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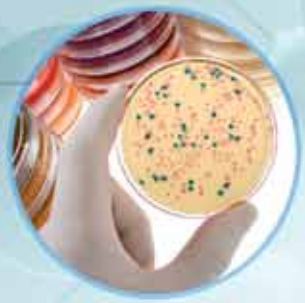

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WHAT IS GOING ON WITH BRAZILIAN ANALYTICAL CHEMISTRY?

In the latest year several important and renowned Brazilian analytical chemists passed away. The last passing Professor Adilson Curtius, occurred recently. Many researchers in Analytical Chemistry are well recognized in the community, but only some of them exhibit a natural leadership in the area. Analytical Chemistry in Brazil has grown in a significant way, as observed in the Brazilian Analytical Chemistry meetings and the number of papers published in the specialized journals. The consequence of this is the development of Analytical Chemistry in all directions by individual initiatives. In terms of science, this fact should be considered good, because scientists should have freedom to investigate anything they intend. Nevertheless, it is important to have goals to spend a lot of funds in researches, keeping in mind the necessity to bring some benefits to the society. We have to consider that the government and companies are investing funds expecting for the science progress to improve the quality of life. The definition of the right strategies is complex and requires leaders, usually senior researchers, who might show the most important directions to follow.

What we can see among the young researchers is the attempt to publish and of course doing investigations that can be published in international journals. Most of them are putting in practice what they learned in their PhD and post doctorate programs, but without creating and/or innovating. Besides educating people to do research to publish, we need to show to students and young researchers what our society and/or nation need to improve the quality of life of the population. In the latest years we have seen the appearance of many journals to fulfill the interest of some groups, creating their own impact index to show that they are producing impact researches.They are just using numbers to cause impact without considering quality, making up the science. Probably, we are reaching the limit to believe in the publication system and we need to think about how to keep the credibility of a scientific paper. Thus, the quality of the human resources formation is the most important point in the role of the universities.

The establishment of the right strategy is not an easy task, but it is important to have a forum of discussions involving academia, industries, government and society conducted by the leaderships. Brazilian Journal of Analytical Chemistry has just come to proportionate the discussion in Analytical Chemistry giving opportunities to people discuss and put their opinion about the topics related to the area, not only publishing papers.

At the moment when new young researchers reach a leadership position it is important to think about the role of the old generation and search for activities to put Analytical Chemistry in the highlighted position. We have to take this opportunity to stress the role of people that had initiative to create Brazilian Analytical Chemistry Meeting, for example, like Prof. Curtius, and what it represented to Analytical Chemistry. Thus, the responsibility is ours in putting Analytical Chemistry in a top level as taught by the old leaders.

Professor Lauro Tatsuo Kubota
Editor-in-chief

Editorial Board

Summary

Expedient

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From Laser Desorption to Image Generation

In the early 1960s a technique based on the ionization of organic molecules by irradiation with a high-intensity laser pulse was developed. This process successfully produced ions of different structures, which could be analyzed by means of a mass spectrometer. This technique was designated laser desorption. In 1987, Michael Karas and Franz Hillenkamp succeeded in demonstrating the application of an organic matrix in laser desorption studies targeting the analysis of high-molar mass compounds. The matrix should display strong absorbance at the selected wavelength and be easily to sublime.

MALDI started being used in 2005 for the analysis of substances of lower molar mass. One decade after its first prototypes, MALDI *imaging* mass spectrometry became a unique technique among the several procedures that were employed in proteomics and biomarker studies targeting the investigation of a variety of diseases. This technique is based on the possibility of MALDI spatial acquisition with good resolution, and it relies on the fact that the ion intensities (X axis in the spectrum) can be correlated with a color scale. The tissue is then photographed, and further superimposition of ion intensity data onto the irradiated area gives an indication of the amount of a certain analyte that is present in a determined space region.

In the coming years the MALDI-*imaging* technique will significantly alter clinical practice, including complementing histopathology-based diagnosis with metabolic profile analysis, early detection of disorders, selection of therapeutic approaches, and real-time evaluation of therapeutic efficiencies and toxicities, among other benefits. In Brazil, where there is a traditional community of researchers working in the area of natural products, there might be a great impact on studies aiming at a better understanding of secondary metabolite control and compartmentalization. From 1968, when the natural products chemist Prof. Dr. Otto Richard Gottlieb wrote his first book on mass spectrometry, to date there has been rapid progress in spectrometric techniques, and each new development furnishes novel information about secondary metabolism dynamics. Will the MALDI-*imaging* system pave the way for the establishment of new rules for natural products functioning? We can only hope that this major technological advance can provide answers to various hypotheses, such as some put forward by the great researcher Otto R. Gottlieb.

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In memory of Adilson José Curtius

Professor Adilson José Curtius died at the age of 67 on November 5, 2012, from intestinal complications due to a treatment against leukemia. Professor Adilson was born in a small town in the interior of Santa Catarina, named Trombudo Central. He was educated at home, and at age 10 began his studies in Rio do Sul, at the Don Bosco College, and later at the College of Santa Catarina in Florianópolis. He graduated in Chemical Engineering from UFRGS (Universidade Federal do Rio Grande do Sul) in 1968, obtained a master's degree in Chemical Engineering from the Lehigh University (1971) and a PhD. in Inorganic Analytical Chemistry from PUC-Rio (Pontifícia Universidade Católica do Rio de Janeiro) in 1974. He obtained a post doctorate in Germany (1986) and was a professor at PUC-Rio and UFRRJ (Universidade Federal Rural do Rio de Janeiro) until 1994. Recently, he was a volunteer professor at the Federal University of Santa Catarina (UFSC).

He specialized in atomic spectrometry, especially in ICP-MS (inductively coupled plasma mass spectrometry) and AAS (atomic absorption spectrometry), and developed analytical methods for the determination of trace elements in geological, biological and industrial samples. In 1974,coming from the UFSM-RS (Universidade Federal de Santa Maria) as a post-graduate student of the Department of Chemistry at PUC-Rio, I met Professor Adilson at the public defense of his doctoral thesis, and soon realized that I had met a great person that later became my colleague and director of the Department of Chemistry at PUC-Rio. Professor Adilson had a very active participation as a member of the scientific community, contributing to the SBQ (Sociedade Brasileira de Química), the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) in Brazilian government. Whenever we played,we argued about "who was born in a smaller town", me, in Tupanciretã-RS, or him, from Trombudo Central-SC.

Most of my professors in the Department of Chemistry at PUC-Rio came from Germany, among them the noted Professor Klaus E. Wagener, professor Adilson's PhD advisor in 1974. The other teachers present at the time were professors Norbert Fritz Miekeley, of Radioanalytics, and Erich L. Minzl,of Advanced Analytical Chemistry. Professor Minzl mastered the knowledge and use of the first atomic absorption equipment of our Department. I believe this brand new unit at the time was the influence of professor Adilson, with his interest in this research area of analytical chemistry, atomic absorption spectrometry.

Receiving the early guidance of professor Adilson in this area were my fellow masters, Jandira da Silva e Souza, Ana Maria Teixeira Costa Horta and his sister Ana Lucia Soldan. In the following years, professor Adilson led a large number of researchers, during his time at PUC-Rio, who later came to occupy prominent positions in their universities or research centers in the area of Analytical Chemistry. During his time at the UFSC in Florianópolis, professor Adilson guided a large number of new doctors, including professor Tatiana Dillenburg Saint'Pierre, our current professor of Analytical Chemistry here at PUC-Rio.

Professor Adilson was a professor at PUC-Rio, from 1971 until the mid-90s, when, for family reasons, he moved to UFSC in Florianópolis. In the early '80s, he was one of the founders of the First and Second National Meetings of Analytical Chemistry, which became known as ENQA, and then, alongside professor Bernhard Welz, he organized an international symposium that became known as the Rio Symposium on Atomic Spectrometry. Professor Adilson certainly was a leading expert in atomic absorption spectrometry, was a 1A CNPq researcher, member of the Brazilian Academy of Science and author of numerous scientific papers. He was recognized as an outstanding researcher in our country, as well as internationally, and was recently honored by his hometown community.

Dr. Pércio Augusto Mardini Farias
Associate Professor at PUC-Rio

Interview

Francisco Radler de Aquino Neto has always been teaching, since graduation. The full professor in the Chemistry Institute of Universidade Federal do Rio de Janeiro (UFRJ) attended his postdoctoral program at Strasbourg, in the Université Louis Pasteur, and in Cambridge University. At UFRJ, he coordinates nothing less than eight different laboratories, ranging from technological support to chromatography, geochemistry, calibration, residue analysis. There he has been doing research even before graduating, in undergraduate research programs. He has oriented undergraduate, graduate and postdoctoral students in more than 188 finished research projects, and 37 are in progress under his supervision. The broad scientific content in his research line is evident: the fields of knowledge range from the oil industry to the doping control in sports, from air quality to toxicology, from forensics to pharmacology. From this observation, one could assume that his contribution is shallow: on the contrary, Aquino Neto shows us, in this very interesting interview, his consistent view of the need of quality in analytical chemistry in Brazil, discussing the certification of laboratories, the experience of studying abroad and the teaching of chemistry in elementary school with a critical view.

Noteworthy is the number of undergraduate students who you have oriented in research projects. Since when do you develop this work and how the undergraduate research programs have evolved in Brazil, in your opinion?

I started to do that during my graduate program, when nearly all colleagues in the Shalechemistry Project (PXQ) at Chemistry Institute, Universidade Federal do Rio de Janeiro (UFRJ), had undergraduate research project (URP) students under their responsibility. Of course, this has been intensified after my return from postdoctoral fellowship, in 1982. The helping factors were: 1. The possibility to obtain scholarships specifically for URP, 2. The possibility to obtain scholarships for these students by means of the so-called "integrated support" by the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico). In this modality of funding, the main researcher could obtain financial support for the research and for members of the research team, and 3. More recently, the opening, by FAPERJ (Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro), of a scholarship modality named "Scientists of our State", which grants a monthly value to the best scientific projects each year and which can be 100% used for URP students grants.

From the late 80's, I was also favored by the laboratory model of sustainability (which provides high technological content services), which guarantees that the lab has its own resources, also used to hire technicians. As these are still undergraduate students in many cases, they can also perform URP.

This is why the number of students/trainees in URP under my guidance is so high. I remember the fantastic

> **Technicians and trainees who are also engaged in undergraduate research programs are split between those directly inserted in better-paid positions in the productive sector and the academic field**

Moscow Olympic Games: 30 years event

initiative of professor Leopoldo de Meis, in UFRJ, of gathering tens of URP students as well as my own undergraduate experience. Prof. Claudio Costa Neto organized a task force of 30 undergraduate students, to synthesize hydrocarbon standards in the Shalechemistry Project (PXQ). At that opportunity, more than 70 substances were prepared in less than one year.

About the URP programs in Brazil, unfortunately I have only my local view. In this respect I consider a breakthrough the PIBIC Program by CNPq (the Institutional Program of URP scholarships at CNPq) and related programs. It has democratized the access to grants for beginners, who still have less competitive curricula to meet the distributive model that classifies, numerologically, the researchers in this country.

How does the career of students of chemistry who do undergraduate research develop? What does this experience mean in their lives?

The URP student usually advance to graduate programs, and a good number of them enters the career of Universities' teaching staff. However, with the recent investments in traditional institutions of research and development and in the chemical industry (particularly oil), these most financially attractive options have drained this manpower. Technicians and trainees who are also engaged in URP programs are split between those directly inserted in a better-paid position in the productive sector and the academic field.

What does the experience of orienting a URP student represent in the life of a professor?

This is probably the best material to work with. These students still have few training vices, and they can be guided according to the didactic and scientific beliefs of the adviser.

Is doping control an area of outstanding performance in analytical chemistry? Can it be an important field of activity in the professional market?

Doping control is a very narrow niche

of professional performance, considering that there are only 33 laboratories accredited by the World Anti-Doping Agency (WADA) worldwide. In Brazil alone there are over 12,000 clinical laboratories and dozens of forensic laboratories, and an intrinsic quality must be achieved by these laboratories to pass this "funnel". The advanced training provided by this activity is a possibility asset and would be a good choice for those who like to dwell in the frontiers of analytical chemistry.

In this background, we have become the trainers of skilled professionals who are hugely demanded by the market. Thus, we "lose" many of our employees to more advantageous positions, not only in the private sector, but also in the R&D public system itself, which is in frank recovery, including salary levels – in contrast to the freezing careers in the university.

What is the main difficulty in doping control?

I would say that doping control is a major, if not the greatest, analytical challenge of our times. First, because it is necessary to work with the ISO17025 accreditation by Inmetro (Instituto Nacional de Metrologia, Qualidade e Tecnologia) and by WADA criteria, which are even more rigorous. Besides, the universe of organic molecules covers the entire molecular spectrum. Thus, there are 15 different classes of drugs involved (for now) going from simple things like methylhexanamine to steroids, polysaccharides, proteins

Event in Ghent, Belgium

such as albumin, erythropoietin, etc. There is a whole body of knowledge on pharmacology and toxicology involved with doping control, and the chemists need to deal with phase 1 and phase 2 metabolites, dietary supplements, pro-drugs, natural products and the possibility of cases of abuse employing exogenous administration of endogenous substances – not mentioning the backyard laboratories synthesizing molecules to cheat the system.

What is the reason for the research laboratories in Analytical Chemistry in Brazil not being accredited/certified in their overwhelming majority? In your opinion, should they be accredited?

Every lab should be accredited. When we started to carry out the doping control in 1989, the rationale we used to obtain the approval of the Organic Chemistry Department to perform this activity was that the University should get exposed, and be subjected to evaluation criteria of ordinary mortals. The proposal was that only with an evaluation of third party, we could beat our chests and say that excellence lies in the Academy. But the fact is that this illusion was dispelled immediately. When we do research and publish, we are unaware of how fragile is our

"Doping control is a very narrow niche of professional performance, with only 33 accredited laboratories worldwide. And it is maybe the greatest analytical challenge of our times."

> **One challenge in the analytical capacity of biological samples today is how to deal with the action of specialized groups in synthetizing molecules that escape the current forms of control and known targets**

guarantee of the accuracy of the measurements and identifications that we perform, and the qualifying criteria we employ. Only with a system of accreditation as ISO17025, with multicenter trials, with the use of certified reference materials and other guarantees of traceability of measurements is that the quality of work can be guaranteed.

For you to get the whole picture, to keep a WADA accreditation we are subject to three sets of six test samples per year (which may include zero, one or more substances or metabolites of banned substances). In addition, there are "educational samples", used to calibrate the performance of laboratories. But the most difficult is the following: we have to undergo two double-blind tests annually; that is, in the accreditation testing, sets of samples we receive from our customers include "disguised" control samples from WADA . Therefore, each of the more than 5000 samples we analyze each year (each processed by seven different screenings, therefore, 35,000 analysis procedures) must be executed with the same rigor, because it may be a WADA control sample!

Back to your question: everyone should be accredited, but it is a process that requires enormous effort and cost to be implemented and constant vigilance.

What are the difficulties and challenges in biological samples analysis?

Generically, the analysis of biological samples requires learning

Lecturing in the event in Ghent

the peculiarities and the diversity of biological matrices, in which the transformation of substances through the metabolic processes would be the biggest challenge. In relation to doping control in sport, specifically, the challenges are the diversity of active ingredients (many of them have never reached the pharmaceutical market, but are inspired by early publications and patents on drugs with pharmacological activity), metabolism, influence of different matrices (in doping control in sports, currently, only urine and blood) and more recently the action of specialized groups in synthesizing molecules that escape the current form of control, which is the analysis oriented to target molecules (target-oriented analysis). These issues lead the analytical chemistry activity to the limit of the existing analytical capacity (molecular). Several laboratories (including ours) are already looking for options to perform comprehensive analyzes that do not seek only specific and known targets, but which can pinpoint suspect foreign substances on a population average basis.

Large companies have developed programs for prevention and treatment of substance abuse. These programs require rapid tests, sensitive, and prefer-

Speaking in an event in Rio de Janeiro (Rio Metrologia)

ably specific. What has been the development of techniques in this area?

It is a system infinitely simpler than the doping control in sports. Drug abuse programs in companies allow the analysis of a few substances and in many cases simple tests of classes of prohibited substances. If there is a suspicion, then a more elaborate process of confirmation can be undertaken. Therefore the vast majority of the evaluations are performed by diagnostic kits, which makes these tests a simple routine. In doping control in sports, each sample is considered a "research topic".

On the other hand, in Brazil, these tests represent a growing market, where the problem of analytical quality is not crucial. Much more important is the issue of traceability of the collected sample. Blood and urine samples can easily be fraudulent, because there is no rigorous collection, such as in doping control in sports. Even hair can be manipulated to escape detection of certain substances.

Your resume shows a broad area of professional and academic activity, which involves spectrometry, chromatography, magnetic resonance spectroscopy, oil, doping/drugs, environment ... What is your view on the specialization?

Scientific and technological worlds need both approaches. Experts will make us go a long way in the specific knowledge and the foundations of science and technology. The "generalists" will use that information and integrate it with others, building new frameworks for the application of knowledge, therefore, one does not evolve without the other, and it is difficult to establish a quantity for each. The healthiest strategy is always let the professionals to develop according to their own beliefs, skills and interests, as they will give their best. But it is clear that a global perspective on the needs of the country should allow part of the effort to finance RD&I to be focused on encouraging the development of the poorest S&T areas and even the least developed re-

"Experts will make us go a long way in the specific knowledge and the foundations of science and technology. The "generalists" will use that information and integrate it with others, building new frameworks for the application of knowledge. One does not evolve without the other"

“There is no way to pre-select successful persons or projects. But the rate of failure in our graduate programs is minimal, because we all do everything possible so that the student gets his/her PhD”

gions of the country, as has been done by funding agencies in recent years.

At the moment the Brazilian government gives priority to Science Without Borders program, many students have the opportunity to go abroad. In your opinion, what benefits it will bring to the country? Is the internship abroad essential for the formation of the student?

Knowing other models of education, being away from their daily activities, allowing for more intense dedication to work and integration with other cultures are essential activities for intellectual, social and professional growth of all. So in theory the program is exemplary.

What we need to understand, but the Brazilian “system” of human resources training “does not understand” is that there is no way to preselect successful persons or projects. If we look at our graduate programs, the rate of failure is minimal. We all do everything possible so that the student gets his/her PhD. This is because the system, stupidly, does not want to lose money in its numerological analyzes and every student must complete the undergraduate course so that he/she inflates, no matter what, the governmental statistics.

For me, the Science Without Borders will be considered successful only if we accept the notion that the use of these professionals in the country has a success rate of 15-20%. And we understand that the “loss” of 80 to 85% of grantees is in fact the “investment” needed for such a program to succeed.

It is easy to see your concern with the practical application of chemistry in society. Do you believe that this concept (that possibility) is presented to students in elementary and high school today?

Everybody has a cause to fight. Mine has always been doing research that is very close to immediate use. My understanding is that a poor country like Brazil needs immediate solutions. Countries like the U.S. can afford to invest to collect (more) in 30 or 40 years from now. We have not yet started to collect, and, we have to do it now. The people deserve the consideration of our science community. However, I do just one important observation: this attitude of mine, of focusing in practical application of results, as all attitudes of common sense, does not preclude the need for basic research in Brazil. Returning to one of your questions, let people follow their vocations for them to give their best and let’s have a funding system that makes efforts in specific fields without compromising individual initiatives, but rather inducing those who are interested, to migrate to the needy sectors of our technical-scientific production.

About the school, surely popularization of science is not made effectively. The spread of science as a way to enhance our lives and careers is not effectively communicated to the youth, as teachers have not received adequate training to perform this task, which, by the

way, is not easy. Recycling courses for teachers should be a priority in our universities, coupled with a "scholarship" for employees, which, unlike the other grants provided by our governments, would not be a form of welfare as it would have direct impact on the qualification of the active professional.

In your view, what are the main trends of analytical chemistry in Rio de Janeiro and in Brazil: are they the same or different? And what about Brazil compared to the rest of the world?

Rio de Janeiro (RJ) has always had a calling for more specialized services and I believe it will grow in coming years for chemical analysis as well. Another aspect of the latest scientific development of RJ is the installation of research centers of multinationals in Brazil, changing the paradigm that they only had their centers in other countries. Only in Fundão, six research centers are being installed.

Of course, with the growth of the pole supporting the offshore oil industry, as well as the installation of the new Petrochemical Complex (additional to the Duque de Caxias), should attract even more activities of petrochemical and related analyzes. This combined with the research centers of multinationals in other areas of knowledge (L'Oreal, GE, etc.) should expand chemical activities with high technological content in the state. This, in parallel with streamlining and modernization of Centres of Excellence as the Inmetro, Fiocruz (Fundação Oswaldo Cruz), etc., and the advanced UFRJ campi, is already having a positive impact on this trend. RJ also has the first Proteomics Network in the country and this area, that in my opinion is no more "biochemistry", but it is simple chemistry, and should also prosper.

In the rest of Brazil and the world, I dare not to opine, as I confess my ignorance on the details of evolution of these segments. Based on the numbers we obtain from suppliers of equipment, and seeing the initiatives of the main suppliers of raw materials and equipment to open their branches in Brazil while continuing to operate through representatives, I could only assume that Chemistry in this country should be in exponential evolution, as to attract these investors, that certainly came in search of business opportunities.

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Analysis of Δ9-THC in cosmetics by high performance liquid chromatography with UV-Vis detection

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Abstract

A chromatographic methodology for Δ9-THC analysis in cosmetics is described. The HPLC-UV-Vis technique was employed for quantification of Δ9-THC in the 1.0 to 100 ppm working range using an optimized mobile phase consisting of methanol/water (4:1). In these experimental conditions, Δ9-THC presented a retention time of 23.7 minutes at the optimized wavelength of 209 nm. The analytical curve presented a linear correlation coefficient of $3.136x10^{-6}$ and the limits of detection and quantification were 0.746 and 2.487 ppm, respectively. This methodology was employed to quantify Δ9-THC in hemp oil-based cosmetics and it was possible to detect this cannabinol in concentrations ranging from 0.728 to 2.672 ppm.

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1. Introduction

Psychotropics are divided into in three large groups: psycholetics (or tranquilizers), psychanaletics (or stimulants) and psychodisletics (or psychedelic). In this context, hemp is placed in the first group, since it acts as a psychic tone reducer, reduces vigil, narrows the range of intellectual power and depresses emotional tensions. Generally speaking, it acts as a suppressor of the central nervous system (CNS) [1].

Δ9-tetrahydrocannabinol (Δ9-THC), the major psychoactive component of Cannabis sativa L, was synthesized and isolated for the first time in the year of 1964. It is now considered to be the component that is mainly responsible for the psychoactive properties of hemp, and its concentration in the plant is directly correlated to the potency of the effects of Cannabis sativa L. on the CNS. The concentration of this substance in the plant can vary depending on the environmental conditions under which the hemp was cultivated (soil fertility, climate, temperature, harvesting time, brightness, etc.), and marijuana may present a Δ9-THC content varying from 0.5% to 40%. Other factors can also alter the amount of Δ9-THC present, e.g., the storage conditions that are used, plant development and stage, and procedures used for plant drying [2]. Figure 1 illustrates the chemical structure of Δ9-THC.

Some studies have been devoted to identifying the active metabolite in hemp users [3,4]. The metabolite most often studied is the 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid, which is generally determined in human plasma and urine. Such studies are very important in the areas of biology and medicine.

