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12th Rio Symposium on Atomic Spectrometry

September, 17 - 21, 2012
Foz do Iguaçu – Brazil

Deadline for Early Registration and Submission of Abstracts - June, 4, 2012

Scope and Topics

The organizers are pleased to invite you to participate in the 12th Rio Symposium on Atomic Spectrometry to be held in Foz do Iguaçu, PR, Brazil. Major discussion topics are:

- Atomic absorption spectrometry
- Optical emission spectrometry
- Mass spectrometry
- Chemical vapor generation
- Instrumental developments
- Speciation analysis
- Electrothermal atomization
- Laser ablation
- LIBS
- Sample preparation
- Chemometrics in spectrometry
- Electrothermal vaporization

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Brazilian Journal of Analytical Chemistry

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Editorial

The Brazilian Meeting on Analytical Chemistry (ENQA 2011) reached its 16th edition in the International Year of Chemistry as a mature event for discussion of a diversity of subjects of interest to the analytical community, including researchers, graduate and undergraduate students as well as professionals from the productive sector. More than recognizing the development of Analytical Chemistry in Brazil, ENQA 2011 was a forum for discussion of scientific trends in the field, giving evidence of gaps that need additional development and showing that we have done a lot but still have a long way to go. The event held in Campos do Jordão-SP successfully showed that Analytical Chemistry frontiers really do not exist and that multidisciplinary work is essential to overcome the continuously renewed challenges. In this context, we have the Brazilian Journal of Analytical Chemistry in its 7th Edition with the mission to integrate academic and industrial worlds, being a discussion forum of the importance of Analytical Chemistry for the development of Brazil and for improving life quality (BrJAC, Editorial number 1).

This special issue brings five selected papers presented at ENQA 2011 together with a very interesting interview with Professor Ivano Gutz and viewpoints critically analyzing Analytical Chemistry in Brazil prepared by Professors Montserrat Fillela and Joaquim A. Nóbrega. Some of the current difficulties can be overcome by education and modern alternatives are highlighted in the point of view from Professor Cinthia Larive. Letters express impressions about ENQA 2011 by Professors Érico M. M. Flores and Clésia Nascentes, as the Director of the Division of the Analytical Chemistry of the Brazilian Chemical Society and as a member of the Organizing Committee of the upcoming ENQA 2013, respectively. A conference report discusses the impressive numbers of ENQA 2011 as well as its main highlights.

I would like to acknowledge Professors Pedro V. Oliveira, Lauro T. Kubota, Joaquim A. Nóbrega and Emanuel Carrilho, who worked together to prepare this BrJAC Special Issue and I invite you to enjoy it.

Fábio R. P. Rocha
Guest Editor

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Summary

Expedient

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16th Brazilian Meeting of Analytical Chemistry (ENQA): A Successful Scientific Meeting

Nowadays, the Brazilian Meeting of Analytical Chemistry (Encontro Nacional de Química Analítica, ENQA) is a well established and the most important meeting of Analytical Chemistry in South America where is possible to have a forum for presentation and discussion of the main aspects and trends related to analytical chemistry. In this sense, ENQA is a biennial meeting that helps to increase the development of analytical chemistry in Brazil and in other countries. Its relevance has been recognized by many international researchers and chemistry associations. Although ENQA is a Brazilian meeting, every edition has been attended by a number of foreign scientists and students.

The last edition, 16th ENQA, was organized by a group of researchers from University of São Paulo, Brazil (USP) and coordinated by Dr. Pedro V. Oliveira by the same university. In addition to the excellent scores (more than one thousand of inscriptions, 921 presented works including dozens of conferences and mini-conferences, several courses, etc), it had the presence of important Brazilian researchers and also from other countries. The covered topics were of current concern and it can be considered as one of the most successful editions of ENQA with special emphasis to the multidisciplinary aspects of analytical chemistry.

It is difficult to select some special points take into account that the overall meeting was a success. The presence of many important Brazilian and international researchers was one of the important aspects related to the 16th ENQA. The conferences of Dr. Alan Walcarius (France) and Dr. Cynthia K. Larive (USA) have shown the new developments of electroanalysis and the trends for leaning analytical chemistry, respectively. Dr. Montserrat Filella (Swiss) and Dr. Patrícia Smichowski (Argentine) presented a critical evaluation of the main aspects regarding the speciation analysis. Dr. Jorge C. Yanez Solorza (Chile) and Dr. Rodolfo G. Wuilloud (Argentine) described respectively the applications for forensic chemistry and the use of ionic liquids for extraction, two relevant topics in analytical chemistry. However, in my opinion, the conference of Dr. Boris L´Vov (Russia) was a kind of gift especially for the young generation of analytical chemists that could watch a deep and amazing discussion of the effort directed to absolute analysis using solid sampling systems. On the other hand, the conference of Dr. Reinhard Niessner (Germany), editor of Analytical Chemistry journal, has demonstrated as analytical chemistry is important to the development of analyzers suitable for many applications in health and industrial fields.

One of the special features of 16th ENQA was the excellent attention to the graduation and post-graduation students, providing the necessary help to allow their participation in a high level meeting as ENQA. It is necessary to keep all the efforts because these new generations of young scientists will be asked to solve the new requirements for the analysis of electronic devices, advanced materials, health, biochemistry, biotechnology and fuels. For the future editions, I would like to suggest special care to these topics in addition to the other relevant ones but keeping the focus in the high scientific level as it has occurred in 16th ENQA and other previous editions.

Finally, I would like to congratulate the organizers by this excellent edition of ENQA and all the staff that directed an enormous effort in order to keep the necessary friendly and high scientific level environment for all the participants.

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A look at the Brazilian Meeting in Analytical Chemistry

As a member of the Organizing Committee of the 17th ENQA, it is a great pleasure to have the opportunity to express my opinion on the 16th ENQA.

The Brazilian Meeting in Analytical Chemistry have represented, over the past 30 years, a place for the integration of professionals connected with Analytical Chemistry, fostering the dissemination of recent developments and discussion of relevant topics in this area. Since 1995 (8th ENQA), I have participated in this event and I am very glad to have been part of the impressive growth and consolidation of this important Brazilian meeting over these 16 years. In 1995 there were 390 participants and 352 abstracts were presented. In the 16th ENQA, we had 1200 participants and 916 abstracts presented. These numbers make the ENQA one of the major Brazilian scientific events in the area of Chemistry. It should be pointed out that the ENQA is one of the only Brazilian events that have special editions of important scientific journals dedicated to publication of selected works presented. Analytical Chemistry is also the first area of Chemistry that has a specific Brazilian scientific journal, the Brazilian Journal of Analytical Chemistry. Clearly this reflects the development and maturity of Analytical Chemistry in Brazil. All these facts make us proud and certain that we are on the proper road.

What can I say about the 16th ENQA? In my opinion, it was a perfect event. The topic chose, "Analytical Chemistry without borders" triggered discussions and reflections on the role of Analytical Chemistry in various sectors of society, as well as on the scientific advances in the area. Even with the record number of participants and abstracts presented, the organization of the event was flawless and it evolved smoothly. There was a wealth of scientific activities, with plenary and keynote lectures on various topics headed by renowned Brazilian and foreign speakers. The massive participation of conference attendees is evidence of the great interest aroused by these speakers and their chosen topics. The poster sessions were another outstanding part of the event: 916 posters were presented into 3 sessions and in all of them there was a sound discussion forum on Analytical Chemistry and its multiple strategies. In addition, the site chosen for the event was outstanding: a small, hospitable city that offered enjoyable opportunities for conference attendees to meet, along with the scientific and social activities of the event.

I am certain that the success of the 16th ENQA is primarily due to the dedication of the Organizing Committee, which took care of all details and did everything possible to guarantee that the 4 days of the event would be a space for high level scientific discussions and learning, and a unique opportunity to establish and strengthen professional and personal relationships. Therefore, I congratulate and thank the Organizing Committee of the 16th ENQA for their commitment and dedication. They may be certain that it was worthwhile!!!

As a member of the Organizing Committee for the 17th ENQA, I am aware of the great challenges involved in organizing this event to maintain the high level reached to date. We will make the greatest efforts for this to occur, and of course we want to invite all of you to join us in 2013. We will be waiting for you in Belo Horizonte!!!

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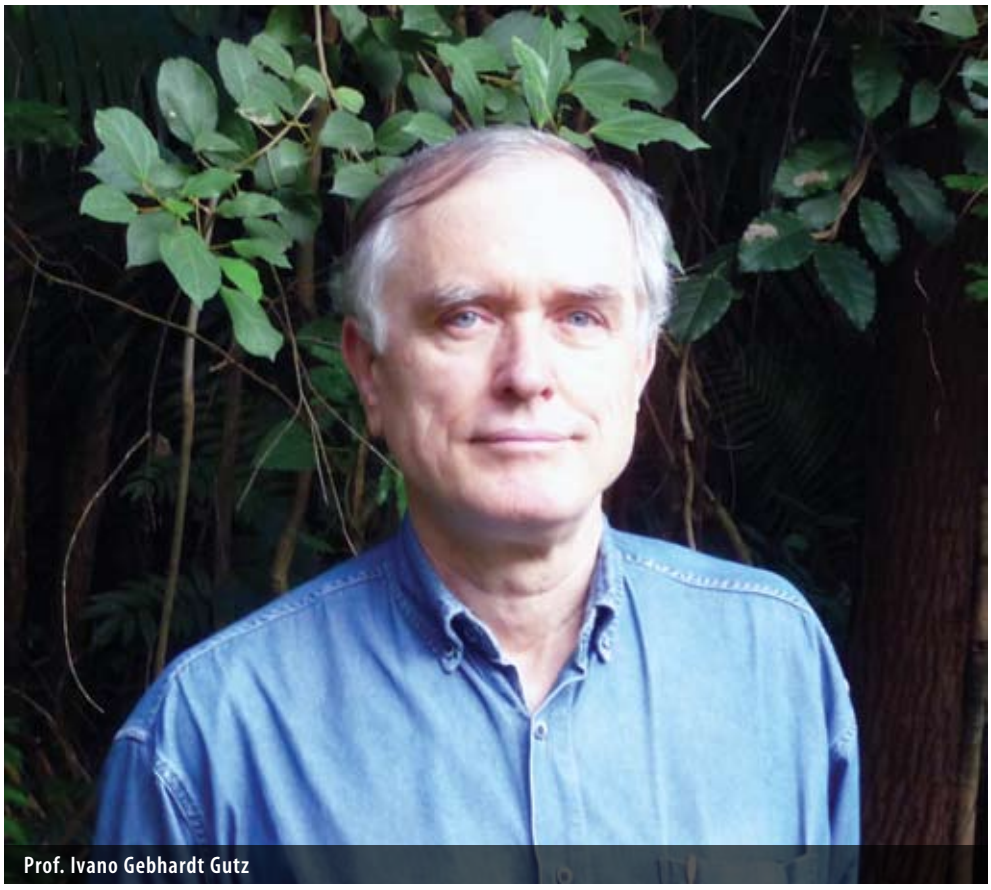

Prof. Ivano Gebhardt Gutz

He was only seventeen when he first started teaching. So interested he was in science, technology and in unravel the mechanisms of production, that even younger than all technicians and managers in the company, Ivano Gebhardt Rolf Gutz was invited by a large textile industry in Brazil to teach process technology to the new apprentices. At that time, a fruitful marriage between science and teaching began and, as we can see in this interview, only death will make them apart: after 35 years of a very intense professional life, with a nomination for the Brazilian Academy of Science, professor Gutz takes care of his health in order to keep teaching, researching and producing knowledge.

This was one of the most difficult interviews to edit in this scientific journal, because the broad, and at the same time deep view of the work field that professor Gutz shows us was very hard to cut down to 10 pages: it is really a brilliant essay on the state-of-the-art of Brazilian Analytical Chemistry, both from the science and the professional perspectives. Enjoy!

How do you see the development of the National Meetings of Analytical Chemistry (ENQA) in Brazil?

I can still remember the first National Meeting of Analytical Chemistry, or ENQA, held at PUC (Pontifícia Universidade Católica) of Rio de Janeiro in 1982, dedicated to the memory of Fritz Feigl, the greatest analytical chemist to live in Brazil so far. At that time, almost one half of the 130 or so communications were from PUC-Rio and from the Chemistry Institute of São Paulo University (IQ-USP-São Paulo). Authors from IQ-UNESP-Araraquara (Universidade Estadual de São Paulo), IQ-Unicamp (Universidade Estadual de Campinas), CNEM-Rio (Comissão Nacional de Energia Nuclear) and UFBA-Salvador (Universidade Federal da Bahia) were the next most frequent. Having attended all 16 meetings until now, in a few words, I would highlight as significant developments over time the ever-growing number of participants and communications, the expansion of topics covered and the multiplication of the geographical origins of the authors, now widely spread across the country but only occasionally from abroad, the enthusiastic and substantial participation of young researchers and graduates and the expanding range of suitable venues for the event.

What would be your critical evaluation of the 16th ENQA? What should be improved for the next events?

The event was better than ever before, with increased quality, diversity, size and reputation. The program has captivated the attendees from the opening ceremony until the closing session, with a surprisingly full auditorium. Each ENQA has a new flavor. During this 16th meeting, like in all previous ones – with exception of the 13th and 15th, that were carried out jointly with the 1st and 3rd CIAQA (Congresso Iberoamericano de Química Analítica) –, participation of foreign scientists was insightful, but limited mainly to the invited speakers. A higher participation is not mandatory for the success of a national meeting, but it would raise more critical discussions and promote greater cross-fertilization of ideas. The prevalence of Portuguese as spoken language is a barrier and perhaps the suggestion given by an invited speaker, to enforce the preparation of posters and slides in English, should be considered henceforth.

In a somewhat larger scale than before, but not disproportionate, instrument manufacturers participated as sponsors, exhibitors and providers of technical presentations in the 16th ENQA. However, idea hunters from these or other companies looking around and contacting authors of commercially promising innovations in techniques, instrumentation or methods were unnoticeable or missing, like in former ENQAs. Is our science still uninteresting for them, or too many communications were overwhelmingly oriented to applications, or are there other factors?

What is the position of analytical chemistry in the setting of national and international scientific production?

Most states of Brazil have at least one university with an active research laboratory in analytical chemistry that is expected to grow in the forthcoming years, continuing to change the geographical distribution

that currently indicates that authors from 50 institutions publish regularly although one quarter of all papers have at least one author from the University of São Paulo and that one author from the State of São Paulo appears in one half of all papers.

Analytical chemistry is well placed in the national scenario of scientific output in chemistry. In the Web of Science database we observe that only physical chemistry and multidisciplinary chemistry are better placed in publication count during the last 10 years, with organic chemistry appearing as the fourth. For sake of comparison, in the United States, organic chemistry reverses its position with analytical chemistry in the third position.

The United States publish about 10 times more than we do in science as a whole and in chemistry in particular, in a per capita basis. I take South Korea as a second example because, half a century ago, they were nearly at the "limit of detection", just like we were, but they have already reached standards of highly developed countries in many aspects and, in the last decade, their per capita index of publications was already about 7 times higher than ours for science and also for chemistry. In analytical chemistry, these ratios fall to one-half of the values mentioned already for both countries, but it should not be understated that the Americans in special but also the Koreans publish in periodicals with higher impact index. The exaggerated weight of publications and citations in the American science model is a matter of discussion, but when we look for technological development in terms of patenting and licensing of products and processes, we are behind the United States and South Korea not by one, but by two or more orders of magnitude.

What are the main trends in the area of analytical chemistry? And in electroanalytical chemistry?

These questions demand a full article after a week of thinking. I will instead give some random points of view. To offer more and more analytical sensors and systems that work autonomously and continuously outside of the laboratory is a challenging current trend. This goes from environmental monitors and stations for air and water to personal monitors, if possible, directly regulating the release of a reagent or drug. Two inspiring examples are the electrochemical oxygen sensor installed in the exhaust pipe of most cars, that provides feedback to the processor in order to inject the optimum amount of fuel, and the amperometric enzymatic glucometer, valuable for 0.5 billion diabetics, now available in a subcutaneous version that lasts for a week and is coupled with an insulin-release pump.

Speciation is a trend that persists for some decades in voltammetry and is being extended, with more complex hyphenated separation and spectrometric techniques, to a wider range of applications, now including larger molecules. This connects with the second part of the question. The richness of electroanalytical chemistry is not only the diversity of available techniques, with or without electrolysis, and electrodes, bare or modified, centimetric or nanometric in size, placed in a river or a brain cell, to detect and quantify a very wide range of organic and inorganic compounds at low

concentrations and with reasonable selectivity. Current research usually addresses selectivity questions at the sensor level, using enzymes or other molecular recognition schemes, combined or not with polymers or carbon nanotubes.

Questions that still demand much research are how to extend the shelf life of enzyme-based sensors or biomimetic ones, and the operational life of the sensor implanted in humans or exposed to complex matrixes. Electrochemical sensors are amenable to miniaturization down to the nano scale, stimulating new research, especially when platforms with multisensing capabilities are aimed, for example, the so-called electronic noses and tongues. Another approach for multiple analytes is to couple electrochemical detectors, mainly amperometric ones, with liquid chromatography, flow analysis and capillary electrophoresis. Contactless conductivity detectors are gaining space whenever applicable because the electrodes never need polishing or electrochemical activation, nor need to be disposable. Prototypes of one chip multipotentiostats for arrays of electrodes are around for some time but now a low cost one-chip potentionstat is available, simplifying miniaturization and embedding of the electronics with USB or Bluetooth connectivity into the body of sensors.

A current trend is also to investigate the utility of the multifaceted electrochemical resources to assist other analytical techniques and systems in one or more tasks like electrochemical preconcentration and derivatization, electrodissolution of metallic samples, electrosmotic fluid pumping, electrochemical and photoelectrochemical pre-treatment for organic material destruction, electrodialytic extraction, controlled electrochemical generation or cleaning of reagents and buffers, separation of ionic species by differential electromigration in capillary electrophoresis, microssensing by electrochemical microscopy, electrodeposition of nanowire connections, electrospray ionization of samples for mass spectrometry, electrochemical nanomotors to move molecular recognition species through the sample, etc. For example, we have recently proposed the first interface of a voltammetric thin layer flow cell with the technique of capillary electrophoresis with contactless conductometric detection. We proved the concepts of pre-capillary accumulation and release, pre-capillary derivatization of neutral species and we are studying electrocatalytic processes with direct analysis of reaction products.

Have the post-graduation programs enabled the renewal of researchers in the field of analytical chemistry with quality? Is there a need that should be supplied?

Postgraduate studies are in expansion in our country and this includes analytical chemistry. The rate exceeds significantly the population growth and we may be now close to 0.1% of researchers with a PhD in our population, but we need to triple this index to enter the top-20 group of nations with more scientists in their population. Besides the creation and expansion of programs, the increase in the number of scholarships and special funding allocation for projects in the North and Northeast regions, to reduce geographical distribu-

tion heterogeneity of research and formation activities, there is also a long-lasting pressure to shorten the graduation time of masters and PhDs and to publish and patent their findings. At the beginning, there was room for this strain for faster turnover, that included a reduction in the scholarship duration, but perhaps it was pushed somewhat too far if we consider the variable level of the candidates admitted by the system, sometimes in detriment of the depth and comprehensiveness of the thesis and papers, when not of the quality. As the absorption of PhDs outside the academy is growing, it is still far from that observed in most developed countries where it reaches two thirds or so of the total.

What is the importance of undergraduate research programs in analytical chemistry? What are the characteristics of these students? How do you evaluate the initiative to allow the access of high school students to research activities?

I consider the undergraduate research as the most important mean of familiarizing students with research activities, since, during the typical undergraduate courses, few lab classes offer individual projects or in small groups. Participation in activities of scientific research of new ideas and concepts, or at least, improvement or search for original applications may prove to be exciting and challenging for some. But they can also be frustrating for other students, as they realize the difficulties to overcome, the intelligence and persistence necessary to achieve significant advances in science, including analytical chemistry. The experience is valid, and not only for those talented for a career in research, because they can become familiar with the scientific methods and new analytical techniques even if they later opt for another professional practice. The time to join in the initiation depends on both the student and the type of activity that some laboratory research can offer, and there are certainly students who can start it even in high school. In some countries, there are examples of approved doctoral students at 20 years of age.

Your enrollment at the Olympics in Chemistry has been remarkable. Is it already possible to see tangible results in relation to the interest of high school students for chemistry? How is the performance of students from public schools in the Olympics?

We started the Chemistry Olympiad of the State of São Paulo in 1997 and repeat it every year since. We count on a team of 50 to 55 PhDs in chemistry that helps voluntarily to apply two consecutive evaluations to the students selected by the schools based on essays about a theme that is announced yearly in 9 thousand folders and banners displayed in schools and in the website AllChemy. E-mails are also sent to 7 thousand teachers, school directors, journalistic reporters and former winners of the Olympiad. There are 1.6 million high school students in the State of São Paulo and the team and I believe that our efforts are contributing to foster the interest in chemistry and helping teachers to motivate their students. The testimony of the 140 finalists, selected from many thousands for the final examination, and some 100 teachers and parents that accompany them for the full day of activities at our Institute is very favorable, stimulating us to continue. The closing

ceremony with distribution of 40 medals to the winners is also very enthusiastic.

Many of participants of the Olympiad who decide to study chemistry, chemical engineering or pharmacy also declare that the Olympiad called their attention to chemistry and that their participation in one or more stages was unforgettable. One of my doctorate students mentions that the Olympiad was decisive in his choice to study chemistry. Obviously, we have only the testimony of those who advanced to the final phase and their teachers, and cannot extrapolate to the 1.6 million. The number of candidates per admission remained reasonably stable at our Institute at least, while the number of chemistry courses has grown a lot in the period.

"No student from public schools has reached the minimum level to represent Brazil in the International Olympics of Chemistry."

The winners of the São Paulo State finals do well at the National and Latin American Chemistry Olympiads, gaining many gold medals, and in the last three years, they started to bring bronze medals also from International Chemistry Olympiad, played by students from some 70 countries.

The sad finding of the Olympiads in São Paulo and in Brazil as a whole is that, even with more students enrolled in public schools, the difference in favor of the private schools is increasing. There are 6 levels of evaluation and some students from public technical schools have reached the 5th level, but no one succeeded yet in the 6th, the level that defines the Brazilian representatives for the two international Olympiads.

The gap between public and private Brazilian basic education is one of the largest revealed by the Program for International Student Assessment (PISA), that samples both public and private schools in adequate proportion and quantifies the scores of 15-year-old students every three years in some 65 participating countries. Regrettably, the performance of our students as a whole places Brazil consistently among the worst 10% of all countries. Another drama that called my attention in the PISA database for the 2009 evaluation is that less than 0.6% of the Brazilian students of the age of 15 years are top performers in science, whereas the 30 best-ranked countries have 5% to 20%! As can be seen, there is much more to do besides Olympiads to change this unfavorable scenario.

How was the experience of being elected for the Brazilian Academy of Sciences? How do you evaluate the performance of ABC? Is analytical chemistry well represented in this important institution?

The Brazilian Academy of Sciences is a prestigious institution with a long and relevant role in the discussion of national science policies, cooperation with international organisms like ICSU (International Council for Science) and TWAS (the Academy of Sciences for the Developing World), organization of scientific events and groups with broader or interdisciplinary interests like health, the

environment and energy sources, to mention only some activities. I felt honored and humbled to be elected by my peers as a member of the Academy. This is certainly a rewarding recognition for anyone and so many more deserve it already in our steadily growing national scientific community. Any expansion of the membership of the Academy will thus be welcome. Earlier full admission of bright young scientists would add to the momentum of the Academy and also speed up the correction of unbalances like the underrepresentation of analytical chemistry in the chemistry sciences.

What is your opinion about BrJAC? Is it important to have a periodical devoted specifically to analytical chemistry? Are there any prospects for growth in the publication field?

In a country capable of generating one thousand or more communications related with analytical chemistry every year, as can be inferred by summing up the contributions to conferences like ENQA, RASBQ (the annual meeting of the Brazilian Chemistry Society), SIBEE (Brazilian Symposium in Electrochemistry and Electroanalysis), COLACRO (the Latin American Congress of Chromatography and Related Techniques), Rio Symposium, EspeQBrazil and others, there is room for a Brazilian periodical specialized in analytical chemistry like BrJAC, edited in English language with no publication fees for authors and freely available on the internet, thanks to the sponsors. The distinguished chief editor of BrJAC and his qualified and representative editorial board are on the right track and I wish them success in establishing a journal that highlights and honors the Brazilian analytical chemistry.

Is the scenario for analytical chemists that work in Brazil different from abroad? How is the job market inside and outside of academia in Brazil? For example, where do your former students work?

One significant difference is that in Brazil, an academic career in a university is still the predominant option of professionals with a doctorate or post-doctorate degree, a trend that is somewhat less accentuated in analytical chemistry than in other areas of chemistry. In developed countries, this is a minor fraction, although in some, half of the PhDs may be enrolled in the public sector. Brazil also still loses more brains than it drains from elsewhere. Our higher authorities are becoming aware that, in a knowledge-based global society, the chronic fault of scientists and researchers, including doctors in analytical chemistry, is a weakness to be overcome in order to preserve Brazil's economic importance, maintain jobs by becoming technologically more competitive and improve the quality of life of the population without endangering the rich environment.

Almost all multinational companies with activities in Brazil have their main research centers overseas, still mainly in the United States and Europe but with an accelerated displacement trend to Asia, where the youngsters are graduating massively in all branches of engineering and science, while so many in Brazil chose humanities, for example, law schools. Our local companies are introducing or already have research and development departments, but, typically, they are undersized and have not enough masters and doctors – if any –, nor critical mass and experience in generating innovative and competitive

products for the global market. There are exceptions already and the situation is gradually improving with help also of tax incentives to increase the propensity of firms to invest more in research and development (R&D) but, once more, many companies abroad are in advantage by investing 9% or more of its revenues in R&D and operating in countries that fund science research with about 3% of the gross product. Some leading companies employ thousands of PhDs and have tens of thousands other workers engaged in R&D.

Anyway, in Brazil, masters and PhDs in Analytical Chemistry seem to be the most demanded in chemistry because, besides the academia, they are required by research institutions like Embrapa (Empresa Brasileira de Pesquisa Agropecuária), IPEN (Instituto de Pesquisas Energéticas e Nucleares), CNEN (Comissão Nacional de Energia Nuclear) and Cenpes-Petrobras (Centro de Pesquisas Leopoldo Américo Miguez de Mello, from Petrobras), besides pharmaceutical and chemical industries, government control and regulating agencies, especially those related to the environment, food and health, forensic services, analytical instrumentation companies and private chemical analysis laboratories, consulting groups, etc.

"We have to overcome the lack of high-level scientists and researchers in analytical chemistry in Brazil in order to preserve our economic importance, maintain jobs by becoming technologically more competitive and improve the quality of life of the population without endangering the rich environment."

Regarding the graduates in electroanalytical chemistry, despite the lack of high-tech firms researching and producing advanced electrochemical sensors and analytical systems in Brazil, there are local manufacturers of the most demanded electroanalytical instruments and electrodes and at least one has an active R&D sector. There is a large commercial market for advanced and automated traditional electroanalytical techniques and methods to be supplied and supported in new applications in the field, process and laboratory. It is also well known that electrochemical analysis demands more brain than investments in equipment. Therefore, the well-formed researchers in this field adapt quite easily to any other activities in analytical chemistry, electrochemistry or instrumentation.

Most of my former master students have also completed a doctorate. About one third of them was hired by private companies and is working mainly in R&D activities or as managers. About 50% are engaged in universities, four at USP and the other at Unifesp (Universidade Federal de São Paulo), UFABC (Universidade Federal do ABC), ITA (Instituto Tecnológico da Aeronáutica), UFU (Universidade Federal de Uberlândia), UB (Universidade de Brasília), UMC (Universidade de Mogi das Cruzes), UFPR (Universidade Federal do Paraná), UFRPE (Universidade Federal Rural de Pernambuco) and Mackenzie. Others have chosen institutes like INPE and INPI (Institu-

to Nacional de Propriedade Industrial) and one or two are working abroad.

Is there space for entrepreneurship in Brazilian analytical chemistry?

For startups, I see more chances for entrepreneurship in analytical services firms than in manufacturing analytical instruments or supplies, besides some niches. Not so much in clinical analysis and healthcare, a branch of very high and ever growing demand, best served with expensive automated analyzers of high throughput, that is becoming dominated by large laboratories providing combined imaging and other medical diagnostics services; but in chemical analysis of specialties like foods, water, fuels, pharmaceutical products, doping, industrial supplies and products, emission and end product control and testing by consumer right entities, environmental control, treatment and remediation of damages.

New opportunities are sometimes driven by existing or new regulatory issues and obligations, even from abroad, in transnational commerce. Calibration, auditing, validation and certification services, consulting with expertise in installation of analytical laboratories are other examples of growing areas. Production of reference materials, standards, certain reagents and solutions, especially those with short shelf life or high local demand, may be viable. The production of analytical instrumentation with special interest for local industries or other niches like in situ measurement or screening may also attract new entrepreneurs.

However, the history of such manufacturing in Brazil is punctured by limited innovation and more failures than successes, so that only few have resisted for more decades. While some of the surviving ones gave up and simply put their brand on Asian O&M products or act as representatives, others still produce simple to medium complexity equipment for widely accepted techniques, not necessarily with state-of -the-art technology.

They can strengthen their position engaging qualified personal in using their manufacturing capacity of small series of instruments to offer "tailor made" solutions, like the adaptation of sensors, calibration modes, mechanization of sampling or simple automation to suit specific application for local use or exportation. Anyway, an increasing number of local or multinational instrumentation companies demand masters and PhDs in analytical chemistry for support and application development, although very few have true research laboratories here.

Various difficulties on entrepreneurship in Brazil cannot be addressed in a few words: the lack of a local production or fast supply of small lots of electronic and mechanical components or sub-units at reasonable prices, the increasing competition of Asian manufacturers besides traditional ones and the current global trend of acquisition of smaller companies by huge ones.

What is your routine as a full professor in São Paulo University? How is your day to day? What are your motivations?

What has never changed in my routine since

I was hired at IQ-USP in 1978 is my enthusiasm and zest for challenging research and experimentation, teaching and interaction with students. A daily privilege that I value highly is to share laboratories and ideas in a group that lasts for decades, formed with the younger, creative, hardworking, autonomous and reliable colleagues and friends Lúcio Angnes and Claudimir do Lago. I have much and many to thank, but I will restrict myself to Eduardo Neves, my supervisor and friend and to his supervisor, who started scientific research in analytical chemistry at IQ-USP, Paschoal Senise, for the reasons and emotions that I have already expressed or published after they died.

