BrJAC

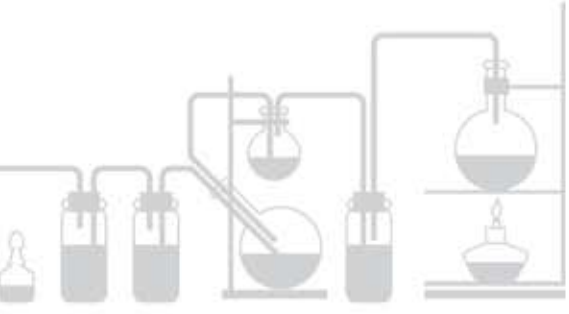
Brazilian Journal of Analytical Chemistry

BrJAC 2010; 1(0)
April/May/June 2010

June, 18th
Professional Chemist's Day

Chemists are paid to be creative, careful and productive.
So are we.
Wheaton Brasil congratulates all chemist professionals for their contributions to everyday living.

GLASS.
THE PACKAGING
THAT PROTECTS
WHAT IS
INSIDE AND
OUTSIDE IT.



WHEATON BRASIL
glass packaging

www.wheatonbrasil.com.br

Brazilian Journal of Analytical Chemistry

DKK Comunicação
April/May/June 2010


Brasil. Líder mundial na busca por petróleo em águas profundas e ultraprofundas. Os avanços da ciência brasileira melhoram a sua vida e ajudam o país a crescer.
A ciência ajuda o Brasil a ir longe. Ou melhor, ir fundo.
Vem aí a 4ª Conferência Nacional de Ciência, Tecnologia e Inovação. Ela vai apontar os caminhos para a ciência continuar ajudando o país a crescer com sustentabilidade. Participe. Acesse o site e saiba mais.
4ª CNCTI
Conferência Nacional de Ciência, Tecnologia e Inovação
"Política de Estado de Ciência, Tecnologia e Inovação para o Desenvolvimento Sustentável"
26 a 28 de maio | Brasília-DF.
Informações e inscrições: www.mct.gov.br
Coordenação:
cgee
Ministério da Ciência e Tecnologia
BRASIL
UM PAÍS DE TODOS
GOVERNO FEDERAL
Fonte: Site Petrobras

Editorial

We are launching **BrJAC** – *Brazilian Journal of Analytical Chemistry* to open a discussion about the real role of the Analytical Chemistry for the development of the country and bring the improvement of the life quality.

BrJAC is an Analytical Chemistry journal whose goal is to debate, discuss, show trends and needs with opinion editorials and interviews with renowned investigators, besides publishing scientific papers from the academic and industry, fulfilling the idealistic purpose of a group of people to achieve actual academic-industrial integration towards innovation and technical-scientific development.

BrJAC arises in a very important moment when technological innovation assumes an increasingly bigger role in public policies planning and the business, signaling that both the government and the business community are already getting uncomfortable with Brazil's position of 42nd in a 48 countries ranking, ahead of Mexico, South Africa, Argentina, India, Latvia and Romania only, according to the study by OECD – the Organization for Economic Cooperation and Development – published in November 2009.

Also, according to IEDI – Institute for Industrial Development Studies – among the 4.4 million companies operating in Brazil, only 30 thousand declare themselves innovative and only 6 thousand perform research and development activities.

It seems that finally both the government and the business community are convinced that the moment is crucial needing the investment in innovation, to face the challenge of changing this scenario and accelerate the growth and development of our Country.

It is "sine qua non" to create consciousness of improving the employment of PhD by enterprises, to be the only way to increase the number of companies which might be considered ahead of innovation. More than the enterprises working side by side with the universities, it is very important to bring high level professionals not only to be employed directly by the industries, but also able to create new technological based companies.

Nowadays in Brazil more than 80% of the students getting their PhD remain at the academic environment (Universia Brasil, 2009), while in developed countries the ratio is just the opposite. Thus, the challenge is to change this scenario and **BrJAC** is being developed to be a medium, which is willing to collaborate with this work through disclosure of discussions, needs, tendencies and opinions.

Now, it is our responsibility to create conditions for **BrJAC** to fulfill its role of a journal specialized in Analytical Chemistry, with efficacy and fidelity to our purposes. So, you can submit your papers, needs, opinions and appraisal for publishing according to the Publication Rules included in this issue.

If, like us, you dream about a strong Analytical Chemistry and real academic-industrial integration, join to this project. It will be a pleasure to have you with us.

Professor Lauro Tatsuo Kubota
Editor-in-chief

Atuação Responsável®
Compromisso com a sustentabilidade

A sustentabilidade tem a Química como parceira

As atividades e produtos gerados pela indústria química são essenciais na construção de um modelo socialmente justo, ambientalmente equilibrado e economicamente próspero, capaz de satisfazer as necessidades das gerações atuais sem comprometer o atendimento às necessidades das gerações futuras. Os produtos químicos auxiliam no aumento da produção de alimentos, no fortalecimento da saúde, na melhoria do conforto, na recuperação de áreas degradadas, na preservação do meio ambiente e nos avanços do conhecimento.

Química. Para o seu bem-estar

ABIQUIM
Associação Brasileira da Indústria Química

www.abiquim.org.br

Fábrica C

Editorial Board

Faturamento anual de

R$ 28,2 BILHÕES

Petroquímica brasileira

A PRIMEIRA A ASSINAR A "DECLARAÇÃO INTERNACIONAL DE PRODUÇÃO MAIS LIMPA" DA ONU.

RESINAS TERMOPLÁSTICAS | POLIETILENO, POLIPROPILENO, PVC

PETROQUÍMICOS BÁSICOS Nº 1

A MAIOR PRODUTORA DE RESINAS TERMOPLÁSTICAS DAS AMÉRICAS

Plástico verde, feito a partir do etanol da cana-de-açúcar.

VINTE E NOVE

unidades industriais no Brasil e nos EUA.

Braskem

O mundo, as pessoas e a Braskem

Summary

O LABCOM® - Laboratório de Combustíveis e Derivados de Petróleo da Escola de Química da UFRJ foi criado em 1999, para integrar a Rede de Laboratórios do Programa Brasileiro de Monitoramento da Qualidade dos Combustíveis, da ANP - Agência Nacional do Petróleo, Gás Natural e Biocombustíveis.

Possui infraestrutura para prestação de serviços e suporte a projetos de PD&I (Pesquisa, Desenvolvimento e Inovação) e atividades disciplinares da graduação e pós-graduação.

O LABCOM® mantém uma relação de **parceria** com o **CENPES/PETROBRAS** desde 2001, desenvolvendo análises físico-químicas em petróleo e seus derivados e, a partir de 2006, passou a integrar o grupo de laboratórios externos ao CENPES, que compõem a sua Rede de Análises.

Além disso, o **LABCOM®** mantém convênio com o Instituto Nacional de Metrologia, Normalização e Qualidade Industrial – INMETRO para a formação de Recursos Humanos no campo da metrologia química e cooperação técnico científica

O LABCOM® participa também de dois grandes projetos: o **PGI – Programa de Gestão Integrada da Rede de Análises,** com foco no aprimoramento analítico quanto aos requisitos de Qualidade e SMS - Segurança, Meio Ambiente e Saúde, na área de petróleo e derivados e o **Projeto CELAB - Confiabilidade em Ensaios Laboratoriais de Biocombustíveis.**

Coordenação Geral: Prof. Dr. Luiz Antonio d' Avila

RoçaDesign

Coordenação Geral de Acreditação

Certificado de Acreditação

Acreditação nº CRL 0202

LABORATÓRIO DE COMBUSTÍVEIS E DERIVADOS DE PETRÓLEO
ESCOLA DE QUÍMICA - UNIVERSIDADE FEDERAL DO RIO DE JANEIRO
CENTRO DE TECNOLOGIA, BLOCO K 02 – ILHA DO FUNDÃO
RIO DE JANEIRO – RJ

A Coordenação Geral de Acreditação do Inmetro – Cgcre/Inmetro – concede acreditação ao Laboratório acima identificado, segundo os requisitos estabelecidos na ABNT NBR ISO/IEC 17025:2005. Esta acreditação constitui a expressão formal do reconhecimento da sua competência para realizar os ensaios constantes no Escopo de Acreditação.

LABCOM®- Laboratório de Combustíveis e Derivados de Petróleo

Centro de Tecnologia Bl. K 02 - Ilha do Fundão - RJ - Brasil - CEP: 21945-900 • Tel/Fax: (21) 2562-8794 - labcom@eq.ufrj.br I www.labcom.org.br

Expedient

BrJAC
Brazilian Journal of Analytical Chemistry

BrJAC is a trade mark of DKK Comunicação. Quartely publication dedicated to Science, Technology and Innovation professionals of universities and industries of Brazil and abroad.

Published by
DKK Comunicação

Publisher
Carlos Roberto Rodrigues
crrodrigues@dkk.com.br

Scientific Journalist
Adimilson Cerqueira
(MTB 21.542 – SP)
dualup@uol.com.br

Translator
Yara Tavares Forneris

Coordinator
Regina Suga
reginasuga@dkk.com.br

Art Director
Wagner G. Francisco

Letters to
DKK Comunicação

Rua Embuaçu, 625 - Sala 06
Vila Mariana – São Paulo – Brazil
Zip Code 04118-080
Phone +55 11 5574-1010
brjac@brjac.com.br
www.brjac.com.br

Official Medium:

Analitica Latin America

2º Congresso Analitica Latin America

Support:

ABIQUIM
Associação Brasileira da Indústria Química

Nürnberg Messe

Ministério da Ciência e Tecnologia
Brasil
Um País de Todos
Governo Federal

Rhodia

Chemistry is our world, Responsibility is our way

O mundo evolui, nossa química também

Da constante busca por fontes renováveis, nasce uma nova química. A Rhodia apresenta **Augeo**, uma linha de produtos elaborada com base em fontes renováveis e alinhada às necessidades do mercado.

O primeiro produto dessa nova linha é o **Rhodia Augeo SL 191**, um solvente de lenta evaporação com performance superior aos glicóis éteres e seus acetatos de fonte petroquímica.

Entre em contato conosco e descubra como a Rhodia pode contribuir para aumentar a performance de sua aplicação. **+ 55 11 3741-7505 | www.rhodia.com.br**

Letters

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!

It is with great enthusiasm that I greet the release of the Brazilian Journal of Analytical Chemistry (BrJAC). This journal, a pioneer in Latin America, is further evidence of strength and dynamism of Brazilian Science, that every day reverses historical trends and puts Brazil today in the center of attention and in a increasingly position of a greater highlights and leadership in C&T. Not only in the production of Science and Technology in first rate, but now also in the scientific publication, through a growing number of journals indexed, well-structured and directed the audience, in concrete action for integration and development.

BrJAC also shows the unrest that the Analytical Chemistry in Brazil lives, and his increasingly pronounced ability to produce techniques and analytical methods, innovative and efficient, which shows not only the academic interest but practical utility and direct in various branches of the Brazilian industry and its laboratories of chemical, biochemical, clinical, forensic, food, pharmaceuticals, among many other.

BrJAC arises, then, in right time, striving to be another mean of international disclosure of our science, also try to encourage the academy-industry integration increasingly strong in Brazil. BrJAC will endeavor to boost this integration and put new scientific information and the analytic needs of the national industry and laboratories in direct mutual communication and analysis, and the natural exchange projects and personal ideas.

We invite everyone, therefore, to participate in this great task, to make BrJAC a reference in Analytical Chemistry in Brazil and abroad, and a facilitator vehicle of a greater integration, which will certainly lead to a further development of our science and our Industry.

BrJAC, success and very welcome!

Prof. Dr. Marcos Nogueira Eberlin
President IMSF, Vice-President BrMASS
Coordinator ThoMSon Mass Spectrometry Laboratory
Institute of Chemistry
UNICAMP - State University of Campinas

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

20 a 22
setembro de 2011

Transamerica Expo Center
São Paulo - SP

Na Analitica sua empresa fica frente-a-frente com os principais fornecedores e compradores dos setores de tecnologia laboratorial, biotecnologia e controle de qualidade dos segmentos: farmacêutico, cosmético, alimentício, petroquímico, químico, entre outros.

www.analiticanet.com.br

Evento Paralelo:

2º CONGRESSO ANALITICA LATIN AMERICA

Organização:

NÜRNBERG MESSE

A LOOK AT ANALYTICAL CHEMISTRY

The first impression of Professor Carol Hollingworth Collins is that of a person in love with Analytical Chemistry. And the last impression, too. Her academic and professional background – as wide as her passion – developed during 50 years of work in the United States, Belgium, Taiwan, the Philippines, and Indonesia. She settled in Brazil in 1974, the year she came to State University of Campinas to work in Radiochemistry. She became a Brazilian citizen and a full professor of Analytical Chemistry at Unicamp Chemical Institute, a position she held until her retirement. "Retired, but not inactive," she hastens to say. Currently, she dedicates most of her time correcting and translating to English over 200 articles every year written by her Chemistry Institute colleagues, for publication in journals indexed abroad. During this interview she recalls a little of the history of Analytical Chemistry and talks about the future trends of this science.

Photo by Adimilson Cerqueira

Professor Carol Hollingworth Collins, in her office at State University of Campinas: more than 200 articles corrected per year

IS IT POSSIBLE TO DEFINE ANALYTICAL CHEMISTRY?

No. Overall, Analytical Chemistry is the development and application of methods for determination of organic and inorganic constituents of samples. I use the word determination because it may be qualitative or quantitative, but this is what differentiates analytical chemists from physical chemists whose main function is to develop equipment.

18 de junho, Dia do Químico

A Associação Brasileira de Cosmetologia
parabeniza todos os químicos pelo seu dia.

abc
associação brasileira de cosmetologia

When did the first researches in this area begin?
Analytical Chemistry was virtually the first science within the wide Chemistry area, obviously not with that name. It was born with the alchemists around 1600, with the beginning of the first pharmaceutical formulas which mixed natural products. From these products originated the first medical drugs, extracted from plants and, sometimes, even from inorganic matter. In reality, it started as a branch of Organic Chemistry which we can define today as Analytical Chemistry.

When did the greatest advance start occurring?
No doubt since the 1950s. The number of procedures employed in Analytical Chemistry determinations increased more than tenfold in comparison with the previous period. I used the first spectrophotometer purchased by my university in the US in 1951, during the last year of my Bachelor´s degree. The equipment already existed before that, although not in small universities. But the development did not occur in Chemistry only, but in all areas, as a function of the advance in the computing area. For instance: computers, which were initially built during the 1940s for military applications, were restricted to government labs in the Soviet Union, the US and Europe and occupied entire rooms. When we received the first mass spectrometer at Unicamp, it came coupled to a minicomputer the size of a counter. So, I would say that the greatest advance was in this field, since today you only have to turn a computer on and it will do the readings you need.

Don't you believe in man's increasing dependence on machine?
Any researcher sees these technologies literally as a means to help them make their projects real. Today there is less human influence on processes, which is positive since there are fewer errors, but it is important to say that human participation is essential for data analysis and interpretation. A very well trained, experienced professional is necessary to develop the programs that allow us to correctly interpret the data. A sharp mind is required to benefit most from the technology in order to improve results in any area, be it Analytical Chemistry, Medicine, Engineering and so on. The computer provides the results, but the correct application of these results depends on the mastery of man over the tool he uses day by day.


Photo by Adimilson Cerqueira

Carol Hollingworth Collins lives in Brazil since 1974

In the academic environment this dependence is a little lower than in the industrial sector, where processes are more automated.

Which event do you deem was most relevant?
In the chromatography area, where I now work, I believe it was the acceptance, in 1930, by organic chemists, that Chromatography does work. In 1906, a Russian botanist, Michael Tswett, published several papers describing a process he employed to separate organic compounds extracted from plants. The chemists did not accept his theory, saying that what he did was Biology, not Chemistry; however, his findings were very important, and were finally accepted by organic chemists during the 1930´s. By the end of the 1930s, inorganic separations had begun, mainly for isolation of the rare earth elements and, later, of uranium and occasionally plutonium. Then the study of chromatography in the separation by adsorption of organic compounds, as well as ion exchange chromatography for the separation of inorganic compounds, allowed a huge leap in research.

Concerning professionals, is the academic formation appropriate?

Undergraduate training is relatively standardized all over the world and involves qualitative and quantitative evaluation and use of the various instruments available. At the graduate level, Analytical Chemistry is so diversified that a generic evaluation would be hard to make. Also, there is an aggravating problem: no dialogue exists among researchers and few know what is being done in the different research lines. An electrochemist does not talk like someone who studies spectrophotometry; and when one talks about spectrophotometry the studies are quite diverse, from UV and visible spectrphotmetry, through the infra-red region, then atomic absorption and ICP systems. We also have mass spectrometry, which studies organic compounds, whose researchers do not dialogue with those who do ICP, who study inorganic compounds. A good example is right in the room next door from where we are talking now: those working in the liquid chromatography lab do not allow access to those working in the gas chromatography lab for fear that they will push the wrong button (laughs) and vice versa.

> **"I would like to see Analytical Chemistry less vulnerable to influences that I consider negative, such as immediacy, for instance. I would like to see things being done more calmly."**

Is the number of graduate programs satisfactory?

In 1981 we had the first Brazilian meeting on Analytical Chemistry in Rio de Janeiro. At that time only six universities had graduate programs – two for Master's degree and four with Master's and Doctor's degrees. Today there are more than 40 graduate programs in Brazilian Universities. It is an in impressive advance, more than in any other area of Chemistry and presents an interesting contrast to the U.S. and Europe, where Analytical Chemistry is subsumed into Physical Chemistry or Organic Chemistry, with very few graduate programs offering degrees in Analytical Chemistry, which I view as a great mistake.

