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A VALIDATED METHOD FOR ANALYSIS OF TOXIC HEAVY METAL IN FOOD SPICES BY ICPMS

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Abstract

Spices are considered effective remedies in treatment of various diseases worldwide for thousands of years. Nowadays theses spices have become a quite serious and important issue to be attention. One major concern is presence of heavy metals. The present study was designed to estimate the concentration of toxic heavy metals in food spices of Hamedan city, for the contaminates of Arsenic, Mercury, Tin, Copper, Zinc, Cadmium, and Lead. Total 22 highly used spices with three replications were tested by using ICP-MS. Samples were digested by Microwave digester system (MDS). Results show that maximum samples were meeting the requirement for As, Hg, Cd, Sn, and Pb but some samples were beyond the acceptable criteria for Cu and Zn. Method was optimized and validated for the analysis of heavy metal as per the ICH requirement. To validate the matrix effects repeatability, reproducibility, recovery and overall uncertainty were calculated. Recovery was ranged between 80 to 120% with RSD less than 20.0 %. The linear calibration curves were established using concentration of 0.5, 1.0, 5.0, 10, 20, 50 and 100 ppb of each element with linearity (r^2 >0.998). In conclusion, present study can offer the capability to performed ppb levels of multi-elements measurement with ICP-MS and microwave digester and can be effectively used for determination of heavy metal in spices samples.

Key words: ICP-MS, FSSAI, Validation, MDS, Food Spice and Toxic Heavy metal.

Introduction

The herbal medicines are commercially used in many countries for the reasons such as increasing general interest in natural treatments. Use of different herbal compounds and products has increased in recent years for their useful effects on human health, relative low price and the knowledge deficiency of people about their likely harmful effects. Various vegetal spices are widely applied in human diet all over the world (Saeed *et al*., 2010). The most famous of them are including Turmeric, Cinnamon, Red pepper, Black pepper, sumac and all dried mint. Backside sings of these spices as the flavor for making color and odor in the food, they are also used for their enormous benefits for the human health. For example:- Turmeric has anti-cancer effects, repairs skin damages; Cinnamon regulates blood sugar and overweight; Red pepper has anti-constipation, removes abscess, toothache, different sunburns and eye problems; Sumac blocks bleeding, diarrhea, treat ear infections and eye trachoma, inhibits chickenpox and decreases blood cholesterol, improves nervous system and decreases allergic symptoms (Gupta *et al*., 2003; Nordin and Selamat, 2013). Simultaneously with growing improvements in human life in different sections such as agriculture, industry, transportation and mines, different pollutants like heavy metal have entered the environment, finally penetrating into the food chain and the bodies of consumers. Heavy metals are a group of minerals pollutants that have occupied a considerable part of environment pollution (Viuda-Martos *et al*., 2011). Admittedly, the extent of contamination of the spices with heavy metal varies from one plant to another. Reasons for this variation have been revealed by different studies, which determined the level of Cd, Fe, Cu, Zn, and Pb in spices, aromatic and medicinal plants from different regions and confirmed that the tendency to accumulate heavy metal in spices depends plant origin (Martena *et al*., 2010). These elements are toxic for the living organisms even in low concentration and not decomposed in their bodies. Metabolism and removing these toxic elements in the body and their absorption is slower, leading the toxicity, diseases and even death of living organisms depending on their entered values. Since spice and different herbal flavors are frequently and daily use in the diets, therefore determining the level of these elements is significantly important. Heavy metal can have a direct impact on human body health and in case of imbalance of their values in food materials, the food can turn in to a harmful factor for human body (Robert *et al*., 2008). Heavy metal toxicity can affect mental development and central nervous system function, alter the blood composition, and disturb the functioning of organs like the kidneys, lungs, and liver (Fiamegos *et al*., 2016). Therefore, the risks associated with metal contamination in foodstuffs are of great concern. Since no study has been conducted on measuring concentration of heavy metals in food spices of Hamedan city, this research aimed to detect the concentration of heavy metal (Zinc, copper, mercury, arsenic, Cadmium, antimony and Lead) in the mostly consumed spices and flavors collected in Hamedan city during 2019 and comparing them with global standards.

