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2º CONGRESSO ANALITICA LATIN AMERICA

20 A 22 setembro 2011

Transamerica Expo Center
São Paulo - SP / Brasil

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Participe do 2º Congresso Analitica Latin America

Se aproxima a data para a realização do 2º Congresso Analitica Latin America 2011 e da feira Analitica Latin America, dois renomados eventos para a interação do meio acadêmico com o setor industrial.

O 2º Congresso Analitica trará palestras, simpósios e mesas redondas, além de área para exposição de painéis, contemplando pesquisas em universidades, centros de pesquisas e indústrias como forma de disseminar informações e incrementar as interações acadêmico-industriais. Nesta edição do congresso, pela segunda vez consecutiva, foi aberta a inscrição para envio dos trabalhos científicos. Todos os trabalhos enviados estão sendo analisados e no dia 15 de agosto os aprovados serão divulgados para exposição durante os dias 20 e 21 de setembro, em espaço reservado na feira Analitica Latin America. O autor do melhor trabalho receberá como prêmio uma viagem com tudo pago para a Pittcon 2012, o segundo lugar receberá uma inscrição para o Enqa 2011 e hospedagem para o período do evento em Campos do Jordão e o terceiro lugar, uma inscrição para o Enqa 2011. Os três ganhadores receberão ainda um ano de cortesia da revista BrJac – Brazilian Journal of Analytical Chemistry.

Simultaneamente ao congresso acontecerá a 11ª edição da feira Analitica Latin America – Feira Internacional de tecnologia para laboratórios, análises, biotecnologia e controle de qualidade. Evento que em sua última edição reuniu cerca de 10.000 visitantes e 500 marcas em exposição.

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Evento Paralelo: 11ª EDIÇÃO analitica LATIN AMERICA – FEIRA INTERNACIONAL DE TECNOLOGIA PARA LABORATÓRIOS, ANÁLISES, BIOTECNOLOGIA E CONTROLE DE QUALIDADE

Apoio: AIQ 2011 Ano Internacional da Química; Sociedade Brasileira de Química; International Year of Chemistry 2011; CRQ-IV; abc associação brasileira de cosmetologia; ABIQUIM Associação Brasileira da Indústria Química; Sociedade Brasileira de Bioquímica e Biologia Molecular – SBBq; Ministério da Ciência e Tecnologia; GOVERNO FEDERAL BRASIL PAÍS RICO É PAÍS SEM POBREZA

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Brazilian Journal of Analytical Chemistry

DKK Comunicação
January / February / March 2011

ERRATUM

DA SILVA, W.T.L.; THOBIE-GAUTIER, C.; ELMURR, N.; REZENDE, M.O.O. Humic acid-metal ions affinity through modified carbon paste electrode. Study by anodic stripping voltammetry. Brazilian Journal of Analytical Chemistry, v. 1, p. 104-109, 2011.

TABLE I
Table I: Characteristics of HA from peat utilized in this Study.
Change for
Table I: Characteristics of HA from peat utilized in this Study. Adapted from Da Silva et al., 2002 [8].

FIGURE 4
Figure 4: Anodic Stripping voltammetry of the Cu adsorbed on CPE-HA surface. Scan rate: (a) 5 mV s-1 and (b) 100 mV s-1.
Change for
Figure 4: Anodic Stripping voltammetry of the Cu adsorbed on CPE-HA surface. Scan rate: (a) 5 mV s-1 and (b) 100 mV s-1. Thobie-Gautier et al., 2003 [2], with permission of the publisher.

Editorial

Toward the growth

Analytical Chemistry is a multidisciplinary scientific research area by nature. Qualitative and quantitative information of chemical species present in a given material are important for any field of scientific knowledge, such as technological, commercial, industrial, etc. However, the modern life has forced the development of analytical techniques and methods to solve new challenges and for this the meanings of the qualitative and quantitative terms suffered transformations that reflect the scientific knowledge and technological progress. While techniques and classical methods require an approach more based on fundamental chemistry, the current necessities to know the chemical species in which analytes are present in a sample and the increasingly necessity for determinations at low concentration in complex samples require a more comprehensive advance, which extrapolates the lab environment. Over the years, instrumentation, automation, electronics, computing, mathematics, chemometrics and new materials have became important areas of development in Analytical Chemistry. This wider vision encompasses the search for qualitative and quantitative answers, however, incorporating and making use of inter and multidisciplinary expertise, able to enhance its efficiency in the most different aspects, such as accuracy, precision and speed. Within this new vision, Analytical Chemistry and its technologies are addressing the challenges in the various social and economic sectors in Brazil (e.g. environment, new materials and nanotechnology, arrays of biological interest, foods, pharmaceuticals, agrochemicals and quality control of products and industrial processes). In this way, we would like to invite you to send your contributions to BrJAC, which will improve the scientific relationship between academy and industrial sector and will transform the dream of this periodic in a reality. In the issue number 3 we are presenting an Interview with Prof. Ramon Murray Barnes (University Research Institute for Analytical Chemistry, USA), who believes in the Brazilian Analytical Chemistry and re-edition of two paper published in the launching issue.

Pedro Vitoriano de Oliveira
Editor

O Encontro da Tecnologia Analítica na América Latina

11ª EDIÇÃO

analitica
LATIN AMERICA

FEIRA INTERNACIONAL DE TECNOLOGIA PARA LABORATÓRIOS, ANÁLISES, BIOTECNOLOGIA E CONTROLE DE QUALIDADE

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O evento é o local de encontro com os principais fornecedores e compradores dos setores de tecnologia laboratorial, biotecnologia e controle de qualidade. Na última edição o evento contou com **500 marcas** em exposição e **10 mil visitantes** de mais de **20 países**.

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Summary

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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!

Some challenges for analytical chemistry in Brazil

The *Zeitschrift für analytische Chemie* (also known as the *Fresenius' Zeitschrift für Analytische Chemie* or *Fresenius' Journal of Analytical Chemistry*), founded in 1862, was the world's first journal entirely devoted to analytical chemistry and many of Fresenius's original papers were published in this journal. On 14 December 2001, this German journal and other two journals, Analusis and Química Analítica, became Analytical and Bioanalytical Chemistry. In 1876, the Society for Analytical Chemistry of the Royal Society of Chemistry published the first issue of the *Analyst* journal. In the United States, *the Journal of Analytical Chemistry* was founded in 1887, and merged with the *Journal of the American Chemical Society* in 1893. Another journal of analytical chemistry was started in 1929 (the *Analytical Edition of Industrial and Engineering Chemistry*) and, in 1948, a new name was adopted and it was renamed Analytical Chemistry. The Russian periodical publication *Zhurnal Analiticheskoi Khimii* (in English as *Journal of Analytical Chemistry*) has been published in Moscow since 1946. The histories of some journals and chemical societies devoted to analytical chemistry, their importance in the development of this area in their respective countries and interdisciplinary cooperation between researchers in the field have been discussed elsewhere[1].

According to the International Monetary Fund (IMF)[2], Brazil ranks as the seventh largest economy in the world with gross domestic product (GDP) of roughly US$ 2 trillion. The research in chemistry occupies the 16th place[3] and in analytical chemistry the 12th place with 4367 indexed articles published in the period 1996-2009, indicating that the field of analytical chemistry has evolved significantly and, among other fields, comprises the most prominent field. However, we can go much further and continue to invest in research institutions focused on priority areas and aimed at resolving national issues. Across the whole field of chemistry there are many paths open for chemists to design and develop studies and/or synthesis of various pharmaceuticals, biotechnology, biotherapy discovery, vaccine development, purification and characterization of biological drugs, clinical and toxicological studies of substances. Moreover, we cannot forget that the Earth's climate has experienced profound changes caused by mankind (anthropogenic effects). So, we need to develop clean (green) technologies, methods of treatment and/or reduction of waste in all processes encompassing aspects of chemistry. In addition to the oil industry (black gold), we need to work on the development of alternative resources as sources of clean energy. The Brazilian ores can be used as a strategy for the development of industries as well as in the process technologies (e.g. electronic industry). We need to develop new technologies to create several products, and not simply sell our mineral wealth in raw form, without adding value. Analytical Chemistry will be of great value for all these areas for the formulation of appropriate methods for each of these studies. This is a great and unique moment for Brazil and the Brazilian Journal of Analytical Chemistry (BrJAC) will be of paramount importance in the congregation of analytical chemists working in academy, companies and research centers for solving local problems and conducting high-quality and innovative investigations. Finally, I would like to thank you very much for giving me the opportunity to participate in this important task and please pass on my compliments to all involved.

References
1. C. A. Russell; K. Roberts, *Chemical History Reviews of the Recent Literature*, RSC, London, 2005.
2. http://en.wikipedia.org/wiki/List_of_countries_by_GDP_(nominal)
3. SCImago Journal & Country Rank

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BRAZILIAN ACHIEVEMENTS AND FRONTIERS IN ANALYTICAL CHEMISTRY

The International Year of Chemistry, 2011, is a good opportunity to discuss what achievements of analytical chemistry have benefited humankind, both in daily living and health. For this, the Brazilian Journal of Analytical Chemistry invited Professor Ramon Barnes to tell us about our last conquers and what we, Brazilians, still should work on to foster analytical chemistry and spectrochemistry nationally.

Ramon Barnes is coming for a Plenary Conference in the 2nd Analitica Latin America Congress, to be held in September 20 to 22. Professor Barnes is an old friend of Brazil: he has been coming to our country for more than two decades to meet with Brazilian researchers, and thus, he speaks knowingly. The conversation ranged from a critical view of the analytical chemistry research in Brazil to the future steps we should take to broaden and strengthen the relationship with industry. His counsel is precious.

Barnes received a Ph.D. in analytical chemistry from the University of Illinois, in 1966, and he was a postdoctoral research fellow at Iowa State University, in 1968 and 1969. From 1969 to 2000 he taught analytical chemistry and maintained an international research program at the University of Massachusetts (UMASS Amherst), where he is now Professor Emeritus. He has published more than 300 papers, edited four books, and continues to have an active interest in fundamentals and applications of inductively coupled plasma (ICP) discharges for spectrochemical analysis. Professor Barnes is the chairman of the Winter Conference on Plasma Spectrochemistry, held since 1980.

Professor Barnes is also a director of the University Research Institute for Analytical Chemistry, (URIAC) which is a non-profit corporation that fosters science education, research, and study in spectroanalytical chemistry. The research institute provides specialty plasma spectrochemical analysis, development of methods, training, consulting, and applied research. Its areas of work are ICP atomic emission spectrometry and ICP mass spectrometry for ultratrace metal and stable isotope analyses in environmental, forensic, drug development, medicine, public health, and semiconductor manufacturing. Through URIAC, Professor Barnes has edited the ICP Information Newsletter, since 1975, one of the first science journals in the area.

What is your impression about the lay public's recognition and appreciation of chemistry research in your country? Do you think that the lay (non-scientist) do know and recognize the advances in the field? Could you give some examples of the contributions of the analytical chemistry to our life?

In general I believe that the lay public is unaware of chemical research, its advances, and contributions/achievements that have benefited mankind. Only a natural disaster, like an earthquake, oil spill, pipeline rupture, or a mine explosion, a chemical or nuclear plant disaster, a drug abuse situation, or a terrorism threat push chemistry into media headlines and attract public attention.

Public recognition and awareness lags scientific discovery until popular media, like TV programs such as the CSI-type, legislation and government regulations (e.g., "heavy metal" poisoning, like lead in paint/soil; arsenic and its organic forms in fish and food, or mercury and its organic forms in sediments), or catastrophes such as the recent disaster in Japan force attention and highlight environmental, food, or drug contamination, for example. Otherwise, commercial products based on chemical discoveries like lithium ion batteries in hybrid cars, cheap photovoltaic solar cells, computer chips based on ultrapure, high-density semiconductors, high-contrast MRI reagents, and advanced clinical testing, are accepted without much public attention to the underlying chemistry and especially analytical chemistry required for their production.

Analytical chemistry, unlike many other branches of the chemistry, is very much involved with the everyday functioning of our society. From the development and characterization of dyes and polymers in the 19th and 20th centuries to the current refinement of semiconductor reagents and their process control, analytical chemistry has played a critical role. Almost every commercial product relies on chemical analysis to guarantee purity and to meet critical specifications. Importing/exporting natural and man-made materials also relies on analytical chemical methods to establish composition, identify contamination sources, and verify origin (provenance).

In summary analytical chemistry is a creative science essential for sustainability and improvements to our way of life. It has made our foods and water cleaner, our workplace and external environment healthier, our health and well being better, our commercial products, energy generation, and transportation more reliable and safer, and our life/entertainment more pleasurable. Analytical chemical research is critical for solving our most vexing global problems involving energy, food, health, materials, water, and more.

"Only a natural disaster, like an earthquake, oil spill, pipeline rupture, or a mine explosion, a chemical or nuclear plant disaster, a drug abuse situation, or a terrorism threat push chemistry into media headlines and attract public attention."

What achievements you believe were the most important in analytical chemistry research worldwide recently? What were the milestones?

Milestone achievements in analytical chemistry have been considered by Warner, Voress and Lee in their book*, in 1994, and include many instrumental innovations (e.g., plasma spectrochemistry), measurement principles and methodology developments (e.g., species-specific and non-specific isotope dilution analysis), data capturing and treatment innovations (e.g., chemometrics), and new chemical reagents and their reactions and applications (e.g., element-tagging antibodies for indirect detection of specific proteins; labelled antibodies for an ICP-MS-linked immunoassay).

Typically the theory of modern analytical techniques

[*] To read more: "Milestones in Analytical Chemistry," Mary Warner, Louise Voress, Grace K. Lee, Felicia Wach, Deborah Noble (eds.), ISBN 0-8412-2855-8, 350 pages, ACS Publication, Washington, DC (1994).

has developed before the hardware, and often as much as a decade can pass between discovery and commercialization. After more than 40 years the inductively coupled plasma (ICP) has set new standards for accurate and precise measurements of elements, isotopes, and metal-containing compounds in agrochemicals, forensics, manufacturing electronics, medicine, metallomics, and nanotechnology. Combined with laser ablation, ICP emission and mass spectroscopies have revolutionized biogeosciences, geochemistry, paleoceanography, sclerochronology, and medical imaging. Remote sensing with laser-induced breakdown spectroscopy has moved elemental analysis from the laboratory to Mars. Ambient desorption/ionization mass spectrometry has stimulated the direct mass analysis of solid, liquid, and gaseous samples. Applications of glow discharges have introduced quantitative surface profiling and novel imaging capabilities of various advanced materials.

In the next years, what is the main target to the analytical chemists in your point of view?

In the near future, analytical chemists will continue their quest for new measurement theory, novel instrumentation, quantitative applications of new physical and chemical phenomena (e.g., nanomaterials), and improved performance (e.g., lower limits of detection for ultra-trace quantities, improved accuracy, species-specific and interference-free determinations, selectivity, specificity). For instance, Freddy Adams* suggested that the milestones of detection limits will decrease from the attogram (ag, 10^{-18} g) level in the 1990's, and zeptogram (zg, 10^{-21} g) levels in the 2000's to yoctogram (yg, 10^{-24} g) levels in the 2010's, for example, with ultra-sensitive nanotube mass sensors providing single yg mass resolution at room temperature. Another trend is micro total analysis systems (μTSA) wherein a chip-size microdevice (i.e., lab-on-a-chip) automates and includes all necessary steps for chemical analysis of a sample (e.g., sampling, sample transport, filtration, dilution, chemical reactions, separation and detection).

"The main challenges is to achieve and then maintain an international level of analytical chemistry research coupled with industrial adoption and support of new analytical chemistry methodology as well as appropriate national legislation for consumer and environmental protection and safety."

We are aware of your big interest in Brazil and we appreciate the many contributions you brought to us. What do you think are the most important research lines here and what would be the best contribution of Brazilian researchers to analytical chemistry?

Analytical chemistry in Brazil has grown remarkable during the past decade from a few, very prominent institutions and researchers, to numerous educational and research programs in new federal and state universities. Brazilian analytical chemistry has a well-established reputation, and its heritage is apparent in these active research programs, Brazilian and international conference presentations, and literature publications in respected journals. For example, Ferreira et al.** reviewed current developments in Brazilian analytical chemistry (circa 2004 - 2008) including automation, chemometrics, chromatography, electrochemistry, environmental analysis, sample treatment, and spectrochemistry, and they highlighted contributions from the major Brazilian groups.

What appears to me as remarkable is that some former-student authors in these reviewed papers have since developed their own very productive research

[*] To see a presentation about detection limits in analytical chemistry, visit: http://www.udias.be/userfiles/file/adams_udias312.pdf.

[**] To access the studies by Fatibello-Filho et al.. and Ferreira et al.. cited by professor Barnes, please visit: www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-40422002000800010 and http://jbcs.sbq.org.br/jbcs/2009/vol20_n10/01-09765RV.pdf.

programs and groups. Subsequently, Rocha et al.* surveyed recent Brazilian work (circa 2008-2009) for the analysis of foods, fuels, materials, pharmaceuticals, biological and environmental samples, as well as chemometrics, instrumentation and automation, and they cited more than 300 references. The authors concluded, "analytical chemistry in Brazil is healthy and growing, and is ready to support the new role of the country as an emerged power." I cannot agree more strongly with this prediction.

What are the windows of opportunity, unexplored fields in analytical chemistry in Brazil? What areas should we lay our eyes on? What is the main challenge?

It appears to me that active research fields in chemometrics, electrochemistry, separation sciences, inorganic and organic spectrochemical analysis, and sample treatment, among others are now topics considered by Brazilian research teams. Application areas including advanced materials, bioanalytical chemistry and metallomics, biotechnology and biofuels, forensics, petroleum materials, and pharmaceuticals, natural products (nutraceuticals/dietary supplements) and their metabolites have joined important areas of consumer and environmental protection as key development fields in Brazil.

The biennial Brazilian analytical chemistry meeting (Encontro Nacional de Química Analitica) and recent special-topics meetings on forensics and speciation feature these areas. Brazil is a country of remarkable natural terrestrial and marine resources and analytical chemistry opportunities. The main challenges is to achieve and then maintain an international level of analytical chemistry research coupled with industrial adoption and support of new analytical chemistry methodology as well as appropriate national legislation for consumer and environmental protection and safety. From the practical viewpoint, Brazilian researchers still lack some of the most advanced tools of plasma spectrochemistry, and perhaps a consortium of universities and research institutes ought to be established to create and maintain a high-resolution, multi-collector, ICP mass spectrometry facility, for example, for geochronology, mineral provenance, and paleoenvironmental and medical research.

"Universities appear to me to be relatively slow to recognize the value of a systematic patent program among academic researchers, and they should be encouraged to organize intellectual property offices."

Brazilian scientific production in the last decade has grown compared to the 1990s. Partnerships with international scientific centers have helped a lot in this. However, the number of patent registries is far from being similar from those of other countries. What do you think would be the reason that Brazilian research does not result in products? How does this apply to analytical chemistry?

The commercial side of an economic development typically drives patent applications, and the number of patents reflects industrial and governmental research and development supplemented by an appropriate academic intellectual protection program. As original research and development increase in Brazil, the number of patents will follow. Universities appear to me to be relatively slow to recognize the value of a systematic patent program among academic researchers, and they should be encouraged to organize intellectual property offices. Government patent agencies also should make applications convenient and expedite application processing. A variety of analytical chemistry concepts, measurement approaches and techniques, instruments, and processes are potentially patentable, and the opportunity to safeguard Brazilian innovations should not be overlooked.

[*] To acces this article, please visit: http://jbcs.sbq.org.br/jbcs/2009/vol20_n10/01-09765RV.pdf.

In your opinion, what is the future of the spectroscopic techniques? And what is the main challenge in terms of the scientific development at the moment in this area?

Spectroscopy measures the interaction of the atoms and molecules with electromagnetic radiation, and it consists of many different atomic and molecular techniques such as atomic absorption spectrophotometry, atomic emission spectroscopy, ultraviolet-visible spectrophotometry, x-ray fluorescence spectroscopy, infrared spectroscopy, Raman spectroscopy, Fourier transform interferometry, mass spectrometry, nuclear magnetic resonance spectroscopy, photoelectron spectroscopy, Mössbauer spectroscopy, etc. Without doubt most of these spectroscopic techniques will continue their mainline role in analytical chemistry. Major developments in laser spectroscopy and inorganic and organic mass spectrometry are expected to show the biggest growth, I suspect. Research areas of particular interest include biotransformation and speciation of nanoparticles, chemical speciation in ecotoxicology, new speciation reference materials, metalloproteomics and health aspects of transgenic food crops, to name a few.

"A critical need exists for more analytical chemistry education and research in universities and other educational institutions worldwide. "

A critical need exists for more analytical chemistry education and research in universities and other educational institutions worldwide. Owing to Brazil's potential growth, more formal programs and increased funding in analytical chemistry for departments, endowed chairs, sponsored lectureships are required to maintain the pool of professional analytical chemists for Brazil's emerging industries, academic research, and consumer protection. Brazilian analytical chemists currently collaborate with colleagues in Austria, Canada, France, Germany, Italy, the United States, the United Kingdom, and others. This collaboration should be encouraged, and enhanced international postgraduate student scholarships and scholar exchanges as well as joint international projects should be funded. They will enrich the educational experience, strengthen training in new technologies and theory, and built the critical mass in analytical chemistry to match Brazil's future.

What is your impression about the Brazilian analytical spectroscopy? What are the main contributions from Brazilian researchers in this area?

The Brazilian community of academic, government, and industrial research involved in spectrochemical analysis is substantial. Numerous contributions are reported at international spectroscopy meetings, and increasing number of papers from Brazilian authors are appearing in international journals. An example is the XXXVII Colloquium Spectroscopicum Internationale (CSI) that will be held in Armação de Búzios, Brazil, August 28 to September 02, 2011*. This is the first time that this important international meeting will convene in Latin America. The topics include atomic plasma spectrometry, molecular spectrometry, X-ray spectrometry, hyphenated techniques, laser spectroscopy, imaging techniques, nuclear techniques, gamma spectroscopy, material sciences, environmental and geochemical analysis, archaeometry and cultural heritage, biological applications, organic and inorganic mass spectrometry, food analysis, clinical and pharmaceutical analysis, and speciation analysis. Based upon Brazilian participation in the recent biennial Rio Symposium on Atomic Spectrometry, initiated in Brazil in 1988, I expect a substantial contribution in each of these areas from Brazilian colleagues.

[*] To see details about the event, visit: http://csixxxvii.org/.

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Chlorine determination in crude oil fractions after digestion using microwave-induced combustion

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Abstract
In this study, a procedure for chlorine determination by ion chromatography (IC) in crude oil fractions (atmospheric distillation residue and gas oil) was proposed after digestion using microwave-induced combustion (MIC). The proposed procedure by MIC was performed in closed quartz vessels under oxygen pressure (20 bar) and using 50 μl of 6 mol l^{-1} NH_4NO_3 for the ignition step. Some parameters of the combustion process were evaluated, as the kind and the suitability of the absorbing solution (H_2O, $(NH_4)_2CO_3$ and NH_4OH), oxygen pressure and sample mass. Certified reference materials (CRM) and spiked samples were used to evaluate the accuracy. The agreement using CRM was higher than 97% and the recoveries using reflux step were in the range of 98 to 102% using 25 $mmol^{-1}$ NH_4OH as absorbing solution. For results comparison, Cl was also determined by ICP-MS and no statistical difference was observed in comparison with results obtained by IC. The limit of detection (LOD, 3σ) for Cl obtained by IC and ICP-MS was 1.2 and 6.6 μg g^{-1}, respectively. The residual carbon content in digests obtained after MIC procedure was lower than 1%. Using the proposed procedure sample digestion was complete in less than 30 min and up to eight samples could be digested that is an important aspect for routine analysis.

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Keywords: chlorine, microwave-induced combustion, atmospheric residue, gas oil, crude oil fractions.

1. Introduction

Crude oil is composed of a mixture of hydrocarbons with a variable amount of sulfur, nitrogen, oxygen, metals, water and salt (generally as NaCl).[1,2] In order to obtain petroleum fractions with higher commercial value, as kerosene, diesel and fuel oils, crude oil is distilled. However, crude oil must contain a low salt concentration, in general lower than 50 mg l^{-1} to allow its use in refineries.[3] Even after treatment for salt removal before the refining process, variable amounts of salt commonly remains in treated crude oil increasing the risk of corrosion during processing and also resulting in changes of quality for some products, as petroleum coke, atmospheric and vacuum residue of crude oil distillation. Therefore, the determination of chlorine must be performed in routine analysis in crude oil refining products due to their important role on the quality of crude oil.[4,5]

The gas oil fraction could be obtained from atmospheric and vacuum distillation units (atmospheric and vacuum gas oil, respectively).[4] The main use of gas oil is as fuel for diesel engines and as feedstock for craking and hydrocracking units. After atmospheric distillation, a residue is obtained (commonly named as atmospheric residue, RAT) and it is normally used as feed to the vacuum distillation unit, catalytic cracking or steam cracking process.[2,4]

The processing of crude oil residues has gained interest due to depletion of the light crude oils reserves. Heavy crude oils yield more high-boiling temperature residues, such as atmospheric and vacuum residues, which need to be refined to yield better commercial value of products.[6] In this sense, heavier fractions of crude oil, as RAT and gas oil, are now being explored as potential feedstock for conversion process in petroleum industry and for this purpose it is necessary to control the presence of impurities in this kind of material, especially chlorine.[1,7] On the other hand, literature data are very scarce concerning the atmospheric residue or gas oil digestion for further chlorine determination. There are only few works related to characterization or other analytes determination in gas

oil, as S, Ni and V [8-10] and crude oil fractions [11-13]. However, chlorine determination in this kind of samples was not currently described in literature.

Significant improvement has been achieved in sample digestion techniques over the last years mainly due to the development of microwave-assisted digestion in pressurized vessels.[14,15] The use of conventional techniques based on microwave-assisted acid digestion presents some drawbacks for some matrices despite the spread and successful application to many other samples. In general, the relatively high stability of some organic materials (as, e.g., crude oil fractions) impairs an efficient and complete digestion.[16] In addition, the use of concentrated acids presents some problems for further chlorine determination, as possible analyte losses even using closed vessels for sample digestion [17,18] and interferences in the determination step by ion chromatography (IC).[19-21]

In this sense, microwave-induced combustion (MIC) was proposed in the last years for organic samples digestion based on the sample combustion in closed vessels pressurized with oxygen after ignition by microwave radiation. After combustion, analytes can be absorbed in a suitable solution.[16] This method was successfully applied for decomposition of biological samples [22], elastomers [23,24], carbon nanotubes [25] and fossil fuel samples, as coal [26], petroleum coke [27,28], heavy crude oil [17,29] and its derivates [10,30] using diluted solutions for most of applications. Concerning halogens determination, alkaline media or even water could be used as absorbing solution with good recoveries.[27,29]

In this work, microwave-induced combustion is proposed for the first time for atmospheric residue and gas oil digestion for further chlorine determination by IC. For results comparison, Cl determination was also performed by inductively coupled plasma mass spectrometry (ICP-MS). Parameters related to the influence of sample mass, maximum pressure achieved during the combustion process and oxygen pressure were investigated. The type (H_2O, $(NH_4)_2CO_3$ and NH_4OH) and the concentration of absorbing solutions were also investigated. As no certified reference materials (CRM) are available for chlorine in a similar matrix, a CRM of coking coal and fuel oil were used to evaluate the accuracy and spiked samples were also performed.

2. Experimental

2.1. Instrumentation

A multiwave 3000 microwave sample preparation system [31] equipped with high pressure quartz vessels was used for MIC procedure. The vessels have internal volume of 80 ml and they support maximum pressure and temperature of 80 bar and 280 °C, respectively. The software version was changed to v1.27-Synt to run with a maximum pressure rate of 3 bar s^{-1} during the digestion.

An ion chromatographic system (Metrohm, Herisau, Switzerland) equipped with a pump (IC liquid handling unit), compact autosampler (model 813) and conductivity detector (model 819) was used for chlorine determination using a Metrosep A Supp 5 column (150 x 4 mm i. d.) composed by polyvinyl alcohol with quaternary ammonium groups and with particle size of 5 μm and a guard column (Metrosep A Supp 4/5 Guard) with the same packing material and particle size of analytical column. The operational conditions were set according to previous work [27] and are shown in Table I.

For results comparison, an inductively coupled plasma mass spectrometer (PerkinElmer-SCIEX, Model Elan DRC II, Thornhill, Canada) equipped with a concentric nebulizer (Meinhard Associates, Golden, USA), a baffled cyclonic spray chamber (Glass Expansion, Inc., West Merbourne, Australia) and a quartz torch with a quartz injector tube (2 mm i.d.) was used for the determination of Cl. Argon 99.996% (White Martins - Praxair, São Paulo, Brazil) was used for plasma generation, nebulization and auxiliary gas. Instrumental performance optimization, including nebulizer gas flow rate, RF power and ion lens voltage, was performed and operational conditions were set according previous work.[26] The operational conditions for Cl determination by ICP-MS are also shown in Table I.

Table I. Operational conditions of Cl determination by IC and ICP-MS

Parameter	ICP-MS	IC
RF power (W)	1400	-
Plasma gas flow rate (l min^{-1})	15.0	-
Auxiliary gas flow rate (l min^{-1})	1.2	-
Nebulizer gas flow rate (l min^{-1})	1.15	-
Spray chamber	Cyclonic	-
Nebulizer	Concentric	-
Sampler and skimmer cones	Pt	-
Ion lens (V)	Auto lens "on"	-
Dwell time (ms)	50	-
Isotope (m/z)	^{35}Cl	-
Mobile phase	-	3.2 mmol l^{-1} Na_2CO_3 1.0 mmol l^{-1} $NaHCO_3$
Flow rate, ml min^{-1}	-	0.7
Sample loop (μl)	-	100
Column	-	Metrosep A Supp 5, 150 x 4 mm i.d

Residual carbon content determination in digests obtained by MIC was carried out in a model Spectro Ciros CCD simultaneous spectrometer with axial view configuration.[32] Plasma operating conditions and selected wavelength are described in reference [26].

2.2. Reagents and sample preparation

All reagents used were of analytical grade. Purified water with a Milli-Q system (18.2 MΩ cm, Millipore, Billerica, USA) was used to prepare the mobile phase,

reagents and standards. Ammonium nitrate was dissolved in water and this solution was used as igniter for MIC procedure. Small discs of paper (15 mm of diameter, about 12 mg) with low ash content (Black Ribbon Ashless, Schleicher and Schuell, Dassel, Germany) were used for ignition step and to aid the combustion process. Ammonium nitrate solution (6 mol l^{-1}) was also used to aid the combustion process and it was prepared by salt dissolution in water. The paper was previously cleaned with ethanol and water for 30 min in an ultrasonic bath. Commercial polyethylene films (thickness 0.02 mm, 10 mg), used to wrap the samples were cleaned in the same way of paper. In addition, the paper, polyethylene and all the glass materials used were washed with hot water before using.

