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Non-targeted analysis of wastewater treatment plant effluents by high performance liquid chromatography–time slice-solid phase extraction-nuclear magnetic resonance/time-of-flight-mass spectrometry

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ABSTRACT

Extracts of effluents from two different wastewater treatment plants (WWTP) in Switzerland taken during the application period of pesticides were examined by coupling an HPLC–MS system to a nuclear magnetic resonance spectrometer using a post column peak trapping device. By trapping 1 min portions of the chromatogram onto post column solid phase extraction cartridges (time slice-SPE-NMR) a comprehensive overview of proton carrying constituents could be achieved. Non-supervised statistical analysis of the NMR spectra obtained by this approach revealed NMR resonances pointing to contaminants present in decreasing proton concentration in the extracts. Comparison of exact mass data acquired during the trapping process to these NMR resonances enabled the identification of the pesticides Linuron, Metazachlor, Ethofumesate, Isoproturon, Metamitron, Propazine and Chloridazon. Desaminometamitron, a known transformation product of Metamitron could also be identified together with unexpected highly concentrated C8, C10 and C12 fatty acids and their glycerol mono- and di esters. Other compounds identified were a drug metabolite (3-Carboxymefenamic acid), a sun screen agent (Ensulizole: 2-Phenyl-1H-1,3-benzodiazole-6-sulfonic acid) and industrial chemicals (Benzotriazole, N-Benzyl-indole). In addition, a number of well-resolved proton spectra cannot be attributed to a mass response showing the need of further investigations using 2D-NMR and different ionization techniques.

1. Introduction

The occurrence of pesticides (herbicides, fungicides and insecticides) and other xenobiotics (pharmacologically active compounds, endocrine disrupting agents) and their corresponding transformation products (TPs) in the water compartment, particularly ground water, surface water and wastewater effluents, is still of growing concern and represents a major challenge for the preservation and sustainability of the environment. The decision as to which of these compounds should be included in monitoring programs is difficult as there are thousands of potential TPs resulting from the transformation of hundreds of parent pesticides and thousands of other xenobiotics in the environment.

Beside GC–MS, high-performance or ultra-performance liquid chromatography (HPLC/UPLC) coupled to tandem mass spectrometry (MS/MS) are most widely used for sensitive and selective quantification of targets that show specific mass transitions in the selected reaction monitoring mode (SRM) [1]. Alder et al. could show the superiority of LC–MS compared to GC–MS for the analysis of priority pesticides. Only for one group (organochlorine pesticides) being very non-polar, GC–MS achieved a better performance [2]. Nevertheless, the LC–MS/MS approach has several limitations, such as the limited amount of compounds which can be detected, the increasing possibility of finding common transitions for coeluting, isobaric substances and finally the "blindness" of SRM methods to compounds which are not included in the target set. As a consequence, no information on non-targets/unknowns will be available using this technique. Therefore, there is a strong need for methods allowing rapid and reliable screening of a large number of compounds.

Together with time-of-flight instruments, Orbitrap ion cyclotron mass spectrometers provide a high resolution and mass accuracy and have been used for both targeted analysis and for the structural elucidation of unknowns [3]. While linear ion traps typically achieve a resolution 10000 and time-of-flight instruments up to 40,000, Orbitrap mass spectrometer reach a resolution of up to 100,000 with scan rates above 3 s per spectrum.

Apart from this, HPLC–time-of-flight mass spectrometry (HPLC–TOF-MS) has become a cost-effective technique for performing routine accurate mass analysis and has successfully been

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applied to the analysis of pesticides and TPs in river water and wastewater samples [4–11]. Moreover, HPLC coupled to hybrid quadrupole-TOF (HPLC–Q-TOF) has been exploited in the elucidation of photo- and biological TPs of single pesticides [12–16]. The advantages of HPLC–(Q)-TOF-MS have been described in several reviews [17–19,3,20]. In summary: the sensitive, full-scan, high-mass resolution and accuracy provided by these instruments are very well suited for the screening and confirmation of pesticides. Additionally, TOF-MS capability of inspecting full-scan data post-acquisition is of particular interest for the identification of unexpected xenobiotics and their TPs or metabolites. Currently, two major strategies are followed in HPLC–(Q)-TOF-MS: target screening followed by an automated target-database search (i) and non-target screening using HPLC–Q-TOF-MS for studying fragmentation patterns of unknowns and subsequent structure elucidation [10]. In this context, mass accuracy, isotopic pattern, retention time of the eluting compound and fragmentation during MS/MS play an important role in the process of identification.

Proton detected nuclear magnetic resonance (^{1}H NMR) has been used in the past in a completely non-targeted approach for the investigation of environmental samples originating from former ammunition production sites [21]. Even though it has an intrinsically low sensitivity, it offers the great advantage that the molar response of a magnetically active nucleus in the spectrum is completely independent of the physical and chemical properties of the analyte. Therefore, the concentration of a contaminant present in environmental extracts can be calculated or at least estimated provided that the compound of interest is harboring the nuclei under investigation and the mass of the analyte is given. The mass of unknowns can be easily obtained from its mass spectrum. From accurate mass measurements the molecular formula can be calculated allowing database search for well-known chemicals.

