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Comprehensive two-dimensional liquid chromatography coupled to high resolution time of flight mass spectrometry for chemical characterization of sewage treatment plant effluents☆

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ABSTRACT

For the first time a comprehensive two-dimensional liquid chromatography (LC × LC) system coupled with a high resolution time-of-flight mass spectrometer (HR-ToF MS) was developed and applied for analysis of emerging toxicants in wastewater effluent. The system was optimized and validated using environmental standard compound mixtures of e.g. carbamate pesticides and polycyclic aromatic hydrocarbons (PAHs), to characterize the chromatographic system, to test the stability of the retention times and orthogonality. Various stationary phases in the second dimension were compared for the LC × LC analysis of silicon rubber passive sampler extracts of a wastewater effluent. A combination of C18 and Pentafluorophenyl (PFP) was found to be most effective. Finally, the hyphenation of LC × LC with HR-ToF MS was optimized, including splitter settings, transfer of data files between the different software packages and background subtraction using instrument software tools, after which tentative identification of 20 environmental contaminants was achieved, including pesticides, pharmaceuticals and food additives. As examples, three pesticides (isoproturon, terbutryn and diazinon) were confirmed by two-dimensional retention alignment.

1. Introduction

Due to massive human activities such as agriculture, wastewater discharges and industrial manufacturing, potentially harmful chemicals reach the environmental. These chemicals are large in number and possess diverse physicochemical properties, ranging from non- or weakly polar compounds such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) to strongly polar compounds such as novel pesticides, pharmaceuticals and personal care products (PPCPs).

Consequently, the analysis of environmental contaminants has always been a challenge and due to the increase in the use and production of chemicals, development of methods for environmental analysis are rapidly expanding. Gas chromatography (GC) in combination with mass spectrometry (MS) has proven its suitability, resulting in the routine separation and identification of environmental contaminants, greatly owing to the well-established mass spectral databases such as the National Institute of Standards and Technology (NIST) database. In the early 1990s, comprehensive two-dimensional gas chromatography (GC × GC) was developed, demonstrating outstanding capability to separate complex environmental samples due to greater peak capacity [1]. Yet, GC and GC × GC are not capable of direct analysis of non-volatile and thermo-labile compounds, unless derivatization steps are introduced, which sometimes are time-consuming.

As an alternative approach to GC, high performance liquid chromatography (HPLC) has also found wide application in the field of environmental chemical analysis over the last decades. Besides the 'classical' reversed phase liquid chromatography (RPLC), in recent years hydrophilic interaction liquid chromatography (HILIC) has been established as a complementary method in environmental analysis. HILIC is especially powerful for separating polar compounds that are usually not retained in RPLC, such as pharmaceuticals [2], organophosphorus pesticides [3], drugs of abuse [4], etc. Besides, by coupling HPLC with modern high resolution mass

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spectrometry (HRMS), non-target screening can be performed for identification and structure elucidation of unknown compounds [5]. Moreover, in the field of effect-directed analysis (EDA) [6], which combines fractionation procedures, effect-based testing and chemical analysis, the use of LC techniques for fractionation of extracts is highly favored over GC due to the complexity of collecting gaseous fractions, although innovation in GC fractionation has been reported [7].

There is no universally applicable separation technology for analyzing all sorts of contaminants, as different classes of compounds require a different approach. However, there is a great demand to apply separation approaches that are able to provide greater separation power, to cover a wider spectrum of analytes and to deliver high throughput.

Comprehensive two-dimensional liquid chromatography (LC × LC) is an emerging technique that has been applied in areas of notable sample complexity, due to its greater peak capacity and tunable selectivity by different stationary phase combinations. Ideally, the peak capacity of LC × LC can achieve the product of each individual peak capacity of the first and second dimension separation, provided that the two dimensions are orthogonal [8]. According to the physicochemical properties of the compounds to be analyzed, the mechanisms of the two separation dimension can be chosen from a variety of stationary phases: RPLC, normal phase liquid chromatography (NPLC), size exclusion chromatography (SEC), ion exchange chromatography (IEC) and HILIC [9]. Examples of LC × LC applications consist of the analysis of synthetic polymers [10], oligonucleotides [11], peptides [12] and proteins [13], pharmaceuticals [14], natural products such as food [15] and Chinese medicines [16] and many other compounds [9].

It is surprising that to our knowledge no LC × LC analysis of contaminants in environmental sample has been reported, although the technique provides almost all the advantages and perfectly meets all the analytical challenges: higher peak capacity, multi-selectivity, suitability for emerging thermo-labile and polar compounds, possibility of post-column fractionation, etc. The objective of this study was to establish and validate an LC × LC-UV/HRMS platform capable of screening and analyzing environmental contaminants in exceedingly complex environmental samples (e.g. wastewater effluent, sediment and biota samples). In our study, the LC × LC system was first validated by environmental standard mixtures. The LC × LC-ToF MS system was then optimized and used for comprehensive analysis of a passive sampler extract of effluent from a wastewater treatment plant (WWTP) in The Netherlands.

