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Brazilian Journal of Analytical Chemistry

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THE ANALYTICAL CHEMISTRY: ALWAYS VIGOROUS AND EXCITING

It is grateful to know that another issue of the Brazilian Journal of Analytical Chemistry is ready, and dealing about exciting themes. Besides important articles referring themselves about chemometrics, hydride generation, mass spectrometry, and others, Interview, Letter and Point of View sections also contribute to give a great dynamism to this issue of BrJAC, make it even more attractive. As examples, Ultrasound techniques are passionately emphasized in the Point of View by Prof. Luis Capelo, and challenging and intriguing questions are addressed regarding this technique. In the Letter written by Prof. Mauro Bertotti, he comments about some aspects related to education, teaching and research when focusing on undergraduate analytical chemistry. Again, challenging questions were pointed out as key components in research and teaching devoted to analytical chemistry. Finally, the interview with Prof. Fatibello, emphasizes his history of life and carrier, putting in evidence his pioneering and expertise in the electrochemistry area, and sensors in Brazil, and how it was (and continue to be) sprayed in the world. In fact, he was the founder of the first analytical chemistry laboratory at the São Carlos Federal University (UFSCar).

In view of the structure of this issue, and the way of how each theme and article paper is addressed by the authors, it is easy to realize that Analytical Chemistry is not only a discipline, but also an exciting part (or the entire part) of our scientific lives. So, I hope that the readers, after appreciating this issue, may enjoy each subjected developed, as well as be engaged by its atmosphere. We need a community that effectively contributes for the growing, upgrading and strengthening of the Analytical Chemistry, today and always. Inside this issue, we have some good examples for attaining these objectives. Enjoy reading.

Marco Aurélio Zezzi Arruda
Editor

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Summary

Expedient

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Letter

This section is reserved for you to send comments, suggestions or reviews about the articles or published reports by BrJAC. You may also submit comments on issues related to the Analytical Chemistry in Brazil and abroad. Join us in this project! Be part of that!

Undergraduate Analytical Chemistry: Education, Teaching and Research

Analytical Chemistry (AC) is concerned with providing information about the chemical and structural composition of a sample of matter and the field is founded on the conversion of a measured physical property of the species being examined to a usable signal. This definition can be broadened by considering AC as the science of creating and making use of the concepts, ideas and instrumental strategies for measuring the features of substances and chemical systems. Taking into account this broader definition and the remarkable growth of knowledge, it is essential to provide students with core ideas that can yield an organizational structure for the acquisition of new information.

In an attempt to decide what subject matter should be included in the AC curriculum, it seems clear that concepts and techniques such as matter and measurements, aqueous reactions and solution stoichiometry, acid base titrations, statistical analysis, sample handling and instrumental methods of analysis (separation, spectroscopic and electroanalytical techniques) are quite relevant. In a typical AC course, these subjects are also presented and discussed in experimental practices, but the laboratory has been poorly used as a learning resource because students tend to work at best conditions and the experiments are usually designed to demonstrate the principles of a technique, method or instrument and to incorporate practices whose main focus is to support content from the lecture. Activities on how scientists ask questions and seek answers are much less emphasized.

One of the most important contributions of AC may be to find ways to keep students engaged in their learning. We need undergraduate AC courses with goals that go beyond specific content and laboratory skills. For instance, by balancing courses with instructional tools that motivate students to learn in environments where they find more realistic problems which trigger their intellectual curiosity, imagination, creativity and initiative and challenge them to think critically and logically. These environments should be designed for students to improve the ability to take directions and to equip them with strong problem-solving skills required to face the problems they will find in the future, which will probably be very different to those we find now. This can be achieved, for example, by including short projects of limited scope in the course, in an attempt to teach students about the process of designing and conducting open-ended inquiries. A training based on such approach can help to prepare students for a better performance in their professional career and in the development of competences through systematic studies with a scientific base.

Science is a body of knowledge on the current understanding of the world and its mode of operation requires a set of practices to establish, expand and continuously discuss that knowledge. As science strongly depends on measurements and analytical chemists are generally involved with making measurements by using established and/or innovative methods, AC is a key component of chemistry research and education to respond questions such as "What is a comprehensive and accurate description of the chemical system or process, how the information can be obtained and what is its meaning?". Accordingly, the way AC courses are presented to our students will provide them the tools and skills to achieve their educational goals, as well as to define the future of the discipline and its relationship with other chemical sciences.

Mauro Bertotti
Departamento de Química Fundamental
Instituto de Química – Universidade de São Paulo

Interview

Orlando Fatibello Filho

For this edition of BRJAC, we invited professor Orlando Fatibello to talk about the history of electroanalytical chemistry in Brazil. It is a privilege to hear about this from him, because professor Fatibello is one of the pioneers in the area in our country. The founder of the first analytical chemistry laboratory at the São Carlos Federal University (UFSCar), he describes his field of work as a growing, promising research area, because electroanalytical methods commonly present lower consumption of chemicals, shorter analysis times, allow the in situ determination of analytes in colored solutions and/or with material in suspension, simultaneous determination of inorganic and/or organic analytes and speciation.

With new ideas for investigation arising in rounds of beer with friends or during a shower, professor Fatibello is a tireless worker. Working at the UFSCar for almost 38 years, he has supervised over 60 graduate students, many of whom are now university professors. Professor Fatibello made a large contribution to the Brazilian rank of publications in analytical chemistry, and has also published interesting papers on the field development too. Following this interview, the reader can get a very nice picture of the hystorical development of electroanalytical chemistry in Brazil, largely concentrated in São Paulo state but now spreading all over the country.

Patricia Logullo

Why do some fields, such as electroanalytical chemistry, tend to be concentrated in some regions, such as São Paulo State? Is there a concentration of specialties in some regions? Is this because of the proximity of agriculture centers? Instrument availability? Once initiating a research line with new techniques, a researcher prefers to explore one technique deeply, i.e., organizing the infrastructure (materials, laboratory, personnel) and the supervision of graduate students using the same line? Is this a vicious or a virtuous cycle?

Initially, I would like to thank the editors and staff of BrJAC for the honorable invitation and for the competent job they do in editing this journal.

The production of electroanalysis researchers in Brazil was 30% of the scientific publication of analytical chemists between 1990 and 2007[1,2] Brazil occupies the 14th position in the ranking of publications in chemistry in the period of 1996 to 2012. Analytical Chemistry occupies the honorable 13th position, ahead of many countries in Europe, Asia and Oceania. The Brazilian Analytical Chemistry ranks first (13th overall position), ahead of Electrochemistry (15th position), Physics and Theoretical Chemistry (17th), Organic Chemistry (17th), Inorganic Chemistry (19th) and side by side with spectroscopy (13th).[1]

We could not identify the number of papers in electroanalytical in this database[1]. Thus, researching the literature[2] and the Web of Science (2004-2013), we observe that the Brazilian production in electroanalytical chemistry is oscillating between 30 and 32% in the last decade.

To respond to you about why electroanalytical chemistry concentrated in São Paulo, I will have to describe briefly how it all started. I suggest readers to consult the work of dear, missed Prof. Paschoal Senise[3] for details. The University of São Paulo was established in 1934 and Chemistry course began in 1935 at the Faculty of Philosophy, Sciences and Letters of USP, in Alameda Glete, with the important participation of Prof. Heinrich Rheinboldt and his assistant, Professor Heinrich Hauptmann, the main teachers who created and organized the course in the typical "German format".

Prof. Senise graduated, together with three colleagues, in the first class, that of 1937. After he completed his doctoral degree course in 1942, under the supervision of Prof. Rheinboldt, Prof. Senise became a pioneer of analytical chemistry at USP. In the period between 1950 and 1952, Prof. Senise completed a postdoctoral internship with Profs. P. W. West and Paul Delahay at the University of Louisiana, in Baton Rouge, LA, USA. With the first supervisor, he worked with microanalysis, and the second, in electroanalysis. These research lines were then implanted in the USP, and of the 10 doctoral students that he supervised, Oswaldo E. S. Godinho, Jaim Lichtig and Eduardo Neves continued working and supervising students in electroanalysis. Prof. Godinho worked at Universidade Estadual de Campinas (UNICAMP) and guided some students in the area, and Prof. Lichtig, in the Institute of Chemistry (IQ), in USP, also supervised some students in the field of electroanalysis. However, it was Prof. Neves who had greater projection and could supervise around 60 MSc and PhD students, many of them in electroanalytical chemistry. He is considered the patron of electroanalytical chemistry.[4] Considering the academic genealogy of Prof. Neves, there are more than 400 master's students and doctoral students of the second generation and over a thousand of them currently; and students from a sixth generation in the area have been identified. Many of the second generation students remained in the state of São Paulo, in the IQ-USP, or in the IQSC-USP, UFSCar, UNESP-Ar, FFCLRP and other institutions.

In 1968, as an Assistant Professor, Prof.Neves, who was accredited as a supervisor in the institution, proposed the creation of a graduate course in the IQ-USP, named "Some Aspects of Electroanalytical Chemistry". This theoretical and experimental course was taught by him for more than two decades and also in collaboration with colleagues (such as Prof. Gutz).[4] Hundreds of graduation students attended the course, which also contributed to the diffusion/dissemination of electroanalytical chemistry.

In October 1978, Prof. Neves, along with Prof. Tibor Rabóczkay, organized the first Brazilian Symposium on Electrochemistry and Electroanalytical (SIBEE I) at IQ-USP. The SIBEE II, as well as the V and VI SIBEEs, took place in São Paulo (SP), in the IQ. An article from Professors Luis A. Vaca and Roberto Tokoro[5] presents a historical review, evolution and growth of these two areas of research. The SIBEEs III and IV occurred in the city of São Carlos (SP), the SIBEE VII in Ribeirão Preto (SP), the VIII in Campinas (SP, UNICAMP), the SIBEE IX, along with the CIBAE (Eletrochemistry Iberoamerican Congress) in Águas de Lindóia (SP) and the SIBEE X in São Carlos again, on the premises of UFSCar. The first time the SIBEE left the State of São Paulo was in 1999: the SIBEE XI was carried out in Maragogi (AL). From there, other SIBEEs were being organized in other states. The offering of SIBEEs biannually certainly gives an important contribution to the development of electrochemical/electroanalytical in São Paulo State and Brazil, and the first 10 SIBEEs, that happened in São Paulo, must have had the greatest impact on the growth of those areas in the State of São Paulo.

The dissemination and discussion of papers in the National Meeting of Analytical Chemistry (ENQAs) were also of great value, as shown in several publications. [2, 5, 6, 7, 8] Funding for research projects and scholarships for master's, doctoral and post-doctoral students by FAPESP was very important, as well as by the national agencies CNPq and FINEP, and, regarding qualification of teachers and students, the CAPES. We cannot forget the PADCT projects and the important contribution of the Brazilian Chemical Society (SBQ) with the Annual Meetings and the official journals: Química Nova and JBCS. Furthermore, we recognize

"Prof. Senise became a pioneer of analytical chemistry at USP, where he implanted the research lines of microanalysis and in electroanalysis. Of the 10 doctoral students that he supervised, Oswaldo E.S. Godinho, Jaim Lichtig and Eduardo Neves continued working and supervising students in electroanalysis. Professor Neves is considered the patron of electroanalytical chemistry. Along with Prof. Tibor Rabóczkay, he organized the first Brazilian Symposium on Electrochemistry and Electroanalytical (SIBEE I) at IQ-USP."

“In the Lattes Platform, we can find 358 doctors who selected the electroanalytical area for work, 162 (45.2%) from São Paulo, 27 (7.5%) from Minas Gerais and 25 (7.0%) from Paraná. There is a good availability of equipment, materials and infrastructure in virtually all regions of Brazil, and with the policy of recruiting young and competent doctors in several Brazilian universities, especially in the federal universities, the training efforts in the area has been also setting out the state of São Paulo, evidencing the geographical distribution of these professionals in the whole country. We consider this to be a virtuous cycle.”

the importance and the support provided by the Division of Analytical Chemistry for more than two decades in the organization of the Annual Meetings.

In a query to the CNPq's Lattes Platform,[9] we found 358 doctors who selected the electroanalytical area for work. Of these, 162 (45.2%) are from the State of São Paulo, followed by Minas Gerais, with 27 researchers (7.5%) and Paraná, with 25 researchers (7.0%). The Brazilian production in electroanalytical chemistry is around 3.4% of the world production and the State of São Paulo produced around 67.8% of the output in the last five years. As the State of São Paulo has 45.2% of Brazilian researchers, we conclude that it is the state with the highest *per capita* productivity. The production of the researchers from the State of São Paulo was 72.7% from January 1990 to October 2007.[6] This apparent decrease in productivity of the São Paulo State researchers is due to the sharp increase in the productivity of other states. The southern and southeastern Brazil states have substantial production, but the productivity of some states of the North, Northeast and Midwest have grown sharply in the last decade.

There is a good availability of equipment, materials and infrastructure in virtually all regions of Brazil, and with the policy of recruiting young and competent doctors in several Brazilian universities, especially in the federal universities, the training efforts in the area have been also setting out the state of São Paulo, evidencing the geographical distribution of these professionals in the whole country. We consider this to be a virtuous cycle.

In your recently published article about the advances in analytical chemistry in Brazil, you mentioned that seven Brazilian groups were developing new biosensors, immunosensors and genosensors for determination of analytes, many of them using vegetable crude extracts and plant tissues instead of purified enzymes. This was highlighted in a seminar you presented in 2012. Is this a typical Brazilian innovation (an adaptive resource facing importation difficulties)? What have been the results in this field of research since then?

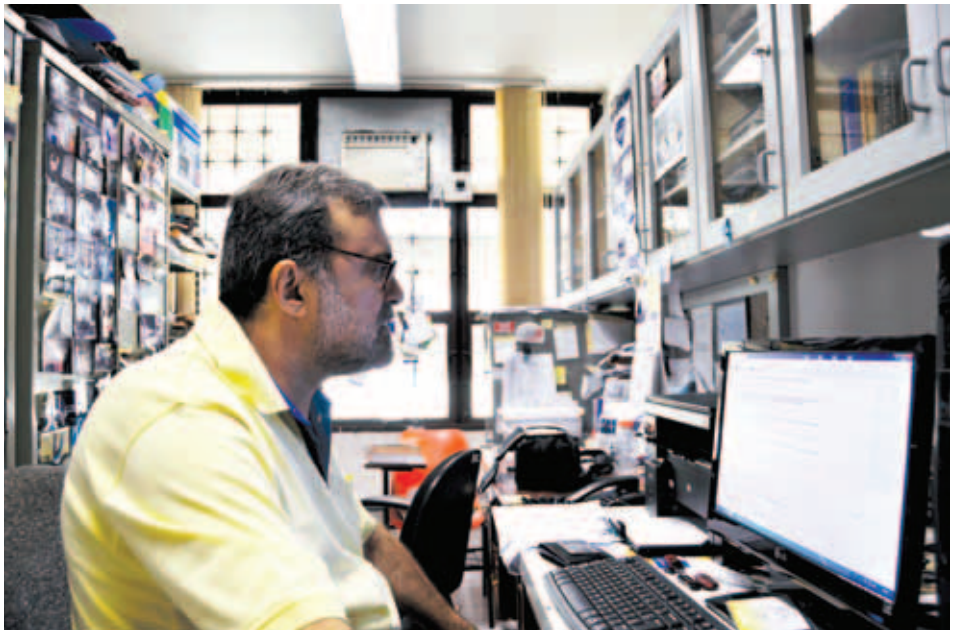
Today, there are a greater number of research groups in Brazil working in the construction and application of biosensors, immunossensors, and electrochemical genossensors for various analytes. I would say that we have research groups in all regions of Brazil working with these types of devices. We must not forget the pioneer, Prof. Graciliano de Oliveira Neto, who did his postdoctoral studies with Prof. G. G. Guilbault, in the Department of Chemistry, University of New Orleans (LA, USA) in 1979-1980. Prof. Graciliano introduced me to Prof. Guilbault and I did my postdoc with that same group of American researchers between 1987-1989.

The first article on biosensors from a Brazilian (Prof. Graciliano) was published in 1986 in Analytica Chimica Acta. Prof. Hideko Yamanaka and Prof. Graciliano published the first scientific dissemination article in the Química Nova journal in 1988, and Prof. Capelato and I published the first review article about biosensors in Química Nova in 1992. Prof. Lauro Kubota, BrJAC Editor, works in the area thanks to the influence of Prof. Graciliano, just like many others throughout the country. Prof. Kubota, despite having started working in the area a few years after our generation, is the researcher in the area with the highest productivity and international recognition.

In Brazil, Prof. Graciliano was the first to work with plant tissues as enzyme sources in the construction of electrochemical biosensors, and my group with crude extracts. The advantages and disadvantages of working with these biological materials can be found in our articles and in particular in the article published in 2004 in Química Nova.[10] The use of crude plant extract in the construction of biosensors was an innovation when we proposed it, and now there are many research groups in Brazil and abroad working with this type of material.

What is your view about the adoption of the new electroanalytical techniques by the Brazilian industry? Out of the research field, are the new evidences being adopted? Are new patents being registered in Brazil? What is the economical trend?

There is not much news in terms of developed electrochemical techniques, except



for the new sensors and/or carbon-based biosensors, various composites, polymer films containing nanomaterials, bismuth film, among others. The boron-doped diamond electrode has been gaining a wide space due to its electrochemical properties, such as low and stable background current, extraordinary morphological and macro structural stability at high temperatures, weak adsorption of molecules or ions, high long-term stability and wide potential window in aqueous and non-aqueous medium. Prof. Kubota has probably more than 20 patents, a good number of them on the development of bio(sensors). He is probably the Brazilian researcher with the largest number of patents on the subject. However, I am not aware if there is any product in the industry.

Focusing only in my research lab (LABBES), I can tell you that one doctoral student, Vagner Bezerra, and FAC Industry, have built a potentiostat/galvanostat (PG) with electrochemical cell in flux thermostated (EFC), printed electrode (SPE) with a coupled boron-doped diamond (SPE-BDD) and a flow system with multicommutation. In addition, wireless communications, including Wi-Fi, Bluetooth and GPRS (3G), solar energy plates and a GPS receiver were used to determine *in loco* the electroactive metal ions by square-wave anodic stripping voltammetry (SWASV). Measurements *in loco* allow us to obtain quick results, usually with fewer analytical steps and reduced possibilities of contamination and/or changes in the samples, compared to conventional collection methods with subsequent laboratory analysis.

Globally, there are lots of equipment and sensors producers that have been distributing portable or reduced size instruments, such as the serum glucose meter (glucometer) for diabetic patients and the lambda probe (a sensor based on zirconium dioxide) for oxygen concentration measurement in the exhaust of vehicles, comparing with the concentration of oxygen in atmospheric air for communication to the central electronic unit (CEU), for a better control of the ratio of fuel and oxygen in the air. There are also many other examples of electroanalytical instruments that are traded by several industries: sensors for gases, multiparameter meters for pH determination *in loco*, conductivity, O_2, Cl^-, NO_3^-, Ca^{2+}, E_h, turbidity, temperature, salinity, total dissolved solids. Some of the measurement devices of the water distribution in water treatment plants are electroanalytical too. There are other potentiometric sensors for gases in solution, such as NH_3, CO_2, H_2S, SO_3 among others, and amperometric for O_2 and H_2O_2, that are widely employed.

In the food industry, we must highlight a

> **“The boron-doped diamond electrode has been gaining a wide space due to its electrochemical properties, such as low and stable background current, extraordinary morphological and macro structural stability at high temperatures, weak adsorption of molecules or ions, high long-term stability and wide potential window in aqueous and non-aqueous medium.”**

“I believe there are many opportunities for the development of electronic nose and tongue, which have many potentiometric, piezoelectric, amperometric, or voltammetric thermodynamically reversible sensors, for successful operation.”

pH meter (the pH is conditional) for meat, with a stainless steel penetration probe, a biosensor to monitor the quality of fish and meat and a glutamate biosensor. There are many more examples: the glass electrode is used in potentiometric titrations to determine the acidity of wines, vinegars and other food products. The conductometric titration is also much used for the same purpose. In the manufacture of fermented drinks (beer and wine), alcohol and processes involving fermentation, various sensors are employed for pH, $CO_2(g)$ $O_2(g)$, glucose and ethanol. For the control of several analytes in the environment, there are bio(sensors) or bio(chips) for metal species, anions, organochlorines, organophosphates and carbamates, viruses and bacteria, among others.

I believe there are many opportunities for the development of electronic nose and tongue, which have many potentiometric, piezoelectric, amperometric, or voltammetric thermodynamically reversible sensors, for successful operation. There is a shortage in the domestic market, for example, of robust sensors and/or actuators that can be employed in the monitoring of substances of interest in the food, alcohol-chemistry, pharmaceutical, petrochemical and general chemistry industries. Human resources for the development of the automation systems are already available in Brazil, for mastering the technology of manufacturing of these sensors and/or actuators.

Electroanalytical methods are also useful in the speciation of organic and inorganic analytes and a current trend is the use of electrochemical detectors in paper, such as point-of-care testing, where it is possible to separate the analyte(s) of interest in the detection device.[11]

You began your professional career in a private industry, but you were very early involved with teaching. What is your opinion about the evolution of Brazilian undergraduate research programs, especially in the analytical chemistry field? Is the number of students increasing? Are they being hired in private companies or mainly becoming researchers in universities? What can an under graduation student expect today for his/her future?

Yes, it is true. My interest in chemistry began during my first job in São Paulo, in the Sociedade Paulista de Indústrias Químicas, SAPIQ. SAPIQ was a midsize chemical industry that dominated the market of dehydrated vegetable oils, such as castor, linseed and cotton, lead, zinc or cobalt naphthanates and resins, used as driers for various types of paints. In 1966, at age of 14, I was hired as an office-boy and archivist. In SAPIQ my vocation was awakened. For details see my interview for the SBQ website.[12]

I was hired, along with three other colleagues/friends, in December 1976 by the Department of Chemistry (DQ) at UFSCar. During the undergraduate program I had already decided to teach and to research in analytical chemistry, and I believe that I have been making a significant contribution in this area, encouraging and teaching students for over 38 years.

The undergraduate courses in chemistry are constantly evolving and the same happens in analytical chemistry. In addition to the chores and/or activities required for the course, there is a growing involvement of students in research in the laboratories under the supervision of teachers in the area of analytical chemistry, with and without scholarships from support agencies. The number of students is growing at a significant rate in DQ-UFSCar, and the same is possibly happening throughout the country. Of over 60 students from DQ supervised by me, a significant fraction followed academic careers, and today many of them are university professors. There are also those who were hired by the private initiative. The future is bright and the Brazilian market still lacks good professionals. There is plenty of space and opportunities for good students.

Three years ago, in an interview, you advised students to dedicate themselves more to studying and to engage in scientific projects more. Do you believe chemical students receive the support they need to develop? Or is there a cultural problem?

In that interview I have advised the students to study more, to be more critical and increasingly engage in research activities. I

have also warned them of the importance of participating in conferences and/or scientific meetings. Moreover, they should invest in computing, Portuguese and English courses, and dedicate themselves to the dissemination of knowledge by written, electronic and oral media. These various activities should be pleasant and joyful – otherwise they will be unhappy and it will compromise their performance and/or professional career. If a student is not getting along well with the course, or not having satisfaction with it, I suggest looking for the course coordinator, or a teacher of the student's trust to expose the problems. If they conclude that the student has no aptitude, skill or interest in it, the student should, the sooner the better, to change to another course, and not extend the decision, because this would cause more disappointment.

Today the conditions found in universities are much better than in the past. A few years ago, making imports of reagents took months or years; today we have the reagent in our laboratories in days. In some countries, in hours.

