HILIC workflow strategy for the hidden target screening of very polar compounds in surface waters

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Abstract

Various workflows have been published for the workout of datasets for the Non-Target screening of water samples for TOrCs. The main aim is to provide a better and more complete view of the organic content in different water bodies, as well as identify new compounds of interest. This study was realized by analyzing river water samples with the established RPLC-HILIC-ToF/MS system and by using the STOFF-IDENT compound database. Its main aim is to demonstrate and discuss an efficient strategy for the non-target screening of aqueous environments, as a mean to facilitate the process of identification of very polar compounds. The samples were prepared using polarity extended SPE and analysed by the established RPLC-HILIC-ToF/MS system. The analysis data were then processed following a non-target screening workflow for very polar compounds. After importing the data in STOFF-IDENT database, a proposed list of possible compounds in the samples was created. Using reference standards of the proposed compounds and MS/MS fragmentation data, it was possible to positively identify nine polar compounds, of which six have not been reported previously in water surface samples.

Keywords: Polar TOrCs, HILIC, RPLC-HILIC-ToF.MS, Non-Target Screening, STOFF-IDENT,

1. Introduction

Trace Organic compounds (TrOCs) in water can be biogenic or anthropogenic. The anthropogenic TrOCs enter the environment through various point and diffuse sources, and can be regulated or unregulated organic compounds, metabolites and transformation products. These compounds can range a lot in terms of polarity. Reverse Phase Liquid Chromatography (RPLC) is the most common and widely used tool for the separation of non-polar and mildly polar compounds. However, for the separation of very polar compounds, techniques like the Hydrophilic Interaction Liquid Chromatography (HILIC) are needed. Using a serial RPLC-HILIC system coupled with ToF-MS the analytical screening of samples comprised of solutes with variability in structure and polarity can be achieved (Greco *et al*. 2013, 2014). Full-spectrum acquisitions in non-target screening approaches are producing large datasets with the detected features (i.e. an exact mass at a certain Retention Time, RT) of the samples. Different workflows have been published, proposing ways to cope with the collected amount of data in an automatic, time efficient and reproducible way, which can be applied to samples with various matrices (Gago-Ferrero *et al*. 2015, Hernández *et al*. 2005, Krauss *et al*. 2010, Letzel *et al*. 2015). The main aim is to provide a better and more complete view of the organic content in different water bodies, as well as identify new compounds of interest.

2. Materials & Methods

2.1. Samples

Three 24h composite samples were collected using time proportional samplers; upstream and downstream of a WWTP and wastewater effluent. The WWTP serves a daily average of 22700 population equivalents (p. eq.) and has a mean flow rate of 6500 m3/d. Upstream of the WWTP, the river passes mainly through rural areas, with one additional smaller WWTP (800 p.eq.) discharging into it.

2.2. Sample Pretreatment and Analysis

The samples were prepared using polarity extended SPE. The method consists of a combined RP-HILIC SPE. C18 cartridges were used for the concentration of the non-polar compounds and ZIC-HILIC for the concentrations of the polar and very polar ones. The chromatographic seperation of the samples was performed by a serial RPLC-HILIC system. The development and robustness of the method has been described previously in detail (Greco *et al*. 2013, 2014) . The RPLC column was a Poroshell 120 EC-C18 (50.0×3.0 mm, 2.7 μm) (Agilent Technologies, USA), and the HILIC was a ZIC®-HILIC column (150 × 2.1 mm, 5 μm, 200 A°) (Merck Sequant, Sweden). The two columns are connected in series (fig. 1).

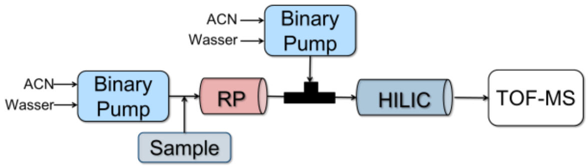

Figure 1. The serial RPLC-HILIC-ToF/MS.

For the Non-Target screening, the serial RPLC-HILIC system was coupled with an Agilent series 6230 API-TOF-

MS system, equipped with a Jet Stream ESI interface (Agilent Technologies, Santa Clara, CA, MS, USA). For the MS/MS evaluation, the RPLC-HILIC system was coupled to a QTrap 5500 Mass Spectrometer (Sciex, Framingham, MA, USA) equipped with a TurboVTM source.

2.3 STOFF-IDENT Compound Database

STOFF-IDENT database (BETA version, update 04/12/2015, http://bb-x-stoffident.hswt.de) was used to match the sample features with possible anthropogenic, water-relevant compounds.

2.4 Hidden Target Screening Workflow

The strategy followed for the identification of possible compounds in a sample's polar fraction (HILIC results) is divided in five steps, beginning from the sample analysis and leading to the final identification of individual compounds. In Step 1 a sample feature list (RT, m/z or molecular formula) is created after the implementation of the feature extraction algorithm and blank correction. The list is then imported in STOFF-IDENT database (Step 2), and a results list is created containing the compounds that were matched with the features. In Step 3 the proposed compounds list can be filtered by excluding the compounds with LogD values higher than zero. Step 4 is a manual re-evaluation of the original feature peaks. Finally, in Step 5 the proposed compounds can be verified or rejected using reference standards and MS/MS data.

3. Results & Discussion

The workflow for identifying polar compounds described in 2.4 was applied on the polar fraction of all three samples. The purpose of the Step 1 of the workflow is to evaluate the raw data that is produced through the sample analysis. The prepared data should be free of analysis artifacts (blank's peaks and instrument noise), containing the analytes that were separated by the HILIC column. After applying Step 2, usually the number of proposed compounds by SI can be larger than the number of the matched features. This can happen because of the isomers existing in the database. Since the search in SI is done by matching molecular formulas/masses, all compounds meeting the search criteria will be in the results list. The names of the proposed compounds together with existing data on them (physicochemical properties, SMILES, class, etc.) will appear in the results list. The additional data on the compounds can then be used for filtering further the results. As described in Step 3, the list is filtered further by applying the LogD filter. The proposed compounds with a positive LogD(pH7) are highly unlikely to be correct, and can be excluded from the results list Step 4 serves as a quality check to ensure that the initial features that were matched with a compound in the database originate from acceptable peaks. It is possible that from the initial data handling, features were extracted from areas without a peak. Finally, in Step 5 the remaining proposed compounds by SI can be verified or not, using reference compounds and MS/MS data. A tentative first evaluation of the accuracy of SI proposed compounds is made by comparing the m/z and RT of the standards solutions against the ones from the samples. Further certainty can be achieved by confirming the results through further comparison to MS/MS fragmentation patterns. MS/MS data can also resolve issues that occur due to isomers and co-elution of analytes. After applying the workflow strategy on the surface water samples, the initial large number of features was reduced gradually. Some of the final proposed SI results where then tested against reference compounds. Finally, it was possible to successfully verify the presence of nine polar and very polar compounds.

4. Conclusion

This study's NTS workflow offers a new approach for the detection and identification of very polar and polar compounds. Although the analytical methods, instrumentation and data analysis tools used were ones available in this laboratory, the proposed strategy can be adjusted and applied to fit into the existing procedures of any laboratory involved in Non-Target Screening analysis.

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