# BrJAC

## Brazilian Journal of Analytical Chemistry

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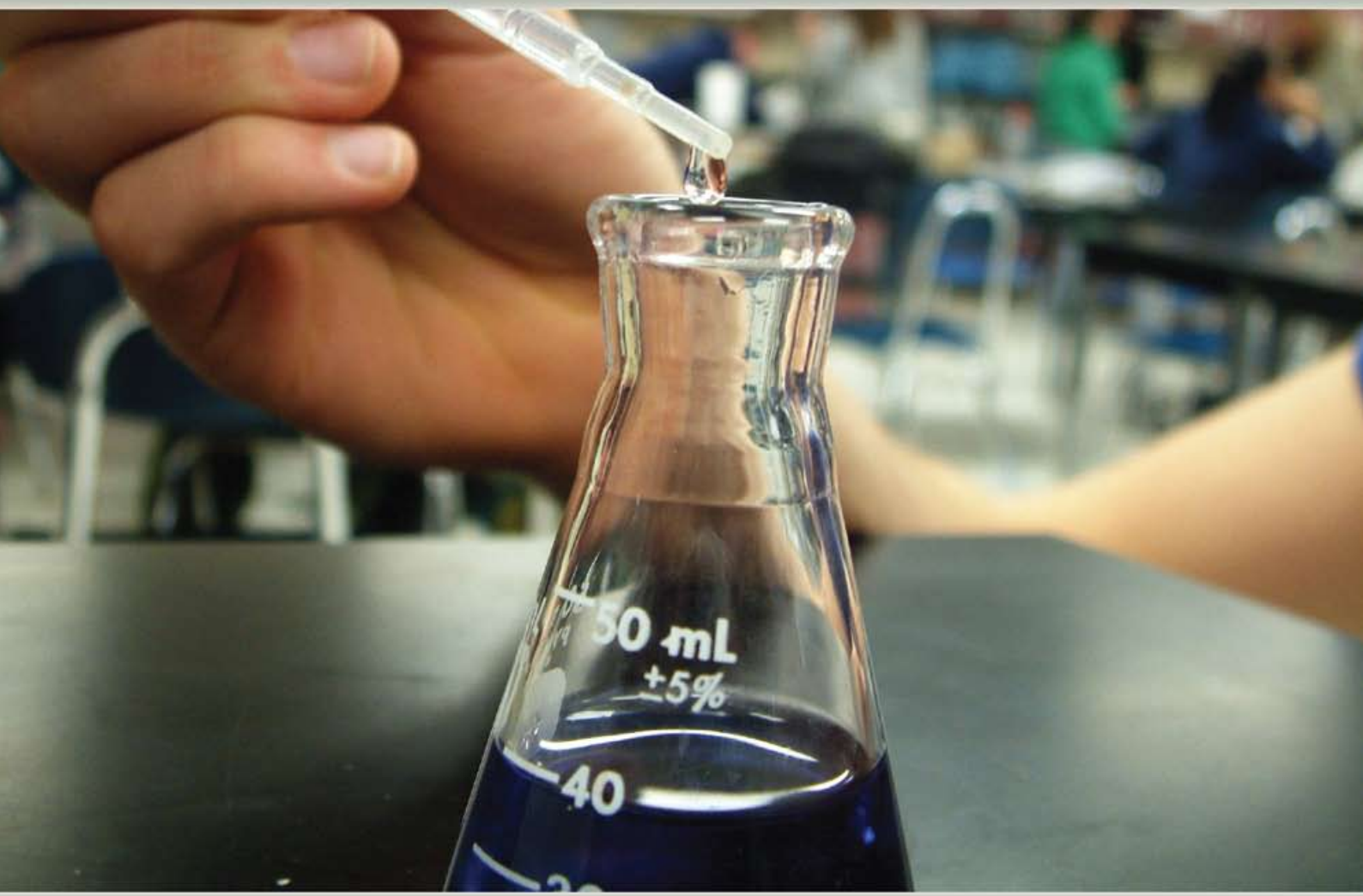


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# Editorial

## The springboard for development

After launching issue number zero of BrJAC, which was given an excellent reception by the community, issue number 1 is planting the seed of our dream of fostering integration between academia, research centers, and industry at this time of growth for graduate programs at the international level. While in 2007 top grades were given to 237 courses covering several knowledge areas, in 2010 over 61 courses joined these in top marks, bringing the total to 298 courses, which represents 11% of all courses.

In addition, Brazil has jumped from 16th to 13th in the ranking on scientific production, contributing 2.12% to total global production; this puts the country ahead of Russia and the Netherlands, which have a longer tradition in scientific activities.

Another evolutionary parameter that deserves mention is the fact that in 2009 Brazil awarded 2.34 times more PhD degrees than ten years ago – 11,368 and 4,853, respectively. Moreover, graduate studies programs have been decentralized, increasing the number of courses by 31.3% in the North and 35.3% in the Northeast.

These advances in the country's graduate studies can be seen in the triennial assessment of 4,099 master's and PhD degree courses offered by Capes – Coordination for the Improvement of Higher Education Personnel, from the Ministry of Education, published in September.

These indicators are very meaningful because, in addition to the maturing of our scientific production system, they show the potential that science holds as a springboard for the development and economic growth.

That is why developing mechanisms for scientific knowledge to dialog with industries and research centers are top priorities. Determination in pursuing the achievement of real academic-industrial integration is needed, based on successful experiences in the agriculture and livestock, petrochemicals, and aeronautics areas.

The confidence that everyone places in the idea of an exclusive publication for Analytical Chemistry shows the important role that BrJAC plays in the search for a reality.

**Professor Lauro Tatsuo Kubota**
Editor-in-chief

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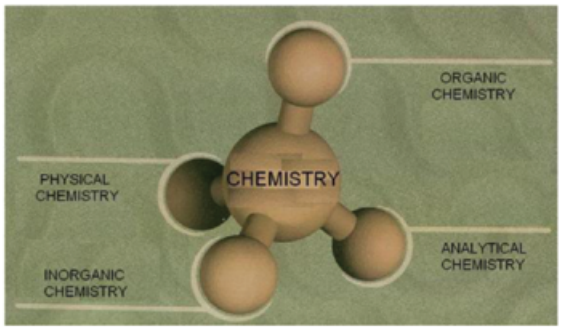


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# Letters

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

## Science and Technology: The foundation of a developed country

As a new member of the advisory board of the BrAJC, it is a great pleasure to have the opportunity to collaborate with this outstanding scientific discussion panel and interact with preeminent scientists from Brazil and abroad.

Brazilian analytical chemistry market is one of the most dynamic in the world, with annual growing several times higher than national GDP. The demand for quality services is colossal, especially for high regulated products as pharmaceutical ingredients and finished products.

This exponential growing is not accidental. No doubt analytical chemistry is strategic for the country and must be faced by our authorities with the highest priority. From the food safety to cutting edge pharmaceuticals, analytical chemistry excellence is one of the keys for the development of Brazil.

In this special context, no other activity is more necessary than provide extensive development of human resources in the analytical chemistry field. The development of the competences that will allow future scientists to deeply understand multidisciplinary science frameworks is crucial. Especially the astonish advances in computer sciences will dramatically change the analytical processes as we know today. Planning and interpretation of data will demand much more time than execution of experiments. The interface computer-instrument is only in the Stone Age and possibilities that are now out of our imagination will very soon knock the door of our laboratories.

Brazil is already a major player in the pharmaceutical global market and will very soon develop large scale R&D capabilities for new pharmaceutical entities. Biotechnology will dramatically change the world as we know today and Brazil has the unique opportunity to jump to the vanguard of this emergent field.

In order to execute this scientific revolution, it is essential that scientists have access to the ultimate analytical instrumentation. Taxes exemptions, special credit lines, attractive scholarships for researchers are just some other critical elements necessary for a successful science and technology police. Taxes system should urgently be reviewed. As example, Brazil authorities should deeply consider if to tax a mass spectrometer by 40% of their FOB cost is a practice that adds value to the economy or just cause the opposite effect. Today, several companies are just not allowed to innovate since the taxes barriers eliminate the possibility to invest in equipments required for true innovation.

I truly believe that the creation of BrJAC is a milestone since this journal will not only enlighten us with the work of our brilliant chemists, but will also become a central forum to discuss this relevant and obligatory theme that is the Brazilian police for science and technology.

Long life to BrJAC !

**Luiz Rogerio M. Silva**
Manager – Reference Standards Laboratory
United States Pharmacopeia – Brazil site

# FLOW INJECTION ANALYSIS: A BRAZILIAN TRIUMPH

By Adimilson Cerqueira

***A resume does not say everything about a person. This is the conclusion drawn by those who personally know Elias Ayres Guidetti Zagatto, Professor at the Center for Nuclear Energy in Agriculture, University of São Paulo (CENA/USP) and Member of the Brazilian Academy of Sciences. After analyzing his resume it is normal that one would expect him to be a flashy guy, almost arrogant, supported by his brilliant academic career and many awards around the world. In practice, Zagatto is a simple guy who likes a good conversation and does not miss the opportunity to say that he doesn't know everything and still has much to learn from life and people. He received BrJAC for an interview about the changes towards a cleaner chemistry, the interaction between industry and university and the highlight of Brazil on the international scene, especially with regard to the pioneering application of flow injection analysis on a large scale basis.***

Elias Ayres Guidetti Zagatto, professor at the Center for Nuclear Energy in Agriculture of University of São Paulo

**Which factors most influenced your education?**
I've always been fascinated by the area of the earth sciences and relevant aspects in the field of soil, geology and chemistry were been carried out at the College of Agriculture "Luiz de Queiroz", University of São Paulo, ESALQ/USP, in Piracicaba. I walked at graduation in order to devote myself to agronomy. In the meantime, the Centre for Nuclear Energy in Agriculture (CENA), linked to the International Atomic Energy Agency (IAEA) was created in Piracicaba. This international institute aimed to implement nuclear analytical techniques in agriculture research and I got an internship. Despite being an agronomist, I was much more interested in research in the field of chemistry. During my graduation, I engaged myself in the Introductory Course on Nuclear Energy in Agriculture at CENA, which gave me an opportunity for improving my education. In 1974, I started the M.Sc. course. As CENA did not offer a graduate course at that time, I studied at ESALQ/USP, and this explains my M.Sc. degree in agronomy. The experimental part of my thesis was carried out at the Atomic Energy Institute (today IPEN) in São Paulo. Later, I spent a 10-month period in Denmark working with the early development of the modern technique Flow Injection Analysis, which had been applied for large scale analyses in a pioneering way in Brazil. At that time, the institute was becoming independent of IAEA, that was about to leave Brazil, and would be integrated with the University of São Paulo. Taking the advice from my boss, the late Prof. Henrique Bergamin Filho, I decided to get my doctorate in analytical chemistry. Once I returned from Denmark, the first step was to take the exam to enter the PhD. Thank God, I did well and received my Ph.D. degree at Unicamp in 1981. So the fact that I was originally an agricultural engineer does not mean my vocation was agronomy. I always preferred chemistry and one of the highest honors I've ever had was to have been Director of the Division of Analytical Chemistry of the Brazilian Chemical Society.

**Traditionally, chemistry has always been associated with aggression to the environment – the general perception of people. Has this culture changed?**
We are experiencing a paradigm shift. Several reagents, efficient enough for certain classical analysis, are now banned because they harm the environment and can contaminate laboratories. There is a modern trend towards a clean chemistry. This perhaps explains the success of the flow injection analytical systems. A very small sample volume is used and that volume is generally handled in a thin tube. This tube can be regarded as a closed lab, a white room. There is no contact between the sample and the environment and vice versa. After sample quantification, the waste is directed towards a bottle for further treatment and disposal. That is, we do an analytical chemistry literally without the problem of waste. This will be my flag during next years: to sensitize the international community to rescue reagents that have been banned and had an excellent performance in analytical chemistry, but sell them in minimum quantities, say, less than 10 milligrams, to be used specifically in flow analysis. It is also important that the analytical methods spend less reagents and, especially, that they can make an *in situ* or even *in vivo* quantitative determination

**Flow injection analysis was originally applied to large-scale analysis in Brazil and is now accepted worldwide. Is this technique really innovative?**
Very innovative and the concept is far stronger. I was recently informed that there are several manufacturers of the analyzer in the world and that the number of scientific articles published in the area exceeds 18 000, including 10-12 text books. And most importantly, the theme flow analysis is already part of the Euro Curriculum. This is something very important and makes us particularly proud as Brazilians. One should also mention that Celio (Prof. Celio Pasquini, Unicamp) developed a new concept in flow analysis relying on mono-segmented flow. This is unprecedented worldwide. In 1994, Boa (Prof. Boaventura F. Reis, CENA/USP) proposed the concept of multicommutation, which also has a strong impact in the area. In 2000, the group of Prof. Mário (Mário C.U. Araújo, UFPb) proposed the flow batch system that combines the advantages of flow analysis and batch analysis. This has been highly valued by the international community. Flow injection analysis has touched the industry, resulting in things that were unthinkable 20 years ago. One of the most amazing innovations was made by a British colleague, Paul Worsfold, who implemented a flow system in a torpedo to make *in situ* analysis of sea-waters. It is something absolutely fantastic. I am very happy to see that although this technique does not have a clear inventor, it is certain that the large-scale use was pioneered in Brazil. This is recognized by the world.

**It is said that a researcher's work never ends with himself and that always arise and other research sources. Is science endless?**
Science is one thing that always moves forward. What's remarkable about this is that if we plot in a graph scientific advances on the ordinate axis and time on the abscissa axis, the resulting curve is asymptotic, approaching saturation. When it seems that there is nothing new and equilibrium is reached, an additional idea comes and starts another asymptotic trend. So, science development generally goes in jumps. In English there is a perfect term for this, which is stair like, or going up like a staircase. In this sense, the old analyzers in flow injection analysis were stepwise improved and several jumps were linked to Brazil. Even more remarkable is that the Brazilian contribution is always recognized. Any review article, any congress in the area and any seminal paper mention some works made by Brazilian fellows. Another interesting aspect is that flow analysis is one of the few research areas where the Brazilian industry participates. There are several published papers whose authors are affili-

ated with a private or public company devoted to analytical chemistry instrumentation and applications. I think analytical chemistry should be more focused on solving real problems. The community now involved with master, doctoral and postdoctoral programs has to be very confident and very sure that the developed research will be useful to the community, no matter when. In this regard, I consider that the ability to self-evaluation and redirecting, within limits, is fundamental to the researcher.

**Is university-company integration vital?**

Yes and in this regard, I would like to highlight FAPESP, which has in recent years encouraged this integration. Unfortunately, there is a common belief that the company is very fast and the university is very slow and bureaucratic. This is not so real, and we must face the problem focused on both sides. I think the companies should be encouraged to call the university to assist them to solve their problems, thus gathering additional information. This will come naturally, as the university becomes more and more competitive. We are in a process of pronounced increase in the number of universities and, unfortunately, this has led to a decline in competitiveness. Perhaps this is necessary in the current situation of our country. However, within a few years, based on what occurred in other countries, there will be an internal competition within the university, as the laboratories responding better to the demands will survive whereas those that do not respond will have to be restructured. Moreover, I believe it is very important to deflate the idea that research gives injury. Some investigations, especially basic research, really need a financial support, but this may not hold in all situations. On average, research may generate financial resources. So we have to seriously consider topple a myth. The research makes a profit, yes.

**Is there a conflict of interest between research and industry?**

Not specifically in the area of analytical chemistry, because we are producing concepts, instrumentation and methodology that typically are not of immediate use. As an example, we can imagine that the user of an analytical procedure will not have conflict with the procedure proposer in view of the time gap involved. There is perhaps a misunderstanding that analytical methods focused on oceanography should be published in journals devoted to marine chemistry, methods applied to agronomy have to be published in newspapers related to agronomy, methods related to the clinic must be published in medical journals and methods that start from absolute concepts and innovations in chemistry to be published in the journals of analytical chemistry. I think that these borders could be safely broken. Often, an analyst who has devoted most of his time in a niche could have a fantastic performance in other similar niches. Of course, we have to have a relatively focused academic, industrial or scientific career , always looking for improvement toward a

## *Nice Stories*

*Once upon a time, a kid was born as the third son of a traditional family and, after a few months, started playing as all kids do. There were great expectations about what he should perform as a citizen. However, as time went by, it was realized that this kid was handicapped; therefore he could not act as the other kids do. After a lot of support, care and education, he came to be more "normal" being then more widely accepted by the community. Surprisingly, his performance came closer to (and even surpassed) those of the "normal" kids.*

*The secret of this story? Work, faith and love.*

*Once upon a time, spectrophotometry was conceived as the first instrumental analytical technique and proved to be an excellent alternative to gravimetry and volumetry. There were great expectations about its performance but, in spite of its initial effervescent development, it could no longer perform as some other advanced analytical techniques do, because it required intensive solution management (pipetting, diluting, reagent adding, pH adjusting, cuvette filling / emptying / drying). As time went by, spectrophotometry was considered "handicapped". With the mechanization of the analyses, its performance was improved, and flow-based spectrophotometry was accepted worldwide. Surprisingly, its figures of merit came closer to (and even surpassed) those of modern instrumental analytical techniques. Hyphenated techniques involving spectrophotometry are nowadays experiencing an amazing increase. Other derived techniques that run better on a flowing basis (e.g. flow enthalpimetry, chemiluminescence) are being developed.*

*The secret of this story? Flow Analysis.*

***Elias Zagatto***

target, but we cannot be in a straightjacket. This border is very difficult. We cannot be fully focused but we also cannot be fully dispersed.

**The technology monitors the speed of academic ideas or is there a mismatch?**
There is always a mismatch. Even when a scientific discovery falls into the media and the public is fascinated with the unexpected result, usually the academy already knew it two or three years in advance. This is inevitable, as the scientist cannot be induced to premature conclusions or syntheses, and the industry also cannot risk putting a product in the market without proper maturation. So, there is a gap between the development of science and the dissemination of science. I believe that this gap should be maintained. But it should also be reduced and, in this sense, the media plays a crucial role.

**Someone once said that science produces more questions than answers. Do you agree with that statement?**
Absolutely. The more we study, the more we see how we know nothing. This is important and this should be in the unconscious mind of every business person, industrialist, scientist, teacher, and every student. I think that is inherent to the human being.

**Do you feel the concept of limit is lacking to the researcher? Self-reliance is detrimental to science?**
Yes, and this is deleterious. I strongly advocate the establishment of research groups. Not huge or formal groups, but two or four researchers working together. I see the effort that CNPq is doing with the Directory of Groups in Brazil with joy but with certain sadness when I realize that most of these groups consist of a teacher and their students. I consider integration and multi-disciplinarity as something very important, especially in studies involving applied research. This is a *sine qua non* condition. There are studies requiring field experiments and other activities outside the laboratory environment. These would not be feasible without a great team around. Hopefully, there are highly integrated Brazilian research teams in specific areas such as *e.g.* natural products and environmental sciences. I think it is also important to analytical chemistry to reflect about multi-disciplinarity.

**In the past research centers linked to several Brazilian and foreign institutions competed and kept up their research findings. Has this scenario changed?**
I believe this outlook is improving a lot, but it is important to stress that in a scientific interaction, the institutions that interact should have more or less the same ballast, scientific profile and performance. Otherwise, we fall into troubles highlighted in La Fontaine's fable of carrying stones and eggs in one basket. When we have a large university and a small research center working together, the university adopts a professorial stance and the center almost has to say *amen* to everything. This is very likely to occur both in small and large countries, or established and new universities. On the other hand, when we have universities or research institutes or industries from the same level, they add knowledge and efforts towards the mutual target and become more profitable.

**In your view, is science simple?**
Absolutely. Perhaps it is difficult to understand, but the originated products have to be as simple as possible. The flow analysis system fascinated the world for its simplicity. It is really a fantastic thing. I had the opportunity to visit a company that manufactures the flow injection analyzer in Sweden and noticed a disparity between low production cost and high sales value, and asked why. They replied that if they sell at a price that's worth, no laboratory manager would buy it. It is so simple, so cheap and so charming that the person stops believing if it does not price competitive with other instruments.

**Looking forward, what are your expectations?**
We have to see the future optimistically. The situation in Brazil, particularly, will be very good. We will have a strong industrial demand as a consequence of the economic jump we had in recent years. It will really require skilled personnel thus it involves personnel training. The analytical chemistry will play an important role in this matter. The second point is that large laboratories will be replaced by smaller units, as in the far future *in situ* analysis will predominate. We can have a micro laboratory in the most inaccessible place and send the analytical results via satellite to a central laboratory. This approach is already taking place in medical diagnosis and environmental chemistry. In view of its territorial extension, Brazil will have a beautiful space. There is another point that makes us forsee a bright future, but unfortunately at the expense of much infighting. As a consequence of the popularization of the university, later it will have to re-elite to find place in the sun. It's not a problem now, but within a few years, this place will be hard won and then we'll have competition that obviously we don't have today. Countries like the U.S. and Korea have already gone through this. I see it as inevitable and believe it is not a threat, but a good thing. The young people should be prepared for that. The classic idea that the person with a college degree already has a job for life will certainly be modified.

**Would you like to add some comments?**
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# Determination of saturates, aromatics and ethanol in brazilian commercial gasoline with low olefin content using $^1$H NMR spectroscopy

**Fabrício de Oliveira Ferreira[A], Danilo Luiz Flumignan[A], Márcia Nasser Lopes[A], Ermelindo Flumignan[B], José Eduardo de Oliveira[A]**
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**Abstract** – In this work, a Hydrogen Nuclear Magnetic Resonance ($^1$H NMR) method has been developed to determine aromatics and ethanol in Brazilian commercial gasoline with low olefin content. The proposed method involves subdividing an $^1$H NMR spectrum into regions, each of which is assumed to be associated with a specific type of structural group (OH, CH, $CH_2$ and $CH_3$). The method is based on the assignment of overlapping regions of $^1$H NMR spectra due to the signals of naphthene (N), iso and normal paraffins (P) and ethanol (E). Each $^1$H NMR spectrum was divided into 8 regions and the integration was correlated to the percentage of the substances to be determined. The results of the analysis by $^1$H NMR were compared with analysis of GC-FID obtained with the PONA system. The proposed technique of $^1$H NMR was shown to be an appropriate method for this sample type.

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**Keywords:** 1. Commercial Gasoline; 2. Ethanol; 3. $^1$H NMR spectroscopy.

## 1. Introduction

Gasoline is a complex mixture of several hundreds hydrocarbon components (paraffins, naphthenes, olefins and aromatics). The relative proportions of these compounds depend on the petroleum and the refining process used. Gasoline is one of the most widely used fossil fuels in the world and there is great interest on the part of society concering to its quality. In order to guarantee gasoline quality and conformity to the specifications, specific components such as paraffins, isoparaffins, olefins, naphthenes and aromatics (PIONA), must be measured accurately. Such analysis is carried out by separating the hydrocarbons compounds into single components or components groups, followed by subsequent identification and quantification. This is performed by chromatographic methods, e.g. gas chromatography (GC) [1-7], supercritical fluid chromatography (SFC) [8, 9], liquid chromatography (LC) with fluorescent indicator adsorption (FIA) [10], infrared spectroscopy (IR) [11-14] and mass spectroscopy (MS) [15]. In recent years, nuclear magnetic ressonance (NMR) [16-24] has become a powerful tool for the analysis of gasoline. $^1$H NMR measurements have a short analysis time of only a few minutes per sample.

One of the main characteristics of gasoline which is related to the performance of the internal combustion engine is its octane rating, or its octane number. To improve the octane number, two oxygenated-type chemicals can be added to the gasoline: alcohols or aliphatic ethers.

Since 1977, different volumes of ethanol have been added to the gasoline sold in Brazil. The proportion of ethanol in automotive gasoline has varied from 20 to 25% v/v over the last five years. The ethanol concentration is established by the National Petroleum Agency (ANP). From an environmental point of view, the addition of ethanol is of fundamental importance because it reduces the emission of pollutants into the atmosphere and it also has the advantage of being a renewable fuel.

There are numerous standard test methods from the American Society for Testing and Materials (ASTM) being used in the petroleum industry all over the world for analyzing gasoline products. Each country has its own specifications, that is, EN-228 in Europe, ASTM D4814 in the USA, JIS K 2202 in Japan and IS 2796 in India. In Brazil, this specification is established by the National Petroleum Agency (ANP) [25], designated as Brazilian Regulation number 309, of 27 December of 2001. The properties included in the Brazilian specifications are: color, aspect, anhydrous ethanol content (AEAC-ethanol), density, distillation curves, MON, AKI, vapor pressure, current gum, sulfur content, benzene, aromatic hydrocarbon and olefins.

The National Petroleum Agency (ANP) classifies au-

tomotive gasoline as: Type A - gasoline without oxygenated compounds present; and, Type C - commercial gasoline, which is a blend of gasoline A and ethanol. Only gasoline C is available at the gas stations. Due to the high ethanol content in gasolines, there is a need to develop specific methodologies to analyze the gasoline consumed in Brazil.

In this paper, the relationships established by Sarpal et al. [21] are used as the starting point and a fast and simple method is developed to determine aromatic hydrocarbon content, as well as ethanol content, without the need of any chemical pretreatment of the sample or the addition of a reference compound.

## 2. Experimental

### 2.1. Gasoline Samples

Thirty-three commercial gasoline samples (regular and podium, which differ only by the octane number), having low olefin content, were provided by CEMPEQC - Center of Monitoring and Research of Fuel Quality, a laboratory responsible for monitoring the quality of automotive fuels, in particular, gasoline, ethanol and diesel oil. Gasoline samples, collected randomly from different gas stations in São Paulo state, Brazil, were stored in polyethylene terephthalate flasks and transported in refrigerated boxes, following ANP regulatory procedures. When arriving at the lab, 90 mL samples were immediately collected in 100 mL amber PET flasks with sealing caps and stored in a freezer to avoid volatilization and keep their integrity. Moreover, gasoline A, obtained from REPLAN refinery in Paulínia-SP, and ethanol p.a. from Merck were used as reference samples for the calibration curve.

All gasoline samples were previously analyzed by several properties as stated in ANP Regulation nº 309, namely, atmospheric distillation temperatures at 10%, 50% and 90% recovery volumes, final boiling point and distillation residue (ASTM D86), density (ASTM D4052), motor octane number – MON (ASTM D2699), research octane number – RON (ASTM D2700), anti-knock index (MON + RON / 2), benzene content (% v/v) (ASTM D6277), ethanol content (NBR 13992) and hydrocarbons (saturates, olefins and aromatics – by Fluorescent Indicator Adsorption correlated to ASTM D1319).

Anhydrous ethanol content in gasoline samples was determined by the aqueous extraction method, by using 50 mL of sample and 50 mL of aqueous 10% NaCl solution (w/v). Distillation temperature profiles were performed in an automatic distiller unit, NDI440 v.1.70C (Normalab, Lintot, France) and density measurements was obtained by an automatic digital densimeter, Anton Paar v.4.600.b (Anton Paar, Graz, Austria). The other parameters were obtained by using a portable Grabner IROX2000 v.2.02 infrared analyzer (Grabner Instruments, Vienna, Austria) via FTIR spectra in the range 3500–650 $cm^{-1}$ at a nominal resolution of 4 $cm^{-1}$. To avoid cell manipulation and to increase the sample throughput, 7.5 mL of sample were introduced in the FTIR spectrophotometer by using the internal equipment pump. The samples were drawn in directly from the sample container through flexible tubing. To avoid out gassing, the samples were drawn carefully into an internal chamber and then pressurized through the absorption cell equipped with two Zn/Se windows, with a mean path length of 23 μm. After the measurement the samples were transferred into the connected disposal container.

### 2.2. NMR Spectrometry

All $^{1}H$ NMR spectroscopic spectra were acquired at room temperature on a Varian (Palo Alto, CA, USA) INOVA spectrometer, using a 5 mm single cell $^{1}H/^{13}C$ inverse detection flow probe. For each analysis, 30 μL of gasoline sample was dissolved in 600 μL of deuterated chloroform ($CDCl_3$). The $^{1}H$ NMR spectrum was obtained at 500 MHz for $^{1}H$ observation, using $CDCl_3$ as the solvent and tetramethylsilane (TMS) as the internal standard. The spectra were obtained using 45° rf pulse (4.1 μs), a spectral width of 4725 Hz, 64 transients with 64 000 data points, an acquisition time of 2 min and relaxation delays of 1 s. Thirty-two scans were accumulated for each spectra and processed with 32 000 data points using an exponential weighing factor corresponding to a line broadening of 0.1 Hz. $^{1}H$ NMR chemical shifts are reported in parts per million (ppm) relative to residual proton signals of $CDCl_3$ at 7.24 ppm. The FIDs were zero filled and Fourier transformed. The phase and baseline were manually corrected in all spectra.

### 2.3. GC-FID Analysis

General chromatographic PONA analysis was performed using a Shimadzu GC-17A with a flame ionization detector (FID), equipped with an automatic injector model AOC-20i and interfaced to a workstation using software from GCSolutions. The mass percent of each group (paraffins, olefins, naphthenes and aromatics) was calculated using the PONA Solution software from the chromatographic profile.

GC-FID system was equipped with a CBP1-PONA fused-silica capillary column (50 m x 0.15 mm x 0.42 μm; Shimadzu, Kyoto, Japan) with dimethylpolysiloxane as the stationary phase and helium as the carrier gas at a constant flow rate of 20 mL $min^{-1}$. Sample aliquots of 0.5 μL were injected in split mode (1:250) without solvent delay. Injector and detector temperature were maintained at 250 °C and the oven temperature was programmed as follows: the column was kept at 35 °C for 20 min and then heated to 155 °C at 3 °C $min^{-1}$.

Finally, the temperature was raised up to 215 °C at 6 °C $min^{-1}$ and kept constant for 15 min.

### 2.4. GC-MS Analysis

A Shimadzu GC-17A-GCMS-QP5050A gas chromatograph coupled to the mass spectrometer was used to identify the gasoline components with the objective of calibrating the PONA solution in the GC-FID, to analyze the number of substituted α carbons in the aromatic ring and for the determination of the main iso-paraffins content in the gasoline samples. The mass spectra obtained were researched and correlated with NIST Library 98 Edition to identify and confirm the substances. GC-MS tests conditions were the same used for GC-FID.

## 3. Results and Discussion

The 500 MHz $^1$H NMR spectra of a commercial gasoline sample with low olefin content is given in Figure 1. The proposed NMR method is based on the signal positions of the structural groups (OH, CH, $CH_2$ and $CH_3$) for each function (paraffins, naphthenes, aromatics and ethanol), elimination of signal overlap of the structural groups for each function and calculation of the total molar mass of the structural groups of the sample ($T_M$), aromatics ($A_M$) and ethanol ($E_M$), obtained from the intensity of the spectrum integrals. The software ACDLabs/Spec Manager v4.09 was used [26] to obtain the values of the integrals. The weight percentages of aromatic (A) and ethanol (E) are calculated using the relationships given by Sarpal et al. [21], adapting them to our system.

The $^1$H NMR spectrum were divided into 08 spectral regions, namely A to J (Figure 1). The chemical shift region was assigned to one or more structural groups (OH, CH, $CH_2$ and $CH_3$). The attributions were based on research found in the Aldrich library of $^1$H FT-NMR spectra Volume I and II [27] and in published papers [16-24].

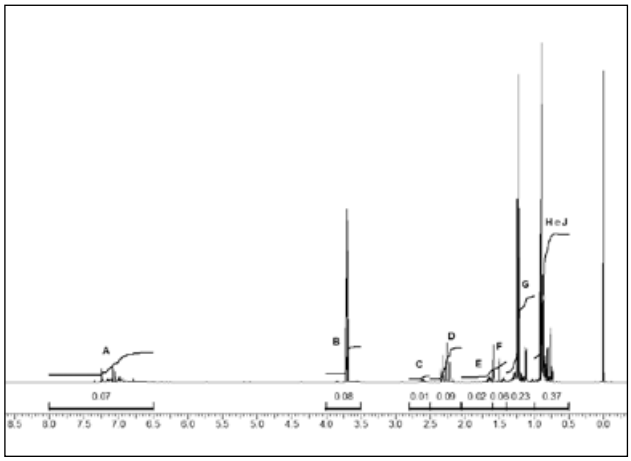


**Figure 1. $^1$H NMR spectrum of a commercial gasoline with low olefin content, chemical shift (δ) and integral values are indicated in the spectrum (500 MHz, $CDCL_3$).**

The region of 6.50 - 8.00 δ (A) is characteristic of aromatics. The signals due to α-substituent groups of the aromatics appear in the 2.50 - 2.80 δ (C) and 2.05 - 2.50 δ (D) regions and are due to $CH_2$ and $CH_3$ protons, respectively.

The signals due to ethanol generally appear in the 1.00-1.40 δ (G) region for $CH_3$ protons, the 3.50 - 4.00 δ (B) region for the $CH_2$ protons and the 1.40 - 1.60 δ (F) region for the OH proton.

The region between 0.50 - 2.05 δ (E, F, G, H and J) is highly overlapped and contains signals mainly due to naphthenes, ethanol and iso-paraffins. A broad assignment in terms of $CH_n$ (n = 1, 2 and 3) is possible. For example, the region 0.50 - 1.00 δ (J) is due to $CH_3$ groups of saturated (naphthenes + paraffins) and the 1.00 - 1.40 δ (G) region is due to $CH_2$ groups of iso-paraffins and ethanol. The 1.40 - 2.05 δ (E, F and H) region is overlapped and consists of $CH_2$, and CH of naphthenes, iso-paraffins and OH protons of ethanol. The sharp signals appearing at 1.43 δ and 1.46 δ can be assigned to $CH_2$ protons of cyclohexane and cyclopentane respectively.

Although, the region of 1.40 - 2.05 δ seems to be characteristic for naphthenes, it cannot be used directly to estimate total naphthenic and ethanol content because of the following two factors:

a) considerable part of the signal intensity of naphthenes falls in the 0.50 - 1.40 δ region and severely overlaps with the signal intensity due to normal and iso-paraffins and $CH_3$ protons of the ethanol. For the quantitative estimation of ethanol content, the contribution of all the ethanol must be extracted from the other overlapped regions as well. This will lead to the resolution of signal intensity due to ethanol in the $^1$H NMR spectrum of the gasoline sample.
b) 1.40 - 2.05 δ region also contain signals due to methine (CH) protons from iso-paraffins and hydroxy proton (OH) from the ethanol.

Therefore, to determine total saturated (naphthene + paraffin) content, it is necessary to determine the quantitative average extent of overlapping of the structural groups (OH, CH, $CH_2$ and $CH_3$) of standard ethanol and of the iso-paraffin mixture.

The contribution of the iso-paraffin $CH_2$ group was determined by subtracting the contribution of the ethanol $CH_3$ group with proton signals present in the same region of chemical shift. The extent of the $CH_3$ signals overlapping the ethanol with the $CH_2$ signals of the iso-paraffins was obtained by integrating the region between 1.00-1.40 δ and subtracting the value corresponding to the signals of the protons of the ethanol $CH_3$ region which has the same value as 3/2 of the integral (I) in the region between 3.50 - 4.00 δ. This corre-

sponds to the signal of the ethanol $CH_2$ protons where there is no overlapping. In this way, we obtained the following relationship:

$$I_{1.0–1.4} - \frac{3}{2} I_{3.5–4.0} \quad (1)$$

$$G = I_{1.0–1.4} - \frac{3}{2} B \quad (2)$$

the value "3/2" comes from the relationship of the ethanol protons ($CH_3CH_2OH$) which is given by:

$$I_{CH3} = \frac{3}{2}(I_{CH2}) = 3(I_{OH}) \quad (3)$$

The extent of the naphthene and iso-paraffin signal overlappings was determined using the relationship given by Sarpal et al. [21]. This relationship is obtained from the spectrum of a mixture of iso-parafin standards.

As shown previously, the contribution of iso-paraffins in the 1.40 - 2.05 δ region must be subtracted before this region is used for estimation of total saturated content in a mixture. The simple spectrum of the isoparafin mixture spreads in a narrow region of 1.60 - 0.50 δ and can be divided into three distinct bands: 0.50 to 1.00δ, 1.00 to 1.40δ and 1.40 to 1.60 δ. These regions are due to $CH_3$, $CH_2$ and CH protons, respectively, and are regions of interest due to CH protons.

Sarpal et al. [21] have observed that around 10% of the intensity of the 0.50 to 1.00 δ region signal is approximately equal to the intensity of the 1.40 to 1.60 δ region signal, i.e:

$$I_{1.4–1.6} = \frac{I_{0.5–1.0}}{10} \quad (4)$$

The above relationships were used to subtract the overlapping of $CH_2$ proton signals caused by naphthenes, from the CH proton signals caused by iso-paraffins in the 1.40 - 2.05 δ region of the $^1$H NMR spectrum. The 0.50 - 1.00 δ region was named 'J', therefore, the intensity J/10 (named 'H') is subtracted from the intensity of region 1.40 - 1.60 δ to obtain the true contribution of saturates. The intensity $I_{1.4-1.6}$ minus 'H' and the value corresponding to the signal from the ethanol's OH proton, which has a value equal to 1/2 of the integral in the 3.50 - 4.00 δ region (Equation 3), was named 'F' and is given by cyclopentane and cyclohexane $CH_2$ protons. This way, we obtain the following relationships starting from Equation 4:

$$J = I_{0.5–1.0} \text{ and } H = \frac{J}{10} \quad (5)$$

$$F = I_{1.4–1.6} - H - \frac{1}{2} B \quad (6)$$

the 1/2 comes from the relationship of the ethanol protons, Equation 3.

After all the $^1$H NMR spectrum assignments (Table I and Figure 1) are completed and the extent of average overlap has been taken into account, the hydrocarbon composition can be estimated in terms of total aromatic and ethanol content.

