# REACTION OF PHENAZONE-TYPE DRUGS AND METABOLITES WITH CHLORINE AND MONOCHLORAMINE

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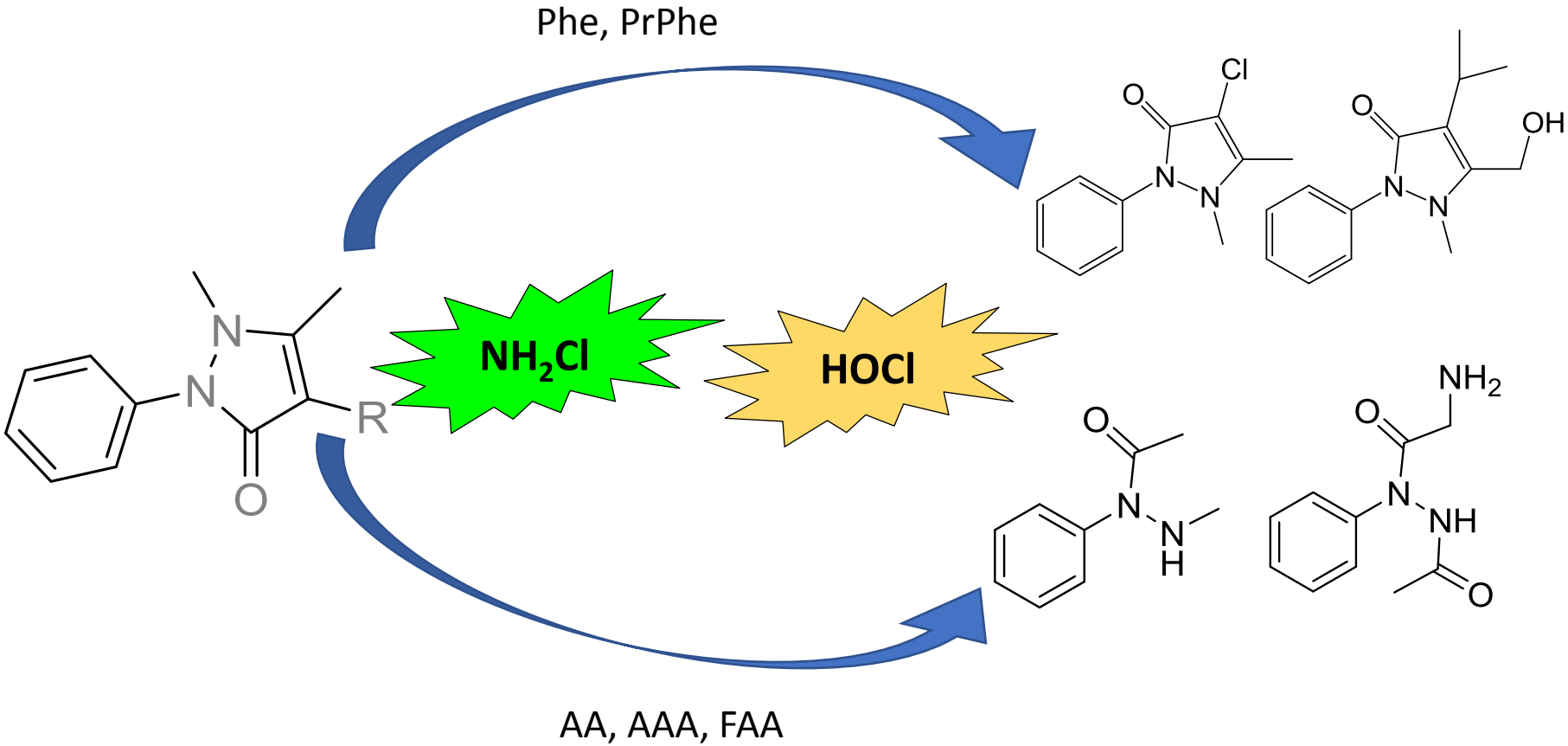
*Graphical Abstract



# REACTION OF PHENAZONE-TYPE DRUGS AND METABOLITES WITH CHLORINE AND MONOCHLORAMINE

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**ABSTRACT**

This work studies the chlorination and monochloramination reaction kinetics of two phenazone-type drugs (phenazone – Phe and propyphenazone – PrPhe) and three metabolites of phenazone-type drugs (4-formylaminoantipyrine – FAA, 4-aminoantiyprine - AA and 4-acetoamidoantipyrine - AAA). Kinetics were faster with chlorine (apparent second-order constants between 100 and 66,500 times higher) than with monochloramine. For FAA and AAA, no significant reaction was observed during monochloramination. Further, apparent rate constants decreased as the pH increased from pH 5.7 to 8.3, except during chlorination of AA. The transformation products (TPs) formed were also elucidated by liquid chromatography-high resolution mass spectrometry. The main transformation pathway for Phe and PrPhe consisted of halogenations, hydroxylations and dealkylations, while AAA and FAA were firstly transformed to AA, then followed by pyrazole ring opening and hydroxylations. The extend of the reaction was also tested in real water samples, where, in general, slower reaction kinetics were obtained during monochloramination, while the chlorination reaction showed similar half-lives to ultrapure water. Finally, acute and chronic toxicity of the TPs were estimated using two quantitative structure-activity relationship (QSAR) software (ECOSAR and TEST), showing that some TPs could be more toxic than their precursor compounds.

Keywords: Pharmaceuticals; transformation products; chlorination; monochloramine; metamizole; quadrupole-time of flight mass spectrometry

## 1. INTRODUCTION

Phenazone-type drugs (also called pyrazolone drugs) are pharmaceuticals with analgesic and antipyretic effect that are used worldwide. This group of drugs includes chemicals such as phenazone (also called, aminoantipyrine, Phe), propyphenazone (PrPhe), aminopyrine and metamizole (also called dipyrone), as their main representatives. Although in some countries the use of metamizole has been banned (eg. USA or UK) or regulated (e.g. Belgium) [1], in other countries it is still available by prescription and available over the counter in yet others. In Spain, the sales of metamizole during the last 10 years have increased a 67% in the last decade (from 3.28 in 2010 to 5.48 in 2019 defined daily doses (DDD) per 1000 inhabitants per day) [2].

After oral intake, in humans, aminopyrine and metamizole are rapidly metabolised in a non-enzymatic process to 4-methylaminoanitipyrine (4-MAA). Then, in the liver, 4-MAA is metabolised to 4-formylaminoantipyrine (FAA) and 4-aminoantiyprine (AA), the last one being further transformed to 4-acetoamidoantipyrine (AAA) [3-5]. So, after oral application, the major metabolites of metamizole and aminopyrine are detected in urine in the following ratios, as percent of original dose: 20-48% for AAA, 11-29% for FAA, 4-9% for AA, 2-4% for 4-MAA [3, 5-8].

Residues from the intake of phenazone-type drugs and their metabolites are excreted by humans and discharged into the sewer system. Several investigations have shown some evidence that these substances are not completely eliminated during wastewater treatment [6, 9-11]. Hence, they may also reach groundwater aquifers or surface water [12] which are finally a source of drinking water [13, 14]. For instance, the metabolites of metamizole, AAA and FAA, have been found in the Elbe river at concentrations up to 939 ng $L^{-1}$ which were higher than those of the drugs Phe (up to 85 ng $L^{-1}$) and PrPhe (up to 32 ng $L^{-1}$) [12]. In other German rivers, PrPhe was found at concentrations as high as 100 ng $L^{-1}$ and AA up to 630 ng $L^{-1}$ [15].

A common step in drinking water production is the use of disinfection methods to reduce the levels of microorganisms or contaminants in drinking water, by using e.g. chlorine, ozone, chloramines, chlorine dioxide, hydrogen peroxide, ferrate or UV

radiation [16]. Yet, such disinfectants can generate transformation products (TPs) which could be more toxic than the precursor compounds themselves [16, 17]. Among those disinfection techniques, the commonest one is chlorination, which is used in more than 90% of Western Europe because of its low cost [18-22]. Chlorine (typically dosed as free chlorine or sodium hypochlorite) is a powerful non-specific oxidant that can remove several compounds, but on the other hand, it generates several toxic, and regulated, disinfection by-products (DBPs), such as haloacetic acids or trihalomethanes [23, 24]. Hence, chloramines has been proposed as an alternative to chlorination, producing less DBPs [23-25].

In the case of phenazone-type drugs, photodegradation [26], ozonation [27, 28], UV/chloramine treatment [29] and mainly chlorination [20-22, 30-32] have been previously reported. Conversely, for the above-mentioned metabolites few studied have been published dealing only with ozonation [27] and photochemical treatment [26], while their reaction with chlorine has not been studied so far. Thus, the aim of this work was to investigate the reaction of aminopyrine and metamizole metabolites (AA, FAA and AAA) with chlorine and the reaction of Phe, PrPhe and the three metamizole metabolites with monochloramine. For those compounds reacting to a significant degree, the pH and, also bromide content in the case of chlorination, were also considered. Further, TPs were tentatively identified by high-performance liquid chromatography-high resolution mass spectrometry (HPLC-HRMS). Finally, a preliminary ecotoxicity estimation of the TPs was performed by Quantitative Structure-Activity Relationship (QSAR) using both the Toxicity Estimation Software Tool (T.E.S.T.) and ECOSAR from the USA Environmental Protection Agency (US-EPA).

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and stock solutions

Table 1 shows the name of the phenazone-type drugs and metabolites considered, their abbreviation, structure, and some physico-chemical properties. Phe, AA, FAA and AA were obtained from Sigma-Aldrich (Steinheim, Germany), and PrPhe from LGC Promochem (Wesel, Germany). Stock solutions were prepared by weight in methanol

(MeOH) (Merck, Darmstadt, Germany) and diluted as necessary. Ultrapure water used for the experiments was obtained in the lab from a Milli-Q water generator (A10 Gradient System, Millipore, Billerica, MA, USA). Sodium hypochlorite (6-14%), potassium bromide, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, sodium hydroxide, ammonia and formic acid were supplied by Sigma-Aldrich. Ammonium chloride and ascorbic acid were from Merck.

The exact nominal free chlorine content of the sodium hypochlorite solutions was regularly determined in the laboratory using a spectrophotometric method based on the hypochlorite ion absorbance at $\lambda$ = 292 nm ($\varepsilon$ = 350 L $mol^{-1}$ $cm^{-1}$) after pH > 8 adjustment with sodium hydroxide, to ensure all the chlorine will be present in the solution as $OCl^-$ ion ($pK_{a\ [HOCl]}$ = 7.2) [33].

Monochloramine ($NH_2Cl$) solution (2 mM) was produced daily in the laboratory by addition of NaOCl drop by drop to a $NH_4Cl$ solution, previously adjusted to a pH above 8.5 [34-36], with vigorous magnetic agitation. The concentration of the generated chloramines ($NH_2Cl$ and $NHCl_2$) was determined by a spectrophotometric method using their molar extinction coefficients at two wavelength [35, 37]: $\lambda$ = 255 nm ($\varepsilon$ $NH_2Cl$ = 369 mol $L^{-1}$ $cm^{-1}$ ; $\varepsilon$ $NHCl_2$= 136 mol $L^{-1}$ $cm^{-1}$) and $\lambda$ = 295 nm ($\varepsilon$ $NH_2Cl$ =15 mol $L^{-1}$ $cm^{-1}$; $\varepsilon$ $NHCl_2$= 289 mol $L^{-1}$ $cm^{-1}$).

### 2.2. Real samples

Surface water samples were collected from a small creek (pH 6.5, dissolved organic carbon (DOC): 8.7 mg $L^{-1}$, bromide: 0.035 mg $L^{-1}$; $UV_{254}$: 5.95x$10^{-2}$) not affected by urban activities, and from a river (pH 5.8, DOC: 22 mg $L^{-1}$, bromide: 0.045 mg $L^{-1}$; $UV_{254}$: 0.117) after receiving the discharge of a wastewater treatment plant (WWTP), ca. 5 Km downstream. All samples were collected in amber bottles and stored at 4 ºC until used. Before their use, samples were filtered through 0.45 µm nitrocellulose filters (Millipore) to remove particle matter.

### 2.3. Chlorination and chloramination experiments

Experiments were performed in 16 mL amber closed vials at room temperature (20 ± 2 ºC). The reaction kinetics were evaluated in ultrapure water at three different pH values (5.7, 7.0 and 8.3) buffered with a $KH_2PO_4/K_2HPO_4$ 0.03 M solution, spiked with the target analytes at 1 µg $mL^{-1}$ (4-5 µM). After the addition of chlorine or chloramine, aliquots of 1 mL were taken at different reaction times and the reaction was stopped with 0.6 µg $mL^{-1}$ of ascorbic acid. In chlorination experiments, free chlorine concentration was set to 10 µg $mL^{-1}$ (0.141 mM). Such pH values and chlorination dosages represent typical values for drinking water [38, 39]. Additionally, KBr was added at 100 ng $mL^{-1}$ (0.84 µM) in some experiments, which reacts with HOCl to produce hypobromous acid, to consider bromination in bromide-containing surface water [40]. Chloramination experiments were performed with 4 µg $mL^{-1}$ (0.0777 mM) of monochloramine, which is the maximum residual disinfectant concentration allowed by the US-EPA [41]. Finally, experiments without chlorine nor chloramine were prepared as a control.

### 2.4. HPLC-HRMS analysis

The system used for samples analysis was a 1200 Series LC (Agilent Technologies, Santa Clara, CA, USA) equipped with a membrane degasser, a binary high-pressure gradient pump, a thermostated LC column compartment and an autosampler interfaced to an Agilent 6520 Series Accurate Mass quadrupole-time-of-flight (QTOF)-MS equipped with a Dual Electrospray ion source. Separation was carried out on a 100 mm x 2 mm (particle size: 3 µm) Synergi Fusion RP (Phenomenex, CA, USA) at a flow rate of at 0.2 mL $min^{-1}$ and 35 °C. Mobile phase consisted of Milli-Q water (A) and MeOH (B) both containing 5 mM of ammonium acetate. The gradient was as follows: 0 min, 5% B; 10-12 min, 100% B; 12.10 min -25 min, 5% B. Injection volume was set to 10 µL.