To escape São Paulo's time consuming traffic, in 1992, when I became a professor, I moved to a nice apartment nearby the university campus. Since then my wife and I have a daily early morning walk in the campus. Despite of my preferential activities, I have cooperated with one hundred or more workgroups, committees, bureaucratic tasks, institutional projects with new buildings, etc., and have been elected and worked seriously as head, member or representative in many academic and administrative positions at USP and various outside. But now, after 35 years of activity, I am learning to say "no" to some tasks

that do not fit in my schedule, in order to stay at the Institute not much more than ten hours a day, leaving time for other interests, like, for example, hearing music and sharing with my wife the pleasure of playing with my little granddaughter before my daughter and son-in-law come to pick her up. In short, I could not be happier with the chosen carrier and workplace, I esteem our national analytical and electrochemical communities and hope to remain healthy to continue.

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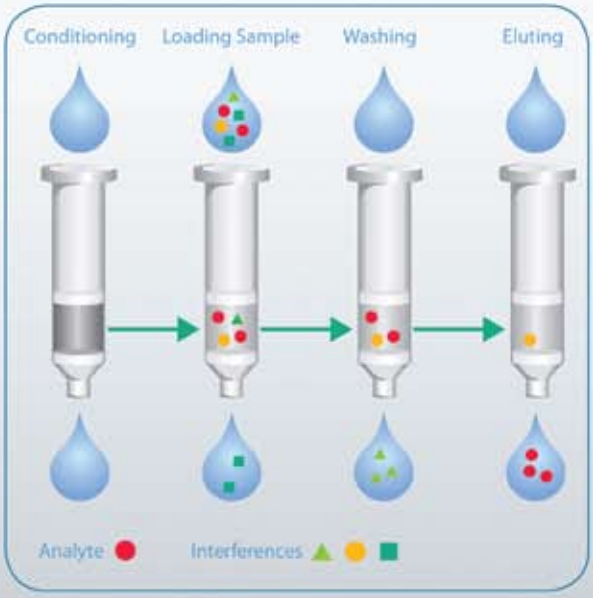
A Typical Four-Step SPE Method



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Conference Report
Brazilian 16th National Meeting on Analytical Chemistry

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Abstract
The Brazilian 16th National Meeting on Analytical Chemistry (16th ENQA) was held on 23-26 October 2011 in Campos do Jordão, with 1141 participants, including professors, researchers, graduate and undergraduate students, industry professionals and technicians from Brazil and other countries. The theme *Analytical Chemistry without Frontiers* covered the interdisciplinarity of Analytical Chemistry and the exchange of knowledge between Brazilian researchers and those from abroad, aiming to resolve new challenges from society and the productive sector. The scientific program involved 5 short-courses, 1 workshop, 6 plenary lectures, 16 short-conferences, 64 oral presentations, 3 symposia and 921 poster presentations. Two special issues of journals were dedicated to papers presented at the 16th ENQA: Microchemical Journal and the present issue of the Brazilian Journal of Analytical Chemistry.

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1. Introduction
The first Brazilian National Meeting on Analytical Chemistry (ENQA) was organized by the Pontifical Catholic University of Rio de Janeiro (PUC/RJ), in 1982, with the second also at PUC/RJ in 1983. Since then the event has occurred every two years in different Brazilian states as a way to disseminate Analytical Chemistry in Brazil. The national meetings have contributed to improve the quality and quantity of the scientific production of Brazilian Analytical Chemistry [1]. ENQA's success can be demonstrated by the increasing number of participants from the diverse regions of Brazil and abroad, by the excellence of the presentations, and by the high interest in the papers published in special issues dedicated to the previous events (11th ENQA: 2001, Journal of the Brazilian Chemical Society, vol. 14, n. 2, 2003; 13th ENQA: 2005, Microchemical Journal, vol. 82, n. 2, 2006; 14th ENQA: 2007, Analytical Letters, vol. 41, n. 9, 2008; 15th ENQA: 2009, Microchemical Journal, vol. 96, n. 1, 2010). Papers from selected works presented in 16th ENQA will be published in a special issue of Microchemical Journal and in the present issue of the Brazilian Journal of Analytical Chemistry.

The Brazilian National Meeting on Analytical Chemistry is an event of the Analytical Chemistry Division of the Brazilian Chemical Society (SBQ). The 16th ENQA was held on 23-26 October 2011 in Campos do Jordão Convention Center, located in the city of Campos do Jordão, São Paulo State, Brazil. In the 16th edition various aspects of the Analytical Sciences were discussed, for the purpose of directing attention to the multidisciplinary research carried out in the area, encouraging the dissemination of knowledge, in order to meet the most diverse sectors of Brazilian society. In this context, the theme proposed was "*Analytical Chemistry without Frontiers*", which covers the interdisciplinarity of Analytical Chemistry and also represents the exchange of knowledge from Brazilian researchers and those from abroad, aiming to overcome the new challenges from society and the productive sector. With this theme it was possible to join specialists and to show how Analytical Chemistry is linked to the environment, forensic and biological sciences, food chemistry, pharmacology, technological products, instrumentation, agrobusiness, petrochemistry, education, metrology and chemometrics, among others. Additionally, it was an important moment for reflection and discussion on Analytical Chemistry as part of the International Year of Chemistry 2011 celebration, especially because Brazil is experiencing an important period of development.

2. Committee
The organizing committee of the 16th ENQA was composed by Cassiana S. Nomura, Pedro V. Oliveira and Renato S. Freire from Chemistry Institute of São Paulo University (IQ-USP), Fábio R.P. Rocha from the Nuclear Energy in Agriculture Center of São Paulo University (CENA-USP) and Paulo R.M. Correia from the School of Arts, Sciences and Humanities of São Paulo University (EACH-USP).

The executive committee was formed by Analytical Chemistry professors of the IQ-USP: Cassiana S. Nomura, Claudimir L. Lago, Ivano G.R. Gutz, Jivaldo R. Matos, Jorge C. Masini, Lilian R.F. Carvalho, Lúcio Angnes, Marina F.M. Tavares, Mauro Bertotti, Nina Coichev, Pedro V. Oliveira, and Silvia H.P. Serrano.

The scientific committee was composed by professors from different Brazilian universities with the objective to represent the diversity of areas and regions: Auro A. Tanaka (UFMA), Celio Pasquini (UNICAMP), Clésia C. Nascentes (UFMG), Éder T.G. Cavalheiro (IQSC-USP), Emanuel Carrilho (IQSC-USP), Érico M.M. Flores (UFSM), Francisco J. Krug (CENA-USP), Iolanda C. Vieira (UFSC), Jailson B. de Andrade (UFBA), Joaquim A. Nóbrega (UFSCar), José de Anchieta Gomes Neto (UNESP-Araraquara), Lauro T. Kubota (UNICAMP), Márcia A.M.S. Veiga (FFCLRP-USP), Marco T. Grassi (UFPR), Maria das Graças A. Korn (UFBA), Maria do Carmo H. da Silva (UFV), Maria Fernanda Pimentel (UFPE), Maria Valnice B. Zanoni (UNESP-Araraquara), Mário C.U. Araújo (UFPB), Orlando Fatibello Filho (UFSCar), Ricardo E. Santelli (UFRJ), Sérgio L.C. Ferreira (UFBA), and Solange Cadore (UNICAMP). The scientific committee actively participated with suggestions for the scientific program aiming at homogeneous representation of the several sub-areas of Analytical Chemistry and also in the evaluation of the abstracts submitted for presentation.

The secretariat and graduate students from IQ-USP and Federal University of ABC were fundamental to help the organizing committee before, during and after the conference: Fatima Mazzine and Fernanda Dib (secretaries), Alexandre M. Fioroto, Daniel M. Silvestre, Gislayne Kelmer, Maciel S. Luz, Rodrigo Chelegão, Samara Garcia, Sidnei G. Silva and Vivian M. O. Carioni.

3. Participants

The event gathered 1,141 conferees, including 167 professors from different universities of Brazil and abroad, 17 high school teachers, 62 researchers, 79 professionals from various industries, 434 graduate and 292 undergraduate students and representatives from different companies. They came from various states of Brazil (Table I) and abroad: Portugal (3), Argentina (3), Chile (3), Spain (2), Russia (1), Switzerland (1), USA (1) and Uruguay (1).

Table I. Origin of the conferees of the 16th ENQA

State	Conferees	State	Conferees
Alagoas	14	Paraíba	34
Amazonas	2	Pernambuco	40
Bahia	115	Piauí	10
Ceará	30	Paraná	34
Distrito Federal	21	Rio de Janeiro	116
Espírito Santo	30	Rio Grande do Norte	4
Goiás	21	Rio Grande do Sul	100
Maranhão	12	Santa Catarina	17
Minas Gerais	136	Sergipe	20
Mato Grosso do Sul	7	São Paulo	298
Pará	11		

4. Pre-meeting activities

Pre-meeting activities involved five short-courses (453 participants) and one workshop with 197 participants. The themes of the short-courses were *"Chromatography", "Applications of isotope dilution in analytical processes", "Introduction to thermal analysis", "Multivariate Calibration"* and *"Fundaments and Applications of Mass Spectrometry"*, presented respectively by the Professors Renato Zanella (UFSM), Maria Fernanda Georgina Gine Rosias (CENA-USP), Éder Tadeu Gomes Cavalheiro (USP), Jez Willian Batista Braga (UnB) and Fábio Cezar Gozzo (UNICAMP).

The workshop discussed "*Graduate programs in Analytical Chemistry in Brazil: challenges and tendencies*", which was coordinated by Professor Solange Cadore (State University of Campinas).

The opening ceremony left its marks due to the excellent Plenary Lecture "*Laser Light or Antibody - Two Friends to Analysts"* presented by Prof. Dr. Reinhardt Niessner from Technical University of Munich (Munich, Germany) and the awards dedicated to the memory of Prof. Paschoal Américo Senise. These prizes were awarded to Prof. Orlando Fatibello Filho (UFSCar), lauded by Prof. Ivano G. R. Gutz (IQ-USP); Prof. Francisco Radler de Aquino Neto (UFRJ), lauded by Prof. Ricardo E. Santelli (UFRJ); Prof. Jailson Bittencourt de Andrade (UFBA), lauded by Prof. Sérgio L. C. Ferreira (UFBA); Prof. Francisco José Krug (CENA-USP), lauded by Prof. Joaquim A. Nóbrega (UFSCar); and Prof. Boris L'Vov (San Petersburg University) lauded by Prof. Solange Cadore.

5. Plenary sessions, short conferences, symposia, round tables and posters

The scientific program presented 5 plenary lectures, 16 short-conferences, 64 oral presentations, 3 symposia and 921 poster presentations.

The plenary lectures and short conferences were selected to show the Analytical Chemistry state-of-art in Brazil, from the inspiring point of view of the meeting's theme "*Analytical Chemistry without Frontiers*". The following exciting plenary lectures were offered for the participants:

Plenary session 1 –"*Analytical Chemistry in Brazil: Evolution and Overview*", by Prof. Joaquim A. Nóbrega from the Federal University of São Carlos (São Paulo, Brazil). He showed that Brazilian scientific production has grown significantly and the same trend is observed in Chemistry and Analytical Chemistry. As a consequence, the vital need for training of human resources is agreed.

Plenary session 2 – *"Active Learning Materials for Analytical Chemistry Education"*, by Prof. Dr. Cynhia K. Larive from the University of California (California, USA). She showed that the availability of digital resources is having a tremendous impact on teaching and learning, as well as on the practice of Analytical Chemistry. Creative uses of electronic resources can provide a true enhancement to

the educational process. After her conference, the Brazilian users of the site www.asdlib.org increased significantly (from 45 to 380 users in the same period).

Plenary session 3 – "*Electro-assisted Generation of Highly Ordered Mesoporous Silica Thin Films with Oriented Pore Channels: Interest in Electroanalysis*", by Prof. Dr. Alan Walcarius from the University of Nantes (Nantes, France). This lecture highlighted how the template technology offers the benefits in terms of designing new types of porous electrodes, mostly based on thin films, functionalized or not, and discussed their interest to electroanalytical chemistry.

Plenary session 4 – "*Forensic Chemistry and Analytical Chemistry: Are there Borders?*", by Dr. Adriano Otávio Maldaner from the Brazilian Federal Police (Brasilia, Brazil). He showed several interesting examples of how Analytical Chemistry is an important tool to solve problems related to forensic science.

Plenary session 5 – "*Speciation of Hg and High Exposure Associated with Consumption of Contaminated Fish*", presented by Prof. Dr. Olaf Malm from the Federal University of Rio de Janeiro (Rio de Janeiro, Brazil). In his lecture, he showed the importance of investigating the chemical species to evaluate the real impact of environmental contamination.

The 16 short conferences were presented by invited speakers over four days. Most of the speakers were researchers or professors of Analytical Chemistry. Each day, 4 lectures on different themes were presented simultaneously. After the short conference a sequence of oral presentations took place. Sixty four works were chosen for oral presentation, most of them presented by undergraduate or graduate students. Figure 1 shows the word clouds obtained from the titles of the oral and poster presentations.

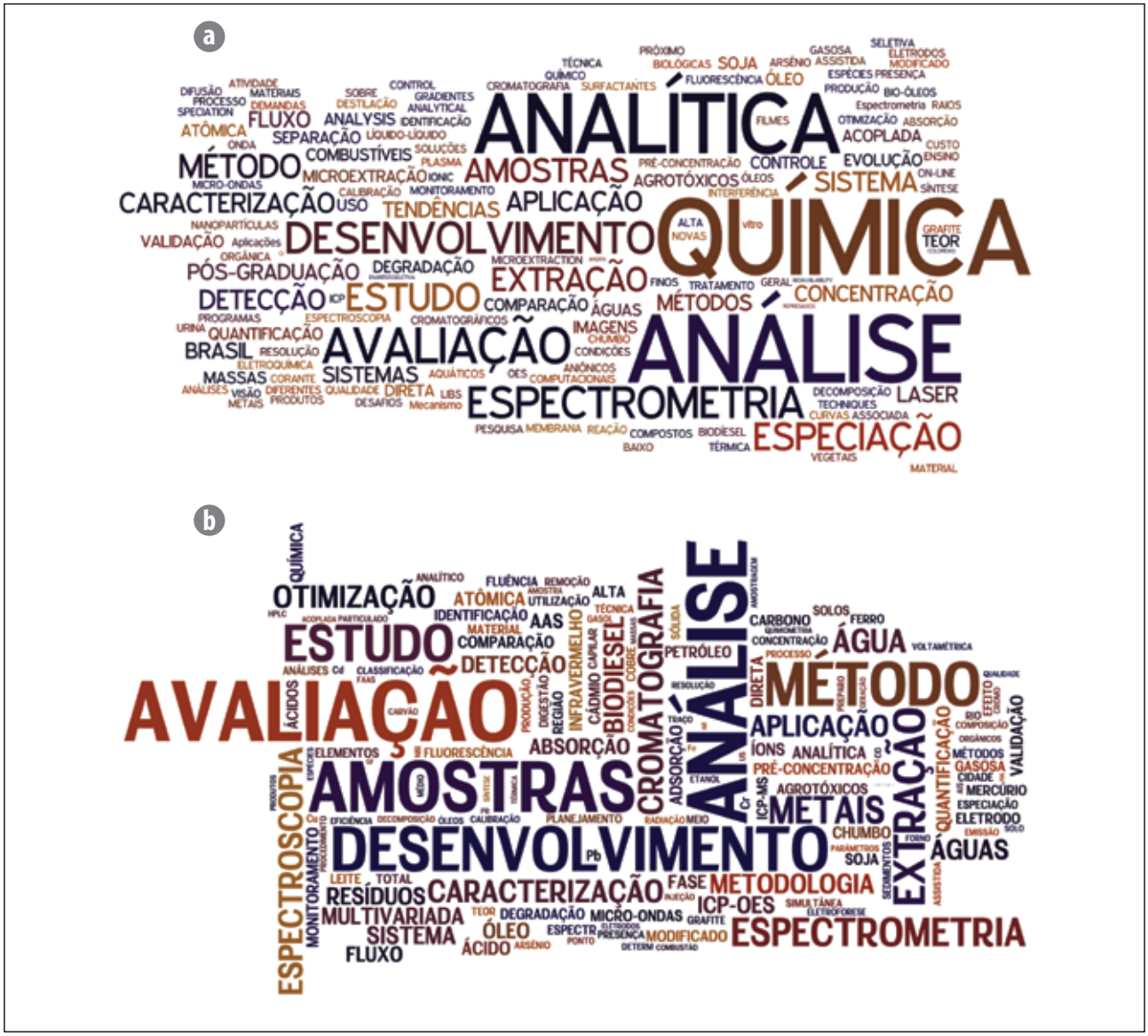

Figure 1. Word clouds referring to the presentations at the 16th ENQA. Word size is proportional to its frequency in the titles of (a) oral and (b) poster presentations.

The 921 abstracts for the poster sections were divided into 3 days, according to the chosen area: agrochemical and agronomic materials, bioanalytical chemistry, chemical speciation and metalomics, chemometrics, drugs and pharmaceutical samples, education in Analytical Chemistry, electrochemistry, environmental samples, flow analysis, food and biological materials, fuels, instrumentation, sample preparation, spectroanalysis, nanomaterials, process and metrology, thermal analysis, and others. All posters were evaluated by a 47 member committee and the top ten were awarded prizes. The first place was awarded with a grant to attend the PittCon Conference & Expo 2012 (Orlando, Florida, USA) and the others with books.

Three symposia were part of the 16th ENQA: "*Analytical Chemistry and (Bio)Fuel*", coordinated by Prof. Jailson B. Andrade (UFBA); "*Direct Solid Analysis – Trends and Demands*", coordinated by Prof. Francisco José Krug (CENA-USP) and "*Chemical Speciation*", coordinated by Prof. Érico M. M. Flores (UFSM).

The last activity in the 16th ENQA was the round table "*Analytical Chemistry: Challenges and Tendencies*" with 5 speakers, each one presenting a short viewpoint to initiate the discussion: "*Analytical Chemistry Evolution*" by Prof. Celio Pasquini (UNICAMP); "*Microfluids and Miniaturized Systems of Analysis: New Tendencies and Bioanalytical Applications*" by Prof. Emanuel Carrilho (IQSC-USP); "*Chromatography and Mass Spectrometry in Organic Analytical Chemistry and its Impact in the Chemistry of the 3rd Millennium*" by Prof. Francisco R. A. Neto (UFRJ); "*Thinking about Analytical Chemistry in Undergraduate Teaching*" by Prof. Mauro Bertotti (IQ-USP); and "*Evaluation of the Scientific Production of Analytical Chemistry in Brazil*" by Prof. Sérgio L. C. Ferreira.

Conclusions

The 16th ENQA had as main objective the discussion, dissemination and exchange of knowledge among professionals, researchers and graduate and undergraduate students, working in Analytical Chemistry and related areas, with emphasis on scientific and technological advances. The topics discussed involved advances in instrumentation and automation, processes in Analytical Chemistry, metrology, chemometrics, flow analysis, thermal analysis, electroanalytical, spectroanalysis, mass spectrometry, separation techniques, sample preparation, chemical speciation, education in Analytical Chemistry, and applications in different areas (food and biological materials, agronomic, environmental, bioanalytical, fuels and refined products, drugs and medicine, materials and nanomaterials). We believe these aims were satisfied taking into account the opinions from conferees from several places, including those presented publicly at the closing ceremony.

Additionally, during the scientific and social activities of the 16th ENQA it was possible to establish or strengthen professional and personal relationships with participants, opening new possibilities for scientific collaboration.

Acknowledgments

We are grateful to the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ) , and The National Institutes of Advanced Analytical Science and Technology and Science and Technology for Bioanalytics for financial support. The Organizing Committee also wish to acknowledge the financial support of the commercial institutions (exhibitors and sponsors): Agilent Technologies Brasil, Anacom Científica, Charis Technologies, DKK Comunicação, Dionex Brasil Instrumentos Científicos Ltda., Importadora e Exportadora de Medidores Polimate Ltda., Marte Científica, Metrohm Pensalab Instrumentação Analítica Ltda., Nova Analítica Importação e Exportação Ltda., NürnbergMesse Brasil, Perkin Elmer do Brasil, Sigma-Aldrich, Shimadzu do Brasil Com. Ltda., Superlab, SBS – Special Bood Services, and Thermo Scientific. We also would like to thank all conferees of the 16th ENQA.

Reference

1. Ferreira, S.L.C.; de Jesus, R.M.; Cavalcante, D.D.; Leão, D.; Portugal, L.A.; dos Santos, W.N.L.; de Araújo, N.M.L.; dos Santos, A.M.O.; Santos, N.C.F.; *Braz. J. Anal. Chem*. 2010, 1, 75-83.

Optimization of the thermal immobilization of poly (methyloctadecylsiloxane) and poly (dimethylsiloxane) onto silica stationary phases using central composite design and response surface methodologies

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Abstract
The thermal immobilization of poly(methyloctadecylsiloxane) and poly(dimethylsiloxane) onto silica was optimized by experimental design and response surface methodology and produced stationary phases that showed good values of efficiency (up to 60 000 plates/m) and appropriate asymmetry factors. The procedure was optimized as functions of efficiency and asymmetry factor of the neutral compound naphthalene by the realization of 11 experiments including a triplicate central point. The model was statistically evaluated using analysis of variance.

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Keywords: RP-HPLC, Stationary phases, Polysiloxanes, Chromatographic silica, Thermal immobilization, Response surface methodology.

Introduction
Since the beginnings of high performance liquid chromatography (HPLC), about 40 years ago, the interactions of an analyte with the reversed-phase (RP) sites of a packing are not the only factor responsible for retention. In addition, the silanols still available on the silica support may interact with analytes containing basic functional groups and can create excessive retention or tailing [1,2]. However, the pharmaceutical and bioanalytical fields require the use of stationary phases (SP) that give symmetrical and efficient peaks for quantification [3] and this sometimes indicates the use of mobile phases in pH range 2 < pH > 8, where silica-based stability is limited. In acid solutions hydrolysis of the Si-C bond, responsible for attachment of the silane agent to the silica surface, occurs, resulting in stationary phase degradation. Above pH 8 silica dissolution is accelerated, causing the destruction of the silica backbone of the stationary phase [4,5].

Polysiloxanes immobilized onto the surface of porous silica particles have proven to be good stationary phases for separation of diverse samples and for concentration and clean-up using solid phase extraction [6,7], having several advantages that make them very attractive for use in RP-HPLC, such as better protection of residual silinols (silica) or Lewis acidic sites (zirconia, titania), minimizing ion exchange interactions, especially with basic solutes like pharmaceuticals, and giving more effective protection of the support matrix against the chemical attack of alkaline mobile phases, beyond the ease of preparation [8].

Experimental design and optimization are tools that are used to systematically examine different types of problems that arise within, e.g., research, development and production [9]. The conventional approach for the optimization of a multivariable system is usually one-variable-at-a-time. This can be very time consuming and, where interactions exist between the variables, it is unlikely to find the true optimum. Response surface methodology (RSM) is a very useful tool for this purpose as it provides statistical models, which help in understanding the interactions among the parameters that should be optimized [10,11].

In this work, the process of thermal immobilization of a sequence using poly (methyloctadecylsiloxane) (PMODS) and poly(dimethylsiloxane) (PDMS) onto silica to prepare a stationary phase was optimized using central composite design and RSM [12,13], evaluated in terms of chromatographic performance (efficiency and asymmetry factor). These results were compared with a SP prepared by immobilization of PMODS onto silica.

Methodology
Materials and instrumentation
Methanol (99.9 %), chloroform (99.8 %) and *n*-hexane

(95%) were from Tedia (Fairfield, USA). Toluene (99.9 %) was from J.T Baker (Phillipsburg, USA). The mobile phases were prepared with ultrapure water from a Millipore Direct- Q™ system (Billerica, USA).

Kromasil silica, from Akzo Nobel (Bohus, Sweden), with a mean particle size of 5 µm, mean-pore diameter of 11.4 nm and a specific surface area of 310 m^2/g was used as support for preparation of the SP. PMODS polymer, average molar mass (Mw) 11 000, and PDMS polymer, average molar mass (Mw) 14 000, were obtained from United Chemical Technologies (Bristol, USA).

The test solutes were naphthalene (> 98.5 %) from Vetec (Duque de Caxias, Brazil), benzene (99 %) from Synth (Diadema, Brazil), and benzonitrile (> 98.0 %), N,N-dimethylaniline (> 98 %) and phenol (> 99.5 %) from Fluka (Buchs, Switzerland). Uracil (98 %) and acenaphthene (99%) were from Aldrich (Milwakee, USA).

A Haskel (Burbank, USA) packing pump was used for column packing.

A Shimadzu modular HPLC system (Kyoto, Japan) equipped with a LC-10AD liquid chromatographic pump, a SPD-10A UV-VIS (254 nm) detector with 8 µL cell, a CTO- 10A column oven and a Rheodyne 8125 injector (Cotati, USA) with 5 µL loop was used for chromatographic analysis. All data were acquired and processed using Chrom Perfect for Windows, version 5.5.6 and Report-Write Plus software from Justice Innovations (Mountain View, USA).

STATISTICA 6.0 from STatSoft (Tulsa, USA) was used for treating the experimental design and statistics data.

Preparation of the stationary phases

Stationary phases were prepared with a PMODS loading of 0.5 g of PMODS/g silica. A 10% w/v solution of PMODS in *n*-hexane was added to the appropriate quantity of silica, previously dried at 150 °C for 24 h. This mixture was slowly stirred at room temperature for 3 h, and then placed in a fume hood for evaporation of the solvent at room temperature (for 4 days), giving Si(PMODS). After, 0.5 g of PDMS/g Si(PMODS) was prepared with the same procedure. This was then placed in a fume hood for evaporation of the solvent at room temperature (at least 10 days). Si(PMODS) SP was prepared using the loading of 0.8 g PMODS/gSi by the same procedure.

Optimization of thermal immobilization

To optimize the immobilization of the polymers onto silica stationary phases, a central composite design (CCD) with RSM was used to evaluate efficiencies and asymmetry factors, as functions of the variation of time and temperature. Portions of 2.5 g of SP obtained after solvent evaporation were placed individually in an oven at the specified conditions as summarized in Table I. The immobilizations were performed under a nitrogen atmosphere, in duplicate.

Table I. Conditions for thermal immobilization (for the variables time and temperature) evaluated by central composite design with the respective chromatographic parameters for each procedure.

EXPERIMENT	VARIABLES		CHROMATOGRAPHIC PARAMETERS	
	TIME (h)	TEMPERATURE (°C)	N/m[a]	As[b]
1	8	100	44700	0.6
2	8	120	41500	1.0
3	16	100	49400	1.0
4	16	120	46100	0.7
5	12	110	55000	0.8
6	12	110	49000	1.3
7	12	110	44000	1.4
8	12	96	52100	0.6
9	12	124	49600	1.0
10	6.4	110	50100	0.9
11	17.6	110	52600	0.8

(a) Calculated for the naphthalene peak. Efficiencies were calculated using: $N = 5.54 (t_R/w_h)^2$, where w_h is the peak width at 50% of the peak height.
(b) Calculated for the naphthalene peak. Asymmetry factors (As) were calculated using the relation $As = rw_{10\%}/lw_{10\%}$

Solvent extraction of excess of polymer

The excess of PMODS and PDMS was extracted after each thermal immobilization. The tubes containing the SP were connected to a Waters 510 pump (Milford, USA) for extraction of non-immobilized polymer by passing hexane at 0.6 mL/min for 3 h at 50 °C. The SP was dried with nitrogen for 3 hours.

Column packing

Columns (60 mm x 3.1 mm id) were made from type 316 stainless steel tubing. The internal surface was polished using a technique developed in our laboratory. The columns were slurry packed using 5 % (w/v) slurries of the SP in chloroform. A constant packing pressure of 5000 psi (34.5 MPa) was used with a Haskel packing pump. Methanol was used as propulsion solvent. The pressure was maintained until the passage of 80 mL methanol to assure good packing and removal of excess polymer. Columns were conditioned for 3 h with 60:40 (v/v) methanol-water mobile phase at 0.5 mL/min prior to the chromatographic tests. The excess SP after packing was used to determine the % carbon on a Model CHN–2400 Perkin–Elmer Elemental Analysis System (Shelton, USA).

Results and discussion

Chromatographic parameters for thermal immobilization of the SP immobilized under different conditions are summarized in Table I. Better chromatographic performances were obtained for the SP produced in experiments 5, 8 and 11, where columns with high efficiencies (higher than 52 000 plates per meter) and symmetrical peaks were obtained for naphthalene. The repeatability of the procedure of immobi-

lization was evaluated from the triplicate experiments performed at the center point (experiments 5, 6 and 7). The estimated RSD was 11.2 % for column efficiency. Figure 1 shows the response surface diagrams of efficiency and asymmetry factor for the naphthalene peak, used to determine the optimal region of both time and temperature to be used for the subsequent preparation of optimized SP. In order to represent the surface responses, quadratic models for both the efficiency and asymmetry factor were used, as given by:

$$\textbf{Efficiency (N m}^{-1}\textbf{ X 1000)} = 49.4 + 1.61t - 1.26T - 0.47t^2 - 0.72T^2 - 0.025tT$$

Equation I

$$\textbf{Asymmetry (As)} = 1.17 - 0.01t + 0.08T - 0.16t^2 - 0.18T^2 - 0.17tT$$

Equation II

where t is time (h) and T is temperature (° C).

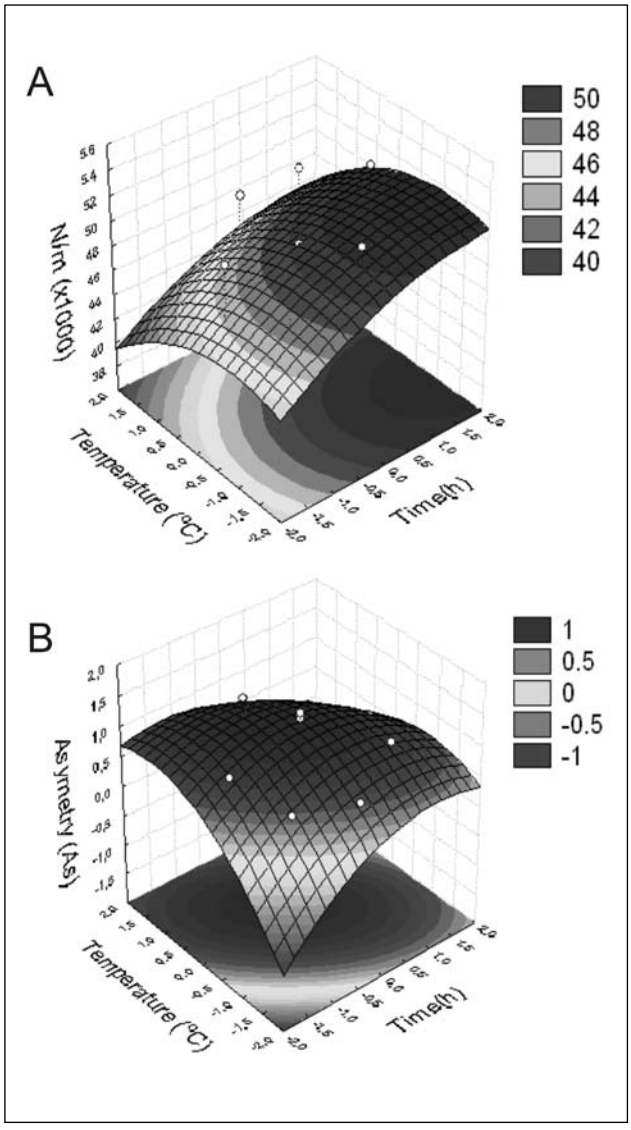

Figure 1. Response surfaces for thermal immobilization of the Si(PMODS/PDMS) stationary phase: (A) efficiency and (B) asymmetry factor.

