Treatment of High Resolution Mass Spectrometry data set from NORMAN network inter-laboratory trial using Non-Target Screening strategies

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Table of contents

Introduction 1

I. Principles 4

A. Chromatography 4

B. High Resolution Mass Spectrometry 4

1. Ionisation 4

2. QToF technology 5

C. Data Acquisition 6

D. Data treatment – The workflow 7

II. Bibliography 8

A. Measurement Instruments 8

1. Liquid and Gaseous chromatography 8

2. Ionisation 9

3. HRMS instruments 10

B. Quality Insurance 10

1. Lockmass and retention time control 10

2. Quality control 10

C. Acquisition during HRMS analyses 11

D. Data treatment - Software 12

E. Non target Screening – The workflow 12

3. Data processing and cleaning 13

4. Identification 14

F. Conclusion about Non-Target Screening 18

III. Materials and methods 19

A. NORMAN Data set 19

B. Softwares – Mzmine vs Workflow4Metabolomics 20

C. Workflow – Steps and parameters 20

D. Retention time indexation 22

E. Exposure time impact on Passive Samplers 24

F. DWTP treatment evaluation – Heatmap and PCA 25

IV. Results 26

A. Open sources software – Mzmine vs Workflow4Metabolomics 26

B. Workflow – Impact on features number 27

C. Laboratories comparisons 28

D. Harmonization with RTI 30

E. Exposure time influence on Passive Sampling 33
F. DWTP treatment evaluation – Heatmap and PCA 36
V. Discussion 38
A. Open sources software 38
B. Retention time Indexation 38
C. Inter laboratories list combination 39
D. Analysis harmonization 39
E. Exposure time impact on Passive Sampler 40
F. DWTP treatment evaluation 41
Conclusion 42
References 44
Annexes 48
Abstract / Résumé ..... Last page

List of Tables and Figures

Figure 1: Picture of QToF HRMS instrument coupled with liquid chromatograph
Figure 2: Scheme of QToF HRMS compartments*
Figure 3: a) TIC and b) mass spectrum at 5.70min of a PS exposed 2 days in river water analysed by lab n°10
Figure 4: Steps of a possible data treatment workflow for HRMS analysis
Figure 5: Comparison of water samples exposed to different treatments through advanced processing of high resolution mass spectrometry data [39]
Figure 6: Heat maps of spatial distribution trend of the four major types of pollutants in Dianshan Lake [57]
Figure 7: Detailed workflow on MZmine2 to treat data from the 3 selected lab (13, 16 & 17)
Figure 8: Curve of standard Rt mean in function of standard elution time inside each lab
Figure 9: Volume of organic solvent injected during a run per lab
Figure 10: Selected groups of compounds to study repartition in Rt and m/z in order to compare exposure time
Figure 11: Upset plot of river samples features from laboratory 13 after data processing from W4M and MZmine
Figure 12: M/z in function of Rt for features of a PS exposed 2 days in a river water (sample n°121) and analysed by 4 laboratories (n°10, 13, 16 and 17)
Figure 13: Venn Diagrams of features in PS exposed 4 days in river water (sample n°141) for laboratories 13, 16 and 17, after 3 Rt indexation methods
Figure 14: Venn Diagram of features after KRT indexation method on the 500 most intense features for PS exposed 4 days in river water (sample n°141)
Figure 15: Venn Diagrams of features in PS exposed 4 days in river water (sample n°141), after RT indexation with KRT and SSM methods and using either the 6 standards spiked in samples or the 12 standards in RTImix.
Figure 16: Upset plot of features list from a) combination of laboratories 13 and 16 lists, b) laboratory 16, c) laboratory 13
Figure 17: Part IV .E Boxplots legend
Figure 18: m/z repartition of compounds in river water samples from laboratory n° 16 depending on their exposure time
Figure 19: Rt repartition of compounds in river water from laboratory n°16 depending on exposure time
Figure 20: a) m/z and b) Rt repartition of compounds in drinking waters from laboratory n°16 depending on exposure time
Figure 21: m/z repartition of compounds in river water from laboratory n°16 depending on their intensity trend
Figure 22: Rt repartition of compounds in river water from laboratory n°16 depending on their intensity trend
Figure 23: a) m/z and b) Rt repartition of compounds in drinking waters from laboratory n°16 depending on their intensity trend
Figure 24: Heatmap representing total intensity of groups of compounds depending on their affinity to PS across time and reaction to DWTP from lab n°13
Figure 25: Principal Components Analysis for samples from laboratory n°13 and n°16

Table 1: Information about vials sent by NORMAN network for LC-HRMS analysis
Table 2: List of laboratories selected for the study
Table 3 : Number and ratio of features with W4M or MZmine workflows, for laboratories 13 and 16.
Table 4: Number of features remaining and percentage of feature reduction after each step of the workflow

Table 5: Measured m/z of spiked standards measured in the blank sample for the 4 laboratories
Table 6: Number of features detected in each sample with a S/N threshold of 200cps

Glossary of abreviation

APCI	Atmospheric Pressure Chemical Ionisation
APPI	Atmospheric Pressure PhotoIonisation
CEC	Chemical Emerging Contaminant
Da	Daltons
DGT	Diffusive Gradient in Thin film
DWTP	Drinking Water Treatment Plant
EI	Electron Impact
ESI	ElectroSpray Ionisation
FTICR	Fourier- Transform Ion Cyclotron Resonance
GC	Gas Chromatography
HESI	Heated ElectroSpray ionisation
HILIC	High Polar Chemicals Hydrophilic Interaction Chromatography
HRMS	High Resolution Mass Spectrometry
KRT	Kovàts Retention Time (indexation method)
LC	Liquid Chromatography
m/z	Mass to charge
MS1	Mass spectrometry level 1
MS2, MS/MS	Mass spectrometry level 2
NTS / NTA	Non Target Screening / Non Target Analysis
PCA	Principal Analysis Components
POCIS	Polar Organic Chemical Integrative Samplers
PS	Passive Sampler
QC	Quality Control
QqQ	Triple Quadrupole
qTof	Hybrid Quadrupole Time of Flight
Rt	Retention time
SWATH	Sequential windowed Acquisition of all THeoretical fragment ions
SSM	Spicked Standard's means (method)
TIC	Total Ion chromatography
Tof	Time of Flight (spectrometer)
TP	Transformation Product
UHPLC	Ultra-High Pressure Liquid Chromatography
VOS	Volume of Organic solvent injected (method)
W4M	Workflow4Metabolomic
WBE	Wastewater Based Epidemiology

Introduction

European Union announced that tap water is safe for drinking everywhere in Europe [1]. Health of all population depends on water. This is why it is important to monitor it. Nevertheless, some pollutants present in trace amount in, can be toxic and cause troubles for drinking water. These are micropollutants. They are mainly attributed to human activities like agriculture, industries and household. Wastewater Treatment Plants (WWTP) treat household wastewater but they cannot removed all contaminants. Here, focus is on small molecules <2000. Some of them are well known and already monitored, as pesticides or antibiotics are. While others belong to the Contaminants of Emerging Concern (CEC). The number of CEC ending in environment is growing and there is a lack of knowledge about them. Lots of them are unknown. Moreover, these chemicals can be degraded and result in transformation products (TP). TPs can be even more dangerous than parent compounds [2]. Even if thousands of chemicals are already registered (U.S. EPA's CompTox Chemicals Dashboard counts 875 000 chemicals), there are discovery of new CEC every year. CEC are distributed in a wide range of categories containing sub categories. There are pesticides included fungicides, insecticides and herbicides; pharmaceuticals like antidepressants, hormones and antibiotics; personal care products which count cosmetics, perfume, sunscreen agent, etc. In addition, new categories come to lengthen the list [3]. Several studies across the world show levels of CEC in surface or ground waters that exceed recommendation from World Health Organisation. It touches every continents and every kind of contaminants [2, 3]. EU keeps a watch list to follow the knowledge about CEC [1]. As scientists learn about a CEC, it comes to complete the already existing lists, monitoring well-known contaminants. To respond to European requirements, chemical analysis appears of major interest to find new CEC and understand their fate in environment. One technology is widely used to analyse chemical compounds in water: the High Resolution Mass Spectrometry (HRMS), coupled with liquid or gas chromatography [2].

Before analysing water, the first step is to sample water. There are three ways: grab, active and passive sampling. First method consists of one punctual grab sample of water. It is cheap but not representative of the average water contamination. Active sampling is done by automatic displays which collect a fixed volume regularly and mix it to get an average of concentration per time or per flux [4]. Biggest disadvantage of grab and active sampling is that concentration of trace contaminants could be too low to be detected with analysis tools. For these contaminants, passive sampling is suitable. Indeed, there is a pre-concentration of contaminant during the sampling in water. There are several wide-used devices, as example for hydrophilic molecules: Chemcatcher, Diffuse Gradient in Thin films (DGT) and Polar Organic Chemical Interactive Samplers (POCIS) [5]. All of them are composed of at least a receiving phase, the sorbent, and a membrane. The receiving phase catches the compounds, fixes them, while the membrane protects the sampler from biofouling, and plays the role of first selective barrier. In this report, data come from Passive Sampler (PS) close to POCIS. POCIS was firstly developed by Alvarez D et al [6]. Originally, external diameter is 7 cm and 3.3 cm internal. This results in an exchange surface of 18 cm^2 with a standard mass of sorbent of 100mg.

In literature, Menger F et al [7] claim PS is a good way to collect information about contaminants of emerging concerns and HRMS is efficient for analysing it. HRMS can detect compounds within a concentration of parts per trillion. It gives spectra of mass for the compounds present in the water sample, according to the chromatographic retention time. Thousands of different molecules can be seen in one water sample. Recently, some authors have used HRMS on passive samplers, mostly for semi-quantification and suspect screening as Soulier C et al [8] or Guibal R et al [9]. There is a lack of

study doing Non-Target Screening (NTS) on passive sampler. Only recent studies, like Zhang X et al [10], are using NTS methods on PS like Kendrick mass deflect or Principal Component Analysis (PCA). In this case, to detect halogenated pesticides in the Canadian Ontario Lake using a PS made of low-density polyethylene sheets.

Non Target Screening is a HRMS strategy. There is a distinction between target and Non-Target Screening (NTS). NTS includes suspect screening, structural elucidation, cluster analysis, etc. Target screening can be done as well with low-resolution spectrometer. It targets selected compounds and use standards of these selected compounds for their identification and quantification in water samples. Suspect screening is done only with high-resolution instruments. Operators compare a list of compounds from a database (including m/z, names, fractioned mass spectrum of molecule, etc.) with data given by the mass spectrometer. It is called "suspected" because operators suspect the presence of molecules from information in the database. Molecules absent from the database cannot be identified. To confirm an identity, scientist need to compare it with a standard (it needs target analyses). It is the most popular HRMS strategy [11]. With the rise of big database, it is becoming possible to screen larger panels of contaminants [12]. Structural elucidation uses analysis of compounds fragmentation to find out their compositions and structure . Fingerprint explorations is the most challenging. It aims to treat the data without a priori. That means operator do not look directly for contaminants. The goal is to put in light as much relevant molecules as possible and then use several tools to identify groups of contaminants, prioritize or classify them, find new ones, gather them in clusters, etc. Fingerprinting can be combined to structural elucidations and suspected strategies for contaminants identification.

As NTS is seeking for all compounds in a sample, resulted data are challenging. Indeed, a sample gives thousands of features. A feature is a signal corresponding to an ion, characterized by a mass to charge (m/z), a Retention time (Rt) and an intensity. It is not possible to look finely all the information. It would be a tremendous task. Robust and reliable workflows need to be developed to eliminate features which are not relevant without discard potential molecules of interest. The main issue is that several softwares exist to do the job and differences have been noticed between softwares. Horenk L et al [13] treat the same set of data with different softwares, from vendors or in open sources. Results are not the same, some tools are more efficient. In addition, for a single tool, the parameters can be changed and then it results in a change of the identified chemicals list at the end. Because of non-standardized workflows, it is difficult to compare results and studies, even between two searchers from the same organisation.

Even more, laboratories are using different HRMS instruments, this increases all the more differences in the obtained results. Moreover, HRMS instruments also bring challenges. There are differences between spectrometers from different suppliers and even between different or even the same models from a single supplier. The resolution will not be the same and so are the data. Because there are several parameters to optimize, even within the same spectrometer models, results can vary. Hence, for one sample, there is no insurance that results will be identical. There is a strong need of harmonization in HRMS and even more in NTS acquisition and workflows [14].

That is why the NORMAN network launched an inter laboratory trial. NORMAN is a European network which aim to promote exchange of information between laboratories and harmonisation of measurement technics in order to create synergies in the field of Chemical Emerging Contaminants. The trial consisted of sending 6 water samples from POCIS-like passive samplers [15] to 21 laboratories all across Europe. Passive samplers were exposed to an input and an output of Drinking Water Treatment Plant in Czech Republic. Both, input and output were exposed during two different periods. Hence, there is possibility to study differences between two exposure times and the impact of DWTP.

Laboratories had to follow a pre-defined protocol of analysis in order to facilitate as much as possible the comparison between their data.

This report will focus only on four laboratories with the same brand of mass spectrometers, instruments from Waters Corporation. The research questions are

On NTS strategy aspects: NORMAN data set combine results of analysis coming from several laboratories, so many differences are expected. Data treatment has to deal with all subtleties and particularities of each labs. That is why it is the most challenging step. In NTS there is no consensus about the method to apply and here data should be processed with open sources software. This study try to answer these questions:

How to build a NTS workflow using open source softwares?

What knowledge on HRMS data can we obtain from this inter laboratory trial using NTS strategies?

On environmental aspects: Data set contains River water and drinking water sampled by PS expose 2 and 4 days. So, information about drinking water treatment and exposure time of PS are available for investigation. For the moment this study focuses on:

What is the influence of the exposure time for the passive sampling of organic micropollutants in water?

First task of the project will be to get an overview of different tools (software, coding package, platform, etc.) already used for data processing. Then, efficiency of two tools (one software and one platform) will be compared. The better tool will help to design a workflow to treat data with a NTS strategy. The workflow created will process a data set including analysis from 4 laboratories. Differences of methods and results will be highlighted to figure out the major issues for harmonization and which results are the most robust. A way to create a unique list of result based on lists coming from different laboratories will be tested. Secondly, we will focus on differences between sampler exposure time in order to understand the dynamic inside PS and find optimal time of expositions for environmental studies using PS.

To do so, in a first part, we explain principle of HRMS analysis and the difference between workflows. In a second part, we draw up an inventory of the actual technics through bibliography. Then methods and material used to complete this study are explained in a third part. In a fourth part, Results are presented to help answering the different questions. To finish, in a fifth part, we discuss about the results to highlight the major issues and findings of this study.

I. Principles

A. Chromatography

A liquid or gas can be directly injected in HRMS but for complexes matrixes, HRMS is always coupled with chromatography. It is a way of separation for the analytes. It is an old technology. Indeed, Mikhail Tswett who wanted to separate plant pigments into distinct bands invented it in 1903 [16]. If compounds arrive all together in the mass spectrometer, it is very difficult to decrypt for complex matrices as environmental samples with diverse organic matter content. The data will be a mess of mass in disorder. Chromatography allows to reduce per unit of time the quantity of organic analytes reaching the mass spectrometer and it gives a retention time (Rt). Rt is coupled with the mass given by HRMS, enabling to facilitate the molecule identification. Additionally, chromatography reduces the chance of Ion suppression during ionisation step, which will be detailed further [17].

There are two categories of chromatography that match well with HRMS: Liquid and gas chromatography [16]. Gas chromatography (GC) heat the sample and carry it with a gas. Mobile phase are gas like nitrogen or argon and they pass through a column containing a stationary phase. GC is efficient for non-polar and semi-polar analytes [17]. These compounds are found in lower concentrations in aqueous matrix (hydrophobic). In contrary, Liquid chromatography (LC) is better for polar and semi-polar chemicals (hydrophilic). Here, the mobile phase is not gaseous but liquid. The retention time in LC depends on the interaction with both the mobile phase and the retention phase.

The LC mobile phase is usually a mix between water and methanol and/or acetonitrile. Generally, there is a gradient for analyte elution with time (from the weakest to the strongest eluting power). As example, with a reverse stationary phase there is almost 100% of water at the beginning of the gradient and almost 100% of organic solvent at the end. When the batch is finished, a strong eluting solvent like acetone or isopropanol flushes the column to prevent carry over (i.e. cross contamination between samples) during the following chromatography [14]. This is principle of chromatography for LC-HRMS but solvents can be completely different for non-aqueous matrix or chromatography not coupled with mass spectrometer.

B. High Resolution Mass Spectrometry

1. Ionisation

To undergo High Resolution Mass Spectrometry (HRMS), molecules should be ionized. Hence, purpose of ionisation is to transform molecules in solution into ions in a gaseous phase. Species not ionised will not be detected in HRMS. [18]. For Electrospray Ionisation (ESI), it happens in three stages: droplet formation, droplet atrophy and ion formation in gaseous phase. Molecule keeps its complete structure and could take one or few charges [19]. First, a needle sprays solution droplets. Droplets are charged due to the counter electrode. Electrode impact the ionisation. It means that if electrode is negatively polarized, ions formed will be negative. If polarization is positive, ions will have positive charges. There is a drying gas which pass through the system (e.g heated nitrogen). It reduces the size of droplets by removing the solvent. So, charges are getting closer. The surface charge density increase until a critical point, the limit of Rayleigh. At this point, repulsion forces are bigger than cohesion and ions are ejected in the gas phase. Then ions are collected in a sampling skimmer cone which sends them inside the mass analyser [18].

2. QToF technology

After molecules are separated by chromatography and ionized, their mass can be measured with high resolution thanks to spectrometry. Several devices can achieve HRMS. The most powerful in term of mass resolution is the Fourier- Transform Ion Cyclotron Resonance (FTICR). But due to its high cost and complexity, it is rarely used for organic micropollutants including CECs in environment. Resolution is the ratio heigh/width of a peak, where the heigh is the intensity and the width is a m/z decay. The mass spectrometer called Orbitrap has a good resolution, up to 1 000 000 but it processes the measurement slowly compared to the Q-tof (up to 80 000 for the best) [7]. Even if Q-tof has a lower resolution, it can still be able to give a mass accurate to 0.0001 Da [17].

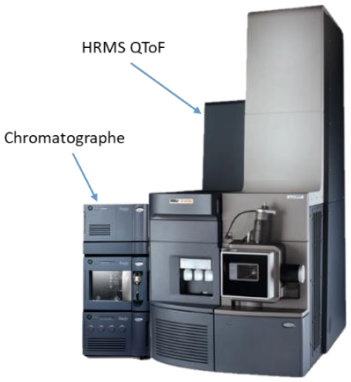


Figure 1: Picture of QToF HRMS instrument coupled with liquid chromatograph*

QToF is a hybrid machine that combines the mass accuracy of a Time of Flight spectrometer (ToF) and a triple quadrupole (QQQ). The third quadrupole is replaced by a TOF. It results in an instrument with a high column vertical or horizontal. **Figure 1** present an LC-HRMS QToF from the brand Waters Company.

Before the quadrupole, there is a collision cooling step ("StepWave ion guide" in **Figure 2**) to orientate ions in same directions. It is a multipole, which focus the ions in the axis and reduce their velocities after the ESI [20]. Quadrupoles are mass filter made of four round rods settled parallel and ions pass between. In the first quadrupole, two rods apply a potential U and the two others are linked to a radio frequency. The rod charged contrarily attracts ions pushed inside. Before the ions strike the rode, the polarity changes. Hence, the ion will oscillate and pass through the quadrupole. Depending on the potential and the rate of polarity change, only an ion in a precise range of m/z will be able to pass through. That is how quadrupole select ions depending on their m/z [21].

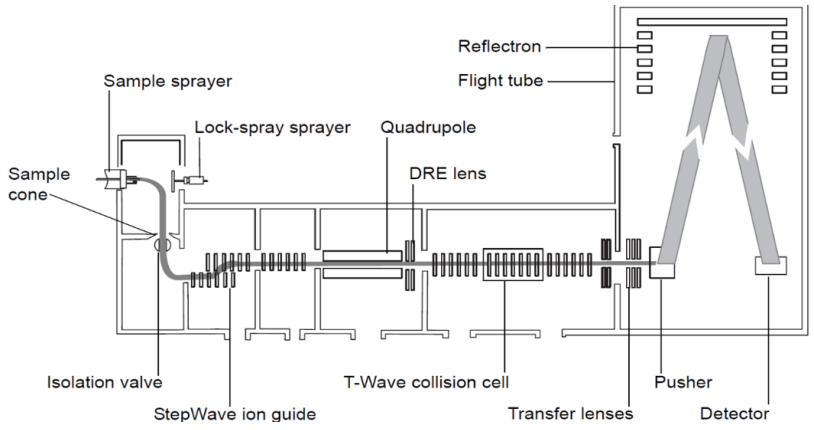


Figure 2: Scheme of QToF HRMS compartments*

*Documents provided by Waters Corporation internal documentary

The second quadruple is not a real quadrupole, but a collision cell ("T-wave collision cell" in **Figure 2**). It aims to fragment the ions. Ions get kinetic energy after collision by a gas, generally nitrogen, argon or helium. This energy is absorbed and it results in a breaking bond [22]. Knowing how an ion fragments itself allows to make the difference between two isomers. Indeed, isomers have the same mass, so neither first quadrupole nor ToF can differentiate these molecules. Fortunately, isomers do not give the same fragmentation pattern [17, 20, 22]. In the collision cell, there are two challenges. Fragment should leave the cell easily, to avoid the possibility to find it in the fragmentation spectra of the following molecules. Then, it is important to put the right amount of energy in the collision gas to fragment the molecule. Each molecule has its own collision energy. That is why a collision energy ramp is used [17].

Then, ions arrive in the third compartment, the TOF, where the exact m/z will be measured by the detector. The principle of Time Of Flight (TOF) analyser is based on the famous equation (1) :

Equation 1: $$E = m \,.\, C^2$$ [21]

With E, the energy of impulsion given to the ion (in joules J). M, the masse of the ion in (Kilograms Kg) and C, the velocity (in meter per second m/s²). ToF is a column where the ion is driven. First, ions are pushed by the pusher in the column with a high voltage potential. Knowing the voltage gives the energy. Then, ions go through the column until the top where a mirror ("reflectron" in **Figure 2**) reflects them and they go back to end on a detector. As the height of the vertical column is known and the detector gives the time, it is easy to calculate the velocity and then obtain ion mass. However, this instrument calculates the mass to charge ratio (m/z) and the column has a region for acceleration (x) and another for free fly (s). Hence, the real equation (2) is:

Equation 2: $$m/z = t_f^2 \,.\, 2 \,.\, E \,\frac{s}{(2s+x)}$$ [21]

With m/z the mass to charge in dalton (Da), t_f the time of flight in second (s), s the length of acceleration region in column and x the length of free flight in column both in meter (m)

C. Data Acquisition

It is described above that ions can be filtered by the first quadrupole and fragmented in the collision cell. If the HRMS is settled on single MS mode, the first quadrupole lets all the ions go and the collision cell does not fragment anything. In this way, all the ions are visible. Nevertheless, first quadrupole can be used to set a limit of mass to select small or big molecules [23]. The other acquisition method is tandem MS mode or MS/MS. It is the name of acquisition when specific ions are selected by their mass and fragmented. It is a method used to identify isotopes or for structural elucidation. It helps when it is not possible to identify a component only with its mass. The way it fragmentates itself gives more clues in order to find the formula [14, 17, 20, 22].

The spectrometer gives information as mass spectra. At t-time, all mass detected are shown on a graph with the intensity (y) and m/z (x). Intensity means the number of contact per second (cps) and per molecule on the detector. The delay between two spectra is settled by the operator. The shorter the delay between two spectra, the lower the measured intensities. A good compromise needs to be found between enough time to detect molecule (sensitivity) and not too much to better distinguish various molecules. Indeed if all molecules arrive at the same time, chromatogram shows only one huge and

large peak. It is impossible to separate the different signal. All m/z spectra can be combined to give the Total Ion Chromatography (TIC). It counts the total amount of ions on each spectra and add them in function of their retention time. **Figure 3** gives an example of a TIC and a mass spectrum.