Materials and Methods

Samples and chemicals

Total 22 spices, 5 different brand with three replications (in total 129 samples) including Cinnamon, Turmeric, Black pepper, Sumac, Red pepper, and dried mint were purchased

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randomly from the stores in the period of 2019. A powder was obtained for whole spices by crushing & grinding. No further drying was applied to these samples, because in the same form they are ingested. All samples are stored at 4^0C till further analyses. All used containers and glasses were immersed in 0.1 Normal Nitric acids for 24h. Then, all containers were rinsed with distilled water and dried in electrical oven. HPLC and AR grade analytical solvents were used in the analysis and purchased from Merck, Delhi, India). The Multi-Element reference standards were obtained from Sigma- Aldrich (Sigma- Aldrich, St. Louis, MO, USA**)**. Apparatus; Blender (Inter science, Japan), Vortex mixture (Jain Sci. India), Weighing balance (Jain Brother) Centrifuge, Sigma 2-16 K (SV Instrument, Delhi, India) was used. Microwave assisted digestion was carried out using Microwave reaction system (Multiwave 3000Solv, Anton Paar, Europe). Agilent 7700x Series ICP-MS was used for quantitative analysis of heavy metal. For preparation of mixed standard concentration Reference standard was diluted 0.5, 1.0, 5.0, 10, 20, 50 and 100 ppb of each element and make up with 2% nitric acid.

Microwave Digestion System and Instrument conditions

Microwave digestion was carried out using Microwave reaction system (Multiwave 3000Solv, Anton Paar, Europe). Accurately weigh 1.0 ±0.1gm of each sample. Add 5 ml of HNO_3 and 2 ml of 30 % H_2O_2 in the digestive vessel (AOAC Official Method, Ed. 20th 2016.). Samples were digested using the following microwave program: Power- 850 (W), Ramp time (min) 20, Temp (^{o}C) 180 ± 5, hold time (min) 10. After finish the process make up the volume to 10 ml with deionized water. Filter the samples with 0.45 µ membrane filter papers and introduce the filtrate into the ICP/MS for analysis. ICP-MS system was setup according to the AOAC Official Method Ed. 20th 2016). The calibration graph of mix metal was constructed using seven different concentrations of standards mix solution. The ICP-MS System was calibrated by the method of external standards with Rh, Re as the internal standard. The reagent blank solution contained 1% of concentrated HNO_3. Mixed standard solutions containing 27 elements (Agilent), were prepared in reagent blank solutions. The back ground interferences from the plasma gases, air entrainment and solvent were corrected by subtraction of reagent blank signal. The isobaric spectral interferences originating from the polyatomic ion species involving the sample matrix element was eliminated by selecting a suitable isotope, corrected or reduced by applying interference correction equation (Sample Application Handbook, Agilent 7700x Series ICP-MS) results were expressed as µg per gm.

Quality parameter of method

To evaluate the method performance the following parameters were checked: sensitivity, linearity, precision, recovery, accuracy and selectivity. The sensitivity of method was evaluated by determining the limit of detection (LOD) and limit of quantitation (LOQ) by measuring the magnitude of analytical background by injecting the blank. In this study, LOD was determined by injection a series of solutions until the height of the peak signal to baseline noise ratio (S/N) was 3:1, while limit of quantitation (LOQ) values were taken at S/N 10:1. The linearity of the method was investigated by spiking blank samples with known concentrations of standard at five-concentration level. Reagent blank was used to check for contamination.

In order to check the accuracy of digestion was evaluated through recovery study by spiking the samples with all reference standard at three different concentrations (0.1 mg kg^{-1}, 0.05 mg kg^{-1} and 0.025 mg kg^{-1}) and three replicates for each concentration were together with a calibration curve to perform and established the repeatability (intra-day precision) and intermediate precision (inter-day precision). The precision is represented by the intra- and inter-day relative standard deviation (%RSD). The within-day accuracy and precision were determined with three replicate on a single day, while the between-day accuracy and precision was carried out over five consecutive days. The different spiking levels were carried out to reflect the sensitivity of the detector towards the different concentration. The accuracy/recovery was determined as the mean of the measured value relative to the theoretical spiked values and is reported in percentage (%).

Statistical analysis

All the data was reported as mean ± SD. Analysis of variance was performed using the ANOVA procedure. Statistical analysis performed according to SAS software. Differences at $P < 0.05$ were considered statistically significant.

