D4RUNOFF

Data driven implementation of hybrid nature-based solutions for preventing and managing diffuse pollution from urban water runoff

D1.1 Preliminary versions of suspect screening and NTS analysis

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R=Document, report; **DEM**=Demonstrator, pilot, prototype; **DEC**=website, patent fillings, videos, etc.; **OTHER**=other
PU=Public, **SEN**=Sensitive, limited under the conditions of the GA

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Executive Summary

Developing standard operating protocols (SOPs) for runoff analysis – to be used both within D4RUNOFF and beyond – is among the main aims of WP1. It is an iterative process that draws on previous studies and methodologies to develop and improve workflows sample preparation, chemical analysis, data processing, and cheminformatics.

This document presents the preliminary versions of suspect screening and non-target screening (NTS) workflows, outlined in figure 1.1.

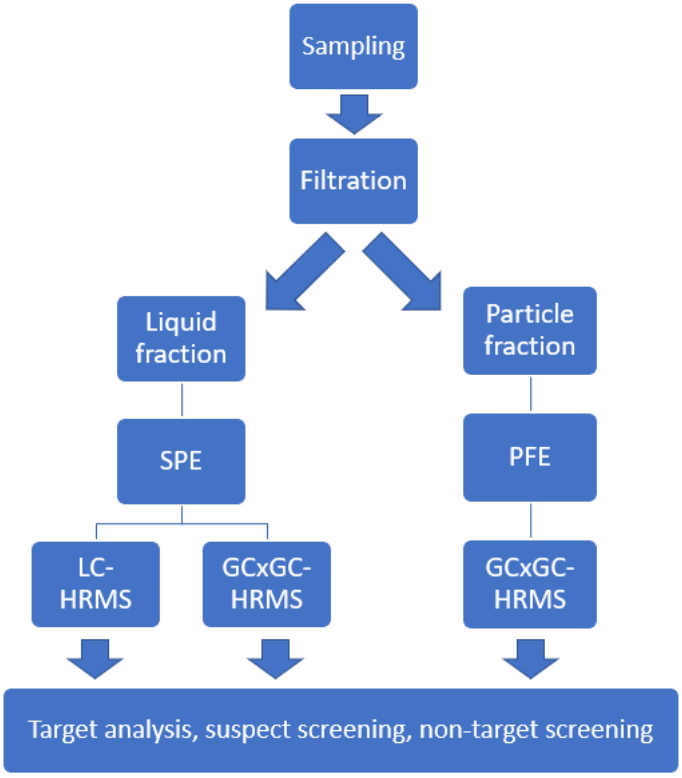

Figure 1.1: Overview of workflows for chemical analysis.

The workflows have already been tested on pilot samples collected in M3 of the project. This ensures their usefulness for runoff analysis and provides a good starting point for further developments during the project.

While this draft is therefore based on extensive experience with the methodologies, each step in the workflows will be further developed during the project. Toward the end of WP1, fully detailed standard operating protocols for suspect screening and NTS will be delivered as part of D1.2 and D1.5.

Addition to revised version

In the revised version, we have addressed the revisions comments:

- p. 2: The document history should not present the authors of different versions but the workflow from the draft version through the QA procedure (internal review) up to

the final version. Please revise accordingly. The list of authors is presented in the box above and thus does not have to be repeated in the version history.
    - **Thank you for the clarification. We have changed and updated the document history and hope it now contains the correct information.**

- The sampling as an important step should also be included to safeguard the overall procedure (type of sampling bottle, required amount, preparation of bottles, transport of samples, etc.).
    - **Thank you for highlighting the importance of sampling. We agree with the comment and have added a description of the sampling approach and procedure in section 2.1. Further descriptions of sampling have been added in deliverable 1.2 which present the final SOPs for suspect screening and NTS, and deliverable 1.3 which presents the inventory of runoff pollutants determined with the methods described in this document, as well as additional methods.**

- Acc. to the GA, D1.1 includes the collection of water samples in the 3 case studies but was not presented. The analytical results from the case studies should be added and discussed.
    - **Results from the analysis of runoff samples have been added with a particular focus on the method development steps, particularly recovery results (section 2.2.2), chromatographic separation (section 2.3.1), and preliminary results from target analysis in section 2.4.1.**
    - **Results related to runoff pollutants are described in deliverable 1.3 and have therefore been given less attention in the present deliverable to avoid repetition.**

- The deliverable lacks a conclusions / outlook chapter at the end of the Del.
    - **A conclusion and outlook section has been added in section 3.**

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1 INTRODUCTION

1.1 Purpose of the document

The purpose of the document is to provide a workflow draft for sample preparation, chemical analysis, data processing, and cheminformatics in suspect screening and NTS. This will give an overview of the workflows and guide developments for runoff analysis in WP1 and WP5.

1.1.1 Scope of the document

The scope of the document is to give a detailed description of the current versions of suspect screening and NTS workflows. The focus is on the core methods for the different steps in the analysis, particularly solid-phase extraction (SPE) and pressurized fluid extraction (PFE) for sample preparation, and liquid chromatography high-resolution mass spectrometry (LC-HRMS) and two-dimensional gas chromatography high-resolution mass spectrometry (GC×GC-HRMS) for separation and detection.

Additional methods might be employed to improve the detection of particular compound groups. This includes vacuum-assisted evaporative solvent extraction (VEC), supercritical fluid chromatography high-resolution mass spectrometry (SFC-HRMS), and inductively coupled plasma mass spectrometry (ICP-MS). However, these workflows are not described at length in this document since the methods are still to be developed based on the needs and initial results from WP1.

2 Preliminary workflows

2.1 Sampling

2.1.1 Sampling approach

A detailed sampling protocol has been provided in D1.3. In short, runoff samples are collected either as composite samples or time-series during a suitable rain event, i.e. with sufficient intensity to cause runoff from surfaces (>5 mm over a two-hour period) and preferably with a proceeding dry-period of minimum 3 days for accumulation onto surfaces between rains.
The majority of samples are composite samples collected as 500 mL sub-samples over 2 hours (at 0, 15, 30, 45, 60, 75, 90, and 120 minutes) and pooled to ensure representativeness. However, some time-series samples are also collected to provide information about the temporal distribution of pollutants during a single rain event. These samples are also collected over 2 hours but analyzed individually. For method development purposes, only composite samples have been analyzed.
Field blanks samples will be made to determine the background contamination from the sampling protocol. These samples will consist of pure water (LC-MS grade) with 300 mg/L $CaCl_2$ (to mimic the ionic strength of runoff) and undergo the same steps during sample handling and preparation.

