Fate of carbamazepine during water treatment

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SUPPORTING INFORMATION

Sample preparation

Wastewater samples were filtered through Machery-Nagel GF-2 microfibre glass and 1.2 µm cellulose nitrate filters (Sartorius, Goettingen, Germany). Solid phase extraction (SPE) was performed at a neutral pH using an Oasis® HLB reversed-phase sorbent (Waters, Corp., Milford, MA, USA). For preconcentration and clean-up of 200 mL samples, the SPE cartridges were first conditioned with 3 mL of ethyl acetate, 3 mL methanol, equilibrated with 3 mL of tap water and enriched at a flow-rate of 4–5 mL min^{-1}. Each cartridge was then washed with water (3 mL), dried for 30 min under vacuum and eluted with 1 mL acetone, 1 mL of 7/3 ethylacetate/acetone mixture and 1 mL of ethylacetate. The combined eluant was evaporated to dryness with nitrogen. The extracts were then dissolved in either 0.5 mL ethylacetate for analysis by gas chromatography – mass spectrometry (GC-MS) or in 0.5 mL of 2/8 methanol/water for liquid chromatography – mass spectrometry (LC-MS) analysis. For derivatisation, 30 µL MTBSTFA was added to 0.5 mL of the ethylacetate sample and left to react at 60°C for 12 hours.

Instrumental analysis

GC-MS. GC-MS analyses were made using a Varian 3800 GC hyphenated with an Ion trap Saturn 2000 mass spectrometer (GC-IT). 10 µL samples were injected (split-splitless) using a PTV injector at 80 °C for 0.30 min before being increased at 200°C/min to 300°C and held for 5 min. For separation a Zebron ZB-5 HT INFERNO 30 m × 0.25 mm × 0.25 µm (Phenomenex) column was used. The GC temperature programme is as follows: 1 min at 80°C, increased to 225°C at 25°C/min and held for 1 min, increased to 231°C at 1°C/min, then to 280°C at 10°C/min and finally to 320°C at 45°C/min and held for 3 min. The total runtime was 22.59 min. The IT mass analyser was operated in electron ionisation (EI) mode, and the masses scanned between *m/z* 40 to 650. For the further mass fragmentation multiple reaction monitoring (MRM), tandem MS (MS/MS) and multiple MS (MS^n) ion preparation modes in the resonant waveform were used.

Quantitation was achieved using a HP6890 series gas chromatograph with a mass selective detector (GC-MSD). The GC oven was programmed as follows: an initial temperature of 65 °C was held for 2 min, then ramped at 30 °C/min to 180 °C, at 10 °C/min to 210 °C, at 20 °C/min to 240 °C, held for 1 min and then at 30 °C/min to 300 °C (5 min). The total runtime was 18.33 min. A DB-5MS 30 m × 0.25 mm × 0.25 µm (J&W) capillary column was used, with He as the carrier gas (37 cm s^{-1}). One µL samples were injected at 250 °C in splitless mode, and the transfer line was maintained at 280 °C. The MSD was operated in EI mode with selected ion monitoring. The following fragment ions were monitored: 179 for ACIN, *m/z* 252 and 193 for ACON-MTBS and *m/z* 293 and 195 for CBZ-MTBS derivative.

LC-MS. The LC-MS analyses were performed using a Waters Acquity ultra performance liquid chromatograph (Waters Acquity UPLC®, Waters Corp., Milford, MA, USA), coupled to a quadrupole – time-of-flight mass spectrometer (LC-QqTOF). The UPLC system was equipped with a binary solvent delivery system and an autosampler. The injection volume was 2 µL. Separation was achieved using a 5 cm long Waters Acquity UPLC® BEH Shield RP18 1.7 µm column with a 2.1 mm internal diameter. Compounds were analysed under positive ion conditions and were eluted from the column using water (A) and methanol (B) as mobile phases. The elution gradient was linearly increased from 20 % to 100 % B in 8 min, and kept isocratic for 1 min, decreased back to 20 % in 1 min and then finally kept isocratic for 1 min.

The total runtime was 11.00 min. Flow rate was 0.2 mL min^{-1} and the column temperature was 35 ºC. The UPLC system was interfaced to a hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer (QqTOF Premier, Waters, Milford, Massachusetts, USA). The instrument was equipped with an electrospray ionisation interface operating in the positive ion mode (ESI(+)). The capillary voltage was 2.8 kV, while the sampling cone voltage was 40 V. Source and desolvation temperatures were set to 100 and 280 °C, respectively. The nitrogen desolvation gas flow rate was 620 L/h. ESI(+)TOF-MS spectra were acquired over an *m/z* range of 50 - 1000 in two parallel experiments performed at collision energies of 5 and 25 eV. The ESI(+)TOF-MS/MS experiments were performed at different collision energies between 10 to 30 eV. Data were collected in the centroid mode, with a scan accumulation time set at 0.2 s and an interscan delay of 0.025 s. The data station operating software was MassLynx v4.1. The instrument was calibrated over a mass range of 50 – 1000 Da using a sodium formate calibration solution. Reproducible and accurate mass measurements, at a mass resolution of 10000, were obtained using an electrospray dual sprayer with leucine enkephalin ($[M+H]^+$ = 556.2771) as the reference compound. The latter was introduced into the mass spectrometer alternating with the sample *via* a Waters LockSpray device.

