Use of quadrupole time-of-flight mass spectrometry for proposal of transformation products of the herbicide bromacil after water chlorination

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ABSTRACT

The herbicide bromacil has been extensively used in the Spanish Mediterranean region. Although plant protection products containing bromacil have been withdrawn by the European Union, this compound is still frequently detected in surface and ground water of this area. However, the fast and complete disappearance of this compound has been observed in water intended for human consumption, after being subjected to chlorination. There is a concern about the possible degradation products formed, since they might be present in drinking water and might be potentially hazardous. In this work, the sensitive full-spectrum acquisition, high resolution and exact mass capabilities of hybrid quadrupole time-of-flight (QTOF) mass spectrometry have allowed the discovering and proposal of transformation products (TPs) of bromacil in water subjected to chlorination. Different ground water samples spiked at 0.5 μg/mL were subjected to the conventional chlorination applied for drinking waters, sampling 2-mL aliquots at different time intervals (1, 10 and 30 minutes). The corresponding non-spiked water was used as control sample in each experiment. Afterwards, 50 μL was directly injected in an ultra high pressure liquid chromatography (UHPLC)-electrospray (ESI)-(Q)TOF system. The QTOF instrument enabled the simultaneous recording of two acquisition functions at different collision energies (MS^E approach): the low energy (LE) function, fixed at 4 eV, and the high energy (HE) function, with a collision energy ramp from 15 to 40 eV. This approach enables the simultaneous acquisition of both parent (de)protonated molecules and fragment ions in a single injection. The low mass errors observed for parent compounds (detected in LE function) allowed the assignment of a highly probable molecular formula. Fragment ions as well as neutral losses were investigated in both LE and HE spectra to elucidate the structure of the TPs found. For those compounds showing poor fragmentation, product ion scan (MS/MS) experiments were also performed. After processing data with specialised software

(MetaboLynx), four bromacil TPs were detected and elucidated. Up to our knowledge, two of them had not been reported in the literature.

Keywords: herbicide bromacil, quadrupole time of flight mass spectrometry, transformation/ degradation products, water chlorination, elucidation.

INTRODUCTION

Nowadays, there is a growing concern about the presence of herbicides and their transformation products in the environment. In the Spanish Mediterranean region, an important agricultural area with predominance of citric crops, the herbicide bromacil has been extensively used for many years. Although plant protection products containing bromacil have been withdrawn by the European Union [1], it is still detected in environmental water [2]. The occurrence of bromacil in the aquatic environment has been confirmed in our recent analysis of ground and surface water of the Valencian region. In several occasions, bromacil concentrations were $\geq 0.1 \mu g/L$, the maximum allowed for drinking water.

Several treatment processes have been investigated for reducing pesticide concentrations in water and to minimize the potential health risks associated with the exposure to these chemicals by the consumption of contaminated waters. The most widely treatment applied to water intended for human consumption is chlorination. Preliminary experiments made at our laboratory showed the fast and complete disappearance of this compound after water chorination; therefore, there is an interest to know the possible degradation products formed, since they might be present in drinking water collected from ground or surface water contaminated by this herbicide, and the products might be potentially toxic.

Some works have reported the degradation of bromacil, after ozonation and photodegradation. One of the first works was reported by Acher et al [3], who identified the degradation by-products of bromacil by ozonation. The products were isolated and their structures elucidated by mass spectrometry, various ^{13}C and ^{1}H NMR spectroscopy, as well as chemical methods. Three main products were identified: two debrominated products, 3-sec-butyl-5-acetyl-5-hydroxyhydantoin and 3-sec-butylparabanic acid, and a dibromohydrin, 3-sec-butyl-5,5-dibromo-6-methyl-6-hydroxyuracil. A fourth product was not identified. Due to the formation of a HOBr adduct of bromacil, the biodegradability and phytotoxicity were not

improved after ozonation. Hapeman et al. [4] suggested that the conversion of bromacil by ozonation was mainly due to direct ozone attack mechanism and not to a hydroxyl radical mechanism. A kinetic model to predict the conversion of bromacil and the formation of the three by-products, during ozonation and O_3/H_2O_2 process, was developed by Torrents et al [5].More recently, a series of studies have reviewed by Ikehata et Gamal El-Din [6] on the degradation of bromacil by ozonation.

Photodecomposition of bromacil has also been investigated in aqueous solutions (using a mercury vapor-lamp with its main output at 254 nm) [7] although the products were not identified. Experiments simulating natural conditions by exposure of aqueous solutions to direct solar irradiation (summer) for 4 months yielded only 2.2% of a single dealkylated photoproduct, indicating that bromacil is very stable toward sunlight [8]. Acher et al [3] performed a comparative study of several oxidation methods of aqueous bromacil solutions. Ozonation [3], UV photolysis at 254 nm and sensitized sunlight photodegradation were examined. The main products found in UV photolysis were 3-sec-butyl-6-methyluracil and a dimer compound. By sensitized sunlight photodegradation, 3-sec-butyl-5-acetyl-5-hydroxyhydantoin and the dimer compound were identified. Recently, our group [9] investigated the degradation of bromacil in aqueous media after being irradiated with a UV-mercury lamp (254 nm). Two TPs were identified: 1-sec-butylurea and N-sec-(butyl(carboxy)carbamoyl)formamide.

In degradation experiments, the use of powerful identification tools such as quadrupole time-of-flight (QTOF) mass spectrometry is of great interest. The inherent high sensitivity of the TOF analyser in full acquisition facilitates the investigation of degradation/transformation processes of organic pollutants at relevant environmental levels (at ppb levels). Additionally, its elevated resolution (>10000 at full width half maximum, FWHM) and high mass accuracy (< 5ppm), allow us to obtain a predicted empirical formula with high reliability. In addition, an

interesting feature (MS^E) is feasible nowadays with modern QTOF instruments, which allows acquiring simultaneously two full spectrum acquisition functions with different collision energies. Using the low energy (LE) function, the information obtained corresponds normally to non-fragmented ions, related to the parent molecule. With the high energy (HE) function, more abundant fragmentation of the (de)protonated parent compound is obtained. Thus, the MS^E approach enables the simultaneous acquisition of both parent (de)protonated molecules and fragment ions in a single injection [10-13]. However, this approach is not a true product ion scan (where only the selected precursor ion is fragmented). If needed, product ion scan experiments at accurate mass can also be performed with a QTOF instrument to help in the elucidation process of TPs.

When investigating TPs in degradation experiments, it is important to have available specialised software to facilitate the process. In some cases, low abundant compounds might not be apparent by visual inspection and, therefore, powerful software with chromatographic peak deconvolution capabilities is required for automated component detection. Several software packages using different peak detection algorithms are available, and they are usually offered by the MS manufacturer. Such algorithms search extracted mass chromatograms for metabolites based on predicted or unpredicted molecular changes relative to the parent compound and thus aid in the detection and identification of unknowns, particularly those buried in baseline noise. The software compares mass spectral chromatograms of a control sample versus an analyte sample (i.e. metabolised, stressed or treated sample) and automates the detection, identification and reporting of metabolites/TPs [11].

In this paper, we investigate the formation of degradation/transformation products of bromacil in ground water after being subjected to chlorination. Ultra high pressure liquid chromatography (UHPLC)-QTOF MS has been applied for this purpose together with the

specialized software available in our instrument. Four bromacil TPs have been found and elucidated.

EXPERIMENTAL

Reagents and chemicals

Bromacil reference standard with purity 99% was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Stock solution (around 500 µg/mL) was prepared by dissolving reference standard in acetone and stored in a freezer at -20 ºC. Working solution was prepared by diluting stock solution with acetonitrile.

HPLC-grade water was obtained by purifying demineralised water in a Milli-Q plus system from Millipore (Bedford, MA, USA). Acetone (pesticide residue analysis), HPLC-grade methanol (MeOH), sodium hydroxide and formic acid (98–100%) were acquired from Scharlau (Barcelona, Spain). Leucine enkephalin, used as the lock mass, and imazalil, used during mass axis calibration, were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Dr. Ehrenstorfer, respectively.

Instrumentation

A Waters Acquity UPLC system (Waters Corporation, Milford, MA, USA) was interfaced to a hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (Q-oaTOF Premier, Waters Corporation, Manchester, UK), using an orthogonal Z-spray-electrospray (ESI) interface operating in both positive and negative ion modes. The UPLC separation was performed using an Acquity UPLC BEH C18 1.7 µm particle size analytical column 100 × 2.1mm (Waters Corporation, Milford) at a flow rate of 300 µL/min. The mobile phases used were A=H_2O with 0.01% HCOOH and B =MeOH with 0.01% HCOOH. The percentage of

organic modifier (B) was changed linearly as follows: 0 min, 10%; 9 min, 90%; 11 min, 90%; 11.1 min, 10%; 14min, 10%. Injection volume was 50 µL.