Sample preparation is done at GEUS and UCPH as quickly as possible after collecting samples. For samples collected within Denmark, this can generally be done within 24 hours or less, but for samples collected other places in Europe, the fastest delivery option is selected to ship the samples to Copenhagen. This generally ensures that samples are processed no later than 72 hours after being collected. To increase the stability of the samples during transportation, insulated transportation boxes with cooling elements have been made by GEUS, which will keep samples cold for several days (a more detailed description is provided in D1.3).

2.1.2 Sampling materials

Depending on the sampling location, different materials will be needed to collect samples. The following section describes the main materials used for collecting samples (only materials that are in direct contact with samples are included) and procedures to clean equipment before and between sampling.

At most stations, samples can be collected manually from the surface flow. Here the following materials are used:

- 8 x 0.5 L borosilicate glass bottle with PBT lids and PTFE inserts
- 1 x 100 mL borosilicate glass bottle with PBT lids and PTFE inserts
- Vinyl or nitrile gloves

At some stations, sampling is done from manholes that require additional materials:

- Sub Surface Grab Sampler II (WHEATON®) with accompanying glass bottle

The Grab Sampler is cleaned by rinsing thoroughly with methanol and MilliQ water before sampling.

Finally, a few samples are collected with automated samplers equipped with glass containers are used which can be cleaned with the cleaning steps described below.

Glassware is cleaned with the following steps:

1) Rinse glassware thoroughly with tap water and wash in dishwasher (using LaboClean FLA and Neodisher Z soaps)
2) Soak glassware in nitric acid bath (approx. 9%) for 2h
3) Fill three separate baths with deionized water and one with Millipore water
4) Rinse glassware in each of the baths two times
5) Cover with alumina foil and heat to 550 ºC (some types of glass cannot sustain that temperature - mainly glass bottles for Sub Surface Grab Samplers and containers automated samplers - and instead undergo a prolonged heating to 450 ºC)

Lids for sampling bottles are cleaned by the following steps:

1) Remove PTFE inserts from lids
2) Rinse thoroughly with tap water and wash in dishwasher (using LaboClean FLA and Neodisher Z soaps)
3) Rinse thoroughly with ethanol
4) Air-dry before use

2.1.3 Method development samples

Method development was done using samples collected at the three case studies (Odense, Pontedera and Santander) as well as additional samples collected in Copenhagen. Sampling station were selected to represent different types of runoff areas that would pose different challenges to the analytical workflow (e.g. low concentrations of compounds from areas with low pollution level and high matrix samples from areas with high levels of organic contaminants).

2.2 Sample preparation

2.2.1 Filtration

Filtration will be done to separate the liquid and particle fractions, which will be analyzed separately with the methods described in 2.1.2 and 2.1.3 to determine chemicals of emerging concern (CECs) both in the runoff water and sorbed to the particles.

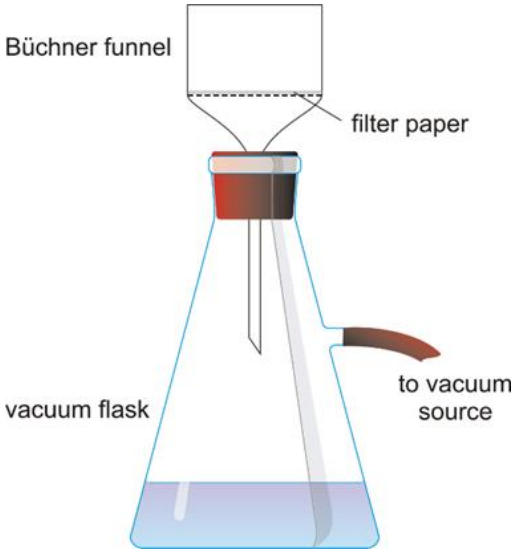

Figure 2.1: Setup used for filtration.

Equipment

- Filtering (vacuum) flask (2L) – use cleaning procedure (5)
- Büchner glass funnel (90 mm in diameter) – use cleaning procedure (5)
- Whatman GF/F 0.7 µm glass microfiber filter – use cleaning procedure (6)
- Whatman GF/A 1.6 µm glass microfiber filter – use cleaning procedure (6)
- Rubber stopper
- Vacuum pump
- Hose
- Milli-Q water
- 20% formic acid (HPLC-grade) in LC-MS grade water
- 2% formic acid (HPLC-grade) in LC-MS grade water
- 20% ammonium hydroxide in LC-MS grade water
- 2% ammonium hydroxide in LC-MS grade water
- Acid-washed 1L bottles – use cleaning procedure (2)
- Heat-treated Pasteur pipettes – use cleaning procedure (4)

First, pH is adjusted to 6.5 with 20% formic acid and ammonium hydroxide. Use 2% solutions for blank samples.

For filtration, a vacuum pump and Whatman glass microfiber filters with diameter 1,6µm and 0.7µm will be used. To avoid contamination, glass microfiber filters are cleaned with dichloromethane according to procedure (6).

To do the filtration,

1) A cleaned Büchner funnel is placed on top of the rubber stopper and filtration bottle so that it closes tightly
2) Place clean 1.6 µm glass fiber filter on the Büchner funnel so that the filter closes tightly and start vacuum pump
3) Use Pasteur pipette to wet the filter, taking from the liquid fraction of the sample – avoid stirring to keep sediment at the bottom
4) Continue pumping for 1 minute
5) Weigh an empty alumina cup

6) Turn off vacuum pump and place filter on alumina cup
7) Weigh the alumina cup with unused filter
8) Place filter back on the Büchner funnel and start vacuum pump
9) Re-wet filter with Pasteur pipette so that it closes tightly, before the rest of the sample is poured into the Büchner funnel – remember to stir sample to get the sediment from the bottom
10) After filtration, let the pump run for 1 minute
11) Transfer the filter to the same alumina cup and weigh it with used filter
12) Wrap used filter in alumina foil and store at -20°C until further processing

The filtration process is repeated for the same sample using a 0.7 µm filter, but a new setup (clean filtration bottle and Büchner funnel).

After filtration, transfer sample to acid-washed 1L bottle – store dark at 5°C and process within 24 hours.

