Ozonation products from trace organic chemicals in municipal wastewater and from metformin: peering through the keyhole with supercritical fluid chromatography-mass spectrometry

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Abstract

Ozonation is an important process to further reduce the trace organic chemicals (TrOCs) in treated municipal wastewater before discharge into surface waters, and is expected to form products that are more oxidized and more polar than their parent compounds. Many of these ozonation products (OPs) are biodegradable and thus removed by post-treatment (e.g., aldehydes). Most studies on OPs of TrOCs in wastewater rely on the reversed-phase liquid chromatography- mass spectrometry (RPLC-MS), which is not suited for highly polar analytes. In this study, supercritical fluid chromatography combined with high resolution MS (SFC-HRMS) was applied in comparison to the generic RPLC-HRMS to search for OPs in ozonated wastewater treatment plant effluent at pilot-scale. While comparable results were obtained from these two techniques during suspect screenings for known OPs, a total of 23 OPs were only observed by SFC-HRMS via non-targeted screening. Several SFC-only OPs were proposed as the derivatives of methoxymethylmelamines, phenolic sulfates/sulfonates, and metformin; the latter was confirmed by laboratory-scale ozonation experiments. A complete ozonation pathway of metformin, a widespread and extremely hydrophilic TrOC in aquatic environment, was elaborated based on SFC-HRMS analysis. Five of the 10 metformin OPs are reported for the first time in this study. Three different dual-media filters were compared as post-treatments, and a combination of sand/anthracite and fresh post-granular activated carbon proved most effective in OPs removal due to the additional adsorption capacity. However, six SFC-only OPs, two of which originating from metformin, appeared to be persistent during all post-treatments, raising concerns on their occurrence in drinking water sources impacted by wastewater.

Keywords

Organic micropollutants, transformation products, tertiary treatment, biological activated carbon, wastewater treatment plant

Abbreviations

BV	Bed Volume
EBCT	Empty Bed Contact Time
GAC	Granular Activated Carbon
HRMS	High Resolution Mass Spectrometry
MSA	Methane Sulfonic Acid
OPs	Ozonation Products
QTof	Quadrupole Time-of-flight
RPLC	Reversed-Phase Liquid Chromatography
RT	Retention Time
S/A	Sand and Anthracite
S/BAC	Sand and Biological Activated Carbon
SFC	Supercritical Fluid Chromatography
TrOCs	Trace Organic Chemicals
UPLC	Ultra Performance Liquid Chromatography
WWTP	Wastewater Treatment Plant

1. Introduction

Trace organic chemicals (TrOCs), such as pharmaceuticals, personal care products, and industrial compounds, in effluents of wastewater treatment plants (WWTP) pose threats to receiving aquatic ecosystems and impact downstream water quality, which is of particular concern if the wastewater-impacted water bodies are used as drinking water sources via indirect potable reuse (Reemtsma et al., 2006; Loos et al., 2013; Luo et al., 2014). Ozonation and subsequent post-treatments are increasingly implemented as additional wastewater treatment steps to improve the abatement of TrOCs (Huber et al., 2005; Hollender et al., 2009; Zimmermann et al., 2011). Ozone is also a common disinfectant and oxidant (e.g., removal of odour and taste) applied in drinking water production (Hoigné, 1998; von Gunten, 2003).

TrOCs are generally not completely mineralized during ozonation, but transformed to smaller and more polar ozonation products (OPs) via reactions with ozone and hydroxyl radicals ($^{\bullet}$OH, formed from ozone decomposition) (von Sonntag and von Gunten, 2012; Prasse et al., 2015). If released into the environment, OPs might exhibit an elevated persistence and mobility in water cycle due to their high polarity (Reemtsma et al., 2016). Moreover, several studies have reported the increased toxicity of WWTP effluent after ozone treatment (Völker et al., 2019; Schneider et al., 2020), indicating that ozonation can result in the generation of toxic compounds, such as the carcinogenic *N*-nitrosodimethylamine, an OP of a nontoxic metabolite of the fungicide tolylfluanid (Schmidt and Brauch, 2008; von Gunten et al., 2010). Also, ozone treatment leads to the formation of bioavailable organic matter (e.g., assimilable organic carbon), which promotes bacterial regrowth and is especially critical in drinking water applications (Wert et al., 2007). The ozone treatment therefore should only be implemented combining with subsequent post-treatments to eliminate OPs (Völker et al., 2019). Many OPs are oxygen-rich (e.g., aldehydes, ketones,

carboxylic acids) and readily biodegradable by the subsequent biofiltration steps (Hammes et al., 2006; Hübner et al., 2014), leading to the reduction in overall toxicity of ozonated water (Mišík et al., 2011; Stalter et al., 2011). Nevertheless, there is also evidence that some OPs can be persistent during biological post-treatment, such as hydroxylamines and *N*-oxides (Hübner et al., 2015; Knopp et al., 2016). As a result, the characterization and persistence assessment of OPs during post-treatment of ozonated water have received considerable attention.

Target and suspect-screening by liquid chromatography-high resolution-mass spectrometry (LC-HRMS) can be applied to monitor the removal of TrOCs and their known OPs during wastewater ozonation (Deeb et al., 2017; Bourgin et al., 2018). However, the monitoring lists of chemicals in these approaches are very limited due to the lack of corresponding reference standards or mass spectral database (Schymanski et al., 2014). In particular, the targeted or suspect analysis of OPs requires the pre-selection of OPs either by laboratory ozonation experiments or by *in silico* prediction based on ozonation mechanisms, which are not available for many TrOCs (Parry and Young, 2016; von Gunten, 2018). Non-target screening by means of HRMS can provide another possibility to comprehensively characterize a wide range of substances in complex water matrix and to prioritize the compounds of interest. The high accuracy and high mass resolution enable the tentative identification of previously unknown compounds (Schymanski et al., 2014; Hollender et al., 2017). Non-target screening with reversed-phase liquid chromatography coupled to mass spectrometry (RPLC-MS) has been successfully applied to analyze TrOCs in different water compartments (e.g., surface water, riverbank filtrate, wastewater) (Gago-Ferrero et al., 2015; Albergamo et al., 2019; Carpenter et al., 2019) and examine their abatement during water treatment (e.g., advanced oxidation, biodegradation) (Parry and Young, 2016; Nürenberg et al., 2019). However, studies on non-targeted analysis of transformation products are still rare. Schollée et al.

(2018) recently performed the RPLC-HRMS based non-target screening to trace OPs during wastewater ozonation and subsequent post-treatments. An important aspect to be considered is that the scope of substances captured by non-target screening is limited by sample enrichment and chromatographic separation steps (Hollender et al., 2017). Particularly, the analysis of highly polar compounds by generic RPLC-MS remains challenging because of their low retention on classical RPLC stationary phases (e.g., C_{18}) (Reemtsma et al., 2016). Many OPs thereby might have been overlooked during the generic RPLC-HRMS based non-targeted analysis, requiring the application of complementary/alternative chromatography, such as hydrophilic interaction chromatography (HILIC) (Gago-Ferrero et al., 2015) or supercritical fluid chromatography (SFC).

