BTO 2018.017 | February 2018

BTO report

Transformation products in the water cycle (II)

KWR Watercycle Research Institute

BTO

Transformation products in the water cycle

BTO 2018.017| February 2018

Project number
400554-220

Project manager
Stefan Kools

Client
BTO - Thematical research - Emerging substances

Quality Assurance
Pim de Voogt

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Sent to
This report is distributed to BTO-participants and is public.

Year of publishing
2018

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Keywords

Transformation products, Organic micro-pollutants, Non-target screening, mass spectrometry, data analysis, chemical water quality

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BTO *Managementsamenvatting*

Transformatieproducten in de waterketen

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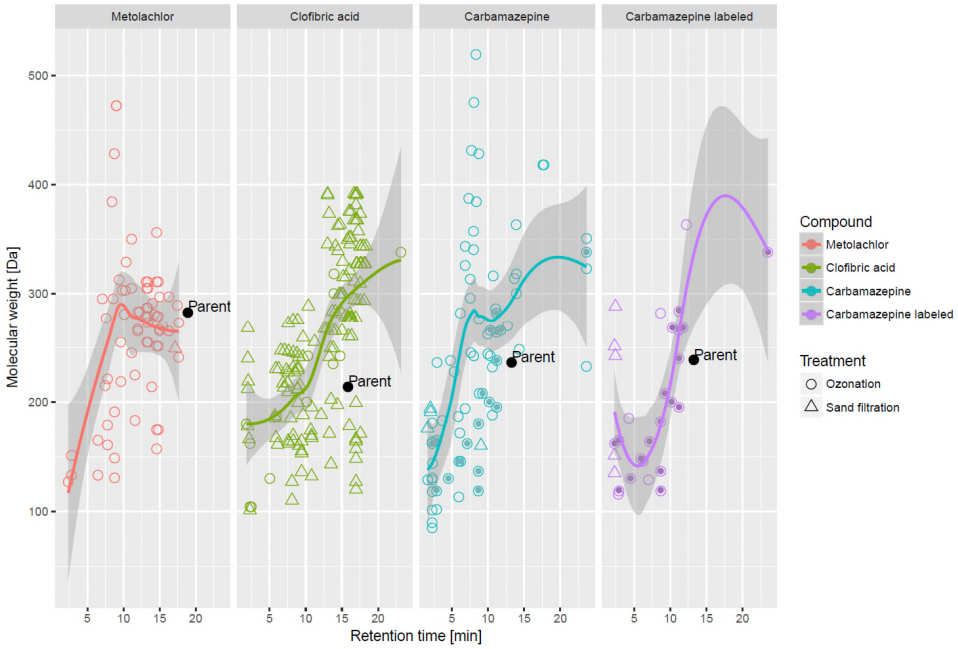
Transformatieproducten worden in de waterketen gevormd bij zowel biologische als technologische processen. Voor hun voorkomen in drinkwater(bronnen) is nog vrij weinig belangstelling en transformatieproducten bieden veel uitdagingen bij chemische analyse en identificatie. Dat verklaart waarom nog weinig studies naar transformatieproducten bestaan. Transformatieproducten zijn zeker relevant voor de drinkwatersector, zo blijkt uit interviews. Op basis van recente literatuur is een workflow ontwikkeld voor de efficiënte en semi-automatische monitoring van de vorming van transformatieproducten. Deze workflow is experimenteel getest voor één conventionele en één geavanceerde zuiveringstechniek (respectievelijk snelle zandfiltratie en ozonisatie). De transformatieproductenvorming van de organische microverontreinigingen carbamazepine, clofibrinezuur en metolachloor is tijdens deze processen gemonitord. De resultaten tonen aan dat de degradatie van de moederstoffen en de transformatieproductenvorming behandeling- en stof-specifiek zijn. Voorspellingen van transformatieproducten op basis van literatuurgegevens en geautomatiseerde modellen maken het identificeren van onbekende transformatieproducten makkelijker. De chemische structuur van het merendeel van de transformatieproducten kan echter niet worden geïdentificeerd en voor de meeste geïdentificeerde transformatieproducten ontbreekt een toxicologische risicobeoordeling.



Moleculaire massa's en retentietijden van moederstoffen en hun transformatieproducten gevormd door ozonisatie en zandfiltratie.

Belang: transformatieproducten ontstaan in milieu en waterbehandeling, worden niet gemonitord

Transformatieproducten vormen een relevante fractie van de organische microverontreinigingen in de waterketen. Ze kunnen meer polair en meer persistent zijn dan hun moederstoffen en daardoor ook moeilijker uit het water te verwijderen zijn. Transformatieproducten vormen een uitdaging bij chemische analyse: er is pas een deel van de transformatieprocessen bekend en de producten die daarbij ontstaan zijn vaak onbekend. Daarom zijn voor het monitoren van transformatie-producten geavanceerde methoden nodig, zoals LC-HRMS non-target screening. Bij de risico-beoordeling en in wettelijke regelingen is nog weinig aandacht voor transformatieproducten. Voor de bescherming van de waterketen en drink-waterbronnen is het belangrijk om meer inzicht te krijgen in de vorming en het voorkomen van transformatieproducten en handvatten te ontwikkelen om hun aanwezigheid aan te tonen en de risico's die dat meebrengt te duiden.

Aanpak: interviews met drinkwaterbedrijven, literatuurstudie en laboratoriumexperimenten

Door interviews met de betrokken partijen als drinkwaterbedrijven en Riwa Rijn is inzicht verkregen in de vragen rond transformatie-producten binnen de drinkwatersector. Op basis van beschikbare (literatuur)kennis is een roadmap met analytisch-chemische mogelijkheden is voor detectie en identificatie van transformatieproducten opgesteld. Laboratoriumexperimenten zijn uitgevoerd om deze roadmap te valideren en vragen uit de sector te beantwoorden. Daarbij is de transformatieproductenvorming gevolgd voor drie organische microverontreinigingen (carbamazepine, clofibrinezuur en metolachloor) onder invloed van één conventionele zuiveringstechniek (snelle zandfiltratie) en één geavanceerde oxidatieve zuiveringstechniek (ozonisatie).

Resultaten: efficiënte workflow voor monitoring transformatieproducten op laboratoriumschaal

Transformatieproducten blijken van belang voor de drinkwaterbedrijven en bieden een uitdaging bij de zuivering. Zoals ook uit de toegenomen hoeveelheid publicaties in 2016 en 2017 blijkt, is meer kennis over en aandacht voor transformatieproducten nodig. De laboratoriumexperimenten tonen aan dat de afbraak van moederstoffen zeker niet altijd leidt tot mineralisatie (afbraak tot anorganische componenten), maar dat de moederstoffen tijdens de behandeling vaak worden omgezet in een groot aantal verschillende organische omzettings-producten, die per behandelingstechniek en per stof sterk verschillen in kwantiteit en aantal. De opheldering van de chemische structuur van de transformatieproducten blijft een arbeidsintensief proces, dat ondersteund en versneld kan worden met voorspellingssoftware en databanken voor suspect screenings. Voorspellingen van transformatieproducten op basis van literatuur-gegevens en geautomatiseerde modellen maken het identificeren van onbekende transformatie-producten makkelijker. De chemische structuur van het merendeel van de transformatie-producten kan echter niet worden geïdentificeerd en voor de meeste geïdentificeerde transformatieproducten ontbreekt een toxicologische risicobeoordeling. Op basis van recente literatuur is een workflow ontwikkeld voor efficiënte en semi-automatische monitoring van de vorming van transformatie-producten.

Implementatie: transformatieproductenmonitoring in pilot en real scale setting.

Het is aan te bevelen de ontwikkelde workflow projectmatig met andere stoffen / zuiverings-technieken in te zetten en daarmee een rol te spelen in de uitbreiding van bekende transformatie-producten en hun voorkomen in stof-databanken en spectral libraries. Dit kan bijvoorbeeld bij de verschillende technieken die nu worden getoetst in het DPWE-onderzoek naar de robuustheid van de zuivering.

Rapport

Dit onderzoek is beschreven in het rapport *Transformation products in the water cycle (II)* (BTO-2018.017). Hiermee is een vervolg gegeven aan het werk beschreven in BTO rapport *Literature survey of transformation products in the water cycle*, BTO 2015.060.

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Abstract

Transformation products (TPs) are formed in the water cycle through both biological and technological processes. Despite the TPs' potentially altered toxicity compared to their parent compounds, transformation processes are not routinely monitored, and in particular those induced by drinking water treatment remain elusive. This lack of information is mainly due to the technical challenges in analyzing TPs, which are often unknown, polar compounds occurring in low concentrations. Their analysis thus requires non-target high-resolution tandem mass spectrometry (HR MS/MS) methods combined with novel data analysis approaches and toxicological risk assessment. Here, we addressed the challenges of TP analysis and the scarcity of TP research concerning studies in drinking water in particular, building on the insights gained from previous work. We assessed the relevance of transformation products for the drinking water sector through interviews with the concerned parties. A roadmap was drawn on how to efficiently and semi-automatically monitor TP formation in drinking water treatment and identify TPs, based on recent literature. Following this roadmap and addressing the sector's reported needs, we then performed a lab-scale pilot to monitor TP formation of the three organic micropollutants carbamazepine, clofibric acid and metolachlor during the rapid sand filtration and ozonation, two readily applied biotic and abiotic drinking water treatments, respectively. The experimental results showed that degradation of the parent compounds and TP formation were treatment and compound specific. *In silico* TP prediction and literature mining enabled suspect screening of the non-target data and thereby significantly facilitated TP identification. However, the majority of TPs remains structurally unidentified, and for the majority of identified TPs toxicological risk assessment is missing. In a follow-up study, the workflow developed will be applied to non-target data from pilot-scale experiments as part of the project DPWE robuustheid zuivering and will allow TP monitoring in actual drinking water production.

Contents

1 Introduction

Transformation products (TPs) encountered in the water cycle are organic chemicals formed during biotic and abiotic processes, with potentially altered toxicity compared to their parent compounds[1]. Despite this having been described over thirty years ago in waste water treatment[2], research on TPs in the water cycle is still considered an emerging field. In particular, transformation processes during drinking water treatment remain elusive, mainly due to the technical challenges to identify TPs. The high number of mainly unknown compounds, and their often low concentrations require non-target high-resolution tandem mass spectrometry (HR MS/MS) methods combined with novel data analysis approaches. Their often increased polarity compared to their parents potentially requires liquid chromatography (LC) online separation methods that are not based on reverse phase (RP) columns[3-4]. The combination of these novel methods enables non-target screening based identification which has been shown to be more selective than model-based prioritization[5-6]. Following identification, the toxicity of the identified TPs can then be determined to evaluate their significance in risk assessment[7-8]. However, research on the identified TPs' toxicity exceeded the scope of this project.

In this report, we address the challenges of TP analysis and the scarcity of TP research concerning studies in drinking water in particular, building on the insights gained from the BTO project *Literature survey of transformation products in the water cycle*, BTO 2015.060 [1]. We first assess the perception of transformation products within the drinking water sector through interviews with the concerned parties in Chapter 2. Based on the identified subjects, we then summarize the state-of-the-art of analytical methods for HR-MS/MS based TP identification and propose a workflow for their tracing tailored to the needs of the drinking water sector in Chapter 3. A lab-scale experiment for TP assessment implements these methods in Chapter 4, the results of which allow us to give personalized advice and recommendations for further development in the field in Chapter 5.

Research questions & activities

What are the relevant questions and issues concerning transformation products for the drinking water sector? – Interviews with drinking water companies

Based on literature, what are feasible approaches to identify transformation products using prediction and HRMS analyses in lab-scale and full-scale water cycle samples? – Roadmap covering analytics and data interpretation

Using controlled laboratory conditions and defined quantities of known parent compounds, which transformation products are generated by the drinking water treatment technologies ozonation and rapid sand filtration? - Lab-scale experiments are conducted to benchmark the roadmap

2 Perception of transformation products within Dutch drinking water companies

First, the relevance of TPs for the drinking water sector was assessed through interviews with representatives of several drinking water companies, and Riwa Rhine, as shown in Table 1. Cheryl Bertelkamp and Roberta Hofman-Caris generated the list of interview questions (see Appendix I), and carried out the interviews.

TABLE 1: INTERVIEWS WITH PEOPLE FROM DRINKING WATER COMPANIES AND RIWA RIJN

Company	interviewee	date
RIWA Rhine	Gerard Stroomberg	21-04-2017
Vitens	Bernard Bajema	01-06-2017
WLN (on behalf of WBG and WMD)	Jan v.d. Kooi	08-06-2017
WLN (on behalf of WBG and WMD)	Jantinus Bruins	20-07-2017
Dunea	Karin Lekkerkerker-Teunissen	02-07-2017
PWN	Bram Martijn and Ruud v.d. Neut	07-07-2017
Brabant Water	Mark van Huijkelom	10-07-2017

In the most general sense, TPs can be defined as compounds that are formed during degradation of parent compounds. Biodegradation products can also be referred to as metabolites. Some drinking water companies reported measuring the concentrations of selected known TPs (from biodegradation processes) in their sources, but not after treatment, while others stated that presence of TPs is not specifically determined.

As most companies don't apply (advanced) oxidation processes (AOP) (yet), the general perception is that the presence of TPs in a water source is caused by external factors, and not by the treatment process(es) applied during drinking water production. As most companies apply rapid and/or slow sand filtration, biodegradation during these processes may also cause TP formation. However, biotic TP formation is overall not considered a problem. This is partly due to a lack of awareness that biodegradation can result in TP formation, and partly stems from the general assumption of the interviewees that biotransformation products are less toxic than those formed through abiotic processes. There is no scientific evidence for this assumption, nor for the opposite. Many biotransformation products have similar or less toxic potency. This especially holds for chemicals with a specific biological mode of action such as pesticides biocides or pharmaceuticals, as transformation might change or remove the "toxicophore" (the part of the molecule that results in its specific biological effect), but there are also examples where the (bio)TPs are more potent than their parents [9] .

Especially drinking water companies that use groundwater as the primary water source state that TPs are a minor point of interest. In general they view the contamination of groundwater

with micropollutants as relatively limited. However, there are cases when contaminants and TPs are observed, sometimes even years after the prohibition of the use of the contaminating compound. These cases are then either alleviated by closing down the well or by withdrawing water from the affected well without using it for drinking water production. Thereby the polluted water can be prevented from entering neighboring wells. The attention is thus focused on protection of the wells, rather than on remediation or extension of the treatment process. The latter may be considered as "fatalistic thinking" according to an interviewee. Instead, the polluter should be held responsible for solving the problem. Moreover, the implementation of an additional treatment step for drinking water production could be considered as a permit to cause more pollution of ground- or surface water, as one interviewee feared.

Drinking water companies that use surface water as a source have to apply more extensive treatment processes. These may induce TP formation via biotic processes, i.e. through biofilms present on membranes, microorganisms in sand filters or filters with activated carbon, or abiotic processes, i.e. (advanced) oxidation processes (AOPs). The awareness of biotic TP formation varied between the interviewees. As AOPs are known to form abiotic TPs, some drinking water companies are reluctant to implement them. Others apply a single AOP treatment or a combination thereof. PWN uses advanced oxidation based on UV/H_2O_2. Dunea uses an additional advanced oxidation treatment step consisting of O_3/H_2O_2 prior to UV/H_2O_2 treatment, to increase micropollutant removal efficiency. Theoretically it is possible to completely mineralize contaminants by means of AOP. In practice, however, this isn't realized as it requires high amounts of both energy and chemicals. Instead, contaminants are oxidized to a certain level, under the assumption that the TPs formed are present in lower concentrations, and more readily biodegradable in a subsequent treatment step, such as activated carbon filtration or dune infiltration, according to an interviewee. However, whether or not dune filtration performs equally well as activated carbon filtration remains to be tested experimentally. In case of the two step AOP applied by Dunea it is possible that the TPs formed during the first oxidation step can be mineralized during the second oxidation step. Even though this has not been the primary reason for the implementation of this process, it might constitute an additional advantage, the interviewee stated. Pretreatment of the surface water, e.g. by ion exchange processes which remove part of the natural organic matter (NOM), can increase the efficiency of the subsequent AOP, and limit TP formation. In general, however, according to Dunea and PWN AOP during drinking water production improves the final water quality to a significant extent, mainly by removing the parent compounds.

The micropollutants found in surface water can stem from waste water treatment plant (WWTP) effluents. In order to decrease the degree of surface water contamination, WWTPs can be extended with an additional treatment step. European countries are beginning to implement different treatments, for instance Switzerland applies ozonation. As contaminant and organic matter concentrations in WWTP effluents are relatively high, ozonation may result in the formation of relatively high concentrations and amounts of TPs. However, TPs are not routinely monitored, merely the removal of parent compounds is measured. Germany, on the other hand, is extending its WWTPs with additional activated carbon treatment. The extent of biodegradation and photolysis by sunlight of both parent compounds and TPs in water, however, is still largely unknown. There are also examples of metabolites being reverted to their parent compounds through biodegradation, for instance hydroxide metabolites can be reverted to the sulphate group-containing parent compounds by certain microorganisms.

There is consensus that analytical techniques to detect and identify micropollutants in water have significantly improved during recent years. As a result, more micropollutants are observed, also in low concentrations, in drinking water sources and in drinking water itself. This, however, doesn't necessarily mean that the drinking water safety is an issue. From that point of view not all drinking water companies welcome the development of analytical techniques that can detect even lower concentrations of contaminants.

The interviewees stated that TP analysis is challenging. Especially in the case of oxidation of compound mixtures by radicals, a broad range of TPs can be formed, as reactive species may also react with each other. In lab-scale experiments, TP formation from defined single, parent compounds in a water matrix can be studied in detail. The resulting information can then be translated into quantitative structure activity relationships (QSARs) to predict TPs specific for a certain process and parent compound. However, the presence of NOM as well as mixtures of parent compounds might lead to other TPs elusive to the lab-scale set-up, but relevant in drinking water production.

Only a selection of TPs is analyzed on a regular basis by the drinking water companies. The argument is raised that as TP formation strongly depends on the specific local and temporal circumstances, it might not be necessary to measure and identify all TPs. Therefore, some drinking water companies prefer the application of effect assays such as bioassays. According to an interviewee these can give an indication whether the (mixture of) compounds present affects living organisms, although translation of these results to effects on human health may still be a difficult task and the suite of bioassays used do not cover all relevant biological processes and endpoints. In order to obtain more information, bioassays can be combined with non-target screening methods. Thereby the biological effects observed can be attributed to the presence of certain compounds in the water.

The drinking water companies concur that in order to gain more information on TPs present in sources for drinking water and their possible effects on human health, it is necessary to obtain more information about potential parent compounds contaminating the water sources through for instance industrial discharges or the application of pesticides. Water authorities do not always have this information, which hinders the prediction of which parent compounds and consequently TPs can be expected in a given water sample. However, this information is essential to determine whether there is a problem, how the problem can be solved and, even more importantly, how the problem might be prevented. Gerard Stroomberg (RIWA-Rhine), highlights a recent example from the Rhine River Basin where farmers along the Saar and Moselle where encouraged to change the timing of the application of isoproturon which dramatically reduced the number and duration of intake stoppages of Rhine water at Nieuwegein. For certain compounds, such as some pesticides, it is known when and where they are applied, and then measures may be taken directly in the application, thus preventing the compounds from entering surface or groundwater.

In brief, it can be concluded that all companies are confronted with TPs to some extent. Their presence is mainly considered a problem in surface waters, and less in groundwater. Attention is largely focused on TPs already present in the source water, and less on TPs formed during treatment processes. The general perception is that TPs formed through biological processes are less harmful than those formed chemically. In some companies, potential TP formation prevents the application of AOP. Others which apply AOPs stress the fact that water quality can be improved significantly by AOP, as the controlled TP formation results in smaller and more biodegradable compounds, that can be removed by biodegradation.

To be able to assess the health risks related to potential toxic effects of TPs, it is necessary to know more about the parent compounds present in water sources, their degradation routes, and the relationship between the presence of certain compounds and responses observed in effect assays.

3 Roadmap to assess transformation products in the water cycle

3.1 Analytical challenges inherent to TP identification

The interviews conducted and summarized in Chapter 2 revealed that TPs are considered relevant by the drinking water sector, but remain an under-studied issue. The lack of TP monitoring, and consequently the limited awareness of their occurrence in water sources as well as their formation during drinking water treatment, seemed partly due to the analytical challenges of TP identification. These challenges include the high number of mainly unknown compounds, their often low concentrations and increased polarity, and require non-target screening methods in combination with novel data analysis approaches and potentially LC separation tailored to polar compounds [3-4].

Here, we would like to resolve some of the challenges by providing a roadmap covering the chemical analytical possibilities to gain insight into TPs in the water cycle. We first summarize the state-of-the-art of the analytical methods, and then propose a workflow for TP tracing tailored to the needs of the drinking water sector.

Increased polarity of TPs compared to parent compounds

Transformation processes often lead to TPs being smaller and more polar than their parent compounds [1, 9]. This poses an additional analytical challenge, as very polar compounds are not retained by C18 based reversed phase (RP) LC columns. Other separation methods are required such as HILIC or mixed bed columns [4].

3.2 Two complementary approaches for TP identification

Greatly simplified, there are two complementary strategies to monitor TP formation and identify TPs. Both take advantage of the relationships between parent compound and TPs. The choice of application depends on whether or not the parent compounds are known. An overview of the strategies is depicted in Figure 1.

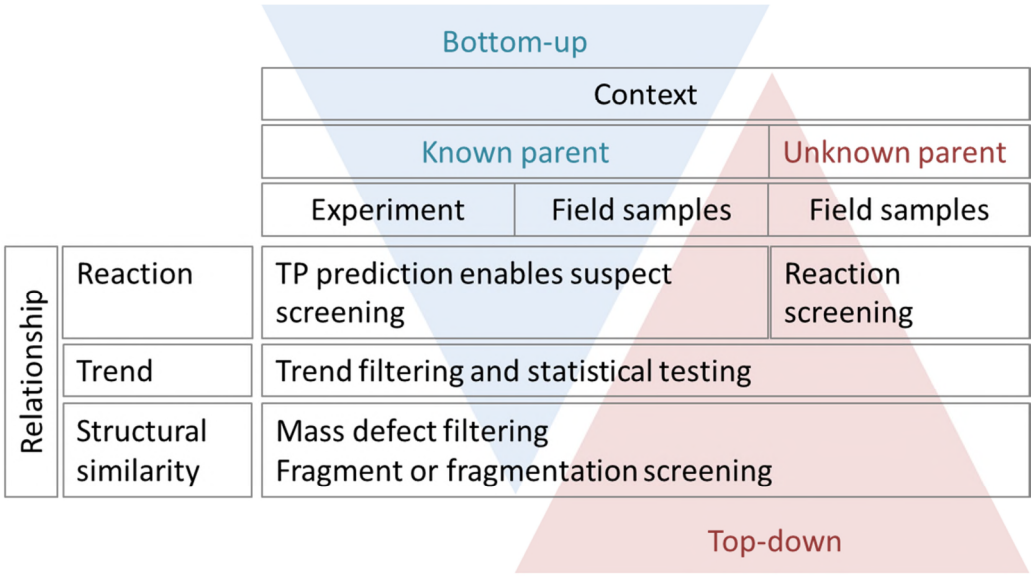
- Known parent, bottom-up approach

In the case of a known parent compound – the so-called bottom-up approach, TPs can be predicted and subsequently screened for via suspect screening.

- Unknown parent, top-down approach

In case of an unknown parent – the so-called top-down approach, the focus lies on (statistical) analysis of patterns and trends, such as changes of intensity between samples, mass shifts indicative of transformation processes, and structural / fragmentation similarity.)

FIGURE 1. OVERVIEW OF STRATEGIES. ADAPTED FROM [10]



3.2.1 Feature reduction in non-target screening

LC-HRMS based non-target screening of water samples generates thousands of peaks representing an accurate mass and retention time (RT), also referred to as "features". After data pre-processing these features can be translated into potential molecular formula(s) which in turn can be related to potential chemical structures, the multitude of which often prevents compound identification. The number of unknowns thus has to be reduced to a more manageable number, which can be done by tailored data acquisition, filtering and prioritizing at various step of the data analysis [11]. These approaches are not specific for TP analyses. However, they allow reduction of feature numbers and thus reduction of the data complexity. This in turn reduces the number of (tentatively identified) parent compounds for which TPs can be predicted, and therefore facilitates higher identification rates with lower false discovery rates.

More detailed information on data acquisition, data curation, feature building, and molecular formula generation can be found in the BTO report 2017.073 (Tools voor de identificatie van onbekende verbindingen met hoge resolutie massaspectrometrie data).

3.2.2 The bottom-up approach: Suspect screening for predicted TPs of known parent compounds

The reduced dataset of features can be searched against suspect lists comprising both potential parent compounds and expected TPs known from batch studies, literature, home-made databases as well as public compound libraries such as NORMAN SusDat[1], STOFF-IDENT[2], DAIOS[12] and comprehensive chemical databases such as ChemSpider[3] and Comptox (EPA). In case of known or tentatively identified parent compounds, TPs can also be predicted using *in silico* transformation algorithms implemented in for instance enviPATH, tailored to environmental analyses [13], and XCMSonline, a metabolomics software suite[14]. Further prediction software include CATABOL[4], PathPred[5], Meteor[6], CRAFT Chemical

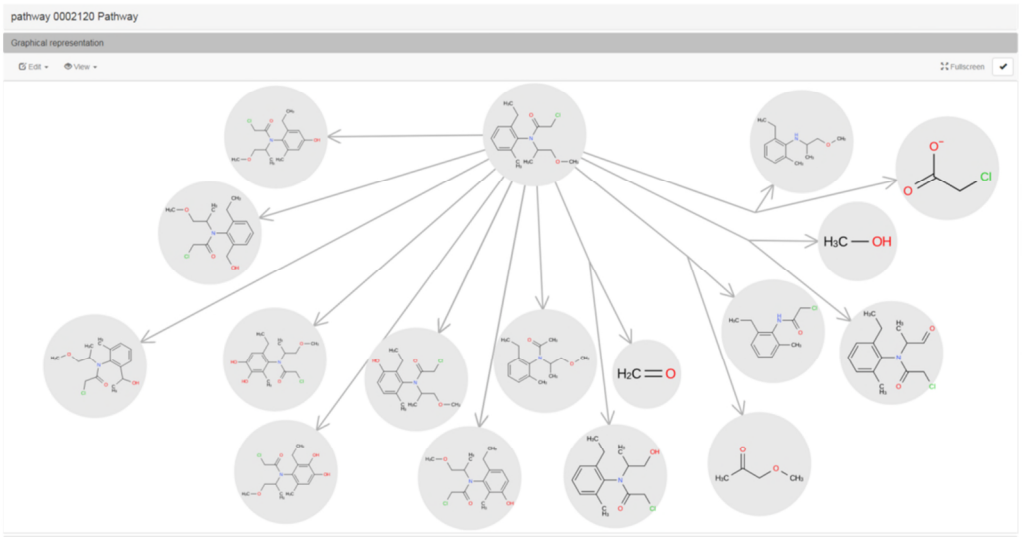
[1] http://www.norman-network.com/?q=node/236

[2] https://www.lfu.bayern.de/stoffident/
[3] http://www.chemspider.com/
[4] http://oasis-lmc.org/products/models/environmental-fate-and-ecotoxicity/catabol-301c.aspx
[5] http://www.genome.jp/tools/pathpred/
[6] http://www.lhasalimited.org/products/meteor-nexus.htm

Reactivity and Fate Tool[7], and the ozone reaction prediction software[15]. Interestingly, prediction software for mammalian biotransformation reactions can be applied to microbial systems as biotransformation reactions have been shown to overlap, for instance biotransformation reactions of amine-containing xenobiotics were similar in mammalian and microbial systems[16]. However, caution should be exercised as phase II transformations are highly relevant in mammals, leading to more polar and larger conjugates, but may not occur in microbial degradation.

The Environmental Contaminant Biotransformation Pathway Resource **EnviPATH**, successor of The University of Minnesota Biocatalysis/Biodegradation Database and Pathway Prediction System (UM-BBD/PPS), is a free and open access database of microbial biotransformation pathways of primarily xenobiotic chemicals, that predicts biodegradation based on relative reasoning and machine learning models.

The user enters a SMILES code directly or a chemical structure via the visual editor at https://envipath.org. A pathway is then predicted for the structure, unless a pathway for that compound is already stored. In the latter case, the stored pathway from the database is retrieved. The pathway and predicted TPs output is illustrated in the figure below for metolachlor. The results can be downloaded as a .csv file and added to a suspect list for suspect screening of the LC-HRMS data.



ENVIPATH PREDICTION FOR METOLACHLOR TP FORMATION

It has to be noted that with increasing size of the suspect screening database, i.e. increase in search space, more false positive identifications occur [17]. Therefore, levels of stringency beyond accurate mass need to be applied to minimize false positives, such as retention time (RT), isotope profiles, ionization efficiency, and the use of MS2 fragmentation data for identification. RT time plausibility can be assessed through comparison of the experimental RT with *in silico* predicted RT based on the compound's logK_{ow}[18], or quantitative structure-

[7] https://www.mn-am.com/products/craft

retention and activity relationship (QSRR/QSAR) models [19-20], as well as experimental retention time indices, such as the Kreti and Letzel index[21-22]. The plausibility of detection in positive and/or negative ionization mode can also be used for ionization efficiency filtering, i.e. compounds with amino but not acidic groups can exclusively be detected in positive ionization mode, and strong acids and sulphonates in negative ionization mode, respectively[23]. Additionally, MS2 fragmentation data is instrumental in structurally identifying a compound and increasing the level of confidence[24]. To this end, fragmentation patterns of TP features can be compared with spectra from experimental spectral libraries such as MassBank[8] and mzCloud[9], and/or *in silico* mass fragmentation databases such as MetFrag[10]. As the identification of unknowns is the goal of BTO project Massaspectrometrie: (i) Tools voor ID van onbekenden, TG NMS 15-04-06, these strategies will not be discussed here in further detail.

A distinct bottom-up strategy for TP identification makes use of parent compound labelling, particularly with stable isotopes of the elements carbon (^{13}C), hydrogen (^{2}H), oxygen (^{18}O), nitrogen (^{15}N) and sulphur (^{34}S). A labelled parent compound will be transformed into a labelled TP, given that the labelled residue is still present in the TP. As both labelled and unlabelled compounds exhibit the same physico-chemical properties, they are not separated by chromatography, but can be distinguished by their mass difference when mixed in one sample or analyzed sequentially. This allows efficient discrimination of TPs from other compounds, efficient compound annotation through constraints for enhanced formula assignment, and reliable quantification. This strategy is routinely applied in metabolomics[25-26], where a tailored software, X13CMS, is available, to analyze the resulting HRMS data[27], and has been exploited previously at KWR to trace nitrogenous TPs formed through medium pressure UV water treatment[28].

3.2.3 The top-down approach: using patterns and trends to identify TPs

If both TPs and their parent compounds are unknown, a top-down approach based on patterns in the data can be applied, given that a “before” and “after” sample are provided. While still in the early stages of development for environmental TP analyses, applicable methods have been established in the field of metabolomics. TPs are by definition formed from parent compounds, which entails that their signal in LC-HRMS experiments increases over time, i.e. between before” and “after” samples, while that of their parent compounds decreases. These signal intensity patterns are utilized in trend filtering and TP identification based on statistical analyses[29]. In particular, significance testing based on Student’s t-test and fold changes can reveal features that are significantly different between samples [30], effectively visualized in a volcano plot [31], (see 4.2.4 for a more detailed description). In addition, multivariate analysis (MVA) methods such as the linear projection models principal component analysis (PCA), hierarchical clustering, and partial least squares (PLS) can be used to characterize and group co-varying features [32]. In PCA, the scores plot can reveal how samples group together, and the loading plot how features relate to each other. However, if there are fewer samples than dimensions, i.e. fewer spectra than features, PCA is technically not possible. It should also be noted that MVA require prior normalization and scaling [33]. Nevertheless, MVA has successfully been applied to classify non-target features into either parent compounds or TPs [34] .

An ever increasing wealth of **R packages** exist that facilitate and accelerate statistical analyses of non-target HRMS data, including:

[8] www.massbank.jp
[9] www.mzcloud.org
[10] https://msbi.ipb-halle.de/MetFragBeta/

- 'prcomp' and 'princomp' for PCA
- 'lattice' for visualization of univariate and multivariate data
- 'clust' for clustering methods
- 'muma' for univariate and multivariate data analysis, including Welch Test, Shapiro Test, Mann-Whitney Tests, fold changes, volcano plots, box plots as well as PCA, PLS-DA, and OPLS-DA

As well as **R graphical user interfaces (GUI)** that have (some of) the functions listed above:

- 'enviMass' for trend detection in LC-HRMS measurement sequences (http://www.looscomputing.ch/eng/enviMass/overview.htm)
- 'rattle' for univariate analysis and clustering

These methods do, however, risk to overlook certain TPs when peak intensities in HRMS experiments are not inherently quantitative as has been described previously[35]. Furthermore, a one-to-one relationship between parent compound and TP cannot necessarily be expected. Rather, one parent compound can form multiple TPs[16], and one TP can come from multiple parent compounds[36]. In addition, transformation of a primary TP can occur and lead to the formation of secondary – and even tertiary, etc. - TPs[37]. Lastly, a TP can transform back to its parent compound, for instance conjugation can be followed by deconjugation, and the resulting trend reversal poses a challenge for statistical hypothesis tests[38].

An alternative to trend filters are logical filters that filter TPs in regard to their molecular weight, which is assumed to be smaller than that of the parent compound, and their polarity, which is assumed to be increased compared to the parent compound and reflected in the shorter LC RTs [9, 37]. However, these trends can prevent identification of TPs such as those formed through conjugation, and caution should be exercised applying them. Moreover, metabolic logic, that is the detection of mass shifts indicative of transformation processes can be used to identify parent compound – TP pairs by looking for mass shifts corresponding to known biotransformation reactions. A list of relevant mass shifts can be found in in 0. The R package 'RMassScreening' that implements metabolic logic can be downloaded at https://github.com/meowcat/RMassScreening (Michele Stravs, personal communication). Finally by combining MVA and metabolic logic, theoretical TP masses can be calculated for the features classified as parent compounds by PCA, based on known biotransformation reactions, and screened for in a suspect screening as described in the bottom-up approach in 3.2.2.

Another set of strategies to analyze non-target data and identify TPs takes advantage of the information inherent to a compound, i.e. its mass defects, isotopic patterns and structural composition, and assumes that parent compound and TP information is to a certain extent alike, or similar. In mass defect filtering, the difference between the accurate and the nominal mass of an ion, the so called mass defect, is used to identify multi-isotopic elements, in particular halogens, in HRMS data. It can thereby facilitate TP identification by exposing features with specific functional groups or elements [39-42]. Similarly, isotopic pattern

filters can be used to identify TPs based on the isotopic patterns of their parent compounds[43]. Finally, the structural similarity of parent compounds and their TPs can reveal TPs [44]; as TPs maintain a structure similar to the parent compound, the stability or reactivity of certain parts of the molecule is similar and they thus exhibit common characteristic fragment ions. This is exploited in Fragment Ion search (FISh) scoring in MassFrontier and Compound Discoverer (both Thermo Fisher Scientific) and Spectral Trees in mzCloud.

