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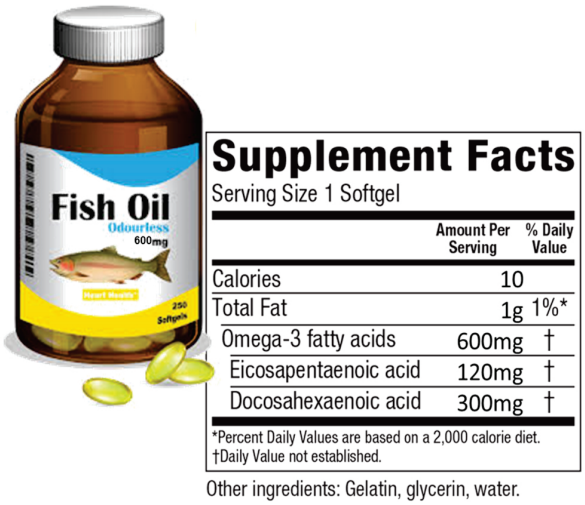
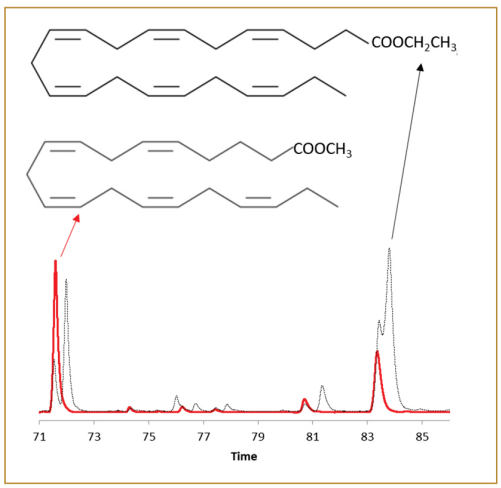
Omega 3 Fish Oil Evaluation





Evaluation of Delivery Form of Eicosapentaenoic and Docosahexaenoic Acids During Quality Control of Fish Oil Supplements

Tatiane Lima Amorim, Miguel Angel de la Fuente,
Marcone Augusto Leal de Oliveira, Pilar Gómez-Cortés

July – September 2020 Volume 7 Number 28

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The *Braz. J. Anal. Chem.* (BrJAC) is a peer-reviewed, open access, scientific journal intended for professionals and institutions acting mainly in all branches of analytical chemistry.

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Scope

BrJAC is dedicated to the diffusion of significant and original knowledge in all branches of Analytical Chemistry. BrJAC is addressed to professionals involved in science, technology and innovation projects in Analytical Chemistry at universities, research centers and in industry.

BrJAC is a quarterly journal that publishes original, unpublished scientific articles, reviews and technical notes that are peer reviewed in the double-blind way. In addition, it publishes interviews, points of view, letters, sponsor reports, and features related to analytical chemistry. Once published online a DOI number is assigned to the paper.

Manuscripts submitted for publication in BrJAC cannot have been previously published or be currently submitted for publication in another journal. For manuscript preparation and submission, please see the Guidelines for the Authors section at the end of this edition.

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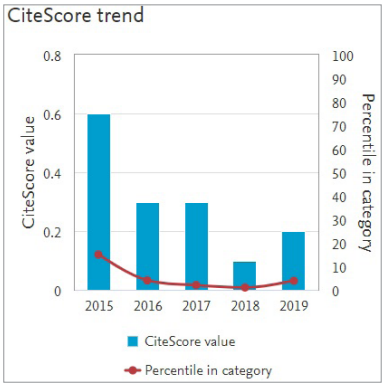

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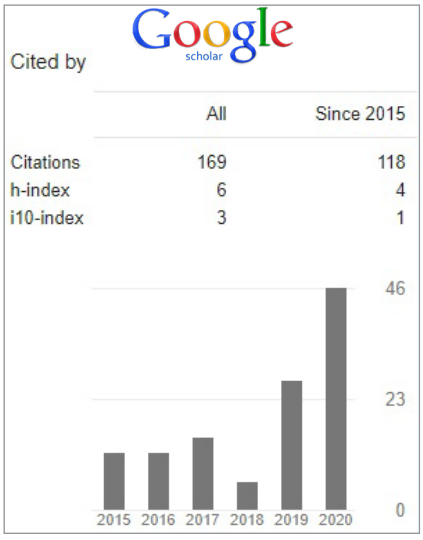

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EDITORIAL

Use of Measurement Uncertainty in Compliance Assessment with Regulatory Limits

Elcio Cruz de Oliveira

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Technical Consultant, Petrobras Transporte S.A. – TRANSPETRO ✉ Rio de Janeiro, RJ, Brazil

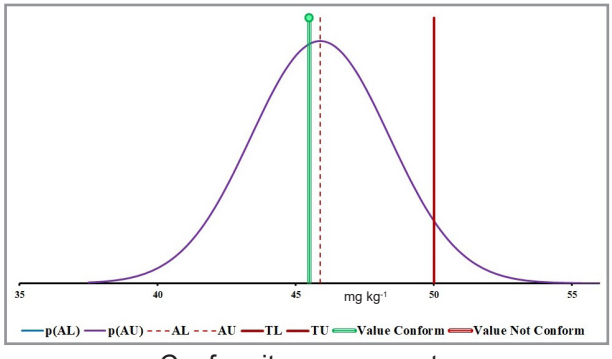
Measurement results, especially in Analytical Chemistry, must be presented in order to guarantee reliability, so that the information can be evaluated and compared with values provided in standards or regulatory limits. These measurement processes must be unambiguous so that, when reproduced anywhere in the world, metrologically compatible results are achieved.

To use a result and decide whether it indicates conformity or non-conformity with a specification, the recent literature recommends considering measurement uncertainty as the main indicator of the quality of any experimental result [1].

I highlight the importance of conformity assessment vis-à-vis the concepts of decision rules. Such rules are based on the level of acceptable probability for a wrong decision on the acceptance or rejection of a product, based on the measurement result accompanied by its uncertainty, reference risk (consumer, producer or shared), specification limits and guard band for conformity assessment against regulatory limits.

The greater the value of measurement uncertainty (sampling uncertainty plus analytical uncertainty) [2], the greater the proportion of samples that will be judged incorrectly. However, the lower the value of this variability, the greater the cost of the analysis. Thus, ideally, the quality of the measurement process should have an uncertainty value in order to balance the costs of analysis and incorrect decisions.

For instance, in the area of oil and gas, Brazil's National Agency of Petroleum, Natural Gas and Biofuels (ANP) regulates the quality of fuels. In the case of commercial gasoline, the upper limit for the specification of the sulfur mass fraction is 50 mg kg^{-1}. Considering a typical result of this critical parameter analyzed by ultraviolet fluorescence as 45.5 mg kg^{-1}, for a measurement uncertainty of 5 mg kg^{-1} (k = 2; 95.45%) and a risk of 5% for the consumer, is this item considered to conform or not?



Conformity assessment

p(AL) - probability density at the lower acceptance limit
p(AU) - probability density at the upper acceptance limit
AL - Lower acceptance limit
AU - Upper acceptance limit
TL - Lower tolerance limit
TU - Upper tolerance limit

Final conformity assessment: This item is conform to an effective Consumer's Risk of 5%.

Cite: de Oliveira, E. C. Use of Measurement Uncertainty in Compliance Assessment with Regulatory Limits. *Braz. J. Anal. Chem.,* 2020, 7 (28), pp 1-2. doi: http://dx.doi.org/10.30744/brjac.2179-3425.editorial.ecoliveira.N28

Finally, in order to leave no doubt concerning the decision rules, a clear and unequivocal method of decision-making should be stated, including the parameters mentioned above in detail. For advanced approaches, I recommend including repeated measurements, the detection and treatment of outliers and multivariate acceptance limits [3].

I am completely confident that BrJAC is in the correct way in order to become a renowned journal, since our publications are shedding light on the importance of Analytical Chemistry related to industrial activities, which can no longer be pushed aside. This issue highlights quality control and the optimization of industrial processes; I invite you to send us manuscripts correlating analytical data to regulatory limits and specifications. Let's think outside the box and understand that this approach continues be Analytical Chemistry!

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Elcio Cruz de Oliveira has a degree in Chemistry from the Rio de Janeiro State University (1990), a Master's degree in Metrology from the Pontifical Catholic University of Rio de Janeiro (2001) and a Doctoral degree in Analytical Chemistry from the Rio de Janeiro Federal University (2008). He is currently a Professor of the Postgraduate Programme in Metrology, Metrology for Quality and Innovation, Pontifical Catholic University of Rio de Janeiro, and a Researcher in Petrobras Transporte S.A. – TRANSPETRO. His research activities include all areas of chemical metrology, but mainly related to the oil & gas industry.

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INTERVIEW

A Model Professor Committed to Science and the Motivation of Young Researchers

Érico Marlon de Moraes Flores
Full Professor
Department of Chemistry, Federal University of Santa Maria
Santa Maria, RS, Brazil

Prof. Dr. Érico Marlon de Moraes Flores has a degree in Industrial Chemistry from the Federal University of Santa Maria (UFSM), Santa Maria, RS, Brazil, a master's degree in Chemistry from the same institution, and a doctorate in Metallurgical, Mining, and Materials Engineering from the Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil. He is currently Full Professor of the Department of Chemistry at UFSM and has been Director of the International Support Secretariat as Advisor to the UFSM Rector since 2018. He has been a Fellow of the Royal Society of Chemistry since 2016 and full member of the National Council of Brazilian Pharmacopeia (Brazilian Health Regulatory Agency, ANVISA) since 2002. He works mainly in research and technological development involving atomic spectrometry and the use of alternative energies, such as ultrasounds and microwaves for sample preparation, with applications in several laboratories and in the intensification of industrial processes. He has also worked in the quality control of pharmaceutical products, food, and nanomaterials and in extraction and analytical development for the determination of rare earth elements.

Prof. Érico Flores was Director of the Analytical Chemistry Division of the Brazilian Chemical Society (DQA-SBQ) from 2010 to 2012, its Deputy Director for two periods, from 2000 to 2002 and 2008 to 2010, and Secretary of the SBQ Regional of Rio Grande do Sul, Brazil. He was also Scientific Director of the Research Support Foundation of the State of Rio Grande do Sul (FAPERGS) from 2014 to 2019 and elected Vice-President of the IUPAC for the 2018–2019 biennium. He is a Full Member of the Brazilian Academy of Pharmaceutical Sciences, and since 2018 he has been Deputy Topic Leader of the Expert Working Group/EWG-Q3D, Elemental Impurities, appointed by ANVISA at the International Conference of Harmonization. He has received several awards at national and international scientific events, has given several lectures and courses in Brazil and in more than 20 countries, and has participated in the organizing and/or scientific committees of several national and international events.

Prof. Flores has more than 340 scientific articles published in international journals, with more than 6700 citations and an H-index of 40, in addition to several chapters of international books and a book published by Elsevier. He has national and international technological innovation patents and one in Germany, with the product sold in several countries. He has supervised more than 50 master's and 30 doctoral students. In the editorial field, Prof. Flores contributes as a reviewer to more than 40 scientific journals and is currently Executive Editor of Ultrasonics Sonochemistry (since 2018) and a member of the Editorial Board of the *Journal Analytical Atomic Spectrometry* (since 2012), *Atomic Spectroscopy* (2020), and the *Brazilian Journal of Analytical Chemistry* (2020).

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Would you tell us where you were born and how your childhood was?

I was born in Caxias do Sul, RS, Brazil, in 1966, as the second of three brothers. When I was a little over a year old, my father, who was a military man, was transferred to Santana do Livramento, RS, where we lived for 4 years, and shortly before I turned 6 years old, we came to the city of Santa Maria, RS, where I have lived since now. Despite the financial difficulties, I can say that I had a relatively peaceful childhood, which was due to the great sacrifice of my parents so that my brothers and I could have a good education. Today, I can clearly see the importance of parents encouraging their children to study with pleasure and to gain a good understanding of life.

What early influences encouraged you to study science? Did you have any influencers, such as a teacher?

Shortly before completing elementary school, I already liked the exact sciences, especially mathematics; although, I was almost obsessed with history, an area that I still like a lot and that is almost a hobby in the few spare hours I have. But it was at the beginning of high school that I really identified myself with chemistry and physics, and I was lucky to have excellent teachers in those areas. It would be unfair to mention just one influencer because at that time I had several teachers who were very committed to their students' educations. However, my main motivator was certainly my mother, who literally studied mathematics, physics, and chemistry during high school with me.

When did you decide to study chemistry? What motivated you? How was the beginning of your career?

As with most of my colleagues at the end of high school, I was very hesitant about which career to choose. Until the last day of registration, I could not decide between Chemical Engineering and Industrial Chemistry courses. I ended up choosing the latter, which I joined in 1983. At the time, the Industrial Chemistry course at the Federal University of Santa Maria (UFSM) was an effervescence course because it counted on the presence of professors from Germany in undergraduate classes through an agreement between UFSM and the German Technical Cooperation Agency (GTZ). It was during a General Chemistry class on the structure of the atom with the excellent professor Lademir D'Avila Cruspeire that I convinced myself that I would continue in the Industrial Chemistry course. I graduated in 1986, when I was 20 years old. During graduation, I was lucky to connect with extremely dedicated professors. I worked as a volunteer in two laboratories – one of organic chemistry and the other of inorganic chemistry – and in my second year of graduation I was selected to be a monitor in the Industrial and Environmental Chemistry Sector of the Department of Chemistry at UFSM. There, I had a fertile environment to work and learn, and I was fortunate to work with Prof. Dr. Berenice Roth and Dr. Leopoldina Keller, in addition to Prof. Dr. Ayrton F. Martins, who was a great motivator and who later would be my master's and doctoral advisor – I was his second master's student and his fourth doctoral student.

Even during my master's degree, when I was only 24 years old, I was already a substitute professor of Inorganic Chemistry at the Department of Chemistry, and the following year I was also a substitute professor in the Department of Physics. When I was 26 (1992), I passed a contest to become a permanent professor at the Department of Chemistry at the UFSM, where I have been a professor and researcher ever since.

What has changed in the student profiles, ambitions, and performance since the beginning of your career?

Over time, I think that the vision of science and the world has become not only broader but more profound and tolerant. However, my main ambitions remain the same. Although I have increasingly held management positions, I am still fascinated by teaching classes for undergraduate and postgraduate students. I always try to find time to talk to students and to discuss Chemistry, History, Society, and, of course, Analytical Chemistry.

Could you briefly comment on recent developments in analytical chemistry, considering your contributions?

There have been substantial advances in all areas of Analytical Chemistry, making it difficult to mention a single subarea. However, in general, there is a clear trend towards the development of faster and less invasive methods, with better detectability and selectivity. This is a growing need in the industry, and, in this regard, the development of portable systems that allow analysis *in loco* or *in situ* is a reality since they facilitate decision making in a safer and faster way. Another important aspect that is increasingly present in analytical protocols refers to the use of methods that are consistent with the principles of Green Chemistry, which presupposes, among other aspects, the use of increasingly smaller volumes and concentrations of reagents and, obviously, lower generation of laboratory waste (and, consequently, less need for waste treatment for disposal or eventual reuse).

What are your lines of research? You have published many scientific papers. Would you highlight any?

Despite mainly researching the development of analytical methods by atomic spectrometry, sample preparation procedures, the quality control of high purity materials, and the analysis of environmental samples, food, effluents, and various industrial matrices, in recent years I have dedicated myself to the development of systems that use alternative energies (microwaves and ultrasound) and that can be applied in the intensification of industrial processes in several areas, such as processing oil and derivatives and food and industrial waste.

Among the more than 340 scientific articles published in international journals, I could highlight my first article published in the *Journal of Analytical Atomic Spectrometry* (JAAS, 1997), resulting from my doctoral thesis. This article was written in partnership with my advisor, Prof. Dr. Ayrton F. Martins, and with my friend and also colleague at the time, Prof. Dr. Sergio R. Mortari. This was my first international article, which received much praise for the simplicity of the generation and introduction of hydrides in an atomizer, and this allowed greater visibility of our, at the time, still small, research group. After that article, many others were published, but it is worth highlighting our first article published in *Analytical Chemistry* (ACS, 2004) in partnership with professors Juliano S. Barin, Guenther Knapp, João Alfredo Medeiros, and José Neri G. Paniz. This article, the result of Prof. Juliano S. Barin's Master's thesis at UFSM, was a pioneer in demonstrating the feasibility of a combustion method involving a new principle, microwave ignition, which ended up being patented. The respective product is still commercialized in more than 20 countries and also used as reference method in pharmacopoeias and as reference method for the establishment of international certified reference materials.

… "one of the biggest challenges for scientific research in Brazil is to transform the knowledge generated into applications that impact the daily lives of different sectors of society, from the economy to social and environmental well-being."

Do you keep yourself informed about the progress of research in chemistry? What is your opinion about the current progress of chemistry research in Brazil? What are the recent advances and challenges in scientific research in Brazil?

Yes, I try to keep myself updated with the news and trends in Analytical Chemistry. Analytical Chemistry, although it is an ancient scientific area, took longer to establish in Brazil. However, it is currently one of the most vigorous fields and it has researchers with great international recognition, which can be seen by the growing number of Brazilian researchers acting as editors or in editorial boards of high impact international journals. There have been substantial advances in all areas of Analytical Chemistry, largely due to advances in microelectronics and new materials with properties that allow the construction of detectors, reactors, instruments, etc., with many advantages over the instruments produced a few years ago. Despite all these advances, one of the biggest challenges for scientific research in Brazil is to transform the knowledge generated into applications that impact the daily lives of different sectors of society, from the economy to social and environmental well-being. For this, we

will have to advance even more in the popularization of science, with the convergence of many academic themes with different demands from society, linking aspects related to technological innovation and entrepreneurship. Thus, different products, processes, and services can be generated through scientific research, which can impact society at the local, regional, national, or even international level.

For you, what have been the most important recent achievements in analytical chemistry research? What are the landmarks?

There have been many notable achievements, and it is impossible to select all without risking forgetting many, but I believe that recent developments in sample preparation methods with the application of systems that combine microwave and ultraviolet energies; the integration of nanomaterials with nucleic acids in biosensors; 3D printed biosensors for optical and electroanalytical applications; microstructure-based techniques for the analysis and manipulation of individual cells; infrared thermal imaging for fast and portable enthalpimetric analysis and the consequent appearance of different spectrometric methods for the generation of chemical images can be cited as interesting novelties.

There are in Brazil, and in the world, several conferences on Chemistry. To you, how important are these meetings to the scientific community? How do you see the development of national chemistry meetings in Brazil?

Despite the growing trend towards virtual meetings, there is a certain consensus that they are still unable to compete with all the advantages and dynamics of face-to-face meetings. In this regard, meetings of great international recognition are organized in Brazil, such as the biennial "Encontro Nacional de Química Analítica" (ENQA), which is in its 20th edition, and the Analitica Latin America Expo&Congress, which is one of the largest Analytical Chemistry meetings in the world today, with thousands of participants in all editions. Both congresses and exhibitions are events that allow the exchange and updating of information. I believe that this face-to-face model, despite recent restrictions due to the COVID-19 pandemic, should return strongly in the coming years.

You have already received some awards. What is the importance of these awards in the development of science and new technologies?

I think that the awards, in addition to the expected joy of those who receive them, are a motivator for students, especially the youngest. Regardless of value, the meaning of the award will always be a healthy stimulus for new generations as well as for scientists with a well-established career. This stimulus obviously has an important role in sharpening the creativity and advances of chemistry.

For you, what is the importance of the national funding agencies for the scientific development of Brazil?

National and state agencies are essential for scientific development. Of course, some adjustments and improvements are always welcome, and it is known that these agencies are constantly improving. However, it is amazing that, despite the obvious advantages of this type of investment, it has been increasingly necessary to clarify and convince most Brazilian managers and politicians of the need to invest in science, technology, and innovation. Such investment is always reverted in improvements for the society, and this has been demonstrated by all industrialized countries. I would like our government officials to take more account of the need to finance science and technology at levels that are at least similar to those of more developed countries.

At the moment, the situation for scientific research in Brazil is one of decreasing investment. How do you see this situation, and what would you say to young researchers?

This is a very difficult time for Brazilian science. The cuts in investments in science and technology have been brutal, and I don't remember so much disinvestment, at least in the last 20 years. This causes

disenchantment and discouragement in all researchers and brain drain to other countries, but it is mainly in the younger generations that this feeling is perceived. Brazil's investment in S&T is one of the lowest among developing countries, which is unacceptable for a country the size of Brazil and with a structure of highly qualified laboratories. I sincerely hope that this obscurantism in terms of understanding the importance of S&T has does not take long to pass. For now, I recommend that young people try to endure this situation and make sure that we will again have investments compatible with what is expected for Brazil.

What advice would you give to a young scientist who wants to pursue a career in analytical chemistry?

I believe that I would give the same advice that I've given to those who wanted to pursue other areas of science, that is, always keep up to date and always try to read what has already been published so as not to risk trying to rediscover old news. In addition, always have a look at other areas of science that may contribute to your specialty, never give up the care in carrying out everything you do and, especially, always be in a good humor. It is not possible to develop good science without a certain dose of good humor. Difficulties always occur, but with optimism and good humor things become easier, and it is not so difficult to deal with ridiculous setbacks, such as scientific denialism and obscurantism, which, from time to time, always end up appearing.

…"never give up the care in carrying out everything you do and, especially, always be in a good humor. It is not possible to develop good science without a certain dose of good humor."

How would you like to be remembered?

For always trying to improve our society by generating and spreading knowledge and for motivating students to believe in their potential and to pursue an academic or entrepreneurial career, but always based on real science never in pseudo or quasi-science. In addition, for having contributed to the consolidation of the following thought: "*if with science we can have problems, without it we would probably not be here today*".

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POINT OF VIEW

Analytical Chemistry and Materials Science: The Perfect Symbiosis to Improve Existing or New Methods

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A simple definition of analytical chemistry is a difficult task due to its broad scope in science. However, one of the most accepted definitions is "the art and science of determining the composition and structure of matter, by using knowledge of chemistry, statistics, computers and instrumentation" [1]. Of course, misleading definitions can be found by some scientists, which suggests that analytical chemistry is the simple application of chemical knowledge. If this is true, any chemist who makes qualitative and quantitative measurements can be considered an analytical chemist, a honest mistake. Hence, analytical chemistry may also be defined as what analytical chemists do, i.e., studies are performed to develop new analytical methods or to improve already established methods in order to solve analytical problems with different kinds of samples with outstanding precision, accuracy, detectability, selectivity, low cost, and less time.

Over the years, new analytical methods and techniques have been based on chemistry concepts and instrumentation involving optical, electrochemical, and separation phenomena. Classical colorimetric methods, analytical methods using bare electrodes, such as mercury, gold, carbon, and platinum, and atomic spectroanalytical techniques have been widely exploited by analytical chemists. However, until recently, analytical difficulties and challenges hold on, such as chemical speciation studies, analysis of biological samples without matrix effects, enantiomeric analysis, and analysis without sample pretreatment, which require deeper and broader knowledge of the analytical chemist. In this sense, connecting materials science research with analytical chemistry has increased, and this is key to improving new or existing techniques for overcoming analytical challenges [2,3]. Notwithstanding, in my opinion, analytical chemists should expand their knowledge in materials science to avoid mere application without scientific rigor. The ability to control the physico-chemical properties of materials by studying different synthesis processes, as well as minimum knowledge of characterisation tools, are highly recommended for better insight into the structure and applicabiltiy of materials for analytical purposes. Considering that the analyses are carried out at the molecular level, the physico-chemical nature of elements, the material morphology, and the kind of interface of materials as signal transducer of sensors or in chromatography stationary phases facilting selective partition also are parameters that play a crucial role to overall performance of analytical techniques. Additionally, it is highly necessary to understand a set of techniques, such as Fourier Transform-Infrared (FT-IR), Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), X-ray Diffraction, Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC), Nuclear Magnetic Resonance (NMR), X-ray Photoelectron Spectroscopy (XPS), Atomic Force Microscopy (AFM), elemental analysis, porosimetry, etc.

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In Brazil, the number of research groups composed of analytical chemists developing stationary phases for chromatographic applications, new material-based optical and electrochemical electrodes, and solid-phase-extraction-based methods has been increasing. A wide range of materials can be cited, such as carbon-based nanomaterials, including carbon nanotubes, black carbon, and graphene; metallic nanoparticles; chemically imprinted polymers; hybrid materials; metallic quantum dots; carbon quantum dots; magnetic particles; metal–organic frameworks; and others. Several successful studies have been carried out by Brazilian analytical chemists for the analysis of complex samples. In the study reported by Barbosa et al. [4], oxidised carbon nanotubes covered with layers of bovine serum albumin, so-called restricted-access carbon nanotubes (RACNTs), were employed to preconcentrate Pb^{2+} directly from untreated human blood serum while excluding all serum proteins, with further metal determination by Thermospray Flame Furnace Atomic Absorption Spectrometry (TS-FF-AAS). Suquila and Tarley [5] synthesised restricted-access copper-imprinted poly(allylthiourea) for the preconcentration of Cu^{2+} from milk samples using an Flow Injection Analysis-Flame Absorption Atomic Spectrometry (FIA-FAAS) system. Such methods, which were the first to show the development of restricted-access adsorbents for metal ions, are characterised by minimal sample manipulation, cost-effectiveness, and simplicity. Wong and co-workers used a graphite pencil electrode modified with palladium nanoparticles (PdNPs) for the simultaneous determination of tryptophan, carbendazim, and direct yellow 50 in environmental and biological samples [6]. The PdNPs improved the simultaneous and sensitive determination of analytes in environmental and clinical samples. Studies focused on the synthesis of hybrid monolith columns for capillary liquid chromatography, with good mechanical properties, stability in a wide pH range, and little swelling effect, have been reported [7]. Carbon dots synthesised from citrate have been used as a fluorescent probe for quercetin determination in tea and beer samples [8]. Simplicity, high selectivity towards possible interferences that could affect the analysis in real samples, and high sample throughput were the highlights of the proposed method. These examples show that different classifications of materials have triggered the development of new analyses with outstanding analytical performance, and Brazililan scientists have greatly contributed to the development of new knowledge.

In conclusion, in my opinion, it seems clear that materials science must be greatly exploited as a frontier of chemical and analytical chemistry knowledge, but commonly, and as expected, analytical chemists have been the protagonists. Thus, by being a multidisciplinary science, collaborative studies involving material scientists and analytical chemists should be encouraged.

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LETTER

Confocal Raman Microscopy: Tablet Mapping Application for the Pharmaceutical Industry

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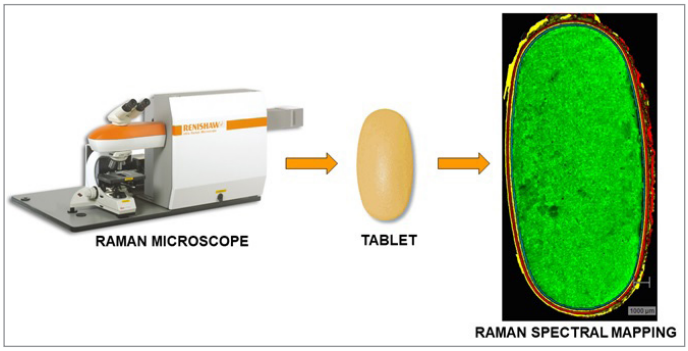

Background

In the United States of America (USA), more than 15 million people perform medical treatments every day using non-steroidal anti-inflammatory drugs (NSAIDs) for pain or inflammation. Although NSAIDs are widely considered effective substances in medical clinics, their administration may lead to the development of gastroduodenal lesions, such as ulcers and erosions [1-5].

Over time, one of the strategies implemented to reduce the gastrointestinal risk associated with the intake of NSAIDs is the prescription of drugs that inhibit stomach acid secretion, ie, the pharmacological class of proton pump inhibitors [1–5]. In addition, the World Health Organization (WHO) classifies chronic inflammatory diseases as a major threat to human health [1–5]. Thus, focusing on improving tolerability to NSAIDs formulations by combining esomeprazole magnesium and naproxen, producing the desired pharmacodynamic response, is becoming more common in the pharmaceutical industry [1–5].

Applying preformulation studies in the early stages of the development of a reference product or generic product enables better knowledge focused on both the optimised manufacturing process and the high potential for clinical success [6–12]. Therefore, an effective strategy is based on the characterisation of the active pharmaceutical ingredient (API) and excipients, providing insight into the physicochemical properties such as incompatibilities, detection of impurities, polymorphic transitions and design of formulations with a pharmacological approach in which esomeprazole magnesium could be added in the coating of the tablet it would be released first in the stomach to reduce the amount of acid produced, while naproxen protected by a layer of excipient resistant to the acidic environment of the stomach, could be released in the small intestine, thus reducing possible damage to the stomach by its action, so contributing only to its therapeutic anti-inflammatory, analgesic and antipyretic effect [6–12]. One of the biggest challenges in the formulation design is to achieve stability in the finished product; therefore, it is important to study the compatibility between more than one API when there is an association in the product beyond the excipients used in the formulation. This enables the selection of the most stable pharmaceutical composition [6–12].

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Chandrasekhara Venkata Raman was an Indian physicist who was awarded the 1930 Nobel Prize in physics for his work on light scattering and the discovery of the Raman Effect [13–20]. When the incidence of photons occurs in a sample, there is a loss or gain of energy. The loss of energy of the incident photon is a stronger effect, and this phenomenon is known as Stokes scattering. In contrast, when the incident photons gain energy, this is known as anti-Stokes scattering [13–20]. Within the pharmaceutical industry, almost all samples have spectra that follow Stoke dispersion, which mainly involves the vibrational modes of the molecules [13–20].

Confocal Raman microscopy (CRM) technology is a powerful ally in the development of pharmaceutical compositions through the investigation of the chemical composition of a sample and the particle size distribution of the components in the formulations and through the characterisation of the homogeneity of the products and the interaction of active substances and excipients. The chemical information obtained by CRM is also useful for the design of new molecules for the development of solids, semi-solids, and liquids, contributing to a comprehensive understanding of a pharmaceutical product and its development [21].

Raman Imaging

A Renishaw inVia Confocal Raman Microscope equipped with 785 nm laser excitation and StreamLine™ fast imaging capability was used to analyse and compare reference and generic tablets.

Reference samples were supplied. Raman spectra were collected from the majority of references (especially the API species) for the purpose of generating chemical images of the different species. Some excipient spectra were used from the Renishaw excipient database.

StreamLine imaging was used to produce Raman map data from the sectioned surface of the tablets. Data was collected from both a small region around the coating (3.5 µm step size) and the entire surface (35.5 µm step size). This provides an ideal balance of high-resolution coating analysis and context with the entire section.

Spectral map data was analysed using a direct classical least-squares method incorporating the reference spectra to produce chemical images of the different species.

For each sample, the chemical images were analysed and compared to provide conclusions and answers to the questions posed regarding the location of specific species.

The tablet was held on a metal microscope slide, and the surface was milled to approximately the centre of the tablet. The specific conditions for collecting map data are shown in Table I.

Equivalent conditions were used between the reference and generic tablets.

Table I: The specific map data collection conditions.

Raman microscope	Coating targeted	Entire surface
Wavelength	785 nm	
Laser power	50%	
Grating	1200 l/mm (1 cm^{-1} spectral resolution)	
Objective	20×	
Scan type	StreamLine™ imaging	
Purpose of scan	Domain size and distribution of API species	
Total time	39 minutes	61 minutes
Area	595 µm (x) x 780.5 mm (y)	9.017 mm (x) x 9.336 mm (y)
Step size	3.5 µm	35.5 µm
Nº of spectra	37,910	66,802

Comparing the Reference Product and Generic Product

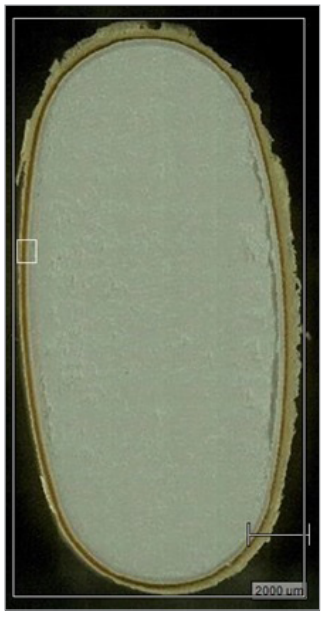

Figure 1. White light montage of the generic tablet.

White light images (montages) of the entire tablet surface were collected and are shown in Figure 1. This makes determination of the context of the analysis locations against the size of the entire sample surface simple (the inset white boxes indicate the analysed areas). Multiple analysis regions can be defined from the same montage and queued to run concurrently.

The Raman images confirm that there are four layers represented in Figure 2. A species similar to esomeprazole magnesium was found in layer 2. Naproxen was found in the core and within layer 4.

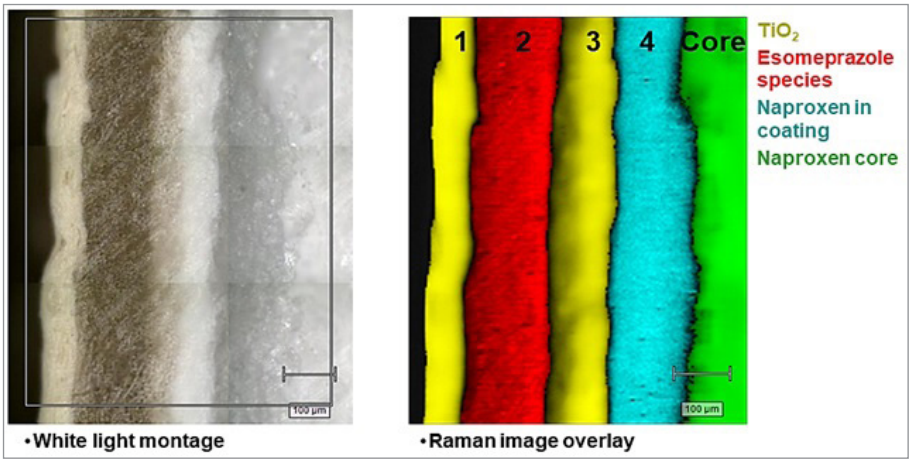

Figure 2. Raman images of the coating region and core of the generic tablet.

As shown in Figure 3, the esomeprazole magnesium Raman spectrum is not identical to the reference spectrum, suggesting that the esomeprazole magnesium exists in the coating as a different form/hydrate. In fact, the esomeprazole magnesium presents pseudopolymorphism, that is, a difference in the crystallographic profile due to a difference in the degree of hydration (anhydrous, dihydrate, and trihydrate forms are available) [22]. The reference spectrum corresponds to the esomeprazole magnesium trihydrate form (CAS Number 217087-09-7).

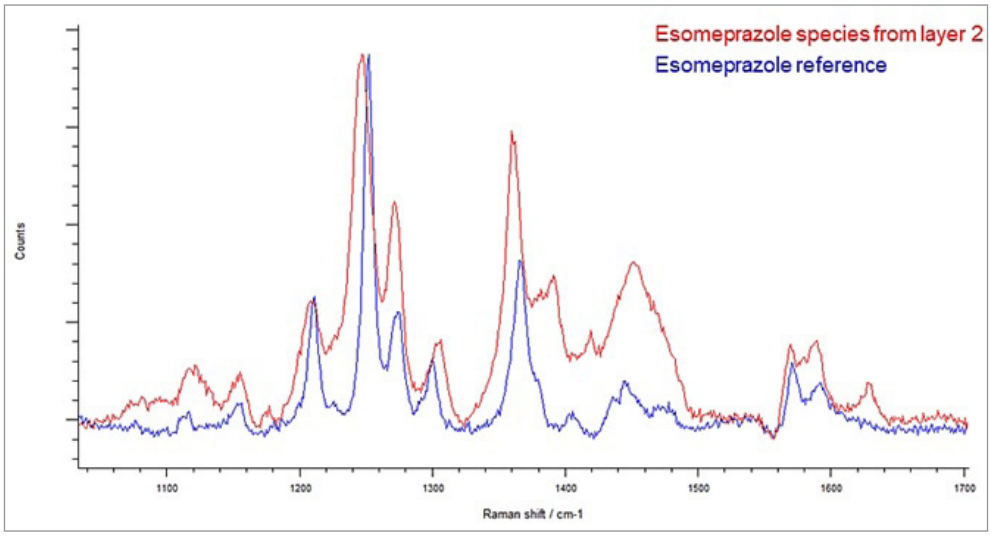

Figure 3. Raman spectra of the layer 2 of the generic tablet.

