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Further transformation of the primary ozonation products of tramadol- and venlafaxine *N*-oxide: Mechanistic and structural considerations

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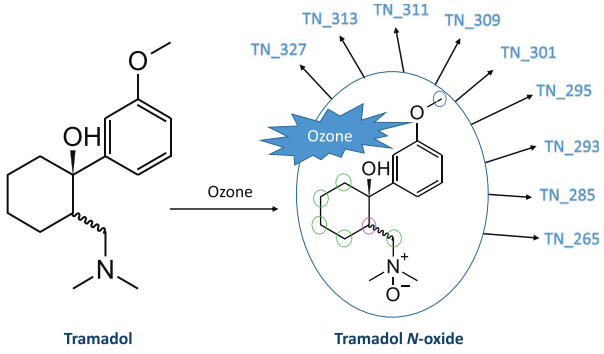
HIGHLIGHTS

- *N*-oxides of tramadol and venlafaxine react further with ozone.
- A multitude of new transformation products are formed.
- Most (18) transformation products are a result of direct reactions with ozone.
- Only five transformation products were formed by OH radicals.

GRAPHICAL ABSTRACT



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ABSTRACT

Ozonation has been used to effectively remove micropollutants from the secondary effluent in several wastewater treatment plants. It is known that ozonation transforms tertiary amine compounds into their respective *N*-oxides, however in an earlier study a mass balance could not be closed at elevated ozone concentrations, leading to the assumption that more ozonation products are possible. This study was conducted to elucidate which (hitherto unknown) ozonation products can be formed from venlafaxine and tramadol when ozonating wastewater. Ozonation experiments were performed with tramadol and venlafaxine *N*-oxide in two different set-ups. Both tramadol- and venlafaxine *N*-oxide degraded during ozonation in pure (deionized) water in both semi-continuous and batch mode ozonation set-ups. 13 and 17 new transformation products were detected from tramadol- and venlafaxine *N*-oxide respectively, using high resolution mass spectrometry with ESI(+) ionization. Empirical chemical formulas were proposed based on the determination of the exact masses and interpretation of the product ion spectra. These transformation products result from the addition of one to three oxygen atoms and removal of C, $-CH_2$, C_2H_2, C_3H_6, etc., from the parent molecule, respectively. Quenching experiments suggested that most of the transformation products originated from the direct reaction with ozone (eight for tramadol *N*-oxide and ten for venlafaxine *N*-oxide), whereas fewer products originated from the reaction with OH radicals (three for tramadol *N*-oxide and three for venlafaxine *N*-oxide). Reaction mechanisms and chemical structures of products are proposed, based on the available active sites and past literature on ozone reaction mechanisms. The experimental results are compared to theory and literature on ozone reactive sites and ozone reaction mechanisms. All in all this shows that there can be multiple ozonation products, and ozonation pathways can be complex, even if initially only one ozonation product is formed.

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1. Introduction

Organic micropollutants such as pharmaceuticals are ubiquitous in the aquatic environment and are of emerging environmental concern (Kasprzyk-Hordern et al., 2008; Kümmerer, 2009; Snyder, 2008). They are present in the aquatic environment, usually in a low concentration range (ng/L–μg/L) (Jiang et al., 2013; Ternes et al., 2003). Consequently, several pharmaceuticals including venlafaxine are included in the watch list of substances for the European Union (EU, 2020). Pharmaceuticals end up in wastewater due to human excretion and improper disposal of unused medication (Margot et al., 2013). While some compounds such as ibuprofen and paracetamol can be removed in conventional wastewater treatment, a multitude of others cannot be significantly removed in the current activated sludge treatment. (Behera et al., 2011; Jelic et al., 2011; Sui et al., 2010). Hence, wastewater treatment effluent discharges are one of the primary sources of occurrence of pharmaceuticals in the aquatic environment (Eggen et al., 2014). To remove these contaminants from wastewater, additional steps using advanced treatment technology are currently being developed and implemented (Morin-Crini et al., 2022; Yang et al., 2011; Eggen et al., 2014). Tramadol and venlafaxine are present in high concentrations (1–10 μg/L (own data)) in wastewater and are among the compounds triggering advanced wastewater treatment, e.g., in Denmark. These are thus taken as model compounds for in depth observations in this study.

Ozonation is one of the most commonly used advanced treatment techniques implemented in wastewater treatment plants for the removal of pharmaceuticals (Bourgin et al., 2018; Hollender et al., 2009; Magdeburg et al., 2012; Zimmermann et al., 2011). Pharmaceuticals with double bonds and electron-rich groups like amines are effectively removed during ozonation by chemical oxidation (Von Sonntag and Von Gunten, 2012). When ozone reacts with tertiary aliphatic amines (such as tramadol and venlafaxine), ozone adducts are formed which subsequently lose singlet oxygen ($^1O_2$) to form *N*-oxides (Von Sonntag and Von Gunten, 2012; Hübner et al., 2015). These, tertiary amine *N*-oxides are rather stable (Lim et al., 2019; Merel et al., 2017). *N*-oxides of wastewater contaminants such as citalopram, venlafaxine, tramadol, clarithromycin, levofloxacin, etc. have been reported after ozonation (De Witte et al., 2009; Hörsing et al., 2012; Lajeunesse et al., 2013; Lange et al., 2006; Zimmermann et al., 2012).

During ozonation, tramadol reacts to tramadol *N*-oxide in wastewater treatment plants with a yield of 25 % (Kharel et al., 2020; Knopp et al., 2016). However, at higher ozone doses tramadol *N*-oxide dissipates, leading to the interpretation that secondary ozonation products are formed.

For this study, the further reactions of ozonation products tramadol- and venlafaxine *N*-oxide are studied as model compounds. Depending on the available reactive sites of the molecule, the *N*-oxides can in theory react with both ozone and hydroxyl radicals depending upon the OH•/O_3 exposure in the type of water selected for reaction (Von Sonntag and Von Gunten, 2012) (Fig. 1). As in all ozonation processes, the formed transformation products (TPs) will be a result of either direct reaction with ozone or a reaction with hydroxyl radicals formed from ozone usually by reactions of ozone with organic material in the water (Von Sonntag and Von Gunten, 2012). Whether a reaction is driven by either agent can be determined by using selective quenchers: reactions with OH radicals (and the respective reaction products) can thus be stopped by adding tertiary butanol (*t*-BuOH) to a parallel reaction (Von Sonntag and Von Gunten, 2012). The general expectation is that ozone itself will only react with the aromatic rings of tramadol *N*-oxide (TRA-NOX) and venlafaxine *N*-oxide (VLX-NOX), respectively, thus methoxybenzene can be used as a proxy to assess the reactions for the reactive moiety. The second order rate constants of ozone with methoxybenzene as a model compound is ~3×10^2 M^{-1} s^{-1} (Hoigné and Bader, 1983) (Fig. 1), whereas the reaction rate constants with aliphatic carbon and/or alcohols as models for the other parts of the molecule is much lower (<10 M^{-1} s^{-1}) (Von Sonntag and Von Gunten, 2012). Opposite to ozone, hydroxyl radicals are non-selective oxidants, exhibiting second order rate constants from 10^8 to 10^9 M^{-1} s^{-1} with most organic compounds, as long as a hydrogen atom is available for abstraction. Thus, hydroxyl radicals can be expected to react with the aromatic rings of the *N*-oxides, as well as with different (tertiary, secondary, primary) aliphatic carbon atoms (Fig. 1).

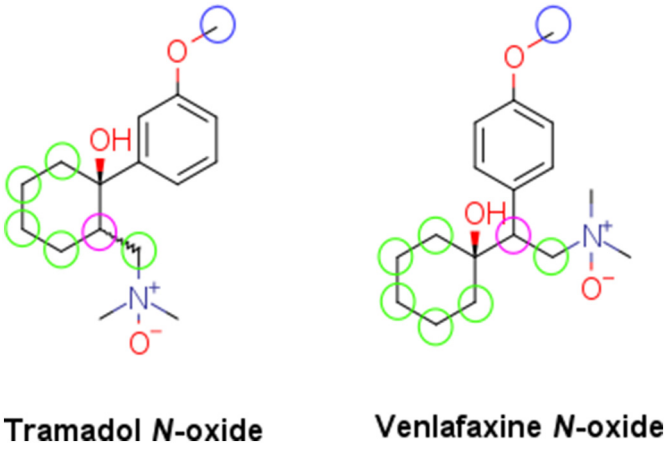

Fig. 1. Structural formulas of tramadol- and venlafaxine-*N*-oxide with possible reactive sites for oxidation. Blue circles: methoxy carbon; pink circles: tertiary carbon; green circles: secondary carbon; aromatic carbon is not marked.

Currently, it is unknown which secondary transformation products are formed from Tramadol *N*-oxide (Lee et al., 2017; Zimmermann et al., 2012) and very little is known about further reaction products of venlafaxine *N*-oxide and often their structures and their reaction pathways are unknown. However, as ozonation technology is implemented in increasing numbers of wastewater treatment plants, to remove parent pollutants and to decrease the toxic load that is introduced into the surface waters, the further reactions of *N*-oxides and the transformation products needs to be resolved to produce a more complete picture on what can be removed by ozonation together with what is emitted and can contribute to the toxic load that was not there before.

Based on Kharel et al., 2020 we hypothesize that the primarily formed *N*-oxides, are successively further oxidized to hitherto unknown stable transformation products.

The objective of the present study is to identify the possible transformation products of tramadol- and venlafaxine *N*-oxide during ozonation in pure water by means of high resolution mass spectrometry (Boix et al., 2016; Bourgin et al., 2013; Ibáñez et al., 2017; Negreira et al., 2015; Bletsou et al., 2015; Krauss et al., 2010; Müller et al., 2011). This exercise is conducted as first step and precondition for future studies in relevant waters (effluents of wastewater treatment plants). Furthermore, this work explores to what extent these observed transformation products can be explained by known reaction pathways of ozone and hydroxyl radicals with the reactive moieties present in both *N*-oxides.

2. Materials and methods

2.1. Chemicals

Tramadol *N*-oxide was purchased (purity 98.7 %) from LGC via Mikrolab Aarhus A/S (Højbjerg, Denmark). Venlafaxine *N*-oxide (purity ≥98 %) was purchased from Toronto Research Chemicals (Toronto, Canada). The stock solutions were prepared in MeOH and stored at −20 °C. Water was LiChrosolv LC-MS grade, methanol of LiChrosolv gradient grade and acetonitrile of LiChrosolv isocratic grade — all three from Merck (Darmstadt, Germany). Deionized (DI) pure water (called "DI water" throughout the text) had a conductivity 0.9 μS/cm and a pH of 7 was produced by a Reverse Osmosis Unit maxi RO (Veolia, Denmark) equipped with an Aquada UV ultraviolet water disinfection (Xylem, Denmark).

