APPLICATION NOTE

Atomic Absorption

Author

Praveen Sarojam

PerkinElmer, Inc.
Shelton, CT 06484 USA

Analysis of Total Mercury in Chinese Spice Mixtures using Flow Injection Cold Vapor Atomic Absorption Spectrophotometry

Introduction

Spices are often used as dietary components to improve color, aroma, palatability and acceptability of food. Most spices are fragrant, aromatic and pungent.[1] Spices and herbs, grown widely in various regions of the world, have been used for several purposes since ancient times – in folk medicine as antiscorbutic, antispasmodic, tonic, carminative agents against bronchitis, ulcers and as diuretics, depuratives and vermifuges. Also, some species are used as tea flavoring agents in several regions.

Natural food spices such as pepper and mustard have been reported to contain significant quantities of some trace metals.[2] These trace metals in spices and medicinal plants play a vital role as structural and functional components of metallo proteins and enzymes in living cells.[1] Heavy metals have important positive and negative roles in human life. Some of the heavy metals are considered essential including iron, zinc and copper,[3] whereas some other metals, like mercury, have toxic roles in biochemical reactions in our body. Mercury is distributed throughout the environment in a number of different forms – it exists mainly as elemental mercury vapor in the atmosphere, while most of the mercury found in water, sediments, soil, plants, and animals is in the inorganic and organic forms (for example methylmercury) of the element.[4] The habitual addition of mercury-contaminated spices

PerkinElmer®
For the Better

to food may result in accumulation of this toxic metal ion in human organs.[1] Developing fetuses are the most sensitive to the toxic effects of methyl mercury – it has been proven that children who are exposed to methyl mercury before birth may be at increased risk of poor performance on neuro-behavioral tasks, such as those measuring attention, fine motor function, language skills, visual/spatial abilities and verbal memory.[5] Contamination with mercury may be accidental (e.g. contamination of environment during plant cultivation) or deliberate (in some cultures, according to traditional belief, specially treated heavy metals are associated with health benefits and are thus an intentional ingredient of traditional remedies).

The India and China regions have a high diversity of plants used as spices, herbs, and traditional medicines. Several spices and herbs are either produced on small farmlands or naturally grow in different regions. There is often little information available about the safety of those plants and their products with respect to mercury contamination. Due to significant amounts of spices consumed, it is important to know the mercury content in these products.

The objective of this work is two-fold: (1) to accurately analyze the total mercury levels that may be present in some major spice brands available in the local markets in China, by using cold vapor atomic absorption spectrophotometry (CVAAS); (2) to cross-reference these measured levels to the recommended limit specified by the U.S. FDA.

Experimental

The determination of mercury by flow injection-cold vapor atomic absorption spectrophotometry (FI-CVAAS) was performed using a PerkinElmer® FIAS-400 system (Shelton, CT, USA), connected to a PerkinElmer AAnalyst™ 800 Atomic Absorption Spectrophotometer equipped with the intuitive WinLab32™ for AA (Version 6.5) software, which features all the tools to analyze samples, report and archive data and ensure regulatory compliance (Figures 1 and 2).
A PerkinElmer high-energy mercury electrodeless discharge lamp (EDL) was used as the line source. The mercury absorbance was measured at 253.7 nm. The flow injection system consists of a six-port injection valve, two peristaltic pumps and a gas/liquid separator. A quartz cell with a path length of 160 mm and a diameter of 7 mm was used as the atomizer. Tygon® pump tubings were used to deliver sample and reagents as well as to remove waste from the gas/liquid separator. The optimized instrumental parameters along with sample and reagent flow rates, the concentration of reagents, argon stripping gas etc. are given in Table 1 (Page 3). The flow-injection program followed for the analysis of mercury is shown in Table 2 (Page 3).

A Multiwave™ 3000 Microwave Sample Preparation System (PerkinElmer/Anton-Paar) was used for the microwave-assisted digestion. This is an industrial-type oven which is equipped with various accessories to optimize sample digestion. The samples were digested in the 8XF100 rotor using eight 100 mL high-pressure vessels made of PTFE-TFM protected with individual ceramic jackets. TFM is chemically modified PTFE that has enhanced mechanical properties at high temperatures compared to the conventional PTFE. This vessel has a working pressure of 60 bars (870 psi) and temperatures of up to 260 °C.