SFC has a unique mobile phase comprising the supercritical CO_2 (non-polar) and polar modifiers (e.g., methanol), and is compatible with both polar and non-polar stationary phases. These features allow SFC to simultaneously separate a large number of analytes with varied polarity, making it a potential alternative to RPLC and HILIC (West, 2018). SFC has been increasingly applied in pharmaceutical industry (Desfontaine et al., 2015) and metabolomics analysis (Shulaev and Isaac, 2018). Promising results on the detection of highly polar TrOCs in environmental waters using SFC-MS were recently documented (Bieber et al., 2017; Schulze et al., 2019). SFC was reported to be clearly superior to RPLC in terms of peak shapes and retention, which considerably facilitates signal detection and integration of polar substances (Schulze et al., 2020).

The aim of this study was to explore the potential of SFC-HRMS to characterize OPs, and to better understand their formation during ozonation and removal by post-treatments. The ozonation and post-treatment of wastewater were conducted using a pilot plant located at a full-scale WWTP. Wastewater samples were analyzed using SFC-HRMS in comparison to RPLC-HRMS. Suspect screening for previously known OPs was performed to examine the consistence of SFC-HRMS

results with RPLC-HRMS output. OPs only detectable by SFC-HRMS and persistent during post-treatments were prioritized through non-target screening, and their structures and origins were tentatively proposed. SFC-HRMS was also employed to study the transformation mechanism of metformin during laboratory (lab)-scale ozonation, and to confirm the presence of metformin OPs during ozonation and subsequent post-treatments of real WWTP effluent.

2. Material and Methods

2.1 Chemical reagents

All chemicals were of analytical grade and used as received without further purification. Carbon dioxide Premium (4.5) was used for SFC. Methanol, acetonitrile, and formic acid were provided by Biosolve (Valkenswaard, Netherlands). Disodium hydrogen phosphate (≥99%) and potassium dihydrogen phosphate (≥99%) were supplied from Merck (Darmstadt, Germany). Potassium indigotrisulfonate (55%) was purchased from abcr (Karlsruhe, Germany).The analytical standards of metformin, methane sulfonic acid, and ammeline were purchased from Th. Geyer (Höxter, Germany). Ultrapure water was obtained from a Merck Milli-Q Integral 5 system (Merck, Darmstadt, Germany).

2.2 Pilot-scale ozonation and post-treatments of wastewater effluent

The municipal WWTP Schönerlinde (Berliner Wasserbetriebe, Berlin, Germany) is designed for approx. 750,000 population equivalents and receives municipal as well as industrial wastewater. A detailed description on the pilot plant was published elsewhere (Sauter et al., 2021). The pilot-scale treatment of secondary effluent consists of an ozonation unit followed by three different deep-bed filtration steps that are operated in parallel. The ozonation unit was operated with a specific ozone dose of 0.8 mg O_3/mg DOC during sampling events. Three different filtration systems were applied as post-treatments: a dual-media filter with sand and biological activated

carbon (S/BAC), a dual-media filter with sand and anthracite (S/A), and the dual-media sand/anthracite filter connected to a post granular activated carbon filter (S/A+ post-GAC). The influent and effluent of ozonation unit, as well as the effluents of three post-treatments were collected as 24 hour composite samples. The sampling campaign took place at two different times (06/2019 and 07/2019, one month in between). The treated bed volumes (BVs) of the filters were 63373 (S/BAC), 59363 (S/A), and 13019 (post-GAC) in 06/2019, and 66310 (S/BAC), 62613 (S/A), and 13912 (post-GAC) in 07/2019. The filters were operated with the following target empty bed contact time (EBCT) (for the dual-media filters EBCT relates to the upper filter layer only): S/BAC: 15 min, S/A: 15 min, post-GAC: 30 min. S/BAC and S/A filters are operated as coagulation/filtration steps by dosing $FeCl_3$ solution in filter influent.

2.3 Lab-scale ozonation of metformin

The stock solution of ozone was prepared by sparging the ice-cooled ultrapure water with ozone containing oxygen, which was produced using an ozone generator (CMG, 10-5, INNOVATEC// Gerätetechnik). The ozone stock solution was standardized by its UV absorption at 258 nm (ε= 3000 M^{-1} cm^{-1}) (Elovitz and von Gunten, 1999). Experiments were performed in amber glass vials at room temperature (23 ± 2 °C). Ozonation was initiated by spiking the pre-determined volume of ozone stock solution into 10 mM phosphate buffer solution (pH 7 and 8.5) containing 20 µM of metformin. Two different initial ozone doses, 0.5 and 5 mg/L, were chosen to maximize the formation of the primary and secondary OPs of metformin for better identification. The residual ozone concentration in reaction solution was determined following the indigo method (Bader and Hoigné, 1981). Samples were directly analyzed using SFC-HRMS and RPLC-HRMS after complete depletion of ozone (approx. 2 hours).

2.4 Sample preparation

Two mL of wastewater samples were filtered using syringe filters (0.45 µm, RC membrane, Minisart® RC4, Sartorius) and filled into 2 mL glass vials, and used for direct injection to RPLC-HRMS. Azeotrope evaporation was used as enrichment procedure prior to SFC-HRMS analysis. An aliquot of 4 mL of the filtered wastewater sample was mixed with 21 mL acetonitrile (a ratio for the minimum azeotrope mixture) in a falcon tube. This mixture was then evaporated to dryness at 40 °C under a stream of nitrogen. The residue was reconstituted in 200 µL acetonitrile/ultrapure water (90:10), resulting in a sample-to-extract enrichment factor of 20. The solution was centrifuged in an Eppendorf tube at 14000 min^{-1} for 30 min and only the supernatant was transferred to a glass vial to ensure that any precipitate was removed before injection. Control samples were prepared by enriching the ultrapure water under the same conditions.

2.5 Mass-spectrometric analysis

For SFC analysis, a method previously developed for highly polar compounds was used (Schulze et al., 2020). For RPLC, a column normally employed for polar compounds was selected with 0.1% formic acid in mobile phase, a common organic modifier applied in many screening studies (Schymanski et al., 2015). The analysis was performed on two different systems as follows:

RPLC-HRMS. For the reversed-phase analysis, an ACQUITY ultra performance liquid chromatography (UPLC) connected to a Xevo G2-XS quadrupole time-of-flight (QTof) mass spectrometry (Waters, Eschborn, Germany) was used. The injection volume was 100 µL. The UPLC separation was achieved using an ACQUITY UPLC HSS T3 column (100 × 2.1, 1.7 µm) at a flow rate of 0.45 mL min^{-1}. The column temperature was set to 45 °C. The mobile phase consisted of (A) water (0.1% formic acid) and (B) methanol (0.1% formic acid). The following gradient was applied: 0–0.25 min, 2% B; 12.25–15 min, 99% B; 15.1–17 min, 2% B.