Absorbing solutions of $(NH_4)_2CO_3$ (10 to 100 mmol l^{-1}) were prepared before use by the correspondent salt dissolution in water (Merck). Ammonium hydroxide (10 to 100 mmol l^{-1}) solutions were prepared after dilution of commercial reagent (25%, m/m, Merck) in water.

The accuracy of the proposed procedure was evaluated using spikes and also analysis of CRM provided by IRMM BCR 181 (Coking coal) and by NIST SRM 1634c (Trace elements in residual fuel oil). Sodium carbonate and $NaHCO_3$ were used to prepare the mobile phase and H_2SO_4 solution for suppression column regeneration. Stock standard solution of chlorine was prepared by dissolving sodium chloride in water. The standard solutions were prepared by sequential dilution of stock solution in water.

Atmospheric residue samples were heat up in an oven at 100 °C for 1 h. The gas oil samples were used at room temperature. The samples were wrapped in polyethylene films and sealed under heat as described in previous work.[17]

2.3. Microwave-induced combustion

Microwave-induced combustion was performed using sample masses of RAT and gas oil in the range of 100 to 500 mg. Sample and a small disc of filter paper were positioned in the quartz holder and 50 µl of 6 mol l^{-1} ammonium nitrate solution were added to the paper. The quartz holder was placed inside the quartz vessel, previously charged with 6 ml of absorbing solution. Vessels were pressurized with oxygen at 20 bar for 1 min. The rotor with vessels was placed inside the microwave cavity and the microwave heating program was started. The microwave heating program used was 1400 W for 5 min and 0 W for 20 min (cooling step). After the end of the irradiation program, the pressure of each vessel was released and the resultant solutions were transferred to polypropylene vessels and diluted with water to 30 ml for further analysis. After each run, holders were soaked in concentrated HNO_3 for 10 min followed by rinsing with water.

3. Results and discussion

3.1. Evaluation of the operational conditions of the combustion process

According to previous works using MIC to digest samples of organic matrix similar to RAT and gas oil [17,30], the use of 20 bar of oxygen was suitable to obtain a complete combustion with no apparent residues. It is important to notice that for less viscous samples, such as gas oil, the operational conditions of digestion by MIC were not investigated in previous works.[17,30] Therefore, an initial study was performed in order to evaluate the operational conditions of the combustion processes for RAT and gas oil digestion. It is important to point out that as a similar way for light crude oil [29], it was necessary to use higher amounts of polyethylene to wrap the gas oil samples due to their lower viscosity.

Initial studies were performed in order to evaluate the behavior of RAT and gas oil digestion by MIC and to check possible risks of explosion. It was observed that a complete and stable combustion always occurred when 20 bar of O_2 using 100 mg of sample. In view of this, further studies were performed using 20 bar of oxygen and sample masses ranging from 100 to 500 mg. It was observed that using 100 mg of RAT and gas oil, the maximum pressure achieved was about 28 bar and even using sample masses up to 500 mg, the maximum pressure achieved during combustion was lower than 60% of the maximum pressure recommended by the manufacturer. Therefore, MIC digestion for RAT and gas oil samples was performed using 20 bar of oxygen and 500 mg of sample. Under these conditions, sample combustion started about 6 s after the microwave irradiation, the combustion time was about 25 s and the temperature achieved was always higher than 1400 °C. This high temperature assured a complete decomposition of organic matrix minimizing the RCC in digests.

3.2. Influence of absorbing solution

The choice of absorbing solution is extremely important in order to achieve good analyte recoveries and it must be compatible with the determination technique.[16] In general, for halogens absorption, concentrated acid solutions could cause risks of analyte losses by volatilization and also interferences in the determination step could be observed. In addition, literature data recommend the use of water or alkaline solutions for further halogens determination after digestion by MIC for different matrices, as coal [26], petroleum coke [27] and heavy crude oils [17]. In this sense, tests were carried out using water, $(NH_4)_2CO_3$ (10, 25, 50 or 100 mmol l^{-1}) and NH_4OH (10, 25, 50 or 100 mmol l^{-1}) as absorbing solution with reflux step. Spike recoveries were evaluated for each absorbing solution for Cl determination as shown in Figure 1. This study was performed using RAT sample.

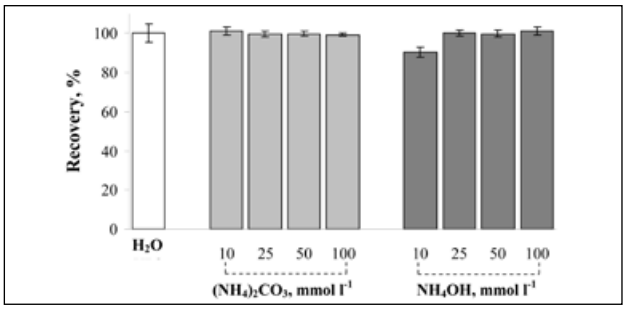

Figure 1. Influence of absorbing solutions for Cl determination using H_2O, $(NH_4)_2CO_3$ and NH_4OH (10, 25, 50 to 100 mmol L^{-1}) with reflux step. Determination by IC; error bars are the standard deviation, n = 3)

Recoveries better than 98% were obtained for Cl using a reflux step after the combustion using water, $(NH_4)_2CO_3$ and NH_4OH solutions for all concentrations studied (exceptionally for 10 $mmol^{-1}$ NH_4OH, Cl recovery was about 90%). Relative standard deviation (RSD) values for MIC with reflux were lower than 3%, however using water RSD values were higher. Taking into account that diluted NH_4OH is a suitable solution for IC and ICP-MS, a 25 mmol l^{-1} NH_4OH solution was arbitrarily selected for further Cl determination allowing quantitative recoveries for Cl. Blanks for Cl using 25 mmol l^{-1} NH_4OH were always lower than 25 µg l^{-1}.

3.3. Chlorine determination in atmospheric residue and gas oil samples after MIC

After RAT and gas oil samples digestion, the resultant solutions were analyzed by IC and also by ICP-MS and results are shown in Table II.

Table II. Chlorine concentration in atmospheric residue and gas oil samples after MIC digestion

Sample	Chlorine, µg g^{-1}	
	IC	ICP-MS
RAT 1	4052 ± 215	4066 ± 356
RAT 2	408 ± 10	415 ± 25
RAT 3	8.27 ± 0.23	8.31 ± 0.41
RAT 4	25.6 ± 1.1	25.4 ± 1.6
RAT 5	57.9 ± 3.3	57.6 ± 4.2
Gas oil 1	4.21 ± 0.13	< 6.6
Gas oil 2	15.8 ± 0.78	15.6 ± 0.82
Gas oil 3	< 1.2	< 6.6
Gas oil 4	< 1.2	< 6.6
Gas oil 5	< 1.2	< 6.6
BCR 181[a]	1382 ± 23	1384 ± 31
NIST 1634c[b]	44.4 ± 1.1	44.3 ± 1.9

Certified values: [a] 1380 ± 50 µg g^{-1}, [b] 45 µg g^{-1} (informed value).

The concentration of chlorine in RAT samples varied from 8 to 4050 µg g^{-1}. In general, Cl concentration in gas oil samples was lower than the LODs obtained by IC and also by ICP-MS, with the exception for gas oil samples 1 and 2. No statistical difference (t-test, confidence level of 95%) was observed between results obtained using IC and ICP-MS. The limit of detection (LOD) for Cl by IC and ICP-MS was 1.2 µg g^{-1} and 6.6 µg g^{-1}, respectively. A typical chromatogram obtained by IC for Cl determination in RAT sample is shown in Figure 2.

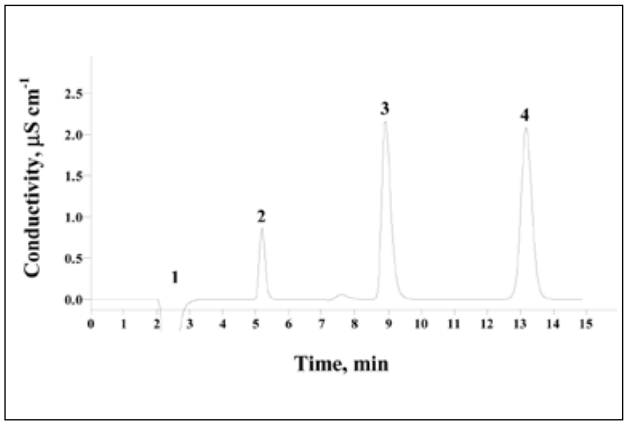

Figure 2. Chromatogram obtained after MIC digestion of RAT: (1) system peak, (2) chlorine, (3) nitrate and (4) sulfate.

The retention time for Cl was about 5 min and other signals, correspondent to nitrate and sulfate, were also observed in the chromatogram. This fact could be explained since these types of samples generally contain high sulfur concentration, as also described in literature. [10] In addition, the high nitrate concentration could be explained by the addition of ammonium nitrate as igniter and also due to the cleaning step of the vessels that is performed using nitric acid. However, despite the presence of these ions in digests, no interference on Cl signals was observed.

In order to check the accuracy, MIC was applied for CRMs of coking coal and fuel oil. After digestion, Cl was determined by IC and ICP-MS. Results are also shown in Table 2. No statistical difference (t-test, confidence level of 95%) was observed for Cl concentration obtained by IC and ICP-MS. In addition, the agreement with the certified value was better than 97% for Cl in all the evaluated CRMs.

3.5. Residual carbon content

The efficiency of the sample decomposition by MIC was evaluated by the residual carbon content (RCC) determination in digests. In this work, RCC was evaluated in samples digested by MIC following the procedure reported in previous work [26]. Residual carbon content were always below 1% for atmospheric and gas oil digests obtained by MIC due to high temperature reached (about 1400 °C) during the combustion. In this condition practically all the organic compounds could be completely decomposed.

4. Conclusions

The proposed procedure by MIC was suitable for RAT and gas oil digestion for further Cl determination by IC. It was possible to use a diluted medium (25 mmol l^{-1} NH_4OH solution) to obtain quantitative recoveries for Cl using a reflux step after the combustion. In addition, digests obtained by MIC were suitable also for determination by ICP-MS. The results obtained for different CRMs under the selected conditions were in agreement with certified values. The proposed MIC procedure allows the combustion of relatively high sample mass (500 mg) without exceeding 60% of the maximum operating pressure (80 bar), combining good performance for atmospheric and gas oil digestion, safety and relatively high sample throughput. Therefore, based on its particular characteristic, MIC combined to IC or ICP-MS determination can be proposed for chlorine determination in RAT and gas oil samples complying with the recent requirements of crude oil industry.

5. Acknowledgements

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Determination of ciprofloxacin by synchronous scanning room-temperature phosphorimetry

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Abstract.
Determination of ciprofloxacin was achieved by synchronous scanning room-temperature phosphorimetry using either $CdCl_2$ or $Th(NO_3)_4$ as phosphorescence inducers. The method was optimized by using the univariate approach, in order to find intense analytical signal from ciprofloxacin, followed by a 2^3 factorial design in order to verify interaction among relevant variables, to check robustness for each variable and to perform final adjustment of parameters. Absolute limit of detection (ALOD) for ciprofloxacin was below 10 ng with a linear signal response extending to at least 415 ng of the analyte. Accuracy was evaluated using commercial and simulated pharmaceutical formulations with recoveries between 97 and 103%. The interferences due the presence of moxifloxacin and gatifloxacin were evaluated and selective conditions of analysis established. Further studies indicated the potential application of the method in urine samples.

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Keywords: Ciprofloxacin, Cellulose substrate, Synchronous scanning phosphorimetry.

1. Introduction

Ciprofloxacin (Figure 1) is a broad spectrum antibacterial drug used in human and veterinary medicine and it is one of the most extensively used fluorquinolones[1,2] even when compared with those recently designed for better performance in medicine (e.g. gatifloxacin and moxifloxacin). Fluorquinolones are a class of synthetic antimicrobial drugs obtained by the modification of the quinolone structure placing in it a fluorine atom at a specific position, producing increased antimicrobial activity, improved pharmacokinetic performance and less intense collateral effects[1]. The molecular target of fluorquinolones is the DNA gyrase that regulates DNA replication and therefore stops bacterial growth. They are very active against gram-negative bacteria, but they are also active against gram-positive cocci[3]. They are prescribed for a myriad of infections such as bladder infection, ophthalmic infection, and also for some cases of sexually transmitted diseases[4].

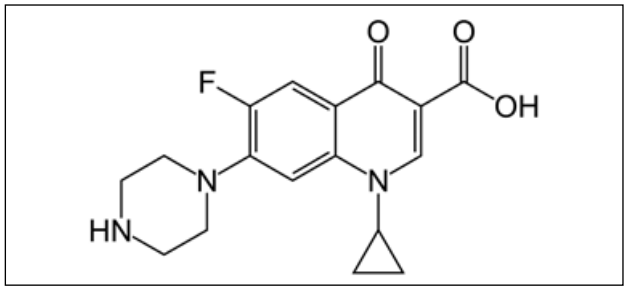

Figure 1. Ciprofloxacin.

Several analytical methods have been developed for the determination of fluorquinolones. Belal *et al.*.[5] present a comprehensive review on this subject, indicating that adsorptive stripping voltammetry[6], UV-vis absorption spectrophotometry[7] and spectrofluorimetry[8] are not selective enough to discriminate a single fluorquinolone in mixtures containing other ones without the use of separation procedures or application of chemometric calibration algorithms. In order to achieve selectivity, separation of components based on the use of liquid chromatography[9] or capillary electrophoresis[10] have been used prior to detection.

Solid surface room-temperature phosphorimetry (SSRTP) is an analytical technique that may allow the selective detection of one substance in the presence of other ones of similar chemical structure. Such selectivity might be achieved by the proper choice of the heavy atom phosphorescence inducer. SSRTP has been applied for the determination of a few fluorquinolones (norfloxacin, ciprofloxacin, ofloxacin, lomefloxacin and fleroxacin)[11]. From the several tested heavy atom phosphorescence inducers, Cd (II) was found to enable most intense analytical signals. However, the authors have been not successful in achieve conditions to perform selective determination in mixtures of fluorquinolones. The selective and sequential determination of norfloxacin and levofloxacin in the presence of other fluorquinolones has been proposed recently based on both the correct choice of the selective phosphorescence inducer and the use of synchronous scanning of spectra[12].

The purpose of the present work is to demonstrate the applicability of the SSRTP for the determination of ciprofloxacin in pharmaceutical formulations. In addition, efforts were made aiming the selectivity towards gatifloxacin and moxifloxacin.

2. Experimental

2.1. Apparatus

Phosphorescence measurements were performed on a luminescence spectrometer Perkin–Elmer LS-55 (Perkin-Elmer, CT, USA) coupled to a solid surface analysis apparatus modified to allow a flow of purging gas (nitrogen dried by passing it through a silica gel bed) on the sample holder. A delay time of 3 ms, gate time of 3 ms and spectral bandwidth of 10 nm were employed. Spectra from ciprofloxacin was obtained by synchronous scanning using $\Delta\lambda$ = 237 nm in the presence of Th (IV) as heavy atom enhancer, and $\Delta\lambda$ = 170 nm in the presence of Cd (II) as heavy atom enhancer. A laboratory made photochemical reactor, described elsewhere[13], was employed to treat the paper substrates in order to reduce their natural phosphorescence background. A pHmeter (MS Tecnopon, model MPA-210, Sao Paulo, Brazil) was also employed. Software Statistica 8.0 (Statsoft Brazil, Sao Paulo, Brazil) was used as statistic analysis tool.

2.2. Reagents

All experiments were performed with analytical grade chemicals and ultrapure water. Whatman N° 42 filter paper (Whatman, Kent, UK) was used as solid substrate. Ciprofloxacin was purchased from Fluka (Buchs, Germany). Moxifloxacin and gatifloxacin were extracted from pharmaceutical tablets and purified. $TlNO_3$ were purchased from Acros Organics (Geel, Belgium), Ethanol, acetic acid, boric acid, sodium hydroxide, phosphoric acid, $Pb(NO_3)_2$, KI, sodium dodecyl sulfate (SDS) were obtained from Merck (Darmstadt, Germany), $AgNO_3$, $CdCl_2$ and Hg_2Cl_2 were from VETEC (Rio de Janeiro, Brazil). Thorium nitrate was from Carlo Erba (Milan, Italy). A commercial pharmaceutical formulation Cloridrato de ciprofloxacino, SEM Industria Farmaceutica Ltda, containing 250 mg of ciprofloxacin per tablet) was purchased in local drugstores.

2.3. Standards and solutions

Ciprofloxacin stock solutions ($1x10^{-3}$ mol L^{-1}) were prepared in acetone/water 50/50% v/v and used to prepare more diluted standard working solutions. The final working solutions were made in acetone/water 25/75% v/v. When necessary, Britton-Robinson buffer 0.04 mol L^{-1} was used to adjust the pH of the solution. In such cases, 20% in volume of the buffer was used replacing part of the water content. The stock solutions of SDS (0.25 mol L^{-1}) and heavy atom salts (0.25 mol L^{-1} of $TlNO_3$, 1.0 mol L^{-1} of KI, 0.5 mol L^{-1} of $AgNO_3$, 0.2 mol L^{-1} of $HgCl_2$, 0.5 mol L^{-1} of $Pb(NO_3)_2$ $CdCl_2$ 0.5 mol L^{-1} and 0.5 mol L^{-1} of $Th(NO_3)_4$) were prepared in water and, when necessary, used to prepare more diluted solutions. If necessary, the pH of the solutions was adjusted to avoid metal hydrolysis. The pharmaceutical formulations were prepared by pulverizing ten medicine tablets followed by dissolution of portions of the resulting powder with acetone. The solutions were vacuum filtered in a Buchner device using quantitative filter paper which was carefully washed with acetone. For some experiments, a known amount of ciprofloxacin was mixed with either moxifloxacin or gatifloxacin pharmaceutical formulation powders before dissolution in acetone. Urine samples were diluted by the addition of acetone/buffer aqueous solution in order to get a ten-fold dilution (in volume) and in a final aqueous solution containing 25% acetone, in volume, and 20% of buffer, in volume, at a specific pH value.

2.4. Procedures

Substrate (filter paper) background reduction consisted of washing paper strips with boiling water in a Soxhlet apparatus for 2 h. After dried under an infrared lamp, the paper was exposed to ultraviolet radiation for another 2 h. These solid substrates were cut in circles (18 mm in diameter) to be used during the analysis. The surface of these cellulose substrates were modified by spotting 5 µL of SDS solution (0.25 mol L^{-1}) on the center of the circle. After dried, each of the all employed solutions was spotted also in the centre of the substrate in the following order: 5 µL of heavy atom solution and 5 µL of the analyte solution using a 1-10 µL adjustable microliter pipette. When performing multiple additions of the SDS solution or the heavy atom salt solutions, a first 5 µL addition was made on the centre of the substrate which was dried, under an infrared light, before the addition of the second 5 µL aliquot, also on the centre of the substrate. The spotted substrates were vacuum-dried at room temperature for 2 h and were then placed in a desiccator until the measurements were carried out. The desiccator was covered with aluminum foil to shield substrates from ambient light. In order to make the analytical measurement, these circles were placed on a clean sample holder and inserted in the front surface instrument accessory. Sample compartment was continuously purged with dry nitrogen gas for 3 min prior to each measurement.

3. Results and discussion

3.1 Room temperature phosphorescence of *ciprofloxacin, gatifloxacin and moxifloxacin*

De-oxigenated environment and immobilization of the analyte (for instance, in a cellulose substrate) are fundamental conditions to allow the observation of

phosphorescence since they minimized non-radiative deactivation of the excited triplet state caused by dynamic quenching and vibrational relaxation. The use of the external heavy-atom effect may induce or significantly amplify phosphorescence by enhancing both the rate of intersystem crossing (excited singlet state – excited triplet state transition) and the phosphorescence rate constant. The selective nature of such effect has made SSRTP a useful analytical tool for the determination of trace amounts of substances of biological, environmental and pharmaceutical interest[14,15].

Room-temperature phosphorescence of ciprofloxacin, gatifloxacin and moxifloxacin were studied in SDS-modified filter paper using different heavy atom salts as potential phosphorescence inducers. Surfactants such as SDS create conditions for a better interaction between the analyte and the substrate and the approximation between the phosphor and the heavy atom enhancer. It also impedes the migration of phosphors into the internal layers of the cellulose substrate becoming readily accessible to the excitation radiation. The analytes (5 µL of a $5x10^{-5}$ mol L^{-1} solution) were placed on the center of the substrate from an acetone/water 25/75% v/v carrier solution. It can be seen in Table I that phosphorescence from ciprofloxacin is induced in the presence of all heavy atoms tested. In contrast, phosphorescence from gatifloxacin was observed only in the presence of Tl (I). In the case of moxifloxacin, phosphorescence was observed in the presence of all heavy atoms but not in the presence of Cd (II). However, when Th (IV) was used as the phosphorescence inducer for moxifloxacin, two important characteristics were found when compared with the ones of ciprofloxacin in similar conditions: (i) the maximum wavelengths of the excitation and emission phosphorescence bands of moxifloxacin was significantly different from the ones observed for ciprofloxacin and ii) for moxifloxacin, the lowest phosphorescence was found in the presence of Th (IV) while the maximum phosphorescence from ciprofloxacin was found in the presence of the same heavy atom. From these preliminary results, $CdCl_2$ and $Th(NO_3)_4$ were chosen as phosphorescence inducers based on their potential selective discrimination of ciprofloxacin towards gatifloxacin and moxifloxacin.

Literature indicates significant differences on the phosphorescence intensities when fluorquinolones such as levofloxacin and norfloxacin are placed on the cellulose substrates from analyte carrier solutions with different pH values[12]. Therefore, phosphorescence from ciprofloxacin, in the presence of either $CdCl_2$ or $Th(NO_3)_4$, was measured after ciprofloxacin was spotted onto SDS-treated filter paper from carrier solutions with pH adjusted from 2 to 12 (adjusted by using Britton-Robinson buffer in the aqueous phase of the solvent system). In substrates containing $Th(NO_3)_4$, the best signal was observed at pH 5, close to the one of the original acetone/water 25/75% v/v ciprofloxacin solution (pH 5.4). Therefore, no buffered solutions were used when analyzing ciprofloxacin in such conditions. In contrast, basic solutions of ciprofloxacin (pH 10) resulted in intense phosphorescence when placed in substrates containing $CdCl_2$ (Figure 2).

Table I. Effect of several heavy atom saltsa on the phosphoresce of ciprofoxacin, moxifloxacin and gatifloxacin (5x10-5 mol L-1) on cellulose substrate modified with SDS.b

Fluorquinolone	Analyte carrier solution	Net RTP (arbitrary units) $\lambda_{exc}/\lambda_{em}$ (nm)				
		$TlNO_3$	$AgNO_3$	$Pb(NO_3)_2$	$CdCl_2$	$Th(NO_3)_4$
Ciprofloxacin	Acetone/water	67 263/525	39 290/506	90 288/481	55 282/443	119 288/447
Gatifloxacin	Acetone/water	56 262/502	-	-	-	-
Moxifloxacin	Acetone/water	77 298/509	50 297/513	63 298/498	-	33 303/402

[a]Heavy atom salt solutions: $TlNO_3$ 0.25 mol L^{-1}; $AgNO_3$ 0.5 mol L^{-1}; $Th(NO_3)_4$ 0.5 mol L^{-1}; $CdCl_2$ 0.5 mol L^{-1}. [b]SDS 0,25 mol L^{-1}.

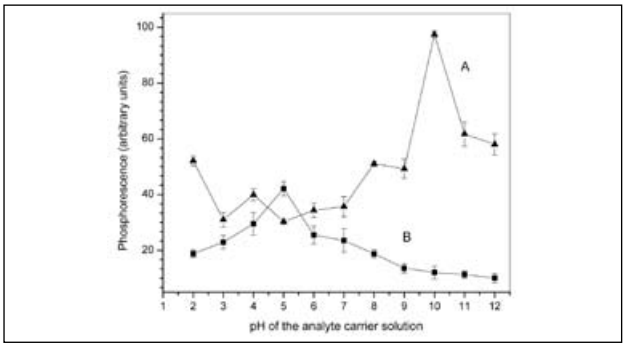

Figure 2. Ciprofloxacin room-temperature phosphorescence induced by (A) $CdCl_2$ and (B) $Th(NO_3)_4$ in function of the pH of the analyte carrier solution.

3.2. Maximization of room-temperature phosphorescence of ciprofloxacin

Room-temperature phosphorescence from ciprofloxacin was maximized by performing univaried studies from which variable levels were chosen to perform a further two level factorial design (2^3) in order to identify any interaction among the chosen variables, to make the final adjustment of experimental conditions and to evaluate the robustness of each of the variables.

The external heavy atom effect can be maximized by adjusting the amount of the heavy metal salt on the substrate where the analyte is placed[16]. In order to do that, a single 5µL volume of either $Th(NO_3)_4$ or $CdCl_2$ solutions of different concentrations were used to obtain amounts between 12 and 600 µg of $Th(NO_3)_4$ or amounts between 9 and 460 µg of $CdCl_2$ on the center of the substrate. In both cases, the maximum phosphorescence was observed using the higher amount of these salts (Figure 3). Due to the limited solubility of the salts in water, higher amount of salt could only be derived on the substrate by adding higher volumes of solutions. However, using such approach, the desired amount of salt would not be concentrated in the centre of the substrate because of the spreading of solution in the substrate. The alternative approach is to use a multiple additions of 5 µL of the most concentrated solution. In this case, the second addition is made only after the first amount of solution is dried under an infrared lamp. The evaluation considering multiple additions of the heavy atom salt solution is included in the factorial design.

In order to complete the optimization, a two level factorial design was performed using three variables: (i) pH of the analyte carrier solution to be delivered in the centre of the substrate, (ii) mass of the heavy atom salt on the centre of the substrate and (iii) mass of SDS on the centre of the substrate.

The presence of 360 µg of SDS (5 µL of a 0.25 mol L^{-1} solution) on the center of the surface of the cellulose substrate was found to improve three times the phosphorescence from ciprofloxacin in the presence of $Th(NO_3)_4$, when compared to the signals achieved in substrates containing no surfactant. In the presence of $CdCl_2$ as the phosphorescence inducer, a twofold ciprofloxacin signal improvement was achieved in substrates containing SDS. For the factorial design, the lowest level (-) was set to be one single addition of SDS solution (360 µg) while the highest level (+) was 720 µg of SDS obtained from two additions of the SDS solution on the centre of the substrate. For the heavy atom salts, a single (-) and two sequential additions of 5 µL of either $Th(NO_3)_4$ 0.50 mol L^{-1} or $CdCl_2$ 0.50 mol L^{-1} were used. Therefore, the factorial design included 600 µg (-) and 1200 µg (+) of $Th(NO_3)_4$ or 460 µg (-) and 920 µg (+) of $CdCl_2$. Finally, for the pH a small range was chosen for the experiment in order to test the robustness of the parameter. Therefore, for the phosphorescence of ciprofloxacin induced by Cd (II), the lowest level (-) was set to be pH 10 and the high level (+) was pH 10.5 while for the phosphorescence of ciprofloxacin induced by Th (IV) the lowest level (-) was set to be pH 5 and the high level (+) was pH 5.5.

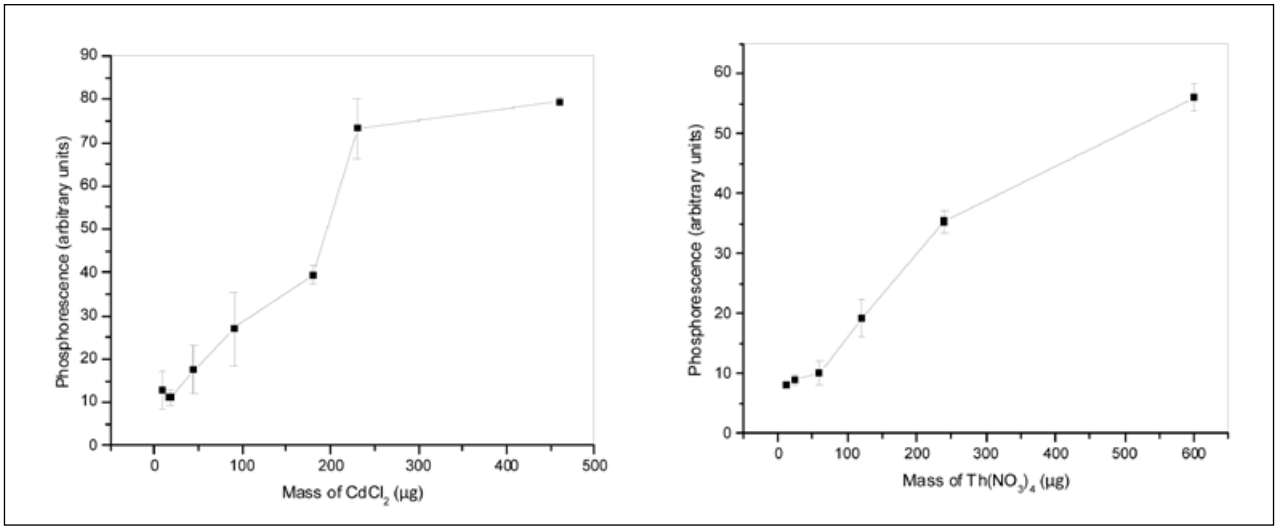

Figure 3. Effect of the mass of the heavy atom salt on the phosphorescence of ciprofloxacin in cellulose substrate.

The result of this 2^3 factorial planning was evaluated through Pareto charts which indicated that none of the interactions among variables are relevant, and there are robust conditions in the chosen range for all of the variables (no statistical differences between the results achieved using the chosen experimental levels). Table II shows the optimized experimental conditions for maxi-

mum room-temperature phosphorescence of ciprofloxacin induced by Th (IV) and induced by Cd (II) whose spectra are indicated in Figure 4.

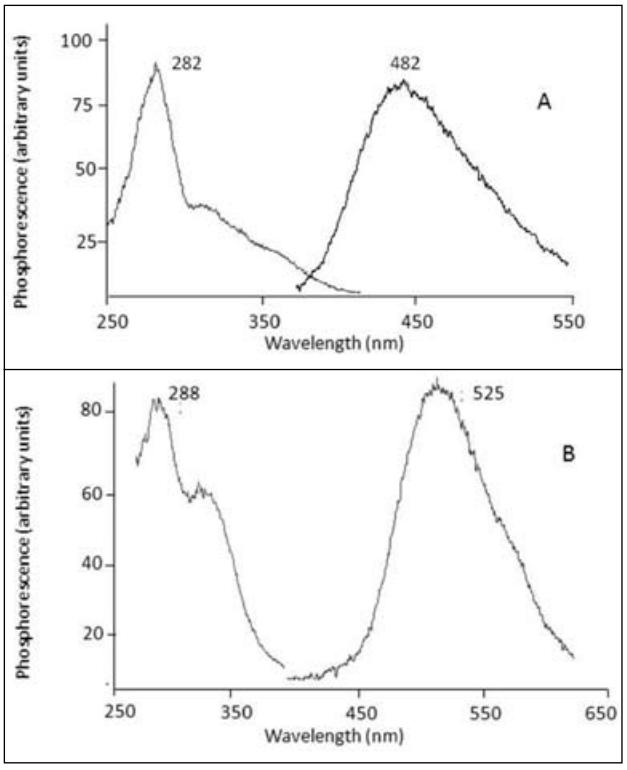

Figure 4. Ciprofloxacin room-temperature phosphorescence excitation and emission spectra in cellulose substrates under the optimized conditions using (A) $CdCl_2$ and (B) $Th(NO_3)_4$.