The difference found would be justified because the generic product most likely used an organic solvent to incorporate the API into the coating, producing a solvate, or caused desolvation, reducing the degree of hydration of esomeprazole magnesium from trihydrate to dihydrate or anhydrous forms.

The naproxen Raman spectra are identical to the reference. The presence of increased background and other Raman band features enables the naproxen coating to be differentiated from the naproxen core (Figure 4).

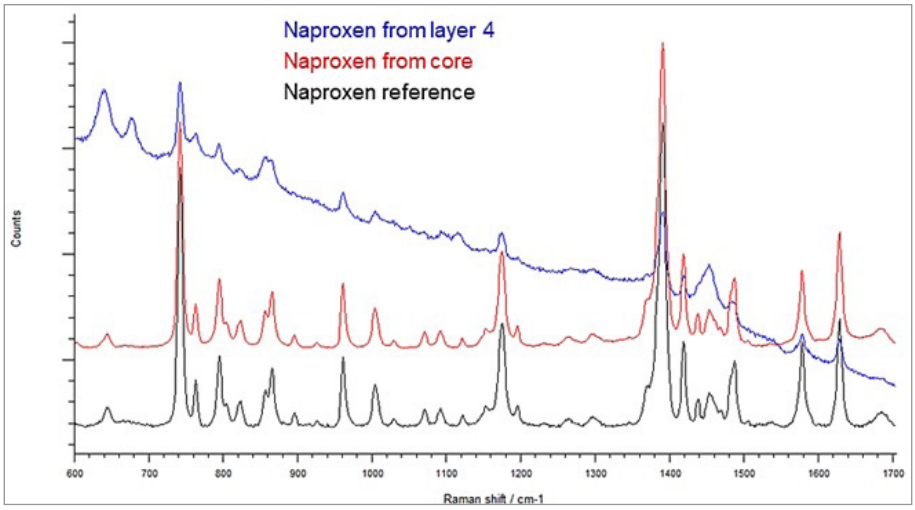

Figure 4. Raman spectra of the layer 4 of the generic tablet.

Analysis of the entire section shows that the core is made predominantly of naproxen. During the sample preparation stage (milling), some parts of the coating became fragmented. The left and lower regions are believed to best represent the real coating. A comparison of the coating regions, collected at the different spatial resolutions, is shown in Figure 5.

Figure 6 shows that both images reveal the same number of layers with the same content. Using a smaller step size from 35.5 µm to 3.5 µm which is smallest spacing between acquisition points on the sample, increases the spatial resolution, enabling more detail to be seen.

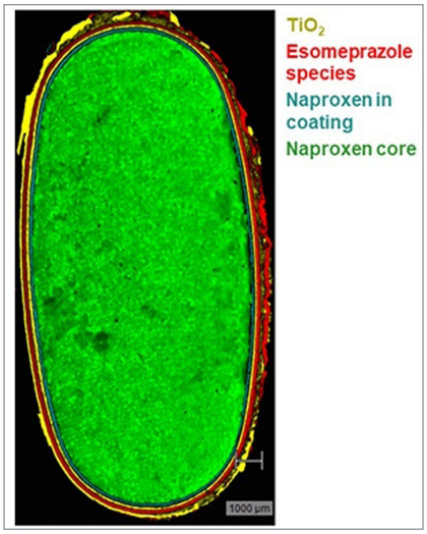

Figure 5. Raman images of the entire section of the generic tablet.

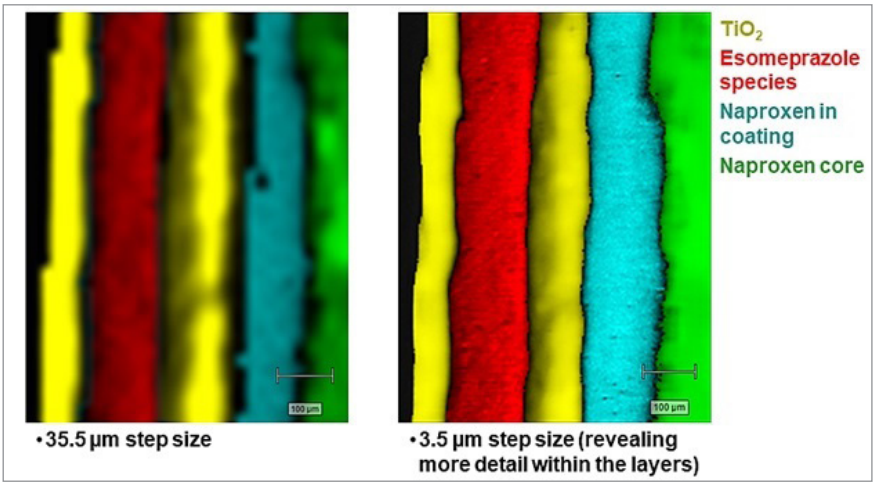

Figure 6. Raman images of the coating region and core of the generic tablet with 35.5 µm versus 3.5 µm of step size.

Raman images were produced in the same way for the reference tablet. The coating images confirm that there are four layers, equivalent to that of the generic sample, shown in Figure 7. The esomeprazole magnesium found in layer 2 exactly matches the supplied reference material (unlike the generic product). Naproxen was found in the core and within layer 4, and this species was equivalent between samples and the reference material.

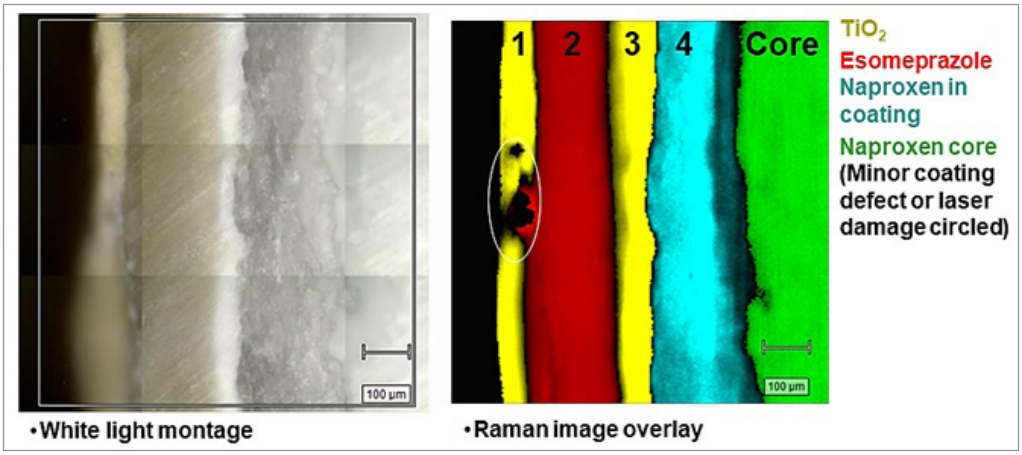

Figure 7. Raman images of the coating region and core of the reference tablet.

Figure 8 shows that the esomeprazole magnesium Raman spectrum is equivalent to the reference (with the addition of some coating excipients) and different from the generic product. Analysis of the entire section showed that the core is made predominantly of naproxen.

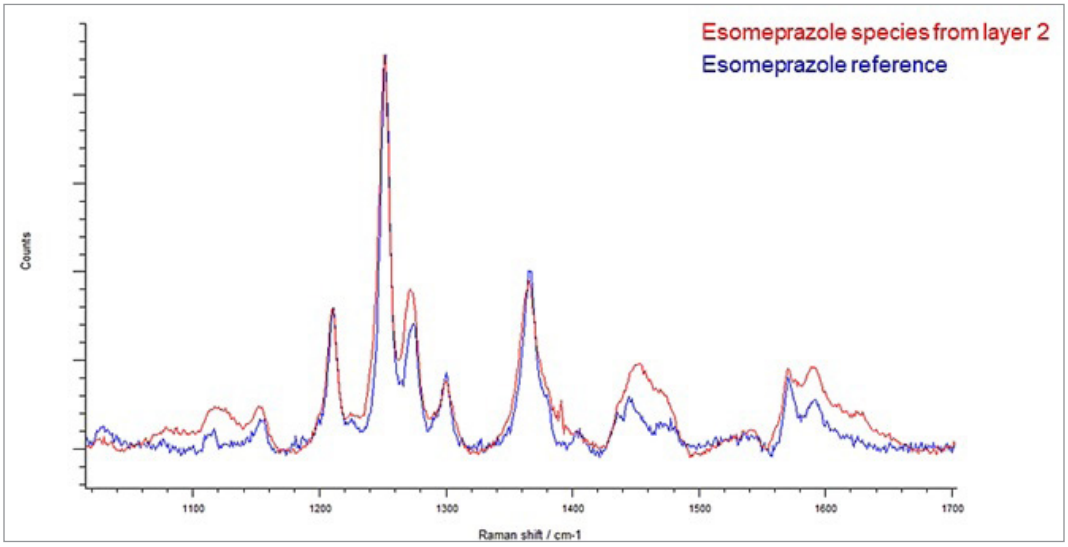

Figure 8. Raman spectra of the layer 2 of the reference tablet.

A comparison of the coating regions collected at the different spatial resolutions is shown in Figure 9.

Both images reveal the same number of layers with the same content. Using a smaller step size from 35.5 µm to 3.5 µm which is smallest spacing between acquisition points on the sample, increases the spatial resolution, enabling more detail to be seen in Figure 10.

Generally, both samples exhibit the same number of coatings, similar coating thicknesses, and the same constituents (excipient species were not specifically targeted in this work), according to Figure 11. The only significant difference observed was the form of the esomeprazole magnesium species. This

species matched the pure reference and the reference tablet and was different from the generic product.

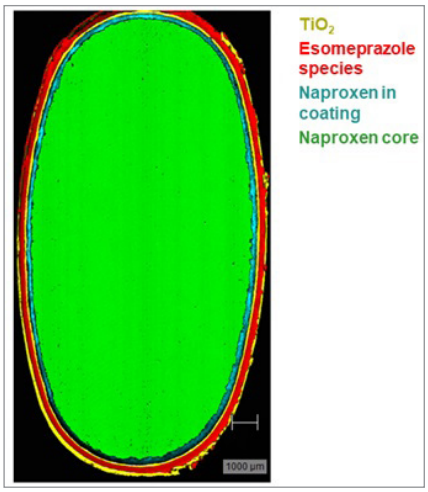

Figure 9. Raman images of the entire section of the reference tablet.

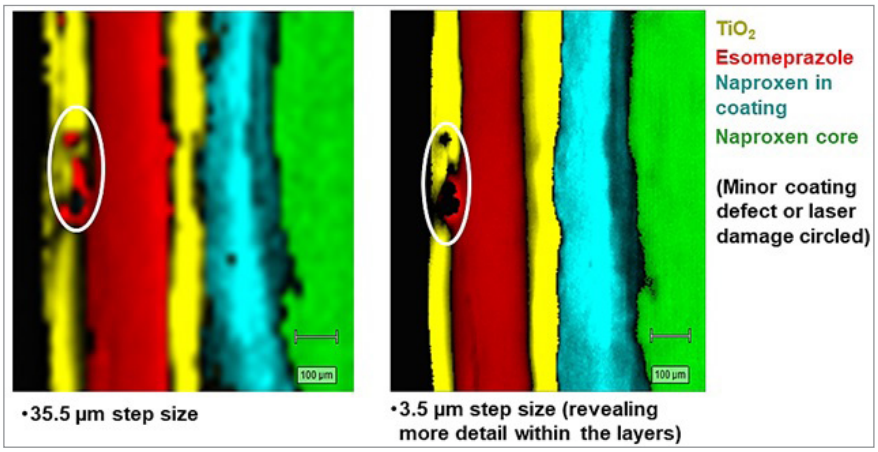

Figure 10. Raman images of the coating region and core of the reference tablet with 35.5 µm versus 3.5 µm of step size.

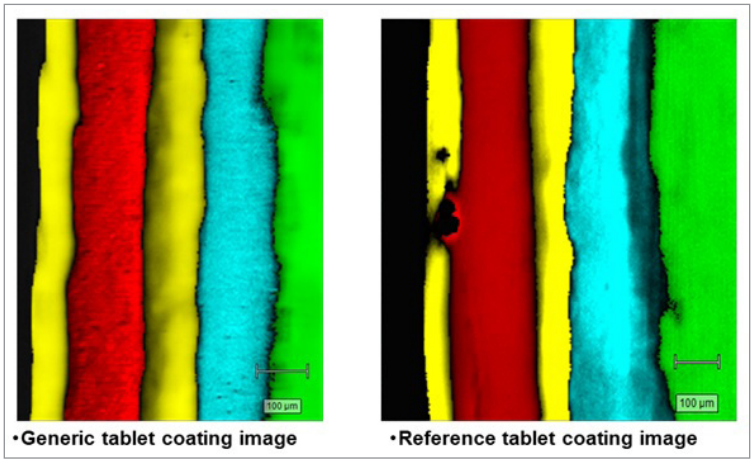

Figure 11. Raman images of the coating region and core of the generic tablet versus reference tablet.

Summary

The inVia Confocal Raman Microscope was used to analyse and compare two different pharmaceutical tablets, showing how this instrument can be applied in real-world scenarios in the development of pharmaceutical formulations. Raman imaging was used to create high-quality data from which detailed chemical images could be produced. inVia was capable of imaging the tablets at a variety of different spatial resolutions. The 20× objective is ideal for tablet imaging since it can provide a range of spatial resolution values (ranging from ~3 µm to greater than 100 µm). This is achieved by analysing the same sample depth, enabling simple image comparison.

In particular, the component analysis method enables images to be generated very quickly, targeting specific components, with the ability to reveal ‘unknown’ species for subsequent work. The high specificity and sensitivity of this method enables different forms of species to be revealed, as well as the generation of detailed chemical images.

The following questions were answered during this analysis, showing the value of the Raman microscope as a tool for product analysis and comparison:

i. Determine the layer(s) that contains esomeprazole magnesium.
Both samples have this present in only one layer. The forms of esomeprazole magnesium appear different between the samples.

ii. Confirm the number of layers since there are some differences in the literature.
Both samples have four clear layers surrounding the core.

iii. Determine the composition of the different layers.
The API species within the layers and the core were determined. Coating thicknesses could be established. Some excipient species were also found; although, this work did not specifically analyse these.

iv. Determine the particle size of naproxen, esomeprazole magnesium, and the major excipients.
Domain sizes are typically quantified using particle statistics with Renishaw WiRE software. Raman images are required to show discrete domains of each species to enable such quantification. In both samples, no discrete API domains were found to enable such particle analysis. The core is primarily comprised of naproxen, which practically forms one continuous domain. The coatings appear relatively homogenous, with no apparent separation of the API species into discrete domains.

v. Map the distribution of the API and excipients across the different layers.
The API species are uniformly distributed in the relevant coatings for both tablets supplied.

vi. Compare the distribution of the reference product core with the generic product core.
No differences were observed between the tablet cores.

vii. Compare the polymorphic form of esomeprazole magnesium and naproxen used in the reference product with the reference APIs. Check the hydration state of both APIs.
The naproxen spectra appear the same between the different tablets and match the reference naproxen supplied. The spectrum of the esomeprazole magnesium in the generic tablet was different from the reference tablet and the supplied reference material. This most likely results from a change in polymorphic form/hydration state.

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ARTICLE

Evaluation of Delivery Form of Eicosapentaenoic and Docosahexaenoic Acids During Quality Control of Fish Oil Supplements

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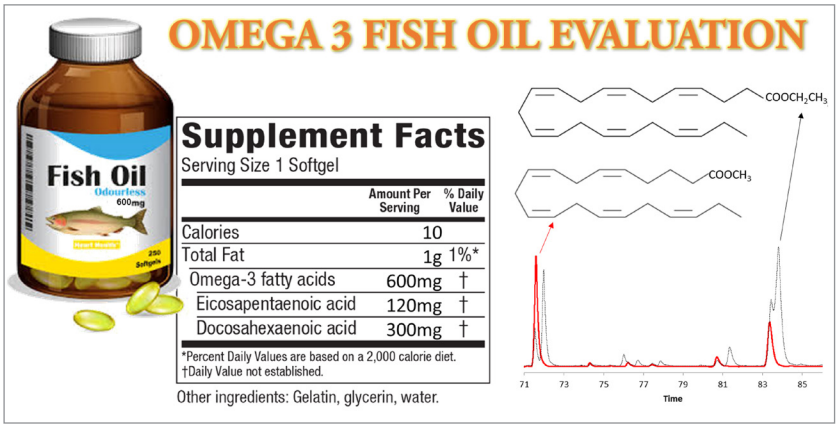

Fish oils (FO) omega-3 supplements containing eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids are extensively consumed due to their beneficial health effects. Fatty acids (FA) are mainly available as triacylglycerols (TAG) and ethyl esters (EE) in FO supplements, and EE is known to be less bioavailable. Then, the evaluation of FA in FO should comprise not only omega-3 content but also the delivered form. This research aimed to approach in detail a set of chromatographic analytical methods employing TLC, GC-FID, and GC-MS for FO quality control, considering both FA contents and form. TAG and EE FA were differentiated by GC-FID, due to the difference in the retention times of compounds, and also by GC-MS, as a result of the different *m/z* spectra observed. TLC also distinguished both FA forms, but a mixture of TAG and EE in FO concentrates was not observed, as evidenced by GC techniques. Ten FO supplements available in the Brazilian market were analyzed, and the FA profiles of natural and concentrated FO were compared. EPA + DHA label claim compliance was also accessed. Their contents varied from 78 to 113% of labeled content, and only one supplement had FA in EE form. Brazilian FO supplements analyzed were a source of EPA and DHA and most products (except one sample) were accurately labeled according to current Brazilian regulation, which permits a variation in 20% of the declared content. Furthermore, the methodologies approached can be used in future researches considering FO analyses and bioavailability studies approaching the different FA forms.

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Keywords: thin-layer chromatography; gas chromatography; ethyl esters fatty acids, triacylglycerols; natural fish oil; concentrated fish oil.

INTRODUCTION

Innumerable health effects of omega-3 fatty acids (FA) consumption have been largely recognized [1], especially related to the ingestion of the polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), available in marine species. In the last decade, the omega-3 supplement market has grown exponentially since consumers want to reach the minimum daily recommendation of 250 mg of EPA and DHA to obtain beneficial health-effects. Fish oil (FO) is a valuable source of EPA and DHA, being commonly commercialized in its natural or concentrated form [2]. Natural FO usually contains EPA and DHA as triacylglycerols (TAG) at different concentrations depending on the fish species, which usually is not superior to 30% of total fat. Concentrated FO are mostly available as ethyl esters (EE) since TAG resynthesis after EE distillation is costly and frequently bypassed by manufacturers [3].

The functionality of EPA and DHA as TAG or other chemical forms in FO supplements has been subject to considerable debate. In the end, a better bioavailability of the TAG form has been reported when compared to EE [4]. Currently, the delivery form of dietary FO supplements, whether TAG, EE, or other, is not legally required on the packaging in Brazil and it should be revised since consumers should be aware of which form they are choosing to understand the wide range of prices of these supplements and their different absorption forms within the body.

Moreover, inaccurate listing of EPA and DHA contents in the labels is also a core quality control issue. Recent studies worldwide evidenced that FO omega-3 supplements did not fully meet labeled EPA and DHA levels, which seriously infringe on the rights and interests of the consumers [5–7]. Regarding supplements available in the Brazilian market, Galuch et al. [8] reported that among the fifteen FO supplements analyzed (sampled in Paraná State), eleven were in the TAG form and two in the EE form. Besides, two brands were discovered with the addition of large amounts of soybean oil, which leads the final consumer to ingest low-cost oil supplements believing that they are consuming adequate doses of EPA and DHA. Overall, these findings warn the real need to intensify the inspection of dietary FO supplements worldwide. Omega-3 supplements have become one of the most popular dietary products due to the low fish intake in many populations, but these products should be more strictly controlled to ensure both quality and label declaration accuracy.

Within this context, this work aimed to apply a set of chromatographic methods described in the literature by thin-layer chromatography (TLC), gas chromatography with flame ionization detector (GC-FID) and mass spectrometry (GC-MS) for the separation of omega-3 FA in the form of TAG and EE in FO supplements. The GC method was proposed by Gallardo et al. (2014) [9] and was applied to evaluate the effects of olive and FO on the lipid composition of ewe milk, and subsequently in the muscle and subcutaneous adipose tissues of lambs suckling such milk. However, only TAG were determined. On the other hand, the TLC method was described by Srigley and Rader (2018) [10] for the identification of EE in krill oil. In the present study, ten Brazilian FO were analyzed to show the applicability of the developed methods for TAG and EE omega-3 separation, in FO supplements. The FA profiles of natural and concentrated FO were compared and discussed. Besides, EPA + DHA label claim compliance was also evaluated, which would be of great interest in a globalized market. Since the FA delivered form in supplements impacts the bioavailability of omega-3 FA in the body, this topic needs to be further addressed in the literature, and we discussed the chromatographic methods approached in detail, also showing identified chromatograms and mass spectra, which could serve for new researchers in this field. The described methodologies can be used for evaluating FO supplements quality, considering both FA composition and form. Moreover, these methods could also be investigated in future works for the determination of TAG and EE EPA and DHA in bioavailability studies.

MATERIALS AND METHODS

Samples

Ten bestsellers dietary FO supplements commercialized in capsules were acquired from local markets (Juiz de Fora, Minas Gerais, Brazil, October 2018). Nine of the products were marketed for the general population (Samples 1-9), and one product was marketed toward pregnant women (Sample 10). All products declared contents of both DHA and EPA; however, no information about the delivery form of FA was provided in the labels.

Reagents and analytical standards

All reagents used for the analysis were of analytical grade. Chloroform, diethyl ether, ethanol, glacial acetic acid, methanol, hexane, potassium hydroxide (KOH) and sodium bisulfate ($NaHSO_4$) were purchased from Panreac Química S.A. (Madrid, Spain). Sigma-Aldrich (St. Louis, MO, USA) provided 2',7'-dichlorofluorescein. FA methyl esters (ME) standards were acquired from Nu-Chek Prep Inc. (Elysian, MN, USA).

Thin-layer chromatography

Samples were analyzed by TLC, according to Srigley & Orr-Tokle (2018) procedure [10]. Commercial FO samples were solubilized in chloroform (25 mg mL^{-1}) and spotted on a silica gel G plate 20x20 cm from Merck (Darmstadt, Germany). A mixture of hexane/diethyl ether/glacial acetic acid in the proportion of 100:20:2 (v/v/v) was used as the mobile phase. After developing, plates were sprayed with a 0.1% ethanolic 2',7'-dichlorofluorescein solution, and bands were visualized under ultraviolet light. TAG and EE were identified based on the calculated retention factors.

Sample preparation for gas chromatography

TAG were converted into FAME by base-catalyzed methylation before GC analysis. Omega-3 marine oils were withdrawn from the capsules with a glass syringe, and 25 mg of fat were weighed in a microtube, where 200 µL of hexane were added. After agitation, 50 µL of a methanolic KOH solution (2 mol L^{-1}) were added, and the microtube was vortexed for 1 min. After 5 min of resting, 125 mg of $NaHSO_4$ were added, and the microtube was centrifuged for 5 min (10000 rpm at 0 ºC). Finally, the solution was transferred to a GC vial and diluted in a proportion of 1:2 with hexane.

Instrumentation for gas chromatography

An Agilent 6890N GC instrument (Palo Alto, CA, USA) equipped with FID detector, autosampler, and a CP-SIL 88 capillary column (100 m, 0.25 mm x 0.2 µm, Varian, Middelburg, The Netherlands) was used to quantify FA in FO samples. The temperature program was: the initial temperature was 45 °C; after 4 min, the temperature was raised at 13 °C min^{-1} to 165 °C and hold for 35 min, then increased to 215 °C at 4 °C min^{-1} and kept for 30 min [9]. An injection volume of 1.0 µL and a split ratio of 1:100 were employed. FID detector and injector temperature were fixed at 250 °C. Identification of EPA and DHA ME were performed in GC-FID by comparison with the retention times of FAME individual standards. Other FAME were identified by comparison with the retention times of FAME mixtures (GLC 80, 403, 409, 411, 603, 642, 643) from Nu-Chek Prep Inc. A GC-MS (electron impact ionization source and single quadrupole mass analyzer, Agilent 6890A GC, MS 5973N; Palo Alto, CA, USA) equipped with the same CP-SIL 88 capillary column was used to corroborate the identification of FAME performed by GC-FID and identify EPA and DHA EE. The filament trap current was 400 µA at 70 eV; ion source and interface temperatures were 150, and 230 °C, respectively, and the mass range was 30-400 *m/z*. Chromatographic conditions were similar to those described by GC-FID but with a split ratio of 1:20. Wiley 275 and NIST 05 libraries were used to confirm mass spectra, which were also compared with those from The LIPID MAPS® Lipidomics Gateway. The general FA composition of commercial FO was quantified by area normalization. In addition, calibration curves for EPA and DHA were carried out by ordinary least squares regression.

RESULTS AND DISCUSSION

Fatty acid composition and form of natural and concentrated fish oil supplements

Figure 1 shows the different profiles of FA obtained by GC-FID for a natural FO supplement (Figure 1-A, Sample 1) and a concentrated FO supplement (Figure 1-B, Sample 10). It can be observed that the natural FO presented a large number of major peaks besides EPA and DHA, with a high amount of saturated fatty acids (SFA), such as C14:0 and C16:0, monounsaturated fatty acids (MUFA), such as C16:1 and C18:1, and some characteristics PUFA, such as C16:2, C16:3, C16:4, C18:4, C21:5, and C22:5 also reported by other researchers [11,12]. Several isomers were also separated in this natural FO (C16:1, C18:1, C18:2, C18:3, 20:4, and 22:5). The isomeric separation was possible due to the employment of a 100 m cyanopropyl phase capillary column, which permits to obtain detailed information on positional and geometrical isomers of FO [13]. In the natural FO, all FA were originally present as TAG (Figure 1-A). This is the molecular form primarily present in fats and oils from both animal and plant species. In the chromatogram, all TAG are observed as MEs, since a methylation reaction was employed to convert TAG into this more volatile form.

In Figures 1-B and -C, it can be observed that the concentrated supplement presented EPA and DHA EE as major peaks. However, a small amount of TAG can be noticed because the industrial process of molecular distillation is not 100% efficient. EE are mostly found as major FA in concentrated FO since they are formed by the esterification of TAG with ethanol for the FA distillation. As the molecular process of TAG resynthesis is highly costly, it is generally bypassed by manufacturers [3], which leads to concentrate supplements mainly composed by EE FA in the market.

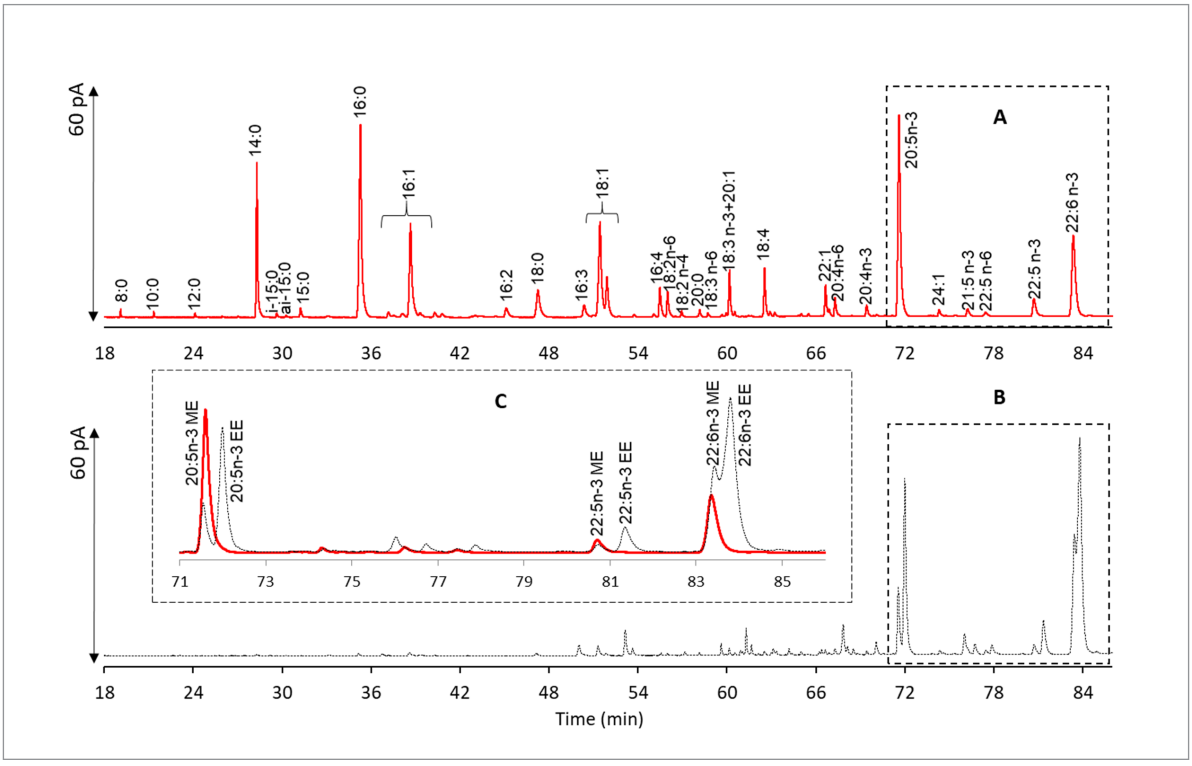


Figure 1. Chromatograms of natural fish oil (A, fatty acids originally as triacylglycerols, solid red line) and concentrated fish oil (B, major fatty acids as ethyl esters (EE), dotted black line). Both samples are superimposed and amplified (C) to show the elution order of methyl esters (ME) and EE omega-3 moieties. In A, all peaks are in the ME form. In B, major fatty acids are in EE form, although small amounts of ME can be observed due to the incomplete conversion of TAG to EE during supplements concentration. Triacylglycerols were analyzed as fatty acids ME after analytes derivatization.

Table I shows the FA composition of the ten FO supplements analyzed in this study, reporting EPA and DHA contents, total SFA, including branched-chain SFA, total MUFA, and PUFA. Samples 1 to 9 presented a very similar FA profile: SFA varied from 28.4 to 30.8%, MUFA from 24.2 to 28.2%, and PUFA from 41.1 to 46.1%. Considering EPA and DHA levels, always below ~30% of total fat, it can be inferred that these supplements were natural FO encapsulated without further concentration processes (samples contained from 16.2 to 19.7% of EPA and from 9.4 to 12.4% of DHA). Besides, these FO presented FA in TAG form. Sample 10 was a concentrated omega-3 supplement marketed for pregnant women, in which EPA and DHA contents accounted for 66.7% of total fat (22.6% EPA and 44.1% DHA). In comparison with the other samples, it presented a very different FA profile, with low SFA (only 1.9%) and MUFA (only 3.2%) levels. The total PUFA content was 77.8%. The GC chromatogram of this concentrated sample showed that omega-3 FA were mainly in the EE form (Figure 1), accounting for 73.2% of EPA and 73.0% of DHA as EE moieties.

Table I. Fatty acid composition of commercial fish oils omega-3 dietary supplements analyzed by gas chromatography (data presented as % of total fatty acids)

Sample	ΣSFA[a]	ΣMUFA[b]	ΣPUFA[c]	EPA[d]	DHA[d]
1	29.1	28.0	41.6	18.3	10.1
2	30.0	27.0	42.0	16.4	10.2
3	30.3	26.2	42.4	16.3	9.7
4	28.4	24.2	46.1	17.4	11.6
5	28.8	24.2	45.7	19.7	12.4
6	28.8	27.3	42.8	16.2	9.4
7	30.8	27.0	41.1	17.9	10.0
8	28.9	26.4	43.3	17.0	10.1
9	28.7	28.2	42.0	18.0	10.6
10	1.9	3.2	77.8	22.6[e]	44.1[f]

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

[a]ΣSFA was calculated by area normalization as the sum of 8:0, 10:0, 12:0, iso 13:0, anteiso 13:0, 13:0, iso 14:0, 14:0, iso 15:0, anteiso 15:0, 15:0, iso 16:0, 16:0, iso 17:0, 17:0, iso 18:0, 18:0, 19:0, 20:0, 21:0 and 22:0.

[b]ΣMUFA was calculated by area normalization as the sum of 14:1, 16:1, 17:1, 18:1, 19:1, 20:1, 22:1 and 24:1.

[c]ΣPUFA calculated by area normalization as the sum of 16:2, 16:3, 16:4, 18:2, 18:3, 18:4, 20:2, 20:3, 20:4, 20:5, 22:2, 22:4, 22:5 and 22:6.

[d] calculated by external calibration. [e]16.4% was in the form of ethyl esters and 6.1% in the form of triacylglycerols, representing 73.2% of total EPA as ethyl esters; [f]32.2% was in the form of ethyl esters and 11.9% in the form of triacylglycerols, representing 73.0% of total DHA as ethyl esters.

Evaluation of Brazilian fish oil supplements label accuracy

Table II presents the comparison between the determined and labeled EPA and DHA contents in all samples analyzed considering the daily serving size recommended for each supplement. The natural FO supplements recommended three capsules as daily serving size, equivalent to 3000 mg, while the concentrated supplement intake recommendation was just one capsule, equivalent to 600 mg. When comparing labeled and analytically determined EPA and DHA contents, only sample 5 presented at least 100% of DHA claimed content and samples 1, 5, 9, and 10 at least 100% of EPA claimed content (Figure 2).

However, when considering a tolerance of 20% in the reported nutritional value, as permitted by current Brazilian legislation [14], almost all samples were rightly labeled (except sample 6 which presented DHA < 80% of labeled content). EPA content varied from 90% to 113%, considering the declared contents, and DHA concentration varied from 78% to 103%. Figure 2 shows the percentage of label declaration achieved for each sample analyzed.

