A DISSERTATION ON

DETERMINATION OF MINERALS & HEAVY METALS IN DIFFERENT NUTRACEUTICAL PRODUCT AND IT'S EFFECT ON HUMAN HEALTH

SUBMITTED TO THE
DEPARTMENT OF BIOSCIENCES
INTEGRAL UNIVERSITY, LUCKNOW

IN PARTIAL FULFILMENT
FOR THE
DEGREE OF MASTER OF SCIENCE
IN
BIOTECHNOLOGY
BY

Ishrat Sheikh
Roll No. 2000102155
M.Sc. Biotechnology (IV Semester)
Department Of Biosciences
Integral University, Lucknow

Under the supervision of

Mr. Haseeb Alam
HEAD OF DEPARTMENT
ATOMIC ABSORPTION SPECTROSCOPY
FARE Labs Pvt. Ltd, Gurgaon

INTEGRALUNIVERSITY
Established Under U.P. Act No 09 of 2004 by state Legislation
ApprovedbyUniversityGrantsCommission
PhoneNo.:
+91(0552)2890812,2890730,3296117,6451039
KursiRoad,Lucknow-226026Uttar Pradesh (INDIA)

TO WHOM IT MAY CONCERN

This is to certify that **Ms. ISHRAT SHEIKH** student of M. Sc. Biotechnology,(IV semester),Integral University has completed her four months dissertation work entitled **" DETERMINATION OF MINERALS & HEAVY METALS IN DIFFERENT NUTRACEUTICAL PRODUCTS AND IT'S EFFECT ON HUMAN HEALTH"** successfully. She has completed this work from the FARE Labs Pvt. Ltd., Gurgoan, under the supervision of **Mr. HASEEB ALAM**. The dissertation was compulsory part of her M.Sc. Degree.

I wish her good luck and a bright future.

Dr. SNOBER S.MIR

HEAD OF THE DEPARTMENT

DEPARTMENT OF BIOSCIENCES

E-mail: info@integraluniversity.ac.in Web: www.integraluniversity.ac.in

FARELABS
FOOD ANALYSIS & RESEARCH LABORATORY
TESTING, CALIBRATION, PROFICIENCY TESTING, R&D & TRAINING SERVICES

FARE LABS Private Limited
L-17/3, DLF Phase-II, IFFCO Chowk, M.G. Road,
Gurgaon-122002, Haryana, INDIA
Phone : +91-124-4223207, 4034205
Fax : +91-124-4036038, Cell : +91-95992 21227
E-mail : farelabs@farelabs.com
Website : www.farelabs.com

02 June 2022

TRAINING CERTIFICATE

This is to certify that **Ishrat Sheikh,** D/o Seikh M.D Nasimuddin from, **Intergral University** MSc in Biotechnology has successfully completed her Major Project in **"Determination of Minerals & Heavy Metals in different nutraceutical products and its effects on human health."** from 17th Jan-2022 to 02 June 2022 at **FARE Labs Pvt. Ltd.** and has been awarded excellent grade on the basis of her performance.

She has accomplished the Major Project successfully. We have found her sincere and devoted during the training.

FARE LABS PVT. LTD. L-17/3 DLF Ph-II GURGAON

Kanishka Sharma
Human Resource Department
FARE Labs Pvt. Ltd.

DECLARATION

This is to declare that the dissertation work embodied in the training report **"Determination Of Minerals And Heavy Metals In Different Nutraceutical Products And It's Effect On Human Health"** to be submitted to the Master of Science in Biotechnology of Integral University, Lucknow, Uttar Pradesh, India is original and is the result of investigations carried out by the candidate under the supervision of **Mr Haseeb Alam,** Head & Scientist Grade C , AAS Department, and , **Komal Srivastav**, AAS Department, Fare Labs Pvt. Ltd., Gurgaon, for the time period of time Janauary' 2022-June' 2022. This work has not been submitted in part or in full for any other degree or diploma of this, or any other institution. The extent of information derived from the existing literature has been indicated in the body of the thesis, at appropriate places, giving the source of information. I have completed all pre submission requirement as per the University rules.

Ishrat Sheikh

M.sc. Biotechnology

ACKNOWLEDGEMENT

The submission of my Master's thesis gave me immense pleasure, satisfaction and unique sense of accomplishment. I convey my humble gratitude to God and then my parents and my other family members for their prayers, love, care and sacrifices for educating and preparing me for my future. They have made this thesis a reality by having faith in me and by instilling in me the courage to strive and achieve higher goals in life**.** With the help, consuelling and support of many people, this report has finally completed. At this point I would like to express our sincere gratitude to **Dr.Meenakshi Tripathi(Quality Manager)** for the great supervision , for the patience she alwys had despite her many other duties and responsibilities, and for provinding good working place.

I would like to acknowledge my profound and heartfelt gratitude and sincere thanks to my guide **Mr. Haseeb Alam(Head of Atomic Absorption Spectroscopy)**, FARE Labs Private Limited for his invaluable advice, continued guidance, constructive encouragement and sound support throughout the period of this research project work. And for providing me a opportunity to work on this research topic and providing facilities during the work. His erudition and rich experience shaped the course of the research and sharpened its outcome. I am sincerely thankful to him for his able guidance and pain taking effort in improving my understanding of this project.
I would like to thank **Mr. D.Mathur(Director), Mr.C.S.Joshi(Director),** FARE Labs Pvt.Ltd. for letting me take experience in AAS Department and learn Analysis and Detection of metals in food and water. I would like to show gratitude from letting me gain knowledge from outside of my department.

I would also like to thank **Dr. Mohd Salman Khan(Coordinator of Biotechnology Department)** and to all the faculty members of Department of Biosciences.

Thanky you,
Ishrat Sheikh

CONTENT

About The Laboratory : FARE Labs Food Analysis & Research Laboratory

FARE LABS: AN OVERVIEW:

• Founded in 1999, FARE LABS Private Limited is a leading laboratory institution of India. It is based in Gurgaon, and is promoted by Mr. Dwijendra Mathur and Mr. Chandra Shekhar Joshi, a team of chemical engineers with a combined industry work experience of over 51 years.

• FARE LABS possesses world-class competencies in Analytical Testing and Research & Development. In addition, it has a strong Consultancy and Training division to provide holistic solutions to its clients.

• Accredited by NABL (National Accreditation Board of Laboratories) & a certified SIRO (Scientific & Industrial Research Organization) institution, FARE Labs has world-class competencies in Analytical Testing and Research & Development.

OUR MISSION:

• To become a source of national pride by contributing to the world of scientific and industrial research and development in the diverse areas of chemical, biotechnological and environmental technology

OUR VISION:

• To generate technology and industrial community on a continuous basis, and accordingly identify focus areas for research and development.

• To assess the needs of scientific process improvement related knowledge for the benefit of industry, environment and public at large.

OUR VALUES:

• Integrity, accountability and client confidentiality.

• Attention to issues of domestic and global relevance.

OUR SERVICES:

• Analytical Testing

• Research & Development

• Consultancy & Training

LIST OF ABBREVIATIONS:

AAS: Atomic Absorption Spectroscopy

ICP-MS: Inductively Coupled Plasma-Mass Spectrometry

MS: Mass Spectrometry

CRM: Certified Reference Material

NIST: National Institute of Standards and Technology

Pb: Lead

Ni: Nickel

Cu: Copper

Na: Sodium

Fe: Iron

Zn: Zinc

Conc.: Concentration

ppm: Parts per million

ppb: Parts per billion

RF: Radio frequency

HVT Tubes: High Voltage Transformer Tubes

LIST OF FIGURES:

ABSTRACT

Using AAS and ICP-MS, this paper presents a straightforward approach for determining elements in nutraceutical food samples. 0.25 gm & 0.5 gm of nutraceutical food samples were carefully weighed into a Teflon digestion vessel prior to the examination. After that, 6-8 mL concentrated nitric acid and 2 mL hydrogen peroxide were added. A microwave digestion method was used to decompose the materials. Six standard reference materials from the National Institute of Standards and Technology (NIST) were analysed to verify the suggested method's accuracy and precision. Then using the analytical results we can tell the concentration of some of the minerals and heavy metals present in the samples.

Key words: AAS, ICP-MS, Microwave Digestion, CRM, Nutraceutical & Functional Foods, Minerals, Heavy Metals.

INTRODUCTION

The term **Nutraceutical** is a hybrid or contraction of nutrition and pharmaceutical. Reportedly, it was coined in 1989 by DeFelice and the Foundation for Innovation in Medicine. Restated and clarified in a press release in 1994, its definition was “any substance that may be considered a food or part of a food and provides medical or health benefits, including the prevention and treatment of disease. Such products may range from isolated nutrients, dietary, supplements and diets to genetically engineered ‘designer’ foods, herbal products, and processed foods such as cereals, soups, and beverages.

According to the International Food Information Council (IFIC), functional foods are “foods or dietary components that may provide a health benefit beyond basic nutrition.” The International Life Sciences Institute of North America (ILSI) has defined functional foods as “foods that by virtue of physiologically active food components provide health benefits beyond basic nutrition.” Health Canada defines functional foods as “similar in appearance to a conventional food, consumed as part of the usual diet, with demonstrated physiological benefits, and/or to reduce the risk of chronic disease beyond basic nutritional functions.” The Nutrition Business Journal classified functional food as “food fortified with added or concentrated ingredients to functional levels, which improves health or performance. Functional foods include enriched cereals, breads, sport drinks, bars, fortified snack foods, baby foods, prepared meals, and more.”

The interest in nutraceuticals and functional foods continues to grow, powered by progressive research efforts to identify properties and potential applications of nutraceutical substances, and coupled with public interest and consumer demand. The principal reasons for the growth of the functional food market are current population and health trends. Across the globe, populations are aging. Life expectancy continues to rise, as does the contribution made by older individuals to the total population. Also, obesity is now recognized as a global issue as its incidence continues to climb in countries throughout the world. In the U.S., approximately 62% of the adult population is classified as overweight (based on body mass index (BMI)), and more than half of those adults are classified as obese. Heart disease continues to be a primary cause of death, responsible for 32% of deaths in the U.S., and cancer, osteoporosis, and arthritis remain highly prevalent. As of this writing, the International Obesity Task Force reports that the incidence of obesity in the majority of European countries has increased by 10 to 50% in the last 10 years. People can optimize the health-promoting capabilities of their diet by way of supplementation and by consuming foods that have been formulated or fortified to include health-promoting factors. Another reason for the growing trend in

functional foods is public education. People today are more nutrition-savvy than ever before, their interest in health-related information being met by many courses of information.

Minerals in Nutraceutical & Functional Foods:

Minerals are stable chemicals that arise obviously in natural form. They are solid or metastable at room temperature (25°C). Minerals are normally crystalline compounds with a reasonably described chemical composition and a particular crystal structure. Some of the minerals are Ca, Na, Fe, Zn, K, Mg discovered in a healthful herbal diet. The frame wishes severe minerals; those are known as essential minerals. Fundamental minerals are partitioned up into extensive minerals (macro-minerals) and comply with minerals (micro-minerals). These gatherings of minerals are further extensive, but comply with minerals are required in littler sums than actual minerals.

All Nutraceutical foods have excessive power values, fats and protein portions. Fortified foods consists of Minerals like iron, calcium, sodium and zinc. A truthful ingesting habitual in most cases offers the bulk of the simple minerals. We must pick functional foods which are better in fiber. Fiber is an essential nutrient that is beneficial in weight reduction and coronary heart disease. Eating lots of functional foods is beneficial to health.

Minerals are naturally occurring inorganic elements, with a definite chemical composition and an ordered atomic structure. More than 50 chemical elements are found in the human body, which are required for growth, repair and regulation of vital body functions. Many of the essential minerals are widely distributed in foods, and most people eating a balanced diet are likely to receive adequate intakes.

The major minerals include calcium, phosphorus, sodium, potassium and magnesium. The trace elements are required in quantities of less than a few milligrams per day e.g. iron, iodine, fluoride, zinc, copper, cobalt, chromium, manganese, molybdenum, selenium, nickel, tin, silicon and vanadium. There are some trace contaminants with no known function e.g. lead, mercury, barium, boron and aluminium. The daily requirement of minerals by the human body varies from grams for sodium and potassium, through milligrams per day (e.g. iron, zinc), to micrograms per day for many of the trace elements.

Heavy Metals in Nutraceutical & Functional Food:

Heavy metals are herbal factors characterized via way of means of their as an alternative excessive atomic mass and their excessive density. Although happening in

low awareness at ppb ranges, they may be observed throughout the crust of our planet, have a density of at the leas 5g/cm³. Quantification of heavy metals entails properly established strategies together with AAS, ICP MS, ICP OES. Nutraceutical & Functional Foods are made for public intake and are broadly famous everywhere in the world, mainly with youngsters. Nutraceutical & Functional Foods are derived from plants, consequently hint steel infection of the Nutraceutical & Functional Foods itself has its foundation in the vegetable rely from which it's far produced. The expression "Overwhelming steel" alludes to any concoction element that has a usually excessive thickness and is risky or poisonous even at low fixation. The Heavy Metals incorporate; Mercury (Hg), Cadmium (Cd), Lead (Pb), Arsenic (As), Copper (Cu), Nickel (Ni) amongst others.

Contamination of foods by heavy metals has a number of different sources. The most significant ones are: contamination of the soil from which foods are produced; residual muds; chemical fertilizers and pesticides used in agriculture; the use of other materials; etc. there is a wide range of foods contaminated by heavy metals, including products of plant origin (cereals, rice, wheat, edible roots, mushroomms, etc.) as well as foods of animal origin (fish, crustaceans, mollusks).