**Table I. Attributions from different regions of the $^1$H NMR spectrum and the integral region used to analyze gasolines (δ in relation to internal standard TMS).**

| Chemical shift (δ) | Attributions | Integral regions |
|---|---|---|
| 6.50 – 8.00 | CH of the aromatic rings | A |
| 3.50 – 4.00 | $CH_2$ of the ethanol | B |
| 2.50 – 2.80 | $CH_2$ of the α-substituints in the aromatic | C |
| 2.05 – 2.50 | $CH_3$ of the α-substituints in the aromatic | D |
| 1.60 – 2.05 | $CH_2$ of the naphthenes | E |
| 1.40 – 1.60 - H - ½B | $CH_2$ of the cyclohexane and cyclopentane + CH of the iso-paraffins + OH of the ethanol | F |
| 1.00 – 1.40 - 3/2B | $CH_2$ of the iso-paraffins + $CH_3$ of the ethanol | G |
| 0.50 – 1.00 = J/10 | CH of the iso-paraffins | H |
| 0.50 – 1.00 | $CH_3$ of the naphthenes and of the paraffins | J |

The first step involves the estimation of the total relative number of carbons ($T_C$) and the sample's total molecular-group weight ($T_W$). $T_C$ was calculated by dividing each individual region in the $^1$H NMR spectrum by the number of protons causing the signal. $T_W$ was calculated by multiplying the values of $T_C$ with the respective molar mass of the groups (i.e. 12 for C, 13 for CH, 14 for $CH_2$, and 15 for $CH_3$). Therefore, $T_C$ and $T_W$ are computed as follows:

$$T_C + \frac{A}{1} + \frac{B}{2} + \frac{C}{2} + \frac{D}{3} + \frac{E}{2} + \frac{F}{2} + \frac{G}{2} + \frac{H}{1} + \frac{J}{3} + Arq \quad (7)$$

where A, B, C, etc. are the integral intensities of the various specified regions given in Table I, Arq are the substituted aromatic quartenary carbons and do not show up in the $^1$H NMR spectrum as there are no protons attached to such carbons. However, their contribution is given by the following simple relationships:

$$Arq = \frac{C}{2} + \frac{D}{3} \quad (8)$$

$$T_W = 13\frac{A}{1} + 45\frac{B}{2} + 14\frac{C}{2} + 15\frac{D}{3} + 14\frac{E}{2} + 14\frac{F}{2} + 14\frac{G}{2} + 13\frac{H}{1} + 15\frac{J}{3} + 12.Arq \quad (9)$$

The value 45(B/2) comes from the relationship of the protons of the ethanol, Equation 3.

$$45\left(\frac{B}{2}\right) = 15(I_{CH3}) + 14(I_{CH2}) + 17(I_{OH}) = 22.5(B) \quad (10)$$

Substituting the value of Arq the equation is simplified as shown here:

$$T_W = 13(A + C + H) + 7(E + F + G) + 22.5(B) + 9(D) + 5(J) + 7(B) \quad (11)$$

### 3.1. Determination of Aromatics

To estimate the total aromatic content (A) in the sample, the group molar mass of the aromatic ($A_M$) was calculated. This is expressed by Equation 12,

$$A_W = 13\left(\frac{A}{1}\right) + n(14)\left(\frac{C}{2}\right) + 15\left(\frac{D}{3}\right) + 12(Arq) \quad (12)$$

where, 'n' is the average chain length of the groups linked to the aromatic ring. This value was determined as 2 (two), using gas chromatography coupled with mass spectrometry (GC-MS).

The aromatic content (A) in %w/w was estimated by Equation 13.

$$A = \left(\frac{A_W}{T_W}\right).100\% \quad (13)$$

### 3.2. Determination of Ethanol

To estimate the total ethanol content (E) in the sample, the group molar mass of the ethanol ($A_M$) was calculated. This is expressed by Equation 14.

$$E_W = 15(I_{CH3}) + 14(I_{CH2}) + 17(I_{OH}) \quad (14)$$

Substituting the relationship of Equation 3 in Equation 14, we obtain:

$$E_W = 22.5(B) \quad (15)$$

The ethanol content (E) in %w/w was estimated by Equation 16.

$$E = \left(\frac{E_W}{T_W}\right)100\% \quad (16)$$

### 3.3. Determination of Saturates

To estimate the total saturates content (S) in the sample, just substract the sum between aromatics (A) and ethanol (E) contents, which was estimated by Equation 17.

$$S = 100 - (A + E) \quad (17)$$

### 3.4. Analytical Curves for Ethanol Obtained using GC-FID

The calibration curve was obtained by carrying out triplicate analysis of mixtures of the gasoline A and ethanol pa reference samples. These samples were prepared from by blending both products in different percentages to have the following ethanol content in volume percent: 20%, 22%, 24%, 26%, 28% and 30%. The linear regression equation was: ethanol = 0.5893 + 8.8512 x $10^{-5}$ area of chromatographic peak ($R^2$ = 0.9989; n=6).

### 3.5. Comparison of the Results with the GC-FID Method

The gasoline samples compositions, determined by NMR, were compared with the GC-FID method. The ethanol content was obtained via the calibration curve. The results are presented in Table II and compared using the *t*-test paired showing a 95% inter-

**Table II. Results of the analyses using the FTIR, GC and NMR methods.**

| Sample | Olefin (%w/w) | Aromatics (%w/w) | | | Ethanol (%v/v) | | |
|---|---|---|---|---|---|---|---|
| | GC | FTIR | GC | NMR | FTIR | GC | NMR |
| 1* | 0.00 | 26.3 | 28.19 | 28.15 | 23.6 | 24.38 | 24.95 |
| 2* | 0.00 | 27.3 | 28.48 | 28.59 | 24.7 | 23.95 | 24.99 |
| 3* | 0.00 | 25.7 | 28.85 | 27.74 | 23.2 | 24.59 | 24.97 |
| 4* | 0.00 | 26.9 | 28.20 | 28.09 | 23.3 | 24.22 | 24.96 |
| 5* | 0.00 | 27.1 | 28.22 | 29.52 | 24.3 | 24.31 | 24.94 |
| 6* | 0.00 | 28.7 | 27.90 | 28.95 | 24.9 | 24.34 | 25.26 |
| 7* | 0.11 | 24.1 | 23.90 | 23.66 | 24.8 | 24.14 | 24.50 |
| 8* | 0.11 | 23.0 | 23.70 | 23.40 | 24.5 | 24.29 | 24.59 |
| 9* | 0.12 | 23.2 | 23.70 | 23.73 | 24.9 | 23.62 | 24.48 |
| 10 | 0.38 | 9.1 | 10.23 | 7.99 | 26.9 | 25.91 | 24.87 |
| 11 | 0.43 | 10.7 | 9.27 | 10.76 | 25.2 | 25.34 | 25.11 |
| 12 | 0.45 | 19.6 | 23.22 | 22.15 | 25.9 | 25.59 | 25.03 |
| 13 | 0.78 | 8.3 | 7.60 | 7.12 | 21.0 | 21.23 | 20.56 |
| 14 | 0.90 | 10.6 | 8.51 | 8.47 | 25.3 | 25.03 | 24.93 |
| 15 | 1.03 | 10.8 | 5.95 | 5.16 | 21.9 | 19.91 | 20.69 |
| 16 | 1.24 | 9.6 | 7.09 | 9.71 | 27.5 | 26.14 | 26.37 |
| 17 | 1.27 | 9.7 | 7.52 | 8.41 | 25.9 | 25.35 | 25.73 |
| 18 | 1.46 | 16.1 | 13.32 | 11.34 | 27.2 | 25.81 | 24.43 |
| 19 | 1.48 | 15.7 | 10.43 | 9.59 | 26.3 | 25.07 | 24.64 |
| 20 | 1.59 | 7.9 | 6.42 | 8.36 | 24.6 | 22.47 | 23.23 |
| 21 | 1.66 | 7.1 | 6.24 | 7.63 | 25.0 | 25.25 | 25.39 |
| 22 | 1.67 | 9.7 | 10.17 | 10.12 | 21.4 | 21.31 | 20.94 |
| 23 | 1.82 | 19.9 | 21.21 | 20.47 | 22.0 | 24.00 | 24.06 |
| 24 | 1.91 | 6.6 | 4.78 | 6.59 | 21.7 | 23.27 | 22.96 |
| 25 | 1.96 | 12.0 | 7.34 | 9.91 | 25.1 | 25.90 | 25.77 |
| 26 | 1.99 | 10.0 | 6.75 | 9.19 | 25.3 | 25.16 | 25.67 |
| 27 | 2.13 | 14.6 | 7.62 | 10.59 | 25.4 | 23.59 | 24.27 |
| 28 | 2.27 | 9.9 | 7.58 | 10.11 | 25.9 | 25.35 | 26.28 |
| 29 | 2.28 | 10.6 | 7.65 | 10.49 | 25.3 | 24.56 | 24.97 |
| 30 | 3.13 | 12.3 | 6.30 | 5.52 | 23.5 | 24.65 | 25.00 |
| 31 | 3.56 | 15.7 | 17.78 | 15.94 | 21.9 | 20.95 | 20.77 |
| 32 | 4.14 | 14.9 | 12.27 | 13.08 | 25.4 | 24.21 | 24.33 |
| 33 | 5.47 | 11.5 | 9.41 | 12.02 | 25.1 | 24.05 | 24.97 |

* Podium Gasoline.

val confidence (Table III and IV). It can be seen that there is no significant difference between the GC and NMR methods when analyzing aromatics and ethanol. However, a significant difference exists between the GC and FTIR methods when analyzing aromatics, mainly when the olefin content increases. This occurs because the area designated to aromatic hydrogen begins to have interference from olefinic hydrogens.

**Table III. Comparison between the GC-NMR and GC-FTIR methods for aromatics, using *t*-test paired.**

| | $t_{calculated}$ | $t_{table}$ | Standard Deviation (SD) |
|---|---|---|---|
| GC-NMR | -1.917 | 2.042 | ± 1.521 |
| GC-FTIR | -2.625 | 2.042 | ± 2.613 |

**Table IV. Comparison between the GC-NMR and GC-FTIR methods for ethanol, using *t*-test paired.**

| | $t_{calculated}$ | $t_{table}$ | Standard Deviation (SD) |
|---|---|---|---|
| GC-NMR | -1.947 | 2.042 | ± 0.596 |
| GC-FTIR | -1.848 | 2.042 | ± 1.033 |

## 4. Conclusion

A fast and simple $^1$H NMR spectroscopy method was developed to measure the concentration of aromatics and ethanol in commercial Brazilian gasolines, having a low olefin content (<5.47% w/w). The main advantages are that the total analysis time per sample is short and all concentrations are determined in one $^1$H NMR experiment. However, several disadvantages in this technique, like equipment price and cost analysis, must be considered. The method demands no chemical pretreatment of the sample and requires only a small volume of sample (50 μL). This method can be applied to podium and regular commercial gasoline. Further work is in progress to extend the methodology for complete PONA (Paraffins, Olefins, Naphthenes and Aromatic) analysis and such results will be used to calculate octane number (MON and RON) using chemometrics methods.

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# On-line molecularly imprinted solid phase extraction for a fast low-pressure separation of chlorpromazine and perphenazine from urine

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## Abstract

A selective on-line molecularly imprinted polymer (MIP) analysis was carried out allowing a simple and fast low-pressure separation procedure for chlorpromazine and perphenazine. A 1:9 (v/v) HCl (pH 2.0):methanol washing solution was employed to improve the selectivity, avoiding some concomitant molecules being bound to the MIP, either onto the specific binding site (molecules similar to the phenothiazines) or onto the polymer surface (non-similar molecules present in the urine samples). The analytical curve ranged from 5.0 to 50 μmol $L^{-1}$ with a correlation coefficient higher than 0.996 (five concentration levels, n = 3) for both analytes. The limit of quantification (LOQ) and the analytical frequency were 5.0 μmol $L^{-1}$ and 6 $h^{-1}$ (time of LLE not considered) for both chlorpromazine and perphenazine. Intra (n = 5) and inter-day (n = 3) precisions were lower than 12%, and the accuracy was checked by analyzing fortified urine samples by the proposed and chromatographic methods. No significant differences at the 95% confidence levels were found, according to Student's t-test.

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**Keywords**: molecularly imprinted polymer, chlorpromazine, perphenazine, low-pressure liquid chromatography, urine

## Introduction

Human fluids are complex samples, frequently requiring clean-up procedures before their analysis in order to circumvent problems with interferences, even when taking into account selective techniques such as chromatography. For this task, solid phase extraction (SPE) is an attractive strategy for sample clean up due to its simplicity, low consumption of organic solvents and ease of automation.[1] When considering SPE, molecularly imprinted polymers (MIP) have been receiving special attention.[2]

MIPs are polymers with recognition abilities derived from the impression of the target template molecule (analyte).[2] They were proposed by Wulff and Sarhan[3] and present binding sites with a pre-determined selectivity for a specific analyte. The synthesis is carried out using a template molecule that forms a complex with the functional monomer. After polymerization, the template is removed, and the functional groups are held in specific positions by the polymeric structure.[4,5] MIPs have been explored in analytical chemistry as sensors,[6] stationary phases for HPLC[7] and capillary electrochromatography,[8] in solid phase extraction (SPE)[9] and, most recently, allied to mechanized system.[10,11]

When a MIP is used as sorbent in SPE, commonly termed MISPE (molecularly imprinted solid phase extraction), it is possible that molecules similar to the template (e.g. molecules of the same classes as phenothiazines, benzodiazepines, etc) are extracted from the sample. This selective extraction is an important advantage in the analytical pathway because fast and low pressure systems can presumably be successfully employed for analyte separation, due to the absence of concomitants. The fast separation system can be obtained with shorter analytical columns, or using analytical columns with larger particles. Additionally, those techniques which do not present good selectivity, such as spectrophotometry, can also be successfully applied in such contexts, as we have previously demonstrated[10,11]. As advantages, smaller runs, improved the analytical frequency, as well as use of on-line systems can be designed.[2,10,11] Then, the welcome characteristics of these systems could perfectly well be employed in monitoring some important substances used in different medical treatments. As an example, phenothiazines can be

pointed out in psychiatry treatment. Phenothiazines are drug antagonists of dopamine receptors, widely used as antipsychotic, antiparkinsonian, and antihistaminic pharmaceuticals. On the other hand, their overdose can cause coma, miosis and respiratory depression, among other disorders.[12] This fact is important since the availability and the frequent therapeutic use of phenothiazines by psychiatric patients result in a great number of suicide attempts, and in serious cases of intoxication.[13] It is important to point out that a MIP of chlorpromazine was employed successfully in an on-line system for its extraction from urine samples followed of chemiluminescence analysis.[14]

Based on this, we have carried out the development of an on-line MIP system for chlorpromazine and perphenazine extraction from urine samples, followed of low-pressure and high-speed separation and quantification of these drugs using spectrophotometry.

## Experimental

### Apparatus

The on-line extraction and the low-pressure separation system were comprised of a FIAlab-3200 instrument (FIAlab® Instruments, Bellevue, USA), an automated injector commutator 2:3:2 constructed from Teflon®, two 12 VDC/100 psi solenoid valves (Cole-Parmer Instrument CO, Vernon Hills, USA), an USB-4000 spectrophotometer (Ocean Optics, Columbia, USA) with a 1-cm optical pass flow cell and an autosampler.[15] Teflon® tubes (0.8 mm i.d.) were used as transmission lines, and Tygon® and Viton® tubes were used for propelling the solutions. Data acquisition as well as the control of the injector-commutator, autosampler and solenoid valves was done by the FIAlab software.

### Reagents and solutions

The solutions were prepared with analytical grade chemicals and deionized water (>18.2 MΩ cm) obtained from a Milli-Q water purification system (Millipore, Bedford, USA). The glassware was kept in an aqueous 10% (v/v) hydrochloric acid solution for 24 h with posterior cleaning using ultra-pure water. The $HNO_3$ was distilled in a quartz sub-boiling still (Marconi, Piracicaba, Brazil).

Chlorpromazine as template, methacrylic acid as functional monomer, ethylene glycol dimethacrylate as crosslinking reagent, and 2,2´-azobisisobutyronitrile as initiator (all from Sigma-Aldrich, Steinheim, Germany) were employed for the MIP synthesis. Chloroform and acetonitrile (both HPLC grade, 99.9%) solvents were obtained from Merck (Darmstadt, Germany).

Chlorpromazine and perphenazine (Sigma-Aldrich, Steinheim, Germany) stock solutions (1 mmol $L^{-1}$) were prepared by dissolving an appropriate mass in HPLC grade methanol, 99.9 % (J. T. Baker, Phillipsburg, USA).

A washing solution of 1:9 (v/v) HCl (pH 2.0):methanol was used, with the HCl purchased from Merck (Darmstad, Germany).

The stationary phase was composed of C18 (particles ≤50 μm) obtained from commercial cartridges of SPE (Varian, Harbor City, USA). For packing, an alcoholic suspension of C18 particles (100 mg $mL^{-1}$ in ethanol) flowed through the column at 0.5 mL $min^{-1}$.

### MIP synthesis

The synthesis of the chlorpromazine imprinted polymer was based on non covalent interactions between the template and the functional monomer. The procedure was similar to that described by Niu *et al.*[16] In a 30 mL glass flask, 2 mmol of chlorpromazine and 8 mmol of methacrylic acid were dissolved in 12 mL of chloroform and this solution was sonicated for 4 h at room temperature. Then, 20 mmol of ethylene glycol dimethacrylate and 50 mg of 2,2´-azobisisobutyronitrile were added. The flask was purged (5 min) with nitrogen and sealed. A water-bath was used to promote temperature control (24 h at 60 °C). After polymerization, the glass flask was broken, and the polymer was mechanically ground in a mortar. A steel sieve was used to select particle sizes (≤75 μm). To remove the template, 500 mg of MIP were packed into a cartridge and submitted to five washing steps with 50 mL of 4:1 (v/v) methanol:acetic acid. The fifth fraction was monitored spectrophotometrically and no signal was observed. Finally, the polymer was dried at 60°C and stored at room temperature.

### Sample preparation

The urine samples of smokers and of non-users of the drugs were submitted to liquid-liquid extraction (LLE) employing hexane as extractor solvent, according to Madej *et al*.[14] A volume of 5 mL of urine was mixed in a test tube with 1 mL of 4 mol $L^{-1}$ NaOH and 250 μL of a chlorpromazine and perphenazine standard solution. Then, 5 mL of 1:99 (v/v) isoamylic alcohol:hexane were added, the tube was vortexed (20 s) and centrifuged at 1000 *g* for 5 min. The organic phase was transferred to another test tube and evaporated under a $N_2$ atmosphere. The residue was dissolved into 5 mL of phosphate buffer (0.01 mol $L^{-1}$, pH 7.0) and analyzed by the proposed method. The urine sample handling was approved by the Ethics Committee of the Medical Science Faculty of the University of Campinas (CAAE: 0411.0.146.000-07).

### On-line extraction and chromatographic separation system

The system (Fig. 1) comprises an injector/commutator, a syringe pump (10 mL), a peristaltic pump, two solenoid valves, a MIP column, an analytical column and an au-

tosampler. Each cycle was divided into eight stages, as shown in Table I.

**First stage:** the injector is switched to the sampling position.

**Second stage:** 5 mL of standard/sample (chlorpromazine and perphenazine) flows (using a peristaltic pump) through the MIP column at 1.6 mL $min^{-1}$.

**Third stage:** Solenoid valve 1 (V1) is turned on and 1 mL of the washing solution (WS = 9:1 (v/v) HCl (pH = 2.0):methanol) flows through the MIP column at 1.6 mL $min^{-1}$, promoting the elution of the concomitants.

**Fourth stage:** V1 and the peristaltic pump are turned off and the injector is switched to the elution position (MIP column is inserted into the analytical pathway).

**Fifth stage:** Solenoid valve 2 (V2) is turned on and the syringe pump aspirates 3 mL of mobile phase (pure methanol) at 30 mL $min^{-1}$.

**Sixth stage:** V2 is turned off and the syringe dispenses the 3 mL of mobile phase at 3 mL $min^{-1}$ to the analytical pathway. At this moment the methanol promotes the elution of the analytes from the MIP column, which are carried to the analytical column (C18), where the separation begins.

**Seventh stage:** V2 is turned on again, and the syringe aspires 10 mL of methanol (mobile phase) at 30 mL $min^{-1}$.

**Eighth stage:** Data acquisition is started at 250 nm. V2 is turned off, the syringe is emptied and the separation is concluded. This stage corresponds to that shown in Fig. 1.

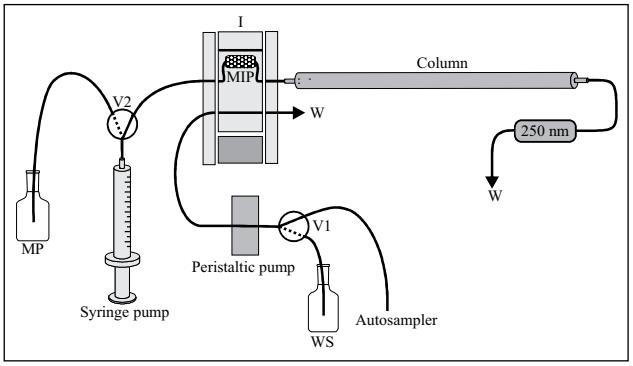


**Figure 1. System used for on-line extraction and separation comprised of a syringe pump (10 mL), peristaltic pump, injector commutator (I), autosampler, two solenoid valves (V1,V2), a column (15 mm x 3 mm i.d.) with 25 mg of MIP and an analytical column (250 mm x 4.6 mm i.d.) filled with C18 (≤ 53 μm particle size). Pure methanol was used as mobile phase (MP) and 1:9 (v/v) HCl (pH 2.0):methanol was employed as washing solution (WS). The analytical signal was monitored at 250 nm, and the remaining solutions were driven to waste (W). Dashed lines in the valves indicate the alternative positions.**

**Table I. Working sequence of the solenoid valves, injector commutator and pumps. On = turn on, Off = turn off, SP = sampling position, IP = injection position, A = syringe is aspirating, D = syringe is dispensing.**

| Stage | Valves | | Injector | Pump | | Time/ s |
|---|---|---|---|---|---|---|
| | V1 | V2 | | Syringe | Peristaltic | |
| 1 | Off | Off | SP | Off | Off | 10 |
| 2 | Off | Off | SP | Off | On | 188 |
| 3 | On | Off | SP | Off | On | 70 |
| 4 | Off | Off | IP | Off | Off | 10 |
| 5 | Off | On | IP | A | On | 6 |
| 6 | Off | Off | IP | D | Off | 60 |
| 7 | Off | On | IP | A | Off | 20 |
| 8 | Off | Off | IP | D | Off | 200 |

### Chromatographic system

The HPLC analysis were done using a PerkinElmer Series 200 chromatograph equipped with a Browlee C18 analytical column, 5 μm, 250x4.6 mm (PerkinElmer, Shelton, USA). The injected volume was 100 μL, and 9:1 (v/v) methanol:phosphate buffer (30 μmol $L^{-1}$ $K_2HPO_4$, pH 5.6) at 1.2 mL $min^{-1}$ was used as mobile phase.

## Results and discussion

### Optimized variables of the system

The absence of memory effect from the synthesis was demonstrated by percolating a blank urine sample through the MIP column, being that the resulting chromatogram did not present any peaks at the retention times of chlorpromazine and perphenazine.

Physical and chemical variables were optimized using a standard solution containing 50 μmol $L^{-1}$ of both chlorpromazine and perphenazine. Integrated absorbance was employed as analytical signal.

The mobile phase composition was appraised in terms of the nature of the solvent. Initially, pure acetonitrile was evaluated, but the analytes were not eluted from the MIP column. On the other hand, pure methanol allows an efficient elution of the analytes (ca. 18 min), and this condition was selected for future experiments. It is important to point out that no methanol:water mixtures were able to elute the analytes from the MIP.

Although there was a complete separation of the analytes using these conditions, broad signals were always observed for chlorpromazine and perphenazine, requiring *ca.* 5.5 and 9.0 min for the signal to return to the baseline. The analytical frequency of the system was improved by increasing the flow rate of the mobile phase. This was easily attained, since 50 μm-C18 particles were used in the analytical column, and the increase in the flow rate did not result in high pres-

sures, as would occur with conventional HPLC particles. However, poorer efficiencies when using larger particle diameters in an analytical column are commonly found. A selective extraction step was then necessary, such as that obtained with MIP. The mobile phase flow rate was evaluated from 1.8 to 3.0 mL $min^{-1}$, and increasing the flow to 3.0 mL $min^{-1}$, the total time was reduced from 18 to 10 min. On the other hand, some leakage problems were observed when flow rates higher than 3.0 mL $min^{-1}$ were employed. The flow rate of 3.0 mL $min^{-1}$ was then selected as the working condition.

The binding between the analyte and the MIP depends on the pH of the sample solution, which determines the ionization of the analytes (weak bases), as well as of the MIP binding sites. Additionally, for complex matrices such as biological samples, buffered media are often necessary. Thus, a 0.01 mol $L^{-1}$ phosphate buffer solution was employed to dissolve the residue obtained from liquid-liquid extraction of the urine samples, since this concentration presents good buffering capacity, as demonstrated by Figueiredo *et al*.[11] The pH of the buffer was evaluated from 6.0 to 8.0 and no significant difference in the analytical signals for pH 6.0 and 7.0 (integrated areas of 0.216 and 0.244 $min^{-1}$ for chlorpromazine and perphenazine, respectively) were found. A decrease in the analytical signal was observed at pH > 7.0. Additionally, phosphate buffer decreased the retention time for both analytes, as demonstrated in Fig. 2, and the running time was reduced from 10 to 6 min when a buffer medium at pH 7.0 was used in the sample preparation step. This fact can be attributed to modifications in the nature of interactions between analytes and MIP due to the presence of phosphate ion, as well as by the pH adjustment. Thus, it can be stated that buffer addition was important to improve not only the analytical frequency but also the detectability. A pH of 7.0 was selected for the remaining experiments. At this pH, the propylamine group of chlorpromazine (pKa 9.3)[17] and the carboxyl group of the methacrylic acid (pKa 4.7)[18] are positively charged and uncharged, respectively. Thus, an electrostatic interaction between the chlorpromazine and MIP could explain the performance of the method. A similar explanation can be used for perphenazine.

In SPE procedures using MIP, the selectivity is highly dependent on the washing solution. This solution is used to eliminate foreign molecules that are concomitantly retained in the MIP. Since, the separation step is not as efficient as in conventional HPLC, due to the larger sizes of the stationary phase particles, a sufficient washing step is necessary to avoid problems such as the overlap of the observed peaks with peaks from other compounds (concomitants). The washing solution was evaluated by increasing the methanol content in the water until a concentration which promoted elimination of the concomitants without removing the analytes from the MIP. Both 1:4 and 1:9 (v/v) proportions of water:methanol were evaluated, and no significant differences in the analytical signals were observed for these solutions. Thus, a 1:9 (v/v) water:methanol ratio was selected as the working condition. The aqueous fraction of this solution (corresponding to 10% (v/v)) was changed to a solution of HCl with pH from 1.5 to 7.0 being evaluated. For pH 2.0 there was a decrease of *ca*. 17% in the analytical signal when compared to pH 3.0. For pH from 3.0 to 7.0, no difference in the analytical signal was observed. Then, pH 2.0 was selected for future experiments as a compromise between selectivity and sensitivity.

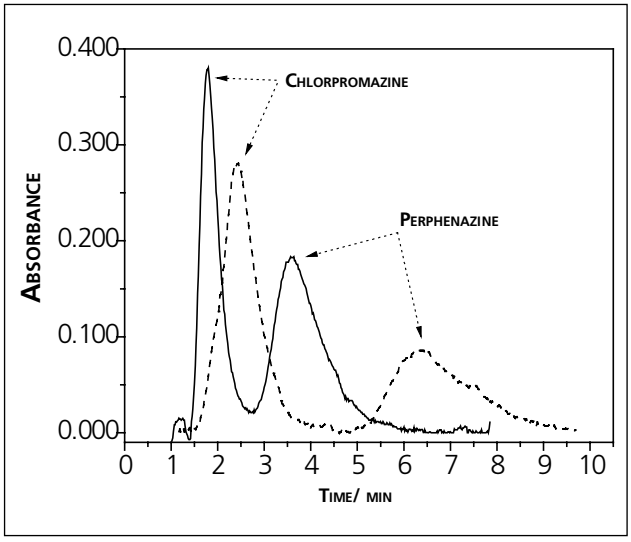


**Figure 2. Chromatograms obtained for chlorpromazine and perphenazine (both at 50 μmol $L^{-1}$) standard solutions in buffered (pH 7.0) (solid line) and unbuffered (dashed line) media.**

## Figures of merit and applications

Blank and reference solutions were prepared from a pool of seven urine samples of non-users of phenothiazines to reproduce the matrix influence in the analytical signal. Additionally, all urine samples were individually submitted to LLE and analyzed by the proposed method. No signals were observed for any urine sample during the runs.

The analytical curve ranged from 5.0 to 50 μmol $L^{-1}$ and the correlation coefficients were higher than 0.996 (for five concentration levels, n = 3) for both analytes. Limits of quantification (5.0 μmol $L^{-1}$) for both chlorpromazine and perphenazine were attained, and the analytical frequency was 6 $h^{-1}$. Intra and inter-day precisions (Table II) were obtained employing urine samples, using three concentration levels of chlorpromazine and perphenazine (10, 30 and 50 μmol $L^{-1}$) added to urine, and analyzed either on the same day (n = 5) or on different days (n = 3), respectively.

**Table II. Intra and inter-day precision for urine of non-users, fortified with the phenothiazinies at three concentration levels.**

| Levels/ μmol L$^{-1}$ | Precision/ % | | | |
|---|---|---|---|---|
| | Chlorpromazine | | Perphenazine | |
| | Intra-day (n = 5) | Inter-day (n = 3) | Intra-day (n = 5) | Inter-day (n = 3) |
| 10 | 6 | 13 | 9 | 12 |
| 30 | 7 | 9 | 5 | 9 |
| 50 | 6 | 10 | 7 | 12 |

Accuracy was checked by analyzing fortified urine samples by the proposed methods (see section 2.6) and by the conventional method. The results are presented in Table III, and no significant differences were found at the 95% confidence level, according to Student's t-test. Fig. 3 shows the chromatogram for a urine sample having 50 μmol L$^{-1}$ of the reference solutions added.

**Table III. Chlorpromazine and perphenazine determination by the proposed method (n = 3) and by HPLC (n = 3) in fortified urine samples of non-users.**

| Analyte | Sample | Addition/ μmol L$^{-1}$ | Proposed system/ μmol L$^{-1}$ | Recovery/ % | HPLC/ μmol L$^{-1}$ |
|---|---|---|---|---|---|
| Chlorpromazine | 1 | 5 | 4.7 ± 0.7 | 94 | 4.5 ± 0.1 |
| | 2 | 5 | 4.3 ± 0.6 | 86 | NA* |
| | 3 | 10 | 10.9 ± 0.4 | 109 | 9.2 ±0.6 |
| | 4 | 10 | 10 ± 1 | 100 | 9.3 ± 0.1 |
| | 5 | 15 | 14 ± 1 | 93 | NA |
| | 6 | 15 | 15 ± 1 | 100 | NA |
| Perphenazine | 1 | 5 | 5.4 ± 0.5 | 108 | 4.5 ± 0.1 |
| | 2 | 5 | 5.7 ± 0.2 | 114 | NA |
| | 3 | 10 | 9.1 ± 0.5 | 91 | 9.2 ± 0.6 |
| | 4 | 10 | 10 ± 1 | 100 | 9.1 ± 0.2 |
| | 5 | 15 | 14.2 ± 0.6 | 95 | NA |
| | 6 | 15 | 15 ± 1 | 100 | NA |

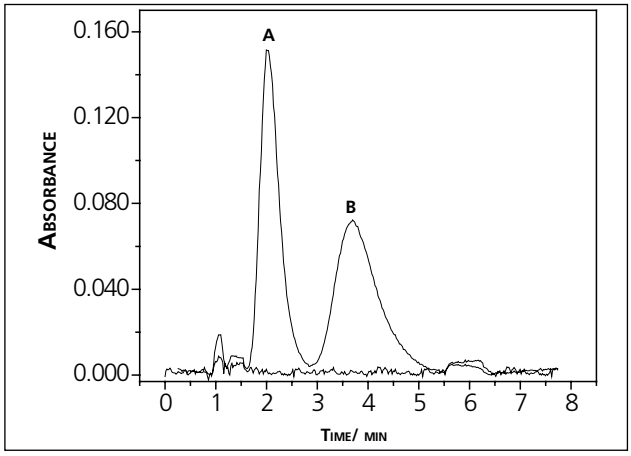


**Figure 3. Chromatograms obtained for a urine sample without addition and with chlorpromazine and perphenazine added, both at 50 μmol L$^{-1}$. A and B correspond to the chlorpromazine and perphenazine signals, respectively.**

## Conclusions

The development of an on-line extraction (using MIP) and a low-pressure separation system for a fast chlorpromazine and perphenazine determination in urine samples was successfully attained. One important characteristic is that when the MIP is used as sorbent in SPE, only a few molecules are extracted. This selectivity allows a faster separation step, since fewer peaks are observed in the chromatograms.

In terms of the proposed methodology, the washing solution was satisfactorily employed to eliminate concomitants nonspecifically bound to the MIP. Additionally, other important figures of merit, such as LOQ, analytical frequency, linear range, selectivity (no interference was observed in the chromatogram of urine samples), precision and accuracy are pointed out in this work.

Finally, besides the novelty of this in the literature, some important advantages of the on-line system proposed can be highlighted such as high-speed analysis of urine samples for quantification of two analytes using a simple technique such as spectrophotometry, low cost of implementation, ease of handling the solutions, high degree of mechanization, and the possibility to work with complex samples, among others. Thus, due to these characteristics, it is presumed that the proposed method can be an interesting alternative for routine analysis of chlorpromazine and perphenazine in urine.

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# Development of environmentally sustainable procedures for the determination of orthophosphate and total iron in fresh water, employing a homemade LED-based photometer and a multicommuted approach

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**Abstract**
In this article, a flow system designed to allow photometric determination of orthophosphate and total iron in fresh water while generating a low volume of waste is described. The procedures for the determination of orthophosphate and iron were based on the molybdenum blue and 1,10-phenanthroline methods, respectively. Accuracy was assessed by comparing results with those achieved using independent procedures. No significant differences at 95% confidence level were observed. Other useful features, including linear responses ranging from 0.1 to 5.0 mg $L^{-1}$ $PO_4^{3-}$ (R = 0.999) and from 0.1 to 2.0 mg $L^{-1}$ Fe (R = 0.999), detection limits of 20 µg $L^{-1}$ $PO_4^{3-}$ and 50 µg $L^{-1}$ $Fe^{3+}$, relative standard deviations of 0.6 % (n = 11) and 0.9 % (n = 11) for $PO_4^{3-}$ and Fe, respectively, and analytical throughput of 76 determinations per hour, were also achieved. Reagent consumption of 0.2 mg ammonium molybdate, 0.12 mg 1,10-phenanthroline and 0.53 mg ascorbic acid per determination, and a waste volume of 0.264 mL per determination were also achieved.

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**Keywords:** Multicommuted flow analysis, LED-based photometer, orthophosphate, iron, water, Green chemistry

## 1. Introduction

Nowadays, attention has been given to the quality of fresh water, which has been accomplished by monitoring the presence of several chemical species, including iron and phosphate. Iron is considered essential for living organisms. Nevertheless, high concentrations cause tissue damage as a result of free radical formation [1,2]. Thus, the World Health Organization (WHO) established a guideline value of 0.3 mg $L^{-1}$ of iron in drinking water [3]. Phosphorus present in rivers and lakes generally results from fertilization of arable land, industrial activities and municipal sources, causing eutrophication, which has been considered as a serious environmental problem [4,5]. For this reason determination of phosphate levels is a requirement when monitoring water quality.

The determination of iron and phosphate in water by spectrophotometry has been carried out by employing analytical procedures based on the flow injection analysis (FIA) process [6,7], presenting as an advantage high throughput, which could be considered a common feature of FIA systems [8,9]. Because reagent solutions are pumped continuously through the flow system, part of them is discarded as waste without mixing with the sample solution, resulting in misuse of this part of the reagent solution. Because of this, the ability to reduce waste generation must be included as a requirement to reduce the pollution caused by the analytical procedure, in accordance with the principles of Green Analytical Chemistry [10].

Sequential injection analysis (SIA) [11,12] and multicommuted flow analysis (MCFA) [13,14] processes present as a common feature low reagent consumption, resulting in reduction of waste generation [15,16,17]. Generally, the carrier solution does not participate in the chemical reaction, but it can be considered as a major contributor to waste generation. For this reason, a strategy to reduce the use of this solution will be incorporated within the current analytical procedure, in order to improve environmental sustainability.

Photometric procedures for the determination of iron and orthophosphate in fresh water will be developed employing a MCFA process designed to use an air stream as carrier. The procedures will be implemented using the molybdenum blue method and the 1,10-phenanthroline method for orthophosphate and iron determination,

respectively [18,19]. Considering that orthophosphate and iron(II) compounds present radiation absorption bands around 660 and 530 nm, respectively, a homemade LED-based photometer will be designed to allow the detection of both analytes using an ON/OFF LED switching strategy. LED based photometers for phosphate determination have been employed using multi-reflection and long pathway flow cells to improve sensitivity [19,20]. In the current work, a LED based photometer will be used to develop analytical procedures for the determination of phosphate and iron in freshwater using a flow system module designed to allow the sequential determination of both analytes, also improving the reagent consumption.

## 2. Experimental

### 2.1. Reagent solutions and samples

All solutions were prepared with analytical grade chemicals. Purified water (conductivity less than 0.1 μS $cm^{-1}$) was used throughout.

A 1000 mg $L^{-1}$ $PO_4^{3-}$ stock solution was prepared by dissolving 0.7163 g of $KH_2PO_4$ (purity 99%) from Merck (Darmstadt, Germany) in 500 mL of water. The solution was stored in a freezer at 4ºC. Working standard solutions with concentrations between 0.1 and 5.0 mg $L^{-1}$ $PO_4^{3-}$ were prepared daily by dilution from the stock solution with a 0.014 mol $L^{-1}$ $HNO_3$ solution. A 1000 mg $L^{-1}$ iron stock solution was prepared by dissolving 2.42 g of $FeCl_3.6H_2O$ (purity 99%) from Merck (Darmstadt, Germany) in 5 mL of concentrated $HNO_3$. After dissolution, the volume was made up to 500 mL by adding water. Working standard solutions with concentrations between 0.1 and 2.0 mg $L^{-1}$ $Fe^{3+}$ were prepared daily by dilution from the stock solution with a 0.014 mol $L^{-1}$ $HNO_3$ solution. A 1.0 % (w/v) ascorbic acid solution was prepared daily by dissolving 1.0 g of reagent (purity 99%) from Sigma-Aldrich (St Louis, USA) in 100 mL of water. A 0.3 % (w/v) ammonium molybdate solution was prepared daily by dissolving 0.3 g of reagent (purity 99%) from Merck (Darmstadt, Germany) in 100 mL of a 0.05 mol $L^{-1}$ $HNO_3$ solution. A 0.25 % (w/v) 1,10-phenanthroline solution was prepared daily by dissolving 0.25 g of reagent (purity 99%) from Sigma-Aldrich (St Louis, USA) in 100 mL of water.

