Marine Biotoxins:
Determination of Spirolide Profiles in Phytoplankton by LC/MS/MS

1. Introduction
2. Search for unknowns
3. Mass spectral characterization
4. Phenotypic profiling of *Alexandrium ostenfeldii* strains
5. Pitfalls

Ecological Chemistry: Allelochemical Effects of Protists

Allelochemistry:

Interaction of *biologically* active components eliciting *specific responses* in target organisms.

These highly specific allelochemical compounds are typically *secondary metabolites* and should be distinguished from low molecular weight inorganic and organic nutrients and complex but poorly defined dissolved organic matter (DOM) that may be utilized as growth substrates by protists.

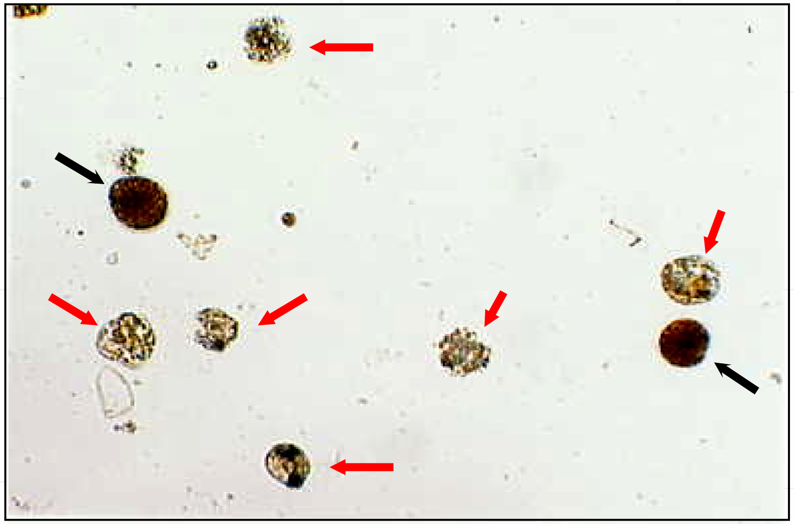
There are both *stimulatory* and *inhibitory* functions to be exploited via production of allelochemicals by protists. Among the putative functions of allelochemicals, their use as agents of chemical defence is most often invoked.

Ecological Chemistry: Allelochemical Effects of Protists

Oxyrrhis marina

Alexandrium ostenfeldii

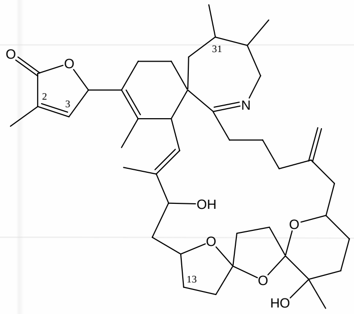
Photos: U. Tillmann



Lytic effect of *Alexandrium*, here the example of Oxyrrhis marina (heterotrophic dinoflagellate).

Black arrows: *Alexandrium*;
Red arrows: remains of *Oxyrrhis*

Ecological Chemistry: Allelochemical Effects of Protists

Organism	Effect	chemical interaction	ecological function
Alexandrium ostenfeldii	Accumulation in marine food webs, poisoning of vertebrates and humans	Spirolides 	?
	Lysis of other protists	?	Defense against predators and/or competitors

The Discovery of Novel „Fast Acting Toxins“

1991-1992

During routine monitoring of shellfish aquaculture sites in Nova Scotia, extracts of the digestive glands of blue mussels (*Mytilus edulis*) from Ship Harbour and sea scallops (*Placopecten magellanicus*) (Graves Shoal) elicited a *unique toxic response* in the DSP mouse bioassay…….

Coincident consumer complaints of *mild illness* after shellfish consumption

“Fast Acting Toxicity”: Lipophilic extracts (DSP toxins)

Symptomology:

not PSP/DSP(!)

