Summary

Nowadays, the major challenge in the field of environmental research is the increasing sample complexity. New chemicals are introduced every day and many end up in environmental matrices. Due to an improved sensitivity, more chemicals can also be detected. The challenge of the analytical environmental scientist, to determine which contaminants are possibly harmful, is therefore becoming more complicated. Non-target analysis is therefore becoming more popular. This study showed that comprehensive two-dimensional liquid chromatography (LC × LC) coupled with high resolution mass spectrometry (HRMS) is a promising tool to assist non-target analysis and effect-directed analysis (EDA) of complex environmental samples.

The background and the objectives of the study have been introduced in Chapter 1. In non-target analysis, the identification of environmental contaminants in complex samples by liquid chromatography coupled with mass spectrometry (LC-MS) is hampered by insufficient separation power. Similarly, although EDA is a useful approach to deal with complex environmental samples (e.g. effluent, sediment, indoor dust and biota) by combining biological analysis, fractionation procedures and chemical analytical methods, the wider application of EDA in environmental research is limited by the resolution of the fractionation and the throughput of the bioassays. LC × LC is an emerging technique that provides high separation power and multiple selectivity by combining two HPLC columns with different stationary phases. Although until now few LC x LC studies were carried out in environmental research, the technique has great potential for analyzing complex environmental samples. Therefore, the objective in this study was to apply LC × LC in both non-target analysis and EDA, to help to unravel the sample complexity in environmental research.

The LC × LC based non-target analysis was successfully applied in characterizing a wastewater treatment plant (WWTP) effluent sample (Chapter 2), as well as indoor sample matrices such as household dust and laundry dryer lint (Chapter 3). LC × LC was coupled with a high resolution time of flight mass spectrometer (ToF-MS) equipped with an electrospray ionization (ESI) source. To adapt the flowrate in the second dimension of LC × LC to the ESI-ToF MS interface, a post-column flow splitter was used.

The repeatability of the retention time, and the orthogonality of the LC × LC separation were characterized. Different combinations of stationary phases were tested. For the WWTP effluent, the stationary phase combination of C18 and PFP showed the best orthogonality. However, due to good solvent compatibility and a complementary separation mechanism, the combination of hydrophilic interaction chromatography (HILIC) and C18 or other reversed-phase liquid chromatography is a potential option for the future. In particular, the LC × LC separation significantly reduced the matrix effect and led to easier identification by high resolution MS based on measured accurate mass and isotopic pattern, which is essential in environmental analysis. The candidate chemical formulas were screened in online databases (e.g. KEGG, Chemspider, METLIN) to find the most probable compounds. Dozens of interesting environmental contaminating compounds were identified in the WWTP effluent, household dust and dry lint samples. To confirm their presence, a two-dimensional retention time alignment approach was developed and applied to the candidate compounds for which analytical standards were available.

To further support the LC × LC based EDA, a FITC-T4/TTR binding assay was miniaturized in a 96 well plate to achieve high throughput determination of thyroid hormone (TH) disruption in environmental samples or sample fractions (Chapter 4). Because of the costly materials and tedious handling procedures, it would have been impossible for the traditional in vitro assays (e.g. radio-ligand binding assay) to be applied in high throughput EDA. The miniaturized FITC-T4/TTR binding assay, in which a fluorescent probe was used, was successfully applied for characterizing eight TH disrupting or potential TH disrupting chemicals from different compound groups such as hydroxylated polychlorinated biphenyls (OH-PCBs), perfluoroalkyl and polyfluoroalkyl substances (PFASs), hydroxylated polybrominated diphenyl ethers (OH-PBDEs) and other brominated flame retardants (BFRs). It was also used to examine the thyroid hormone (TH) disrupting potency of 22 herring gull egg samples from two different locations in Norway. The sensitivity of the assay was on average about one magnitude lower than the classical radio-ligand binding assay, yet the throughput was at least 100-fold enhanced and the costs were substantially reduced. Although the high throughput FITC-T4-TTR assay developed in this study was

designed to fulfill the LC × LC-facilitated EDA approach, the assay is also extremely valuable in high throughput screening of a large quantity of environmental samples. It may be a good alternative to the radio-ligand binding assay for analyzing environmental samples, which are easily obtained in large quantities, such as WWTP effluent, surface water, sediment, soil and household dust.

In Chapter 5 another high throughput assay, the acetylcholinesterase (AChE) inhibition assay, was used in combination with LC × LC-ToF MS facilitated EDA. The enhanced peak capacity in LC × LC enables high resolution post-column fractionation and, therefore, avoids sub-fractionation steps for identifying toxicants in the fractions. As a result of much finer separation and fractionation, the candidate lists of toxicants in each active fraction was significantly shortened and the ion suppression in mass spectrometry was much reduced. By using a post-column flow splitter, MS detection was made possible in parallel to the fractionation procedure. The toxicity in the fractions and their chemical identities could be linked via the retention time and the fraction number. Eventually, by performing high resolution LC × LC fractionation, parallel LC × LC-ToF MS identification and a high throughput bioassay, high throughput EDA has reduced the total analysis time of an EDA study of a complex environmental sample from months to a few weeks (Chapter 5).

Finally, in Chapter 6, the results achieved in this thesis are discussed. The developed LC × LC technique provides enhanced separation power and a higher throughput in non-target and target contaminant analysis. By applying various stationary phase combinations and coupling to different MS interfaces, the potential is even larger than was demonstrated in this thesis. In addition, LC × LC facilitated EDA has a potential application in screening biological active ingredients from complex matrices in other fields. It should also be mentioned that LC × LC may not be suitable in all occasions in environmental research. Because of the intrinsic characteristics of LC × LC, the necessary ultra-fast separation in the second dimension comes at the sacrifice of sensitivity. Therefore, LC × LC works best when the sample is complex enough and highly concentrated. For instance, water samples obtained by passive sampling and large volume solid phase extraction are ideal for LC × LC analysis.