April 30, 2021

PharmacologyOnLine

Archives • 2021 • vol.1 • 487-502

DEVELOPMENT OF A VALIDATED METHOD FOR QUANTITATIVE ANALYSIS OF HEAVY METALS IN HERBAL MEDICINES USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

Indrajit Sen[1], Deepak Shrivastava[2], Manjeet Aggarwal[3] and Rakesh Kumar Khandal[4]*
[1]Dr. A.P.J. Abdul Kalam Technical University, Lucknow, India.
[2]Plastics Technology- School of Chemical Technology, H.B Technical University, Kanpur, India.
[3]National Institute of Food Technology Entrepreneurship and Management, Kundli, Haryana, India.
[4]India Glycol Ltd, Noida, Utter Pradesh, India.

*drrakeshkhandal@gmail.com

Abstract:

Herbal medicines have been using for the treatment, management, and prevention of different types of diseases by majority of the population in India since time immemorial. These medicines are effective besides being easily available, and inexpensive compared with allopathic drugs. In recent times, several cases of heavy metals in herbal medicine have come to light. As a result, regulators have started monitoring for controlling the quality of herbal medicines. The present study pertains to the development of a validated method to determine the content of lead, cadmium, arsenic, and mercury in different herbs e.g. Ajwain (*Trachyspermum copticum*), Ginger (*Zingiber officinale*), Neem tree (*L. Azadirachta Indica*), Cloves (*Syzygium aromaticum*), Turmeric (*Curcuma longa*), Since these herbs are widely used in herbal medicines, the method would be of high value. Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) was used for this purpose. Guidelines of SANTE have been followed to develop and validate this method.

Keywords: *Heavy metals, Herbal medicines, ICP-MS*

Introduction

Medicinal herbs are been used to prepare herbal medicines due to their therapeutic potential and considered to be safe in comparison to allopathic medicines, which are widely used worldwide since a decade (Samali et.al 2017 and Haider et.al 2004) [1,2].

Ajwain or Carom seed is an essential oil containing thymol and used as an ingredient in cough syrups and throat lozenges, due to its germicide and antiseptic properties. It is also benefited to asthma sufferers (Zarshenas et.al 2013 and El-Wahab et.al. 2008) [3,4]. Ginger is the most commonly used herb for indigestion, gastric pains, diarrhea, and stomach cramping, besides other uses. Ginger root is known to contain 6- gingerol, 6- shogaol, and 2,6- paradol that has strong antioxidant activity responsible for its several health benefits (Goroya et.al. 2019, Bua et.al 2016) [5,6]. Nutrients such as insoluble and soluble fibers, essential fats, zingibain, carbohydrates, bisabolenel, starch, oleoresins, essential oil (zingiberole, zingiberene, camphene, borneol, cineol,) protein, and mucilage in ginger are also useful for enhancement of immunity. The therapeutic properties of ginger include stimulating the circulation of the blood effectively, removing various toxins from the body, nourishing the skin, and as an anti-inflammatory medicine. It is also used as a cleansing agent for bowels and kidney (Al-Eed et.al 2002 and Krejpcio et.al. 2007) [7,8].

Neem is used as herbal medicine to cure skin diseases such as ring worm, ulcers, boils, eczema, various skin fungus conditions, including lesions in the mouth, athlete's foot, and vagina, has reported by Augustine et.al 2016 [9]. Akan et.al 2013 has stated that the extract of neem seed has also been used for the treatment of chicken pox and small pox. Patients of Herpes and Hepatitis B viruses are also treated by preparations based on neem. It is also used for the purification of blood and also useful for the treatment of liver and heart diseases [10].

Clove oil has been in use as a natural remedy for maintaining good oral health because of its effect on gingivitis, plaque, and bacteria in the mouth (Krejpcio et.al. 2007) [8]. Inam et.al 2013 reported clove is known for its rich nutritional and medicinal values [11]. The nutrients present in cloves are protein, carbohydrates, antioxidants, dietary fiber, and energy; Minerals include iron, calcium, potassium, phosphorus, magnesium, sodium, and zinc; vitamins such as vitamin E, vitamin C, riboflavin, niacin, vitamin A, thiamin, folic acid and vitamin K. The clove is directly applied to the gums for toothache and also controlling the pain, during dental work and other dental related issues (Gaya et.al. 2016) [12].

Al-Eed et.al 2002 described turmeric is rich in antioxidant content and thus its regular intake protects against free radical damage, blood purification, and also used as an antiseptic [7]. It is also helpful in the treatment of different skin problems like skin rashes, spots, acne etc. It is pretty effective for the treatment of hepatitis, gallbladder problems, infections, indigestion, scabies, lack of appetite, arthritis, alzheimer's disease, asthma, boils, athlete's foot, bursitis, high cholesterol, inflammation, irritable bowel syndrome, intestinal pain, lack of menstruation (Ahmed et.al 2017) [13]. It is also used as an important medicinal ingredient to cure colon cancer, breast cancer, colic, dermatitis, cataracts, diarrhea, fibrosis, gallstones, eczema, gas, hardening of the arteries, heart disease, high triglycerides, lymph gland problems, jaundice, morning sickness, psoriasis, menstrual pain, sprains, wounds, ulcers and yeast infections (Khongkaew et.al. 2020) [13,14].