SFC-HRMS. The SFC separation was performed by coupling an ACQUITY UPC^2 system to

Synapt GS2 QTof (Waters, Eschborn, Germany). The chromatographic separation was performed on a BEH column coupled at 55 °C with flow rate of 1.5 mL min^{-1} and injection volume of 8 µL. The (A) CO_2 and (B) methanol/water gradient containing 10 mM ammonium formate in methanol/water co-solvent was applied as follows: 0–0.5 min, 1% B; 9–12.5 min, 50% B; 12.6–15 min, 1% B. A methanol/water make-up flow containing 0.1% formic acid was used at 0.3 mL min^{-1} to transfer the column effluent into mass spectrometry.

Samples were analysed using above instruments in positive and negative electrospray ionization modes (separate runs) following the same HRMS parameters. A lock-spray containing leucine enkephalin was continuously infused during measurement. The source settings include capillary voltage of 0.7 kV in positive and -2 kV in negative ionisation modes, source temperature at 140 °C, and desolvation temperature at 550 °C. The sampling cone voltage and source offset were set as 20 V and 50 V, respectively. Nitrogen and argon were used as cone and collision gases, respectively. The desolvation gas flow was 950 L h^{-1}. The data was recorded in sensitivity mode (resolution approx. 20000) as centroid data with a 0.15 s scan time over the mass range *m/z* 50 to *m/z* 1200. The MS^E acquisition was performed to simultaneously collect two data sets: a low-collision-energy scan (4 eV) to obtain parent ion information and an elevated-collision-energy scan (15–35 eV) to get all fragment ions. All samples were analysed in triplicate by a random sorting.

2.6 Data processing and analysis

Non-target screening was done by evaluating the SFC-HRMS and RPLC-HRMS data in retention time 1 to 10 min and mass range *m/z* 50 to 1200. MarkerLynx was used to perform the peak picking for the molecular ion trace (function 1) with 0.1 min deviation in retention time and 0.01 Da deviation in exact mass. The marker table with relative intensities (to the total marker intensity) was exported to excel and all further evaluations were performed there. All markers that had a

relative intensity >1 in control samples were excluded. Further reduction was done by removing the markers with >50% standard deviation in three injection replicates. The remaining mass-retention time (*m/z*-RT) pairs were further sorted to keep those with relative intensity >2 and with intensity increase by three times in ozonation effluent compared to influent. These values were tested beforehand to ensure that the known OPs were included in final data set and the reported *m/z*-RT pairs fulfil the signal-to-noise (*S/N*) >10 criteria. The marker tables from the same ionization modes of SFC-HRMS and RPLC-HRMS measurements were grouped together and ordered according to ascending exact mass. Exact mass pairs were determined with a mass accuracy of 0.1 Da, and sorted out to a new table as common OPs, while the remaining *m/z*-RT pairs were treated as SFC-only and RPLC-only OPs. A further manual evaluation in MassLynx was performed to ensure the finally extracted OPs strictly comply with the criteria stated above: to be assigned as an SFC-only OPs each peak was checked for its absence in control samples and RPLC measurements, for proper integration with *S/N* ratio >10, its intensity was enhanced by at least 3 times in ozonation effluent compared to influent, and it was not a fragment ion. Sum formulas were assigned by using a mass tolerance of 5 ppm and an elemental composition of $C_{0\text{-}100}$, $H_{0\text{-}100}$, $N_{0\text{-}20}$, $O_{0\text{-}20}$, $P_{0\text{-}2}$, $S_{0\text{-}2}$, $I_{0\text{-}3}$, $Br_{0\text{-}3}$,$Cl_{0\text{-}3}$, and $Na_{0\text{-}2}$, by checking the isotopic pattern for the absence of Cl and Br, and by taking into account the fragment ions detected by MS^E. Suspect screenings for SFC-HRMS and RPLC-HRMS data were done by using Unifi.

3. Results and Discussions

3.1 Suspect screenings of literature known OPs

A suspect screening for 70 known OPs that were previously found during RPLC-MS analysis of ozonated WWTP effluent (Merel et al., 2017; Bourgin et al., 2018; Schollée et al., 2018) (Table S1, supporting information) was firstly performed using SFC-HRMS and RPLC-HRMS to assess

the comparability of these two techniques. At least 13 of these 70 known OPs were detected by both SFC-HRMS and RPLC-HRMS in the ozonated WWTP effluent (Table 1). This low number may partially be due to the limitation in sensitivity of the employed SFC-HRMS (following azeotropic enrichment) and RPLC-HRMS (direct injection) approaches compared to the analytical methods applied in literature (i.e., solid-phase extraction followed by RPLC-MS). Nine of the known OPs detected in this study were *N*-oxides (Table 1). Interestingly, *N*-oxides behaved similarly during SFC and RPLC separations by eluting later than their parent compounds, with one exception of clarithromycin *N*-oxide. This supports the notion that the elution order in SFC is not simply governed by increasing polarity (Schulze et al., 2020). Notably, all known OPs detected by RPLC-HRMS in this study were also detectable by SFC-HRMS, indicating that SFC-HRMS could produce comparable results to the generic RPLC-HRMS while screening the known substances.

Table 1. Parent compounds (PC) and corresponding OPs **(in bold)** found by suspect screenings of ozonated WWTP effluent and their removal during post-treatments. [a]

PC/ **OP**	Ionization mode	RT in SFC (min)	RT in RPLC (min)	*m/z* $[M+H]^+$	S/BAC	S/A	S/A+ post-GAC
venlafaxine	pos	5.14	6.60	278.2120			
desvenlafaxine	pos	6.43	5.16	264.1964			
venlafaxine *N*-oxide	pos	5.71	7.06	294.2060			
tramadol	pos	5.10	5.41	264.1964			
tramadol *N*-oxide	pos	5.92	5.76	280.1903			
tiapride	pos	6.91	3.59	329.1535			
tiapride *N*-oxide	pos	8.13	3.91	345.1470			
sulpride	pos	8.01	3.18	342.1488			
sulpiride *N*-oxide	pos	9.18	3.60	358.1419			
carbamazepine	pos	3.49	8.32	237.1028	n.d.	n.d.	n.d.
BQM	pos	3.37	7.47	251.0819			
BaQM	pos	5.55	7.29	267.0760			
BaQD	pos	5.43	7.17	283.0720			

hydrochlorothiazide	pos	5.56	3.32	297.9723	n.d.	n.d.	n.d.
chlorothiazide	pos	5.61	3.16	295.9569			
lidocaine	pos	2.51	4.60	235.1810			
lidocaine *N*-oxide	pos	4.62	5.30	251.1760			
citalopram	pos	5.22	6.97	325.1716			
citalopram *N*-oxide	pos	6.30	7.19	341.1665			
fexofenadine	pos	6.88	8.13	502.2948			
fexofenadine *N*-oxide	pos	7.02	8.22	518.2930			
clarithromycin	pos	6.47	9.01	748.4850	n.d.	n.d.	n.d.
clarithromycin *N*-oxide	pos	6.36	9.27	764.4796			
amisulpride	pos	8.30	4.46	370.1801			
amisulpride *N*-oxide	pos	9.05	4.95	386.1750			

[a] The complete list of compounds for suspect screening is provided in the supporting information (Table S1). The categorization within post-treatment is marked as non/low-removal (<20%, dark grey), medium removal (20-70%, grey) or removal (>70%, white). The specific values for percent removal are provided in Table S4.
n.d. means that the compound was not detected in the effluent of ozonation unit and thus not evaluated after post-treatments.