Emission spectrum of the UV lamp

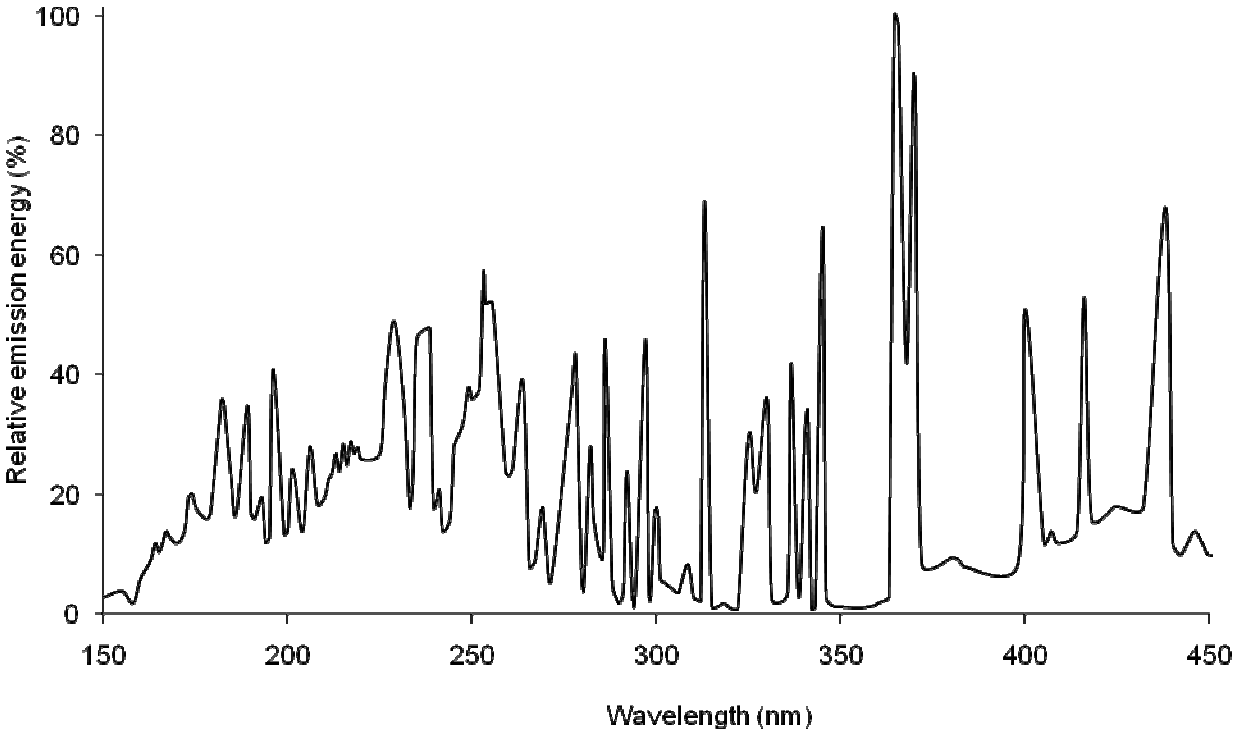

fig S 1: Emission spectrum of a 690 W medium pressure metal-halogen UV lamp (Bau 42, Scan Research A/S, Denmark) emitting polychromatic light down to 185 nm, with an enhanced output compared to standard medium pressure mercury lamps in the 190-250 nm range.

Identification of CARBAMAZEPINE (CBZ)