Heavy metals are natural components of soil hence, the produces from plants may carry them to preparation if not properly processed of heavy metals enter in the human body, they cause toxicity (Bua et.al 2016) [6]. Heavy metals have a relatively high density as well as higher molecular weight and they are toxic at very low concentration. Lead (Pb), Cadmium (Cd), Arsenic (As), and Mercury (Hg) are the common toxic elements that have become a major concern due to their presence as contamination in various herbal preparations, as reported earlier (Al-Eed et.al 2002) [7]. Baranowska et.al. 2002 has described the toxicity of lead in the human body. Lead causes neurological disorders, kidney damage, anemia, lower sperm count, miscarriage, and hepatotoxicity in higher concentration [15]. Acute or chronic contact of cadmium causes anemia, lung and breast cancers,

respiratory distress, hemorrhagic injuries, and cardiovascular disorders, has reported by Akram et.al. 2015 [16]. Behari et.al. 2006 reported the toxicity due to arsenic. It causes peripheral arteriosclerosis, hypertension, neurotoxicity, and skin diseases [17]. Mercury is reported (Sudha et.al. 2014) to causes neurological disorders, paralysis, inflammation of the digestive tract, acrodynia, uremia, and immunotoxicity [18]. Consumption of herbal medicines worldwide, regulatory authorities World health organization (WHO) and Ayush are now more concerned with the efficacy and safety of these herbal medicines and have emphasized on the need of quality and safety of these herbal preparations for checking the presence of these heavy metals i.e. lead, cadmium, arsenic and mercury [19-22].

More than a few analytical methods have been reported till date for the determination of heavy metals in various food and pharmaceutical products. Among these, atomic absorption spectrophotometer (AAS) [7, 8, 11-18] and inductively coupled plasma with optical emission spectrophotometry (ICP-OES) detector [23-24] are the utmost common techniques and are being used on a routine basis for identification and determination of heavy metals. Both these methods are used for qualitative as well as quantitative analysis of heavy metals in various complex matrices. Numerous studies have been done to identify and determine the heavy metals in herbal medicines, spices, and dry fruits by AAS and ICP-OES analytical techniques. Using these methods for the determination of heavy metals at residue levels in complex food and pharmaceutical products may suffer from interferences from components of the matrix affecting selectivity and sensitivity of the method adversely.

This present paper describes the development and validation of a method for the determination of residues of toxic heavy metals mainly lead, cadmium, arsenic, and mercury by using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). For this, five major ingredients Ajwain, Ginger, Neem, Clove, and Turmeric, from six different locations of Delhi (marked as Source D 1, 2,3) and Varanasi (marked as Source V 1, 2, 3) have been chosen for this purpose. Since all these products are used as raw materials for the preparation of herbal medicines, the method validated here would serve the cause for several medicinal preparations.

Materials and Methods

Chemicals and Reagents:

Standards aqueous solution which is a mixture of four heavy metals i.e., 1000 µg/ml of lead, cadmium, arsenic mixture, and 10 µg/ml of mercury (traceable to NIST) were procured from ScharlauChemie, Spain. Ajwain, ginger, neem, clove, and turmeric were procured from the local market of Delhi and Varanasi (India). Ultra-pure grade Nitric acid (HNO_3) and Hydrochloric acid were procured from Merck Specialist Chemical limited, India. Mili Q water was used for the preparation of all samples and standards. All glassware used was of "A" grade and duly calibrated. Calibrated micropipettes were used with the range of 100µl to 10000µl.

Instrumentation:

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) Agilent 7800 model USA, equipped with micro mist nebulizer; quartz spray chamber; torch, nickel cone, and mass spectrometry detector. An autosampler was used to introduced digested samples in the ICP-MS. All the samples were analysed in helium collision mode using ORS4 octapole collision or reaction cell, under optimized autotune conditions of the equipment directly from the MassHunter 4.4 software.

Sample digestion was performed through a closed-vessel microwave digestion system (Anton Paar) with a multiwave pro rotor, temperature, and pressure sensor, provided with an auto pressure vent PTFE vessel. The maximum temperature for this system is 220°C and the sample can be digested with a maximum of 20 bar pressure.

Preparation of Calibration Standard solutions:

Accurately 1ml of mixed standard reference solution (1000 µg/ml) of lead, cadmium, arsenic and were pipetted into a 100 ml volumetric flask respectively and diluted to the volume with 5% nitric acid in mili Q water. 10 µg/ml concentration of mercury reference standard was taken in a 100 ml volumetric flask and diluted to the volume with the mixture of 3% nitric acid and 2% hydrochloric acid in mili Q water. This was taken as the stock solution

used for the preparation of calibration standard solutions and was stored under suitable conditions.

Appropriate aliquots were taken and further diluted with 5% nitric acid in mili Q water so as to give a series of calibration standard solutions having lead, cadmium and arsenic concentration range of 0.1, 0.2, 0.50, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0 and 100.0 µg/l and for mercury the calibration standard solutions concentration range of 0.1, 0.2, 0.50, 1.0, 2.0, 5.0, and 10.0 µg/l, respectively.

Sample preparation:

Sample preparation for heavy metal for analysis consists of three steps: sample homogenization, digestion, and dilution. For digestion, about 0.50 ±0.01g, accurately weighed, dried homogenized powder samples (already milled with Teflon mortar to avoid metal contamination) of each ajwain, ginger, neem, clove, and turmeric were taken in 10.0 ml of concentrated ultra-pure HNO_3 in a tightly closed PTFE vessel and digested in a microwave digester separately. The digestion was carried out in three steps with a constant microwave power of 1000 W; (i) First step:temperature was increased to 110°C in 20 mins with 5 mins held time, (ii) Second step: temperature was increased to 145°C in 10 mins and held at that for 5 mins; and (iii) temperature was increased to 190°C in 10mins and held at that for 10 mins. Cooled down the digester at room temperature and diluted the digested samples with Mili Q water up to 50 ml. The final solution was made with 100-time dilution. Three replicate samples were prepared for each sample for analysis. Recovery study was carried out by fortifying the sample of known concentration with standard solution of 10, 20 and 50 µg/kg.