### 2.2. Samples

Water samples were collected at different sites along the Corumbataí and Piracicaba Rivers. Samples were filtered through a 0.42 μm filter and acidified using concentrated nitric acid, in order to maintain an acid concentration of 0.014 mol $L^{-1}$ $HNO_3$. Samples were stored in amber bottles and maintained at 4ºC. Prior to use, aliquots of 25 mL were removed from the stock, which was processed after equilibration to laboratory temperature (22 ºC).

### 2.3. Apparatus

The equipment set up was constituted of four pinch solenoid valves, normally closed (61P011), and one pinch solenoid valve, normally open (161P021), purchased from NResearch; an IPC4 Ismatec peristaltic pump furnished with Tygon pumping tubes; a Pentium IV microcomputer (Advantech Corp) equipped with an interface card (PCL711S, Advantech Corp); a homemade regulated power supply (12 V) to feed the solenoid valves; reaction coils and flow lines made of PTFE tubing (0.5 mm i.d.); joint devices made of acrylic; and a homemade flow cell in the form of a Z, made of a glass tube with a 2.0 mm inner diameter and a 20 mm length. A homemade electronic interface to drive the solenoid valves and to control LED feeding was assembled using LN2803 integrated circuits, which were coupled to output ports of the PCL711S interface card, using wiring similar to that employed elsewhere [22].

The photometer comprised a green LED (λ = 530 nm, high-bright), a red LED (λ = 660 nm, high-bright), and a photodiode IPL10530DAL (RS Components). The software to control the solenoid valves and to perform data acquisition was written in QuickBASIC 4.5.

### 2.4. LEDs and flow cell assembly

The compounds of orthophosphate/ammonium molybdate and iron(II)/1,10-phenanthroline show light absorption with maxima around 660 nm and 530 nm, respectively. Thus, the arrangement shown in Fig.1 was

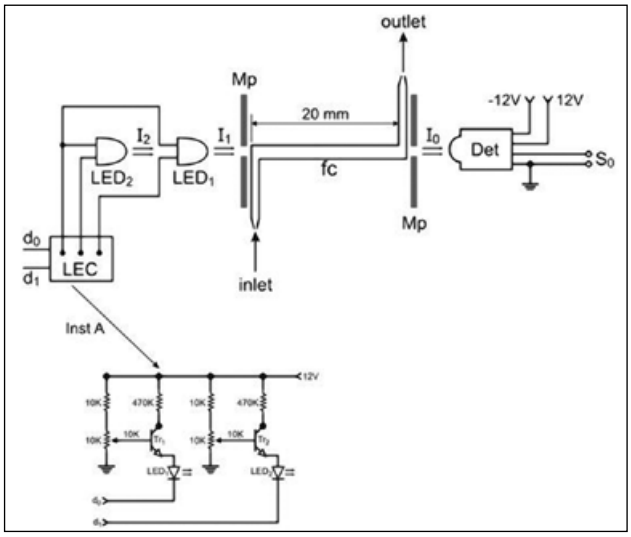


**Figure 1. Representation view of the LEDs and photodetector coupling to flow cell. $LED_1$ and $LED_2$ = light emitting diode, $\lambda_{max}$ at 530 nm and 660 nm, respectively; $I_1$ and $I_2$ = radiation beam emitted by $LED_1$ and $LED_2$, respectively; fc = flow cell, glass tube, 2.0 mm inner diameter; $I_0$ = radiation beam that crossed the flow cell; Mp = metallic plates; Det = photodiode IPL 10530DA (RS); $S_0$ = analytical signal (mV); $Tr_1$ and $Tr_2$ = transistors BC547; $d_0$ and $d_1$ = control lines wired to the ULN2803 integrated circuit. The values of the resistors are expressed in Ohm**

designed to permit their determinations using a single photodetector. The metallic plates (Mp) installed in front of the flow cell observation windows prevent scattered light from reaching the photodetector (Det). The LEDs, the flow cell and the photodiode (Det) were attached to PVC blocks, which were fixed on a PVC holder with screws in order to form a compact unit.

Since the intensities of the radiation beams emitted by each LEDs were different, and a part of the radiation emitted by $LED_2$ was lost by scattering when it crossed $LED_1$, the electronic interface (Inset A) was employed in order to allow the adjustment of the LEDs emission intensities, which was done by rotating the variable resistors (20 kΩ) that were wired to the base of the transistors ($Tr_1$, $Tr_2$).

### 2.5. Flow system

The diagram of the flow system, shown in Fig.2, was designed to work in the pulling mode [14], whereby the solutions are propelled by suction toward the peristaltic pump (pp).

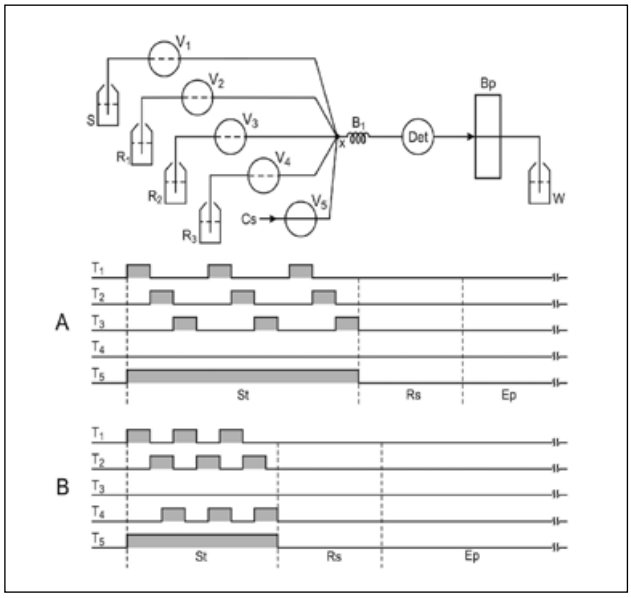


**Figure 2. Flow diagram of the system. S = sample; $R_1$ = 0.3 % (w/v) ammonium molybdate solution in a 0.05 mol $L^{-1}$ $HNO_3$ medium; $R_2$ = 1.0 % (w/v) ascorbic acid solution; $R_3$ = 0.25 % (w/v) 1,10-phenantroline solution; Cs = air stream; $V_1$, $V_2$, $V_3$ and $V_4$ = pinch solenoid valve normally closed; $V_5$ = pinch solenoid valve normally open; x = six-way joint device machined in acrylic; $B_1$ = reactor coil, 20.0 cm length, 0.8 mm i.d.; Det = photometric detector; pp = peristaltic pump, flow rate a 33 $\mu L s^{-1}$; W = waste. $T_1$, $T_2$, ..., $T_5$ = timing course of the valves $V_1$, $V_2$, ..., $V_5$ ON/OFF switching pattern, respectively; A and B = valves switching pattern to perform the determination of orthophosphate and iron, respectively; shadowed surfaces beneath the T-lines indicate that the corresponding valve was switched ON; St, Rs and Ep = sampling, signal reading and flow cell emptying steps, respectively. Continuous line in the symbol of valve $V_5$ indicates that solution flowed through while switched OFF. Interrupted lines in the symbols of valves $V_1$, $V_2$, $V_3$ and $V_4$ mean that solutions only flowed through them when switched ON.**

In this configuration, all valves are switched off and the air stream (Cs) flows through valve $V_5$ and reaction coil ($B_1$), towards the waste (W). When the software was run, the valve-switching sequence labeled in Table I as *a*, *b*, *c*, *d* and *e* was carried out, in order to fill the flow lines with the respective solutions.

### 2.6. Calibration stage

Prior to beginning the analytical run, the photometer calibration stage was performed by the microcomputer as a sequence of steps labeled in Table I as *f*, *g* and *h,* in order to fill the flow cell with blank solution (S). Maintaining the two LEDs switched OFF (lines $d_0$ and $d_1$ at high level), the microcomputer read the dark signal (Dks). Afterwards, the microcomputer sent a control signal through the PCL711S interface card (Table I, $d_0 = 0$) to permit the electric current to circulate through $LED_1$. The intensity of the radiation beam emitted by $LED_1$ was adjusted by means of the variable resistor (20 kΩ), in order to obtain a potential difference of around 2000 mV ($S_{0A}$) in the photometer (Det) output. Afterwards, the microcomputer enabled the electric current circulation through $LED_2$ ($d_1 = 0$). The adjustment of its emission intensity and the reading of signal ($S_{0B}$) were carried out using an operational sequence similar to that described for $LED_1$. The values of Dks, $S_{0A}$ and $S_{0B}$ were used to calculate the absorbance. The control software was designed to permit only one LED to be switched ON at a time.

**Table I. Operational sequence to control the flow system**

| Analyte | Step | Event | $V_1$ | $V_2$ | $V_3$ | $V_4$ | $V_5$ | $d_0$ | $d_1$ | Time (s) | Cycles |
|---|---|---|---|---|---|---|---|---|---|---|---|
| - | a | Standing by | 0 | 0 | 0 | 0 | 0 | 1 | 1 | - | - |
| - | b | Fills flow line S | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 15 | - |
| - | c | Fills flow line $R_1$ | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 15 | - |
| - | d | Fills flow line $R_2$ | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 15 | - |
| - | e | Fills flow line $R_3$ | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 15 | - |
| - | f | Fills flow cell* | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 15 | - |
| - | g | Switches ON $LED_1$ | 0 | 0 | 0 | 0 | 0 | 0 | 1 | - | - |
| - | h | Switches ON $LED_2$ | 0 | 0 | 0 | 0 | 0 | 1 | 0 | - | - |
| - | i | Washes with sample | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 20 | - |
| - | j | Empties flow cell | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | - |
| $PO_4^{3-}$ | 1 | Switches On $LED_1$ | 0 | 0 | 0 | 0 | 0 | 0 | 1 | - | - |
| - | 2 | Inserts sample (S) | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0.4 | |
| - | 3 | Inserts solution ($R_1$) | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0.6 | 6 |
| - | 4 | Inserts solution ($R_2$) | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0.6 | |
| - | 5 | Switches On $LED_2$ | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1.0 | - |
| - | 6 | Read signal | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 30 | - |
| $Fe^{2+}$ | 7 | Switches ON $LED_2$ | 0 | 0 | 0 | 0 | 0 | 1 | 0 | - | - |
| - | 8 | Inserts sample (S) | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0.4 | |
| - | 9 | Inserts solution ($R_1$) | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0.3 | 6 |
| - | 10 | Inserts solution ($R_3$) | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0.2 | |
| - | 11 | Switches ON $LED_1$ | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1.0 | - |
| - | 12 | Read signal | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 20 | - |

The numbers 1 and 0 in the columns indicate that the corresponding valve was switched ON or OFF, respectively. *When the software was run at first time its execution was halted at this step to allow the calibration of the photometer with blank solution. $d_0$ and $d_1$ = control lines to switch ON /OF (0/1) $LED_1$ and $LED_2$, respectively.

### 2.7. Procedure

The software was designed to work according to the sequence of steps summarized in Table I. The microcomputer sent electric pulses through the control interface to switch the solenoid valves $V_1$, $V_2$, and $V_3$ ON/OFF according to the switching pattern shown in the valve-timing course (A) depicted in Fig.2. Under this condition, the reaction coil ($B_1$) and the flow cell were loaded with a solution string comprising slugs of sample (S) and reagent solutions ($R_1$, $R_2$). While the sampling step (St) was being processed, $LED_1$ ($\lambda$ = 530 nm) was maintained powered and the microcomputer read the signal generated by the photodetector (Det) through the analog input of the PCL711S interface card. The microcomputer recognized that the flow cell was filled with sample zone when a signal ($S_0$) higher than 1700 mV was detected. When this condition was attained, the sampling step was ended by the microcomputer, which was done by switching all valves OFF and sending a control signal through the serial interface to stop peristaltic pump rotation. Afterwards, $LED_1$ was switched OFF and $LED_2$ ($\lambda$ = 660 nm) was switched ON, to permit the monitoring of the radiation absorption caused by the orthophosphate compound (Table I, step 5). After reading the signal (step 6), the pump rotation was established again and sample zone was suctioned from the flow cell towards the waste (W). Under this condition, all valves were maintained switched OFF, so that only the air stream (Cs) flowed through reaction coil, in order to empty the flow cell. After this step, the system was ready to carry out the next analytical run.

The determination of iron was carried out by employing an operational sequence similar to that described for the orthophosphate determination. In this case, $LED_2$ ($\lambda$ = 660 nm) was maintained switched ON to allow the microcomputer to recognize when the flow cell was filled with the mix comprising sample and reagent solutions ($R_1$, $R_3$). In this case, the solenoid valves $V_1$, $V_3$ and $V_4$ were switched sequentially to the correct ON/OFF setting, according to valve switching pattern (B) shown in Fig.2. As we can see, while the sampling step proceeded, valve $V_5$ was maintained switched ON in order to interrupt the air stream. The recognition of when the flow cell was filled with a mix of sample and reagent solutions ($R_1$, $R_3$) and the analytical signal reading steps were done in a similar way to that described above for the orthophosphate determination.

Reaction coil length and flow rate were maintained at 20.0 cm and 33 μL $s^{-1}$, respectively. Concentrations of ammonium molybdate and of 1,10-phenanthroline solutions were maintained at 0.3 % (w/v) and 0.25 % (w/v), respectively. The variables investigated in order to find the best working conditions were the concentration of ascorbic acid solution, which was assayed within the range of 0.25 % to 3.0 % (w/v), and ratios between the volumes of sample and reagents solution aliquots. The assays were carried out maintaining valve $V_1$ switched ON at 0.4 s and varying the intervals to switch ON/OFF valves $V_2$ and $V_3$ for orthophosphate or $V_2$ and $V_4$ for iron determination, respectively. In both cases, the time intervals were varied from 0.1 to 1.2 s. These experiments were performed using standard solutions with concentrations ranging from 0.1 to 5.0 mg $L^{-1}$ orthophosphate or 0.1 to 2.0 mg $L^{-1}$ iron.

Intending to prove that the proposed system was reliable, water samples from the Corumbataí and Piracicaba Rivers were analyzed by employing the working conditions summarized in Table I. To allow accuracy assessment, samples were also analyzed by employing the reference method [17] for $PO_4^{3-}$ and Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) for total iron.

## 3. Results and discussion

### 3.1. General comments

In Section 2.4, we described how the LED emission intensities were adjusted to obtain output signals ($S_{0A}$ and $S_{0B}$) around 2000 mV, generated by the photometer, maintaining the flow cell filled with blank solution. Thus, when the flow cell was filled with a mix of sample and reagent solutions, the signal generated was lower than 2000 mV. When the standard solution or sample was processed, the microcomputer read the signal generated by the photometer (ReadSig) and calculated the absorbance by employing the following equation: Abs = - Log(ReadSig - Dks)/( $S_{0A}$ -Dks); where $S_{0A}$ = reference signal and Dks = dark signal, which were obtained in the photometer calibration stage. The absorbance was saved as an ASCII file to allow further processing.

Because a liquid fluid was not used as a carrier stream, the sample zone was not affected by dispersion. In this sense, the volume of the sample zone comprised only the solution volume required to fill the reaction coil (100 μL) and the flow cell (105 μL). This feature was exploited in order to assure reduction of waste volume.

In order to find the appropriate volume of sample zone for filling both reaction and flow cell, the solenoid valves $V_1$, $V_2$ and $V_3$ were switched ON/OFF sequentially, maintaining each one turned ON during a time interval of 0.4 s. Thus, a time interval of 1.2 s elapsed while a sampling cycle was carried out. The microcomputer recognized that the flow cell was filled when 6 sampling cycles were carried out. Since flow rate was maintained at 33 μL $s^{-1}$, and the volume of each solution slug was 13.2 μL, the total volume of sample zone inserted into the reaction coil and flow cell was 237.6 μL.

### 3.2. Effect of ascorbic acid concentration

Ascorbic acid was a common reagent for the deter-

mination of both analytes, so its concentration was the first parameter investigated. The assays were performed using a standard solution with a concentration of 5.0 mg $L^{-1}$ $PO_4^{3-}$ and varying ascorbic acid concentrations from 0.25 to 3.0 % (w/v). The analytical signal attained the maximum value when the ascorbic acid concentration was 1.0 % (w/v). Similar experiments were performed using a 2.0 mg $L^{-1}$ iron(III) standard solution. When the ascorbic acid concentration was equal to or higher than 1.0 % (w/v), the analytical signal displayed similar values. Therefore, this concentration was selected.

### 3.3. Effect of the solution slug volume

In the proposed system, aliquots of sample and reagent solutions were handled according to the basic rule of the multicommutation process [13,14], whereby slugs of sample, ammonium molybdate and ascorbic solutions were inserted into the reaction coil sequentially. Under this condition, the mixing between the solution slugs proceeded by mean of the shared liquid interfaces. This implies that large slug volumes could hamper the mixing process. Since these parameters could affect sensitivity, experiments were carried out maintaining the valve $V_1$ switched ON for 0.4 s and varying from 0.1 to 1.2 s the time intervals during which valves $V_2$ and $V_3$, were kept ON. The main results are shown in Table II, where we can see that several combinations of solution volumes could be selected without risk of impairing sensitivity and linearity, except for those shown on the first line for phosphate and for iron(III). Nevertheless, when slugs of solutions were higher than those shown in Table II, decreases in sensitivity and linearity were observed, which could be caused by incomplete mixing.

**Table II. Effect of the reagent solution volumes**

| Phosphate | | | | | |
|---|---|---|---|---|---|
| R1 (µL) | R2 (µL) | R3 (µL) | Intercept | Slope | Linear Coef.(R) |
| 3.3 | 13.2 | - | 0.0210 | 0.0615 | 0.997 |
| 6.6 | 13.2 | - | 0.0223 | 0.0622 | 0.999 |
| 9.9 | 13.2 | - | 0.0224 | 0.0624 | 0.999 |
| 13.2 | 13.2 | - | 0.0220 | 0.0631 | 0.999 |
| 9.9 | 6.6 | - | 0.0212 | 0.0613 | 0.998 |
| 9.9 | 13.2 | - | 0.0220 | 0.0625 | 0.999 |
| 9.9* | 19.8* | - | 0.0231 | 0.0633 | 0.999 |
| 9.9 | 26.4 | - | 0.0341 | 0.0589 | 0.998 |
| Iron(II) | | | | | |
| 3.3 | | 13.2 | 0.0209 | 0.1458 | 0.997 |
| 6.6 | - | 13.2 | 0.0211 | 0.1531 | 0.998 |
| 9.9* | - | 13.2* | 0.0218 | 0.1549 | 0.999 |
| 13.2 | - | 13.2 | 0.0225 | 0.1547 | 0.999 |
| 9.9 | - | 6.6 | 0.0219 | 0.1557 | 0.999 |
| 9.9 | - | 13.2 | 0.0215 | 0.1552 | 0.999 |
| 9.9 | - | 19.8 | 0.0223 | 0.1548 | 0.999 |

*Selected values. Results are average of the three consecutive measurements. Sample volume was maintained at 13.2 µL

### 3.4. Effect of the stopped flow on the analytical signal

Because the reaction coil was short (20 cm, Fig.2), the sample residence time was too short for chemical reaction development to be completed. To deal with this problem, a strategy of stopping the flow was implemented. After ending the sampling step, the pump was halted during a preset delay time, thereby stopping the flow of sample zone into the reaction coil, so as to allow chemical reaction development to occur. For the results mentioned in the previous sections, the delay time was 40 s. Assays to find the appropriate time interval for chemical reaction development were done by processing a set of iron standard solutions with concentrations ranging from 0.1 to 2.0 mg $L^{-1}$ $Fe^{3+}$. The time interval was varied from 5 to 45 s. Processing the data revealed that a linear relationship (R = 0.999) giving higher sensitivity (slope = 0.1531) was achieved when the time interval was 20.0 s. Under this condition, a relative standard deviation of 0.6 % and a detection limit of 50 µg $L^{-1}$ $Fe^{3+}$ were estimated. Since the linear coefficient, intercept and limit of detection achieved when the time interval was higher than 20 s showed similar values, this time interval was selected, with the aim of improving the sample processing throughput.

For orthophosphate determination, the time interval was varied from 10 to 180 s, and an increase in signal was observed throughout. This effect could be expected considering that the orthophosphate reaction rate is slow. Analyzing the results, we observed that a linearity coefficient (R = 0.999) was maintained. Therefore, the time interval of 30.0 s was selected. Additional experiments showed that by maintaining this operational condition a detection limit of 20 µg $L^{-1}$ $PO_4^{3-}$ and a standard deviation of 0.9 % were achieved, which could be considered appropriate for orthophosphate determination in river water.

### 3.5. Performance and accuracy assessment

Since an air stream was employed as carrier for the sample zone, air bubbles would be expected to be retained in the flow cell, thus impairing the precision of the results. The records of Fig. 3 show that this effect did not occur. These records were obtained using delay times of 30 and 10 s for phosphate and iron, respectively. The profiles of the records related to phosphate indicate that, when the data acquisition step terminated, reaction development was still proceeding.

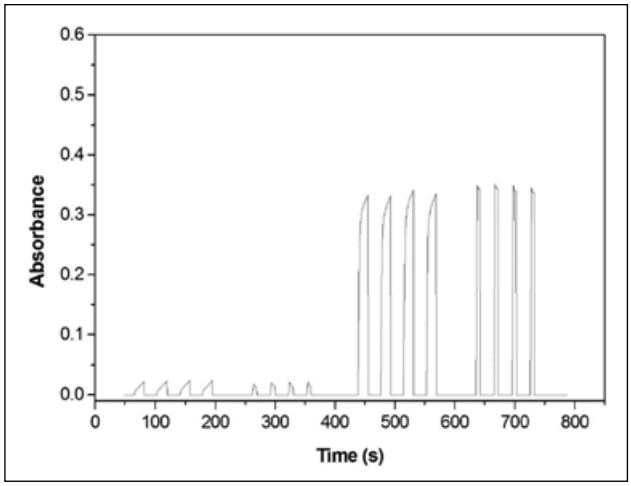


**Figure 3. Typical records of the signals. From left the first and third sets of records correspond to the blank and 5.0 mgL$^{-1}$ of phosphate standard solutions, while the second and fourth sets refer to the blank and 2.0 mgL$^{-1}$ of iron(III) standard solutions.**

The system parameters related to the best operational conditions are summarized in Table I. In order to access the overall performance of the system, a set of iron(III) and orthophosphate standard solutions were processed, yielding the results shown in Table III. These data show that the overall performance of the proposed system for both analytes is comparable to that obtained for the earlier procedures, which were based on the MCFA process [18,23]. Furthermore, the volumes of waste generated per determination were significantly lower than those achieved in the cited works.

**Table III. Analytical performance comparison**

| Parameters | $PO_4^{3-}$ | | | Total Fe | | |
|---|---|---|---|---|---|---|
| | Prop A* | Ref. [18] | Ref. [24] | Prop B* | Ref. [23] | Ref. [25] |
| Liner range (mg L$^{-1}$) | 0.1-5.0 | 0.25-3.0 | 0-2.00 | 0.1-2.0 | 0.5-6.0 | 0.5-6.0 |
| Liner coefficient (R) | 0.999 | 0.999 | 0.998 | 0.999 | 0.999 | |
| Standard deviation (%) | 0.9 | 0.7 | 3.6 | 0.6 | 0.2 | 0.3 |
| Limit of detection (mg L$^{-1}$) | 0.02 | 0.02 | 0.15 | 0.05 | 0.05 | 0.01 |
| Sample (µL)* | 104 | 312 | 500 | 140 | 1500 | 600 |
| Acid ascorbic (mg)* | 1.0 | 3.3 | 60 | 0.8 | 0.6 | 28 |
| Ammonium molybdate (mg)* | 0.3 | 0.3 | 96 | - | - | - |
| 1,10-phenanthroline (mg)* | - | - | - | 0.13 | 0.6 | - |
| Ferrozine (mg)* | - | - | - | - | - | 1.5 |
| Waste generation (µL)* | 238 | 860 | 8000 | 238 | 1600 | 8600 |
| Throughput (h$^{-1}$) | 38 | 56 | 15 | 38 | 40 | 90 |

A and B are the propose procedures for the determination of phosphate an on, respectively. *The values are related to one determination.

Comparing the performances of the proposed procedures with those presented by procedures based on the usual FIA approaches [24,25], we can observe that the comparisons favor the proposed procedures, except for the throughput of the procedure for iron determination [25]. Comparing the performance of the proposed procedure for phosphate determination with the existing procedure employing a LED based photometer [21], we can observe that the linear response range and limit of detection are similar, but the proposed procedure showed better standard deviation, and lower waste generation.

Intending to prove the feasibility of the proposed system, a set of river water samples was analyzed by employing the operational conditions summarized in Table I, providing the results shown in Table IV. With the aim of assessing accuracy, the data obtained employing the proposed procedures were compared with those obtained using independent methods. Applying the paired *t*-test, the values calculated were 1.335 and 0.531 for iron and orthophosphate, respectively. Since the theoretical *t*-value considering nine degrees of freedom is 2.262, the results obtained for both analytes showed no significant difference at the 95 % confidence level.

**Table IV. Results comparison**

| Sample | $PO_4^{3-}$ (mg L$^{-1}$) | | Total Fe (mg L$^{-1}$) | |
|---|---|---|---|---|
| | Ref. method [17] | Proposed method | ICP-OES | Proposed method |
| 1 | 4.43 ± 0.03 | 4.65 ± 0.02 | 1.19 ± 0.02 | 0.96 ± 0.01 |
| 2 | 4.81 ± 0.03 | 4.50 ± 0.01 | 1.20 ± 0.02 | 1.01 ± 0.01 |
| 3 | 1.96 ± 0.02 | 2.09 ± 0.03 | 0.292 ± 0.004 | 0.344 ± 0.003 |
| 4 | 1.40 ± 0.03 | 1.32 ± 0.04 | 0.29 ± 0.01 | 0.364 ± 0.002 |
| 5 | 1.25 ± 0.03 | 1.41 ± 0.05 | 0.27 ± 0.01 | 0.289 ± 0.005 |
| 6 | 2.70 ± 0.03 | 2.76 ± 0.05 | 0.325 ± 0.003 | 0.384 ± 0.006 |
| 7 | 3.86 ± 0.08 | 3.93 ± 0.06 | 0.406 ± 0.004 | 0.496 ± 0.002 |
| 8 | 2.41 ± 0.04 | 2.33 ± 0.06 | 0.31 ± 0.01 | 0.334 ± 0.004 |
| 9 | 2.33 ± 0.02 | 2.43 ± 0.03 | 0.323 ± 0.002 | 0.338 ± 0.003 |
| 10 | 1.35 ± 0.02 | 1.22 ± 0.01 | 0.37 ± 0.01 | 0.407 ± 0.004 |

Results are average of four consecutive measurements

## 4. Conclusion

The LED coupling in tandem and their attachment to the observation window of the flow cell resulted in a compact and downsized detection unit. The photodiode coupling to the other observation window of the flow cell provided a complementary advantage by improving the light detection conditions.

The use of solenoid pinch valves to construct the flow system manifold allows an air stream to be employed as a carrier for the sample zone. Since the flow cell was emptied prior to beginning the analytical run, the air bubbles were not delivered into the sample bulk. Therefore, we can conclude that the use of an air stream as a carrier fluid is a reliable strategy for reducing the

volume of waste generated, which, in the current work, was ten times lower than that observed in a previous paper [17]. This feature could be considered as an effective contribution for the environmental sustainability of the proposed procedure.

The procedures for both analytes resulted in overall performances similar to those achieved when employing procedures based on a multicommuted flow system. Furthermore, the volume of waste generated was lower than that delivered using the earlier flow analysis procedure. This represents an effective contribution to the reduction of pollution caused by an analytical procedure, thereby fulfilling the Green Analytical Chemistry requirement [10].

Useful features such as production of precise and accurate results, low limits of detection for both analytes, low volume of waste generated, and high throughput obtained allow us to conclude that the proposed system shows appropriate versatility and robustness for use in a laboratory devoted to large-scale analysis.

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# PREPARATION OF CERAMICS SPIKED WITH CADMIUM AND LEAD AS SYNTHETIC CALIBRATING MATERIAL

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**ABSTRACT**
Solid sampling techniques have been recommended for analysis of biological and inorganic materials, in order to simplify sample preparation procedures. Besides the several advantages, the main limiting steps of these techniques are the use of appropriate materials for the analytical calibration curve and sample microhomogeneity. Although aqueous reference solutions can be used for calibration, certified reference materials (CRM) are necessary in most cases. However, most commercially available CRMs are homogeneous only for sample mass sizes higher than 100-500 mg, making their use as calibrating material not recommended for microanalysis. Additionally, it is not trivial to find CRMs certified for different concentrations of one specific analyte.
The aim of this work is to propose the use of ceramics spiked with Cd and Pb reference solutions as a synthetic calibrating material for soil and sediment analysis, using solid sampling atomic absorption spectrometry (SS-GFAAS) as a tool for homogeneity investigation. After impregnation (0.5 to 50 mg $kg^{-1}$ for Cd and 10 to 1000 mg $kg^{-1}$ for Pb), each prepared standard had its homogeneity evaluated. The homogeneity factors ranged from 2.2 to 2.8 for Cd and from 5.0 to 13 for Pb. The analytical calibration curves were accomplished by increasing the mass of one calibrating standard and using four calibrating standards containing different concentration of Cd and Pb. Analysis of certified reference materials showed concentrations with no statistical differences at the 95 % confidence level, indicating that synthetic solid material based on spiking ceramics can be recommended for method calibration.

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**KEYWORDS:** synthetic calibrating material, microhomogeneity, solid sampling, environmental sample, microanalysis.

## INTRODUCTION

Solid sampling techniques have been recommended for analysis of biological and inorganic materials, in order to simplify sample preparation procedures and to avoid some inconveniences related to wet decomposition and/or dry ashing procedures. Many atomic spectrometric techniques have been proposed for qualitative and quantitative determinations of elements by direct solid analysis [1,2]. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) [3,4], laser induced breakdown spectrometry (LIBS) [5], X-ray fluorescence spectrometry (XFS) [6], electrothermal vaporization inductively coupled plasma optical emission spectrometry (ETV-ICP OES) [7], and solid sampling graphite furnace atomic absorption spectrometry (SS-GFAAS) [8,9] can be outstanding due to their main advantages: shortening of the sample preparation time; less sample contamination and analyte loss; low reagent consumption and residue generation, an important contribution for clean chemistry; applicability in semi-micro, micro or ultra-microanalysis [2,10-11]. On the other hand, the main limiting steps of solid sampling methods are the use of appropriate materials for construction of the analytical calibration curve and sample microhomogeneity.

Carrying out method calibration using standard aqueous solutions is the most common procedure. However, in solid sampling methods this is not always possible [2]. The behavior of matrix and analyte during the analytical process is determinant in choosing the best way to make the method calibration. Ideally, to overcome matrix interference, the calibrating material must have a composition identical to the sample material, both preferably having similar analyte content [1]. In these situations, certified reference materials (CRM) are the best option.

Aqueous calibrations have been successfully em-

ployed for direct solid analysis by SS-GFAAS [9,12-16]. However, the use of CRMs for method calibration is necessary for a lot of applications [9,15,17,18]. For LIBS, solid standards are imperative in the majority of applications [19]; thus, calibration free procedures have also been investigated [20].

Unfortunately, the practical application of CRMs for calibration presents some difficulties or restrictions. In the case of unusual types of materials, CRMs having equivalent matrix composition may not be available [2]. Beside this, most of the commercially available CRMs present the representativeness and homogeneity only when at least 100 to 500 mg of sample is used [17], their application becoming unsuitable for microanalysis. The National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) has already initiated the production of CRMs for microanalysis purposes. Marine sediment for solid sampling analytical techniques (SRM 2703) is certified for 0.7 mg of sample.

Although efforts have been directed to produce CRMs for microanalysis purposes, material available for microanalysis calibration practically does not exist. Considering the difficulties in finding these materials, many strategies have been adopted to calibrate the methods. Crystalline compounds of Ba, Cr and/or Pb were added to each matrix to calibrate LIBS for soil analysis [21]. Reference solutions applied directly on graphite pellets were used as calibrating materials for Cd determination in medicinal plants using solid sampling flame atomic absorption spectrometry (SS-FAAS) [22]. The determination of Cd in sulfide ores was performed by preparing a solid synthetic reference standard by intensive grinding of solid matrix components, such as FeS, ZnS, PbS, CuS, S with the analyte added as cadmium sulfide [23]. Synthetic cellulose calibrators spiked with appropriate amounts of analyte were used for the multielement analysis of vegetable samples by XFS [24] and by ETV-ICP OES [7]. Filter paper spiked with Cu and Zn was also used as synthetic calibrating material for plant material analysis by SS-GFAAS [25].

Thus, this work proposes to investigate the use of ceramics spiked with Cd and Pb as synthetic calibrating standards for direct environmental materials analyses. This paper deals with the preparation of calibrating standards and their application for direct microanalysis. SS-GFAAS was used as a tool to investigate the microhomogeneity of analyte distribution in the ceramics. Analysis of environmental CRMs was done to check the reliability of the prepared calibrating standards.

## Materials and methods

### Instrumentation

A ZEEnit® 60 atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) equipped with a transversely heated graphite atomizer, an inverse and transversal 2- and 3-field mode Zeeman-effect background corrector, an automatic auto sampler and a manual solid sampling accessory was used. Pyrolytic graphite tube atomizers and boat-type platforms were used throughout. Ceramics and environmental samples were weighed in an Auto Balance AD-4 microbalance (Perkin-Elmer, Norwalk, USA) with a precision of 0.001 mg. The spectrometer was operated with a hollow cathode lamp (applied current = 4.0 mA, spectral bandpass = 0.8 nm, magnetic field strength = 0.8 T) for Cd (wavelength = 222.8 and 326.1 nm) and for Pb (wavelength = 217.0 and 368.3 nm), using the heating program presented in Table I. All measurements were based on integrated absorbance values controlled by Windows NT® software. Argon (99.998 % v/v) (Air Liquid Brazil, São Paulo, Brazil) was used as protective and purge gases. Agata mortar and pestle were used to grind the ceramics and a 250 µm sieve was used to sieve the ground sample.

**Table I. Heating program for Cd and Pb determination by SS-GFAAS**

| Heating Program | | | | |
|---|---|---|---|---|
| Step | Temperature (°C) | Ramp (°C s$^{-1}$) | Hold (s) | Ar flow (L min-1) |
| Drying | 130 | 10 | 15 | 1 |
| Pyrolysis | 600[a], 900[b] | 100 | 20 | 1 |
| Atomization | 2000[a], 2200[b] | 2600 | 4 | 0 |
| Cleaning | 2600 | 1200 | 3 | 1 |

[a]Cd, [b]Pb; * Chemical Modifier: 5 µg Pd

### Reagents

High purity deionized water (resistivity = 18.2 MΩ cm) obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used throughout. Analytical reagent grade $HNO_3$ (Titrisol, Merck, Darmstadt, Germany) was distilled in quartz sub-boiling stills (Marconi, Piracicaba, SP, Brazil) and used for solution preparation. All solutions and samples were stored in decontaminated polypropylene bottles (Nalge Company, Rochester, NY, USA).

Analytical calibration solutions of Cd and Pb were prepared by suitable dilution of stock standard solutions containing 1000 mg l$^{-1}$ of $CdCl_2$ and $Pb(NO_3)_2$ (Titrisol, Merck, Darmstadt, Germany). Ceramics were used to make the synthetic calibrating standard through the quantitative adsorption of Cd and Pb. A solution containing 0.5 mg l$^{-1}$ Pd, prepared by successive dilution of stock solution (1000 mg l$^{-1}$ $Pd(NO_3)_2$ (Suprapur, Merck) was used as chemical modifier.

Certified reference materials were used to check the accuracy of synthetic calibrating material applicability: Marine Sediment (MESS-1 and BCSS-1), Sediment for Solid Sampling (Small Sample) Analytical Techniques (SRM 2703), Urban Particle Matter (SRM 1648a), New York/New Jersey Waterway Sediment (SRM 1944), Toronto Harbour sediment for trace analysis (TH-1 and TH-2), Harbour sediment (PACS-2), Portland cement (CP III).

The ceramics used in the preparation of the calibrating material were taken from a water treatment filter.

### Procedure

The ceramics were ground with mortar and pestle, sieved through a 250 µm sieve and cleaned by adding 1000 ml of 10 % v/v $HNO_3$ to 50 g of this material followed by agitation for 1 h. After that, the same procedure was adopted using deionized water, followed by being filtered and dried for 72 h in an oven at 80°C. The spiking of the analytes was performed by adding a volume of 40 ml of Cd/Pb solution (blank; 0.125/2.5; 1.25/25; 6.25/125 and 12.5/250 mg $l^{-1}$) in 0.1 % v/v $HNO_3$ to 10 g of ceramic powder. After 30 min of agitation, the ceramics were filtered and dried for 48 h in an oven at 60°C until constant mass.

To check the exact concentration of Cd and Pb adsorbed on the spiked ceramics, the calibrating standard was analyzed by slurry sampling GFAAS (SLS-GFAAS). The slurry was prepared by adding 20 mg of non-spiked (blank) and spiked ceramics into 5 ml of solution containing 0.5 % v/v $HNO_3$. Aliquots of 10 µl of slurry were inserted in the graphite furnace together with 10 µl of chemical modifier containing 0.5 g $l^{-1}$ Pd. The analysis was performed using the heating program presented in Table I using aqueous calibration.

### Homogeneity investigation

Solid sampling GFAAS is a good method to be used in the investigation of sample microhomogeneity: (1) it does not require sample pre-treatment, (2) it allows a sufficiently precise analysis of small samples (< 1 mg) and (3) it is fast [9,25].

The homogeneity factor ($H_e$) proposed by Kurfurst was estimated using the equation ($H_e = S_H * (m)^{½}$), in which $S_H$ is the relative sampling error that can be estimated directly from the overall experimental relative standard deviation, RSD, and *m* is the sample mass size (mg), of which 1 mg is commonly used. To estimate the calibrating standard homogeneity, 12 replicates of each standard were analyzed by SS-GFAAS using the heating program presented in Table I. Masses between 80 – 800 µg of spiked ceramics were used for Cd determination and 20 - 200 µg for Pb. When the homogeneity factor is lower than 10 ($H_e$ < 10), the material can be considered sufficiently homogeneous [26].

