Easy and fast method development for the mercury speciation in food by HPLC-ICP-MS

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Introduction

Mercury is highly toxic to living organisms. This element is naturally encountered at relatively low concentration 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 also to its species. Therefore, mercury speciation in food analysis is required to fully estimate its toxicity on the human health.

In this study, the use of HPLC-ICP-MS was evaluated for the speciation analysis of mercury in food samples. The separation of four alkyl mercury species was achieved using a rapid organic solvent gradient. Advances in RF generator technology allowed the switch between aqueous and organic solvent to be made without affecting plasma stability. The method was evaluated with the use of certified reference materials.

Experimental

HPLC conditions

Chromatographic analysis was performed with an Agilent 1260 HPLC binary pump with autosampler. The column was a Zorbax C-18, 4.6 x 50 mm, with 1.8 µm particle size. The chromatographic details are shown in the Table 1. To increase the eluting power of the mobile phase, gradient conditions were used. Figure 1 shows the gradient used during the separation: starting at 2% methanol and increasing to 90%.

Parameter		Value
Flow		1 mL/min
Injection volume		50 µL
Mobile phase:	A channel	0.5 g/L L-Cystein 0.5 g/L L-Cystein, HCL, H_20 pH = 2.3 with HCl
	B channel	Methanol

Table 1 Operating parameters of the 1260 HPLC

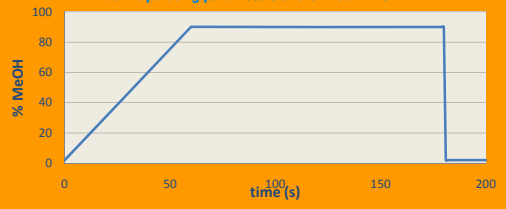

Figure 1 Gradient conditions used during the separation

Experimental

ICPMS conditions

The mercury detection has been made with the 7700x ICP-MS from Agilent Technologies. The addition of oxygen (mix of 20% O_2 in argon) has been used to enable the direct introduction of organic solvent into the ICP-MS. The presence of O_2 will burn the carbon from the solvent and avoid the deposit of graphite on the interface. Platinium cones and a 1.0 mm injector torch have been used on the instrument. The general settings of the 7700x system are detailed in the Table 2.

Parameter	Value
PF power	1600 W
Carrier Gas	0.54 L/min
Make up Gas	0.10 L/min
Option Gas (O_2)	9 %
Spray chamber temperature	-5 °C
Sample depth	8.0 mm

Table 2 General settings of the 7700x ICP MS

Figure 2 Details of the MassHunter software under the HPLC-ICP-MS configuration

The new MassHunter workstation software for the 7700 Series ICP-MS enables the direct control of common Agilent LC & GC modules. Therefore, the 1260 HPLC method and sequence has been directly set up in the ICP-MS MassHunter software (Figure 2). Despite the ease of use for the HPLC-ICP-MS coupling, this feature increases also the safety of such analysis: if one instrument stops the software will automatically stop the second instrument.

Sample preparation

Two certified reference materials (CRMs) were used for the evaluation of the method. BCR-464 Tuna Muscle (IRMM, Belgium) is certified at 5.12 ± 0.16 mg(Hg)/kg for methylmercury, representing 97% of its total mercury content. DOLT-4 Dogfish Liver (NRC, Canada) contains MeHg+ at 1.33 ± 0.12 mg(Hg)/kg (52% of its total mercury content). 150 mg of material is extracted in 20 mL of mobile phase A. Extraction was assisted with the use of a microwave system: hold for 11 min at 140 W. The supernatants are directly injected into the HPLC after filtration through 0.45 µm filters.

Results and Discussion

Performances of the HPLC-ICP-MS system

Inorganic (Hg2+) and methyl-mercury (MeHg+) compounds are the main concerns of such analysis but the method has also been extended to some species than can be locally encountered: ethyl-mercury (EtHg+) and phenyl-mercury (PhHg+). Under the conditions previously detailed, the separation of the four mercury species was obtained in less than 3 minutes (Figure 3). In addition, the plasma remained stable as the methanol content was increased from 2% to 90% in less than 1 minute due to the the fast frequency-matching capability of the 7700 Series ICP RF generator. Likewise at the end of the separation, when the mobile phase switched back to 2% methanol, no plasma stability issues were observed.

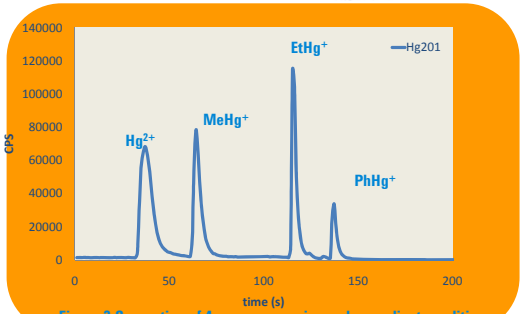

Figure 3 Separation of 4 mercury species under gradient conditions

The system was calibrated with a mix of the four mercury species from 80 ng/L to 8 µg/L (except MeHg+ from 62 ng/L till 6.2 µg/L). All calibrations demonstrated excellent linearity over the calibration range. Background equivalent concentration (BEC) for all four species was lower than 15 ng/L.

Method validation

The two CRMs were extracted and analyzed in triplicate. Results are shown in Table 3. Since the content of inorganic mercury for each sample is not certified, the concentrations in brackets are based on the assumption that only Hg2+ and MeHg+ are present in the sample (no EtHg+ or PhHg+ was observed in the sample chromatograms). For MeHg+, the measured concentrations were in good agreement with the certified concentrations.

	BCR-464		Dolt-4	
	201 Hg^{2+}	201 $MeHg^+$	201 Hg^{2+}	201 $MeHg^+$
Result	0.074	4.93	1.17	1.34
RSD (%)	9	8	8	10
Certified	(0.12)*	5.12 ± 0.16	(1.25)*	1.33 ± 0.12
Recovery (%)	62	96	94	101

Table 3 Results for the analysis of the CRM sample - Concentrations are expressed in mg/kg of mercury
* not certified concentration

As can be seen from table 1, for MeHg+, the measured concentrations were in good agreement with the certified concentrations. Inorganic mercury concentration is not certified but the content in the Dolt-4 sample was in accordance with the estimation of its content.

Conclusions

A fast and effective HPLC-ICP-MS method has been developed for the analysis of mercury species in foods. The method performs the separation of four species in less than three minutes under gradient conditions. BECs were at the low ng/L (ppt) level, and the method was tested on certified samples with excellent agreement for species with certified concenrtations.

In addition, the use of a fast methanol till 90% has been easily obtained thanks to the capacity of the 7700x generator. This feature open new possibilities for HPLC separation with organic solvents linked with an ICP-MS.

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