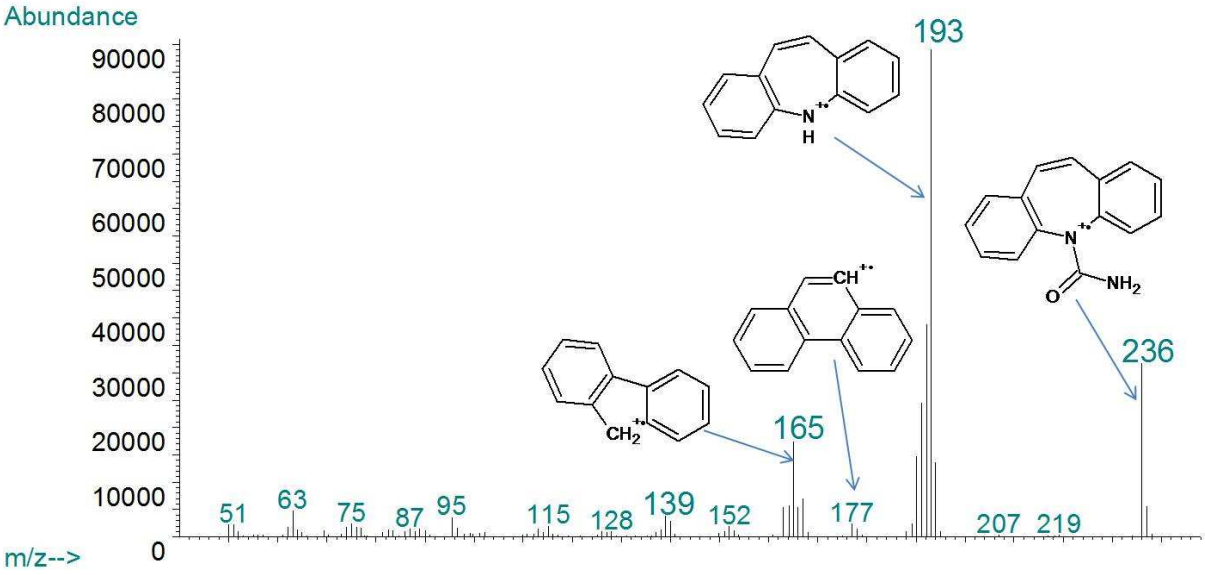

fig S 2: EI-MS spectrum of underivatised CBZ with the proposed ion fragments

In the EI mass spectrum of CBZ (Figure S2) in its underivatised state we observe a molecular ion at *m/z* 236, which is subjected to a cleavage of the NHCO side chain to produce a base peak at *m/z* 193, corresponding to an iminostilbene structural fragment. Further loss of NH forms a phenantrene ring at *m/z* 178 and is followed by the loss of CH to give a 9H-fluoren-9-yl fragment at *m/z* 165. During derivatisation, the carbonyl group of CBZ is transformed into the enol-*tert*-butyl-dimethylsilyl ether, the EI mass spectrum of which shows a molecular ion at *m/z* 350, the typical loss of a *tert*–butyl group at *m/z* 293 and a base peak at *m/z* 193. Correspondingly, the ESI(+)ToF of CBZ shows a protonated molecule at *m/z* 237, a sodium adduct $[M+Na]^+$ at *m/z* 259 and a fragment ion at *m/z* 194, attributed to the protonated iminostilbene fragment. The collision induced fragmentation of *m/z* 237, produces fragment ions at *m/z* 194, *m/z* 179 and *m/z* 165. The accurate mass of the protonated CBZ molecule was 237.1030, equivalent to $C_{15}H_{13}N_2O$ (mass error ± 0.8 ppm).

Identification of IMINOSTILBENE (IMS)

IMS: This is an artefact created by thermal degradation in the GC. The compound was identified based on its EI spectra and has an abundant molecular ion at m/z 193, while the remaining two fragment ions m/z 180 and 165 are just noticeable. Its mass spectrum also matches that of IMS in the National Institute of Standards and Technology (NIST) mass spectral library.

GC-IT extracted mass chromatograms of UV and ClO_2 treated samples

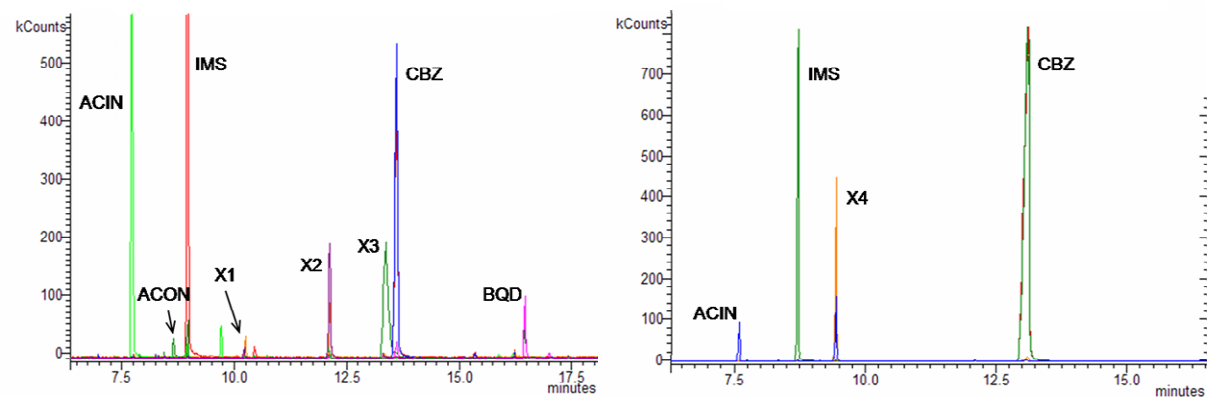

fig S 3: Extracted mass chromatogram showing the residual carbamazepine (underivatised) and its degradation products after 15 minutes of UV treatment (left) and after addition of 13.5 mg L^{-1} ClO_2 (right)

Identification of ACRIDINE (ACIN)

ACIN: Figure S3 is a GC-IT extracted mass chromatogram of a CBZ sample subjected to UV treatment, where CBZ and its UV transformation products are evident. Among the most abundant peaks, is the one eluting at t_R 7.8 min, the EI mass spectrum of this peak shows a base peak corresponding to the molecular ion at *m/z* 179 and fragment ions at *m/z* 151 and 89. The EI-MS/MS produced the same ion fragments. The NIST library matched it to acridine and we confirmed its identity by comparing its retention time and mass fragmentation pattern to that of the authentic compound. In parallel, using MetaboLynx to process the LC-QqTOF data revealed a peak at t_R 5.9 min (Figure S3). Its ESI(+) TOF mass spectrum shows a protonated molecule at $[M+H]^+$ 180, while the ESI(+)-MS/MS fragmentation (collision energy: 30 eV) gives two additional fragments: *m/z* 152 and *m/z* 128. The accurate mass measurement of the protonated molecule at $[M+H]^+$ 180.0814 is only 0.6 ppm from the theoretical mass of the protonated acridine ($C_{13}H_{10}N$: 180.0813), confirming its identity.