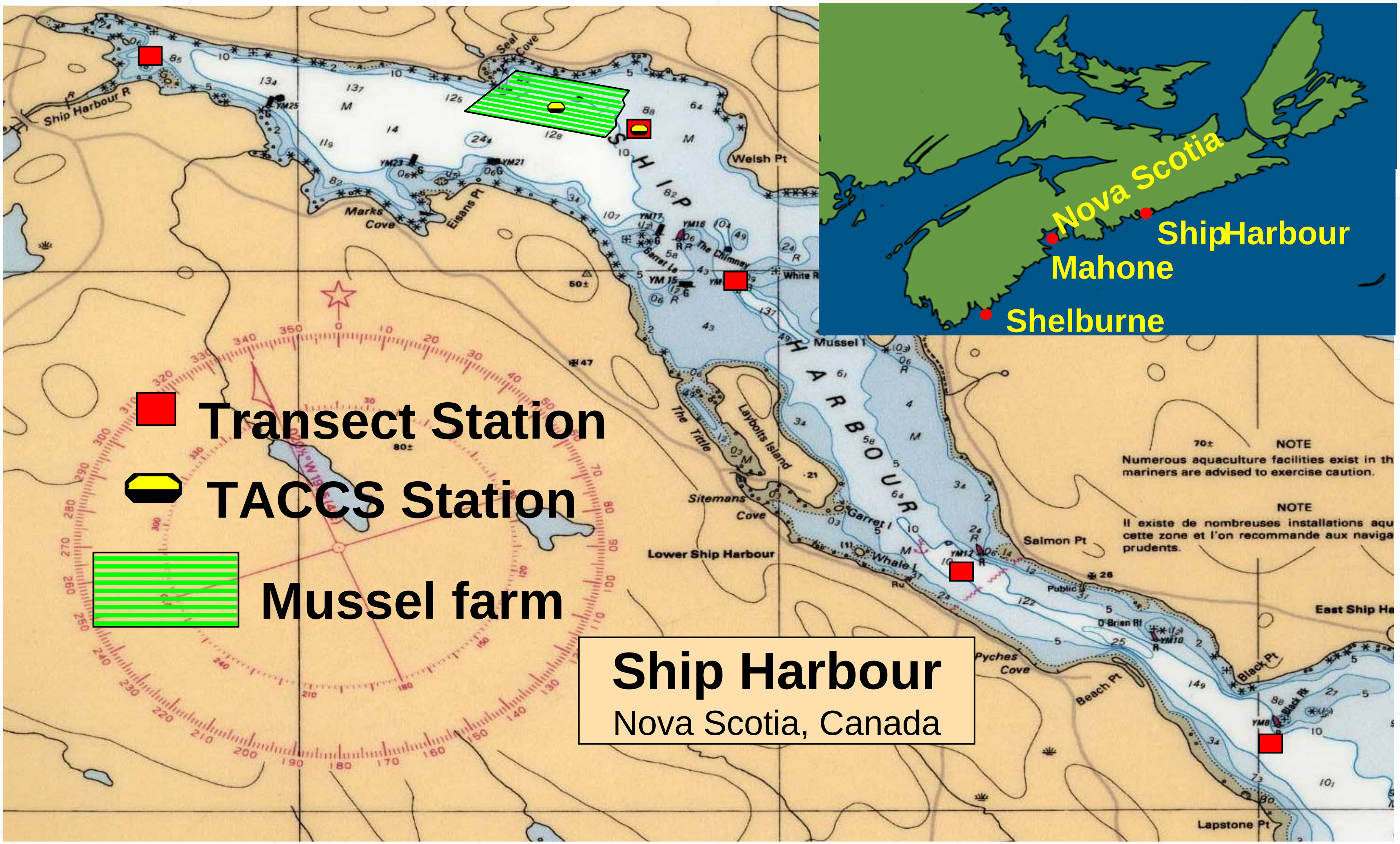
strong convulsions

tail whirling

body arching

rapid death (min)

