Combining the Synergies of Ion Chromatography and Inductively Coupled Plasma to Identify Mercury Contamination in Herbal Medicines

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Key Words

ICS-1600, IC-ICP-MS, Speciation, Mercury, Herbal medicine

Goal

Combining the synergies of speciation by Ion Chromatography and trace elemental analysis by Inductively Coupled Plasma Mass Spectrometry to investigate mercury content in herbal medicines.

Abstract

Combining the synergies of two analytical techniques, speciation by Ion Chromatography (IC) and total multi-elemental analysis by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), were used to solve the source and clinical cause of a mercury poisoning cluster. When multiple patients had clinical symptoms of mercury poisoning and unusually high blood concentrations (mg/kg) of total mercury and lead by ICP-MS, the trail led to adulterated herbal medicines at 1-10 wt% total mercury (by ICP-MS). To resolve this contradiction of 10,000x lower concentrations in the patients' blood after ingesting herbal medicines with 1-10% mercury, mercury speciation was determined by IC with UV detection which found sub-percent concentrations of inorganic mercury and negligible concentrations of the more toxic mercury compound, methylmercury. Although the patients were very sick, these results agreed with the clinical symptoms rather than the total mercury results. However, IC is too specific to conduct a multi-element screening for an unknown contamination and therefore the synergies of IC with ICP-MS were both needed to solve the mercury poisoning cluster.

Introduction

When ion chromatography (IC) meets Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), the whole story comes together. ICP-MS is a multi-element spectroscopy method to determinate total metal concentrations regardless of the metal species. Contrarily, IC determines ionic species with typically conductivity detection but can also be used with other detectors, such as MS or UV/vis detectors. Both analytical techniques provide valuable information but together these techniques can solve the whole problem. In such a case, multiple people became ill in Iowa, U.S.A., resulting in a mercury poisoning cluster. The analytical results of these patients' blood using ICP revealed unusually high concentrations (mg/kg (ppm)) of total lead and total mercury, well above the U.S. EPA and FDA Maximum Contamination Levels (2 µg/L of inorganic mercury in drinking water and 1 mg/kg of methylmercury in seafood).

Both mercury and lead are neurologic toxins and bio-accumulators, targeting brain, and other organs which can cause birth defects and sometimes death. Therefore, not only must the patients be rapidly diagnosed and treated to minimize the damage to their health but the source of their illness must be also rapidly identified to prevent other future cases. As the patients are being treated, the health researchers found that all patients had ingested herbal medicine products from the Republic of India and these products were the source of their illness. Further ICP-MS analysis on the herbal medicines found alarming concentrations of total mercury, 1-10 wt%, and lesser amounts of total lead. These results were very alarming but also very puzzling. Why were the patients' blood mercury levels significantly lower than the amount of mercury ingested from the herbal medicines? As it is well known that the toxicity of mercury is dependent on the species of mercury present, the health researchers contacted Thermo Fisher Scientific to identify the mercury species using IC and complete the story.

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Typically mercury concentrations are very low, µg/L, and therefore sensitive methods such as cold vapor atomic absorption or ICP-MS are used. Sarzanini, etal (Anal. Chim. Acta, 1993, 00, 1–7) previously demonstrated similarly low concentrations of mercury using large volume injections using a cysteine-based eluent on a Thermo Scientific Dionex IonPac CS5A column. However in this case, the mercury compounds are present at mg/L or higher concentrations and therefore it is not necessary to use a large volume injection. Therefore, the methylmercury and inorganic mercury species in the extractions of the herbal medicine samples were separated according to the Sarzanini method using the cysteine-perchlorate eluent and detected by UV absorbance at 210 nm. These IC results demonstrate that advantages of using ion chromatography as part of problem solving to provide mercury speciation results.

