

# Mercury Speciation Determinations in Asian Dietary Supplements

*Terri Christison, Deepali Mohindra, Frank Hoefler, and Linda Lopez, Thermo Fisher Scientific, Sunnyvale, California, USA*



## Overview

**Purpose:** Demonstrate low cost solutions for use in food safety for mercury speciation and counter anion determinations in solutions extracted from dietary supplements on an integrated ion chromatography system.

**Methods:** Mercury species (mg/L) were determined on a mixed anion-cation-exchange column optimized for metal speciation using a cysteine-perchlorate eluent and detected by absorbance at 210 nm. Inorganic anions and organic acids were determined on an anion-exchange high-capacity column optimized for organic acids using electrolytically generated hydroxide eluent and detected by suppressed conductivity detection.

**Results:** The Asian supplements had 0.05 to 0.1 wt% extractable amounts primarily of less toxic inorganic mercury.

## Introduction

Multiple patients had clinical symptoms of mercury poisoning and unusually high blood concentrations (mg/dL) of total mercury after ingesting Asian dietary supplements adulterated with mercury. Testing of the dietary supplements by Inductively Coupled Plasma (ICP) showed they contained 10,000-fold higher concentrations of total mercury (1–10 wt%) than measured in the patients' blood. Because inorganic and organic forms of mercury show disparate toxicity, mercury speciation was required to establish the forms of mercury present in the supplement for determining food safety. Counter-anion determinations were also needed to calculate mass balance. Ion chromatography (IC) is well suited for ion separations and provides a well-established method to determine and quantify metal species and anions.

### Methods

#### Sample Preparation

The Asian dietary supplements were ground to a coarse powder, extracted in 20 mL of eluent with manual agitation for 5 min, filtered, diluted with deionized water prior to injection for mercury speciation determinations. The samples were treated in the same manner and extracted with deionized water for counter anion determinations.



#### Standard Preparation

The methylmercury and ethylmercury standards were prepared from their mercury salts (Aldrich) in methanol, and diluted with water to the working standard concentrations. All other standards were prepared in deionized water.

#### Instrument

##### Method 1: Mercury Speciation By Ion Pairing Chromatography

- Thermo Scientific Dionex ICS-1600 Standard Integrated or Dionex ICS-1100 Basic Integrated IC system
- Thermo Scientific Dionex ICS Series Variable Wavelength Detector (VWD)
- Thermo Scientific Dionex AS-AP Autosampler

##### Method 2: Counter Anion Determinations By Ion Chromatography

- Dionex ICS-2100 Integrated Reagent-Free™ IC (RFIC™) system
- Dionex AS-AP Autosampler

#### Software

- Thermo Scientific Dionex Chromeleon Chromatography Data System

**FIGURE 1. Dionex AS-AP Autosampler (Bottom Left), Dionex ICS Series VWD (Top Left) shown next to Dionex ICS-1600 (Center), and ICS-2100 RFIC (Right) Integrated IC Systems.**



#### **Method 1: Ion Pair Chromatography**

Columns: Thermo Scientific Dionex IonPac CG5A, CS5A, 4 x 250 mm  
Eluent 1.0 mM acetic acid, 1.0 mM sodium perchlorate, 5 mM cysteine, adjusted to pH = 4.0  
Flow Rate: 1.75 mL/min  
Injection Volume: 25  $\mu$ L  
Detection: UV Absorbance, 210 nm

#### **Method 2: Counter Anion Determinations Using Reagent-Free Ion Chromatography**

Columns: Dionex IonPac™ AG11-HC, AS11-HC, 4 x 250 mm  
Eluent Source: Thermo Scientific Dionex EGC III KOH cartridge  
Gradient: 1.0 mM KOH for 8 min, 1–15 mM KOH from 8 to 18 min, 15–20 mM KOH from 18 to 28 min, 30–60 mM KOH from 28 to 38 min, 1 mM KOH from 38 to 45 min  
Flow Rate: 1.5 mL/min  
Injection Volume: 25  $\mu$ L  
Detection: Suppressed conductivity, Thermo Scientific Dionex ASRS 300 Anion Self-Regenerating Suppressor, recycle mode, 223 mA

