Biotransformation of the UV-Filter Sulisobenzone – challenges for the identification of transformation products

Environmental Science & Technology

Supporting Information

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Materials and methods

Standard substances

4-Hydroxy-2-methoxy-5-(oxo-phenylmethyl)benzenesulfonic acid (BP-4) and (2-Hydroxy-4-methoxyphenyl)-phenylmethanone were purchased from TCI Deutschland GmbH (Eschborn, Germany). The surrogate standard Triclosan-d3 was obtained from Dr. Ehrenstorfer GmbH, Augsburg, Germany and DMSO-d6 from Deutero GmbH, Kastellaun, Germany. Acetone, *n*-heptane and dichloromethane (all picograde) were bought from LGC Promochem, Wesel, Germany. Acetonitrile (gradient grade) was purchased from Th. Geyer, Renningen, Germany. Sulfuric acid (98 %, v/v), formic acid (98-100 %), sodium borohydride, sodium chloride, monosodium phosphate, dipotassium phosphate, magnesium sulfate, diammonium phosphate, glycerine, peptone, yeast extract and ammonium actetate were retrieved from Merck KgaA, Darmstadt, Germany. Methanol (LC-MS grade) was purchased from Sigma-Aldrich GmbH, Munich, Germany. Sodium hydroxide was obtained from Carl Roth GmbH & Co. KG, Karlsruhe, Germany.

Quantification of BP-4 and identification of TPs

The LTQ-Orbitrap ESI-source parameters were set as follows: capillary temperature 370 °C, capillary voltage 280 V, heater temperature 300 °C, sheath gas flow rate 50 AU, aux gas flow rate 15 AU, S-lens RF level 56%. External calibration of the MS was completed every three days at latest at a resolution of 60,000 to ensure accurate mass detection. The MS was operated in negative ion mode using the following parameters: after each full scan in the mass range m/z = 50 - 450, MS^2 scans of the most intense ions (isolation width: 1 m/z) were performed with both collision induced dissociation (CID) and high-energy collision dissociation (HCD) using normalized collision energies of 35 % and 60 % or 80 %, respectively. Subsequently, MS^3 scans

of the CID-produced MS^2-fragments were also generated with CID at 35 %. Full scan as well as direct infusion MS^n experiments were performed with a resolution of 60,000, whereas MS^2 and MS^3 experiments during the chromatographic run were performed at a resolution of 30,000. Moreover, dynamic exclusion (masses are excluded from MS^n experiments for 30 s, if three experiments of these have been performed before) was applied, for enabling fragmentation of co-eluting substances. The automatic gain control (AGC) values were set to: ion trap full MS 30,000, ion trap MS^n 10,000, FT full MS 1,000,000 and FT MS^n 50,000. To obtain the sequence of the TPs, the peak areas of the TPs in the total ion chromatogram were compared over the course of the time.

The LC-Qq-LIT-MS source conditions were set as follows: collision gas – high, curtain gas – 30 psi, ion source gas 1 and 2 – 40 psi, source temperature 400 °C, entrance potential -10 V, ion spray voltage -4.5 kV. The two MRM transitions (quantification and confirmation) and the compound specific parameters (declustering potential DP, collision energy CE, cell exit potential CXP) were adjusted according to Wick et al.[1] and are listed in Table S1.

[1] Wick, A.; Fink, G.; Ternes, T. A. Comparison of electrospray ionization and atmospheric pressure chemical ionization for multi-residue analysis of biocides, UV-filters and benzothiazoles in aqueous matrices and activated sludge by liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* **2010**, *1217*, 2088-2103.

Table S1 MRM-transition and MS-parameters for the analysis of BP-4 and BP-4 TPs with LC-Qq-LIT-MS

compound	transition 1 [m/z]	transition 2 [m/z]	dwell time [ms]	DP [V]	CP [V]	CXP [V]
BP-4	306.8/211.0	306.8/226.9	30	-85	-48/-32	-9/-1
TP 192	190.7/110.9	190.7/162.8	30	-50	-22/-18	-7/-9
TP 204	202.8/111.0	202.8/168.1	30	-50	-26/-26	-7/-7
TP 206	204.7/124.9	204.7/109.9	30	-50	-24/-24	-7/-7
TP 208	206.7/174.8	206.7/146.8	30	-55	-14/-18	-9/-9
TP 218	216.8/110.0	216.8/148.7	30	-50	-36/-14	-7/-7
TP 220	218.8/110.9	218.8/203.8	30	-70	-54/-26	-5/-3
TP 236	234.7/155.0	234.7/153.1	30	-50	-25/-25	-7/-7
TP 252	250.8/206.8	250.8/110.0	30	-30	-14/-38	-5/-7
TP 310	308.8/291.0	308.8/240.8	30	-50	-22/-18	-11/-1

DP: declustering potential, CP: Collision potential, CXP: cell exit potential

Isolation of TPs and determination of mass balance

Calculation of the mass balance was performed using a HPLC-UV detector at a defined wavelength of 246 nm. This was done in accordance with the absorption characteristics of BP-4 as well as of the major TPs formed. Additionally, quantum mechanical ZINDO-single point energy calculations upon AM1 optimized molecular structures using Gaussian 09 have been performed in order to obtain the respective theoretical UV spectra.

Experiments concerning environmental detection

Definite portions of the isolated TP 208 and two TP mixtures were spiked to MilliQ- and surface water as well as WWTP effluent and raw wastewater. Analysis was performed via: method on LC-Qq-LIT as described (as well as on Agilent 1260 infinity, Waldbronn, Germany with AB Sciex QTrap 5500, Darmstadt, Germany with the same parameters). Furthermore, the injection volume during both analyses methods was increased to 900 µg/L to perform large-volume injection (LVI) (change of Agilent 1200 autosampler (1260 infinity autosampler) to Agilent 1100 autosampler with 900 µL sample loop). Freeze-drying (like described above) of water samples spiked with TPs was tested as enrichment method. Since RP cartridges do not retain the TPs, half of the samples tested were cleaned via RP cartridges before being freeze-dried. Afterwards, analyses were performed via LC-Qq-LIT (MRM) with the RP-method and additional LVI.

High-performance thin layer chromatography followed by testing of bacterial toxicity

The following equipment was used for the chromatographic separation: Automatic TLC Sampler ATS4, Automated Multiple Development-System AMD2, Chromatogram Immersion Device III, TLC Visualizer, TLC Scanner 4 and a Bioluminizer (Camag, Muttenz, Switzerland). Chromatography was performed on 10 cm x 20 cm silica gel 60 F254 HPTLC plates (Merck, Darmstadt, Germany). The following analytes were used in solution: benzophenone-3 as known bacteria-toxic control (1 mg/mL in methanol), benzophenone-4 (1 mg/mL in methanol), TP 208 (in DMSO-d6), TPmix1 (in DMSO-d6), TPmix2 (in DMSO-d6), a freeze-dried sample (incubated for 21 d) of a batch experiment a) containing a mixture of the TPs (dissolved in MilliQ-water) and DMSO-d6. The liquids were applied to the TLC-plates as 8 mm bands by using a CAMAG ATS4 application device. An automated development was performed in an

AMD2 chamber using the following gradient of methanol (MeOH), ethylacetate (EE) and n-hexane (H) [vol%]: migration distance 20 mm = 100% MeOH, 0% EE, 0% H dried for 3 min; 23 mm = 70% MeOH, 30% EE, 0% H (3 min); 26 mm = 65% MeOH, 35 % EE, 0% H (3 min); 29 mm = 60% MeOH, 40% EE, 0% H (2 min); 32 mm = 55% MeOH, 45% EE, 0% H (2 min); 35 mm = 30% MeOH, 70% EE, 0% H (2 min); 38 mm = 10% MeOH, 90% EE, 0% H (2 min); 40 mm = 0% MeOH, 100% EE, 0% H (2 min); 42 mm = 0% MeOH, 50% EE, 50% H (2 min); 44 mm = 0% MeOH, 20% EE, 80% H (2min) and 46 mm = 0% MeOH, 0% EE, 100% H (2 min). A picture of the developed TLC-plate was taken under UV-light at 254 nm in order to detect UV-active compounds and control the separation of the compounds on the TLC-plate. UV-active spots were marked, scraped off the TLC-plate, suspended in 1 mL methanol and stored cooled after centrifugation (3 min, 14500 rpm) until LC-HR-MS analysis to confirm the substance responsible for the spot.