IC Method and Conditions

The ICP-MS method was done by another organization according to EPA recommended methods for mercury determinations and therefore will only be discussed in general terms. In the IC method described here, a 25 µL sample of inorganic mercury (Hg++), and methylmercury were separated using a mixed eluent (1.0 mM acetic acid, 1.0 mM sodium perchlorate, 5 mM cysteine adjusted to pH = 4.0) at 1.75 mL/min on a 4 mm Thermo Fisher Scientific Dionex IonPac CS5A mixed cation/anion exchange column set. The analytes were detected by UV absorbance at 210 nm as they eluted from the column. The samples and standards were introduced by the Thermo Fisher Scientific Dionex AS-AP autosampler on to the Thermo Fisher Scientific Dionex ICS-1600 Integrated IC System for chromatographic analysis. To run IC-ICP as an integrated method, it is recommended to modify the IC method with a nitric acid eluent to make it more compatible with ICP-MS.

Preparing Standards and Samples

Inorganic mercury standards were prepared from ACS grade mercuric nitrate (Mallinchrodt) in deionized water. The methylmercury and ethylmercury standards were prepared from their mercury salts (Aldrich) in methanol, and diluted with water to the working standard concentrations. The herbal medicines 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.

IC Results

In this method, the mercury species is reportedly separated as a mercury-cysteine complex by the cysteine perchlorate-based mobile phase on the mixed mode Dionex IonPac CS5A column. The Dionex IonPac CS51 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. To verify the linearity of this method, replicate injections of four standards from 1 to 10 mg/L were evaluated. All three mercury species evaluated had linear responses with concentration ($r^2 > 0.999$).

The method was applied to the diluted extracts of the samples which showed variable results of inorganic mercury from 3–15 mg/L, calculated to 400 to 2200 mg/kg in/on the tablets (or 0.04 to 0.2 wt%) (Figures 1–3, Table 1). Additionally, the extractions of the samples had considerable amount of insoluble material settling to the bottom of the vials which could be insoluble mercury sulfide compounds or just other insoluble materials. Methylmercury was not detected.

Table 1. Results of mercury determinations in contaminated herbal medicines

Sample	Inorganic Mercury			Methyl-mercury
	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

As a final note, it was reported later that the herbal medicines were hand rolled with cinnabar, a mercury containing ore, during tablet preparation to make the tablets more visually attractive. The use of cinnabar, a mercury sulfide ore, has the characteristics found in the analysis. Cinnabar is insoluble in neutral aqueous solutions and soluble in strongly acidic solutions and therefore would only contribute mercury results in the total mercury determinations. As cinnabar is a mercury sulfide ore, it would also be less toxic than the methyl and ethylmercury compounds.