Table II. Label declaration and determined eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid contents in the Brazilian omega-3 fish oil dietary supplements analyzed

		Labeled content		Determined content	
Sample	**Daily serving size (mg)**	**EPA (mg serving^{-1})**	**DHA (mg serving^{-1})**	**EPA (mg serving^{-1})**	**DHA (mg serving^{-1})**
1	3000	540	360	549	303
2	3000	540	360	492	306
3	3000	540	360	489	291
4	3000	540	360	522	348
5	3000	540	360	591	372
6	3000	540	360	486	282
7	3000	540	360	537	300
8	3000	540	360	510	303
9	3000	540	360	540	318
10	600	120	300	136	265

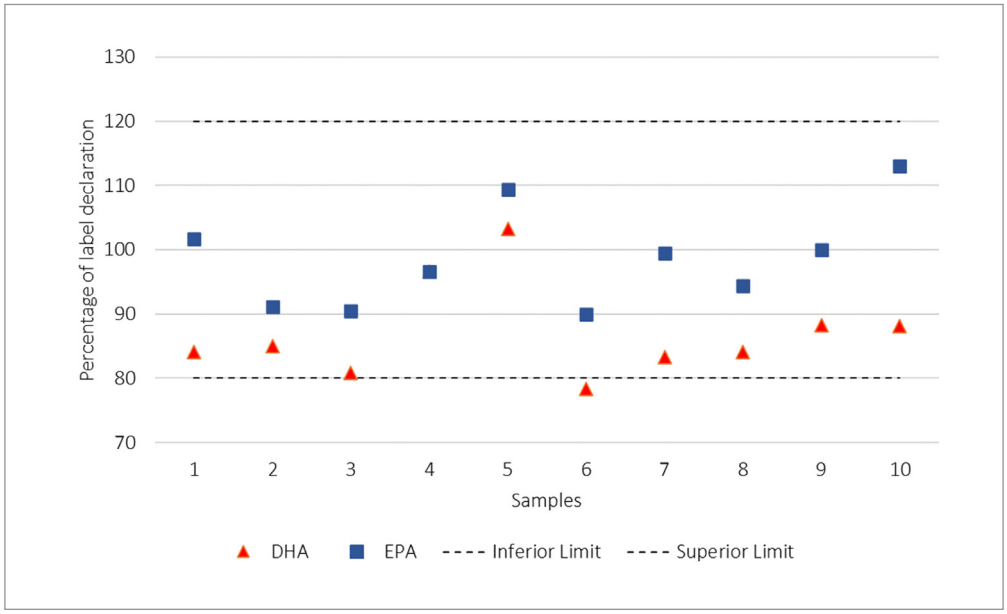
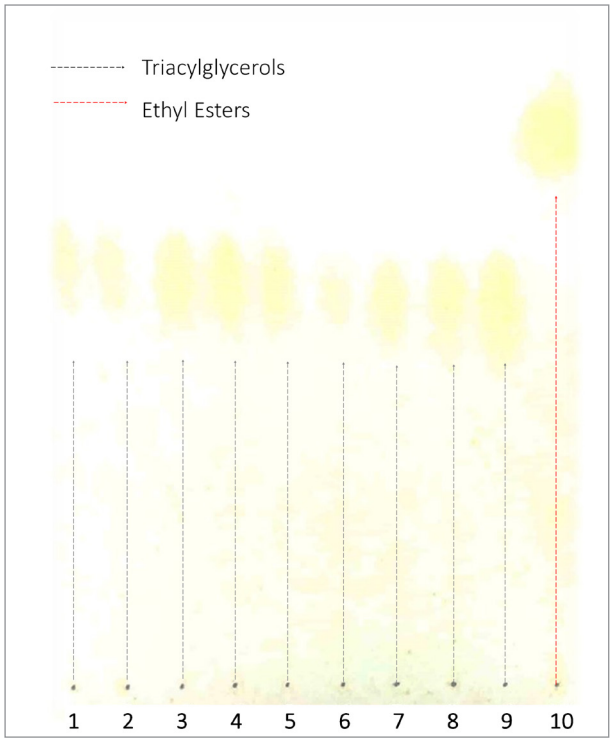


Figure 2. Percentage of eicosapentaenoic (■) and docosahexaenoic (▲) acids content on the label declaration for all samples analyzed. Dotted lines express legal tolerance limits.

Against the evidences reported by Galuch et al. (2018) [8] that some FO supplements acquired in the Brazilian market (samples acquired in Maringá City, Paraná State) were not a source of EPA and DHA and were adulterated with soybean oil, most FO analyzed within this data set (acquired in Juiz de Fora city, Minas Gerais State), have shown to meet their label claims for EPA and DHA contents, considering the Brazilian current legal tolerance limits. These findings are also contrary to the evidences shown by several international studies, which demonstrated the lack of rigor in the labeling of FO dietary supplements. In the United States, Srigley & Rader (2015) [7] detected that almost 20% of the marine oil supplements did not meet their respective label declarations and Kleiner et al. (2015) [15] verified that among all samples considered, over 70% of the supplements tested did not contain the stated labeled amount of EPA or DHA. In New Zealand, it was observed that only 9% of the FO supplements analyzed contained quantities of EPA and DHA that were equal or higher than their labeled contents [5] and, more recently, it was revealed that 9% of the FO samples did not comply with the declarations [16]. In South Africa, only 52% and 35% of the FO supplements contained the labeled amount of EPA and DHA, respectively [17], while, in Austria, more than 10% of the omega-3 supplements did not meet omega-3 labeling [18]. A further study from Australia reported misdeclaration of 42% for EPA and 10% for DHA [19], and, in Korea, 100% of the FO samples were mislabelled according to analytically determined contents [6]. Overall, these findings highlight the quality of FO Brazilian supplements, but also warn the real need to intensify the inspection of dietary FO supplements worldwide in order to guarantee the rights and interests of the consumers.

Evaluation of the delivered fatty acid form in fish oil supplements

As evidenced in Figure 1, GC-FID was successful in the discrimination between ME and EE because their retention times were slightly different. Omega-3 ME eluted just before the EE homologs (Figure 1C). However, these assignments were also corroborated by GC-MS (Mass spectra of EPA and DHA TAG and EE are available in the supporting information), which permitted to distinguish FA forms in the different supplements due to a CH_2 group missing in the ME form (*m/z* = 14). EPA ME presented M+ at *m/z* = 316, while the EPA EE homolog showed M+ at *m/z* = 330. Correspondingly, DHA ME presented M+ at *m/z* = 342, and DHA EE exhibited M+ at *m/z* = 356. Thus, both techniques can be employed to TAG and EE discrimination in FO supplements, under the analytical conditions described.



FA form could also be evaluated by TLC, as shown in Figure 3. This method distinguished TAG from EE samples based on the difference in their retention factors. TAG samples resulted in a retention factor = 0.6 and EE samples resulted in a retention factor = 0.8. However, it is worth noticing that Sample 10 was not observed as a mixture of TAG and EE, as evidenced by GC. Only a spot corresponding to the EE form was visualized. Then, in order to obtain a complete profile of TAG and EE forms in FO samples and also to quantify these forms in supplements, GC techniques are recommended.

Figure 3. Thin-layer chromatography analysis of fish oil supplements containing fatty acids as triacylglycerols (retention factor = 0.6) and ethyl esters (retention factor = 0.8).

CONCLUSIONS

A set of chromatographic techniques (TLC, GC-FID, and GC-MS) was approached in detail in this study applied to FO analyses, considering both FA contents and form. The profiles of natural and concentrated FO with FA present in the form of TAG and EE were compared and discussed, and identified chromatograms were shown, as well as the mass spectra of EPA and DHA methyl and ethyl esters. This information can be useful to new researches, considering FO analyses and also future investigations about the bioavailability of FA forms, which needs to be further approached in the literature. It was possible to differentiate TAG and EE FA by GC-FID, due to the difference in the retention times of both forms, and also by GC-MS, as a result of the different *m/z* spectra observed. TLC was also able to distinguish FA forms, but a mixture of TAG and EE in FO concentrates was not observed, as evidenced by GC techniques. Besides, label accuracy of Brazilian supplements was also evaluated, which could be of interest in a globalized market. All FO analyzed were a source of EPA and DHA, containing from 90% to 113% EPA and from 78% to 103% DHA, according to the omega-3 contents declared by manufacturers.

Acknowledgments

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Conflicts of interest

All the authors declare no conflict of interest.

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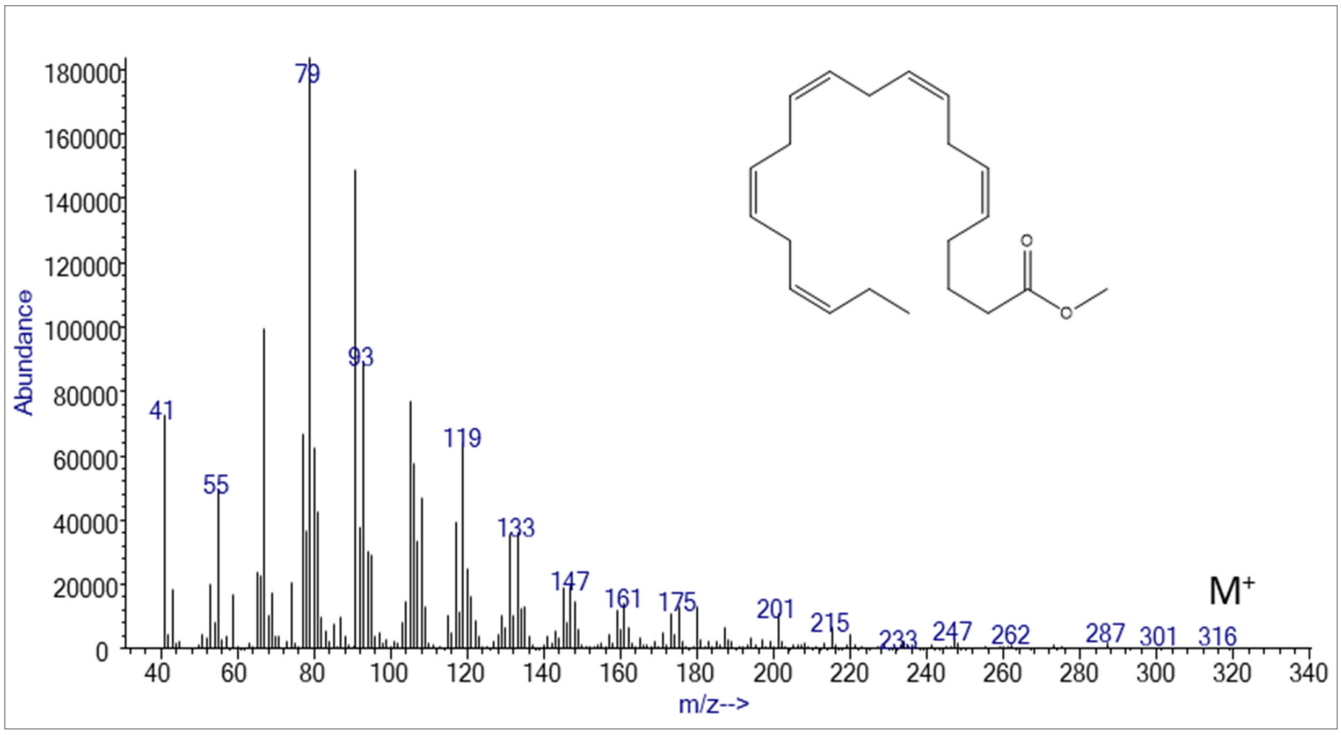
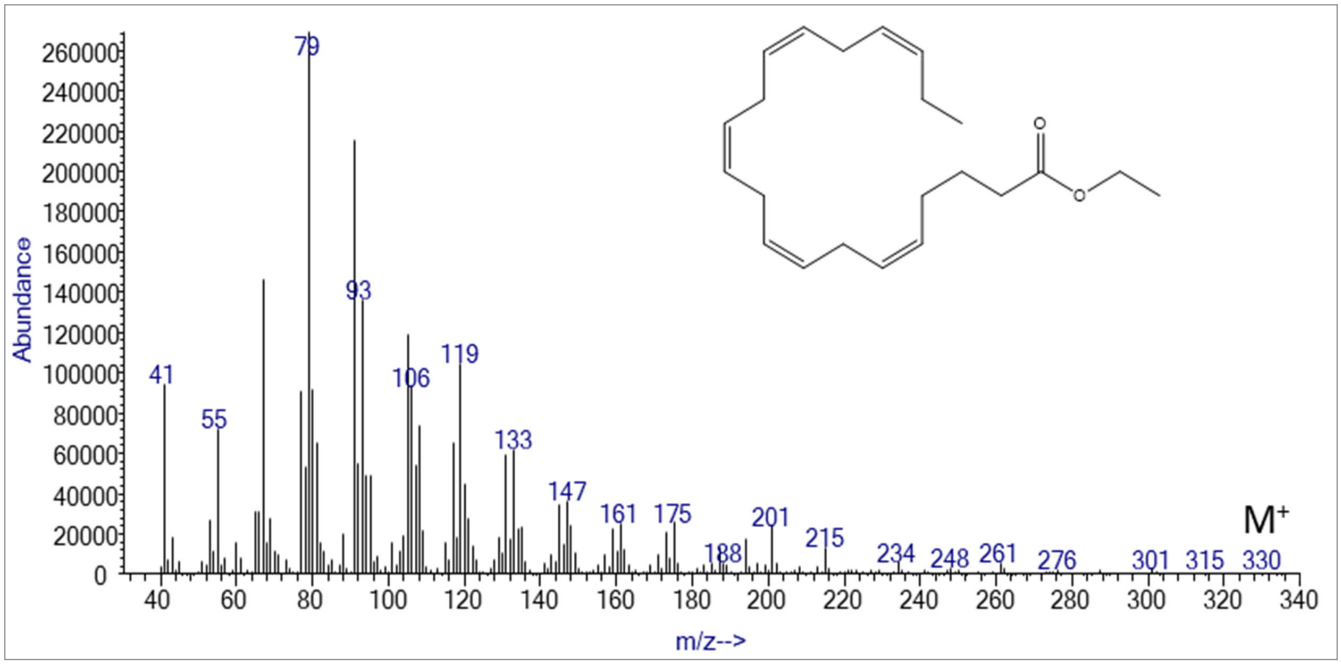
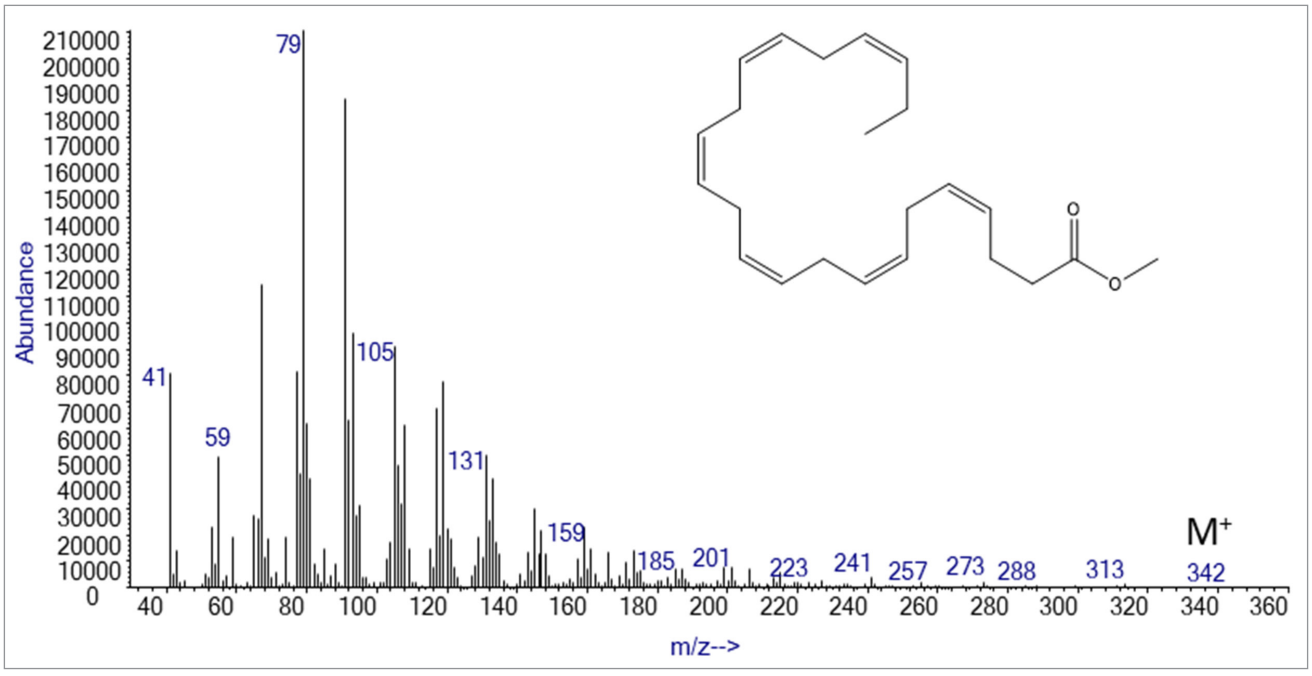
SUPPORTING INFORMATION



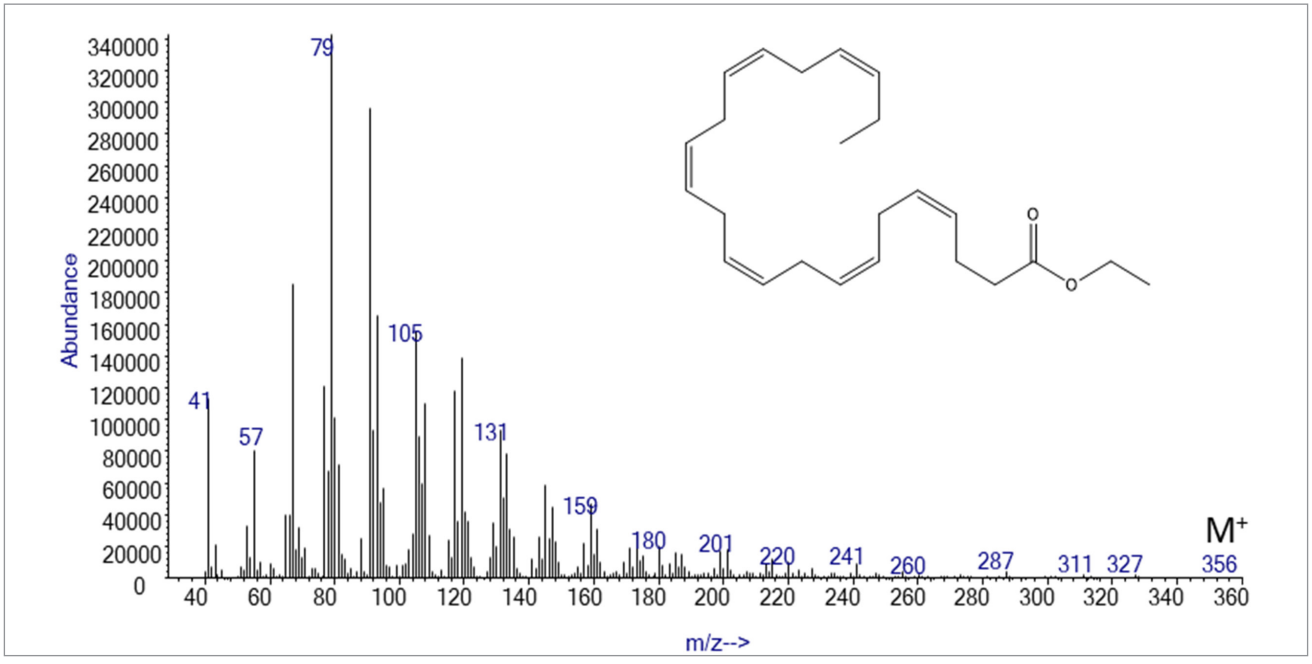
Supporting information – **Figure 1.** Mass spectra of eicosapentaenoic acid methyl ester.



Supporting information – **Figure 2.** Mass spectra of eicosapentaenoic acid ethyl ester.



Supporting information – **Figure 3.** Mass spectra of docosahexaenoic acid methyl ester.



Supporting information – **Figure 4.** Mass spectra of docosahexaenoic acid ethyl ester.

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ARTICLE

Isotherm and Kinetic Modeling of Cd(II) Uptake from Aqueous Solutions using an Agri-Waste Biosorbent Jute Stick Powder

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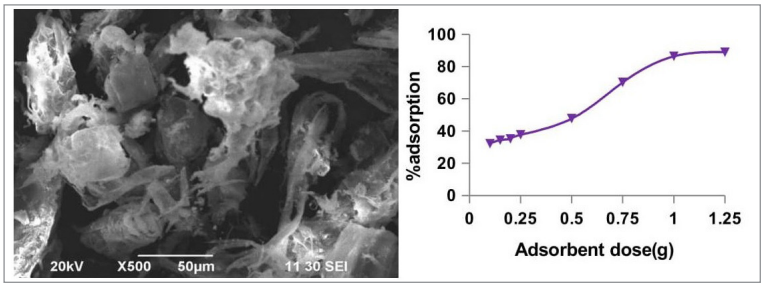

The removal of cadmium(II) from aqueous solutions in a batch system was conducted by using an agri-waste biomass Jute Stick Powder (JSP). To optimize parameters like metal concentration, solution pH, adsorbent amount, contact time and agitation speed, batch experiments were conducted. Further experiments were performed, using optimized conditions at pH 4.5, adsorbent dosages of 0.5 g/100 mL, a contact time of 60 min and shaking speed of 180 rpm. Favorable adsorption occurred at around pH 4.5, with a maximum adsorption capacity of 10.003 mg g^{-1}. Detailed analysis has been conducted by testing kinetic models such as pseudo-first-order and pseudo-second-order models to determine the sorption rate and mechanism. The adsorption process was confirmed by various isotherm models and experimental data for Cd(II) biosorption fitted well to the Freundlich isotherm model at room temperature and under optimum conditions. Positive values of the thermodynamic parameter ΔG assumed that the metal adsorption process was spontaneous. JSP can be used repeatedly more than three times with a small efficiency loss. Experimental data plotted to the isotherm and kinetic models confirms that JSP can be used as a potential biosorbent for the successful removal of Cd(II) ions from waste water.

Key words: Jute Stick Powder (JSP), lingo-cellulosic, cadmium(II), Freundlich isotherm, pseudo-first-order.

INTRODUCTION

Heavy metals are a threat to human life and the environment. Toxic heavy metals, such as cadmium (Cd), lead (Pb), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), chromium (Cr) and copper (Cu), are discharged into the environment by both anthropogenic activities and natural circumstances. A very important problem worldwide is water contamination with heavy metals. These heavy metals are persistent in nature and non-biodegradable, causing toxicity via bioaccumulation in animal cells. Cadmium is one of the most toxic heavy metals, having a half-life of 10–30 years. It enters the food cycles and remains

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in the kidney, liver and other vital organs for a long time. Once taken up by the blood, the majority of cadmium is transported to proteins, inducing the production of Metallothionein in the liver. Kidney damage, bone and renal disease, lung and renal dysfunction, cancer and cardiovascular diseases, hypertension, and chronic anemia are common diseases caused by cadmium toxicity [1]. Electroplating, smelting, alloy manufacturing, pigments, plastic, battery, mining and refining processes are the main sources of cadmium pollution in the environment. Hence, it is necessary to remove cadmium from industrial water before discharge into the water body.

To remove toxic metals from water, a number of conventional methods are available, including ion exchange, reverse osmosis, precipitation, solvent extraction, membrane technologies, electrochemical treatment, and adsorption. The adsorption process employed for materials of biological origin is called biosorption; this is a very attractive method to researchers currently. Because conventional methods have some drawbacks such as high operational costs, the requirement for new reagents and the production of sludge as a secondary pollutant which needs to be treated, they can be alternated with biosorption, which is cost effective, easy to operate and environmentally friendly. Widely used cheap agricultural wastes to remove Cd from aqueous solutions include rice straw [2], leaf biomass of the sodom apple [3], tea waste [4], rice bran [5], and loquat leaves [6]. Jute stick is one of the most abundant lingo-cellulosic agricultural waste by-products of jute fiber, which is the main cash crop of Bangladesh. In continuation of our previous project, JSP has been applied for the removal of Cr(III) and Pb(II) ions from aqueous solutions [7,8].

The aim of this work is to study the biosorption of Cd(II) ions onto jute stick powder as a low cost and extensively available biomass. Different biosorption controlling factors such as the initial solution pH, temperature, contact time, and metal ion concentration on the adsorption isotherm were examined to optimize the adsorption process. Sorption capacity was tested by plotting some isotherm models and sorption mechanism was studied by plotting kinetic equations.

MATERIAL AND METHODS

Preparation of biosorbent

After the separation of jute fibers, the inner woody fiber stick is left, which is then dried in the sunlight. These jute stick biomasses were collected and chopped into small pieces. After that, the biomass was washed with water to remove any adhering substances and dried at 80 °C in an oven for 12 hours. Finally, it was powdered by grinding manually by a kitchen grinder. To obtain a uniform size particle, the powder was sieved and 80–100 mesh size fractions were used for subsequent study.

Preparation of synthetic waste water

The stock solution (1000 mg L^{-1}) of Cd(II) was prepared by dissolving 2.751 g of cadmium nitrate tetrahydrate [$Cd(NO_3)_2.4H_2O$] in 1000 mL of double-distilled water. The working solutions were prepared by the dilution of the stock solution and were used throughout the experiment. The pH of the waste water was adjusted by using 0.1 M NaOH and 0.1 M HCl solution and was tested by a digital pH meter (HANNA, HI2209). All of the chemicals were of analytical grade and purchased from Merck Company (Germany).

Batch biosorption studies

Equilibrium isotherm studies

Equilibrium isotherm studies were conducted using 100 mL fixed volume solutions with a range of concentrations in 250 mL conical flasks mixed with an optimum biosorbent dose of 0.5 g and then agitated for 60 minutes at shaking speed 180 rpm by a reciprocating orbital shaker. After completion of shaking the solutions were filtered by Whatman™ filter paper. Biosorption experiments were conducted at room temperature (28 °C) and the concentration of the metal was determined by an atomic absorption spectrophotometer (AA7000, SHIMADZU, Japan) with Cd hollow cathode lamp and air acetylene flame. It is important to consider that all of the experiments were replicated three times and the mean of the data was used to determine the result.

Effect of contact time

Batch biosorption experiments were conducted at different time periods (5, 15, 25, 35, 60, 90 and 120 min) at pH 4.5, the initial biosorbent concentration of 0.5 g in 100 mL solution, metal ion concentration of 50 mg L^{-1}, and shaking speed of 180 rpm at 28 °C. After shaking on a reciprocal shaker for a definite time period, the solution of the specified flask was taken out and filtered. The concentration of metal was determined by AAS.

Effect of pH

To determine the influence of pH on the sorption of Cd(II) by batch process, 100 mL of 50 mg L^{-1} metal solution was placed in a conical flask. Using 0.1 M HCl and 0.1 M NaOH pH of the solution was adjusted and kept at pH 1 to 10; the solutions were taken as 0.5 g JSP in 10 flasks. Then, the flasks were shaken at rpm 180 for 60 minutes. Next, the residue was filtered and the final concentration of metal ions was determined by AAS.

Influence of adsorbent dose

The effect of the biosorbent dose on the adsorption capacity was investigated at 28 °C using a range of adsorbents (0.10 g to 1.25 g) at a constant pH of 4.5 and metal concentration of 50 mg L^{-1} in 100 mL of solution. Then, the mixtures were shaken at 180 rpm for 60 minutes. When equilibrium was reached, the solutions were filtered. The concentration of metal ions was then determined by AAS.

Calculation of Biosorption

The amount of metal adsorbed by the biosorbent was calculated based on the mass balance for the biosorbent in the system as the metal uptake (biosorption capacity, q) to judge the quality of that biomass. Biosorption capacity (q) and metal sorption (%) are calculated according to Equations 1 and 2.

$$q_e = \frac{V(C_i - C_f)}{S} \quad (1)$$

$$\%Sorption = \frac{C_i - C_f}{C_i} \times 100 \quad (2)$$

Where q_e (mg g^{-1}) = metal ion uptake capacity, C_i (mg L^{-1}) = initial concentration of metal in solution before the sorption analysis, C_f (mg L^{-1}) = final concentration of metal in solution after the sorption analysis, S (g) = dry weight of the biosorbent, and V (L) = solution volume. The difference between the initial and final concentration of metal ions was assumed to be bound to the biosorbent.

Mathematical modeling

Isotherm models

At the equilibrium of an adsorption process, the distribution of adsorbate molecules between the liquid and the solid phase can be described mathematically by various adsorption isotherms [9]. Experimentally collected data were analyzed by using four adsorption isotherms: the Langmuir, Freundlich, Temkin and Dubinin–Radushkevich (D–R) models. By calculating the isotherm parameters, some information such as adsorption mechanism, favorability of adsorption process and adsorbate-adsorbent affinity may be interpreted.

The Langmuir isotherm [10] describes equilibrium distribution of metal ions between the solid and liquid phases, the formation of an adsorbate monolayer, and uniform energies of adsorption onto the surface, is expressed by the mathematical relation in Equation 3.

$$\frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{q_m K_L C_e} \quad (3)$$

Where C_e is the equilibrium concentration of Cd(II) (mg L^{-1}), q_e is the quantity of Cd(II) adsorbed on to the adsorbent at equilibrium (mg g^{-1}), q_m is the maximum monolayer adsorption capacity of adsorbent (mg g^{-1}) and K_L is the Langmuir adsorption constant (L mg^{-1}). The plot of $1/q_e$ against $1/C_e$ gives a straight line with a slope and intercept of $1/q_m.K_L$ and $1/q_m$, respectively. An important characteristic of the Langmuir isotherm is expressed by a dimensionless constant equilibrium parameter R_L, which is calculated from the value of K_L in a range of initial concentrations (20 mg L^{-1} to 100 mg L^{-1}). The R_L value indicates the favorability of adsorption processes and is expressed by Equation 4.

$$R_L = \frac{1}{1 + K_L C_0} \quad (4)$$

According to Mckay et al. [16], the R_L value indicates the adsorption nature to be either unfavorable if R_L>1, linear if R_L = 1, and favorable if 0< R_L<1.

The Freundlich isotherm [17] is an empirical model which is applicable to adsorption processes that occur on heterogonous surfaces. The linear form of the isotherm as an expression of heterogeneity and the exponential distribution of active sites and their energies can be given as follows:

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (5)$$

Where K_F is a constant describing the adsorption capacity (L g^{-1}) and n is an empirical parameter related to the adsorption intensity which can be determined from the plot of $\log q_e$ against $\log C_e$. According to Kadirvelu and Namasivayam [18], a value of n between 1 and 10 represents a beneficial adsorption process.

The Temkin isotherm assumes the effects of indirect adsorbate/adsorbent interactions on the adsorption process ignoring extremely low and very high concentrations in the uniform distribution of bounding energy up to some maximum bonding energy [19]. The linear form of Temkin isotherm model is given by the following equation:

$$q_e = B \ln A_T + B \ln C_e \quad (6)$$

Where, q_e is the amount of adsorbate adsorbed at equilibrium (mg g^{-1}); C_e is concentration of adsorbate in solution at equilibrium (mg L^{-1}), B is a constant related to the heat of adsorption which is defined by the expression B = RT/b, b_T is the Temkin constant (J/mol), T is the absolute temperature (K), R is the gas constant (8.314 J/mol/K), and A is the Temkin isotherm constant (L g^{-1}). From the plot of q_e vs. $\ln C_e$, B and A can be calculated from the slopes (B) and intercepts (BlnA) respectively.

The Dubinin Radushkevich (D-R) model gives information about biomass porosity as well as the adsorption energy which assumes that the adsorption process is physical or chemical in nature [20]. The model also presumes that adsorption is a multilayer character involving Van Der Waal's forces, applicable for physical adsorption processes following pore filing mechanism [21]. The D-R isotherm is expressed as follows:

$$\ln q_e = \ln q_m - K_D \varepsilon^2 \quad (7)$$

$$\varepsilon = RT \ln\left(1 + \frac{1}{C_e}\right) \quad (8)$$

Where q_m and K_D are the constants of D-R isotherm model, respectively, the maximum adsorption capacity (mol g^{-1}) and the adsorption energy. The constant K_D is related to the mean free energy of sorption

per mole of the adsorbent as it is moved from infinite distance in the solution to the surface of the biomass, E, which can be calculated using the following equation:

$$E = \frac{1}{(2K_D)^{\frac{1}{2}}} \quad (9)$$

The values of q_m and K_D may be obtained from the slope and the intercept of lnqe versus ε^2 plot.

Kinetic models

To analyze possible Cd(II) metal adsorption rate controlling steps and possible mechanism kinetic models such as pseudo-first-order, a pseudo-second-order model was studied.

The pseudo first-order kinetic model of Lagergren [22] is given by Equation 10:

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \quad (10)$$

Where, q_e (mg g^{-1}) and q_t (mg g^{-1}) are the adsorption amount at equilibrium and time t (min), respectively. k_1 (min^{-1}) is the rate constant in the pseudo-first-order adsorption process. The constants were determined experimentally by plotting log (q_e-q_t) versus t.

The pseudo-second-order kinetic model [23] can be presented as shown in Equation 11:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (11)$$

Where k_2 (g mg^{-1} min^{-1}) is the rate constant of the second-order equation. A linear graph was obtained from the plot of t/q_t versus t. The pseudo-second-order rate constants k_2 and q_e were determined from the intercept and slope of the plot.

RESULTS AND DISCUSSIONS

Characterization of biosorbent

Constituents of JSP are mainly α-cellulose, hemicellulose, and lignin, containing some characteristic functional groups which are responsible for metal binding. To identify the functional groups in the JSP, FTIR experiments for raw and Cd(II) loaded JSP were performed in the same conditions. In the FTIR spectrum of raw JSP, a broad intense peak at 3296.52 cm^{-1} was assigned to stretching of OH groups. A high concentration of intermolecular H-bonded phenol and alcohol is responsible for this broad band. The bands at 2872.13 cm^{-1} are most probably due to the contribution from the C—H stretching of cellulose and hemicelluloses. The peak number at 1745 cm^{-1} is identical to C-O stretching of the carboxyl and acetyl groups in hemicelluloses of the JSP. The absorption band at around 1592 cm^{-1} is attributed to C=O bonds [24]. For the contribution from various vibration modes, most bands are complex below the spectral region of 1400 cm^{-1} which is more difficult to analyze. A weak band at 1376 cm^{-1}, which is seen in the spectrum due to symmetric C–H deforming, may be attributed to lignin, α-cellulose, or xylan. Again, the peaks observed at 1059 cm^{-1} and 1035.92 cm^{-1} are due to aromatic C–H in plane deformation, C–O deformation, C-C stretching and C-OH bending for primary alcohol in lignin and polysaccharides. A medium band peak 1234.50 cm^{-1} in the spectrum is ascribed to C-O stretching of acetyl in xylan [25]. The figure shows that over the spectral region 1000–1300 cm^{-1}, carbohydrate originating vibrations are associated. However, peaks at 2333.00 and 2360.01 cm^{-1} in the spectrum might be due to noises obtained for flaws in the machine.

The FTIR spectra of metal Cd(II) loaded JSP show that the spectral band at 3296.52 cm^{-1} became less intense and shifted to 3294.57 cm^{-1}, which confirms the exchange of protons with metal ions. The peaks obtained for raw JSP at 2872.13, 1745.27, 1598.27 and 1059.93 cm^{-1} etc. had shifted to 2845.13, 1743.31, 1592.31, and 1057.04 cm^{-1}, respectively, due to Cd(II) ion adsorption. The shifting of these spectral bands may be attributed to the changes in counter-ions associated with carboxylate and hydroxylate anions, suggesting that acidic groups, carboxyl and hydroxyl, are predominant contributors to metal ion uptake [26,27].

Figure 1 shows the scanning electron micrographs (SEM) of raw JSP and cadmium-loaded JSP at different magnifications. The presence of a few macropores of various irregular sizes at the surface of thread-nodule like JSP particles is clearly observed in the SEM image [Figure 1(a, b)]. After the adsorption of cadmium, significant changes in the surface morphology were noted. For example, uneven and irregular macropores in the surface and mineral matter might be trapped within these macropores [Figure 1(c, d)]. The presence of very distinguished multiple layers in SEM images of metal-loaded JSP could be taken as a sign for the effective adsorption of Cd(II) ions.