Vibrio fischeri bacteria kits (Hach-Lange, Düsseldorf, Germany) were stored frozen and rehydrated before testing by incubation in the below mentioned medium with a rotary shaker (200 rpm) for 48 h. The LB medium used for growth of the bacteria consists of 30 g NaCl, 6.1 g $NaH_2PO_4*H_2O$, 2.75 g $K_2HPO_4*3H_2O$, 0.204 g $MgSO_4*7H_2O$, 0.5 g $(NH_4)_2HPO_4$, 3 mL glycerine, 5 g peptone from casein and 0.5 g yeast extract. The components were dissolved in 1 L MilliQ water and the pH value was adjusted to pH 7 with NaOH. After autoclavation for 20 min at 121°C, the medium was stored in the refrigerator until usage.

The developed TLC-plate with the analytes was automatically immersed into a solution containing the bioluminescent bacteria *V. fischeri* prior to monitoring the bioluminescence using a cooled CCD camera with an exposure time of 60 s.

Results and Discussion

Transformation of BP-4 in laboratory batch systems

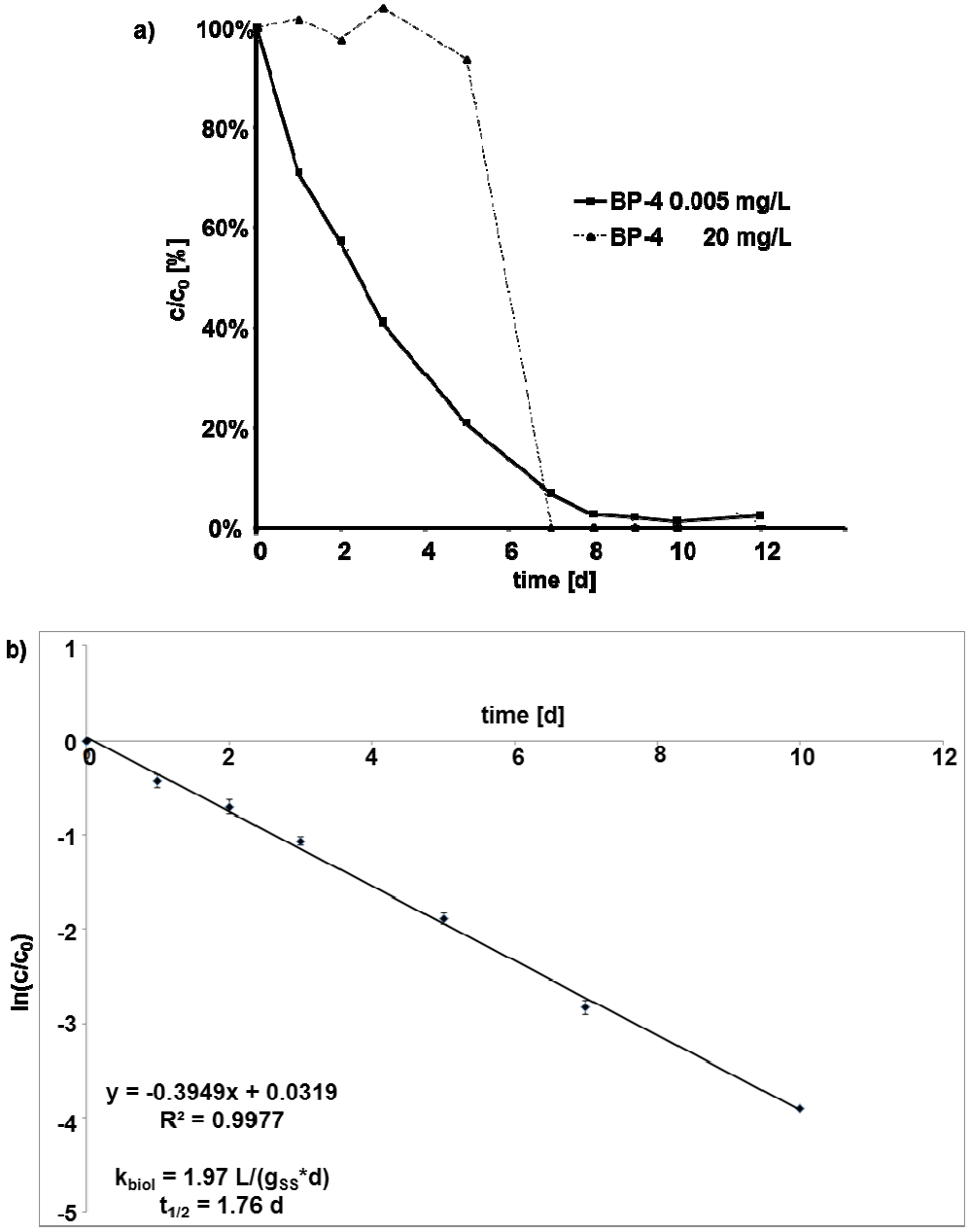

Fig. S1 Degradation of BP-4 in an aerobic batch experiments with diluted activated sludge in the course of the time (k_{biol} = rate constant, SS = content of suspended solids (0.2 g_{SS}/L), $t_{1/2}$ = half-life) a) Comparison of degradation of BP-4 spiked at 5 µg/L and 20 mg/L; b) line: fit of the pseudo first order kinetic of BP-4

Mass balance

Measurements of the UV-absorption using a multichannel diode array detector showed no change in the position of the absorption maximum of the TPs in the course of the time (Fig. S2 b), indicating similar absorption behaviour of all major TPs. Furthermore, we found that 246 nm represents the longest wavelength absorption maximum. The calculated longest wavelength transitions have been compared to the longest wavelength transition of BP-4. Although the latter is found at 287 nm in the experimental spectrum, including a broad shoulder at 322 nm, comparability to the measurement at 246 nm is maintained due to an almost equal absorptivity of both bands (Fig. S2 a).

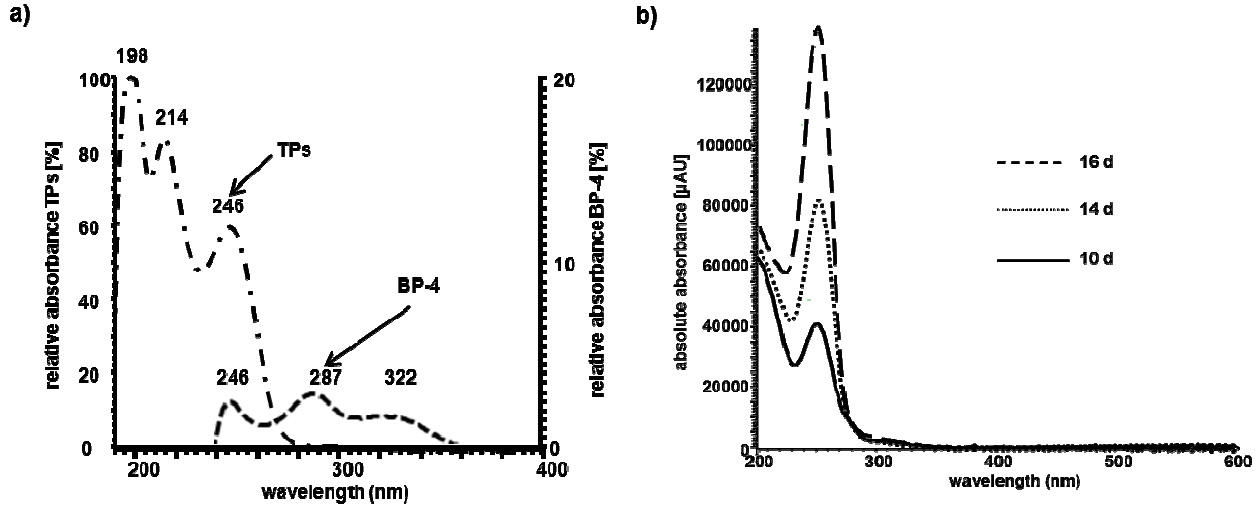

Fig. S2 recorded with HPLC-UV: a) absorption wavelengths of BP-4 and the sum of TPs (recorded in the freeze-dried batch experiment spiked with 500 mg/L of BP-4); b) absorption wavelengths of the TPs at different sampling times of the batch experiment spiked with 500 mg/L