Many high-density metals are not especially toxic. Several of them are actually essential elements for human beings, though at certain concentrations they may be toxic in some of their forms. However, there is a series of elements that, regardless of their form, could represent a major environmental problem. These are commonly referred to with the generic term “heavy metals.”

The danger of heavy metals is especially severe, because they are not chemically or biologically degradable. Once released into the environment, principally due to industrial or mining activities, they can remain for hundreds of years, polluting the soil and accumulating in plants and organic tissues. Moreover, their concentration in living beings increases as they move up the food chain.

Nutraceutical & Functional Foods:

Fortified Foods:

Food Fortification is the process of adding micronutrients (essential trace elements and vitamins) to food . It can be carried out by food manufacturers, or by governments as a public health policy which aims to reduce the number of people with dietary deficiencies within a population. The addition of micronutrients to staples and condiments can prevent large-scale deficiency diseases in most cases.

As defined by the World Health Organization (WHO) and the Food & Agricultural Organization of the United States (FAO), fortification refers to "the practice of

deliberately increasing the content of an essential micronutrient, i.e. vitamins and minerals (including trace elements) in a food, to improve the nutritional quality of the food supply and to provide a public health benefit with minimal risk to health", whereas enrichment is defined as "synonymous with fortification and refers to the addition of micronutrients to a food which are lost during processing".

Fortified Rice is an example of fortified food in which fortification of iron on rice happens. Fortified rice is made as per the standards fixed by the Food Safety and Standards Authority of India (FSSAI) **.** It has prescribed blending rice with three micronutrients - Iron, Folic Acid and Vitamin B12.

Soya Protein:

Soy protein is generally regarded as the storage protein held in discrete particles called protein bodies which are estimated to contain at least 60–70% of the total soybean protein. In finely ground meat products soy protein gels to form a matrix entrapping moisture and lipid droplets resulting in improved emulsion stability (Mittal and Usborne, 1985).

Soy proteins are applied in a wide range of food products, therefore improved functionality and sensory characteristics are always needed.

Protein Powder is a popular nutritional supplement. Protein is an essential macronutrient that helps build muscle, repair tissue, and make enzymes and hormones. Using protein powder may also aid weight loss and help people tone their muscles.

Micro nutrients Peanut Paste:

Peanuts or “groundnuts” as they are known in some parts of the world are the edible seeds of a legume. India is second largest producer of peanuts in world, with total production of approximately 7.131 million metric tons per year (USDA, PS&D database 1996–2000). Peanut (*Arachis hypogaea*) is technically considered as pea and belongs to the family (*fabaceae*) of bean/legume. Although a legume; it is generally included amongst the oilseeds due to its high oil content. Peanuts are rich in protein, oil and fibers (Suchoszek-Lukaniuk et al. 2011). Apart from oil, peanuts are widely used for production of peanut butter, confections , roasted peanuts, snack products, extenders in meat product formulation, soups and desserts.

Protein, fats, and fiber are the major components that make up peanuts. All these components are present in their most beneficial forms. The protein is plant-based: the fat is unsaturated, and the fiber is complex carbohydrate which are all proved to be the best for human nutrition.

Poshan Shakti is a micro nutrient peanut paste is a smooth, homogeneous and thick peanut paste which contains 500 kcal of total energy providing women and children in need all the nutrition requirements for a healthy life.

Nutritional supplement:

Nutritional supplements are products that are added to a regular diet in order to meet an individual's dietary requirements. These requirements are based on age, gender, level of physical activity, etc. And because these factors are different for each person, supplementation can be implemented in a variety of different ways in order to provide the necessary macronutrients (carbohydratess, proteins, and fats), fiber, vitamins, minerals, etc.

Multivitamin or multimineral tablets are used to provide vitamins that are not taken in through the diet. Multivitamins are also used to treat vitamin deficiencies (lack of vitamins) caused by illness, pregnancy, poor nutrition, digestive disorders, and many other conditions.

OBJECTIVE

1. **To determine the concetration of minerals and heavy metals in different nutraceutcal food products.**
2. **To determine concentration of minerals & heavy metals by Atomic Absorption Spectroscopy & Inductively Coupled Plasma- Mass Spectrometery.**
3. **To determine whether it is safe for human consumption or not.**

REVIEW OF LITRATURE

Megan Lofaso et. al. in 2021, did an experiment driven by a demand for health and wellness products worldwide, the dietary supplement industry continues to expand with an economic impact >$100 billion in the USA alone. However, the industry is plagued by a lack of regulation and incidents of contamination, including with toxic heavy metals that can put consumers at potential risk. In this study, eight trace elements (Cd, Pb, Fe, Co, Mn, V, Cu, and Cr), including heavy metals (Cd and Pb), were determined in whey and vegan protein powder by inductively coupled plasma mass spectrometry (ICP-MS) after microwave-assisted digestion using nitric acid and hydrogen peroxide. They run the samples in triplicate along with blanks and a reference material. Mean concentrations (µg/g ± 1SD) in the vegan protein powder were Fe (133±1) > Cu (1.4±5.4) > Cr (0.226±0.0414) > Pb (0.038±0.013) > Cd (0.033±0.003). This was higher than the whey protein powder: Fe (11.5±4.3) > Cu (1.91±2.2) > Cr (0.0491±0.0505) > Pb (0.017±0.005) > Cd (0.010±0.001). These levels correspond to amounts per serving that were below the US FDA recommended daily allowance for both the whey and vegan protein powders. However, the vegan protein powder had concentrations of Mn and Fe that could exceed the FDA criteria and be a risk to the consumer if they ingest more than the recommended daily serving (which is common for body builders) or attain these metals from other dietary sources. They intended analyze additional samples to confirm this finding and to determine how widespread the issue is, but unfortunately, the ICP-MS became inoperable and is in need of repair. Instead, on suggestion from the Honor's College, He conducted a deep literature review on the subject of heavy metal contamination in the supplement industry and current guidelines. Herein he also provide his overview and recommendations on this subject. Their purpose of this research was to determine the concentration of trace metals in protein powders bought from a local store and to assess the risk associated with consumption of potentially toxic levels of metals. Since the supplement industry is not heavily regulated, trace amounts of toxic metals are in the supplements, which can lead to harmful side effects. In addition, the performed an extensive literature search to assess the current state of the industry, with emphasis on heavy metal contamination.

David Romero et. al in 2019, did an experiment that allows them to observe the environmental pollution that allows heavy metals to interact with ecosystems, bioaccumulating and passing through the food chain. Animals and humans can consume contaminated species and reach toxic and harmful concentrations in their organisms. While there are international regulatory frameworks for heavy metal contents, these are not always known or suitable for local conditions. This situation calls

for the development of locally-applicable analytical methods for the determination of heavy metal concentrations in common vegetal and animal food products. Two established methods (AOAC 999.11, based on sample drying and calcination, and IPN AC-06-00, based on microwave-assisted acid digestion) were comparatively tested at the CESAQ-PUCE laboratory in Quito, Ecuador, to determine their suitability. Sample matrices used were non-industrial, non-organic tomato, lettuce, and beef commonly found in local markets. Heavy metals tested were cadmium, nickel, and lead. Test guidelines and comparative parameters were based on AOAC (2002) and included quantification limits, repeatability variation coefficients, intermediate precision percentages, accuracy and calculated expanded uncertainties. Unlike method AOAC 999.11, method IPN AC-06-00 performance for all parameters was within the range of recommended expected values as per AOAC, and was therefore deemed more suitable to be applied under the local CESAQ-PUCE laboratory conditions. The validation of method IPN AC-06-00 demonstrated its local applicability. In addition, IPN AC-06-00 can be used by similar laboratories to assess contaminants concentrations and improve the baseline information concerning human exposure to toxic metals.

Louise A. Berner et. al. in 2014 conducted an experiment on fortified foods being the major contributors to nutrient intake in diets of US children, even in an era of obesity and dietary excess, numerous shortfall micronutrients have been identified in the diets of US children and adolescents. To help tailor strategies for meeting recommendations, it was important to know what foods contribute greatly to micronutrient intakes because the data are lacking on specific contributions made by added nutrients. Their aims were to examine the impact of fortification on nutrient adequacy and excess among US children and adolescents and to rank food sources of added nutrient intake and compare rankings with those based on total nutrient intake from foods. It helps then gain knowledge about nutrient intakes and sources which then can help them put dietary advice into a practical context. Continued monitoring of top food sources of nutrients and nutrient contributions from fortification will be important.

Martinez Navarrete et. al in 2002, did an experiment on iron deficiency and iron fortified foods as iron is a mineral that is necessary for producing red blood cells and for redox processes. Iron deficiency is considered to be the commonest worldwide nutritional deficiency and affects approximately 20% of the world population. Lack of iron may lead to unusual tiredness, shortness of breath, a decrease in physical performance, and learning problems in children and adults, and may increase your chance of getting an infection. This deficiency is partly induced by plant-based diets, containing low levels of poorly bio-available iron. The most effective technological approaches to combat iron deficiency in developing countries include supplementation targeted to high risk groups combined with a program of food fortification and dietary strategies designed to maximize the bio-availability of both the added and the intrinsic food iron. In this paper,

different aspects related to iron-fortified foods is reviewed. These include used iron compounds, considering its bioavailability and organoleptic problems, food vehicles and possible interactions.

Thomas Walter et al in 1993, did the experiment on effectiveness of iron fortified infant cerreal in prevention of iron iron deficiency anemia as iron deficiency continues to be a common problem among infants throughout the world. Iron-fortified formula is effective in preventing iron deficiency but the benefit of iron-fortified cereal is controversial. They compared iron-fortified rice cereal to unfortified rice cereal in infants who were exclusively breast-fed for more than 4 months and to iron-fortified formula in infants who were weaned to formula before 4 months of age. The design was double blind in respect to the presence or absence of fortification iron in the cereal or formula and included 515 infants who were followed on the protocol from 4 to 15 months of age. Rice cereal was fortified with 55 mg of electrolytic iron per 100 g of dry cereal and infant formula with 12 mg of ferrous sulfate per 100 g of dry powder, levels approximating those in us e in the United States. Measures of iron status were obtained at 8, 12, and 15 months. Infants with hemoglobin levels of <105 g/L were excluded from the study and treated. In the end the concluded that iron-fortified infant rice cereal can contribute substantially to preventing iron deficiency anemia.

Ning Xing et. al. did an experiment regarding deficiency of iron as iron deficiency is a common nutritional disorder worldwide. Iron fortification of food is an effective strategy to control iron deficiency anemia (IDA), however, traditional iron fortificants usually provoke undesirable organoleptic changes or have limited colloid stability. In this research, they investigated iron reducibility of soy protein amyloid fibrils made from soy protein isolates (SPI), soy β-conglycinin (7S) and soy glycinin (11S), and explored their applications in iron fortification. All three protein fibrils showed iron reducibility. The reducibility was utilized to generate fibril-iron nanoparticle composites. The iron reducibility was affected by fibril concentration, degree of fibrillation and reducing amino acid composition. They identified 11S had the most significant effect on reducing Fe (III) to more bioavailable Fe (II) state, whereas 7S showed the optimal result for generation of iron nanoparticle on fibrils *in situ*. The resulted fibril-iron nanoparticle hybrids showed high dispersibility in various liquid foods, without distinct color change.

Fernando Gil et. al. in 2021 did an experiment on toxic elements of nutraceuticals foods as the observe an increasing growth in popularity of over-the-counter health foods, nutraceuticals, and medicinal products from plants or other natural sources has been observed in developed countries with the belief that they could be more effective than conventional treatments. Nutraceuticals and food ingredients (NFI) from herbal products may be contaminated with pesticides, heavy metals and metalloids, mycotoxins, and radioactivity, and they may also be adulterated with drugs. This toxic contamination, as

well as any other that can be produced during any stage of production, may change their quality and safety. The population and healthcare professionals should be better informed regarding the concept and usefulness of nutraceuticals and also regarding the potential adverse effects associated with their possible contamination. They conducted the experiment to prevent and screen for contamination, and to ensure safety and conformity to quality standards, NFI should be included in an appropriate regulatory framework.

K Bu el. al. in 2013, conducted an experiment in which they determine concentrations of twelve elements (Mg, Al, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd) in six herbal supplements, Korean Panax Ginseng (*Panax ginseng*), Golden Seal (*Hydrastis canadensis*), Ginger Root (*Zingiber officinale*), St. John's Wort (*Hypericum perforatum*), Green Tea (*Camellia sinensis*) and Valerian Root (*Valeriana officinalis*), by both laser ablation-inductively coupled plasma mass spectrometry (LA-ICPMS) and conventional closed-vessel digestion solution nebulization-ICPMS (SN-ICPMS). For LA-ICPMS, powder from supplement capsules and leaf reference materials were pressed into pellets, the later being used for calibration and quality assurance.They performed laser ablation using line scans with a scan rate of 30 μm min^{-1}, a frequency of 20 Hz and a spot size of 100 μm; ^{13}C served as the internal standard. For LA it was found that low resolution ($m/\Delta m \approx 400$) yielded good recoveries for the reference materials and results comparable to SN-ICPMS for most elements, except for Ca, which was better determined in medium resolution ($m/\Delta m \approx 400$ 0). Overall, this study shows that LA-ICPMS can serve as an alternative way for determining the concentration of elements in herbal supplements in a rapid and pragmatic fashion.