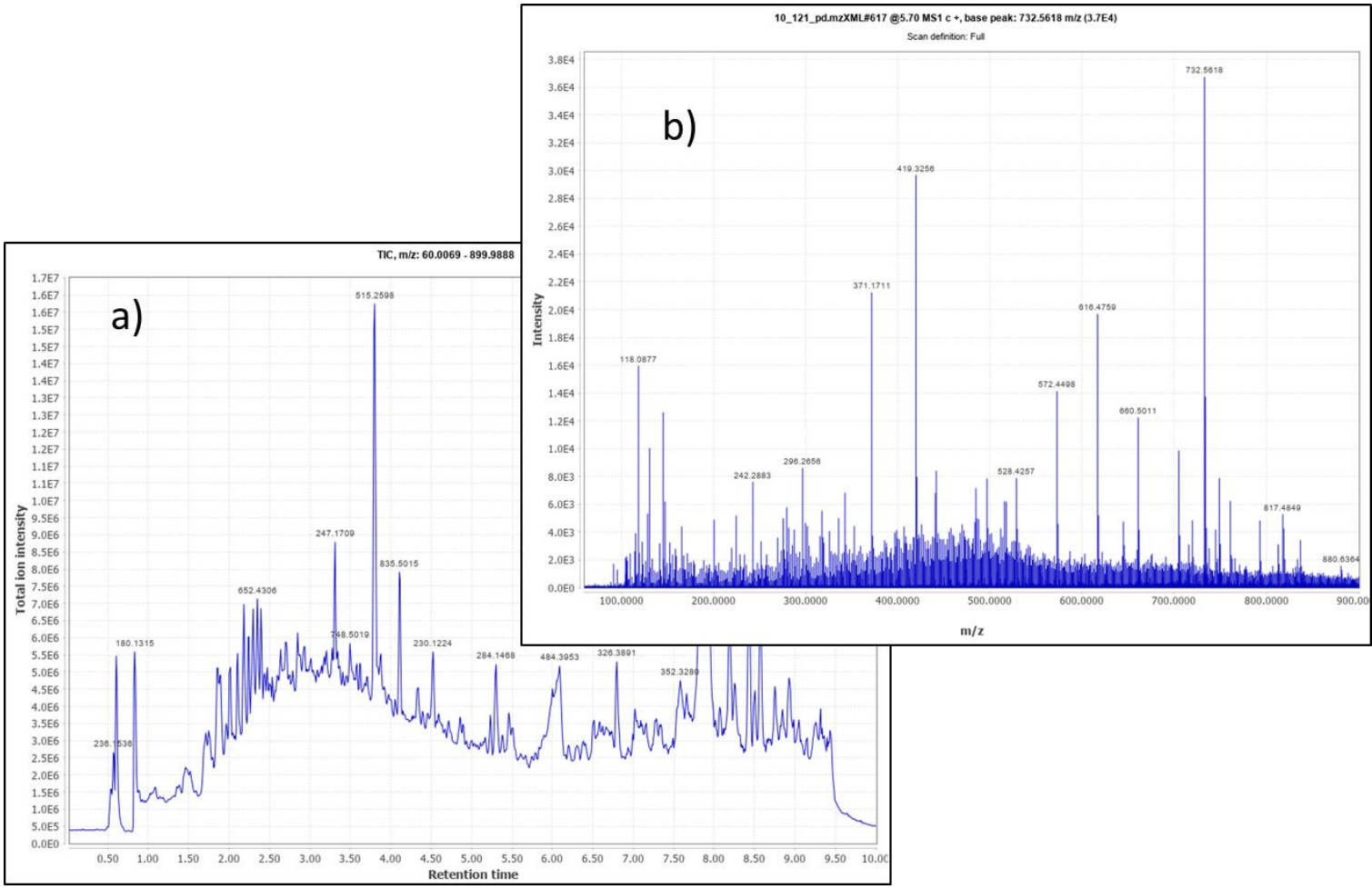


Figure 3: a) TIC and b) mass spectrum at 5.70min of a PS exposed 2 days in river water analysed by lab n°10

D. Data treatment – The workflow

HRMS counts target and Non-Target Screening (NTS). Target screening is looking for a known chemical inside a sample. Indeed, it compares a standard to the sample during the analysis and it allows to quantify this chemical in the sample [11]. No need for a QToF nor an orbitrap spectrometer to do that, a low resolution mass spectrometer is enough [14].

Different approaches exist to treat data for NTS. It depends on the sample analysed and the purpose of the study. There are three main NTS strategies: suspect screening, structural elucidation and fingerprint clustering. Data processing is composed of several successive steps to get a list of features, clean it and identify the relevant features and the potential contaminants associated with these features [11]. To obtain the list of features, softwares and parameters can differ between studies but principles are the same [24].

First step is peak picking, including several actions optional or not. The first one is mass detection from mass spectra that gives a list of m/z, Rt and intensity combinations [11]. Settlement of parameters is

very important because it can eliminate the noise at this step. Noise is a background signal digitized by the detector, even present in blank. These signals with low intensity is constantly deflecting. Operator has to settle a minimum intensity threshold under which, all m/z are not taken in account. It is then possible to use signal to noise ratio to filter features regarding their intensity.

Optionally, shoulder peak filter algorithms are used to check the peak shapes. If peaks are not Gaussian nor Lorentzian, they are discarded because they can interfere during other steps [25]. A next step, also optional, is the smoothing. It improves the shape of the peak and helps to reduce the noise. However, it distorts the peak original shape, lowers the intensity and it results in a loss of accuracy. That is why there are several levels of smoothing and it is important not to smooth too strongly to avoid feature loss. Smoothing can be done with algorithm like Savitsky-Golay smoothing as example [26]. Moreover, some chemicals can be eluted at the same time, leading to peaks overlapping in the chromatogram. Deconvolution allows the separation of these peaks. Deconvolution tools need to know the number of masses and peaks. That is why previous peak filtering and smoothing are most of time necessary [26].

When peak picking is accomplished, a cleaning is necessary. In one hand, NTS aims to explore without a priori the highest amount of features and molecules. In the other hand, it is not possible to study all the features per sample (from several hundreds to few thousands for a water sample). Cleaning is a way to reduce the number of features without losing information on molecules.

A common used cleaning is isotopes grouping. Each molecule has isotopes that have a mass decay of a proton (1.007 Da). No information about molecules is lost if all features of a same isotope are gathered in the most intense masses. That is what isotopes grouping tools do [27]. It is the same with adducts grouping. Furthermore, chemical groups coming from ionisation, solvents or others, can bond to a molecule, leading to adducts. When the chemical group and then its mass are known, , the mass decay software can recognize feature belonging to the same molecule and group them [27]

II. Bibliography

This part focus on different instruments and strategies used for Non target screening analysis using HRMS technology. Results from HRMS analysis are used in a large range of sectors. It is widely used in medicine to find metabolites in cells, this science is called metabolomics. It is also helpful in pharmaceutics field and in food monitoring. In this study, it is especially about water samples like WWTP output, drinking water, surface water, etc.

A. Measurement Instruments

1. Liquid and Gaseous chromatography

As said earlier, chromatography can be liquid or gaseous. Because it is better for non-polar molecules, articles using GC are mainly focused on sediments [28, 29]. Hence, Ayala Cabrera JF et al [23] used GC coupled with an orbitrap to detect polychlorinated naphthalenes in marine sediments. GC can be used for others non-aqueous matrices like animal samples. Abdel Malak I et al [28] analysed industrially used specific flame-retardants in catfish (*Silurus spp*.) tissues. Thanks to this technic, they could quantify the amount of monitored Dechlorane related compounds in catfish's lipid weight. Both studies [23, 28] used helium as carrier gas and a temperature system rising gradually until 275-280°C.

For water samples, all articles selected for this bibliography used liquid chromatography, with reversed (non-polar) stationary phase. It is because LC is more efficient for polar molecules and compounds ending up in water are mostly polar. In addition, reversed phase column is convenient for aqueous-based matrix [16]. Silica based column and especially octadecyl (C18) is in the huge majority of LC-HRMS experimentations [24, 30, 31]. For laboratories, this column has good robustness and reproducibility [17]. For very polar compounds, HILIC phases (High Polar Chemicals Hydrophilic Interaction Chromatography) are used in complementary or instead C18, because it is more efficient for highly polar compounds. HILIC column are compatible with HRMS and aqueous sample [17]. Bride E et al [31] used an HILIC column in complement, to improve their retention time prediction model. Other reverse phase columns exist. As example, they are used by Samanipour S et al [32] to monitor hazardous compounds in drinking water.

More recently, it is possible to hear about Ultra High Pressure Liquid Chromatography (UHPLC). Mobile and stationary phases are generally the same as classic LC. Only the size of the column changes, it is shorter. It means the resistance is bigger so the pressure is higher. It involves shorter retention times and sharper peaks. There are less chances of co-elution [7]. That is why the majority of recent studies are using this column also filled with C18 [27, 33, 34, 35, 36, 37, 38, 39, 40, 41]. To separate even better the molecules, two dimensions chromatography offer a god tool. Zhang X et al [10] have done their chromatography with two GC from Agilent.

Commonly, scientists add a small amount of acid, like formic acid, in mobile phase to avoid moisture and other contamination [42]. But, this can further affect ionisation.

2. Ionisation

To undergo HRMS, molecules should be ionized. In GC, one ionisation is mainly used. It is Electron Impact ionization (EI), classify as hard ionization. EI is used in GC because it helps analysing volatile and semi volatile compounds. In LC, soft ionization are used. Electrospray ionisation (ESI), Atmospheric pressure chemical ionisation (APCI) and Atmospheric Pressure PhotoionIsation (APPI) belong all to soft ionization and can be used in LC. Hard ionization method will require more energy than soft [17].

ESI is widely used with LC, as it is more efficient for water matrices [7, 38]. Indeed, most of the studies used ESI [33, 34, 35, 37, 43]. During this ionisation, temperatures are comprised between 30 and 40°C. But ESI has one major disadvantage, which is ion suppression or enhancement. If chemicals arrive from chromatography at the same time, they can compete for charges. Hence, all the molecules will not be ionised. It involves a reduction of signal during following steps. Ions suppression can be caused by matrix interferents or a too high amount of molecules inside the source. APCI and APPI show less ions suppression than ESI. A way to control ion suppression is to use standards which elute at the same time, or with similar molecular structures as the studied molecules [17]. But, this implies scientist already knows which molecule he wants to study (target analyses).

ESI can be enhanced by increasing the temperature in the source. It is then called, Heated Electrospray Ionisation (HESI). In 2020, Beckers LM et al [30] ionised their river water samples with HESI. Temperatures can reach 600°C in the source. With the high temperature, liquid droplet evaporate quicker, so there are more analytes ionized. [44].

3. HRMS instruments

There are three kinds of HRMS instrument. From the biggest resolution to the lowest there are Fourier Transform InfraRed spectrometer (FTIR), Orbitrap and Qtof spectrometer [30]. FTIR is to expensive for environmental studies. That is why, articles mentioned only Orbitrap and Qtof spectrometers. Orbitrap is developed by the brand ThermoFisher and is used in few laboratories [24, 30, 42]. It offers a better resolution than a QToF spectrometer. But because it is cheaper, Qtof are mostly used for environmental analysis. Several brands produce QToF spectrometers. As example, Agilent provided instruments for Merel S et al [39] and Barcelo D et al [45]. Sciex instruments analysed samples for Backe WJ et al [27] and Samanipour S et al [31]. While the majority of selected articles were mentioning Waters company QToF [8, 10, 31, 33, 34, 35, 36, 38].

Notice that it is not an exhaustive list and other brands commercialize HRMS instrument, as Bruker do.

B. Quality Insurance

1. Lockmass and retention time control

In HRMS, mass is the most important parameter and the instrument should stay well calibrated. The range is comprised between 50 and 2000 Da. To ensure a good precision, a “lock mass” is injected during the analysis. It is a pure compound, with a well-known mass. The instrument knows the value of the lockmass and corrects itself if there is a variation with the measured mass. Several chemicals are used as lockmass: as example, Tröger R et al [35] used leucine enkephalin, Zhang X et al [10] used fluorine containing compound. Mass correction is generally done during the acquisition. Nevertheless, it is also possible to keep the data without correction and apply it after acquisition, during the data processing. To do so, analyst will need the lockmass spectra which is the third acquisition level.

As Retention time is also important for the identification of chemicals, it should stay precise too. Algorithms like PeakGroups help to correct retention time. It compares same features between different sample injections and it aligns retention times thanks to a local non-linear regression [34]. Several softwares for data processing propose this tool, as XCMS packages [33].

Intensity deviation is another important parameter to monitor. Intensity can allow approximating the relative quantity of compounds between samples. But it is not a necessary parameter for contaminant identification.

2. Quality control

Between laboratories, instruments differs but also quality controls strategies. Quality controls (QC) are needed to correct deviation of intensity and also masses and retention times during a single analytical sequence (intra-sequence) or between several analytical sequences (inter-sequence). QC are samples injected regularly with the real samples. Different kinds of QC are developed to be as close in composition as possible to the analysed samples. Standards of target molecules are used even if it cannot be representative of all unknown chemicals explored by NTS HRMS [14]. Correction inside a batch is called intra-sequence correction. It is also possible to use QC to compare two different sequences (inter-sequence) [34]. QC should be the same and representative of both batch analysed. Lebre S [34] try to create synthetic QC for this purpose.

Pooled QC or QCpool are made up of aliquots of all samples analysed in each analytical sequence. This QC is specific of NTS analyses. In this QC there should be all the features presents in other samples. QCpool is injected regularly so it enables an intra sequence correction. Indeed, analyst can see

variation of Rt or intensity in QCpool along the sequence. If he knows which peak in QCmix correspond to a peak in a sample, he can apply the good correction on sample's peak for Rt or/and intensity.

QCmix is a blank with several standards inside. It is the same for all injections of a laboratory. It enable to compare and correct inter sequences analysis. Moreover, QC mix acquisition can be checked to see if the HRMS analysis went well compared to QCmix acquisition from previous analysis

Other QC consist on spiking all samples of the analytical sequence with deuterated compounds, not found in real samples and used as surrogates. This QC allows monitoring of intra-sequence Rt, mass and intensity variation. [34]

When thinking about minimizing deviation, it is important to think about the frequency of QC and the size of a sequence [42]. Vuillermoz-Bellod M [33] showed that a sequence should not exceed 100 samples because beyond that, HRMS loses 40% of sensitivity. Lebre C [34] gives a limit of 150 samples and recommends to inject QCpool every 5 or 10 injections.

False positive features can appear in some samples. It is due to carry over (cross contamination between samples). It is explained by a previous injection with chemical that stay in the injection device or inside the column and that get out with the next sample. To cope with that, cleaning blanks are prepared with strong eluting solvents to clean the system and remove all recalcitrant molecules [42]. Analyst also inject these blank regularly inside the sequence to prevent intra sequence carryover. For inter sequence carry over, solvent with a high percentage of organic is injected at the end of sequence during few minutes.

C. Acquisition during HRMS analyses

Acquisition can be done with positive or negative ionisation. Visible components will not be the same. For a majority of compounds, ionisation is positive [24, 30, 31, 33, 34, 37, 39, 46]. Nevertheless, it is possible to cover a wider range of compounds by performing a second analysis in negative mode [27, 39, 41, 48]. To keep the ionisation step efficient, it is suitable to modify chromatographic solvents, like Backe JW [27] who replaced formic acid in positive ionisation by acetic acid and ammonium dioxide in negative ionisation. Double acquisition takes more time for analysis and data treatment, but it makes more chemicals visible.

There are two MS/MS categories, Data Dependent Acquisition (DDA) and Data Independent Acquisition (DIA).

DDA was more popular [4]. Indeed, it is easier to handle these data. In DDA, a range of specific m/z is settled and only parent ions in this range are fragmented. For example, Beckers LM et al [30] decided to fragmentate only the 10 most intense features. MS2 is the name of fragment's spectra obtained.

DIA is more difficult to interpret but it is better for non a priori analysis as NTS-HRMS does. It fragments all ions. Each brand has its own name for DIA fragmentation method. Waters Corporate calls it MSe [37]. In fact, it combines high-energy scans and low energy scans. During low energy, molecules stay intact. While, fragments are visible in the high collision energy scan. MS^e is done for all compounds, so more data are produced than with MS1 or MS2 and it is more complex to analyse. Moreover, small compounds often interfere with fragmented ions. To disable non-relevant signals interference, sequential windowed acquisition of all theoretical fragment ions (SWATH) was developed by Sciex [14, 27, 38]. It cuts the m/z in several range. One range for each precursor gives the opportunity to assign fragments to the parent ions [14, 40]. So all studies doing structural elucidation use DDA or DIA [27, 30, 37, 38, 39, 41, 45, 47,]

Another more innovative kind of MS2 is MS^n. Only feasible with an orbitrap HRMS. This technic can fragment specific ions and can go further by fragmenting specific fragments [48].

D. Data treatment - Software

Once samples are analysed by GC or LC-HRMS, data should be treated. There are two different ways: Using vendors or open sources softwares.

Each brand provides their own vendor softwares. CompoundDiscover is developed by ThermoFisher and is used for data processing [13, 46]. Laboratories working with Agilent brand can treat their data with Agilent Mass Profiler Professional Software [39, 41] or Mass Hunter [45] which is also provided by Agilent. The brand Sciex gives the software Sciex OS. Backe WJ et al [27] or Samanipour S et al [32] have treated their data with the version 1.3 of this software. Another software for data processing is UNIFY from Waters company [35, 37]. Acquisition on Waters' HRMS is done with MassLynx [34]. This list of softwares is not exhaustive and continuously updated.

Vendor softwares allow data processing fast and easily (almost automatic). But it is difficult to check, understand and upgrade the algorithms behind. The workflow is not customisable. That is why an increasing number of open tools are created. The first gap is the data format. Indeed, vendor softwares are made to work with data coming from instruments of the same brand, with the format conversion made automatically. Open tools softwares need a format like .mzML or .mzXML. MSconvert is often used for the data conversion [13, 30, 33, 34, 38]. It is a free software, user friendly, which gives options to select MS level, scans, range of RT, etc. [49]

MZmine is another free software that can process data. Beckers LM et al [30] used it and Hohrenk L et al [13] compared it with other vendor softwares. Other studies proceed with the coding language R [13, 33, 34, 47]. Freely available package was developed on this purpose; it is called "XCMS" [50]. In 2012, a collaborative programme created an online platform: Workflow4Metabolomics. Thanks to this platform, scientists can treat their data with XCMS package without coding. It is how Vuillermoz-Bellod M [33] and Lèbre S [34] have processed their results. Scientist community keep developing new tools. For example, SPIX is a software developed on Matlab [51] or PatRoon , a package on R for NTS [52].

The main issue is that several softwares exist to do the job and differences have been noticed between softwares. Hohrenk L et al [13] treat the same set of data with different software, vendors and open source. Results are not the same. Some tools are more efficient. CompoundDiscover found 88% of injected standards while XCMS online only found 64%. MZmine2 and enviMass were also compared with the two precedent software and around 10% of features were common to all the software.

E. Non target Screening – The workflow

Workflow is the set of steps to be completed for the realisation of a process. In this part, the process includes data acquisition / conversion / selection / interpretation. There is none standardized workflow and workflow depend on the objective of the study, available material and time. But some steps are almost mandatory and could be grouped in several categories. **Figure 4** and the following text present a workflow for NTS.

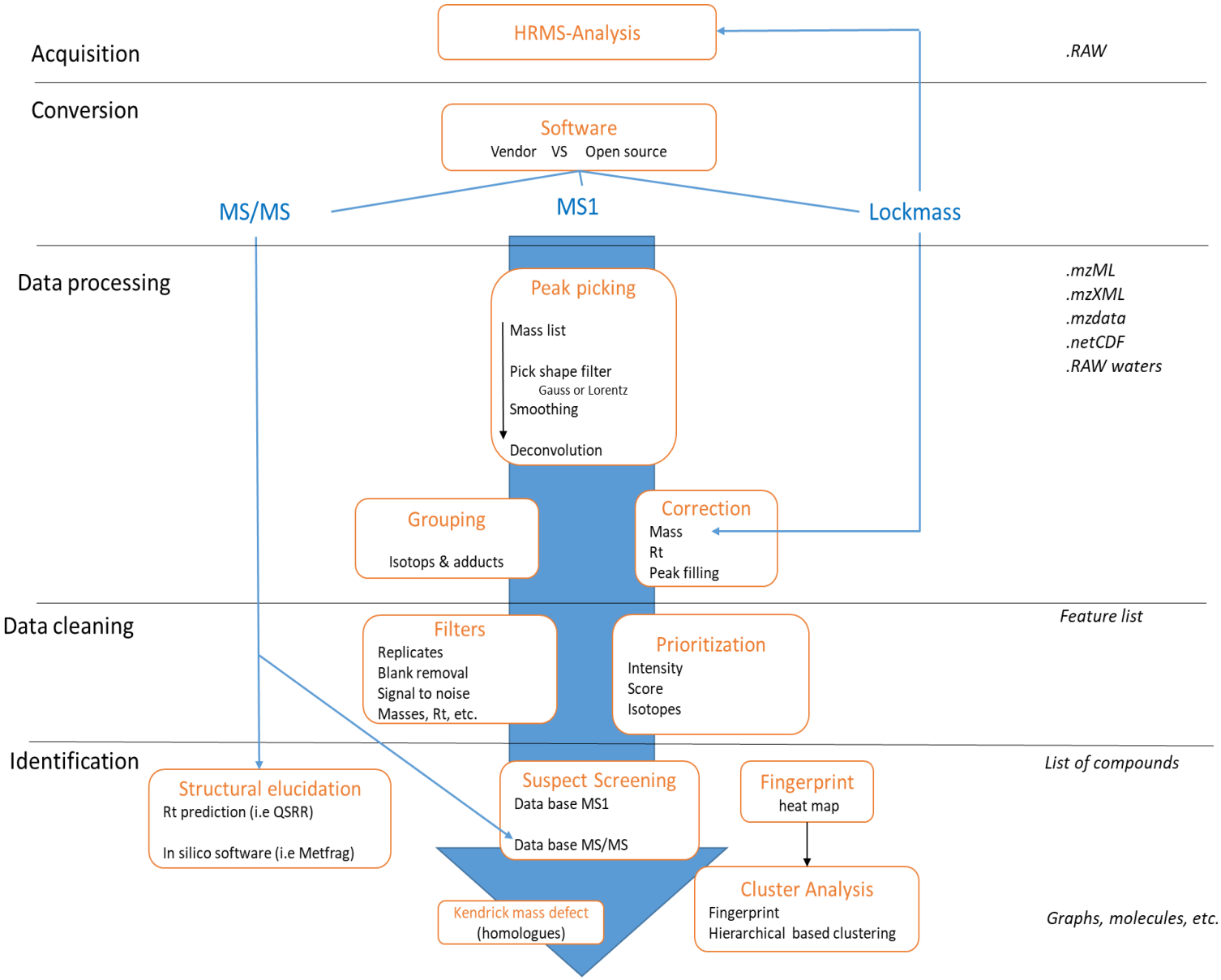


Figure 4: Steps of a possible data treatment workflow for HRMS analysis

3. Data processing and cleaning

Peak Picking is the key point of the workflow. It allows to define a list of features characterized by m/z, retention time and intensity [14, 17, 24] . There are different strategies to select features. A threshold can be set. All signals which have an intensity below this threshold do not count. The threshold value depends on instrument sensibility. For instance, Bijlsma L et al [37] settled an intensity threshold at 200 cps. Scientists using Orbitrap spectrometers have to set up a value even higher due to the high resolution of these instruments. Beckers LM et al [30] selected a threshold with MZmine at 5000 cps.

Another approach is to define a ratio between the signal and the noise. For example, Vuillermoz-Bellod M [33] used a signal to noise ratio of 3. It means that all peaks in the same region of elution which have not at least 1/3 of the intensity of the highest peak, are removed. This method can be combined with the intensity threshold, as Samanipour S et al [32] have done with an intensity at 1000cps and a signal to noise at 5. These criteria depends on the instruments and also on the algorithms used to detect features. In literature, there are several algorithms of detection: Centwave used with XCMS package

[13, 33], Centroid algorithm from MZmine [30], EnviPick function from EnviMass [47], Continuous wavelet transform algorithm [13], etc.

For the purpose of having a list of compounds from the list of features, analysts have to deal with **adducts and isotopes**. Indeed, several features are relative to the same compounds. Isotopes and adducts signals can be removed as done by Backe JW et al [27]. Adducts can also be simply annotated [34]. Isotopes can be useful for identification, using isotopic pattern [7]. It worked well for molecules with bromine or chlorine inside, in the study of Zhang X et al [10].

Filters are useful to clean data. A lot of cleaning strategies can be used. It includes S/N already mentioned above during peak picking. Di Marcantonio C et al [46] excluded all features that are not 10 times higher in samples than in the blank: it is a common strategy called blank removal [35, 42]. Another strategy is to replicate the sample and discard features which are not representative of replicate. As example, Lèbre S [34] has removed features not present in all triplicates for each sample. Analyst can also look at the retention time to remove features which have a very wide peak. It may be a contamination that is detected during the run. That is why Beckers LM et al [30] removed all peaks larger than 5 minutes.

Prioritization is a way to reduce the data set complexity and highlight features that seem more interesting a priori [7]. Easiest prioritization is based on intensity. Backe JW [27] focus on the most intense features which are present in the majority of samples. Schulze B et al [16] consider that it is a dangerous strategy because some relevant compounds are present in trace amount, whereas they are worth looking into for environmental or health questions. That is why, scientists developed scoring tools to give a score on the features or on groups of features (compounds) and prioritize them based on several criteria. Dürig W et al [36] invented SusTool for priorization after identification with suspect screening. It is an example showing steps order is not fixed and some can be moved also after or during identifications. It considers 15 parameters that can be excluded or included and with customisable threshold values. Among these parameters belong toxicity, volatility, biodegradation, emission index, etc. It allows a more objective prioritization. Samanipour S et al [32] prioritize features from isotopic patterns and studying irregular feature in sample that can testify of a punctual contamination or a default in water treatment.

Last step is **alignment**. It consists to apply a tolerance of mass (around 5 and 10ppm) and retention time to harmonize features between the different samples analysed [43].

4. Identification

Once relevant and clean feature list is done, the purpose is to identify contaminants among the hundreds or thousands of remaining features. It is worth noting that strategies explained above can be combined according to the objectives of the operator.

Keep in mind that the ultimate goal is to monitor water quality and find new contaminants. Meng D et al [53], drew up a map of the contamination of Dianshan lake in china. It takes in account pesticide, drugs, plastic additives and surfactants. It works for all kind of water sample, from surface water to drinking. Ruff M et al [54] have found new contaminant in the Rhine River, which are the muscle relaxant tizanidine and the solvent 1,3-dimethyl-2-imidazolidinone. Last example, with the combination of others sectors, it wides the possibilities. Here, Samanipour S et al [33], combine machine learning and HRMS analysis to automatize the monitoring of a drinking water, this tool detects

anomaly in drinking water, this anomaly can be contaminations. An agent can check them and tells if it is a real contamination or not. It saves time because analyst can focus only on detected peaks.

These three study use a different NTS strategy: Samanipour S et al [32] test his model with suspect screening, Ruff M et al [53] use structural elucidation to find new contaminants and Meng D et al [54] draw a map of contamination which is a fingerprint.

Suspected identification

In suspected analysis, operators have an a priori idea of types of molecules they are looking for [40]. Because suspect screening is more developed than other strategies, it concerns a majority of scientific papers [8, 30, 47, 45, 46]

The feature and molecule masses are used for identifications. Fortunately, there are databases listing masses for hundreds of thousands of molecules. Some databases are sector specific like EAWAG database which target biocatalyst and biodegradation products in water. Some are specific to human body metabolites or to country or world region, etc. It is the case of SPIN (Substances in Preparation in the Nordic Countries) or National Research Data Infrastructure Initiative for Chemistry in Germany. Finally, some institutes, or networks or web sites aim to build universal databases or group of databases with as much gathering information as possible: Chemspider, Pubchem, US EPA databases [53], Databases from NORMAN Network [12], HBM4UE [14], etc.

