Supporting Information

Discovery of Chlorine Exposure Signatures in Plant Material Using Targeted and Comparative Mass Spectrometry Methods

Jayda E. Meisel*, Cory Fix, Jerry Casbohm, Amy Hill, James Ficker, Christina Saeger, Sarah Dreher, Michael Murray, Craig Shepherd, Kristyn Johnson, and Mark Bauer

[1]Battelle Memorial Institute. 505 King Avenue Columbus, Ohio 43201

*Corresponding Author

Table 1 Condensed Table of Unknowns from Chlorine Gas-Exposed Pine & Ivy Sample Extracts

Compound No.	Matrix[7]	Tentative Identification	CASRN	Confirmed?
1	PN	Chloroaniline[1]	95-51-2 108-42-9	Yes
2		1-chloromethyl-2-chloro-4-(2-hydroxypropyl-2)-cyclohexanol[2]	126297-16-3 126297-15-2	Yes
3		1,7,7-trimethyl-2,6-dichloro norbornane[2]	3212-36-0	Yes
4		2-chloro-N,N-dimethyl-ethanamine	107-99-3	Yes
5		2,2-dichloroethylbenzene[3]	1331-29-9 4412-39-9	No
6		2,2-dichloroacetamide	683-72-7	Yes
7		3-chloro-4-hydroxy-benzaldehyde[4]	56962-11-9	Yes
8		2,4,6-trichlorophenol	88-06-2	Yes
9		3-chlorophenol[5]	108-43-0	Yes
10	EI	Unknown 1245[6]	NA	No

1. Based on retention time, the unknown compound is likely 2-chloro or 3-chloroaniline; ion ratio suggests 3-chloroaniline
2. Not commercially available; synthesized for confirmation
3. Not commercially available; not synthesized for confirmation
4. Based on ion ratios and retention time, this unknown compound is likely 3-chloro-4-hydroxy-benzaldehyde; NIST library match was initially 2-chloro-4-hydroxy-benzaldehyde
5. Levels are not consistently 3x the blank across all replicates; possible isomers include 3-chlorophenol and 4-chlorophenol; observed retention times and ion ratios suggest this is likely 3-chlorophenol
6. Unknown did not confirm against a standard of cyclohexane carbonyl chloride; tentative identification is incorrect and identity remains unknown
7. EI = English Ivy (*Hedera helix*), PN = pine needles (*Pinus strobus*)

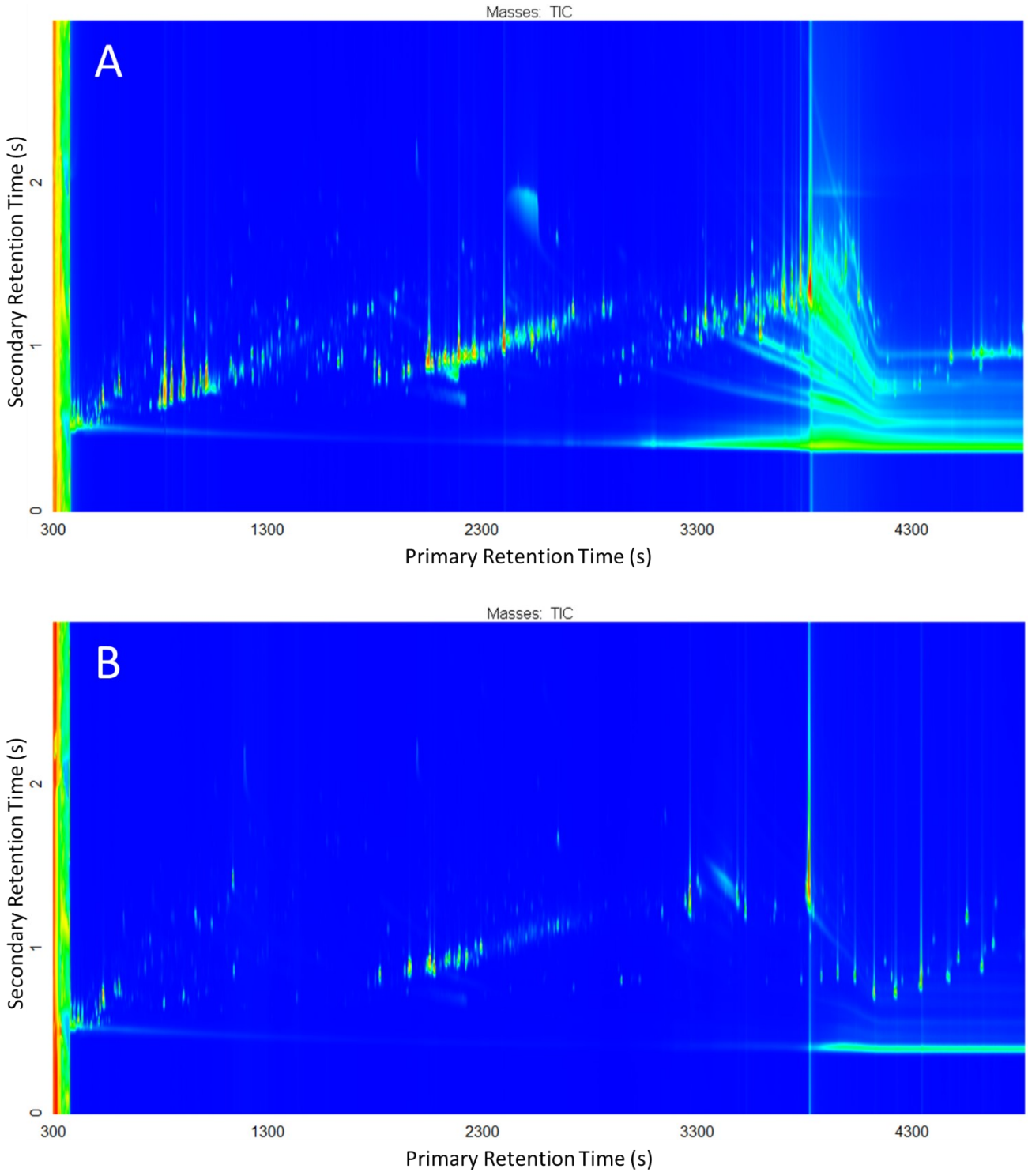
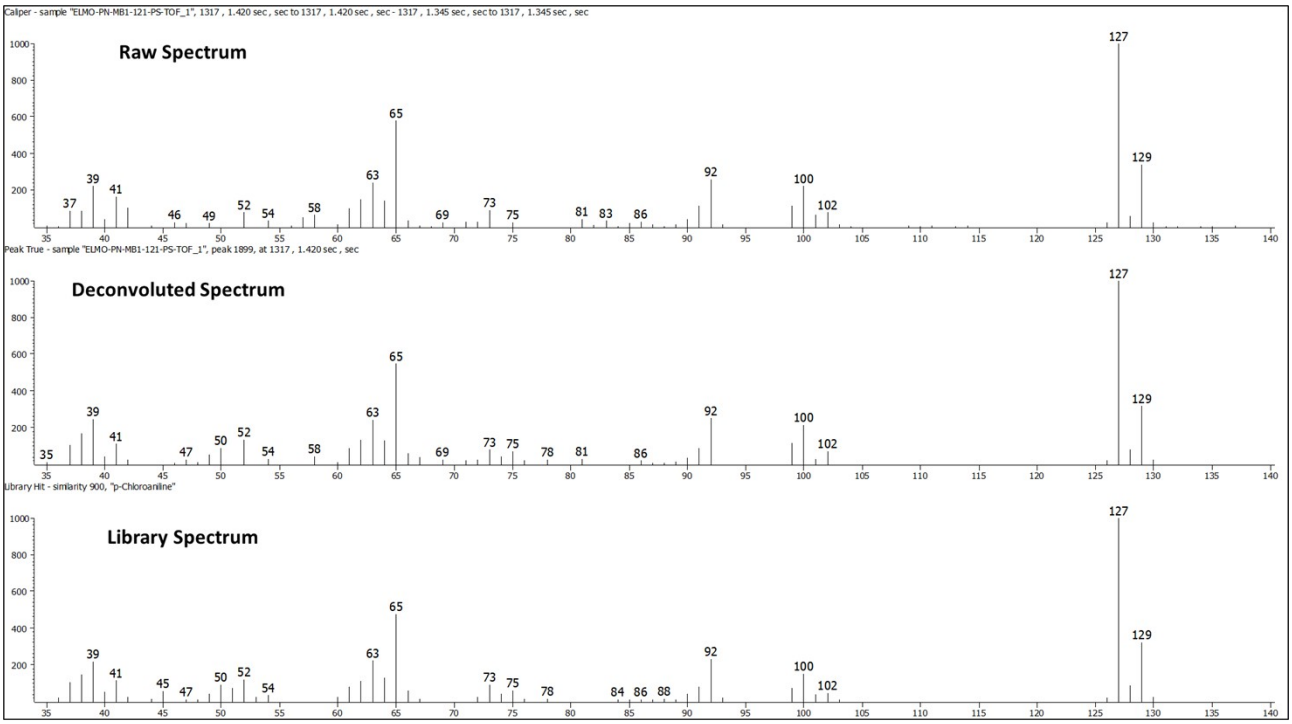
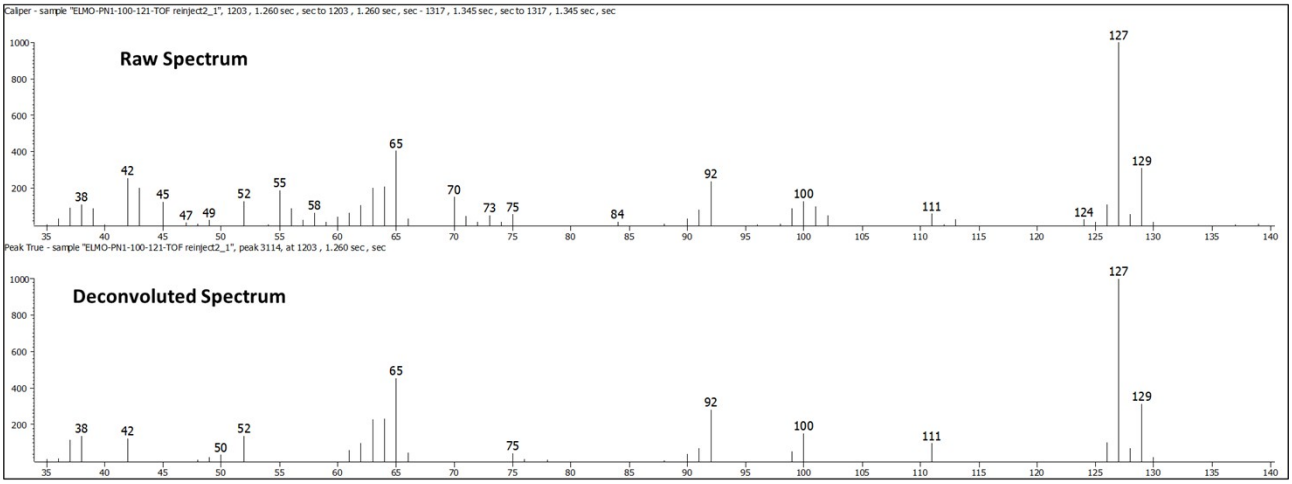


Figure 1 GCxGC-TOFMS contour plot chromatogram examples of an extracted pine needle (A) and ivy (B) sample

