Benefits of HPLC-ICP-MS coupling for mercury speciation in food

Application note

Food testing

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Introduction

Mercury is one of the more toxic elements to living organisms. It is encountered at relatively low concentrations in the environment but with its amplification through the food chain, the final concentration in some foods can be relatively high. In addition, its toxicity is not only linked to its total concentration but is also dependent on the species. Mercury speciation in food analysis is necessary to fully estimate the toxic potential of this element to humans.

Due to the low detection limits required for the analysis of Hg species, GC-ICP-MS has been the preferred method. However, with the sensitivity improvements in modern ICP-MS systems, the use of HPLC has become practical for the separation. Coupling HPLC and ICP-MS is particularly attractive due to the simple and fast connection between the techniques. Furthermore, in contrast to a GC analysis, sample extracts are directly injected into the system and no species derivatization is required, greatly simplifying the sample preparation and reducing the cost of the total analytical procedure.

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In this study, the use of HPLC-ICP-MS coupling was evaluated for the speciation analysis of mercury in food samples. Inorganic (Hg^{2+}) and methyl-mercury ($MeHg^+$) compounds are the main concerns from a toxicological standpoint, but the method was also extended to some other species that might be encountered locally, such as ethyl-mercury ($EtHg^+$) and phenyl-mercury ($PhHg^+$). The separation was achieved with the use of an organic solvent gradient to accelerate the species separation. The unique, fast frequency matching, solid state RF-generator used in the Agilent 7700/7800 ICP-MS system permits the switch between aqueous and organic solvents easily and without affecting plasma stability. The method has been validated using certified reference materials (CRMs). The result is a complete, fully integrated and validated solution for the analysis of mercury species in foodstuffs using a hyphenated HPLC-ICP-MS system from Agilent Technologies.

Experimental

Instrumentation

Chromatographic separation was achieved using an Agilent 1260 HPLC. The 1260 HPLC is capable of operating at pressures up to 600 bar, allowing rapid resolution liquid chromatography using columns with very small particle diameters. The column used in this work was a Zorbax C-18, 4.6 x 50 mm, with particles at 1.8 μm (Agilent). Chromatographic details can be found in Table 1. The separation is an optimization of previous works published by Hight *et al*. [1].

Table 1. General operating settings of the Agilent 1260 HPLC system

Parameter	Value
Flow rate	1 mL/min
Injection volume	50 μL
Mobile phase: A channel	0.5 g/L L-Cystein
B channel	0.5 g/L L-Cystein, HCl, H_2O, pH = 2.3 with HCl Methanol

Mercury was measured at the preferred isotope of ^{201}Hg using an Agilent 7700x ICP-MS. Oxygen (a mix of 20% O_2 in argon) was added to the carrier gas using the optional 5th mass flow controller of the 7700x. Addition of oxygen to the plasma ensures that organic solvents can be introduced directly into the ICP-MS without risk of carbon deposition on the interface cones. Platinum cones, which resist oxidation from the added oxygen, and a 1.0 mm inner diameter (id) injector torch were used, which are typical for organic analysis. The operating parameters of the 7700x ICP-MS system are detailed in Table 2.

Table 2. General operating settings of the Agilent 7700x ICP-MS

Parameter	Value
RF power	1600 W
Carrier gas flow rate	0.54 L/min
Make-up gas flow rate	0.10 L/min
Option gas (20% O_2 in Ar)	0.06 L/min
Spray chamber temperature	-5 °C
Sample depth	8.0 mm

The MassHunter workstation software of the 7700/7800 ICP-MS enables the direct control of common Agilent LC and GC modules, allowing the 1260 HPLC method and sequence to be directly set up in the ICP-MS MassHunter software (Figure 1). In addition to the ease-of-use for the HPLC-ICP-MS coupling, this feature increases the safety of such analysis, as the bidirectional communication ensures that if either the HPLC or the ICP-MS stops, the software will automatically stop the other.

Sample preparation

Two CRMs were used for the validation of the method. BCR-464 from IRMM (Belgium) is made from tuna muscle and it is certified at 5.12 ± 0.16 mg(Hg)/kg for its methyl-mercury content, which represents 97% of its total mercury content. Dolt-4 from NRC (Canada) is made from dogfish liver and contains a $MeHg^+$ content of 1.33 ± 0.12 mg(Hg)/kg (52% of its total mercury content).