3.3 Evaluation of interferences from moxifloxacin and gatifloxacin in the ciprofloxacin phosphorescence

An interference study was performed in order to evaluate the influence of increasing concentrations of gatifloxacin and moxifloxacin on the ciprofloxacin phosphorescence induced by either $Th(NO_3)_4$ or $CdCl_2$. In order to perform such evaluation, phosphorescence from ciprofloxacin (I_{CIP}) was compared to the ones observed from synthetic mixtures containing ciprofloxacin and increasing concentrations of either one of the other two fluorquinolones ($I_{(CIP + GAT)}$ or $I_{(CIP + MOX)}$). Results in Table III indicate that when using Th (IV) and synchronous scanning with $\Delta\lambda$ = 237 nm, the selective determination of ciprofloxacin can be performed in samples containing either gatifloxacin or moxifloxacin if the molar proportion of them does not exceed two times the one of ciprofloxacin. For the other mixtures, severe non-spectral interferences were found as indicated by the $I_{CIP}/I_{(CIP + GAT)}$ and $I_{CIP}/I_{(CIP + MOX)}$ values higher than the unit. However, such non-spectral type of interference may be promptly corrected by using analyte addition technique. In contrast, using Cd (II) and synchronous scanning with $\Delta\lambda$ = 170 nm, non spectral interferences are found even in samples containing equimolar quantities of ciprofloxacin and the interferent fluorquinolone (gatifloxacin or moxifloxacin).

Since up to 30% of the ingested quantity of fluorquinolones is eliminated in urine in the original form, a study to evaluate the potential detection of ciprofloxacin in urine was performed. The results indicated that urine matrix imposes non-spectral interferences on the phosphorescence signal of ciprofloxacin, therefore, cleaning up procedures to reduce protein content of the biological sample were applied (protein precipitation with methanol or ammonium sulfate), however, such approaches did not solve the interference problem. Interferences were promptly minimized a ten-fold dilution of the sample using acetone/water solvent system. The dilution of the sample is in part compensated by the good detectability of the method. Potential interferences from ciprofloxacin metabolites were not evaluated due to the lack of standards.

3.4. Analytical figures of merit

The analytical figures of merit for ciprofloxacin were obtained under the experimental conditions optimized indicated in Table II. In the presence of Th (IV) as phosphorescence heavy atom inducer, the absolute limit of detection (ALOD) of 9.6 ng was calculated while the absolute limit of quantification (ALOQ) was 31.7 ng. Using Cd (II) as the heavy atom inducer, the ALOD and ALOQ were respectively 7.1 and 23.6 ng. ALOD and ALOQ were calculated based on the following equations: $3s_b\ m^{-1}\ V\ MM$ and $10s_b\ m^{-1}\ V\ MM$, where s_b is

Table II. Optimized conditions for the determination of ciprofloxacin using SSRTP.

Parameter	Heavy atom salt $\lambda_{exc}\backslash\lambda_{em}$	
	$CdCl_2$ 282\452 nm	$Th(NO_3)_4$ 288\525 nm
$\Delta\lambda$	170 nm	247 nm
Buffer	pH 10	Non buffered
Mass of salt on substratum	460 µg	600 µg
Mass of SDS on substratum	360 µg	360 µg

the standard deviation from 16 blank determinations, m are the slopes of the analytical curves, MM is the molar mass of ciprofloxacin and V is the analyte volume deposited on the substrate (5 µL).

The analytical curves showed linear dynamic ranges that extended from the ALOQ to at least 415 ng of ciprofloxacin. The analyte curve equations were $Y = 0.41\ ng^{-1}\ X + 44$ for substrates containing $Th(NO_3)_4$ and $Y = 1.11\ ng^{-1}\ X + 59$ for substrates containing $CdCl_2$. The curves presented a homoscedastic behavior and their determination coefficients were close to the unity (R^2>0.99). Evaluations of the repeatability were performed using two different masses of the analyte (83 and 331 ng). The relative standard deviation of the measured values varied from 3.2 to 6.6% what can be considered satisfactory for measurements from solid substrates.

3.5. Application of the method

The proposed SSRTP method was applied for the quantification of ciprofloxacin in one commercial pharmaceutical formulation. Other two simulated formulations were also analyzed. These simulated samples were prepared by mixing a known amount of ciprofloxacin standard with the pulverized pharmaceutical formulation of either gatifloxacin or moxifloxacin in order to get ciprofloxacin/gatifloxacin or ciprofloxacin/moxifloxacin proportions of either 1/1 or 1/2 w/w. The recovery values were calculated based on the ciprofloxacin quantity indicated on the medicine instruction, which agreed with experimental results found using a reference HPLC with fluorescence detection[17]. Recovery tests were made using each one of the heavy atom salts as phosphorescence enhancer in order to get a comparison of performance. The tabulated values are averages of three different determinations performed in three different days and a Student t-test (at 95% confidence level) was used to statistically compare the experimental result with the reference one.

The determination of ciprofloxacin in the pharmaceutical formulation was successfully achieved with recoveries of 97.9 ± 4% (using $Th(NO_3)_4$) and 103.8 ± 1% (using $CdCl_2$) indicating no interference problems imposed by the matrix components. For synthetic mixtures, results in Table IV indicates that the use of Th (IV) allowed accurate ciprofloxacin determinations in 1/1 and 1/2 w/w ciprofloxacin/gatifloxacin or ciprofloxaxin/moxifloxacin mixtures. Spectral interferences were found when larger proportions of moxifloxacin and gatifloxacin were present as indicated by the percent recoveries significantly higher than 100%. On the other

Table III. Evaluation of interferences of gatifloxacin and moxifloxacin in the ciprofloxacin phosphorescence using synchronized scanning.

Mixture (molar proportion)	$Th(NO_3)_4$	$CdCl_2$
Ciprofloxacin/Gatifloxacin	$I_{CIP}/I_{(CIP + GAT)}$	
1/1	0.99	1.86
1/2	0.98	2.49
1/5	3.12	2,49
1/10	4.14	3.31
Ciprofloxacin/Moxifloxacin	$I_{CIP}/I_{(CIP + MOX)}$	
1/1	1.00	1.49
1/2	0.97	2.11
1/5	0.66	2.85
1/10	0.47	2.85

Table IV. Recovery tests using simulated pharmaceutical formulations using synchronous scanning SSRTP.

Analyte	Concomitant florquinolone	Analyte/concomitant florquinolone proportion	Analyte recoveries[a]	
			$Th(NO_3)_4$	$CdCl_2$
Ciprofloxacin	Gatifloxacin	1/1	103 ± 8.8%	56.4 ± 4.7%
Ciprofloxacin	Gatifloxacin	1/2	97 ± 11%	22 ± 8%
Ciprofloxacin	Moxifloxacin	1/1	94.3 ± 3.6%	61 ± 12%
Ciprofloxacin	Moxifloxacin	1/2	99 ± 7.4%	36.3 ± 3.8%

[a] Average of three determinations (n=3).

hand, $CdCl_2$ could not allow satisfactory results even in 1/1 w/w mixtures of ciprofloxacin/gatifloxacin and ciprofloxacin/moxifloxacin.

Tests using urine samples fortified with ciprofloxacin was also performed. Recoveries between 98.6 and 107.1% were achieved in urine samples ten times diluted in the acetone/water solvent system. These results indicated the potential feasibility of the method in such biological sample.

4. Conclusions

The developed SSRTP based allowed accurate and precise determination of ciprofloxacin. The method presented satisfactory figures of merit with ng level detectability achieved by using SDS-treated cellulose substrates and heavy atom salt phosphorescence inducers ($Th(NO_3)_4$ or $CdCl_2$). Synchronous scanning allowed a certain degree of selectivity towards gatifloxacin and moxifloxacin. In the selective point of view, the presence of Th (IV) allowed better results. The method can be readily used to quantify ciprofloxacin in pharmaceutical formulations and this study also indicates that SSRTP can be used as a simple approach to detect counterfeit medicines that have been adulterated by replacing new generation and expensive fluorquinolones (gatifloxacin and moxifloxacin) with ciprofloxacin which is a cheaper active components. Trace-level determination in urine samples is possible.

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Closed-vessel microwave-assisted digestion using a diluted oxidant mixture for trace element determination in petrochemical samples by axially-viewed ICP OES

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Abstract
A simple, robust and reliable analytical method for the determination of metals (Ag, Al, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Na, Ni, Pb, V and Zn) and nonmetals (P and S) in petrochemical samples (petroleum, diesel oil, biodiesel, and oil sludge waste) by axially-viewed inductively coupled plasma optical emission spectrometry (ICP OES) is proposed. A diluted oxidant mixture (4 mL HNO_3 + 2 mL H_2O_2 + 2 mL H_2O) was used to achieve almost complete oxidation of the organic matrix in a closed-vessel microwave oven. The residual carbon (3-9 % w/w) supported the effectiveness of the sample digestion with the diluted oxidant mixture. The accuracy of the analytical method was confirmed by analysis of a standard reference material (residual oil - NIST SRM 1634c) and another reference material (hydrocarbon oil - Alfa Aesar JM-21). The reference values showed good agreement at a 95% confidence limit (Student's *t*-test).

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Keywords: petroleum, diesel oil, biodiesel, sludge waste, ICP OES, diluted oxidant mixture, microwave

Introduction

The development of analytical methods for elemental determination in petrochemical products has been arousing interest, mainly due to their prominence, in terms of quality control, economics and environmental factors [1-3]. Crude oil contains several metals as organometallic complexes and information on these trace elements is important for geological (crude oil origin) [4,5] and environmental studies (anthropogenic emission of petroleum-based fuels) [6,7]. The poisoning of catalysts in the petroleum cracking process and corrosion of equipment, owing to the presence of these trace metals in oils, has also been widely reported [8,9]. Therefore, with the exception of certain additives, it is desirable that petrochemical products have low metal concentrations, aiming at a highly sensitive and precise method for quality control and for emission controls of these elements to the atmosphere.

Elemental determination in petrochemical products by spectroscopic techniques is not easy, mainly because of the high complexity and organic nature of their matrix [1,10]. Despite this, inductively coupled plasma optical emission spectrometry (ICP OES) is the technique recommended by the American Society of Testing Materials (ASTM D 4951) [11] and the European Committee for Standardization (CEN-EN 14 107) [12]. This analytical technique is a well-established multielementar one and presents a wide linear dynamic range, good analytical detectivity and high sample throughput [13]. However, ICP OES usually requires the conversion of organic samples into a clear solution with low residual carbon content [14].

In general, digestion procedures are useful for petrochemical samples as a pre-treatment before elemental determination by ICP OES [1]. Microwave-assisted sample preparation has been used for a wide range of applications [15]. Sample preparation procedures using digestion with diluted oxidant mixtures are still little explored for petrochemical samples. Some favorable characteristics of the digestion with diluted reagents are low blank values, low standard deviations and less contamination. Particularly for ICP OES, it is important to point out the minimization of interferences caused by concentrated acids, mainly during sample nebulization, and the reduction of chemical attack on equipment parts, such as the nebulizer, nebulization chamber and torch [14]. Finally, this procedure promoted the application of clean analytical methods with low residue generation, and is economically viable due to its low

consumption of reagents and the low costs associated with the analysis and residue treatment.

Taking into account these advantages, the aim of this work was to propose a fast, robust, and reliable method for the determination of metals (Ag, Al, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Na, Ni, Pb, V and Zn), and non-metals (P and S) in petrochemical samples (petroleum, diesel, biodiesel and oil sludge waste) using a closed-vessel microwave oven and diluted oxidant mixture and determination by axially-viewed ICP OES.

Experimental

Instrumentation

A Spectro Ciros^{CCD} ICP optical emission spectrometer (Spectro Analytical Instruments, Kleve, Germany) equipped with axially-viewed plasma was used throughout this work. The spectrometer is equipped with a simultaneous CCD solid state detector that allows measurements from 120 to 825 nm. The polychromator was thermostated at 34°C and purged with argon. An end-on gas interface was used in the axially viewed configuration to minimise interference caused by self-absorption and recombination, to provide a wide linear dynamic range and low background. The introduction system was composed of a double pass (Scott-type) spray chamber and a cross-flow nebulizer. The injector tube diameter of the torch was 2.0 mm. The selection of spectral lines was based on the spectrometer database, after scanning the emission lines obtained from a digested sample and standard solutions containing 10 mg L^{-1} of each analyte. The background correction was manually selected for all emission lines chosen for the quantitative measurements. The setup of ICP instrumental conditions for analysis is depicted in Table I.

Table I. Instrumental parameters for elemental determinations using axially-viewed ICP OES

Power (W)	1400
Nebulizer	Cross-flow (Spectro)
Spray chamber	Double pass, Scott-type
Outer gas flow (L min^{-1})	12
Intermediate gas flow (L min^{-1})	1.0
Nebulizer gas flow (L min^{-1})	0.9
Sample uptake rate (mL min^{-1})	1.5
Analytical wavelength (nm):	
Ag (I) = 328.068	Mn (I) = 257.618
Al (I) = 167.078	Mo (I) = 203.844
Ba (I) = 455.404	Na (I) = 588.995
Ca (II) = 396.847	Ni (II) = 232.003
Cd (II) = 228.802	P (I) = 178.287
Cr (I) = 205.552	Pb (II) = 283.305
Cu (II) = 327.396	S (II) = 180.731
Fe (I) = 259.948	V (I) = 311.071
Mg (I) = 285.213	Zn (I) = 213.056

(I) = atomic line; (II) = ionic line

The sample digestion with the diluted oxidant mixture was carried out by using a closed-vessel microwave system (Multiwave 3000, Anton Paar, Austria), equipped with 16 fluoropolymer vessels and ceramic vessel jackets. They support maximum temperatures and pressures of 240 °C and 4 MPa, respectively. The internal temperature and pressure are monitored only in one controlling vessel using a sensor-protecting glass tube that enters directly in contact with the digestion solution. The external temperature is controlled in all vessels using an IR sensor, which measures each digestion vessel through ports in the rotor base.

Reagents and samples

All solutions were prepared from analytical reagent grade chemicals and using high-purity deionized water obtained from a Milli-Q water purification system (Millipore, Belford, MA, USA). Nitric acid 65% (w/v) and H_2O_2 30% w/v (Merck, Darmstadt, Germany) were used for sample digestion. Analytical-grade Tritisol solutions of 1000 mg L^{-1} of Ag ($AgNO_3$), Al ($Al(NO_3)_3$), Ba ($BaCl_2$), Ca ($CaCl_2$), Cd ($CdCl_2$), Cr ($CrCl_3$), Cu ($CuCl_2$), S ($NaSO_4$), Fe ($FeCl_3$), P (KH_2PO_4), Mg ($MgCl_2$), Mn ($MnCl_2$), Mo ($MoCl_5$), Na (NaCl), Ni ($NiCl_2$), Pb ($Pb(NO_3)_2$), V (VCl_3) and Zn ($ZnCl_2$) from Merck were used to prepare the reference analytical solutions. For determination of residual carbon in digested sample solutions, stock solutions were prepared with urea (CH_4N_2O, Reagen, Brazil) in an aqueous medium, as previously described in the literature [16].

Three soya biodiesel (B1, B2 and B3) and four diesel (D1, D2, D3 and D4) samples purchased at different gas stations in the city of Sao Paulo were used in this work. One petroleum (P1) and one oil sludge waste (W1) were obtained from the Chemical Engineering Department of the University of São Paulo. One standard reference material (SRM) of residual oil (NIST SRM 1624c) and one multielement reference material prepared by addition of 100 (μg g^{-1}) of elements in a hydrocarbon oil matrix (JM-21 from Specpure-Alfa Aesar) were used to check the accuracy of the analytical method.

Procedure

The Mg II 280.265 nm/Mg I 285.208 nm emission line intensity ratio was used to check the robustness of the ICP [17,18]. The observation zone was automatically set by the program supplied with the Smart Analyzer Vision 1.50.0534 spectra using the Mn II 257.610 nm emission line. The radio frequency power was varied from 1200 to 1700 W and the nebulizer gas flow rate was adjusted to 1.0 (L min^{-1}). In order to check matrix effects on the detectivity and selectivity a scanning of the emission lines from a standard solution containing 10 (mg L^{-1}) of each analyte and petroleum digested sample was obtained. With this experiment it was possible to

choose the best analytical emission line and the signal-to-background ratio for all elements. After this, the background correction was manually selected for all emission lines for the quantitative measurements.

The limits of detection (LOD) were calculated using the background equivalent concentration (BEC) and signal-to-background ratio (SBR), according to IUPAC recommendations [19]: BEC = C_{rs}/SBR; SBR = $I_{rs} - I_{blank}$/I_{blank}; LOD = 3 x BEC x RSD/100; where, C_{rs} is the concentration of multi-elemental reference solution (10 mg L^{-1}), I_{rs} and I_{blank} are the emission intensities for the multi-elemental reference (10 mg L^{-1}) and blank solutions, and RSD is the relative standard deviation for ten consecutive measurements of a blank solution. The limits of quantification (LOQ) were calculated as 10 x LOD.

About 0.1 g from each sample was digested using the diluted oxidant mixture (4 mL HNO_3 + 2 mL H_2O_2 + 2 mL H_2O). The heating program was performed in three steps (temperature, °C; ramp, min; hold, min): 1 (80; 5; 2); 2 (140, 5, 2); and 3 (190, 10, 35). There is a fourth step for cooling down the system through forced ventilation during 20 min. After digestion, the samples and blank solutions were transferred to volumetric polypropylene tubes (Axygen Scientific, California, USA) and made up to 30 mL with deionized water. The digestion procedure was done in triplicate for each sample. The reference materials were also digested following the same procedure described by the samples.

The analytical curves were prepared with the following reference solutions: blank 1.0 mg L^{-1} HNO_3; 1.0 – 100 mg L^{-1} of Al, Ba, Ni, and V; 0.5 – 50 mg L^{-1} of Ag, Cd, Cr, Cu, Fe, Mn, Mo, Pb and Zn; 5.0 – 500 mg L^{-1} Ca, Mg, Na and P; and 5.0 – 500 mg L^{-1} S. All these solutions were prepared in 1.0 mg L^{-1} HNO_3.

Results and discussion

Analytical performance of ICP

The term robustness was proposed to represent the efficiency of energy transfer and response of the plasma to change in atomization and excitation conditions, and the chemical composition of the aspirated solution. The best intensities to background ratio was obtained when 1400 W was applied. Under these conditions the Mg II/Mg I ratio was 4.9. This ratio was multiplied by 1.8 to compensate the different wavelength response intensities taking into account the use of an Echelle grating and a solid state detector [20]. The found value of 8.8 can be considered robust for ICP operation.

Residual carbon determination

The decomposition of the organic matrix present is critical for various analytical techniques. For this reason, the determination of the residual carbon content is important to evaluate the effectiveness of the sample decomposition procedure. This determination can be done as described in the literature, using urea for preparing aqueous standards for calibration, and ICP OES for measuring the atomic emission signal for C at 193.091 nm [16]. Considering that petrochemical samples are constituted almost of 100% (w w^{-1}) of carbonic compounds, except the oil sludge waste, which contains 16% (w w^{-1}), the residual carbon percentage obtained for the digested solutions of petroleum, diesel, biodiesel and oil sludge waste were 8, 9, 8 and 3% (w w^{-1}), respectively. The low content of residual carbon found in all digested samples confirmed the high efficiency of the proposed sample pre-treatment procedure, using the diluted oxidant mixture with the closed-vessel microwave oven.

The effectiveness of the described closed-vessel microwave digestion procedure is due to the high pressure and temperature achieved in the closed-vessels. In spite of being diluted and in small quantities, the oxidant mixture of HNO_3 + H_2O_2 presents a high oxidizing power, increasing the pressure and temperature inside the closed-vessels during sample digestion [14]. Furthermore, water is a superb dipole molecule suited to interact efficiently with microwave radiation and to increase the heating caused by it, rapidly enhancing the oxidizing power of the HNO_3 + H_2O_2 mixture.

Table II. Method validation using reference materials (NIST SRM 1634c and JM-21) and limits of quantification (LOQ) for the elements studied.

Element	Concentration (μg g^{-1}) ± SD (n=3)				
	JM-21 (found)*	NIST SRM 1634c (reference)	NIST SRM 1634c (found)	LOQ (μg g^{-1})	LOQ (mg L^{-1})
Ag	87±1	ND**	<LD	0.54	0.0018
Al	114±3	ND**	13±2	15.8	0.0527
Ba	124±1	1,8***	1.9±0.1	0.24	0.0008
Ca	110±4	ND**	120±2	0.24	0.0008
Cd	116±1	ND**	<LD	0.42	0.0014
Cr	112±1	ND**	0.71±0.08	0.75	0.0025
Cu	99±1	ND**	1.4±0.4	0.15	0.0005
Fe	128±1	ND**	42±1	0.66	0.0022
Mg	124±1	ND**	11±1	0.45	0.0015
Mn	116±2	ND**	0.56±0.01	0.09	0.0003
Mo	121±1	ND**	<LD	2.19	0.0073
Na	88±2	37±2***	27±2	4.26	0.0142
Ni	118±2	17.54±0.21	17±1	0.27	0.0009
P	123±10	ND**	22±2	14.3	0.0478
Pb	97±6	ND**	<LD	0.84	0.0028
S	ND**	2***	2.3	0.36	0.0012
V	118±1	28.19±0.40	31±1	0.93	0.0031
Zn	120±4	ND**	3.1±0.2	0.27	0.0009

* All reference values are 100 (μg g^{-1}); **ND = not determined; ***Not certified

Analytical figures of merits

The petrochemical samples pre-treatment in a closed-vessel microwave oven allowed reduction of reagent volumes and an increased throughput, since the simultaneous digestion of 16 samples in one heating cycle (79 min of total heating cycle) is possible. The diluted oxidant mixture resulted in digested samples with low acid concentration (1.9 mol L^{-1}), therefore being less aggressive for the instrumental parts of the detection technique.

The quality of the results obtained by using the entire analytical procedure was checked by analyzing two RMs, which were submitted to the same experimental steps adopted for the samples. The comparison between experimental and certified values for all analytes is presented in Table II. They are in good agreement, considering Student's *t* test at a significance level of 95%.

The limits of detection (LOD) and quantification (LOQ) for all analytes are also presented in Table II. Considering a sample mass of 100 mg and a final volume of 30 ml, limits of quantification (10 x LOD) were at a level of 0.09 μg g^{-1} for Mg to 15.8 μg g^{-1} for Al. The average RSD for repeatability of calibration solution measurements were in the range of 1.1% to 6.7%.

Analytical results

The Table III shows the concentration values obtained for the digested solutions of petrochemical samples. Only the Ag concentration was below the detection limit for all samples. For petroleum, diesel and biodiesel, Cd, Mo and Pb concentrations were below the detection limit. The S concentrations in petroleum, diesel and oil sludge waste samples were very high when compared with the concentrations in biodiesel samples.

In the oil sludge waste sample high concentrations of all elements were found. The high concentration of elements attested to the necessity to do a pretreatment of the residue after the extraction of about 16% (w w^{-1}) of oil content.

Conclusions

A simple, robust and reliable analytical procedure for digestion of petrochemical samples, aiming for the elemental determination of Ag, Al, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Na, Ni, P, Pb, S, V and Zn by ICP OES was developed. The oxidizing effectiveness of the organic content present in petrochemical matrices by using a closed-vessel microwave system and a diluted oxidant mixture (4 mL HNO_3 + 2 mL H_2O_2 + 2 mL H_2O) was evaluated and confirmed by RMs analysis. The low residual carbon concentrations found for the digested solutions corroborate the usefulness of the devised sample pretreatment procedure.

The good results allow recommending the proposed method as a simple and accurate procedure for the routine determination of these elements in petroleum, diesel oil, biodiesel, and oil sludge waste samples.

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Table III. Elemental concentrations in the petrochemical samples: P1 = petroleum; W1 = oil sludge waste; D1, D2, D3 and D4 = diesel; and B1, B2 and B3 = biodiesel

	Concentration (μg g^{-1}) ± SD (n=3)								
	P1	W1	D1	D2	D3	D4	B1	B2	B3
Ag	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD	<LD
Al	15±1	3925±38	10±1	2.8±0.1	3.8±0.3	367±5	5.9±0.1	4.2±0.1	1.5±0.1
Ba	<LD	1967±22	<LD	0.28±0.01	0.12±0.01	<LD	<LD	0.53±0.03	0.41±0.05
Ca	95±9	7769±13	<LD	109±3	112±7	<LD	<LD	<LD	4.2±1.9
Cd	<LD	2.6±0.2	<LD	<LD	<LD	<LD	<LD	<LD	<LD
Cr	0.37±0,16	270±2	0.36±0.08	0.35±0.19	0.29±0.14	0.44±0.08	0.63±0.06	<LD	<LD
Cu	<LD	238±2	0.74±0.31	<LD	<LD	<LD	<LD	<LD	<LD
Fe	12±1	26613±132	10±1	1.9±0.2	3.6±0.3	3.1±0.3	0.95±0.24	3.5±0.8	0.44±0.11
Mg	8.1±0.3	1874±10	<LD	4.9±0.5	9.5±0.7	19±1	<LD	<LD	<LD
Mn	0.29±0.01	314±4	0.16±0.01	0.10±0.01	<LD	0.10±0.01	<LD	<LD	<LD
Mo	<LD	11,3±0,5	<LD	<LD	<LD	<LD	<LD	<LD	<LD
Na	195±3	6976±118	18±3	11±1	6.8±1.6	7.9±1.4	2.5±1.2	<LD	<LD
Ni	16±2	63±1	<LD	<LD	<LD	<LD	<LD	<LD	<LD
P	28±2	227±2	31±3	34±3	26±4	35±2	36±3	24±21	25±3
Pb	<LD	171±6	<LD	<LD	<LD	<LD	<LD	<LD	<LD
S	5378±143	19982±130	641±9	1299±9	1118±18	648±6	378±6	334±3	351±3
V	19±1	23±1	<LD	<LD	<LD	<LD	<LD	<LD	<LD
Zn	1.1±0.4	1927±9	0.66±0.39	11±1	0.42±0.14	<LD	<LD	0.95±0.10	0.41±0.10

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PESTICIDE RESIDUES ANALYSIS IN POTATOES BY GAS CHROMATOGRAPHY

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ABSTRACT
This paper describes a gas-chromatography with electron capture detection (GC-ECD) method for determination of some pesticides in potato samples. The extraction of the pesticides was carried out by solid–liquid extraction with clean-up by precipitation at low temperature, without additional steps for removal of interferences. The method requires 3 g of potato and an extraction mixture (6.5 mL acetonitrile, 1.0 mL water and 2.5 mL ethyl acetate) that was established by experimental design. The method was efficient with recoveries of 93.8% for chlorpyrifos, 94.4% for λ-cyhalothrin, 82.1% for cypermethrin and 97.9% for deltamethrin. The quantification limits were 18.7 to 26.4 μg kg^{-1} for the four pesticides. The parameters evaluated in the process of validation, such as selectivity, detection limit, quantification limit, linearity, precision and accuracy, indicated that the methodology is efficient for extraction of the studied pesticides from potatoes. Furthermore, the method was simple, easy to execute, and used only small quantities of organic solvent. The optimized SLE-LTP methodology was applied for the analysis of potatoes randomly purchased from local retail stores and potato samples from a crop treated with commercial insecticide products containing the active ingredients chlorpyrifos and cypermethrin.

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KEYWORDS: Pesticides; CG-ECD; potato

1. Introduction

The potato (*Solanum tuberosum L.*) is one of the most widely consumed food products in the world, being surpassed only by wheat, rice and corn [1]. Various insect species with subterranean habitats have caused severe damage to potato crops, producing perforations and piercings that depreciate their commercial value [2]. Wireworms (*Conoderus* sp.) and cucumber beetle (*Diabrotica* sp.) are the main pests of potatoes [2, 3], and the use of insecticides such as the organophosphate chlorpyrifos [3] and the pyrethroids cypermethrin, deltamethrin and λ-cyhalothrin [4] is necessary. These are the principal active ingredients permitted for use on potato crops in Brazil.

The rational application of these products can significantly reduce problems related to the contamination of foods, water and soils, as well as the intoxication of human beings [5]. However, it has been observed that in order to guarantee the adequate control of pests, diseases and invasive weeds, pesticides have sometimes been applied indiscriminately [6]. The utilization of these compounds in excessive quantities may cause development of resistant species, harming of untargeted species and human intoxication [5], as well as leaving residue levels in foods higher than the maximum residue limits established by regulatory agencies [4, 7].

The maximum residue limits (MRL) acceptable in potatoes for chlorpyrifos, deltamethrin, cypermethrin and λ-cyhalothrin established by ANVISA (National Health Surveillance Agency) [4] are 1.0, 0.01, 0.05 and 0.05 mg kg^{-1}, respectively.

Analysis of pesticide residues is traditionally performed using gas chromatography (GC) or high performance liquid chromatography (HPLC) [8, 9, 10, 11]. The viability of GC analysis depends on an adequate method for sample preparation and generally these steps consist of extraction of the analyte from the matrix, purification for removal of impurities, concentration of the analytes and finally instrumental analysis [10, 12].

Extraction techniques for multi-residue pesticide screening in foods have evolved, with new techniques being developed, including solid phase micro-extraction (SPME) [13,14], matrix solid-phase dispersion (MSPD) [10, 15, 16] and others. However, solvent extraction continues to be the most utilized technique since it is simple and easy to execute. However, various papers have described the optimization of outcomes and minimization of consumption of solvents and clean up steps utilized in this technique [17, 18, 19, 20].

With the purpose of minimizing the number of sample manipulation steps, our research group has developed methodologies for the determination of pesticide residues in different matrices such as water [17], milk [18] and tomato [20] using the method known as liquid-liquid extraction with the low temperature partition technique (LLE-LTP) for liquid matrices and solid-liquid extraction with the low temperature partition technique (SLE-LTP) for solid matrices. The SLE-LTP method was originally utilized in the analysis of residues of organochlorine and organophosphorus pesticides for the removal of fat from extracts of plants, animal tissues and foods [21, 22]. The extraction technique is based on the partition of analytes between a solid matrix and a water-miscible organic phase, at room temperature (25 °C). By decreasing the temperature to – 20 °C, the liquid aqueous phase is solidified, entrapping the solid matrix, and the liquid supernatant consists of organic solvent containing the analytes. This organic extract obtained can be directly analyzed by gas chromatography, with no need for cleanup [20]. This technique was shown to be simple, of low cost and with recovery rates between 80-120% for most compounds, with a minimum consumption of solvent and absence of purification steps before chromatographic analysis of the extracts.