Column efficiencies higher than 50 000 N/m are represented in darkest region of the Figure 1A. Higher column efficiencies were obtained when the immobilization step was carried out at low to medium temperatures with medium to high immobilization times. In Figure 1B, the same dark region revealed good values of symmetry. The optimal region was determined by applying the derivatives (dy/dx_1 and dy/dx_2) to the equations. The values obtained were t = 1.76 and T = –0.91. Substituting these values into equation I, the value calculated corresponding to an efficiency of 52 600 N/m refers to a time of 8 hours and 36 minutes and to a temperature of 127.6 °C. Immobilizations using these conditions gave efficiency (n = 2) of 60 000 N/m and asymmetry factor of 1.0, better values than predicted by the model. Figure 2A shows the chromatogram for a separation of benzonitrile, benzene, toluene and naphthalene. Uracil, an unretained compound, was used to determine the mobile phase elution time. The separation of a second mixture, having phenol, a acidic compound, and N,N-dimethylaniline, a basic compound, is shown in Figure 2B. The value of asymmetry N,N-dimethylaniline,1.6, is quite acceptable.

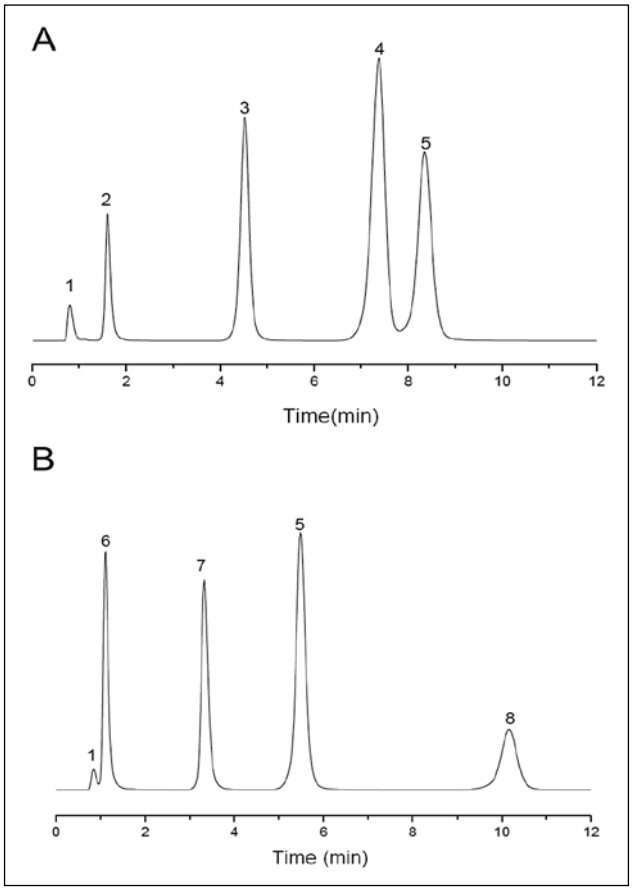

Figure 2. Chromatogram showing the separation of several test compounds on the Si(PMODS/PDMS) SP prepared with the best immobilization conditions (127.5 °C and 8.36 h). Mobile phase: methanol:water (60:40 v/v) at 0.3 mL/min; Injection volume: 5 µL; UV detection at 254 nm. (1) Uracil, (2) Benzonitrile, (3) Benzene, (4) Toluene, (5) Naphthalene, (6) Phenol, (7) N,N-Dimethylaniline and (8) Acenaphthene.

The validity of the model for efficiency was verified by the analysis of variance (ANOVA). The values are presented in Table II. The ANOVA results for efficiency showed that the quadratic regression for efficiency is statistically significant, since the ratio between of mean square of regression and mean square of residue (MSreg/MSres = 17.56) was higher than the tabulated F ($F_{5.5,95\%}$ = 5.05)[14]. The quadratic model did not show lack of fit since the ratio between of mean square of lack of fit and mean square of pure error (MSlof/Mpe = 0.76) was smaller than the tabulated F ($F_{3.2,95\%}$ = 19.16)[14]. This implies that the quadratic model adjusted to efficiency data for the thermal immobilization of PMODS and PMDS onto silica was satisfactory to explain the variation of the results. As related in Table II, approximately 94 % of variations can be explained by Equation I. Figure 3A shows a plot of predicted values versus observed values and Figure 3B shows plot of residuals for the model optimized for efficiency. The general impression is that the residuals scatter randomly on the display, suggesting that the variance of the original observations is constant for all values of efficiency [15].

Table II. Analysis of variance (ANOVA) table for the quadratic model ajusted to the efficiencies of naphthalene.

VARIATION	SUM OF SQUARE (SS)	DEGREES-OF-FREEDOM (df)	MEAN SQUARE (MS)
REGRESSION	157.28	5	31.45
RESIDUE	8.99	5	1.79
LACK OF FIT	69.66	3	23.22
PURE ERROR	60.67	2	30.33
TOTAL	166.26	10	
VARIANCE EXPLAINED (%): 94.6			

A similar analysis of ANOVA data for asymmetry (Table III) showed that the quadratic regression for asymmetry is not statistically significant, since the ratio between of mean square of regression and mean square of residue (MSreg/MSres = 2.5) was smaller than the tabulated F ($F_{5.5,95\%}$ = 5.05) [14]. On the other hand, the quadratic model did not show lack of fit since the ratio between of mean square of lack of fit and mean square of pure error (MSlof/Mpe = 0.1) was smaller than the tabulated F ($F_{3.2,95\%}$ = 19.16)[14]. This implies that the quadratic model adjusted to asymmetry data for the thermal immobilization of PMODS and PMDS onto silica was not satisfactory to explain the variation of the results. As related in Table III, approximately 73 % of variations can be explained by equation II.

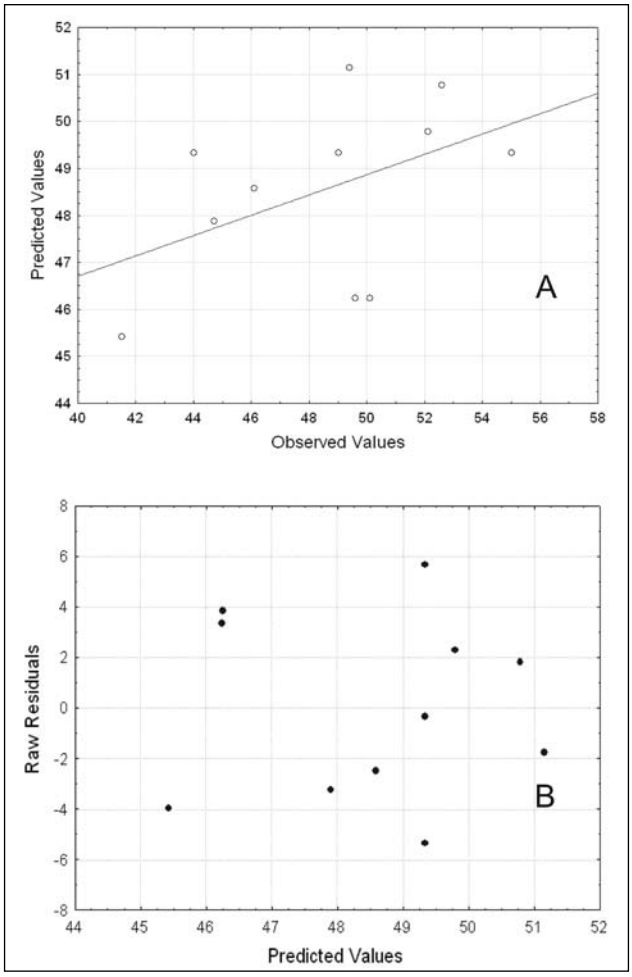

Figure 3. Predicted versus observed values (A) and residuals plot (B) for the efficiency model.

Table III. Analysis of variance (ANOVA) table for the quadratic model ajusted to the asymmetry of naphthalene.

VARIATION	SUM OF SQUARE (SS)	DEGREES-OF-FREEDOM (df)	MEAN SQUARE (MS)
REGRESSION	0.50	5	0.1
RESIDUE	0.18	5	0.04
LACK OF FIT	0.03	3	0.01
PURE ERROR	0.21	2	0.10
TOTAL	0.68	10	
VARIANCE EXPLAINED (%): 73.5			

The same optimized immobilization conditions were used to prepare a PMODS-SiO_2. Figure 4A shows the chromatogram for a separation of benzonitrile, benzene, toluene and naphthalene and Figure 4B shows the chromatogram for a separation for neutral, basic and acidic compounds on the Si (PMODS) stationary phase. Table IV compares the chromatographic parameters of the optimized Si(PMODS/PDMS) phase with the Si(PMODS) phase using the same immobilization conditions. The use of the sequence PMODS and PDMS improved chromatographic performance, as

seen by the higher values of efficiency and asymmetry for both the neutral compound (naphthalene) and the basic compound (N,N-dimethylaniline), suggesting that the coverage of the silica support was better using the procedure with the sequential immobilization. Mobile phase composition was chosen based on % carbon (Table IV) and flow-rates were previously optimized by van Deemter curves for each column.

Table IV. Percent carbon and chromatographic parameters for the SP prepared by the sequence PMODS/PDMS and the SP using only PMODS, both immobilized onto silica supports.

CHROMATOGRAPHIC PARAMETER	Si(PMODS/PDMS)[a]	Si(PMODS)[b]
% CARBON	15.0	22.5
EFICIENCY (N/m) (NAPHTHALENE)	61 300	52 800
EFICIENCY (N/m) (N-N-DIMETHYANILINE)	37 500	40 000
ASYMMETRY (NAPHTHALENE)	0.9	1.3
ASYMMETRY (N-N-DIMETHYANILINE)	1.5	1.2

(a) Mobile phase: methanol:water (60:40, v/v) at 0.3 mL/min; injection volume: 5 µL; UV detection at 254 nm.
(b) Mobile phase: methanol:water (70:30, v/v) at 0.3 mL/min; injection volume: 5 µL; UV detection at 254 nm.

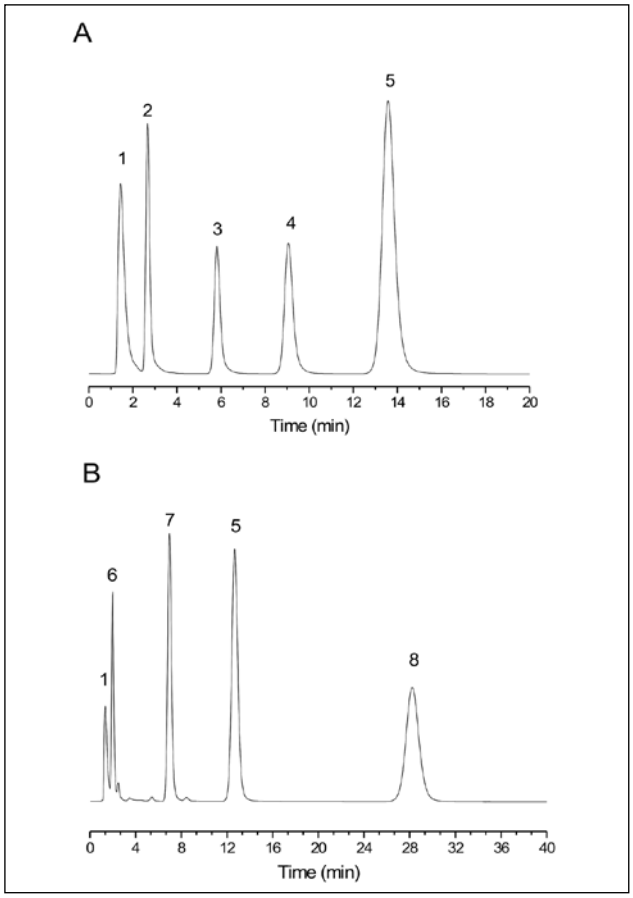

Figure 4. Chromatogram showing the separation of several test compounds on SP prepared with the best immobilization conditions (127.5 °C and 8.36 h) for the Si(PMODS) SP. Mobile phase: methanol:water (70:30 v/v) at 0.3 mL/min; Injection volume: 5 µL; UV detection at 254 nm. (1) Uracil, (2) Acetophenone, (3) Benzene, (4) Toluene, (5) Naphthalene, (6) Phenol, (7) N,N-Dimethylaniline and (8) Acenaphthene.

Conclusion

The process of thermal immobilization of PMODS and PDMS onto silica to prepare a SP was optimized by central composite design and surface response methodology. The results obtained for efficiency and asymmetry of naphthalene, performing only 11 experiments with triplicate central point, were used to determine the best immobilization conditions, based on time and temperature. The results of separation of a mixture of aromatic hydrocarbons presented good efficiencies and symmetrical peaks (for naphthalene) and sequential immobilization of PMODS and after PDMS appeared to give better coverage of the silica support.

Acknowledgments

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References

1. Neue, U.D.; Tran, K.V.; Méndez, A.; Carr, P.W. *J. Chromatogr. A*. 2005, 1063, 35.
2. Kirkland, J.J. *J. Chromatogr. A*. 2004, 1060, 9.
3. Stella, C.; Rudaz, S.; Gauvrit, J. Y.; Lantéri, P.; Huteau, A.; Tchapla, A.; Veuthey, J.L. *J. Pharm. Biomed. Anal.* 2007, 43,89.
4. Claessens, H.A.; van Straten, M.A. *J. Chromatogr. A*. 2004, 1060, 23.
5. Faria, A.M.; Collins, K.E.; Collins, C.H. *J. Chromatogr. A*. 2006, 1122, 114.
6. Jardim, I.C.S.F.; Collins, K.E.; Collins, C.H. *Microchem. J.* 2004, 77, 191.
7. Borges, E.M.; Silva, C.G.A.; Collins, C.H. *Microchem. J.* 2010, 96, 120.
8. Faria, A.M.; Jardim, I.C.S.F.; Collins, C.H. *J. Braz. Chem. Soc.* 2009, 20, 1385.
9. Lundstedt, T.; Seifert, E.; Abramo, L.; Thelin, B.; Nyström, A.; Pettersen, J.; Bergman, R. *Chemom. Intel. Lab. Syst*. 1998, 42, 3.
10. Melo, A.; Aguiar, A.; Mansilha, C.; Pinho, O.; Ferreira, I.M.P.V.O. *Food Chem*. 2012, 130, 1090.
11. Leardi, R. *Anal. Chim. Acta* 2009, 652, 161.
12. Box, G.E.P.; Hunther, W.E.; Hunther, J.S.; *Statistics for Experiments: an Introduction to Design, Data Analysis and Model Building,* Jonh Wiley & Sons: New Jersey, 1978.
13. Manson, R.L.; Gunst, R.F.; Hess, J.L.; *Statistical Design and Analysis of Experiments with Applications to Engineering and Science,* Jonh Wiley & Sons: New Jersey, 2003.
14. Bruns, R.E.; Neto, B.B.; Scarminio, I.S.; *Como Fazer Experimentos*, 2nd ed.; Editora da UNICAMP: Campinas, 2003.
15. Myers, R.H.; Montgomery, D.C.; *Response Surface Methodology, Process and Product Optimization Using Designed Experiments,* 2nd ed.; Jonh Wiley & Sons: New Jersey, 2002.

Principal component analysis (PCA) of the fragmentation patterns of anabolic steroids by tandem mass spectrometry with electrospray ionization

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Abstract

Anabolic steroids have been used for a long time as doping substances in order to enhance muscle gain. Their analysis by doping control laboratories has been performed mainly by gas chromatography coupled to mass spectrometry (GC-MS) so far and their behavior in this technique is thus widely known. However, there is a trend that doping control laboratories incorporate liquid chromatography coupled to tandem mass spectrometry in their routine analyses and little is known about fragmentation patterns for these substances when electrospray ionization and collision induced dissociation is applied. This work aims at determining statistically which fragments are determinant to each steroid class and proposing formation pathways for some of these fragments. Solutions of the steroids were prepared in methanol : water (1:1) with 0.1 % formic acid and were directly injected into an electrospray ionization tandem mass spectrometer (QqLIT). The mass spectra generated had their relative abundances calculated and some key fragments were selected to compose a data matrix for principal component analysis. This analysis showed statistical separation of the theoretical groups of steroids, revealing which fragment ions are the most important when considering differences between steroid classes.

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Keywords: Mass spectrometry; electrospray ionization; doping control; LC-MS/MS; Principal component analysis; pattern recognition; anabolic steroids.

1. Introduction

Amongst the substances generally used as doping, one can find anabolic steroids which have been used in sports since the early 1950s and form the most abused class of substances in doping. Since then, their undesirable effects have already been known. Doping is characterized by the use of a substance or a method which is capable of leading an athlete to a performance beyond what could supposedly be achieved by training [1]. Analysis of anabolic steroids, however, was complicated at first because of the lack of techniques that could determine their presence unequivocally in samples from athletes. As these detection capabilities were improved, athletes started to use different substances in order to avoid detection [2]. In the beginning, testosterone esters were employed and later, the development of the so called "designer steroids" was started.

Designer steroids have special structural features which were incorporated on purpose in order to avoid detection by the most used analysis technique, namely gas chromatography coupled to mass spectrometry (GC-MS). These special structural features include, for example, the presence of multiple conjugated double bonds which lead to the formation of various derivatives during the derivatization reaction, making it difficult to detect low concentrations of these substances [3,4,5]. As an alternative to this issue, many laboratories have been using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) to detect designer steroids and there is a trend in doping control that the analyses of some other steroids are also performed using this technique [6,7]. There is poor or no fragmentation of the compounds when analyzed by electrospray ionization, which makes it necessary to use collision induced dissociation (CID) spectra when one aims at identifying an unknown substance.

Steroid analysis has been performed for a long time using GC-MS and thus there is a lot of information on their chromatographic behavior and their fragmentation profile, including statistical analyses [8]. Some authors have already studied the fragmentation profile of steroids using electrospray ionization and tandem mass spectrometric detection [6,7,9]. However, when analyzing an unknown steroid by LC-MS/MS, it is still very difficult to determine its structure because little information is available on the fragmentation behavior.

Principal component analysis is used for visualization of huge amounts of data in a meaningful way. It is a multivariate technique and it is largely used with gas chromatography, inductively coupled plasma emission, infrared and ultraviolet spectroscopies [10].

The objective of this work is to clarify the fragmentation patterns observed in different groups of steroids by using their mass spectra in principal component analysis, thus leading to easier identification of unknown steroids analyzed by LC-MS/MS.

2. Experimental

2.1. Chemicals and reagents

The following chemicals and reagents were used: methanol pesticide grade from Tedia (Fairfield, OH, USA). Formic acid (96%) from Sigma (St. Louis, MO, USA). 17α-Methyltestosterone and boldenone from Steraloids (Newport, RI, USA). All other steroid standards used were purchased from the Australian National Metrology Institute (NMIA).

Stock solutions of the analytes were prepared in methanol at a concentration of 1 mg/mL. These solutions were further diluted to appropriate working solutions at 1.0 µg/mL. All solutions were sealed and kept at −20 °C until use.

2.2. Sample preparation

1.0 mL of a 1.0 µg/mL solution (1.0 µg) of each steroid was added to a clean glass tube. These solutions were evaporated under mild nitrogen flow and then resuspended with 1 mL of a mixture of methanol : water (1:1) with 0,1 % of formic acid.

2.3. LC-MS/MS conditions and analysis

The analyses were performed in an ABSciex 4000 Qtrap system equipped with an electrospray source. Source temperature was set to 500 °C; spray voltage: 4000 V; curtain gas pressure: 15 psi; ion source gas 1: 50 psi; ion source gas 2: 50 psi; collision gas pressure: high. All analyses were performed in the positive ion mode. Declustering potential: 80 V; entrance potential: 10 V. System was set to use a QqLIT configuration (enhanced product ion scan). The collision energy applied was 30 eV. Infusion pump was set to a flow rate of 10 µL/min. Each compound had its mass spectrum acquired for 0.5 min and then the 0 – 0.5 min range was averaged.

2.4. Principal component analyses

The principal component analyses were performed using Statistica 8.0 (Statsoft). Instead of using the entire mass spectra in the construction of the model, the most important ions were chosen, based on their relative abundances and chemical information. The initial data matrix was constructed with 21 samples and 39 variables (ions). The maximum number of factors selected was 6 and the minimum eigenvalue was set to 0. The scores of the 6 principal components were analyzed by combining them one by one in bidimensional graphics. In order to build the data matrix, relative intensities of characteristic fragment ions were calculated for each compound. A null (0) value was attributed in the matrix for all compounds which did not show the presence of a determined ion. Careful observation of the data matrix allowed detection of some ions that were present in almost all steroids studied. Since these ions were present in most of the compounds, they probably do not contribute for separation between classes, but only add noise to the model. These variables were removed and the PCA was repeated, showing better results.

3. Results and discussion

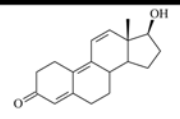
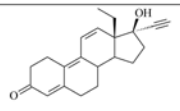
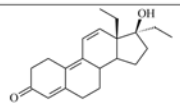
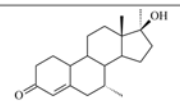
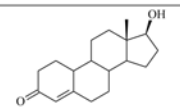
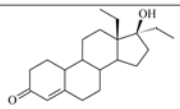
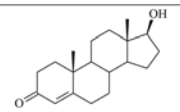
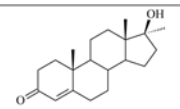
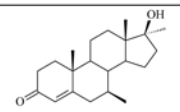
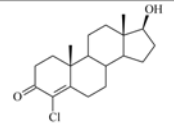
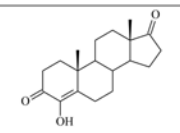
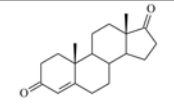
Initially, it was observed that the hydroxy and unconjugated keto steroids did not have satisfactory formation of protonated molecules in positive electrospray analysis. This fact had already been described by Pozo *et al.* who showed that these groups of steroids only have abundant formation of adducts or $[M+H-nH_2O]^+$ [6]. The use of these ions as parents for the formation of CID mass spectra would prejudice our statistical analysis as it would lead to completely different fragmentation pathways and thus these steroids were excluded from our analyses.

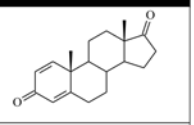
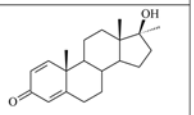
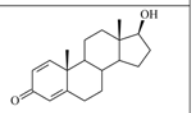
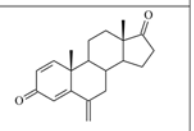
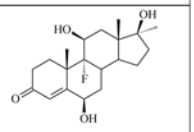
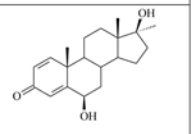
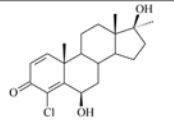
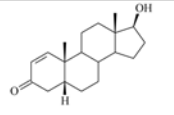
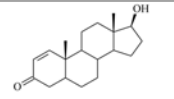
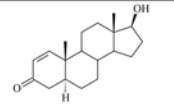
The remaining steroids were grouped in theoretical classes according to their structures. This classification is presented in Table I.

For the selection of the most relevant ions, it was necessary to study the fragmentation pattern of some compounds/groups in order to understand their relevance. In general, the protonated molecules, and the ions of 1, 2 and 4 neutral water losses were considered important markers. Neutral losses of 58, 60, 64, 74, 76 and 100 Da were also included in the matrix as variables.

For the first studied group of compounds, the estra-4,9,11-trien-3-keto steroids, ions *m/z* 241 and 239 were selected as important markers for gestrinone and tetrahydrogestrinone, according to Guan *et al.* [11]. Ion *m/z* 199 was also observed in the three compounds of the group and was thus considered another important marker for this group. Figure 1 shows the proposed fragmentation pathway for the formation of this ion and *m/z* 227.

Table I. Theoretical classification of the steroids according to the structures

Class	Steroid	Structure
Estra-4,9,11-trien-3-keto	17β-Trenbolone	
	Gestrinone	
	Tetrahydrogestrinone	
Androst-4-en-3-keto (19-Nor)	Mibolerone	
	Nandrolone	
	Norbolethone	
Androst-4-en-3-keto	Testosterone	
	17α-methyltestosterone	
	Calusterone	
	Clostebol	
	Formestane	
	4-androstenedione	

Class	Steroid	Structure
Androsta-1,4-dien-3-keto	Boldione	
	Methandienone	
	Boldenone	
	Exemestane	
Androsta-1,4-dien-3-keto-6-hydroxy	6β-hydroxy-fluoxymesterone	
	6β-hydroxy-methandienone	
	6β-hydroxy-turinabol	
Androst-1-en-3-keto	1-androstenedione	
	1-testosterone	
	5α-androst-1-en-3,17-dione (boldenone metabolite)	

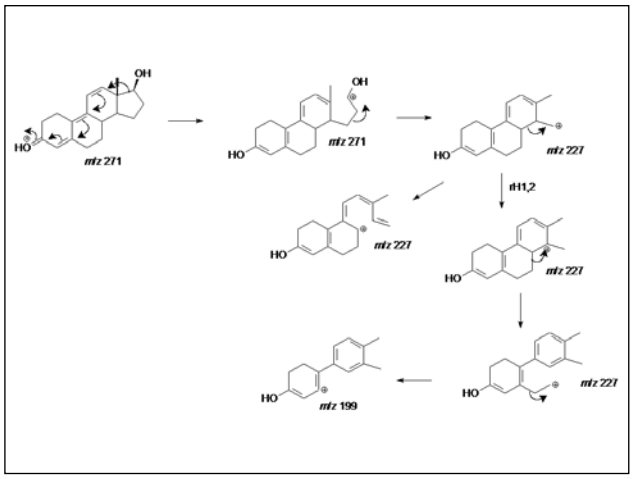


Figure 1. Proposed fragmentation pathway from trenbolone to yield ions *m/z* 199 and 227

The collision induced dissociation mass spectrum of trenbolone is presented in figure 2 as an example of the mass spectra of this class in which we note one neutral water loss and ions *m/z* 199, 227 and 159 used in the data matrix.

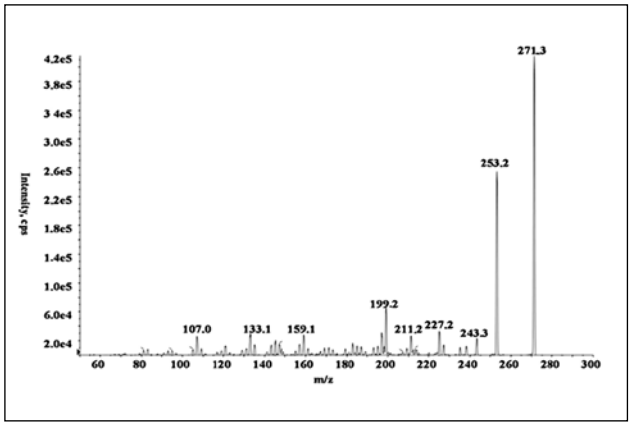


Figure 2. CID mass spectrum of trenbolone

For the androst-4-en-3-ketosteroids (19-Nor), a characteristic fragment ion is observed at *m/z* 97 and was already discussed by Guan *et al.* [11]. Another important ion observed for this group of substances is *m/z* 145 and its proposed fragmentation pathway is shown in figure 3 while the CID mass spectrum of nandrolone is shown in figure 4.

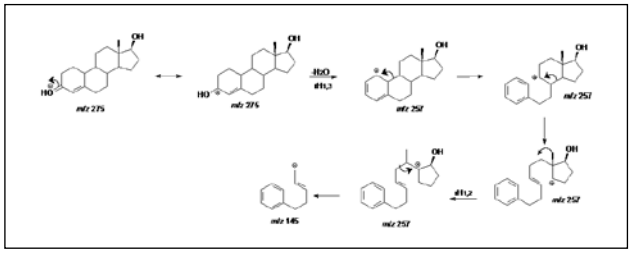


Figure 3. Proposed fragmentation pathway of nandrolone yielding ion *m/z* 145

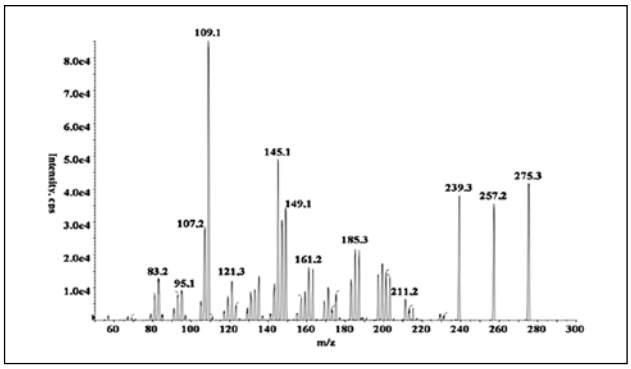


Figure 4. CID mass spectrum of nandrolone

The androst-4-en-3-ketosteroids constituted the class with the greatest number of studied compounds. For this class, the main observed ions are *m/z* 97 and 109. Formation of *m/z* 97 has been proposed by Pozo *et al*. [12] and Williams *et al*. [13] showed the formation of *m/z* 109. Both of them propose unusual pathways for the formation of the ions, based on isotopically labeled experiments. Formestane and clostebol were included in this group in the theoretical classification and since these compounds have substituent groups in C4 which shift the masses of these fragments, the corresponding ions (*m/z* 113 and 125 for formestane and *m/z* 132 and 144 for clostebol) were put in the data matrix together with *m/z* 97 and 109. This procedure can be done because the objective of the work is the analysis of the fragmentation pathways instead of solely the *m/z* ratios. The substitutions that these compounds present do not affect the fragmentation pattern, but elevate the measured masses for the fragments. Hence, the fragments with higher masses were included in the same variable because they correspond to the same pathway. Another important characteristic observed in this group are the two subsequent neutral water losses. Figure 5 shows the CID mass spectrum of testosterone as an example of the class.

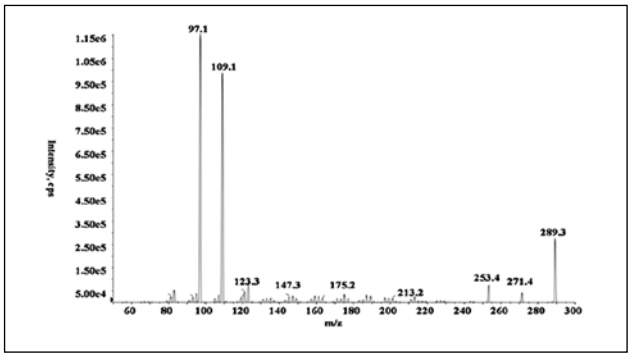


Figure 5. CID mass spectrum of testosterone

The androsta-1,4-dien-3-ketosteroids have boldenone as their most known compound, which is a product of a dehydration reaction of testosterone. The compounds in this class present very characteristic fragment ions at *m/z* 121

and 135 and had their fragmentation proposed by Guan *et al.* [11] and Thevis *et al.* [14] respectively. Figure 6 shows the CID mass spectrum of boldenone.

