High-resolution MS and MSⁿ investigation of UV oxidation products of phenazone-type pharmaceuticals and metabolites

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Abstract

The occurrence of phenazone-type analgesics, such as aminopyrine, metamizole, phenazone and propyphenazone, has been reported in the effluent of wastewater treatment plants in $\mu\text{g/L}$ concentrations. The presence of the main metabolites of aminopyrine and metamizole – acetamido antipyrine and formyl aminoantipyrine – has even been detected in sub $\mu\text{g/L}$ concentrations in surface water and water bodies used to produce drinking water. This points at their high persistence and the need for adequate removal strategies.

The degradation of phenazone, propyphenazone, acetamido antipyrine and formyl aminoantipyrine by UV radiation was investigated under laboratory conditions. An elucidation approach based on high-resolution mass spectrometry resulted in the identification of 11 degradation products. A mechanism of ring opening via the oxidation of the N-N bond of the pyrazolone ring was observed as well as the more typical oxidation of carbon-carbon double bonds. Aside from the degradation products, the capacity of formyl aminoantipyrine to produce trimers and dimers was demonstrated. The dimers were shown to be persistent despite continuous UV radiation.

The toxicity of the degradation products was assessed by quantitative structure-activity relationships. It was shown that, when the carbon-carbon double bond is partially oxidized to an epoxy, the toxicity towards fish and daphnids is increased with respect to the parent compound.

Keywords: phenazone-type pharmaceuticals, photolysis, pyrazolone, QSAR, transformation products.

1. Introduction

Understanding the processes behind the transformation and transfer of pharmaceutical contaminants in the environment is receiving a lot of attention for numerous reasons. First, a wide knowledge regarding the occurrence and fate of pharmaceutical compounds in the environment has been gathered and published in the past decade (the so-called emerging contaminants are not so emerging anymore). A number of excellent reviews are available that describe the occurrence of these micropollutants in terms of compound type, for instance hypertensive compounds [1], matrix type [2], geographical position [3] and effects on the environment [4].

Secondly, processing techniques have evolved to allow the treatment of wastewater effluents - a major source of contamination of the aquatic environment - by advanced techniques or tertiary techniques, such as ozonation (O_3), UV radiation, ozone combined with hydrogen peroxide (O_3/H_2O_2) or UV (O_3/UV) or both ($O_3/H_2O_2/UV$), Fenton treatment (Fe^{2+}/H_2O_2), photo- and electro-Fenton, etc. These techniques are efficient in removing parent pharmaceuticals but are also responsible for the production of transformation products (TPs) derived from the parent molecules [5-9]. These TPs can be less harmful, as harmful or even more harmful than the parent compound they originate from [10].

This additional dimension of complexity brought to a field that was already complex – several thousands of different pharmaceutical molecules are sold in Europe – has been tackled by the tremendous improvement of analytical hardware and methods. The use of liquid chromatography (LC) in combination with high-resolution mass spectrometry (MS), such as time-of-flight (TOF) or Orbitrap MS, is becoming more and more important in environmental analysis [11]. It allows screening complicated matrices for low amounts of compounds as well as identifying unknown compounds such as degradation and transformation products

[6,12,13]. The use of GC/MS has also been reported [14], but seems less advantageous in this case since mostly polar compounds are expected from oxidation.

Pyrazolone compounds are pharmaceuticals that are used for their analgesic properties. Phenazone - which was the most widely used pharmaceutical in the world until aspirin was discovered - propyphenazone, aminopyrine and metamizole (or dipyron) are examples of active compounds. The latter is extensively used as over-the-counter medicine in Brazil and Russia. Dipyron and aminopyrine generate formyl aminoantipyrine (FAA) and acetylaminoantipyrine (AAA) as metabolites at the end of their degradation pathway [15].

Environmental contamination by pyrazolone compounds has been widely reported [11,16-23], since phenazone and propyphenazone do not adsorb onto sludge [24]. In terms of quantities, the main transformation products acetamidoantipyrine (AAA) and formyl aminoantipyrine (FAA) are typically reported in the µg/L range in wastewater treatment effluents [25].

The current study aimed at understanding the UV degradation of four pyrazolone compounds: phenazone, propyphenazone, AAA and FAA (structures in Table 1) and identifying the formed transformation products by applying high-resolution MS. Focus in this study was on UV degradation, since micropollutants are susceptible to this type of degradation in environmental water streams through solar radiation, while it is also utilized as a tertiary treatment step in wastewater treatment plants. The environmental relevance of the formed TPs was assessed by their ecotoxicity.