Results and Discussion

The concentrations of heavy metals in the analyzed spices are given in table 1. Data is reported on the basis of mean ± standard deviation. Almost results in the spices samples decreased in following sequence: Zn>Cu>As>Cd>Sn=Hg=Pb. Arsenic is one of the most toxic trace elements. Inorganic As including As (III) and As (V) is more toxic than organic arsenic species. Exposure to inorganic As is associated with skin lesions and increased risk of developing cancer of the skin, lungs, liver, and kidney (Dos *et al*., 2015). The concentrations of arsenic were found within the range of specified value according to FSSAI & Codax standard. Zinc is an integral component of a wide variety of different enzymes and plays catalytic, structural, and/or regulatory roles (Maiga *et al*., 2005). Zinc deficiency has been known to cause growth retardation and hypogonadism. Several mechanisms of growth retardation and hypogonadism due to zinc deficiency have been suggested. Zinc affects growth hormone (GH) metabolism. Conversely, Growth Hormones affects zinc metabolism. Zinc deficiency may also affect bone metabolism and gonadal function (Nishi, 1996). Just as inordinately high amounts of zinc could be more deleterious than nutritious, Therefore many agency set permissible limit/acceptable criteria. In this study, the Zn value was assessed for the different brand of turmeric 12.88 ±0.04 as minimum value and mustard seed 108.63 ± 0.06 as maximum value. The sample of *trachyspermum ammi, Brassica nigra* were not meeting the requirement according to the FSSAI requirement while, rest of the sample were below the limit and may be considered tolerable. The highest value for Cu was registered for Myristica fragrans while, highest value for Cd was for *Cinnamon* 22.45±0.04; 1.06± 0.04 respectively. However, the result for antimony, mercury and lead were found within the requirement according to the FSSAI and codex alimantarius commission. Lead exposure has been shown to cause severe anemia, permanent brain damage, neurological disorders, reproductive problems, diminished intelligence and a host of other diseases. According to the Agency for Toxic Substances and Disease Registry, a division of the U.S. Public Health Service, the major exposure of lead to the general population in food is through fruits, vegetables and grains (McNamara, 2008, Marian *et al*., 2010). However, the results of this study show that there are relatively significant levels of lead in the spices sampled.

Concentration of heavy metal is directly may affect by the origin of plant. Contamination of water, air pollution, industrial activities, and uses of fertilizers can contribute for the presence or amount of heavy metal. Plants absorb metal ions from the soil through their roots. The concentration of heavy metal is not uniformly distributed throughout the plant. In general the root contains the highest levels of heavy metal, followed by vegetative tissue, which has the higher concentration than seeds (Maiga *et al*., 2005). The degree of element uptake by plant root is depends on many factor including the soil, pH content, the presence or absence of competing ions, rooting depth, age and seasonal growth effects, chemical form of the trace elements present (Nordin and Selamat 2013). Any comparison cannot make for this study because of the sample type, sample collection point and different brand.

Quality parameter of method

Several parameters have been taken into account and evaluated for the validation of the analytical methods for quantitative determination of toxic metals in spices.

Linearity

Linearity was assessed by using calibration solutions, calibration curves y= ax+ b were determined; y is the signal intensity, x is the known concentration of the given analyte in the calibration solution. The linearity of the method was obtained by least-squares linear regerration analysis of the peak area versus analytes concentration in triplicates. The correlation coefficient (r^2) is shown in table-2.

LOD and LOQ

The Limit of detection (LOD) is the lowest concentration of the analyte in a sample, which can be detected but not necessarily quantified. The LOQ is the lowest concentration of the analytes in a sample, which can be quantified with an acceptable degree of accuracy and precision. To determine the limit of detection, Ultra pure water of 18.2 MWcm-1 was aspired and signal intensities for blank were recorded. A solution of 10 µg/l for As, Ni, Cd, Hg, Pb, Cr was aspired and the signal intensities for these analytes were recorded (table 3). The limit of detection was calculated by the equation (1), where: SD blank is the standard deviation for the signal recorded on the blank for the element studied, conc sample is the concentration [µg/l] of the analyte in the sample, I sample, I blank are the signal intensities recorded for the sample and blank respectively.

$$LOD = 3.SD_{blank} * conc_{sample} / [I_{sample} - I_{blank}] \qquad \text{Equation (1)}$$

The limit of quantification (LOQ) is the lowest concentration that can be quantitatively determined with an acceptable level of repeatability accuracy. The quantification limit is generally considered to be approximately ten times the minimum detection limit. In order to verify that, three standard solution of 0.01, 0.05, 0.1 µg/l of multielement standard were prepared and aspired in the ICP-MS inlet system. The maximum measurement limit is conditioned by the dynamics of the spectrometer detectors and limited by the requirement that the total amount of the dissolved solid must not exceed 0.2% in the sample solution (unless clogging of the nebulizer nozzels would lead to instabilities and loss of sensitivity).