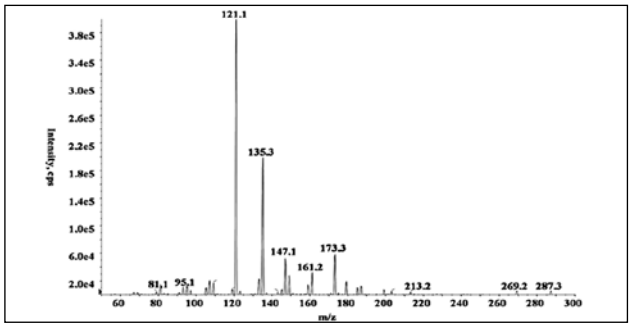


Figure 6. CID mass spectrum of boldenone

Androsta-1,4-dien-3-keto-6-hydroxysteroids were organized in another class, although they could be analyzed as a subclass of the androsta-1,4-dien-3-ketosteroids. The C6 hydroxyl gives this group some interesting fragmentation properties because they exhibit neutral water loss in this position, which leads to a fragmentation pathway that cannot be observed in the androsta-1,4-dien-3-ketosteroids. Figure 7 shows this fragmentation pathway for the formation of ion *m/z* 173. One might note that the androsta-1,4-dien-3-ketosteroids also have *m/z* 173 in their mass spectra, however the relative intensity is much higher when the C6 hydroxyl is present, suggesting a different formation pathway.

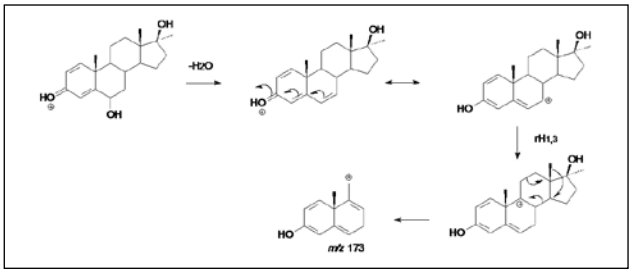


Figure 7. Fragmentation pathway of 1,4-dien-3-keto-6-hydroxysteroids yielding ion *m/z* 173

The CID mass spectrum of 6-hydroxy-methandienone is shown in figure 8.

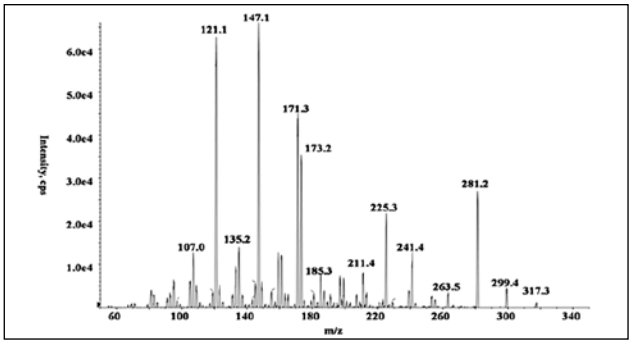


Figure 8. CID mass spectrum of 6-hydroxy-methandienone

Finally, the androst-1-en-3-ketosteroids exhibit two subsequent neutral water losses and a very characteristic fragment ion at *m/z* 69 which was only observed in this group. Ions *m/z* 203 and 185 were also observed. The proposed fragmentation pathways for this group are shown in figure 9 represented by 1-androstenedione.

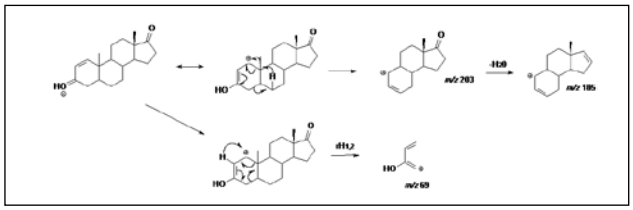


Figure 9. CID fragmentation pathways of androst-1-en-3-ketosteroids represented by 1-androstenedione

The CID mass spectrum of 1-androstenedione is presented in figure 10.

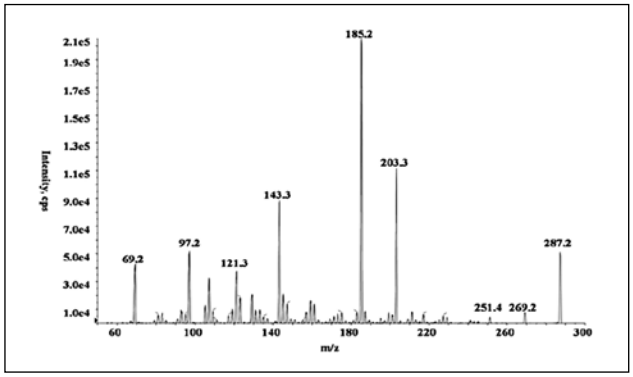


Figure 10. CID mass spectrum of 1-androstenedione

After analyzing the mass spectra and fragmentation patterns, the data matrix was constructed by organizing the selected relevant fragment ions and their relative abundances for each analyzed steroid.

The 15 possible combinations of the 6 used principal components were analyzed and the best class separation was observed when principal components (PC) 2 and 4 were combined. The eigenvalues and explained variance are shown in table II. The factor score plot for this combination is presented in figure 11.

Table II. Explained variance and eigenvalues for each principal component

PC	Eigenvalue	% variance	Cumulative Eigenvalue	Cumulative % variance
1	7.741967	20.92423	7.74197	20.92423
2	6.316532	17.07171	14.05850	37.99594
3	4.938677	13.34778	18.99718	51.34372
4	3.264203	8.82217	22.26138	60.16589
5	2.372416	6.41194	24.63380	66.57783
6	2.222568	6.00694	26.85636	72.58477

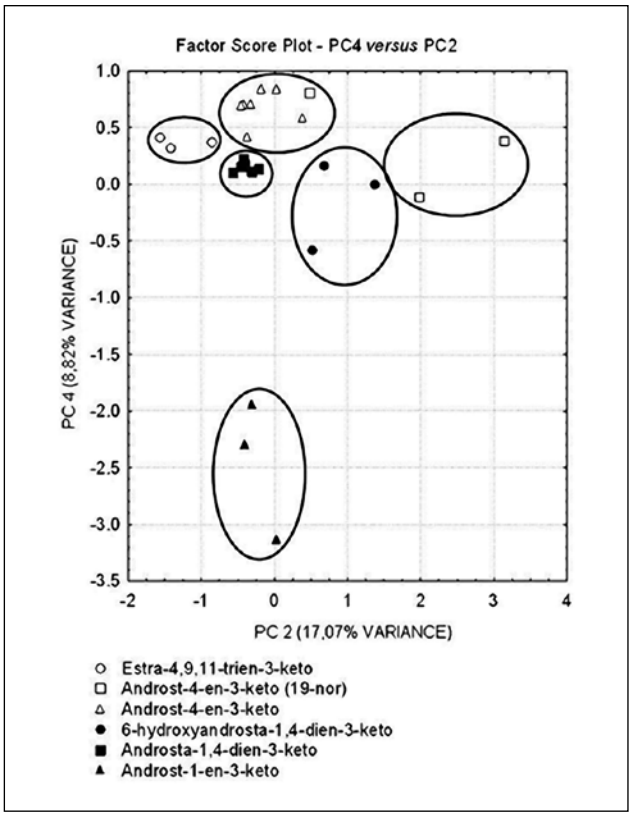


Figure 11. Factor score plot: PC4 against PC2

In this plot, there is satisfactory separation between the 6 groups of steroids. The structural feature that changes the fragmentation behavior the most is the double bond between carbon atoms 1 and 2 and it can be seen that this is the most separated group. One 19-norsteroid (mibolerone) was classified in a different group than initially expected and ended up in the androst-4-en-3-ketosteroids group. This is acceptable as this steroid is 19-nor but also 4-en-3-keto, sharing fragmentation properties with both groups. The androsta-1,4-dien-3-keto group is the least disperse one, meaning that these steroids have the most similar fragmentation behaviors, which can be explained by their mass spectra where ions *m/z* 121 and 135 are the dominant ones.

The plot of factor loadings enables the analysis of the contribution of each variable to the separation of samples. This plot is shown in figure 12.

The greatest contributions for separation in PC4 comes from ions *m/z* 69, 185, 187, 203 and 205. All of these ions are characteristic of the androst-1-en-3-ketosteroids, which explains why this is the most separated group of substances. Ion *m/z* 69 had its fragmentation proposed above and is only present in this class. Ions *m/z* 239, 241 and $[M+H-60]^+$ contributed the most to the separation of the estra-4,9,11-trien-3-ketosteroids. Ion *m/z* 145 also had its fragmentation proposed above and is an important marker for 19-norsteroids. Ions *m/z* 97 and 109 lead the androst-4-en-3-ketosteroids to the positive side of PC4 (upper side of the graph) and are thus confirmed as the best markers for this class as already expected. Ions *m/z* 147 and 173 are the main markers which separate the androsta-1,4-dien-3-ketosteroids from their C6 hydroxylated analogues as the 6-hydroxysteroids have much greater abundances for these two ions.

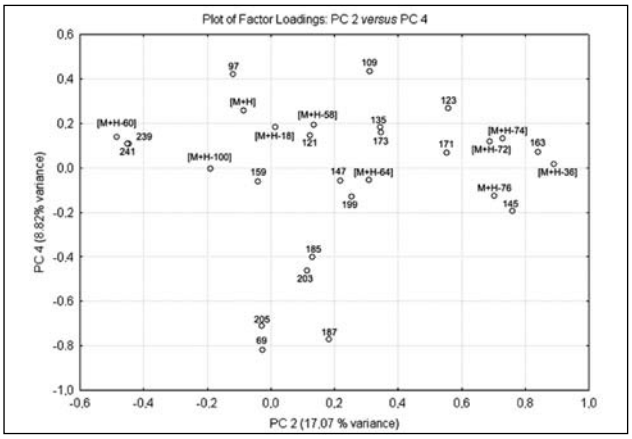


Figure 12. Plot of factor loadings: PC4 against PC2

After this analysis, the data matrix was screened seeking for noisy information. The use of the protonated molecule in the matrix was considered noise addition to the matrix, since most of the steroids have the protonated molecule present and its structural information contributes very little. Another PCA was performed without this variable and its factor scores plot is presented in figure 13.

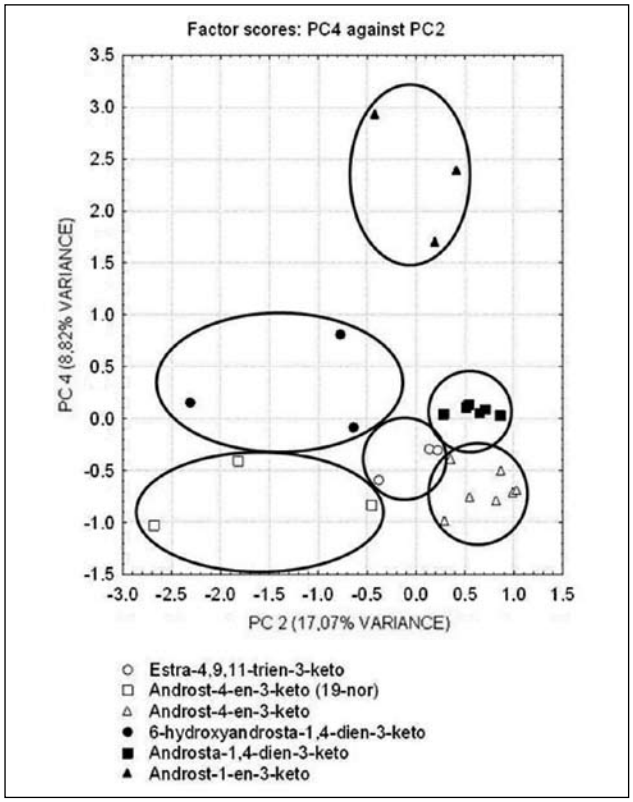


Figure 13. Factor scores plot after removing protonated molecules

Class separation was still achieved when the principal component analysis is performed without the protonated molecules with the additional advantage that mibolerone was separated from the androst-4-en-3-ketosteroids. Ions *m/z* 97 and 109 are now leading to a contribution to the negative side of PC4 and positive side of PC2 (lower right side of the graph) and steroids are shifted to this side when these ions have greater abundances.

The 19-norandrost-4-en-3-keto and the 6-hydroxyandrosta-1,4-dien-3-keto classes present dispersion of the data within the class that is bigger than the dispersion between classes. This happens because these classes have structural properties closely related to other ones: androst-4-en-3-keto and androsta-1,4-dien-3-keto, respectively. For this reason, some substances on the 19-norandros-4-en-3-ketosteroids class share some fragmentation pathways with the androst-4-en-3-ketosteroids while others do not, which generates greater dispersion within the class. The same happens to the pair of classes 6-hydroxyandrosta-1,4-dien-3-ketosteroids and androsta-1,4-dien-3-ketosteroids.

4. Conclusions

Principal component analysis was successfully applied to the separation of the classes of steroids according to their fragmentation pattern. This technique allowed the recognition and confirmation of fragment ions which are characteristic to each class of steroids.

Important ion markers for some groups of steroids were identified and their formation and fragmentation pathways were discussed and are presented in the work.

A statistical analysis of the fragmentation patterns of steroids was successfully performed and the data presented in this work can be of great help in identification of unknown steroids when analyzed by electrospray ionization and collision induced dissociation.

5. References

1. Aquino Neto, F.R.. *Rev. Bras. Med. Esporte.* 2001, 7 (4), 134-148.
2. Bowers, L.D.; Clark, R.V.; Shackleton, C.H.L.. *Steroids.* 2009, 74, 285-287.
3. Catlin, D.H.; Ahrens B.D.; Kucherova Y.. *Rapid Commun. Mass Spectrom.* 2002, 16, 1273-1275.
4. Catlin, D.H.; Sekera, M.H.; Ahrens, B.D.; Starcevic, B.;Chang, Y.C.; Hatton, C.K.. *Rapid Commun. Mass Spectrom.* 2004, 18, 1245-1249.
5. Sekera, M.H.; Ahrens, B.D.; Chang, Y.C.; Hatton, C.K.; Catlin, D.H.. *Rapid Commun. Mass Spectrom.* 2005, 19, 781-784.
6. Pozo, O.J.; Van Eenoo, P.; Deventer, K.; Delbeke, F.T.. *TrAC Trends Anal. Chem.* 2008, 27(8), 657-671.
7. Pozo, O.J.; Deventer, K.; Van Eenoo, P.; Delbeke, F.T.. *Anal. Chem.* 2008, 80, 5, 1709-1719.
8. Fragkaki, A.G.; Angelis, Y.S.; Tsantili-Kakoulidou, A.; Koupparis, M.; Georgakopoulos, C.. *Int. J. Mass Spectrom.* 2009, 285 (1-2),58-69.
9. Guan, F.; Uboh, C.E.; Soma, L.R.; You, Y.; Liu, Y.; Li, X.. *J. Mass Spectrom.* 2010, 45, 1261-1269.
10. Massart, D.L.;. Vandeginste B.G.M.; Buydens L.M.C.; De Jong S.; Lewi, P.J.; Smeyers-Verbeke, J. Handbook of Chemometrics and Qualimetrics: Part A. in Data Handling in Science and Technology, vol. 20. Amsterdam, Elsevier Science, 1998, 519-556.
11. Guan, F.; Soma, L.R.; Luo, Y.; Uboh, C.E.; Peterman, S.. *J. Am. Soc. Mass Spectrom.* 2006, 17, 477–489.
12. Pozo, O.J.; Van Eenoo, P.; Deventer, K.; Grimalt, S.; Sancho, J.V.; Hernández, F.; Delbeke, F.T.. *Rapid Commun. Mass Spectrom.* 2008, 22 (24), 4009-4024.
13. Williams, T.M.; Kind, A.J.; Houghton, E.; Hill, D.W.. *J. Mass Spectrom.* 1999, 34 (3), 206-216.
14. Thevis, M.; Schänzer, W.. *J. Am. Soc. Mass Spectrom.,* 2005, 16 (10), 1660-1669.

Selective determination of enoxacin by solid surface room-temperature phosphorimetry

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Abstract

Solid surface room-temperature phosphorimetry was used to quantify enoxacin. Phosphorescence (340/460 nm) was induced by using $Pb(NO_3)_2$ in cellulose substrates.The absolute limits of detection were 0.6 and 1.1 ng (for 5 µL sample volume) calculated respectively by measuring phosphorescence obtained using normal scan mode and second derivative scans. These values indicate excellent ultra-trace capability of the method. The detectability can be improved at least 10 times using pre-concentration on a C18 SPE cartridge.The linear response covered the range up to at least 124 ng. A detailed metrological study was made to calculate the combined uncertainty associated to the enoxacin phosphorescence measurement. Satisfactory analyte recoveries were obtained for urine samples (96%) and for simulated counterfeit pharmaceutical formulations (from 90 to 103%). Improvement in selectivity in determinations in more complex matrices was made by using the higher order (2^{nd}) derivative of the emission spectra ($\lambda_{isodifferential}$ = 448 nm). The results demonstrated the applicability of the method due to its simplicity, low cost, effectiveness and selectivity.

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Keywords: enoxacin, solid surface room-temperature phosphorimetry, second order derivatization, urine, counterfeit drug

1. Introduction

Enoxacin,1-ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid, see Figure 1, is a synthetic antibacterial agent used in human and veterinary medicines mainly to treat urinary infections and sexually transmitted diseases [1]. The presence of the piperazinyl substituent in the 7 position of the quinolone ring and the lack of substituents in position 5 make enoxacin more efficient (selective) against Gram-negative bacteria. The presence of the piperazinyl group also improves pharmacokinetic of the drug and the stability of their metabolites [2]. As with any other fluoroquinolone, enoxacin action involves the inhibition of two important enzymes for the bacterial chromosome replication process, which are DNA girase (topoisomerase II) and topoisomerase IV [3]. Enoxacin is administrated, generally, in two daily doses of 400 mg, presenting fast oral absorption (0.5 to 2.5 h) with $t_{1/2}$ from 4.2 to 6.8 h [4,5]. The oral bioavailability of this fluoroquinolone is about 80 to 100%. Less than 30% is bound with plasmatic protein [6] and about 45% of the dose is excreted in urine in its unchanged form [5].

Most of the methods used to determinate enoxacin are based on high performance liquid chromatography (HPLC). Samanidou *et al.*, for instance, developed a chromatographic (UV photometric detection) method to determinate enoxacin and other three fluoroquinolones in medicines in blood serum [7]. The limit of detection was 0.02 ng (in 20 µL sample) and recoveries varied from 91 to 103%. Enoxacin in pharmaceutical formulations and human serum was also determined by Tuncel *et al.* who proposed a capillary electrochromatographic method with UV absorption photometri detection with satisfactory results [8]. Luminescence based methods have also been proposed for the determination of enoxacin in pharmaceutical formulations and in urine. Fluorescence intensity was improved

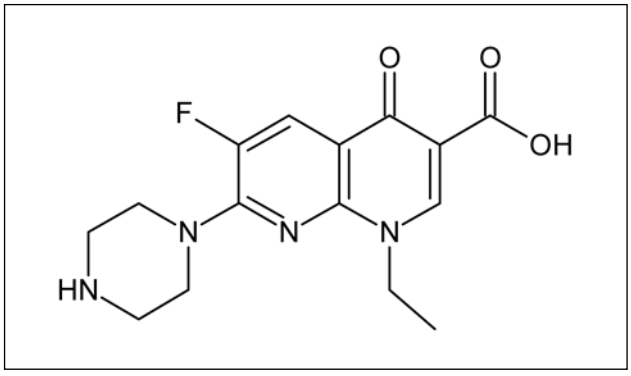

Figure 1. Chemical structure of enoxacin.

after sensitization with Tb^{3+} [9,10] or by using a surfactant organized environment [11]. Reported limits of detection were in the μg L^{-1} level.

Phosphorimetric methods present advantages in terms of labor and operational cost and enable the selective determination of very similar substances without prior separation by exploring slight differences in their phosphorescence spectral characteristics [12]. In solid surface room-temperature phosphorimetry (SSRTP) the analytical signal is measured from analytes immobilized in substrates. The right choices of the experimental and instrumental conditions when detecting traces of luminescent contaminants in a matrix containing other luminescent species improve selectivity and detectability [13,14]. However, the uncertainty of the luminescence measurement must be carefully evaluated in order to enable reliable results, especially, when inhomogeneous substrates like cellulose (high background fluctuations) are used [15].

In this work, the phosphorescence from enoxacin was studied using a solid substrate (cellulose) as support aiming to achieve experimental conditions to enable a sensitive phosphorimetric method for the determination of this fluoroquinolone in pharmaceutical formulations (simulated counterfeit drug) and in urine. Sensitivity was improved by using the selective heavy atom effect and selectivity improved by using second order spectrum derivativation. Precision was evaluated through a detailed uncertainty study. The proposed SSRTP method is simple enough to enable efficient and low cost screening of pharmaceutical samples.

2. Experimental

2.1. Instrumentation

Phosphorescence measurements were made on a Perkin-Elmer LS-55 luminescence spectrophotometer (Perkin-Elmer, Norwalk, USA) coupled to a solid surface analysis apparatus modified to allow a flow of purging dry nitrogen gas on the sample holder. Phosphorescence measurements were made using excitation at 340 nm and detection at 460 nm (or at 480 nm after second order derivatization of the emission spectrum). The instrument was operated with 1500 nm min^{-1} scan rate, 0.10 ms delay time and 3 ms gate time. Spectral bandpass was set to 15 nm. A pHmeter (MS Tecnopon, model MPA-210, São Paulo, Brazil) was used.

Liquid chromatographic analysis were made on an Agilent Infinity 1200 series HPLC system (Santa Clara, CA, USA) equipped with a binary pump and a fluorescence detector set at 340/420 nm. Degassing of mobile phase solvents was made off-line in a 9 L ultrasonic bath, Model NSC2800 (Unique, São Paulo, Brazil). Separation was made on an Agilent 5 μm particle size C18 stationary phase chromatographic column (4.6 x 150 mm). The column was kept inside an oven set at 20 ^{0}C.

A laboratory-made photochemical reactor was used for the treatment of cellulose substrate in order to reduce its background. The photochemical reactor was constructed by using an oven unit cabinet that was adapted by placing on the top of its internal part, six mercury vapor sterilization lamps (6 W each) with most intense UV wavelengths at 253 and in the 296-313 nm range. A Soxhlet apparatus and a 150 W infrared lamp (Philips, Brazil) were used in the cellulose cleaning process. The Statistica 7.0 (Statsoft-Inc. USA) software package was used for statistical calculations.

2.2 Reagents and materials

All chemical reagents were of analytical grade. Ultrapure water (18.2 MΩ cm^{-1}) from an ultra purifier water system (Milli-Q system, gradient A10, Millipore, USA) was used to prepare all aqueous solutions. Enoxacin was from Sigma-Aldrich, USA. Methanol, perchloric acid, boric acid, phosphoric acid, acetic acid, sodium hydroxide and sodium dodecyl sulfate (SDS) were obtained from Merck (Germany). Acetonitrile and methanol (HPLC-grade) were from Merck, Brazil. Sodium hydrogen phosphate heptahydrate was from Vetec (Brazil). A nalidixic acid based oral drug (Naluril® with 500 mg of nalidixic acid) from Sandoz, Argentina, was used as a matrix to test the determination of enoxacin. Thallium, thorium and lead nitrates were from Acros (USA). Filter paper (Whatman N° 42) was used as cellulose substrate after a treatment to reduce its background. Nitrogen (99.996%) was from White Martins, Brazil, and it was further purified by passing it through an ammonium metavanadate solution and dried over a silica gel bed. Urine was obtained from a healthy volunteer.

2.3 Procedures

Enoxacin stock solutions (5.0 x 10^{-3} mol L^{-1}) were freshly prepared in methanol because of the good solubility of the analyte in this solvent and volatility that ensures fast evaporation after deposition on a solid surface. Standard solutions with lower concentrations (below 8.0 x 10^{-5} mol L^{-1}) were made by further dilution of the stock in a solvent system composed of Britton-Robinson buffer pH 9.0:methanol (1:1 v/v). The Britton-Robinson buffer was prepared by mixing acetic acid, boric acid and phosphoric acid aqueous solutions. The pH of the buffer was adjusted by drop additions of 1.0 mol L^{-1} sodium hydroxide solution.

The simulated pharmaceutical formulation samples were prepared by mixing a specific amount of enoxacin into a mass of the nalidixic acid based commercial oral drug (Naluril; 500 mg), which was fortified with enoxacin with different molar proportions (1:2 and 1:5 v/v). The phosphorescence measurements were made at 448 nm of the 2nd derivative emission spectra and 460 nm of the normal emission spectra. Derivative spectra were directly obtained from the normal spectra through mathematical conversion made by equipment software using $\Delta\lambda$ = 1 nm. For interference tests, samples were prepared by mixing a specific amount of enoxacin with nalidixic acid. The pharmaceutical formulation (containing nalidixic acid

- Naluril®) and urine were fortified with a specific amount of enoxacin to perform recovery tests. Aliquots of 300 µL of analyte spiked urine were treated with 300 µL of a 7% v/v aqueous perchloric acid solution. After vortex mixing for 10 s, samples were centrifuged for 2 min at 3000 rpm [16]. Pre-concentration of enoxacin in urine samples was made by solid phase extraction using a C18 cartridge. The cartridge was conditioned with 2 mL of water and then with 4 mL of methanol. Then, 50 mL of sample was passed through the cartridge, forced by a gentle vacuum, followed by water for cleaning. Then, the analyte was eluted with 2.5 mL of methanol into a 5 mL volumetric flask, which has its volume adjusted to the mark with Britton-Robinson buffer (pH 9.0). A pre-concentration factor of 10 times was achieved.

To perform phosphorescence measurements, 5.0 µL of the heavy atom was spotted at the center of the substrate. Then, 5.0 µL of sample, standard or blank was deposited on the same spot. The substrate was left to dry (2 h) in a desiccator under vacuum before measurement. In order to reduce the background signal of cellulose, the filter paper was washed with hot water in a Soxhlet system for 2 h. Then, the paper sheet was exposed to an infrared lamp to dry for 30 min. The filter paper was cut in circles (1.8 cm diameter) before exposing them to UV (2 h) inside the photochemical reactor.

Before signal measurement, the substrate was placed in a solid surface luminescence measurement apparatus. Excitation radiation was focused in the center of the substrate, where the sample was spotted, and luminescence was collected at an angle of 90°. Before measurement the solid surface was coupled to an apparatus which was purged with dry and deoxygenated nitrogen gas (1 L min^{-1} flow rate) for 2 min to minimize quenching effects from oxygen and air moisture. The laboratory relative humidity was set to a constant 30% and maintained by the use of a dehumidifier device. The waste generated (heavy atom solutions and substrates containing heavy atom salts) were sent to a specialized waste management company (Saniplan Engineering and Environmental Services Ltda) that processed these materials on the premises established by the Rio de Janeiro State Environmental Agency (State Institute for the Environment, INEA/RJ).

Analyte recoveries achieved with the proposed phosphorimetric method were compared with the ones achieved by the HPLC method with fluorescence detection adapted from Davis *et al.* [17]. Separation was made using an isocratic elution (1 mL min^{-1} flow rate) with mobile phase consisting on a mixture of phosphate buffer 10 mmol L^{-1}, pH 4.0: acetonitrile (15:85, v/v). The retention time for enoxacin was 3.7 min.

3. Results and discussion

3.1 Preliminary studies

The external heavy atom effect is an essential artifice to induce or amplify phosphorescence from chemical species as it stimulates singlet-triplet forbidden transitions and decreases the triplet-ground state lifetime. However, since phosphorescence lifetimes of substances such as fluorquinolones is in the µs range, they need to be immobilized in a substrate in order to protect the excited molecule from quenching processes that usually prevail in solution when luminophores do not complete their radiative deactivation in the ns range (as is the case of fluorescence that can be easily measured in solution because of the allowed nature of the singlet-triplet transitions). Therefore, a systematic study was made to evaluate the effect of several heavy atoms salts in the substrate containing enoxacin. For these preliminary studies, the heavy atom salts, $CdCl_2$,$TlNO_3$, $Th(NO_3)_4$, $AgNO_3$ and $Pb(NO_3)_2$, and their amounts in the substrate were chosen based on previous experiences to induce room-temperature phosphorescence (RTP) from fluoroquinolones and other substances [15,18,19]. The study was made with the addition of 333 µg of $TlNO_3$, 414 µg of $Pb(NO_3)_2$, 9 µg of $AgNO_3$, 505 µg of $CdCl_2$ or 690 µg of $Th(NO_3)_4$ on the center of the substrate, and then 160 ng of enoxacin (5.0 µg of a $1x10^{-4}$ mol L^{-1} solution).

In all cases two excitation bands of similar intensities (with maximum intensities about 275 and 340 nm) and only one emission band (maximum intensity about 460 nm) were observed. The analytical signal was significantly more intense by using $Pb(NO_3)_2$. Phosphorescence of low intensity was observed from substrates containing enoxacin in the presence of $TlNO_3$ and $Th(NO_3)_4$. In the presence of either $AgNO_3$ or $CdCl_2$, the measured signal was no different than the one of the background substrate.

Substrate background varies in intensity as a function of the wavelength no matter which heavy atom inducer is employed. The background measured at 460 nm was about four times less intense when excitation was made at 340 nm than at 275 nm (the maximum excitation wavelengths for enoxacin in the presence of $Pb(NO_3)_2$). Therefore, in order to achieve improved detection limits, the excitation wavelength chosen for the method was 340 nm.

3.2.Maximization of the phosphorescence from enoxacin

Enoxacin presents several groups that may be influenced by the variation of the pH of the carrier solution. On the other hand, such speciation in solution influences the analyte interaction with cellulose, affecting the phosphorescence from molecules immobilized on the solid surface. In order to evaluate how the pH of the carrier solution affects the solid-surface room-temperature excitation of enoxacin, solutions were prepared in solvent systems containing 0.04 mol L^{-1} Britton-Robinson buffer (pH values from 2.0 to 12.0).

In substrates containing Th(IV), phosphorescence from enoxacin was of low magnitude and constant over the range of pH values tested. In the presence of either Tl(I) or Pb(II), enoxacin phosphorescence was low in the acid pH range of the

carrier solution and increased sharply in basic media, reaching a maximum signal with solutions at pH 9.0. The phosphorescence (345/460 nm) profiles are shown in Figure 2.

