A novel 3-dimensional fractionation approach for effect-directed analysis of antiandrogenic compounds in a river water extract

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The occurrence of feminized fish in river water has been associated not only with estrogenic but also antiandrogenic compounds, which so far remain widely unknown. Effect-directed analysis (EDA) is a powerful tool for the identification of potentially harmful compounds in environmental compartments like sediments or water. The success of EDA is essentially influenced by the fractionation procedure. Classically, fractionation is performed in a sequence of fractionation, biotesting and selection of active fractions for the next fractionation step until the complexity has been reduced to a small number of compounds, for which structure elucidation is carried out. Often this process is tedious and time-consuming

In this study we aimed at the identification of the causes of antiandrogenic activity in a small, wastewater-impacted river in Germany. To this end, we developed a novel fractionation approach using three orthogonal columns in parallel as a time-efficient alternative, and applied it on an SPE extract from the river water.

For the selection of suitable LC stationary phases a set of 52 known or suspected androgens and antiandrogens was used. The retention times of these compounds were determined on 15 different reversed-phase stationary phases owning widely differing chemistries. Retention data were analyzed using principal component analysis, spearman rank correlation and two-dimensional plots of the retention data. This resulted in the selection of three columns (octadecyl, aminopropyl- and pentafluorophenyl-modified silica) allowing for the best orthogonal separation. Currently these columns are used for the fractionation of the river water sample extract. For antiandrogenicity detection, the cell-based antiAR-CALUX assay is performed at a non-cytotoxic concentration range to identify the most active fractions from each LC separation. These fractions will be analyzed by LC-high resolution mass spectrometry. Candidate peaks shared by antiandrogenic acitve fractions from the different separations will be selected and subjected to structure elucidation.