3.3 Assignment of transformation reactions

Ultimately, the potential TPs identified with (a combination of) bottom-up and top-down approaches can be assessed in terms of which transformation reactions resulted in their formation. The assignment of reactions to observed parent–TP pairs also provides a certainty with which a transformation reaction can be attributed. This certainty is composed of the confidence in TP identification, and the confidence with which TP formation from the corresponding parent compound can be attributed to a plausible reaction. According to Gulde et al. reactions can then be classified as certain, likely, possible, and unknown [16].

3.4 Workflow

As outlined in detail above, there are two complementary approaches to identify TPs, a bottom-up approach where parent compounds are known and transformation products are predicted and then experimentally detected typically through a suspect screening, and a top-down approach where both parent compound and TP are unknown and identification is achieved based on trend and statistical analyses. We here propose a workflow (Figure 2) that synergistically combines both approaches to monitor transformation processes and identify TPs in a lab-scale experiment under well-defined conditions. The advantages of such a controlled system are manifold; the higher spike-in concentrations, the possibility to use stable isotopically labelled parent compounds to facilitate detection of TPs and the availability of appropriate controls facilitates tracing of TP formation [28, 45-48]. The TPs of a given parent compound identified in such a laboratory study can then be integrated in suspect and target lists for future analysis of environmental samples, and health risk assessments.

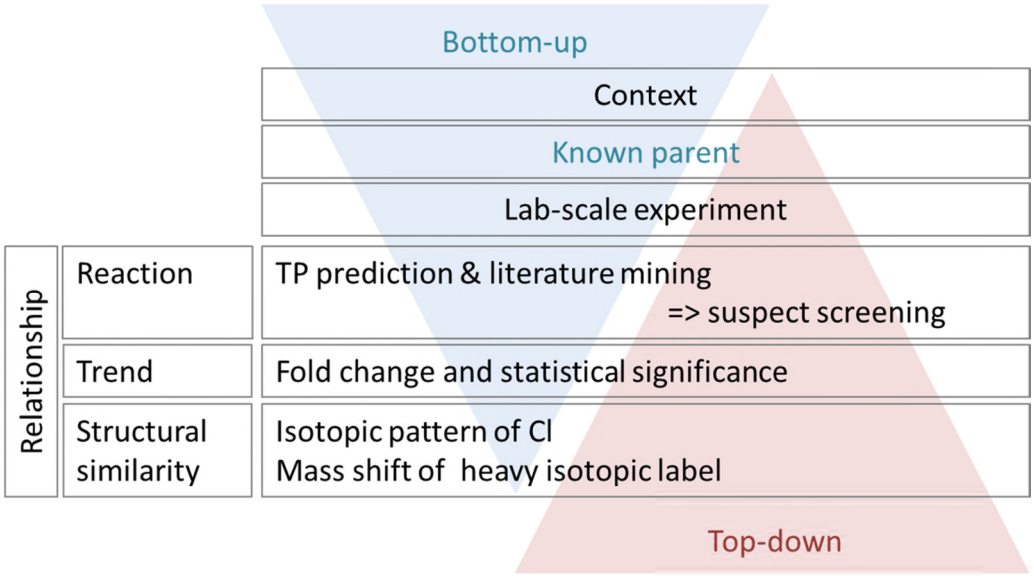


FIGURE 2. WORKFLOW COMBINING BOTTOM-UP AND TOP-DOWN STRATEGIES TO MONITOR TRANSFORMATION PROCESSES AND IDENTIFY TPS IN A LAB-SCALE EXPERIMENT

4 Lab-scale experiment: Assessing transformation product formation in drinking water treatment

4.1 Rationale for selection of experiments

To benchmark the roadmap described in Chapter 3, a lab-scale experiment applying bottom-up and to a lesser extent also top-down approaches to study TP formation and identification was performed using controlled laboratory conditions and defined concentrations of known parent compounds. As outlined in Chapter 3, these conditions facilitate TP identification through:

- providing **before and after treatment samples**. During the data analysis one can thus focus on the differences between the two and disregard all information that is the same in both.

- **known parent compounds**. These allow prediction of potential TPs based on literature and models, and consequently a suspect screening of the data against a list of predicted TPs.

- the possibility to use **high concentrations of spiked-in parent compounds**. Thereby also TPs formed at low rates can be detected.

- **presence of a halogen**. The distinct isotopic pattern of the halogenated parent compound and TPs, as well as the negative mass defect can support screening approaches. However, this advantage may be lost in the TP, for instance through dehalogenation during biotransformation.

- **inclusion of a label**. Labelled parent compounds will form labelled TPs which are readily detected based on their mass shift.

The experiments, in particular the applied water treatment technologies and the parent compounds studied, were selected based on the outcome of the interviews conducted and summarized in Chapter 2, to ensure relevance for the drinking water sector. Furthermore, emphasis was placed on the development of a generic approach that could be implemented across other drinking water treatments and for other parent compounds. As environmental TPs can be formed by either abiotic or biotic processes, which in turn require different prediction software, a treatment representing each process was applied. The selected treatment can then serve as a model for other treatments with the same transformation process. The parent compounds were chosen based on their relevance for the drinking water sector, their occurrence in drinking water sources, the availability of an isotopically labelled parent, or the presence of a halogen. Furthermore, we considered whether they could be sensitively detected with RP-LC-HRMS. As TPs are generally expected to be more polar and thus elute earlier than their parent compounds during RP-LC, we ensured that the parent compounds allowed some flexibility in terms of RT.

4.1.1 Rapid sand filtration as a model for biotransformation

To accommodate a technology used by drinking water companies with groundwater as a water source and to address the topic of (aerobic) microbial biotransformation, which were both issues raised by the initial interviews, rapid sand filtration was selected as the biotic process for the lab-experiment since spiking and analysis in real soils systems is not feasible. Rapid sand filtration is a process that is implemented in almost every drinking water treatment plant in The Netherlands. In addition, it facilitates the biological degradation of a number of organic micropollutants (e.g. pyrazole[49-50], caffeine, dimethoate, gemfibrozil [51]).

4.1.2 Ozonation as an example for abiotic transformation processes

Ozonation was selected as the abiotic process to study in the lab-experiment, in particular because it represented an AOP and thus one of the main points of concern during the interviews. Dunea is running ozonation on pilot-scale for drink water production with surface water as the water source, and Waternet is using ozonation after dune infiltration. The technology is known to result mainly in transformation of the chemical structure of compounds rather than their mineralization, and the biological effects of formed TPs have been of concern [52-56]. There are two distinct reactions occurring during ozonation: the direct reaction of the ozone molecule with a target compound, and the decomposition of ozone in aqueous medium. With the latter, hydroxyl radicals are produced which can in turn react with the target compound. In practice, both direct and indirect reactions take place simultaneously. It should be noted that the published prediction software for ozonation TPs by Lee et al. which derived 340 individual reaction rules from literature data mining to predict the TPs of micropollutants, does not predict hydroxyl radical-induced transformation products [15].

4.1.3 Parent compounds: incorporating halogens and labels

The relevance and suitability of the selected parent compounds is summarized in Table 2.

	Compound	Sand filtration breakdown	Ozonation breakdown	Available with stable isotope label	Halogen	Molecular weight	RT (min)
1	Carbamazepine	No	Yes	Yes	No	236.0947 (238.0952 labelled)	13.3
2	Clofibric acid	Medium	oxidized by hydroxyl radicals	No	Cl	214.0394	15.9
3	Metolachlor	Medium	oxidized by hydroxyl radicals	No	Cl	283.1334	19.0

TABLE 2. SELECTED PARENT COMPOUNDS, RELEVANCE AND SEPCIFICATIONS.

The anti-epileptic and neuropathic pain medication carbamazepine (Figure 3, 1a) is one of the pharmaceuticals most frequently detected in the aqueous environment[57-58]. Its human metabolites are known. The compound is persistent in sand filtration [59], but readily reacts with ozone. Ozone TPs are well studied [60], and can thus be used as positive controls to evaluate the experimental set-up. In addition, the availability of the isotopically labelled standard carbamazepine-(carboxamide-^{13}C,^{15}N) (Figure 3, 1b), which has a ^{13}C and a ^{15}N incorporated at atoms that remain in the known ozone TPs of carbamazepine, the suitable

RT in RP experiments, and its omnipresence in the environment makes carbamazepine an ideal candidate for the lab-pilot.

Clofibric acid (Figure 3, 2) and metolachlor (Figure 3, 3) are both herbicides that have been detected in groundwater and surface waters. Clofibric acid is also a human metabolite of the cholesterol-lowering pharmaceutical clofibrate. It is a medium biodegradable pollutant [51], with a number of known biotic [61-63] and abiotic [64] TPs. Metolachlor is susceptible to both biotic and abiotic degradation [65-71], and its TPs have been shown to be more toxic than the parent compound [72-73]. The lack of available labelled standards for both clofibric acid and metolachlor is compensated by the presence of chlorine atoms in the compounds exhibiting a distinct isotopic pattern and a negative mass defect, a suitable RT in RP-LC experiments, and their presence in the KWR suspect list.

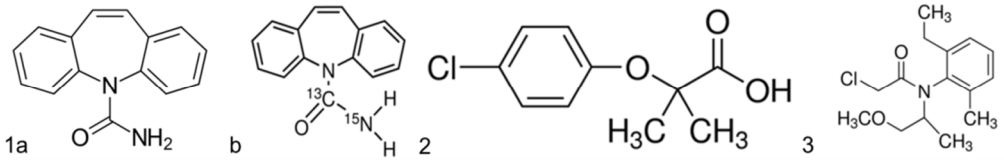


FIGURE 3. PARENT COMPOUND STRUCTURAL FORMULAS. 1A. CARBAMAZEPINE 1B. CARBAMAZEPINE-(CARBOXAMIDE-13C,15N) 2. CLOFIBRIC ACID 3. METOLACHLOR

4.2 Experimental set up (materials and methods)

4.2.1 Rapid sand filtration (RSF) experiments

For the RSF experiments, a RSF filter was sampled at the WRK pre-treatment plant of Waternet in Nieuwegein, the Netherlands (Figure 4). Real sand filter sand was used in order to supply the right microbial community for RSF experiments. This sand sample was mixed and transported in a closed PE bucket and cooled at 4 ˚C until use. Influent water of the sand filters was sampled weekly at the WRK water plant as well, using a 600 L stainless steel tank to transport the water to KWR. The water was filtered on site by 10 µm cartridge filters to remove particles that could block the laboratory columns filled with RSF material. After transport, the water was transferred to two separate 550 L RVS tanks to be used with the column setup.

FIGURE4A. SAMPLING OF RSF FILTERS AT WRK NIEUWEGEIN (WATERNET). B. COLUMN SETUP USED IN RSF LAB-SCALE EXPERIMENTS.

On the day following the sampling, the sand was flushed with drinking water (KWR, Nieuwegein) to remove the main part of dirt which was present between the grains. The sand was then transferred to glass columns (3,5 cm internal diameter, 100 cm height) to a final height of 80 cm, i.e. 770 mL, as a slurry to prevent air bubble entrapment. Two columns were filled following this procedure. To prevent clogging of the filters, the columns were then backwashed to remove residual dirt and air. Backwashing led to ~ 20 % expansion of the sand bed. The two column setup shown in Figure 4 was used to study the breakdown and potential formation of TPs by bacteria present in the RSF material. It allowed investigation of two parent compounds in parallel, i.e. in a first round metolachlor in one and clofibric acid in the other column, in the second round carbamazepine with and without label.

For the RSF experiments, two 550L stainless steel tanks were filled with WRK water. Parent compounds were added to a final spike-in concentration of 10 µg/L, and the water stirred for one hour with a mechanic stirrer. A flow of 4.8 L/h (velocity of 5.0 m/h) was set for both columns. For the first round of experiments, clofibric acid was added to one, and metolachlor to the other tank, respectively. The experiments lasted for 5 days, from Monday 26th to Friday 30th of June 2017. Samples of the influents and effluents were taken at day 0 (after 8 hours of flow[11]) and day 4[12] of experiments for analyses of parent compound

[11] 8 hours * 4,8 L/h = 38.4 L / (pi*(1,75/100)2*0,8) = 437 treated bed volumes
[12] 4*24hours *4.8L/h = 460.8 L / (pi*(1,75/100)2*0,8) = 5238 treated bed volumes

breakdown and TP formation. After the first round of experiments, the columns were flushed for 7 days with WRK water (no spike-in). For the second round of experiments, carbamazepine was added to one, and labelled carbamazepine to the other tank. The second round of experiments started Monday 10th of July 2017 and ran through to Friday 15th of July 2017. Again, samples were taken on day 1 and 5 samples for subsequent chemical analysis.

DATE	NO	SAMPLE NAME	TYPE	MATRIX
	1	METOLACHLOR	STD	UP
	2	CLOFIBRIC ACID	STD	UP
	3	CARBAMAZEPINE	STD	UP
	4	CARB LABEL	STD	UP
	5	BLANK MQ	BLANK	UP
23-06-17	6	WEEK 1 BLANK BEFORE FILTER	BLANK	SW
23-06-17	7	WEEK 1 BLANK AFTER FILTER	BLANK	SW
26-06-17	8	WEEK 2 ROUND 1 DAY 0 SPIKE-IN METOLACHLOR BEFORE FILTER	METOLACHLOR	SW
26-06-17	9	WEEK 2 ROUND 1 DAY 0 SPIKE-IN METOLACHLOR AFTER FILTER	METOLACHLOR	SW
26-06-17	10	WEEK 2 ROUND 1 DAY 0 SPIKE-IN CLOFIBRIC ACID BEFORE FILTER	CLOFIBRIC ACID	SW
26-06-17	11	WEEK 2 ROUND 1 DAY 0 SPIKE-IN CLOFIBRIC ACID AFTER FILTER	CLOFIBRIC ACID	SW
30-06-17	12	WEEK 2 ROUND 1 DAY 4 SPIKE-IN METOLACHLOR BEFORE FILTER	METOLACHLOR	SW
30-06-17	13	WEEK 2 ROUND 1 DAY 4 SPIKE-IN METOLACHLOR AFTER FILTER	METOLACHLOR	SW
30-06-17	14	WEEK 2 ROUND 1 DAY 4 SPIKE-IN CLOFIBRIC ACID BEFORE FILTER	CLOFIBRIC ACID	SW
30-06-17	15	WEEK 2 ROUND 1 DAY 4 SPIKE-IN CLOFIBRIC ACID AFTER FILTER	CLOFIBRIC ACID	SW
10-07-17	16	WEEK 4 BLANK BEFORE FILTER	BLANK	SW
10-07-17	17	WEEK 4 BLANK AFTER FILTER	BLANK	SW
10-07-17	18	WEEK 4 ROUND 2 DAY 0 SPIKE-IN CARBAMAZEPINE BEFORE FILTER	CARBAMAZEPINE	SW
10-07-17	19	WEEK 4 ROUND 2 DAY 0 SPIKE-IN CARBAMAZEPINE AFTER FILTER	CARBAMAZEPINE	SW

10-07-17	20	WEEK 4 ROUND 2 DAY 0 SPIKE-IN CARB LABEL BEFORE FILTER	CARB LABEL	SW
10-07-17	21	WEEK 4 ROUND 2 DAY 0 SPIKE-IN CARB LABEL AFTER FILTER	CARB LABEL	SW
14-07-17	22	WEEK 4 ROUND 2 DAY 4 SPIKE-IN CARBAMAZEPINE BEFORE FILTER	CARBAMAZEPINE	SW
14-07-17	23	WEEK 4 ROUND 2 DAY 4 SPIKE-IN CARBAMAZEPINE AFTER FILTER	CARBAMAZEPINE	SW
14-07-17	24	WEEK 4 ROUND 2 DAY 4 SPIKE-IN CARB LABEL BEFORE FILTER	CARB LABEL	SW
14-07-17	25	WEEK 4 ROUND 2 DAY 4 SPIKE-IN CARB LABEL AFTER FILTER	CARB LABEL	SW

TABLE 3. OVERVIEW OF RSF EXPERIMENTAL CONDITIONS AND SAMPLING

4.2.2 Ozonation experiments

With a BMT-laboratory setup (see Figure 5) several ozonation experiments were conducted with water spiked with the selected parent compound. Table 4 summarizes the experimental conditions.

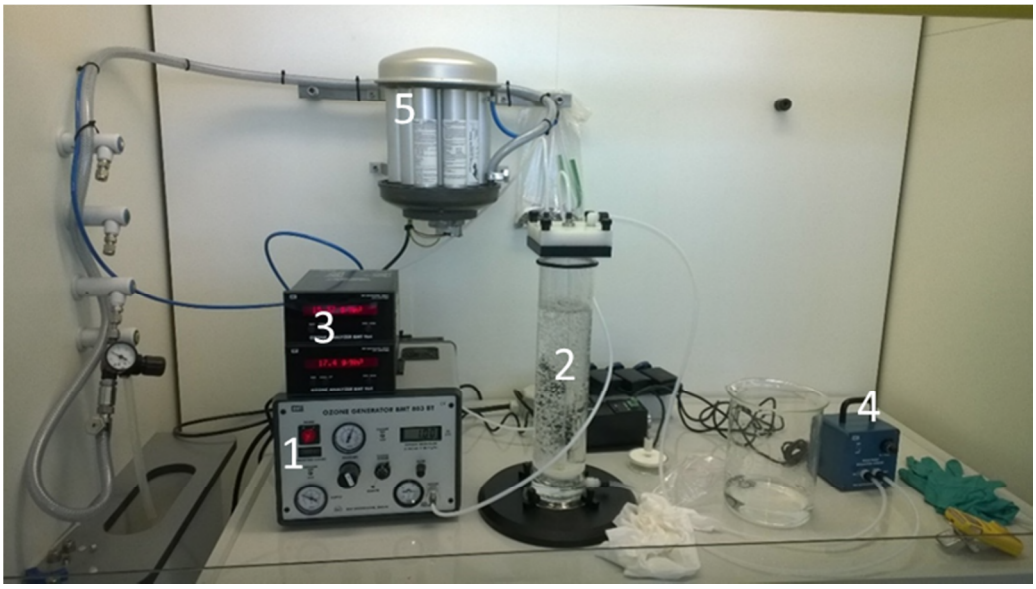


FIGURE 5. OZONATION SET-UP INCLUDING 1. OZONE GENERATOR WITH GAS CONTROL, 2. REACTION CHAMBER, 3. OZONE ANALYZER, 4. PUMP, 5. OXYGEN SUPPLY

1 L of water, containing a specific parent compound in a defined spike-in concentration, was poured into the reactor of the ozone setup. Then the ozone generator was started with a continuous flow of oxygen from an oxygen concentrator at 1 N-L/min. Influent and effluent ozone concentrations in the gas were continuously monitored with ozone-in-gas meters to measure the ozone consumption by the water in the reactor in time. Effluent gas with ozone

is treated using a heated catalyst to prevent ozone escaping to the environment, the setup was placed in a fume hood. Prior to the experiments, the ozone consumption of the empty system (without water) was measured and was used to correct the measured concentrations with a filled system. This blank value was established at 0.3 g ozone/m^3 (or 0.3 mg ozone/L). With a small pump the water was continuously recirculated from top to bottom in the opposite direction of the gas flow. A tap was mounted to enable sampling from the recycling stream. After 1 and 6 min, respectively, a sample was taken (60 mL) using the sample tap. After this the experiment was stopped, the reactor flushed and filled with the new water batch. During each experiment ozone concentrations and gas flow were monitored every minute to be able to calculate the ozone consumption.

In blank experiments water samples were exposed to oxygen from the oxygen concentrator without starting the ozone generator. The water used for the standard solutions was ultrapure water (UP, Veolia), the surface water used for the conversion experiments was water from the Waternet Leiduin plant before ozonation.

It has to be noted that a brief (few seconds) spike of ozone (concentration up to 120 g ozone/m^3) occurred at the start of every experiment which could not be prevented using the described set-up. In addition, the set-up generated relatively high ozone concentrations also at the lowest possible settings. This occurred during all experiments and probably influenced the results. Preventing this peak and the ability of using lower ozone concentrations should be studied before starting new experiments with this setup.

no	sample bottle	sample prep	ozonation sample name	type	matrix
O1	metolachlor std	std samples need to be diluted from 10mg/L to 10ug/L (1:1000 in UP)	metolachlor	std	UP
O2	clofibric acid		clofibric acid	std	UP
O3	carbamazepine		carbamazepine	std	UP
O4	carb label		carb label	std	UP
O5	blank UP	UP	blank UP	blank	UP
O6	SW	SW	SW blank no ozone	blank	SW
O7	SW	SW	SW blank oxygen	blank	SW
O8	SW	SW	SW blank low ozone	blank	SW
O9	SW	SW	SW blank high ozone	blank	SW
O10	metolachlor 10	metolachlor 10: 1mL 10mg/L stock in 1000mL SW -> 10ug/L	metolachlor 10ug/L no ozone	sample	SW
O11	metolachlor 10		metolachlor 10ug/L low ozone	sample	SW
O12	metolachlor 10		metolachlor 10ug/L high ozone	sample	SW
O13	metolachlor 100	metolachlor 100: 10mL 10mg/L stock in 1000mL SW -> 100ug/L	metolachlor 100ug/L no ozone	sample	SW
O14	metolachlor 100		metolachlor 100ug/L low ozone	sample	SW
O15	metolachlor 100		metolachlor 100ug/L high ozone	sample	SW
O16	clofibric acid 10	clofibric acid 10: 1mL 10mg/L stock in 1000mL SW -> 10ug/L	clofibric acid 10ug/L no ozone	sample	SW
O17	clofibric acid 10		clofibric acid 10ug/L low ozone	sample	SW

O18	clofibric acid 10	clofibric acid 100: 10mL 10mg/L stock in 1000mL SW -> 100ug/L	clofibric acid 10ug/L high ozone	sample	SW
O19	clofibric acid 100		clofibric acid 100ug/L no ozone	sample	SW
O20	clofibric acid 100		clofibric acid 100ug/L low ozone	sample	SW
O21	clofibric acid 100		clofibric acid 100ug/L high ozone	sample	SW
O22	carbamazepine 10	carbamazepine 10: 1mL 10mg/L stock in 1000mL SW -> 10ug/L	carbamazepine 10ug/L no ozone	sample	SW
O23	carbamazepine 10		carbamazepine 10ug/L low ozone	sample	SW
O24	carbamazepine 10		carbamazepine 10ug/L high ozone	sample	SW
O25	carbamazepine 100	carbamazepine 100: 10mL 10mg/L stock in 1000mL SW -> 100ug/L	carbamazepine 100ug/L no ozone	sample	SW
O26	carbamazepine 100		carbamazepine 100ug/L low ozone	sample	SW
O27	carbamazepine 100		carbamazepine 100ug/L high ozone	sample	SW
O28	carb label 10	carb label 10: 1mL 10mg/L stock in 1000mL SW -> 10ug/L	carb label 10ug/L no ozone	sample	SW
O29	carb label 10		carb label 10ug/L low ozone	sample	SW
O30	carb label 10		carb label 10ug/L high ozone	sample	SW
O31	carb label 100	carb label 100: 10mL 10mg/L stock in 1000mL SW -> 100ug/L	carb label 100ug/L no ozone	sample	SW
O32	carb label 100		carb label 100ug/L low ozone	sample	SW
O33	carb label 100		carb label 100ug/L high ozone	sample	SW
O34	carb mix 10	carb mix 10: 1mL 10mg/L stock carbamazepine plus 1mL 10mg/L stock carb label in 1000mL SW -> 10ug/L	carb mix 10ug/L no ozone	sample	SW
O35	carb mix 10		carb mix 10ug/L low ozone	sample	SW
O36	carb mix 10		carb mix 10ug/L high ozone	sample	SW
O37	carb mix 100	carb mix 100: 10mL 10mg/L stock in 1000mL SW -> 100ug/L	carb mix 100ug/L no ozone	sample	SW
O38	carb mix 100		carb mix 100ug/L low ozone	sample	SW
O39	carb mix 100		carb mix 100ug/L high ozone	sample	SW

TABLE 4. SUMMARY OF SAMPLES AND EXPERIMENTAL CONDITIONS IN OZONATION EXPERIMENTS

Under the conditions shown in Table 4 an average ozone consumption of 5.60 ± 0.32 mg/L (low ozone) and 11.78 ± 0.65 mg/L (high ozone) in surface water was measured. The ozone consumption thus exceeds the 0.7-2.0 mg/L ozone dosage that is routinely applied at Waternet, which could result in the formation of more and/or other TPs. The consumption however is depending on the concentration of organics in the water that will consume ozone, like humic acids and the dosed compounds. Compounds dosed were in concentration levels of 10 and 100 µg/L while humic acids were present measured as TOC at a

concentration level of 2.2mg C/L. In the ultrapure water about 5-10 µg C/L was present (as measured by the Veolia system).

4.2.3 LC-HRMS based non-target screening

A Tribrid Orbitrap Fusion mass spectrometer (ThermoFisher Scientific, Bremen, Germany) provided with an electrospray ionisation source was interfaced to a Vanquish HPLC system (ThermoFisher Scientific). For the chromatographic separation an XBridge BEH C18 XP column (150 mm × 2.1 mm I.D., particle size 2.5 µm) (Waters, Etten-Leur, The Netherlands) preceded by a 2.0 mm × 2.1 mm I.D. Phenomenex SecurityGuard Ultra column. Phenomenex, Torrance, USA) maintained at a temperature of 25 °C was used. The gradient started with 5% acetonitrile, 95% water and 0.05% formic acid (v/v/v), increased to 100% acetonitrile with 0.05% formic acid in 25 min, and was held constant for 4 min at a flow rate of 0.25 mL/min. 100 µL of sample was used for injection. With every batch run mass calibration was performed using Pierce ESI positive and negative ion calibration solution. The vaporizer and capillary temperature were maintained both at 300 °C. Sheath, auxiliary and sweep gas was set to arbitrary units of 40, 10 and 5. The source voltage was set to 3.0 kV in the positive mode, and -2.5kV the negative mode respectively. The RF lens was set to 50 %. Full scan high accuracy mass spectra was acquired in the range of 50-1000 m/z with the resolution set at 120,000 FWHM and quadruple isolation were used for acquisition with a 5 ppm mass window. Data dependent acquisition was performed using a High Collision Dissociation (HCD) energy at 35% and an FT resolution of 15,000 FWHM.

4.2.4 Data processing and analysis

The acquired data was processed using Compound Discoverer 2.1 (Thermo Fisher) for peak picking, componentization, chlorine pattern scoring, suspect screening and automatic MS2 fragment searches in mzCloud. An overview of the Compound Discoverer workflow is depicted in 0. A summary of the data processing parameters and Compound Discoverer 2.1 settings used to analyze metolachlor data is provided in Appendix IV. Settings for the analysis of clofibric acid and carbamazepine data were the same, apart from the mass list node that enables suspect screening. For each parent compound, suspect screening was performed against an in-house curated TP suspect list specific for the selected parent compound via the mass list node, as well as the EAWAG Biocatalysis/Biodegradation and EPA DSSTox databases via the ChemSpider node. The in-house suspect lists were generated through literature mining for known environmental TPs and metabolites[59-73], entries in the NORMAN SusDat [13] and the STOFF-IDENT [14] databases, and *in silico* prediction using EnviPATH [15]. The suspect lists for potential TPs of metolachlor, clofibric acid and carbamazepine are provided in Appendix V, VI, and VII, respectively.

After processing, significance testing and fold change filtering (see text box) was applied to identify potential TPs, and illustrated using Volcano plots [31] in Compound Discoverer 2.1. These scatter plots display the log2 fold change (log2FC) and the negative log10-transformed p-values of features. They are an effective and easy to interpret presentation of the changes between before and after treatment groups, and thus TP formation.

[13] http://www.norman-network.com/?q=node/236
[14] https://www.lfu.bayern.de/stoffident/
[15] https://envipath.org/

Significance testing and fold change filtering

- Filter 1: after / before water treatment: log2FC >1, p<0.05[16]

Significant increase in response indicates that the feature is formed during water treatment.

- Filter 2: spike-in / no spike: log2FC>2, p<0.05[17]

Significantly higher response in the spike-in compared to the no-spike sample indicates that the feature originates from the parent compound.

As the selected parent compounds can be present in the source water, but at concentrations 100x lower than the lowest spike-in concentration, this filtering step does not compromise TP identification, and greatly reduces data complexity.

The TPs thereby identified were further inspected using MS1 full scan data in regard to available suspect screening matches, and if applicable presence of a halogen or label. For features matching a suspect list entry, identification was attempted using MS2 fragmentation data for spectral library searches against mzCloud[18], FiSH scoring in Compound Discoverer 2.1 (Thermo Fisher Scientific), and MetFrag queries[19], including MassBank of North America searches[20].

4.3 Results and discussion

4.3.1 Degradation of the parent compounds

First, the parent compound degradation with the different treatments was assessed. The consistent retention times and the high mass accuracy resulting in good reproducibility between experiments facilitated comparison of peak intensities of the parent compounds between samples. The log2FC between parent compound peak areas in the before and after treatment samples measured in triplicate for statistical power could thus be determined. We defined a significant increase as a log2FC >0.25, a significant decrease as a log2FC< -0.25, both with p<0.05. These cut-offs reflect a roughly 20% difference, which seemed appropriate considering the measurement accuracy. Parent compounds with corresponding ionization modes, molecular weight, RT and log2FC across the different experimental conditions are summarized in Table 5.

[16] p-value with Benjamini-Hochberg correction

[17] p-value with Benjamini-Hochberg correction

[18] https://www.mzcloud.org/
[19] https://msbi.ipb-halle.de/MetFragBeta/
[20] http://mona.fiehnlab.ucdavis.edu/

not significant no change according to cut-off , but statistically significant (-0.25< log2FC < 0.25. p< 0.05) significant increase (log2FC > 0.25. p< 0.05) significant decrease (log2FC <-0.25 p< 0.05)	Feature sand filtration	Feature ozonation	Sandfiltration		Ozonation			
			day 0	day 4	10ug/L spike-in		100ug/L spike-in	
Change of parent compound abundance during drinking water treatment. + / - indicates ionisation mode. Log2FC (after / before treatment). NB: log2FC 0.25 ~ 20%increase	(molecular weight / retention time)	(molecular weight / retention time)	Effluent / influent	Effluent / influent	low O3 / no O3	high O3 / no O3	low O3 / no O3	high O3 / no O3
Metolachlor (+)	283.13343 / 18.905	283.13346 / 18.898	0.25	-0.35	-5.08	-5.69	-11.33	-12.30
Clofibric acid (-)	214.03942 / 15.858	214.03957 / 15.874	-0.24	-0.20	-6.56	-12.59	-5.06	-11.54
Carbamazepine (+)	236.09488 / 13.263	236.09471 / 13.264	0.37	-0.02	-14.03	-14.01	-10.37	-15.68
Carbamazepine-(carboxamide-^{13}C,^{15}N) (+)	238.09527 / 13.259	238.09511 / 13.261	0.05	-0.17	-13.84	-13.87	-14.58	-15.06

TABLE 5. PARENT COMPOUND CHANGES, EXPRESSED IN LOG2FC UNITS BETWEEN AFTER / BEFORE TREATMENT. (+ / -) INDICATES IONISATION MODE. RETENTION TIME IS IN MINUTES. NB: LOG2FC -0.25 ~ 20% DECREASE OF PARENT COMPOUND.

No significant decrease of parent compounds was observed in sand filtration experiments. This was expected for carbamazepine, which initially showed a slight signal increase (~30%). However, after four days there was no longer any difference in carbamazepine signal between influent and effluent samples. This might be due to sorption or/and charging. As carbamazepine is a hydrophobic compound and neutral at pH7, the column might need to first stabilize. Metolachlor and clofibric acid are known to be degraded in soil, witha DT50 of months, and weeks to month, respectively. A significant decrease of these parent compounds was thus expected, but not observed. This could result from the continuous flow of spiked-in parent compound which might mask the decrease in concentration. As biodegradation can depend on the bacterial population present, it could be that no degradation was observed due to the lack of appropriate microorganisms in the RSF sand[74-80]. In the case of clofibric acid, a significant change between effluent and influent was observed, albeit the log2FC was smaller than the arbitrarily defined cut-off of -0.25. This could indicate low rates of degradation and thus potential TP formation. A data-driven or inert tracer based cut-off could alleviate this issue in future experiments.

In contrast, all parent compounds showed a significant decrease in ozonation experiments, with an overall stronger decrease in parent compound signal at higher ozone concentrations. Interestingly, in the case of metolachlor ozone degradation seemed to be dependent on the spike-in concentration, with the lower spike-in concentrations showing less decrease in peak intensities. In contrast, the extent of clofibric acid degradation by ozonation seemed to be dependent on the ozone concentration, with higher ozone concentrations leading to a stronger decrease. The decrease of both labelled and not labelled carbamazepine was similar under all experimental conditions, and more pronounced than that of metolachlor and clofibric acid. The two distinct reactions occurring during ozonation might be responsible for these results, i.e. direct and indirect reaction of ozone with the target molecule. Carbamazepine is known to react directly with ozone, however, metolachlor and clofibric acid react indirectly through the hydroxyl radicals that are generated by decomposition of ozone in aqueous medium [15].

4.3.2 Formation of TPs

Next, it was determined whether the degradation of parent compounds resulted in mineralization or TP formation. Moreover, identification of TPs, potentially specific for the different treatment conditions was attempted. Therefore, peak areas of all features were compared between the before and after treatment groups. Subsequently, filtering steps were applied to reduce the peak number to those peaks that are potential TPs. As TPs are formed

during treatment, only peaks that showed a log2FC >1 ($p<0.05$) between treatments were kept. Additionally, peaks with a log2FC <2 between spike-in and no spike samples were discarded, as these peaks could potentially be TPs that are formed from a parent compound present in the source water, and thus not derived from the spiked-in parent compounds. The benefits of these filtering steps are illustrated in the volcano plots in Figure 6 and Figure 7.. These plots show the features detected in metolachlor sand filtration and ozonation experiments, respectively, with the log2FC plotted on the x-axis against the –log10 of the p-value on the y-axis. The colored squares highlight significant increase in red and decrease in green of a feature. The red features in the red square are thus potential TPs. In addition, blue features have a log2FC>2 ($p<0.05$) between spike-in and no spike samples. Hence these features pass both filters and are therefore potential TPs of the spiked-in parent compound, i.e. here metolachlor TPs. Interestingly, although no substantial degradation of metolachlor had been observed in sand filtration experiments (Table 5), a TP is observed.