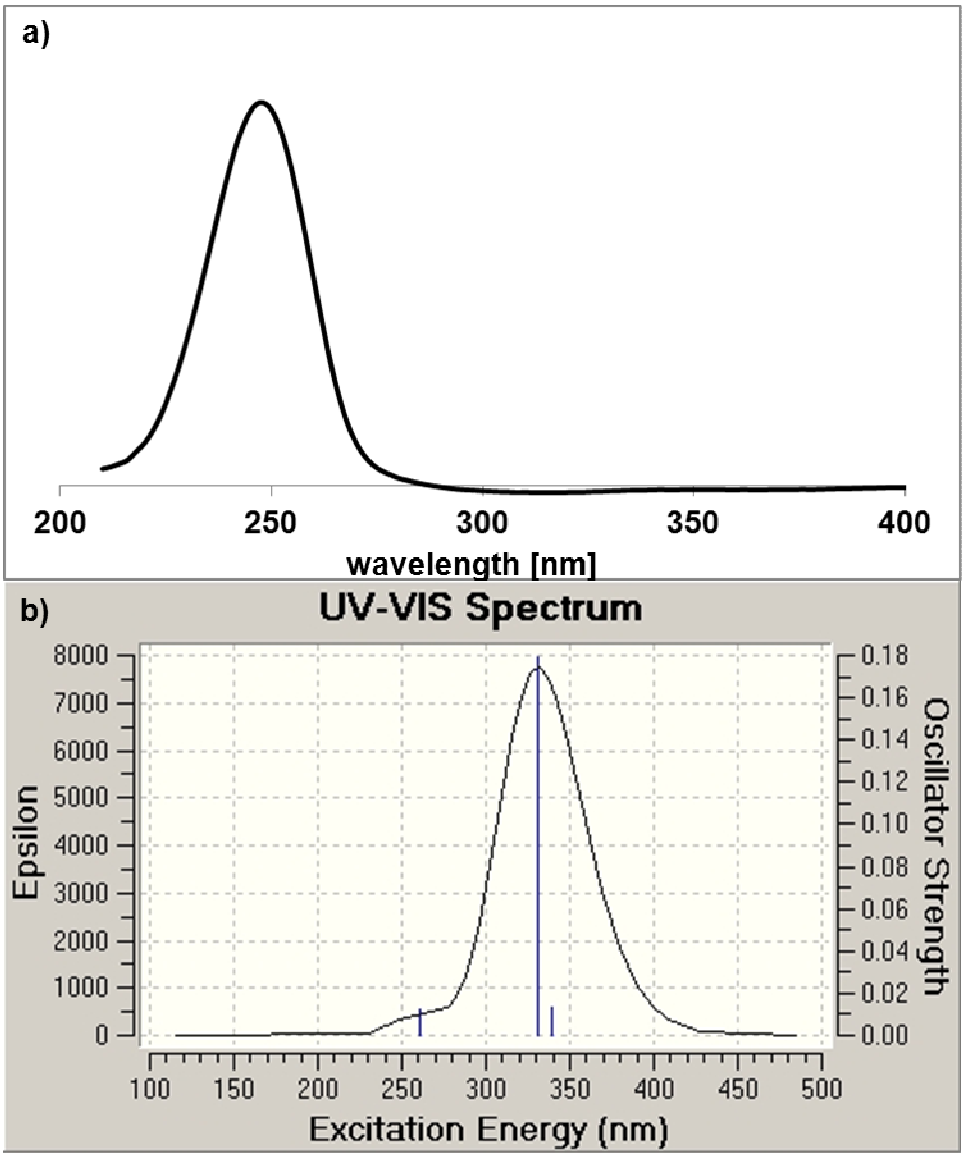

Fig. S3 a) Experimentally obtained absorption spectrum of TP 208 (HPLC-DAD): maximum 246 nm; b) calculated absorption spectrum of TP 208 (AM1, ZIndo(n=6))

In order to check the calculated spectra, comparisons to experimental data have been performed. Fig. S3 a) shows the experimental UV-spectrum of TP 208, whereas the latter (Fig. S3 b) reveals an overestimation of the position of the transition band of about 80 nm. This deviation can be attributed to a systematic error in the calculation of the absolute molecular orbital-energy values

due to the semi-emprirical calculation method. It can be seen in Figures S3 - S5 that this applies to all calculations performed within this study. Nevertheless, it can be seen in Fig. S3 and S4 that the predicted absorption coefficients of the respective longest wavelength transition in BP-4 and the two main TPs (TP 208 and TP 192) resemble each other, indicating that the transition dipoles responsible for the respective absorption band are almost equal. Therefore, a direct quantitative comparison of the HPLC-UV chromatograms is reasonable in this case and the closed mass-balance (calculated from the HPLC-UV chromatograms) at the end of the treatment confirms the results of the mass spectrometry analysis (manuscript, Fig. 1) revealing that TP 208 and TP 192 represent the two main transformation products.