### Calibration

The calibration curves were made using two different approaches. The first was based on increasing masses of one calibrating standard. Masses from 30 to 300 µg of the calibrating standard, containing 0.44 ± 0.05 mg $kg^{-1}$ of Cd and 506 ± 21 mg $kg^{-1}$ of Pb were directly weighted onto the boat-type platform and inserted into the graphite furnace. The second was based on weighing similar masses (approximately 250 µg) of calibrating standards, containing different concentrations of Cd and Pb. Due to the difficulties in weighing exactly 250 µg, the ratio between absorbance and sample mass in mg (*A* / *m*) was used instead of absorbance values.

## Results and discussion

The concentrations of Cd and Pb in each calibrating material achieved after analysis by SLS-GFAAS are presented in Table II. The adsorption percentage varied from 70 to 98 %. Similar values of recovery were observed in previous studies [25].

**Table II. Percentages of adsorption and concentrations of Cd and Pb in ceramics by slurry sampling GFAAS**

| | Cd | | |
|---|---|---|---|
| | SLS-GFAAS (mg $kg^{-1}$) | Added mass (mg $kg^{-1}$) | Adsorption percentage (%) |
| Blank | 0.013 ± 0.007 | 0 | - |
| Standard 1 | 0.44 ± 0.05 | 0.5 | 85 |
| Standard 2 | 4.7 ± 0.2 | 5 | 94 |
| Standard 3 | 23.3 ± 1.1 | 25 | 93 |
| Standard 4 | 47.1 ± 1.8 | 50 | 94 |
| | **Pb** | | |
| | SLS-GFAAS (mg $kg^{-1}$) | Added mass (mg $kg^{-1}$) | Adsorption percentage (%) |
| Blank | 34 ± 2 | 0 | - |
| Standard 1 | 41 ± 4 | 10 | 70 |
| Standard 2 | 132 ± 9 | 100 | 98 |
| Standard 3 | 506 ± 21 | 500 | 94 |
| Standard 4 | 995 ± 36 | 1000 | 96 |

### Homogeneity investigation

Homogeneity is one of the most important prerequisites for a reference material, especially for small sub-sample analysis, as commonly practiced in direct solid sampling analysis. The results of the homogeneity investigation of Cd and Pb in spiked ceramics are presented in Table III. Sample mass sizes of approximately 40 µg to 700 µg were used to evaluate the Cd and Pb distribution, depending on their concentration.

Results showed that all standards presented homogeneity factors of about 10 ($H_E$ < 10) for Cd and Pb, indicating acceptable homogeneity for microanalysis.

**Table III. Homogeneity results for Cd and Pb in ceramics**

| | Cd | | Pb | |
|---|---|---|---|---|
| | m (mg) | $H_E$ | m (mg) | $H_E$ |
| Standard 1 | 0.662 ± 0.142 | 2.2 | 0.157 ± 0.049 | 13 |
| Standard 2 | 0.081 + 0.021 | 2.4 | 0.173 + 0.074 | 11 |
| Standard 3 | 0.085 + 0.026 | 2.6 | 0.101 + 0.091 | 5.0 |
| Standard 4 | 0.077 + 0.024 | 2.8 | 0.052 + 0.027 | 7.6 |

### Calibration

Analytical calibration curves for Cd and Pb made by increasing the mass of one calibrating standard are shown in Figures 1a (y = 1.942 ± 0.0386 x, $R^2$ = 0.9956 ± 0.0087) and 2a (y = 0.00222 ± 0.00004 x, $R^2$ = 0.9962 ± 0.0079) respectively. The analytical calibration curves using four calibrating standards containing different concentration of Cd and Pb are shown, respectively, in Figures 1b (y = 0.00883 ± 0.00006 x, $R^2$ = 0.9999 ± 0.0033) and 2b (y = 0.00190 ± 0.00006 x, $R^2$ = 0.9983 ± 0.0539) for Pb.

Analytical calibration curves were also performed with aqueous solutions that showed the same behaviors as those obtained using solid calibrating standards for Cd (y = 0.00900 ± 0.00009 x, $R^2$ = 0.9992 ± 0.0045) and for Pb (y = 0.00193 ± 0,000024 x, $R^2$ = 0.9991 ± 0.0033). In this specific case, the determinations of Cd and Pb by SS-GFAAS could be done using aqueous calibrations.

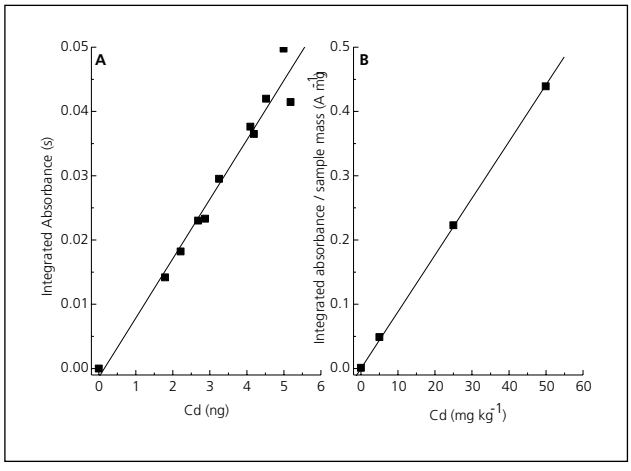


**Figure 1. Analytical calibration curves for Cd: (a) increasing sample mass of one calibrating standard, and (b) four calibrating standards containing different concentrations of Cd**

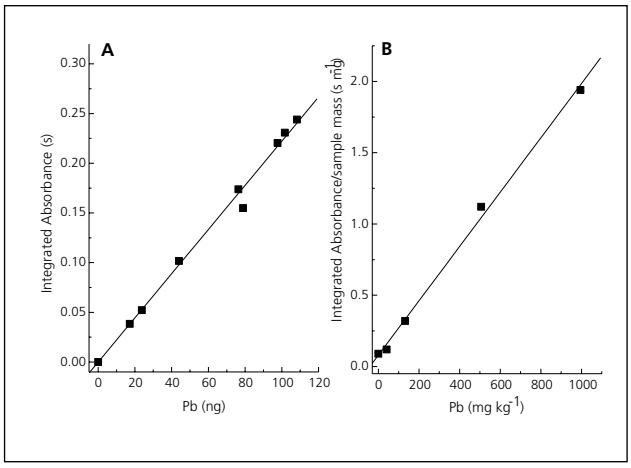


**Figure 2. Analytical calibration curves for Pb: (a) increasing sample mass of one calibrating standard, and (b) four calibrating standards containing different concentrations of Pb**

**Table IV. CRM analysis using ceramics impregnated with Cd and Pb standards for analytical calibration curves: 1). increasing sample mass of calibrating standard containing 0.44 ± 0.05 mg kg$^{-1}$ of Cd and 506 ± 21 mg kg$^{-1}$ of Pb; 2). similar sample masses of calibrating standards containing different concentration of Cd and Pb**

| | Cd (mg kg$^{-1}$) | | |
|---|---|---|---|
| | CRM | Certified value | Found Value |
| Type 1 | MESS-1 | 0.59 ± 0.10 | 0.25 ± 0.04 |
| | BCSS-1 | 0.25 ± 0.04 | 0.26 ± 0.03 |
| | SRM2703 | 0.811 ± 0.076 | 0.77 ± 0.04 |
| | CPIII* | 0.40 ± 0.01 | 0.35 ± 0.09 |
| Type 2 | SRM 1648a | 73.7 ± 2.3 | 79.9 ± 11.5 |
| | PACS 2 | 2.11 ± 0.10 | 2.3 ± 0.3 |
| | SRM 1944 | 8.8 ± 1.4 | 9.8 ± 1.8 |
| | TH-1 | 5.41 ± 1.83 | 6.2 ± 0.4 |
| | TH-2 | 5.22 ± 0.46 | 4.9 ± 0.6 |
| | Pb (mg kg$^{-1}$) | | |
| Type 1 | MESS-1 | 34.0 ± 6.1 | 35.1 ± 8.9 |
| | BCSS-1 | 22.7 ± 3.4 | 23.4 ± 1.3 |
| | TH-1 | 257 ± 69 | 267 ± 25 |
| | TH-2 | 194 ± 14 | 198 ± 22 |
| | PACS 2 | 183 ± 8 | 191 ± 29 |
| | SRM 1944 | 330 ± 48 | 341 ± 37 |
| Type 2 | TH-1 | 257 ± 69 | 246 ± 5 |
| | TH-2 | 194 ± 14 | 184 ± 16 |
| | PACS 2 | 183 ± 8 | 188 ± 20 |
| | SRM 1944 | 330 ± 48 | 301 ± 32 |

[a] Calibration type 1 (using increasing sample mass of one calibrating standard)

[b] Calibration type 2 (using four calibrating standards containing different concentration of Cd and Pb)

### Analysis of CRMs

The applicability of the proposed ceramics spiked with different concentration of Cd and Pb as a synthetic calibrating standard was checked by the analysis of environmental CRMs. The CRM results using calibration with increasing mass of one standard (type 1) and using four

standards (type 2) are shown in the Table IV. The values found are in agreement with the certified concentrations at the 95 % confidence level (student t-test).

## Conclusion

The practical application of CRMs for method calibration presents some difficulties or restrictions. Most of the commercially available CRMs present representativeness and homogeneity when using at least 100 to 500 mg of sample, their application becoming unsuitable for microanalysis purposes. Moreover, the availability of matrices containing a variety of analyte concentrations is a real necessity for some methods. In these cases, the use of synthetic calibrating material is a good alternative.

Thus, the preparation of a synthetic solid calibrating material has been proposed. It is believed that the success of applying ceramics spiked with Cd and Pb for environmental sample analysis is due to the similarity of sample and calibrating material matrix. Both have silicate in their composition. The homogeneity investigation showed that the standards produced present homogeneity factors of about 10, indicating they are sufficiently homogeneous to be applied in microanalysis.

This is an initial investigation of the production of synthetic calibrating standards for direct solid sampling microanalysis. The homogeneity study suggests that this material could be employed in other solid sampling analytical methods such as LA-ICP-MS, LA-ICP OES and LIBS, among others. These tests will be presented in the near future.

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# SIMPLE AND FAST METHOD FOR THE DETERMINATION OF NA AND K IN RAW GLYCERIN FROM BIODIESEL PRODUCTION BY FLAME ATOMIC EMISSION SPECTROMETRY

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**ABSTRACT**
A simple and fast method for the determination of Na and K in raw glycerin samples by flame atomic emission spectrometry is proposed. The sample is prepared by a simple dilution with 30% n-propanol in water. Hydrochloric acid and Cs are also added to the measuring solution. Flame atomic emission spectrometry was used for quantification. Calibration was carried out against aqueous standard solutions in the same medium as for sample preparation. The linear correlation coefficients of the calibration curves were higher than 0.998. The limits of quantification were 0.08 mg $g^{-1}$ and 0.02 mg $g^{-1}$ for K and Na, respectively. Six raw glycerin samples were analyzed and the results obtained were in the range from < 0.08 mg $g^{-1}$ to 92 ± 7 mg $g^{-1}$ for K and in the range from 0.36 ± 0.03 mg $g^{-1}$ to 19 ± 3 mg $g^{-1}$ for Na. Accuracy was assured by comparing the results with those obtained after acid digestion of the samples in a microwave oven. The concentrations obtained could indicate the catalyst used in the biodiesel production.

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**KEYWORDS**: Sodium and potassium; Raw glycerin; Flame atomic emission spectrometry; Biodiesel; Dilution with n-propanol

## 1. INTRODUCTION

The need for energy in our industrialized world and the pollution problems related to petroleum fuel consumption, have promoted the development of renewable energy sources that result in less environmental impact [1]. Gasoline and diesel obtained from petroleum are major emission sources of gases responsible for the greenhouse effect. In addition, the high consumption of fuels and the corresponding enormous extracted volumes of petroleum along the years have resulted in a significant reduction of the available sources. Because of these facts, several biofuels are now produced from renewable sources, such as biomass, biogas, ethanol and vegetable oils [2].

Produced from vegetable oils or from animal fat, biodiesel is a biodegradable non-toxic fuel, with low emission of pollutants during its combustion [3]. The production process usually involves the transterification of the vegetable oil or animal fat, catalyzed by a strong acid or base in alcoholic medium, giving alkyl esters derived from fatty acids and glycerin, as a co-product [4-6]. The vegetable and animal oils and fats are composed of triacylglycerides that have fatty acids with long chains linked to the glycerol molecule with an ester bond [7].

By the definition adopted by the Programa Brasileiro de Biodiesel: "biodiesel is the fuel obtained by the mixture, in different proportions, of diesel and alkyl esters of vegetable oil or animal fat" [8]. According to the Agência Nacional do Petróleo, Gás Natural e Biocombustíveis (ANP), Resolution nº 7, from 19.3.2008, biodiesel (B100) is defined as the fuel composed of alkyl esters from long chain fatty acids derived from vegetable oil or animal fat", and biodiesel (B-X) as the commercial fuel composed of (100-X) % v/v of diesel oil and X% v/v of biodiesel (B100) [8]. Since 2005, biodiesel has been added to diesel. Currently, according to ANP, LEI nº 11.097 of 2005, the minimum percentage of biodiesel in diesel is 5% v/v, required for all diesel fuel commercialized in Brazil [9].

About 10% w/w of the total product obtained in the transesterification reaction that leads to the biodiesel formation is glycerin [10]. Glycerin may be used in several products, mainly in the pharmaceutical industry, in products for mouth hygiene, and for processing tobacco and urethane foams. Its use in the

food, drug and cosmetics industries has increased [11]. However, its use and cost depend on its purity, usually higher than 95%. Raw glycerin has a price about 3 times lower than bidistilled glycerin and, on the average, 500 times lower than pharmaceutical glycerin [10,11]. The glycerin used in the pharmaceutical industry is of high purity, usually higher than 99.5% and is more expensive than the others. Raw glycerin has a purity of about 70%. The main impurities, such as catalyst, fatty acids and alcohol, originate from the biodiesel production process and the raw material and catalysis type used [10,12].

The chemical characterization of glycerin was studied by Thompson and He [13], who have determined the concentrations of the macroelements in different glycerin samples. The obtained results show that the average content of carbon is 25% w/w and the concentrations of Ca, K, Mg, P, S were in the range 4-163 ppm. Sodium was more concentrated, around 1% w/w, because sodium methylate was used as catalyst [13], indicating that part of it remains in the glycerin phase. This means that the control of Na and K concentrations in glycerin is important, as their hydroxides are frequently used during biodiesel production.

Alkaline metals are easily and efficiently atomized in flames. Flame atomic emission spectrometry (FAES) is of low cost and easily performed, being widely used especially for Na and K. After forming a microemulsion, Na and K in biodiesel have been determined by FAES [14]. It seems that this technique could also be applied to raw glycerin samples, after being submitted to a convenient sample preparation procedure. The objective of this work is the development of a method for the determination of Na and K in glycerin by FAES, after dilution with n-propanol. To the best of our knowledge the procedure has not been proposed before.

## 2. Experimental

### 2.1. Instrumentation

The measurements were carried out in an atomic absorption spectrometer, model AAnalyst 100 (Perkin Elmer, Norwalk, CT, USA), in the flame emission mode. The wavelengths were 766.5 nm for K and 589 nm for Na and a 0.2 nm spectral slit was used for both analytes. The gas flame mixture contained 6.0 L $min^{-1}$ air and 3.0 L $min^{-1}$ acetylene (99.6%, from White Martins, São Paulo, SP, Brazil). The analytical signals were evaluated by the peak height. The sample was aspirated by a pneumatic nebulizer, through a 30 cm long PTFE capillary. For sample digestion, a microwave oven, Milestone model MLS 1200 mega (Sorisole, Italy), was employed.

The surface tension was measured with a tensiometer (Kruss GmbH) model K 8 equipped with a Pt-Ir-20 ring and a thermostat for the sample flask kept at 25.0 ± 0.1°C. The viscosity was measured with a viscosimeter (Shott AVS 350) coupled to a capillary tube (Cannon-Fenske nº75), using a solvent flow time of about 129 s, in a thermostated bath (Schott CT 52) kept at 25.0 ± 0.1°C.

### 2.2. Reagents and samples

All reagents were of analytical grade. Water was distilled and deionized (to a resistivity of 18.2 MΩ cm) in a Milli-Q Plus Millipore system (Bedford, MA, USA). Hydrochloric acid (Merck, Darmstadt, Germany) and nitric acid (Carlo Erba, Milan, Italy) were sub-boiling distilled in a quartz distiller (Kürner Analysentechnik, Rosenheim, Germany). Cesium chloride (Merck) was used as ionization suppressor. n-Propanol (99.5%) was from Nuclear (São Paulo, Brazil). 1000 µg $mL^{-1}$ standard solutions of K and Na (Merck), hydrogen peroxide (Merck) and glycerin (99.5%) (Nuclear) were also used.

Six raw glycerin samples, co-products of biodiesel production by the alkaline catalysis process, were analyzed. The samples were obtained by the transesterification of different oils: samples A, B, E and F from residual vegetable oils from frying and samples C and D from commercial soy bean oils.

### 2.3. Sample preparation

An aliquot of about 0.5 g of raw glycerin was weighed in a 50 ml PTFE flask and the volume was made up with deionized water. A clear yellowish and alkaline solution (pH > 10) was obtained. This dilution is necessary because the raw glycerin samples contain large amounts of the catalyst used in the production of biodiesel. From this solution, aliquots of 20 to 1000 µL were withdrawn and diluted to 10 mL, in order to have a final measuring solution containing 30% v/v n-propanol, 1% v/v HCl and 0.2% w/v Cs in water. For glycerin diluted only in water, the addition of hydrochloric acid precipitates fatty acids, justifying the use of a co-solvent such as n-propanol, to guarantee total solubilization of the sample. Calibration solutions contained 30% v/v n-propanol, 1% v/v HCl and 0.2% w/v Cs in water. Cesium was added in order to reduce the ionization of analytes in the air/acetylene flame.

In order to verify the accuracy of the proposed method, the results were compared with those obtained after microwave-assisted sample digestion. An aliquot of 0.5 g of glycerin was mixed with 4 mL of nitric acid and 2 mL of hydrogen peroxide and submitted to the following power program: 250 W for 2 min, 0 W for 2 min, 250 W for 6 min, 400 W for 5 min and 600 W for 5 min. These conditions were

based on those given in the manual of the microwave oven. The clear sample solution, after digestion, was properly diluted to 20 mL with deionized water in a PTFE flask before being measured. Calibration was carried out against aqueous standard solutions, containing 0.2% w/v Cs.

## 3. Results and discussion

Due to the presence of impurities, such as soaps and fatty acids, the determination os Na and K in glycerin requires a specific sample treatment, as acids cannot be used when the sample is directly diluted in water. The addition of acids would turn the soaps insoluble due to hydrolysis. The addition of n-propanol completely dissolves the raw glycerin, even after the addition of an acid. However the addition of an organic solvent, either pure or mixed with water, may dramatically change the physical properties of the solution and can cause interference during the analysis.

### 3.1. Solvent for the sample

Glycerin samples can be dissolved in water containing different proportions of n-propanol. Pneumatic nebulization is one of the techniques most used for sample introduction in atomic spectrometry. During nebulization, an aerosol with poly-dispersed droplets (primary aerosol) is formed. The physical properties of the solution have a great influence on the aerosol formation and on the transport of the analyte and of the solvent to the flame, when atomic absorption spectrometry is used [15]. The signal intensity is determined by the tertiary aerosol, which reaches the flame and is more homogeneous and less dispersed. Organic solvents, pure or mixed with water, are frequently used in atomic spectrometry, to separate interferents, concentrate the analytes or increase the signal intensity [15]. Viscosity, surface tension and solvent volatility have a great influence on the pneumatic nebulization process, for organic solvents, pure or mixed with water. In general, the average particle size produced in aerosol formation decreases as the volatility of the solvent increases [15]. The addition of n-propanol to an aqueous solution chances the physical properties of the medium, especially the viscosity and the surface tension. Different proportions of n-propanol were added to water and the viscosity and the surface tension of the resultant solutions were measured and are shown in Figure 1. This Figure also show the Na and K signal intensities for 0.5 mg $L^{-1}$ in different n-propanol concentrations.

As the concentration of n-propanol increases, the surface tension decreases and the viscosity increases. Concerning the viscosity, lower signal intensity would

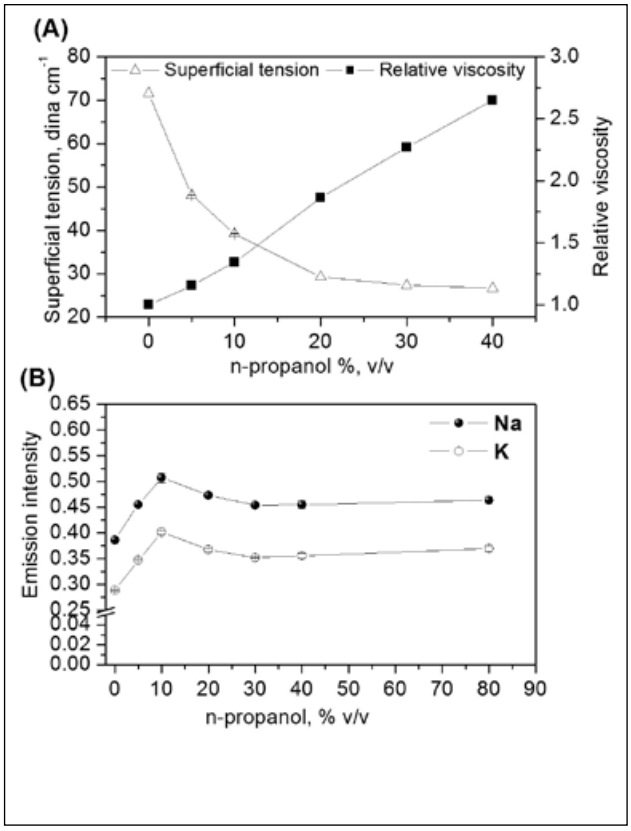


**Figure 1. Effect of n-propanol (A) on the viscosity and surface tension of water (25.0 ± 0.1 °C) and (B) on Na and K signal emission intensity for a solution containing 0.5 mg $L^{-1}$ of the analytes.**

be expected for higher n-propanol concentrations, as lesser amounts of sample would reach the flame. However, for both analytes, the signal intensity increases as the n-propanol concentrations increases (Figure 1B) up to 10% v/v n-propanol. For higher n-propanol concentrations, the signal intensity decreases up to 30% v/v n-propanol, remaining almost constant for higher concentrations of the solvent. Probably, the surface tension is the predominant effect up to 10% v/v n-propanol. The decrease in the signal intensity from 10% to 30% v/v n-propanol is probably due to the increase in viscosity. For concentrations higher than 30% v/v n-propanol, the signal intensity remains constant, independent of the solvent concentration. As the surface tension decreases, more droplets of smaller size are formed and less of the sample is eliminated by drop condensation in the nebulization chamber. By adopting a mixture of n-propanol (30% v/v) as the solvent, the sensitivities were increased for both elements (21% for Na and 30% for K), when compared to water only. Higher sensitivities would be obtained by using 10% v/v n-propanol. However, the samples do not dissolve efficiently in that mixture. The calibration was carried out with aqueous standard solutions

containing the same proportion of n-propanol as in the sample solution.

### 3.2 Stability of Na and K in the solvent

The final glycerin solution is an aqueous solution containing 30% v/v n-propanol, 1% v/v HCl, 0.2% Cs w/v and the glycerin aliquot. After preparing the raw glycerin solution in a polypropylene flask, the Na and K signal intensities were measured every 10 min up to 120 min. For comparison purpose, the signal intensities were measured for an aqueous solution containing 0.5 mg $L^{-1}$ of Na and K. As shown in Figure 2, the analytes were stable in the medium of 30% v/v n-propanol during the investigated period of time.

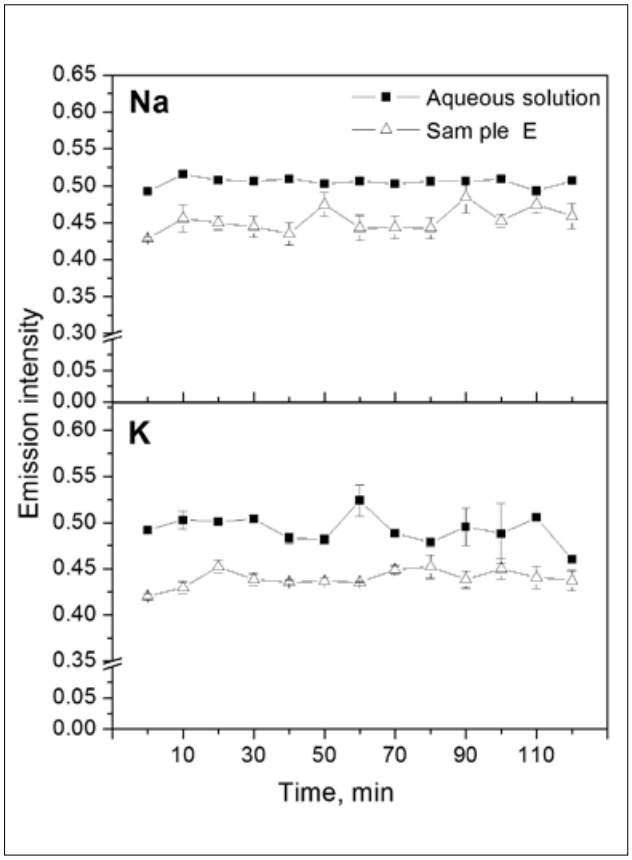


**Figure 2. Signal intensity stability for Na and K: (-■-) raw glycerin diluted with 30% v/v n-propanol in water and (-Δ-) aqueous standards containing 0.5 mg $L^{-1}$ of Na and K.**

### 3.3 Figures of merit

The figures of merit are shown in Table I for both analytes. For comparison purposes, the slopes of the calibration curves using aqueous standards are also presented. It is shown that the signal intensities increase in the presence of 30% v/v n-propanol, probably due to the lower surface tension of the measuring solution, as already discussed. The proposed method requires calibration in 30% v/v n-propanol, also containing 1% v/v HCl and 0.2% w/v Cs, as in the samples solutions.

**Table I. Figures of merit for the determination of Na and K in raw glycerin by FAES, after dilution with 30% v/v n-propanol in water, n=3.**

| | Na | | K | |
|---|---|---|---|---|
| Calibration (0.1 – 1.0 mg $L^{-1}$) | In water | 30% v/v n-propanol | In water | 30% v/v n-propanol |
| Slope (L $mg^{-1}$) | 0.399 | 0.505 | 0.417 | 0.600 |
| R | 0.9992 | 0.9998 | 0.9990 | 0.9984 |
| LOQ* (mg $g^{-1}$) | 0.07 | 0.02 | 0.1 | 0.08 |
| RDS (n=3) % | 5 | 3 | 5 | 6 |

* LOQ values for 500 mg of sample diluted 1000 times (in two steps).

Good linear correlation coefficients, R>0.998, were obtained, especially considering the flame emission technique used is usually of reduced linearity. The precision, estimated by the relative standard deviation (RSD), was below 6%, being appropriate for the analysis of raw glycerin. The limit of detection (LOD) was defined as 3 times the standard deviation of 10 measurements of the blank divided by the curve slope. The limit of quantification (LOQ) was taken as 3.3 times the LOD. For the sample, the LOQ depends on the dilution, the values for the adopted procedure being: 0.02 mg $g^{-1}$ for Na and 0.08 mg $g^{-1}$ for K, considering 500 mg of the sample diluted 1000 times (in two steps), which was adequate for raw glycerin samples produced by alkaline catalysis.

### 3.4 Accuracy validation

As no certified reference material for glycerin is available, the accuracy was checked by comparing the results obtained after total acid digestion in a microwave oven with those obtained after dilution with 30% v/v n-propanol. Calibration in the same medium, according to the preparation procedure, was carried out. The analysis of three raw glycerin samples, produced by using alkaline catalysis, lead to the results shown in Table II, which are in agreement, according to the student-t test for a 95% confidence level, except for one result. In sample A, the concentration of K was below the quantification limit and the comparison is not conclusive for this case. This experiment demonstrates that the proposed method leads to accurate results.

**Table II. Na and K concentrations (average ± confidence interval, n=3) in raw glycerin samples, A, C and E, diluted with 30% v/v n-propanol in water or acid digested in a microwave oven.**

| Sample | Na (mg g$^{-1}$) | | K (mg g$^{-1}$) | |
|---|---|---|---|---|
| | Acid digested | Diluted 30% v/v n-propanol | Acid digested | Diluted 30% v/v n-propanol |
| A | 17 ± 2 | 19 ± 3 | 0.08 ± 0.01 | < 0.08 |
| C | 0.33 ± 0.06 | 0.36 ± 0.03 | 30 ± 2 | 29 ± 6 |
| E | 0.69 ± 0.05 | 0.70 ± 0.03 | 30 ± 6 | 27 ± 3 |

### 3.5 Analytical Application

Raw glycerin samples were produced by alkaline catalysis using Na and K hydroxides. Four of the six analyzed samples were obtained by transterification of residual vegetable frying oil of unknown origin and two samples were produced from industrialized soy bean oils. The raw glycerin samples were analyzed following the proposed procedure, that is, sample dilution in 30% v/v n-propanol, calibration in this same medium and detection by FAES, using Cs as ionization suppressor. The Na and K concentrations obtained are shown in Table III.

**Table III. Na and K concentrations (average ± confidence interval; n=3) in raw glycerin samples obtained from biodiesel production using NaOH or KOH as catalysts.**

| Sample | Na (mg g$^{-1}$) | K (mg g$^{-1}$) |
|---|---|---|
| A | 19 ± 3 | < 0.08 |
| B | 0.48 ± 0.06 | 33 ± 2 |
| C | 0.36 ± 0.03 | 29 ± 6 |
| D | 16 ± 1 | < 0.08 |
| E | 0.70 ± 0.03 | 27 ± 3 |
| F | 10 ± 1 | 92 ± 7 |

The two metal concentrations show great variations in the different samples. The Na concentrations varied from 0.7 mg g$^{-1}$ to 16.3 mg g$^{-1}$. For K, the lowest concentrations were below the quantification limit, but above the detection limit. The highest K concentration was 92.4 mg g$^{-1}$. The relatively high concentrations of Na and K in sample F, may be due to the material used, residual oil from frying. For the other samples, the catalyst used in the biodiesel production, if sodium or potassium hydroxide, seems to control the residual Na or K concentrations in the raw glycerin, even for the ones produced from frying oils. It is clear that samples A and D were produced using sodium hydroxide, while samples B, C and E used potassium hydroxide in their production. The high K concentration in sample F indicates that potassium hydroxide was used for producing this sample, even if the Na concentration is also relatively high, probably due to the frying oil used as raw material.

## 4. Conclusions

The determination and control of residual Na and K concentrations in glycerin is very important to allow its application as a component of different products. The proposed analytical procedure, which requires a simple sample dilution in 30% v/v n-propanol prior to determination by FAES, using calibration standards solutions prepared in the same medium, is easy, accurate and requires common non-expensive instrumentation. The procedure can be easily applied to routine analysis of raw glycerin.

## Acknowledgements

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# Non-targeted screening and accurate mass confirmation of 510 pesticides on a high resolution LC-MS Orbitrap system

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**Abstract**
As agricultural trade grows and food safety concerns mount, stricter pesticide regulations are being enforced around the world. Increased pesticide testing and reductions in maximum permissible residue levels have driven demand for fast, sensitive and cost-effective analytical methods for high-throughput screening of multi-class pesticides in food. Detection of 510 pesticides at low ppb levels was achieved within 12 minutes using a benchtop LC-MS system powered by Orbitrap technology. The high resolving power of the Orbitrap platform enabled accurate mass confirmation of all compounds, including isobaric pesticides. LOQs for the majority of pesticides in a standard mixture and in spiked matrix were lower than the maximum residue levels established by the EU and Japan. The method developed is suitable for the routine monitoring of targeted and non-targeted pesticides by regulatory laboratories.

**Keywords:** Pesticide Analysis, High Resolution MS, High Mass Accuracy, Orbitrap Technology

## Introduction

In 2007, the United States Environmental Protection Agency (EPA) completed a ten-year reassessment of 9721 pesticide tolerances to meet more stringent safety standards and recommended the revocation or modification of thousands of uses of pesticides in food [1]. China published national standard GB 2763-2005 in 2005, which established 478 maximum residue levels (MRLs) for 136 pesticides [2]. Japan's Positive List System, introduced in 2006, established MRLs for hundreds of agricultural chemicals, including approximately 400 pesticides, in food and set a uniform limit of 10 ppb to chemicals for which MRLs have not been determined [3]. Regulation (EC) No. 396/2005 of the European Parliament, implemented in 2008, harmonized all pesticide MRLs for European Union (EU) member states and set default limits of 0.01 mg/kg for all pesticide/commodity combinations for which no MRLs have been set [4]. A pesticide safety review of about 1000 active substances on the market was mandated by EU Directive 91/414/EEC and, upon completion in 2009, led to the approval of only about 250 substances, effectively setting the permissible levels of over 700 de-listed pesticides to the default limit [5]. The EU and Japanese regulations are among the most stringent in the world and have fueled the need for faster and more sensitive analytical methods for cost-efficient, high-throughput screening of multi-class pesticide residues.

Pesticides in food were traditionally monitored and quantified using gas chromatography (GC) coupled with either selective detectors (e.g., electron capture) or mass spectrometry (MS). GC-MS continues to be widely used in pesticide analysis because it is highly selective, provides confirmation of multiple classes of pesticides in a single analytical run, and is relatively inexpensive and easy to operate. However, GC-MS cannot detect polar, thermally unstable or low volatility compounds without derivatization. Recent improvements in liquid chromatography (LC) throughput and MS detection capabilities have led to a surge in the use of LC-MS-based techniques for screening, confirmation and quantitation of ultra-trace levels of multi-class pesticide residues, including those that are not GC-amenable. LC-triple quadrupole tandem MS (LC-MS/MS) enables highly selective and sensitive quantification and confirmation of hundreds of target pesticides in a single run, but this approach requires extensive compound-dependent parameter optimization and cannot be used to screen for untargeted pesticides. Full scan approaches using high performance time-of-flight (TOF) or Orbitrap mass spectrometers coupled to ultra-high pressure LC (UHPLC) facilitate rapid and sensi-

tive screening and detection of LC-amenable pesticide residues present in a sample [6]. The superior resolving power of the Orbitrap mass spectrometer (up to 100 000 FWHM) compared to TOF instruments (10 000–20 000) ensures the high mass accuracy required for complex sample analysis and enables confident discrimination of co-eluting, isobaric compounds [7, 8]. A wide in-scan dynamic range (3-4 orders of magnitude) facilitates the detection of trace levels of compounds in the presence of highly abundant matrix interferences. High scan speeds and polarity switching ensure full compatibility with UHPLC and high-throughput methods.

In this work, a UHPLC system coupled to a high resolution benchtop Orbitrap mass spectrometer was used and the rapid screening and accurate mass confirmation of 510 pesticides at low ppb levels was achieved [9-12]. Full scan UHPLC-single stage Orbitrap MS can be used to screen a virtually limitless number of pesticides and, unlike MS/MS methods, does not require compound-dependent parameter optimization.

## Materials and Methods

### Sample Preparation

Pesticide standards were obtained from the U.S. Food and Drug Administration (FDA). A stock solution of a mixture of 510 pesticides was prepared at a concentration of 3 mg/L. Calibration solutions, with concentrations of 1-250 µg/L, were prepared by serial dilution of the stock solution in 50:50 (v/v) acetonitrile/water. Spiked spinach samples were prepared for analysis using a modified QuEChERS method (Figure 1). QuEChERS, an acronym for Quick, Easy, Cheap, Effective, Rugged, and Safe, is a sample preparation procedure used to extract pesticides from food [13]. Malathion D6 was used as an internal standard for calibration.

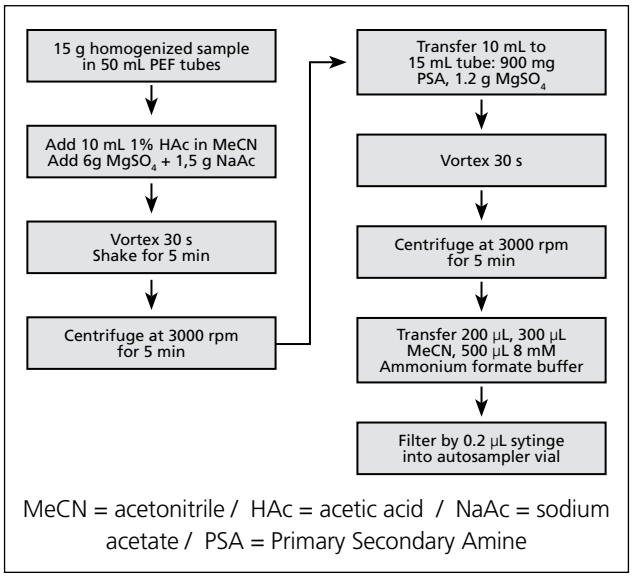


**Figure 1. Schematic of the modified QuEChERS workflow used to extract pesticides from spinach matrices**

## Experimental Conditions

### Instrumentation

LC-MS analysis was performed using a Thermo Scientific Accela U-HPLC system with a CTC Analytics PAL autosampler coupled to an Exactive benchtop Orbitrap mass spectrometer. Data acquisition was performed using Thermo Scientific Xcalibur software. Thermo Scientific Pathfinder software was used for data processing.

**Table I. LC-MS Parameters**

| | | | |
|---|---|---|---|
| Column: | Thermo Scientific Hypersil GOLD aQ C18 column (100 x 2.1 mm, 1.9 µm particle size) | | |
| Mobile Phase: | A: Water with 0.1% formic acid and 4 m mol/L ammonium formate<br>B: Methanol with 0.1% formic acid and 4 m mol/L ammonium formate | | |
| Flow Rate: | 300 µL/min | | |
| Column Temperature: | Ambient | | |
| Sample Injection Volume: | 10 µL | | |
| Gradient: | Time (min) | %A | %B |
| | 0 | 100 | 0 |
| | 1 | 100 | 0 |
| | 8 | 0 | 100 |
| | 12 | 0 | 100 |
| | 12.5 | 100 | 0 |
| | 14 | 100 | 0 |
| Full mass scan positive/negative ion mode (mass range = 100 to 1500) | | | |
| Resolution: | 50 000 | | |
| Automatic Gain Control (AGC) Target Value: | 10e6 | | |
| Heated Electrospray Ionization Source Conditions: | | | |
| Spray Voltage: | 2200 V | | |
| Capillary Temperature: | 280 °C | | |
| Sheath Gas: | 32 au | | |
| Auxiliary Gas: | 7 au | | |
| Vaporizer Temperature: | 200 °C | | |

## Results and Discussion

UHPLC improves chromatographic resolution, speed and sensitivity, and when coupled to MS, facilitates rapid, high-throughput analysis of challenging samples. Using UHPLC-single stage Orbitrap MS, a mixture of 510 pesticides representing a broad spectrum of chemical classes was separated and detected within 12 minutes (Table II). High resolution (50 000) and high mass accuracy (< 5 ppm without internal calibration for most compounds) enabled identification of all analytes (Table II). Separation of isobaric pesticides was achieved only at the high resolving powers provided by Orbitrap MS, as demonstrated in Figure 2.