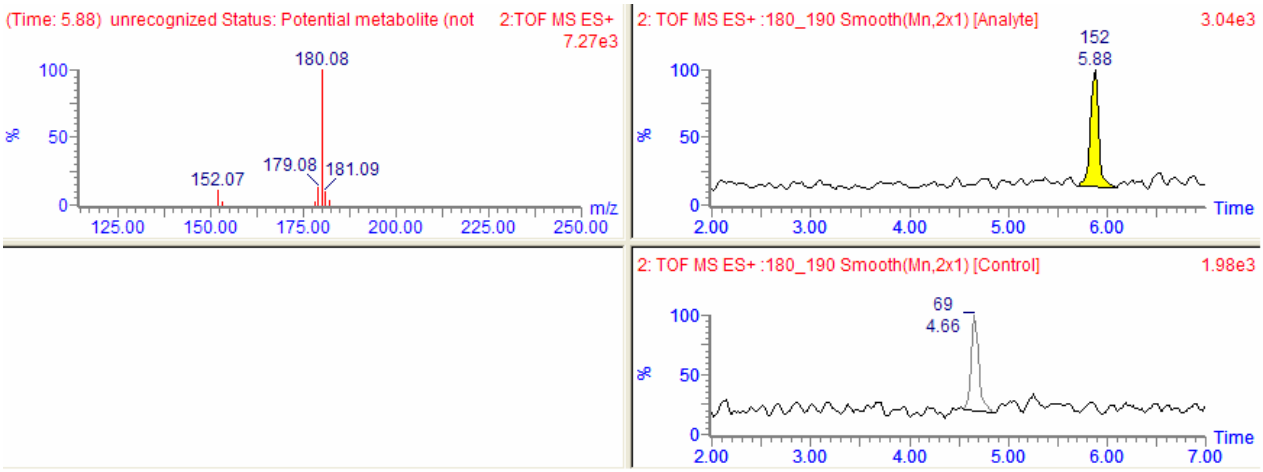

fig S 4: A report segment from MeatboLynx data processing: a chromatogram of a newly-formed compound (top / right), its ESI(+) mass spectrum (top / left) and its absence in the chromatogram of a control sample (bottom / right)

Identification of ACRIDONE (ACON) and X5

Figure S3 shows a second TP at t_R 8.7 min. The base peak is attributable to the molecular ion at *m/z* 195 and fragment ions at *m/z* 167 and 139. The mass increase of 16 Da corresponds to ACIN with one added oxygen atom and is the result of either replacing a hydrogen atom with a hydroxyl group or replacing a double bond with a ketone functional group. A search of the NIST library supports this assumption yielding a good match to 9(10H)-acridinone. This was confirmed using the authentic compound. During derivatisation the ether forms on the 9-hydroxy group of the ACON 'enol' tautomer. The EI mass spectrum of ACON-MTBS shows a molecular ion at *m/z* 309 and, likewise CBZ-MTBS, the loss of a *tert*-butyl fragment to give a base peak at *m/z* 252. In addition, the subsequent losses of methyl groups at *m/z* 236 and 222 were evident. In the LC-MS chromatogram ACON eluted at t_R 5.1 min. Its ESI(+)TOF spectrum shows the protonated molecule at $[M+H]^+$ 196, a sodium adduct $[M+Na]^+$ 218 and a potassium adduct at $[M+K]^+$ 234, while ESI(+)-MS/MS (collision energy: 30 eV) produces an abundant fragments ion at *m/z* 167 and m/z 178 (Figure S4, bottom). The mass of the protonated molecule at $[M+H]^+$ 196.0765 (± 1.5 ppm) confirms it as being acridone: $C_{13}H_{10}NO$.