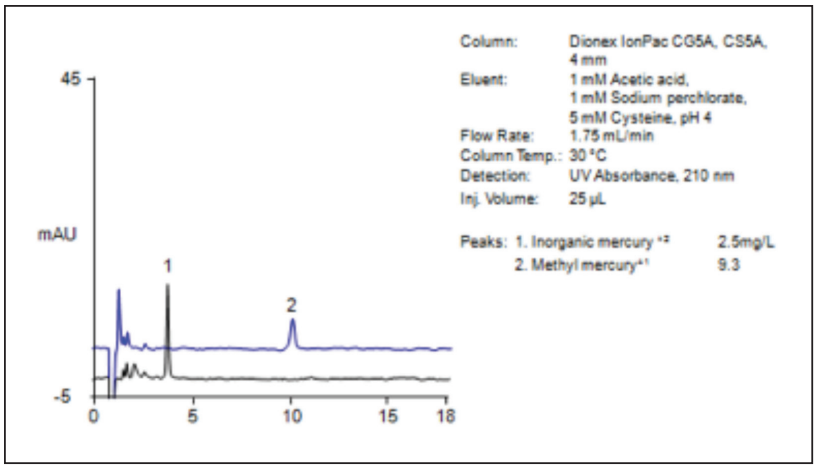

Figure 1. Inorganic Mercury and Methylmercury Standards

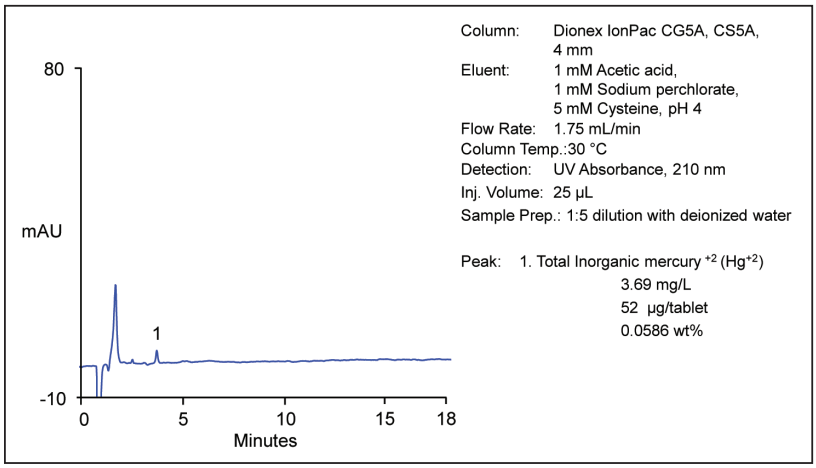

Figure 2. Sample 1 Extract Exhibiting High Inorganic Mercury Concentrations

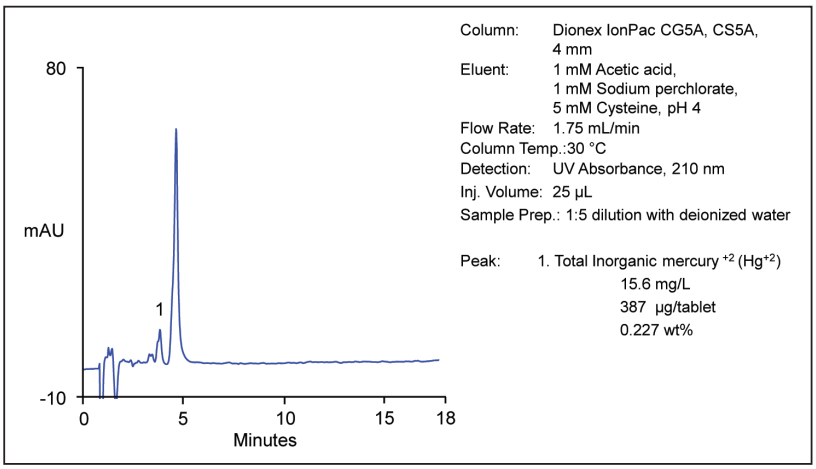

Figure 3. Sample 2 Extract Exhibiting Very High Inorganic Mercury Concentrations

Conclusion

Combining synergistic methods, such as IC and ICP-MS, can provide the total solution and solve complex problems. In this mercury poisoning cluster example, the IC analysis provided mercury speciation which defines to potential total toxicity based on the toxicity of each species, whereas the ICP-MS analysis provided a fast multi-element screening, revealed that mercury caused the clinical symptoms, and determined the total mercury contamination. While both methods provide valuable information, neither method can provide the results for the total solution. In this case, IC and ICP-MS were used individually at different locations however these instruments can be interfaced to run serially with only small modifications.

Ordering Information

Item	Description	Dimensions	P/N
IC System	Thermo Fisher Dionex ICS-1600 Integrated system	-	069655
Autosampler	Thermo Fisher Dionex AS-AP autosampler	-	074921
Detector	Thermo Fisher Dionex IC Series VWD Variable Wavelength Detector 3400	4 channels	070221
Detector cell	Thermo Fisher Dionex PEEK semi-micro flow cell	2 µL, 7 mm	6074-0300
Column, Guard	Thermo Fisher Dionex IonPac CG5A	4 x 50 mm	046104
Column, Separation	Thermo Fisher Dionex IonPac CS5A	4 x 250 mm	046100
Software	Thermo Fisher Dionex Chromeleon Data System		

Certified System ISO 9001
Thermo Electron Manufacturing Ltd (Cambridge) is ISO Certified.

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