Good linearity in detector response was observed over the range of 1-250 µg/L, with correlation coefficients greater than 0.99 for the majority of pesticides (Table II).

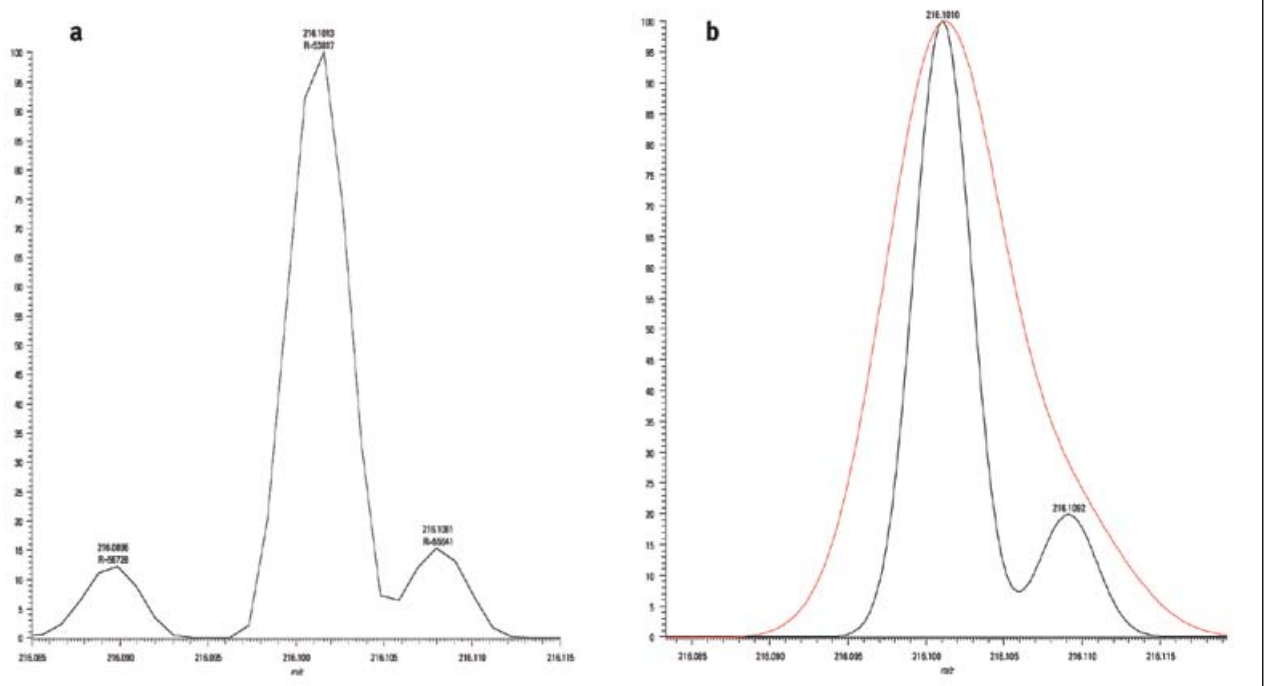


**Figure 2. The high resolving power of the benchtop Orbitrap mass spectrometer enabled separation of the [M+H]+ ion of atrazine (m/z = 216.1012) from the $[M_{+NH4}]$+ ion of cymoxanil (m/z = 216.1088). (a) Mass spectra of the two isobaric pesticides at a resolution of 50 000. (b) Simulated mass spectra of the isobaric pesticides at resolutions of 25 000 (red line) and 50 000 (black line).**

**Table II. Molecular formula, MS polarity mode, theoretical and experimental mass, mass deviation, limit of quantitation (LOQ) and correlation coefficients ($R^2$) data for 510 Pesticide Standards**

| Compound | Formula | Polarity | Theoretical Mass (*m/z*) | Experimental Mass (*m/z*) | Mass Deviation (ppm) | LOQ (µg/L) | R2 |
|---|---|---|---|---|---|---|---|
| Abamectin B1a | C48H72O14 | + | 890.526 | 890.5261 | 0 | 10 | 0.9898 |
| Abamectin B1b | C47H70O14 | + | 876.5104 | 876.5138 | 3.8 | 10 | 0.9315 |
| Acephate | C4H10NO3PS | + | 184.0192 | 184.0193 | 0.8 | 1 | 0.9994 |
| Acequinocyl | C24H32O4 | + | 402.2639 | 402.2638 | 0.2 | 1 | 0.9886 |
| Acetamiprid | C10H11ClN4 | + | 223.0745 | 223.0747 | 0.7 | 1 | 0.9989 |
| Acibenzolar S-methyl | C8H6N2OS2 | + | 210.9994 | 211.0004 | 4.4 | 10 | 0.9936 |
| Acifluorfen | C14H7ClF3NO5 | - | 359.9892 | 359.9896 | 1.1 | 1 | 0.9961 |
| Aclonifen | C12H9ClN2O3 | + | 282.064 | 282.065 | 3.6 | 25 | 0.9812 |
| Acrinathrin | C26H21F6NO5 | + | 559.1662 | 559.1664 | 0.3 | 1 | 0.9931 |
| Akton | C12H14Cl3O3PS | + | 374.954 | 374.9536 | 1 | 25 | 0.9859 |
| Alachlor | C14H20ClNO2 | + | 270.1255 | 270.1255 | 0.3 | 1 | 0.9890 |
| Alanycarb | C17H25N3O4S2 | + | 400.1359 | 400.1369 | 2.5 | 1 | 0.9049 |
| Aldicarb | C7H14N2O2S | + | 208.1114 | 208.1116 | 0.6 | 1 | 0.9989 |
| Aldicarb sulfone | C7H14N2O4S | + | 223.0747 | 223.0747 | 0.3 | 1 | 0.9987 |
| Aldicarb sulfoxide | C7H14N2O3S | + | 207.0798 | 207.0798 | 0.1 | 1 | 0.9998 |
| Allethrin | C19H26O3 | + | 303.1955 | 303.1957 | 0.6 | 1 | 0.9983 |
| Allidochlor | C8H12ClNO | + | 174.068 | 174.068 | 0 | 1 | 0.9936 |

| Compound | Formula | Polarity | Theoretical Mass (m/z) | Experimental Mass (m/z) | Mass Deviation (ppm) | LOQ (µg/L) | R2 |
|---|---|---|---|---|---|---|---|
| Ametryn | C9H17N5S | + | 228.1277 | 228.1278 | 0.4 | 1 | 0.9979 |
| Amicarbazone | C10H19N5O2 | + | 242.1612 | 242.1612 | 0.1 | 1 | 0.9986 |
| Aminocarb | C11H16N2O2 | + | 209.1285 | 209.1285 | 0.3 | 1 | 0.9997 |
| Aminopyralid | C6H4Cl2N2O2 | - | 204.9577 | 204.9571 | 2.9 | 1 | 0.9629 |
| Amitraz | C19H23N3 | + | 294.1965 | 294.1965 | 0.1 | 1 | 0.9725 |
| Ancymidol | C15H16N2O2 | + | 257.1285 | 257.1284 | 0.4 | 1 | 0.9954 |
| Anilazine | C9H5Cl3N4 | - | 272.9507 | 272.9572 | 2.4 | 1 | 0.9653 |
| Anilofos | C13H19ClNO3PS2 | + | 368.0305 | 368.0304 | 0.3 | 1 | 0.9986 |
| Anilofos | C13H19ClNO3PS2 | + | 368.0305 | 368.0304 | 0.3 | 1 | 0.9971 |
| Antimycin A | C28H40N2O9 | - | 547.2661 | 547.2668 | 1.2 | 1 | 0.9928 |
| Aramite | C15H23ClO4S | + | 352.1344 | 352.1345 | 0.4 | 1 | 0.9946 |
| Aspon | C12H28O5P2S2 | + | 379.0926 | 379.0927 | 0.1 | 1 | 0.9853 |
| Asulam | C8H10N2O4S | + | 248.07 | 248.07 | 0 | 1 | 0.9986 |
| Atrazine | C8H14ClN5 | + | 216.1011 | 216.1012 | 0.8 | 1 | 0.9991 |
| Azaconazole | C12H11Cl2N3O2 | + | 300.0301 | 300.0302 | 0.1 | 1 | 0.9940 |
| Azadirachtin | C35H44O16 | + | 738.2968 | 738.2968 | 0 | 1 | 0.9904 |
| Azafenidrin | C15H13Cl2N3O2 | + | 338.0458 | 338.0458 | 0 | 1 | 0.9932 |
| Azamethiphos | C9H10ClN2O5PS | + | 324.9809 | 324.981 | 0.2 | 1 | 0.9991 |
| Azinphos methyl oxon | C10H12N3O4PS | + | 302.0359 | 302.0359 | 0.1 | 1 | 0.9969 |
| Azinphos-ethyl | C12H16N3O3PS2 | + | 346.0444 | 346.0443 | 0.1 | 1 | 0.9906 |
| Azinphos-methyl | C10H12N3O3PS2 | + | 318.0131 | 318.0129 | 0.3 | 1 | 0.9957 |
| Azoxystrobin | C22H17N3O5 | + | 404.1241 | 404.124 | 0.2 | 1 | 0.9948 |
| Barban | C11H9Cl2NO2 | + | 275.0349 | 275.0355 | 2.4 | 10 | 0.9953 |
| Benalaxyl | C20H23NO3 | + | 326.1751 | 326.175 | 0.4 | 1 | 0.9986 |
| Benazolin | C9H6ClNO3S | + | 243.983 | 243.9827 | 1.2 | 1 | 0.9863 |
| Bendiocarb | C11H13NO4 | + | 224.0917 | 224.0919 | 0.8 | 1 | 0.9993 |
| Benfluralin | C13H16F3N3O4 | + | 353.1431 | 353.143 | 0.2 | 1 | 0.9883 |
| Benfuracarb | C20H30N2O5S | + | 428.2214 | 428.2211 | 0.6 | 1 | 0.9956 |
| Benodanil | C13H10INO | + | 323.988 | 323.9879 | 0.4 | 1 | 0.9925 |
| Benoxacor | C11H11Cl2NO2 | + | 260.024 | 260.024 | 0.1 | 10 | 0.9989 |
| Bensulide | C14H24NO4PS3 | + | 415.0943 | 415.0944 | 0.1 | 1 | 0.9872 |
| Bentazone | C10H12N2O3S | + | 241.0641 | 241.0643 | 0.5 | 1 | 0.9982 |
| Benthiavalicarb | C15H18FN3O3S | + | 340.1126 | 340.114 | 4.2 | 1 | 0.9047 |
| Benzoximate | C18H18ClNO5 | + | 364.0946 | 364.0944 | 0.7 | 1 | 0.9965 |
| Bifenazate | C17H20N2O3 | + | 301.1547 | 301.1546 | 0.1 | 1 | 0.9892 |
| Bifenox | C14H9Cl2NO5 | + | 359.0196 | 359.0193 | 0.8 | 10 | 0.9668 |
| Bifenthrin | C23H22ClF3O2 | + | 423.1333 | 423.1322 | 2.6 | 10 | 0.9729 |
| Binapacryl | C15H18N2O6 | + | 340.1503 | 340.1496 | 2.2 | 10 | 0.9688 |
| Bispyribac-sodium | C19H17N4NaO8 | + | 453.1017 | 453.1018 | 0.1 | 10 | 0.9843 |
| Bitertanol | C20H23N3O2 | + | 338.1863 | 338.1861 | 0.5 | 1 | 0.9916 |
| Boscalid | C18H12Cl2N2O | + | 343.04 | 343.0399 | 0.1 | 1 | 0.9797 |
| Brodifacoum | C31H23BrO3 | - | 521.0758 | 521.0755 | 0.5 | 1 | 0.9905 |
| Bromadiolone | C30H23BrO4 | - | 525.0707 | 525.0706 | 0.1 | 1 | 0.9879 |
| Bromoxynil | C7H3Br2NO | - | 273.8509 | 273.8506 | 1 | 1 | 0.9990 |
| Bromuconazole(cis-) | C13H12BrCl2N3O | + | 375.9614 | 375.9613 | 0 | 1 | 0.9961 |
| Bromuconazole(trans-) | C13H12BrCl2N3O | + | 375.9614 | 375.9613 | 0 | 1 | 0.9912 |
| Bufencarb | C13H19NO2 | + | 222.1489 | 222.149 | 0.6 | 1 | 0.9965 |
| Bupirimate | C13H24N4O3S | + | 317.1642 | 317.1641 | 0.2 | 1 | 0.9978 |
| Buprofezin | C16H23N3OS | + | 306.1635 | 306.1632 | 0.7 | 1 | 0.9974 |
| Butachlor | C17H26ClNO2 | + | 329.199 | 329.1989 | 0.3 | 10 | 0.9928 |
| Butafenacil | C20H18ClF3N2O6 | + | 492.1144 | 492.1144 | 0.1 | 1 | 0.9981 |
| Butocarboxim | C7H14N2O2S | + | 208.1114 | 208.1116 | 0.6 | 1 | 0.9971 |
| Butoxycarboxim | C7H14N2O4S | + | 223.0747 | 223.0747 | 0.3 | 1 | 0.9990 |
| Butralin | C14H21N3O4 | + | 296.1605 | 296.1604 | 0.4 | 1 | 0.9983 |
| Butylate | C11H23NOS | + | 218.1573 | 218.1575 | 0.9 | 1 | 0.9993 |
| Cadusafos | C10H23O2PS2 | + | 271.095 | 271.0948 | 0.8 | 1 | 0.9874 |
| Carbaryl | C12H11NO2 | + | 202.0863 | 202.0855 | 3.9 | 1 | 0.9923 |
| Carbendazim | C9H9N3O2 | + | 192.0768 | 192.0767 | 0.4 | 1 | 0.9986 |
| Carbetamide | C12H16N2O3 | + | 237.1234 | 237.1235 | 0.6 | 1 | 0.9982 |
| Carbofuran | C12H15NO3 | + | 222.1125 | 222.1126 | 0.4 | 1 | 0.9980 |
| Carbofuran, 3OH- | C12H15NO4 | + | 255.1339 | 255.1338 | 0.5 | 1 | 0.9986 |
| Carboxin | C12H13NO2S | + | 236.074 | 236.074 | 0.3 | 1 | 0.9972 |
| Carfentrazone-ethyl | C15H14Cl2F3N3O3 | + | 429.0703 | 429.0702 | 0.1 | 1 | 0.9957 |
| Carpropamid | C15H18Cl3NO | + | 334.0527 | 334.0526 | 0.1 | 1 | 0.9982 |
| Chinomethionate | C10H6N2OS2 | + | 252.026 | 252.0267 | 2.7 | 10 | 0.9963 |
| Chlorantraniliprole | C18H14BrCl2N5O2 | + | 481.9781 | 481.978 | 0.1 | 1 | 0.9718 |

| Compound | Formula | Polarity | Theoretical Mass (m/z) | Experimental Mass (m/z) | Mass Deviation (ppm) | LOQ (µg/L) | R2 |
|---|---|---|---|---|---|---|---|
| Chlorbromuron | C9H10BrClN2O2 | + | 292.9687 | 292.9688 | 0.2 | 1 | 0.9958 |
| Chlorbufam | C11H10ClNO2 | + | 241.0738 | 241.073 | 3.5 | 1 | 0.9864 |
| Chlordimeform | C10H13ClN2 | + | 197.084 | 197.084 | 0.1 | 10 | 0.9973 |
| Chlorfenvinphos | C12H14Cl3O4P | + | 358.9768 | 358.9767 | 0.2 | 1 | 0.9976 |
| Chlorfluazuron | C20H9Cl3F5N3O3 | + | 556.9968 | 556.9968 | 0.1 | 1 | 0.9963 |
| Chloroxuron | C15H15ClN2O2 | + | 291.0895 | 291.0893 | 0.8 | 1 | 0.9978 |
| Chlorpropham | C10H12ClNO2 | + | 214.0629 | 214.0632 | 1.3 | 10 | 0.9910 |
| Chlorpyrifos | C9H11Cl3NO3PS | + | 349.9336 | 349.9336 | 0 | 1 | 0.9951 |
| Chlorpyrifos oxon | C9H11Cl3NO4P | + | 333.9564 | 333.9564 | 0.1 | 1 | 0.9903 |
| Chlorpyrifos-methyl | C7H7Cl3NO3PS | + | 321.9023 | 321.9022 | 0.1 | 25 | 0.9763 |
| Chlorthiamid | C7H5Cl2NS | + | 222.9858 | 222.9852 | 2.8 | 10 | 0.9857 |
| Chlorthion | C8H9ClNO5PS | + | 314.9966 | 314.9971 | 1.6 | 25 | 0.9812 |
| Chlorthiophos | C11H15Cl2O3PS2 | + | 360.965 | 360.9643 | 1.9 | 25 | 0.9632 |
| Chlortoluron | C10H13ClN2O | + | 213.0789 | 213.079 | 0.6 | 1 | 0.9976 |
| Clethodim | C17H26ClNO3S | + | 360.1395 | 360.1395 | 0.2 | 1 | 0.9923 |
| Clofentezine | C14H8Cl2N4 | + | 320.0464 | 320.045 | 4.5 | 10 | 0.9935 |
| Clothianidin | C6H8ClN5O2S | + | 250.016 | 250.016 | 0.2 | 1 | 0.9916 |
| Coumaphos | C14H16ClO5PS | + | 363.0217 | 363.0217 | 0.1 | 1 | 0.9983 |
| Coumaphos oxon | C14H16ClO6P | + | 347.0446 | 347.0446 | 0 | 1 | 0.9951 |
| Crotoxyphos | C14H19O6P | + | 332.1258 | 332.1255 | 0.7 | 1 | 0.9982 |
| Crufomate | C12H19ClNO3P | + | 309.1129 | 309.112 | 3.1 | 1 | 0.9914 |
| Cumyluron | C17H19ClN2O | + | 303.1259 | 303.1258 | 0.2 | 1 | 0.9989 |
| Cyanazine | C9H13ClN6 | + | 241.0963 | 241.0963 | 0.2 | 1 | 0.9951 |
| Cyazofamid | C13H13ClN4O2S | + | 342.0786 | 342.077 | 4.6 | 1 | 0.9895 |
| Cyclanilide | C11H9Cl2NO3 | - | 271.9887 | 271.9891 | 1.8 | 1 | 0.9991 |
| Cycloate | C11H21NOS | + | 216.1417 | 216.1418 | 0.4 | 1 | 0.9913 |
| Cyclohexamide | C15H23NO4 | + | 299.1965 | 299.1966 | 0.3 | 1 | 0.9977 |
| Cycluron | C11H22N2O | + | 199.1805 | 199.1805 | 0.1 | 1 | 0.9922 |
| Cyflufenamid | C20H17F5N2O2 | + | 413.1283 | 413.1282 | 0.2 | 1 | 0.9977 |
| Cyfluthrin | C22H18Cl2FNO3 | + | 451.0986 | 451.098 | 1.3 | 10 | 0.7124 |
| Cyhalothrin | C23H19ClF3NO3 | + | 467.1344 | 467.1339 | 1 | 1 | 0.9859 |
| Cymoxanil | C7H10N4O3 | + | 216.1091 | 216.1088 | 1.3 | 1 | 0.9885 |
| Cypermethin | C22H19Cl2NO3 | + | 433.108 | 433.108 | 0 | 10 | 0.9859 |
| Cyphenothrin | C24H25NO3 | + | 393.2173 | 393.2173 | 0 | 1 | 0.9959 |
| Cyproconazole | C15H18ClN3O | + | 292.1211 | 292.1211 | 0.2 | 1 | 0.9978 |
| Cyprodinil | C14H15N3 | + | 226.1339 | 226.1339 | 0.3 | 1 | 0.9967 |
| Cyprosulfamide | C18H18N2O5S | + | 375.1009 | 375.1009 | 0.1 | 1 | 0.9977 |
| Cyromazine | C6H10N6 | + | 167.104 | 167.1039 | 0.2 | 1 | 0.9445 |
| Daimuron | C17H20N2O | + | 269.1648 | 269.1646 | 0.7 | 1 | 0.9992 |
| Dazomet | C5H10N2S2 | + | 163.0358 | 163.0358 | 0.1 | 1 | 0.9451 |
| DEF (Tribufos) | C12H27OPS3 | + | 315.1034 | 315.1033 | 0.3 | 1 | 0.9840 |
| Deltamethrin | C22H19Br2NO3 | + | 521.007 | 521.0073 | 0.5 | 1 | 0.9986 |
| Demeton S-methyl | C6H15O3PS2 | + | 231.0273 | 231.0275 | 0.9 | 1 | 0.9966 |
| Demeton S-sulfone | C6H15O5PS2 | + | 263.0171 | 263.0173 | 0.8 | 10 | 0.9914 |
| Demeton-O | C8H19O3PS2 | + | 259.0586 | 259.0586 | 0.1 | 1 | 0.9960 |
| Demeton-S (Disulfoton oxon) | C8H19O3PS2 | + | 259.0586 | 259.0586 | 0.1 | 1 | 0.9960 |
| Desmedipham | C16H16N2O4 | + | 318.1448 | 318.1448 | 0 | 1 | 0.9975 |
| Desmetryn | C8H15N5S | + | 214.1121 | 214.1122 | 0.6 | 1 | 0.9986 |
| Dialifor | C14H17ClNO4PS2 | + | 411.0363 | 411.0363 | 0.1 | 1 | 0.9984 |
| Diallate | C10H17Cl2NOS | + | 270.0481 | 270.0482 | 0.5 | 1 | 0.9636 |
| Diamidafos (Nellite) | C8H13N2O2P | + | 201.0787 | 201.0787 | 0 | 1 | 0.9986 |
| Diazinon | C12H21N2O3PS | + | 305.1083 | 305.1081 | 0.9 | 1 | 0.9983 |
| Diazinon hydroxy | C12H21N2O4PS | + | 321.1032 | 321.1031 | 0.6 | 1 | 0.9985 |
| Diazinon oxon | C12H21N2O4P | + | 289.1312 | 289.1311 | 0.2 | 1 | 0.9385 |
| Dicapthon | C8H9ClNO5PS | + | 314.9966 | 314.9971 | 1.6 | 25 | 0.9812 |
| Dichlofluanid | C9H11Cl2FN2O2S2 | + | 349.9961 | 349.9961 | 0.2 | 1 | 0.9930 |
| Dichlorfenthion | C10H13Cl2O3PS | + | 314.9773 | 314.9768 | 1.5 | 10 | 0.9966 |
| Dichlormid | C8H11Cl2NO | + | 208.0291 | 208.0292 | 0.6 | 1 | 0.9923 |
| Dichlorvos | C4H7Cl2O4P | + | 220.9532 | 220.9533 | 0.4 | 10 | 0.9920 |
| Diclobutrazol | C15H19Cl2N3O | + | 328.0978 | 328.0978 | 0.1 | 1 | 0.9949 |
| Dicrotophos | C8H16NO5P | + | 238.0839 | 238.0839 | 0.2 | 1 | 0.9991 |
| Diethofencarb | C14H21NO4 | + | 268.1543 | 268.1543 | 0.1 | 1 | 0.9994 |
| Difenacoum | C31H24O3 | + | 445.1798 | 445.1798 | 0.1 | 1 | 0.9972 |
| Difenoconazole | C19H17Cl2N3O3 | + | 406.072 | 406.0719 | 0.3 | 1 | 0.9914 |
| Diflenoxuron | C16H18N2O3 | + | 287.139 | 287.1389 | 0.6 | 1 | 0.9938 |
| Diflubenzuron | C14H9ClF2N2O2 | - | 309.0248 | 309.0246 | 0.6 | 1 | 0.9985 |

| Compound | Formula | Polarity | Theoretical Mass (m/z) | Experimental Mass (m/z) | Mass Deviation (ppm) | LOQ (µg/L) | R2 |
|---|---|---|---|---|---|---|---|
| Dimepiperate | C15H21NOS | + | 264.1417 | 264.1429 | 4.9 | 1 | 0.9994 |
| Dimethachlor | C13H18ClNO2 | + | 256.1099 | 256.1098 | 0.3 | 1 | 0.9921 |
| Dimethametryn | C11H21N5S | + | 256.159 | 256.1588 | 0.8 | 1 | 0.9983 |
| Dimethenamid | C12H18ClNO2S | + | 276.082 | 276.0818 | 0.5 | 1 | 0.9977 |
| Dimethoate | C5H12NO3PS2 | + | 230.0069 | 230.007 | 0.3 | 1 | 0.9993 |
| Dimethomorph | C21H22ClNO4 | + | 388.131 | 388.131 | 0 | 1 | 0.9970 |
| Dimethylvinphos. Z- | C10H10Cl3O4P | + | 330.9455 | 330.9455 | 0.1 | 1 | 0.9950 |
| Dimetilan | C10H16N4O3 | + | 241.1295 | 241.1295 | 0.1 | 1 | 0.9990 |
| Dimoxystrobin | C19H22N2O3 | + | 327.1703 | 327.1702 | 0.4 | 1 | 0.9905 |
| Diniconazole | C15H17Cl2N3O | + | 326.0821 | 326.0821 | 0.2 | 1 | 0.9899 |
| Dinotefuran | C7H14N4O3 | + | 203.1139 | 203.1139 | 0.1 | 1 | 0.9957 |
| Dioxacarb | C11H13NO4 | + | 224.0917 | 224.0919 | 0.8 | 1 | 0.9978 |
| Dioxathion | C12H26O6P2S4 | + | 474.0426 | 474.0426 | 0 | 1 | 0.9900 |
| Diphenamid | C16H17NO | + | 240.1383 | 240.1383 | 0.1 | 1 | 0.9992 |
| Diphenylamine | C12H11N | + | 170.0964 | 170.0965 | 0.3 | 1 | 0.9952 |
| Dipropetryn | C11H21N5S | + | 256.159 | 256.1588 | 0.8 | 1 | 0.9983 |
| Disulfoton | C8H19O2PS3 | + | 275.0358 | 275.0355 | 0.9 | 1 | 0.9935 |
| Ditalimfos | C12H14NO4PS | + | 300.0454 | 300.0452 | 0.6 | 1 | 0.9967 |
| Dithianon | C14H4N2O2S2 | + | 314.0052 | 314.0064 | 3.6 | 10 | 0.9235 |
| Dithiopyr | C15H16F5NO2S2 | + | 402.0615 | 402.0617 | 0.3 | 10 | 0.9866 |
| Diuron | C9H10Cl2N2O | + | 233.0243 | 233.0244 | 0.6 | 1 | 0.9947 |
| DNOC | C7H6N2O5 | - | 197.0204 | 197.0205 | 1.5 | 1 | 0.9948 |
| Dodemorph | C18H35NO | + | 282.2791 | 282.279 | 0.6 | 1 | 0.9946 |
| Doramectin | C50H74O14 | + | 916.5417 | 916.5418 | 0.1 | 10 | 0.9888 |
| Edifenphos | C14H15O2PS2 | + | 311.0324 | 311.0322 | 0.6 | 1 | 0.9952 |
| EPN | C14H14NO4PS | + | 341.0719 | 341.0721 | 0.3 | 1 | 0.9983 |
| Epoxiconazole | C17H13ClFN3O | + | 330.0804 | 330.0803 | 0.2 | 1 | 0.9953 |
| Eprinomectin B1a | C50H75NO14 | + | 914.526 | 914.526 | 0 | 1 | 0.9852 |
| Eprinomectin B1b | C49H73NO14 | + | 900.5104 | 900.5131 | 3 | 10 | 0.9738 |
| EPTC (eptam) | C9H19NOS | + | 190.126 | 190.1261 | 0.2 | 1 | 0.9938 |
| Esprocarb | C15H23NOS | + | 266.1573 | 266.1572 | 0.4 | 1 | 0.9981 |
| Etaconazol | C14H15Cl2N3O2 | + | 328.0614 | 328.0613 | 0.3 | 1 | 0.9980 |
| Ethaboxam | C14H16N4OS2 | + | 321.0838 | 321.0839 | 0.3 | 1 | 0.9907 |
| Ethalfluralin | C13H14F3N3O4 | + | 334.1009 | 334.0994 | 4.6 | 1 | 0.9845 |
| Ethidimuron | C7H12N4O3S2 | + | 265.0424 | 265.0422 | 0.6 | 1 | 0.9805 |
| Ethiofencarb | C11H15NO2S | + | 226.0896 | 226.0898 | 0.8 | 1 | 0.9987 |
| Ethiolate | C7H15NOS | + | 162.0947 | 162.0947 | 0.2 | 1 | 0.9960 |
| Ethion | C9H22O4P2S4 | + | 384.9949 | 384.9948 | 0.1 | 1 | 0.9914 |
| Ethion monoxon | C9H22O5P2S3 | + | 369.0177 | 369.0177 | 0 | 1 | 0.9975 |
| Ethiprole | C13H9Cl2F3N4OS | + | 414.0165 | 414.0164 | 0.1 | 1 | 0.9817 |
| Ethirimol | C11H19N3O | + | 210.1601 | 210.1602 | 0.4 | 1 | 0.9984 |
| Ethofumesate | C13H18O5S | + | 304.1213 | 304.1213 | 0.1 | 1 | 0.9986 |
| Ethoprop | C8H19O2PS2 | + | 243.0637 | 243.0637 | 0 | 1 | 0.9865 |
| Ethoxyquin | C14H19NO | + | 218.1539 | 218.1541 | 0.7 | 1 | 0.9967 |
| Etobenzanid | C16H15Cl2NO3 | + | 340.0502 | 340.0502 | 0.1 | 1 | 0.9969 |
| Etofenprox | C25H28O3 | + | 394.2377 | 394.2379 | 0.6 | 1 | 0.9928 |
| Etoxazole | C21H23F2NO2 | + | 360.177 | 360.1769 | 0.1 | 1 | 0.9976 |
| Etrimfos | C10H17N2O4PS | + | 293.0719 | 293.0718 | 0.6 | 1 | 0.9982 |
| Famoxadone | C22H18N2O4 | + | 392.1605 | 392.1603 | 0.4 | 1 | 0.9937 |
| Famphur | C10H16NO5PS2 | + | 343.0546 | 343.0531 | 4.4 | 1 | 0.9973 |
| Famphur oxon | C10H16NO6PS | + | 327.0774 | 327.0775 | 0.2 | 1 | 0.9955 |
| Fenamidone | C17H17N3OS | + | 312.1165 | 312.1163 | 0.6 | 1 | 0.9986 |
| Fenamiphos | C13H22NO3PS | + | 304.1131 | 304.113 | 0.3 | 1 | 0.9944 |
| Fenamiphos sulfone | C13H22NO5PS | + | 336.1029 | 336.1029 | 0.1 | 1 | 0.9924 |
| Fenamiphos sulfoxide | C13H22NO4PS | + | 320.108 | 320.1079 | 0.2 | 1 | 0.9936 |
| Fenarimol | C17H12Cl2N2O | + | 331.04 | 331.0399 | 0.3 | 1 | 0.9825 |
| Fenazaquin | C20H22N2O | + | 307.1805 | 307.1805 | 0.1 | 1 | 0.9881 |
| Fenbuconazole | C19H17ClN4 | + | 337.1215 | 337.1214 | 0.1 | 1 | 0.9970 |
| Fenhexamid | C14H17Cl2NO2 | + | 302.0709 | 302.0709 | 0.2 | 1 | 0.9965 |
| Fenitrothion | C9H12NO5PS | + | 295.0512 | 295.0517 | 1.6 | 10 | 0.9971 |
| Fenoxanil | C15H18Cl2N2O2 | + | 346.1084 | 346.1083 | 0.1 | 1 | 0.9914 |
| Fenoxycarb | C17H19NO4 | + | 302.1387 | 302.1386 | 0.5 | 1 | 0.9943 |
| Fenpiclonil | C11H6Cl2N2 | + | 254.0246 | 254.0246 | 0.3 | 1 | 0.9817 |
| Fenpropathrin | C22H23NO3 | + | 350.1751 | 350.1759 | 2.4 | 1 | 0.9954 |
| Fenpropimorph | C20H33NO | + | 304.2635 | 304.2633 | 0.5 | 1 | 0.9919 |
| Fenpyroximate | C24H27N3O4 | + | 422.2074 | 422.2074 | 0.2 | 1 | 0.9966 |

| Compound | Formula | Polarity | Theoretical Mass (m/z) | Experimental Mass (m/z) | Mass Deviation (ppm) | LOQ (µg/L) | R2 |
|---|---|---|---|---|---|---|---|
| Fensulfothion | C11H17O4PS2 | + | 309.0379 | 309.0378 | 0.3 | 1 | 0.9969 |
| Fenthion | C10H15O3PS2 | + | 279.0273 | 279.0286 | 4.5 | 1 | 0.9941 |
| Fenthion oxon | C10H15O4PS | + | 263.0501 | 263.0501 | 0.1 | 1 | 0.9975 |
| Fenthion sulfone | C10H15O5PS2 | + | 328.0437 | 328.0439 | 0.6 | 1 | 0.9993 |
| Fenthion sulfoxide | C10H15O4PS2 | + | 295.0222 | 295.022 | 0.6 | 1 | 0.9957 |
| Fenuron | C9H12N2O | + | 165.1022 | 165.1022 | 0.4 | 1 | 0.9998 |
| Fenvalerate | C25H22ClNO3 | + | 437.1627 | 437.1629 | 0.7 | 10 | 0.9919 |
| Fipronil | C12H4Cl2F6N4OS | - | 434.9314 | 434.9316 | 0.4 | 1 | 0.9968 |
| Flonicamid | C9H6F3N3O | - | 228.039 | 228.0384 | 2.6 | 1 | 0.9989 |
| Florasulam | C12H8F3N5O3S | + | 360.0373 | 360.0374 | 0.2 | 1 | 0.9956 |
| Fluazinam | C13H4Cl2F6N4O4 | - | 462.9441 | 462.945 | 1.9 | 1 | 0.9946 |
| Flubendiamide | C23H22F7IN2O4S | - | 681.016 | 681.0154 | 0.9 | 1 | 0.9917 |
| Flucarbazone | C12H11F3N4O6S | + | 414.069 | 414.069 | 0 | 1 | 0.9924 |
| Fluchloralin | C12H13ClF3N3O4 | + | 373.0885 | 373.0894 | 2.4 | 10 | 0.9605 |
| Flucythrinate | C26H23F2NO4 | + | 469.1933 | 469.1933 | 0.2 | 1 | 0.9932 |
| Fludioxonil | C12H6F2N2O2 | + | 266.0736 | 266.0736 | 0.1 | 1 | 0.9749 |
| Flufenacet | C14H13F4N3O2S | + | 364.0737 | 364.0736 | 0.4 | 1 | 0.9980 |
| Flufenoxuron | C21H11ClF6N2O3 | + | 489.0435 | 489.0436 | 0.1 | 1 | 0.9929 |
| Flumetralin | C16H12ClF4N3O4 | + | 422.0525 | 422.0537 | 2.8 | 25 | 0.9917 |
| Flumetsulam | C12H9F2N5O2S | + | 326.0518 | 326.0516 | 0.6 | 1 | 0.9988 |
| Flumioxazin | C19H15FN2O4 | + | 355.1089 | 355.1089 | 0 | 10 | 0.9677 |
| Fluometuron | C10H11F3N2O | + | 233.0896 | 233.0897 | 0.4 | 1 | 0.9983 |
| Fluopicolide | C14H8Cl3F3N2O | + | 382.9727 | 382.9728 | 0.2 | 1 | 0.9911 |
| Fluorochloridone | C12H10Cl2F3NO | + | 329.043 | 329.0431 | 0.4 | 1 | 0.9837 |
| Fluorodifen | C13H7F3N2O5 | + | 346.0645 | 346.0652 | 2 | 10 | 0.9963 |
| Fluoxastrobin | C21H16ClFN4O5 | + | 459.0866 | 459.0865 | 0.3 | 1 | 0.9983 |
| Fluquinconazole | C16H8Cl2FN5O | + | 376.0163 | 376.0163 | 0 | 10 | 0.9939 |
| Fluroxypyr | C7H5Cl2FN2O3 | - | 252.9588 | 252.9581 | 2.7 | 10 | 0.9928 |
| Flusilazole | C16H15F2N3Si | + | 316.1076 | 316.1076 | 0.1 | 1 | 0.9932 |
| Flutolanil | C17H16F3NO2 | + | 341.1471 | 341.1471 | 0 | 1 | 0.9948 |
| Flutriafol | C16H13F2N3O | + | 302.11 | 302.11 | 0 | 1 | 0.9942 |
| Fluvalinate ? | C26H22ClF3N2O3 | + | 520.1609 | 520.1613 | 0.7 | 10 | 0.9968 |
| Fonophos | C10H15OPS2 | + | 247.0375 | 247.0375 | 0.2 | 1 | 0.9165 |
| Fonophos O-analog | C10H15O2PS | + | 231.0603 | 231.0601 | 0.8 | 10 | 0.9526 |
| Forchlorfenuron | C12H10ClN3O | + | 248.0585 | 248.0585 | 0.1 | 1 | 0.9967 |
| Formasafen | C15H10ClF3N2O6S | - | 436.9827 | 436.9817 | 2.2 | 1 | 0.9972 |
| Formetanate | C11H15N3O2 | + | 239.1503 | 239.1503 | 0.1 | 1 | 0.9981 |
| Fosthiazate | C9H18NO3PS2 | + | 284.0539 | 284.0538 | 0.2 | 1 | 0.9958 |
| Fuberidazole | C11H8N2O | + | 185.0709 | 185.0708 | 0.9 | 1 | 0.9972 |
| Furalaxyl | C17H19NO4 | + | 302.1387 | 302.1386 | 0.5 | 1 | 0.9943 |
| Furathiocarb | C18H26N2O5S | + | 383.1635 | 383.1635 | 0.1 | 1 | 0.9980 |
| Griseofulvin | C17H17ClO6 | + | 353.0786 | 353.0787 | 0.2 | 1 | 0.9968 |
| Halofenozide | C18H19ClN2O2 | - | 329.1062 | 329.1063 | 0.3 | 1 | 0.9984 |
| Haloxyfop-methyl | C16H13ClF3NO4 | + | 376.0558 | 376.0556 | 0.4 | 1 | 0.9965 |
| Heptenophos | C9H12ClO4P | + | 251.0235 | 251.0235 | 0.2 | 10 | 0.9983 |
| Hexaconazole | C14H17Cl2N3O | + | 314.0821 | 314.082 | 0.4 | 1 | 0.9947 |
| Hexaflumuron | C16H8Cl2F6N2O3 | - | 458.9743 | 458.9745 | 0.4 | 1 | 0.9834 |
| Hexazinone | C12H20N4O2 | + | 253.1659 | 253.1658 | 0.5 | 1 | 0.9975 |
| Hexythiazox | C17H21ClN2O2S | + | 353.1085 | 353.1084 | 0.4 | 1 | 0.9807 |
| Hydramethylnon | C25H24F6N4 | + | 495.1978 | 495.1976 | 0.3 | 1 | 0.9965 |
| Imazalil | C14H14Cl2N2O | + | 297.0556 | 297.0555 | 0.4 | 1 | 0.9960 |
| Imazamox | C15H19N3O4 | + | 306.1448 | 306.1447 | 0.5 | 1 | 0.9962 |
| Imazapyr | C13H15N3O3 | + | 262.1186 | 262.1185 | 0.3 | 1 | 0.9972 |
| Imazaquin | C17H17N3O3 | + | 312.1343 | 312.1341 | 0.5 | 1 | 0.9970 |
| Imibenconazole | C17H13Cl3N4S | + | 410.9999 | 411 | 0.2 | 1 | 0.9909 |
| Imidacloprid | C9H10ClN5O2 | + | 256.0596 | 256.0595 | 0.5 | 1 | 0.9983 |
| Imiprothrin | C17H22N2O4 | + | 319.1652 | 319.1651 | 0.4 | 1 | 0.9663 |
| Inabenifide | C19H15ClN2O2 | + | 339.0895 | 339.0895 | 0 | 1 | 0.9974 |
| Indanofan | C20H17ClO3 | + | 341.0939 | 341.0938 | 0.4 | 1 | 0.9824 |
| Indoxacarb | C22H17ClF3N3O7 | + | 528.078 | 528.0779 | 0.2 | 1 | 0.9922 |
| Ioxynil | C7H3I2NO | - | 369.8231 | 369.8237 | 0.2 | 1 | 0.9955 |
| Ipconazole | C18H24ClN3O | + | 334.1681 | 334.1679 | 0.4 | 1 | 0.9968 |
| Iprobenfos | C13H21O3PS | + | 289.1022 | 289.1021 | 0.1 | 1 | 0.9977 |
| Iprovalicarb | C18H28N2O3 | + | 321.2173 | 321.2171 | 0.4 | 1 | 0.9993 |
| Isazophos | C9H17ClN3O3PS | + | 314.049 | 314.0489 | 0.3 | 1 | 0.9988 |
| Isocarbamid | C8H15N3O2 | + | 186.1237 | 186.1237 | 0 | 1 | 0.9967 |