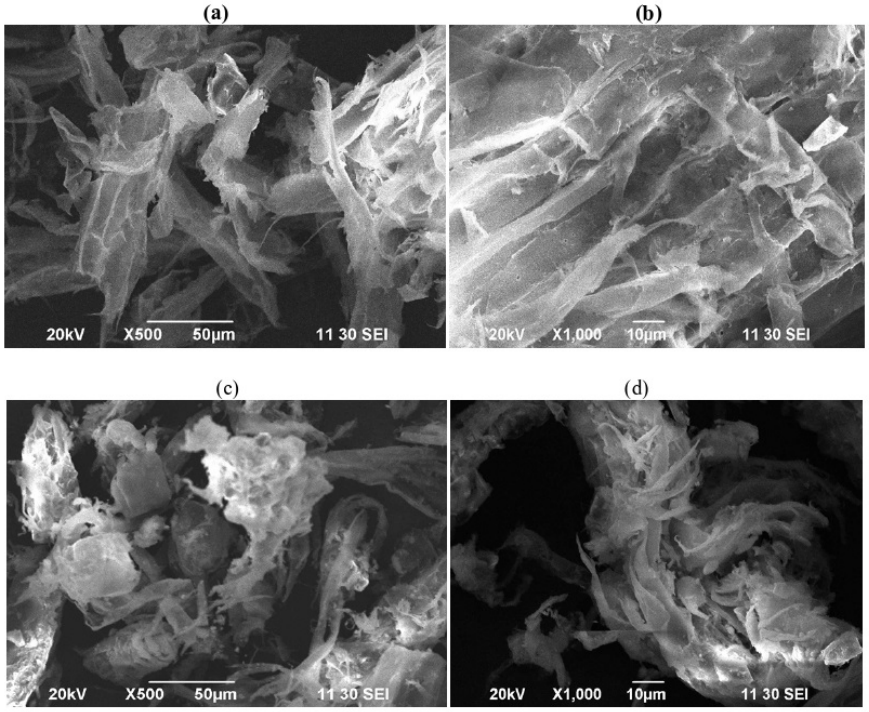

Figure 1. SEM images: (a, b) is for raw JSP and (c, d) is for Cd(II) ions loaded JSP.

Optimization of influencing parameters

Influence of pH

pH is considered a key controlling parameter in the bio-sorption process of metal ions from aqueous solutions. The experimental data plotted in Figure 2(a) indicate that the adsorption of Cd(II) ions from the aqueous solution was very low at low pH values and increased sharply with the increase in pH. The removal of Cd(II) was about 0.892% at pH 2.0 and reached a maximum value (54.29%) at about pH 4.0. Further increases in pH from 4 to 10 lead to slight decreases in Cd(II) removal efficiency. Módenes et al. [9] reported that metal precipitation began to occur at pH 5.5. At low pH values, the uptake of Cd(II) was very low, because higher concentrations of H^+ ions competed with Cd(II) ions for the exchangeable sites on the biosorbent surface and heavy metal ions were completely free in the extreme acidic conditions. With the increasing pH electrostatic attraction between the Cd(II) cation and negatively charged jute stick, the surface was increased. At higher pH, electrostatic repulsion decreases due to a reduction of positive

charge density on the sorption sites, thus resulting in an enhancement of metal adsorption [10]. A smaller decrease in removal at pH levels from 5 to 10 can be explained by the fact that the mobility of some elements may be reduced, leading to the precipitation of insoluble $Cd(OH)_2$ at higher pH values. Therefore, the optimum pH of 4.5 is considered for all experiments of Cd(II) adsorption by JSP.

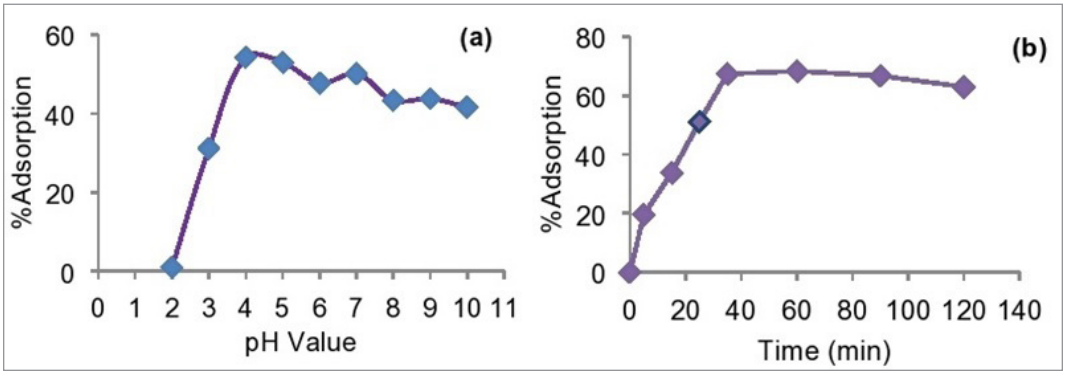

Figure 2. (a) Effect of pH on % removal and (b) Effect of contact time on % removal for biosorption of Cd(II) using JSP as biosorbent (Ci = 50 mg L^{-1}; dose = 0.5 g/100 mL).

Influence of contact time

For the design of batch biosorption studies, the rate of biosorption is important. Figure 2(b) shows that the percentage biosorption of cadmium metal ions increases sharply to 67.34% with a rise in contact time up to 40 minutes at room temperature (28 ºC). Equilibrium was reached quickly within 35–40 minutes for Cd(II) biosorption and slightly increased or decreased afterwards. It is observed that a further increase in time did not cause a significant enhancement in the biosorption of the cadmium ions because, at that time, there is saturation of the active sites on the biosorbent. The maximum amount of cadmium (68.29%) was adsorbed within 60 minutes, possibly as a result of an increased number of vacant sites available on the adsorbent surface. The adsorption process became slower with the progressive occupation of these sites, causing a decrease in the sorption capacity after 60 minutes since there were no more vacant sites for the metal ions to occupy [11]. Therefore, the optimum contact time was selected as 60 minutes for further experiments.

Influencing of biosorbent amount

Figure 3(a) shows that the adsorption of Cd(II) ions onto JSP increases with an increase in adsorbent dosage from 0.10 g to 1.25 g, with a percentage removal from 32.316% to 88.914%. This is because the ability of ion exchange sites, surface areas and the number of available adsorption sites for metal adsorption were higher as the dosage increased. However, an excessive increase in the adsorbent amount could cause a reduction in removal efficiency due to the formation of aggregates. Similar results were reported by Matakaet et al. [12], using *Moringastenopetala* and *Moringaoleifera* seed powders. By increasing *Moringastenopetala* powder dosage from 0.5 to 2.5 g/100 mL, the Cd(II) removal percentage was increased from about 20% to 58%. On the other hand, Figure 3(b) illustrates a decreasing order in the amount of metal adsorbed (mg) in each gram of adsorbent with the increase in adsorbent dosage from 0.10 to 1.25 g/100 mL. During the adsorption process, some adsorption sites may remain unsaturated, which can be attributed to the insufficiency of metal ions in solution with respect to available binding. Moreover, due to the increased biomass, the amount of unsaturated active sites was increased. However, it is notable that in Figure 3(b), with the increase of adsorbent dosages, the adsorption capacity was supposed to decrease sharply. However, this did not happen as there might have been some instrumental faults during the collection of datadata.

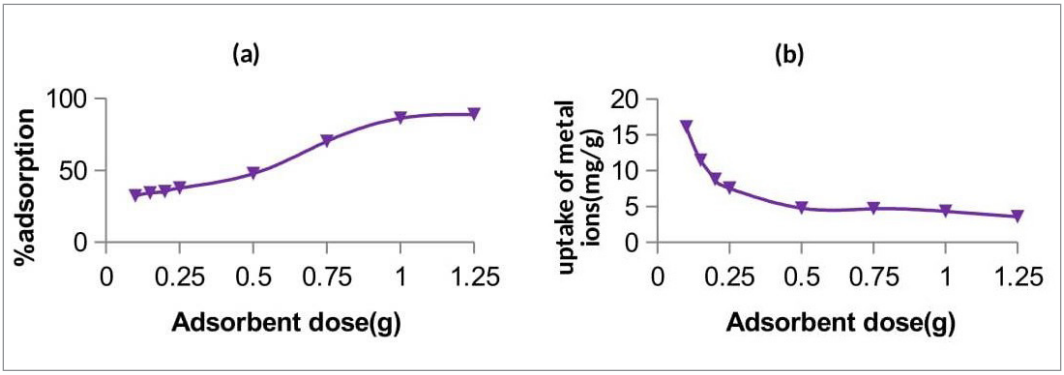

Figure 3. Influence of different adsorbent quantity on adsorption: (a) % adsorption and (b) adsorption capacity (mg g^{-1}) of JSP (pH = 4.5, Contact time = 60 min, Initial conc. of cadmium = 50 mg L^{-1}).

Influence of shaking speed on % adsorption

It was observed that with the increasing of agitation speed the percent removal increased while other parameters were kept constant. The adsorption efficiency was 63.85% at 80 rpm, reached at a maximum point 70.4% while it was 120 rpm and then gradually decreased to 67.8% at 230 rpm. Because low speed could not spread the particles properly in the water by providing active binding sites for biosorption of metal ions. On the other hand, the high rpm value vigorously spreading the particles of jute stick powder in the water and did not allow sufficient time to bind with metal ions. With the incensement of rpm value the equilibrium reached quickly due to attaining of proper contact between metal ion and JSP in solution. Although mass transference speed increased with high shaking speed adsorption capacity was not increased tremendously due to accumulation of biosorbent at the bottom of water which buried the active binding sites. Similar result was reported by other authors described in literature [13].

Influence of initial cadmium concentration of solution

It was found that the adsorption percentage decreased from 64.44% to 50.02% with an increase in the initial concentration of cadmium from 20 to 100 mg L^{-1}. The reason for this could be that the ratio of metal ions to active sites is low at low initial metal concentrations and a higher percentage was removed. In contrast, this ratio is relatively high at high initial metal concentrations. In other words, if the number of metal ions was much higher than the number of active sites available for adsorption, then a lower percentage would be removed. Therefore, the % adsorption was rather low at high initial metal concentrations. On the other hand, the migration of Cd(II) ions into vacant sites of the sorbent increased due to the high initial Cd(II), resulting in the agglomeration of adsorbent particles at higher concentrations. It is leading to a decrease in the total surface area of the adsorbent particles available for adsorption.

Adsorption isotherm

The adsorption studies were conducted by changing the initial metal ion concentrations of Cd(II) at a fixed adsorbent dosage. The Freundlich, Langmuir, Temkin and D-R isotherms are shown graphically in Figure 4.

Langmuir isotherm constants q_m and K_L and the coefficient of determination (R^2) are represented in Table I. Figure 4(a) shows that these isotherms were found to be linear, with high R^2 values. The sorption constant, K_L and the saturated monolayer sorption capacity, q_m onto JSP were satisfactory. The values of R_L indicated that the adsorption process was favorable because it was within the range $0<R_L<1$. The low value of K_L obtained from the Langmuir isotherm indicated that Jute Stick powder has a high affinity for cadmium ion.

Table I. Langmuir, Freundlich, Temkin and Dubinin–Radushkevich isotherm constants for biosorption of Cd(II) onto the JSP

Isotherm Model	**Constants**				
Langmuir	q_{max} (exp.) (mg g^{-1})	q_{max} (cal.) (mg g^{-1})	K_L (L mg^{-1})	R_L	R^2
	10.004	14.28	0.030	(0.246-0.620)	0.988
Freundlich	-	-	n (g L^{-1})	K_F (mg g^{-1})	R^2
	-	-	1.485	0.674	0.992
Temkin	-	-	B_T (J mol^{-1})	A_T (L g^{-1})	R^2
	-	-	708.789	0.259	0.926
D-R	-	q_m (mol g^{-1})	K_D (mol^2/J^2)	E (J mol^{-1})	R^2
	-	8.0285	1.00E-05	0.223	0.865

Figure 4(b) shows that plot of the Freundlich isotherm is a linear graph with a high coefficient of determination (R^2=0.992). The values of Freundlich isotherm constant n and K_F, representing the adsorption intensity and adsorption capacity (L g^{-1}) are calculated from the slope and intercept of the curve presented in Table II. The results show that the n value (1.485) is between 1 and 10, which represents a beneficial adsorption process [18].

Figure 4(c) illustrates the linear plot of Temkin isotherms, with a coefficient of determination of R^2= 0.926. Temkin constants A_T and B_T, which are related to the binding energy of the adsorbate and adsorbent, are summarized in the Table I indicating a physical adsorption. The values are at a satisfactory level, which confirms the adsorption of Cd(II) ions onto the JSP biosorbent [28].

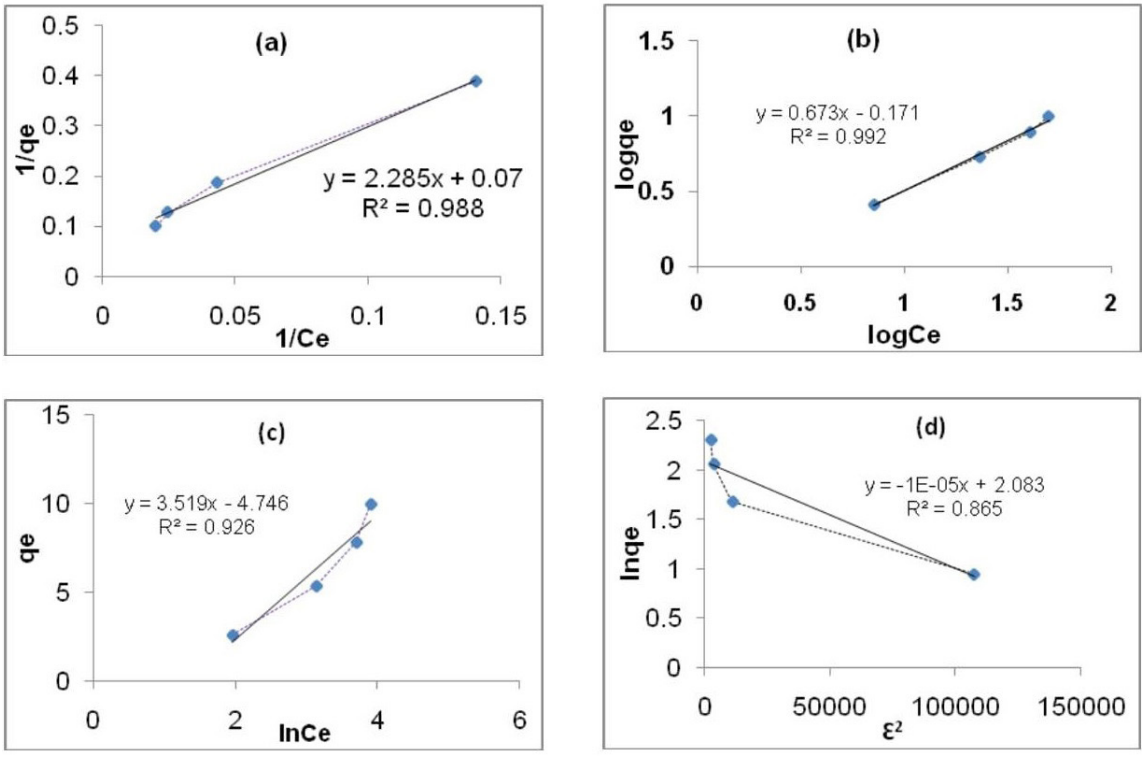

Figure 4. Plot of Adsorption isotherm models: (a) Langmuir (b) Freundlich (c) D-R and (d)Temkin for biosorption of Cd(II) onto the JSP (pH 4.5, biosorbent dose conc.: 0.5 g/100 mL, contact time: 60 min, temperature: 28 ºC.

Table II. First and second-order kinetic model parameters for adsorption of Cd(II) onto JSP

Kinetic model	Parameters			
Pseudo-first order	q_e, exp. (mg g^{-1})	q_e, calc. (mg g^{-1})	k_1 (min^{-1})	R^2
	6.83	7.585	0.0575	0.985
Pseudo-second order	q_e, exp. (mg g^{-1})	q_e, calc. (mg g^{-1})	k_2(g/mg/min)	R^2
	6.83	7.87	0.0122	0.749

The D-R isotherm constant q_m and K_D are given in the Table I. The value of apparent energy (E) of adsorption found 0.223 kj mol^{-1} indicating a physical adsorption process between JSP and Cd(II) ions [29]. From the plot, the coefficient of determination R^2 is found to be 0.865, indicating that this isotherm did not provide a very good fit to the experimental data.

It is concluded from the constant values and coefficient of determination that the Freundlich isotherm was the best fit for cadmium biosorption using Jute stick powder. It is in close agreement with the results shown by many authors discussed in literature [30]. The data found from the Freundlich isotherm suggest that the adsorption mechanism was physical and chemical processes, where Cd(II) ions are transported to the interior porous surface of JSP. It is assumed that chemical attraction to the heterogeneous surface with Cd(II) ions involves some energy change. After attaining thermodynamic equilibrium, no further net adsorption occurs between the solution and the adsorbent [31].

Adsorption Kinetics

The pseudo-first order and pseudo-second order kinetic models were investigated within the contact time of 0 to 120 minutes while keeping other parameters constant, such as the concentration of Cd(II) 50 mg L^{-1}, adsorbent dose 0.5 g/100 mL, pH 4.5 and shaking speed 180 rpm. Figure 5(a, b) shows the plot of both kinetic models and Table II summarizes the rate constant and other parameters. The results show that the equilibrium sorption capacities qe(cal.) determined using the pseudo-first-order and pseudo-second-order models are almost in agreement with the experimental qe(exp) values. However, the values of rate constant and coefficient of determination (R^2 = 0.985) of pseudo-first-order are higher than those of the pseudo-second-order model. The mechanism of adsorption is assumed to involve Cd(II) being adsorbed by proper diffusion, making a layer through the boundary of the JSP surface. Besides, chemisorption also happened in the removal of Cd(II), but physisorption might be predominant over chemisorption [32]. Therefore, the pseudo-first-order kinetic model was the best described model for the adsorption of Cd(II) onto the JSP biosorbent.

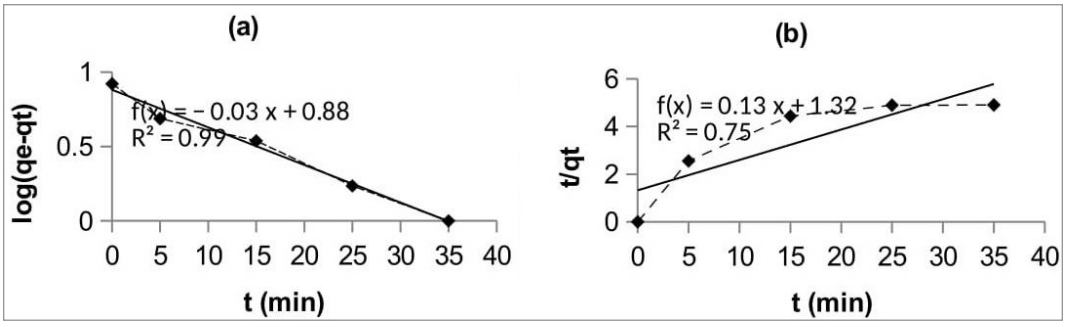

Figure 5. Plot of adsorption kinetic models: (a) pseudo-first-order equation, (b) pseudo-second-order equation, for biosorption of Cd(II) by JSP.

Equilibrium sorption spontaneity studies

In the experimental working process, all experiments were carried out at room temperature (28 ºC), maintaining the optimum temperature. In order to reveal the spontaneity of the equilibrium adsorption process of Cd(II) onto the JSP, the thermodynamic parameter Gibbs free energy change was calculated using Equation 13. The biosorption process is considered equilibrium reactions between the adsorbate and adsorbent. For such equilibrium reactions, the distribution constant, Kc, is used to evaluate the thermodynamic parameter (ΔG°) since it depends on temperature. It is calculated using Equation 12:

$$K_C = \frac{\mathrm{Cad}}{\mathrm{Ce}} \qquad (12)$$

Where Kc is related to change in free energy expressed by Equation 4.

$$\Delta G^\circ = -RT\ln K_c \qquad (13)$$

Where, Kc is the thermodynamic equilibrium constant and is used to calculate Gibbs free energy; C_{ad} = mg of adsorbate adsorbed per liter; C_e = the equilibrium concentration of solution, mg L^{-1}; R is the universal gas constant (8.314 J/mol/K) and T is the reaction temperature in Kelvin (301 K). Table III shows the calculated Gibbs free energy change with negative value for a range of initial concentrations, confirming the feasibility and spontaneous nature of the biosorption process.

Table III. For different initial concentration of Cd(II), calculated values of Gibbs free energy

Initial concentration C_0 (mg L^{-1})	Equilibrium concentration C_e (mg L^{-1})	Amount adsorbed C_{ad} (mg L^{-1})	ΔG°_{ad} (J mol^{-1})
20	7.111	12.889	-488.02
50	23.164	26.836	-368.16
80	38.79	41.21	-151.42
100	49.976	50.024	-2.4

Desorption of metal ions and reusability of biosorbent

It is important to desorb the metal ions and regenerate the adsorbent used to make the process economically feasible. The desorption studies were carried out by batch process using 0.1 M HCl, 0.1 M HNO_3, 0.1 M NaOH, 0.1 M NaCl, and distilled water. The percentage recovery of Cd (II) obtained with HCl 0.1M (86.49%) was higher than with the other solutions. It was observed that 0.1 M NaOH and distilled water desorbed Cd(II) ions to a very small amount. The desorption of metal ions with acidic solution is greater, indicating that adsorption in low pH is less and metal ions are exchanged by hydrogen ions. Similar results were also reported by several researchers using different agro-based biosorbents [33]. As 0.1 M HCl is most efficient to regenerate the biosorbent, it was used to investigate the desorbing agent four times. For the study of desorption, 0.5 g of Cd(II) loaded JSP was kept in contact with 100 mL of 0.1 M HCl for 60 min and the desorbed acidic solution was subjected to atomic absorption spectrometry to determine the metal concentration. Five successive cycles of adsorption and desorption were investigated and the efficiency was found to be almost unchanged for the first three cycles and then gradually reduced for the fourth and fifth cycles (Figure 6). The regenerated JSP could be used three times with only minor efficiency loss. Functional groups described in IR analysis and structural porosity are important for the adsorption and desorption processes. These adsorbing sites in the structure of JSP may cause damage for recurrent use with a desorbing agent (0.1 M HCl) resulting in less efficiency after three or four times.

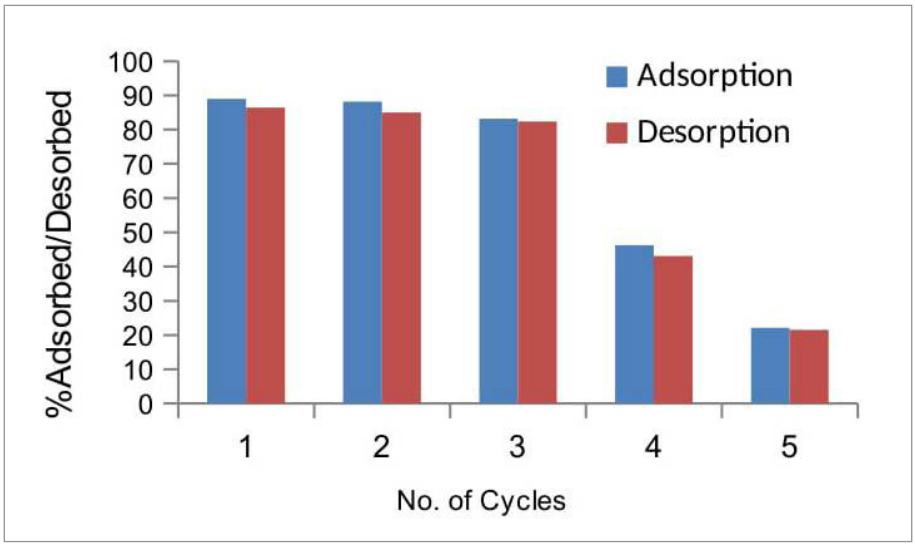

Figure 6. Biosorption and desorption cycles for Cd(II) onto JSP using 0.1 M HCl as desorbing agent.

CONCLUSION

The biosorption of Cd(II) by Jute Stick powder biomass is found to be influenced by the solution pH, biosorbent dose, contact time, temperature and initial metal ion concentration. The sorption data were fitted to Langmuir, Freundlich, Temkin and Dubunin–Radushkevich isotherms, of which, the Freundlich adsorption model was found to be the best fit with the aspects of highest regression value and other parameters. The kinetic studies indicate that the biosorption process follows pseudo-first-order and pseudo-second-order models, but that the pseudo-first-order model was better for interpreting kinetic data. The biosorbent Jute Stick powder can be regenerated and reused successfully more than three times. The values of the thermodynamic parameters show the spontaneous nature of the biosorption of Cd(II) from aqueous solution. Compared to the other low cost agricultural biosorbents from the literature (Table IV), the adsorption capacity of JSP is high. This is because the current biosorbent consists of an adequate number of acidic groups, and carboxyl and hydroxyl groups from the IR study. Jute Stick has no or little economic value, and is readily available, which strengthens its future use as a cost-effective adsorbent for the elimination of toxic heavy metals from aqueous solutions.

Table IV. A comparison of different low cost adsorbents in terms of Cd(II) metal ion adsorption capacities (q_{max})

Biosorbent	pH	q_{max}	Reference
Tea waste	5	1.76	[4]
Almond shell	5–6	7.19	[34]
Caulerpa lentillifera	5	4.7	[35]
Gas industry sludge-based adsorbent	5	25	[36]
Chemically modified Sorghum bicolor	5–6	17.2	[35]
JSP	4–5	10	This study

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ARTICLE

Selective Potentiometric Sensors for the Determination of Quetiapine Fumarate in Pharmaceuticals and Spiked Human Urine

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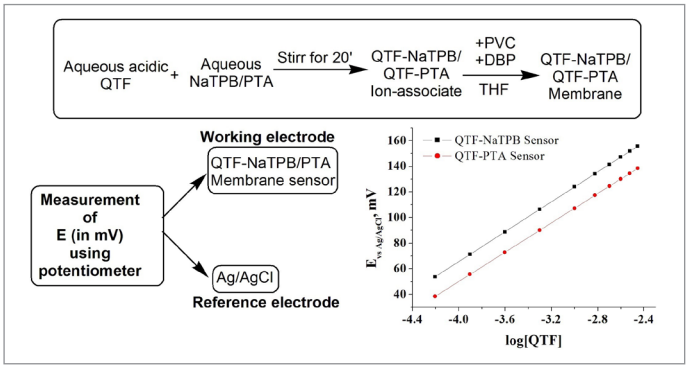

Quetiapine fumarate (QTF), chemically known as 1-[2-(2-hydroxyethoxy)-ethyl]-4-(dibenzo[b,f][1,4] thiazepin-11-yl)-piperazinium hemifumarate, is one of the derivatives of dibenzothiazepine. It is used as an atypical antipsychotic drug and is prescribed for the treatment of schizophrenia and bipolar disorders. Fabrication and the application of two selective potentiometric sensors for determination of QTF in pharmaceuticals and spiked human urine are presented. The membrane sensors are fabricated by preparing ion pair complexes of QTF with sodium tetraphenyl boron (NaTPB) and phosphotungstic acid (PTA). Using the ion-associates of QTF-NaTPB and QTF-PTA, Sensor I and Sensor II, respectively, were designed in polyvinyl chloride matrix using dibutyl phthalate as a plasticizer in THF. The fabricated Sensor I and II are applicable for the quantification QTF over the concentration range from 6.25×10^{-5} to 3.5×10^{-3} M QTF. The operative pH ranges for the determination of QTF were found to be in the range from 1.5 to 2.20 and from 1.00 to 1.6, for Sensor I and II with the Nernstian slopes of 58.34±1.04 and 57.23±0.78 mV/decade, respectively. The regression coefficient values of 0.9992 and 0.9982 show good correlation between the measured potentials and concentrations using Sensor I and II, respectively. The limit of detection (LOD) values for the fabricated sensor are calculated and reported. The experimental conditions have been optimized to reach the effective performance characteristics of the sensors. Standard-addition procedure is followed to study the effect of additives in tablets and foreign species in spiked human urine. The results revealed no such variations due to presence of additives or foreign species or endogenous species. The fabricated sensors are subjected to validation to check accuracy, precision, robustness and ruggedness. The mean accuracy for the determination of QTF is very close to 100%. The developed and validated sensors have yielded excellent results.

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Keywords: Potentiometric sensor, quetiapine fumarate, determination, pharmaceuticals, spiked human urine.

INTRODUCTION

Quetiapine fumarate (QTF), chemically known as 2-[2-(4-dibenzo[b,f][1,4]thiazepin-11-yl-1-piperazinyl) ethoxy] ethanol hemifumarate (Figure 1), is an atypical antipsychotic drug used for the treatment of schizophrenia and acute episodes of bipolar disorder [1-3].

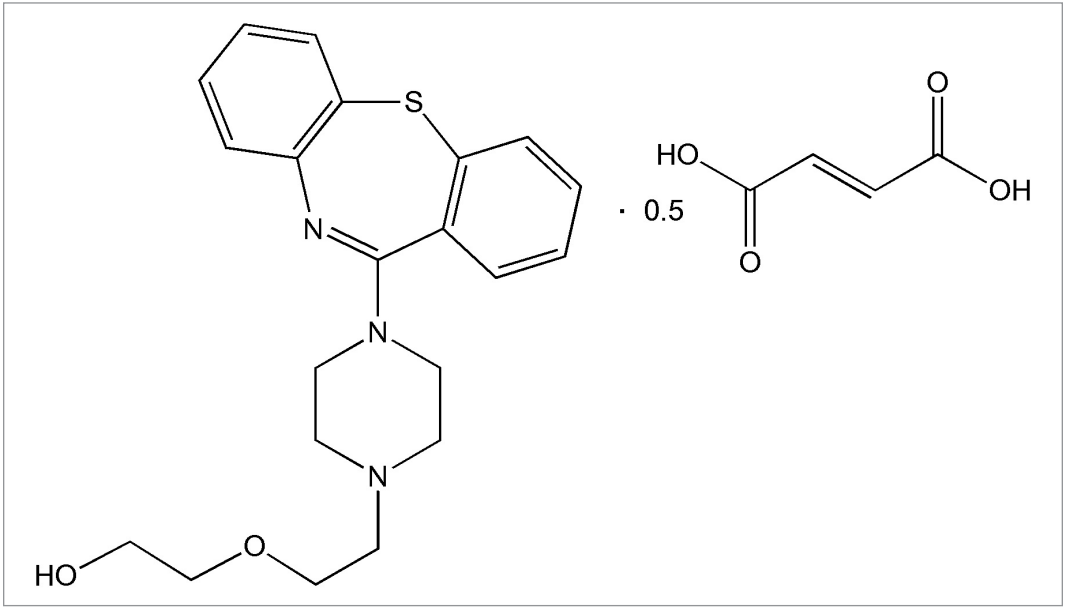


Figure 1. Structure of QTF.

QTF is not official in any Pharmacopeia. Many analytical methods are reported by different workers for the determination of QTF in pure form, formulations and in biological materials. High-performance liquid chromatography (HPLC) [4-12], ultra-performance liquid chromatographic with tandem mass spectrometry (UPLC-MS) [13,14], HPLC with different detection systems such as chemiluminescence [15], electrospray ionization mass spectrometry (ESI-MS) [16-19] and tandem mass spectrometry [20-24], and gas chromatography [25,26] techniques have been used for the assay of QTF in body fluids. However, QTF has been determined in pharmaceuticals by titrimetric [27,28], potentiometric [29], polarographic [30], differential pulse and square wave voltammetric [31], capillary zone electrophoretic [32,33], high performance thin layer chromatographic (HPTLC) [34-36], HPLC [12,37-41], UPLC [42] and spectrophotometric [28,32,43-49] techniques.

Of the various analytical methods mentioned above, the instruments required to determine QTF in pharmaceuticals by voltammetric [30,31], capillary zone electrophoretic [32,33], high performance thin layer chromatographic (HPTLC) [34-36], HPLC [12,37-41] and UPLC [42] are highly sophisticated. Moreover, a highly skilful operator or an expertise is required to carry out the analysis. The titrimetric [27,28] and the conventional potentiometric [29] techniques, although simple, are applicable for macro-size samples. Also, they consume larger volumes of organic solvents such as $CHCl_3$ and glacial acetic acid. The spectrophotometric methods are also limited in use because they require organic and toxic solvents, extraction requirements and the maintenance of one or many stringent experimental conditions. Because of one or more limitations of the reported analytical methods, attempts are being made to develop new, simple, rapid and highly selective analytical methods for quantification of QTF in pharmaceuticals and in spiked human urine.

Potentiometry with membrane sensor electrode, also known as an ion-selective electrode (ISE), for

analysis of organic compounds, is a highly simple technique because only the measurement of potential is involved. The selective functioning of the ISE is decided by the composition of the membrane. Therefore, membrane sensors are going to be better tools for the quantification of compounds in a hassle-free manner and without compromising selectivity and sensitivity.

The literature survey presented above indicated only one report [50] on potentiometric sensor for determination of QTF in biological and pharmaceutical samples. Fabrication of a coated wire electrode using tetraphenyl borate as ion pair complexing agent, PVC as supporting matrix, 2-nitrophenyl-octyl ether as solvent mediator and potassium tetrakis-(4-chlorophenyl) borate as lipophilic additive was described. This electrode was found to respond to QTF with a Nernstian slope of 57 mV/decade and is selective for functioning up to 30 days only. Moreover, the detailed validation results are absent from the report.

Therefore, an attempt has been made to develop two potentiometric membrane sensors to determine QTF in pharmaceuticals and spiked human urine. The membrane sensors have been fabricated by using the ion pair complexes formed between protonated QTF ($QTFH^+$) in acidic medium and either sodium tetraphenyl borate (Na^+ TPB^-) or phosphotungstic acid anion (PTA^-) as ion-pairing agents in THF solvent using polyvinyl chloride as matrix and dibutyl phthalate (DBP) as a plasticizer. Different parameters have been optimized to improve the selectivity of membranes for the accurate and precise determination of QTF. The fabricated sensors have been used to determine QTF in pharmaceuticals to assure the selectivity of sensors to determine active component in the presence of many unknown inactive ingredients present in tablets. The sensors have also been used to determine QTF in spiked human urine to ascertain their applicability for physiotherapeutic administration of drug so that the simple means of technique can be provided to quantify QTF.

MATERIALS AND METHODS

The chemicals and reagents used were of analytical grade. Distilled water was used throughout the work. The pure QTF (99%) was kindly provided by Cipla India Ltd, Bangalore, India. It was used without further purification. Quitipin tablets (200 mg QTF/tablet) (Sun Pharmaceuticals Laboratories Ltd., Mumbai, India) were purchased from local commercial sources. Sodium tetraphenyl boron (NaTPB), phosphotungstic acid (PTA), tetrahydrofuran (THF), dibutyl phthalate (DBP), bibutyl sebacate (DBS), dioctyl phthalate (DOP), o-nitrophenyl octylether (NPOE) and polyvinyl chloride (PVC) were supplied from S. D. Fine Chem Ltd, Mumbai, India. Concentrated sulfuric acid (H_2SO_4) (98% v/v, Sp. gr. 1.84) was supplied by, S. D. Fine Chem Ltd, Mumbai, India. Urine sample was collected from a 22 year-old male healthy volunteer.

The solutions of 5.0 mM each of NaTPB and PTA, 0.1 M H_2SO_4, 0.5 M each of NaOAc, Na_2CO_3, $NaHCO_3$, NaOH, CH_3COOH, glycine, $AgNO_3$, talc, Arginine, KCl, glucose, KOH, KNO_3, KH_2PO_4, H_3PO_4, $NaNO_2$, oxalic acid, sucrose, talc, urea, cadmium chloride and cobalt chloride (all from S.D. Fine Chem Ltd., Mumbai, India) were prepared in bi-distilled water.

