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 β-andenomimetics in natural water samples by SPE-GC technique

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Environmental water sample matrices, especially sewage and marine-water samples are complex and often contain interfering elements that can mask or interfere with the analysed pharmaceuticals. In this situation 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 and purification of the analytes prior to their analysis is necessary. The solid phase extraction (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 ion-exchange (Strata C-X, Oasis MCX) and a three-function sorbent (Strata Screen C) for extraction of six β-blockers (acebutolol, atenolol, metoprolol, nadolol, propranalol, pindolol,) and two β-adrenomimetics (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 SPE conditions will be discussed.

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TU 082

Mutistep 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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Effected Directed Analysis (EDA) approach aims to identify adverse pollutants by reducing the complexcity of environmental matrices. Single hyperfractionation combined to bioassays is usefull to isolate known active chemicals and to direct chemical analyses to these "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 4 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-like, anti-androgenic and dioxin-like activity was fractionated following 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 fenofibrate were detected only in F2. Finally, steroids, bisphenol A and clotrimazole 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 activity found in the crude extract, sugggesting the occurrence 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 biological activities. Based on this first isolation directed hyperfractionation has then been undergone, RP-HPLC hyperfractionation on C18 has been calibrated for the separation of 35 EDCs with broad range of chemical properties and will be readly used for the isolation of active chemicals in the polar and semi-polar pre-fractions.

TU 083

Towards a common mass spectra database for the identification of unknowns in 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 outcome of theses studies. Gas chromatography - electron ionisation mass spectrometry (GC-EI-MS) is frequently used to generate mass spectra due to its potential to produce many fragments and therefore unique and/or easily 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. QToF-MS, ToF-MS, FTICR, Orbitrap) in combination with liquid chromatography and soft ionisation techniques such as electro spray 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 formulae. However 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 EI-MS spectra.

Our aim is to improve the identification of unknowns in environmental samples using a common and open access mass spectra database including MS data from all instrument types and with sophisticated 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 EI-MS, 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:

[1] http://www.massbank.jp
[2] Horai H et al. (2010), J. Mass. Spec. 45, 703-714

TU 084

Construction of a water toxicity sensor based on luminescent bacteria

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To ensure safe drinking water it is critical to have a reliable toxicity monitoring system. Although there are several chemical and biological detection methods, there is no suitable system yet for the real-time monitoring of toxicants in water, taking endpoints into account with human relevance. This gap may be partly bridged by a sensor that applies genetically modified bacteria that respond to specific groups of toxicants by emitting luminescence. These bacteria carry a plasmid with a promoter-gene that is known to be activated in case of exposure to certain types of toxicants, for example DNA damaging agents or heavy metals. This promoter gene is coupled to a luminescence gene-set, so that luciferase is formed when the promoter is activated. The resulting production of light can then be detected and used as a measure of the toxic stress the bacteria were exposed to.

A new prototype of a flow-through sensor for on-line water monitoring based on these modified bacteria 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 stations 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 085

Toxicity of coastal waters: use of a quick algal bioassay

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Primary production by microalgae embodies the carrying capacity of marine ecosystems and is primarily linked to nutrient availability and light. However, recent studies indicate that certain industrial chemicals may have a direct impact on coastal plankton communities and hence on the carrying capacity of estuarine and marine ecosystems. At the same time the frequency and intensity of toxic algal blooms in the coastal zone are increasing globally, resulting in increased levels of toxins prospected to affect coastal ecosystems. These different chemical stressors are hypothesized to disturb regulatory mechanisms within algal communities, modifying the competitive abilities of individual species and resulting in shifts from highly nutritious to unfavourable algal species that destabilize the food chain. It remains however difficult to quantify the toxic effects of these chemicals: the relative contribution of anthropogenic and natural chemicals on the total chemical pressure is unknown. Also insight in the potential synergistic action of toxicants and toxins is lacking, while in the field many confounding factors (e.g. changing nutrient and light regimes) may mask effects.

The first step to unravel the complex interaction between algae and toxic pressure is to provide knowledge on chemical compounds causing phytotoxic effects. In this study we use passive samplers which extract the freely dissolved concentration in the water during a period of 6 weeks to take episodic events into account. The concentrated extracts are tested in an algal bioassay with different marine algal species (e.g. Dunaliella tertiolecta, Phaeodactylum tricornutum) to include differences in algal sensitivity. Use of Pulse Amplified Modulation (PAM) fluorometry provides a quick (4.5h) method to determine toxicity to algae based on changes in photosynthetic efficiency. An Effect Directed Analysis (EDA) will be performed to unravel which chemical compounds are responsible for the toxic effect on the algae. In 2010-2011 passive samplers are exposed at Hansweert (Westerscheldt, The Netherlands) and collected every 6 weeks to include the seasonal dynamics of both anthropogenic as well as natural compounds. Here, first results of this sampling campaign are presented and discussed. The results of the EDA analysis will be used in experiments where mixture toxicity, multi stress and community effects are taken into account to describe the overall toxic effect under relevant field conditions.

TU 086

Dissolved and intracellular microcystins in lake waters during a Planktothrix rubescens algal bloom: HPLC quantification and crustacean acute toxicity test

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Microcystins, highly toxic cyclic peptides, are a group of hepatotoxins produced by a number of aquatic species of cyanobacteria, such as Microcystis, Anabaena and Planktothrix. Worldwide contamination in water has prompted the development of detection methods for their identification and quantification. A massive seasonal development of Planktothrix rubescens in a reservoir destined for crop irrigation located in Southern Italy has lead to quantify algal toxin content in the lake water to verify the possible health risk. Microcystins dissolved into the water were separated from intracellular ones by filtering raw samples. Filters were extracted by methanol/water solutions after frozen/defrozen treatment over night. Water samples were concentrated 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 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