

## 1. Summary of Phase II

The project **Analytical eco-scale methods for the determination and speciation of Hg by UV photo-induced derivatization and detection using a full-miniaturized experimental spectrometric system (ECOSPEC)** aims at developing and validation of high sensitivity, excellent green methods for the quantification of total Hg and its speciation as inorganic and organic forms. According to the achievement plan of the project, the 2<sup>nd</sup> phase titled **Validation and demonstration of utility and capability of the new eco-scale methods for the determination and speciation of mercury and preparation of the analytical procedures** had the following specific objectives:

- Validation of the eco-scale excellent green methods by UV-PVG- $\mu$ CCP-OES by analyzing certified reference materials, comparison to traditional methods and related European legislation (completed)
- Implementation of the validated eco-scale methods using UV-PVG- $\mu$ CCP-OES for the analysis of real samples
- Preparation of internal standard operating procedures for the methods using UV-PVG- $\mu$ CCP-OES and implementation within the accredited laboratory of the partner
- Demonstration of utility of the new, validated eco-scale method using UV-PVG- $\mu$ CCP-OES for the determination and speciation of Hg from real samples (environmental samples and food)
- Dissemination of results through ISI quoted papers

Two eco-scale methods of general green profile were validated in terms of sample preparation, derivatization procedure and miniaturized instrumentation for the determination of total Hg in seafood, food of vegetal and animal origin and water using UV-PVG- $\mu$ CCP-OES at TRL4 achieved in the 1<sup>st</sup> phase of the projects. The most distinctive characteristic of these methods is the use of a single reagent (formic acid) for the extraction and as medium for the photo-induced cold vapor generation. An amount of 0.2 g lyophilized food sample was subjected to ultrasound assisted extraction in 10 ml formic acid 98-100% for 3 h at 50 °C. An aliquot volume of 0.2 – 1 ml extract was diluted to 50 ml and adjusted to contain 0.6 mol l<sup>-1</sup> formic acid, then subjected to on-line UV photo-induced derivatization (500 W lamp power) at 10 ml min<sup>-1</sup> flow rate and 5 s exposure time. Emission of Hg 253.652 nm was measured with the low resolution Maya2000 Pro microspectrometer in the  $\mu$ CCP (15 W, 100 ml min<sup>-1</sup> Ar). Water samples were filtered (0.45  $\mu$ m) and adjusted to contain 0.6 mol l<sup>-1</sup> formic acid. In the case of food and water samples with low Hg content, a preconcentration from 25 ml sample was achieved on a gold filament microcollector. The limit of detection obtained without/with preconcentration were 3.5/0.1 ng l<sup>-1</sup> Hg in solution and 9/0.25  $\mu$ g kg<sup>-1</sup> in the solid. The UV-PVG- $\mu$ CCP-OES method fulfils the demands of the European legislation in Decision 2007/333/EC in terms of determination of total Hg in seafood. The method was validated by analyzing certified reference materials of fish, food of animal and vegetal origin, and water, and recovery of Hg without/with preconcentration was 101 $\pm$ 7%/101 $\pm$ 12% in food and -/97 $\pm$ 12% in water. The proposed method was compared to traditional methods using SnCl<sub>2</sub> for CV generation and detection by atomic fluorescence spectrometry (SnCl<sub>2</sub>-CV-AFS) and inductively coupled plasma optical emission spectrometry (SnCl<sub>2</sub>-CV-ICP-AES), and it was found that UV-PVG- $\mu$ CCP-OES provides similar precision and recovery and better sensitivity.

An eco-scale method based on UV-Vis photochemical induced vapor generation and detection using the experimental model at TRL 4 was also validated for Hg speciation in seafood. The characteristic of the method is once more the use of a single reagent (formic acid) for the extraction of Hg species from solid and as medium for the photo-induced cold vapor generation. An amount of 0.2 g lyophilized sample was subjected to ultrasound assisted extraction in 10 ml formic acid 98-100% for 3 h at 50 °C, then the on-line UV-Vis photo-induced derivatization in 0.6 mol l<sup>-1</sup> HCOOH was carried out. In 0.6 mol l<sup>-1</sup> HCOOH and UV exposure both Hg<sup>2+</sup> and CH<sub>3</sub>Hg<sup>+</sup> species are converted with same efficiency to cold vapor, while natural room light (Vis) achieved selectively derivatization of Hg<sup>2+</sup>. Total Hg and Hg<sup>2+</sup> species were quantified against Hg<sup>2+</sup> external standard

solution by measuring emission at 253.652 nm in a low power/low Ar consumption microplasma with Maya Pro2000 (Ocean Optics) microspectrometer.