To have a more specific database, it is possible to build homemade databases. As example, it can be useful when looking for transformation products. Bijlsmas L et al [37] have investigated the fate of an insecticide inside farmed Atlantic salmons with an homemade database of 60 molecules. They used a tool to predict metabolites with a given molecule.

Once the database is built or chosen, comparison of masses between feature list and molecule database allow the identification of molecules. A tolerance on the mass precision need to be settled. 2mDA or 5ppm is a reasonable tolerance [43]. But, the correspondence between feature and molecule mass is not sufficient to be sure of the compound presence. It can be a coincidence that a molecule from the database has almost the same m/z as a feature. For clarification and homogenisation, Shymanski E.L et al [55] have proposed a confidence scale called “Schymansky scale”. It gives a score from 1 the highest confidence to 5. To increase confidence, scientists need to show similarity between fragmentation spectra, retention time from prediction models (e.g QSRR), isotopes patterns, adducts signals, etc. Finally, to strictly confirm an identification, scientists need to purchase a standard of the identified molecule and check that it has the same MS1, MS2 and Rt.

As just said, to increase the confidence in suspect screening, MS/MS is used. MS and MS/MS spectra have to be compiled inside molecule databases, generally the same than the mass databases previously mentioned (e.g Chemspider, MZcloud, MassBank, METLIN, etc) [9]. Nevertheless, MS/MS spectra can variate depending on the instrument. Homemade databases allow overpassing this issue.

Structural elucidation

In addition of databases, some specific softwares are able to predict fragmentation from a precursor without experimentation. It uses information from databases or fragmentation rules from literature. Some open source softwares do that, like Metfrag or CSI:FingerID. However, vendor softwares do it too, for example Waters proposes MassFragment on UNIFI. [40]

Being able to elucidate structure is useful to identify new molecules, as transformation products (TP). And it also helps to find structure of already known compounds. For example, Chevalier M.L. et al [29] studied transformation products of chlordecone insecticide with a GC-MS and a Nuclear magnetic resonance spectrometer to elucidate the structure.

Clustering & fingerprinting

Some studies present results as feature clusters and fingerprints, without compounds identification. Features with same tendency are gathered in same groups. Meng D

Merel S et al [39] used this strategy through heatmap presented in **Figure 5**. Heatmap fingerprint means that a colour from blue to red is given to the feature according to its intensity. One row represent a feature. The colour can change across the time and it makes the tendency more visible. In their study, they have evaluated the degradation of trace organic compounds with UV processes in function of UV dose.

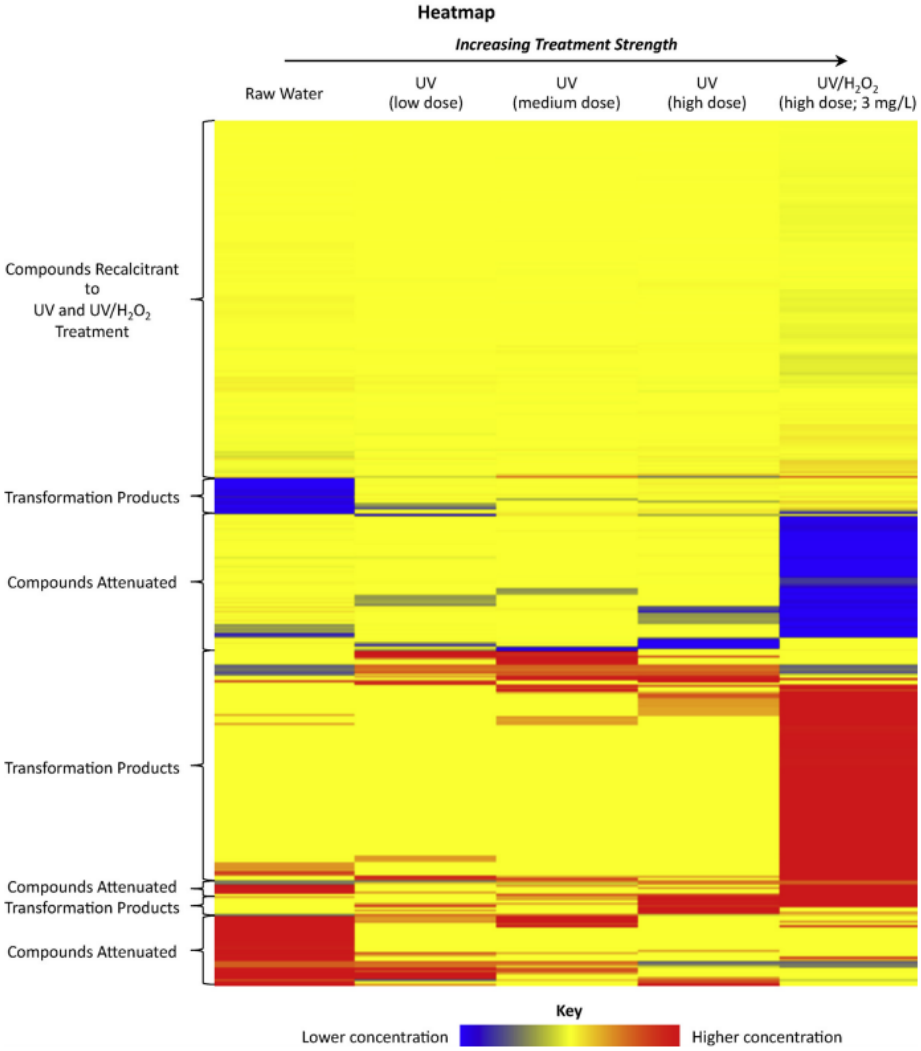


Figure 5: Comparison of water samples exposed to different treatments through advanced processing of high resolution mass spectrometry data [39]

Meng D et al [53] use another kind of heatmap, taking in account geographic repartition of contaminants in Dianshan Lake. Maps are illustrated in the following **Figure 6**.

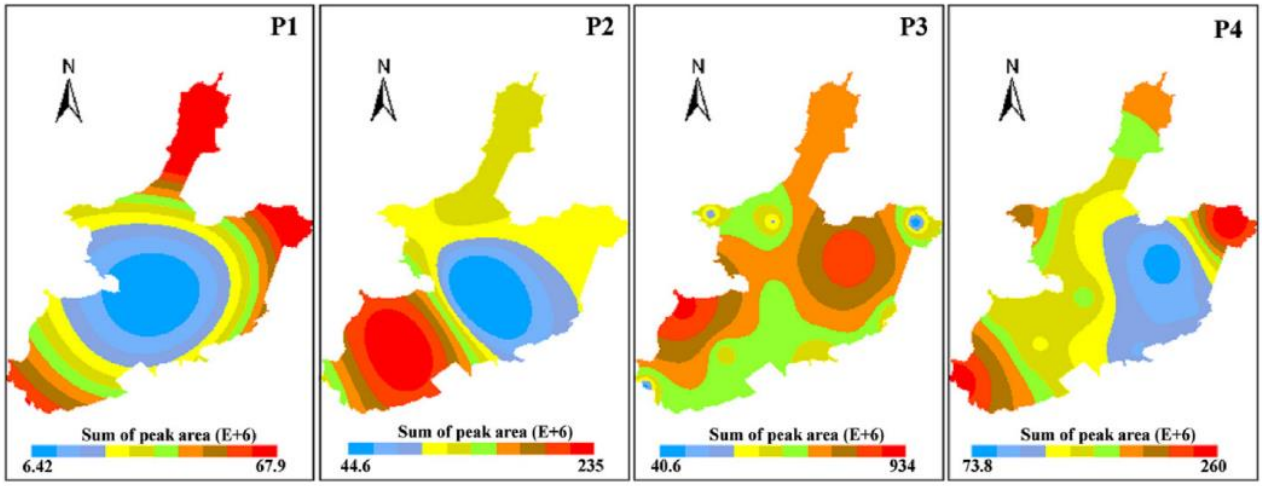


Figure 6: Heat maps of spatial distribution trend of the four major types of pollutants in Dianshan Lake [57]

Generally, Clustering and fingerprint are achieved simultaneously. Compounds are clustered depending on their tendency. Tendencies can be intensity or presence-absence across time, geographic area, processes, etc. Computational tools are able to recognize tendencies, automatically cluster compounds and in parallel draw up fingerprint from a list of feature. Beckers L M et al [30] have clustered features from water sampled along the Bode river. Clusters allowed to distinguish several relevant sources of contamination like WWTP inputs, diffuse and random inputs and native compounds of the river. It is an interesting strategy because it allows prioritizing and sorting features according to their appearance or disappearance in the various water samples.

Samanipour S et al [32] combines machine learning to create an intelligent programme able to find irregularity in samples

Kendrick mass defect for homologues grouping

Kendrick mass decay is useful to study homologues. Homologues are molecules almost similar. There is only one or several chemical groups added or removed. It is the same principle as for adducts and isotopes grouping. Scientist has to find the mass decay corresponding to the targeted chemical group. This strategy is efficient for larger molecules like polymers, in the m/z range of acquisition . Zhang X et al [10] have identified halogenated compounds in Ontario Lake with the Kendrick mass defect on chlorine and bromine.

F. Conclusion about Non-Target Screening

From this bibliographic study, there are ideas that come out:

- NTS is a strategy aiming to see as much chemicals as possible. HRMS is the technology of choice to do it because it enables to see thousands of compounds inside a water sample.

- NTS particularity is the big amount of data acquired, making data treatment the step most challenging. Key point is to find a robust workflow which fit well with data.

- There are a lot of different instruments and methods used to do HRMS analysis.
  → Data are difficult to compare.

- A lot of different softwares and parameters are available for data treatment.
  → it results in a multitude of workflows applicable.

- Number of open sources tools for data treatment is increasing and constantly developed by their users.

- In one hand, a harmonisation of workflow is needed in this sector to allow the comparison of studies, monitoring from peers and repeatability. In the other hand, diversity is necessary to keep exploring and make the field evolving , according to Pourchet M et al [14]

For all this reasons, this study will explore different open sources tools and methods to harmonize data coming from separated laboratories. Then, a workflow will be build, hoping it will help to answer environmental questions about exposure time of passive samplers.

III. Materials and methods

A. NORMAN Data set

The NORMAN Network launched an inter-laboratory trial in which 6 different vials were sent to 21 laboratories across 11 countries. There is one RTImix containing 16 known chemicals described in the **Annexe 1**. Then, sampling was done with Passive Samplers (PS). PS are Horizon Atlantic® HLB-L disks (47 mm diameter). The samplers were put in a dynamic PS device in order to increase the sampling rate. Briefly, four samples were extracts from Passive Samplers (PS) exposed for 2 and 4 days at the input and the output of a drinking water treatment plant in Czech Republic. The blank is a PS that was not exposed to water, it only underwent the extraction. The 6 vials are summarized in the **Table 1**, with their equivalent volume of water extracted. PS where stored in freezer at -20°C until extraction. They did an extraction of the PS and proceed to a dilution by 40. Extraction was made with 200 mL of acetone during 24h, three time. Then, they followed the US EPA method 357036 to do the solvent exchange with methanol.

Table 1: Information about vials sent by NORMAN network for LC-HRMS analysis

Name	Water filtered	Exposure time	Equivalent volume after dilution
001	Procedural blank		
121	River water	2 days	4.8 L
141	River water	4 days	8.7 L
221	Drinking water	2 days	4.0 L
241	Drinking water	4 days	7.4 L
RTImix	16 standards		

All the samples except the RTImix were spiked with 6 isotope-labeled standards: Caffeine-$^{13}C_3$, Nicotine-D_4, Cotinine-D_3, Simazine-D_{10}, Carbamazepine-D_{10}, and Diuron-D_6.

Once vials were ready, they were shipped to the laboratories that had to analyse them following a pre-defined method and their own method. Pre-defined methods is a set of instrument parameters imposed by NORMAN network. In this study, only data from the pre-defined method will be considered, because methods need to be the same in order to compare the results. Moreover, only laboratories with Waters Corporate's QToF will be selected to avoid any variability due to the instrument brand. In the **Table 2** there is the list of chosen laboratories with their ID number for the experiment. Detail of LC-HRMS parameters for the four laboratories are presented in **Annexe 2**. After, processing the LC-HRMS analysis, all participants used MSconvert to put their data in mzXML format.

Table 2: List of laboratories selected for the study

ID	Institute	Place
10	INRAE, UR RiverLy	Villeurbanne, France
13	Man-Technology-Environment Research Centre, School of Science and Technology	Örebro, Sweden
16	Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences	Uppsala, Sweden
17	Environmental and Public Health Analytical Chemistry, Research Institute for Pesticides and Water, University Jaume I	Castellón, Spain

B. Softwares – Mzmine vs Workflow4Metabolomics

The inter-laboratory trial gathers analysis coming from several HRMS brands. Hence, for a better harmonization, all data were converted in an open format (.mzXML). This format only allows open source softwares for data processing. Two major open source softwares found in bibliography are MZmine 2 [58] and the R package XCMS [50]. A first test is made to compare and choose the better tool for the following parts.

Data for comparison come from the laboratory n°13 and 16. Samples are treated then aligned with the two workflows. To detect and highlight feature in common, a script in R was developed. The script is shown in **Annexe 3**. XCMS package was used through the online platform Workflow4Metabolomics and software MZmine2. Parameters were chosen to be close from each other's. Parameters values for both workflows are detailed in the **Annexe 4**. Focus is only on MS1. Number of features and quality of alignment are observed through diagram made by the R package "UpsetR". Coding with R was performed with R studio software. No need to use MSconvert because data were already converted in the good format.

C. Workflow – Steps and parameters

Finally, MZmine2 was selected for next parts. Laboratory n°10 has a mass deviation because correction with lockmass has not been done during the acquisition. So lab n°10 is excluded. Data from laboratories n°13, 16 & 17 were treated for following parts.

The exact workflow with all the parameters set in MZmine2 is described by the **Figure 7**. Step labelled with an orange star were not performed to get the feature list. They were done to get the list of compounds. Differences between both list are quickly discussed in part IV.A . For the next parts, focus is made only on the list of compounds. Second part of the workflow made with R studio correspond to the following part which is the laboratories comparison with Rt Indexation

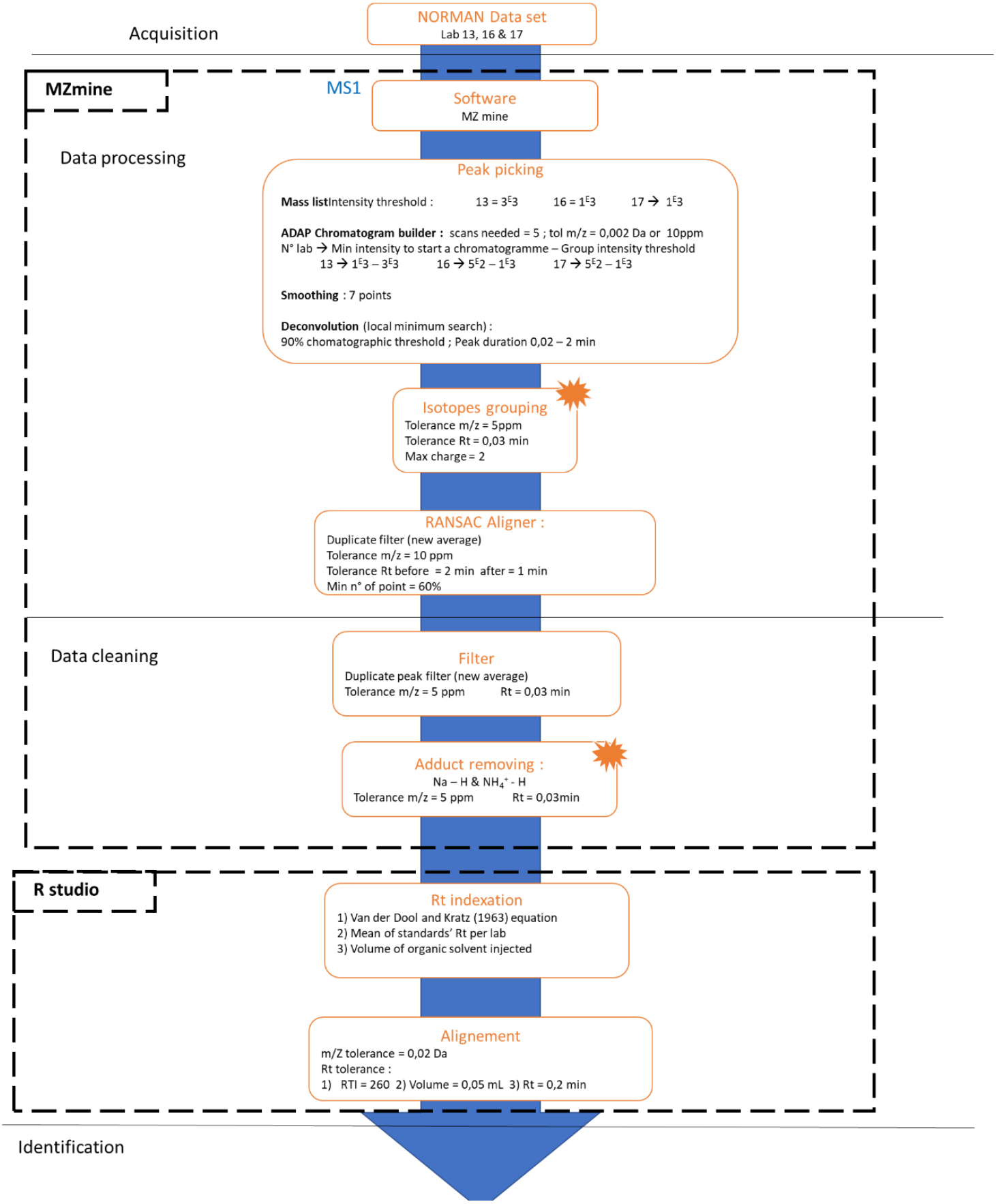


Figure 7: Detailed workflow on MZmine2 to treat data from the 3 selected lab (13, 16 & 17)

D. Retention time indexation

Chromatography method differ between laboratories. To be able to compare lists, an indexation of Retention time is necessary. A new retention time index is set for laboratories n°13, 16 &17. Comparison of common features after indexation is made with the sample of water river exposed 4 days. This sample was chosen because it should be the one with most compounds inside. Indeed, it is the longest exposition to a non-treated water.

Three indexation methods were tested:

- **Using the equation of RTI found in literature - KRT**

Celma A et al [59] used an indexation equation developed by Kovàts in 1958 and improved by Van der Dool and Kratz in 1963. This equation was invented for Liquid Chromatography. See equation (3):

Equation 3: $RTI = 100 \times (n + \frac{Rt_a - Rt_n}{Rt_{n+1} - Rt_n})$

With Rt_a = Retention time of analyte;
Rt_{n+1} = Retention time of standard eluting after the analyte
Rt_n = Retention time of standard eluting before the analyte
n = Order of elution of the standard eluting before (from 1 to the number of total standard)

This method is based on the standards from RTImix. 4 of the 16 standards were removed because they did not have the same order of elution between laboratories. Detail about standards used and order of elution are available in **Annexe 1**.

- **Regarding the Rt mean of spiked standard - SSM**

This is an original method, which has never been used in literature to our knowledge. It is based on the 6 spiked standards. For each standards the mean retention time across the laboratory is calculated. Then retention time of all compounds are normalised according to this standard curve, visible in **Figure 8**.

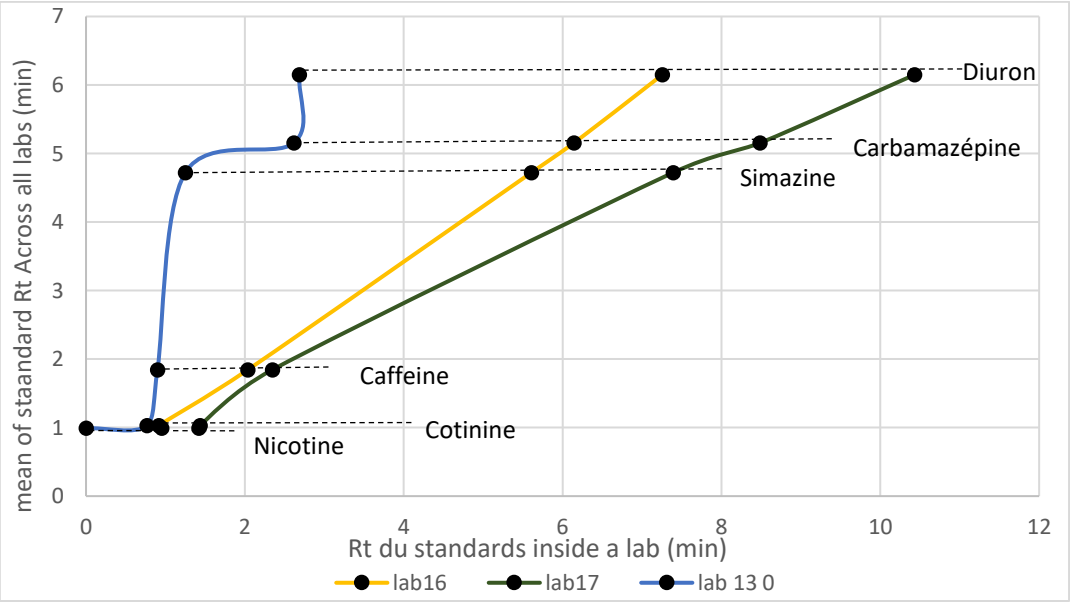


Figure 8: Curve of standard Rt mean in function of standard elution time inside each lab

Equation 4 is used to take in account the different run time and standard time of elution for each laboratories.

Equation 4:

$$RTI_a = {Rt_a}/{Run\ time} * \frac{\overline{Rt_{n+1}} - \overline{Rt_n}}{Rt_n - {Rt_{n+1}}/{Run\ time}} + P$$

With

Rt_a = Retention time of analyte

Rt_{n+1} = Retention time of standard eluting after

$\overline{Rt_{n+1}}$ = mean of Rt across the laboratories for the standards eluting after

Rt_n = Retention time of standard eluting before

$\overline{Rt_n}$ = mean of Rt across the laboratories for the standards eluting before

P = y - intercept

- **Depending of the volume of organic solvent pumped - VOS**

Last method used is inspired by a model of Rt prediction developed by Bride E et al [30]. This method follows the volume of organic solvent injected in the chromatography during the run time and settle a Rt in mL of organic solvent injected. RTI is the volume at the time equal to the Rt of the analyte. To do that, curve of solvent injected in function of time were drawn in **Figure 9**. It was calculated with the information of the **Annexe 2**.

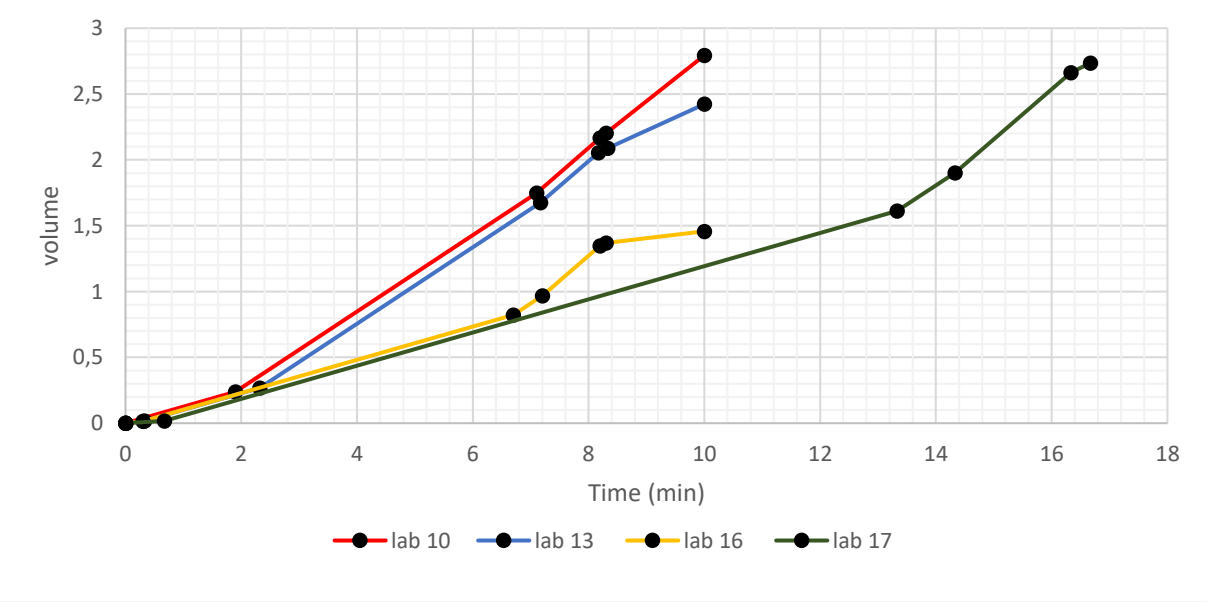


Figure 9: Volume of organic solvent injected during a run per lab

RT indexation was calculated and added to the list with R studio scripts. All three scripts are joined in **Annexe 5**. Then, list of compounds with the new Rt undergo the script to detect features in common (**Annexe 3**). Evaluation of RT indexation methods is done according to the number of compounds in common across the three laboratories. To do so, the R package "Venndiagram" draw Venn diagram to show the results. Tolerance of m/z is 10 ppm and in Retention time 0.2 min is commonly used (e.i

Backe JW [26]). 0.2 represents 2% of run time for labs 10, 13 and 16. So, tolerance for methods about solvent gradients is 2% of total volume injected, which gives 0.05ml. Tolerance for the Kovàts equation is 2% of highest score, which give 26. Finally for the standards mean methods 2% represents 0.2 min too.

E. Exposure time impact on Passive Samplers

To study the impact of exposure time, three groups of features were made: Compounds only found in input sample exposed 2 days, compounds only found in input sample exposed 4 days and compounds common for both of them. Those are the groups circled in red in **Figure 10**. Then, mass to charge and retention time repartition are observed and compared through boxplots. One boxplot for m/z and a second for Rt. To validate the result, same boxplots are drawn with compound specific to outputs samples exposed 2 days, 4 days and in common to both exposure times. These ones are groups circled in blue in **Figure 10**.

