Toxicity of coastal waters: use of a quick algal bioassay
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Optimization of the SPE step in the analysis of β-blockers and β- and δ-endorphinics in natural water samples by SPE–GC 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 pharmacueticals. In this study direct analysis of these samples may not be possible. Additionally, the low concentrations in which the pharmaceuticals are generally found can be a major challenge for the analyst prior to separation. 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 different SPE sorbents: C18 bonded silica gel (Strata C18), copolymers (Osiris HLB, Strata X, and LiChrosorb EN), functionalized copolymers (Isolute ENV+), mixed-mode (diphenyl/phenylmethyl), and also a safe drinking water sorbent (Strata SCX). For extraction of six β-blockers (acetobutolol, atenolol, metoprolol, nadolol, propranolol, and pindolol), and two β-endorphinics (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 protein binding 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

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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The identification of endocrine disrupting chemicals (EDCs) 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 aim 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 EDC’s 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) - or a 2 step elution – heptane and dichloromethane (50/50, v/v) have been chosen for further investigations. For these investigations, 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, and β-receptor activities was fractionated under 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 clotrimazol 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 PAF-like activity was detected in F1, F2 and 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, suggesting 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 separations were performed on a C18 reversed-phase column (MCX) and a three-functional fractionation (MCX-NH2). This methodology can be used to isolate the active chemicals in the polar and semi-polar 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 identification of the investigated compounds. Good mass spectra can be used for the ready identification of the compounds and will be used for the isolation of the active chemicals in the polar and semi-polar fractions.

TU 084

Construction of a water toxicity sensor based on luminescent bacteria

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Toxicity of coastal waters: use of a quick algal bioassay

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The only microcystin detected was [d-Asp3] microcystin and was found in the crude extract, whereas no other microcystin congeners were detected. 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 clotrimazol 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 PAF-like activity was detected in F1, F2 and 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, suggesting 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 separations were performed on a C18 reversed-phase column (MCX) and a three-functional fractionation (MCX-NH2). This methodology can be used to isolate the active chemicals in the polar and semi-polar fractions.

TU 086

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

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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, concentrations in water have been investigated for many years. To date, most microcystin analyses are performed as liquid chromatography (HPLC)-DAD. The only microcystin detected was [d-Asp3] microcystin-LR. This microcystin 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-LR was measured. As predictable, endocellular toxin was 99.95% of the total microcystin content; the endocellular