Clean the filtration bottles, bucket funnel and rubber stopper; first with MilliQ water, then with ethanol and finally again with MilliQ water with 2% formic acid. A new sample can then be filtered.

2.2.2 Solid-phase extraction (SPE)

Extraction of the liquid fraction from 2.1.1 is done on a PromoChrom Technologies Ltd. automated SPE-03 system.
6 mL cartridges with 250 mg Supelclean ENVI-Carb pre-packed material are used, with an additional layer on top of 550 mg 1:1 bulk material mixture of Oasis HLB and Isolute ENV+. Cartridges, frits and sorbent materials are cleaned with methanol to remove potential impurities, according to cleaning procedures (7), (8), and (9) in Annex A.

No internal standards are used in the SPE method. The reason for this is to be able to use the samples later for toxicity tests.

The system is cleaned before each batch with a pre-set program named “D4R sys clean”.
Place sampling tubes in the respective solvents to rinse each channel with:

1) 20 mL methanol
2) 50 mL 2% formic acid in MilliQ water
3) 25 mL LC-MS grade water

The extraction is done with a pre-set program named “D4R sample”.
In summary the method consists of placing cartridges upside-down – using plastic connectors – and conditioning with

1) 5 mL methanol
2) 10 mL LC-MS grade water

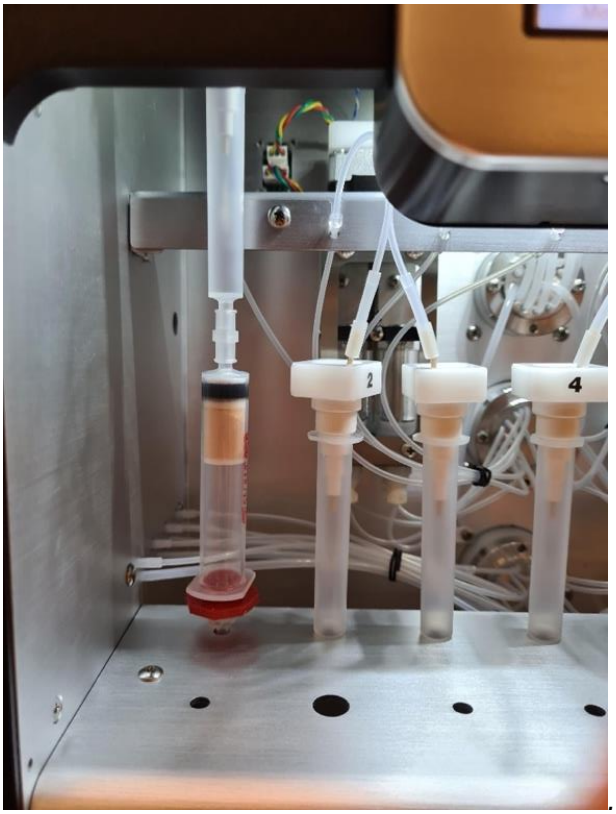

Figure 2.2.1: Placing the cartridge upside-down for conditioning and elution.

After conditioning, the program stops and cartidges are flipped to “regular” position for sample loading.
A total of 500 mL are loaded at a flow rate of 6 mL/min. Make sure that all hoses reach the bottom of the sample containers. After loading, the cartridges are dried with nitrogen for 60 minutes and the program stops.

To elute, turn cartridges upside-down and place 16 mL centrifuge tubes in the rack underneath – remember to mark which sample elutes where. The program will elute 11 mL methanol before drying the cartridges with nitrogen for 1 minute.

Following extraction, the samples are evaporated to a final volume of 1 mL (500 times pre-concentration) in a Biotage TurboVap at 40 °C. Use an initial 0.5 L/min nitrogen stream, increase the flow, if necessary, as the evaporation proceeds.
The final volume is determined by doing a visual comparison with a similar centrifuge vial filled with 1 mL methanol to compare.
After evaporation, the samples are centrifuged before being divided with heat-treated Pasteur pipette into heat-treated vials – cleaning procedure (4) – and stored at -20 °C until analysis.

The recovery of the SPE method was validated with spike experiments. Runoff samples of 2 L each were combined to a 6 L composite sample. The sample was split into 6 x 1 L samples with a pipette while stirring to ensure equal splitting of samples. Samples were then filtered and split into two groups of three samples each: One group was spiked before SPE (named Prespiked) whereas the other was not. After SPE extraction and evaporation, each of the three non-spiked extracts were split into two: Postspiked samples, which were spiked with

the same analyte mix to a final concentration similar to the pre-spiked samples, and Nonspiked. All samples were then analyzed on LC-HRMS, and the recovery was calculated with the following formula:

$$Recovery = \frac{Postspiked - nonspiked}{Postspiked - nonspiked} * 100\ \%$$

The recovery results are presented in figure 2.2.2. Of the 84 detected analytes, recovery above 80 % was found for 39 analytes, whereas 11 were less than 50 % recovered. As the figure shows, some analytes showed very low recovery. These included very polar analytes, such as Acesulfame (logD -1.5 at pH 6.5) and PFBA (logD -1.2 at pH 6.5). The results therefore shows that the SPE method is unable to retain very polar analytes that are easily soluble in the water sample and therefore not retained on the SPE cartridge. Challenges with analyzing very polar pollutants are well known (see Reemtsma et al., 2016), and alternative approaches able to analyze these compounds (e.g. VEC for sample preparation and SFC for separation) will be investigated during the project.