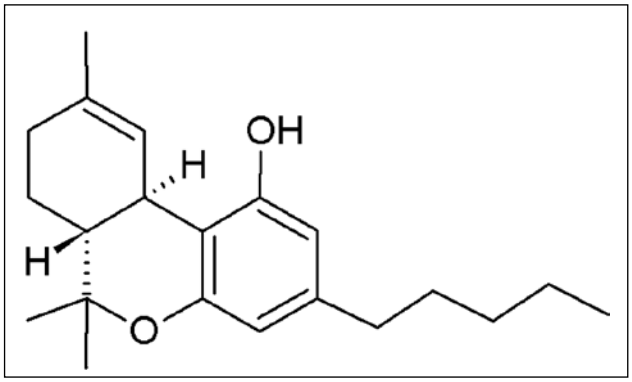

Figure 1. Chemical structure of Δ9-THC.

The therapeutic applications of hemp have also been studied in recent years [5-8]. The pharmaceutical industry uses synthetic Δ9-THC in medicines. An example is Dronabinol®, commonly used in the treatment of some pathological conditions, such as progressive anorexia in patients with AIDS, in some countries. However, recent studies have pointed out that the prolonged use of Dronabinol® can cause gynecomastia [9].

Δ9-THC analysis has also been accomplished in food samples [10,11]. For example, Zoller et al. have deter-

mined Δ9-THC in food, teas, and eatable oils samples by HPLC-UV. These papers are important for studies about intoxications caused by foods, which could culminate in death in chronic cases.

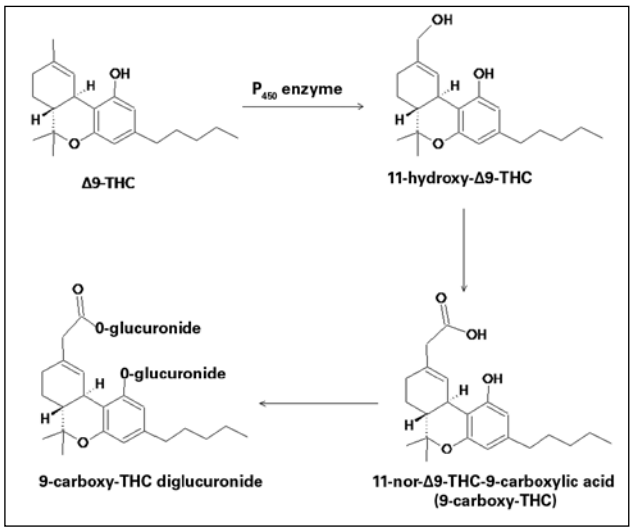

Figure 2. Δ9-THC biotransformation in the human organism

The direct analysis of drugs and the fast detection of their principal metabolites has prompted the development of new analytical methodologies [12-17]. Among these techniques are HPLC and vibrational spectroscopic methods. In this context studies on hemp, i.e., the fibers of marijuana and hemp applications, such as its use in the composition of fabrics and footwear, have also been reported [18]. Furthermore, the Δ9-tetrahydrocannabinol content of commercially available hemp products has been determined [19].

HPLC-UV and HPLC-fluorescence have been used for assaying Δ9-tetrahydrocannabinol in dietary products [20] and the simultaneous determination of three canabinoids in hemp seed oil by HPLC has been reported [21].

Despite the interesting properties of hemp-based commercial products, it is crucial to study their toxicological potential in terms of Δ9-THC residues, since this cannabinoid is considered an illicit substance in several countries. Therefore, the aim of this work is to investigate the presence of this species in hemp-oil-based cosmetics, using HPLC with UV-Vis detection.

2. Experimental

2.1 Reagents

Methanol from Merck (HPLC grade) and an analytical Δ9-THC standard solution from Cerilliant (1000 ppm in methanol) were employed in this work. Deionized water was obtained from a Milli-Q Water System (Millipore). The solutions used for construction of the analytical curve were achieved by dilution of the analytical Δ9-THC standard solution.

The construction of analytical curves provides reliable answers in chemical analysis, especially in chromatographic methods. In the linear range of concentration the detector response is directly proportional to sample concentration and the results outside the linear range are not reliable.

In order to construct the analytical curves, seven standard Δ9-THC samples with concentrations of 1.0, 5.0, 10.0, 20.0, 30.0, 50.0, and 100.0 ppm were obtained by dilution of aliquots the analytical standard Δ9-THC solution in methanol.

Commercial samples of cosmetics (shampoo, hair conditioner, and spray) are easily obtained via the Internet. The samples were prepared at a cosmetic:methanol (m/m) ratio of 1:4, and all samples were filtered prior to the chromatographic analysis.

A hemp extract in methanol, used in the experiment, was provided by the Institute of Criminalistics of Ribeirão Preto - State of São Paulo - Brazil.

2.2 Instruments

A Shimadzu HPLC chromatograph equipped with a Shimadzu SPDM-10AVP Diode Array Detector linked to the software data system Class VP was used for Δ9-THC determination in samples of hemp-oil-based cosmetics. Twenty microliters of the target sample were injected into the chromatograph, equipped with a Shimadzu ODS chromatography column (C18, 250mm x 4.6mm i.d., 5µm).

2.3 Experimental Parameters

The mobile phase consisted of a mixture of 4:1 (v/v) methanol:water. The analyses were accomplished at 25 ºC, at a mobile phase flow rate of 1.0 mL/min (pressure = 150 kgf/cm^2).

The analysis was run for 30 minutes, in order to ensure total Δ9-THC detection.

2.4 Validation

Linearity was examined using standard Δ9-THC methanol solutions with concentrations of 1.0, 5.0, 10.0, 20.0, 30.0, 50.0, and 100.0 ppm. The recovery tests were carried out for 3 aliquots of the studied samples, which were previously fortified with Δ9-THC until a final increase of 1ppm to these samples.

2.5 Comparative Tests

All the commercial samples of hemp-based cosmetics were submitted to colorimetric tests with Fast Blue B Salt. The samples were diluted using 1:4 ratio of cosmetics/methanol and placed in a petri dish, followed by spraying with Fast Blue B salt aqueous solution.

3. Results and Discussion

The optimization study for the spectrophotometric detection of Δ9-THC indicated an optimum wavelength of 208 nm, as shown in Figure 3.

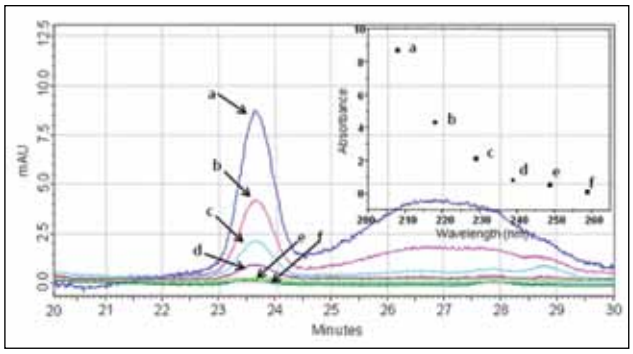

Figure 3. Optimization studies for the spectrophotometric detection of Δ9-THC (standard solution with a concentration of 1 ppm). (a) 208nm; (b) 218nm; (c) 229nm; (d) 239nm; (e) 249nm; (f) 259nm.

The HPLC analytical curve was based on the relation between peak area and Δ9-THC concentration in the standard solutions. Good linearity was obtained from 1.0 to 100.0 ppm.

The equation that describes the analytical curve for Δ9-THC analysis is:

$$[\Delta 9\text{-THC}] = 3.14 \times 10^{-6} \cdot X \ ,$$

where X is the peak area and [Δ9-THC] is the cannabinoid content, in ppm.

The average value of the standard deviation obtained from the points of the analytical curve (7.2×10^{-7} milliabsorbance units) was employed to calculate both limits of detection and quantification, calculated as 0.687 and 2.293 ppm, respectively. The analytical frequency was two analysis / hour.

The chromatogram for the Δ9-THC standard solution (Figure 4) demonstrated that this compound has a retention time around 23.7 minutes in the experimental conditions employed here.

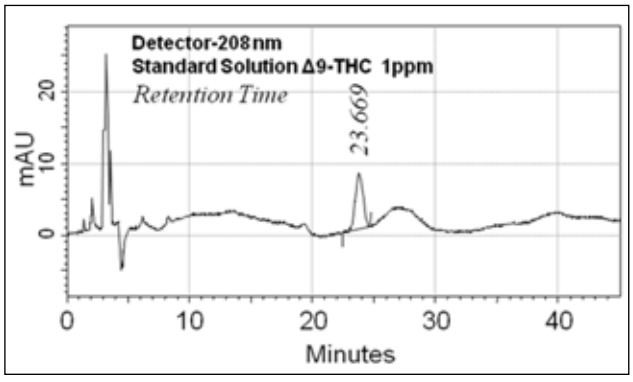

Figure 4. Δ9-THC standard solution (concentration = 1 ppm)

Using the experimental conditions described in the experimental section, a sample of each cosmetic was prepared in triplicate, which generated a total of 9 samples that were further analyzed by HPLC.

On the basis of the results obtained, the presence of Δ9-THC was verified in all the hemp oil-based cosmetics utilized in this work.

Figure 5 depicts the chromatograms confronting the samples and standard solution. The results indicate the presence of Δ9-THC in the three samples of cosmetics analyzed here.

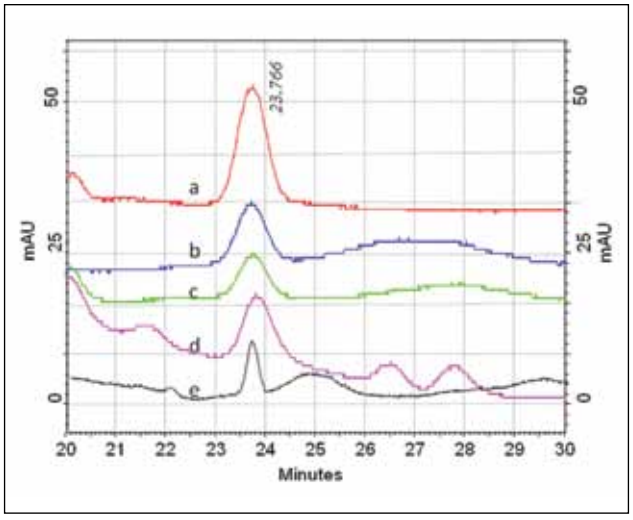

Figure 5. Chromatograms of the 3 samples of cosmetics, (a) spray; (b) hemp extract; (c) hair conditioner; (d) shampoo; (e) standard Δ9-THC solution – 1ppm.

According to Table I, all the samples studied presented good CV values (ranging from 1.1 to 6.9), which demonstrates the applicability of this methodology for the intended purposes.

Table I. Δ9-THC analysis of in commercial samples of marijuana-based cosmetics.

Sample	Concentration (ppm)	SD	CV (%)
Shampoo	1.24	0.04	3.3
Hair conditioner	0.73	0.05	6.9
Spray	2.67	0.03	1.1

In comparison with colorimetric tests used for cannabinoids, it was possible to observe that in the commercial samples the cannabinolic species is present in a concentration range lying below the LOD for the colorimetric technique, since the latter gave negative results for Δ9-THC.

The presence of broad peaks after Δ9-THC elution (Figure 5) indicates that other matrix compounds present in the samples may provide undesirable adsorptions in the stationary phase. In order to increase the lifetime of the chromatographic column, prior extraction stages (liquid-liquid or solid-liquid) are recommended if this technique is chosen for routine analysis.

Conclusions

In agreement with the chromatograms presented and the experimental methodology used in the experiment, the presence of Δ9-THC was detected in all the hemp oil-based cosmetics studied in this work.

The results obtained indicate that it is possible to detect Δ9-THC by HPLC-UV-Vis analysis, even though the colorimetric tests give negative results for this species.

Table II. Comparative results obtained for Δ9-THC detection in cosmetics samples by different techniques.

Sample	Colorimetric Test	HPLC
Seized sample hemp	POSITIVE	POSITIVE
Shampoo	NEGATIVE	POSITIVE
Hair conditioner	NEGATIVE	POSITIVE
Spray	NEGATIVE	POSITIVE

This analysis is important because these products could be banned in countries where hemp consumption is prohibited, since they contain the major psychoactive component of marijuana.

Ackowledgements

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References

1. Luchiari, E. E., et al. Comentários sobre a nova Lei sobre Drogas. Campinas, Millenium Editora, 2007, p. 3.
2. Passagli, M. et al. Toxicologia Forense: Teoria e Prática. Campinas, Millenium Editora, 2007, pp. 156 ; 161.
3. Mercoline, L.; Musenga, A.; Comin, I.; Baccini, C.; Conti, M.; Raggi, M. A. *J. Pharm. Biomed. Anal*. 2008, 47, 156.
4. Gustafson, R. A.; Levine, B.; Stout, P. R.; Kleite, K. L.; George, M. P.; Moolchan, E. T.; Huestis, M. A. *Clin. Chem*. 2003, 49:7, 1114.
5. van der Kooy, F.; Pomahacova, B.; Verpoorte, R. *Inhal.Toxicol.* 2009, 21, 87.
6. Narang, S.; Gibson, D.; Wasan, A. D.; Ross, E. L.; Michna, E.; Nedeljkovic, S. S.; Jamilson, R. N. *J. Pain* 2008, 9, 254.
7. Haney, M.; Hart, C. L.; Vosburg, S. K.; Comer, S. D.; Reed, S. C.; Foltin, R. W. *Psychofarmacology* 2008, 197, 157.
8. ElSohly, M. A.; deWit, H.; Wachtel, S. R.; Feng, S.; Murphy, T. P.. *J. Anal. Toxicol.* 2001, 25, 565.
9. Allen, R. C.; Wallace, A. M.; Royce, M. R. *Am. J. Med.* 2007, 120, e1.
10. Zoller, O.; Rhyn, P.; Zimmerli, B. *J. Chromatogr. A.* 2000, 872, 101.
11. Pellegrini, M.; Marchei, E.; Pacifici, R.; Pichini, S. *J. Pharm. Biomed. Anal.* 2005, 36, 939.
12. Sprogöe, K.; Staerk, D.; Jäger, A. K.; Adsersen, A.; Hansen, S. H.; Witt, M.; Landbo, A-K. R.; Meyer A. S.; Jaroszewski, J. W. *J. Nat. Prod.* 2007, 70, 1472.
13. Lurie, I. S. *J. Chromatogr. A.* 2005, 1100, 168.
14. Jung, J.; Kempf, J.; Mahler, H.; Weinmann, W. *J. Mass. Spectrom*. 2007, 42, 354.
15. O´Neal, C. L.; Crouch, D. J.; Fatah, A. A. *Forensic Sci. Int.* 2000, 109, 189.
16. Toonen, M. A. J.; Maliepaard, C.; Reijmers, T. H.; van der Voet, H.; Mastebroek, H. D.; van den Broeck, H. C.; Ebskamp, M. J. M.; Kessler, W.; Kessler, R. W. *Euphytica* 2004, 140, 39.
17. Le Troedec, M.; Sedan, D.; Peyratout, C.; Bonnet, J. P.; Smith, A.; Guinebretiere, R.; Gloaguen, V.; Krausz, P. *Composites A: Appl. Sci. Manuf.* 2008, 39, 514.
18. Kojoma, M.; Seki, H.; Yoshida, S.; Muranaka, T.. *Forensic Sci. Int*. 2006, 159, 132.
19. Holler, J.M.; Bosy, T.Z.; Dunkley, C.S.; Levine, B.; Past, M.R.; Jacobs, A. *J. Anal. Toxicol.* 2008, 32 (6), 428.
20. Zhang, G.; Guo, J.-N.; Xu, Z.-M.; Gao, J.; Bi, K.-S. *Chin. Pharm. J.* 2003, 38 (3), 214.
21. Chao, W.; Hong-li, Y.; Qing, L.; Xiao-hui, C.; Tian-hong, Z.; Kai-shun, B. *Chin. J. Pharm. Anal.* 2010-09.

Optimization of solid-phase extraction of non-coloured phenolic compounds from fortified wines using response surface methodology

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Abstract

Non-coloured phenolic compounds were determined by high performance liquid chromatography in samples of fortified wines. A solid-phase extraction procedure was optimized for pretreatment of the sample, whose optimization used surface response methodology. The variables evaluated were sample volume, elution solvent volume and flow rate. Application of the surface response methodology revealed that the conditions for extraction were 5 mL of sample, 20 mL of elution solvent and flow rate 0.06 mL s^{-1}. Verification tests gave percent recoveries in the interval between 71.06 and 119.40%. The optimized method was applied to samples of white and red fortified wines with good results for determination of gallic acid, (+) catechin, caffeic acid, *p*-coumaric acid, ferulic acid and quercetin, indicating the suitability of the model employed and the applicability of surface response methodology in optimization of the extraction conditions.

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Keywords: non-coloured phenolic compounds; fortified wines; surface response methodology; HPLC-UV/Vis

1. Introduction

Fortified wines are partly fermented wines and, in their production, the must is fortified with spirits of grape origin when approximately half of the original sugars have been converted to alcohol [1, 2]. Many fortified wines are aged in wooden casks and in bottles, developing characteristics essential to their flavours and aromas [3]. Fortified wine is a complex matrix, which, besides water, sugar and alcohol, contains a great variety of inorganic and organic components [4]. These compounds includes polyphenols such as phenolic acids, trihydroxystilbene, flavonols, and flavan-3-ols, as well as polymers defined as procyanidins and anthocyanins [5, 6, 7]. These compounds have beneficial health effects including anti-inflammatory, antiviral, anti-carcinogenic and antioxidant activities [7, 8, 9, 10].

The importance of phenolic compounds in wine is very well established due to their influence on colour, flavour and astringency [11, 12, 13]. Non-coloured phenolic compounds include benzoic and cinnamic acids and aldehydes, usually called low molar mass phenols. Although present in small amounts in wines, these compounds play an important role in terms of the sensory quality, and may contribute, through an additive effect, to the bitterness and harshness [13] as well as affect the colour of wine since, during the aging process of wines, new, more stable, pigments are formed from the reaction between anthocyanins and flavonols [14].

One of the most commonly used analytical techniques for the determination of phenolic compounds in highly complex samples, such as wine, is high performance liquid chromatography (HPLC). Considering the great variety of compounds present and the wide variations in their levels, sample preparation is essential to ensure the identification and quantification of these compounds, even when this technique is used in association with detection methods with high discrimination power [15]. In fortified wines the pretreatment of the sample is crucial since the high content of ethanol, sugars and other compounds usually present in wine matrices may decrease the detectability of the analytical technique, causing irreversible damage to the analytical column, and variation in the flow rates and peak shapes [16, 17].

The search for faster and cleaner (using less organic solvent) extraction techniques, more efficient methodologies (higher recoveries and reproducibility, lower limits of detection and quantification) and ease of automation has been the subject of several studies [13, 18, 19, 20]. An alternative to the above-mentioned techniques is solid-phase extrac-

tion (SPE), which offers several advantages, including better selectivity and ease of automation [15, 21].

Sample preparation by solid-phase extraction is required due to the difficulties encountered in interpreting chromatograms and identifying the polyphenolic compounds. Depending on the nature of the compounds, the selectivity and accuracy of the method can be low, requiring optimization of the extraction procedure in order to obtain the best results [15].

In general, optimization of an extraction process can be achieved by employing statistical methods such as response surface methodology. This is often used because it allows an evaluation of the interactions between various factors. The response surface methodology is an effective statistical method based on a multivariate non-linear model that has been widely used for the optimization of complex processes, as well as for simultaneously estimating the effects of several process variables and their interactions on the response variables [12].

The aim of this paper is to propose an optimized process for the solid-phase extraction of non-coloured phenolic compounds and to identify and quantify six phenolic compounds in fortified wines by HPLC-UV/Vis.

2. Experimental

2.1 Reagents and standards

Acetonitrile, methanol and ethyl acetate (HPLC grade) were obtained from Merck (Darmstadt, Germany) and hydrochloric acid, acetic acid, tartaric acid and ethanol were analytical reagent grade. The water used was passed through a Milli-Q system (Millipore, Bedford, MA, USA). All solvents used as the mobile phase were previously filtered through 0.45 µm membranes (Millipore) and degassed prior to use. The solid-phase extraction (SPE) cartridges used were Spe-ed C_{18} (500 mg sorbent mass and 6 mL reservoir volume) from Applied Separations (Allentown, PA, USA).

Standards of caffeic acid, ferulic acid and quercetin were obtained from Fluka (Steinheim, Germany) and standards of (+)catechin, *p*-coumaric acid and gallic acid from Sigma-Aldrich (St. Louis, MO, USA). All standards were prepared in stock solutions of synthetic wine with 18% (v/v) ethanol and 5 g L^{-1} tartaric acid in Milli-Q water.

2.2 Samples

To optimize the extraction of non-coloured phenolic compounds, samples of fortified wine made from Cabernet Sauvignon and Merlot grapes, vintage 2005, produced in São Joaquim, SC, Brazil, were analyzed. The proposed methodology was applied to samples of fortified white and red wines of *Vitis vinifera* L., produced in traditional Brazilian wine-producing regions, from the red varieties Cabernet Sauvignon/Merlot, Cabernet Sauvignon/Tannat and Touriga Nacional and white varieties Goethe and Moscato Giallo.

2.3 Experimental design for optimization of extraction of non-coloured phenolic compounds

To optimize the conditions for the extraction of the non-coloured phenolic compounds from fortified wines response surface methodology [23, 24] was used. A central composite design (CCD) consisting of 17 experimental runs, including three replications at the central point, was chosen to evaluate the combined effect of the independent variables. The ranges and the center point values of the three independent variables were based on the results of preliminary experiments (sample volume, 5-10 mL; elution solvent volume, 10-30 mL; and flow-rate 0.03-0.09 mL s^{-1}). Three levels were adopted and coded as -1, 0 and +1. The experiments were performed in random order to minimize the effects of unexplained variability in the observed responses due to systematic errors [25]. The independent variables were sample volume (X_1, mL), elution solvent volume (X_2, mL), and flow-rate (X_3, mLs^{-1}) while the response variable was the total phenolic compounds expressed as total area of the chromatographic peaks (mAU). The response function (Y) was partitioned into linear, quadratic, and interactive components [24, 25, 26, 27]. Experimental data were fitted to the second-order regression equation (Eq. 1):

$$(1)\quad Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_3 + \beta_{23} X_2 X_3 + \varepsilon$$

were Y is the prediction response, β_0 the intercept; β_1, β_2, β_3 linear coefficients; β_{11}, β_{22}, β_{33} squared coefficients; β_{12}, β_{13}, β_{23} interaction coefficients; X_i and X_j the coded levels of variables X_i and X_j; and ε is the residual. The software Statistica, version 7.0 (Statsoft, Inc. Tulsa, OK), was used to determine the analysis of variance (ANOVA) and the coefficient of determination (R^2) to evaluate the goodness of fit of the model.

2.4 Sample pretreatments

The preconditioning of the SPE cartridge was conducted with 2 mL of ethyl acetate, 2 mL of methanol and 2 ml of 0.01mol L^{-1} HCl. Due to the high cost of the SPE cartridge, each one was used 6 times. Before analysis, the reused SPE cartridge was preconditioned with 20 mL of ethyl acetate, 20 mL of methanol and 20 mL of 0.01mol L^{-1} HCl. The sample was added to the preconditioned cartridge and a solution of 0.01mol L^{-1} HCl (3 mL) was then used to elute contaminants. The non-coloured phenolic compounds were eluted with ethyl acetate and this solution was evaporated under reduced pressure at 30ºC. The concentrate was dissolved in 2 mL of methanol, filtered through a Millipore membrane (0.45µm) and analyzed using HPLC-UV/Vis.