Imagine what it was to do a literature search a few years ago: we had to handle the Chemical Abstract, read each summary and take notes of the journal where the article was published. Then, depending on the journal, we had to wait months to receive it via COMUT (a Brazilian bibliographic exchange program). As a rule, we had to expect two to three months to receive a copy of an article. Today, in minutes we do a literature search and get the articles needed to develop our research.

To publish an article before the arrival of the Internet in Brazil (1991) was no easy task. We had to type the manuscript (I worked in the era of the typewriter), send three printed copies via airmail. The editor would receive the manuscript and send it to the referees by regular mail too. After some 2-4 months, you received the referees' report, made the corrections (and in the case of the typewriter, you had to type the entire manuscript again) and sent it back via airmail to the editor. If no further corrections were needed, you could receive the acceptance communication after 2-3 months. If further corrections were needed, then 2-3 months would pass, and so forth. Publishing more than four articles per year was not easy or trivial task. Today the situation is highly favorable and students receive all necessary support from educational institutions and professionals.

Eduardo Neves wrote that scientific creative ideas often come up when scientists are not in the lab, but having beer with friends, talking in long walks or simply relaxing. Do you agree with this? Tell us

“Today the conditions found in universities are much better than in the past. A few years ago, making imports of reagents took months or years; today we have the reagent in our laboratories in days. In some countries, in hours. Imagine what it was to do a literature search a few years ago: we had to handle the Chemical Abstract, read each summary and take notes of the journal where the article was published. Then, depending on the journal, we had to wait months to receive it via COMUT (a Brazilian bibliographic exchange program). As a rule, we had to expect two to three months to receive a copy of an article. Today, in minutes we do a literature search and get the items needed to develop our research.”

“Many ideas come when we are outside of the lab, talking to students and fellows. We are always tuned in our work and producing new ideas. We are different from a typical worker who works on average 8 hours a day, and who can forget about work when out of service. Many of these ideas come in any circumstance: having a beer with friends, bathing, having meals and so on. Often I wake up during the night with a new idea, and I use to keep a small notebook by my bed to record them, not risking to lose it. For example, I am officially on vacation right now, but I am here writing answers for this interview. Good science is produced working seriously and competently, but always aware of what is being produced in the same area of expertise elsewhere and also hearing the suggestions of students and colleagues.”

about one idea that came up to you in such circumstances. Or do you believe that good science is produced only from hard work?

I had the privilege of being supervised by Prof. Neves, “our eternal Guru” (to paraphrase Prof. Gutz, my scientific friend and brother). I think just like him: many ideas come when we are outside of the lab, talking to students and fellows. We are always tuned in our work and producing new ideas. We are different from a typical worker who works on average 8 hours a day, and who can forget about work when out of service. Many of these ideas come in any circumstance: having a beer with friends, bathing, having meals and so on. Often I wake up during the night with a new idea, and I use to keep a small notebook by my bed to record them, not risking to lose it. For example, I am officially on vacation right now, but I am here writing answers for this interview. Good science is produced working seriously and competently, but always aware of what is being produced in the same area of expertise elsewhere and also hearing the suggestions of students and colleagues. Albert Einstein once said that geniality is 1% inspiration and 99% perspiration. I agree with him: the success of a person has a very high component of perspiration and very little of inspiration; there are few privileged people who do not follow these standards. I work a lot to have some career success, at least 50 hours a week on average. Thus, I believe that success depends largely on the commitment to the job, and, as thought by our Guru Dudu, we cannot disconnect from science or from the work we do. With few exceptions, any place is a place to think and/or discuss our work.

What is your opinion about the quality of scientific journals dedicated to chemistry in Brazil and in Latin America? Do you believe that they have good standards of editing and quality control of the manuscripts?

The journals of the Brazilian Chemical Society (Química Nova, Química Nova na Escola and the Journal of the Brazilian Chemical Society) are the best in Latin America and have excellent quality standards. BrJAC is starting very well and should also be one of the best in Latin America soon, since it has a competent Editor and Editorial Board, and a very high quality and international projection.

The first teams of electroanalytical chemists were already working since the 1970's in São Paulo State. However, it was only in the 1990's that the Division of Electrochemistry and Electroanalysis was established in the Brazilian Chemistry Society. What is the reason for this delay?

The reasons and explanations of what happened are well discussed in the Avaca and Tokoro article.[5] I invite our readers to know that article if interested. I do not think there was a delay in the creation of Electrochemistry and Electroanalytical Division. It was created at the right time, because we had a good contingent of colleagues working in these areas, with a good portion of competent and experienced professionals who have helped us a lot at that time.

The development of electrodes for detection of analytes in small samples seems to have received much attention in the last years, and they are present in your research line too. What have been the main applications of the scientific advances in this area in the food, pharmaceutical and other industries? Is the adoption by the industry quick or does it take many years? Is it expensive?

One of the advantages of electroanalytical chemistry is the possibility to build the potentiometric, amperometric / voltammetric electrode etc. in size needed to solve an analytical problem. Besides, it can be adapted for use in electrochemical cells of varying volumes, in flow injection systems, chromatography, capillary electrophoresis, among others. In our research group, we came to develop flexible electrodes with the active material placed inside a polypropylene tube of 0.8-mm internal diameter, for the pH measurements in hard-to-reach places. With that same tube, we built a flexible biosensor containing immobilized soybean tissue on a composite of epoxy and graphite powder (there is also the possibility to immobilize carbon nanotubes and metallic nanoparticles), as the source of the urease enzyme, and used it for urea determination. This same strategy can be developed in the construction of other sensors and/or biosensors.

The application of bio(sensors) and nano(bio) electrochemical sensors is already a reality in many industries, in environmental control and even in outer space spaceships and/or in the diagnosis of astronauts.

There are sensors used in gas leak detection, such as $H_2(g)$ $NH_3(g)$, $N_2H_4(g)$ of $N_2O_4(g)$ $NO_2(g)$ and others, in the work environment. In clinical analysis and/or point-of-care hospitals, there are electrodes for glucose, cholesterol, uric acid, lactate, ketones, creatinine, nitric oxide, among others. There are sensors for $O_2(g)$ and $CO_2(g)$ in the blood and others. The 1st Automatic Radiometer performs the determination of blood gases of patients in a short time, since the equipment is next to the patient's bed in an intensive care unit (ICU) of the hospital.

Nanoelectrodes to detect traces of explosives (nitroaromatics, nitrosamines, nitroesters, organic peroxides, among others) have been studied for some time and much of these studies results are being applied in the laboratory and in the field, with reports of prototypes to be launched later this year in the American and European markets. The pentaerthyritol tetranitrate (PETN), 2,4,6-trinitrotoluene (TNT) and cyclotrimethylenetrinitramine (RDX) have been determined at concentrations of the order of parts per trillion in the air, and there are industries interested in marketing these products.

Antibodies have been employed in various nanoimunosensors, devices with high selectivity and sensitivity and low detectability, due to the high affinity of the antibody for antigen, and these have been successfully used in medical diagnostics, environmental monitoring (e.g. to detect herbicides and pesticides), in public health and safety control. There are many miniaturized and portable systems, of fast and low cost, employing, for example, microfluidic platforms, on-chip sensors, integrated or not to the lab-on-chip (LOC) systems, micro-total analysis systems (μ-TAS) and field effect transistors (FET) sensors. There are many very competent Brazilian groups working with most of these systems, but we need to increase the critical mass of researchers.

Printed electrodes containing various inorganic, organic and biological modifiers (enzymes, plant tissues, animal tissues, cells, organelles, DNA, RNA, antibodies, peptides, etc.) have been manufactured and also applied to the determination of analytes in various matrices, and the big challenge for Brazilian and worldwide researchers is to increase the stability and lifetime of these devices, as well as increasing the shelf-life. The great advantage of the development of these sensors is their mass, and often inexpensive production.

“The application of bio(sensors) and nano(bio) electrochemical sensors is already a reality in many industries, in environmental control and even in outer space spaceships and/or in the diagnosis of astronauts.”

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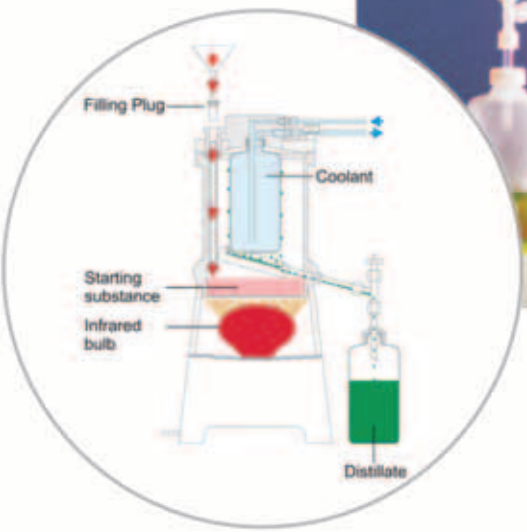
Acid purification apparatus BSB-939-IR

Sub-boiling apparatus for production of fresh, high-purity acids for ultra trace analysis

distillacid

This unique subboiling system is employed to produce high-purity acids for use in trace analysis by acid distillation. The acid is always fresh and is therefore guaranteed to be of the desired purity. Contaminated acids can be re-cleaned.

Contact-free heating of the acids by means of an infrared lamp allows an equilibrium between the absorbed IR radiation and the liquid's evaporation heat to be established. This equilibrium state is reached at approx. 10°C/50°F below the individual acid's boiling point. This allows the acid to evaporate slowly for a gentle distillation.



By cleaning more economical low purity acids, you can save up to 90% of the cost of analytically pure acids. As a rule, the system therefore pays for itself within the first year.

Direct quantification of trace element concentrations in spring waters by ICP-MS

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Abstract

A direct quantification of trace element concentrations in spring water samples using inductively coupled plasma mass spectrometry (ICP-MS) was evaluated with respect to the selection of isotope, detection limits, accuracy, precision, and spectral interferences. The measurements were carried out for each of the following isotopes: ^{107}Ag, ^{27}Al, ^{75}As, ^{137}Ba, ^{9}Be, ^{209}Bi, ^{111}Cd, ^{59}Co, ^{52}Cr, ^{133}Cs, ^{63}Cu, ^{71}Ga, ^{115}In, ^{7}Li, ^{55}Mn, ^{60}Ni, ^{208}Pb, ^{85}Rb, ^{82}Se, ^{86}Sr, ^{203}Tl, ^{238}U, ^{51}V, ^{66}Zn, ^{232}Th, ^{89}Y and the rare earths: ^{139}La, ^{140}Ce, ^{141}Pr, ^{143}Nd, ^{152}Sm, ^{151}Eu, ^{157}Gd, ^{159}Tb, ^{163}Dy, ^{165}Ho, ^{167}Er, ^{169}Tm, ^{174}Yb, ^{175}Lu. Without pre concentration or separation, the method presented selectivity, detectivity and accuracy for the selected isotopes varying between 92 and 108% with relative standard deviations <5 % and detection limits varying from 0.4 ng L^{-1} (Cs) to 0.5 µg L^{-1} (Zn) and 0.2 ng L^{-1} (Lu) to 2.7 ng L^{-1} (Yb) for the rare earth elements. These detection limits are appropriate and below the guideline levels set by Brazilian Ministry of Health (2914-12/2011) for all the elements. The method was applied to the analysis of spring water samples collected at sources spread throughout the historical towns in the state of Minas Gerais, Brazil. In the past and even today these sources played essential and strategic roles in supplying these towns with potable water. The popular idea that natural spring water is "clean" is misleading since many toxic elements may be naturally present. The arsenic level in one of the analyzed sources was higher than the guideline level, demonstrating that possible health risks for humans may not be excluded.

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1. Introduction

Water is a basic necessity, an essential element for life as well as the world's most valuable asset in future resources. Although almost two thirds of our planet is covered with water, only a tiny fraction of less than 1% is available for the needs of mankind as pure and healthy drinking water. Of this, almost all available resources are stored underground (about 99%) from where it must be tapped for drinking water supply as well as for agricultural, industrial and environmental purposes. Hosted in various types of aquifers, groundwater appears at the surface in the form of springs feeding streams and saturated wetlands. While still underground, it provides an estimated 25 to 40% of all drinking water on the planet. It may interact with various minerals in the aquifer and become enriched in several elements, some of which are good for health although some are less so and some can be even toxic if critical concentrations are exceeded [1].

Ground and surface waters are among the most important media that act as a bridge between rock and soil geochemistry and human physiology. In addition to the anthropogenic sources, the natural baseline geochemistry of groundwater and surface water resulting from interaction with rocks also creates widespread health and acceptability problems in many regions of the world [2]. The levels of trace elements in groundwater and their significance in terms of health and environmental protection are of particular importance since safe drinking water is an extreme necessity in developing countries.

An increasing use of rare earth elements (REEs) for different applications increases their release into the environment where REE traces are bio-accumulated by organisms. Their bioavailability and toxic properties are currently under investigation and REEs are known to activate or inhibit metabolism or enzyme activity. As a consequence, REEs are

already part of national and EU legislation [3]. The average of uranium in the earth's crust corresponds to 2 µg uranium per g of soil, being concentrated mainly in the acidic magmatic rocks with lesser amounts in basic minerals and sediments [4]. Soluble U(VI) carbonate complexes produced in soil can be transferred into natural water. High values of uranium in drinking water and foodstuffs may lead to harmful effects in human beings. Uranium has both chemical and radiological toxicity, being the kidney and lung the two important target organs [5, 6].

The determination of trace elements in natural water is of increasing importance for routine monitoring of environmental pollution and studies on the ecological and physiological role of the essential and toxic elements. Most trace elements regulated by brazilian and international regulations can be properly measured by inductively coupled plasma mass spectrometry (ICP-MS), since this multi-element technique is able to provide, in a short analysis time, the detention limits, precision and accuracy required for diverse applications [7]. In non-contaminated waters, uranium, thorium and the REE (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu) concentrations are typically in the ng/L range, except for some geothermal and acid waters that have REE concentrations in the µg/L range. The low concentration of these elements in waters implies an analytical challenge even for a technique such as ICP-MS.

In this work a direct quantification of Ag, Al, As, Ba, Be, Bi, Cd, Co, Cr, Cs, Cu, Ga, In, Li, Mn, Ni, Pb, Rb, Se, Sr, Tl, U, V, Zn and Th, Y and the REEs: La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, in natural water samples using one ICP-MS was evaluated with respect to isotope selection, detection limits, accuracy, precision, matrix effects, linear calibration range for each isotope and spectral interferences. The method was applied to the analysis of spring water samples collected in sources spread throughout historical towns in the state of Minas Gerais, Brazil. In former times these sources played an essential and strategic role in supplying these towns with potable water. Even today this water is used by both the local population and visitors who trust its quality.

2. Experimental

2.1. Instrumentation

All measurements were carried out using a Perkin Elmer ELAN DRC-e ICP-MS spectrometer equipped with auto sampler (AS-93plus), sea spray nebulizer, cyclonic spray chamber and quadrupole analyzer consisting of 4 cylindrical bars of 20 cm length and 1 cm diameter, allowing mass determinations in the $1 < m/z < 240$ range in less than 0.1 s. Standard and sample solutions were aspirated into the argon plasma via a peristaltic pump with 1 mL/min carrier flow rate. Data were acquired using a Dell computer with a PerkinElmer-SCIEX quantitative ELAN Version 3.4 software.

2.2. Reagents and standard solutions

A multi-element standard solution (Smart Tune solution - N8125040 - PerkinElmer) was used for daily performance evaluation, lens voltage calibration and nebulizer gas flow (NEB) optimization. Solutions, standards, and dilutions were prepared with ultra-pure water (18.2 MΩ cm) obtained from a Milli Q Element System (Millipore, Belford, MA, USA). Ultrapure 69.5% (w/w) HNO_3 (Fluka) was used for the preparation of all standard solutions and in the preservation and dilution of the samples. Argon gas (99.999% purity), supplied by White Martins, was used for plasma, sample nebulization and as auxiliary gas.

Multi-element Standard 2 PerkinElmer N9300232 solution (STD 2) containing REEs, Th, Y (10.0 mg/L) and Multi-element Standard 3 PerkinElmer N9301720 solution (STD 3) containing Ag, Al, As, Ba, Be, Bi, Cd, Co, Cr, Cs, Cu, Ga, In, Li, Mn, Ni, Pb, Rb, Se, Sr, Tl, U, V, Zn (10.0 mg/L) were used for preparing the calibration curves and all the solutions and samples were prepared in 1% HNO_3 for ICP-MS. Appropriate dilutions from the stock multielement standard solutions (STD 2, STD 3) were made every three days. Since the samples to be analyzed are primarily ground waters, the elements of interest were calibrated at levels typical of the samples analyzed.

Two certified reference materials, TM-DWS.2, lot 1010, a trace element fortified sample from the Environment Canada and synthetic water SPS- SW1 Batch 116 from Spectrapure Standards (Oslo, Norway) were used for recovery and precision studies.

Germanium (Aldrich 356247), rhodium (Fluka 83722), yttrium (Fluka 95802), indium (Fluka, 8372) and holmium (PerkinElmer N9300123) were used as internal standards in concentrations of 20 µg/L (Rh, Y, Ho, In) and 100 µg/L (Ge) in 2% (v/v) nitric acid.

2.3. Sample collection

The spring water samples used for this study were collected in the historical and touristic towns of Minas Gerais State, Brazil, in June and July, 2011. After collecting the samples were filtered in the field through Millipore 0.45 µm filters and immediately acidified with nitric acid (pH <2) and stored in the acid pre-cleaned, low density polyethylene bottles. Total dissolved solids (TDS) of the water samples ranged from 4.2 mg/L (Lajes) to 79.5 mg/L (Kaquende), which is relatively low for analysis using the Sea Spray concentric nebulizer, since this nebulizer is ideal for samples with high dissolved solids conferring significant sensitivity gains.

2.4. Procedure

Before starting the analytical measurements, the plasma instrument is allowed to equilibrate for 30 min and then the daily performance check of the ICP-MS ELAN DRC-e is evaluated using the Smart Tune solution - N8125040 - PerkinElmer (10 µg/L Ba, Be, Ce, Co, In, Mg, Pb, Rh, U in 1% HNO_3). During the daily performance the instrument settings are optimized for sensitivity, interferences (oxides,

double charging), background and precision. Detectivity is checked through the intensities (cps) of ^{24}Mg (> 50 000), ^{115}In (> 250 000) and ^{238}U (> 200 000) and precision (< 2%) through the relative standard deviation (RSD) of these measurements. Oxides of Ce, mass 156 (corresponding to $^{140}Ce^{16}O^{+}$) and doubly charged Ba mass 69 (corresponding to $^{138}Ba^{2+}$) should be below 3%. The background for mass 220 is typically below 25 and the RSD should be below 5%.

Besides the daily performance evaluation, ELAN (Version 3.4) software performs lens voltage calibration and nebulizer gas flow-rate (NEB) optimization that allows the correct gas flow rate to be chosen. The optimum gas-flow settings are then stored in the analytical method for use in the routine analysis. The instrumental settings and optimized operating conditions for the determination of the elements of Group 1 (Ag, Al, As, Ba, Be, Bi, Cd, Co, Cr, Cs, Cu, Ga, In, Li, Mn, Ni, Pb, Rb, Se, Sr, Tl, U, V, Zn) and elements of Group 2 (REEs, Th, Y) are summarized in Table I.

Table I. ELAN DRC-e instrumental settings and operating conditions.

ICP-MS operating conditions		
	Elements group 1	**Elements group 2**
RF power	1400 W	1400 W
Nebulizer	Sea Spray	Sea Spray
Nebulizer gas flow-rate	0.78 L/min (set for <3% oxides)	0.82 L/min (set for <3% oxides)
Auxiliary gas flow-rate	1.10 L/min	1.10 L/min
Sample introduction flow- rate	1mL/min	1mL/min
Plasma gas flow-rate	16 L/min	16 L/min
Lens setting	AutoLens	AutoLens
Spray chamber	Cyclonic Spray	Cyclonic Spray
Interface cones	Nickel	Nickel
Lens voltage	6.5 V	7.5 V
Analog stage voltage	-1650 V	-1937
Pulse stage voltage	1200 V	900
Mass spectrometer acquisition settings		
Dwell time	50 ms	50 ms
Points per peak	1	1
Sweeps per reading	20	20
Readings per replicate	1	1
Replicates	3	3
Scan mode	Peak hopping	Peak hopping
Detector mode	Dual	Dual

The internal standards were added on-line to standards and samples using a separate feeding tube on the peristaltic pump (Trident Internal Standard Kit- PerkinElmer). The elements were measured in 2 groups: Group 1 (Ag, Al, As, Ba, Be, Bi, Cd, Co, Cr, Cs, Cu, Ga, In, Li, Mn, Ni, Pb, Rb, Se, Sr, Tl, U, V, Zn) and Group 2 (REEs, Th, Y). Germanium, Y, Rh and Ho (Table II) and In and Rh (Table III) were tested for Group 1 and Group 2, respectively.

In order to improve sample throughput and decrease the time and cost of analysis, the elements were run in the standard mode in two multielemental analyses: elements of Group 1 and elements of Group 2. The measurement data were interpolated from a calibration curve of aqueous standards covering a concentration range from 0.5 to 50.0 µg/L (Group 1) and a concentration range from 0.01 to 1.0 µg/L (Group 2) prepared with the same reagents as the samples.

2.5. Interferences

Several interference sources may cause inaccuracies in the determination of trace elements by ICP-MS. One way to remove these interferences is using a reaction cell (DRC) or collision cell accessory prior to analysis. These accessories chemically remove the interferences from the ion beam before they enter the analyzer quadrupole. In this work a methodology without DRC was established with the objective to obtain a reliable, single, easy to use, low cost and robust analytical method.

The major elements in environmental water that suffer from interferences include Cr, Ni, As, Se and V and their associated interferences are ArC^{+}, CaO^{+}, $ArCl^{+}$, Ar_2^{+}, ClO^{+}. These interferences can be overcome in most cases by monitoring an alternative isotope or by applying elemental correction. For these isobaric polyatomic ion interferences, commonly formed in the plasma or interface system from support gases or sample components, appropriate elemental correction equations were used in the method: ^{51}V (-3.127*(ClO53-(0.113*Cr52)), ^{75}As (-3.127*(ArCl77-(0.815*Se82), ^{82}Se (-1.007833*Kr83), ^{86}Sr (-1.505657*Kr83), ^{111}Cd (Cd111-1.073*(MoO108-(0.712*Pd106)), ^{115}In (-0.014038*Sn118), ^{208}Pb (+1*Pb206+1*Pb207). The common ^{82}Kr that affects the determination of arsenic and selenium was reduced with the use of high purity argon (99.999%).

REEs analysis by ICP-MS is known to be hampered by unwanted spectral and non-spectral interferences [3]. Isobaric interferences, polyatomic ions and to a small extent (for Y) multiple charged ions are the most important spectral interferences, which are usually overcome by mathematical correction procedures. Non-spectral interferences can be divided in reversible, which can occur while the sample is being measured, and irreversible matrix effects: clogging of the nebulizer and sampling orifices or deposition on the torch or in the ion lens stack. The errors associated with non-spectral interferences can be eliminated by appropriate calibration procedures, adapted sample preparation or limitation of the amount of sample delivered to the nebulizer, plasma and sampling devices, for example through the application of flow injection [8]. The mass spectra are also plagued by other spectroscopic interferences, e.g., oxides and hydroxides, which pose serious problems for the determination of middle and heavier rare earth elements by ICP-MS, particularly when the concentration ratio of lighter to heavier rare earths is high. In addition, Ba is one of the most abundant elements in many samples and its oxides and hydroxides also interfere with the determination of some of the rare earth elements, e.g., Eu [9].