Apparatus

A digital dual channel potentiometer (PICO Chennai-32, India), Ag/AgCl reference electrode with the internal solution containing the saturated solutions of KCl and AgCl and copper and aluminum wires were used for potential measurements. A multichannel pH meter (Labtronics Ltd, Mumbai, India) was used for pH measurements. An Elico Conductivity meter (Hyderabad, India) was used to measure conductance.

Preparation of standard QTF solution

A standard 5.0 mM solution of QTF was prepared by accurately dissolving a calculated quantity of pure drug in 100.0 mL of 0.1 M H_2SO_4 in a volumetric flask.

General procedure

Procedure for fabrication of sensors

Twenty five mL each of 5.0 mM QTF and 5.0 mM NaTPB or PTA solutions were transferred into a beaker

and stirred for 30 minutes on a magnetic stirrer. The content was filtered through Whatmann Nº 40 filter paper. The precipitate was dried for 24 h at room temperature. A 40 mg of clean and dried precipitate was taken in a Petri Dish of 5 cm width, 300 mg of PVC and 100 mg of DBP were added, and the content was dissolved in 10 mL of THF. After mixing, the content was allowed to evaporate under room temperature for 24 hours. The thin layer of dried membrane was fused to one end of a Pyrex glass tube with the aid of THF. The tube was dried and filled with 3-5 mL of 5 mM QTF solution. A pure aluminum wire (2.0 mm i.d x 20 cm length) was tightly insulated leaving 1.0 cm at the ends for connection. One end of the wire was inserted into the solution of the tube and the other terminal was connected to the potentiometer. The QTF-NaTPB (Sensor I) and QTF-PTA (Sensor II) sensors were then soaked in the standard 5 mM QTF solution at least for 1.75 and 2 h, respectively, before use for the measurement of potential.

The sensors fabricated above were used for potential measurements. The systematic representation of the potentiometric electrochemical cell could be depicted as follows:

$$Ag\text{-}AgCl_{Ref} \| QTF\text{-}NaTPB/PTA_m \mid [QTF]_{Int} \mid Al\ wire$$

where Ref is reference electrode, m is membrane and $[QTF]_{Int}$ meant for QTF internal solution of fixed concentration.

The Nernst equation was related between the potential and concentration of QTF [51] in the electrochemical cell used for potentiometric determination, can be written as:

$$E_{Cell} = K + 0.05916 \log[QTF]_{Sample}$$

where K accounts for the potential of the reference electrode, liquid junction potentials, the asymmetry potential, the activity coefficient of QTF and the concentration of QTF in the internal solution. Thus, this equation is to show the linear relationship between E_{cell} and concentration of QTF in the solution with the Nernstian slope of ~60 mV. Under this background, potentiometry, as a simple technique, was employed to quantify QTF.

Preparation of calibration curve

Different volumes (0.125, 0.250, 0.500, 1.000,……7.000 mL) of 5.0 mM standard QTF solutions were placed into a series of 10 mL volumetric flasks with the help of a micro-buret. The pH of each solution was brought in between 1.5 and 2.2 with 0.5 M NaOAc solution or 0.1 M H_2SO_4. The volume of each flask was then adjusted to 10 mL with water, mixed the content well and transferred into a series of 25 mL beakers. The conditioned membrane sensor (Sensor I) and Ag-AgCl reference electrode were immersed into the solution and recorded the potential of each solution using a pre-calibrated potentiometer. However, to measure potential using Sensor II, the pH of the solution was maintained in between 1 and 1.6 and the procedure followed intact.

The calibration graphs of measured potential *versus* log [QTF] were prepared. The concentration of the unknown was found by using calibration graphs or regression equation derived using potential *versus* log [QTF] data.

Procedure for tablets

Ten QTF tablets were weighed, transferred into a clean dry mortar and powdered. A portion of the tablet powder equivalent to 500 mg of QTF was transferred into a 100 mL volumetric flask and shaken with 70 mL of 0.1 M H_2SO_4 for 20 minutes. The content, after diluting to the mark with the same solvent, was mixed and filtered through Whatmann No. 41 filter paper. A suitable aliquot was used and measured the potential by following the procedure as described under preparation of the calibration curve. The concentration of QTF was calculated using the calibration curve or regression data.

Procedure for spiked urine sample

A 25.0 mg sample of pure drug was accurately weighed and dissolved in 10 mL of $CHCl_3$ in a 50 mL beaker and the solution was transferred into a 125 mL separating funnel. One milliliter of urine sample was added followed by 20 mL of water. The content was extracted with 10 mL portions of $CHCl_3$. The triplicate extract was collected in a beaker containing anhydrous Na_2SO_4 and the solvent was evaporated to dryness on water bath. The resulted residue was dissolved in 0.1 M H_2SO_4 and the volume brought up to 50 mL with the same solvent. A suitable aliquot was then transferred into a 25 mL beaker, the pH was brought to a value between 1.5 and 2.2 (Sensor I) or 1.00 and 1.60 (Sensor II) and the solution was diluted to 10 mL with water. After mixing, the potential was measured using either QTF-NaTPB (Sensor I) or QTF-PTA (Sensor II) sensor as a function of Ag-AgCl reference electrode. The concentration of QTF in the solution was calculated with the help of calibration curve or regression equation.

Procedure for interference study

Into a series of 25 mL beakers, 2.0 mL of 5.0 mM pure drug solution and 6 mL of 0.1 M H_2SO_4 were taken. One milliliter of 0.5 M solution of interferent was added, the pH was brought to the optimum value mentioned in the preparation of the calibration curve, the content was diluted to mark with water and after mixing, and the potential of each solution was measured using the electrochemical cell assembled for preparation of calibration curve.

Procedure for determination of selectivity coefficient of sensors (Study of interference)

The interference study was performed using the solutions of fixed quantity of intereferent and varying amounts of analyte at optimum pH. Into a series of 10 mL beakers, varying volumes (0.25 to 3 mL) of 5 mM solution of QTF were transferred and 1.0 mL of 0.5 M interferent was added to each beaker. The pH in each solution was adjusted to the required level, as described above, and the final volume was brought to 10 mL with water. The potential of each solution was measured. The procedure was repeated for each intereferent separately.

The graph of measured potential *versus* log [QTF] was prepared. The point of intersection between two linear portions in the plot was located. At the point of intersection the value of selectivity coefficient ($K_{QTF.I}$) was calculated by using the formula [51]:

$$K_{QTF.I} = \frac{[QTF]_E}{[I]_E^{z_{QTF}/z_I}} = \frac{[QTF]_{Int}}{[I]_{add}^{z_{QTF}/z_I}}$$

where z_{QTF} and z_I are the charges of the analyte and interferent, respectively, and $[QTF]_E$ and $[I]_E$ are the concentrations of analyte and interferent yielding identical cell potentials. $[QTF]_{int}$ is the QTF concentration in the internal solution and $[I]_{add}$ is the concentration of interferent added to the QTF solution.

Procedure for determination of stoichiometry of ion-pair complexes

A 10 mL aliquot of 0.01 M QTF was transferred into a clean beaker and placed on a magnetic stirrer. The conductivity cell was immersed into the solution and the titration was carried out by adding 0.01 M NaTPB or PTA. The recorded conductance values were plotted against the molar ratio of titrant and the composition of each ion-association complex was examined.

RESULTS AND DISCUSSION

The chemical reaction between protonated QTF ($QTFH^+$) solution in acid medium with anion of either NaTPB or PTA yields the respective electrically neutral 1:1 ion-association complex. The aqueous insoluble ion-association complex of $QTFH^+$-NaTPB or $QTFH^+$-PTA is useful as a recombinant material to fabricate the membrane sensor for determination of QTF concentration. The membrane is formed effectively

with uniform thickness all over its area when PVC and DBP were used as the matrix and plasticizer, respectively. The membranes constructed here should therefore selectively response to QTF, as the artificial ion-selective sensors. The potentiometry will enable their use to confirm the selective functioning by generating the potential difference due to the different concentrations of QTF solutions at opposite sides of the membrane [51].

Composition of ion-association complex

Preliminary experiments were carried out to deduce the reaction stoichiometry between QTF and NaTPB or PTA in the formation of ion-pair complexes. Conductometric experiments yielded satisfactory results. The stoichiometric ratio between either QTF and NaTPB or QTF and PTA was determined by conductometric titration of QTF with either NaTPB or PTA as titrant [52]. Figure 2, obtained by plotting the graphs of conductance *versus* molar ratio of either NaTPB or PTA, revealed the stoichiometry of 1:1 with respect to QTF and either NaTPB or PTA, which was indicated by the appearance of the equivalence point of the titration at the molar ratio of QTF with either NaTPB or PTA of 1.0. The conductance values at the beginning of the titration were absolutely due to the free cationic protonated QTF ($QTFH^+$). After commencing the addition of titrant there were gradual increases in the conductance up to the equivalence point. Due to a decrease in concentration of $QTFH^+$ by involving in ion-association reaction, the solution becomes diluted and hence the trend was as seen. The conductance beyond the equivalence point was assumed as presence of excess titrant in the presence of ion-associate at high concentrations. The reaction between protonated QTF ($QTFH^+$) and anionic species of NaTPB or PTA, $NaTPB^-$ or PTA^- is expected to take place as presented in Scheme 1. This influences the almost Nernstian response by the membranes while measuring the potential. Slopes of 58.34±1.04 and 57.23±0.78 mV/decade, respectively, for QTF-NaTPB and QTF-PTA sensors, satisfied the said Nerstian response. Therefore, the reagents NaTPB and PTA were tested as active materials for development of selective membrane sensors for the determination of QTF.

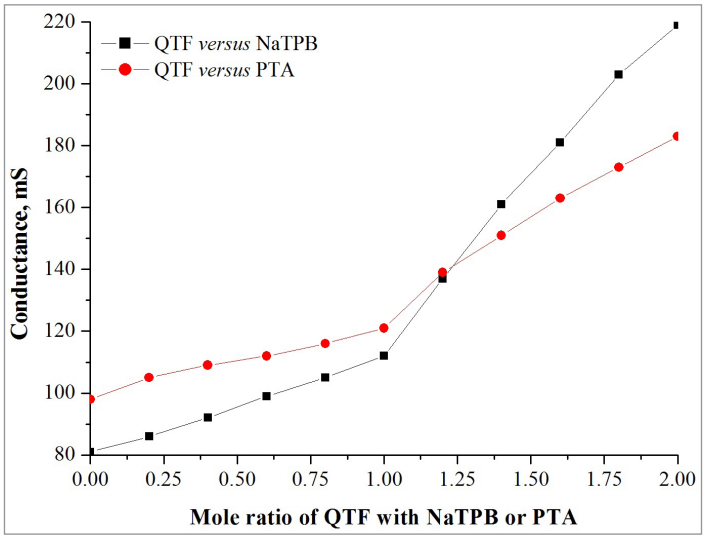
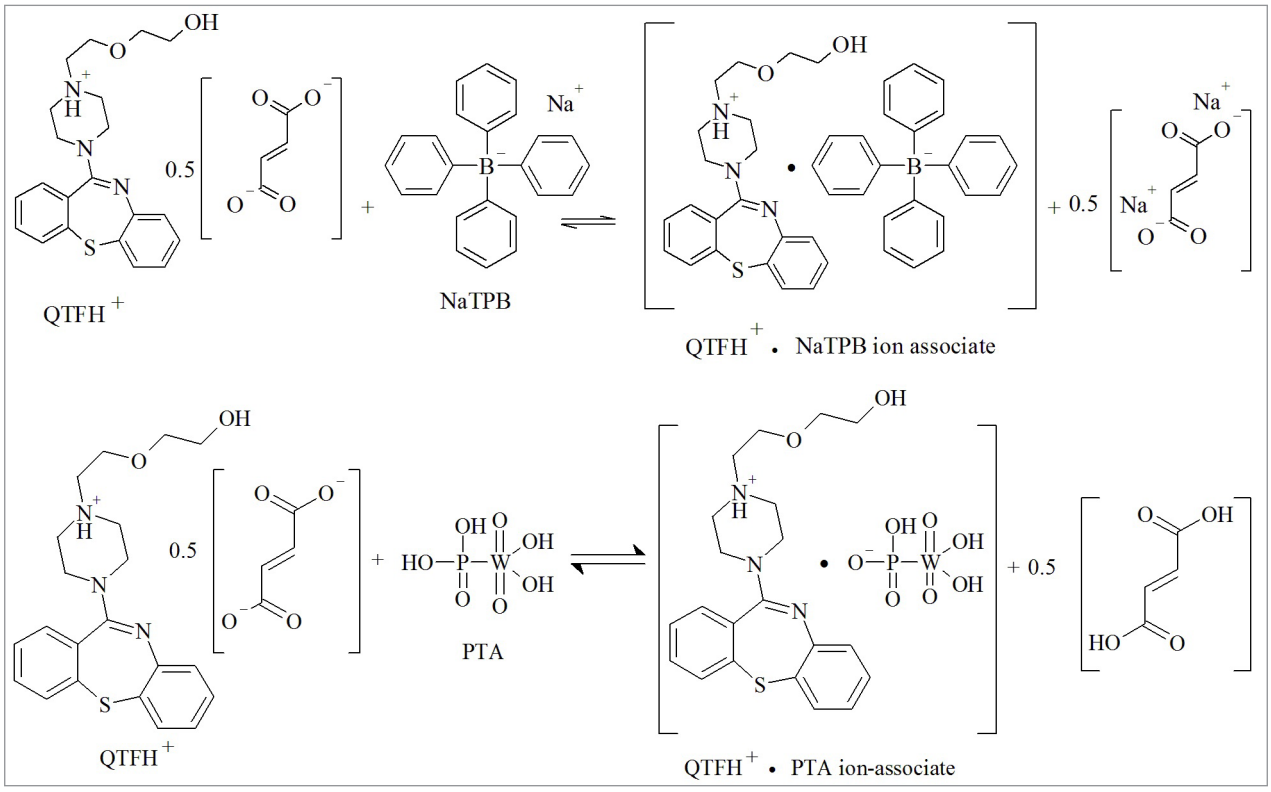


Figure 2. Variation of conductance as the function of mole ratio of QTF with either NaTPB or PTA in the formation of $QTFH^+$-NaTPB/$QTFH^+$-PTA ion-association complexes.



Scheme 1: Pathway of reaction in the formation of $QTFH^{+}$-$NaTPB^{-}$/PTA^{-} ion-association complex.

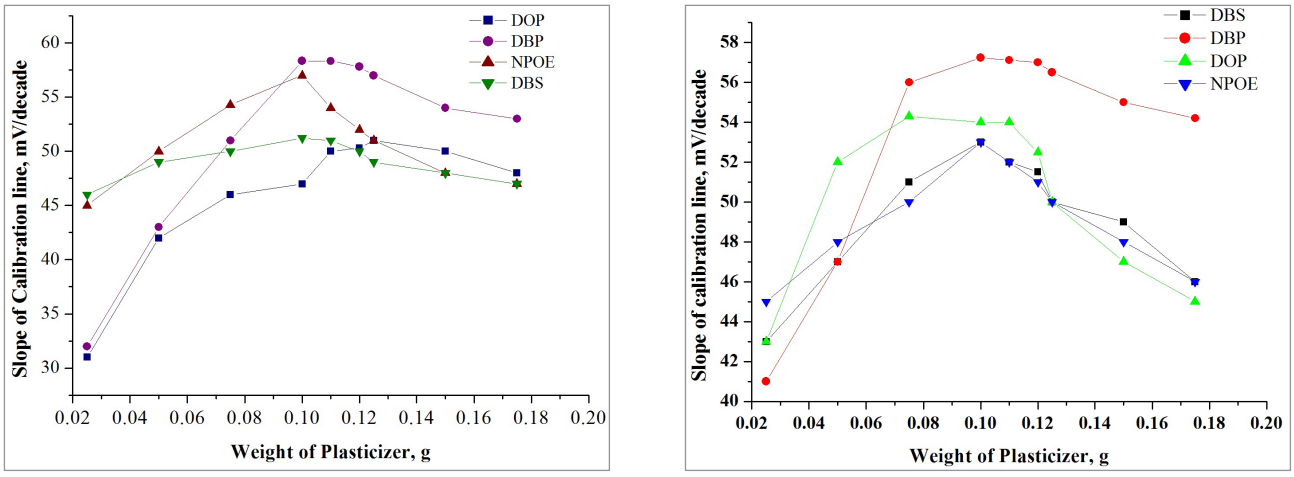
Optimization of variables

Membrane components

At the beginning of the preparation of membranes, different amounts of materials such as ion-associate, PVC and plasticizer were used and the effective functioning for sensing QTF was evaluated by potentiometry. In the preparation of QTF-NaTPB or QTF-PTA sensors, the results of preliminary investigations showed that the membrane prepared using 40 mg of ion-associate, 300 mg of PVC and 100 mg of DBP in 10 mL of THF was to the most convenient for use with the thickness of 0.5 mm. The membranes prepared with lower quantities of ion-associate, PVC or plasticizer were found to have an inappropriate thickness and not to effectively function. At volumes of THF larger than 10 mL, little variation was seen in the sensing ability of the membrane. The membranes were found to have dried completely in 24 h after pouring to Petri Dish; thus, the standing evaporation time was fixed as 24 h. Thus, the procedure followed to prepare the membranes, as described above, was found as optimized. The fabricated membranes obtained results in excellent agreement with respect to linearity of the calibration curve with good Nernstian behavior.

Plasticizer

The sensing membranes were prepared separately by adding different amounts of dibutyl sebacate (DBS), dibutyl phthalate (DBP), dioctyl phthalate (DOP) and o-nitrophenyl octylether (NPOE) as plasticizers. The membranes prepared using 100 mg of DBP for Sensor I and II were found to behave in a Nernstian manner. The sensors were found to perform satisfactorily with respect to stable potential readings, ease of conditioning and less response time. Therefore, DBP was used as plasticizer in fabricating Sensor I and II for determination of QTF. The variation of Nernstian slope of the calibration line for membrane sensors fabricated using different amounts of different plasticizer is presented in Figure 3.



(a) (b)

Figure 3. Effect of type and quantity of plasticizer in the fabrication of: a) QTF-NaTPB and b) QTF-PTA sensors.

QTF internal solution

The effect of the concentration of internal QTF solution on the potential response of the sensors was studied. Different trials were carried out after filling the electrode with different concentrations of QTF solution and measuring the potential of the QTF standard solutions of concentrations mentioned in preparation of calibration curves. Excellent linearity (Figure 4) between potentials and the logarithmic concentration of QTF solutions were obtained with acceptable Nernstian slope only at a QTF concentration of 5 mM in the internal solution. At the other QTF concentrations in the internal solution, the linearity and correlations between QTF concentrations and potential values were not in good agreement. Therefore, 5 mM QTF solution was used as an internal solution for both sensors.

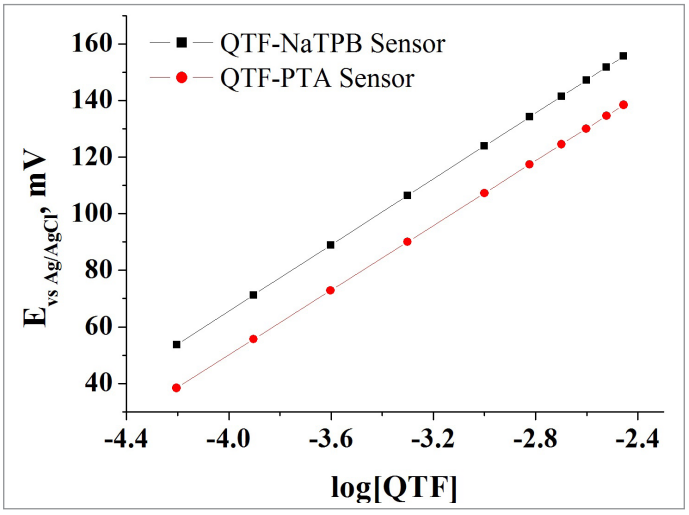


Figure 4. Calibration curves prepared by plotting the potentials of 0.125, 0.25, 0.50, 1.0,……7.0 mL of 5 mM standard QTF solutions (equivalent to 6.25×10^{-5} to 3.5×10^{-3} M) of pH adjusted to either between 1.5 and 2.2 with QTF-NaTPB sensor or between 1 and 1.6 with QTF-PTA sensor.

Choice of conducting wire

Copper (Cu), silver (Ag) and aluminum (Al) wires with proper insulation to required length were used as conducting wires to immerse into the internal solution of QTF in the membrane sensors and potentials of standard QTF solutions measured. Satisfactory results were obtained with Al when compared to others. Moreover, the use of cheaper and PVC-insulated Al wire was very widely used in constructing the ion-selective electrodes for determining aromatic amines [53], cationic surfactants [54], triarylmethane dyes [55], 4-aminoacetanilide [56], septonex, pilocarpine, ethylmorphine, homatrophine and cinchocaine [57], procaine and trimecaine [58], atrophine [59], cefditoren [60], etc. Therefore, an Al wire was used as medium of conductor in measuring potentials of QTF solutions using both the proposed sensors.

Soaking time

Sensor activation is an important parameter in potentiometry with membrane sensors. The surfaces of sensors were effectively activated by soaking in standard solution of analyte and the potential of the solution was expected to become constant. It was obtained that after immersing the respective sensor into analyte solution the potentials were become constant after 1.75 and 2 h for QTF-NaTPB and QTF-PTA sensors, respectively. Thus, these times were fixed as time duration required to make the active surface of the membrane ready for effective use at 25 °C. The effect of soaking time on the potential using QTF-NaTPB and QTF-PTA sensors are presented in Figure 5. This study also revealed and recommended that the sensors may be kept dry and packed in an opaque closed vessel whenever they are out of use.

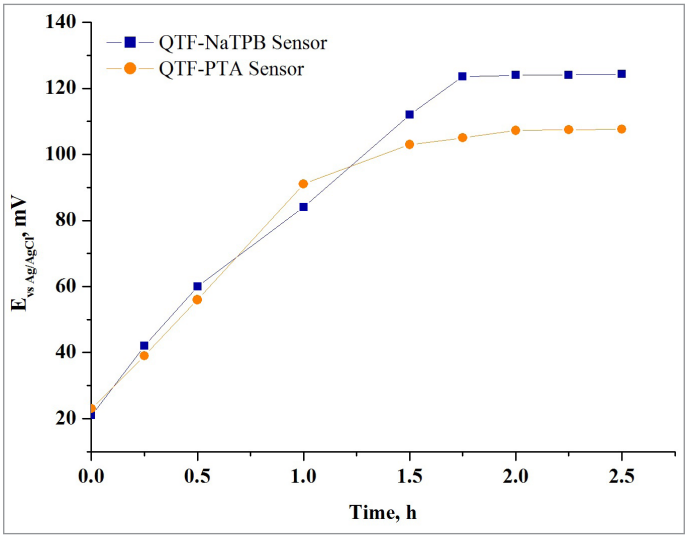


Figure 5. Effect of soaking time on potential of 1 mM QTF solution of pH adjusted in between 1.5 and 2.2 while measuring with QTF-NaTPB sensor and in between 1 and 1.6 while measuring with QTF-PTA sensor.

Fixing of pH

The effect of pH while measuring the potential of QTF solutions was evaluated by potential measurement. The measurements were performed by potentiometry in the pH range from 0.5 to 8.0 using Sensor I and II separately. The pHs of solutions were brought to the required values by adding either 0.1 M H_2SO_4 or 0.5 M NaOAc or 1:5 NH_3 solutions. The resulted effect on potential was recorded and the consequent plot is presented in Figure 6. Less stable and lower potential values were observed at a pH less than 1.5 and greater than 2.2 with QTF-NaTPB sensor. The same trend was observed with QTF-PTA sensor at pH less than 1 and greater than 1.6. Thus, this study revealed that the pH ranged between 1.5 and 2.2 or 1 and 1.6 is required to be maintained for the measurement of potential of QTF solutions using Sensor I or II.

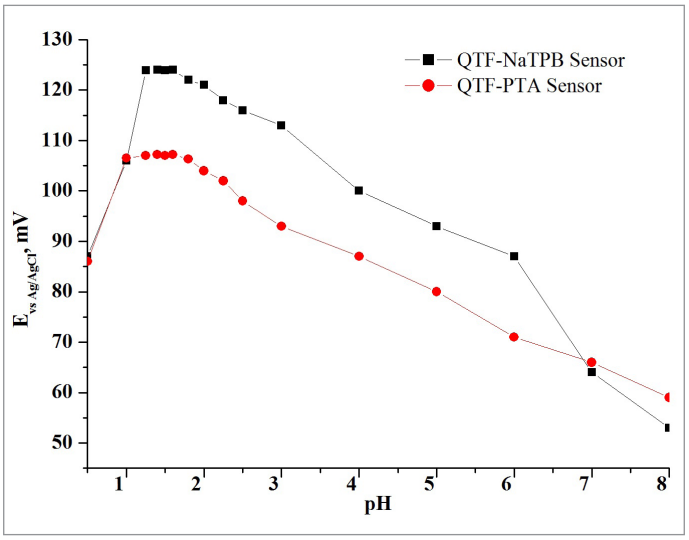


Figure 6. The effect of pH on potential of 1 mM QTF solution measured using QTF-NaTPB and QTF-PTA sensors.

Response time

The experimental response time [60] for the proposed sensors was evaluated and found to be 8.0 and 12.0 s for QTF-NaTPB and QTF-PTA sensors, respectively.

Life time of sensors

The life times of QTF-NaTPB and QTF-PTA Sensors were evaluated to assess their stable and uncompromised performance ability. It was confirmed from the study that both the sensors resulted in Nernstian slopes without deviation from the actual optimum values for at least 60 days. This revealed that the sensors could be used continuously for up to 60 days. However, after 60 days, their characteristics significantly drifted away from the Nernstian behavior (Figure 7). Therefore, the average life time of QTF-NaTPB and QTF-PTA Sensors were proposed as 60 days.

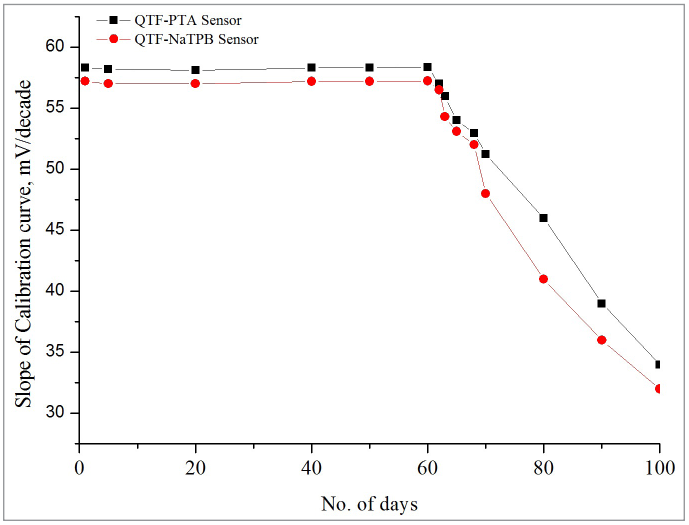


Figure 7. Life time of QTF-NaTPB and QTF-PTA Sensors.

Selectivity coefficients of sensors

The selectivity coefficients ($K_{QTF.I}$) of QTF-NaTPB and QTF-PTA Sensors were investigated in the presence of inorganic and organic compounds as spikes. The $K_{QTF.I}$ values of QTF-NaTPB and QTF-PTA Sensors in the presence of various compounds have been determined experimentally by preparing a series of solutions, each of which contains the same concentration of interferent, $[I]_{add}$, but a different concentration of QTF and measuring the cell potential using respective sensor. A plot of potential *versus* the log [QTF] concentration has two distinct linear regions [51]. When the analyte's concentration is significantly larger than $K_{QTF.I}[I]_{add}$, the measured potential is a linear function of log [QTF], in the presence of interferents, as given by equation [51]:

$$E_{cell} = K + 0.05916\log([QTF]_{sample} + K_{QTF.I}[I]^{\frac{Z_{QTF}}{Z_I}})$$

where $[QTF]_{sample}$ and [I] are the concentrations of QTF of charge Z_{QTF} and interferent of charge Z_I in the solutions, respectively.

If $K_{QTF.I}[I]$ is significantly larger than the QTF's concentration, however, the cell potential remains constant. The concentration of analyte and interferent at the intersection of these two linear regions is used to calculate $K_{QTF.I}$ [51].

The values of $K_{QTF.I}$, presented in Table I, revealed that Na^+, oxalic acid and sucrose showed significant interference with QTF while using the QTF-NaTPB Sensor. Although, sucrose is a non-ionic compound, the detectability of Sensor I was found for it. Thus, sucrose is treated as non-ionic interferent in the determination of QTF. On the other end, K^+ and H^+ ions were proved as interferents while measuring the potential of QTF with QTF-PTA sensor. The results presented below as values of $K_{QTF.I}$, less than unity indicated that the proposed sensors are suitable to determine QTF in the presence of interferents.

Table I. Selectivity coefficients ($K_{QTF.I}$) of sensors

Interferent Species	Selectivity coefficient, $K_{QTF.I}$*	
	QTF-NaTPB Sensor	QTF-PTA Sensor
Cd^{2+}	0.398	0.417
K^+	0.042	1.450
H^+	0.035	1.110
Na^+	1.340	0.053
H_3PO_4	0.179	0.258
CH_3COOH	0.048	0.072
Oxalic acid	1.020	0.094
Sucrose	1.120	0.431
Talc	0.229	0.629

*Mean value of three determinations

Validation of sensors

The fabricated QTF-NaTPB and QTF-PTA sensors were validated according to IUPAC recommendations [62,63] and ICH Guidelines [64]. The validation results for individual parameters are presented in the following sections.

Linearity of calibration curve, regression data and performance characteristics

The sensors provide a rapid, stable and linear response over the QTF concentration ranges presented in Table II. The calibration lines (Figure 4) with slopes of 58.34±1.04 and 57.23±0.78 mV/decade, very close to 59.2 mV, indicated the Nernstian behavior of QTF-NaTPB and QTF-PTA sensors. The regression equations for QTF-NaTPB and QTF-PTA sensors were found to be Y = 58.34X+283 and Y = 57.23X+279, respectively. The regression coefficient values of 0.9992 and 0.9982 for QTF-NaTPB and QTF-PTA sensors, respectively, showed very good linearity between measured potentials and log[QTF]. Stable potential readings but variations by ±1 mV were seen during the period of 60 days of usage of sensors. The limit of detection (LOD), calculated according to IUPAC recommendations [62,65], from the intersection of the two extrapolated linear portions of the calibration curve and other performance measurement values for QTF-NaTPB and QTF-PTA sensors are presented in Table II.

Table II. Performance characteristics of sensors with regression data

Parameters	QTF-NaTPB Sensor	QTF-PTA Sensor
Linear range, M	6.25×10^{-5} to 3.50×10^{-3}	
Limit of detection (LOD), M	1.56×10^{-5}	2.16×10^{-5}
Slope (m), mV/decade	58.34	57.23
Intercept (b), mV	283	279
Correlation coefficient	0.9992	0.9982

Intra- and inter-day precision and accuracy

Pure QTF solutions of three different concentrations within the calibration range were prepared in seven replicates each. Intra-day variations were evaluated by calculating the %RSD for each concentration of QTF found. The values are presented in Table III. The pure QTF solutions of three different concentrations in five replicates were prepared and analyzed during different days for study of inter-day variations. The %RSD values for the found QTF amounts were calculated and presented in Table III. The accuracy was evaluated by calculating the amount of QTF found in intra- and inter-day basis. The relative error (RE), the metric for accuracy, was calculated for each concentration of QTF found. The obtained %RSD values ranged between 3.16 and 5.29%, indicating the satisfactory precision of the results. The %RE, which is an index of accuracy, ranged from 2.00 to 4.0 and indicated the acceptable accuracy in functioning of sensors. These results are summarized in Table III.

Robustness and ruggedness

The robustness of the proposed sensors was examined by deliberately changing the working pH. The solutions of 0.5, 1 and 1.5 mM QTF were used in the study. The %RSD values were calculated for the obtained results. The pH was varied by 0.2 units at before and after the range of values for each sensor [1.5(±0.2) to 2.2(±0.2)] for QTF-NaTPB and 1.0(±0.2) to 1.6(±0.2) for QTF-PTA sensors. For these variations, the %RSD values calculated were ranged between 2.52 and 4.52.

The ruggedness was checked by inter-analysts and inter-instrumental performance. The inter-analysts and inter instrumental RSD values of ≤3.5% showed robust functioning of QTF-NaTPB and QTF-PTA sensors. The %RSD of robustness and ruggedness studies are presented in Table IV.

Table III. Results of precision and accuracy

Sensor	QTF taken, mM	Intra-day variations			Inter-day variations		
		QTF found*, mM	%RSD	%RE	QTF found$, mM	%RSD	%RE
QTF-NaTPB	0.50	0.51	4.23	2.00	0.52	4.34	4.00
	1.00	1.03	3.16	3.00	1.04	4.77	4.00
	1.50	1.46	3.22	4.00	1.47	4.81	2.00
QTF-PTA	0.50	0.49	3.98	2.00	0.48	5.29	4.00
	1.00	1.04	4.67	4.00	1.04	5.00	4.00
	1.50	1.55	3.56	3.33	1.45	4.68	3.33

*Mean value of seven measurements; $Mean value of five measurements.

Table IV. Results of robustness and ruggedness (expressed in %RSD)

Sensor	Concentration of QTF, mM	%RSD Values for varied parameters		
		Robustness (by varying pH)	Ruggedness	
			Inter-potentiometric	Inter-analysts
QTF-NaTPB	0.50	2.59	2.11	3.14
	1.00	3.45	3.09	3.12
	1.50	4.51	3.50	2.93
QTF-PTA	0.50	2.52	3.29	2.44
	1.00	3.98	2.76	3.12
	1.50	4.52	2.77	2.94

Application of sensors to tablet analysis

The tablet extracts in three different concentrations were subjected to analysis by potentiometry using the proposed sensors. Five replicates each of 0.5, 1.0 and 1.5 mM QTF-containing tablet extracts were used to measure the potentials with proposed sensors by following the procedure described under '*procedure for tablets'*. The amount of QTF found with percentage recoveries was calculated for each concentration. The obtained results were statistically compared with the results of reference method [32]. In the reference method, a methanolic solution of QTF was measured at 246 nm by UV spectrophotometry. The mean percent recoveries of QTF from tablets were found as 97.32 and 96.21 with RSD value of 1.78 and 2.17%, for QTF-NaTPB and QTF-PTA, respectively. The accuracy and precision were evaluated by applying Student's *t*- test and variance ratio F- test, respectively. The calculated *t*- and F- values are less than the tabulated values, so it was clear from the assessment that the proposed sensors yielded accurate and precise results. The results are presented in Table V.

Table V. Results of analysis of QTF tablets using proposed sensors and statistical comparison of the results with the reference method

Tablet analyzed	mg of QTF/Tablet	Found*		
		%Label claim±SD		
		Reference method	Sensor	
			QTF-NaTPB	QTF-PTA
Quitipin	200.00	98.98±1.58	97.32±1.78 *t* = 1.56 F = 1.27	96.21±2.17 *t* = 2.33 F = 1.89

*Mean value of five determinations. The tabulated *t*- and F- values at 95% confidence level for four degrees of freedom are 2.77 and 6.39, respectively.

Recovery study

The standard addition procedure was followed to further ascertain accuracy of the sensor. The solutions were prepared by spiking pure drug into a pre-analyzed tablet powder at three different levels and potential was measured using each sensor separately. Into five replicates of 3 mL tablets, extracts of 5 mM QTF, 1, 2 and 3 mL of 5 mM QTF from pure drug were spiked, pH was adjusted to the optimum value and, after diluting to 10 mL, the potential measured and the amounts of QTF were calculated. The percent recovery of pure QTF was computed. The percentage recovery of QTF from tablets close to 100% revealed that good and acceptable recovery values were obtained. The results of recovery study are summarized in Table VI.