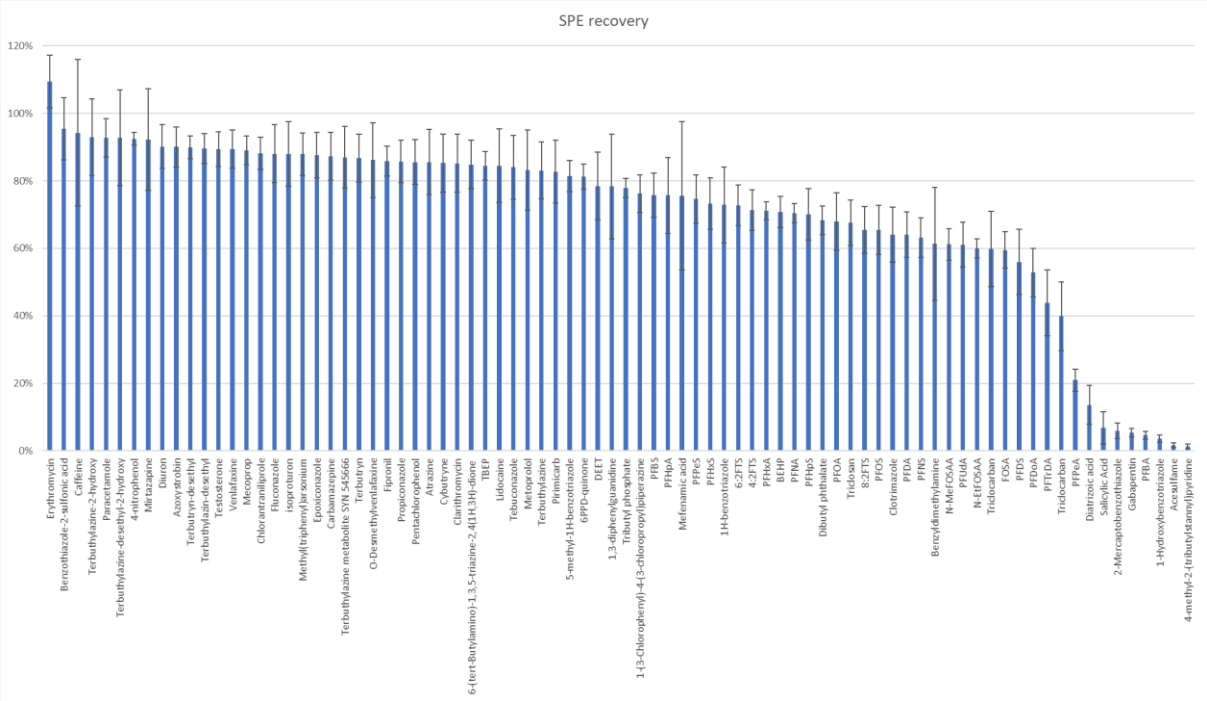

Figure 2.2.2: SPE recovery in percentage determined with spike experiment. Sorted by recovery.

2.2.3 Pressurized fluid extraction (PFE)

Integrated extraction and cleanup of the used filters from 2.1.1 will be performed with a Dionex ASE 200 accelerated solvent extractor. The materials are cleaned according to cleaning procedure (10) in Annex A.
Internal standard mix is added before the extraction, consisting of approximately 500µg/L of each compound (preliminary list of compounds table 5.2 in Annex B). The exact composition will be decided based on initial results.

For the extraction,

1) A 33 mL stainless steel extraction cell is packed from the bottom with two cellulose filters and 4 gram of activated silica gel
2) Stormwater filters are cut into pieces and placed in the cartridge
3) 50 µL internal standard mix is added directly on top of filters
4) 2 g hydromatrix added on top of filters and pressed firmly
5) The cell is filled Ottawa sand until approximately 1 cm from the top
6) Extraction cells are closed tightly and inserted in the autosampler
7) Place 60 mL amber vials with soft lids below – remember that each cartridge is extracted into two separate vials
8) Remember to check the placement of cartridges and vials, plus that nitrogen flow is on
9) Rinse the system three times, then start the sequence

The extraction is done with 9:1 n-pentane and dichloromethane according to the method below:

- Pressure: 1500 psi
- Preheat time: 0 min
- Static time: 5 min
- Flush volume: 70%
- Purge time: 60 sec

After extraction, samples are evaporated:

1) Evaporate to approximately 15 mL at 40°C under a gentle stream of nitrogen – use a vial with 15 mL water for comparison
2) Pour the second extract into the first extract for each sample – wash vial walls with approximately 2 mL solvent three times to transfer quantitatively
3) Add 2 mL isooctane as keeper and concentrate to approximately 4 mL under the same conditions – use a vial with 4 mL water for comparison
4) Transfer extract to 8 mL amber vial – wash three times with approximately 1mL solvent to transfer quantitatively
5) Evaporate to 2 mL – use a vial with 2 mL solvent for comparison
6) Store at -20°C until analysis.

2.2.4 Alternative sample preparation methods

In addition to SPE and PFE, an alternative workflow using VEC will be tested on the liquid fraction to assess the performance compared to SPE, especially the recovery of very polar compounds.
The workflow consists of pre-concentrating the filtered sample from 2.1.1 with a Büchi Syncore Polyvap instrument. The exact method will be optimized according to the need of the project.

2.2.5 Blank samples

To monitor the performance of the sample preparation workflow, blank samples will be run with every batch to identify potential variances and contamination. For this purpose, three types of blank samples will be used:

- Field blanks, which mimic all the steps from sampling to analysis
- Laboratory blanks, which go through the steps from extraction to analysis
- Analytical blanks, which are used to assess the performance of the chemical analysis

2.3 Chemical analysis

2.3.1 Liquid chromatography high-resolution mass spectrometry (LC-HRMS)

The LC-HRMS analysis will be used to analyze the SPE extract from 2.1.2.

Before analysis, SPE enriched water samples are diluted to relative enrichment factor (REF 50), by diluting the samples 10 times in MeOH. The samples will be spiked with internal standard solution at a concentration of approximately 50 µg/L (preliminary list of internal standard compounds in Annex B).
For target analysis and retention time alignment, calibration standards (preliminary list in Annex B) with 50 ug/L internal standards and varying concentrations of analytical standards, from approximately 0,1-100 ug/L, will be run with every batch. The exact concentrations depend on the initial results.

The LC-HRMS analysis is carried out on an Acquity Ultra-Performance Liquid Chromatograph equipped with a Synapt G2S quadrupole time of flight mass spectromer (Waters).
For separation, an Acquity UPLC BEH C18 100mm column (Waters) with 1.7 µm particle size and 2.1 mm inner diameter will be used.

A 25-minute gradient elution will be performed with solvents consisting of:

A: H_2O with 0,1% formic acid
B: Acetonitrile with 0,1% formic acid

For the gradient, which is shown in figure 2.3, 99% A and 1% B will be kept for the first minute, before an isocratic hold at 70% A and 30% B for 3 minutes. Then going with a linear gradient to 1% A and 99% B over 16 minutes and keeping these setting for 5 minutes. Finally, the composition will return to 99% A and 1% B to condition for the remaining 4 minutes. This gradient was developed to elute the compounds found in the runoff samples. An example of the total ion chromatogram (TIC) and LC gradient is shown in figure 2.3. As the figure shows, peaks are distributed throughout the chromatographic separation (except for the first two minutes during which the eluent was directed to waste to avoid contaminating the HRMS).

