PEAKTRAMS: AN AUTOMATED COMPUTATIONAL APPROACH FOR THE SIMULTANEOUS DETECTION OF FEATURES IN REVERSE PHASE AND HILIC HRMS SCREENING

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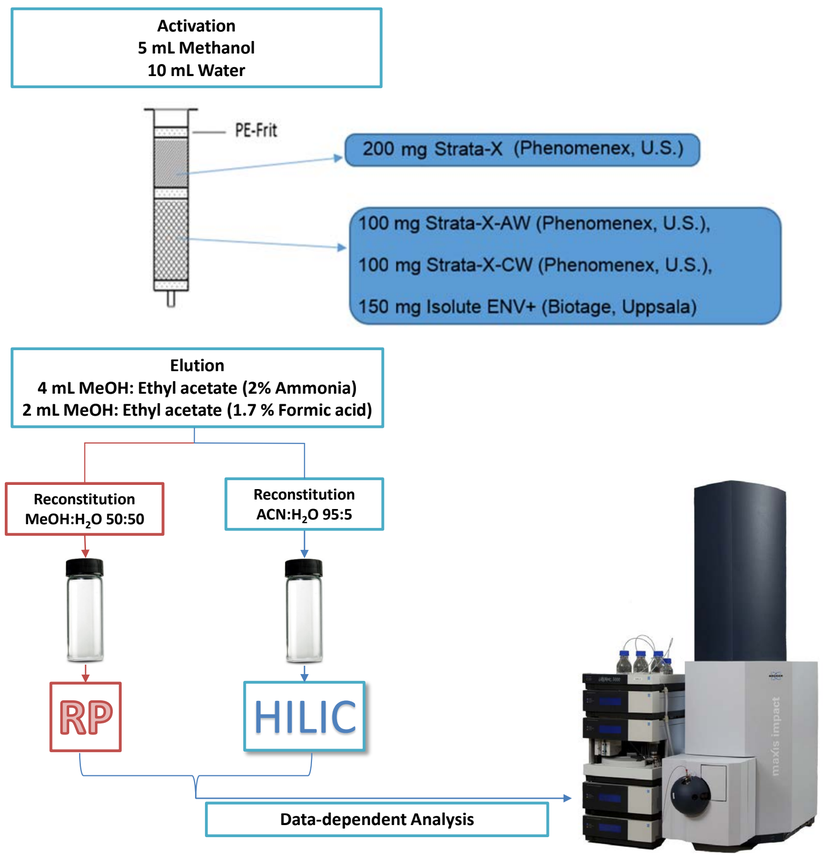
Abstract

Comparison of chromatograms obtained in reserved phase (RP) liquid chromatography and hydrophilic interaction liquid chromatography (HILIC) can provide valuable information for the identification and confirmation of suspect and non-target compounds. The plausibility of the obtained chromatographic retention times (RTs) in both modes as well as the comparison of the MS/MS spectra are strong points to be considered. This work presents the development of a novel automatic approach for the identification of common peaks between RP and HILIC chromatograms. The core of the program is written in R-project while a simple and user friendly graphical user interface (GUI) was built in JAVA.

The first step consists of the introduction of the target chromatograms of the same sample (one obtained by RP and one by HILIC) plus the corresponding blank chromatograms. Blank subtraction was performed first using an algorithm to find in each scan the common *m/z* features (with a given mass accuracy). This algorithm also considers the RTs (a tolerance interval is applied), so the subtraction takes place even with slight drifts in the RTs between target and blank chromatograms. After blank subtraction, two different lists are obtained with the detected peaks in both RP and HILIC modes. Subsequently, *m/z* values are compared and matches are listed.