2.2. Ozonation

2.2.1. Semi-continuous mode (for identification of TPs)

Tramadol- and venlafaxine *N*-oxide methanolic standards each were placed in separate Erlenmeyer flasks. Successively, the methanol was evaporated completely overnight and 100 mL of deionized water were added to yield a final concentration of 10 mg/L. Ozone was generated using a lab ozone generator (1000BT-12, Enaly, China, capacity 1 g O_3/h) fed with dry air. Ozone was continuously bubbled into the Erlenmeyer flask using a gas wash bottle adapter with a sintered glass disc. 2 mL samples were taken before start (0 min) and during ozonation at 0.5, 1, 1.5, 2, 3, 5, 10 and 20 min ozonation time. To assess the applied ozone dose indigo was ozonated likewise under time control in a successive identical experiment (Bader and Hoigné, 1981). Briefly, indigo dye and phosphate buffer (pH 2) were added in deionized water in their respective proportion to obtain a final volume of 100 mL. The dosing times of 0.5, 1, 1.5, 2, 3, 5, 10 and 20 min correspond to 1.6, 3.2, 4.7, 6.3, 9.5, 15.8, 31.6 and 63.2 mg/L ozone. This corresponds to 0.2, 0.3, 0.5, 0.6 0.9, 1.6, 3.2 and 6.3 mg O_3/mg DOC in the samples due to the compound concentration of 10 mg/L.

2.2.2. Batch experiment

An ozone generator (GM1, Primozone, Sweden) fed with oxygen was used to produce ozone stock solutions of concentration 28–70 mg O_3 L^{-1} by sparging ozone into DI water in a 2 L glass vessel, which was placed in an ice bath. Ozone concentrations were determined by the Indigo method (Bader and Hoigné, 1981).

The experiments were conducted in a series of glass vials. 52.6 and 164.8 μL standard of tramadol- and venlafaxine *N*-oxide in methanol was spiked in all vials to achieve the final concentration of 1 mg/L for *N*-oxide in the sample (final reaction volume 3 mL). Even though the resulting methanol concentrations are relatively high and will to some extent also quench the OH radicals, this should be OK for a mechanistic experiment like this — though it would be prohibitive for a quantitative experiment. The experiments were performed in DI water with four different ozone doses, with and without the addition of tertiary butanol (*t*-BuOH, 7.14 g/L) to quench emerging OH radicals.

2.3. Characterization of TPs by means of HPLC coupled to high resolution mass spectrometry (LC-HRMS/MS)

The samples were analyzed by high performance liquid chromatography coupled to high resolution mass spectrometry (HPLC-HRMS) (TripleTOF 6600, ABSCIEX, Framingham, MA, USA). Electrospray ionization was used for identification in both positive and negative polarization. However, negative polarization did not result in new knowledge on top of the positive, thus these data are not presented. Details on chromatography (Text S1.1), MS method (Text S1.2) and post processing of data (Text S1.3 and Text S1.4) are provided in the supplementary material.

Briefly, samples were analyzed by injecting 5 μL of the respective samples without pre-concentration into the HPLC column (waters acquity BEH shield RP18 2.1 × 30 mm with 1.7 μm particles equipped with a 2.1 × 5 mm precolumn of the same material) while the MS was operated with Information-Dependent Acquisition (IDA) to automatically generate product ion spectra. The identification of transformation products formed during the studies relied on i) processing data in the MetabolitePilot® 2.2 software (AB Sciex) for automatic peak recognition and integration. MetabolitePilot compares LC-MS runs from a sample from, e.g., start (or before start of the reaction) and after a given time. Signals that have increased are annotated automatically and are linked to the structure and mass spectral data of the parent, and possible reaction products are suggested based on molecular ion and product ion spectrum of significant increased peaks after reaction. ii) extracting the product ion spectra of possible transformation products and manually interpreting the detected fragments, mass deviation was calculated with the tool from Warwick University (https://warwick.ac.uk/fac/sci/chemistry/research/barrow/barrowgroup/calculators/mass_errors/), iii) plotting of extracted chromatographic peak area of transformation products from Sciex OS (AB Sciex) in different samples with Origin 2021 (OriginLab Corporation), and iv) perform a structure search of the suggested structures in chemical abstracts online via Scifinder for verification.

3. Results and discussion

The removal of tramadol- and venlafaxine *N*-oxide during ozonation in deionized water (without hydroxyl radical scavenger, i.e. *t*-BuOH) in semi-continuous experiments is shown in Fig. 2. The degradation of both compounds relates similarly towards the increasing ozone dose. Samples from different ozone dosage were used to identify transformation products.

The transformation products detected by HPLC-HRMS screening were labelled with the initial letter from the parent *N*-oxide followed by its nominal molecular mass (e.g., TN_295 for a tramadol *N*-oxide transformation product with a molecular mass of 295 Da, and VN_307 for a venlafaxine *N*-oxide transformation product with a molecular mass of 307 Da). If several isomers of a transformation product were detected, they were nominated with a suffix a, b, c, etc. (following the elution order) at the end of transformation products name.

To test whether the reactions occurred by direct reactions with the ozone molecule or by oxidations via OH radicals, batch experiments with and without quenchers (*t*-BuOH) were conducted. Thus, e.g., TN_293e resulted from direct reaction with ozone while its isomer TN_293a from OH radicals as seen in Fig. 3. The results from batch experiments were used to explain the reaction mechanism for the formation of transformation products in Section 3.1.

The chemical formulas of potential transformation products formed from tramadol- and venlafaxine *N*-oxide, respectively, are listed in Table 1 and Table 2. In principle, a multitude of different transformation products are detected: some originating from classical oxidation reactions (additional hydroxy- and carbonylic functions) some from removal of alkyl groups.

3.1. Mass spectrometric characterization and mechanistic considerations of ozonation products with structural suggestion

3.1.1. Single additions of oxygen to N-oxides of tramadol and venlafaxine

The elemental composition of the oxidation products was established by HPLC-HRMS scans and is given in Table 1 and Table 2 for tramadol and venlafaxine *N*-oxide, respectively. More structural information was gained from the product ion scans. Product ion spectra (MS/MS spectra) of the parent tramadol *N*-oxide ($C_{16}H_{25}NO_3$) and one of its oxidation products

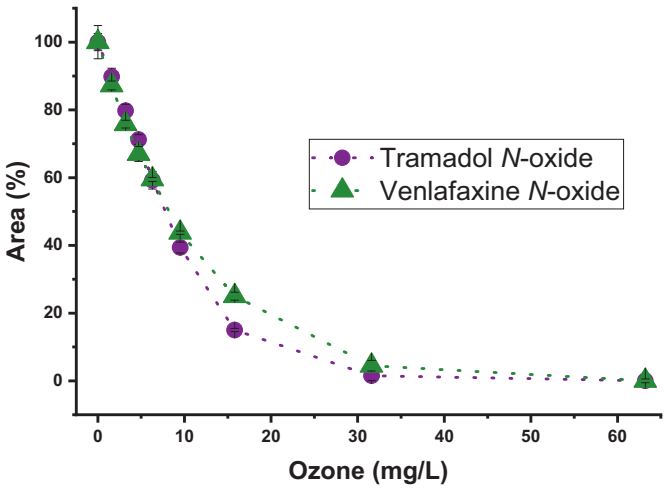

Fig. 2. Removal of tramadol- and venlafaxine *N*-oxide during ozonation in deionized water in semi-continuous experiment. (Concentration of *N*-oxide = 10 mg/L, DOC value of deionized water = 1 mg/L).

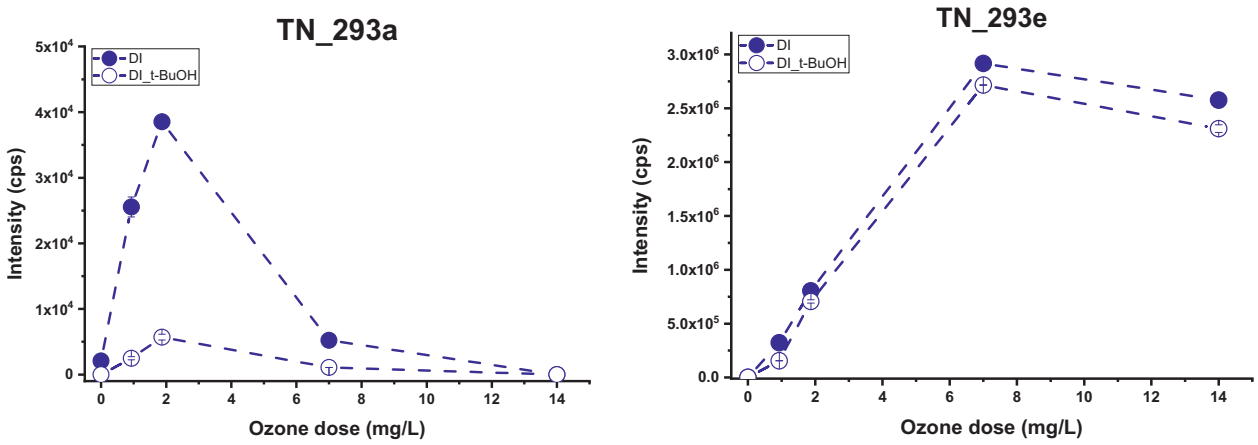

Fig. 3. Effects of quenching with *t*-BuOH as OH radical quencher on a compound resulting from reactions with OH radicals (TN:293a) and one that is formed by direct reactions with the ozone molecule (TN_293e) as examples. All reactions are in DI water.

($C_{16}H_{25}NO_4$: TN_295a) are shown in Fig. 4 as examples, while the mass spectrometric data of all transformation products of tramadol *N*-oxide are shown in the supplementary materials (S2.3–S2.14). In Fig. 5 the parent venlafaxine *N*-oxide ($C_{17}H_{27}NO_3$) and one of its oxidation products ($C_{17}H_{27}NO_4$: VN_307d) are shown as examples, while the mass spectrometric data of all transformation products of venlafaxine *N*-oxide are shown in the supplementary materials (S3.1–S3.18). Structures and fragmentation patterns for all compounds are suggested in the respective figures.

The analysis of product ion mass spectra for all transformation products was performed to ensure that it originated from the respective parent compound (and not from any environmental or instrumental background). For TN_295a, characteristic fragments, e.g., $C_3H_8N^+$, $C_7H_7O^+$, $C_8H_9O^+$ and $C_8H_7O2^+$ in the product ion spectrum (ESI(+)) are similar to those found for the parent compound tramadol *N*-oxide, while, fragments like $C_5H_{10}N^+$, $C_{16}H_{24}NO_3^+$ and $C_{16}H_{26}NO_4^+$ are typical for TN_295a (Fig. 4). Similarly, in VN_307d, fragment $C_8H_9O^+$ (which is a significant fragment in the MS2 spectrum) is also contained in the product ion spectrum of the parent VLX-NOX. On the other hand, fragment $C_3H_8N^+$ is typical for VN_307d, and is not present in the parent *N*-oxide (Fig. 5). Mass spectrometric data for all transformation products/isomers are presented in detail in supplementary material (SI Tables S2–33).

Table 1
Signals obtained after ozonation of tramadol *N*-oxide detected by HPLC-HRMS with electrospray (+) ionization. (Note: the exact mass detected, theoretical mass and the mass deviation of each transformation products isomers are presented in supplementary material (SI S2). None of these products have been reported in the literature before except as discussed in the text).