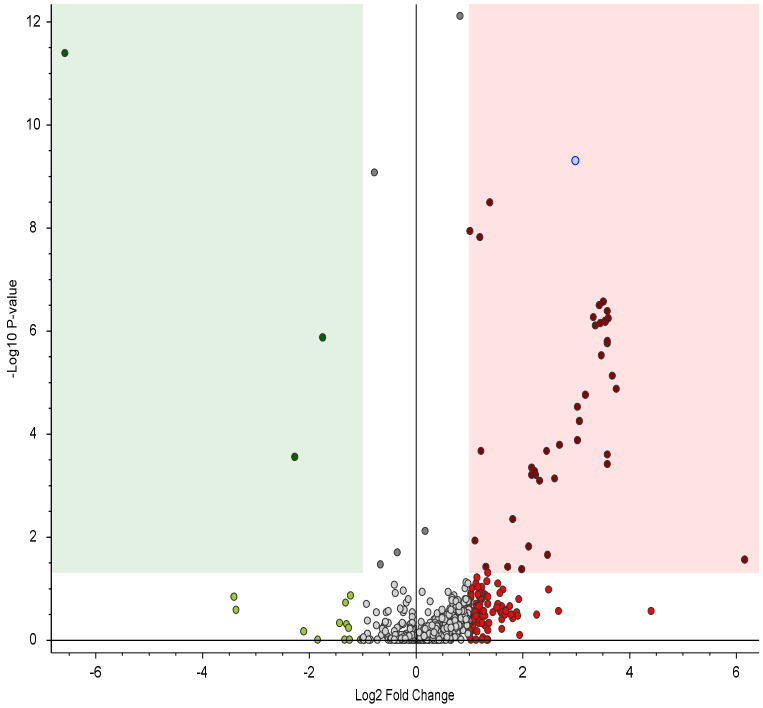


FIGURE 6. VOLCANO PLOT OF METOLACHLOR FEATURES IN SAND FILTRATION EXPERIMENTS. DAY 0. FEATURES ARE PLOTTED AS DOTS ACCORDING TO FOLD CHANGE BETWEEN TREATMENTS AND SIGNIFICANCE VALUE. COLORED RECTANGLES INDICATE SIGNIFICANCE ($P<0.05$) AND LOG2FC BETWEEN EFFLUENT AND INFLUENT SAMPLES <-1 (GREEN), OR LOG2FC >1 (RED). LOG2FC BETWEEN SPIKE-IN AND NO SPIKE >2 INDICATED IN BLUE.

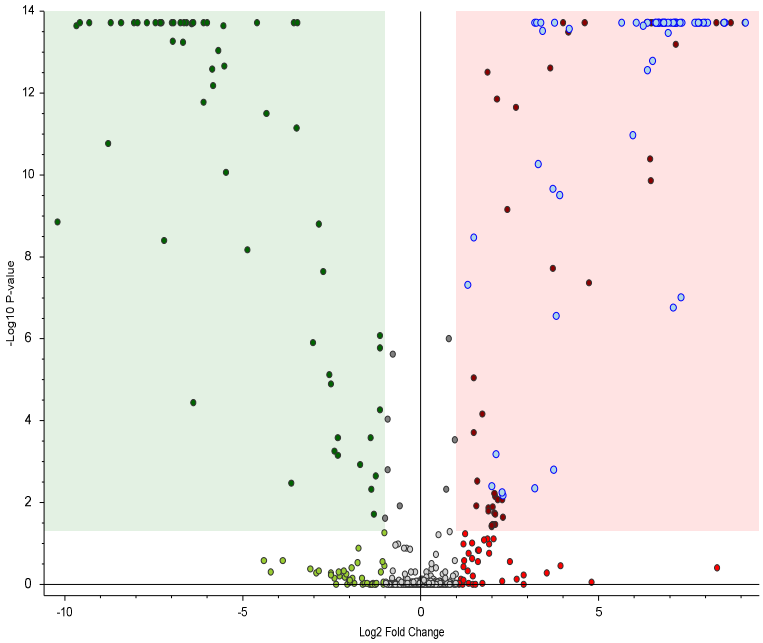


FIGURE 7. VOLCANO PLOT OF METOLACHLOR FEATURES IN OZONATION EXPERIMENTS. 100UG/L SPIKE-IN, LOW OZONE CONCENTRATION. FEATURES ARE PLOTTED AS DOTS ACCORDING TO FOLD CHANGE BETWEEN TREATMENTS AND SIGNIFICANCE VALUE. COLORED RECTANGLES INDICATE SIGNIFICANCE (P<0.05) AND LOG2FC BETWEEN EFFLUENT AND INFLUENT SAMPLES <-1 (GREEN), LOG2FC >1 (RED). . LOG2FC BETWEEN SPIKE-IN AND NO SPIKE >2 INDICATED IN BLUE.

4.3.2.1 Metolachlor: 1 biotic RSF TP versus 68 abiotic ozonation TPs

The results of the filtering steps are summarized in Tables 6 and 7 for metolachlor. A total of 214 TPs were identified across all experiments (>90% in positive ionization mode), 124 of which exhibited an isotopic pattern suggesting the presence of a chlorine atom. 69 of these features were unique, and 40 were dechlorinated. Sand filtration led to the formation of a single, dechlorinated TP already on day 0, which persisted through day 4. The sand filtration TP matched with the suspect screening candidate deschlormetolachlor, which is listed in SusDat and STOFF-IDENT, and was predicted by EnviPATH. MS2 fragmentation data based FiSH scoring allowed to confirm the structural identification (see 0I, confidence level 2 according to Schymanski [24]). All 194 other TPs were formed through ozonation, with more than half comprising the chlorine atom. There was substantial overlap in TP formation with the different spike-in and ozone concentrations. On average a TP was detected in three different experimental conditions (see 0IX). The majority of TPs could not be assigned to a suspect. Merely a single abiotic TP could be matched to a suspect list entry, namely an EnviPATH predicted compound with the SMILES CCC1=C(C(=CC=C1)C)N(C(C)CO)C(=O)CCl and corresponding chemical formula C14H20Cl1N1O2. As EnviPATH is a prediction tool for biodegradation reactions, an overlap between ozonation TPs and predicted biodegradation TPs was not per se expected. However, MS2 based FiSH scoring (see Appendix X) and MetFrag fragmentation (data not shown) confirmed the EnviPATH predicted compound (confidence level 2), while it rejected the two known compounds alachlor and acetochlor which have the same accurate mass, but no fragmentation peak at m/z 176.14305.

Metolachlor			potential TP pos	potential TP neg	with Cl pos	with Cl neg
sand filtration	10ug/l spike-in	day 0 day 4	1 1	0 0	0 0	0 0
ozonation	10ug/l spike-in	low O3 high O3	57 28	5 3	38 15	1 0
	100ug/L spike in	low O3 high O3	62 47	5 5	38 30	1 1
			196	18	121	3
total number			214		124	
unique features			69		40	

TABLE 6. COUNTS OF POTENTIAL METOLACHLOR TRANSFORMATION PRODUCTS.FILTERERING PARAMETERS WERE: LOG2FC BEFORE/AFTER TREATMENT >1, LOG2FC SPIKE-IN/NO SPIKE >2, AND P< 0.05.POS AND NEG ARE POSITIVE AND NEGATIVE IONISATION.

Metolachlor			match with suspect list	tentative ID
sand filtration	10ug/l spike-in	day 0 day 4	249.17233 / 17.148	Deschlormetolachlor (CAS 126605-22-9; Norman SusDat, StoffIDENT, EnviPATH)
ozonation	10ug/l spike-in	low O3 high O3	269.11772 / 15.421	CCC1=C(C(=CC=C1)C)N(C(C)CO)C(=O)CCl (EnviPATH)
	100ug/L spike in	low O3 high O3		

TABLE 7. FEATURES WITH ACCURATE MASS MATCHING A POTENTIAL TP OF THE METOLACHLOR SUSPECT LIST AND TENTATIVE IDENTIFICATION. THE 3RD COLUMN INDICATES MOLECULAR WEIGHT AND RT IN MINUTES.

4.3.2.2 Clofibric acid: abundance of biotic TPs & increase of biodegradation over time

An overview of clofibric acid TP formation is shown in Table 8 and Table 9, and a detailed list of identified clofibric acid TPs can be found in 0I. 194 TPs were formed in all experiments together, roughly one third of the TPs was detected in negative ionization mode, the mode of ionization of the parent compound. There was minor overlap between experimental conditions, 161 of the 194 TPs were unique. This is due to the fact that about 80% of all TPs were detected in sand filtration experiments, and 3 out of 4 on day 4 of the biodegradation time course. Only 5 of the 173 biotic TPs were chlorinated, indicating that dechlorination was one of the main biodegradation pathways occurring. Ozonation resulted in the formation of 13 unique TPs, only one of which was chlorinated.

Clofibric acid			potential TP pos	potential TP neg	with Cl pos	with Cl neg
sand filtration	10ug/l spike-in	day 0	8	7	0	0
		day 4	107	51	0	5
ozonation	10ug/l spike-in	low O3	5	2	1	0
		high O3	3	1	1	0
	100ug/L spike in	low O3	1	3	0	0
		high O3	0	6	0	0
			124	70	2	5
total number			194		7	
unique features			161		6	

TABLE 8. COUNTS OF POTENTIAL CLOFIBRIC ACID TRANSFORMATION PRODUCTS. FILTERERING PARAMETERS WERE: LOG2FC BEFORE/AFTER TREATMENT >1, LOG2FC SPIKE-IN/NO SPIKE >2, AND P< 0.05.POS AND NEG ARE POSITIVE AND NEGATIVE IONISATION.

As was the case for metolachlor TPs, suspect list hits for clofibric acid TPs were limited. Again one single biotic and one single abiotic TP[21] could be matched, specifically the EnviPATH predicted structure CC(C)OC1=CC=CC=C1 in sand filtration, and alpha-hydroxyisobutyric acid in ozonation. Interestingly the abiotic TP had been described in biodegradation experiments previously [61], and was predicted by EnviPATH. MS2 based FiSH scoring and MetFrag queries (0) structurally confirmed alpha-hydroxyisobutyric acid (confidence level 2).

Clofibric acid			match with suspect list	tentative ID
sand filtration	10ug/l spike-in	day 0		
		day 4	136.08849 / 9.425	CC(C)OC1=CC=CC=C1 (EnviPATH)
ozonation	10ug/l spike-in	low O3		
		high O3		
	100ug/L spike in	low O3	104.04685 / 2.299; 104.04685 / 2.456	Alpha-Hydroxyisobutyric (Salgadoac et al., EnviPATH)
		high O3		

TABLE 9. FEATURES WITH ACCURATE MASS MATCHING A POTENTIAL TP OF THE CLOFIBRIC ACID SUSPECT LIST AND TENTATIVE IDENTIFICATION. THE 3RD COLUMN INDICATES MOLEDUCLAR WEIGHT AND RT IN MINUTES.

4.3.2.3 Carbamazepine: labelling facilitates efficient TP detection

Finally, carbamazepine experiments were performed in duplicate, with carbamazepine (Figure 3, 1a) and carbamazepine-(carboxamide-^{13}C,^{15}N) (Figure 3, 1b), the results of which are summarized in Tables 10 and 11, respectively. Experiments without label resulted in a total of 135 potential TPs and 78 unique features, experiments with label in 81, of which 29 unique, respectively. TPs were detected to similar extents using positive and negative ionization mode. The discrepancy in TP abundance between the non labelled and labelled compound, which in theory should form the exact same TPs without and with the label, may be related to difficulties in peak picking for the labelled TPs. As the monoisotopic peak is missing, the shift in isotopic distribution might hinder peak picking. This could potentially

[21] alpha-hydroxyisobutyric acid was matched twice to two isobaric features with RTs differing by less than 0.2 minutes

be resolved in future experiments by manually adding the isotopic pattern of the label to the pattern recognition node in Compound Discoverer 2.1. Alternatively, slightly different experimental conditions across experiments, such as actual ozone concentrations, bacterial populations in the RSF, etc. might be responsible for the different results and consequently limited overlap in carbamazepine TPs.

Carbamazepine			potential TP pos	potential TP neg
sand filtration	10ug/l spike-in	day 0 day 4	2 0	2 0
ozonation	10ug/l spike-in	low O3 high O3	14 4	14 11
	100ug/L spike in	low O3 high O3	44 9	27 8
			73	62
total number			135	
unique features			78	

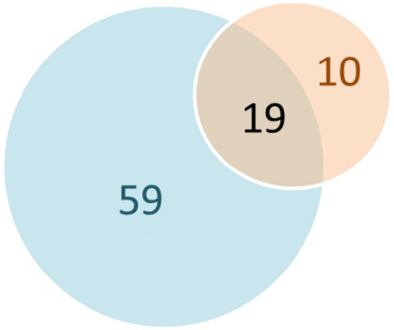
TABLE 10. COUNTS OF POTENTIAL CARBAMAZEPINE TRANSFORMATION PRODUCTS. FILTERERING PARAMETERS WERE: LOG2FC BEFORE/AFTER TREATMENT >1, LOG2FC SPIKE-IN/NO SPIKE >2, AND P< 0.05.POS AND NEG ARE POSITIVE AND NEGATIVE IONISATION.

Carbamazepine labelled			potential TP pos	potential TP neg
sand filtration	10ug/l spike-in	day 0 day 4	1 3	2 2
ozonation	10ug/l spike-in	low O3 high O3	10 4	13 9
	100ug/L spike in	low O3 high O3	8 6	11 12
			32	49
total number			81	
unique features			29	

TABLE 11. COUNTS OF POTENTIAL LABELLED CARBAMAZEPINE TRANSFORMATION PRODUCTS. FILTERERING PARAMETERS WERE: LOG2FC BEFORE/AFTER TREATMENT >1, LOG2FC SPIKE-IN/NO SPIKE >2, AND P< 0.05.POS AND NEG ARE POSITIVE AND NEGATIVE IONISATION.

In addition to the statistical testing and fold change filtering, the labelling strategy allows to use an additional, more stringent criterion, i.e. the overlap between the two experimental groups, to filter for features representing TPs. An added benefit here that could also be achieved without labelling, is that the experiment is performed in duplicate which by itself will lead to more stringent results, and more confident TP identification. In the carbamazepine experiments, 19 TPs overlapped as illustrated in Figure 8. Of these, 8 TPs showed the characteristic 2Da mass shift, indicating that the labelled residues were still present in the compound. None of the biotic TPs showed overlap between groups, in line with what was observed for metolachlor and clofibric acid, indicating that sand filtration is

more susceptible to slight changes in experimental conditions, and thus less reproducible. In case of the abiotic TPs of carbamazepine, 19 of the 24 labelled TPs from ozonation overlapped, representing 83% overlap.



no label	78	
label	29	
overlap *	19	11 same M 8 2Da mass shift

FIGURE 8. OVERLAP OF TPS FROM CARBAMAZEPINE WITHOUT AND WITH LABEL IN ALL EXPERIMENTS

All overlapping features are listed in Table 12. The eight TPs exhibiting the 2Da mass shift were manually inspected for peak duplets in the raw data of the mixed label experiment (see 0I). For four of the twenty overlapping TPs, suspect list matches were found, for two of them multiple suspects were possible based on the accurate mass alone. MS2 based FiSH scoring and MetFrag queries (0) enabled unambiguous identification of the three TPs 1-(2-benzoic acid)-(1H,3H)-quinazoline-2,4-dione (BaQD) [60, 81], 1-(2-benzaldehyde)-(1H,3H)-quinazoline-2,4-dione (BQD) and 1-(2-benzoic acid)-4-hydro(1H,3H)-quinazoline-2-one (BaQM, Azaïs, Mendret et al. 2017) with confidence level 2. The fragmentation spectra of the fourth TP with a suspect list match could be equally well explained by *in silico* fragmentation spectra of acridone and 9-hydroxy-acridine, which are tautomers and have both been previously reported TPs of carbamazepine [82] (Appendix XV).

TABLE 12. OVERLAP TRANSFORMATION PRODUCTS OF CARBAMAZEPINE WITHOUT AND WITH LABEL.THE FIRST COLUMN INDICATES NO LABEL (BLUE) AND LABEL (ORANGE).IN THE SECOND COLUMN, FEATURES WITH 2DA MASS SHIFT ARE LISTED IN PURPLE, FEATURES WITH SAME MASS IN BLUE.THE TOTAL NUMBER OF DETECTED TPS ARE SHOWN WITH A COLOR GRADIENT WITH SINGLE HITS IN RED AND MOST HITS IN GREEN. TPS DETECTED IN THE DIFFERENT OZONATION CONDITIONS ARE MARKED WITH GREEN CELLS.

	Feature (Molecular weight / Retention time)	total number detected	pos low t1	pos low t6	pos high t1	pos high t6	neg low t1	neg low t6	neg high t1	neg high t6	Suspect list match	MS2 match
	119.037 / 8.676	4	1	1	1	1	0	0	0	0	no match	
	119.03698 / 8.674	4	1	1	1	1	0	0	0	0		
	119.07336 / 2.899	2	0	0	1	1	0	0	0	0	no match	
	119.0734 / 2.894	1	0	1	0	0	0	0	0	0		
	130.06241 / 4.747 // 130.06242 / 4.624 // 130.06243 / 4.531	4	0	0	0	0	1	1	1	1	no match	
	130.06245 / 4.499	4	0	0	0	0	1	1	1	1		
	137.04709 / 8.682 // 137.04751 / 8.676	7	1	1	1	1	1	0	1	1	no match	
	137.0471 / 8.658 // 137.04753 / 8.673	7	1	1	1	1	1	0	1	1		
	146.04782 / 5.965	2	1	0	1	0	0	0	0	0	no match	
	148.04822 / 5.967	2	1	0	1	0	0	0	0	0		
	146.0574 / 6.219	2	0	0	0	0	0	0	1	1	no match	
	146.05737 / 6.232	3	0	0	0	0	1	1	0	1		
	162.04267 / 7.132	3	1	0	1	1	0	0	0	0	no match	
	164.04276 / 7.14 // 164.04309 / 7.13	6	1	0	1	1	1	0	1	1		
	162.11565 / 2.283	3	1	1	1	0	0	0	0	0	no match	
	162.11558 / 2.287	1	1	0	0	0	0	0	0	0		
	165.07852 / 2.83 // 165.07877 / 2.895	3	0	0	1	1	0	0	1	0	no match	
	165.07878 / 2.895	1	0	1	0	0	0	0	0	0		
	180.053 / 8.685	4	0	0	0	0	1	1	1	1	no match	
	182.05339 / 8.655	4	0	0	0	0	1	1	1	1		
	195.068 / 11.198	3	0	0	0	0	1	0	1	1	acridone, 9-hydroxy-acridine	FISh scoring - no match. MetFrag 2.0 search against acridone 1/4 fragments match.
	195.06801 / 11.192	2	0	0	0	0	1	0	1	0		
	200.05025 / 10.172	3	0	0	0	0	1	1	1	0	no match	
	200.05022 / 10.184	2	0	0	0	0	0	1	0	1		
	208.01903 / 9.23	1	0	0	0	0	0	0	1	0	no match	
	208.01901 / 9.234	1	0	0	0	0	1	0	0	0		
	238.07397 / 11.2	3	0	0	0	0	1	0	1	1	no match	
	240.0744 / 11.192	3	0	0	0	0	1	0	1	1		
	264.05294 / 11.193	3	1	0	1	1	0	0	0	0	no match	
	266.05342 / 11.19	3	1	0	1	1	0	0	0	0		
	266.06868 / 10.307	2	1	0	1	0	0	0	0	0	1-(2-Benzaldehyde)-(1H,3H)-quinazoline-2,4-dione (BQD); BaQM	BaQM (Azais et al.)
	268.06918 / 10.303	2	1	0	1	0	0	0	0	0		
	266.06871 / 11.78	2	1	0	1	0	0	0	0	0	1-(2-Benzaldehyde)-(1H,3H)-quinazoline-2,4-dione (BQD); BaQM	BQD (McDowell et al., Azais et al.)
	268.06914 / 11.775	2	1	0	1	0	0	0	0	0		
	282.06345 / 11.194	4	1	1	1	1	0	0	0	0	1-(2-benzoic acid)-(1H,3H)-quinazoline-2,4-dione (BaQD)	BaQD (McDowell et al., Azais et al.)
	284.06392 / 11.19 // 284.06432 / 11.193	7	1	0	1	1	1	1	1	1		
	337.86582 / 23.606	1	0	0	0	0	0	1	0	0	no match	
	337.86583 / 23.489	1	0	0	0	0	0	1	0	0		

Features that overlap in carbamazepine without and with label experiment after

4.4 Conclusions: suitability and performance of the developed workflow

The applied workflow combining bottom-up and top-down approaches allowed monitoring of TP formation in a lab-scale experiment. TP identification based on log2FC filters and statistical significance between before and after treatment, and spike-in and no spike samples was efficient in revealing an abundance of drinking water treatment-specific TPs. Interestingly, an absence of significant parent compound degradation could also be accompanied in TP formation. The distinct isotopic pattern of chlorine in metolachlor and clofibric acid samples, as well as the 2Da mass shift of the label in carbamazepine experiments further facilitated TP identification. Monitoring three different parent compounds and two drinking water treatments in parallel allowed to assess similarities and differences between their biotic and abiotic TPs. Prior to the start of the lab-scale experiment, it was known that carbamazepine readily reacted with ozone and was not susceptible to biodegradation. Correspondingly the majority of carbamazepine TPs was formed during ozonation. However, sand filtration did result in the formation of a small number of TPs, which was surprising, but in line with previous research reporting minor degradation of carbamazepine in laboratory scale experiments[83-84], and the isolation of bacterial strains able to biodegrade the pharmaceutical [85]. Metolachlor formed one single biotic TP, which was dehalogenated. Clofibric acid TPs, in contrast, were mainly formed during sand filtration, and less than 5% contained a chlorine atom. Dehalogenation thus seemed an ubiquitous process in the biodegradation experiments performed, which is in line with dehalogenation being a thermodynamically favorable reaction[86]. In particular TPs formed by biodegradation varied between experiments, performing experiments in duplicates or triplicates would be more meaningful, but was logistically not possible for the sand filtration set-up. Another significant difference between the two halogenated parent compounds metolachlor and clofibric acid was that 2/3 of the metolachlor ozonation TPs still contained the chlorine atom, while only a single clofibric acid TP did. The structural positioning of the chlorine atom strongly influences its breakdown during ozonation experiments and thus TP formation.

As described in 3.2.3, top-down approaches can include logical filters concerning molecular weight and RT to identify TPs (Helbling, Hollender et al. 2010, Escher and Fenner 2011); the hypothesis being that TPs are more polar than their parent compound, and therefore elute earlier in RP-LC runs, and that they are smaller than their parent compounds. However, when the sand filtration and ozonation data sets were examined in regards to molecular weight and RT distribution as illustrated in Figure 9, metolachlor was the only parent compound of which all TPs had shorter RT, and molecular weight distribution of TPs spanned from roughly 1/3 to 2x that of the parent compounds (cf. Figure 10). Visual examination showed that the metolachlor sand filtration TP was smaller than the mean ozonation TP. Accordingly statistical testing showed that carbamazepine sand filtration TPs were significantly smaller than ozonation TPs (see 0I). However, the small number of sand filtration TPs renders generalization difficult. In the case of clofibric acid where on the contrary an abundance of sand filtration TPs were identified, there was no significant difference between treatment groups. These results emphasize that filters have to be carefully selected when designing the data processing workflow. Application of a logical filter for decreased molecular weight and shorter RT here would have led to a substantial loss of identified TPs.

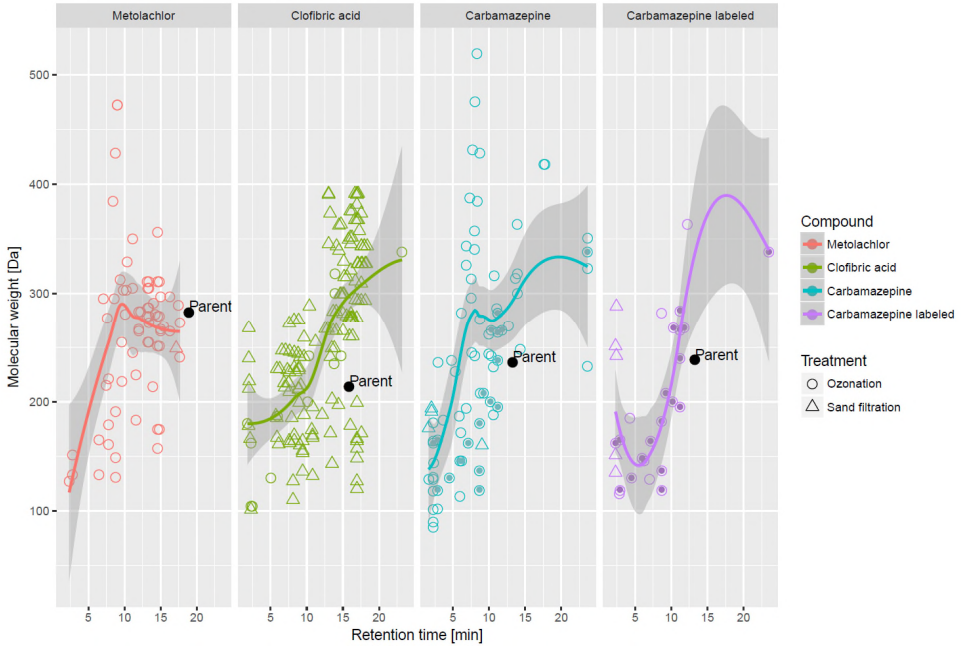


FIGURE 9. COMPARISON MOLECULAR WEIGHT AND RT OF PARENT COMPOUNDS AND THEIR TPS. TPS DETECTED IN BOTH CARBAMAZEPINE LABELED AND UNLABELED EXPERIMENTS ARE REPRESENTED WITH FILLED DOTS.

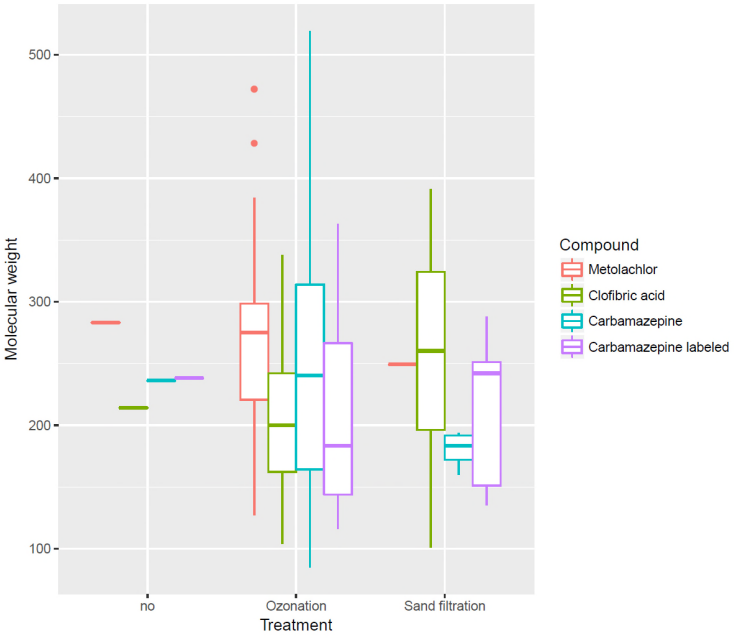


FIGURE 10. MOLECULAR WEIGHT DISTRIBUTION OF PARENT COMPOUNDS (NO TREATMENT GROUP) AND TPS OF OZONATION AND SAND FILTRATION.

5 Perspective on transformation products: challenges and future research

5.1 Future challenges

Substantial advances in TP identification have occurred through application of analytical methods combining so called "bottom-up" and "top-down" approaches, i.e. the prediction of transformation processes to create suspect lists with TPs of known parent compounds that can be searched in non-target LC-HRMS data, and statistical methods to identify patterns and similarities between unknown parent compounds and their TPs. The methods developed and applied to a lab-scale experiment representing relevant drinking water treatment technologies and parent compounds allowed the detection of a multitude of TPs. However, despite current advancements in the non-target screening based identification of unknown compounds, the number of TP features remaining unidentified exceeds the number of annotated features by far. This is in particular due to the fact that TPs are often lacking from suspect lists and spectral databases. The results of the lab-scale experiments emphasize this issue, with only a minority of TPs matched to a suspect, despite the effort spent on manually creating appropriate suspect lists and the selection of 3 separately tested chemicals. More comprehensive databases will likely alleviate this issue in the future. In particular, STOFF-IDENT has increasingly been adding TPs to its database. Furthermore, dissemination of spectra in MassBank is facilitated by the R package 'RMassBank'[22] and the creation of the hashed identifier for mass spectra, called SPLASH [87], finally allows Google searches for spectra. However, the multitude of chemicals present at low concentrations will challenge the application of these techniques outside the well-defined lab and pilot scale testing for the near future because of insufficient resolution and challenging data processing and analysis. With the developments and improvements in LC-HRMS instrumentation and data analysis in mind, we envision that the monitoring of TPs can become a routine task in water analyses on the long run. Potentially, specific TPs could be traced instead of their parent compounds in target screenings of drinking water as has been shown in waste water for sulfamic acid (SA), a TP of the artificial sweetener acesulfame (ACE) [88] Moreover, by identifying TPs and connecting them to their parent compounds, sources of contaminants can be determined and potentially regulated. Overall, these actions would allow a more comprehensive assessment of drinking water quality.

5.2 Recommendations for future research

From both the conducted interviews and the experimental results it was evident that TP formation during drinking water treatment was a relevant topic for the drinking water companies and needed more attention, a fact that is also reflected in the exponential increase of (peer reviewed) publications on the topic in recent years shown in Figure 11.

[22] http://www.bioconductor.org/packages/devel/bioc/html/RMassBank.html

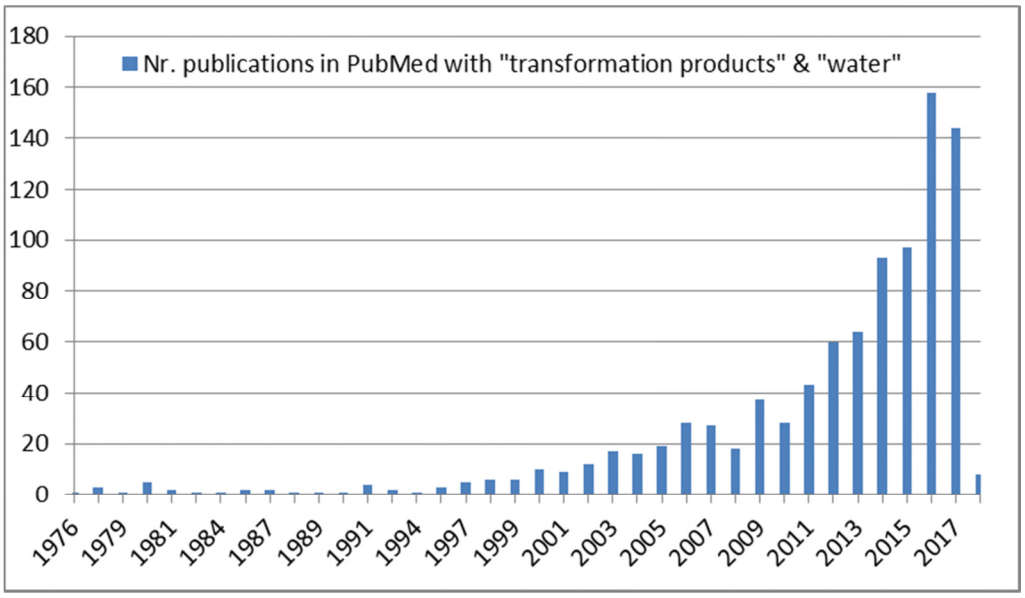


FIGURE 11. NUMBER OF PUBLICATIONS LISTED IN PUBMED WITH THE QUERY "TRANSFORMATION PRODUCTS" AND "WATER"

We suggest the following potential directions for further research that are particularly relevant for the drinking water sector:

1. From a general water quality point of view, the contribution of TPs in relation to parents to the total chemical contamination/toxicology needs to be assessed. This relates to treatment efficiency research and formation of compounds. Traditionally, treatments are studied and compared based on removal rates. TPs shed a different light on this issue as removal rates are merely reduction rates of the parent compound, not complete removal. (How) does testing treatment from a different paradigm including TPs, change the water quality?
2. From a technological point of view, mass balances could reveal the extent of parent compound reduction due to TP formation. Furthermore, it should be evaluated if / how the process parameters of drinking water treatment can be manipulated to stimulate the degradation of formed TPs in biotic as well as AOP processes. As TPs will be formed during drinking water production, we see this as a pragmatic solution to manage the TP-associated risks. Along these lines, it would be interesting to compare TP formation across different ozone consumption and dosage conditions, including those conditions used in practice by the drinking water utilities.
3. From a microbiological point of view, it would be interesting to inquire which bacterial populations are responsible for successful biodegradation and to correlate the presence of certain populations with the formation of specific TPs.
4. From a health risk assessment point of view, it is necessary to evaluate the toxicity of formed TPs, for instance by coupling non-target screening methods with effect directed analysis. The bioassay response can then be connected to TPs detected in LC-HRMS experiments, which will give valuable insight into which TPS are (more) toxic and which treatments lead to their formation.
5. From a regulatory / management point of view, it will be interesting to study the sources, parent compounds, conditions and treatments that result in TPs of concern for drinking water production (and other uses of water) in order to prevent their emission/formation. Ultimately a joint database for relevant parent compounds and

TPs could be created to increase transfer of TP knowledge and experiences for this purpose.

5.3 Conclusions and outlook

The BTO project 400554-220 “Transformation products in the water cycle” allowed us to develop and test an efficient workflow to monitor TP formation and identify drinking water treatment specific TPs on a lab-scale. It was apparent that the degradation of parent compounds did not per se lead to mineralization of the compound, but rather to an abundance of TPs, in often low concentrations. Some of these TPs were bigger and less polar than their parent compounds, which was somewhat unexpected. The identification of peaks representing TPs was straightforward and semi-automatic with the developed workflow, based on statistical testing and peak area filters. The suspect screening based on TP suspect lists manually curated from literature mining and prediction tools was efficient for TPs in the lists. However, the majority of TPs identified did not match suspect list entries. Furthermore, the structural identification of these features, as well as of isobaric suspects remained labor and time intensive. The follow-up BTO project “Non-target screening: Automated and confident identification of unknown compounds” will target and hopefully alleviate these issues. Finally, the developed workflow in combination with the top-down approaches from the roadmap will be applied to non-target data from pilot-scale experiments as part of the project “DPWE robuustheid zuivering” and will allow TP monitoring in actual drinking water production.

6 Acknowledgements

The contributions of Gerard Stroomberg (RIWA Rijn), Jan van der Kooi and Jantinus Bruins (WLN), Bram Martijn and Ruud van der Neut (PWN), Karin Lekkerkerker (Dunea), Mark van Huijkelom (Brabant Water), and Bernard Bajema (Vitens) are greatly appreciated.

We thank Eric Baars of Waternet for help with sand and water sampling at the WRK plant.