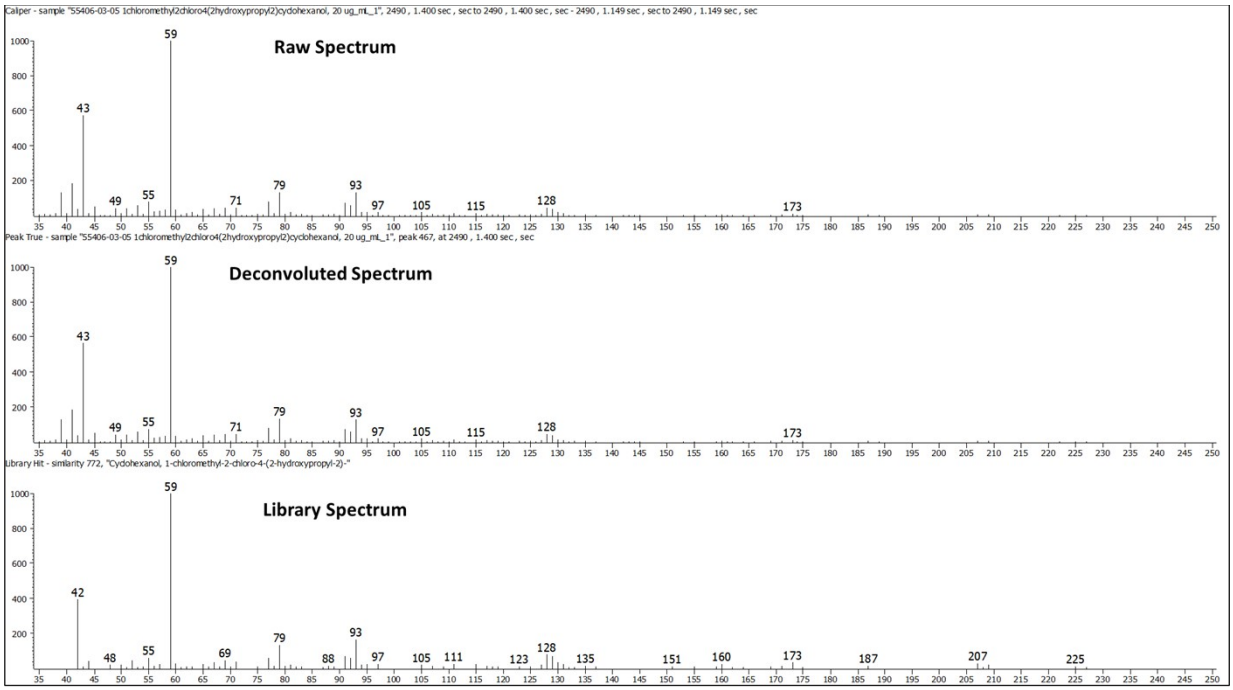


p-Chloroaniline standard

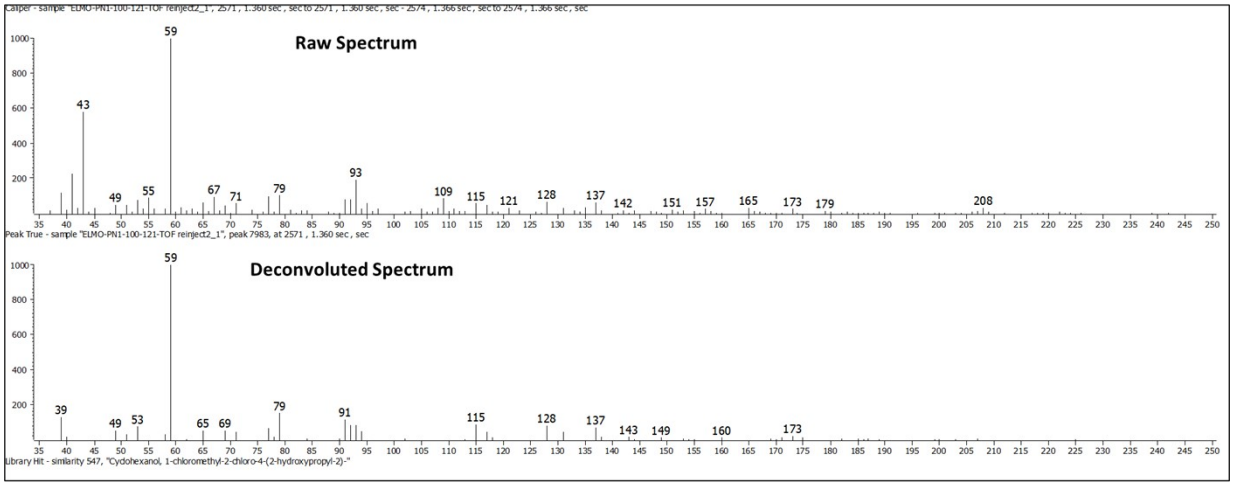


Compound 1 detection in pine sample

Figure 2 Mass spectra for 4-chloroaniline standard (top) and pine sample detection of Compound 1 (bottom)

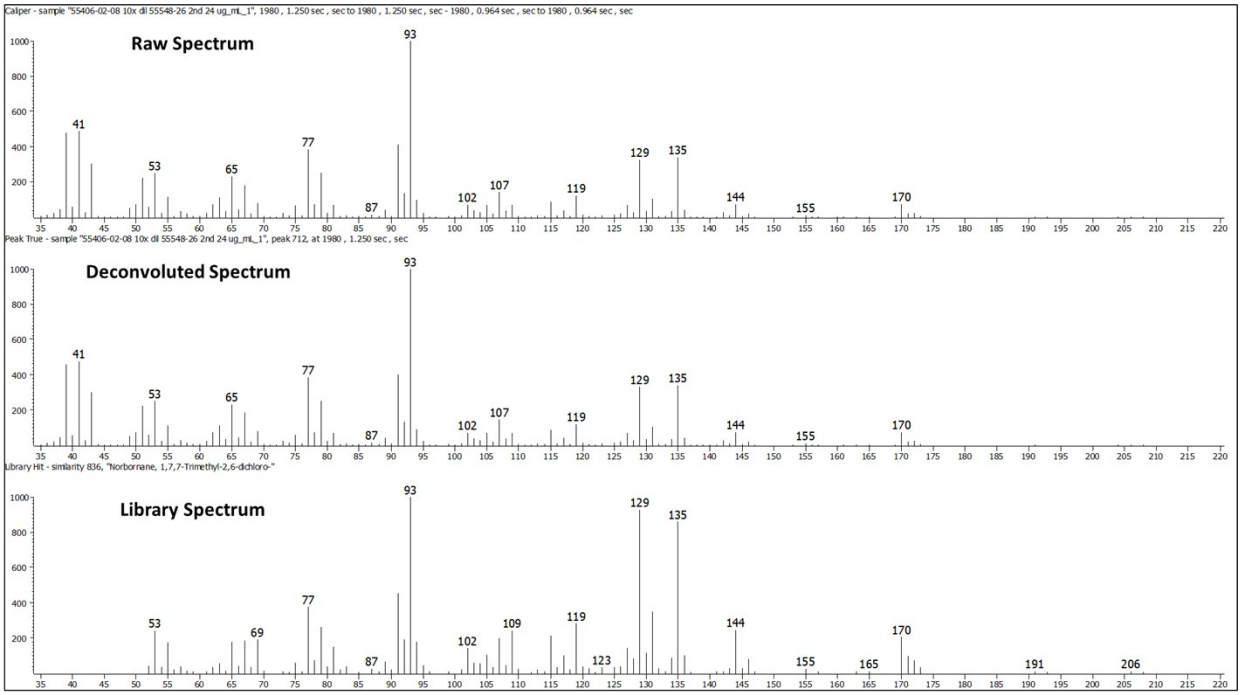


1-Chloromethyl-2-chloro-4-(2-hydroxypropyl-2)-cyclohexanol standard

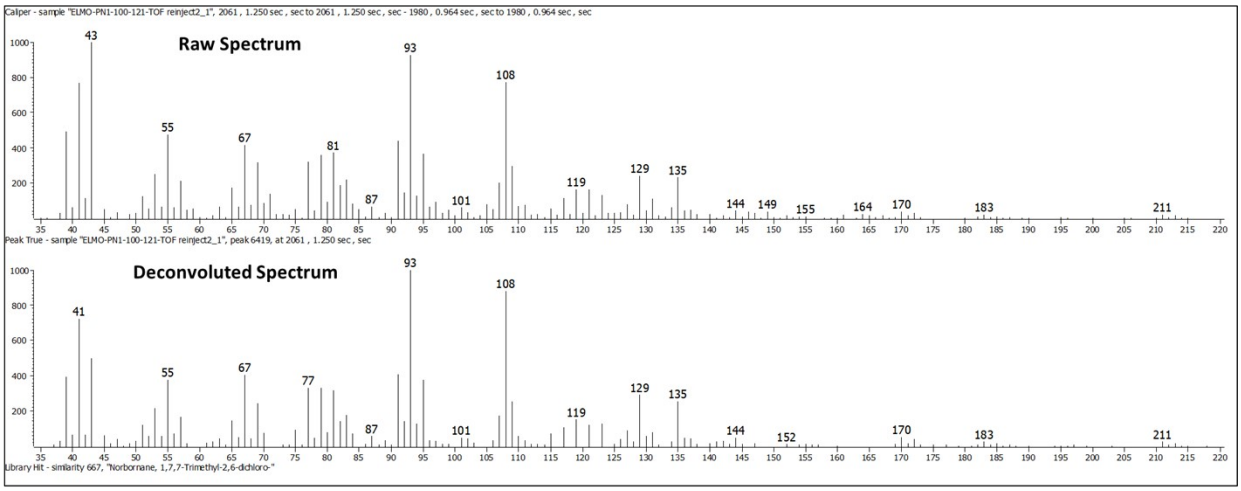


Compound 2 detection in pine sample

Figure 3 Mass spectra for 1-chloromethyl-2-chloro-4-(2-hydroxypropyl-2)-cyclohexanol standard (top) and pine sample detection of Compound 2 (bottom)

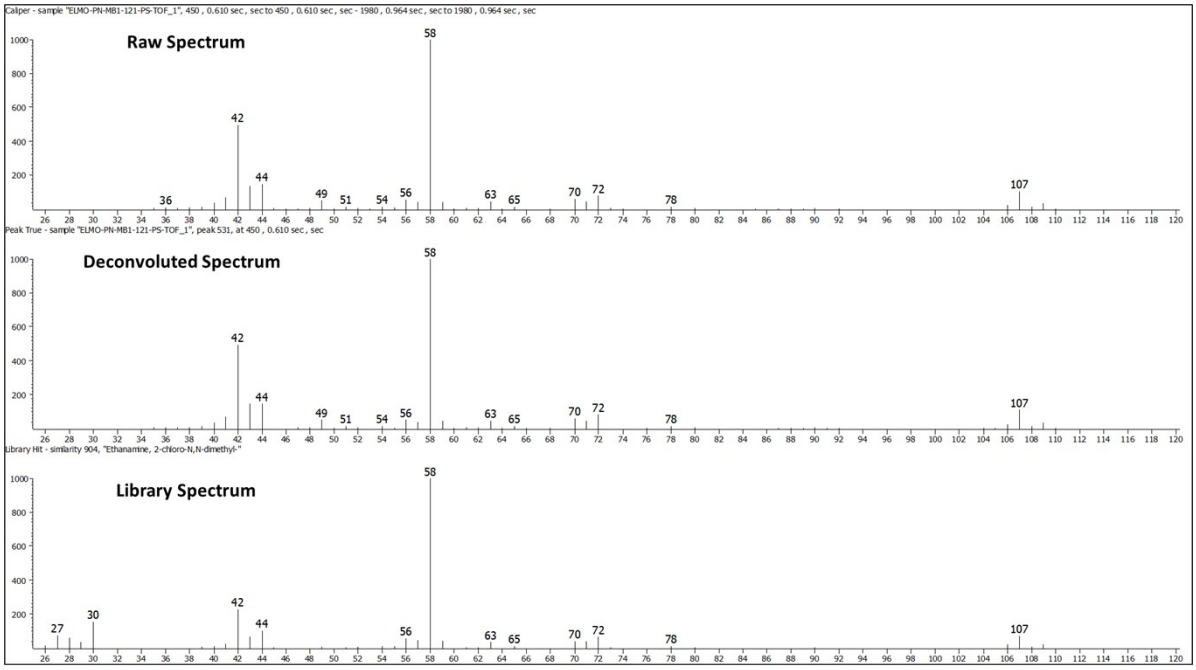


1,7,7-Trimethyl-2,6-dichloro norbornane standard

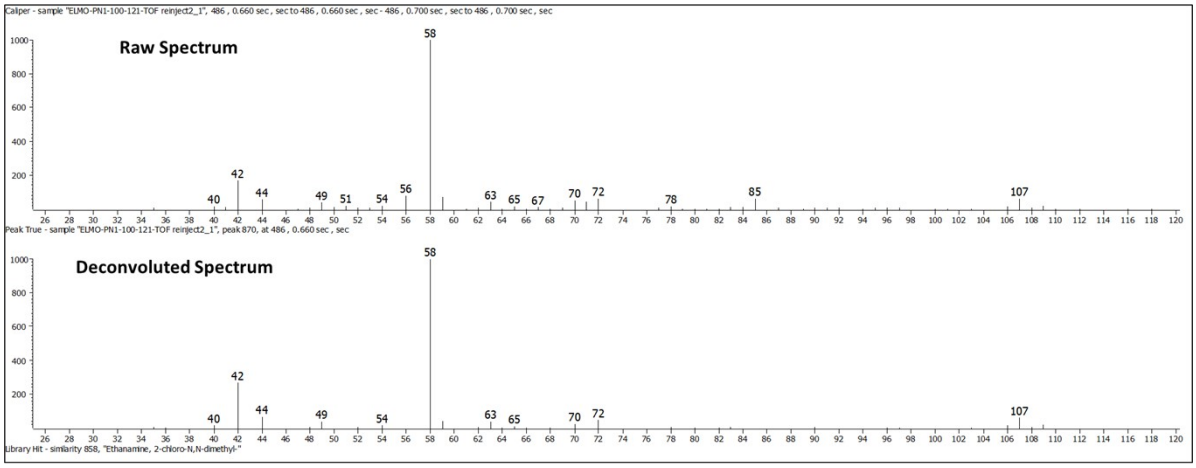


Compound 3 detection in pine sample

Figure 4 Mass spectra for 1,7,7-trimethyl-2,6-dichloro norbornane standard (top) and pine sample detection of Compound 3 (bottom)