2. Experimental

2.1. Sample collection and preparation

The sample used to demonstrate the suitability of LC × LC for environmental contaminant analysis was collected from WWTP Cuijk (The Netherlands) using silicon rubber passive samplers (six blades on holder, 20 g total weight) [17]. The samplers were deployed at the sedimentation pond that receives the effluent from the WWTP. The silicon rubbers were collected after six weeks of exposure and thereafter cleaned with water from the sampling site in order to remove sedimentation and bio-fouling. Cleaned samplers were transported to the lab in plastic containers, and stored at −20 °C until extraction.

When preparing the sample, the silicon rubber blades were cut into small pieces and collected in pre-cleaned thimbles used in the Tecator® Soxtec Avanti extraction system. The extraction was performed with 80 ml of a methanol:acetonitrile (1:2, v/v) mixture and 5–6 boiling stones. The extraction program was 120 min of boiling at 120 °C, 30 min of rinsing, 5 min of recovery and 1 min drying. After cooling the extracts were filtered over Duran® glass fibre filters (100–160 μm) and collected in 250 ml glass bottles. Extraction jars were rinsed twice with 10 ml of extraction mixture. Extracts were evaporated by a TurboVap® at 45 °C to approximately 5 ml. The extracts were transferred (rinsing twice with 5 ml extraction mixture) to conical tubes and evaporated under nitrogen to exactly 10 ml. Before injecting into the LC × LC-ToF MS system, the extracts were diluted by a factor of 10 (v/v) in acetonitrile and Milli-Q water (1:1, v/v).

2.2. Chemicals

Methanol and acetonitrile were of HPLC grade supplied by Sigma-Aldrich (Zwijndrecht, The Netherlands). Water was obtained from a Milli-Q Reference A^+ purification system (Millipore, Bedford, MA, USA). Formic acid as LC-MS eluent additive was purchased from Sigma-Fluka (Zwijndrecht, The Netherlands).

The EPA 531.1 carbamate mixture was purchased from Sigma-Fluka. The EPA 8270C multiple component standard mixture solution was from Chiron (Trondheim, Norway). The triazine and urea pesticide mixture was obtained from LGC Standards (Teddington, UK). Details of all standard mixtures were given in supplementary information. All standards were diluted to 1 μg/ml prior to injection.

2.3. Instrumentation

The LC × LC system consisted of an Agilent 1100 auto sampler, an Agilent 1100 HPLC binary pump for the first dimension, an Agilent 1290 infinity UHPLC binary pump for the second dimension and an Agilent 1290 infinity thermostatted column compartment (TCC) with a 2-position/4-port duo valve installed as the 2D interface (Agilent Technologies, Waldbronn, Germany). Two sampling loops (60–120 μl) were applied to collect the eluent from the first dimension and thereafter via valve switching, deliver the collected eluent to the second dimension separation in the next sampling cycle. The 2D-LC add-on for Chemstation version B.04.03 (Agilent Technologies) was used to control the LC × LC modulation. The detection was done using an Agilent 1260 Infinity VWD detector (Agilent Technologies) and a Bruker micrOTOF™ Time of Flight (ToF) mass spectrometer with an electrospray interface (ESI, Bruker Daltonics, Bremen, Germany), by splitting the flow after the second dimension using a QuickSplit™ adjustable flow splitter (Richmond, CA, USA). The Bruker micrOTOF™ was initiated (start and stop signal) by external control via a serial port of the auto-sampler and the MS data were recorded by Bruker OtofControl 3.0. Two-dimensional data (both from UV and MS) evaluation was done with the software GC Image 2.3b4 (Lincoln, NE, USA). Compound screening and identification were carried out using the instrument software packages *DataAnalysis* version 4.1 and *MetaboliteDetect* version 2.0 (Bruker Daltonics).

In the first dimension of the LC × LC system, a ZORBAX Eclipse Plus (1.8 μm, 2.1 × 150 mm ID) C18 Rapid Resolution HD column (Agilent Technologies, USA) was used. An Agilent Poroshell 120 (2.7 μm, 50 × 4.6 mm ID) Phenyl Hexyl column (Agilent Technologies, USA), an Agilent ZORBAX (1.8 μm, 50 × 3.0 mm ID) HILIC Plus column (Agilent Technologies, USA) and a Phenomenex Kinetex (2.6 μm, 50 × 4.6 mm ID) PFP column (Phenomenex, USA) were used for the second dimension.

2.4. Methods

2.4.1. LC × LC-ToF MS conditions

The chromatographic conditions of the LC × LC experiments are listed in Table 1. Shifted gradients were applied to provide better