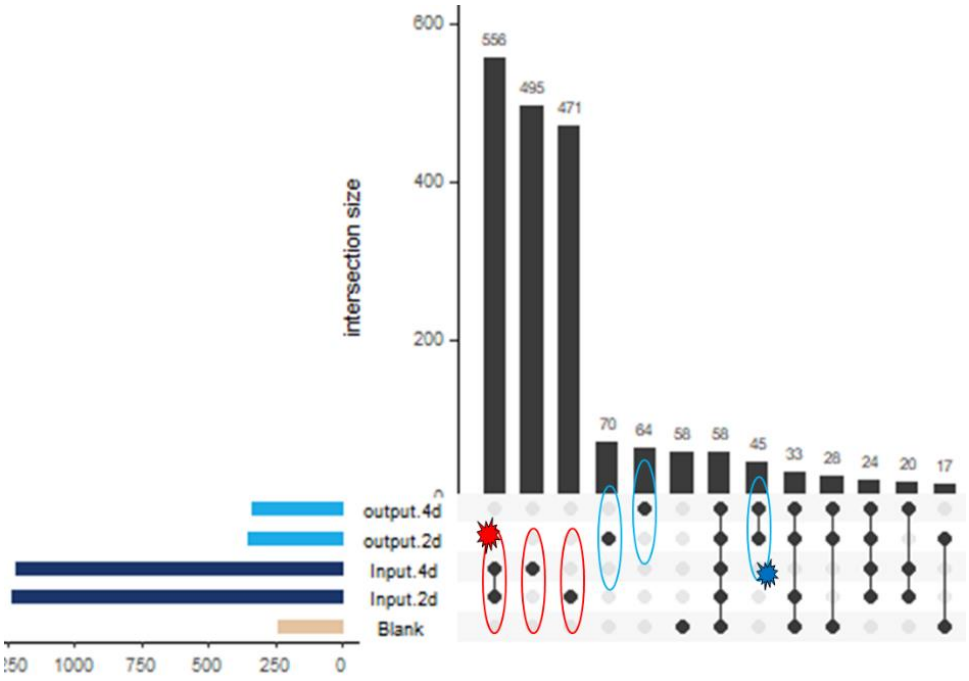


Figure 10: Selected groups of compounds to study repartition in Rt and m/z in order to compare exposure time

A second study on exposure time impact is done looking solely compounds in common to both exposure times. At first, compounds in common to both intakes are selected, as labelled with a red star in **Figure 7**. This time, three other groups are created according to intensity trend: intensity increasing between two and four days, intensity decreasing or intensity stagnating. Intensity differences should be at least 10% higher or lower, if not it belongs to stagnating group. Same boxplots are drawn to look to m/z and Rt repartition. For validation, same boxplots are made with the feature in common of compound list from outputs, as labelled by a blue star on **Figure 10**.

Three list undergone this process: compound list of lab n°13, compound list of lab n°16 and compound list from combination of both lab. Results will be presented with list from lab 16.

Groups were selected with R script in Rstudio. “Ggplot” R package draw the boxplot. Moreover, Tukey’s HSD test is done to detect significant differences between groups. This statistical test is realised with

R package “stat”. Letters were added directly to boxplots. Different letter tells there is a significant difference between group repartitions.

F. DWTP treatment evaluation – Heatmap and PCA

Heatmap

To do a heatmap, features are grouped in three categories: Compounds degraded, compounds persistent, compounds created by the DWTP depending if they are found in river water only, drinking water only or both. Inside these three groups, compounds are divided depending of their exposure time and intensity trends across time. Sub-categories are compounds:

- Only found in 2 days exposed PS
- With an Intensity decreasing between 2 and 4 days exposure time PS
- With an intensity stagnating between 2 and 4 days exposure time PS
- With an intensity increasing between 2 and 4 days exposure time PS
- Only found in 2 days exposed PS

Heatmap will not be coupled with a cluster analysis. For each sub categories, logarithm of the sum of all intensities is calculated. A heatmap is drawn with the r package “ggplot2”.

PCA

The R package “Factoshiny” is used to draw Principal component analysis (PCA). It is a geometric and statistical tool. When doing PCA, scientist should do previously test like analysis of covariance (ANCOVA). It is not the case in this study. Hence, there are a lot of variable that co-vary, so it distorts the PCA. Variables are the features and individues are samples from laboratory n°13 and n°16. **Annexe 6** represents the graph of PCA variables and it tells there are a lot of variables going in the same direction (e.i a lot of co variation). That is why this PCA should be taken lightly.

IV. Results

A. Open sources software – Mzmine vs Workflow4Metabolomics

All samples from laboratories 13 and 16 were studied with Workflow4Metabolomics (W4M) and MZmine2. Workflow from W4M gives less features than MZmine, regarding both laboratories. Nevertheless, ratio of feature numbers between samples is more informative than feature number. **Table 3** summarize the number of features and ratio between samples for both laboratories for data treated with the two workflows. With MZmine's workflow, there are more differences between samples. River water includes three times more features than blank, and drinking water is slightly more charged than blank (around 20% more). Both results from W4M and MZmine make sense. But, because MZmine results are more contrasted, this software and workflow look preferable for data treatment.

Table 3: Number and ratio of features with W4M or MZmine workflows, for laboratories 13 and 16.

		Water sampled	**Blank**	**RTImix**	**River water**		**Drinking water**	
		Exposure time			**2 days**	**4 days**	**2 days**	**4 days**
lab 16	**W4M**	**N° of features**	1949	1633	3779	3472	2678	2365
		Ratio 1/Blank	1	0.84	1.94	1.78	1.37	1.21
	Mzmine	**N° of features**	2158	1974	6233	6179	2936	2171
		Ratio 1/Blank	1	0.91	2.89	2.86	1.36	1.01
lab 13	**W4M**	**N° of features**	1002	1022	2931	2620	1215	1206
		Ratio 1/Blank	1	1.02	2.93	2.61	1.21	1.2
	Mzmine	**N° of features**	1723	2422	5541	5411	2127	2236
		Ratio 1/Blank	**1**	**1.41**	**3.22**	**3.14**	**1.23**	**1.3**

Then, it is also interesting to compare features alignment. Features in the same type of samples can be compared (sample n°121 and n°141): W4M allows to obtain more common features between the two river samples. This is also shown in **Figure 11** with 78% of overlapping with W4M against 66% for MZmine. Same plot was made for drinking waters and for laboratory 16, available in **Annexe 7**. It confirms the fact that W4M allows to obtain more features in common between similar samples, hence, seems more efficient and robust on this point.

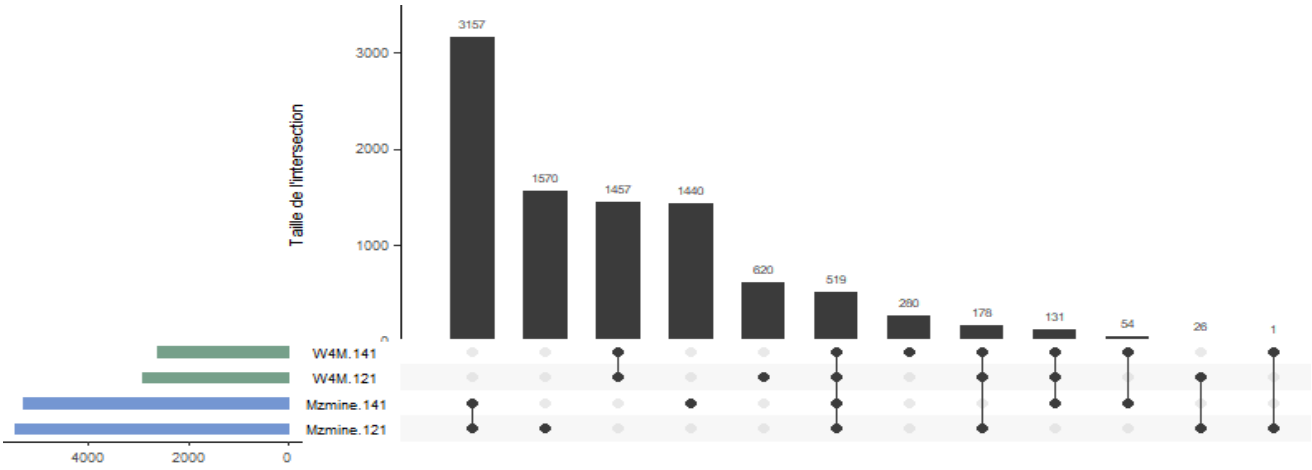


Figure 11: Upset plot of river samples features from laboratory 13 after data processing from W4M and MZmine

Main advantages emerge while using each tools. In one hand, MZmine is easy to use, and chromatograms of featurest are available at each steps of the workflow. There are more parameters to choose and they are well explained. In general, MZmine is visual and intuitive. In the other hand, W4M works on a platform, so there is no need of powerful computer to proceed heavy data. Without background knowledge in LC-HRMS, W4M is easier because scientist can just follow the steps with the default parameters. Nevertheless it may be less accurate because parameters are less close to the data so it could extract more noise or delete features representing real compounds.a

For the following parts of this study, MZmine is chosen. The choice is not based on performance because this parts says that it is difficulte to distinguish them. The choice is on MZmine because it is more visual, there are more optional steps and parameters to try. It seems to be a good tool to learn how to treat HRMS data.

B. Workflow – Impact on features number

Processing data with workflow of part III.D gives information about the number of features removed by each steps. **Table 4** presents number of features for water river (121) exposed 2 days in 3 laboratories, and the mean percentage of features reduced taking into account the 6 samples .

Table 4: Number of features remaining and percentage of feature reduction after each step of the workflow

Workflow steps	Lab 13		Lab 16		Lab 17	
	N° of features in river sample exposed 2 days (n°13_121)	Mean % for all samples of features reduction	N° of features in river sample exposed 2 days (n°16_121)	Mean %for all samples of features reduction	N° of features in river sample exposed 2 days(n°17_121)	Mean % for all samples of features reduction
Chromatogramme	7869		12846		5630	
Deconvolution	5541	27.33	6233	55.78	4877	10.63
isotopes grouping	4522	19.33	5336	16.59	3953	20.06
Duplicate filter	4521	0.11	5335	0.04	3952	0.03
Adduct grouping	4459	1.6	5288	1	3854	2.74

Numbers and percentages vary a lot according to the laboratory. Deconvolution seems to be the step removing the highest amount of features, followed by isotopes grouping. Duplicate filtering changes almost nothing, it allows to clean only one or two features per sample. Then, adduct grouping represents 1 to 3% of feature number reducing but here there is only two adducts searched [Na-H] and [NH4 – H]. The FTMS shoulder peak step does not figure in the **Table 4** neither in the workflow since it never removed any signals.

C. Laboratories comparisons

One feature is characterized by a Rt and m/z value. All features can be represented as illustrated in **Figure 12**.

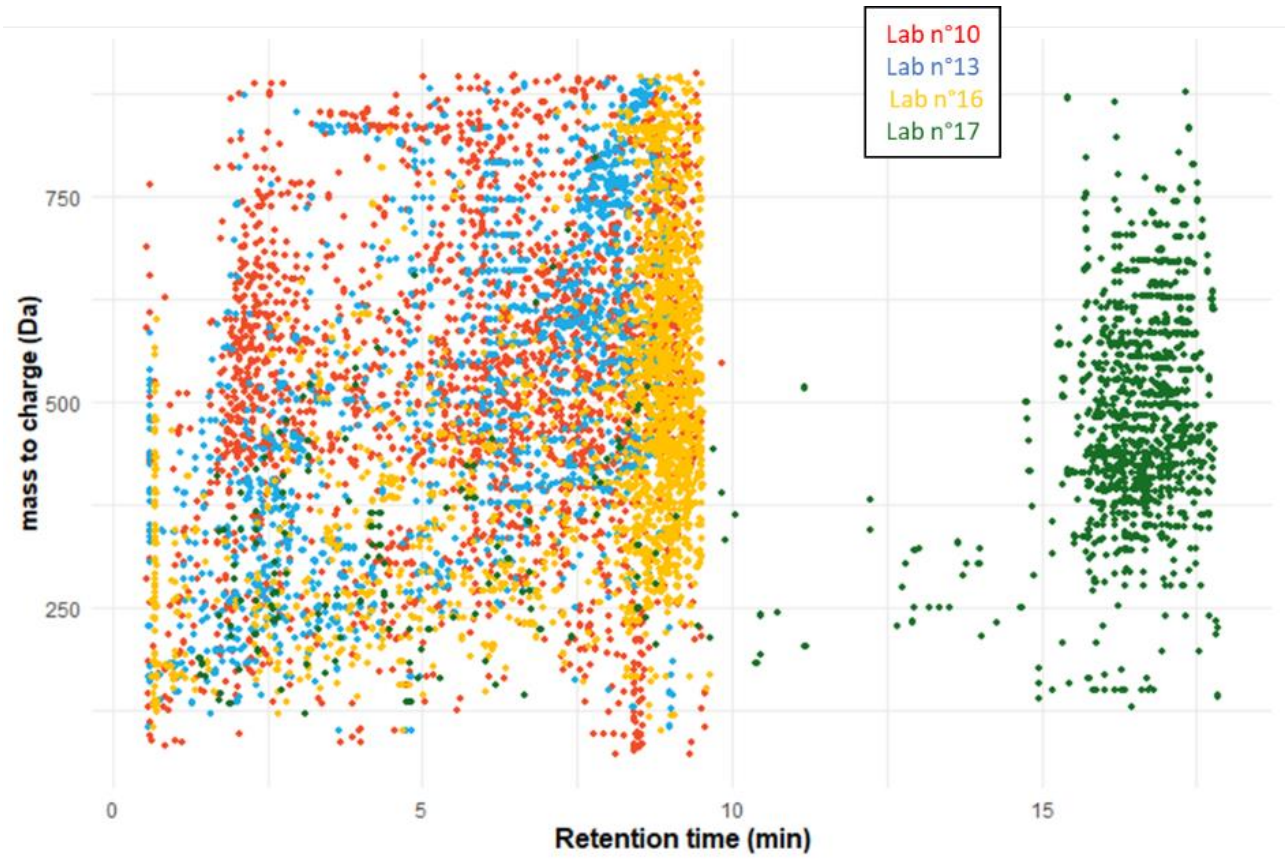


Figure 12: M/z in function of Rt for features of a PS exposed 2 days in a river water (sample n°121) and analysed by 4 laboratories (n°10, 13, 16 and 17)

Figure 12 shows that lab n°17 remains far away compared to the other 3 laboratories, with compounds eluting later (until 18 min). For laboratories n°10, 13 and 16, compounds seem to be well distributed in m/z and during the 10 first minutes of chromatographic separation, even if for laboratory n°16, a lot of compounds elute at the end. Lab n°17 is atypical because almost all features elute at the end. This figure gives an idea on the number of feature: There are less compounds in red and green than in yellow.

Each laboratory has a particularity, which compromises the possibility to compare the data, as explained hereafter.

Lab 10 - lockmass not adjusted

After data treatment, the 6 spiked standards can be studied to evaluate the analysis reliability. Scientist should find the standards m/z with a tolerance of 5ppm (10 can still be acceptable) between measured and theoretical values. **Table 5** shows these results and the associated error.

Table 5: Measured m/z of spiked standards measured in the blank sample for the 4 laboratories

		Lab n°10		Lab n°13		Lab n°16		Lab n°17	
Deuterated compounds	**Theoritical m/z**	**measured m/z**	**Error ppm**	**measured m/z**	**Error ppm**	**measured m/z**	**Error ppm**	**measured m/z**	**Error ppm**
Cafeine 13C3	198,.0977	198.1031	**27.2593**	198.0982	2.5240	198.0969	4.0384	198.0980	1.5144
Diuron D6	239.0619	239.0682	**26.3530**	239.0619	0	239.0619	0	239.0621	0.8366
Cotinine D3	180.1210	180.1260	**27.7591**	180.1209	0.5552	180.1207	1.6655	180.1212	1.1104
Simazine D10	212.1481	212.1536	**25.9253**	212.1490	4.2423	212.1474	3.2996	212.1476	2.3568
Carbamazépine D10	247.1650	247.1709	**23.8707**	247.1656	2.4275	247.1649	0.4046	247.1663	5.2596
Nicotine D4	167.1480	167.1505	**14.9568**	167.1477	1.7948	167.1475	2.9914	167.1494	8.3758

For all laboratories except n°10, standards m/z are found in an acceptable range. Laboratories 13 and 16 seem very precise with an error measured lower than 1ppm. However, laboratory n°10 systematically measures standards m/z with an higher error, probably because of a lack of lockmass correction. The laboratory strategy was probably to correct mass deviation *a posteriori*, but , in the NORMAN dataset, this correction is not possible. Hence, these data will not be used in the following parts. Indeed, the features alignment with other laboratories and identification of features / compounds are not allowed with this decay.

Lab 13 - high sensibility and resolution

QTOF instruments have not the same sensibility and resolution. That is why the workflow needs to be adapted. **Table 6** shows the number of features detected with an identical S/N threshold (200 cps) for the four labs.

Table 6: Number of features detected in each sample with a S/N threshold of 200cps

	Blank (n°001)	**River water 2 days (n°121)**	**River water 4 days (n°141)**	**Drinking water 2 days (n°221)**	**Drinking water 4 days (n°241)**
lab 10	1407	2362	2583	1609	1730
lab 13	**3840**	**11261**	**11691**	**7928**	**7979**
lab 16	1972	4998	4904	3980	3471
lab 17	2366	2973	2900	2344	2328

Laboratory 13 stands out by its number of features higher than the others. A 4 times fold compared to laboratories 10 and 17, and 2 times compared to laboratory 16. Notice that for all laboratories, except n°17, number of features makes sense. Indeed, as expected river has more features than drinking waters, which has more than blank.

Lab 16 - parasite signal

Laboratory 16 shows an atypical signal in his Total Ion Chromatogram (TIC). Indeed, in TIC of river water PS exposed 4 days, the only visible peak is at 8.63 min with a m/z of 563.7015 Da (**Annexe 8**). It is due to the high intensity of this signal, $5x10^{17}$ cps. This intensity is too high to be caused by a molecule in water sample. It is due to the acquisition step, because it is present only in the scan n°1905. Fortunately, it does not disturb the data processing and at the end of the workflow, this feature is eliminated. When MZmine build the chromatogram at the end of peak picking, it does not count signals represented by less than 5 consecutive scans.

Lab 17 - chromatographic method not adequate

Laboratory 17 looks more challenging to compare with other laboratories. The chromatographic separation is different, during over 20 minutes contrary to the 10 minutes set in the ILS (according to a predefined chromatographic method). It is explained by the size of the column which is 150mm length with a flow rate of 0.3mL/min, in opposition of a column of 100mm crossed by solvent injected at 0.4mL/min. In **Annexe 2**, chromatographic parameters are detailed.

D. Harmonization with RTI

3 methods for retention time indexation were tested and evaluated : Volume of Organique Solvant method (VOS), Spiked standard's mean method (SSM) and Kovàts equation of RTI (KRT). Evalution is based on Venn Diagrams which show the alignment efficiency with the new Rt. Venn diagram are compiled in **Figure 13**.

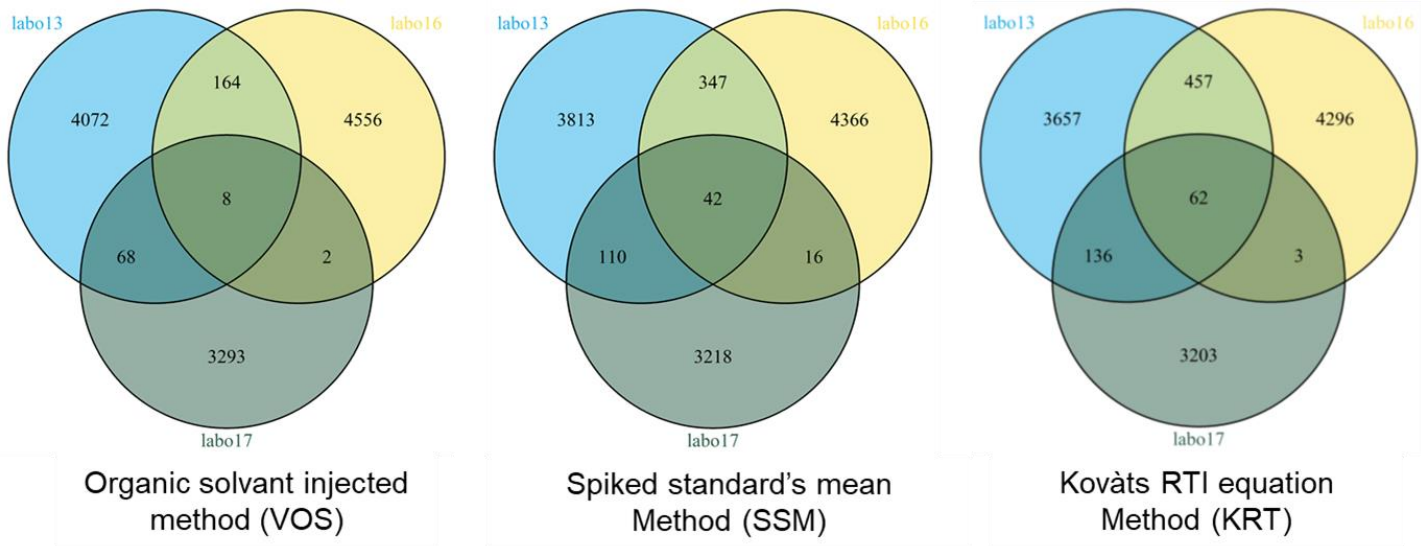


Figure 13: Venn Diagrams of features in PS exposed 4 days in river water (sample n°141) for laboratories 13, 16 and 17, after 3 Rt indexation methods

Considering that we should obtain the higher number of features in common between laboratories as possible, KRT method is more efficient than VOS and SSM. Moreover, we studied the repartition of Rt and m/z for the common features and for the 3 methods, informations are gathered in the **Annexe 9**.

It appears that for VOS and SSM, common features are smaller molecules eluting with short Rt. In contrary, the third method (KRT) covers molecules with a wider range of Rt and m/z.

This test tells also that features from laboratories 13 and 16 match relatively better, with 10-14% in common. A new feature list could be considered merging both and used for further data treatments.

Another test was done with KRT method applied on the 500 most intense features. **Figure 14** illustrates the results.

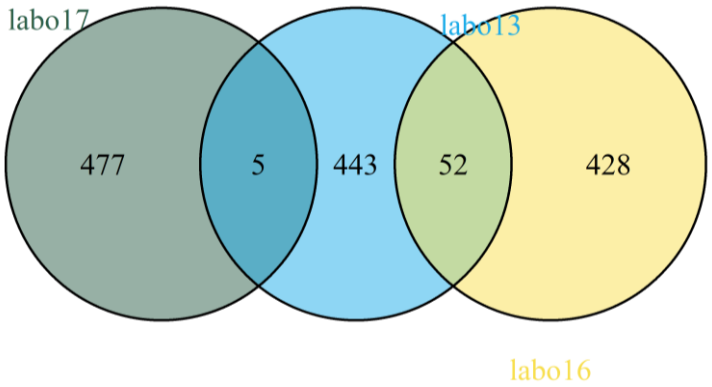


Figure 14: Venn Diagram of features after KRT indexation method on the 500 most intense features for PS exposed 4 days in river water (sample n°141)

Feature concordance between the 2 laboratories is not better when considering intense signals (only 10% of overmatch). HRMS are very sensitive instrument and high signals can comes from contaminations specific to each laboratory (bottles, solvent, micro-pipettes, etc.). Moreover, micropollutants are present at trace concentrations in waters, then, it is important to look after all signals, also the less intense ones.

A last test consisted of a comparison of two RTI indexation methodsKRT and SSM, using either spiked standards or RTImix's standards. These new results are illustrated in Figure 15. Even if there are more compounds in the RTImix, the SSM method is more accurate with the 6 spiked standards in all samples than with the 12 in the RTImix. In contrary, KRT indexation method is more efficient when using the 12 standards in the RTI mix. Both methods have their own advantages and can be complementary. This test shows that SSM methods should be used with spiked standards while KRT should used a mix of standards. Moreover, the 3 RTI indexation methods takes in account a different information. There is no need of standard when using the VOS method. The Kovàts method does not require spiking the sample. The SSM method works without additional sample to inject.