Is there integration between the academic sector and the industrial sector?

The barrier between academics and industry has always been huge. In the United States, for example, rarely does the industry interact with academics, although this is better than in the past, since the sources of public sector resources are decreasing, making American universities and companies feel the need to approach each other. In Brazil, where public financing still meets the most of the demand, university-level research depends on public resources and there is not too much interest in approaching industry. Also, there is a cultural issue with Brazilian scientists, who see this relationship as somewhat unethical. There are a few joint projects, but contact is still very slight. I think that higher integration would be beneficial to all of us.

Which is the preferred profile of professionals in industry?

That depends on its needs. The industry will often hire people with a bachelor's degree to receive specific training on a given equipment; however, for other more complex activities, such as chromatography or ICP, companies prefer specialists with master's and doctor's degrees, since most of them have practical experience in the different types and brands of equipment in the market. This is usually a differential at the time of hiring.

In your opinion, what change is needed to make the future of Analytical Chemistry more promising?

I would like to see Analytical Chemistry less vulnerable to influences that I consider negative, such as immediacy, for instance. We live in an instant world that requires immediate application for everything. The time to complete a master's degree is two years, and a little more generous to complete a doctor's degree, which is three to four years. We need to have a project with immediate answers, and most research institutes require that their researchers and their graduate students publish partial results of their work during its development. Also, there is a generalized hurry, sometimes even a requirement, to publish before defending a master's or doctor's thesis. I would like to see things being done more calmly. I see research lines as a building, where each researcher adds a brick to improve it. ■

LARP

LABORATÓRIO DE ANÁLISES
DE RESÍDUOS DE PESTICIDAS

O LARP atua em programas regulares de monitoramento de resíduos de pesticidas, drogas veterinárias e outros contaminantes em alimentos (com destaque para leite, frutas e cereais) e em amostras ambientais (como água de irrigação, de rio e água potável).

Nossos serviços são prestados para produtores rurais, cooperativas, órgãos de fiscalização, companhias de exportação, instituições de ensino, pesquisa e extensão ou qualquer pessoa ou instituição interessada em analisar resíduos de pesticidas, drogas veterinárias e outros contaminantes.

O Laboratório de Análises de Resíduos de Pesticidas (LARP), faz parte do Departamento de Química, do Centro de Ciências Naturais e Exatas da Universidade Federal de Santa Maria e foi criado em 2001.

Determinação de resíduos de pesticidas drogas veterinárias e outros contaminantes em vários tipos de amostras:

Ambientais (solo, sedimento, água potável, água de rio ou do cultivo de arroz irrigado entre outras)

Alimentos (frutas, vegetais, carnes, leite e cereais)

Possui banco de padrões certificados

No LARP são desenvolvidos trabalhos de mestrado e doutorado, na área de Química Analítica, envolvendo a determinação de resíduos de pesticidas, drogas veterinárias e outros contaminantes.

TECNOLOGIA DE PONTA para realização das análises: Cromatografia Gasosa com detectores ECD, NPD e MS/MS; Cromatografia Líquida com detectores UV-vis., fluorescência, arranjo de diodos e MS/MS.

Equipe formada por mestres e doutores em Química Analítica.

Coordenadores do LARP:
Prof. Dr. Renato Zanella
Profa. Dra. Martha Bohrer Adaime

Universidade Federal de Santa Maria
Laboratório de Análises de Resíduos de Pesticidas (LARP)
Departamento de Química, Prédio 17, 2° Andar, Sala 1306
Fone/Fax: (55) 3220 8011 • Fone: (55) 3220 9531
CEP: 97105-900 • Santa Maria, RS, Brasil
larp@www.ufsm.br
www.ufsm.br/larp

Universidade Federal de Santa Maria
1960

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

Juliana Severo Fagundes Pereira[a], Diogo Pompéu de Moraes[a], Edson Irineu Muller[a], Juliano Smanioto Barin[a], Liange de Oliveira Diehl[a], Márcia Foster Mesko[b], Valderi Luiz Dressler[a], Érico Marlon de Moraes Flores[a,*]

a) Department of Chemistry , Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil
b) Chemistry and Geoscience Institute, Universidade Federal de Pelotas, 96010-610, Pelotas, RS, Brazil

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.

***Corresponding author:**
Phone/Fax: +55 55 3220 9445,
flores@quimica.ufsm.br

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 ºC, 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 dis-

solved 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.

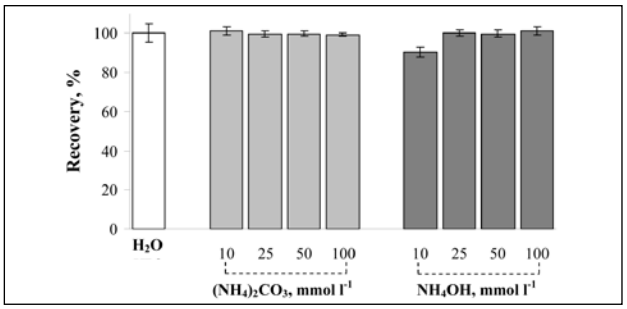


Figure 1. Influence of absorbing solutions for Cl determination using H_2O, $(NH_4)_2CO_3$ and NH_4OH (10, 25, 50 to 100 mmol L^{-1}) with reflux step. Determination by IC; error bars are the standard deviation, n = 3)

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.

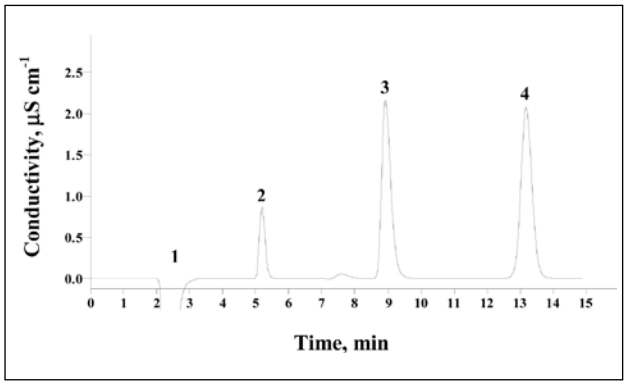


Figure 2. Chromatogram obtained after MIC digestion of RAT: (1) system peak, (2) chlorine, (3) nitrate and (4) sulfate.

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

The authors are grateful to Fapergs, CNPq and CAPES for supporting this study and also to CENPES/PETROBRAS S. A. for supporting and donation of the samples. The authors also thank to Metrohm AG (Switzerland and partner in Brazil) for IC support and to Dr. Marco Aurélio Zezzi Arruda (UNICAMP) for lending the infrared thermometer.

6. References

1. Speight, J. G. *Handbook of Petroleum Analysis*; New Jersey: John Wiley & Sons, 2001, pp. 519.
2. Matar, S. *Chemistry of Petrochemical Processes*; Texas: Gulf Publishing Company, 2000, pp. 392.
3. Xu, X.; Yang, J., Gao, J.; *Petrol. Sci. Technol.*, 2006, 24, 673.
4. Speight, J. G. *Handbook of Petroleum Product Analysis*; New Jersey: John Wiley & Sons, 2002, pp. 454.
5. Jayaraman, A.; Saxena, R. C.; *Corros. Prevent. Contr.*, 1995, 42, 123.
6. Ahmaruzzaman, M.; Sharma, D. K.; *Energy Fuels*, 2007, 21, 891.
7. Sharma, B. K.; Sarowha, S. L. S.; Bhagat, S. D.; Tiwari, R. K.; Gupta,S. K.; Venkataramani, P. S.; *Petrol. Sci. Technol.*, 1999, 17, 319.
8. Maciel, R.; Batistella, C. B.; Sbaite, P.; Winter, A.; Vasconcelos, C. J. G.; Maciel, M. R. W.; Gomes, A.; Medina, L.; Kunert, R.; *Petrol. Sci. Technol.*, 2006, 24, 275.
9. Ostermannn, M.; Berglund, M.; Taylor, P. D. P.; *J. Anal. At. Spectrom.*, 2002, 17, 1368.
10. Mello, P. A.; Pereira, J. S. F.; Moraes, D. P.; Dressler, V. L.; Flores, E. M. M.; Knapp, G.; *J. Anal. At. Spectrom.*, 2009, 24, 911.
11. Bressani, F. A.; Silva, H. O.; Nóbrega, J. A.; Costa, L. M.; Nogueira, A. R. A.; *Quím. Nova*, 2006, 29, 1210.
12. Dos Santos, D. S. S.; Teixeira, A. P.; Barbosa, J. T. P.; Ferreira, S. L. C.; Korn, M. D. A.; Teixeira, L. S. G.; *Spectrochim. Acta Part B*, 2007, 62, 1072.
13. Santelli, R. E.; Oliveira, E. P.; De Carvalho, M. D. B.; Bezerra, M. A.; Freire, A. S.; *Spectrochim. Acta Part B*, 2008, 63, 800.
14. Nogueira, A. R. A.; Moraes, D. P.; Flores. E. M. M.; Krug, F. J.; Knapp, G,; Nóbrega, J. A.; Barin, J. S.; Mesko, M. F. *Decomposições Assistidas por Radiação Microondas* in: Krug, F. J. (Ed.) *Métodos de Preparo de Amostras – Fundamentos sobre Preparo de Amostras orgânicas e inorgânicas para análise elementar*; Piracicaba: Seção Técnica de Biblioteca – CENA/USP, 2008, pp. 276-325.
15. Trevisan, L. C.; Donati, J. L.; Araujo, A. R. *Microwave-assisted procedures for sample preparation: Recent developments*, in: Arruda, M. A. (Ed.) *Trends in Sample Preparation*; New York: Nova Science Publishers, 2006, pp. 29-52.
16. Flores, E. M. M.; Barin, J. S.; Mesko, M. F.; Knapp, G.; *Spectrochim. Acta Part B*, 2007, 62, 1051.
17. Pereira, J. S. F.; Mello P. A.; Moraes D. P.; Duarte F. A.; Dressler, V. L.; Knapp, G.; Flores, E. M. M.; *Spectrochim. Acta Part B*, 2009**,** 64, 554.
18. Fecher, P. A.; Nagengast, A.; *J. Anal. At. Spectrom.*, 1994, 9, 1021.
19. Kaiser, E.; Rohrer, J. S.; Jensen, D.; *J. Chromatogr. A*, 2001, 920, 127.
20. Novic, M.; Dovzan, A.; Pihlar, B.; Hudnik, V.; *J. Chromatogr. A*, 1995, 704, 530.
21. Kaiser, E.; Rohrer, J. S.; Watanaber, K.; *J. Chromatogr. A*, 1999, 850, 167.
22. Duarte F. A.; Pereira J. S. F.; Barin, J. S.; Mesko, M. F.; Dressler, V. L.; Flores, E. M. M.; Knapp, G.; *J. Anal. At. Spectrom*, 2009, 24, 224.
23. Moraes, D. P.; Mesko, M. F.; Mello, P. A.; Paniz, J. N. G.; Dressler, V. L.; Knapp, G; Flores, E. M. M.; *Spectrochim. Acta Part B*, 2007, 62, 1065.
24. Moraes, D. P.; Pereira, J. S. F.; Diehl, L. O.; Mesko, M. F.; Dressler, V. L.; Paniz, J. N. G.; Knapp, G.; Flores, E. M. M.; *Anal. Bioanal. Chem.*, 2010, DOI 10.1007/s00216-010-3478-1.
25. Mortari, S. R.; Cocco, C. R.; Bartz, F. R.; Dressler, V. L.; Flores, E. M. M.; *Anal. Chem.* (2010), doi:10.1021/ac100429v.
26. Flores, E. M. M.; Mesko, M. F.; Moraes, D. P.; Pereira, J. S. F.; Mello, P. A.; Barin, J. S.; Knapp, G.; *Anal. Chem.*, 2008, 80, 1865.
27. Pereira, J. S. F.; Diehl, L. O.; Duarte, F. A.; Santos, M. F. P.; Guimarães, R. C. L.; Dressler, V. L.; Flores, E. M. M.; *J. Chromatogr. A*, 2008, 1213, 249.
28. Mello, P. A.; Giesbrecht, C. K.; Alencar, M. S.; Moreira, E. M.; Paniz, J. N. G; Dressler, V. L.; Flores, E. M. M.; *Anal. Lett.*, 2008, 41, 1623.
29. Pereira, J. S. F.; Moraes, D. P.; Antes, F. G.; Diehl, L. O.; Santos, M. F. P.; Guimarães, R. C. L.; Fonseca, T. C. O.; Dressler, V. L.; Flores, E. M. M.; *Microchem. J.*, (2010), doi:10.1021/ef900707n.
30. Pereira, J. S. F.; Mello, P. A.; Duarte, F. A.; Santos, M. F. P.; Guimarães, R. C. L.; Knapp, G.; Dressler, V. L.; Flores, E. M. M.; *Energy Fuels*, 2009, 23, 6015.
31. Anton Paar GmbH; software version v1.27-Synt; *Microwave Sample Preparation System*, Graz, Austria, 2003.
32. Spectro Analytical Instruments, Kleve, Germany.

Analysis of street Ecstasy tablets by thin layer chromatography coupled to easy ambient sonic-spray ionization mass spectrometry

Bruno D. Sabino,[a,b] Morena L. Sodré,[a] Emanuele A. Alves,[a] Hannah F. Rozenbaum,[a] Fábio O. M. Alonso,[a] Deleon N. Correa,[c] Marcos N. Eberlin,[c]*, Wanderson Romão[c]*

a) Institute of Criminalistic Carlos Éboli, Rio de Janeiro, RJ, Brazil, 20060-050, Rua Pedro I, 28, Rio de Janeiro, RJ, Brazil
b) National Institute of Metrology, Standardization and Industrial Quality, Av. N. Sra. das Graças, 50 Xerém, 22250-020 - Duque de Caxias - RJ - Brasil
c) ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas - UNICAMP, 13084-971, Campinas, SP, Brazil

***Corresponding authors:**
Marcos N. Eberlin and Wanderson Romão
wandersonromao@gmail.com/ Phone + 55 19 3521 3049
eberlin@iqm.unicamp.br/ Phone: + 55 19 3521 3073

Abstract

Ecstasy is a famous illicit drug with varying drug composition, but it usually contains 3,4-methylenedioxymethamphetamine (MDMA) as the main active ingredient. The common procedure to identify ecstasy tablets uses testing kits, but its low specificity may lead to false positives. Thin layer chromatography (TLC) is used worldwide in forensic investigations due to its simplicity, low-cost and versatility but may also lead to false positives. In this study, TLC separation of seven common ecstasy drugs: MDMA, metamphetamine, 3,4-methylenedioxyethylamphetamine (MDEA), 3,4-methylenedioxyamphetamine (MDA), amphetamine, caffeine and lidocaine was attained, and twenty five apprehended street ecstasy tablets analyzed by TLC. Easy ambient sonic-spray ionization mass spectrometry (EASI-MS) was then performed directly on the surface of each TLC spot for MS characterization. The combination of TLC with EASI-MS is shown to provide a relatively simple and powerful screening tool for forensic analysis of street drugs with fast and indisputable results.

Keywords: ecstasy tablets; MDMA; illicit drug; TLC; EASI-MS;

Introduction

Ecstasy, also known as "candy", "XTC" and "Adam", is a popular illicit drug sold worldwide in the form of colored tablets with varying logos and shapes. Ecstasy most often contain 3,4-methylenedioxymethamphetamine (MDMA, Figure 1), but 3,4-methylenedioxyamphetamine (MDA) or 3,4-methylenedioxyethylamphetamine (MDEA) are also found particularly in samples known as "Eve" tablets. These amphetamines display close chemical compositions and biological effects.

Renfroe and co-workers [1] were the first to report the chemical composition of ecstasy tablets. They analyzed, from 1972 to 1985, hundreds of tablets of ecstasy sent anonymously to their laboratory. All samples sent before 1975 were found to contain only MDA. The first tablet with MDMA was found in 1975, the second in 1976 and, during the next years the number of tablets with MDMA increased progressively. In the beginning of the 80's, MDMA was the main drug found in ecstasy tablets. Other amphetamine analogues, such as methamphetamine (Figure 1) and other psychoactive substances including ketamine have also been found in ecstasy tablets. Other drugs such as caffeine, amphetamine, lidocaine, and adulterants have been found in ecstasy tablets.

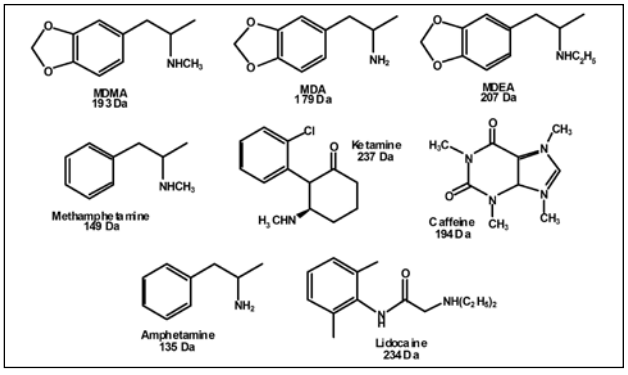


Figure 1. Structures and MW of drugs normally found in ecstasy tablets.

Forensic laboratories analyze ecstasy tablets mainly using ecstasy testing kits, which are often based on the Marquis or Simon tests and develop specific colors such as dark blue or black. These tests display, however, low selectivity leading sometimes to false-positives [2]. Ad-

ditional techniques have therefore been employed to confirm the kit results such as gas chromatography (GC), GC coupled to mass spectrometry (GC-MS) [3], high performed liquid chromatography (HPLC) [4] and HPLC coupled to mass spectrometry (HPLC-MS) [5]. These instrumental techniques naturally require more skilled operators and are much more effort and time consuming.