“Fast Acting Toxicity”: Occurrence



"Fast Acting Toxicity": Occurrence

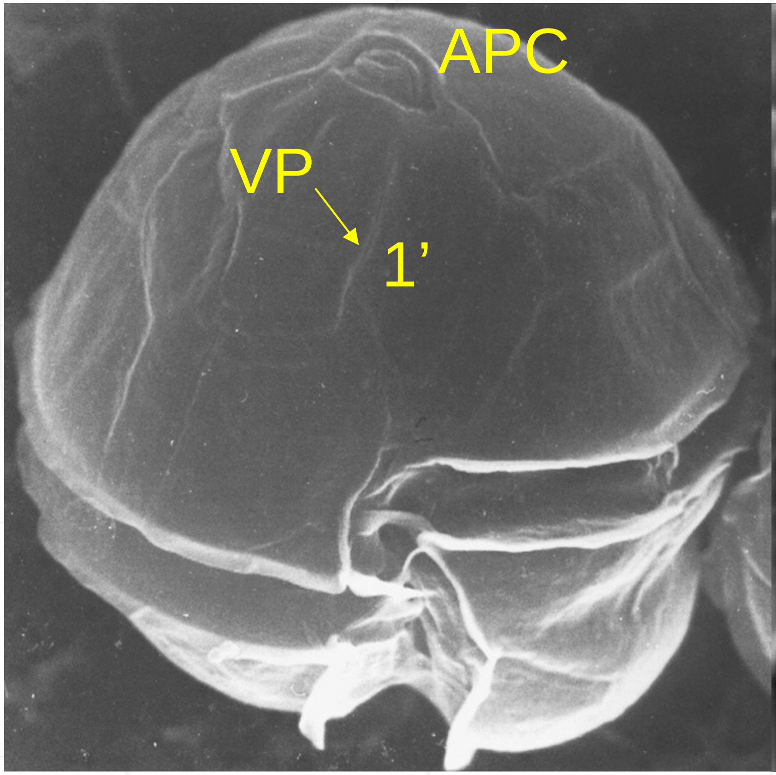
Limfjorden, Denmark

Later also found in:

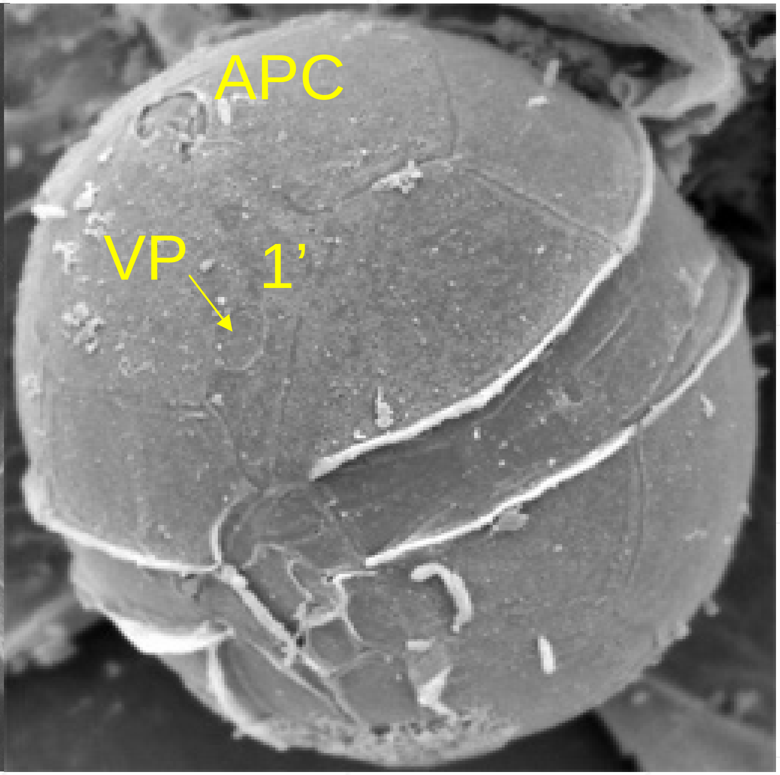
Norway,

Scotland

Scanning electron micrographs of vegetative cells

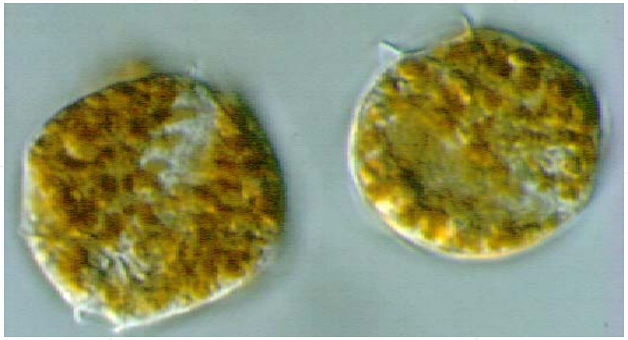


Alexandrium tamarense



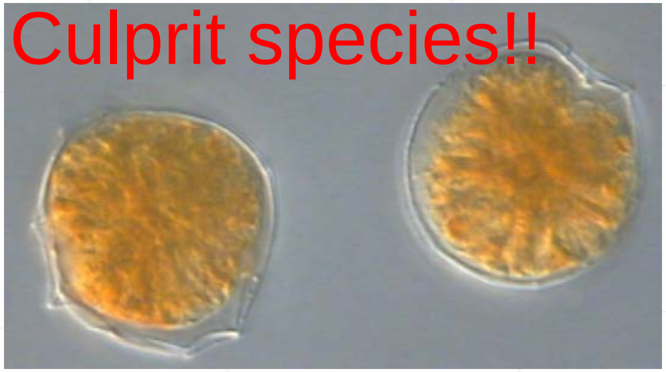
Alexandrium ostenfeldii

Confirmation of spirolides in cultured isolates from Nova Scotia



