NORMAN Cross-working group activity Nontarget Screening (NTS): Workshop NTS in biota

Wiebke Dürig [1], Lutz Ahrens [1], Juliane Hollender [2]

[1] Swedish University of Agricultural Sciences (SLU), Aquatic Sciences and Assessment, Uppsala, Sweden

[2] Eawag, Swiss Gederal Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland

Introduction

As part of the NORMAN Joint Programme of Activities (JPA), a workshop on nontarget screening (NTS) in biota with 41 participants was held in Uppsala, Sweden, on 16th and 17th of October 2018. The workshop was initiated by the activity leaders Wiebke Dürig and Lutz Ahrens from the Swedish University of Agricultural Sciences (SLU) to increase awareness of suspect and nontarget screening with biological matrices.

Program

The meeting was a lunch-to-lunch workshop, starting with nine short presentations of experts within and outside of the Norman Network (Jan Koschorreck, UBA; Sara Danielsson, SNHM; Wim Cofino, Quasimeme; Nikiforos Alyizakis, EI; Pawel Rostkowski, NILU; Roman Grabic, University of South Bohemia; Pablo Gago Ferrero, ICRA; Juliane Hollender, Eawag; Peter Haglund, University Umeå). The presentations covered a broad range of topics (environmental specimen banks, interlaboratory study designs, sample freezing platform, sample preparation techniques, suspect screening workflows and nontarget screening workflows for both LC and GC-HRMS). The aim of the presentations was to summarize the current knowledge in the field of suspect and NTS in biota and stimulating discussion. Afterwards, the participants were divided into 3 discussion groups (*i)* selection of species/tissues, *ii)* sample preparation, *iii)* suspect/NTS workflow) with each 2-3 discussion group leaders. The main aim of the discussion groups was to give recommendations on how to design an interlaboratory study on suspect and NTS in biota in 2019/2020.

Outcomes

<u>Selection of species/tissues</u>

The species/tissues discussion group discussed the importance of species selection and tissue selection considering trophic levels, biota monitoring programs and regulation. In addition, the group pointed out that harmonization of methodology (e.g. sample extraction) is important for digital freezing (storage of the chromatograms) is relevant. Individual species should be selected with consistency with regards to reproductive status, age, sex, conditions etc. To minimize the variation between individuals as many individuals as possible should be pooled together. A suitable tissue for suspect and NTS was suggested to be muscle, liver, blood, eggs, brain and bile, however, the selection is highly dependent on the aim of the study. Focusing on the possibility of an interlaboratory study design whole fish homogenates were suggested. With regards to a collaborative trial the lipid content, available amount

of material for both GC and LC analysis should be considered. The group suggested in the end that a collaborative trial should be consisting of two parts: Firstly, prepared extracts should be send out to compare workflows and secondly, extracts and homogenate samples should be send out to compare sample preparation techniques of the participating labs.

Sample preparation

The sample preparation discussion group pointed out that the tissue amount for sample preparation can vary from 0.1-1 g up to 5-70 g depending on the type of sample, fat content of the species and analytical method. With regards to sample preparation for suspect and NTS the group agreed that solvent extraction might be the best option (polar as nonpolar solvents should be selected to cover both GC and LC approaches). QuECHERS was discussed and agreed that it is too difficult to select salts for such a wide range of compounds included in suspect and NTS. Clean-up procedure of the extracts should be as minimal and simple as possible (e.g. GCP). For protein and low fat removal the best option would be freezing to denaturalize the proteins. Ultimately, two approaches for low fat and high fat biota samples were suggested as illustrated in Figure 1.

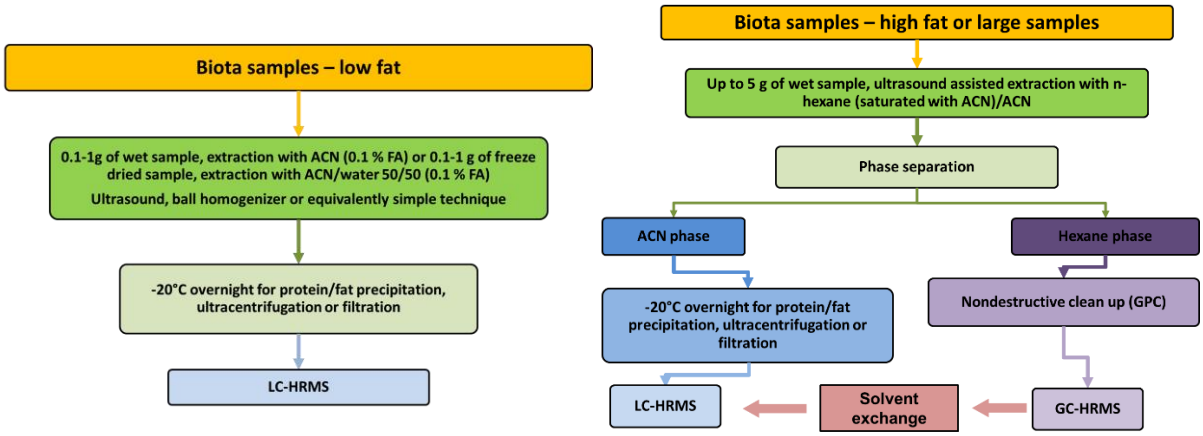

Figure 1: Suggested sample preparation for biological matrices with low and high fat content with regards to suspect and nontarget screening analysis for both LC and GC analysis (Illustration from Pawel Rostkowski and Roman Grabic).

Suspect/NTS workflow

The suspect and NTS workflow group suggested to use whole fish with intermediate lipid content and compare contaminated vs. non-contaminated (viz. upstream and downstream) samples. They suggested to spike downstream samples with ca. 50 compounds that are not highly investigated and unknown to the participants to evaluate their workflow and methods. In addition, 10 compounds should be spiked and known in advance so that the participants can verify if their approach is working. For identification of suspects and nontarget compounds it was suggested to use a 3-to-10-fold change approach, meaning only features/compounds with a 3-10 times higher intensity in the downstream samples compared to the upstream samples will be investigated. With regards to suspect screening it was mentioned that the NORMAN suspect list of ca. 40 000 compounds with fragments and retention time indexes is quite useful. Retention time predictions could be provided if the participant also analyze a calibration solution. Another important discussion point in this group was how to

communicate the confidence level of identified compounds. In the light of nontarget screening it was suggested to focus on the 10 most intense halogenated compounds within the samples. As the samples will be of biological origin endogenous compounds have to be considered. With the help of a database, endogenous compounds could be removed from the data set. Data independent analysis is recommended for wide scope suspect screening and data dependent analysis acquisition is recommended for nontarget analysis. The group also agreed that both ionization modes should be included and guidance for collision energies for each vendor should be provided. For uniform data handling, the participants should upload their data in mzML format.

Future work:

From this workshop, it was concluded that a collaborative trial with biological samples in the light of suspect and nontarget screening is highly appreciated. About 10 labs indicated during the workshop to be interested in joining such a trial. SLU (Lutz Ahrens, Oksana Golovko, Wiebke Dürig), Stockholm University (Jon Benskin, Merle Plassmann), Umeå University (Peter Haglund) and Environmental Institute (EI) (Nikiforos Alygizzakis) decided to take the lead in organizing the trial during 2019/2020. An application for this will be handed in to the NORMAN network for the NORMAN JPA 2019.