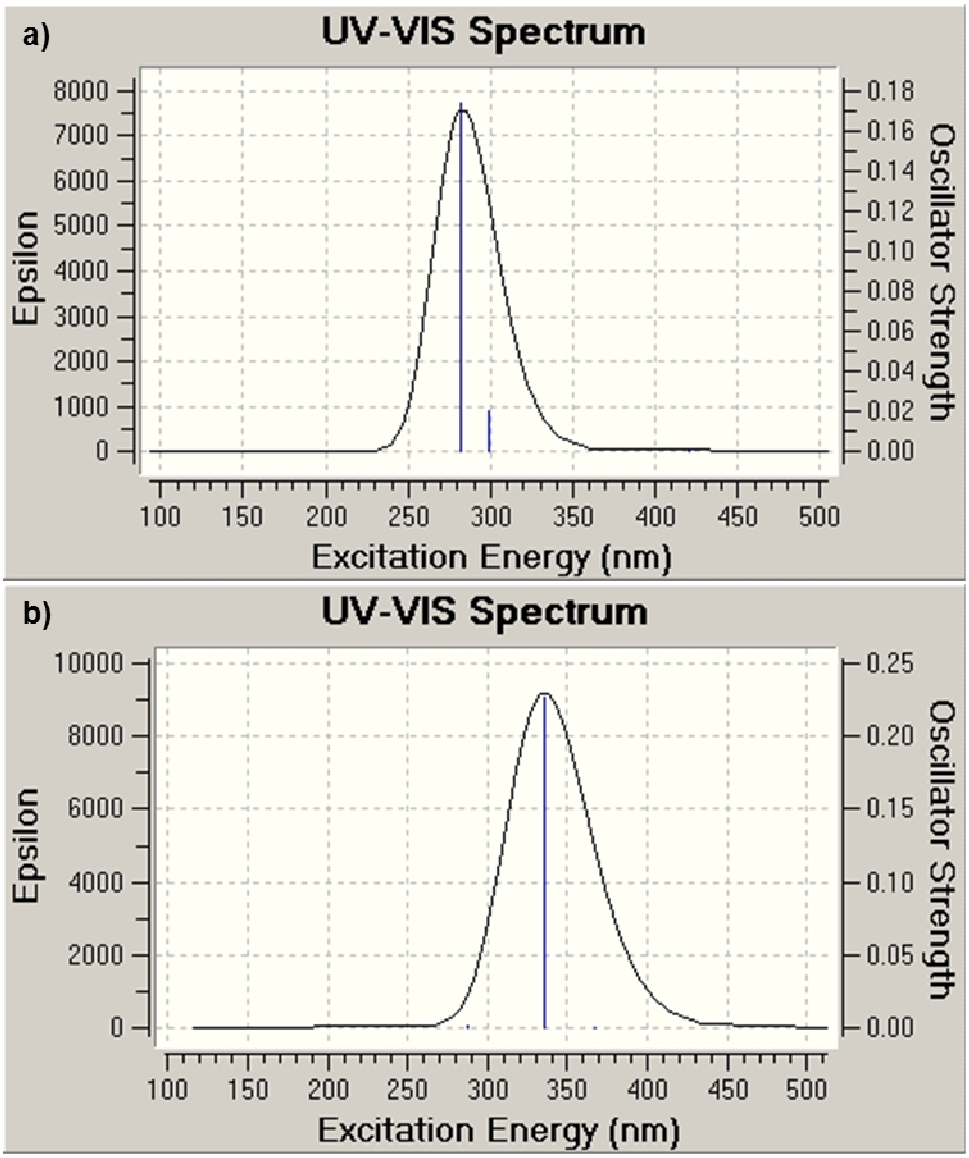

Fig. S4 Calculated absorption spectra of a) BP-4 (AM1, ZIndo(n=6)); b) TP192 (AM1, ZIndo(n=6))

Calculations of the remaining TP structures (Fig. S5) show a similar absorption behaviour of all TPs, which again justifies the aforementioned measurements at a fixed wavelength. However, absorption coefficients of the minor TPs are predicted to be lower. This might result in a slight underestimation of the amount of detected TPs between day 5 and day 15 and therefore provides an additional explanation for the fact that mass-balance is not completely closed during that time.

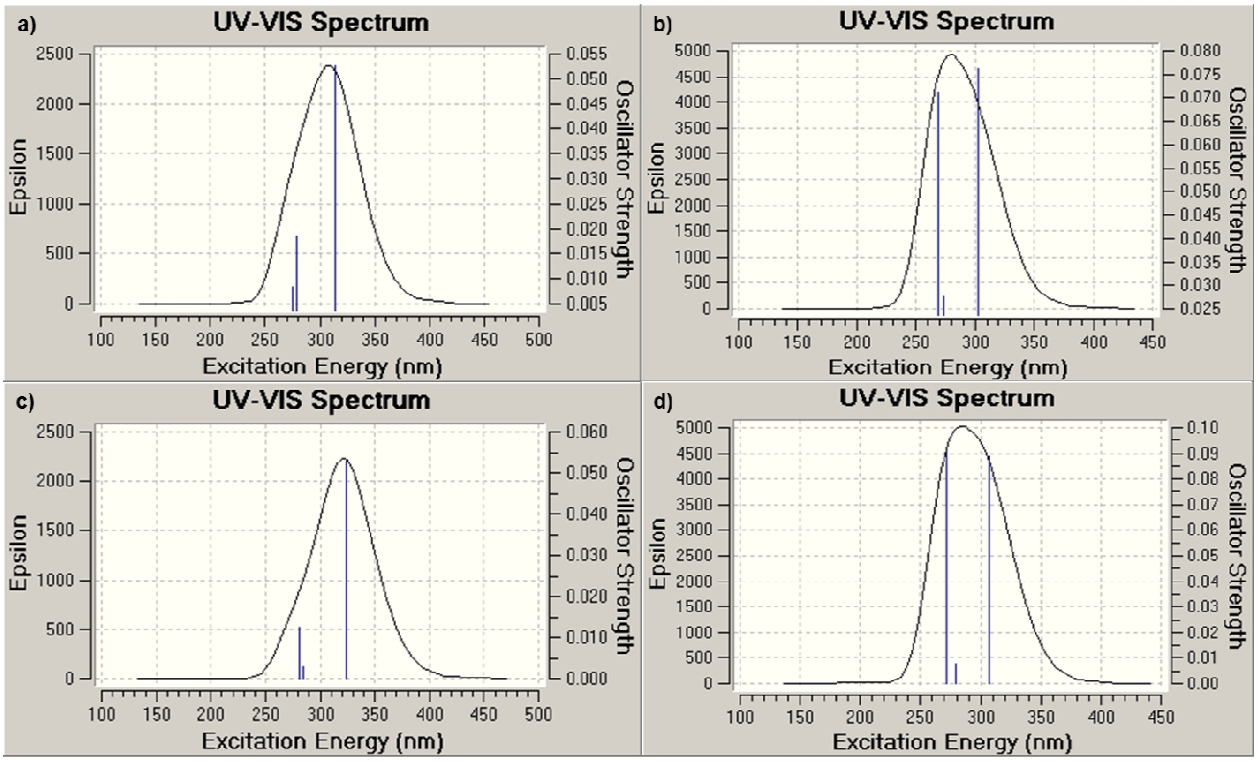

Fig. S5 Calculated absorption spectra of a) TP236 (AM1, ZIndo(n=6)); b) TP220 (AM1, ZIndo(n=6)); c) TP252 (AM1, ZIndo(n=6)); d) TP206 (AM1, ZIndo(n=6))

Identification of transformation products

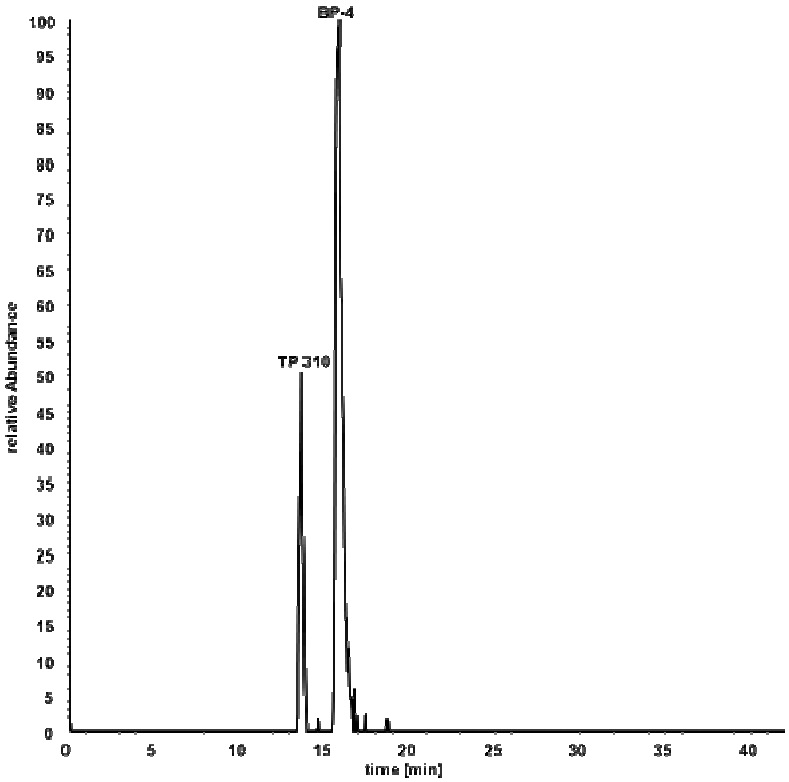

Fig. S6 Comparison of the retention times of TP 310 and BP-4

The MS^3 spectrum of F1/TP 310 showed three fragments, that are helpful for structure elucidation: m/z 263, $C_{13}H_{11}O_4S$ (F2/TP 310) correspondent to a loss of CO; m/z 248, $C_{12}H_8O_4S$ (F3/TP 310) representing the cleavage of $\cdot CH_3$ and CO; m/z 227, $C_{14}H_{11}O_3$ (F4/TP 310) indicating the cleavage of SO_2. F2/TP 310 obviously reveals the phenolic function, which is also present in BP-4, by the very common cleavage of CO out of this group. The cleavage of a methyl radical followed by the release of CO leading to the formation of F3/TP 310 are characteristic for O-bound methyl groups and confirmed that the methoxy moiety remained unaltered. The formation of F4/TP 310 can be explained by the cleavage of SO_2 from the sulfonic acid substituent. Therefore, the MS^3 fragmentation pattern confirmed that the pattern of