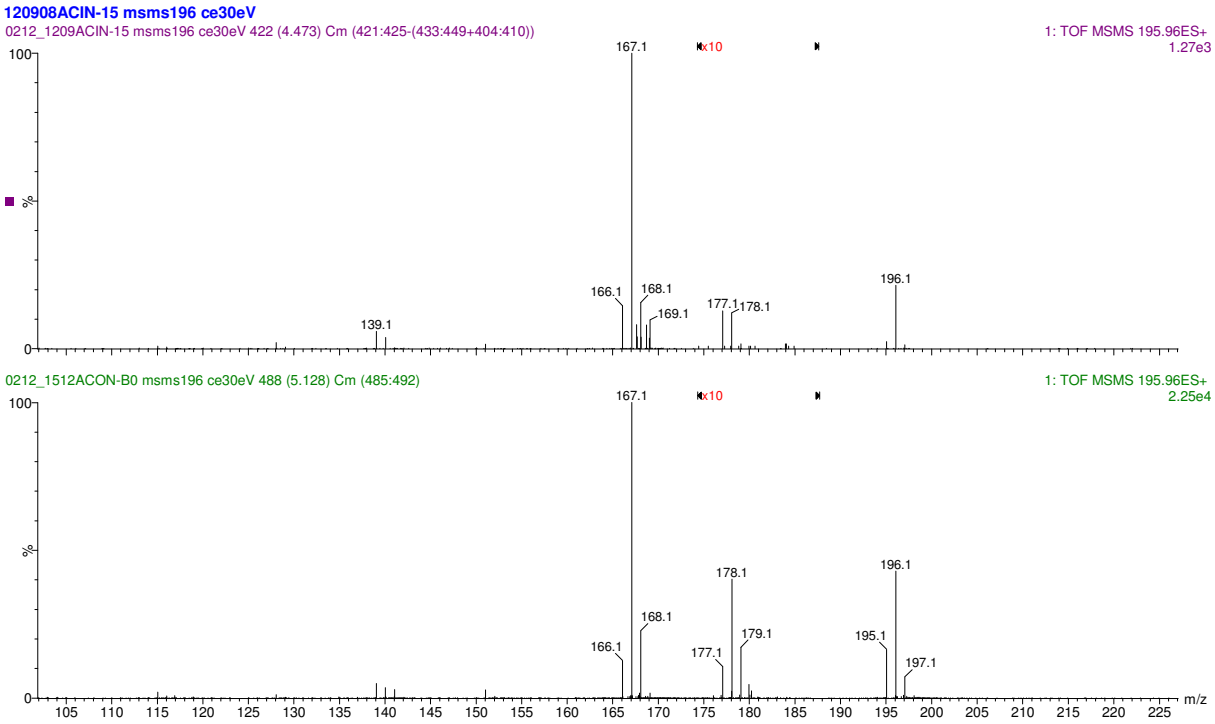

fig S 5: Comparison of ESI(+) TOF-MSMS spectra of hydroxyacridine, X5 (top) and acridone (bottom)

X5: The peak X5, highlighted by MetaboLynx, elutes at t_R 4.5 min, where its ESI(+)TOF spectrum shows a protonated molecule at $[M+H]^+$ 196. The elemental composition gives the formula $C_{13}H_{10}NO$ (mass error 2.6 ppm). From the identical ESI(+)TOF-MS/MS fragmentation of ACON and X5, we can assign the latter, tentatively, as its structural isomer hydroxy-acridine. The position of the –OH group was not determined. However, taking into account similar mass fragmentation and that oxidation at position 9 is favourable due to the low electron density at this carbon on the acridine ring, we assume that X5 is the 9-hydroxy-derivative of acridine.

Identification of X1

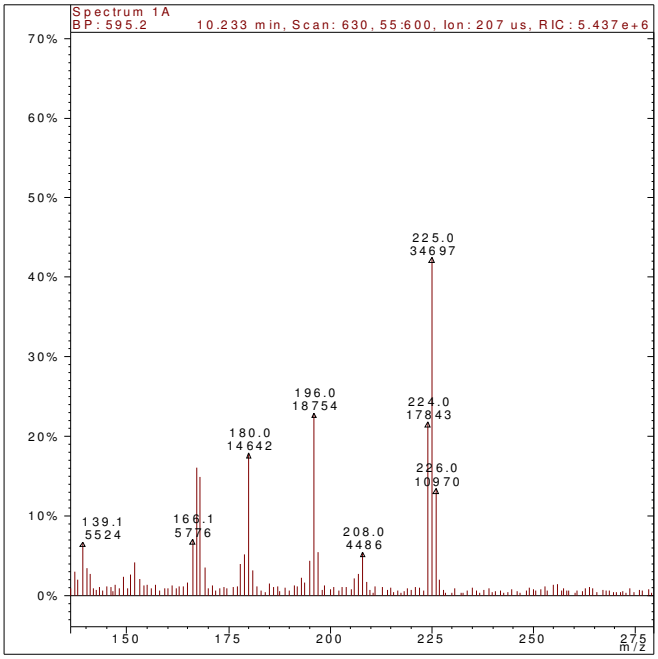

fig S 6: GC-IT-MS spectrum of X1, postulated chemical structure: hydroxy (9H,10H)-acridine-9-carbadehyde

X1: X1 (Figure S3) elutes at 10.2 min. Its EI mass spectrum shows a molecular ion at *m/z* 225 (100 %), *m/z* 208 (12 %) corresponding to the loss of a hydroxyl group and *m/z* 196 (53 %) - a mass loss generated by the cleavage of an aldehyde group from the molecular ion. The lower ion fragments, *m/z* 180 and 167 arise from the cleavage of an oxygen atom and a 9-CH group from the acridine structure. The EI-MS/MS fragmentation also confirms this, allowing us to tentatively assign its chemical structure as hydroxy-(9*H*,10*H*)-acridine-9-aldehyde. Other structural isomers are also possible and further investigation is needed to confirm with certainty its structure.