The extraction procedure was based on a previous study [2]. Briefly, 150 mg of material was extracted in 20 mL of mobile phase A. The extraction was microwave assisted: 11 min at 140 W. The supernatants were injected directly into the HPLC after a filtration at 0.45 μm.
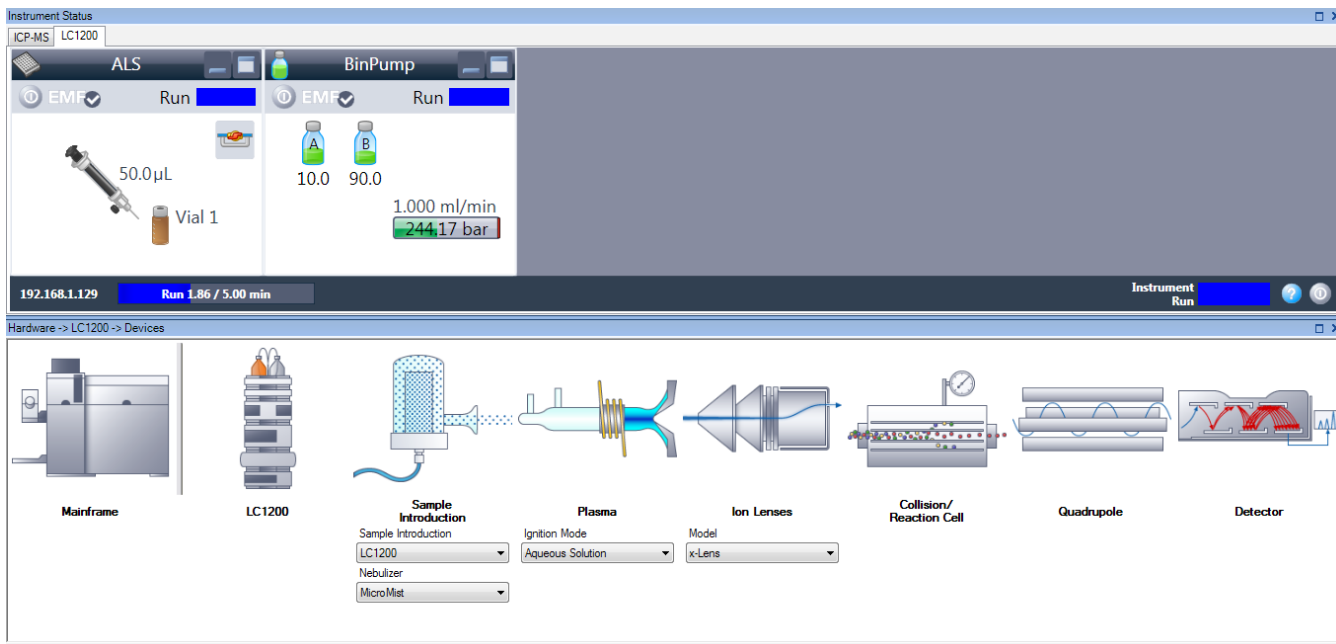
Figure 1. ICP-MS MassHunter software screenshot showing details of the HPLC-ICP-MS configuration

Results and discussion

Chromatographic optimization

The initial method to separate the mercury species used isocratic conditions with 2% methanol (B channel) being added to the L-Cystein buffer (A channel). The resulting chromatogram is shown in Figure 2.
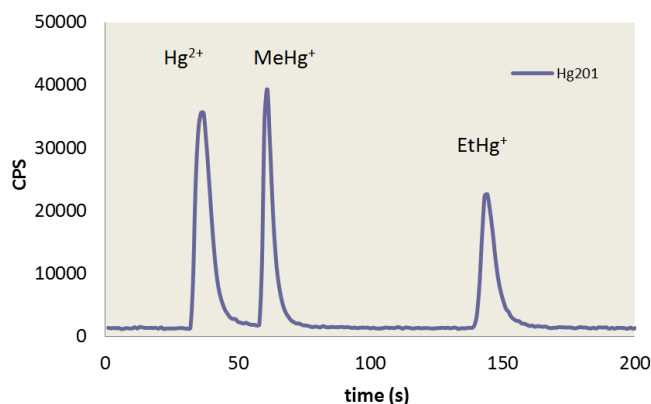