Table II. Isotopes monitored and their respective abundances (At %) for Group 1 in the certified reference material TM-DWS2, experimental values (Exp. Value), certified values (Cert. Value) and recovered certified values obtained without internal standards (IS) and with internal standards (Ge, Y, Rh, Ho).

Analyte	At (%)	Certified Reference Material TM-DWS2- Environment Canada						
		Exp. Value (µg/L)	RSD (%)	Cert. Value (µg/L)	Recovered Certified Values (%)			
					Ge Ho (IS)	Ge Y Ho (IS)	Ge Rh Ho (IS)	Without (IS)
^{6}Li	7.5	42.9	7.6	20.2 ± 0.26	212.6	212.6	212.6	212.1
^{7}Li	**92.5**	19.9	1.6	20.2 ± 0.26	**98.4**	98.4	98.4	97.3
^{9}Be	**100**	13.8	1.3	13.4 ± 0.11	**102.7**	102.7	102.7	102.2
^{27}Al	**100**	63.0	1.3	58.6 ± 0.48	**107.5**	107.5	107.5	107.0
^{51}V	**99.75**	43.6	1.5	44.5 ± 0.29	**98.1**	98.1	98.1	95.8
^{52}Cr	**83.79**	43.8	1.6	44.4 ± 0.27	**98.6**	98.6	98.6	98.1
^{53}Cr	9.5	44.9	2.3	44.4 ± 0.27	101.7	101.7	101.7	96.6
^{55}Mn	**100**	46.9	3.4	47.3 ± 0.25	**99.1**	99.1	99.1	96.8
^{59}Co	**100**	64.0	2.7	64.4 ± 0.39	**99.4**	99.4	99.4	97.1
^{60}Ni	**26.22**	82.2	3.9	82.5 ± 0.42	**99.7**	99.7	99.7	93.2
^{62}Ni	3.63	79.4	2.7	82.5 ± 0.42	96.2	96.2	96.2	96.5
^{63}Cu	**69.17**	168	2.4	167 ± 1.07	**100.8**	100.1	100.1	100.0
^{65}Cu	30.83	168	4.9	167 ± 1.07	100.8	100.8	100.8	100.4
^{66}Zn	**27.9**	359	4.5	379 ± 2.57	**94.8**	94.8	94.8	94.4
^{68}Zn	18.8	361	3.8	379 ± 2.57	94.8	94.8	94.8	94.3
^{69}Ga	60.12	5.58	1.2	0.045[a]	12401	12401	12401	12056
^{71}Ga	**39.89**	0.036	6.0	0.045	**78.9**	78.9	78.9	76.7
^{75}As	**100**	4.29	1.2	4.18 ± 0.049	**98.8**	98.7	102.7	98.8
^{77}Se	7.63	7.95	3.1	8.65 ± 0.13	91.9	88.9	91.7	91.9
^{82}Se	**8.73**	8.23	0.4	8.65 ± 0.13	**95.1**	92.4	95.5	95.1
^{85}Rb	**72.17**	0.43	0.5	0.42[a]	**100.2**	100.7	100.8	100.2
^{86}Sr	**9.89**	238	0.9	244 ± 1.38	**97.5**	94.6	94.9	97.5
^{88}Sr	82.58	212	0.1	244 ± 1.38	86.8	84.6	84.8	86.8
^{107}Ag	**51.84**	9.87	2.0	9.94 ± 0.08	**99.3**	96.3	96.6	99.3
^{109}Ag	48.16	10.0	4.1	9.94 ± 0.08	100.8	97.9	98.2	100.8
^{111}Cd	**12.8**	4.2	3.7	4.2 ± 0.04	**99.9**	96.9	97.3	99.9
^{114}Cd	28.73	4.4	2.5	4.2 ± 0.04	104.1	101.0	101.4	104.1
^{115}In	**95.7**			b				
^{133}Cs	**100**			b				
^{135}Ba	6.59	144	1.4	146 ± 0.76	98.3	98.3	98.3	103.9
^{137}Ba	**11.23**	146	1.1	146 ± 0.76	**100.0**	100.0	100.0	105.6
^{203}Tl	**29.25**	8.09	1.1	8.32 ± 0.085	**97.2**	97.7	92.0	97.2
^{205}Tl	70.48	7.70	3.7	8.32 ± 0.085	92.6	92.6	90.2	92.6
^{206}Pb	24.1	7.42	0.8	7.87 ± 0.08	94.2	94.2	89.1	94.2
^{207}Pb	22.1	7.76	3.8	7.87 ± 0.08	98.6	98.6	93.2	98.6
^{208}Pb	**52.4**	7.82	2.4	7.87 ± 0.08	**99.3**	99.3	93.4	99.3
^{209}Bi	**100**	14.78	0.3	14[a]	**105.6**	104.2	102.9	105.6
^{238}U	**99.28**	13.5	0.5	14.2 ± 0.11	**95.0**	95.0	92.5	95.0

a- Information Value
b- Not available

Before obtaining the quantitative data of this paper a semi-quantitative analysis (10% of the samples) was carried out using a TotalQuant Method. This technique enables determining concentrations (semi-quantitative results) of up to 81 elements in a single measurement. The range of the interfering element concentrations considered was quite low.

3. Results and Discussion

3.1 Selection of isotopes and internal standards

3.1.1 Group 1 elements (Ag, Al, As, Ba, Be, Bi, Cd, Co, Cr, Cs, Cu, Ga, In, Li, Mn, Ni, Pb, Rb, Se, Sr, Tl, U, V, Zn)

To achieve good detectivity and precision for the determination of all elements, isotopes with high natural abundance should be selected, provided they are free of isobaric or oxide interference [10]. Based on this, elements from Group 1 were monitored in the certified reference material TM-DWS.2 (a trace element fortified sample) not only for those isotopes with high natural abundance but also for isotopes with lesser abundances (Table II). The isotopes were selected through the highest recovered certified values and most of them (in bold face in Table II), were in agreement with previously defined data [11, 12, 13]. The best recovered certified values obtained for Li, Ga, Sr and In, not mentioned in previous papers [11, 12, 13], were obtained for the isotopes ^{7}Li, ^{71}Ga, ^{86}Sr, ^{115}In.

Since an internal standard is the most used and most attractive method for the correction of non-spectral interferences in routine analyses [3], Ge, Y, Rh and Ho were tested regarding their suitability as internal standards in the analysis of TM-DWS.2. Germanium (100 µg/L) was added to the internal standard mixture for As, Se and Zn determination since the ionization potential of Ge is much closer to As, Se and Zn and matches far better with the ionization interferences. This interference may occur in samples containing significant levels of easily ionizable elements like Na and K [12].

The use of internal standards was tested in four combinations (Table II): the first without internal standards (IS); the second using germanium for the elements Li, Be, Al, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, As, Se, rhodium for Rb, Sr, Ag, Cd, In, Cs, and holmium for Ba, Tl, Pb, Bi, U; the third using the same combination only substituting Rh for Y; the fourth using germanium for the elements Li, Be, Al, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, and holmium only for Ba. The best results were obtained with the fourth combination (Ge, Ho) with the recovered certified values for the selected isotopes varying from 94.8% (Zn) to 107.5% (Al), as shown in Table II. These values are considered as acceptable recoveries for U.S.EPA Method 200.8 [13], which requires the measured values to be within ±10% of the stated value. Since the analyzed Ga concentration was below the detection limit (0.04 µg/L), a low recovery value was obtained (78.9%).

3.1.2 Group 2 elements (REEs, Th, Y)

The selection of isotopes for REE determination by ICP-MS was performed in agreement with previous works [3, 9, 14 and 15] and by analyzing the certified reference material, synthetic water SPS- SW1, Batch 116, at different m/z in order to evaluate the isotopes with the highest recovered certified values. The selected isotopes, in bold face in Table III, presented recovered certified values ranging from 91.2% (Ce) to 101.8% (Er), except for Dy (160%).

In many of the reports on rare earth elements determination by ICP-MS [3, 9, 14, 15], ^{143}Nd (12.18%) or ^{146}Nd (17.20%) isotopes have been used for Nd determination [9]. As reported, ^{143}Nd showed no interference, but ^{146}Nd presented interference from ^{130}BaO. ^{147}Sm (14.99%) is the best isotope for the determination of Sm due to extremely small contributions from ^{130}BaOH [9]. Also, ^{152}Sm, with nearly double the abundance as those of ^{147}Sm and ^{149}Sm, can also be used and would improve the detectivity by a factor of 2.

As seen in Table III, both Eu isotopes, with recovery values of 99.6 and 98.2%, respectively, may be used for Eu determination. ^{157}Gd, with a recovery value of 99.6%, is the most common choice for Gd and ^{167}Er is normally used due to very small interferences from ^{151}EuO in its determination. For Yb determination, ^{171}Yb to ^{174}Yb isotopes have been used in different publications [3, 9]. Hence, with a recovery value of 98.8 and high abundance ^{174}Yb may be the best choice.

For the other REEs, the selection of isotopes is straightforward. ^{139}La (99.91%), ^{140}Ce (88.48%) and ^{175}Lu (97.4%) are the isotopes with the highest abundances. ^{141}Pr, ^{159}Tb, ^{165}Ho and ^{169}Tm are mono-isotopic elements (100%). Pr shows no interference and Ho and Tm show negligible interference contributions from SmO and EuO, respectively. ^{159}Tb shows major interference from ^{143}NdO, and the contribution depends on the Nd/Tb concentration ratio in the sample [9]. In this work, all Dy isotopes monitored (^{161}Dy, ^{162}Dy, ^{163}Dy, ^{164}Dy) showed interferences demonstrated by the high recovery values (160 %). Oxides such as SmO and NdO are potential interferences for ^{163}Dy that may cause the discrepancy on this recovery percentage. Good recovery values (90-95%) were obtained from a single Dy standard (0.05 µg/L) purchased from GFS Chemicals.

Also, the appropriate internal standards for the analysis of the REEs, Y and Th were evaluated through comparison of the results obtained from the recovery values by analyzing the certified reference material, synthetic water SPS-SW1, Batch 116, with In and Rh as internal standards. As observed in Table III, both Rh and In can be used as internal standards. Through these internal standards better certified recovery values were obtained for the determination of the REEs and Y and Th.

3.2 Performance characteristics

The instrument detection limits (IDLs) were calculated as the concentration equal to the analyte signal which is equal

Table III. Isotopes monitored and their respective abundances (At %) for Group 2 (REE, Th and Y) in the reference sample SPS SW1 and recovered certified values obtained without internal standard (IS) and with internal standards (In, Rh).

Isotopes	At (%)	Reference Material SPS SW1 Batch 116 -Spectrapure Standard					
		Experimental Value (µg/L)	RSD (%)	Certified Value (µg/L)	Recovered Certified Values (%)		
					Without IS	In (IS)	Rh (IS)
^{89}Y	**100.0**	0.48	4.5	0.50 ± 0.01	**95.6**	98.0	98.4
^{139}La	**99.9**	0.48	1.7	0.50 ± 0.01	**95.4**	97.4	97.4
^{140}Ce	**88.5**	0.46	1.6	0.50 ± 0.01	**92.2**	94.4	95.0
^{141}Pr	**100.0**	0.47	1.0	0.50 ± 0.01	**93.4**	95.2	95.2
^{142}Nd	27.13	0.48	2.1	0.50 ± 0.01	96.8	98.8	99.0
^{143}Nd	**12.18**	0.49	4.9	0.50 ± 0.01	**98.0**	100.2	100.2
^{144}Nd	23.8	0.48	2.6	0.50 ± 0.01	96.2	98.2	98.2
^{145}Nd	8.30	0.45	7.3	0.50 ± 0.01	90.8	92.8	92.8
^{146}Nd	17.19	0.46	3.0	0.50 ± 0.01	92.6	94.6	94.6
^{147}Sm	**15.0**	0.46	3.1	0.50 ± 0.01	**92.2**	94.0	94
^{149}Sm	13.8	0.46	5.6	0.50 ± 0.01	91.0	92.8	92.8
^{151}Eu	**47.8**	0.50	4.3	0.50 ± 0.01	**100.0**	101.6	101.6
^{152}Sm	**26.7**	0.49	3.9	0.50 ± 0.01	**98.0**	99.0	99.2
^{153}Eu	52.2	0.49	2.8	0.50 ± 0.01	98.0	100.2	100.2
^{157}Gd	**15.65**	0.50	4.6	0.50 ± 0.01	**99.6**	101.8	101.8
^{158}Gd	24.84	0.50	4.8	0.50 ± 0.01	99.4	101.4	101.2
^{159}Tb	**100.0**	0.48	2.8	0.50 ± 0.01	**95.6**	97.6	97.6
^{160}Gd	21.86	0.48	7.8	0.50 ± 0.01	96.6	98.4	98.6
^{161}Dy	**18.90**	0.82	3.3	0.50 ± 0.01	**163.4**	167	167
^{162}Dy	25.50	0.82	4.4	0.50 ± 0.01	164.8	168	168
^{163}Dy	24.90	0.82	5.1	0.50 ± 0.01	163.2	166	166
^{164}Dy	28.2	0.79	2.8	0.50 ± 0.01	158.4	162	161
^{165}Ho	**100.0**	0.47	2.8	0.50 ± 0.01	**93.8**	95.6	95.6
^{166}Er	33.6	0.48	3.5	0.50 ± 0.01	96.0	97.8	97.8
^{167}Er	**22.95**	0.51	4.5	0.50 ± 0.01	**101.8**	103.8	104.0
^{168}Er	26.8	0.48	2.6	0.50 ± 0.01	95.4	97.2	97.2
^{169}Tm	**100.0**	0.47	4.6	0.50 ± 0.01	**93.8**	95.8	95.6
^{171}Yb	14.3	0.46	1.7	0.50 ± 0.01	92.8	94.6	94.6
^{172}Yb	21.9	0.49	4.3	0.50 ± 0.01	97.8	99.8	99.6
^{173}Yb	16.12	0.49	9.3	0.50 ± 0.01	98.4	100.6	100.6
^{174}Yb	**31.8**	0.49	5.8	0.50 ± 0.01	**98.8**	101.0	101.0
^{175}Lu	**97.41**	0.49	2.5	0.50 ± 0.01	**98.0**	98.8	98.8
^{232}Th	**100.0**	0.51	2.8	0.50 ± 0.01	**101.2**	103.2	103.2

to three times the standard deviation of a series of ten replicate measurements of the calibration blank (1% HNO_3) signal at the selected analytical masses. The method detection limits (MDLs) for the determination of dissolved analytes in waters were determined using the blank solution fortified with analytes at concentrations between two and five times the estimated IDL [11, 12]. The resulting IDLs and MDLs for the elements of Group 1 and Group 2 are given in Table IV. With no preconcentration or separation, detection limits for the REEs found were 0.2 ng/L (Lu) to 2.7 ng/L (Yb) and for Group 1, detection limits ranged from 0.4 ng/L (Cs) to 0.5 µg/L (Zn).

Two certified reference materials, TM-DWS.2, lot 1010, a trace element fortified sample from the Environment Canada, and synthetic water SPS- SW1, Batch 116, from Spectrapure Standards (Oslo, Norway) were used for recovery and precision evaluations. The reference materials TM-DWS2 and SPS- SW1 were 2 and 10 fold diluted, respectively. With these dilution factors, the element concentrations were at the same levels as the spring water samples analyzed and below the maximum contaminant level specified by the Decree -2914- 11/2011 of the Brazilian Ministry of Health, allowing assessing the accuracy of the method at this level. The data of Tables II and III show that the experimental values for all analytes, except Dy, are within 10% of the certified values, which is considered an acceptable recovery for U.S EPA Method 200.8 [13].

Table IV. Instrument detection limits (IDLs) and method detection limits (MDLs) obtained for the analytes of Group 1 and Group 2.

Elements Group 1			Elements Group 2		
Analyte	IDL (µg/L)	MDL (µg/L)	Analyte	IDL ng/L	MDL ng/L
^{7}Li	0.01	0.01	^{89}Y	0.7	0.8
^{9}Be	0.005	0.02	^{139}La	0.3	0.7
^{27}Al	0.12	0.2	^{140}Ce	1.3	1.3
^{51}V	0.005	0.1	^{141}Pr	0.2	1.4
^{52}Cr	0.02	0.12	^{143}Nd	1.3	2.6
^{55}Mn	0.01	0.04	^{147}Sm	0.8	1.2
^{59}Co	0.0003	0.001	^{151}Eu	0.4	0.5
^{60}Ni	0.013	0.02	^{157}Gd	0.6	1.2
^{63}Cu	0.006	0.01	^{159}Tb	0.07	0.3
^{66}Zn	0.34	0.5	^{163}Dy	0.8	1.4
^{71}Ga	0.001	0.04	^{165}Ho	0.4	0.5
^{75}As	0.01	0.06	^{167}Er	0.9	1.1
^{82}Se	0.03	0.1	^{169}Tm	0.1	0.3
^{85}Rb	0.0005	0.0005	^{171}Yb	1.0	2.7
^{86}Sr	0.03	0.08	^{173}Yb	0.5	0.6
^{107}Ag	0.0001	0.004	^{174}Yb	0.6	0.5
^{111}Cd	0.001	0.01	^{175}Lu	0.15	0.2
^{115}In	0.001	0.002	^{232}Th	0.2	0.3
^{133}Cs	0.00005	0.0004			
^{137}Ba	0.008	0.014			
^{203}Tl	0.001	0.002			
^{208}Pb	0.008	0.01			
^{209}Bi	0.0005	0.001			
^{238}U	0.002	0.002			

The relative standard deviations (RSD, n=3) used to assess the precision of the determination of the selected isotopes (Group 1) in the reference sample TM-DWS2 were smaller than 3.5%, including elements commonly subjected to polyatomic ions interferences, such Cr, Ni, As, Se and V (Table II). For the determination of the REEs, Th and Y in SPS- SW1 reference material, precision was less than 5% (Table III).

To test the recovery and matrix effects of the direct determination of the elements of Group 1, two real spring water samples from different towns and TM-DWS2 reference material were spiked with 10 µg/L of Group1elements. The results for these samples, as well as the recoveries for these spikes, are presented in Table V. These results show that the major matrix species are present at different levels in the samples, as evidenced by the differing Ca, Na, K and Mg concentrations (Table V). Nevertheless, spike recoveries are within ± 10% for all elements present in the samples at concentrations under 50 µg/L.

3.3 Application: spring water analysis

The method was applied to the analysis of spring water samples collected from sources spread throughout the historical towns of Ouro Preto, Mariana, Sabará and Diamantina in the state of Minas Gerais, Brazil. In the past, these sources played an essential and strategic role in supplying these towns with potable water. Even today this water is used by both the local population and visitors who believe in its quality. The range of analytical results of the elements Ag, Al, As, Ba, Be, Bi, Cd, Co, Cr, Cs, Cu, Ga, In, Li, Mn, Ni, Pb, Rb, Se, Sr, Tl, U, V, Zn, Th and Y are shown in Fig. 1 and the results of the REEs are presented in Fig. 2.

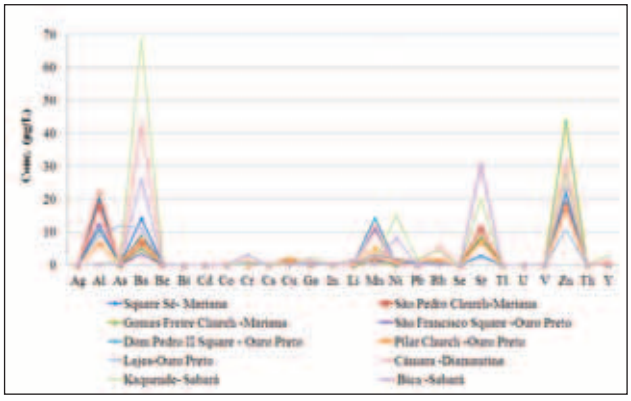

Figure 1. Analytical results of Ag, Al, As, Ba, Be, Bi, Cd, Co, Cr, Cs, Cu, Ga, In, Li, Mn, Ni, Pb, Rb, Se, Sr, Tl, U, V, Zn, Th and Y in spring waters samples.

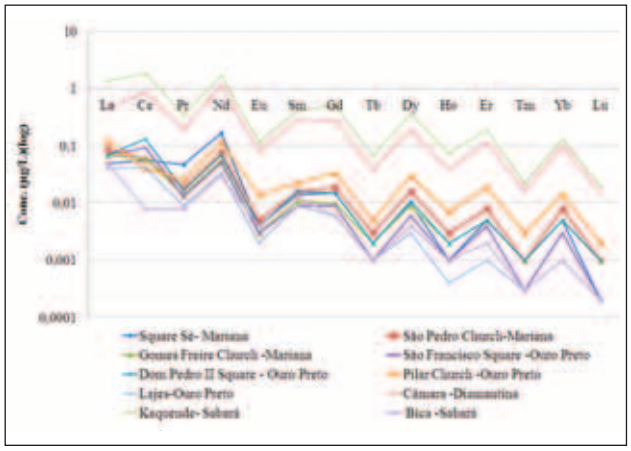

Figure 2. Analytical results of rare earth elements in spring water samples.

In some sources, Al, As, Ba, Mn, Ni, Rb, Sr and Zn were the elements (Group 1) that presented the highest values (Fig.1). However, only arsenic (Lajes) presented a level higher than the one established by brazilian legislation (Ministry of Health 2914 - 11/2011) and WHO-2008. As seen in Fig. 2, the highest levels of REEs were found in Kaquende and Câmara spring waters. It was also verified that the concentrations of light rare-earth elements (LREE) (La, Ce, Pr, Nd and Sm) are greater than those of heavy rare earth elements (HREE) (Gd, Tb, Dy, Ho, Er and Yb) in all samples.

The patterns of concentrations of REEs obtained in these samples demonstrated the Odd-Harkins law, in which the concentrations of even atomic number (Ce, Nd, Sm, Gd, Dy, Er Yb) are more abundant than those of odd atomic number (La, Pr, Eu, Tb, Ho). This pattern is an important tool to understand the geochemical processes as well as to detect anomalous data that may be due to natural process, contamination (anthropogenic, in field or laboratory) or analyti-

Table V. Recoveries of spiked aliquots on the TM-DWS2 reference material and on two real spring water samples from different towns.

Analyte	TM DWS2 Certified Value	Spring samples		Spike	Spike Recovery		
		Kaquende	Lajes		TM DWS2	Kaquende	Lajes
	Conc. (µg/L)	Conc. (µg/L)	Conc. (µg/L)	(µg/L)	(%)	(%)	(%)
Li	20.2 ± 0.26	2.10	0.56	9.95	96.7	101.6	108.1
Be	13.4 ± 0.11	0.16	<0.02[a]	9.93	105.9	109.8	109.1
Al	58.6 ± 0.48	9.42	14.5	9.99	91.0	93.7	100.6
V	44.5 ± 0.29	<0.1[a]	<0.1[a]	10	98.8	97.1	94.7
Cr	44.4 ± 0.27	0.24	0.30	10	95.8	94.4	100.3
Mn	47.3 ± 0.25	1.38	3.26	9.96	90.5	96.8	95.8
Co	64.4 ± 0.39	0.02	0.02	10.2	88.7	96.5	95.6
Ni	82.5 ± 0.42	17.1	1.66	10	96.9	89.9	90.3
Cu	167 ± 1.07	0.82	0.52	9.98	b	90.3	90.5
Zn	379 ± 2.57	27.4	9.86	10	b	94.3	90.0
Ga	0.045	<0.04[a]	<0.04[a]	10.1	96.9	95.5	95.6
As	4.18 ± 0.049	1.76	11.9	9.92	100.1	104.9	100.9
Se	8.65 ± 0.13	0.22	0.11	10	95.1	100.5	102.2
Rb	0.42	5.06	0.62	10.1	96.2	102.3	100.7
Sr	244 ± 1.38	21.7	2.61	9.97	93.9	102.2	104.3
Ag	9.94 ± 0.08	0.01	0.01	9.99	101.8	100.6	104.7
Cd	4.2 ± 0.04	0.09	0.01	10	102.9	104.6	104.1
In	c	<0.012[a]	<0.012[a]	10.1	107.0	107.6	108.4
Cs	c	0.51	0.03	10	106.1	103.8	107.3
Ba	146 ± 0.76	68.2	16.2	9.87	116.2	60.6	86.3
Tl	8.32 ± 0.085	0.03	0.007	9.94	97.2	101.2	105.4
Pb	7.87 ± 0.08	2.75	1.27	10	93.5	102.5	103.9
Bi	14	0.013	0.042	9.99	90.5	95.3	98.2
U	14.2 ± 0.11	0.022	0.015	10.1	96.9	99.9	107.4
Na	c	7550	390				
Mg	c	4630	192				
K	c	<100[a]	330				
Ca	c	4064	<100[a]				
Fe	224 ± 1.8	7.98	7.38	10.1	91.4	95.3	109.8

a - < Sample concentration below established method detection limit
b - Spike concentration <10% of sample background concentration
c - Not available

cal error [16]. All samples analyzed presented these typical patterns, suggesting good analytical accuracy.