Desolvation gas as well as nebulising gas was nitrogen (Praxair, Valencia, Spain). The gas flow was set at 600 L/h. TOF-MS resolution was approximately 10,000 FWHM at *m/z* 556.2771. MS data were acquired over an *m/z* range of 50-1000. The microchannel plate (MCP) detector potential was set to 1950 V. A capillary voltage of 3.5 kV and cone voltage of 25 V were used in both positive and negative ionisation modes. Collision gas was argon 99.995% (Praxair, Valencia, Spain). The interface temperature was set to 350 ºC and the source temperature to 120 ºC. The column temperature was set to 60 ºC.

For MS^E experiments, two acquisition functions with different collision energies were created: the first one, the low energy function (LE), selecting a collision energy of 4 eV, and the second one, the high energy (HE) function, with a collision energy ramp ranging from 15 to 40 eV. The LE and HE functions settings were both a scan time of 0.1 s and an inter-scan delay of 0.05 s. For MS/MS experiments, a collision energy ramp from 15 to 40 eV was also used. The automated attenuated function was also selected to correct for possible peak saturations (extended mode). Calibrations were conducted in both ionisation modes from *m/z* 50 to 1000 with a 1:1 mixture of 0.05 M NaOH :5% HCOOH diluted (1:25) with acetonitrile:water (80:20) plus 500 µg/L imazalil (*m/z* $[M+H]^+$ 297.0561), at a flow rate of 10 µL/min. For automated accurate mass measurement, the lock-spray probe was used, using as lockmass a solution of leucine enkephalin (2 µg/mL) in acetonitrile:water (50:50) at 0.1% HCOOH pumped at 30 µL/min through the lock-spray needle. The protonated and deprotonated molecule of leucine enkephalin were used for recalibrating the mass axis and ensuring a robust accurate mass measurement along acquisition time in ESI+ (*m/z* 556.2771) and ESI- (*m/z* 554.2615), respectively. Cone voltages were selected to obtain adequate signal intensities for this compound (~ 400 counts).

It should be noted that all the exact masses shown in this work have a deviation of 0.55 mDa from the "true" value, as the calculation performed by the MassLynx software uses the mass of hydrogen instead of a proton when calculating $[M+H]^+$ exact mass. However, because this deviation is also applied during mass axis calibration, there is not negative impact on the mass errors presented in this article.

Analytical procedure

Three ground water samples from the province of Castellon (Eastern Spain) were collected in glass bottles (1 L) during the second week of May 2010. Samples were immediately stored at 4ºC until analysis.

50-mL of each water sample were spiked with bromacil at 0.5 µg/mL, by adding 0.5 mL of 50 µg/mL standard solution in acetonitrile. Additionally, 50-mL of non-spiked water were used as control samples. All experiments were also carried out at 20 µg/mL to facilitate the detection of minor TPs.

Sample aliquots of 2 mL were collected at different times. The first 2-mL aliquot was collected before chlorination (t=0). After chlorination (by adding 30 µL of 1% commercial sodium hypochlorite), 2-mL water aliquots were taken at different intervals (1, 10 and 30 minutes). Analysis were performed immediately by injecting directly 50 µL in the UPLC-QTOF MS system in full scan acquisition mode.

Data processing

MS data were acquired in centroid mode and processed using MetaboLynx software (MassLynx v 4.1) (Waters Corporation, Manchester), which has been proved to be highly useful in previous studies on pesticide metabolites/TPs performed at our laboratory [9, 14-16].

RESULTS AND DISCUSSION

MS^E experiments performed (at the high energy function) lead to fragmentation spectra similar to those of MS/MS experiments but also conserve the isotopic pattern of the fragments, as well as adduct and/or dimer information, as shown in **Figures 1** and **2**. In addition, both the (de)protonated molecule and fragment ion data are available in a single injection, without the need of selecting the precursor ion. Using this approach, the QTOF instrument is used in TOF mode, but promoting fragmentation in the collision cell in the HE function. In order to avoid spectrum interferences that would complicate the identification process, recognizing which ions are fragments, and which are not, becomes mandatory. In this sense, UHPLC turned valuable for choosing perfectly co-eluting ions.

Once acquired, data of this work were processed using the available processing package, which compares two LC-MS data files (sample and control). The differences resulting from the presence of new compounds, which should be, in principle, due to the transformation processes occurring in the sample, are highlighted. **Figure 3a** shows Total Ion Chromatograms (TICs) corresponding to the control (top) and the sample (bottom) after 1 minute of chlorination. At a first glance, there are no differences between them and not visible differential peaks are observed. However, after processing data using MetaboLynx, four TPs were found (**Table 1**). As an example, **Figure 3b** shows the narrow-window eXtracted Ion Chromatograms (nw-XICs), at *m/z* 175.1113, corresponding to the transformation product numbered as 3 (TP 3).

On the basis of their accurate masses, possible elemental compositions for the TPs detected were calculated using the elemental composition calculator tool, obtaining mass errors as shown in Table 1. Maximum and minimum parameter settings for all compounds were restricted as a function of the structure of bromacil ($C_9H_{13}BrN_2O_2$): C:0-11, H:0-20, O:0-10, N:

0-10. The number of Br was determined from the observed isotopic pattern and added if required (in this case, none of the TPs maintained the bromine atom in its structure). The applied double-bond equivalent (DBE) filter, which gives information about aromaticity of the structure, was set between 3.5 and 10. Additionally, the option ‘‘even-electrons ions only’’ was selected for the (de)protonated molecule.

Accurate masses of the neutral losses observed in both HE and LE spectra were investigated in order to reduce the number of possible molecular formulae and facilitate the prediction of a plausible structure. For the elemental composition calculation of fragment ions, the parameter settings were restricted by the calculated elemental composition of the (de)protonated TP, while for neutral losses no restrictions were used. In this case, the option ‘‘both odd and even-electrons ions only’’ was selected for calculating their elemental compositions. With these restrictions, the first proposed elemental composition was the right option in all cases. For those compounds which presented a poor MS^E fragmentation, product ion scan experiments (MS/MS experiments) were also carried out, to promote the production of more abundant fragment ions.

The results obtained in our experiment are summarized in **Tables 1** and **2**. These tables show MS data for the 4 TPs detected and for their main fragments, respectively. Mass errors of both (de)protonated molecule and fragment/product ions are shown. As it can be seen, most of deviations were lower than 2 mDa. After 30 minutes, TP1 still remained visible with a similar area, whereas the areas of the other TPs decrease considerably. Therefore, TP1 would be the most important from an environmental point of view.

Figure 4 shows the nw-XICs for parent bromacil and their TPs in positive and negative ionisation modes. As it can be seen, the presence of herbicide bromacil was not detected even only one minute after chlorination, while the peaks corresponding to three TPs (ESI+) and one more TP (ESI-) were clearly observed.

For identification of the new TPs, the fragmentation patterns of both the protonated and the deprotonated parent compound were firstly studied**. Figure 1** shows LE and HE functions of the parent bromacil in both positive and negative ionisation modes, as well as proposed fragment ions using MassFragment software. However, due to the important change in the structure of TPs, the fragmentation of the bromacil was not useful for elucidating them.

Proposal of transformation product 1.

Figure 5 illustrates the elucidation process for TP1. The accurate mass of the protonated molecule, with retention time 2.49 min, was measured to be *m/z* 117.1025. This mass differed 0.3 mDa from *m/z* 117.1028, which corresponds to an elemental composition of $[M+H]^+$ $C_5H_{13}N_2O$. After observing the structure of bromacil, it seems that the ring has been opened. As this compound presented poor fragmentation in both LE and HE spectra, MS/MS experiments were performed using the same collision energy ramp than in MS^E mode as well as at different fixed collision energies (10, 20, 30 and 40 eV) trying to obtain more abundant fragmentation. However, poor fragmentation still occurred (see **Figure 5**), although two low abundant product ions could be now observed. Product ions showed *m/z* 61.0388 (CH_5N_2O, ΔmDa=-1.4, as regards the theoretical exact mass) and 57.0693 (C_4H_9, ΔmDa=1.1, corresponding to the sec-butyl group) and were in accordance with the structure proposed in **Figure 5**. It is interesting to remark that this TP had been previously reported by our own group when studying the photodegradation of bromacil [9]. In that occasion, a poor fragmentation was also observed.