Table VI. Results of recovery study for accuracy assessment by standard-addition procedure

Sensor	QTF from tablet extract, mM	Pure QTF added, mM	Total QTF found, mM	%QTF recovered*	%RSD
QTF-NaTPB	1.50	0.50	1.99	99.00	2.13
	1.50	1.00	2.48	97.90	1.98
	1.50	1.50	2.94	96.20	2.77
QTF-PTA	1.50	0.50	1.98	95.20	2.45
	1.50	1.00	2.47	97.20	3.11
	1.50	1.50	2.98	98.53	2.89

*Mean value of three measurements.

Spiked human urine analysis

To ensure the suitability of developed sensors for physiotherapeutic administration of QTF by its quantification, spiked human urine sample was analyzed. A drug free urine was collected from healthy male volunteer, filtered and used for analysis by following the procedure described under 'procedures' section. After obtaining the extract from spiked human urine and after suitable dilution, completed the measurement of potential using the sensors by potentiometry. The percentage recovery of QTF from urine was calculated and reported with RSD values. The percent recovery of QTF ranged from 95.2 to 99.2 with RSD of less than 4% (Table VII) indicated the non-interference from the endogenous substances in urine. Therefore, these results held the applicability of QTF-NaTPB and QTF-PTA sensors for the analysis of urine samples to determine QTF.

Table VII. Results of analysis of spiked human urine

Sensor	[QTF] in urine, mM	[QTF] found, mM	%QTF recovered±SD*
QTF-NaTPB	1.00	0.990	99.00±1.23
	1.50	1.469	97.90±2.31
	2.00	1.924	96.20±3.67
QTF-PTA	1.00	0.952	95.20±3.44
	1.50	1.488	99.20±2.87
	2.00	1.971	98.53±2.11

*Mean value of three measurements.

CONCLUSIONS

In this study, for the first time, the fabrication, optimization and application of membrane-based potentiometric sensors using sodium tetraphenyl boron (NaTPB) and phosphotungstic acid (PTA) for the selective and rapid determination of quetiapine fumarate (QTF) was described. Membranes formed by the aggregation of QTF with either NaTPB or PTA exhibit required features for use as electrochemical sensors. Simple experimental design, ease of use, better performance characteristics, selective, robust and rugged

functioning ability are the hallmarks of the sensors. The potentiometric determination of QTF using these sensors requires a very simple instrument, so the technique is highly cost-effective. The maintenance of stringent experimental conditions is not required in the analysis. The superiority in reference to the performance characteristics of the proposed analytical methods to determine QTF over all other reported methods is highlighted in the Supplementary Material. The sensors are applicable for the determination of QTF in a wide linear range with a Nernstian response and low detection limits. The statistical comparison of results of determination of QTF using proposed sensors with those of reference method [32] revealed the selectivity and suitability of the electrodes for the accurate and precise determination of QTF in real samples. The results of the recovery study also revealed the inactive role of excipients in tablets in the determination of QTF. The sensors are also applicable for the determination of QTF in spiked human urine. The proposed potentiometric procedures to determine QTF using QTF-NaTPB and QTF-PTA sensors were found to be specific and relevant for their adoption in routine quality control and physiotherapeutic administration laboratories.

Acknowledgements

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Supplementary Material

Comparison of performance characteristics between reported and proposed analytical methods

Technique and reagents employed	Methodology	Linear range for detection of QTF	Limit of detection (LOD)	Remarks	Reference No.
A reversed-phase high performance liquid chromatography (RPHPLC). Column: 250 mm×4.6 mm i.d., 5 µm particle size Zorbax SB-Phenyl. Mobile phase (MP): Mixture of acetonitrile and 0.02 M phosphate buffer (50:50) (pH=5.5)	Elution of QTF with UV detection made at 254 nm	0.08–20 µg mL^{-1}	0.03 µg mL^{-1}	Require sophisticated instrument and larger volumes of toxic organic solvents. Applicable to tablets and human plasma.	4
Extraction and HPLC Column: A narrow bored ZORBAX Stable bond phenyl (SB-Ph) column (150 × 2.1 mm, 5 µm) MP: 20 mM Phosphate buffer (pH 7.4), methanol and acetonitrile (40:50:10, v/v/v). The injection solvent: Phosphate buffer (pH 7.4), methanol and acetonitrile (60:30:10, v/v/v)	Liquid-liquid extraction of quetiapine and its 7-hydroxylated and 7-hydroxylated, N-dealkylated metabolites from human plasma, and UV (at 225 nm) and electrochemical detection of QTF made	500–5000 ng mL^{-1}	-	Require sophisticated instrument. Tedious extraction procedure involved. Applicable to plasma samples.	5
HPLC method Column: ODS Hypersil C18 MP: Acetonitrile–water–tetramethylethylenediamine (37.5:62.1:0.4, v/v/v), (pH 6.5)	Separation of quetiapine from clozapine, norclozapine, perazine and olanzapine in blood samples. UV detection made at 254 nm	20–370 ng mL^{-1}	-	Applicable for separation of QTF in blood samples. Require sophisticated instrument.	6
HPLC-UV method Column: Reversed-phase C8 column (150 mm x 4.6 mm i.d., 5 µm) MP: Acetonitrile (30%) and a 10.5 mM, pH 3.5 phosphate buffer containing 0.12% triethylamine (70%) Flow rate: 1.2 mL min^{-1}	Sample pretreatment was carried out by an original solid-phase extraction (SPE) procedure. UV detection of eluate at 245 nm	2.5–400 ng mL^{-1}	-	Applicable for simultaneous determination of fluvoxamine and QTF in human plasma samples. Require sophisticated instrument.	7
HPLC-UV method Column: C8 (150x4.6 mm i.d., 5 µm) MP: Mixture of acetonitrile, methanol and pH 1.9 phosphate buffer	Pre-treatment of sample for SPE. Detection of HPLC eluate at 254 nm	4–400 ng mL^{-1}	-	SPE and sophisticated instruments required. Applicable to human plasma samples.	8

Comparison of performance characteristics between reported and proposed analytical methods (Continuation)

Technique and reagents employed	Methodology	Linear range for detection of QTF	Limit of detection (LOD)	Remarks	Reference No.
HPLC-UV method Column: Nucleosil 100-Protect 1 MP: Acetonitrile (60%)-25 mM potassium dihydrogenphosphate buffer (40%) (pH 7.0) Flow rate: 1.0 mL min^{-1}	Separation and elution of 18 antidepressants and detection of QTF at 230 nm	5–1000 ng mL^{-1}	-	Sophisticated instrument required. Applicable to serum analysis.	9
HPLC method Column: Silica MP: A 99:1 mixture of methanol and ammonium acetate (pH 5.0) Flow rate: 1.0 mL min^{-1}	Separation by SPE and elution by HPLC. UV detection at 257 nm	50-5000 nM	10 nM	SPE and HPLC techniques are sophisticated. Applicable to serum samples.	10
HPLC method	Elution of QTF by HPLC	-	-	Sophisticated instrument required. Applicable to serum samples.	11
HPLC method Column: ODS (250 mm × 4.6 mm i.d., 5 µm) Chromatopack MP: Mixture of acetonitrile and 0.1% phosphate buffer (pH 3.1) (40:60) Flow rate: 1.0 mL min^{-1}	Elution and detection at 240 nm	0.09 – 18 µg mL^{-1}	0.03 µg mL^{-1}	Sophisticated instrument required. Applicable to spiked human urine samples and pharmaceuticals.	12
UPLC–ESI-MS/MS method Column: Acquity UPLC™ BEH C_{18} column (100 mm × 2.1 mm i.d., 1.7 µm) MP: 62: 38 Acetonitrile and ammonium acetate at a final concentration of 30 mmol/l	Separation and determination of quetiapine, perospirone, aripiprazole and quetiapine sulfoxide in in vitro samples. Tandem quadrupole mass spectrometric detection made.	0.05 – 5 µg L^{-1}	< 0.005 µg L^{-1}	Highly sophisticated instrument required. Not applicable to determine QTF in pharmaceuticals.	13
UPLC–MS/MS method	Separation and detection of quetiapine and its two active metabolites, 7-hydroxyquetiapine and 7-hydroxy-Ndealkylquetiapine.	-	-	Highly sophisticated instrument required. Applicable for assay of QTF in rat plasma and cerebrospinal fluid.	14

Comparison of performance characteristics between reported and proposed analytical methods (Continuation)

Technique and reagents employed	Methodology	Linear range for detection of QTF	Limit of detection (LOD)	Remarks	Reference No.
HPLC with tris(2,2'-bipyridyl) ruthenium(II) chemiluminescence detection method Column: Chromolith Performance RP-18e 100 mm × 4.6 mm (Analytical) and 5 mm monolithic (guard column) MP: Mixture of methanol and trifluoro acetic acid	HPLC separations with tris(2,2 –bipyridyl) ruthenium(II) chemiluminescence detection and estimation of quetiapine. The observation of major metabolites of QTF	20 µL injection: 1×10^{-7}–1×10^{-4} M 100 µL injection: 1×10^{-8}–1×10^{-4} M	7×10^{-8} M 2×10^{-10} M	Sophisticated instrument required. Applicable to analyze body fluids.	15
HPLC-MS/MS-solid phase extraction	SPE separation followed by elution by HPLC and detection by electrospray characterization technique	1.1×10^{-9} to 4.3×10^{-7} M	3.3×10^{-10} M	Sophisticated instrument required. Applicable to human serum samples.	16
HPLC electrospray ionization mass spectrometry Column: C18 (2.0 mm x 125 mm, 3 µm MP: Formic acid (2.70 mol/l), ammonium acetate (10 mmol/l)-acetonitrile (53:47) Flow rate:16 ml/min	Simultaneous determination of clozapine, olanzapine, risperidone and quetiapine by the use of high-performance liquid chromatography-electrospray ionization mass spectrometry	20–1000 ng mL^{-1}	-	Sophisticated technique employed. Suitable to determine QTF simultaneously with other drugs.	17
HPLC-electrospray ionization mass spectrometry	High-performance liquid chromatography-electrospray ionization mass spectrometry assay of QTF	-	-	Sophisticated technique employed. Applicable for assaying QTF in body fluids.	18
Liquid chromatography- electrospray ionization mass spectrometry Column: Zorbax SB-C18 (150 mm × 4.6 mm, 5 µm) MP: Mixture of acetonitrile and 0.2% aqueous solution of formic acid FR: 0.6 mL min^{-1}	Extraction of QTF from the samples containing eight antipsychotic drugs: chlorpromazine, haloperidol, zuclopenthixol, clozapine, risperidone, quetiapine, aripiprazole or olanzapine and some active metabolites. The determination of QTF by electrospray ionization mass spectrometry	1-1000 ng mL^{-1}	-	Highly sophisticated instrument required.	19

Comparison of performance characteristics between reported and proposed analytical methods (Continuation)

Technique and reagents employed	Methodology	Linear range for detection of QTF	Limit of detection (LOD)	Remarks	Reference No.
HPLC-MS/MS method determination	HPLC-MS/MS determination of QTF by tandem mass spectrometric detection	-	-	Sophisticated technique employed. Applicable for assaying QTF in plasma.	20
SPE & HPLC-MS/MS method	Extraction by SPE, analysis of QTF by HPLC-MS/MS and detection by tandem mass spectrometric detection	1.0–382.2 ng mL^{-1}	-		21
Liquid-liquid extraction and HPLC-tandem MS Column: Zorbax C8, 50 × 4.6 mm MP: 10 mM Ammonium acetate and acetonitrile	Sample preparation by solvent extraction, separation by LC and tandem mass spectrometric detection of QTF	0.25–500 ng mL^{-1}	-	Highly sophisticated LC-MS/MS in positive electrospray ionization technique using multiple reaction monitoring system required. Applicable to rat plasma.	22
SPE and LC-MS/MS technique	Sample preparation by SPE, elution by LC and detection tandem mass spectrometry	-	-	Highly sophisticated LC-MS/MS technique required. Applicable to human plasma.	23
SPE and LC-MS/MS technique Column: C_{18} MP: 85:15 (*v*/*v*) acetonitrile–5 mM ammonium formate, pH adjusted to 4.5 with formic acid FR: 0.5 mL min^{-1}	Extraction of plasma sample by SPE and LC-MS detection of QTF by tandem mass spectrometry	1–240 ng mL^{-1}	-		24
GC-MS technique	Detection of QTF by GC-MS	-	-	Sophisticated instrument required.	25
GC-MS technique Column: Capillary fused silica (DB-5 MS) (30 m x 0.25 mm ID x 0.25 µm film) MP: He gas	GC-MS detection of QTF in the scan mode from 33-460 m/z	-	-		26
Ion-pair titrimetric assay of QTF using NaTPB and SDS	Solvent extraction-titration of QTF with: i. NaTPB	4-18 mg	-	Titrimetry require large sized samples. Less sensitive.	27
	ii. SDS	5-25 mg	-		

Comparison of performance characteristics between reported and proposed analytical methods (Continuation)

Technique and reagents employed	Methodology	Linear range for detection of QTF	Limit of detection (LOD)	Remarks	Reference No.
Titrimetry and spectrophotometry	Non-aqueous titration of QTF with 0.01 M perchloric acid in acetic acid medium	2.0–20.0 mg	-	Least sensitive and applicable to macro-size samples.	28
	Measurement of QTF in 0.1 M acetic acid spectrophotometrically at a wavelength of 222 nm	1.25–15.0 μg mL^{-1}	0.07 μg mL^{-1}	Measurement made at shorter wavelength.	
Potentiometric method	Potentiometric titration of QTF with 0.01 M perchloric acid in acetic acid medium	2-20 mg	-	Less sensitive. Applicable to macro-size samples.	29
Polarographic method: Dropping mercury working electrode (DME), Ag/AgCl reference electrode, and a graphite rod as the auxiliary electrode	The study of voltammetric characterization of QTF by i. direct current, differential pulse and	8-44 μg mL^{-1}	0.06 μg mL^{-1}	Analysis prone to get interfered by atmospheric components.	30
	ii. alternating current polarography	4-44 μg mL^{-1}	0.04 μg mL^{-1}		
Differential pulse (DP) and Osteryoung square wave (OSW) voltammetry	Study of electrochemical characterization of QTF by voltammetric techniques using glassy carbon disc electrode	4.0 × 10^{-6} to 2.0 × 10^{-4} M	4.0 × 10^{-8} M (DPV) and 1.33 × 10^{-7} M (OSWV)		31
Capillary zone electrophoretic (CZE) and spectrophotometric methods for determination of QTF	CZE operation with uncoated fused-silica capillary and a pH 2.5, 50 mM phosphate buffer. UV detection made at 205 nm, the separation voltage was 15 kV, and a complete electrophoretic run lasts less than 2.5 min	5-50 μg mL^{-1}	0.05 μg mL^{-1}	Nonselective electrophoretic and spectrophotometric techniques employed. Applicable to determine QTF in tablets.	32
	UV spectrometric measurement of QTF at 246 nm in methanol	5-25 μg mL^{-1}	1.5 μg mL^{-1}		
CZE analysis of QTF using a 35 cm (75 μm id) fused silica capillary and UV detection at 214 nm at 10 kV	Separation of four atypical antipsychotics: clothiapine, clozapine, olanzapine, and quetiapine	0.050–0.250 mg mL^{-1}	-	Applicable to determine QTF in combination formulations.	33

Comparison of performance characteristics between reported and proposed analytical methods (Continuation)

Technique and reagents employed	Methodology	Linear range for detection of QTF	Limit of detection (LOD)	Remarks	Reference No.
HPTLC technique TLC Plate: Pre-coated silica gel 60 F254 aluminum plates MP: mixture of methanol and toluene (4:3%v/v). Densitometric evaluation	Elution study of QTF by HPTLC and detection by densitometry at 235 nm	100-500 ng/spot	30 ng/spot	Sophisticated instrument required. Applicable to determine QTF in tablets.	34
NPHPTLC technique TLC Plate: silica F254 plates MP: tetrahydrofuran-phosphate buffer, pH 9.0, [5:5 (v/v)]. Densitometric and video densitometric evaluation	Elution study of QTF by NPHPTLC and detection by densitometry (DM) and video densitometry (VDM) at 243 nm	0.2-1.2 µg/spot	0.02 µg/spot (DM) 0.04 µg/spot (VDM)		35
RPHPTLC technique TLC Plate: HPTLC RP8 F254 plates MP: hexane-dioxane-propylamine [1:9:0.4 (v/v)]. Densitometric and video densitometric tric evaluation	Elution study of QTF by RPHPTLC and detection by DM and VDM at 254 nm	0.1-1.1 µg/spot	0.01 µg/spot (DM) 0.02 µg/spot (VDM)		
HPTLC technique TLC Plate: silica gel plates MP: toluene-methanol 8:2 (v/v). Densitometric detection	Determination of QTF by HPTLC and detection by DM at 254 nm	-	-		36
HPLC method Column: X-bridge C18 (150 x 4.6 mm, 3.5 µm id) MP: 5 mM Ammonium acetate (MP- A) and acetonitrile (MP- B) FR: 1 mL min^{-1}	HPLC elution and UV detection at 220 nm	-	-	Sophisticated instrument required. Applicable for stability indicating and impurity profile study of QTF in pharmaceuticals.	37
HPLC method Column: Pack-C8, 150 mm long, 4.6 mm i.d., 5 mm particle diameter MP: Mixture of phosphate buffer and acetonitrile in the ratio of 90 : 10 v/v (pH 6.7) (MP- A) and acetonitrile (MP- B) FR: 1.5 mL min^{-1}	RP-HPLC elution of QTF and detection at 225 nm. Spectroscopic characterization of impurities	-	-	Sophisticated analytical technique required. Applicable for impurity profile and characterization studies.	38

Comparison of performance characteristics between reported and proposed analytical methods (Continuation)

Technique and reagents employed	Methodology	Linear range for detection of QTF	Limit of detection (LOD)	Remarks	Reference No.
RP-HPLC method Column: C18, RRHD 1.8 µm (50 mm x 2.1 mm) MP: 0.1% aqueous triethylamine (pH 7.2) (solvent-A) and mixture of acetonitrile and methanol in the ratio of 80:20 (v/v) (Solvent-B) FR: 0.5 mL min^{-1}	RP-HPLC elution of QTF and detection at 252 nm	62.5 to 187.5 µg mL^{-1}	-	Sophisticated instrument required.	39
RP-HPLC method	Elution of QTF by HPLC	-	-	Sophisticated instrument required. Applicable to tablets.	40
HPLC method Column: C18 (50 × 4.6 mm with 1.8 µm particles) MP: Mixture of 10 mM potassium dihydrogen orthophosphate (pH 7.0) buffer, methanol and acetonitrile (450:300:250) (v/v). FR: 1.0 mL min^{-1}	Stability of quetiapine hemifumarate through stress studies using LC elution and UV detection made at 225 nm.	Up to 150 µg mL^{-1}	-	Sophisticated instrument required. Applicable for stability indicative and impurity profile studies.	41
Isocratic reversed phase ultra-performance liquid chromatography (RP-UPLC) Column: AQUITY UPLC (2.1 x 50 mm, 1.8 µm) MP: 30:70 (v/v) mixture of potassium dihydrogen phosphate and dipotassium hydrogen phosphate (mobile phase A) (pH 6.5) and methanol (mobile phase B)	Elution of QTF and UV detection at 252 nm	1.0–15.0 µg mL^{-1}	0.04 µg ml^{-1}	Require sophisticated instrument and larger volumes of toxic organic solvents. Applicable to tablets.	42
Spectrophotometric method	Measurement of QTF at 290 nm in water	6-54 µg mL^{-1}	-	Measurement made at shorter wavelength.	43

Comparison of performance characteristics between reported and proposed analytical methods (Continuation)

Technique and reagents employed	Methodology	Linear range for detection of QTF	Limit of detection (LOD)	Remarks	Reference No.
Extractive spectrophotometric method with bromocresol green	Measurement of absorbance of ion-pair of QTF and bromocresol green after extracted into chloroform at 415 nm	5-25 µg mL^{-1}	0.29 µg mL^{-1}	Toxic organic solvents are required. Tedious extraction procedure is involved.	44
Extractive spectrophotometric method with quinolone yellow	Measurement of absorbance of ion-pair of QTF and quinolone yellow dye after extracted into chloroform at 420 nm	2.5-25 µg mL^{-1}	0.11 µg mL^{-1}		45
Extractive spectrophotometric method with calmagite	Measurement of absorbance of ion-pair of QTF and calmagite dye after extracted into dichloromethane at 490 nm	3-30 µg mL^{-1}	0.27 µg mL^{-1}		46
Extractive spectrophotometric method with bromocresol purple (BCP) and bromocresol green (BCG)	Measurement of absorbance of ion-pair complex of QTF with either BCP at 406.5 nm or BCG at 416 nm in chloroform.	0.5-20 µg mL^{-1}	0.12 µg mL^{-1} (BCP method); 0.16 µg mL^{-1} (BCG method)		47
Extraction-free spectrophotometric methods with bromophenol blue (BPB) and thymol blue (TB)	Measurement of ion-association complexes formed between QTF in 1,4-dioxane and BPB in acetone at 410 nm	1-25 µg mL^{-1}	0.21 µg mL^{-1}	Organic solvents are required. Not applicable to spiked human urine.	48
	Measurement of ion-association complexes formed between QTF in TB in acetone at 380 nm	1.5-30 µg mL^{-1}	0.54 µg mL^{-1}		
Ultraviolet spectrophotometric methods	Measurement of absorbance of QTF solution in either 0.1 N HCl at 209 nm or in methanol at 208 nm	1.25-12.5 µg mL^{-1}	0.02 µg mL^{-1}	Toxic organic solvent required. Shorter wavelengths employed in the measurement.	49

Comparison of performance characteristics between reported and proposed analytical methods (Continuation)

Technique and reagents employed	Methodology	Linear range for detection of QTF	Limit of detection (LOD)	Remarks	Reference No.
Potentiometry with coated wire electrode	Coated wire electrode construction using NaTPB ion pair complexing agent, PVC as supporting matrix, 2-nitrophenyl octyl ether as mediator of solvent and potassium tetrakis (4-chlorophenyl) borate as lipophilic additive	1×10^{-5} to 1×10^{-2} mol L^{-1}	3.2×10^{-6} mol L^{-1}	Coated wire electrode is used. Usable only up to 30 days. Sensor is incompletely validated.	50
Potentiometry with membrane sensors/ ISEs	Potentiometric determination of QTF using: i. QTF-NaTPB ISE	6.25×10^{-5} to 3.5×10^{-3} M	1.56×10^{-5} M	Low cost and easy to handle instrument is needed. No stringent experimental conditions are involved. The methods employ ecofriendly and low cost materials and less energy is consumed in the analysis. Methods are adequately sensitive. Analytical procedures are free from stringent experimental conditions such as extraction and sample preparation by elimination of interferences.	**Proposed work**
	ii. QTF-PTA ISE		2.16×10^{-5} M		

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ARTICLE

Fourier Transform Infrared Spectrophotometry: An Eco-Friendly Green Tool for Simultaneous Quantification of Aspirin and Omeprazole in Pharmaceutical Formulation

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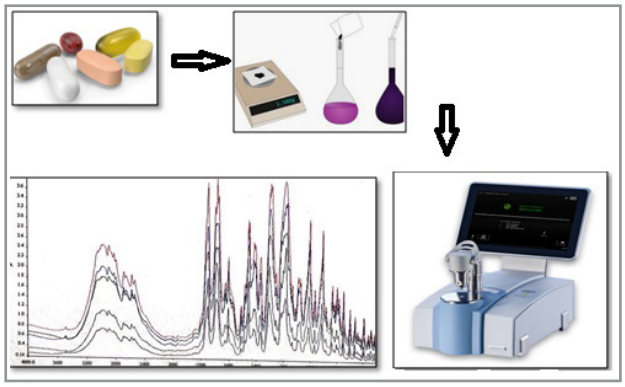

An eco-friendly method for quantification of Aspirin and Omeprazole in pharmaceutical solid dosage form has been developed using Fourier transform infrared spectrophotometry. The proposed method avoids the use of solvents which are commonly used for other methods of quantification e.g. Liquid Chromatography, UV spectrophotometry, etc. The developed method has been validated for the quantification of Aspirin and Omeprazole in a marketed formulation as per ICH Topic Q 2 (R1) guidelines. The method is based on Beer-Lamberts' law. For the proposed method C=O stretch at 1754 cm^{-1} was selected for Aspirin between 1750 – 1730 cm^{-1} and C=N stretch at 1627 cm^{-1} was selected for Omeprazole in the range of 1690 – 1620 cm^{-1}. Linearity was obtained in the concentration range of 10 – 50 mg g^{-1} and 5 – 25 mg g^{-1} with an R^2 value of 0.999 and 0.997 for Aspirin and Omeprazole respectively. The % recovery was calculated with intra and inter day precision study.

Keywords: FTIR spectrophotometry, Aspirin, Omeprazole, C=O stretching peak, C=N stretching peak.

INTRODUCTION

Yosprala® (Aralez Pharmaceuticals R&D Inc.), a fixed dose combination of 81 mg Aspirin and 40 mg of Omeprazole, which was approved by USFDA in 2016 [1], was selected for the proposed method. Aspirin produces gastric ulcer as a side effect in some patients with the age > 55 years, so Omeprazole is added to reduce the side effect of Aspirin in this combination. Aspirin ($C_9H_8O_4$), chemically 2-(acetyloxy) benzoic acid is used as an anti-inflammatory, antipyretic and analgesic. It decreases the formation of precursors of thromboxane and prostaglandin from arachidonic acid by inhibiting the activity of COX-1 and COX-2 enzymes [2]. Omeprazole ($C_{17}H_{19}N_3O_3S$), chemically 5-methoxy-2-[[(4-methoxy-3, 5-dimethyl pyridine-2yl) methyl] sulfinyl]-1H-benzoimidazole is a proton pump inhibitor used in gastric and duodenal

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ulcers, nonsteroidal anti-inflammatory drug (NSAID) associated ulceration and gastroesophageal reflux disease (GERD) [3]. The structural formulas of Aspirin and Omeprazole are presented in Figures 1 and 2 respectively.

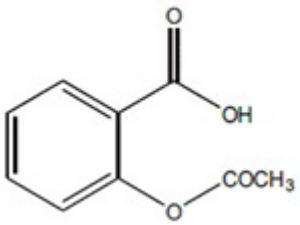

Figure 1. Structural formula of Aspirin.

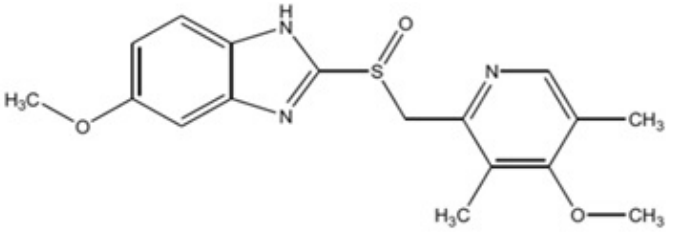

Figure 2. Structural formula of Omeprazole.

Omeprazole is used to inhibit acid secretions [4]. Studies have reported analytical methods for quantitative estimation in which Omeprazole can be determined in a solution form e.g UV [5] and HPLC [6-8]. One UV spectrophotometric method for simultaneous quantification of Aspirin and Omeprazole is reported [8] and one HPLC method [10] is reported. The earlier reported methods required the preparation of solutions using different solvents. Fourier Transform Infrared (FTIR) spectrophotometry uses molecular vibrations with the help of which Aspirin and Omeprazole can be quantified in a solid form [11-15]. The present study was aimed to develop an FTIR spectrophotometric method which is more advantageous, green, simple and rapid as compared to the other available methods. Individual identical Infrared (IR) spectra are given in Figures 3 and 4.

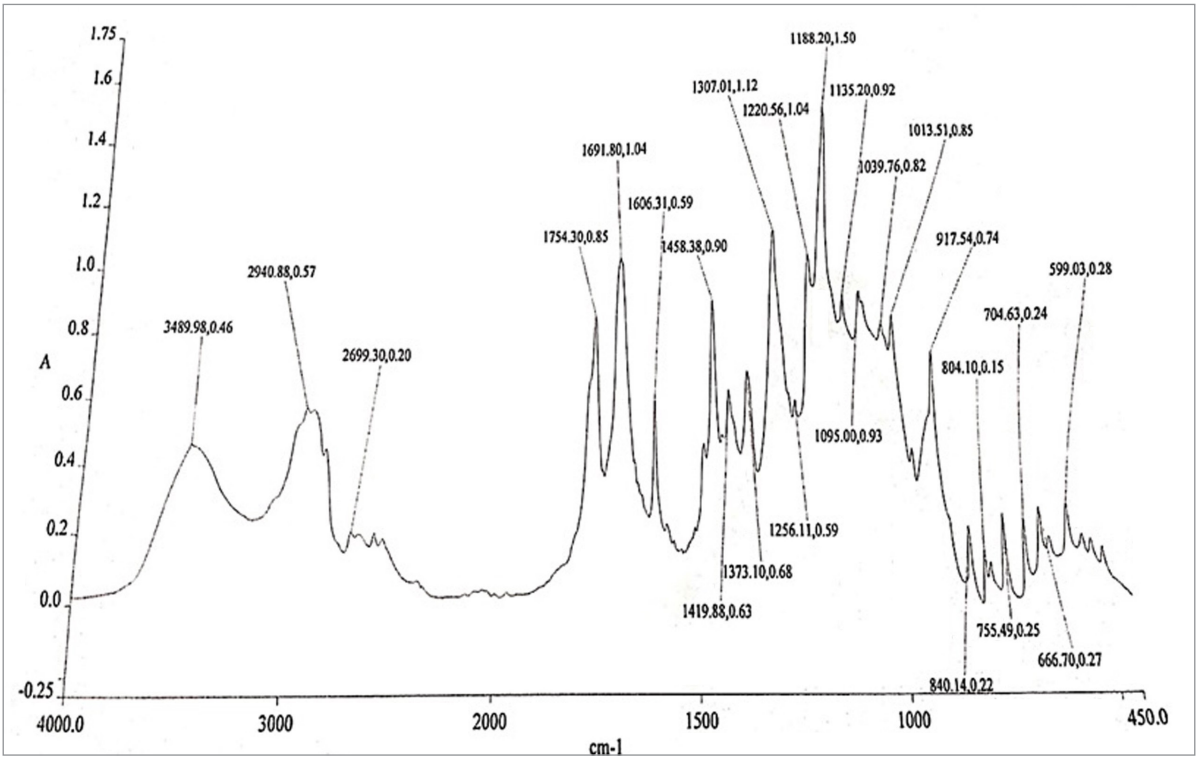

Figure 3. Spectra of standard Aspirin.

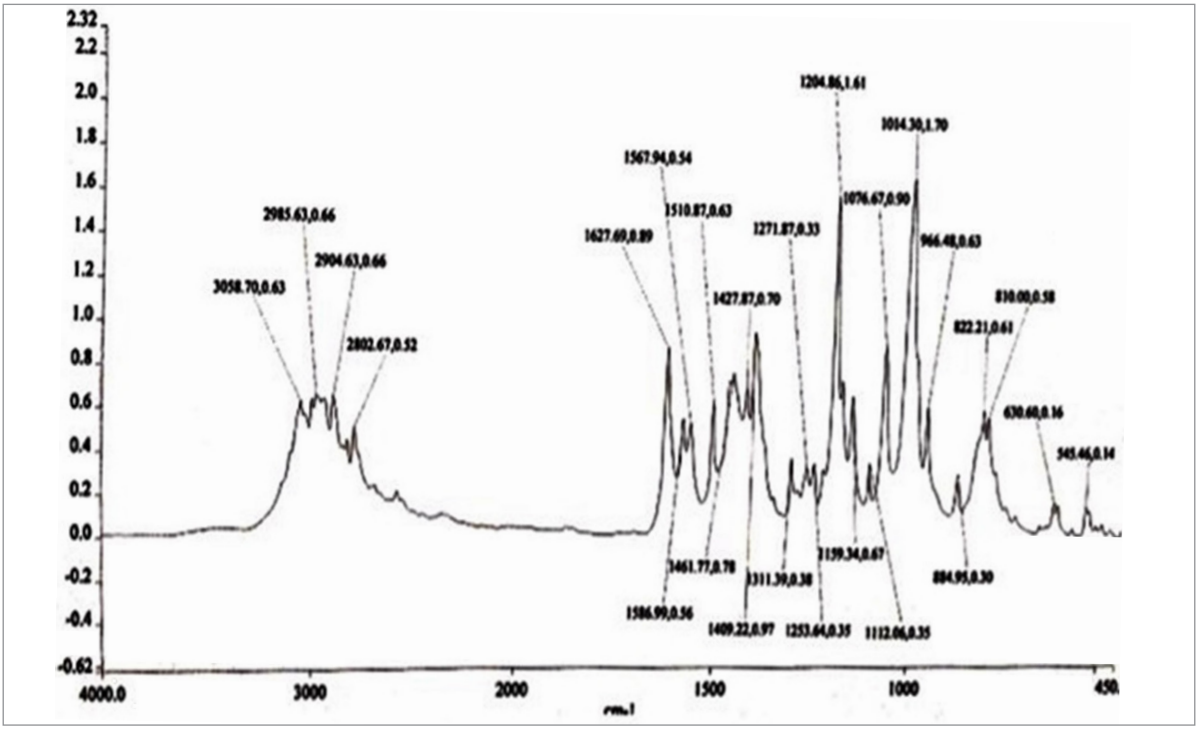

Figure 4. Spectra of standard Omeprazole.

MATERIALS AND METHODS

Chemicals and Reagents

The standard sample of Aspirin was procured from Sidmak, Valsad, India and Omeprazole from Mangalam drugs, Vapi. Potassium bromide was procured from the Axiom chemicals PVT. Ltd., Vadodara, India.

Instrument

The analysis was performed on Bruker Optics Alpha-Fourier transform infrared spectrophotometer. For the collection and analyses of data, OPUS software was used. The optimised IR conditions are given in Table I. The interpretation of the Aspirin IR spectra is given in Table II and the interpretation of the Omeprazole IR spectra is given in Table III.

Table I. Optimised infrared conditions for proposed study

Parameters	Optimised conditions
Method of making pellets	Direct mixing (press pellet) method
Mode of measurement	Absorbance mode
Final mass of pellet	200 mg
Peak selection	1754 cm^{-1} for aspirin and 1627 cm^{-1} for omeprazole
No. of scans	16 scans
Resolution	4 cm^{-1}

Table II. Interpretation of IR spectrum of Aspirin

Wave number (cm^{-1})	Functional group
3489	O-H streching
2700	C-H streching
1754	C=O streching (ester)
1691	C=O streching (carboxylic acid)
1458	CH_3 bending
1306	C-O stretching
917, 840	C=C bending in ring

Table III. Interpretation of IR spectrum of Omeprazole

Wave number (cm^{-1})	Functional group
2903	C-H streching
1627	C=N streching
1271	C-N streching
1204	C-O streching
101	S=O stretching
1567	N-H bending
965, 629	C=C aromatic ring

Calibration Curve

To plot a calibration curve, solid pellets of Aspirin and Omeprazole were prepared by the Pressed Pellet Technique using Polymer Press. Five different concentrations were selected in the range of 10 – 50 mg g^{-1} and 5 – 25 mg g^{-1} for Aspirin and Omeprazole respectively. Appropriate quantities (2, 4, 6, 8 and 10 mg) of Aspirin and (1, 2, 3, 4 and 5 mg) of Omeprazole were separately mixed with potassium bromide and triturated to get a homogenous solid solution which was converted to solid pellets ensuring the final mass of 200 mg for each pellet. Each concentration was used in replicates of six to record and analyze the data. For quantification, absorbance of C=O stretch cantered at 1754 cm^{-1}, between wave number of 1750 – 1730 cm^{-1} was selected for Aspirin and absorbance of C=N stretch cantered at 1627 cm^{-1}, between 1690 – 1620 cm^{-1} was used for Omeprazole. Results are reported in Figure 5.