#### **Safety Warnings**

Mercury salts and organic mercury compounds are very toxic and sometimes can be fatal. Additionally, perchlorate used in the eluent is classified as a hazardous chemical because it interferes with iodide uptake thereby causing hypothyroidism. Review material safety data sheets for handling precautions, safe storage, and disposal methods, and review local regulations prior to ordering reagents or analyzing samples.

## Results

### Method 1: Ion Pairing

The mercury species is separated as a mercury-cysteine complex by ion pair chromatography using the cysteine perchlorate-based mobile phase on the mixed mode Dionex IonPac CS5A column. The Dionex IonPac CS5A has both anion- and cation-exchange properties making it ideal for the separation of transition metal species. As the analytes elute from the column, they are detected by UV at 210 nm. All three mercury species evaluated had linear responses replicate injections of four standards from 1 to 10 mg/L with concentration ( $r^2 > 0.999$ ).

The ground samples were extracted with eluent to ensure that the ions were soluble throughout the chromatographic separation.

Figure 2 shows the separation of methylmercury and inorganic mercury standards. The chromatograms show that inorganic mercury ( $\text{Hg}^{2+}$ ) is well resolved from methylmercury. Ethylmercury is not shown.

**FIGURE 2. Separation of mercury standards.**

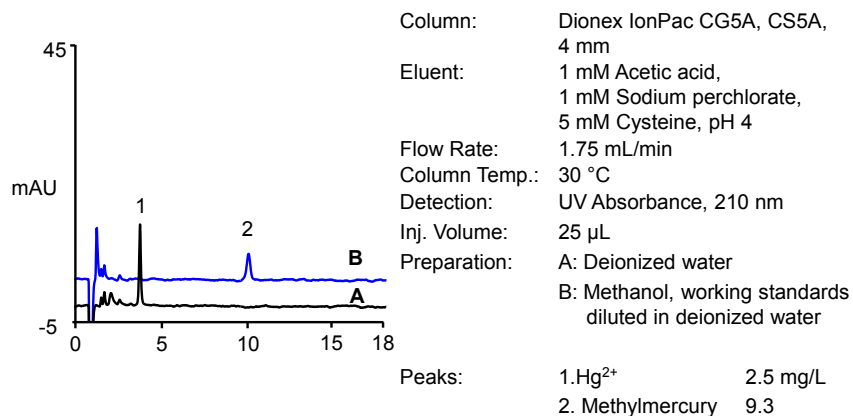
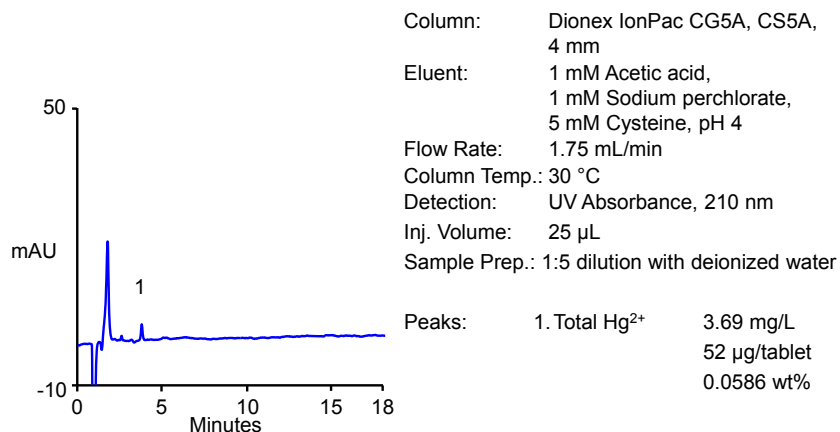


Figure 3 shows the separation of inorganic and organic mercury in dietary supplement 1 after extraction in 20 mL of the acetic acid, sodium perchlorate, and cysteine eluent. This sample has moderately high concentrations of inorganic mercury as compared with the other four samples. No methylmercury or ethylmercury was detected.