Figure 2. Separation of mercury species under isocratic conditions

As can be seen, under isocratic conditions, the separation of three mercury species was obtained in less than 3 minutes, and in less than 2 minutes for Hg^{2+} and $MeHg^+$. However, the phenyl-mercury is bound to the column and cannot be detected. Therefore, to increase the elution power of the mobile phase, a gradient elution was proposed. Figure 3 shows the gradient used during the separation: starting at 2% methanol, ramping to 90% over 1 minute.
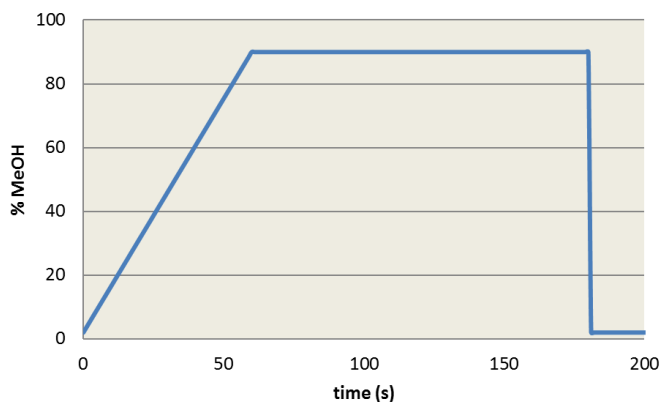

Figure 3. Gradient conditions used during the separation

With this gradient elution, all 4 mercury species were separated successfully in less than 3 minutes (Figure 4). In addition, the use of the methanol gradient did not perturb the plasma stability because of the fast frequency-matching capability of the 7700/7800 ICP-MS plasma RF generator. At the end of the separation, when the mobile phase is rapidly returned to 2% methanol, no issues with plasma stability were encountered.
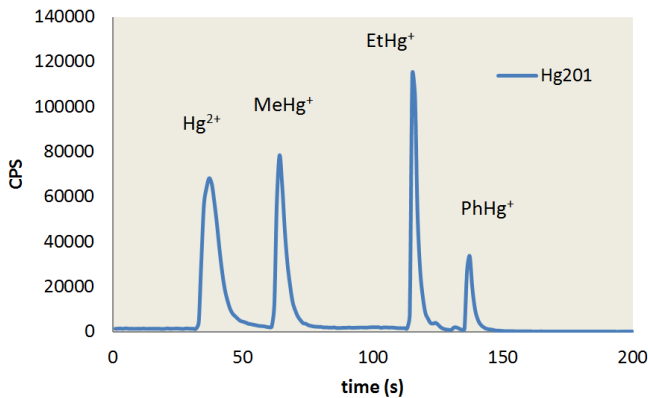
Figure 4. Separation of mercury species under gradient conditions

Performance of the HPLC-ICP-MS system

Before the analysis of the sample extracts, the system was calibrated with a mix of the four mercury species from approximately 100 ng/L (ppt) up to 10 µg/L (ppb). Calibration curves can be seen in Figure 5.

The calibration curves highlight the linearity of the analysis: each species is perfectly calibrated over its range of concentrations. In addition, the background equivalent concentration (BEC) of the mobile phase is shown in Figure 5. For all species, the BEC value was lower than 20 ng/L. These low BEC values underline the small contamination from the mobile phase and emphasize the low mercury detection capability of the system.