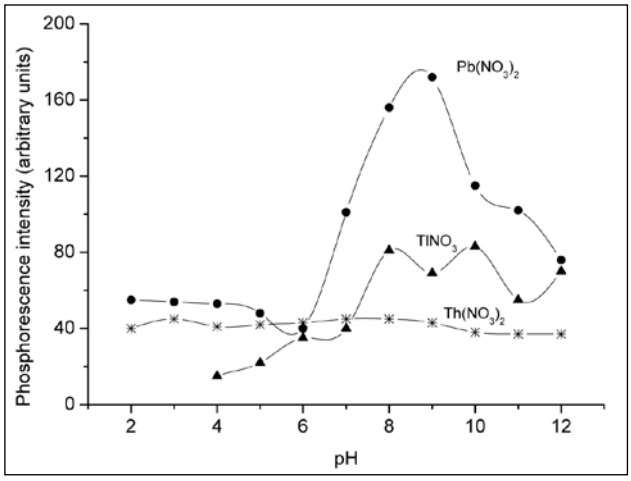

Figure 2. Effect of the pH of the analyte carrier solution on the phosphorescence of enoxacin in solid substrates containing different phosphorescence inducers.

The enoxacin molecular structure presents a carboxylic acid and secondary amine (of the piperazinyl group) which may receive and donate protons as the pH of the carrier solution is changed. When pH value exceeds its first pK_a value, the carboxylic acid deprotonates and its negative charge contributes to increasing the phosphorescent signal as it promotes conjugation with the π electrons of the quinolonic ring. At higher pH values, the protonated amine from the piperazine ring loses the proton and phosphorescence increases as it benefits from the presence of the free electron pair in the molecule.

One instrumental optimization was made, the delay time that enables best phosphorescence acquisition. The delay time range tested varied from 0.05 to 3 ms and, as expected, higher signal was achieved by using the lower delay time (5 times more intense than the one observed with 3 ms). However, the large standard deviation observed indicated influence from residual fluorescence from the substrate. In order to minimize this effect, a delay time of 0.1 ms was chosen. The gate time of the detector (from 3 to 9 ms) seemed to not affect the signal magnitude. Therefore, the 3 ms gate time was selected since it tended to enable spectra with reduced background.

The results obtained in the univariate studies indicated that $Pb(NO_3)_2$ was the best heavy atom inducer for enoxacin. Two main factors may influence the magnitude of the phosphorescence signal: (i) pH of the analyte solution and (ii) mass of the $Pb(NO_3)_2$ deposited on the substrate. Therefore, a final adjustment of conditions was made by using a two level central composite experimental design. This multivariate approach allowed: (i) the evaluation of possible interactions between these main factors, (ii) to evaluate the robust range for each factor when choosing the final condition for the method and (iii) to obtain the best conditions for maximum signal.

Replicates (n = 5) were made only at the central point (0.0). For the pH of the analyte carrier solution, the chosen levels, around the pH that enabled the maximum phosphorescence, were: 8.3 (+ √2), 8.5 (+1), 9.0 (0), 9.5 (-1) and 9.7 (-√2). The chosen levels for mass of the $Pb(NO_3)_2$ deposited on the substrate (with codified level in parenthesis) were: 188 µg (+√2), 400 µg (+1), 911 µg (0), 1422 µg (-1) and 1633 µg (-√2). This range of values was chosen as they can be delivered onto the center of the substrate by using 5 µL of $Pb(NO_3)_2$ solutions. Only one addition of 5 µL was used in order to avoid spreading of solution away from the center of the substrate.

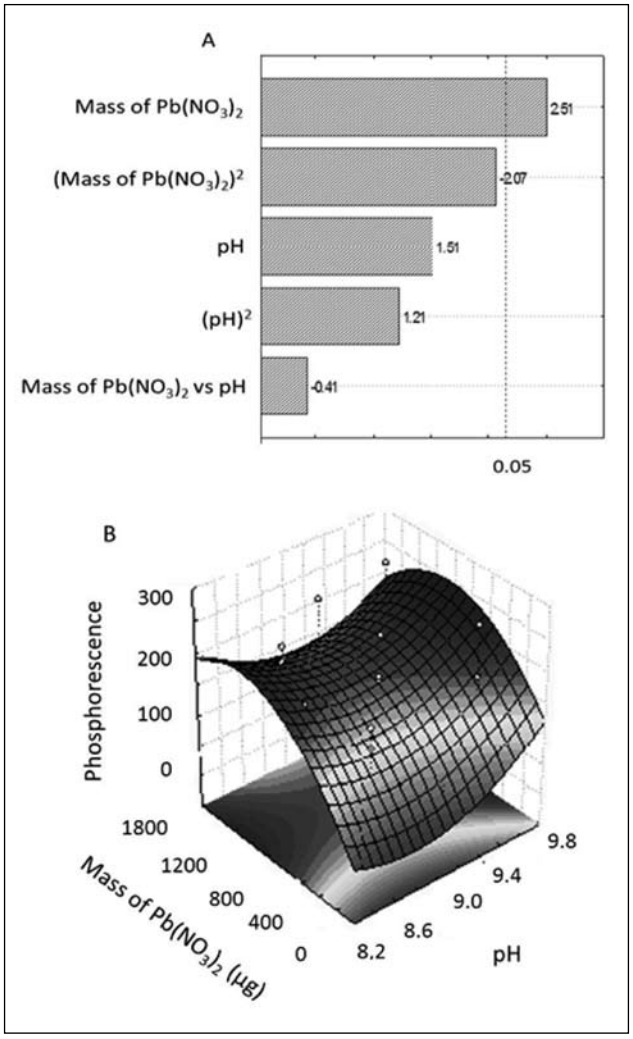

Figure 3. A) Pareto´s chart for the circumscribed central composite design using three replicates per point and five replicates for the central point (factors: amount of $Pb(NO_3)_2$ and pH). B) Response surface.

According to the results in Pareto´s chart (Figure 3A) no relevant interaction occurs between the two factors. It also shows the effect of the mass of $Pb(NO_3)_2$ surpass-

ing the critical level (with a positive statistical parameter) indicating that a possible adjustment of mass is need to enable maximum signal. However, the response surface (Figure 3B) and a previous univariate study indicate that the presence of about 1000 µg of $Pb(NO_3)_2$ enabled maximum signal. In addition signal variations tend to increase at higher masses of the phosphorescence enhancer, therefore, the adjustment of this parameter was found not necessary. A robust behavior in terms of the pH value was achieved since the measured phosphorescence intensities are statistically the same over the entire tested range. The values of 1197 µg of $Pb(NO_3)_2$ and pH 8.8 were chosen based on the results from the multivariate optimization. Figure 4A presents phosphorescence spectra of enoxacin under the optimized conditions.

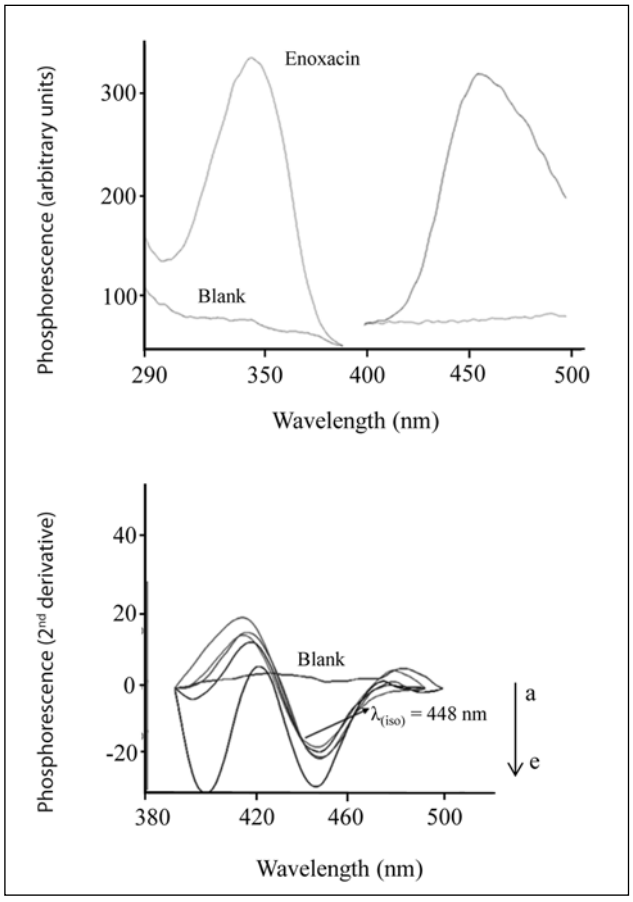

Figure 4: A) Phosphorescence spectra for enoxacin in paper substrate with $Pb(NO_3)_2$ under the optimized conditions. B) The second derivative for the enoxacin phosphorescence using measurement at the isodifferential wavelength $\lambda_{(iso)}$ of 448 nm. Enoxacin/nalidixic acid molar proportions: a) 1/0; b) 1/1; c) 1/2; d) 1/5; e) 1/10.

3.3.Interference test

The evaluation of the selectivity of the method was tested in the presence of a quinolone, nalidixic acid. This agent is commonly used for antibacterial treatments before the use of more specific and/or stronger fluoroquinolones such as enoxacin. In addition, nalidixic acid may be used as a cheaper component replacing fluoroquinolones in counterfeit formulations. The test was performed by using synthetic mixtures containing different enoxacin/nalidixic acid concentration ratios. The selectivity was evaluated by the ratios between the phosphorescence measured from an enoxacin (I_{eno}) solution, called reference standard, and from synthetic mixtures of enoxacin (in the same concentration as the reference standard) mixed with increasing concentrations of nalidixic acid ($I_{eno}/I_{(eno + nalid. ac.)}$). In mixtures containing concentrations up to two times more nalidixic acid than enoxacin, no interference was observed as the $I_{eno}/I_{(eno + nalid. ac.)}$ ratio is equal to unity. However, as the concentration of nalidixic acid is increased, phosphorescence from the interferent becomes relevant and makes the $I_{eno}/I_{(eno + nalid. ac.)}$ value decrease to 0.9 and to 0.65 respectively in samples containing concentrations of nalidixic acid five and ten times higher than the concentration of enoxacin).

In order to improve the capability to detect enoxacin in substandard formulations containing nalidixic acid, the second derivative of the phosphorescence emission spectra was used. The second derivative was found to enable good resolution for the enoxacin phosphorescence in an environment with contributions from nalidixic acid and from the substrate (Figure 4B). Measurements at the isodifferential wavelength (λ_{iso}), 448 nm, enabled the detection of enoxacin free of interference in samples containing concentrations of nalidixic acid five times higher than the concentration of enoxacin.

3.4.Analytical figures of merit

Analytical figures of merit were obtained for enoxacin in the conditions set for its determination by using both normal scanning and second derivative scanning since luminescence scanning strategies and wavelength set for measurement are different for each situation.

The analytical response in normal scanning is represented by the linear equation $y = 4.41 \times 10^6 x + 17.55$ (where y is the measured phosphorescence net intensity and x is the concentration of the analyte calculated by the linear model) and it was obtained by simple linear regression due to the homoscedastic nature of variances. This linear response ($R^2 = 0.997$) covered the enoxacin concentration range between the LOQ and 124 ng (7.7×10^{-5} mol L^{-1}).The linear equation of the curve made by using second derivative scanning was $y = -1.06 \times 10^6 x + 0.25$ ($R^2 = 0.997$).

Detection capability was reported in terms of mass values in the substrate (absolute limits of detection, ALOD, and quantification, ALOQ) and also (in parenthesis) the values in terms of the concentration of the analyte in the solution placed on the center of the substrate reported either as limit of detection, LOD, or limit of quantification, LOQ. The ALOD of 1.1 ng (7.0×10^{-7} mol L^{-1}) at λ_{em} = 460 nm

and 0.6 ng ($3.7x10^{-7}$ mol L^{-1}) at λ_{iso} = 448 nm were achieved. ALOD was calculated as C_{xb+3sb} V M, where C_{xb+3sb} is the analyte concentration, in mol L^{-1}, that enabled a signal magnitude equal to the blank average signal (x_b) plus three times the standard deviation from 10 blank determinations (s_b), M is the molar mass of enoxacin and V is the volume of the analyte solution deposited on the substrate (5 µL). The LOD was calculated based on C_{xb+3sb}. The ALOQ (LOQ) values were 5 ng (3.1×10^{-6} mol L^{-1}) at λ_{em} = 460 nm and 2.4 ng (1.5×10^{-6} mol L^{-1}) at λ_{iso} = 448 nm. These values were calculated based on the $C_{xb+10sb}$ V M and $C_{xb+10sb}$ criteria. The improvement in ALOD and ALOQ by using the second derivative spectra was due to the decrease of the variances of the blank signal.

Precision was evaluated through a metrological study. Since there is no equation to account, in detail, all sources of uncertainty associated to the phosphorescence measurement, an incomplete model was used. In this model, relevant sources that contribute to the uncertainty associated to the phosphorescence measurement of enoxacin were divided into four different main groups: i) repeatability (u_r); ii) reproducibility (u_R); iii) analytical curve (u_{curve}); and iv) preparation of solutions (u_s) [20]. This study was based on measurements at 340/460 nm after normal scanning of the phosphorescence.

The u_r was estimated by the relative standard deviation (RSD) of consecutive phosphorescence measurements (n=10) of the same analyte solution spiked onto different cellulose substrates. The study was performed with three different amounts of enoxacin deposited onto the substrates. The results achieved were about 7% at 12 ng, 5% at 48 ng and 8% at 120 ng levels. The u_{ip} value was calculated based on the analysis of variance (ANOVA) comparing two different analysts, each one preparing ten different substrates with the same analyte solution. The result was below 10% no matter the amount of enoxacin in the substrate (see Table I).

In order to calculate u_s, only the analyte solutions were taken into consideration because of the robust condition obtained for the setting of the pH of the analyte carrier solution and the phosphorescence enhancer solution. In this case, in order to minimize the contribution of this source of uncertainty, analyte solutions were prepared by weighing masses of analyte (or sample) and also of the solvent system in order to eliminate the relatively high uncertainties associated to the use of volumetric measurements. The u_s value was calculated as quadratic combinations of the expanded uncertainties from the balance, U_{bal}. The uncertainty was obtained from $u_{bal} = U_{bal}/k$, where U values were obtained from calibration certificates and k = 2 (the chosen coverage factor, 95.4%). The final u_s value must be converted into concentration (mol L^{-1}) in order to perform final quadratic summation for the combined uncertainty. This transformation was made by multiplying the u_s in mass by the sensitivity coefficient as follows: $u_{s(conc)} = (u_{s(mass)}/M_{eno})/V$, where, $u_{s(mass)}$ and $u_{s(conc)}$ values expressed, respectively, in mass (g) and in concentration (mol L^{-1}); M_{eno} was the molar mass of enoxacin in g mol^{-1} and V was the volume of solution in L.

Table I. Calculated uncertainties associated with the measurement of luminescence of enoxacin

	[Enoxacin] mol L^{-1} (mass)		
	8.0×10^{-6} (12ng)	**3.0×10^{-5} (48ng)**	**8.0×10^{-5} (125ng)**
Repeatability			
u_r *(mol L^{-1})*	5.3×10^{-7}	1.4×10^{-6}	6.4×10^{-6}
Between Analysts			
U_{ip} *(mol L^{-1})*	1.0×10^{-7}	8.0×10^{-7}	2.0×10^{-6}
Analytical curve			
Sensitivity (u_m)	1.2×10^{10}	7.3×10^{9}	
Linear coefficient (u_b)	3.5	1.1×10^{1}	
u_{curve} *(mol L^{-1})*	6.5×10^{-7}	1.4×10^{-6}	
Preparation of solution [a]	**Mass**	**Volume**	
Balance ($U_{bal(k=2.00;\ 95\%)}$)	2.0×10^{-5}		
microliter pipettes ($U_{mp(k=2.32;\ 95\%)}$)	-	8.0×10^{-7}	
volumetric flask ($U_{vf(k=2.18;\ 95\%)}$)	-	3.0×10^{-6}	
u_s *(mol L^{-1})*	3.2×10^{-8}	2.2×10^{-6}	
Combined Uncertainty			
u_c *(mol L^{-1})*	8.5×10^{-7}	3.1×10^{-6}	7.2×10^{-6}
	1.4ng	4.9ng	11.5ng
%	11%	11%	9%
Expanded Uncertainty			
$U_{(k=2;\ 95\%)}$ *(mol L^{-1})*	1.7×10^{-6}	6.1×10^{-6}	1.4×10^{-5}

In order to obtain u_{curve}, the phosphorescence for each of the enoxacin standards (from 2 to 124 ng) was measured three times. The standard deviations for both the sensitivity of the curve (s_m) and linear coefficient (s_b) were obtained from the linear regression and their respective uncertainties u_m and u_b were calculated as $u_b = \sqrt{s_b^2}$ and $u_m = \sqrt{s_m^2}$. From these results, u_{curve} was calculated by using the expression: $u_{curve} = [(c_{ib}^2 \times s_b^2) + (c_{im}^2 \times s_m^2) + (2 \times c_{ib} \times c_{im} \times u_b \times u_m \times r)]^{1/2}$, where c_{ib} and c_{im} were sensitivity coefficients and r is the correlation coefficient. The sensibility coefficients c_{ib} and c_{im} are used to make uniform dimensional units of the uncertainties, allowing the quadratic summation to be performed. Their values were obtained from $c_b = -1/m$ and $c_m = (y-b)/m^2$, that were achieved from the partial derivative of

the calibration curve equation in respect to the linear coefficient (*b*) and in respect to the linear coefficient (*m*).

The values for each of the uncertainties described above are listed in Table I. The combined uncertainty (u_c) was calculated by the square root of the quadratic summation of the four uncertainty values (Equation 1) considering that the contributing groups are independent from each other on the overall variability of the measurement.

$$u_c = (u_r^2 + u_R^2 + u_s^2 + u_{curve}^2)^{1/2} \quad (1)$$

The combined uncertainty (u_c) obtained was equivalent to 11, 11 and 9% of the original phosphorescence measured from 1.4; 4.9 and 11.5 ng of enoxacin, respectively. These uncertainty values are satisfactory considering that the measurement is made from solid substrates, due to fluctuations in phosphorescence background, even in substrates from the same lot and cut from the same paper sheet, and to small variations in the position where the analyte solution was spotted on the substrate which causes variations in the amount of analyte effectively interacting with the incident excitation light.

The expanded uncertainty (*U*) was obtained by multiplying u_c by a coverage value (k), in this case 2, in order to express the uncertainty at a confidence interval at some probability level (95% in this case). The expanded uncertainty associated to the phosphorescence measurement of enoxacin can be seen in Table I.

3.5. Application of the method

The method was tested through recovery studies in samples prepared by fortifying a nalidixic acid sample with enoxacin. Analysis were made in solutions containing two different levels of analyte concentration: 5.0×10^{-6} and 8.0×10^{-5} mol L^{-1}. The average recoveries for enoxacin were satisfactory for 2nd derivative and the results obtained were between 90 and 103% (n = 5). These results were in the acceptable range and in agreement (at 95% confidence level) with the ones achieved by using the reference HPLC method [17]. Urine samples were also analyzed. The samples were fortified at a 6×10^{-7} mol L^{-1} level and submitted to pre-concentration on a C18 SPE cartridge. Average recoveries of 96 ± 2% (n =5) was achieved.

4. Conclusion

Room temperature phosphorimetry was successfully applied in the determination of enoxacin. The multivariate optimization of parameters allowed the best signal to be achieved. The application of the method enabled satisfactory results in the analysis of simulated counterfeit drugs and in fortified urine samples. For the simulated drug containing nalidixic acid as interferent, the second derivative approach minimized interferences and also allowed better ALOD values (due to the lower noise of the blanks). For urine, the use of SPE allowed a 10 fold analyte concentration and elimination of interferences from the matrix. Precision of the method was accessed by calculating the combined uncertainty and the results were less than 11%.

Acknowledgments

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Reference

1. D.C. Hooper, *Biochim. Biophys.Acta.*1998, 1400, 45-61.
2. A. Bryskier, J.F. Chantot, *Drugs*. 1995,49, 16-28.
3. J.M. Blondeau, *Surv.Ophthalmol*. 2004, 49, S73-S78.
4. R.D. Toothaker, *Clin. Pharmacokinet*. 1989, 16, 52-58.
5. A.A. Somogyi, F.J. Bochner, *J. Clin. Pharmacol*.1988, 28, 707-713.
6. M. Neuman, *Clin. Pharmacokinet*.1988, 14, 96-121.
7. V.F Samanidou, C.E. Demetriou, I.N. Papadoyannis, *Anal. Bioanal. Chem*. 2003, 375, 623-629.
8. M. Tuncel, D. Dogrukol-AK, Z. Senturk, S.A. Ozkan, H. Aboulenein, *J. Liq. Chromatogr.* 2001, 24, 2455-2467.
9. F.T. You, L.P. Jin, H.C. Zhao, *Anal.Commun*. 2008, 69, 1130-1135.
10. Z.K. Su, L. Wang, G.P. Ma, J.T. Hu, W. Jiang, *J. Indian Chem Soc*. 2002, 79, 444-446.
11. L.M. Du, X.M. Cao, H.Y. Wu, *Chin. J. Anal. Chem*. 2003, 31,1330-1332.
12. T. Vo Dinh, T., *Room temperature phosphorimetry for chemical analysis. Chemical analysis series*, Vol. 101, J. Wiley & Sons, New York, 1984.
13. T. VoDinh, J.R. Hooyman, *Anal. Chem*. 1979, 51, 1915-1921.
14. F.F.C. Marques, C.F. Souza, F.S. Figueiredo, R.Q. Aucelio, *Anal. Lett*. 2008, 41, 1640-1649.
15. P.M.S. Maia, A.L.M.C Cunha, F.F.C. Marques, C.F. Souza, F.S. Figueiredo, R.Q. Aucelio, *Microchem. J*. 2010, 96, 108-113.
16. T. Kobayashi, M. Homma, K. Momo, D. Kobayashi, Y. Kohda, *Biomed. Chromatogr*. 2011, 25, 435-438.
17. J.D. Davis, L. Aarons, J.B. Houston, *J. Chromatogr. B*. 1993, 621, 105-109.
18. I.S. Nava-Junior, R.Q. Aucelio, *Spectrochim. Acta A*. 2009, 72, 429-435.
19. I.S. Nava-Junior, R.Q. Aucelio, *Braz. J. Anal. Chem*. 2010, 1, 18-24.
20. A.L.M.C. Cunha, F.F.C. Marques, R.L., Ziolli, R.L., R.Q. Aucélio, *Metrologia*. 2008, 45, 474.

Combination of FTIR, liquid-liquid aqueous partition and chemometric methods in the identification of tampered gasoline.

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Abstract

Due to dependence on the use of gasoline, traders see an opportunity to increase their profit by adding solvents such as ethanol, kerosene, turpentine and diesel. Attenuated total reflectance with Fourier transform infrared spectroscopy (ATR-FTIR) is a fast and nondestructive technique and requires little sample. In this work, the applicability of ATR-FTIR for detection of adulteration of Brazilian gasolines using liquid-liquid aqueous separation and chemometric tools was evaluated. Gasolines from 15 gas stations from the cities of Tietê, Ibaté and São Carlos (State of São Paulo) were acquired. Three data sets were generated: pure gasolines, polar phase and aqueous phase. The partition procedure aids in the identification of possible adulterants, since ethanol, present in Brazilian gasoline, presents strong peaks in the IR spectra. By IR spectra analysis methanol was found in the polar fraction of a sample, a novelty in the "gasoline tampering procedure". The same partition procedure was applied on samples spiked with methanol (MTH), ethanol (ETH), turpentine spirit (TUS) and toluene (TOL) from 0 to 25% yielding twelve datasets. It was found that identification of adulterated gasolines can be performed using spectra without partition but the quality of PCA separation is increased when pure, nonpolar and polar fraction spectra are concatenated, due the increase of chemical information. PLS models presented standard errors of cross-validation of 1.36%, 4.10%, 1.92% and 2.34% for MTH, ETH, TUS and TOL, respectively. The tampered sample presented 8.93% of methanol, using the developed model.

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Keywords: Tampered gasoline, ATR-FTIR, aqueous partition, multivariate calibration

1. Introduction

According to article 177 of the Brazilian Constitution, the prospecting, exploitation, refining, transportation, import and export of oil and its derivatives is a monopoly of the Brazilian government. However, private or state companies may be contracted to perform these tasks under a concession contract [1].

Gasoline is a fuel originating from the processing of petroleum, composed of paraffinic, olefinic, naphthenic and other aromatic compounds, as well as traces of oxygen, nitrogen and sulfur compounds. The proportion of these constituents in gasoline is related to the origin of the petroleum used. This mixture needs to be adequate for spark-ignition engines resulting in a proper energetic efficiency and low emission of pollutants [2,3].

Several industrial processes are employed in order to obtain a gasoline that will satisfy the quality criteria established in each country. Distillation, cracking, reform, alkylation, polymerization and isomerization are some process used in the achievement of the desired product [2]. The addition of additives is a common practice in order to reduce maintenance costs or increase engine performance. For example, the addition of anhydrous ethyl alcohol to Brazilian gasoline aims to increase the octane rating of the fuel (and it lowers the price of the fuel).

The proportion of anhydrous ethanol in Brazilian gasoline is regulated by the Ministry of Agriculture, Livestock and Supply (MAPA), since in Brazil ethanol comes almost exclusively from sugarcane. When this work was conducted, there was 25% ethanol in Brazilian gasoline; however, due to a MAPA decision on 1 September 2011, this percentage has been decreased to 20% [4].

Gasoline is so widely used that it has become a strategic product economically and politically. Thus, marketers see an opportunity to increase their profits by addition of adulterants. The adulteration of gasoline is made through the addition of miscible substances such as toluene, xylene, turpentine spirits and by the addition of an excess of ethanol and other solvents, according to resolution number 274

of 1 November 2011 of the Brazilian National Petroleum Agency (ANP) [5]. All of these substances are less expensive than gasoline, thus they decrease the cost of the product. However, the adulterated fuel is seriously detrimental to the operation and life of the engines, which begins to present increases in fuel consumption, high corrosion rates and consequent failure of engine components. Moreover, due to irregular combustion, a drastic increase in the release of harmful gases occurs, such as CO, unburned hydrocarbons, NO_x and SO_x, as well as heavy metals, depending on the adulterant used.

To avoid the adulteration procedure, ANP monitors gas stations, performing quality tests. The test takes into account physicochemical technical parameters such as: waste after evaporation, vapor pressure, density, octane rating, corrosivity, all described according to resolution ANP number 309 of 27 December 2001. However, most of these parameters were set based on good performance and operation of internal combustion engines, disregarding deliberate tampering.

Currently, all "type A" gasoline (denomination of the fuel before ethanol addition) comes from Petrobrás S./A., with a few exceptions due to that which are imported (special gasolines). Thus, distributors buy this product from Petrobrás S./A. and the anhydrous ethanol is added according ANP specification, yielding "type C" gasoline [6].

Fourier transform infrared spectroscopy (FTIR) is able to identify chemical bonds present in organic compounds. Therefore, the technique produces a specific spectrum for every molecule, which is called a fingerprint. This technique yields a very small amount of waste, needs only a milliliter of the sample and pretreatment of the sample is not necessary, resulting in a very convenient technique. In addition, FTIR provides a rich amount of information in a short period of data collection [7]. Due the fact that all Brazilian gasoline is produced by Petrobrás S/A and contains low aromatic content and high branched hydrocarbon content, the gasoline present a specific IR fingerprint, which can be used to detect chemical adulterations in the fuel. Any adulterant added will cause a variation in this fingerprint, and this spectral variation is easily detected by using chemometric methods such as principal component analysis (PCA) [8].

FTIR is commonly performed in the evaluation and characterization of fuels. An example of FTIR associated with multivariate calibration in the evaluation of petroleum is presented by Mohammad *et. al* [9,10]. FTIR has been allied with gas chromatography by Brudzewski *et. al*. to predict gasoline quality [11]. Teixeira *et. al.* got good results by employing the FTIR technique to identify gasoline samples adulterated with diesel, kerosene, turpentine spirit or thinner by using soft independent modeling of class analogy (SIMCA) and partial least squares (PLS) [12]. Felício *et. al*. used PLS to compare parameters of gasoline and gas oil [13]. Gaydou *et. al.* used concatenated spectra from near and mid infrared analysis and PLS models to detect adulteration of diesel and biodiesel blends by vegetal oil [14]. On the other hand, physical-chemical methods can be employed to find adulterations. Wiedemann *et. al.* employed physicochemical parameters and gas chromatographic data in order to detect the type of solvent used in adulteration, using hierarchical clusters analysis (HCA) [15]. Oliveira *et. al.* used data from distillation curves to identify adulterated Brazilian gasoline samples by applying SIMCA [16]. Barbeira *et. al*. applied physicochemical properties allied with multivariate analysis to identify the source of fuel [17]. Comprehensive two-dimensional gas chromatography combined with multivariate models were used by Pedroso *et. al.* in order to detect adulterated gasolines [18]. Hydrogen nuclear magnetic resonance was used by Monteiro *et. al.* to identify gasoline adulterations [19]. One of the most traditional adulteration methods is the addition of excess ethanol. In this context, Ye *et. al.* employed Raman spectroscopy for quantitative detection of ethanol in gasoline samples [20]. Others fuel properties such as density and octane rating were employed by Honorato *et. al.* to investigate spiked gasoline samples [21].

In this work, ATR-FTIR spectra were used to detect adulterations in "type C" gasolines from 15 gas stations. In order to produce clear spectra and increase the chemical information by a spectra concatenation procedure, aqueous partition was made, eliminating the interference of ethanol, which is naturally present in Brazilian fuel. PCA were performed to verify the formation of clusters between adulterated or non-adulterated samples. PLS calibration was performed to verify the power of prediction of the models, which were verified by a cross-validation method.

2. Methodology

Fifteen samples from different gas stations and cities in the State of São Paulo (Tietê, São Carlos and Ibaté) were purchased and stored in PET bottles. Firstly, the IR spectra of the original samples were collected and analyzed by PCA. A sample that did not present characteristics of tampering was purposely tampered, from 5 to 25%, using the adulterants: methanol (MTH), hydrated ethanol (ETH), turpentine spirit (TUS) and toluene (TOL). Spectra from this sample after adulteration were collected again. Reverse osmosis water was used to perform the aqueous partition in Falcon tubes, by using 10 ml of sample and adding 5 ml of water. The spectra of the aqueous phase and the nonpolar phase were collected from all samples and from purposely tampered samples. All of these spectra were acquired by using a BOMEM Model Michelson 100 FTIR spectrometer, using the attenuated total reflectance accessory (ZnSe), from 4000 to 800 cm^{-1}, 4 cm^{-1} spectral resolution and 32 scans. The dataset was exported to Matlab 2011a (Mathworks, Natick, MA, U.S.A) software in order to perform the data treatment.

Some pretreatments were performed to decrease spectral noise and to optimize the PLS models: spectra were transformed to absorbance, the base line shift was correct

by using wavenumbers without absorptions, a smoothing was performed by moving average (n = 4), then data was normalized and the first derivative spectra were obtained.