Instrument conditions:

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) conditions:

The ICP-MS operating conditions for the determination of Pb, Cd, As and Hg were: High purity (99.99%) argon was used as plasma, auxiliary, and nebulizer gas. The gas flows were kept at 15.0 l/min for plasma, 0.9 l/min for auxiliary, 0.9 l/min for nebulizer, and 0.15 l/min for dilution. The nebulizer pump was kept at 0.10 rps speed. The Radiofrequency (R.F) power 1550 W; helium collision gas flow rate 4.3 ml/min; spray chamber temperature 2°C; sample depth 8.0 mm; sample introduced flow rate 0.5 ml/min; extract lens 2 voltage -200V; omega lens voltage 9.6V; cell entrance -40V; cell exit -60V; octapole RF 200V. Masses for the element as taken for the analysis were As (75); Cd (111); Hg (202) and Pb (208). The instrument was also tuned before studies to obtained highest signal to noise ratio for 7Li, 59Co, 80Y and 205Tl along with the ratios 140Ce 16O/ 140Ce < 3.0% and 70Ce++/140Ce <3.0%.

Results and Discussion

Method validation:

The validation of method for the ICP-MS was carried out according to the SANTE guidelines, considering selectivity, sensitivity linearity, limit of detection (LOD), limit of quantification (LOQ), precision or repeatability and recovery or accuracy [25]. Ten calibration standards for Pb, Cd, As and seven calibration standards for the Hg were evenly spread over the concentration range of interest and encompassing the maximum residual levels reflecting Ayush / WHO limits were analyzed [19-21].

The method is validated to quantitatively determine heavy metals at low levels as prescribed by WHO, FSSAI and Ayush for these metals in herbal preparations. The unique features of the method are as follows a) hominization of samples, b) digestion c) dilution of samples. Previously numerous methods have been published for sample preparation with the open wet digestion method. At the time of using the open wet digestion method, there were chances to lose some fraction of low boiling heavy metals like mercury. Here the closed vessel digestion was done and hence, the possibility of loss of heavy metals during sample preparation. The calibration standards were run each with six replicates and the linearity of the method was assessed by the means of Pb, Cd, As and Hg calibration curves built following the linear least-squares regression method. Linearity of the method was obtained excellent for all interested heavy metals with correlation coefficient (r) of more than 0.999 (Table 1). The results of the linearity study for all metals are shown in Figure 1-4. LOD for the method was determined by considering the signal to noise (S/N) ratio of 3:1 with respect to the

background noise obtained from the sample blank which is 5 µg/kg whereas LOQ was determined similarly by considering signal to noise ratio (S/N) ratio of 10:1, is 10 µg/kg. The precision or repeatability study was calculated by the data of measurements for both intra-day and inter-day repeatability and reproducibility by measuring the three concentrations 0.10 µg/kg, 0.20 µg/kg, and 0.50 µg/kg in seven replicates are presented in table 2. The recovery or accuracy of the method was measured to study the effect of matrix on the determination of Pb, Cd, As, and Hg. Samples of Ajwain, Ginger, Neem, Clove, and Turmeric were spiked with the known concentration of Pb, Cd, As and Hg standard solutions at different levels i.e., 10.0 µg/kg, 20.0 µg/kg, and 50.0 µg/kg respectively and after the digestion the samples were diluted 100 times with mili-Q water. The final concentration of these solutions was made 0.10 µg/kg, 0.20 µg/kg, and 0.50 µg/kg and was analysed on the same day and onthree subsequent days by three different analysts. The results are given in table 3. The recoveries of Pb, Cd, As and Hg in samples were found to be in the range between 90.60% to 100.44%, 94.70% to 101.21%, 92.0 % to 100.26%, 89.60% to 99.52 % respectively for the ICP-MS method. The percent recovery in all the cases was within the acceptable limits of 70% to 120% as per regulatory guidelines [25]. Now, this ICP-MS method proficiently competeswith all the criteria established by the Association of Official Analytical Chemists (AOAC 2019) for the confidence of an analytical method [26, 27], as well as all needs of ISO/IEC 17025-2017 to generate valid results [28]. Shim et.al (2019) [29] had published their study to determination of heavy metals in spices and validate their method using ICP-MS and Kilic et.al. (2020) [30] had also described the detailed methods for the determination of trace element contaminants in herbal teas using ICP-MS by different sample preparation method. Our validation data were in agreement with their findings and confirming that ICP-MS has admirable selectivity and sensitivity for simultaneous multi-element detection.

Heavy metal analysis:

The sample analysis results obtained for Pb, Cd, As and Hg using ICP-MS are tabulated in table 4. Lead was found in all samples of ajwain, ginger, neem, clove, and turmeric from all the sources. The concentration of lead in the samples was obtained in the range of 0.1749 mg/kg to 2.3447 mg/kg and was found within the maximum residual limits prescribed by the WHO/Ayush i.e. 10.0 mg/kg. All samples showed the presence of cadmium in the range of 0.0222 mg/kg to 0.0788, which were also found in the range of maximum residual limits as per WHO/Ayush i.e. 0.3 mg/kg. Samples showed the presence of arsenic 0.0169 mg/kg to 0.1341 and mercury 0.0214 mg/kg to 0.0755, the levels were within the maximum residual limits i.e. 3.0 mg/kg and 1.0 mg/kg respectively [19,21]. The concentration level of heavy metals shown in turmeric is higher as compared to other samples like ajwain, ginger, neem, and clove. The contamination of heavy metals in herbal medicinal plants could be either due to intake by the roots or due to the surface deposit from the environmental pollution.