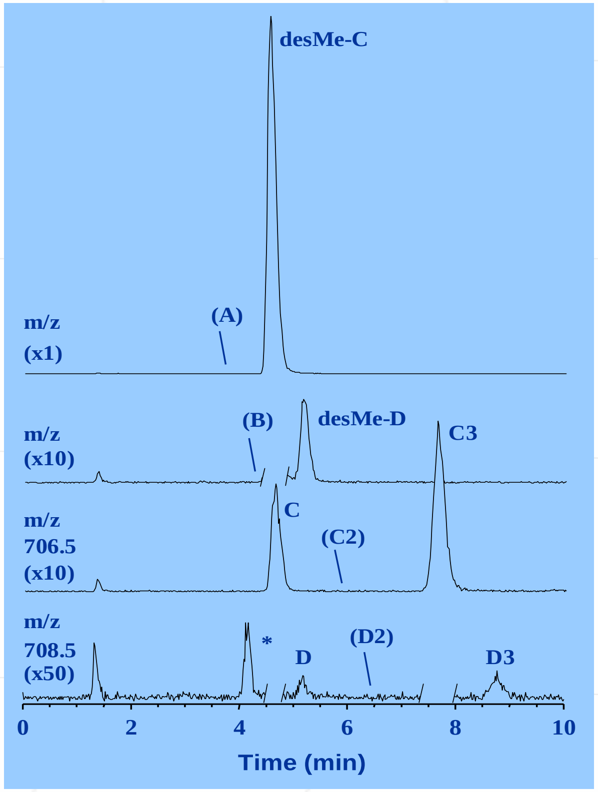
Alexandrium tamarense

Produces PSP toxins, but no spirolides



Alexandrium ostenfeldii

Produces spirolides, but no PSP toxins

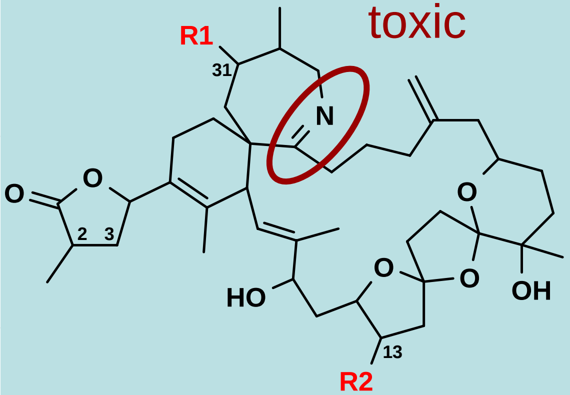


Cause of “Fast Acting Toxicity”

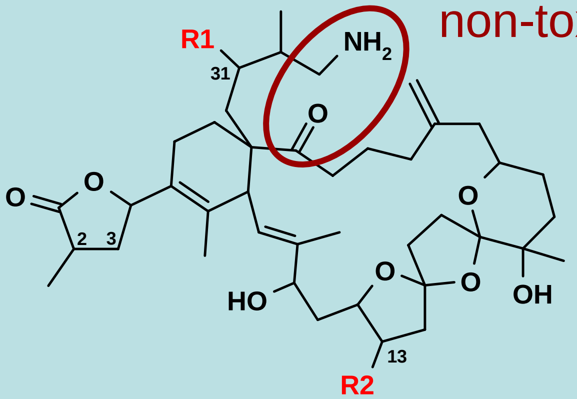
Novel compounds identified as “spirolides”