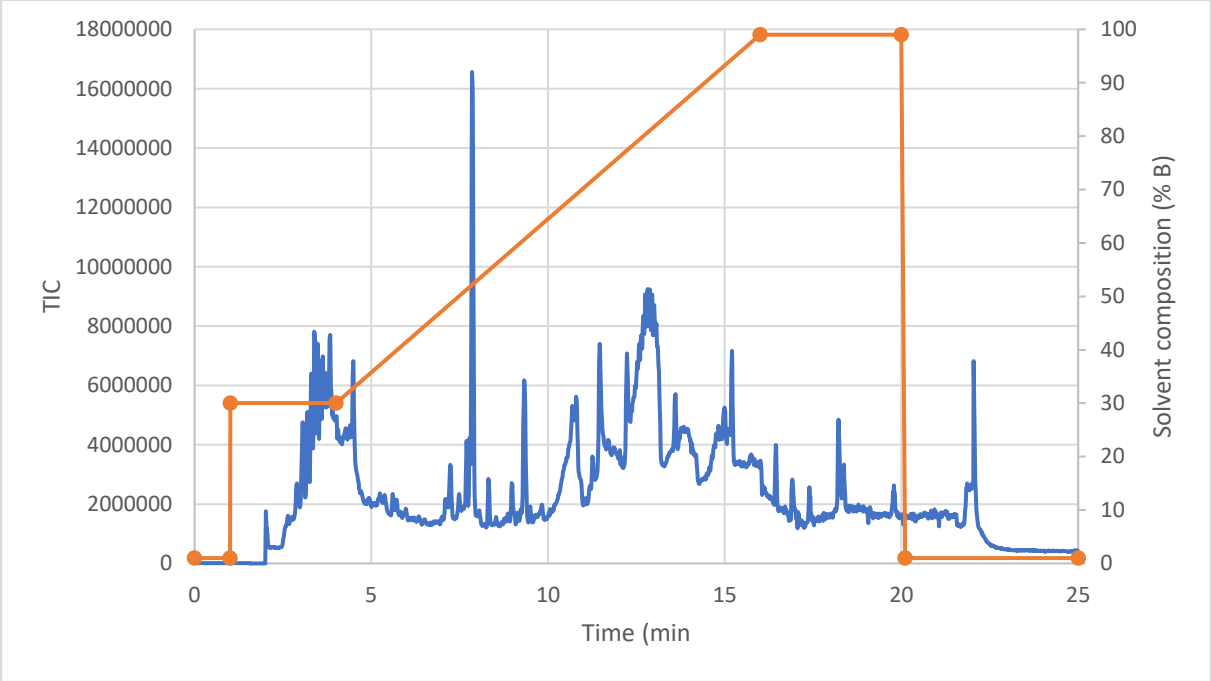

Figure 2.3: LC-gradient with %B (acetonitrile with 0,1% formic acid).

Before each batch, a system suitability test (SST) sample will be run to assess the performance of the system.
Quality control (QC) samples will be developed by combining runoff samples to quantify system performance. They will be run for approximately every 5 injections to determine drift in retention time and mass accuracy, and analytical blanks – consisting of pure methanol – will be run to check for contamination and carry-over between injections.

Each sample will be run in triplicate both in positive and negative electrospray ionization (ESI) mode. During the analysis, lock spray calibration will be performed using leucine-enkephalin with masses 556.2771 Da and 554.2615 Da for positive and negative ionization, respectively.
The MS-detection will be done with data independent acquisition (DIA) using alternating collision energy in MS^E mode. The low collision energy will provide information about the precursor ions, while the high collision energy will provide information about product ions that will be used for identification.
Start parameters of 10 to 40eV collision energies and a scan time of 0.35 seconds are used. These will be optimized according to the compounds detected in the samples.

2.3.2 Comprehensive two-dimensional gas chromatography high-resolution mass spectrometry (GC×GC-HRMS)

The GC×GC-HRMS will be used to analyze both SPE and PFE extracts.

GC×GC-HRMS analysis will be performed on an Agilent 7890B GC system coupled to an Agilent 7200 Accurate Mass QTOF MS. Separation is done with an orthogonal column set, consisting of a non-polar and a mod-polar column with helium as carrier gas.

For SPE samples, the extracts will be derivatized using N-Methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) before injection. After spiking with internal standard mixture, the samples will first be evaporated to dryness and then reconstituted in 25 µl pyridine. Immediately before injection, 25 µl MSTFA will be injected into the sample vial, and the vial will be heated to 70 °C for 90 minutes before injection. This will ensure complete reaction with polar functional groups, which will increase the range of compounds that can be detected.

PFE samples will not be derivatized, since the non-polar compounds sorbed to the particles do not require derivatization for detection. Instead, extracts will be spiked with internal standards and analyzed directly.

Separation will be done with 1.0 µl splitless injection and a temperature gradient program, with the second oven offset with +30 °C from the primary oven.
The primary oven temperature will start at 105 °C for 5 minutes, ramped to 315 °C at a rate of 4.5 °C/min, with a final hold of ten minutes, giving a total run time of 72 minutes. Ionization will be done using an electron ionization source with electron energy of 70 eV.

2.3.3 Additional platforms for chemical analysis

In addition to LC-HRMS and GC×GC-HRMS analysis, other platforms will also be tested to assess their contribution, especially SFC-HRMS and LC-ICP-MS.
SFC-HRMS will potentially perform better than LC-MS for very polar compounds. A workflow will be developed depending on the needs of the project.
Furthermore, a LC-ICP-MS workflow will be developed for analysis of CECs containing heteroatoms susceptible to detection with ICP, including As, Sb, Br, and Cl.

2.4 Quantification workflow

2.4.1 Target analysis

Quantification of target analytes will be performed based on the response factor from reference standards and internal standards.

For LC-HRMS data, data acquisition of target analytes will be performed with TargetLynx (Waters). Compounds will be identified based on comparison with an analytical standard. The retention time needs to be within a specified window (will be decided based on the initial results), and the monoisotopic mass with a *m/z* window of 0.01 Da. The integration will be checked manually to avoid false positives. For reliable quantification, the target compounds will be corrected with internal standards.