substituents at the aromatic system of TP 310 is similar to that of BP-4 supporting the assumption that TP 310 is the benzhydrol-derivative of BP-4. To further confirm not only the substituent pattern, but the backbone structure of TP 310, further MS experiments were performed on the synthesis product (Fig. S7). In the MS^4 spectrum of F4/TP 310, $\cdot CH_3$ is split off to form m/z 212, $C_{13}H_8O_3$ (F5/TP 310), this reaction again representing the methoxy function. Further experiments on F5/TP 310 showed m/z 184, $C_{12}H_8O_2$ (F6/TP 310) in the MS^5. Finally, when performing MS^6 on F6/TP 310, the result is m/z 139, $C_{11}H_7$ (F7/TP 310) by extrusion of CO and $\cdot OH$. Here, a very stable gas phase structure is built out of the cyclisation of the former two aromatic rings. Especially when taking a look at chemical structures of the MS^5/MS^6 fragment ions, the last two steps are the final proof for the two ringed structure of TP 310 in the beginning. Hence, by the multiple fragmentation experiments, the chemical structure of TP 310 is unambiguously demonstrated. A similar intermediate is known from ketoprofen[2,3].

[2] Quintana, J. B.; Weiss, S.; Reemtsma, T. Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor. *Wat. Res.* **2005**, *39*, 2654-2664.

[3] Kosjek, T.; Perko, S.; Heath, E.; Kralj, B.; Zigon, D. Application of complementary mass spectrometric techniques to the identification of ketoprofen phototransformation products. *J. Mass Spectrom.* **2011**, *46*, 391-401.

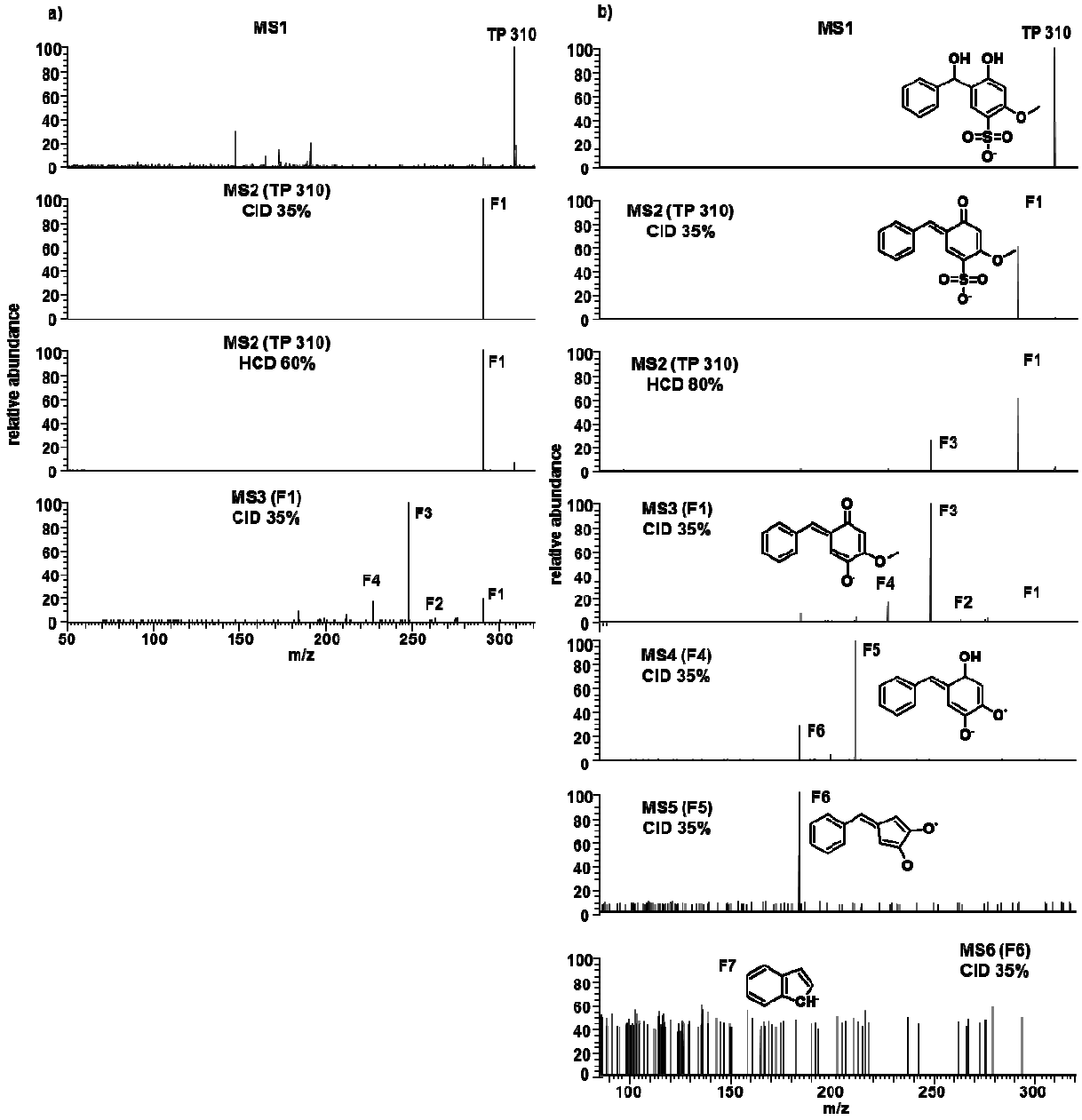

Fig. S7 MS^{1-6} Fragmentation of TP 310 (left = batch experiment, right = synthesis)

Table S2 MS fragmentation details of BP-4 TPs (M is the respective precursor ion) (LC-LTQ-Orbitrap-MS, direct infusion)

compound	MS^n n =	precursor ion m/z / sum formula	product ion m/z / sum formula	proposed fragmentation
TP 192	1	190.97 / $C_5H_3O_6S$		
	2		55.02 / C_3H_3O	$[M - H - CO - CO_2 - SO_2]^-$
			79.96 / SO_3	$[M - H - C_5H_3O_3]^-$
			83.01 / $C_4H_3O_2$	$M - H - CO – SO_3]^-$
			99.01 / $C_4H_3O_3$	$[M - H - CO - SO_2]^-$
			111.01 / $C_5H_3O_3$	$[M - H – SO_3]^-$
			118.98 / $C_3H_3O_3S$	$[M - H - CO - CO_2]^-$
			146.98 / $C_4H_3O_4S$	$[M - H - CO_2]^-$
			162.97 / $C_4H_3O_5S$	$[M- H - CO]^-$
	3	162.97 / $C_4H_3O_5S$	55.02 / C_3H_3O	$[M - CO_2 - SO_2]^-$
			79.96 / SO_3	$[M - C_4H_3O_2]^-$
			99.09 / $C_4H_3O_3$	$[M - SO_2]^-$
			118.98 / $C_3H_3O_3S$	$[M - CO_2]^-$
TP 204	1	202.97 / $C_6H_3O_6S$		
	2		123.01 / $C_6H_3O_3$	$[M - H - SO_3]^-$
			139.00 / $C_6H_3O_4$	$[M - H - SO_2]^-$
TP 206	1	204.98 / $C_6H_5O_6S$		
	2		80.97 / HO_3S	$[M - H - C_6H_4O_3]^-$
			97.03 / $C_5H_5O_2$	$[M - H - CO - SO_3]^-$
			110.00 / $C_5H_2O_3$	$[M - H - CH_3 - SO_2]^{\cdot-}$
			125.02 / $C_6H_5O_3$	$[M - H - SO_3]^-$
			141.02 / $C_6H_5O_4$	$[M - H - SO_2]^-$
			160.99 / $C_5H_5O_4S$	$[M - H - CO_2]^-$
			176.99 / $C_5H_5O_5S$	$[M - H - CO]^-$
TP 208	1	207.00 / $C_6H_7O_6S$		
	2		79.96 / SO_3	$[M - H - C_6H_7O_3]^-$
			80.97 / HO_3S	$[M - H - C_6H_6O_3]^-$
			83.01 / $C_4H_3O_2$	$[M - H - CH_4O - CO - SO_3]^-$
			96.96 / HO_4S	$[M - H - C_6H_6O_2]^-$
			110.00 / $C_5H_2O_3$	$[M - H - CH_3 - H_2O - SO_2]^{\cdot-}$
			111.01 / $C_5H_3O_3$	$[M - H - CH_4O - SO_2]^-$
			125.03 / $C_6H_5O_3$	$[M - H - H_2O - SO_2]^-$
			127.04 / $C_6H_7O_3$	$[M - H - SO_3]^-$
			143.04 / $C_6H_7O_4$	$[M - H - SO_2]^-$
			146.98 / $C_4H_3O_4S$	$[M - H - CH_4O -CO]^-$
			174.97 / $C_5H_3O_5S$	$[M - H - CH_4O]^-$
			188.99 / $C_6H_5O_5S$	$[M - H - H_2O]^-$
	3	111.01 / $C_5H_5O_3$	83.01 / $C_4H_3O_2$	$[M - CH_2O]^-$
		125.03 / $C_6H_5O_3$	82.01 / $C_4H_2O_2$	$[M - CH_3 - CO]^{\cdot-}$