- macrocyclic imines
- structural similarity to pinnatoxin & gymnodimine
- pharmacologically active/inactive forms

toxic

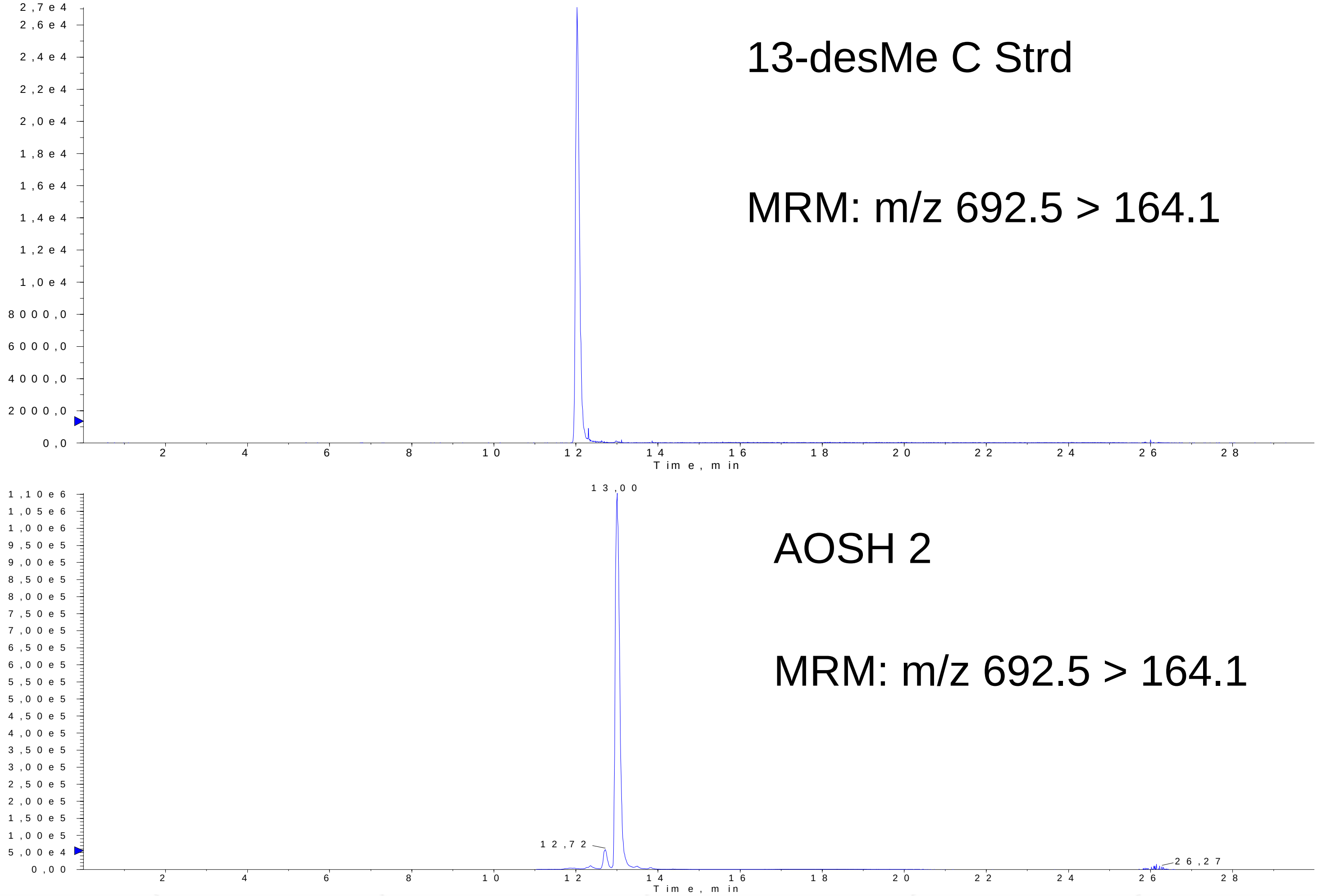


non-toxic



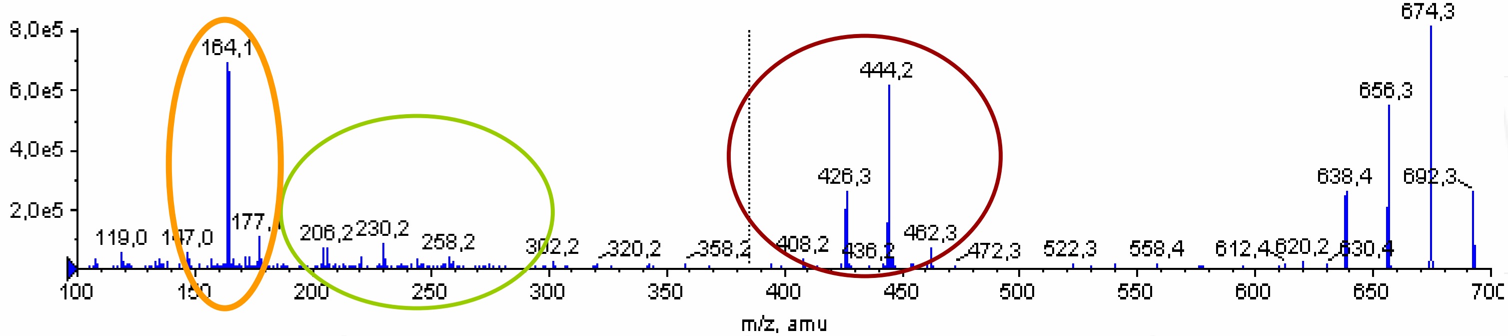
	R1	R2		MW
A	H	CH_3	$\Delta^{2,3}$	691.5
desMeC	CH_3	H	$\Delta^{2,3}$	691.5
B	H	CH_3		693.5
desMeD	CH_3	H		693.5
C	CH_3	CH_3	$\Delta^{2,3}$	705.5
D	CH_3	CH_3		707.5
E	H	CH_3	$\Delta^{2,3}$	709.5
F	H	CH_3		711.5