Thin layer chromatography (TLC) is a classical, simple, low-cost, fast, and versatile separation technique [6] and has been widely used in forensic investigations. A variety of developing reagents are also available, such as ninhydrin and the Marquis reagent for anphetamines [7]. The main drawbacks of TLC are limited resolving power and lack of a unquestionable method for structural characterization. Recently, a new class of ionization techniques for ambient mass spectrometry [8-11] has been developed. These techniques allow desorption, ionization, and MS characterization of analytes directly from their natural surfaces and matrixes [12], becoming therefore an attractive solution for direct characterization of TLC spots. Among these techniques, easy ambient sonic spray ionization (EASI) is likely the simplest, gentlest, and most easily implemented [13]. An EASI source can be constructed and installed in a few minutes from simple MS laboratory parts (Figure 2) requiring no voltages, no UV lights, no laser beams, no corona or glow discharges, and no heating, and as shown recently, even with no pumping systems [14]. EASI relies on the forces of a high velocity stream of N_2 (or even air) to accomplish analyte desorption and supersonic spray ionization (SSI) [15]. EASI has already been successfully tested with different analytes in different matrices and in various applications such as aging of ink writings on paper surfaces [16], perfumes [17], surfactants [18], biodiesel [19], propolis [20], cloth softeners [21]. EASI has been coupled to membrane introduction mass spectrometry [22], TLC [23], HPTLC [24] and has applied molecularly imprinted polymers as selective surfaces [25].

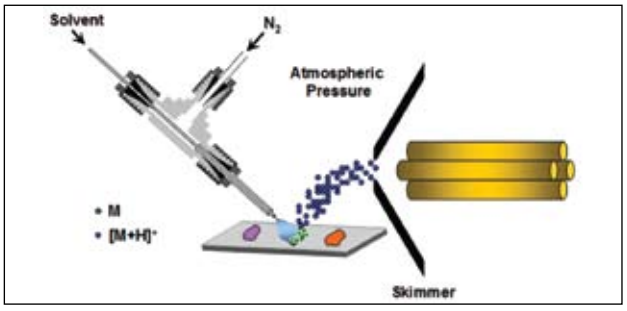


Figure 2. Schematic of TLC/EASI-MS. Supersonic spray produces a bypolar stream of very minute charged droplets (blue spray) that bombard the TLC silica surface causing desorption and ionization of the analyte molecules that rest on the target spot (green dots). Analytes are ionized often as $[M = H]^+$ or $[M – H)^-$, or both. EASI is assisted only by compressed nitrogen or air, and causes no oxidation, electrical, discharge, or heating interferences.

In this work, the coupling of TLC and EASI-MS has been tested in a "real world" forensic application. First, TLC separation has been optimized for seven standards of drugs normally found in street ecstasy tablets. A total of 25 street ecstasy tablets apprehended by the Rio de Janeiro Police Department were then analyzed by TLC/EASI-MS.

Experimental

Reagents and Samples

HPLC and P.A. grade methanol (CH_3OH), chloroform ($CHCl_3$), isopropanol ($CH_3CH(CH_3)OH$), acetic acid (CH_3COOH), and ammonium hydroxide (NH_4OH) were obtained from Merck. Twenty five street ecstasy tablets were provided by the Rio de Janeiro Civil Police. MDMA, MDEA, MDA, ketamine, caffeine, methamphetamine, and amphetamine standards solutions (1 mg mL^{-1}) were purchased from Radian (Austin, TX, USA).

Ecstasy Tablets

The ecstasy tablets were provided by the Carlos Éboli Institute of Criminalistic. The Rio de Janeiro police apprehended these tablets during the years of 2008 and 2009. The tablets displayed diameter, thickness, and weight of ca. 0.79 ± 0.11 cm, 0.44 ± 0.15 cm, and 260 ± 56 g, respectively, with a variety of shapes, logos, and colors. Tablets were pulverized and a 10 mg of the sample was partially dissolved in 10 ml of methanol. After centrifugation, the upper layer was transferred to a glass vial and analyzed by TLC.

TLC

Precoated plates (silica gel 60 GF 254, Merck, 6100 Darmstadt, Germany) were used. These plates were dried for 30 min at 80 °C and then stored in a desiccator. A volume of ca 3 µl of a sample or standard solution were carefully applied to the TLC plate, which were developed in an horizontal chamber (Camag, Switzerland). The total developing distance was 8 cm. Four different solvent systems were tested as eluents: $CHCl_3/CH_3OH$ (50/50 v/v); $CHCl_3/CH_3OH/CH_3COOH$ (20/75/5 v/v); CH_3OH/NH_4OH (98/2 v/v); and $CH_3CH(CH_3)OH/NH_4OH$ (95/5 v/v). After experimental development, the plates were dried at 100 °C for 15 min. Spots were detected under ultraviolet (UV) radiation at 254 nm.

Limit of detection (LOD)

The LOD of MDMA in the TLC plates used was set as the minimum compound concentration that could be visualized by UV with an acceptable level of precision of ≤ 15% and accuracy of ± 15% in 10 replicates.

EASI-MS

Experiments were performed on a single quadrupole mass spectrometer (LCMS- 2010EV -Shimadzu Corp.,

Japan) equipped with a home-made EASI source, which is described in detail elsewhere [15]. Acidified methanol (0.1% in volume of formic acid) at a flow rate of 20 μL min^{-1} and compressed N_2 at a pressure of 100 *psi* were used to form the supersonic spray. The capillary-surface entrance angle was of ca 45°. Each TLC spot was directly analyzed by EASI-MS, without any sample preparation. Spectra were collected on each spot for about 10 s.

Gas Chromatography coupled to Mass Spectrometry (GC/MS).

GC/MS was conducted using a Thermo Scientific (Austin, Texas) Focus gas chromatograph coupled to an ITQ 700 Thermo mass selective detector. The mass spectra scan rate was 3 scans s^{-1}. The GC was operated in the splitless mode with a carrier gas (helium grade 5) flow rate of 1.5 mL min^{-1}. The mass spectrometer was operated using 70 eV electron ionization (EI) and a source temperature of 250 °C. Both the GC injector and the transfer line were maintained at 250 °C. The mass spectra reported were obtained after background subtraction and by averaging ca five scans. Samples (caffeine standard solution and tablets) were diluted in HPLC grade methanol to give a final concentration of 1 mg mL^{-1}, and 1 μL was introduced via manual injection. The GC temperature program used consisted of an initial temperature of 130 °C for 1 min then increased to 280 °C at 17 °C min^{-1} and held for 11 min.

Results and Discussion

TLC separation of the seven common ecstasy tablet components was evaluated using four different solvent systems as eluents (Table I). $CHCl_3/CH_3OH$ (50/50 v/v) was inefficient since it caused spot tailing for most standards and ecstasy samples tested. $CHCl_3/CH_3OH/CH_3COOH$ (20/75/5 v/v) provided well defined spots for both the samples and standards, but MDMA, metamphetamine, amphetamine, and ketamine presented too close Rf values (0.62-0.71). The best results were obtained for $CH_3CH(CH_3)OH/CH_3OH$ (95/5 v/v) and, most particularly, for CH_3OH/NH_4OH (98/2 v/v) (Figure 3). Although close Rf values for MDMA (main drug expected in ecstasy tablets) and metamphetamine were observed, good separation and resolution was observed for MDA, MDEA, amphetamine, ketamine, and caffeine (Figure 3 and Table I).

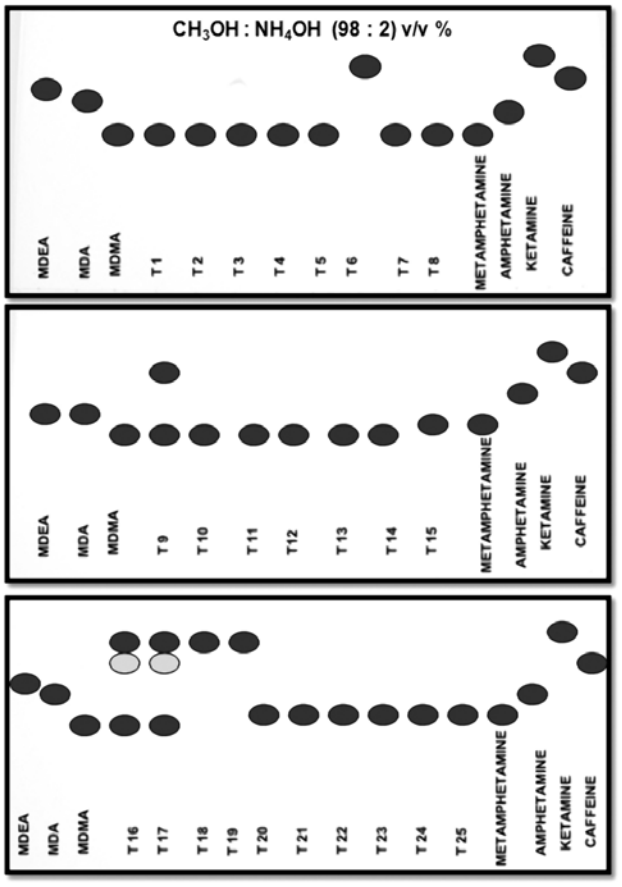


Figure 3. TLC data for the seven common ecstasy components tested as well as for the 25 samples of apprehended street ecstasy tablets using $CH_3OH:NH_4OH$ (98:2) v/v as the eluent. Spots developed by UV are represented by dark black or gray (less intense) ovals.

TLC/EASI-MS

For TLC, we selected therefore CH_3OH/NH_4OH (98:2) v/v as the best eluent and the components of each spot (Figure 3) were then subjected to desorption, ionization, and *m/z* measurements by EASI-MS in the positive ion mode using acidified methanol as the spray solvent.

Table I. Rf values for the seven drug standards as a function of different TLC eluents

Compound	$CHCl_3:CH_3OH$ (50:50) v/v	$CHCl_3:CH_3OH:CH_3COOH$ (75:20:5) v/v	$CH_3OH:NH_4OH$ (98:2) v/v	$CH_3CH(CH_3)OH:NH_4OH$ (95:5) v/v
MDEA	0.62	0.74	0.71	0.87
MDA	0.48	0.60	0.67	0.81
MDMA	0.37	0.64	0.56	0.62
Metamphetamine	0.35	0.62	0.57	0.62
Amphetamine	0.71	0.66	0.66	0.70
Ketamine	0.86	0.71	0.84	0.80
Caffeine	0.84	0.94	0.77	0.70

Figure 4 shows the "on-spot" EASI-MS acquired directly from the surface of the TLC spots of each of the seven standards used. Note the unambiguous characterization of each drug, mostly as a single ion (which facilitates spectra interpretation and analyte characterization) corresponding to their protonated molecules, that is, $[M + H]^+$. MDEA was the only drug that was also detected as $[MDEA + H_20 + H]^+$ and $[MDEA + Na]^+$. Signal-to-noise ratio was quite high for all standards except caffeine (Figure 4). LOD was evaluated for TLC of MDMA and found to be of 3 ± 0.3 µg. For the caffeine spot, the low sensitivity of EASI-MS seems to result from its polarity and high affinity to silica that hampered desorption from the TLC plate by the EASI droplets containing acidified methanol. We are currently searching for an EASI-spray solvent or mixture of solvents that could provide proper desorption and ionization of caffeine from the silica in TLC spots.

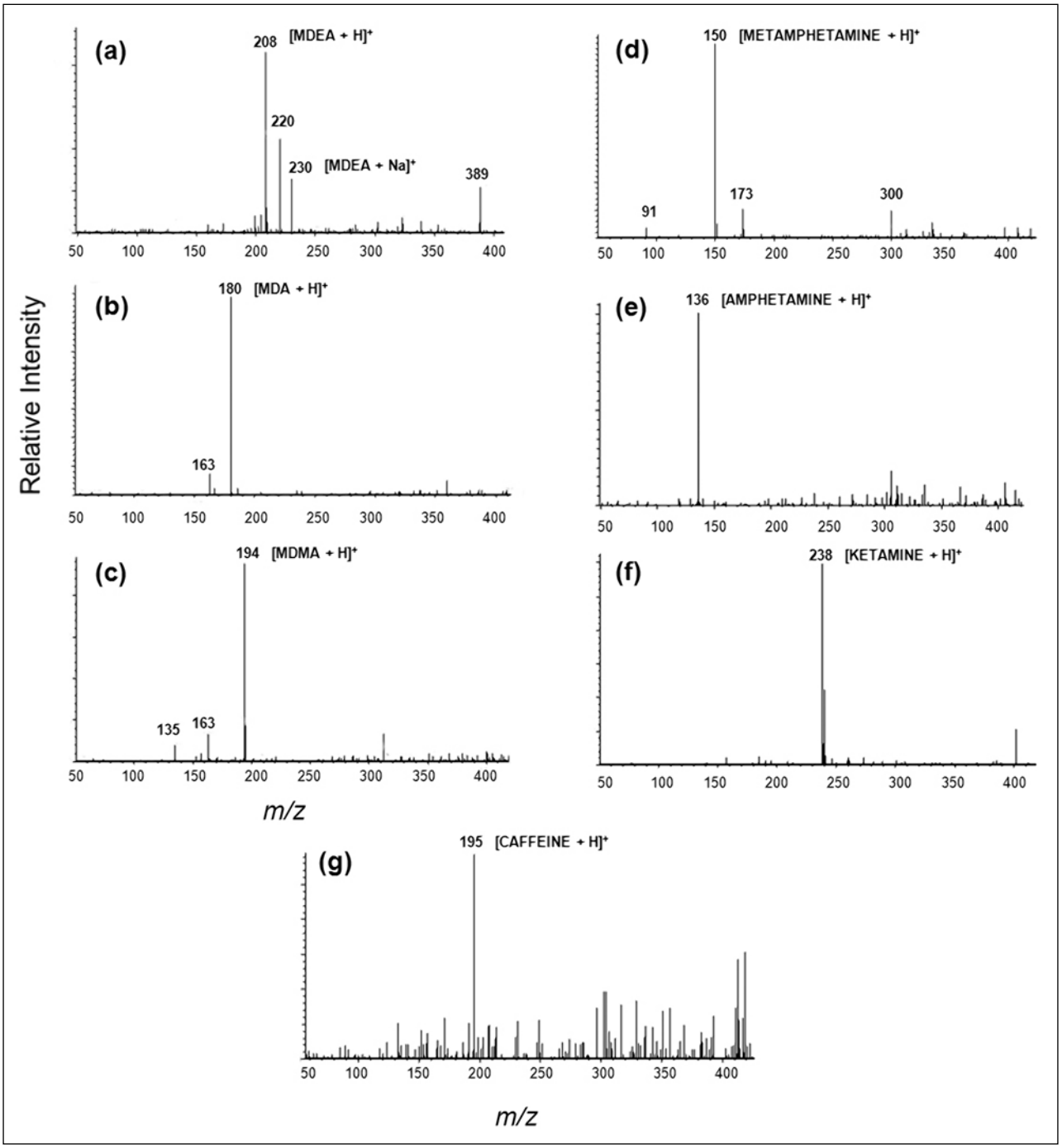


Figure 4. EASI-MS collected directly on the surface of the TLC spots corresponding to the seven common ecstasy components tested: (a) MDEA, (b) MDA, (c) MDMA, (d) metamphetamine, (e) amphetamine, (f) ketamine, and (g) caffeine.

Figure 5 shows the EASI-MS for the single TLC spot of sample T1 (Figure 3), a representative street sample of ecstasy. Note there could be doubt about the composition of this spot based on TLC alone, since both MDMA and metamphetamine displayed quite close Rf values (Figure 3). But the presence of MDMA (*m/z* 194) is unmistakably confirmed by EASI-MS. This result illustrates the importance of the TLC/EASI-MS coupling for rapid and unambiguous analysis of ecstasy tablets. Sample T6 also provided a dubious case since its single TLC spot, judging by the Rf value, could be interpreted as corresponding to either ketamine or caffeine. EASI-MS of this T6 spot (not shown) displayed very low overall abundance (mostly noise) and failed to detect therefore the intense protonated molecule of *m/z* 238 expected for ketamine (Figure 4). Since EASI-MS sensitivity to caffeine using acidified methanol as the spray solvent was found to be very poor (Figure 4), the spot was assigned to caffeine. GC/MS data (not shown) confirmed that the main constituent of T6 was indeed caffeine.

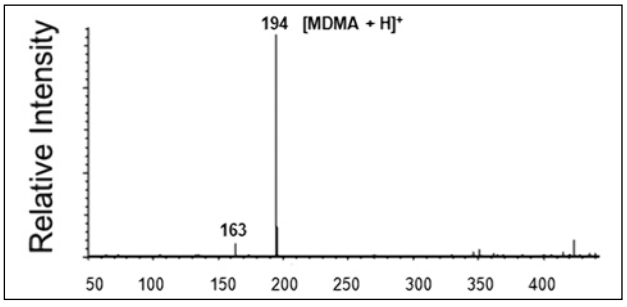


Figure 5. EASI-MS collected directly on the surface of the single TLC spot of sample T1.

Figure 3 shows that most ecstasy tablets displayed a single TLC spot with Rf values (and EASI-MS data) corresponding to MDMA. Tablets T18 and T19 displayed, however, a single spot corresponding, as far as only TLC and Rf values are concerned, to ketamine. But EASI-MS analysis for T18 (Figure 6) and T19 clearly points to an erroneous TLC attribution since the $[M + H]^+$ ion of *m/z* 235 indicates lidocaine as the main spot constituent. Both T18 and T19 samples displayed similar shape, logo, dimension, and mass indicating common origin.