Conclusion

An ICP-MS method has been developed for selective, sensitive, repeatable, and accurate determination of heavy metals in herbal medicine. The method yields high precision (RSD 1.02 to 4.18%), accuracy (recovery 89.60 to 101.2%), LOD 5.0 µg/kg, and LOQ 10.0 µg/kg. The masses of heavy metals show As (75); Cd (111); Hg (202) and Pb (208). In spite of using a simple digestion method for sample preparation, no interferences were observed from the matrix. Data of sample analysis indicated that the presence of various toxic metals in the ajwain, ginger, neem, clove, and turmeric from the different locations of Delhi and Varanasi market, has been well within the allowed limit. The use of ICP-MS to determine such toxic metals up to trace levels (µg/kg) and thus indicate compliance to the regulations of permissible maximum residual limits as per the various regulatory authorities.

References

1. Samali A, Mohammed MI, Ibrahim MB. Analysis of heavy metals concentration in Kano herbal preparations for major disease conditions. Chemsearch Journal. 2017;8(2):22-8.
2. Haider S, Naithani V, Barthwal J, Kakkar P. Heavy metal content in some therapeutically

important medicinal plants. Bulletin of environmental contamination and toxicology. 2004 Jan 1;72(1):119-27..
3. Zarshenas MM, Moein M, Samani SM, Petramfar P. An overview on ajwain (Trachyspermum ammi) pharmacological effects; modern and traditional. Journal of natural Remedies. 2013 Dec 3;14(1):98-105.
4. El-Wahab A, Mohamad A. Effect of some trace elements on growth, yield and chemical constituents of Trachyspermum ammi L.(AJOWAN) plants under Sinai conditions. Research Journal of Agriculture and Biological Sciences. 2008;4(6):717-24.
5. Goroya KG, Mitiku Z, Asresahegn YA. Determination of concentration of heavy metals in ginger using flame atomic absorption spectroscopy. African Journal of Plant Science. 2019 Jun 30;13(6):163-167.
6. Bua DG, Annuario G, Albergamo A, Cicero N, Dugo G. Heavy metals in aromatic spices by inductively coupled plasma-mass spectrometry. Food Additives & Contaminants: Part B. 2016 Jul 2;9(3):210-216.
7. Al-Eed MA, Assubaie FN, El-Garawany MM, El-Hamshary H, El-Tayeb ZM. Determination of heavy metal levels in common spices. J Appl Sci. 2002;17:87-98.
8. Krejpcio Z, Krol E, Sionkowski S. Evaluation of Heavy Metals Contents in Spices and Herbs Available on the Polish Market. Polish Journal of Environmental Studies. 2007 Jan 1;16(1).
9. Augustine AU, Onwuka JC, Albert CQ. Determination of heavy metal concentration in Neem (Azadirachta indica) leaves, bark and soil along some major roads in Lafia, Nasarawa State Nigeria. Journal of Environmental Chemistry and Ecotoxicology. 2016 May 31;8(5):38-43.
10. Akan JC, Inuwa LB, Chellube ZM, Lawan B. Heavy metals in leaf, stem bark of neem tree (Azadirachta indica) and roadside dust in Maiduguri Metropolis, Borno State, Nigeria. Environment and Pollution. 2013 Jan 1;2(1):88.
11. Inam F, Deo S, Narkhede N. Analysis of minerals and heavy metals in some spices collected from local market. J. of. Phar. and Bio. Sci. 2013;8(2):40-43.
12. Gaya UI, Ikechukwu SA. Heavy metal contamination of selected spices obtained from Nigeria. Journal of Applied Sciences and Environmental Management. 2016;20(3):681-688.
13. Ahmed M, Khaleeq A, Huma R, Munir S. Atomic absorption and inductively coupled plasma–optical emission spectroscopic method for determination of micronutrients and toxic metals in Curcuma longa L. to characterize human health toxicity. Spectroscopy Letters. 2017 Sep 14;50(8):432-439.
14. Khongkaew P, Phechkrajang C, Cruz J, Cárdenas V, Rojsanga P. Quantitative models for detecting the presence of lead in turmeric using Raman spectroscopy. Chemometrics and Intelligent Laboratory Systems. 2020 Mar 6:103994.
15. Baranowska I, Srogi K, Wlochowicz A, Szczepanik K. Determination of heavy metal contents in samples of medicinal herbs. Polish Journal of Environmental Studies. 2002 Jan 1;11(5):467-472.
16. Akram S, Najam R, Rizwani GH, Abbas SA. Determination of heavy metal contents by atomic absorption spectroscopy (AAS) in some medicinal plants from Pakistani and Malaysian origin. Pakistan journal of pharmaceutical sciences. 2015 Sep 1;28(5):1781-1787.
17. Behari JR, Prakash R. Determination of total arsenic content in water by atomic absorption spectroscopy (AAS) using vapour generation assembly (VGA). Chemosphere. 2006 Mar 1;63(1):17-21.
18. Sudha K, Bhavani C, Vivek C. Heavy metal analysis from traditionally used herb Ceropegia juncea (Roxb.). IOSR J Pharm. 2014;4(12):7-11.
19. FAO/WHO.1984. Joint FAO/WHO Food standers Program, Codex Alimentarius Commission Contamination. CAC/Vol.XV11.FAO, Roma and WHO, Geneva.
20. Codex Alimentarius International Food Standards, General Standard for