Proposal of TP 2.

Figure 6 shows the elucidation process for TP2. The accurate mass of the protonated molecule, with retention time 2.53 min, was found to be *m/z* 233.1142. This mass differed 0.5

mDa from *m/z* 233.1137, which corresponds to an elemental composition of $[M+H]^+$ $C_9H_{17}N_2O_5$. This could be due to the loss of bromine (Br isotopic pattern was not observed) and an increase of three atoms of oxygen and two atoms of hydrogen, suggesting that an oxidative process had occurred.

The LE spectra of TP2 ($[M+H]^+$ $C_9H_{17}N_2O_5$, *m/z* 233.1142, ΔmDa=0.5) already showed fragment ions at *m/z* 215.1028 (ΔmDa=-0.6, as regards the theoretical exact mass), 197.0938 (ΔmDa=1.2), and 177.0526 (ΔmDa=1.5), which resulted from losses of one molecule of water, two molecules of water and the butene group, respectively, from the parent ion *m/z* 233.1142. Fragment ions at *m/z* 159.0411 (ΔmDa=0.5) and 141.0304 (ΔmDa=0.4) were also observed, which resulted from losses of one and two molecules of water, respectively, from the ion *m/z* 177.0526. A fragment ion at *m/z* 169.0968 was also observed, corresponding to the direct loss of water plus formic acid from the parent ion. As it has been previously commented, when working with MS^E approach, it is important to recognize which ions are fragments, and which are not. **Figure 6** shows nw-XICs for protonated molecule and several fragment ions. As it can be see, all ions perfectly coelute.

On the basis of this information, two possible structures for this TP were proposed. The structures suggested are supported by our experimental accurate data. Both structures would match with the butene loss, which indicates that no oxidation has been carried out in this group. Moreover, it would not involve the reduction of a carbonyl group (usually, the chlorination is an oxidative process) and therefore ring and/or lateral chain would have been oxidized. This TP was only observed in ESI positive mode. In this case, although it is very difficult to predict whether it should also show up in negative-ion mode, having an –N-COOH group looks quite logical its no detection in negative-ion mode. The ring opening was the only way to explain a DBE of 3 in the molecule without producing a reduction. Moreover, these structures would also explain the fragment ion at *m/z* 169.0968 as a formic acid loss from *m/z* 215.1028 At this point,

it would be interesting to make additional experiments with, for example, labeled standards or trying to synthesize this compound to have better support on the structure suggested. Up to our knowledge, this TP has not been previously reported in the literature.

Proposal of TP 3.

Regarding TP3 (retention time 5.32 min), accordingly to the accurate mass measurement, the elemental composition of the protonated molecule was found to be $C_7H_{15}N_2O_3$ (ΔmDa=3). This implies the loss of two carbon atoms respect to bromacil and the increase of one atom of oxygen, apart from the bromine loss. In this case, two fragment ions were also observed in the LE spectra. The fragment at *m/z* 119.0473 ($C_3H_7N_2O_3$, ΔmDa=1.6) was due to a butene loss, therefore indicating that this group was still present in this TP. The structure suggested is shown in **Figure 7,** which also would explain the fragment at *m/z* 76.0408 (ΔmDa=0.9). The loss of two carbon atoms would surely be produced by a rearrangement in the ring during bromine loss. Up to our knowledge, this TP has not been previously reported in the literature.

Proposal of TP 4.

This TP was only observed in ESI negative mode. The elemental composition of the deprotonated molecule corresponding to TP4 (3.96 min) was found to be $C_7H_9N_2O_3$ (ΔmDa=1). Comparing this elemental composition with the deprotonated molecule of bromacil ($C_9H_{12}BrN_2O_2$) also a loss of two atoms of carbon was observed, suggesting the same rearrangement than in TP3. The increase in one atom of oxygen together with the decrease in the number of hydrogens suggested an oxidation to ketones. We propose the structure shown in **Figure 8,** which also explains the negative ionization because of the two keto groups

surrounding the NH, which makes the hydrogen very acidic. This structure has some similarities to barbiturates (also ring with C=O and NH, and one alkyl-substitued N), which also primarily show up in negative-ion mode rather than on positive ion mode. So, it seems reasonable that this compound was not detected in positive-ion mode. This TP had already been reported by Acher et al [3] and Hapemman et al [4]. In this case, no fragment ions were obtained after performing MS/MS experiments or after increasing the ramp in the HE function. Therefore, little information was available for a further confirmation of its identity.

As a summary, **Figure 9** shows the structures suggested for different bromacil TPs formed in water after being subjected to ozonolysis or photodegradation (reported in the literature) and after chlorination experiments (results of this paper). Three TPs have been reported in ozonolysis experiments. One of these compounds has also been detected in the chlorination process (TP4). Regarding photodegradation, five TPs had been identified until now, and again one of these TPs has been found in the chlorination experiments [TP1]. Although standards for reported TPs are not commercially available, it seems that TP1 is the major TP detected after the chlorination process. Two additional TPs have been identified in the chlorination experiments (TPs 2 and 3). Up to our knowledge, they have not been previously reported in the literature.

CONCLUSIONS

This work has demonstrated that hybrid quadrupole time of flight (QTOF) mass spectrometry is a valuable technique for proposal of transformation products of selected organic contaminants in degradation experiments. In this article, herbicide bromacil TPs have been investigated in groundwater after being subjected to chlorination. The complete disappearance of bromacil was observed, even only one minute after chlorination. Combining MS^E and MS/MS experiments has allowed the detection and identification of four TPs after direct injection of aqueous sample extracts (control and spiked), without the need of laborious sample pre-treatment, and minimising possible losses of analytes along the analytical procedure. On the basis of the results obtained, in those waters contaminated by bromacil and subjected to chlorination, it would be more interesting the investigation of the presence of the TPs reported in this work than the bromacil parent, due to its rapid degradation.

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TABLES

Table 1. Exact mass measurements and mass errors for the bromacil TPs detected

Proposed compound	ret time (min)	Ionisation modes	Elemental composition $[M+H]^+$/ $[M-H]^-$	Experimental mass $[M+H]^+$/ $[M-H]^-$	theoretical mass $[M+H]^+$/ $[M-H]^-$	Deviation (mDa)
parent	5.6	ESI+	$C_9H_{14}BrN_2O_2$	261.0243	261.0239	0.4
		ESI-	$C_9H_{12}BrN_2O_2$	259.0089	259.0082	0.7
TP1	2.5	ESI+	$C_5H_{13}N_2O$	117.1025	117.1028	-0.3
TP2	2.5	ESI+	$C_9H_{17}N_2O5$	233.1142	233.1137	0.5
TP3	5.3	ESI+	$C_7H_{15}N_2O_3$	175.1113	175.1083	3
TP4	3.9	ESI-	$C_7H_9N_2O_3$	169.0603	169.0613	-1

Table 2. Transformation products identified in the degradation study of bromacil. Mass fragments and mass errors for the proposed compounds obtained by UHPLC-ESI-(Q)TOF MS.

Compound	Ionization mode	MS mode	Theoretical mass fragments	Deviation (mDa)	Fragment ions or losses	
bromacil	ESI+	MS^E	204.9613	1.1		-butene
			187.9347	1.2	$C_5H_3NO_2Br$	
			161.9554	1.6	C_4H_5NOBr	
			131.9449	1.5	C_3H_3NBr	
	ESI-	MS^E	202.9456	-1.0		-butene
			159.9398	-0.3	C_4H_3NOBr	
			78.9183	2.1	Br	
TP1	ESI+	MS/MS (117)*	61.0402	-1.4		-butene
			57.0704	1.1	sec-butyl	
TP2	ESI+	MS^E	215.1034	-0.6		$-H_2O$
			197.0926	1.2		$-2H_2O$
			177.0511	1.5		-butene
			169.0977	-0.9		$-H_2O$-HCOOH
			159.0406	0.5		-butene-H_2O
			141.0300	0.4		-butene-$2H_2O$
TP3	ESI+	MS^E	119.0457	1.6		-butene
			76.0399	1.1	$C_2H_6NO_2$	
TP4	ESI-	MS^E	-			

*Precursor ion

FIGURE CAPTIONS

Figure 1. LE and HE spectra of bromacil for both ionization modes.

Figure 2. MS/MS spectra of bromacil in both ionization modes, using as precursor ions the (de)protonated molecule and the peak corresponding to the isotopic pattern.