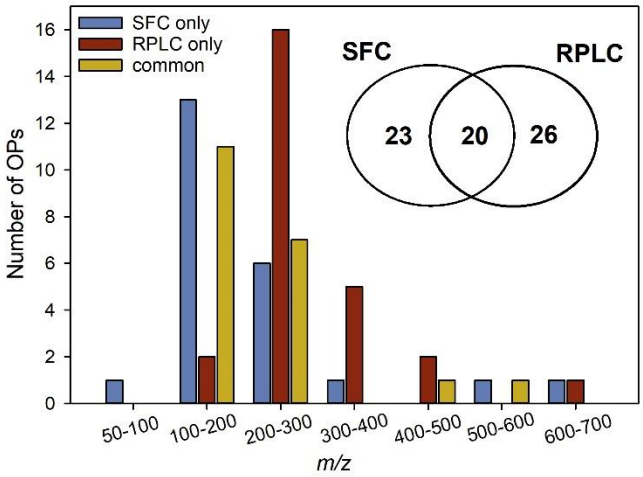
3.2 Non target-screenings by SFC-HRMS and RPLC-HRMS

Non-target screening was applied to WWTP effluent before and after ozonation using SFC-HRMS and RPLC-HRMS, to explore whether SFC-HRMS could provide additional information on OPs of TrOCs. One may expect that highly oxidized and consequently very polar OPs may be overlooked by RPLC-HRMS screening.

The comparison of *m/z*-RT pairs (hereafter named features) before and after ozonation was done without any attempt to identify for both setups of SFC and RPLC. The features present in instrumental blanks were firstly subtracted and only those picked in all three injection replicates (from one sampling) were used for following data processing. It should be noted that OPs are not necessarily new compounds that are absent before ozonation. Rather, some OPs are also formed as human metabolites or by microorganisms in wastewater treatment, such as 5-

hydroxydiclofenac, chlorothiazide, tramadol *N*-oxide, and clarithromycin *N*-oxide (Bourgin et al., 2018). For this reason, a signal increase by a factor of 3 after ozonation was used to select OPs from SFC-HRMS and RPLC-HRMS data separately, which were then subsequently compared. Chromatographic RT could, obviously, not be used to decide on the similarity of features from both data sets. Thus, the comparison of OPs detected by SFC-HRMS and RPLC-HRMS was based on exact masses of molecular ions and verification by comparing the presence or absence of fragment ions.

The SFC-only *m/z*-RT pairs were found to be 77 in positive and 42 in negative ionization modes. Manual correction of features was done to comply with the intensity and blank criteria (i.e., S/N>10), which resulted in many false positives and 23 SFC-only OPs in total. The same was done for RPLC-only OPs as well as for the common OPs. Comparable numbers of RPLC-only (26), SFC-only (23), and common (20) OPs were found (Figure 1, venn diagram). It is important to note that a higher number of detected peaks does not necessarily reflect a better performance of an instrument here. The applied criteria on peak picking and intensity comparison were quite strict to exclude many uncertain peaks. The final OPs cover the whole mass range (*m/z* 100−700), but the maximum numbers of SFC-only and common OPs are located in *m/z* range of 180−220, whereas the RPLC-only OPs are mainly found in the range of *m/z* 280−320 (Figure 1). These results suggested that SFC is advantageous for OPs of lower molecular weight, which are possibly highly polar and thus not retained during RPLC separation. RPLC retention is not only due to hydrophobic interaction (which one may describe by Log*D*), but also by van-der Waals interaction. The van-der Waals interaction becomes more important when the stronger hydrophobic interaction decreases, i.e. with decreasing (or negative) Log*D*. Thus, especially the small polar compounds are not retained by RPLC.



285

286 **Figure 1.** Allocation of the number of OPs in different mass ranges (*m/z* 100−700). OPs were
287 detected by non-target screenings of ozonated WWTP effluent using SFC-HRMS and RPLC-
288 HRMS. The "common" in legend refers to the common OPs detected by both SFC-HRMS and
289 RPLC-HRMS. Venn diagram shows the share of total numbers.

290 Four of the 20 common OPs (Table S2), i.e., chlorothiazide (originating from the ozonation of
291 hydrochlorothiazide), BQM (from carbamazepine), tramadol *N*-oxide (from tramadol), and
292 lidocaine *N*-oxide (from lidocaine), were already known from the suspect screening as shown in
293 Table 1. The 9 additional OPs found during suspect screening (Table 1) were not extracted by the
294 non-targeted data processing here, likely due to their too low intensity to meet the peak-picking
295 requirement (relative intensity >2). One of the common OPs ($[M-H]^-$, $C_7H_5O_3S$, *m/z* 184.9906, Table
296 S2) was proposed to be the hydroxylated thiosalicylic acid, which may originate either from
297 thiomersal or thioindigo, or related dyes. No literature data was available to confirm this
298 assumption. The other 15 common OPs (Table S2) could not be identified either due to the missing
299 fragment ions for proposing the basic structures or no assignment of elemental composition within

the selected criteria. They elute in the whole RT range in RPLC and SFC chromatograms, with *m/z* ranging from 146 to 567.

A total of 26 OPs were detected only by RPLC, with RT in the range of 2.4−9.5 min and *m/z* 201−688, respectively (Table S3). The assigned sum formulas of these compounds allow for many different structures, thus preventing their tentative identification. Interestingly, one of the RPLC-only OPs (i.e., $[M+H]^-$, $C_{13}H_{13}N_3O_6I_3$, *m/z* 687.7953, Table S3) was probably derived from the iodinated contrast agents (e.g., iohexol). One possible explanation for the non-detection of these OPs by SFC could be a stronger matrix effect, which may have prevented the automatic extraction of these signals.

The SFC-only OPs (23 in total, Table 2) cover the whole SFC chromatogram (2.5−11 min), and no clear trend was observed by comparing the RT and molecular mass. Furthermore, the H/C *vs.* O/C shows no significant difference within the proposed formulas of SFC-only, RPLC-only, and common OPs (Figure S1). Thus the retention behaviour of compounds in SFC is a result of rather complex relationships between molecular mass, formula, and polarity.