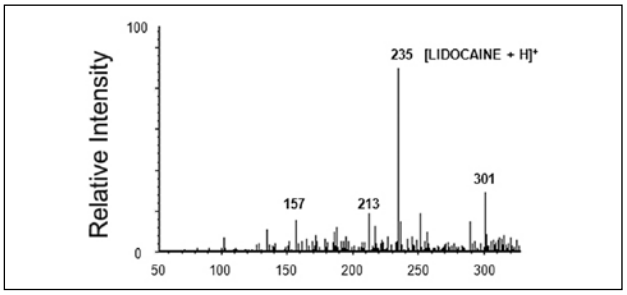


Figure 6. EASI-MS collected directly on the surface of the single TLC spot for tablet T18. A similar spectrum was collected for T19.

In contrast to most ecstasy tablets showing a single TLC spot, samples T9, T16 and T17 displayed two or three spots. Some of these spots (Figure 3) have Rf values corresponding to caffeine, and this attribution was confirmed by GC/MS (data not shown). A third spot observed for T16 and T17 with the highest Rf value could be attributed to ketamine. But again EASI-MS discarded ketamine, showing very low ion abundance and mostly noise. These spots were therefore labeled as "unknown". Tablets T16 and T17 also displayed similar shapes, logos and colors, indicating common origin.

Conclusions

Validation of methods used to detect drugs using TLC analysis is crucial to generate undisputable results, particularly in forensic investigations. TLC is a simple, low-cost, versatile, and popular technique used widely in forensic screening of illicit drugs, but may lead to false positives or erroneous attributions due to limited resolution and lack of an undisputable and selective method for structural characterization particularly for unexpected components. EASI-MS performed directly on the surface of TLC spots provides rapid and secure MS characterization. The coupling of TLC with EASI-MS constitutes therefore a valuable tool in forensic investigations, as demonstrated herein for a "real world" case involving the analysis of apprehended street ecstasy tablets. Although a few cases have required more elaborated GC/MS analysis, or a few spots remained identified, rapid screening of samples by TLC/EASI-MS provided secure identification for most samples, greatly speeding the overall analysis time and increasing its accuracy. EASI is the simplest ambient ionization technique currently available for ambient mass spectrometry [9], being easily implemented in all API mass spectrometers. Miniature mass spectrometers able to operate with ambient ionization techniques are also being made more compact and robust, and with diminishing costs [26, 27]. Therefore, the use of such compact and affordable instruments would allow widespread use of the EASI-MS technique in most forensic laboratories. TLC is also the simplest and the most popular separation technique in forensic investigations. The coupling of TLC to EASI-MS provides therefore a suitable technique for simple, rapid and secure forensic investigations. The favorable characteristics of TLC/EASI-MS indicate many advantageous applications in forensic analysis.

Acknowledgements

The authors thank the Rio de Janeiro Police Department, Institute of Criminalistics for providing the apprehended ecstasy samples, and the Brazilian science foundation's FAPESP, CNPq, FAPERJ and FINEP for financial assistance.

References

1. Renfroe, C. L.; *J. Psychoactive Drugs,* 1986, 18, 363.
2. Jeffrey, W. *Clarke's Analysis of Drugs and Poisons in Pharmaceuticals, Body Fluids and Postmortem Material*. Moffat, A.; Osselton, M.; Widdop, B.; Galichet, L.,eds.; London, UK: *Pharmaceutical Press*, 2004.
3. Sherlock, K.; Wolff, K.; Hay, A. W.; Conner, M. *J. Accid. Emerg. Med.*, 1999, 16, 194.
4. Sadeghipour, F.; Veuthey, J. L. *J. Chromatogr. A,* 1997, 787, 137.
5. Bogusz, M. J. *J. Chromatogr. B*, *Biomed. Sci. Appl.,* 2000, 748, 3.
6. Kato, N.; Pharm, B.; Fujita, S.; Pharm, B.; Ohta, H.; Fukuba, M.; Pharm, M.; Toriba, A.; Hayakawa, K. *J. Forensic Sci.,*, 2008, 53, 1367.
7. Zakrzewska, A.; Parczewski, A.; Kazmierczak, D.; Ciesielski, W.; Kochanaa, J. *Acta Chim. Slov.,* 2007, 54, 106.
8. Ifa, D. R.; Wu, C.; Ouyang, Z.; Cooks, R. G. *Analyst,* 2010, 135, 669.
9. Alberici, R. M.; Simas, R. C.; Sanvido, G. B.; Romão, W.; Lalli, P. M.; Benassi, M.; Cunha, I. B. S.; Eberlin, M. N. *Anal Bioanal. Chem.*, 2010, in press.
10. Harris, G. A.; Nyadong, L.; Fernandez, F. M. *Analyst*, 2008, 133, 1297.
11. Chen, H.; Gamez, G.; Zenobi, R. *J. Am. Soc. Mass Spectrom.,* 2009, 20, 1947.
12. Ifa, D. R.; Gumaelius, L. M.; Eberlin, L. S.; Manicke, N. E.; Cooks, R. G. *Analyst*, 2007, 132, 461.
13. Haddad, R.; Sparrapan, R.; Kotiaho, T.; Eberlin, M. N. *Anal Chem.,* 2008, 80, 898.
14. Santos, V. G.; Regiani, T.; Dias, F. F. G.; Romão, W.; Klitzke, C. F.; Coelho, F.; Eberlin, M. N. *Angew. Chem. Int. Ed.*, 2010, submitted.
15. Haddad, R.; Sparrapan, R.; Eberlin, M. N. *Rapid Commun. Mass Spectrom.,* 2006, 20, 2901.
16. Lalli, P. M; Sanvido, G. B.; Garcia, J. S.; Haddad, R.; Cosso, R. G.; Maia, D. R. J.; Zacca, J. J.; Maldaner, A. O.; Eberlin, M. N. *Analyst*, 2010, 135, 745.
17. Haddad, R.; Catharino, R.R.; Marques, L. A.; Eberlin, M. N. *Rapid Commun. Mass Spectrom.,* 2008, 22, 3662.
18. Saraiva, S. S.; Abdelnur, P. V.; Catharino, R. R.; Nunes, G.; Eberlin, M. N. *Rapid Commun. Mass Spectrom.*, 2009, 23, 357.
19. Abdelnur, P. V.; Eberlin, L. S.; Sá, G. F.; Souza, S V.; Eberlin, M. N. *Anal. Chem.* 2008, 80, 7882.
20. Sawaya, A.C.H.F.; Abdelnur, P. V.; Eberlin, M. N.; Kumazawa, S.; Ahn, M.-R.; Bang, K-S.; Nagaraja, N.; Bankova, V. S.; Afrouzan, H. *Talanta*, 2010, 81, 100.
21. Saraiva, S. A.; Abdelnur, P. V.; Catharino, R. R.; Nunes, G.; Eberlin, M. N. *Rapid Commun. Mass Spectrom.*, 2009, 23, 357.
22. Haddad, R.; Sparrapan, R.; Kotiaho, T.; Eberlin, M. N. *Anal. Chem.*, 2008, 80, 898.
23. Haddad, R.; Milagre, H. M. S.; Catharino, R. R.; Eberlin, M. N. *Anal. Chem.* 2008, 80, 2744-2750.
24. Eberlin, L.S.; Abdelnur, P. V.; Passero, A.; de Sa, G. F.; Daroda, R. J.; de Souza, V.; Eberlin, M. N. *Analyst*, 2009, 134, 1652.
25. Figueiredo, E. C.; Sanvido, G. B.; Arruda, M. A. Z.; Eberlin, M. N. *Analyst*, 2010, 135, 726.
26. Gao, L.; Cooks, R. G.; Ouyang, Z. *Anal. Chem.* 2008, 80, 4026.
27. Syms, R. *Anal. Bioanal. Chem.* 2009, 393, 427-429.

Determination of Hg species in edible mushrooms using reversed phase-liquid chromatography-chemical vapor generation-inductively coupled plasma mass spectrometry

Camila Pilz[a], Fabiane Goldschmidt Antes[a], Clarissa Marques Moreira[a], Paola de Azevedo Mello[a], Fábio Andrei Duarte[b], Dirce Pozebon[c], Érico Marlon de Moraes Flores[a], Valderi Luiz Dressler[a*]

a) Department or Chemistry, Universidade Federal de Santa Maria, 97105-900 - Santa Maria, RS, Brazil.
b) School of Chemistry and Food, Universidade Federal do Rio Grande, Campus Carreiros, 96201-900 - Rio Grande, RS – Brazil.
c) Department of Chemistry, Universidade Federal do Rio Grande do Sul, 91500-970 - Porto Alegre, RS, Brazil.

Abstract

A method for Hg speciation in edible mushroom is described. Mercury species were determined using reversed phase liquid chromatography (LC) combined with chemical vapor generation (CVG) and inductively coupled plasma mass spectrometry (ICP-MS). Conventional extraction, ultrasound (US) and microwave (MW) radiation were evaluated for Hg species extraction using water, hydrochloric acid or L-cysteine solutions as extraction media. Extraction time, temperature (for MW extraction), concentration of HCl and L-cysteine solutions and US amplitude were investigated. Hg species interconversion was observed in HCl media, whereas the extraction was not quantitative if only water was used. Better results were obtained by using 1.0% (m/v) L-cysteine and US amplitude set at 10%. Accuracy was evaluated by the analysis of certified reference material (CRM) and analyte recovery from spiked samples. The agreement of the obtained results for CRM sample was in the range of 92 to 96% for methylmercury (CH_3Hg^+) and 95 to 102% for total Hg. Analyte recoveries from spiked samples were in the range of 93 to 109% for Hg^{2+} and 96 to 113% for CH_3Hg^+. Ultrasound assisted extraction was considered more efficient, simple and faster than conventional and MW assisted extraction. The proposed method was applied for Hg speciation in edible mushrooms and the main species was Hg^{2+}.

***Corresponding author:**
Fax: +55 55 3220 9445
valdres@quimica.ufsm.br

Keywords: Mercury, Speciation, Liquid chromatography-inductively coupled plasma mass spectrometry

Introduction

Edible mushroom may contain relatively high levels of Hg, which can be a result of natural uptake of the element due to its growing in polluted areas or use of Hg-based fungicides. Mushroom uptakes Hg and it has been used as bioindicator for environmental pollution for Hg and to assess the impact on public health.[1,2] Because of the health risks to Hg exposure, the United States Environmental Protection Agency (USEPA) established the reference value for methylmercury (CH_3Hg^+) as 0.1 μg kg^{-1} body weight/day. Additionally, the World Health Organization (WHO) set the tolerable value to 1.6 μg kg^{-1} body weight/week (0.23 μg kg^{-1} body weight/day). However, the maximum acceptable content of Hg in mushroom has not been established up to now.[3] All Hg species are cosidered toxic, while CH_3Hg^+ is one the most dangerous and usually present in the enviroment.[4] Therefore, the identification and quantification of Hg species in edible mushroom is of great interest.

Although improvements in instrumentation have been made in recent years, accurate Hg species determination may be difficult and non-quantitative analyte recovery and Hg species interconversion during sample preparation can occur.[5,6] Sample preparation is considered of primary concern in Hg speciation analysis and it needs to be carefuly evaluated for each kind of sample in order to avoid analyte losses, contamination and species interconversion.[7] Studies dealing with procedures of Hg extraction as well as extractant solutions are de-

scribed in the literature.[5] Acid and alkaline solutions, thiol compounds, cysteine, thiosulfate and mercaptoethanol have been used.[7-11] Compounds containing sulfur are usually very effective owing to the high affinity of Hg to sulfur, which promotes Hg species releasing from sample matrix.[12,13]

Microwave (MW)[7,14-16] and ultrasound (US)[8,17] have been evaluated to promote Hg species extraction from different biological matrices. Both systems promote and accelerate analyte extraction. The extraction efficiency varies with the irradiation time, temperature, characteristics of sample and extraction media. Usually, extraction using MW and US are advantageous in comparison to conventional extractions (mechanical shaking and heating) in terms of time, efficiency and reagent consumption.[6,8,18]

For Hg species determination, hyphenated techniques such as gas chromatography (GC)[14] and liquid chromatography (LC) coupled to a selective detector[19-21] have been frequently used. Most of LC methods are based on the use of silica-C_{18} as stationary phase, whereas the mobile phase contains methanol, or chelating or ion-pair reagent (especially 2-mercapthethanol or L-cysteine).[5,22] Liquid chromatography or GC coupled to inductively coupled plasma mass spectrometry (ICP-MS)[7,12,13,16,23] has been used for Hg speciation analysis and good limits of detection (LOD) and specificity are obtained. In addition, cold vapor generation (CVG) can be combined to LC-ICP-MS, wich improves the LOD.[15,24,25]

Although different methods have been proposed for Hg speciation in biological tissues, particularly in fish and seafood, little information is available about Hg speciation in edible mushroom. Thus, the main purpose of this study was to develop a method for Hg speciation in edible mushroom focused on sample preparation. Conventional extraction (mixture of sample plus extracting solution and let to stand) and also the use of US and MW radiation combined with different extractant solutions were studied. LC-CVG-ICP-MS was used for Hg species separation and quantification. Accuracy was evaluated using certified reference material and recovery tests.

EXPERIMENTAL

Instrumentation

The LC system used for mercury species separation consisted of a quaternary pump (Model Series 200, PerkinElmer, USA) equipped with a Rheodyne six-port injector valve, a 200 µL-sample loop and a silica-C_{18} column (Discovery C_{18} HPLC column, 250 mm x 4 mm, 5 µm, Supelco, USA). The mobile phase flow rate was 1.0 mL min^{-1} using isocratic conditions. The separation column was connected to a continuous cold vapor generation system. The Hg vapour produced was introduced directly into the ICP.

Mercury determination was carried out by means of an inductively coupled plasma mass spectrometer (PerkinElmer SCIEX, Model ELAN DRC II, Canada), equipped with a quartz torch (injector tube 2 mm i.d.) and platinum cones. Parameters of ICP-MS were adjusted in order to obtain the highest signal to background ratio for Hg (using ^{202}Hg). Operational conditions of the LC-CVG-ICP-MS system are summarized in Table I and a scheme of the proposed system is shown in Fig. 1.

TABLE I. LC-CVG-ICP-MS OPERATIONAL CONDITIONS

ICP-MS	
RF power, W	1250
Plasma gas flow-rate, L min^{-1}	15
Auxiliary gas flow-rate, L min^{-1}	1.2
Nebulizer gas flow-rate, L min^{-1}	1.10
Dwell time, ms	250
LC	
Column	C_{18} (250 mm x 4 mm, 5 µm)
Mobile phase (L-cysteine), % (m/v)	0.10 (pH 4.0)
Mobile phase flow rate, mL min^{-1}	1.0
Injected volume, µL	200
CVG-ICP-MS	
Carrier solution (HCl), mol L^{-1}	1.0 (8.0 ml min^{-1})
Reductant solution ($NaBH_4$), % (m/v)	0.2 (3.7 ml min^{-1})

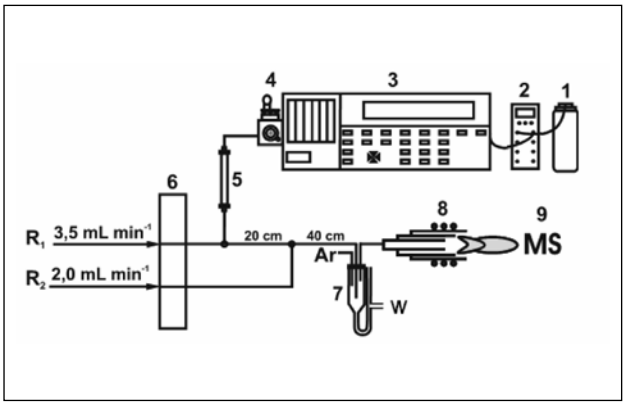


FIGURE 1. SQUEMATIC DIAGRAM OF THE LC-CVG-ICP-MS SYSTEM. 1: MOBILE PHASE (L-CYSTEINE); 2: VACUUM DEGASSER; 3: LC PUMP; 4: INJECTOR; 5: COLUMN (SI-C_{18}); 6: PERISTALTIC PUMP; 7: GAS-LIQUID SEPARATOR (L = 10 CM; I.D. = 15 MM); 8: ICP; 9: MASS SPECTROMETER; R_1: HCL (1.0 MOL L^{-1}); R_2: $NaBH_4$ (0.25% M/V).

A Multiwave 3000 microwave oven (Anton Paar, Austria) was used for sample digestion and mercury species extraction. A Fisher Sonic Dismembrator (Fisher

Scientific, Model 100, 20 kHz, 100 W, USA) with an ultrasonic probe (1/8", full wave titanium probe solid, 127 mm long) was used for Hg species extraction. A pH meter (Metrohm, Switzerland) and a centrifuge (Nova Técnica, Brazil) were also used.

Reagents, standards and mobile phase

Distilled and deionized water was obtained by using a Milli-Q system (Millipore Corp., USA). Concentrated HNO_3 and HCl (Merck, Germany) were purified in a sub-boiling system (Milestone, Model Duopur, Italy). L-cysteine with purity higher than 98.5% (Vetec, Brazil) was used and solutions were prepared in water and used as extracting solution and as mobile phase. Sodium tetrahydroborate (Vetec, Brazil) solutions (prepared in 0.2% NaOH - Merck) and ammonium hydroxide (Merck) were used for mercury reduction and pH adjustment, respectively.