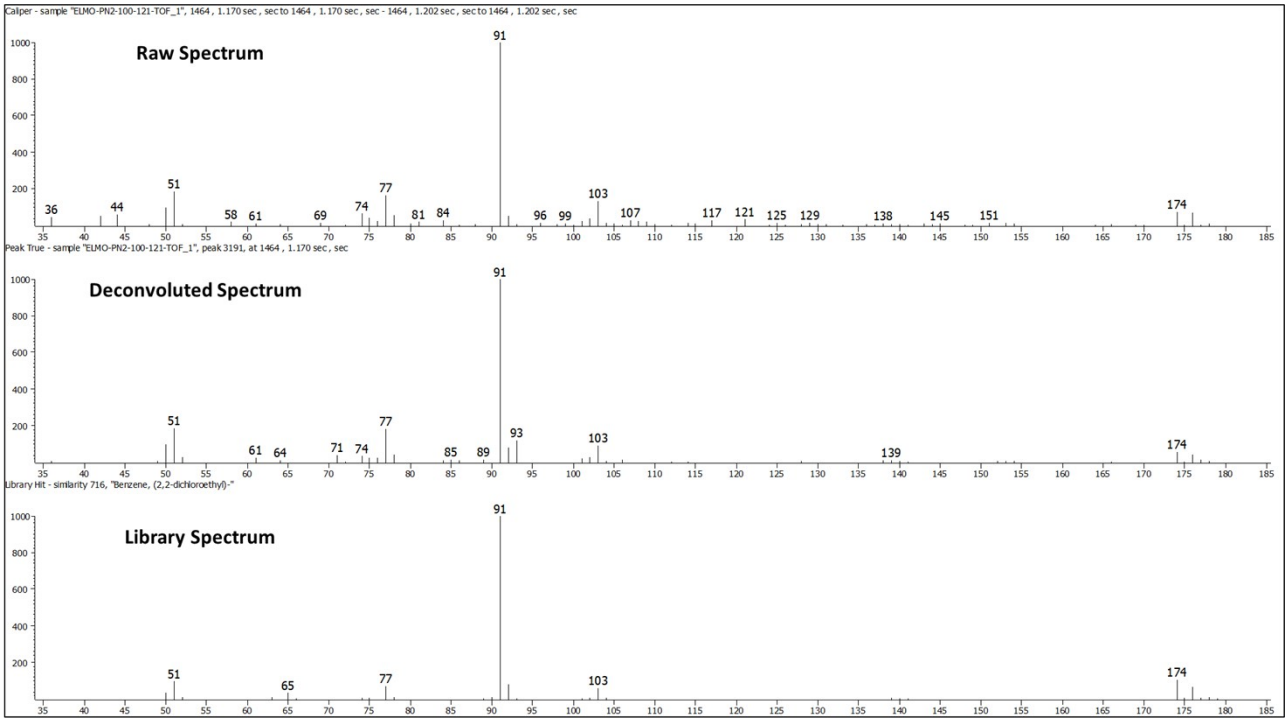


2-Chloro-N,N-dimethyl-ethanamine standard



Compound 4 detection in pine sample

Figure 5 Mass spectra for 2-chloro-N,N-dimethyl-ethanamine standard and pine sample detection of Compound 4 (bottom)



2,2-Dichloroethyl benzene (Compound 5) detection in pine sample

Figure 6 Mass spectra for pine sample detection of 2,2-dichloroethylbenzene (Compound 5)

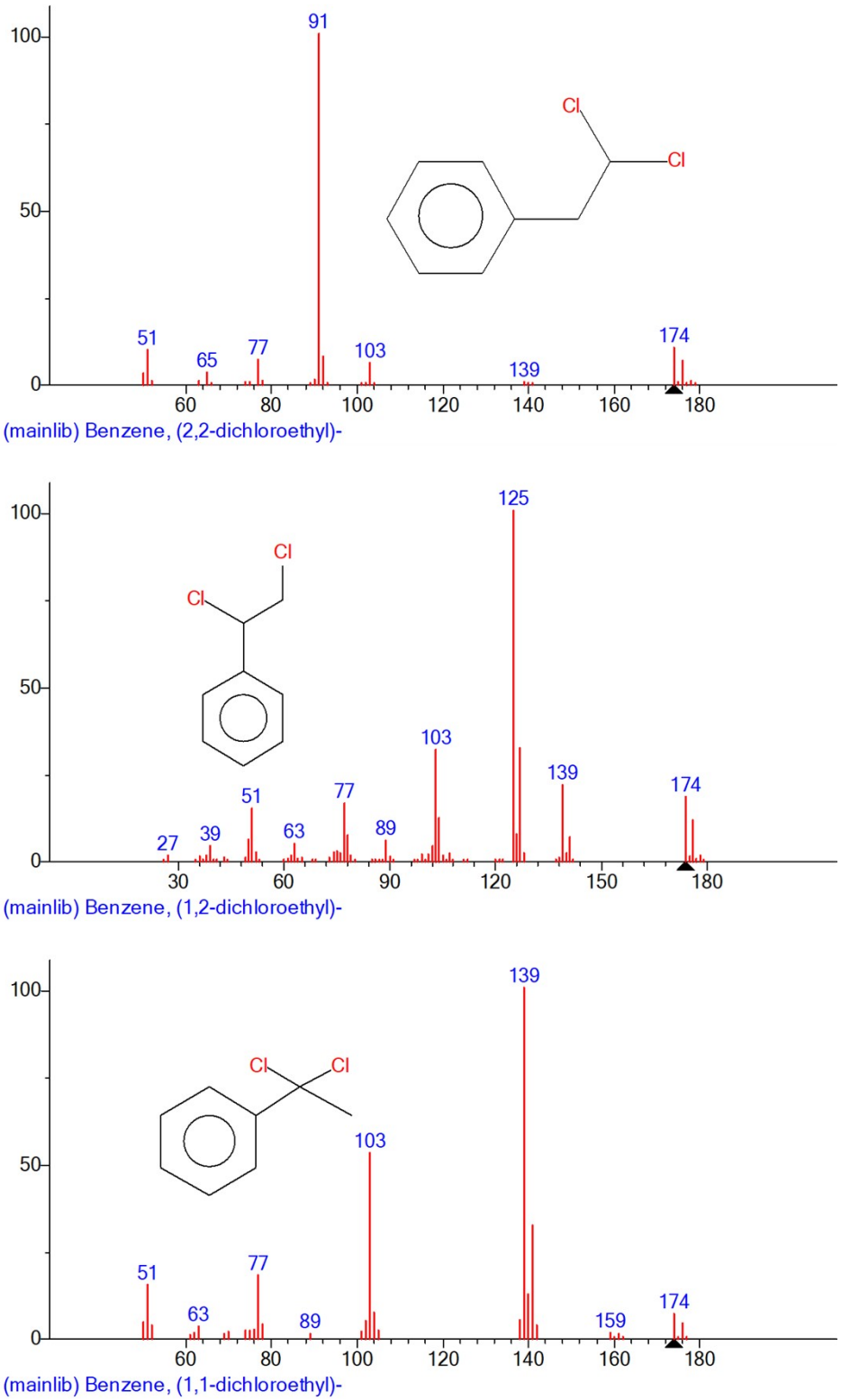
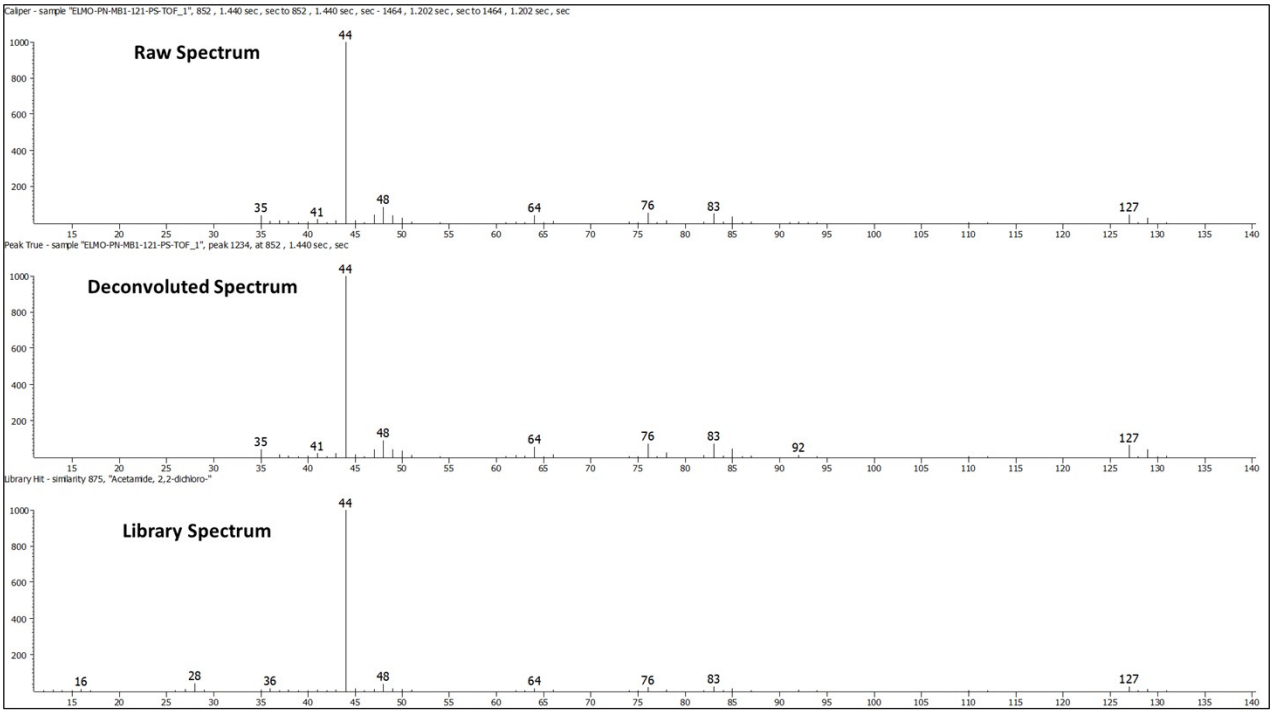
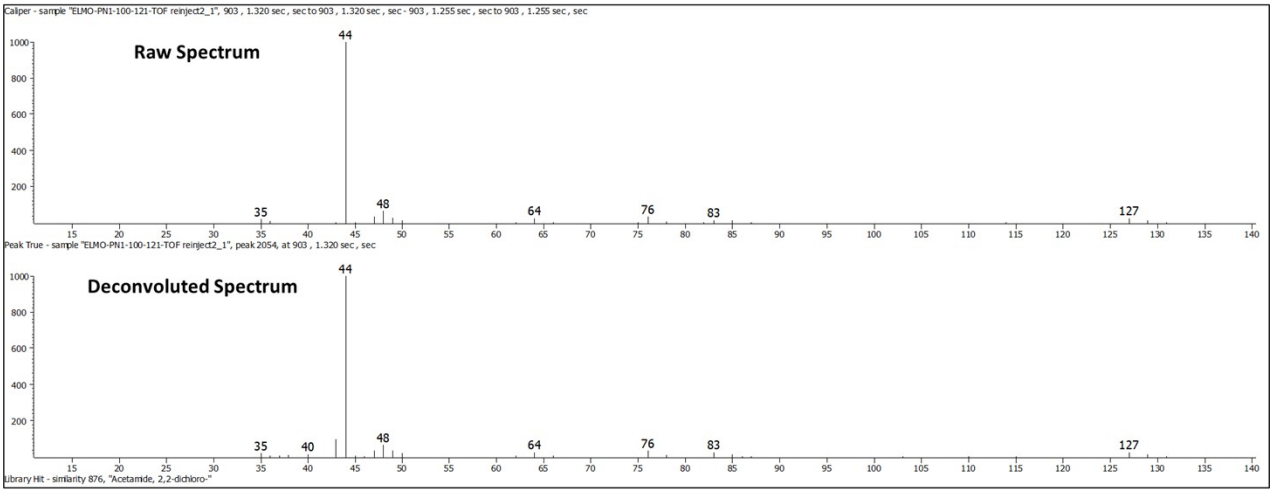