Table 2. SFC-only OPs found by non-target screening of ozonated WWTP effluent using SFC-HRMS and their removal during post-treatments. [a]

OP	Enhanced/ Formed	Ionization mode	RT in SFC (min)	*m/z* $[M+H]^+$ or $[M-H]^-$	Formula	Fragments	S/BAC	S/A	S/A+ post-GAC
1	+	pos	2.55	212.130	$C_{11}H_{18}NO_3$	126.091 ($C_7H_{12}NO$)			
2	+	pos	2.88	183.100	$C_{10}H_{15}O_3$ *				
3	+	pos	3.19	196.130	$C_{11}H_{18}NO_2$*				
4	+	pos	3.69	126.080	$C_4H_7N_5$	84.053 ($C_3H_6N_3$), 70.224 ($C_2H_4N_3$)			
5	+	pos	4.10	112.060	$C_3H_6N_5$	70.049 ($C_2H_4N_3$)			

6	+	pos	4.15	150.080	$C_5H_{12}NO_4$					
7	+	pos	4.32	207.050	$C_7H_{11}O_7$ or $C_{11}H_{11}O_2S$ *					
8	+	pos	4.45	127.070 (155.068)	$C_4H_7N_6O$	127.072 ($C_3H_7N_6$), 85.051 ($C_2H_5N_4$)				
9	++	pos	4.86	199.094	$C_6H_{11}N_6O_2$	183.098 ($C_6H_{11}N_6O$)				
10	++	pos	4.87	241.140	$C_9H_{17}N_6O_2$	223.129 ($C_9H_{15}N_6O$)				
11	+	neg	4.89	574.450						
12	++	neg	4.93	604.460 (618.475)	$C_{38}H_{60}N_5O_2$	604.459 ($C_{37}H_{58}N_5O_2$), 590.442 ($C_{36}H_{56}N_5O_2$)				
13	++	pos	5.05	143.080	$C_6H_{11}N_2O_2$					
14	+	neg	5.57	94.980	CH_3O_3S					
15	++	pos	5.59	171.100	$C_5H_{11}N_6O$ *					
16	++	neg	5.76	204.980	$C_6H_5O_6S$					
17	++	neg	6.27	240.960	$C_6H_6O_6SCl$					
18	+	pos	6.30	208.040	$C_{10}H_{10}NO_2S$	186.051 ($C_9H_9NO_2Na$), 165.038 (C_9H_9OS), 163.012 (C_9H_7OS)				
19	++	pos	6.82	182.050 (224.059)	$C_7H_{14}NO_5S$	182.048 ($C_5H_{12}NO_4S$)				
20	+	pos	7.83	399.140	$C_{16}H_{24}N_4O_6P$	199.017 ($C_8H_8O_4P$), 224.012 ($C_9H_7NO_4P$), 323.092 ($C_{13}H_{16}N_4O_4P$), 363.123 ($C_{16}H_{20}N_4O_4P$), 381.134 ($C_{16}H_{22}N_4O_5P$)				
21	+	pos	8.90	174.100	$C_{10}H_{12}N_3$ *					
22	+	pos	8.99	162.110	$C_7H_{16}NO_3$	134.117 ($C_6H_{16}NO_2$)				

23	+	pos	10.55	158.120	$C_8H_{16}NO_2$	58.064 (C_3H_8N)	

[a] Multiple formula assignments are possible for the compounds designated by (*). The origins of the OPs **in bold** were tentatively proposed (detailed in the main text, Section 3.2) and their extracted ion chromatograms/mass spectra are provided in Figures S2-S13. The behaviour during ozonation is stated as enhanced (+, already present in ozonation influent and intensity increased during ozonation) or as formed (++, newly generated during ozonation). The categorization within post-treatment is marked as non/low-removal (<20%, dark grey), medium removal (20-70%, grey) or removal (>70%, white). The specific values for the percent enhancement/formation during ozonation and the percent removal during post-treatments are provided in Table S5.

316

317 The possible parent compounds of the SFC-only OPs (Table 2) may be found by taking into
318 account the well-known ozonation reactions such as the addition of an oxygen atom, which leads
319 to hydroxylation or *N*-oxides (Schollée et al., 2018). For example, one possible parent compound
320 of OP_1 (i.e., $[M+H]^+$, $C_{11}H_{18}NO_2$, *m/z* 196.134, formula difference to OP_1: -O) was present in
321 both data sets of RPLC (3.18 min) and SFC (3.16 min) (Figure S2) before ozonation, and absent
322 in ozonated wastewater. Its formula, $C_{11}H_{17}NO_2$, hits total 10,243 possible molecules in
323 Chemspider (https://www.chemspider.com, 07/2020). The substance with the highest reference
324 number was 2,2'-[(4-methylphenyl)imino]bisethanol (4-tolyldiethanolamine), a high production
325 volume (10-100 t/year, ECHA) and environmentally persistent chemical according to the OECD
326 criteria (https://echa.europa.eu/de/registration-dossier/-/registered-dossier/27362/5/3/2).
327 However, the reference standard did neither fit the RT, nor the fragment ions of the proposed
328 parent of OP_1 (Figure S3).
329 One possible parent formula of OP_22 (i.e., $[M+H]^+$, $C_7H_{18}NO_2$, *m/z* 148.132, formula difference
330 to OP_22: $-O+H_2$) was present in WWTP effluent (RT= 5.14 min in SFC and 0.96 min in RPLC)
331 and completely removed after ozonation (Figure S4). The 3 top candidates in Chemspider were
332 tert-butylaminopropane-1,2-diol, N-propyldiethanolamine, and diethanolisopropylamine.
333 However, all 3 compounds are neither high production volume chemicals according to ECHA, nor

was the loss of -CO during fragmentation of OP_22 (i.e., fragment ion, $[M+H]^+$, $C_6H_{16}NO_2$, *m/z* 134.117, Figure S4) indicative enough to support any of these structures.

Several SFC-only OPs are nitrogen rich compounds, including OP_4 ($[M+H]^+$, $C_4H_7N_5$, *m/z* 126.080, Figure S5) and OP_5 ($[M+H]^+$, $C_3H_6N_5$, *m/z* 112.060, Figure S6) with 5 nitrogen atoms. They were proposed to be derived from metformin ($C_4H_{11}N_5$). Lab-scale ozonation of metformin was conducted in this study and confirmed that metformin is likely a precursor of OP_4 and OP_5 (see Section 3.4).

OP_9 ($[M+H]^+$, $C_6H_{11}N_6O_2$, *m/z* 199.094, Figure S7) comprises 6 nitrogen atoms and seems to be an *N*-oxide due to the facile loss of an –O during in-source fragmentation. However, the corresponding parent compound (i.e., $[M+H]^+$, $C_6H_{11}N_6O$, *m/z* 183.098) did not exist in WWTP effluent before ozonation; therefore OP_9 is possibly a secondary OP formed from an intermediate during ozonation, such as the methoxymethylmelamine derivatives. Similarly, OP_8 ($[M+H]^+$, $C_4H_7N_6O$, Figure S8), 10 ($[M+H]^+$, $C_9H_{17}N_6O_2$, Figure S9), and 15 ($[M+H]^+$, $C_5H_{11}N_6O$, Figure S10) also contain 6 nitrogen atoms, and might share the same origin (e.g., methoxymethylmelamines). However, there are no characteristic fragment ions to verify the melamine structure for OP_10 and 15. Notably, OP_8 ($[M+H]^+$, $C_4H_7N_6O$, *m/z* 155.068, Figure S8) is likely to be one of the transformation products of hexamethoxymethylmelamine as its fragmentation pattern is comparable to literature results (Alhelou et al., 2019). OP_8 was already present in WWTP effluent before ozonation (probably formed by biotransformation), but its intensity significantly increased upon ozonation.