| Compound | Formula | Polarity | Theoretical Mass (m/z) | Experimental Mass (m/z) | Mass Deviation (ppm) | LOQ (µg/L) | R2 |
|---|---|---|---|---|---|---|---|
| Isocarbophos | C11H16NO4PS | + | 307.0876 | 307.0876 | 0.1 | 1 | 0.9941 |
| Isofenfos | C15H24NO4PS | + | 346.1236 | 346.1236 | 0.2 | 1 | 0.9911 |
| Isofenfos O-analog | C15H24NO5P | + | 330.1465 | 330.1473 | 2.6 | 10 | 0.9344 |
| Isoprocarb | C11H15NO2 | + | 194.1176 | 194.1177 | 0.8 | 1 | 0.9978 |
| Isopropalin | C15H23N3O4 | + | 310.1761 | 310.1761 | 0.2 | 1 | 0.9932 |
| Isoprothiolane | C12H18O4S2 | + | 291.0719 | 291.0718 | 0.6 | 1 | 0.9961 |
| Isoproturon | C12H18N2O | + | 207.1492 | 207.1492 | 0.2 | 1 | 0.9939 |
| Isoxaben | C18H24N2O4 | + | 333.1809 | 333.1809 | 0.1 | 1 | 0.9982 |
| Isoxadifen-ethyl | C18H17NO3 | + | 296.1281 | 296.1281 | 0 | 1 | 0.9968 |
| Isoxaflutole | C15H12F3NO4S | + | 377.0777 | 377.0779 | 0.4 | 1 | 0.9919 |
| Isoxathion | C13H16NO4PS | + | 314.061 | 314.0608 | 0.7 | 1 | 0.9895 |
| Ivermectin B1a | C48H74O14 | + | 892.5417 | 892.5415 | 0.2 | 10 | 0.9915 |
| Ivermectin B1b | C47H72O14 | + | 883.4814 | 883.4818 | 0.4 | 50 | 0.9695 |
| Kresoxim-methyl | C18H19NO4 | + | 314.1387 | 314.1386 | 0.2 | 1 | 0.9969 |
| Lactofen | C19H15ClF3NO7 | + | 479.0827 | 479.0828 | 0.1 | 1 | 0.9883 |
| Linuron | C9H10Cl2N2O2 | + | 249.0192 | 249.0191 | 0.3 | 1 | 0.9977 |
| Lufenuron | C17H8Cl2F8N2O3 | + | 510.9857 | 510.9833 | 4.7 | 1 | 0.9808 |
| Malathion | C10H19O6PS2 | + | 348.0699 | 348.07 | 0.4 | 1 | 0.9950 |
| Malathion O-analog | C10H19O7PS | + | 315.0662 | 315.0661 | 0.2 | 1 | 0.9948 |
| Mandipropamid | C23H22ClNO4 | + | 412.131 | 412.131 | 0.1 | 1 | 0.9978 |
| Mefenacet | C16H14N2O2S | + | 299.0849 | 299.0848 | 0.4 | 1 | 0.9985 |
| Mefluidide | C11H13F3N2O3S | + | 328.0937 | 328.0937 | 0.1 | 1 | 0.9987 |
| Mepanipyrim | C14H13N3 | + | 224.1182 | 224.1184 | 0.6 | 1 | 0.9887 |
| Mephospholan | C8H16NO3PS2 | + | 270.0382 | 270.038 | 0.6 | 1 | 0.9915 |
| Mepronil | C17H19NO2 | + | 270.1489 | 270.1487 | 0.4 | 1 | 0.9938 |
| Mesotrione | C14H13NO7S | + | 340.0486 | 340.0502 | 4.9 | 1 | 0.9952 |
| Metaflumizone | C24H16F6N4O2 | - | 505.1105 | 505.1106 | 0.1 | 1 | 0.9745 |
| Metalaxyl | C15H21NO4 | + | 280.1543 | 280.1542 | 0.6 | 1 | 0.9988 |
| Metazachlor | C14H16ClN3O | + | 278.1055 | 278.1054 | 0.3 | 1 | 0.9984 |
| Metconazole | C17H22ClN3O | + | 320.1524 | 320.1523 | 0.4 | 1 | 0.9881 |
| Methabenzthiazuron | C10H11N3OS | + | 222.0696 | 222.0698 | 0.9 | 1 | 0.9982 |
| Methacrifos | C7H13O5PS | + | 258.056 | 258.0559 | 0.1 | 1 | 0.9958 |
| Methamidophos | C2H8NO2PS | + | 142.0086 | 142.0087 | 0.4 | 1 | 0.9990 |
| Methidathion | C6H11N2O4PS3 | + | 319.9957 | 319.9956 | 0.2 | 1 | 0.9971 |
| Methiocarb | C11H15NO2S | + | 226.0896 | 226.0898 | 0.8 | 1 | 0.9987 |
| Methomyl | C5H10N2O2S | + | 163.0536 | 163.0534 | 0.9 | 1 | 0.9991 |
| Methoprotryne | C11H21N5OS | + | 272.154 | 272.1537 | 1 | 1 | 0.9978 |
| Methoxyfenozide | C22H28N2O3 | + | 369.2173 | 369.2172 | 0.2 | 1 | 0.9935 |
| Metobromuron | C9H11BrN2O2 | + | 259.0077 | 259.0077 | 0.2 | 1 | 0.9948 |
| Metofluthrin | C18H20F4O3 | - | 359.1276 | 359.1277 | 0.2 | 1 | 0.9887 |
| Metolachlor | C15H22ClNO2 | + | 284.1412 | 284.1411 | 0.1 | 1 | 0.9981 |
| Metominostrobin(E-) | C16H16N2O3 | + | 285.1234 | 285.1232 | 0.7 | 1 | 0.9957 |
| Metosulam | C14H13Cl2N5O4S | + | 418.0138 | 418.0137 | 0.3 | 1 | 0.9924 |
| Metoxuron | C10H13ClN2O2 | + | 229.0738 | 229.074 | 0.6 | 1 | 0.9995 |
| Metrafenone | C19H21BrO5 | + | 409.0645 | 409.0643 | 0.4 | 1 | 0.9963 |
| Metribuzin | C8H14N4OS | + | 215.0961 | 215.0963 | 0.7 | 1 | 0.9969 |
| Mevinphos | C7H13O6P | + | 242.0788 | 242.0788 | 0.1 | 1 | 0.9977 |
| Mexacarbate | C12H18N2O2 | + | 223.1441 | 223.1443 | 0.7 | 1 | 0.9991 |
| Milbemectin A3 | C31H44O7 | + | 546.3425 | 546.3421 | 0.8 | 10 | 0.9819 |
| Milbemectin A4 | C32H46O7 | + | 560.3582 | 560.3584 | 0.4 | 1 | 0.9905 |
| Molinate | C9H17NOS | + | 188.1104 | 188.1104 | 0.2 | 1 | 0.9881 |
| Monocrotophos | C7H14NO5P | + | 224.0682 | 224.0685 | 1 | 1 | 0.9989 |
| Monolinuron | C9H11ClN2O2 | + | 215.0582 | 215.0583 | 0.7 | 1 | 0.9977 |
| Moxidectin | C37H53NO8 | + | 640.3844 | 640.3847 | 0.5 | 1 | 0.9966 |
| Myclobutanil | C15H17ClN4 | + | 289.1215 | 289.1214 | 0.1 | 1 | 0.9940 |
| Naled | C4H7Br2Cl2O4P | + | 395.8164 | 395.8164 | 0.1 | 10 | 0.9908 |
| Naphthol | C10H8O | + | 145.0648 | 145.0648 | 0.2 | 1 | 0.9939 |
| Napropamide | C17H21NO2 | + | 272.1645 | 272.1644 | 0.5 | 1 | 0.9933 |
| Naptalam sodium | C18H12NNaO3 | + | 331.1053 | 331.1067 | 4.2 | 1 | 0.9931 |
| Neburon | C12H16Cl2N2O | + | 275.0713 | 275.0711 | 0.5 | 1 | 0.9941 |
| Nitenpyram | C11H15ClN4O2 | + | 271.0956 | 271.0948 | 3.2 | 1 | 0.9876 |
| Nitralin | C13H19N3O6S | + | 346.1067 | 346.1083 | 4.6 | 1 | 0.9824 |
| Nitrothal-isopropyl | C14H17NO6 | + | 313.1394 | 313.1385 | 3.5 | 10 | 0.8345 |
| Norflurazon | C12H9ClF3N3O | + | 304.0459 | 304.0458 | 0.3 | 1 | 0.9858 |
| Novaluron | C17H9ClF8N2O4 | - | 491.005 | 491.0053 | 0.6 | 1 | 0.9902 |
| Noviflumuron | C17H7Cl2F9N2O3 | - | 526.9617 | 526.9613 | 0.7 | 1 | 0.9759 |

| Compound | Formula | Polarity | Theoretical Mass (m/z) | Experimental Mass (m/z) | Mass Deviation (ppm) | LOQ (µg/L) | R2 |
|---|---|---|---|---|---|---|---|
| Nuarimol | C17H12ClFN2O | + | 315.0695 | 315.0693 | 0.5 | 1 | 0.9907 |
| Octhilinone (2-Octyl-4-isothiazoline-3-one) | C11H19NOS | + | 214.126 | 214.1262 | 0.8 | 1 | 0.9977 |
| Ofurace | C14H16ClNO3 | + | 299.1157 | 299.1156 | 0.2 | 1 | 0.9974 |
| Omethoate (Dimethoate oxon) | C5H12NO4PS | + | 214.0297 | 214.0298 | 0.4 | 1 | 0.9997 |
| Orbencarb | C12H16ClNOS | + | 258.0714 | 258.0712 | 0.6 | 1 | 0.9969 |
| Oryzalin | C12H18N4O6S | - | 345.0874 | 345.0876 | 0.5 | 1 | 0.9895 |
| Oxadiazon | C15H18Cl2N2O3 | + | 362.1033 | 362.1032 | 0.1 | 1 | 0.9969 |
| Oxadixyl | C14H18N2O4 | + | 279.1339 | 279.1339 | 0 | 1 | 0.9994 |
| Oxamyl | C7H13N3O3S | + | 237.1016 | 237.1017 | 0.5 | 1 | 0.9997 |
| Paclobutrazol | C15H20ClN3O | + | 294.1368 | 294.1367 | 0.3 | 1 | 0.9955 |
| Parathion | C10H14NO5PS | + | 309.0669 | 309.0679 | 3.2 | 10 | 0.9645 |
| Parathion methyl oxon | C8H10NO6P | + | 265.0584 | 265.0585 | 0.5 | 10 | 0.9903 |
| Parathion oxon | C10H14NO6P | + | 293.0897 | 293.0896 | 0.3 | 1 | 0.9928 |
| Pebulate | C10H21NOS | + | 204.1417 | 204.1417 | 0.1 | 1 | 0.9929 |
| Penconazole | C13H15Cl2N3 | + | 284.0716 | 284.0715 | 0.4 | 1 | 0.9931 |
| Pencycuron | C19H21ClN2O | + | 329.1415 | 329.1414 | 0.5 | 1 | 0.9986 |
| Pendimethalin | C13H19N3O4 | + | 282.1448 | 282.1448 | 0.2 | 10 | 0.9949 |
| Penoxsulam | C16H14F5N5O5S | + | 484.0709 | 484.071 | 0.3 | 1 | 0.9928 |
| Penthiopyrad | C16H20F3N3OS | + | 360.1352 | 360.1352 | 0.1 | 1 | 0.9935 |
| Permethrin(cis-) | C21H20Cl2O3 | + | 408.1128 | 408.1129 | 0.2 | 1 | 0.9935 |
| Permethrin(trans-) | C21H20Cl2O3 | + | 408.1128 | 408.1129 | 0.2 | 1 | 0.9935 |
| Phenmedipham | C16H16N2O4 | + | 318.1448 | 318.1448 | 0 | 1 | 0.9975 |
| Phenothrin | C23H26O3 | + | 368.222 | 368.2222 | 0.6 | 1 | 0.9944 |
| Phenthoate | C12H17O4PS2 | + | 321.0379 | 321.0378 | 0.4 | 1 | 0.9929 |
| Phenylphenol(o-) | C12H10O | + | 188.107 | 188.107 | 0.2 | 1 | 0.9854 |
| Phorate | C7H17O2PS3 | + | 261.0201 | 261.02 | 0.3 | 10 | 0.9812 |
| Phorate oxon | C7H17O3PS | + | 230.0974 | 230.0982 | 3.5 | 1 | 0.9973 |
| Phorate oxon sulfone | C7H17O5PS2 | + | 277.0328 | 277.0327 | 0.5 | 1 | 0.9979 |
| Phorate oxon sulfoxide | C7H17O4PS2 | + | 261.0379 | 261.0377 | 0.8 | 1 | 0.9995 |
| Phorate sulfone | C7H17O4PS3 | + | 310.0365 | 310.0363 | 0.6 | 1 | 0.9951 |
| Phorate sulfoxide | C7H17O4PS2 | + | 261.0379 | 261.0377 | 0.8 | 1 | 0.9995 |
| Phosalone | C12H15ClNO4PS2 | + | 385.0207 | 385.0206 | 0.3 | 1 | 0.9945 |
| Phosmet | C11H12NO4PS2 | + | 318.0018 | 318.0018 | 0.1 | 1 | 0.9938 |
| Phosphamidon | C10H19ClNO5P | + | 317.1028 | 317.1026 | 0.4 | 1 | 0.9936 |
| Phoxim | C12H15N2O3PS | + | 299.0614 | 299.0613 | 0.4 | 1 | 0.9963 |
| Picloram | C6H3Cl3N2O2 | + | 240.9333 | 240.9331 | 0.7 | 10 | 0.9594 |
| Picoxystrobin | C18H16F3NO4 | + | 368.1104 | 368.1104 | 0.1 | 1 | 0.9981 |
| Pinoxaden | C23H32N2O4 | + | 401.2435 | 401.2434 | 0.3 | 1 | 0.9968 |
| Piperonyl butoxide | C19H30O5 | + | 356.2432 | 356.2433 | 0.3 | 1 | 0.9872 |
| Piperophos | C14H28NO3PS2 | + | 354.1321 | 354.132 | 0.3 | 1 | 0.9932 |
| Pirimicarb | C11H18N4O2 | + | 239.1503 | 239.1503 | 0.1 | 1 | 0.9992 |
| Pirimiphos-ethyl | C13H24N3O3PS | + | 334.1349 | 334.1348 | 0.2 | 1 | 0.9977 |
| Pirimiphos-methyl | C11H20N3O3PS | + | 306.1036 | 306.1034 | 0.7 | 1 | 0.9952 |
| Pretilachlor | C17H26ClNO2 | + | 329.199 | 329.1989 | 0.3 | 1 | 0.9928 |
| Probenazole | C10H9NO3S | + | 224.0376 | 224.0378 | 0.9 | 1 | 0.9989 |
| Prochloraz | C15H16Cl3N3O2 | + | 376.0381 | 376.0379 | 0.4 | 1 | 0.9933 |
| Profenophos | C11H15BrClO3PS | + | 372.9424 | 372.9424 | 0.1 | 1 | 0.9939 |
| Prohexadione | C10H12O5 | - | 211.0612 | 211.0613 | 0.4 | 1 | 0.9936 |
| Promecarb | C12H17NO2 | + | 208.1332 | 208.1333 | 0.4 | 1 | 0.9972 |
| Prometon | C10H19N5O | + | 226.1662 | 226.1664 | 0.7 | 1 | 0.9991 |
| Prometryn | C10H19N5S | + | 242.1434 | 242.1434 | 0.2 | 1 | 0.9985 |
| Propachlor | C11H14ClNO | + | 212.0837 | 212.0839 | 0.8 | 1 | 0.9962 |
| Propamocarb | C9H20N2O2 | + | 189.1598 | 189.1597 | 0.5 | 1 | 0.9992 |
| Propanil | C9H9Cl2NO | - | 215.9988 | 215.9987 | 0.4 | 1 | 0.9855 |
| Propargite | C19H26O4S | + | 368.189 | 368.1891 | 0.1 | 1 | 0.9961 |
| Propazine | C9H16ClN5 | + | 230.1167 | 230.1168 | 0.5 | 1 | 0.9976 |
| Propetamphos | C10H20NO4PS | + | 299.1189 | 299.1188 | 0.3 | 1 | 0.9929 |
| Propham | C10H13NO2 | + | 180.1019 | 180.1019 | 0.1 | 1 | 0.9131 |
| Propiconazole | C15H17Cl2N3O2 | + | 342.0771 | 342.077 | 0.1 | 1 | 0.9885 |
| Propisochlor | C15H22ClNO2 | + | 284.1412 | 284.1411 | 0.1 | 1 | 0.9981 |
| Propoxur | C11H15NO3 | + | 210.1125 | 210.1126 | 0.7 | 1 | 0.9949 |
| Prothioconazole | C14H15Cl2N3OS | - | 342.024 | 342.0245 | 1.4 | 1 | 0.9864 |
| Prothoate | C9H20NO3PS2 | + | 286.0695 | 286.0693 | 0.8 | 1 | 0.9982 |
| Pymetrozine | C10H11N5O | + | 218.1036 | 218.1037 | 0.5 | 1 | 0.9985 |

| Compound | Formula | Polarity | Theoretical Mass (m/z) | Experimental Mass (m/z) | Mass Deviation (ppm) | LOQ (µg/L) | R2 |
|---|---|---|---|---|---|---|---|
| Pyracarbolid | C13H15NO2 | + | 218.1176 | 218.1177 | 0.6 | 1 | 0.9986 |
| Pyraclofos | C14H18ClN2O3PS | + | 361.0537 | 361.0537 | 0.1 | 1 | 0.9969 |
| Pyraclostrobin | C19H18ClN3O4 | + | 388.1059 | 388.1057 | 0.5 | 1 | 0.9951 |
| Pyraflufen-ethyl | C15H13Cl2F3N2O4 | + | 430.0543 | 430.0527 | 3.7 | 1 | 0.9833 |
| Pyrasulfotole | C14H13F3N2O4S | - | 361.0475 | 361.0476 | 0.2 | 1 | 0.9926 |
| Pyrazone (Chloridazon) | C10H8ClN3O | + | 239.0694 | 239.0687 | 3.1 | 50 | 0.9448 |
| Pyrazophos | C14H20N3O5PS | + | 374.0934 | 374.0933 | 0.3 | 1 | 0.9958 |
| Pyridaben | C19H25ClN2OS | + | 365.1449 | 365.145 | 0.3 | 1 | 0.9881 |
| Pyridalyl | C18H14Cl4F3NO3 | + | 489.9753 | 489.9755 | 0.4 | 1 | 0.9958 |
| Pyridaphenthion | C14H17N2O4PS | + | 341.0719 | 341.0721 | 0.3 | 1 | 0.9938 |
| Pyridate | C19H23ClN2O2S | + | 379.1242 | 379.1242 | 0.2 | 1 | 0.9902 |
| Pyrifenox | C14H12Cl2N2O | + | 295.04 | 295.0397 | 0.7 | 1 | 0.9979 |
| Pyrimethanil | C12H13N3 | + | 200.1182 | 200.1183 | 0.2 | 1 | 0.9977 |
| Pyriproxyfen | C20H19NO3 | + | 322.1438 | 322.1438 | 0 | 1 | 0.9977 |
| Pyroquilon | C11H11NO | + | 174.0913 | 174.0913 | 0.5 | 1 | 0.9992 |
| Pyroxsulam | C14H13F3N6O5S | + | 435.0693 | 435.0693 | 0.1 | 1 | 0.9962 |
| Quinalphos | C12H15N2O3PS | + | 299.0614 | 299.0613 | 0.4 | 1 | 0.9963 |
| Quinclamine | C10H6ClNO2 | + | 208.016 | 208.0158 | 1 | 1 | 0.9879 |
| Quinoxyfen | C15H8Cl2FNO | + | 308.004 | 308.0039 | 0.4 | 1 | 0.9980 |
| Resmethrin | C22H26O3 | + | 339.1955 | 339.1955 | 0.1 | 1 | 0.9948 |
| Rotenone | C23H22O6 | + | 395.1489 | 395.1489 | 0.1 | 1 | 0.9948 |
| Saflufenacil | C17H17ClF4N4O5S | + | 518.0883 | 518.0883 | 0 | 1 | 0.9868 |
| Schradan | C8H24N4O3P2 | + | 287.1396 | 287.1389 | 2.7 | 1 | 0.9937 |
| Secbumeton | C10H19N5O | + | 226.1662 | 226.1664 | 0.7 | 1 | 0.9991 |
| Sethoxydim | C17H29NO3S | + | 328.1941 | 328.1939 | 0.5 | 1 | 0.9977 |
| Siduron | C14H20N2O | + | 233.1648 | 233.165 | 0.5 | 1 | 0.9996 |
| Simazine | C7H12ClN5 | + | 202.0854 | 202.0855 | 0.3 | 1 | 0.9963 |
| Simeconazole | C14H20FN3OSi | + | 294.1432 | 294.1431 | 0.5 | 1 | 0.9949 |
| Simetryn | C8H15N5S | + | 214.1121 | 214.1122 | 0.6 | 1 | 0.9986 |
| Spinetoram | C42H69NO10 | + | 748.4994 | 748.4992 | 0.3 | 1 | 0.9878 |
| Spinetoram 1 | C43H69NO10 | + | 760.4994 | 760.4995 | 0.1 | 1 | 0.9934 |
| Spinosad A | C41H65NO10 | + | 732.4681 | 732.468 | 0.2 | 1 | 0.9960 |
| Spinosad D | C42H67NO10 | + | 746.4838 | 746.4836 | 0.3 | 1 | 0.9932 |
| Spirodiclofen | C21H24Cl2O4 | + | 428.139 | 428.1389 | 0.2 | 1 | 0.9991 |
| Spiromefisen | C23H30O4 | + | 388.2482 | 388.2482 | 0 | 1 | 0.9934 |
| Spirotetramat | C21H27NO5 | + | 374.1962 | 374.1963 | 0.3 | 1 | 0.9990 |
| Spiroxamine | C18H35NO2 | + | 298.2741 | 298.2739 | 0.4 | 1 | 0.9910 |
| Sulcotrione | C14H13ClO5S | + | 346.0511 | 346.0519 | 2.6 | 10 | 0.9706 |
| Sulfentrazone | C11H10Cl2F2N4O3S | + | 386.9892 | 386.9906 | 3.8 | 1 | 0.9906 |
| Sulfotep-ethyl | C8H20O5P2S2 | + | 323.03 | 323.03 | 0.1 | 1 | 0.9950 |
| Sulfuramid | C10H6F17NO2S | - | 525.9775 | 525.9779 | 0.7 | 1 | 0.9828 |
| Sulprofos | C12H19O2PS3 | + | 340.0623 | 340.0636 | 3.7 | 1 | 0.9950 |
| Tebuconazole | C16H22ClN3O | + | 308.1524 | 308.1522 | 0.7 | 1 | 0.9924 |
| Tebufenozide | C22H28N2O2 | + | 353.2224 | 353.2223 | 0.3 | 1 | 0.9946 |
| Tebufenpyrad | C18H24ClN3O | + | 334.1681 | 334.1679 | 0.4 | 1 | 0.9968 |
| Tebupirimphos | C13H23N2O3PS | + | 319.124 | 319.124 | 0.1 | 1 | 0.9953 |
| Tebuthiuron | C9H16N4OS | + | 229.1118 | 229.1119 | 0.5 | 1 | 0.9947 |
| Teflubenzuron | C14H6Cl2F4N2O2 | - | 378.967 | 378.9675 | 1.3 | 1 | 0.9785 |
| Tefluthrin | C17H14ClF7O2 | + | 419.0643 | 419.0635 | 1.9 | 50 | 0.9203 |
| Tembotrione | C17H16ClF3O6S | + | 458.0647 | 458.0649 | 0.5 | 10 | 0.9866 |
| Temephos | C16H20O6P2S3 | + | 484.0236 | 484.0236 | 0.1 | 1 | 0.9953 |
| Tepraloxydim | C17H24ClNO4 | - | 340.1321 | 340.1322 | 0.2 | 1 | 0.9947 |
| Terbacil | C9H13ClN2O2 | - | 215.0593 | 215.0596 | 1.3 | 1 | 0.9911 |
| Terbufos | C9H21O2PS3 | + | 289.0514 | 289.052 | 2 | 1 | 0.9928 |
| Terbufos oxon sulfoxide | C9H21O4PS2 | + | 289.0692 | 289.0691 | 0.4 | 1 | 0.9927 |
| Terbufos sulfone | C9H21O4PS3 | + | 338.0678 | 338.0678 | 0.1 | 1 | 0.9963 |
| Terbumeton | C10H19N5O | + | 226.1662 | 226.1664 | 0.7 | 1 | 0.9991 |
| Terbuthylazine | C9H16ClN5 | + | 230.1167 | 230.1168 | 0.5 | 1 | 0.9976 |
| Terbutryn | C10H19N5S | + | 242.1434 | 242.1434 | 0.2 | 1 | 0.9985 |

Chromatograms and calibration curves for eight representative pesticides are shown in Figure 3. For the concentration range studied (1-250 µg/L), limits of quantitation (LOQs) were estimated from triplicate injections (CV < 15%) of standard solutions at concentration levels corresponding to a signal-to-noise ratio of 10. As shown in Table II, LOQs ranged from 1-50 µg/L, and for 499 pesticides, LOQs were at or below 10 µg/L, the MRL imposed by EU and Japanese regulations.

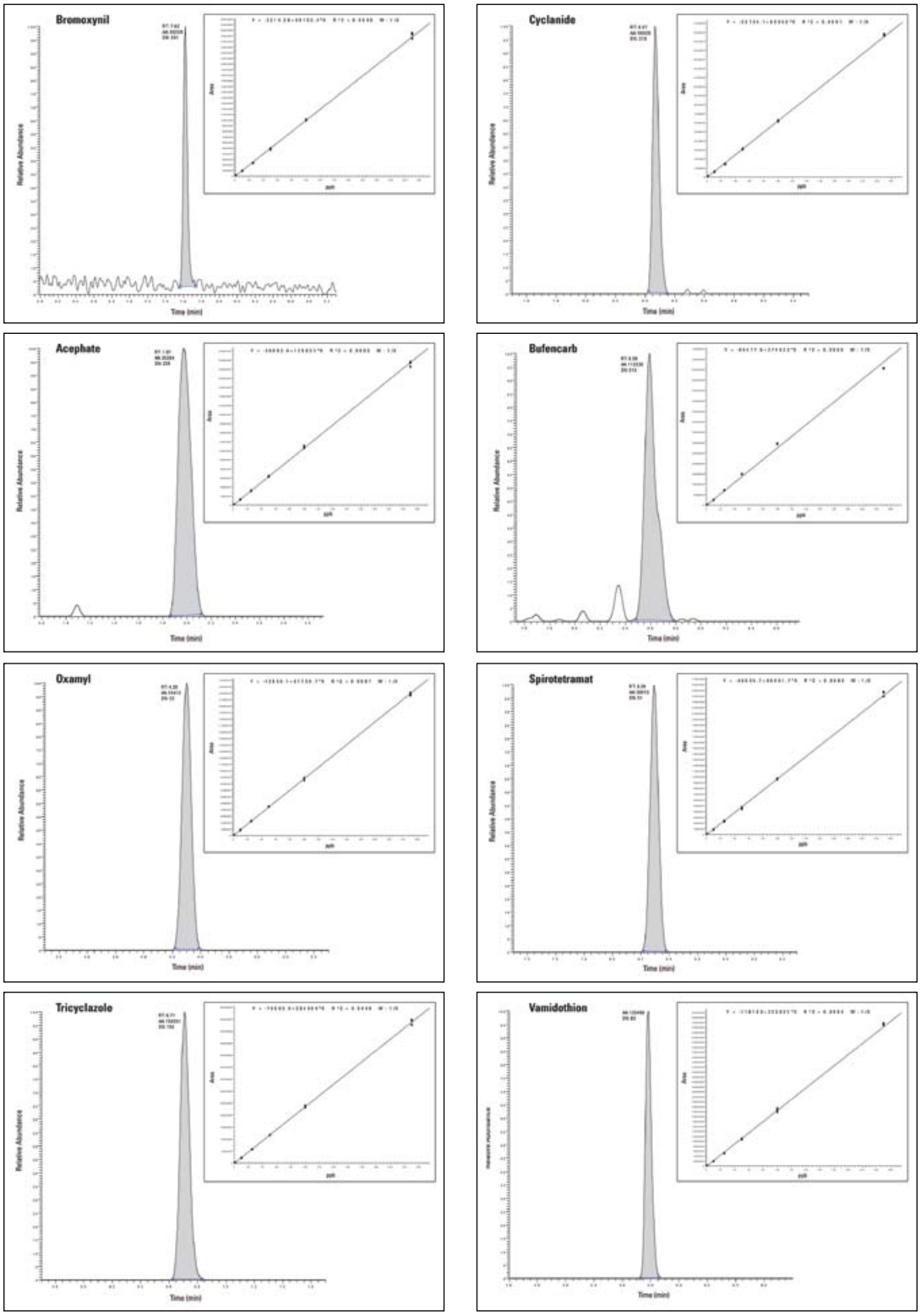


**Figure 3. Extracted ion chromatograms (at 1 µg/L level) and calibration curves (1-250 µg/L) of eight pesticides.**

To evaluate the applicability of this technique to complex food samples, U-HPLC-single stage Orbitrap MS was used to screen for pesticides extracted from a spiked spinach matrix. An extraction procedure based on fast and efficient QuEChERS methodology was used to facilitate rapid high-throughput multiresidue analysis. Table III summarizes this and mass spectral data obtained for a representative set of extracted pesticides. Extracted ion chromatograms and calibration curves for six pesticides extracted from the spiked spinach matrix are depicted in Figure 4. The detection and quantitation capabilities of this method were assessed using the EPA method detection limit (MDL) procedure [14]. For all pesticides, limits of detection (LODs) and LOQs were lower than 1 µg/L (Table III).