4. Conclusions

It has been demonstrated that the method established is suitable for direct determination of trace levels of Ag, Al, As, Ba, Be, Bi, Cd, Co, Cr, Cs, Cu, Ga, In, Li, Mn, Ni, Pb, Rb, Se, Sr, Tl, U, V, Zn, Th, Y and of REEs in natural waters. Without preconcentration or separation, the method developed in this work presented selectivity, detectivity, accuracy for the selected isotopes varying between 92 and 108%, relative standard deviations <5 %, and detection limits varying from 0.4 ng/L (Cs) to 0.5 µg/L (Zn) and 0.2 ng/L (Lu) to 2.7 ng/L (Yb) for the REEs. These detection limits are appropriate and well below the guideline levels set by brazilian legislation (Ministry of Health 2914- 11/2011) and WHO-2008 for all the elements. Therefore the method can be widely applied to determine trace elements in environmental water samples. The REEs patterns of the spring water samples reflect underlying water-rock interactions.

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Simultaneous determination of arsenic, antimony and selenium in agronomic samples by hydride generation and optical emission spectrometry

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Abstract

A continuous-flow hydride generation system was developed and used with inductively coupled plasma optical emission spectrometry (ICP OES) for simultaneous determination of As, Sb and Se in agronomic samples. A factorial design was applied in order to establish the best chemical and instrumental conditions. The factors chosen for evaluation were: reagent concentration, plasma power, carrier gas flow rate, and the viewing height of the plasma. The LODs of the method were 0.124, 0.168, and 0.141 μg L^{-1} for As, Sb, and Se, respectively. The RSD (relative standard derivation) was lower than 1.8%, and the sampling throughput was 40 samples h^{-1}. To check the method accuracy, eight different kinds of agronomic samples - forage, corn silage, mineral salt, and bovine-derived samples (blood, viscera, carcass, meat, and semen) - were spiked with different analyte concentrations (at the μg L^{-1} level). Recoveries ranged from 90 to 102%.

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Keywords: hydride generation; inductively coupled plasma optical emission spectrometry; agronomic samples; factorial design.

1. Introduction

Increasingly, to achieve maximum productivity with low cost, farmers use chemical parameters to evaluate and monitor the development of crops and animals. Beef cattle require at least 17 minerals in their diet [1]. These include the macro minerals (calcium, magnesium, phosphorus, potassium, sodium, chlorine and sulfur) and trace minerals (chromium, cobalt, copper, iodine, iron, manganese, molybdenum, nickel, selenium and zinc). Others, including arsenic, lead, silicon, antimony, aluminum and vanadium, have also been considered, in a narrow concentration range as the essential and toxic levels are very close.

Several of these minerals are present in living tissues at such small concentrations that it is often not possible to detect them by most analytical techniques. In this sense, the determination of arsenic, antimony, and selenium in some agronomic samples of animal feed, corn silage, mineral salts, and bovine blood, carcasses, viscera, meat and semen is an essential requirement to ascertain maximum productivity. Selenium in bovine meat, blood, and viscera samples can be used as an indicator of animal development; in semen, selenium can be an indicator of fertility and provides parameters for discrimination of origin or race [1]. The determination of arsenic and antimony in food samples (*in natura* or after industrialization) is important, considering the toxicity of the elements. Contamination of tissues may occur through arsenic-containing pesticides/herbicides or rations [2]. The presence of 1.0, 2.0, and 0.30 μg g^{-1} of As, Sb and Se, respectively, in food are the maximum levels allowed by the Brazilian Agency of Health and Safety (ANVISA) [3].

Usually, the detectivity of inductively coupled plasma optical emission spectrometry (ICP OES) is not sufficient to determine arsenic, antimony, and selenium in agronomic samples at the low concentrations (trace) relevant to health criteria. Hydride generation (HG) is a technique commonly employed to enhance the detectivity of such elements in spectrometric methods [4]. The HG technique is based on the production of a volatile hydride as the result of the reaction between the acidified sample and a reducing agent [5]. To increase detectivity hydride generation and hydride transport to the atomizer should be as rapid as possible, in order to avoid dilution by the carrier gas [6].

Results of previous studies [7,8] of simultaneous determination of elements that form volatile hydrides and are detected by ICP OES, up to the present day [9] indicate that better detectivity is still needed through the development of novel designs of reaction-separation systems. The best chemical and instrumental conditions for hydride generation can be quite different for each element, since the acid con-

centration in the sample solution can often have a marked effect on detectivity.

Many hydride forming elements exist in two oxidation states that are not equally amenable to tetrahydroborate reduction [10]. The variation in the redox state of the concerned elements requires considerable effort in developing a suitable sample treatment procedure. In hydride generation processes using sodium tetrahydroborate, arsenic (III) and antimony (III) can react more easily than their pentavalent forms. Potassium iodide is commonly used as an auxiliary reducing agent to convert As(V) and Sb(V) to As(III) and Sb(III), respectively [11], but it also causes reduction of Se(VI) to Se(0), which does not react to produce volatile hydrides [12].

The use of flow injection analysis (FIA) is an alternative to solve the problem of the reduction of Se(VI) to Se(0) by KI [13]. By using FI [12, 13], it is possible to promote on-line generation of hydrides simultaneously, reducing Se(VI) to Se(IV) by HCl while allowing to reduction of As(V) and Sb(V) by KI in a prior step. Another advantage is that hydride transport to the atomizer is faster, increasing sample throughput. Small sample volumes and a small manifold are needed, reducing the contact time between the reagents and minimizing interference effects.

To improve chemical and instrumental conditions for simultaneous determination of As, Sb and Se by using hydride generation - inductively coupled plasma optical emission spectrometry (HG-ICP OES), univariate methodologies have been used [14-17]. Particularly in the hydride generation process, which involves several chemical and instrumental variables, the univariate approach of optimization may lead to too much experimentation and erroneous conclusions when the effect of one particular variable on the response also depends on others variables [18]. To circumvent these drawbacks, factorial design based on a multivariate optimization approach is the most useful tool for obtaining the optimum conditions.

In this work, an analytical method for simultaneous determination of total As, Sb and Se was developed. For this purpose, a FIA system for hydride generation was designed, which was associated with ICP OES for analytic detection. To establish the best conditions of system operation and the interaction between them, a factorial design based on multivariate optimization was applied. The analytical method was evaluated for determination of As, Sb and Se in agronomic samples.

2. Experimental

2.1. Reagents and solutions

All reagents used were of analytical-reagent grade and all the solutions were prepared using distilled and deionized water having an electric conductivity lower than 0.1 mS cm^{-1} (Milli-Q®, Millipore, Bedford, MA, USA). A 5 mg L^{-1} multielemental reference solution of As(V), Sb(V) and Se(VI) was prepared daily from 1 000 mg L^{-1} individual element stock solutions (Teclab, São Paulo, Brazil). Reference solutions containing 0, 20, 40, 60, 80, 100, and 120 μg L^{-1} of As, Sb and Se were prepared by dilution of the multielemental reference solution in 6.0 mol L^{-1} hydrochloric acid (HCl). Sub-boiling distilled HCl was used (Milestone, Sorisole, Italy). A solution containing 1.1% (w/v) sodium tetrahydroborate ($NaBH_4$) in 0.4% (w/v) sodium hydroxide was prepared daily by dissolving 1.1 g of $NaBH_4$ (Nuclear, São Paulo, Brazil), and 0.4 g of NaOH pellets (Vetec, São Paulo, Brazil) in 100 mL of water. A 8.3% (w/v) solution of potassium iodide (KI) was prepared by dissolving 8.3 g of KI (Synth, São Paulo, Brazil) in 100 mL of water. The glassware and plasticware were soaked overnight in 10% (v/v) HNO_3 and subsequently rinsed three times with distilled water before use.

2.2. Apparatus

A 6750 freezer mill (Spex Certiprep, USA) was used for cryogenic grinding of samples. Microwave-assisted acid decomposition of these materials was carried out in a microwave oven (Multiwave, Anton Parr, Austria) equipped with 6 TFM vessels for sample preparation. The FIA setup consisted of an IPC-8 Ismatec peristaltic pump furnished with a Tygon pumping tube; reaction coils; a connection device machined in acrylic; and a commutator injector [19]. The system was connected to a homemade glass gas-liquid separator (120 mm long x 15 mm internal diameter) for As, Sb and Se determination, and connected with a Varian (Mulgrave, Australia) Vista simultaneous (ICP OES) spectrometer, with radial viewing configuration.

2.3. Sample preparation

Bovine semen, blood, viscera, carcass, meat, ration, forage, and mineral salt samples were investigated. All samples were previously freeze-dried, ground in a cryogenic mill, and stored in a freezer (-20°C). A 0.2 g portion of the samples was weighed and introduced into a PFA vessel, with 2 mL of a 5 mol L^{-1} HNO_3 solution and 2 mL of 30% (w/v) H_2O_2 (Merck). The vessel was closed and placed in the rotor inside the microwave oven. Thereafter, the following power/time program was applied: step 1: 250 W for 2 min; step 2: 0 W for 3 min; step 3: 550 W for 4 min; step 4: 750 W for 5 min; step 5: 1000 W for 5 min; and step 6: 0 W for 15 min (vent step). For each decomposition batch, four vessels (one blank and three samples replicates) were simultaneously run. In this way, each material was digested in triplicate. The decompositions were made with dilute nitric acid (HNO_3) to minimize the interference of volatile nitrogen oxides in the hydride generation step [20].

After the decomposition procedure, each digest was transferred to a 10-mL volumetric flask and the volume was adjusted with 6.0 mol L^{-1} HCl. Subsequently, the solution was heated in a water bath for 15 min to reduce Se(VI) to Se(IV). Increasing the heating time did not improve the Se(VI) reduction. Then, the samples were submitted to a 5 L h^{-1} nitrogen flow for 2 min to eliminate residual chlorine and NO_x. This procedure for chlorine and NO_x elimination was also applied to the reference solutions.

2.4. Hydride generation flow system and procedure

A homemade glass gas-liquid separator, shown in Fig. 1, was used to separate the formed hydrides that were transported to the ICP by argon. The nebulizer gas from the ICP OES equipment was connected to the input at the lower end of the gas-liquid separator to carry the formed hydrides to the ICP. The remaining liquid solution in the gas-liquid separator was removed through two outlets, which was aspirated by the peristaltic pump of the ICP OES equipment.

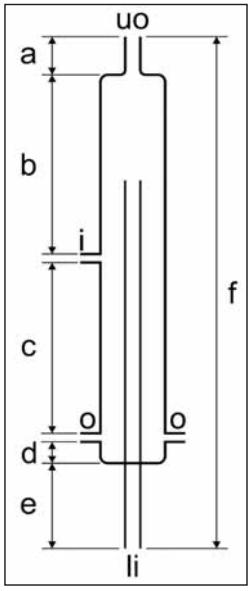

Figure 1. Schematic diagram of the hydride generation separator. (f), vertically cylinder from a single piece of glass, 120 mm long, 15 mm i.d.; a = 15 mm; b = 40 mm; c = 40 mm; d = 3 mm and e = 20 mm. (i) inlet through which a continuous flow of liquid and gas are introduced, 1 mm i.d.; (o), outlet for discharge of the liquid, 1 mm i.d.; (li), entrance for the carrier gas, 3.5 mm; (uo), upper end connected to the ICP OES to transport the hydrides, 2.5 mm i.d

The diagram of the flow system, designed and used to implement the simultaneous determination of As, Sb, and Se by HG-ICP OES, is shown in Fig. 2. It indicates the injector commutator (I) in the injection position. In this position, the sample, $NaBH_4$, and KI solutions were pumped simultaneously (P_p). At the first confluence point (x_1), the acidified sample received $NaBH_4$, which was then completely mixed in the reaction coil (B_1) to generate the hydrides.

In this system, the auxiliary reducing agent (KI) enters at confluence point (x_2) [12], which is then mixed with the reductant ($NaBH_4$) and sample solution in the reaction coil (B_2). This way, reduction of Se(IV) to Se(0) is avoided; Se(IV) is converted to SeH_2 by $NaBH_4$ and only As(V) and Sb(V) are reduced to As(III) and Sb(III), respectively, producing AsH_3 and SbH_3. Then, the liquid solution and the generated hydrides flow toward the gas-liquid separator (A). Within the gas-liquid separator, the flow of argon gas transports the hydrides of Se, As, and Sb to the ICP. In the next step, the commutator injector is moved to the sampling position; the $NaBH_4$ and KI solutions are pumped to their recovery vessels (V_1 and V_2 respectively) and the sample (S) is directed to its vessel. In the meantime, the analytical path is washed with water to remove any residues.

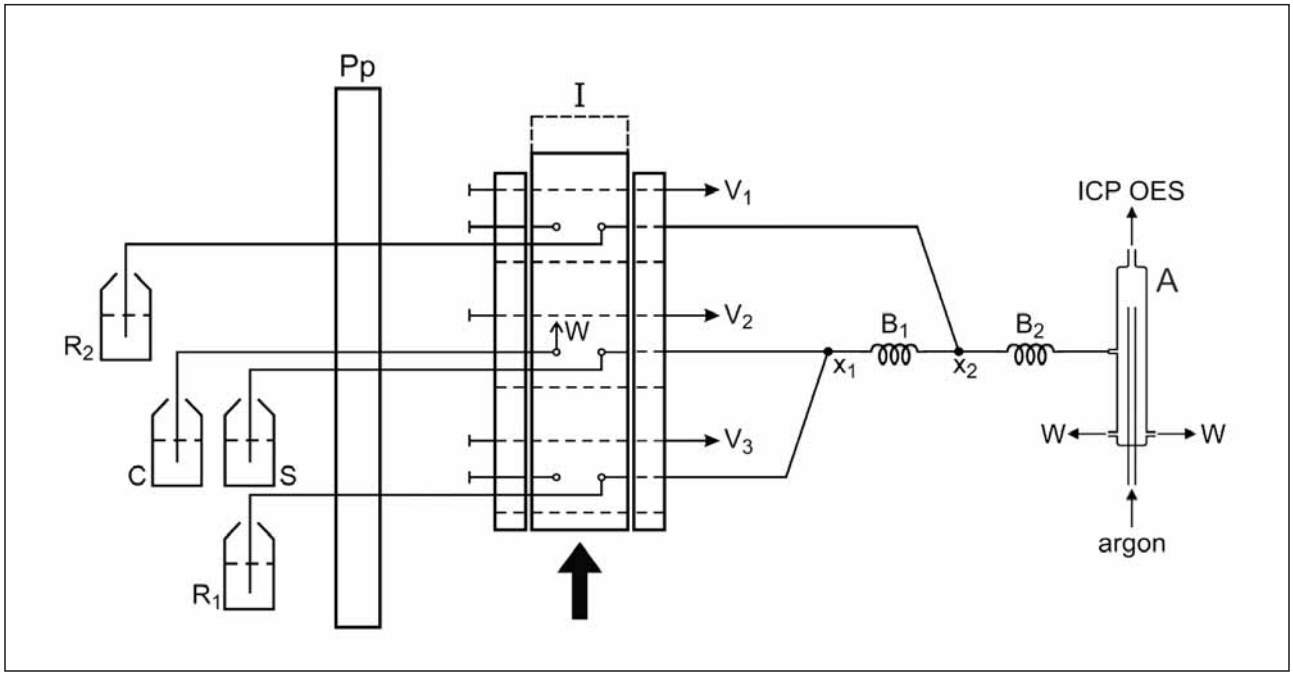

Figure. 2. Diagram of flow system. Pp = peristaltic pump; I = injector commutator; C = washing solution, water (1.0 mL min^{-1}); S = sample or standard solution in 6 mol L^{-1} HCl medium (1.42 mL min^{-1}); R_1 and R_2 = 1.1 % (w/v) $NaBH_4$ in 0.1 mol L^{-1} NaOH (1.42 mL min^{-1}) and 8.3 % (w/v) KI (0.88 mL min^{-1}), respectively; V_1 to V_3 = circulating solution through storing vessels; (A), gas liquid separator; W, waste (3.0 mL min^{-1}); B_1 and B_2 = reaction coils of Teflon tubing, 0.8 mm i.d. and 50 and 25 cm long, respectively; x_1 and x_2 = confluences machined in acrylic; continuous and dotted lines in the proportional injector indicate the fluid pathway when the injector is in the injection position and the sampling position, respectively. The arrows indicate that the proportional injector is in the injection position.

2.5. Optimization strategy

The operational conditions of the proposed hydride generation system and ICP OES parameters to determine As, Sb and Se in agronomic samples were established by applying a factorial design based on multivariate optimization. For monitoring the response, a 100 μg L^{-1} multielemental reference solution of As(V), Sb(V) and Se(VI) was used. The established ICP OES operational parameters and hydride generation conditions are given in Table I.

Table I. Optimized ICP OES operating parameters and continuous flow-hydride generation conditions.

ICP OES	
RF power (kW)	1.3
Plasma (L min^{-1})	15
Auxiliary (L min^{-1})	1.5
Viewing height (mm)	8.0
Wavelengths (nm)	As, 193.696 Sb, 217.582 Se, 196.026
Hydride generation conditions	
Sample acidity (HCl, mol L^{-1})	6.00
Sample flow rate (mL min^{-1})	1.42
KI flow rate (mL min^{-1})	0.88
$NaBH_4$ flow rate (mL min^{-1})	1.42
$NaBH_4$ concentration (% w/v)	1.10
KI concentration (% w/v)	8.30
Carrier gas flow rate (L min^{-1})	0.60
Reaction coil (cm)	B1 - 50 B2 - 25

In the first step of the multivariate optimization the response for three hydride generation factors and three ICP OES instrumental factors were evaluated in order to determine which variables deserved future studies. As hydride generation factors, the HCl, KI, and a BH_4^- concentrations were evaluated. For this, a 2^3 full factorial design with three replicates was conducted, resulting in 24 experiments. The values of the variables for each level were 3.0 and 6.0 mol L^{-1} HCl (A), 0.5 and 1.5% (w/v) $NaBH_4$ (B), and 4.0 and 8.0% (w/v) KI (C). The instrumental factors evaluated for ICP OES were radiofrequency (RF) power, carrier gas flow rate, and viewing height of the ICP. In this case, a 2^3 full factorial design with three replicates was also used, resulting in 24 experiments. The values of the variables for each level were 0.50 and 0.30 L min^{-1} for the carrier gas flow rate (A), 1.0 and 1.3 L min^{-1} for the RF power (kW) (B), and 8.0 and 12 mm for the viewing height (C).

After identifying the significant factors, a 2^2 full factorial design and a star central composite design were used, allowing fitting a quadratic function. Table II summarizes the coded factors and levels used in the experimental design.

Table II. Factors and levels of the 2^2 full factorial design and star central composite used for As, Se and Sb determination (n = 3).

Factors	Levels		
	Low (-)	Central (0)	High (+)
B: BH_4^- (%, w/v)	0.3	0.7	1.1
C: KI (%, w/v)	6.3	8.5	10.6
B: Carrier gas flow rate (L min^{-1})	0.4	0.9	1.3
C: Viewing height of the ICP (mm)	7	10	12

3. Results and Discussion

3.1. Optimization of hydride generation conditions

Full factorial experiments in completely randomized designs are extremely useful when one is exploring the factor space to identify the region where the optimum response can be located. Although these designs are highly efficient, they must be used with some caution because they allow the fitting of only first-order models and cannot detect curvature [21]. Fig. 3A shows the Pareto chart for the emission signal value for each element according to a 2^3 full factorial design performed with three replicates.

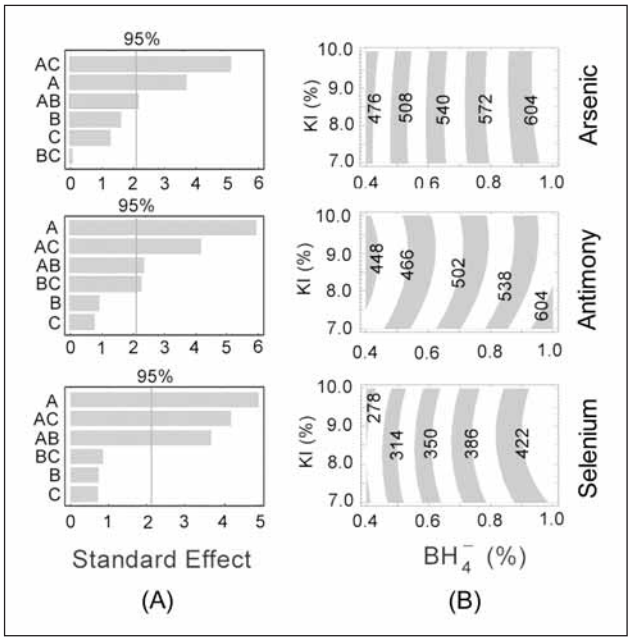

Figure 3. Optimization of hydride generation conditions. (A): Pareto's charts of the hydride generation conditions on the simultaneous determination of As(III), Sb(III) and Se(IV) at the 95 % confidence level. A: HCl; B: BH_4^-: C: KI, AB: HCl - BH_4^- interaction; AC: HCl - KI interaction and BC: BH_4^- - KI interaction. (B): central composite design contour plots for BH_4^- and KI concentrations. The values in the graph are the respective emission intensities.

In Figure 3 one capital letter represents the effect of one hydride generation factor, while two capital letters together represent the interaction effect of the two factors on the monitored response. The concentration of the HCl solution

was identified as factor A, the $NaBH_4$ concentration factor as B, and the concentration of the KI solution as factor C.

The Pareto chart shows each of the estimated effects in decreasing order of magnitude. The length of each bar is proportional to the standardized effect, which is the estimated effect divided by its standard error. This is equivalent to computing a *t*-statistic for each effect. The vertical line can be used to judge which effects are statistically significant. Any bar that extends beyond the line corresponds to effects that are statistically significant at the 95% confidence level.

Among all studied elements, the factors tetrahydroborate (B) and potassium iodide (C) concentrations were not significant in the concentration range studied for each factor. Only the HCl concentration had a significant effect on all elements investigated. As expected, the $NaBH_4$ reaction for hydride generation requires the presence of an acidic medium. Therefore, the $NaBH_4$ factor was not revealed as significantly important, but its interaction with HCl influenced the hydride generation efficiency.

Figure 3A also shows that the KI-HCl interaction was significant: the HCl concentration was associated with that of KI for an efficient reduction of As(V) and Sb(V) to As(III) and Sb(III), respectively, while Se(VI) was not reduced to Se(0). Therefore, in the present method the reduction of Se(IV) to Se(0) was circumvented by adding the KI solution after that of $NaBH_4$ [12]. Interaction between BH_4^- and KI on the Sb signal was also revealed as important.