Figure 3. (a) Total Ion Chromatograms and (b) eXtracted Ion Chromatograms at *m/z* 175.1113, for control (top) and analyte samples (bottom) (T=1 min).

Figure 4. UHPLC-(Q)TOF MS eXtracted Ion Chromatograms in positive and negative ionization mode (LE funtion) corresponding to parent bromacil and the four TPs detected in ground water spiked with bromacil at 20 μg/mL: (a) before chlorination and (b) one minute after chlorination

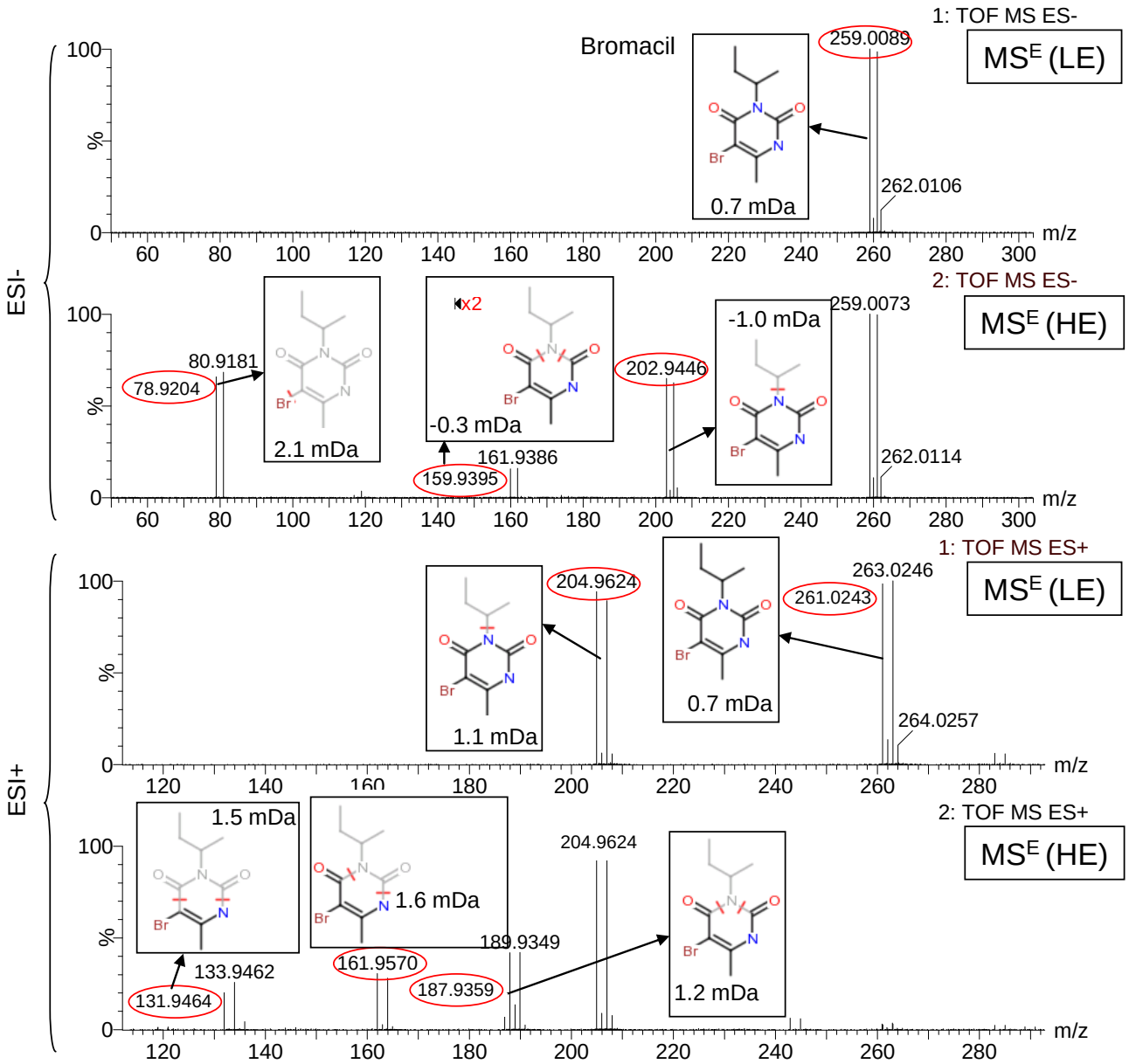
Figure 5. UHPLC-QTOF MS chromatogram, full scan spectra (LE) and product ion spectra of TP1 in positive ionization mode.

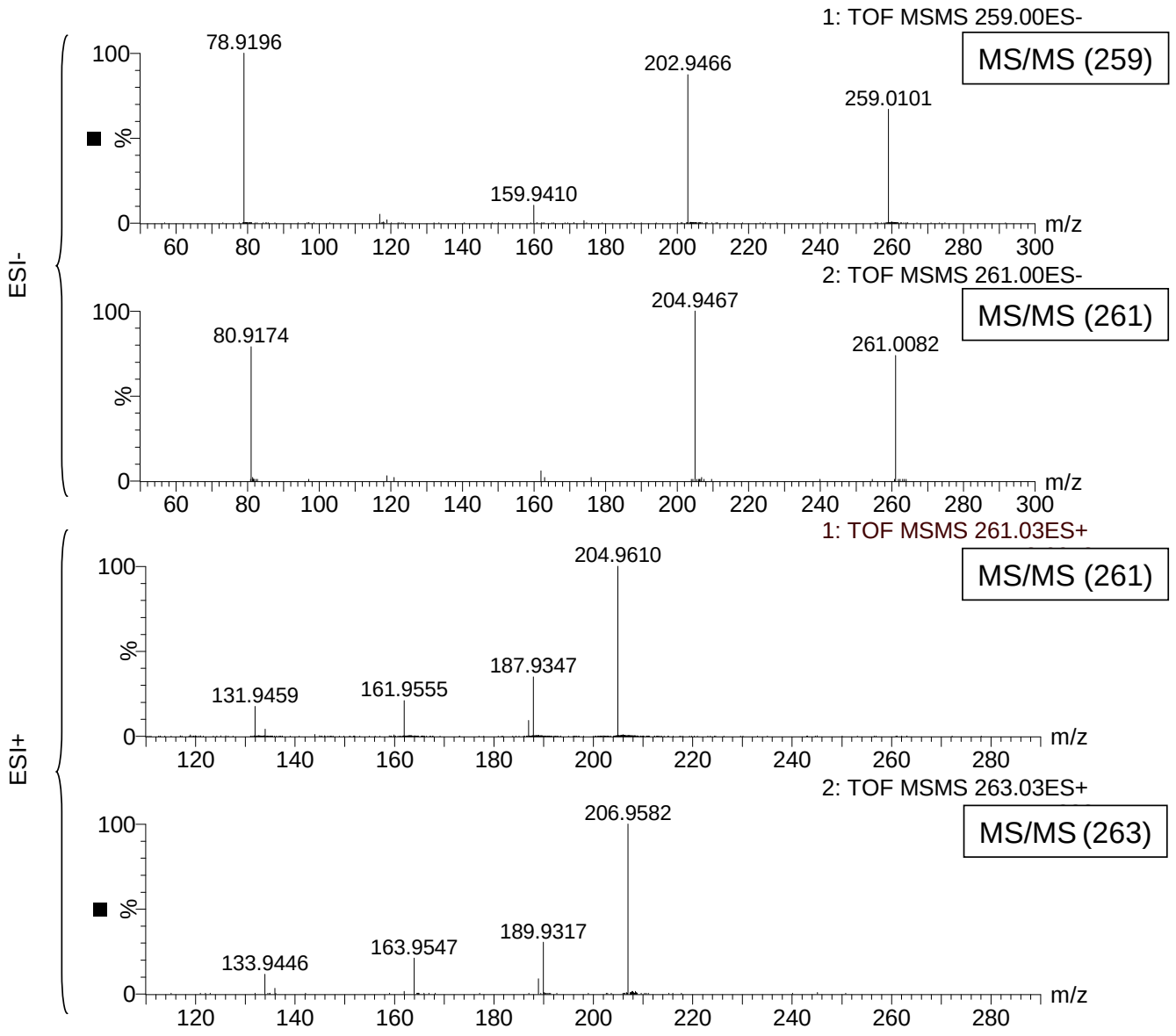
Figure 6. UHPLC-(Q)TOF MS chromatogram and full scan spectra (LE) of TP2 in positive ionization mode. XICs at 20 mDa mass window for the protonated molecule and the two fragment ions observed in LE function.