Compounds and its TPs	Change of elemental composition	Chemical formula	Reaction driven by
TRA-NOX		$C_{16}H_{25}NO_3$	Parent compound
TN_327	+3*O	$C_{16}H_{25}NO_6$	O_3
TN_315	+3*O, –C	$C_{15}H_{25}NO_6$	315a: $O_3$315c: OH• 315b: not resolved
TN_313	+3*O, $–CH_2$	$C_{15}H_{23}NO_6$	O_3
TN_311	+2*O	$C_{16}H_{25}NO_5$	311b, c, e, f, g: O_3 311a, d: not resolved
TN_309	+2*O, –2*H	$C_{16}H_{23}NO_5$	O_3
TN_301	+3*O, $–C_2H_2$	$C_{14}H_{23}NO_6$	O_3
TN_299	+3*O, $–C_2H_4$	$C_{14}H_{21}NO_6$	O_3
TN_295	+O	$C_{16}H_{25}NO_4$	295h: O_3/295a–g, i: OH•
TN_293	+O, –2*H	$C_{16}H_{23}NO_4$	293a, b: O_3/293c, d, e: OH•
TN_285	+2O, $–C_2H_2$	$C_{14}H_{23}NO_5$	O_3
TN_269	+2O, $–C_3H_6$	$C_{13}H_{19}NO_5$	O_3
TN_265	$–CH_2$	$C_{15}H_{23}NO_3$	OH•
TN_253	+O, $–C_3H_6$	$C_{13}H_{19}NO_4$	O_3

The extracted ion chromatogram (XIC) of $C_{16}H_{25}NO_4$ (TN_295, *m/z*: 296.1856 ± 0.0005) obtained from ozonation in DI water revealed at least 9 chromatographic peaks of isobaric compounds as shown in Fig. 6 (TN_295a-i, SI Table S3). These can be attributed to introducing single oxygen atoms at different carbon atoms of the parent molecule (Fig. 1). Thus, the reaction generated 9 isomers. The exact position of the attached oxygen in the respective isomers could not be revealed simply from the product ion spectra.

Quenching experiments revealed: Only one isomer of this reaction was formed by direct reactions with ozone (TN_295h) (Table 1) and all other isomers were formed by reactions with hydroxyl radicals (TN_295a,b,c,d, e,f,g and VN_309a,b,c,d,e,f,g,i) during the reactions in DI water (Table 2).

Single addition of oxygen in venlafaxine *N*-oxide also resulted in detection of 9 isomers as demonstrated by XIC of $C_{17}H_{27}NO_4$ (VN_309, *m/z*: 310.2013 ± 0.0005) (SI Table S24).

3.1.1.1. Mechanistic considerations. The high number of isomers can easily be explained by the high number of reactive sites for both O_3 and OH-radicals in the respective molecules (Fig. 1), but when applying more theoretical know-how the following can be concluded:

1) The potential reactive sites in the molecules are indicated in Fig. 1. Both ozone and hydroxyl radicals can lead to hydroxylation of the aromatic ring, leading to phenols (Fig. 7 reveals possibilities to form four isomers for tramadol *N*-oxide +O and two for venlafaxine *N*-oxide +O). The proposed phenols are ozone-reactive, and further reactions with HO• are unlikely as ozone reactive species will react more rapidly directly with ozone than with OH radicals.
2) One more (+O) isomer could be explained by a reaction that Zhang et al. (2016) reported on the formation of phenyl formate from methoxybenzene via hydroxyl radicals, and an intermediate formation of a phenoxymethanol (+O) moiety is imaginable (see Fig. 8(a)).
3) More (+O) isomers can only be explained by an attack of HO• on the primary, secondary and tertiary carbon on the aliphatic part (reactive sites shown in Fig. 1) of the molecule (proposed reactions pathways are shown in Fig. 8), opening the possibility for several isomers more (including stereoisomers and diastereomers). These reaction pathways would be in competition and in parallel of HO• attacking the aromatic part of the molecule. The rate constants of HO• with aliphatic alcohols and methoxybenzene are reasonably close, so that both parts of the molecule might be attacked. Data from quenching experiments supports this hypothesis for both TN_295 and VN_309 isomers, as the intensity of all isomer signals except TN_295h are decreased after quenching. This is indicating towards all isomers are transformation products with OH radicals, while TN_295h results from direct reactions with the ozone molecule. Possible products are shown in Fig. 8 for tramadol. Products

of aliphatic hydroxylation should exhibit unchanged (low) reactivity towards ozone, thus further reactions with both HO• and O_3 have to be considered. While 9 isomers are detected, the same number of isomers is suggested by theory (Figs. 1, 7, 8). Usually the spectra are so similar (SI) that it is difficult to discriminate them, with the exception shown in Fig. 4 in which the significant fragment of 84.080 seem to enable the localization of the OH group.

3.1.2. Tramadol- and venlafaxine N-oxide plus two oxygen atoms (TN_311 and VN_325)

The presence of oxidation products with two oxygen atoms added was established by mass spectra revealing the ESI(+) signal for a molecule with mass 312.1805 Da, which agrees to the elemental composition of $C_{16}H_{25}NO_5$ being generated during the ozonation from tramadol *N*-oxide as well as the respective analogue from venlafaxine *N*-oxide (Table 1 and Table 2). The respective product ion spectra are shown and interpreted in the SI (Tables S12 and S28). At least 7 isomers for the tramadol derivative (TN_311a-g in SI Table S12) were detected and 8 for venlafaxine (VN_325a–h in SI Table S28). Most isomers of TN_311 (TN_311b,c,e,f,g and VN_325a, b,c,d,h) were observed to be products from the direct reaction with ozone during the quenching experiments (no influence of *t*-BuOH). Several possibilities for this reaction exist:

(a) double hydroxylation of the aromatic ring, forming a dihydroxybenzene or a di-enone (Fig. 9). Methoxyhydroquinone was a reported direct ozonation product of methoxybenzene. Thus, the methoxybenzene moiety might have been similarly dihydroxylated, potentially forming the corresponding hydroquinone, catechol or resorcinol from a phenol intermediate (Mvula et al., 2009), thus the substituted methoxylated hydroquinone might be a product, but also the corresponding catechols or resorcinols (Ramseier and Gunten, 2009; Tentscher et al., 2018). Some of the isomers are formed to a lower extent at higher ozone doses, indicating further degradation with increasing ozone dose. Dihydroxybenzenes are highly reactive towards ozone ($k_{o3} = 3.1 * 10^5\ M^{-1}\ s^{-1}$ (Gurol and Nekouinaini, 1984; Turhan and Uzman, 2008).

(b) ring-opening at different positions through the Criegee reaction, forming a di-aldehyde (Fig. 10, Mvula et al., 2009). Ring-opening products are alpha-beta unsaturated carbonyl compounds. Using p-benzoquinone ($k_{o3} = 2.5 * 10^3\ M^{-1}\ s^{-1}$, (Mvula and Von Sonntag, 2003)) as a proxy, these should be slightly more reactive towards ozone than the parent methoxybenzene ring ($\sim 3 \times 10^2\ M^{-1}\ s^{-1}$, Hoigné and Bader, 1983). With the high number of detected isomers (7) it seems difficult to relate the theoretical findings to the details of the mass spectra (which are usually very similar (SI)).

Table 2
Signals obtained after ozonation of venlafaxine *N*-oxide detected by HPLC-HRMS with electrospray (+) ionization. (Note: the exact mass detected, theoretical mass and the mass deviation of each transformation products isomers are presented in supplementary material (SI S3). None of these products have been reported in the literature before.

Compounds and their TPs	Change of elemental composition	Chemical formula	Reaction driven by
VLX-NOX		$C_{17}H_{27}NO_3$	Parent compound
VN_357	+4*O	$C_{17}H_{27}NO_7$	VN_357d, f, g: O_3 VN_357a, b, c, e, f: not resolved
VN_341	+3*O	$C_{17}H_{27}NO_6$	O_3
VN_339	+3*O, −2*H	$C_{17}H_{25}NO_6$	VN_339a, c, d: O_3 VN_339g: OH• VN_339b, e, f, h: not resolved
VN_329	+3*O, –C	$C_{16}H_{27}NO_6$	O_3
VN_327	+3*O, $–CH_2$	$C_{16}H_{25}NO_6$	O_3
VN_325	+2*O	$C_{17}H_{27}NO_5$	325a–d, h: O_3 325e, f, g: not resolved
VN_323	+2*O, −2*H	$C_{17}H_{25}NO_5$	VN_323a, c, d: O_3 VN_323d: not resolved
VN_315	+3*O, $–C_2H_2$	$C_{15}H_{25}NO_6$	VN_315b: O_3 VN_315a: not resolved
VN_313	+3*O, $–C_2H_4$	$C_{15}H_{23}NO_6$	VN_313b, c: O_3 VN_313a: not resolved
VN_309	+O	$C_{17}H_{27}NO_4$	309h: O_3 309a–g, i: OH•
VN_307	+O, −2*H	$C_{17}H_{25}NO_4$	307e: O_3 307a, b, c, d: OH•
VN_297	+2*O, $–C_2H_4$	$C_{15}H_{23}NO_5$	O_3
VN_299	+2*O, $–C_2H_2$	$C_{15}H_{25}NO_5$	O_3
VN_295	+O, $–CH_2$	$C_{16}H_{25}NO_4$	VN_295a, d: O_3 VN_295b, c, e, f: not resolved
VN_279	$–CH_2$	$C_{16}H_{25}NO_3$	OH•
VN_267	+O, $–C_3H_6$	$C_{14}H_{21}NO_4$	O_3
VN_265	$–C_2H_4$	$C_{15}H_{23}NO_3$	O_3

3.1.3. Tramadol- and venlafaxine N-oxide plus 2 oxygen atoms and loss of 2 hydrogen atoms (TN_309 and VN_323)

The presence of oxidation products with two oxygen atoms added and two hydrogen atoms substracted was proven by mass spectra revealing the ESI(+) signal for a molecule with mass 310.1649 Da which agrees to the elemental composition of $C_{16}H_{23}NO_5$ Da for TN_309 and the respective analogues for VN_323 (Table 1, Table 2). The respective product ion spectra are shown and interpreted in the supplementary materials (SI Table S9 and S27). These compounds are formed when two oxygen atoms are added and two hydrogen atoms are lost from the molecule creating a) a carboxylic acid functional group in one carbon atom or b) a carbonylic function is established on one C and a hydroxylic function at another Carbon atom. For TN_309, only one isomer was detected (TN_309, SI S2.6), while for VN_323, least 4 isomers were found (VN_323a–d, Table S27) which is due to the different reactive sites of the two molecules (Fig. 1).

TN_309 and VN_323a,c,d were observed to be products from direct reaction with ozone in quenching experiments (no influence of quenching) (Table 1 and Table 2). Expected reactions are the transformation of the aromatic ring to a p-benzoquinone (as observed for methoxybenzene, (Zhang et al., 2016)), but also o-benzoquinone is possible (1 isomer for venlafaxine *N*-oxide, 2 for tramadol *N*-oxide); as shown in Fig. 9. For hydroxyl radical, it would be possible to form TN_309 and VN_323, from a combination of the formation of a ketone (either phenyl formate or the aliphatic side chain) in combination with a hydroxylation reaction (Fig. 8).

3.1.4. Tramadol- and venlafaxine N-oxide plus 3 oxygen atoms (TN_327 and VN_341)

The presence of oxidation products with three oxygen atoms was proven by mass spectra revealing the ESI(+) signal for a molecule with mass 328.1755 Da, which agrees to the elemental composition of $C_{16}H_{25}NO_6$ for TN_327, while the respective data for the venlafaxine analogues are also detected (Table 1, Table 2). The respective product ion spectra are shown and interpreted in the SI (Table S15).