Rodrigo Mendas Pereira el.al. did an microwave-assisted digestion using diluted acid under oxygen pressure is proposed for sample preparation of sports supplements and subsequent determination of Ca, Cu, Fe, K, Mg, Mn, Na, Zn, P and S by inductively coupled plasma-optical emission spectrometry (ICP-OES). In this sense, 1350 mg of sport supplement were digested in a microwave oven using only 6 mL of 5 mol L^{-1} HNO_3 under 5 bar of oxygen pressure during 15 min. They evaluated an accuracy of proposed method by recovery tests using a standard solution and using a certified reference material. Suitable recoveries (ranging from 86% to 108%) were obtained in both evaluations for all analytes. Results were compared with those obtained by conventional microwave-assisted digestion (without oxygen pressure) for further analytes determination by ICP-OES, and no statistical differences were observed (Student's *t*-test, confidence level of 95%, $p > 0.05$). The precision of proposed method was evaluated in terms of repeatability (RSDs ≤3%) and intermediate precision (RSDs ≤7%), and suitable results were obtained for all analytes. In addition, the limits of detection for all analytes using proposed method were lower than those obtained using conventional digestion in view of use a high sample mass and a diluted acid solution for

sample digestion. The use of diluted acid reduces risks to analyst – since it eliminates the concentrated acid handling; reduces the consumption of reagents and, consequently, the waste generation, in accordance with the principles of green chemistry. Using the proposed method was possible for them to perform the determination of ten elements in a single analysis with suitable precision and accuracy. Six sport supplements were analyzed and the results are alarming because in most cases they were in disagreement with those informed on the label. Thus, a suitable quality control of sports supplements should be performed and the proposed method is a green, safe, accurate, precise and sensitive alternative for this purpose.

Marek Biziuk et. al did an experiment regarding the problem of the presence of mineral components in food is complicated. Anthropogenic pollution of drinking water supplies and resources indispensable for food production has become a fact of life. The main route of introduction of metals to the human organism is through ingestion of food and drinking water, but the inhalation route can sometimes be significant. There is a need for continuous monitoring of the degree of pollution of food and potable and surface waters by inorganic (and organic) compounds from anthropogenic sources. The sampling and analysis of food should be carried out according to the standards published by the National Standard Committee, the International Standard Organization or the European Committee of Standardization. They determind concentration of mercury in food products using mercury specific atomic fluorescence spectrometry after mineralization of the samples and with or without pre concentration of mercury on a gold trap.

A Filipiak et. al. in 2015 did an experiment on the potentially toxic metals content was determined in selected plants, used in Traditional Chinese Medicine (*Angelica sinensis*, *Bacopa monnieri*, *Bupleurum sinensis*, *Curcuma longa*, *Cola accuminata*, *Emblica officinalis*, *Garcinia cambogia*, *Mucuna pruriens*, *Ocimum sanctum*, *Panax ginseng*, *Pueraria lobata*, *Salvia miltiorrhiza*, *Schisandra sinensis*, *Scutellaria baicalensis*, *Siraitia grosvenorii*, *Terminalia arjuna* and *Terminalia chebula*), and some European herbs (*Echinacea purpurea*, *Hypericum perforatum*, *Vitis vinifera)*. They mineralized the samples in a closed microwave system using HNO_3 and the concentrations of Cd, Pb, Al, As, Ba, Ni and Sb were determined by ICP-MS method. Some relevant aspects of potential toxicity of metallic elements and their compounds were also discussed. Results of metal content analysis in dietary supplements available on Polish market, containing studied plants, are presented as well. The results were analyzed by principal component analysis (PCA) and cluster analysis.

TECHNIQUES AND INSTRUMENT USE

Sampling:

The object of this step is to obtain a small and representative portion from the large sample in such a way that any subsequent test on the sample will give a reproducible value. For fresh foods, the homogenization process is like macerating in a blender whereas dry products are normally ground mechanically and then mixed and the powder is sieved before analysis. Contamination during this step can be avoided with the use of stainless steel equipment. Hard foods, such as, chocolates are sampled by grating/chopping finely by ANALYSIS OF METALS 2015 3 hand. Meat and meat products are thoroughly minced and then ground in a mortar and in this case, too small quantities should not be taken for analysis. Fats are melted before analysis. Wet foods such as pickles, etc should be homogenized in high-speed blender. Liquids are normally sampled after they have been thoroughly mixed by repeated slow inversion of container. After the sample is properly homogenized and reduced to usable form, it should be stored in an air tight container. If the sample received for analysis is too large, it has to be reduced to a more convenient size (for homogenization purpose) by repeated quartering in which the sample is arranged in a flat heap, opposite two quarters are rejected and remaining two quarters are mixed and again subjected to quartering. This process is continued till a convenient quantity of sample remains for homogenization by grinding etc. The edible portion of the sample of food has to be taken for preparation of sample for analysis. E.g. fish, etc.

Importance of Homogenization: Sample which is to be investigated must be homogenous, so a uniform measure of test can be taken for examination. It enables blender to test well. Homogenization expands reproducibility and precision of result. A homogenized sample is equal in composition throughout, so that removing a fraction does not alter the overall molecular make-up of the sample remaining, and is identical to the fraction removed. Consequently samples were crushed in a spotless and dry processor and put away in a crisp ziplock pack. Rest classes of the test were at that point founded in powder structure.

Labeling of samples-Each sample container after filling shall be sealed and marked with full details of sampling, the number of packages sampled, the date of sampling, and other particulars of the consignment. Laboratory samples shall be packed in rigid airtight and moisture-tight containers fitted with airtight and moisture-tight closures. The containers shall be completely filled and the closures shall be sealed to avoid any change in the original sample.

Storage-Laboratory samples which are not analyzed immediately should be stored under conditions that minimize decay. Fresh products should be stored in the refrigerator, but typically no longer than 5 days. Dried products may be stored at room temperature, but if storage time is expected to exceed two weeks, they should be sub-sampled and stored in the freezer.

SAMPLE PREPRATION:

DIGESTION (closed) is a simple sample preparation technique suitable for determination of metal contaminants. This method is a streamlined approach that makes it easier and less expensive for analytical chemists to examine metal contaminants in food. Once the samples are digested they will be further processed for analysis on AAS & ICP-MS.

MICROWAVE DIGESTER :

Microwave-assisted sample preparation techniques are becoming widely used in analytical laboratories all over the world. Microwave radiation can greatly speed up the extraction and the so-called microwave assisted extraction (MAE) is thus established.
In principle, only samples or solvents containing dipolar materials or microwave absorbents can be affected by microwaves which heat the extraction body from inside to outside in a very short time, much different from the common heating methods**.** MAE can be conducted with an open or closed microwave system. A closed-vessel offers a special way to regulate the extracting temperature by simply adjusting the vessel pressure. The main advantage of MAE lies in its wide applicability for fast extractions of analytes including some thermally unstable substances. The closed digestion technique involves placing the sample in a vial (or vessel), usually constructed of a fluorinated polymer, such as polytetrafluoroethylene (PTFE) or teflon. After adding the digestion reagents, the vessel is tightly sealed and placed in the microwave oven for irradiation by microwave energy.

Fig 1: Microwave Digester

PROCEDURE:

- 0.5-1.0 gm of test was gauged (in view of test nature) into the HVT (High Venting Technology) Vessel.
- 5 ml nitric corrosive was included in the vessel.
- 2ml of hydrogen peroxide (proportion of HNO_3 and H_2O_2 change from framework to lattice and as indicated by test weight was included a similar vessel.

Fig 2: Taflon Vessels

- Vessels were left for 10 minutes (so as to perform self assimilation by corrosive).
- The tops were shut appropriately and the vessel was kept in the digester in a Multiwave 3000 ECO Anton Paar machine.
- After assimilation volume cosmetics of the test were finished with Milli Q water and later examples were sifted whenever required.

ATOMIC ABSORBTION SPECTROSCOPY:

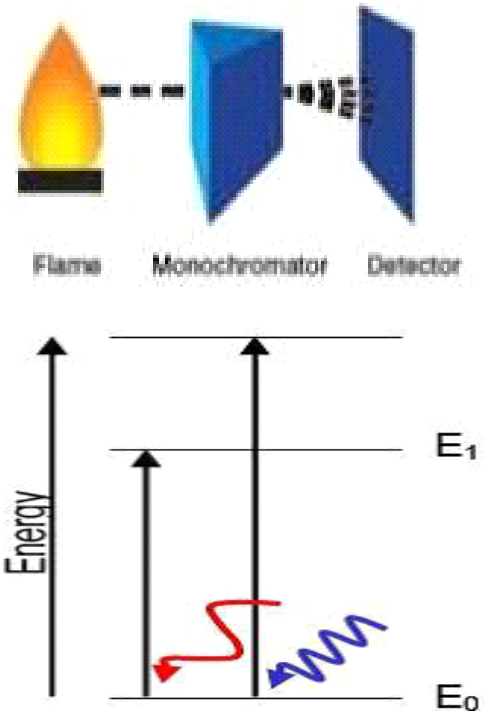
PRINCIPLE OF AAS:

This technique uses basically the principle of Beer lambert's law, that “The amount of light absorbed by an element in a sample is directly proportional to the concentration of that element in the sample” or free atoms (gas) generated in an atomizer can absorb radiation at specific frequency. Atomic absorption spectroscopy quantifies the absorption of ground state atoms in the gaseous state. The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels. The analyte concentration is determined from the amount of absorption. Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration. Calibration curve is used to plot the relationship b/w absorbance and the concentration.

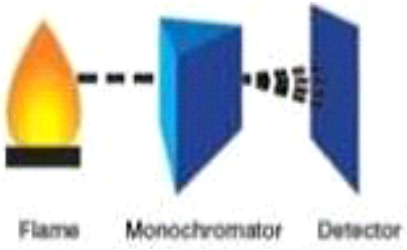
Fig 3: AAS instrument in the laboratory

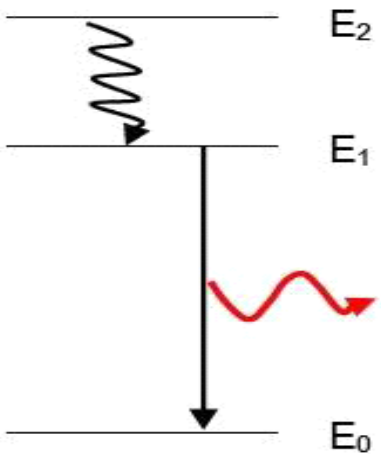
Phenomenon of AAS:

- Absorption
- Emission
- Flouresence



Atomic absorption



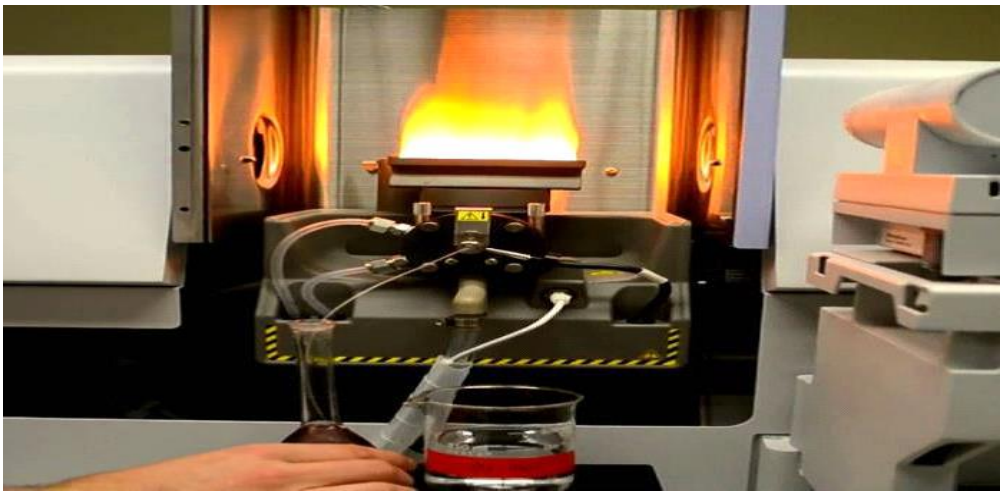


Atomic emission

The components of AAS are:

- Sample insertion system
    1. Nebulizer
    2. Spray chamber

- Atomizer (Flame atomizer)

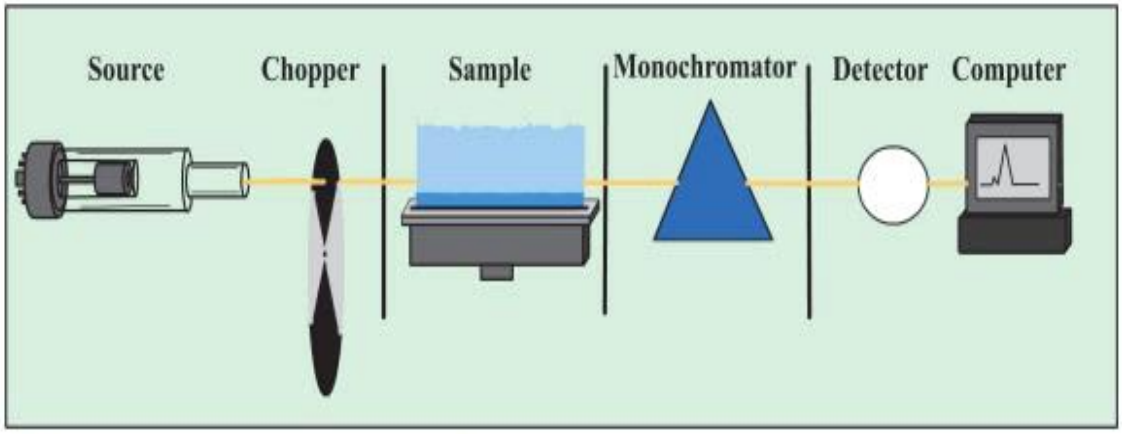


- Radiation source (Hollow cathode lamps)

- Monochromator (Prism)

- Detector
    - **Gases used: Acetylene+ air at 2600K**

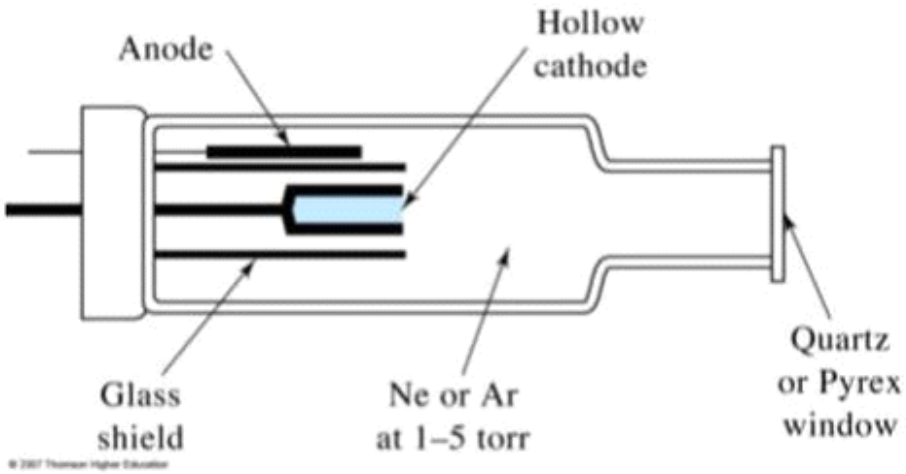
In which acetylene is used as a fuel and air as oxidant.