Figure 7 Reference mass spectra (NIST) for dichloroethylbenzene isomers

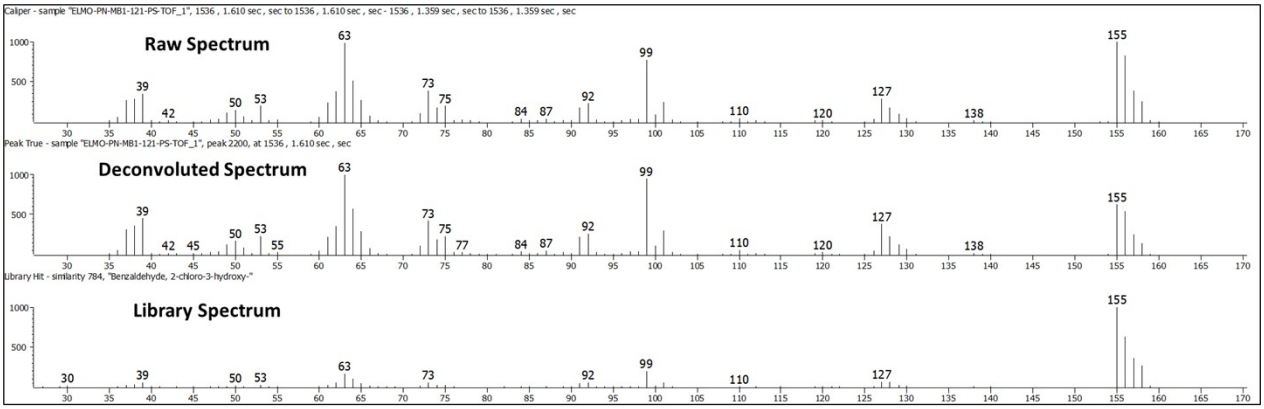


2,2-dichloro-acetamide standard

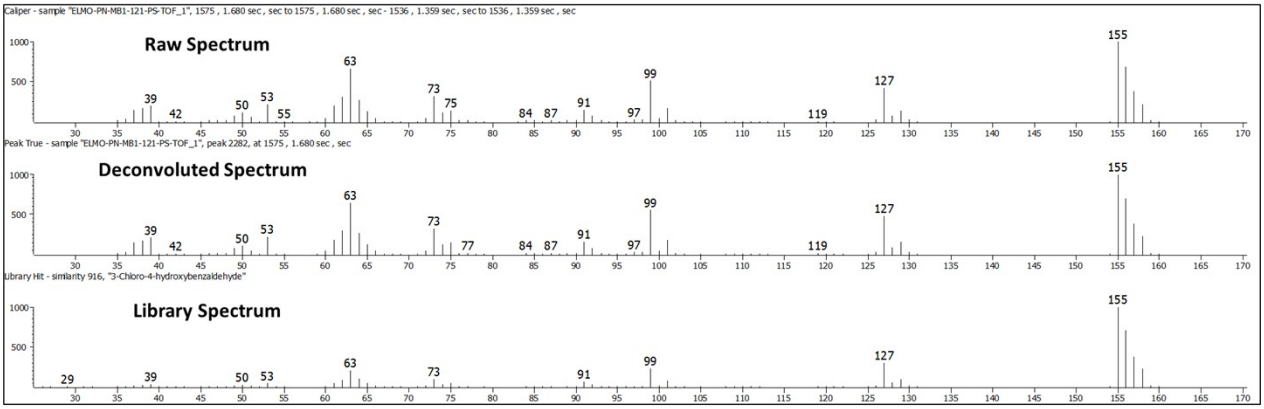


Compound 6 detection in pine sample

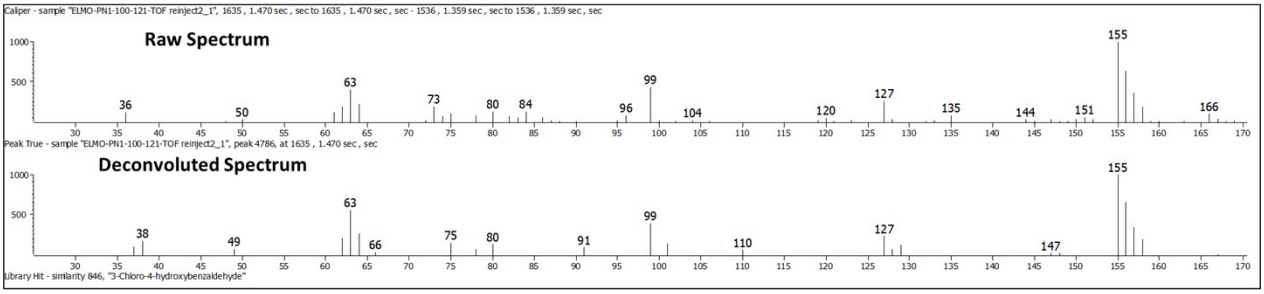
Figure 8 Mass spectra for 2,2-dichloro-acetamide standard (top) and pine sample detection of Compound 6 (bottom)



2-chloro-3-hydroxy-benzaldehyde standard

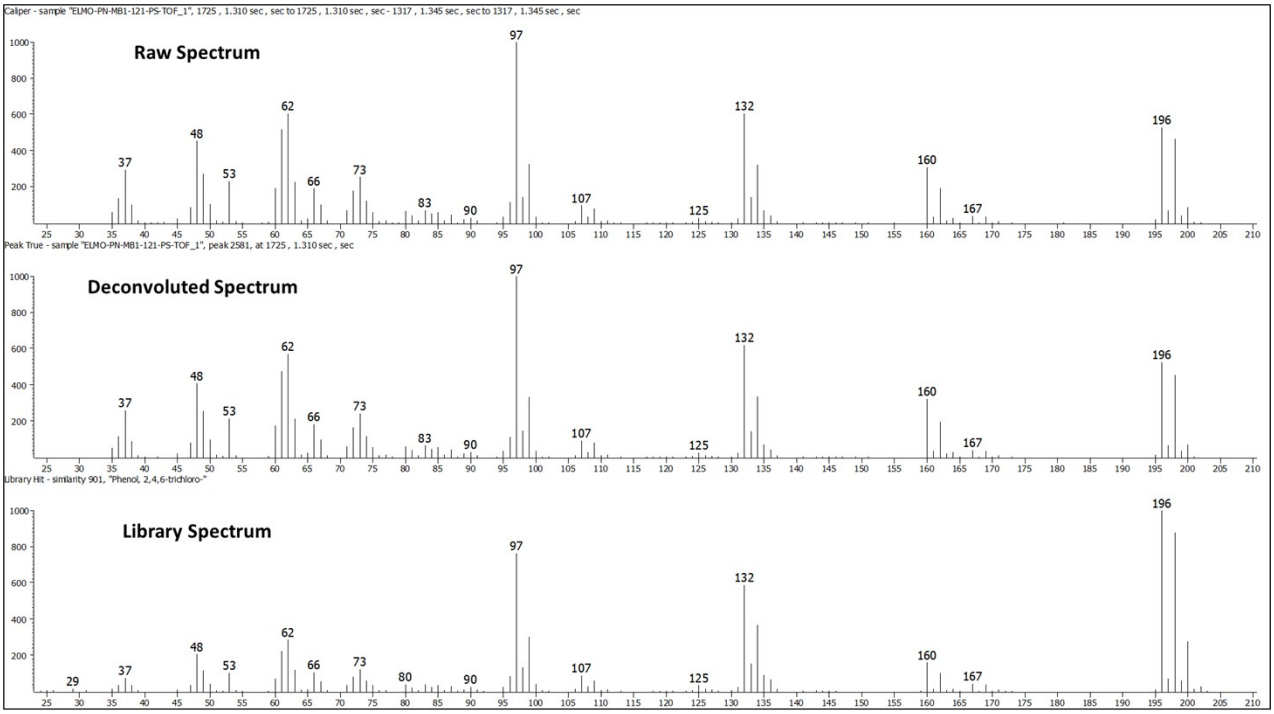


3-chloro-4-hydroxy-benzaldehyde standard

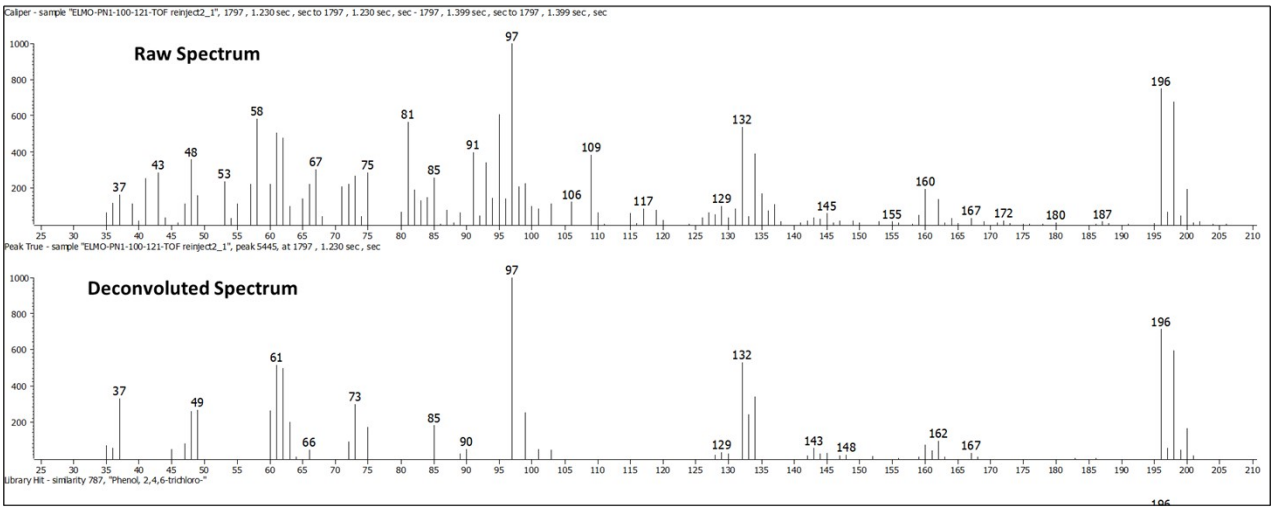


Compound 7 detection in pine sample

Figure 9 Mass spectra for 2-chloro-3-hydroxy- (top) and 3-chloro-4-hydroxy-benzaldehyde (middle) standards and pine sample detection of Compound 7 (bottom)

