Originalbeiträge

Expanding the scope of wastewater monitoring by SFC-HRMS analysis

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Abstract

Broad screening approaches for monitoring wastewater are normally based on reversed phase liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). This method is not sufficient for the very polar micropollutants. In this study, we were using supercritical fluid chromatography (SFC) to analyze very polar and unrecognized micropollutants in wastewater. We tentatively identified 85 compounds, whereas 29 have only rarely/never been detected in waste-water effluent. The analysis of the same samples with LC showed clearly how SFC increases the ionization efficiency for low molecular weight micropollutants (m/z < 300 Da), which significantly improved the mass spectra for identifying very polar compounds.

Introduction

Reversed phase LC-MS (RP-LC-MS) is so far still the method of choice for water analysis, especially wastewater monitoring. RP-LC-MS is highly versatile and can be used to analyze a wide variety of compounds with log D between -1 and 7. The use of hydrophilic interaction chromatography (HILIC) extends the polarity range for compounds with log D between 0 and -7[1]. However, HILIC shows the superior performance mostly for selected compound classes, depending on the stationary phase selection. A universal method for polar compounds is lacking. Thus, alternative chromatography techniques are needed. SFC is especially promising as it has been shown to cover the combined polarity range of RP-LC and HILIC[2]. However, the application of SFC in environmental analysis is still in its infancy[3,4]. Recent studies show especially the ability of detecting more polar compounds and their transformation products (TPs) in water[5] by SFC.

Methods

Flow proportional 24-hour wastewater influent and effluent samples were collected at eight Danish WWTPs during summer 2020: three WWTPs (W1, W2, W3) located in the densely populated areas of Copenhagen, three (W4, W5, W6) in the municipality of Odense, and two (W7, W8) in towns of northern Funen. The sample preparation is described elsewhere[6]. In brief, wastewater samples were filtered through a 0.7 µm glass microfiber filter and enriched with an automated multi-layer solid-phase extraction (SPE) system SPE-03 from PromoChrom Technologies Ltd. The SPE cartridges were self-packed multi-layer cartridges containing Supelclean ENVI-Carb (200 mg) as the bottom layer below a mixture (400 mg (±10 mg); ratio 1:1:0.8) of Oasis Weak Anion Exchange (WAX), Cationic Exchange (WCX) and Isolute ENV+. The samples were analyzed in methanol with an enrichment factor of 50.

For chemical analysis, an electrospray ionization – quadrupole time of flight mass spectrometer (ESI-QTOFMS) (G2-Si Synapt MS, Waters) was connected to either LC or SFC. The ESI-QTOFMS parameters were the standard parameter for calibration of the system, without further optimization for LC or SFC. Before the analysis on both platforms, the reference mass (leu-enkephalin, m/z 556.2771) showed similar intensities (±15% variation) with direct flow injection. RP BEH C18 1.7 µm column (2.1×100mm) was used for LC and UCP$^{2®}$ BEH 1.7 µm column (3×100mm) for SFC. The injection volume was 2 µL for both systems. The data analysis was conducted with the UNIFI software and in-house R-scripts.

Matrix effects were evaluated by post column infusion[6]. For SFC, five compounds with a broad variety of physical-chemical properties (melamine, metoprolol, losartan, tebuconazole and citalopram) were spiked with a concentration of 10 µg/L in the make-up solvent flow (0.1 mL/min). Over the entire chromatogram, the retention time dependent matrix effect was evaluated by the median intensity difference of the five compounds between blank and a pooled effluent sample.

Results

Suspect screening with SFC
The 85 tentatively identified micropollutants were detected in positive ESI (70 compounds), negative ESI (11 compounds) or both ionization modes (4 compounds). We could confirm 53 compounds with analytical standards (confidence level 1), for 24 compounds the suspect MS/MS spectra matched a library spectra (confidence level 2a). The 85 compounds were spread in a polarity range from log D of -5.6 at pH=7 for metformin to log D of 4.9 for boscalid. In our study, the median log D was 0.25, and significant lower as the median log D of 5.4 from different water regulation[3]. The group of pharmaceuticals showed the highest number of detected compounds (43). Ten compounds were detected as drugs of abuse or drug metabolites. Further compounds were assigned to industrial, household, natural and agricultural origin. For 18 of the 85 micropollutants, we found <3 references about their occurrence in wastewater effluents, and for 11 micropollutants we could not find wastewater related references at all (17α-hydroxy pregnanolone, 1H-indole-3-carboxamide, apixaban, cyclohexylamine, picolinic acid, rivaroxaban, benzoguanamine, diethanolamine, hypoxanthine, urocanic acid,

phthalimide). In Figure 1 the normalized intensity distribution of highlighted compounds is shown in a heatmap.