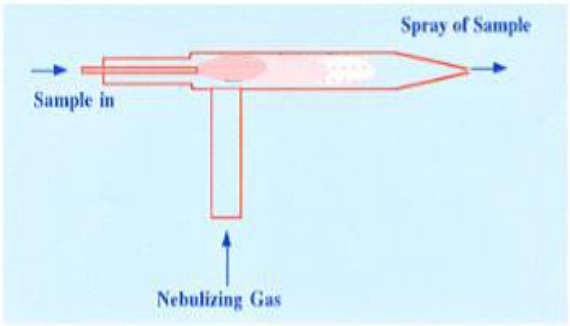
Basic operation:

Radiation or light source Hollow cathode lamp: Hollow cathode lamps are the most common radiation light sources in AAS. It contains a tungsten anode and a hollow cylindrical cathode made of the element to be determined. These are sealed in a glass tube filled with an inert gas (neon or argon) at low pressure. The glass chamber has a quartz or UV glass window for ideal transmittance of the produced radiation. A red gleam is seen in lights loaded up with neon, while argon filled lights have a blue sparkle. Each element has its own unique lamp which must be used for that analysis. A high voltage is applied across the anode and cathode, resulting in an ionization of the fill gas. The gas ions are accelerated towards the cathode and, upon impact on the cathode, sputter cathode material that is excited in the glow discharge to emit the radiation of the sputtered material. In the majority of cases single element lamps are used. Multi element lamps are available with combinations of compounds of the target elements pressed in the cathode. Multi element lamps produce slightly less sensitivity than single element lamps and the combinations of elements have to be selected carefully to avoid spectral interferences.

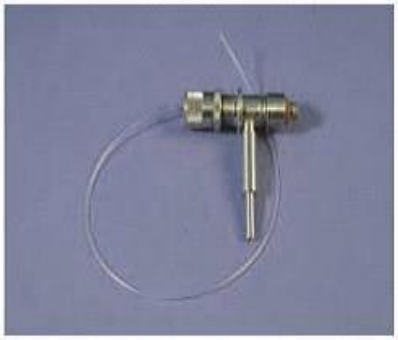
Ionized atoms hit cathode and form a cloud this process takes place in hollow cathode lamps is known as **sputtering.**



Nebulizer: Nebulizer refers to an apparatus that converts liquids into a fine mist. Nebulizers generally use gas flows to deliver the mist. Analytical nebulizers are a special category in that their purpose is to deliver a fine mist to spectrometric instruments for elemental analysis. They are necessary parts of ICP MS and AAS. When gas at higher pressure exits from a small hole (orifice) into gas at a lower pressure, it forms a gas jet into the lower pressure zone, and pushes the lower pressure gas away from the orifice. This creates a current in the lower pressure gas zone, and draws some of the lower pressure gas into the higher pressure gas jet. At the orifice, the draw of the lower pressure gas creates considerable suction .In all pneumatic induction nebulizers, the suction near the orifice is utilized to draw the liquid into the gas jet. The liquid is broken into small droplets in the process and gets introduced into the flame.

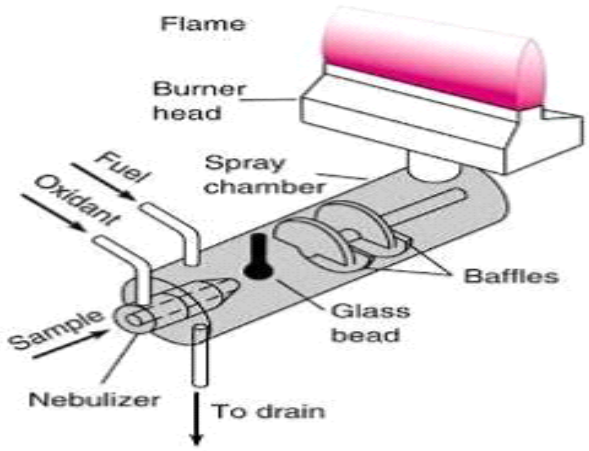


AAS Nebulizer Schematic


AAS Nebulizer

Burner heads: There are four burner sets out accessible toward use with the double choice burner framework. They are altogether made of strong titanium which is erosion

safe and free of the majority of the components regularly dictated by nuclear retention. The 10 cm burner head is intended to be utilized with the air-acetylene fire. Due to its long burner wavelength, it gives the best affectability to air-acetylene components. The 5 cm nitrous oxide burner head is required for nitrous oxide acetylene tasks. On numerous spectrometer models, it can likewise be utilized with air-acetylene or air-hydrogen. The three-space burner head is intended to be utilized when examining tests with high convergences of disintegrated solids. The three-opening burner head isn't good with all gas control frameworks. A 5 cm air-acetylene burner head is accessible for applications in which diminished affectability is required. On numerous spectrometer models, it very well may be pivoted 90° to give diminished affectability, and it has a wide opening to forestall stopping up. This burner head can be utilized distinctly for air-acetylene tasks.



Details of burner head

Atomizers: The atomizers most commonly used nowadays are flames (spectroscopic), electro thermal (graphite tube) atomizers. Other atomizers such as glow discharge atomization, hydride atomization or cold vapor atomization, might be used for special purposes.

Flame: The oldest and most commonly used atomizers in AAS are flame, principally the air-acetylene flame with a temperature of about 2300°C and the nitrous oxide (N_2O)-acetylene system flame with a temperature of about 2700°C. Liquid or dissolved samples are typically used with flame atomizers.

The sample solution is aspirated by a pneumatic analytical nebulizer, transformed into an aerosol, which is introduced into a spray chamber, where it is mixed with the flame gases and conditioned in a way that only the finest aerosol droplets (<10μm) enter the flame. On the top of the spray chamber is a burner head that produces a flame that is laterally long and a few mm deep. The radiation beam passes through this flame at its longest axis, and the flame gas flow-rates may be adjusted to produce the highest concentration of free atoms. The burner height may also be adjusted, so that the radiation beam passes through the zone of highest atom cloud density in the flame, resulting in the highest sensitivity. The process in flame include the stages of:

● Desolvation(drying): In this the solvent is evaporated and the dry sample nano-particles remain.

● Vaporization: In this the solid particles are converted into gaseous molecules.

● Atomization: In which the molecules are dissociated into free atoms.

● Ionization: Where may be in part converted to gaseous ions (depending upon the ionization potential of the analyte atoms and the energy available in a particular flame).

Monochromator (Prism) –the tarnsmitted light falls on prism. Monochromator is used to select the specific wavelenghts which is absorbed by the sample and exclude the other wavelenghts.

INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETRY:

Principle:

ICP is an analytical technique used to measure and identify elements within a sample matrix based on the ionization of the elements withing the sample. Mass Spectrometer separates the ions out by their mass-to-charge ratio after going through the ICP, and the detector counts the number of selected ions per second which allows the instrument to determine the concentration of each chosen element.

Fig 4: ICP-MS in the Laboratory

Mass spectrometer is an instrument in which ions are analyzed according to their mass to-charge ratio, and in which the number of ions is determined electrically. For the most part, there are four basic components that are standard in all mass spectrometers.These are i) sample inlet ii) ionization source iii) mass analyzer and iv) ion detector. Although there are many variations of mass spectrometers the process by which all sample molecules are analyzed is similar regardless of instrument configuration. Sample molecules are introduced into the instrument through a sample inlet. Once inside the instrument, the sample molecules are converted to ions in the ionization source, before being electrostatically propelled into the mass analyzer. Ions are then separated according to their m/z within the mass analyzer. The detector converts the ion energy into electrical signals, which are then transmitted to a computer.

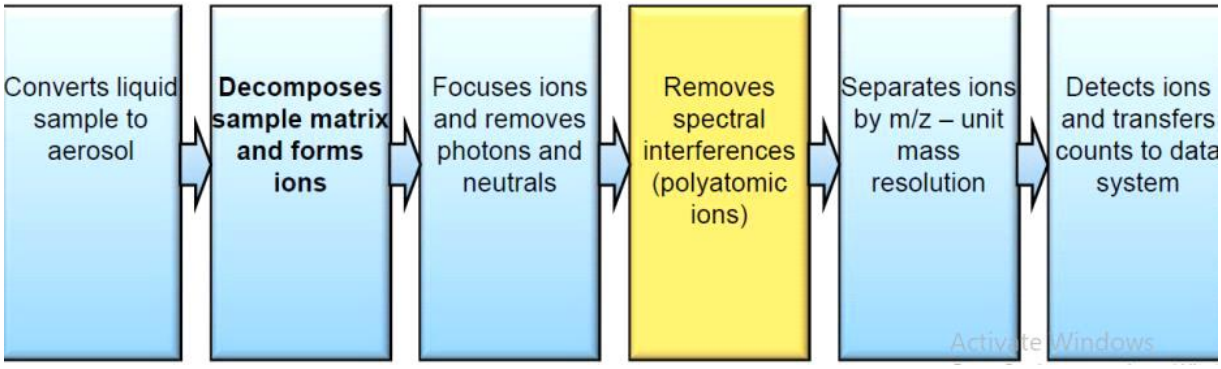
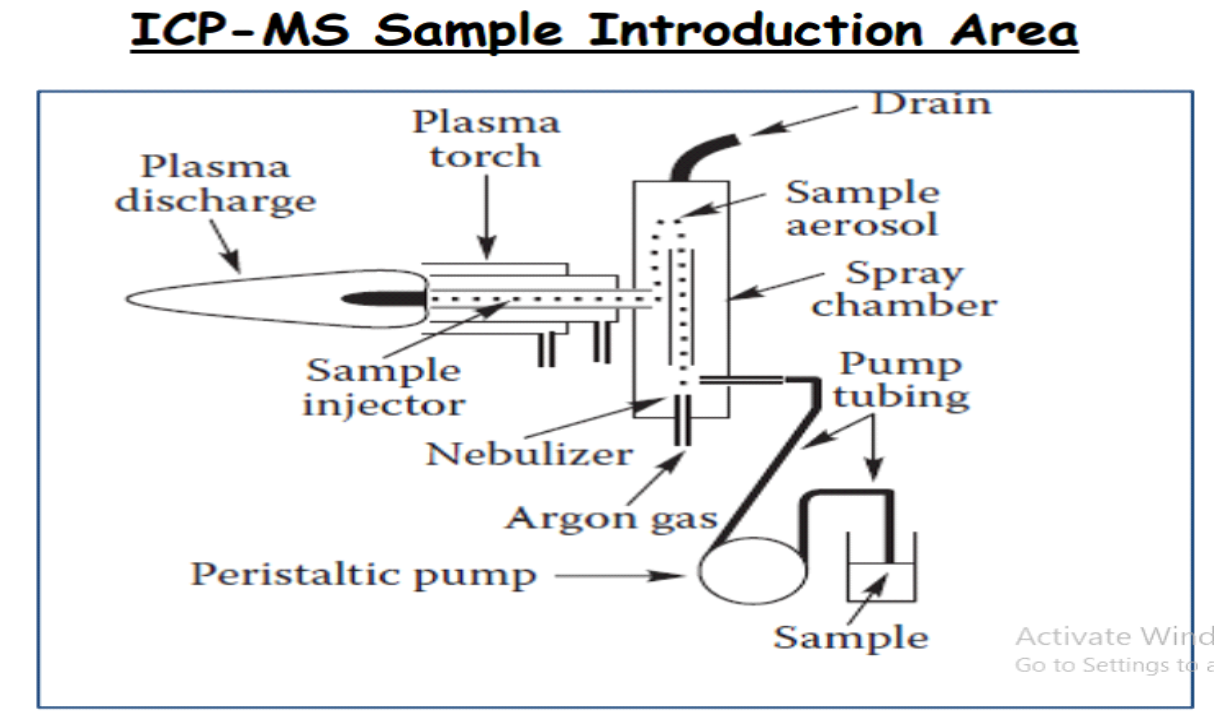


Fig 5: Basic Instrumental Components of ICP-MS



Components of ICP-MS:

- Sample introduction system

- ICP Torch
- ICP interface region
- ICP Ion Focussing Region
- Collision/ reaction cell
- Quadrupole
- Detector
    - Gas used – argon
    - **Sample introduction system** consist of Peristaltic Pump: Ensures constant flow of liquid.
    - Nebulizer: Liquid is converted into a fine aerosol.
    - Spray Chamber: Spray Chamber only allows small droplets to enter the plasma.
    - **ICP Torch** :The plasma generated in the ICP torch creates a very hot zone temperature of approximately 6000 °C, The plasma is generated by passing argon through a series of concentric quartz tubes (the ICP torch) that are wrapped at one end by a radio frequency (RF) coil. Energy supplied to the coil by the RF generator couples with the argon to produce the plasma.
    - **ICP interface region** –Function is to export the ions produced in argon plasma and transport them to the mass spectrometer.
    - Two metallic (Ni or Pt) cones with small orifices- Sampler and Skimmer Cones directs the expanded gas jet of ions into the MS.
    - **ICP Ion Focussing Region**: Role of ion focussing is to transport maximum number of analyte ions from the hostile environment of plasma to the MS via the interface.