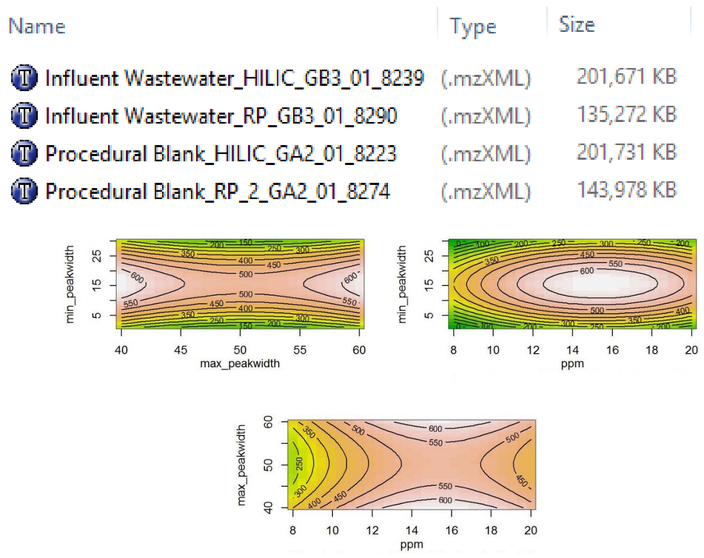
The developed workflow was validated with solvent standards and with spiked wastewater samples with a mixture of compounds with a wide range of physicochemical properties. Successful results were obtained for 26 out of the 27 evaluated substances, allowing the recording of the corresponding RTs in both RP and HILIC mode.

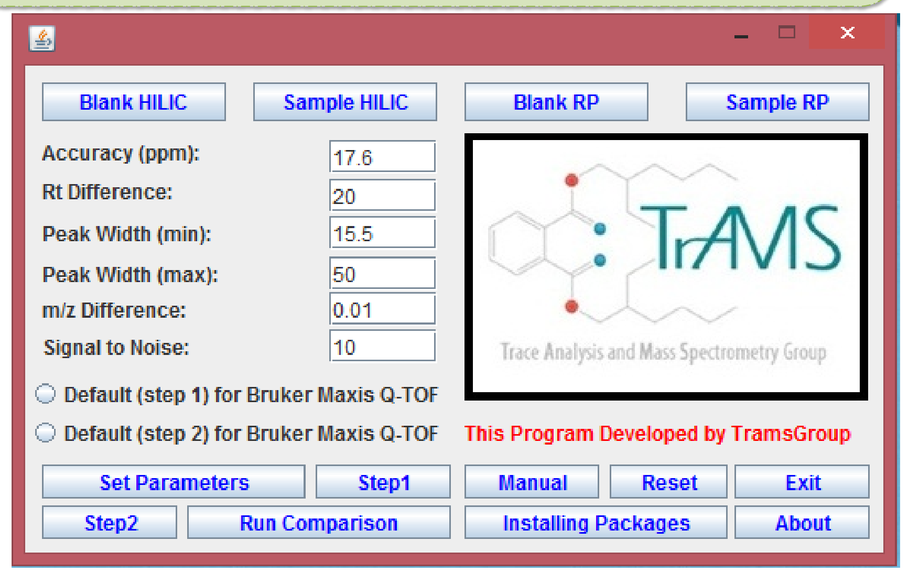
Analytical Protocol



Programming and GUI

Output data were converted to mzXML without zlib compression using ProteoWizard Software®. Scan by scan subtraction of samples minus respective blank samples in both chromatographies was performed. Subsequently centWave peak picking algorithm with optimum input parameters (mass accuracy and peak width) was implemented to the acquired data files. Optimum peak picking parameters were selected using IPO R-package (Libiseller et al., 2015, BMC Bioinformatics). Common features in blank and sample are removed. Finally, features observed in RP and HILIC are matched based only on mass accuracy. In case two or more peaks exist within mass tolerance in EICs, output of the procedure gives the opportunity to the user to manually select the correct matched pair of peaks.