Two isomers were detected for the tramadol derivatives (TN_327a,b, SI Table S15) and four for the venlafaxine one (VN_341a-d, SI Table S32). All of these were products from direct reaction with ozone in the quenching experiment. A possible pathway is a hydroxylation reaction (forming a phenol), followed by a Criegee reaction (Fig. 11). Since there are few isomers (only two in case of TN_327), it is implied that the hydroxylation has a dominating effect on the site of the second attack. Another possibility is a Criegee reaction resulting in an aldehyde and a carboxylic acid, adding an additional oxygen atom (Fig. 10, left, (b)).

3.1.5. Tramadol- and venlafaxine N-oxide plus single oxygen atom and loss of two hydrogen atoms (TN_293 and VN_307)

The presence of oxidation products with one oxygen atom added and two hydrogens lost was proven by mass spectra revealing the ESI(+) signal for a molecule with mass 294.1700 Da, which agrees to the elemental composition of $C_{16}H_{23}NO_4$ for TN_293 while the respective signals for the venlafaxine analogues were also detected. The respective product ion spectra are shown and interpreted in Fig. 5 (VN_307d) and the supplementary materials (S2.10) (for TN_293).

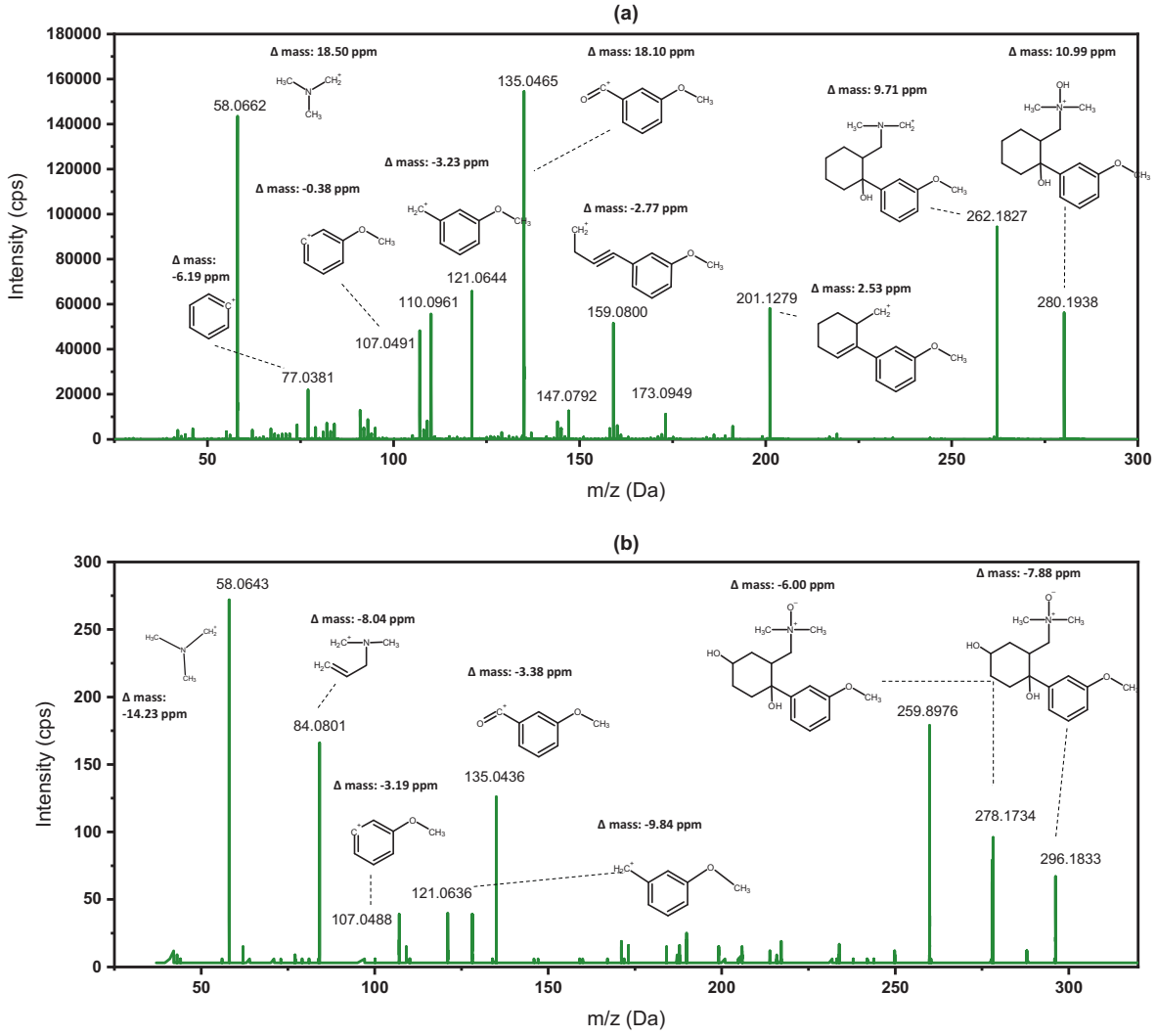

Fig. 4. Product ion spectra of (a) tramadol *N*-oxide and (b) TN_295a with tentative interpretation Fragment 84 seems to indicate towards a C—C bond breakage that did not occur in the parent pointing towards a hydroxy group in the suggested position. Most probably this compound is formed via hydroxyl radicals as suggested in Fig. 1. The details of interpretation of the MS data is given in Table 3a and 3b.

At least five isomers were detected for the tramadol derivative (TN_293a-e, SI Table S8) and five resulting from venlafaxine derivative (VN_307a–e, SI Table S23). The five MS^2 spectra of the a, b, c, d, e isomers of VN_307 are shown in SI Table S23. The exact position of the attached single oxygen and loss of 2 hydrogens in the molecule is difficult to propose from the most abundant fragments as the product ion spectra are very similar for all isomers.

(TN_293a, TN_293b and VN_307a, b, c, d) originate from reactions with hydroxyl radicals while (TN_293c, d, e and VN_307e) from direct reactions with the ozone molecule. Possible pathways from hydroxyl radical reactions are the formation of phenyl formate (reported by (Zhang et al., 2016) for methoxybenzene), or alternatively the formation of a carbonyl (keto or aldehyde function) group at the aliphatic side chain, which would be a product of a second reaction with HO• (Fig. 8). The formation of a carbonyl group on the aliphatic side chain does not alter the reactivity towards ozone, so that further reactions with both HO• and O_3 have to be considered. The phenyl formate moiety should be less ozone-reactive

Table 3a
Elemental composition of the fragmentation pattern of Tramadol *N*-Oxide (Fig. 4).

Name	Chemical formula	Theoretical *m/z* [M + H]$^+$	Measured *m/z* [M + H]$^+$	Retention time (min)	Mass deviation (ppm)
Tramadol *N*-oxide	$C_{16}H_{25}NO_3$	280.190720	280.1938	4.39	10.99
Product ion spectrum of tramadol *N*-oxide	C_3H_8N		58.0662		18.50
	C_6H_5		77.0381		−6.19
	C_7H_7O		107.0491		−0.38
	C_8H_9O		121.0644		−3.23
	$C_8H_7O_2$		135.0465		18.10
	$C_{11}H_{11}O$		159.0800		−2.77
	$C_{14}H_{17}O$		201.1279		2.53
	$C_{16}H_{24}NO_2$		262.1827		9.71

Table 3b
Elemental composition of the fragmentation pattern of Tramadol *N*-oxide–oxide (TN_295a) (Fig. 4).

Name	Chemical formula	Theoretical *m/z* [M + H]$^+$	Measured *m/z* [M + H]$^+$	Retention time (min)	Mass deviation (ppm)
Tramadol *N*-oxide	$C_{16}H_{25}NO_3$	280.190720	280.1938	4.39	10.99
Product ion spectrum of tramadol *N*-oxide	C_3H_8N		58.0662		18.50
	C_6H_5		77.0381		−6.19
	C_7H_7O		107.0491		−0.38
	C_8H_9O		121.0644		−3.23
	$C_8H_7O_2$		135.0465		18.10
	$C_{11}H_{11}O$		159.0800		−2.77
	$C_{14}H_{17}O$		201.1279		2.53
	$C_{16}H_{24}NO_2$		262.1827		9.71

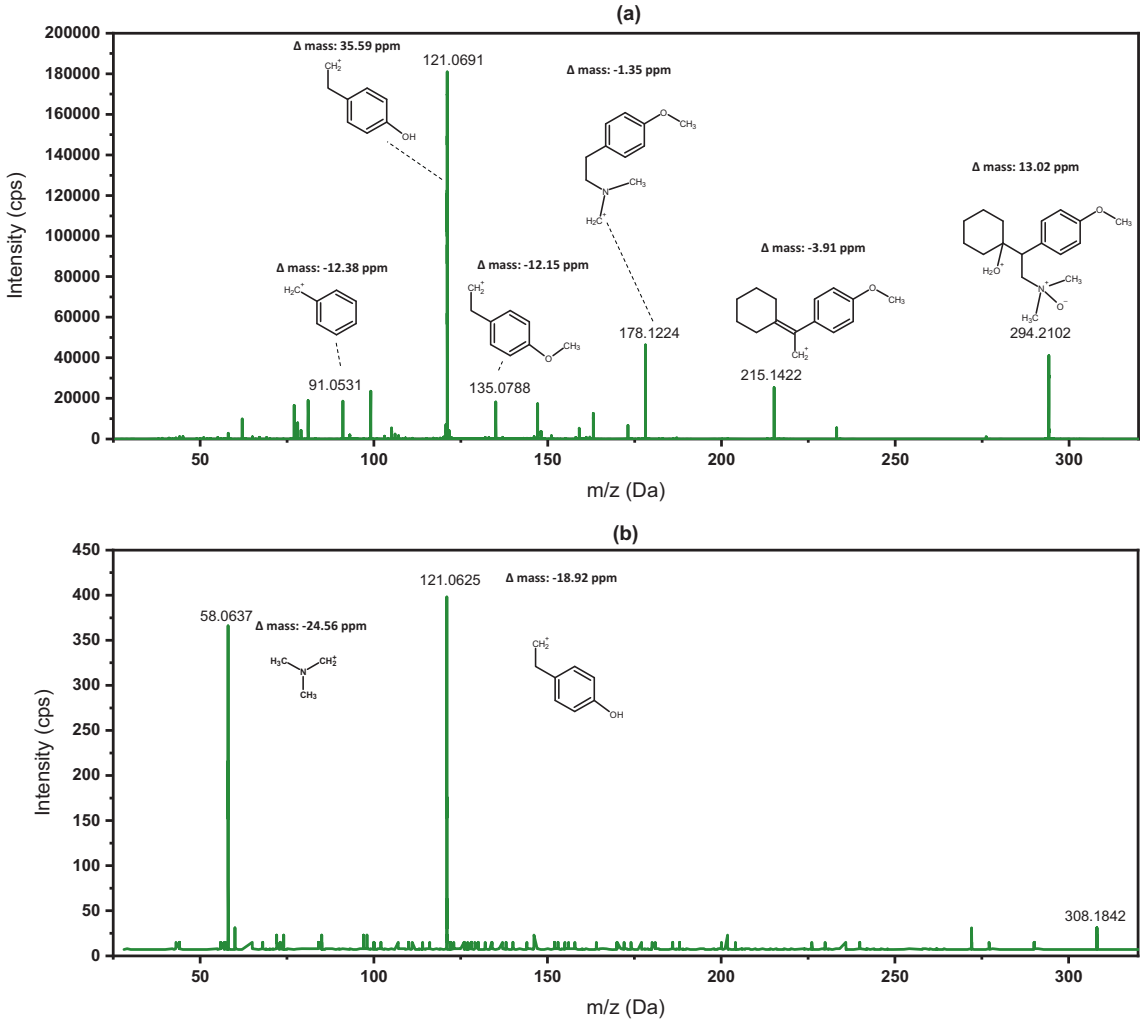

Fig. 5. Product ion spectrum of (a) venlafaxine *N*-oxide and (b) VN_307d with tentative interpretation as example for identification of transformation products.