PCA were performed by using the singular value decomposition (SVD) function present in Matlab software. PLS models were created by using PLS_Toolbox 6.7 (Eigenvector Research, Inc, Wenatchee, WA, USA) for Matlab.

3. Results and Discussion

3.1 Analysis of samples without the addition of adulterants

Figure 1a shows the spectra obtained from the analyses of fifteen samples from different gas stations, without addition of adulterants and without partition. Figures 1b and 1c show the apolar and polar phase for these fifteen samples after the aqueous partition procedure. Figure 1a shows peaks related to C-C, C-O, C-H and O-H vibrations, from branched alkanes and from ethanol. One sample shows a peak which was not present in the other samples in the region between 950 and 1150 cm^{-1}. After aqueous partition, in the nonpolar phase presented in Figure 1b, all samples present very similar spectra, which is due the presence of hydrocarbons. The difference between the samples was present again in the polar phase (Figure 1c), this indicates that there were other water soluble solvents besides ethanol in the sample.

PCA was performed on spectra from the original samples (scores shown in Figure 2a) and from nonpolar (scores shown in Figure 2b) and aqueous phases (scores shown in Figure 2c). After these analyses, all the spectra were concatenated (pure + aqueous + nonpolar) and another PCA was performed (scores shown in Figure 2d). Figure 2 shows that the sample indicated by the symbol "+" was noticeably far from the other samples, which form a cluster. These figures show that the possible adulterant in sample "+" was not a nonpolar solvent, as previously verified in Figure 1, since this sample is far from the others, as shown in Figures 2a and 2c. Figure 3 shows the loadings of PC1 and PC2 from PCA performed on the original samples (Figure 3a, related to Figure 2a) and polar phase (Figure 3b, related to Figure 2c). Figure 3a shows a great contribution of PC2 loadings in the spectrum of sample "+", but not in the other ones (they present score values close to zero in PC2). By analysis of loadings in PC2 in Figure 3a, the sample "+" presents a IR peak shifted between 1050 and 1000 cm^{-1} (because PC2 is important only to sample "+" according Figure 2a), probably due the addition of a soluble adulterant, since this sample is far from others in Figure 2c. Figure 2c shows that in the aqueous phase PC1 is the principal response for the separation of sample "+" from the others. The loadings related to scores from figure 2c are shown in Figure 3b, where only ethanol contribution could be noticed (which is naturally present in the "type C" Brazilian gasoline). In this case it is worth noting that the PC1 score, which is mainly related to ethanol, presents a smaller absolute value for sample "+" than the other samples, which indicates that there is less ethanol in this sample.

In the nonpolar phase, there was no separation of sample "+", as shown in Figure 2b, which indicates that the compositions of hydrocarbons in the samples were very similar, without evidence of tampering using a nonpolar solvent.

In Figure 2d, which unifies the spectra from the samples without partition, polar phase and nonpolar phase, there was separation of sample "+", which was expected due to the previous separation by samples without partition and from the polar phase.

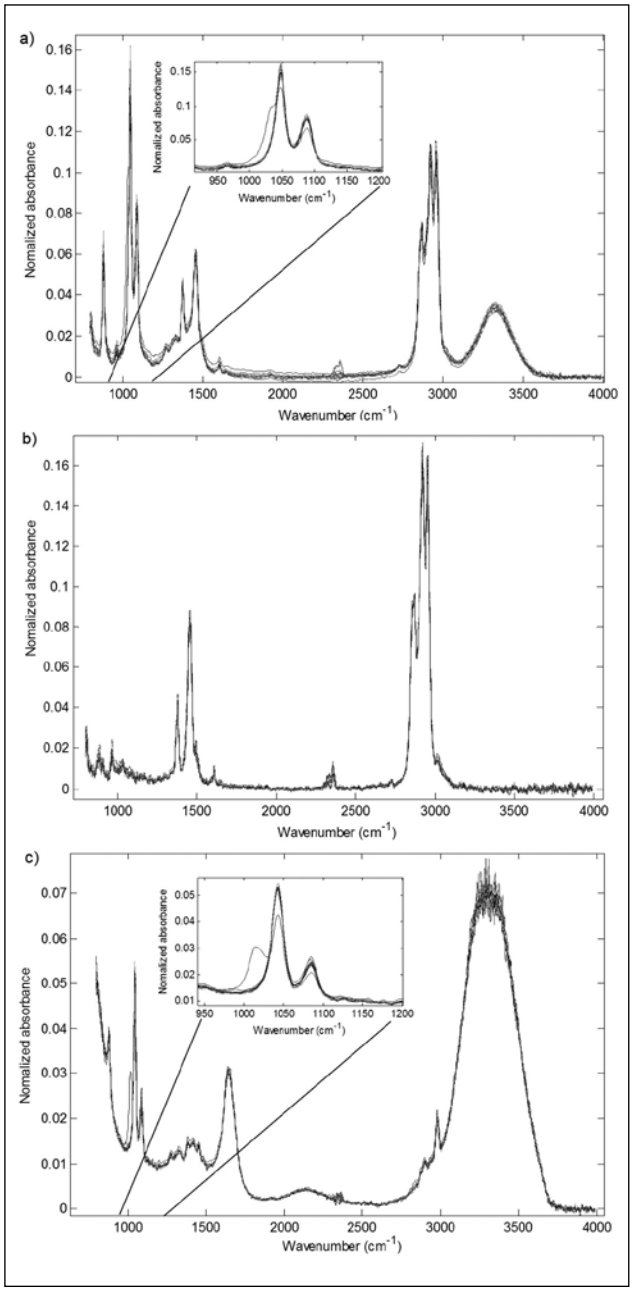

Figure 1. Spectra obtained from the analyses of fifteen samples from different gas stations: a) without partition; b) apolar phase after aqueous partition and; c) polar phase after aqueous partition.

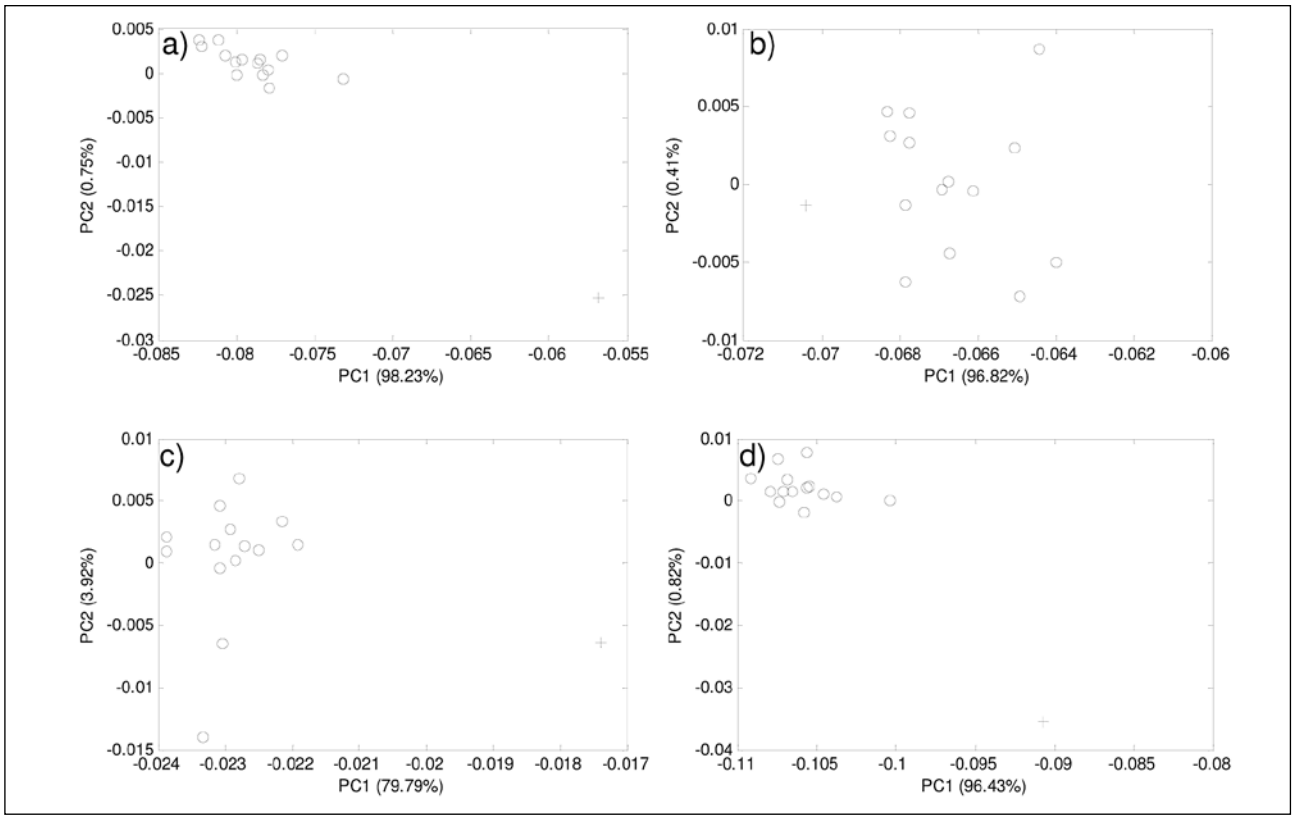

Figure 2. Scores (PC1 x PC2) from PCA performed on spectra from: a) original samples, b) nonpolar phase, c) aqueous phase and d) concatenated spectra (pure + aqueous + nonpolar)

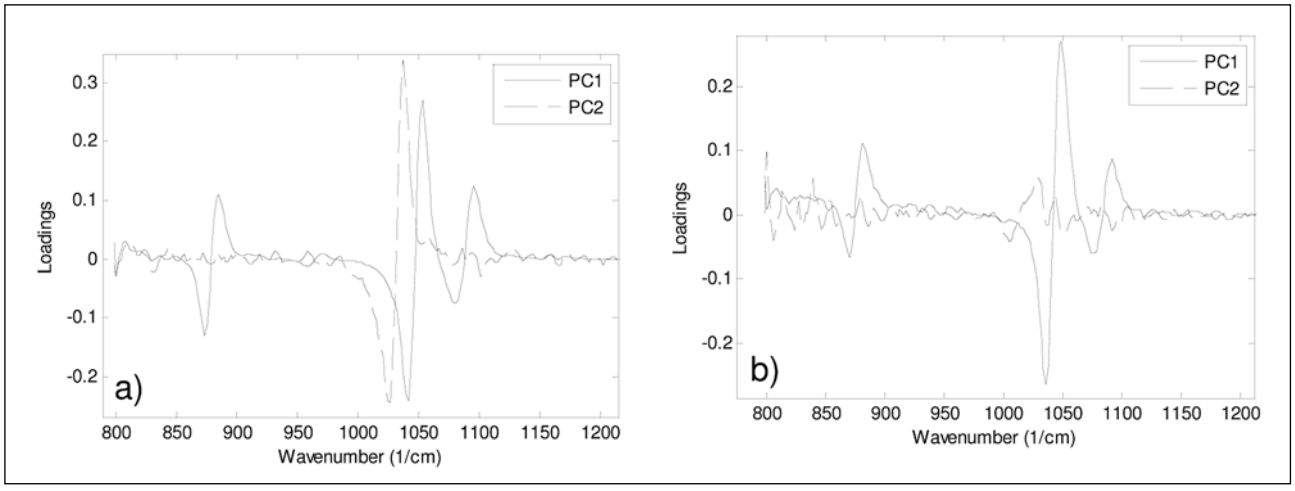

Figure 3. Loadings (PC1 and PC2) from PCA performed on spectra from: a) original samples and b) aqueous phase.

A polar adulterant was not expected due to the "graduated cylinder test", which is performed in gas stations in order to verify the ethanol content in type C gasoline. The volume of polar compounds was in accordance with the regulations (25% v/v, but using anhydrous ethanol). Therefore, this solvent is probably cheaper than anhydrous ethanol and was used instead of ethanol to decrease fuel cost. By some IR tests, using water soluble organic solvents, it was easily found that methanol was being used instead of ethanol, at least partially.

3.2 Analysis of samples with the addition of adulterants

In order to evaluate if IR spectroscopy could identify and quantify some kind of adulterants in gasoline, samples with methanol (MTH), ethanol (ETH), turpentine spirit (TUS) and toluene (TOL), from 0 to 25%, were analyzed following the same aqueous partition procedure. Figure 4 shows the scores for these data sets and Figure 5 shows the PC1 loading from the concatenated data sets.

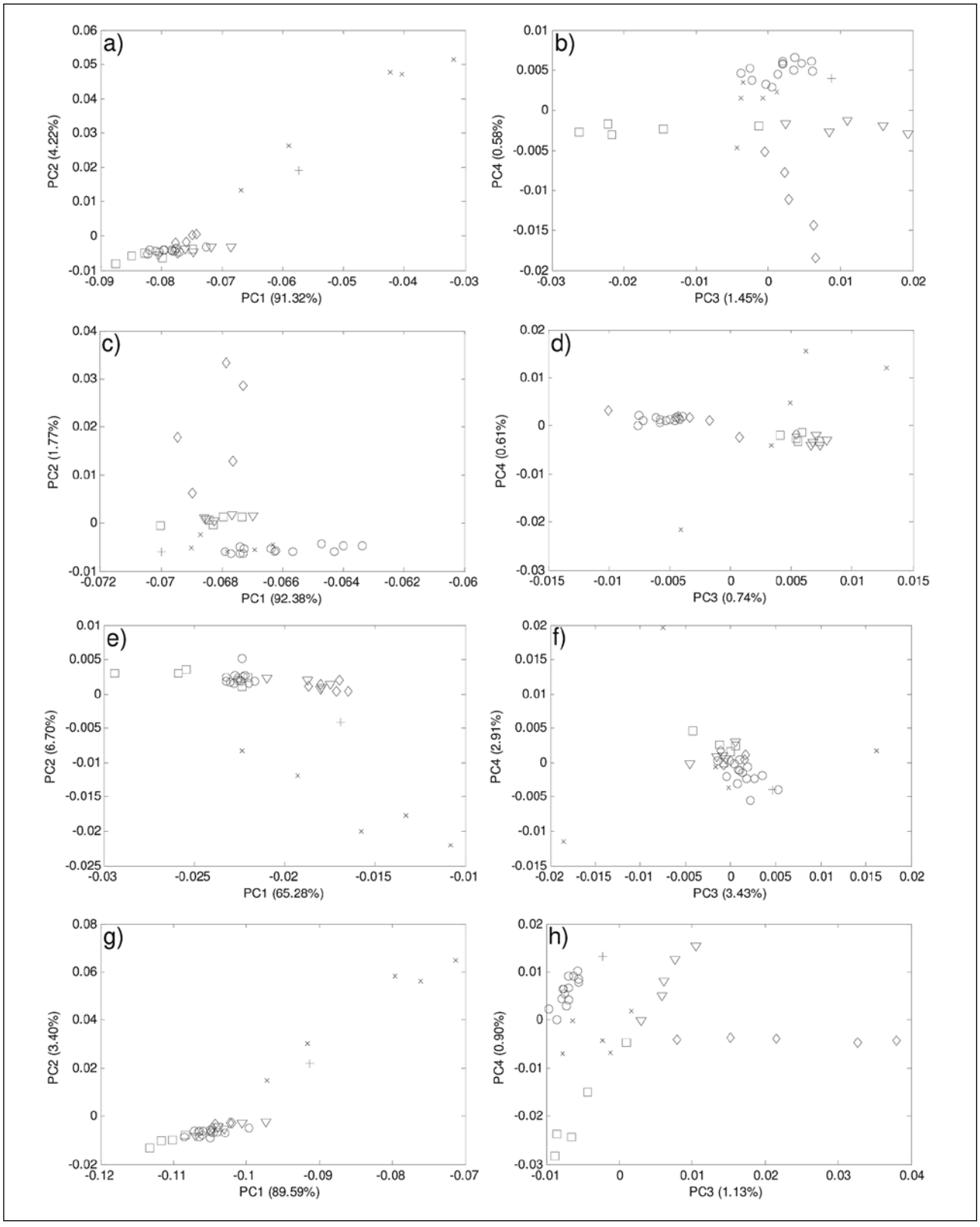

Figure 4. PC1 vs. PC2 and PC3 vs. PC4 PCA scores from: a) and b) original and spiked samples; c) and d) nonpolar phase of original and spiked samples; e) and f) aqueous phase of original and spiked samples and; g) and h) original and spiked sample with concatenated spectra (pure + nonpolar + aqueous). Symbols: 'O' original samples, '+' tampered original sample, '×' samples spiked with methanol, '□' samples spiked with ethanol, '◊' samples spiked with toluene and '▽' samples spiked with turpentine spirit.

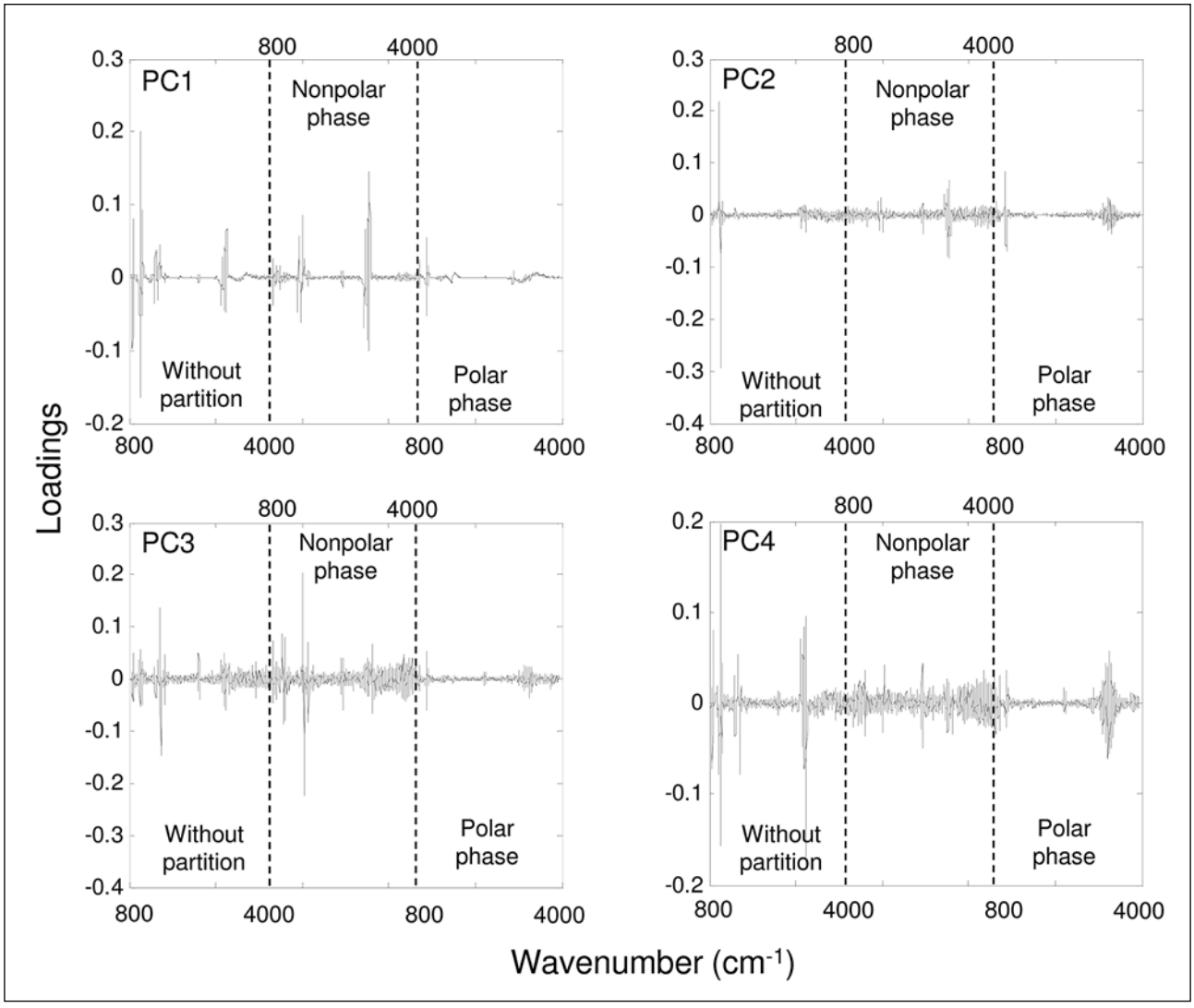

Figure 5. PC1 loadings from original and spiked sample with concatenated spectra (pure + nonpolar + aqueous). These loadings are related to scores from Figure 4 g) and h).

Figures 4 a) and 4 b) shows that, even without using the partition procedure, all adulterations can be detected (PC1 vs. PC2 for MTH and PC3 x PC4 for ETH, TUS and TOL). Due to the fact that TUS and TOL are hydrocarbons and ETH is already present in type C Brazilian gasoline, the changes in gasoline spectra from the addition of these adulterants are small. This fact justifies why the separation is seen in the PC3 x PC4 scores plot.

When the partition procedure is performed, the cluster formation changes. Figures 4 c) and 4 d) show the scores plot for the nonpolar phase of the aqueous partition. In this phase, MTH has been removed, so it was not possible to verify its separation on a PC1 x PC2 scores plot. A MTH separation can be noticed in PC3 x PC4 scores plot probably due to traces of methanol in the nonpolar phase or due to the removal of hydrocarbons from the nonpolar phase to polar phase by MTH. Still in the nonpolar phase, Figures 4 c) and 4 d), TOL is clearly separated by PC2 and TUS and ETH by PC3.

In the aqueous phase there was a clear separation in the PC1 x PC2 scores plot only due to MTH addition, in Figure 4 e). This was expected since TUS and TOL are not water soluble. However, an ETH separation could not be visualized. Figure 4f) does not present any cluster formation.

Figures 4 g) and 4 h) shows the score plots for the concatenated spectra. In these figures, MTH is separated by the PC1 x PC2 scores plot and TUS, ETH and TOL by PC3 x PC4, similarly to the scores plots from samples without partition, since concatenated spectra present the spectral information from samples without partition and the nonpolar and polar phases. Figure 5 shows that the main variables in the first PC (which explained the most part of the variance) for concatenated spectra were around 1050, 1450 and 2950 cm^{-1}. The same variables were responsible for the separation in the others PCs.

3.3 Quantification errors by PLS and cross-validation

In order to verify the possibility of quantification of adulterants by PLS and FTIR, a cross-validation procedure was made. Cross-validation was chosen due to the small number of points in the calibration set, however it can be compared with prediction errors from an external data set since not every sample is present in the calibration set when it is predicted. This procedure was performed for samples without partition and using nonpolar phase, aqueous phase or concatenated spectra. In order to increase the reliability of data, an interval selection was performed, from 800 up to 1568 cm^{-1} and 2823 up to 3034 cm^{-1}, in which were present most of the chemical contribution. Even using only six standards (from 0 to 25%), relatively low quantification errors were obtained. Table I shows the root-mean-square errors of the cross validation (RMSECV) and root-mean-square errors of the calibration (RMSEC) for every data set.

Table I. RMSECV and RMSEC (in brackets) values for quantification of ETH, TOL, TUS and MTH, by PLS, using two latent variables.

	Without partition	Nonpolar phase	Aqueous phase	Concatenated spectra
ETH	4.10 (1.49)	10.65 (0.68)	7.32 (1.02)	5.02 (0.81)
TOL	1.92 (0.13)	2.04 (1.18)	11.97 (0.76)	2.03 (0.70)
TUS	2.34 (0.28)	9.38 (0.36)	6.20 (1.09)	3.59 (0.22)
MTH	2.44 (0.53)	12.55 (1.95)	1.36 (0.11)	2.90 (0.20)

For ETH, TOL and TUS, 4.10%, 1.92% and 2.34% RMSECV values were obtained, respectively, by the use of two latent variables, and using the spectra without partition. For MTH, the best calibration model was found using the aqueous phase from the partition procedure, which presents 1.36% RMSECV value by the use of two latent variables. The smallest error of quantification was found for MTH, probably due to the fact that it is not present in type C gasoline, unlike ethanol and aliphatic or aromatic compounds.

The RMSEC values are naturally lower than RMSECV values, since predicted samples are present in the calibration model, yielding overfitting. This overfitting is more significant when few samples are used to build calibration models, thus, these values cannot be used to estimate the quality of the models obtained in this work.

Other figures of merit were calculated using PLS in its pseudo-univariate form, applying the net analytical signal (NAS), i.e., the fraction of signal which comes from the analyte [22,23]. The NAS and the other figures of merit were calculated using the PLS_Toolbox 6.7 and Figure 6 shows the linear adjustment between concentration of adulterant and NAS for each of them.

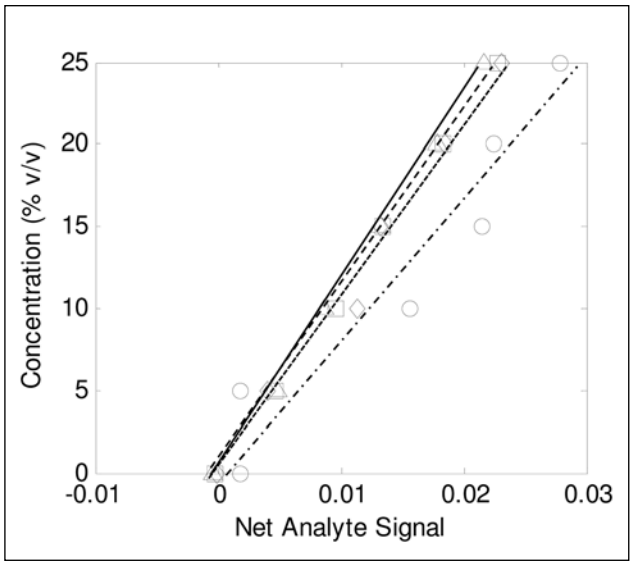

Figure 6. Linear approach between NAS and concentration (% v/v) of adulterant for the best models. Symbols of adulterants: 'O' and dashed-dotted line for ethanol, '□' and small dashes for toluene, '◊' and larger dashes for turpentine spirit and 'Δ' and continuous line for methanol.

Table II presents some figures of merit obtained from NAS for the best calibration models for each adulterant. R^2 values are according with RMSECV values, where higher R^2 are observed when lower errors are present. The sensitivity (SEN) found by NAS for multivariate calibration models is proportionally inverse to the analytical sensitivity [23], thus, lower values for SEN indicates higher analytical sensitivity, agreeing with other figures of merit, where MTH shows the best model and ETH the worst model. For multivariate calibration, selectivity (SEL) can be understood as how much overlap apppears between the spectrum of the analyte and the matrix spectrum. SEL will be unitary if there is no overlap and will be zero if there is total overlap. In this case MTH presents the greatest selectivity, as expected since methanol presents peaks in FTIR that are not present in type C Brazilian gasoline. For the other adulterants, the selectivity values were low and similar, since all of them present peaks found in Type C gasoline.

Table II. Inclination, intersection, R^2, sensitivity (SEN) and selectivity (SEL) estimatives for the best models, using NAS approach.

	Inclination	Intersection	R^2	SEN	SEL
ETH	810.16	0.19	0.9173	0.0027	0.1753
TOL	1092.10	0.00	0.9993	0.0021	0.1501
TUS	1071.50	0.02	0.9858	0.0021	0.1581
MTH	1133.50	0.04	0.9982	0.0020	0.5229

By using the developed model to methanol and using two latent variable, it was found 8.93% of methanol in the tampered sample "+" of Figure 2.

5. Conclusions

An aqueous partition procedure aids mainly in the identification of adulterants by removal of ETH from type C gasoline, since the spectrum of ethanol (in type C gasoline) is more complex than those from the hydrocarbons present in gasoline. In this paper, the adulterant remained in the aqueous phase. Although the information contained in the spectra from non-partitioned samples were similar to the information contained in the spectra from nonpolar plus aqueous phase, they are not the same, since when ETH is removed from type C gasoline, there is a change in the chemical environment causing peak shifting of the compounds in the polar and non polar phase, adding new information to improve chemometric models.

By IR spectra analyses from original samples and from the polar fraction of the partition procedure, methanol was identified as an adulterant, a novelty in the "gasoline tampering procedure". This solvent is cheaper than anhydrous ethanol, used in type C Brazilian gasoline.

The PCA performed on the pure and adulterated samples data sets, using spectra from samples without partition and concatenated spectra, shows the best separation to identify adulteration with MTH, TOL, TUS or ETH. Concatenated spectra present the spectral information from samples without partition plus the information from nonpolar and polar phase. Due to this fact, separations were similar. For the same reason, RMSECV values were similar for samples without partition and for concatenated spectra in the quantification procedure. For methanol, the best RMSECV value was obtained from the aqueous phase showing that the partition procedure can increase the possibility of quantification of some compounds in gasoline by using FTIR. The estimated figures of merit for the multivariate models were calculated using NAS, and they agree with the RMSECV values found for the models.

The use of ATR-FTIR and aqueous partition from samples is shown to be fast, simple and reliable for the identification of gasoline adulteration. Unlike distillation tests, FTIR finds the fingerprint of the combinations of the substances present in gasoline, so tamperings that are not identified by the traditional distillation techniques can be quickly identified by FTIR and appropriate chemometric methods.