In negative ionisation mode, OP_14 ($[M-H]^-$, CH_3O_3S, *m/z* 94.980, Figure S11) was confirmed to be methane sulfonic acid (MSA) using analytical standard. It was a prominent OP significantly enhanced during ozonation; however its origin remains unclear. Previous studies suggested that

the reduced sulfur containing compounds such as dimethyl sulfide (present in cooked vegetables or seafood) can be oxidized to MSA by ozonation (Devulapelli and Sahle-Demessie, 2008). Furthermore, MSA was reported to be formed by ozonation of dimethyl sulfoxide (Wu et al., 2007). OP_16 ($[M-H]^-$, $C_6H_5O_6S$, Figure S12) and OP_17 ($[M-H]^-$, $C_6H_6O_6SCl$, Figure S13) might be the substituted phenyl or alkyl sulfates/ sulfonates based on their exact masses and molecular formulas, but this could not be substantiated as the fragment ions were missing possibly due to the already too low intensity of molecular ions.

3.3 Behaviour of OPs during post-treatments

The post-treatment subsequent to ozonation is necessary to eliminate OPs with the goal of minimizing the ecotoxicological effects of ozonated wastewater (Hollender et al., 2009; Prasse et al., 2015). The removal of OPs that were detected by suspect and non-target screenings of ozonated WWTP effluent in this study was investigated during 3 different post-treatments based on dual-media filters: S/BAC, S/A, and S/A+ post-GAC. The removal efficiency for each OP was evaluated based on the evolution of its peak intensity.

The behaviour of known OPs as well as of the SFC-only OPs during post-treatments are presented in Table 1 and 2, respectively. Generally, medium (20-70%) to no removal (<20%) was observed for most OPs during S/BAC and S/A treatments, whereas S/A+ post-GAC showed far better removal (>70%). The treated BVs of S/BAC and S/A here were about 60,000 (see Section 2.2), where the abatement of most micropollutants had already reached a steady-state on these filters (Sauter et al., 2021). This indicates that biodegradation is of limited efficacy for the OPs detected in this study. The S/A filter was less effective in OP removal than S/BAC, which was related to the non-adsorptive characteristic of anthracite (Sauter et al., 2021). The third post-treatment S/A+ post-GAC refers to S/A and post-GAC filters that were connected in series. The post-GAC applied

here was relatively fresh (~ 13,000 BV) compared to the other filters (~ 60,000 BV). Thus, the better performance of S/A+ post-GAC than S/BAC and S/A can be attributed to its higher remaining adsorption capacity.

N-oxides are known to be non-biodegradable (Merel et al., 2017; Schollée et al., 2018). Bourgin et al. (2018) reported that the biological post-treatments are often better for the abatement of parent compounds than their *N*-oxides. This was further confirmed by our results. All known *N*-oxides were not completely removed by S/BAC and S/A but successfully eliminated by S/A+ post-GAC with predominant adsorption (Table 1 and Table S4). Particularly, the *N*-oxides of citalopram, sulpiride, tramadol, and tiapride were less eliminated compared to their parent compounds within S/BAC and S/A filters. The carbamazepine OPs, BaQM and BaQD, were found to be the most persistent substances within all known OPs in Table 1. A partial removal of BaQM (30 to 50%) by S/A+ post-GAC was observed, whereas BaQD appeared to remain stable or even being formed during all post-treatments. Hübner et al. (2014) also reported that a sand column failed to eliminate BaQD although a complete abatement of BaQM was achieved.

Only 8 and 5 out of 23 SFC-only OPs were removed (>70% removal) by S/BAC and S/A, respectively (Table 2 and Table S5). Thereby, 10 and 14 of the SFC-only OPs were classified as persistent (<20% removal) during S/BAC and S/A, respectively, revealing that the specific biodegradation conditions in this study were not sufficient to remove all SFC-only OPs. BAC was known to perform better than sand in eliminating biodegradable organic matter (Reungoat et al., 2011; Sauter et al., 2021). Thus, the SFC-only OPs persistent to S/BAC here are expected to remain stable during sand filtrations following the ozonation of wastewater or drinking water under comparable operating conditions. The majority of SFC-only OPs (17 out of 23), including the methoxymetheylmelamine related OPs (i.e., OP_8, 9, 10 and 15), were completely removed or

remained to less than 30% during S/A+ post-GAC. The adsorption therefore can be an option for the elimination of most SFC-only OPs. However, approximately 50% of OP_4 (i.e., OP of metformin) and OP_18 were still present even after the S/A+ post-GAC treatment, while another OP of metformin (i.e., OP_5), the tentatively proposed phenolic and alkyl sulfates or sulfonates (i.e., OP_16 and_17), as well as OP_7, appeared to persist or tend to increase during S/A+ post-GAC. The electrostatic and hydrophobic interactions play important role in the adsorption process of activated carbon (Margot et al., 2013). These results suggested that some SFC-only OPs were extremely hydrophilic or negatively charged (e.g., in the case of OP_16), which prevented their adsorption even by fresh GAC.

3.4 Ozonation of metformin

As mentioned in Section 3.2, two of the SFC-only OPs (i.e., OP_4 and 5, Table 2) were proposed to be metformin products. Moreover, about 0.94 µg/L of metformin was present in WWTP effluent in this study, which was reduced to 0.57 µg/L after ozonation (~40% removal), suggesting that metformin was degraded to OPs. Thus, lab-scale ozonation was conducted in this study to confirm the identification of metformin OPs in real wastewater ozonation. The antidiabetic drug metformin is one of the most prescribed pharmaceuticals worldwide with an average oral dose of 2 g per day (Straub et al., 2019). There is no significant metabolism of metformin in human body, therefore the majority is excreted as unchanged in urine and feces (Straub et al., 2019). Considerable amount of metformin (up to several tens µg per L) can be still present in wastewater effluent due to its high influent concentration, even though a significant removal could be achieved by conventional treatments (Scheurer et al., 2009; Scheurer et al., 2012). Metformin is an extremely hydrophilic compound (Log*D* = -5.62 at pH 7.4), and thus mobile in aquatic environment. Previous screening studies reported the widespread presence of metformin in surface water, ground water, and

drinking water (Caldwell et al., 2019, and references therein). Despite the frequent occurrence of metformin in the environment, studies on its fate during wastewater and drinking water treatment processes are challenging due to the lack of suitable enrichment and analytical approaches for metformin as a highly polar compound (Scheurer et al., 2009). Thus, SFC-HRMS was expected to provide a complete picture of metformin transformation pathway during ozonation.