Ecotoxicity can be predicted by Quantitative Structure-Activity Relationship (QSAR) models, that predict ecotoxicity according to a given molecular structure. These models have been used by Miao and coworkers to assess the ecotoxic character of the degradation products of aminopyrine after ozonation [6]. The obtained results indicated an increased toxicity for many degradation products, although the overall toxicity of the samples, experimentally tested by bioluminescence inhibition, decreased. Contrarily, Gomez and coworkers [9] demonstrated an increased toxicity for the entire sample after the photolysis of pyrazolones

(methylaminoantipyrine, AAA and FAA) and reported N-phenylacetamide as the most abundant transformation product.

All transformation products encountered in this study were assessed for toxicity by QSAR modeling to understand what the transformation from parent to degradation compound implies in terms of toxicity. The relevance of this information is high as pyrazolones are highly mobile in the environment and are definitely “drinking water relevant” [26].

2. Materials and methods

2.1 Chemicals and instrumentation

Phenazone and AAA were obtained from Sigma-Aldrich (Steinheim, Germany). Propyphenazone and FAA were purchased from Mikromol (Luckenwalde, Germany). Milli-Q water was prepared in the lab using a Milli-Q gradient water purification system from Millipore (Bedford, MA, USA). Methanol (MeOH) used as eluent for LC/MS was LC/MS grade and purchased from LGC Promochem (Wesel, Germany).

Liquid chromatography was conducted using an HTC-Pal cooled autosampler (CTC Analytics, Zwingen, Switzerland) and a Finnigan Surveyor MS Pump Plus (Thermo Fisher, San Jose, USA). Mass spectrometry was carried out on a LTQ Orbitrap XL mass analyser (Thermo Fisher, Bremen, Germany) and operated by Xcalibur 4.0 software.

2.2 UV degradation experiments

UV degradation experiments were conducted in a 1 L glass vessel equipped with a double-layer plunging recipient hosting a generic 15 W low-pressure mercury UV (254 nm) lamp. The vessel was filled with 800 mL of a 100 mg/L solution of each of the four pyrazolone

compounds: FAA, AAA, propyphenazone and phenazone. A magnetic stirrer was used and aluminium foil was wrapped around the vessel.

The experiment was started as the lamp was ignited and samples were taken at 0 s, 10 s, 20 s, 30 s, 40 s, 50 s, 60 s, 90 s, 2 min, 5 min, 10 min, 20 min. The samples were transferred in vials and analyzed by LC/MS. Experiments were conducted at pH 7.

2.3 LC/MS methodology

All pyrazolone compounds and their respective TPs were separated on a Hypersil Gold aQ column (125 × 2.1 mm, 5 µm) (Thermo Fisher, Waltham, MA, USA). A gradient program was employed using MeOH and water as eluents A and B, respectively. Acetic acid (0.1%) and ammonium acetate (2 mM) were added to both eluents. The mobile phase composition changed from 20% A to 90% A in 12 min, was subsequently held constant at 90% A for 10 min, brought back to 20% A in 1 min and held constant at 20% A until the end of the method (total run time: 30 min). The flow rate was 0.2 mL/min and the injection volume 10 µL. The mass analyzer was set in positive atmospheric pressure chemical ionization (APCI) mode. This allowed identifying all transformation products encountered in each sample according to their mass/charge ratio (m/z). Since reference standards for the pyrazolone compounds were available, their concentrations could be determined by LC/MS via external calibration. The validation of the method is given in the Supplementary Information (section S-1). For the TPs, however, no reference standards were available. Therefore, the degradation of the parent compounds and their TPs is reported as the actual area count with respect to the maximum observed area count during the course of the UV degradation experiment and should merely be interpreted as semi-quantitative.

To elucidate the structure of the transformation products, fragmentation (MS^n) experiments were subsequently conducted on the most degraded sample of each parent

compound by syringe infusion using either collision-induced dissociation (CID) or high-energy collisional dissociation (HCD) at increasing energy values.

Infusion experiments allowed optimizing the energy settings for each observed transformation product to build their MSⁿ tree in a much faster way compared to running a separate LC run for each energy setting. Exact masses allowed the determination of one molecular formula using Xcalibur 4.0 software.