6. Acknowledgments

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7. References

1. http://www.planalto.gov.br/ccivil_03/Constituicao/ConstituicaoCompilado.htm), accessed January 2012.
2. http://www.ibp.org.br/services/DocumentManagement/FileDownload.EZTSvc.asp? DocumentID=%7B3D3E7B70-B8F1-42A7-AFF3-C48611D53170%7D&ServiceInstUID=%7BA792040F-E3D5-4209-91CB-5DD99CEADB13%7D, accessed January 2012.
3. Takeshita, E. V.; Master's Degree Thesis, Universidade Federal de Santa Catarina, Brazil, 2006.
4. http://extranet.agricultura.gov.br/sislegis/action/detalhaAto.do?method=consultar LegislacaoFederal, accessed January 2012.
5. http://nxt.anp.gov.br/NXT/gateway.dll?f=templates&fn=default.htm&vid=anp:10.1048/ enu, accessed January 2012.
6. http://fatosedados.blogspetrobras.com.br/2011/04/07/preco-da-gasolina-mitos-e-verdades/, accessed January 2012.
7. Silverstein, R. M.; Webster, F. X.; Kiemle, D.; *Spectrometric Identification of Organic Compounds*, 7th ed.; Hoboken; John Wiley & Sons, 2005, 78 - 80.
8. Pereira, R.C.C.; Skrobot, V.L.; Castro, E.V.R.; Fortes, I.C.P.; Pasa, V.M.D.; *Energy Fuels* 2006, 20, 1097.
9. Al-Ghouti, M.A.; Al-Deghs, Y.S.; Amer, M.; *Talanta* 2008, 76, 1105.
10. Soares, I.P.; Rezende, T.F.; Fortes, I.C.P.; *Energy Fuels* 2009, 23, 4143.
11. Brudzewski, K.; Kesik A.; Kolodziejczyk, K.; Zborowska, U.; Ulaczyk, J.; *Fuel* 2006, 85, 553.
12. Teixeira, L.S.G.; Oliveira, F.S.; Santos, H.C.; Cordeiro, P.W.L.; Almeida, S.Q.; *Fuel* 2008, 87, 346.
13. Felício, C.C.; Brás, L.P.; Lopes, J.A.; Cabrita, L.; Menezes, J.C.; *Chemom. Intell. Lab. Syst.* 2005, 78, 74.
14. Gaydou, V.; Kister, J.; Dupuy, N.; *Chemom. Intell. Lab. Syst.* 2011, 106, 190.
15. Wiedemann, L.S.M.; d'Avila, L.A.; Azevedo, D.A.; *Fuel* 2005, 84, 467.
16. Oliveira, F.S.; Teixeira, L.S.G.; Araujo, M.C.U.; Korn, M.; *Fuel* 2004, 83, 917.
17. Barbeira, P.J.S.; Pereira, R.C.C.; Corgozinho, C.N.C.; *Energy Fuels* 2007, 21, 2212.
18. Pedroso, M.P.; Godoy, L.A.F.; Ferreira, E.C.; Poppi, R.J.; Augusto, F.; *J. Chromatogr., A* 2008, 1201, 176.
19. Monteiro, M.R.; Ambrozin, A.R.P.; Lião, L.M.; Boffo, E.F.; Tavares, L.A.; Ferreira, M.M.C.; Ferreira, A.G.; *Energy Fuels* 2009, 23, 272.
20. Ye, Q.; Xu, Q.; Yu, Y.; Qu, R.; Fang,Z.; *Opt. Commun.* 2009, 282, 3785.
21. Honorato, F.A.; Neto, B.B.; Pimentel, M.F.; Stragevitch, L.; Galvão, R.K.H.; *Fuel* 2008, 87, 3706.
22. Olivieri, A.C.; Faber, N.M.; Ferré, J.; Boqué. B.; Kalivas, J.H.; Mark, H.; *Pure Appl. Chem.*, 2006, 87, 633.
23. Braga, J.W.B; Poppi, R.J.; *Quim. Nova*, 2004, 27, 1004.

Simultaneous determination of pesticide and antibiotic residues at trace levels in water samples by SPE and LC-MS/MS

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Abstract

A reliable multiresidue method for determination of pesticide and antibiotic residues based on solid-phase extraction (SPE) was developed for the determination of 87 compounds in surface water by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Parameters that might influence the extraction efficiency such as sample loading volume, flow rate and eluent volume were optimized. The experimental results showed excellent linearity for the most of compounds ($r^2 > 0.99$) over the range of 1.0 to 250.0 μg L^{-1}. Most of the analyzed compounds showed limits of detection (LOD) and quantification (LOQ) between 0.01 and 0.04 μg L^{-1}, respectively. Recoveries achieved with spiked water samples were in the range of 70.0 to 120.0% for 86.2% of the analyzed compounds in the three fortification levels (1.0, 2.5, 5.0 μg L^{-1}), with good precision. The multiresidue method developed could be used to determine multiclasses of pesticides and antibiotics simultaneously in superficial water samples with less cost, shorter analytical time and less solvent consumption.

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Keywords: SPE, LC-MS/MS, pesticides, veterinary drugs, water samples

1. Introduction

In past decades the development and use of pesticides and antibiotics played important roles in increasing agricultural and animal productivity [1,2]. Because pesticides and antibiotics can be toxic, persistent, carcinogenic and present bioaccumulation, their presence in water for human consumption must be controlled [3-5]. As a consequence of the low levels established by some countries, the development of sensitive, fast, and efficient methods of analysis remains of great interest [6-8].

For monitoring purposes and for pesticide and antibiotic environmental fate studies, accurate and cost-effective analytical methods are important. They usually combine an extraction technique with a separation technique followed by a powerful quantification technique such as mass spectrometry (MS) in order to determine the low levels of concentration usually found in environmental water samples [9].

In order to simultaneously quantify any existing pesticides and antibiotics, which usually present a variety of physical-chemical properties, liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is the current state of the art analytical technique [10,11]. It has clear advantages over traditional detection methods such as LC-UV, whose main drawbacks are the lack of selectivity and sensitivity. Compared to gas chromatography coupled to mass spectrometry (GC-MS), the advantage is the broader number of substances that can be analyzed [9,12].

LC-MS/MS is a highly selective technique and allows the accurate and precise determination of substances through the detection of fragments from previously selected precursor ions [13,14]. However, the ionization process, which is crucial for quantitative measurements, is affected by the sample matrix [15,16]. Ion suppression is attributed to the competition that occurs between matrix components (e.g., humic acids and ions) and analytes for ionization or access to the droplet surface for gas phase emission. This phenomenon is known and different procedures for eliminating or mitigating these effects, e.g., the use of pre-column procedures for matrix elimination, matrix matched calibration, and quantification using standard addition, have been reported [15,16]. These methods are only partly effective, except standard addition, which is, however, very time consuming. Despite ion suppression being a well know topic, we can affirm that matrix ion suppression is seldom emphasized in the literature when it comes to the trace level quantification of pollutants in water [15,16].

A traditional approach to sample preparation in water analysis is liquid-liquid extraction (LLE). This method is time and solvent-consuming, and laborious. Nowadays, sample enrichment is a pre-requisite for reaching low detection

limits of a few ng L^{-1}, which is achieved using solid phase extraction (SPE) in the case of aqueous samples [17, 18]. SPE is one of the preferred extraction techniques for isolating and enriching polar analytes in complex aqueous samples [19-24]. One of the main advantages of SPE is the wide range of sorbents available, which covers a broad range of analyte properties including the polar and non-polar analytes [25-27].

This paper describes a method for multiresidue determination of 87 compounds (pesticides and antibiotics) in surface water. Most of the compounds are medium-polar and polar compounds; therefore, reversed-phase LC is the separation method of choice. Regarding detection, the use of tandem MS enables the combination of screening and confirmation in one procedure.

2. Experimental

2.1. Standards, reagents and solvents

All standards were purchased from Dr. Ehrenstofer (Augsburg, Germany) with purity between 80.0 and 99.9%. The stock solutions of individual compounds at 1000 mg L^{-1} were prepared in HPLC grade acetonitrile. The working standard mixture, containing 5 mg L^{-1} of each compound, was prepared in acetonitrile for use as a spiking solution and for the analytical curves.

Acetonitrile and methanol (HPLC grade), as well as glacial acetic acid were purchased from J. T. Baker (Phillipsburg, USA). Ammonium formiate was obtained from Sigma-Aldrich (Steinheim, Germany). Purified water was provided by a Direct UV3 system from Millipore (Molshein, France).

The sorbents for SPE, Strata C_{18}, Strata SDB-L and Strata-X, in cartridges of 200 mg/3 mL, were purchased from Phenomenex (Torrance, CA, USA).

2.2. Equipment

Method validation was performed using a high-performance liquid chromatography system interfaced to a triple quadrupole mass spectrometer, Varian 320-MS (Walnut Creek, CA, USA). The instrument was equipped with 212-LC quaternary pump, 410 autosampler with 20µL sample loop and column oven. A nitrogen generator, Domnick Hunter LC/MS 12/2 (Gateshead, England), was used to deliver the nebulizer and desolvatation gas to the mass spectrometer. The software MS Workstation Version 6.0 was used to control the instrument and to evaluate the chromatographic and mass spectrometric data.

A Denver Instrument APX-200 analytical balance (Arvada, USA), Microtécnica Biomixer QL 901 vortex (Boqueirão, Brazil), Milli-Q Direct UV3® water purification system (Millipore, Bedford, MA, USA), and Transferpette®*S* Brand (Wertheim, Germany) were used. For the SPE system a model TE-058 vacuum pump (Piracicaba, SP, Brazil) and a Varian manifold (Palo Alto, CA, USA) with capacity for 20 samples were employed.

2.3. LC Conditions

Reverse-phase HPLC analyses were performed using a Varian Pursuit XRs column (C18, 100 x 2.0 mm, 2.8 µm) and Pursuit XRs metaguard column (C18, 10 x 4.6 mm, 5.0 µm). The mobile phase used was 5 mmol L^{-1} aqueous ammonium formiate (A) and methanol (B) at the flow rate of 150 µL min^{-1}. The initial composition was 25% B, which increased linearly to 95% B in 15 min, holding until 20 min and then returning to the initial composition (25% B) in 5 min and hold by 5 min. Samples extract volumes of 5 µL were injected into the system.

2.4. MS/MS Conditions

MS/MS conditions, performed with an electrospray ionization source (ESI) operated in positive and negative mode, were optimized in order to obtain the maximum intensity of the precursor ions as follows: dry gas pressure, 20 psi; nebulizing gas pressure, 40 psi; drying gas temperature, 250 °C; API house temperature, 50 °C; needle voltage, 5000 V. Argon was used as a collision gas at the pressure of 1.8 mton and Multiple Reaction Monitoring (MRM) transitions was employed in this work. Table I shows the pesticides and antibiotics analyzed by LC-MS/MS using positive and negative ESI ionization mode, MRM acquisition mode, precursor and product ions, collision energy for the two monitored transitions and retention time of the compounds studied.

Table I. LC-MS/MS conditions for the determination of pesticides and antibiotics with precursor and product ions and collision energies for both transitions monitored.

			First transition (Quantification)			Second transition (Confirmation)		
t_R (min)	Compound	Ionization mode	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)
5.87	Salbutamol	+	240.0	147.9	13.0	240.0	222.0	7.0
6.56	Sulfathiazole	+	256.0	155.9	12.5	256.0	91.9	23.0
6.78	Oxamyl	+	237.0	71.9	5.5	237.0	89.9	5.0
7.26	Sulfamethazine	+	279.0	185.9	15.0	279.0	155.9	17.0
7.34	Mevinphos	+	242.1	126.8	14.5	242.1	192.9	7.5
7.35	Monocrotophos	+	224.0	192.9	7.0	224.0	126.9	13.5

t_R (min)	Compound	Ionization mode	First transition (Quantification)			Second transition (Confirmation)		
			Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)
7.85	Imidacloprid	+	256.1	209.0	14.0	256.1	175.0	17.0
7.94	Methiocarb sulfoxide	+	242.0	185.0	8.5	242.0	170.0	14.0
8.05	Bentazone	-	239.0	131.7	25.5	339.0	196.6	19.0
8.25	Vamidathion	+	288.0	145.9	10.0	288.0	117.8	20.0
8.28	Metsulfuron-methyl	+	382.0	167.5	12.0	382.0	198.9	19.5
8.39	Carbofuran-3-OH	+	238.0	163.0	12.0	238.0	220.0	6.0
8.52	Methiocarb sulfone	+	275.0	258.0	9.5	275.0	201.0	19.5
8.65	Dimethoate	+	230.0	198.9	8.5	230.0	124.8	19.5
8.69	Trichlorfon	+	257.0	108.8	15.5	257.0	220.8	11.0
8.80	Sulfadimethoxine	+	311.0	155.9	17.5	311.0	107.9	25.5
9.00	Thiacloprid	+	252.9	125.8	18.0	252.9	185.9	12.5
9.37	Chloramphenicol	-	321.0	151.7	16.5	321.0	256.7	10.5
9.42	Carbendazim	+	192.0	160.0	15.0	192.0	131.9	27.0
10.40	Cyanazine	+	241.0	214.0	14.0	241.0	131.9	21.5
11.03	Propoxur	+	210.0	110.9	10.0	210.0	168.0	5.0
11.10	Carbofuran	+	222.0	165.0	7.5	222.0	122.9	16.5
11.28	Simazine	+	202.0	131.9	19.5	202.0	123.9	18.0
11.68	Thiodicarb	+	355.0	87.9	9.0	355.0	107.9	11.5
11.73	Carbaryl	+	202.0	144.9	8.0	202.0	126.9	26.5
11.75	Carboxym	+	236.0	142.8	10.5	236.0	86.8	21.0
11.98	2,4 D	-	219.0	160.6	11.5	219.0	159.9	16.0
12.26	Monolinuron	+	215.0	125.8	15.0	215.0	147.9	12.0
12.42	Pirimicarb	+	239.0	182.0	12.5	239.0	108.8	15.0
12.55	Paraoxon	+	293.0	219.9	16.0	293.0	275.9	8.5
12.92	Metalaxyl	+	280.0	220.0	12.0	280.0	248.0	9.0
12.94	Pendimethalin	+	216.0	174.0	15.5	216.0	103.8	26.0
12.94	Atrazine	+	216.0	173.9	13.5	216.0	103.8	24.5
13.25	Chlorimuron	+	415.0	186.0	15.0	415.0	277.9	15.0
13.42	Fluroxypyr	-	252.8	194.5	11.5	252.8	232.6	6.0
13.42	2,4,5 T	-	252.8	160.6	11.5	252.8	158.6	28.0
13.47	Bispyribac-sodium	+	453.0	296.6	17	453.0	179.0	18.5
13.71	Azinphos methyl	+	318.0	131.9	11.0	318.0	160.0	5.0
13.77	Azoxystrobin	+	404.0	372.0	14.5	404.0	328.9	30.0
13.90	Clomazone	+	240.0	124.9	18.0	240.0	88.8	39.5
14.54	Linuron	+	249.1	159.9	16.0	249.1	181.9	13.5
14.60	Boscalid	+	343.0	306.9	17.5	343.0	270.9	30.5
14.67	Terbutylazine	+	230.0	174.0	12.0	230.0	131.9	20.5
14.73	Pyrimethanil	+	200.0	106.9	21.0	200.0	140.0	14.0
14.79	Flutolanil	+	324.2	261.9	16.5	324.2	241.9	23.0
14.90	Malathion	+	331.0	126.9	9.0	331.0	284.9	8.0
15.05	Mepronil	+	270.0	228.0	15.0	270.0	118.9	20.5

			First transition (Quantification)			Second transition (Confirmation)		
t_R (min)	Compound	Ionization mode	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)
15.10	Triadimefon	+	294.0	224.9	12.0	294.0	197.0	14.5
15.12	Pyridaphenthion	+	341.0	189.0	20.0	341.0	204.9	20.0
15.18	Myclobutanil	+	289.1	69.9	11.5	289.1	124.9	25.0
15.28	Propyzamide	+	255.9	189.9	10.5	255.9	172.9	18.0
15.32	Chlorprofam	+	214.0	171.9	6.0	214.0	153.9	14.0
15.32	Triazophos	+	314.0	162.0	16.0	314.0	118.9	32.0
15.40	Bromuconazol	+	378.0	158.9	23.5	378.0	160.9	23.5
15.50	Iprovalicarb	+	321.0	203.0	8.0	321.0	118.9	14.0
15.59	Dichlofluanid	+	350.0	223.8	14.0	350.0	332.8	7.5
15.60	Triadimenol	+	296.0	69.9	5.5	296.0	227.1	9.0
15.64	Fluquinconazole	+	376.0	306.8	24.0	376.0	348.9	18.5
15.76	Fenarimol	+	331.0	267.9	22.5	331.0	258.9	24.5
15.77	Tetraconazole	+	372.0	158.9	24.0	372.0	124.8	19.5
15.83	Mecarbam	+	330.0	226.9	7.0	330.0	198.9	13.0
15.98	Epoxiconazol	+	330.0	120.9	17.5	330.0	122.9	12.5
16.33	Fipronil	-	435.0	329.7	15.5	435.0	249.6	26.5
17.19	Fenthion	+	279.0	246.9	11.5	279.0	169.0	14.5
17.23	Diazinon	+	305.1	169.0	17.5	305.1	153.0	17.0
17.24	Propiconazole	+	342.0	158.9	25.5	342.0	69.0	14.0
17.35	Tebuconazole	+	308.0	69.9	14.0	308.0	124.9	33.0
17.38	Pyraclostrobine	+	388.0	194.0	9.0	388.0	163.0	22.0
17.60	Prochloraz	+	376.0	307.8	11.0	376.0	265.8	16.5
17.61	Bitertanol	+	338.0	269.1	8.0	338.0	251.0	9.5
17.63	Metaconazole	+	320.1	69.9	15.5	320.1	124.9	31.5
17.75	Pirazofos	+	374.0	222.0	19.0	374.0	194.0	30.0
17.85	Pirimiphos methyl	+	306.0	164.0	19.5	306.0	147.9	12.0
17.88	Tolclofos	+	300.9	268.7	15.5	300.9	124.8	13.5
17.97	Profoxidin	-	464.0	280.0	14.0	464.0	238.0	17.5
18.00	Difenoconazole	+	406.0	250.9	24.0	406.0	336.9	15.5
18.07	Trifloxystrobin	+	409.0	186.0	14.0	409.0	206.0	11.0
18.52	Triflumizole	+	346.0	278.0	10.5	346.0	220.2	13.5
18.94	Profenofos	+	375.0	304.7	19.5	375.0	346.7	12.0
19.37	Buprofezin	+	306.0	201.0	9.5	306.0	115.9	14.0
19.45	Terbufos	+	288.9	102.9	5.0	288.9	232.9	5.0
19.62	Ethion	+	384.9	198.9	7.0	384.9	170.9	13.0
20.25	Chlorpyrifos ethyl	+	349.9	197.8	16.0	349.9	321.7	10.5
20.42	Propargite	+	368.0	231.1	9.5	368.0	175.1	12.0
21.82	Fenpropimorph	+	304.6	147.0	24.5	304.6	129.9	20.5
22.92	Monesin	+	693.5	461.0	45.0	693.5	675.5	28.5
24.63	Salinomycin	+	773.5	431.2	45.0	773.5	531.7	40.5

2.5. Water Samples

Due to the difficulty of finding real samples without the presence of compounds under study, method validation was performance using Milli-Q water (resistivity 18.2 MΩ cm at 25 °C), in order to validate the method for the determination of pesticide and antibiotic residues by SPE and LC-MS/MS. After that, the method was applied to real samples.

2.6. Preliminary Tests of SPE

The SPE method was optimized for the extraction of pesticides and antibiotics, in order to obtain satisfactory recovery values. In preliminary experiments the extraction efficiencies of some solid-phase extraction cartridges at different elution conditions using water blank samples spiked with the selected compounds at 2 μg L^{-1} were tested. The cartridges were Strata C_{18}, Strata SDB-L and Strata-X, tested with a elution solvent of 1:1 (v/v) methanol:acetonitrile mixture without and with acidification (1% (v/v) acetic acid) for all the compounds. The analytical protocol used for the SPE extraction is presented in Figure 1.

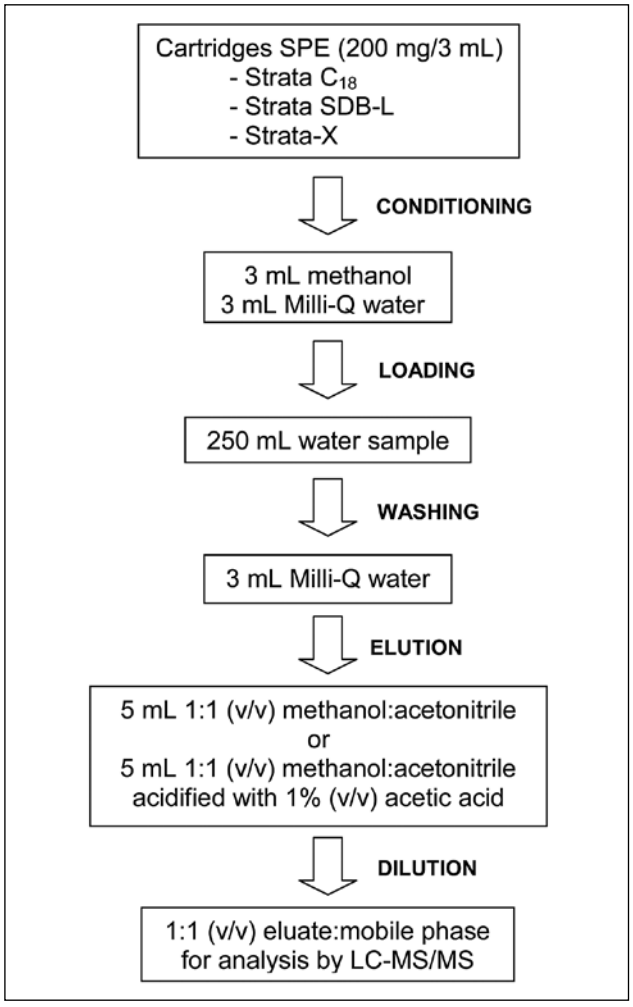

Figure 1. Scheme for simultneous extraction of 81 pesticides and 6 antibiotics in water using SPE with subsequent analysis by LC-MS/MS.

2.7. Sample fortification

Ultrapure water was spiked by adding an appropriate volume of a mixed standard solution containing all compounds studied at concentrations of 5 mg L^{-1}. The water samples were spiked at different concentration levels (1.0; 2.5 and 5.0 μg L^{-1}) in triplicate and the recovery and precision were evaluated.

2.8. Sample preparation

The sample was filtered through a 0.45 μm membrane to eliminate particulate matter, and a filtered volume of 250 mL was used for the SPE step. The sample was percolated into the Strata-X cartridge at a rate of 2 mL min^{-1}. The cartridge was conditioned using 3 mL of methanol and 3 mL of Milli-Q water. This procedure was conducted at low pressure (less than 100 bar). For the elution of compounds 5 mL of 1:1 (v/v) methanol:acetonitrile containing 1%(v/v) of acetic acid was used. The final extract was diluted 1:1 (v/v) in the mobile phase composed of 1:1 (v/v) of aqueous ammonium formiate (5 mmol L^{-1}) and methanol.

2.9. Method evaluation

To ensure that a new analytical method generates reliable and interpretable information about the sample, it should be submitted to a validation procedure in order to ensure that the method is suitable for the intended use. Parameters such as linearity, selectivity, accuracy (through recovery tests), repeatability, limit of detection (LOD) and limit of quantification (LOQ), known as analytical performance parameters, were evaluated. The linearity of the calibration curves was evaluated at concentrations of 1, 5, 10, 25, 50, 100, 200 and 250 μg L^{-1} with injections of each level in triplicate. The calibration standards were prepared in ultrapure water and analyzed by LC-MS/MS. The LOD and LOQ values for each compound was estimated from the signal to noise ratio calculated by the software of the equipment, considering at least 3 and 10 times the ratio of the signal to the noise, respectively. The method accuracy and precision were evaluated by analysis of ultrapure water spiked at 3 different concentration levels (1.0, 2.5 and 5.0 μg L^{-1}) with the studied pesticides and antibiotics. The accuracy (mean recovery) and precision (RSD%) values were obtained from LC-MS/MS analyses in triplicate of each fortification level.

3. Results and discussion

3.1. Optimized SPE method

Among the extraction materials tested were an apolar sorbent (Strata C_{18}) and two polymeric sorbents (Strata SDB-L and Strata-X). The Strata C_{18} cartridge has a C_{18} sorbent that offers strong hydrophobic retention with polar interactions from active silanol groups, offering possible places for non-polar interactions. In the same way, Strata SDB-L cartridges offer hydrophobic selectivity, because it contains a rugged polymer (styrene-divinylbenzene) sor-

bent. On the other hand, Strata-X cartridge is a functionalized polymeric sorbent that contains N-vinylpyrrolidone, which offers multiple modes of retention for stronger analyte-sorbent interactions, through π-π bonding, hydrogen bonding, dipole-dipole interactions and hydrophobic interactions. So, Strata-X increases the ability of retaining a variety of polar and non-polar compounds. This can be seen by the recovery results obtained when different sorbents (Strata C_{18}, Strata SDB-L and Strata-X) were used with blank samples spiked at 50 µg L^{-1}. Of the 87 compounds analyzed, extraction with Strata C_{18} showed recoveries between 70 and 120% (established acceptable range) for 14% of compounds and with Strata SDB-L recoveries were 17%. These results were obtained probably because of the wide range of polarity of the compounds analyzed, and the fact that these two sorbents are better used for sorption of more non-polar compounds. Meanwhile, when using Strata-X, recovery results between 70 and 120% were obtained for 90% of the analytes, demonstrating that the presence of polar and non-polar groups in the structure of the sorbent retains analytes that have great variety of polarities.

Also crucial were the elution conditions for the Strata-X cartridge, which were optimized by using acidified or non-acidified eluent, in a level of 2 µg L^{-1}. While the elution using 5 mL of a mixture 1:1 (v/v) of methanol:acetonitrile acidified with 1% (v/v) acetic acid, resulted in good recovery for 90% of compounds (Figure 2A), the use of a non-acidified mixture presented this range of recovery only for 15% of the analytes (Figure 2B). This probably happens because most of the analytes have acidic character, so they are better eluted in an acidic medium.

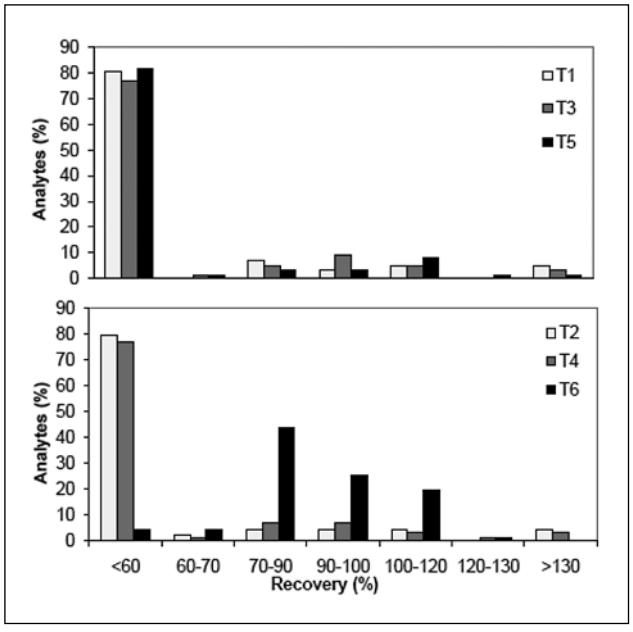

Figure 2. Percentage of analytes for different recovery ranges obtained with different conditions: with eluent not acidified (T1: C18, T3: SDB-L and T5: Strata-X cartridges) and with eluent acidified (T2: C18, T4: SDB-L and T6: Strata-X cartridges).

As the best results were obtained with Strata-X and with the acidified eluent, this method was validated for the extraction of the 87 analytes under study.

3.2. Method validation

3.2.1 Selectivity, calibration curve, LOD and LOQ

Method selectivity was evaluated through the injection of a sample without the presence of the compounds under study, comparing with a concentration level of 2 µg L^{-1}, in order to verify the presence of any interference at the same retention time or at the ions of quantification and confirmation of each analyte, according to Figure 3.

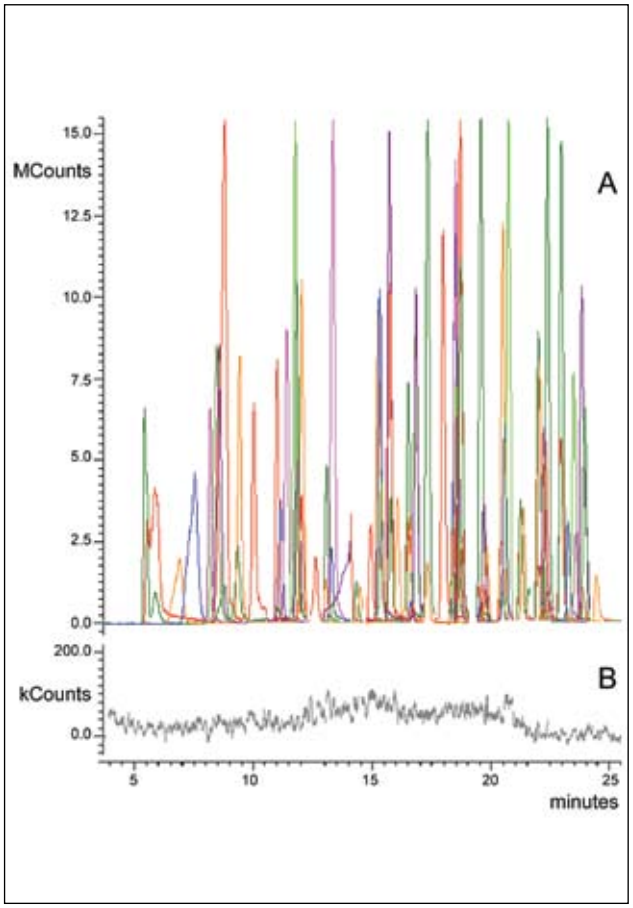

Figure 3. LC-MS/MS total chromatogram of A) a standard solution of mixed pesticides and antibiotics (50 µg L^{-1} of each compound) and B) water blank.

Most of the compounds presented excellent linearity within the studied concentration range, from instrumental LOQ values of each compound up to a concentration of 250 µg L^{-1}. However, some compounds exhibit good linearity, from instrumental LOQ values of each compound up to a concentration of 200 µg L^{-1}, as shown in Table II. Most of the compounds showed determination coefficient (r^2) values greater than 0.99, only the compounds sulfathiazole, bentazone, cyanazine, dichlofluanid, terbufos and salinomycin presented r^2 values between 0.98 and 0.99 (Table II).

Table II. LOD, LOQ, determination coefficients and linearity ranges obtained by LC-ESI-MS/MS.