Ozonation of metformin was conducted in buffered pure water and samples were analyzed with SFC-HRMS in comparison to RPLC-HRMS. Metformin was eluted in void volume of RPLC (RT = 0.71 min, Table 3) but well retained in SFC (RT = 7.72 min, Table 3). Metformin with its amine substituents is protonated at neutral pH (p*K*a=10.3 and 12.3) (Scheurer et al., 2009), showing low reactivity towards ozone (1.2 M^{-1} s^{-1} at pH 7) (Jin et al., 2012). It was partly removed (~50%, Figure S14) by 5 mg/L of ozone at pH 7, and almost completely degraded while pH was increased to 8.5. These results suggested that •OH also contributed to the transformation of metformin ($k_{\bullet OH+metformin}$ =1.4 × 10^9 M^{-1} s^{-1}) (Wols et al., 2013) as alkaline pH values promote the formation of •OH during ozonation (von Gunten, 2003). This was further supported by additional experiments conducted in the presence of •OH scavenger, where 1 mol/L of *tert*-butanol reduced the removal of metformin (e.g., by a factor of 100 at pH 8.5) by largely consuming •OH in the solution (Figure S14).

Table 3. OPs of metformin detected by SFC-HRMS and RPLC-HRMS. The mass spectra and fragments of compounds were obtained from SFC-HRMS and are provided in the supporting information (Figures S16-S26).

Compound	RT[a] (SFC)	RT[a] (RPLC)	*m/z* $[M+H]^+$	Error (ppm)	Formula $[M+H]^+$	Fragments (*m/z*)	reference

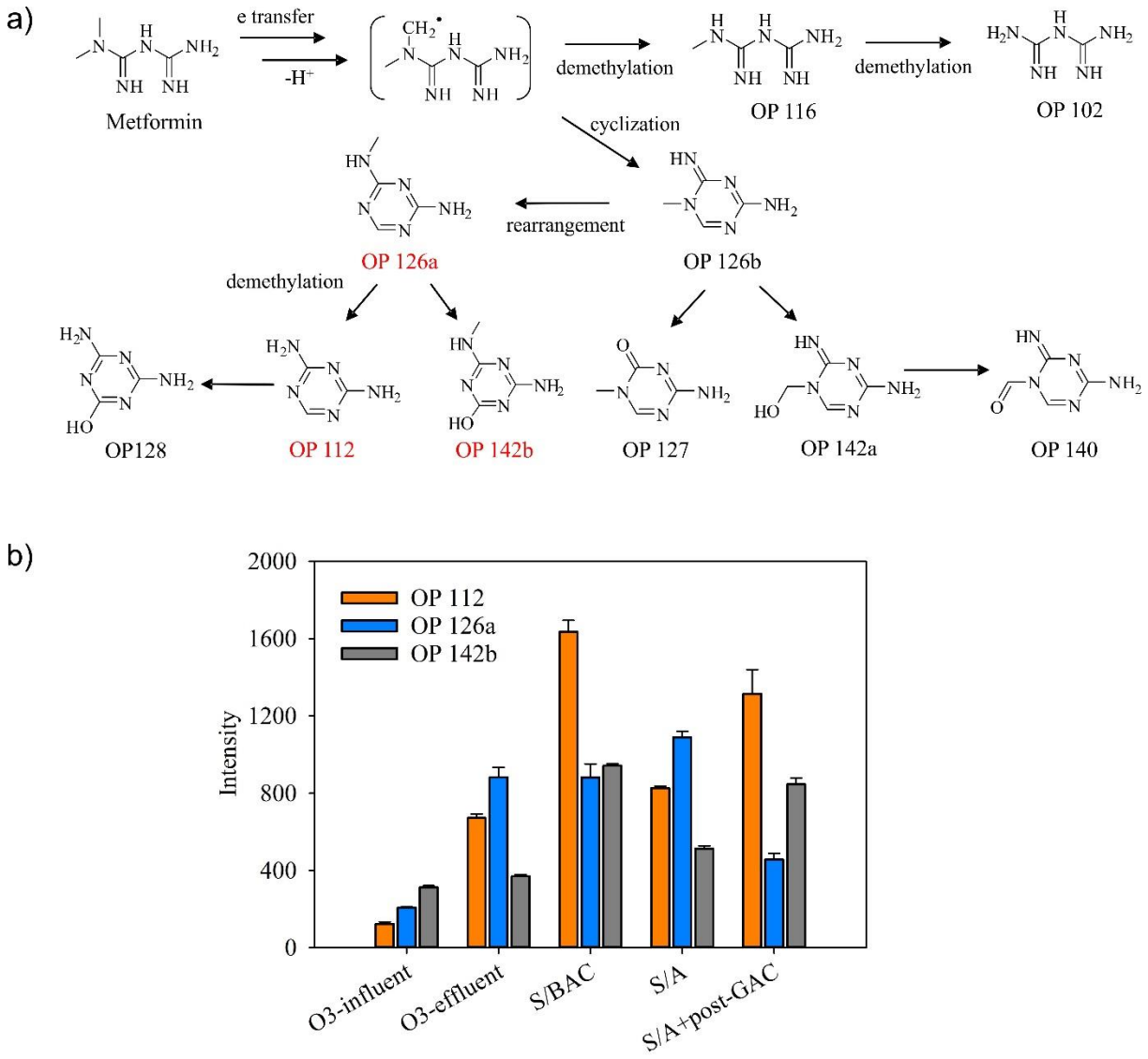
Metformin	7.72	0.71	130.1093	-4.6	$C_4H_{12}N_5$	113.0822 ($C_4H_9N_4$) 88.0869 ($C_3H_{10}N_4$) 85.0505 ($C_2H_5N_4$)	
OP 140	3.54	2.14	140.0572	-3.6	$C_4H_6N_5O$	112.0623 ($C_3H_6N_5$)	this study
OP 126a[b]	3.71	0.91	126.0780	-2.4	$C_4H_8N_5$	82.0398 ($C_3H_4N_3$) 84.0553 ($C_3H_6N_3$) 70.0400 ($C_2H_4N_3$)	d,e, f
OP 112[b]	4.08	0.66	112.0623	-0.9	$C_3H_6N_5$	70.0399 ($C_2H_4N_3$)	f
OP 142a	4.48	n. d.	142.0729	-2.8	$C_4H_8N_5O$	124.0621 ($C_4H_6N_5$) 112.0620 ($C_3H_6N_5$)	this study
OP 127	4.53	0.86	127.0620	-3.9	$C_4H_7N_4O$	86.0349 ($C_2H_4N_3O$) 83.0240 ($C_3H_3N_2O$)	this study
OP 142b[b]	5.75	0.72	142.0729	-2.8	$C_4H_8N_5O$	100.0509 ($C_3H_6N_3O$) 99.0654 ($C_3H_7N_4$) 86.0349 ($C_2H_4N_3O$)	this study
OP 128	5.87	0.61	128.0572	-3.1	$C_3H_6N_5O$	86.0347 ($C_2H_4N_3O$)	this study
OP 102	7.77	0.56	102.0780	-14.7	$C_2H_8N_5$	c	d
OP 116	7.57	0.55	116.0936	-8.6	$C_3H_{10}N_5$	99.0654 ($C_3H_7N_4$) 74.0705 ($C_2H_8N_3$)	d,e,f, g
OP 126b	8.35	0.6	126.0780	-3.2	$C_4H_8N_5$	82.0399 ($C_3H_4N_3$) 84.0552 ($C_3H_6N_3$)	d,e,f, g

Experimental conditions: metformin: 20 µM, ozone: 0.5 and 5 mg/L, 10 mM phosphate buffer at pH 7 and 8.5.
[a] retention time in min.
[b] also found from ozonated wastewater in this study.
[c] signal too weak to perform MS/MS.
[d] hydroxyl radical oxidation (Collin et al., 2004), [e] hydroxyl radical oxidation (Trouillas et al., 2013), [f] electrochemical oxidation (Tisler and Zwiener, 2018), [g] ozonation and photocatalysis (Quintão et al., 2016).