For the QTOF-MS, nitrogen (99.9%), used for nebulising and drying gas, was provided by a nitrogen generator (Erre Due Srl, Livorno, Italy). Nitrogen (99.9995%) used for collision-induced dissociation was supplied by Praxair Spain (A Coruña, Spain). The electrospray ion source was operated in positive (no TPs were detected in negative) mode with the following parameters being applied: gas temperature: 350 °C; drying gas: 7 L $min^{-1}$; nebulizer: 42 psig, capillary: 4000 V; fragmentor: 120 V; skimmer voltage: 65 V; and

octapole RF peak: 750 V. Instrument was operated in the 2 GHz (extended-dynamic range) mode, which provides a Full Width at Half Maximum (FWHM) resolution of ca. 4,500 at m/z 121 and ca. 11,000 at 922 m/z. A reference solution was also continuously infused using a second nebulizer of the Dual- Electrospray ion source (5 psig), to recalibrate the QTOF using two masses (*m/z* 121.0509 and 922.0098) and maintain the mass accuracy. Instrument control, data acquisition and evaluation were performed with the MassHunter software (Agilent Technologies). Quantification was carried out in single MS mode from the accurate mass extracted chromatogram (10 ppm mass accuracy window).

Determination of TPs was performed as described elsewhere [42] using the algorithm "*Find by molecular feature*" from the MassHunter software, which generates a list of possible entities for each chromatogram, which were exported as CEF files. Such files were then transferred to the MassProfiler Professional software (Agilent Technologies) which compares the control group (aliquots at time 0 s) and aliquots collected at different times, excluding those entities which were not observed at least in the three replicates of any reaction time. Then, empiric formulae were produced for the TPs using the "*generate formula*" tool of the MassHunter software, which considers the accurate mass and isotopic distribution, providing a score (100 is a perfect match). Cut-off values were set at mass error <5 ppm and score > 80. Finally, MS/MS analysis were acquired, using different collision energies (10, 20 and 40 V), and interpreted in order to tentatively elucidate the structure of the TPs.

### 2.5. Toxicity assessment

An *in-silico* preliminary estimation of the ecotoxicity of the investigated substance and their TPs was performed by two Quantitative Structure-Activity Relationship (QSAR) software, viz the US-EPA Toxicity Estimation Software Tool (T.E.S.T.) version 4.1 and ECOSAR version 1.11. In T.E.S.T., toxicity values can be estimated with different QSAR methodologies and a large number of molecular descriptors such as structural or electronic parameters. The 48-hour *daphnia magna* $LC_{50}$, 96-hour *fathead minnow* $LC_{50}$ and oral rat $LD_{50}$ endpoints were estimated by the consensus method, which uses an average value of the calculated toxicities by five different QSAR methodologies [42]. In ECOSAR, toxicity values were estimated using different linear regression models for each

chemical class. The 48-hour *daphnia magna* $LC_{50}$, 96-hour fish $LC_{50}$, 96-hour green algae $EC_{50}$ and chronic toxicity values were estimated [42].

## 3. RESULTS AND DISCUSSION

### 3.1. Preliminary experiments

Preliminary chlorination and chloramination tests were performed in order to assess the reactivity of the selected compounds in presence of chlorine and monochloramine. Since we had previously investigated Phe and PrPhe chlorination [22], and considering the similarity in terms of structure of AA, FAA and AAA, chlorination was performed under similar conditions, with 10 μg $mL^{-1}$ of free chlorine in 10 mL of ultra-pure water at neutral pH (7) containing 1 μg $mL^{-1}$ of compound (AA, FAA or AAA). After 30 min, AA, AAA and FAA were completely removed.

Chloramination reactions for Phe, PrPhe, AA, AAA and FAA were performed by using 1 μg $mL^{-1}$ of each compound in 10 mL of ultrapure water at neutral pH with 4 μg $mL^{-1}$ of $NH_2Cl$. After 72 h, Phe, PrPhe and AA were completely removed, while for AAA and FAA signal decrease was below 10%. So, AAA and FAA were deemed stable and their chloramination was no further studied.

### 3.2. Reactions kinetics

The influence of the pH over the reaction kinetics was studied considering three levels, i.e. pH 5.7, 7 and 8.3. Aliquots were taken at different reaction times from 0 s to 5 min for chlorination and up to 72 h for chloramination. The ratio of the areas obtained at a certain time divided by those obtained at 0 s, against the reaction time were fitted to an exponential model, where the exponent is the pseudo-first order velocity constant (k') (k' values compiled in Table S1, examples of the fittings presented in Figure 1), from which the second order constant (k) was also determined (Table S2). Then, half-lives were calculated as $t_{1/2} = \ln 2/k'$ (Table 2).

Phe, PrPhe and AA reaction with chlorine is faster than in the case of AAA and FAA (Table 2), which may explain why these last two chemicals did not significantly react with monochloramine. Furthermore, as shown in Table S2, reaction kinetics are significantly

faster with HClO than $NH_2Cl$, e.g. k at pH 7 with HClO were between 97 and 63,000 times higher than with $NH_2Cl$.

When bromide was added in chlorination experiments, a Student's t test showed no statistically significantly different half-lives as compared to samples that did not contain such anion for AA (p-value 0.3144) (Table 2), similarly to what we had previously observed for Phe and PrPhe [22]. On the other hand, AAA and FAA showed statistically faster reactions with bromide (Student's t test p-values 1.4E-05 and 3.5E-07, respectively). This may be due to the fact that the first step of FAA and AAA rection is its hydrolysis to produce AA (see 3.3 and Figure 3) mediated by an electrophilic attack of HClO/HBrO, which would be faster with HBrO due to its higher electrophilic character [43].

As regards the pH (Table 2), the $t_{1/2}$ in the reaction of AA with HClO was not statistically affected (ANOVA p-value 0.9783). On the other hand, the reaction of AAA (Figure 1a) and FAA with HClO, as for Phe and PrPhe [22], are faster at lower pH values, being the differences observed statistically significant (ANOVA p-values <0.0001). This could be explained by the speciation of HOCl/$ClO^-$, considering that HOCl is a much electrophilic than $ClO^-$, which could be counterbalanced by the speciation of the primary amine moiety only present in AA structure. On the other hand, chloramination of Phe, PrPhe and AA was affected by the pH (ANOVA p-values <0.0001), being faster at lower pH values (Figure 1b). This could be attributed to, as mentioned in the literature [44, 45], monochloramine autodecomposition at lower pH values leading to the formation of dichloramine and HOCl. Given the fact that the TPs observed during chlorination and chloramination are the same (see below), this may point to the fact that HClO (formed from $NH_2Cl$ autodecomposition) is also the species reacting during monochloramination, as has already been by Duirk et al [46] for iodinated X-ray contrast media.

### 3.3. Transformation products

A summary of the detected TPs (proposed formulas, mass errors and scores) is shown in Table S3 (Phe and PrPhe) and Table 3 (AA, AAA and FAA). Phe and PrPhe chlorination TPs have already been described by Rodil et al. [22], so only chloramination TPs were investigated here. Chloramination of Phe and PrPhe yielded six and five TPs, respectively

(Table S3). All TPs were the same as those reported during chlorination [22], except Cl-PrPhe, which could not be detected in our former work. This could be due to the much faster chlorination kinetics, so that this TP undergoes a rapid reaction with chlorine to yield other TPs, being in this way not detectable in our former chlorination study [22]. The spectrum of Cl-PrPhe is presented in Figure S1, whereas the spectra of the remaining chloramination TPs also observed during chlorination are reported elsewhere [22]. Similarly to chlorination, Cl-Phe and OH-PrPhe are the main TPs produced by chloramination from Phe and PrPhe, respectively (Figure 2a and 2b) [22].

Regarding AA, FAA and AAA, a summary of the TPs detected during chlorination is presented in Table 3 (HRMS data) and Figure 3 (proposed structures). Six TPs were tentatively identified, five of them being common to the 3 compounds and the remaining one being AA (TP-204), produced during AAA and FAA chlorination as an initial step of the reaction (Figure 3). As it can be observed in Table 3, the empirical formula could be proposed with a high degree of certainty, with score values higher than 97% and mass errors lower than 5 ppm. The proposed structures shown in Figure 3 are based on the interpretation of the MS/MS spectra which are presented in the Supplementary Information (Figure S2). Besides AA, one product (TP-191) presents a DBE (double-bond equivalents) of 7. This TP is produced by substitution of the primary amine by a hydroxy group and demethylation of AA (Figure 3). On the other hand, the remaining TPs present lower DBE values due to pyrazole ring opening. TP-166 and TP-208 showed similar MS/MS spectra (Figure S2g and S2d), with the only difference of the first loss of $C_2H_2O$ in the case of TP-208, corresponding to a ketone group. This loss is also observed for TP-165 (Figure S2h), which also possess a ketone group. On the other hand, TP-194 spectrum (Figure S2e) exhibits a loss of CO, typical of an aldehyde. Only one of these TPs, TP-165, has been reported previously in ozonation and photodegradation studies, for which two different structures were proposed: 2-methyl-1-phenylhydrazide acetic acid [47] and 1-methyl-2-phenylhydrazide acetic acid [13, 26, 27, 48]. Unfortunately, its MS/MS spectrum was not reported in any of those publications. In this work, we propose the first one, 2-methyl-1-phenylhydrazide acetic acid, as the most probable structure, as it is more compatible with the precursor structure, though the MS/MS spectrum is not conclusive (Figure S2h). The chlorination time-profile plot representing

the formation of TPs is presented in Figure 4, where the most intense product for FAA and AAA is in fact AA, followed by TP-208 and TP-165 (being the most intense products observed during AA chlorination).

In the case of AA chloramination, the same 5 TPs obtained during chlorination were observed. However, the formation profile of the TPs along time is different, the main product being by far TP-165 (Figure 2c).

### 3.4. Reaction on real sample matrices

Chlorination and chloramination reactions were also studied with two real water samples, one with low anthropogenic impact (creek) and one impacted from a wastewater discharge (river). AA, AAA and FAA reaction with chlorine in creek (pH 6.5) and river (pH 5.8) water showed no statistically different half-lives (Students-t test p-values >0.1590) than in ultrapure water at pH 7 and 5.7 respectively, and the reaction was complete for the three compounds (Table 4). This is likely due to the very high reactivity of these compounds. Actually, no significant change in chlorine content was observed in the treated surface water samples after 5 min of reaction. Furthermore, four of the six TPs were detected in the samples, and only TP-166 and TP-165 were not observed during chlorination of real samples.

Phe and PrPhe reaction with monochloramine was slower in real samples (Student's t-test p-values <0.0088), with apparent half-lives slightly higher in creek water (17.5 and 7.6 h, respectively) and significantly higher in river water (27 and 21 h, respectively, Table 5) than those obtained in ultrapure water at similar pH (7 and 5.7, respectively, Table 2). Moreover, even at longer times (up to 72 h) reaction was not complete and about 70% and 45% Phe and PrPhe remained in the solution, respectively. Regarding TPs, only Cl-Phe and Cl-Phe-Me were detected for Phe, and OH-PrPhe and $OH_2$-PrPhe for PrPhe (Table 4). This may be due to the fact that competition with organic matter (particularly for higher DOC samples) can take place, due to the much slower monochloramination kinetics.

Similarly, the half-lives of AA with monochloramine are significantly longer in both creek and river samples (Student's t-test p-values 0.0003 and 0.0004, respectively) (Table 4) than those obtained in ultrapure water at pH 7 and pH 5.7, respectively (Table 2). The

reaction was complete at 72 h, remaining less than 1% of AA. Conversely to chlorination, TP-165 and TP-166 were found after the reaction of AA with monochloramine in real water samples.

### 3.5. (Eco)Toxicity estimation

In order to obtain a preliminary estimation of the (eco)toxicity of the TPs, the US-EPA T.E.S.T. and ECOSAR software were used as described in 2.5, the results obtained being compiled in Table 5. As already reported in the literature, significant differences were observed for toxicity prediction using both software [42], so these data shall be used only as a preliminary screening for toxicity.

According to the results (Table 5),Cl-Phe, Br-Phe and Cl-Phe-Me (Phe TPs) and Cl-PrPhe, $Cl_2$-PrPhe and Cl,Br-PrPhe (PrPhe TPs) would present higher acute (lower $LC_{50}$ or $EC_{50}$) and chronic (lower chronic value - ChV) toxicities for the three considered trophic levels (fish, daphnid and algae) than their precursor compounds. In the case of AA and according to ECOSAR data, FAA, AAA, TP-191 and TP-165 would also exhibit higher toxicity than AA, while the T.E.S.T. software results estimated that AAA would be more toxic for *Fathead Minnow* and TP-191 and TP-166 for *Daphnia magna* than AA. According to the ECHA guidance [49], precursors and TPs, which showed oral rat toxicities $LD_{50}$ values in the 392-1476 mg kg body weight$^{-1}$, would be classified as Category 4, except TP-165 and TP-166 which would be classified as Category 3 (oral rat toxicities $LD_{50}$ values of 157 and 229 mg kg body weight$^{-1}$, respectively).

## 4. CONCLUSIONS

The metabolites of metamizole, AA, FAA and AAA, undergo a rapid reaction during chlorination (half-lives lower than 3 minutes) while only AA significantly reacts with monochloramine (AA chloramination half-lives between 0.2 and 3.9 h). The reaction kinetics, in excess of oxidant, followed pseudo-first order kinetics with half-lives affected by the pH, the reaction being slower at higher pH values, except in the case of AA, whose chlorination kinetics is not significantly affected in the 5.7-8.3 pH range. Several TPs were tentatively identified to be formed mainly via pyrazole ring opening and hydroxylations in the case of metamizole metabolites, and via halogenations,

hydroxylations and dealkylations for Phe and PrPhe. Also, we have shown that this reaction takes place with real water matrices and that some of these TPs are predicted to be more toxic than the original compounds. However, toxicity prediction seems to have a large uncertainty and experimental ecotoxicological experiments should be conducted in the future.