Figure 1. PerkinElmer FIAS 400 Flow Injection System for atomic spectroscopy.

Figure 2. PerkinElmer AAnalyst 800 Atomic Absorption Spectrophotometer.

Figure 3. PerkinElmer/Anton-Paar Multiwave 3000 Microwave Sample Preparation System.

Table 1. **Optimized experimental conditions of FIAS 400 and AAnalyst 800.**	
Element	Hg
Wavelength	253.7 nm
Slit	0.7H nm
Mode	AA
Calibration	Linear through zero
Lamp	EDL
Current	185 mA
Standards	2.5, 5.0 & 10.0 µg/L
Correlation Coefficient	0.9999
Spike	1.0 µg/L
Read Time	15.0 sec
Carrier Solution	3% (V/V) HCl
Carrier Flow Rate	9-11 mL/min
Reductant	0.2% $NaBH_4$ in 0.05% NaOH
Reductant Flow Rate	5-6 mL/min
Carrier Gas	Argon
Carrier Gas Flow	50-60 mL/min
Sample Volume Injected	500 µL

Table 2. **Flow injection program used for mercury analysis.**				
Step	**Time (sec)**	**Pump 1 Speed (rpm)**	**Pump 2 Speed (rpm)**	**Valve**
Prefill	15	100	120	Fill
1	10	100	120	Fill
2	15	120	0	Inject, Read
3	0	0	0	Fill

Standards, Certified Reference Materials

PerkinElmer NIST® traceable mercury single-element calibration standards for atomic spectroscopy were used as the stock standard for preparing the working standards. Working standards were prepared by serial volume/volume dilution in polypropylene vials (Sarstedt®, Germany) which contained 5% volume/volume nitric acid and 1-2 drops (about 25 µL) of 5% weight/volume potassium permanganate from Merck® (Darmstadt, Germany) in order to ensure preservation of the element in solution. ASTM® Type I water (from a Millipore® filtration system, Millipore® Corporation, Billerica, Massachusetts, USA) acidified with Suprapur® nitric acid used for preparing the diluent for standards was from Merck®. Micropipettes (Eppendorf®, Germany) with disposable tips were used for pippetting solutions. NIST® 1573e certified reference material for trace metals in tomato leaves and GBW 09101 certified reference material for trace metals in human hair were used to validate the method developed.

A single-element ICP standard for mercury in nitric acid (Spex. Certiprep.®, New Jersey, USA), prepared at midpoint of the calibration curve, was used as the quality control (QC) check standard.

Reagent Preparation

Carrier solution: Prepared by adding 15.0 mL of Suprapur® hydrochloric acid (Merck®) and making up to 500 mL in Class I standard flask, by using ASTM® Type I water.

Reducing agent: Dissolved 0.25 g of Suprapur® sodium hydroxide (Merck®) and 1 g of Suprapur® sodium boro-hydride in ASTM® Type I water, and made up to 500 mL in Class I standard flask. All the samples prepared in boro-silicate vessels were stabilized by the addition of 1-2 drops of a 5% weight/volume potassium permanganate solution.

Sample and Certified Reference Material Preparation

Four powdered spices and herb-mixture samples of famous brands available in the local markets in China (five-spice mix, ground szechuan, five-spice powder and curry powder), were bought from a Chinese supermarket and were used without any pre-treatments. ~0.5 g of each sample, accurately weighed in duplicate, was transferred to the digestion vessels of the microwave digestion system and the sample digestion was done in accordance with the program given in Table 3. The digested samples were diluted with 5% nitric acid and made up to 20 mL in Class I borosilicate standard flasks. The certified reference materials (CRMs) were also digested in a similar manner. Sample blanks were also prepared in a similar manner with each batch of digestion.

Table 3. **Program used for the digestion of spices and herbs with Multiwave.**				
Sequence	**Power**	**Ramp Time (min)**	**Hold Time (min)**	**Fan**
1	1200	15	15	1
2	0		15	3
Weight Taken	~500 mg	HNO_3	5.0 mL	
H_2O_2	1.0 mL	Rate	0.5 bar/sec	
Pressure	55 Bars			

Prior to the analyses, the flows of the 0.2% weight/volume sodium borohydride reductant and 3% volume/volume hydrochloric acid carrier solution was adjusted and set at 5-6 mL/minute and 9-11 mL/minute respectively. The argon gas flow was set at about 50-60 mL/minute. The waste flow from the gas/liquid separator was adjusted to the rate such that the liquid leaves the gas/liquid separator effectively, without any of it getting into the transfer tubing to the

quartz cell. The sensitivity was checked using a 10 µg/L mercury standard solution. 500 µL sample volume was used in every analysis. The results were obtained using peak height calculation with 2 seconds BOC (baseline offset correction) time and 19-point peak smoothing algorithm. Each result was calculated as a mean of two replicate determinations.