Sample analysis

The two CRM extracts were analyzed using the optimized gradient elution method. Three replicate samples were extracted for each CRM. Extraction blanks were also prepared and their analysis didn't show any measurable contamination. Table 3 shows the results for the samples.
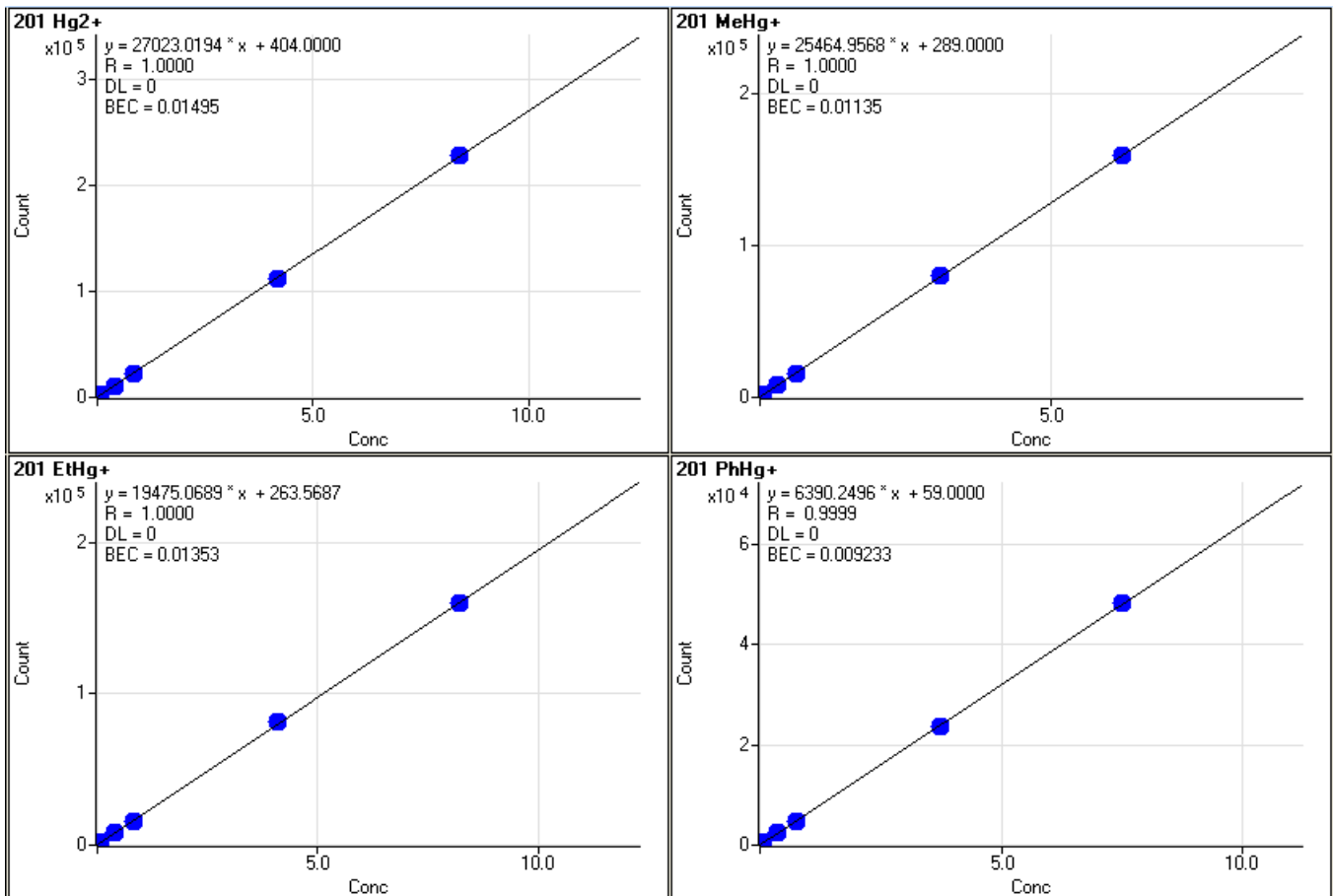

Figure 5. Calibration curves for the analysis of the four mercury species

Table 3. Results for the analysis of the CRM samples — concentrations are expressed in mg/kg of mercury

* Not certified concentration

	BCR-464	Dolt-4	
	201 $MeHg^+$	201 Hg^{2+}	201 $MeHg^+$
Result	4.93	1.17	1.34
RSD (%)	8	8	10
Certified	5.12 ± 0.16	(1.25)*	1.33 ± 0.12
Recovery (%)	96	94	101

The content of inorganic mercury for these samples is not certified; therefore it is difficult to evaluate the accuracy of the measured value. Only the MeHg and total Hg concentrations are certified. The bracketed concentration shown in Table 3 is based on the assumption that only Hg^{2+} and $MeHg^+$ are present in the Dolt-4 sample (no $EtHg^+$ or $PhHg^+$ were observed in the sample chromatograms). Under this assumption, the inorganic mercury result of the Dolt-4 sample is in accordance with the expected value.

For $MeHg^+$, the measured values agreed well the certified concentrations in both samples, validating the efficiency of the new method for the determination of $MeHg^+$ in these food samples. In particular for the Dolt-4 sample where the $MeHg^+$ content represents only about 52% of the total mercury, excellent agreement of the measured concentration with the certified value proves the ability of the sample preparation to conserve this species during extraction.

Conclusions

A fast and effective gradient HPLC-ICP-MS method has been developed for the analysis of mercury species in food samples, giving complete separation of the four species of interest in less than three minutes. The method has been tested on certified samples and has been demonstrated to provide good Hg species recoveries compared to the certified values, where available. In addition, if phenyl-mercury does not need to be monitored, the method can be further simplified by using an isocratic separation.

By permitting the rapid gradient from aqueous to 90% methanol, the fast frequency matching RF generator of the 7700/7800 ICP-MS allows the separation of the mercury species with no adverse effect on the stability of the plasma. This feature will open new possibilities for HPLC separations using organic solvents linked to ICP-MS.

Acknowledgements

References

1. Hight, S. C. & Cheng, J. (2006). *Anal. Chim. Acta. 567*:160–172.

2. Sannac, S., Labarraque, G., Fiscaro, P., Pannier, F. & Potin Gautier, M. (2009). *Accred. Qual. Assur. 14*:263–267.

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Results presented in this document were obtained using the 7700x, but performance is also verified for the 7800 ICP-MS.

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