

## OP 5.4

### Use of double spike speciated isotope dilution to investigate the differences of reactivity between freeze dried and cryogenic biological standard reference materials for mercury speciation analysis

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The origins and the processes driving the inadvertent transformations of inorganic mercury (iHg) and methylmercury (MeHg) in cryogenically stored and homogenized fresh-frozen (FF) versus freeze-dried (FD) biological Standard Reference Materials (SRM) were investigated using alkaline digestion, derivatization and GC/ICP-MS analysis. Labile enriched <sup>201</sup>iHg and <sup>202</sup>MeHg isotopic standards together with their cysteine-complexed molecular analogs (<sup>201</sup>Hg(Cys)<sub>2</sub> and <sup>202</sup>MeHgCys) were used in a double spike speciated isotope dilution (SID) model to study the role and influence of the complexing ligands/radicals originally associated with mercury species in these materials, on the equilibration, the reactivity and the transformation processes between endogenous and isotopic species during the main analytical steps.

The results revealed that a negligible methylation occurred in both materials, whereas a significant demethylation yield was only detected in the cryogenically stored fresh-frozen materials. Systematic investigation of the analytical steps revealed that this apparent demethylation yield, as given by the double-SID model, resulted from the possible influence of demethylating agents. However, a significant fraction of the demethylation yield was found to be potentially biased and resulted from a lack of equilibration between labile spiked iHg species and their endogenous analogs, indicating probable different complexation/lability patterns in the FF material after the extraction step. This effect was not observed in the FD material. The derivatization reaction was found to drive these non-quantitative equilibrium conditions by fractionating labile and/or weakly bound mercury species relative to strongly bound mercury complexes, leading to potentially biased iHg isotopic ratios. These differences of reactivity between FD and FF biological materials might reflect the effects of freeze-drying procedures on the stability and reactivity of mercury species and/or on the demethylating agents. Although the effect of this preparation step on organometallic species stability has been poorly described, it is known to denature proteins by inducing structural perturbations (unfolding/conformation changes). These conditions are likely to modify the binding/complexation of mercury species and potentially change the reactivity of the materials.