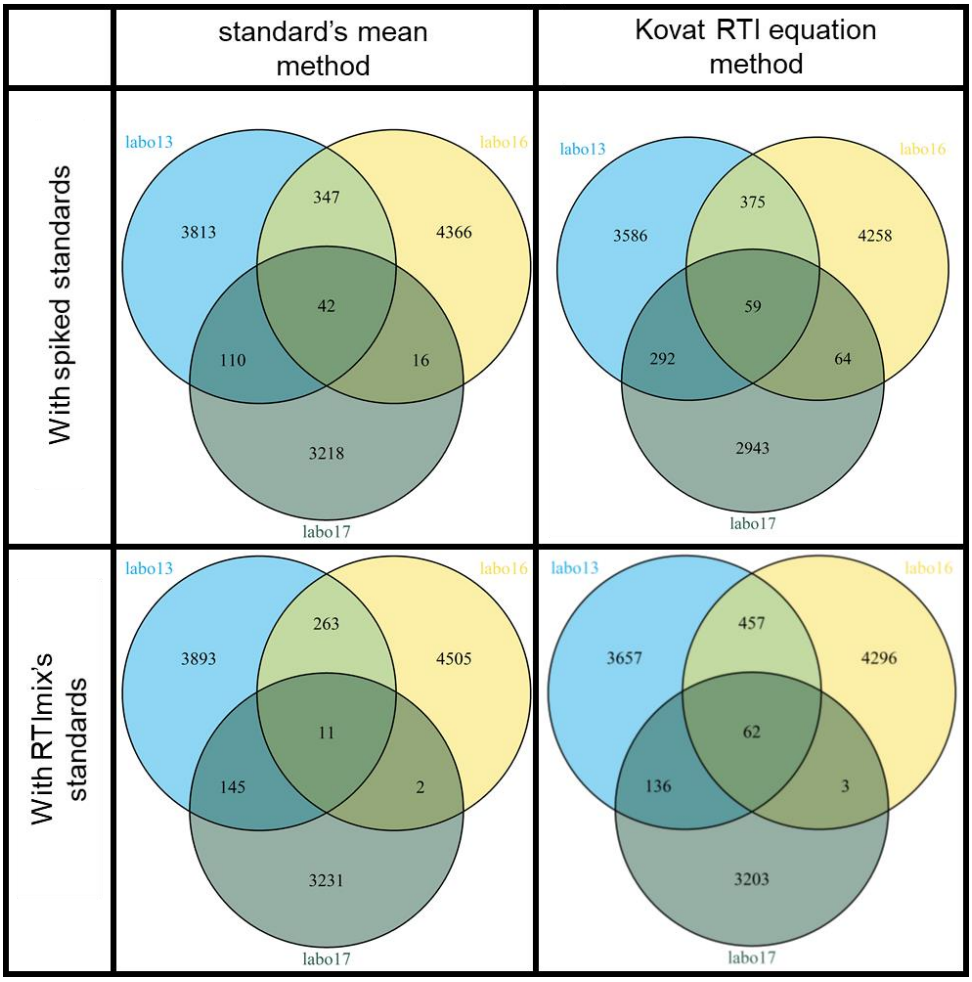
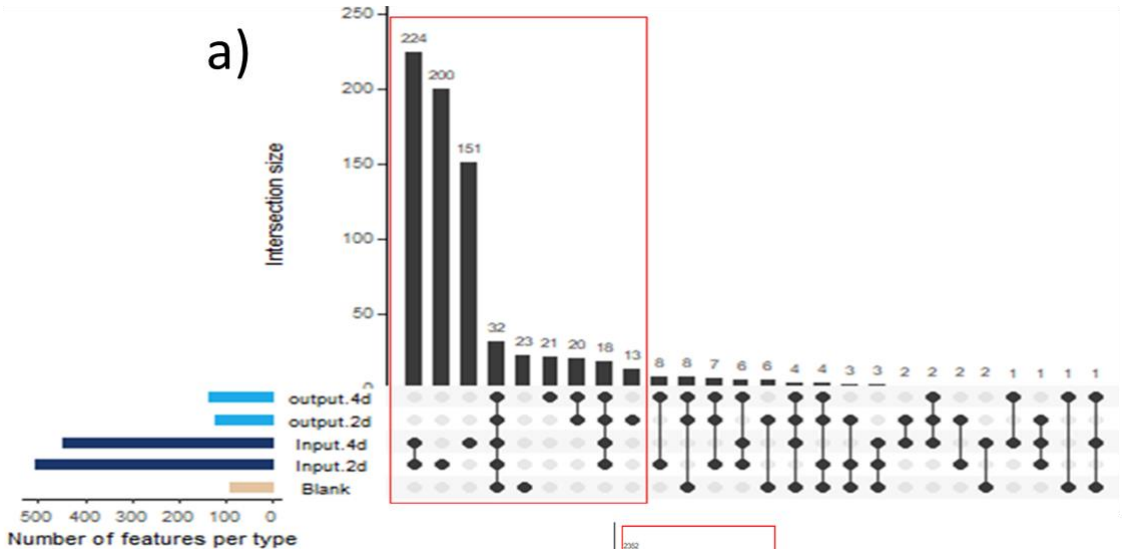


Figure 15: Venn Diagrams of features in PS exposed 4 days in river water (sample n°141), after RT indexation with KRT and SSM methods and using either the 6 standards spiked in samples or the 12 standards in RTImix

Since laboratories 13 and 16 have a good overlapping score and chromatographic and spectrometric parameters chosen by scientists are really close. , a list can be created picking exclusively features in common (see in **Figure 16**). When studying all samples using the features in this new list, there is 9-12% of overlapping, representing 846 features in common.



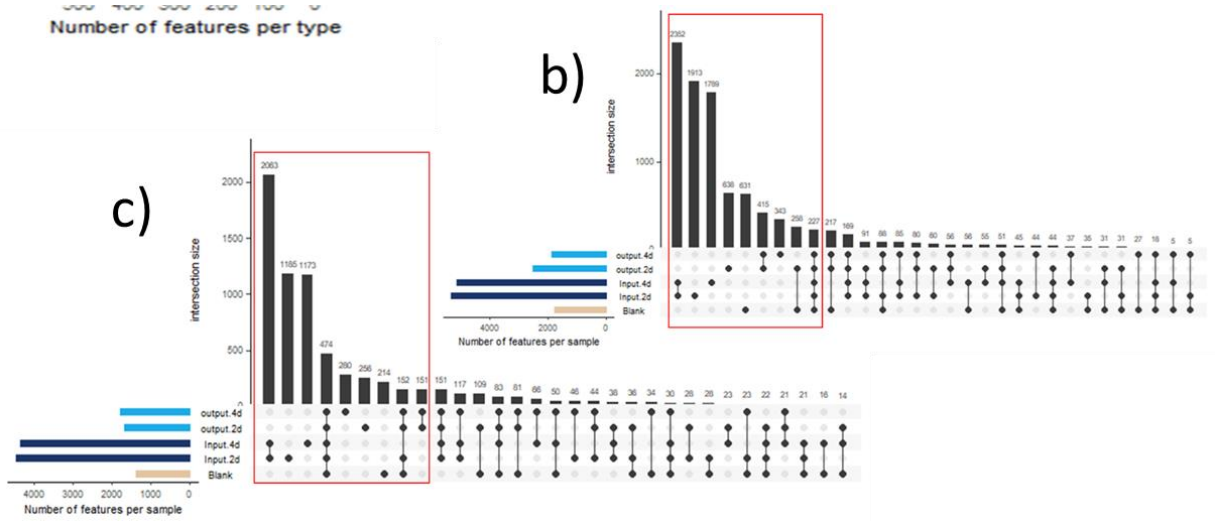


Figure 16: Upset plot of features list from a) combination of laboratories 13 and 16 lists, b) laboratory 16, c) laboratory 13

The majority of features comes from river sample (input of drinking treatment plant). **Figure 16** shows that tendencies are the same when considering separately laboratory 13 and 16. The red square in **Figure 16** highlight the major groups of features and they are the same. Indeed, in all Upset plot of **Figure 16** (a, b and c), there are lots of features common to both inputs, less features specific to each input and even less features common to all five samples.

E. Exposure time influence on Passive Sampling

To learn more about the influence of PS exposure time on the compounds (nature and quantity) sampled inside the PS, we studied m/z and Rt of features. This repartition is show with boxplot, all boxplots will follow the same legend presented in **Figure 17**. While looking to m/z repartition in **Figure 18**, it appears clearly that compounds retained after 2 days have a m/z smaller than compounds retained after 4 days. Even if m/z is not exactly the same as mass, it means that compounds are bigger in PS exposed 4 days than 2 days. In **Figure 18**, means of each group shows that m/z is increasing with time exposure. Indeed, for laboratory n°16, mean is 385 Da for 2 days, 463Da for both exposure times and 529 Da for 4 days.

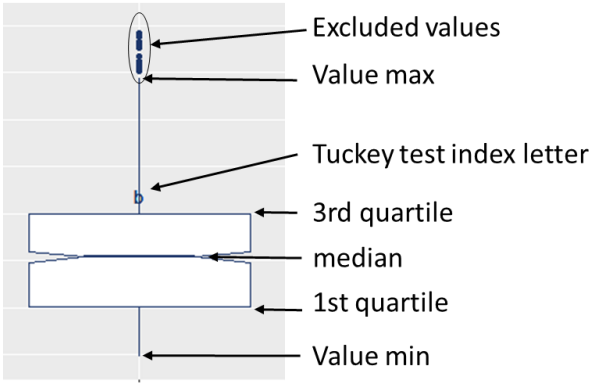
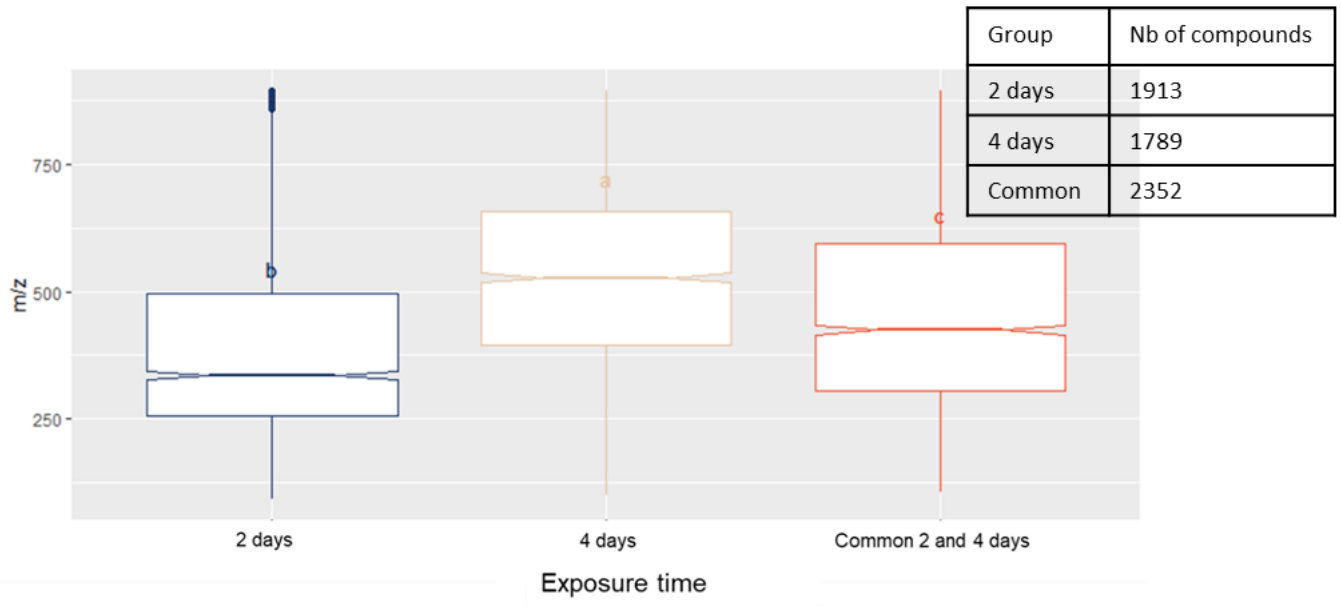


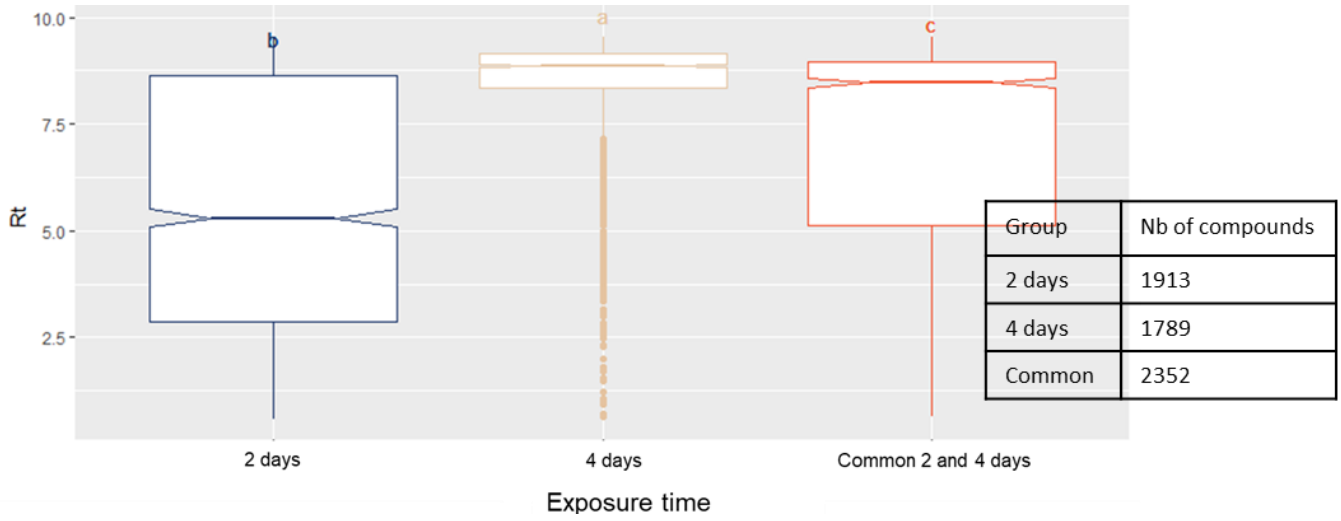
Figure 17: Part IV .E Boxplots legend



Group	Nb of compounds
2 days	1913
4 days	1789
Common	2352

Figure 18: m/z repartition of compounds in river water samples from laboratory n° 16 depending on their exposure time

Considering Rt, there are also differences between 2 and 4 days exposure, as **Figure 19** shows. Compounds accumulated in PS during 4 days have a higher Rt, corresponding to more hydrophobic compounds in our chromatographic conditions. In **Figure 19**, means of each group shows that hydrophobicity is increasing with time exposure ((Rt mean at 5.42 min for 2 days, at 6.97min for both exposure times and at 8.11min for 4 days).



Group	Nb of compounds
2 days	1913
4 days	1789
Common	2352

Figure 19: Rt repartition of compounds in river water from laboratory n°16 depending on exposure time

For drinking waters (output of drinking water treatment plant), the same boxplots were drawn. Compared to river water (input of treatment plant), drinking water should have less compounds inside. **Figure 20** shows the same trends in Rt and m/z evolution for output, between 2 and 4 days of PS exposure. Boxplots of m/z and Rt from all feature lists (lab 13, lab 16 and lab 13&16) gave the same results (gathered in the **Annexe 10, 11, 12, 13** with the number of features for all plots in **Annexe 14**).

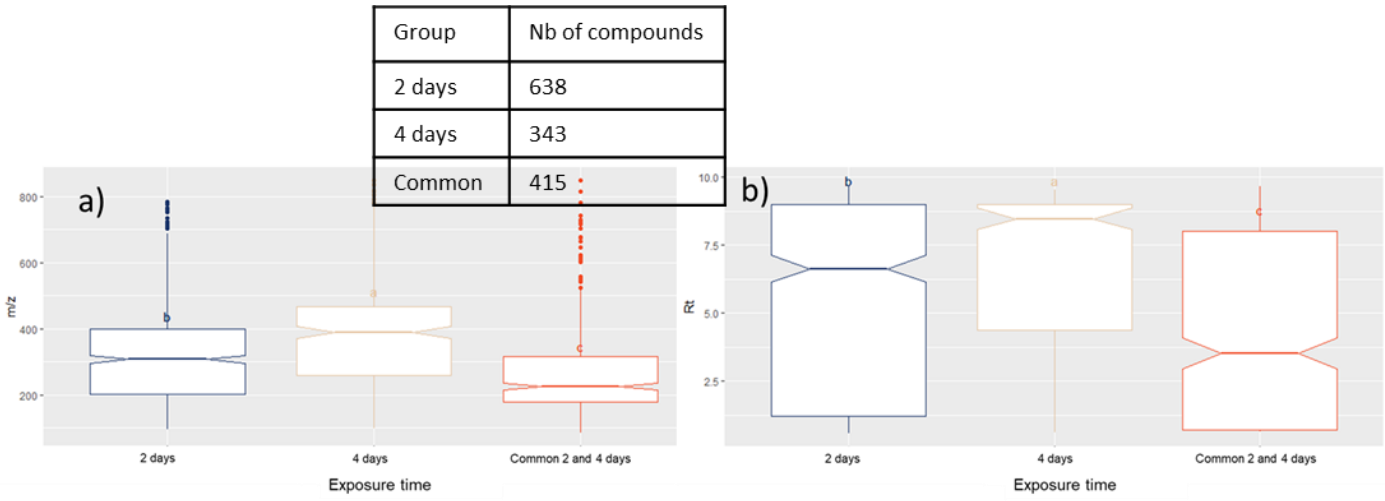


Figure 20: a) m/z and b) Rt repartition of compounds in drinking waters from laboratory n°16 depending on exposure time

In a second approach, only features present at 2 and 4 days exposure were considered. Intensities are studied and three groups are created: Compounds with an increasing intensity, compounds which their intensity decrease and those stagnating. M/z repartitions of these groups are illustrated in **Figure 21**.

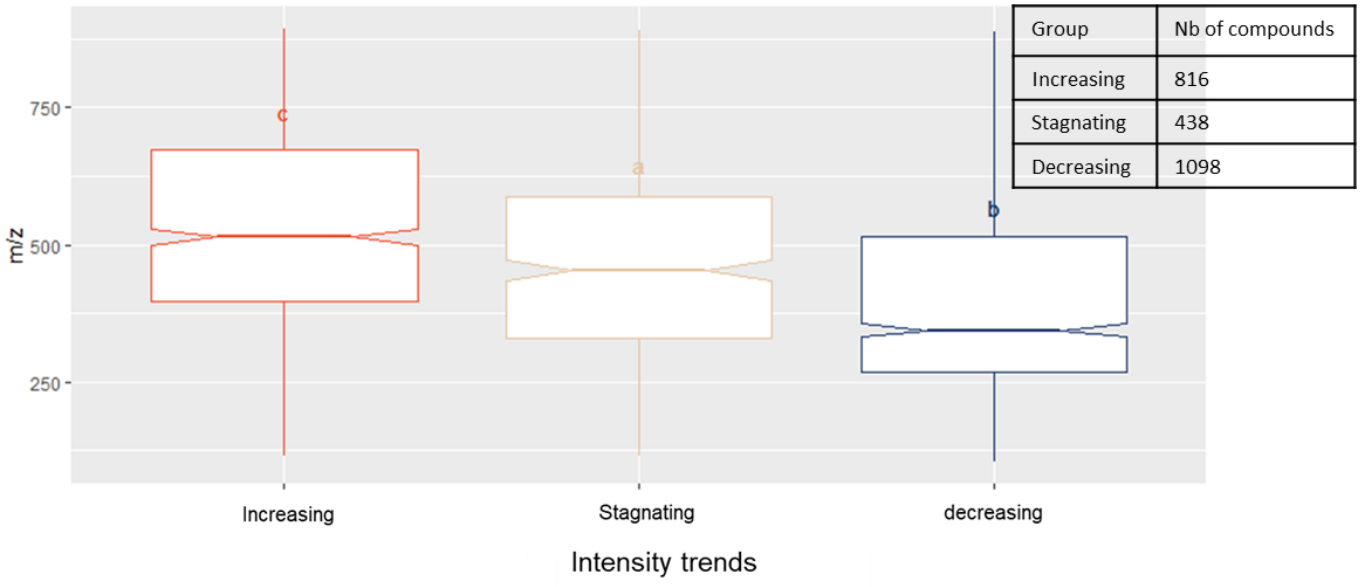
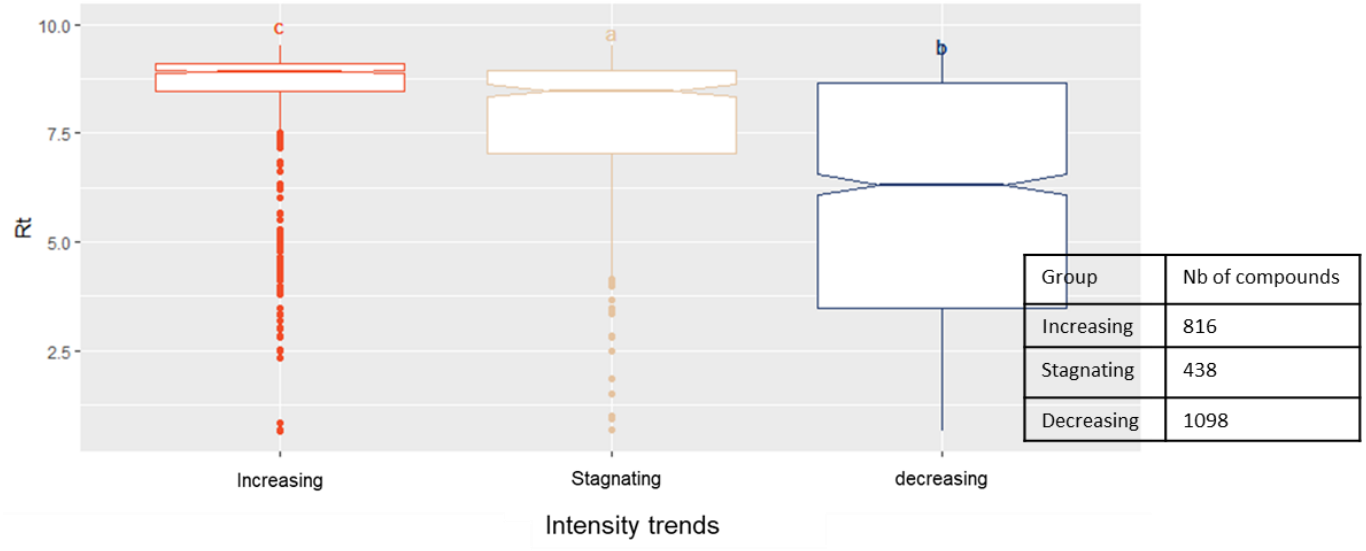


Figure 21: m/z repartition of compounds in river water from laboratory n°16 depending on their intensity trend

The group where tendency is increasing has a higher m/z than the decreasing group. Tukey's HSD test shows differences for the group which stagnate, mean of this group is between the increasing and decreasing groups. M/z means of features is 529Da for the increasing group, 477Da for the stagnating group and 407Da for the decreasing group.



Group	Nb of compounds
Increasing	816
Stagnating	438
Decreasing	1098

Figure 22: Rt repartition of compounds in river water from laboratory n°16 depending on their intensity trend

About Rt, the group with increasing intensities has the highest Rt mean, as shows in **Figure 22**. Mean of Rt is 5.88 min for group with increasing intensities, 7.61 min for stagnating group and 8.07 min for decreasing group.

Group	Nb of compounds
Increasing	77
Stagnating	83
Decreasing	255

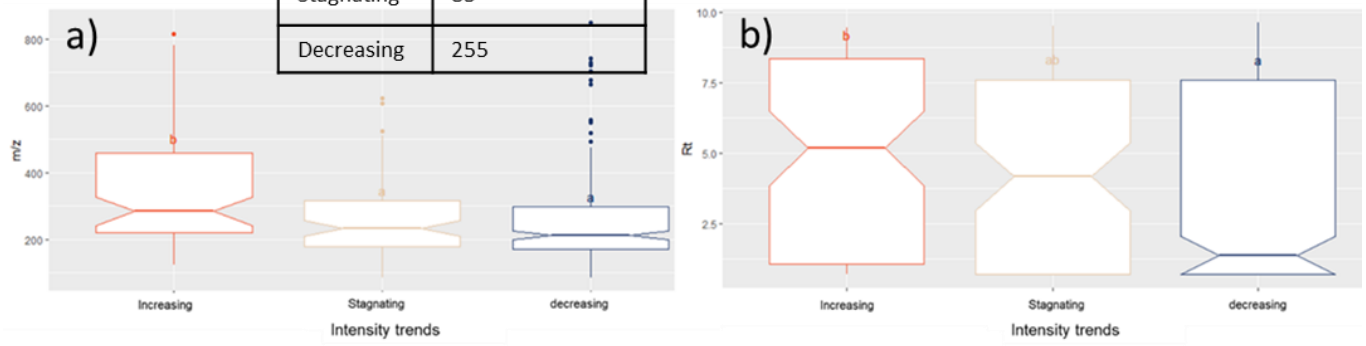


Figure 23: a) m/z and b) Rt repartition of compounds in drinking waters from laboratory n°16 depending on their intensity trend

Finally, drinking water samples were also studied and trend is the same, see **Figure 23**.

F. DWTP treatment evaluation – Heatmap and PCA

Heatmap is under the shape of a fifteen tiles table. Tile's colour go from white to red as the logarithm of intensity sum is increasing. So, colour is influenced by intensity of each compounds but also by the number of compounds belonging to the group. Heatmap is illustrated in **Figure 24** for the laboratory n°16. **Annexe 15** gather heatmaps for laboratory n°13 and list made after combination of lab n°13 and 16. Colour patterns look similar except that in lab n°13 persistent compounds have a slightly higher overall intensity.

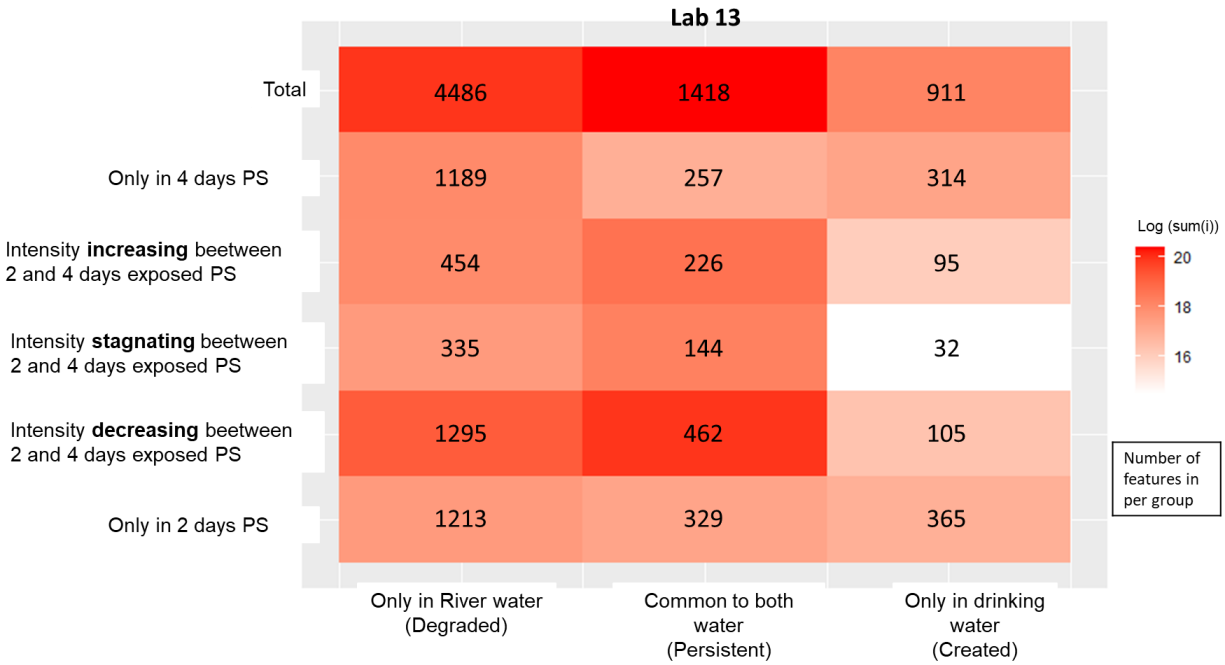


Figure 24: Heatmap representing total intensity of groups of compounds depending on their affinity to PS across time and reaction to DWTP from lab n°13

The row "total" is fading. It means that there are more degradation than contamination going through the process and there is even less creation of chemicals in water during the process. Molecules degraded are well divided regarding sub-categories The DWTP treat as well small and big or hydrophobic and hydrophilic molecules. About chemicals created in the DWTP, is it mostly compounds found in 2 days exposure time PS or with an intensity decreasing between 2 and 4 days exposed PS. So, molecules created by the treatment are mostly small and hydrophilic compounds.