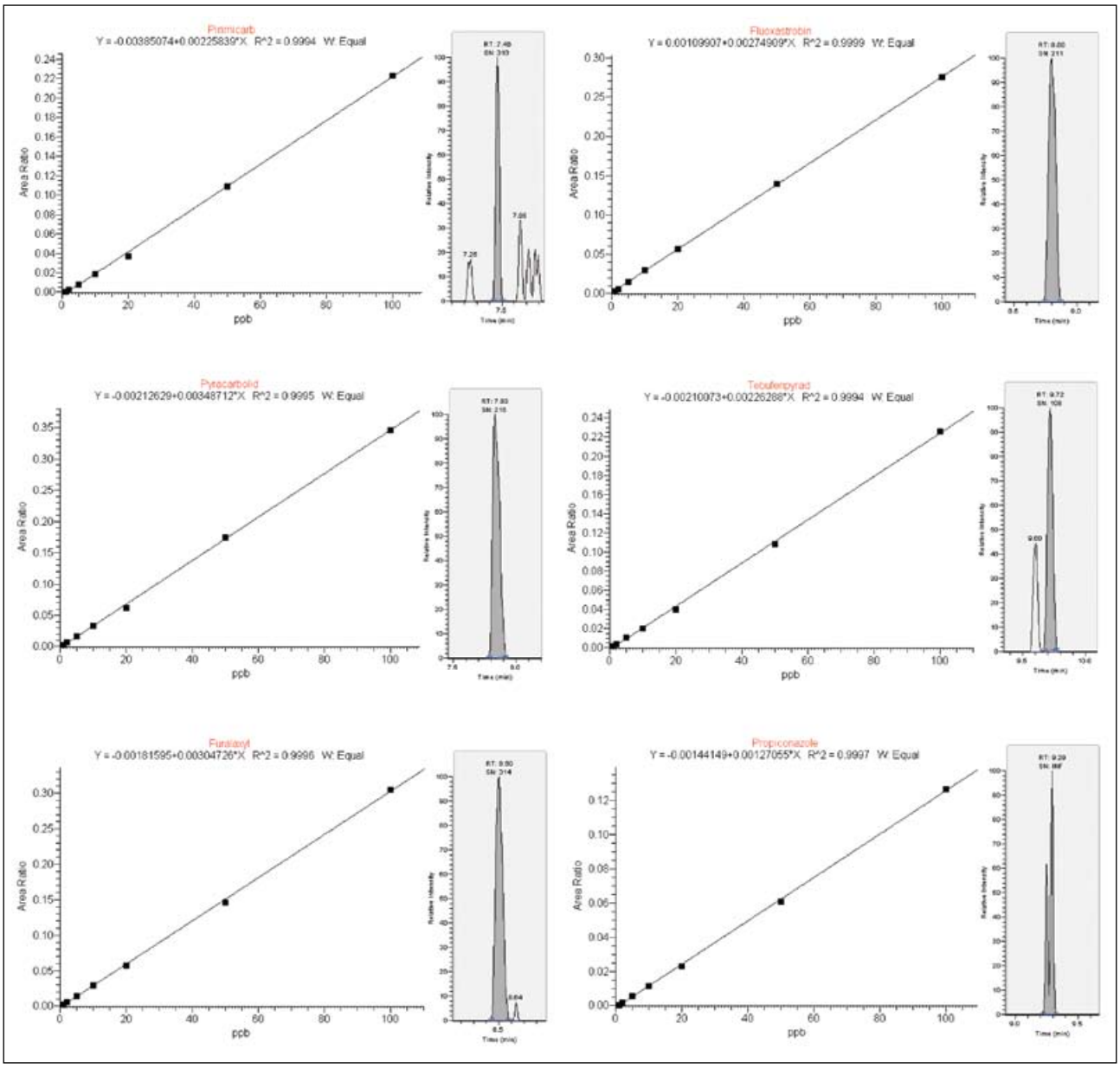


**Figure 4. Extracted ion chromatograms (at 1 µg/L) and calibration curves (1-250 µg/L) of six pesticides extracted from spiked spinach sample.**

**Table III. Molecular formula, theoretical and experimental mass, mass deviation, limit of detection (LOD) and limit of quantitation (LOQ) for representative pesticides extracted from spinach matrix. All MS data was obtained with Orbitrap MS operating in positive ion mode. LODs and LOQs were assessed using the EPA method detection limit (MDL) procedure [14].**

| Compound | Formula | Theoretical Mass (*m/z*) | Experimental Mass (*m/z*) | Mass Deviation (ppm) | LOD (µg/L) | LOC (µg/L) |
|---|---|---|---|---|---|---|
| Azoxystrobin | C22H17N3O5 | 404.1241 | 404.12466 | 1.4 | 0.2 | 0.7 |
| Bendiocarb | C11H13NO4 | 224.09173 | 224.09169 | 0.2 | 0.2 | 0.7 |
| Benthiavalicarb | C18H24FN3O3S | 382.15952 | 382.1597 | 0.5 | 0.2 | 0.7 |
| Benzoximate | C18H18ClNO5 | 364.09463 | 386.07663 | 0.2 | 0.2 | 0.5 |
| Bifenazate | C17H20N2O3 | 301.15467 | 301.15457 | 0.3 | 0.3 | 0.9 |
| Bupirimate | C13H24N4O3S | 317.16419 | 317.16431 | 0.4 | 0.2 | 0.5 |
| Buprofezin | C16H23N3OS | 306.16346 | 306.16354 | 0.3 | 0.2 | 0.6 |
| Butafenacil | C20H18ClF3N2 | 492.11437 | 492.11469 | 0.6 | 0.3 | 0.9 |
| Carbaryl | C12H11NO2 | 219.1128 | 219.1127 | 0.5 | 0.3 | 0.9 |
| Carbendazim | C9H9N3O2 | 192.07675 | 192.07684 | 0.5 | 0.2 | 0.7 |
| Carbofuran | C12H15NO3 | 222.11247 | 222.11241 | 0.3 | 0.2 | 0.7 |
| Carboxin | C12H13NO2S | 236.07398 | 236.07358 | 1.7 | 0.2 | 0.5 |
| Chlortoluron | C10H13ClN2O | 213.07892 | 213.07925 | 1.6 | 0.2 | 0.6 |
| Clethodim | C17H26ClNO3S | 360.13947 | 360.13962 | 0.4 | 0.2 | 0.6 |
| Clofentezine | C14H8Cl2N4 | 303.01988 | 303.01993 | 0.2 | 0.1 | 0.4 |
| Cyazofamid | C13H13ClN4O2S | 342.0786 | 342.077 | 4.7 | 0.3 | 0.8 |
| Cycluron | C11H22N2O | 199.18049 | 199.18054 | 0.3 | 0.2 | 0.7 |
| Cyproconazole | C15H18ClN3O | 292.12112 | 292.12115 | 0.1 | 0.2 | 0.7 |
| Cyprodinil | C14H15N3 | 226.13387 | 226.13385 | 0.1 | 0.2 | 0.7 |
| Diclobutrazol | C15H19Cl2N3O | 328.09779 | 328.09781 | 0 | 0.2 | 0.5 |
| Dicrotophos | C8H16NO5P | 238.08389 | 238.08391 | 0.1 | 0.3 | 0.8 |
| Difenoconazol | C19H17Cl2N3O3 | 406.07197 | 406.07251 | 1.3 | 0.2 | 0.6 |
| Dimethoate | C5H12NO3PS2 | 230.0069 | 230.00685 | 0.2 | 0.3 | 0.8 |
| Dimethomorph | C21H22ClNO4 | 388.13101 | 388.13113 | 0.3 | 0.3 | 0.9 |
| Dimoxystrobin | C19H22N2O3 | 327.17032 | 327.17047 | 0.5 | 0.2 | 0.5 |
| Dinotefuran | C7H14N4O3 | 203.11387 | 203.11389 | 0.1 | 0.2 | 0.7 |
| Dioxacarb | C11H13NO4 | 203.11387 | 224.09169 | 0.2 | 0.2 | 0.7 |
| Emamectin B1b | C49H75NO13 | 886.53112 | 886.53168 | 0.6 | 0.3 | 0.8 |
| Epoxiconazole | C17H13ClFN3O | 330.08039 | 330.08029 | 0.3 | 0.2 | 0.6 |
| Etaconazole | C14H15Cl2N3O2 | 328.06141 | 328.06143 | 0.1 | 0.3 | 0.9 |
| Ethiofencarb | C11H15NO2S | 226.08963 | 226.08969 | 0.3 | 0.3 | 0.9 |
| Etoxazole | C21H23F2NO2 | 360.17696 | 360.17715 | 0.5 | 0.1 | 0.4 |
| Famoxadone | C22H18N2O4 | 392.16048 | 397.11591 | 0.1 | 0.2 | 0.7 |
| Fenamidone | C17H17N3OS | 312.11651 | 312.11652 | 0 | 0.2 | 0.6 |
| Fenazaquin | C20H22N2O | 307.18049 | 307.18039 | 0.3 | 0.3 | 0.8 |
| Fenbuconazole | C19H17ClN4 | 337.12145 | 337.12128 | 0.5 | 0.2 | 0.6 |
| Fenoxycarb | C17H19NO4 | 302.13868 | 324.12073 | 0.3 | 0.1 | 0.4 |
| Fenpropimorph | C20H33NO | 304.26349 | 304.26349 | 0 | 0.1 | 0.3 |
| Fenpyroximate | C24H27N3O4 | 422.20743 | 422.20789 | 1.1 | 0.3 | 0.9 |
| Fenuron | C9H12N2O | 165.10224 | 165.10239 | 0.9 | 0.3 | 0.9 |
| Flufenacet | C14H13F4N3O | 364.07374 | 364.07401 | 0.7 | 0.2 | 0.6 |
| Fluometuron | C10H11F3N2O | 233.08962 | 233.08958 | 0.2 | 0.2 | 0.7 |
| Fluoxastrobin | C21H16ClFN4O5 | 459.0866 | 459.08704 | 0.9 | 0.3 | 0.8 |
| Flusiazole | C16H15F2N3Si | 316.10761 | 316.10776 | 0.5 | 0.2 | 0.7 |
| Flutolanil | C17H16F3NO2 | 324.12059 | 324.12073 | 0.4 | 0.3 | 0.9 |
| Flutriafol | C16H13F2N3O | 302.10995 | 302.10999 | 0.1 | 0.1 | 0.3 |
| Forchlorfenuron | C12H10ClN3O | 248.05852 | 248.05832 | 0.8 | 0.2 | 0.6 |
| Formetanate | C11H15N3O2 | 239.15025 | 239.15018 | 0.3 | 0.2 | 0.5 |
| Fuberidazole | C11H8N2O | 185.07094 | 185.07108 | 0.7 | 0.3 | 0.9 |
| Furalaxyl | C17H19NO4 | 302.13868 | 324.12073 | 0.3 | 0.1 | 0.4 |
| Hexaconazole | C14H17Cl2N3O | 314.08214 | 314.08206 | 0.3 | 0.2 | 0.7 |
| Hydramethylnon | C25H24F6N4 | 495.19779 | 495.19824 | 0.9 | 0.2 | 0.6 |
| Imazalil | C14H14Cl2N2O | 297.0556 | 297.05566 | 0.2 | 0.2 | 0.6 |
| Iprovalicarb | C18H28N2O3 | 321.21727 | 321.21744 | 0.5 | 0.1 | 0.4 |
| Isoproturon | C12H18N2O | 207.14919 | 207.14932 | 0.6 | 0.2 | 0.5 |
| Mefenacet | C16H14N2O2S | 299.08487 | 299.08484 | 0.1 | 0.2 | 0.7 |
| Mepanipyrim | C14H13N3 | 224.11822 | 224.11821 | 0.1 | 0.2 | 0.7 |

| Compound | Formula | Theoretical Mass (m/z) | Experimental Mass (m/z) | Mass Deviation (ppm) | LOD (µg/L) | LOC (µg/L) |
|---|---|---|---|---|---|---|
| Mepronil | C17H19NO2 | 270.14886 | 270.14886 | 0 | 0.1 | 0.1 |
| Metalaxyl | C15H21NO4 | 280.15433 | 280.15445 | 0.4 | 0.2 | 0.5 |
| Methabenzhiazuron | C10H11N3OS | 222.06956 | 222.06952 | 0.2 | 0.1 | 0.4 |
| Methamidophos | C2H8NO2PS | 142.00861 | 142.00865 | 0.3 | 0.2 | 0.5 |
| Methiocarb | C11H15NO2S | 226.08963 | 226.08969 | 0.3 | 0.3 | 0.9 |
| Methomyl | C5H10N2O2S | 163.05357 | 163.05357 | 0 | 0.2 | 0.6 |
| Methoprotryne | C11H21N5OS | 272.15396 | 272.15393 | 0.1 | 0.2 | 0.6 |
| Methoxyfenozide | C22H28N2O3 | 369.21727 | 369.21738 | 0.3 | 0.1 | 0.2 |
| Neburon | C12H16Cl2N2O | 275.07125 | 275.07126 | 0 | 0.3 | 0.8 |
| Oxadixyl | C14H18N2O4 | 279.13393 | 279.13397 | 0.1 | 0.1 | 0.4 |
| Penconazole | C13H15Cl2N3 | 284.07158 | 284.07153 | 0.2 | 0.3 | 0.8 |
| Pinoxaden | C23H32N2O4 | 401.24348 | 401.24393 | 1.1 | 0.1 | 0.1 |
| Pirimicarb | C11H18N4O2 | 239.15025 | 239.15018 | 0.3 | 0.2 | 0.5 |
| Promecarb | C12H17NO2 | 208.13321 | 208.13329 | 0.4 | 0.2 | 0.5 |
| Prometon | C10H19N5O | 226.16624 | 226.16623 | 0 | 0.2 | 0.5 |
| Prometryn | C10H19N5S | 242.14339 | 242.14348 | 0.4 | 0.2 | 0.5 |
| Propamocarb | C9H20N2O2 | 189.15975 | 189.15988 | 0.7 | 0.1 | 0.4 |
| Propargite | C19H26O4S | 189.15975 | 368.18933 | 0.9 | 0.2 | 0.6 |
| Propiconazole | C15H17Cl2N3O2 | 342.07706 | 342.077 | 0.2 | 0.3 | 0.9 |
| Pyrimethanil | C12H13N3 | 200.11822 | 200.11826 | 0.2 | 0.2 | 0.6 |
| Pyriproxyfen | C20H19NO3 | 322.14377 | 322.14392 | 0.5 | 0.2 | 0.6 |
| Quinoxyfen | C15H8Cl2FNO | 308.00397 | 308.00394 | 0.1 | 0.2 | 0.6 |
| Rotenone | C23H22O6 | 395.14891 | 395.14923 | 0.8 | 0.2 | 0.6 |
| Siduron | C14H20N2O | 233.16484 | 233.16492 | 0.3 | 0.3 | 0.9 |
| Simetryn | C8H15N5S | 214.11209 | 214.11174 | 1.6 | 0.2 | 0.4 |
| Spiroxamine | C18H35NO2 | 298.27406 | 298.27417 | 0.4 | 0.2 | 0.5 |
| Tebuconazole | C16H22ClN3O | 308.15242 | 308.15234 | 0.2 | 0.2 | 0.5 |
| Tebufenozide | C22H28N2O2 | 353.22235 | 353.22247 | 0.3 | 0.1 | 0.2 |
| Tebufenpyrad | C18H24ClN3O | 334.16807 | 334.16821 | 0.4 | 0.2 | 0.7 |
| Terbumeton | C10H19N5O | 226.16624 | 226.16623 | 0 | 0.2 | 0.5 |
| Terbutryn | C10H19N5S | 242.14339 | 242.14348 | 0.4 | 0.2 | 0.5 |
| Tetraconazole | C13H11Cl2F4N | 372.02881 | 372.02902 | 0.6 | 0.3 | 0.8 |
| Thiabendazole | C10H7N3S | 202.04334 | 202.04344 | 0.5 | 0.2 | 0.6 |
| Thiamethoxam | C8H10ClN5O3S | 292.02656 | 292.02655 | 0 | 0.3 | 1 |
| Thiobencarb | C12H16ClNOS | 258.07139 | 280.05246 | 3.1 | 0.3 | 0.8 |
| Triadimefon | C14H16ClN3O2 | 294.10038 | 294.10031 | 0.2 | 0.3 | 0.8 |
| Tricyclazole | C9H7N3S | 190.04334 | 190.04356 | 1.2 | 0.1 | 0.4 |
| Trifloxystrobin | C20H19F3N2O4 | 409.13697 | 409.13745 | 1.2 | 0.2 | 0.6 |
| Triflumizole | C15H15ClF3N3O | 346.09285 | 346.09302 | 0.5 | 0.1 | 0.2 |
| Triticonazole | C17H20ClN3O | 318.13677 | 318.13687 | 0.3 | 0.3 | 0.8 |
| Uniconazole | C15H18ClN3O | 292.12112 | 292.12115 | 0.1 | 0.2 | 0.6 |
| Vamidothion | C8H18NO4PS2 | 288.04876 | 288.04883 | 0.2 | 0.2 | 0.5 |
| Zoxamide | C14H16Cl3NO2 | 336.03194 | 336.03189 | 0.1 | 0.3 | 0.9 |

## Conclusion

A rapid and robust U-HPLC Orbitrap MS method for multiresidue pesticide screening was developed and validated. Screening of 510 pesticides at low µg/L levels was achieved within 12 minutes, and the high mass resolution and accuracy of the Orbitrap mass spectrometer enabled identification of all compounds. LOQs for the majority of pesticides in a standard mixture and in spiked matrix were lower than MRLs established by the EU and Japan. The U-HPLC Orbitrap MS platform is ideally suited for the routine monitoring of targeted and non-targeted pesticides by regulatory laboratories.

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# Overview of the Brazilian publications in analytical chemistry

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## Abstract

In this paper, Brazilian scientific production in analytical chemistry was researched. The work searched 45 international journals that publish research in analytical chemistry. The Web of Science database was used for data collection. The period studied was from 1945 to November, 2009. This search revealed 4461 papers, 3582 being articles (80.3%), 667 proceedings papers (14.9%), 102 notes (2.3%), 67 reviews (1.5%), and 43 letters, corrections, editorial materials etc. (0.9%). These publications resulted in 46,740 citations, with an average of 10.51citation per item and an H index of 65. This number is the greatest among the countries in South America and also compared to other countries including some of the European Community. This investigation shows that the journals more frequently chosen by Brazilians for publishing their work are: Analytica Chimica Acta (11.7%) and Talanta (10.0%). The evaluation of the distribution of the published papers for each Brazilian State showed that São Paulo leads the research in Analytical Chemistry in Brazil. However, the States of Bahia, Rio de Janeiro, Santa Catarina, Rio Grande do Sul, and Minas Gerais also have an expressive scientific output in analytical chemistry. Finally, it may be concluded that analytical chemistry research is growing throughout most Brazilian regions. Some papers are randomly cited in order to highlight some of the research in Analytical Chemistry performed by Brazilian groups.

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## 1. Introduction

Several papers have evaluated the evolution of the Brazilian analytical chemistry. One of the first was published by Neves in 1984[1], discussing mainly the situation of the area at the Universidade de São Paulo. Some publications were cited in order to characterize the research lines of this institution. In 1985, Senise presented a general description of the area in Brazil and he identified several research groups at 10 Brazilian universities of the followings Brazilian States: Bahia, Ceará, Distrito Federal, Minas Gerais, Paraná, Rio de Janeiro, and São Paulo[2]. Another paper, published one decade later evaluated quantitatively analytical chemistry developed in Brazil in the period from 1974 to 1994. The search involved 18 analytical chemistry journals, and also included 2 general journals published by the Brazilian Chemical Society, i.e., Química Nova and Journal of the Brazilian Chemical Society. This paper demonstrated that the publication of analytical chemistry in this period was mainly done in international journals[3]. De Andrade et al.[4] presented in 2004 an overview about the research on analytical chemistry in Brazil. The authors observed great evolution of this area and they suggested some possible routes for the future of the area in Brazil[4]. These data were updated in 2008 focusing on the period from 2004 to 2007. Relevant contributions performed by Brazilian analytical research groups were presented and criticized. This paper included 303 references[5]. Finally, in 2009 a paper was published showing that analytical chemistry in Brazil is healthy and growing and is exerting a positive effect on the economical development of the country[6].

Actually, analytical chemistry is one of the major areas of Chemistry in Brazil, with research groups working on the following representative research topics:

### Automation

It is one of the most consolidated research areas of analytical chemistry in Brazil. Methods involving flow injection analysis (FIA), sequential injection analysis (SIA) and other flow systems have been proposed for the determination of inorganic and organic species employing several instrumental techniques for detection[7-21].

### Chemometrics

The research activities involving chemometrics in Brazil

started in São Paulo State, however, in recent years it can be observed that researchers of several other Brazilian states are working on related topics. Papers were published about multivariate designs and optimization, classification and calibration, as well as the development of new chemometric methods[22-50].

#### Separation techniques

Chromatography in all its various form is intensely developed and used in Brazil. There are several consolidated research groups in a number of Brazilian States. This area has a huge impact in analytical laboratories in chemical industries for quality control and there are companies in different areas, such as petrochemical, foods and pharmaceuticals, which extensively use separation techniques[51-73].

Additionally, if we consider concentration and separation procedures there are several Brazilian research groups developing procedures for determination of inorganic and organic species in a plethora of sample types. These procedures have been established using separation techniques such as solid phase extraction (SPE), liquid-liquid extraction (LLE), cloud point extraction (CPE), and co-precipitation, and include knotted reactor systems, frequently associated with spectroanalytical techniques[74-80]. The papers listed show part of the contribution of Brazilian groups in this research line**.**

#### Spectroanalytical methods

There are many active groups in spectroanalysis in Brazil. Research involving conventional techniques, such as flame atomic absorption spectrometry (FAAS), electrothermal atomic absorption spectrometry (ET AAS), hydride generation atomic absorption spectrometry (HG AAS), cold vapor atomic absorption spectrometry (CV AAS), inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS), and other more recent techniques, for instance, high resolution-continuum source atomic absorption spectrometry (HR-CS-AAS) and laser induced breakdown spectroscopy (LIBS), are exhaustively used and some recent applications and developments can be seen in the cited papers[81-120].

#### Sample preparation

Brazilian groups have leadership in this area, particularly for total inorganic analysis based on microwave-assisted procedures. Systems involving microwave-induced combustion have also been developed. Additionally, procedures using ultrasound are also being investigated and several important contributions are described. The cited papers highlight some recent developments on this--topic[81-127.]

Recently, a book was edited emphasizing recent contributions in sample preparation strategies[131].

#### Electroanalytical methods

Electroanalytical chemistry is one of the most traditional sub-areas in Brazil and several research groups are developing new applications. Developments with various electroanalytical techniques, such as amperometry, chronoamperometry, chronocoulometry, conductometry, polarography, potentiometry, voltammetry, and related techniques have been reported[122-150].

#### Instrumentation

Several Brazilian groups in analytical chemistry have research activities involving the development of apparatus and low-cost equipment employing different detection techniques[151-161].

## 2. Methodology

### 2.1. Bibliographic search

This search was performed employing the Web of Science database. A total of 45 selected journals in analytical chemistry were selected as listed below. Search for each Brazilian State was done using the name of the state and its abbreviation as keywords, for example, for Bahia: Bahia or BA.

#### Selected analytical chemistry journals and its classification

**General Journals**

Analyst, Analytica Chimica Acta, Analytical and Bioanalytical Chemistry, Analytical Chemistry, Analytical Letters, Analytical Sciences, Canadian Journal of Analytical Sciences and Spectroscopy, Chemia Analityczna, Current Analytical Chemistry, Fresenius Journal of Analytical Chemistry, Journal of Food Composition and Analysis, Journal of Hazardous Materials, Journal of Analytical Chemistry, Microchemical Journal, Microchimica Acta, Quimica Analitica, Talanta and Trac-Trends in Analytical Chemistry.

**Spectroscopy journals**

Applied Spectroscopy Reviews, Atomic Spectroscopy, Journal of Analytical Atomic Spectrometry, Spectrochimica Acta Part B and Spectroscopy Letters.

**Electroanalytical journals**

Biosensors & Bioelectronics, Electroanalysis and Journal of Electroanalytical Chemistry.

**Separation journals**

Chromatographia, Electrophoresis, HRC-Journal of High Resolution Chromatography, Journal of Chromatographic Science, Journal of Chromatography A, Journal of Liquid Chromatography & Related Technologies, Journal of Separation Science and Separation Science and Technology.

**Environmental Chemistry journals**

Atmospheric Environment, Journal of Environmental Monitoring and International Journal of Environmental Analytical Chemistry.

**Automation journals**

Journal of Automated Methods & Management in Chemistry, Journal of Automatic Chemistry and Laboratory Robotics.

**Others journals**

Chemometrics and Intelligent Laboratory Systems, Journal of Chemometrics, Journal of Radioanalytical Chemistry, Journal of Radioanalytical and Nuclear Chemistry and Ultrasonics Sonochemistry.

## 3. Results and Discussion

### The publication of analytical chemistry in Brazil

The Web of Science was searched from 1945 to November, 2009, involving the 45 chosen journals as "publication name" and Brazil as "address". This search revealed 4461 papers, being 3572 articles (80.3%), 667 proceedings papers (15.0%), 102 notes (2.3%), 66 reviews (1.5%), and 40 letters, corrections, editorial material etc. (0.9%). These publications led to 46,740 citations, an average of citation per item of 10.51 and an H index of 65. Figure 1 shows the number of published items for the last 20 years. It can be seen that publication in analytical chemistry in Brazil is growing every year.

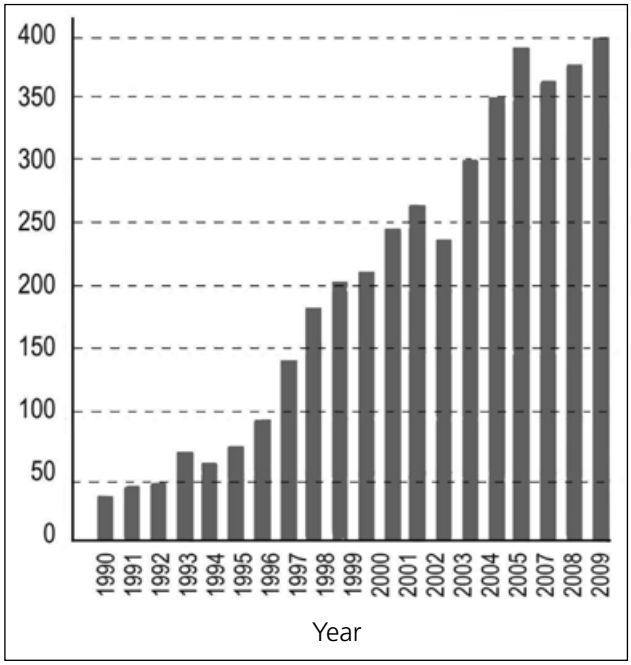


**Figure 1. Evolution of the number of papers published in the last 20 years.**

Figure 2 shows that the number of citations increases geometrically every year. These data also demonstrated that the publication of review papers for Brazilian groups should be incremented taking into account their importance in the literature.

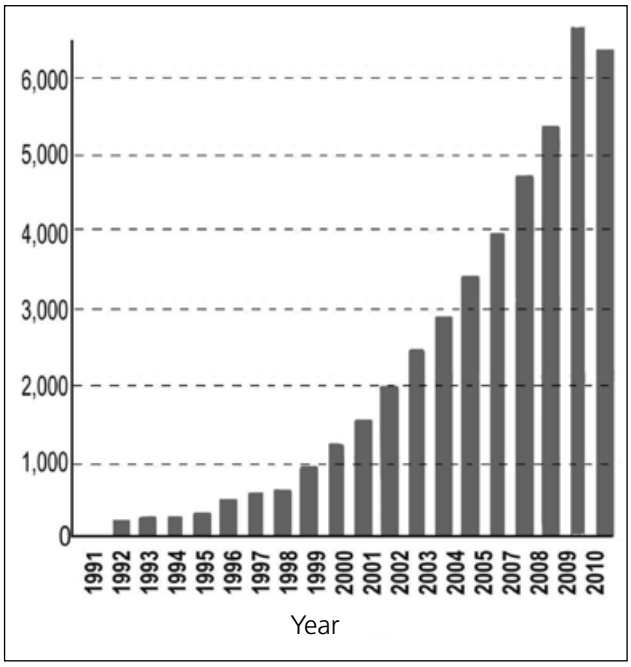


**Figure 2. Evolution of the number of citations of the papers published of the selected journals.**

In 1988 23 papers were published in the selected journals. Of these, 82% (19 papers) originated from São Paulo State. In 1998, 194 papers were published and 74% of these (144 papers) had authors from São Paulo State. In 2008, 384 papers were published, and 56% (214 papers) originated from São Paulo State. All these numbers confirm that publication is growing throughout the country. This evolution is a consequence of research funding by federal and state agencies. Particularly, some government actions have contributed decisively in the last 30 years to this strong development. The influence that the creation, in 1984, of the strategic program named PADCT (Action Program for Scientific and Technological Development), which established Chemistry and Chemical Engineering as priority areas for research funding, must be pointed out. In 1998, several other actions were taken to increase research funding in Brazil. The National Council for Scientific and Technological Development *(CNPq)* has been applying large volumes of resources in research and development in all areas of Brazil. Finally, the creation of the "*Portal da CAPES*" (www.periodicos.capes.gov.br) allowed easy access to information throughout the country, which became one of the greatest examples of free access to scientific publications provided by government funds [165].

The publication of analytical chemistry in each Brazilian State was also evaluated. The search was performed using the same journals as "publication source" and for

address the state name and state abbreviation (e.g. for Sao Paulo, Brazil and Sao Paulo or Brazil and SP). Data were collected for number of papers, number of citations, average citations per item published and H index (see Table I).

**Table I. Scientific production of Analytical Chemistry in some Brazilian States**

| State | Number of Papers | Number of Citations | Citation per papers | H index | Total of researchers with CNPq* | Category I researchers with CNPq* |
|---|---|---|---|---|---|---|
| São Paulo | 2,764 | 31,605 | 11.4 | 59 | 66 | 30 |
| Rio de Janeiro | 493 | 5,059 | 10.3 | 31 | 10 | 4 |
| Rio Grande do Sul | 404 | 3,028 | 7.5 | 24 | 15 | 4 |
| Bahia | 266 | 3,724 | 14.0 | 33 | 10 | 4 |
| Santa Catarina | 264 | 3,068 | 11.6 | 28 | 6 | 2 |
| Minas Gerais | 261 | 1,612 | 6.2 | 20 | 11 | 0 |
| Paraná | 195 | 1,340 | 6.9 | 17 | 5 | 0 |
| Maranhão | 58 | 722 | 12.4 | 17 | 2 | 0 |
| Pernambuco | 111 | 919 | 8.3 | 16 | 2 | 0 |
| Distrito Federal | 76 | 708 | 9.3 | 16 | 2 | 1 |
| Paraíba | 74 | 685 | 9.3 | 15 | 4 | 1 |
| Mato Grosso | 44 | 444 | 10.1 | 11 | 0 | 0 |
| Ceará | 40 | 188 | 4.7 | 8 | 2 | 0 |
| Alagoas | 27 | 131 | 4.9 | 7 | 0 | 0 |
| Pará | 15 | 114 | 7.6 | 7 | 1 | 0 |
| Rio Grande do Norte | 13 | 47 | 3.6 | 3 | 1 | 1 |
| Amazonas | 7 | 57 | 8.1 | 4 | 0 | 0 |

* Data collected in March, 2009.

These results demonstrated clearly that São Paulo State leads the research in analytical chemistry in Brazil. The number of universities and research centers developing research in analytical chemistry in this state justify these results. Moreover, the efficient and continuous support of the São Paulo Research Foundation (*Fundação de Amparo à Pesquisa do Estado de São Paulo, FAPESP)* has contributed to the dissemination of research in this State. However, it can also be seen that Rio de Janeiro, Bahia, Rio Grande do Sul, Santa Catarina, Minas Gerais and others also have a significant publication in analytical chemistry from Brazil. The number of citations and H index of these states clearly support this fact.

CNPq in 1976 established a fellowship program for researchers from all areas of science. Actually, these fellowships have two categories which are: Researcher 2 and Researcher 1, being that the category 1 has classification of 1D, 1C, 1B and 1A, from bottom to top, considering the scientific production of the researchers. In March, 2009, the total number of fellowships awarded was 11,456, with 604 for chemistry and 142 for analytical chemistry. Table I also shows the distribution of fellowship by Brazilian state. It can be observed that São Paulo has the largest fellowship number among the Brazilian states.

Another investigation evaluated the distribution of the papers published in Brazil in the 45 selected journals. Figure 3 shows the classification of these journals in terms of impact factor. It can be seen that most of the journals have impact factors between 2 and 3 (28%). Journals with impact factors from 3 to 4 and from 1 to 2 published 23 and 22% of the papers, respectively.

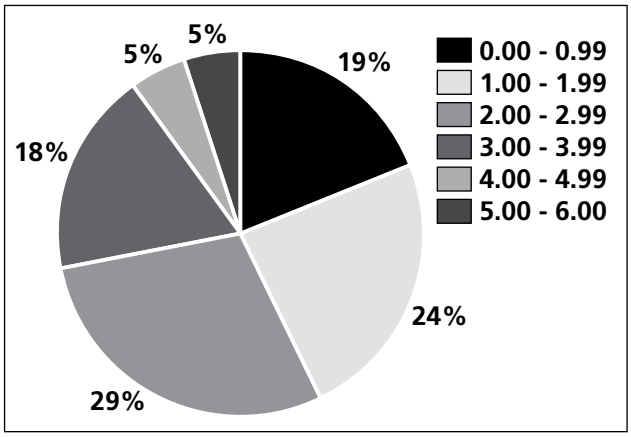


**Figure 3 - Classification of the selected journals in terms of impact factor**

The distribution of the 4461 papers, considering the impact factor of the 45 selected journals, was also evaluated. Figure 4 shows the classification of these papers in terms of impact factor of the selected journals. This demonstrates that most of the papers were published in journals with impact factors varying from 3 to 4 (38%, 1710 papers). Finally, it can be concluded that 70% (3148 papers) of the publications were in journals with impact factors greater than 2.

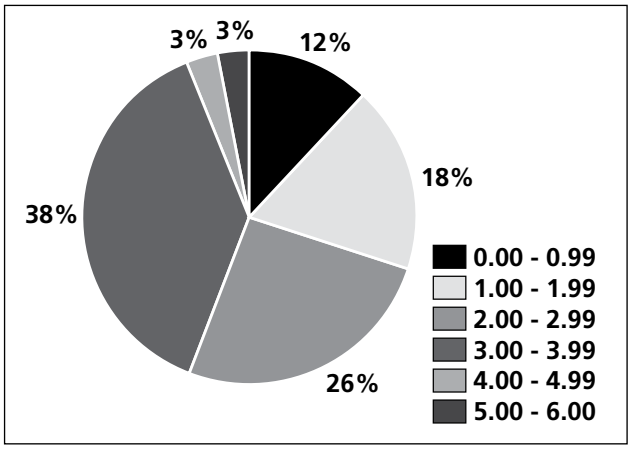


**Figure 4 - Classification of the papers published in terms of impact factor of the selected journals**

Table II presents the top 20 journals more frequently accessed by Brazilians groups for publication of their papers.

**Table II. Distribution of the papers among the selected journals**

| Journal | Number of Paper | % |
|---|---|---|
| Analytica Chimica Acta | 518 | 11.61 |
| Talanta | 445 | 9.98 |
| Journal of Radioanalytical and Nuclear Chemistry (JRNC) | 290 | 6.50 |
| Journal of Electroanalytical Chemistry (JEC) | 284 | 6.37 |
| Analytical Letters | 230 | 5.16 |
| Journal of Chromatography a | 217 | 4.86 |
| Journal of Hazardous Materials | 196 | 4.39 |
| Spectrochimica Acta Part B - Atomic Spectroscopy | 190 | 4.26 |
| Analyst | 165 | 3.70 |
| Electroanalysis | 144 | 3.23 |
| Atmospheric Environment | 137 | 3.07 |
| Chromatographia | 127 | 2.85 |
| Journal of Analytical Atomic Spectrometry | 119 | 2.67 |
| Spectroscopy Letters | 114 | 2.56 |
| Microchemical Journal | 111 | 2.49 |
| Analytical Chemistry | 99 | 2.22 |
| Analytical Sciences | 92 | 2.06 |
| Journal of Liquid Chromatography & related technologies | 90 | 2.02 |
| Analytical and Bioanalytical Chemistry | 89 | 2.00 |
| Journal of Food Composition and Analysis | 71 | 1.59 |

The 5 most used journals are: Analytica Chimica Acta (11.61%), Talanta (9.98%), Journal of Radioanalytical and Nuclear Chemistry (6.50%), Journal of Electroanalytical Chemistry (6.37%), and Analytical Letters (5.16%). Again, these results confirm the good performance of the Brazilian publications in analytical chemistry. These results also demonstrated that there is a balance between publication in major sub-areas, such as eletroanalytical methods, spectroanalytical methods and separation methods. The Brazilian journals Química Nova and Journal of Brazilian Chemical Society (JBCS) were not included in this search because both publish papers in all areas of chemistry. Food Chemistry, Analytical Biochemistry, Electrochimica Acta, Water Research, Journal of Thermal Analysis and Calorimetry, Journal of Pharmaceutical and Biomedical Analysis, Sensors and Actuators B: Chemical and Journal of Agricultural and Food Chemistry are journals that are frequently used for publication of analytical chemistry papers by Brazilians, but these journals are also used exhaustively by Brazilian researchers from other areas and, consequently, they were not included among the selected journals.

A refinement of the search involving the publication in analytical chemistry in Brazil based on institutions was also performed. Once again, a prevalence of the São Paulo State in publication of papers can be seen. The *Universidade de São Paulo (USP),* including its several institutes, i.e., CENA, Institute of Chemistry in São Paulo, Institute of Chemistry in São Carlos and Department of Chemistry in Ribeirão Preto, has obviously the greatest number of publications. The *Universidade Estadual de Campinas (UNICAMP)* has also a great contribution for Brazilian analytical chemistry. This demonstrates the participation that these institutions have in the training of human resources in analytical chemistry in Brazil.

### Comparison of Brazilian publications in Analytical Chemistry and other countries

A comparison between Brazilian publications in Analytical Chemistry and the publications of some other countries was performed using the 45 selected journals. Results are shown in Table III. It can be seen that Brazil has a good performance in publication, but surely there is room for growth and improvement its overall quality.

**Table III. Scientific production of Analytical Chemistry in Brazil and other countries**

| Country | Number of Paper | Number of Citations | Citation per papers | H Index |
|---|---|---|---|---|
| Brasil | 4 461 | 46 740 | 10.51 | 65 |
| Argentina | 1 427 | 16 620 | 11.65 | 48 |
| Uruguay | 75 | 581 | 7.75 | 13 |
| Chile | 526 | 5 273 | 10.02 | 30 |
| Venezuela | 462 | 5 695 | 12.33 | 34 |
| Canada | 8 481 | 182 754 | 21.55 | 138 |
| Portugal | 1 838 | 18 955 | 10.31 | 47 |
| Australia | 4 267 | 70 658 | 16.56 | 87 |
| Turkey | 2 722 | 25 133 | 9. 23 | 49 |
| South Africa | 1 128 | 12 609 | 11.18 | 44 |
| South Korea | 2 966 | 27 682 | 9.33 | 54 |
| Australia | 4 267 | 70 658 | 16.56 | 87 |
| Costa Rica | 17 | 151 | 8.88 | 7 |
| Egypt | 2 170 | 15 598 | 7.19 | 35 |

### Final Remarks

This paper studied the Brazilian publications in analytical chemistry considering the 45 main journals of this area. The results demonstrated the evolution of this subarea of chemical sciences in practically all of the country. The increase in the number of papers published and also in the cumulative citations clearly observed in the

results show this fact. It was also observed that most of the publications were in journals with impact factors greater than 2 (considering the impact index for 2009). Sao Paulo state has the largest production, but it can be seen that other Brazilian states are catching up and presenting major contributions for continuous development of analytical chemistry in Brazil.

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# SYNTHESIS, CHARACTERIZATION AND KINETIC STUDIES OF MIP - BASED BIOMIMETIC CATALYST FOR SELECTIVE SEROTONIN OXIDATION

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**ABSTRACT**
The aim of this study is the synthesis and characterization of a molecularly imprinted catalytically active polymer for the oxidation of serotonin (5-hydroxytryptamine, 5-HT). Fe(III)protoporphyrin(IX) (Hemin, Fe(III)PPIX) and methacrylic acid (MAA) were chosen as functional monomers, ethyleneglycoldimethyl methacrylate (EGDMA) as cross-linking monomer and 2,2'-azobisisobutyronitrile (AIBN) as initiator. Hemin was introduced in the synthesis of MIP to mimic the catalytic center of natural peroxidase, but also to play an essential role in the molecular recognition. The polymer formed was ground and sieved (106–150 μm) and subsequently treated with a methanol/acetic acid (4:1 v/v) solution, to remove serotonin from the polymer cavity. The characterization of the MIP (both unleached and leached) and NIP materials were carried out by IR, TGA, SEM, surface area and pore size analysis. In addition, selectivity studies were carried out. Based on the Michaelis-Menten approach, kinetic parameters including the values of maximal rate: $V_{max}$ (9.05 μA $cm^{-2}$) and apparent Michaelis–Menten constant, $K_m^{app}$ (818 μmol $dm^{-3}$), were obtained from the Lineweaver–Burk plots for the imprinted polymeric catalyst.

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**KEYWORDS:** Molecularly imprinted polymers; Hemin; Serotonin; Michaelis-Menten

## 1. INTRODUCTION

Molecular recognition for a target substrate and a high catalytic activity of enzymes in a biological system are the great interest. These enzymatic functions are created by well-organized three-dimensional structures. Native enzymes, however, exhibit many difficulties for practical use due to their sensitive properties such as instability against high temperature, organic solvents, and severe pH conditions, etc. Mimicking the highly organized structures in enzymes is one of the most challenging themes. Various complicated organic compounds possessing enzyme-mimic functions have been developed to overcome the drawbacks in enzymes. Recently, considerable attention has been given to the development of the molecular imprinting technique. This is a technology that allows the creation of molecular recognition sites in synthetic polymers, mimicking biological receptors [1-4].

Molecular imprinted materials, usually called molecular imprinted polymers (MIPs), have proven to be a very effective method to create three-dimensional binding sites in polymers [5-8]. It involves arrangement of functional monomers around a template molecule, with a subsequent polymerization trapping template molecules in a highly cross-linked polymer matrix. After removing the templates cavities possessing the shape and the arrangement of the functional groups remain imprinted in the polymer. Application of the molecular imprinting technique varies from polymer catalysts to sensor design, artificial receptors/antibodies and biomaterials for bioseparations [9-11].

The development of an efficient artificial enzyme by molecular imprinting has been one of the most challenging subjects in this area and many efforts have been devoted to achieve this goal [12,13]. In this sense, metalloporphyrins have been used as catalysts in MIP, mimicking the highly selective recognition features of biological receptors, with the advantages of lower price, and higher thermal and chemical stability [14,15]. Comonomers used in the preparation of MIPs are very important to get the properties mentioned above. Thus,

the technique of molecular imprinting can be applied to generate much more stable catalysts that mimic enzymes. For this purpose, in the present work, Fe(III) protoporphyrin(IX) (Hemin, Fe(III)PPIX) was used in the synthesis of MIP to get a synthetic material with peroxidase-like activity. The cavity left by the template can work as a nanoreactor mimicking the active site of peroxidase. Hemin may be found as a common catalyticly active site in hemoproteins like hemoglobin, myoglobin, cytochromes and peroxidases [16,17]. Iron porphyrins are biocatalysts, which catalyze oxidative reactions by decomposing hydrogen peroxide at the expense of various substrates, such as phenols and amides [18].