**FIGURE 3. Determination of organic and inorganic mercury in dietary supplement Number 1.**



The results of the mercury speciation determinations are summarized in Table 1. Sample 2 had the highest concentration and sample 1 the lowest. Only very high concentrations of inorganic mercury were found; no organic mercury was detected. Although the results are very high (400 to 2200 mg/kg), the effect could be more devastating if ethylmercury and methylmercury were present at these concentrations.

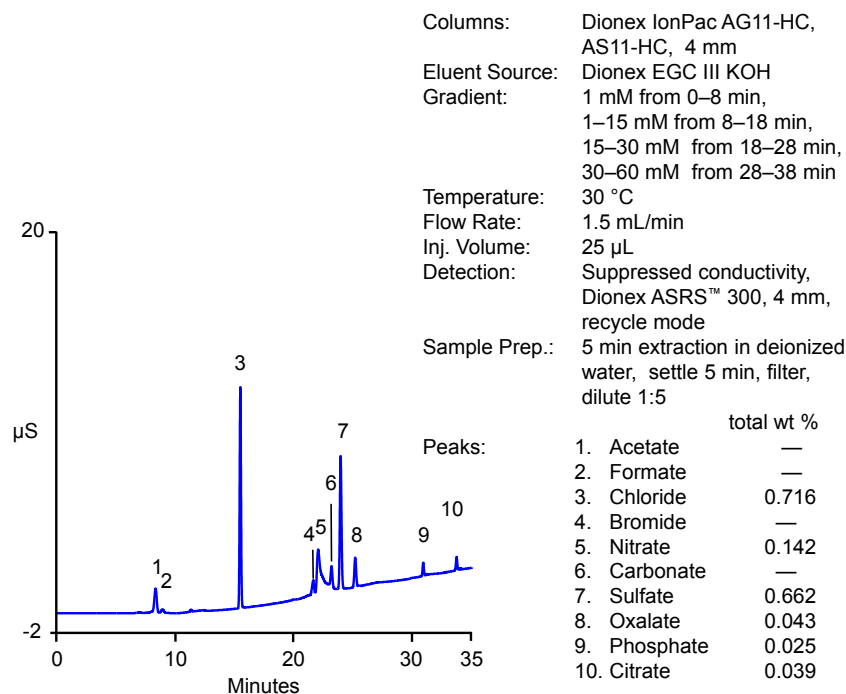
**Table 1. Summary of mercury speciation determinations.**

Sample	Inorganic Mercury			Methylmercury / Ethylmercury
	Measured (mg/L)	Calculated (wt %)	Calculated (µg/tablet)	
Control	--	--	--	--
1	3.69	0.0586	52	ND
2	15.6	0.2265	387	ND
3	4.82	0.0437	117	ND
4	5.31	0.0473	123	ND
5	11.3	0.0708	243	ND

### Method 2: Counter Anion Determinations

Counter anion determinations were needed to conduct a mass balance of the mercury speciation in the dietary supplement samples. We selected the high capacity Dionex IonPac AS11-HC anion-exchange column optimized for organic acid determinations for this application because this column provides the best separations when the samples are uncharacterized. Inorganic anions and organic acids in these samples were separated using a shallow potassium hydroxide gradient to resolve as many peaks as possible. The eluent was electrolytically generated without solution preparation by just adding water. The chromatographic separation of a water extract from Sample 5 is shown in Figure 4. The sample has high amounts of chloride and sulfate (~ 700 mg/kg) with < 50 mg/kg of nitrate, oxalate, phosphate, and citrate.