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**Figures Caption**

Figure 1. Reaction kinetics plots at different pH values, for (a) AAA with chlorine (RSD<10%) and (b) AA with monochloramine (RSD<15%).

Figure 2. Time course of TPs response during chloramination for (a) Phe (RSD<12%), (b) PrPhe (RSD<18%) and (c) AA (RSD<16%). Conditions: 4 μg $mL^{-1}$ (0.0777 mM) of monochloramine, target analytes at 1 μg $mL^{-1}$ (4-5 μM) and pH 5.7.

Figure 3. Proposed reaction pattern of metamizole metabolites during chlorination.

Figure 4. Time course of TPs response during chlorination for (a) AA (RSD<12%), (b) AAA (RSD <14%) and (c) FAA (RSD>12%). Conditions: 10 μg $mL^{-1}$ (0.141 mM) of free chlorine, target analytes at 1 μg $mL^{-1}$ (4-5 μM) and pH 7.

495 **Table 1.** Structures and some physico-chemical properties of the phenazone-type drugs and metabolites
496 considered.

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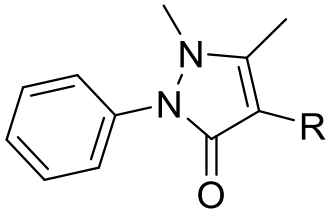
| Structure | Compounds (abbreviation) | R | CAS Nº | log P[a] | $pK_a$[a] |
|---|---|---|---|---|---|
|  | Phenazone (Phe) | H | 60-80-0 | 0.44 | 0.65 (basic) |
| | Propyphenazone (PrPhe) | $CH(CH_3)_2$ | 479-92-5 | 1.72 | 1.46 (basic) |
| | Aminoantipyrine (AA) | $NH_2$ | 83-07-8 | -0.26 | 4.07 (basic) |
| | 4- Formylaminoantipyrine (FAA) | NHCHO | 1672-58-8 | -0.06 | 1.07 (basic)<br>12.72 (acid) |
| | N-acetylaminoantipyrine (AAA) | $NHCOCH_3$ | 83-15-8 | -0.89 | 1.07 (basic)<br>12.84 (acid) |

[a] Values obtained from ACD/Labs

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**Table 2**. Chlorination and chloramination half-lives (compounds concentration: 1 µg $mL^{-1}$), calculated from k' values presented in Table S1.

| | 10 µg $mL^{-1}$ $Cl_2$ ($t_{1/2}$ in s) | | | | 4 µg $mL^{-1}$ $NH_2Cl$ ($t_{1/2}$ in s) | | |
|---|---|---|---|---|---|---|---|
| | 0 ng $mL^{-1}$ $Br^-$ | | | 100 ng $mL^{-1}$ $Br^-$ | | | |
| **pH** | **5.7** | **7.0** | **8.3** | **7.0** | **5.7** | **7.0** | **8.3** |
| **Phe** | 0.9[a] | 1.8[a] | 4.1[a] | 1[a] | 6840 ±360 | 52920 ± 1080 | 230040 ± 7200 |
| **PrPhe** | 0.4[a] | 0.5[a] | 0.9[a] | 1.1[a] | 2160 ± 144 | 21240 ± 180 | 105480 ± 6480 |
| **AA** | 3.7 ± 1.2 | 3.6 ± 1.1 | 3.5 ± 1.2 | 5 ± 1.8 | 720 ± 72 | 4320 ± 360 | 14040 ± 72 |
| **FAA** | 50 ± 2 | 52 ± 0.4 | 59 ± 0.5 | 12 ± 1 | - | - | - |
| **AAA** | 25 ± 2 | 43 ± 2 | 163 ± 2 | 10 ± 1 | - | - | - |

[a] Values from [22]

508

509 **Table 3**. LC-QTOF identification data on f AA, FAA and AAA and their TPs detected during chlorination experiments.

| TPs | Precursor | $t_R$ (min) | Experimental m/z | Proposed formula | Calculated m/z | Error (mDa) | Mass error (ppm) | DBE | Score |
|---|---|---|---|---|---|---|---|---|---|
| AAA | - | 9.81 | 246.1229 | $C_{13}H_{15}N_3O_2$ | 246.1237 | 0.80 | 3.28 | 8 | 97.37 |
| FAA | - | 9.49 | 232.1080 | $C_{12}H_{13}N_3O_2$ | 232.1081 | 0.05 | 0.23 | 8 | 99.99 |
| AA | AAA, FAA | 11.39 | 204.1131 | $C_{11}H_{13}N_3O$ | 204.1131 | 0.04 | 0.19 | 7 | 99.99 |
| TP-208 | AA, AAA, FAA | 10.89 | 208.1084 | $C_{10}H_{13}N_3O_2$ | 208.1084 | -0.35 | -1.67 | 6 | 99.43 |
| TP-194 | AA, AAA, FAA | 9.98 | 194.0922 | $C_9H_{11}N_3O_2$ | 194.0921 | 0.30 | 1.57 | 6 | 99.54 |
| TP-191 | AA, AAA, FAA | 10.17 | 191.0819 | $C_{10}H_{10}N_2O_2$ | 191.0819 | -0.40 | -2.08 | 7 | 99.21 |
| TP-166 | AA, AAA, FAA | 10.16 | 166.0970 | $C_8H_{11}N_3O$ | 166.0983 | 0.49 | 2.96 | 5 | 98.69 |
| TP-165 | AA, AAA, FAA | 10.19 | 165.1023 | $C_9H_{12}N_2O$ | 165.1022 | -0.06 | -0.37 | 5 | 99.98 |

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Table 4: Half-lives obtained in real water samples spiked with 1 μg $mL^{-1}$ of the phenazone type drugs and metabolites and 10 μg $mL^{-1}$ of free chlorine or 4 μg $mL^{-1}$ of monochloramine, and TPs observed.

| | | 10 μg $mL^{-1}$ $Cl_2$ ($t_{1/2}$ in s) | | 4 μg $mL^{-1}$ $NH_2Cl$ ($t_{1/2}$ in h) | |
|---|---|---|---|---|---|
| **Precursor** | | **Creek** | **River** | **Creek** | **River** |
| **Phe** | | -[a] | -[a] | 17.5 ± 1 | 27 ± 5 |
| **PrPhe** | | -[a] | -[a] | 7.6 ± 0.3 | 21 ± 7 |
| **AA** | | 4.0 ± 0.4 | 6.3 ± 0.6 | 17 ±0.6 | 2.6 ± 0.3 |
| **FAA** | | 58 ± 6 | 51 ± 5 | - | - |
| **AAA** | | 47 ± 5 | 25 ± 1.6 | - | - |
| | | **10 μg $mL^{-1}$ $Cl_2$ TPs** | | **4 μg $mL^{-1}$ $NH_2Cl$ TPs** | |
| **Precursor** | **TP** | **Creek** | **River** | **Creek** | **River** |
| **Phe** | **Cl-Phe** | Yes[a] | Yes[a] | Yes | Yes |
| | **Cl-Phe-Me** | Yes[a] | No[a] | No | Yes |
| **PrPhe** | **OH-Prophe** | No[a] | Yes[a] | Yes | Yes |
| | **$(OH)_2$-Prophe** | Yes[a] | Yes[a] | Yes | Yes |
| **AA** | **TP-191** | Yes | Yes | No | No |
| | **TP-194** | No | No | Yes | Yes |
| | **TP-166** | No | No | Yes | No |
| | **TP-165** | No | No | Yes | Yes |
| **FAA** | **TP-204/AA** | Yes | Yes | - | - |
| | **TP-208** | Yes | Yes | - | - |
| | **TP-194** | Yes | Yes | - | - |
| **AA** | **TP-191** | Yes | Yes | - | - |

[a] Previously reported in chlorination experiments from reference [22]

Table 5. Ecotoxicological data of phenazone type drugs and metabolites, and TPs predicted by the US EPA TEST and ECOSAR software.

| Compound | ECOSAR | | | | | | | TEST | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Chemical class | Acute toxicity (mg $L^{-1}$) | | | Chronic toxicity (mg $L^{-1}$) | | | 96h *Fathead Minnow* $LC_{50}$ (mg $L^{-1}$) | 48 h *Daphnia magna* $LC_{50}$ (mg $L^{-1}$) | Oral rat $LD_{50}$ (mg kg body weight$^{-1}$) | Toxicity/Mutagenicity |
| | | Fish (96h $LC_{50}$) | *Daphnid* (48h $LC_{50}$) | Algae (96h $EC_{50}$) | Fish | *Daphnid* | Algae | | | | |
| **Phe** | Hydrazine | 2.32 | 3.47 | 1.31 | 4.85 | 1.06 | 0.14 | 36.82 | 24.71 | 819.50 | Toxicant / Negative |
| **PrPhe** | Hydrazine | 1.12 | 2.33 | 0.92 | 1.73 | 0.48 | 0.11 | 10.35 | 13.23 | 667.93 | Toxicant / Negative |
| **Cl-Phe** | Hydrazine | 1.72 | 3.07 | 1.18 | 3.05 | 0.76 | 0.14 | 10.67 | 24.45 | 870.33 | Toxicant / Negative |
| **Br-Phe** | Hydrazine | 2.26 | 3.90 | 1.50 | 4.15 | 1.01 | 0.17 | 9.64 | 17.33 | 1476.20 | Toxicant / Negative |
| **Cl-,OH-Phe** | Hydrazine | 7.48 | 7.79 | 2.82 | 21.5 | 3.62 | 0.29 | 18.34 | 50.83 | 1118.78 | Toxicant / Negative |
| **Cl2,OH-Phe** | Hydrazine | 3.24 | 4.93 | 1.86 | 6.66 | 1.47 | 0.21 | N/A | N/A | N/A | N/A |
| **Cl-Phe-Me** | Hydrazine | 1.97 | 3.26 | 1.24 | 3.75 | 0.88 | 0.14 | 34.61 | 24.95 | 757.93 | Toxicant / Negative |
| **Cl,OH-Phe-Me** | Hydrazine | 8.61 | 8.30 | 2.97 | 26.5 | 4.22 | 0.29 | 53.71 | 131.74 | 461.78 | Toxicant / Negative |
| **OH-PrPhe** | Hydrazine | 3.07 | 4.56 | 1.72 | 6.42 | 1.40 | 0.19 | 7.26 | 27.82 | 841.61 | Toxicant / Negative |
| **OH2-PrPhe** | Hydrazine | 5.00 | 6.33 | 2.34 | 12.1 | 2.34 | 0.25 | N/A | N/A | N/A | N/A |
| **OH3-PrPhe** | Hydrazine | 21.5 | 15.9 | 5.51 | 84.1 | 11.0 | 0.52 | N/A | N/A | N/A | N/A |
| **OH-PrPhe-Me** | Hydrazine | 3.54 | 4.87 | 1.82 | 7.94 | 1.64 | 0.19 | 28.07 | 45.37 | 612.39 | Toxicant / Negative |
| **Cl,OH-PrPhe-Me** | Hydrazine | 1.66 | 3.22 | 1.26 | 2.74 | 0.73 | 0.15 | 28.33 | 12.55 | 392.30 | Toxicant / Negative |
| **Cl-PrPhe** | Hydrazine | 0.636 | 1.78 | 0.725 | 0.758 | 0.262 | 0.092 | 5.18 | 3.03 | 807.92 | Toxicant/Negative |
| **Cl2-PrPhe** | Hydrazine | 0.41 | 1.42 | 0.59 | 0.39 | 0.16 | 0.08 | 8.08 | 5.79 | 517.63 | Toxicant / Negative |
| **Cl,Br-PrPhe** | Hydrazine | 0.43 | 1.55 | 0.65 | 0.41 | 0.17 | 0.09 | 7.43 | 6.86 | N/A | Toxicant / Negative |
| **AA** | Hydrazine | 898 | 84.1 | 112 | 109 | 5.47 | 31.2 | 62.45 | 48.68 | 588.54 | Toxicant / Negative |
| **FAA** | Hydrazine | 3.13 | 4.51 | 1.69 | 6.74 | 1.44 | 0.19 | 108.01 | 146.47 | 699.38 | Toxicant / Positive |
| **AAA** | Hydrazine | 6.05 | 6.91 | 2.53 | 16.0 | 2.88 | 0.26 | 4.51 | 74.35 | 1122.95 | Toxicant / Negative |
| **TP-208** | Hydrazine | $7.13 \times 10^3$ | 575 | $1.04 \times 10^3$ | $1.41 \times 10^3$ | 32.0 | 260 | 373.40 | 130.56 | 637.63 | Toxicant / Negative |
| **TP-194** | Hydrazine | $1.32 \times 10^4$ | $1.00 \times 10^3$ | $2.02 \times 10^3$ | $3.04 \times 10^3$ | 53.0 | 484 | 292.15 | 66.76 | 843.24 | Toxicant / Negative |
| **TP-191** | Hydrazine | 6.71 | 6.68 | 2.40 | 20.1 | 3.27 | 0.24 | 82.83 | 17.04 | 446.96 | Toxicant / Negative |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **TP-166** | Hydrazine | $5.24x10^3$ | 422 | 757 | $1.10x10^3$ | 23.7 | 189 | 227.85 | 29.08 | 229.47 | Toxicant / Negative |
| **TP-165** | Hydrazine | 3.06 | 3.90 | 1.44 | 7.57 | 1.43 | 0.15 | 220.73 | 59.02 | 156.72 | Toxicant / Negative |

N/A Not related to existent chemical class.

