Advanced bioactivity screening analytics for rapid identification of environmental toxicants

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2018

document version
Publisher's PDF, also known as Version of record

8.1 Summary

This thesis describes the development of new analytical methods for the detection and identification of bioactive substances in environmental extracts. These analytical methods are based on integrating chromatography, mass spectrometry and bioassays. The rationale for developing and applying new analytical methods in environmental screening settings lies in current bottlenecks in traditional effect-directed analysis (EDA) or bioassay guided fractionation (BGF) approaches, such as their success rate and labor intensity. Although many EDA approaches have resulted in the detection of environmental toxicants, they are typically very low throughput and require multiple fractionation cycles for analyte isolation. During this process sample losses may occur and eventually full structural identification of the bioactives is often not achieved. An overview of the current technology and approaches used for the profiling of bioactive mixtures for environmental and food research but also pharma settings are discussed in chapter 2.

Chapter 3 describes the development of a liquid chromatography (LC) based high resolution fraction collection platform. The platform is based on the chromatographic separation of a bioactive mixture after which the column eluate is split towards a fraction collector for subsequent bioassay analysis and MS for bioactive identification. This analytical method was designed for the efficient identification of endocrine disrupting toxicants in environmental mixtures. Because fraction collection is performed at a high frequency, the chromatographic separation is essentially maintained thereby potentially preventing the need for repeated fractionation cycles and increasing EDA throughput. Selection of the reporter gene assay for estrogen receptor activity enabled sensitive and selective detection of estrogenic and anti-estrogenic compounds commonly encountered in environmental extracts at low levels. As such, it provided information on the functional activity of the bioactives. The strength of the platform lies in its ability to reconstruct bioassay chromatograms by plotting the assay response of each fraction against its corresponding fraction time. Subsequent comparison with the parallel acquired MS chromatogram allowed for rapid pinpointing of bioactives in complex chromatograms. Since only a small percentage of the fractions is used for bioassay testing multiple biological endpoints could be tested using a battery of assays. Compared to traditional EDA studies this approach significantly increased throughput which was also shown in chapter 4 where successful detection of estrogenic compounds in plastic casings from electronic products (television, router, decorative items, printer) was demonstrated using the LC fractionation platform described in chapter 3. A total of eight products were analysed and estrogenic

activity could be detected in four samples. Because additives have shown to successfully migrate from plastic casings into the environment this may be an important source of estrogenic compounds.

In chapter 5, the development of analytics involving a fraction collector based on solenoid valve technology is described for accurate and contact-free high-resolution fraction collection of LC separations. Fraction collection in conventional LC is mostly based on the collection of droplets released by gravitational force from a fraction collection tip. In this scenario, repeatable fractionation may not be possible when small fractions need to be collected and the droplet release rate has to be increased. This can result in alternating fractions containing different volumes of LC effluent thereby affecting the repeatability of bioassay analysis. The implementation of a solenoid valve enabled non-gravitational ejection of very small droplets at a high frequency, thereby improving the fraction collection accuracy. To this end mechanical and electronic modifications to existing robotics were made. A mounting unit for the solenoid valve and a low dead volume unit to connect the solenoid valve to the LC tubing was designed. Also, an electronic signal converter was developed for accurate control of the pulse width and solenoid-valve supply voltage. Droplet ejection of the platform was tested with multiple LC solvents, and mixtures thereof, while covering a wide range of flow rates. Results showed there is little variation in the collected volumes and therefore the fraction collection was very repeatable, also at low flow rates. Peak broadening induced by the system was negligible. The potential of the system for bioactivity screening was successfully demonstrated by fractionating mixtures containing estrogenic and androgenic compounds, and a spiked environmental extract. All bioactives present in the analysed mixtures were successfully identified. Repeated fractionation showed that analytes were consistently collected in the same fractions indicating the system can be used for analyte purification and enrichment. The latter was also performed to demonstrate the feasibility of analyte enrichment for NMR analysis.

While bioactive isolation is predominantly performed with LC fractionation, GC remains the method of choice for many compound classes and is the preferred technique to study the occurrence and fate of many classes of environmental pollutants. However, compared to LC fractionation, collecting fractions from a GC separation is much more challenging. In contrast to available devices capable of collecting only a few fractions after GC, in chapters 6 and 7 a fractionation technique is described for the collection of complete GC separations. The platform is based on the post-column infusion of a vaporized trap solvent that condenses once directed outside the GC oven via an exit capillary allowing the collection of trap solvent droplets containing separated compounds. Parallel flame ionization detection (FID) (chapter 6) and MS (chapter 7) detection created

the possibility to directly correlate fractions to chromatographic peaks. The specific advantage of the MS detector is the availability of electron-ionization (EI) spectral databases useful for analyte identification. The technique's feasibility for toxicity screening purposes was demonstrated for environmental profiling of acetylcholinesterase inhibiting and anti-androgenic compounds thereby demonstrating its potential impact on EDA research. The technique proved to perform repeatable fractionation cycles (30x) without significant band broadening and could be used for analyte enrichment purposes.