			110.00 / $C_5H_2O_3$	$[M - CH_3]^{\cdot-}$
		143.04 / $C_6H_7O_4$	83.01 / $C_4H_3O_2$	$[M - CH_4O - CO]^-$
			111.01 / $C_5H_3O_3$	$[M - CH_4O]^-$
		174.97 / $C_5H_3O_5S$	79.96 / SO_3	$[M - C_5H_3O_2]^-$
			83.01 / $C_4H_3O_2$	$[M - CO - SO_2]^-$
			95.01 / $C_5H_3O_2$	$[M - SO_3]^-$
			111.01 / $C_5H_3O_3$	$[M - SO_2]^-$
			146.98 / $C_4H_3O_4S$	$[M - CO]^-$
		188.99 / $C_6H_5O_5S$	82.01 / $C_4H_2O_2$	$[M - CH_3 - SO_3]^{\cdot-}$
			97.03 / $C_5H_5O_2$	$[M - CO - SO_2]^-$
			110.00 / $C_5H_2O_3$	$[M - CH_3 - SO_2]^{\cdot-}$
			125.03 / $C_6H_5O_3$	$[M - SO_2]^-$
			173.96 / $C_5H_2O_5S$	$[M - CH_3]^{\cdot-}$
	4	146.98 / $C_4H_3O_4S$	83.01 / $C_4H_3O_2$	$[M - SO_2]^-$
		125.03 / $C_6H_5O_3$	110.00 / $C_5H_2O_3$	$[M - CH_3]^{\cdot-}$
		173.96 / $C_5H_2O_5S$	82.01 / $C_4H_2O_2$	$[M - CO - SO_2]^-$
			110.00 / $C_5H_2O_3$	$[M - SO_2]^-$
TP 218	1	216.98 / $C_7H_5O_6S$		
	2		81.03 / C_5H_5O	$[M - H - CO - CO_2 - SO_2]^-$
			96.96 / HO_4S	$[M - H - C_7H_4O_2]^-$
			110.00 / $C_5H_2O_3$	$[M - H - CH_3 - CO - SO_2]^{\cdot-}$
			125.02 / $C_6H_5O_3$	$[M - H- CO - SO_2]^-$
			138.00 / $C_6H_2O_4$	$[M - H - CH_3 - SO_2]^{\cdot-}$
			153.02 / $C_7H_5O_4$	$[M - H - SO_2]^-$
			172.99 / $C_6H_5O_4S$	$[M - H - CO_2]^-$
			173.96 / $C_5H_2O_5S$	$[M - H - CH_3 - CO]^{\cdot-}$
			188.99 / $C_6H_5O_5S$	$[M - H - CO]^-$
	3	125.02 / $C_6H_5O_3$	82.01 / $C_4H_2O_2$	$[M - CH_3 - CO]^{\cdot-}$
			110.00 /$C_5H_2O_3$	$[M - CH_3]^{\cdot-}$
		153.02 / $C_7H_5O_4$	110.00 / $C_5H_2O_3$	$[M - CH_3 - CO]^{\cdot-}$
			125.02 / $C_6H_5O_3$	$[M - CO]^-$
			138.00 / $C_6H_2O_4$	$[M - CH_3]^{\cdot-}$
TP 220	1	219.00 / $C_7H_7O_6S$		
	2		79.96 / SO_3	$[M - H - C_7H_7O_3]^-$
			96.96 / HSO_4	$[M - H - C_7H_6O_2]^-$
			124.02 / $C_6H_4O_3$	$[M - H - CH_3 - SO_3]^{\cdot-}$
			139.04 / $C_7H_7O_3$	$[M - H - SO_3]^-$
			203.97 / $C_6H_4O_6S$	$[M - H - CH_3]^{\cdot-}$
	3	139.04 / $C_7H_7O_3$	124.02 / $C_6H_4O_3$	$[M - CH_3]^{\cdot-}$
		203.97 / $C_6H_4O_6S$	124.02 / $C_6H_4O_3$	$[M - SO_3]^-$
			175.98 / $C_5H_4O_5S$	$[M - CO]^-$
TP 236	1	234.99 / $C_7H_7O_7S$		
	2		79.96 / SO_3	$[M - H - C_7H_7O_4]^-$
			80.97 / HO_3S	$[M - H - C_7H_6O_4]^-$