Compounds	LOD_m (μg L^{-1})	LOQ_m (μg L^{-1})	Determination Coefficient (r^2)	Linearity range (μg L^{-1})
Salbutamol	0.01	0.04	0.9993	1-200
Sulfathiazole	0.06	0.2	0.9893	5-250
Oxamyl	0.01	0.04	0.9933	1-200
Sulfamethazine	0.01	0.04	0.9951	1-200
Mevinphos	0.01	0.04	0.9992	1-200
Monocrotophos	0.01	0.04	0.9996	1-200
Imidacloprid	0.06	0.2	0.9984	5-200
Methiocarb sulfoxide	0.01	0.04	0.9991	1-200
Bentazone	0.01	0.04	0.9868	1-200
Vamidothion	0.01	0.04	0.9945	1-250
Metsulfuron-methyl	0.01	0.04	0.9906	1-250
Carbofuran-3-OH	0.01	0.04	0.9924	1-250
Methiocarb sulfone	0.01	0.04	0.9984	1-200
Dimethoate	0.01	0.04	0.9990	1-200
Trichlorfon	0.01	0.04	0.9984	1-200
Sulfadimethoxine	0.01	0.04	0.9991	1-200
Thiacloprid	0.01	0.04	0.9947	1-250
Chloramphenicol	0.01	0.04	0.9962	1-250
Carbendazim	0.01	0.04	0.9926	1-250
Cyanazane	0.06	0.2	0.9883	5-250
Propoxur	0.01	0.04	0.9995	1-200
Carbofuran	0.01	0.04	0.9997	1-200
Simazine	0.06	0.2	0.9997	5-200
Thiodicarb	0.01	0.04	0.9967	1-250
Carbaryl	0.01	0.04	0.9970	1-250
Carboxym	0.01	0.04	0.9968	1-200
2,4-D	0.01	0.04	0.9961	1-200
Monolinuron	0.01	0.04	0.9991	1-250
Pirimicarb	0.01	0.04	0.9981	1-250
Paraoxon	0.01	0.04	0.9959	1-250
Metalaxiyl	0.01	0.04	0.9983	1-250
Pendimethalin	0.01	0.04	0.9933	1-250
Atrazine	0.01	0.04	0.9933	1-250
Chlorimuron	0.01	0.04	0.9982	1-250
Fluroxypyr	0.06	0.2	0.9998	5-200
2,4,5 T	0.06	0.2	0.9987	5-250
Bispyribac-sodium	0.01	0.04	0.9965	1-250
Azinphos methyl	0.01	0.04	0.9971	1-250
Azoxistrobin	0.01	0.04	0.9926	1-250
Clomazone	0.01	0.04	0.9992	1-250
Linuron	0.06	0.2	0.9938	5-250
Boscalid	0.01	0.04	0.9984	1-250
Terbutylazine	0.01	0.04	0.9949	1-250
Pyrimethanil	0.01	0.04	0.9968	1-250
Flutolanil	0.01	0.04	0.9938	1-250
Malathion	0.01	0.04	0.9980	1-250
Mepronil	0.01	0.04	0.9954	1-250
Triadimefon	0.01	0.04	0.9992	1-250
Pyridaphenthione	0.01	0.04	0.9992	1-250
Myclobutanil	0.06	0.2	0.9972	5-250
Propyzamide	0.01	0.04	0.9941	1-250
Chlorpropham	0.06	0.2	0.9919	5-250
Triazophos	0.01	0.04	0.9978	1-250
Bromuconazole	0.06	0.2	0.9945	5-250
Iprovalicarb	0.01	0.04	0.9985	1-250
Dichlofluanid	0.01	0.04	0.9803	1-200
Triadimenol	0.06	0.2	0.9988	5-250
Fluquinconazole	0.01	0.04	0.9980	1-250
Fenarimol	0.01	0.04	0.9940	1-250
Tetraconazole	0.01	0.04	0.9987	1-250
Mecarbam	0.01	0.04	0.9949	1-250
Epoxiconazol	0.01	0.04	0.9995	1-250
Fipronil	0.01	0.04	0.9953	1-250
Fenthion	0.06	0.2	0.9994	5-200
Diazinon	0.01	0.04	0.9985	1-250
Propiconazole	0.01	0.04	0.9949	1-250
Tebuconazole	0.06	0.2	0.9945	5-250
Pyraclostrobine	0.01	0.04	0.9994	1-250
Prochloraz	0.01	0.04	0.9967	1-250
Bitertanol	0.06	0.2	0.9992	5-250
Metaconazole	0.01	0.04	0.9983	1-250
Pirazofos	0.01	0.04	0.9980	1-250
Pirimiphos methyl	0.01	0.04	0.9985	1-250
Toclofos	0.06	0.2	0.9958	5-250
Profoxydim	0.06	0.2	0.9919	5-250
Difenoconazole	0.01	0.04	0.9989	1-250
Trifloxystrobin	0.01	0.04	0.9992	1-250
Triflumizole	0.01	0.04	0.9985	1-250
Profenofos	0.01	0.04	0.9995	1-250
Buprofezin	0.01	0.04	0.9995	1-250
Terbufos	0.06	0.2	0.9854	5-250
Ethion	0.01	0.04	0.9987	1-250
Chlorpyrifos ethyl	0.06	0.2	0.9979	5-250
Propargite	0.01	0.04	0.9998	1-250
Fenpropimorph	0.01	0.04	0.9995	1-250
Monesin	0.12	0.4	0.9951	10-250
Salinomycin	0.06	0.2	0.9830	5-250

The limits of detection (LOD) were established by considering the relation 3 times the signal to noise ratio (S/N) of the blank sample at the retention time of each compound. The limits of quantification (LOQ) were calculated by considering the relation 10 times the S/N ratio. Table II shows the obtained method LOD and LOQ, represented as LOD_m and LOQ_m, for each compound. Considering a 25-fold SPE pre-concentration step, the LOD_m and LOQ_m for most of the compounds were 0.01 and 0.04 μg L^{-1}, respectively, but for sulfathiazole, imidacloprid, cyanazine, simazine, fluroxypyr, 2,4,5 T, linuron, myclobutanil, bromuconazole, triadimenol, fenthion, tebuconazole, bitertanol, toclofos, profoxydim,

terbufos, salinomycin and chlorpyrifos ethyl the values LOD_m and LOQ_m were 0.06 and 0.2 µg L^{-1}, respectively. Only for the antibiotic monesin were the LOD_m and LOQ_m 0.12 and 0.4 µg L^{-1}, respectively.

3.2.2. Recovery study and precision (repeatability)

The recovery and precision results obtained with Strata-X cartridges for the three concentration levels (1.0, 2.5, 5.0 µg L^{-1}) of the pesticides and antibiotics under study are shown in Table III.

Table III. Values of recovery (%) and RSD (%) obtained for the compounds under study spiked at three concentration levels.

Compounds	Spike levels (µg L^{-1})					
	5.0		2.5		1.0	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Salbutamol	105,3	4,0	112,3	10,2	114,8	6,4
Sulfathiazole	127,9	3.4	129,3	2.0	102,3	4.7
Oxamyl	112,8	5.5	104,2	12.3	115,0	2.9
Sulfamethazine	105.3	1.3	107.5	3.6	108.1	13.1
Mevinphos	100.3	2.3	98.5	5.0	104.2	4.7
Monocrotophos	100.3	5.4	104.6	1.8	110.0	6.2
Imidacloprid	117.6	3.3	100.0	8.0	121.1	1.5
Methiocarb-sulfoxide	102.1	3.9	102.7	1.4	81.9	5.9
Bentazone	73.0	4.7	85.7	1.5	82.8	5.9
Vamidothion	67.4	1.6	68.6	4.8	80.6	11.4
Metsulfuron-methyl	117.6	1.9	110.7	4.2	105.7	6.1
Carbofuran-3-hidroxy	122.7	3.6	122.5	7.2	119.5	3.7
Methiocarbsulfone	102.9	3.1	106.0	0.3	77.7	6.3
Dimethoate	100.0	3.2	100.8	1.9	105.5	1.1
Trichlorfon	100.9	4.9	102.5	4.0	92.0	6.4
Sulfadimethoxine	113.3	7.5	106.0	0.3	101.8	7.9
Thiacloprid	100.7	3.7	98.7	3.0	106.7	5.1
Chloramphenicol	80.3	3.8	87.7	7.9	102.1	9.2
Carbendazim	97.5	4.9	95.3	4.8	88.7	5.3
Cyanazane	101.7	4.8	100.2	12.2	115.5	8.2
Propoxur	95.8	4.5	87.9	2.7	93.1	3.2
Carbofuran	95.8	1.0	94.9	1.8	95.7	3.4
Simazine	111.6	5.6	110.9	3.8	95.0	18.4
Tidicarbe	83.8	5.5	85.9	6.7	88.1	9.1
Carbaryl	94.7	2.6	93.7	4.6	97.1	5.0
Carboxym	64.0	0.2	69.8	3.0	69.2	1.7
2,4-D	96.2	2.0	104.8	8.4	78,5	7.5
Monolinuron	91.3	6.6	89.7	3.0	89.8	8.7
Pirimicarb	91.2	5.4	91.6	3.7	97.5	3.6
Paraoxon	93.9	1.0	91.4	4.0	101.8	2.2
Metalaxiyl	92.7	2.2	94.5	3.3	95.2	4.1
Pendimethalin	92.0	3.9	94.9	1.8	98.8	5.1
Atrazine	92.0	3.9	94.9	1.8	98.8	5.1

Compounds	Spike levels (µg L^{-1})					
	5.0		2.5		1.0	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Chlorimuron	99.6	2.9	94.0	3.2	83.2	16.2
Fluroxypyr	97.2	6.6	94.6	5.0	84.7	20.0
2,4,5T	94.2	4.7	91.7	7.3	79.5	19.5
Bispyribac-sodium	100.2	8.5	88.2	0.8	74.1	19.0
Azinphos methyl	87.6	5.1	85.3	5.3	89.4	5.6
Azoxistrobin	95.2	4.8	93.6	3.6	95.5	2.6
Clomazone	94.3	2.5	94.3	1.4	95.5	1.6
Linuron	89.6	13.0	93.7	4.5	106.9	1.8
Boscalid	84.7	3.0	93.6	5.7	94.4	5.4
Terbutylazine	89.7	2.3	94.0	0.9	98.9	7.7
Pyrimethanil	84.2	1.8	76.0	6.6	84.7	10.3
Flutolanil	91.4	4.2	94.5	1.3	103.6	2.4
Malathion	88.4	7.5	87.4	1.4	92.5	7.4
Mepronil	91.6	5.3	88.8	1.9	90.6	5.8
Triadimefon	86.7	3.1	79.0	2.4	97.1	6.4
Pyridaphenthione	92.0	3.3	90.3	3.9	98.4	9.5
Myclobutanil	85.5	3.6	88.3	3.8	99.9	7.4
Propyzamide	93.5	1.3	101.6	2.9	107.1	7.1
Chlorpropham	96.3	2.8	105.9	11.9	114.9	8.8
Triazophos	88.4	6.3	86.8	1.9	92.5	5.7
Bromuconazole	92.2	1.2	87.6	10.1	94.2	0.4
Iprovalicarb	94.1	4.3	95.6	1.2	100.6	9.1
Dichlofluanid	91.8	4.3	95.0	4.3	27.7	6.8
Triadimenol	81.5	2.3	74.2	6.4	75.2	5.4
Fluquinconazole	94.9	14.6	88.6	6.8	53.4	4.2
Fenarimol	96.5	10.5	89.3	3.6	101.4	11.6
Tetraconazole	90.9	3.2	87.1	3.2	103.1	8.7
Mecarbam	89.9	6.9	89.2	1.2	78.5	11.5
Epoxiconazol	94.7	4.1	86.0	2.3	99.8	6.8
Fipronil	85.4	7.3	94.6	1.8	108.1	3.3
Fenthion	87.2	8.7	79.5	8.0	93.5	10.1
Diazinon	91.3	6.6	92.8	3.3	92.8	2.1
Propiconazole	86.2	5.5	82.5	2.2	95.5	4.0
Tebuconazole	96.6	21.8	111.0	10.8	123.1	8.4
Pyraclostrobine	83.9	6.0	75.1	2.4	75.9	8.5

Compounds	Spike levels (μg L^{-1})					
	5.0		2.5		1.0	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Prochloraz	90.6	7.2	80.4	3.4	96.2	7.7
Bitertanol	91.2	8.2	82.0	5.8	88.7	6.9
Metaconazole	88.4	8.3	90.5	5.7	86.6	5.6
Pirazofos	84.1	4.6	79.6	6.6	90.4	3.9
Pirimiphos methyl	87.5	6.8	76.8	5.5	91.4	3.9
Toclofos	130.2	17.8	127.1	19.4	72.0	6.6
Profoxydim	73.6	14.9	76.0	11.9	81.9	19.4
Difenoconazole	87.9	7.4	78.1	4.7	89.3	6.5
Trifloxystrobin	90.0	14.6	85.2	3.3	85.0	7.3
Triflumizole	83.4	11.9	81.8	1.7	93.3	7.3
Profenofos	97.6	11.0	88.2	4.8	96.0	3.7
Buprofezin	92.0	13.0	81.8	4.5	84.9	9.0
Terbufos	94.0	8.4	75.3	7.3	81.9	12.3
Ethion	65.8	8.3	60.9	4.6	65.6	8.7
Chlorpyrifos ethyl	55.6	2.2	47.7	12.3	65.5	10.4
Propargite	54.6	1.5	51.2	9.0	47.4	8.6
Fenpropimorph	78.4	10.5	68.9	5.7	65.0	9.4
Monesin	69.5	5.8	55.8	9.2	80.5	17.9
Salinomycin	73.2	9.5	70.5	9.1	85.8	15.2

The results showed recovery percentages between 70 and 120% for 87.4% of the compounds in the three fortification levels. Only 8.0% of the compounds exhibited recoveries below 70% for at least one level of fortification and 4.6% of the compounds presented values higher that 120%. According to Table III precision values were satisfactory for the majority of the compounds studied since RSD% values were lower than 20%.

4. Application of the developed method

The developed method was applied to the determination of pesticide and antibiotic residues in two water samples (A and B), obtained from the Jacuí river in the city of Ernestina-RS. In both samples residues were quantified above LOQ_m for the insecticides propoxur (27.2 and 51.0 μg L^{-1}) and buprofezin (17.1 and 104.0 μg L^{-1}) and for the herbicide clomazone (59.7 and 23.2 μg L^{-1}). However, only sample A presented the herbicide simazine at 77.2 μg L^{-1}. In the samples analyzed the presence of antibiotics was not detected. Figure 4 shows the MRM chromatograms of a water sample analyzed by LC-MS/MS, containing residues of the pesticides propoxur, clomazone, buprofezin and simazine, with the transitions employed for quantification and confirmation.

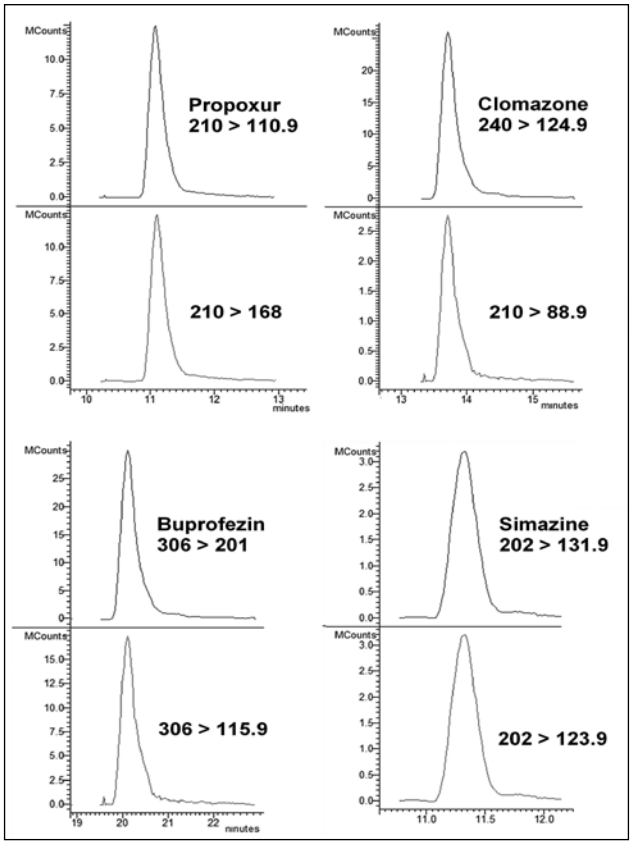

Figure 4. MRM chromatograms obtained from positive samples analyzed by LC-MS/MS, containing residues of the pesticides propoxur (51.0 μg L^{-1}), clomazone (59.7 μg L^{-1}), buprofezin (104.0 μg L^{-1}) and simazine (77.2 μg L^{-1}).

5. Conclusions

MS/MS coupled to liquid chromatography provides the highest degree of certainty in the identification of target compounds. The selection of specific fragmentations for MRM in the MS/MS mode, with optimization of cone voltage and collision energy, guarantee a high degree of selectivity as well as additional sensitivity enabling the determination of trace levels of pesticides and antibiotics in water samples. LC-MS/MS showed good linearities for the studied pesticides, as well as excellent precision. The Strata-X cartridges allowed good recoveries (70 to 120%) for 86% of the pesticides and antibiotics analyzed. LOD and LOQ values for most of the compounds were 0.01 and 0.04 μg L^{-1}, respectively. This method has been successfully applied for the determination of pesticides and antibiotics residues in river water samples. The pesticides propoxur, clomazone, buprofezin and simazine were present at concentration levels between 17.1 to 104.0 μg L^{-1} in superficial water samples, but no residues of the evaluated antibiotics were found.

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References

1. McGlinchey, T.A.; Rafter, P.A.; Regan, F.; McMahon, G. P. *Anal. Chim.Acta*, 2008, 624, 1.
2. Stolker, A.A.M.; Brinkman, U.A.Th. *J. Chromatogr. A*, 2005, 1067, 15.
3. Torre, A., Iglesias, I., Carballo, M.; Ramírez, P., Muñoz, M.J. *Sci. Total Environ.,* 2012, 414, 672.
4. Baran, W.; Adamek, E.; Ziemianska, J.; Sobczak, A. *J. Hazard. Mater., 2011, 196, 1*.
5. Villanneau, E.; Saby, N.P.A.; Marchant, B.P.; Jolivet, C.C.; Boulonne, L.; Cara, G.; Barriuso, E.; Bispo, A.; Briand, O.; Arrouays, D. *Sci. Total Environ*., 2011, 409, 3719.
6. L.; Vanhaecke, L.; Reybroeck, W.; Ooghe, S.; Croubels, S. *J. Chromatogr. A*, 2009, 1216, 7964
7. De Brabander, H.F.; Noppe, H.; Verheyden, K.; Vanden Bussche, J.; Wille, K.; Okerman, *J. Chromatogr. A*, 2009, 1216, 7964.
8. Shaaban, H.; Górecki, *Anal. Chim. Acta*, 2011, 702, 136.
9. Gentili, A.; Perret, D.; Marchese, S. *TrAC*, 2005, 24, 705.
10. Zhou, L.J.; Ying, G.G.; Zhao, J.L.; Yang, J.F.; Wang, L.; Yang, L.; Yang, B.; Liu, S. *Environ. Pollut*., 2011, 159, 1877.
11. Ok, Y.S.; Kim, S.C.; Kwon, R.K.; Lee, S.S.; Moon, D.H.; Lim, K.J.; Sung, J.K.; Hur, S.O.; Yang, J.E. *Environ. Monit. Assess*., 2011, 174, 693.
12. Kienhuis P.G.M.; Geerdink, R.B. *TrAC*, 2000, 19, 249.
13. Zwiener, C.; Richardson, S.D. *TrAC*, 2005, 24, 613.
14. Jones-Lepp, T.L.; Momplaisir, G.M. *TrAC*, 2005, 24, 590.
15. Trufelli, H.; Palma, P.; Famiglini, G.; Cappiello, A. *Mass Spectrom. Rev.*, 2011, 30, 491.
16. Cappiello, A.; Famiglini, G.; Palma, P. Trufelli, H. *J. Liq. Chromatogr. Relat. Technol.,* 2010, 33, 1067.
17. Viglino, L.; Aboulfadl, K.; Prevost, M.; Sauve, S. *Talanta*, 2008, 76, 1088.
18. Majzik E.S.; Toth, F.; Benke, L.; Kiss, Z. *Chromatographia*, 2006, 63, S105.
19. Sabin, G.P.; Prestes, O.D.; Adaime, M.B.; Zanella, R. *J. Braz. Chem. Soc.,* 2009, 20, 918.
20. Demoliner, A.; Caldas, S.S.; Costa, F.P.; Gonçalves, F.F.; Clementin, R.M.; Milani, M.R.; Primel, E.G. *J. Braz. Chem. Soc*., 2010, 21, 1424-1433.
21. Primel, E.G.; Zanella, R.; Kurz, M.H.S.; Gonçalves, F.F.; Machado, S.O.; Marchezan, E. *Quim. Nova*, 2005, 28, 605.
22. Zanella, R.; Primel, E.G.; Gonçalves, F.F.; Martins, M.L.; Adaime, M.B.; Marchezan, E.; Machado, S.L.O. *J. Braz. Chem. Soc*., 2008, 19, 987.
23. Caldas, S.S.; Demoliner, A.; Primel, E.G. *J. Braz. Chem. Soc*., 2008, 19, 987.
24. Caldas, S.S.; Demoliner, A.; Primel, E.G. *J. Braz. Chem. Soc*., 2009, 20, 125.
25. Zanella, R.; Primel, E.G.; Machado, S.L.O.; Goncalves, F.F.; Marchezan, E. *Chromatographia,* 2002, 55, 573.
26. Carvalho, J.J.; Jerónimo, P.C.A.; Gonçalves, C.; Alpendurada, M.F. *Anal. Bioanal. Chem.,* 2008, 392, 955.
27. Kowal, S.; Balsaa, P.; Werres, F.; Schmidt, T. C. *Anal. Bioanal. Chem.,* 2009, 395, 1787.

Point of View

Analytical chemistry in Brazil, open to the world?

Attending a scientific conference in Brazil, like the last ENQA in Campos do Jordão, is a revitalising experience for somebody coming from 'old' Europe, where the environment scientists are working in is increasingly harsh and competitive due to the combined effects of the never-ending economic crisis and the long transition from the post-war where governments acted as patrons to one where the onus is on scientists to constantly seek funding. Perhaps my initial, somewhat naïve, impression was influenced by the myth of America as a land of promise, and discussions with Brazilian colleagues on 'day-to-day' science tempered it. Nevertheless, seeing so many motivated young scientists, such a high percentage of women, and the energy and commitment of the organisers has borne out my initial positive impression.

A second striking impression is that Brazilians share a strong feeling of developing a 'national science'. Of course, the size of the country and the strength of its economy allows for this. It is no secret that scientific research is often driven not solely by curiosity but often by local interests. Even when pure curiosity was probably a stronger driving force in science than nowadays, discoveries were linked to national characteristics. Consider, for example, the high number of chemical elements discovered in Sweden thanks to the importance of the mining industry at the time the discoveries were made. The link between national 'interests' and scientific research peaked during the Second World War and the Cold War, when military and economic interests drove research both in the USA and the USSR, but it is still present, with China verging on overtaking the USA as a scientific power. It is thus understandable that in an emerging economy such as Brazil, research is closely tied to local characteristics and needs, such as the agribusiness industry (more than 35 % of Brazilian exports) or oil production (Petrobas is Latin America's largest company). This is particularly apparent in a discipline such as analytical chemistry which, by its very nature, spans basic and applied science and is crucial to 'getting the science right' in many different fields.

However, while it is not independent of geopolitical realities, science is by definition a human endeavour that does not respect borders; free communication of scientific ideas and results is part and parcel of scientific research. Research, whether guided by pure curiosity or by commercial interests, builds on the knowledge of those who came before us, irrespective of their origin. As Isaac Newton reminded Robert Hooke, "If we have seen further, it is by standing on the shoulders of giants". For both sharing our own results and keeping up with advances, a lingua franca in science, such as English is nowadays, should be considered an asset rather than an imposition or a nuisance, as I got the impression is sometimes the case in Brazil today. I believe that it is absurd to resist the use of English in science, be it on the grounds of 19th century nationalism, 20th century anti-imperialism or 21st century anti-globalization. In my own country I was myself forced to attend school and university in a language that was not my mother tongue, and so am very sensitive to the importance for the speakers of any language of being able to use it in any field of human endeavour, including science. However, there is no contradiction in using English to share knowledge with the research community while using your Portuguese, and my Catalan, in other key aspects of science such as informing political decisions, providing education and popularising science. Such an approach will be of benefit to both our countries and to ourselves.

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Analytical Chemistry in Brazil: growing and moving forward

It is worldwide acclaimed that Brazil is one of the biggest economies in the world in the beginning of the 21st century and this remains as a clear trend for the decades ahead. Certainly analytical chemistry has a critical role for supporting this growing economy and there is a critical demand for new analytical procedures and instrumentation for quality control of processes and products. Just thinking about two critical needs, all food chain from farm to fork and all exportation of agricultural and industrialized products are seriously dependent on chemical analysis. The 16th Brazilian Meeting in Analytical Chemistry with around 1200 attendees and 1000 oral and poster presentations was a superb demonstration of the Brazilian strength in this area and its maturity to support our economical development. The quality of the research in this area was recently demonstrated by Ferreira et al. by searching the Web of Science Database for papers produced by Brazilian authors from 1945 to 2009 and showing that 70% of these papers were published in journal with impact factors greater than 2 [1]. However, we have big challenges and many serious situations, for instance 52% of teachers of chemistry in elementary and high schools do not have a major degree in chemistry and 87% of population from 18 to 30 years-old do not have access to universities. So, we may say that a lot was successfully done but we still have a huge step towards a society with equal opportunities for all citizens. No doubts analytical chemistry community is ready to support this giant step and we may emphasize some needs such as the development of local instrumentation, a strong and permanent support to a national center for production of certified reference materials, and induction for fast evolution of specific areas, as for example speciation analysis, and simple and straightforward tools for health diagnostics. However, all continuous development must be carefully and gradually done. Recently Senise pointed out that "Today, there are many more PhDs than years ago, and I think this number has to grow. We have to go further, but with quality. There must be more investment in qualification and research. And we cannot do it in a hurry: in science, maturation takes time and hard work." [2]. Similar views were mentioned by Collins: "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" [3].

Once again it may be concluded that "analytical chemistry in Brazil is healthy and growing, and is ready to support the new role of the country as an emerged power" [4]. This aspect was endorsed by Barnes by saying that "I cannot agree more strongly with this prediction" [5]. Certainly we have a wonderful horizon for growing and our biennial meetings at ENQAs are a privileged platform for seeing the scenario and meeting each other. I am looking forward to meeting you all in Minas Gerais at the 17th ENQA. Let us keep working and moving forward! It has been fun and full of achievements.

As a final note, I kindly suggest you may (re)think about the distinction of analytical chemistry and chemical analysis [6] and how the understanding of these parallel routes can make us even stronger and better understood.

References

1. Ferreira, S. L. C.; de Jesus, R. M.; Cavalcante, D. D.; Leão, D.; Portugal, L. A.; dos Santos, W. N. L.; de Araújo, N. M. L.; dos Santos, A. M. P.; Santos, N. C. F.; *Braz. J. Anal. Chem*. 2010, 1, 75.
2. Senise, P. E. A.; *Braz. J. Anal. Chem.* 2011, 1, X.
3. Collins, C. H.; *Braz. J. Anal. Chem*. 2010, 0, XV.
4. Rocha, F. R. P.; Pereira-Filho, E. R.; Nóbrega, J. A.; *J. Braz. Chem. Soc.* 2009, 20, 1759.
5. Barnes, R. M.; *Braz. J. Anal. Chem.* 2010, 1, XI.
6. Senise, P. E. A.; *Quim. Nova* 1993, 16, 257.

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Internet-based Analytical Chemistry Teaching Resources

In October I had the opportunity to attend ENQA 2011 in Campos de Jordão. The meeting was a wonderful introduction to the vibrant analytical chemistry community of Brazil. At the conference, I spoke about the Analytical Sciences Digital Library (ASDL) a freely accessible resource for students, educators and practitioners of analytical chemistry available at www.asdlib.org. During the 2 months prior to the ENQA conference, the ASDL website was accessed by only 45 users in Brazil, placing it 23rd in terms of countries accessing ASDL. I was very pleased that following the ENQA meeting for same period 380 Brazilian users accessed materials in the ASDL collection, and Brazil ranked 4th in countries accessing the site. It was gratifying to see that so many scientists found ASDL to be useful!

With the ubiquitous nature of the Internet, electronic resources are easily available and at no cost. Many of the resources available on the ASDL website can be used for learning about instrumentation and the application of instrumental methods. A particularly valuable resource is David Harvey's e-text, Analytical Chemistry 2.0. This comprehensive text covers instrumental topics including spectroscopy and chromatography, as well as the basics: statistical treatment of data, standardization/calibration, equilibrium chemistry, gravimetric and titrimetric methods. Unlike many web-based resources Analytical Chemistry 2.0 has the look and feel of a "real" textbook. Each of the 15 chapters, available as a free downloadable pdf, includes detailed graphics, example problems and embedded hyperlinks that provide functionality not available in a printed textbook. Although the language of instruction in Brazil is Portuguese, science students, especially graduate students, need to be able to use the English language, and ASDL resources can help hone their language skills while teaching basic analytical chemistry concepts.

At the ENQA conference I had several discussions with faculty about the use of problem-based or active learning methods for teaching analytical chemistry. I was pleased to learn than many Brazilian professors also embrace this approach. A major focus of ASDL is the development of resources to support instruction using active learning (http://www.asdlib.org/ActiveLearning.php). Engaging students in practical, real world applications is a great way to teach basic concepts of analytical chemistry and, because of their interest in the problem, students are more likely to retain the knowledge gained. It can be difficult as an instructor to give up the controlled environment of the lecture for the chaos that comes from having students engaged in group problem solving, however the rewards for both faculty and students make the risk worthwhile. Another limitation of problem-based or active learning methods is that they are time consuming. In transitioning to this teaching style, faculty may discover that they have to sacrifice coverage of some topics, however, since we can now access facts and details about instruments and methods anywhere at any time via the Internet, this type of knowledge is no longer of great value. Instead of teaching facts that will soon be forgotten, we should focus on teaching our students how to use knowledge to solve problems – a skill that will make them valuable to their future employers and to society.

Prof. Cynthia K. Larive
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XVI BMIC / III LABIC – XVI Brazilian Meeting on Inorganic Chemistry / III Latin American Meeting on Biological Inorganic Chemistry
Florianópolis, SC – August 13-17, 2012
www.bmic2012.ufsc.br

The 30th World Congress of Biomedical Laboratory Science
Berlim, Germany - August 18-22, 2012
http://www.ifbls-dvta2012.com/

ANNUAL ISE MEETING – 63rd Annual Meeting of the Internacional Society of Electrochemistry
Prague, Czech Republic - August 19-24, 2012
http://event12.ise-online.org/index.php

4th International IUPAC Conference on Green Chemistry
Foz do Iguaçu, PR - August 25-29, 2012
http://www.iupac.org/publications/ci/2011/3302/ca9_250812.html

ENTEQUI – 5º Encontro Nacional de Tecnologia Química
Maceió, AL – August 27-28, 2012
http://www.abq.org.br/entequi/

Rio Oil & Gas Expo and Conference
Rio de Janeiro, RJ – September 17-20, 2012
http://www.riooilgas.com.br

ITP 2012 – 19th International Symposium, Exhibit & Workshops on Electro- and Liquid Phase-separation Techniques
Baltimore Inner Harbor - Maryland, USA - September 30–October 3, 2012
http://itp2012.okstate.edu/index.shtml

CBQ – 52º Congresso Brasileiro de Química
Recife, PE – October 14-18, 2012
http://www.abq.org.br/cbq/

BrazMedChem 2012 – The 6th Brazilian Symposium on Medicinal Chemistry
Canela, RS – October 28-31, 2012
http://www6.ufrgs.br/brazmedchem2012/index.php

6th Congress of the International Society of Nutrigenetics/ Nutrigenomics (ISNN)
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http://www.isnnbrazil.org.br/

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6. Regitz, M. in *Multiple Bonds and Low Coordination in Phosphorus*

Chemistry; Regitz, M.; Sherer, O.J., eds.; George Thieme Verlag: Stuttgart, 1990, Chap. 2.

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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:***

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

- ***Theses:***

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

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

11. Ferreira, A. B.; Brito, S. L.; *Resumos da 20ª Reunião Anual da Sociedade Brasileira de Química,* Pocos de Caldas, Brazil, 1998.

- ***Internet pages:***

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

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