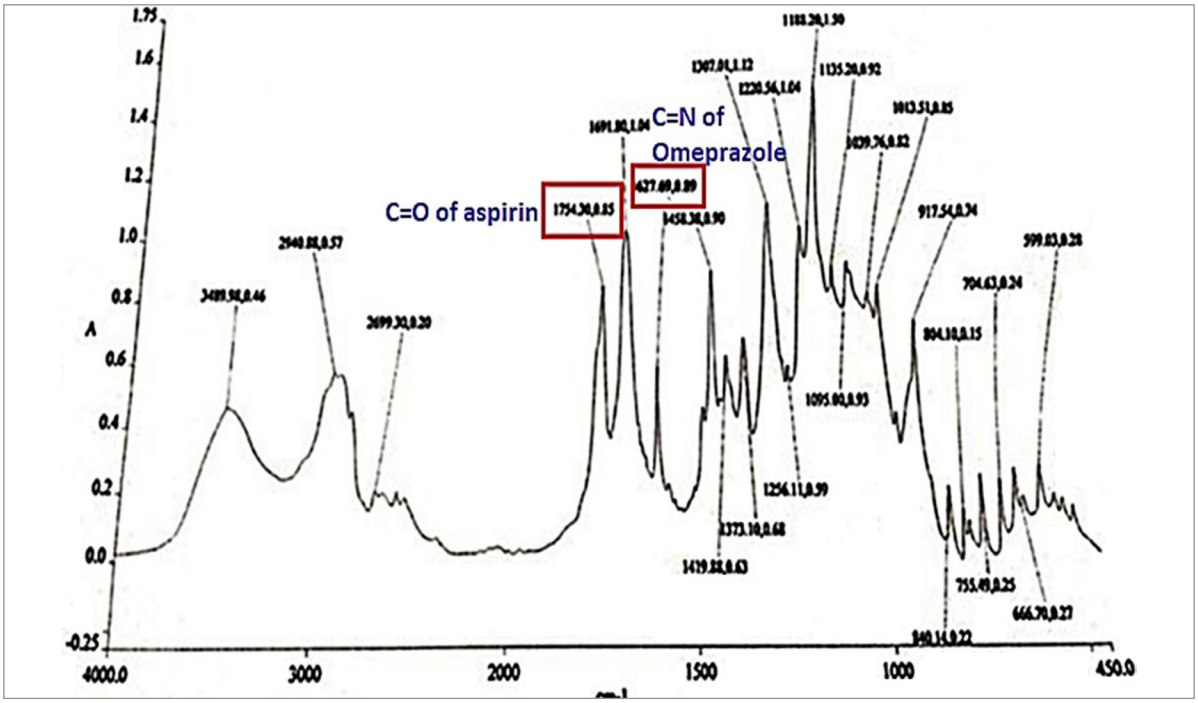

Figure 5. Spectra of standard Aspirin (10 mg g^{-1}) and Omeprazole (5 mg g^{-1}).

Method Validation

Parameters like linearity, specificity, precision, accuracy and robustness were evaluated for validation of the developed method.

Specificity

The wave number (cm^{-1}) selected for the study was specific for Aspirin and Omeprazole. Results are reported in Figures 6 and 7.

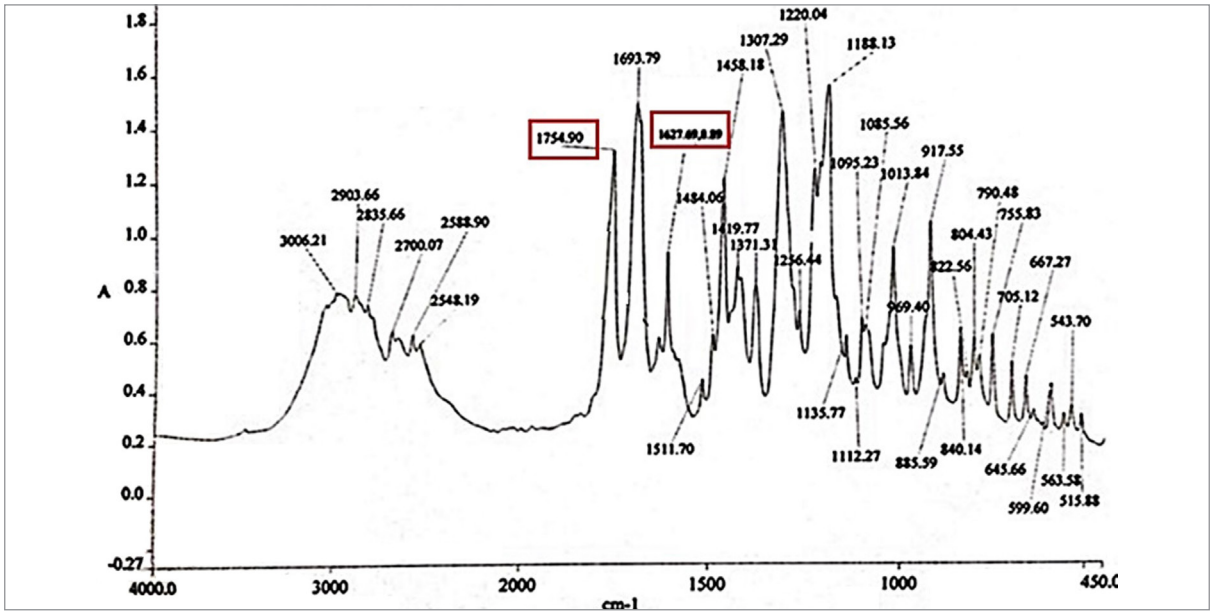

Figure 6. Spectra of test Aspirin (10 mg g^{-1}) and Omeprazole (5 mg g^{-1}).

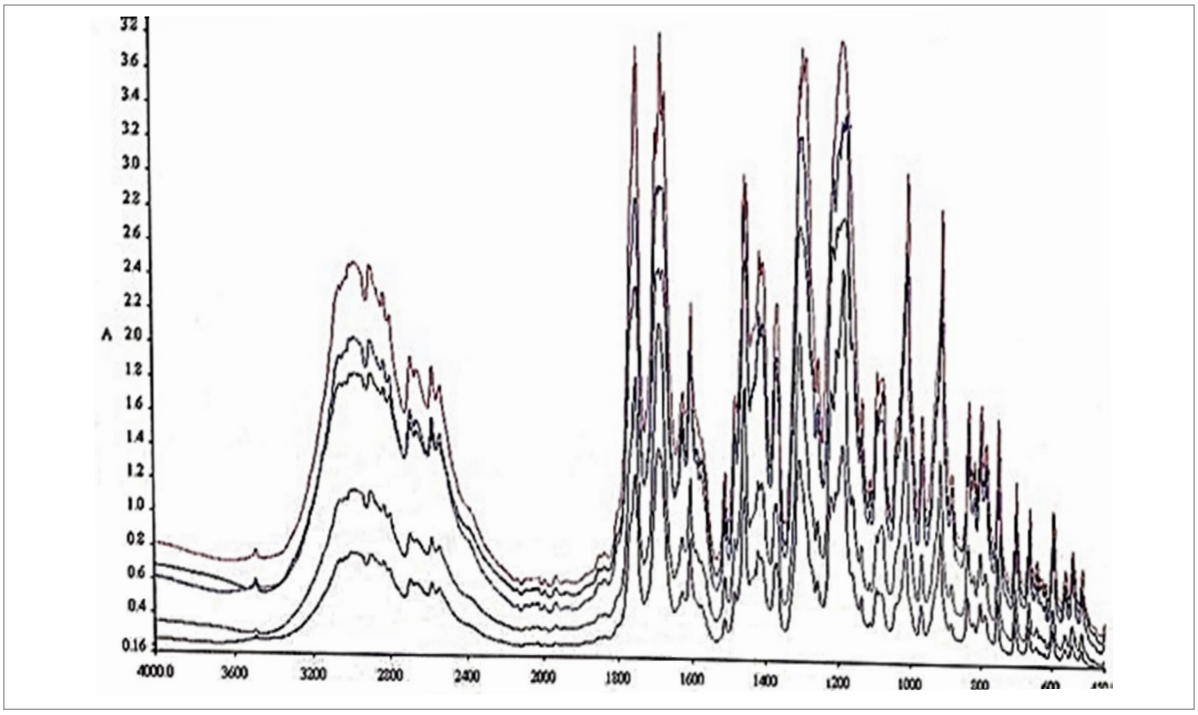

Figure 7. Overlain spectra of Aspirin (10 – 50 mg g^{-1}) and Omeprazole (5 – 25 mg g^{-1}) of linearity.

Linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The linearity study was performed by plotting absorbance *vs*. concentration in the range of 10 – 50 mg g^{-1} for Aspirin and 5 – 25 mg g^{-1} for Omeprazole at selected wave numbers respectively. As it follows Beer-Lambert's law, an increase in absorbance with concentration for the selected peak was analysed. LOD and LOQ were calculated in the same concentration range. Results were reported in Figures 8 and 9 and Table IV.

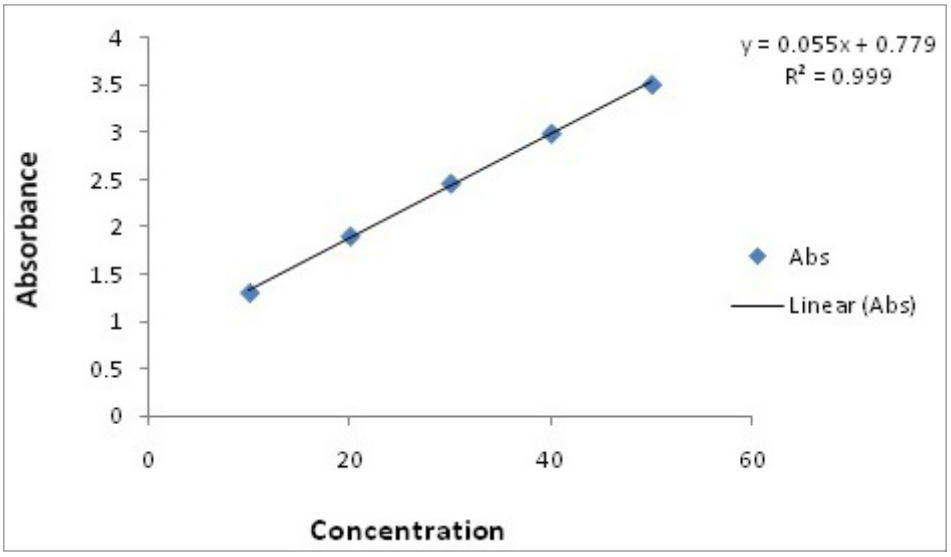

Figure 8. Calibration curve of Aspirin (10 – 50 mg g^{-1}).

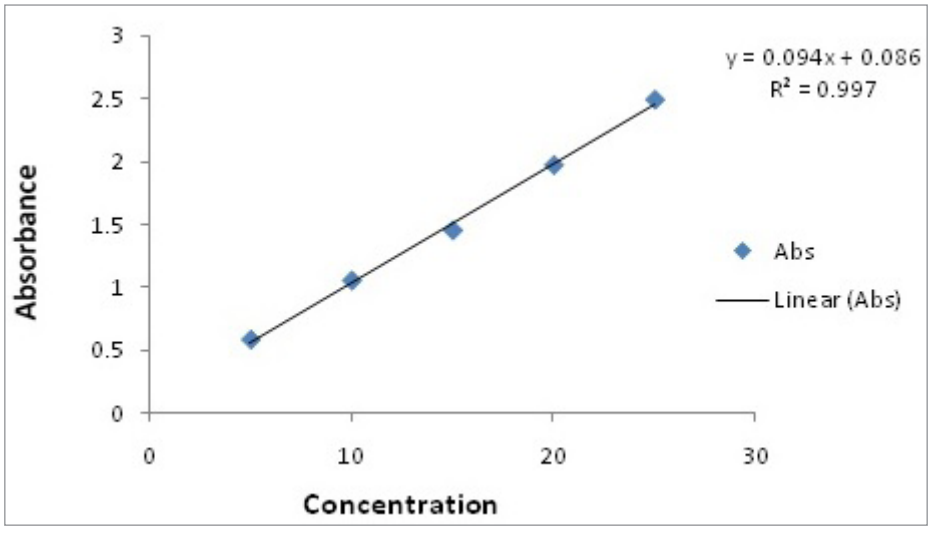

Figure 9. Calibration curve of Omeprazole (5 – 10 mg g^{-1}).

Table IV. LOD and LOQ data of Aspirin and Omeprazole by FT-IR method

Parameters	Results of ASP	Results of OME
S.D. of Y intercept of calibration curve	0.0115	0.015
Mean slope of the calibration curve	0.055	0.095
LOD	0.69	0.05
LOQ	2.09	0.15

Accuracy

Accuracy was recorded at 3 different concentration levels (50%, 100%, 150%). Known amounts of standard Aspirin (15, 30 and 45 mg g^{-1}) and Omeprazole (7.5, 15 and 22.5 mg g^{-1}) were added to a test sample containing 10 mg g^{-1} of Aspirin and 5 mg g^{-1} of Omeprazole. The results of this study are ported in Table V. The percentage recovery for both drugs was calculated at each level using the formula: (Measured value/ True value) x 100.

Table V. Accuracy data of Aspirin and Omeprazole by FT-IR method

Drug	Level (%)	Sample (mg g^{-1})	Standard (mg g^{-1})	Spiked (mg g^{-1})	Found (mg g^{-1})	% Recovery (Avg.)
Aspirin	50	10	15	25	24.57	98.29
	100		30	40	40.34	100.85
	150		45	55	54.53	99.15
Omeprazole	50	5	7.5	12.5	12.71	101.70
	100		15	20	19.65	98.27
	150		22.5	27.5	27.5	100

Precision

Precision was carried out by repeatability and intermediate study at 3 different concentration levels (50%, 100%, and 150%) for a test sample. The concentrations of Aspirin and Omeprazole were kept the same as the accuracy study (15, 30 and 45 mg g^{-1} for Aspirin and 7.5, 15 and 22.5 mg g^{-1} for Omeprazole.) %RSD was calculated. Results of the precision studies are reported in Table VI.

Table VI. Results of the precision studies of the proposed FT-IR method

Drug	Concentration (mg g^{-1})	Intraday (at 10 am) (%RSD)	Intraday (at 4 pm) (%RSD)	Interday (Day 2) (%RSD)	Interday (Day 3) (%RSD)
Aspirin	15	0.0293	0.0783	0.0228	0.4963
	30	0.0227	0.0359	0.0412	0.0239
	45	0.0061	0.0046	0.5207	0.0991
Omeprazole	7.5	0.0483	0.1158	0.0587	0.1302
	15	0.0943	0.7878	0.0864	0.0555
	22.5	0.0710	0.7687	0.3034	0.0903

Robustness

The robustness study was performed by changing parameters such as different analysts (analyst 1, analyst 2), different solvent (NaCl) and changing scanning time (16 s, 24 s). To perform this study, the same standard solutions with 30 mg g^{-1} Aspirin and 15 mg g^{-1} Omeprazole (six measurements were taken), and a sample solution of the same concentration (two measurements were taken) were tested and the %RSD was calculated for both the drugs. The results of this study are reported in Table VII.

Table VII. Results of the Robustness studies by changing solvent and scan time

	Average			
Parameters	**Absorbance of Aspirin**	**Absorbance of Omeprazole**	**% RSD of Aspirin**	**% RSD of Omeprazole**
Solvent	2.4700	1.4614	0.028	0.1995
Scanning time	2.4701	1.4577	0.030	0.0258
Analyst	2.4681	1.4561	0.1091	0.0488

Analysis of tablets

Twenty tablets were accurately weighed and made into the powder form by trituration. Quantity of tablet powder equivalent to 10 mg g^{-1} of Aspirin was taken and diluted with potassium bromide to get a pellet with about 200 mg. The pellets were analyzed to determine the % of Aspirin and Omeprazole. The results are reported in Table VIII.

Table VIII. Analysis of Marketed Formulation by FT-IR Method

Formulation	**Drug**	**Concentration (mg g^{-1})**	**Mean of absorbance**	**Label claim (mg)**	**Amount obtained (mg)**	**% Assay**
Yosprala	Aspirin	30	2.9665	81	80.93	99.92
	Omeprazole	15	1.4575	40	40.01	100.04

RESULTS AND DISCUSSION

This method is based on the measurement of absorbance for Aspirin at C=O stretching vibration centred at 1754 cm^{-1}, which is typically in the range of 1750 – 1730 cm^{-1} and for Omeprazole at C=N stretch vibration at 1627 cm^{-1} between 1690 – 1630 cm^{-1}. The proposed developed method was validated as per ICH Topic Q 2 (R1) guidelines [16]. The validation was started by specificity study and the results are described in the Figures 6 and 7. Figure 6 presents the spectra of the standard mixture of Aspirin and Omeprazole and Figure 7 presents the spectra of the formulation. By comparing both the spectra it was observed that there was no interference from other excipients at wave number of 1754 cm^{-1} for Aspirin and 1627 cm^{-1} for Omeprazole. The overlain plot was obtained in the range of 10-50 mg g^{-1} and 5-25 mg g^{-1} for Aspirin and Omeprazole respectively. The linear regression coefficient correlation value for Aspirin was found to be 0.999 and for Omeprazole was found to be 0.997 whose values for both drugs meet the acceptance criteria. The precision study was performed at intraday as well as inter day. The calculated % RSD for intermediate precision and repeatability was within the acceptance limit of ± 2.0%. The accuracy study was performed by recovery study. The accuracy was found to be within the acceptance criteria of 98 – 102%. The result of the accuracy study was described in Table V. The results of the robustness studies show that the developed method is consistent for small changes. The developed and validated method was applied to the assay of marketed formulation of Yosprala (81 mg Aspirin and 40 mg Omeprazole) and the results were in agreement with the reported values.

CONCLUSION

The proposed FTIR spectrophotometric method for the simultaneous determination of Aspirin and Omeprazole was found to be a novel, simple and rapid method. The proposed method was found to be Eco-friendly as well as environmentally friendly compared to the UV and HPLC methods, as it requires only one specifically selected solid solvent. This method was found to be less time consuming compared to other analytical methods. This method can be used as a green tool and can be applied to other pharmaceutical ingredients too.

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FEATURE

PDF

The University of Campinas will have a Water Research Center

The scarcity of drinking water is a problem that affects everyone across the world. Despite having 12% of the total fresh water available on the planet, which, according to the Food and Agriculture Organization of the United Nations (FAO), is the largest reserve of fresh water in the world, Brazil already feels the impact of the pollution of rivers and the unrestrained consumption of treated water.

Data from the United Nations (UN) indicate that more than 2.7 billion people are expected to suffer from water shortages in 2025 if the planet's consumption continues at the current levels. The data are even more frightening when we analyze the current situation, where about 2.2 billion people worldwide do not have safe managed water services, according to a report by the World Health Organization (WHO) and United Nations Children's Fund (UNICEF) 2019.

According to a report in 2019 by the World Health Organization and UNICEF, about 2.2 billion people in the world do not have safe managed water services. Image: *Divulgação*

In order to develop advanced research to deal with the needs related to water quality, sewage treatment, and water security in general, the city of Campinas (State of São Paulo, Brazil) will create an inaugural research center as a result of a partnership between the Campinas Water Supply and Sanitation Society (Sanasa), São Paulo Research Foundation (Fapesp) and the University of Campinas (Unicamp). This research center, whose name is the Brazilian Water Research Center (BWRC), will be implemented on the Unicamp campus, close to the Science and Technology Park, in an area of 5,000 square meters, with investments in the order of R$ 130 million.

In addition to the advanced lines of research that will be developed at the BWRC, some technical aspects, such as losses in water distribution and telemetry, and diversified themes such as formal and informal education, the development of technological products, and the formal regulation of the area, will also play a role in the activities of this research center, as a social commitment to provide benefits to consumers and the environment.

Lauro Tatsuo Kubota, Full Professor at the Institute of Chemistry at Unicamp and coordinator of the BWRC, points out: "With a focus on changing the culture of populations and disseminating the idea that water is a finite consumption good, as a commodity that, despite being essential for the maintenance of life, it is moving towards having a value regulated by the circumstances of availability, that is, of the supply/demand relationship. Therefore, the BWRC will act to change culture, education and public policies locally and in underdeveloped or developing countries".

Aerial view of the Unicamp campus in Campinas (SP, Brazil). Photo: *Reprodução*/EPTV

Partnership Sanasa–Unicamp

The Campinas region is considered one of the most populous in the state of São Paulo and is already very concerned with water supplies. “The city of Campinas could be one of the pioneers in Brazil to obtain the benefits of holistic management of water resources, fostered by the new technologies and culture that will be developed by the BWRC” explained Adriana V. Isenburg, Demand Coordinator for Sanasa and BWRC Technical Support.

Arly de Lara Romêo, president of Sanasa, has a special interest in water desalination processes, which, according to him, are the future of public treated water supplies, making cities independent of rivers as a source of water for the population. “The BWRC will discuss and develop research on major topics, such as desalination, telemetry, development of cheaper filter membranes, to extend the results to the whole country, “said Romêo.

Still according to Romêo, another line of research will be the replacement of chlorine by ozone in the treatment of water. Chlorine and other chemicals decrease the levels of pollutants in the water of treatment plants, swimming pools and fish farming tanks. Ozone is also used in the treatment of water and, because of its oxidizing action, it is able to disinfect water in a shorter time of contact with the infective agents, therefore, providing a faster treatment than other disinfectants.

The partnership between Unicamp and Sanasa in the area of Research, Development and Innovation has been established for a long time, which demonstrates the company’s concern regarding the use of innovative and more efficient technologies in the treatment of water and sewage, in addition to new analytical methods for proving the quality of water supplied to the population.

According to Dr. Cassiana Montagner, Professor at the Chemistry Institute at Unicamp and Coordinator of research lines at BWRC, Sanasa’s role in this triple partnership “Sanasa-Unicamp-Fapesp” is to stimulate the generation of more efficient and sustainable water treatment and distribution technologies, and assimilate improved analytical techniques that guarantee quality. Regarding the treatment of effluents, the company is interested in technological innovation that contributes to the recovery of water resources. In addition, Sanasa invests in training specialized professionals, at both the undergraduate and postgraduate levels, ready to work in the water supply and basic sanitation market, and in the training of citizens who exercise conscious use of water. To this end, the company has developed educational programs in elementary schools and media awareness campaigns, in addition to maintaining two museums in Campinas, an old

museum in “Torre do Castelo” with objects related to water distribution, and a new “Museu da Água” in the “Centro de Conhecimento da Água”, which presents water as an element that guarantees life and environmental balance on the planet.

Campinas Water Supply and Sanitation Society, Sanasa.

Results and Deadlines

According to Prof. Kubota, there are already institutional commitments between Fapesp-Unicamp-Sanasa that will be evaluated annually. However, as the BWRC brings together several researchers who already have research in progress in the area, the expectation is that the first results may appear soon.

The BWRC project involves different units at Unicamp; as Kubota explained: “There is no way to precisely define the role of each Unicamp unit in the project because our themes are multi and interdisciplinary. Our competencies will be added to those of other partners in Brazil and abroad, to carry out priority researches strategically defined by the BWRC management committee. Our focus will be on the development of scientific and technological solutions and on the innovative ways of implementing these solutions. Even the promotion of new municipal, state and national public policies will be contemplated. The training of human resources with a multi and interdisciplinary perspective will also be one of the great motivations for the actions of the BWRC, which aims to train specialists focused on problem solutions”.

In addition to the Institute of Chemistry, the BWRC involves the Campinas Agronomic Institute, the Environmental Company of São Paulo State (CETESB) and the following Unicamp units: Institute of Biology, Institute of Education, Institute of Geosciences, Institute of Computing, Faculty of Electrical and Computer Engineering, Faculty of Mechanical Engineering, Faculty of Chemical Engineering, Faculty of Technology, Faculty of Civil Engineering, Architecture and Urbanism, Faculty of Agricultural Engineering, Pluridisciplinary Center for Chemical, Biological and Agricultural Research, and the Meteorological and Climate Research Center Applied to Agriculture.

“We believe that, together, it will be possible to innovate both in the way of doing science and in the way of training professionals, as we will have a partnership with experienced and very well trained specialists from Sanasa who will participate in the research with a pragmatic look, so that the innovations generated have short-term applicability and economic viability” concluded Prof. Kubota.

SPONSOR REPORT

PDF

This section is dedicated for sponsor responsibility articles.

Analysis of Elemental Contaminants in Beverages using the Thermo Scientific iCAP PRO X Duo ICP OES

Nora Bartsch – *Thermo Fisher Scientific, Bremen, Germany*

This report was extracted from the Thermo Scientific Application Note Nº 44421

GOAL

This report describes the high performance of the Thermo Scientific™ iCAP™ PRO X Duo ICP OES when analyzing elemental contaminants in different types of beverages. The vertical duo view offers optimal method conditions using axial plasma view for traces and radial plasma view for major elements.

Keywords: Beverages, Contaminants, EN 1134:1994

INTRODUCTION

The analysis of elements in beverages is a routine practice to ensure consumer safety and product quality. A typical analysis would include both the measurement of toxic elements as well as those which provide nutritional benefit to the consumer. Historically, the analytical technique of choice was atomic absorption spectroscopy (AAS). However, due to the limitations of AAS, such as its ability to measure only one element at a time, there has been a shift towards inductively coupled plasma–optical emission spectroscopy (ICP OES).

Within the European Union, guidance on the analysis of beverages is provided by the means of the Reports on tasks for Scientific Cooperation (SCOOP, Task 3.2.11) and the Committee on Toxicity (COT; Chemicals in Food, Consumer Products and the Environment, 2004). The elements typically analyzed as a result of this guidance are outlined in Table 1.

Table 1. Elements commonly analyzed in beverages

Major nutrients	Contaminants
Ca, Mg, K, Na	Al, As, Cd, Cu, Fe, Mn, Pb, Sn, Zn

This report will detail a procedure for the analysis of fruit juice (and other beverages) by ICP OES. Typically, this was carried out using the standard method EN 1134:1994 Method for determination of sodium, potassium, calcium and magnesium contents of fruit and vegetable juices by atomic absorption spectrometry. The method has been adapted to remove the digestion stage as ICP OES has the ability to process the sample directly, saving time and reagents. In addition, ICP OES is a multi-element technique and therefore a greater range of elements can be analyzed without increasing the time of analysis.

INSTRUMENTATION

The Thermo Scientific iCAP PRO X Duo ICP OES was used for the direct analysis of a range of beverages. This truly simultaneous instrument achieves powerful analyte detection and provides a highly cost-effective solution for routine analysis of liquids in laboratories with standard sample throughput requirements. A Teledyne CETAC ASX-560 Autosampler was used for the sample transport into the system. The Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software simplifies

method development and provides easy options for post-analysis data manipulation.

Sample and standard preparation

Two different beverages were analyzed:

- Apple Juice (consumed undiluted)
- Cola (consumed undiluted)

To reduce the influence of the dissolved CO_2 gas on nebulization and transport, the samples were degassed in an ultrasonic bath. Samples were then prepared by shaking the individual containers and weighting 10 g of each into a glass volumetric flask. A duplicate was prepared for each beverage, which was spiked with the same 1000 mg L^{-1} elemental solutions at same concentrations as used to make the calibration standard 2. All samples were diluted to 50 mL volume with deionized water.

Calibration standards and the spiked blank solution were prepared by diluting 1000 mg L^{-1} single element solutions from Spex CertiPrep (SPEX CertiPrep Group, Metuchen, US), the final concentrations are listed in Table 2. All solutions were made up to a volume of 50 mL in 0.5% high purity nitric acid to improve the long-term stability of the solutions.

Table 2. Calibration standard concentrations (mg L^{-1}) and blank spike concentration (µg L^{-1})

Element	Blank	Standard 1	Standard 2	Standard 3	Blank Spike
Al	0	0.5	1	2	1
As	0	0.5	1	2	5
Ca	0	2	5	10	1
Cd	0	0.5	1	2	1
Cu	0	0.5	1	2	2
Fe	0	0.5	1	2	1
K	0	2	5	10	30
Mg	0	2	5	10	5
Mn	0	0.5	1	2	1
Na	0	2	5	10	10
Pb	0	0.5	1	2	5
Sn	0	0.5	1	2	5
Zn	0	0.5	1	2	1

Method development

A method was created in the Qtegra ISDS Software. Wavelengths were selected as they were free from interferences and offered the sensitivity required for the analysis. The standard sample introduction kit was used for the analysis as per the recommendations in the method notes. The instrument was calibrated and the samples analyzed in a single run. Table 3 shows the parameters used for the method.

Table 3. Method parameters

Parameter	Setting	
Pump tubing	Sample Drain	Tygon® orange/white Tygon® white/white
Pump speed	45 rpm	
Spray chamber	Glass cyclonic	
Nebulizer	Glass concentric	
Nebulizer gas flow	0.5 L min^{-1}	
Coolant gas flow	12 L min^{-1}	
Auxiliary gas flow	0.5 L min^{-1}	
Center tube	2 mm	
RF power	1150 W	
Repeats	3	
Exposure time	Axial 15 s	Radial 15 s

RESULTS

The results obtained in the analysis of the different beverage samples are shown in Table 4 and are further highlighted in Figure 1. The spiked samples were analyzed in the same run as the other samples and all recoveries were within the acceptable range of ±15%.

Method detection limits (MDLs) were established for the trace elements by analyzing the spiked blank solution (see Table 2) with ten replicates. The standard deviation of the result of the spike blank was added to that of the instrument detection blank and was multiplied by 3 to give a detection limit in mg L^{-1} that equates to a confidence interval of approximately 99.7%.

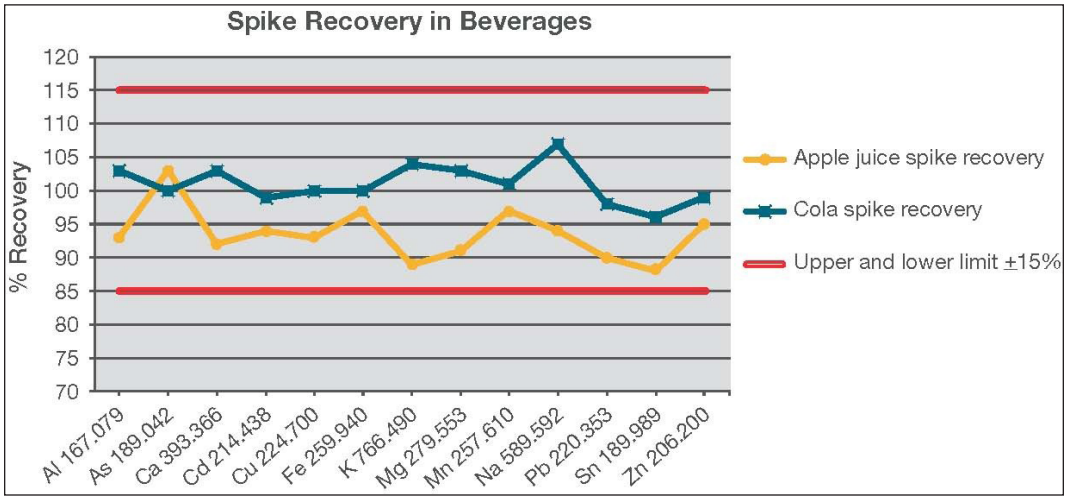

Figure 1. Percentage recovery of spikes in analyzed beverages.

Table 4. Results of the analysis of the diluted beverages

Analyte wavelength (nm)	Plasma view	Spike value (mg L^{-1})	Apple juice measured (mg L^{-1})	Spiked apple juice measured (mg L^{-1})	Apple juice spike recovery (%)	Cola measured (mg L^{-1})	Spiked cola measured (mg L^{-1})	Cola spike recovery (%)	MDL (µg L^{-1})
Al 167.079	Axial	1	0.12	1.05	93	0.002	1.03	103	0.12
As 189.042	Axial	1	0.01	1.04	103	0.002	1.00	100	4.17
Ca 393.366	Radial	5	11.41	16.01	92	4.12	9.25	103	0.94
Cd 214.438	Axial	1	<DL	0.94	94	4.9	0.99	99	0.17
Cu 224.700	Axial	1	0.02	0.94	93	<DL	1.00	100	0.74
Fe 259.940	Axial	1	0.13	1.10	97	0.02	1.00	100	0.98
K 766.490	Radial	5	166.51	170.95	89	12.52	17.71	104	260
Mg 279.553	Radial	5	8.77	13.32	91	0.997	6.16	103	15
Mn 257.610	Axial	1	0.08	1.05	97	<DL	1.01	101	0.26
Na 589.592	Radial	5	3.74	8.44	94	14.01	19.34	107	14
Pb 220.353	Axial	1	<DL	0.90	90	<DL	0.98	98	2.4
Sn 189.989	Axial	1	0.01	0.89	88	0.001	0.96	96	1.0
Zn 206.200	Axial	1	0.02	0.97	95	<DL	0.99	99	0.022

All of the trace contaminants detrimental to human health (As, Cd and Pb) were measured and found to be below the regulation limits (Table 5).

Table 5. Regulation maximum allowed concentrations in various beverages

Element	Maximum (mg L^{-1})
As	<0.2
Cd	<0.1
Cu	<2
Fe	<7
Pb	<0.5
Sn	<100

The results show that the robust RF generator and the standard sample introduction kit easily handle the range of sample densities and viscosities. An internal standard may be added to account for matrix effects, if the recoveries degrade below regulation levels. An online internal standard mixing kit may also be used for ease of use or to reduce labor for higher sample numbers. By connecting an additional pump tube and adding the internal standard on-line, continuous accurate dilution of the sample is assured. Qtegra ISDS Software allows the analyst the ability to turn the internal standard correction on or off pre- or post-analysis, saving valuable method development time as only one analysis is required to generate two sets of results.

CONCLUSION

The Thermo Scientific iCAP PRO X Duo ICP OES and Qtegra ISDS Software features make the analysis of beverages rapid and analyst friendly allowing both experienced and inexperienced users alike to vastly reduce the method development time for these types of samples, resulting in cost-effective analyses. In addition to the time saved on method development, removal of the digestion stage and the use of internal standards produce an easy to use, versatile method capable of analyzing a wide variety of food and beverage samples.

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Full-Scan Fragmentation Options for the Detection of Food Contaminants by an Affordable LC-Q-Orbitrap MS

Paul Zomer[1], Hans Mol[1], Olaf Scheibner[2], Maciej Bromirski[2]

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[2]Thermo Fisher Scientific, Bremen, Germany

This report was extracted from the Thermo Scientific Technical Note Nº 64394

GOAL

To compare two different scan options of a quadrupole-Orbitrap™ system, both offering full mass range fragmentation techniques, and to optimize performance in terms of sensitivity and selectivity.

Keywords: Q Exactive Focus, pesticides analysis, mycotoxin analysis, veterinary drugs analysis, high resolution, data independent acquisition, sensitivity, selectivity.