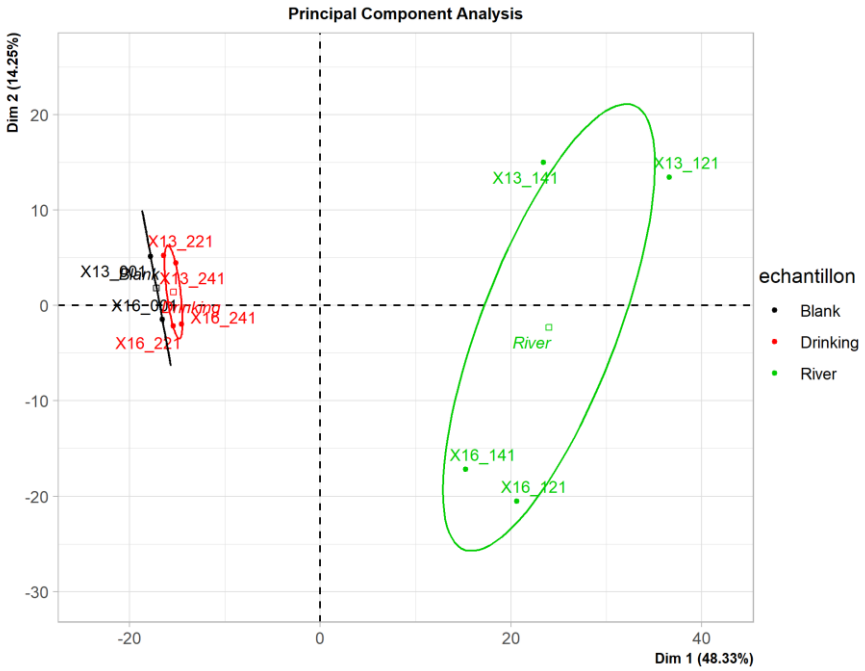


Figure 25: Principal Components Analysis for samples from laboratory n°13 and n°16

V. Discussion

A. Open sources software

The ratio of features extracted by both software in **Table 3** are sound. Moreover, percentage of overlapping between W4M and MZmine correspond to the percentage found in Hohrenk L et al [13]. Indeed, there is between 10% and 20% of features in common for samples treated with both workflow, according to **Figure 11** and **Annexe 7.** Moreover, it is hard to conclude on the best alignment. MZmine may find less features in common because alignment algorithm is less efficient, but in reality, it could be because there is no more features in common. Perhaps, features visible with MZmine but not with W4M are features specifics to one sample. A hypothesis is that all study could have been done with W4M or MZmine as well and it will end up with the same results. To be sure, this study should have been done in parallel with both tools but it is very time consuming. That is why, tool should be chosen at the beginning.

It should be noticed that comparison gets difficult because MZmine enables an easy isotopes and adducts removal as well as various filters like duplicates filter. W4M has less optional steps. For comparison, same workflow was applied without these steps for both softwares. But customisable parameters are not the same, so it is impossible to create exactly the same workflow on both tools. For example, W4M uses signal to noise for peak detection while MZmine uses a threshold that the user has to define. In fact, this experiment does not test the softwares efficiency but only the workflow applied with them. Workflow can change depending on the objective of a given study and the specific question the user is trying to answer using a given set of HRMS data. One software can be better on a special kind of data while the other software will be more efficient for data from another analysis.

For next parts MZmine workflow is chosen and processed with adducts, isotopes and duplicates removal. Objectively, this study could have been realised using other open sources softwares or packages. One purpose of this study was also to explore the different possibilities for data treatment without using vendor software. Indeed, in this context it is not possible to use it because data where given in a converted format. Open sources software allow a self-development by their users. It also enables communication and exchange of data. Moreover, some studies put their data online, give their workflow and therefore enable people to reuse or verify their experimentations.

B. Retention time Indexation

A Retention Time Indexation (RTI) is a way to harmonize Rt between different datasets, particularly if they come from different batches of data acquisition or from different laboratories. It is a way to compare them but as already said, chromatography method must be the same or almost.

An indexation with organic solvent volume is interesting because it does not require any standards and it gives information about hydrophobicity too. Here, this method does not show good results. It is due to a lack of knowledge about chromatography instrument. Indeed, size of column and dead space need to be known. Size of column is sometimes mentioned in article supporting information. NORMAN network gave all information about chromatography column but nothing for the dead volume which is instrument specific and can vary greatly while using the exact same chromatographic method. It is the time taken by solvent to arrive to the detector, going through all the empty tubes and volumes like solvent pump. It is different even between same brand instruments and can vary from few seconds to few minutes. That is why model of RTI presented here is not efficient.

Method with spiked standard has a potential because, all informations were available. Moreover, standards are directly in the sample. So it is close from sample, there is no decay in Rt. Major issue is that there is standards measured in every sample and little variations can appear across samples from same lab. Here, mean across the samples of one laboratory was calculated for all standard.

Last method shows the best results. To settle this method a mix prepared in advance is injected. It is a promising method for laboratory harmonization. Scientist can agree on a mix, prepare it and store it in order to inject it with all sequences. Data are acquired in same time as analysis. There is no need to spike all samples, it is a gain of time. Nevertheless, there is few needs: Standards must be the same in all RTImix and they need to cover a range in m/z and Rt as wide as the analysis acquisition. Spiked standard method has the same needs.

Matching results seem very low but it fits with Hohrenk L et al [13] results. Indeed if they have found only 10 to 20% of feature overlapping using different software, it is easy to imagine that the score will not be higher between different LC-HRMS instrument belonging to different laboratories. Here between lab n°13 and n°16 the overlapping on sample 141 is 10-12%. When comparing features in list gathering all samples, matching score were close, 9-11%. So, 10% appear to be a well representative matching score for both laboratories.

C. Inter laboratories list combination

Naturally, Kovatz RTI equation is chosen to harmonize Rt from lab 13 and 16 and combined the two list. To combine list, there are several ways. It is possible to add all features. Here 90% are laboratory specific, so list would have counted around 16 000 features. On the contrary, in this study, only identic features were added to the list but it reduces a lot the number. After concatenating, the list in common counts 846 features and ratio between samples change. The less feature there are in a sample, the more is lost after concatenation. In drinking water samples, 5-8% are kept while 9-11% are kept from river water samples.

It is a big issue to analyse data. If number of features or compounds is very low, data are less significant and it is getting more difficult to statistically analyse the data. That is why exposure time analysis could be done with this list only concerning input water samples. In one hand, when using list of compounds from output samples, the number of compounds is too low to get significant results. On the other hand, it reduces the list, it is a kind of prioritization and in suspect screening it may be useful. Further studies need to be done to know if this list of compounds is well representative of water analysed. It could emerge a new way of selection. To my knowledge, there is no studies using two different laboratories to build a list and process it.

Seeing Upset diagram in **Figure 16** and boxplot in **Annexe 10**, almost same results are visible with this new list compared to list from lab n°13 or lab n°16 alone. It confirms the potential utility of this combination. It would have been interesting to find more laboratories with similar data to apply the same strategy with more laboratory analysis. It can be a way to analyse data and have conclusion supported by different analysis but working on only one set of data. For the moment, improvements in Rt indexation and harmonization between laboratory is still needed.

D. Analysis harmonization

Many different waters across the world have been analysed, as seen in bibliography. Harmonization of LC-HRMS technics and NTS data treatment is required to use and compare analysis. Environmentally,

it can be useful to monitor and learn about water quality from across the world. Moreover, data set from old analysis should be retreated now. Indeed, knowledge about micropollutants is always evolving and new CEC are found every year. So, Scientist may have missed some CEC in analysis performed few years ago.

First issue encountered was the lockmass correction. In laboratory n°10, mass was not corrected during the acquisition. It is not an issue as long as lockmass data are available to apply a mass correction *a posteriori*. For a workflow suitable to all data, scientist have to choose the same process, all laboratories correct m/z during acquisition or none. Molecule used for lockmass correction can be different, it does not affect the confidence put in correction but it will require more work to correct a posteriori all data from different HRMS instruments. In addition, Lockmass correction is easy to proceed during acquisition or with vendor software but with open source software it is much more complex. m/z is the most important value and tolerance associated is very low (5 to 10ppm). Hence, scientists need to be confident in tools used in mass correction. In this study it is possible to observe the mass repartition across laboratories because they used the same range, 60 – 900Da.

A complete harmonization of workflow is not possible. Indeed, some parameters are specific to each instrument. Noise removing threshold differs as the resolution of the instrument is changing. To get comparable number of features, noise threshold is chosen for each laboratory. If mass resolution is unknown, several test are require to find threshold value, which give an acceptable number of features. Number depend on the water analysed. Scientists expect more features in a water from Wastewater treatment plant than tap water for example. For further study, it could be interesting to find a method to choose a threshold value which is not subjective. For the moment, it is chosen by the analyst without knowing precisely the amount of noise will be removed and how many features will be visible at the end.

The most challenging point in this study is the chromatographic methods and how to harmonize retention time. This study shows that there is big differences between laboratories who use almost the same methods (lab n°13 and 16). When method is completely different (e.g lab n°17), harmonization is not possible with tools presented in this paper. So, in order to compare results between different analysis, chromatography needs to be the same to have robust couple of m/z and Rt. m/z only is not enough to do suspect or NTS with a high level of confidence. Even with m/z and Rt, to identify compounds, scientists need to bring additional information to improve confidence level. This information can be Rt prediction, isotopic pattern, fragments spectrum, etc.

E. Exposure time impact on Passive Sampler

The main idea of results from part IV. E is that the longer PS are exposed, the bigger will be the Rt and m/z of molecules captured. It means that molecule will be larger and more hydrophobic. This is because compounds analysed are small molecules (60-900 Da) so they tend to be only mono-charged. That is why m/z and molecule size is linked. But a compound with lower m/z can be bigger if it has several charges. Secondly, Rt indicates hydrophobicity of molecule because of the type of chromatographic column used. It is also assumed that intensity is in linked with concentration in water. Unfortunately, it is not always the case. During ionisation, a highly concentrated compound can be less ionised, due to his properties. So, less ions of this molecules will go through the spectrometer and their will be less detected. It results in a loss of intensity.

These results show the same trend between compounds present only in 4 days exposed sampler and compounds with an increasing intensity between 2 and days and vice versa with compounds decreasing and present only in PS exposed 2 days.

Theoretically, a POCIS type passive sampler does not lose compounds. Molecules accumulate inside until a point where it becomes saturated. But yet in this study, compounds are lost between 2- and 4-days exposure and intensities are decreasing for some other compounds. Two hypothesis can explain that. First one is that there is actually a competition inside PS and compounds can take place of others. Second hypothesis question the analysis itself. May be, when compounds are accumulating, other compounds at trace level are still here but close to the noise. Data treatment needs to be precise to not remove them with the noise.

Keep in mind, that 2 and 4 days exposure are not the real exposure time. PS were put in a dynamic pumping system to sample a large volume of water quickly. Exposure time is not significant without the flow rate. A river water and a water who has undergone a DWTP process do not have the same flow rate so we should think in volume. Two days exposure time PS represent a volume of 180L while 4 days represent around 320L.
For a better understanding of PS dynamic in environment, further experimentation need to be realised with more exposure time and perhaps in controlled conditions to apply different concentrations of pollutants. It would enable to choose the right time of exposition if an idea of studied contaminant's concentration is available. It will help environmental studies.

F. DWTP treatment evaluation

Heatmap shows total intensity. Nevertheless, water quality is not equal to number and amount of compounds in water. Some compounds can be at trace level but highly toxic. Persistent compounds are mainly gathered in the three sub-categories in middle (increasing, stagnating and decreasing intensities). This category are made with features presents in both exposure time PS. So, it can be compounds resisting well to DWTP or compounds with a lot of affinity with PS which explain why they are found in both water and both exposure time PS. About created compounds, there are a majority of small compounds. It may be because they come from fragmentation of degraded contaminants.

The major result of PCA plot is that drinking water is close to the blank. It is comforting because blank should be very poor in chemicals. It is just an extraction of PS which were not exposed to any water. But the PCA analysis has many variables co variating. As river water is far from the other samples, it makes the other samples looks close. With PCA and heatmap, it is not possible to conclude on water quality because no chemical had been identified neither quantify but both Figure tell that DWTP decrease the overall number and intensity of compounds inside river water.

Conclusion

This study is about data treatment in Non Target Screening of LC-HRMS analysis. Samples analysed are POCIS like Passive Samplers exposed 2 and 4 days to surface water and drinking water. Data set comes from an inter-laboratory trial. It means that same samples were analysed by 21 laboratories belonging to NORMAN network. All of them should have done the same methodology for LC-HRMS analysis. To process these data, open sources software must be used. But yet, it has been proved that results are different when processing data with different software. Two data treatment tools were tested: MZmine and Workflow4Metabolomics. As input parameters are different between the two software, it is impossible to do an objective comparison. Even if there is only 9-20% of similarity between MZmine and W4M, both tools could have been chosen for data treatment of this study.

Once workflow were developed and data treated, first task was to observe particularity of each laboratories. Four were chosen and each one have his own issue, even with a common pre-defined method prescribed by NORMAN. Two up to four needed to be excluded because comparison was impossible due to a different data acquisition (lab n°10) or chromatographic method (lab n°17). Then, Retention Time Indexation methodologies were developed and compared, in order to find the best way to harmonize Rt between labs and make possible a combination of both list (from lab n°13 and n°16). RTI model were based on three different informations: Volume of organic solvent injected, spiked standards and RTImix of 16 standards. RTI equation developed by Kovàts in 1958 still appears to be the best, with 10-14% of overlapping between lab N°13 and n°16. A new list of compounds were created with the common features after harmonization with RTI. This list gave similar results than list from laboratories n°13 and n°16 independently.

The ultimate goal of this study was to understand the impact of exposure time on PS. Compounds were grouped in several lists to observe their m/z and Rt repartition. Groups were: compounds only present in 2 days exposed PS, in 4 days exposed PS, compounds with increasing intensity between 2 and 4 days, intensity stagnating between two exposure time and intensity decreasing from 2 days to 4 days. Results are clear, m/z and Rt are higher for compounds presents only in 4 days exposure time PS than 2 days exposed PS. Same conclusion between compounds with intensity increasing compared to decreasing. It means that longer the PS is exposed, bigger are the molecules trapped and so they are more hydrophobic. It asks the questions about competitively inside the passive sampler. There should be no competition but results show that perhaps some molecules are taking smaller and more hydrophilic molecule's places. About exposure time impact on PS, same results were obtained with river water and drinking water. Hence, exposure time brings bias regarding molecules observed after in HRMS.

As only two exposure times are tested, conclusion are limited. It would be interesting to do experimentations with POCIS like PS in controlled conditions, making vary more exposure times. Environmental studies will take benefit of a better knowledge on passive water sampling. Moreover, scientists need to continue their efforts to find a way to harmonize Retention time. Because for the moment, tools available are not very efficient. Using data from several laboratories, analysis could open new doors for water monitoring worldwide.

Personal conclusion

First of all, this experience shows me the research field, which I didn't know coming from an Engineering school. I learn the different steps we should pass through to achieve a study. I also have the luck to be inside a laboratory doing public researches. It presents me the overall organization of this structure. I find communication is good inside the unit. In addition, I see that it gathers people with many different competencies and we can easily find help on diverse topics, for laboratory manipulations, statistical studies, coding scripts, etc.

I was working only on data treatment. It is a very good training because you need to know and understand how LC-HRMS works since the beginning, which is sampling. Through data treatment, all subtleties of this technology are visible. Nevertheless, it become frustrating to analyse data that I have not produced. I am looking forward to do complete HRMS analysis from the sample preparation until identification, passing by LC-HRMS injection.

Field studied here is attractive. Indeed, there is a lot of different problematics in water and many ways to study it. So, this experience reinforces the idea that I want to work in water sector and I want to try the research sector. This internship is linked with another one to prepare a thesis, which aims to study micropollutants in Rhone sediments using GC-HRMS. Fortunately, I am selected to run this thesis. So, I will continue in the same unit. It allows me to continue what I have done during this experience. I see this experience like a trial for the thesis.

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Annexes

Annexe 1: Table of standards in RTImix

Annexe 2: LC-HRMS parameters

Annexe 3: R Script to select common feature between two lists

Annexe 4: MZmine and W4M workflow parameters

Annexe 5: R scripts to apply the three different RTI on a feature list

Annexe 6: Plot of PCA variables

Annexe 7: Upset plot W4M vs MZmine for a) lab 13 river water b) lab 13 drinking water c) lab 16 river water d) lab 16 drinking water

Annexe 8: TIC lab 16 parasité

Annexe 9: M/z Rt repartition of common features of RTI

Annexe 10: Boxplots of m/z and Rt repartition of compounds presents in river water depending of their exposure time

Annexe 11: Boxplots of m/z and Rt repartition of compounds presents in drinking water depending of their exposure time

Annexe12: Boxplots of m/z and Rt repartition of compounds presents in river water depending of their intensity trend between 2- and 4-days exposure time

Annexe 13: Boxplots of m/z and Rt repartition of compounds presents in drinking water depending of their intensity trend between 2- and 4-days exposure time

Annexe 14 : table of number of feature for all compounds depending on their affinity to PS across time and reaction to DWTP

Annexe 15 : Heatmap representing total intensity of groups of compounds depending on their affinity to PS across time and reaction to DWTP from a) lab n°16 and b) lis of lab n°13 and 16 combined

Annexe 16 : Fiche de compétence

Annexe 1: Table of standards in RTImix

	M/z (Da)	Rt labo 10 (in min)	Rt labo 13 (in min)	Rt labo 16 (in min)	Rt labo 17 (in min)	Oder of elution
Histamine	112.0869	0.51	0.56	0.6	1	1
Guanylurea	103.0614	0.55	0.6	0.63	1.05	2
Chlormequat	122.0731		0.62	0.67	1.12	3
Vancomycin	724.7224	0.83	0.77	0.96	1.57	4
Methamidophos	142.0086	0.9	0.8	1	1.58	5
Cefoperazone	646.1497	2.38	1.64	3.61	4.7	6
Dichlorvos	220.9532	3.32	2.69	5.91	8.12	7
Rifaximin	786.3596	6.14	3.76	8.48	14.22	8
Spinosad A	732.4681	4.25	4.55	8.55	14.87	9
Emamectin B1a	886.5311		5.35	8.77	15.38	10
Avermectin B1a	890.526	7.89	6.35	8.8	16.4	11
Ivermectin B1a	892.5417	9.07	7.29	8.80E+00	17.32	12

Annexe 2: LC-HRMS parameters of NORMAN preconized pre-defined methods for the four Waters Company labs

ID	Name	Model	column	column dimension	injection volume	column t°	composition mobile phase	mobile phase gradient programme	flow rate	scan range	resolving power & referenced m/z	Ionization
10	Irstea	UPLC H-Class / Xevo-G2S-QTOF	Waters HSS T3	100; 2.1; 1.8	5	50	A=5mMaqueous ammonium formate + 0,1% formic acid; B=acetonitrile + 0,1% formic acid	13%B(0);13%B(0,1);13%-50%B over 1,9min; 50%-5%B over 5,2min; 5%B(8,2); 5%-87%B over 0,1min; 87%B(10)	0.4	60-900	25,000 @m/z 556,2771	ESI (P)
13	Örebro University	G2-XS-qToF	Acquity UPLC BEH C18	100; 2.1; 1.7	10; 5 (blank)	30	A=water+5mM ammonium formate B=ACN + 0.1% formic acid	13%B(0); 13%B-87%A over 0.33min; 50%B-50%A over 2.32min; 95%B-5%A over 7.17min; 95%B-5%A over 8.17: 13%B-87%A over 8.33min; 87B-13%A over 10min	0.4	60-900		ESI (P)
16	SLU	G2-S QTOF	Acquity HSS-T3 C18	100; 2.1; 1.8	5	40	A=5mM ammonium formate (in water, pH=3); B=acetonitrile+0.1% formic acid	13%B for 0.3min(0-0.3); 13-50%B over 6.4min(0.3-6.7);50%-95%B in 0.5min(6.7-7.2);95% B for 1min (7.2-8.2);95%-13%B in 0.1 min(8.2-8.3); 13%B for 1.7min (8.3-10)	0.4	60-900	35,000 @m/z 300; 32,000 @m/z 550	ESI (P)
17	University Jaume I	QTOF Xevo G2	Cortecs C18	150; 2.1; 2.7	5	40	A=water+5mM (at pH3); Acetonitril+0.1%HCOOH	13 %B for 0.67 min(0-0.67);13 %B-50% over 12.66 min (0.67-13.33);50%B-95% over 1 min(13.33-14.33);95%B for 2 min (14.33-16.33); 95%B-13% over 0.33 min (16.33-16.67); 13%B for 3.33 min (16.67-20)	0.3	60-900	20,000 @m/z 556	ESI (P)

Annexe 3: R Script to select common feature between two lists

Préparation des fichiers sous la forme : nom du feature | m/z | Rt | Intensité |

```{r preparation, include=TRUE}
rm(list=ls(all=TRUE))
library(dplyr)

dossier <- "chemin du dossier de travil"
setwd(dossier)
noms <- c("name", "mz", "RTI", "lab13", "lab16", "lab17")
serie_2 <- read.csv2("1ere liste a aligner.csv",dec = ",", header = TRUE)
serie_2 <- serie_2[,c(5,1,4,3)]
colnames(serie_2) <- noms[1:(4)]
serie_1 <- read.csv2("2eme liste à aligner.csv",dec = ",", header = TRUE)
serie_1 <- serie_1[,c(5,1,4,3)]
colnames(serie_1) <- noms[c(1:3,5)]
```

Création de la fonction cherchant les features en commun d'une liste à l'autre

```{r fonction}

comp_feature <- function(mz2_RT2, mz1, RT1, Dmz, DRT){
  if((abs(mz2_RT2[2]-mz1)<Dmz)&(abs(mz2_RT2[3]-RT1)<(DRT))){
    return(mz2_RT2)
  }else{
    return(c(NA, NA, NA))
  }
}
```

Préparation des liste résultat. La liste "result_final" combine les liste 1 et 2, les m/z et Rt gardé sont ceux de la liste 2 en cas de feature en commun. La liste "result_commun" rassemble les features en communs avec tous leurs détails.

```{r pressure, echo=FALSE}
result_final <- cbind(serie_2, matrix(NA, nrow = nrow(serie_2), ncol = 1))
colnames(result_final) <- noms[1:5]
nbcommun = 0 #compte le nombre de feature total en commun
commun = matrix(NA, ncol= 40, nrow = 0)
colnames(commun) = c(noms[1:4], rep(noms[1:3], times = 12))
commun <- as.data.frame(commun)
```

Application de la fonction sur les listes. La fonction va passer en revu la liste 1 et chercher les feature en commun a la liste 2.

```{r Boucle, echo=FALSE}
for (i in 1:length(serie_1$mz)) {
  print(i)
  # Modifier dans la ligne suivante, les valeurs de Dmz et DRT de comparaison souhaitées
  result <- apply(serie_2[,1:3], 1, comp_feature, mz1=serie_1[i,2], RT1=serie_1[i,3], Dmz=0.002, DRT=0.2)
  result2 <- t(result[,which(is.na(result[1,]) == FALSE)])
  result2 <- as.data.frame(result2)
  colnames(result2) <-noms[c(2,3,4)]
  correspondance <- serie_1[i,]
  if (nrow(result2)>0){
    nbcommun <- nbcommun + nrow(result2)
    addligne <- as.data.frame(correspondance)
    for (j in 1:nrow(result2)){
    result_final[result2[j,1], ncol(result_final)] <- correspondance[,4]
    addligne <- cbind(addligne,result2[j,1:3])

    }
    addligne <- cbind(addligne, t(rep(NA, times = 40-ncol(addligne))))
    colnames(addligne) <- colnames(commun)
    commun <- rbind(commun, addligne)
  }else{
    addligne <-  as.data.frame(c(correspondance[1,1:3], c(rep(NA, times = (ncol(result_final)-4)), correspondance[1,4])))
    colnames(addligne) <- noms[1:5]
    result_final <- rbind(result_final, addligne)}
   }
```

Préparation des documents à exporter

```{r résultat, echo=FALSE}

new <- as.data.frame(matrix(NA, ncol = ncol(commun), nrow = 1))
new[,2] <- nbcommun
colnames(new)<- colnames(commun)
commun <- rbind(commun, new)
result_final[is.na(result_final)] <- 0
commun[is.na(commun)] <- 0
write.table(result_final, file="result_final.csv", col.names=T, row.names=F, sep=";", dec = ",")
write.table(commun, file="result_commun.csv", col.names=T, row.names=F, sep=";", dec = "," )
```

Annexe 4: MZmine and W4M workflow parameters

for lab n°13	W4M	Mzmine
Peak picking	**Centwave algorithm** S/N = 10 Peak width = 3 – 120s Noise filter = 3000	**Centroïde** Noise threshold = 3000 Smoothing with 7 points Min of scans detected = 5
Alignement	**Join aligner** m/z tol = 10 ppm Rt tol = 2 min Min n° of sampes = 1	**RANSAC aligner** m/z tol = 10 ppm Rt tol = 2 min Min number of sample = 1