2.5 HPLC-UV/Vis analysis

The analysis of non-coloured phenolic compounds was performed using a previously developed methodology and validated for samples for dry wine [48] using a Shimadzu HPLC instrument equipped with a quaternary system pump, model LC-10AT, a DGU-14A degasser, a UV/Vis detector model SPD-10AV and a Rheodyne injector with a 20 µL loop. The HPLC system was controlled by CLASS VP 6.1 software, with a communicator (model SCL-10A). The stationary phase consisted of a HiChrom (Berkshire, UK) reversed-phase column (250 x 4.60 mm, 5 µm). A guard column was used to protect the analytical column. Chromatographic separations of the compounds were performed with a gradient elution of mobile phase A (pure water containing acetic acid, pH 2.65) and mobile phase B (80% acetonitrile, 20% eluent A): 0-35 min, 0-30% B; 35-40 min, 30-50% B; 40-45 min, 50-100% B; 45-50 min, 100-50% B; 50-55 min, 50-30% B; 55-60 min, 30-0% B. The flow rate was 1.2 mL min^{-1} and detection was performed at 280 nm. The identification of non-coloured phenolic compounds was obtained by comparing the retention times of the peaks of the samples and standards and the quantification was carried out by external standardization.

2.6 Method assessment

Calibration curves were constructed for the phenolic compounds caffeic acid, *p*-coumaric acid, (+) catechin, quercetin and ferulic acid. The calibration curve for gallic acid was constructed separately due to its reactivity with other analytes. Each calibration curve was constructed with seven data points in concentrations ranging from 0.3 – 150 mg L^{-1} and with three replicates for each point. For all points of the calibration curve the extraction of non-coloured phenolic compounds was performed using the optimum values obtained through response surface methodology. Quantification was performed by external standardization by evaluating the area of the relevant chromatographic peak. For all compounds linear correlations were obtained with R^2 values greater than 0.99.

The accuracy was determined by measuring recovery after addition of standards in a concentration of 15.0 mg L^{-1} in fortified wine samples and was expressed as percentage of recovery and relative standard deviation for the different concentrations.

3. Results and Discussion

3.1 Optimization of the solid-phase extraction of non-coloured phenolic compounds

The response obtained for 17 different combinations was analyzed using CCD. The values for the independent variables (X_1, X_2 and X_3) and the response variable are shown in Table I. The data evaluation pertaining to this experiment was carried out by integrating and evaluating the peak areas corresponding to six compounds with different retention times. The response variable was expressed as the sum of the chromatographic peak areas of the six phenolic compounds analyzed.

Table I. Central composite design arrangement and response.

Run	Factor 1 (X1) Sample Volume (mL)	Factor 2 (X2) Elution Solvent Volume (mL)	Factor 3 (X3) Flow Rate mL s^{-1}	Non-coloured phenolic compounds (total area)	
				Observed	Predicted
1	5	10	0.03	322435	423164.6
2	5	10	0.09	306294	313629.1
3	5	30	0.03	263935	273043.7
4	5	30	0.09	208922	255748.6
5	10	10	0.03	273598	306935.3
6	10	10	0.09	242890	313945.2
7	10	30	0.03	302658	375486.8
8	10	30	0.09	495303	474737.2
9	3.3	20	0.06	462409	403417.6
10	11.7	20	0.06	544355	489735.4
11	7.5	3.2	0.06	494790	406955.1
12	7.5	36.8	0.06	441695	415918.9
13	7.5	20	0.01	313046	221922
14	7.5	20	0.11	237663	213351
15	7.5	20	0.06	541247	586798.5
16	7.5	20	0.06	604600	586798.5
17	7.5	20	0.06	592546	586798.5

Experimental values were the measured response data for a particular run, and the predicted values were obtained from the model and generated using approximation functions [28, 29]. As can be seen, the predicted value obtained was quite close to the experimental values, indicating that the model developed was successful in describing the correlation between the factors of the solid-phase extraction procedure and the response variable. The non-coloured phenolic compound contents expressed as the sum of the chromatographic peak areas of the six compounds analyzed, varied from 208922 to 604600 mAU according to variations in the extraction conditions.

The application of the response surface methodology describes, on the basis of parameter estimates, an empirical relationship between the response variable (non-coloured phenolic compound contents) and the test variables. By applying multiple regression analysis to the experimental data, the response variable and the test variables are related by the following the second-order polynomial equation (Eq. 2), in which the insignificant factors are excluded:

(2) $$Y = 523558 + 2668X_2 - 2571X_3 - 47582X_2^2 - 118243X_3^2$$

The quadratic effects of the elution solvent volume

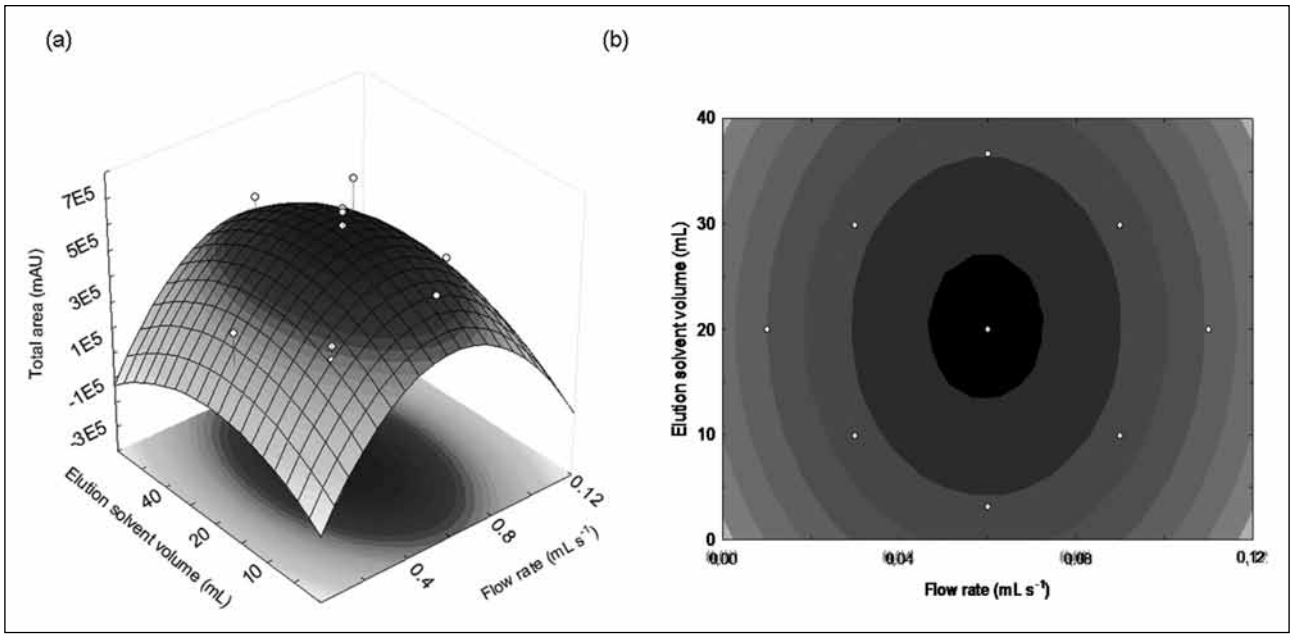

Figure 1. Three dimensional response surface plot (a) and contour curves (b) of the variation in the total area of chromatographic peaks of six non-coloured phenolic compounds as a function of elution solvent volume (mL) and flow rate (mL s-1) with the sample volume set at 7.5 mL.

and the flow rate were statistically significant while the linear and quadratic effects of the sample volume and the effect of the interactions of the three independent variables were not significant. Despite the statistical insignificance, the linear effects of elution solvent volume and flow rate were not removed from the model in order to give hierarchical support [34].

The regression coefficients of intercept, linear, quadratic, and interaction terms in the experimental model were calculated and their levels of significance were determined using the analysis of variance (ANOVA). Table II shows the ANOVA summary for the response surface quadratic model used to estimate the solid-phase extraction of non-coloured phenolic compounds in fortified wines as a function of the three independent variables.

The testing of the model adequacy is an important part of the data analysis procedure, since an inadequate fit would lead to poor or misleading results [24, 31, 32]. The quality of the developed model was evaluated based on the coefficient of determination (R^2), which indicates the proportion of variability in the response variable. The closer R^2 is to unity, the better the empirical model fits the experimental data and therefore the greater the precision. The smaller the value of R^2 the less relevant the dependent variables in the model are in terms of explaining the variation in the response [25, 33, 34, 35, 36]. According to Sin et al. [25], R^2 values higher than 0.8 indicate that the regression model adequately describes the behavior of the system under study. The coefficient of determination (R^2) obtained in our study was 0.8293. The regression model had low dispersion, explaining 82.9% of the total observed variation in the response.

The lack of fit was not significant at the 5% level of probability of error, indicating that the model is suitable for application to the range of the variables that determine the response [22, 37]. The ANOVA results demonstrated that the model is significant as evidenced by the value of calculated F (3.78) and the value of reduced error probability ($p = 0.047$).

For any of the terms in the models, a large F value and a small P value would indicate a more significant effect on the respective response variables [38, 39]. Based on the F values shown in Table II, it can be observed that the highest value was 26.84, indicating that the quadratic effect of flow rate was more significant for the variable response when compared to the other factors.

The effect of two independent variables (elution solvent volume and flow rate) on the response variable, setting the third independent variable (sample volume) at the central experimental point, is shown in Figure 1.

The efficiency of the solid-phase extraction of non-coloured phenolic compounds was dependent mainly on the solvent volume used for elution (quadratic effect) and the flow rate (quadratic effect), resulting in a curvilinear effect for the surface response (Figure 1a). The extraction yield for non-coloured phenolic compounds is low when

the solvent volume and the flow rate are less than 15 mL and 0.05 mL/s, respectively, or greater than 25 mL and 0.07 mL/s, respectively (Figure 1b).

Table II. Analysis of Variance (ANOVA) for response surface quadratic model of the solid-phase extraction of non-coloured phenolic compounds in fortified wines.

	Sum of Square	Degrees of Freedom	Mean Square	F-Value	p-Value
Model	245420275828	9	27268919536.44	3.78	0.046708
X_1	9005106658	1	9005106658	1.25	0.300876
X_2	97111128,2	1	97111128.2	0.01	0.910916
X_3	89623347,2	1	89623347.2	0.01	0.914403
X^2_1	27807638519	1	27807638519	3.85	0.090446
X^2_2	43491099722	1	43491099722	6.03	0.043806
X^2_3	193728739362	1	193728739362	26.84	0.001280
X_1X_2	23908831128	1	23908831128	3.31	0.111567
X_1X_3	6791426785	1	6791426785	0.94	0.364357
X_2X_3	4254154920	1	4254154920	0.59	0.467757
Residual	50525336860	7	7217905266		
Lack of fit	48262840551	5	9652368110	8.53	0.108263
Pure Error	2263496309	2	1131748154		
Total	295945612688	16			

R^2_{adj}= 0.60977; R= 0.91064; R^2= 0.82927

The three dimensional response surface plot (Figure 1a) shows a parabolic shape indicating that the extraction of non-coloured phenolic compounds from fortified wines using the technique of solid-phase extraction on a C_{18} cartridge with 500 mg of sorbent is maximal with 20 mL of elution solvent (ethyl acetate) at a flow rate of 0.06 mL s^{-1}. The sample volume was not significant in the model studied. This can be attributed to the low amount used in the experiment (5 mL).

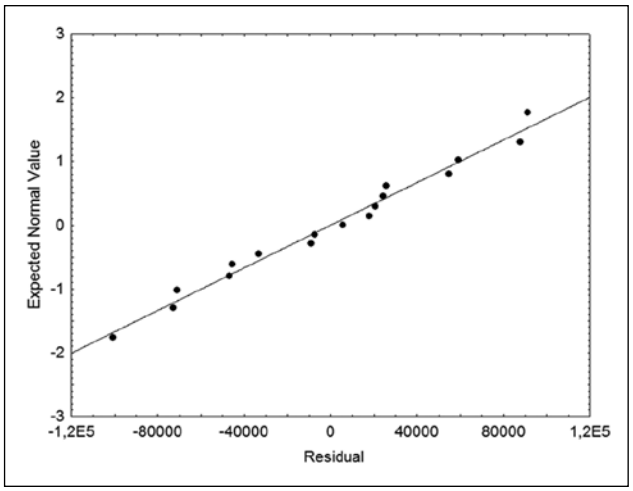

Figure 2. Normal probability plot residuals for the total area of chromatographic peaks of six non-coloured phenolic compounds.

The normal probability plot of the residuals for the content of non-coloured phenolic compounds is shown in Figure 2. It can be observed that the data distribution is random and the residuals generally fall on a straight line without giving discrepant values. The variances are homogeneous, implying that the errors are normally distributed and the assumption of normality is satisfied, proving that the model is suitable for the study.

3.2 Analytical Performance

The qualitative variables, including linearity, limits of detection (LOD) and quantification (LOQ) and accuracy were determined to assess the analytical performance of the proposed model.

Calibration curves were linear over the concentration ranged studied and all compounds showed determination coefficients (R^2) greater than 0.99 (Table III).

Table III. Linearity, limits of detection and quantification and accuracy for the studied on non-coloured phenolic compounds in fortified wines, obtained by HPLC with sample pretreatment by SPE.

Compound	t_R (min)	Linearity		LOD mg L^{-1}	LOQ mg L^{-1}	Recovery (%) ± RSD
		Equation of linear regression	R^2			
Gallic acid	9.3	Y = 30469x- 46040	0.9914	0.05	0.16	119.4 ± 11.97
Catechin	22.3	Y =12036x + 5613.8	0.9979	0.04	0.12	117.3 ± 2.06
Caffeic acid	24.7	Y = 54017x + 27286	0.9980	0.02	0.06	81.83 ± 5.84
Coumaric acid	29.4	Y = 78779x + 46923	0.9979	0.08	0.24	71.06 ± 0.02
Ferulic acid	33.7	Y = 47844x + 25800	0.9980	0.04	0.13	75.73 ± 0.02
Quercetin	45.0	y = 25553x – 1910.9	0.9976	0.05	0.15	99.71 ± 0.03

The limits of detection (LOD) and quantification (LOQ) were determined based on the standard deviation of the response of the blank (n = 7) and the slope of the calibration curve for each point. A solution of synthetic wine (18% (v/v) ethanol and 5g L^{-1} tartaric acid in Milli-Q water adjusted to pH 3.2 with 2 mol L^{-1} NaOH) was used as the blank. The LOD values ranged from 0.02 to 0.08 mg L^{-1}, and the LOQ from 0.06 to 0.24 mg L^{-1}. These values are in agreement with limits obtained by other researchers for the determination of phenolics in red wines and fortified wines [5, 6, 7, 40].

To assess the accuracy of the method the solid-phase extraction of fortified wine samples spiked with standard solutions of the six non-coloured phenolic compounds, at a concentration of 15 mg L^{-1}, was carried out. To calculate the recovery values the concentration of the each phenolic compound in the pure sample was subtracted from the corresponding value obtained for each sample spiked with the standard. The recovery values ranged from 71.06 to 119.4% and the relative standard deviation (RSD) from 0.02 to 11.97% (Table III). The recovery ranges are in agreement with those of other researchers who reported the quantification of these compounds [15, 21, 41, 42]. Low percentages of recovery were cited by Guillén et al. (15) for *p*-coumaric acid and ferulic acid, as occurred in this study. According to these authors (15), an alternative to increase the recovery can be the addition an ion-forming reagent to the conditioning solution of the cartridge.

These results suggest that the proposed method is a potential tool for analysis of samples of fortified wines when the objective is the determination of non-coloured phenolic compounds.

3.3 Determination of non-coloured phenolic compounds in real samples of fortified wine

The proposed method was applied to determine the concentration of non-coloured phenolic compounds in samples of fortified white and red wines. Eleven samples of fortified wines made from different varieties of grapes were analyzed in triplicate. All samples were pretreated by solid-phase extraction using the optimal points obtained in the optimization process and later analyzed by HPLC-UV/VIS. The chromatogram of a sample of fortified wine after pretreatment by SPE is shown in Figure 3.

The concentrations of phenolic compounds in the samples analyzed (Table IV) are in agreement with those reported in the literature for white and red wines of different varieties [14, 43, 44]. Fortified wines have concentrations of non-coloured phenolic compounds equal to, or often higher than, those of traditional wines. This can occur because even with the reduced time of maceration used in the preparation of fortified wines the extraction of phenolic compounds is very efficient because of the concentration of ethanol [2].

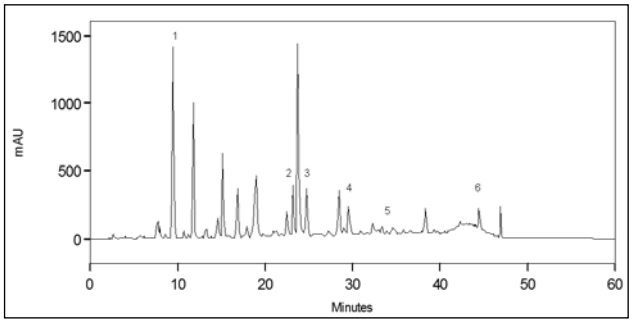

Figure 3. Typical chromatographic profile of a sample of fortified wine after pretreatment by solid-phase extraction. The peaks correspond to: 1, gallic acid; 2, (+)catechin; 3, caffeic acid; 4, *p*-coumaric acid; 5, ferulic acid and 6, quercetin.

Table IV. Concentration of non-coloured phenolic compounds ($mgL^{-1} \pm SD$) in samples of fortified wines.

Variety	Catechin	Caffeic acid	*p*-Coumaric acid	Ferulic acid	Quercetin	Gallic acid
Goethe	3.69 ± 0.07	2.94 ± 0.01	1.40 ± 0.01	1.28 ± 0.04	2.61 ± 0.14	6.76± 0.19
Moscato 1	19.31 ± 0.29	6.93 ± 0.04	2.56 ± 0.43	0.96 ± 0.03	8.38 ± 0.10	51.54 ± 0.31
Moscato 2	5.39 ± 0.06	5.98 ± 0.30	1.60 ± 0.18	0.85 ± 0.01	3.01 ± 0.13	149.99 ± 9.76
Moscato 3	5.01 ± 1.18	8.12 ± 0.09	4.24 ± 0.07	0.96 ± 0.07	8.71 ± 1.04	16.92 ± 0.01
Cab. Sauvignon/Merlot 1	116.98± 6.67	0.67 ± 0.02	0.72 ± 0.04	0.78 ± 0.02	11.57 ± 0.44	94.55 ± 3.86
Cab. Sauvignon/Merlot 2	46.39 ± 2.06	0.79 ± 0.03	0.64 ± 0.03	0.63 ± 0.02	4.34 ± 0.09	137.98 ± 7.75
Cab. Sauvignon/Merlot 3	12.01 ± 0.19	27.07 ± 2.83	2.69 ± 0.12	0.85 ± 0.02	10.73 ± 1.67	58.50 ± 5.29
Cab. Sauvignon/Merlot 4	10.22 ± 0.30	19.71 ± 2.62	8.62 ± 0.76	0.95 ± 0.03	1.67 ± 0.15	302.30 ± 0.71
Cab. Sauvignon/Tannat 1	221.35± 8.11	5.60 ± 0.09	1.68 ± 0.03	2.44 ± 0.05	26.41 ± 0.33	1062.31±56.59
Cab. Sauvignon/Tannat 2	216.19± 3.75	1.64 ± 0.02	1.09 ± 0.01	2.40 ± 0.08	22.62 ± 0.09	552.24 ± 34.70
Touriga Nacional	22.37 ± 0.34	21.56 ± 0.15	4.34 ± 0.20	1.93 ± 0.03	6.30 ± 0.01	64.28 ± 3.44

Values are expressed as mean ± standard deviation of triplicate determinations.

Gallic acid was the most abundant compound found in the wines analyzed, as previously reported for fortified wines [2, 11, 45]. The main sources of gallic acid and catechin are grape seeds and higher concentrations of these compounds in wines may be due to the application of certain practices involving maceration, pressing or high temperatures during winemaking [46]. These compounds may also originate from their extraction from wood or the degradation of tannins during wine ageing [11]. Differences in the concentrations of coumaric and ferulic acids were observed in wines according to the grape variety used in the winemaking, as described previously [14, 47]. Differences observed among wines also occur according to the pH, ethanol content, temperature and time of fermentation and the maceration process [46].

Conclusions

The extraction of non-coloured phenolic compounds from fortified wines using a solid-phase extraction technique was optimized efficiently using surface response methodology. The procedure for solid-phase extraction (SPE) can be carried out in a single step requiring smaller amounts of sample (5 mL) and lower consumption of organic solvents (20 mL) than other traditional sample pretreatment techniques, which makes the procedure an attractive alternative for the routine analysis of fortified wines by HPLC. The optimized method was shown to give reproducible results with good recoveries and low limits of detection and quantification.