Contaminants and Toxins in Food and Feed, CODEX STAN 193-1995, Amended in 2015.
21. Voluntary Certification Scheme for AYUSH Products, Section III, Annexure C, Page 1-2.
22. Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011, Version-IV (26.04.2019).
23. Gomez MR, Cerutti S, Sombra LL, Silva MF, Martínez LD. Determination of heavy metals for the quality control in argentinian herbal medicines by ETAAS and ICP-OES. Food and Chemical Toxicology. 2007 Jun 1;45(6):1060-1064.
24. Gonçalves DA, de Souza ID, Rosa AC, Melo ES, Goncalves AM, de Oliveira LC, do Nascimento VA. Multi-wavelength calibration: Determination of trace toxic elements in medicine plants by ICP OES. Microchemical Journal. 2019 May 1;146:381-386.
25. Guidance document on analytical quality control and method validation procedures for Plants, Animals, Food and Feed Section, Phytopharmaceuticals – Residues SANTE/12682/2019 , Implemented by 01.01.2020.
26. Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis, Official Methods of Analysis of AOAC INTERNATIONAL (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA. Available at: http:// www.eoma.aoac.org/app d.pdf.
27. Guidelines for Standard Method Performance Requirements, Officials Methods of Analysis of AOAC INTERNATIONAL (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA. Available at: http:// www.eoma.aoac.org/app_f.pdf
28. General requirements for the competence of testing and calibration laboratories, INTERNATIONAL STANDARD, ISO/IEC-17025, Third Edition 2017-11, Reference number ISO/IEC 17025:2017(E).
29. Shim J, Cho T, Leem D, Cho Y, Lee C. Heavy metals in spices commonly consumed in Republic of Korea. Food Additives & Contaminants: Part B. 2019 Jan 2;12(1):52-58.
30. Kilic SE, Soylak MU. Determination of trace element contaminants in herbal teas using ICP-MS by different sample preparation method. Journal of food science and technology. 2020 Mar;57(3):927-933.

Table- 1: Selectivity, LOD & LOQ for the proposed method for lead, cadmium, arsenic and mercury.

Heavy metals	Mass	Calibration (R^2)	LOD (µg/kg)	LOQ (µg/kg)
Lead	208	0.9998	5.0	10.0
Cadmium	111	1.0000	5.0	10.0
Arsenic	75	1.0000	5.0	10.0
Mercury	202	0.9994	5.0	10.0

Table- 2: Intra-day and Inter-day precision data for the proposed method for lead, cadmium, arsenic and mercury residues in samples of ajwain, ginger, neem, clove and turmeric.

Concentration µg/kg	Sample	Day 1		Day 2		Day 3		Intra-assay	
		Concn*. obtained (µg/kg) n=7	% RSD	Concn. obtained (µg/kg) n=7	% RSD	Concn. obtained (µg/kg) n=7	% RSD	Concn. obtained (µg/kg) n=7	% RSD
Lead (Pb)									
10	Ajwain	9.85	4.18	9.94	2.89	9.85	2.65	9.89	2.69
	Ginger	9.61	3.66	9.71	3.71	9.83	2.38	9.48	3.55
	Neem	9.67	3.51	9.14	3.20	9.34	2.76	9.57	2.79
	Clove	9.89	2.59	9.62	2.79	9.56	3.61	9.63	2.16
	Turmeric	9.94	3.44	9.96	3.92	9.58	3.31	9.79	2.07
20	Ajwain	19.40	2.11	19.90	2.55	19.15	3.92	19.80	2.71
	Ginger	19.75	2.45	19.45	2.87	20.05	3.15	19.15	2.62
	Neem	19.46	2.31	19.76	2.74	19.23	3.21	19.61	3.46
	Clove	19.04	2.07	20.01	2.46	19.18	2.43	19.25	3.11
	Turmeric	19.99	2.94	20.13	2.64	19.88	2.31	19.94	2.10

50	Ajwain	49.70	2.85	49.83	3.42	49.72	1.25	50.22	3.35
	Ginger	46.07	2.51	45.30	2.64	47.60	2.19	48.11	2.16
	Neem	49.24	2.11	48.95	2.55	47.15	3.92	47.80	2.71
	Clove	49.75	2.25	49.45	2.87	50.05	3.51	49.15	2.62
	Turmeric	48.96	3.95	49.61	2.46	49.13	1.24	49.02	2.71
Cadmium (Cd)									
10	Ajwain	9.82	3.82	9.91	3.07	9.63	2.39	9.66	1.83
	Ginger	9.49	3.75	9.79	2.88	9.91	3.17	9.74	2.89
	Neem	9.62	1.49	9.67	3.19	9.84	2.44	9.96	3.79
	Clove	9.87	2.37	9.61	2.04	9.76	3.21	9.90	3.01
	Turmeric	9.94	1.98	9.98	2.16	9.96	2.94	9.83	3.48
20	Ajwain	18.94	2.34	19.72	2.73	19.16	1.68	19.43	3.80
	Ginger	19.24	3.46	18.96	3.19	19.01	3.49	19.49	2.91
	Neem	19.38	3.81	19.82	3.37	19.47	2.94	19.88	1.99
	Clove	19.57	2.01	19.94	3.19	19.87	1.79	19.07	2.08
	Turmeric	19.99	2.94	20.13	2.64	19.88	2.31	19.94	2.10
50	Ajwain	49.30	2.83	48.30	3.05	48.90	2.34	50.60	3.56
	Ginger	49.90	3.59	49.80	3.00	50.50	2.45	49.20	3.95
	Neem	48.35	1.88	47.35	1.52	48.80	2.22	49.15	1.96
	Clove	49.70	2.97	49.05	1.99	48.75	2.86	49.13	2.34
	Turmeric	49.11	**1.02**	49.62	1.07	49.31	1.46	49.85	1.79
Arsenic (As)									
10	Ajwain	09.57	3.89	9.78	3.09	9.99	2.70	9.71	3.32
	Ginger	9.59	2.96	9.63	3.19	9.97	3.11	9.59	2.13
	Neem	9.97	1.86	9.64	2.35	9.75	3.49	9.89	1.87
	Clove	9.84	2.19	9.97	2.48	9.76	2.88	9.91	1.64
	Turmeric	9.92	2.30	9.83	3.76	9.95	2.14	9.76	2.79
20	Ajwain	19.85	2.18	18.50	2.61	18.45	2.95	18.95	2.64
	Ginger	19.80	3.73	19.25	3.26	18.40	2.61	19.15	3.16
	Neem	19.77	2.96	19.40	2.91	18.94	2.07	19.08	2.13
	Clove	18.87	2.47	18.98	1.85	19.19	2.16	19.67	2.45
	Turmeric	19.29	3.12	19.84	1.23	19.04	2.10	19.98	3.09