Annexe 5: R scripts to apply the three different RTI on a feature list, a) KRT , b) SSM c) VOS

a) KRT

````
library and filds required
```{r setup, include=TRUE}

rm(list=ls(all=TRUE))
library(dplyr)
library(ggplot2)
library(tidyverse)
setwd("chemin du dossier de travail")

featurelist <- read.csv2("la feature liste (ici contient 5 echantillons) .csv",header = TRUE)
lab <- 4 #in which column is written the standards Rt for this labo
liste <- featurelist[, c(2,3,5:9)]
liste <- arrange(liste, liste[,2])
liste <- cbind(liste, c(1:(nrow(liste))))
rm(featurelist)

#call the table of Rt of standards in RTImix
setwd("C:/Users/tom.ducrocq/Documents/Traitement données/comparaisonLabo")
RTImix <- read.csv2("RTImix.csv", dec = ",",header = TRUE)
RTImix <- RTImix[,c(1,2,(2+lab),ncol(RTImix))]
```

Function calculating the Rt in volume of organic solvent injected
```{r function}
  RTI <- function(resultat ,N, RTj, RTn, RTn1) {Resultat <- (100*( N  + (RTj - RTn)/(RTn1 - RTn)))
  return( Resultat)}
```

Function calculating the Rt in volume of organic solvent injected
```{r loop}
simpleliste <- liste
  simpleliste <- cbind(arrange(simpleliste, simpleliste[,2]),NA,c(1:nrow(simpleliste)))
  RTImix[nrow(RTImix),3] <- simpleliste[nrow(simpleliste), 2]


  p = 1

  for (k in 1:nrow(simpleliste)){
    RTj <- simpleliste[k,2]
    if (RTj >= RTImix[p+1,3]){
      Resultat <- RTI(N = RTImix[p,ncol(RTImix)], RTj = simpleliste[k,2], RTn = RTImix[p,3], RTn1 = RTImix[p+1,3])
      p <- p+1
      }else{ Resultat <- RTI(N = RTImix[p,ncol(RTImix)], RTj = simpleliste[k,2], RTn = RTImix[p,3], RTn1 = RTImix[p+1,3])
    }
    simpleliste[k,4] <- Resultat}
```

Create a new liste in CSV format
```{r result}

colnames(simpleliste[,c(1,2,4,5)]) <- c("mz", "Rt", "RTI", "place")
nom <- paste("240821_tom_RTI","lab",as.character(lab), ".csv")
write.table(simpleliste, file=nom, col.names=T, row.names=F, sep=";", dec = ",")
```
````

b) SSM

library and filds required

```r
rm(list=ls(all=TRUE))
library(dplyr)
library(ggplot2)
library(tidyverse)

setwd("chemin du dossier de travail")
RT_standards <- read.csv2("doc des Rt de chaque standards (lignes) par laboratoire (colonne).csv",header = TRUE) #retention time of standards
(colum = labo, row = standards )

featurelist <- read.csv2("liste à indexer.csv",header = TRUE)
lab <- 4 #in which column is written the standards Rt for this labo
liste <- featurelist[, c(2,3,5:9)]
liste <- arrange(liste, liste[,2])
liste <- cbind(liste, c(1:(nrow(liste))))
rm(featurelist)
RT_standards <- RT_standards[,c(lab+1, ncol(RT_standards))]
```

Function calculating the Rt in volume of organic solvent injected

```r
RTi <- function(resultat ,RTj, RTn, RTn1, Sn, Sn1) {

  A <- (Sn1-Sn)/(RTn1-RTn)
  B <- Sn -(A*RTn)
  resultat <- ((RTj)*A)+B
      return( resultat)}
```

Function calculating the Rt in volume of organic solvent injected

```r
simpleliste <- liste
simpleliste <- cbind(arrange(simpleliste, simpleliste[,2]),NA,c(1:nrow(simpleliste)))

p = 1
for (k in 1:nrow(simpleliste)){
  if( simpleliste[k,2] >= RT_standards[p+1,1]){
    resultat <- RTi(RTj = simpleliste[k,2], RTn = RT_standards[p+1,1],  RTn1 = RT_standards[p+2,1], Sn = RT_standards[p+1,2],  Sn1 =
RT_standards[p+2,2] )
p <- p+1}else {resultat <- RTi(RTj = simpleliste[k,2], RTn = RT_standards[p,1],  RTn1 = RT_standards[p+1,1], Sn = RT_standards[p,2],  Sn1 =
RT_standards[p+1,2] )}
  simpleliste[k,4] <- resultat}
```

Create a new liste in CSV format

```r
colnames(simpleliste[,c(1,2,4,5)]) <- c("mz", "Rt", "RTI", "place")
nom <- paste("240821_tom_RTI","lab",as.character(lab), ".csv")
write.table(simpleliste, file=nom, col.names=T, row.names=F, sep=";", dec = ",")
```

b) VOS

library and filds required

```r
rm(list=ls(all=TRUE))
library(dplyr)
library(ggplot2)
library(tidyverse)

setwd("chemin du dossier de travail")


featurelist <- read.csv2("liste à indexer.csv",header = TRUE)
lab <- 4 #in which column is written the standards Rt for this labo
liste <- featurelist[, c(2,3,5:9)]
liste <- arrange(liste, liste[,2])
liste <- cbind(liste, c(1:(nrow(liste))))
rm(featurelist)

#call the  coordinates for organic solvent injection plot
table <- read.csv2("coordonnée de la courbe représentatnt V de solvant injecté au cours du temps.csv",header = TRUE)
grad <- table[,((lab-1)*4+1):((lab-1)*4+4)]
colnames(grad) <- c("V orga", "t", "A","B")
```

Function calculating the Rt in volume of organic solvent injected

```r
RTi <- function(resultat ,RTj, RTn, RTn1, Sn, Sn1) {

  A <- (Sn1-Sn)/(RTn1-RTn)
  B <- Sn -(A*RTn)
  resultat <- ((RTj)*A)+B
      return( resultat)}
```

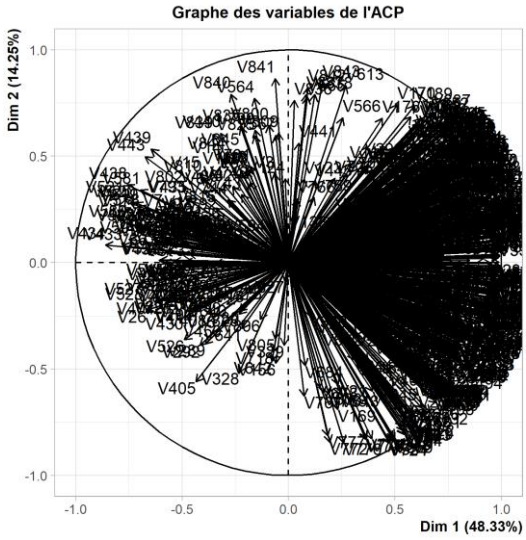
Function calculating the Rt in volume of organic solvent injected

```r
simpleliste <- liste
simpleliste <- cbind(arrange(simpleliste, simpleliste[,2]),NA,c(1:nrow(simpleliste)))
p = 1

for (k in 1:nrow(simpleliste)){

  Rt <- simpleliste[k,2]
  if (Rt > grad$t[p+1]){p <- p+1
    resultat <- grad[p,1]}else{resultat <- RTi(Rt = simpleliste[k,2], A = grad$A[p], B = grad$B[p])}
  simpleliste[k,4] <- resultat}
```

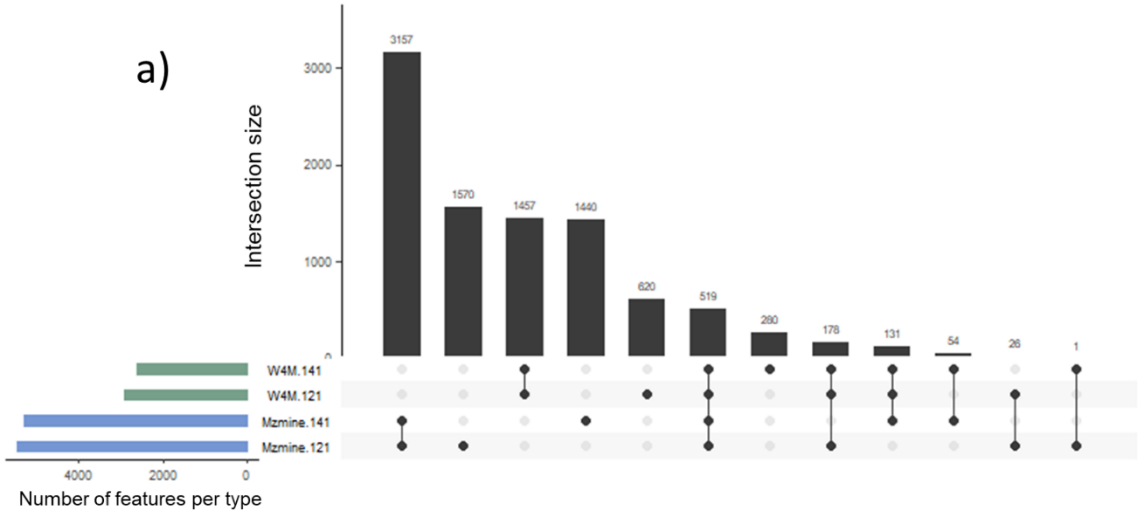
Create a new liste in CSV format

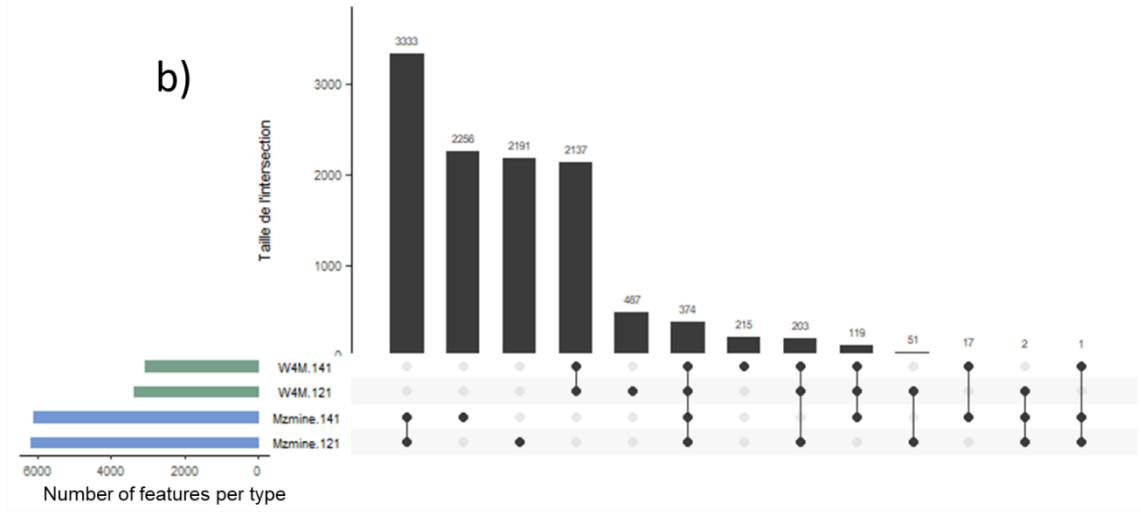
```r
colnames(simpleliste[,c(1,2,4,5)]) <- c("mz", "Rt", "RTI", "place")
nom <- paste("240821_tom_RTI","lab",as.character(lab), ".csv")
write.table(simpleliste, file=nom, col.names=T, row.names=F, sep=";", dec = ",")
```

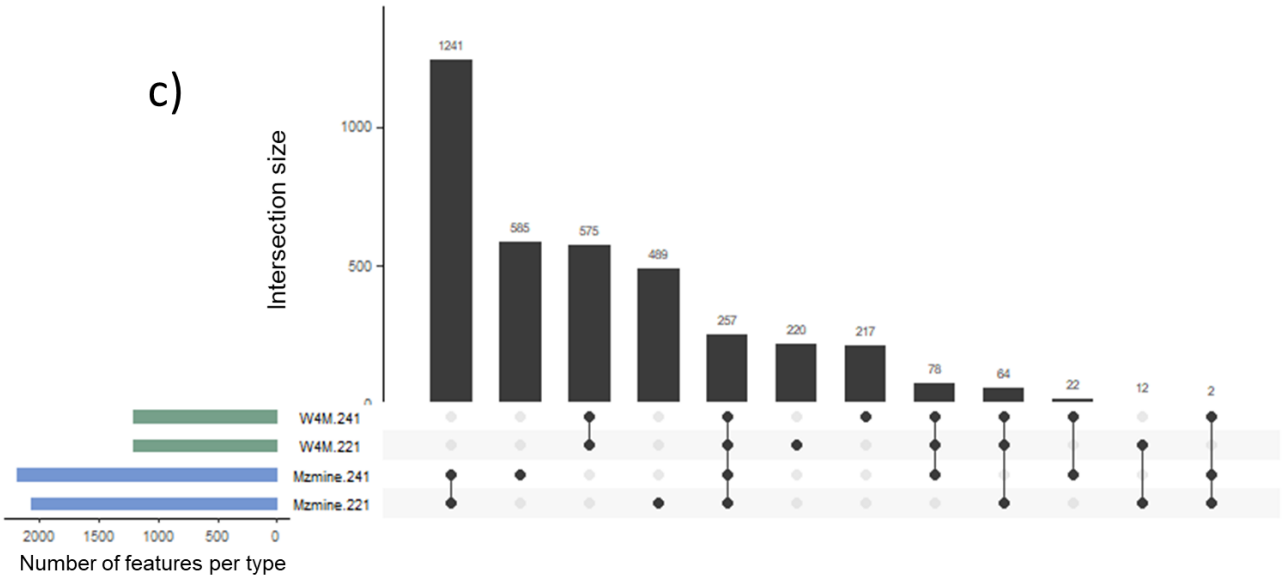
Annexe 6 : Plot of PCA variables

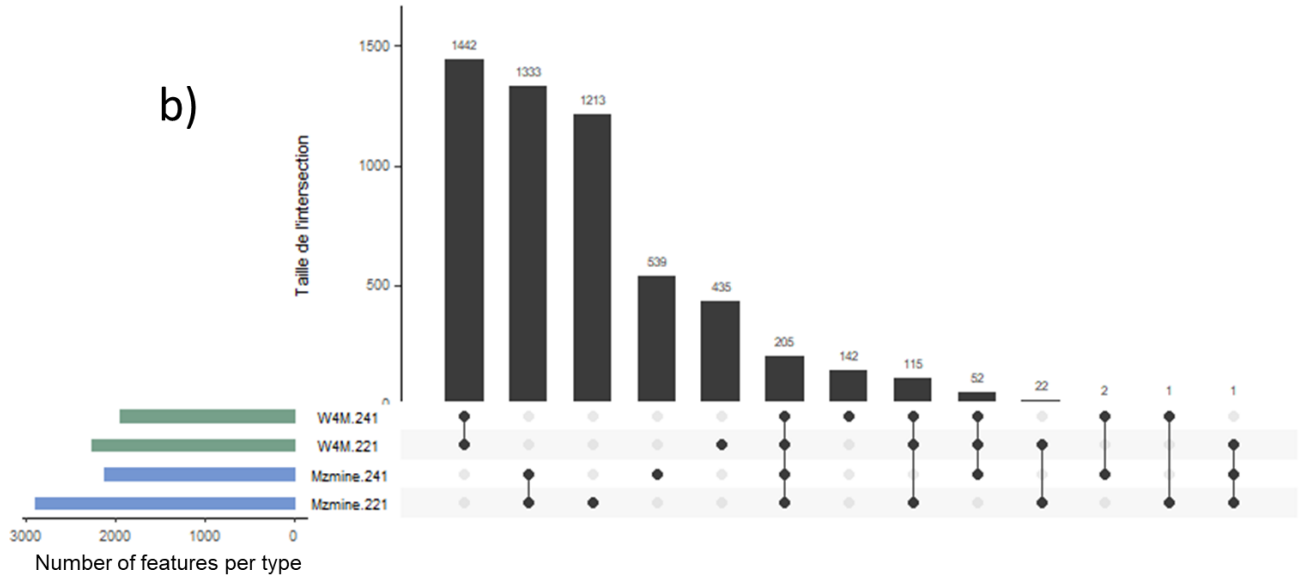


Annexe 7: Upset plot W4M vs MZmine for a) lab 13 river water b) lab 13 drinking water c) lab 16 river water d) lab 16 drinking water

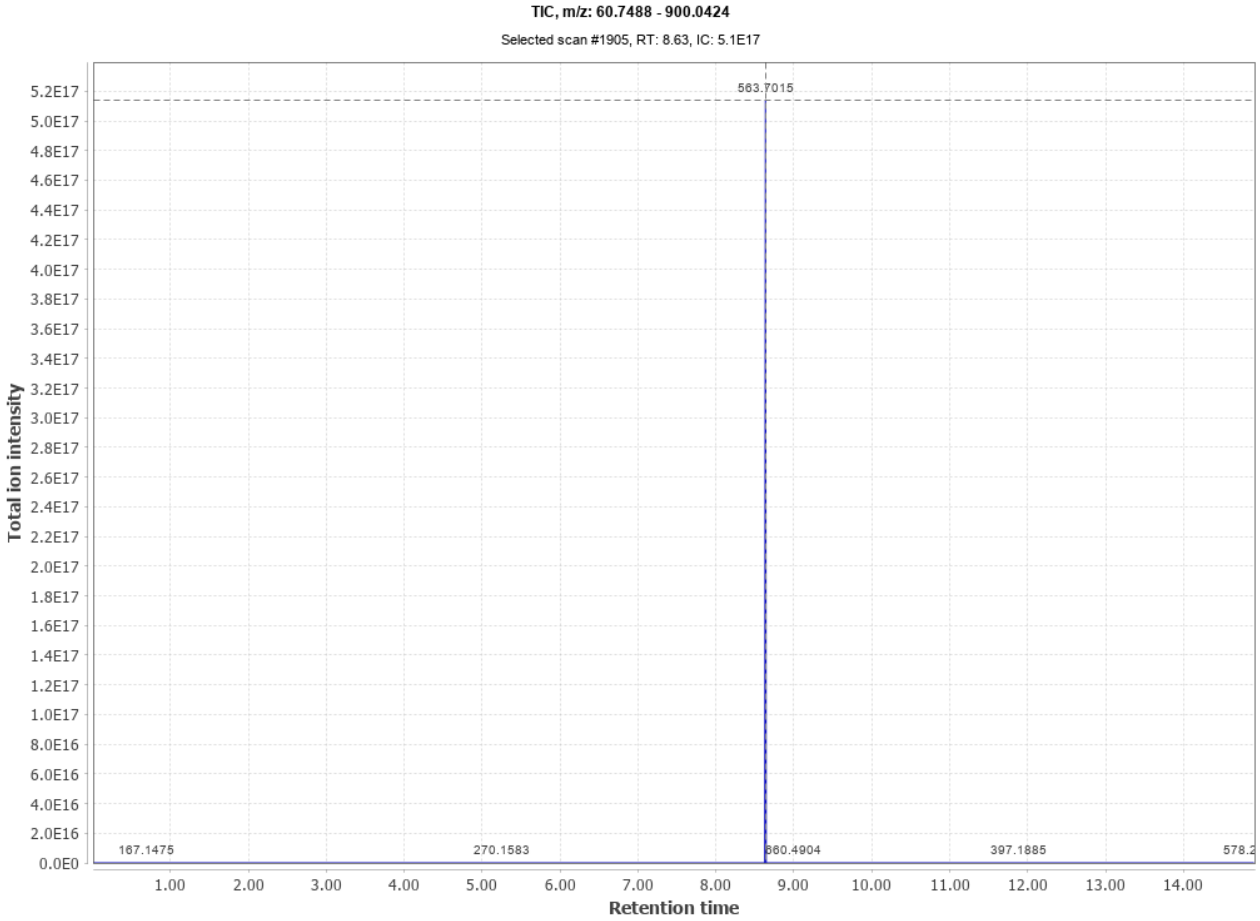





c)
Intersection size
1241
585
575
489
257
220
217
78
64
22
12
2
1000
500
0
W4M.241
W4M.221
Mzmine.241
Mzmine.221
2000
1500
1000
500
0
Number of features per type


b)
Taille de l'intersection
1442
1333
1213
539
435
205
142
115
52
22
2
1
1
1500
1000
500
0
W4M.241
W4M.221
Mzmine.241
Mzmine.221
3000
2000
1000
0
Number of features per type

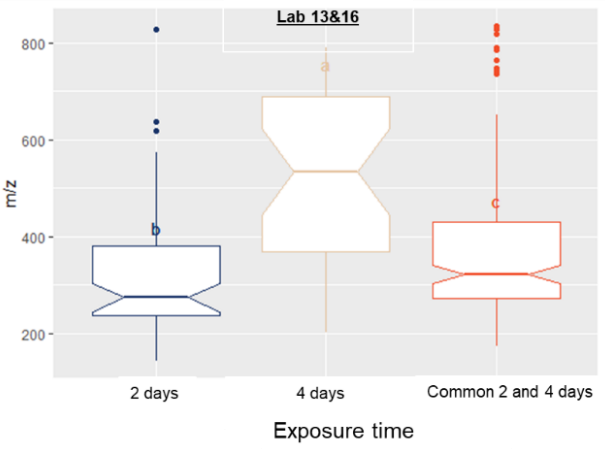
Annexe 8: TIC of samples from river water exposed 2 days analysed by laboratory 16, mass spectrum scan n°1905

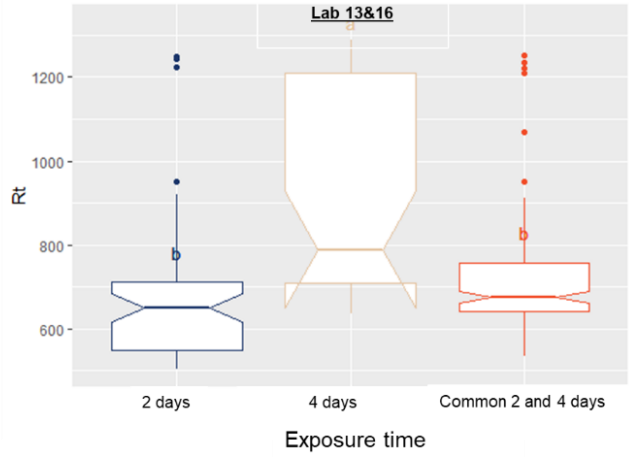


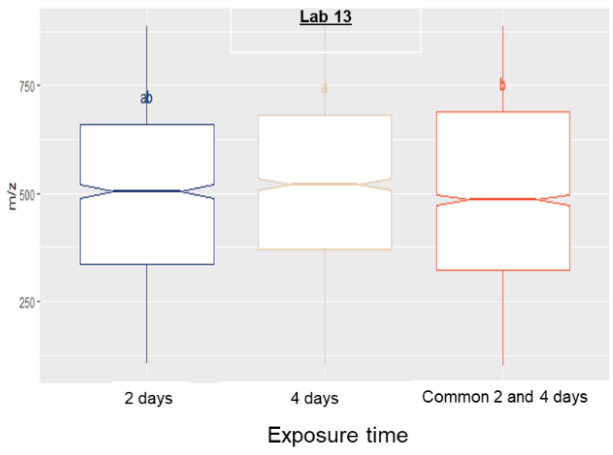
Annexe 9: M/z and Rt repartitions values for common features after the 3 RTI methods

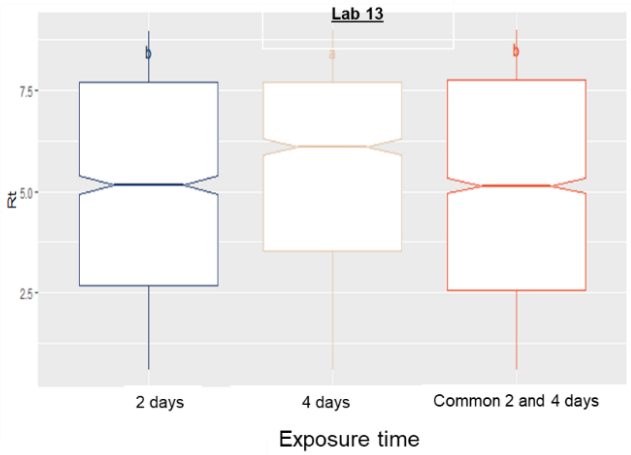
	VOS		SSM		KRT	
	m/z	RTI (ml)	m/z	RTI (min)	m/z	RTI (no unit)
min	167.1474	0.0529608	167.1474	0.8119169	133.0774	270.6667
Q1	167.1514	0.05433693	167.1527	0.8147451	249.1873	664.1526
médiane	262.1797	0.06473571	249.6141	5.1674378	332.3339	796.5732
Q3	344.2349	0.1227332	287.0928	5.4230608	403.8358	1112.9357
max	388.2637	0.1457367	387.2041	10.307479	628.5156	1298.4701

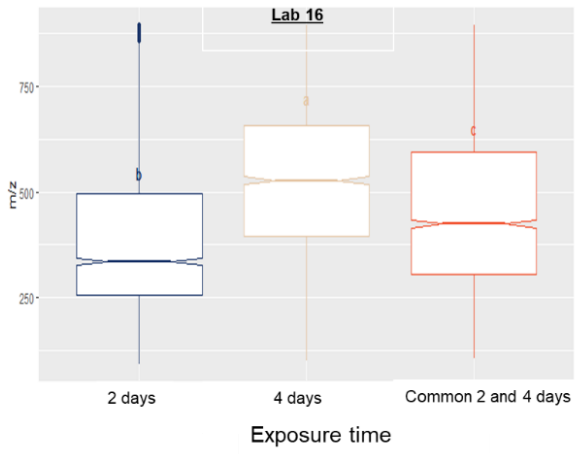
Annexe 10: Boxplots of m/z and Rt repartition of compounds presents in **river** water depending of their **exposure time**