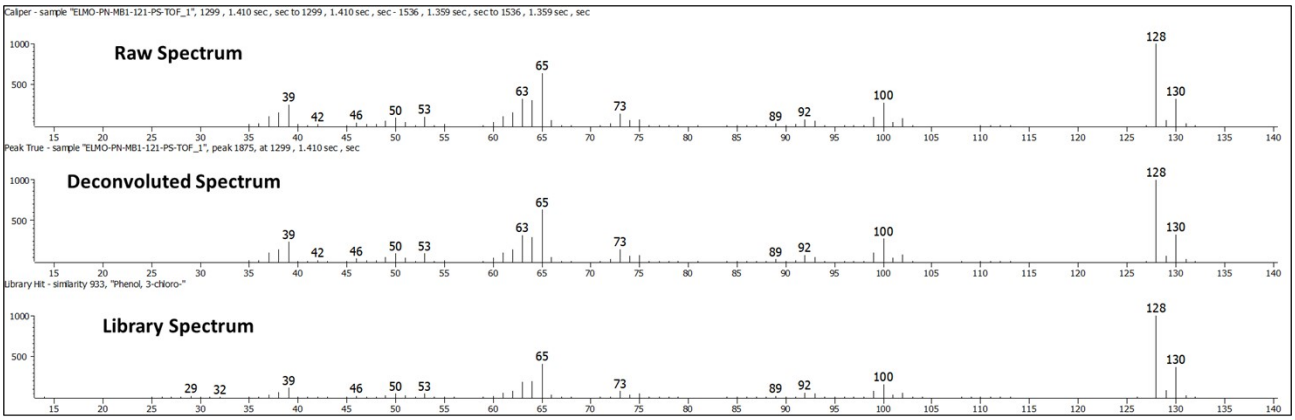


2,4,6-Trichlorophenol standard

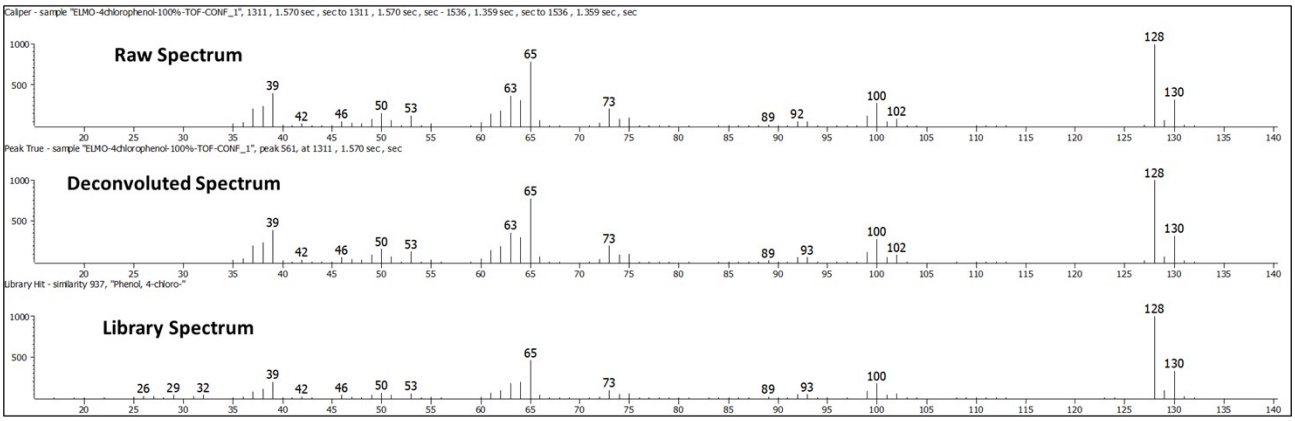


Compound 8 detection in pine sample

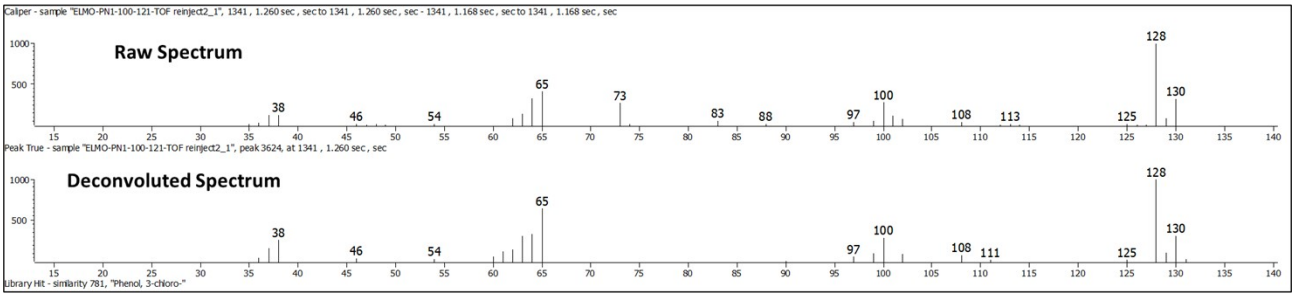
Figure 10 Mass spectra for 2,4,6-trichlorophenol standard (top) and pine sample detection of Compound 8 (bottom)



3-Chlorophenol standard



4-Chlorophenol standard



Compound 9 detection in pine sample

Figure 11 Mass spectra for 3-chlorophenol (top) and 4-chlorophenol (top) standard and pine sample detection of Compound 9 (bottom)

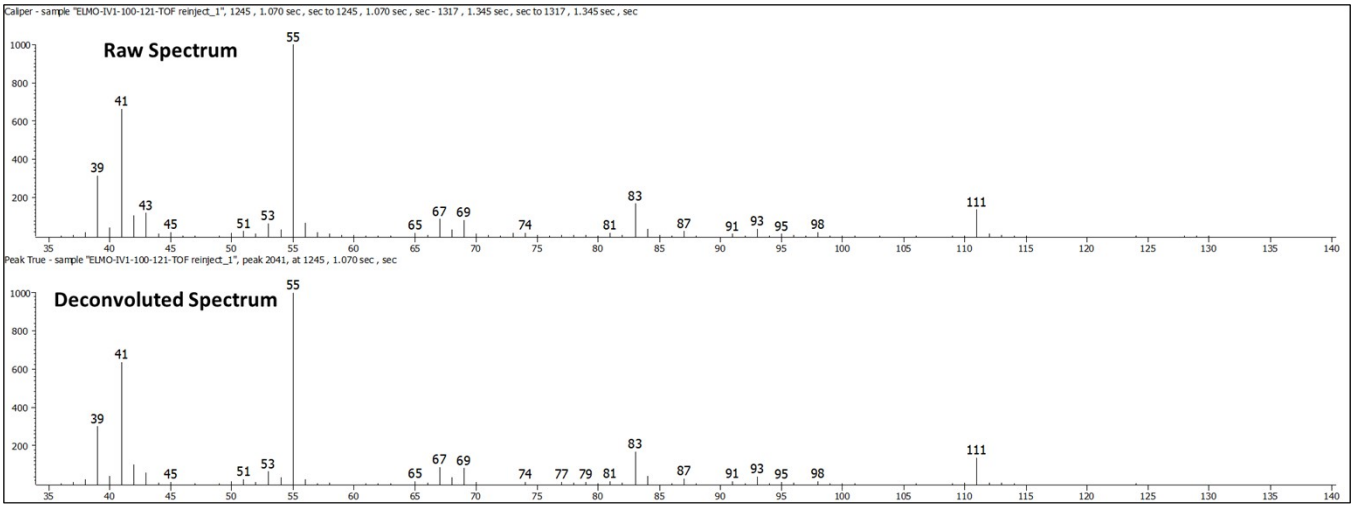


Figure 12 Mass spectra for ivy sample detection of Compound 10

Additional Mass Spectra from Unknowns

LC Compound 1

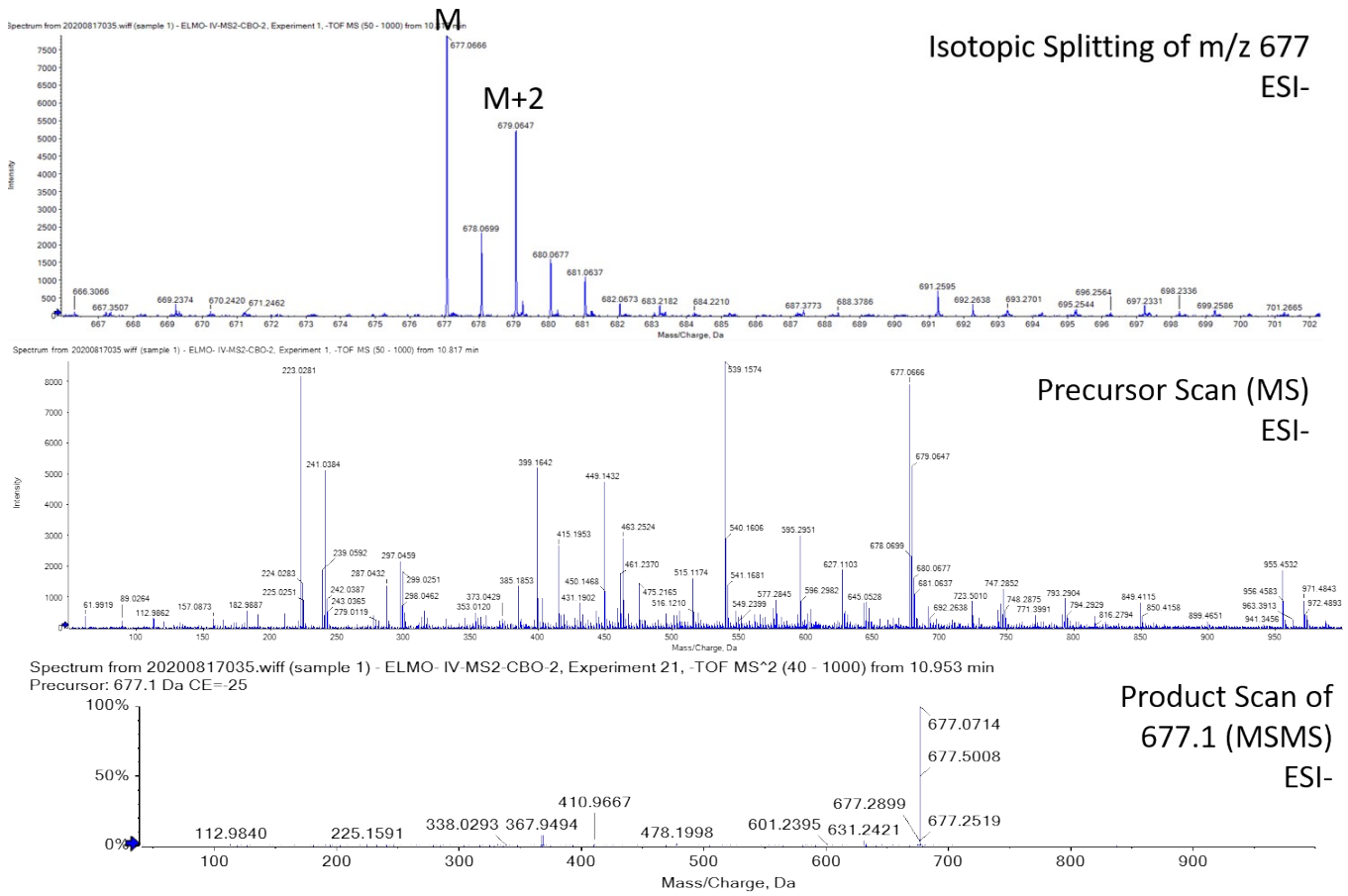


Figure 13 Precursor and product ion scans for m/z 677 in extracts from bleach-treated ivy

