Toxicity of coastal waters: use of a quick algal bioassay

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TU 081
Optimization of the SPE step in the analysis of β-blockers and aromatase inhibitors in natural water samples by SPE-GE technique
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Environmental samples, especially sewage and marine-water samples are complex and often contain interfering elements that can mask or interfere with the analysed pharmaceuticals. In this study direct analysis these samples may not be possible. Additionally, the low concentrations in which the pharmaceuticals are generally found cause that an initial stage of concentration of the analytes prior to analysis is necessary. The selected adsorption step (SPE) is the most common sample preparation technique used in environmental areas.

Choice of sorbent is a crucial in SPE because it can control such parameters as selectivity, affinity and capacity. This choice depends strongly not only on the target analytes and the interactions of the chosen sorbent through the functional groups of the analytes, but also on the kind of sample matrix and its interactions with both the sorbent and the analytes. This work describes the application of the different kinds of SPE sorbents: C18 bonded silica gel (Strata C18), copolymers (Oasis HLB, Strata X, and Lichrolut EN), functionalized copolymers (Isolute ENV+), mixed-mode sorbent (mixed-mode chiral bonded silica sorbent Silica MCM-41), a safe drinking water column, a C18 sorbent (Strata C18) for extraction of six β-blockers (acebutolol, atenolol, metoprolol, nadolol, propranolol, pindolol), and two β-aromatase inhibitors (terbutaline, salbutamol) from natural water samples. Parameters such as pH of the loading samples, the amount and the kind of solvents used in conditioning, washing and eluting steps, were selected and optimized. The obtained extracts were evaporated to dryness, subjected to silylation using BSTFA, and finally analysed by GC-FID technique. The recovery of the analytes form natural water samples in the mentioned above condition will be discussed.

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TU 082
Mustard fractionation based on normal phase SPE and reverse phase HPLC (RP-HPLC) for isolation of endocrine disrupting chemicals in environmental extracts
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Mustard fractionation (EDA) approach aims to identify adverse pollutants by reducing the complexity of environmental matrices. Single hyperfractionation combined to biosays is powerful to find active chemicals and to direct chemical analyses to the “classical” pollutants. However, although the emergence of promising chemical tools (e.g. Orbitrap), identification of unknown active chemicals is still time and cost consuming due to the complexity of each active fraction (e.g. mixture effect). Hence, further fractionation steps are often needed. The aims of this study was to develop and to test the use of a first pre-fractionation step on SPE that will be followed by a RP-HPLC fractionation. First the separation of 12 EDCs have been evaluated with several elution conditions. Silica cartridges with a step elution - heptane, heptane/dichloromethane (50/50, v/v), ethyl acetate and methanol/water (50/50, v/v) - allowing the best and reproductible isolation of chemicals, have been chosen for further investigations. For these conditions, recoveries were assessed for the mixture alone and for a blank sediment extract spiked with this mixture. Finally, a natural sediment known to exert estrogenic, PXR(α/β) and AhR activity in the mussel Mytilus galloprovincialis was fractionated with these conditions. Good mixture recoveries (74-110 %), were obtained. The fractionation F1 contained only the PCBs and the PAHs, while 4-tert-octylphenol, triphenyl phosphate and fenobrate were detected only in F2. Finally, steroids, bisphenol A and clortimazole were found in F3 while F4 contained more polar chemicals.

Fractionation on natural sediment allows isolation of TCDD-like activity in F1 and F2 while PAH like activity was detected in F1, F2 and in F3. Then estrogenic compounds were only detected in F2 and F3. Interestingly, the sum of the estrogenic activity found in these 2 fractions is higher than the active in the crude extract, with a decrease in the occurence of anti-estrogenic chemicals. Finally, PXR-like activity was mainly detected in F3.

This pre-fractionation protocol allows, in the present case study, the isolation of several biologically active activities. Based on this first isolation directed hyperfractionation has then been undergone, RP-HPLC (hyperfractionation step) and a three fractionation step SPE (silica MCM-41) and a broad range of chemical properties and will be used to be the suitability of the isolation of active chemicals in the polar and semi-polar fractions.