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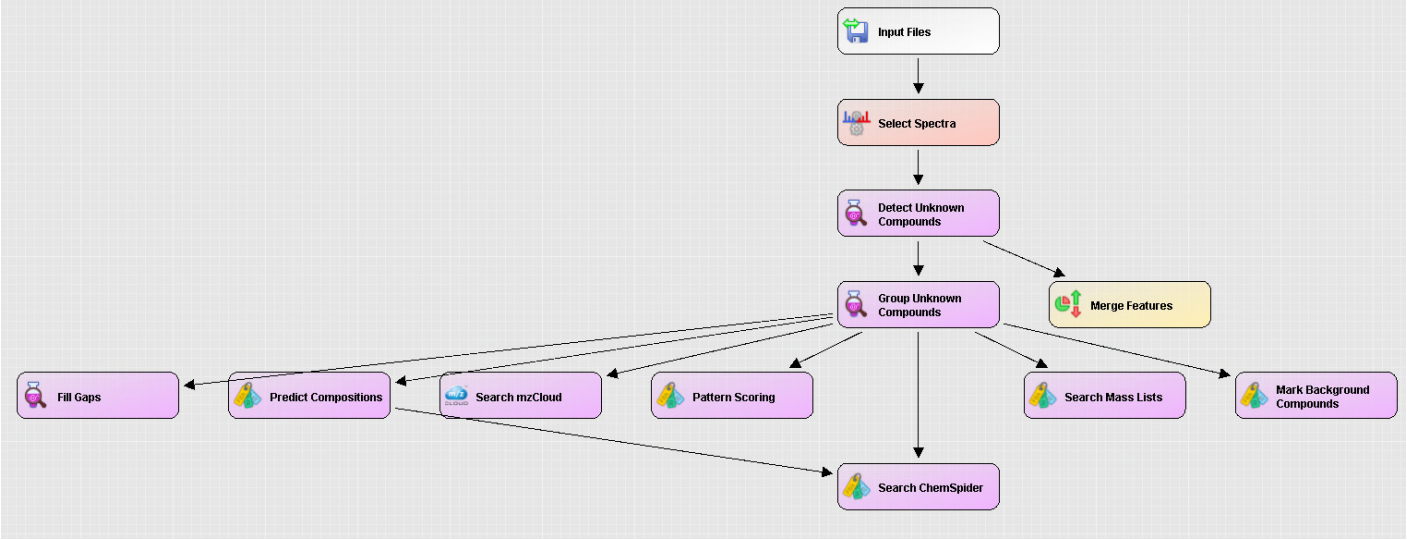
Appendix I. List of interview questions

1. Do you analyze transformation products in the influent and effluent water?
2. If so, what type of transformation products do you find, and what are the concentrations?
3. Do you know anything about the formation of transformation products in the treatment process? Has research been done into this subject?
4. If so, can you tell us something about transformation products, e.g. in sand filters and activated carbon (where transformation products are formed via biodegradation)?
5. In case advanced oxidation is applied do you know which transformation products are formed during the process, and what the concentrations are?
6. Do you consider the formation of transformation products an important topic? Is this something you would like to limit or prevent, e.g. by adjusting reaction conditions or pretreatment of the water?
7. Do you consider transformation products, formed during biological processes, to be different from transformation products formed during oxidation processes?
8. Do you think it important to limit the formation of transformation products?
9. Is the removal of transformation products an important issue within your company?
10. Do you think it important to obtain more information about which transformation products are being formed, and what kind of effects these may have?

Appendix II. Transformation reactions and their induced mass shifts. adapted from [34]

	transformation	atomic loss/gain	mass difference
1	hydroxylation	+ O	+15.9949
2	demethylation	- CH_2	−14.0157
3	deethylation	- C_2H_4	−28.0313
4	dehydrogenation	+ H_2	−2.0157
5	hydrogenation	- H_2	+2.0157
6	dehydration	- H_2O	−18.0106
7	chlorine reduction	- Cl / + H	−33.9611
8	acetylation	+ C_2H_2O	+42.0106
9	deacetylation	- C_2H_2O	−42.0106
10	glucuronidation	+ $C_6H_8O_6$	+176.0320
11	deglucuronidation	- $C_6H_8O_6$	−176.0320
12	sulfonation	+ SO_3	+79.9568
13	desulfonation	- SO_3	−79.9568

Appendix III. Compound Discoverer workflow



Appendix IV. Summary of data processing parameters and Compound Discoverer 2.1 settings.

Processing node 1: Select Spectra
---
1. General Settings:
- Precursor Selection: Use MS(n - 1) Precursor
- Use New Precursor Reevaluation: True
- Use Isotope Pattern in Precursor Reevaluation: True
- Store Chromatograms: False

2. Spectrum Properties Filter:
- Lower RT Limit: 0
- Upper RT Limit: 0
- First Scan: 0
- Last Scan: 0
- Ignore Specified Scans: (not specified)
- Lowest Charge State: 0
- Highest Charge State: 0
- Min. Precursor Mass: 100 Da
- Max. Precursor Mass: 5000 Da
- Total Intensity Threshold: 0
- Minimum Peak Count: 1

3. Scan Event Filters:
- Mass Analyzer: (not specified)
- MS Order: Any
- Activation Type: (not specified)
- Min. Collision Energy: 0
- Max. Collision Energy: 1000
- Scan Type: Any
- Polarity Mode: (not specified)

4. Peak Filters:
- S/N Threshold (FT-only): 1.5

5. Replacements for Unrecognized Properties:
- Unrecognized Charge Replacements: 1
- Unrecognized Mass Analyzer Replacements: ITMS
- Unrecognized MS Order Replacements: MS2
- Unrecognized Activation Type Replacements: CID
- Unrecognized Polarity Replacements: +
- Unrecognized MS Resolution@200 Replacements: 60000
- Unrecognized MSn Resolution@200 Replacements: 30000

Processing node 3: Detect Unknown Compounds
---
1. General Settings:
- Mass Tolerance [ppm]: 5 ppm
- Intensity Tolerance [%]: 30
- S/N Threshold: 3
- Min. Peak Intensity: 50000
- Ions:
  [M+Cl]-1
  [M+H]+1
  [M+K]+1

[M+Na]+1
[M+NH4]+1
[M-H]-1
- Base Ions: [M+H]+1; [M-H]-1
- Min. Element Counts: C H
- Max. Element Counts: C90 H190 Br3 Cl4 K2 N10 Na2 O15 P2 S5

2. Peak Detection:
- Filter Peaks: True
- Max. Peak Width [min]: 0.8
- Remove Singlets: True
- Min. # Scans per Peak: 5
- Min. # Isotopes: 1

Processing node 4: Group Unknown Compounds
---
1. Compound Consolidation:
- Mass Tolerance: 5 ppm
- RT Tolerance [min]: 0.1

2. Fragment Data Selection:
- Preferred Ions: [M+H]+1; [M-H]-1

Processing node 7: Fill Gaps
---
1. General Settings:
- Mass Tolerance: 5 ppm
- S/N Threshold: 1.5
- Use Real Peak Detection: True

Processing node 20: Search mzCloud
---
1. Search Settings:
- Compound Classes: All
- Match Ion Activation Type: True
- Match Ion Activation Energy: Match with Tolerance
- Ion Activation Energy Tolerance: 20
- Apply Intensity Threshold: True
- Precursor Mass Tolerance: 10 ppm
- FT Fragment Mass Tolerance: 10 ppm
- IT Fragment Mass Tolerance: 0.4 Da
- Identity Search: HighChem HighRes
- Similarity Search: None
- Library: Reference
- Post Processing: Recalibrated
- Match Factor Threshold: 20
- Max. # Results: 10

Processing node 21: Pattern Scoring
---
1. General Settings:
- Isotope Patterns: Cl
- Mass Tolerance: 5 ppm
- Intensity Tolerance [%]: 30
- SN Threshold: 3
- Min. Spectral Fit [%]: 0

Processing node 5: Mark Background Compounds
---
1. General Settings:
- Max. Sample/Blank: 5
- Max. Blank/Sample: 0

- Hide Background: True

Processing node 6: Predict Compositions
---
1. Prediction Settings:
- Mass Tolerance: 5 ppm
- Min. Element Counts: C H
- Max. Element Counts: C30 H50 Cl4 N10 O10 P2 S5
- Min. RDBE: -1
- Max. RDBE: 40
- Min. H/C: 0.1
- Max. H/C: 3
- Max. # Candidates: 10
- Max. # Internal Candidates: 200

2. Pattern Matching:
- Intensity Tolerance [%]: 30
- Intensity Threshold [%]: 0.1
- S/N Threshold: 3
- Min. Spectral Fit [%]: 10
- Min. Pattern Cov. [%]: 90
- Use Dynamic Recalibration: True

3. Fragments Matching:
- Use Fragments Matching: True
- Mass Tolerance: 5 ppm
- S/N Threshold: 3

Processing node 22: Search ChemSpider
---
1. Search Settings:
- Mass Tolerance: 5 ppm
- Database(s):
EAWAG Biocatalysis/Biodegradation Database
EPA DSSTox
- Max. # of results per compound: 100
- Max. # of Predicted Compositions to be searched per Compound: 5
- Result Order (for Max. # of results per compound): Order By Reference Count (DESC)

2. Predicted Composition Annotation:
- Check All Predicted Compositions: True

Processing node 23: Search Mass Lists
---
1. Search Settings:
- Input file(s): metolachlor.csv
- Mass Tolerance: 5 ppm
- Show extra Fields as Columns: False
- Consider Retention Time: False
- RT Tolerance : 0.05

Processing node 14: Merge Features
---
1. Peak Consolidation:
- Mass Tolerance: 5 ppm
- RT Tolerance [min]: 0.1

Processing node 15: Differential Analysis
---
1. General Settings:
- Log10 Transform Values: True

Processing node 24: Assign Compound Annotations
---
1. General Settings:
- Mass Tolerance: 5 ppm

2. Data Sources:
- Data Source #1: mzCloud Search
- Data Source #2: Predicted Compositions
- Data Source #3: MassList Match
- Data Source #4: ChemSpider Search

Processing node 25: Descriptive Statistics

No parameter

Appendix V. Metolachlor suspect list

name	formula	Monoisotop mass	CAS	SMILES	source
metabolite CGA 50720 of S-Metolachlor ([(2-ethyl-6-methylphenyl)carbamoyl]formic acid)	C11H13N1O3	207.0895	152019-74-4	CCc1cccc(C)c1NC(=O)C(O)=O	Norman, Stoffident
CCC1=C(C(=CC=C1)C)NC(=O)CCl	C11H14Cl1N1O1	211.0764		CCC1=C(C(=CC=C1)C)NC(=O)CCl	Norman, Stoffident
metabolite CGA 37735 of S-Metolachlor (N-(2-ethyl-6-methylphenyl)-2-hydroxyacetamide)	C11H15N1O2	193.1103	97055-05-5	CCc1cccc(C)c1NC(=O)CO	Norman, Stoffident
metabolite CGA 368208 of S-Metolachlor ([(2-ethyl-6-methylphenyl)carbamoyl]methanesulfonic acid)	C11H15N1O4S1	257.0722	1173021-76-5	CCc1cccc(C)c1NC(=O)CS(O)(=O)=O	Norman, Stoffident
metabolite CGA 50267 of S-Metolachlor (N-(2-Ethyl-6-methylphenyl)alanine)	C12H17N1O2	207.1259	82508-03-0	CCc1cccc(C)c1N[C@@H](C)C(O)=O	Norman, Stoffident
CCC1=C(C(=CC=C1)C)NC(C)COC	C13H21N1O1	207.1623		CCC1=C(C(=CC=C1)C)NC(C)COC	Norman, Stoffident
metabolite CGA 357704 of S-Metolachlor (2-[1-carboxy-N-(2-ethyl-6-methylphenyl)formamido]propanoic acid)	C14H17N1O5	279.1107	1217465-10-5	CCc1cccc(C)c1N(C(C)C(O)=O)C(=O)C(O)=O	Norman, Stoffident
CCC1=C(C(=CC=C1)C)N(C(C)C=O)C(=O)CCl	C14H18Cl1N1O2	267.1026		CCC1=C(C(=CC=C1)C)N(C(C)C=O)C(=O)CCl	EnviPATH

Metolachlor-Morpholinone	C14H19N1O2	233.1416	120375-14-6	CCc1cccc(C)c1N1C(C)COCC1=O	Norman, Stoffident
S-Metolachlor NOA 413173 (2-[N-(2-ethyl-6-methylphenyl)-2-sulfoacetamido]propanoic acid)	C14H19N1O6S1	329.0933	1418095-19-8	CCc1cccc(C)c1N(C(C)C(O)=O)C(=O)CS(O)(=O)=O	
CCC1=C(C(=CC=C1)C)N(C(C)CO)C(=O)CCl	C14H20Cl1N1O2	269.1183		CCC1=C(C(=CC=C1)C)N(C(C)CO)C(=O)CCl	EnviPATH
Metolachlor OXA ([(2-ethyl-6-methylphenyl)(1-methoxypropan-2-yl)carbamoyl]formic acid)	C15H21N1O4	279.1471	152019-73-3	CCc1cccc(C)c1N(C(C)COC)C(=O)C(O)=O	Norman, Stoffident
Metolachlor	C15H22Cl1N1O2	283.1339	51218-45-2	CCc1cccc(C)c1N(C(C)COC)C(=O)CCl	Norman, Stoffident
CC1=CC=CC(=C1N(C(C)COC)C(=O)CCl)C(C)O	C15H22Cl1N1O3	299.1288		CC1=CC=CC(=C1N(C(C)COC)C(=O)CCl)C(C)O	EnviPATH
CCC1=C(C(=CC=C1O)C)N(C(C)COC)C(=O)CCl	C15H22Cl1N1O3	299.1288			EnviPATH
CCC1=CC=C(C(=C1N(C(C)COC)C(=O)CCl)C)O	C15H22Cl1N1O3	299.1288			EnviPATH
CCC1=CC=CC(=C1N(C(C)COC)C(=O)CCl)CO	C15H22Cl1N1O3	299.1288			EnviPATH
CCC1=CC(=CC(=C1N(C(C)COC)C(=O)CCl)C)O	C15H22Cl1N1O3	299.1288			EnviPATH
CCC1=C(C(=CC(=C1N(C(C)COC)C(=O)CCl)C)O)O	C15H22Cl1N1O4	315.1237			EnviPATH
CCC1=C(C(=C(C(=C1)O)O)C)N(C(C)COC)C(=O)CCl	C15H22Cl1N1O4	315.1238			EnviPATH

Deschlormetolachlor	C15H23N1O2	249.1729	126605-22-9	CCc1cccc(C)c1N(C(C)COC)C(C)=O	Norman, Stoffident
metolachlor-hydroxy	C15H23N1O3	265.1678			Norman
Metolachlor ESA [(2-ethyl-6-methylphenyl)(1-methoxypropan-2-yl)carbamoyl]methanesulfonic acid	C15H23N1O5S1	329.1297	171118-09-5	CCc1cccc(C)c1N(C(C)COC)C(=O)CS(O)(=O)=O	
C=O	C1H2O1	30.0106		C=O	EnviPATH
CO	C1H4O1	32.0262		CO	EnviPATH
C(C(=O)[O-])Cl	C2H2Cl1O2	92.9743		C(C(=O)[O-])Cl	EnviPATH
CC(=O)COC	C4H8O2	88.0524		CC(=O)COC	EnviPATH

Appendix VI. Clofibric acid suspect list

name	formula	monoisotop mass	CAS
Lactic acid (LA)	$C_3H_6O_3$	90.03169	50-21-5
4-chlorophenol (4-CP)	C6H5ClO	128.003	106-48-9
CC(C)OC1=CC=CC=C1	C9H12O	136.0888	
1-chloro-4-isopropoxybenzene	C9H11ClO	170.04985	51241-43-1
CC(CC(=O)[O-])OC1=CC=CC=C1	C10H11O3	179.0708	
CC(C)(C(=O)[O-])OC1=CC=CC=C1	C10H11O3	179.0708	
CC(CO)OC1=CC=C(C=C1)Cl	C9H11ClO2	186.0448	
(Z)-4-chloro-5-oxohex-2-enedioic acid	C6H5ClO5	191.9826	
EnviPATHstr0041047	C10H11O4	195.0657	
CC(C)OC1=CC=C(C(=C1O)O)Cl	C9H11ClO3	202.0397	
CC(C)(C(=O)[O-])OC1=C(C(=CC=C1)O)O	C10H11O5	211.0606	
clofibric acid	C10H11ClO3	214.0397	882-09-7
CC(C=O)(C(=O)[O-])OC1=CC=C(C=C1)Cl	C10H8ClO4	227.0111	
EnviPATHstr0041048	C10H10ClO4	229.0268	
EnviPATHstr0041046	C10H10ClO5	245.0217	
CC(CC(=O)[O-])OC1=CC=C(C(=C1O)O)Cl	C10H10ClO5	245.0217	
2-(4-Chloro-2,3-dihydroxyphenoxy)-2-methylpropionic acid	C10H11ClO5	246.0295	
CC(C)(C(=O)[O-])OC1=C(C(=C(C=C1O)Cl)O)O	C10H10ClO6	261.0166	
CC(CO)(C(=O)[O-])OC1=CC=C(C(=C1O)O)Cl	C10H10ClO6	261.0166	

CC(C)(C(=O)[O-])OC1=CC(=C(C(=C1O)O)O)Cl)O	C10H10ClO6	261.0166	
CC(C)(C(=O)[O-])O/C(=C/C=C(\C(=O)[O-])/Cl)/C(=O)[O-]	C10H8ClO7	274.9959	
α-Hydroxyisobutyric acid (AHIBA)	$C_4H_8O_3$	104.0473	594-61-6
cis-3-Hexenyllactate	C9H16O3	172.1099	61931-81-5
BroxTP1: Clofibric acid + O + SO3	C10H11O7SCl	309.9914	
BroxTP2: Glucuronide conjugated 4-chlorophenol	C12H11O7Cl	302.0193	
BroxTP3: Cyclic structure of clofibric acid + sulfate + cysteine	C12H14NO8S2Cl	398.9849	
BroxTP4a: 4-Chlorophenol + O + SO3	C6H5O5SCl	223.9546	
BroxTP4b: 4-Chlorophenol + O + SO3	C6H5O5SCl	223.9547	
BroxTP5: 4-Chlorophenol + SO3	C6H5O4SCl	207.9597	
BroxTP6a: 4-Chlorophenol + O + SO3 + CH3	C7H7O5SCl	237.9703	
BroxTP6b: 4-Chlorophenol + O + SO3 + CH3	C7H7O5SCl	238.9703	
BroxTP7: Cyclic structure of clofibric acid + glucuronic acid	C15H17O9Cl	376.0561	
BroxTP8a: Glucuronide conjugated clofibric acid (acyl glucuronide)	C16H19O9Cl	390.0718	
BroxTP9: Clofibric acid–aminomethanesulfonic acid	C11H14NO5SCl	307.0281	
BroxTP10: Cyclic structure of clofibric acid + sulfate	C9H10O6SCl	280.9887	
BroxPrecusor: Clofibric acid	C10H11O3Cl	214.0397	
BroxTP8b: Glucuronide conjugated clofibric acid (acyl glucuronide)	C16H19O9Cl	390.0718	
BroxTP11: Clofibric acid + O	C10H11O4Cl	230.0346	
BroxTP12: Clofibric acid-taurine	C12H16O5NSCl	321.0438	
BroxTP8c: Glucuronide conjugated clofibric acid (acyl glucuronide)	C16H19O9Cl	390.0718	
BroxTP13: −	C16H23N2O6SCl	406.0965	
BroxTP14: Clofibric acid + carnitine	$C_{17}H_{24}O_5NCl$	357.1343	

Appendix VII. Carbamazepine suspect list

name	formula	monoisotop mass	CAS	source
Kosjek: acridine	C13H9N	179.0735	260-94-6	Kosjek: photolysis, ClO2oxidation, biodegradation
Kosjek: acridone	C13H9NO	195.0684	578-95-0	Kosjek: photolysis, biodegradation of ACIN
Kosjek: 9-hydroxy-acridine	C13H9NO	195.0684		Kosjek: ClO2treatment of ACIN
Kosjek: iminostilbene	C14H11N	193.0891	256-96-2	Kosjek: thermal degradation in GC liner. EnviPATH predicted
Kosjek: hydroxy-(9H,10H)-acridine-9-carbaldehyde	C14H11NO2	225.079		Kosjek: photolysis
C1=CC2=C(C=C1)NC3=C(C=CC=C3)CC2	C14H13N	195.1048		EnviPATH
Kosjek: acridine-9-carbaldehyde	C14H9NO	207.0684	885-23-4	Kosjek: ClO2 oxidation
Kosjek: acridone-N-carbaldehyde	C14H9NO2	223.0633		Kosjek: photolysis
Kosjek: 1-(2-benzaldehyde)-(1H,3H)-quinazoline-2,4-dione	C15H10N2O3			Kosjek: photolysis
C1=CC2=C(C=C1)N(C3=C(C=CC=C3)C=C2)C(=O)[O-]	C15H10NO2	236.0712		EnviPATH
C1=CC2=C(C=C1)N(C3=C(C=C2)C=CC(=C3O)O)C(=O)[O-]	C15H10NO4	268.061		EnviPATH
C1=CC2=C(C=C1)N(C3=CC=C(C(=C3C=C2)O)O)C(=O)[O-]	C15H10NO4	268.061		EnviPATH
C1=CC2=C(C=C1)N(C3=C(C=C2)C=C(C(=C3)O)O)C(=O)[O-]	C15H10NO	268.061		EnviPATH

	C15H12N2O4			
Carbamazepine	C15H12N2O	236.095	298-46-4	SusDat_SA1012
C1=CC2=C(C=C1)N(C3=C(C=CC=C3)C=C2)C(=O)N	C15H12N2O	236.095		EnviPATH
Carbamazepine-10,11-epoxide	C15H12N2O2	252.0899	36507-30-9	SusDat_SA1142
2-Hydroxycarbamazepin	C15H12N2O2	252.0899	68011-66-5	SToffIDENT
oxcarbazpine	C15H12N2O2	252.0899	28721-07-5	SToffIDENT
3-Hydroxycarbamazepin	C15H12N2O2	252.0899	68011-67-6	SToffIDENT
C1=CC2=C(C=C1)N(C3=C(C=C2)C=C(C(=C3)O)O)C(=O)N	C15H12N2O3	268.0848		EnviPATH
C1=CC2=C(C=C1)N(C3=CC=C(C(=C3C=C2)O)O)C(=O)N	C15H12N2O3	268.0848		EnviPATH
C1=CC2=C(C=C1)N(C3=C(C=C2)C=CC(=C3O)O)C(=O)N	C15H12N2O3	268.0848		EnviPATH
C1=CC2=C(C=C1)N(C3=C(C=CC=C3)CC2)C(=O)[O-]	C15H12NO2	238.0868		EnviPATH
C1=CC(=C(C=C1)NC(=O)[O-])C=CC2=CC=CC(=C2O)O	C15H12NO4	270.0766		EnviPATH
Dihydro-Carbamazepin	C15H14N2O	238.1106	3564-73-6	SToffIDENT, EnviPATH
C1=CC2=C(C=C1)N(C3=C(C=CC=C3)C(C2)O)C(=O)N	C15H14N2O2	254.1055		EnviPATH
10,11-Dihydroxy-10,11-dihydrocarbamazepin	C15H14N2O3	270.1004	35079-97-1	SToffIDENT

trans-10,11-Dihydroxy-10,11-dihydrocarbamazepin	C15H14N2O3	270.1004	58955-93-4	SToffIDENT
10,11-Dihydroxy-10,11-dihydrocarbamazepin	C15H14N2O3	270.1004	35079-97-1	Kern 2009
trans-10,11-Dihydroxy-10,11-dihydrocarbamazepin	C15H14N2O3	270.1004	58955-93-4	PubChem
C(=O)(N)[O-]	CH2NO2	60.0086		EnviPATH
N	H3N	17.0265		EnviPATH
Carbamazepine-9-carboxaldehyde	$C_{15}H_{12}N_2O_2$	252.0899		SusDat_SA7186
1-(2-benzaldehyde)-4-hydro-(1*H*,3*H*)-quinazoline-2-one (BQM)	$C_{15}H_{10}N_2O_2$	250.0742		McDowell: ozonation; Azais
1-(2-Benzaldehyde)-(1*H*,3*H*)-quinazoline-2,4-dione (BQD)	$C_{15}H_{10}N_2O_3$	266.0691		McDowell: ozonation; Azais
1-(2-benzoic acid)-(1H,3H)-quinazoline-2,4-dione (BaQD)	$C_{15}H_{10}N_2O_4$	282.0641		McDowell: ozonation; Azais
BaQM	$C_{15}H_{10}N_2O_3$	266.0691		Azais

Appendix VIII. Potential transformation products of metolachlor.

Features with log2FC before / after treatment >1; spike-in / no spike >2. p-value < 0.05.

	Feature (Molecular weight / Retention time)	Molecular Weight	RT	Cl	total times detected	Sand filtration		Ozonation							
						pos d0	pos d4	pos low t1	pos low t6	pos high t1	pos high t6	neg low t1	neg low t6	neg high t1	neg high t6
	Metolachlor (283.13343 / 18.905 // 283.13346 / 18.898)	283.13343 // 283.13346	18.905 // 18.898												
TP smaller than parent compound	127.06323 / 2.352	127.06323	2.352		1	0	0	0	0	0	1	0	0	0	0
	131.07335 / 8.769	131.07335	8.769		3	0	0	1	0	1	1	0	0	0	0
	133.02933 / 6.472	133.02933	6.472	Cl	4	0	0	1	1	1	1	0	0	0	0
	133.02941 / 2.8	133.02941	2.8	Cl	2	0	0	0	0	1	1	0	0	0	0
	149.08387 / 8.763	149.08387	8.763		4	0	0	1	1	1	1	0	0	0	0
	151.03983 / 2.814	151.03983	2.814	Cl	2	0	0	0	0	1	1	0	0	0	0
	157.08891 / 14.578	157.08891	14.578		1	0	0	0	0	1	0	0	0	0	0
	161.04748 / 7.791	161.04748	7.791		4	0	0	1	1	1	1	0	0	0	0
	165.05547 / 6.472	165.05547	6.472	Cl	4	0	0	1	1	1	1	0	0	0	0
	175.09946 / 14.579	175.09946	14.579		4	0	0	1	1	1	1	0	0	0	0
	175.09946 / 14.821	175.09946	14.821		4	0	0	1	1	1	1	0	0	0	0
	179.05802 / 7.79	179.05802	7.79		4	0	0	1	1	1	1	0	0	0	0
	183.04487 / 11.586	183.04487	11.586	Cl	2	0	0	1	0	1	0	0	0	0	0
	191.0944 / 8.764	191.0944	8.764		3	0	0	1	1	1	0	0	0	0	0
	214.12033 / 13.912	214.12033	13.912		1	0	0	0	0	1	0	0	0	0	0
	215.11545 / 7.456	215.11545	7.456		4	0	0	1	1	1	1	0	0	0	0
	219.05275 / 9.655	219.05275	9.655		4	0	0	0	0	0	0	1	1	1	1
	221.06837 / 7.819 // 221.06852 / 7.79	221.06837 // 221.06852	7.79		8	0	0	1	1	1	1	1	1	1	1
	225.05535 / 11.586	225.05535	11.586	Cl	2	0	0	1	0	1	0	0	0	0	0
	241.08675 / 17.651	241.08675	17.651	Cl	2	0	0	1	0	1	0	0	0	0	0
	245.06834 / 11.136	245.06834	11.136		4	0	0	1	1	1	1	0	0	0	0
	251.07084 / 14.579	251.07084	14.579	Cl	4	0	0	1	1	1	1	0	0	0	0
	251.07085 / 14.82	251.07085	14.82	Cl	4	0	0	1	1	1	1	0	0	0	0
	255.02942 / 9.626	255.02942	9.626	Cl	4	0	0	1	1	1	1	0	0	0	0
	255.06574 / 13.371	255.06574	13.371	Cl	3	0	0	1	0	1	1	0	0	0	0
	255.06575 / 13.222	255.06575	13.222	Cl	3	0	0	1	0	1	1	0	0	0	0
	265.08645 / 11.972	265.08645	11.972	Cl	4	0	0	1	1	1	1	0	0	0	0
	265.08648 / 14.993	265.08648	14.993	Cl	3	0	0	1	1	1	0	0	0	0	0
	265.08648 / 16.275	265.08648	16.275	Cl	2	0	0	1	0	1	0	0	0	0	0
	267.08318 / 11.971	267.08318	11.971		3	0	0	1	0	1	1	0	0	0	0
	267.08351 / 14.993	267.08351	14.993		3	0	0	1	1	1	0	0	0	0	0
	269.11772 / 15.421*	269.11772	15.421	Cl	1	0	0	1	0	0	0	0	0	0	0
	273.07633 / 13.371	273.07633	13.371	Cl	3	0	0	1	0	1	1	0	0	0	0
	273.11273 / 17.652	273.11273	17.652	Cl	2	0	0	1	0	1	0	0	0	0	0
	277.09457 / 7.629	277.09457	7.629		3	0	0	1	0	1	1	0	0	0	0
	279.06557 / 13.133	279.06557	13.133	Cl	3	0	0	1	0	1	1	0	0	0	0
	279.06561 / 13.376	279.06561	13.376	Cl	3	0	0	1	0	1	1	0	0	0	0
	279.06562 / 14.579	279.06562	14.579	Cl	4	0	0	1	1	1	1	0	0	0	0
	279.0657 / 14.822	279.0657	14.822	Cl	4	0	0	1	1	1	1	0	0	0	0
	281.11781 / 14.259	281.11781	14.259	Cl	1	0	0	1	0	0	0	0	0	0	0
	281.12626 / 10.089	281.12626	10.089		3	0	0	0	0	0	0	1	0	1	1

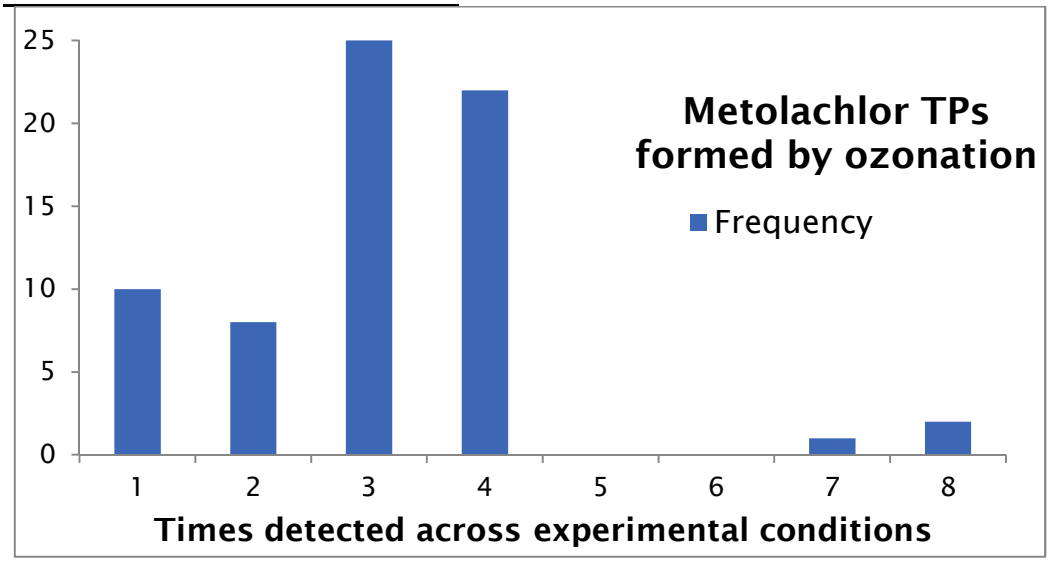
TP bigger than parent compound	283.09695 / 11.972	283.09695	11.972	Cl	4	0	0	1	1	1	1	0	0	0	0
	283.097 / 12.256	283.097	12.256	Cl	3	0	0	1	0	1	1	0	0	0	0
	287.09182 / 13.221	287.09182	13.221	Cl	3	0	0	1	0	1	1	0	0	0	0
	287.09182 / 13.372	287.09182	13.372	Cl	4	0	0	1	1	1	1	0	0	0	0
	289.26113 / 17.442	289.26113	17.442		1	0	0	1	0	0	0	0	0	0	0
	291.11002 / 14.02	291.11002	14.02		3	0	0	1	0	1	1	0	0	0	0
	295.10486 / 8.594	295.10486	8.594		4	0	0	1	1	1	1	0	0	0	0
	295.10494 / 7.033 // 295.10544 / 7.071	295.10494 // 295.10544	7.071		8	0	0	1	1	1	1	1	1	1	1
	297.11253 / 14.994	297.11253	14.994	Cl	3	0	0	1	1	1	0	0	0	0	0
	297.1126 / 16.268	297.1126	16.268	Cl	2	0	0	1	0	1	0	0	0	0	0
	303.08668 / 10.264	303.08668	10.264	Cl	3	0	0	1	0	1	1	0	0	0	0
	303.08669 / 9.829	303.08669	9.829	Cl	3	0	0	1	0	1	1	0	0	0	0
	305.08925 / 11.137	305.08925	11.137		3	0	0	1	0	1	1	0	0	0	0
	305.10232 / 13.221	305.10232	13.221	Cl	3	0	0	1	0	1	1	0	0	0	0
	305.10232 / 13.372 // 305.10264 / 13.373	305.10232 // 305.10264	13.373	Cl	7	0	0	1	1	1	1	1	0	1	1
	311.09174 / 14.58	311.09174	14.58	Cl	4	0	0	1	1	1	1	0	0	0	0
	311.09175 / 13.132	311.09175	13.132	Cl	3	0	0	1	0	1	1	0	0	0	0
	311.09175 / 13.377	311.09175	13.377	Cl	3	0	0	1	0	1	1	0	0	0	0
	311.09191 / 14.822	311.09191	14.822	Cl	3	0	0	1	0	1	1	0	0	0	0
	313.07105 / 9.412	313.07105	9.412	Cl	4	0	0	1	1	1	1	0	0	0	0
	329.10235 / 10.369	329.10235	10.369	Cl	3	0	0	1	0	1	1	0	0	0	0
	350.14691 / 11.137	350.14691	11.137		4	0	0	1	1	1	1	0	0	0	0
	356.14931 / 14.578	356.14931	14.578	Cl	3	0	0	1	0	1	1	0	0	0	0
	384.23506 / 8.401	384.23506	8.401		1	0	0	0	0	1	0	0	0	0	0
	428.26131 / 8.726	428.26131	8.726		1	0	0	0	0	1	0	0	0	0	0
	472.28751 / 9.005	472.28751	9.005		1	0	0	0	0	1	0	0	0	0	0
	472.28751 / 9.005	472.28751	9.005		1	0	0	0	0	1	0	0	0	0	0
	249.17233 / 17.148**	**249.1729**	**17.148**		2	1	1	0	0	0	0	0	0	0	0

*** CCC1=C(C(=CC=C1)C)N(C(C)CO)C(=O)CCl (EnviPATH)**

****Deschlormetolachlor (CAS 126605-22-9; Norman SusDat, StoffIDENT, EnviPATH)**

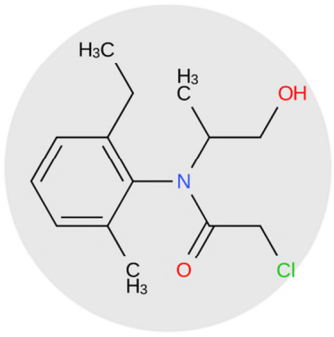
Appendix IX. Metolachlor TP distribution in regards to experimental conditions

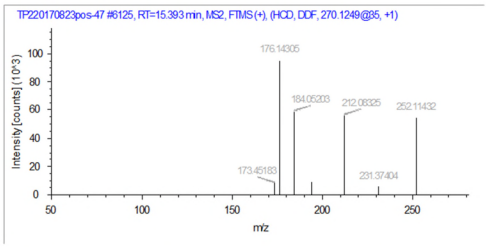
Metolachlor TP formed by number of experimental conditions	
Mean	3.117647
Standard Error	0.172175
Median	3
Mode	3
Standard Deviation	1.41979
Sample Variance	2.015803
Kurtosis	3.250483
Skewness	1.075793
Range	7
Minimum	1
Maximum	8
Sum	212
Count	68
Largest(1)	8
Smallest(1)	1
Confidence Level(95.0%)	0.343662

