NORMAN Collaborative Trial

Non-target screening of organic substances in river water samples

Background

This Collaborative Trial is organised by the NORMAN Association (www.norman-network.net) as part of its Joint Programme of Activities for the year 2013. The activity is a follow-up action to the NORMAN-JRC workshop in Stresa (2010), where one of the main conclusions was that **comparison and harmonisation of non-target screening methods** in Europe are needed.

The scientific and technical preparation of the exercise, the collection and evaluation of the results, the preparation of the evaluation report, the organisation of the related discussion workshop and the dissemination of results will be taken care of by the Environmental Institute (EI) - Slovak Republic, in close cooperation with UFZ and LfU - Germany, Eawag - Switzerland and Umea University - Sweden.

This Collaborative Trial is carried out in synergy with the international Joint Danube Survey 3 (JDS3) organised by the International Commission for the Protection of the Danube River (ICPDR) taking place in August/September 2013. The Joint Danube Surveys are organised once in six years and provide a lot of additional chemical (target screening), biological, ecotoxicological and hydromorphological data, which may assist in interpreting the results.

Objectives

The main objective of the exercise is to draft a recommendation by the NORMAN Association on the use of **non-target and suspect screening for the identification of the Water Framework Directive (WFD) river basin specific pollutants**.

This recommendation will be based on in-depth discussion of the outcomes of the trial at the planned workshop.

Specific objectives of the Collaborative Trial are analysis of samples using MS techniques established in each of the participating laboratories and declaration of:

1. **How many substances are present in the sample, and**
2. **How many of them can be provisionally identified by suspect and non-target screening.**

The Collaborative Trial should provide in-depth information on the methodologies used by participating laboratories. It will be carried out for the first time in the area of environmental analysis worldwide and therefore aims to result in a common publication in a prestigious refereed journal.

Set up

The Collaborative Trial will be carried out with a sample from the Danube river using the liquid chromatography-high resolution-mass spectrometry (**LC-HR-MS**) and gas chromatography-mass spectrometry (**GC-MS**) methodologies available in participating laboratories.

The sample was taken in the area of downstream Ruse/Giurgiu (border between Romania and Bulgaria), known for the specific problem of untreated waste water being released into the Danube.

A list of previously determined and suspect substances in the Danube by both LC-HR-MS and GC-MS techniques will be provided to the participants beforehand so that participants start with the same knowledge.

An evaluation workshop will be organised in 2014[1] to provide in-depth discussions of the results with the participating laboratories and drafting of recommendations for further improvement actions.

Samples

Participants will receive the following items:

An aliquot of a surface water sample from the Danube river obtained by large volume sampling **of 1000 l of water sample** (without Suspended Particulate Matter above 0.65 µm) through a series of three solid phase extraction cartridges capturing wide range of polarity (neutral, acidic, basic) substances. The three sorbents will be freeze-dried and eluted separately with the most suitable solvent. All three extracts will be combined into 1000 ml combined extract. An **aliquot corresponding to 2 l water sample as a 2 ml extract** will be delivered to each participating laboratory. The sample will be accompanied with clear instructions for its reconstitution into organic solvent or water.

A standard mixture of compounds for calculation of retention time index will be provided in Certan vials to all participating laboratories using LC-HR-MS. The mixture should be injected into the analytical system **with the same conditions as used for analysis of the reconstituted Danube water sample** and the **compounds should be identified and their retention times recorded**. Other retention time prediction methods can be used in parallel.

A standard mixture of substances **for calculation of Kovat's indices** will be provided for all laboratories using GC-MS systems. The mixture must be injected into the analytical system **with the same conditions as used for analysis of the reconstituted Danube water sample** and the **retention times should be recorded**.

Timing

The water sample was taken on **18 September 2013** and delivered to the UFZ laboratory in a cooling box maintaining temperature during the transport below 10°C.

The extraction/freeze drying of the large volume extraction sample were carried out by UFZ after the sample was delivered to the laboratory. The sample aliquots with accompanying standard mixtures will be dispatched on 3 December 2013 with the expected **date of arrival to the participating laboratories on 4 / 5**

[1] The date and venue of the workshop will be confirmed during the course of the exercise. One option is that the workshop will be organised by JRC in Ispra as part of the NORMAN-JRC Collaboration Agreement.

December 2013.

The deadline for submitting the final results is 28 February 2014!!

Time schedule of the exercise

***25 November 2013**: Deadline for registration in the Collaborative Trial*
***3 December 2013**: Shipment of samples to participants*
***28 February 2014**: Deadline for submission of the results by the participants*
***30 April 2014**: Reporting and distribution of draft report to the participants*
***2014 (date and venue to be confirmed**): Discussion of the results in the Non-target screening workshop*

Participants
Any laboratory equipped with LC-HR-MS and/or GC-MS instruments for the analysis of unknown environmental pollutants is invited to participate in this Collaborative Trial. Participants will preferably be members of the NORMAN Association. However, participation of external laboratories is very much welcome.

Explicitly, any laboratory performing non-target screening in Europe and other countries were invited to participate without fees. Participants are automatically welcome to join the workshop.

Participation and registration
*If you wish to participate, please **fill out the registration form** and send it by e-mail to Ildiko Ipolyi at Environmental Institute (ipolyi@ei.sk) before 25 November 2013. Participation in this Collaborative Trial is free of charge.*

Reporting of results
Results shall be submitted in the reporting format described in the annex and sent by 28 February 2014 to Ildiko Ipolyi at Environmental Institute ipolyi@ei.sk.