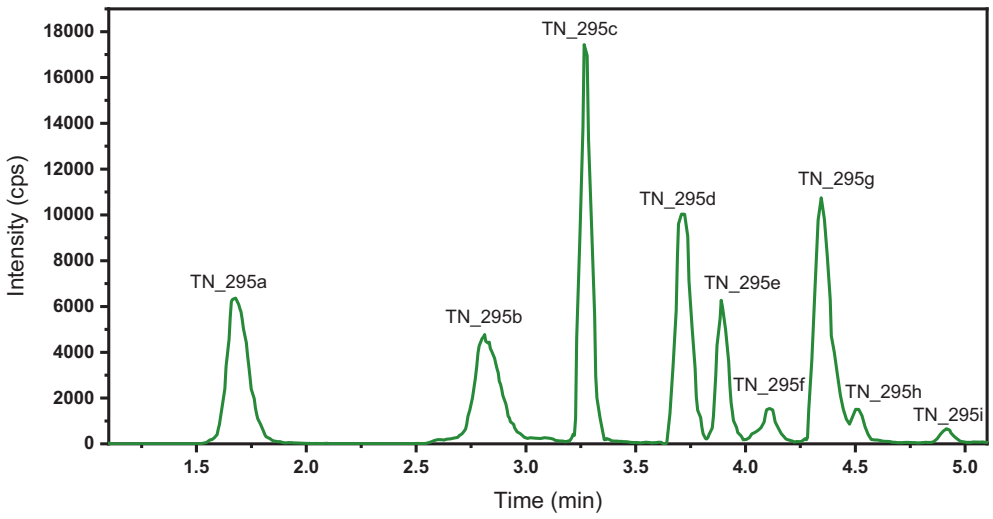

Fig. 6. Extracted ion chromatogram (XIC) of $C_{16}H_{25}NO_4$ (*m/z*: 296.1856 ± 0.0005 Da, TN_295) demonstrating the formation of nine isomers (a–i) of tramadol *N*-oxide plus oxygen at 6.3 mg/L ozone.

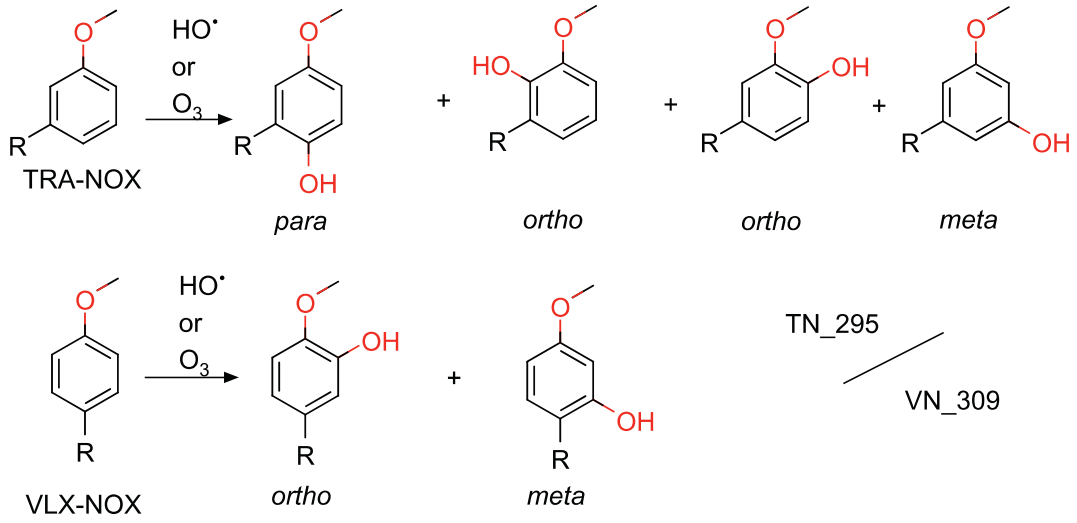

Fig. 7. Possible O-insertion reactions at aromatic carbon, which lead to phenols as proposed intermediates.

than the parent methoxybenzene group, and may predominantly react with hydroxyl radical. Phenyl formate could hydrolyze to phenol (Fig. 8). Products from the direct reaction with ozone remain unexplained. Ozone can react with aliphatic alcohols through H-abstraction or O_3-insertion (into the C—H bond), but this reaction is 2–3 orders of magnitude slower than the reaction with methoxybenzene, and thus the yields of reactions with aliphatic moieties of the N-oxides could amount to ~0.1–1 % of that of products from the methoxybenzene moiety.

3.1.6. Tramadol- and venlafaxine N-oxide minus CH_2 (TN_265 and VN_279)

The presence of transformation products with CH_2 lost, was proven by mass spectra revealing the ESI(+) signal of 266.1751 Da, which agrees to the elemental composition of $C_{15}H_{23}NO_3$ for TN_265, while the respective data for the venlafaxine analogues are also detected. The respective product ion spectra are shown and interpreted in the SI (Tables S5 and S19).

Both TN_265 and VN_279 are formed as single isomers by the loss of a methylene group from TRA-NOX and VLX-NOX (TN_265, VN_279).

Demethylation seems to be a result of reaction with hydroxyl radical as observed in the quenching experiment. A possible formation pathway for the reaction with hydroxyl radical is the initial formation of phenyl formate (see +O; −2*H) and the subsequent hydrolysis to the phenol form (Fig. 8). The resulting phenol should be highly ozone-reactive, and no further reactions with hydroxyl radical should be anticipated. The only other possible site for demethylation would be the *N*-oxide moiety, forming the hydroxylamine, but this does not seem reasonable.

3.1.7. Tramadol- and venlafaxine N-oxide plus 3 oxygen and loss of –CH_2 (TN_313 and VN_327)

The presence of transformation products with 3 oxygen atoms added and CH_2 lost was proven by mass spectra revealing the ESI(+) signal for a molecule with mass 314.1598 Da which agrees to the elemental composition of $C_{15}H_{23}NO_6$ for TN_313 while the respective data for the venlafaxine analogues are also detected. The respective product ion spectra are shown and interpreted in the SI (Table S13).

TN_313 (one isomer: SI S2.4) and VN_327 (five isomers: VN_327a-e, SI Table S29) are formed by the addition of 3 oxygen atoms and loss of a methylene group in tramadol and venlafaxine *N*-oxide all of which were products from direct reaction with ozone. This oxidation product should thus not be a result of hydroxyl radical-initiated CH_2 loss at the methoxy group. CH_2 loss through ozonation might be possible if a methyl ester is created by a Criegee reaction, which could then hydrolyze to the carbonic acid (Fig. 10, left, (b)). The other possibility to form the ester with 3*O is the Criegee reaction of a phenol, currently shown in Fig. 11, and further hydrolysis of the ester may lead to the additional loss of CH_2. The fragmentation in Table S29 agrees with this explanation, however with the detected numbers of isomers more detailed considerations are difficult.

3.1.8. Tramadol- and venlafaxine N-oxide plus 3 oxygen atoms and loss of C_2H_2 (TN_301 and VN_315)

The presence of transformation products with 3 oxygen atoms added and C_2H_2 lost was proven by mass spectra revealing the ESI(+) signal for a molecule with mass 302.1598 Da, which agrees with the elemental composition of $C_{14}H_{23}NO_6$ for TN_313 while the respective data for the venlafaxine analogues are also detected. The product ion spectra are shown and interpreted in the SI Table S12).

One isomer of the tramadol derivative (TN_301, SI S2.7) and two for venlafaxine (VN:315a–b, SI Table S26) were detected. TN_301 and VN_315b resulted from direct reaction with ozone. Carbon loss can be explained through consecutive Criegee reactions (Fig. 12), or through the same reaction as $+O_2$, $–C_2H_2$ but with Criegee reaction resulting in a carboxylic acid, adding an extra oxygen (not shown).

3.1.9. Tramadol- and venlafaxine N-oxide plus 2 oxygen atoms and loss of -C_2H_2 (TN_285 and VN_299)

The presence of transformation products with 2 oxygen atoms added and C_2H_2 lost was proven by mass spectra revealing the ESI(+) signal for a molecule with mass 286.1649 Da, which agrees with the elemental composition of $C_{14}H_{23}NO_5$ for TN_385 while the respective data for the venlafaxine analogues are also detected. The product ion spectra are shown and interpreted in the supplementary materials (S2.11). Three different isomers were formed from the tramadol derivative and three from venlafaxine (TN_285a–c, SI Table S7, VN_299a–c, SI Table S22). All of these were products from the direct reaction with ozone. The isomers also appear to be primary products. For more than one carbon atom to leave the molecule, a primary ring-opening reaction could be followed by a secondary Criegee reaction at the α,β-unsaturated carbonyl, yielding two isomers with this mass difference (Fig. 10).

3.2. Mass spectrometric characterization of ozonation products without mechanistic explanation

The following products were observed by their mass spectra with and without fragmentation. Their elemental change and pattern in quenching experiment are described below. However, their formation cannot be mechanistically explained or corroborate with the existing literature. Nonetheless, some suggestions for the formation of some products are given.