In the current work, a new type of MIP with considerable peroxidase-like activity was prepared with hemin as a co-monomer and serotonin (5-hydroxytryptamine, 5-HT) as a specific template/substrate. The synthesized materials were characterized using different techniques: BET surface area and pore size analysis, scanning electron microscopy (SEM), IR spectral analysis and thermogravimetry (TGA). Finally, parameters such as the specificity of the imprinted polymers towards serotonin and the apparent Michaelis–Menten constant were evaluated.

## 2. Experimental

### 2.1. Chemicals

The following reagents were used for the synthesis of serotonin-imprinted polymers by bulk polymerization: serotonin (5-HT) as template, hemin and methacrylic acid (MAA) as monomers, ethylene glycol dimethacrylate (EGDMA) as the cross-linking reagent, and 2,2´-azobisisobutyronitrile (AIBN) as initiator, all purchased from Sigma-Aldrich (Steinheim, Germany). Methanol and acetic acid were purchased from Tedia (Rio de Janeiro, Brazil). All solvents and other chemicals were of analytical grade. Tris-HCl purchased from Merck (Darmstadt, Germany) was used without further purification. In the selectivity studies the following structurally analogous phenol compounds were employed: 3,4-dihydroxy-α-(methylaminomethyl) benzyl alcohol (epinephrine), 3,4-dihydroxyphenethylamine hydrochloride (dopamine), (*R*)-4-(2-amino-1-hydroxyethyl)-1,2-benzenediol (norepinephrine) and 4-hydroxyaniline (4-aminophenol), all purchased from Sigma-Aldrich (Steinheim, Germany).

### 2.2. Preparation of molecularly imprinted polymers

The procedure adopted for the synthesis of the polymer based on the non-covalent approach, as well as the experimental details have been described in previous work [15]. The pre-polymerization mixture comprised of 5-HT (45.0 µmol) as template dissolved in 2 mL of chloroform/DMSO (1:1) in a thick-walled glass tube with 30.0 µmol of hemin and 450.0 µmol of MAA as monomer. Then, 7.95 mmol of cross-linking EGDMA, and 0.18 mmol of AIBN as initiators were added to mixture (see Figure 1). The mixture was purged with nitrogen in a glass tube for 15 min. The polymerization reaction was carried out at 60 °C for 24 h. The prepared polymer was ground and sieved by passing the milled polymer through a steel sieve to get particle sizes between 106 and 150 µm. After ending this step, the removal of the template from the polymer was carried out by using methanol/acetic acid (4:1 v/v) solution according to the previous work [15]. Finally, the polymer was dried at room temperature for 12 h and stored for further use.

The imprinting effect in MIP was evaluated by preparing corresponding blank polymer (*NIP – Non Imprinted Polymer*), which underwent the same protocol, but without adding 5-HT.

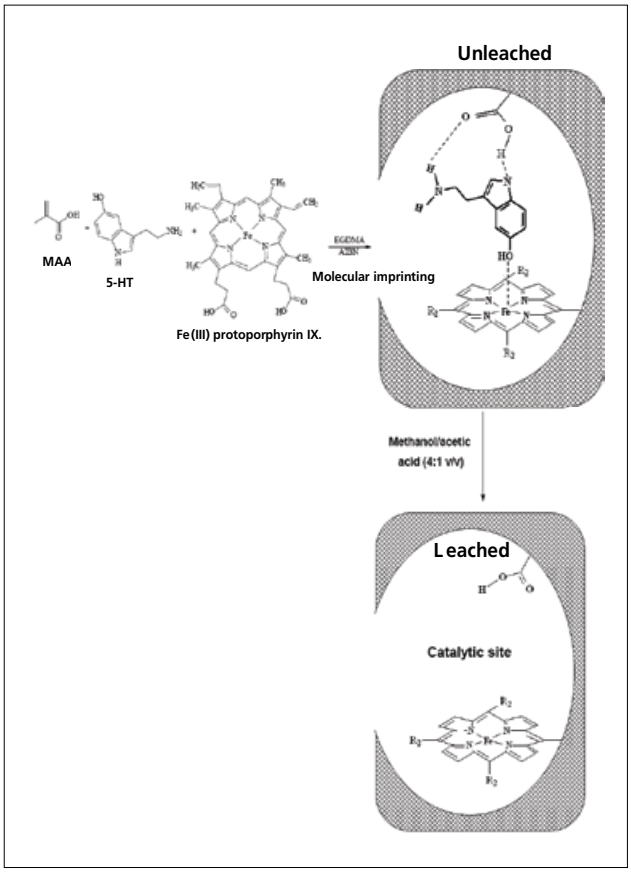



**Figure 1. The schematic representation of molecular imprinting and recognition for the 5-HT imprinted polymers.**

### 2.3. Characterization of MIPs

Surface area and pore volume of the MIPs were measured using an ASAP 2010 equipment (Micromeritics, Atlanta, USA). A quantity of 100 mg of dry polymer was used and degassed at 100°C under nitrogen flow for approximately 4 h prior to the measurement. The specific surface area was calculated using the Brunauer-

Emmett-Teller (BET) method. The pore size distribution was determined by the Barrett–Joyner–Halenda (BJH) method [19].

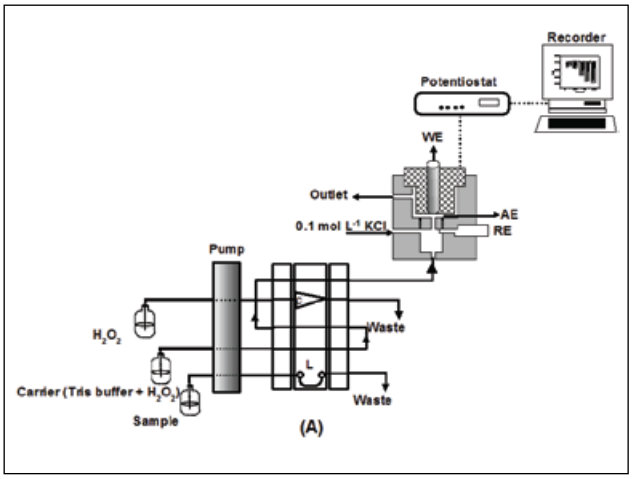
The morphological characteristics of MIP were evaluated by scanning electron microscopy (SEM), using a JEOL JSM-6360 LV scanning electron microscope (Tokyo, Japan) with an electron acceleration voltage of 20 kV. The samples were previously coated by a thin gold layer in a Bal-Tec MED 020 equipment.

IR spectra of grounded polymer were recorded on a Bomem Michelson MB-102 spectrometer using KBr pellets in the wavelength range of 400-4000 $cm^{-1}$.

The thermal analysis of polymer was carried out on a thermogravimetric analysis instrument model 2950 (TGA). About 5 mg of the grounded polymer was heated at a heating rate of 5°C $min^{-1}$ from ambient temperature up to 600°C under nitrogen atmosphere (flow rate = 20 mL $min^{-1}$) and the corresponding TG curve was obtained.

### 2.4. Amperometric measurements

Experiments were performed with an Autolab® PGSTAT-12 potentiostat (Eco Chemie B.V., The Netherlands). A wall-jet electrochemical cell containing a glassy carbon electrode as the working electrode, Ag/AgCl electrode as the reference and a platinum wire as the auxiliary electrode was used for the measurements (Figure 2). The solutions were propelled by a peristaltic pump from Ismatec (Zurich, Switzerland), with silicone tubes. A home-made injector of Teflon® (PTFE, polytetrafluoroethylene) was used for the sampling and injection steps. The minicolumn (3 cm in length) made of polyethylene with glass wool in both ends of the minicolumn was used to hold the MIP during the sampling and injection steps [10]. All experiments were carried out at room temperature by applying the intended operating potential. Currents were allowed to reach a stable baseline prior to the amperometric monitoring.



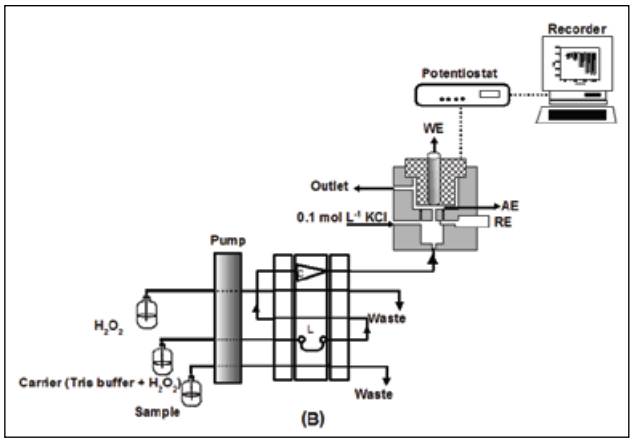




**Figure 2. Schematic diagram of the flow injection system for amperometric determination of 5-HT. (A) Sampling position. (B) Injection position. L = eluent loop; C = mini-column packed with 35 mg of MIP; WE = working electrode (glassy carbon); AE = auxiliary electrode (platinum); RE = reference electrode (Ag/AgCl).**

### 2.5. Catalytic recognition measurement of imprinted polymers

The catalytic recognition of MIP was studied using 500 µmol $L^{-1}$ of 5-HT in a flow injection analysis system coupled to amperometric detection with an applied potential of –100 mV *vs.* Ag/AgCl. The sampling step (Figure 2A) was carried out by percolating 310 µmol $L^{-1}$ peroxide solution through 35 mg of MIP packed into a minicolumn at a flow rate of 2.0 mL $min^{-1}$. During this step, the peroxide solution at pH 8.0 buffered with 0.1 mmol $L^{-1}$ Tris-HCl used as carrier solution flows towards the wall-jet electrochemical cell. After this step, the injector was switched to the injection position (Figure 2B), in which 200 µL from the sample loop were displaced by the carrier solution at a flow rate of 2.0 mL $min^{-1}$. The responses were evaluated by their peak heights, which were proportional to the analyte concentration in the sample.

### 2.6. Kinetic analysis

In order to verify the behavior of the system, kinetic parameters such as Michaelis–Menten constant ($K_m$) and maximum rate ($V_{max}$) were also evaluated by employing the Lineweaver–Burk graph [20].

## 3. Results and discussion

### 3.1. Preparation of the molecularly imprinted polymer

MIP as an artificial receptor was synthesized, as shown in the Experimental section, by bulk polymerization. In the present study, hemin was used as the catalytic center to mimic the active site of peroxidase. Due to the unique structural features in the hemin molecule, as well as the interactions between the functional monomer (MAA) and the template (5-HT), it was possible to prepare this new type of biomimetic nanoreactor for peroxidase based on molecular imprinting technology (Figure 1). Generally speaking, the most commonly used

functional monomer is MAA, which can form hydrogen bonds with template molecules prior to polymerization. The resulting specific and positioned interactions contribute to the MIP's selectivity.

As cross-linking reagents, EGDMA was used, since it has been used widely and successfully in the synthesis of MIP [7,21]. This compound has vinyl groups that are reactive with free radicals, cations or anions, participating in the polymerization reaction. The radical polymerization continues until the consumption of double bonds of the reactive system [22]. Thus, EGDMA acts as a structural monomer since it creates a three-dimensional polymeric structure that preserves the functional groups of the monomer in a fixed position complementary to the template molecule, ensuring rigidity and stability to the binding sites [21]. Therefore, EGDMA should be used in excess of the amount of functional monomer, so that the stability of the product is guaranteed [23].

The initiation of the polymerization process is only possible when free radicals are present in the reaction medium [24]. In this sense, the presence of AIBN as initiator was required as this substance can polymerize methyl methacrylate under thermal or photochemical conditions to give poly(methyl methacrylate) [25].

In addition, the porogens chosen for the synthesis were a mixture of DMSO and chloroform (1:1, v/v). These porogens were chosen due to their ability to dissolve 5-HT (and the other components of the polymerization mixture).

### 3.2. Characterization of unleached and leached imprinted polymer materials

#### 3.2.1. BET analysis

Porosity is an important factor as it can change the surface area of the material and thus increase the selective removal of serotonin ion from complex matrices. Typically, pore sizes have been separated into three categories by the IUPAC: micropores (<2 nm), mesopores (2–50 nm) and macropores (>50 nm) [23]. The average pore size diameter of the prepared NIP and MIP were 5.05 and 16.96 nm, respectively. The specific surface area (6.9 $m^2\ g^{-1}$) and porosity (0.03 $cm^3\ g^{-1}$) of the NIP are much smaller than the specific surface area (166 $m^2\ g^{-1}$) and the porosity (0.21 $cm^3\ g^{-1}$) of the MIP. As understood, the presence of template serotonin molecules in the polymerization medium resulted in a particles having larger surface area. This was confirmed with inactive synthesized MIP (molecularly imprinted polymer without hemin) particles, where the specific surface area and porosity were as 170 $m^2\ g^{-1}$ and of 0.20 $cm^3\ g^{-1}$, respectively, very similar to those observed for MIP.

#### 3.2.2. SEM analysis

The scanning electron microscope (SEM) was used to characterize MIP and NIP morphologically (Figure 3). The images show evident differences in the morphologies of the polymers. For both polymers, agglomerates of microparticles of different sizes were obtained. The particles present porous surfaces that play an important role in the adsorption process. Also, the roughness of the particle surface should be considered as a factor providing an increase in the surface area. In addition, these large mesopores reduce diffusion resistance and facilitate the mass transport because of high internal surface area.

The shape of the polymer NIP was more uniform, compact and smooth than the shape of polymer MIP, which had an irregular, rough morphology. The regular structure of the non-imprinted polymer is probably due to the lack of specific binding sites having been created for the templates.

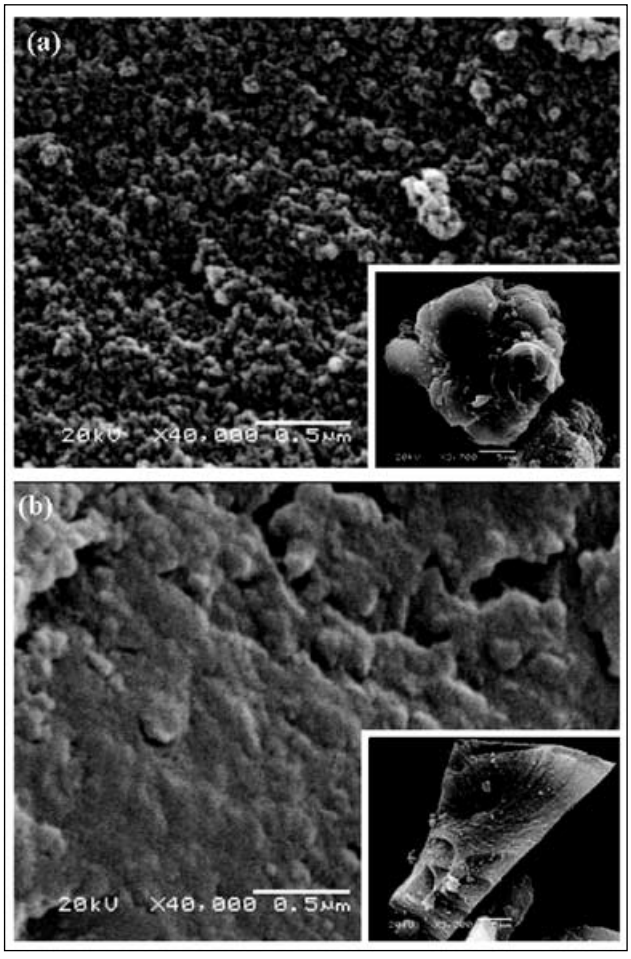



**Figure 3. Scanning electron micrographs of (a) MIP and (b) NIP particles.**

#### 3.2.3. IR spectra analysis

The IR spectra of unleached and leached serotonin imprinted polymer materials were recorded using the KBr pellet method (Figure 4). As observed, no band is

present in the region of 1648–1638 $cm^{-1}$ indicating the absence of vinyl groups in polymeric materials. This confirms the complete polymerization of vinyl groups. Meanwhile, other observations can be noticed, the IR spectrum of MIPs (3447, 2949, 1729, 1160 $cm^{-1}$) shows typical porphyrin pattern, which is consistent with the porphyrinic backbone of hemin. The IR spectrum of unleached-MIP (Figure 3a) (3447, 2949, 1729, 1160 $cm^{-1}$) is very similar to that of the leached-MIP (Figure 3b), indicating the similarity of the molecular structure [13].

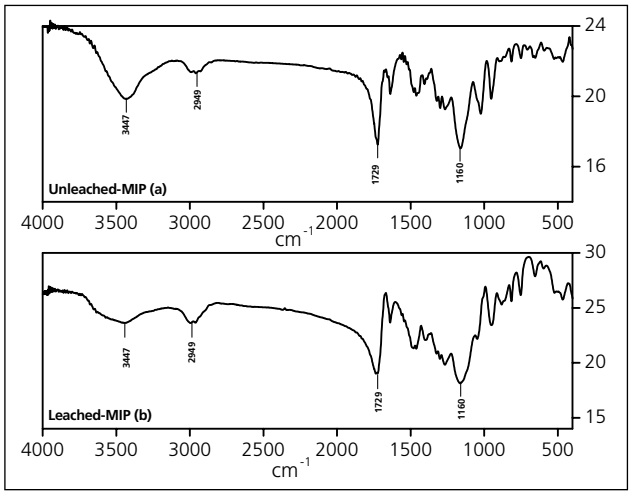



**Figure 4. IR spectra of unleached and leached 5-HT imprinted polymer.**

### 3.2.4. Thermogravimetry

Figure 5 shows the TGA plots of unleached and leached serotonin imprinted polymer particles. TGA plots with similar characteristics were obtained for unleached and leached polymer particles. These observations indicate the thermal stability of both leached and unleached serotonin imprinted polymer particles with decomposition above 250°C. Further, in the case of unleached serotonin imprinted polymer particles, the change in TGA plots at temperatures above 117°C is due to the decomposition of serotonin and possibly to the loss of residual solvent.

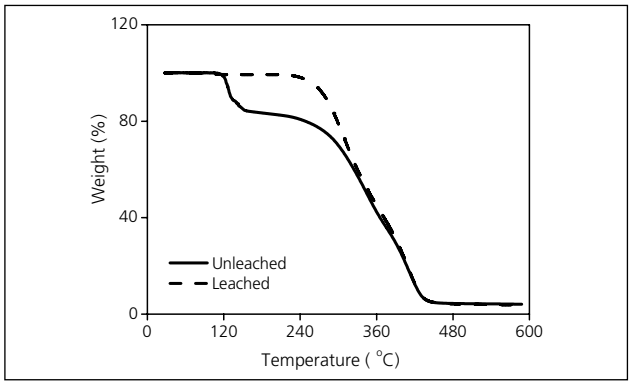



**Figure 5. TGA plots of unleached (A) and leached (B) 5-HT imprinted polymer.**

### 3.3. Catalytic recognition the MIP

Investigations were performed in order to evaluate the recognition ability of the MIP. In this way, the MIP sites were first activated by percolating a peroxide solution through 35 mg of MIP packed into a minicolumn (3 cm length). At the same time, the sample loop (200 µL) was filled with a standard solution of 5-HT (substrate) at 500.0 µmol $L^{-1}$ at pH 8.0, buffered with 0.1 mmol $L^{-1}$ Tris-HCl. By switching the injector position, 5-HT is inserted into the carrier flow, which undergoes oxidation at the active sites of the MIP (see Figure 6).

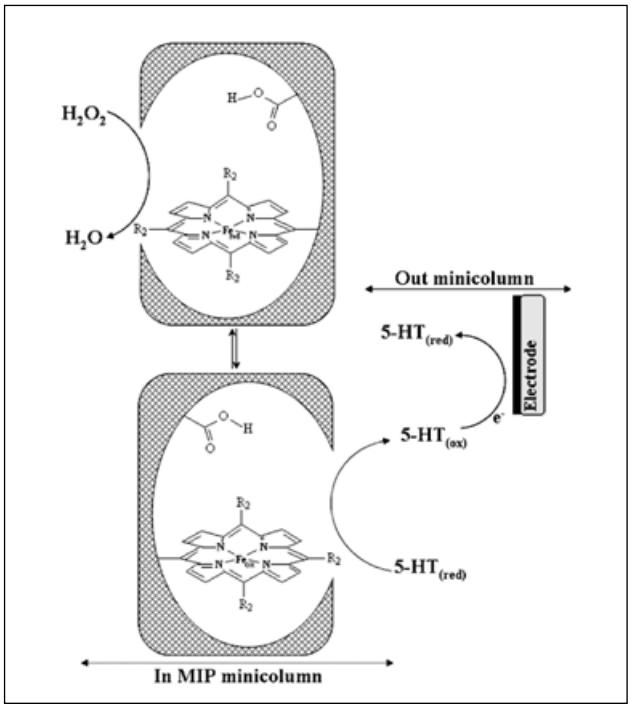



**Figure 6. Proposed mechanism of MIP activation in the column.**

Therefore, the MIP column is regenerated while the product of the oxidation of 5-HT is monitored by reducing it on the electrode. According to Figure 7, it was possible to observe a small reduction current without using the column, due to the interaction between peroxide and analyte. In addition, when the NIP or the inactive MIP was used in the column, a very small reduction current was observed, suggesting that the oxidation of 5-HT is not significant, even passing through the column. The low signal from the NIP can be attributed to the lack of molecular recognition of the template, because the NIP does not possess the selective cavities that would allow it to act as an active site, and/or to the low surface area. The behavior observed with inactive MIP showed that porosity (0.20 $cm^3\ g^{-1}$) and surface area (170 $m^2\ g^{-1}$) are not the principal characteristic responsible for the oxidation rate of 5-HT. A high reduction current was observed when the MIP was packed into the minicolumn, confirming the imprinting effect of the catalyst in the MIP. This is

confirmed by the catalytic effect of the prosthetic group and molecular recognition of 5-HT by the selective cavities imprinted in MIP.

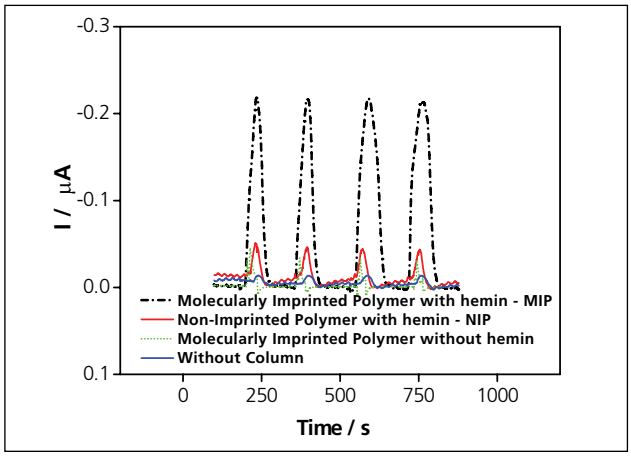



**FIGURE 7. AMPEROGRAMS FOR THE DETECTION OF THE PRODUCT OF THE OXIDATION OF 5-HT. MEASUREMENTS WERE CARRIED OUT IN 500 μMOL $L^{-1}$ 5-HT, [$H_2O_2$] = 310 μMOL $L^{-1}$, SAMPLE VOLUME = 200 μMOL, BUFFER SOLUTION FLOW-RATE = 2.0 ML $MIN^{-1}$, TRIS BUFFER SOLUTION = 0.1 MMOL $L^{-1}$, PH = 8.0, AND A MINICOLUMN PACKED WITH 35 MG OF THE MIP WAS USED.**

### 3.4. ANALYTICAL FEATURES AND KINETIC ANALYSIS OF THE IMPRINTED POLYMER

Under optimized conditions, a calibration curve was obtained for 5-HT concentrations ranging from 1.0 to 1000 μmol $L^{-1}$. The sensitivity was 0.4 nA/μmol $L^{-1}$, and a satisfactory correlation coefficient (r > 0.999) was observed. The detection and quantification limits expressed according to IUPAC recommendations were 0.30 and 0.98 μmol $L^{-1}$ [15].

The kinetic parameters of the imprinted polymer were evaluated based on the Michaelis–Menten kinetics. Figure 8 represents the Lineweaver–Burk plot for the MIP in the presence of different concentrations of 5-HT in 0.1 mmol $L^{-1}$ Tris-HCl buffer at pH 8.0. It is seen that a non linear dependence of the cathodic current on 5-HT concentration in the whole range from 50 to 1500 μmol $L^{-1}$ is obtained (insert Figure 8), as would be anticipated from a Michaelis-Menten type process.

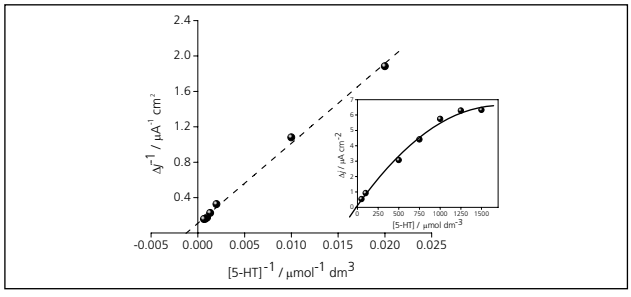



**FIGURE 8. LINEWEAVER–BURK PLOT FOR THE MIP IN THE PRESENCE OF DIFFERENT CONCENTRATIONS OF 5-HT IN 0.1 MMOL $L^{-1}$ TRIS-HCL BUFFER AT PH 8.0. INSERT: TYPICAL DYNAMIC PROFILE OF THE CATALYTIC REACTION OF 5-HT CATALYZED BY THE PEROXIDASE-LIKE MIP.**

The apparent $K_m$ value represents the affinity of the polymer for the substrate and a low value indicates high affinity. In the present case, $K_m$ for the MIP represents the affinity of the active site for the substrate 5-HT. Thus, in the Michaelis–Menten model initial rates of reaction are plotted against the substrate concentration (5-HT) based on a hyperbolic relation as follow:

$$\Delta j_s = \frac{V_{max}[5-HT]}{\left([5-HT]+K_m^{app}\right)} \quad (1)$$

In order to have a linear relation, the Lineweaver–Burk equation can be written as [26,27]:

$$\frac{1}{\Delta j_s} = \frac{1}{V_{max}} + \frac{K_m^{app}}{V_{max}[5\text{-}HT]} \quad (2)$$

where $j_s$ is the current density in the steady-state after the addition of substrate, *[5-HT]* is the substrate concentration in the bulk solution, $V_{max}$ is the maximum rate measured under saturated substrate conditions (i.e. maximum current density measured under saturated substrate conditions), and $K_m^{app}$ is the apparent Michaelis-Menten. A low $K_m$ value indicates a strong substrate affinity. A value of 818 μmol $dm^{-3}$ and 9.05 μA $cm^{-2}$ for $K_m$ and $V_{max}$ were obtained, respectively. The obtained $K_m$ value is smaller than those observed for peroxidase $K_m$ (1.5 mmol $L^{-1}$ for oxidation of phenolic compounds) [28], it indicates a potential for application of MIP-5-HT catalyst for analytical purposes, for example, serotonin determination in biological samples. However, in native enzymes many difficulties in practical use exist due to their sensitive properties such as instability against high temperature, organic solvents, and several different pH conditions, etc. Furthermore, the $K_m$ value for imprinted polymer is lower than those reported values in the literature for systems containing naturally immobilized peroxidases [29-36], giving evidence for the higher sensitivity and stability of the imprinted polymer. These results show that imprinted polymer could be used as a peroxidase mimicking catalyst, indicating that it could be an alternative for the peroxidase enzyme.

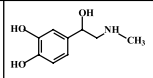
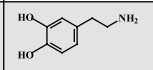
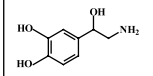
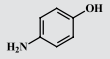
### 3.5. REPEATABILITY AND STABILITY OF THE MIP

The stability of the MIP was investigated. After successive measurements for a long period, no significant change was observed after 100 determinations. The precision of the responses expressed in terms of the relative standard deviation (RSD), were 1.3 and 1.7% for n = 6 analyses of standard solutions containing 50 and 750 μmol $L^{-1}$ 5-HT, respectively. This shows that MIP has good stability and reproducibility.

### 3.6. Selectivity studies of the cavities of the MIP

It is known that biomimetic polymers based on molecular imprinting have an inherent characteristic that is the selectivity. In order to verify the selectivity of biomimetic polymer for serotonin, the relative analytical signals of the MIP was obtained using some structurally related substrates (Table I). The selected substrates, including epinephrine, dopamine, norepinephrine and 4-aminophenol, have hydroxyl groups, which are also found in the template. The ability of these substrates to form hydrogen bonds with the selective cavity of the MIP would be similar to that of the template. However, the chemical structures of the substrates are different from the template in terms of size and shape. Thus, it is difficult for them to fit into the cavities formed by the extraction of the template. Notably, the MIP developed in this work exhibited substrate specificity toward the template molecule (5-HT), comparing the results shown in Table I. Therefore, the selectivity tests have further confirmed that the major contribution to the recognition ability of the imprinted polymers was the stereo-shape effect inherent in the MIP and the hydrogen bonding was not the main interaction between the template and the functional group.

**Table I. Relative analytical signals (in percentage) obtained for serotonin in the presence of molecules with similar structures.**

| Compounds | Structures | Mole ratio* | | |
|---|---|---|---|---|
| | | 1:1 | 1:5 | 1:10 |
| Epinephrine |  | 99.8±0.3 | 98.3±5.8 | 98.1±1.2 |
| Dopamine |  | 98.8±1.4 | 99.5±0.9 | 98.6±0.8 |
| Norepinephrine |  | 99.2±1.8 | 98.7±2.4 | 98.8±3.5 |
| 4-Aminophenol |  | 99.4±0.8 | 98.2±0.8 | 98.6±1.5 |

*Relative response (%) obtained with the MIP. Mole ratio = [5-HT]: [interfering compounds]. Values ± S.D. for three measurements.

## 4. Conclusions

Synthesis of a molecularly imprinted polymer as an artificial receptor that could specifically recognize and bind serotonin was achieved. In this sense, the results of this work demonstrated that the functions exerted by hemin as co-monomer can somewhat mimic the controlled interactions between metalloporphyrin and the surrounding protein in natural peroxidase, thus leading to considerable catalytic recognition along with ideal substrate specificity. In this context, hemin has proven to not only serve as the catalytic center but also to play an essential role in molecular recognition because of its unique and potentially useful framework for the artificial receptor. Physical and chemical characteristics of the polymer, such as pore surface area and pore size, SEM, FT-IR, and TGA, were found to be crucial for the application of the MIP that was demonstrated to be remarkably stable against mechanical stress, elevated temperatures and high pressure. Furthermore, the polymers can be used repeatedly, in excess of 100 times, without loss of the "memory effect", thus indicating good stability. Interference studies performed using phenolic compounds with similar structures to serotonin demonstrated the high selectivity of the MIP.

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# A sustentabilidade tem a Química como parceira

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

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Fábrica C

# Point of View

## The partnership university-industry and innovation in Brazil

Collaborative scientific research involving Brazilian public universities and sponsoring corporations, in Brazil, is taking on increased importance in the scope of this partnership. Public policies and new legislation aimed to increase the country's capacity to innovate, together with the growing challenges facing Brazilian corporations to develop innovative products, processes and services to compete in the markets, are the driving force behind this renewed importance. The challenge of both Brazilian universities' and corporations' strategies and efforts is to effectively contribute to build in the country a productive structure to support the internal economic and social development needs, and to compete internationally. The benefits and difficulties of this partnership are already well known in the countries that practice it more intensely, and that lead the economic paradigm where knowledge and innovation are central. Our experience is still incipient, and a more converging perception of the new potentialities and difficulties of a university-industry partnership is still building up following new policies and legislation. The recent Federal Innovation Law of 2004, together with new incentives and flexibilities, also introduced a new element of tension and potential conflict in those collaborative partnerships. The legal requirement that public universities should seek to protect its intellectual property rights (IPR) in all their activities, has magnified an issue of potential conflict in sponsored collaborative research and in its negotiarion with the business partners. Industry and business corporations seek to increase their competitiveness, and accordingly, prefer full property and secrecy or confidentiality, whereas academic institutions are committed to disseminating knowledge. In my view, the Innovation Law's requirement that all public universities and research institutions should establish units or offices of technology innovation represents an encouraging step towards the successful resolution of this issue how to reach a fair IPR treatment in sponsored research agreements.

**Roberto de Alencar Lotufo**
Full professor of Faculdade de Engenharia Elétrica e de Computação
State University of Campinas - UNICAMP

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## Eleita a Empresa Mais Inovadora do Brasil

***Prêmios Finep 2007 e Protec 2009***

*Sempre um passo à frente...*

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A recent survey developed by Accenture consulting and involving more than 250 CEO´s (Chief Executive Officer) showed **Innovation** will be at the core of Europe´s recovery. More than two thirds of the CEO´s surveyed said their companies were looking at innovation as a key to putting their businesses back on track. In the US 84% of CEO´s surveyed by Business Week & BCG (Boston Consulting Group) consider innovation important or extremely important in their ability to reap benefits of an economic recovery.

With the global advent, the innovation is also highly considered for most of companies in Brazil. We see this theme in a lot of magazines articles, books and even Awards have being created to disseminate this organizational phenomenon which involves strategy, discipline, people, knowledge and research, it is really a cultural change process. In order to achieve entirely the technological innovate goals many companies are establishing partnerships with academy and in some cases creating your own "academy" supported by experts from referential universities. This reveals the importance of academy and industry interaction and the awareness of the private sector about the value and knowledge contribution from academy expertise.

BrJAC comes to add a valuable contribution in the whole cooperation process between industry and academy and certainty in the technological innovation of our country. Most relevant is the noble role this cooperation brings: the quality life improvement and a better world. As a company which serves both academy and industry and make efforts to put their knowledge together in an ethical environment we are so proud to be part of this !

**Denise Estela Schwartz**
President Brazil & General Manager South America
PerkinElmer do Brasil

# Events 2010 / 2011

## FIMAI/SIMAI
**FEIRA INTL. DO MEIO AMBIENTE INDUSTRIAL E SUSTENTABILIDADE / SEMINÁRIO INTL. DO MEIO AMBIENTE INDUSTRIAL E SUSTENTABILIDADE**
São Paulo, SP – November 9th to 11th, 2010
http://www.crq4.org.br/default.php?p=eventos.php&id=41

## 2º ENQFor
**2º ENCONTRO NACIONAL DE QUÍMICA FORENSE**
Ribeirão Preto, SP – December 8th to 11th, 2010
http://sites.ffclrp.usp.br/enqfor/index.html

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

## PITTCOM 2011 - BRAZILIAN SESSION
**DEVELOPMENT OF INSTRUMENTATION AND CHEMOMETRY IN BRAZIL**
Atlanta, Georgia, USA - March 16th, 2011

## II SIGERA
**II SIMPÓSIO INTERNACIONAL SOBRE GERENCIAMENTO DE RESÍDUOS AGROPECUÁRIOS E AGROINDUSTRIAIS**
Foz do Iguaçu, PR – March 15th to 17th, 2011
http://www.sbera.org.br/sigera2011

## BIOCOM
**4º SIMPÓSIO NACIONAL DE BIOCOMBUSTÍVEIS**
Rio de Janeiro, RJ – April 11th and 12th, 2011
http://www.abq.org.br/biocom/

## SBBq
**XL REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE BIOQUÍMICA E BIOLOGIA MOLECULAR**
Foz do Iguaçu, PR – April 30th to May 3rd, 2011
www.sbbq.org.br

## VI CONGRESSO RIO AUTOMAÇÃO
**DESAFIOS DA INSTRUMENTAÇÃO E AUTOMAÇÃO NO PRÉ-SAL E NA CADEIRA DO PETRÓLEO E GÁS**
Rio de Janeiro, RJ – May 16th-17th, 2011
www.ibp.org.br

## 34ª RASBQ
**34ª REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE QUÍMICA**
Florianópolis, SC – May 23th to 26th, 2011
http://www.sbq.org.br/34ra/

## METALLOMICS 2011
**3rd INTERNATIONAL SYMPOSIUM ON METALLOMICS**
Münster, Germany - June 15 th - 18 th, 2011
www.metallomics2011.org

## SIMPEQUI
**9º SIMPÓSIO BRASILEIRO DE EDUCAÇÃO QUÍMICA**
Natal, RN – July 17 th to 19 th, 2011
http://www.abq.org.br/simpequi/

## II ICAP
**INTERNATIONAL CONGRESS ON ANALYTICAL PROTEOMICS**
Ourense – Espain - July 18th to 20th, 2011
http://sing.ei.uvigo.es/ICAP

## ENTEQUI
**4º ENCONTRO NACIONAL DE TECNOLOGIA QUÍMICA**
Rio de Janeiro, RJ – August 21st to 23rd, 2011
http://www.abq.org.br/entequi/

## PRE-SYMPOSIUM CSI
São Pedro, SP - August 24th to 26th, 2011
www.csixxxvii.org

## XXXVII CSI
**COLLOQUIUM SPECTROSCOPICUM INTERNATIONALE**
Búzios, RJ - August 28th to September 2nd, 2011
www.csixxxvii.org

## XVIII SIBEE
**SIMPÓSIO BRSILEIRO DE ELETROQUÍMICA E ELETROANALÍTICA**
Bento Gonçalves, RS - August 28th to September 1st, 2011.
http://www6.ufrgs.br/xviii-sibee/Index.html

## ANALÍTICA LATIN AMERICA
**2º CONGRESSO LATIN AMERICA**
**11th ANALITICA LATIN AMERICA**
São Paulo, SP – September 20th to 22nd
http://www.analiticanet.com.br/

## 16º ENQA
**16º ENCONTRO NACIONAL DE QUÍMICA ANALÍTICA**
Campos do Jordão, SP – October 23rd to 26th, 2011
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# Release

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

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

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**Equipe formada por mestres e doutores em Química Analítica.**

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

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  8. Sheldrick, G. M.; SHELXL-93; *Program for Crystal Structure Refinement,* Göttingen University, Germany, 1993.
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  9. Velandia, J. R.; *Doctorate Thesis*, Universidade Federal Rural do Rio de Janeiro, Brazil, 1997.
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  10. Ferreira, A. B.; Brito, S. L.; *Resumos da 20ª Reunião Anual da Sociedade Brasileira de Química,* Poços de Caldas, Brazil, 1998.
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