LC-NMR is a well-known technique and is utilized for many applications, e.g. natural product analysis [22,23], pharmaceutical analysis [24] and environmental analysis [25,26]. With the advance of cryogenically cooled NMR probe heads and post column SPE prior to the elution to the NMR tube or flow probe head, detection limits have been dramatically improved to the mid- to upper ng-range of analyte injected on column depending on the size of the molecule [27]. One way to operate such a system is to trap time intervals of the chromatographic run on post column SPE cartridges. This allows a comprehensive overview of all proton carrying contaminants present in the extract of an aqueous sample comparable to an onflow LC-NMR run [28]. The advantage of the time slice SPE-NMR technique is a higher sensitivity in the final NMR spectrum combined with NMR spectra acquired under well-defined conditions, e.g. in deuterated solvents. Comparison to spectra of reference compounds measured in the same solvent allows unequivocal identification of the analyte. Here we describe the use of such an LC-SPE-NMR/MS system in the time slice mode for the first time.

2. Experimental

2.1. Reagents and chemicals

LiChrosolv LCMS-grade acetonitrile and water was obtained from Merck (Darmstadt, Germany). Formic acid was from Fluka (Buchs, Switzerland). Deuterated formic acid (95%) and methanol (99.8%) were obtained from Deutero GmbH (Kastellaun, Germany). Pesticide standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany). 2-Phenyl-5-benzimidazolesulfonic acid was obtained from Sigma–Aldrich (Steinheim, Germany).

2.2. Effluents of wastewater treatment plants (WWTP)

Effluent samples from the WWTP Ins and Lyss, located in an area rich of intensive agriculture, were obtained during the main application period of pesticides (March–July 2009) as follows: 2 weeks composite samples were prepared by pouring every day approximately 50 ml of a 24 h composite sample into a 1.5-L glass bottle and immediately freezing it at −20 °C. In this way, 600–700 ml of a 2 weeks composite sample was obtained and a total of 9 of such samples during the whole pesticide application period. These samples were pre screened for major pesticides such as Chlorotoluron, Isoproturon and Metolachlor using HPLC–tandem mass spectrometry (HPLC–MS/MS). Experimental conditions and pesticides analyzed are summarized in Table S1 in the supplementary information (SI). Finally, the three samples exhibiting the highest pesticide concentrations were pooled to one (1.8–2 L) and further used for SPE clean-up. Before SPE samples were thawed and decanted from remaining particles.

2.3. Solid phase extraction

Solid phase extraction was done after acidification with 2 mL of glacial acid and stabilization with 2 mg of sodium azide on Phenomenex Strata X SPE cartridges filled with 500 mg of sorbent (Aschaffenburg, Germany). Prior to loading, the SPE material was washed with 5 mL of methanol/acetonitrile 1:1 followed by conditioning with 5 mL of ultra pure water. 900–1000 mL of each pooled sample were passed through the sorbent bed at a flow rate of 5–6 mL/min. Therefore, two SPE cartridges per pooled sample were used. After loading, the sorbent bed was washed with 5 × 1 mL of 5% methanol/water and dried for 30 min by passing air through the cartridge. Elution of the analytes was done with 5 × 1 mL of methanol/acetonitrile 1:1. After combining the two extracts for each individual sample, the extracts were evaporated to dryness with argon gas.

The extracts were re-constituted in 200 μL of deuterated methanol for analysis by LC-SPE-NMR/MS. Prior to the injection on the HPLC column both extracts were measured in a 3 mm NMR tube using the NMR spectrometer described in Section 2.5.

The trapping efficiency of the SPE was studied by spiking tap water and matrix ($n = 2$) at the 1 and 10 μg/L level with pesticides and applying the SPE extraction procedure as described above. Recoveries were calculated and are presented in Table S2 (SI).

2.4. HPLC-time slice-SPE/TOF-MS analysis

The system consisted of an Agilent 1200 HPLC system (quaternary pump, auto sampler, diode array detector, Waldbronn, Germany), a Bruker/Spark Prospekt 2 SPE cartridge exchanger (Emmen, The Netherlands) and a MicrOTOF mass spectrometer from Bruker Daltonics (Bremen, Germany). The scan range was between m/z 50 and 1000. The calibration was done with a 20 mM lithium formate solution that was introduced to the ion source with the help of a divert valve at the beginning of each chromatographic run. Separation was done on an Agilent Eclipse C18 250 mm × 4 mm, 5 μm particle size (Agilent, Waldbronn, Germany) with a flow rate of 0.5 mL/min. The chromatography was done with a solvent composition starting with 100% A (H_2O 0.1% formic acid-d_2) and 0% B (acetonitrile 0.1% formic acid-d_2) which was held for 5 min for multiple injection. After 5 min the composition changes to 90% A using a step gradient. Within 80 min the composition changes linearly to 0% A. 12.5 μL was injected 4 times on-column using an injection program.

Starting from 15 min, each minute of the chromatogram was guided to a post column SPE cartridge after adding a make-up of 1.5 mL/min (mobile phase A) to the effluent from the column