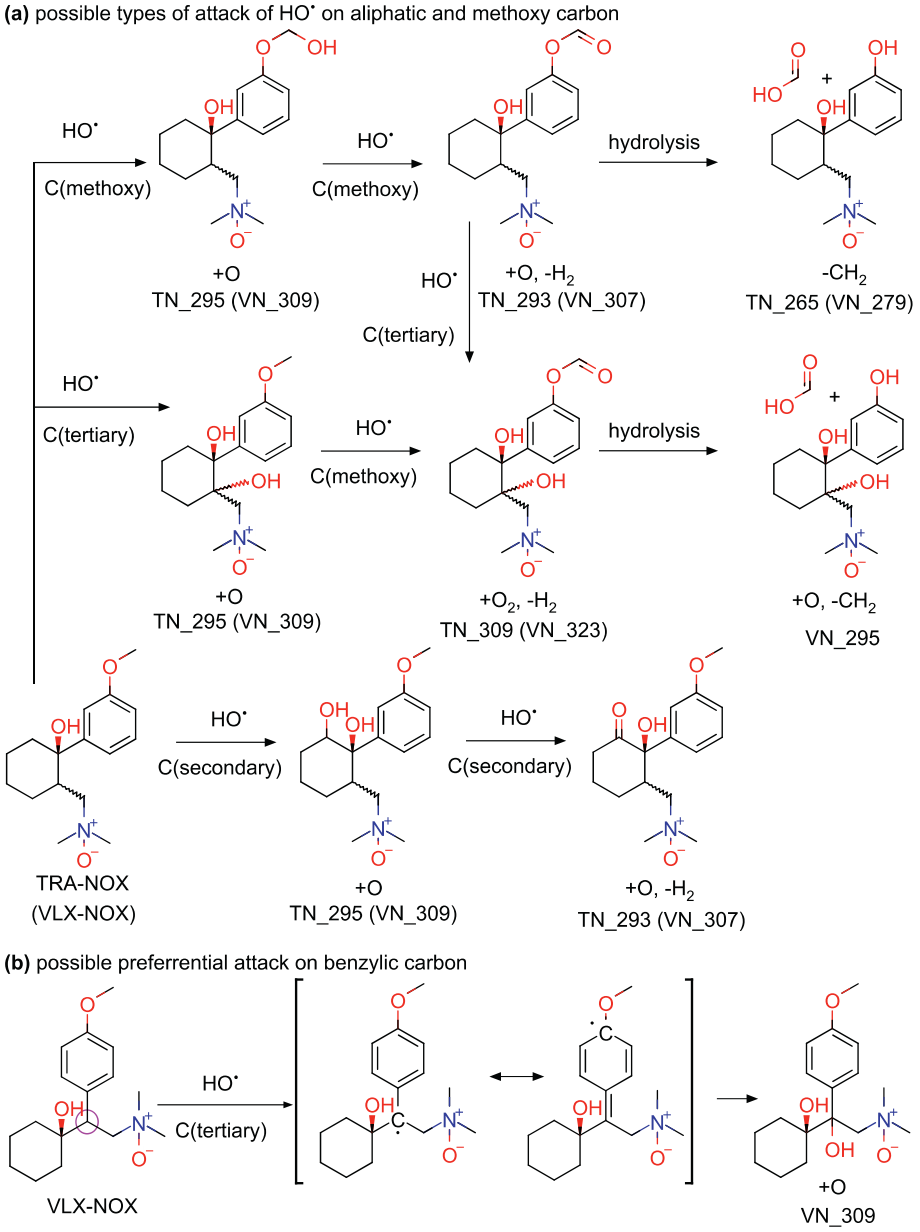

Fig. 8. Proposed reactions of HO• with different types of aliphatic sites in tramadol-*N*-oxide. The reaction with C(methoxy) to phenyl formate was based on the corresponding product that had been observed for anisole in Zhang et al. (2016). Only the reaction with one possible secondary carbon is shown. Possible reactions of venlafaxine-*N*-oxide are analogous. A reaction of O_3 or HO• with the aromatic ring could happen for any proposed intermediate, and would lead to O_3-reactive products.

3.2.1. Common change in elemental composition (tramadol and venlafaxine derivatives)

3.2.1.1. Addition of three oxygen atoms and loss of single C. Three isomers of a transformation product of both tramadol and venlafaxine *N*-oxide were detected. TN_315a–c, *m/z*: 316.1755 ± 0.0005 (SI Table S14) and VN_329a–c, *m/z*: 330.1911 ± 0.0005 (SI Table S31). TN_315a was product from direct reaction with ozone, TN_315c from reaction with hydroxyl radical while TN_315b could not beresolved. However, all isomers of VN_329 were observed to be products from direct reaction with ozone. The loss of "C" (without H) implies a loss of e.g. a CH_2 group with addition of hydrogen to other parts of the molecule. This could occur by reactions with OH radicals e.g., with a double bond, formally adding H_2O, and resulting in an alcohol. If the demethylation of the methoxy group is relatively slow, then these products should not be a follow-up product of CH_2 loss at the methoxy group. However, if this is formed through the phenyl formate hydrolysis, the rest of the molecule could react, and CH_2 loss could occur afterwards.

3.2.1.2. Addition of three oxygen atoms and one methylene group. Six isomers of TN_299 and three of VN_313 are formed from the parents. The data for TN_299a–f, *m/z*: 300.1442 ± 0.0005 are presented in SI S2.8), while the data for VN_313a–c, *m/z*: 314.1598 ± 0.0005 is presented in SI S3.10). Several isomers (TN_299a,c,d and VN_313c) were observed to originate from direct reaction with ozone in the quenching experiments (Table 1). It would require an additional ketone formation (+O, $-H_2$) in combination with a $+O_2$, $-C_2H_2$ transformation. Note that +O, $-H_2$ in combination with a $+O_2$, $-C_2H_2$ transformation was observed during ozonation, but no mechanism can be proposed by the authors. For an isomer, formed by reactions with OH radicals, the ketone could be formed on the aliphatic side chain, combined with the $+O_2$, $-C_2H_2$ (Figs. 10, 12) transformation through ozone.

3.2.1.3. Adding one oxygen atom and loss of the C_3H_6 group. In both tramadol- and venlafaxine- *N*-oxide lead to formation of four isomers TN_253 and two VN_267. TN_253a–d, *m/z*: 254.1387 ± 0.0005 is presented in SI Table S4

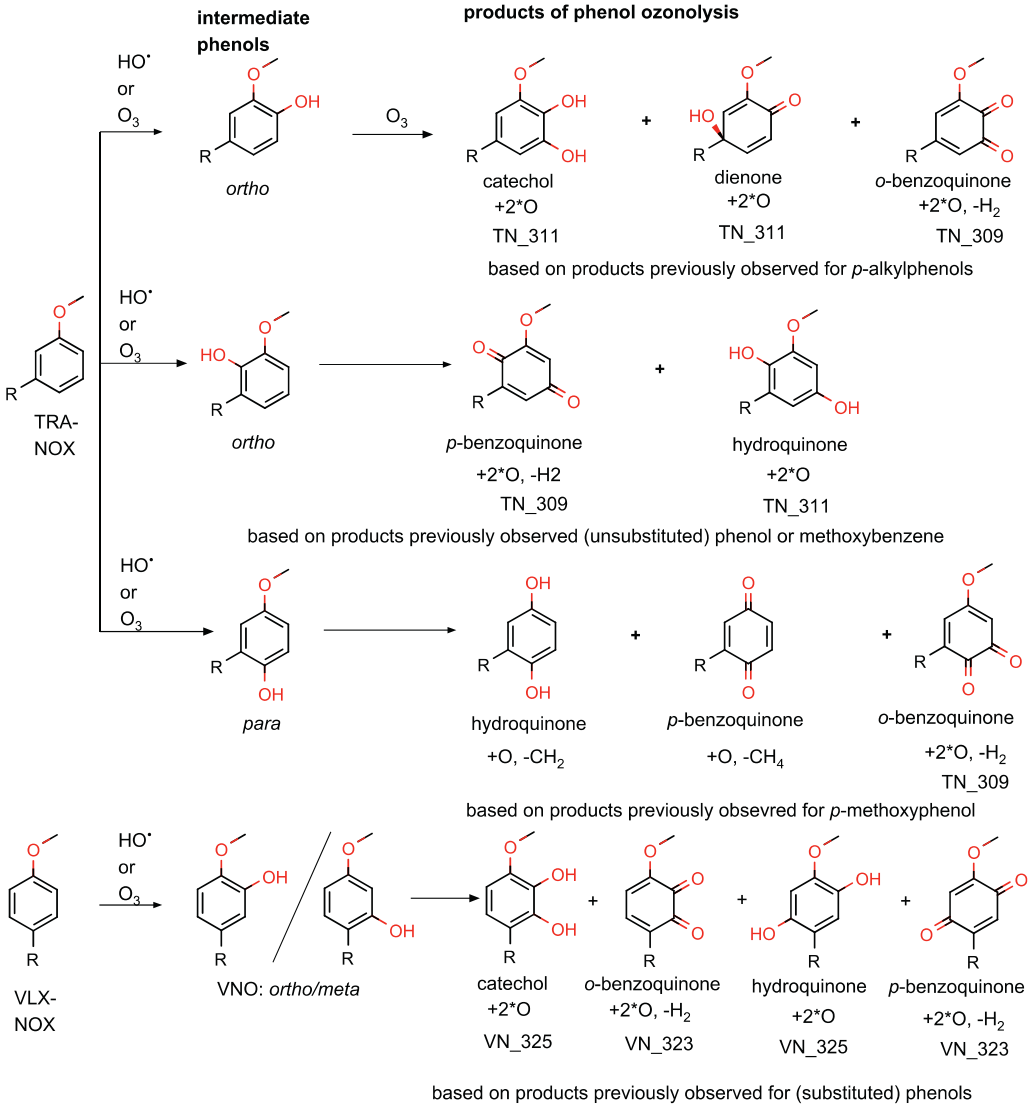

Fig. 9. Proposed reactions of O_3 with tramadol- and venlafaxine-*N*-oxide without aromatic ring opening reaction, based on products previously observed for methoxybenzene (Mvula et al., 2009) and substituted phenols (Ramseier and Gunten, 2009; Tentscher et al., 2018).

and VN_267a–b, *m/z*: 268.1543 ± 0.0005 in SI Table S18). All isomers of TN_253 and VN_267 were observed to be products from direct reaction with ozone in quenching experiments.

3.2.2. Changes in elemental composition specific to either tramadol or venlafaxine

3.2.2.1. Addition of 2 oxygen atoms and loss of C_3H_6 from tramadol N-oxide. Two isomers of transformation products were formed (TN_269a–b, *m/z*: 270.1336 ± 0.0005, SI Table S6) by direct reaction with ozone in quenching experiments. Ozonation can explain a $+O$, $-C_3H_4$ pattern through two Criegee reactions (Fig. 10), and that seems to be the most realistic possibility to loose three carbon atoms. To get from that to $+O_2$, $-C_3H_6$ would require an additional $+O$, $-H_2$ reaction. This would be possible from the formation of a ketone on the aliphatic side chain through hydroxyl radical (Fig. 8), but the quenching experiment data does not seem to support the involvement of hydroxyl radical. Note that there is an unexplained $+O$, $-H_2$ reaction attributed to ozone that could possibly be combined with the $+O$, $-C_3H_4$ pattern. A different possibility would be via a hydroquinone: two subsequent Criegee reactions can lead to a maleic acid moiety, which could condensate to maleic anhydride.

3.2.2.2. Loss of C_2H_4 from venlafaxine N-oxide. VN_265, *m/z*: 266.1751 ± 0.0005 (SI Table S17) is formed by direct reaction with ozone.

3.2.2.3. Addition of four oxygen atoms to venlafaxine N-oxide. Forms 7 isomers of (VN_357a–g, *m/z*: 358.1860 ± 0.0005, SI Table S33) by direct reaction with ozone. The formation of hydroquinone followed by Criegee alkene cleavage could lead to the formation of this product.

3.2.2.4. Addition of one oxygen atom and loss of CH_2 from venlafaxine N-oxide. Results in six isomers of VN_295 (VN_295a–f, *m/z*: 296.1856 ± 0.0005, SI Table S20) by direct reaction with ozone.

3.2.2.5. Addition of two oxygen atoms and loss of C_2H_4 from venlafaxine N-oxide. Forms four isomers (VN_297a–d, *m/z*: 298.1649 ± 0.0005, SI Table S21) by direct reaction with ozone except VN_297c which is a product from a hydroxyl radical reaction.

3.2.2.6. Addition of 3 oxygen atoms and loss of 2 hydrogen atoms from venlafaxine N-oxide. Forms eight isomers of VN_339 (VN_339a–h, *m/z*: 340.1755 ± 0.0005, SI S3.4). VN_339a, c, d were products from direct reaction with ozone while VN_339g was formed by reactions with hydroxyl radicals.