A solution containing 1000 mg L^{-1} Hg (as Hg^{2+}) in 2% (v/v) HNO_3 was purchased from Merck. This solution was sequentially diluted in 1.0 mol L^{-1} HCl in order to prepare the reference solutions of Hg^{2+}. A solution containing 1000 mg L^{-1} of Hg (as CH_3HgCl) was prepared by dissolving CH_3HgCl salt (Aldrich, USA) in methanol. Intermediate solutions of Hg^{2+} and CH_3HgCl (1 mg L^{-1}) were prepared by dilution in 1.0 mol L^{-1} HCl. Working solutions used for calibration curve were prepared fresh daily by diluting the Hg stock solutions in the mobile phase solution. All the solutions were stored in polypropylene vessels, which were previously cleaned by immersion in 20% (v/v) HNO_3 solution by 24 h and rinsed with water and were kept at 4 °C in the dark before use.

The mobile phase used for mercury species separation by LC was adapted from reference [13] which contains 0.10% (m/v) L-cysteine at pH 4.0, adjusted with NH_4OH.

Sample preparation

Five species of edible mushroom were purchased at the local market and freeze-dried. Samples were ground in a cryogenic mill (SPEX, SamplePrep, USA), transferred to polypropylene flasks and stored 4 °C in the dark until analysis. Particle size of the powdered samples was lower than 100 µm.

Total mercury determination

About 500 mg of dried sample were transferred to quartz vessels of microwave oven and 6 mL of HNO_3 were added. The mixture was irradiated for 20 min at 1000 W (ramp of 10 min) and 0 W for 20 min (cooling step). The maximum temperature and pressure were 280 °C and 80 bar, respectively. After cooling, digests were transferred to graduated polypropylene vessels and the volume completed to 30 mL. Certified reference material (Dogfish Liver, DOLT-3 from National Research Council Canada - NRCC) and blanks were analyzed in parallel using the same conditions selected for samples. Total mercury concentration in digested samples was determined by CVG-ICP-MS.

Procedures for Hg species extraction

Ultrasound, microwave and conventional procedures were evaluated for Hg species extraction from CRM and mushroom samples. Standard Hg^{2+} and CH_3Hg^+ solutions addition were also tested in order to evaluate possible interconversion or losses of the Hg species.

About 500 mg of dried and powdered mushroom were accurately weighed and transferred to polypropylene vessels (15 mL) or quartz flasks of the microwave oven. Water (6 mL), HCl (6.0 mL of 1.0, 3.0 and 6.0 mol L^{-1}) and L-cysteine (6 mL from 0.1 to 3% m/v) were added to each aliquot of the sample. Subsequently, the mixture was submitted to the following treatments: a) shaken during 2 min and standing for 12 h, or b) US treatment up to 3 min using US amplitude of 10, 20 and 30%, or c) irradiation with microwave at 500 W for 5 min (ramp of 5 min) at 60, 80, 100 and 120 °C. In the following step, the mixture was transferred to polypropylene vessels and the pH adjusted to 4.0 using 10% (v/v) NH_4OH. Final volume of the mixture was completed to 20 mL with water. Then, the mixture was centrifuged by 10 min at 3000 rpm and filtered through a 0.45 µm-glass fiber filter. Aliquots of obtained solutions were immediately injected in the chromatograph. To evaluate the accuracy, analyte recovery tests and certified reference material were used. The certified sample and solutions spiked with the analytes were submitted to the same procedure used for the mushroom samples.

Results and discussion

Total Hg determination

Total Hg concentration in the certified sample and mushroom was determined by CVG-ICP-MS after digestion in microwave oven. The obtained results are summarized in Table II. No statistical difference was obtained between the results after microwave digestion and value of certified reference material. Total mercury found in mushroom samples was used as reference to the value found when the speciation of Hg was performed.

Mercury species extraction assisted by US

Effects of the extracting media composition (water, HCl and L-cystein), sonication at different US amplitudes, time of sonication, as well as the mass of sample were investigated. Solutions containing Hg^{2+}or CH_3Hg^+ or a mixture of both were evaluated in parallel to check possible interconversion of the Hg species. It was ob-

served that Hg species interconversion occurred in HCl media, mainly when the mixture was sonicated for a period longer than 1 min (the period of sonication was extended up to 6 min). With higher HCl concentration (3.0 and 6.0 mol L^{-1}) CH_3Hg^+ was almost completely converted to Hg^{2+}, even using low US amplitude (10%) and time of sonication (1 min). With 1.0 mol L^{-1} HCl the interconversion of CH_3Hg^+ to Hg^{2+} was complete at 20% of US amplitude. These results are not in agreement with previously published results where US bath was used instead of US probe.[9] The main reason for the different results found in the present work could be due to the energy delivered to the solution that is higher when a US probe is used when compared to US baths.

No mercury species conversion was observed using water and L-cysteine medium. However, only L-cysteine was able to extract mercury species quantitatively from mushroom samples. It can be explained by the high affinity of Hg to the sulphydryl group of L-cysteine that improves Hg species extraction.[5] It was observed that Hg species were well extracted with 1.0% (m/v) L-cysteine under sonication at 10% US amplitude. In order to evaluate the effect of the sonication time, the concentration of L-cysteine solution (1% m/v) and the US amplitude (10%) were kept constant, while the period of time of sonication was varied from 0.5 to 3 min. The effect of the time of sonication on Hg^{2+} and CH_3Hg^+ in standard solution was also evaluated where no analyte losses or interconversion of Hg species have occured. The same was observed for Hg in the certified sample and mushroom samples. Therefore, the time of sonication for Hg species extraction was kept in 1 min.

Due to the low concentration of Hg in mushroom samples (Table II), the effect of the sample mass on Hg species extraction was evaluated in order to improve the LOD of the method. Volumes of 1% (m/v) L-cysteine solution (1.0, 3.0 and 6.0 mL) were used for analyte extraction keeping the sample mass up to 500 mg. No mercury species interconversion was observed, but the analysis became difficult for volumes lower than 3.0 mL mainly due to the high viscosity of the mixture. Quantitative Hg species recovery was observed up to sample masses of 500 mg, using 6 mL of extractant solution. This condition was used for further experiments.

Mercury species extraction assisted by MW radiation

Temperature can affect Hg species stability and also analyte extraction. Therefore, the microwave oven conditions were adjusted for a maximum temperature of 120 °C using water, HCl and L-cysteine as extractants. Mercury species interconversion has occured in 6.0 mol L^{-1} HCl media, even at 60 °C. They were stable in 1.0 mol L^{-1} HCl in the investigated temperature range, while CH_3Hg^+ was converted in 3.0 mol L^{-1} HCl at temperatures higher than 100 °C. As cited, CH_3Hg^+ was stable in 1.0 mol L^{-1} HCl, but it was observed that the extraction of Hg species in mushroom and certified sample was not quantitative.

Concerning the other extractant solutions, Hg species interconversion in water was observed only at 120 °C, while no interconversion was observed in any condition for L-cysteine. Besides, L-cysteine has demonstrated to be the best media for Hg species extraction from mushroom. As previously mentioned, the good performance of L-cysteine is due to the strong afinity between sulfur and Hg.[26,27] According to the results obtained in this step of the study, 1% (m/v) L-cysteine, heating at 60 °C for 5 min (ramp of 5 min) were stablished for Hg species extration assisted by MW radiation.

Mercury species extraction by using conventional extraction

Conventional extraction of Hg species was evaluated by using the same sample mass, volume of extractants and respective concentrations used for US and MW procedures. The extracting solution was spiked with Hg^{2+} and CH_3Hg^+ and the mixture was let to stand up to 12 h at room temperature. It was observed that Hg species losses or interconversion have occurred only in 6.0 mol L^{-1} HCl. For Hg species extraction from the certified sample or mushroom, the mixture was manually shaken for 2 min and then allowed to stand for 12 h at room temperature.[13] However, as previously found extraction with 1% (m/v) L-cysteine solution was more efficient.

Determination of inorganic and methylmercury in mushrooms

In order to evaluate the accuracy of the extraction procedures, a certified reference sample was analyzed and recovery tests of Hg species performed. Results found in the speciation analysis step were also compared with the total Hg concentration found in the digested samples (Table II). For this purpose the sum of Hg species concentration was calculated and compared with the total Hg concentration in order to check the mass balance. According to Table II, the concentrations of Hg species found in the reference sample submitted to conventional extraction, or US or MW assisted were in good agreement with the certified values. However, when MW radiation was used for Hg species extraction from mushroom, the sum of the concentrations of Hg species was generally lower than the total Hg concentration determined by CVG-ICP-MS. In the case of conventional extraction and US application, the sum of Hg species was always higher than 92% of the total Hg found by CVG-ICP-MS.

Chromatograms obtained for samples submitted to the three different extraction procedures using 1% (m/v) L-cysteine are presented in Fig. 2. As can be seen, only Hg^{2+} and CH_3Hg^+ were detected in mushroom. The retention time for both Hg species was the same in mushroom and certified sample. It was also observed that complete separation of the Hg species was achieved in 5 min, for samples and standard solutions.

Conclusions

It was demonstrated that Hg species in HCl medium are easily interconverted or not quantitatively extracted by using conventional, US or MW assisted extraction. Hg species are more stable in water and L-cysteine. In water, CH_3Hg^+ is only converted to inorganic mercury when submitted to MW irradiation at temperature higher than 120 °C. No interconversion of

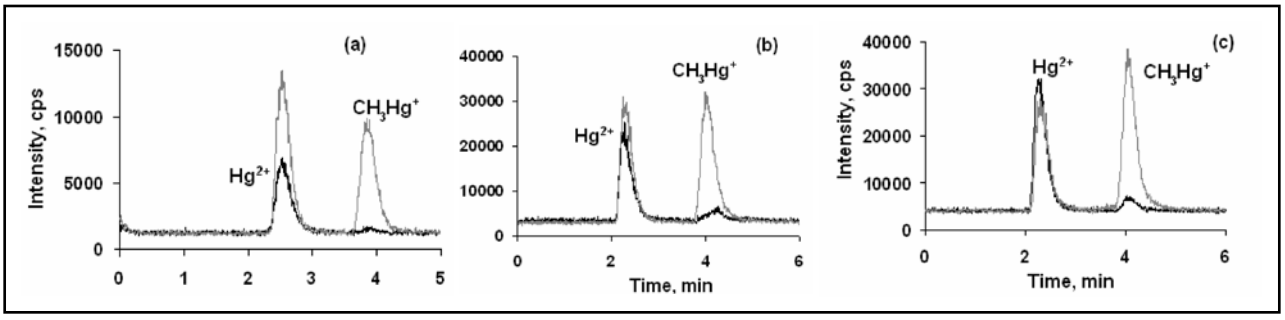


Figure 2. Chromatograms of a standard solution (1.0 µg L^{-1} Hg) and mushroom sample. (a) US assisted extraction; (b) MW assisted extraction; (c) conventional extraction. Gray line: standard solutions; black line: mushroom sample.

The concentration of mercury species was determined by external calibration, using peak area signal intensity for each Hg species. Calibration curves for both Hg species were linear up to 5.0 µg L^{-1} of Hg. Detection limits (based on 3σ of the baseline noise, in peak area) of Hg^{2+} and CH_3Hg^+ were 0.41 and 0.35 ng g^{-1} (as Hg), respectively. Detection limit was estimated considering 500 mg of sample in 20 mL extraction solution and a volume of 200 µL of solution injected in the chromatograph.

Hg species occurs when US and 1.0% (m/v) L-cysteine were selected. In this case, Hg recovery from mushroom and certified sample (dogfish liver) was higher than 92%. However, recovery of Hg was only 62% when MW assisted extraction and L-cysteine 1% (v/v) were used. Therefore, it was concluded that US assisted extraction using L-cysteine can be recommended for Hg speciation analysis in mushroom. Extraction of Hg species was quantitative, no interconversion of Hg species was observed and the time of extraction (1

Table II. Concentration (in ng g-1) of mercury species and total mercury in mushroom and certified sample (DOLT-3) using conventional extraction, US and MW radiation with 1% (m/v) L-cysteine. Analyte measurements by LC-CVG-ICP-MS. Results are the average and standard deviation from 3 consecutive determinations.

	US (20%, 1 min)			MW (100 °C, 10 min)			Conventional Extraction			Digestion
Sample	CH_3Hg^{+**}	Hg^{2+}	Hg_{Total}^{***}	CH_3Hg^{+**}	Hg^{2+}	Hg_{Total}^{***}	CH_3Hg^{+**}	Hg^{2+}	Hg_{Total}^{***}	Hg_{Total}
Agaricus bisporus	4.85 ± 0.59	35.2 ± 0.1	40.0 ± 0.6 (100%)	7.73 ± 0.30	22.5 ± 0.1	30.2 ± 0.3 (76%)	4.21 ± 0.07	34.7 ± 1.3	38.9 ± 1.3 (97%)	39.9 ± 2.0
Pleurotus citrinopileatus	7.20 ± 0.96	19.1 ± 1.0	26.3 ± 1.4 (92%)	8.52 ± 1.30	17.0 ± 0.8	25.5 ± 1.5 (88%)	5.36 ± 0.47	20.2 ± 1.4	25.6 ± 1.5 (88%)	28.4 ± 1.2
Pleurotus eryingii	7.31 ± 0.33	10.6 ± 0.6	17.9 ± 0.5 (93%)	6.67 ± 0.25	8.39 ± 0.15	15.1 ± 0.3 (73%)	4.65 ± 0.51	18.6 ± 0.7	23.2 ± 0.9 (111%)	19.2 ± 2.3
Pleurotus ostreatus	5.18 ± 0.41	16.9 ± 1.43	22.1 ± 1.5 (92%)	3.20 ± 0.34	11.3 ± 0.4	14.5 ± 0.5 (59%)	1.95 ± 0.15	21.1 ± 1.6	23.0 ± 1.6 (94%)	24.4 ± 0.5
Pleurotus djamor	5.38 ± 0.32	17.9 ± 1.1	23.3 ± 1.2 (92%)	3.55 ± 0.53	7.15 ± 0.66	10.7 ± 0.8 (42%)	2.50 ± 0.33	22.5 ± 0.8	25.0 ± 0.9 (98%)	25.6 ± 0.8
DOLT-3*	1465 ± 47 (92%)	1646 ± 58	3111 ± 75 (95%)	1482 ± 90 (93%)	1646 ± 93	3128 ± 129 (96%)	1403 ± 46 (88%)	1917 ± 97	3320 ± 107 (102%)	3258 ± 85

* Certified value: Hg_{Total} 3370 ± 140 ng g^{-1}; CH_3Hg^+ 1590 ± 120 ng g^{-1}
** As Hg
*** Sum of CH_3Hg^+ e Hg^{2+} concentration

min of sonication) was considered suitable for routine analysis.

Acknowledgements

The authors are grateful to CNPq (Conselho Nacional de Desenvolvimento Científico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior) for financial support.

References

1. L. Cocchi, L. Vescovi, L. E. Petrini and O. Petrini, *Food Chem.*, 2006, 98, 277.
2. S. G. Capar, W. R. Mindak and J. Cheng, *Anal. Bioanal. Chem.*, 2007, 389, 159.
3. C. W. Levenson and D. M. Axelrad, *Nutr. Rev.*, 2006, 64, 139.
4. G. S. Silva, M. C. Bisinoti, P. S. Fadini, G. Magarelli, W. F. Jardim, A. H. Fostier, *J. Braz. Chem. Soc.*, 2009, 20, 1127.
5. L. H. Reyes, G. M. M. Rahman, T. Fahrenholz and H. M. S. Kingston, *Anal. Bioanal. Chem.*, 2008, 390, 2123.
6. E. L. M. Flores, J. N. G. Paniz, E. M. M. Flores, D. Pozebon, V. L. Dressler, *J. Braz. Chem. Soc.*, 2009, 20, 1666.
7. M. Leermakers, W. Baeyens, P. Quevauviller and M. Horvat, *Trends Anal. Chem.*, 2005, 24, 383.
8. L. H. Reyes, G. M. M. Rahman and H. M. S. Kingston, *Anal. Chim. Acta*, 2009, 631, 121.
9. S. Río-Segade and C. Bendicho, *J. Anal. At. Spectrom.*, 1999, 14, 263.
10. A. I. C. Ortiz, Y. M. Albarrán and C. C. Rica, *J. Anal. At. Spectrom.*, 2002, 17, 1595.
11. O. Alp and N. Ertas, *J. Anal. At. Spectrom.*, 2009, 24, 93.
12. L. E. Kaercher, F. Goldschmidt, J. N. G. Paniz, E. M. M. Flores and V. L. Dressler, *Spectrochim. Acta, Part B*, 2005, 60, 705.
13. W. Meng, F. Weiyue, S. Junwen, Z. Fang, W. Bing, Z. Motao, L. Bai, Z. Yuliang and C. Zhifang, *Talanta*, 2007, 71, 2034.
14. S. C. Hight and J. Cheng, *Anal. Chim. Acta*, 2006, 567, 160.
15. J. J. B. Nevado, R. C. R. Martín-Doimeadios, F. J. G. Bernardo and M. J. Moreno, *J. Chromatog., A*, 2005, 1093, 21.
16. C. Chiou, S. Jiang and K. S. K. Danadurai, *Spectrochim. Acta, Part B*, 2001, 56, 1133.
17. L. Chang, S. Jiang and A. C. Sahayam, *J. Chromatogr., A*, 2007, 1176, 143.
18. F. A. Duarte, C. A. Bizzi, F. G. Antes, V. L. Dressler, E. M. M. Flores, *Spectrochim. Acta, Part B*, 2009, 64, 513.
19. M. V. B. Krishna, M. Ranjit, D. Karunasagar and J. Arunachalam, *Talanta*, 2005, 67, 70.
20. E. M. M. Flores, B. Welz and A. J. Curtius, *Spectrochim. Acta, Part B*, 2001, 56, 1605.
21. R. C. Campos, R. A. Gonçalves, G. P. Brandão, M. S. Azevedo, F. Oliveira, J. Wasserman, *Spectrochim. Acta. Part B*, 2009, 64, 506.
22. E. M. Krupp, B. F. Milne, A. Mestrot, A. A. Meharg and J. Feldmann, *Anal. Bioanal. Chem.*, 2008, 390, 1753.
23. B. Vallant, R. Kadnar and W. Goessler, *J. Anal. At. Spectrom.*, 2007, 22, 322.
24. G. M. M. Rahman, T. Fahrenholz and H. M. S. Kingston, *J. Anal. At. Spectrom.*, 2009, 24, 83.
25. C. Wan, C. Cheen and S. Jiang, *J. Anal. At. Spectrom.*, 1997, 12, 683.
26. L. Lin, L. Chang and S. Jiang, *J. Agric. Food Chem.*, 2008, 56, 6868.
27. F. M. M. Morel, A. M. L. Kraepiel and M. Amyot, *Annu. Rev. Ecol. Syst.*, 1998, 29, 543.