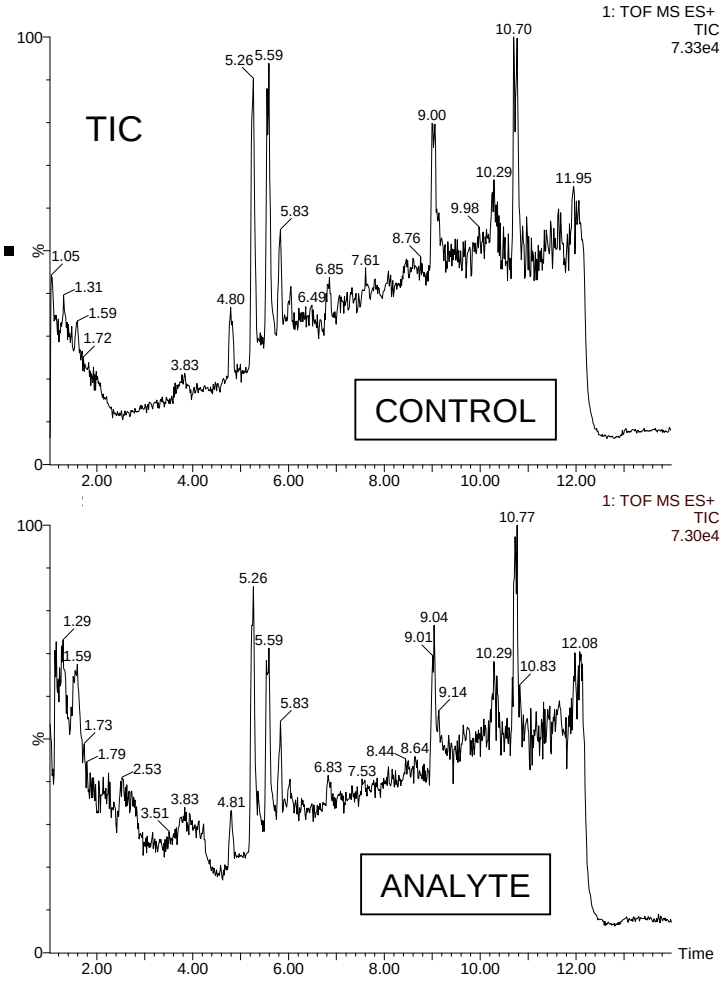
Figure 7. UHPLC-(Q)TOF MS chromatogram and full scan spectra (LE) of TP3 in positive ionization mode. XICs at 20 mDa mass window for the protonated molecule and different fragment ions observed in LE function.

Figure 8. UHPLC-(Q)TOF MS chromatogram and full scan spectra (LE) of TP4 in negative ionization mode.

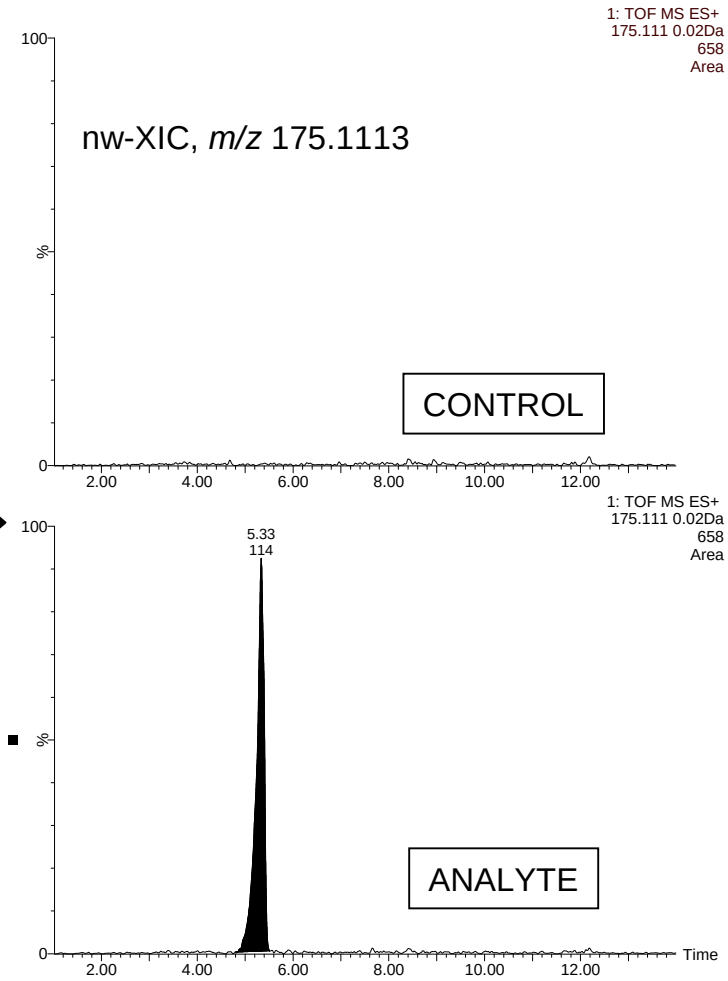
Figure 9. Bromacil TPs identified in different degradation experiments: (ch) TPs detected after water chlorination (this work), (ph) TPs detected in photodegradation experiments (254 nm), (ph*) TPs detected in sensitized sunlight photodegradation (400-700 nm), (oz) TPs detected after ozonolysis.


ESI-
ESI+
Bromacil
1: TOF MS ES-
MS^E (LE)
259.0089
262.0106
0.7 mDa
2: TOF MS ES-
MS^E (HE)
80.9181
78.9204
2.1 mDa
x2
-0.3 mDa
161.9386
159.9395
202.9446
-1.0 mDa
259.0073
262.0114
1: TOF MS ES+
MS^E (LE)
204.9624
1.1 mDa
263.0246
261.0243
0.7 mDa
264.0257
2: TOF MS ES+
MS^E (HE)
1.5 mDa
133.9462
131.9464
1.6 mDa
161.9570
187.9359
189.9349
204.9624
1.2 mDa
m/z
%