As the HCl concentration factor presented a positive effect, increasing the acid concentration increased the emission signal of the elements. Consequently, searching for a higher emission signal, one might theorize that using a HCl concentration higher than 6 mol L^{-1} would favor hydride generation. However, the acid is also responsible for the production of H_2, which is important for atomization/excitation process but, in excess, can cause instability of the plasma or even its extinction [22]. Thus, a 6 mol L^{-1} HCl solution was established for further studies.

After identifying the significant factors and establishing the acidity of the medium, other experiments based on response surface methodology were carried out to investigate the dependence of the response on the remaining factors and to estimate the position of maximum response, or more realistically, a region close to optimal response [23]. A more accurate analysis with the remaining factors (KI and BH_4^- concentrations) was performed in order to estimate the position of maximum response for each element. The results are given in Figure 3B in the form of response surface contour plots.

The highest emission signal intensities for all investigated elements were obtained for 1.0% (w/v) BH_4^-. For higher concentrations of BH_4^-, the reaction became turbulent due to increased H_2 production. With respect to KI, changing its concentration had no effect on the As and Se emission. However, for Sb the highest emission signal was obtained with KI concentration ranging from 7.0 to 8.0% (w/v). These results are in agreement with the full factorial results, which showed no statistical significance for the KI-BH_4^- association effect. Therefore, to maintain the simultaneous generation of As, Sb and Se hydrides, 8.3% (w/v) KI and 1.1% (w/v) $NaBH_4$ were selected for further studies.

3.2. Optimization of the ICP OES instrumental conditions

A 2^3 full factorial design with three replicates was employed to evaluate the ICP OES instrumental conditions and to estimate all factor effects, in the same way as performed for hydride generation. The results are given in Figure 4A. The radiofrequency (RF) was nominated as factor A, the carrier gas flow rate as factor B, and the viewing height of the ICP as factor C. One and two capital letters together represent the interaction effect of one and two factors, respectively, on the monitored response.

As demonstrated in Figure 4A, all studied factors as well as their interaction effects are significant on the emission signal of the elements at 95% confidence level. Among the calculated effects, the RF power has positive influence on the response of all studied elements.

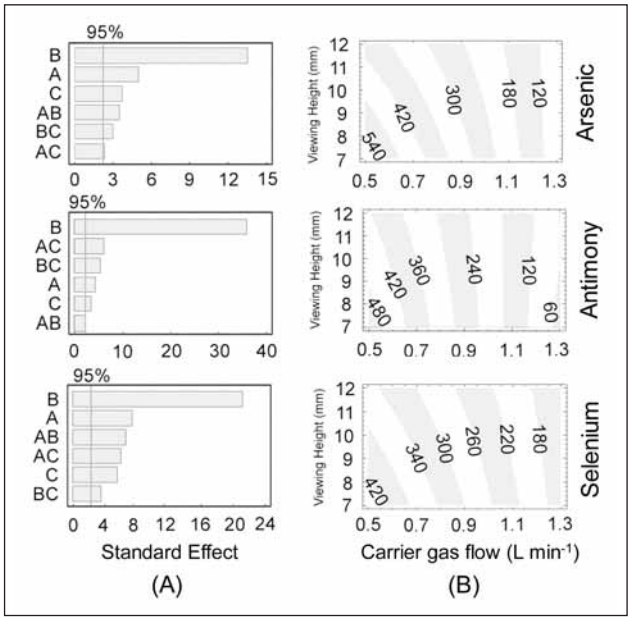

Figure 4. Optimization of the ICP OES instrumental conditions. (A): Pareto's charts of ICP OES instrumental conditions for simultaneous hydride generation of As(III), Sb(III) and Se(IV) at the 95 % confidence level. A: carrier gas flow rate; B: RF power; C: plasma viewing height; AB: carrier gas flow rate - RF power; AC: carrier gas flow rate - plasma viewing height; BC: RF power - plasma viewing height. (B): central composite design contour plots for ICP OES instrumental conditions. The values in the graph are the respective emission signal values.

The analytes have a relatively high excitation potential and therefore, more energy is required [24]. Thus, the emission signal increases with the RF power increase at a constant carrier gas flow rate and constant viewing height of the ICP.

An increase in the carrier gas flow rate and in the plasma viewing height caused a decrease in the response for all studied elements. All factors had a significant influence on the response but that of the carrier gas flow rate was higher. An increase in the carrier gas flow rate led to more efficient transport. However, the time of residence of atoms in the plasma is reduced [10], diminishing the percentage of analyte excitation. Although H_2 is important in the atomization/excitation process, an excess of H_2 can change the characteristics of the plasma, including its excitation capacity, and stability. In addition to a lower carrier gas flow rate, a lower plasma viewing height is necessary to obtain better detectivity.

In the present study, a RF power increase enhanced the evaluated response. Nevertheless, the maximum RF power used was very close to the maximum allowed by the equipment. Thus, a further increase of RF power would not represent a significant gain in detectivity. Therefore, the RF power was fixed at its maximum value (1.3 Kw) and a response surface methodology was used to study the remaining factors: carrier gas flow rate and plasma viewing height. The results are shown in Figure 4B, in the form of response surface contour plots for each element.

As expected, lower values of carrier gas flow rate and plasma viewing height promoted the highest signal intensities for all elements. The obtained response surfaces suggested that ICP OES was robust [25].

3.3. Interferents

Transition metals have been reported as potential interferents to the formation of hydrides [11]. In the present study, the effects of transition metals were verified by mixing 15 μg L^{-1} As(V), Sb(V) and Se(VI) in 6 mol L^{-1} HCl solution with 15,000 μg L^{-1} of Zn, Fe, Mn, Al, Cu, and Ni. The percentage of recovery of each element is presented in Figure 5.

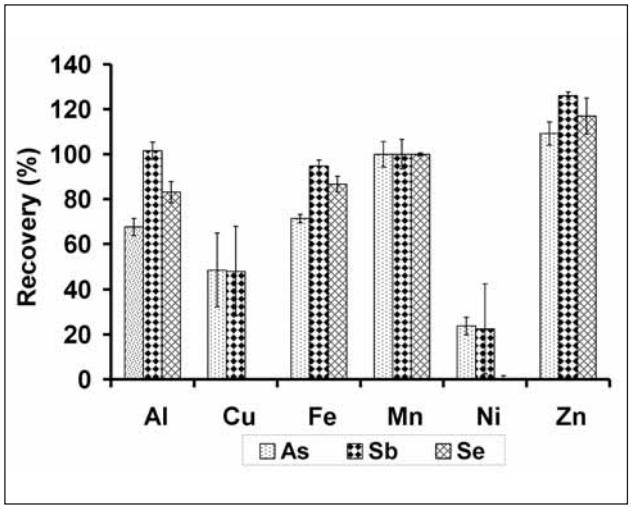

Figure 5. Effect of 15 mg L^{-1} of concomitant elements on the recoveries of As, Sb and Se determined using HG-ICP OES.

As Figure 5 shows, interference exist, as already observed for other hydride generation systems described in the literature [11,26]. The interference caused by the concomitants occurs mainly in the liquid phase [27]. Selenium reacts faster than As or Sb with BH_4^- to form SeH_2, so Se interferes in As and Sb determinations. On the other hand, there is a competition between concomitants and the analytes for BH_4^- that is necessary for hydride generation [28]. The most severe interference was observed for Se in the presence of Cu and Ni, due the interaction of these elements with SeH_2, which produce copper selenides and nickel selenides, causing signal suppression of Se [26].

Interference of Al and Fe on As and Se was observed. Arsenic and Se recoveries were 72% and 84%, respectively. This probably occurred due to the formation of metal colloids/metal borides and their interaction with As and Se [2,26]. Manganese did not interfere whereas Zn interfered with Sb and Se determinations, increasing the signals of these two elements. This interference can also be attributed to effects caused in the ICP; the concentration of Zn was 10,000 times higher than those of Sb and Se. However, it is necessary to emphasize that the transition metals (present as concomitants in the sample) were added in excess to verify the possibility of interference.

Other interferences in the determination of As, Sb, and Se were caused by the presence of strong oxidants [29, 11, 26], such as Cl_2 and NO_x, present in the final sample solution. Residual NO_x resulted in a brown solution color, mainly due to the presence of NO and NO_2 molecules. Residual chlorine caused the back oxidation of Se(IV) to Se(VI) [30]. With the objective of eliminating these interferents, a degassing step [30] on the digested samples was carried out in the present study.

3.4. Performance of the proposed method

The performance of the proposed method was verified by analyzing a set of multielemental standard solutions of As(V), Sb(V), and Se(VI) under the conditions cited in Table I. The figures of merit for As, Sb, and Se were calculated according to IUPAC [31]. They are compared with previously published ones and are summarized in Table III.

The results shown in Table III demonstrate the feasibility of the method. The most important analytical characteristics are low reagent consumption (0.023 g $NaBH_4$ and 0.0109 g KI) and waste generation of (5.0 mL per determination) and high sampling throughput (40 determinations per hour). This demonstrates the method contributes to the environment sustainability.

3.5. Analysis of agronomic samples

The method developed was applied for quantification of As, Sb, and Se in samples of animal feed (forage, corn,

Table III. Comparison of figures of merit. LOD: limit of detection; LOQ: limit of quantification; and RSD: relative standard deviation.

Parameters	Proposed Method			Reference [32]			Reference [33]		
	As	Sb	Se	As	Sb	Se	As	Sb	Se
Concentration Range ($\mu g\ L^{-1}$)	20 - 120			-			1 - 5		
Slope	3.21	2.18	4.55	62.1	27.2	37.9	255	164	207
Intercept	6.45	8.60	3.12	0.038	0.017	0.029	-120	130	120
R^2	0.9988	0.9913	0.9925	-			0.9991	0.9990	0.9985
LOD ($\mu g\ L^{-1}$)	0.12	0.17	0.14	2	2.4	2.6	0.4	0.3	0.3
LOQ ($\mu g\ L^{-1}$)	1.24	1.68	1.41	20	24	26	4	3	3
RSD	1.8	2.0	1.0	8	7	3	2.0	2.0	3.0
Sampling throughput/ h^{-1}	40			-			40		

silage, mineral salt) and bovine blood, carcass, viscera, meat, and semen. The accuracy of the method was demonstrated by the recovery of As, Sb, and Se spiked in samples of forage, viscera, and semen in order to obtain 30 $\mu g\ L^{-1}$ of the analytes. The recoveries of As, Sb, and Se spiked in the samples are given in Table IV.

Recoveries were satisfactory with exception of Se in viscera, where the element recovery was 89.5%. This probably occurred because those samples contained high amount of protein and fat, which interfered in the Se determination. However, the relative standard deviation (RSD) was below 5.3% for all spiked samples.

Table IV. Analyte recovery (n =4) in agronomic samples spiked with 30 $\mu g\ L^{-1}$ of As, Sb and Se.

	Recovery (%)					
Samples	**As**		**Sb**		**Se**	
Forage	97.7	± 1.4	93.9	± 5.3	102.0	± 5.1
Viscera	98.6	± 2.4	97.8	± 2.8	89.5	± 3.3
Semen	94.6	± 4.3	94.3	± 2.5	94.7	± 3.8

In all the samples, As and Sb were not detected (< LOD), except As in the mineral salt, with a concentration of 2.48 $\mu g\ kg^{-1}$. This was probably due to the salt origin - it is extracted from the sea where there are large sources of organic arsenic - and when it was subjected to acid digestion, these were completely oxidized to As(V).

Table V shows the values obtained for Se in the analyzed samples. With the exception of the carcass and forage samples, Se was detected in the samples.

Table V. Concentration of Se found in agronomic samples (n=3).

Samples	Se	
	Conc.	RSD
	($\mu g\ kg^{-1}$)	
Forage	< LOD	-
Corn silage	106.3	5.0
Mineral salt	4301.7	5.1
Blood 1	436.4	3.9
Blood 2	424.8	4.3
Carcass 1	< LOD	-
Carcass 2	< LOD	-
Viscera 1	155.6	4.2
Viscera 2	103.6	5.8
Meat 1	173.8	4.7
Meat 2	183.6	5.1
Semen	530.3	4.0

4. Conclusions

The proposed flow system and developed method proved to be adequate for simultaneous determination of As, Sb, and Se in agronomic samples. With this system, reagent consumption and waste generation are effectively reduced. The accuracy shown by the recovery tests was good for As, Sb and Se in all samples, with the exception of Se in bovine viscera, owing to higher protein and fat contents.

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Application of chemometric methods for the quantitative analysis of antihypertensive drugs with severely overlapping fluorescence spectra

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Abstract

Two antihypertensive drugs, metoprolol (MET) and pindolol (PIN), were quantitatively analyzed in synthetic mixtures by the application of chemometric methods to their synchronous fluorescence spectral data. The fluorescence spectral profiles of these drugs were highly overlapping; thus, a quantitative analysis based on an univariate method was not possible. Chemometric multivariate calibration methods were found to be a good alternative for the simultaneous quantitative analysis of multicomponent samples with severely overlapping spectral features. The observed emission maxima for MET and PIN were at 302 nm and 310 nm respectively. A method was developed based on the synchronous fluorescence of analytes within the concentration range of 0-0.5 mg/L for MTP and 0-5 mg/L for PIN. Multivariate calibration methods such as multivariate curve resolution (MCR), partial least squares regression (PLSR) and principal component regression (PCR) were performed for the analysis of the spectral data. The proposed method was successfully applied for the determination of the drugs in serum samples. The results of the method were validated statistically and found to be satisfactory.

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1. Introduction

Simultaneous quantitative determination of drugs in pharmaceutical formulations and biological fluids using a simple, sensitive and economical method will be highly beneficial for the routine quality control and laboratory testing. Metoprolol (MET) and pindolol (PIN) are two antihypertensive drugs with the IUPAC names, 1-[4-(2-methoxy ethyl) phenoxy]-3-[(1-methyl ethyl) amino]-2-propanol and 1-(1H-indol-4-yloxy)-3-[1(1-methyl ethyl)amino]-2-propanol, respectively [1,2]. Both drugs belong to a class of beta-adrenergic receptor blockers and are generally given to patients suffering from cardiovascular illness [3,4]. These are used as doping agents in sports and are listed as forbidden substances by the International Olympic Committee [5].

Spectroscopic and chromatographic methods are reported in literature which deals with the quantitative determination of PIN in combination with other drugs [6,7]. PIN was quantitatively determined in oxidized form by dichromate in sulphuric acid media by sequential injection analysis and chemometric optimization [8]. Binary mixtures of the drugs, metoprolol and propranolol, were simultaneously determined by HPLC and UV spectrophotometry with the aid of multivariate methods [9]. Metoprolol was also determined by fluorescence [10], RP- UPLC [11], LCMS [12] and GCMS [13] methods.

Fluorescence spectroscopy is used widely to study drug interactions, qualitatively and quantitatively, because of their intrinsic fluorescence. For multicomponent samples, energy transfer, quenching and spectral overlap are comparable along with fluorescence emission. The problems that arises in conventional fluorescence spectroscopy can be annulled to an extent by synchronous fluorescence spectroscopy (SFS). In SFS, simultaneous scanning of both excitation and emission monochromators is carried out, while maintaining a constant wavelength interval ($\Delta\lambda$) between them [14].

A direct correlation between the intensity of fluorescence and the concentration is not valid for a multicomponent sample with severely overlapping spectral profiles. When more than one species fluoresces and interference from the matrix exists, then the analysis of the data at λ_{max} alone is not sufficient for calibration purposes. Intelligent ways to reduce the noise which oth-

erwise impairs the information part has to be adopted for a better analysis. This can be accomplished using multivariate calibration methods [15]. Multivariate calibration involves a calibration step in which a mathematical model is built, using a chemical data set, **X** (e.g., absorbance values), and a concentration data set, **Y,** followed by validation and prediction steps [16,17]. A detailed simultaneous quantitative analysis of PIN and MET in synthetic binary mixtures and serum samples is the main focus of this work. It involves the development of a rapid, economical and sensitive calibration model based on the synchronous fluorescence of drug combinations followed by the validation of model. This model will be used for the quantitative prediction of drug mixtures in serum. The established method will be verified with respect to % recovery, % relative error and root mean square error of prediction (RMSEP) values.

2. Experimental

2.1. Apparatus

Emission spectra for PIN and MET were measured with a Jasco fluorimeter equipped with a 150W xenon lamp. Excitation and emission monochromator slit widths were adjusted at 5 and 5 nm, respectively. SF spectra were obtained within the range 280 - 340 nm with a scan speed of 1000 nm/minute. Spectral data obtained in ASCII format was plotted in Origin 6. All multivariate calibration methods were developed on PLS toolbox 7.5 working on a MATLAB platform.

2.2. Materials

Metoprolol tartrate and pindolol in pure form were purchased from Sigma Aldrich. Bovine serum albumin fraction V was obtained from HI Media. Metoprolol tartrate tablets were purchased from a local pharmacy. All solvents used throughout this study were of spectroscopic reagent grade. Triply distilled water (TDW) was used for the study.

2.3. Preparation of solutions for calibration and validation

Metoprolol stock solutions of 200 mg/L were prepared by dissolving 0.0050 g of material in ethanol and making volume up to 25 mL. The pindolol drug stock solution of 50 mg/L was prepared in 100 mL calibrated volumetric flask by dissolving 0.005 g in an ethanol-water mixture. Solutions of concentrations in the working range, *i.e.,* 0.027 to 0.50 mg/L for MET and 0.350 to 4.575 mg/L for PIN, were prepared from the stock solution with suitable dilutions using triply distilled water. 37 different combinations with varying drug concentrations were prepared. Solutions were stored at lower temperature and protected from light. The concentration of MET and PIN, employed in the calibration dataset and validation dataset, are given in tables I and II respectively.

Table I. Concentration data (in mg/L) of the different mixtures of MET and PIN used in the calibration set for the development of calibration model

Sample Nº.	MET mg/L	PIN mg/L	Sample Nº.	MET mg/L	PIN mg/L	Sample Nº.	MET mg/L	PIN mg/L
1	0.027	3.73	11	0.168	2.56	21	0.347	4.29
2	0.032	1.47	12	0.192	3.73	22	0.354	1.92
3	0.05	3.18	13	0.203	4.575	23	0.373	0.69
4	0.062	4.575	14	0.21	0	24	0.389	3.18
5	0.084	4.29	15	0.245	3.18	25	0.405	1.15
6	0.09	0.69	16	0.252	2.56	26	0.432	1.92
7	0.097	2.56	17	0.264	0.35	27	0.446	3.73
8	0	1.15	18	0.293	4.575	28	0.454	1.47
9	0.14	0.69	19	0.308	3.18	29	0.48	0
10	0.152	4.29	20	0.336	1.47	30	0.5	2.56

Table II. Concentration data (in mg/L) of the different mixtures consisting of MET and PIN used in the validation set

Sample Nº.	Metoprolol mg/L	Pindolol mg/L	Sample Nº.	Metoprolol mg/L	Pindolol mg/L
1	0.044	1.92	5	0.280	3.73
2	0.128	1.92	6	0.324	0.69
3	0.175	0.35	7	0.365	1.15
4	0.228	1.47			

2.4. Preparation of solutions for the analysis of drugs in serum

The evaluation of robustness of the method was carried out by performing drug analysis in the presence of serum. A stock solution of bovine serum albumin of 660 mg/L was prepared by weighing 0.066 g and transferring quantitatively into a 100 mL volumetric flask. This was diluted up to the mark with triply distilled water. The working concentration of serum was maintained constant, *i.e.,* 0.66 mg/L. Different drug mixtures contains MET concentrations ranging from 0.050 mg/L to 0.446 mg/L and PIN concentrations ranging from 0.350 mg/L to 3.730 mg/L were prepared in this serum concentration.

2.5. Theory

Principal component regression (PCR) and partial least squares regression (PLSR)

The synchronous fluorescence data is processed using multivariate calibration methods, where a relation between fluorescence intensities and component concentrations are established. This factor-based method involves the development of a calibration model followed by the validation of the model using some known concentrations. This model can then applied for predicting the concentrations of components in unknown samples [18]. In this study, two multivariate methods, principal component regression (PCR) and partial least squares regression (PLSR) are employed.

In PCR, a principal component analysis (PCA) is performed followed by a regression analysis. PCA converts the

huge number of variables to a very few principal components without loosing much information in the data. Thus, the measured variables get converted to new ones, *i.e.,* scores of latent variables. In the second step, a multiple linear regression (MLR) is performed on the scores obtained in the PCA step. PLSR is another regression approach where the above mentioned two steps are done on the data in a single step [19-20].

The multivariate calibration model developed will be investigated further and parameters such as correlation coefficient (R^2) and root mean square error (RMSE) will be examined. RMSE is a way of expressing the difference between the predicted and reference value for a set of samples. RMSE is defined as,

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(y_{pred} - y_{ref})^2}{n}} \quad \text{(eq. I)}$$

Where y_{pred} and y_{ref} are the predicted and reference values, respectively, of sample 'i' in the calibration set (or validation set or prediction set) and 'n' is the number of samples used. RMSE values calculated for calibration data set are expressed as root mean square error of calibration (RMSEC), which indicates the robustness of the model developed. The efficiency of the model to predict an unknown sample is expressed in terms of root mean square error of cross validation (RMSECV). It is generally performed by a method known as *leave one out* cross validation. When the model is applied to a new set of data it is possible to calculate a root mean square error of prediction (RMSEP) provided the reference values for the new data set are known [15,20-23].

Multivariate curve resolution (MCR)

Multivariate curve resolution alternating least squares (MCR–ALS) analysis is a factor analysis method in which the two or higher way array of spectroscopic data set is decomposed into the pure component spectra and the relative concentration profiles [24]. The spectroscopic data is arranged in the form of a data matrix, D, in which the rows are the different individual spectra measured for the analysed samples and the columns are the spectroscopic data at each wavelength. PCA is performed first on the data matrix to get an idea regarding the number of components required to explain the maximum variation. A bilinear relation between the experimental data, the concentrations and the pure spectra of the components is assumed, where the individual response of each analyte is additive. In matrix form, this bilinear model is expressed in the following way:

$$D = CS^T + E \quad \text{(eq II)}$$

MCR-ALS decomposes the bilinear datamatrix, D, into the 'true' pure response profiles associated with the data variance in the rows and columns, and represented by matrices C (concentration data) and S^T (spectral data), respectively. C and S are calculated by the method of least squares, and the iterative process is repeated till the model converges.

3. Results and Discussions

3.1. Fluorescence spectra

Figure 1 represents the excitation and emission spectrum of MET and PIN. Metoprolol shows excitation and emission maxima at 275 nm and 302 nm, respectively, while pindolol exhibits excitation maximum at 280 nm and emission maximum at 310 nm. Their spectral profiles are highly overlapping as is clear from the narrow difference in emission maxima. There can be appreciable amount of energy transfer also possible along with other non- radiative processes. Hence, conventional fluorescence methods cannot be used for the direct and simultaneous analysis of these drug compounds. We can rely more on synchronous fluorescence spectroscopic method because it provides simplified and narrowed spectrum with appreciable spectral resolution [14].