References

1. Esteves, V., Lima, S.S.F., Lima, D.L.D., Duarte, A.C. *Anal. Chim. Acta*. 2004, 513, 163.
2. García-Viguera, C.; Bakker, J.; Bellworthy, S. J.; Reader, H. P.; Watkins, S. J.; Bridle, E. P. *Z. Lebensm. Unters. Forsch A.* 1997, 205, 321.
3. Ho, P.; Hogg, T. A.; Silva, M. C. M. *Food Chem.* 1999, 64, 115.
4. Fabani, M.P., Arrúa, R.C., Vásquez, F., Diaz, M.P., Baroni, M.V., Wunderrlin, D.A. *Food Chem.* 2010, 119, 372.
5. Nave, F.; Cabrita, M. J.; Costa, C. T. *J. Chromatogr., A.* 2007, 1169, 23.
6. Masa, A.; Vilanova, M. *Food Chem.* 2008, 107, 273.
7. 7. Rastija, V.; Srecnik, G.; Medic-Saric, M. *Food Chem.* 2009, 115, 54.
8. López, M.; Martínez, F.; Del Valle, C.; Orte, C.; Miró, M. *J. Chromatogr., A.* 2001, 922, 359.
9. Villaño, D.; Fernández-Pachón, M. S.; Troncoso, A. M.; García-Parrilla, M. C. *Food Chem.* 2006, 95, 394.
10. Gürbüz, O.; Göcmen, D.; Dagdelen, F.; Gürsoy, M.; Aydin, S.; Sahin, I.; Büyükuysal, L.; Usta, M. *Food Chem.* 2007, 100, 518.
11. Rodríguez-Delgado, M. A.; Malovaná, S.; Pérez, J. P.; Borges, T.; García Montelongo, F. J. *J. Chromatogr., A.* 2001, 912, 249.
12. Valentão, P.; Seabra, R. M.; Lopes, G.; Silva, L.; Martins, V.; Trujillo, M. E.; Velázquez, E.; Andrade, P. B. *Food Chem.* 2007, 100, 64.
13. Cabrita, M. J.; Torres, M.; Palma, V.; Alvez, E.; Patão, R.; Costa Freitas, A. M. *Talanta*. 2008, 74, 1281.
14. Dopico-García, M. S.; Fique, A.; Guerra, L.; Afonso, J. M.; Pereira, O.; Valentão, P.; Andrade, P. B.; Seabra, R. M. *Talanta*. 2008, 75, 1190.
15. Guillén, D. A.; Barroso, C. G.; Pérez-Bustamante, J. A. *J. Chromatogr., A.* 1996, 730, 39.
16. Rodríguez-Bencomo, J. J.; Conde, J. E.; García-Montelongo, F.; Pérez-Trujillo, J. P. *J. Chromatogr., A.* 2003, 991, 13.
17. Alves, R. F.; Nascimento, A. M. D.; Nogueira, J. M. F. *Anal. Chim. Acta.* 2005, 546, 11.
18. Oszmianski, J.; Ramos, T.; Bourzeix, M. *Am. J. Enol. Viticult.* 1988, 39, 259.
19. Ricardo da Silva, J. M.; Rosec, J. P. H.; Bourzeix, M.; Heredia, N. *J. Sci. Food Agric.* 1990, 53, 85.
20. Chilla, C.; Guillén, D. A.; Barroso, C. G.; Pérez-Bustamante, J. A. *J. Chromatogr., A.* 1996, 750, 209.
21. García-Falcón, M.. S.; Pérez-Lamela, C.; Martínez-Carballo, E.; Simal-Gándara, J. *Food Chem.* 2007, 105, 248.
22. Ku, C. S.; Mun, S. P. *Biores. Technol.* 2008, 99, 8325.
23. Montgomery, D. C. *Design and Analysis of Experiments.* 5 ed. New York: Wiley, 2001.
24. Meyers, R.; Montgomery, D. C. *Response Surface Methodology.* New York: Wiley, 2002.
25. Sin, H. N.; Yusof, S.; Sheikh Abdul Hamid, N.; Abdul Rahman, R. *J. Food Eng.* 2006, 74, 352.
26. Henika, R. G. *Food Technol.* 1982, 36, 96.
27. Giovanni, M. *Food Technol.* 1983, 37, 41.
28. Körbahti, B. K.; Rauf, M. A. *Chem. Eng. J.* 2008, 136, 25.
29. Zhang, A.; Peng, J.; Qu, W.; Zhang, L.; Zhang, Z.; Li, W.; Wan, R. *J. Taiwan Inst. Chem. Eng.* 2009, 40, 542.
30. Aliakbarian, B.; De Faveri, D.; Converti, A.; Perego, P. *Biochem. Eng.* 2008, 42, 34.
31. Lyana-Pathirana, C.; Shahidi, F. *Food Chem.* 2005, 93, 47.
32. Körbahti, B. K.; Rauf, M. A. *J. Hazard. Mater.* 2009, 161, 281.
33. Ravikumar, K.; Ramalingam, S.; Krishnan, S.; Balu, K. *Dyes Pigment* 2006, 70, 18.
34. Lee, W. C.; Yusof, S.; Hamid, N. S. A.; Baharin, B. S. *J. Food Eng.* 2006, 73, 55.
35. Fan, G.; Han, Y.; Gu, Z.; Chen, D. *LWT-Food Sci. Technol.* 2008, 41, 155.
36. Wang, L.; Yang, B.; Du, X.; Yang, Y.; Liu, J. *Innov. Food Sci. Emerg. Technol.* 2008, 9, 604.
37. Zulkali, M. M. D.; Ahmad, A. L.; Norulakmal, N. H. *Biores. Technol.* 2006, 97, 21.
38. Quanhong, L.; Caili, F. *Food Chem.* 2005, 92, 701.
39. Yuan, Y.; Gao, Y.; Mao, L.; Zhao, J. *Food Chem.* 2008, 107, 1300.
40. De Quirós, A. R. B.; Lange-Yusty, M. A.; López-Hernández, J. *Food Res. Int*. 2009, 42, 1018.
41. Sladkovský, R.; Solich, P.; Urbánek, M. *J. Chromatogr., A.* 2004, 1040, 179.
42. Muñoz, S.; Mestres, M.; Busto, O.; Guash, J. *Anal. Chim. Acta.* 2008, 628, 104.
43. Del Álamo, M,; Casado, L.; Hernández, V.; Jiménez, J. J. *J. Chromatogr., A.* 2004, 1049, 97.
44. Castillo-Muñoz, N.; Gómez-Alonso, S.; García-Romero, E.; Hermosín-Gutiérrez, I. *J. Agric. Food Chem.* 2007, 55, 992.
45. La Torre, G. L.; La Pera, L.; Rando, R.; Lo Turco, V.; De Bella, G.; Saitta, M.; Dugo, G. *Food Chem.* 2008, 110, 729.
46. Bravo, M. N.; Silva, S.; Coelho, A. V.; Vilas Boas, L.; Bronze, M. R. *Anal. Chim. Acta.* 2006, 563, 84.
47. Cantos, E.; Espín, J. C.; Tomás-Barberán, F. A. *J. Agric. Food Chem.* 2002, 50, 5691.
48. Burin, V. M.; Arcari, S. G.; Costa, L. L. F.; Bordignon-Luiz, M. T. *J. Chromatogr. Sci.* 2011,. 49, 647.

Effect of the particle size of the quinoa sample (*Chenopodium quinoa* Willd) on the validation process of the QuEChERS method for seven pesticides using GC-ECD

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Abstract

Controlling the particle size in the sample preparation process improves the uniformity and representativeness of the sample, enabling more accurate results on analysis. The effects of three types of mills (M1, M2 and M3) were tested in samples preparation of quinoa grains for method validation of seven pesticides (chlorothalonil, heptachlor, captan, α-endosulfan, dieldrin, β-endosulfan and endosulfan sulfate), using QuEChERS and GC-ECD. The method achieved a good chromatographic separation for the seven compounds in 12 minutes. The QuEChERS method was adjusted by changing the type of solvent used for the extraction process to a mixture of ethyl acetate with 1% glacial acetic acid. According to the results, the sensitivity of QuEChERS was influenced by particle size, showing an increase of matrix effects for four of the seven compounds studied and the particle size of 10 µm using cryogenic grinding improved the recovery percentages of method.

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Keywords: quinoa; QuEChERS; pesticides; gas chromatography; matrix effect

1. Introduction

One of the critical points in the analysis of chemical compounds at low concentrations is the type of analytical tool to be used; also the preparation of solid samples is an important stage. A method of analysis requires small amounts of sample, usually between 10 to 500 µg. However, it is essential that the ground material present homogeneous distribution of particle size [1]. Homogenization is an important step in the process of sample preparation for direct analysis of solids and is easily reached by milling procedures. In general, the narrower the particle size range distribution and the smaller the diameter (< 10 µm) improves homogeneity of the sample [2]. Grinding is necessary because finely ground samples are more homogenous and can be subdivided while maintaining representativeness if their particles were carefully homogenized [3].

The choice of milling system to be employed may vary depending on the sample properties such as hardness and fat and fiber content. Cryogenic grinding [4] has as its fundamental principle the increase in hardness of the material and insertion of faults in the crystal structure, which facilitate the process of grinding [4-5]. Since its proposition, cryogenic grinding has been widely used in different types of samples [6-7]. It is a technique that uses liquid nitrogen to freeze the samples before milling, making them brittle and thus reducing the energy required in mechanical mills or the stress required of the analyst when grinding in a mortar. With optimization of methodology a larger number of samples can be processed and more homogenous particles can be obtained [7].

Pesticides residues can occur in food products, especially in fruits and vegetables. Authorities have established controls at Maximum Residue Levels (MRL) or tolerances to protect the environment and, especially, consumer health [8-9-26]. Determinations of pesticide residues in food samples have been necessary due to the toxicity and stability of these xenobiotics [2]. For this purpose, the European Union developed a series of laws that regulate the presence of pesticide residues in food. Actually more than 1100 substances are registered as pesticides [9].

Quinoa (*Chenopodium quinoa* Willd), shown in Figure 1, is a grain cultivated for over 5000 years in the Andes of South America and was an important food for the Inca people due its high nutritional value [10-11]. Its cultivation has been introduced on small scales in other countries in South America, United States, Denmark, among others. The interest in this culture is due to the fact that can be cultivated in soils poor in nutrients and with low water availability. On the other hand, the productivity is low when compared to wheat or corn, ranging to 0.42 t/ha. The quinoa grains are

considered as a rich food, due to their high protein content and quality, while still possessing essential aminoacids and no gluten [12-13]. In 2009, Peru was the major world producer of quinoa, with 53.83% of all world production [7]. Control of pests and diseases in this culture is commonly performed using pesticides.

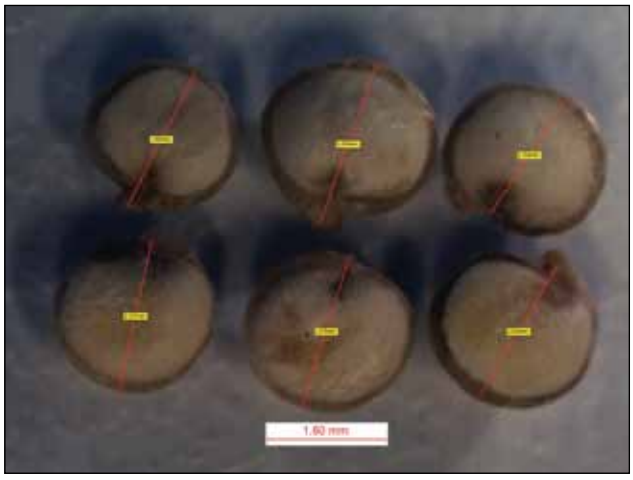

Figure 1. Quinoa grains (*Chenopodium quinoa* Willd)

For pesticide residues analyses in quinoa samples, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method was used. Since its development, the QuEChERS method has revolutionized the form of pesticide multiresidue analysis [14-27]. This method quickly became the most used for laboratories to analyze pesticide residues and was recently established as the official multiresidue method in Europe (European Norm EN 15662: 2009-02: Foods of plant origin determination of pesticide residues using GC-MS or LC-MS/MS following acetonitrile extraction partition and cleanup by dispersive solid phase extraction – DSPE and QuEChERS method).

This method can be used with simultaneous analysis by gas chromatography coupled to a mass spectrometer detector (GC-MS) and high performance liquid chromatography coupled to a mass spectrometer detector (HPLC-MS). Compared to traditional methods of analysis, the QuEChERS method has several advantages, such as high recoveries (> 85%) and results with high accuracy. Moreover, this method is completely robust, since the "clean up" of the extract is done to remove organic acids, reagent costs are low and few devices are needed for sample preparation [16].

Gas chromatography (GC) is a technique that, for more than a half century, has been used extensively in many areas of science. This separation technique is widely used because of its ability to determine the number of components and the proportion of each analyte in a sample. When coupled to MS it has the ability to determine the nature and chemical structure of the separated and quantified compounds. All these aspects depend on the use of spectroscopic detectors, such as the mass spectrometer [17]. The mass spectrometer coupled to gas chromatography is considered a good technical base to offer better chromatographic resolution [18]. However, the main factor limiting the QuEChERS method is that it was developed to be used with mass detectors, with few studies using gas chromatography coupled to an electron capture detector (GC-ECD).

Therefore, the objective of this study was to evaluate the effect of particle size in samples of quinoa grains, subjected to different types of grinding with a normal food processor and two sequences of cryogenic grinding in the process of method validation for determination of seven pesticides (chlorothanlonil, heptachlor, captan, α-endosulfan, dieldrin, β-endosulfan and endosulfan sulfate) using the QuEChERS method.

2. Methodology

2.1. Reagents and chemicals

Pesticide standards (chlorothalonil, heptachlor, captan, α-endosulfan, β-endosulfan, endosulfan sulfate and dieldrin), with more than 98.9% of purity were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and ChemService (West Chester, PA, USA). Stock solutions were prepared in HPLC grade toluene and stored at -18°C. Working standard mixtures were obtained with appropriate dilution from stock solutions before use. Ethyl acetate and toluene were of pesticide grade (J. T. Baker), and acetic acid P.A., CH_3COONa, anhydrous $MgSO_4$ (J.T Baker) and PSA (Varian) were suitable for residue analysis. The quinoa sample without pesticides residues was obtained in Brazil, coming from the company "Vitalina Natural Products" and was used as the blank for method validation.

2.2. Equipment

For identification and quantification of the pesticides evaluated a gas chromatographic system was used (Agilent, model 7890A) coupled to an electron capture detector (ECD), using an Agilent HP column (30m x 320 µm x 0.25 µm). For acquisition and data integration a workstation (Agilent Technologies ChemStation, B.04.02) was used and the statistical processing was performed using Microsoft Excel 2007. The temperature program applied and ECD conditions were: 180-230 °C at 10 °C min^{-1}, 230 °C for 5 min, 230-280 °C at 20 °C min^{-1} and 280 ºC for 5 min, carrier gas (N_2) at a constant flow rate of 40 mL min^{-1} and a detector temperature of 300 °C. The injection system was "pulsed splitless" at 300 ºC with gas flow of 59 mL min^{-1}. The chromatographic run was performed in 12 minutes.

2.3. Sample preparation

Quinoa samples were subjected to three grinding pro-

cesses; the first milling (M1) was performed using a simple food processor, Oster brand at a power of 600 W (Figure 2). The second (M2) and third (M3) milling systems were done with a cryogenic mill, SPEX model 6800; for M2 the program was 5 minute pre-freezing, two mills of two minutes each one with one minute between each grinding (Figure 3), M3 had 5 minutes of pre-freezing, 15 mills of two minutes each with one minute between each grinding (Figure 3).

Figure 2. Particle size after grinding with simple food processor, Oster brand 600 W of power supply (M1).

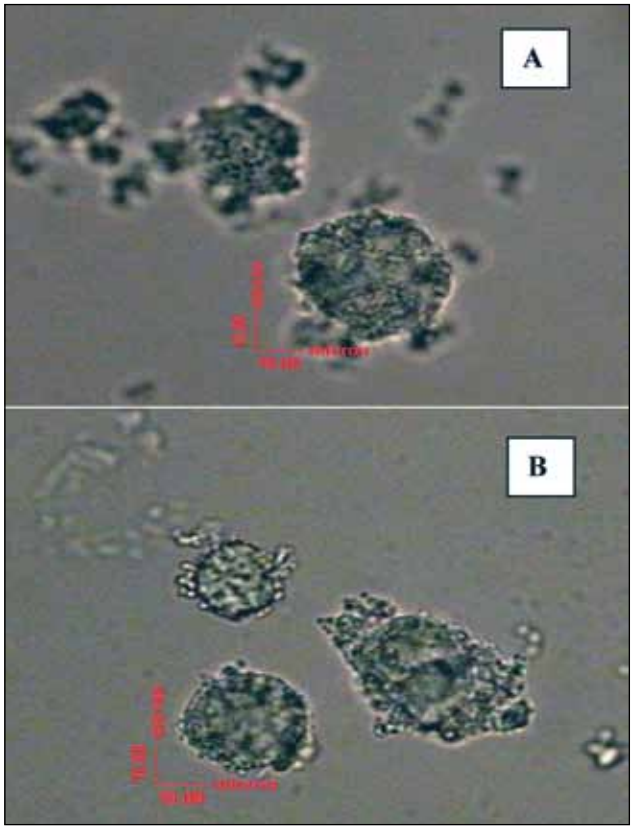

Figure 3. Particle sizes of samples processed with the first cycle cryogenic grinding (M2) (A) and the second cycle of cryogenic grinding (M3) (B).

2.4. Extraction

For the pesticide extraction, an adaptation of the QuEChERS method was performed [26]. Weighing 5 g of previously ground quinoa sample that was placed into a polypropylene tube with 50 mL conical base with lid, containing 6 g of $MgSO_4$ and 1.5 g of CH_3COONa. Then 10 mL of ultrapure water was added to the mixture with 15 mL of ethyl acetate with 1% acetic acid (extraction solution). The tube was hand-shaken vigorously for 1 minute and centrifuged for 5 minutes at 5000 revolutions per minute (rpm). For the process of clean-up 1 mL of the supernatant was placed in a polypropylene tube of 2 mL containing 50 mg of PSA and 150 mg of $MgSO_4$, with vigorous hand-shaking for 30 seconds, then centrifuging for 5 minutes at 5000 rpm. 500 µL of the extract was transferred into a glass vial of 2 mL, wich was completed with 500 µL of ethyl acetate. 1 µL from the final extract was injected into the GC-ECD chromatographic system.

3. Results and discussion

3.1. Validation of analytical method

Validation process of the analytical method was conducted under the guidance of INMETRO [19]. A quinoa sample without pesticide residues was used as a blank. Curves were executed in the matrix extract and solvent (Figures 4, 5, 6 and 7), with seven concentrations and three replicates of each. Recoveries and relative standard deviation (RSD) were determined for all compounds (chlorothalonil, heptachlor, captan, α-endosulfan, β-endosulfan, dieldrin and endosulfan sulfate) at three fortification levels, 0.08, 0.20 and 0.40 mg kg^{-1} and three replicates each level.

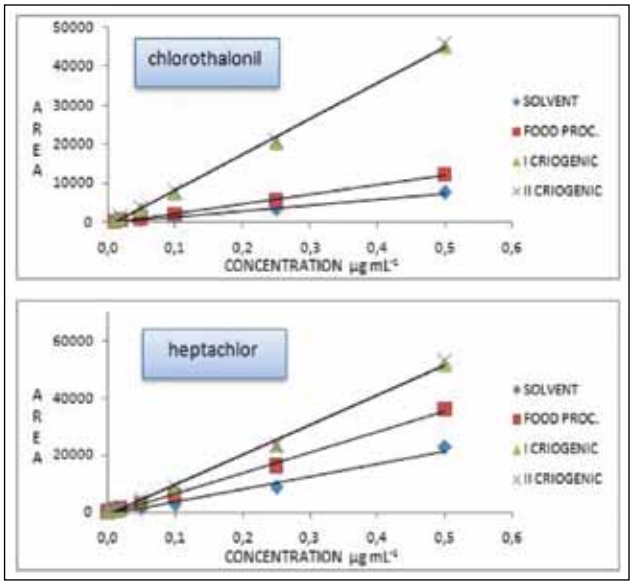

Figure 4. Analytical curves for the compounds chlorothalonil and heptachlor between each treatment

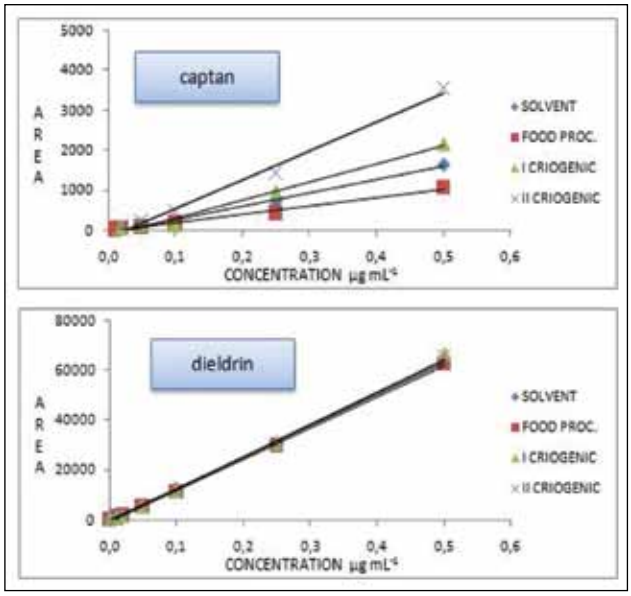

Figure 5. Analytical curves for the compounds captan and dieldrin between each treatment.

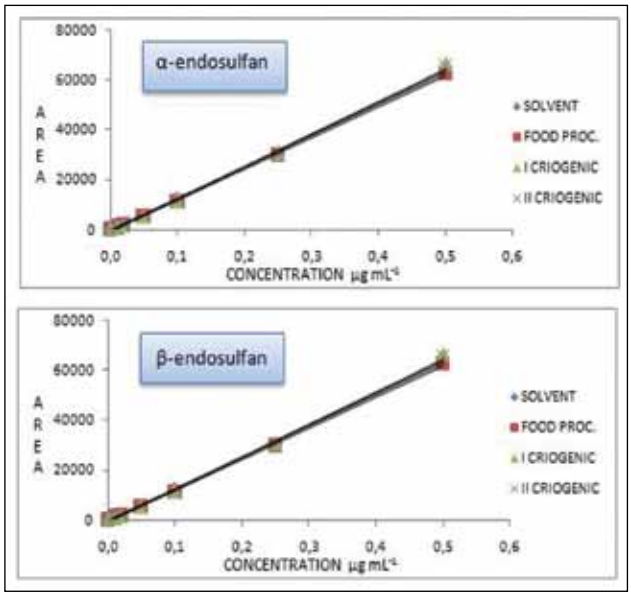

Figure 6. Analytical curves for the compounds α-endosulfan and β-endosulfan between each treatment.

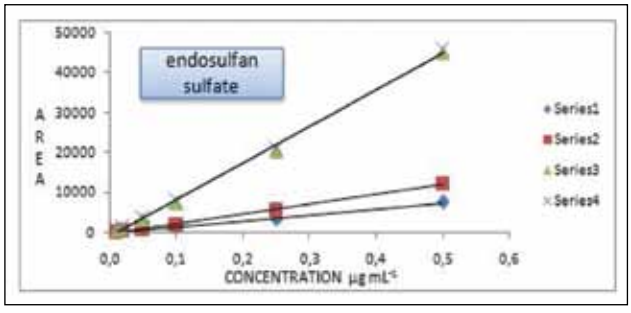

Figure 7. Analytical curves for the compounds endosulfan sulfate between each treatment.

3.2. Selectivity of pesticides in GC-ECD

The separation of compounds in the chromatographic system was performed by injecting standards individually to determine the retention time of each. In order to evaluate the chromatographic separation of the pesticides under study, a standard mixed solution was prepared at a concentration of 0.2 µg mL^{-1}, obtaining good chromatographic separation of the seven compounds, as shown in Figure 8.

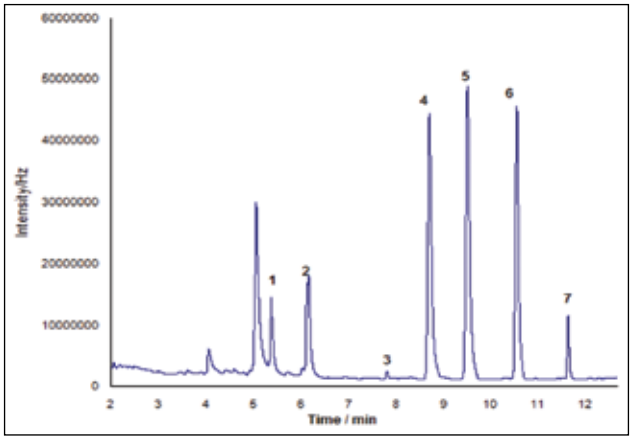

Figure 8. Chromatogram showing the separation of seven compounds analyzed, (1) chlorothalonil, (2) heptachlor, (3) captan, (4) α-endosulfan, (5) dieldrin, (6) β-endosulfan and (7) endosulfan sulfate, respectively. Conditions described in methods.

3.3. Linearity

All compounds showed a correlation coefficient (r^2) ≥ 0.99 in both solvent and matrix extract. The presence of a matrix effect was observed for the compounds chlorothalonil (Figure 4), heptachlor (Figure 4), captan (Figure 5) and endosulfan sulfate (Figure 7). Usually this effect is observed only with organophosphorus compounds [20-21]. However, the present results differ from those found by other authors, because chlorothalonil is a chloronitrilo compound, heptachlor and endosulfan sulfate are organochlorines and captan is a phthalimide, thus, showing that the matrix effect is not unique to organophosphorus compounds. It was observed that the types of grinding influence the presence and intensity of this effect. The presence of a matrix effect can lead to false estimates of the presence of a compound during pesticide residues analysis [21].