All information obtained from the fragments in MSⁿ led to a “reconstruction” of the structure of the transformation product observed in MS. This reconstruction was facilitated by the fact that the structure of the parent compound was known, making the transformation products “known unknowns” as they are unknown compounds originating from a known compound. This approach has been reported previously [6,10,27]. The exact masses of the transformation products identified in this study and their MSⁿ fragments (where available) are given in Section S-2 in the supporting information, together with their proposed molecular formula, ring double bond (RDB) values and mass accuracy (in ppm). The structures of the transformation products are given in Fig. 1.

2.4 Estimation of ecotoxicity via Quantitative Structure-Activity Relationship (QSAR)

QSAR were used to assess the ecotoxic potential of the identified structures with the help of the EPIWIN software that can be consulted free of charge at the following website: <http://www.epa.gov/opptintr/exposure/pubs/episutedl.htm>.

The software provides LC50 values for fish (96 h) and for Daphnid (48 h) as well as EC50 – the half maximal effective concentration– (96 or 144 h) for green algae.

3. Results and discussion

3.1 Identification of photolysis degradation products

Acetamido antipyrine (AAA) is the most abundant compound in the environment among the pyrazolones and was therefore studied first. Figure 2 shows the degradation of AAA as a function of the UV exposure time, expressed as the actual observed area count with respect to the maximum observed area count ($\text{Area}/\text{Area}_{\text{max}}$). From this figure, it is evident that AAA is effectively degraded by UV radiation after 300 s as the $\text{Area}/\text{Area}_{\text{max}}$ curve asymptotically reaches 0 after 300 s. However, as the concentration of AAA decreases, the formation of a second compound is evident. This compound with an m/z of 192.06590 was named TP1 (structure see Fig. 1). The molecular formula of TP1 was deduced from the instrument software based on the similarity with the parent compound (mass accuracy below 2 ppm). The structure of the compound was subsequently derived from the observed fragments and the ring-double bond equivalent. TP1 is formed through a pyrazolone ring opening mechanism, which is presented in Figure 3. This mechanism was previously described by Gómez and coworkers [10] for methylaminoantipyrine (MAA, an intermediate in the metabolic pathway of metamizole) and is adapted to the molecules studied here. The same ring opening mechanism has also been observed during the ozonation of phenazone by Favier and coworkers [27].

Figure 2 shows that TP1 is degraded once it reaches its maximum concentration at about the same time that half of AAA is degraded (about 75 seconds in the experiment). The area count of AAA at the maximum concentration amounted 280×10^6 , while 76×10^6 area counts were recorded at the maximum concentration of TP1.

FAA shows a very different behavior towards UV oxidation compared to AAA. Follow-up of the concentration of FAA throughout the experiment shows a decrease in FAA until complete disappearance. However several compounds result from this degradation. It was found that while the FAA peak reduced over time, two peaks with the same m/z ratio appeared as illustrated by the extracted ion chromatogram in Figure 4. In this figure, it is demonstrated how the original FAA peak, with a retention time of 4.96 min, disappears while two new peaks appear at retention times of 7.23 and 8.83 min.

The emergence of peaks with the same m/z as the parent compound at different retention times indicates that the observed ions are produced by fragmentation during the ionisation in the source of the mass spectrometer. This happens when the ionisation energy is high enough to fragment a particularly unstable molecule. The mass spectra of the peaks at 7.23 min and 8.83 min moreover suggest that the observed molecules are the dimer and the trimer of FAA (Figure 5).

Figure 5a shows the peak with m/z 232.10889 and its sodium adduct (m/z 254.08995) eluting at 7.23 min. Additionally, a peak with m/z 463.21051 is visible. The formula determined for this compound ($C_{24}H_{26}O_4N_6$) is rigorously twice that of FAA ($C_{12}H_{13}O_2N_3$). In addition the sodium adduct of the dimer (m/z 485.19052) is also present in the spectrum. For Figure 5b the same peaks are present at an elution time of 8.83 min, but an additional mass is observed with m/z 694.30920. The corresponding formula is $C_{36}H_{39}O_6N_9$, which corresponds to three times the formula of FAA, hence the presence of the trimer is demonstrated. Once again a sodium adduct of this polymer is present at m/z 716.29035 (not visible in Figure 5b). As expected, MS² experiments on the dimer and the trimer gave the same m/z 232.10809 fragment corresponding to the $[M+H]^+$ of FAA. However, MS³ experiments yielded different results (Supplementary Information section S-2).