LC Compound 2

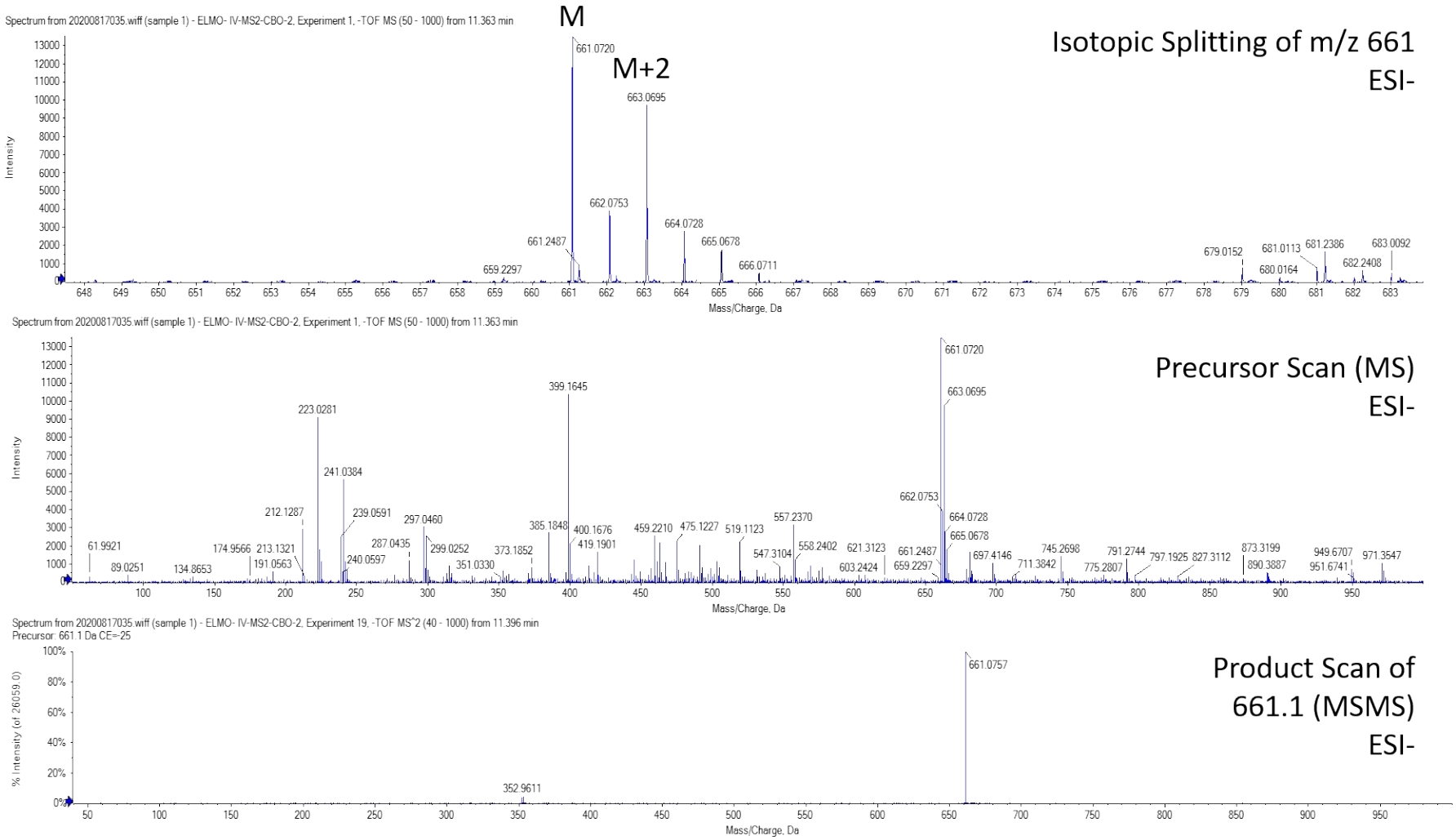


Figure 14 Precursor and product ion scans for m/z 661 in extracts from bleach-treated ivy

LC Compound 3

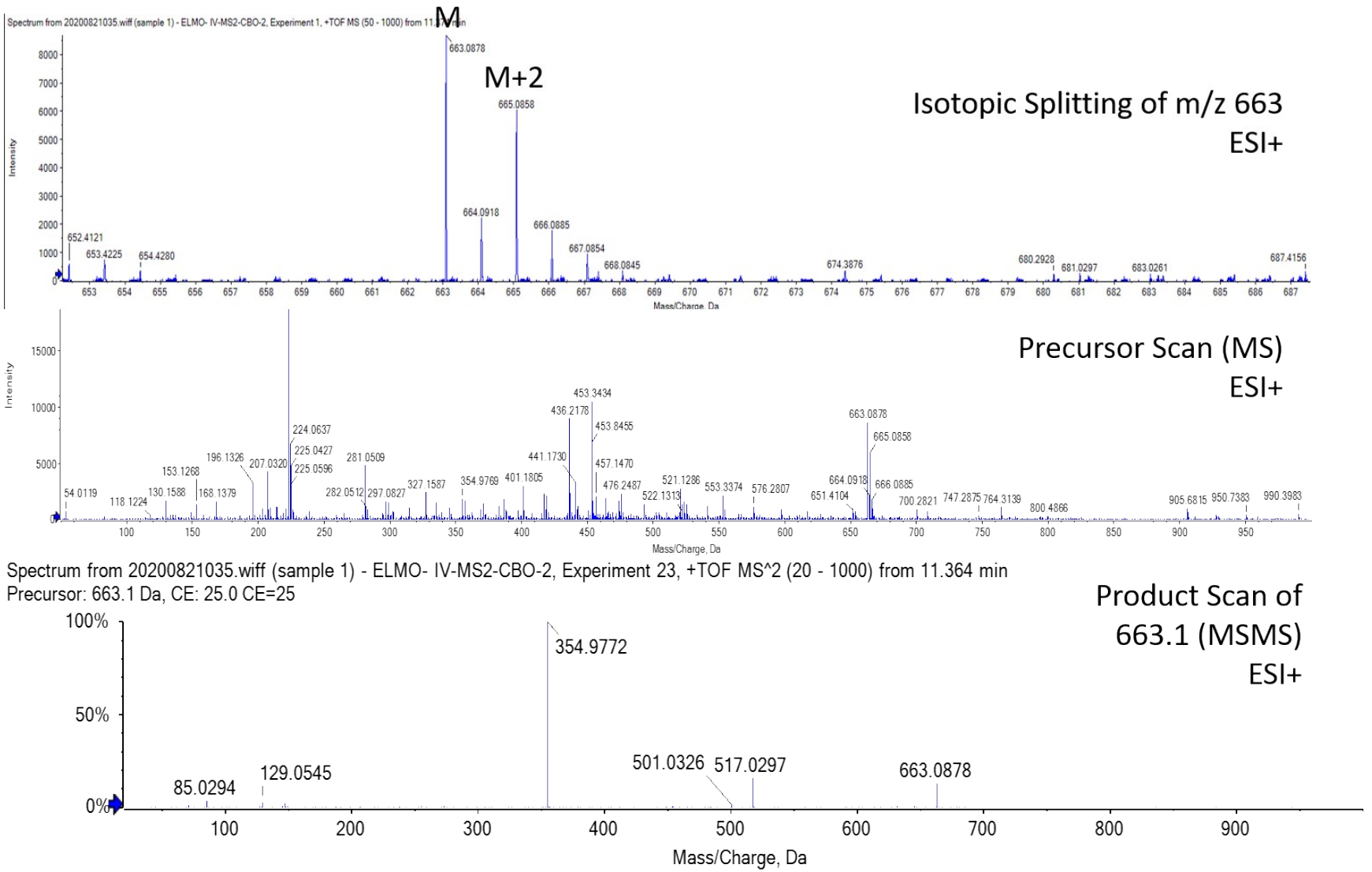


Figure 15 Precursor and product ion scans for m/z 663 in extracts from bleach-treated ivy

LC Compound 4

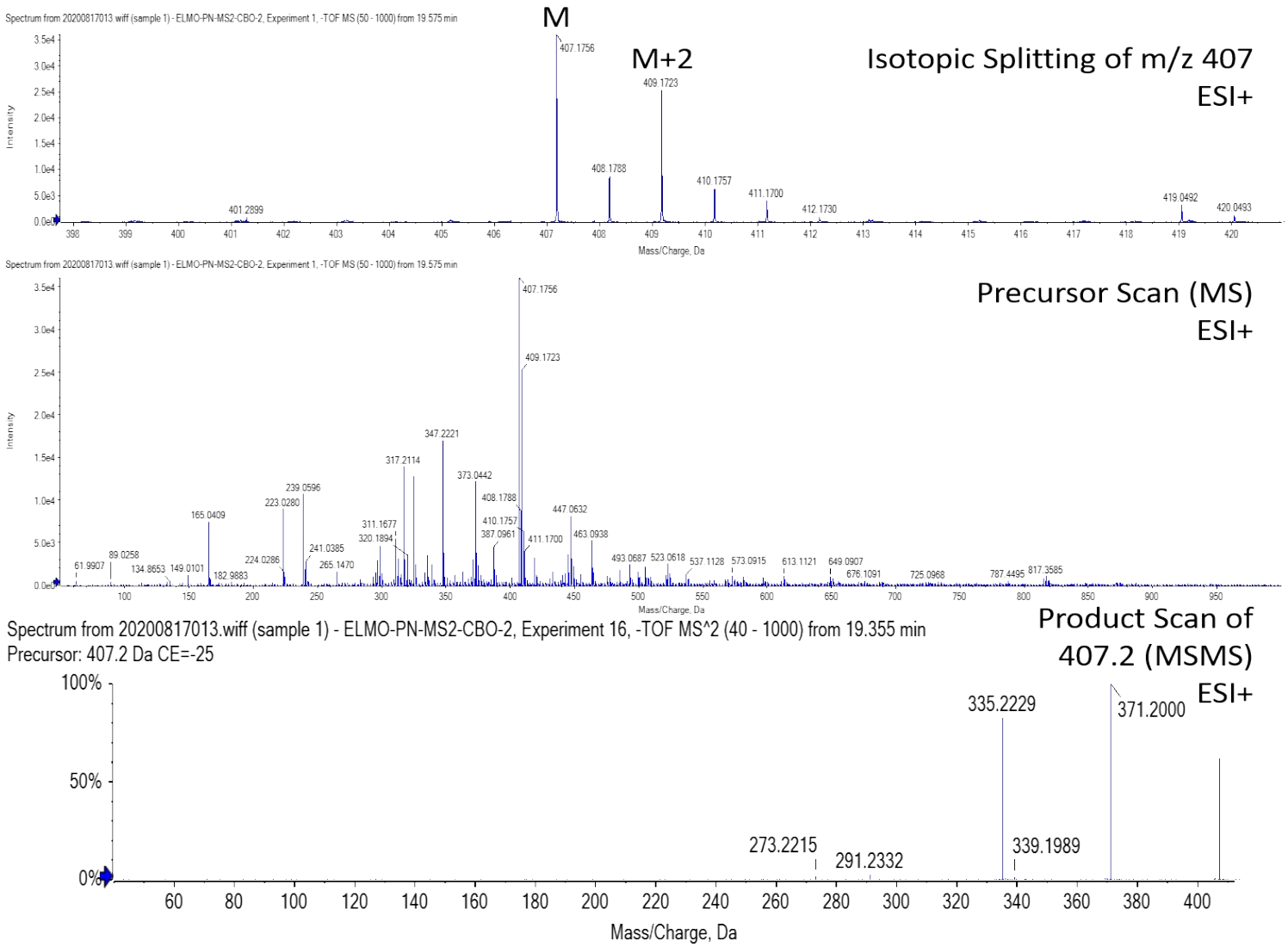


Figure 16 Precursor and product ion scans for m/z 407 in extracts from bleach-treated pine needles