The aim of this work was to optimize and validate the solid-liquid extraction technique with low temperature partitioning (SLE-LTP) for the analysis of residues of the pesticides chlorpyrifos, λ-cyhalothrin, cypermethrin and deltamethrin in potatoes by gas chromatography with electron capture detection (GC-ECD).

2. Experimental

2.1. Reagents and solutions

Solutions of the studied active principles were prepared from standards of chlorpyrifos (99.0 % w/w – Chem Service), λ-cyhalothrin (86.5 % w/w – Syngenta), cypermethrin (92.4 % w/w – Chem Service) and deltamethrin (99.0 % w/w – Chem Service) using acetonitrile as a solvent (Mallinckrodt/HPLC). A bifenthrin (92.2 % w/w – FMC do Brazil) solution, used as an internal standard for the chromatographic analysis, was also prepared in acetonitrile. The solutions were prepared considering the purity of the standards.

2.2. Potato samples

For optimization and validation of the SLE-LTP technique for residue analysis of the pesticides chlorpyrifos, λ-cyhalothrin, cypermethrin and deltamethrin, samples of potatoes free of pesticides were used. These samples were provided by the Associação de Bataticultores do Sul de Minas Gerais (ABASMIG) and the Empresa de Assistência Técnica e Extensão Rural (EMATER-MG).

2.3. Solid-liquid extraction with low temperature purification

2.3.1 Experimental planning of mixtures

For extraction and analysis of the active principles of interest, experimental planning was performed of mixtures limited to a sub-region of the experimental range for mixtures of the three components. The upper and lower limits for each solvent of the extraction mixture were: 10% ≤ Water ≤ 20%; 65% ≤ Acetonitrile ≤ 75% and 15% ≤ Ethyl acetate ≤ 25%. Within these limits, the extraction mixtures maintained the necessary characteristics for the SLE-LTP technique where a single phase is formed and part of the mixture remains in a liquid state at -20 °C. The volumetric proportions of the components used in each of the planning tests are presented in Table I.

Table I. Composition of the extraction mixtures employed in the SLE-LTP of potato samples fortified with the active principles of interest

	Components			
Extraction mixture	Water	Acetonitrile	Ethyl acetate	Total volume of the mixture
	(%)	(%)	(%)	(mL)
1	20	65	15	10.0
2	10	75	15	10.0
3	10	65	25	10.0

In a transparent glass flask with a plastic stopper (30.0 mL capacity), 3.0 g of mashed potato (free of pesticides) was fortified with 0.1 mL of the standard solution containing the four pesticides at a concentration of 50 mg L^{-1}. Then, water, acetonitrile and ethyl acetate were added in accordance with the volumes presented in Table I. The mixture obtained was agitated at 170 rotations per minute (rpm) in an orbital shaker (Tecnal TE - 420) for 10 minutes at 28 °C. The resulting mixture was placed in a freezer at a temperature of approximately -20 °C for 12 hours. After phase separation by the freezing of the aqueous phase and potato pulp, the organic liquid phase was passed through a paper filter containing 1.5 g of anhydrous sodium sulfate (Nuclear - PA), previously washed with acetonitrile left in the freezer at -20 °C. The volumes of the extracts obtained were adjusted in volumetric flasks to 10.0 mL with acetonitrile and stored in glass flasks at -20 °C until analysis by GC-ECD. For GC-MS analysis the extracts (10.0 mL) were dried in a rotary evaporator and reconstituted in 1.0 mL of acetonitrile. Extracts from pesticide-free potato samples (blank) were obtained in a similar way.

2.3.2. Chromatographic analysis

Quantitative and qualitative analysis of the active ingredients was performed on a Shimadzu GC-17A gas

chromatograph equipped with an Agilent Technologies HP-5 capillary column with a stationary phase composed of 5% phenyl and 95% dimethyl-siloxane (30 m x 0.25 mm i.d., 0.1 μm film thickness) and an electron capture detector (ECD). The analytical chromatographic conditions were: injector temperature of 280 °C, column temperature of 200 °C with heating rate of 10 °C per minute until reaching 290 °C, maintaining this temperature for 2 min. The detector (ECD) temperature was 300 °C. The injection volume was 1.0 μL, with carrier gas (N_2) flow of 1.2 mL min^{-1} and split ratio of 1:5.

For confirmation of the results, gas chromatography with mass spectrometry (Shimadzu GC-MS-QP5050A) was used, equipped with an AOC-5000 auto-injector and a HP-5 capillary column (Agilent Technologies), with stationary phase of 5% phenyl and 95% dimethyl-siloxane (30 m x 0.25 mm i.d., 0.1 μm of film thickness), and helium as the carrier gas (1.2 mL min^{-1}). The chromatographic conditions were: injector temperature of 280 °C, initial column temperature of 200 °C with heating rate of 10 °C min^{-1} until reaching 290 °C, keeping this temperature for 5 minutes. The transferline temperature was 290 °C and a splitless injection was used. The injection volume was 1.0 μL. The mass detector was operated with electron ionization impact (70 eV), with a scan range from 30 to 600 *m/z*. The SIM mode was also used. Identification of the components in the extracts was performed by comparison to spectral data in the library (Wiley 330,000) and with standard solutions.

2.3.3. Univariate modifications

The optimized extraction mixture for the planning of some experimental parameters was tested with the objective of improving its extraction efficiency. The time of ultrasound (1, 3, 5, 10 and 15 minutes), ionic strength effect (evaluated by replacing the water added to the mixture extraction by an equal volume of 0.2 mol L^{-1} NaCl solution, so as not to disturb the single phase equilibrium), and fortification time (0, 3, 5, 7, 24, 168 and 432 hours) were evaluated for the extraction of pesticides in potato.

2.4. Method validation

The analytical performance parameters used for validation of the separation method and optimized analysis were: selectivity, linearity, detection and quantification limits, precision (repeatability and intermediate precision) and accuracy (recovery tests and comparison of methods). The procedures performed were based on recommendations of the National Institute of Metrology, Standardization and Industrial Quality [23], National Health Surveillance Agency [4] and International Conference on Harmonisation [24].

Selectivity was evaluated by comparison of chromatograms from extracts obtained from potato samples free of the pesticides chlorpyrifos, λ-cyhalothrin, cypermethrin and deltamethrin to those obtained from fortified samples submitted to the optimized SLE-LTP method.

The linear response of the electron capture detector was determined by the injection of standard solutions of the pesticides chlorpyrifos, λ-cyhalothrin, cypermethrin and deltamethrin in acetonitrile with increasing concentrations (6.0, 10.0, 25.0, 50.0, 125.0, 250.0, 375.0, 500.0, 650.0 and 750.0 μg L^{-1}), using bifenthrin (500 μg L^{-1}) as an internal standard.

The limit of detection for the proposed method was determined by considering the signal producing three times the baseline noise obtained for pesticide-free potato samples (blank) and analyzed by GC-ECD. The limit of quantification was determined by considering a signal at least 10 times greater than the noise.

Repeatability of the SLE-LTP method was determined by performing the extraction of the pesticides in fortified potato samples (0.05 μg g^{-1}) with seven repetitions. Determination of the intermediate precision was performed in triplicate by the same analyst using extracts of the four pesticides in the fortified potato samples (0.05 μg g^{-1}) at four different time periods (1, 7, 30 and 60 days).

Accuracy was evaluated in triplicate for recovery tests in three concentrations (0.05; 0.1 and 0.5 μg g^{-1}) and by comparison of the results with those obtained by the multi-residue method utilized by the IMA (Instituto Mineiro de Agropecuária, the government institute responsible for pesticide residue control) for analysis of pesticide residue in diverse food products such as potatoes, carrots, strawberries and others. This method is based on Welfare and Sport Analytical Methods for Pesticide Residues in Foodstuffs, described by the Ministry of Public Health of the Netherlands [25]. In this method 15.0 g of sample are extracted with 40.0 mL of acetone and 60.0 mL of dichloromethane and hexane (1:1) for 10 minutes in an ultrasonic bath. After extraction, 10 mL of the extract is filtered and evaporated to complete dryness, and residues are recovered in 1.0 mL hexane for the chromatographic analysis.

2.5. Application of the optimized methodology to potato samples

The optimized and validated method was applied to samples harvested in the southern region of Minas Gerais state, Brazil. Seven samples from a crop which received the recommended doses of commercial insecticides containing chlorpyrifos and cypermethrin were submitted to the optimized methodology. This methodology was also applied to samples of potatoes purchased from local retail stores (Viçosa, MG, Brazil). For each sample, about one kilogram of potatoes was

taken to the laboratory and stored in a freezer until the analysis was performed. Samples containing pesticides above the MLR were analyzed by GC-MS to confirm the results. Furthermore, samples of potatoes in which pesticide residues were detected were peeled and the pulp and peel analyzed separately. A portion of the residue containing samples was also submitted to a cooking process and then peeled. The peel and pulp were then analyzed.

All analyses were performed in triplicate.

3. Results and discussion

3.1. Chromatographic Analysis

Figure 1 represents the chromatogram of the GC-ECD analysis of a standard solution of the pesticides in acetonitrile at the established conditions. The peaks with t_R equal to 4.1, 6.9, 8.1 and 9.5 minutes correspond to chlorpyrifos, λ-cyhalothrin, cypermethrin and deltamethrin, respectively. The second peak, with a retention time of 6.3 minutes corresponds to bifenthrin, the internal standard. The presence of a second peak for each of the pyrethroids is related to the conversion of isomers during injection into the chromatograph [25]. Therefore, the sum of the peak areas of both isomers was considered for quantification of each pyrethroid.

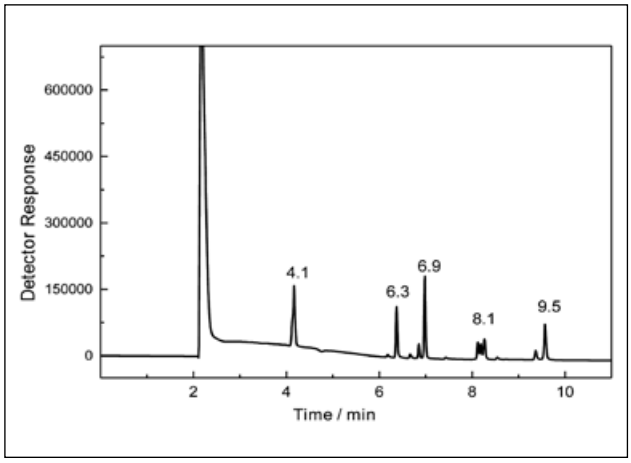

Figure 1. Chromatogram of a 500.0 μg L^{-1} standard solution of the active ingredients in acetonitrile, where t_R = 6.3 min: internal standard, t_R = 4.1 min: chlorpyrifos, t_R = 6.9 min: λ- cyhalothrin, t_R = 8.1 min: cypermethrin and t_R = 9.5 min: deltamethrin.

Quantification of pesticides in the extracts was performed by employing the internal standardization method. Calibration curves of each pesticide were constructed for the concentration interval of 6.0 to 750.0 μg L^{-1}, obtaining correlation coefficients (r) greater than 0.99.

3.2. Optimization of the SLE-LTP method

In the optimization process of the SLE-LTP technique, analyses were performed of the pesticides chlorpyrifos, λ-cyhalothrin, cypermethrin and deltamethrin in potatoes. Some factors which influenced the results of the technique, such as type of extraction mixture, time of ultrasound, the ionic strength effect and fortification time were evaluated using fortified potato samples.

The experimental planning of the mixtures was performed on the basis of volumetric proportions of water, acetonitrile and ethyl acetate, determined by the upper and lower limits of each solvent in the extraction mixture established in preliminary studies (Figure 2). Response surface models, obtained with the assistance of the Statistica 6.0® program (StatSoft) as functions of the pseudocomponents (Figure 2), show the recovery percentage estimates obtained for each pesticide.

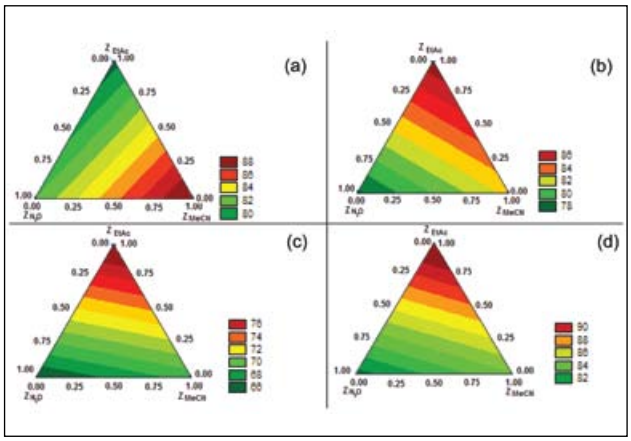

Figure 2. Response surface models showing recovery percentage estimates for chlorpyrifos (a), λ- cyhalothrin (b), cypermethrin (c) and deltamethrin (d), as functions of the pseudo-components (Z_i). Z_{H2O} = mixture 1, Z_{MeCN} = mixture 2, Z_{EtAc} = mixture 3, Table I.

It is noted for clorpyrifos that the best response was obtained with pseudocomponent 2, Z_{MeCN}, composed of 1.0 mL water, 7.5 mL acetonitrile and 1.5 mL ethyl acetate, with 88% recovery percentage. For the pyrethroids, the best response was obtained with pseudocomponent 3, composed of 1.0 mL water, 6.5 mL acetonitrile and 2.5 mL ethyl acetate. In Figures 2b, 2c and 2d, the recovery percentages obtained when employing pseudo-component 3 were 86% for λ- cyhalothrin, 76% for cypermethrin and 90% for deltamethrin. More polar extraction mixtures favored the extraction of chlorpyrifos and less polar mixtures the extraction of pyrethroids. However, as the response obtained with pseudocomponent 3 was more suitable for extraction of most insecticides, it was selected as the extraction mixture. After defining the best extraction phase, some experimental parameters were tested with the objective of improving extraction efficiency.

The time of ultrasound was evaluated in this study. The recovery percentage of the pesticides in all

evaluated intervals was greater than 70%. An increase in extraction efficiency was observed for the four active principals after 6 minutes of sonication, with 10 minutes being the chosen time for the extraction stage.

The variation of ionic strength produced by using 0.2 mol L^{-1} aqueous NaCl solution did not affect the percentage of pesticides extracted.

The "interaction" time of the pesticides with the matrix during the fortification process was also evaluated. The evaluated times were 0, 3, 5, 7, 24, 168 and 432 hours after application of the standard solution containing the four active principles to the potato pulp. The greatest recovery percentages were obtained within the interval of 0 to 7 hours following fortification. After this period the stability of the compounds, principally the pyrethroids, in the matrix was low, with a rapid degradation (75%) of deltamethrin observed one day after fortification, and approximately 50% of λ- cyhalothrin and cypermethrin and 25% of chlorpyrifos were also degraded during this time. After one week degradation became much slower and no significant changes were observed between the results obtained after 168 and 432 hours after fortification. This behavior of the pesticides in potatoes is similar to that which occurs when the pyrethroids deltamethrin, λ- cyhalothrin, permethrin and cypermethrin were introduced in water samples [17]. The fortification time of the samples employed in the studies was 3 hours.

The optimized method consists of adding 1.0 mL of distilled water, 6.5 mL of acetonitrile and 2.5 mL of ethyl acetate to the 3.0 g potato pulp sample. The system is exposed to ultrasound for 10 min and frozen at -20 °C for 12 h. The liquid phase containing the extracted material is filtered through a layer of anhydrous sodium sulfate (1.5 g), recovered to 10 mL and analyzed by gas chromatography.

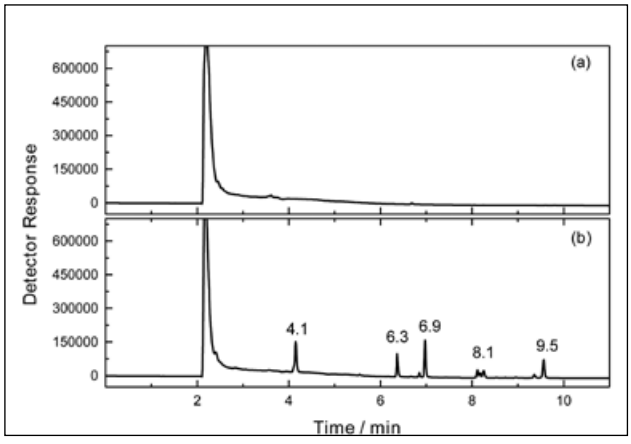

Figure 3. (a) – Chromatogram of an extract obtained from potato pulp free of the active ingredients and (b) – chromatogram of an extract from potato pulp fortified with 500 μg L^{-1} of the studied pesticides, in which: t_R = 6.3 min: internal standard, t_R = 4.1 min: chlorpyrifos, t_R = 6.9 min: λ-cyhalothrin, t_R = 8.1 min: cypermethrin e t_R = 9.5 min: deltamethrin.

3.3. Method Validation

Selectivity was evaluated by comparing the chromatograms of the matrix free of pesticides with extracts from the matrix fortified with the compounds (Figure 3). No interferences were observed with retention times near those of the compounds of interest. An important advantage of the solid-liquid extraction technique with low temperature purification is that the extract obtained is free of matrix interferences. Similar results were encountered for the extraction of pesticides in water [17] and other food products [18, 19].

In the study of linearity (concentration interval of 6 to 750 μg L^{-1}), coefficients of determination greater than 0.99 were obtained for all pesticides studied.

The detection and quantification limits for the proposed method are shown in Table II.

Table II. Linear (s) and angular (S) coefficients of the analytical curves, detection (DL) and quantification (QL) limits and maximum residue limit (MRL) for the four studied pesticides.

Pesticides	s	S	DL (μg kg^{-1})	QL (μg kg^{-1})	MRL (μg kg^{-1})
Chlorpyrifos	0.00357	0.00414	5.7	18.8	1000
λ- Cyhalothrin	0.00487	0.00401	7.9	26.1	50
Cypermethrin	0.00303	0.00225	7.9	26.1	50
Deltamethrin	0.00146	0.00168	5.7	18.8	10

The detection limits for each pesticide, determined using the proposed method, were between 5.7 and 7.9 μg kg^{-1} (1.7 and 2.4 μg L^{-1} in the extract) and quantification limits between 18.8 and 26.1 μg kg^{-1} (5.7 and 7.9 μg L^{-1} in the extract). Therefore, it is important to mention that the quantification limit of the proposed method was able to evaluate these active ingredients below the range of their MRL, with the exception of deltamethrin. In the case of chlorpyrifos, the quantification limit of this method showed to be notably lower than the MRL.

The precision of the SLE-LTP method was evaluated by levels of repeatability and intermediate precision for the analytes at a concentration of 50 μg kg^{-1}. Estimates of the relative coefficient of variation (CV) based on seven repetitions varied between 2.4 and 2.8%. Intermediate precision of the method was evaluated within the same laboratory on different days (1 day, 1 week, 1 month and 2 months) by the same analyst. The CV values obtained for all compounds varied from 1.2 to 5.5%.

In the study of accuracy, recovery tests were performed by adding pesticides to mashed potato samples at concentrations nearly 2, 5 and 20 times (15.0, 30.0 and 150.0 μg L^{-1}) the encountered quantification limit (QL). The method was efficient with recoveries of 93.8% for chlorpyrifos, 94.4% for λ-cyhalothrin, 82.1% for cypermethrin and 97.9% for deltamethrin. The method presented good accuracy since all obtained recovery percentages at the three levels did not differ significantly among themselves at the level of 95% probability in accordance with the t-test, and the coefficients of variation (CV) were less than 5%, independent of the concentration.

Considering the MRL of the pesticides of interest in potatoes established by ANVISA (1, 0.05, 0.05 and 0.01 μg g^{-1} for chlorpyrifos, λ -cyhalothrin, cypermethrin and deltamethrin, respectively) and recovery percentages obtained at the level of fortification of 0.05 μg g^{-1} (15.0 μg L^{-1} in the extract), it was concluded that all pesticides were efficiently extracted from the matrix.Despite the good efficiency of the extraction of deltamethrin, the LQ of this compound was two times greater than the MRL established by ANVISA.

The results obtained by employing the SLE-LTP method in fortified potato samples were compared to the results obtained by the method utilized by IMA (Instituto Mineiro de Agropecuária) for extraction of the pesticides chlorpyrifos, λ-cyhalothrin, cypermethrin and deltamethrin in food products (Table III).

Table III. Recovery percentages (%R), standard deviation estimate and coefficient of variation, CV(%), of the pesticides chlorpyrifos, λ-cyhalothrin, cypermethrin and deltamethrin in fortified potato samples, employing the SLE-LTP method and the method utilized by IMA.

	Chlorpyrifos		λ- Cyhalothrin		Cypermethrin		Deltamethrin	
	%R	CV(%)	%R	CV(%)	%R	CV(%)	%R	CV(%)
SLE-LTP	93.8	2.4	94.4	2.4	82.1[a]	2.6	97.9	2.8
IMA Method	98.5	4.4	87.0	5.3	81.9[a]	4.5	80.0	3.2

(a) results statistically similar at a 95% probability level according to the t-test.

Analyzing the degree of proximity between the results obtained by the two methods (Table III), it was observed that λ-cyhalothrin and deltamethrin were best extracted by the SLE-LTP technique, while the recovery percentages obtained for cypermethrin were statistically the same. However, the two methods did not show significant dispersion between the two sets of results.

The multi-residue method employed by the laboratories of IMA basically consist of a solid-liquid extraction using acetone, dichloromethane and hexane as solvent extractors. This method requires a large volume of organic solvent (100.0 mL) and various sample preparation steps, characteristics that increase the cost of analysis and decrease laboratory productivity. The use of dichloromethane, a solvent incompatible with the electron capture detector, requires an evaporation stage and changing of solvent. Dichloromethane also presents high toxicity, inflammability and environmental risks. Acetonitrile employed in the SLE-LTP is toxic as well, but according to Maštovská and Lehotay [26], the negative impacts caused by this solvent to human health and to the environment are lower than those caused by chlorinated solvents such as dichloromethane. According to the US Environmental Protection Agency (EPA), acetonitrile is not classified as carcinogenic to human beings and is less persistent in the environment. Its low volatility also reduces exposure of analysts to vapor during sample preparation.

Advantages of the IMA method include its use of an extract concentration factor of 10, which may contribute to better detectability and the fact that it can be used with different matrices (carrots, strawberries, potatoes, etc.) for analysis of a wide range of pesticides of different chemical classes.

In contrast, the SLE-LTP technique presents a lower consumption of solvent (10.0 mL), and does not require an evaporation step and solvent change, reducing the risks of contamination and loss of analytes of interest. This provides more precise results which can be observed (Table III) from the coefficients of variation which are slightly lower when the SLE-LTP methodology is employed.

3.4 Application of the optimized SLE-LTP methodology to potato samples harvested in the southern region of Minas Gerais state, Brazil.

Pyrethroid residues were not detected in any of the analyzed samples.

In three samples from a crop that received the recommended doses of commercial insecticides containing chlorpyrifos and cypermethrin, chlorpyrifos residues (50 μg kg^{-1}, 230 μg kg^{-1} and 6.5 μg kg^{-1}) were detected at levels below the MRL established for potato by Brazilian Legislation (1000 μg kg^{-1}).These results were confirmed by GC-MS for two samples. In addition to retention time, the ion *m/z* values of 97, 197, 314 and 349 were also used for identification of chlorpyrifos.

In samples of potatoes purchased from local retail stores in Viçosa, MG, Brazil clorpyrifos residues were detected at levels below the QL.

Samples of potatoes in which quantifiable pesticide residues were detected were peeled and the pulp and peel analyzed separately. Residues of chlorpyrifos were not detected in the pulp. However, in the peel, chlorpyrifos concentrations higher than 970 μg kg^{-1} were

found, a value four times higher than that observed in whole potato samples.

Submitting samples with chlorpyrifos residues to a cooking process did not eliminate the contamination. High temperatures did not degrade the pesticide. The levels found in the peel after cooking were 860 and 350 μg kg^{-1} for those samples in which contamination was detected.

4. Conclusions

The SLE-LTP methodology was shown to be efficient for analysis of the pesticide residues studies with recovery percentages between 80-120%. Other advantages of this technique included low solvent consumption, relatively pure extracts and the fact that no clean-up steps are required before chromatographic analysis.

The parameters evaluated in the process of validation, such as selectivity, detection limit, quantification limit, linearity, precision and accuracy, indicate that the methodology is efficient for extraction of chlorpyrifos, λ-cyhalothrin, cypermethrin and deltamethrin residues from potatoes with detection limits lower than the MRL established for these pesticides in this food product.

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Determination of flunarizine in plasma by liquid chromatography-electrospray tandem mass spectrometry

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Abstract

This study presents a sensitive and selective liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantification of flunarizine (FLU) in human plasma. The mass spectrometer was operated in the selected reaction monitoring (SRM) mode using the protonated molecule to ionic fragment combinations of m/z 404.50 > 203.00 (FLU) and m/z 369.40 > 167.00 for cinarizine (internal standard-IS) with short run times (2.5 min). The peak areas ratio of the analyte and IS were used for quantification of FLU. The limit of quantification (LOQ) was 0.30 ng mL^{-1}. The assay exhibited a linear dynamic range of 0.30-150.00 ng mL^{-1} with a determination coefficient (r^2) of at least 0.98. The precisions were less than 8.42% and 6.36% for the intra-batch and inter-batch variation at 0.90, 60.00 and 120.00 ng mL^{-1}, respectively. This method is suitable for bioequivalence studies accordingly to FDA guidelines.

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Keywords: flunarizine; cinarizine; LC-MS/MS; bioequivalence; liquid-liquid extraction.

Introduction

Flunarizine, 1-[bis (4-fluorophenyl)methyl]-4-(3–phenyl–2–propenyl) piperazine (CAS 52468-60-7] is a derivative of cinarizine discovered at Janssen Pharmaceutica in 1967 (Figure 1). Both cinnarizine and flunarizine are calcium channel blocking drugs, but flunarizine is 2.5 to 15 times stronger than cinarizine in the frequency of drug-induced Parkinsonism [1].

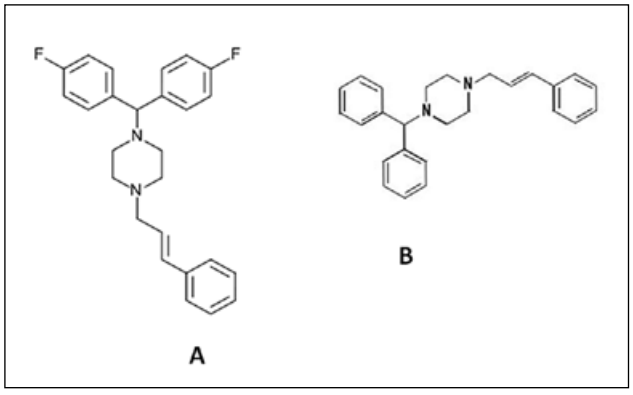

Figure 1. Chemical structure of (A) flunarizine and (B) cinarizine.

It is effective in the prophylaxis of migraine, occlusive peripheral vascular disease, and vertigo of central and peripheral origin and as an adjuvant in the therapy of epilepsy. Flunarizine is well absorbed after oral administration, reaching peak plasma levels within 2-4 hours and reaching steady state at 5-6 weeks. After extensive hepatic metabolism, flunarizine and its metabolites are excreted through the feces via the bile. The mean terminal elimination half-life is about 18 days. Plasma protein binding is 99% [2-5].

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the state of–the-art in quantification of drugs in biological matrices due to improved detectivity, selectivity and speed of this technique. The LC-MS/MS system used in this study is one of the most utilized analytical tools for determining pharmacokinetic parameters of a drug with high precision and reliability [6, 7].

Other techniques have been used previously to analyze flunarizine in a variety of matrices. These methods include gas chromatography coupled to mass spectrometry (GC-MS) [8,9] and LC-MS [10]. Some of these techniques were not developed for use in biological matrices, while others have either a very high limit of quantification (LOQ) or are much too complex, limiting their application for a large number of samples. The use of LC-MS/MS to determine flunarizine levels above the lower limit of quantified plasma levels is already described, but the

applicability of the process is questionable since it has a long run time and low recoveries [11].

Thus, the aim of this study was to describe a sensitive and selective liquid chromatography-electrospray (ES) tandem mass spectrometry method for flunarizine quantification in biological samples (human plasma), enabling a low limit of quantification and a short run time.

Methodology

Chemicals

Flunarizine (FLU) was obtained from SIMS Trading (Firenze, Florence, Italy), and cinarizine (IS) was obtained from the European Pharmacopeia (Strasbourg, France). Sodium hydroxide and HPLC grade hexane were purchased from Merck (Darmastadt, Germany), and ammonium acetate and acetonitrile were from J.T. Baker (Phillipsburg, NJ, USA). Water was purified using a Milli-Q water purification system from Millipore (Bedford, MA, USA). All other chemicals were of analytical grade and used without further purification.

LC–MS-MS analysis—apparatus and chromatographic conditions

Mass spectrometric detection was performed using a Varian LC 1200 triple quadrupole mass spectrometer, equipped with electrospray source. The temperatures of the desolvation gas and source block were 270°C and 42°C, respectively. Nitrogen was used as both a nebulizer (55 psi) and desolvation gas (26 psi). The electrospray source was operated in the positive ionization mode (ES+) at 5000 V, in selected reaction monitoring mode (SRM), and m/z 404.50 > 203.00 and m/z 369.40 > 167.00 channels were used for quantification of FLU and IS, respectively. Collision energy and collision gas pressure (argon) were 15.50 V and 4.20 mtorr, respectively.

An aliquot (10 μL) of the extracted plasma reconstituted in methanol was injected onto a Polaris C18 (50 mm x 20 mm, 5 μm) column in a Varian Prostar LC. Separation and elution were achieved using acetonitrile:ammonium acetate, 10 mmol L^{-1} (9:1, v/v) as the mobile-phase at a flow-rate of 0.22 mL/min. The chromatography was performed at ~20°C, with a total injection time of 2.50 min (run time of 2.0 min, autosampler of 0.5 min).

Sample preparation

The samples were stored as 300 μL aliquots at -20 °C and these were thawed to room temperature before processing. Fifty microliters of 0.05 mol L^{-1} NaOH and 25 μL aliquot of internal standard (IS) solution (1 μg/mL of cinarizine) were added to 300 μL of plasma, and the tube was briefly shaken. The mixture was then vortex-mixed with 1000 μL of hexane/ethyl acetate (1:1, v/v) for 10 min at 1800 rpm. The tube was centrifuged for 5 min at 14000 rpm, and the organic phase (800 μL) transferred to another tube and evaporated to dryness under a nitrogen stream at room temperature. The residue was dissolved in 600 μL of acetonitrile. The sample was transferred to the glass autosampler vial and 10 μL was injected into the chromatographic system.