The m/z 232.10809 of the dimer gave a fragment with an m/z 175.08699 with the following formula: $C_{10}H_{10}ON_2$. From the trimer, the MS³ experiment induced a fragment with m/z 139.05049, corresponding to $C_6H_6O_2N_2$. The fragmentation routes are thus different, suggesting the observed m/z 232.10809 on the MS² spectra of the dimer and the trimer present differences in terms of position of the positive charge, which then leads to different fragmentation routes.

Figure 6 shows that the disappearance of FAA is linked to the formation of the trimer. The latter, after reaching its maximum, gradually diminishes while the dimer is forming. It is clear from this figure that the polymerized FAA is more resistant to UV degradation, especially in the dimer form.

UV photodegradation experiments on FAA have also been conducted by Gómez et al. [10], using simulated solar UV radiation over 209 hours of exposure. In this study, the formation of the dimer and trimer were mentioned and these compounds were observed for an initial concentration of FAA of 10 mg/L. The dimer was found to be persistent even after 209 hours of solar UV radiation [10]. Yuan et al. observed dimerization of phenazone after direct photolysis for an initial concentration of phenazone of 1 mg/L [14]. These results indicate that the occurrence of these dimers and trimers is not merely an artefact of the high concentrations used in this study to identify the transformation products, although their contribution might be lower at lower concentrations.

Besides these major transformation products, other degradation compounds, present in smaller amounts, were identified. First of all, a compound with $m/z=192.06554$ corresponding to TP1 was detected. This compound was already observed during the degradation of AAA by UV, but was also observed when degrading FAA by ozone [27]. During the experiment, TP1 reached its maximum concentration at about 1.5 min into the experiment before decreasing and finally disappearing completely. In addition, the precursor of TP1, named TP2 (see Figure 1 and Figure 3) was detected in amounts about 20-fold lower than TP1. The mass to charge ratio of TP2 is 210.07597 and its formula: $C_{10}H_{11}O_4N$.

In addition, the epoxy TP3 (see Figure 1) was detected, and at the same retention time, a compound that could be its precursor with an m/z of 266.11361 and the following formula: $C_{12}H_{15}O_4N_3$ (TP4). Similarly as for the FAA dimer, TP3 and TP4 reach their maximum concentration at the end of the experiment as shown in Figure 6. Thus, these compounds can be considered as persistent.

The results of the degradation of phenazone under UV light brought a similar impression as for FAA. The extracted ion trace of phenazone showed two additional peaks after some time of reaction in the same manner as for FAA. The first impression was that a dimer or a trimer was being formed. But when extracting the ion traces corresponding to those, no peaks resulted. The mass spectra of the additional peaks revealed in addition to the m/z

for phenazone, mass-to-charge ratios of $m/z = 201.10258$ and $m/z = 243.11328$, respectively. The software proposed the following formulas for these compounds: $C_{12}H_{13}ON_2$ and $C_{14}H_{15}O_2N_2$, respectively. The first one corresponds to the addition of only one carbon atom (C) to the phenazone structure as well as an additional insaturation. The second results from the addition of C_3H_2O to phenazone. No further fragmentation data were available for these compounds, therefore no formal structural identification was conducted. Recorded concentration profiles (data not shown) indicated that the formation of the two degradation compounds was not simultaneous and they were fully degraded after 20 min of UV radiation.

Several degradation products were observed when exposing propyphenazone to UV radiation. First of all, evidence of an opening of the pyrazolinone ring by oxidative cleavage of the N-N bond is patent. TP5 ($m/z = 218.11756$) and its precursor TP6 ($m/z = 236.12818$) (structures in Figure 1) were identified. In addition, TP7 ($m/z = 247.14100$) and TP8 ($m/z = 263.13815$) were detected, be it in very low amounts for the latter. They result from the partial or complete oxidation of the C=C double bond [27].

Figure 7 shows that TP5, TP6 and TP7 reach a maximum concentration before being further degraded. However, whereas TP6 is completely degraded at the end of the experiment, TP5 and TP7 are still at around 80% of their maximum concentration, showing a quite persistent character.