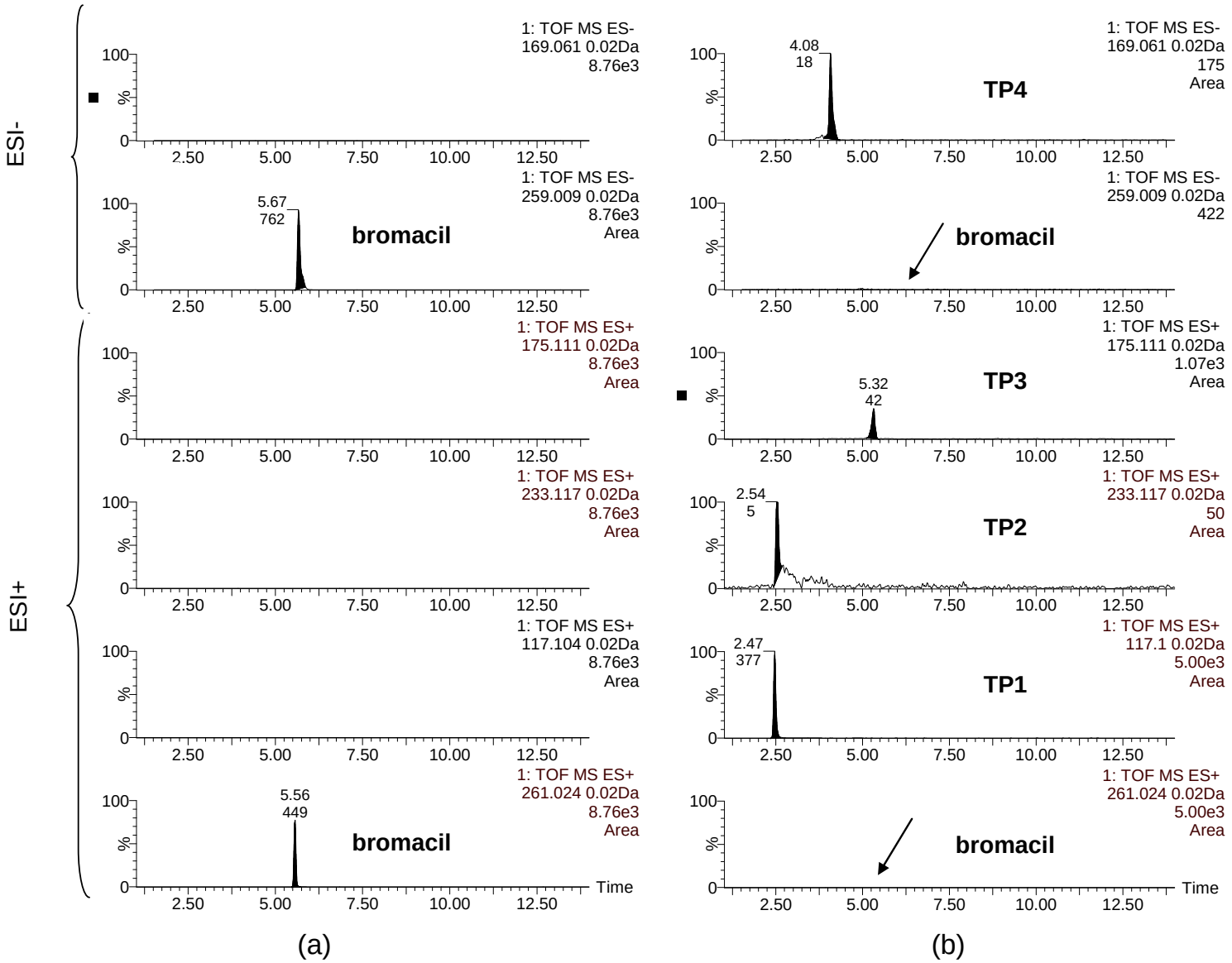

ESI-
1: TOF MSMS 259.00ES-
MS/MS (259)
78.9196
202.9466
259.0101
159.9410
100
%
0
m/z
60 80 100 120 140 160 180 200 220 240 260 280 300
2: TOF MSMS 261.00ES-
MS/MS (261)
204.9467
80.9174
261.0082
100
%
0
m/z
60 80 100 120 140 160 180 200 220 240 260 280 300
ESI+
1: TOF MSMS 261.03ES+
MS/MS (261)
204.9610
187.9347
131.9459
161.9555
100
%
0
m/z
120 140 160 180 200 220 240 260 280
2: TOF MSMS 263.03ES+
MS/MS (263)
206.9582
189.9317
163.9547
133.9446
100
%
0
m/z
120 140 160 180 200 220 240 260 280