Melitracen, the tricyclic antidepressant, was detected in the effluent of wastewater treatment plant 3 (W3) only. A R&D facility of a company that produces melitracen is located in the catchment area of W3. Further compounds produced by this company were detected with highest peak intensities in W3 as well (azoxystrobin, chlorprothixene). Thus, an industrial point source of the antipsychotics is highly likely.

The industrial compound cyclohexamine showed the highest peak intensity in W1, which indicates discharge from a specific producer. 17α-hydroxy pregnanolone is a major progestin metabolite. We detected the compound in all W2 effluent samples but not in the corresponding influent samples. Thus, our results indicate that 17α-hydroxy pregnanolone is a transformation product (TP) of other steroid precursors formed in the WWTP.

Perfluorobutanesulfonic acid (PFBS) was detected in W5 only, which could be related to a landfill draining percolating rainwater. Both W5 sampling days were influenced by some precipitation events in the previous days. Trifluoromethylsulfonic acid (TFMS), an ultra-short chain PFAS, was detected in W5 and W4, with the highest peak intensity on the only rainy sampling day of W4.

The samples from the capital region showed higher peak intensities of drug of abuse related compounds. Interestingly, in the capital region a high peak intensity was observed for 1H-indole-3-carboxamide, which is the core structure for the new cannabinoid classes of synthesized indole-3-carboxamide cannabinoid derivatives[7]. Thus, we assume that this compound was detected also in relation to drug consumption.

The oral anticoagulant apixaban and rivaroxaban are used for venous thromboembolism and stroke treatment, with continuously increasing use[8]. Both compounds were detected in effluent wastewater from all eight WWTPs.
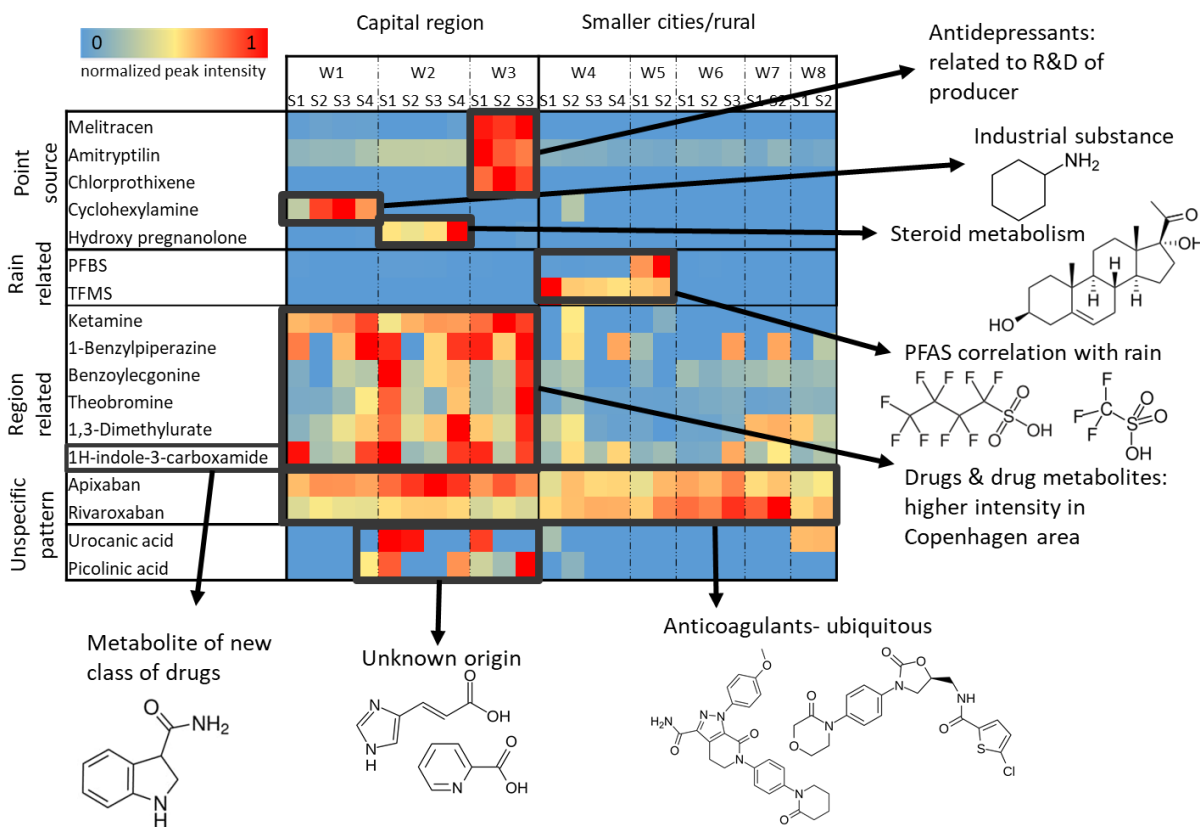