For GC×GC-HRMS data, data acquisition of target analytes will be performed with Mass Hunter (Agilent) and interpretation of the chromatograms using GC Image. Selected qualifying and quantifying ions will be used for integration of each standard and internal standard, and the integration will be checked manually by inspecting selected ion chromatograms.

Quantification will be done with an in-house Excel worksheet using calibration with the most appropriate fit for each analyte.

Preliminary results from target analysis are presented in figure 2.4.1. This shows characteristic pollutants compositions at different sites, particularly typical wastewater compounds (including caffeine and the pharmaceutical O-Desmethylvenlafaxine) at SA5, which is a combined sewer overflow station at the case study in Santander. Since this sample is known to contain wastewater, it is not surprising to find these compounds in relatively high concentrations. More surprising is the occurrence of the same compounds in samples OD1 (from the case study in Odense) and CO1 (from the inner city in Copenhagen) which do now have any known input of wastewater. Since both these two samples were collected during a heavy rain event, a possible explanation could be that sewage water overflowing from the drains were mixed with the surface runoff. Such occurrence will be further investigated during WP1.
Another characteristic group of compounds are plasticizers and industry chemicals (including the tyre-related compound 6PPD-Quinone and rubber compounds 1,3-Diphenylguanidine (DPG) and 1H-benzotriazole). These sampling stations represent high vehicle traffic and highly urbanized areas, which are possible explanations for the observed contaminants. The specific sources will be further investigated in the project.

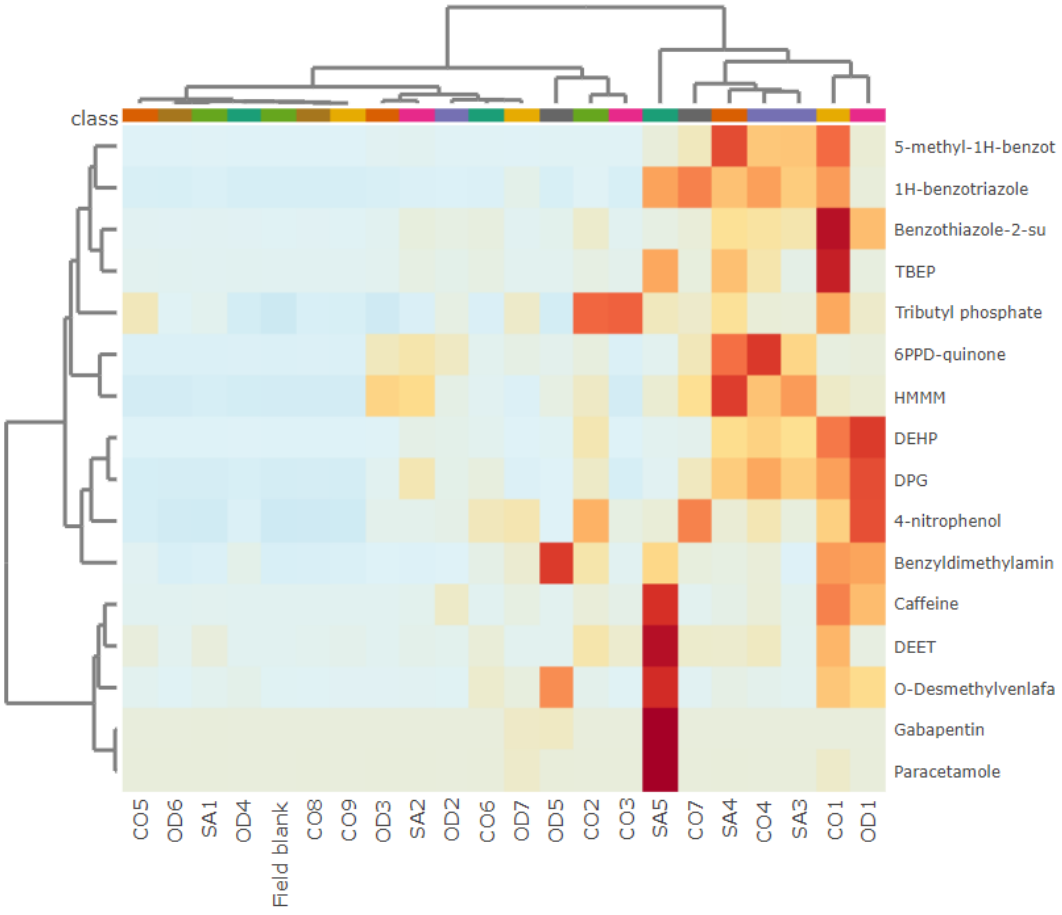

Figure 2.4.1: Heatmap of target analytes detected in runoff samples (auto-scaled).

2.5 Suspect screening and NTS workflows

2.5.1 Suspect screening

The suspect screening workflow is based on a list of approximately 1600 suspect CECs that have previously been detected in stormwater analysis and/or are of specific relevance because of environmental toxicity and persistence. With InChIKey, metadata for each compound is retrieved, including Smiles, XlogP, Henry's law constant, monoisotopic mass, and predicted no-effect concentration (PNEC), which makes it possible to group compounds based on their chemical and toxicological characteristics. Furthermore, mass spectra will be retrieved from the NORMAN Substance Database (SusDat) and MassBank of North America (MoNA), or predicted *in-silico* to generate enable identification based on fragmentation pattern and retention time.

Concentration estimation will be done based on in-house developed workflows. For LC-HRMS, concentration will be semi quantified based on a quantitative structure property relationships (QSPR) model built with around 300 relevant compounds. The exact workflows are still under optimization and testing.

2.5.2 Identification workflow for LC-HRMS

Identification of compounds will be performed with the software MS-Dial, which is well suited for handling the HRMS data from data independent acquisition (DIA) that is generated with MS^E.
An overview of the workflow is presented in figure 2.4. In summary, it consists of data-processing steps (blue boxes) to improve reliability, prioritization (green box) to identify priority features, and identification of priority compounds (red boxes). The parameters for each step will be developed based on initial results.