The proposed method was compared with Thermal Desorption Atomic Absorption spectrometry (TD-AAS) that allows direct total mercury determination in solid and  $\text{CH}_3\text{Hg}^+$  species after double liquid-liquid extraction according to the protocol recommended by European Commission. The method proposed by us provided detection/quantification limits of  $9/27 \mu\text{g kg}^{-1}$  total Hg and  $4.8/14.4 \mu\text{g kg}^{-1} \text{Hg}^{2+}$ . Study of accuracy against certified reference materials gave recovery of  $99\pm 6\%$  total Hg,  $99\pm 9\%$   $\text{Hg}^{2+}$  and  $99\pm 10\%$   $\text{CH}_3\text{Hg}^+$ , while precision assessed from measurements on real samples were 2.6-10.2%, 2.0 – 13.4% and 5.3-14.5% respectively, similar to those in the reference method. Our proposal provides a greenness profile in terms of sample preparation, derivatization and quantification using miniaturized instrumentation. Coupling of the UV-Vis photochemical reactor with plasma microtorch is an ideal approach for the development of an eco-scale method for Hg speciation in seafood with major advantages against classical methods.

The new validated methods were used for the analysis of real samples and implemented in the Laboratory for environmental analysis (LAM) within The Research Institute for Analytical Instrumentation (ICIA) Cluj-Napoca, partner in the PED project.

Four standard operating procedures were prepared:

1. Determination of total Hg in water by photochemical cold vapor generation and detection by optical emission spectrometry in a capacitively coupled plasma microtorch (UV-PVG- $\mu\text{CCP}$ -OES)
2. Determination of methylmercury in seafood by photochemical cold vapor generation and detection by optical emission spectrometry in capacitively coupled plasma microtorch (UV-PVG- $\mu\text{CCP}$ -OES)
3. Speciation of mercury as  $\text{CH}_3\text{Hg}^+$  and  $\text{Hg}^{2+}$  in seafood by cold vapor generation in  $\text{HCOOH}$  or  $\text{SnCl}_2$  medium and detection by optical emission spectrometry in capacitively coupled plasma microtorch ( $\text{SnCl}_2$ -CV- $\mu\text{CCP}$ -OES/UV-PVG- $\mu\text{CCP}$ -OES)
4. Mercury speciation as  $\text{CH}_3\text{Hg}^+$  and  $\text{Hg}^{2+}$  in seafood by photochemical cold vapor generation in the presence and absence of UV irradiation and detection by capacitively coupled plasma microtorch optical emission spectrometry (UV-Vis-PVG- $\mu\text{CCP}$ -OES).

A demonstration was organized on the utility of the validate eco-scale methods for the determination and speciation of Hg in food and environmental samples. The event was held on 15.03.2018 at ICIA Cluj-Napoca, Partner within the project. A number of 18 representatives of several entities (County Council Cluj, Technical University Cluj-Napoca, Babeş-Bolyai University, National Institute for Research of Development of Isotopic and Molecular Technologies Cluj-Napoca (INCDTIM), Someş-Tisa Water Basin Administration Cluj-Napoca, Someş Water Company S.A. Cluj-Napoca and Research Institute for Analytical Instrumentation) participated at the event. It was emphasized novelty and originality of the new eco-scale methods and the main results. Several demonstrations were carried out to highlight the utility of the methods for the analysis of food and environmental samples. A demonstration report was prepared and signed.

**Dissemination of results:** 1. 3 ISI quoted papers (**7.671 total impact factor and 3.675 relative influence score**); 2. 1 participation in international conference (1 poster, The 45<sup>nd</sup> International Conference of the Slovak Society of Chemical Engineering, Tatranske Matliare, May 2018)

**Degree of achievement of objectives.** The objectives of the execution phase concerning *Validation and demonstration of utility and capability of the new eco-scale methods for the determination and speciation of mercury and preparation of the analytical procedures* **were exceeded under the implementation plan.**

**Results:** 1. Progress report; 2. Eco-scale validated methods for the determination and speciation of Hg using the new UV-PVG- $\mu\text{CCP}$ -OES system as TRL4; 3. Standard operating procedures; 4. A number of 3 papers published in ISI journals; 5. Participation in an international conference (Slovakia, May 2018).