Figure 1: Heatmap of the normalized peak intensity of rarely described compounds in wastewater effluent of different sampling days (S1-S4) in the eight investigated WWTPs (W1-W8). Blue= not detected, red= highest peak intensity

Comparison suspect screening LC/SFC
All effluent samples were also analyzed by LC-HRMS. From the 70 compounds detected with SFC in the positive ESI mode, 48 were also detected with LC-HRMS. Only four compounds showed higher intensities with LC than SFC (venlafaxine, EDDP, dehydro-lidocaine and trimethoprim) and 12 compounds showed only slightly higher intensities with SFC

(≤factor 2). For the remaining 32 compounds, the ionization efficiency increased with up to 87 times in SFC compared to RP-LC. This was especially pronounced for compounds with m/z <300 (Figure 2A). The compounds solely detected with SFC were mainly below m/z 200. We suspect that even more of the compounds detected by SFC-MS could have been detected with LC-MS but could not be confirmed because of

too low intensities of the fragments in the high energy spectra. The desolvation efficiency and ionization in ESI is higher for SFC due to high CO_2 content and low water content in the mobile phase [9,10]. However, the improved ionization leads also to higher matrix effects (Figure 2B). In our pooled waste-water effluent samples (enrichment factor 50), the retention time dependent matrix effect for LC was most of the time <-10%. For SFC, the matrix effect was between -10 % and -37 %. For both methods, the highest matrix effect was detected between 3-5 min, due to the highest number and concentration of compounds eluting in this RT window[6]. For LC and SFC the elution gradient was set to around 20 % eluent B within this window. The compounds eluted within this window were different for both methods. The higher matrix effect in SFC could lead to underestimation of analyte concentrations, due to signal suppression. This means, more effort is needed to produce reliable data with SFC.
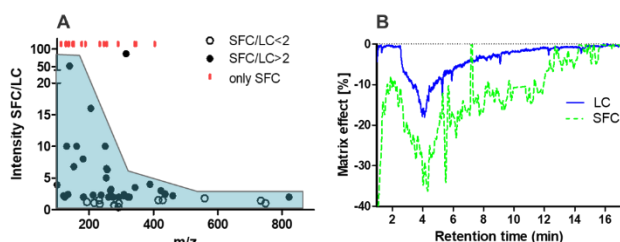

Figure 2: 50 times enriched pooled wastewater effluent sample: A) Comparison of the peak intensity ratio of suspect compounds between analysis with SFC and LC, sorted by the m/z value. Compounds detected only with SFC are shown as red dash at the respective m/z. B) Retention time dependent matrix effect for the pooled wastewater sample obtained by post column infusion of five representative compounds.

From the eleven newly detected compounds in our study, five compounds could not be detected with LC (benzoguanamine, diethanolamine, hypoxanthine, urocanic acid and phthalic-mide), 17α-hydroxy pregnanolone and 1H-indole-3-carbo-xamide showed 90 and 10 times higher peak intensities with SFC than LC, respectively, and the remaining four compounds were detected with between 2 and 5 times higher intensity in SFC. This shows the additional need for SFC for a broader screening of wastewater as it can be obtained with RP-LC-HRMS only.

Conclusion

This study shows the importance of extending the screening of wastewater effluent using SFC for identifying new compounds. This could be achieved by a chromatographic SFC method focused on a broad polarity range, including especially very polar compounds, and the higher ionization efficiency by SFC in comparison to LC.

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