**FIGURE 4: Determinations of inorganic anions and organic acids in a water extract of dietary supplement Number 5.**



The counter anions results are summarized in Table 2. The counter anions are primarily chloride and sulfate. Sample 2 has the highest results.

**Table 2. Summary of counter anion results.**

Counter Anion (wt %)	Sample #				
	1	2	3	4	5
Acetate	0.012	0.389	0.014	0.008	--
Formate	0.002	0.019	--	--	--
Chloride	0.080	1.624	0.128	0.254	0.716
Bromide	--	--	0.093	0.192	--
Nitrate	0.102	0.507	0.061	0.201	0.142
Sulfate	0.240	1.818	0.196	0.554	0.662
Oxalate	0.003	0.026	0.005	0.011	0.043
Phosphate	--	0.005	--	0.070	0.025
Citrate	--	0.050	0.018	0.042	0.039

## Conclusion

- These tests identified that the majority of the soluble mercury in the dietary supplements was inorganic mercury ( $\text{Hg}^{2+}$ ), corresponding to the sub-percent amounts found in the patients.
- IC with Inductively Coupled Plasma (ICP) provides a total solution by combining speciation with total elemental content.
  - ICP analysis screens samples for unknown metal contamination especially when only one species is present, but also provides the total amount of an element when more than one species is present.
  - IC analysis separates metal species thereby providing important mercury speciation when multiple species are present.
- Mercury speciation determinations define the potential toxicity in food sources.
- Inorganic anions and organic acids needed for mass balance calculations are easily and accurately determined by anion-exchange chromatography with a Reagent-Free IC system.
- For more information on this method and mercury analysis methods, see references 1–4.

## References

1. Sarzanini, C.; Sacchero, G.; Aceto, M.; Aboilino, O.; Mentasti, Ed. Ion Chromatographic Separation and On-Line Cold Vapour Atomic Absorption Spectrometric Determination of Methylmercury, Ethylmercury and Inorganic Mercury, *Anal. Chim. Acta*, **1993**, *00*, 1–7.
2. Dickson, H.R.; Price, R. Application Note AN40992 Accurate Analysis of Low Levels of Mercury in Fish by Vapor Generation AA, Thermo Fisher Scientific, Cambridge, UK, 2010.
3. Christison, T.; Hoefler, F.; Lopez, L. Application Note AN43130 Combining the Synergies of Ion Chromatography and Inductively Coupled Plasma to Identify Mercury Contamination in Herbal Medicines, Thermo Fisher Scientific, Sunnyvale, CA, 2012.
4. Dionex Application Note AN 131, Determination of Transition Metals at PPT Levels in High Purity Water and SC-2 (D-Clean) Baths, LPN 1058, Thermo Fisher Scientific, Sunnyvale, CA, 1998.

For research purposes only, not for clinical diagnostics.

[www.thermofisher.com/dionex](http://www.thermofisher.com/dionex)

©2016 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific Inc. products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

**Australia** +61 3 9757 4486  
**Austria** +43 1 616 51 25  
**Benelux** +31 20 683 9768  
+32 3 353 42 94  
**Brazil** +55 11 3731 5140

**China** +852 2428 3282  
**Denmark** +45 36 36 90 90  
**France** +33 1 39 30 01 10  
**Germany** +49 6126 991 0  
**India** +91 22 2764 2735

**Ireland** +353 1 644 0064  
**Italy** +39 02 51 62 1267  
**Japan** +81 6 6885 1213  
**Korea** +82 2 3420 8600  
**Singapore** +65 6289 1190

**Sweden** +46 8 473 3380  
**Switzerland** +41 62 205 9966  
**Taiwan** +886 2 8751 6655  
**UK** +44 1276 691722  
**USA and Canada** +847 295 7500

**Thermo**  
S C I E N T I F I C  
Part of Thermo Fisher Scientific