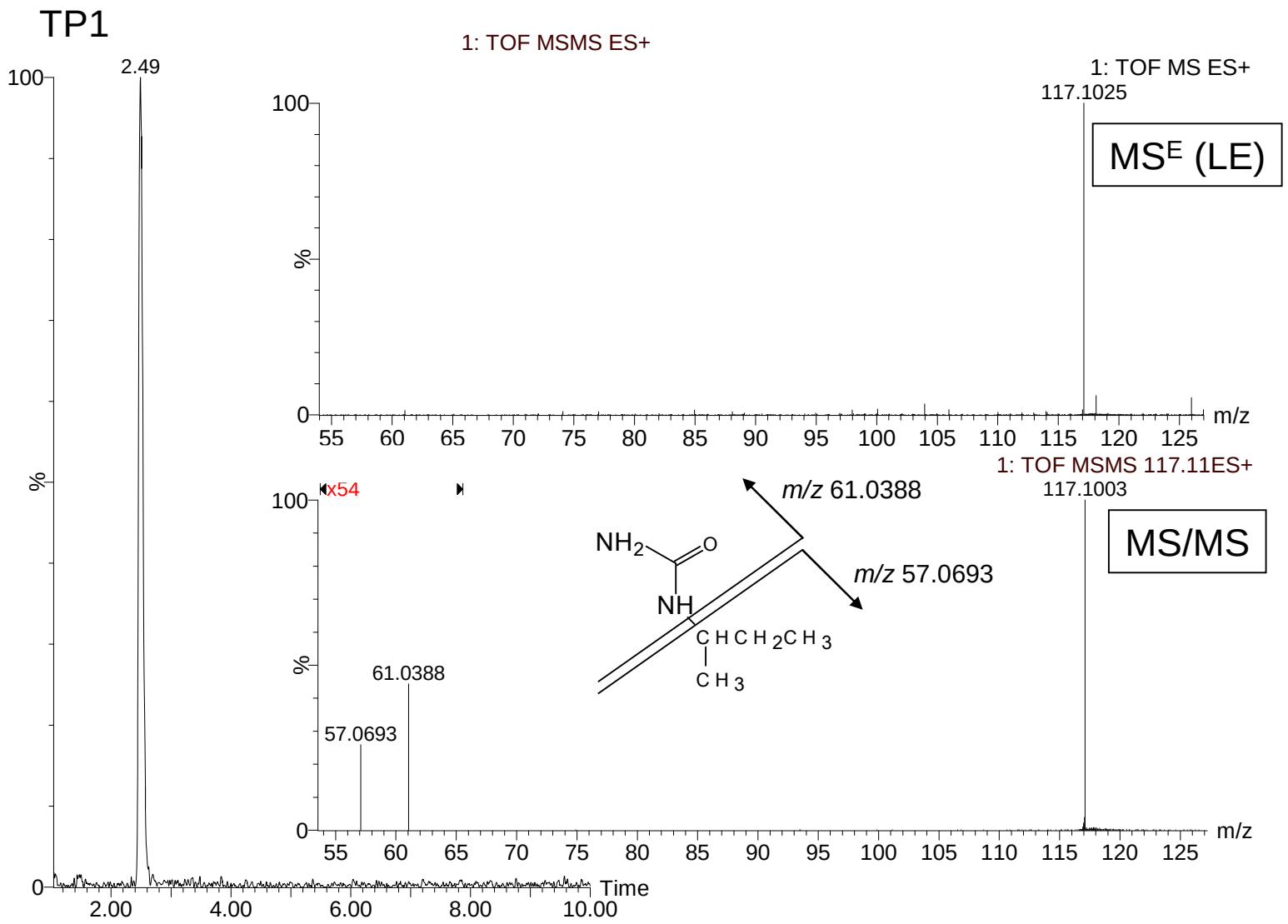


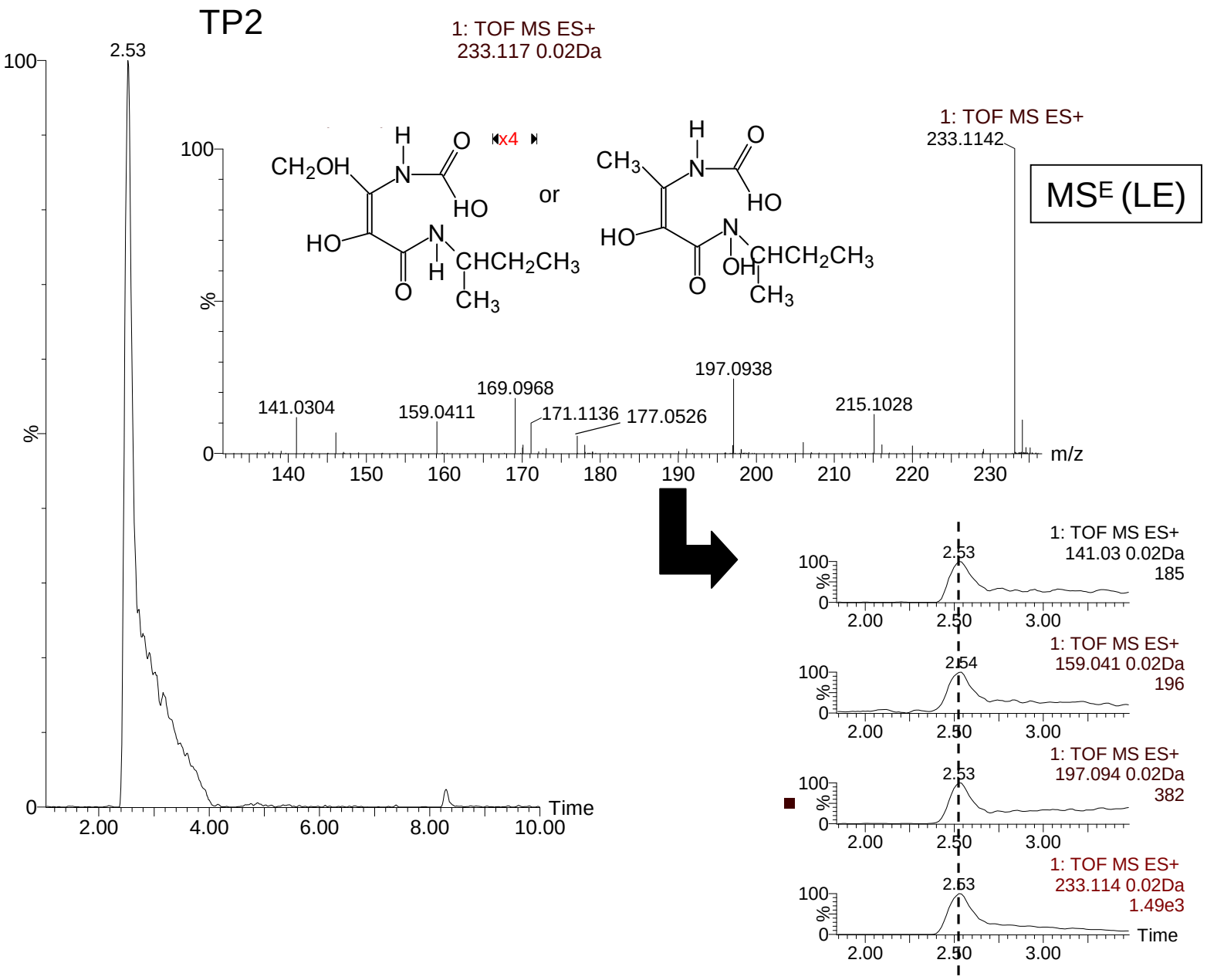
MetaboLynx

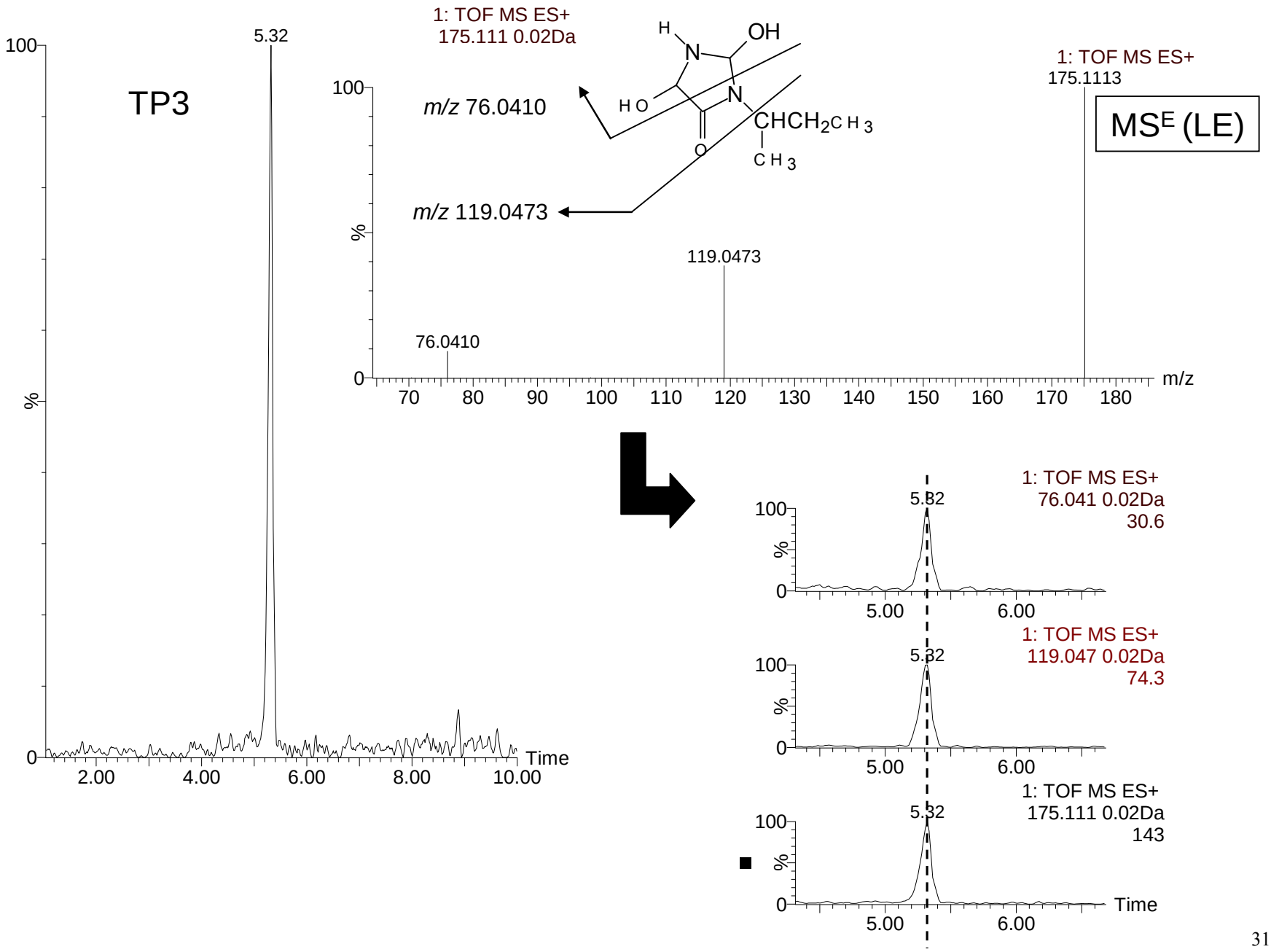


(a) (b)

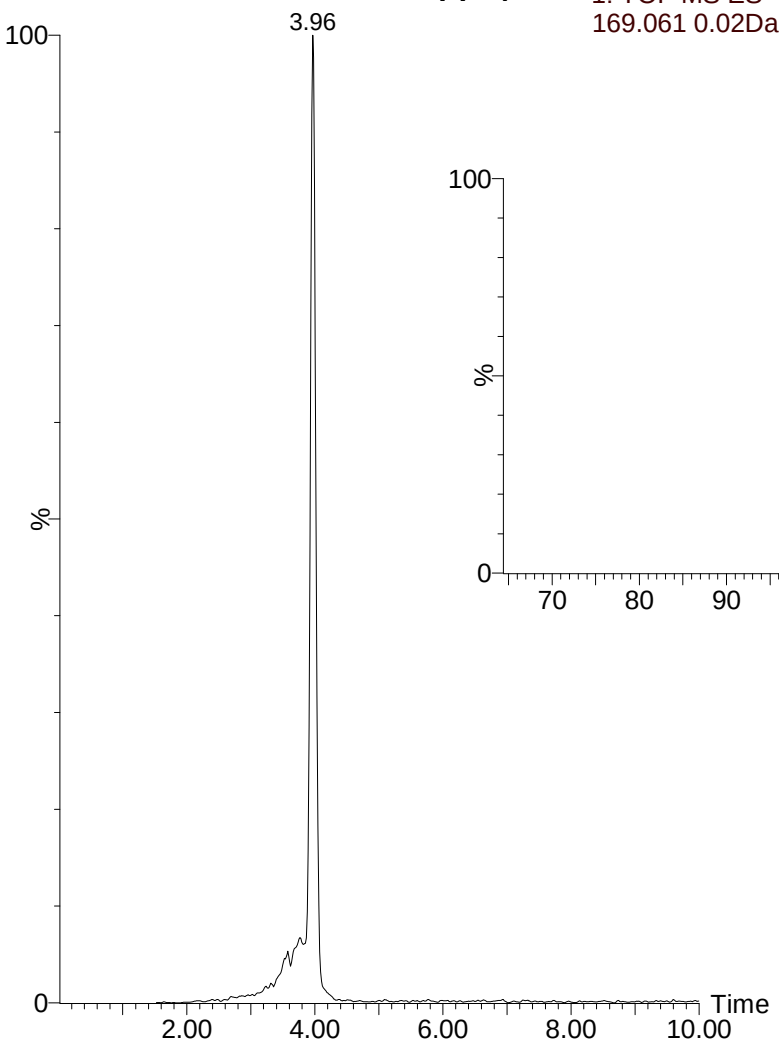

ESI-
ESI+
1: TOF MS ES-
169.061 0.02Da
8.76e3
1: TOF MS ES-
259.009 0.02Da
8.76e3
Area
5.67
762
bromacil
1: TOF MS ES+
175.111 0.02Da
8.76e3
Area
1: TOF MS ES+
233.117 0.02Da
8.76e3
Area
1: TOF MS ES+
117.104 0.02Da
8.76e3
Area
1: TOF MS ES+
261.024 0.02Da
8.76e3
Area
5.56
449
bromacil
Time
100
%
0
2.50
5.00
7.50
10.00
12.50
(a)
4.08
18
TP4
1: TOF MS ES-
169.061 0.02Da
175
Area
1: TOF MS ES-
259.009 0.02Da
422
bromacil
1: TOF MS ES+
175.111 0.02Da
1.07e3
Area
5.32
42
TP3
1: TOF MS ES+
233.117 0.02Da
50
Area
2.54
5
TP2
1: TOF MS ES+
117.1 0.02Da
5.00e3
Area
2.47
377
TP1
1: TOF MS ES+
261.024 0.02Da
5.00e3
Area
bromacil
Time
(b)


