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-adrenoceptors in natural water samples by SPE-UC technique

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Environmental contamination of aquatic ecosystems by beta-blockers is a significant problem due to sewage and marine water samples being complex and often containing interfering elements that can mask or interfere with the analytes. A straightforward strategy is to directly analyze the complete sample; however, this may not be possible due to the concentration and purification of the analytes prior to their analysis is necessary. The SPE step (SPE) is the most common sample preparation technique used in environmental areas. Choice of sorbent is a crucial point 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 resins (Toko MCD), a safe drinking water sorbent (Syracord Cx) for extraction of six β-blockers (acbetalol, atenolol, metoprolol, nadolol, propranolol, pindolol) and two β-adrenoceptors (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 analyzed 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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Butterfly 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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Directed Extraction (EDA) approach aims to identify adverse pollutants by reducing the complexity of environmental matrices. Single hyperfractionation combined to biosays is useful to identify 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 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)) were tested. However, the resulting isolation of each compound has been chosen for further investigations. For these conditions, recoveries were assessed for the mixture alone and for a blank sample extract spiked with this mixture. Finally, a natural sediment known to exert estrogenic, PXR, Ah receptor, a PPAR receptor, and a combination of these activities, was fractionated. 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 clomiphene were found in F3 while F4 contained more pollutant 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 of the crude extract, which is due to 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 (preparation of a natural matrix) and a three-fraction protocol has been developed for further investigations. 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 active compounds: the relative contribution of anthropogenic and natural chemicals on the total chemical pressure is unknown. Also insight in the potential synergistic action of toxins and toxins is lacking, while in the field many confounding factors (e.g. changing nutrient and light regimes) are the main effectors. 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 between 20 and 200 events into account. Extracted dissolved algaic toxins 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 of 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 Harzgouw (Westerscheld, 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 seasonal monitoring 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.