Determination of ciprofloxacin by synchronous scanning room-temperature phosphorimetry

I.S. Nava-Júnior and R.Q. Aucelio*

**Department of Chemistry, Pontifícia Universidade Católica do Rio de Janeiro (PUC-RIO), Rio de Janeiro, RJ, Brazil, 22453-900*

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.

***Corresponding author:**
aucelior@rdc.puc-rio.br
Fax: +55 21 3527 1637

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].

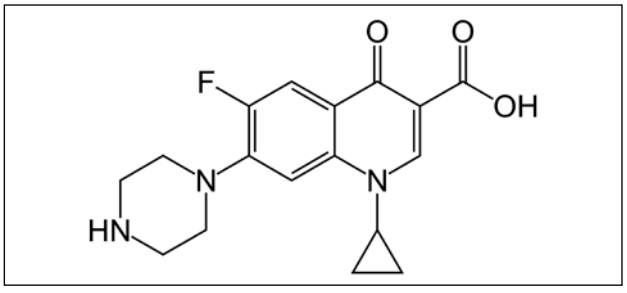


Figure 1. Ciprofloxacin.

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 salts[a] 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}.

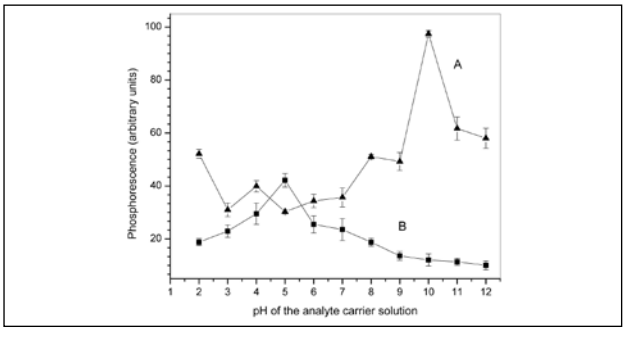


Figure 2. Ciprofloxacin room-temperature phosphorescence induced by (A) $CdCl_2$ and (B) $Th(NO_3)_4$ in function of the pH of the analyte carrier solution.

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 two-fold 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.

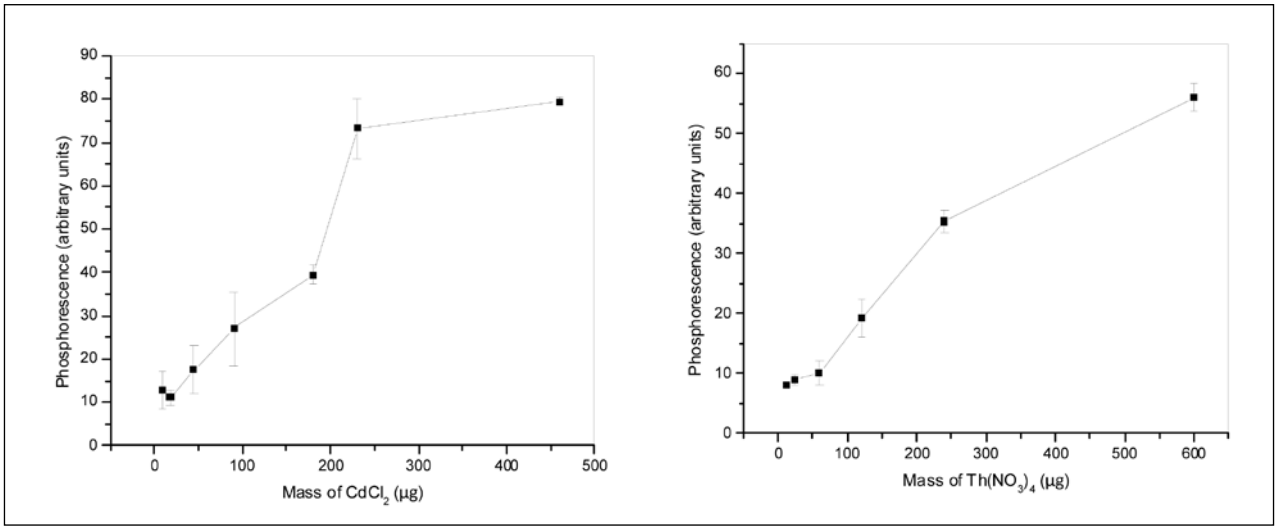


Figure 3. Effect of the mass of the heavy atom salt on the phosphorescence of ciprofloxacin in cellulose substrate.

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

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

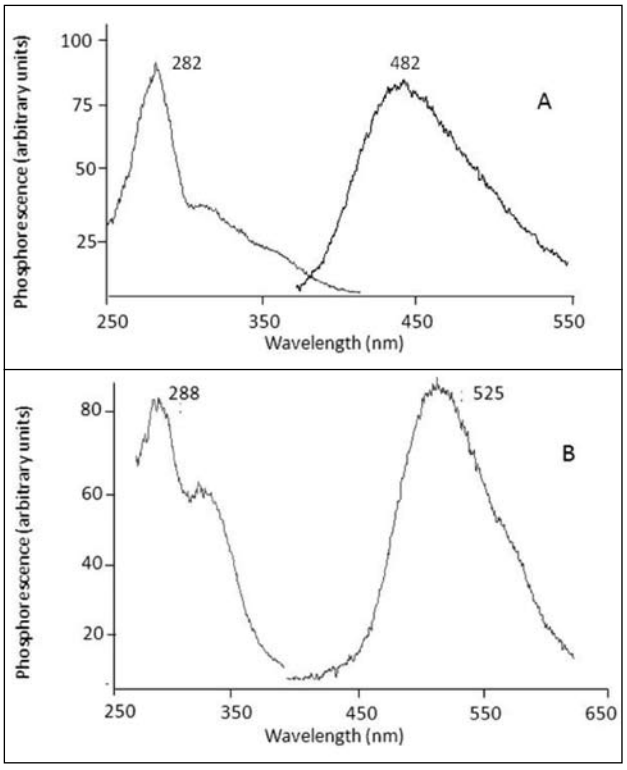


Figure 4. Ciprofloxacin room-temperature phosphorescence excitation and emission spectra in cellulose substrates under the optimized conditions using (A) $CdCl_2$ and (B) $Th(NO_3)_4$.

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

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

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 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

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).

gatifloxacin were present as indicated by the percent recoveries significantly higher than 100%. On the other 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.

Acknowledgements

The authors thank the Conselho National de Desenvolvimento Cientifico e Tecnologico-CNPq for scholarships and for financial support. Scientific funding from MCT-FINEP and FAPERJ are also acknowledged. Software licenses for Statistica version 8 was kindly provided by Statsoft Brazil.

References

1. Rubinstein, E.; *Chemotherapy*, 2001, 47(Suppl. 3), 3.
2. Van Banbeke, F.; Michot, J.M; Van Eldere, J.; Tulkens, J.; *Clin. Microbiol. Infec.* 2005, 11, 513.
3. Blondeau, J.M.; *Surv. Ophtalmol*. 2004, 49(Suppl. 2), S73.
4. Hopper, D.C.; *Biochim. Biophys. Acta*, 1998, 1400, 45.
5. Belal, F.; Al-Majed, A.A.; Al-Obaid, A.M.; *Talanta* 1999, 50, 765.
6. O'Dea, P.; Costa-Garcia, A.; Miranda-Ordieres, A.J.; Tunon-Blanco, P.; Smyth, M.; *Electroanal.* 1991, 3, 337; O'Dea, P.; Costa-Garcia, A.; Miranda-Ordieres, A.J.; Tunon-Blanco, P.; Smyth, M.; *Electroanal.* 1990, 2 637.
7. Xuan, C.S.; Wang, Z.Y.; Song, J.L.; *Anal. Lett*. 1998, 31, 1185; El-Walily, A.F.M.; Belal, S.F.; Bakry, R.S.; *J. Pharm. Biomed. Anal.* 1996, 14, 561.
8. Murillo Pulgarín, . J.A.; Alañón Molina, A.; Muñoz Fernández, L.; J. Agric. Food Chem. 2008, 56, 8838; Shtykov, S.N.; Smirnova, T.D.; Bylinkin, G.; Kalashnikova, N.V.; Zhemerichkin, D.A.; *J. Anal. Chem*., 2007, 62, 136; Navalón, A.; Ballesteros, O.; Blanc, R.; Vílchez, J.L.; *Talanta*. 2000, 52, 845.
9. Yu-Lin, F.; Dong, C.; *J. Chromatogr. Sci*. 2004, 42, 474; Turiel, E.; Martin Esteban, A.; Tadeo, J.L.; *Anal. Chim. Acta* 2006, 562, 30.
10. Michalska, K.; Pajchel, G.; Tyski, S.; *J. Chromatogr. A*. 2004, 1051, 267.
11. Li, J.; Li, J.; Shuang, S.; Dong, C.; *Anal. Chim. Acta*. 2005, 548, 134.
12. Nava-Jr, I.; Aucélio, R.Q.; *Spertrochim. Acta Part A*. 2009, 72, 429.
13. Cardoso, C.E.; Martins, R.O.R.; Telles, C.A.S.; Aucélio, R.Q.; *Microchim. Acta*, 2004, 146, 79.
14. Vo-Dinh, T. em *Room Temperature Phosphorimetry for Chemical Analysis, Chemical Analysis series*, v. 68, first ed., J. Wiley & Sons, New York, 1984, cap 1.
15. Marques, F.F.C.; Figueiredo, F.S.; Aucélio, R.Q.; *Quím. Nova*, 2008, 31, 204.
16. Vo-Dinh, T.; Hooyman, J.R.; *Anal. Chem.* 1979, 51, 1915.
17. Lacroix, P.M.; Curran, N.M.; Sears, R.W.; *J. Pharm. Biomed. Anal.* 1996, 14, 641.

Chromium speciation in cement extracts and airborne particulates using HPLC coupled with ICP-MS

Authors: Shona McSheehy[a], Fabienne Seby[a], Martin Nash[b]

a) Group of Biogeochemistry, Ultra Trace and Isotopic Analysis, Laboratoire de Chimie-Analytique, Bio-Inorganique et Environnement, University of Pau, France;
b) Thermo Fisher Scientific, Cambridge, UK

Abstract

A HPLC-ICP-MS method was developed to analyze trivalent and hexavalent chromium in cement extracts and airborne particulates. Method validation was performed using a NIST Certified Reference Material (CRM-545) and agreement between the measured and certified values was achieved. The determined method detection limits (MDLs) were 0.10 µg/kg for Cr^{VI} and 0.64 µg/kg for Cr^{III}. The determined limits of quantification (LOQs) were 0.35 µg/kg for Cr^{VI} and 2.13 µg/kg for Cr^{III}. The developed methodology allowed for sensitive and accurate chromium speciation in real samples of cement and dust extracts.

Keywords: Chromium speciation, airborne particulates, cement extract, HPLC, ICP-MS

Introduction

Oxidation state and chemical form are important factors which influence the toxicity, bioavailability and mobility of chromium. For example, trivalent chromium (Cr^{III}) is essential for many biochemical mechanisms in contrast to hexavalent chromium (Cr^{VI}) which is highly toxic due to its high oxidation potential and ability to attack the skin, respiratory and digestive systems [1]. There is an increasing requirement for methodologies to enable sensitive, quantitative chromium speciation analyses in order to determine concentrations of toxic and/or nontoxic species and to better understand the implications of total chromium concentrations [2]. Speciation analysis using inductively coupled plasma mass spectrometry (ICP-MS) as detector is an important tool because of its characteristics, such as extremely low detection limits (LOD) for almost all elements, wide linear range, the possibility for multi-elemental analysis and the possibility to apply isotope dilution mass spectrometry (IDMS) [3].

Chromium is employed in a number of industrial applications (e.g. chromium plating, stainless steel production, paint, pigment and cement manufacture) and occupational exposure issues have prompted the implementation of a number of directives for the protection of employees in the workplace. According to the European Commission (EC) directive 2003/53/EC [4], wet cement should contain no more than 2 ppm hexavalent chromium and according to the directive 2000/53/EC [5], no more than 2 g of hexavalent chromium can be used in anti-corrosion coatings on road vehicles. Additionally, the 'Occupational Health and Safety Administration' (OSHA) have recently proposed a permissible exposure limit of 0.5 µg/m^3 Cr^{VI} in workplace atmospheres [6].

This work describes the use of an HPLC-ICP-MS instrument to enable the determination of chromium species in cement extracts and airborne workplace particulates. Chromium species were separated on-line prior to ICP-MS detection using a cation exchange stationary phase in conjunction with a 100 % aqueous acidic mobile phase. The HPLC-ICP-MS methodology was validated using a CRM (NIST CRM-545, welding dust). Method detection limits (MDLs) and limits of quantification (LOQ) were determined using the 3σ and 10σ models respectively based on repeat injections of the calibration blank (n=5).

Sample Preparation

Cement extracts were prepared according to the Technical Regulations for Dangerous Materials method TRGS 613 (Germany). 10.0 g of sample were mixed with 40 mL of water for 15 min at 300 rpm using a mechanical shaker. The extracts were centrifuged at 3000 rpm for 20 minutes and then filtered through a 0.45 µm filter. Samples were diluted appropriately with ultra-pure water and aliquots then used for the speciation analysis.

A surface area (static) dust sample and filters containing airborne particulate matter collected from a stainless steel manufacturing plant were prepared according to ISO 16740:2005, which specifies a method for the determination of the time-weighted average mass concentration of Cr^{VI} in workplace air [7]. Sequential sample preparation methods are specified for the extraction of

soluble and insoluble hexavalent chromium. The surface area dust sample and filters (Teflon) were firstly extracted with 10 mL $(NH_4)_2SO_4$-NH_4OH buffer (0.5 M) at pH 8 for 1 hour at ambient temperature. The first extractant was decanted into a clean dry vial and the samples were subsequently extracted with 6 mL 2% NaOH / 3% Na_2CO_3 for 1 hour in an ultrasonic bath. The second extractant was decanted into a clean dry vial. All extracted samples were diluted appropriately with ultra-pure water prior to analysis. The CRM BCR-545 (welding dust) was extracted according to the method outlined in the certification report supplied with the CRM. The filter was leached with 10 mL 2% NaOH / 3% Na_2CO_3 buffer for 30 min in an ultrasonic bath heated at 70 °C. The sample was then centrifuged for 2 min at 2500 rpm and diluted with ultra-pure water prior to analysis.

Instrument Configuration

A Surveyor Plus HPLC system with autosampler was coupled to the XSeries 2 ICP-MS, all instruments from Thermo Fisher Scientific. The ICP-MS was operated under standard hot plasma conditions using a one-piece quartz torch with 1.5 mm ID injector. The spray chamber was cooled to 2°C with a Peltier cooling device. PlasmaLab and Xcalibur software packages (Thermo Fisher Scientific) were used in conjunction with an External Trigger Card to enable automated HPLC accessory control using bi-directional communications and intelligent peak integration facilities. The associated HPLC parameters and analytical conditions for HPLC-ICP-MS are shown in Table I.

Table I. HPLC-ICP-MS conditions

Column	Dionex IonPac® CS5A (250 x 2.0 mm, 5µm)
Injection volume	20 µL
Flow rate	0.7 mL min^{-1}
Gradient elution	0.35 to 1.2 M HNO_3
Forward Power	1400 W
Nebulizer Gas Flow	0.9 L min^{-1}
Auxilliary Gas Flow	0.85 L min^{-1}
Cool Gas Flow	14.5 L min^{-1}
Data Acquisition Mode	PlasmaLab Transient Time Resolved Analysis (TRA)
Isotopes (dwell times, ms)	^{13}C (10 ms)
	^{51}V (10 ms)
	^{52}Cr (200 ms)
	^{53}Cr (10 ms)
Channels per AMU	1
Timeslice duration	263 ms
Transient acquisition time	450 s (per sample)
Spray chamber	Glass impact bead
Nebulizer	Glass concentric
Cones	Xt

Results and Discussion

The HPLC methodology enabled separation of Cr^{VI} and Cr^{III} species with retention times of 90 and 395 seconds respectively (Figure 1 (a.)). External calibration curves were generated in PlasmaLab using a blank and Cr^{VI} and Cr^{III} calibration standards at 0.5, 1, 2, 10 and 25 ng/g (Figure 2). Quantification of Cr^{VI} and Cr^{III} species was achieved in several samples using the external calibration curves presented in Figure 2 and fully quantitative data processing was achieved using the software's automated peak integration tools. Method validation was performed through triplicate analyses of one extract of CRM BCR-545 (welding dust). The associated quantitative data is presented in Table II and there is good agreement between the measured and certified values for Cr^{VI} (40.2 mg/g).