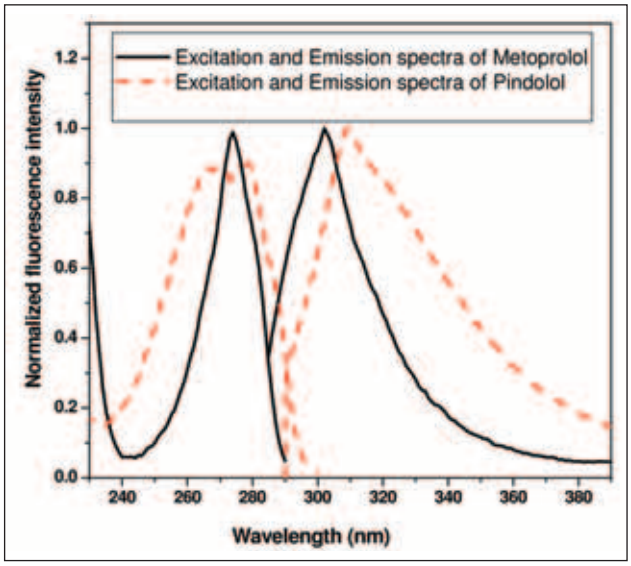

Figure 1. Excitation and emission spectra of Metoprolol (thick line) and Pindolol (dashed line)

3.2. Constant wavelength synchronous fluorescence spectroscopy (SFS)

This serves as a simple, effective and sensitive method of obtaining data for quantitative determination in a single measurement (with proper selection of Δλ). The crucial step in SFS measurement is the selection of wavelength interval or Δλ. For this purpose scans were made from Δλ = 5 nm to 100 nm with 5 nm intervals. It was observed that Δλ 30 nm provided maximum fluorescence intensity and simplified spectra. Hence Δλ of 30 nm was used for the whole analysis (From the contour map of the drug mixtures it was

confirmed that Δλ 30 nm is suitable for the simultaneous analysis.). 37 samples prepared for the experiment containing various concentrations of MET and PIN were divided into a calibration set of 30 samples and a validation set of 7 samples. Concentration of MET and PIN in the 30 samples constituting the calibration dataset is tabulated (Table I).

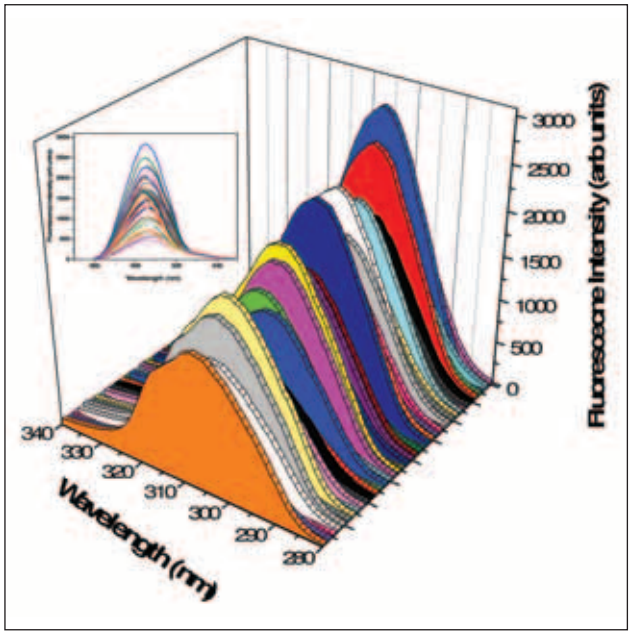

Figure 2. Synchronous fluorescence spectra of samples containing various concentrations of Metoprolol and Pindolol (calibration dataset) at Δλ 30 nm. (Inset: a two dimensional picture of SF spectra)

Figure 2 represents the SF spectra of the 30 samples of the calibration dataset. The scan range chosen for the experiment was from 280 nm to 340 nm. The spectral features are simple, but spectral resolution is not evident. The spectral profiles of MET and PIN are heavily overlapped and the individual peaks are indistinguishable. As is clear from Figure 2, the samples differ from one another only in terms of the fluorescence intensity. The synchronous fluorescence spectra does not provide or elucidate any individual information since information of both components are embedded in it. To recognize if multivariate methods are able to detect the existence of both components in the SF spectra, multivariate curve resolution was performed prior to PLSR and PCR analysis.

3.3. Multivariate curve resolution (MCR)

Since the spectral profiles were complex and difficult to interpret, the MCR-ALS method was applied to extract further information. Resolution of spectra was achieved using MCR-ALS. The number of significant factors, N, which indicates the number of components involved, was extracted by principal component analysis. Figure 3 shows the extracted spectral profiles of components A and B (dotted line) and the experimentally obtained spectral profiles of the pure components (solid line), which give a clear correspondence. The extracted spectral profile of component A and B matches exactly with the emission spectra of MET and PIN respectively. Even though severe spectral overlap persists, MCR, being a powerful tool for spectral resolution, identifies the two components present in the mixture qualitatively.

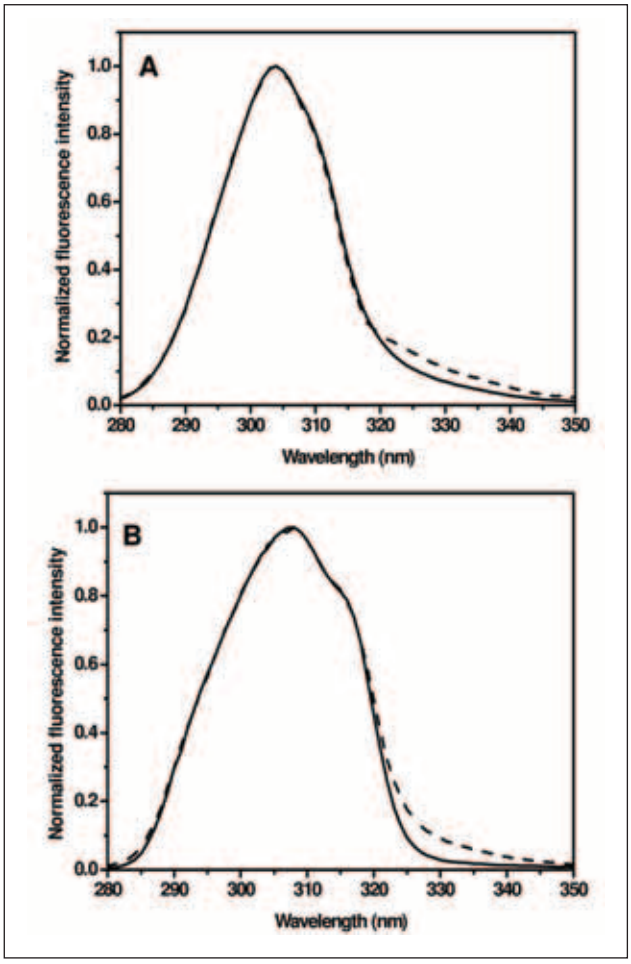

Figure 3. Results of the Multivariate Curve Resolution (MCR-ALS) on the synchronous spectroscopic data of the synthetic samples (A) Metoprolol (B) Pindolol (solid line: pure component emission; dotted line: recovered from MET-PIN combination using MCR method)

3.4. Partial least squares regression (PLSR) and principal component regression (PCR)

Many regression methods which are intrinsically linear have been proposed for multicomponent analysis, among which the most popular ones are PLSR and PCR. These efficient chemometric multivariate calibration methods are a good alternative for quantitative analysis, hence can be applied on the fluorescence data.

3.5. Pre- processing methods

The first step involved in the multivariate analysis is the selection of the best preprocessing method. For the analysis of a two way data set using multivariate analysis, data were arranged in the form of a matrix where rows contain samples and columns consist of SF intensities. Mean centering was

used as the pre-processing techniques. The results are presented in Table III. From the table it is clear that the data without any pre-processing yield a high RMSE values compared to MNCN. Hence, the further analysis and model development was done with MNCN as the pre-processing method.

Table III : Comparison of preprocessing methods adopted for PLSR and PCR methods for the calibration and validation of MET-PIN synthetic binary mixtures (RMSE values are in mg/L)

Component	Statistical parameters	NONE		MNCN	
		PLSR	PCR	PLSR	PCR
MET	R^2	0.990	0.989	**0.992**	**0.992**
	No of factors	6	6	**6**	**6**
	RMSEC	0.0136	0.0144	**0.0128**	**0.0131**
	RMSECV	0.0169	0.0168	**0.0159**	**0.0161**
PIN	R^2	0.986	0.987	**0.988**	**0.990**
	No of factors	3	5	**3**	**5**
	RMSEC	0.1717	0.1633	**0.1671**	**0.1473**
	RMSECV	0.1935	0.2006	**0.2120**	**0.2017**

3.6. Cross validation method

This is used to obtain an estimate of the predictive ability of a multivariate regression model. Cross validation predicts the optimum number of factors or principal components or latent variables required to develop the calibration model without over fitting or under fitting the data. Cross validation for the calibration set was performed by the *leave one out* method. The regression model was calculated using 29 samples leaving out one sample at a time and predicting the concentration of the left out sample. The prediction error of this process was calculated from the difference between the predicted and true value. This procedure was then repeated leaving out every sample in the calibration set once and the summed prediction error was calculated. The root mean square error of cross validation (RMSECV) is plotted against the number of components (Figure 4). This is known as a PRESS (Prediction error sum of squares) plot. The inset on Figure 4 is the magnified part of the plot to recognize the optimum number of components.

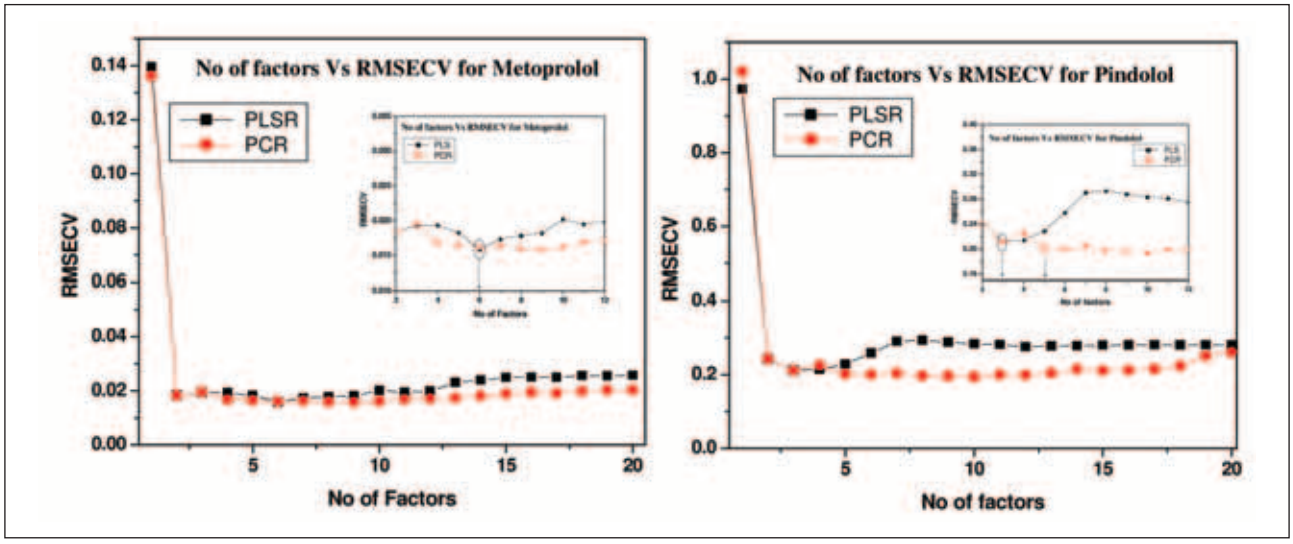

Figure 4. Plot of number of factors versus RMSECV values for MET and PIN for both PCR and PLSR analysis (Inset: magnified from number of factors 2 to 12)

The number of factors giving a minimum RMSECV value was chosen as the best number of factors for developing the model. The optimum number of factors observed from figure 4 was 6 for MET and 3 for PIN by the PLSR method. By PCR methods the optimum numbers of components were found to be 6 and 5, respectively, MET and PIN.

3.7. Loadings plot

The first six loadings (PLSR and PCR) obtained from chemometric analysis are plotted in figure V (A and B). The profile of the first loading is relatively the same as the synchronous fluorescence spectra of the mixture of MET and PIN (Figure 2). The second loading has positive values at wavelength intervals greater than 310 nm, whereas negative values below the specified wavelength. First and second loadings in PLSR and PCR are almost the same and capture about 90 % of the variance. Fourth and sixth loadings are almost identical. The fifth as well as the sixth loadings of the MET and PIN have reverse directions (orthogonal). These observations may be due to the differences between the spectra of the two drugs at the specified wavelength regions. Loadings data recommend significant information for calibration of a given species, but these features are not necessarily only from that species, because of which numbers of factors are more than expected.

The regression coefficients are plotted in figure 5 (C and D). The prediction ability with seven factors is slightly poorer than the ability of the one that is obtained with six factors.

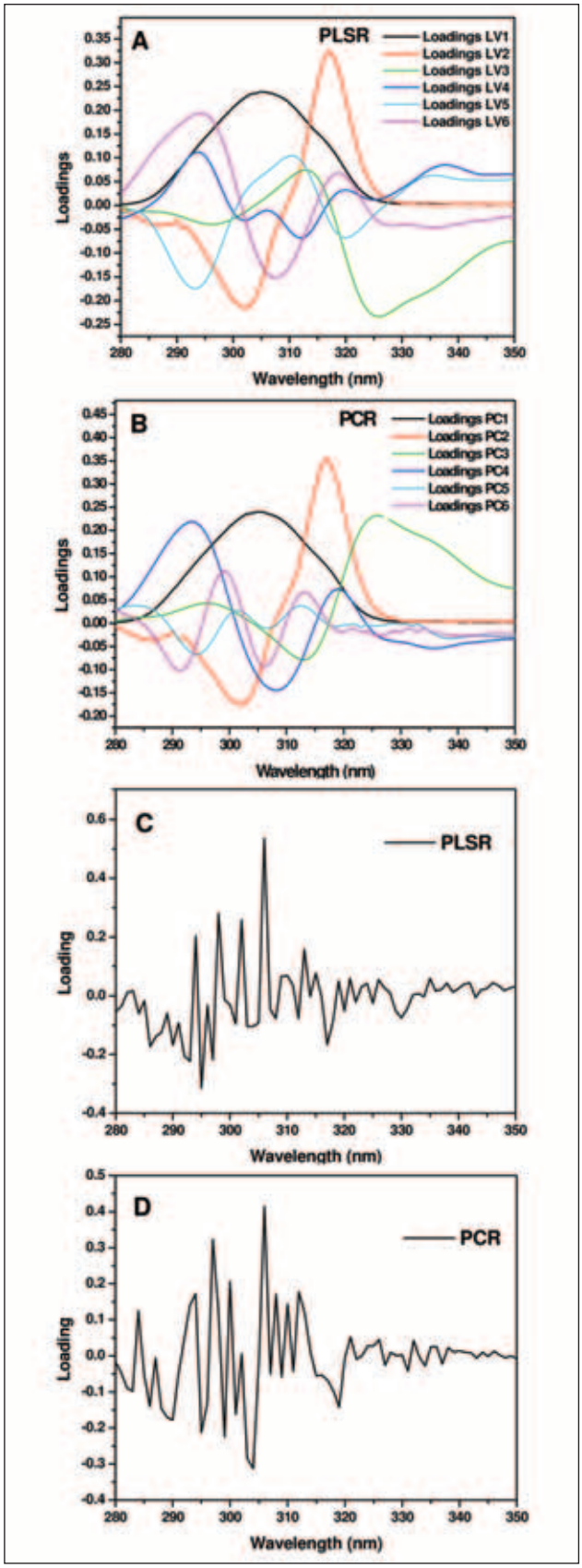

Figure 5. First six loadings for Metoprolol and Pindolol (A, B) and regression coefficient obtained after six factors (C, D) by PLSR and PCR methods.

3.8. Model development

The reference values of concentration were plotted against the concentration values predicted by the PCR and PLSR models. Both PCR and PLSR models showed excellent correlation between the measured and predicted concentrations. Correlation coefficients obtained are around ~ 0.992, with less prediction error as shown in figure 6.

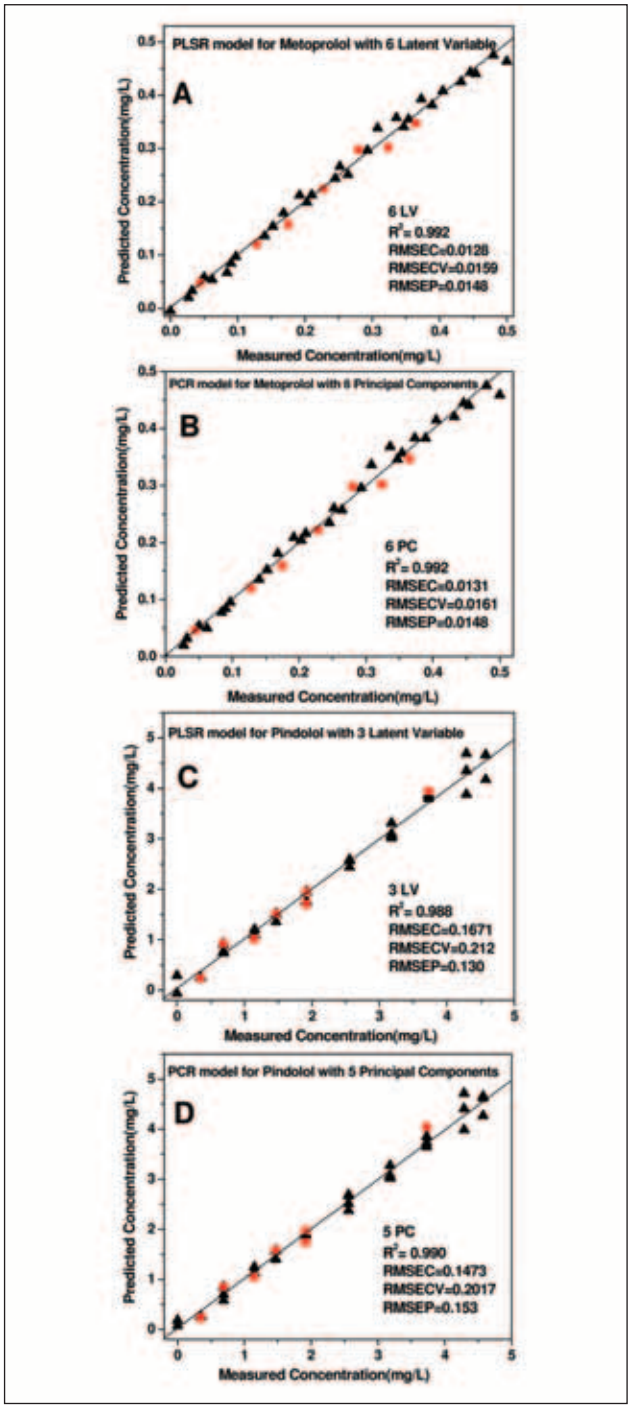

Figure 6. Measured versus predicted concentrations of MET PIN synthetic mixtures (calibration dataset) based on cross validation (A) MET by PLSR model (B) MET by PCR model (C) PIN by PLSR model (D) PIN by PCR model. (▲ Calibration dataset and ■ Prediction dataset)

Table IV. Determination of MET-PIN in synthetic mixtures in order to check the validation of the developed model

		PLSR				PCR			
Added Conc. mg/L		Found Conc. mg/L		Relative error %		Found Conc. mg/L		Relative error %	
MET	PIN	MET	PIN	MET	PIN	MET	PIN	MET	PIN
0.044	1.92	0.048	1.945	9.09	1.30	0.046	1.97	4.55	2.60
0.128	1.92	0.120	1.713	6.25	10.78	0.120	1.737	6.25	9.53
0.175	0.35	0.157	0.310	10.29	11.43	0.158	0.382	9.71	9.14
0.228	1.47	0.224	1.515	1.75	3.06	0.222	1.585	2.63	7.82
0.280	3.73	0.298	3.946	6.43	5.79	0.298	4.042	6.43	8.36
0.324	0.69	0.302	0.755	6.79	9.42	0.302	0.779	6.79	12.90
0.365	1.15	0.347	1.019	4.93	11.39	0.347	1.057	4.93	8.09
RMSEP		0.0148	0.130			0.0148	0.153		

RMSEC and RMSECV values obtained are very low which indicates the strength of the developed model and its predictive capability.

3.9. Validation of the model

The developed model was validated using 7 samples of the synthetic mixtures. Concentration of both MET and PIN fits in the calibration data range. Recovery % and % relative error were calculated for each component. The recovery range for MET was found to be 89-109 % and 90-106 % for PLSR and PCR methods respectively. In the case of pindolol, the recovery range was 88-109 % and 90-112 % for PLSR and PCR methods respectively. Average percentage relative error was ~7 %. The results are given in Table IV.

The root mean square error of prediction was found to be 0.0148 mg/L for metoprolol and 0.130 mg/L and 0.153 mg/L for pindolol. This shows the robustness of the developed model for prediction purposes.

3.10. Prediction of concentration of MET and PIN in serum

To determine the concentration of MET and PIN in the presence of serum, a series of solutions with various concentrations of MET and PIN were prepared in serum. Concentration of serum was kept constant at 0.660 mg/L. The concentrations of both drugs were predicted using the developed model. Table V represents the predicted concentration of MET and PIN in serum. The recovery % for each sample was calculated and recovery ranges of 94-108 % for MET-PLSR method, 86-97 % for MET-PCR method, 85-111 % for PIN-PLSR method and 85-108 % for PIN-PCR method were obtained. The average relative error was found to be ~7.1 %. Lower RMSEP values indicate the efficiency of the developed model to predict MET and PIN concentrations even in the presence of strong background signals of serum fluorescence. The developed methods are able to determine selectively the drug components present in the sample.

Table V. Added and found concentrations of MET and PIN in serum with their recovery.

		PLSR				PCR			
Added Conc. mg/L		Found Conc. mg/L		Relative error %		Found Conc. mg/L		Relative error %	
MET	PIN	MET	PIN	MET	PIN	MET	PIN	MET	PIN
0.050	3.180	0.054	2.71	8.00	14.78	0.043	2.73	14.00	14.15
0.073	0.350	0.069	0.37	5.48	5.71	0.066	0.32	9.59	8.57
0.168	2.560	0.173	2.72	2.98	6.25	0.161	2.75	4.17	7.42
0.324	0.690	0.35	0.76	8.02	10.14	0.291	0.73	10.19	5.80
0.354	1.920	0.338	1.94	4.52	1.04	0.335	1.99	5.37	3.65
0.446	3.730	0.438	4.16	1.79	11.53	0.433	4.05	2.91	8.58
RMSEP				0.0132	0.2699			0.0172	0.2410

4. Conclusions

Multivariate chemometric methods extend the applicability of the fluorescence method for the analysis of multicomponent samples. Here, a synchronous fluorescence spectroscopic analysis is performed for the direct and simultaneous analysis of MET and PIN combination in synthetic mixtures and in serum samples. The method developed was simple and fast and is sensitive up to mg/L levels of the components present. The % recovery obtained was more than 85 % and average % relative error was ~7 %. The low RMSEP values obtained show the capability of the model to predict MET and PIN in the presence of serum.

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Sequential preconcentration using cloud point extraction: determination of vanadium and molybdenum in water and pharmaceutical samples using Flame Atomic Absorption Spectrometry

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Abstract

In the present study is proposed a method for sequential preconcentration of vanadium and molybdenum with cloud point extraction and determination using FAAS. The optimization process was carried out using a two-level full factorial design. The parameters optimized were the concentrations and volumes of complexing reagents,1-(2-pyridylazo)2-naphthol(PAN) and 8-hydroxyquinoline (8-HQ), concentrations and volumes of non-ionic surfactants (Triton X-114 and Triton X-100), solution pH and sample volume. An enrichment factor of 10 and 27, and limits of detection of 80 and 40 μg L^{-1}, were obtained for V and Mo, respectively. Measurements in presence of Fe (II) (possible interfering) in proportions higher than 1:10 (V: Fe and Mo: Fe) were performed and the absorbance signals of V and Mo decrease about 30 and 50%, respectively. The procedure was applied for determination of these elements in pharmaceutical formulation samples and accuracy was assessed by addition-recovery experiments.