TP1
2.49
100
%
0
2.00
4.00
6.00
8.00
10.00
Time
1: TOF MSMS ES+
100
%
0
55 60 65 70 75 80 85 90 95 100 105 110 115 120 125
m/z
1: TOF MS ES+
117.1025
MS^E (LE)
1: TOF MSMS 117.11ES+
117.1003
MS/MS
x54
61.0388
57.0693
m/z 61.0388
m/z 57.0693
NH2
O
NH
C H C H 2C H 3
C H 3

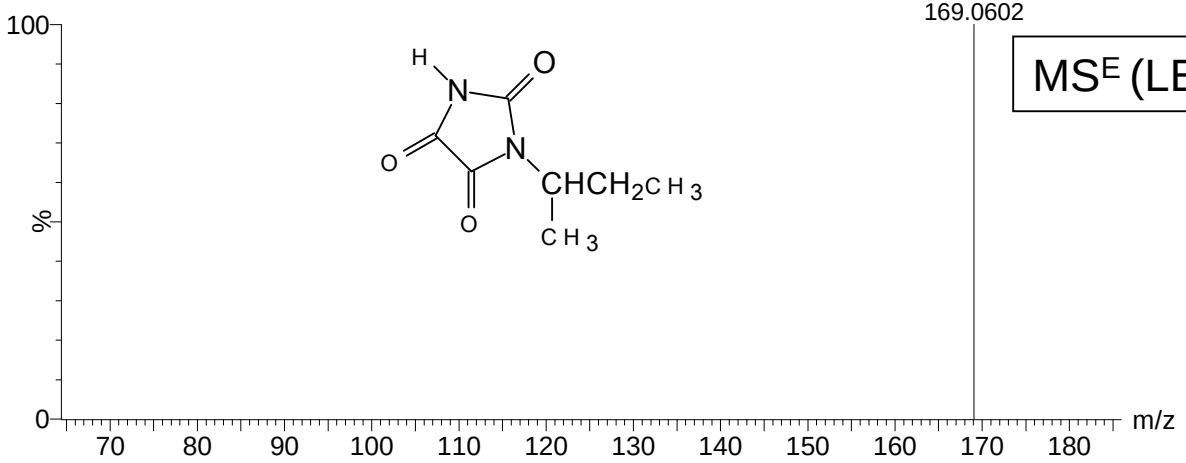

TP2
1: TOF MS ES+
233.117 0.02Da
2.53
100
%
0
2.00
4.00
6.00
8.00
10.00
Time
x4
or
1: TOF MS ES+
233.1142
MS^E (LE)
141.0304
159.0411
169.0968
171.1136
177.0526
197.0938
215.1028
140
150
160
170
180
190
200
210
220
230
m/z
1: TOF MS ES+
141.03 0.02Da
185
1: TOF MS ES+
159.041 0.02Da
196
1: TOF MS ES+
197.094 0.02Da
382
1: TOF MS ES+
233.114 0.02Da
1.49e3


TP3
5.32
100
%
0
2.00
4.00
6.00
8.00
10.00
Time
1: TOF MS ES+
175.111 0.02Da
m/z 76.0410
m/z 119.0473
H
N
OH
HO
N
O
CHCH2CH3
CH3
119.0473
76.0410
70
80
90
100
110
120
130
140
150
160
170
180
m/z
1: TOF MS ES+
175.1113
MSE (LE)
1: TOF MS ES+
76.041 0.02Da
30.6
1: TOF MS ES+
119.047 0.02Da
74.3
1: TOF MS ES+
175.111 0.02Da
143
5.00
6.00

TP4

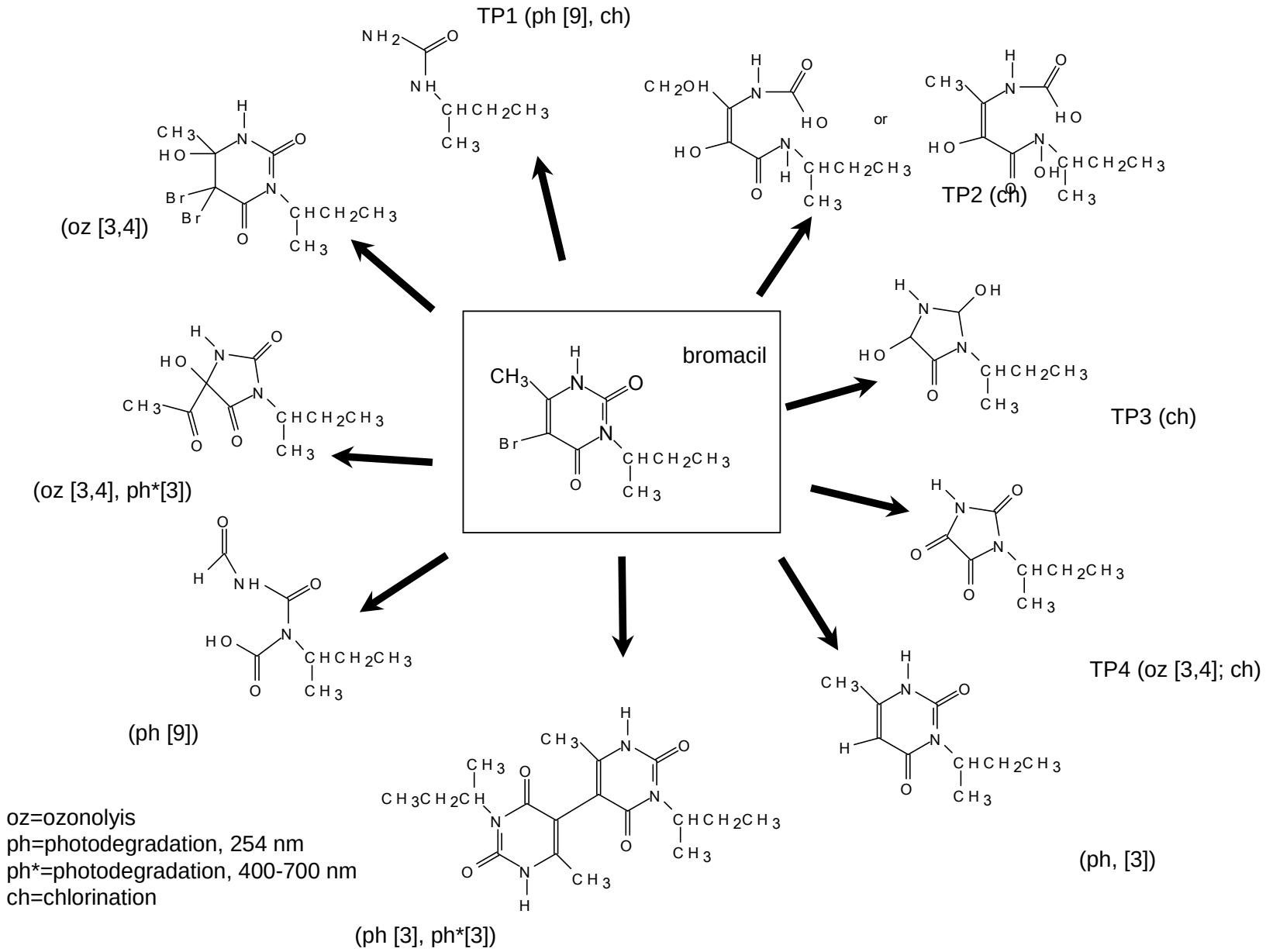


1: TOF MS ES-
169.061 0.02Da



1: TOF MS ES-
169.0602

MS^{E} (LE)


TP1 (ph [9], ch)
or
TP2 (ch)
(oz [3,4])
bromacil
TP3 (ch)
(oz [3,4], ph*[3])
TP4 (oz [3,4]; ch)
(ph [9])
(ph, [3])
(ph [3], ph*[3])
oz=ozonolyis
ph=photodegradation, 254 nm
ph*=photodegradation, 400-700 nm
ch=chlorination