- **Collision Reaction Cell** -The CRC devices in commercial ICP-MS instruments have been designed to remove polyatomic species.

- **Quadrupole** -The quadrupole mass analyzer filters out non-analyte, matrix, and interfering ions, allowing only desired analyte ions of a single mass-to-charge ratio (m/z) to be transmitted to the detector. It has four electrically charged rods through which Rf and DC voltages will be applied to direct the analyte having similar mass to charge ratio.

- Detector - Detector is an Electron Multiplier Device which can generate a measurable signal pulse from the impact of a single ion.

CRM (CERTIFIED REFERENCE MATERIAL) :

ISO 17025 defines a reference material as material that is “sufficiently homogenous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process. Properties can be quantitative or qualitative (e.g., identity of substances or species).” A CRM is different than a reference material in that it is accompanied by a certificate, has metrological traceability, and each property value has an uncertainty value assigned to it. Once property values are assigned to a CRM, the values are “stored” by the CRM and then transferred when the CRM itself is transported from one location to another.

CRM are used for standard preparation.

Fig 6: Certified Reference Material

Preparation of Standards :

- Choose a clean volumetric glass of appropriate capacity for reference standard stock solution preparation.
- Ensure the micropipette & Volumetric glass is clean and calibrated.
- Take the reference standard and note down description (e.g. Purity and expiry date).
- Pipette the standard (5 ml) in 50 ml volumetric glass from 1000 ppm standard and make up to 50 ml by 2% Nitric Acid (For 100 ppm Standard)
- Pipette the standard (5 ml) in 50 ml volumetric glass from 100 ppm standard and make up to 50 ml by 2% Nitric Acid (For 10 ppm Standard)
- Pipette the standard (5 ml) in 50 ml volumetric glass and make up to 50 ml by 2% Nitric Acid (For 1 ppm Standard).

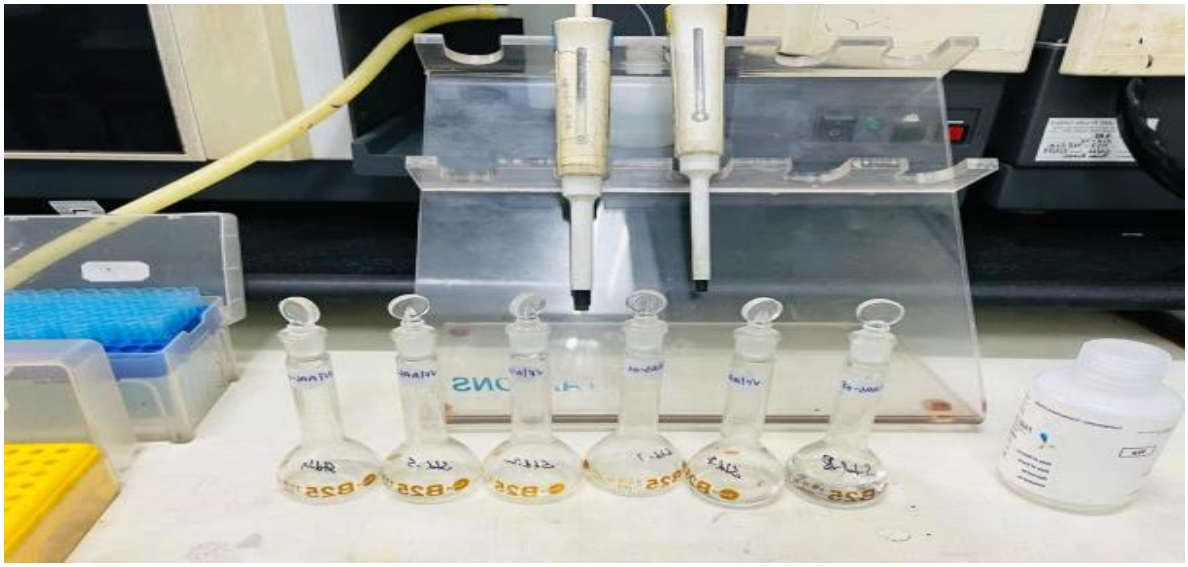


Fig 7: Standards

Calibration Standard Preparation (ICPMS) :

Calibration Level	Concentration in ppb	Vol. (µl) of tertiary Standard to be taken (From 1 ppm)	Make up vol. (ml)
Level – 1	Cal. blank	0	50
Level – 2	0.5	25	50
Level – 3	1.0	50	50
Level – 4	5.0	250	50
Level – 5	10	500	50
Level – 6	20	1000	50
Level – 7	50	2500	50
Level – 8	100	5000	50

Calibration Standard Preparation (AAS) :

Calibration Level	Concentration in ppm	Vol. (ml) of tertiary Standard to be taken	Make up vol. (ml)
Level - 1	Cal. blank	0	50
Level – 2	0.2	0.05	50
Level - 3	0.5	0.25	50
Level - 4	1.0	0.5	50
Level - 5	2.0	1.0	50
Level -6	5.0	2.5	50
Level -7	10.0	5.0	50

In ICP-MS & AAS. The extracted residues are examined by ICP-MS on the basis of mass to charge ratio (m/z) and AAS bases on atoms (and ions) can absorb light at a specific, unique wavelength. When this specific wavelength of light is provided, the energy (light) is absorbed by the atom.

Instrumental conditions:

Agilent 7700 ICP-MS Conditions for Metal Contaminants:

Equipment	ICP-MS
Make & Model	AGILENT- 7700
Software	Mass Hunter

ICP-MS CONDITIONS & Analysis –

- RF Power - 1500w
- Sample depth - 8 mm
- Carrier gas (Argon) - 0.99 L/min
- Makeup gas (Argon) – 15 L/ min
- Nebulizer Flow – 0.2 L per min.
- Peristaltic pump - 0.3 rpm
- Spray chamber - 0.02 rps
- Spray chamber temp - 2°c
- Reaction Gas (Helium) - 4.0 ml/min
- Integration time - 0.1 to 0.3 sec

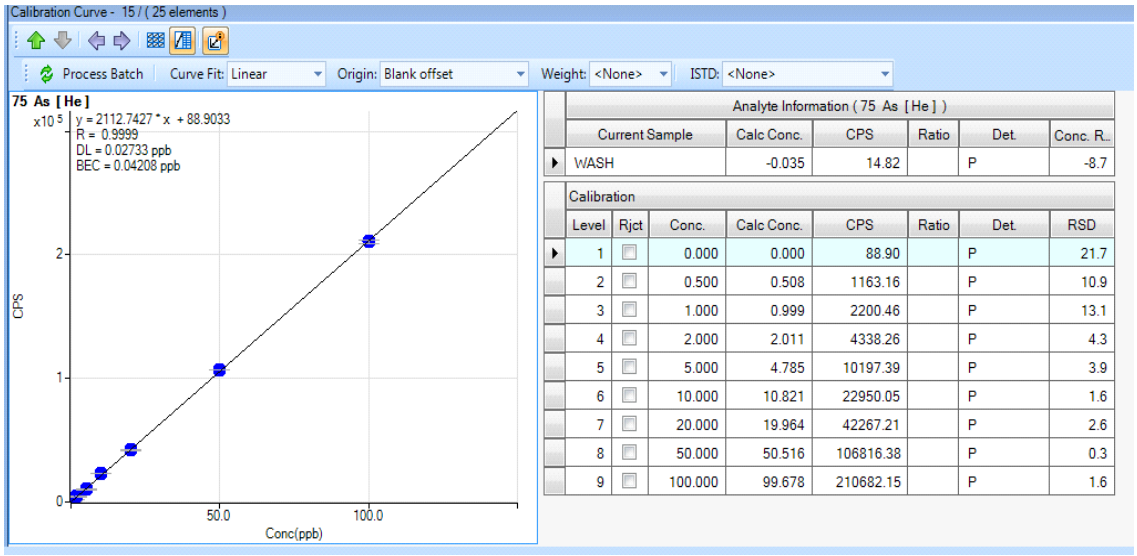


Fig 8: ICP-MS

CALIBRATION CURVE OF AAS

Sample Analysis on Instrument

Calibration curve are prepared in 2% nitric acid for at least 5 points including blank and treated in the same way like the samples.

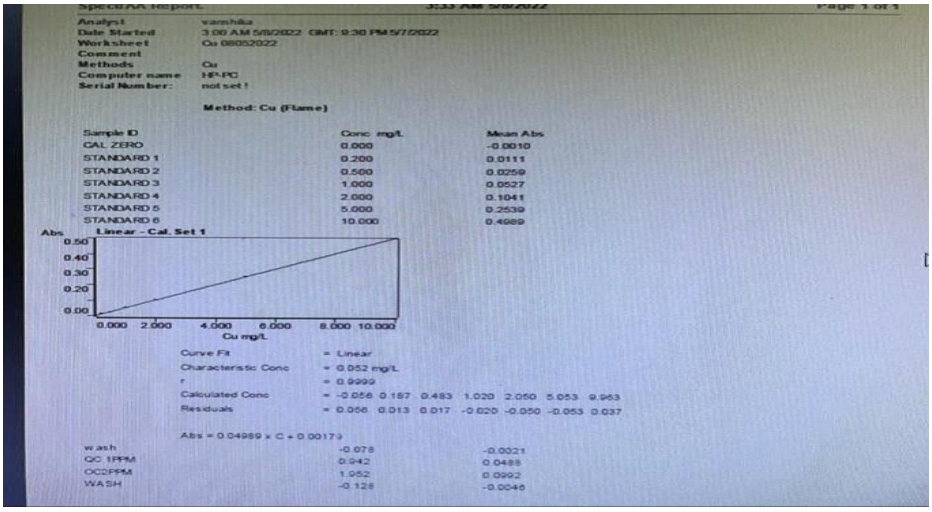


Fig 9: AAS

MATERIALS & METHODS

This section describes the experimental arrangement and procedure adopted for analysis of heavy metals and minerals in various food samples. All the nutrients were analysed by using AAS (Atomic absorption sepectroscopy) and ICP-MS (Inductively coupled plasma mass spectrometry).

SAMPLE COLLECTION: For analysis of heavy metals and minerals by AAS and ICPMS, various food samples of different categories and brands were randomly gathered from local market. The samples were:

Fig 10: Fortified Rice

Fig 11: Protein Powder

Fig 13: Poshan Shakti

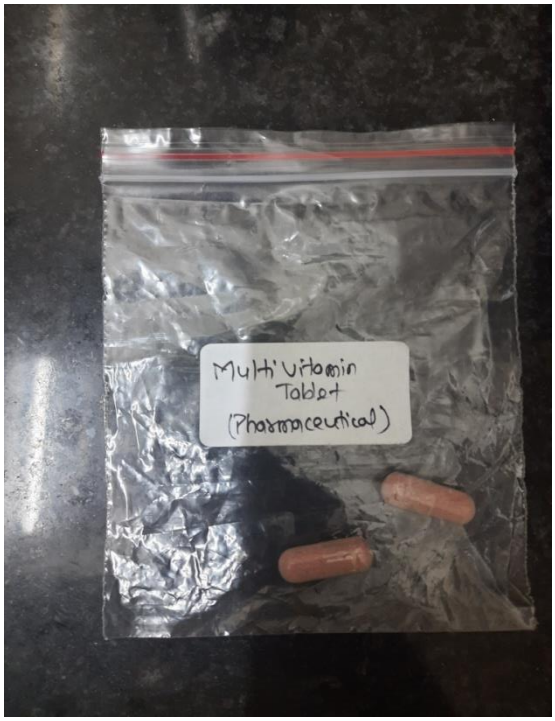


Fig 14: Multivitamin Tablets

Instrument / Equipment:

- ICP-MS & AAS
- Microwave Digester
- Volumetric Glass
- Teflon Tubes
- Butter paper
- Centrifuge: Rotor head with holding capacity: 2 mL, 10 -15 mL and 50 mL centrifuge tubes
- Weighing Balance
- Vortex shaker
- Micro pipettes

Reagents/Chemicals:

- Nitric Acid 69-71% Supra pure.
- Hydrogen Peroxide 30% Supra pure
- Whatmann Filter Paper No.-42
- MQ Water

Extraction Procedure:

- Weigh a known amount of sample in HVT-50 vessel.

Fig 15: Weighing Balance

- Add 6-8 ml conc. HNO3 and 2 ml H2O2 and leave for 10 minutes for reaction and then close the vessel tightly.

Fig 16: Nitric Acid

Fig 17: Hydrogen Peroxide Solution

- Place the vessels in the microwave digester and start the program.

Temp	Ramp	Hold	Power
170	15.00	10.00	800 w
175	15.00	10.00	800 w

- Once the digestion is completed, take out the vessels and open it in the fume hood.