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Keywords: sequential cloud point extraction, vanadium, molybdenum, flame atomic absorption spectrometry

1. Introduction

The extensive use of V and Mo compounds in the chemical industry and the high concentrations of these elements in petroleum have contributed to increase their availability in the environment, growing the interest in studying the effects of these elements. Their physiological effects are quite diverse; for example, an animal diet low in V can present several deficiencies such as poor bone formation and growth retardation. Furthermore, several studies have shown the importance of V as an inhibitor of the biosynthesis of cholesterol, plasma triglycerides and in the effects of insulin in the human organism [1]. The estimated daily intake of V ranges from 10 to 60 mg [2, 3], the concentrations in human blood and serum are between 1 nmol L^{-1} and 10 nmol L^{-1} and are slightly lower in urine [4].

Molybdenum is an element found in trace levels in soils, being essential for growth of many biological organisms. In plants it operates actively in the processes of nitrogenase, nitrate reductase, sulfate oxidase, and is also related to the transport of electrons in several biochemical reactions and in N fixation [5]. Molybdenum deficiency in human diet can inhibit cell growth but a high concentration of this element in blood can increase gout risk and disseminated sclerosis [6]. This biological functioning diversity shows the importance of the determination of these elements at low concentrations in several matrices [7,8].

The determination of refractory metals such as V and Mo can be accomplished through various instrumental techniques such as inductively coupled plasma optical emission spectrometry (ICP OES), electrochemical methods (voltametry and polarography) [9,10] and atomic absorption methods, such as graphite furnace atomic absorption spectrometry (GFAAS) and flame atomic absorption spectrometry (FAAS) [11,12]. Atomic absorption spectrometry is one of the most widely used techniques for the determination of trace elements. Its main characteristics are the versatility and low cost (acquisition, operation and maintenance) [13]. However, some limitations are the poor sensitivity and interferences related to matrix effects [14].

Among the various procedures used to overcome these drawbacks, the use of preconcentration / separation methods is a versatile alternative [15,16]. The preconcentration steps have become a complement of the techniques already discussed. If the species of interest is in a very low concentration (below the limit of detection) the preconcentration step becomes mandatory [17]. The preconcentration method using cloud point extraction (CPE) stands out as the most efficient alternative compared to other conventional methods of extraction / preconcentration, whereas it produces high extraction efficiency, high preconcentration factors and doesn't require toxic reagents

(mainly organic solvents), contributing to green chemistry concepts [18].

The method of CPE is based on the formation of an aqueous surfactant (surfactant) which turns turbid with the addition of a suitable substance or by changing some of its properties such as temperature or pressure. At this point the solution separates into two phases, one aqueous of large volume containing a small amount of surfactants, named poor phase, and another phase, which is the rich phase and characterized by high concentrations of the analyte [19].

In this study, V and Mo were simultaneously extracted using CPE in water and pharmaceutical formulations and were sequentially determined by FAAS. The determination of these elements in water samples is of great importance since little is known about its effects in humans when administered for long periods, thus further studies are needed to fully elucidate the molecular mechanism of these beneficial effects.

The goal of this study was to combine the method of CPE technique in determination of V and Mo using FAAS.

2. Experimental

2.1. Reagents

All reagents used were of analytical grade. Solutions were prepared with ultrapure water obtained from a Milli-Q® purification system (Millipak-40 Filter Unit 0.22 µm NPT, Bedford, MA, USA) with resistivity higher than 18.2 MΩ cm. Analytical reference solutions were prepared by subsequent dilutions of 1000 mg L^{-1} stock standard solutions of V and Mo (Quemis High Purity, Hexis, Jundiaí, SP, Brazil).

The chelating agents 8-hydroxyquinoline (8-HQ) (Vetec, Rio de Janeiro, RJ, Brazil) and 1-(2-pyridylazo)2-naphthol (PAN) (Sigma Aldrich, St Louis, USA) were prepared daily by dissolving the appropriate amount of the reagents in 10 mL of ethanol and stored in a brown glass flask. Acetate buffer (2.0 mol L^{-1}) was prepared to adjust sample pH to 3.8 and 4.5. The non-ionic surfactants Triton X-114 and Triton X-100 were obtained from Sigma Aldrich and prepared by dilution of the commercially available product. Ethanol 99.5 % (v v^{-1}) (Tec Lab, São Paulo, SP, Brazil) and methanol (Tedia, Rio de Janeiro, RJ, Brazil) were used for dilution of the phase containing the analyte.

2.2. Samples and samples preparation

The mineral water and pharmaceutical (V chelate) samples were purchased in the local market of São Carlos – state of São Paulo (Brazil). Water samples were used without further preparation and the pharmaceutical sample was ground manually using a mortar and a pestle of agate until it became homogeneous. Before cloud point extraction procedure, 100 mg sample were weighed, transferred to tubes and 5 mL of HNO_3 1 mol L^{-1} added. These tubes were placed on a shaker (Barnsteady, Iowa, USA) for 10 minutes and then they were placed in an ultrasound (Barnstead/Lab-Line Aqua Wave 9374) for more 10 minutes. After the extraction procedure, it was centrifuged (Hermle/Labnet Z200A, Germany) for 5 minutes at 3000 rpm to separate the supernatant from the residue.

2.3. Cloud point extraction procedure

The CPE analytical procedure was optimized using two 2^4 experimental designs for each element separately, with the objective of identifying the experimental conditions that provided the highest responses in integrated absorbance. The conditions used for CPE were optimized individually for Mo in the studies of Oviedo et al. [20, 21]. The cloud point extraction procedure for V was accomplished using a 2^4 factorial design in order to study the following variables: type of complexing agent (PAN, 1-(2-pyridylazo)-2-naphthol and 8-HQ 8-quinoline), volume of solutions (0.5 to 1 mL) and concentration (0.1 and 0.5% v m^{-1}) of complexing agents, and pH (4.5 and 3.75). Subsequently another 2^4 experimental design was carried out, in which the variables were analyzed, complexation time (10 and 20 min), volume (0.5 to 1 mL), concentration (5 to 10% v^{-1}) and the type of surfactant agent (Triton X-100 and Triton X-114).

Adopting the best experimental conditions for the

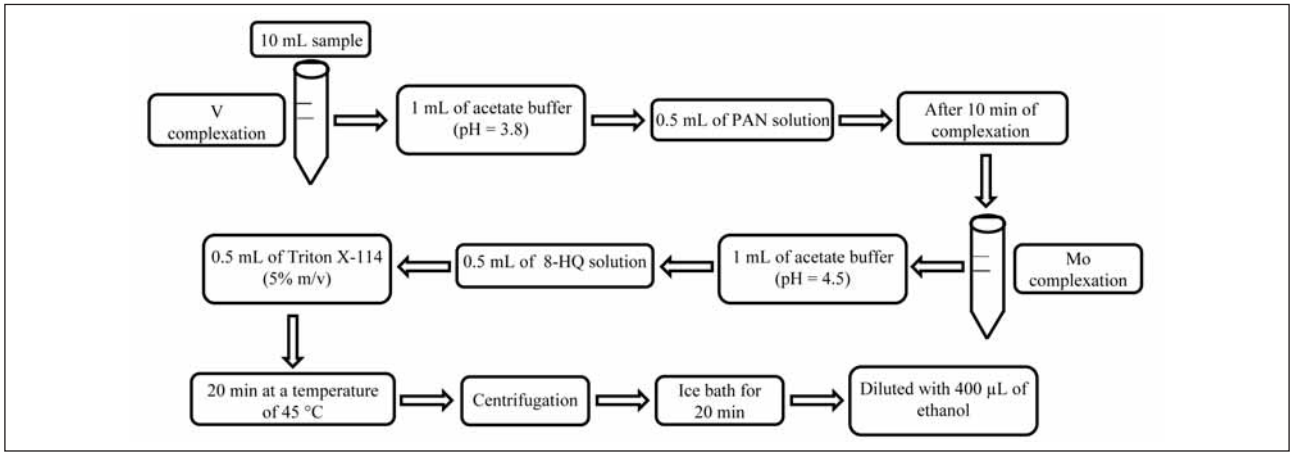

Figure 1. General procedure applied for sequential CPE of V and Mo.

extraction of V and Mo individually, a procedure aiming at the simultaneous extraction for these elements was performed. The general procedure is depicted in Figure1.

In the CPE procedure, 10 mL of a solution containing simultaneously V and Mo in different concentrations (from 0.5 to 8 mg L^{-1}) were placed in a 15 mL plastic tube. Then, 1 mL of acetate buffer (pH=3.8) and 0.5 mL of PAN solution, used as a chelating agent specific for V, were added to the solution. After 10 min of complexation, 1 mL of acetate buffer (pH=4.5) and 0.5 mL 8-HQ solution, a chelatin agent for Mo, were added. After more 10 min of complexation, 0.5 mL of Triton X-114 5% (m v^{-1}) surfactant were added and the plastic tubes were placed in a thermostatic bath for 20 min at a temperature of 45 °C. All pH measurements were carried out using a PHS-3B digital pH meter (Phtek, China), and a centrifuge was used to accelerate phases separation. Then the tubes were cooled in an ice bath for 20min and after separation, the phase containing predominantly water (poor phase) was removed with a micro-pipette and the surfactant-rich phase containing the analytes of interest was diluted with 400 µL of ethanol, and led to FAAS to reading.

2.4. V and Mo determination

The study was performed with a Varian AA240FS atomic absorption spectrometer (Mulgrave, Australia) equipped with a deuterium arc lamp background corrector. Hollow cathode lamps of V (318.5 nm) and Mo (313.3 nm) were used as primary radiation sources. Lamp currents of 20 and 7 mA with a spectral bandwidth of 0.5 nm were used for V and Mo, respectively. A nitrous oxide-acetylene flame was used with flow rates of 11.0/7.6 L min^{-1}, respectively, and burner height was kept at 10 mm.

Peak area was used to monitor the analytical signal for discrete nebulization. A 200 µL sample volume was manually introduced with a pipette into a small polytetrafluoroethylene (PTFE) funnel connected to the tip of the nebulizer aspiration tube for discrete nebulization.

3. Results and discussion

3.1. Study of injection volume on FAAS

Due to the small volume of the rich phase, FAAS measurements were performed with the aid of a 1000 µL automatic micropipette tip connected directly to the inlet of the nebulizer through a capillary polytetrafluoroethylene (PTFE) and fixed in a universal holder. The signal obtained was integrated absorbance. Due to the small volumes of the rich phase obtained in the cloud point extraction procedure, the study of different injection volumes in the spectrometer was performed and is shown in Figure 2. An injection volume of 200 µL presented lower relatives standard deviations (3.0% for V and 1.2 % for Mo).

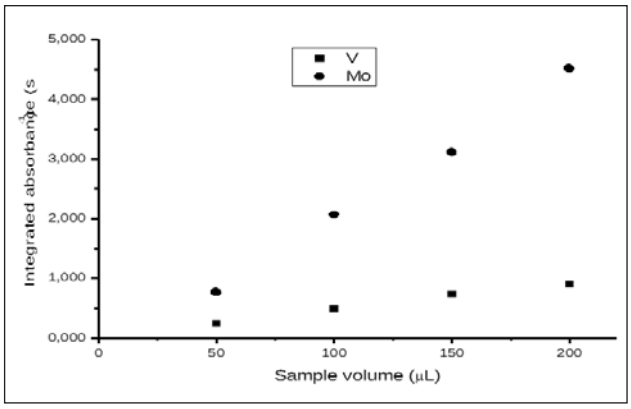

Figure 2. Analytical signals (Integrated absorbance s^{-1}) obtained for V and Mo with different injection volumes (from 50 to 200 µL).

3.2. Optimization of cloud point extraction procedure

The responses of individual experimental designs used in the optimization to V are shown in Table I and II.

The best conditions in the first experimental designs were observed in the Experiment 6, where PAN was the most appropriate complexing agent (0.5 % concentration, volume of 0.5 mL and 1.0 mL of the buffer solution of acetic acid / acetate sodium with pH = 3.75).

Table I. Factorial design 2^4 to study the variables: type, volume and concentration of complexing agent and pH.

Experiment	Complexing agent	Vol. complexing agent	[Complexing agent]	pH	E.F.
1	-1 (8-QH)	-1 (0.5 mL)	-1 (0.1 % m v^{-1})	-1 (3.75)	1.00
2	1 (PAN)	-1	-1	-1	3.27
3	-1	1 (1 mL)	-1	-1	0.85
4	1	1	-1	-1	0.39
5	-1	-1	1 (0.5 % m v^{-1})	-1	0.77
6	1	-1	1	-1	3.95
7	-1	1	1	-1	0.89
8	1	1	1	-1	2.40
9	-1	-1	-1	1 (4.5)	0.80
10	1	-1	-1	1	0.49
11	-1	1	-1	1	3.20
12	1	1	-1	1	0.29
13	-1	-1	1	1	1.47
14	1	-1	1	1	2.07
15	-1	1	1	1	0.81
16	1	1	1	1	3.06

Table II. Factorial design 2^4 to the study of variables: volume of surfactant agent, complexation time, type and concentration of surfactant agent.

Experiment	$Vol_{surf.}$	Time of Complexing	Type of surfactant	[surfactant]	E.F.
1	-1 (0.5 mL)	-1 (10 min)	-1 (Triton X-114)	-1 (10% v v^{-1})	1.88
2	1 (1 mL)	-1	-1	-1	5.05
3	-1	1 (20 min)	-1	-1	2.68
4	1	1	-1	-1	5.66
5	-1	-1	1 (Triton X-100)	-1	1.70
6	1	-1	1	-1	4.77
7	-1	1	1	-1	2.35
8	1	1	1	-1	4.70
9	-1	-1	-1	1 (5% v v^{-1})	1.38
10	1	-1	-1	1	6.90
11	-1	1	-1	1	2.63
12	1	1	-1	1	5.13
13	-1	-1	1	1	1.45
14	1	-1	1	1	5.13
15	-1	1	1	1	2.54
16	1	1	1	1	5.46

From this best response a second factorial design 2^4 (Table II) was performed. The best conditions for the pre-concentration was observed in the experiment 10 in which was achieved by adding 1.0 mL of Triton X-114 surfactant 5 % (w / v) and 10 min complexation. Under such conditions the pre-concentration factor was approximately 7-fold.

The cloud point extraction optimization was performed for each element individually and the best conditions are shown in Table III. All determination in the subsequent samples preparation were made using these optimized conditions.

Table III. Optimized parameters for CPE.

Parameters	V	Mo
Chelating reagent	PAN	8-HQ
Chelating reagent volume (mL)	0.5	0.5
Chelating reagent concentration (%)	0.1	0.5
Surfactant volume (mL)	0.5	0.5
Sample volume (mL)	10	10
Solution pH	3.75	4.5
Surfactant	X-114	X-114
Surfactant concentration (%)	5	5

3.3. Interference of Fe in cloud point extraction

The Fe can react with the complexing and decreases the efficiency of extraction [18,22,23]. This element can present also a potential interference in pharmaceutical formulations samples [3, 24]. A study of possible Fe interference in three different ratios (1:1, 1:10 and 1:100) was conducted and Table IV shows the obtained results when V and Mo concentrations were fixed at 10.0 mg L^{-1}.

It was observed that the presence of Fe caused interference in the pre-concentration procedure of V and Mo above 1:10 ratio (V:Fe and Mo:Fe), and it was observed around 30 and 50% reduction in the signal of V and Mo, respectively.

Table IV. Study of possible Fe interference in cloud point extraction procedure for Mo and V.

Analyte	Analyte and Fe ratio	Recovery (%)
Mo	1:1	99 ± 8
	1:10	51 ± 8
	1:100	24 ± 6
V	1:1	90 ± 9
	1:10	68 ± 6
	1:100	34 ± 6

3.4. Figures of merit

The figures of merit of the developed procedure were evaluated by calculating the limits of detection (LOD) and quantification (LOQ) defined as: LOD = 3σ/s and LOQ = 10σ/s, where s is the slope (sensitivity) of analytical curves, and σ is the standard deviation of 10 consecutive measurements of the blank.

Table V presents the figures of merit of the proposed procedure. The values were obtained by the ratio between the slope values of the two standard calibration curves (with and without CPE).The same linear range for all curves was used in order to compare the different ways the of extraction procedure (with and without cloud point).

An enrichment factor of 10 and 27, and limits of detection of 80 and 40 μg L^{-1} were obtained for V and Mo, respectively.

Table V. Figures of merit of the developed procedure.

Parameters	Mo		V	
	Aqueous standard solution procedure	CPE procedure	Aqueous standard solution procedure	CPE procedure
LOD (μg L^{-1})	400	40	800	80
LOQ (μg L^{-1})	1200	150	2603	2690
Sensitivity s (L $\mu g^{-1})^{-1}$	27	740	11	110
R^2	0.996	0.997	0.992	0.998

3.5. Application to samples

The procedure was applied for the determination of these elements in mineral water samples and accuracy was assessed by addition-recovery experiments. The concentrations added of V and Mo were 1.63 mg L^{-1} and the recoveries ranged from 98-103 % for V and 106-115 % for Mo.

The developed procedure was applied for the deter-

mination of V and Mo in a pharmaceutical sample. Molybdenum was below the LOD and the V recovery obtained was107± 4 % (precision around 4 %) when compared with the reference V value.

4. Conclusions

The cloud point extraction procedure was efficient for sequential extraction of V and Mo. The employment of discrete nebulization as an alternative for sample introduction into the flame was efficient and sensitive for the determination of V and Mo in mineral water and pharmaceutical samples. The study of Fe interference shows that this element caused a signal reduction of 30 and 50 % for V and Mo, respectively, when the ration analyte: Fe is higher than 10.

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Point of View

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The best technology I have ever worked with

Ultrasound or ultrasonic?, not clear which one suits best the work done in analytical chemistry that uses cavitation. Anyhow, magic starts as soon as we join anyone with this other two words: sample treatment. Sample treatment is my passion; it has been like this since I started to work in analytical chemistry. Sample treatment complexity can be reduced shortening the time required to handle the sample, by reducing the number of steps to treat it or by a combination of both. Whatever strategy chosen, advances in technology are always welcome, as they use to be the force that pushes advances in sample treatment forward. The piezoelectric effect, heart of transducers and therefore of ultrasonic devices, was first reported by Pierre Curie in 1880. Soon after, transducers were applied in many fields of research. By using the searching engine SCOPUS with the words ultrasound and sample treatment, the oldest manuscript retrieved dates back to 1953. The work deals with the use of ultrasonic energy to fragment tobacco mosaic virus [1]. It is likely that other works can be traced back even before. Starting my PhD thesis in 1996 (43 years later), the gold device for ultrasonic applications in sample treatment at that time was the ultrasonic probe, titanium made. This is an extraordinary tool to speed the liquid-liquid or solid-liquid extraction of many analytes, such as metals or organic compounds. The technique is so efficient that guide-lines provided in the USEPA extraction method 3550B recommend the use of focused ultrasound (FU), i.e., probe sonication, for the solid–liquid extraction of Polycyclic Aromatic Hydrocarbons, PAHs, from sediments [2]. The extraordinary thing is that this method was made even better when it was adapted to the analytical minimalism concept [3]. Many ultrasonic-based applications are indeed done nowadays in thousand of laboratories on a standard base worldwide. Some of such applications have changed in many areas the way we work. For instance, the remarkable achievement of speeding protein identification protocols from tens of hours to tens of minutes using focused ultrasonic energy has settled this method as the less time consuming for massive protein identification of large sets of samples [4]. The combination of ultrasonic energy and enzymes to extract metals while preserving the species they are involved in has become ultimately the gold standard for Se, As, and Hg speciation [5].

As technology evolved, new ultrasonic devices have been presented to the analytical community. Currently, ultrasonic probes can be used to treat a sample volume as small as 10 µL. Amazingly, ultrasonic energy can be applied now with glass probes, avoiding this way the contamination caused by metal impurities present in titanium made probes. But technology, like the human imagination, can not be stopped. Both are different expressions of the same thing, the human ability of dreaming. And through evolution, technology can offer today in day indirect ultrasonication with a level of ultrasonic energy so intense, that most of the processes of interest in sample treatment can be boosted using it. It is surprising that trough indirect ultrasonication as much as 96 samples can be treated at the same time using 96 well plates, with as low as 5 µL of sample volume. Furthermore, treatment can be done with the vials sealed, avoiding cross contamination. By sealing the vials, dangerous samples can be also treated. Moreover, on-line approaches allow now handling samples in an automated fashion [6]. On my opinion, for many protocols, ultrasonic energy eliminated handling time from the complex equation that sample treatment is.

Has ultrasonic energy finished of surprising us? No. When it seemed that no new surprises would appear with ultrasonic energy and sample treatment as the key players, DNA fragmentation with ultrasonic energy is now gaining momentum in genetics [7] and ultrasonic sample treatment for tissues in imaging mass spectrometry has changed the way tissues are treated [8]. Unbelievable, is it not?

What can we still await from ultrasonic energy in sample treatment ahead? Can we still be surprised? Ask this question yourself. Go to your lab, read your protocols, wherever a liquid-liquid or solid-liquid extraction process takes place, wherever a chemical breaking process takes place, wherever a heating process takes place, replace the standard method by ultrasonic energy, you will be surprised.

Last but not least, I would like to thank Professor Marco Arruda and the Brazilian Society of Chemistry for allowing me to publish this point of view about ultrasonic energy and sample treatment in the Brazilian Journal of Analytical Chemistry.

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Brazilian Session at Pittcon - Helping the internationalization of the Brazilian Analytical Chemistry

The Brazilian Session of Pittcon 2014 was definitely a greast success. I do not have exact numbers of participants, but during the interval, I rapidly went through several sessions and noticed that there was a few parallel sessions with greater attendance than ours. My participation was very fortuitous because I had to replace the Professor Clesia Nascentes of the Federal University of Minas Gerais, which would make an excellent overview of Analytical Chemistry in the country, since she was the Chairwoman of the last National Meeting of Analytical Chemistry - ENQA. As such, she was holding a full X-ray of all the most active groups in analytical chemistry in the country. Trying to be faithful to her presentation, I based such map as a starting point of my own presentation and tried to make a mix with the main theme of the session, which was the Federal Program of the Science without Borders. Making this parallel, I showed the origins of analytical chemistry in the country and its highlights how the masters Fritz Feigl, Pascoal Senise, Eduardo Neves, and all the legacy they left after them justify where we are thanks to them. We also present the recent Federal Government initiatives to stimulate the Science and Technology of the country as the creation of the National Institutes of Science and Technology - INCTs. In particular giving a highlight two INCTs dealing directly with the development of analytical chemistry. Thus, it was possible to make a quick history of Brazilian analytical chemistry and current opportunities for collaboration and internationalization of research groups that develop cutting-edge analytical chemistry.

With this stage set, much has been said about the internationalization of science and the importance of relations between countries in the construction of knowledge. The Pittcon is a great arena that provides a broad exchange of knowledge and a great prospect of the global situation of Analytical Chemistry, especially about research in the countries that are represented there. It is really important to Brazil to be present at this huge event, and that was a great honor and responsibility for me to be there as a representative of the country. While there is a knowledge that Pittcon is a grand fair, it is even more spectacular when seen from the inside.

Every expectation of being in touch with analytical chemists of the highest level, and meeting the newest technologies available in the market was met. During the event, I got to know more about prominent researchers, many of the caliber of a Nobel prize, as professors George Whitesides, Chad Mirkin, Steven Carr, James Jorgenson, Franz Hillenkamp, Martin Vestal, Peter Roepstorff, Allen Bard, to name a few. Besides proximity and interaction with these exponents, I could connect with many other analytical and bioanalytical chemists from countries like Germany, Hungary, Sweden, Japan, and of course, the United States.