3.4. Limits of detection and quantification

The limit of detection (LOD) represents the lowest concentration of the substance in question that can be detected, but not necessarily quantified, using a particular experimental procedure, according to the "International Conference on Harmonization" [22] and DOQ-CGCRE-008 [19]. Using the method based on the analytical curve, the LOD was obtained [24]. Table I shows LOD and Limit of Quantification (LOQ), which were influenced by the types of grinding. Even if slope of the curve is a parameter for calculating

them, there is a negative influence with regard to this parameter, making the method less efficient with respect to its ability for detection and quantification of compounds at low concentrations.

Table I. Limits of detection (LOD) and quantification (LOQ) for each compound according each type of grinding.

Pesticide	Limits for quinoa matrix with different kinds of grinding (mg kg^{-1})					
	M1		M2		M3	
	LOD	LOQ	LOD	LOQ	LOD	LOQ
Chlorothalonil	0.049	0.060	0.138	0.140	0.049	0.050
Heptachlor	0.049	0.051	0.047	0.049	0.049	0.052
Captan	0.077	0.115	0.106	0.127	0.130	0.144
α-endosulfan	0.024	0.025	0,031	0.031	0.034	0.034
Dieldrin	0.023	0.023	0,030	0.031	0.035	0.035
β-endosulfan	0.019	0.020	0,025	0.025	0.027	0.027
Endosulfan sulfate	0.040	0.043	0,039	0.051	0.035	0.046

3.5. Recovery

Results show that the method was less efficient when the sample was subjected to normal grinding (M1), in comparison to the other treatments (Table II). According to the European Comission [9] recoveries at concentrations above 0.1 mg kg^{-1} and under or equal to 1.0 mg kg^{-1} have to be in the range of 70 to 110% with RSD of 15%. The type of grinding of samples has a direct relationship to compound recovery, achieving good recoveries for six of the seven compounds studied. However, endosulfan sulfate showed low recoveries, which may be caused by the release by the sample matrix of interferences that are active in some area generated in active matrix, which then prevent the release of this compound [20]. There was also no recovery for captan at the first two levels of spike with the M1 grinding system. With the M2 and M3 milling recovery occurred in the second and third level. This can occur with some samples due to the fact that captan is known to be difficult to recover and can have a high matrix effect [20].

Santana (2006) [24] presented one of the key projects for improvement of different materials applying another methods of grinding. This author mentions a fine particle can be considered to be a particle whose floatability is prevented by its size. In other words, the fine size range starts at the point where a decrease in recovery starts as a function of particle size. However, this reduction may not occur.

The particle size of quinoa initially presented a diameter of approximately 1.6 mm. With the M1 grinding it became 1.25 mm while grinding with the M2 and M3 systems resulted in 10 mm diameters. However, the milling process directly influenced recoveries of the analytes under study. Recoveries were improved in the treatments with cryogenic grinding (M2 and M3), observing uniform recoveries in the first level of spiked solution (0.08 mg kg^{-1}, Table II. In the matrix spiked at 0.2 mg kg^{-1} (Table III), grinding M3 showed better recoveries for all pesticides, as well as for the fortification at 0.4 mg kg^{-1}. Thus, the M3 system was the level that best showed recoveries for the analytes under study (Table IV).

Grains of smaller diameter showed greater recoveries for the seven pesticides evaluated due to the fact that it increased the contact area with the finer particles. There is also the fact that the samples are at lower temperatures, which favors the preservation of analytes in the quinoa particles.

Table II. Recoveries of analytical methodology after spiking at 0.08 mg kg^{-1}, for gridings M1, M2 and M3.

Pesticide	Recoveries of spike (n=3) at 0.08 mg kg^{-1}					
	M1		M2		M3	
	Average	CV(%)	Average	CV(%)	Average	CV(%)
Chlorothalonil	67.1	1.0	101.7	6.5	119.2	6.4
Heptachlor	96.4	8.0	116.7	11.1	145.1	2.7
Captan	-	-	-	-	201.7	2.5
α-endosulfan	59.8	2.0	84.3	5.6	115.0	3.0
Dieldrin	57.7	3.0	82.5	7.7	112.2	3.6
β-endosulfan	55.1	3.0	78.3	6.8	103.8	3.3
Endosulfan sulfate	50.0	11.0	9.5	67.6	33.9	11.3

Table III. Recoveries of analytical methodology after spiking at 0.2 mg kg^{-1}, for gridings M1, M2 and M3.

Pesticide	Recoveries of spike (n=3) at 0.2 mg kg^{-1}					
	M1		M2		M3	
	Average	CV(%)	Average	CV(%)	Average	CV(%)
Chlorothalonil	32.7	8.0	50.8	2.9	62.3	4.2
Heptachlor	63.6	8.0	57.4	2.2	93.3	0.4
Captan	-	-	52.9	3.9	88.6	2.6
α-endosulfan	43.8	4.0	56.3	6.1	79.0	0.1
Dieldrin	41.9	5.0	54.3	6.2	76.9	0.2
β-endosulfan	42.6	6.0	54.4	6.3	76.1	0.1
Endosulfan sulfate	73.7	8.0	26.4	12.7	45.7	1.3

Table IV. Recoveries of analytical methodology after spiking at 0.4 mg kg^{-1}, for gridings M1, M2 at M3.

Pesticide	Recoveries of spike (n=3) at 0.4 mg kg^{-1}					
	M1		M2		M3	
	Average	CV(%)	Average	CV(%)	Average	CV(%)
Chlorothalonil	20.0	7.0	52.5	1.9	62.2	3.4
Heptachlor	45.0	4.0	54.9	5.2	74.3	10.2
Captan	-	-	47.3	12.7	51.9	4.5
α-endosulfan	35.7	4.0	51.0	3.2	75.0	2.9
Dieldrin	34.7	4.0	50.2	3.1	68.9	8.1
β-endosulfan	35.7	4.0	51.2	2.9	68.2	8.1
Endosulfan sulfate	102.8	13.0	50.9	3.5	69.3	5.4

The size of the particles had greater uniformity with the cryogenic grinding and this directly influenced the quality of

results and the validation of the QuEChERS method, which can show matrix effects due to larger particle sizes from other types of mills. Smaller grain diameters, for the seven pesticides evaluated, showed greater recoveries of analytes due to the fact that the contact area increased with the finer grind. The fact that the samples are at lower temperatures also favored the preservation of analytes in ground particles. Therefore, from the data analyzed studies of three mills (M1, M2 and M3), its suggested to use quinoa particles at size of 10 mm to improve the sensitivity of the QuEChERS methodology, enabling more precise estimates of the results. Using a conventional milling machine Oster (M1), methodology validation is compromised because analytes do not reach internationally acceptable levels of recovery.

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References

1. Ebdon, L.; Foulkes, M.; Sutton, K. *J Anal. At. Spectrosc.*, 1997, 12, 213-229.
2. Bro-Rasmussen, F. *Sci. Total Environ.*, 1996, 188, 45–60.
3. Krug, F. *Métodos de preparo de amostras*. 1. ed. Piracicaba: Ed. do autor, 2008.
4. Iyengar, G.V.; Kasperek, K. *J. Radioanal. Chem.* 1977, 39, 301-316.
5. Koglin, D.; Backhaus, F.; Schladot, J.D. *Chemosphere*, 1997, 34, 2041-2047.
6. Nomura, C.S.; Silva, C.S.; Nogueira, A.R.A.; Oliveira, P.V. *Spectrochim. Acta Part B*, 2005, 60, 673-680.
7. Santos Júnior, D.; Tomazelli, A.C.; Krug, F.J.; Nóbrega, J.A. *Revista Analytica*, 2003, 40-44.
8. http://www.fas.usda.gov/htp/MRL.asp, acessed June 2011.
9. http://ec.europa.eu/sanco_pesticides/public/index.cfm, acessed in June 2011.
10. Galway, N.W.; Leakey, C.L.A.; Price, K.R.; Fenwick, G.R. *Food Sci Nutr.* 1990, 42, 245-261.
11. Kumpun, S.; Girault, J.P.; Dinan, L.; Blais, C.; Maria, A.; Dauphin-Villemant, C.; Yingyongnarongkul, B.; Suksamrarn, A.; Lafont, R. *J. Steroid Biochem. Mol. Biol.*, 2011, 126, 1-9.
12. Dini, G.; Tenore, C.; Dini, A. *J. Nat. Prod.* 2002, 65, 1023–1026.
13. Dini, G.; Tenore, C.; Dini, A. *Food Chem.* 2005, 92, 125–132.
14. Lehotay, S.J.; Mastovska, K.; Lightfield, A. R. *J. AOAC Int.*, 2005, 88, 615-629.
15. Lehotay, S.J; Anastassiades, M; Majors, R.E. *LC GC Eur.*, 2010, 23, 418.
16. Lehotay, S. J. in *Quick, easy, cheap, effective, rugged and safe approach for determining pesticide residues*, eds.; Martínez Vidal, J.L.; Garrido Frenich A.: Wyndmoor, 2005, 239-261.
17. Baker, J. *Mass spectrometry*. 2. ed. New York: John Wiley and Sons, 1999.
18. Grob, R. *Modern Practice of Gas Chromatography*. 3. ed. New York: Wiley: 1999.
19. Mandalakis, M.; Apostolaki, M.; Stepahou, E.G. *J. Chromatogr. A*, 2010, 1217, 143–150.
20. INMETRO. Coordenação Geral de Acreditação. *DOQ-CGCRE-008* - Orientação sobre validação de métodos analíticos. Documento de caráter orientativo. Revisão 03. Rio de Janeiro, 2010. 20 p.
21. Erney, D.R.; Gillespie, A.M.; Gilvydis, D.M. *J. Chromatogr. A*, 1993, 638, 57-63.
22. Poole, C.F. *J. Chromatogr. A*, 2007, 1158, 241–250.
23. FDA. International Conference on Harmonization (ICH). *Guidance for Industry. Q2B:* Validation of analytical procedures: Methodology. Silver Spring, MD, 1995.
24. Santana, R.C.; *Master of Science*, Universidade Federal de Uberlândia, Brazil, 1997.
25. Ribani, M.; Bottoli, C.B.G.; Collins, C.H.; Jardim, I.C.S.F.; Melo, L.F.C. *Quím. Nova*, 2004, 27, 771-780.
26. http://faostat.fao.org/site/567/default.aspx#ancor, acessed in June 2011.
27. Anastassiades, M.; Lehotay, S.J.; Stajnbaher, D.; Schenck, F.J. *Journal of AOAC International*, 2003, 86, 412-431.

Methodology for lanthanide elements quantification in NiMH batteries

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Abstract

This work describes a method for lanthanide quantification in NiMH (Nickel-metal hydride) batteries using inductively coupled plasma optical emission spectrometry (ICP OES). Sample decomposition using *aqua regia*, calcination and fusion were evaluated, with direct decomposition using *aqua regia* and heating being found to be more appropriate. Analyte recovery in solutions with different concentrations of interfering matrix was used to evaluate the method. Lanthanide recoveries in the range 96 to 106% and relative standard deviation (RSD) lower than 7% were observed. Possible spectral interferences were evaluated and spectral lines selected for lanthanide quantification. The limits of detection (LODs) of La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu were 0.38, 0.50, 0.50, 0.12, 0.50, 0.30, 1.65, 0.42, 0.18, 0.75, 0.75, 0.50, 0.50 and 0.38 μg g^{-1}, respectively. The proposed method was applied for lanthanide quantification in samples of NiMH batteries where the concentrations of La, Ce, Pr, Nd and Sm were about 5, 5, 0.4, 1 and 0.1%, respectively, while those of Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu were lower than 0.01% or not detected. The proposed method is useful for quality control of commercialized NiMH batteries and to give support in recycling process of NiMH batteries, with respect to lanthanide concentrations.

Keywords: ICP OES, lanthanide elements, NiMH battery, sample preparation

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1. Introduction

The lanthanide elements have similar physical and chemical properties [1] and are recognized as versatile elements in view of their broad applicability (e.g., in microelectronics, optics, materials science, nuclear reactors, batteries, biomolecular research, medicine, and agriculture) [2]. Data about accumulation of lanthanides in biota and their respective toxic effects are still scarce [3]. Nevertheless, some biological effects have already been observed, such as interference in algae cell processes [4], effects on growth and reproduction of worms [5] and oxidative stress or injury in several organs (e.g. lung, liver, kidney and spleen) of mice [6-9]. The reasons for this are still not sufficiently understood and additional studies are necessary.

Nowadays, production of environmentally acceptable batteries (without toxic elements such as Cd, Hg and Pb in their composition) used for handy electronic devices (cameras, toys, watches, computers, cell phones, remote controls, CD players, alarms, etc.) are required. In this sense, rechargeable batteries such as NiMH (Ni-metal hydride) replaced NiCd batteries in the market [10-12]. Besides being environmentally acceptable, rechargeable NiMH batteries have higher energy capacity, longer cycle of use, and longer shelf life [11] than NiCd batteries.

Several studies report the presence of valuable metals in NiMH batteries such as Co, Mn and the lanthanides [13-16]. Most of the elements of the lanthanide series are rare in nature and have high economic value. Therefore, recycling of NiMH batteries containing lanthanides is very important in view of the limited natural sources of these elements. In this respect, various researchers have investigated processes for battery recycling and recovery of metallic constituents from these batteries [14-18]. However, in order to determine the efficiency of recovery, accurate quantification of the targeted elements is necessary. Nevertheless, lack of detailed information is evident in several applied and reported methods [13, 15, 16, 18, 19]. Therefore, the development of methods for metal (including lanthanide elements) quantification in batteries and products obtained in recycling processes is very important.

The determination of lanthanide elements in NiMH batteries (or in sub products generated in the recycling process) may be challenging due to the complexity of the matrices involved. The determination can be carried out using techniques such as neutron activation analysis (NAA), X-ray fluorescence (XRF), inductively coupled

plasma optical emission spectrometry (ICP OES), and inductively coupled plasma mass spectrometry (ICP-MS). NAA is a very sensitive technique, but a long period of irradiation is necessary to measure the fourteen lanthanide elements, in addition to the high cost of the NAA equipment and low sample throughput. XRF can be employed for direct determination of lanthanides but typical limits of detection (LODs) for lanthanides are not satisfactory for their determination at trace levels using this technique [20]. Graphite furnace atomic absorption spectrometry (GFAAS), despite the lower LODs and worldwide use, is not feasible for lanthanides determination, in light of the formation of thermally stable oxides and refractory carbides that leads to severe memory effects, low tube lifetime and high temperature of atomization [21]. However, these effects are reduced when a metallic atomizer is used instead of a graphite tube [1] but the use of metallic atomizers is restricting. In the case of flame atomic absorption spectrometry (FAAS) the LODs are too high owing to production of refractory oxides of the lanthanides in the flame.

The ICP OES and ICP-MS techniques have been successfully applied for lanthanide determination [2, 3, 13, 15, 16, 18-20] in view of good sensitivity. Spectral and isobaric interferences are observed in ICP OES [22-31] and ICP-MS [32] measurements, respectively, nevertheless these techniques are advantageous for lanthanide determination and accurate results can be obtained by using appropriate nebulizers and/or selection of spectral lines free of overlap [2,32]. Matrix interferences are more pronounced in ICP-MS than in ICP OES due to deposits of salts and oxides on the interface and ion lens. Thus, depending on the complexity of sample matrix, ICP OES may be more suitable for lanthanide determination, despite having lower sensitivity than ICP-MS and being prone to spectral interference.

According to what has been discussed, this work aims to develop a method for lanthanide quantification in NiMH batteries. All lanthanide elements are determined using ICP OES; sample preparation procedures are investigated (i), interferences are discussed (ii), and the spectral lines of lanthanides are selected (iii).

2. Experimental

2.1. Instrument and materials

An ICP OES spectrometer (Optima 2000 DV from PerkinElmer) was used for lanthanide quantification. Argon (purity of 99.998%) from White Martins/Praxair (Brazil) was used as nebulizer gas, plasma gas and auxiliary gas, while nitrogen 99.996% (White Martins/Praxair) was used as purging gas. The operation conditions and accessories employed are summarized in Table I. They were used following recommendations of the instrument manufacturer, or selected during the development of the method or from previous work [2]. A digestion unit (Quimis, Brazil) and a heating block equipped with screw cap- PTFE flasks (TE 0070 from Tecnal, Brazil) were employed for sample decomposition. A muffle furnace (Bravac, Brazil) was used for sample ashing. Graduated (50 mL) polypropylene vials were used to store the sample solutions.

Table I. Operating conditions and accessories employed for lanthanide determination in NiMH batteries by means of ICP OES.

Parameters and accessories	Setting
RF power (W)	1500
Argon flow rate (L min^{-1})	Plasma gas: 15; auxiliary gas: 0.2 and nebulizer gas 0.8
Purge gas (mL min^{-1})	2.5
Spray Chamber	Cyclonic
Nebulizer	GemCone®
Injector tube	Alumina (2 mm i.d.)
Resolution	High
Signal processing mode	Peak area (7 points per peak)
Sample uptake rate (mL min^{-1})	1.5
Plasma viewing	Axial and radial (Co, Mn, Ni, Zn, La, Ce, Pr and Nd)
Spectral lines selected (nm) - all lines are ionic	
La 398.852; Ce 413.380; Pr 414.311; Nd 406.109; Sm 359.260; Eu 381.967; Gd 335.047; Tb 350.917; Dy 353.170; Ho 345.600; Er 337.271; Tm 346.220; Yb 328.937; Yb 369.419; Lu 261.542; Co 228.616; Mn 257.610; Ni 231.604; Zn 206.200	

2.2. Reagents and solutions

All reagents were of analytical-grade. High-purity water (18.2 MΩcm) obtained from a Milli-Q water purification system (Millipore) was utilized throughout the work. Nitric acid (65% in mass) and hydrochloric acid (37% in mass) from Merck (Darmstadt, Germany) were employed for preparation of samples and calibration solutions. Calibration solutions were prepared in 5% (v/v) HNO_3 by serial dilution of monoelemental stock solutions (Tritisol/Merck) containing 1000 mg L^{-1} of each lanthanide element, or Co, Mn, Zn and Ni). A 1000 mg L^{-1} Mg solution (Tritisol/Merck) was used for plasma robustness investigation. For sample fusion, $Li_2B_4O_7.7H_2O$ (from Merck) was used.

Calibration curves were obtained using at least five calibration solutions. The concentration of calibration solutions of La, Ce, Nd and Pr ranged from 0.050 to 50 mg L^{-1} whereas those of Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu ranged from 2.5 to 200 μg L^{-1}. For Co, Zn, Ni and Mn, the concentration of the calibration solutions ranged from 25 to 1200 μg L^{-1}. External calibration was employed and the correlation coefficient of calibration curves was typically 0.999.

2.3. Samples

The NiMH batteries are basically composed of a positive electrode (cathode), a negative electrode (anode) coated with a metal grid, a separator between the electrodes and a metal casing [33,34]. The cathode is constituted of nickel coated with nickel hydroxide whereas the anode consists of ZrNi, $TiNi_2$, $LaNi_5$, or Ti_2Ni. These batteries are nominated as AB, AB_2, AB_5 and A_2B, respectively [35]. Other elements (mainly Mg, Fe, Cr, Al, Co, Mn and V) are also present in the anode [35] where their concentrations vary according to the type of battery. Nowadays, AB_5 is the type of NiMH battery most employed. Due to the high costs involved in La extraction and purification, mischmetal is alternatively used in AB_5 batteries. Mischmetal is a mixture of lanthanide elements extracted from the ore where these elements are naturally present in higher concentration. Mischmetal consists of 50-55% of Ce, 18-28% of La, 12-18% of Nd, 4-6% of Pr and other lanthanides in smaller quantities, as well as other elements present as impurities [36].

The external metal casing and the metal grid are made of steel (Ni-Fe alloy) [33]. Polyamide or polypropylene is used as separator [34] that retains the electrolyte (usually an aqueous KOH solution) [10], avoiding electrode contact and battery self-discharge. The discharge/charge insert process in NiMH batteries occurs according to reactions (1), (2) and (3) [11,37]. During discharge, the nickel oxyhydroxide is reduced to nickel hydroxide (reaction (1)) and the metal hydride MH is oxidized to metal M (reaction (2)). Lanthanum (pure or in mischmetal) in the anode acts as a reservoir to produce the MH used in reaction (2). The overall reaction on discharge is represented by reaction (3). The process is reversed on battery charging.

$$NiOOH + H_2O + e^- \rightarrow Ni(OH)_2 + OH^- \quad (1)$$

$$MH + OH^- \rightarrow M + H_2O + e^- \quad (2)$$

$$MH + NiOOH \rightarrow M + Ni(OH)_2 \quad (3)$$

2.4 Sample identification and decomposition procedures

Five used rechargeable AB_5 batteries of different size (AAA, AA and D) and from different manufacturers were analyzed. These batteries were nominated as samples 1, 2, 3, 4 and 5. The batteries were opened mechanically and the different parts (external metal casing, positive and negative electrodes, metal grid and the separator) were separated and weighed. The mass of each part and the total mass are summarized in Table II.

Table II. Identification and mass of the analysed battery samples.

	Sample Identification and Mass (g) of Respective Parts				
Battery parts	**1 (AA)**	**2 (AAA)**	**3 (D)**	**4 (D)**	**5 (AAA)**
External metal casing	6.61	2.74	14.58	12.45	3.98
Cathode (positive electrode)	11.78	4.80	15.51	19.00	7.34
Anode (negative electrode)	9.21	3.96	13.68	11.18	4.88
Metal grid	1.98	1.18	2.52	3.73	1.38
Separator	2.62	1.01	4.02	4.25	1.58
Total mass	32.20	13.69	50.31	51.61	19.16

The negative electrode (without the metal grid) was taken, macerated and homogenized by grinding in an agate mortar and then stored in a polypropylene flask. The negative electrode (a dark-colour paste) was the only part analyzed since the lanthanides added for battery production are present in this part of the battery. The following decomposition procedures were investigated. The decomposition procedures were evaluated by using sample 3 since its anode had higher mass than the anode of the others (Table II).

Procedure A: 0.2 g of sample was transferred to an open glass tube (2 cm i.d. x 24 cm high) to which 4 ml of *aqua regia* were added. The mixture was let standing at room temperature for 4 h to aid the decomposition of the sample. After that, the mixture was heated at 70 ºC for 4 h. Then, after reaching the room temperature the solution was transferred to a graduated flask and the volume was made up to 50 mL using water.

Procedure B: 1.5 g of sample were transferred to a porcelain crucible that was then placed in a muffle furnace where the sample was sequentially heated at 250 ºC for 30 min, 500 ºC for 1 h and 700 ºC for 1 h. After cooling to room temperature, 0.2 g of the powdered sample was submitted to decomposition according to procedure *A*.

Procedure C: 0.2 g of sample powder obtained according to procedure *B* was transferred to a PTFE flask to which 4 ml of *aqua regia* were added. This mixture was allowed to stand for 16 h at room temperature. After this period, the flask was closed and the mixture heated in a metallic block at 100 ºC for 1 h followed by 150 ºC for 3 h. After reaching room temperature, the flask was opened and the digest transferred to a graduated flask and the volume made up to 50 mL using water.