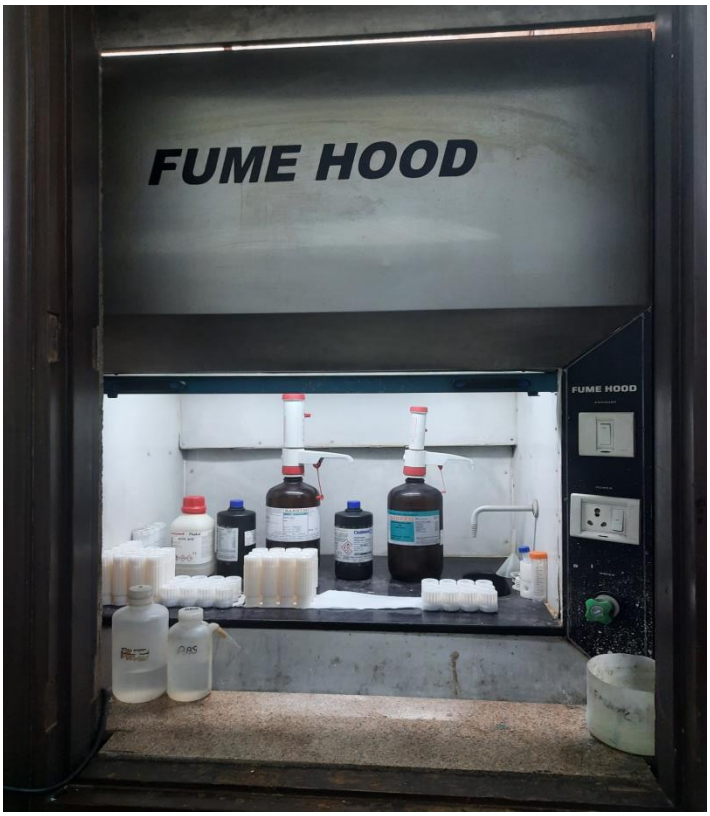


Fig 18: Fume Hood

- Make up the volume with Millipore water as per the requirement for the analysis.

Fig 19: Digested Samples for AAS

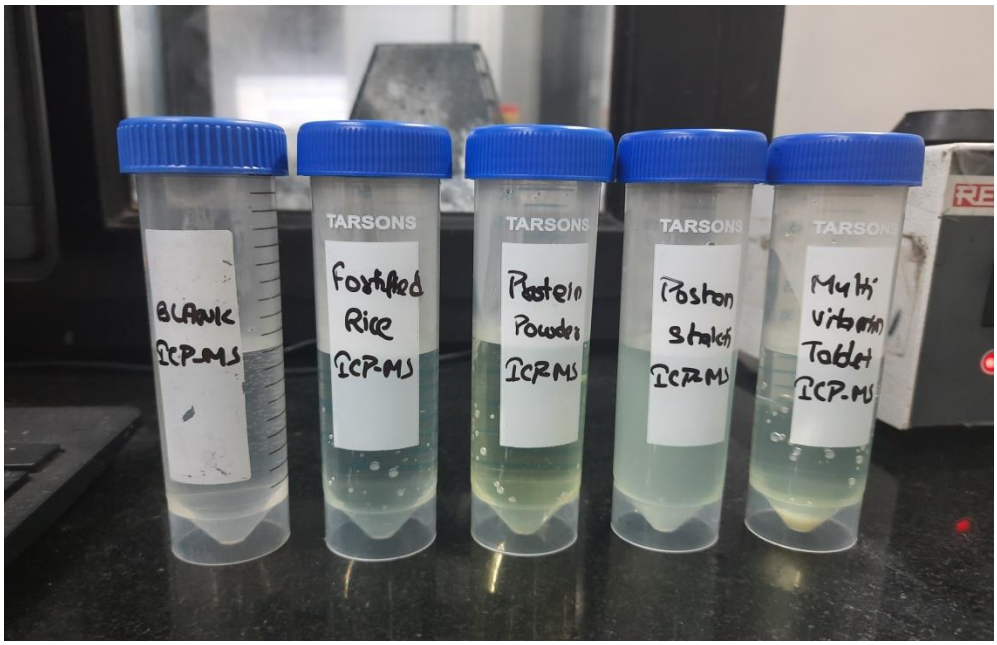


Fig 20: Digested Samples for ICP-MS

- Centrifuge at 6000 rpm at 4°C for 10 minutes Store Centrifuge tube at -20°C for 1 Hour.

- Filter the digested samples using Whatmann Filter Paper.

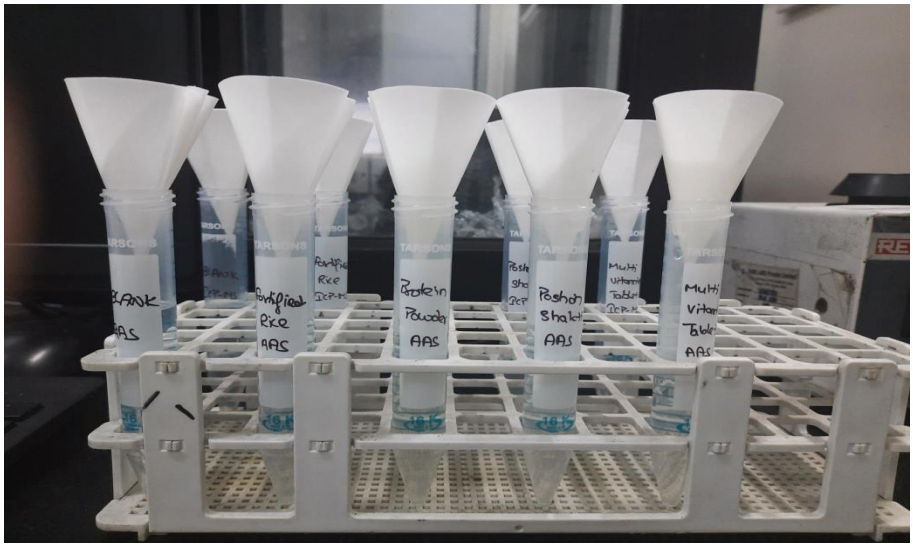


Fig 21: Filtered Samples for AAS

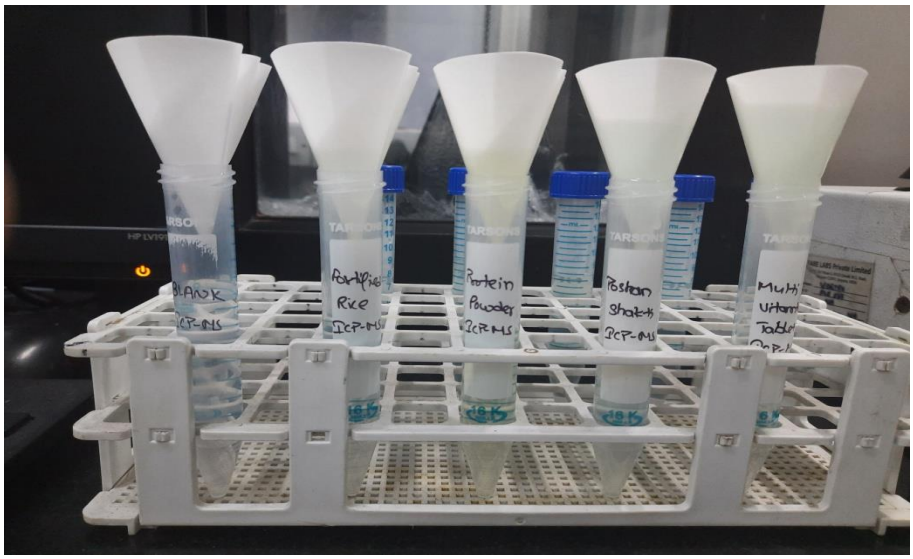


Fig 22: Filtered Samples for ICP-MS

- Go for further dilutions as required.
- Now introduce the sample in the instrument and start the instrumentation procedure.

PRECAUTIONS:

- Wear safety goggles
- Wear lab coat
- Wear gloves when necessary
- Don’t eat at your workstation
- Clean up your workplace
- Weighing balance should be calibrated

RESULTS

This section deals with the results obtained from experimental analysis of various samples by AAS as well as ICP-MS.

Minerals such as Sodium, Iron, Zinc and heavy metals such as Lead, Copper, Nickel analyses have been performed on 4 types of nutraceutical food samples.

The result are mentioned in the table below:

RESULT CALCULATION:

$$\textbf{Concentration(ppm)} = \frac{\textbf{(conc. of sample - conc. of blank)} * \textbf{Volume} * \textbf{Dilution.}}{\textbf{(weight of sample)}}$$

- **Concentration of Iron:**

SAMPLE ID	ELEMENT	WEIGHT	CONC.	BLK CONC.	Makeup vol.	Final result (mg/kg)	Final result (mg/100gm)
Fortified Rice	Fe	0.2563	32.73	1.540	50	6091.80	609.18
Protein Powder	Fe	0.2509	5.15	1.540	50	719.41	71.94
Poshan Shakti	Fe	0.2526	11.03	1.540	50	1878.46	187.85
Multi-vitamin Tablet	Fe	0.2507	12.66	1.540	50	2217.79	221.78

Table 1. Concentration of Iron

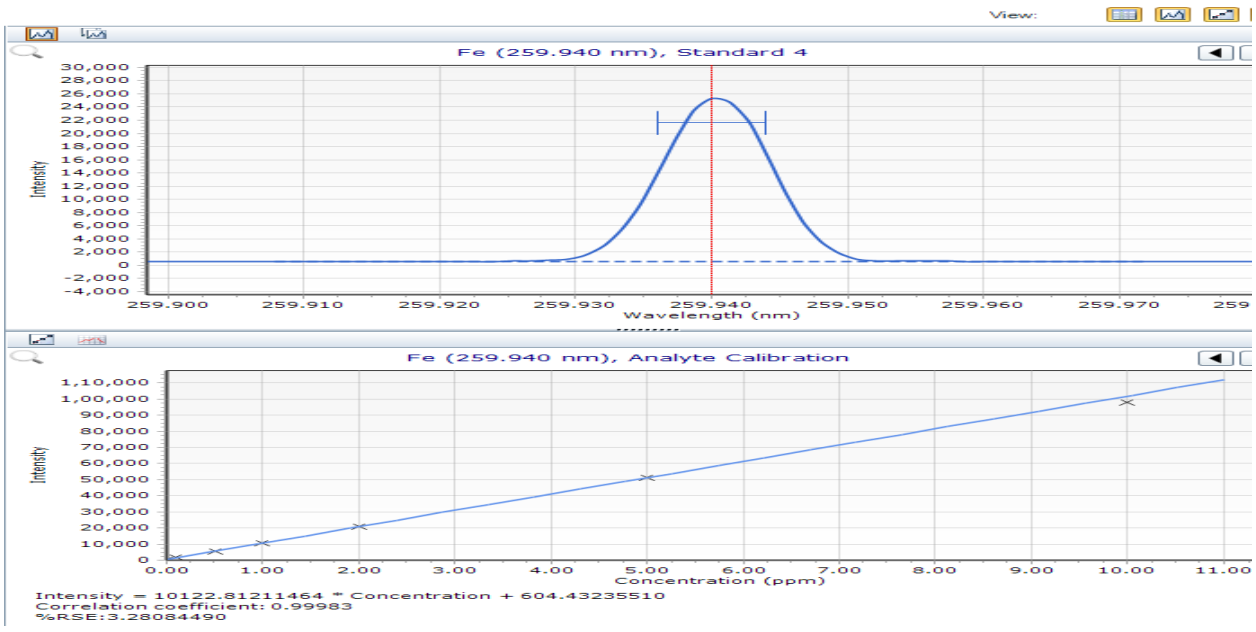


Fig: Graph of Iron

- **Concentration of Sodium:**

SAMPLE ID	ELEMENT	WEIGHT	CONC.	BLK CONC.	Makeup vol.	Final result (mg/kg)	Final result (mg/100gm)
Fortified Rice	Na	0.2585	2,13	1.820	50	59.96	6.00
Protein Powder	Na	0.2526	7.02	1.820	50	1029.30	102.93
Poshan Shakti	Na	0.2522	14.99	1.820	50	2611.02	261.10
Multi-vitamin Tablet	Na	0.2510	12.14	1.820	50	2055.78	205.58