517

Figure1

Click here to access/download;Figure;Figure 1.pptx

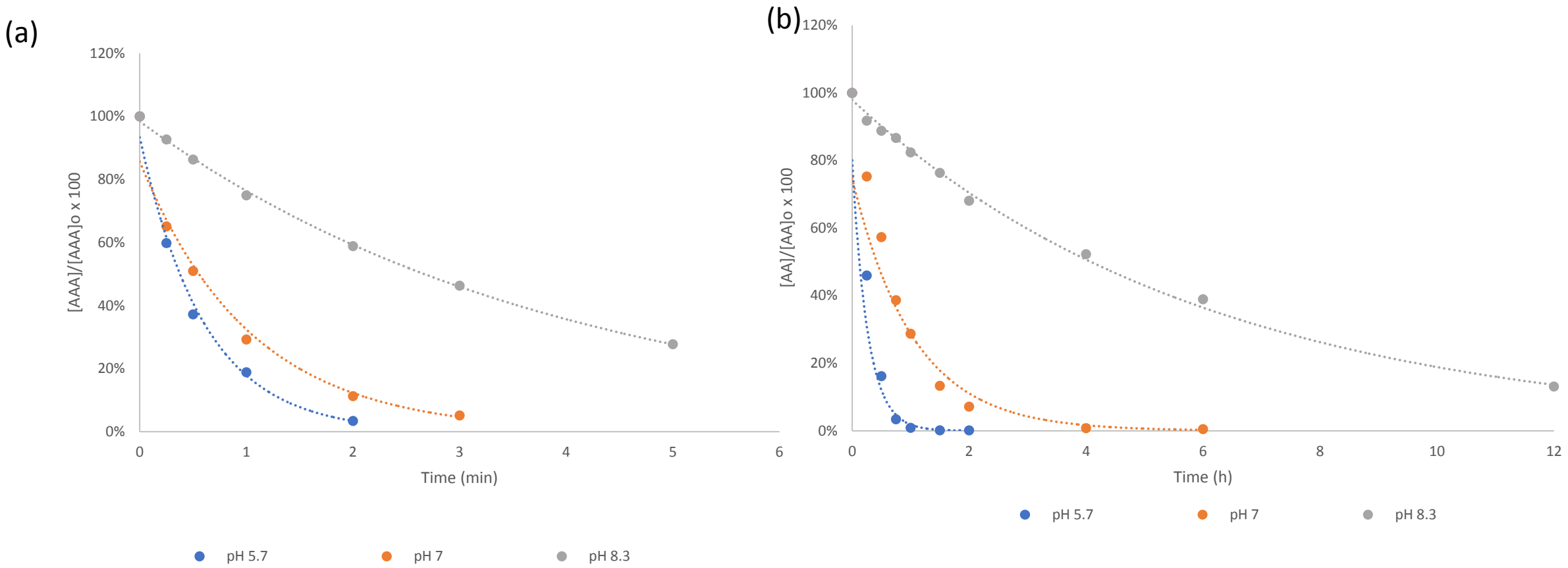
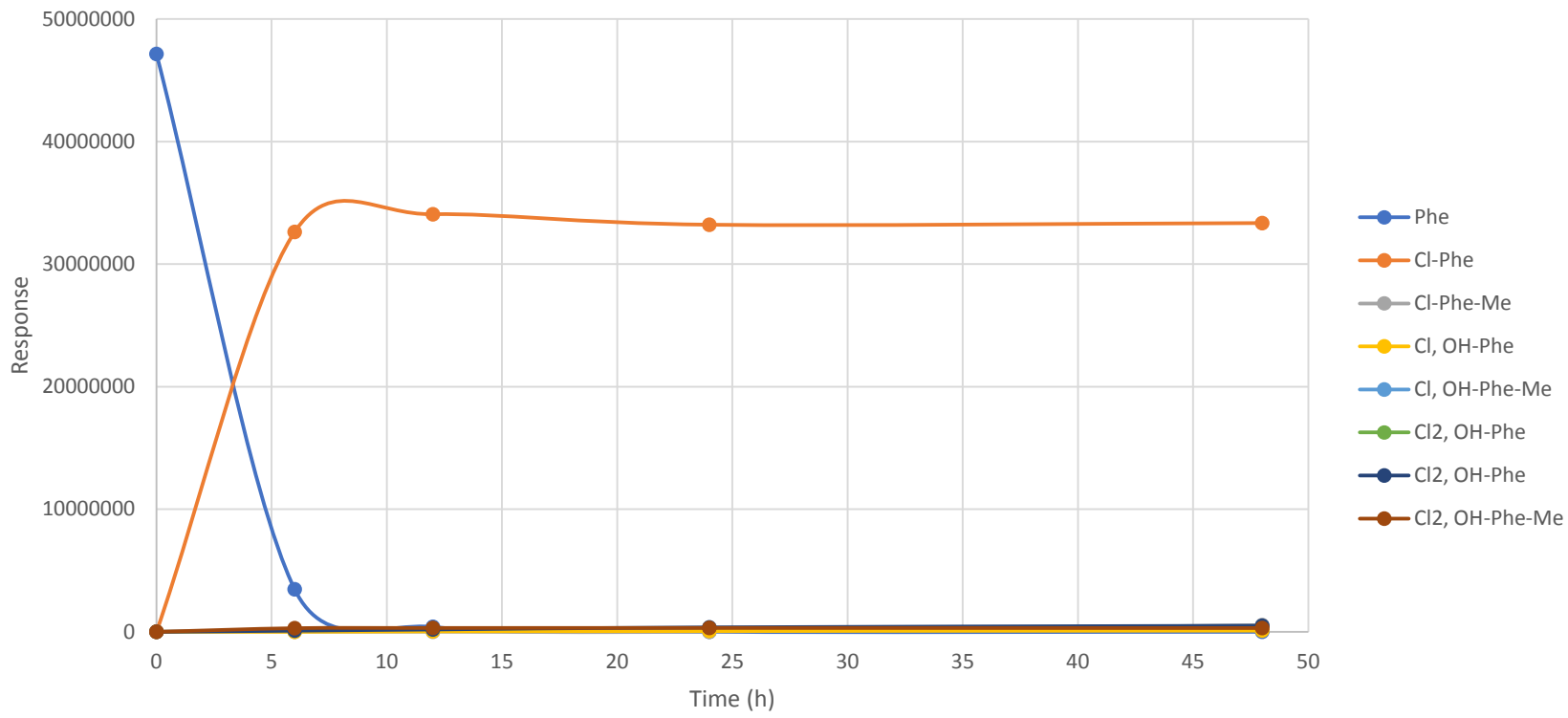



Figure 1

(a)

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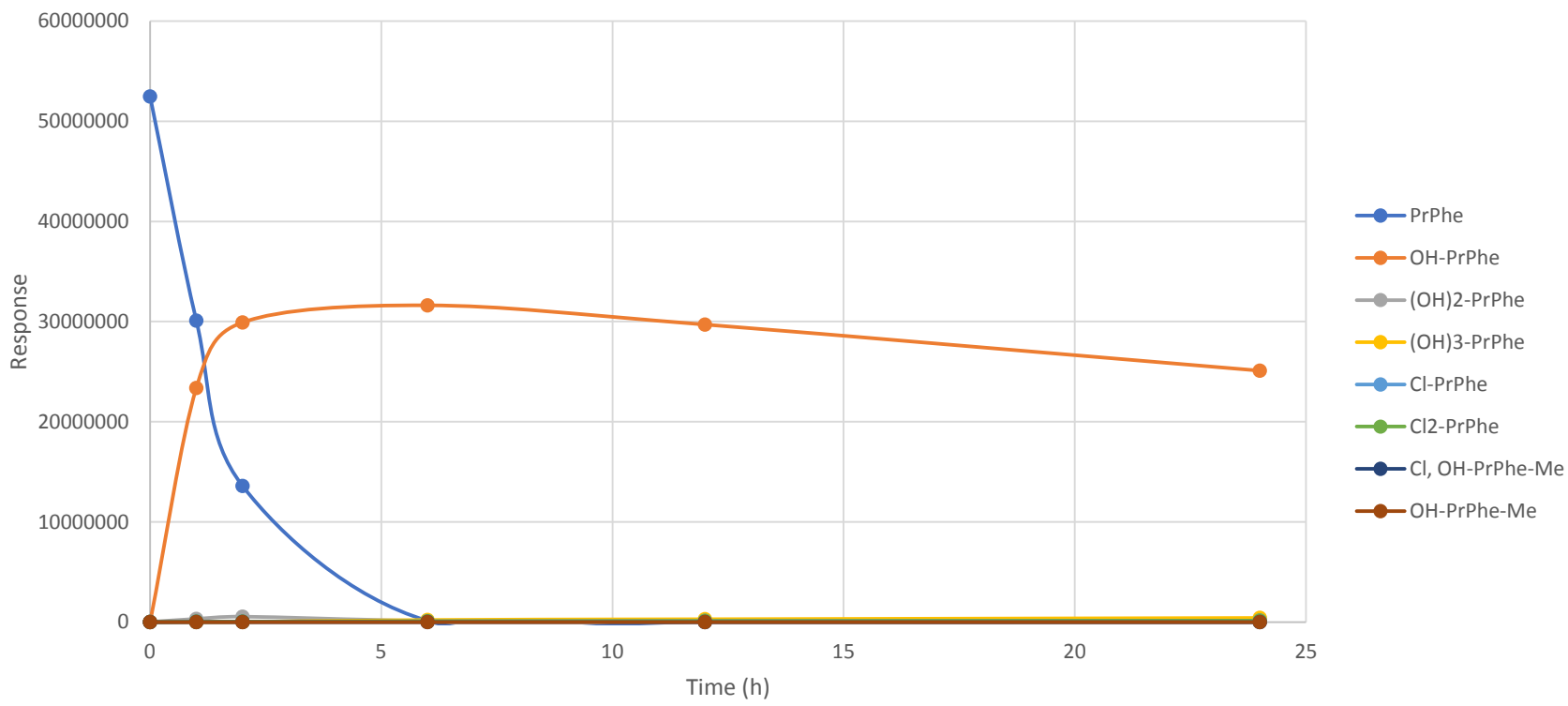