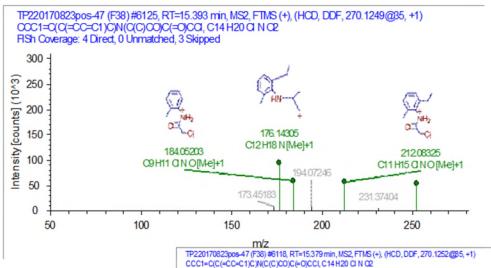


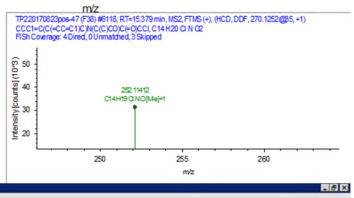
Appendix X. Structural identification of metolachlor TP. MS2 fragmentation data based FiSH scoring

alachlor and acetolchlor cannot explain peak @ 176.14305

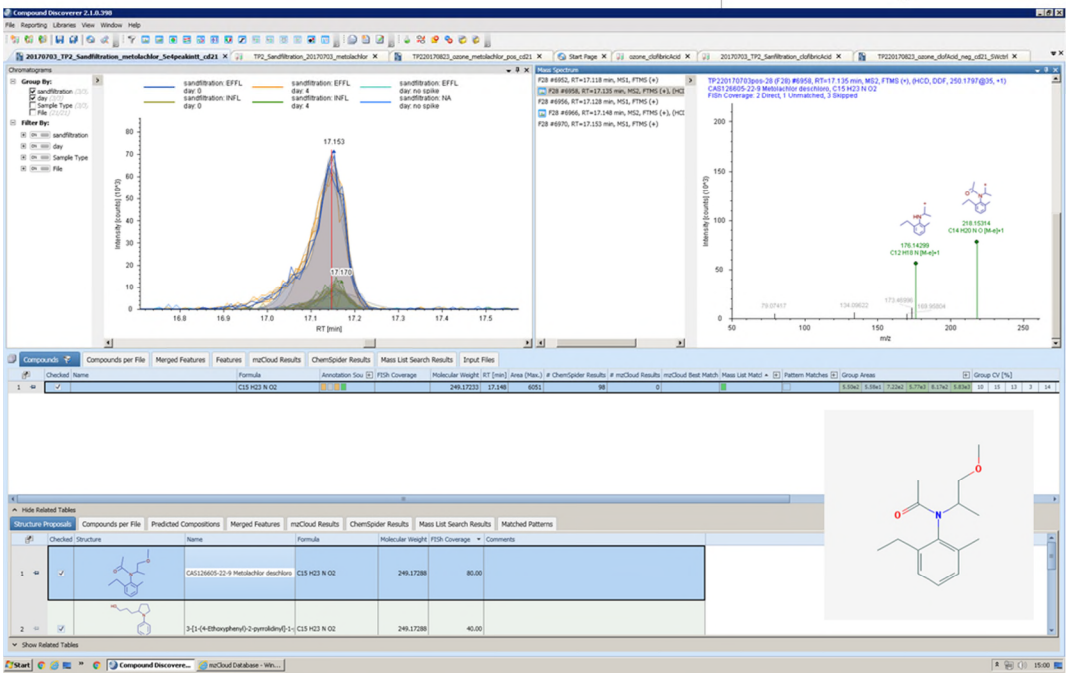








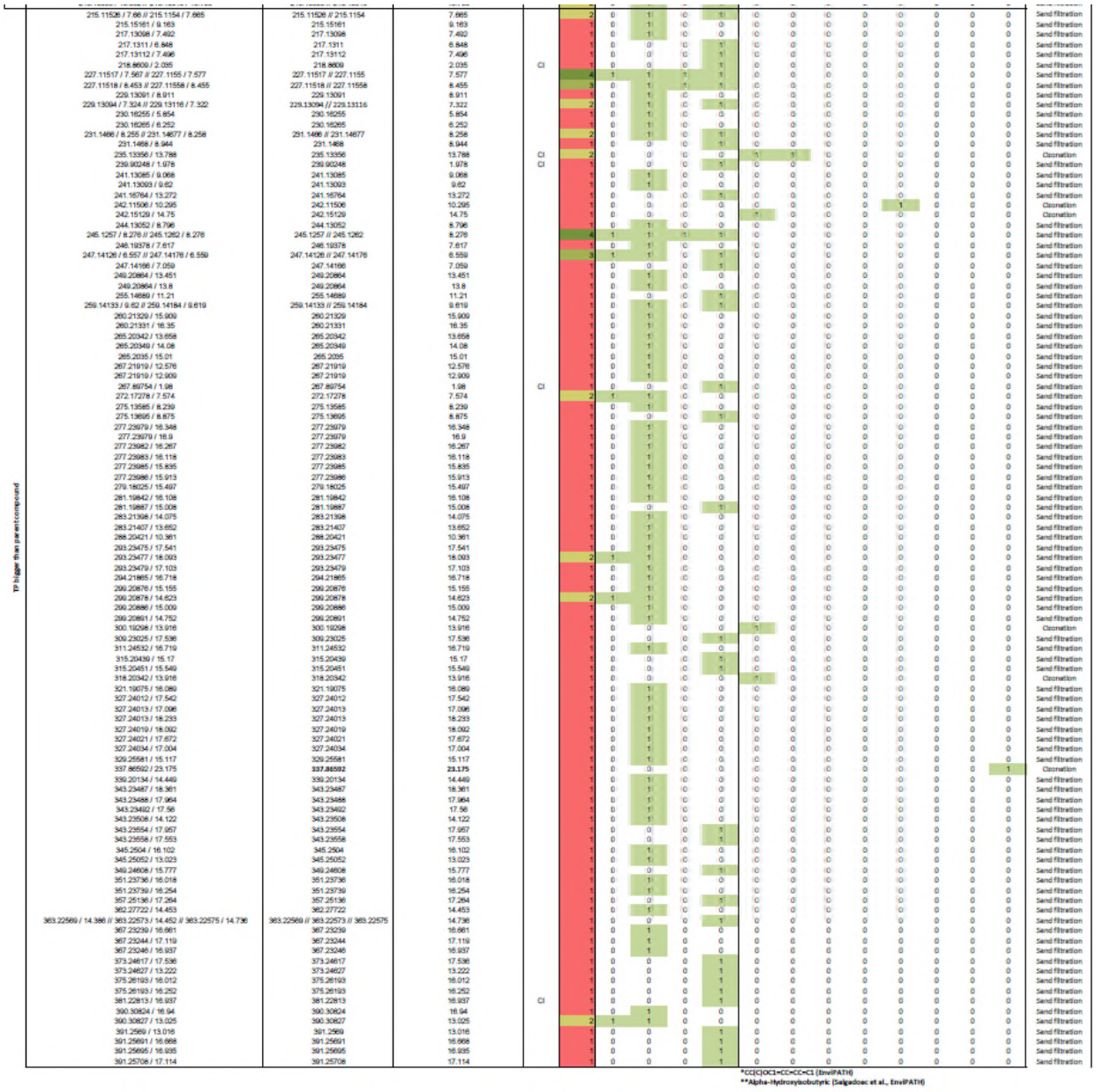
	Name	Formula	Molecular Weight	ΔMass [ppm]	Reference List Name	feature
ozone						
	CCC1=C(C(=CC=C1)C)N(C(C)CO)C(=O)CCl	C14 H20 Cl N O2	269.1183	2.01	metolachlor	
pos low t1						269.11772 / 15.421



Appendix XI. Potential transformation products of clofibric acid.

Features with log2FC before / after treatment >1; spike-in / no spike >2. p-value < 0.05.

	Feature (Molecular weight / Retention time)	Molecular Weight	RT	Cl	total times detected	Sand filtration				Ozonation								treatment
						pos d0	pos d4	neg d0	neg d4	pos low t1	pos low t6	pos high t1	pos high t6	neg low t1	neg low t6	neg high t1	neg high t6	
	Clofibric acid (214.03942 / 15.858 // 214.03957 / 15.874)	214.03942 // 214.03957	15.858															no
TP smaller than parent compound	101.12045 / 2.284	101.12045	2.284		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	104.04685 / 2.299**	104.04685	2.299**		2	0	0	0	0	0	0	0	0	0	0	1	1	Ozonation
	104.04685 / 2.456**	104.04685	2.456**		2	0	0	0	0	0	0	0	0	0	0	1	1	Ozonation
	110.07298 / 8.098	110.07298	8.098		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	120.09364 / 16.942	120.09364	16.942		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	127.09947 / 16.939	127.09947	16.939		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	127.09948 / 8.099	127.09948	8.099		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	130.06244 / 4.949 // 130.06244 / 5.041	130.06244 // 130.06244	5.041		4	0	0	0	0	0	0	0	0	1	1	1	1	Ozonation
	132.09365 / 10.801	132.09365	10.801		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	136.08849 / 9.425*	136.08849	9.425*		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	143.13075 / 13.497	143.13075	13.497		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	148.0885 / 16.943	148.0885	16.943		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	153.1151 / 9.427	153.1151	9.427		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	155.13051 / 9.425	155.13051	9.425		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
	157.1099 / 16.94	157.1099	16.94		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	160.10931 / 9.035	160.10931	9.035		2	0	0	1	1	0	0	0	0	0	0	0	0	Sand filtration
	161.10485 / 6.107	161.10485	6.107		2	1	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	162.06768 / 7.659	162.06768	7.659		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	162.11559 / 2.286	162.11559	2.286		3	0	0	0	0	1	1	1	0	0	0	0	0	Ozonation
	164.08331 / 8.27	164.08331	8.27		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	164.08332 / 7.568	164.08332	7.568		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	164.08339 / 9.426	164.08339	9.426		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	164.08344 / 16.94	164.08344	16.94		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	165.94181 / 2.159	165.94181	2.159		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
	166.09892 / 16.946	166.09892	16.946		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	167.09426 / 5.676	167.09426	5.676		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	167.13068 / 10.803	167.13068	10.803		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	169.14618 / 10.796	169.14618	10.796		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
	169.1463 / 8.259	169.1463	8.259		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	171.12562 / 17.561	171.12562	17.561		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	171.19839 / 13.272	171.19839	13.272		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	174.12506 / 10.685	174.12506	10.685		2	0	0	1	1	0	0	0	0	0	0	0	0	Sand filtration
	177.9636 / 1.955	177.9636	1.955		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
	178.09904 / 16.02	178.09904	16.02		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	180.06307 / 1.793	180.06307	1.793		1	0	0	0	0	0	0	0	0	0	0	0	1	Ozonation
	183.12546 / 17.118 // 183.1256 / 16.944	183.12546 // 183.1256	16.944		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	185.1048 / 7.909	185.1048	7.909		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
	185.10484 / 5.856	185.10484	5.856		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	187.06316 / 5.938	187.06316	5.938		1	0	0	0	0	0	1	0	0	0	0	0	0	Ozonation
	187.12046 / 5.727	187.12046	5.727		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	188.08328 / 9.62	188.08328	9.62		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	188.14083 / 12.131	188.14083	12.131		2	0	0	1	1	0	0	0	0	0	0	0	0	Sand filtration
	195.12557 / 10.802	195.12557	10.802		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	197.14133 / 14.384	197.14133	14.384		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	199.12041 / 8.957	199.12041	8.957		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
	199.12044 / 9.424 // 199.12044 / 9.426	199.12044 // 199.12044	9.426		2	0	1	0	1	0	0	0	0	0	0	0	0	Sand filtration
	199.12054 / 16.94	199.12054	16.94		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	200.05025 / 10.137	200.05025	10.137		1	0	0	0	0	0	0	0	0	0	0	0	1	Ozonation
	202.15649 / 13.495	202.15649	13.495		2	0	0	1	1	0	0	0	0	0	0	0	0	Sand filtration
	203.11523 / 5.573 // 203.11537 / 5.581	203.11523 // 203.11537	5.581		2	0	1	0	1	0	0	0	0	0	0	0	0	Sand filtration
	209.1048 / 7.569	209.1048	7.569		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	211.12048 / 8.866	211.12048	8.866		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	211.90745 / 1.983	211.90745	1.983	Cl	1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
	212.10453 / 8.866	212.10453	8.866		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
	213.09979 / 6.958	213.09979	6.958		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
	213.13606 / 10.802 // 213.13615 / 10.795	213.13606 // 213.13615	10.795		2	0	1	0	1	0	0	0	0	0	0	0	0	Sand filtration



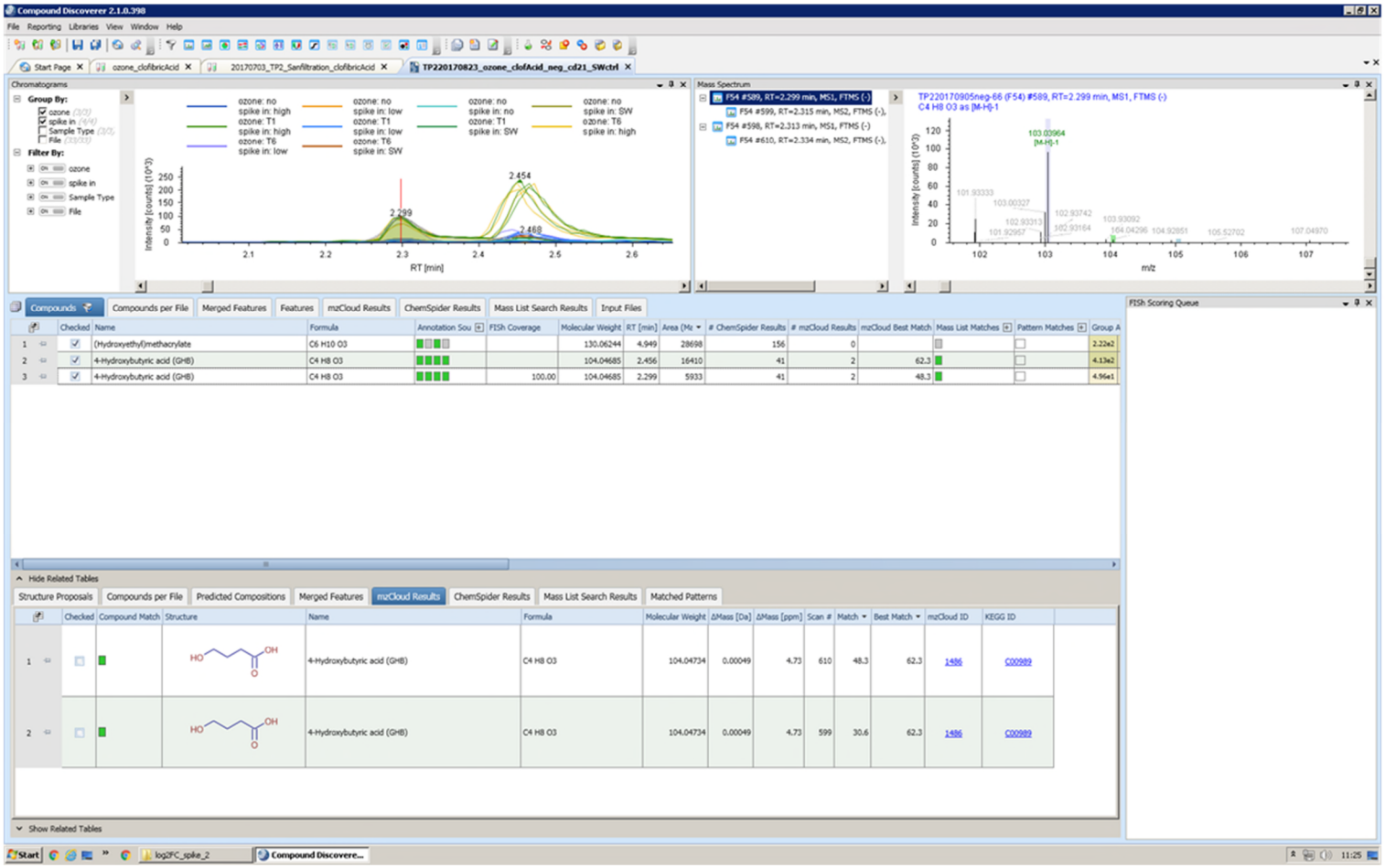
TP bigger than parent compound																	
215.11526 / 7.66 // 215.1154 / 7.665	215.11526 // 215.1154	7.665		2	0	1	0	1	0	0	0	0	0	0	0	0	Sand filtration
215.15161 / 9.163	215.15161	9.163		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
217.13098 / 7.492	217.13098	7.492		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
217.1311 / 6.848	217.1311	6.848		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
217.13112 / 7.496	217.13112	7.496		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
218.8609 / 2.035	218.8609	2.035	Cl	1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
227.11517 / 7.567 // 227.1155 / 7.577	227.11517 // 227.1155	7.577		4	1	1	1	1	0	0	0	0	0	0	0	0	Sand filtration
227.11518 / 8.453 // 227.11558 / 8.455	227.11518 // 227.11558	8.455		3	0	1	1	1	0	0	0	0	0	0	0	0	Sand filtration
229.13091 / 8.911	229.13091	8.911		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
229.13094 / 7.324 // 229.13116 / 7.322	229.13094 // 229.13116	7.322		2	0	1	0	1	0	0	0	0	0	0	0	0	Sand filtration
230.16255 / 5.854	230.16255	5.854		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
230.16265 / 6.252	230.16265	6.252		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
231.1466 / 8.255 // 231.14677 / 8.258	231.1466 // 231.14677	8.258		2	0	1	0	1	0	0	0	0	0	0	0	0	Sand filtration
231.1468 / 8.944	231.1468	8.944		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
235.13356 / 13.788	235.13356	13.788	Cl	2	0	0	0	0	1	1	0	0	0	0	0	0	Clorination
239.90248 / 1.978	239.90248	1.978	Cl	1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
241.13085 / 9.068	241.13085	9.068		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
241.13093 / 9.62	241.13093	9.62		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
241.16784 / 13.272	241.16784	13.272		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
242.11506 / 10.295	242.11506	10.295		1	0	0	0	0	0	0	0	0	1	0	0	0	Clorination
242.15129 / 14.75	242.15129	14.75		1	0	0	0	0	1	0	0	0	0	0	0	0	Clorination
244.13052 / 8.796	244.13052	8.796		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
245.1257 / 8.278 // 245.1262 / 8.278	245.1257 // 245.1262	8.278		4	1	1	1	1	0	0	0	0	0	0	0	0	Sand filtration
246.19378 / 7.617	246.19378	7.617		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
247.14126 / 6.557 // 247.14176 / 6.559	247.14126 // 247.14176	6.559		3	1	1	0	1	0	0	0	0	0	0	0	0	Sand filtration
247.14166 / 7.059	247.14166	7.059		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
249.20864 / 13.451	249.20864	13.451		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
249.20864 / 13.8	249.20864	13.8		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
255.14689 / 11.21	255.14689	11.21		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
259.14133 / 9.62 // 259.14184 / 9.619	259.14133 // 259.14184	9.619		1	0	1	0	1	0	0	0	0	0	0	0	0	Sand filtration
260.21329 / 15.909	260.21329	15.909		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
260.21331 / 16.35	260.21331	16.35		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
265.20342 / 13.658	265.20342	13.658		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
265.20349 / 14.08	265.20349	14.08		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
265.2035 / 15.01	265.2035	15.01		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
267.21919 / 12.576	267.21919	12.576		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
267.21919 / 12.909	267.21919	12.909		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
267.89754 / 1.98	267.89754	1.98	Cl	1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
272.17278 / 7.574	272.17278	7.574		2	1	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
275.13585 / 8.239	275.13585	8.239		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
275.13695 / 8.875	275.13695	8.875		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
277.23979 / 16.348	277.23979	16.348		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
277.23979 / 16.9	277.23979	16.9		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
277.23982 / 16.267	277.23982	16.267		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
277.23983 / 16.118	277.23983	16.118		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
277.23985 / 15.835	277.23985	15.835		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
277.23986 / 15.913	277.23986	15.913		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
279.18025 / 15.497	279.18025	15.497		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
281.19842 / 16.108	281.19842	16.108		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
281.19887 / 15.008	281.19887	15.008		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
283.21398 / 14.075	283.21398	14.075		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
283.21407 / 13.652	283.21407	13.652		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
288.20421 / 10.361	288.20421	10.361		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
293.29475 / 17.541	293.29475	17.541		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
293.29477 / 18.093	293.29477	18.093		2	1	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
293.29479 / 17.103	293.29479	17.103		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
294.21865 / 16.718	294.21865	16.718		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
299.20878 / 15.155	299.20878	15.155		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
299.20878 / 14.623	299.20878	14.623		2	1	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
299.20886 / 15.009	299.20886	15.009		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
299.20891 / 14.752	299.20891	14.752		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
300.19298 / 13.916	300.19298	13.916		1	0	0	0	0	1	0	0	0	0	0	0	0	Clorination
309.23025 / 17.536	309.23025	17.536		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
311.24532 / 16.719	311.24532	16.719		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
315.20439 / 15.17	315.20439	15.17		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
315.20451 / 15.549	315.20451	15.549		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
318.20342 / 13.916	318.20342	13.916		1	0	0	0	0	1	0	0	0	0	0	0	0	Clorination
321.19075 / 16.089	321.19075	16.089		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
327.24012 / 17.542	327.24012	17.542		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
327.24013 / 17.096	327.24013	17.096		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
327.24013 / 18.233	327.24013	18.233		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
327.24019 / 18.092	327.24019	18.092		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
327.24021 / 17.672	327.24021	17.672		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
327.24034 / 17.004	327.24034	17.004		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
329.25581 / 15.117	329.25581	15.117		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
337.86592 / 23.175	**337.86592**	**23.175**		1	0	0	0	0	0	0	0	0	0	0	0	1	Clorination
339.20134 / 14.449	339.20134	14.449		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
343.23487 / 18.361	343.23487	18.361		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
343.23488 / 17.964	343.23488	17.964		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
343.23492 / 17.56	343.23492	17.56		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
343.23508 / 14.122	343.23508	14.122		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
343.23554 / 17.957	343.23554	17.957		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
343.23558 / 17.553	343.23558	17.553		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
345.2504 / 16.102	345.2504	16.102		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
345.25052 / 13.023	345.25052	13.023		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
349.24608 / 15.777	349.24608	15.777		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
351.23736 / 16.018	351.23736	16.018		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
351.23739 / 16.254	351.23739	16.254		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
357.25136 / 17.264	357.25136	17.264		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
362.27722 / 14.453	362.27722	14.453		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
363.22569 / 14.386 // 363.22573 / 14.452 // 363.22575 / 14.736	363.22569 // 363.22573 // 363.22575	14.736		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
367.29239 / 16.661	367.29239	16.661		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
367.29244 / 17.119	367.29244	17.119		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
367.29246 / 16.937	367.29246	16.937		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
373.24617 / 17.536	373.24617	17.536		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
373.24627 / 13.222	373.24627	13.222		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
375.26193 / 16.012	375.26193	16.012		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
375.26193 / 16.252	375.26193	16.252		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
381.22813 / 16.937	381.22813	16.937	Cl	1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
390.30824 / 16.94	390.30824	16.94		1	0	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
390.30827 / 13.025	390.30827	13.025		2	1	1	0	0	0	0	0	0	0	0	0	0	Sand filtration
391.2569 / 13.016	391.2569	13.016		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
391.25691 / 16.668	391.25691	16.668		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
391.25695 / 16.935	391.25695	16.935		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration
391.25708 / 17.114	391.25708	17.114		1	0	0	0	1	0	0	0	0	0	0	0	0	Sand filtration

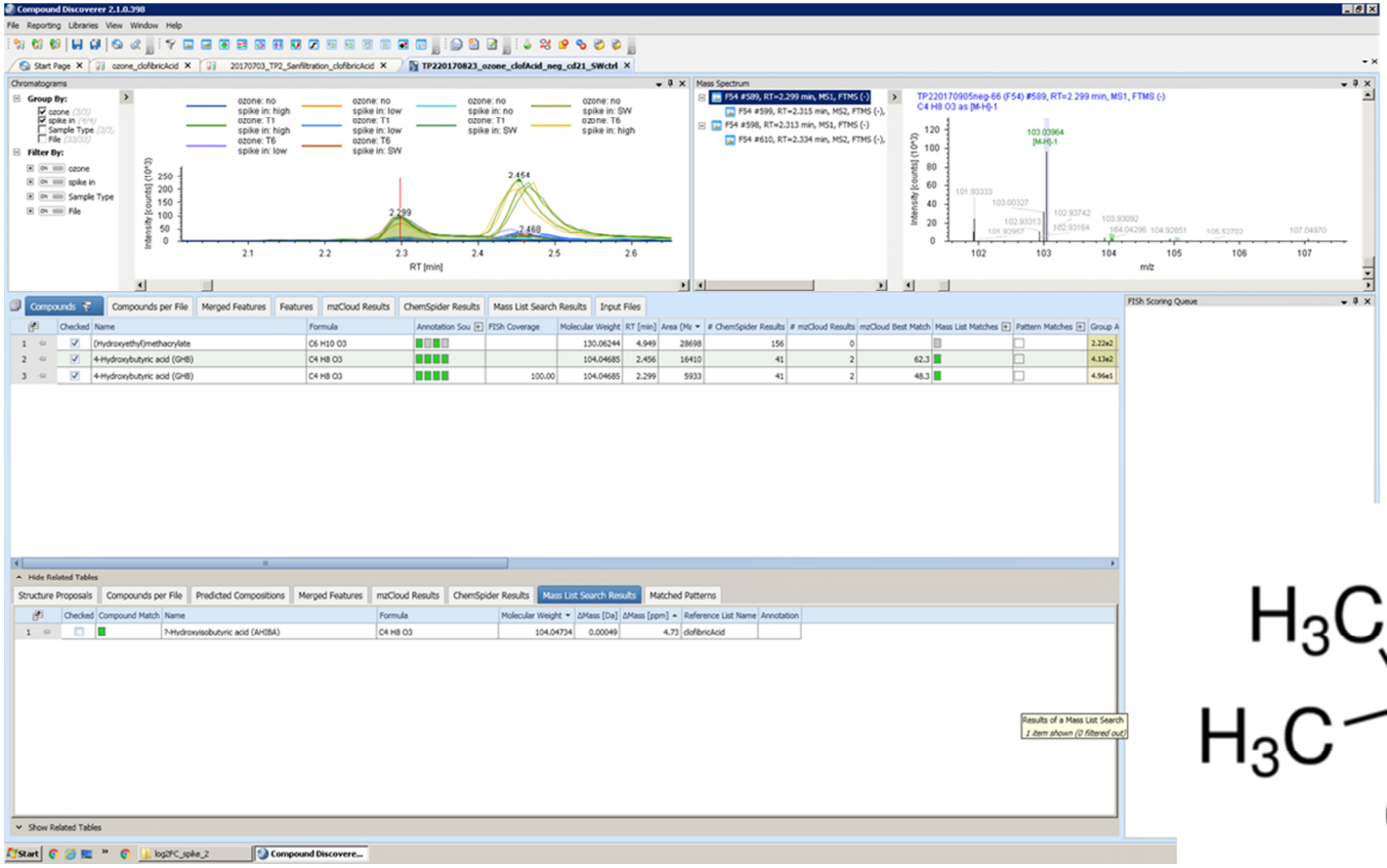
*C(C)OC1=CC=CC=C1 (EnviPATH)

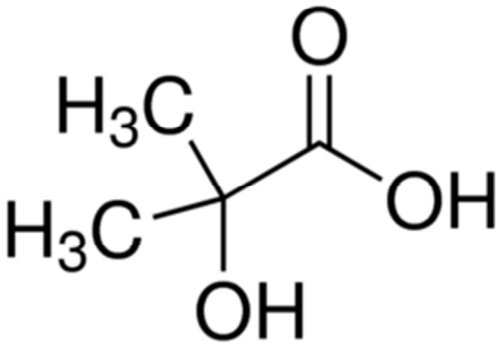
**Alpha-Hydroxyisobutyric (Salgadoac et al., EnviPATH)

Appendix XII. Structural identification of clofibric acid TPs

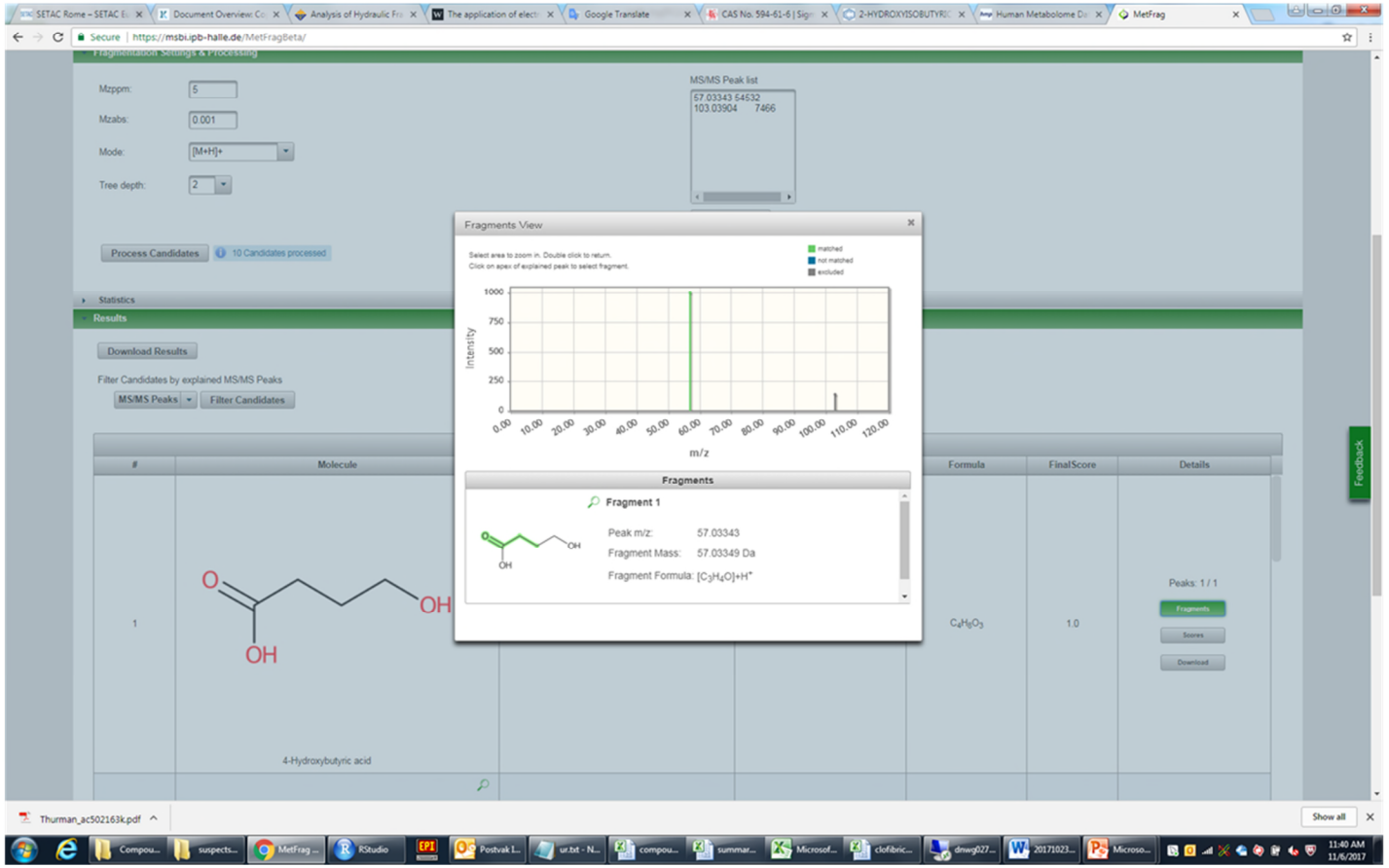
mass list hits



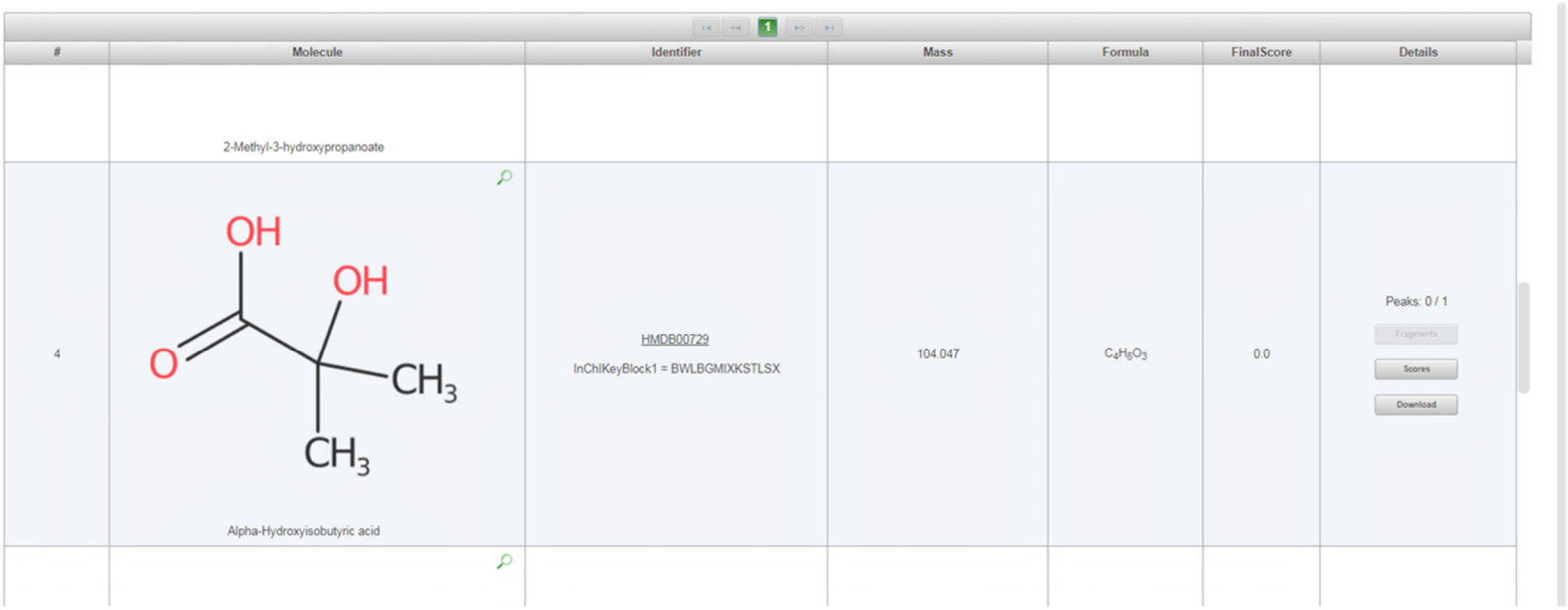

Compound Discoverer 2.1.0.398
Chromatograms
Mass Spectrum
RT [min]
m/z
Compounds
Compounds per File
Merged Features
Features
mzCloud Results
ChemSpider Results
Mass List Search Results
Input Files
FISh Scoring Queue
Structure Proposals
Predicted Compositions
Matched Patterns
Results of a Mass List Search
1 item shown (0 filtered out)


O
H3C
OH
H3C
OH

Metfrag search against HMDB - 4-Hydroxybutyric acid



no match with Alpha-Hydroxyisobutyric acid in Metfrag



#	Molecule	Identifier	Mass	Formula	FinalScore	Details
	2-Methyl-3-hydroxypropanoate					
4	Alpha-Hydroxyisobutyric acid	HMDB00729 InChIKeyBlock1 = BWLBGMIXKSTLSX	104.047	$C_4H_8O_3$	0.0	Peaks: 0 / 1 Fragments Scores Download

match with Alpha-Hydroxyisobutyric acid in HMDB

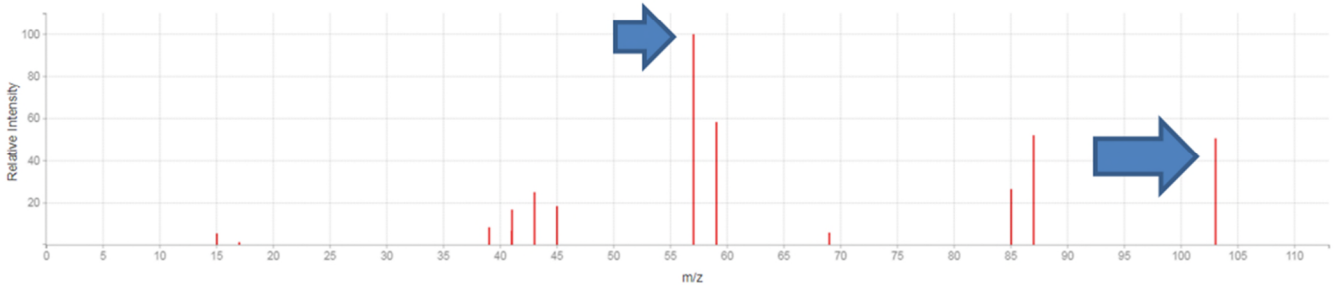
- http://www.hmdb.ca/spectra/ms_ms/17677

Predicted LC-MS/MS Spectrum - 40V, Negative (HMDB0000729)

Spectrum Details

HMDB ID: HMDB0000729
Compound name: Alpha-Hydroxyisobutyric acid
Spectrum type: Predicted LC-MS/MS Spectrum - 40V, Negative
Splash Key: splash10-0a4i-9100000000-237ae993c0cc1090dd96 View in MoNA
Notes: This is a predicted spectrum and it should only be used as a guide, further evidence is required to confirm identification.