Table 2. Concentration of Sodium

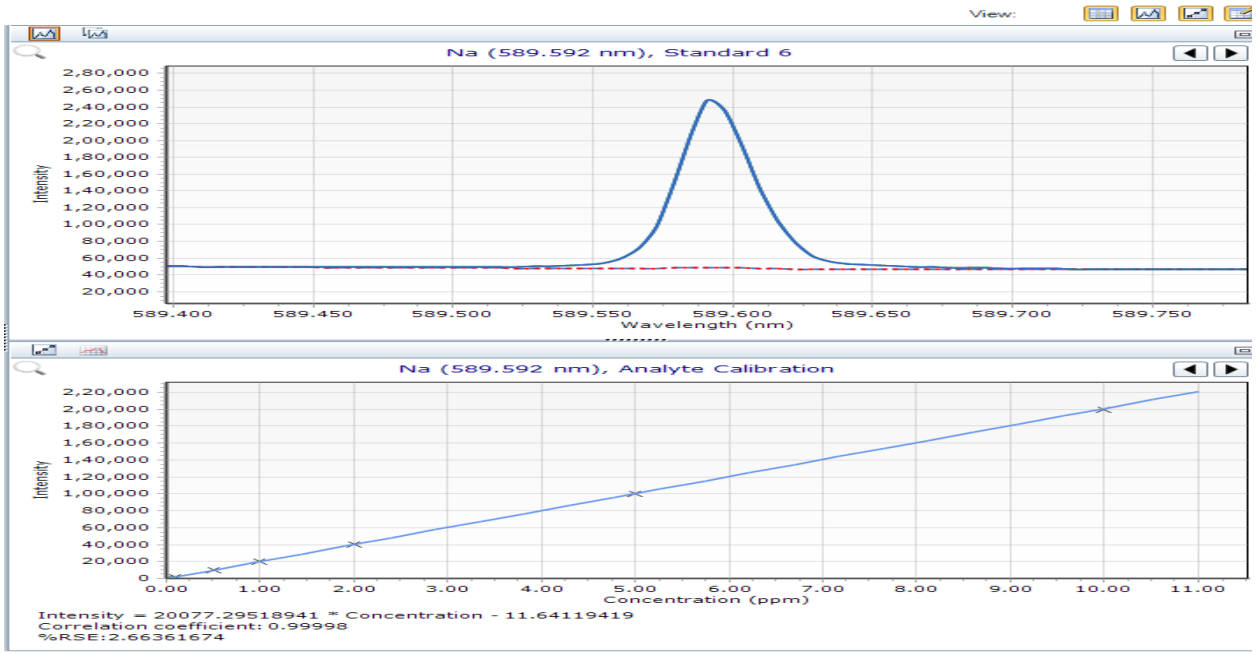


Fig: Graph of Sodium

- **Concentration of Zinc:**

SAMPLE ID	ELEMENT	WEIGHT	CONC.	BLK CONC.	Makeup vol.	Final result (mg/kg)	Final result (mg/100gm)
Fortified Rice	Zn	0.2522	7.79	0.460	50	1453.21	145.32
Protein Powder	Zn	0.2509	17.49	0.460	50	3393.78	339.38
Poshan Shakti	Zn	0.2522	22.24	0.460	50	544.50	54.45
Multi-vitamin Tablet	Zn	0.2538	44.88	0.460	50	8778.66	887.87

Table 3. Concentration of Zinc

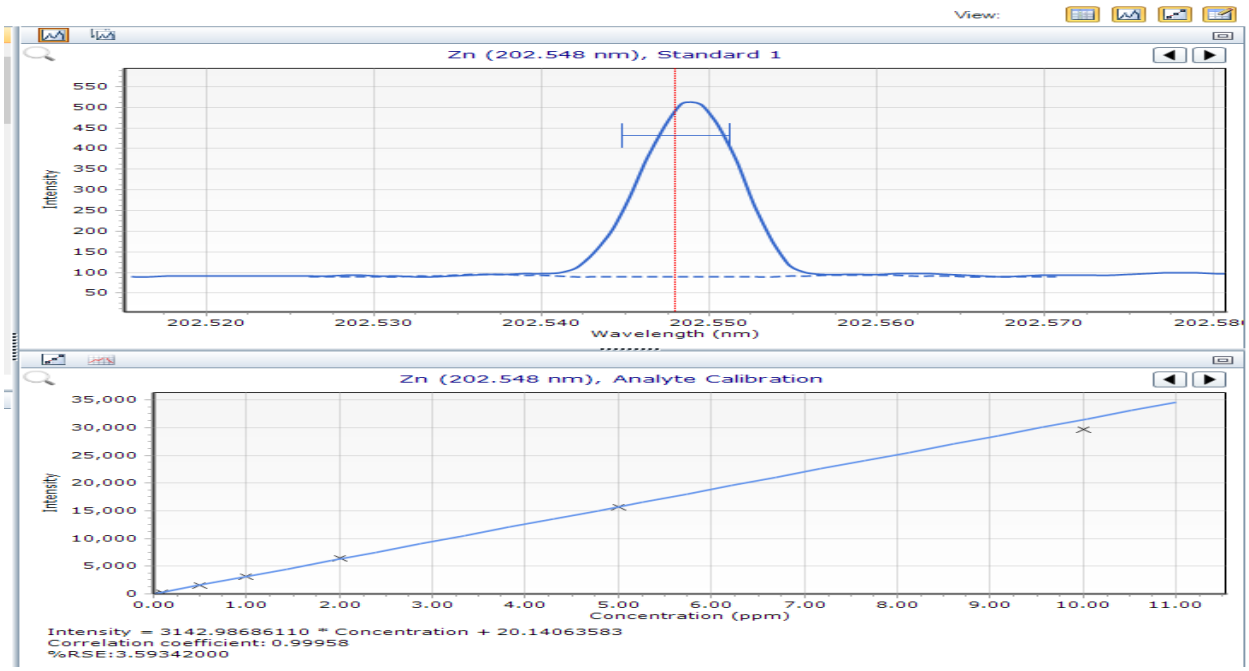


Fig: Graph of Zinc

- **Concentration of Copper:**

SAMPLE ID	ELEMENT	WEIGHT	CONC.	BLK CONC.	Makeup vol.	Final result (mg/kg)	Final result (mg/100 gm)
Fortified Rice	Cu	0.5053	0.04	0.00	25	0.04	00.4
Protein Powder	Cu	0.5064	0.08	0.00	25	0.08	00.8
Poshan Shakti	Cu	0.5013	0.30	0.00	25	0.30	03.0
Multi-vitamin Tablet	Cu	0.5088	0.05	0.00	25	0.05	00.5

Table 4. Concentration of Copper

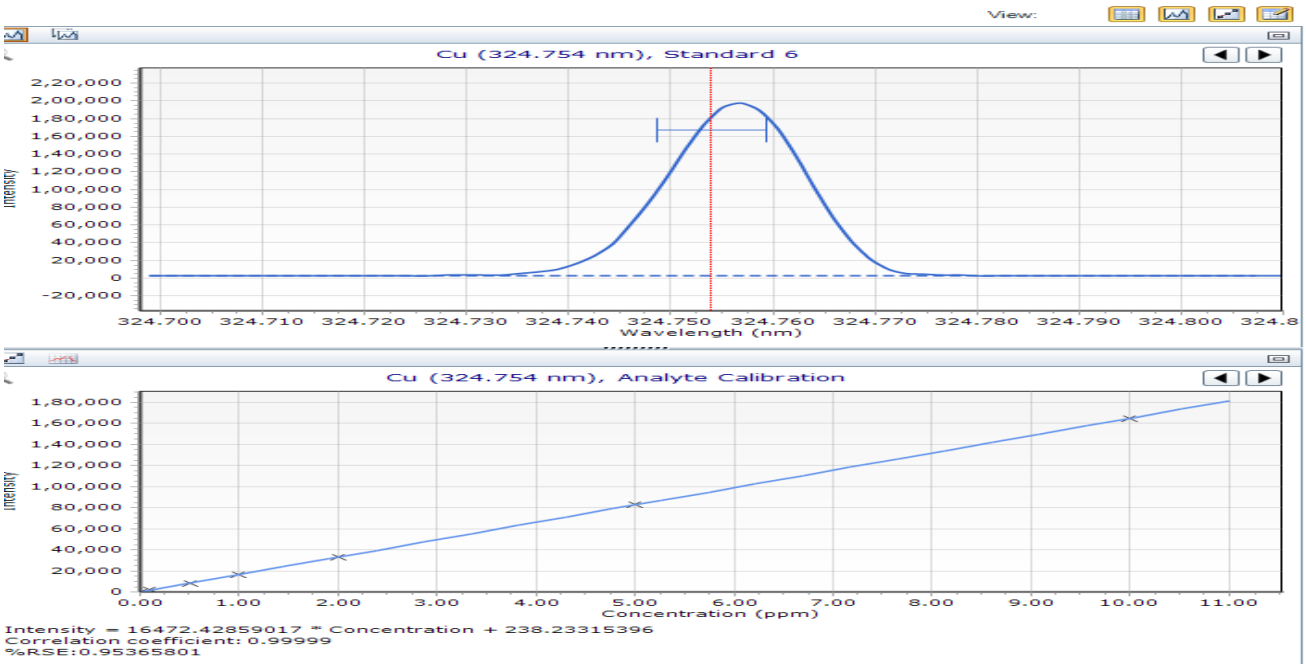


Fig: Graph of Copper

- **Concentration of Lead:**

SAMPLE ID	ELEMENT	WEIGHT	CONC.	BLK CONC.	Makeup vol.	Final result (mg/kg)	Final result (mg/100gm)
Fortified Rice	Pb	0.5053	0.01	0.010	25	0.00	0.00
Protein Powder	Pb	0.5064	0.01	0.010	25	0.00	0.00
Poshan Shakti	Pb	0.5013	0.01	0.010	25	0.00	0.00
Multi-vitamin Tablet	Pb	0.5088	0.01	0.010	25	0.00	0.00

Table 5. Concentration of Lead

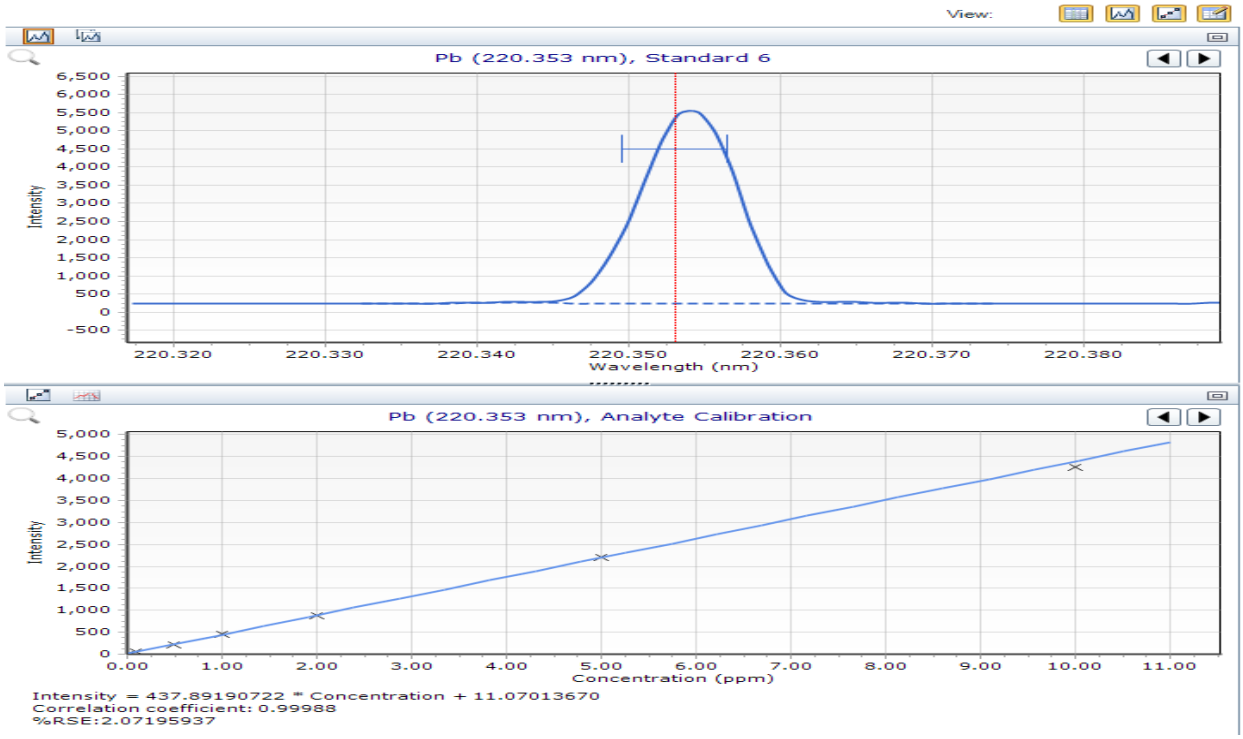


Fig: Graph of Lead

- **Concentration of Nickel:**

SAMPLE ID	ELEMENT	WEIGHT	CONC.	BLK CONC.	Makeup vol.	Final result (mg/kg)	Final result (mg/100gm)
Fortified Rice	Ni	0.5053	0.00	0.000	25	0.00	0.00
Protein Powder	Ni	0.5064	0.00	0.000	25	0.00	0.00
Poshan Shakti	Ni	0.5013	0.00	0.000	25	0.00	0.00
Multi-vitamin Tablet	Ni	0.5088	0.00	0.000	25	0.00	0.00