Preparation of standards and quality control (QC)

Stock solutions of FLU (100000, 1000, 100, and 10 ng/mL) and IS (100 μg/mL) were prepared in acetonitrile. Aliquots of the 1000, 100, and 10 ng/mL solutions were used to spike blank human plasma in order to obtain calibration standards of 0.30, 1.50, 5.00, 15.00, 50.00, 75.00 and 150.00 ng/mL. Three levels of quality controls fixed at 0.90, 60.00, and 120.00 ng/mL (low, medium and high) were prepared using the same blank plasma. All spiked plasma was stored at -20 °C.

Recovery

The extraction efficiency of FLU from human plasma was determined using quality control samples. The recovery at three concentrations was determined by comparing peak areas obtained from plasma and standard solution spiked with the blank plasma residue. The recovery of (IS) was also tested using the same procedure.

Limit of quantification

The limit of quantification was defined as the lowest concentration at which precision and accuracy, expressed by relative standard deviation (R.S.D.) were within 20% of the true value.

Analytical curves

The analytical curves were constructed using values ranging from 0.30 to 150.00 ng/mL of FLU in human plasma. Calibration curves were obtained by weighted linear regression (weighting factor: 1/x), and the ratio of FLU peak area to IS peak area was plotted versus the FLU concentration in nanograms per milliliter. The suitability of the calibration model was confirmed by back-calculating the concentrations of calibration standards.

Accuracy and precision

Quality controls of FLU (0.90, 20.00 and 40.00 ng/mL) were determined using the corresponding standard curves. The method´s accuracy was shown as error (RE) and calculated based on the difference between the mean calculated and nominal concentrations, whereas precision was evaluated by calculating the within- and between-run relative standard deviations.

Freezing and thawing stability

Freezing and thawing stability for flunarizine in plas-

ma samples in three cycles was studied with control concentrations in quintuplicate. Samples were frozen at - 20°C and thawed in three cycles of 24, 36 and 48 h. In addition, the long-term stability of FLU in QC samples was also evaluated by analysis after 134 days of storage at - 20°C. Autosampler stability was studied over a 12-24 h storage period in the autosampler rack with control concentrations.

Results

Selectivity

The analysis of flunarizine and cinarizine using the selective reaction monitoring (SRM) function was highly selective, with neither interfering compounds nor significant ion supression from endogenous substances observed at the retention times for flunarizine and IS, as shown in Figure 2.

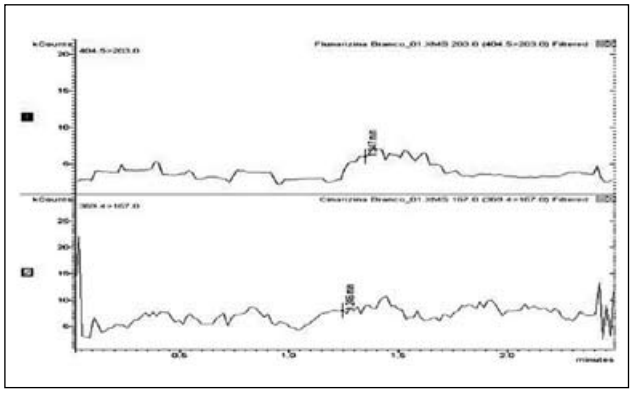

Figure 2. Representative SRM chromatograms of flunarizine and cinarizine in blank human plasma.

Figure 3 shows the mass spectrum of FLU in the sequential mode: m/z 404.60 generating ion fragments at m/z 202.90 at collision energies of 20 eV. The intensities of other fragments are negligible.

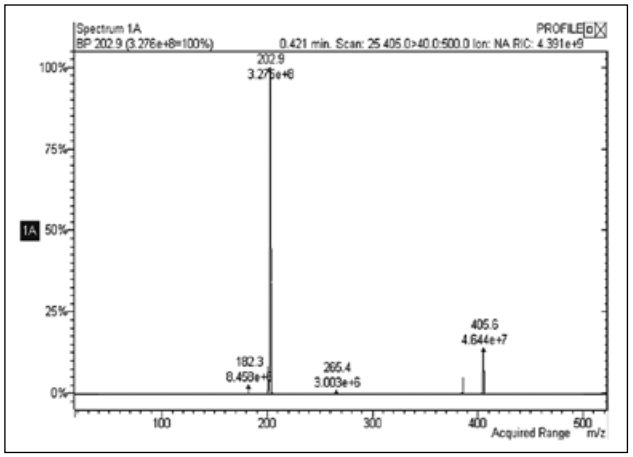

Figure 3. MS-MS spectra of flunarizine base peak at m/z 404.6 and daugther peak m/z 202.9. Collision energy 20eV.

Chromatograms obtained from plasma spiked with flunarizine (0.30 ng/mL) are shown in Figure 4.

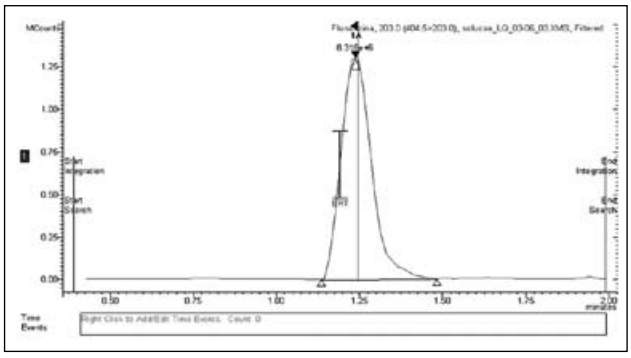

Figure 4. Chromatogram of flunarizine obtained from plasma spiked with 0.30 ng/mL.

The matrix effect was evaluated directly by extracting blank plasma and then spiking it with the analyte at the LOQ concentration. There was no difference observed in the signal for the solution and the spiked extract at the LOQ concentration.

Linearity, precision and accuracy

Calibration curves were plotted as the peak area ratio (flunarizine/cinarizine) versus flunarizine concentration. The assay was linear in the concentration range of 0.30-150.00 ng/mL; R.S.D.s were less than 10%. The relative error of the mean of measured concentrations ranged from 7.33 to 9.00%. The determination coefficients (r^2) were greater than 0.98 for all curves (Table I).

Table I. Calibration curve from batch one during method validation.

Spiked plasma concentration (ng/mL)	Concentration measured (mean) (ng/mL)	R.S.D. (%)[a] (n=3)	Relative error [b]
0.30	0.29	1.72	-3.33
1.50	1.58	6.83	5.33
5.00	5.30	5.20	6.00
15.00	15.37	1.23	2.46
50.00	47.78	2.74	-4.44
75.00	69.48	2.07	-7.36
150.00	148.56	6.83	- 0.96

[a] standard deviation / mean concentration measured
[b] [(Mean concentration measured – spiked plasma concentration) / spiked plasma concentration] × 100.

Precision and accuracy for this method were controlled by calculating the intra-batch and inter-batch variation at three concentrations (0.90, 60.00 and 120.00 ng/mL)

Table II. Precision and accuracy (analysis with spiked plasma samples at three different concentrations)

Spiked plasma concentration (ng/mL)	Within-run			Between-run		
Relative (%) (ng/mL)	Concentration Mesaured (mean ± S.D.)	R.S.D. (%) [a] (n=5)	Relative error (%)[b]	Concentration Mesaured (mean ± S.D.)	R.S.D. (%) [a] (n=5)	Relative error (%)[b]
0.90	0.98 ± 0.08	8.41	9.55	0.93 ± 0.05	5.56	3.77
60.00	61.28 ± 1.45	2.38	2.15	63.81 ± 2.22	3.48	6.36
120.00	113.33 ± 4.69	4.14	-5.55	118.72 ± 5.05	4.25	-1.06

[a] Standard deviation / mean concentration measured

[b] [(Mean concentration measured - Spiked plasma concentration) / Spiked plasma concentration] x 100.

Table III. Freeze and thaw stability of the samples

Sample Concentration (ng/mL)	Initial (0h)		Cycle 1 (24h)		Cycle 2 (36h)		Cycle 3 (48h)	
	Concentration measured (mean ±SD) (ng/mL)	R.S.D[a] (%) (n=5)	Concentration measured (mean ±SD) (ng/mL)	R.S.D[a] (%) (n=5)	Concentration measured (mean ±SD) (ng/mL)	R.S.D.[a] (%) (n=5)	Concentration measured (mean ±SD) (ng/mL)	R.S.D.[a] (%) (n=5)
0.90	0.85 ± 0.05	6.91	0.89 ± 0.10	11.26	0.89 ± 0.06	7.60	0.93 ± 0.06	6.52
60.0	56.87 ± 4.61	8.11	57.01 ± 5.09	8.94	59.99 ± 5.58	9.31	57.22 ± 2.24	3.92
120.0	109.53 ± 5.81	5.31	104.02 ± 1.81	1.75	104.48 ± 2.18	2.09	115.47 ± 3.63	3.15

[a] Standard deviation / mean concentration measured.

of QC samples in five replicates. As shown in Table II, the intra-batch R.S.D.s and Res were less than 8.41% and the inter-batch ones were less than 6.36%. These results indicate that the method is reliable and reproducible within its analytical range.

Freezing and thawing stability

The results of freeze-thaw stability studies are shown in Table III. Quantification of the analyte in plasma subjected to a number of freeze-thaw (-20°C to room temperature) cycles showed that the analyte is stable after three cycles. No degradation of the analyte occurred after a 24 h storage period in the auto sampler rack, with final concentrations of flunarizine ranging from 94.75 to 103.85% of the theoretical values. In addition, the long-term stability of flunarizine in QC samples after 134 days of storage at -20°C was also evaluated. The concentrations ranged from 91.28 to 96.23% of the theoretical values.

Recovery

The recovery levels of FLU, which were determined at three different concentrations (0.90, 60.00 and 120.00 ng/mL) were 90.10, 90.00 and 85.50%, respectively; the overall average recovery was 88.90%.

Discussion

Among the different possible detection techniques that can be coupled to LC, mass spectrometry has been unparalleled for performing bioanalytical determinations with maximum selectivity and detectivity [12-14].

The rough ion source MS optimization was performed by direct infusion of solutions of both flunarizine and cinarizine into the ESI source of the mass spectrometer. The critical parameters in the ESI source includes the needle (ESI) voltage, which is directly related to charged droplet formation and to the amount of gaseous ions formed. Capillary voltage (the tube that connects the atmospheric pressure side to the vacuum side) is related to gaseous ion guidance on the inside of the last barrier between atmospheric pressure and the high vacuum of the mass spectrometer. Other parameters, such as the nebulizer and the desolvation gases, are meant to be optimized in order to obtain better spray shape, resulting in better ionization and droplet drying for, in our case, the protonated ionic flunarizine and cinarizine molecules.

A collisionally activated dissociation (CAD) product ion spectrum for flunarizine and IS yielded high-abundance ions of m/z 203.0 and m/z 167.0. After the SRM channels were tuned, the mobile phase was changed to obtain a fast and selective LC method. A better signal was obtained using acetonitrile:ammonium acetate, 10 mmol L^{-1} (9:1, v/v) as mobile phase.

The method´s selectivity was analyzed by the matrix effect directly by extracting blank plasma and then

spiking it with the analyte at the LOQ concentration. There was no difference observed in the signal for the solution and for the spiked plasma extract at the LOQ concentration and the signal observed in blank plasma was below 20% of the analyte signal at the LOQ concentration.

The recovery for sample preparation, which involved a liquid-liquid extraction with hexane/ethyl acetate (1:1, v/v), was calculated by comparing the peak area ratios of flunarizine in plasma samples with the peak area ratios of solvent samples, and it was estimated at control levels of analyte.

There have also been several other reports on analytical techniques to measure flunarizine in human plasma. In this study an analytical method of flunarizine determination using LC–MS-MS has been developed. This new technique presented some advantages over others previously described, such as: simple sample preparation (liquid-liquid extraction), small sample volume of 300 µL, low limit of quantification (0.3 ng/mL) and short run time allowing the analysis of a large number of samples per day. Moreover, this new method has an analytical frequency very suitable for large numbers of samples such as demanded by bioequivalence analysis, and the quantification limit is better.

Conclusion

The described method has proven to be rapid and robust, with each sample requiring less than 2.5 min of run time. This assay method is also highly specific due to the inherent selectivity of tandem mass spectrometry. The technique possesses significant advantages over others previously described for the measurement of flunarizine in biological fluids while the detectivity of the assay is sufficient to accurately follow this drug's pharmacokinetics.

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Chemometric and analytical strategies for the study of soils from maritime Antarctica

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Abstract

In this study, some analytical and chemometric strategies were used for the comparison of chemical features of soil samples collected near the Brazilian Antarctic Station and sites further from it. As a preliminary analysis, synchrotron radiation X-Ray Fluorescence was employed. Besides, the soil characteristics: total organic carbon, soil pH and bioavailable/pseudototal concentrations of Cd, Cr, Ni and Pb were determined. In order to achieve both high analytical throughput and sensitivity over the conventional monoelement flame AAS, Slotted Tube Atom Trap (STAT) was combined with FS-FAAS for the sequential determination of Cd and Pb in the aqua regia soil extracts. Thermospray Flame Furnace AAS was also used for Cd and Pb quantification in DTPA soil extracts using the monoelement mode. Furthermore, a sequential extraction procedure was applied for Cu, Mn and Zn determination. Copper, Mn, Ni and Zn were determined by FAAS in the fast-sequential mode which allows reduction in the analysis time and minimization of sample consumption and waste generation. By the use of Principal Component Analysis and Hierarchical Cluster Analysis, a clear differentiation was observed between the sampling sites. This finding indicates anthropogenic disturbance by the scientific station, mainly through Pb, Cr and Ni deposition. However, the concentrations for the most impacted sites do not exceed guidelines for soil quality, except for Cr. Nevertheless, mitigation and treatment of contaminated areas could be important for preservation of the local ecosystem.

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Keywords: Soil contamination; Antarctica; Fast-sequential AAS; Chemometrics; STAT-FAAS; TS-FF-AAS

1. Introduction

Due to its pristine condition, Antarctica permits researchers to observe an environment almost free of human activities. The study of changes in this environment is necessary to distinguish between natural and anthropogenic inputs, and to create a historical database around studied sites. Therefore, Antarctica offers a good comparison for more populated places to measure contaminant changes in the world, because there is no industrial activity and low human occupation on the Antarctic continent [1]. However, after the first expeditions to this continent, human impact has increased, particularly due to tourism and the establishment of scientific stations by several countries.

The Antarctic Treaty was signed by 12 countries in 1959, and in 1991, this treaty was improved by the Madrid Protocol. The Protocol on Environmental Protection to the Antarctic Treaty (Madrid Protocol) provides the basis for environmental protection and management in Antarctica. All nations with scientific bases on this continent must comply with the rules of environmental impact during their presence in that region [2]. In 2009, 53 scientific stations were spread out over the continent [3].

In 1975, the Brazilian government signed the Antarctic Treaty, and since 1982, regular Brazilian scientific expeditions have been sent to Antarctica. In 1984, the first modules of the Antarctic Scientific Station Comandante Ferraz (Ferraz Station) were installed at Admiralty Bay, King George Island, Maritime Antarctica [4].

Following the proposal established in the Madrid Protocol, Admiralty Bay is currently classified as an Antarctic Specially Managed Area (ASMA). This classification implies a number of requirements in multidisciplinary studies to assess impacts by scientific

research, tourism and other human activities, and to provide information for environmental monitoring [5].

Scientific programs installed in the Antarctic stations have developed a wide range of environmental projects targeting the measurement of anthropogenic contaminants on that continent [6-11]. Soils and sediments are the principal matrices used in these studies.

Soils are considered to be important contaminant sinks in natural ecosystems. Therefore, chemical analyses of these matrices are of special concern in environmental studies [12,13]. A number of analytical procedures can be used to discriminate between anthropogenic pollution and background content in soils. Sequential extraction procedures (SEPs) are often utilized to obtain a diagnostic view of the chemical distribution patterns of elements in soils [14-19]. Initially developed by Tessier et al.. [19], SEPs prescribe the use of different extractors with growing chemical strength and reactivity. This approach provides information about the distribution of elements in labile and more recalcitrant phases [14]. Single extractants (SEs) such as EDTA, DTPA, diluted HCl, NH_4NO_3, $CaCl_2$, and $NaNO_3$ are already used in soil analysis [20-23]. These SEs have some advantages with respect to SEPs because they are simpler, faster, cost-effective and less subject to analyte losses and contamination [23-25]. However, it is of the utmost relevance to determine soil properties such as cation exchange capacity, pH, organic matter content and granulometry, because these variables are closely linked to the availability of metallic elements in the soil solution [22].

In this context, for an adequate environmental study, it is necessary to manage a large amount of data from a lot of environmental samples, justifying on this way, the use of chemometric tools for data processing and exploitation.

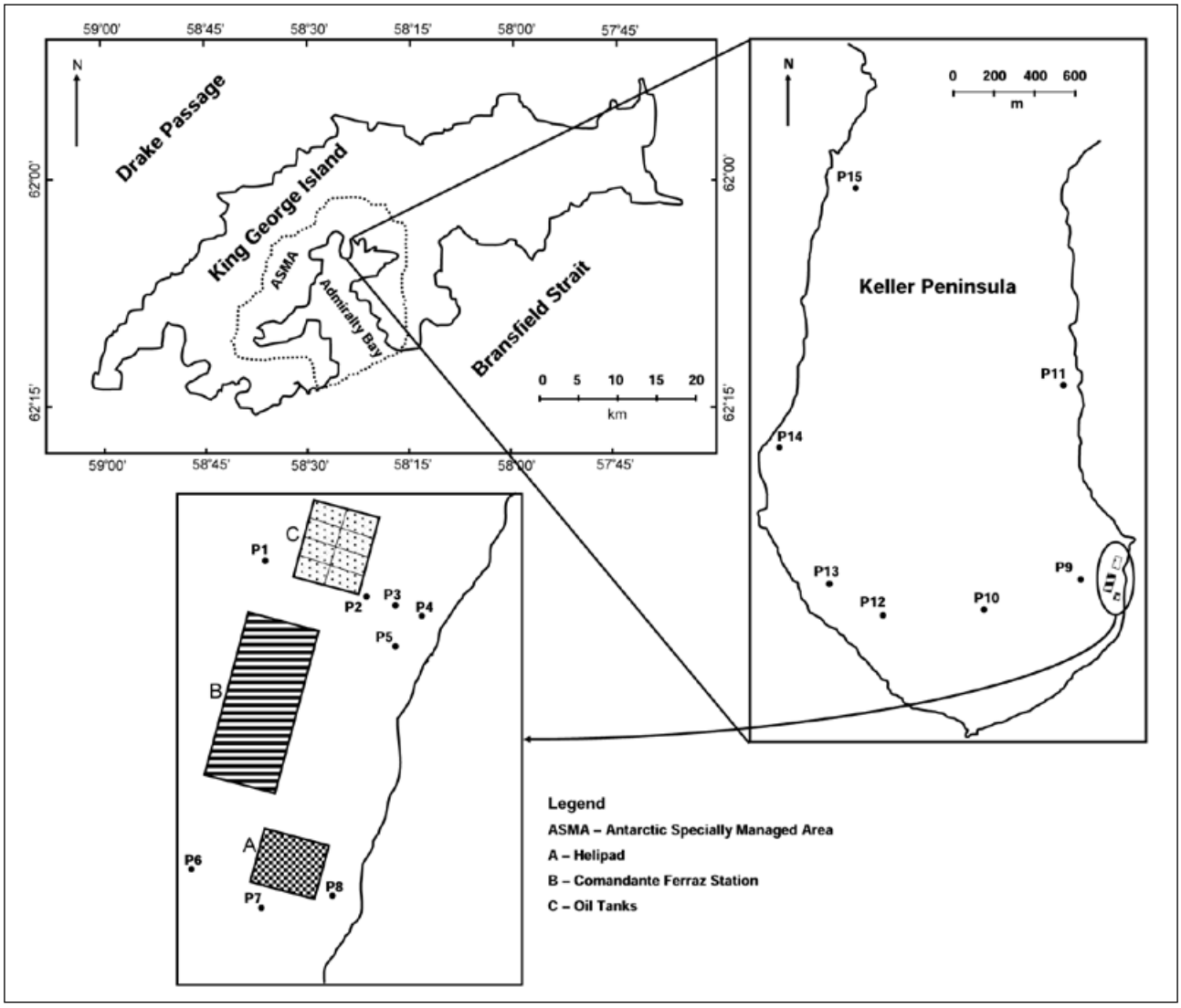

Figure 1. Collection points (P#) in Keller Peninsula, King George Island.

This study compared the chemical characteristics of soil samples collected in regions around the Ferraz Station and at sites far from it. Several analytical procedures were developed and used to evaluate the concentrations of toxic elements (commonly named heavy metals) in these soil samples and to investigate some soil characteristics related to the availability of the studied analytes. Atom trap techniques, such as STAT-FAAS (Slotted Tube Atom Trap - FAAS) and TS-FF-AAS (Thermospray Flame Furnace AAS) were used to achieve better detectivity for Cd and Pb quantification. Moreover, high analytical throughput was obtained by the use of FAAS in the fast-sequential mode. X-ray fluorescence (XRF) spectra were also obtained using synchrotron light and chemometric tools like Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were used to extract useful information from the data.

2. Materials and methods

2.1 Collection and preparation of samples

The collection of soil samples was performed between December 2007 and February 2008. Samples were collected at 15 sites (totaling 49 samples) located at King George Island, Admiralty Bay, Maritime Antarctica. Figure 1 and Table I provide detailed information about the locations of the collection sites (P#).

The sampling was performed at depths up to 60 cm for sites located around Ferraz and further (more than 150 m) from the station. The collection procedure was performed using a representative portion of soil (around 2 kg of each sample) from each 10 cm of depth (points far from the station) or each 20 cm of depth (points around the station). At points around Ferraz (P1 - P8, see Fig. 1), soil samples were collected from places near oil tanks and the helipad (see more details in Fig. 1 and Table 1). This sampling strategy was performed taking into account the logistic work in Antarctica and pedogenetic differentiation between the soil samples. Thus, the soil samples further from Ferraz station have more discernible and developed horizons, so it was important to collect them in a more systematical way (10 by 10 cm). On the other hand, soil samples around Ferraz were clearly impacted (attested to by visual field observations). Thus, they were collected in a deeper profile, until 40 cm (2 samples of 20 cm depth to accomplish an adequate number of samples for storage and transportation to Brazil).

The samples were collected and stored in plastic bags that were kept refrigerated until they were processed in the laboratory. The soils were dried at 60 °C, keeping the fraction finer than 2 mm (excluding gravel and stones), milled with a knife mill (A11 basic Analytical mill, IKA®, USA), sieved at 212 μm and again dried at 60 °C until they reached constant weight.

Table I. Description of the sampling sites

Points (see locations at Figure 1)	Number of collected soil samples	Maximum Depth (cm)	Identification of the selected samples for the quantitative determinations	Description of the collection sites
P1	01	40	1	Control point
P2	02	40	2A and 2B	Immediately below the oil tanks
P3	02	40	3A and 3B	Affected by the oil tanks
P4	02	40	4A and 4B	Affected by the oil tanks
P5	02	40	5A and 5B	Affected by the oil tanks
P6	01	40	6	Control point
P7	02	40	7A and 7B	Affected by the helipad
P8	02	40	8A and 8B	Entrance of the boats garage
P9	05	50	9A and 9B	Undisturbed point
P10	03	30	10A and 10B	Undisturbed point
P11	06	55	11A and 11B	Undisturbed point
P12	05	45	12A and 12B	Undisturbed point
P13	06	60	13A and 13B	Undisturbed point
P14	04	40	14A and 14B	Undisturbed point
P15	06	60	15A and 15B	Undisturbed point

A – lower depth
B – higher depth

2.2. Sample preparation for SR-XRF analysis

The dried samples were pressed in a pneumatic press under the same conditions (7 tons for 30 seconds) in order to obtain thin pellets of approximately 1 mm thickness. These pellets were fixed in aluminum supports with adhesive tape (3M®, Sumaré, São Paulo State, Brazil).

2.3. Reading of the samples using SR-XRF

Initial soil analyses were performed using X-ray fluorescence (XRF) technique with the synchrotron radiation excitation source (SR-XRF) of the Brazilian Synchrotron Light Laboratory (LNLS) located at Campinas, São Paulo State, Brazil. X-ray fluorescence measurement conditions were: beam diameter 200 μm, one point per sample irradiated with a reading time of 200 s and a Fe filter with 6 mm thickness applied between the sample and the beam. This Fe filter was used to

minimize detector saturation by Fe signal due to the naturally strong presence of Fe in the soil samples. The PCA calculations were made with the help of Pirouette 4.0, rev. 2 software (Infometrix, Bothell, USA). These preliminary analyses were done for all collected samples to determine the general tendency of the data before applying quantitative measurements. Some of these samples were randomly selected and analyzed in duplicate.

2.4. Reagents and solutions

All reagents were of analytical grade, and sub-boiling distilled acids (HCl and HNO_3) were used to prepare the aqua regia extraction solution. Deionized water was used in all solutions. All flasks and glassware were cleaned and then decontaminated by immersion in HNO_3 (10 % v/v) overnight. Standard stock solutions of the metals (1000 mg L^{-1}) were used to prepare the analytical curves.

2.5. Aqua regia extraction for Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn determination

Extraction with aqua regia was applied instead of total soil digestion to permit access to the maximum content of elements actually available to plants [26]. The extraction was performed according to the German norm, DIN 38414-S7 [27]. Three samples of 300 mg dried soil were weighed into digestor block tubes, and 3 mL of aqua regia were added. The mixture was allowed to stand at room temperature overnight. The tubes were then transferred to a digestor block (Solab, Piracicaba, Brazil), and a warming step of 3 hours at 120 °C was performed using a reflux apparatus. Finally, the extracts were transferred to previously decontaminated tubes, and the final volumes were made up to 10 mL.

2.6. Application of the modified BCR (Community Bureau of Reference) sequential extraction procedure for Cu, Mn and Zn determination

The sequential extraction procedure (SEP) was performed as described by Scancar et al.. [28]. Three samples of 500 mg dried soil were weighed into centrifuge tubes, and 10 mL of CH_3COOH (0.11 mol L^{-1}, pH = 2.8) was added. The mixture was shaken in a horizontal shaker end-over-end (Barnstead, Dubuque, IA, USA) overnight. The second step of the sequential extraction was performed with 10 mL of NH2OH·HCl (0.5 mol L^{-1}, pH = 1.5), and the third step was performed with 1 mL of H_2O_2 (30% v/v, pH = 2) and 9 mL of CH_3COONH_4 (1 mol L^{-1}, pH=2). Steps 1, 2 and 3 were achieved under agitation using the same conditions over 10 h. In the last step, an aqua regia extraction was performed on the residues of the third stage. After each step, the supernatant was separated by centrifugation and transferred to decontaminated flasks.

2.7. DTPA extraction for Cd and Pb determination

The DTPA extraction solution was prepared as 0.005 mol L^{-1} diethylenetriamine-pentaacetic acid (DTPA), with 0.01 mol L^{-1} $CaCl_2$ and buffered at pH 7.30 with triethanolamine [29]. Three samples of 1 g of each soil sample were extracted with 5 mL DTPA solution in a horizontal shaker end-over-end (Barnstead) over 2 h. The supernatant was separated by centrifugation, and it was transferred to previously decontaminated flasks.

2.8. Spectrometric determination of metals by AAS

Aqua regia, DTPA and SEP extractions were performed on the most superficial soil samples of the 15 sites, totaling 28 soil samples. Besides the samples collected around the station for quantitative analyses, samples further from Ferraz were also analyzed, but only up to a depth of 20 cm. Thus, 14 samples of each site, both around Ferraz and further from it, were quantitatively analyzed.

Spectrometric determination of the elements Cd, Cr, Cu, Mn, Ni, Pb and Zn in aqua regia, SEP and DTPA extracts was performed using three different atomic absorption-based methods: FAAS (Flame Atomic Absorption Spectrometry), STAT-FAAS (Slotted Tube Atom Trap - FAAS) and TS-FF-AAS (Thermospray Flame Furnace Atomic Absorption Spectrometry). The more sensitive approaches, STAT-FAAS and TS-FF-AAS, were used to reach a high power of detection for Cd and Pb quantification. All determinations were performed using a flame atomic absorption spectrometer (AA240FS; Varian, Mulgrave, Australia) equipped with a deuterium lamp for background correction. Copper, Mn, Ni and Zn determination in the aqua regia and SEP extracts was performed by FAAS operation in sequential mode using an air-acetylene flame at wavelengths of 324.7, 279.5, 232.0 and 213.9 nm, respectively. Chromium determination by FAAS (357.9 nm) in the aqua regia extracts was performed separately, because this analyte was determined using a nitrous oxide-acetylene flame. Cadmium (228.8 nm) and Pb (283.3 nm) determinations in the DTPA extracts were performed using TS-FF-AAS. To enhance detectivity, a Ni tube (10cm length) was placed above an air-acetylene flame to increase the residence time of the free atoms in the measurement zone. Using this approach sample introduction was performed via a ceramic capillary (0.5 mm i.d.) achieving complete introduction [30, 31]. The TS-FF-AAS arrangement was mounted as described by Miranda and Pereira-Filho [32] and slightly modified by Souza et al.. [33]. An important modification proposed by Souza et al.. [33] was the use of a carrier solution composed of a mixture of 0.5 % w/v EDTA and 0.1 % v/v Triton X-100 to avoid capillary clogging.