Procedure D: 0.2 g of sample was transferred to a platinum crucible to which 1 mL of HNO_3 was added and the mixture allowed to stand for 5 min. Subsequently,

the crucible containing the sample was placed on a hot plate and heated at 100 ºC to evaporate the acid. After cooling to room temperature, 0.5 g of $Li_2B_4O_7.7H_2O$ was added to the sample in the crucible that was subsequently placed in a muffle furnace. Then, the temperature inside the furnace was increased to 1100 ºC and maintained at this temperature for 1 h. After reaching room temperature, the outside of the crucible was carefully washed with purified water and immersed in 40 mL of 10% (v/v) HNO_3 in a beaker and heated to 100 ºC on a hot plate. After dissolution of the transparent melt, the crucible was carefully washed with purified water and then removed from the solution. The solution in the beaker was heated until the volume was reduced to 10 mL. Then, the solution was transferred to a graduated polypropylene vial and the volume made up to 50 mL using water.

Procedure adapted from ASTM E1277-08: 0.2 g of the sample was weighted in an open glass tube (2 cm i.d. x 24 cm high) to which 1.5 ml of water was added. Next, 1.5 ml of HCl and 0.1 ml of HNO_3 were sequentially added and the mixture heated during 4 h at 70 ºC. The solution obtained was transferred to graduated flask and the volume made up to 50 ml using water. This procedure is recommended for aluminium-mischmetal alloy analysis in *ASTM E1277-08* [38]. In the present study, the ASTM procedure was adapted just by adding a heating step.

2.5. Dilution of sample solution and recovery tests

The sample solutions obtained according to the procedures above described were further diluted in 5% (v/v) HNO_3. They were diluted 50-fold for quantification of Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu (present in lower concentrations) and diluted 1000-fold for La, Ce, Nd, Pr, Co, Mn, Ni and Zn (present in higher concentrations). Additional dilutions of the sample 3 solution were made to evaluate matrix effects as further discussed in the text. Recovery tests were carried out by spiking the solution of sample 3 decomposed according to procedure A. Plasma robustness (Mg(II) 280.271 nm/Mg(I) 285.213 nm emission ratio) was evaluated using the sample solution diluted 10, 20, 50, 100 or 1000-fold and spiked with Mg in order to obtain 1 mg L^{-1} of this element, since Mg was not present in the sample solution.

Memory effect and plasma stability were also checked by analyzing a 10 µg L^{-1} lanthanides solution between each 10 sample readings (the solution of the sample was 50-fold diluted). The recovery of lanthanides in the 10 µg L^{-1} solution should be at least 95%. Otherwise, the sample introduction system was washed out for 2 min and the sample re-run and/or a new calibration carried out.

3. Results and discussion

3.1 Selection of spectral lines and matrix interference

Considering the mutual interference among the lanthanides elements [22-31] and others present in the samples [22-25, 31], the selection of spectral lines free of interference is mandatory. Mutual interferences depend on the electronic configuration of the atoms and the electronic transitions involved. The *s-p* transition produces stronger emission than those corresponding to *p-d* and *d-f* transitions, which may lead to spectral interference in ICP OES. Cerium, Pr, Nd, Sm, Eu, Gd and Er have partially filled *f*-orbitals that give rise to richer emission spectra than the other lanthanides [25,28,29]. Lanthanum exhibits negligible interference [25] since La^{3+} has a configuration similar to Xe, decreasing the degree of excitation. Lutetium is a lanthanide element with full *f*-orbitals and this configuration also decreases the number of emission lines in comparison with the other lanthanide elements [28]. Anyway, the mutual interference among the lanthanides depends on their concentration in the analyzed sample. It is worth citing that more severe spectral interferences between lanthanides were observed in the past, using older ICP OES spectrometers. Current spectrometers have polychromators or monochromators with higher capability for spectral resolution and some spectral interferences are no longer observed.

Investigations of interferences were carried out by spiking the sample solution with all lanthanides. Before spiking, the sample solution was diluted 10, 20, 50 and 100-fold. The recoveries are depicted in Figure 1. The spectral lines shown were selected by considering quantitative recoveries, absence of overlapping peaks and/or higher sensitivity. In Figure 1 it is possible to observe a similar profile for most elements shown; the lanthanides recoveries for solutions diluted 10 or 20-fold are, in general, overestimated due to matrix interference. On the other hand, recoveries range from 95 to 105% when the sample solution is 50 or 100-fold diluted. Different behavior is observed for La, Ce, Pr and Nd, which were measured by using the radial view of the plasma. The recoveries of these elements are not superstimated even when the sample solution is ten-fold diluted. As will be seen later, the concentrations of Co, Mn, Ni, Zn, La, Ce, Pr and Nd (major elements) in this sample were 7.76, 4.28, 51.8, 0.042, 4.90, 5.23, 0.392 and 1.19%, respectively. The radial view was used to measure these elements since their signals were high.

According to the results obtained in this step of the study, the spectral lines (in nm) chosen for further measurements of Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu were Sm(II) 359.260, Eu(II) 381.967, Gd(II) 335.047, Tb(II)

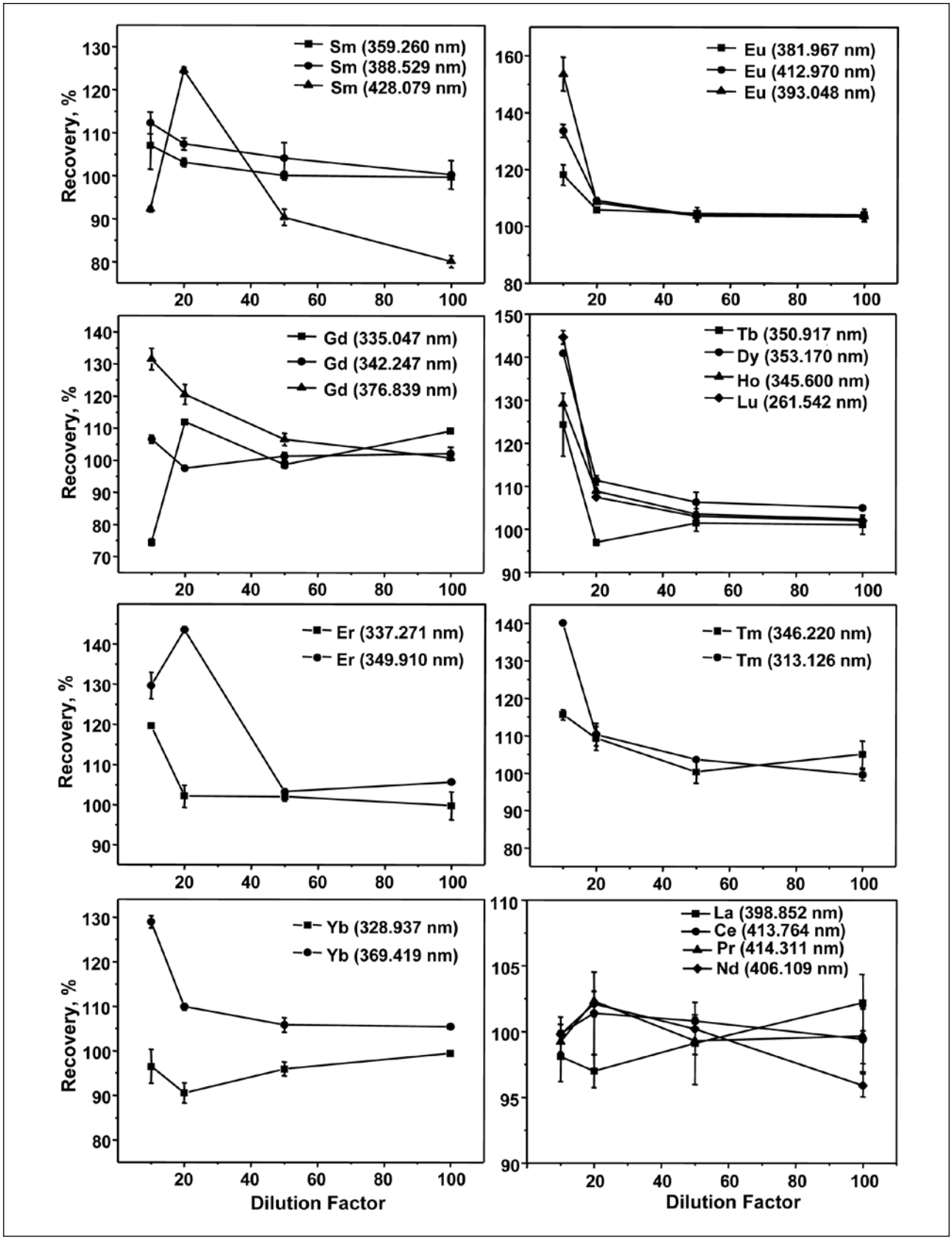

Figure 1. Lanthanide recoveries as a function of sample dilution. Sample 3 was decomposed according to procedure A and spiked with the lanthanides; 20 µg L^{-1} for Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu; 1.0 mg L^{-1} for La, Ce, Nd and Pr. Error bars represent the standard deviation of three replicates of the sample.

350.917, Dy(II) 353.170, Ho(II) 345.600, Er(II) 337.271, Tm (II) 346.220, Yb(II) 328.973 and Lu(II) 261.542. With respect to La, Ce, Nd and Pr, the sample solution could be much more diluted (100-fold for radial view and 1000-fold for axial view) for quantification of these elements in the analyzed samples, reducing matrix interference. The spectral lines (in nm) La(II) 398.852, Ce(II) 413.380, Pr(II) 414.311 and Nd(II) 406.109 were chosen for quantification of La, Ce, Pr and Nd owing to higher sensitivity, good recovery and the absence of spectral interferences (overlap was not observed).

3.2 Evaluation of sample decomposition procedures

The concentrations of lanthanides found in the sample submitted to different decomposition procedures are shown in Table III. Procedure *A* was chosen because higher concentrations for most detected elements were found and the precision was better (the standard deviations are in general lower). Note that procedure *D* gives the worst results for Ce, Er and Gd. This could be due to losses during the fusion step (at 1100 °C) or flux ($Li_2B_4O_7$) interference in the plasma, despite the sample solution being diluted at least 50-fold. Recovery tests were made for these three elements to check whether matrix interference or losses in the fusion step was the reason for the lower concentrations found. The recoveries of Ce, Gd and Er were near 100%, indicating that analyte loss had occurred during sample fusion using $Li_2B_4O_7$ at 1100 °C.

According to the Student-t test (95% confidence level) the results obtained by procedures B and C do not differ significantly. Therefore, the decomposition can be carried out in open or closed vessels. In order to verify if 4 h of contact of sample with *aqua regia* was sufficient (in procedure A), the mixture was left in contact overnight (16 h). The results obtained did not differ significantly, indicating that 4 h of contact was sufficient to extract the lanthanides.

Black insoluble residue was observed in the solution of the sample submitted to decomposition according to procedure A. This residue was probably carbon, which is usually present in battery electrodes [39]. However, according to the literature [39, 40], the residue does not adsorb other elements present in the solution. The solutions obtained according to procedures *B* and *C* (the sample was heated to 700 °C before being treated with *aqua regia*) did not contain residues. However, the lanthanide concentrations found were slightly lower and losses may have occurred during sample calcination. According to the Student-t test (95% confidence level), the results obtained using procedures A, B, C are similar to those obtained using the ASTM procedure and any of them can be employed. In the case of procedure D, only the concentration of Ce was significantly different.

Table III. Concentrations of lanthanides found in the anode of a NiMH battery (sample 3) submitted to different decomposition procedures. Uncertainties are the standard deviations of three determinations (three replicates of the sample).

Element	Concentration, μg g^{-1}				ASTM- E1277
	Procedure A	Procedure B	Procedure C	Procedure D	
La[a]	4.96 ± 0.07	4.39 ± 0.07	4.28 ± 0.10	4.92 ± 0.04	4.87 ± 0.12
Ce[a]	5.08 ± 0.11	4.43 ± 0.03	4.29 ± 0.10	2.55 ± 1.61	5.03 ± 0.15
Pr[a]	0.394 ± 0.004	0.344 ± 0.006	0.336 ± 0.008	0.403 ± 0.005	0.396 ± 0.010
Nd[a]	1.189 ± 0.002	1.045 ± 0.013	1.026 ± 0.016	1.173 ± 0.011	1.176 ± 0.042
Sm	904 ± 1	807 ± 38	827 ± 15	885 ± 20	896 ± 12
Eu	63.8 ± 1.8	57.4 ± 1.0	57.5 ± 0.4	61.8 ± 1.8	61.9 ± 2.3
Gd	47.7 ± 2.0	45.8 ± 0.6	45.5 ± 1.9	31.9 ± 4.5	45.2 ± 1.8
Tb	154 ± 3	142 ± 3	142 ± 1	61.5 ± 50.0	152 ± 5
Dy	< 0.175	< 0.175	< 0.175	< 0.175	< 0.175
Ho	10.4 ± 0.2	9.0 ± 1.0	9.7 ± 0.9	11.9 ± 1.0	10.8 ± 0.5
Er	12.2 ± 0.9	10.3 ± 1.4	9.5 ± 0.8	< 0.75	11.5 ± 0.7
Tm	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Yb	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Lu	< 0.375	< 0.375	< 0.375	< 0.375	< 0.375

[a] in % (g/g); value preceded by < is the respective the LOD (in μg g^{-1})

3.3. Precision, accuracy, limits of detection and quantification

Considering the inexistence of certified reference material (CRM) of NiMH batteries with respect to lanthanide elements concentration, analyte recovery test was used to evaluate matrix interference and precision. In addition to the recovery tests indicated by Figure 1, the solution of sample 3 was diluted 50-fold and spiked in order to obtain 5 or 20 μg L^{-1} for Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu. For La, Ce, Pr and Nd, the solution of sample 3 was spiked in order to obtain 1.0 or 20 mg L^{-1}. Satisfactory recoveries were obtained (typically 95-105%) for a sample solution diluted 50-fold and spiked with all lanthanides. The exceptions were Dy and Ho in the sample solution spiked with 5 μg L^{-1}, whose recoveries were about 114 and 112%, respectively. The relative standard deviation (RSD) for all lanthanides was, in general, lower than 7%.

Plasma robustness (signal of Mg II 280.271 nm/signal of Mg I 285.213 nm) under the operation conditions used (see Table I) was checked for sample solutions diluted 10, 20, 50 and 100-fold. The Mg(II)/Mg(I) ratio found for these solutions were 11.8, 12.2, 12.9, 13.3, respectively. The plasma is considered robust if the ratio Mg(II) 280.271 nm/Mg(I) 285.213 nm is 10 or greater. Therefore, the lanthanide determinations were carried out under robust plasma conditions. Plasma robustness is important for atomization and ionization processes [41] that are mainly influenced by the RF power applied and nebulizer gas. High RF and low nebulizer gas flow rate reduce self-absorption, molecular compounds generation [42] and the effects

of easily ionizable elements such as Na [43]. However, plasma robustness does not necessarily prevent severe matrix interference. According to Figure 1, the recovery of the lanthanides is quantitative when the dilution factor of the sample solution is above 20 even using robust plasma conditions.

The instrumental limits of detection (LODs) were calculated according to following equations:

$$BEC = C_a/SBR \quad SBR = (I_a - I_{blank})/I_{blank} \quad LOD = 3 \times BEC \times RSD/100$$

where, *BEC* is the background equivalent concentration; C_a is the analyte concentration in the reference solution (10 µg L^{-1}); *SBR* is the signal to background ratio, I_a and I_{blank} are the signal intensities of the analyte in the reference solution and blank, respectively, and RSD the relative standard deviation of 10 consecutive measurements of the blank solution. The LOD of the method was calculated taking into account 0.2 g of sample in 50 mL with a 50-fold dilution. The LODs and BEC calculated are summarized in Table IV.

Table IV. Background equivalent concentration (BEC) and limit of detection (LOD) of the method developed for lanthanide elements determination in NiMH batteries.

Analyte (wavelength, nm)	LOD (µg L^{-1})	LOD[a] (µg g^{-1})	BEC (µg L^{-1})
La (398.852)	0.03	0.38	0.05
Ce (413.380)	0.04	0.50	0.21
Pr (414.311)	0.04	0.50	0.16
Nd (406.109)	0.01	0.12	0.12
Sm (359.260)	0.04	0.50	0.11
Eu (381.967)	0.023	0.30	0.09
Gd (335.047)	0.13	1.65	0.22
Tb (350.917)	0.033	0.42	0.14
Dy (353.170)	0.014	0.18	0.11
Ho (345.600)	0.06	0.75	0.56
Er (337.271)	0.06	0.75	0.20
Tm (346.220)	0.04	0.50	0.33
Yb (328.937)	0.04	0.50	0.18
Lu (261.542)	0.03	0.38	0.14

[a] 0.2 g of sample in 50 mL and dilution factor of 50 were considered

3.4. Sample analyses

Five spent AB_5 batteries were analyzed and the concentrations of lanthanides found in the anode are shown in Table V. Considering the entire battery (see the mass in Table II) the concentrations of all quantified elements are about 4 times lower. As expected, La, Ce, Pr and Nd are the elements present in higher concentrations because they are major constituents of the mischmetal used in AB_5 battery production. Futhermore, concentrations of Yb, Sm, Eu, Gd, Tb are also relevant. The Ni, Co, Mn and Zn concentrations are also shown in Table V to highlight the complexity of the sample matrix involved.

Table V. Lanthanides concentrations and major elements found in the anode of NiMH batteries.

Element	Concentration, %				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Lanthanides					
La	3.22 ± 0.19	3.22 ± 0.45	4.90 ± 0.05	14.1 ± 0.5	10.3 ± 0.2
Ce	0.81 ± 0.03	0.88 ± 0.12	5.23 ± 0.06	9.47 ± 0.23	6.31 ± 0.14
Pr	0.043 ± 0.003	0.11 ± 0.01	0.392 ± 0.004	0.59 ± 0.01	0.41 ± 0.01
Nd	0.323 ± 0.022	0.46 ± 0.06	1.189 ± 0.002	2.34 ± 0.11	1.87 ± 0.03
Sm	87.42 ± 4.76*	0.015 ± 0.002	0.088 ± 0.004	0.079 ± 0.002	0.052 ± 0.001
Eu	< 0.30	< 0.30	61.07 ± 0.31*	49.77 ± 0.99*	0.122 ± 0.001
Gd	< 1.65	28.05 ± 1.95*	58.82 ± 7.78*	33.82 ± 0.36*	0.161 ± 0.012
Tb	31.55 ± 0.79*	35.39 ± 2.65*	0.0154±0.0003	0.0164±0.0002	0.360 ± 0.019
Dy	< 0.175	< 0.175	< 0.175	< 0.175	< 0.175
Ho	< 0.75	< 0.75	29.01 ± 0.15*	< 0.75	0.023 ± 0.001
Er	< 0.75	< 0.75	18.01 ± 0.83*	0.0108±0.0008	< 0.75
Tm	30.94 ± 1.98*	26.2 ± 1.5*	< 0.5	< 0.5	< 0.5
Yb	0.318 ± 0.004	< 0.5	< 0.5	< 0.5	< 0.5
Lu	< 0.375	< 0.375	< 0.375	< 0.375	< 0.375
Other major elements, %					
Co	6.00 ± 0.15	6.23 ± 0.12	7.76 ± 0.05	7.50 ± 0.12	4.45 ± 0.19
Mn	1.43 ± 0.07	1.63 ± 0.01	4.28 ± 0.32	4.65 ± 0.05	4.35 ± 0.14
Ni	55.3 ± 1.4	56.8 ± 0.5	51.8 ± 0.4	51.7 ± 0.2	50.1 ± 1.5
Zn	1.61 ± 0.03	2.12 ± 0.14	0.042 ± 0.001	0.073 ± 0.015	0.050 ± 0.002

*: in µg g^{-1}; value preceded by < is the respective the LOD (in µg g^{-1})

The presence of La, Ce, Nd and Pr as major elements in the anode of NiMH batteries has been reported previously [17, 18]. In general, major elements and lanthanide elements determinations were carried out using AAS [13, 17], X-ray diffraction (XRD) [14, 17, 18, 33, 44, 45], X-ray fluorescence [18, 33, 44, 45] and/or ICP OES [13, 15, 16]. Nevertheless, the determination of the other lanthanide elements in NiMH batteries has not been reported previously.

Ananth *et Yo. al.* [45] reported that different La/Ce ratios in the anode result in different crystalline structures that lead to distinct performances in the discharge-charge cycle of the AB_5 batteries. They observed that the battery has better capacity and performance if the La/Ce ratio is 12. These lanthanides allow formation of sites for storage and diffusion of hydrogen, resulting in better performance. No study reported the influence of minor lanthanides elements (Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu), but it is possible that they play an important role.

Conclusions

To conclude, a method for lanthanide determination in NiMH batteries was developed, which allows quantifi-

cation of these elements by ICP OES. The method developed can be useful in recycling processes of NiMH batteries containing lanthanides as well as for battery quality control. This study also revealed the satisfactory performance of ICP OES for lanthanide elements determination in a quite complex matrix.

The proposed method is potentially useful for quality control of commercialized NiMH batteries and may give support in the recycling processes of NiMH batteries, with respect to lanthanide elements concentrations.