ANNEX

Reporting format for the results to be submitted by the participants

Reporting format for LC-HR-MS data

Overall description
Analytical system
Manufacturer of the instrumentation
Model number

Sample analysis
Analytical column – dimensions/packing
Sample injection volume
Column temperature
Composition of the mobile phase
Mobile phase gradient programme
List of internal/external standards with their concentrations

Interface
Type of interface (e.g. ESI/APCI)
Interface settings

Mass spectrometry
Full scan chromatograms obtained in positive and negative ion mode – **to be provided together with the results** (further instructions on the format will be provided via e-mail)
Resolving power (with referenced m/z)
Collision energies used for generation of product ion spectra (e.g. MS/MS)
Parent mass lists including:

(i) The masses of all detected target compounds;
(ii) All masses assigned to a suspect detected in the chromatogram;
(iii) All masses of peaks picked for identification in non-target screening;
(iv) Retention times of all masses (not just targets);
(v) Intensities of all detected masses.

Data evaluation
Workflow for data evaluation:

(i) Target screening – software;
(ii) Confirmation of identity of detected targets – procedure;
(iii) Suspect and non-target screening – software, procedures and databases used;
(iv) Retention time prediction method using standard mixture given or own method (if applicable);
(v) Fragmentation prediction method (if applicable).

Reporting in Excel file
No./ RT [min]/ Name of the compound (for targets/suspects/tentatively identified unknowns only)/ Exact mass/ SMILES (canonical SMILES where possible) **or**

InChI Keys/ Intensity (to be normalised including intensity 'cut-off'; exact guide to be provided)/ Estimated concentration (ug/l)/ Category (T/S/N/U))*

**Category - clearly distinguished whether the substance was identified using TARGET (T), SUSPECT (S), NON-TARGET (N) SCREENING or remained unknown (U).*

***TARGET** – substance WITH a standard available in the laboratory, ability to report provisional (semi)quantitative results;*

***SUSPECT** – substance WITHOUT a standard available in the laboratory, suspected to be present in the sample, searched based on the availability of a mass spectrum in mass spectral libraries or prior knowledge;*

***NON-TARGET** – substance not foreseen to be in the sample and identified using mass spectral elucidation tools;*

***UNKNOWN** – substances present in the sample remaining unknown.*

Reporting format for GC-MS data

Overall description
Analytical system
Manufacturer of the instrumentation
Model number

Sample analysis GC
Analytical column – type/dimensions
Sample injection type/volume
Carrier gas
Temperature programme
List of internal/external standards with their concentrations

Sample analysis GCxGC (if applicable)
Second column – type/dimensions
Temperature bias between columns (if any)
Modulation period/data collection frequency

Mass spectrometry
Full scan chromatograms obtained in the electron impact (EI) mode – **to be provided together with the results.**
Full scan chromatograms obtained in the positive chemical ionisation mode (PCI) – **to be provided together with the results (optional).**
Full scan chromatograms obtained in the negative chemical ionisation mode (NCI) – **to be provided together with the results (optional).**

Data evaluation
Workflow for data evaluation:

(i) Confirmation of identity of detected targets/suspects – procedure
(ii) Library searching – databases
(iii) Identification of compounds absent in libraries (incl. features with poor library hits) – software, procedures and used databases
(iv) Retention time prediction method (if applicable)
(v) Fragmentation prediction method (if applicable)

No./ RT [min]/ Name of the compound/ Nominal mass (if applicable)/ CAS No./ SMILES (canonical SMILES where possible) **or** InChI Keys/ Intensity (to be normalised including intensity 'cut-off'; exact guide to be provided)/ Estimated concentration (ug/l)/ Category (T/S/N/U))*

**Category - clearly distinguished whether the substance was identified using TARGET (T), SUSPECT (S), NON-TARGET (N) SCREENING or remained unknown (U).*

TARGET *– substance WITH a standard available in the laboratory, ability to report provisional (semi)quantitative results;*
SUSPECT *– substance WITHOUT a standard available in the laboratory, suspected to be present in the sample, searched based on the availability of a*

mass spectrum in mass spectral libraries or prior knowledge;
***NON-TARGET** – substance not foreseen to be in the sample and identified using mass spectral elucidation tools;*
***UNKNOWN** – substances present in the sample remaining unknown.*

The participants are kindly asked to read the instructions carefully before starting the analysis.

Description of samples and instruction for their use

Aliquot of the 1000 l river water sample in solvent

You will receive a dried aliquot of the river water sample - labelled *'NORMAN CT-1'* - for this exercise. The material, sampled and prepared in collaboration with the JDS3, is a 1000 l river water sample without SPM. The full amount of the obtained 1000 ml combined extract is used in this Collaborative Trial. Each participant receives an aliquot of 2 ml of the extract (corresponding to 2 litres of water). The aliquot is dried with nitrogen and delivered in glass vials.

The procedure for the reconstitution will be provided to all participating laboratories before sample delivery.

Instructions for use

In order to minimize the risks of analyte decomposition, the samples have to be analysed within **48 hours after reconstitution** and **latest on 20 December 2013**.
Please record the **date and time of sample reconstitution** and the **date of analysis**. You will be asked to report this information together with the results.

Store the samples unopened at + 4°C until use.
Let the samples equilibrate to room temperature after reconstitution and before starting the analysis.

Caution

It is strongly recommended to run blanks in order to make sure that the used glassware is free of contamination.

Instruction for the analysis of the NORMAN CT-1 samples

The analyses have to be carried out under repeatability conditions in the same laboratory, by the same operator, applying the same instrumentation and analytical method; and maximum on two consecutive days [ISO 3534 – 1: 1993].

A procedural blank will be provided by UFZ Leipzig.

There is no expressed need for replicates, however, laboratories are encouraged to use their usual procedures.

Reporting of results

Results shall be submitted in the annexed reporting format by 28 February 2014 to ipolyi@ei.sk.