The STAT-FAAS was applied for Cd and Pb determination in the aqua regia extracts. The STAT-FAAS proposed by Watling et al.. [34] consists of an atom trap

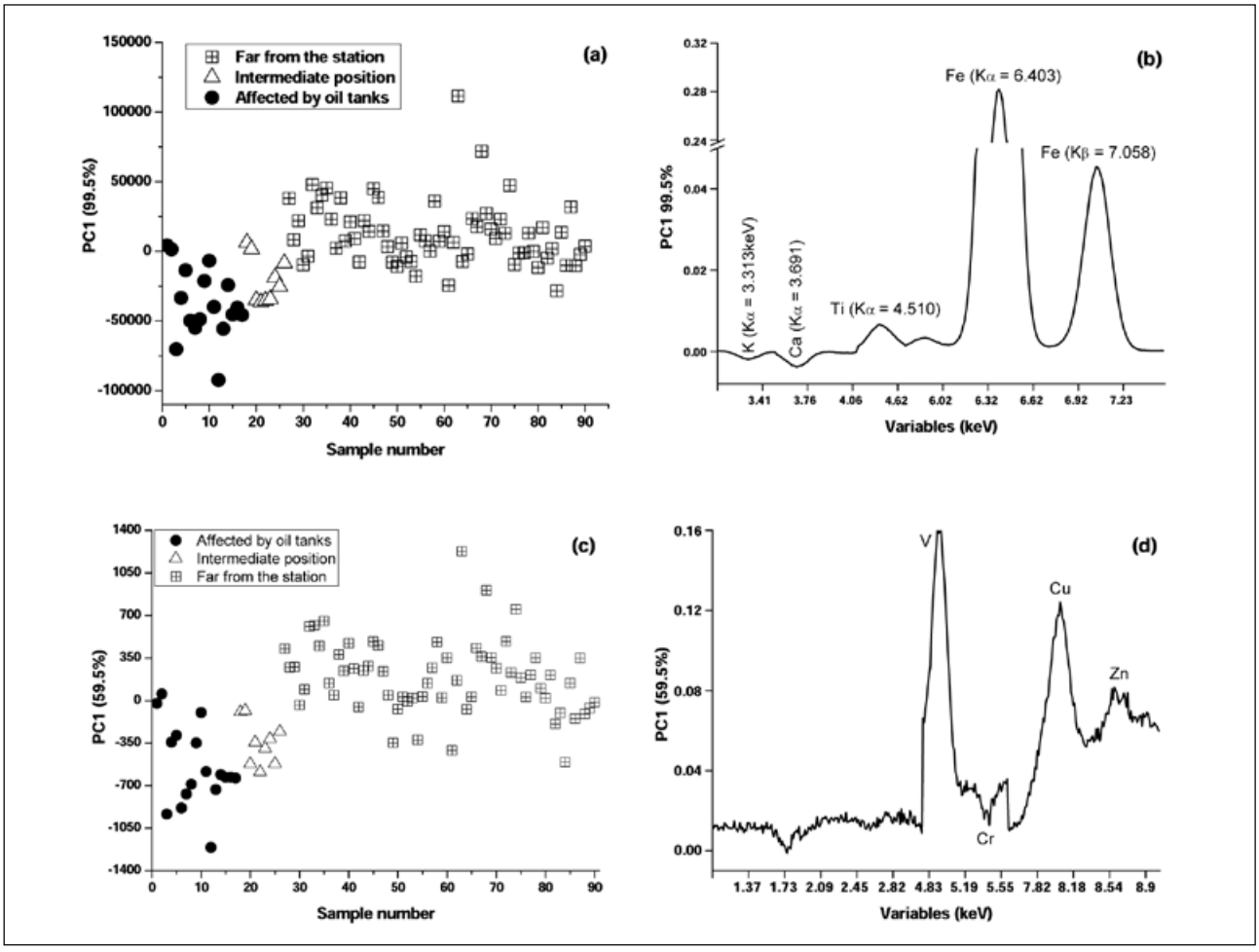

Figure 2. Score (a) and loading (b) plots using all XRF data. Score (c) and loading (d) plots excluding Ca, Fe, K and Ti signals.

technique that allows improvement of the detectivity of AAS by increasing the residence time of the atoms, similar to TS-FF-AAS [35]. However, in STAT-FAAS, there is no gain in detectivity arising from sample introduction because it operates with a conventional AAS nebulizer. In this instrumental approach, a Ni tube (10 cm length) is placed above the air-acetylene flame. This tube is designed with a 5 cm central slot that permits the entrance of combustion gases.

2.9. Soil sample complementary analysis: pH in water, total organic carbon and granulometry

For pH measurements, 10 g of the soil sample (sieved to <2 mm) was transferred to 50 mL centrifuge tubes, and 20 mL of deionized water was added [36]. The system was shaken for 30 minutes in a horizontal shaker end-over-end. Finally, the pH was measured using a glass electrode after soil particle sedimentation. The total organic carbon content was estimated using the Walkley & Black method [37]. Granulometry was determined using the pipette method described by EMBRAPA [38].

All data obtained from the chemical extractions and complementary analyses were treated with HCA and PCA using the software Pirouette 4.0.

3. Results and discussion

3.1. Preliminary analysis with SR-XRF

All data obtained by X-ray fluorescence were organized in a matrix of 90 rows (number of samples) and 2048 columns (energy intensities ranging from 0 to 25 keV). This matrix was mean-centered, and the PCA was applied to it. The scores and loadings of the PCA plot are shown in Fig. 2a and 2b.

The PCA analysis allowed the identification of contrasting materials in these Antarctic soils, mostly based on Fe content. Lower Fe contents were consistently observed in soils from the marine terraces, where the oil tanks are located.

A new PCA calculation was performed without the elements with the highest signals (Ca, Fe, K and Ti). The same trend of grouping was observed after the elimination of the Ca, Fe, K and Ti fluorescence signals (see the fig. 2a and 2c). The samples under influence of the oil tanks were associated with the presence of Cr (Fig. 2d). Samples further from the station were characterized by high V and Cu contents.

High Cu content in King George Island soils has already been detected in past studies, and is related to chalcopyrite (a Cu mineral) mineralization in volcanic rocks of the area [39].

Vanadium is associated with petroleum contamination [40], although high V contents were observed in soils obtained at a great distance from the station. This behavior can be explained by the geochemical affinity of this element for Fe oxides [41], especially because samples distant from Ferraz have high Fe content (see Fig. 2b).

Although Zn is commonly associated with atmospheric deposition coming from burning diesel [42], in this study no clear evidence of high Zn content in the soils around the station was observed (see fig. 2d).

Claridge et al.. [43] studied polluted soils for anthropogenic activity in the vicinity of the American Antarctic Station (McMurdo) and found evidence of contamination by heavy metals, such as Cu, Pb and Zn, although the observed levels did not present a threat to the Antarctic environment.

Chromium has been associated with petroleum contamination [44], and soils collected in the vicinity of oil tanks can be seriously polluted by fuel. Chromium can also be associated with paints used to protect the station's oil tanks [9].

Chromium species can be adsorbed onto soil components, preventing leaching of Cr into the groundwater or uptake into plants, thus reducing bioavailability. These soil components can be macromolecular clay compounds or humic acids recognized to form stable Cr (III) compounds [45]. It is important to highlight that Antarctic soils usually have low clay (generally ranging to 1 – 5%) and organic matter contents [43]. Santos et al.. [46] studied soil chemical characteristics of marine terraces around the Ferraz Station and found low organic matter levels, with an average of 2.60 % m/m.

3.2. Recovery of studied elements

In order to verify the accuracy of the extraction and quantification of metals, a certified reference material, BCR 146 R (sewage sludge from industrial origin) was used. The accuracy of the proposed methods falls in the range of 89 – 102 %.

In spite of the recognized irreproducibility inherent to SEPs [47], the sum of element concentration in the fractions were in good agreement with the pseudototal concentration, with recoveries of 96 ± 6; 97 ± 11 and 87 ± 16 % for Cu, Mn and Zn, respectively.

3.3. Use of atom trap techniques and Fast-Sequential Flame Atomic Absorption Spectrometry (FS-FAAS) for soil extract analysis

Thermospray Flame AAS was employed for determination of Cd and Pb in the DTPA soil extracts, achieving adequate detectivity in quantifying these elements. In this particular case, the use of a carrier composed of 0.5 % w/v EDTA and 0.1 %v/v Triton X-100 to prevent capillary clogging during sample introduction was important. Unfortunately, it was not possible to use the fast sequential mode for Cd and Pb determination because this approach requires sample introduction without an aqueous carrier and capillary clogging was systematically observed.

Moreover, the aqua regia soil extracts could not be determined by TS-FF-AAS even in monoelement mode, because high background signals were observed. This behavior was due to the high concentration of chloride salts inside the atomizer and the undissociated matrix species derived from them can generate molecular species at a much higher degree than commonly observed in FAAS [48]. Then, in order to achieve both high analytical throughput and detectivity over the conventional mono-element flame AAS, Slotted Tube Atom Trap (STAT) was combined with FS-FAAS for the sequential determination of Cd and Pb in the aqua regia soil extracts. In table II, the figures of merit of the spectrometric methods employed in this work are shown.

Table II. Figures of merit for the employed spectrometric methods

Elements	Type of Flame	Method	Maximum Achievable Analytical Throughput (determinations per hour)	LOD (µg L-1)
Cd and Pb	Air-Acetylene	FS-FAAS	386	3.6 and 86
Cd and Pb	Air-Acetylene	STAT-FAAS	386	0.7 and 31
Cd and Pb	Air-Acetylene	TS-FF-AAS	109	0.3 and 8.8
Cu and Ni	Air-Acetylene	FS-FAAS	363	8.5 and 27
Mn and Zn	Air-Acetylene	FS-FAAS	379	9.9 and 2.6
Cr	Acetylene-N_2O	FAAS	257	60

Two groups of elements were formed for determinations by FAAS in the fast-sequential mode: 1) Mn and Zn and 2) Cu and Ni; based in the same dilution factor required by both groups of elements in the AAS determinations. In table II, a comparison between FAAS, STAT-FAAS and TS-FF-AAS in the fast-sequential mode can be seen. For the calculation of maximum achievable analytical throughput, the following conditions were used: 1 second of pre-read delay; 3s of integration time per each element and 33 nm s^{-1} as wavelength scan speed of the equipment, and, for routine analysis,

use of an autosampler that spends at least 10 s for each sample. The STAT-FAAS became an interesting strategy for fast sequential determination of Cd and Pb, because it combines high analytical throughput (the same observed by FS-FAAS) and good LODs (limits of detection, defined by IUPAC as three times the standard deviation of the blank (n=10) divided by the slope of the calibration curve) with intermediate values between the conventional FAAS and TS-FF-AAS. The poorer performance of TS-FF-AAS in the fast-sequential mode, in relation to analytical throughput, for Cd and Pb determination, is due to the signal stabilization time (60 s for each sample) before starting the measurements.

3.4. Chemometric treatment of soil chemical analyses: metal concentrations and soil characteristics

For the HCA, the data were organized in a matrix with 28 rows (number of samples) and 29 columns (investigated variables, expressed as the average of three replicates). The variables investigated were the pseudototal concentrations of Cd, Cr, Cu, Mn, Ni, Pb and Zn; the bioavailable concentrations of Cd and Pb; the percentage at each step of the SEP and the sum of the three initial fractions related to the labile portion of Cu, Mn and Zn; and finally, soil characteristics (pH, TOC and granulometry, including the percentages of sand, silt and clay), totaling 29 variables. The data were autoscaled and, for dendrogram calculation, the Euclidean distance and incremental linkage methods were used.

The HCA results are shown in Fig. 3. The dendrogram exposed remarkable differences between soil samples around the station (especially those impacted by the oil tanks) and the samples further from Ferraz, as seen by the formation of two groups of samples at each sampling region. The control points around Ferraz Station, points 1 and 6 (see fig. 1), had chemical characteristics similar to those points further from Ferraz, as seen from their position in the dendrogram (Fig. 3). These results confirm the preliminary analysis performed with the combination of SR-XRF and PCA, which identified differences between the samples of the 2 sites.

For PCA application, some of the variables were discarded to facilitate the analysis interpretation. In the same fashion as for HCA, fractions 1, 2 and 3 of SEP for each element (Cu, Mn and Zn) were combined. All data were then organized in a matrix with 28 rows (number of samples) and 17 columns (related to the variables). This matrix was autoscaled to give the same importance to all variables, and the PCA was applied. The scores and loadings of the PCA plot are shown in Fig. 4a and 4b. A large separation was observed between the sampling sites (Fig. 4a, scores plot) and the variables related to this behavior, as seen in Fig. 4b. Points directly impacted by the oil tanks had the highest concentrations of Cr, Ni and Pb.

Table III confirms that the points directly affected by Ferraz oil tanks had higher concentrations of Cr, Ni and

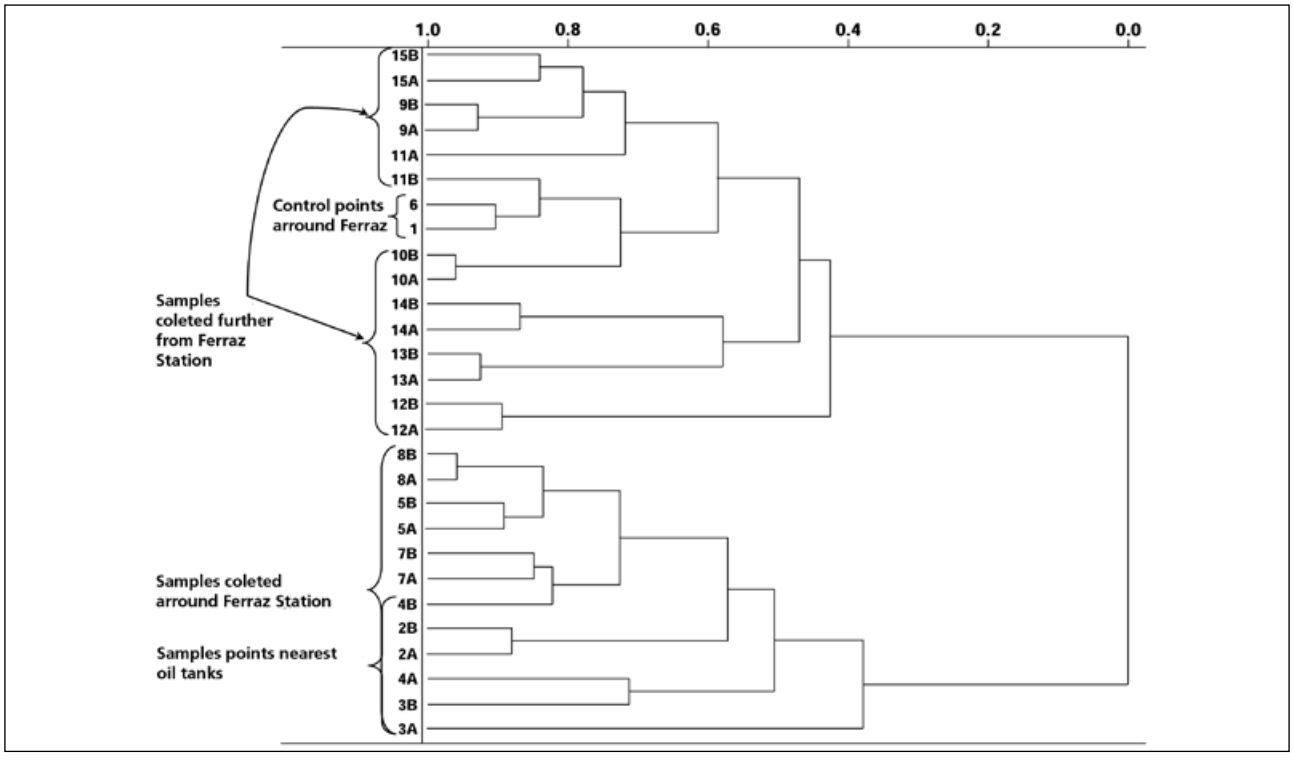

Figure 3. HCA (dendrogram) with all data obtained from the quantitative determination of metals and soil characteristics.

Table III. Comparative table with concentrations of Cr, Ni and Pb in soil samples of selected papers.

Place / Matrix	Reference	Cr (mg/kg)		Ni (mg/kg)		Pb (mg/kg)	
		Range	Average	Range	Average	Range	Average
Near Ferraz oil tanks[1]	This study	31.2 – 300.1	190.3	2.6 – 36.1	12.3	7.6 – 105	38.2
Far from the station[1]	This study	15.4 – 272.3	68.3	4.2 – 16.8	8.5	5.65 – 9.05	7.45
Ferraz Station soils[2]	Santos et al.. [10]	Unavailable	40	Unavailable	5.1	Unavailable	11.5
Shanghai urban soils[3]	Shi et al.. [50]	25.5 – 233.3	107.9	4.95 – 65.7	31.14	13.7 – 192	70.7
Vietnam rural soils[4]	Thuy et al.. [51]	80.8 – 116.7	98.5	11.0 – 31.5	21.6	0.8 – 3.2	1.9
Vietnam urban soils[4]	Thuy et al.. [51]	23.2 – 174.5	92.2	9.4 – 67.9	22.6	0.4 – 20.1	3.6
Marambio Station[5]	Chaparro et al.. [9]	Unavailable	22	Unavailable	5.1	Unavailable	10.2
Mcmurdo Station (gray soil)[6]	Crockett [8]	95 – 249	172	77 – 119	98	1.0 – 10.6	5.8
Mcmurdo Station (red soil)[6]	Crockett [8]	154 – 480	317	137 – 215	176	3.7 – 6.3	5.0
Guideline value[7]	DEFRA [52]	200		75		450	
Prevention value[8]	CETESB [49]	75		30		72	

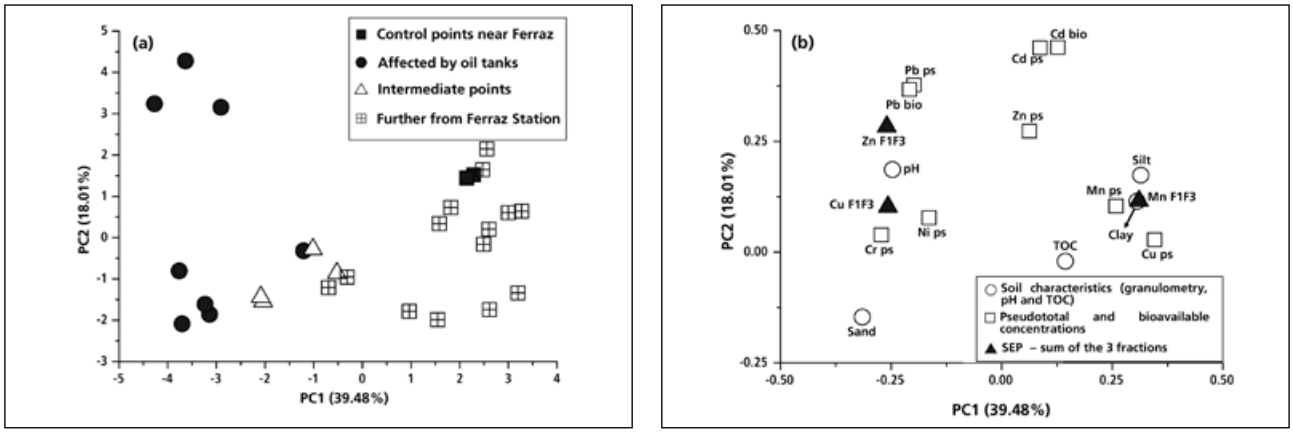

Figure 4. PCA graphs of selected data in a scores plot (a) and loadings plot (b).

Pb. Lead was enriched by about 5-fold at points around the diesel tanks, compared to points further from the station. However, the highest concentrations for Pb were still below the guideline value for soil quality and slightly higher than the prevention value, according to CETESB [49], the environmental agency of São Paulo State, Brazil. The Cr concentrations at the most impacted sites reached more than 300 mg kg^{-1}, higher than the guideline value for soil quality. Finally, Ni was slightly enriched in the most affected samples, exceeding the value prescribed by CETESB [49]. Table III also shows a comparison between concentrations of Cr, Ni and Pb in this work and some selected soil samples around the world.

Inherent characteristics of soil samples such as granulometry, pH and TOC are important variables related to the observed discrimination between the sampling sites. In the samples collected around Ferraz, granulometry and TOC, in particular, are closely related to the highest bioavailable concentrations of Pb and to

greater concentrations in the labile portions of the SEP for Cu and Zn (CuF1F3 and ZnF1F3 in Fig. 4b). This can be confirmed by the lower clay (4.75 % m m^{-1}, on average) and TOC content (0.38 % m m^{-1}, on average) of the soils impacted by the oil tanks around Ferraz. On the other hand, the soil samples collected further from Ferraz have 5-fold higher clay (22.3 % m m^{-1} on average) and almost double TOC contents (0.74 % m m^{-1} on average). Soil organic matter plays an important role in soil chemistry, acting as a sorbing metal phase that reduces metal bioavailability [53]. Simas et. al [54], studying some soil samples collected from the same Peninsula as this work, found clay minerals of the illite group, a class of clays with a high cation exchange capacity. More recent installations have used clays as landfill chemical barriers due their strong interactions with metallic cations present in leachates [55, 56].

4. Conclusions and outlook

The use of atom trap techniques (STAT-FAAS and TS-FF-AAS) allowed to attain better limits of detection for Cd and Pb in relation to conventional flame AAS. Furthermore, the fast-sequential determination of metals (by FAAS and STAT-FAAS) permitted reduction in the analysis time and minimization of sample consumption and waste generation. The combination of PCA with SR-XRF enabled to perform a geochemical exploratory study of Antarctic soils, providing reliable information for the environmental monitoring of the studied sites. The information obtained using this combination was confirmed by quantitative measurements performed using spectrometric techniques. Furthermore, chemometric tools were used for the treatment of data obtained from soil characteristics, in addition to the information on SEP and the pseudototal and bioavailable concentrations of metals. The use of such tools was implemented as a novel alternative for environmental assessment of areas with anthropogenic inputs of contaminants. Although the most impacted sites contain anomalous concentrations of Cr, Ni and Pb, the concentrations do not exceed guidelines for soil quality, except for Cr. Nevertheless, mitigation and treatment of contaminated areas could be important for preservation of the local ecosystem.

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A MULTICOMMUTATED FLOW SYSTEM FOR PERACETIC ACID DETERMINATION

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Abstract

Peracetic acid (PAA) has been used as a pre-disinfectant in water supplies to improve the quality of raw water, reducing the amount of pathogens and the formation of chlorinated byproducts. A rapid analytical procedure is desirable for the analysis of PAA, since its diluted solutions are unstable. A flow procedure based on a multicommuted flow injection analysis process (MCFIA) to determine PAA is proposed. The method is based on the reaction of PAA with an iodide solution liberating iodine. The liberated iodine oxidizes the DDPD (the methyl-substituted form of N, N-diethyl-p-phenylenediamine) with the use of a commercial *kit,* Vacu-vials® (CHEMetrics, Inc). The compound was monitored at 565 nm. Three solenoid micro-pumps were used to insert samples of the carrier solution (water) and reagent (*kit* Vacu-vials®). The linear response ranged from 0.5 to 5.0 mg L^{-1} PAA (R = 0.9995). The coefficient of variation was estimated as 4.0 % (n = 8) for a sample of 3 mg L^{-1} PAA. The detection and quantification limits were 0.10 mg L^{-1} and 0.33 mg L^{-1}, respectively. The sampling throughput of 80 determinations per hour consumed 80 µL of reagent solution (this volume is 25 times smaller than the volume used in bench scale measurements with a kit) and a waste generation of 1.50 mL per determination.

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1. Introduction

Peracetic acid (ethaneperoxoic acid, PAA) was patented in 1950 by Greenspan and Margulies for treating fruit and vegetables to reduce spoilage from bacteria and fungi [1]. The technical synthesis of PAA comprises a reaction of hydrogen peroxide with acetic acid in the presence of a strong mineral acid, typically sulphuric acid, as a catalyst [2,3,4]

PAA is efficient at low concentrations against a broad spectrum of microorganisms, including gram-positive and gram-negative bacteria, yeasts, molds and algae under a wide variety of conditions [4,5]. It is an important oxidant in industrial processes, such as for disinfection in breweries and dairies, for the bleaching of paper and textiles, and in the sterilization of medical instruments [6]. Due to its bactericidal, virucidal, fungicidal and sporicidal effectiveness, the use of PAA as a disinfectant for wastewater effluents has recently been attracting attention [7-9]. PAA has been tested and used for wastewater disinfection in England, Finland, Italy, Brazil and Canada [9].

In addition, it is well-known that PAA is a more efficient oxidant and disinfectant than chlorine or chlorine dioxide [9]. In drinking water the utilization of this acid is reported as a prior-disinfectant to improve the quality of water, reducing the quantity of pathogens and the potential formation of chlorine by-products when chlorine is used as disinfectant [10]. Chlorine is maintained as a secondary disinfectant only to maintain residual disinfectant.

The advantages for wastewater disinfectation are: ease of implementing treatment, a broad spectrum of activity even in the presence of heterogeneous organic matter, the absence of persistent toxic or mutagenic residuals or by-products, and the no quenching requirement (i.e., no dechlorination), little dependence on pH and a short contact time [9].

An early approach to PAA determination involves a two-step titration. The H_2O_2 present is titrated at a low temperature with a standard solution of potassium permanganate ($KMnO_4$) in the first step. Potassium iodide is then rapidly added and the iodine liberated by the PAA is titrated with a standard thiosulfate solution. This procedure is called the potassium permanganate – thiosulfate titration method and has been used as the reference method for monitoring industrial PAA applications [6,11,12].

There are other methods to determine PAA in disinfectant samples [2], in synthetic samples [3], in brewery cleaning-in-place disinfection processes [4,11,13], in tap water [6] and in wastewater samples previously treated with PAA [14] using spectrophotometry [2,4,6,14-16], potentiometry [3], gas chromatography [17] and high-performance liquid chromatography [11,13].

An analytical method for the analysis of PAA requires high selectivity and little cross-reaction with the coexistent H_2O_2. The coexistence of PAA with H_2O_2 is unavoidable owing to the synthesis of PAA from H_2O_2 and acetic acid, as mentioned above, and its continuous decomposition [3]. A rapid analytical technique is also desirable for the analysis of PAA, since diluted solutions are unstable and they have to be recalibrated frequently using titration or other absolute determination methods [3,11]. But, a rapid analytical technique is difficult using a manual procedure.

By employing automatic procedures, however, these goals may be attained using those based on a sequential injection analysis (SIA) [18] or a multicommuted flow injection analysis process (MCFIA) [19-21].

Solenoid micro-pumps were introduced to replace peristaltic pumps as an alternative for fluid propulsion in a flow system [22]. A flow system based on micro-pumps has been designed employing one propelling device for each solution, controlled by a microcomputer, thus permitting solution flow rates that can be individually varied by the software [23].

The inherent features of the MCFIA, such as versatility, low reagent consumption, and robustness, are also enhanced using a solenoid micro-pump to propel sample and reagent solutions [18, 24-28].

In this work, we describe the development of a multicommuted flow injection procedure for the spectrophotometric determination of peracetic acid in disinfectants employing solenoid micro-pumps to propel reagent solutions contained in Vacu-vials® ampoules (CHEMetrics, Calverton, VA, U.S.). The detection was measured at 565 nm.

2. Experimental

2.1. Safety note

PAA is strong oxidizer, is incompatible with easily oxidized substances and forms explosive mixtures. These solutions are severely irritating to eyes, skin and mucous membranes.

2.2. Reagents and solutions

All the solutions were prepared from analytical reagent grade chemicals using ultrapure water (18 MΩ cm^{-1}) obtained by means of a Milli-Q (Millipore Corp., USA) water purification system.

The PAA solutions were prepared from their stock solutions (15 %, w/v). The concentration of the PAA stock solution, obtained from Microbiana Descontaminações Ltda (São Paulo, SP – Brazil), was analyzed to be 169.7 ± 2.2 g L^{-1} using the conventional method proposed by Greenspan and Mackellar [12]. An aqueous stock solution of 100 mg L^{-1} peracetic acid was prepared. The concentrations of calibration solutions varied from 0.5 to 5.0 mg L^{-1} of PAA. Sample solutions of PAA were prepared by diluting the appropriate volume of PAA with water.

Vacu-vials® ampoules (CHEMetrics, Calverton, VA, U.S.) containing approximately 2 mL of DDPD solution were used as the reagent. The DDPD solution is a methyl-substituted form of DPD (N, N-diethyl-p-phenylenediamine) which is kept in a sealed glass tube under vacuum.

The activator solution was 15 g L^{-1} potassium iodide. Three drops of the activator solution were added to 25 mL of the calibration and PAA sample solutions.

All disinfectant samples contained peracetic acid (approximately 15%, v/v), acetic acid (16%, v/v) and hydrogen peroxide (23 %, v/v), although the package label did not specify a PAA concentration. The disinfectant samples were diluted to the desired concentration with water prior to analysis.

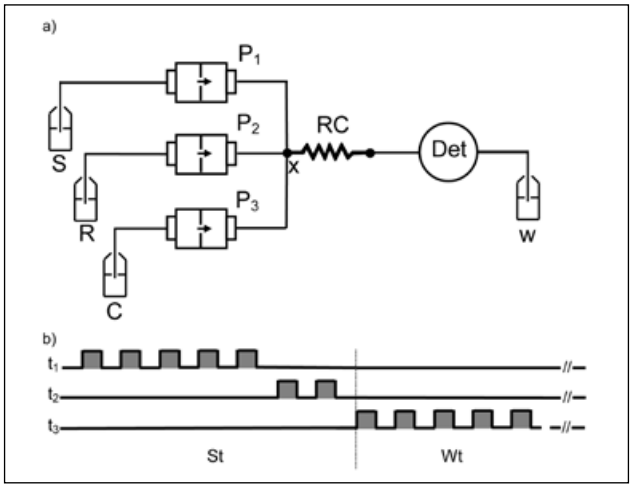

Figure 1. Flow diagram of the multicommuted flow system. a) Diagram of the flow system. P_1, P_2 and P_3: solenoid micro-pumps; S: sample; R: reagent solution; C: carrier solution (H_2O), RC: reaction coil, 0.8 mm i.d., 100 cm; x: confluence; Det: detector, 565 nm; W: waste. b) Switch time course of micro-pumps, respectively t_1, t_2 and t_3. St: sampling step; Wt: washing step. The shadow areas beneath of lines indicate that the solenoid micro-pumps were switched ON.

2.3. Apparatus

The flow system is schematically outlined in Figure 1.

The FI manifold was composed of two 8 µL per stroke solenoid micro-pumps (P1 and P2), and one 10 µL per stroke solenoid micro-pump (P3) type Bio-chem 090SP (Boonton, NJ, USA); a microcomputer furnished with an electronic interface card PCL711S (American Advantech Co.); a 700S Femto spectrophotometer equipped with a flow cell, a 10 mm optical path and an 80 µL inner volume; a homemade electronic interface [29] to drive the solenoid micro-pumps; and a four-way acrylic connector. The reaction coil and flow lines were made from 0.8 mm i.d PTFE tubing. The control of the micro-pumps and data acquisition were performed by a microcomputer running with software written in Quick BASIC 4.5. The micro-pumps were configured to function as micro-injectors and the microcomputer managed the drive, adding the same aliquots of reagent solution in the sequence required by the chemical reactions involved.

2.4. Procedure

The proposed method is based on the reaction of PAA with I^- solution in excess. The concentration of I^- in the sample solution should be high enough to dissolve the I_2 resulting from the reaction of PAA with I^-. The I_2 liberated reacts with I^- forming I_3^-, it oxidizes the DDPD, forming a purple colored complex with maximum absorbance at 565 nm. The reaction rate of PAA and I^- is 5 orders of magnitude greater than that of H_2O_2 with I^- [30].

Figure 1a shows the flow diagram of the system. All pumps are switched OFF, thus no solution is flowing through the system. When the software was run, the microcomputer sent control signals through the PCL711S interface card to the switch ON/OFF of the solenoid micro-pumps, which carried out the switching pattern depicted in Figure 1b.