Results and Discussions

Problems in the digestion of samples for mercury determination are volatility, mobility, and adsorption on the walls of the containers, as well as possibility of contamination.[6] The spice samples contain a number of organic substances of different types and impurities of sparingly soluble mineral components. Incomplete mineralization of samples during the microwave-digestion process may cause difficulty in transferring analytes into solution and, on the other hand, disturb spectrochemical measurements. The complete decomposition of organic matter could be achieved only under vigorous conditions such as excess of acid mixtures, high temperature and pressure, and long digestion times. In addition, oxidative conversion of all mercury species to mercury (II) in the sample is an elementary step prior to analysis.[7] Thus, digestion of the sample for mercury determination is carried out in an oxidizing environment using a combination of nitric acid and hydrogen peroxide and by utilizing closed vessel digestion to avoid loss of mercury. The oxidative conversion of all mercury species to inorganic mercury is further ensured by the addition of potassium permanganate. On the other hand, there is no need to decompose silicates because mercury cannot form natural silicate minerals due to its large ionic radius. Further preservation of mercury has been done as per the U.S. EPA method for the preservation of lower concentrations of mercury by maintaining an overall concentration of 200 ppb of gold in sample solutions.[8] The commonly observed interference caused by volatile nitrogen oxide was minimized by using a combination of nitric acid and hydrogen peroxide. However, as our experience shows, preservation using gold will help to hold the mercury in solution so that there is no need to analyze the samples immediately after digestion. Allowing some time (with occasional shaking) before analysis (~1 hour) will help to remove all dissolved nitrogen oxide, if present. The instrumental calibration was performed using four points, including the blank. Prior to starting sample analysis, the method detection limit (MDL) was measured using seven reagent blanks and then multiplying the standard deviation with the student t value of 3.14 for a confidence interval of 98% (Table 4).

Table 4. **Method detection limit (MDL).**

Analyte	MDL (µg/kg)
Hg	0.8

The good agreement between the certified value and the result (Table 5) obtained using calibration with aqueous standards suggests that the hydride generation AA technique is quite independent from matrix effects.

Table 5. **Analysis of certified reference materials.**

Metal	NIST® 1573e		GBW 09101	
	Certified Value (µg/g)	Measured Value (µg/g)	Certified Value (µg/g)	Measured Value (µg/g)
Hg	0.034	0.030 ± 0.003	1.06 ± 0.28	1.31 ± 0.11

Furthermore, the excellent spike-recovery results (Table 7 – Page 5) showed that the matrix is not contributing to the final analytical results even at extremely lower concentrations. The long-term stability of the analyte signal has been monitored by analyzing quality control (QC) check standards prepared at the midpoint of calibration, at precise intervals (Table 6 – Page 5). The excellent QC recoveries, with a variation of less than 10%, usually prescribed by the regulatory bodies, showed that there is no drift in analyte signal during the course of analysis. There was virtually no difference between the QC standard which was performed immediately after calibration and the QC standard which was analyzed at the end of analysis with a time difference of three hours.

The extremely low detection limits obtained (Table 4) further demonstrated that the FIAS 400 system can be used together with the AAnalyst 800 to analyze extremely lower concentrations of mercury in difficult matrices.