			95.01 / $C_5H_3O_2$	$[M - H - CH_4O - CO_2 - SO_2]^-$
			111.01 / $C_5H_3O_3$	$[M - H - CH_4O - CO - SO_2]^-$
			125.02 / $C_6H_5O_3$	$[M - H - CO - H_2O - SO_2]^-$
			153.02 / $C_7H_5O_4$	$[M - H - H_2O - SO_2]^-$
			155.03 / $C_7H_7O_4$	$[M - H - SO_3]^-$
			158.98 / $C_5H_3O_4S$	$[M - H - CH_4O - CO_2]^-$
			174.97 / $C_5H_3O_5S$	$[M - H - CH_2O - CO]^-$
			202.97 / $C_6H_3O_6S$	$[M - H - CH_4O]^-$
			216.98 / $C_7H_5O_6S$	$[M - H - H_2O]^-$
	2	153.02 / $C_7H_5O_4$	110.00 / $C_5H_2O_3$	$[M - CH_3 - CO]^{\cdot-}$
			125.02 / $C_6H_5O_3$	$[M - CO]^-$
			138.00 / $C_6H_2O_4$	$[M - CH_3]^{\cdot-}$
		216.98 / $C_7H_5O_6S$	81.03 / C_5H_5O	$[M - CO - CO_2 - SO_2]^-$
			110.00 / $C_5H_2O_3$	$[M - CH_3 - CO - SO_2]^{\cdot-}$
			125.02 / $C_6H_5O_3$	$[M - CO - SO_2]^-$
			138.00 / $C_6H_2O_4$	$[M - CH_3 - SO_2]^{\cdot-}$
			153.02 / $C_7H_5O_4$	$[M - SO_2]^-$
			188.99 / $C_6H_5O_5S$	$[M - CO]^-$
TP 252	1	250.99 / $C_7H_7O_8S$		
	2		79.96 / SO_3	$[M - H - C_7H_7O_5]^-$
			80.97 / HO_3S	$[M - H - C_7H_6O_5]^-$
			83.01 / $C_4H_3O_2$	$[M - H - CH_4O - CO- CO_2 - SO_2]^-$
			95.01 / $C_5H_3O_2$	$[M - H - CH_2O - CO_2 - SO_3]^-$
			99.05 / $C_5H_7O_2$	$[M - H - CO_2 - CO_2 - SO_2]^-$
			110.00 / $C_5H_2O_3$	$[M - H - CH_3 - CO_2 - H_2O - SO_2]^{\cdot-}$
			111.01 / $C_5H_3O_3$	$[M - H - CH_2O - CO_2 - SO_2]^-$
			125.02 / $C_6H_5O_3$	$[M - H - CO_2 - H_2O - SO_2]^-$
			127.04 / $C_6H_7O_3$	$[M - H - CO_2 - SO_3]^-$
			143.04 / $C_6H_7O_4$	$[M - H - CO_2 - SO_2]^-$
			163.01 / $C_5H_7O_5S$	$[M - H - CO_2 - CO_2]^-$
			169.01 / $C_7H_5O_5$	$[M - H - H_2O - SO_2]^-$
			174.97 / $C_5H_3O_5S$	$[M - H - CH_4O - CO_2]^-$
			176.99 / $C_5H_5O_5S$	$[M - H - CH_2O - CO_2]^-$
			188.99 / $C_6H_5O_5S$	$[M - H - H_2O]^-$
			207.00 / $C_6H_7O_6S$	$[M - H - CO_2 - H_2O]^-$
			218.97 / $C_6H_3O_7S$	$[M - H - CH_4O]^-$
	3	207.00 / $C_6H_7O_6S$	143.04 / $C_6H_7O_4$	$[M - SO_2]^-$
			163.01 / $C_5H_7O_4S$	$[M - CO_2]^-$
			176.99 / $C_5H_5O_5S$	$[M - CH_2O]^-$
		143.04 / $C_6H_7O_4$	111.01 / $C_5H_3O_3$	$[M - CH_4O - SO_2]^-$
		125.02 / $C_6H_5O_3$	82.01 / $C_4H_2O_2$	$[M - CH_3 - CO]^{\cdot-}$
			110.00 / $C_5H_2O_3$	$[M - CH_3]^{\cdot-}$

		169.01 / $C_7H_5O_5$	125.02 / $C_6H_5O_3$	$[M - CO_2]^-$
TP 310	1	309.04 / $C_{14}H_{13}O_6S$		
	2		229.09 / $C_{14}H_{13}O_3$	$[M - H - SO_3]^-$
			248.01 / $C_{12}H_8O_4S$	$[M - H - CH_3 - CO - H_2O]^{\cdot-}$
			291.03 / $C_{14}H_{11}O_5S$	$[M - H - H_2O]^-$
	3	291.03 / $C_{14}H_{11}O_5S$	108.02 / $C_6H_4O_2$	$[M - CH_3 - C_7H_4 - SO_3]^{\cdot-}$
			184.05 / $C_{12}H_8O_2$	$[M - CH_3 - CO - SO_2]^{\cdot-}$
			212.05 / $C_{13}H_8O_3$	$[M - CH_3 - SO_3]^{\cdot-}$
			227.07 / $C_{14}H_{11}O_3$	$[M - SO_2]^-$
			248.01 / $C_{12}H_8O_4S$	$[M - CH_3 - CO]^{\cdot-}$
			263.04 / $C_{13}H_{11}O_4S$	$[M - CO]^-$
			276.01 / $C_{13}H_8O_5S$	$[M - CH_3]^{\cdot-}$
	4	227.07 / $C_{14}H_{11}O_3$	184.05 / $C_{12}H_8O_2$	$[M - CH_3 - CO]^{\cdot-}$
			212.05 / $C_{13}H_8O_3$	$[M - CH_3]^{\cdot-}$
		248.01 / $C_{12}H_8O_4S$	79.96 / SO_3	$[M - C_{12}H_8O]^-$
			184.05 / $C_{12}H_8O_2$	$[M - SO_2]^-$
			139.06 / $C_{11}H_7$	$[M - CO - OH]^{\cdot-}$
	5	212.05 / $C_{13}H_8O_3$	184.05 / $C_{12}H_8O_2$	$[M - CO]^-$
		184.05 / $C_{12}H_8O_2$	139.06 / $C_{11}H_7$	$[M - CO - OH]^{\cdot-}$
	6	184.05 / $C_{12}H_8O_2$	139.06 / $C_{11}H_7$	$[M - CO - OH]^{\cdot-}$

NMR-spectra

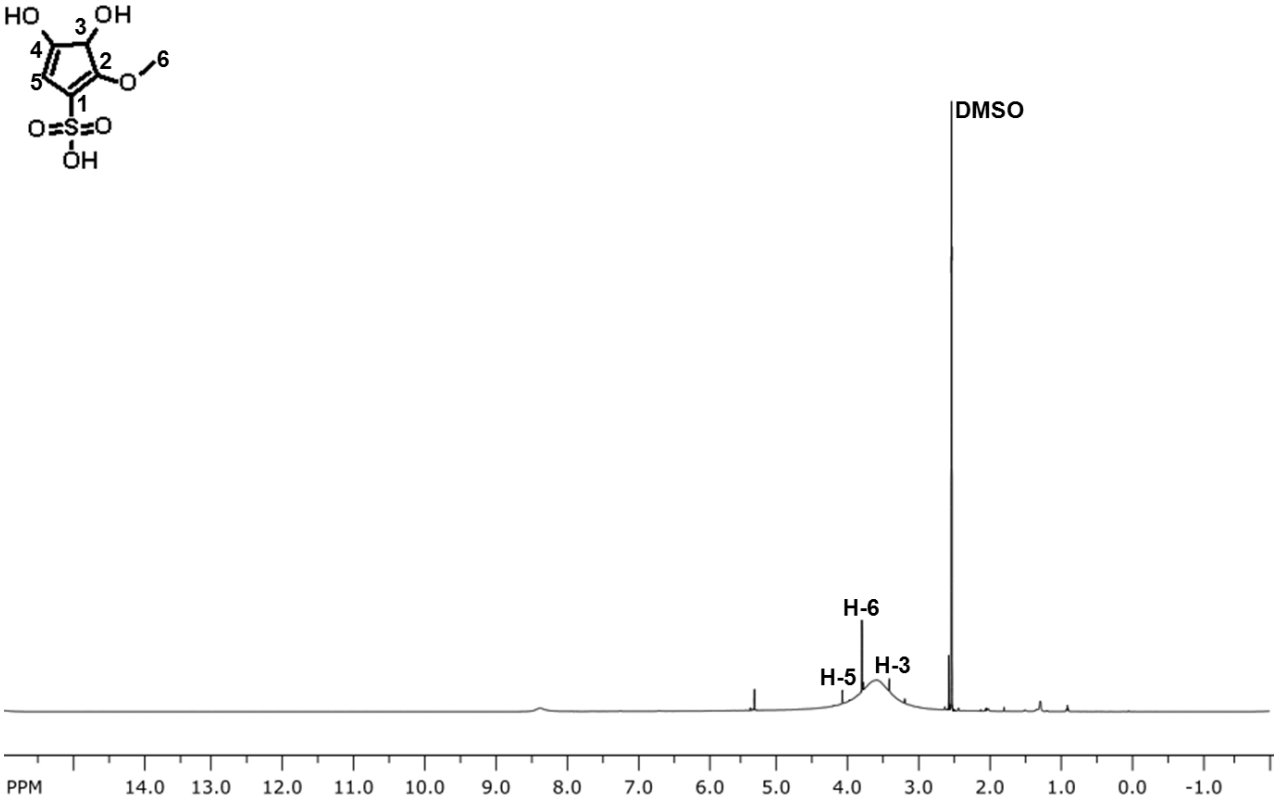

Fig. S8 ^{1}H-NMR spectrum of TP 208 (recorded in DMSO-d6 at 700 MHz and 298.3 K).