Identification of X2

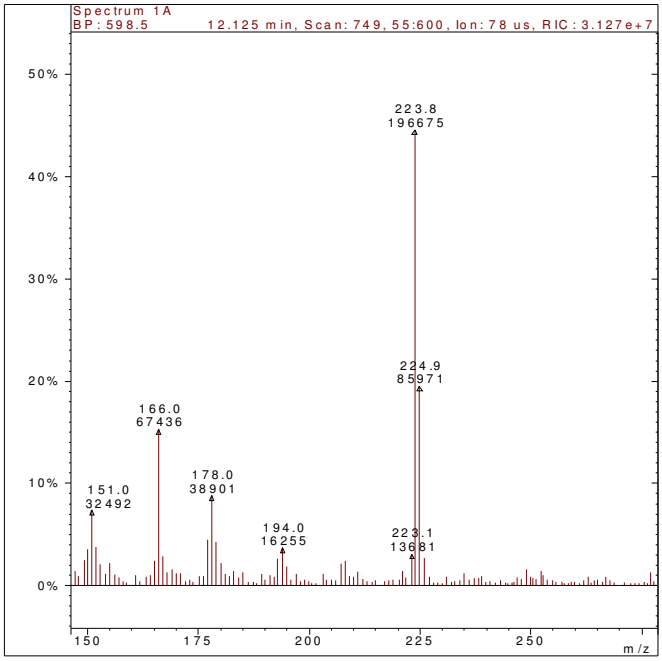

fig S 7: GC-IT-MS spectrum of X2

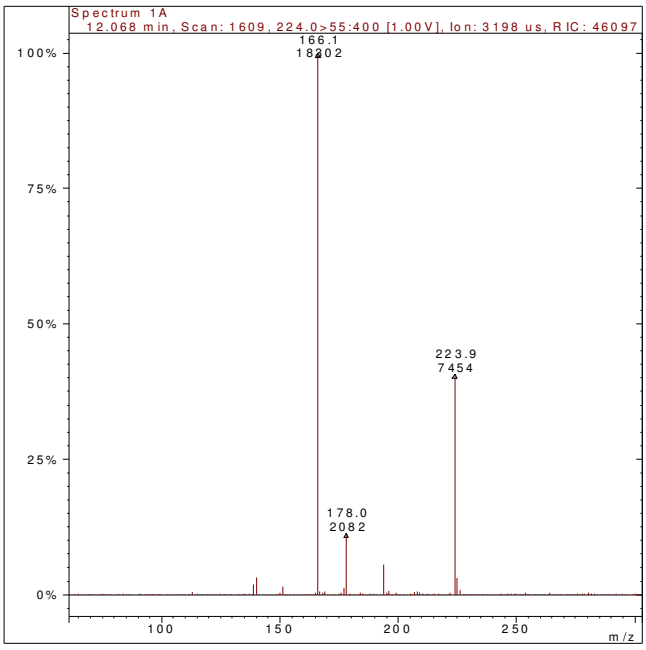

fig S 8: GC-IT-MS/MS spectrum of X2

X2: Its EI-MS and MS/MS mass spectra reveal the fragmentation patterns, suggesting that X2 is a direct CBZ derivative. However, because the highest ion fragment has an even mass, it is likely that that the molecular ion of X2 does not appear in the spectra, which is not uncommon for a “hard” ionisation method, such as EI. A solution would be to use chemical ionisation (CI) to observe the molecular ion. Unfortunately, the inspection of the ESI(+) mass spectra revealed no compound having the related mass fragmentation pattern.

