – June 4, 2021
22nd International Conference on Flow Injection Analysis and related Techniques (22 ICFIA)
Marseille, France
Organization: Aix-Marseille University, CNRS and the Japanese Association for Flow Injection Analysis
https://icfia2020.sciencesconf.org/

June 8 – 10, 2021
FCE Pharma
São Paulo, SP, Brazil
https://www.fcepharma.com.br/o-evento

June 14 – 18, 2021
XVII Italian-Hungarian Symposium on Spectrochemistry – Current Approaches in Health and Environmental Protection
Turin, Italy
https://www.ihss2020.unito.it

June 22 – 25, 2021
17th National Meeting on Mycotoxins (ENM 2021) – Hybrid event in person and online
Rio de Janeiro, RJ, Brazil |
https://www.enmicotoxinas.com.br/

August 22 – 27, 2021
EuroAnalysis 2021: Societal Changes Analytical Solutions – Hybrid event in person and online
Nijmegen, the Netherlands
https://www.euroanalysis2021.nl/

August 30 – September 3, 2021
International Conference on Electronic Materials (2021 IUMRS-ICEM) and the XIX Brazilian Materials Research Society Meeting (XIX B-MRS) – ONLINE
https://www.sbpmat.org.br/19encontro/

September 28 – 30, 2021
Analitica Latin America Conference & Expo
São Paulo, SP, Brazil
https://www.analiticanet.com.br/

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

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

October 18 – 21, 2021
Metrology 2021 – Online
Rio de Janeiro, RJ. Brazil
https://metrologia2021.org.br/

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

October 24 – 27, 2021
20th National Meeting on Analytical Chemistry (20th ENQA) & 8th Ibero-American Congress of Analytical Chemistry (8th CIAQA)
Dall'Onder Grande Hotel, Bento Gonçalves, RS, Brazil
https://enqa2021.com.br/

November 3 – 6, 2021
XXII Brazilian Congress of Toxicology (CBTox 2021)
Balneário Camboriú, SC, Brazil
https://www.cbtox2021.com.br/

November 16 – 19, 2021
60th Brazilian Chemistry Congress
Foz do Iguaçu, PR, Brazil
http://www.abq.org.br/cbq/

December 12 – 16, 2021
XXIII International Mass Spectrometry Conference (IMSC 2021)
Windsor Oceânico Hotel, Rio de Janeiro, RJ, Brazil
https://www.imsc2020.com/

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

Date to be determined

44th Annual Meeting of the Brazilian Chemical Society (RASBQ)
Maceió, AL, Brazil
http://www.sbq.org.br/reunioes-anuais

XVIII Chemometrics in Analytical Chemistry (CAC)
Courmayeur, Italy / Chamonix, France
http://cac2020.sciencesconf.org

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Electronic journals

2. Szafnauer, R.; Hughes, E. *LCGC-North America*, **2020**, *38* (12), pp 664-670. Available from: https://www.chromatographyonline.com/view/a-look-at-improved-aroma-profiling-of-foods-by-high-capacity-sorptive-extraction [Accessed 20 December 2020].

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10. Author, A. A. *J. Braz. Chem. Soc.*, in press.
11. Author, B. B., **2019**, submitted for publication.
12. Author, C. C., **2019**, unpublished manuscript.

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