50	Ajwain	49.50	1.80	50.08	2.97	49.90	2.93	48.90	2.77
	Ginger	48.70	2.11	49.13	3.58	49.60	2.54	48.86	3.06
	Neem	48.85	2.18	48.56	2.61	48.41	2.95	48.19	2.64
	Clove	48.80	3.73	49.25	3.26	49.40	2.61	48.72	3.16
	Turmeric	50.13	3.49	48.21	2.94	49.57	2.37	49.87	1.66
Mercury (Hg)									
10	Ajwain	9.59	2.81	9.57	1.86	9.71	3.87	9.59	2.91
	Ginger	9.46	3.19	9.49	2.33	9.37	3.19	9.79	2.43
	Neem	9.57	3.99	9.43	3.17	9.18	2.43	9.35	2.65
	Clove	9.08	3.09	9.17	3.40	9.64	3.97	9.11	3.22
	Turmeric	9.16	3.10	9.07	2.04	9.27	2.82	9.49	2.73
20	Ajwain	18.28	3.37	18.63	3.92	18.57	3.68	19.28	2.19
	Ginger	19.63	2.23	19.53	2.06	18.49	2.15	19.37	3.29
	Neem	19.03	2.31	18.92	2.85	18.64	3.06	18.24	2.15
	Clove	18.27	2.13	19.39	3.11	18.63	3.87	18.94	3.64
	Turmeric	18.87	2.16	18.61	2.55	18.37	3.13	19.09	1.50
50	Ajwain	47.80	1.95	48.34	3.62	49.60	1.09	47.69	2.17
	Ginger	47.61	1.87	48.90	2.54	47.50	1.12	48.70	2.77
	Neem	46.95	2.47	47.30	3.56	49.05	2.54	49.55	2.08
	Clove	47.30	2.86	46.90	2.17	49.20	2.93	49.01	2.13
	Turmeric	47.62	1.62	48.97	2.13	47.93	1.09	49.76	1.99

***** Concentration

Table- 3: Percent recovery of lead, cadmium, arsenic and mercury from ajwain, ginger, neem, Clove and turmeric samples analysed on different days (n=7).

Concentration µg/kg	Sample	Day-1	Day-2	Day-3	Intra-assay
		% Recovery	% Recovery	% Recovery	% Recovery
Lead (Pb)					
10	Ajwain	98.50	99.40	98.5	98.9
	Ginger	96.10	97.10	98.3	94.8
	Neem	96.70	91.40	93.4	95.74
	Clove	98.90	96.20	95.6	96.30
	Turmeric	99.40	99.60	95.8	97.90
20	Ajwain	97.00	99.50	95.75	99.01
	Ginger	98.75	97.25	100.25	95.75
	Neem	97.30	98.80	96.15	98.05
	Clove	95.20	100.05	95.91	96.25
	Turmeric	97.30	99.35	97.30	95.65
50	Ajwain	99.40	99.66	99.44	100.44
	Ginger	92.14	90.60	95.23	96.22
	Neem	98.48	97.90	94.30	95.64
	Clove	99.50	98.90	100.10	98.30
	Turmeric	97.92	99.22	98.26	98.04
Cadmium (Cd)					
10	Ajwain	98.20	99.1	96.3	96.6
	Ginger	94.90	97.9	99.10	97.4
	Neem	96.20	96.7	98.43	99.60
	Clove	98.70	96.1	97.61	99.00
	Turmeric	99.40	99.80	99.60	98.3
20	Ajwain	94.70	98.60	95.80	97.15
	Ginger	96.20	94.80	95.05	97.45
	Neem	96.90	99.10	97.35	99.4
	Clove	97.85	99.70	99.35	95.35
	Turmeric	99.95	100.65	99.42	99.71

50	Ajwain	98.60	96.60	97.80	101.21
	Ginger	99.80	99.61	101.01	98.40
	Neem	96.70	94.70	97.60	98.32
	Clove	99.40	98.1	97.5	98.26
	Turmeric	98.22	99.24	98.62	99.7
Arsenic (As)					
10	Ajwain	95.70	97.8	99.9	97.1
	Ginger	95.90	96.3	99.7	95.9
	Neem	99.70	96.4	97.5	98.9
	Clove	98.40	99.7	97.6	99.1
	Turmeric	99.20	98.3	99.5	97.6
20	Ajwain	99.25	92.5	92.25	94.75
	Ginger	99.00	96.25	92	95.75
	Neem	98.85	97.0	94.7	95.4
	Clove	94.35	94.9	95.95	98.35
	Turmeric	96.45	99.2	95.2	99.9
50	Ajwain	99.00	100.16	99.8	97.8
	Ginger	97.40	98.26	99.2	97.72
	Neem	97.70	97.12	96.82	96.38
	Clove	97.60	98.5	98.8	97.44
	Turmeric	100.26	96.42	99.14	99.74
Mercury (Hg)					
10	Ajwain	95.90	95.7	97.1	95.9
	Ginger	94.60	94.9	93.7	97.9
	Neem	95.70	94.3	91.8	93.5
	Clove	90.80	91.7	96.4	91.1
	Turmeric	91.60	90.7	89.60	94.9
20	Ajwain	91.40	93.15	92.85	96.4
	Ginger	98.15	97.65	92.45	96.85
	Neem	95.15	94.60	93.2	91.20
	Clove	91.35	96.95	93.15	94.70
	Turmeric	94.35	93.05	91.85	95.45

50	Ajwain	95.60	96.68	99.2	95.38
	Ginger	95.22	97.8	95	97.4
	Neem	93.90	94.6	98.1	99.1
	Clove	94.60	93.8	98.4	98.02
	Turmeric	95.24	97.94	95.86	99.52

Table- 4: Results for the presence of Pb, Cd, As and Hg (mg/kg) in samples of ajwain, ginger, neem, clove and turmeric from all the sources.