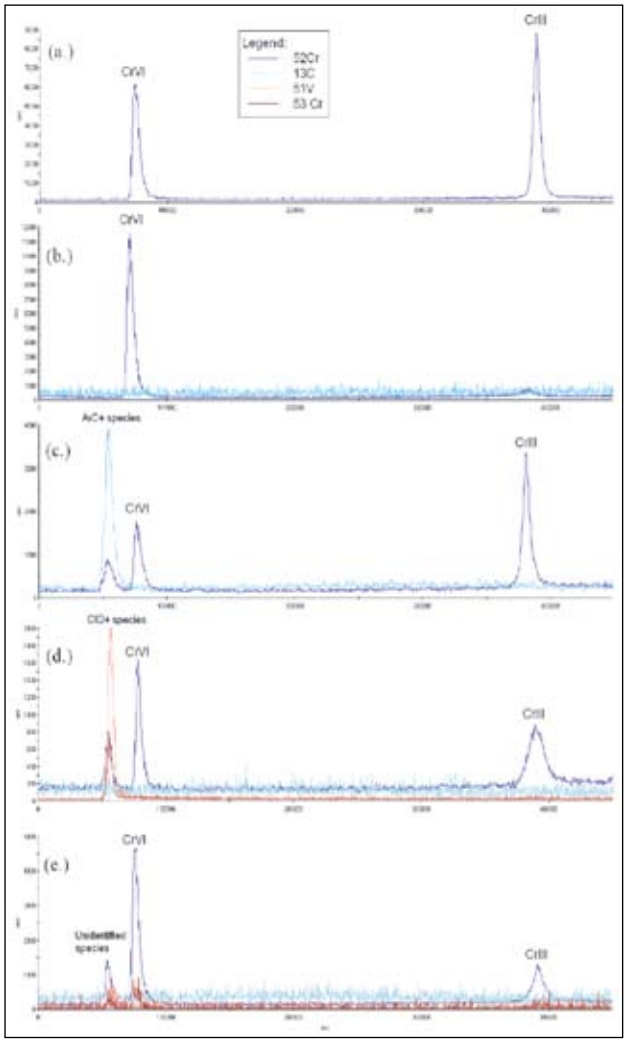


Figure 1.
(a) commercially available Cr^{VI} and Cr^{III} standards at 5 ng g^{-1};
(b) cement extract 3 (x200 dil.);
(c) cement extract 6 (undiluted) - contains lignin sulphonate reducing agent;
(d) extracted surface area dust (x2 dil.) - soluble Cr^{VI};
(e) extracted surface area dust (x20 dil.) - insoluble Cr^{VI}.

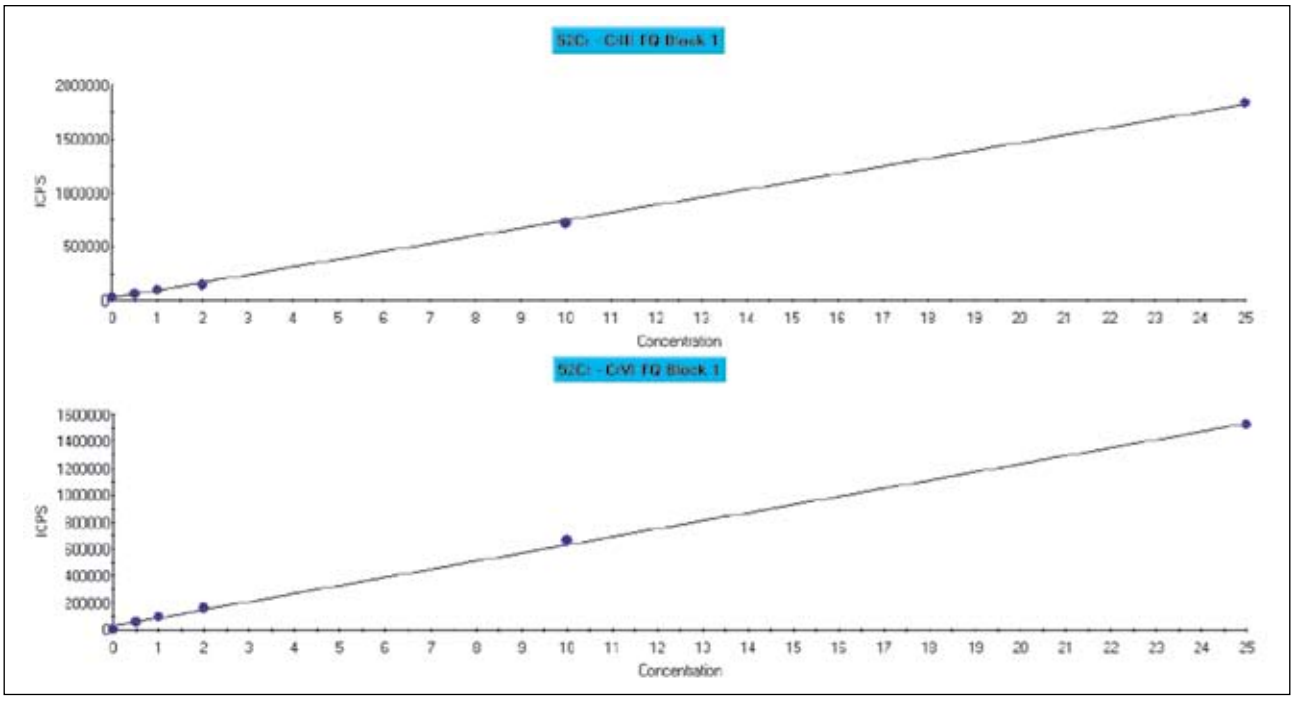


Figure 2. Calibration curves for Cr^{VI} and Cr^{III} calibration standards.

Chromium containing species were determined in six cement extract samples. Figure 1(b.) presents data derived from a cement sample that contains no reducing agents and Figure 1(c.) presents data for a cement extract known to contain lignin sulphonate as a reducing agent. The chromatographic peak observed in the void volume (^{52}M+, Figure 1(c.)) was attributed to the formation of the ^{52}ArC+ polyatomic species and this is confirmed by the overlay of the peak observed for ^{13}C. All of the cement samples analyzed were found to contain Cr^{VI}. However, the reducing agents in some cement samples were found to maintain the level of the water-soluble Cr^{VI} concentration below the maximum permitted value of 2 mg/kg.

Chromatograms for the dust sample extracted following the ISO 16740 protocol for soluble and insoluble Cr are presented in Figure 1(d.) and Figure 1(e.) respectively. A chromatographic peak was observed in the void volume for the extract prepared for soluble Cr^{VI} analysis when examining the ^{52}M+ data. However, concomitant peaks were also observed at ^{51}M+ and ^{53}M+ reflecting the natural isotopic ratio of ^{35}Cl and ^{37}Cl (i.e. as ^{51}ClO+ and ^{53}ClO+ polyatomic species). As a result of this, the ^{52}M+ peak was attributed to formation of the ^{52}ClOH+ species. A ^{52}M+ peak was also identified in the void volume for the extract prepared for analysis of insoluble Cr^{VI} although this peak was not attributed to the formation of ^{52}ArC+ or ^{52}ClOH+ species. Further work is required to confirm the origin of this chromatographic peak.

A series of spike recovery samples were prepared to validate the analytical methodology and ensure that there is no reduction of Cr^{VI} to Cr^{III} due to interactions with the polymeric media in the filters or during extraction process. Spikes of 100 ng Cr^{VI} were added to a filter extract during the extraction process and also to two filters prior to the extraction. The associated spike recovery data is presented in Table II and the complete recovery of the spiked concentrations confirm the accuracy of the methodology.

Table II. HPLC-ICP-MS fully quantitative sample data

CRM 545 WELDING DUST (40.2 ± 0,6 g/kg Cr^{VI})	Cr^{VI} (g/kg)	Cr^{III} (g/kg)
Mean of three replicate analyses of one extract	39.8 ± 0.5 g/kg	-
CEMENT	**Cr^{VI} (mg/kg)**	**Cr^{III} (mg/kg)**
1	5.21	-
2	7.07	-
3	14.53	-
4	11.16	-
5	0.011	0.013
6	1.20	1.80
SURFACE AREA DUST	**Cr^{VI} (pg/kg)**	**Cr^{III} (pg/kg)**
Extraction of soluble Cr^{VI}	70	13
Extraction of insoluble Cr^{VI}	1544	156
FILTERS - SPIKE RECOVERIES (100 ng Cr^{VI})	**Cr^{VI} (ng)**	**Cr^{III} (ng)**
Filter 1 spiked prior to extraction	105	-
Filter 2 spiked prior to extraction	90	-
Filter 3 spiked during extraction	97	-

The MDLs and LOQs for Cr^{VI} and Cr^{III} species were determined in accordance with the 3s and 10s models respectively using fully quantitative analyses of method blanks (n=5) and the associated figures of merit are presented in Table III.

Table III. MDL and LOQ Data

	Cr^{VI} µg/kg	Cr^{III} µg/kg
MDL (3σ)	0.10	0.64
LOQ (10σ)	0.35	2.13

Conclusions

The External Trigger Card and PlasmaLab software features permit automated instrument operation and integration for the routine speciation of chromium using HPLC-ICP-MS. The above described methodology provides a validated solution for rapid and accurate determination of Cr^{VI} and Cr^{III} species, addressing the current requirement for assessment of occupational exposure and monitoring the workplace environment.

References

1. Petrilli F.L., de Flora Silvio, Toxicity and Mutagenicity of Hexavalent Chromium on *Salmonella typhimurium*, *Applied and Environmental Microbiology*, Apr. 1977, p. 805-809.
2. Kota J., Stasicka Z., Chromium occurrence in the environment and methods of its speciation, *Environmental Pollution* Volume 107, Issue 3, March 2000, Pages 263-283.
3. Popp M., Hann S., Koellensperger G., Environmental application of elemental speciation analysis based on liquid or gas chromatography hyphenated to inductively coupled plasma mass spectrometry—A review , *Analytica Chimica Acta*, Article in Press, 2010, doi:10.1016/j.aca.2010.04.036
4. Directive 2003/53/EC of the European Parliament and of the Council, 18 June 2003, *Official Journal of the European Union*, 17.7.2003, item 9, L 178/25.
5. Directive 2000/53/EC of the European Parliament and of the Council, 18 September 2000 on end-of life vehicles, *Official Journal of the European Union*, 21.10.2000.
6. Hexavalent Chromium in Workplace Atmospheres, OSHA Method Number ID-215, T-ID215-FV-01-9806-M.
7. ISO 16740:2005 Workplace air - Determination of hexavalent chromium in airborne particulate matter - Method by ion chromatography and spectrophotometric measurement using diphenyl carbazide

Tornando acessível o saber

PES - Programa Educacional Sindusfarma

Com uma ampla gama de ações voltadas para a difusão do conhecimento e a educação continuada, o Sindusfarma atende à demanda da indústria farmacêutica pelo aperfeiçoamento contínuo de seus profissionais.

Desenvolvendo a capacidade de inovar e investindo no futuro

Saiba mais sobre as atividades do PES no site www.sindusfarma.org.br, enviando um e-mail para pes@sindusfarma.org.br ou pelo tel. (11) 3897-9779

SINDUSFARMA

SINDICATO DA INDÚSTRIA DE PRODUTOS FARMACÊUTICOS NO ESTADO DE SÃO PAULO

Rua Alvorada, 1280 - Vila Olímpia - São Paulo/SP - Fone: 3897-9779 - E-mail: sindusfarma@sindusfarma.org.br

DIVISÃO DE TECNOLOGIA DA INFORMAÇÃO

O **Grupo Vidy** amplia sua atuação no segmento laboratorial! Além de fornecer toda a infra-estrutura para seu laboratório, traz um novo conceito de Gestão Laboratorial a fim de adequar seu laboratório às normas ISO/IEC 17.025, FDA 21 CFR Part 11, GLP, ISO GUM e outras.

O **Grupo Vidy** firmou uma parceria com a **Thermo Fisher Scientific** trazendo soluções em sistemas de gestão laboratorial, tais como SampleManager, Darwin, Nautilus, e outros.

A **Thermo Fisher Scientific** é lider mundial neste segmento, oferecendo soluções multilaboratoriais, convertendo-se em uma referência na prestação de serviços de implantação, formação,

A Divisão de Tecnologia da Informação do **Grupo Vidy** atua com:

Serviços de Software

- Projetos de Sistemas de Gerenciamento Laboratorial
- Estudo para seleção do sistema mais adequado ao seu laboratório
- Projeto e instalação de sistemas LIMS
- Suporte e manutenção

Serviços de Hardware

- 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

Solicite a visita de nosso especialista.

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

SGS

GRUPO VIDY

ThermoFisher SCIENTIFIC

Rod. Regis Bittencourt, 3.360 • Taboão da Serra • SP • 06793-000 • Tel.: +55 11 4787.3122 • Fax: +55 11 4787.3399 • www.vidy.com.br • vidy@vidy.com.br

Point of View

Steps of Development

One important difference between developed and in development societies is the cost and speed of creation and flow of knowledge. Knowledge, and all its consequent innovations in science and technology, is a critical step towards an affluent society. Surely we need institutions for research and development, but simultaneously we must improve the information exchange between productive sectors in academy, industry and society. Information must fly smoothly and in a cost effective way.

All this was already known before our digital era, as clearly stated by David S. Landes in "The Wealth and Poverty of Nations" by pointing out: "the invention of invention, that is, the routinization of research and its diffusion". Nowadays, despite it may be seem just as a truism, diffusion still requires improvements in countries in the stage of development that Brazil is going through. No doubts we are evolving to become one of the most powerful world economies during the coming decades, but we need to speed up the process and disseminate it around all active partners and to the well-being of the society.

The launching of Brazilian Journal of Analytical Chemistry (BrJAC) is a milestone with full potential to expand the flow of knowledge. The integration of academy and industry is a must and BrJAC will certainly play a major role in putting them in contact. Why now? Why not before? Because now we have both the combination of economic strength and innovation in academic research in analytical chemistry in Brazil, as recently stated: "…analytical chemistry is healthy and growing, and is ready to support the new role of the country as an emerged power" (J. Braz. Chem. Soc.,20(10):1759,2009).

Great and successful steps were already surpassed, but there is a long and challenging road ahead. We need more engineers and scientists to grow our market competitiveness. We need all professionals connected in well established networks and with clear targets. Bring your knowledge and share it. Let us join forces and work together!

Joaquim A. Nóbrega
Department of Chemistry
Federal University of São Carlos

KINETEX™

Desempenho Ultra Alto em Qualquer Sistema de Cromatografia Líquida HPLC ou UHPLC

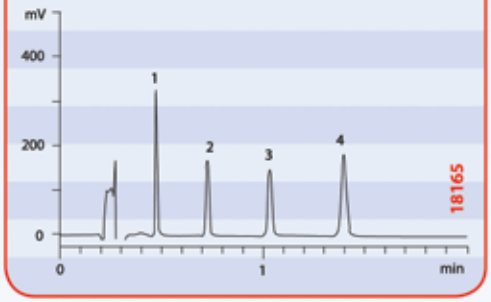
As Colunas Kinetex™ core-shell oferecem em **HPLC** e **UHPLC**:

Ultra Alta Eficiência | Análises mais rápidas | Menor consumo de solvente | Aumento de sensibilidade

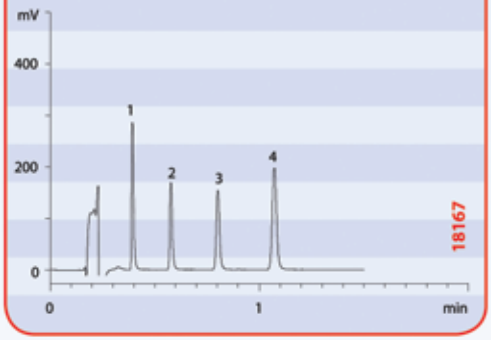
LANÇAMENTO 1,7 µm para UHPLC/UPLC*

Fases Disponíveis

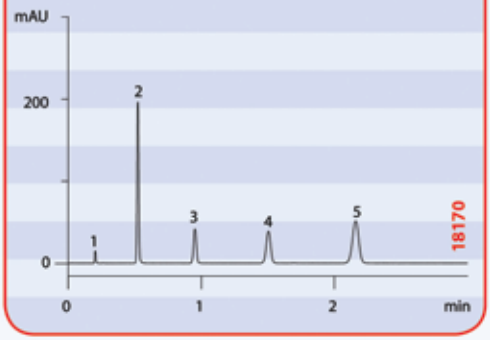
Waters® ACQUITY® BEH 1,7 µm C18
BP = 450 bar, Eficiência = 272.080 p/m



Kinetex™ 1,7 µm C18
Eficiência = 318.680 p/m



Agilent Technologies ZORBAX® 1,8 µm C18
Eficiência = 250.880 p/m



Entre em contato e solicite Notas Técnicas e Aplicações das Colunas Kinetex!

SPE

Extração em Fase Sólida

Faça pré tratamento das suas amostras através do Strata™

RECUPERAÇÃO
Não perca compostos essenciais

CARREGABILIDADE DE ANÁLISES
Evita limitações do volume de carga

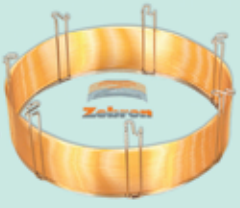
REPRODUTIBILIDADE
Você pode perder tempo reanalisando amostras?