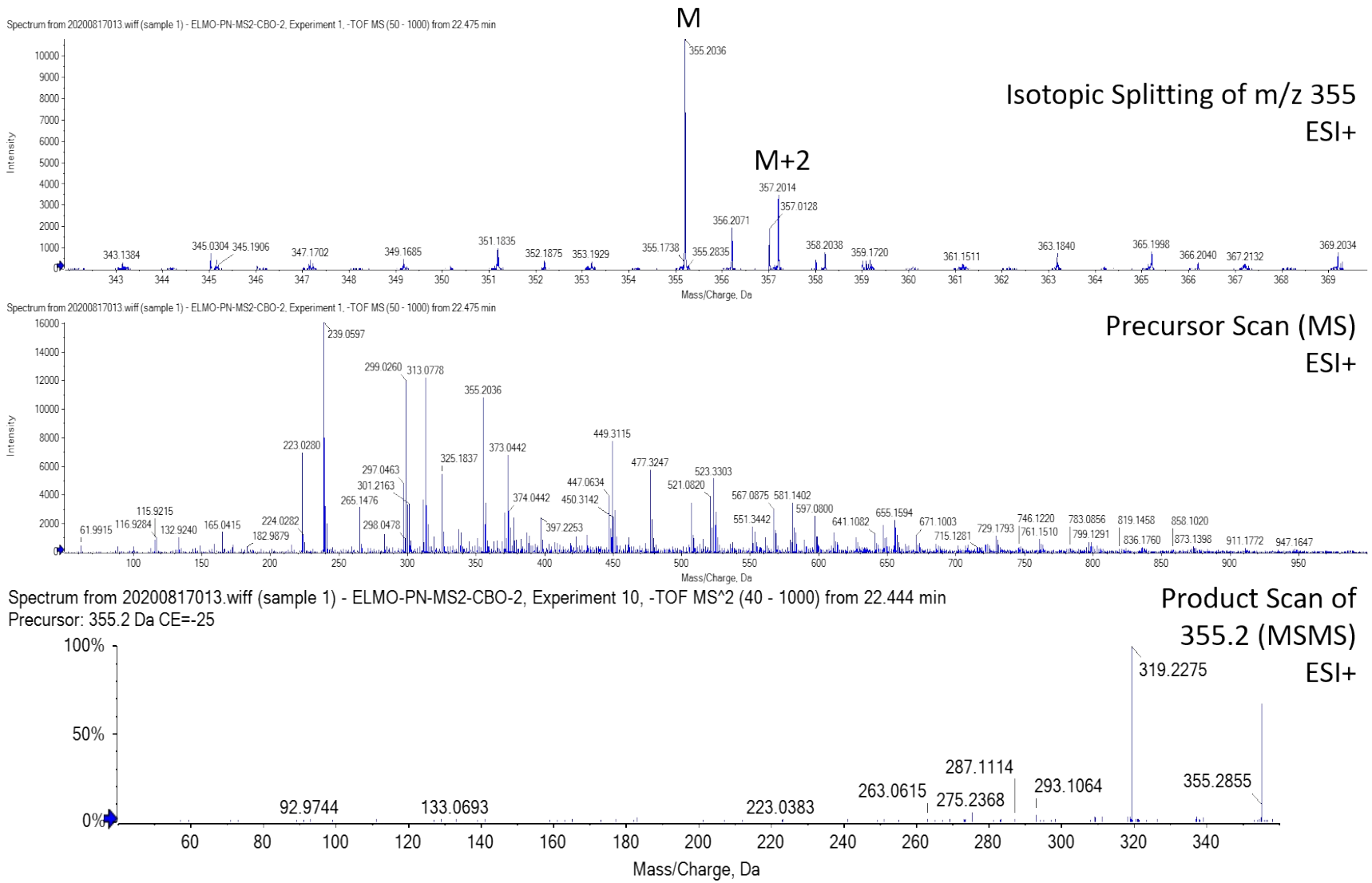


Figure 17 Precursor and product ion scans for m/z 355 in extracts from bleach-treated pine needles

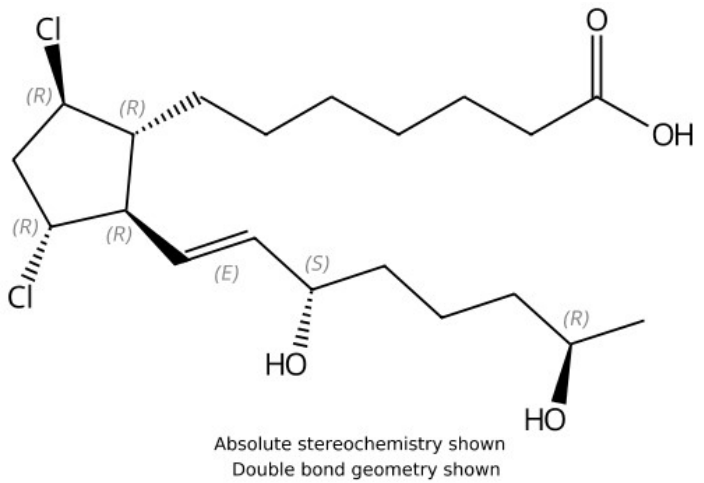


Figure 18 Sirius proposed structure for m/z 407 (CASRN 1350227-80-3, PubChemCID 90843716)

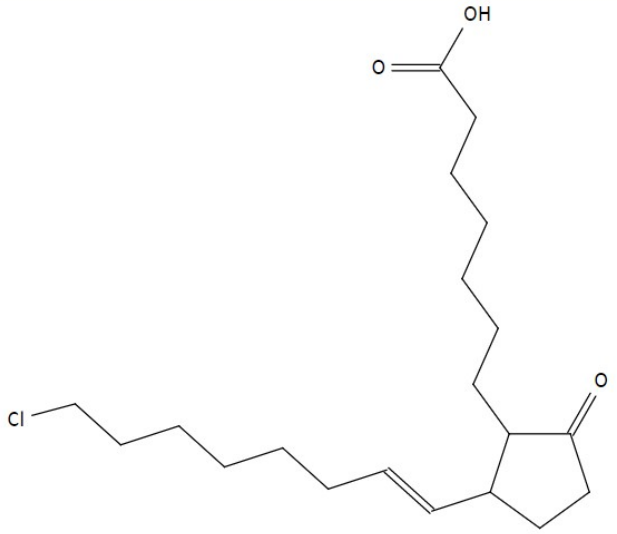


Figure 19 Sirius proposed structure for m/z 355 (PubChemCID 54318964)

Synthesis. Treatment of α-pinene with dry chlorine in the presence of base provided a collection of chlorinated products including compounds with the mass spectrum matching that of dichlorobornanes. Isolation of Compound 3 (1,7,7-trimethyl-2,6-dichloro norbornane, which is also referred to as 2,6-dichlorocamphene) was attained by vacuum distillation, where the compound separated as a solid from the high boiling point fractions. The ^{1}H NMR of the compound (Figure 20) was consistent with that reported in the literature.[1]

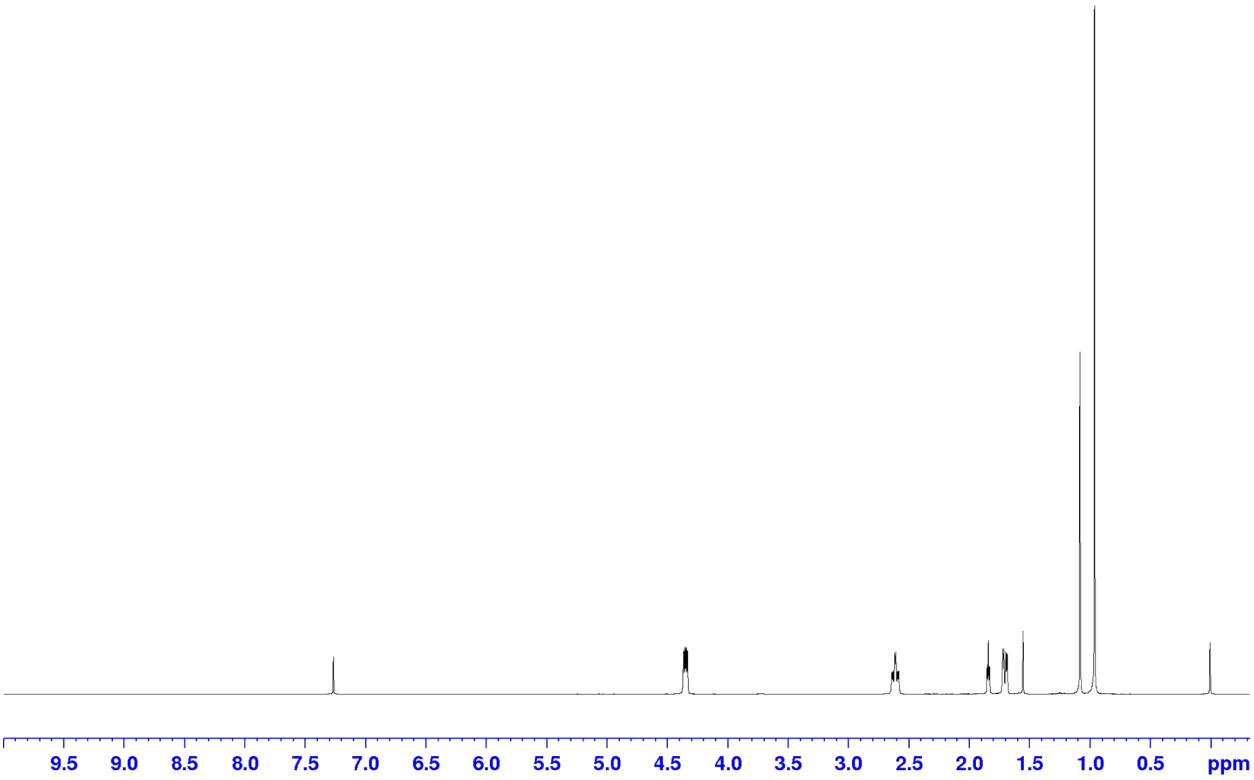


Figure 20. 1H NMR spectrum of 1,7,7-trimethyl-2,6-dichloro norbornane (2,6-dichlorocamphene) Standard

The chlorinated alpha-pinene reaction product contained a complex mixture of products as indicated by the GC-MS analysis of the high boiling point fractions (Figure 21). A product with a mass spectrum similar to Compound 3 (Figure 22) eluted at approximately 9.7 minutes in the chromatogram., however we note that multiple peaks in the chromatogram contained similar spectra.

[1] Parlar, Harun, Siegfried Nitz, Siegmar Gaeb, and Friedhelm Korte. "A contribution to the structure of the toxaphene components. Spectroscopic studies on chlorinated bornane derivatives." *Journal of Agricultural and Food Chemistry* 25, no. 1 (1977): 68-72.

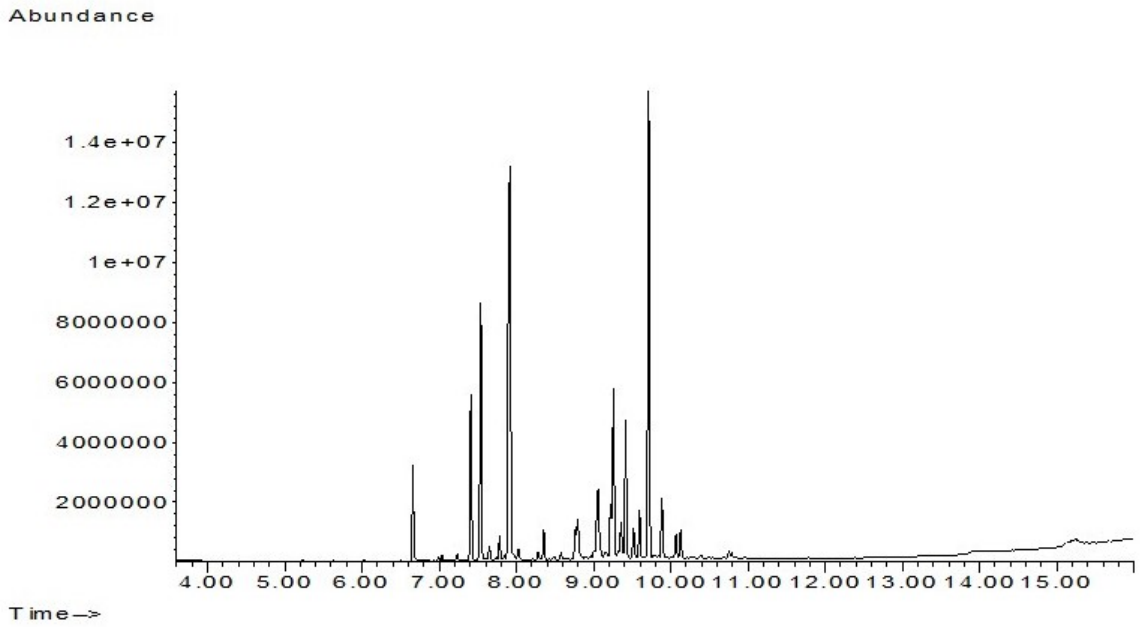


Figure 21. GC-MS Total Ion Chromatogram of the α-Pinene Chlorination High Boiling Point Fractions