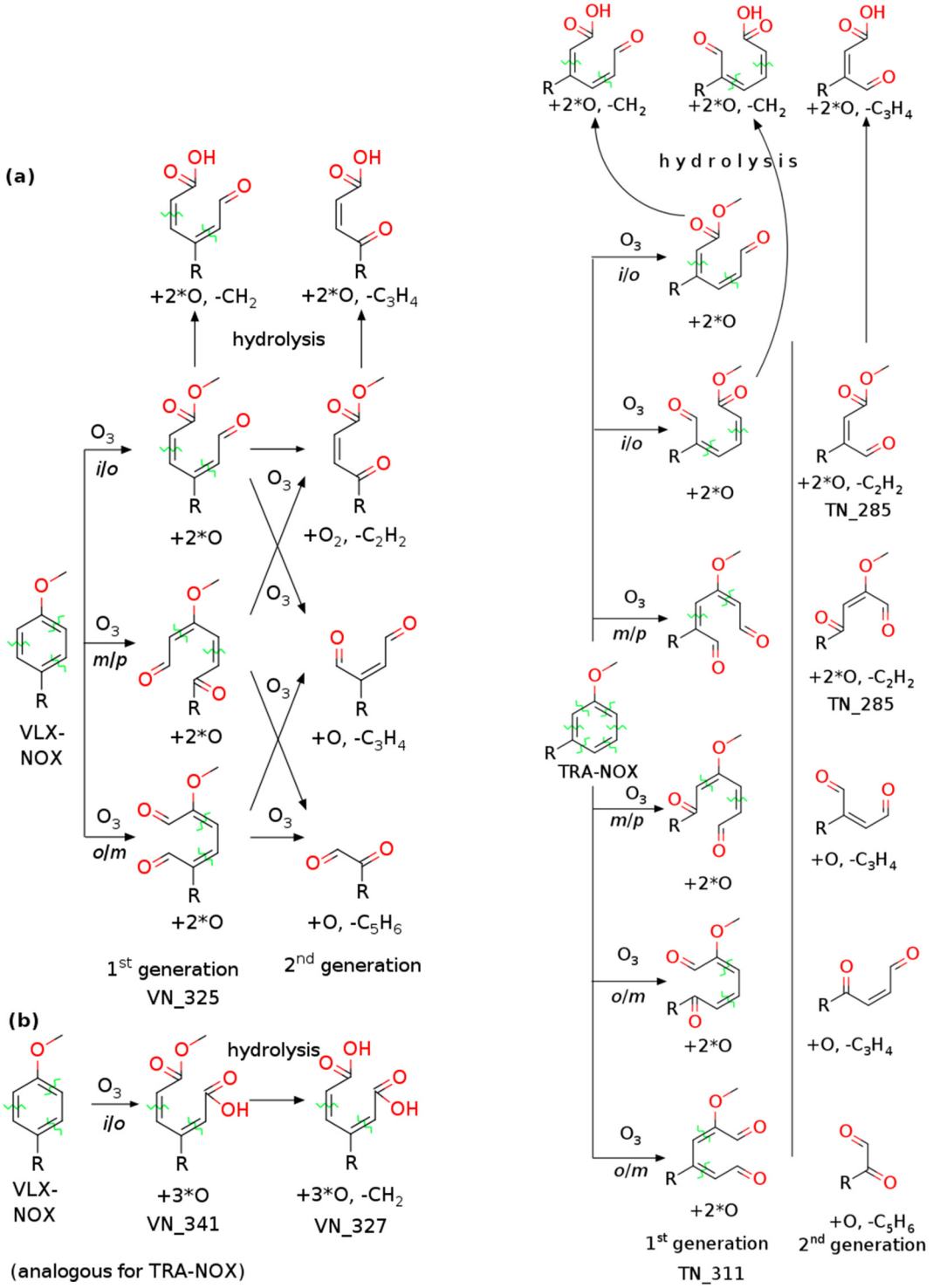

Fig. 10. Proposed Criegee reactions of O_3 with venlafaxine *N*-oxide (Left). Bidentate attack is possible in *ipso/ortho* (*i*/*o*), *meta/para* (*m*/*p*), or *ortho/meta* (*o*/*m*) position relative to the methoxy group. For the model compound methoxybenzene, the ring-opening product from an *i*/*o*-attack was detected (Mvula et al., 2009). The double bonds of the resulting dialdehydes remain O_3-reactive, and a second generation of products is proposed. Esters are proposed to hydrolyze after the reaction, which leads to an additional loss of CH_2. Proposed Criegee reactions of O_3 with tramadol *N*-oxide (Right). Bidentate attack leads to six possible isomers. Connectivity between the first and second generation of products is not shown.

3.2.3. Results from analyzing effluent wastewater from a poststream ozonation plant

Effluent wastewater post ozonation was scanned for the described ozonation products by non-target screening. However, none of the described transformation products could be determined without preconcentration. This is probably due to the fact that the relative high concentrations of the parents are distributed to a multitude of transformation products and a target quantification method would be more adequate. – This would, however need the pure compounds as calibration and tuning standards.

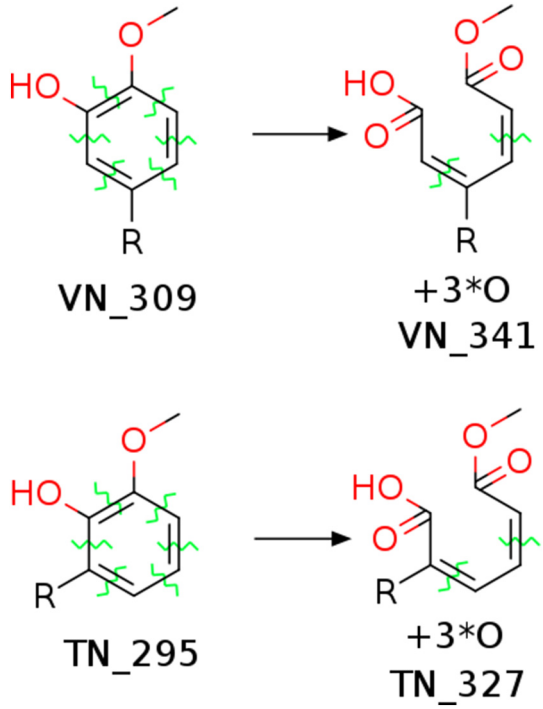

Fig. 11. Criegee mechanism for addition of 3*O. Only one of the possible phenols (see Fig. 7) is shown, and only one possible isomer of VN_341 and TN_327 is shown. Hydrolysis of the ester of the shown isomers may lead to the additional loss of CH_2.

3.2.4. Persistence considerations

While the fate of the initial parent compounds (tramadol and venlafaxine) in classical and advanced wastewater treatment is well known and the fate of the respective *N*-oxides in post treatment is known in principle (Edefell et al., 2021; Kharel et al., 2020; Knopp et al., 2016; Merel et al., 2017; Zucker et al., 2018), there is still a knowledge gap on the fate of the described ozonation products of the *N*-oxides in potential ozonation post treatment.

4. Conclusions

In the bigger pictures this paper demonstrates that even with focusing on only two compounds the number of formed ozonation products is high (30 new compounds). Eight transformation products for tramadol *N*-oxide were formed by direct reactions with ozone and three by hydroxyl radicals while the remaining two were formed by both pathways (Table 1). For venlafaxine *N*-oxide ten transformation products by were formed by direct reactions with ozone, three by reactions with hydroxyl radicals, three transformation products by reaction with ozone and hydroxyls radical (Table 2). However, the reaction mechanism for one transformation product was not clear from quenching experiment.

- 13 new (secondary) ozonation products of tramadol and 17 of venlafaxine as originating from various ozone doses in pure water were detected and characterized by HPLC-MS/MS with electrospray ionization ESI(+).
- All of these oxidation products were formed from or via tramadol- and venlafaxine *N*-oxide
- Reaction mechanisms were proposed for the formation of nine transformation products based on HR-MS data, quenching experiments, number of isomers and previous literature on compounds having similar functional groups.
- This study indicated, that the ozonation of *N*-oxides of tramadol and venlafaxine involves complex reactions for the formation of different transformation products and their isomers.

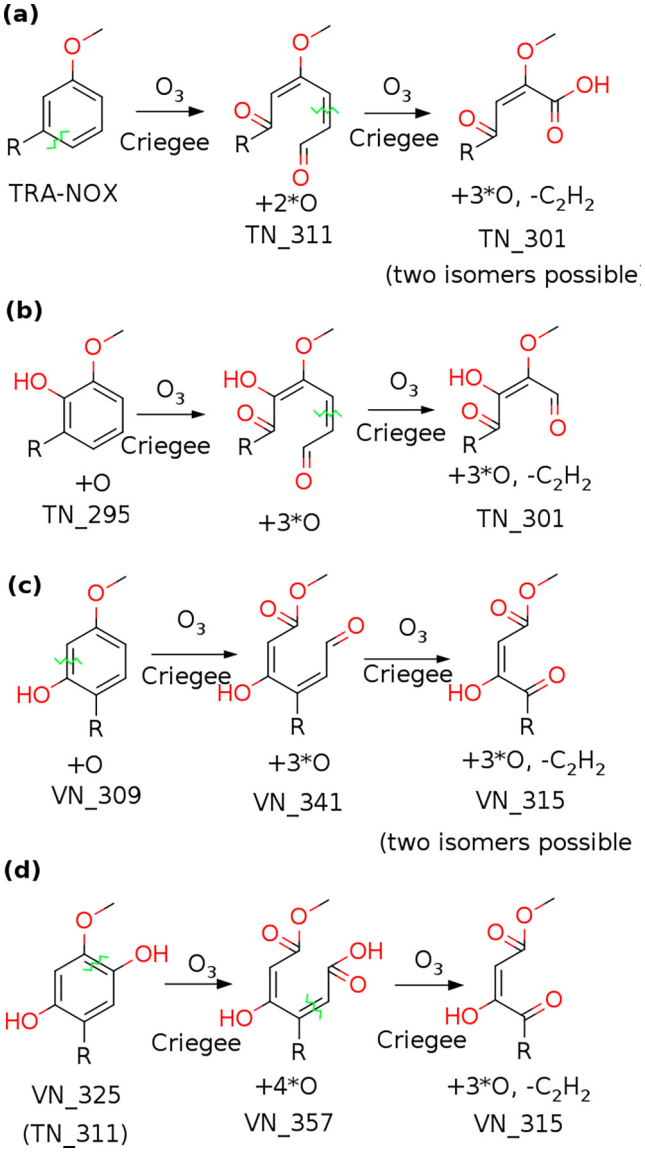

Fig. 12. Two consecutive Criegee reactions could account for the formation of products with +3*O and $-C_2H_2$.

- The formation of these compounds should be included in the assessment of ozonation for wastewater treatment.
- Whether or not the reaction schemes get even more complex in real effluent wastewater remains to be studied in the future.

CRediT authorship contribution statement

Suman Kharel: Investigation, Writing – original draft. **Peter Tentscher:** Methodology, Data curation. **Kai Bester:** Supervision, Methodology, Writing – review & editing, Data curation.

Declaration of competing interest

The authors have declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.157259.

References

Bader, H., Hoigné, J., 1981. Determination of ozone in water by the indigo method. Water Res. 15, 449–456.

Behera, S.K., Kim, H.W., Oh, J.-E., Park, H.-S., 2011. Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea. Sci. Total Environ. 409, 4351–4360.