Figure 7 also shows the $Area/Area_{max}$ versus UV radiation time of a new degradation product (TP9) found during this experiment with an m/z of 217.13400. The corresponding formula is $C_{13}H_{16}N_2O$. This formula suggests that propyphenazone would have lost a $-CH_3$ group from its isopropyl function to form a compound with a structure presented in Figure 1. This compound reaches its maximum concentration at the end of the experiment and is considered quite stable. The breakage of the isopropyl group can be proven by the detection – albeit in a low amount – of the compound TP10 (Figure 1). Its mass to charge ratio is 203.11819 and its formula is $C_{12}H_{14}ON_2$.

Finally, another compound was detected in amounts similar to TP9. This compound (TP11) has a mass to charge ratio of 192.13864 for the following formula: $C_{12}H_{17}ON$. The formula indicates an opening of the pyrazolinone ring (loss of C_2HN from propyphenazone). The ring-double bond equivalent (RDB) of TP11 is 4.5 indicating that only one insaturation is present in the molecule besides the benzene ring. Thus an aliphatic carbon chain is present but no MSⁿ experiment could be conducted to confirm the proposed structure presented in Figure 1.

3.2 QSAR toxicity assessment

Modeling allows one to relatively simply prioritize compounds according to their toxicity. In this study, ecotoxicological data were calculated for the four parent compounds and the observed transformation products. Table 2 summarizes the results obtained from the EPIWIN software. The data were organized by increasing LC50 at 96 h for fish. Parent compounds are indicated in bold. This table clearly illustrates the potentially higher toxicity of some transformation products compared to their parent compounds.

For instance, TP1, which is obtained after the N-N bond oxidative opening of the pyrazolone ring, displays a quite high toxicity - although Daphnid and Green algae data could not be modeled from this particular compound.

These findings, adding to those already available in literature, clearly indicate that the prevention of the contamination of the environment by pharmaceuticals should not only focus on the degradation of the parent compound, since the degradation in this case is a transformation into compounds showing some persistence and a higher ecotoxicological potential. Thus, transformation products should be part of any research aiming at the removal of pharmaceuticals.

4. Concluding remarks

The current study confirms a mechanism of transformation of pyrazolone compounds where the N-N bond is oxidized followed by the loss of water which closes the ring again. Oxidation can also occur at the C=C double bond position as is also observed for oxidation by ozone.

In addition, it was found that pyrazolone compounds do not all behave in the same way under photolysis; FAA is found to produce a trimer and a persistent dimer while phenazone undergoes a yet uncharacterized transformation. Propyphenazone displays a wide range of possible transformations, such as a ring opening at the N-N bond or a successive demethylation mechanism.

A better understanding of the transformation mechanisms, wherein the chain reaction of one compound into another could be elucidated, would be of great value, but would require more complicated studies, including the synthesis or isolation of the TPs for further studies.

The ecotoxicological character of the TPs was established by QSAR modeling and points at important differences in toxicity for some TPs in comparison with their parent compounds. This observation has already been made in other studies as well. Therefore, advanced degradation processes, which are dynamic, should be carefully set up and not only based on the disappearance of the parent compound, but also on the disappearance of the transformation products.

The comprehensive approach discussed in this study, wherein high-resolution mass spectrometry and ecotoxicological modeling are combined, seems promising to achieve faster output in an extensive research field.

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6. Figure Captions

Figure 1: Summary of identified structures of TPs from the degradation of pyrazolones by UV.

Figure 2: Evolution of the ratio of the compound area versus the maximum encountered area ($\text{Area}/\text{Area}_{\text{max}}$) of AAA (♦) and TP1 (■) as a function of the UV exposure time.

Figure 3: General mechanism of N-N ring opening of pyrazolone leading to the formation of TP1.

Figure 4: Extracted ion chromatograms of $m/z = 232.10850$ at (a) $t = 0$ s, (b) $t = 30$ s, (c) $t = 1$ min, (d) $t = 5$ min, (e) $t = 20$ min of the UV degradation experiment.

Figure 5: Mass spectra at (a) 7.23 min and (b) 8.83 min of the FAA sample treated by UV for 20 min.

Figure 6: Evolution of the ratio $\text{Area}/\text{Area}_{\text{max}}$ of FAA (■), dimer (⊗), trimer (·), TP3 (♦) and TP4 (○) as a function of the UV exposure time.

Figure 7: Evolution of the ratio $\text{Area}/\text{Area}_{\text{max}}$ of propyphenazone (♦), TP5 (*), TP6 (●), TP7 (▲), TP9 (·) and TP11 (■) as a function of the UV exposure time.