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References

1. Silva, J.C.J.; Garcia, E.E.; Nogueira, A.R.A.; Nóbrega, J.A. *Talanta* 2001, 55, 847.
2. Bentlin, F.R.S.; Pozebon, D. *J. Braz. Chem. Soc.* 2010, 21, 627.
3. Costas, M.; Lavilla, I.; Gil, S.; Pena, F.; de la Calle, I.; Cabaleiro, N.; Bendicho, C. *Anal. Chim. Acta* 2010, 679, 49.
4. Tai, P.; Zhao, Q.; Su, D.; Li, P.; Stagnitti, F. *Chemosphere* 2010, 80, 1031.
5. Zhang, H.; He, X.; Bai, W.; Guo, X.; Zhang, Z.; Chai, Z.; Zhao, Y. *Metallomics* 2010, 2, 806.
6. Liu, J.; Li, N.; Ma, L.; Duan, Y.; Wang, J.; Zhao, X.; Wang, S.; Wang, H.; Hong, F. *J. Alloys Compd.* 2010, 489, 708.
7. Marubashi, K.; Hirano, S.; Suzuki, K.T. *Toxicol. Lett.* 1998, 99, 43.
8. Weilin, S.; Xiuying, S.; Xiying, M. *J. Rare Earth* 2006, 24, 415.
9. Shimada, H.; Nagano, M.; Funakoshi, T.; Kojima, S. *J. Toxicol. Environ. Health* 1996, 48, 81.
10. Ambrosio, R.C.; Ticianelli, E.A. *Quím. Nova* 2001, 24, 243.
11. Linden, D.; Reddy, T.B.; *Handbook of Batteries*, 3 rd ed.; McGraw-Hill: New York, 2002.
12. http://www.epbaeurope.net. European Portable Battery Associtation. Accessed in March 2012.
13. Tzanetakis, N.; Scott, K. *J. Chem. Technol. Biotechnol.* 2004, 79, 919.
14. Tenório, J.A.S.; Espinosa, D.C.R. *J. Power Sources* 2002, 108, 70.
15. Zhang, P.; Yokoyama, T.; Itabashi, O.; Wakui, Y.; Suzuki, T.M.; Inoue, K.. *Hydrometallurgy* 1998, 50, 61.
16. Zhang, P.; Yokoyama, T.; Itabashi, O.; Wakui, Y.; Suzuki, T.M.; Inoue, K. *J. Power Sources* 1999, 77, 116.
17. Pietrelli, L.; Bellomo, B.; Fontana, D.; Montereali, M.R. *Hydrometallurgy* 2002, 66, 135.
18. Pietrelli, L.; Bellomo, B.; Fontana, D.; Montereali, M. *Waste Manage.* 2005, 25, 221.
19. Vassura, I.; Morselli, L.; Bernardi, E.; Passarini, F. *Waste Manage.* 2009, 29, 2332.
20. Zawisza, B.; Pytlakowska, K.; Feist, B.; Polowniak, M.; Kita, A.; Sitko, R. *J. Anal. At. Spectrom*. 2011, 26, 2373.
21. Tsalev, D.L.; *Atomic Absorption Spectrometry in Occupational Environmental Health Practice*, vol. 3, CRC Press: Boca Raton, FL, 1995.
22. Boumans, P.W.J.M.; Tielrooy, J.A.; Maessen, F.J.M.J. *Spectrochim. Acta B* 1988, 43, 173.
23. Boumans, P.W.J.M.; Vrakking, J.J.A.M.; Herjms, A.H.M. *Spectrochim. Acta B* 1988, 43, 1365.
24. Boumans, P.W.J.M.; He ZhiZhuang; Vrakking, J.J.A.M.; Tielrooy, J.A.; Maesen, F.J.M.J. *Spectrochim. Acta B* 1989, 44, 31.
25. Daskalova, N.; Velichkov, S.; Krasnobaeva, N.; Slavova, P. *Spectrochim. Acta B* 1992, 47, E1595.
26. Velichkov, S.; Daskalova, N.; Slavova, P. *Spectrochim. Acta B* 1993, 48, E1743.
27. Daskalova, N.; Velichkov, S.; Slavova, P. *Spectrochim. Acta B* 1996, 51, 733.
28. Velichkov, S.; Kostadinova, E.; Daskalova, N. *Spectrochim. Acta B* 1998, 53, 1863.
29. Kostadinova, E.; Aleksieva, L.; Velichkov, S.; Daskalova, N.. *Spectrochim. Acta B* 2000, 55, 689.
30. Aleksieva, L.; Daskalova, N.; Velichkov, S. *Spectrochim. Acta B* 2002, 57, 1341.
31. Kolibarska, I.; Velichkov, S.; Daskalova, N. *Spectrochim. Acta B* 2008, 63, 603.
32. Dressler, V.L.; Pozebon, D.; Matusch, A.; Becker, J.S. *Int. J. Mass Spectrom.*2007, 266, 25.
33. Bertuol, D.A.; Bernardes, A.M.; Tenório, J.A.S. *J. Power Sources* 2006, 160, 1465.
34. Müller, T.; Friedrich, B. *J. Power Sources* 2006, 158, 1498.
35. Berndt, D.; *Maintenance-Free Batteries, 2nd ed.*; John Wiley & Sons: New York, *1997*.
36. Ruetschi, P.; Meli, F.; Desilvestro, J. *J. Power Sources* 1995, 57, 85.
37. Kirk, R.E.; Othmer, D.F.; *Batteries, Encyclopedia Chem. Technol.* 1984, 3, 963.
38. American Society for Testing Materials. ASTM E 1277-08; Standard Test Method for Chemical Analysis of Zinc-5% Aluminium-Mischmetal Alloys by ICP Emission Spectrometry, 2008.
39. Recknagel, S.; Richter, A.; Richter, S. *Waste Manage.* 2009, 29, 1213.
40. Rastogi, S.C. *At. Spectrosc.* 1989, 10, 144.
41. Velitchkova, N.; Velichkov, S.; Daskalova, N. *Spectrochim. Acta B* 2007, 62, 386.
42. Garcia, E.E.; Nogueira, A.R.A.; Nóbrega, J.A. *J. Anal. At. Spectrom.* 2001, 16, 825.
43. Mermet, J.M. *Anal. Chim. Acta* 1991, 250, 85.
44. Rodrigues, L.E.O.C.; Mansur, M.B. *J. Power Sources* 2010, 195, 3735.
45. Ananth, M.V.; Raju, M.; Manimaran, K.; Balachandran, G.; Nair, L.M. *J. Power Sources* 2007, 167, 228.

Determination of Sudan II dye in ethanol fuel by chromatographic and electroanalytical methods

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Abstract

Chromatographic and electroanalytical methods were developed to detect and quantify Sudan II (SD-II) dye in fuel ethanol samples. Sudan II is reduced at +0.50 V *vs.* Ag/AgCl on a glassy carbon electrode using Britton-Robinson buffer (pH 4.0) and *N*,*N*-dimethylformamide (70:30, v/v) + sodium dioctyl sulfosuccinate surfactant as supporting electrolyte, due to the azo group. This is the basis for its determination by square-wave voltammetry (SWV). Using the optimized conditions, it is possible to get a linear calibration curve from 3.00×10^{-6} to 1.80×10^{-5} mol L^{-1} (r = 0.998) with limits of detection (LOD) and quantification (LOQ) of 2.05×10^{-6} and 6.76×10^{-6} mol L^{-1}, respectively. In addition, the hydroxyl substituent in the SD-II dye is also oxidized at +0.85 V *vs.* Ag/AgCl, which was conveniently used for its determination by high-performance liquid chromatography coupled to electrochemical detection (HPLC-ED). Under the optimized condition, the SD-II dye was eluted and separated using a reversed-phase column (cyanopropyl, CN) using isocratic elution with the mobile phase containing acetonitrile and aqueous lithium chloride (5.00×10^{-4} mol L^{-1}) at 70:30 (v/v) and a flow rate of 1.2 mL min^{-1}. Linear calibration curves were obtained from 3.00×10^{-7} to 2.00×10^{-6} mol L^{-1} (r = 0.999) with LOD and LOQ of 3.10×10^{-8} and 1.05×10^{-7} mol L^{-1}, respectively. Both methods were simple, fast and suitable to detect and quantify the dye in fuel ethanol samples at recovery values between 83.0 to 102% (SWV) and 88.0 to 112% (HPLC-ED) with satisfactory precision and accuracy.

Keywords: Sudan II; Dye marker; Fuel analysis; Electroanalysis; HPLC-ED.

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1. Introduction

The use of dyes in the fuel industry, in general, is associated with brand protection and destination [1-4]. Some dyes can be called "markers" and are used to guarantee the identity of the specific product or discourage theft, tampering and disclosure of the quality of solvent or fuel. A typical example, prescribed by ANP (Brazilian National Petroleum Agency), is the use of a yellow dye and a red dye to produce an orange coloration in dehydrated fuel ethanol with the purpose of distinguishing it from hydrated ethanol [5]. Fuels are usually taxed according to government regulations, which depend on the types of fuel as well as on their applications [1-4, 6-15].

1-(2,4-Xylidylazo)-2-naphthol, also called Sudan II (SD-II), Figure 1, is a representative of an azo-dye family, a class of substances used as visible markers added to different types of fuels and solvents [1-4]. For this reason, to protect the fuel tax system and prevent fraud, liquid chromatographic and electroanalytical methods must be in place to detect dyes in fuels and distinguish between different varieties of fuel alcohols available on the market, as well as to differentiate between the different dyes available today. Despite the importance of detection and quantification of dyes, few analytical methods have been described using chromatographic and electrochemical techniques for the determination of dye in fuel samples such as, ethanol, gasoline and diesel [4, 6].

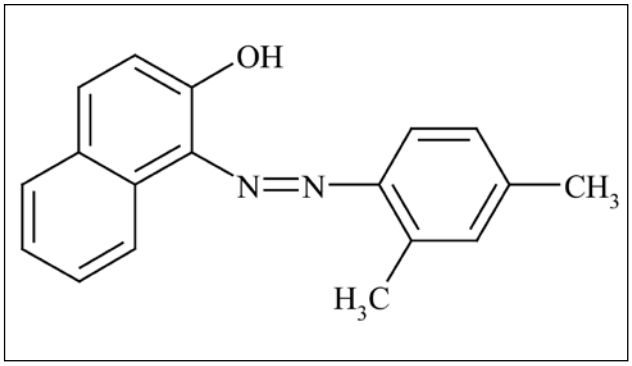

Figure 1. Molecular structure of Sudan II (SD-II).

Our research group has shown that electroanalytical techniques can be successfully applied to quantify quinizarin, QNZ, [16] and solvent blue 14, SB-14, [17, 18] in fuel samples. The methodology allowed detecting these dyes with a glassy carbon electrode and screen-printed carbon electrodes at satisfactory concentration levels. In reported work [18], we investigated the possibility of using surfactants as effective for preventing electrode fouling from the matrix constituents and screen-printed carbon electrode devices as a way of miniaturizing the set of electrodes conventionally used in electrochemical procedures and to propose a simple voltammetric method to determine the SB-14 dye.

Research-using HPLC with UV/vis detection [19] and HPLC with electrochemical detection [20, 21] were also developed. The electrochemical detection system can be recommended for the quantitative determination of dyes in fuels when low-level detection and interference separations are required. Based on what was proposed before, quantitative methods were developed to detect the SB-14, solvent blue 35 (SB-35) and solvent red 24 (SR-24) dyes in fuel ethanol samples and gasoline. The use of the HPLC technique coupled with electrochemical detection for the quantification of the dye-compounds SB-14, SB-35 and SR-24 in fuel samples after a simple and fast pretreatment protocol was also demonstrated [20, 21].

An electroanalytical methodology was also developed and applied to quantify Sudan II (SD-II) in ethanol samples using the square-wave voltammetry technique and detection with a glassy carbon electrode [22]. The methodology was developed using Britton-Robinson buffer and *N,N*-dimethylformamide (1:1, v/v) as supporting electrolyte, which allowed directly detecting the SD-II dye in fuel ethanol samples at a concentration of 0.025 mg L^{-1}. However, the electrode process was complicated due to strong adsorption of the dye and/or electrochemical products on the electrode surface, which promoted passivation and required polishing for cleaning and reestablishing its electroactivity surface.

In the present work, we investigated method improvement by using sodium dioctyl sulfosuccinate surfactant as an antifouling agent to avoid electrode passivation, which allowed lower detection limits and well-defined waves able to be applied in the determination of SD-II dye in fuel ethanol samples. In addition, taking into consideration that the HPLC technique with electrochemical detection system is recommended for the quantitative determination when low-level detection and interference separations are required [23], we also propose the quantification of the SD-II dye in fuel ethanol samples after a simple and rapid procedure using this analytical technique.

2. Experimental section

2.1. Chemicals, reagents and standard solutions

For the chromatographic analysis, acetonitrile (HPLC-grade, Merck), methanol (HPLC-grade, Merck) and ultra-pure water were used throughout the experiment. Moreover, an electrolyte solution of ammonium acetate and lithium chloride were obtained from Sigma-Aldrich Chemical Company (99%, for HPLC) and (Merck), respectively. Finally, the standard Sudan II (SD-II) was obtained from Sigma-Aldrich Chemical Company.

Standard stock solutions of the dye SD-II (1.00×10^{-3} and 1.00×10^{-2} mol L^{-1} for SWV and cyclic voltammetry studies, respectively) were prepared through weighing a sufficient amount of the corresponding solid followed by dissolution (to 10 mL) with acetonitrile (HPLC grade, J.T. Baker). The stock solutions were stored in glass flasks in a freezer and the working standards for the dye in different concentrations were prepared in calibrated flasks of 5.0 mL after dilution of a stock solution aliquot in acetonitrile. Ammonium acetate and lithium chloride were prepared in ultra-pure water (Millipore, Bedford, MA, USA).

For electrochemical measurements, the Britton-Robinson (B-R) (0.10 mol L^{-1}) buffer, at pH range between 2.0 and 9.0, was used as the supporting electrolyte solution and was prepared in the usual way: a mixture of 0.10 mol L^{-1} acetic acid (Merck), 0.10 mol L^{-1} boric acid (Merck) and 0.10 mol L^{-1} orthophosphoric acid (Merck) with the appropriate amount of 1.0 mol L^{-1} sodium hydroxide (Merck) solution to adjust the pH to the required value. The N,N-dimethylformamide (DMF) (Mallinckrodt), used as supporting electrolyte solution, was mixed with B-R (analytical grade). The stock solution of sodium dioctyl sulfosuccinate (DSS, Sigma-Aldrich) surfactant at a concentration of 5.50×10^{-2} mol L^{-1} was prepared in deionized water.

Ethanol fuel samples were collected from a gas station in Araraquara city, São Paulo, Brazil, and an appropriate dye concentration was added.

2.2. Instrumentation

The HPLC system consisted of a binary pump (Varian ProStar, model 210/215), UV-vis detector (Varian ProStar, model 320) and an electrochemical detector (Varian ProStar, model 370). The measurements were performed in amperometric detection mode using a wall-jet cell. Working (geometric area of 0.71cm^2), reference and auxiliary electrodes were glassy carbon, in situ Ag/AgCl and platinum, respectively. The HPLC system was used in an isocratic elution with a mobile phase consisting of organic solvent (acetonitrile, ACN) and aqueous lithium chloride (5.0 mmol L^{-1}) in the required proportions.

Chromatographic conditions (mobile phase composition and flow-rate) were evaluated and optimized in reversed-phase testing with RP-C18 (150 × 4.6 mm i.d., 5 µm, Phenomenex®) and cyanopropyl (LC-Column Shim-pack, Shimadzu ®) (150 × 4.6 mm, 5 µm). For sample analysis, a guard column (SecurityGuard Holder, Phenomenex®, C-18, 4.0 × 3.0 mm) was used.

Electrochemical analysis was performed using an Autolab PGSTAT-30 (Eco Chemie) connected to a microcomputer controlled by General Purpose Electrochemical System (GPES 4.9) software for data acquisition and experimental control. The measurements were performed in a conventional electrochemical cell, maximum capacity, 10 mL. A conventional three-electrode system was used and they were composed of a glassy carbon disc (2.0 mm diameter) set in a Teflon tube as the working electrode, a saturated Ag/AgCl (inside a Luggin capillary containing 3.0 mol L^{-1} KCl) used as the reference electrode and a platinum wire used as the counter electrode.

All pH measurements were made using a combined glass electrode (Orion, Thermo Electron Corporation) connected to the digital pH-meter (Orion, Thermo Electron Corporation) and are expressed as $pH_{apparent}$ (pH*). The deionized water used to prepare the supporting electrolyte solutions was purified with a Milli-Q system and it had a resistivity of 18 MΩ cm^{-1} (model Simplicity 185, Millipore).

2.3. Procedure for voltammetric analysis

The voltammograms were recorded for 10 mL of the supporting electrolyte solution, transferred into the electrochemical cell and deaerated with nitrogen for 10 min. The same procedure was then repeated after the addition of the SD-II dye sample. Each series of measurements were monitored after polishing the glassy carbon electrode with 1.0 µm alumina, rinsing with de-ionized water and acetone. The calibration curve for SD-II was constructed after dilution (in the electrochemical cell containing supporting electrolyte) of the stock solution to obtain concentrations from 3.00×10^{-6} to 1.80×10^{-5} mol L^{-1}.

2.4. Sample preparation and analysis of SD-II dye in ethanol sample

For the chromatographic analysis, two different samples were prepared, in which the ethanol sample (aliquots of 10 mL, collected from a gas station in Araraquara city, SP, Brazil) were spiked with SD-II dye at concentration levels of 0.50 mg L^{-1} (sample A) and 2.00 mg L^{-1} (sample B). These values are lower than those commonly found in this matrix. Prior to the analysis, aliquots containing 0.50 mL of sample A and 0.20 mL of sample B were collected in an 5.00 mL volumetric flask and the volume was completed with acetonitrile. The resultant solutions were filtered through a 0.45 µm PTFE filter membrane (Millipore) prior to injection in chromatographic system. The recovery tests of the SD-II in the spiked samples were performed after its determination using the standard addition method.

For the electrochemical analysis, two different samples were prepared, in which the ethanol sample (aliquots of 10 mL, collected from a gas station in Araraquara city, SP, Brazil) were spiked with SD-II dye at concentration levels of 5.00 mg L^{-1} (sample C) and 10.0 mg L^{-1} (sample D). For the quantitative analysis of samples containing the SD-II dye, aliquots containing 2.0 mL (sample C) and 1.0 mL (sample D) were mixed with, respectively, 8.0 and 9.0 mL of supporting electrolyte solution (B-R buffer pH 4.0 + DMF, 7:3 v/v) and in the presence of the required concentration of the DSS surfactant. The resulting mixture was transferred to the electrochemical cell, submitted to deaeration with nitrogen for 10 min, and directly analyzed using square-wave voltammetry. The recovery tests of the SD-II in the spiked samples were performed after its determination using the standard addition method.

3. Results and discussion

3.1. Optimization of the chromatographic conditions

The separation of the SD-II dye was performed after testing reversed-phase columns (C-18) and a cyanopropyl column with mobile phases composed of acetonitrile (ACN) and aqueous solutions containing ammonium acetate or lithium chloride. This chromatographic condition was chosen as the initial point, which was similar to those already used in previous work on the determination of other dyes [19-21]. SD-II dye is strongly retained on conventional reversed-phase column (C-18) due to its hydrophobicity, requiring a higher percentage of ACN (more than 80%) in the mobile phase composition. However, a mobile phase containing more than 80% of ACN promoted baseline perturbation, requiring a long time for baseline-stability, which caused high resistivity of the medium.

As reported by Kissinger [23] and according to what was discussed in a previous work [20, 21], the gradient mode is not useful in HPLC with electrochemical detection due to the changes in the mobile phase conductivity composition and the difficulty to control baseline stability. As a result, we have found that satisfactory chromatographic conditions could be obtained using the isocratic mode. In liquid chromatography, the analysis time can be reduced by means of smaller columns packed with an appropriate stationary phase. Thus, experiments were conducted using the reversed phase mode and a cyanopropyl column. Satisfactory results were obtained when the optimized conditions were set by adjusting the flow rate, as an attempt to improve tailing peaks, peak shape and to decrease the retention time for the target dye. The percentage of ACN was set at 70% and the flow rate at 1.20 mL min^{-1}. Taking this into consideration, no significant fluctuations in the

background current was observed and the final elution program allowed obtainments of the chromatograms with the analysis taking less than 6.0 min.

Furthermore, to optimize the potential for detection of the target dye, the experiments were carried out recording chromatograms at applied potentials between +0.70 and +1.00 V (Figure 2), of which the baseline stability is a significant factor to control when using HPLC with electrochemical detection. The hydrodynamic voltammograms recorded measuring the current over the range of working electrode potentials indicated that the oxidative current response appeared larger around +0.80 and +0.90 V, showing that the analysis can be obtained by detection at a specific oxidation potential. The dye studied exhibited considerable difference in the current intensity for potential detection smaller than +0.90 V and it was also observed that the background currents evaluated at a potential smaller than +0.85 V were reproducible, presenting values around 5.0 ± 0.5 nA with reduced time for baseline stabilization. Therefore, the suitable voltage for the SD-II detection was attributed as +0.85 V and this potential represented the best compromise between maximum signal, the highest S/N ratio, time baseline-stabilization and analytical applicability.

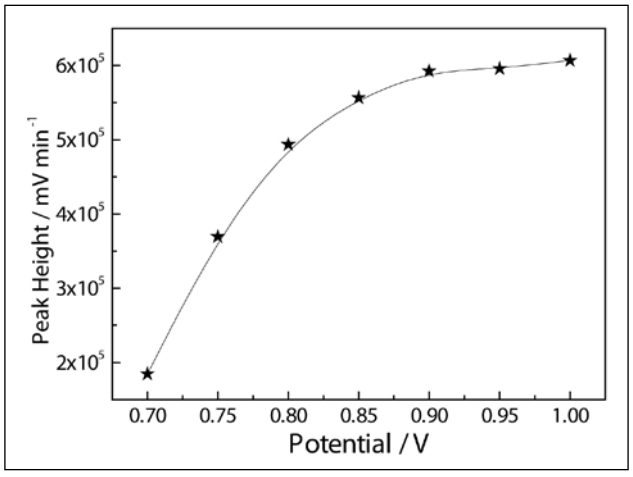

Figure 2. Hydrodynamic voltammogram obtained recording chromatograms for 2.00×10^{-6} mol L^{-1} of the SD-II dye. Conditions: mobile phase acetonitrile / lithium chloride (5.00×10^{-4} mol L^{-1}) in proportions of 70:30 (v/v). Flow rate = 1.20 mL min^{-1} and injection volume of 25 µL.

Under the mentioned conditions, the selectivity of the method was demonstrated by analyzing aliquots of the ethanol fuel in the absence and the presence of the SD-II dye. To certify that the standard dye would be accurately detected in the presence of the matrix (prepared as in experimental section 2.4) 1.50 mg L^{-1} of the dye were added and it was submitted to analysis. In this case, the chromatogram obtained also allowed identification of the SD-II peak and to observe that the method proved to be selective for SD-II, in which there are no interference peaks overlapping in the dye identification.

3.1.1. Linearity, limits of detection and quantification

Chromatograms were recorded in concentration range between 3.00×10^{-7} to 2.00×10^{-6} mol L^{-1} to check system linearity using the optimized conditions previously defined and performed in triplicate injections (each point) of standard solutions in the presence of sample. The representative chromatograms, Figure 3, showed that there are no interference peaks overlapping in the chromatograms obtained. Satisfactory linearity was verified for all the studied concentration levels and the regression equation obtained from plotting peak area against the concentration was $Area = 9.97\times10^{4} + 5.20\times10^{12}\times C$ (mol L^{-1}), with a correlation coefficient of 0.999. From the linear regression obtained in the analytical curve, the standard deviation (intercept error, S_A), was used to estimate the limits of detection (LOD) and quantification (LOQ) through the following equations: $3\times S_A/m$ and $10\times S_A/m$, where m is the slope of the calibration curve [24]. The values obtained for LOD and LOQ were 3.10×10^{-8} mol L^{-1} and 1.05×10^{-7} mol L^{-1}, respectively, which were satisfactory, taking into consideration the purpose of the proposed methodology. Moreover, comparing the LOD and LOQ obtained with the method using HPLC-ED in the determination of SD-II in food samples [25], there is no significant difference between them, confirming the higher detectability of the proposed method.

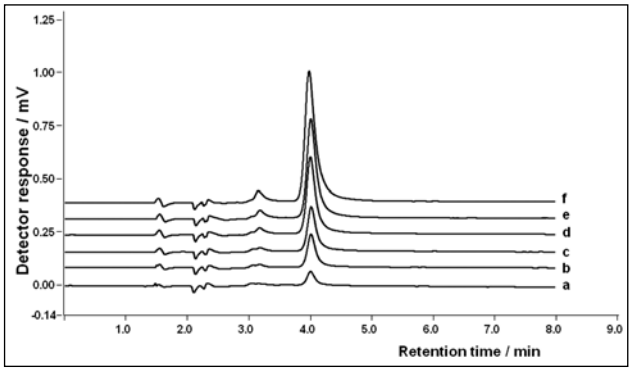

Figure 3. Chromatograms obtained on HPLC-ED system for SD-II detection at concentrations of: (a) 3.00×10^{-7}, (b) 5.00×10^{-7}, (c) 7.00×10^{-7}, (d) 9.00×10^{-7}, (e) 1.00×10^{-6} and (f) 2.00×10^{-6} mol L^{-1}. Conditions: separated on cyanopropyl column (150 × 4.6 mm, 5 µm), $E_{Detec.}$ = +0.85 V, mobile phase acetonitrile / lithium chloride (5.0×10^{-4} mol L^{-1}) in proportions of 70:30 (v/v). Flow rate = 1.20 mL min^{-1} and injection volume 25 of µL.