Bletsou, A.A., Jeon, J., Hollender, J., Archontaki, E., Thomaidis, N.S., 2015. Targeted and non-targeted liquid chromatography-mass spectrometric workflows for identification of transformation products of emerging pollutants in the aquatic environment. TrAC Trends Anal. Chem. 66, 32–44.

Boix, C., Ibáñez, M., Sancho, J.V., Parsons, J.R., de Voogt, P., Hernández, F., 2016. Biotransformation of pharmaceuticals in surface water and during waste water treatment: identification and occurrence of transformation products. J. Hazard. Mater. 302, 175–187.

Bourgin, M., Beck, B., Boehler, M., Borowska, E., Fleiner, J., Salhi, E., Teichler, R., Von Gunten, U., Siegrist, H., McArdell, C.S., 2018. Evaluation of a full-scale wastewater treatment plant upgraded with ozonation and biological post-treatments: abatement of micropollutants, formation of transformation products and oxidation by-products. Water Res. 129, 486–498.

Bourgin, M., Bichon, E., Antignac, J.-P., Monteau, F., Leroy, G., Barritaud, L., Chachignon, M., Ingrand, V., Roche, P., Le Bizec, B., 2013. Chlorination of bisphenol a: non-targeted screening for the identification of transformation products and assessment of estrogenicity in generated water. Chemosphere 93, 2814–2822.

De Witte, B., Van Langenhove, H., Hemelsoet, K., Demeestere, K., De Wispelaere, P., Van Speybroeck, V., Dewulf, J., 2009. Levofloxacin ozonation in water: rate determining process parameters and reaction pathway elucidation. Chemosphere 76, 683–689.

Edefell, E., Falas, P., Kharel, S., Hagman, M., Christensson, M., Cimbritz, M., Bester, K., 2021. MBBRs as post-treatment to ozonation: degradation of transformation products and ozone-resistant micropollutants. Sci. Total Environ. 754, 142103.

Eggen, R.I., Hollender, J., Joss, A., Schärer, M., Stamm, C., 2014. Reducing the Discharge of Micropollutants in the Aquatic Environment: The Benefits of Upgrading Wastewater Treatment Plants. ACS Publications.

EU, 2020. EUROPEAN COMMISSION IMPLEMENTING DECISION 2020/1161, of 4 August 2020, establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council. Off. J. Eur. Union L 257, 32–35.

Gurol, M.D., Nekouinaini, S., 1984. Kinetic behavior of ozone in aqueous solutions of substituted phenols. Ind. Eng. Chem. Fundam. 23 (1), 54–60.

Hoigné, J., Bader, H., 1983. Rate constants of reactions of ozone with organic and inorganic compounds in water—I: non-dissociating organic compounds. Water Res. 17, 173–183.

Hollender, J., Zimmermann, S.G., Koepke, S., Krauss, M., McArdell, C.S., Ort, C., Singer, H., von Gunten, U., Siegrist, H., 2009. Elimination of organic micropollutants in a municipal wastewater treatment plant upgraded with a full-scale post-ozonation followed by sand filtration. Environ. Sci. Technol. 43, 7862–7869.

Hörsing, M., Kosjek, T., Andersen, H.R., Heath, E., Ledin, A., 2012. Fate of citalopram during water treatment with O3, ClO2, UV and Fenton oxidation. Chemosphere 89, 129–135.

Hübner, U., von Gunten, U., Jekel, M., 2015. Evaluation of the persistence of transformation products from ozonation of trace organic compounds–A critical review. Water Res. 68, 150–170.

Ibáñez, M., Borova, V., Boix, C., Aalizadeh, R., Bade, R., Thomaidis, N., Hernández, F., 2017. UHPLC-QTOF MS screening of pharmaceuticals and their metabolites in treated wastewater samples from Athens. J. Hazard. Mater. 323, 26–35.

Jelic, A., Gros, M., Ginebreda, A., Cespedes-Sànchez, R., Ventura, F., Petrovic, M., Barceló, D., 2011. Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. Water Res. 45, 1165–1176.

Jiang, J.-Q., Zhou, Z., Sharma, V., 2013. Occurrence, transportation, monitoring and treatment of emerging micro-pollutants in waste water—a review from global views. Microchem. J. 110, 292–300.

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Res. 42, 3498–3518.

Kharel, S., Stapf, M., Miehe, U., Ekblad, M., Cimbritz, M., Falås, P., Nilsson, J., Sehlén, R., Bester, K., 2020. Ozone dose dependent formation and removal of ozonation products of pharmaceuticals in pilot and full-scale municipal wastewater treatment plants. Sci. Total Environ. 731, 139064.

Knopp, G., Prasse, C., Ternes, T.A., Cornel, P., 2016. Elimination of micropollutants and transformation products from a wastewater treatment plant effluent through pilot scale ozonation followed by various activated carbon and biological filters. Water Res. 100, 580–592.

Krauss, M., Singer, H., Hollender, J., 2010. LC–high resolution MS in environmental analysis: from target screening to the identification of unknowns. Anal. Bioanal. Chem. 397, 943–951.

Kümmerer, K., 2009. The presence of pharmaceuticals in the environment due to human use–present knowledge and future challenges. J. Environ. Manag. 90, 2354–2366.

Lajeunesse, A., Blais, M., Barbeau, B., Sauvé, S., Gagnon, C., 2013. Ozone oxidation of antidepressants in wastewater–Treatment evaluation and characterization of new by-products by LC-QToFMS. Chem. Cent. J. 7, 1–11.

Lange, F., Cornelissen, S., Kubac, D., Sein, M.M., Von Sonntag, J., Hannich, C.B., Golloch, A., Heipieper, H.J., Möder, M., Von Sonntag, C., 2006. Degradation of macrolide antibiotics by ozone: a mechanistic case study with clarithromycin. Chemosphere 65, 17–23.

Lee, M., Blum, L.C., Schmid, E., Fenner, K., Von Gunten, U., 2017. A computer-based prediction platform for the reaction of ozone with organic compounds in aqueous solution: kinetics and mechanisms. Environ Sci Process Impacts 19, 465–476.

Lim, S., McArdell, C.S., von Gunten, U., 2019. Reactions of aliphatic amines with ozone: kinetics and mechanisms. Water Res. 157, 514–528.

Magdeburg, A., Stalter, D., Oehlmann, J., 2012. Whole effluent toxicity assessment at a wastewater treatment plant upgraded with a full-scale post-ozonation using aquatic key species. Chemosphere 88, 1008–1014.

Margot, J., Kienle, C., Magnet, A., Weil, M., Rossi, L., De Alencastro, L.F., Abegglen, C., Thonney, D., Chèvre, N., Schärer, M., 2013. Treatment of micropollutants in municipal wastewater: ozone or powdered activated carbon? Sci. Total Environ. 461, 480–498.

Merel, S., Lege, S., Yanez Heras, J.E., Zwiener, C., 2017. Assessment of N-oxide formation during wastewater ozonation. Environ. Sci. Technol. 51, 410–417.

Morin-Crini, N., Lichtfouse, E., Fourmentin, M., Ribeiro, A.R.L., Noutsopoulos, C., Mapelli, F., Fenyvesi, É., Vieira, M.G.A., Picos-Corrales, L.A., Moreno-Piraján, J.C., Giraldo, L.G., Sohajda, T., Huq, M.M., Soltan, J., Torri, G., Magureanu, M., Bradu, C., Crini, G.C., 2022. Removal of emerging contaminants from wastewater using advanced treatments. A review. Environ. Chem. Lett. 20, 1333–1375. https://doi.org/10.1007/s10311-021-01379-5.

Müller, A., Schulz, W., Ruck, W.K., Weber, W.H., 2011. A new approach to data evaluation in the non-target screening of organic trace substances in water analysis. Chemosphere 85, 1211–1219.

Mvula, E., Naumov, S., von Sonntag, C., 2009. Ozonolysis of lignin models in aqueous solution: anisole, 1, 2-dimethoxybenzene, 1, 4-dimethoxybenzene, and 1, 3, 5-trimethoxybenzene. Environ. Sci. Technol. 43, 6275–6282.

Mvula, E., Von Sonntag, C., 2003. Ozonolysis of phenols in aqueous solution. Org. Biomol. Chem. 1, 1749–1756.

Negreira, N., Regueiro, J., de Alda, M.L., Barceló, D., 2015. Degradation of the anticancer drug erlotinib during water chlorination: non-targeted approach for the identification of transformation products. Water Res. 85, 103–113.

Ramseier, M.K., Gunten, U.V., 2009. Mechanisms of phenol ozonation—kinetics of formation of primary and secondary reaction products. Ozone Sci. Eng. 31, 201–215.

Snyder, S.A., 2008. Occurrence, treatment, and toxicological relevance of EDCs and pharmaceuticals in water. Ozone Sci. Eng. 30, 65–69.

Sui, Q., Huang, J., Deng, S., Yu, G., Fan, Q., 2010. Occurrence and removal of pharmaceuticals, caffeine and DEET in wastewater treatment plants of Beijing, China. Water Res. 44, 417–426.

Tentscher, P.R., Bourgin, M., von Gunten, U., 2018. Ozonation of Para-substituted phenolic compounds yields p-benzoquinones, other cyclic α, β-unsaturated ketones, and substituted catechols. Environ. Sci. Technol. 52, 4763–4773.

Ternes, T.A., Stüber, J., Herrmann, N., McDowell, D., Ried, A., Kampmann, M., Teiser, B., 2003. Ozonation: a tool for removal of pharmaceuticals, contrast media and musk fragrances from wastewater? Water Res. 37, 1976–1982.

Turhan, K., Uzman, S., 2008. Removal of phenol from water using ozone. Desalination 229, 257–263.

Von Sonntag, C., Von Gunten, U., 2012. Chemistry of Ozone in Water and Wastewater Treatment. IWA Publishing.

Yang, X., Flowers, R.C., Weinberg, H.S., Singer, P.C., 2011. Occurrence and removal of pharmaceuticals and personal care products (PPCPs) in an advanced wastewater reclamation plant. Water Res. 16, 5218–5228. https://doi.org/10.1016/j.watres.2011.07.026.

Zhang, N., Geronimo, I., Paneth, P., Schindelka, J., Schaefer, T., Herrmann, H., Vogt, C., Richnow, H.H., 2016. Analyzing sites of OH radical attack (ring vs. Side chain) in oxidation of substituted benzenes via dual stable isotope analysis (δ13C and δ2H). Sci. Total Environ. 542, 484–494.

Zimmermann, S.G., Schmukat, A., Schulz, M., Benner, J., Gunten, U.V., Ternes, T.A., 2012. Kinetic and mechanistic investigations of the oxidation of tramadol by ferrate and ozone. Environ. Sci. Technol. 46, 876–884.

Zimmermann, S.G., Wittenwiler, M., Hollender, J., Krauss, M., Ort, C., Siegrist, H., von Gunten, U., 2011. Kinetic assessment and modeling of an ozonation step for full-scale municipal wastewater treatment: micropollutant oxidation, by-product formation and disinfection. Water Res. 45, 605–617.

Zucker, I., Mamane, H., Riani, A., Gozlan, I., Avisar, D., 2018. Formation and degradation of N-oxide venlafaxine during ozonation and biological post-treatment. Sci. Total Environ. 619–620, 578–586.