TP1		TP2		TP3	
TP4		TP5		TP6	
TP7		TP8		TP9	
TP10		TP11			

Figure 1

Figure 2

Figure 3

RT: 0.00 - 15.00 SM: 3B

Figure 4

Figure 5

Figure 6

Figure 7

Table 1: Structures and physico-chemical characteristics of the selected pyrazolone compounds

	R	Given name	Log k_{ow}^*	Mol. Weight (g.mol ⁻¹)	CAS
	H	Phenazone	0.82	188	60-80-0
	C(CH ₃) ₂	Propyphenazone	1.97	230	479-92-5
	NH(COH)	Formyl-4-aminoantipyrine (FAA)	-0.51	231	1672-58-8
	NH(CH ₃ CO)	4-Acetamidoantipyrine (AAA)	-0.43	245	83-15-8

* calculated with Chemaxon Marvin software (www.chemaxon.com)

Table 2: Toxicity evaluation for parent pyrazolone compounds and their degradation products after UV degradation using QSAR modeling, organized by increasing *Fish* LC50

	Fish LC50 96Hr (ppm)	Daphnid LC50 48Hr	Green Algae EC50 144h	log K _{ow}
TP7	0.1	1.1	0.6	4.2
TP3	0.4	2.2	0.8	3.0
Propyphenazone	0.9	3.5	1.0	
TP1	1.0	/	/	0.5
TP6	1.1	/	/	0.7
TP2	1.2	/	/	-0.3
TP9	1.2	4.2	1.0	1.6
TP10	1.9	5.2	1.0	1.1
Phenazone	3.1	6.8	1.1	
FAA	4.1	8.8	1.4	0.5
AAA	8.3	13.5	1.7	-0.1
TP11	19.4	22.1	14.6 ^a	2.9
TP5	26.1	29.7	19.5 ^a	2.8

^a: EC50 at 96 h; /: not determined

In bold: parent compound

Supplementary Information

High-resolution MS and MSⁿ investigation of UV oxidation products of phenazone-type pharmaceuticals and metabolites

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S-1. Validation of the LC/MS methodology

The LC/MS method described in Section 2.3 was developed for the analysis of the four phenazone-type pharmaceuticals: AAA, FAA, phenazone and propyphenazone. The validation was conducted on peaks extracted by the accurate masses.

S-1.1. Linearity

Six-point calibration curves were generated by injecting standard solutions in the following range: 0, 1, 10, 50, 100, 150 and 200 ng/mL (injection volume: 10 μ L).

Figure S-1 shows the obtained curves. The MS instrument provided an excellent linearity for the four compounds with correlation coefficients (R^2) ranging between 0.9996 and 1.000.

Figure S-1: Linearity of the instrument for the analysis of the four pyrazolones.

S-1.2. Instrumental detection and quantification limits, instrumental reproducibility

Instrumental detection limit (IDL) and instrumental quantification limit (IQL) were determined by injecting increasing amounts of the four compounds starting from low pg levels. The limits were established at the first amount wherein the extracted exact mass trace a peak could be observed with a signal to noise ratio (S/N) above 3 for the IDL and 10 for the IQL. The results are summarized in

Table S-1.

Table S-1: Instrument detection and quantification limits for the four pyrazolones.

	AAA	FAA	Phenazone	Propyphenazone
IDL (pg injected)	10	5	5	5
IQL (pg injected)	50	10	10	10

The instrumental repeatability was established by triplicate injections of a standard mixture at 5 different concentrations (5, 10, 50, 100 and 150 ng/mL). For all compounds and at these five concentrations, the relative standard deviation (% RSD) was below 2%, which is excellent and can be explained by the highly selective character of the high resolution MS instrument.

Where necessary, samples were diluted to obtain a signal within the linear range.