Solicite AMOSTRA GRÁTIS para fazer testes!

Coluna para GC ZEBRON™

Introduzindo NOVAS Fases Zebron

Zebron ZB-XLB-HT Inferno
Zebron ZB-35 HT Inferno
Colunas de polaridade média com limite de temperatura de 400 °C
Para alto peso molecular e pontos de ebulição



Zebron ZB-BAC1
Zebron ZB-BAC2
Separação rápida para álcool no sangue

Zebron ZB-Drug-1
Análises rápidas de alta resolução para drogas de abuso

Zebron ZB-Bioethanol
Reduz em 50% o tempo de análise de Bioetanol

Temos uma ampla linha de Colunas Zebron, por gentileza, consulte nossas fases e dimensões disponíveis.

Allcrom
TUDO PARA CROMATOGRAFIA

Tel.: (11) 3464 8900 Fax: (11) 3464 8901
E-mail: allcrom@allcrom.com.br
www.allcrom.com.br

phenomenex®
...breaking with tradition™
Representada exclusivamente no Brasil pela Allcrom

Preencha cadastro na página www.allcrom.com.br/analyticamagazine.html e receba um brinde especial.

*Todas as Marcas Citadas são de seus respectivos proprietários.

Why running just to run after?

Direct seeding is the most evident example that only innovation is able to lead a country toward economic independence. Brazil use to apply imported technology in every productive field. The first sector to develop its own technology was agriculture in the last century. Only 35 years were enough to bring Brazil to the largest agricultural economy in the world, which is not a very evident statistics. This is because, specially european countries heavly subsidize their agriculture.

According with IMF (2009), Brazil, England, Russia and France are at same GDP level, around US$2,100 billion, which put our country among the six largest economies. For this to reach Germany, however, is a very big jump toward US$2,800 billion. This slope must be scaled by innovation at sectors, which have been seen as too high for Brazil's human resources.

This is not a question of producing aircrafts, ships or trains. This is a question to develop innovation in transportation systems to be able to supply the country with infra-structre without being obliged to follow first world countries step by step. To go forth in development is to invest in domestic inteligence, which is able to create domestic solutions to be copied by less developed countries instead of try to follow those who are ahead. To copy will lead us to the second place in every sector, never to the first position, just because, as soon as a new step is scaled, innovator country will have scaled one more.

Now is the time to decide. What is our goal? If the target is to be the first, academy must be ready to do high level research, while industry need to be prepared do absorb not only this knowledge but to employ those who have just produced this at the university. Perhaps, the most important idea that must be undestood by braziliam society is that the paper of academy is to produce primary research, while the role of industry is to pick this and transform in products, goods and services.

Luiz Alberto Melchert de Carvalho e Silva
Economist and Consultant in Agrobusiness

Events 2010

ABIQUIM
13º CONGRESSO DE ATUAÇÃO RESPONSÁVEL
2ª CONFERÊNCIA LATINO-AMERICANA DE SEGURANÇA DE PROCESSOS
São Paulo, SP – June 21st to 23rd
http://www.abiquim.org.br/congresso/default.asp

BMIC
XV BRAZILIAN MEETING ON INORGANIC CHEMISTRY
II LATIN AMERICA MEETING ON BIOLOGICAL INORGANIC CHEMISTRY
Angra dos Reis, RJ – August 16th to 20th
http://www.proppi.uff.br/bmic/

COBEQ 2010
XVIII CONGRESSO BRASILEIRO DE ENGENHARIA QUÍMICA
Foz do Iguaçu, PR – September 19th to 22nd
http://www.cobeq2010.com.br/

SIMCRO 2010
SIMPÓSIO BRASILEIRO DE CROMATOGRAFIA E TÉCNICAS AFINS
Campos do Jordão, SP – September 14th to 16th
http://www.simcro.com.br/

CBQ
50º CONGRESSO BRASILEIRO DE QUÍMICA
Cuiabá, MT – October 10th to 14th
http://www.abq.org.br/cbq/

FIMAI/SIMAI
FEIRA INTL. DO MEIO AMBIENTE INDUSTRIAL E SUSTENTABILIDADE
SEMINÁRIO INTL. DO MEIO AMBIENTE INDUSTRIAL E SUSTENTABILIDADE
São Paulo, SP – November 9th to 11th
http://www.crq4.org.br/default.php?p=eventos.php&id=41

ESPEQBRASIL 2010
2º ECONTRO BRASILEIRO SOBRE ESPECIAÇÃO QUÍMICA
São Pedro, SP – December 12th to 15th
http://www.espeqbrasil.iqm.unicamp.br/

Releases

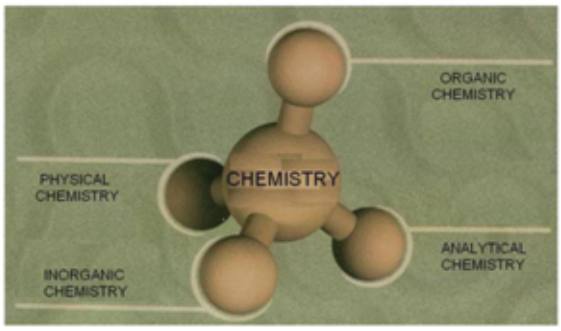
The Newsletter Analitica is a reliable technical-scientific information source to the professionals in analytical chemistry

Contact:
55 11 2162 8091
revista@novanalitica.com.br

Sign up at analiticaweb.com.br/newsletter
to freely download the works of your interest and to receive future editions

With seven monthly issues already published, the electronic Newsletter Analitica is a safe and reliable source of useful information to laboratory professionals.
In each edition of the Newsletter Analitica, experimental works developed by Thermo Scientific experts in chromatography, mass spectrometry, elemental and molecular spectrophotometry are presented. These works are applied to strategic areas such as environment, food safety, biofuels, proteomics, pharmaceuticals, forensics and many others. In general, the works are intended to address current problems and difficulties of the analytical laboratories in universities, research institutes, industries and companies providing services. Methods developed with the latest technology and validated in accordance with international standards, and new solutions to achieve lower limits of detection, high throughput and low consumption of reagents are presented.

Live or recorded Webseminars, events such as national scientific meetings devoted to different areas of knowledge and relevant laboratory news are published too.
Access analiticaweb.com.br/newsletter to search for the previous editions of the Newsletter Analitica and sign up to receive the future editions.



FEDERAL UNIVERSITY OF SÃO CARLOS

GRADUATE PROGRAM IN CHEMISTRY

PPGQ UFSCar

DEPARTMENT OF CHEMISTRY

Professional Master´s in Chemistry

Concentration Areas

- Chemical Technology

- Teaching of Chemistry

A must for R&D professionals working in chemical industries and teachers of chemistry

Information: www.ppgq.ufscar.br

Phone: 16-3351-8209 (Mrs. Ariane), ariane@dq.ufscar.br

Publication Rules

BrJAC, the ***Brazilian Journal of Analytical Chemistry***, is published quarterly in English by DKK Comunicação, in its printed and electronic versions. It is intended for professionals and institutions acting in the Chemistry area.

Papers for publication must be submitted exclusively to ***BrJAC***, in English. Previously published articles will not be accepted.

Categories – ***BrJAC*** publishes articles in the following categories:

a) Unpublished papers: new information on analytical research. Such articles should not exceed 25 (twenty five) pages.
b) Reviews: scientific articles on well established subjects, including a critical analysis of the bibliographical references, and conclusions. Such articles should not exceed 40 (forty) pages.
c) Technical and scientific information: case reports, presentation of new techniques, methods and equipment.
d) Miscellaneous articles: Those that do not fit into any of the above categories but are of acknowledged interest for Analytical Chemistry.
e) Letter to the Editor: comments, praises, criticism, suggestions and consultations to Authors are welcome and will be published at the discretion of the Editor-in-Chief.
f) Editorials
g) Book Comments
h) Presentation of novelties, always relevant to the area.

Manuscript preparation: Authors must present the manuscript in the most clear and succinct manner possible. The Introduction must include in a succinct manner important references, concerning both the nature of the problem being investigated and its background. The manuscript must include Introduction, Experiment, Results/Discussion and Conclusion and should not exceed 25 pages, including tables, figures and diagrams, and all pages must be numbered. All papers must be typed with double spacing using Microsoft Word only. Then a single file must be generated in the Portable Document Format (pdf) including the entire article, to be sent via e-mail (in the near future submission will be online through ***BrJAC***'s site (under construction).

Organization of the electronic file: all parts of the manuscript must be included in one single file organized as follows: cover page, text, bibliographical references, figures (with subtitles), tables and charts (with subtitles).

1st page: submission letter of the material including name, address, phone number and e-mail address of the main author, establishing the exclusiveness of publication in ***BrJAC***, in case the article is published.

2nd page: (cover page) Article's title, which must be short, clear and succinct to facilitate its classification. A subtitle may be used, if needed. This page must include the full name of the author(s), their titles and institutions. Other collaborators' names may be included at the end, in Acknowledgments. In a separate paragraph, indicate the place where the study was performed.

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

4th page: begin the text on the fourth page with the title of the article, without indicating the author(s) or the place where the work was performed. Unpublished articles must include the following chapters: Abstract, Introduction, Methodology, Results, Discussion and Bibliographical References.

Bibliographical References: begin a new page. The article must include only the consulted references, numbered according to their appearance in the text. Other authors' citations already numbered must indicate exclusively the reference numeral: avoid mentioning the name of the respective author. It is not recommended to mention several authors with identical concept (prefer the author who demonstrated it). It is recommended to avoid citations older than 5 (five) years, except in relevant cases. Include references that are accessible to readers. If the citation refers to an article already accepted for publication, mention as "in process of publication" and, if possible, mention the journal and year. Personal communications may be accepted exceptionally.

Please use the following model:

- Journals: name(s) of author(s), first name(s) initial(s) – Title of paper. Name of journal (abbreviated as defined by the *Chemical Abstracts Service Source Index* – see http://www.cas.org/sent.html), year of publication; volume; number of first and last pages.

  1. Barma, T.Y.; Song, B.J.; L..*China Chem. Soc.* 1997, 87, 418.

  If it is difficult to access the Journal, we recommend that you include its *Chemical Abstract* number, as follows:

  2. Chistyvst, L.K.; Andrenev, I.D.; Karol, F.W.; Cyrus, G.H.; *Zabrousk. Tacsh. Wilheim. Lyris.; Huil. Garm. Visijak*. 1987, 28, 816. *(BI 97:89745s).*

  If the paper does not have a full reference, please mention doi as follows:

  3. Striver, J.; Costa, T.C.; Pial, Q.P.; Temiza, V.L.; Vargas, V.N.; *Metalochimica Acta* (2004), doi:20.4598/v. metalacta.2006.15.023.

  We recommend the use of compound references, whenever possible, instead of individual references, following the style below:

  4. Varela, H.; Torresi, R.M.; J. *Eletrochem. Soc.* 2000, 147, 775; Lemos, T.L.G.; Andrade, C.H.S.; Guimarães, A.M.; Wolter-Filho, W.; Braz-Filho, R.; *J. Braz. Chem. Soc.* 1996, 7, 123; Ângelo, A.C.D.; de Souza, A.; Morgon, N.H.; Sambrano, J.R.; *Quim. Nova* 2001, 24, 473.

- Books: name(s) of author(s), first name(s) initial(s) – title of book (initials in capital letters), volume and issue, city where edited, Publisher, year of publication and number(s) of page(s) of citation.

  5. Regitz, M. em *Multiple Bonds and Low Coordination in Phosphorus Chemistry;* Regitz, M.; Sherer, O.J., eds.; George Thieme Verlag: Stuttgart, 1990, Chap. 2.

  Or, in case of "no authors":

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

- Patents: Please inform the *Chemical Abstracts* number in parentheses:
  7. Bash, T.; Dot, K.; Petersen, G.; Orbfield, B.; Cooper, D.; Sabbag, L.; Fce. *Le Kokai Tokkyo Koho 54 95,489* 1975. *(CA 233:P179341v)*
  8. McDonald, L.B.; *US pat. 9,236,006* 1987. *(CA 233:P49542y)*
- Software:
  8. Sheldrick, G. M.; SHELXL-93; *Program for Crystal Structure Refinement,* Göttingen University, Germany, 1993.
- Theses:
  9. Velandia, J. R.; *Doctorate Thesis*, Universidade Federal Rural do Rio de Janeiro, Brazil, 1997.
- Material presented in Congresses:
  10. Ferreira, A. B.; Brito, S. L.; *Resumos da 20ª Reunião Anual da Sociedade Brasileira de Química,* Poços de Caldas, Brazil, 1998.
- Internet pages:
  11. http://www.sbq.org.br/jbcs, accessed June 2001.
- Unpublished material:

Material accepted for publication

12. Magalhães, U. H.; *J. Braz. Chem. Soc.,* in press.

Material submitted for publication, awaiting approval

13. Magalhães, U. H.; *J. Braz. Chem. Soc.,* submitted for publication.

Unpublished material

14. Magalhães, U. H.; unpublished paper.

Material from personal communication

15. Magalhães, U. H., personal communication.

Notes:

1. Unpublished results may be mentioned only with express authorization of the persons involved in their achievement;

2. It is recommended that, whenever possible, authors follow the IUPAC standards, particularly the International System of Units.

Illustrations: the illustrations must be numbered according to the order of appearance in the text. For photographs, figures or graphs, use Arabic numbers. Charts and tables use Roman numbers. Subtitles must be properly numbered. In each illustration, identify the sequence number, name(s) of author(s) and the correct position where the illustration (photograph and drawing) should be inserted. Illustrations must have bases measuring 8.5 cm or 17.7 cm. The same result should not be expressed by more than one illustration. Graphs are preferable. For figures, graphs, diagrams, tables, etc identical to others previously published in literature, the author must ask for permission for publication to the company or scientific society holding the copyrights, and send such permission to the Editor of ***BrJAC*** together with the final version of the manuscript. If the figures are scanned, they must be high resolution (800 dpi/trace bitmap) with .tif or .jpg extension, provided that they are in the dimensions specified by the Editors. Color photographs or drawings (300 dpi/grayscale) must be submitted with .tif/.jpg extension and maximum total width of 8.5 cm in order not to create problems when applied to the Journal pattern. Other extensions possible include .cdr, .eps, .cdx and .opj. In the particular case of schemes containing chemical structures, they must have always the same dimension in order to be uniformly reduced.

Submission of articles: Please send one single file of the full manuscript in the .pdf format.

Manuscripts to be revised: Manuscripts sent to authors for revision must return to the Editor within at most 45 (forty five) days. After this deadline the manuscripts will be considered withdrawn and the system will terminate the process. To be reopened, a new submission must occur, starting a new process.

Revised manuscripts: Submission of a revised manuscript must be done by the same author, following the instructions for document submission: complete .pdf of the revised version and the answers to Editors, detailing the changes made in the new version and justifying the alterations that were suggested in the opinions and were not accepted by the authors. Both files must be sent through the online submission system. As they are received, an e-mail will be sent with the reference number of the paper. The author must be alert to the receipt of this e-mail with the submission code, since non-receipt means that the submission was not completed. The author will have 5 (five) days to complete it; otherwise, the system will not allow the submission and a new process must be started.

Final version: When requested to send the final version, the author will receive specific instructions concerning programs for file submission (text, figures, tables, etc). Pdf files will not be requested anymore at this stage.

Measurement standards: Follow the provisions in the General Table of Units of Measurement.

Abbreviations: Abbreviations are not recommended except those recognized by the International Bureau of Weights and Measures or those recorded and established in scientific publications. In case the abbreviations are many and relevant, use their definitions (Glossary) in a separate note. Abbreviations of terms established by Chemistry must follow the international standards traditionally in use, according to standard abbreviations.

Commercial names: The use of commercial names is not recommended.

Copyright: papers submitted for publication in ***BrJAC***, either from universities, industry or any other public or private institution cannot have been either published or being submitted for publication in another journal simultaneously. When the paper belongs to more than one author, it is clear that the other authors agree with the publication in ***BrJAC*** from the moment of its submission.

When submitting their papers for publication in ***BrJAC***, the authors agree that the copyright will become property of the *Brazilian Journal of Analytical Chemistry* – ***BrJAC*** –, if and when accepted for publication. The copyright comprehends exclusive rights of reproductions and distribution of the articles, including reprints, photographic reproductions, microfilms or any other reproductions similar in nature, including translations. As to the contents of published articles, the sole responsibility is of the authors, and ***BrJAC*** and its Editors, Editorial Board, employees and collaborators are fully exempt from any responsibility concerning data, opinions or unfounded statements.

Final Considerations: ***BrJAC*** reserves the right to make, whenever necessary, small alterations to the manuscripts in order to adapt them to the Journal rules or make them more clear in style, obviously respecting the original contents. Whatever the nature of the submitted manuscript, it must be original in terms of methodology, information, interpretation or criticism. The qualification of the paper will be attested by two or more consultants indicated by the Editorial Office.

A Saúde comemora.

18 de Junho
Dia do Químico

Empresa do Grupo
WHEATON Brasil

Farmacap

Lacrando e protegendo as descobertas do Químico no setor de Saúde, há mais de 20 anos.

www.farmacap.com.br

www.cristalia.com.br

Laboratório Cristália

Eleita a Empresa Mais Inovadora do Brasil

Prêmios Finep 2007 e Protec 2009

Sempre um passo à frente...

CRISTÁLIA
PRODUTOS QUÍMICOS FARMACÊUTICOS LTDA.