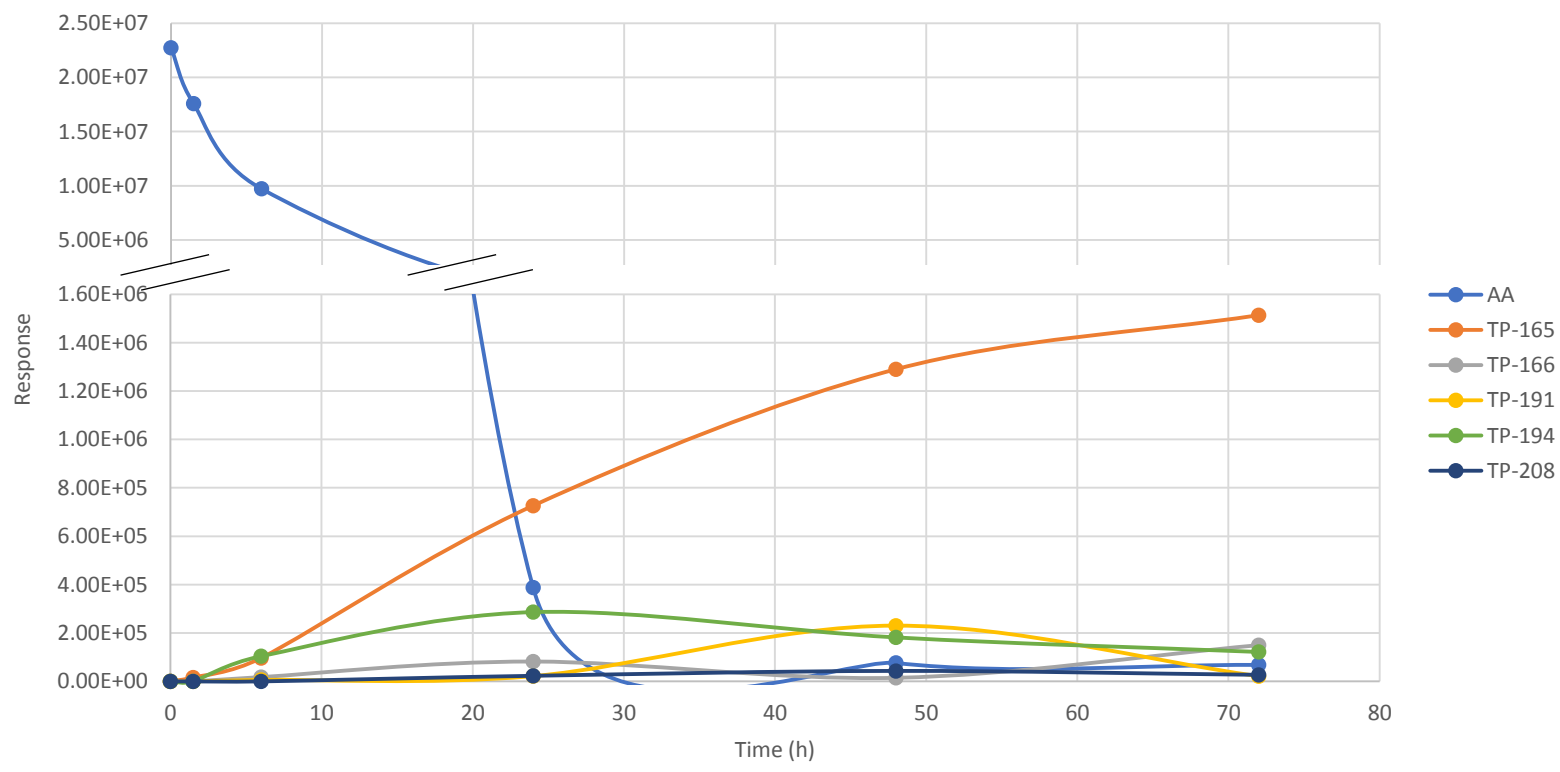




Figure 2

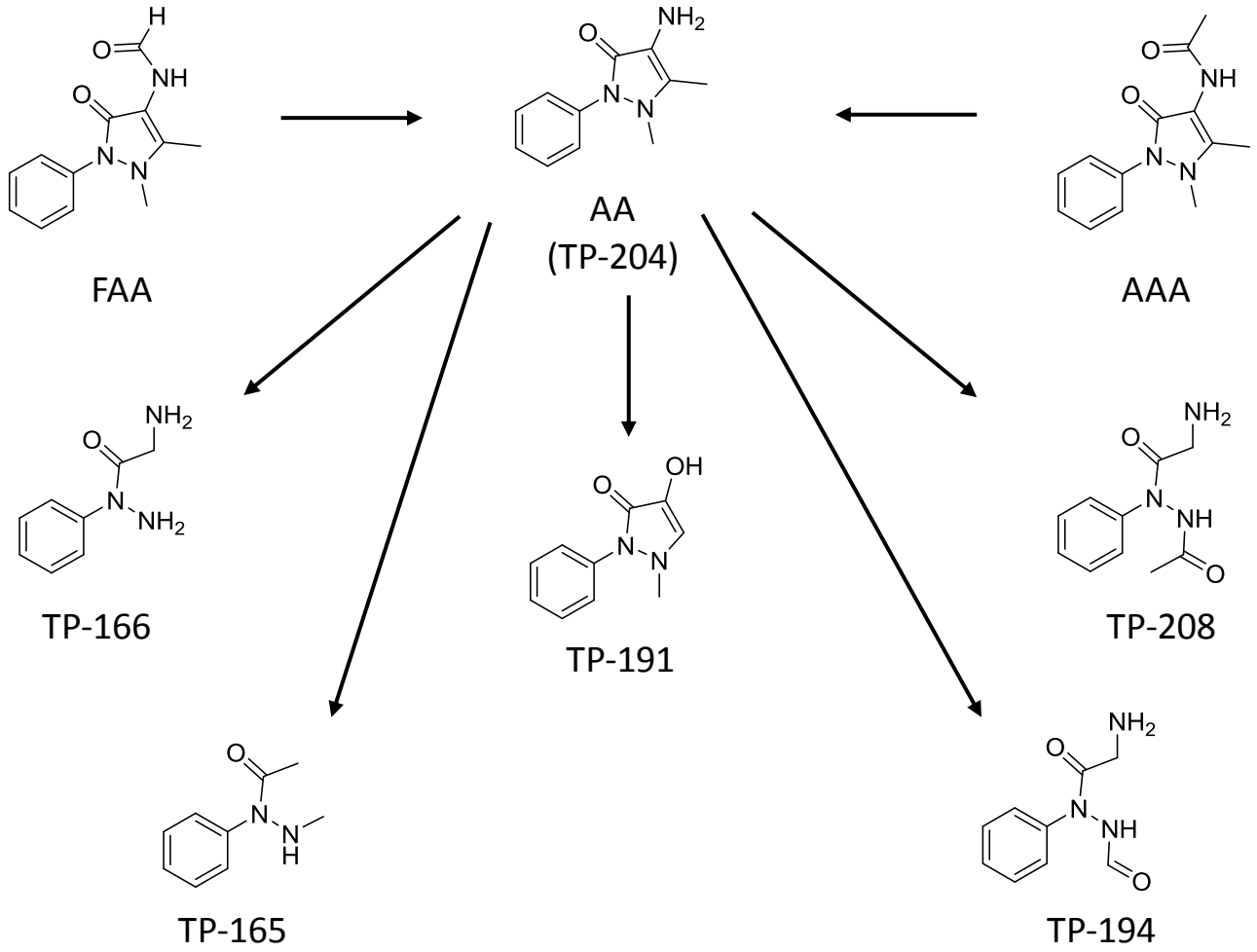
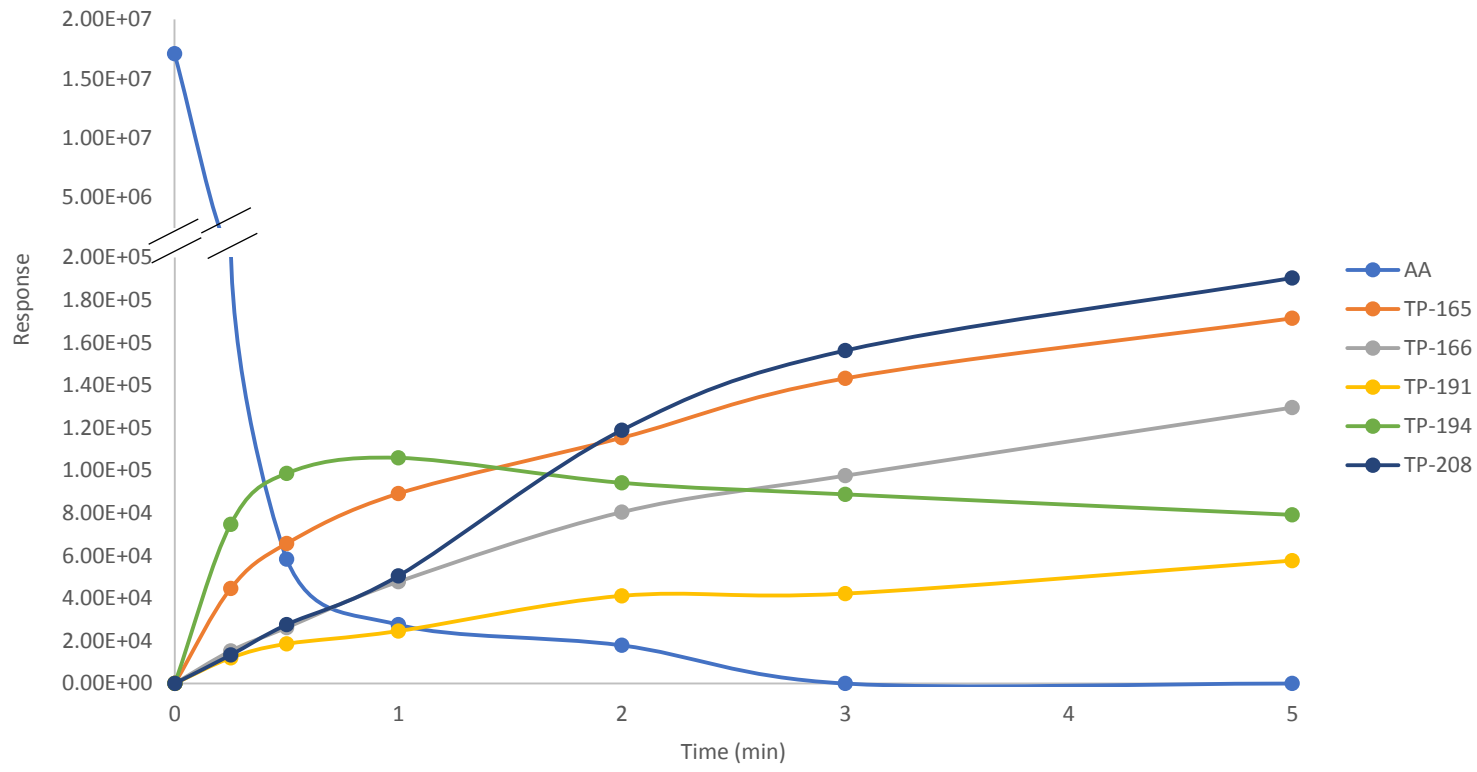



Figure 3

(a)

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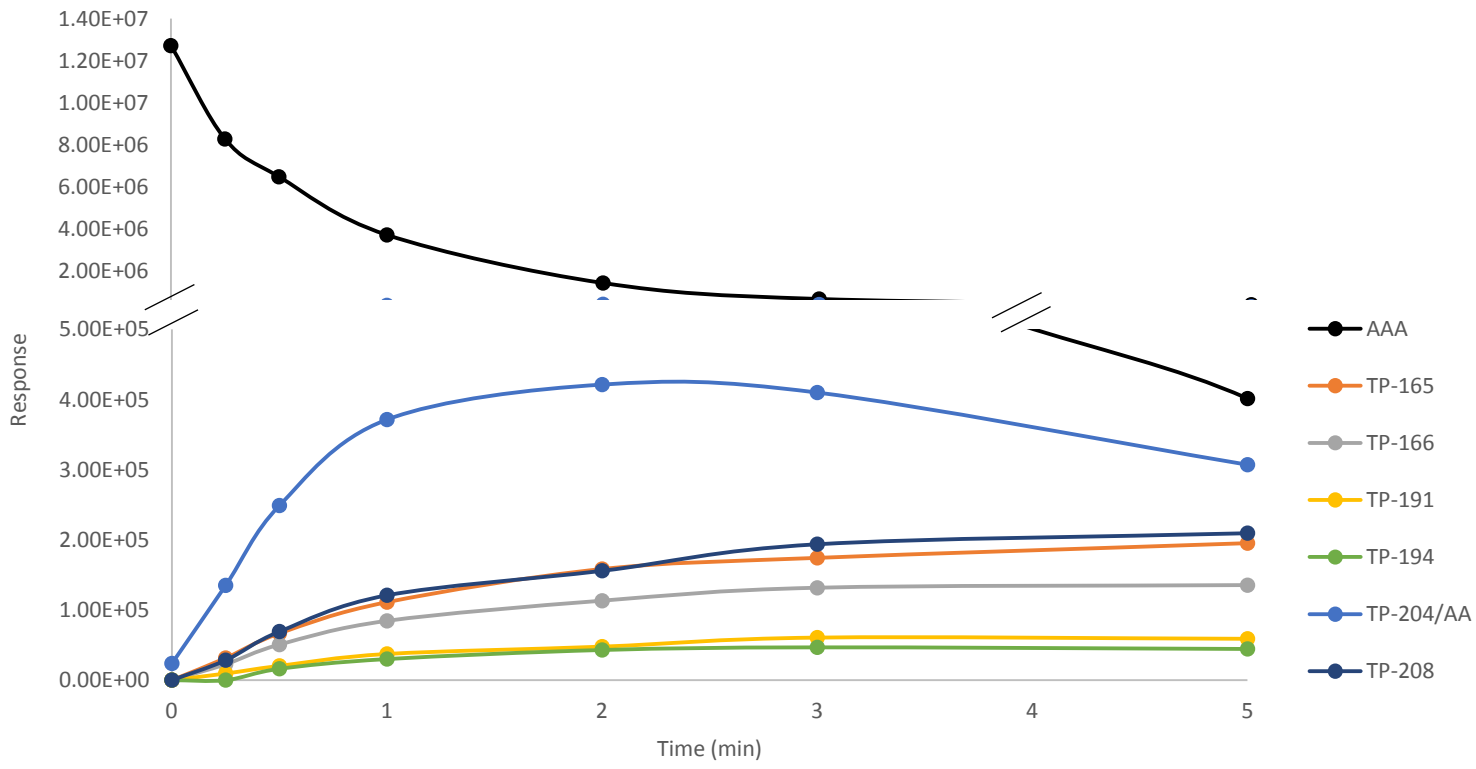


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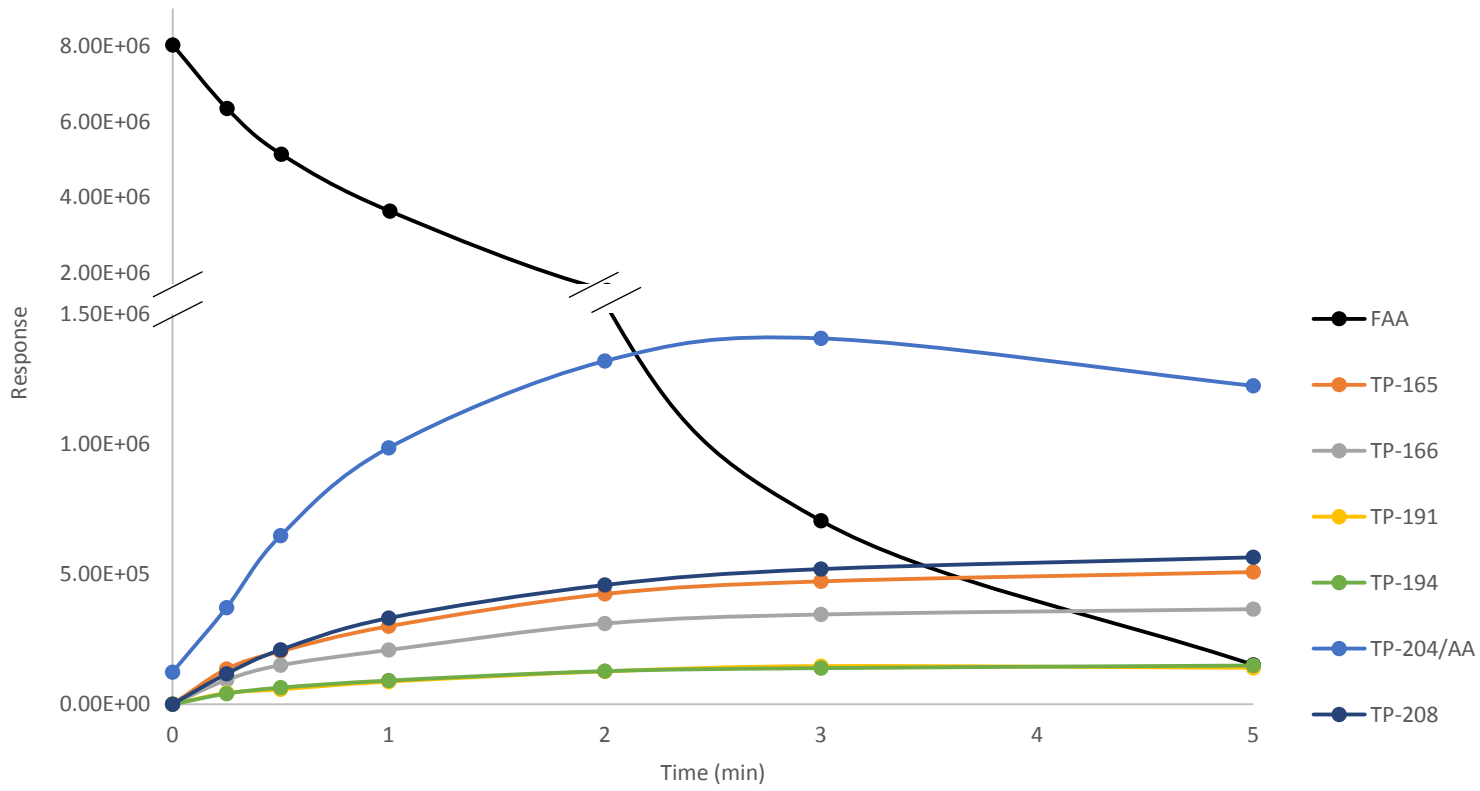