Precision, Recovery and Accuracy

Precision, Recovery and Accuracy expresses the correlation between the arithmetic mean of the measured values and the accepted reference value. Considering the "true" concentration of 2 µg/l, the bias (%) was calculated for the elements taken into consideration (0.8-17%). The repeatability of an analytical method refers to the use of the procedure within a laboratory over a short period of time, carried out by the same analyst with the same equipment. The inter-day accuracy and repeatability were assessed, at three concentration levels with three replicates for each concentration on the same day. While the intermediate precision was based on the mean repeatability values of a set of spiked samples at three concentration levels and analysed daily for a period of 5 days. **Table 3** shows the mean repeatability of the method for the investigated compounds in the spiked samples. Results with less than 20% relative standard deviation (RSD) and 80-120% of recovery were accepted as satisfactory. The results of inter day precision show good robustness of the method with mean a value as % RSD of less than 20 %.

Selectivity

The selectivity of method was assessed by comparing the spectra obtained with or without the analytes in the blank samples. Matrices with each element were injected separately to ensure that no interfering peaks were present. In order to check the reproducibility a standard mixture solution was also analyzed at three different concentrations under the optimum conditions in the experiment. The within-day accuracy was determined with three replicate on a single day, while the between-day accuracy was carried out over five consecutive days. The relative standard deviation (RSD) of the peak area was ranged 0.5-2.5 for intra-day and 2.1-3.6 for inter-day analysis, while and relative standard deviation (RSD) of retention time was 0.05-0.4 for intra -day and 0.25-1.16 for inter-day analysis (RSD < 2.0%).

Overall uncertainties

Due to difficulty in calculating the individual uncertainty contributions a "bottom up" procedure was followed as proposed by the ISO guide (Gum, 1993) and the different contributions were grouped as recommended by the Eurachem/CITAC guide (Eurachem, 1995). The contributions in the MAE–ICP/MS method can be grouped in three terms, permit the calculation of the overall uncertainty according to the following equation:

$$Ur = r \times k \quad \sqrt{(u(CRM))^2 + (u(Rep))^2 + (u(Bias))^2}$$

The first term (*u*CRM) corresponds to the relative uncertainty from the certified reference material used for calibration and the subsequent uncertainties introduced by the balance, volumetric material, etc. during weighing and diluting to the final concentration. The second term (*u* (Rep)) corresponds to the relative uncertainty of contribution due to the precision of the method, also called repeatability uncertainty, which gives a value for the standard uncertainty due to run-to-run variation, day-to-day variation, analyst-to analyst variation and commodity-to-commodity variation of the overall analytical process. (*u* (Bias)) is the relative uncertainty due to *bias* i.e. corresponds to the tolerance that each laboratory establishes for their internal quality controls of the analytical procedure, investigated during the in house validation study using spiked samples (homogenized sample were split and spiked). Finally, *k* and r are the coverage factor and reported result respectively to expand the uncertainty to the desirable level of confidence with desirable units of the measurement. The second and third terms are generally the most important contributions to the overall uncertainties. In the present work, the overall uncertainties were calculated at 0.025, 0.05 and 0.100 µg gm^{-1} level. The *u*CRM was calculated by taking into account of all the dilution steps and the uncertainties from the CRM and all the volumetric material

and balances used to prepare the calibration standards. The second term *u*Rep was calculated from the *n*=6 results (each sample) from the experiment performed under repeatable and reproducible conditions. The third term was calculated considering mean recovery of samples with recovery 80.2% to 120% with RSD less than 20.0 %, tolerance that the laboratory accepts as a maximum for the verification of the daily analysis. Finally, a coverage factor $k = 2$ was used for a confidence interval of 95% as (n =6). As shown in **Table 3.** The uncertainties calculated are within 25%. In our case, the tolerance was stated as 25% for practical purposes; nevertheless, the uncertainties of the method can be reduced with a more exigent tolerance in the daily verification by doing the study at higher concentration.