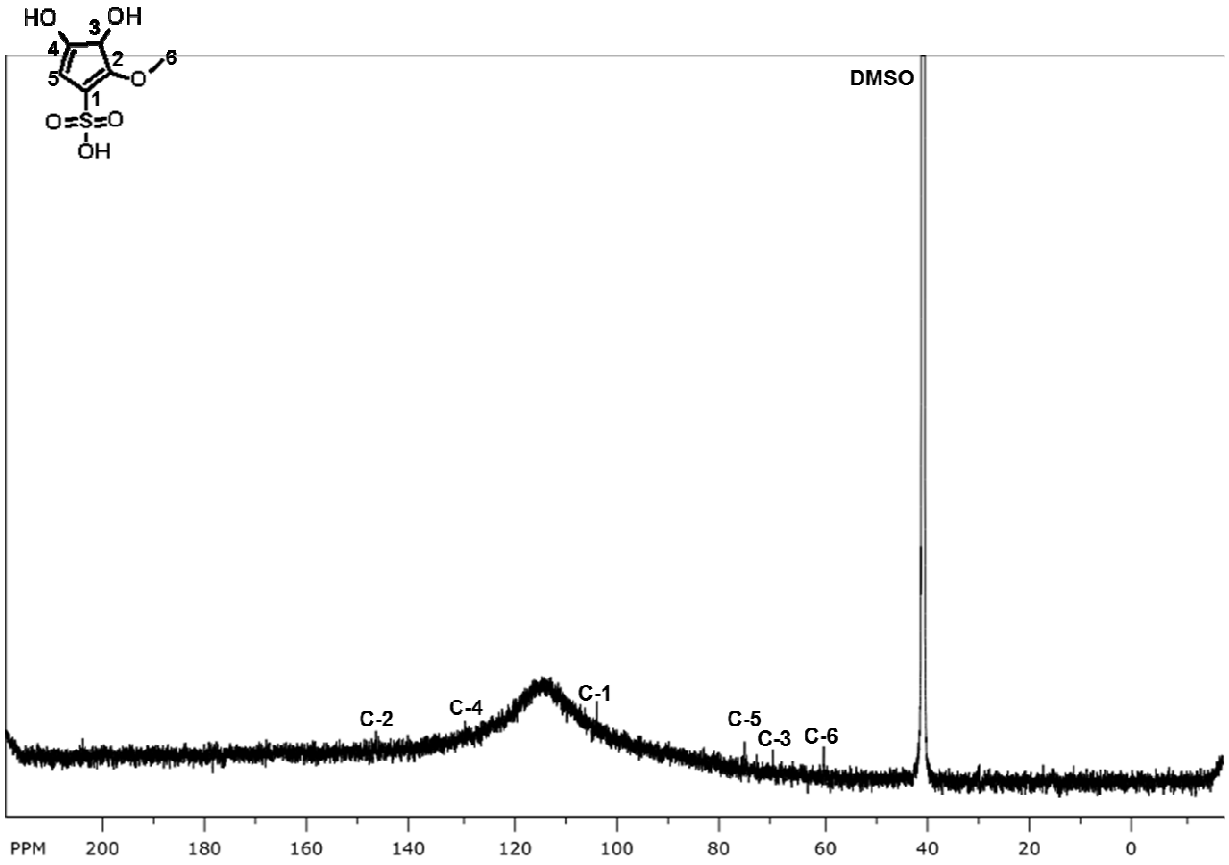

Fig. S9 ^{13}C-NMR spectrum of TP 208 (recorded in DMSO-d6 at 176 MHz and 298.3 K).

Transformation pathway

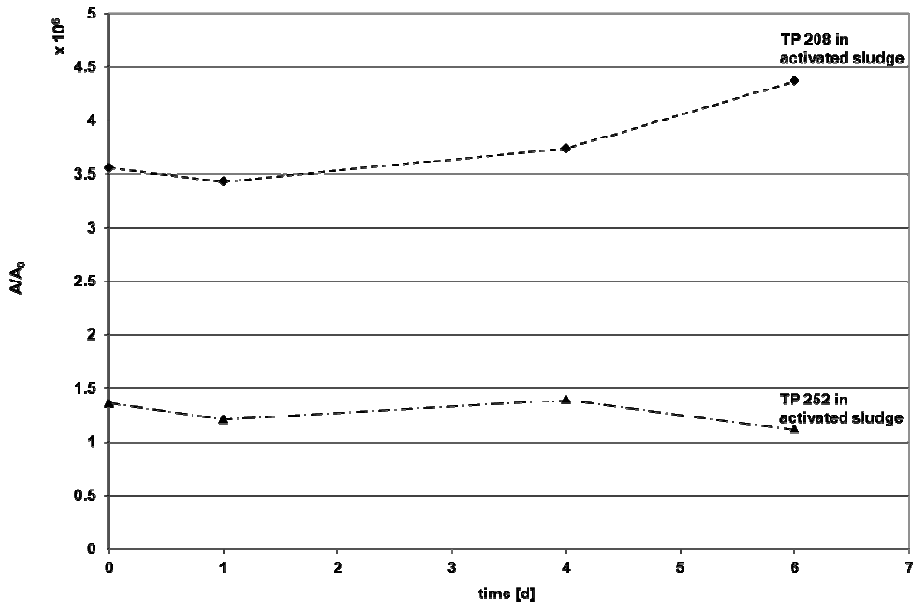

Fig. S10 Biodegradation batch experiments with TP 208 and TP 252

Environmental relevance of TPs

During the SPE experiments (Table S3), it was revealed that the matrix content in the sample led to TP recovery decrease below 1 % (Table S4). Therefore, detection of the TPs in the environment via SPE was impossible. In the samples enriched via freeze-drying, TPs could not be detected at all. Matrix effects and the salt content in freeze-dried samples are supposedly too high and suppress the analyte signals.

Table S3 SPE experiments on BP-4 TPs

cartridge	retardation	elution
		-
Waters Oasis HLB 200 mg / 6 mL	no	- -
J.T. Baker Bakerbond C18 200 mg / 3 mL	no	- -
Biotage Isolute ENV+ 200 mg/ 3 mL	no	-
Waters Oasis WAX 150 mg / 6 mL	partly	acidic: partly, not reproducable basic: not reproducable
Macherey-Nagel Chromabond HR-XAW 200 mg / 3 mL	partly	acidic: partly, not reproducable basic: not reproducable
Waters Oasis MAX 200 mg / 3 mL	yes	acidic: TPs partly destroyed basic: not reproducable
Phenomenex Strata SAX 200 mg / 3 mL	yes	acidic: TPs partly destroyed basic: not reproducable
Macherey-Nagel Chromabond HR-XA 200 mg/ 3 mL	yes	acidic: TPs partly destroyed basic: not reproducable
Supelco Supelclean ENVICarb 500 mg / 6 mL	yes	50 mM ammonium acetate in Dichloromethane:methanol 80:20 v/v
Supelco ENVICarb plus 400 mg / 1 mL reversible	yes	50 mM ammonium acetate in Dichloromethane:methanol 80:20 v/v

Table S4 Recovery experiments of spiked BP-4 TPs with Supleco Supelclean ENVI-Carb cartridges

	A/A_{comp} MilliQ H_2O	A/A_{comp} Rhine water	A/A_{comp} WWTP effluent	A/A_{comp} WWTP influent
TP 192	51 %	2 %	1 %	3 %
TP 204	16 %	< 1 %	< 1 %	< 1 %
TP 206	44 %	2 %	2%	2 %
TP 208	36 %	< 1 %	< 1 %	< 1 %
TP 218	108 %	13 %	6 %	2 %
TP 220	21 %	< 1 %	< 1 %	< 1 %
TP 236	8 %	1 %	< 1 %	< 1 %
TP 252	33 %	< 1 %	< 1 %	< 1 %

A/A_{comp}: MS area of TP related to MS area of a not enriched spiked comparison solution of the BP-4 TPs.