Identical mass transitions – different retention times

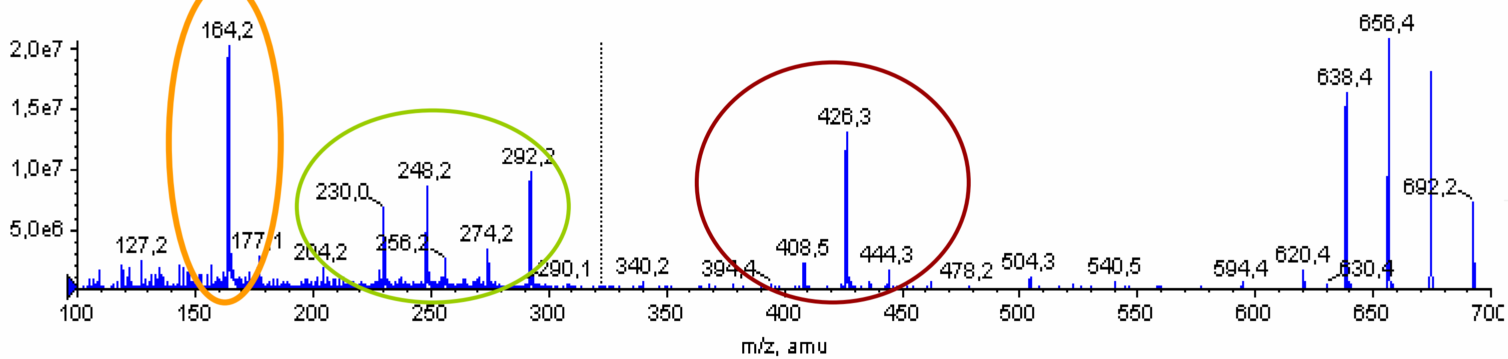


MS/MS spectra of m/z 692.5

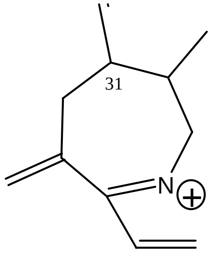
13-desMe C Strd: EPI m/z 692.5



AOSH 2: EPI m/z 692.5



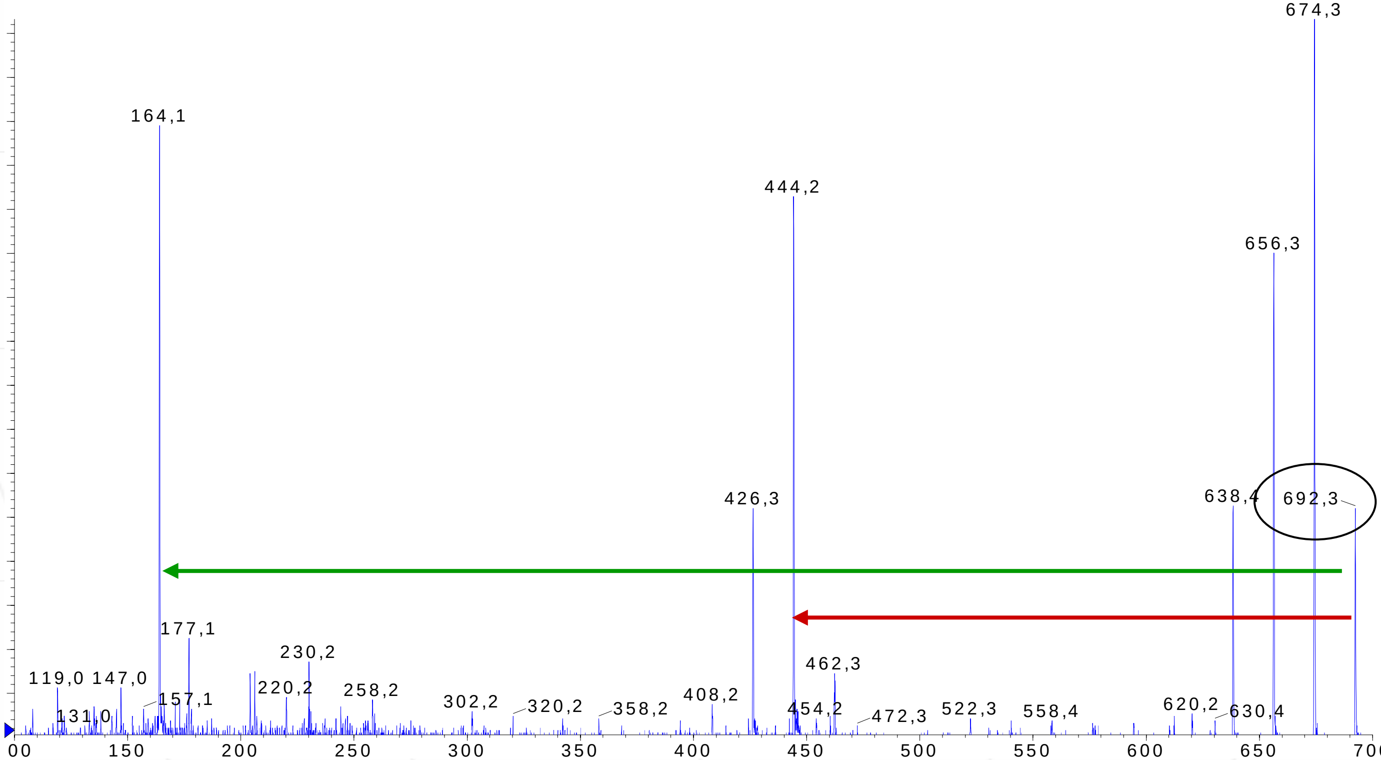
Characteristic spirolide fragment