Samples	Pb mg/kg	Cd mg/kg	As mg/kg	Hg mg/kg
Ajwain				
Source-D1	0.3572	0.0222	0.0755	0.0430
Source-D2	0.9879	0.0505	0.1274	0.0522
Source-D3	0.7889	0.0548	0.0255	0.0369
Source-V1	0.4221	0.0710	0.0862	0.0686
Source-V2	0.8907	0.0579	0.0941	0.0390
Source-V3	0.3598	0.0261	0.0399	0.0329
Ginger				
Source-D1	1.3490	0.0774	0.0689	0.0755
Source-D2	1.3803	0.0788	0.0817	0.0636
Source-D3	0.7701	0.0425	0.0414	0.0749
Source-V1	0.9943	0.0678	0. 1341	0.0645
Source-V2	1.9801	0.0793	0.0552	0.0442
Source-V3	1.2620	0.0473	0.0620	0.0595
Neem				
Source-D1	0.2467	0.0391	0.0419	0.0282
Source-D2	0.3260	0.0425	0.0274	0.0317
Source-D3	0.1577	0.0337	0.0169	0.0508
Source-V1	0.5820	0.0291	0.0719	0.0214
Source-V2	0.5155	0.0301	0.0241	0.0337
Source-V3	0.3953	0.0578	0.0460	0.0375
Clove				
Source-D1	0.2160	0.0272	0.0886	0.0505
Source-D2	0.1749	0.0417	0.0725	0.0662
Source-D3	0.2450	0.0395	0.0238	0.0560
Source-V1	0.5905	0.0235	0.0362	0.0398
Source-V2	0.7316	0.0512	0.0641	0.0616
Source-V3	0.3611	0.0336	0.0398	0.0580

Turmeric				
Source-D1	1.6529	0.0382	0.0921	0.0734
Source-D2	1.0797	0.0476	0.0974	0.0589
Source-D3	1.3524	0.0384	0.0491	0.0497
Source-V1	2.3447	0.0273	0.0785	0.0677
Source-V2	0.9321	0.0527	0.0918	0.0474
Source-V3	1.5312	0.0480	0.0935	0.0689
As per WHO / Ayush guidelines	10	0.3	3.0	1.0

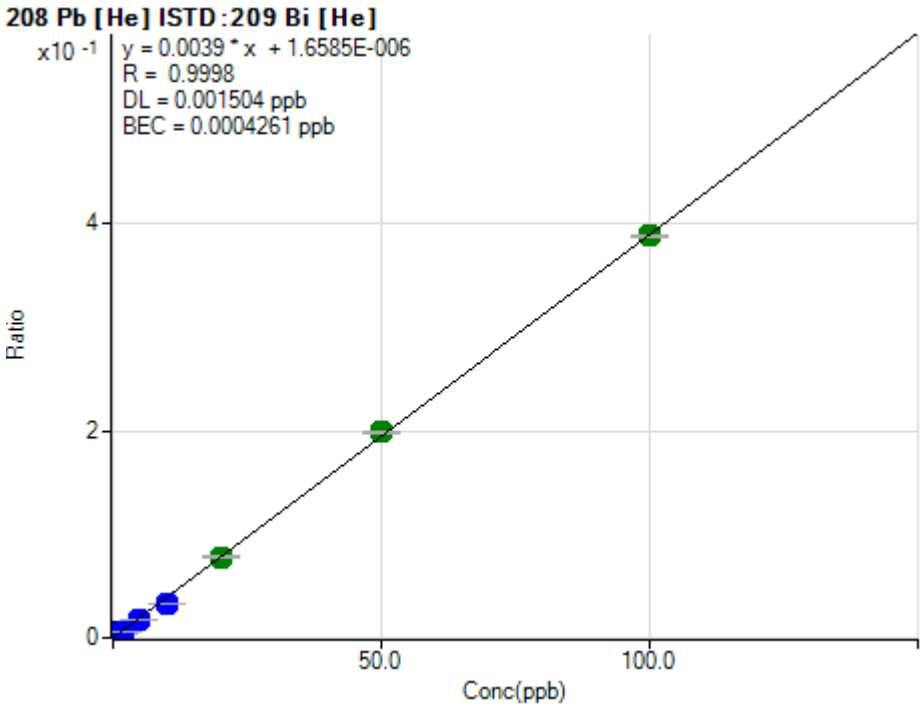

Figure 1: Linearity Curve of Lead (Pb).