S-2. MS and MSⁿ information for all identified TPs

compound	MS	MS ²	MS ³	MS ⁴
TP1	$m/z = 192.06590$ $C_{10}H_{10}NO_3$ RDB = 6.5 $\Delta ppm = 1.980$	$m/z = 174.05544$ $C_{10}H_8NO_2$ RDB = 7.5 $\Delta ppm = 2.786$	$m/z = 146.06035$ C_9H_8NO RDB = 6.5 $\Delta ppm = 2.119$	
		$m/z = 146.06033$ C_9H_8NO RDB = 6.5 $\Delta ppm = 1.982$	$m/z = 128.04980$ C_9H_6N RDB = 7.5 $\Delta ppm = 2.532$	
		$m/z = 94.06516$ C_6H_8N RDB = 3.5 $\Delta ppm = 0.363$		
Dimer of FAA	$m/z = 463.21051$ $C_{24}H_{27}N_6O_4$ RDB = 14.5 $\Delta ppm = 3.627$	$m/z = 232.10889$ $C_{12}H_{14}N_3O_2$ RDB = 7.5 $\Delta ppm = 3.605$	$m/z = 175.08699$ $C_{10}H_{11}N_2O$ RDB = 6.5 $\Delta ppm = 2.287$	
Trimer of FAA	$m/z = 694.30920$ $C_{36}H_{40}N_9O_6$ RDB = 21.5 $\Delta ppm = -0.585$	$m/z = 232.10889$ $C_{12}H_{14}N_3O_2$ RDB = 7.5 $\Delta ppm = 3.605$	$m/z = 139.05049$ $C_6H_7N_2O_2$ RDB = 4.5 $\Delta ppm = 2.057$	
TP2	$m/z = 210.07597$ $C_{10}H_{12}NO_4$ RDB = 5.5 $\Delta ppm = -0.544$			
TP4	$m/z = 266.11368$ $C_{12}H_{16}N_3O_4$ RDB = 6.5 $\Delta ppm = 0.554$			
TP5	$m/z = 218.11756$ $C_{13}H_{16}NO_2$ RDB = 6.5 $\Delta ppm = 1.443$			
TP6	$m/z = 236.12818$ $C_{13}H_{18}NO_3$ RDB = 5.5 $\Delta ppm = 0.932$			
TP3	$m/z = 248.10335$ $C_{12}H_{14}N_3O_3$ RDB = 7.5 $\Delta ppm = 1.540$	$m/z = 127.05050$ $C_5H_7N_2O_2$ RDB = 3.5 $\Delta ppm = 2.330$		
		$m/z = 230.09322$ $C_{12}H_{12}N_3O_2$ RDB = 8.5 $\Delta ppm = 3.550$	$m/z = 215.06976$ $C_{11}H_9N_3O_2$ RDB = 9.0 $\Delta ppm = 3.822$	
			$m/z = 202.09822$ $C_{11}H_{12}N_3O$ RDB = 7.5 $\Delta ppm = 3.619$	$m/z = 159.09230$ $C_{10}H_{11}N_2$ RDB = 6.5 $\Delta ppm = 3.929$

				$m/z = 145.07681$ $C_9H_9N_2$ RDB = 6.5 $\Delta ppm = 5.412$
				$m/z = 118.06541$ C_8H_8N RDB = 5.5 $\Delta ppm = 2.407$
			$m/z = 187.08728$ $C_{11}H_{11}N_2O$ RDB = 7.5 $\Delta ppm = 3.691$	$m/z = 130.06558$ C_9H_8N RDB = 6.5 $\Delta ppm = 3.492$
			$m/z = 173.07158$ $C_{11}H_{11}N_2O$ RDB = 7.5 $\Delta ppm = 3.701$	$m/z = 130.06558$ C_9H_8N RDB = 6.5 $\Delta ppm = 3.492$
				$m/z = 146.06074$ C_9H_8NO RDB = 6.5 $\Delta ppm = 4.105$
			$m/z = 132.04477$ C_8H_6NO RDB = 6.5 $\Delta ppm = 2.875$	
			$m/z = 111.05548$ $C_5H_7N_2O$ RDB = 3.5 $\Delta ppm = 1.716$	
TP7	$m/z = 247.14100$ $C_{14}H_{19}N_2O_2$ RDB = 6.5 $\Delta ppm = 1.439$			
TP8	$m/z = 263.13815$ $C_{14}H_{19}N_2O_3$ RDB = 6.5 $\Delta ppm = -3.302$			
TP9	$m/z = 217.13400$ $C_{13}H_{16}N_2O$ RDB = 6.5 $\Delta ppm = 1.429$			
TP10	$m/z = 203.11819$ $C_{12}H_{19}N_2O$ RDB = 6.5 $\Delta ppm = 1.479$			
TP11	$m/z = 192.13864$ $C_{12}H_{17}NO$ RDB = 4.5 $\Delta ppm = 1.974$			