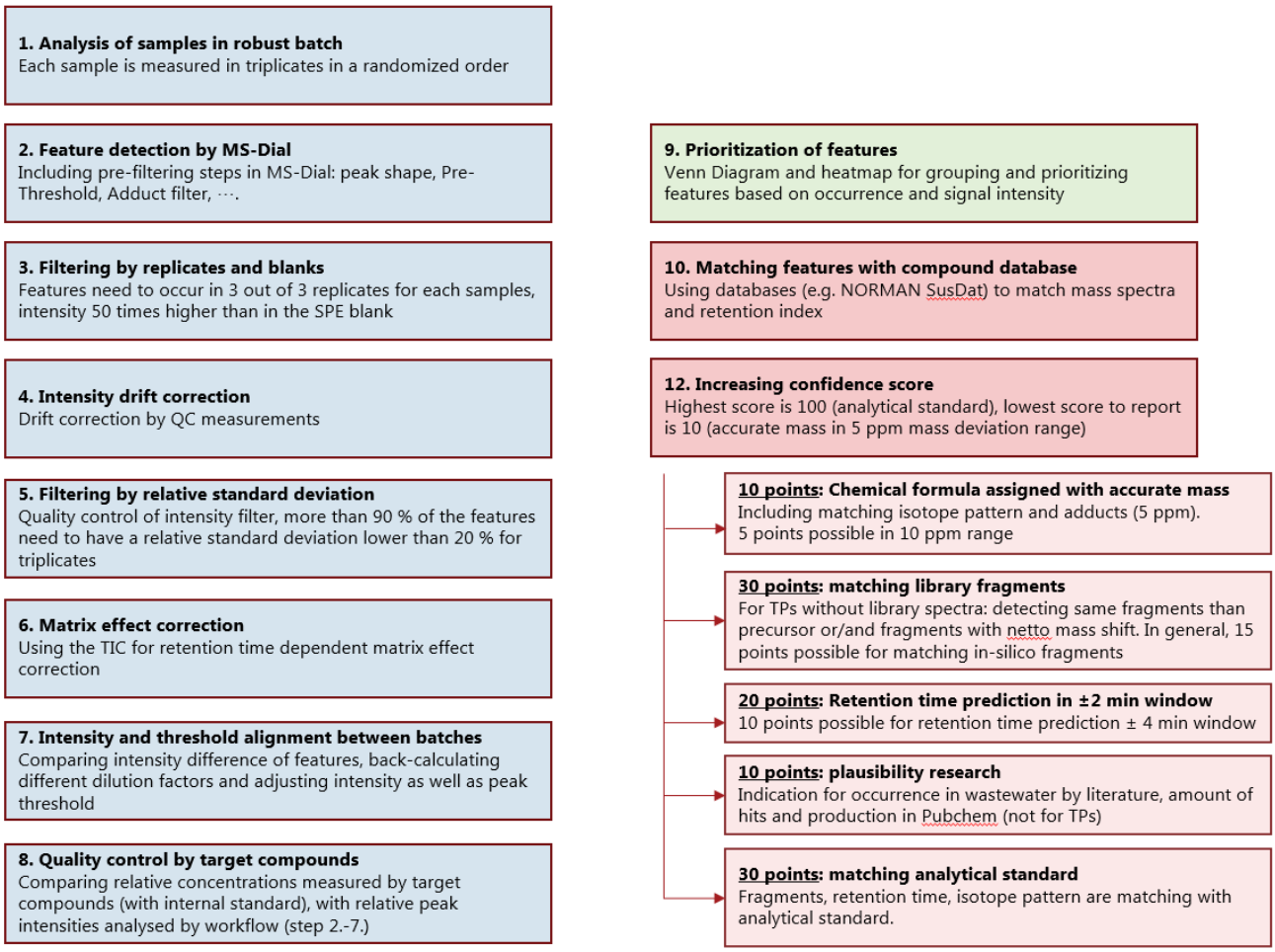

Figure 2.4: Workflow for suspect screening and NTS of LC-HRMS data.

For suspect screening, step 10 will be done using the suspect screening list for candidate compounds, whereas NTS will be done using an extensive library of known compounds, e.g. SusDat or MoNA.
Beside full identification of compounds, identification of compound classes will also be conducted. For example, chlorinated and bromindated compounds can be evaluated based on the characteristic isotope pattern and perfluorinated compounds can be recognized without knowing the exact identity, based on screening for negative mass defects and their frequent occurrence in homologous series.

2.5.3 Identification workflow for GC×GC-HRMS

GC×GC-HRMS instrument data acquisition will be performed via Mass Hunter (Agilent), and the interpretation of the resultant chromatograms using GC Image (GC Image LLC, version: 2.9R1.1). Interactive blob detection is carried out on each chromatogram using a S/N threshold of >10, and blob tables exported containing peak metadata such as 1/2D retention time, peak area/volume, NIST match factor and 1D retention indices (based on an injected n-Alkane sample). Compounds are considered tentatively identified when not also identified within the blank samples, having a NIST match factor of >/=700, and an absolute agreement between reference and calculated retention indices of </= 50.

3 Conclusion and outlook

Analytical workflows have been developed and tested for urban runoff analysis. With the developed methods, we are able to analyze urban runoff samples using both target, suspect and non-target screening analysis methods, which will generate insights into the occurrence of known and unknown runoff pollutants.
Next step will be to apply the methods for analysis of all runoff samples collected in WP1 and to further develop the methods, particularly data-dependent acquisition methods (using e.g. Orbitrap mass spectrometer) with dedicated approaches to isolate precursor ions and generate very high quality mass spectra for compound identification.

4 References

Reemtsma, T., Berger, U., Arp, H. P. H., Gallard, H., Knepper, T. P., Neumann, M., Quintana, J. B., & Voogt, P. De. (2016). Mind the Gap: Persistent and Mobile Organic Compounds - Water Contaminants That Slip Through. *Environmental Science and Technology*, *50*(19), 10308–10315. https://doi.org/10.1021/acs.est.6b03338

D4RUNOFF

5 Acronyms

SPE: Solid-phase extraction
ICP: Inductively coupled mass spectrometry
VEC: Vacuum-assisted evaporative concentration
CEC: Chemicals of emerging concern
LC-HRMS: Liquid chromatography high-resolution mass spectrometry
GC×GC-HRMS: Comprehensive two-dimensional gas chromatography high-resolution mass spectrometry
SFC: Supercritical fluid chromatography
PFE: Pressurized fluid extraction