Identification of X3

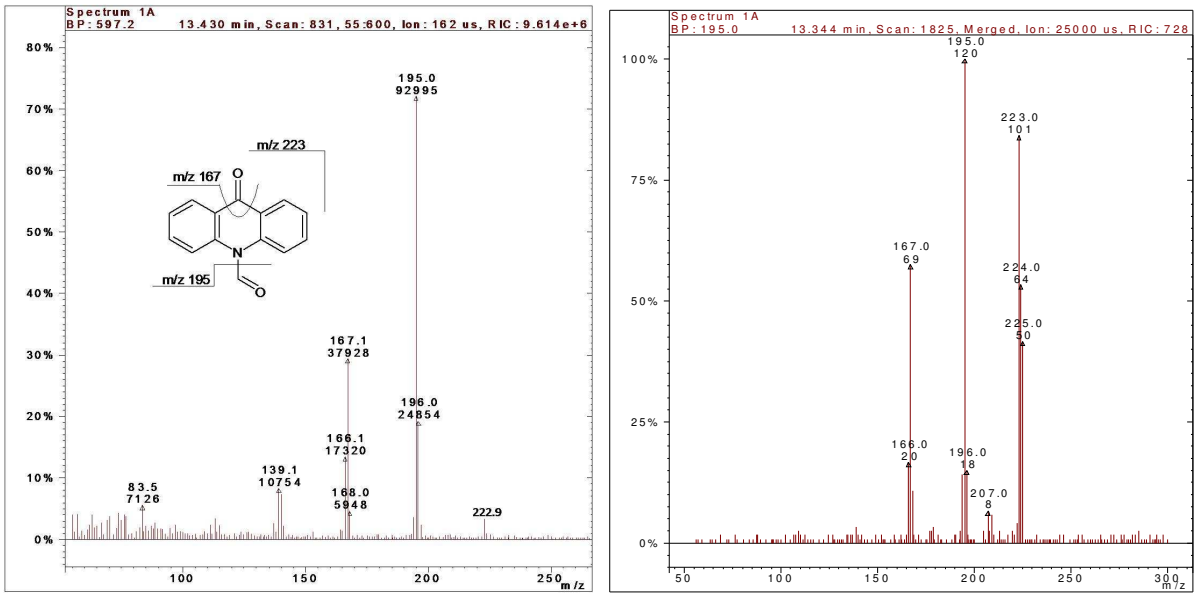

fig S 9: GC-IT-MS (left) and GC-IT-MS^2 (right) spectra of X3, postulated chemical structure: acridone-N-carbaldehyde

X3: X3 elutes at 13.4 min (Figure S3). Its EI mass spectrum shows the molecular ion at *m/z* 223 (4 %), a base peak at *m/z* 195 and fragments at *m/z* 167 (40 %), 139 (11 %) and 84 (7 %). The fragmentation is confirmed with EI-MS/MS using an ion trap mass spectrometer. The fragmentation pattern of X3 (Figure S8, left) resembles closely that of ACON, with the exception of the X3 molecular ion at *m/z* 223. The molecular ion shows a mass increase of 28 Da, i.e. a carbonyl group, which is likely positioned on the heterocyclic nitrogen as the residual from the carbamyl side chain of CBZ. Thus X3 is tentatively assigned as acridone-N-carbaldehyde, which has to our knowledge not yet been identified as a TP of CBZ.

Identification of BQD

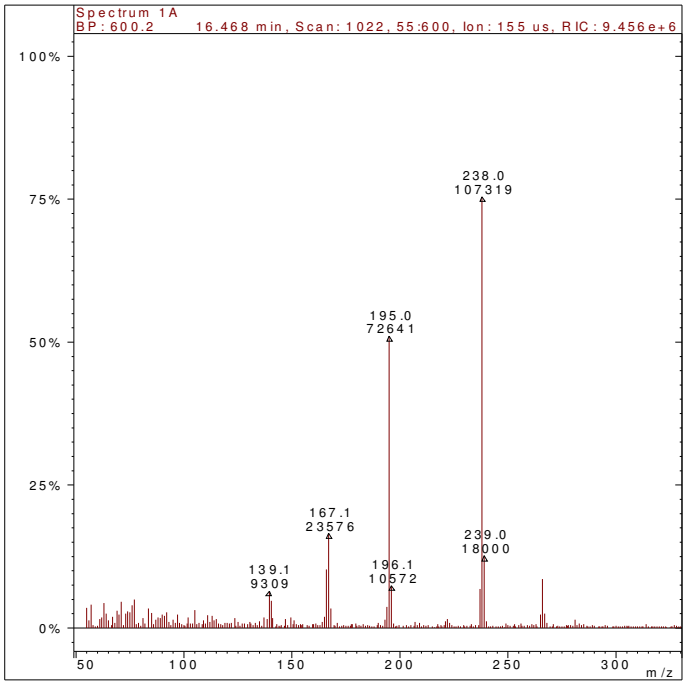

fig S 10: GC-IT-MS spectrum of BQD (1-(2-benzaldehyde)-(1H,3H)-quinazoline-2,4-dione)

BQD: The last UV-breakdown product, sufficiently volatile to be analysed by GC-IT (Figure S3), appears at 16.5 min. The prominent mass fragments are at *m/z* 266 (molecular ion), 238 (base peak), 195, 167 and 140 (Figure S9). The EI-MS^3 acquisition (*m/z* 238 > 195) again resulted in the same ion fragments at *m/z* 167 and *m/z* 140. An identical mass fragmentation was published by McDowell *et al.*[1], who determined three TPs during ozonation of CBZ that contained the quinazoline structural fragment. Hence, based on the matching mass spectra with McDowell *et al.* we assume that this compound is 1-(2-benzaldehyde)-(1*H*,3*H*)-quinazoline-2,4-dione (BQD).

[1] McDowell, D.C.; Huber, M.M.; Wagner, M.; Von Gunten, U.; Ternes, T.A. Ozonation of Carbamazepine in Drinking Water: Identification and Kinetic Study of Major Oxidation Products. *Environ. Sci. Technol.* **2005**, 39, 8014-8022.