Figure 4

**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

**CRediT author statement**

**Benigno José Sieira**: Investigation, Formal analysis, Visualization, Writing - original draft, **José Benito Quintana**: Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition, **Rafael Cela**: Resources, Writing - review & editing, Funding acquisition, **Rosario Rodil**: Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration

Supplementary Information to:

# REACTION OF PHENAZONE-TYPE DRUGS AND METABOLITES WITH CHLORINE AND MONOCHLORAMINE

Benigno J. Sieira, José Benito Quintana, Rafael Cela, Rosario Rodil*

CONTENTS:

Table S1: Pseudo-first order rate constants (k') ($s^{-1}$) obtained during chlorination and chloramination experiments.

| | 10 µg $mL^{-1}$ $Cl_2$ | | | | 4 µg $mL^{-1}$ $NH_2Cl$ | | |
|---|---|---|---|---|---|---|---|
| | 0 ng $mL^{-1}$ $Br^-$ | | | 100 ng $mL^{-1}$ $Br^-$ | | | |
| **pH** | **5.7** | **7.0** | **8.3** | **7.0** | **5.7** | **7.0** | **8.3** |
| **Phe** | 0.77[a] | 0.39[a] | 0.17[a] | 0.69[a] | 9.97E-05 ± 6E-06 | 1.31E-05 ± 3E-07 | 3.06E-06 ± 1E-07 |
| **PrPhe** | 1.73[a] | 1.39[a] | 0.77[a] | 0.63[a] | 3.09E-04 ± 2E-05 | 3.25E-05 ± 3E-07 | 6.67E-06 ± 4E-07 |
| **AA** | 0.18 ± 0.06 | 0.19 ± 0.06 | 0.20 ± 0.07 | 0.14 ± 0.05 | 1.05E-03 ± 1E-04 | 1.64E-04 ± 2E-05 | 4.86E-05 ± 3E-06 |
| **FAA** | 1.36E-02 ± 5E-04 | 1.32E-02 ± 1E-04 | 1.17E-02 ± 1E-04 | 5.78E-02 ± 5E-03 | - | - | - |
| **AAA** | 2.75E-02 ± 2E-03 | 1.60E-02 ± 8E-04 | 4.16E-03 ± 4E-05 | 6.93E-02 ± 7E-03 | - | - | - |

[a] Values from R. Rodil, J.B. Quintana, R. Cela, Transformation of phenazone-type drugs during chlorination, Water Research, 46 (2012) 2457-2468.

Table S2: Second-order rate constants (k) ($M^{-1}$ $s^{-1}$) obtained during chlorination and chloramination experiments.

| | 10 µg $mL^{-1}$ $Cl_2$ | | | | 4 µg $mL^{-1}$ $NH_2Cl$ | | |
|---|---|---|---|---|---|---|---|
| | 0 ng $mL^{-1}$ $Br^-$ | | | 100 ng $mL^{-1}$ $Br^-$ | | | |
| **pH** | **5.7** | **7.0** | **8.3** | **7.0** | **5.7** | **7.0** | **8.3** |
| **Phe** | 5.47E+03 | 2.73E+03 | 1.20E+03 | 4.92E+03 | 1.3 | 0.17 | 0.04 |
| **PrPhe** | 1.23E+04 | 9.85E+03 | 5.47E+03 | 4.48E+03 | 4.1 | 0.42 | 0.09 |
| **AA** | 1.31E+03 | 1.37E+03 | 1.41E+03 | 9.85E+02 | 14 | 2.11 | 0.63 |
| **FAA** | 97 | 94 | 83 | 410 | - | - | - |
| **AAA** | 195 | 114 | 30 | 492 | - | - | - |

Table S3. LC-QTOF data on TPs identification in phenazone and propyphenazone chloramination experiments.

| Experimental m/z | $t_R$(min) | Proposed formula | Difference (ppm / mDa) | Score (%) | TPs identification[a] |
|---|---|---|---|---|---|
| 189.1023 | 10.07 | $C_{11}H_{12}N_2O$ | 0.32 / 0.06 | 99.98 | Phenazone |
| 223.0634 | 10.76 | $C_{11}H_{11}N_2OCl$ | 1.95 / 0.43 | 99.16 | Cl-Phe |
| 209.0486 | 8.82 | $C_{10}H_9N_2OCl$ | 4.72 / 0.98 | 95.62 | Cl-Phe-Me |
| 239.0589 | 7.99 | $C_{11}H_{11}N_2O_2Cl$ | 3.02 / 0.72 | 97.84 | Cl, OH-Phe |
| 225.0432 | 10.05 | $C_{10}H_9N_2O_2Cl$ | 3.11 / 0.71 | 95.23 | Cl, OH-Phe-Me |
| 261.0192 | 10.45 | $C_{10}H_{10}N_2O_2Cl_2$ | -0.04 / -0.01 | 100.00 | $Cl_2$, OH-Phe-Me |
| 275.0349 | 11.99 | $C_{11}H_{12}N_2O_2Cl_2$ | -2.77 / -0.76 | 97.85 | $Cl_2$, OH-Phe |
| 231.1497 | 12.43 | $C_{14}H_{18}N_2O$ | 2.22 / 0.51 | 98.97 | Propyphenazone |
| 247.1447 | 10.82 | $C_{14}H_{18}N_2O_2$ | 2.42 / 0.60 | 98.54 | OH-Prophe |
| 265.1548 | 12.24 | $C_{14}H_{21}N_2O_3$ | 0.50 / 0.13 | 99.93 | $(OH)_2$-Prophe |
| 281.1500 | 11.60 | $C_{14}H_{20}N_2O_4$ | 1.49 / 0.42 | 99.36 | $(OH)_3$-Prophe |
| 265.1111 | 12.62 | $C_{14}H_{17}N_2OCl$ | -0.40/-1.50 | 99.39 | Cl-Prophe |
| 299.0736 | 13.59 | $C_{14}H_{16}N_2OCl_2$ | 7.90 / 2.35 | 83.92 | $Cl_2$-Prophe |

[a] Previously reported and identified in chlorination experiments from R. Rodil, J.B. Quintana, R. Cela, Transformation of phenazone-type drugs during chlorination, Water Research, 46 (2012) 2457-2468.