The micro-pumps P1 and P2 were switched ON/OFF sequentially five and two times, respectively, thus inserting a string comprising five slugs of sample solution in and two slugs of reagent solution into the reaction coil (RC). Afterwards, the micro-pump P3 was switched ON/OFF several times to establish a stream of the carrier solution to displace the sample zone towards the photometer (Det).The mixing of solutions between slugs proceeded while the sample zone was displaced through the reaction coil RC, thus causing a reaction which produced the compound that was monitored at 565 nm. The control parameters are shown in Table I.

Afterwards, the analytical procedure was carried out as depicted in the pump switching pattern. The time interval used to switch micro-pumps P1 and P2 ON/OFF was set at 0.1 and 0.1 s, making the switching frequency 5 Hz. Each micro-pump, P1 and P2, delivered 8 µL per stroke during the sampling step (St, Figure1), inserting 200 µL of the sample and 80 µL of the reagent solution into the reaction coil.

Table I. Solenoid micro-pump switching course for the determination of peracetic acid

Step	Step	P1	P2	P3	Pulses	Cycles
1	Sample or peracetic acid standard introduction	ON/OFF[a]	OFF	OFF	5	5
2	Reagent solution introduction	OFF	ON/OFF	OFF	2	
3	Transport to the detection cell and system washing	OFF	OFF	ON/OFF	150	-

[a]ON/OFF indicate a 0.1 s/0.1 s pulse of the solenoid micro-pump.

Afterwards, micro-pump P3 was switched ON/OFF several times to maintain a frequency of 5 Hz, propelling the carrier solution through the reaction coil (B) to displace the sample zone towards the detector at a flow rate of 42 µL s^{-1}. The signal generated by the spectrophotometer (Det) was read by the microcomputer through a serial interface, and stored as an ASCII file to allow further treatment. While the analytical process was proceeding, a signal plot was displayed on the computer screen as a time function to allow its visualization in real time.

3. Results and discussion

3.1. Effect of reagent slug volume

The reagent volume might affect the detectivity of the procedure. To test this, a set of experiments were carried out in order to ascertain optimum conditions. Slugs of the reagent with volumes in the range of 8 to 40 µL were inserted while the volume of the sample slug was maintained at 40 µL. These assays were carried out using a set of reference solutions with concentrations ranging from 0.5 up to 5.0 mg L^{-1} PAA, carried out by switching ON/OFF micro-pump P_2 one, two, three, four and five times to comprise each sampling cycle, while micro-pump P_1 was switched ON/OFF five times.

When reagent solution slugs of volumes 16 and 24 µL were used (Figure 2), the analytical signals showed an increase of 45 and 62 %, respectively, when compared with the use of a slug volume of 8 µL. Better results (R = 0.992) were achieved, however, when the volume of the reagent slug solution was 16 µL (Figure 2, curve b). These results agree with those suggested in the procedure manual of Vacu-vials®, where a stoichiometric relation between sample and reagent of 5:2 is recommended.

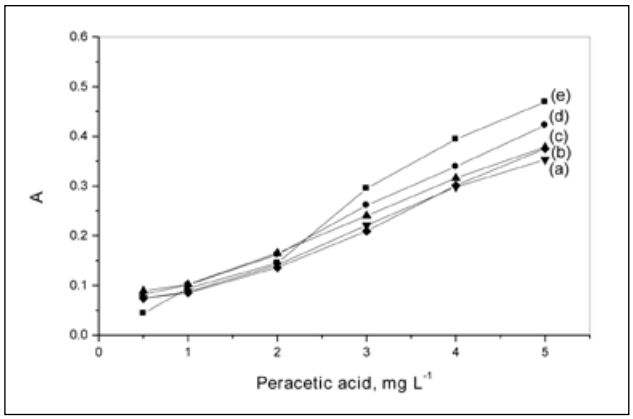

Figure 2. Effect of reagent slug volume on absorbance signal. Experimental conditions: 0.1 s/0.1 s (ON/OFF), 100 cm coil length, 40 µL sample slug volume. Sample/reagent: (a) 5:1 (v/v); (b) 5:2 (v/v); (c) 5:3 (v/v); (d) 5:4 (v/v); (e) 5:5 (v/v).

3.2. Effect of sample slug volume

The recommendations accompanying the Vacu-vials® kit suggest 25 mL for the sample solution in the vial containing the reagent solution. If the volume of reagent is 5 mL, the stoichiometric relation between the sample and reagent solution would be 5:2. The volume of sample solution was tested, by varying the number of pulses of micro-pump P1 switch ON/OFF in order to change the sample slug volume from 8.0 to 80 µL (Figure 3). Better results were obtained when the volume of the sample solution was 40 µL (Figure 3, curve c). These assays were carried out using standard solutions with concentrations within the range of 0.5 to 5.0 mg L^{-1} PAA, obtaining a correlation coefficient of 0.9995 (n = 6).

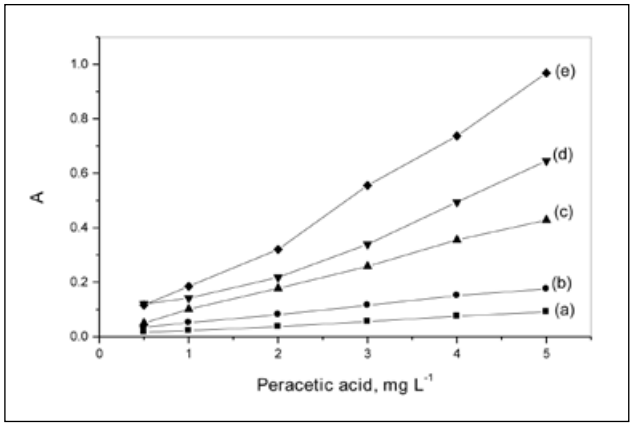

Figure 3. Effect concerning to the ratio between volumes of sample and reagent solutions. Experimental conditions: 0.1 s/0.1 s (ON/OFF), 100 cm coil length. Sample/reagent: (a) 1:2 (v/v); (b) 1:1 (v/v); (c) 2.5:1 (v/v); (d) 3:1 (v/v); (e) 5:1 (v/v).

3.3. Effect of reaction coil length

The results discussed above were obtained using a 100 cm reaction coil. Because the dimension of the reaction coil may affect detectivity, precision and sampling rate, a set of assays was performed varying the length of the coil from 50 to 150 cm. A slightly increased signal magnitude of 6 % was observed for reaction coils that were longer (120 cm). A coil with a length of 150 cm decreased the signal magnitude by 15% when compared with the 100 cm reaction coil. It is possible that the decrease in signal was caused by a dispersion effect, which increases with the length of the reaction coil. Because the best results considering detectivity, linearity and precision, were obtained with a reaction coil with a length of 100 cm, this was the reaction coil used.

3.4. Analytical figures of merit

Using the best operational conditions determined previously (volumes of 200 µL for sample and 80 µL for reagent solutions) additional experiments were performed in order to establish the linear dynamic range and detection limit. The analytical curve equation was Abs = (0.0725 ± 1.12 x 10^{-3}) mg L^{-1} PAA + (0.0017 ± 0.0018) with a correlation coefficient of 0.9995. The limit of detection (LOD) and limit of quantification (LOQ) were 0.10 mg L^{-1} and 0.33 mg L^{-1}, respectively. LOD and LOQ were estimated using the equation: LOD = $3s_b/S$ and LOQ = $10s_b/S$, where S is the slope and s_b is the blank solution standard deviation (n_b = 8). The results obtained by the system were compared with those found using the Vacu-vials® kit and the reference method based on permanganate-thiosulfate titration [12], as shown in Table II.

Table II. Comparison of the analytical features achieved by the proposed method, Vacu-vials® procedure and reference method [12] for peracetic acid determination in disinfectants samples.

Parameters	Proposed method	Kit Vacu-vials®	Reference method
Sample volume (µL)	200	25000	50000
Reagent volume (µL)	80	2000	- a
Linear range (mg L^{-1})	0.5 - 5.0	1.0 - 4.0	150 - 300
LD (mg L^{-1})	0.10	NI	NI
RSD (%)	4.0 (3.0 mg L^{-1})	NI	NI
Analytical throughput (h^{-1})	80	10	2
Waste volume[b] (mL)	1.5	27	> 50

NI: not informed. [a]Varies with the sample concentration. [b]Per determination

The coefficient of variation (obtained using eight replicates of a 3 mg L^{-1} peracetic acid solution) was 4.0%.

The sample consumption was 600 times lower for the flow system than for the reference method. The sampling throughput was 80 injections per hour, for the proposed system, which indicates that laboratory productivity would be enhanced by 3.6 or 18 times, compared to the Vacu-vials® Kit and to the reference method, respectively.

The performance of the multicommuted flow injection analysis process (MCFIA) in real sample analysis was evaluated using three disinfectant samples that were assayed using both the proposed procedure and the reference permanganate titration [12] method. The results are presented in Table III.

Table III. Peracetic acid concentrations (g L^{-1}) in sample disinfectants obtained by the proposed procedure (MCFIA) and the reference method [12].

Samples	Proposed method (g L^{-1})	Reference method (g L^{-1})	Relative Error (%)
1	166.9 ± 16.4	163.4 ± 13.2	- 2.14
2	169.8 ± 18.2	162.1 ± 4.8	- 4.75
3	77.4 ± 6.4	101.3 ± 21.9	23.6

The data reported indicate the presence of peracetic acid concentrations between 77 and 170 g L^{-1} with a RSD between 6.4 and 18.2 % using the method under study, compared with 4.8 to 21.9% for the reference method. The values of relative errors were both positive and negative, indicating that both procedures provide comparable results without systematic errors.

In the reference method, the hydrogen peroxide present is first titrated under cold conditions with a standard solution of potassium permanganate. This titration eliminates the peroxide. Potassium iodide is then rapidly added and the iodine liberated by the peracid is titrated with standard thiosulphate solution [12]. In the proposed method there is no step to eliminate peroxide. As mentioned, the proposed method is based on the reaction of PAA with I^- solution. The concentration of I^- in the sample solution should be high enough to dissolve the I_2 resulting from the reaction of PAA (and H_2O_2) with I^- (reference 3). Probably, in sample 3, there was a high concentration H_2O_2 and this reacted with I^- generating a larger amount of I_2. Thus, the excess of I^- was not enough to solubilize I_2, so that the analytical signal was smaller, resulting in a lower PAA concentration.

The standard addition method was also used to test the accuracy of the assessment samples, yielding the results showed in Table IV. As seen, the recoveries obtained are within the range of 95 to 106 %, which is considered acceptable for this type of sample.

Table IV. Recoveries of peracetic acid in water samples. Results expressed in mg L^{-1}

Samples	Concentration added	Standard addition Method [A]	Recovery (%)
1	1.00	1.01 ± 0.04	101 ± 4
2	1.69	1.79 ± 0.16	106 ± 9
3	1.35	1.32 ± 0.05	97 ± 3
4	1.22	1.17 ± 0.09	95 ± 7
5	2.00	1.94 ± 0.12	97 ± 5

[A] n = 3

4. Conclusions

The proposed methodology (MCFIA) with spectrophotometric detection showed robustness and versatility and offers several advantages over the reference method for routine analyses of peracetic acid samples (permanganate titration) [12] and the Vacu-vials® kit, such as higher analytical frequency and lower cost. The reaction and detection are integrated in the same manifold and all operations can be controlled from the computer, providing simplicity and reliability when compared to permanganate titration. Considering the advantages presented, it is feasible to employ this procedure for the analysis of peracetic acid in any water treatment laboratory.

Acknowledgements

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Determination of volatile organic compounds present in fruit from the Geonoma *gamiova* and Geonoma *pauciflora* palm trees using the SPME technique

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Abstract
The purpose of this study was to collect and identify the greatest possible number of volatile organic compounds produced in the inflorescence of the Geonoma *gamiova* and Geonoma *pauciflora* palm trees, which are plant species endemic to the Atlantic rain forest in the Cubatão region. Sample collection and pre-concentration were carried out using the SPME technique and compounds were analyzed by GC-MS. The samples were extracted via the HS-SPME method using a 100 μm PDMS-coated fiber mounted in a manual syringe holder at 30°C for 30 min**.** The analyses were performed in full-scan mode with a mass range of m/z 40 to m/z 500. The compounds were ionized in the electron ionization ion source and separated by an ion trap mass analyzer. By analyzing the results, we can observe that the fruit from the Geonoma *gamiova* palm features 20 different volatile compounds, which were extracted and identified by the aforementioned techniques, while the Geonoma *pauciflora* palm featured 15 different volatile compounds, which were also extracted and identified. Both palms proved to have five volatile compounds in common: 1,2-benzenedicarboxylic acid, n-capric acid n-heptyl ester, 3,5-bis(1,1-dimethylethyl)-phenol, 2-hexyl-1-octanol.

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Keywords: SPME, volatile organic compounds, GC-MS, Palm tree

Introduction

Headspace solid-phase micro-extraction (HS-SPME) is an efficient technique for the extraction of volatile compounds from plants. HS-SPME has also been successfully used for screening complex volatile mixtures and for complementing information obtained from other methods of extraction, due mainly to its simplicity and reliability (1).

Sample preparation for the analysis of flavors and fragrances normally involves the use of headspace, purge and trap, liquid-liquid extraction, solid phase extraction or simultaneous extraction and distillation techniques, in order to concentrate the analyte in question. These methods have several drawbacks, including a lengthy sample preparation time and excessive use of solvents. Solid-phase micro extraction (SPME) is a sorption and desorption technique, developed by researchers at the University of Waterloo (Ontario, Canada), which eliminates most of the disadvantages pertaining to sample preparation. (2-5).

The SPME technique requires no solvents or complex apparatus and it can be used to concentrate volatile and non-volatile compounds from liquid samples or headspace, providing linear results over a wide range of concentrations (sometimes in parts per trillion) and it can be used with gas chromatography systems coupled to mass spectrometry (1).

The volatile and semi-volatile compounds produced by plants and other living entities are collectively known as biogenic volatile organic compounds (BVOC). They include a large number of organic substances, including isoprene and terpenoid compounds, alkanes, alkenes, carbonyl compounds, alcohols and esters, and they are typically present in the atmosphere in concentrations ranging from parts per trillion (ppt) to parts per billion (ppb). Determining the quantity of BVOC released from living plants is usually carried out by placing whole plants or parts thereof in a chamber made of glass or plastic, from which the organic compounds emitted are collected and analyzed chromatographically. The overall process has several drawbacks; for example, material used for extraction may introduce artifacts into the extracted material, the solvent used to extract the adsorbed material can mask the detection of compounds with low boiling points, and long sampling periods may be necessary to capture sufficient amounts of analyte for analysis (6).

To date, poly(dimethylsiloxane) (PDMS) coating is one of the most widely used coatings for extracting volatile

analytes from environmental samples via absorption. (7) The relative ease of extraction, and the fact that the absorption process is not affected by competition between the absorbed analytes, prompted the development of air sampling methods based on PDMS coating. In addition, the theory behind the equilibrium and non-equilibrium extraction process for absorptive (liquid-phase) PDMS coatings is well-understood and has already been described in the literature (8-12)

The proposed method of SPME analysis of inflorescence offers considerable advantages compared to previously-used methods for this study. The method is robust and precise, and it reduces the influence of stress on the plant during sampling. (1)

The main objective of this study was to collect and identify the largest possible number of volatile organic compounds produced in the inflorescence of the Geonoma *gamiova* and Geonoma *pauciflora* palm trees, plant species endemic to the Atlantic forest in the Cubatão region. The collection and pre-concentration of samples were carried out by the SPME technique and the compounds were analyzed by GC-MS.

Experimental Section

Instruments

A gas chromatograph coupled to an ion trap mass spectrometer (Varian Saturn model 2200), equipped with a Agilent (DB-17MS) 30m x 0.25 mm (Narrowbore) x 0.25 μm column, was used for the chemical analyses.

Oven temperature was programmed as follows: initial temperature, 50°C ; increased to 250°C at 10°C min^{-1}; maintained for 20 min. The total run time was 45 min. Injection temperature 260°C, and interface temperature 360°C. The fiber was inserted into a silanized narrow-bore injector using the splitless mode. Ultrapure nitrogen was used as the carrier gas. The analyses were carried out using an SPME System for GC-MS (Supelco, Bellefonte, PA, USA.), and a 100 μm coating of polydimethylsiloxane (PDMS) on the Fiber.

Sample Collection

Fruit from the Geonoma *gamiova* and Geonoma *pauciflora* palm trees was collected from the Atlantic forest in the Cubatão region. After collection, the fruit was placed in plastic material bags (used for wrapping food) and the bags were sealed.

SPME analysis

The samples were extracted via the HS-SPME method using a 100 μm PDMS-coated fiber mounted in a manual syringe holder at room temperature for 30 min.

Approximately 1.5 grams (3 units) of fruit were placed in a 10 ml vial at room temperature. The fruits were then macerated and the bottle was immediately sealed with a silicone seal, in order ensure uniformity in the gas phase. Subsequently, the SPME system was introduced into the vial and the fiber was exposed to the compounds. The volume of headspace was approximately 5 ml. After 30 minutes the fiber was withdrawn from the vial and immediately introduced into the gas chromatograph injector. The compounds were then thermally desorbed and analyzed by mass spectrometry

The analyses were performed in full-scan mode with a mass range from m/z 40 to m/z 500. The compounds were ionized in the electron ionization ion source and separated by an ion trap mass analyzer.

Results

Preparatory experiments were carried out to determine several operational parameters to be adopted for this study, such as the type of SPME fiber to be employed and optimum extraction times. 65-μm PDMS/DVB and 100-μm PDMS fibers, and 10, 20 and 30 min of extraction time were tested.

Heating before extraction was evaluated, but showed no significant changes. For this reason, it was decided to perform the extraction at room temperature, with the aim of simplifying the experiments.

Exposure times were the first parameter tested. The experiments were performed for exposure times of 10, 20 and 30 min for the Geonoma *gamiova and* for Geonoma *pauciflora*, using the PDMS fiber. It was possible to observe that the exposure time that was able to extract the largest amount of compounds was the time of 30 minutes for both palms trees.

Experiments were also carried out to choose the SPME fiber. The experiments were performed using the 65-μm PDMS/DVB fiber with the exposure time of 30 min for Geonoma *gamiova* palm tree and compared with the results obtained with the100-μm PDMS fiber using the same exposure time.

It was determined that the best fibers for monitoring BVOC produced by the fruit of Geonoma *pauciflora* and Geonoma *gamiova* were those coated with polydimethylsiloxane (PDMS). The amounts extracted were smaller for the other liquid coated fiber analyzed, when compared to the solid-coated fibers, and most of the BVOC peaks could not be discerned from the background signal. During these preliminary experiments, BVOC from mechanically crushed fruit were extracted with PDMS fibers. These extractions were performed immediately after the fruit was mechanically crushed.

Blank analyses of the fiber and of the glass chamber were run before starting each daily sample set. Time between sampling and analysis never exceeded 5 min. Under these conditions, the loss of extracted analytes is expected to be insignificant. (6) Carryover was also tested and the results proved to be negligible. During

the experiments, the fibers were not used more than 10 times each. All experiments were performed 3 times and it was possible to observe good reproducibility of the system

Because it is a complex multicomponent system, it is not possible to say that the analysis conditions used correspond to equilibrium partition. The main object of the study was to analyze the largest possible amount of compounds extracted from fruits. Experiments of linearity, and equilibrium were not performed.

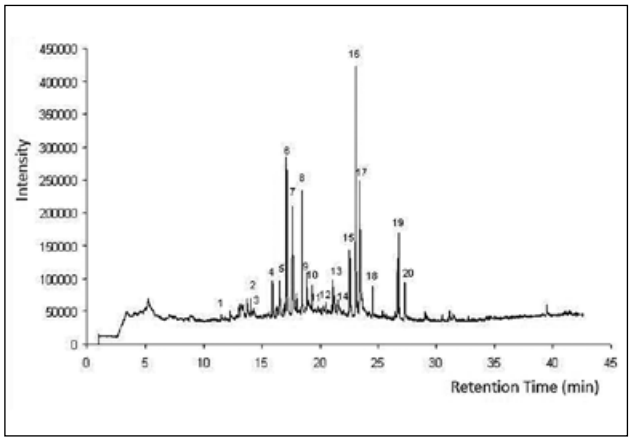

Figure 1. Total ion chromatogram profile of the samples extracted by the SPME technique from the Geonoma gamiova palm tree, using a PDMS fiber and an exposure time of 30 min.

Figure 01 shows the total ion chromatogram profile of the samples extracted from the Geonoma *gamiova* palm tree using the SPME technique with the PDMS fiber and exposure time of 30 min. The spectrum was acquired using GC-IT-MS, operating in full-scan mode with a mass range from m/z 40 to m/z 500. Identification of the compounds was performed by comparing the spectrum of the samples with a mass spectra library (NIST).

The twenty tentatively identified compounds extracted from the fruit of Geonoma *gamiova* can be observed in Figure 01. In Table I, some of the compounds tentatively identified by mass spectrum comparison after extraction from the fruit of the Geonoma *gamiova* palm tree are presented. In this table the retention time of the identified compounds, as well as their mass spectra, can also be observed.

The same experiment was carried out on fruit from the Geonoma *pauciflora* palm tree. The characteristic chromatographic profile is shown in Figure 02; the compounds were also tentatively identified by comparing their mass spectra with a NIST mass spectra library. More than 30 different compounds were extracted; however, some of these peaks could not be identified due to low similarity with the spectra stored in the NIST library.

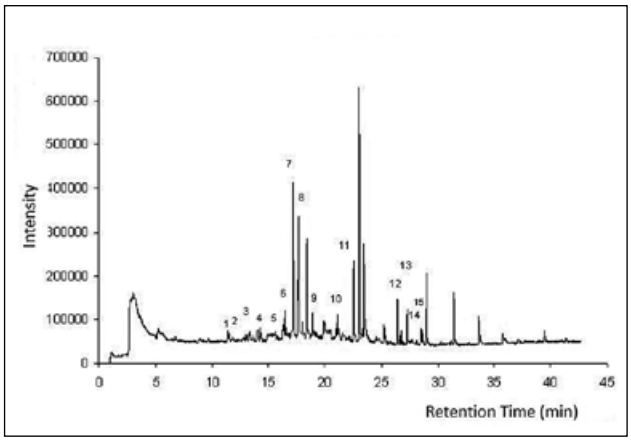

Figure 2. Total ion chromatogram profile of the samples extracted by the SPME technique from the Geonoma pauciflora palm tree, using a PDMS fiber and an exposure time of 30 min.

Table II shows the tentatively identified compounds present in the fruit from Geonoma *pauciflora*, their mass spectra and also their retention time. The two tables show the similarity index for the identification of compounds.

Analyzing the results we can see that the fruit from the Geonoma gamiova palm tree contained 20 different volatile compounds, which could be extracted and tentatively identified by the techniques employed, while the Geonoma pauciflora palm contained 15 different volatile compounds that were extracted and identified. Together, the palms contained five volatile compounds in common: 1,2-benzenedicarboxylic acid, n-capric acid n-heptyl ester, 3,5-bis(1,1-dimethylethyl)-phenol, 2-hexyl-1-octanol.

Conclusion

The SPME technique was very efficient in extracting volatile compounds from the fruit of the Geonoma *gamiova* and Geonoma *pauciflora* palm trees.

SPME is a unique technique for sample preparation because it addresses most of the drawbacks pertaining to the extraction of organic compounds prevalent in other methods, such as the use of solvents or complex apparatus. The volatile compounds present in the fruit were easily concentrated by the SPME fiber system, which made even the analysis of low concentrations of compounds possible.

In addition, the possibility of subsequent analysis by gas chromatography / mass spectrometry, by only carrying out the thermal desorption of compounds in the injector of the chromatograph, made analyzing the complex mixture of compounds present in the fruit of palm trees much easier.

The use of the SPME technique reduced the preparation and pre-concentration time for samples, since all compounds are extracted simultaneously. The

Table I. Retention time, mass spectra, structure and similarity grade of the Identified compounds present in the fruit of the Geonoma gamiova palm tree.

RT (min)	Mass spectra Peaks(intensity)	Compound	Structure	% similarity
13.29	**95** (999), **41** (890), **107** (834), **149** (784), *81* (741), **220** (731), **93** (681), **43** (670), **55** (669), **82** (547)	3-Isopropyl-6,7-dimethyltricyclo[4.4.0.0(2,8)]decane-9,10-diol	$C_{15}H_{26}O_2$	99
14.04	**57** (999), **43** (804), **41** (632), **55** (549), **71** (522), **69** (418), **29** (349), **56** (312), **85** (294), **83** (272)	2-Hexyl-1-octanol	$C_{14}H_{30}O$	93
14.28	**191** (999), **57** (302), **41** (181), **206** (145), **192** (143), **29** (121), **74** (102), **39** (71), **27** (58),**91**(53)	2,4-bis(1,1-dimethylethyl)-phenol	$C_{14}H_{22}O$	94
15.92	**69** (999), **41** (800), **81** (480), **53** (360), **82** (240), **39** (170), **175** (150), **95** (140), **136** (120), **55** (120)	3-(4,8-dimethyl-3,7-nonadienyl)-, (E)-furan	$C_{15}H_{22}O$	97
16.53	**57** (999), **43** (782), **71** (641), **85** (500), **41** (384), **55** (282), **56** (256), **84** (179), **69** (147), **155** (134)	2,6,10-trimethyl-tetradecane	$C_{17}H_{36}$	98
17.16	**233**(999), **57**(582), **18** (382), **29**(377), **41**(178), **247** (168), **234** (165), **262** (116), **17** (101), **91**(99)	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	$C_{17}H_{26}O_2$	97
17.66	**57** (999), **43** (697), **41** (553), **173** (548), **56** (542), **70** (516), **98** (479), **55** (441), **155** (370), **69** (359)	n-Capric acid n-heptyl ester	$C_{17}H_{34}O_2$	95
18.43	**100** (999), **56** (134), **42** (109), **41**(68), **43** (64), **101**(55), **70** (54), **55** (30), **57** (29), **86** (23)	2-(4-morpholyl)-N-(4,5,6,7-tetrahydro-2-cyano-benzothien-3-yl)-acetamide	$C_{15}H_{19}N_3O_2S$	97
18.89	**57** (999), **43** (804), **41** (632), **55** (549), **71** (522), **69** (418), **29** (349), **56** (312), **85** (294), **83** (272)	2-Hexyl-1-octanol	$C_{14}H_{30}O$	95
19.30	**41** (999), **55** (936), **43** (885), **57** (783), **82** (736), **71** (702), **67** (671), **68** (615), **69** (587), **81** (521)	tetradecyl-oxirane	$C_{16}H_{32}O$	99
21.13	**57** (999), **43** (762), **71** (720), **85** (530), **41** (447), **55** (345), **56** (201), **69** (174) **70** (155) **29** (139)	Nonadecane	$C_{19}H_{40}$	97
21.52	**57** (999), **43** (888), **55** (670), **41** (633), **69** (580), **56** (572), **71** (514), **83** (460), **97** (386), **70 (**341)	2-methyl-1-hexadecanol	$C_{17}H_{36}O$	93
21.58	**82** (999), **57** (860), **41** (760), **55** (720), **43** (690), **96** (640), **81** (570), **83** (510), **95** (450), **69** (450)	Pentadecanal-	$C_{15}H_{30}O$	92
22.55	**149** (999), **57** (261), **150** (94), **41**(91), **29** (72), **56**(65), **223** (64), **104** (51), **76** (35),**167** (34)	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	96
23.07	**100** (999), **56** (96), **42** (74), **70** (55), **101** (46), **44** (33), **113** (33), **43** (20), **41**(20), **157**(18)	1-[p-Nitrophenyl]-3-[2-morphaínaethylurea}	$C_{13}H_{18}N_4O_4$	95
23.41	**58** (999), **43** (60), **55** (30), **59** (30), **269** (22), **42** (20), **57** (20), **268** (10), **84** (10),**72** (10)	N,N-dimethyl-hexadecanamine,	$C_{18}H_{39}N$	89
25.33	**43** (999), **57** (898), **71** (571), **41** (490), **85** (408), **55** (321), **69** (162), **56** (139), **210** (106), **99** (103)	9-hexyl-heptadecane	$C_{23}H_{48}$	94
24.52	**272** (999), **106** (750), **257** (548), **105** (54), **91** (521), **94** (458), **120** (451), **19** (416), **93** (416), **41** (389)	13-isopropyl-podocarpa-6,13-diene	$C_{20}H_{32}$	96
26.70	**145** (999), **57** (944), **43** (426), **55** (352), **41** (333), **88** (333), **89** (222), **69** (204), **71** (185), **56** (185)	Hexanoic acid, 2-ethyl-, dodecyl ester	$C_{20}H_{40}O_2$	99
27.29	**100** (999), **30** (370), **43** (119), **29** (109), **41** (106), **55** (103), **56** (65), **101**(60), **44** (51), **27** (51)	6-Undecylamine	$C_{11}H_{25}N$	91

Table II. Retention time, mass spectra, structure and similarity grade of the Identified compounds present in the fruit of the Geonoma pauciflora palm

RT (min)	Mass spectra Peaks (intensity)	Compound	Structure	% similarity
11.37	**88** (999), **101** (700), **43**(570), **55** (500), 41 (480), **57** (400), **69** (226), **73** (224), **71** (190), **70** (152)	Hexadecanoic acid, ethyl ester	C18H36O2	98
11.51	**43** (999), **57** (701), **71** (583), **85** (418), **55** (371), **41** (337), **69** (215), **70** (144), **99** (134), **83** (130)	3 ethyl-5(2-ethylbutyl)-octadecane	C26H54	96
14.03	**57** (999), **43** (804), **41** (632), **55** (549), **71** (522), **69** (418), **29** (349),**56**(312),**85** (294), **83**(272)	2-Hexyl-1-octanol	C14H30O	98
14.27	**191** (999)**, 57** (392), **206** (208), **41** (158), 192 (146). 91 (84), **135**, (71), **107**, (67), **77** (56), **163** (55)	3,5-bis(1,1dimethylethyl-phenol,	C14H22O	97
16.35	**88** (999), **101** (581), **43** (376), **57** (275) **55** (238), **41** (226), **89** (183), **29** (159), **69** (155), **157** (134)	Nonadecanoic acid, ethyl ester	C21H42O2	96
16.50	**57** (999), **43** (710), **28** (560), **41** (450), **71** (300), **29** (280), **55** (280), **85** (160), **56** (160), **98** 140	6-methyl-octadecane	C19H40	97
17.13	**233** (999), **57** (582), **18** (382), **29** (377), **41** (178), **247** (168), **234** (165), **262** (116), **17** (101), **91**(120)	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	C17H26O2	98
17.64	**57** (999), **43** (697), **41** (553), **173** (548) **56** (542), **70** (516), **98** (479), **55** (441),**155** (370), **69** (359)	n-Capric acid n-heptyl ester	C17H34O2	97
18.87	**57 (**999), **43** (762), **71** (720), **85** (530), **41** (447), **55** (345), **56** (201), **69** (174), **70** (155), **29** (139)	Nonadecane	C19H40	95
21.13	**41** (999), **57** (948), **85** (939), **43** (881),**29** (811), **55** (638), **27** (359), **56** (299), **42** (268), **69** (265)	Valeric acid, 2-pentadecyl ester	C20H40O2	98
22.55	**149** (999), **57** (261), **50** (94), **41** (91), **29** (72), **56** (65), **223** (64), **104** (51), **76** (35), **167** (134)	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	C16H22O4	97
26.40	**356** (999), **43** (328), **357** (217), **399** (95), **326** (61), **297** (55), **340** (53), **358** (39), **298** (37),**328** (36)	α-Lumicolchicine	C44H50N2O12	98
27.30	**100** (999), **101** (59), **43** (51), **55** (33) **41** (32), **57**(27), **56** (26), **42** (21), **87**(20), **29**(15)	4-octadecyl-morpholine	C22H45NO	98
28.50	**67** (999), **81** (748), **41** (604), **55** (87), **95** (511), **54** (424), **45** (421), **68** (367), **82** (356)	Linoleic acid ethyl ester	C20H36O2	97
28.61	**55** (999), **41** (720), **69** (640), **43** (560), **83** (480), **98** (460). **67** (430), **57** (420), **264** (390), **81**(380)	9-Octadecenoic acid (Z)-2,3-dihydroxypropyl ester	C21H40O4	96

technique also proved to be a clean and economical method, since no solvents were used, thus reducing both costs and waste.