Identification of X4

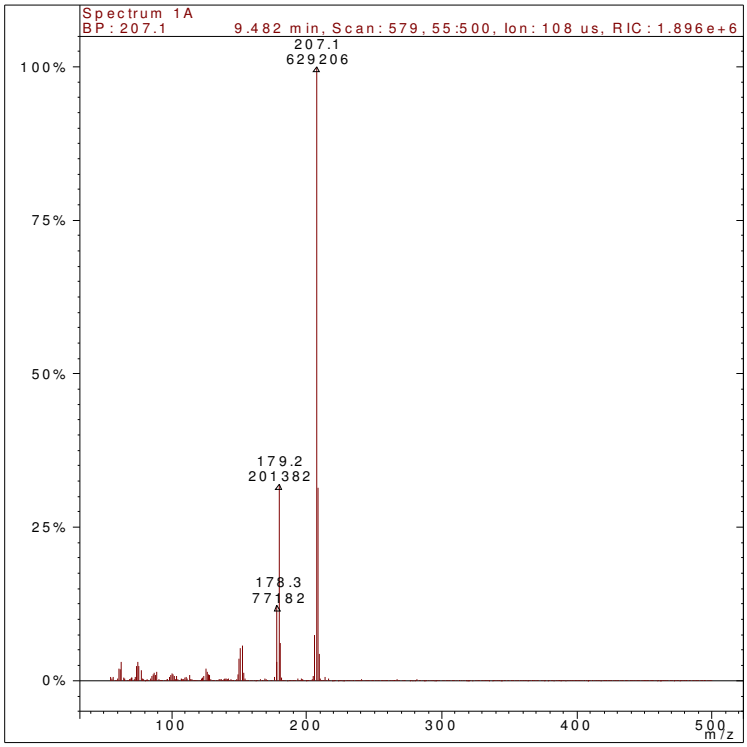

fig S 11: Mass spectrum of the X4, the compound proposed to be acridine-9-carbaldehyde

X4: A comparison between the extracted mass chromatogram (*m/z* 179) of the ClO_2-treated sample (Figure S3) and the control reveals a new peak at t_R 9.5 min. It shows a steady increase with increasing ClO_2 dose. Its mass spectrum has a molecular ion at *m/z* 207 (M^+ = 100%), while the pattern of the remaining ion fragments is identical to ACIN (Figure S10). Although the MS^2 fragmentation of *m/z* 207 does not give any additional structural information, we conclude, that X4 contains the acridine structural fragment, the mass of which is increased by 28 Da, corresponding to a carbonyl bearing compound, i.e. acridine-carbaldehyde.

Formation of CBZ breakdown products during UV treatment

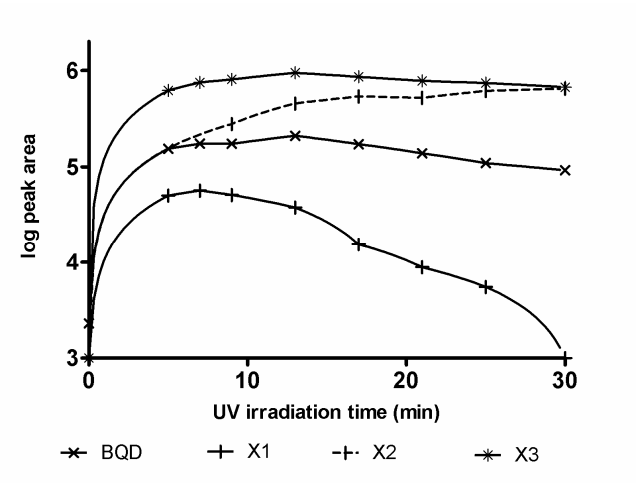

fig S 12: Formation of carbamazepine TPs (BQD, X1, X2, X3) during UV treatment

ClO_2 treatment of CBZ and formation of breakdown products

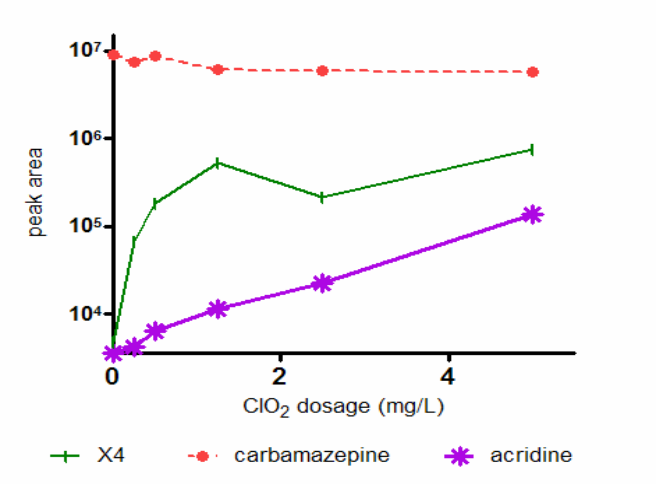

fig S 13: The graph shows the behaviour of CBZ and breakdown products during the ClO_2 treatment of CBZ.

Removal of CBZ, ACIN and ACON in the bench-top bioreactors

Table S1: Removal of CBZ, ACIN and ACON in the bench-top bioreactors

compound	Aerobic			Anoxic		
	% removal	STDEV	n	% removal	STDEV	n
carbamazepine	16	± 6	4	16	± 10	2
acridine	92	± 6	4	90	± 6	2
acridone	40	± 12	3	23	± 1	2