Table 6. Concentration of Nickel

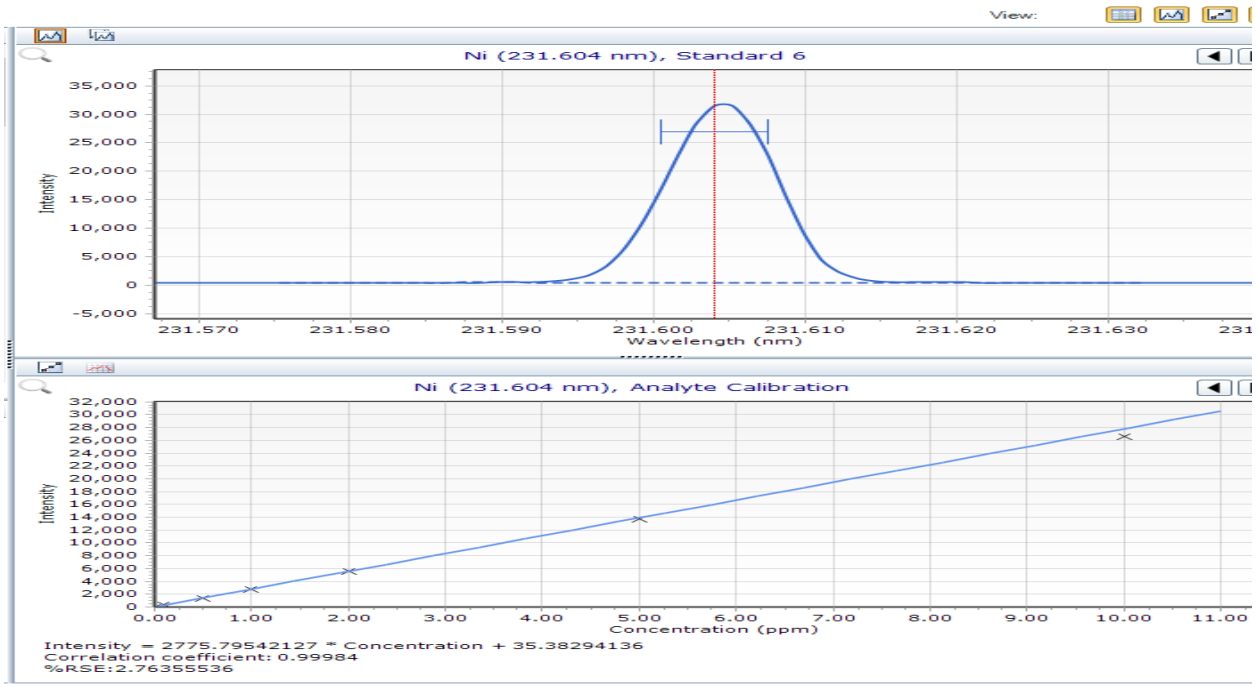


Fig: Graph of Nickel

CONCLUSION

Nutraceutical foods are now new norm of human's day to day life. By doing this study we can now conclude the concentration of minerals and heavy metals present in these foods as well as is it really beneficial for consumption as the companies claim them to be. As we know that minerals like iron, zinc, sodium are very important for our body and its defeciency can led to cause many harmful effects in our body. Iron deficiency is very common now a days, by consuming fortified rice which is fortified by iron we can now minimise the chances of it and it can be very easily become a part of aur normal meal. Due to our busy schedule, we tend to give less thought on taking care of our health which can led to weakness or many harmful effects in our body, that's when multi vitamin come into our daily life consumption, if we take them regularly, it can benefit us in the long run. At the same time metals like lead, copper, nickel, if consumed in high amount, it can cause more destruction in our body than benefit. By doing mineral and heavy metal analysis on these nutraceutical products by AAS and ICP-MS, we can easily detect the concentration of these elements present in the products and if they are okey for consumption.

REFERENCES

AOAC 20th Edition 2016, Chapter

Agilent 7700X ICP-MS Application note 2014.

Lofaso, M. (2021). Determination of Metals in Whey and Vegan Protein Supplements using Inductively Coupled Plasma Mass Spectrometry.

Romero-Estévez, D., Yánez-Jácome, G. S., Simbaña-Farinango, K., Vélez-Terreros, P. Y., & Navarrete, H. (2019). Evaluation of two sample preparation methods for the determination of cadmium, nickel and lead in natural foods by Graphite Furnace Atomic Absorption Spectrophotometry. *Universitas Scientiarum*, *24*(3), 497-521.

Berner, L. A., Keast, D. R., Bailey, R. L., & Dwyer, J. T. (2014). Fortified foods are major contributors to nutrient intakes in diets of US children and adolescents. *Journal of the Academy of Nutrition and Dietetics*, *114*(7), 1009-1022.

Martınez-Navarrete, N., Camacho, M. M., Martınez-Lahuerta, J., Martınez-Monzó, J., & Fito, P. (2002). Iron deficiency and iron fortified foods—a review. *Food Research International*, *35*(2-3), 225-231.

Walter, T., Dallman, P. R., Pizarro, F., Vebozo, L., Peña, G., Bartholmey, S. J., ... & Arredondo, M. (1993). Effectiveness of iron-fortified infant cereal in prevention of iron deficiency anemia. *Pediatrics*, *91*(5), 976-982.

Gil, F., Hernández, A. F., & Martín-Domingo, M. C. (2021). Toxic contamination of nutraceuticals and food ingredients. In *Nutraceuticals* (pp. 1145-1158). Academic Press.

Bu, K., Cizdziel, J. V., & Reidy, L. (2013). Analysis of herbal supplements for selected dietary minerals and trace elements by laser ablation-and solution-based ICPMS. *Microchemical Journal*, *106*, 244-249.

Filipiak-Szok, A., Kurzawa, M., & Szłyk, E. (2015). Determination of toxic metals by ICP-MS in Asiatic and European medicinal plants and dietary supplements. *Journal of Trace Elements in Medicine and Biology*, *30*, 54-58.

Bhat, R., Kiran, K., Arun, A. B., & Karim, A. A. (2010). Determination of mineral composition and heavy metal content of some nutraceutically valued plant products. *Food analytical methods*, *3*(3), 181-187.

Das, L., Bhaumik, E., Raychaudhuri, U., & Chakraborty, R. (2012). Role of nutraceuticals in human health. *Journal of food science and technology*, *49*(2), 173-183.

Lewen, N. (2011). The use of atomic spectroscopy in the pharmaceutical industry for the determination of trace elements in pharmaceuticals. Journal of pharmaceutical and biomedical analysis, 55(4), 653-661.

Anal, J. M. H., & Chase, P. (2016). Trace elements analysis in some medicinal plants using graphite furnace-atomic absorption spectroscopy. Environmental engineering research, 21(3), 247-255.

Bhowmik, S., Datta, B. K., & Saha, A. K. (2012). Determination of mineral content and heavy metal content of some traditionally important aquatic plants of tripura, India using atomic absorption spectroscopy. Journal of Agricultural Technology, 8(4), 1467-1476.

Gupta, S., Pandotra, P., Gupta, A. P., Dhar, J. K., Sharma, G., Ram, G., ... & Bedi, Y. S. (2010). Volatile (As and Hg) and non-volatile (Pb and Cd) toxic heavy metals analysis in rhizome of Zingiber officinale collected from different locations of North Western Himalayas by Atomic Absorption Spectroscopy. Food and Chemical Toxicology, 48(10), 2966-2971.

Durukan, İ., Şahin, Ç. A., Şatıroğlu, N., & Bektaş, S. (2011). Determination of iron and copper in food samples by flow injection cloud point extraction flame atomic absorption spectrometry. Microchemical Journal, 99(1), 159-163.

Fairulnizal, M. M., Vimala, B., Rathi, D. N., & Naeem, M. M. (2019). Atomic absorption spectroscopy for food quality evaluation. In Evaluation Technologies for Food Quality (pp. 145-173). Woodhead Publishing.

Smichowski, P., & Londonio, A. (2018). The role of analytical techniques in the determination of metals and metalloids in dietary supplements: A review. Microchemical Journal, 136, 113-120.

Afkhami, A., Saber-Tehrani, M., Bagheri, H., & Madrakian, T. (2011). Flame atomic absorption spectrometric determination of trace amounts of Pb (II) and Cr (III) in biological, food and environmental samples after preconcentration by modified nano-alumina. Microchimica Acta, 172(1), 125-136.

Giannenas, I., Nisianakis, P., Gavriil, A., Kontopidis, G., & Kyriazakis, I. (2009). Trace mineral content of conventional, organic and courtyard eggs analysed by inductively coupled plasma mass spectrometry (ICP-MS). Food Chemistry, 114(2), 706-711.

Dolan, S. P., Nortrup, D. A., Bolger, P. M., & Capar, S. G. (2003). Analysis of dietary supplements for arsenic, cadmium, mercury, and lead using inductively coupled plasma mass spectrometry. Journal of Agricultural and Food Chemistry, 51(5), 1307-1312.

Mohamed, R., Zainudin, B. H., & Yaakob, A. S. (2020). Method validation and determination of heavy metals in cocoa beans and cocoa products by microwave assisted digestion technique with inductively coupled plasma mass spectrometry. Food chemistry, 303, 125392.

Rao, R. N., & Talluri, M. K. (2007). An overview of recent applications of inductively coupled plasma-mass spectrometry (ICP-MS) in determination of inorganic impurities in drugs and pharmaceuticals. Journal of pharmaceutical and biomedical analysis, 43(1), 1-13.

Satpute, A., & Pandey, A. DETERMINATION OF INORGANIC TOXICANTS IN NUTRACEUTICALS BY INDUCTIVELY COUPLED PLASMA MASS SPECTROSCOPY.

Dehelean, A., Kovacs, E. D., Magdas, D. A., Cristea, G., Voica, C., Feher, I., ... & Zang, Q. (2019). Infant Supplement Quality Evaluation by Inductively Coupled Plasma–Mass Spectrometry, Gas Chromatography, and Isotope Ratio Mass Spectrometry. Analytical Letters, 52(1), 127-138.

Christopher, S. J., & Thompson, R. Q. (2013). Determination of trace level cadmium in SRM 3280 Multivitamin/Multielement Tablets via isotope dilution inductively coupled plasma mass spectrometry. Talanta, 116, 18-25.

Lewen, N. (2011). The use of atomic spectroscopy in the pharmaceutical industry for the determination of trace elements in pharmaceuticals. Journal of pharmaceutical and biomedical analysis, 55(4), 653-661.

Olesik, J. W. (1991). Elemental analysis using icp-oes and icp/ms. Analytical Chemistry, 63(1), 12A-21A.

Thomas, R. (2001). A beginner's guide to ICP-MS. Spectroscopy, 16(4), 38-42.

Thomas, R. (2008). Practical guide to ICP-MS: a tutorial for beginners. CRC press.

Tyler, G., & Jobin Yvon, S. (1995). ICP-OES, ICP-MS and AAS Techniques Compared. ICP Optical Emission Spectroscopy Technical Note, 5.

Olesik, J. W. (1996). Peer reviewed: Fundamental research in ICP-OES and ICPMS. Analytical Chemistry, 68(15), 469A-474A.

Inam, F., Deo, S., & Narkhede, N. (2013). Analysis of minerals and heavy metals in some spices collected from local market. J. of. Phar. and Bio. Sci, 8(2), 40-43.

Subramanian, R., Gayathri, S., Rathnavel, C., & Raj, V. (2012). Analysis of mineral and heavy metals in some medicinal plants collected from local market. Asian Pacific Journal of Tropical Biomedicine, 2(1), S74-S78.

Kılıç Altun, S., Dinç, H., Paksoy, N., Temamoğulları, F. K., & Savrunlu, M. (2017). Analyses of mineral content and heavy metal of honey samples from south and east region of Turkey by using ICP-MS. International Journal of Analytical Chemistry, 2017.

Şahan, Y., Basoglu, F., & Gücer, S. (2007). ICP-MS analysis of a series of metals (Namely: Mg, Cr, Co, Ni, Fe, Cu, Zn, Sn, Cd and Pb) in black and green olive samples from Bursa, Turkey. Food Chemistry, 105(1), 395-399.\.

Pregl, Fritz (1917). Quantitative Micro Analysis of Organic Substances .Berlin: Springer. ISBN 978-3-86444-914-7.

“Fritz Pregl – Nobel Lecture: Quantitative Micro-Analysis of Organic Substances”. Retrieved 2016-07-04.

“McCarthy, G.J.”Walsh, Alan- Biographical entry”. Encyclopedia of Australian Science. Retrived 22 May 2012.

Koirtyohann, S.R. (1991). “A History of Atomic Absorption Spectroscopy” Analytical Chemistry. 63(21): 1024A-1031A. doi:10.1021/ac00021a716.ISSN 0003-2700.

“Sample Atomization- Atomic Absorption Spectroscopy Learning Module. blogs.maryville.edu. Retrived 2017-11-02

Robinson, James; et al. (2005). Undergraduate instrumental analysis, Sixth Edition .New York: Marcel Dekker .p. 493.

A. G. Howard and P. J. Stathm, Inorganic trace analysis, philosophy and practice. John Wiley and sonsLtd. (1997). J. Sneddon, C. Hardaway, K. K. Bobbadi, and A. K. Reddy, Applied Spectroscopy Reviews, 41, 1 (2006).

R. Bock, Handbook of Decomposition Methods in Analytical Chemistry, Willey, New York, (1979), translated and revised by I.L. Marr.

R. Anderson, Sample Pretreatment and Separation, John Wiley and Sons, New York, (1987).

F. E. Smith, E. A. Arsenault, Talanta, 43, 1207 (1996).

A. Serrano, M. Gallego, J. Chromatogr. A 1104 (1-2), 323 (2006).

Food science: Third Addition – By Norman Potter

Hydrogenated Sperm Oil: Sec 173.275, of Ch 1 “Food and Drug Administration, Dept of Health and Human Services”, Code of Federal RegulationIs.

Abdalla A.E, Darwish S.M., Ayad E.H., and EL-Hamahmy R.M. 2007. Egyptian mango by-product 2; antioxidant and antimicrobial activities of extract and oil from mango seed kernel. Food chemistry,1034: 1141-1152.

Abdel Ghany T.M. and Hakamy O.M. 2014. Juniperus procera as food safe additives, their antioxidant, anticancer and antimicrobial activity against some food-borne bacteria. J. Biol. Chem. Research ., 31 2; 668-667.

Abee, T., Krockel, L, and Hill, C., 1995. Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. Int. J. Food microbiol. 28, 169-185.

Atterbury R.J. 2009. Bacteriophage biocontrol in animals and meat products. Microbial biotechnology,2, 601-612.

Barlow, S.M. 1990. Toxicological aspects of antioxidants use food additives. In: Hudson BJF ed Food antioxidants. Elsevier, London, pp 2530-307.

AOAC 2007.01 Edition 21st, 2019.

ISO/EIC 17025:2010

FSSAI Lab manual 9

FSSAI Compendium_Contaminants_Regulations_20_08_2020