3.1.2. Determination of SD-II in fuel ethanol samples (recovery and accuracy)

For the recovery tests, a fuel ethanol sample totally free of the analyzed dye was collected, spiked at two different concentration levels (0.50 and 2.00 mg L^{-1}) and then submitted to the chromatographic analyses using the procedure

previously described in experimental section 2.4. For these studies, more dilute samples (0.50 and 2.00 mg L^{-1}) were used to avoid baseline perturbation due to fluctuations in the background current, which were more evident at concentrations higher than 2.00 mg L^{-1}. In Table I, it is possible to observe a satisfactory relationship between theoretical and experimental values when the recovery test for the dye concentration, accuracy and precision (RSD) [26-28] of the method is performed using this procedure. Accordingly, a matrix effect is not evident under these conditions and the method can be applied to detect and quantify SD-II using the chromatographic technique with electrochemical detection and applying the standard addition method.

Table I. Assay of recoveries for added dye in fuel ethanol samples prepared as in experimental section 2.4 (Sample A and B) and analyzed using the chromatographic method.

Matriz	Nominal (mg L^{-1})	Found (mg L^{-1})	Recovery (%)	RSD [a] (%)	t [b]
Sample A	0.50	0.44	88.0	4.50	3.60
	0.50	0.48	96.0		
	0.50	0.45	90.0		
	0.50	0.47	94.0		
	Average	0.46	92.0		
Sample B	2.00	2.14	107	10.0	0.60
	2.00	2.24	112		
	2.00	1.83	91.7		
	2.00	1.85	92.5		
	Average	2.02	101		

[a] relative standard deviation; [b] Student's *t* test (95% confidence interval; $t_{critical\ (p=0.05)} = 3.18$).

3.2. Voltammetric behavior of the SD-II dye

This study was carried out to develop a rapid and simple method to determine the SD-II dye with the purpose to compare the available results and the analytical performance during the use of electrochemical detection in two different analytical techniques. Based on the study already done about electroanalytical determination of this and other dyes [16-18, 22], the electrochemical condition for the detection of the SD-II dye was adapted to the present work. In Figure 4, it is possible to observe typical cyclic voltammograms obtained for SD-II reduction – using B-R buffer (pH 4.0) and DMF (7:3, v/v) as supporting electrolyte solution – in the absence and presence of DSS surfactant. It can be seen that the electrochemical reduction of the SD-II dye presented one well-defined peak at -0.50 V vs. Ag/AgCl (absence of DSS, Figure 4, voltammogram B) and at around -0.57 V vs. Ag/AgCl (presence of DSS, Figure 4, voltammogram C), which can be attributed to the reduction of the azo group (–N=N–) present in the dye molecule. The reduction peak is narrowed and visibly amplified concomitant to a negative shift of the peak potential, around 0.07 V (Figure 4, voltammogram C), in relation to the recorded voltammograms in the absence of surfactants (Figure 4, voltammogram B). The increase in current intensity and the displacement of the peak potential in the presence of surfactant could be correlated with DSS interaction on the electrode surface, which acts as an anti-fouling agent, assisting in the electron transfer for the SD-II oxidation. However, there was no other modification in the voltammetric behavior that compromises the electrochemical detection and analytical applicability. Additionally, no peak is observed in the reverse scan, indicating that the reduction process is irreversible.

For the recorded voltammograms, without surfactant present, during continuous potential scanning, the peak was markedly lowered, including disappearing, indicating the passivation of the electrode surface, as previously reported for electrochemical oxidation of the SD-II dye [22]. This effect was observed even when polishing the electrode surface after recording each voltammogram. However, when the same study was repeated in the presence of DSS surfactant, the voltammogram profile could be re-established (20 repetition) after 30 seconds of rest period before each series of measurements. Therefore, the surfactant presents anti-fouling capacity under the experimental conditions, improving the viability of the glassy carbon electrode to detect SD-II dye without adsorption complications.

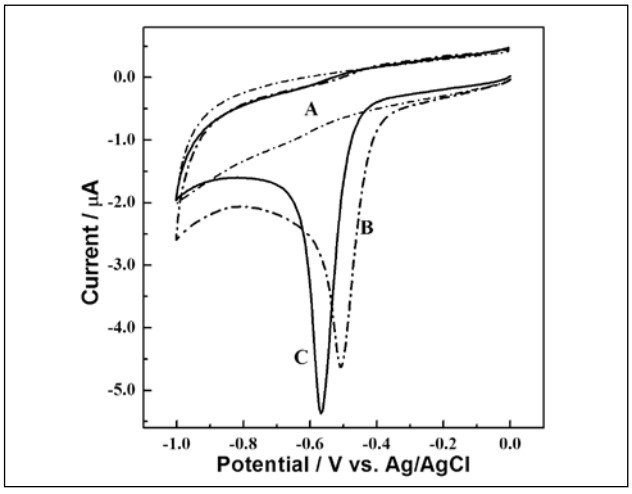

Figure 4. Cyclic voltammograms obtained on the glassy-carbon electrode surface for: (A) Blank, 0.04 mol L^{-1} B-R buffer (pH 4.0) containing 30% of DMF, (B) electrochemical reduction of 5.00×10^{-5} mol L^{-1} SD-II in 0.04 mol L^{-1} B-R buffer (pH 4.0) containing 30% of DMF and (C) electrochemical reduction of 5.00×10^{-5} mol L^{-1} SD-II as in B plus presence of DSS surfactant 1.00×10^{-3} mol L^{-1}. Scan rate of 100 mV s^{-1}.

Square-wave voltammograms were also used to examine the voltammetric behavior of the SD-II dye in the absence of surfactant (Fig. 5A) and in the presence of the anionic surfactant DSS (concentration in electrochemical

cell of 1.00×10^{-3} mol L^{-1}, Fig. 5B). Comparing the recorded voltammograms for 1.00×10^{-5} mol L^{-1} of SD-II, an intense reduction peak current is observed at -0.50 V, whose height is, approximately, two times higher than that given without surfactant, indicating that the target surfactant offers an improvement in detectability as well as the solubility of the dye in the aqueous/organic medium. The current intensity and position (Fig. 5B) were constant in the recorded voltammograms for each experiment and this is an attractive condition for electroanalytical purposes.

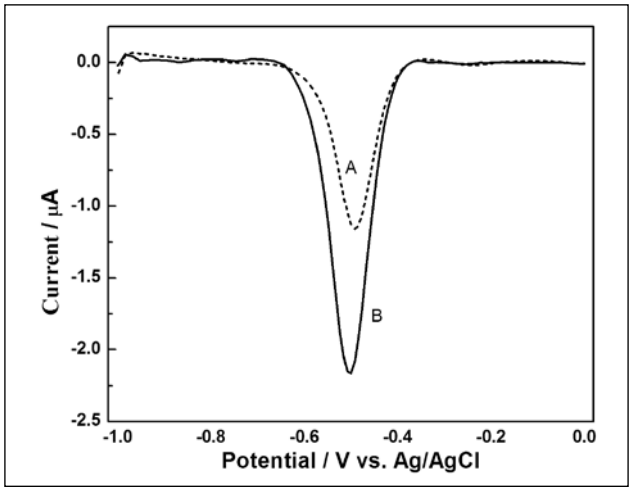

Figure 5. Square-wave voltammograms on the glassy-carbon electrode surface indicating the electrochemical reduction of 1.00×10^{-5} mol L^{-1} SD-II dye in 0.04 mol L^{-1} B-R buffer (pH 4.0) containing 30% of DMF for: (A) absence of DSS surfactant and (B) the presence of 1.00×10^{-3} mol L^{-1} DSS surfactant. Conditions: frequency (f) = 60 Hz, step potential (ΔE_s) = 8 mV and pulse amplitude (E_{sw}) = 50 mV.

Considering these aspects, together with the addition of an organic solvent to modify the composition of aqueous buffer electrolyte, satisfactory results were obtained for a mixture of BR buffer and *N*,*N*-dimethylformamide (DMF) medium. The effect of BR buffer concentration (0.04, 0.08, and 0.12 mol L^{-1}) and the influence of pH in the range of 2.0 and 10.0 were also investigated. The peak was strongly pH-dependent and the best results in terms of peak current intensity, voltammetric resolution and reproducibility were obtained for a mixture of 0.04 mol L^{-1} BR buffer at pH 4.0 and DMF at 7:3 (v/v). In BR buffer at pH values above 4.0, there is no peak current definition; besides, a considerable shifting to more negative potentials with increasing pH was observed, which overlapped with supporting electrolyte reduction. Accordingly, a combination of BR buffer (pH 4.0) and DMF (7:3, v/v) was chosen as the satisfactory supporting electrolyte solution – because it did not compromise the SD-II solubility – being used for further experiments.

To improve the applicability of electroanalytical techniques in the development of methods, the instrumental parameters play an important role in enhancing the detectability and sensitivity of a detection system, since the signal strongly depends on the excitation potential. Thus, prior to use of the square-wave voltammetry technique, some instrumental parameters and experimental conditions were evaluated from the analytical standpoint. Table II presents the interval and the optimized experimental condition adapted to SD-II reduction, where the peak current intensity was recorded accurately. The satisfactory working condition was a compromise between peak current intensity and voltammetric resolution – which was evaluated observing the width of the peak at half height.

Table II. Experimental parameters optimized in the development of the electroanalytical method.

Parameters	Type or range tested	Optimized
Supporting electrolyte	Britton–Robinson (BR) + DMF or Acetonitrile	Britton–Robinson + DMF (7:3, v/v)
Buffer concentration	0.04 – 0.12 mol L^{-1}	0.04 mol L^{-1}
pH (BR buffer)	2.0 – 10.0	4.0
Frequency (f)	30 – 200 Hz	60 Hz
Scan increment (ΔE_s)	1.0 – 12 mV	8.0 mV
Pulse amplitude (E_{sw})	10 – 100 mV	50 mV
Rest period	0 – 30 s	15 s

3.2.1. Linearity, limits of detection and quantification

After optimizing all the operational parameters for SD-II detection, voltammograms were recorded at concentrations from 3.00×10^{-6} mol L^{-1} to 1.80×10^{-5} mol L^{-1} (n = 8) to investigate the possibility of obtaining an analytical curve and using the proposed method for the analytical quantification of the target dye via SWV. According to the results obtained (data not shown), the analytical curve was linear over the studied concentration range, with the equation: I_{pc} (μA) = 0.30 + $4.06\times10^{5}\times$C (μmol L^{-1}), a correlation coefficient of 0.998. From linear regression, the limits of detection (LOD) and quantification (LOQ) were estimated using the statistical treatment $3\times S_A/m$ and $10\times S_A/m$ [24]. The S_A represents the standard deviation (intercept error) and m the slope of the analytical curve. The LOD and LOQ values were 2.05×10^{-6} mol L^{-1} and 6.76×10^{-6} mol L^{-1}, respectively, indicating that the proposed method is satisfactory for SD-II detection under the optimized conditions.

To confirm the precision of the proposed method, a spiked sample containing 1.00×10^{-5} mol L^{-1} of the SD-II dye was submitted to the electroanalytical detection performed without cleaning the electrode surface between the measurements. The relative standard deviation (RSD) for ten measurements was 2.50%, demonstrating that the electrode surface was clean and the current responses were clearly reproducible.

3.3. Analytical application

3.3.1. SD-II quantification using the electroanalytical method

In the beginning, to investigate the method's analytical application for SD-II detection, matrix effects were evaluated with addition-recovery experiments carried out on fuel ethanol samples. The ethanol samples that were collected did not exhibit any SD-II reduction signals and they were spiked to 5.0 mg L^{-1} and 10.0 mg L^{-1} of the dye, as previously mentioned in experimental section 2.4. They were submitted to direct square-wave voltammetric analysis. Next, the optimized procedure was applied for dye detection in fuel ethanol samples using the standard addition method. Figure 6 represents the typical square-wave voltammograms obtained for the spiked sample containing 5.0 mg L^{-1} (Fig. 6 line a) as well as after dye addition (Fig. 6 line b-d), using the standard addition method. From these voltammograms, a well-defined reduction peak for SD-II detection was observed, and it showed that matrix effects were absent in this sample-analysis.

Recoveries ranging from 92.0% to 97.0% were obtained using this procedure (Table III). Thus, the values obtained for the recovery and %RSD are acceptable and are in agreement with the labeled values, indicating the satisfactory precision and accuracy of the proposed methodology. These findings demonstrate that the proposed method can be successfully employed for SD-II determination in fuel ethanol – in a fast and simple manner – without any pre-treatment steps, since matrix effects were not observed for this sample.

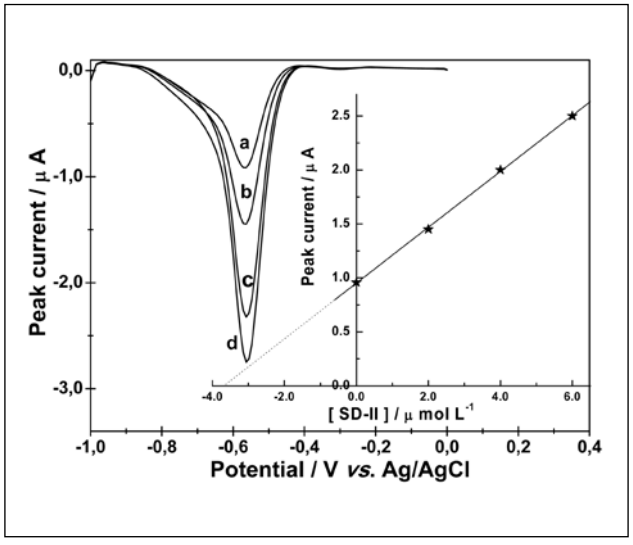

Figure 6. Square-wave voltammograms obtained for the determination of SD-II. (a) Fuel ethanol sample spiked to 5.0 mg L^{-1} of the SD-II dye, (b – d) successive standard additions (20 µL) of SD-II (1.0×10^{-3} mol L^{-1}). Inset: Analytical calibration function, peak current vs. SD-II concentration. Parameters: f = 60 Hz, ΔE_s = 8 mV and E_{sw} = 50 mV.

Table III. Assay of recoveries for added dye in fuel ethanol samples prepared as in experimental section 2.4 (Sample C and D) and analyzed using the electroanalytical method.

Matriz	Nominal (mg L^{-1})	Found (mg L^{-1})	Recovery (%)	RSD [a] (%)	t [b]
Sample C	5.00	4.58	91.6	6.40	2.30
	5.00	5.10	102		
	5.00	4.60	92.0		
	5.00	5.15	103		
	Average	4.85	97.0		
Sample D	10.0	8.30	83.0	6.70	1.20
	10.0	9.75	97.5		
	10.0	9.20	92.0		
	10.0	9.40	94.0		
	Average	9.20	92.0		

[a] relative standard deviation; [b] Student's t test (95% confidence interval; $t_{critical\ (p = 0.05)} = 3.18$).

4. Conclusion

In this work, two methods – based on HPLC coupled with electrochemical detection and on electroanalysis via the SWV technique – were developed for the determination of the dye SD-II in fuel ethanol samples without any pre-treatment. The HPLC method was satisfactory, providing acceptable selectivity and high detectability due to the electrochemical detection, suggesting a suitable alternative for quality control when selectivity and sensitivity are required. Furthermore, this work also shows that square-wave voltammetry is a suitable technique for the determination of SD-II in fuel ethanol samples, demonstrating an analytical performance with suitable recovery values. In addition, the use of the standard addition method permitted sample dilution, minimizing matrix effects. Therefore, the proposed methods are suggested as alternatives for controlling this dye in fuel ethanol samples when demanding quick and low cost analysis.

Acknowledgements

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References

1. The hydrocarbon oil (marking) regulations 2002, Statutory Instrument 2002, n. 1773. http://www.opsi.gov.uk/SI/si2002/20021773.htm. [Accessed April 2012].
2. European Refining & Marketing. http://www.process-nmr.com/pdfs/ERM-V1-10-02.pdf. [Accessed April 2012].
3. Orelup, R.B. U.S. Patent 4735631, 1988.
4. Trindade, M.A.G.; Stradiotto, N.R.; Zanoni, M.V.B. Quim. Nova. 2011, 34, 1683.
5. ANP (Brazilian Regulatory Authority). Resolução ANP n. 36, 6 de dezembro de 2005. http://www.puntofocal.gov.ar/notific_otros_miembros/bra199_t.pdf. [Accessed August 2009].
6. Santos, A.L.; Takeuchi, R.M.; Fenga, P. G.; Stradiotto, N.R. *Electrochemical Methods in Analysis of Biofuels*. In: Ivanov, O., Ed., Ap-

plications and Experiences of Quality Control. Rijeka: Intech, 2011.
7. Nowak, A.V. US Patent 4918020, 1990.
8. Friswell, M. R; Orelup, R.B. US Patent 5156653, 1992.
9. Trindade, M. A. G.; *Doctorate Thesis*, Universidade Estadual Paulista, Brazil, 2009.
10. Barwick, V.J.; Ellison, S.L.R.; Rafferty, M.J.Q.; Gill, R.S. Accred. Qual. Assur. 2000, 5, 104.
11. May, E.M.; Hunt, D.C.; Holcombe, D.G. Analyst. 1986, 111, 993.
12. Sundberg, L.N.; Andrasko, J.; Wistedt, I.; Kopp, I. J Forensic Sci. 1996; 41: 300-3.
13. Henricsson, S.; Westerholm, R. J. Chromatogr. A. 1996, 723, 395.
14. Timkovich, R. Dyes Pigm. 2000, 46, 69.
15. Linsinger ,T.; Koomen, G.; Emteborg, H.; Roebben, G.; Kramer, G.; Lamberty, A. Energ. Fuels. 2004, 18, 1851.
16. Trindade, M.A.G.; Ferreira, V.S.; Zanoni, M.V.B. Dyes Pigm. 2007, 74, 566.
17. Trindade, M.A.G.; Zanoni, M.V.B. Electroanalysis. 2007, 19, 1901.
18. Trindade, M.A.G.; Zanoni, M.V.B. Sens. Actuators B: Chem. 2009, 138, 257.
19. Trindade, M.A.G.; Zanoni, M.V.B.; Matysik, F-M. Fuel. 2010, 89, 2463.
20. Trindade, M.A.G.; Rinaldo, D.; Vilegas, W.; Zanoni, M.V.B. Quím. Nova. 2010, 33, 146.
21. Trindade, M.A.G.; Zanoni, M.V.B.; Matysik, F-M. Electroanalysis**.** 2010, 22, 955.
22. Romanini, D.C.; Trindade, M.A.G.; Zanoni, M.V.B. Fuel. 2009, 88, 105.
23. Kissinger, P.T. *Electrochemical Detectors*. In: Vickrey, T.H., Ed., Liquid Chromatography Detectors. New York and Basel: Marcel Dekker, 1986.
24. Miller, J.C., Miller, J.N. *Statistics for Analytical Chemistry*, 3rd ed. New York: Ellis Horwood, 1993.
25. Chailapakul, O.; Wonsawat, W.; Siangproh, W.; Grudpan, K.; Zhao, Y.; Zhu, Z. Food Chem. 2008, 109, 876.
26. Wood, R. Trends Anal. Chem. 1999, 18, 624.
27. González, A.G.; Herrador, M.A.; Asuero, A.G. Talanta. 1999, 48, 729.
28. Horwitz, W. Anal. Chem. 1982, 54, 67A.

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[1] *O Alcoolismo na Perspectiva da Saúde Pública*, Professor Doutor José Mauro Braz de Lima, da Faculdade de Medicina da UFRJ, Coordenador do Centro de Estudos de Prevenção e Reabilitação do Alcoolismo – CEPRAL.

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Point of View

Analytical Chemistry and Service Providing in Brazil

The Service Provider Laboratory of Analytical Chemistry (SPLAC) is critical in the chain of technological development because it reduces the financial and labor investment to the sponsor. From the technological view, the sponsor gets the resolution of its analytical demand either in development or in quality control, without the need of an internal structure.

Most of the analytical equipment available in Brazil is imported; therefore, it suffers a high tax burden, thus, sales of equipment are reduced and the maintenance of the structure of the reseller is passed to the SPLAC, which have little ease the financial resources from governmental agencies, as a result, the developed countries will have the latest technology. The training of interns, six hours a day, consumes from six to twelve months to get the base domain.

The SPLAC then absorbs all costs of training. This formation occurs by little investment in educational quality and at the end of the cycle certifies the low level of student knowledge.

Thus, there are sponsors who exchange the training for financial attraction to the SPLAC's analysts, having more profit. Whoever loses is SPLAC to reinvest. Who loses is the analyst, who gains new experience, but can be frustrated after realizing the low professional development and the increasing difficulty of the high competitiveness in case he gets back to the job market.

The analytical result is sovereign and cannot be invalidated by a complete forgetfulness of filling in the "log book", that is, excess control reduces productivity, challenges, innovations and increases the reanalysis, deadlines, costs and errors. This excess has created the profession of Quality Analyst with its main qualification in documents. The importance of Quality Assurance is to be intelligent and not to reduce the access of small and medium sponsors who need the analyses to evolve in their products.

In the SPLAC not knowing what the "next" sample is, creates challenges for the experienced analysts. Therefore the university would be ideal because it has resources, but is a difficult partnership.

Identify is a kind of high responsibility and perhaps several techniques. In quantification, sampling and identification are fundamental to the accuracy of the result. The technology in analytical chemistry targets the individualization of techniques in "new equipment", leading to high and varied investments to solve it analytically. As a result, today's professionals lack correlations between these techniques and their theoretical basis.

The SPLAC must have its analytical vocation proven and its existence known by the society and have differentiated accreditations, because the Analytical Chemistry is fundamental to the economy and to quality of life, since what the man made, he will use it for the good or for the evil, but it needs further analyses.

Flavio Leite
T&E Analítica Centro Analítico e Científico

Current status and future trends about miniaturized analytical systems in Brazil

One of the current trends in Analytical Chemistry is associated with the development of miniaturized systems. The miniaturization enables the integration of standard analytical procedures on a chip-based platform with capability of high-throughput analysis. This instrumental advance offers some advantages including short analysis time, reduced volume consumption and portability. These microdevices have been produced in a wide range of materials using conventional or alternative fabrication techniques. The manipulation and the accuracy control of fluids (pL - nL) inside microchannels is a true drawback due to requirements for coupling external elements (for example, pumps and high-voltage power supplies) with microfluidic networks.

In Brazil, the development of microfluidic devices for chemical and biochemical analysis started around ten years ago, where the group of Professor Claudimir L. do Lago was the pioneer. During the last decade, this technology has been spread out to a few groups from different Universities located in several regions of our country. Based on data found in the Web of Science database, Brazilian publications related to microfluidics include the development of new or disposable platforms for separation-based devices, flow analysis systems, sample preparation and purification as well as electroanalytical applications. Moreover, some reports have focused on the fabrication of microfluidic devices for clinical diagnostics, sensors and biosensors. Overall, it can be inferred that Brazilian publications have positively contributed to the advances in this field.

In my point-of-view, microfluidics is one of the most promising areas for a near future once it is not restricted only to Analytical Chemistry. Miniaturization science could be considered an interdisciplinary research field whereas biological, medical and other problems can be solved or better understood on analytical miniaturized systems. Due to its young age, the number of researchers focused on the development of microfluidic devices is still quite limited. For this reason, I would like to encourage undergraduate students as well as young researchers to take a careful looking on the possibilities of applications and technological developments before choosing a specific area to carry out their respective graduation levels.

Finally, I hope microfluidics field can inspire young scientists to contribute to the development of analytical devices with sample-in-answer-out capability to be used readily in the point-of-care testing, for example. This could immediately impact healthcare systems.

Prof. Wendell Karlos Tomazelli Coltro
Institute of Chemistry – Federal University of Goiás

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

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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.

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

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