Conclusion

More and more, our attention turns the medicines offered by nature. Therefore many plants based medicines have been developed. Heavy metal may accumulate in the body and led to health issues. This study may be helpful for the estimation of heavy metal in raw material in daily life and used for the preparation of compound formulation drugs. The results obtained by all experimental study suggested this method has a great potential for the digestion and analysis of heavy metal by ICPMS in spices. The observed concentrations of heavy metal for selected spices were found well under prescribed values except a few. Several statistical parameters have been taken into account and evaluated for validation of method with good recovery, RSD and uncertainty. Results suggest that it should be favored for routine analysis of heavy metal.

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Table 1: Heavy metal concentrations in different brands of spices

Sample Name	Mean concentration ± SD (µg g−1)						
	Cu	Zn	As	Cd	Sn	Hg	Pb
Cuminum Cyminum (Cumin)							
Brand 1	13.80 ± 0.05	53.01 ± 0.12	0.06± 0.01	0.07± 0.04	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 2	15.00 ± 0.14	54.00 ± 0.11	0.05± 0.17	0.06± 0.04	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Coriandrum Sativum (Coriander)							
Brand 1	18.65± 0.23	47.05± 0.24	0.03± 0.22	0.19± 0.24	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 2	18.10± 0.14	47.35± 0.11	0.03± 0.04	0.08± 0.64	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 3	18.22± 0.04	46.25± 0.15	0.02± 0.34	0.11± 0.22	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Cinnamomum Verum (Cinnamon)							
Brand 1	8.50± 0.24	15.66± 0.24	0.05± 0.12	1.06± 0.09	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 2	8.10± 0.74	15.27± 0.12	0.05± 0.07	1.36± 0.04	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)

Sample Name	Mean concentration ± SD (µg g−1)						
	Cu	Zn	As	Cd	Sn	Hg	Pb
Brassica nigra (Mustard seed)							
Brand 5	16.10± 0.04	108.63±0.44	0.05± 0.22	0.12± 0.24	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 3	15.20± 0.04	111.21±0.12	0.05± 0.17	0.10± 0.40	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Piper Nigrum (White Pepper)							
Brand 4	25.17± 0.01	18.25± 0.41	0.02± 0.34	0.09± 0.45	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 5	25.87± 0.22	17.15± 0.19	0.03± 0.14	0.10± 0.34	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Piper Nigrum (Black Pepper)							
Brand 3	17.96± 0.11	24.40± 0.30	0.03± 0.22	0.06± 0.44	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Curcuma longa (Turmeric)							
Brand 1	3.30± 0.24	11.88± 0.24	0.02± 0.22	0.04± 0.19	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 2	2.96± 0.80	10.17± 0.44	0.02± 0.29	0.03± 0.04	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 5	2.10± 0.22	12.33± 0.10	0.02± 0.40	0.04± 0.70	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Trigonella foenum-graecum (Fenugreek)							
Brand 3	17.85± 0.04	46.02± 0.04	0.03± 0.04	0.07± 0.04	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 4	17.85± 0.04	46.02± 0.04	0.03± 0.04	0.07± 0.04	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Trigonella foenum-graecum (Fenugreek Leaf)							
Brand 4	12.67± 0.11	39.11± 0.74	0.30± 0.22	0.11± 0.44	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 2	11.22± 0.56	41.33± 0.55	0.27± 0.09	0.12± 0.87	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Ferula assa-foetida (Asafoetida)							
Brand 3	5.10± 0.34	17.60± 0.22	0.05± 0.14	0.05± 0.44	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Syzygium aromaticum (Cloves)							
Brand 5	9.29± 0.24	44.90± 0.32	0.04± 0.64	0.04± 0.02	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Elettaria Cardamomum (Green Cardamom)							
Brand 1	17.01± 0.03	49.81± 0.64	0.03± 0.02	0.09± 0.04	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 2	15.01± 0.16	48.20± 0.42	0.13± 0.13	0.09± 0.24	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Trachyspermum ammi (Carom)							
Brand 3	16.97± 0.64	91.06± 0.01	0.12± 0.11	0.12± 0.04	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Myristica Fragrans (Mace)							
Brand 5	16.52± 0.05	13.74± 0.14	0.02± 0.74	0.06± 0.25	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Mangifera indica (Dried Mango)							
Brand 3	11.22± 0.24	15.90± 0.44	0.15± 0.14	0.04± 0.14	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 5	11.18± 0.05	16.10± 0.62	0.16± 0.11	0.04± 0.22	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Foeniculum Vulgare (Fennel)							
Brand 3	18.70± 0.26	42.15± 0.10	0.07± 0.52	0.05± 0.02	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 5	17.26± 0.24	44.65± 0.16	0.05± 0.04	0.05± 0.22	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 2	17.67± 0.11	44.95± 0.04	0.04± 0.24	0.05± 0.51	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Capsicum annum (Red Chilli)							
Brand 1	12.94± 0.06	24.15± 0.17	0.01± 0.12	0.12± 0.51	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 2	12.14± 0.52	25.00± 0.27	0.01± 0.4	0.11± 0.55	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 3	13.12± 0.16	24.95± 0.17	0.01± 0.11	0.12± 0.53	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Zingiber Officinale (Dried Ginger)							
Brand 3	19.46± 0.24	33.67± 0.50	0.06± 0.04	0.10± 0.11	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Amomum (Black Cardamom)							
Brand 3	7.58± 0.24	42.07± 0.04	0.12± 0.04	0.07± 0.05	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 2	7.11± 0.54	41.09± 0.14	0.02± 0.14	0.17± 0.22	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)