Beyond personal enrichment, the experience has made possible the access of information to many students and Brazilian researchers, not only to colleagues with whom I have the opportunity to work more closely, but with all those who work with these colleagues, and thereby multiply the interactions and connections. Thanks to Brazilian Session at Pittcon and its theme, it was possible to articulate some new collaborations and attract researchers to spend a sabbatical period at the Institute of Chemistry of São Carlos, already using the resources of the Science Without Borders Program of CNPq.

Having followed Analytical Congress Latin America, which occurs in parallel with Latin America Analytical Fair since its inception, it is possible to note the growing synergy of both towards the consolidation as a "Brazilian Pittcon".

Prof. Dr. Emanuel Carrilho
Bioanalytical, Microfabrication, and Separations Group
Instituto de Química de São Carlos/Universidade de São Paulo

The 5th BrMASS Conference takes Brazil close to the leadership of Mass Spectrometry in Latin America

Prof. Dr. Marcos N. Eberlin
(Photo: Luciene Campos)

Thermo Scientific booth, one of the BrMASS sponsors (Photo: Luciene Campos)

From 7 to 11 December 2013, the 5th BrMASS Conference took place at the Royal Palm Plaza Hotel in Campinas, SP, Brazil. Around one thousand and two hundred people attended this meeting, the 3rd biggest conference in mass spectrometry in the world.

The BrMASS Conference is promoted and coordinated by Prof. Dr. Marcos Nogueira Eberlin, Titular Professor at the Chemical Institute of the State University of Campinas, SP, Brazil, Vice President of the Brazilian Mass Spectrometry Society (BrMASS) and President of the International Mass Spectrometry Foundation, the organization which brings together all the Mass Spectrometry Societies of the world.

"In 2005, I realized that it was necessary to gather all the people interested in using mass spectrometry to discuss collaborative projects and learn about this technique, because people wanted to use it more, but were not sure what it was", said Eberlin.

The event in 2013 had a large and diversified scientific and technical program in which pre-congress courses and lectures were presented by leading names in mass spectrometry, as Raymond E. March, Robert B. Cody, K.W. Michael Siu, Facundo M. Fernandez, Renato Zenobi, among others.

Several pre-congress courses and lectures in parallel sections on the relevant mass spectrometry themes were organized by companies sponsoring the meeting.Thermo Scientific, Waters, AB Sciex, Shimadzu, Bruker, LECO, Peak, Perkin Elmer, Agilent and Allcrom were present at the exhibition area in interesting booths that enriched, even more, the event.

The 5th BrMASS Conference exceeded all expectations from the organizers and exhibitors, mainly in the quantity and quality of the participants.

"The conference's audience is gradually increasing in each edition.This was the fifth edition and the technical-scientific program included thirty courses, user's meetings, and lots of parallel sections "said José Felipe Lugão, from Thermo Scientific.

Moreover, visitors were very impressed with the booths, the exposed materials and the quality of information provided by exhibitors.

All of this may explain the growing success of the BrMASS Conference. The event fully meets the expectations of both researchers and sponsors.

Prof. Eberlin highlighted the dimension of this Conference edition: "The meeting was bigger than expected. Everyone worked to make it better. It was fantastic to see the amount of people who attended this year, all sections were so crowded that it was difficult to walk,"saidEberlin.

Scientific papers

The scientific program attendants presented their research results in mass spectrometry in the Poster Section.

The posters were evaluated by the Scientific Committee and the three best works were awarded at the event closing ceremony.

The best scientific paper done with the Thermo Scientific Orbitrap technology was presented by Gabriela Venturini, a doctorate student at the Heart Institute of the São Paulo University. Her research is on protein biomarkers in atherosclerosis studies. The award for Gabriela was a trip to Bremen, Germany, to attend a mass spectrometry course.

BrMASS Medal

The BrMASS Society has created an award to the researchers who contributed to mass spectrometry.

This award consists of delivering BrMASS medals to the winners. To do so, some names are given by the BrMASS board of directors and then a voting is held to choose three winners.

This time, two winners were Brazilian researchers: Prof. Dr. Norberto Peporine Lopes,Titular Professor at the São Paulo University (USP,RibeirãoPreto), and Prof. Dr. Mario Sergio Palma, Associate Professor at the Paulista State University (UNESP, Rio Claro). Joerg Von Helden, consultant graduated in physics from the University of Mainz, Germany, was the third winner of a BrMASS medal.

The young researcher Norberto Lopes was surprised to be honored: "I'm obviously very happy, but I'm surprised because when we have created this award in the BrMASS Society its main focus was to honor the pioneering researchers," said Lopes.

Norberto Lopes works in Natural Products Chemistry, basically with small molecules.

Noberto Lopes
(source: personal file)

"In my lab we try to understand since the chemical ecology until the medicinal use of the arnica Brazilian plant - arnicas from the mountain. We study several aspects, as pharmaco technical development, formulations, applications, efficiency analysis and chemical ecology," said Lopes.

The researcher Mario Sergio Palma highlighted how the choice of the medal winners is made: "Have the work recognized by our peers is very interesting, especially when we consider the way it is done. Some names are indicated and then the BrMASS Board of Directors evaluates not only one work done, but all the researcher story of life inside the area.Taking into account the importance of the BrMASS Society today, I find extremely relevantto be honored with the BrMASS medal", said Palma.

The laboratory where Mario Palma works is one of the largest labin Brazil.

Mario Sérgio Palma
(source: personal file)

"One of the researches conducted in the laboratory is the study of disease mechanisms, we try to understand how diseases generally occur," said Palma.

According to him, the structures of some types of cancer, such as lung, brain (gliomas) and pancreas cancers are studied in this lab. The goal is to understand how they create a mechanism of resistance to radiotherapy and chemotherapy.

Both Brazilian researchers praise and strongly emphasize the importance of BrMASS Conference to the mass spectrometry.

"The conference brings together researchers from different areas, such as proteomics, natural products, food chemistry and others, which normally I don't find in any other event. Getting together people from completely different areas, but with a common subject, and all interested in the same technique is the main value of the BrMASS Conference, in my point of view," said Lopes.

Mario Palma thinks the BrMASS Conference favors the exchange of experiences with researchers from other countries. "The conference is an opportunity to evaluate and compare what we are doing in Brazil with what is being done in Europe, North America and Asia," he said.

Innovation in Mass Spectrometry

Some of the technological innovations were presented by three large companies that sponsored the BrMASS Conference: Thermo Scientific, Waters and AB Sciex.

Thermo Scientific presented the Orbitrap analyzer which is, according to José Felipe Lugão, Product Manager in Organic Mass Spectrometry (Thermo Scientific / Nova Analítica), an advanced technology, the only effectively new technology for mass spectrometry in the last 25 years.

"The Orbitrap analyzer is really a paradigm shift. It has a different way to obtain the mass spectra and its main benefit is to work with a very high resolution, i.e., with the ability to separate the interfering compounds from the analites in a much easier way,"said Lugão.

Another innovation from Thermo Scientific was the new triple quadrupole LC-MS Series widely used for quantitation. These instruments are used in the monitoring of environmental pollution, particularly water pollution, and in the control ofcontaminants in food.

The big innovation from Waters was the ACQUITY QDa Detector, for everyday applications. According to Luiz Fernando Lopez, General Director at Waters Technologies Brazil, this is an instrument that will bring the mass spectrometry for daily applications without a big financial investment.

Finally, Matthew Goulart Campos, Director at AB Sciex Brazil, highlighted some AB Sciex equipment launched last year, as the triple quadrupole systems and hybrid triple quadrupole-ion trap6500 Series, the TripleTOF® systems, and the new Eksingent HPLC systems.

New BrMASS Directory Board

In the BrMASS Society's meeting at the 5th BrMASS Conference, Prof. Dr. Marcos Eberlin was elected the Executive President of the BrMASS Society and José Manuel Riveros (USP) became the Emeritus President.

See the new BrMASSDirectory and Council Board, at: http://brmass.vdusso.com.br/news/view/317

In another meeting with the Presidents of the Mass Spectrometry Societies from Portugal, Spain, Mexico, Italy, France, Chile, Uruguay and Argentine, a project of an Ibero-American Conference in Mass Spectrometry was presented. This new conference will be organized in conjunction with the 6th BrMASSto be held in 2015.

"With this project, theBrazil's leadership in mass spectrometry will be consolidated in Latin America and among the Iberian countries. This event will allow more and more collaborations and partnerships. It will be the internationalization of mass spectrometry in Brazil," concluded Eberlin.

(Source: Luciene Campos and Lilian Freitas - Visão Fokka – 15/01/2014)

Release

New Evolution 200 UV-Vis Series

Evolution 200 UV-Visible Spectrophotometers

Thermo Fisher Scientific Inc., the world leader in serving science, announces that its Evolution 200 series of next-generation UV-Visible spectrophotometers featuring Thermo Scientific™ Insight™ 2 and CUE (Customized User Environment) software have been named one of the most technologically significant products of the past 12 months. Designed for analyses in the QA/QC, life science, and material science industries, the instruments combine high performance and straightforward software, enabling personalized operation for greater productivity. The Thermo Scientific Evolution 200 series was recognized for the unique nature of the platform for spectroscopy analyses that can be tailored to individual measurements.

Thermo Scientific™ Insight™ 2 software has been designed to improve the user experience by simplifying method creation and results interpretation. The Insight plataform simplify the workflow with automated calculations and result analysis, minimize errors and increase productivity in the laboratory, perform method calculations and analyses. From simple measurements to the most sophisticated research studies, Thermo Scientific™ Insight™ 2 software help the analyst reach answers consistently and with unprecedented speed.

INSIGHT software also includes CUE scripting capabilities, which enables the user to create a dedicated analyzer with a simplified, customized user interface to streamline workflows, enforce proper procedures, and reduce errors. CUE scripts can be locked from further editing, resulting in custom programs that can be run in regulatory environments. This is an industry first.

The Thermo Scientific Evolution 200 instruments feature high quality accessories and application specific technology to meet a wide range of sampling needs, including application focused beam geometry (AFBG) technology that optionally tailors the instrument's optical system to specific applications for microcells, solid sampling and fiber optics. Forward-looking design elements also include a moveable detector, integrated triggering for automation or interaction with other laboratory instruments and a local control module with USB accessory support.

For routine to research analysis, the Evolution 201 and 220 systems offers real innovations that provide enhanced usability and performance - without added complexity. With straightforward software, cutting-edge instrumentation and a wide selection of accessories, the Evolution 201 and 220 work with you to perform experiments the way you want - for the results you need.

The Evolution 260 Bio UV-Vis spectrophotometer was designed for the quickly changing field of life science. With the choice of integrated or computer software control, the Evolution 260 Bio spectrophotometer is always up-to-date and ready for the next challenge. Powerful software, a high-performance spectrophotometer, and an extensive line of accessories combine for a complete solution to move you from samples to answers faster. Thermo Scientific™ Insight™ 2, included with the Evolution 260 Bio system, offers pre-programmed assay methods for increased accuracy and convenience.

For additional information please contact **revista@novanalitica.com.br or visit analiticaweb.com.br**

New Acid Purification Apparatus BSB-939-IR

New Acid Purification Apparatus BSB-939-IR

Nova Analitica presents the acid purification sub-boiling apparatus **BSB-939-IR** from Berghof Products + Instruments, DE. This unique sub-boiling system is employed to produce high-purity acids for use in trace analysis by acid distillation. The acid is always fresh and is therefore guaranteed to be of the desired purity. Contaminated acids can be re-cleaned.

Contact-free heating of the acids by means of an infrared lamp allows equilibrium between the absorbed IR radiation and the liquid's evaporation heat to be established. This equilibrium state is reached at approx. 10°C below the individual acid's boiling point. This allows the acid to evaporate slowly for a gentle distillation.

To minimize corrosion risk and to guarantee high quality of purified acids the corrosion risk has to be minimized. Therefore, the only materials used are plastics (PP, TFM™-PTFE, PTFE, PFA), ensuring that the possibility of corrosion is completely excluded even during long term operation. The distillate comes into contact solely with ultrapure PFA.

All low boiling acids as HF, HNO3 and HCl are suitable for distillation. It is also possible to produce high purity water very easily. Within 24 hours distillation quantities of 1 - 2 liters can be obtained. Generally, the attainable purity grade is significantly better than <1 ppb per element when starting with p.a. purity grade. Multiple distillations will produce still higher purity grades.

By cleaning more economical low purity acids, you can save up to 90% of the cost of analytically pure acids. As a rule, the system therefore pays for itself within the first year.

For additional information please contact
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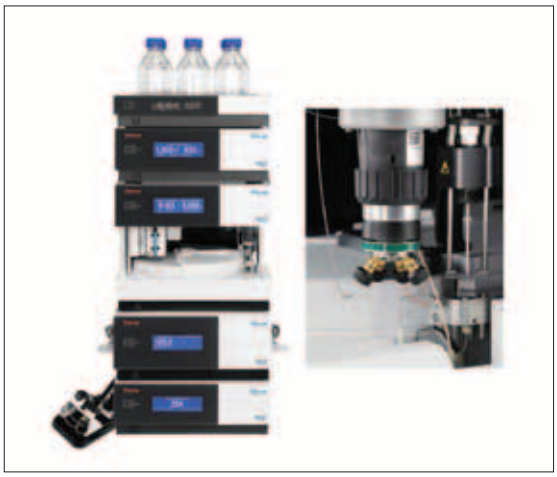

Thermo Scientific™ Dionex™ UltiMate™ 3000 BioRS system

Genuine Bio UHPLC for biomolecule analysis

Ultra high performance liquid chromatography (UHPLC) provides improved separation speed, throughput, and sensitivity by employing stationary phase particles of around 2 µm or smaller. UHPLC has found widespread use in the analysis of small molecules in pharmaceutical, food, and environmental areas.

As of today the analytical benefits where not so applicable to the separation of larger molecules, such as proteins and peptides. Biomolecules have, apart from their size, other differences from small molecules (e.g. charges, complex structures, etc) that make the application of UHPLC not as straightforward as for small molecules. Apart from smaller particles, special solvents and separation principles beyond reversed phase also need to be supported

The Thermo Scientific™ Dionex™ UltiMate™ 3000 BioRS system represents a platform that is designed with multiple modules to allow configuration for in depth multidimensional analysis or high throughput quality control. The extensive column portfolio extends UHPLC capabilities beyond reversed phase by offering IEX or glycan separation materials in smaller particles and longer columns. Additionally, a ready-to-use buffer set is available to perform pH gradient charge variant analysis. The buffer system allows application of linear gradients to achieve a linear pH profile.

With all these capabilities, Thermo Scientific™ Dionex™ UltiMate™ 3000 BioRS is the system that will work in development, characterization, and QC:

- Optimizing Analysis Time and Resolution for Peptide Mapping
- Method speed up for Protein Charge Variant Analysis using IEX
- Proteins Charge Variant Analysis with pH Gradients
- Glycan Separation Resolution when coupling LC to a Mass Spectrometer

For more information:
Contact: Marc Yves Chalom
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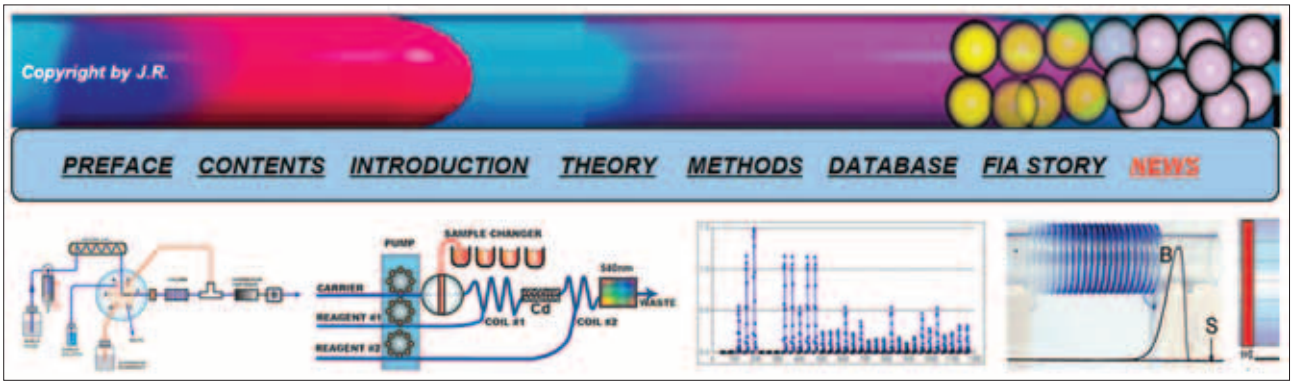

Jarda Ruzicka
Databased by **E.H. Hansen**
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Flow injection analysis

The 5th edition of The Tutorial, now accessible on line comprises. **Preface**, discussing revolution taking place in scientific publishing. **Introduction**, reviews development of methods of flow analysis, beginning with air segmented systems, focuses on flow programming, and concludes with high throughput Sequential Injection technique. **Theory** deals with mixing by dispersion in flow system, emphasizing the essential role of the turbulent flow. **Methods** section deals with Flow Injection, Sequential Injection, Bead Injection and Sequential Injection Chromatography, illustrated by examples of work from world leaders in the field. **FIA Story** is collection of contributions written by those, who participated in inventing, advancing and commercialization of Flow Injection and related techniques. This multicolor quilt of stories is the heritage from which much is to be learned about science, discovery and commerce. **Database** contains over 22000 instantly searchable references. **News** section comprises announcements of forthcoming meetings and publications from the field of analytical chemistry. Contributions related to flow analysis and instrumentation are cordially invited, and will be published free of charge, with the exception of commercials. The website is financially supported by the author.

For additional information please contact
http://www.flowinjectiontutorial.com/

Events 2014

XLIII SBBq
Reunião Anual da Sociedade Brasileira de Bioquímica e Biologia Molecular
Foz do Iguaçu, PR – May 17-20, 2014
http://www.sbbq.org.br/

37ª RASBQ
37ª Reunião Anual da Sociedade Brasileira de Química
Natal, RN – May 26-29, 2014
http://boletim.sbq.org.br/boletim/1077.php

SIMPEQUI
12º Simpósio Brasileiro de Educação Química
Fortaleza, CE - August 6-8, 2014
http://www.abq.org.br/simpequi/

ENTEQUI
7º Encontro Nacional de Tecnologia Química
Vitoria, ES - September 17-19, 2014
http://www.abq.org.br/entequi/

CBQ
54º Congresso Brasileiro de Qiímica
Natal, RN – November 3-7, 2014
http://www.abq.org.br/cbq/

BrazMedChem2014
7º Simpósio Brasileiro em Química Medicinal
Campos do Jordão, SP – November 9-12, 2014
http://brazmedchem.iqsc.usp.br/2014/index.php?lang=pt_BR

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The Introduction must include important references, concerning both the nature of the problem being investigated and its background. The manuscript must include Introduction, Experiment, Results/Discussion, Conclusion and Reference and should not exceed 25 pages, including tables, figures and diagrams. All pages must be numbered. Authors should indicate, by text or marginal notation in the type script where the figures and tables are to be inserted. All papers must be typed with double spacing using Microsoft Word only, preferably at 12 pt but no smaller than 10 pt. A single file must be generated in the Portable Document Format (pdf) including the entire article, to be sent via online through ***BrJAC´s*** site: www. brjac.com.br.

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3rd page: abstract with a maximum of 250 (two hundred and fifty) words; it must include the work´s objective, essential data of the methods employed, and the main results and conclusions. Next, list the main terms or words to be used for bibliographic classification (keywords).

4th page: begin the text on the fourth page with the title of the article, without indicating the author(s) or the place where the work was performed.

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2. Chistyvst, L.K.; Andrenev, I.D.; Karol, F.W.; Cyrus, G.H.; *Zabrousk. Tacsh. Wilheim. Lyris.; Huil. Garm. Visijak.* 1987, 28, 816. *(Bl 97:89745s).*
   - If the paper does not have a full reference, please mention doi as follows:
3. Striver, J.; Costa, T.C.; Pial, Q.P.; Temiza, V.L.; Vargas, V.N.; *Metalochimica Acta* (2004), doi:20.4598/v. metalacta.2006.15.023.
   - We recommend the use of compound references, whenever possible, instead of individual references, following the style below:
4. Varela, H.; Torresi, R.M.; J. *Eletrochem. Soc.* 2000, 147, 775; Lemos, T.L.G.; Andrade, C.H.S.; Guimaraes, A.M.; Wolter-Filho, W.; Braz-Filho, R.; *J. Braz. Chem. Soc.* 1996, 7, 123; Angelo, A.C.D.; de Souza, A.; Morgon, N.H.; Sambrano, J.R.; *Quim. Nova* 2001, 24, 473.

- ***Books:*** name(s) of author(s): surname and initial(s); title of book (initial letters in capital letters), volume (if a series), edition (if not 1st); city where edited; publisher; year of publication and number(s) of chapter or of page(s) cited.

5. Cotton, F. A.; Wilkinson, G.; *Advanced Inorganic Chemistry*, 5th ed.; Wiley: New York, 1988.

- ***Edited book:*** name(s) of author(s), "in" title of book (initial letters in capital letters); name(s) of editor(s); city where edited; publisher; year of publication and number(s) of chapter or of page(s) cited.

6. Regitz, M. in *Multiple Bonds and Low Coordination in Phosphorus Chemistry;* Regitz, M.; Sherer, O.J., eds.; George Thieme Ver-

lag: Stuttgart, 1990, Chap. 2.

- ***Patents:*** Please inform the *Chemical Abstracts* number in parentheses:

7. Bash, T.; Dot, K.; Petersen, G.; Orbfield, B.; Cooper, D.; Sabbag, L.; Fce. *Le Kokai Tokkyo Koho 54 95,489* 1975. *(CA 233:P179341v)*
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- ***Software:***

9. Sheldrick, G. M.; SHELXL-93; *Program for Crystal Structure Refinement,* Gottingen University, Germany, 1993.

- ***Theses:***

10. Velandia, J. R.; *Doctorate Thesis*, Universidade Federal Rural do Rio de Janeiro, Brazil, 1997.

- ***Material presented in Congresses:***

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- ***Internet pages:***

12. http://www.sbq.org.br/jbcs, accessed June 2001.

- ***Unpublished material:***

- Material accepted for publication

13. Magalhaes, U. H.; *J. Braz. Chem. Soc.,* in press.

- Material submitted for publication, awaiting approval

14. Magalhaes, U. H.; *J. Braz. Chem. Soc.,* submitted for publication.

- Unpublished material

15. Magalhaes, U. H.; unpublished paper.

- Material from personal communication

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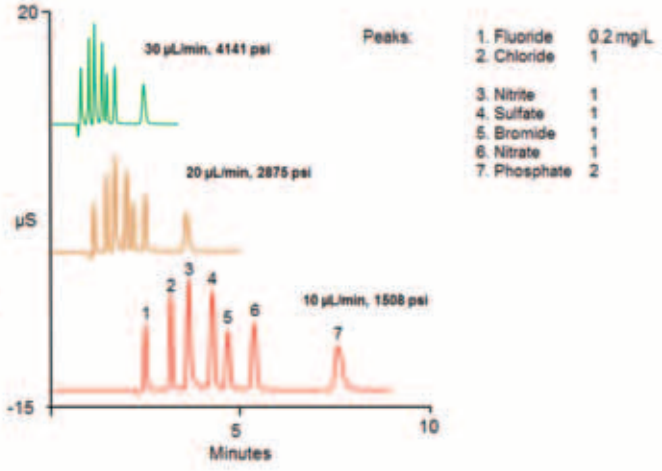

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