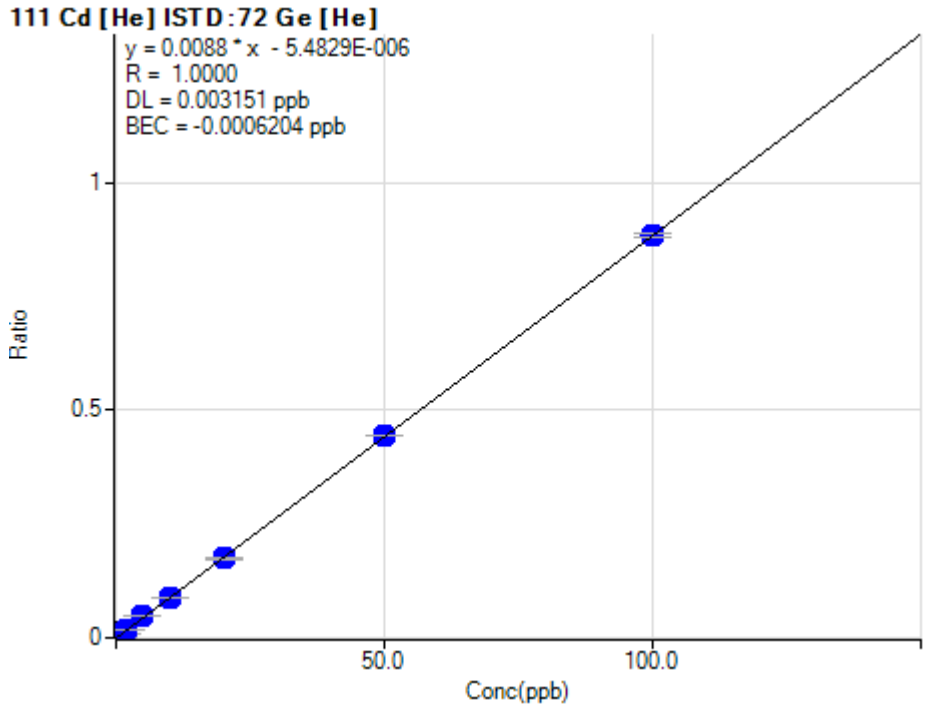

Figure 2: Linearity Curve of Cadmium (Cd).

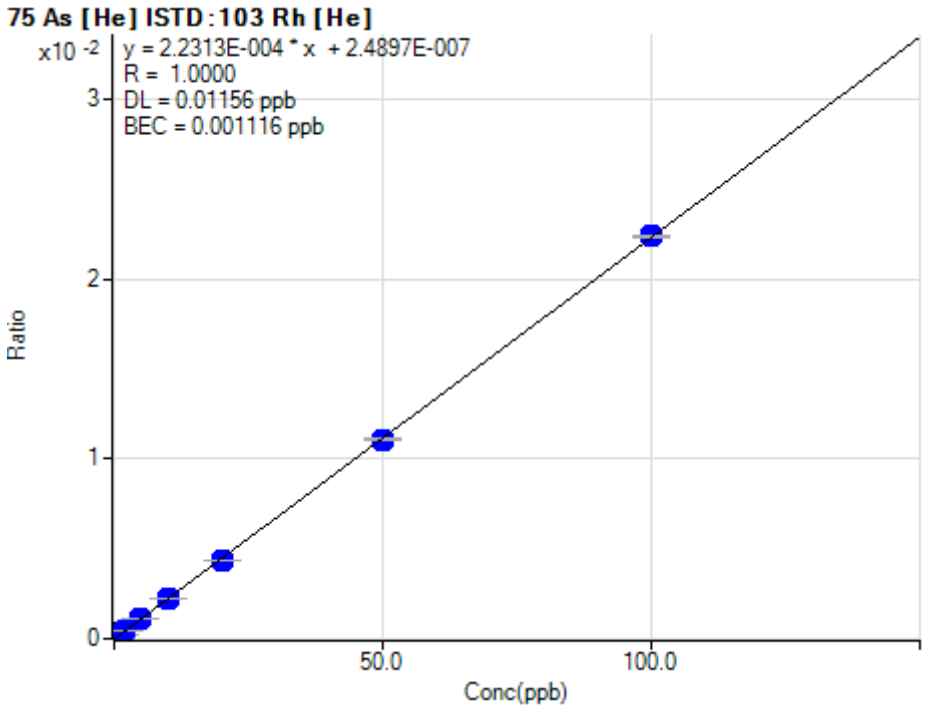

Figure 3: Linearity Curve of Arsenic (As).

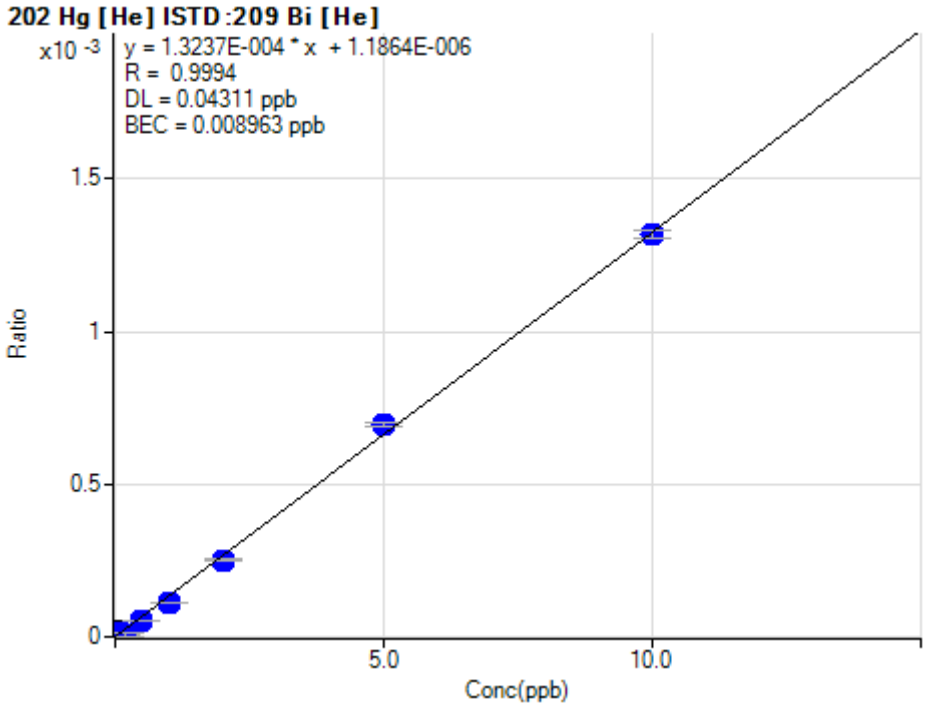

Figure 4: Linearity Curve of Mercury (Hg).