Sample Name	Mean concentration ± SD (µg g−1)						
	Cu	Zn	As	Cd	Sn	Hg	Pb
Laurus Nobilis (Bay Leaf)							
Brand 5	11.20± 0.54	19.21± 0.22	0.05± 0.54	0.04± 0.94	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 3	11.80± 0.92	19.11± 0.04	0.05± 0.21	0.04± 0.44	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Myristica fragrans (Nutmeg)							
Brand 3	25.49± 0.64	24.15± 0.74	0.01± 0.64	0.05± 0.22	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 1	22.45± 0.24	24.85± 0.24	0.01± 0.84	0.05± 0.04	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Lllicium Verum (Star Anise)							
Brand 4	21.22± 0.44	20.83± 0.59	0.08± 0.24	0.05± 0.41	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)
Brand 3	21.72± 0.70	20.13± 0.84	0.08± 0.11	0.04± 0.33	BLQ(0.05)	BLQ(0.05)	BLQ(0.05)

Table 2: Operating parameter Mass spectrometer ICP-MS

Plasma Gas Flow	15 L/m
Nebulizer Gas Flow	1L/m
ICP RF Power	1550
Lens Voltage	6.00-7.00 Voltz
Detector	Dual
Internal Standard	^{103} Rh, ^{187} Re
Nebulizer	Cross Flow
Sample Flow	About 1 ml/min
Spetra Scanning	Peek hopping
Auxiliary	1L/min
Stabilization Time	30 min
Analog Stage Voltage	-2100 Voltz
Dwell Time	100 ms
Integration Time	2000 ms

Table 3: Chosen isotopes and validation parameter of method

Element	Mass	R^2	LOD (µg gm^{-1})	LOQ (µg gm^{-1})	% Recovery ±SD	Intra-day Precision (%RSD)	Inter-day Precision (%RSD)	RSD (%) for retention time	RSD (%) for retention time	RSD (%) for Peak Area	RSD (%) for Peak area	± UM
								Intra-day	Inter-day	Intra-day	Inter-day	
As	75	0.9957	0.05	0.05	82.0 ± 3.5	11.9	12.7	0.36	1.12	2.3	2.7	22.3
Hg	201	0.9929	0.05	0.05	95.2 ± 2.7	10.0	11.1	0.40	1.04	0.6	2.1	20.5
Sn	118	0.9946	0.05	0.05	92.0 ± 4.1	7.2	9.5	0.20	1.02	1.0	2.5	21.6
Cu	63	0.9962	0.05	0.05	80.2 ± 2.6	8.5	10.5	0.15	0.96	2.8	3.5	22.0
Zn	66	0.9982	0.05	0.05	103 ± 7.7	5.3	9.9	0.09	1.16	1.1	2.9	24.6
Cd	111	0.9965	0.05	0.05	97.0 ± 6.4	11.2	12.0	0.15	1.00	2.6	3.6	23.4
Pb	208	0.9967	0.05	0.05	80.4 ± 3.9	8.6	10.1	0.02	0.60	2.0	3.5	22.9

r^2(coefficient of regression), LOD (µg gm^{-1}) (Limit of detection), LOQ (µg gm^{-1}) (Limit of quantification), data are expressed as mean (Recoveries (%) RSD %, n=3 for intraday and n=3 for inter day analysis). Intra-day precision (Repeatability) & Inter-day precision expressed as pooled RSD and overall uncertainties expressed as percent (k=2) calculated at LOQ level of the investigated heavy metal using the optimized MAE-ICP/MS method.