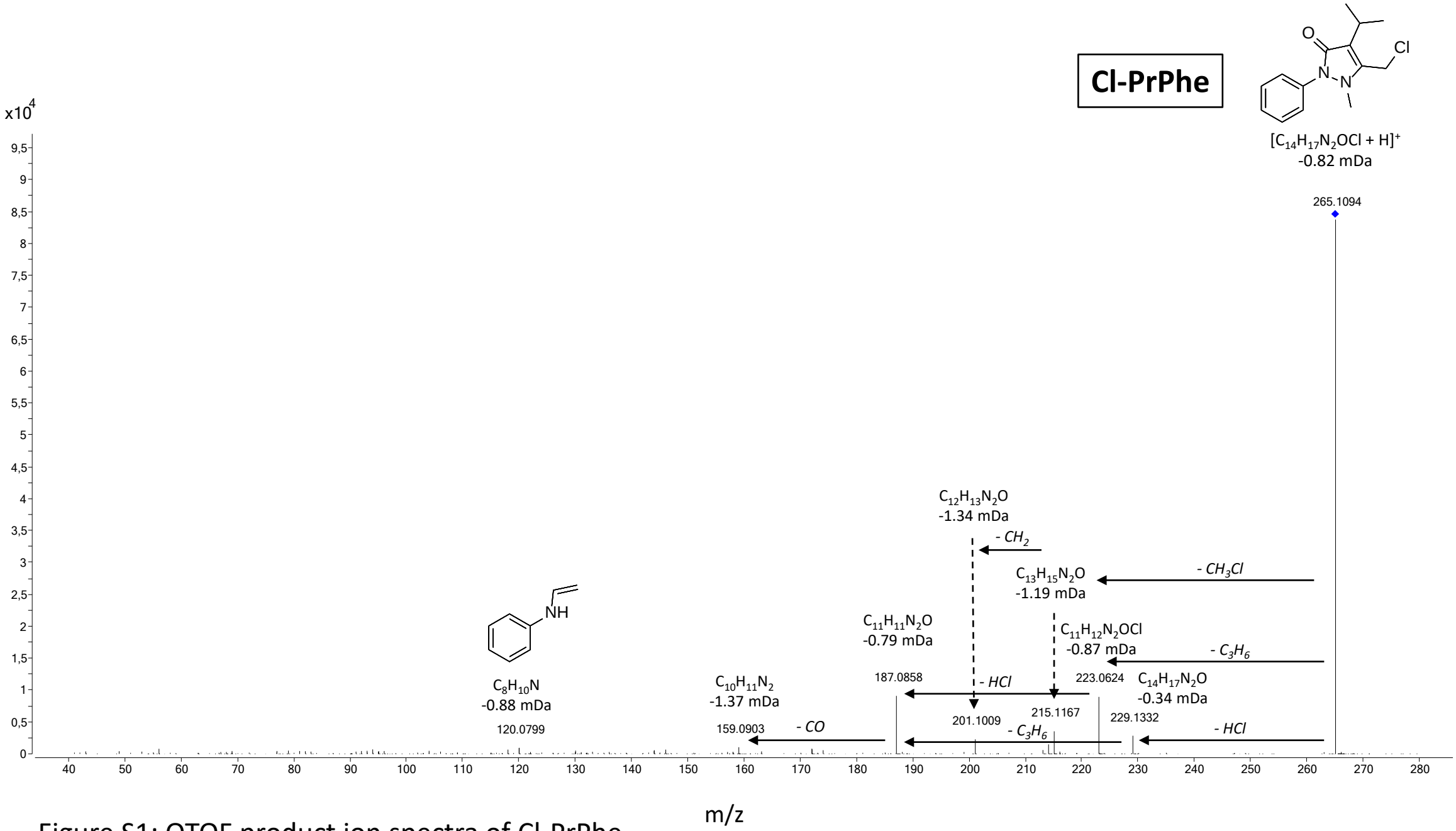


Figure S1: QTOF product ion spectra of Cl-PrPhe

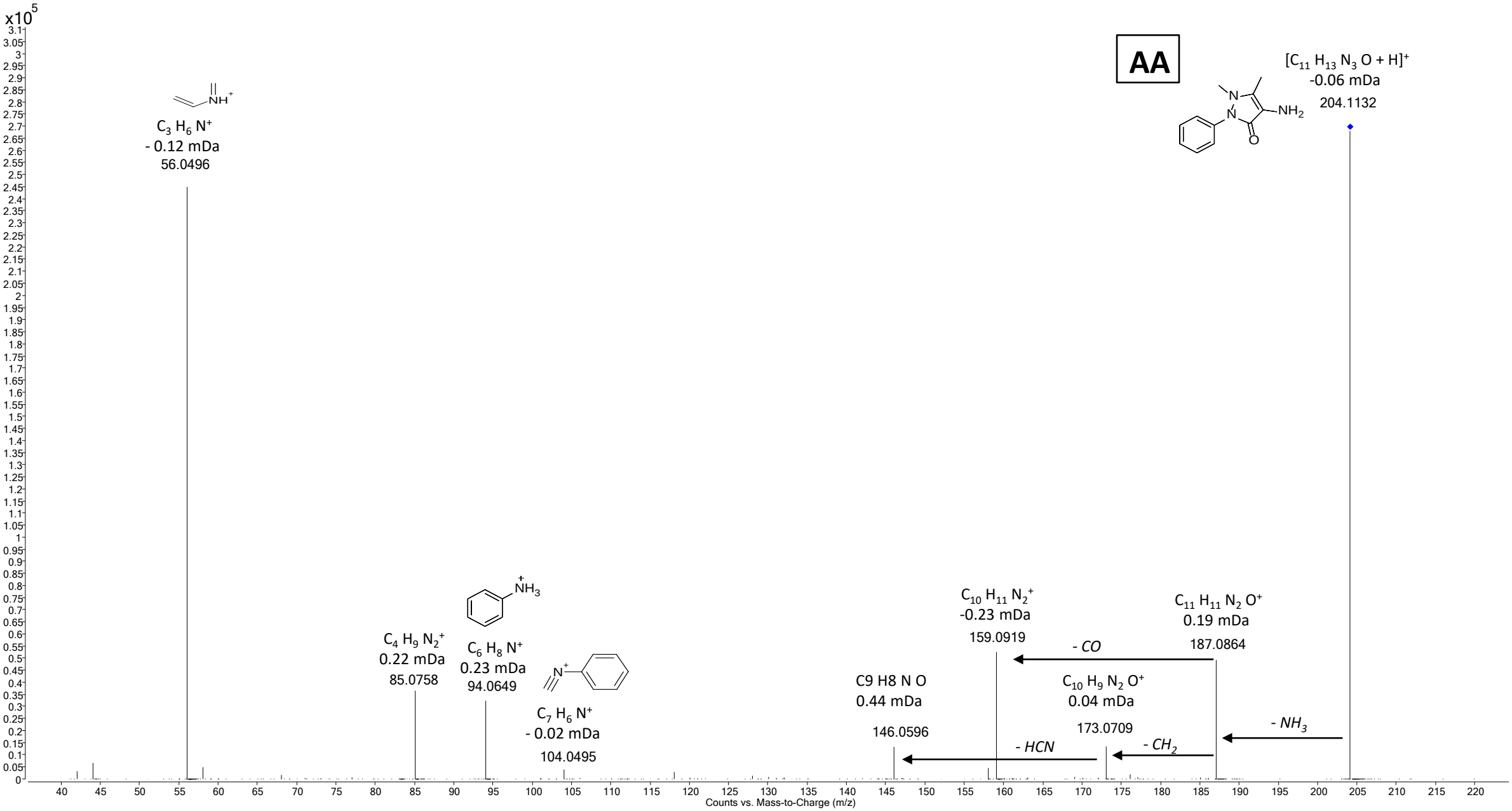


Figure S2: QTOF product ion spectra of AA, AAA and FAA and their TPs: (a) AA

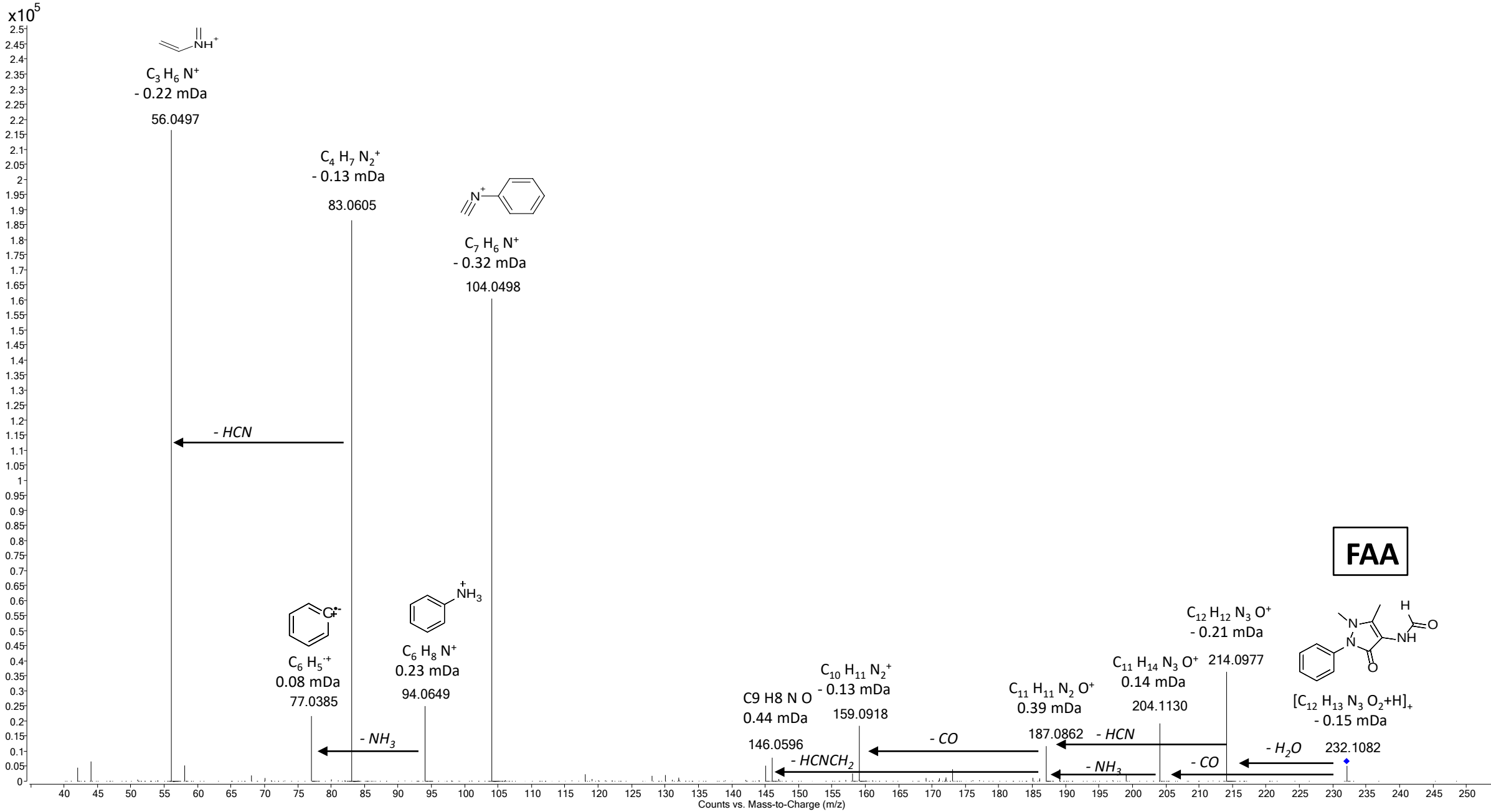


Figure S2: QTOF product ion spectra of AA, AAA and FAA and their TPs: (b) FAA

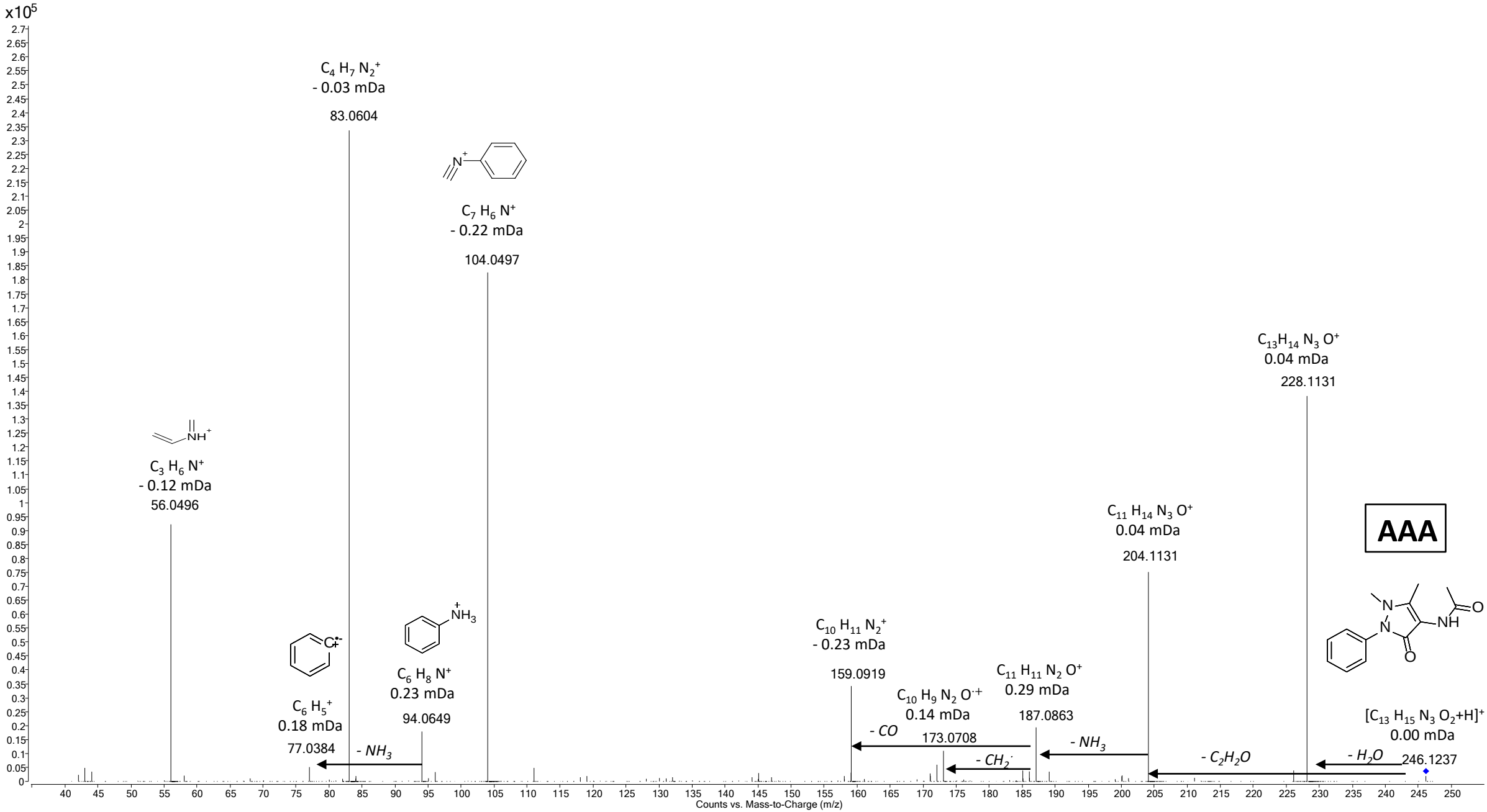


Figure S2: QTOF product ion spectra of AA, AAA and FAA and their TPs: (c) AAA

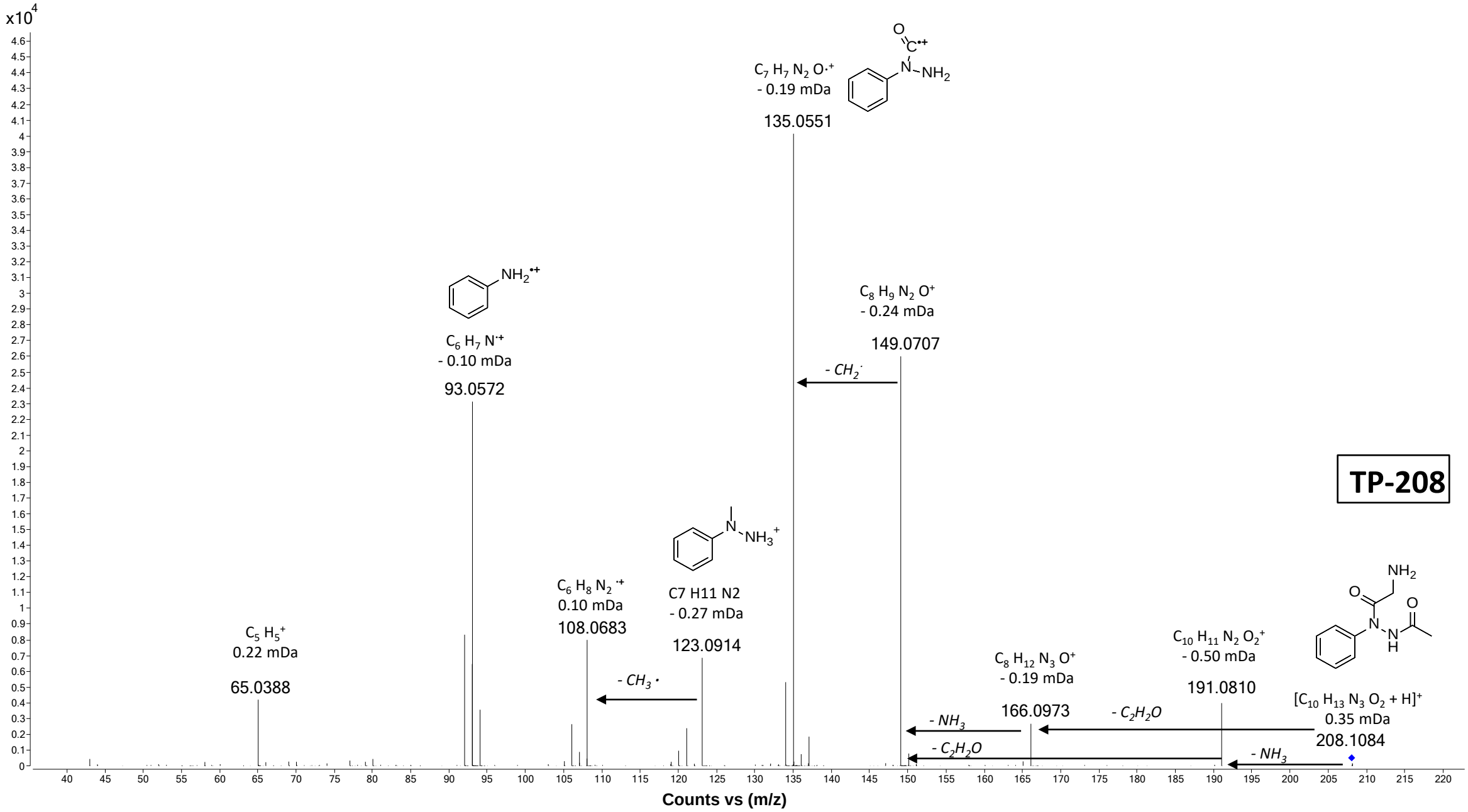