Spectrum View

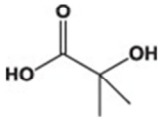


Experimental Conditions

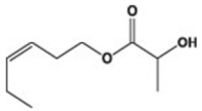
Ionization Mode: Negative
Collision Energy: 40 eV
Instrument Type: QTOF (generic), spectrum predicted by CFM-ID
Mass Resolution: 0.0001 Da
Molecular Formula: C4H8O3
Molecular Weight (Monoisotopic Mass): 104.0473 Da
Molecular Weight (Avergae Mass): 104.1045 Da

sandfiltration pos d4

Biodegradation of clofibric acid and identification of its metabolites.
R.SalgadoacA.OehmenaG.CarvalhoabJ.P.Noron haaM.A.M.Reisa. Journal of hazardous materials 2012



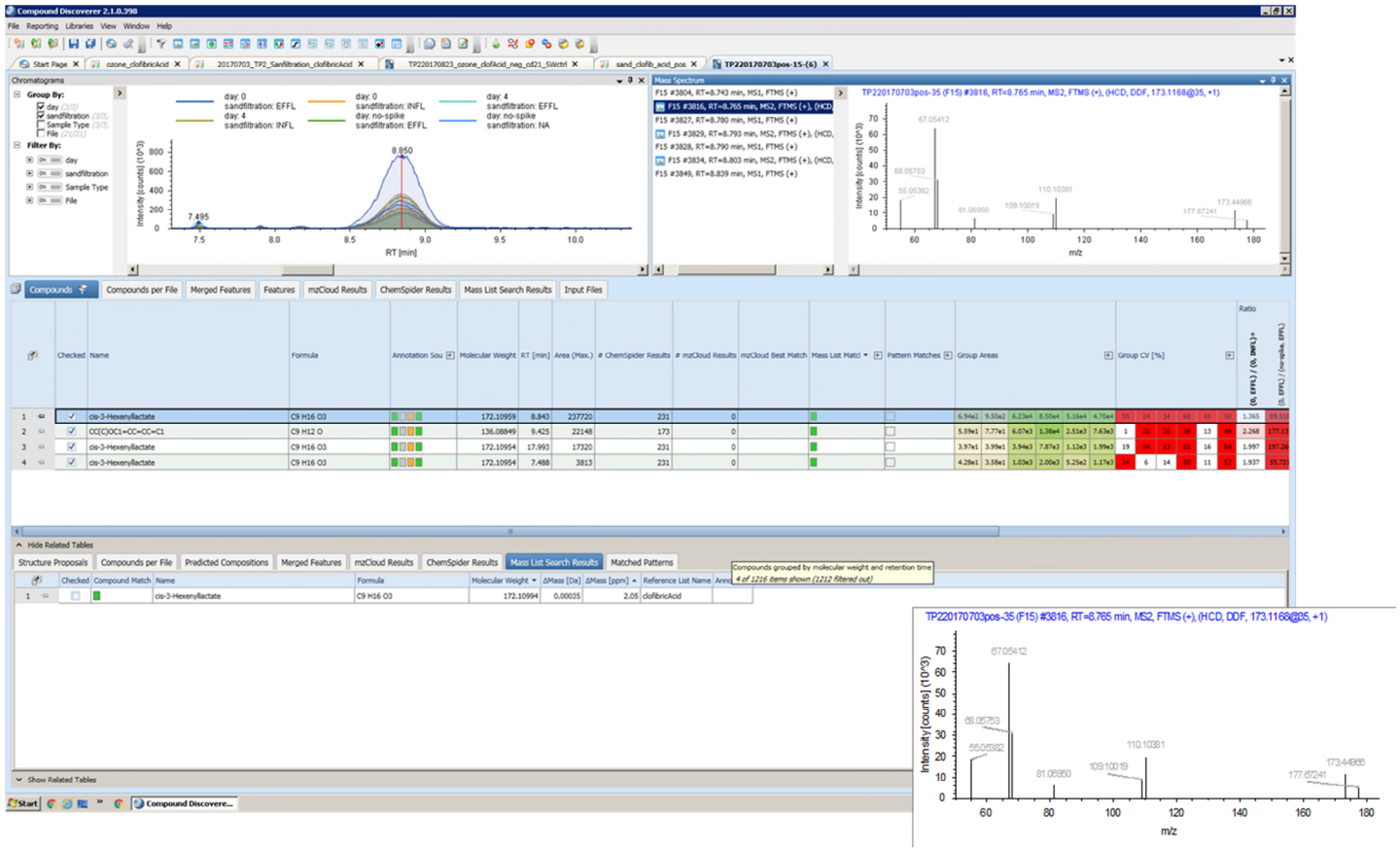
α-Hydroxyisobutyric acid (AHIBA)



cis-3-Hexenyllactate

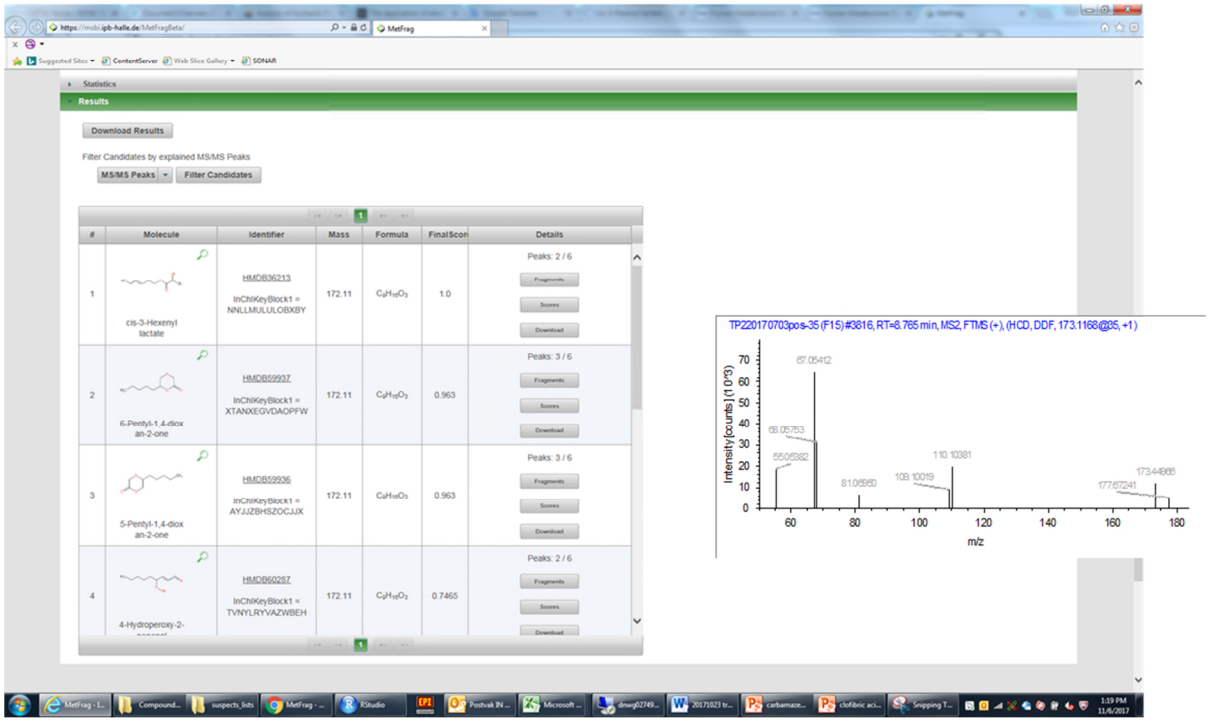
both also predicted with ENviPATH

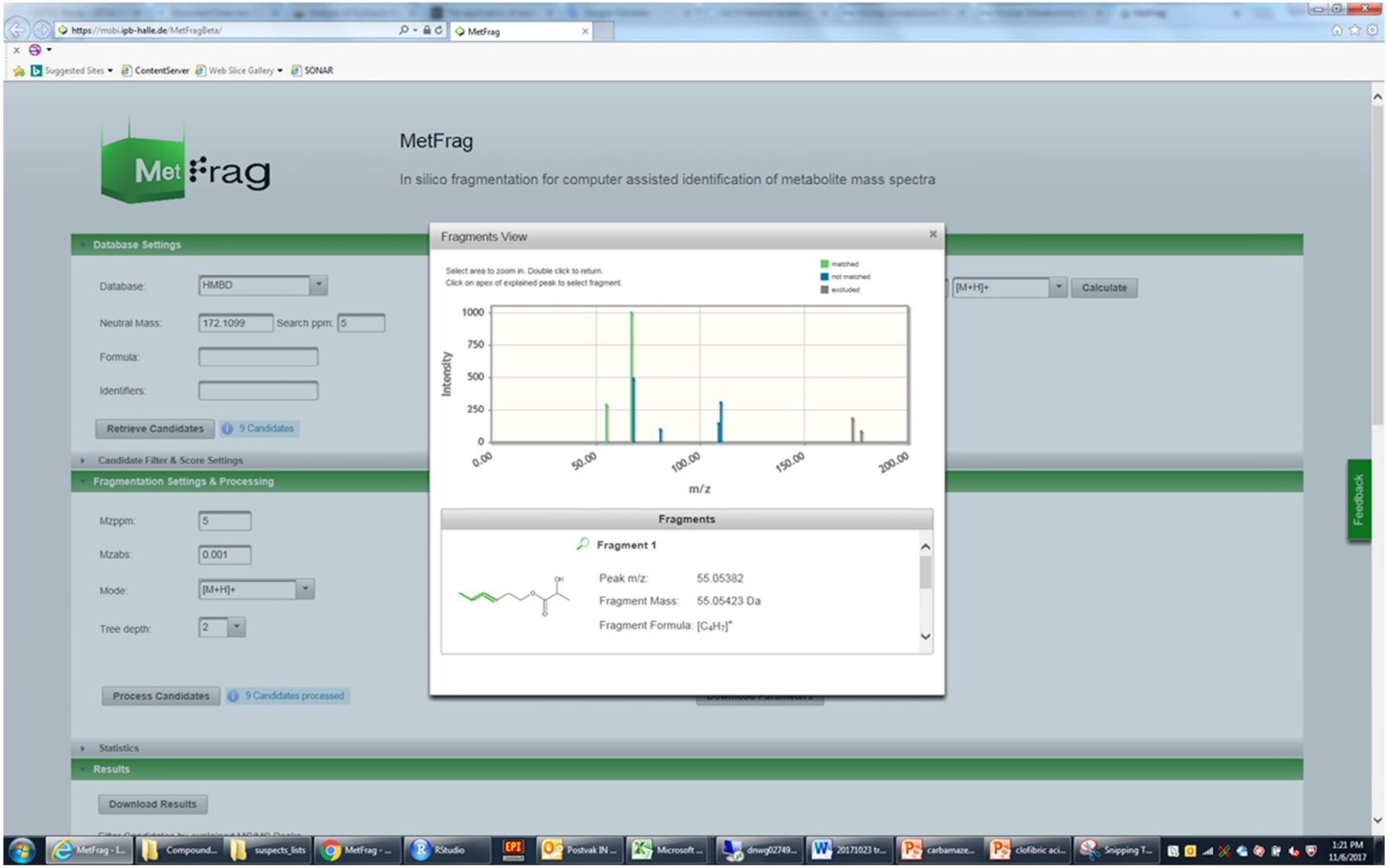
3-hexenyllactate

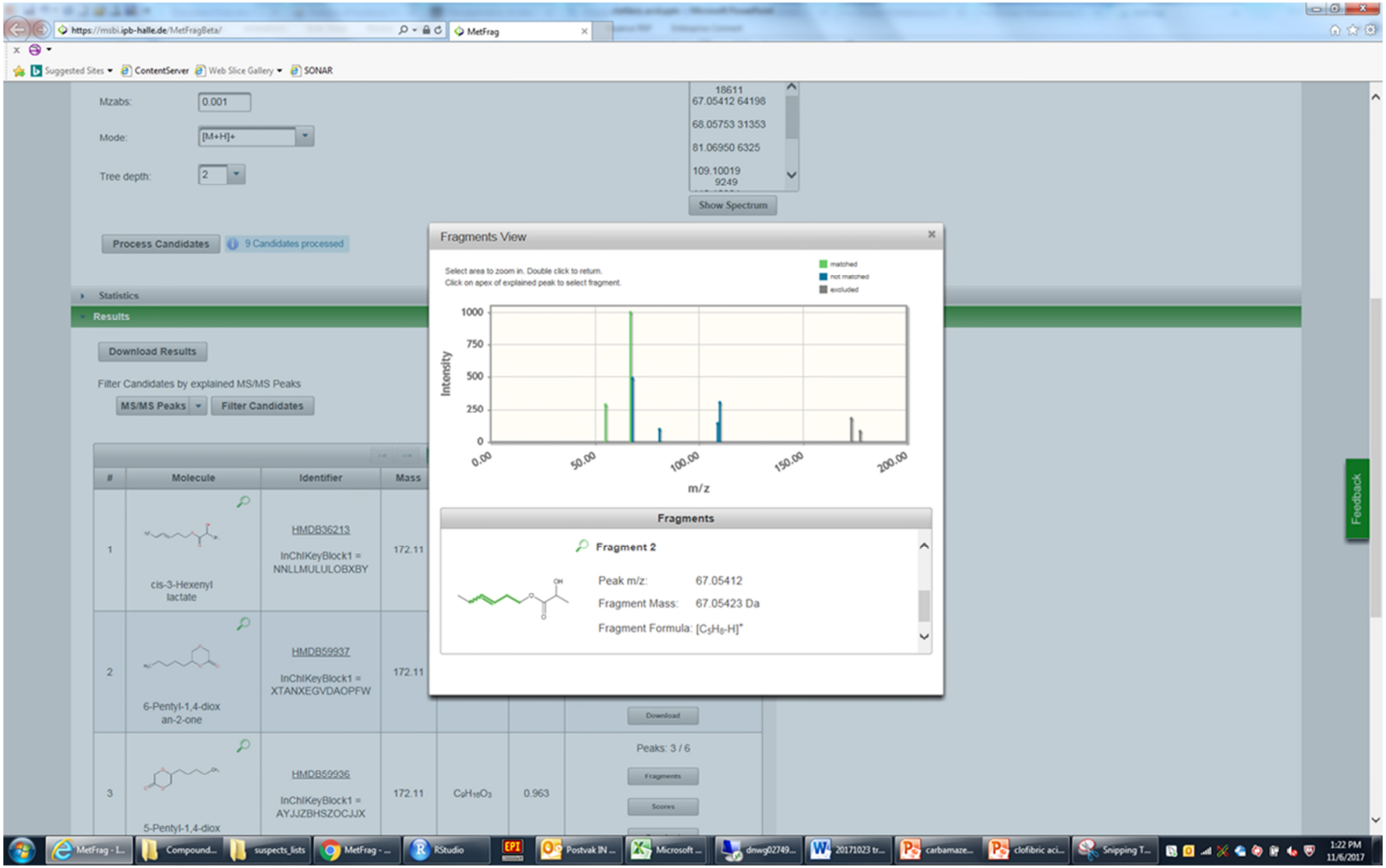


3-hexenyllactate

- http://www.hmdb.ca/metabolites/HMDB0036213
- top hit in metfrag when searched against hmdb




https://msbi.ipb-halle.de/MetFragBeta/
MetFrag
Suggested Sites
ContentServer
Web Slice Gallery
SONAR
MetFrag
In silico fragmentation for computer assisted identification of metabolite mass spectra
Database Settings
Database: HMBD
Neutral Mass: 172.1099 Search ppm: 5
Formula:
Identifiers:
Retrieve Candidates
9 Candidates
Candidate Filter & Score Settings
Fragmentation Settings & Processing
Mzppm: 5
Mzabs: 0.001
Mode: [M+H]+
Tree depth: 2
Process Candidates
9 Candidates processed
Statistics
Results
Download Results
[M+H]+
Calculate
Feedback
Fragments View
Select area to zoom in. Double click to return.
Click on apex of explained peak to select fragment.
matched
not matched
excluded
Intensity
1000
750
500
250
0
0.00
50.00
100.00
150.00
200.00
m/z
Fragments
Fragment 1
Peak m/z: 55.05382
Fragment Mass: 55.05423 Da
Fragment Formula: [C4H7]+
1:21 PM
11/6/2017


https://msbi.ipb-halle.de/MetFragBeta/
MetFrag
Suggested Sites
ContentServer
Web Slice Gallery
SONAR
Mzabs:
0.001
Mode:
[M+H]+
Tree depth:
2
Process Candidates
9 Candidates processed
18611
67.05412 64198
68.05753 31353
81.06950 6325
109.10019
9249
Show Spectrum
Fragments View
Select area to zoom in. Double click to return.
Click on apex of explained peak to select fragment.
matched
not matched
excluded
Intensity
1000
750
500
250
0
0.00
50.00
100.00
150.00
200.00
m/z
Fragments
Fragment 2
Peak m/z: 67.05412
Fragment Mass: 67.05423 Da
Fragment Formula: [C5H8-H]+
Statistics
Results
Download Results
Filter Candidates by explained MS/MS Peaks
MS/MS Peaks
Filter Candidates
#
Molecule
Identifier
Mass
1
cis-3-Hexenyl lactate
HMDB36213
InChIKeyBlock1 = NNLLMULULOBXBY
172.11
2
6-Pentyl-1,4-dioxan-2-one
HMDB59937
InChIKeyBlock1 = XTANXEGVDAOPFW
172.11
3
5-Pentyl-1,4-dioxan
HMDB59936
InChIKeyBlock1 = AYJJZBHSZOCJJX
172.11
C9H16O3
0.963
Download
Peaks: 3 / 6
Fragments
Scores
Feedback
1:22 PM
11/6/2017

Appendix XIII. Potential transformation products of carbamazepine.

Features with log2FC before / after treatment >1; spike-in / no spike >2. p-value < 0.05.

	Feature (Molecular weight / Retention time)	Molecular Weight	RT		Sand filtration				Ozonation							
					pos d0	pos d4	neg d0	neg d4	pos low t1	pos low t6	pos high t1	pos high t6	neg low t1	neg low t6	neg high t1	neg high t6
	Carbamazepine (236.09488 / 13.263 // 236.09471 / 13.264)	**236.09471**	**13.263**													
TP smaller than parent compound	85.08922 / 2.291	85.08922	2.291		0	0	0	0	1	0	1	0	0	0	0	0
	90.04697 / 2.283	90.04697	2.283		0	0	0	0	0	0	1	0	0	0	0	0
	101.12047 / 2.285	101.12047	2.285		0	0	0	0	0	1	0	0	0	0	0	0
	102.04686 / 2.891	102.04686	2.891		0	0	0	0	0	0	1	1	0	0	0	0
	113.08403 / 5.98	113.08403	5.98		0	0	0	0	0	0	1	0	0	0	0	0
	118.04181 / 2.285	118.04181	2.285		0	0	0	0	1	0	1	0	0	0	0	0
	119.037 / 8.676	119.037	8.676		0	0	0	0	1	1	1	1	0	0	0	0
	119.07336 / 2.899	119.07336	2.899		0	0	0	0	0	0	1	0	0	0	0	0
	129.04198 / 1.686	129.04198	1.686		0	0	0	0	0	0	0	0	0	0	1	0
	129.04214 / 2.287	129.04214	2.287		0	0	0	0	0	0	0	0	0	0	1	0
	130.06241 / 4.747 // 130.06242 / 4.624 // 130.06243 / 4.531	130.06241 // 130.06242 // 130.06243	4.531		0	0	0	0	0	0	0	0	1	1	1	1
	131.09457 / 2.294	131.09457	2.294		0	0	0	0	0	0	1	0	0	0	0	0
	137.04709 / 8.682 // 137.04751 / 8.676	137.04709 // 137.04751	8.676		0	0	0	0	1	0	1	1	1	0	1	1
	143.98766 / 2.348	143.98766	2.348		0	0	0	0	0	0	0	0	0	0	1	0
	146.04782 / 5.965	146.04782	5.965		0	0	0	0	1	0	1	0	0	0	0	0
	146.0574 / 6.219	146.0574	6.219		0	0	0	0	0	0	0	0	0	0	1	1
	160.10925 / 9.02	160.10925	9.02		0	0	1	0	0	0	0	0	0	0	0	0
	162.04267 / 7.132	162.04267	7.132		0	0	0	0	1	0	1	1	0	0	0	0
	162.11565 / 2.283	162.11565	2.283		0	0	0	0	1	1	1	0	0	0	0	0
	164.04723 / 2.286	164.04723	2.286		0	0	0	0	1	0	1	0	0	0	0	0
	165.07852 / 2.83 // 165.07877 / 2.895	165.07852 // 165.07877	2.895		0	0	0	0	0	0	1	1	0	0	1	0
	172.01896 / 6.084	172.01896	6.084		0	0	0	0	0	0	0	0	1	0	1	0
	175.88672 / 1.641	175.88672	1.641		1	0	0	0	0	0	0	0	0	0	0	0
	180.053 / 8.685	180.053	8.685		0	0	0	0	0	0	0	0	1	1	1	1
	181.07365 / 2.294 // 181.07376 / 2.284	181.07365 // 181.07376	2.284		0	0	0	0	0	0	1	0	1	0	1	0
	182.99859 / 3.661	182.99859	3.661		0	0	0	0	0	0	0	0	0	0	1	0
	187.06311 / 5.872	187.06311	5.872		0	0	0	0	0	0	1	1	0	0	0	0
	188.10438 / 10.613	188.10438	10.613		0	0	0	0	0	0	0	0	0	0	1	0
	190.90898 / 2.049	190.90898	2.049		1	0	0	0	0	0	0	0	0	0	0	0
	193.94124 / 2.027	193.94124	2.027		0	0	1	0	0	0	0	0	0	0	0	0
	194.08018 / 6.843	194.08018	6.843		0	0	0	0	1	0	1	0	0	0	0	0
	195.068 / 11.198	195.068	11.198		0	0	0	0	0	0	0	0	1	0	1	1
	200.05025 / 10.172	200.05025	10.172		0	0	0	0	0	0	0	0	1	1	1	0
	208.01903 / 9.23	208.01903	9.23		0	0	0	0	0	0	0	0	0	0	1	0
	208.01911 / 8.774	208.01911	8.774		0	0	0	0	0	0	0	0	0	0	1	0
	228.11067 / 5.334 // 228.11079 / 5.335	228.11067 // 228.11079	5.335		0	0	0	0	1	1	1	1	1	1	1	1
	232.13071 / 10.612	232.13071	10.612		0	0	0	0	0	0	0	0	0	0	1	0
	232.93871 / 23.617	232.93871	23.617		0	0	0	0	0	0	0	0	0	1	0	0
	236.07945 / 2.936	236.07945	2.936		0	0	0	0	0	0	1	0	0	0	0	0

TP bigger than parent compound																	
	238.07397 / 11.2	238.07397	11.2		0	0	0	0	0	0	0	0	1	0	1	1
	238.14132 / 4.818	238.14132	4.818		0	0	0	0	0	0	1	0	0	0	0	0
	242.1152 / 10.3	242.1152	10.3		0	0	0	0	0	0	0	0	0	0	1	0
	242.12647 / 8.034	242.12647	8.034		0	0	0	0	0	0	0	0	1	0	1	0
	244.13067 / 9.962	244.13067	9.962		0	0	0	0	0	0	1	0	0	0	0	0
	245.16226 / 7.596	245.16226	7.596		0	0	0	0	0	0	1	0	0	0	0	0
	248.0899 / 14.311	248.0899	14.311		0	0	0	0	0	0	0	0	0	1	0	0
	262.14151 / 9.961	262.14151	9.961		0	0	0	0	0	0	0	0	0	0	1	0
	264.05294 / 11.193	264.05294	11.193		0	0	0	0	1	0	1	1	0	0	0	0
	266.06868 / 10.307	266.06868	10.307		0	0	0	0	1	0	1	0	0	0	0	0
	266.06871 / 11.78	266.06871	11.78		0	0	0	0	1	0	1	0	0	0	0	0
	270.14656 / 12.702	270.14656	12.702		0	0	0	0	0	0	0	0	0	0	1	0
	276.11094 / 8.739	276.11094	8.739		0	0	0	0	0	0	0	0	1	0	1	0
	282.06345 / 11.194	282.06345	11.194		0	0	0	0	1	0	1	1	0	0	0	0
	282.06387 / 11.2	282.06387	11.2		0	0	0	0	0	0	0	0	1	1	1	1
	282.16723 / 6.175	282.16723	6.175		0	0	0	0	0	0	1	0	0	0	0	0
	286.22495 / 11.057	286.22495	11.057		0	0	0	0	0	0	1	0	0	0	0	0
	296.18291 / 7.535	296.18291	7.535		0	0	0	0	0	0	1	0	0	0	0	0
	300.19299 / 13.916	300.19299	13.916		0	0	0	0	0	0	1	0	0	0	0	0
	313.20935 / 7.537	313.20935	7.537		0	0	0	0	0	0	1	0	0	0	0	0
	314.25627 / 13.652	314.25627	13.652		0	0	0	0	0	0	1	0	0	0	0	0
	316.11551 / 10.716	316.11551	10.716		0	0	0	0	0	0	0	0	0	1	0	0
	318.20343 / 13.916	318.20343	13.916		0	0	0	0	0	0	1	0	0	0	0	0
	322.87919 / 23.635	322.87919	23.635		0	0	0	0	0	0	0	0	1	1	0	0
	326.19339 / 6.846	326.19339	6.846		0	0	0	0	0	0	1	0	0	0	0	0
	337.86582 / 23.606	337.86582	23.606		0	0	0	0	0	0	0	0	0	1	0	0
	340.20894 / 8.012	340.20894	8.012		0	0	0	0	0	0	1	0	0	0	0	0
	343.21976 / 6.851	343.21976	6.851		0	0	0	0	0	0	1	0	0	0	0	0
	350.87407 / 23.63	350.87407	23.63		0	0	0	0	0	0	0	0	1	1	0	0
	357.23551 / 8.01	357.23551	8.01		0	0	0	0	0	0	1	0	0	0	0	0
	363.26103 / 13.914	363.26103	13.914		0	0	0	0	0	0	1	0	0	0	0	0
	384.23505 / 8.399	384.23505	8.399		0	0	0	0	0	0	1	0	0	0	0	0
	387.24596 / 7.337	387.24596	7.337		0	0	0	0	0	0	1	0	0	0	0	0
	418.23517 / 17.593	418.23517	17.593		0	0	0	0	0	0	0	0	0	0	1	0
	418.23543 / 17.751	418.23543	17.751		0	0	0	0	0	0	0	0	0	0	1	0
	428.26139 / 8.724	428.26139	8.724		0	0	0	0	0	0	1	0	0	0	0	0
	431.27208 / 7.73	431.27208	7.73		0	0	0	0	0	0	1	0	0	0	0	0
	475.29825 / 8.059	475.29825	8.059		0	0	0	0	0	0	1	0	0	0	0	0
	519.32422 / 8.345	519.32422	8.345		0	0	0	0	0	0	1	0	0	0	0	0

Sand
Ozone
Both

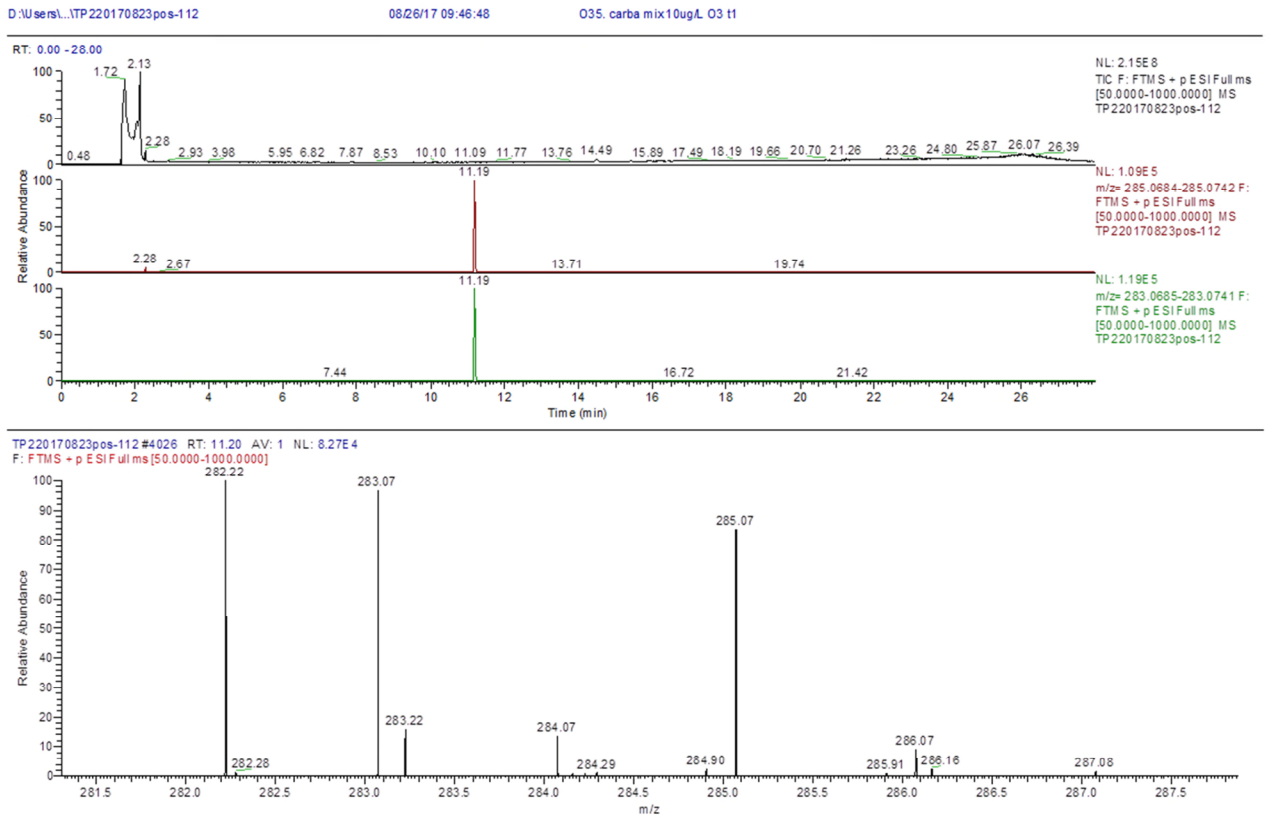
Appendix XIV. Potential transformation products of labelled carbamazepine.