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Determination of chlorophenols in environmental samples by fiber introduction mass spectrometry using a novel sol-gel fiber

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Abstract

A new sol-gel solid phase microextraction fiber, based on aminopropyltrimethoxysilane (APTMS) and polydimethylsiloxane (PDMS), is applied for the extraction of chlorophenols from environmental samples followed by fiber introduction mass spectrometry (FIMS) determination. Sensitive, selective, simple and simultaneous quantitation of several chlorophenols is achieved with no derivatization or pre-separation by monitoring diagnostic fragment ions. Extraction efficiencies were maximized by sample acidification to pH = 1 and by allowing an equilibrium time of ca. 8 min. Analytical curves ranging from 25 to 400 μg L^{-1} were obtained, with correlations higher than 0.93. Limits of detection (LOD) and quantification (LOQ) were in the range of a few μg L^{-1} with recoveries from creek water samples spiked with 100 μg L^{-1} of chlorophenols ranging from 55 to 107%. The APTMS/PDMS sol-gel fiber presented good chemical and mechanical resistance to the extreme desorption conditions of FIMS, with no substantial carry over effects, and could be utilized in more than 200 extraction/desorption cycles.

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1. Introduction

Chlorophenols (CP) have been extensively utilized as disinfectants, herbicides, insecticides, fungicides and for wood conservation. These compounds tend to accumulate in fat tissues of animals, where they may act as endocrine-disrupting agents [1][2]. CP may also give rise to dioxins in milk and beef when cows are fed with CP-contaminated vegetable matter, in a clear risk to human health [3]. Other sources of CP environmental contamination is chlorination of phenolic compounds in waste waters treated with chlorine [4]. Due to their toxicity, CP were considered priority pollutants by both the US Environmental Protection Agency [5] and the European Community [6].

To achieve the necessary detectivity for contaminants in environmental samples, matrix clean-up and analyte pre-concentration are often required. For example, Bagheri et al. [7] developed a solid phase extraction (SPE) device based on a polypyrrol conductive polymer, achieving limits of detection (LOD) of ca. 0.15 μg L^{-1} in river water analyses. A major drawback of traditional extraction techniques such as SPE is, however, the need for great amounts of toxic and expensive solvents; hence alternative extraction techniques have been developed. Solid phase microextraction (SPME), developed in the early 1990s [8], offers a much simplified procedure and in a single step combines sampling, clean-up, extraction and pre-concentration. SMPE is relatively inexpensive, fast, and highly sensitive, and its operational principle is the sorption of analytes by a sorbent coating deposited onto a piece of fused silica fiber. After extraction, the fiber is removed from the sample vial and inserted into the injector of a gas chromatograph or a liquid chromatograph for further separation and quantitation. Chlorophenols, for instance, have been determinated by SPME-GC in honey [9], wine [10] and water [11] whereas SPME coupled to high-performance liquid chromatography was used to determine CP in water [12].

The commercial SPME fibers applied to chlorophenol determination suffer, however, from low thermal stability and bleeding when exposed to organic solvents. There is a need therefore to develop new sorbent coatings to overcome these drawbacks. Sol-gel chemistry is a mild and easy way to obtain glassy materials and has been applied in the preparation of SPME sorbent coatings since 1997 [13]. A wide variety

of new and stable coatings has been produced [14] [15]. The advantages of these sol-gel coatings, such as better chemical and mechanical resistances (including thermal resistance), when compared with the commercial fibers, result from the chemical anchoring of the sorbent phase onto the fused silica fiber. Briefly, sol-gel reactions are based on the hydrolysis and condensation of (generally) silicon alcoxides, leading to a three-dimensional glassy network. Organic compounds containing reactive functionalities (hydroxyl, for example) may take part of the reaction, giving rise to an organically modified silica (ormosil). The surface of the ormosil possesses hydroxyl groups, hence they are able to bind to the surface of the fused silica fiber, which has also superficial hydroxyl groups. The characteristics of an ormosil may be tailored according to the condensable organic compounds added. A sol-gel fiber based on aminopropyltrimethoxysilane (APTMS) and PDMS [16] has been described.

Chromatographic analysis of CP often requires a derivatization step, since their acidic hydrogens give rise to peak tailing with poor detection limits and poor quantitation. The derivatizating agents usually employed are acetic anhydride [17] and reactive silicon compounds such as hexamethyldisilazane [18] and bis(trimethylsilyl)trifluoroacetamide [19], but their use introduces a new step into the analytical process.

We have reported on the coupling of SPME with MS and its direct use with no pre-separation and derivatization steps and termed this new technique as fiber introduction mass spectrometry (FIMS) [20]. In FIMS (Figure 1), the SPME fiber is directly inserted into the mass spectrometer source (between the EI filaments) by means of a proper fiber holder, allowing efficient analyte desorption by filament heating and ionization by the action of the 70 eV electrons. This direct fiber exposure is aimed to eliminate the need of derivatization and pre-separations, thus reducing analysis time, manipulation and separation biases and has been shown to be efficient for multiresidual determination, with the target analytes being monitored by molecular ions or diagnostic fragment ions. Using this technique, we have analyzed phtalates in mineral water samples, achieving limits of detection of 3.6 ng mL^{-1} with a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber [21]. Pesticide determination from *P. edulis* herbs was also performed, and limits of detection of a few ng mL^{-1} were achieved with a sol-gel PDMS/PVA fiber. This fiber displayed good chemical and mechanical resistance to the severe EI desorption conditions, being used for more than 400 extraction/desorption cycles [22].

Herein we describe the use of a new sol-gel fiber based on aminopropyltrimethoxysilane (APTMS) and PDMS [16] for the direct, fast and efficient FIMS analysis of CP in aqueous solutions.

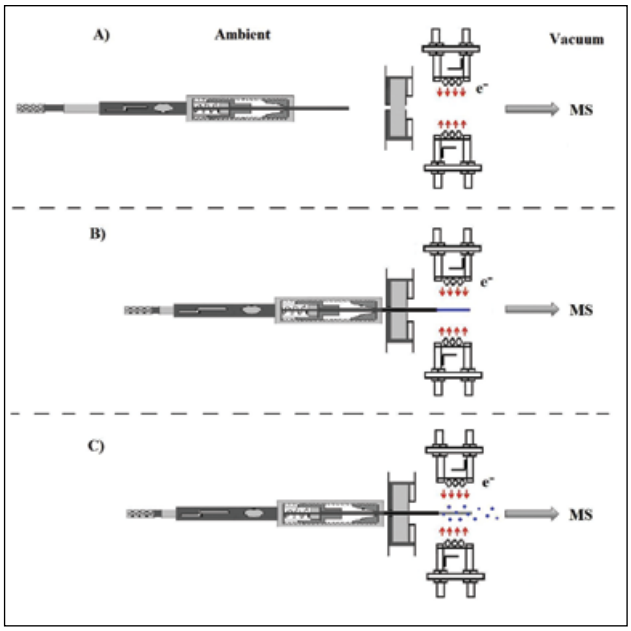

Figure 1. Overview of the main steps involved in FIMS analysis.

2. Experimental

2.1. Chemicals and materials

Optical fibers (ABC-Xtal, Campinas, SP, Brazil) with a 128 µm core were used as support. The sol-gel materials were 3-aminopropyltrimethoxysilane (APTMS - Acros Organics, Geel, Belgium), hydroxyl-terminated polydimethylsiloxane (PDMS-OH - Aldrich, Milwankee, WI, USA), trimethylmethoxylsilane (TMMS - Fluka, Buchs, Switzerland), methyltrimethoxysilane (MTMS - Fluka) and trifluoroacetic acid (TFA - Acros Organics). The CP selected for this work, 4-chloro-3-methylphenol (43CP), 2-chlorophenol (2CP), 2,4,6-trichlorophenol (246CP) and pentachlorophenol (PCP), were obtained from Merck (Schuchardt, Germany). Stock solutions containing 1000 mg L^{-1} of each CP were prepared in HPLC-grade methanol (Merck) and stored in a refrigerator. The extractions were performed in Teflon/silicone septa-capped 15 mL glass vials (Supelco, Bellefont, PA, USA). Analytical grade NaCl and HCl (Aldrich) were also used. Deionized water was generated with a Direct-Q UV water purification system (Millipore, Molsheim, France).

2.2. Samples

Water from Anhumas Creek, a highly polluted water course in the city of Campinas (95 km NW from São Paulo, Brazil), was collected for analysis. This sample was filtered to eliminate suspended solids and stored in sealed glass vials under refrigeration until use. Samples from three wooden railway sleepers, stored in open air near a deactivated railway station in the same city, were also collected. Chunks of the wood samples were brought to the laboratory, broken into small (~ 1 – 2

cm) pieces, mixed with dry ice to avoid loss of volatile species and pulverized in a stainless steel blender (Cole Parmer, Vernon Hills, IL, USA). The samples were ground, sieved (particle size ranging from 75 μm to 100 μm) and stored in closed flasks under refrigeration prior to use.

2.3. FIMS

All FIMS analysis were performed in an Extrel Mass Spectrometer (Pittsburgh, PA, USA) fitted with a high-transmission ¾" quadrupole and an EI ion source. The ion source of the MS was modified as previously described [20] to allow a SPME fiber to be placed directly between the two parallel MS filaments for uniform heating and efficient desorption of the analytes (Figure 1), being ionized by 70 eV EI. The desorption time was 60 s for all experiments and no carryover between runs was observed with this time. The MS gain and electron multiplier voltages were 1 x 10^{10} and 1100 V, respectively. Detection and quantification of the CP were performed by selective ion monitoring (SIM) of diagnostic fragment ions of *m/z* 107 for 4-chloro-3-methylphenol, *m/z* 128 for 2-chlorophenol, *m/z* 196 for 2,4,6-trichlorophenol and *m/z* 266 for pentachlorophenol.

2.4. Preparation of the sol-gel APTMS / PDMS fiber

Two centimeter pieces of the optical fiber were dipped in concentrated sulfuric acid for 3 h for removal of the protective polyimide layer. In sequence, the uncoated fibers were exposed for 1 h to 1 mol L^{-1} NaOH solution, to activate its surface. The activated fibers were subsequently washed with 0.1 mol L^{-1} HCl for removing the excess base, rinsed with distilled water, dried at 70°C and stored in a desiccator. The sol-gel reaction was carried out in a 3 mL disposable polyethylene vial: 300 mg of APTMS, 75 mg of MTMS, 150 mg of PDMS-OH and 125 μL of TFA (with 5 % of water) were mixed. The mixture was vortexed for 2 min; the dry activated fused silica fibers were exposed to the resulting sol for 1 h at lab temperature (22 °C – 27 °C), removed from the sol and stored overnight in a desiccator. This procedure was repeated five times with fresh sol, in order to obtain thicker coatings. The fibers were then exposed to a solution of 20 % methanolic solution of MTMS for 5 minutes, to end-cap residual superficial hydroxyls. Finally, the fibers were mounted on used, discarded commercial (Supelco) SPME assemblies, after careful removal of the original (deteriorated) fiber. Prior to use, the fibers were conditioned at 100°C for 1 h and then at 260°C for 6 h in the injection port of a gas chromatograph under flow of nitrogen (1 mL min^{-1}).

2.5. SPME

Water Samples. The extraction profiles for the CP using the APTMS / PDMS fiber were determined using aqueous solutions containing 100 μg L^{-1} of each analyte as sample. Due to the acidity of the analytes (pK_a ranging from 4.8 to 7.8), and to maintain the CP in their protonated forms so as to maximize their sorption by the fiber coating, the pH of the aqueous samples were adjusted to 1.0 prior to all extractions by dropwise adding of conc. HCl (10 μL to 15 μL). For the extractions, the APTMS / PDMS fiber was immersed in 10 mL of test sample in septum-sealed glass sample vials under magnetic stirring of 1000 rpm, for periods ranging from 1.0 to 20 min. After extraction, the analytes were immediately desorbed inside the MS. Analytical curves were obtained for the concentration range between 25 and 400 μg L^{-1} and the optimized extraction time of 7.5 min allowed assessment of quantitative figures of merit of the method. The LOD and the limits of quantitation (LOQ) were calculated from signal-to-noise ratios (S/N) of 3 and 10, respectively, estimated from data collected from extractions of 25 μg L^{-1} of the CP. The recovery was estimated with creek water samples spiked with 100 μg L^{-1} of each analyte.

Samples of sleeper wood. A suspension of 1.000 g of powdered wood sample in 6 mL of a water / methanol mixture (5:1 v/v) was stirred for 1 h at room temperature. This suspension was filtered, acidified and quantitatively transferred to vials for SPME analysis, which were carried out using the same parameters as above. Quantitation was then performed via analytical curves obtained by extractions of standard solutions of the analytes in water / methanol mixtures.

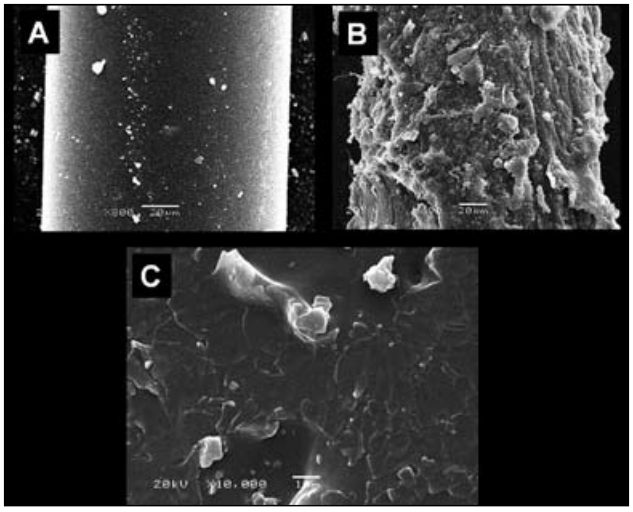

Figure 2. Electron scanning micrography of A) the uncoated fiber with 800× magnification and the APTMS/PDMS sol-gel fiber with B) 600× and C) 10,000× magnification. Adpated from reference [16].

3. Results and discussion

APTMS / PDMS SPME Fiber. Figure 2 shows the morphology of the sorbent coating of the APTMS / PDMS fiber. With magnification of 600×, it is seen that the

surface is very irregular, with the appearance of an agglomerate of globular and/or cylindrical elements of ~ 5 μm or less. Accurate measurement of the coating thickness is not feasible, due to this rough and irregular surface. Comparing the coated fiber (Figure 2B, fiber thickness of 194 ± 4 μm) with an uncoated fused silica fiber (Figure 2A, fiber thickness of 130 μm), the film thickness is calculated to be 32 ± 2 μm. Apart from preventing an accurate estimation of thickness, this irregular surface is an attractive characteristic for a SPME fiber since it leads to an increase in the surface area of the fiber exposed to analytes or to the vaccuum inside the MS ionization chamber, allowing faster mass transfer of analytes to and from the fiber. The microstructure of the coating can be inspected after 10k× magnification (Figure 2C), and the APTMS / PDMS surface appears here as being compact and non-porous. The material seems to be a cluster of scale-like pieces, and no macro- or mesoporous structures are visible. The lack of a pore system can be considered a drawback since irregular surfaces improve the performance of SPME fibers. But, apparently, the analytical performance of the fiber is not affected by this feature, as demonstrated elsewhere [16]. The coating also seems to maintain its integrity even after 200 extraction and desorption cycles. This endurance is relevant, since both processes occur under conditions which are particularly aggressive to SPME fibers: the extraction is performed by its immersion in acidified media, and the fiber is directly exposed to heating from the EI filaments. This robustness, not so typical for conventional fibers, is typical of sol-gel coated SPME fibers, probably due to the chemical bonding between the coating and the fused silica support.

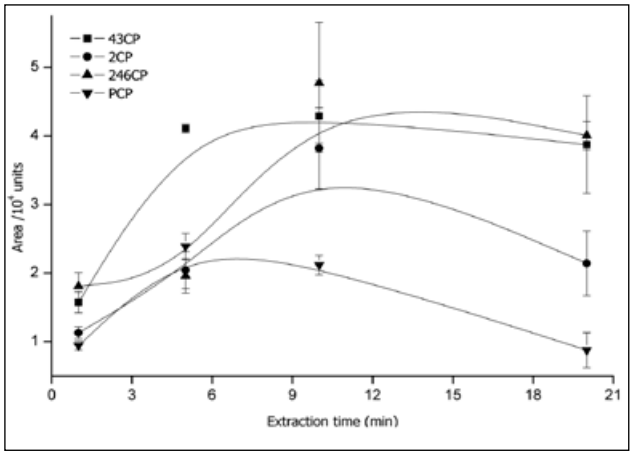

Figure 3. SPME extraction profile of CP from aqueous solutions (100 μg L^{-1}) in pH 1.

Extraction and MS Signal Profiles. Figure 3 shows FIMS (APTMS/PDMS) extraction profiles for all CP investigated. Equilibration is reached for periods ranging from ~6 min (PCP, 43CP) to ~13 min (246CP). These results show that the equilibration is extremely fast, compared to typical values of 60 min or more obtained for CP with commercial SPME fibers. Such fast equilibration is characteristic of sol-gel coated SPME fibers. Since the coatings provided by the sol-gel process are thinner, equilibration with sample is more rapidly achieved, in comparison with thicker coatings from commercial fibers. For these results, a time of 7.5 min was chosen as ideal for direct extraction, with the solution pH adjusted to 1 and with no CP derivatization. The standard relative deviation presented in CP ranged from 1.4% to 25%. Considering the extreme extraction and desorption conditions, and the considerable gains in speed and simplicity, these values seem quite acceptable.

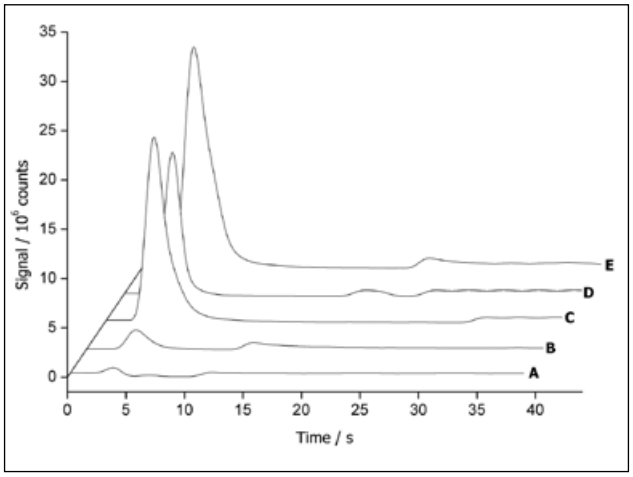

Figure 4. FIMS-APS/PDMS profile for SIM of the ion of *m/z* 128 from 2CP. A: Fiber blank; B: Deionized water blank; C: 200 μgL^{-1} aqueous test sample; D: Natural water spiked with 200 μgL-1 of each CP; E: MeOH/water extract of wooden railway sleeper spiked with 200 μgL-1 of each CP.

Figure 4 shows typical FIMS signal profiles obtained by selective ion monitoring (SIM) of the diagnostic ion of *m/z* 128 for 2CP. Similar profiles were observed for the other CP investigated. Curve **A** is the fiber blank, presenting no relevant signals that could interfere with the analyte signal. Curve **B** is the extraction signal of a deionized water sample: in this case, little signal perturbation is observed likely due to water adsorption by and subsequent desorption from the polar fiber coating. Curves **C** and **D**, intense and quite similar peaks for the ion of *m/z* 128 from 2CP obtained from a 200 μg L^{-1} aqueous solution are seen, with intensities ca. ten times greater than the blank. Curve **E** is for a wood sample spiked with 200 μg L^{-1} of 2CP; note the peak with intensity ca. fourteen times greater than the blank. After analysis, the fiber was re-inserted into the ionization chamber for cleaning. No substantial carryover effects were observed.

An analytical curve was obtained in the interval ranging from 25 to 400 μg L^{-1}. Concentrations lower than 25 μg L^{-1} presented signals with intensities close to the water blank signals and were therefore not studied. Table I shows figures of merit obtained for the analytical curves: good linear correlations were obtained, with a lower value of 0.9301 for 246TCP and a higher value of 0.9907 for 43DCP. Linear equations provided the detectability order: 43CP > PCP > 246PCP > 2CP. Detection and quantification limits (LOD and LOQ) were determined by signal-to-noise ratios of, respectively, 3 and 10, with the signal being estimated from the lower concentration of the obtained analytical curve. LOD values were in the order of μg L^{-1}, being 2 μg L^{-1} for 246CP and 13 μg L^{-1} for 43CP; LOQ was 5 μg L^{-1} for 246 CP and 23 μg L^{-1} for 2CP. Considering the extreme conditions and substantial variations that may occur in the EI source conditions to which the fiber is exposed, and the greater simplicity and speed of the FIMS method, these seems to represent satisfactory results (Table I).

Table I. Analytical curves obtained for the CP determination by FIMS. Linear range: 25 μg L^{-1} to 400 μg L^{-1}. LOD and LOQ – μg L^{-1}; ions of *m/z* 107 for 43CP; *m/z* 128 for 2CP; *m/z* 196 for 246CP and *m/z* 266 for PCP. [a]Uncertainty of a and b are expressed as the corresponding estimates of standard deviations.

CP	Linear equation	Correlation	LOD	LOQ
43CP	$y = 466 \pm 37^a x - 3582 \pm 7626^a$	0.9907	13	22
2CP	$y = 155 \pm 18 x + 8420 \pm 3769$	0.9797	7	23
246CP	$y = 172 \pm 39 x + 21774 \pm 8111$	0.9301	2	5
PCP	$y = 319 \pm 30 x + 3373 \pm 6183$	0.9871	4	8

Table II shows percentages of recovery for a creek water sample spiked with 100 μg L^{-1} of the CP. Recoveries ranged from 55% (246CP) to 107 % (2CP). Wood samples of a deactivated railway were also analyzed for quantitative evaluation, and 43CP, 2CP and 246CP could be determined in μg g^{-1} levels. PCP was not detected.

Table II. Recovery percentages of CP in 100 μg L^{-1} spiked creek water sample (R) and CP quantitative determination in extracts of railway wood samples (μg g^{-1}).
[a]species detected but not quantified; [b]non-detected species. Ions of *m/z* 107 for 43CP; *m/z* 128 for 2CP; *m/z* 196 for 246CP and *m/z* 266 for PCP.

CP	R (%)	W_{sample} 1	W_{sample} 2	W_{sample} 3
43CP	87	a	0.54	0.70
2CP	107	0.73	1.50	0.98
246CP	55	a	0.93	b
PCP	85	b	b	b

4. Conclusions

A newly developed sol-gel SPME fiber has been shown to function properly for the determination of chlorophenols via FIMS, with short extraction times (ca. 7.5 min) and LOD below or close to those required by the US EPA-625 methods [23]. The method also displays high simplicity, selectivity and robustness, and seems to provide a viable alternative for rapid and direct MS determination [24-25] of chlorophenols in water or aqueous extracts.

Acknowledgments

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Point of View

Technical and scientific partnerships require well defined objectives

Partnerships between companies, the academic community and government agencies can generate very positive results for all parties involved. However, to avoid negative surprises during the process and after its conclusion, it is important that partnerships be carefully established and included very clear objectives and goals. It is essential for each party to have well defined roles.

Technical and scientific partnerships should have very clear purposes that generate specific competencies or technological knowledge. In the case of companies, one of the first steps should be to verify the competency gap in order to ensure that the challenges are overcome. By identifying these gaps, partnerships can be formed with people and entities that are references in this competency in order to transfer the required knowledge. In the case of partnerships with universities, an important part of the process is transferring the knowledge generated by the institution. And this knowledge should be transferred to the company's R&D group. Knowledge is directly transferred from universities to companies more effectively by adopting competent intermediation.

Therefore, defining the objectives of this process is extremely important. For example, a university professor expects clear demands from a company. The clearer and better defined a need, project or problem, better is the chance of its success.

The initial definition of scope and the division of intellectual capital, royalties, patents, etc. should be combined at the start of the partnership, not during the process. However, it is important to emphasize that the main role of a university is the development of good professionals and researchers. The company is the one that transforms the knowledge into an interesting product for the market and that also incurs the risks inherent to development.

Today, universities are better understanding the role of supporting a manufacturer in its technological development, while the manufacturer has also put itself in the place of universities in order to better understand the difficulties of the environment, culture and legislation to which they are subject. And the government has worked through its agencies, coordinated by the Ministry of Science and Technology, to remove barriers and facilitate access to the development and tax incentives, to adjust laws, administrative rules and decrees and to disclose information to the Internal Revenue Service, Public Prosecutors' Office, Federal Accounting Court and other institutions.

Antonio Morschbacker
Braskem's Manager of Renewable Technologies

Point of View

Integration Academy / Industry: Quo Vadis?

In the beginning of the twenty-first century science in Brazil is experiencing a period of sustainable growth regarding any evaluation criteria used (e.g. number of publications, number of PhD graduates, international insertion, etc.). However, with respect to the industrial sector it is undergoing a process of "deindustrialization," since the current share of this sector in the economy is only about 23%, which represents a significant drop when compared with the last two decades.

The transition from a model of mineral and agro-based exports system, which currently predominates, to another one based on high-tech companies is too long and tortuous. It requires, among others, an effective interaction between academia and industry.

From the academic side, the challenge is to change the focus in a conventional disciplinary and departmental approach to a system focused beyond the academicals departments, and including "Centers of Excellence", where the multi and interdisciplinary approach are predominant. These "Centers of Excellence" should have some valuable characteristics, such as : i) institutional autonomy; ii) financial sustainability; iii) intelligence and leadership, iv) partnerships with public and private sectors; v) national and international partnerships; vi) an interdisciplinary research agenda, and vii) basic and applied research.

In turn, from the Industrial side, the challenge is create, over the country, research centers, capable of dialogue and practice effective partnerships with the academia, in the model, for example, of the Research Center Leopoldo Américo Miguez de Mello, CENPES - Petrobras. For instance, most of the Petrobras' leadership in deepwater exploration results from the academy/industry interaction. Other companies (e.g. Braskem, Vale and Natura) have increased the interaction academy / company and have achieved relevant results. But, although qualified, the interaction between academia and industry is still small, compared to the country needs, being necessary to be improved.

Scientific research, technology and innovation, require an interdisciplinary approach. Chemistry, a branch of science essentially experimental, has an important role in this interaction through the construction and transformation of molecules and, in particular, their immense responsibility in the establishment, and recognition, of standards and quality assurance of products. In this way, chemistry enables interaction Academy / Company / Society!

The integration Academy / Company is the way to the country turn knowledge into goods and welfare for the population.

Jailson B. de Andrade
Full Professor, UFBA, Coordinator of the INCT for Energy and Environment

Point of View

Good Fast Cheap
Choose any two

This should be the theme of the shell for industrial chemists, as it accurately portrays the daily lives of every laboratory in the industry.

As thought in academy we always known how to start, deploy, and complete an analysis (nothing like using the logical sequence of a survey for cations and anions as learned in qualitative analytical chemistry). With the resources of equipment now available at the university - or at least should be - we also develop a good knowledge of instrumental analysis and, aided by access to computers in existence today, of chemometrics. Nothing like what we had 40 years ago.

Over time we have obtained excellent analytical results more accurate and faster with the use of the latest equipment. However, if it was to be perform, the same analysis, using conventional methods and techniques (such as gravimetric/ titration analysis), we would find ourselves in trouble. Skills were lost and worse, knowledge. However things can be even worse, now there is an aggravating circumstance: that the outcome of the machine is regarded as divine truth.

For new equipment manufacturers they "teach" what buttons to push to get the results, but for the correct interpretation of them, we still relay on academic support. The university must provide the knowledge bases, and show how and where to seek information that may be necessary of professional development of their students.

Some very good journals, for example, Analytical Chemistry, brings excellent articles, but of little use in practice of the laboratory industry. Try just look for one that instructs how to open a sample.

The university has a major role, instill the habit of think.

Vladis Bernal Bassetto.
Bachelor in chemistry, IQ-USP 1974.
Worked as a petroleum chemistry in PETROBRAS from 1975 to 1998.
Consultant from 2001 up to present day.
Voting member of ASTM, status granted in 2003.

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- Outsourcing de computadores, notebooks, servidores, equipamentos de rede
- Outsourcing de impressão: terceirização da infra-estrutura de impressão
- Automação de processos

Conheça como:

- Melhorar a capacidade produtiva do laboratório e aumentar o faturamento
- Reduzir o tempo de entrega de resultados
- Reduzir a incidência de erros por conta de processos manuais de entrada de resultados e duplicação de tarefas
- Reduzir custos operacionais (papel, tempo de equipamentos, retrabalho, etc)
- Adequar os processos às normas de qualidade como ISO/IEC 17.025, FDA 21 CFR Part 11, etc.
- Ter visibilidade e controle sobre as tarefas sendo executadas pela equipe
- Ter controle sobre os equipamentos (calibração, prevenção)
- Manter histórico de todas as análises efetuadas
- Centralização de todas as informações e integração com outros sistemas
- Facilidade na tomada de decisão através de indicadores
- Aumento da qualidade, otimizando o atendimento aos seus clientes

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O Grupo VIDY é certificado ISO 9001:2000, envolvendo Projeto, Construção Civil, Fabricação, Instalação de Laboratórios e Comercialização de Equipamentos e Acessórios para Laboratório

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