INTRODUCTION

The analysis of food toxicants is a challenging task because of the high number of substances that needs to be analyzed. Pesticides alone account for over 800 analytes and many food commodities may contain other types of toxicants such as mycotoxins, plant toxins and/or veterinary drugs. Such a great number of analytes can be difficult to handle in a single run by targeted, triple quadrupole MS/MS measurements since the instrument will reach its limits with respect to scan speed. The use of liquid chromatography with full-scan, high-resolution accurate mass spectrometry (HRAM) as an alternative is therefore gaining in popularity, especially in pesticide analysis. HRAM enables simultaneous screening, quantitative determination, and identification of multiple analytes in one run. For identification, the SANCO guideline on pesticide residue analysis (12571/2013) requires the detection of two accurate mass ions, at least one of which is a fragment. Today's instruments offer different options to obtain the required fragment ion while still maintaining a fully non-targeted measurement. On a Thermo Scientific™ Q Exactive™ Focus™ instrument, besides fragmentation modes with precursor ion selection, full mass range fragmentation modes are available. With these, all possible fragments are recorded over the full chromatographic time range, which offers the advantages of full scan measurements for non-targeted screening and retrospective data analysis, while still complying with the identification criteria set in 12571/2013 (Figure 1). These criteria are the detection of at least two diagnostic ions, including the quasi molecular ion and at least one fragment. One option is all-ion fragmentation (AIF) where all precursor ions are sent to the collision cell and fragmented; then, the resulting fragments are measured in the Orbitrap mass analyzer. Another option is variable data-independent acquisition (vDIA) where the mass range for the precursor ions is split into multiple events [1]. This way, sensitivity is improved through the higher number of analyte precursor ions in the C-trap, and selectivity is improved because fragments originate from a smaller range of precursors.

EXPERIMENTAL

Sample Preparation

Samples were prepared using a modified QuEChERS method. The final concentrations were as follows: 1 g/mL (apple, chicken liver); 0.5 g/mL (wheat, compound feed); 0.1 g/mL (food supplement). Final extracts were diluted 1:1 with water prior injection.

LC-MS/MS

The analyses were conducted on a Thermo Scientific™ UltiMate™ 3000 LC system interfaced via a heated electrospray ionization (HESI-II) source to a Q Exactive Focus mass spectrometer. The LC was equipped with a C18 analytical column (100 x 3 mm, particle size 3 µm). A gradient based on water/methanol containing 0.1% formic acid and 2 mM ammonium formate (Fisher Chemical brand) was used. The injection volume was 5 µL. Figure 1 describes the scan events. Fragmentation was done at normalized collision energy (NCE) settings of 30 and 80 (stepped collision energy) in both modes.

Data Analysis

Thermo Scientific™ TraceFinder™ software was used for data analysis. The analyte detection requirements were one precursor plus one fragment ion at T_R ± 0.5 min with *m/z* ± 5 ppm.

RESULTS AND DISCUSSION

Figure 2 shows the extracted ion chromatograms (XIC) of selected compounds measured both with AIF and vDIA. The vDIA data clearly shows the improvements in sensitivity and selectivity compared with AIF. Although the vDIA method includes more scans per scan cycle, the number of data points per chromatographic peak is still more than sufficient. The usability of the vDIA method was tested by analyzing a mixture of 37 compounds (pesticides, natural toxins, veterinary drugs) in solvent and five matrices at four levels. Table 1 shows the number of detected compounds based on precursor plus fragment. Another important parameter to assess the suitability of a method is the number of false positives. To check this, an internally developed database containing 170 pesticides was used to process samples of the blank matrices used for spiking. Fully automated analyte detection resulted in 4–12 primary detects/sample. With the software used, manual verification of these potential detects was quick and straightforward and for none of the software-detects coinciding peaks for precursor and fragment were observed. Hence, no false positives were found in any of the blanks.

vDIA method is not available in the United States of America.

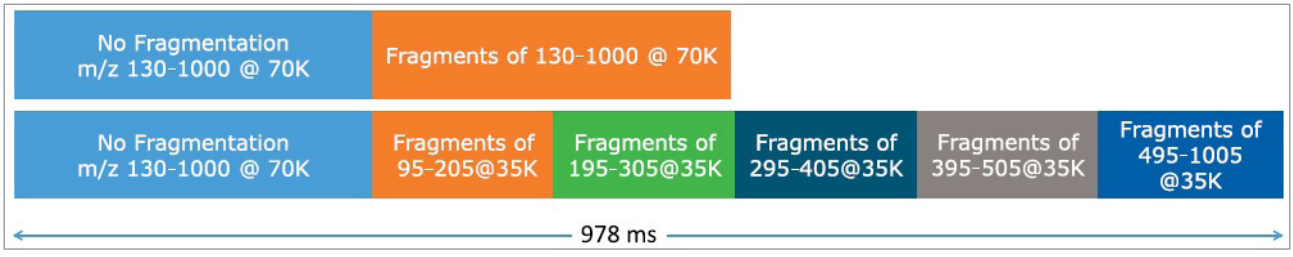

Figure 1. Schematic representation of measured scan event cycles. Option 1: FS+AIF (top bar) Option 2: FS+5 vDIA events (lower bar).

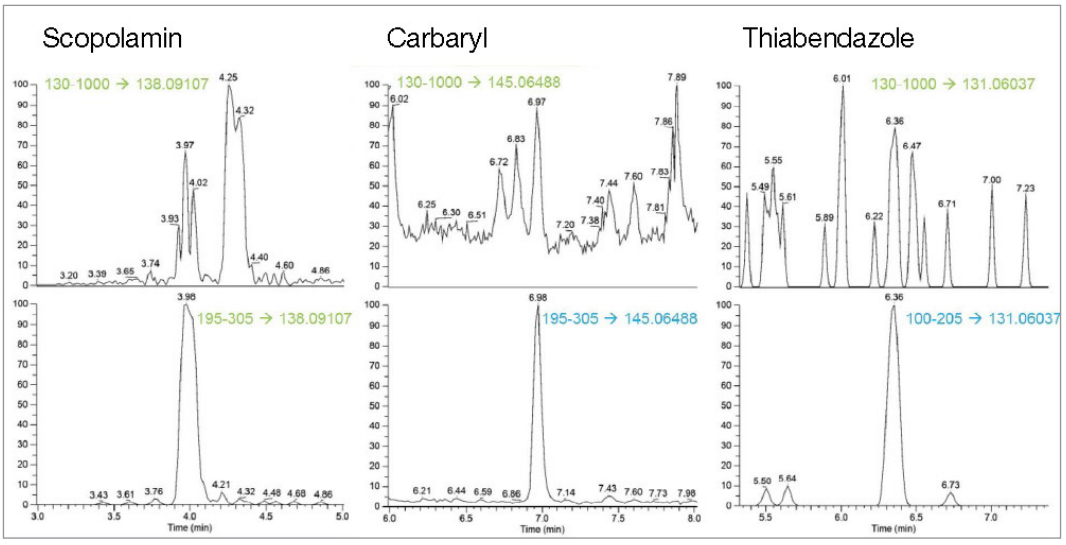

Figure 2. Comparison of XICs of fragment ions, fragmented with AIF (top) and vDIA (bottom). From left to right: scopolamin in wheat, carbaryl in wheat and thiabendazole in compound feed. Spike level: 10 ng/g.

Table 1. Number of compounds out of a total number of 37 automatically detected by TraceFinder software at different levels in five matrices, comparing vDIA mode (left) with AIF mode (right).

vDIA					AIF				
Matrix	1 ng/g	10 ng/g	50 ng/g	200 ng/g	Matrix	1 ng/g	10 ng/g	50 ng/g	200 ng/g
Solvent	33	37	37	37	Solvent	32	37	37	37
Apple	31	37	37	37	Apple	26	35	37	37
Liver	28	35	37	37	Liver	24	35	37	37
Food Supplement*	26	32	37	37	Food Supplement*	14	23	37	37
Wheat	21	33	37	37	Wheat	11	30	36	37
Compound Feed	9	13	24	34	Compound Feed	1	19	21	31

*Spiking levels in food supplement 10x higher.

CONCLUSION

Variable data-independent data acquisition improves sensitivity, selectivity, and the ability to identify target analytes adding extended non-target screening capabilities. The sensitivity, as well as the limited number of false detects obtained by software-based detection and the ease with which to review and discard them, make LC-full-scan analysis with vDIA in high resolution mass spectrometry (HRMS) suited for routine applications.

REFERENCE

1. Scheibner, O.; Kellmann, M.; Yang, C.; Bromirski, M. Thermo Scientific Technical Note 64283; Variable Data-Independent Acquisition (vDIA) Delivers High Selectivity and Sensitivity in Combined Targeted and Untargeted Analyses for Small Molecules, 2014.

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Sample Preparation of Food Samples for Trace Metal Analysis

This report was extracted from a Milestone Industry Report on Ethos UP/Food

INTRODUCTION

Demand for trace metals analysis in the food industry is growing strongly due to stricter food regulations such as the recent Food Safety Modernization Act. ICP has been the standard for metals analysis for food, but as demand for lower levels of detection grows, the industry is experiencing a significant transition to ICP-MS. This transition is placing increased emphasis on the sample preparation method. Traditional sample preparation techniques for food include hot block digestion, closed vessel microwave digestion, and ashing; each of them posing different challenges.

Hot block digestions suffer from long digestions, airborne contamination, poor digestion quality, and poor recovery of volatile compounds. Closed vessel microwave digestion has proven to be an effective technique with fast, complete digestions, a clean environment, and full recovery of volatile compounds.

Milestone's ETHOS UP microwave digestion system incorporates all of the benefits of closed vessel microwave digestion while making sample preparation fast, easy, effective, and of the highest quality.

EXPERIMENTAL

In this industry report, a recovery study on certified reference food materials has been performed to prove the efficacy of ETHOS UP in the sample preparation for metal analysis.

Instrument

ETHOS UP

The ETHOS UP meets the requirements of modern analytical labs. It offers several unique benefits including:

- Increased ease of use and productivity
- Enhanced control in all vessels
- Fast, accurate and traceable
- Superior safety and digestion quality

The ETHOS UP is a flexible and high performing platform used for elemental analysis and routine determinations in many applications. Its construction of stainless steel coated with five PTFE layers and accommodates both high-pressure and high-throughput rotors.

easyTEMP

Milestone's easyTEMP contactless sensor directly controls the temperature of all samples and solutions, providing accurate temperature feedback to ensure complete digestion in all vessels and high safety. The superior temperature measurement of easyTEMP allows the processing of different samples of similar reactivities, thus reducing labor time and increasing overall throughput.

This technology combines the fast and accurate reading of an in-situ temperature sensor with the flexibility of an infrared sensor. The ETHOS UP software provides digestion history traceability and temperature measurement for every sample. The temperature diagram and profiles are displayed real-time, and can be subsequently saved on the ETHOS UP terminal.

Figure 1. Milestone's ETHOS UP.

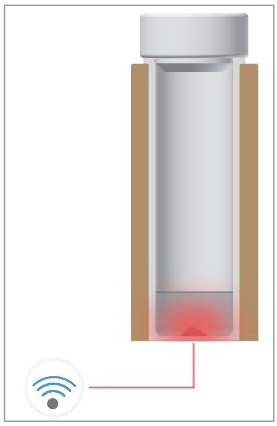

Figure 2. easyTEMP contactless direct temperature sensor.

Figure 3. SK-15 easyTEMP High Pressure Rotor.

SK-15 High Pressure Rotor

The SK-15 rotor perfectly matches the needs of a modern analytical lab to determine trace elements, thanks to its capability for digesting large sample amounts at high temperature (up to 300 °C) and pressure (up to 100 bar).

The 15-position rotor is controlled by a contactless direct temperature sensor that controls the internal temperature of all vessels throughout the digestion cycle. This ensures complete and reproducible digestions of even the most difficult and reactive samples. The SK-15 also features Milestone's patented "vent-and-reseal" technology for controlling the internal pressure of each vessel.

MAXI-44 High Throughput Roto

The MAXI-44 is a high throughput rotor capable of digest a large variety of food samples, improving throughput in the lab.

The MAXI-44 is fully controlled by a contactless temperature/pressure sensor that directly control each vessel. This assures maximum safety and digestion quality.

Figure 4. MAXI-44 easyTEMP High Throughput Rotor

User Interface

The ETHOS UP comes with a dedicated touch screen terminal and easyCONTROL software which incorporates our expertise and know-how in microwave sample preparation. The ETHOS UP user-interface fully control all the digestion parameters, provides complete documentation and expedites the overall digestion procedure. The terminal is equipped with multiple USB and ethernet ports for interfacing the instrument to external devices and the laboratory network. The ETHOS UP controller is user-friendly, icon-driven, multi-language and 21 CFR Part 11 compliant. To find the method which best suits your application simply select from the vast library of pre-stored methods. Included with the ETHOS UP is a unique web-based application: Milestone Connect.

This app allows you to become a part of the Milestone community and gain exclusive access to a robust library of information: lists of parts, technical notes, user manuals, video tutorials, continuously updated application notes and all relevant scientific articles.

Figure 5. easyCONTROL built-in library.

Analytical Procedure

Table 1. Sample amount and acid mixture used for the microwave digestion run

ETHOS UP			
Rotor	**Sample**	**Sample amount**	**Acid mixture**
SK 15 easyTEMP	Lobster hepatopancreas (TORT-3)	1 g	5 mL of HNO_3 65%
	Bovine Liver (NIST 1577c)	1 g	5 mL of HNO_3 65%
	Diary Feed (BCR 708)	1 g	5 mL of HNO_3 65%
Maxi 44 easyTEMP	Lobster hepatopancreas (TORT-3)	0.5 g	5 mL of HNO_3 65%
	Bovine Liver (NIST 1577c)	0.5 g	5 mL of HNO_3 65%
	Diary Feed (BCR 708)	0.5 g	5 mL of HNO_3 65%

SK-15 eT method and microwave run report

Table 2. SK-15 eT microwave program used for digestion of samples

Step	Time	T2	Power
1	00:20:00	210 °C	1800 W
2	00:15:00	210 °C	1800 W

- Final dilution: 50 mL with deionized water

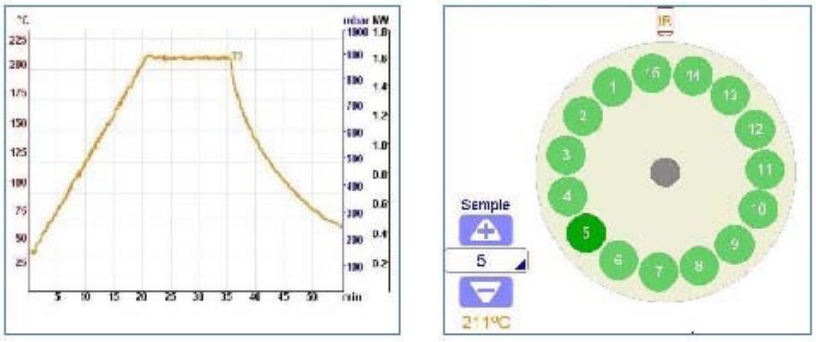

Figure 6. SK-15 Microwave Run Report and Multiple temperature traceability.

MAXI-44 eT method and microwave run report

Table 3. MAXI-44 eT microwave program used for digestion of samples

Step	Time	T2	Power
1	00:20:00	200 °C	1800 W
2	00:15:00	200 °C	1800 W

- Final dilution: 50 mL with deionized water

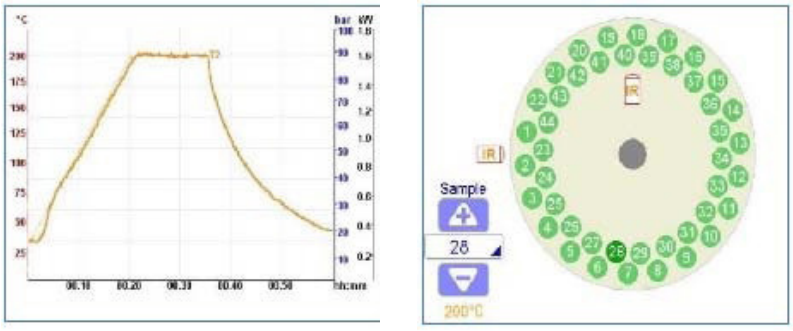

Figure 7. MAXI-44 Microwave Run Report and Multiple temperature traceability

Quantification

ICP-OES Instrumental Parameters: RF power (W): 1300; Plasma flow (L/min): 15.0; Auxiliary Flow (L/min): 1.5; Nebulizer Flow (L/min): 0.75; Replicate read time (s): 10; Instrument stabilization delay (s): 15; Sample Uptake Delay (s): 30; Pump Rate (rpm): 15; Rinse Time (s): 10; Replicates: 3.

RESULTS AND DISCUSSION

The performance of the Milestone's ETHOS UP equipped with SK-15 easyTEMP rotor was evaluated through a recovery study on Lobster hepatopancreas (TORT-3), Bovine liver NIST (1577c), Diary Feed (BCR 708). The sample were digested with Milestone's ETHOS UP and subsequently analyzed via ICP-OES.

The analytical results are shown in Table 4, 5 and 6 with good recoveries of all elements and RSDs below 3%. This demonstrates the robustness and reproducibility of microwave digestion using the ETHOS UP equipped with SK-15 and MAXI-44 easyTEMP technologies.

Table 4. Data of the recovery study on lobster hepatopancreas TORT-3

	Certified value (mg/Kg)	SK-15 eT		MAXI-44 eT	
		Recovery % (n=3)	RSD (%)	Recovery % (n=3)	RSD (%)
As	59.5 ± 3.8	102.8	2.5	108.2	2.9
Cd	42.3 ± 1.8	89.1	2.2	84.2	2.6
Cr	1.95 ± 0.24	96.5	2.9	95.4	2.5
Cu	497 ± 22	103.3	2.2	96.2	1.8
Fe	179 ± 8	93.5	2.9	94.1	2.3
Mn	15.6 ± 1.0	98.5	1.1	96.3	1.9
Ni	5.30 ± 0.24	102.1	1.0	103.2	2.1
Se	10.9 ± 1.0	94.1	1.9	97.8	1.8
Sr	36.5 ± 1.6	100.7	1.5	93.4	2.2
V	9.1 ± 0.4	83.7	2.3	84.5	2.3
Zn	136 ± 6	90.3	2.1	90.2	2.5

Table 5. Data of the recovery study on bovine liver NIST 1577c

	Certified value (mg/Kg)	SK-15 eT		MAXI-44 eT	
		Recovery % (n=3)	RSD (%)	Recovery % (n=3)	RSD (%)
Ca	131 ± 10	92.8	1.2	91.9	1.8
Cu	275.2 ± 4.6	91.6	1.4	91.8	2.3
Fe	197.94 ± 0.65	98.2	2.1	94.3	2.7
Mg	620 ± 42	101.2	1.6	96.2	1.2
Mn	10.46 ± 0.47	95.3	2.3	92.6	2.2
Mo	3.30 ± 0.13	104.6	1.4	99.4	2.3
Zn	181.1 ± 1.0	92.3	1.1	95.1	2.8

Table 6. Data of the recovery study on diary feed BCR 708

	Certified value	SK-15 eT		MAXI-44 eT	
		Recovery % (n=3)	RSD (%)	Recovery % (n=3)	RSD (%)
Ca	4.8 ± 0.5 g/Kg	96.4	1.3	92.7	2.9
Cu	37 ± 4 mg/Kg	97.8	1.8	93.2	1.6
Mg	1.47 ± 0.22 g/Kg	99.6	1.2	95.4	2.4
P	4.7 ± 0.4 g/Kg	95.4	1.6	96.5	3.0

CONCLUSION

The data shown in this industry report demonstrates full recovery of the element reported in the certificates of the reference material.

Highly reactive samples such as food can be completely digested even in large sample amounts along with different samples of similar reactivities. The digestion process was accurately controlled by the easyTEMP sensor, ensuring superior digestion quality and reliable results.

The ETHOS UP provides a complete solution for food laboratories, enabling the processing of high sample amount with SK-15 easyTEMP rotor as well as unmatched throughput with the MAXI-44 easyTEMP rotor.

In addition, microwave digestion using the Milestone ETHOS UP provides the highest level of reproducibility, great ease of use and high productivity.

About Milestone

At Milestone we help chemists by providing the most innovative technology for metals analysis, direct mercury analysis and the application of microwave technology to extraction, ashing and synthesis. Since 1988 Milestone has helped chemists in their work to enhance food, pharmaceutical and consumer product safety, and to improve our world by controlling pollutants in the environment.

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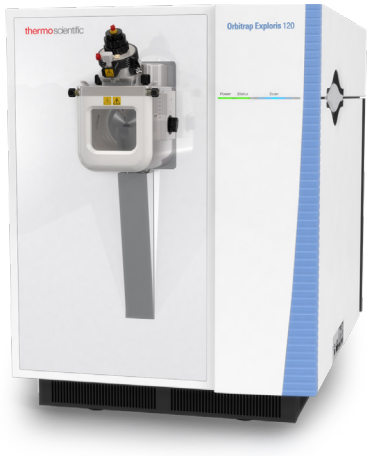
Productivity redefined with the Orbitrap Exploris 120 Mass Spectrometer



The new Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer accelerates your qualitative and quantitative confidence for both method development and everyday testing purposes. With its unique ability to provide consistently accurate data and a design made for operational simplicity, it sets the new standard in instrument productivity and ruggedness.

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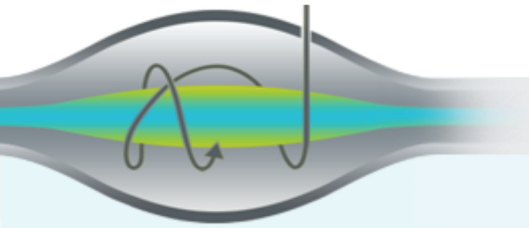

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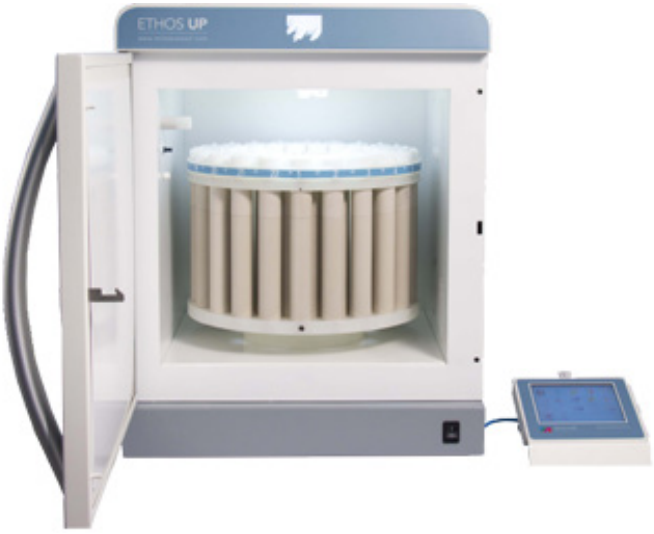

Open the door to a new Milestone! The new ETHOS UP is the most advanced microwave digestion systems Milestone has ever manufactured.

The new Milestone ETHOS microwave cavity has a volume in excess of 70 litres, by far the largest currently available; up to 44 samples can be accommodated, improving productivity and sample preparation throughput.

The new Milestone ETHOS UP is equipped with the most advanced yet easy to use reaction sensors for complete quality control of the digestion conditions.

In combination with our 'vent-and-reseal' vessel technology, the sensors ensure complete and safe digestions without any loss of volatile compounds

Included with the brand-new ETHOS UP microwave digestion system is a unique web based application – Milestone Connect. The app provides up to date information and extended instrument control from outside the laboratory. By adding the IP address of your network, operators will be able to control the ETHOS UP from outside the laboratory with remote monitoring of every sample in the digestion run and other information related to the system on any wifi-enabled mobile device. That ultimately helps to provide high quality sample preparation. The app works on various external devices such as PC, tablets or smartphones connected to the ETHOS UP.

Users will be part of Milestone scientific community and will gain an exclusive access to Milestone contents: application notes, digestion tips and techniques, Milestone library, scientific articles, video tutorials, special offers, news and a help-on-line section.

Milestone know-how and 26-year experience in sample preparation are now available for chemists to provide instant support available 24 hours a day, 7 days a week.

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Productivity UP.
Simplicity UP.
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HIGHEST THROUGHPUT ROTORS

NO METHOD DEVELOPMENT

REMOTE SYSTEM CONTROL

ENHANCED SAFETY FEATURES

JUST WHEN YOU THINK METALS PREP TECHNOLOGY CANT GET ANY BETTER, IT DOES.

The Milestone Ethos UP isnt just intelligent, its the most powerful microwave digestion system on the market today.

Featuring the highest throughput rotors, stainless steel construction and patented vent-and-reseal technology, the Ethos UP ensures market-leading safety and productivity.

Plus, new Milestone Connect offers remote system control, 24/7 technical support and direct access to a comprehensive library of content developed especially for lab professionals.

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We speak to a scientist at the forefront of innovative efforts to scale up coronavirus testing for use at the population level

Working Medical College of Georgia at Augusta University, Dr. Ravindra Kolhe is a trained pathologist whose lab is now focused almost entirely on SARS-CoV-2 testing and COVID-19, and has effectively become a hybrid clinical, diagnostic and translational lab dedicated to scaling up RT-PCR assays for coronavirus testing. With cases rocketing in the US and elsewhere, the stakes could hardly be higher. Read this Editorial here

Webinar: ***Rapid and functional evaluation of virus-neutralizing antibodies using multi-parametric live cell analysis***

Current methods for screening virus-neutralizing antibodies, including the canonical plaque reduction neutralization test, require multiple handling steps and numerous days to complete. This presentation will highlight a more efficient assay that identifies virus-neutralizing antibodies in real-time. Attend this webinar here

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Are we doing enough with testing for COVID-19 to answer the important questions for patients and healthcare practitioners?

In this presentation, Dr. Michael Laposata (University of Texas Medical Branch) reviews the different testing platforms currently used for COVID-19 testing and highlights the challenges faced by healthcare practitioners in reaching accurate and reliable diagnostic conclusions. Laposata provides insight into how to overcome the occurrence of false negatives and false positives, highlights the need for high performing ELISA and chemiluminescence assays without cross-reactivity, and describes how the provision of test result interpretations could greatly reduce diagnostic errors, with significant clinical consequences. Attend this presentation here

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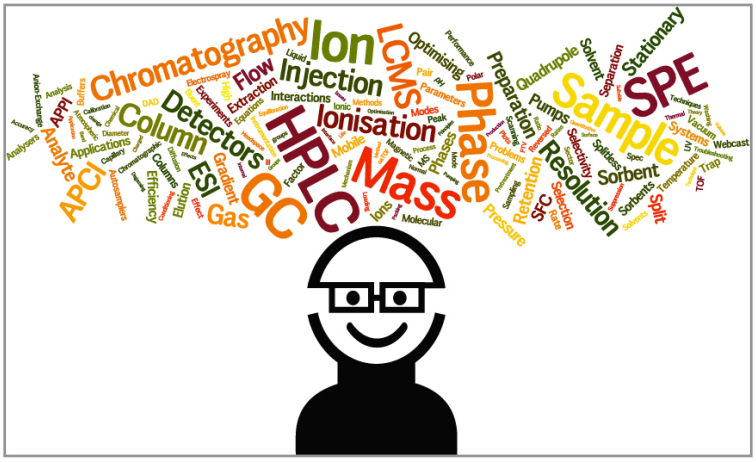

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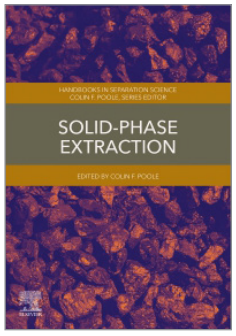
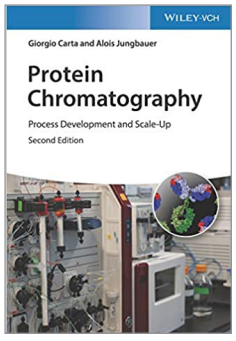
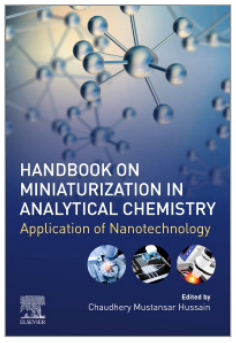


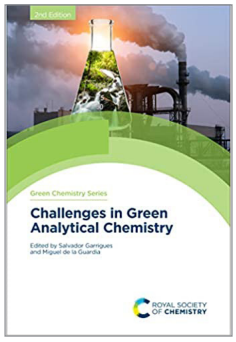
Solid-Phase Extraction – a volume in *Handbooks in Separation Science*
Colin Poole, Editor
September 2019. Publisher: Elsevier
This book thoroughly presents both new and historic techniques for dealing with SPE. It provides all information laboratory scientists need for choosing and utilizing suitable sample preparation procedures for any kind of sample. In addition, the book showcases the contemporary uses of sample preparation techniques in the most important industrial and academic project environments, including SPME, molecularly imprinted polymers, magnetic nanoparticles, and more. Read more …



Protein Chromatography: Process Development and Scale-Up, Second Edition
Giorgio Carta, Alois Jungbauer, Authors
March 2020. Publisher: Wiley
Chapters look at: Downstream Processing of Biotechnology Products; Laboratory and Process Columns and Equipment; Adsorption Equilibrium; Rate Processes; Dynamics of Chromatography Columns; Effects of Dispersion and Rate Processes on Column Performance; Gradient Elution Chromatography; and more. This book will appeal to biotechnologists, analytical chemists, chromatographers, chemical engineers, pharmaceutical industry, biotechnological industry, and biochemists. Read more …



Handbook on Miniaturization in Analytical Chemistry: Application of Nanotechnology
Chaudhery Mustansar Hussain, Editor
July, 2020. Publisher: Elsevier
Covering all stages of analysis, from sample preparation to separation and detection, this book discusses the design and manufacturing technology of miniaturization and includes an entire section on safety risks, ethical, legal and social issues (ELSI), the economics of nanotechnologies, and a discussion on sustainability with respect to nano- and lab-on-chip technologies. Read more …



Challenges in Green Analytical Chemistry
Miguel de la Guardia, Salvador Garrigues, Editors
May, 2020. Publisher: Royal Society of Chemistry
This new edition presents an overview of the latest tools and techniques for improving safety and sustainability in analytical chemistry. Covering topics including solvent selection, miniaturization and metrics for the evaluation of greenness, this book is a useful resource for researchers and application laboratories interested in reducing the risks and environmental impacts of analytical methods. Read more …

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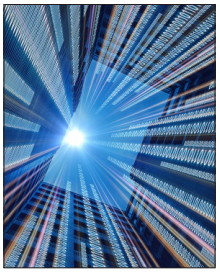

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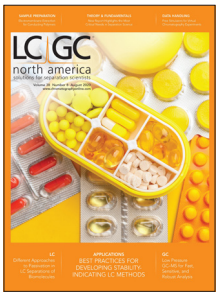

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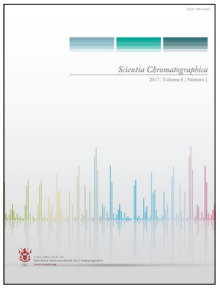

Scientia Chromatographica

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EVENTS – It is suggested to consult the event's official website for updates.

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Belgrade Online — previously 71st Annual Meeting of the International Society of Electrochemistry — "Electrochemistry towards Excellence"
https://annual71.ise-online.org/

Octorber 5 – 16, 2020
VIRTUAL 43rd Annual Meeting of the Brazilian Chemical Society (43rd RASBQ)
http://www.sbq.org.br/43ra/

October 7 – 8, 2020
FCE Pharma Virtual Sessions
https://www.fcepharma.com.br/sessions/

October 20 – 21, 2020
18th Congress on Quality in Metrology (ENQUALAB 2020)
São Paulo, SP
https://www.enqualab.net/

October 26 – 29, 2020
1th Online National Congress on Analytical and Environmental Chemistry (CONQUIAMB)
https://congresse.me/eventos/conquiamb/

November 9 – 11, 2020
National Meeting on Forensic Chemistry (7th EnqFor) & 4th Meeting of the Brazilian Society of Forensic Sciences (SBCF)
Ribeirão Preto, SP, Brazil
https://www.en.enqfor2020.sbcf.org.br/

December 10 – 11, 2020
International Conference on Metrology, Measurement and Inspection (ICMMI 2020)
New York City, USA
https://waset.org/metrology-measurement-and-inspection-conference-in-december-2020-in-new-york

February 07 – 11, 2021
XXIII International Mass Spectrometry Conference (IMSC 2021)
Windsor Oceânico Hotel, Rio de Janeiro, RJ, Brazil
https://www.imsc2020.com/

October 11 – 15, 2021
34th Latin American Congress of Chemistry – CLAQ 2020; 18th Latin American Congress of Chromatography – COLACRO; 10th Colombian Congress of Chromatography – COCOCRO; 4th Colombian Congress of Biochemistry and Molecular Biology - C2B2
Convention Center, Cartagena de Indias, Colombia
https://claq2020.com/en/bienvenida/

October 24 – 27, 2021
20th National Meeting on Analytical Chemistry (20th ENQA) & 8th Ibero-American Congress of Analytical Chemistry (8th CIAQA)
Dall'Onder Grande Hotel, Bento Gonçalves, RS, Brazil
https://enqa2021.com.br/

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Examples of reference formatting

Journals

1. Orlando, R. M.; Nascentes, C. C.; Botelho, B. G.; Moreira, J. S.; Costa, K. A.; Boratto, V. H. M. *Anal. Chem*., **2019**, *91* (10), pp 6471-6478 (https://doi.org/10.1021/acs.analchem.8b04943).
   - *Publications with more than 10 authors, list the first 10 authors followed by a semicolon and et al.*
   - *Titles of journals must be abbreviated as defined by the Chemical Abstracts Service Source Index (http://cassi.cas.org/search.jsp).*

Electronic journals

2. Sapozhnikova, Y.; Hoh, E. *LCGC North Am*., **2019**, *37* (1), pp 52-65. Available from: http://www.chromatographyonline.com/suspect-screening-chemicals-food-packaging-plastic-film-comprehensive-two-dimensional-gas-chromatogr [Accessed 20 January 2019].

Books

3. Burgot, J.-L. *Ionic Equilibria in Analytical Chemistry*. Springer Science & Business Media, New York, **2012**, Chapter 11, p 181.
4. Griffiths, W. J.; Ogundare, M.; Meljon, A.; Wang, Y. Mass Spectrometry for Steroid Analysis. In: Mike, S. L. (Ed.). *Mass Spectrometry Handbook*, v. 7 of Wiley Series on Pharmaceutical Science and Biotechnology: Practices, Applications and Methods. John Wiley & Sons, Hoboken, N.J., **2012**, pp 297-338.

Standard methods

5. International Organization for Standardization. ISO 26603. Plastics — *Aromatic isocyanates for use in the production of polyurethanes — Determination of total chlorine*. Geneva, CH: ISO, **2017**.

Master's and doctoral theses or other academic literature

6. Dantas, W. F. C. *Application of multivariate curve resolution methods and optical spectroscopy in forensic and photochemical analysis*. Doctoral thesis, **2019**, Institute of Chemistry, University of Campinas, Campinas, SP, Brazil.

Patents

7. Trygve, R.; Perelman, G. US 9053915 B2, June 9, **2015**, Agilent Technologies Inc., Santa Clara, CA, US.

Web pages

8. http://www.chromedia.org/chromedia [Accessed 10 January 2019].

Unpublished source

9. Viner, R.; Horn, D. M.; Damoc, E.; Konijnenberg, A. *Integrative Structural Proteomics Analysis of the 20S Proteasome Complex* (WP-25). Poster presented at the XXII International Mass Spectrometry Conference (IMSC 2018) / August 26-31, **2018**, Florence, IT.
10. Author, A. A. *J. Braz. Chem. Soc*., in press.
11. Author, B. B., **2019**, submitted for publication.
12. Author, C. C., **2019**, unpublished manuscript.

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Based on the comments and suggestions of the reviewers and editors a revision of the manuscript may be requested to the authors. A revised manuscript should be submitted by the authors, containing the changes made in the manuscript clearly highlighted. Responses to the reviewers must also be submitted answering in detail to the questions made by them, and describing the changes made in the manuscript.

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