Ten OPs of metformin were detected by SFC-HRMS in positive ionization mode. They were well distributed in the chromatogram with sharp (Gaussian) peak shapes and RTs ranging from 3.54 min to 8.35 min (Table 3). Nine of these compounds were observable in the void volume of the RPLC-HRMS (0.51-0.91 min), but with tailed peak shapes (Figure S15, as *m/z* 126.0780 an example) and overlapping, so that the structure elucidation of these OPs based on fragment ions in RPLC-HRMS would not have been possible. Furthermore, this lack of retention in RPLC would

likely lead to strong signal suppression in real wastewater matrix. In addition, one isomer of *m/z* 142.0729 was not detected by RPLC.

The molecular structures of OPs, which were tentatively identified based on their fragmentation patterns (mass spectra, Figures S16-S26) in SFC-HRMS, supported that the *N*-dimethyl moiety of metformin was the main reaction site during ozonation (Figure 2a). A carbon-centered radical is assumed to be initially formed following the electron transfer and H-abstraction, which either undergoes demethylation to form OP 116 or intramolecular cyclization to produce OP 126b. Subsequent demethylation can transfer OP 116 to OP 102, whereas OP 126b can be isomerized to OP 126a via intramolecular rearrangement (methyl transfer). The cyclization products (i.e., OP 126a and b) were more abundant in total ion chromatogram than the demethylation products (i.e., OP 116 and OP 102). The $^{\bullet}$OH-induced cyclization of metformin to OP 126 isomers was studied in detail previously (Collin et al., 2004; Trouillas et al., 2013). It was reported that the isomerization process (methyl transfer) favors high pH conditions (pH≥7) (Trouillas et al., 2013). The MS/MS spectra of OP 126 isomers in this study exhibited *m/z* 82.0398 and *m/z* 84.0553 as dominant fragment ions. However, the isomer at RT=8.35 min had a unique fragment ion *m/z* 99.0648, corresponding to the loss of –CNH, and tended to be more abundant at pH 7. In contrast, the isomer at RT=3.71 min showed 4-fold higher formation yield at pH 8.5 than that at pH 7. Therefore, the structures of OP 126 isomers at RT=8.35 and 3.71 min were assigned to be before (i.e., OP 126b) and after methyl transfer (i.e., OP 126a), respectively. The further demethylation of OP 126a could lead to the formation of OP 112. Five further oxidation products (i.e., OP 127, 128, 140, 142 a and b) are reported for the first time in this study. Among them, OP 128 was confirmed to be ammeline (4,6-diamino-2-hydroxy-1,3,5-triazine) based on its analytical standard (Figure S23).



475

476 **Figure 2.** a) Proposed transformation pathways of metformin during ozonation (pH 7 and 8.5) and
477 b) Intensity evolution of metformin OPs found from wastewater during ozonation and post-
478 treatment (i.e., OP 112, 126a, and 142b, highlighted in red in (a)). Error bars represent the standard
479 deviations of 3 injection replicates

480 Importantly, the SFC-only OPs, OP_4 and 5 (Table 2) found during non-target analysis of
481 ozonated wastewater, were confirmed to be OP 126a and OP 112 of metformin, respectively, based
482 on the similarity of their RT, exact masses, and fragment ions. OP 126a and OP 112 were already

present in WWTP effluent before ozonation and thus likely formed by biotransformation of metformin or other precursors during wastewater treatment. Nevertheless, a significant increase in their intensities (4-5 fold) was observed upon ozonation (Figure 2b). It should be noted that lab-scale ozonation has confirmed metformin as precursor of these OPs in buffered pure water. However, current study cannot rule out the possibility of other precursors in complex wastewater matrix. As mentioned in Section 3.3, both OP 126a and OP 112 persisted to post-treatments. OP 112 even appeared to increase in S/BAC and S/A+ post-GAC, revealing that it can be continuously formed during post-treatment via biotransformation of OP 126a (in the case of S/A+ post-GAC) or other intermediates (Figure 2b). Another metformin product, OP 142b, was also detected from wastewater effluent. A slightly low intensity increase (1.2 fold) of OP 142b was observed by ozonation (Figure 2b), a reason why it was not extracted during non-targeted analysis mentioned in Section 3.2. However, post-treatment also failed to eliminate this product, but continuously elevated its intensity.

4. Conclusions

- SFC-HRMS and RPLC-HRMS were applied in parallel to comparatively characterize the OPs generated during pilot-scale ozonation of real WWTP effluent. In the ozonated WWTP effluent non-target screening by SFC-HRMS detected a total of 23 OPs that were not found by RPLC-HRMS. These OPs were, on average, lower in molecular weight and thus difficult to retain in RPLC. Several SFC-only OPs were proposed to originate from methoxymethylmelamine, metformin, phenolic and alkyl sulfates/sulfonates.
- In general, only 20% of the SFC-only OPs were removed by more than 70% by subsequent dual-media filters S/BAC and S/A (both 60,000 BV), revealing that the majority of SFC-only OPs were persistent to biodegradation in these systems. An additional filter employing

relatively fresh GAC (S/A+ post-GAC, 13,000 BV) showed far better removal efficiency due to the additional adsorption capacity. Nevertheless, six (out of 23) of the SFC-only OPs still escaped through S/A+ post-GAC.

- Ten OPs generated from metformin were detected from a lab-scale experiment using SFC-HRMS, five of which are reported for the first time. Three metformin OPs were found in ozonated WWTP effluent and appeared to persist to all post-treatments applied in this study.

This study shows that RPLC-HRMS screening can overlook a certain fraction of OPs generated during ozonation of municipal WWTP effluent. Chromatographic methods such as SFC, if combined with HRMS, can extend our view and add considerably to the picture of OPs specifically to those that appear to be biologically stable and hydrophilic, and thus are not completely removed by post-treatments. Due to their persistence and mobility, these OPs may eventually reach the drinking water sources after the discharge of ozonated WWTP effluent.

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Supporting Information

6 tables and 26 figures are available as supplementary material and data.

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