Table 6. **Results of quality control (QC) check standard recoveries.**

Analyte	Prepared Concentration (µg/L) QC Standard	Measured Concentration (µg/L) QC Standard (analyzed at 11:55 am)	% QC Recovery	Measured Concentration (µg/L) QC Standard (analyzed at 15:00 pm)	% QC Recovery
Hg	5.0	4.89	98	4.93	99

Table 7. **Results of post digestion spike recoveries.**

Analyte	Spiked Concentration ion (µg/L)	Measured Concentration (µg/L) of Ground Szechuan	Measured Concentration (µg/L) of spiked Ground Szechuan (I)	% Recovery	Measured Concentration (µg/L) of spiked Ground Szechuan (II)	% Recovery
Hg	1.0	0.322	1.27	94.8	1.32	100

Table 8. **Results of spices and herbs sample analysis.**

Analyte	Five-Spice Powder	Five-Spice Mixture	Ground Szechuan	Curry Powder
Hg (µg/Kg)(FI-CVAAS)	15	16	13	3.0
Hg (µg/Kg)(ICP-MS)*	7.0	38	11	4.0

**Different set of similar samples measured at a different site.*

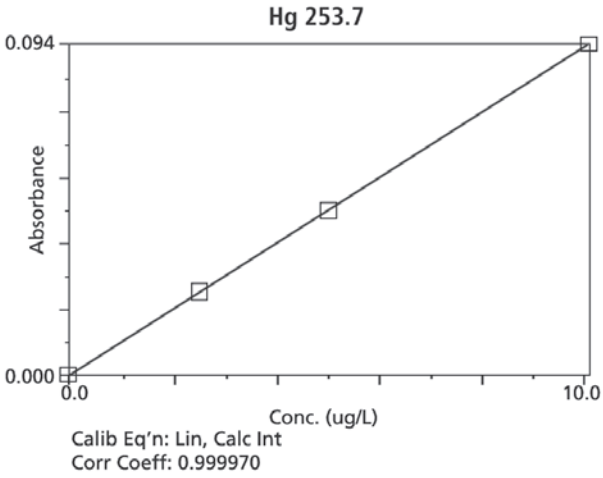
Conclusions

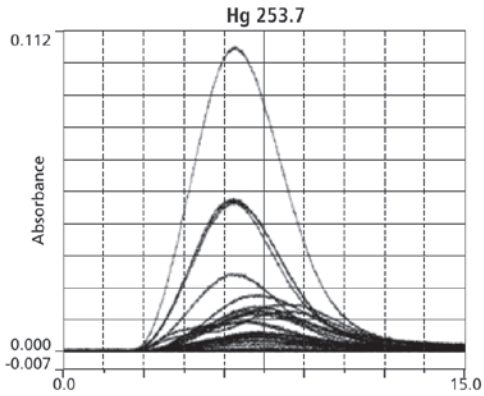
An accurate and reliable microwave-assisted sample-pre-treatment procedure for the determination of mercury in spices using FI-CVAAS is described. Application of concentrated nitric acid along with hydrogen peroxide for mineralization of spices and herbs leads to the complete digestion of samples, which is proven by determined values of mercury in various certified reference materials. Toxicity of medicinal spices and herbs is of much greater concern today than ever before. In recent years, much emphasis is being laid on the toxic metal content of spices and herbs, as several western countries have banned many ayurvedic drugs based on their heavy-metal content exceeding the permissible limits.[9] A cursory look at results (Table 8) shows that the level of mercury did not exceed the permissible limits of 1 mg/kg specified by the U.S. FDA in any of the samples analyzed. The results confirmed that the determination of mercury after acid solubilization of spice mixture samples by microwave digestion can be performed by FI-CVAAS without any interference and the same has been cross checked by analyzing a different set of similar samples with ICP-MS (Table 8) analysis. The slight variations are due to the fact that the analyses were done on a different set of similar samples and not on the same samples.

References

1. H. Mubeen, I. Naeem, A. Taskeen and Z. Saddiqe, New York Science Journal, 2 (5) (2009) 1554-0200.
2. http://en.wikipedia.org/wiki/Spices
3. U. Divrikli, N. Horzum, M. Soylak and L. Elc, International Journal of Food Science and Technology, 41 (2006) 712–716.
4. Effects of Methyl Mercury: http://en.wikipedia.org/wiki/Methylmercury.
5. H. Lippo, T. Jauhiainen and P. Peramaki, Atomic Spectroscopy, 18 (3) (1997) 102-108.
6. I. Baranowska, K. Srogi, A. Włochowicz, K. Szczepanik, Polish Journal of Environmental Studies, 11(5) (2002) 467-471.
7. United States Environmental Protection Agency Mercury preservation techniques.
8. R.P. Choudhury and A.N. Garg, Food Chemistry, 104 (2007) 1454-1463.

Appendix I. Calibration Curve and Atomization Profile





PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

009270_01