Prioritization of common peaks

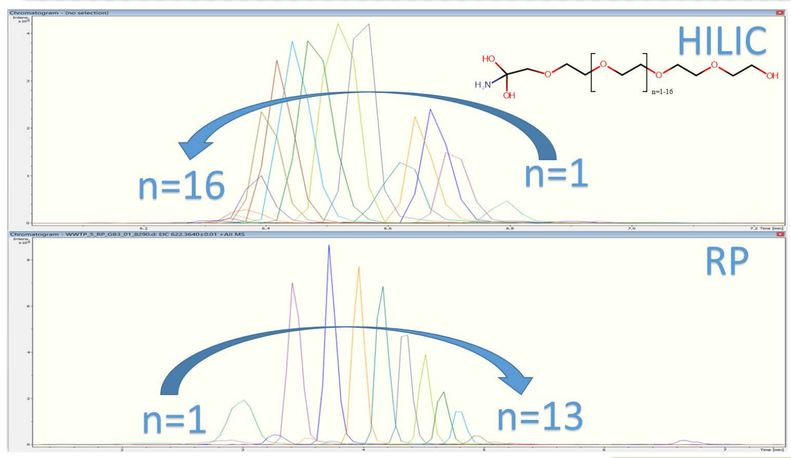
Common peaks are prioritized accordingly;

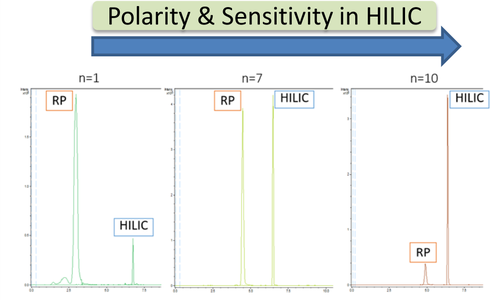
- Sufficient retention in HILIC (ret. time ≥ 1.50 min; k'> 1)
- Higher signal in HILIC

Examples of picked peaks

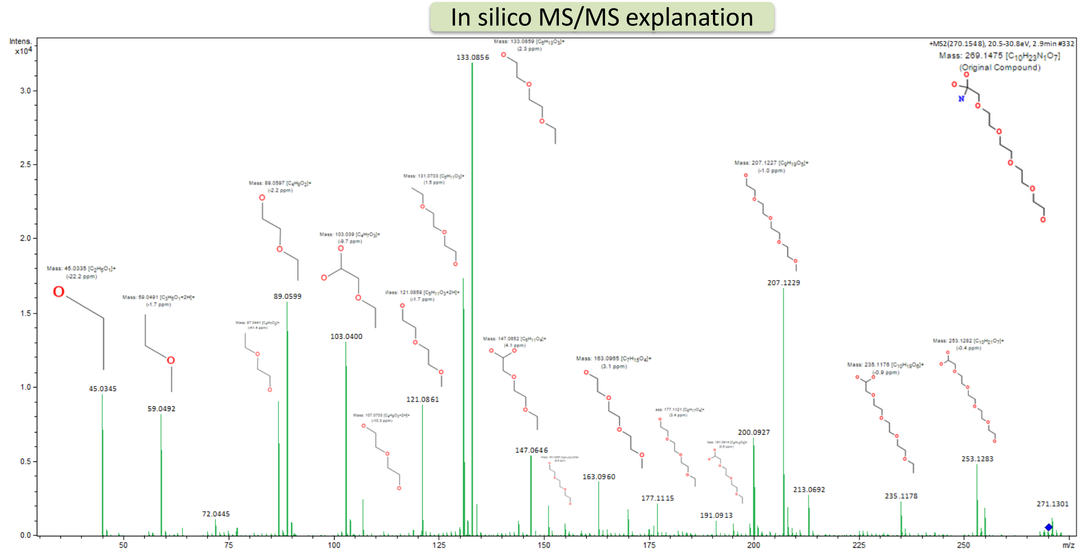
Non-target Identification of a series of homologues contaminants

The following non-target tentative identification (level 2B according to the identification levels proposed by Schymanski et al. 2014, EST) demonstrates the complimentary of HILIC and RP chromatographies. As the polarity of analytes increases they are better retained and the response increases in HILIC .





In silico MS/MS explanation



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