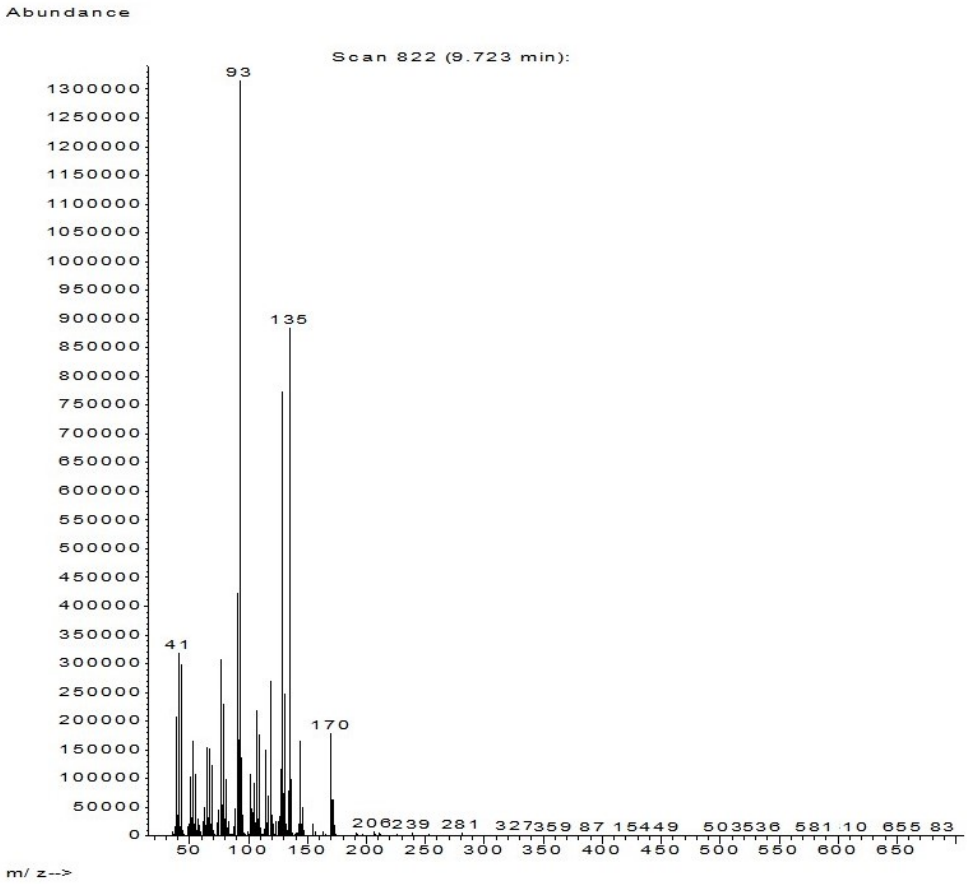


Figure 22. Mass Spectrum of 2,6-Dichlorocamphene

Compound **2** (1-chloromethyl-2-chloro-4-(2-hydroxypropyl-2)-cyclohexanol) is a ring opened product derived from the reaction of β-pinene with chlorine in the presence of water under acidic conditions. The treatment of β-pinene with trichlorisocyanuric acid and water replicated these conditions and produced a collection of dichlorinated species. GC-MS analysis of this mixture identified a compound that eluted at ~11 minutes with a mass spectrum consistent with those of Compound **2**. The crude standard (unpurified) was utilized for confirmation analysis.

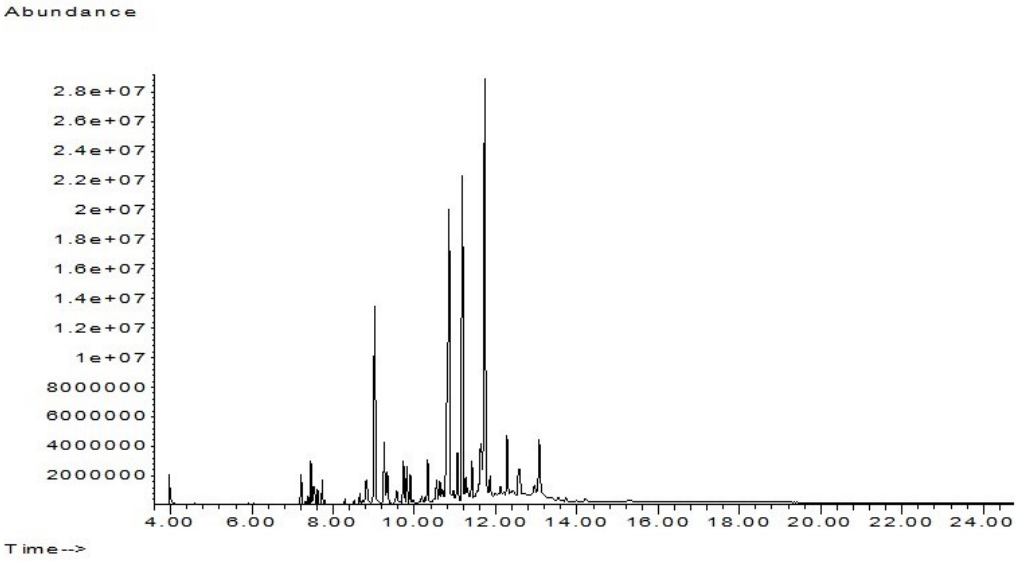


Figure 23. GC-MS Total Ion Chromatogram of the β-Pinene Chlorination Products

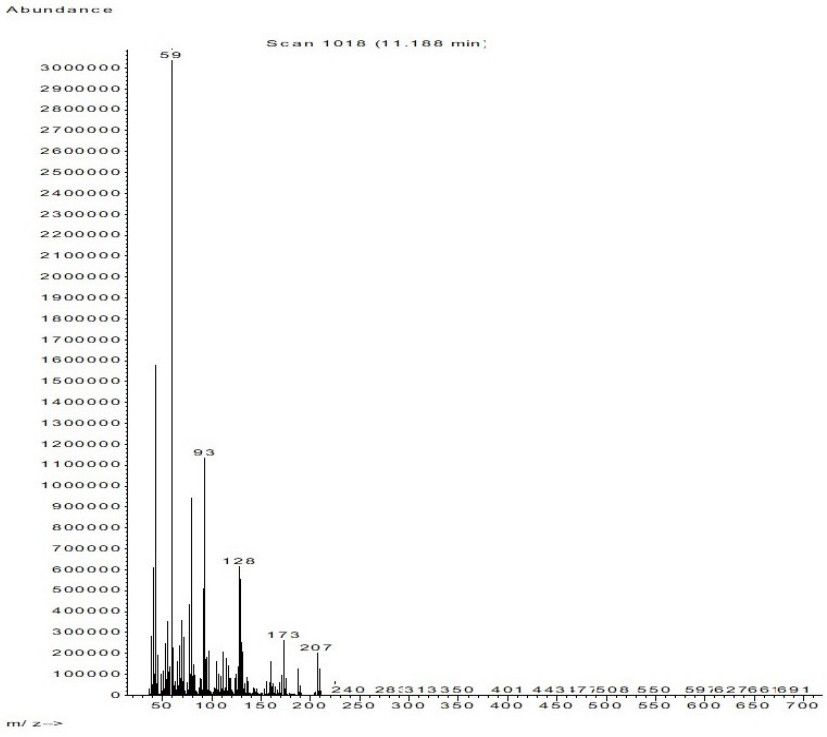


Figure 24. Mass Spectrum of a Dichlorinated Product from the Chlorination of β-Pinene

Table 2. Chlorotyrosine Detection Summary

Sample ID	Pronase Extract (Bound)		Methanol Extract (Free)	
	Cl-TYR	**Cl_2-TYR**	**Cl-TYR**	**Cl_2-TYR**
	Nanograms per mL extract			
Lettuce Unexposed Replicate 1	*ND*	*ND*	**0.40**[4]	*ND*
Lettuce Unexposed Replicate 2	*ND*	*ND*	*ND*	*ND*
Lettuce Bleach-treated Replicate 1	**3.7**	**1.2**	*ND*	*ND*
Lettuce Bleach-treated Replicate 2	**2.3**	**0.27**	*ND*	*ND*
Lettuce Unexposed Replicate 2-PS[1]	**0.78**	**1.0**	**0.98**	**0.96**
Ivy Unexposed Replicate 1	*ND*	*ND*	<LLOQ[3,4]	*ND*
Ivy Unexposed Replicate 2	*ND*	*ND*	<LLOQ[3,4]	*ND*
Ivy Unexposed Replicate 3	*ND*	*ND*	<LLOQ[3,4]	*ND*
Ivy Unexposed Replicate 4	*ND*	*ND*	<LLOQ[3,4]	*ND*
Ivy Bleach-treated Replicate 1	**0.54**	**0.56**	**1.7**	**0.84**
Ivy Bleach-treated Replicate 2	**0.27**	**0.27**	<LLOQ[3,4]	**0.35**
Ivy Bleach-treated Replicate 3	**0.69**	**1.1**	**1.5**	**1.1**
Ivy Bleach-treated Replicate 4	**0.60**	**0.76**	<LLOQ[3,4]	**0.62**
Ivy Chlorine gas treated Replicate 1	**0.45**[4]	*ND*	*ND*	*ND*
Ivy Chlorine gas treated Replicate 2	*ND*	*ND*	*ND*	*ND*
Ivy Chlorine gas treated Replicate 3	**0.56**[4]	*ND*	*ND*	*ND*
Ivy Chlorine gas treated Replicate 4	**0.34**[4]	*ND*	*ND*	*ND*
Ivy Unexposed Replicate 3-PS[1]	<LLOQ[3,4]	<LLOQ[3]	<LLOQ[3,4]	<<LLOQ[5]
Ivy Chlorine gas treated Replicate 1-PS[2]	**5.1**	**3.2**	*ND*	**1.7**
Pine Unexposed Replicate 1	<LLOQ[3,4]	*ND*	*ND*	*ND*
Pine Unexposed Replicate 2	*ND*	*ND*	*ND*	*ND*
Pine Unexposed Replicate 3	*ND*	*ND*	*ND*	*ND*
Pine Unexposed Replicate 4	*ND*	*ND*	*ND*	*ND*
Pine Bleach-treated Replicate 1	**2.2**	**2.1**	**0.60**[4]	**0.66**
Pine Bleach-treated Replicate 2	**0.60**	**0.74**	**0.25**[4]	**0.63**
Pine Bleach-treated Replicate 3	**7.7**	**7.5**	**0.52**[4]	**0.35**
Pine Bleach-treated Replicate 4	**3.6**	**3.9**	**0.77**[4]	**1.1**
Pine Chlorine gas treated Replicate 1	<LLOQ[3]	*ND*	*ND*	<<LLOQ[5]
Pine Chlorine gas treated Replicate 2	<LLOQ[3,4]	*ND*	**0.36**	<<LLOQ[5]
Pine Chlorine gas treated Replicate 3	<LLOQ[3,4]	*ND*	*ND*	*ND*
Pine Chlorine gas treated Replicate 4	<LLOQ[3,4]	*ND*	*ND*	*ND*
Pine Unexposed Replicate 3-PS[1]	**0.25**[4]	**0.40**	<LLOQ[3,4]	<LLOQ[3]
Pine Chlorine gas treated Replicate 3-PS[2]	**4.1**	**3.9**	**3.0**	**1.4**

ND = not detected

[1] Samples post-spiked at 5 ng/mL

[2] Samples post-spiked at 50 ng/mL

[3] Detected below the lower limit of quantification

[4] Ion ratio failure occurs due to interferences

[5] Single ion observed

Table 3. Chloroaniline Ion Ratio and Relative Retention Time Summary

File	m/z 92 Abundance	m/z 100 Abundance	Ion Ratio	RT (s)	RRT
2-Chloroaniline NIST Library	151	65	2.32	1094	0.919
3-Chloroaniline NIST Library	206	116	1.78	1157	0.972
4-Chloroaniline NIST Library	155	107	1.45	1160	0.975
4-Chloroaniline analytical standard*	454	392	1.16	1299	0.993
Unknown*	281	152	1.85	1203	0.871

*Values in the table correspond to observed values; those populated for the NIST library entries for chloroaniline derive from the reference spectra for ion ratio and aggregate non-polar retention indices for analyte and aggregate semi-standard non-polar for naphthalene d8 (1190)

Table 4. Chlorophenol Ion Ratio and Relative Retention Time Summary

File	m/z 65 Abundance	m/z 100 Abundance	Ion Ratio	RT (s)	RRT
2-Chlorophenol NIST Library	NA**	NA**	NA**	979	0.823
3-Chlorophenol analytical standard	662	282	2.35	1290	0.986
4-Chlorophenol analytical standard*	776	282	2.75	1311	1.005
Unknown*	638	287	2.22	1341	0.972

*Values in the table correspond to observed values; those populated for the NIST library entries for chlorophenol derive from the reference spectra for ion ratio and aggregate non-polar retention indices for analyte and aggregate semi-standard non-polar for naphthalene d8 (1190) for retention time

**NA = not applicable; ion ratio of m/z 92 and 100 ruled out the possibility of 2-chloroaniline; NIST RRT values shown for completion