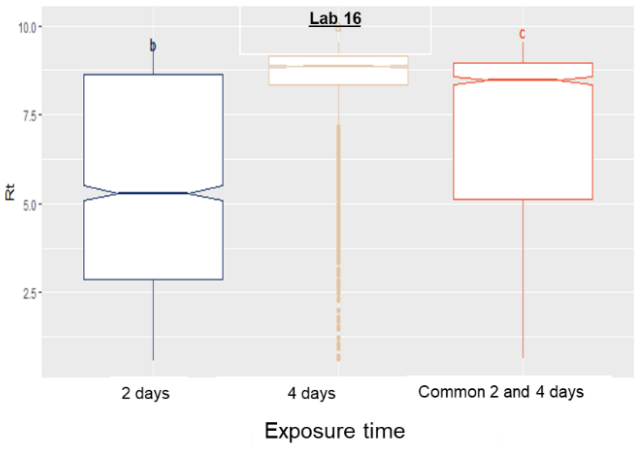




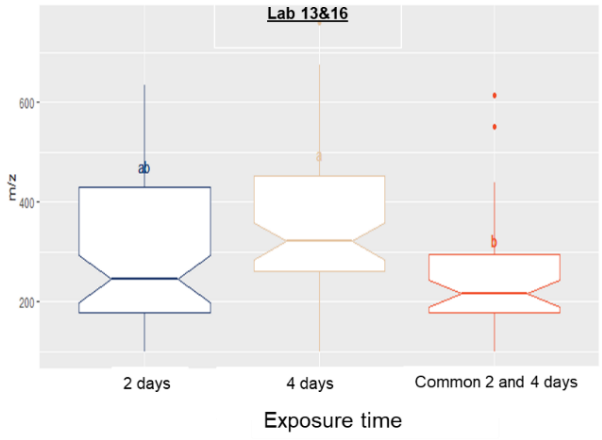


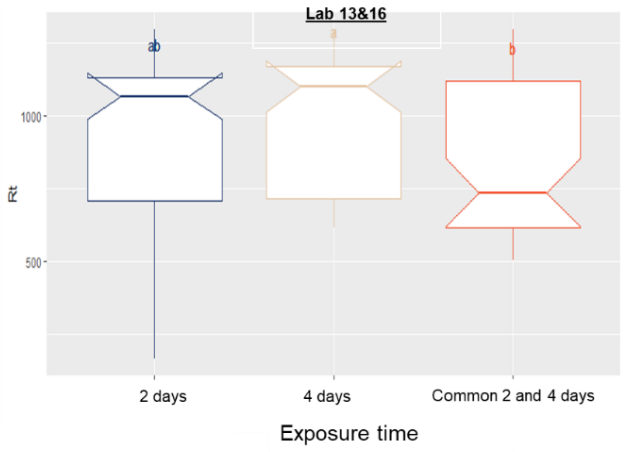


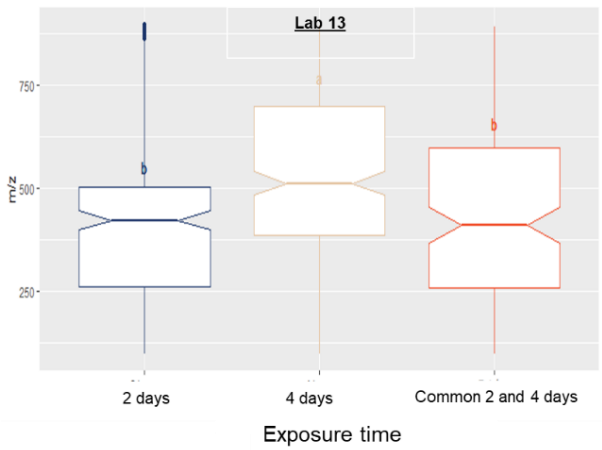


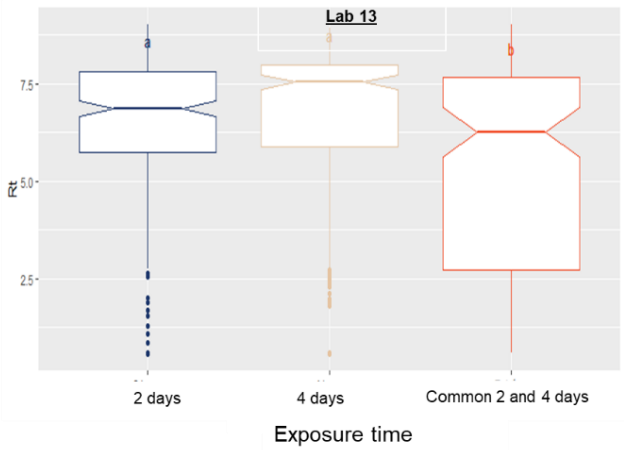


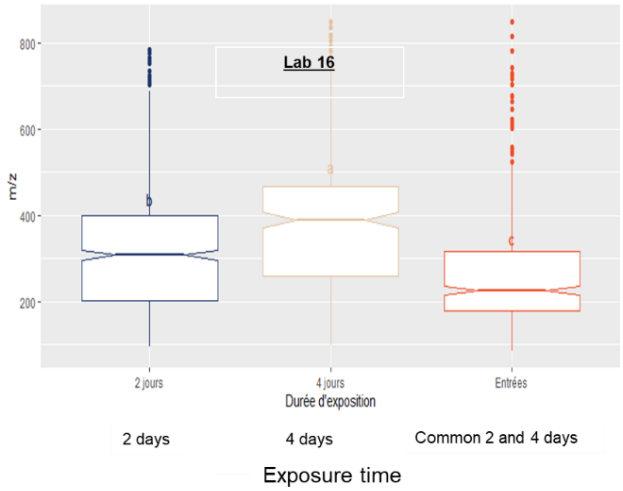
Annexe 11: Boxplots of m/z and Rt repartition of compounds presents in **drinking** water depending of their **exposure time**

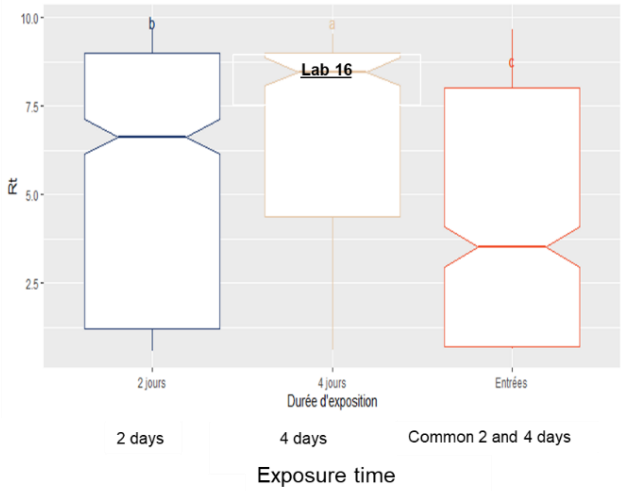




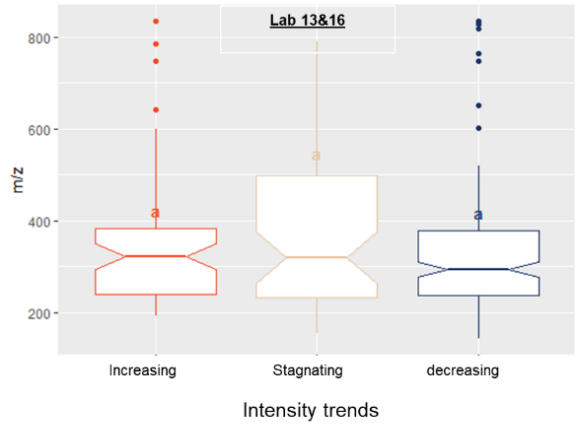


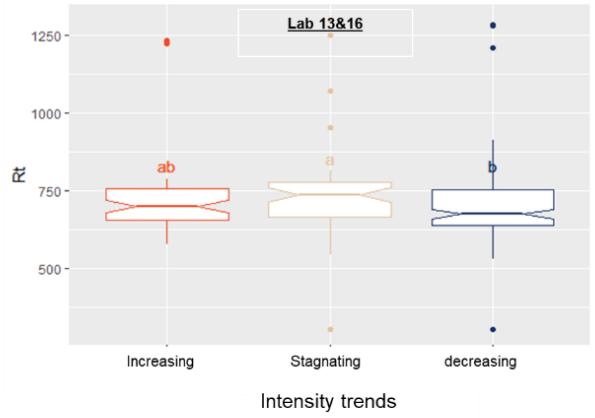


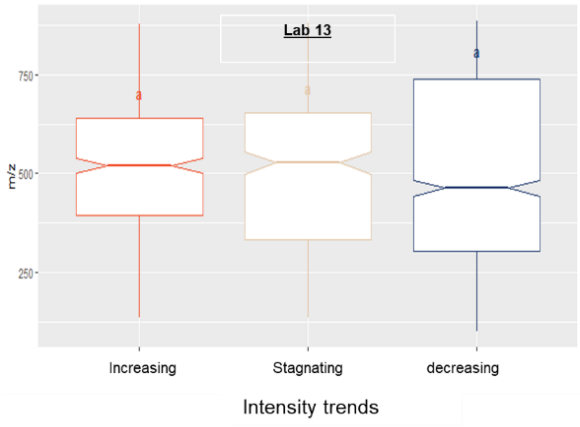


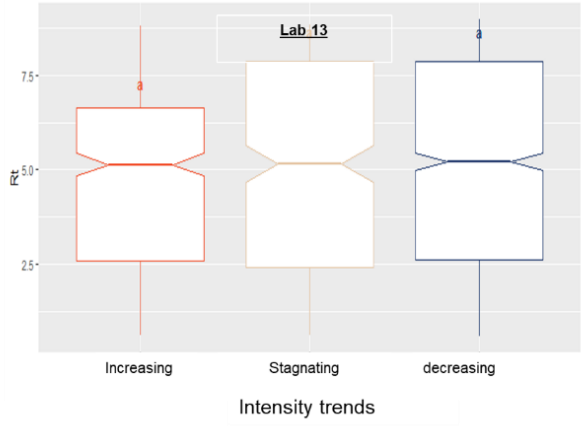


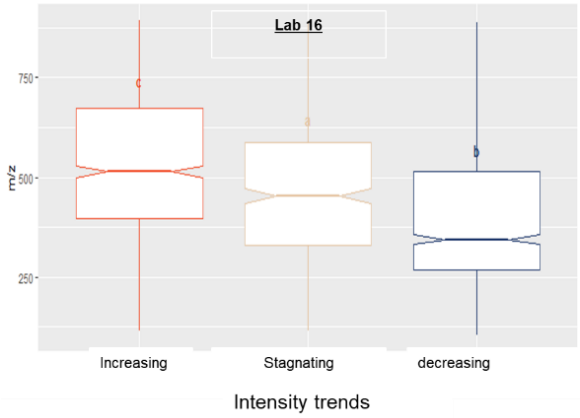
Annexe12: Boxplots of m/z and Rt repartition of compounds presents in **river** water depending of their **intensity trend** between 2- and 4-days exposure time

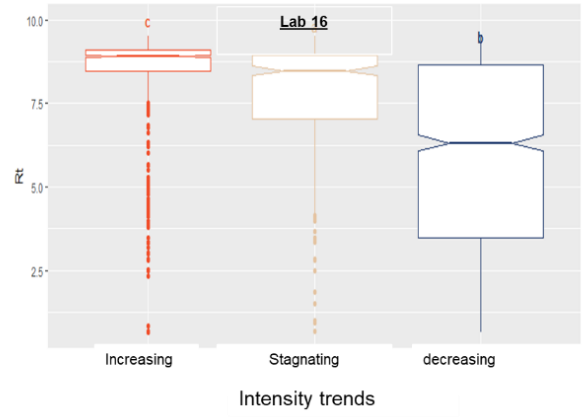




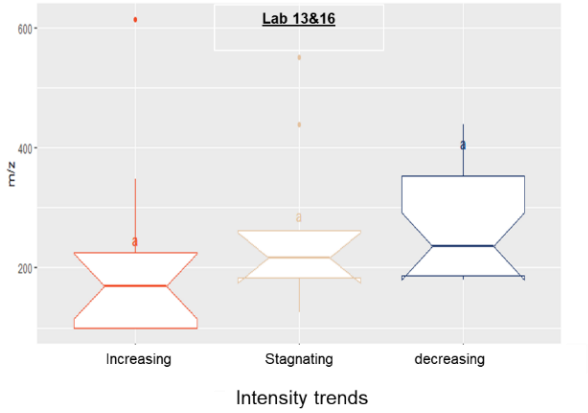


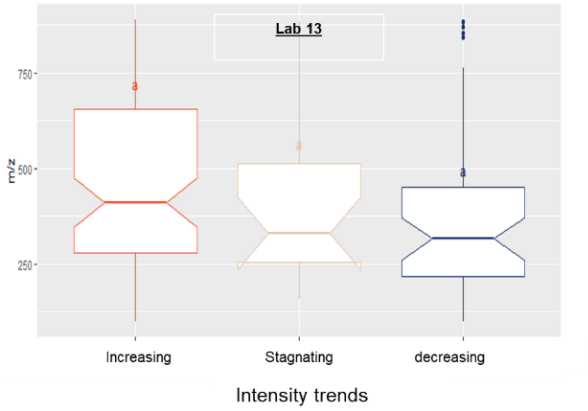


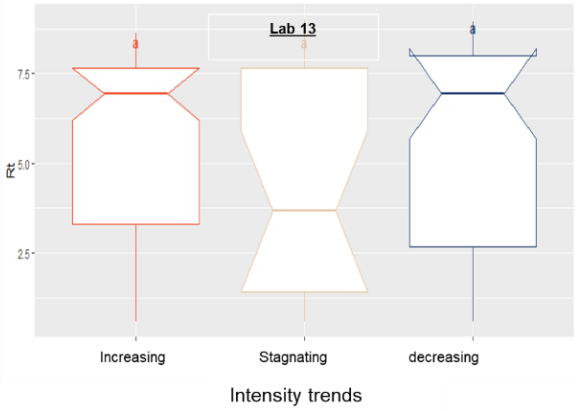


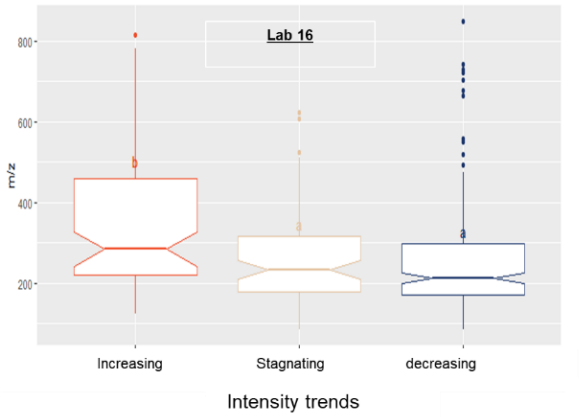


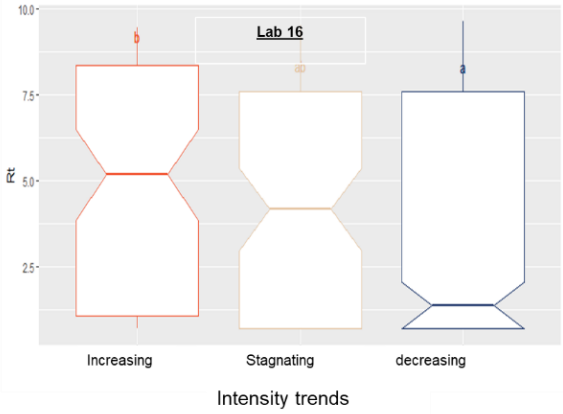
Annexe 13: Boxplots of m/z and Rt repartition of compounds presents in **drinking** water depending of their **intensity trend** between 2- and 4-days exposure time







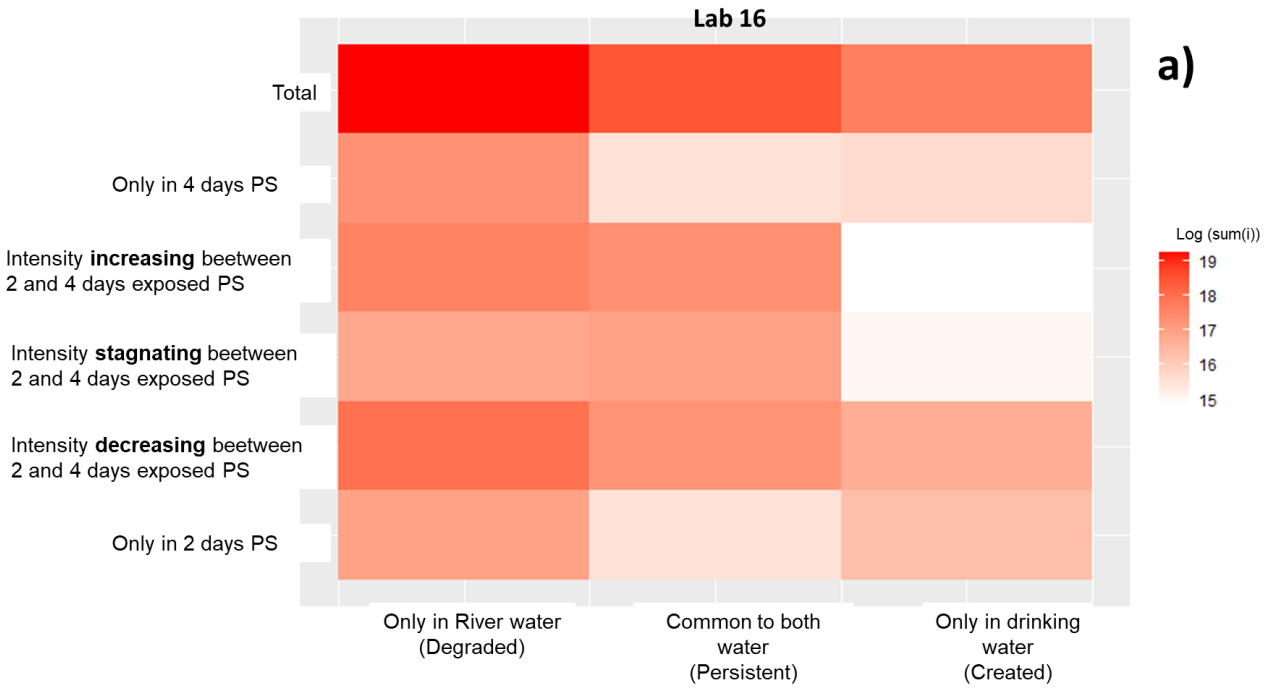


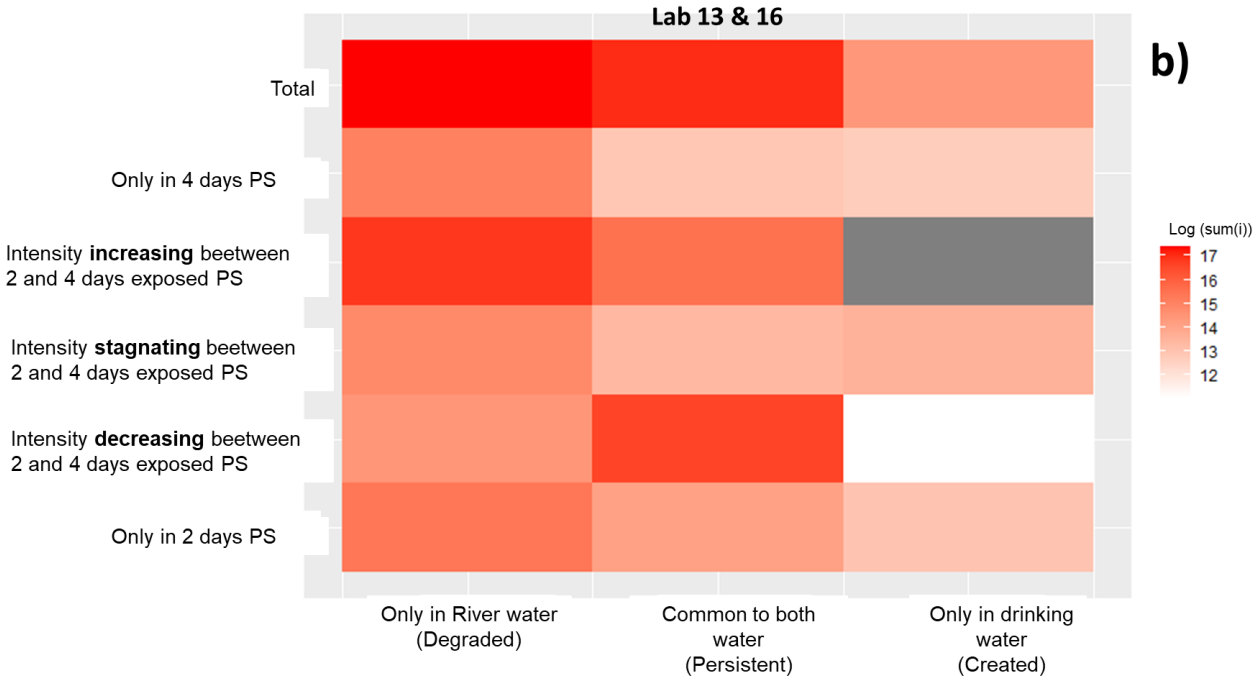


Annexe 14: Table of number of feature per categories

Groups	nb of compounds in river water lab n°13	nb of compounds in river water lab n°16	nb of compounds in river water list from lab n°13 and n°16	nb of compounds in drinking water lab n°13	nb of compounds in drinking water lab n°16	nb of compounds in drinking water list from lab n°13 and n°16
2 days	1185	1913	96	256	638	71
4 days	1173	1789	90	280	343	65
Common	2063	2352	628	151	415	46
Increasing	445	816	156	87	77	14
Stagnating	329	438	77	19	83	9
Decreasing	1289	1098	395	45	255	22

Annexe 15: Heatmap representing total intensity of groups of compounds depending on their affinity to PS across time and reaction to DWTP from a) lab n°16 and b) lis of lab n°13 and 16 combined





Annexe 16: Fiche competence - Following pages

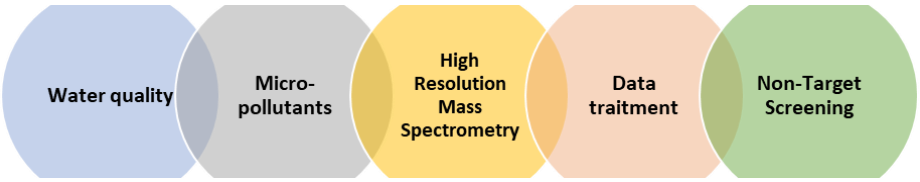
Abstract

There is a need to detect and monitor organic micropollutants in nature for public health care and environment. High Resolution Mass Spectrometry (HRMS) is a technology capable to detect thousands of compounds in a water sample. Non Target Screening (NTS) is a strategy done with HRMS, aiming to observe as much compounds as possible. NTS is challenging because data generated are huge. That is why data treatment is the key point of NTS. However, it exists many different data treatment processes, called workflows. It has been proved that for a same sample analysed, there are variations in results depending on the software used for data treatment [13], or the laboratory doing analysis [14].

Data set used is generated by an inter laboratory trial from NORMAN network and contains results from Liquid chromatography HRMS. POCIS like PS were exposed 2 and 4 days to a river and drinking water. Data of 4 laboratories using Waters Corporate instruments were processed. Each laboratories present a particularity making difficult comparison between all. The most annoying is a change of chromatography method.

For the same data treated with 2 different softwares (MZmine and Workflow4Metabolomics), 9-20% of features are found with both softwares. Then three methods of Retention Time Indexation are tested, based on: Volume of organic solvent injected (VOS) , Spiked standards (SSM), Kovàts equation (KRT) with RTImix. The last method shows the best score with only 10-14% of overlapping between the two closest laboratories. Finally, PS exposure time is analysed. It appears that longer a PS is exposed, bigger and more hydrophobic are the molecules trapped. More experimentations need to be done on PS exposure time and scientist should continue efforts to develop a way to harmonize retention time across different analysis.

Key words



Résumé

C'est important de pouvoir détecter et contrôler les micropolluants organiques dans la nature pour la santé publique et l'environnement. La Spectrométrie de Masse à Haute Résolution (HRMS) est une technologie capable de détecter des milliers de composés au sein d'un échantillon d'eau. L'analyse non ciblée (NTS) est une stratégie en HRMS qui vise à observer le plus de composés possibles. La NTS est un vrai défi car la quantité de données générées est considérable. C'est pour cela que le traitement de la donnée est le point clef de la NTS. Cependant, il existe beaucoup de processus différents pour traiter ces données, on appelle cela un Workflow. Il a été prouvé que pour un même échantillon analysé, les résultats varient en fonction du logiciel utilisé pour le traitement de données [13] et les laboratoires réalisant l'analyse [14].

Le jeu de donnée utilisé est généré par l'essai inter-laboratoire du réseau NORMAN et contient des résultats d'analyse en chromatographie liquide HRMS. Des échantillonneurs passifs de types POCIS ont été exposés 2 et 4 jours à une eau de rivière et une eau potable. Les données de 4 laboratoires utilisant un appareil de Waters Corporate sont choisies. Chaque laboratoire présent une particularité rendant impossible la comparaison des données entre les 4. Le plus dérangeant est le changement de méthode chromatographique.

Pour les mêmes échantillons traités avec 2 logiciels différents (MZmine and Workflow4Metabolomics), 9-20% des features sont trouvés avec les deux logiciels. Ensuite, trois méthodes d'indexation des temps de rétentions sont testées, basées sur : Le volume de solvant organique injecté, les standards spikés, les standards du mix RTI. C'est cette dernière méthode la plus efficace avec seulement 10-14% de composés communs entre les 2 laboratoires les plus proches. Finalement, le temps d'exposition des échantillonneurs passifs est étudié. Il apparait que plus le temps d'exposition est long, plus les molécules contenues dans l'échantillonneurs vont être grosses et hydrophobes. Plus d'expérimentation ont besoin d'être faites sur le temps d'exposition des échantillonneurs passifs et les scientifiques devraient continuer leurs efforts pour trouver un moyen d'harmoniser les temps de rétentions entre différentes séquences

Mots clefs