A fast and sensitive method for determination of methylmercury in human milk and blood using isotopic dilution HPLC-ICP-MS

Research centre for toxic compounds in the environment

Kristýna Maléřová[1], J. Kuta[1], J. Maňoušek[2], S. Adu-Kumi[3]

1) *RECETOX – Research Centre for Toxic Compounds in the Environment; Faculty of Science Masaryk university, Brno, Czech Republic, www.recetox.muni.cz*
2) *Department of Internal Medicine and Cardiology, University Hospital Brno, Brno, Czech Republic*
3) *Environmental Protection Agency, Chemicals Control and Management Centre, Accra, Ghana*

Highlights

- **First method for determination of methylmercury in human milk and blood based on ID-HPLC-ICP-MS:**
- fast and sensitive: up to 60 samples per day, minimal number of sample handling steps
- suitable for routine analysis of the great number of samples
- includes preconcentration and purification of the sample
- limit of the detection was estimated to 0.002 (milk) and 0.008 (blood) $\mu g.L^{-1}$
- repeatability of the procedure is typically in single unit of percent
- accuracy was validated by analyzing CRM

Materials and method

Samples:
stored in plastic container in freezer (-18°C). Swine blood and bovine milk from local traders (optimization) and human blood and breast milk (real samples measurement).

Instrumentation:
Agilent 1100 Series HPLC (Agilent Technologies), with a reverse phase column SYNERGI HYDRO-RP, coupled to Agilent 7700x ICP-MS (Agilent Technologies)

Conclusions

A method for methylmercury determination in human milk and whole blood is based on isotopic dilution high-performance liquid chromatography coupled to mass spectrometry with inductively coupled plasma (ID-HPLC-ICP-MS).
The method contains purification and preconcentration steps. The whole process of extraction is controlled by addition of isotopic enriched methylmercury (^{198}MeHg). The amount of native MeHg in each sample was calculated using the recovery of ^{198}MeHg.

Method was optimized, the accuracy of the procedure was validated by analyzing the certified reference material.
The method is suitable for routine analysis (verification by analyzing 70 blood and 50 samples of breast milk).
Median of the measured concentration were 0.127 $\mu g.L^{-1}$ (milk) and 0.311 $\mu g.L^{-1}$ (blood).

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Introduction

- Methylmercury (MeHg) – a proven neurotoxicant.
- There is increasing demand of MeHg analysis especially after adoption of Minamata Convention on Mercury.
- MeHg exposure can be assessed by the analysis of human body fluids (e.g. blood, human milk).
- The methods for methylmercury analysis are based mostly on gas chromatography, however sample preparation procedures are usually complicated and time consuming.
- There were purposed methods based on high-performance liquid chromatography, which are simpler, but also can be time consuming and do not contain any purification and preconcentration step. These methods are not suitable for routine analysis of large number of samples.

Optimization of the extraction procedure

There are several parameters which could affect the performance: type of added acid and salt, number of toluene extractions and time of handshaking. The method was optimized by using swine blood and bovine milk. The whole process of extraction is controlled by addition of isotopic enriched methylmercury (^{198}MeHg). Following tables describe process of the optimization in case of swine blood.

Type of acid and salt	Mean recovery of ^{198}MeHg [%]
HCl	71.4 ± 3.8
HCl + NaCl	68.6 ± 3.8
HBr	63.6 ± 2.6
HBr + NaBr	75.0 ± 3.4

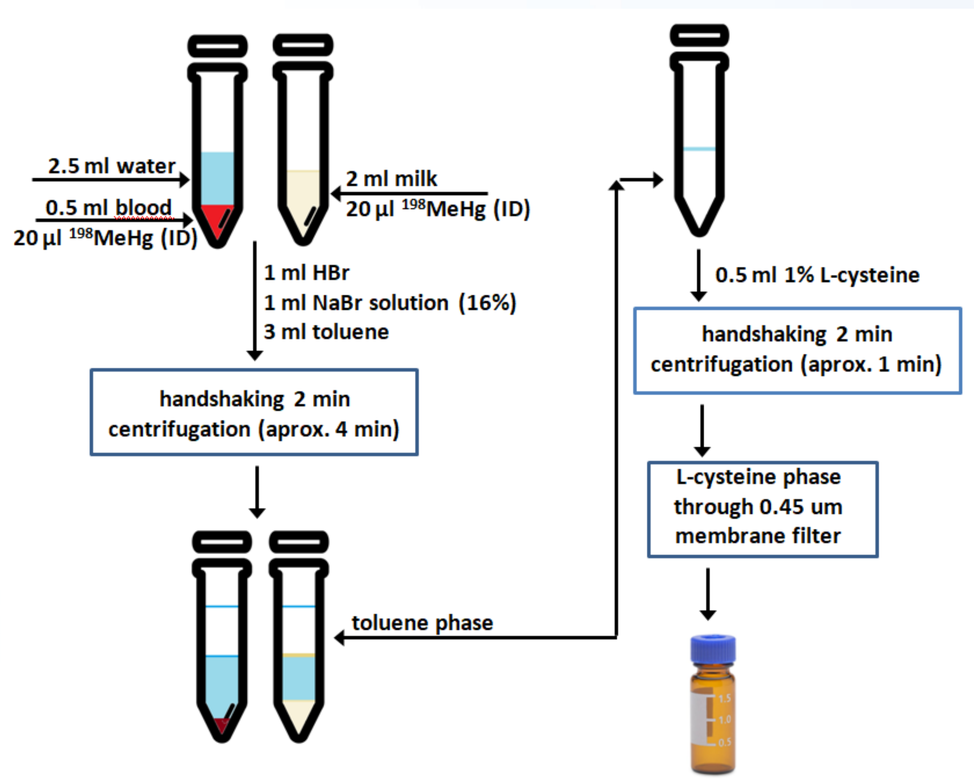
Nr. of toluene extractions	Mean recovery of ^{198}MeHg [%]
1	73.4 ± 1.6
2	82.4 ± 1.9
3	86.8 ± 1.5

Time of handshaking	Mean recovery of ^{198}MeHg [%]
1 min	75.0 ± 3.4
2 min	73.4 ± 1.6
3 min	71.3 ± 1.1

The most effective extraction procedure:

General steps of the sample preparation procedure

1. to release MeHg from protein (HBr)
2. to form MeHg halide in solution (NaBr)
3. to extract MeHg into toluene (proteine removal)
4. to extract MeHg from toluene to L-cysteine solution (fat removal)
5. to analyze L-cysteine solution (by HPLC-ICP-MS)



Application of the method

The accuracy of the procedure was validated by analyzing the certified reference material (NIST 955C Caprine blood)

	Concentration [$\mu g.L^{-1}$]
Declared value	4.5 ± 1.0
Measured value	4.72 ± 0.04

Method was verified by analyzing 70 blood samples from Czech Republic and 50 samples of breast milk from Ghana.

	Median recovery of ^{198}MeHg [%]	10-90 percentil of recovery of ^{198}MeHg [%]	Concentration of MeHg median [$\mu g.L^{-1}$]	Concentration of MeHg range [$\mu g.L^{-1}$]
milk	70.4	60.3 – 80.1	0.127	0.008 – 0.734
blood	72.7	63.9 – 84.3	0.311	0.02 – 1.04

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