	Feature	Molecular Weight	RT	total number detected	pos d0	pos d4	neg d0	neg d4	pos low t1	pos low t6	pos high t1	pos high t6	neg low t1	neg low t6	neg high t1	neg high t6	treatment
	Carbamazepine-(carboxamide-13C,15N) (238.09527 / 13.259 // 238.09511 / 13.261)	238.09527 // 238.09511	13.261														no
TP smaller than parent compound	116.04686 / 2.829	116.04686	2.829	4	0	0	0	0	0	0	0	0	1	1	1	1	Ozone
	119.03698 / 8.674	119.03698	8.674	4	0	0	0	0	1	1	1	1	0	0	0	0	Ozone
	119.0734 / 2.894	119.0734	2.894	1	0	0	0	0	0	1	0	0	0	0	0	0	Ozone
	129.15163 / 7.003	129.15163	7.003	2	0	0	0	0	1	0	0	1	0	0	0	0	Ozone
	130.06245 / 4.499	130.06245	4.499	4	0	0	0	0	0	0	0	0	1	1	1	1	Ozone
	135.05454 / 2.287	135.05454	2.287	1	0	1	0	0	0	0	0	0	0	0	0	0	Sand
	137.0471 / 8.658 // 137.04753 / 8.673	137.0471 // 137.04753	8.673	7	0	0	0	0	1	1	1	1	1	0	1	1	Ozone
	146.05737 / 6.232	146.05737	6.232	3	0	0	0	0	0	0	0	0	1	1	0	1	Ozone
	148.04822 / 5.967	148.04822	5.967	2	0	0	0	0	1	0	1	0	0	0	0	0	Ozone
	151.04945 / 2.284	151.04945	2.284	1	0	1	0	0	0	0	0	0	0	0	0	0	Sand
	162.11558 / 2.287	162.11558	2.287	1	0	0	0	0	1	0	0	0	0	0	0	0	Ozone
	164.04276 / 7.14 // 164.04309 / 7.13	164.04276 // 164.04309	7.13	6	0	0	0	0	1	0	1	1	1	0	1	1	Ozone
	165.07878 / 2.895	165.07878	2.895	1	0	0	0	0	0	1	0	0	0	0	0	0	Ozone
	182.05339 / 8.655	182.05339	8.655	4	0	0	0	0	0	0	0	0	1	1	1	1	Ozone
	185.06839 / 4.257	185.06839	4.257	4	0	0	0	0	0	0	0	0	1	1	1	1	Ozone
	195.06801 / 11.192	195.06801	11.192	2	0	0	0	0	0	0	0	0	1	0	1	0	Ozone
	200.05022 / 10.184	200.05022	10.184	2	0	0	0	0	0	0	0	0	0	1	0	1	Ozone
	208.01901 / 9.234	208.01901	9.234	1	0	0	0	0	0	0	0	0	1	0	0	0	Ozone
TP bigger than parent compound	240.0744 / 11.192	240.0744	11.192	3	0	0	0	0	0	0	0	0	1	0	1	1	Ozone
	242.08983 / 2.393	242.08983	2.393	2	0	0	1	1	0	0	0	0	0	0	0	0	Sand
	251.10182 / 2.287	251.10182	2.287	2	1	1	0	0	0	0	0	0	0	0	0	0	Sand
	266.05342 / 11.19	266.05342	11.19	3	0	0	0	0	1	0	1	1	0	0	0	0	Ozone
	268.06914 / 11.775	268.06914	11.775	2	0	0	0	0	1	0	1	0	0	0	0	0	Ozone
	268.06918 / 10.303	268.06918	10.303	2	0	0	0	0	1	0	1	0	0	0	0	0	Ozone
	281.97905 / 8.659	281.97905	8.659	3	0	0	0	0	0	0	0	0	1	0	1	1	Ozone
	284.06392 / 11.19 // 284.06432 / 11.193	284.06392 // 284.06432	11.193	7	0	0	0	0	1	0	1	1	1	1	1	1	Ozone
	288.09543 / 2.401	288.09543	2.401	2	0	0	1	1	0	0	0	0	0	0	0	0	Sand
	337.86583 / 23.489	337.86583	23.489	1	0	0	0	0	0	0	0	0	0	1	0	0	Ozone
	363.15271 / 12.224	363.15271	12.224	4	0	0	0	0	0	0	0	0	1	1	1	1	Ozone

Appendix XV. Carbamazepine TPs: mass shifts and MS2 based identification

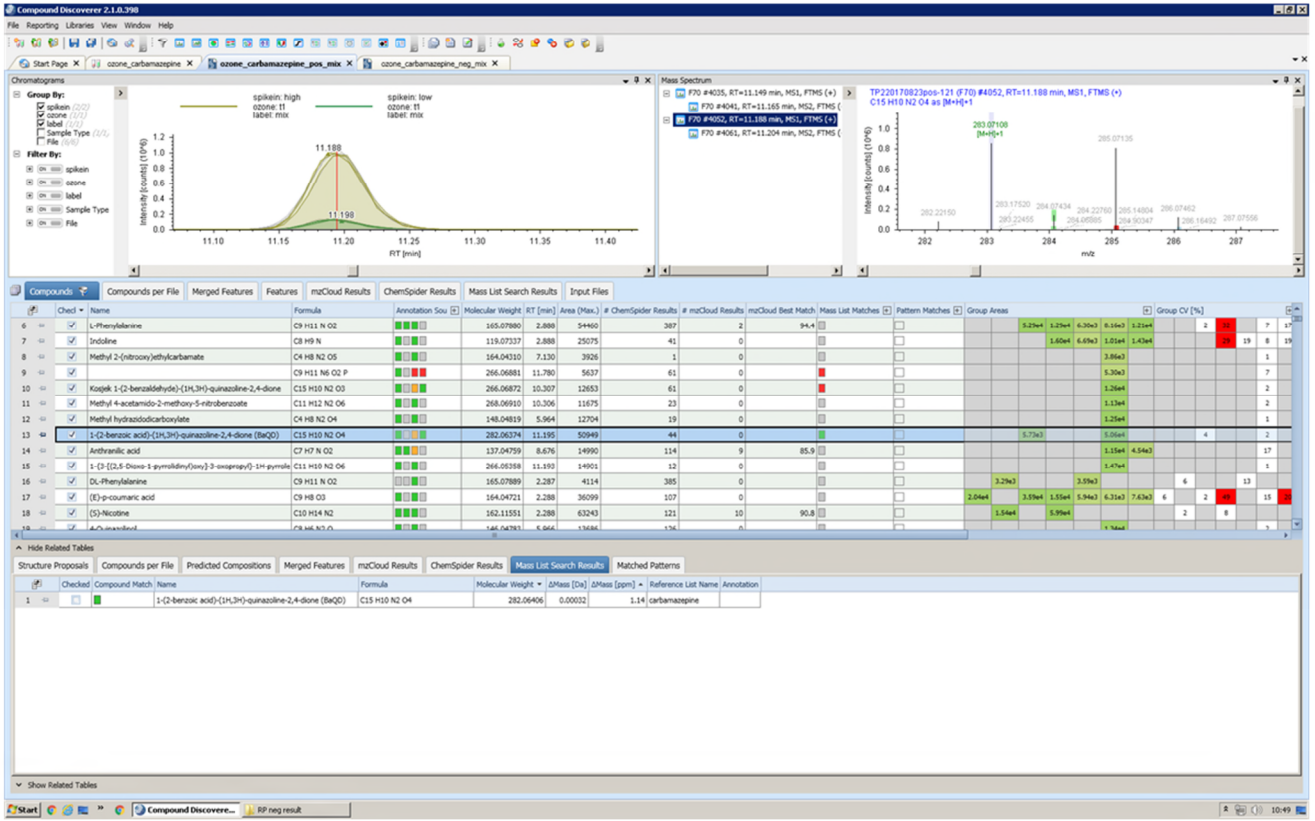
282.06387 / 11.2 284.06392 / 11.19 // 284.06432 / 11.193

Features that overlap in carbamazepine without and with label experiment after filtering (p< 0.05, log2fold increase 1 before vs after treatment); all features were detected in ozone mix experiments.



282.06387 / 11.2
284.06392 / 11.19 // 284.06432 / 11.193

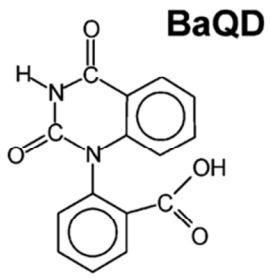
Hit in suspect list of carbamazepine predicted transformation products



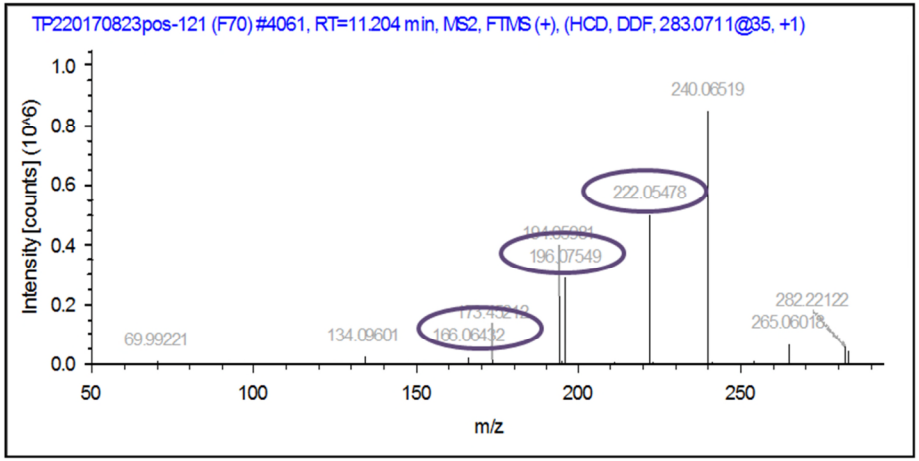
282.06387 / 11.2
284.06392 / 11.19 // 284.06432 / 11.193

Hit in suspect list of carbamazepine predicted transformation products: 1-(2-benzoic acid)-(1H,3H)-quinazoline-2,4-dione (BaQD)

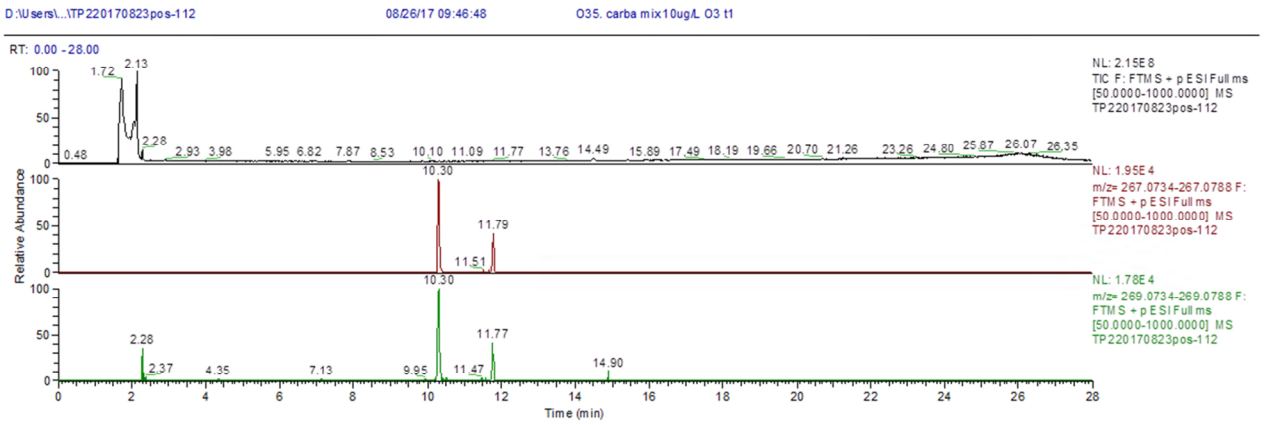
- McDowell et al. *Ozonation of Carbamazepine in Drinking Water: Identification and Kinetic Study of Major Oxidation Products.* ES&T 2005
- Azaïs et al. *Ozonation as a pretreatment process for nanofiltration brines: Monitoring of transformation products and toxicity evaluation.* J Hazard Mater 2017. – m/z MS2 CID: 222.0546; 196.0746; 166.0653

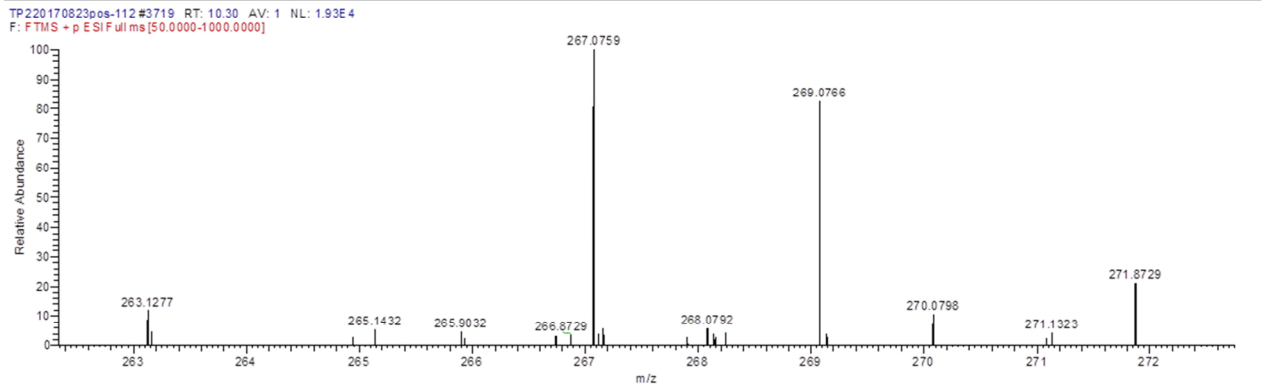


1-(2-benzoic acid)-(1*H*,3*H*)-quinazoline-2,4-dione



266.06868 / 10.307 268.06918 / 10.303	266.06871 / 11.78 268.06914 / 11.775

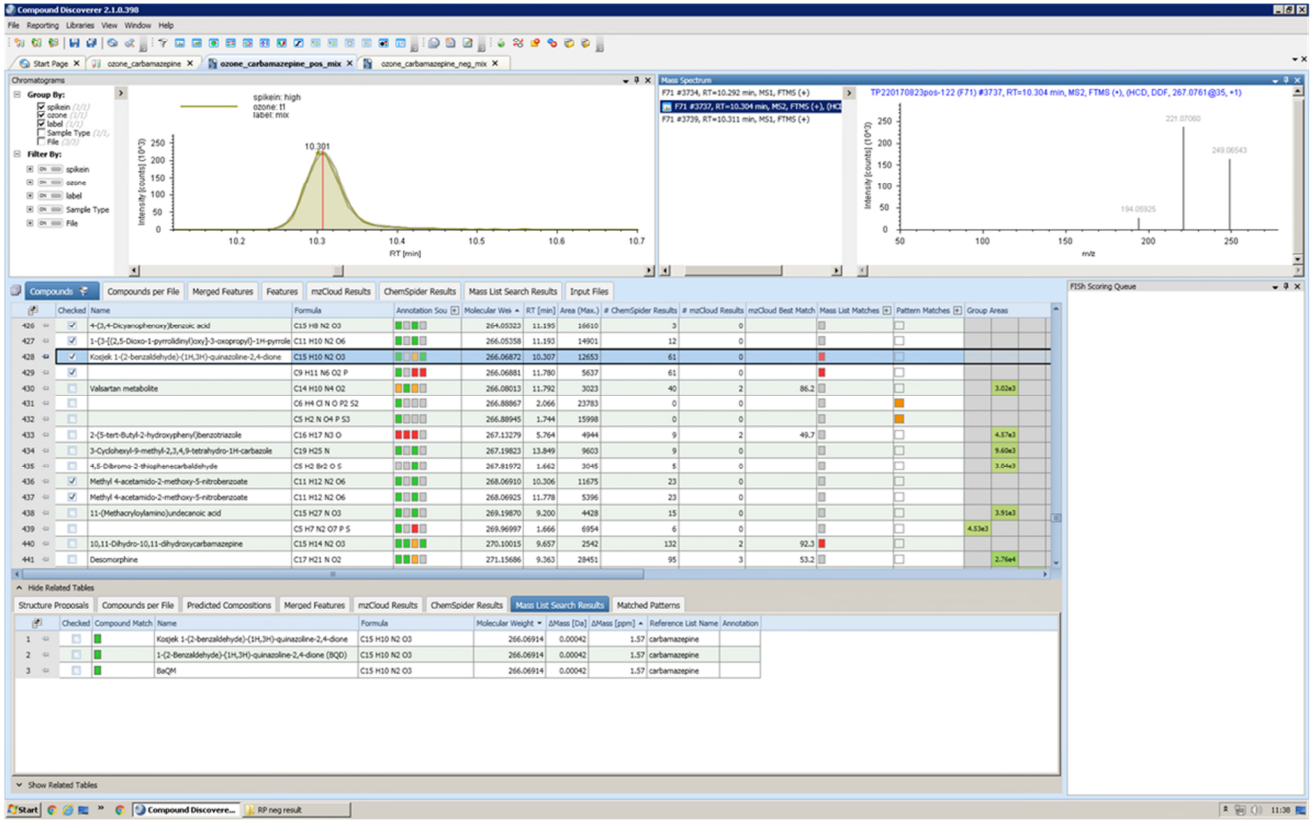




266.06868 / 10.307
268.06918 / 10.303

2 potential suspects in TP suspect list:

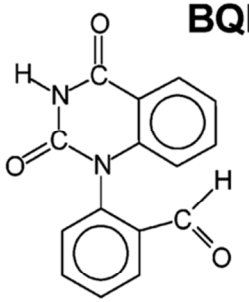
- 1-(2-Benzaldehyde)-(1H,3H)-quinazoline-2,4-dione (BQD)
- BaQM



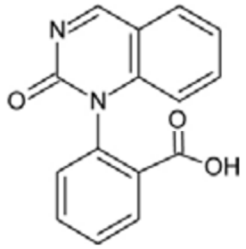
266.06868 / 10.307
268.06918 / 10.303

2 potential suspects in TP suspect list:
- 1-(2-Benzaldehyde)-(1H,3H)-quinazoline-2,4-dione (BQD)
- BaQM

BQD



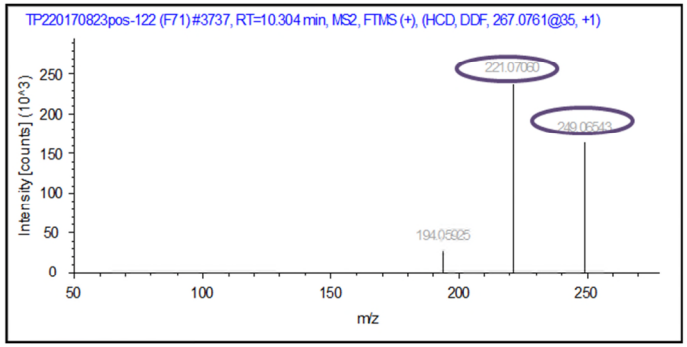
BaQM



Azais et al. 196.0766; 167.0737

no fragments match

Azais et al. m/z MS2: 249.0658; 221.0714

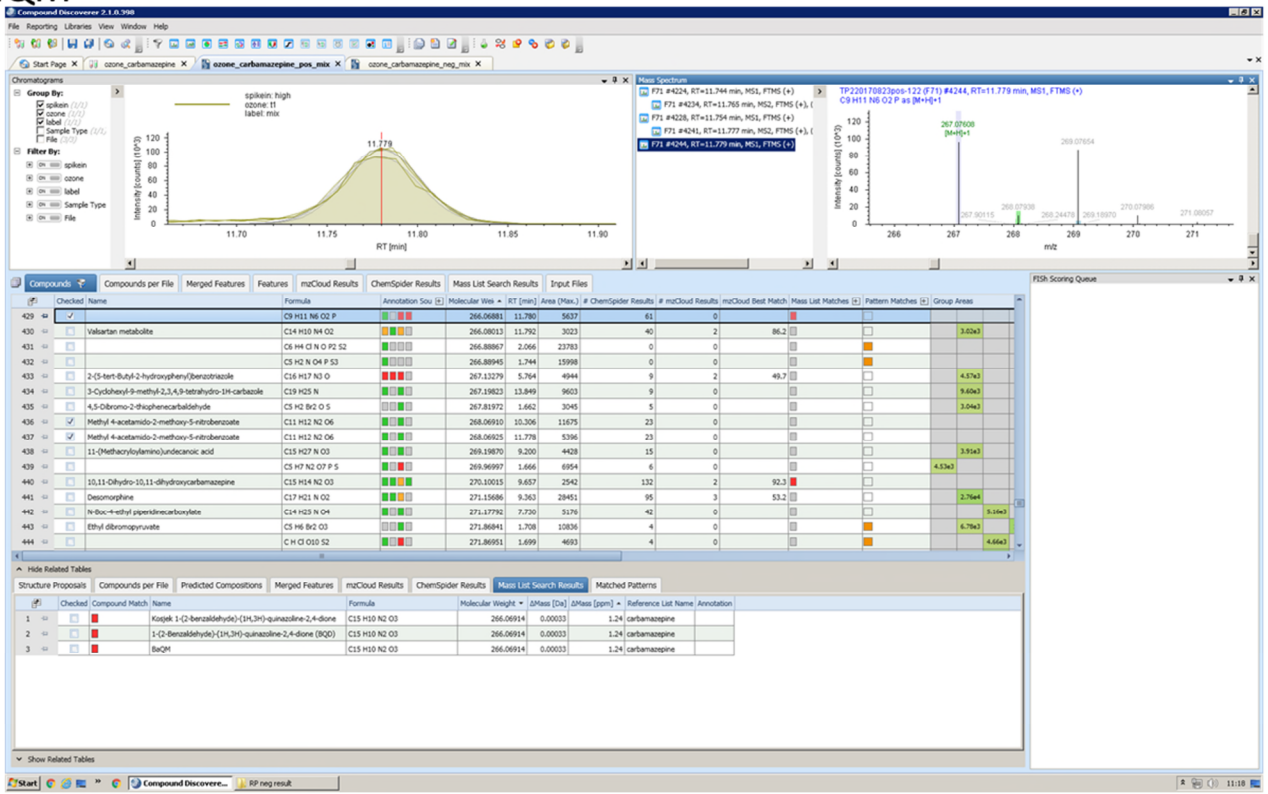


266.06871 / 11.78
268.06914 / 11.775

change in RT, same M

2 potential suspects in TP suspect list:

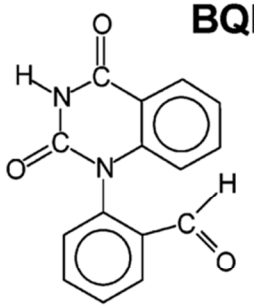
- 1-(2-Benzaldehyde)-(1H,3H)-quinazoline-2,4-dione (BQD)
- BaQM



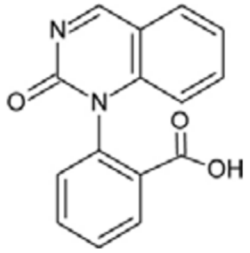
266.06871 / 11.78
268.06914 / 11.775

2 potential suspects in TP suspect list:
- 1-(2-Benzaldehyde)-(1H,3H)-quinazoline-2,4-dione (BQD)
- BaQM