TU 083
Towards a common mass spectra database for the identification of unknown environmental samples
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The identification of unknown compounds in environmental samples isolated during non-target screening or effect-directed analysis (EDA) is often a challenge on the way to the successful outcomes of this type of investigations. In the present study, the isolation of 35 EDCs using GC-HRMS in the polar, semi-polar and non-polar fractions is frequently used to generate mass spectra due to its potential to produce many fragments and therefore unique and/or identifiable spectra. This technique is commonly used and a lot of commercial and a few free mass spectra libraries are available to support identification. The advancement of database search strategies and publishing of online databases has improved tentative identification of many compounds in recent years, but many chemicals and their transformation products are still not included in such databases.

Improvements in the analytical technology and tools such as accurate and multidimensional mass spectrometry (e.g. FTICR, Orbitrap, ESI-FTICR, Orbitrap) in combination with liquid chromatography and soft ionisation techniques such as electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI) allow the analysis of a broad range of compounds including polar one (or polar substances) and the restriction of the elemental composition in many cases to one or few fragment ion series. These databases containing accurate mass spectra generally contain relatively few spectra and are not yet widely used, as many compounds relevant in environmental samples are still absent from these databases. One obstacle is the comparability of mass spectra generated with different settings, ionisation and spectrometric techniques due to increased instrument specificity, compared with the relatively reproducible EIMS spectra. Our aim is to improve the identification of unknown samples using a common and open access mass spectra database including MS data from all instrument types and with stop-hygrophilic data evaluation tools. The web-based database MassBank [1] was developed within a metabolomics consortium [2] and is a possible tool to achieve this target. The database is free and allows the storage of a wide variety of spectra including EIM, ESI-QToF-MSMS and ESI-FTICR-MS. Different tools are available to process the raw data and upload the data to MassBank including a spreadsheet-based record editor for the addition of metadata.

References:

TU 084
Construction of a water toxicity sensor based on luminescent bacteria
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Environmental water sample matrices, especially sewage and marine-water samples are complex and extracted by SPE-C18 cartridges. Toxin content was detected and quantified using high performance liquid chromatography (HPLC-DAD). The only microcystin detected was [d-Asp3]microcystin-RR. It was identified by retention time and spectrum comparing with a certified performance liquid chromatography (HPLC-DAD) sample.

Maximum level as dissolved microcystin was 0.7 µg/L on April 2009 sample. In the same sample, microcystin extracellular concentration was never above the WHO limits for drinking waters (1 µg/L).

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TU 085
Toxicity of coastal waters: use of a quick algal biosensor
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Toxicity testing of coastal waters using a quick algal biosensor is being developed at KWR. The bacteria are fixed on an optic fiber or a glass slide and placed in a continuous water flow. The light generated by the bacteria is then measured by photomultiplier tubes. The current prototype is highly adjustable and allows control of pH, temperature, flow, and pressure. Additionally, it is possible to add nutrients as well as test compounds to the water. This sensor prototype is being tested in both the laboratory and at monitoring sites along Dutch rivers. The ultimate aim is to develop a sensor that measures several types of toxicity and that can be applied continuously in the field, both at surface water inlets and in the distribution network.

TU 086
Dissolved and intracellular microcystins in lake waters during a Plankothrix rubescens algal bloom: HPLC quantification and crustacean acute toxicity test
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Microcystins, highly toxic cyclic peptides, are a group of hepatotoxic proteins produced by a number of aquatic species of cyanobacteria, such as Microcystis, Anabaena and Plankothrix. Worldwide, contamination in water has prompted the development of detection methods for their identification and quantification. A massive seasonal development of Plankothrix rubescens in a reservoir destined for crop irrigation located in Southern Italy has led to quantify algal toxin content in lake water to verify the possible health risk. Microcystins dissolved into the water were easily extractable from intracellular and extracellular reservoirs. Toxins were extracted by methanol/water solutions after frozen/defrosten treatment over night. Water samples were concentrated and extracted by SPE-C18 cartridges. Toxins content was detected and quantified using high performance liquid chromatography (HPLC-DAD). The only microcystin detected was [d-Asp3]microcystin-RR. It was identified by retention time and spectrum comparing with a certified standard. Quantification was made by means of a calibration curve obtained at 238 nm. Microcystin extracellular concentration was never above the WHO limits for drinking waters (1 µg/L). Maximum level as dissolved microcystin was 0.7 µg/L on April 2009 sample. In the same sample, the highest endocellular concentration (30.8 µg/L) of [d-Asp3]microcystin-RR was measured. As predictable, endocellular toxin was 90.95% of the total microcystin content; the endocellular