Figure S2: QTOF product ion spectra of AA, AAA and FAA and their TPs: (d) TP-208

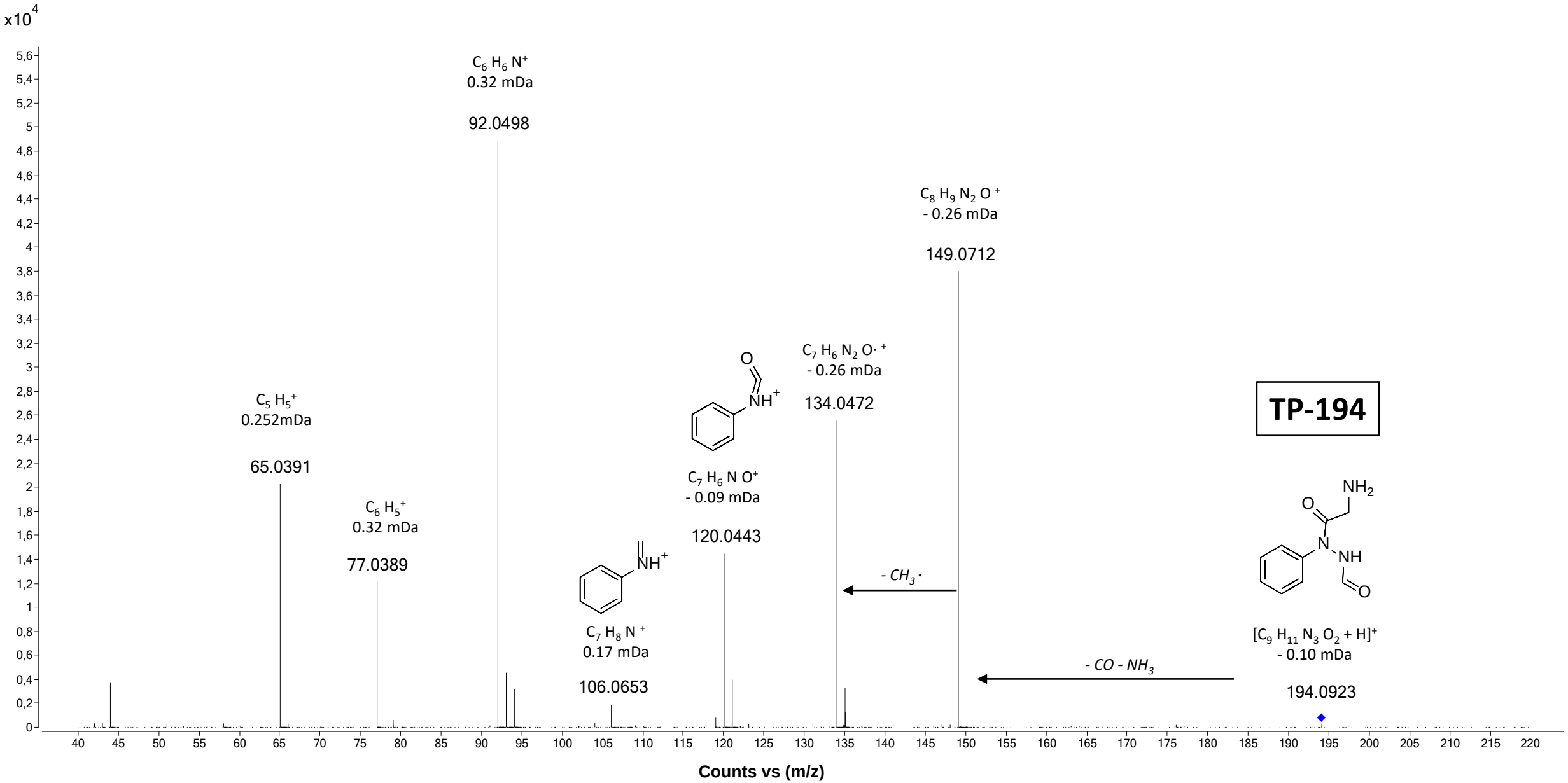


Figure S2: QTOF product ion spectra of AA, AAA and FAA and their TPs: (e) TP-194

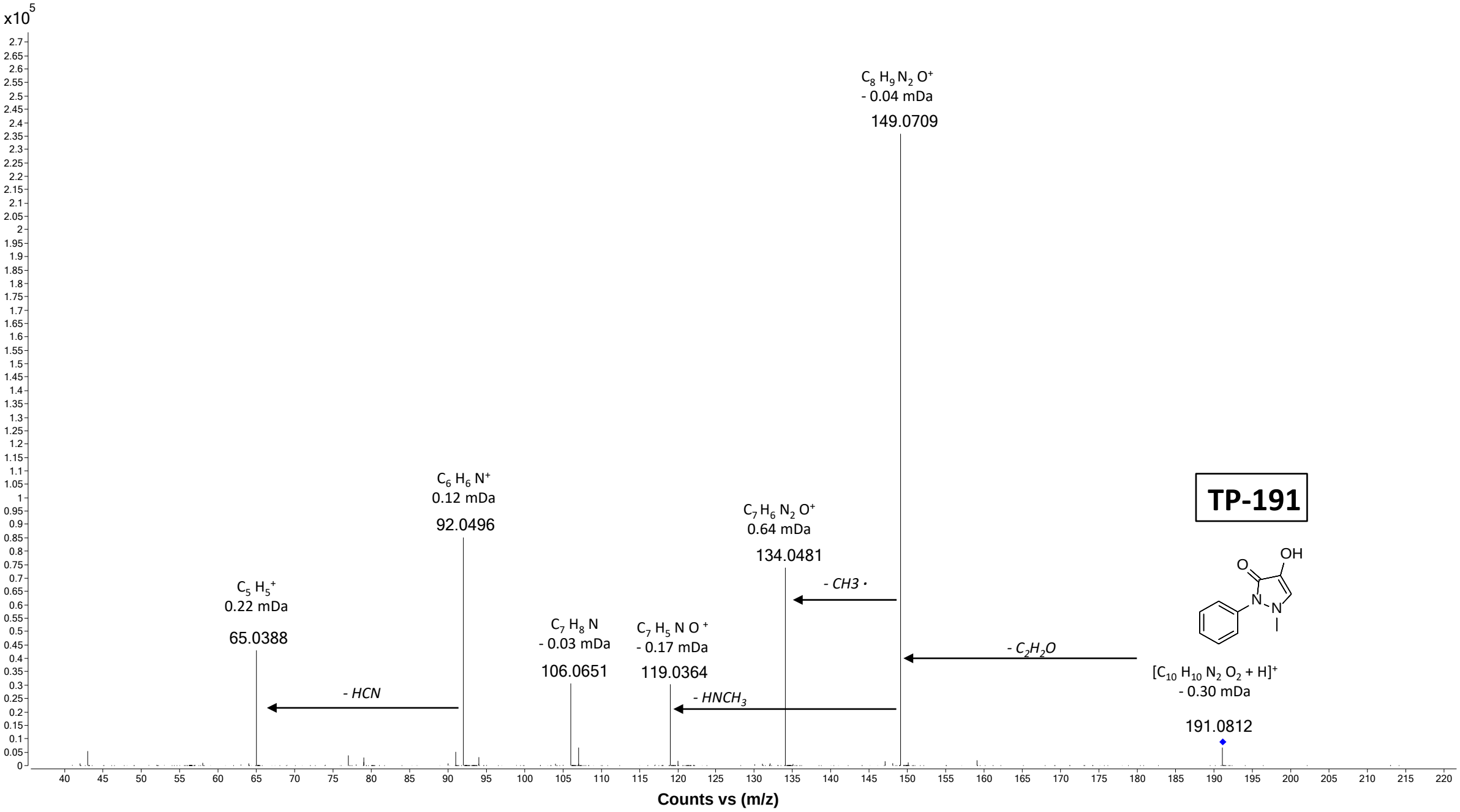


Figure S2: QTOF product ion spectra of AA, AAA and FAA and their TPs: (f) TP-191

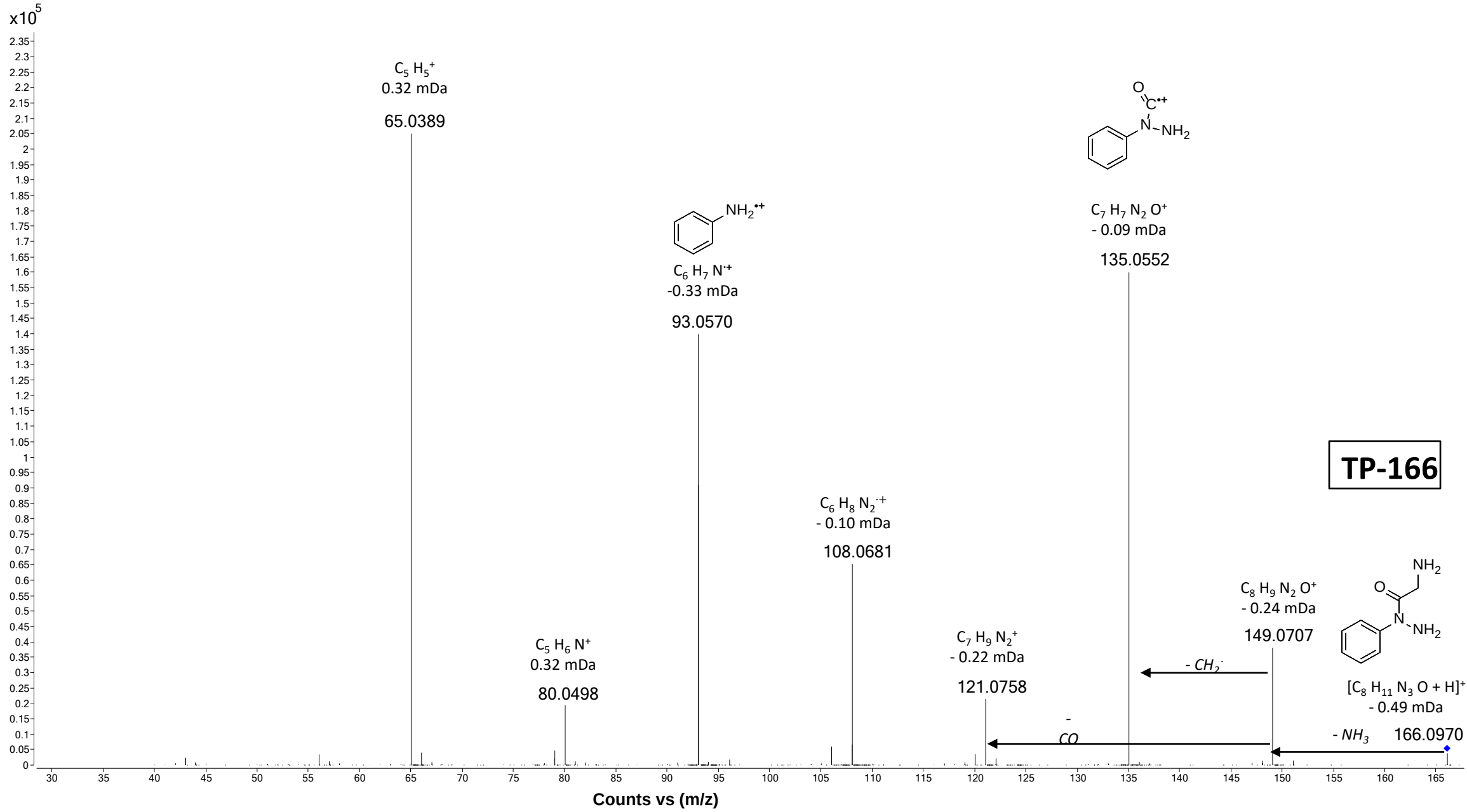


Figure S2: QTOF product ion spectra of AA, AAA and FAA and their TPs: (g) TP-166

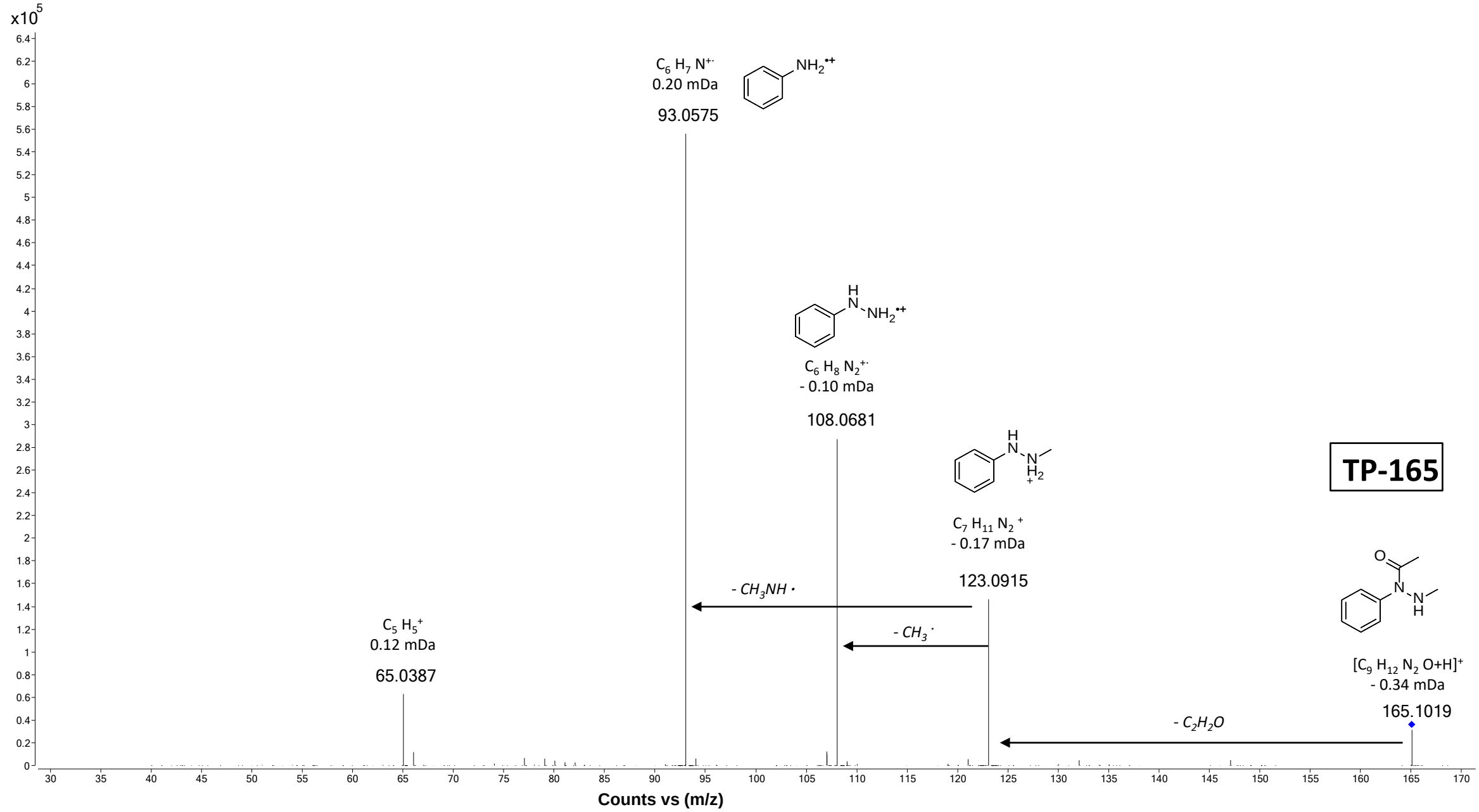


Figure S2: QTOF product ion spectra of AA, AAA and FAA and their TPs: (h) TP-165