NTS: Non-target screening
SST: System suitability test
QC: Quality control

6 Annex A: Cleaning procedures

(1) Standard cleaning procedure for glassware
1) Rinse thoroughly with solvent and allow to dry
2) Wash in dishwasher with LaboClean FLA and Neodisher Z
3) Cover with alumina foil and heat to 550 °C for 4 hours

(2) Acid-wash cleaning procedure
1) Rinse thoroughly with solvent and allow to dry
2) Wash in dishwasher with LaboClean FLA and Neodisher Z
3) Cover with alumina foil and heat to 550 °C for 4 hours
4) Soak in 9% nitric acid bath for 2 hours
5) Fill three separate baths with deionized water and one with Millipore water
6) Rinse glassware twice in each bath
7) Rinse thoroughly with ethanol
8) Air-dry before use

(3) Cleaning procedure for plastic lids and volumetric flasks
1) Rinse with solvent and allow to dry
2) Wash in dishwasher with LaboClean FLA and Neodisher Z
3) Rinse thoroughly with ethanol and air-dry before use

(4) LC vials and Pasteur pipettes
1) Heat to 450°C for 10 hours

(5) Filtering (vacuum) flask
1) Rinse thoroughly with deionized water
2) Wash with ethanol
3) Rinse with 2% formic acid in MilliQ water

(6) Glass microfiber and cellulose filter cleaning
1) Place filters in a beaker and cover with dichloromethane
2) Ultrasonicate 5 min
3) After ultrasonication, decant and add new solvent
4) Ultrasonicate for another 5 min
5) Leave to dry on alumina foil

(7) Supelclean™ ENVI-carb cleaning
1) Place 250 mg ENVI-carb cartridge in opposite direction on SPE system
2) Elute with 20 ml of MeOH
3) Dried with air-purge for 10 min on SPE system

(8) HLB and ENV+ cleaning
1) Weigh a glass beaker
2) Add the the materials 1:1 into the beaker and mix with a glass spoon
3) Add methanol 10x the mass of mixture
4) Place beaker in the ultrasonic bath for 10 min
5) Filter mixture of HLB and ENV+ on a Buchner funnel

6) Transfer sorbent in a new clean beaker with glass spoon
7) Repeat steps of adding MeOH, ultrasonication, filtration
8) Transfer sorbent in a new clean beaker and cover with aluminum foil
9) Leave to dry – NB it takes several days

(9) Frits cleaning

1) Place frits in a beaker and fill methanol to cover
2) Place beaker in ultrasonic bath for 10 min
3) Decant methanol, and fill again with new methanol
4) Place beaker in ultrasonic bath for 10 min
5) Decant methanol and cover with aluminum foil
6) Leave to dry

(10) PLE cleaning

1) Separate the extraction cell completely and remove old sample material from the cylinder
2) Cleaning the cylinder inside with a tissue
3) Rinse all parts in warm tap water – use long brush to clean cartridges
4) Rinse all parts with deionized water
5) Sonicate all parts for 5 min in methanol
6) Sonicate all parts for 5 min in pentane:acetone (1:1)
7) Let all parts dry on alumina foil

7 Annex B: Standards

Table 7.1: Preliminary standard list

Compound
1,3-diphenylguanidine
1,4,6,7-Tetramethylnapthalene
1-Aminobenzotriazole
1-Ethylpyrene
1-Hydroxybenzotriazole
2,4,6-Trichlorophenol
2,4-Dichlorophenol
2-Mercaptobenzothiazole
4-nonylphenol
4-tert-Octylphenol
6-Ethylchrysene
6PPD
6PPD-quinone
9,10-Dimethylphenanthrene
9-Methylphenanthrene
Acenaphthene
Acenaphthylene
Acesulfame
Acetophenone
Amitriptyline
AMPA
Anthracene
Anthraquinone
Atorvastatin
Azoxystrobin
Benz[a]anthracene
Benzo(a)pyrene
Benzo(b)fluoranthene
Benzo(ghi)perylene
Benzo(k)fluoranthene
Benzo[e]pyrene
Benzoic acid
benzothiazole
Benzyldimethylamine
Biphenyl
Bis-(2-ethylhexyl) phthalate (DEHP)
Bisphenol A
Bromacil
Bronopol
Caffeine
Captan

Carbamazepine
Carbazole
Cetirizine
Chrysene
Citalopram
Clarithromycin
Clotrimazole
Clozapine
Coronene
Cotinine
Cyanoguanidine
cybutryne
Cypermethrin
Cyromazine
Daidzein
Decabromodiphenyl oxide
DEET
Desvenlafaxine
Diatrizoic acid
Dibenz[a,h]anthracene
Dibenzothiophene
Dibutyltin dilaurate
Dibutyltindichlorid
Diclofenac
Diethyl phthalate
Di-n-butyl phthalate
Diuron
Erythromycin
Fluoranthene
Fluorene
Gabapentin
Glyphosate
Guanylurea
Hexachlorobenzene
Ibuprofen
Imazalil
imidacloprid
Indeno(1,2,3-cd)pyrene
Indole
Iohexol
isoproturon
Lidocaine
Losartan
Mecoprop
Mefenamic acid

Melamine
Metformin
Metoprolol
Mirtazapine
Naphthalene
Paracetamole
pentachlorophenol
Perylene
PFOA
PFOS
Phenanthrene
Phenol
Propiconazole
Pyrene
Sertraline
Sucralose
Sulfamethoxazole
Sulfapyridine
Tebuconazole
Terbuthylazine
Terbutryn
Tetraconazole
Thiacloprid
Tributyltin
Triclocarban
Triclosan
Venlafaxine

Table 7.2: Preliminary internal standard list

4-tert-Octylphenol-13C6
6PPD-quinone-d5
Acenaphthene-d10
Acenaphthylene-d8
Acetaminophen(methyl-d3)
Anthracene-d10
Benz[a]anthracene-d12
Benzo(a)pyrene-d12
Benzo(ghi)perylene-d12
Benzo(k)fluoranthene-d12
Bisphenol A-d16
Caffeine-(trimethyl-d9)
Chrysene-d12
d6-Phenol

d8-Carbazole
d9-Biphenyl
Dibenzothiophene-d8
Fluoranthene-d10
Fluorened-d10
Indeno(1,2,3-cd)pyrene-d12
Indole-d7
Lidocaine-d10
Naphthalene-d8
Phenanthrene-d10
Pyrene-d10