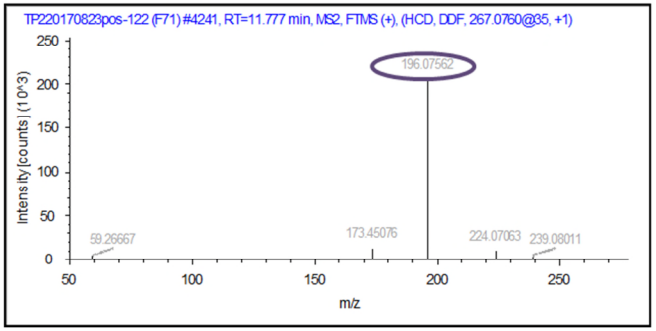
BQD



BaQM



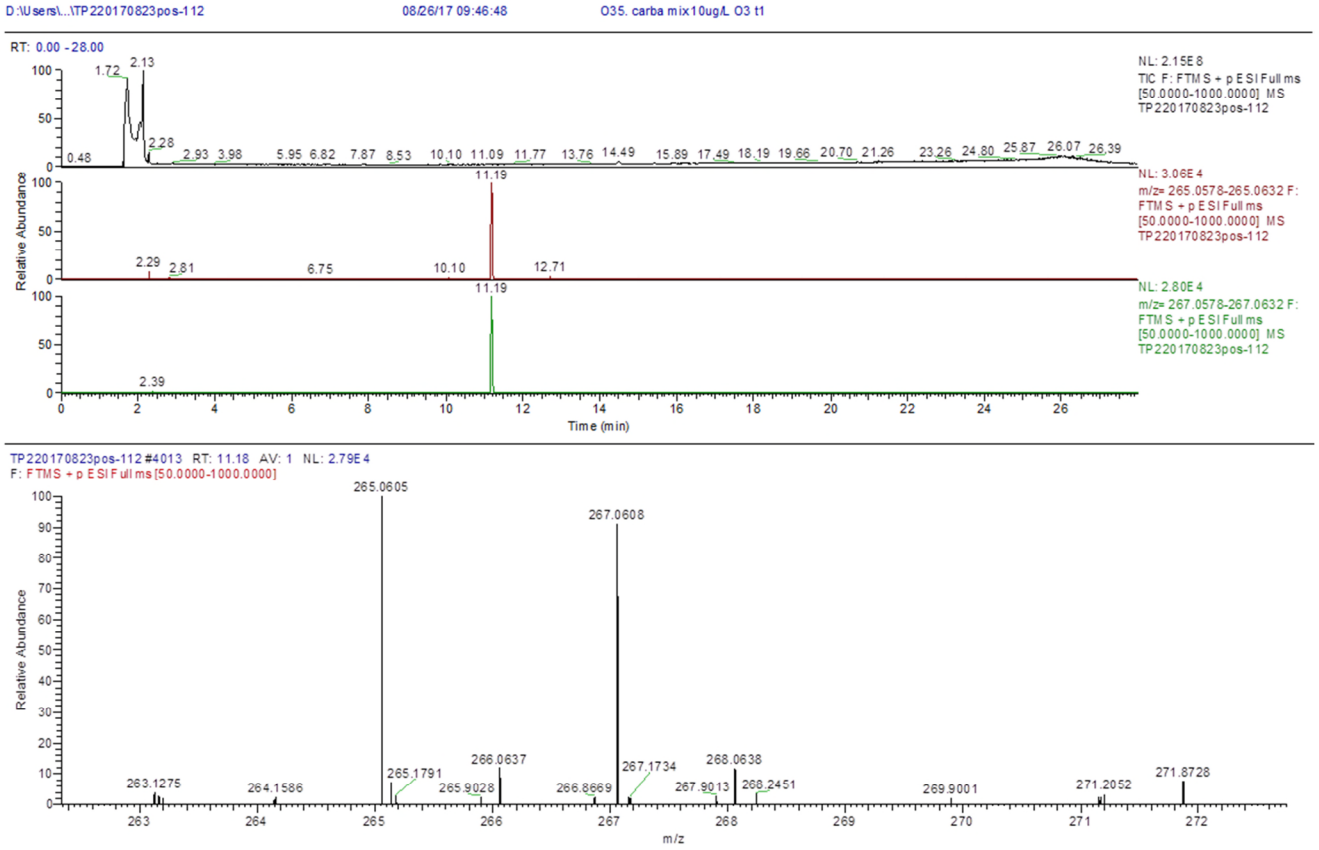
Azais et al. 196.0766; 167.0737



Azais et al. m/z MS2: 249.0658; 221.0714

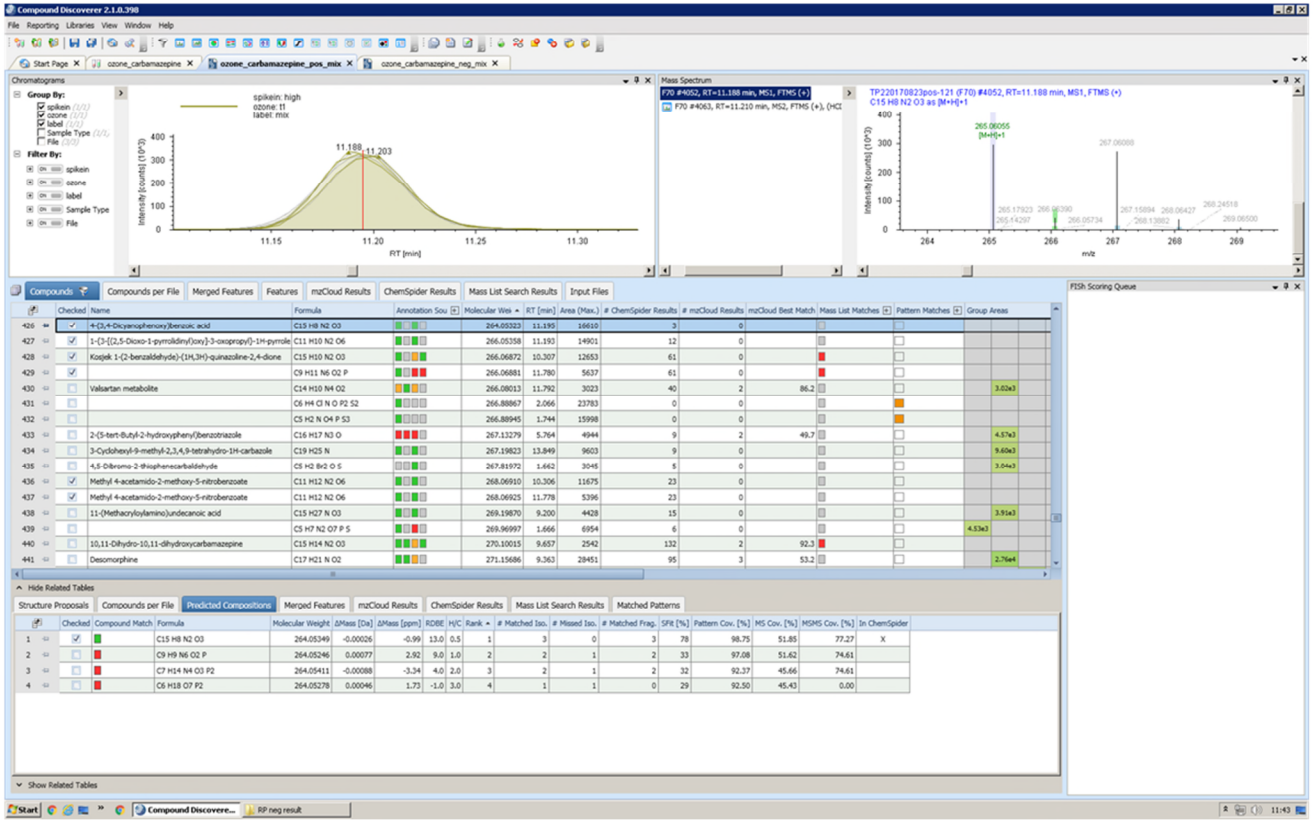
no fragments match

264.05294 / 11.193
266.05342 / 11.19

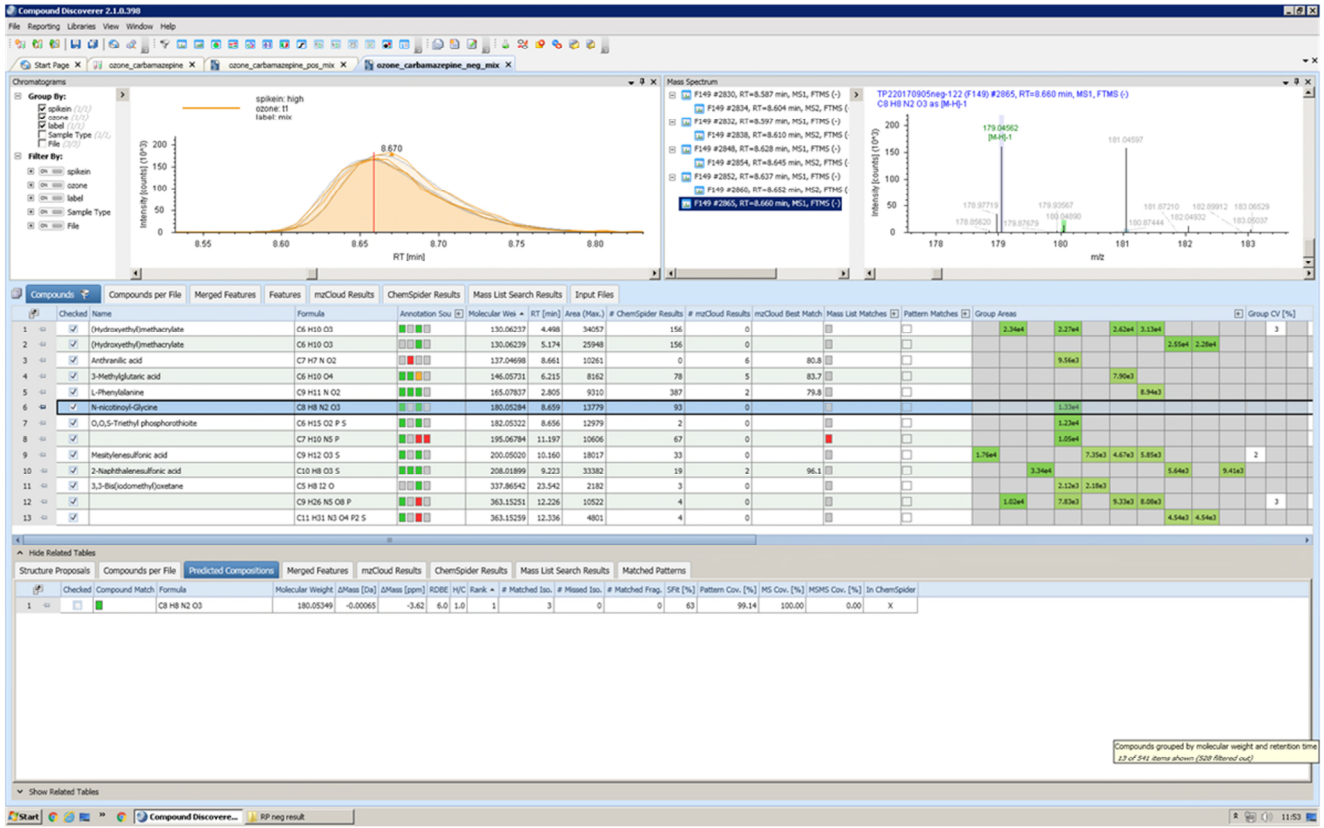


264.05294 / 11.193
266.05342 / 11.19

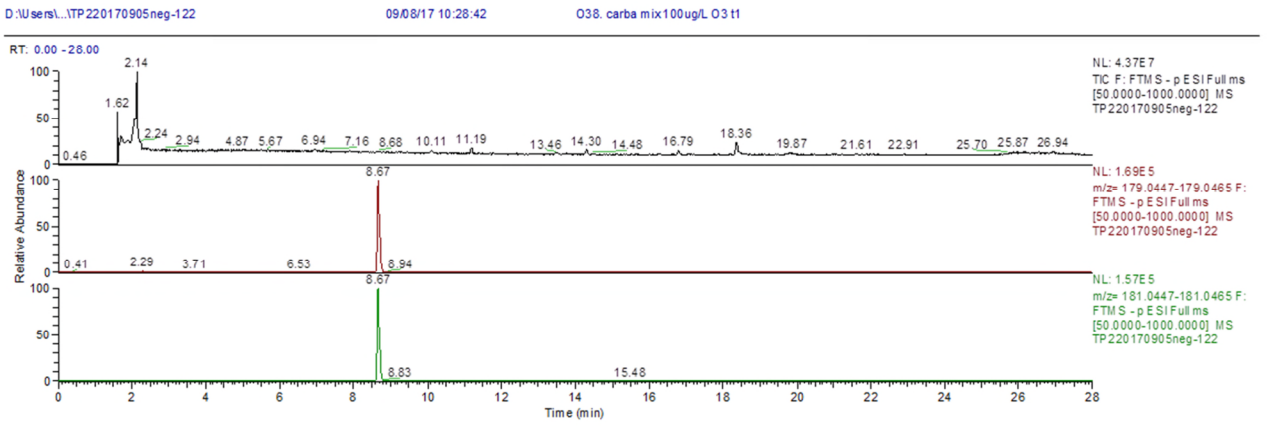
no match with suspect list

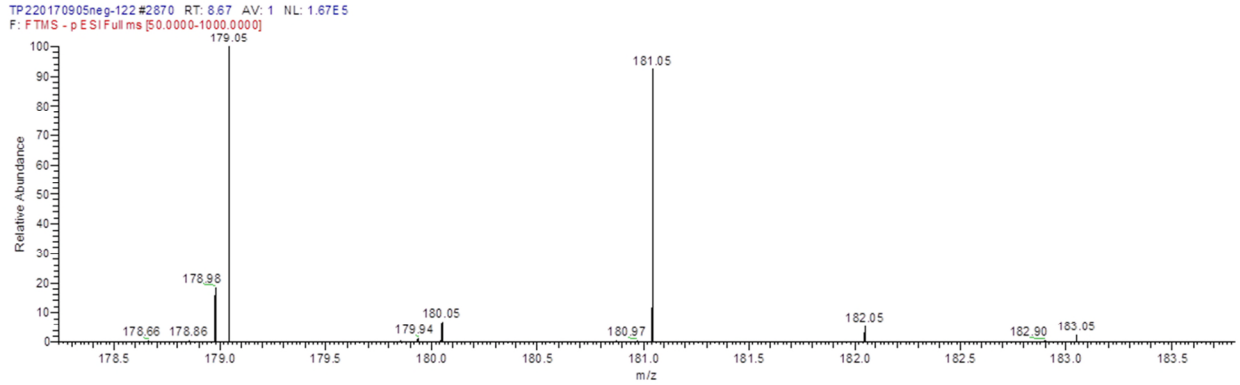


180.053 / 8.685
182.05339 / 8.655

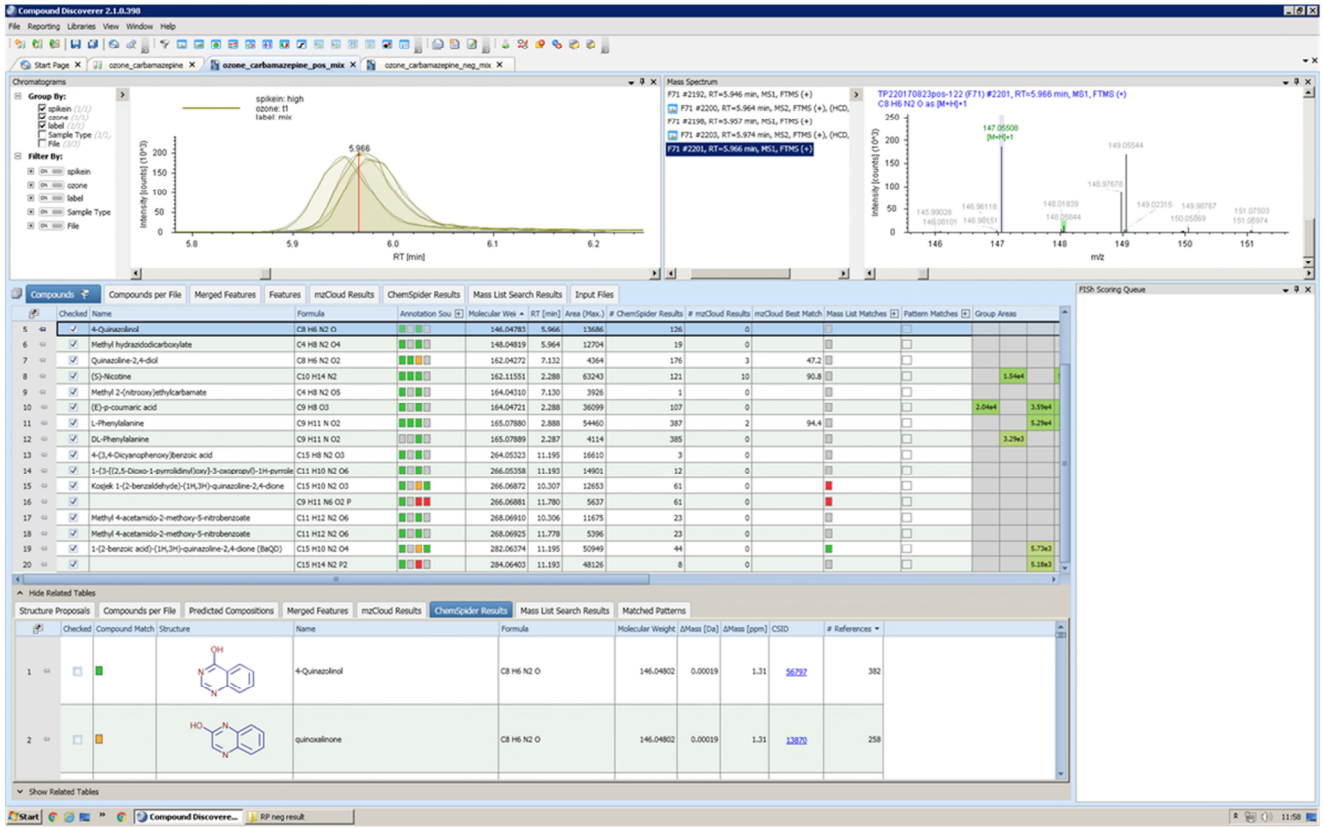


180.053 / 8.685
182.05339 / 8.655

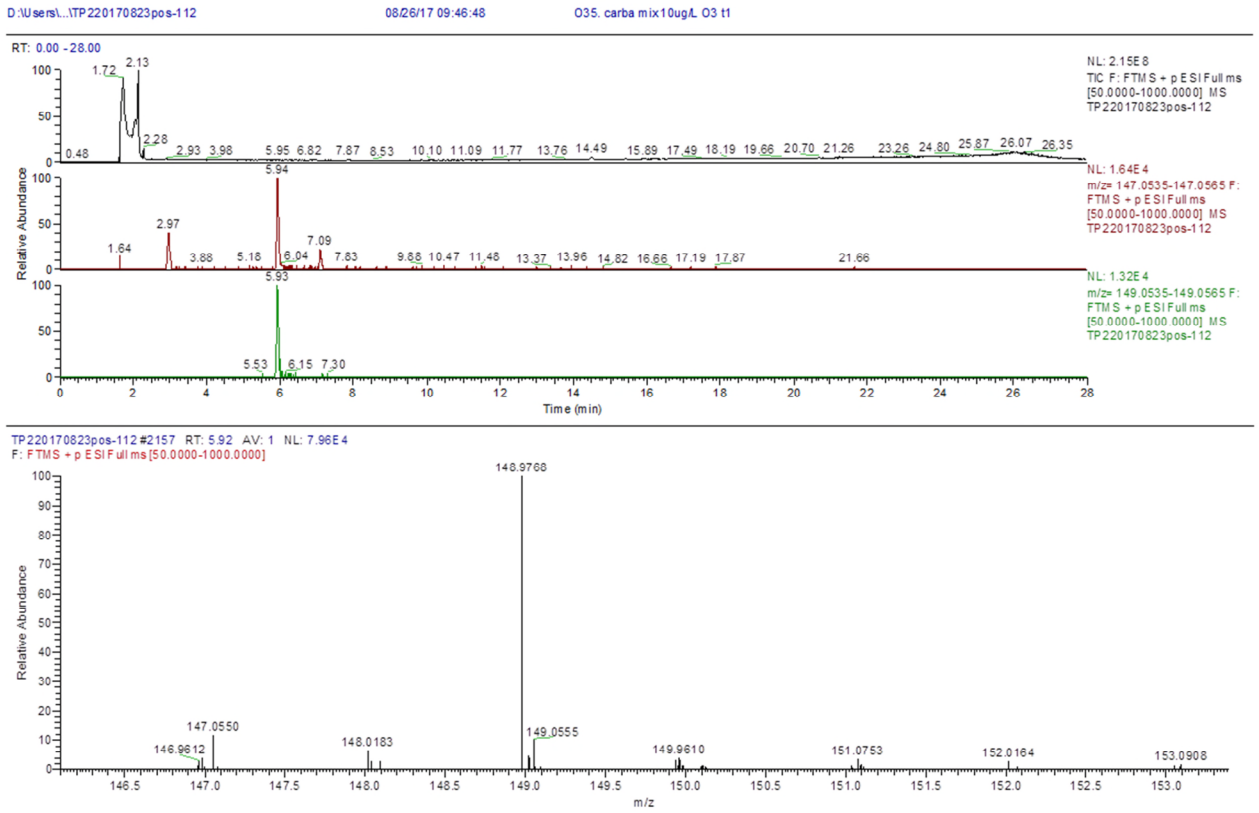




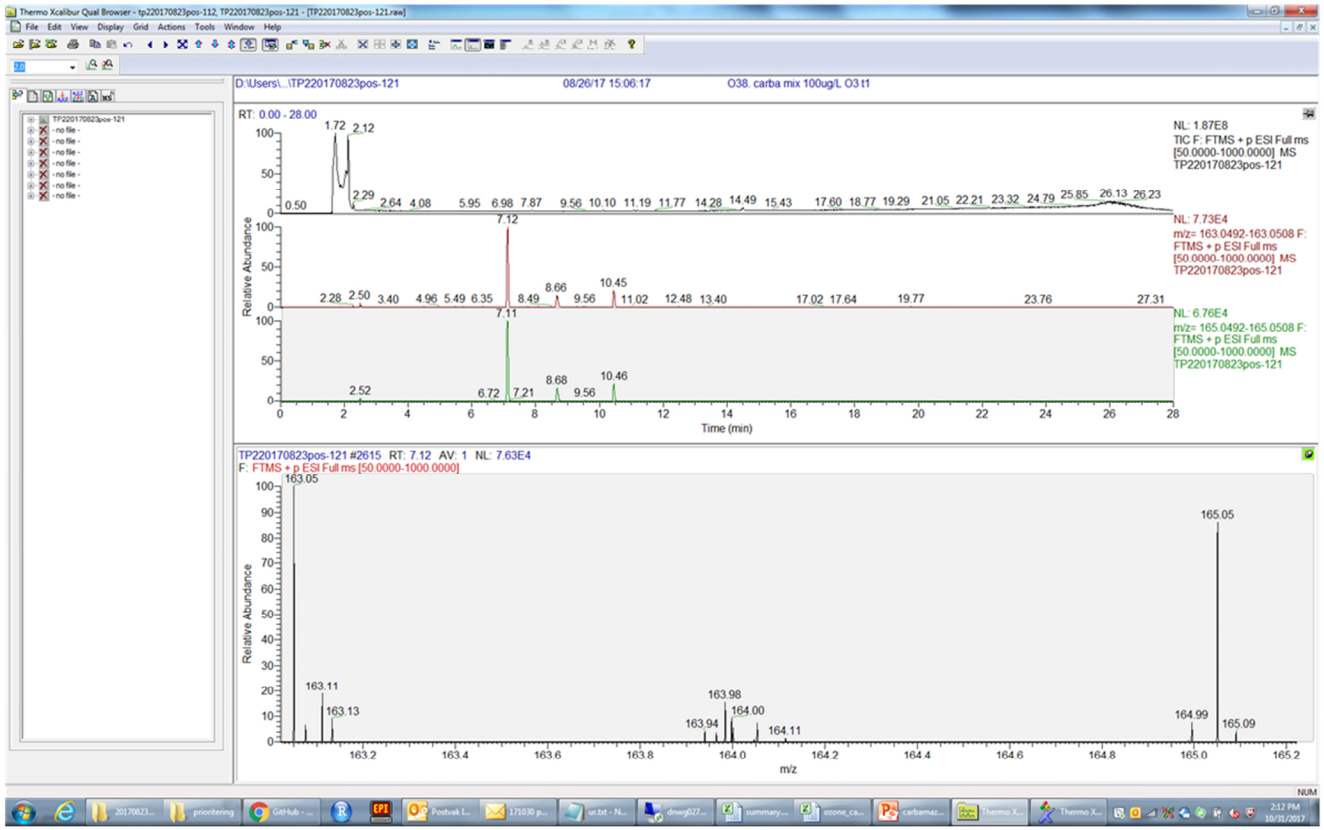
146.04782 / 5.965
148.04822 / 5.967



146.04782 / 5.965
148.04822 / 5.967

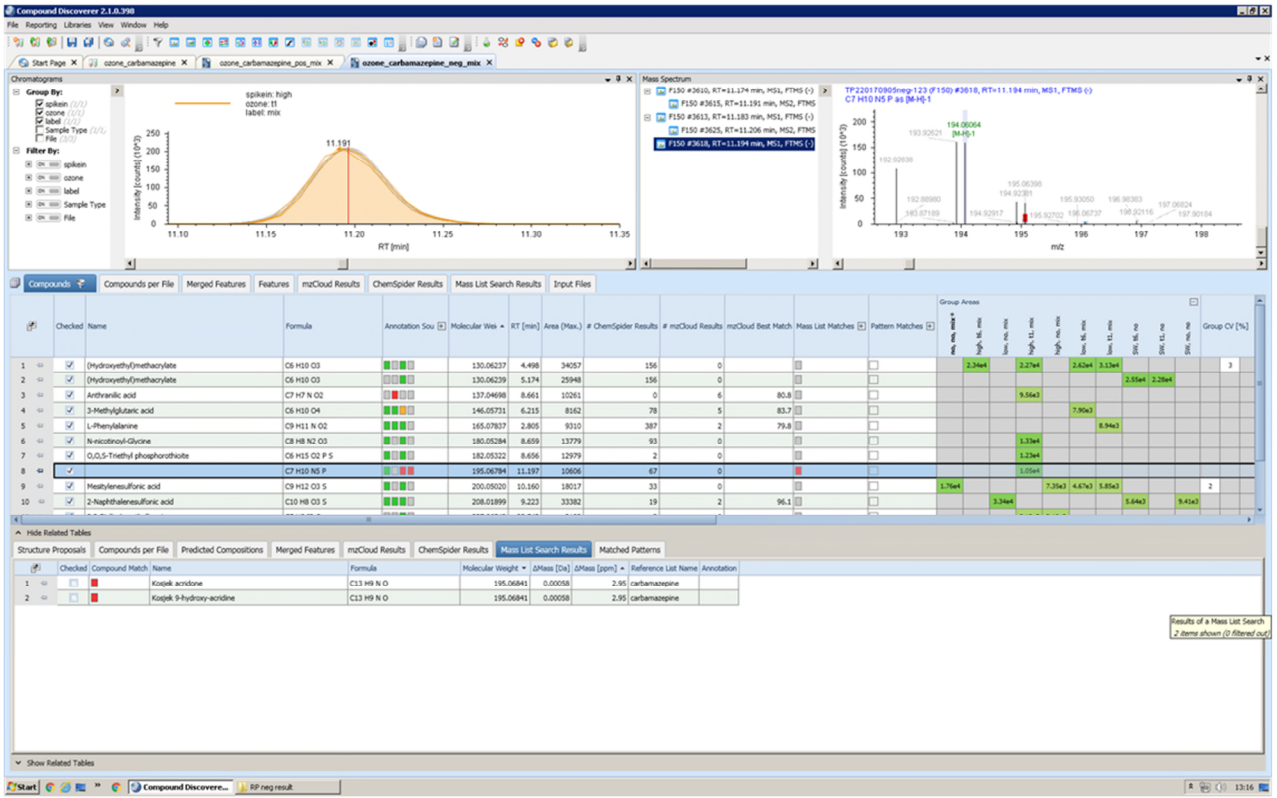


162.04267 / 7.132
164.04276 / 7.14 // 164.04309 / 7.13



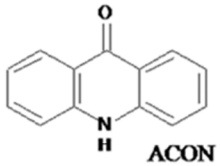
195.068 / 11.198
195.06801 / 11.192

2 potential suspects in TP suspect list:
- acridone
- 9-hydroxy-acridine

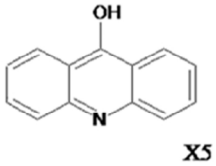


195.068 / 11.198
195.06801 / 11.192

2 potential suspects in TP suspect list:
- acridone
- 9-hydroxy-acridine



ACON

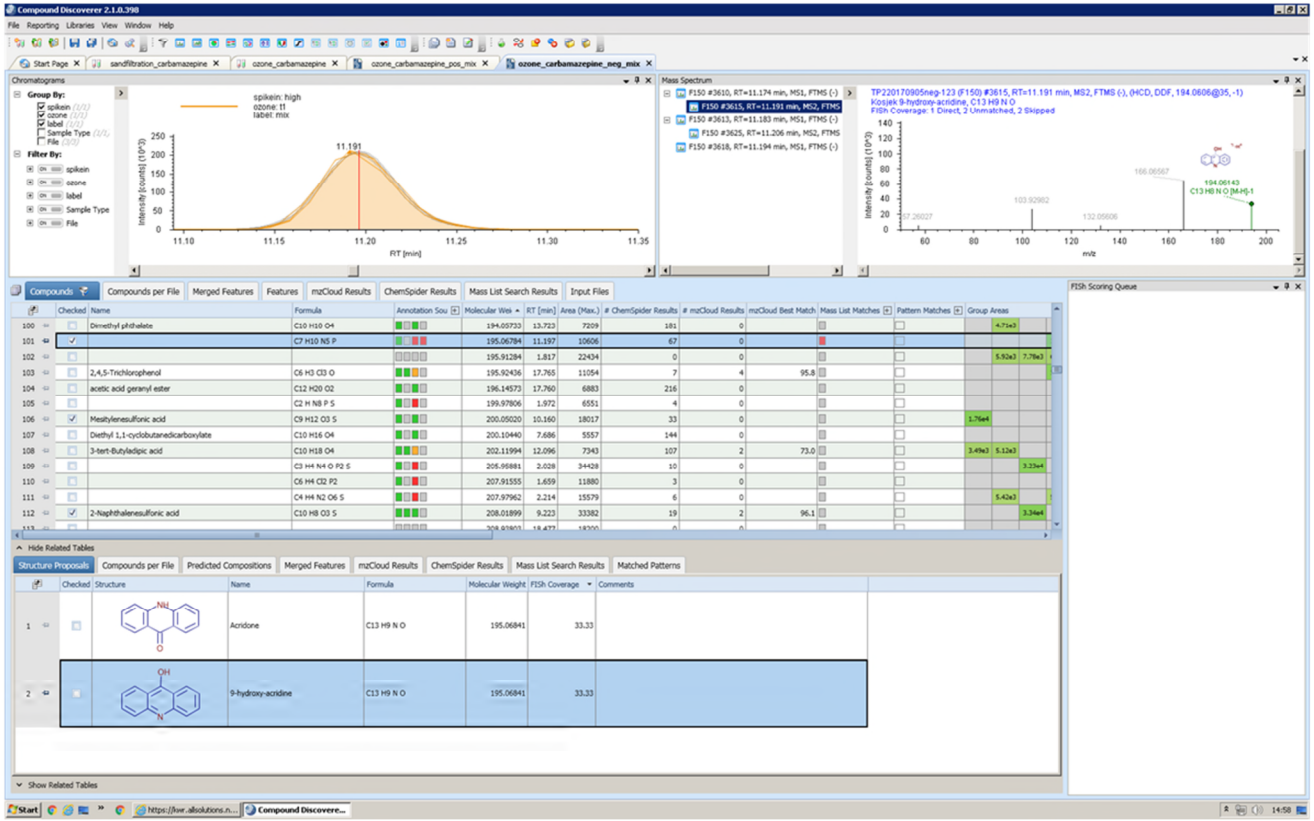


X5

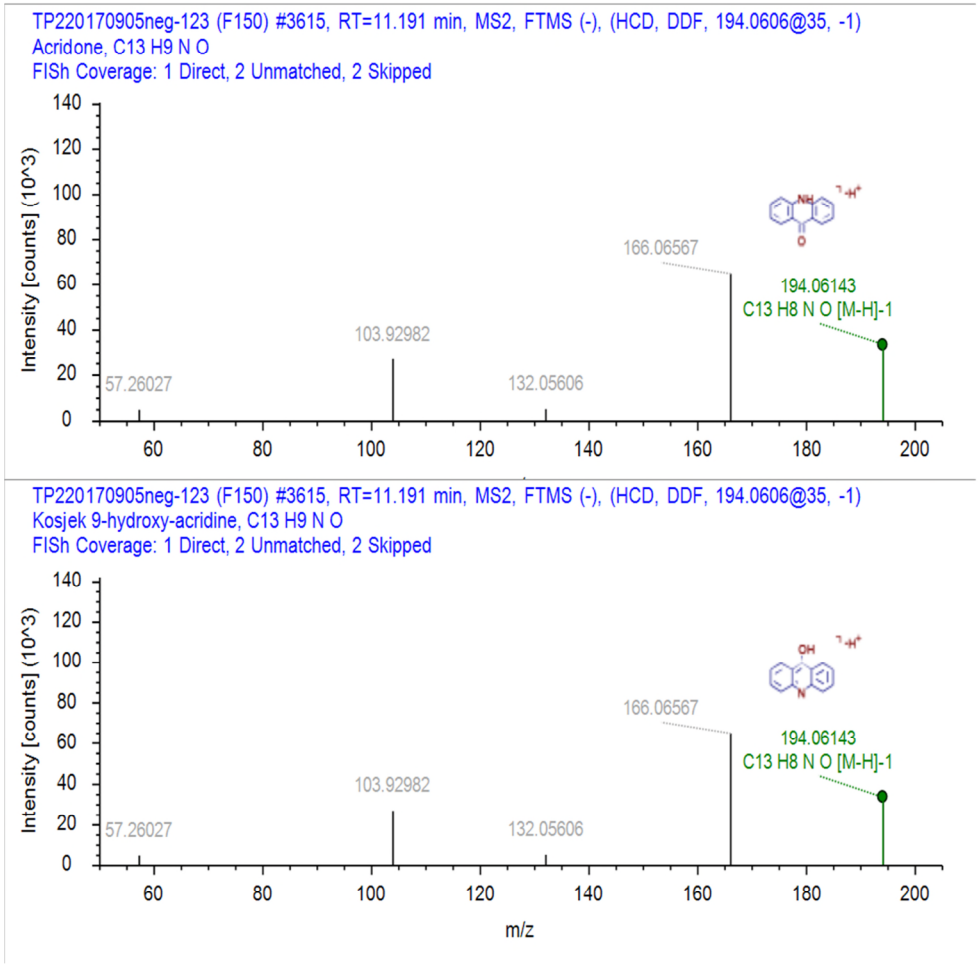
acridone	ACON	$C_{13}H_9NO$	195 ([M + H]$^+$ = 196.0765)	photolysis, biodegradation of ACIN	LC-QqTOF, GC-IT, GC-MSD, NIST library, authentic standard
9-hydroxy-acridine	X5	$C_{13}H_9NO$	195 ([M + H]$^+$ = 196.0767)	ClO_2treatment of ACIN	LC-QqTOF

Kosjek et al EST 2009

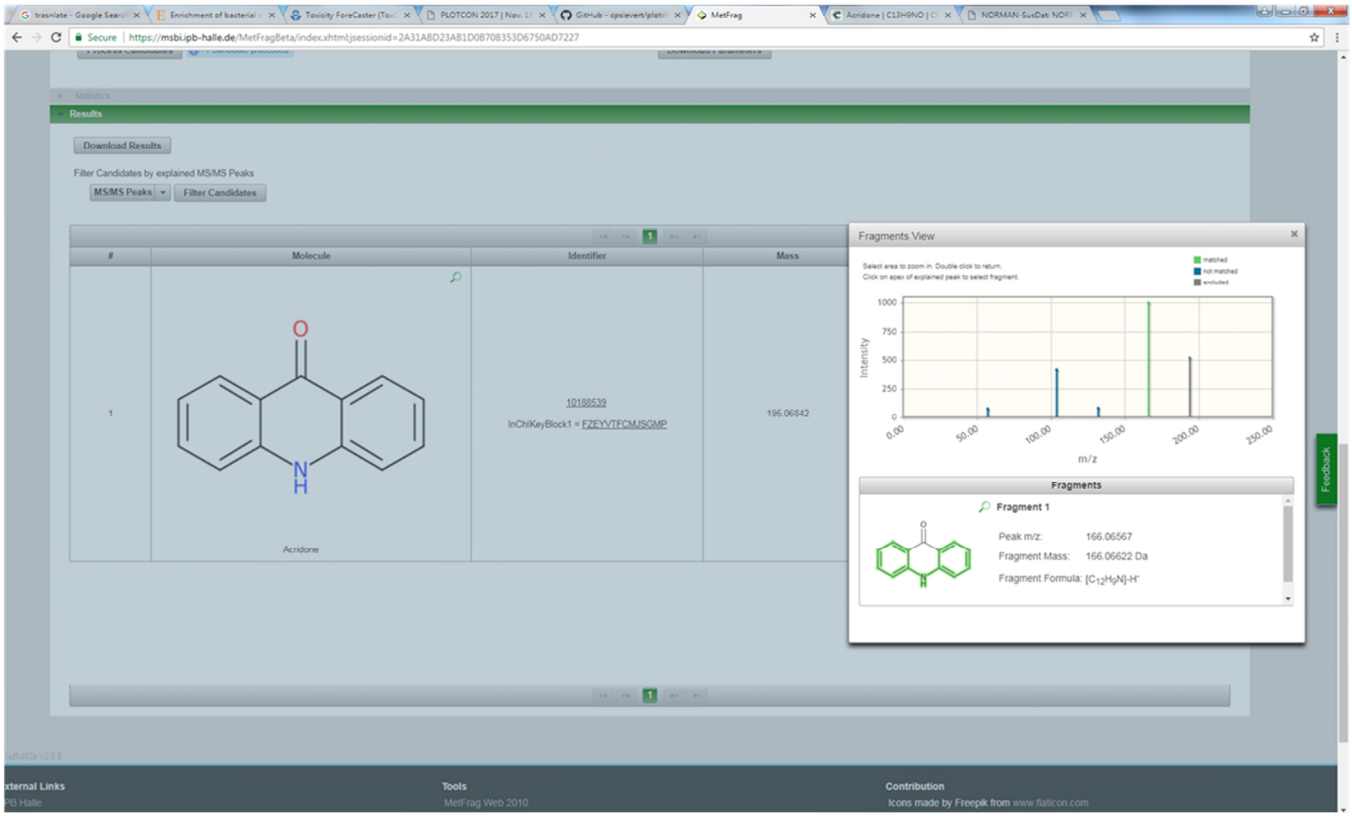
195.068 / 11.198
195.06801 / 11.192



195.068 / 11.198 195.06801 / 11.192



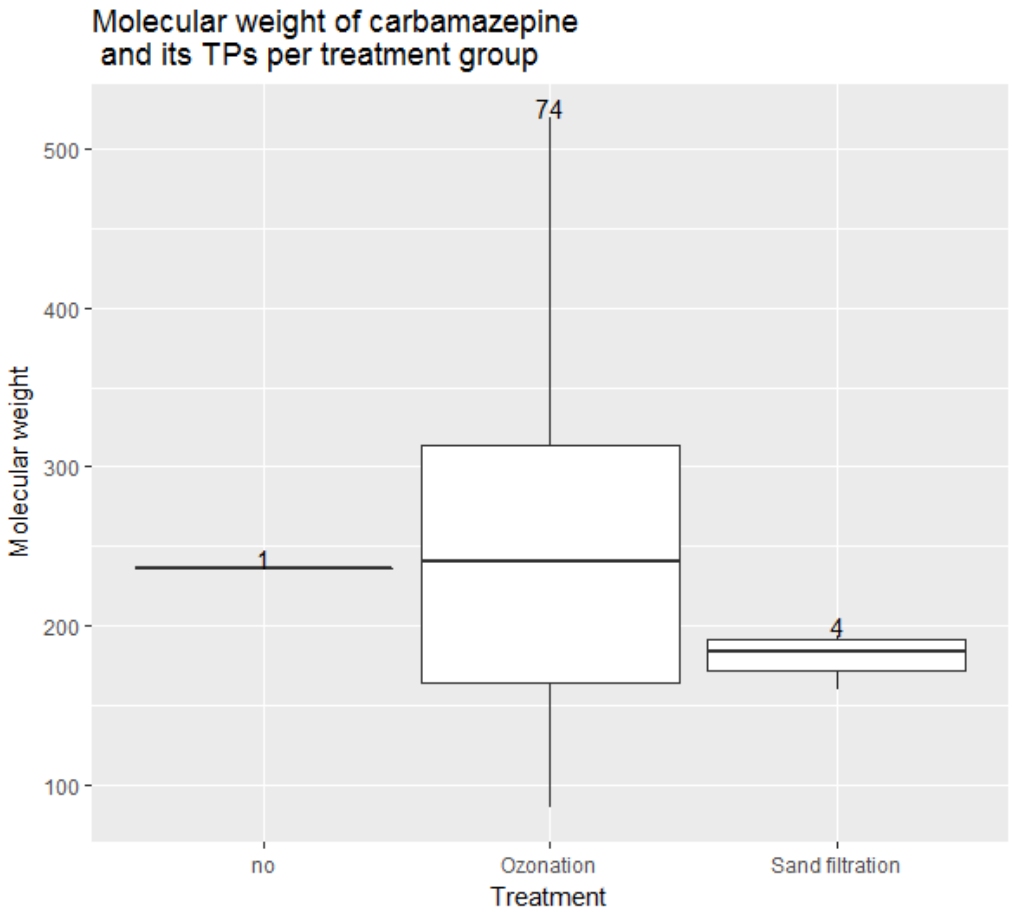
195.068 / 11.198 195.06801 / 11.192



Appendix XVI. Statistical testing between treatment groups

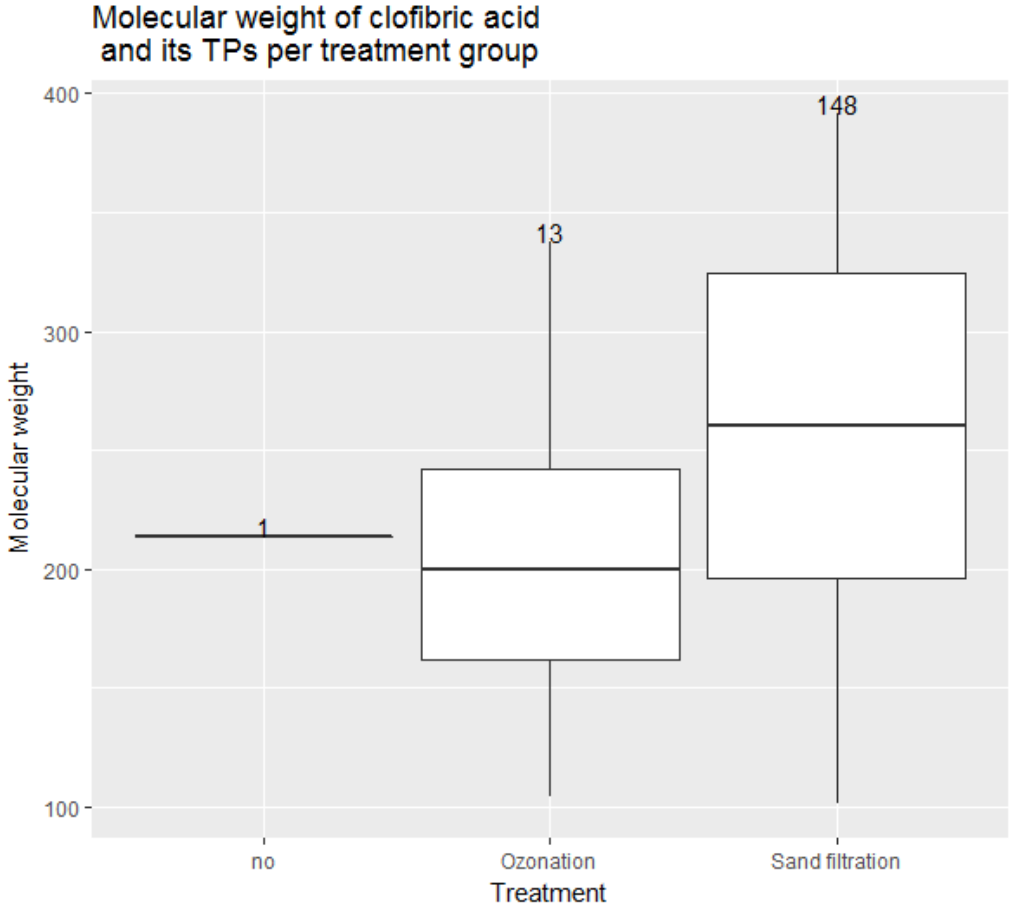
```
Welch Two Sample t-test

All TPs
data: TP$Molecular.Weight by TP$Treatment
t = -1.1625, df = 329.96, p-value = 0.2459
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -27.865992  7.164233
sample estimates:
   mean in group Ozonation mean in group Sand filtration
          243.6318                253.9827
```

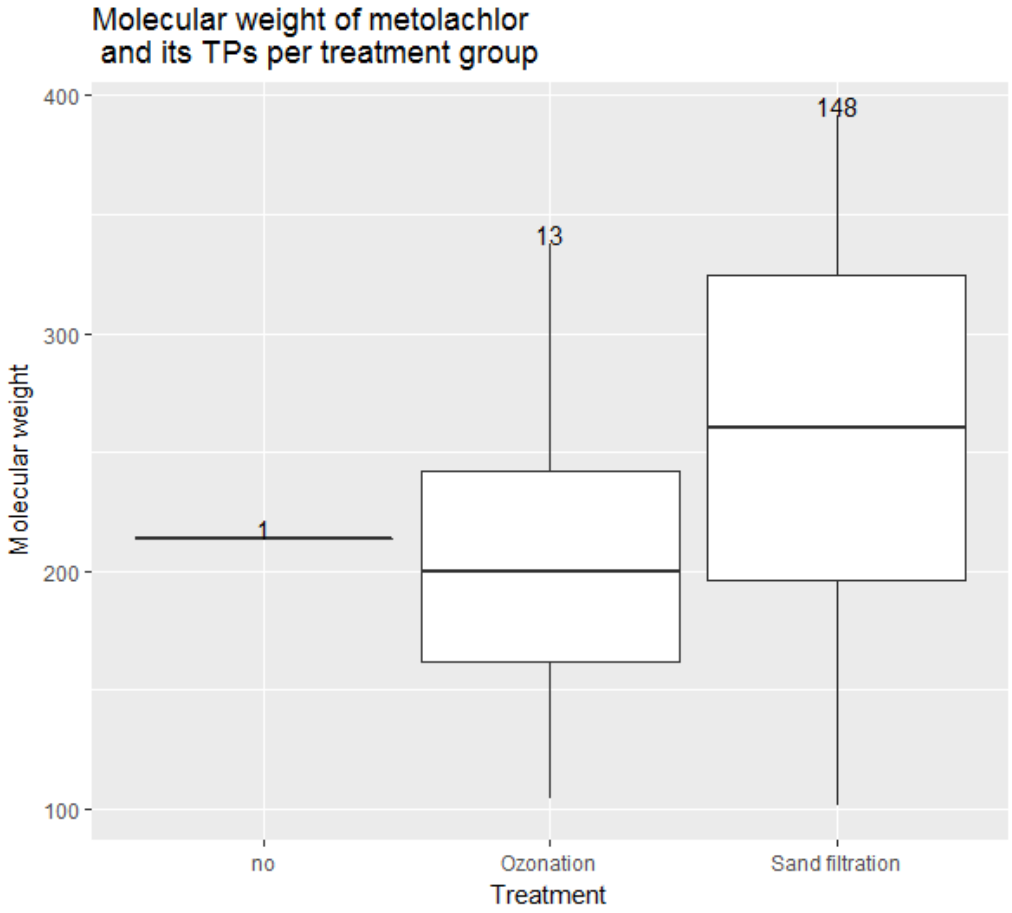



```
Carbamazepine
data: carba$Molecular.Weight by carba$Treatment
t = 4.6399, df = 25.684, p-value = 8.931e-05
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 35.96375 93.23389
```

```
sample estimates:
   mean in group Ozonation mean in group Sand filtration
          244.8104                 180.2115
```




```
Clofibric acid
data: clofibac$Molecular.Weight by clofibac$Treatment
t = -2.0824, df = 14.167, p-value = 0.0559
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -94.315825  1.340613
sample estimates:
   mean in group Ozonation mean in group Sand filtration
          211.0085                 257.4961
```



No testing possible