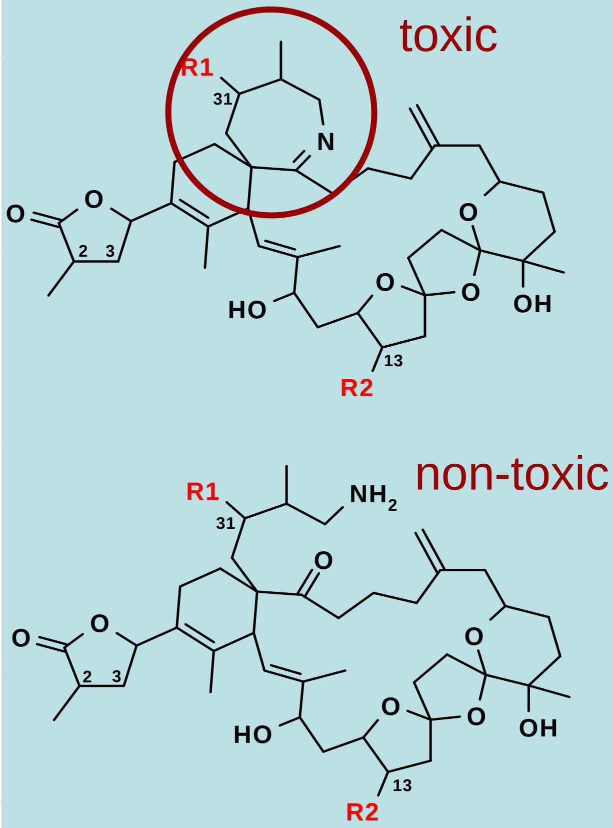
M+H = 692.5

m/z = 444.3

m/z = 164.1



Cyclic imino moiety accounts for toxicity ***and*** forms a characteristic spirolide fragment



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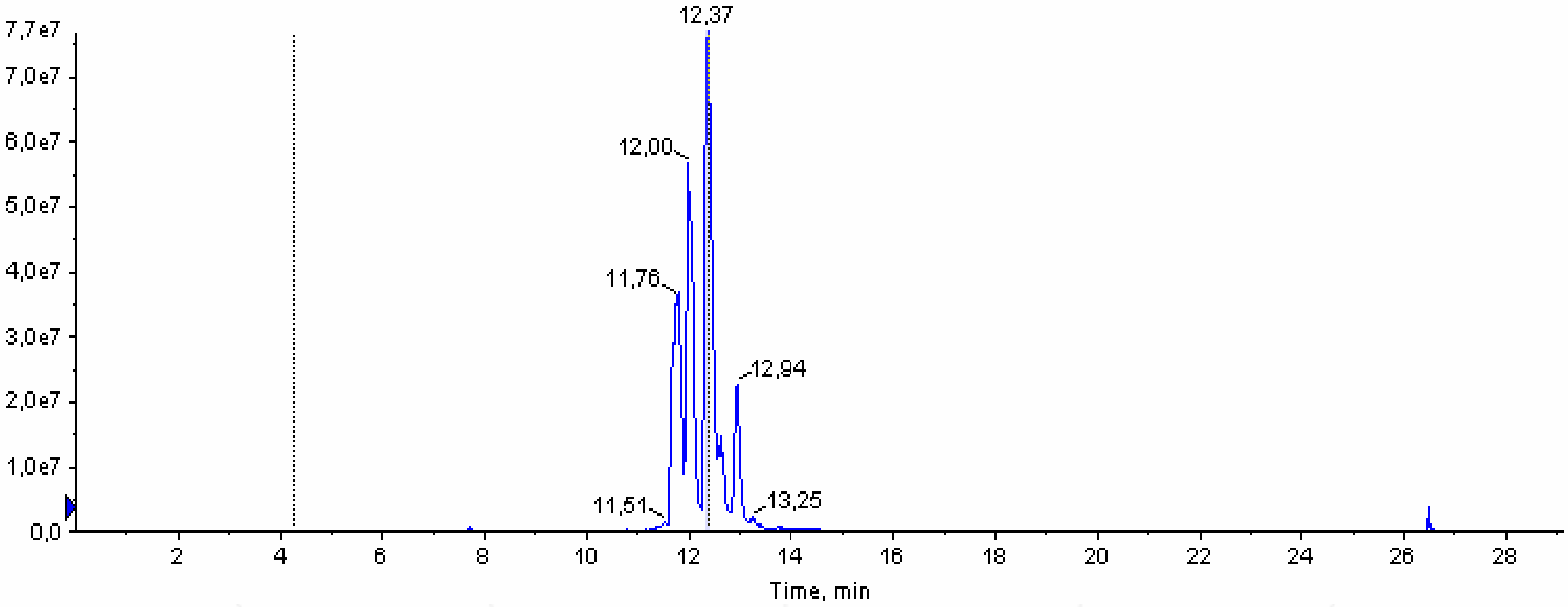
D	CH_3	CH_3		707.5
E	H	CH_3	$\Delta^{2,3}$	709.5
F	H	CH_3		711.5

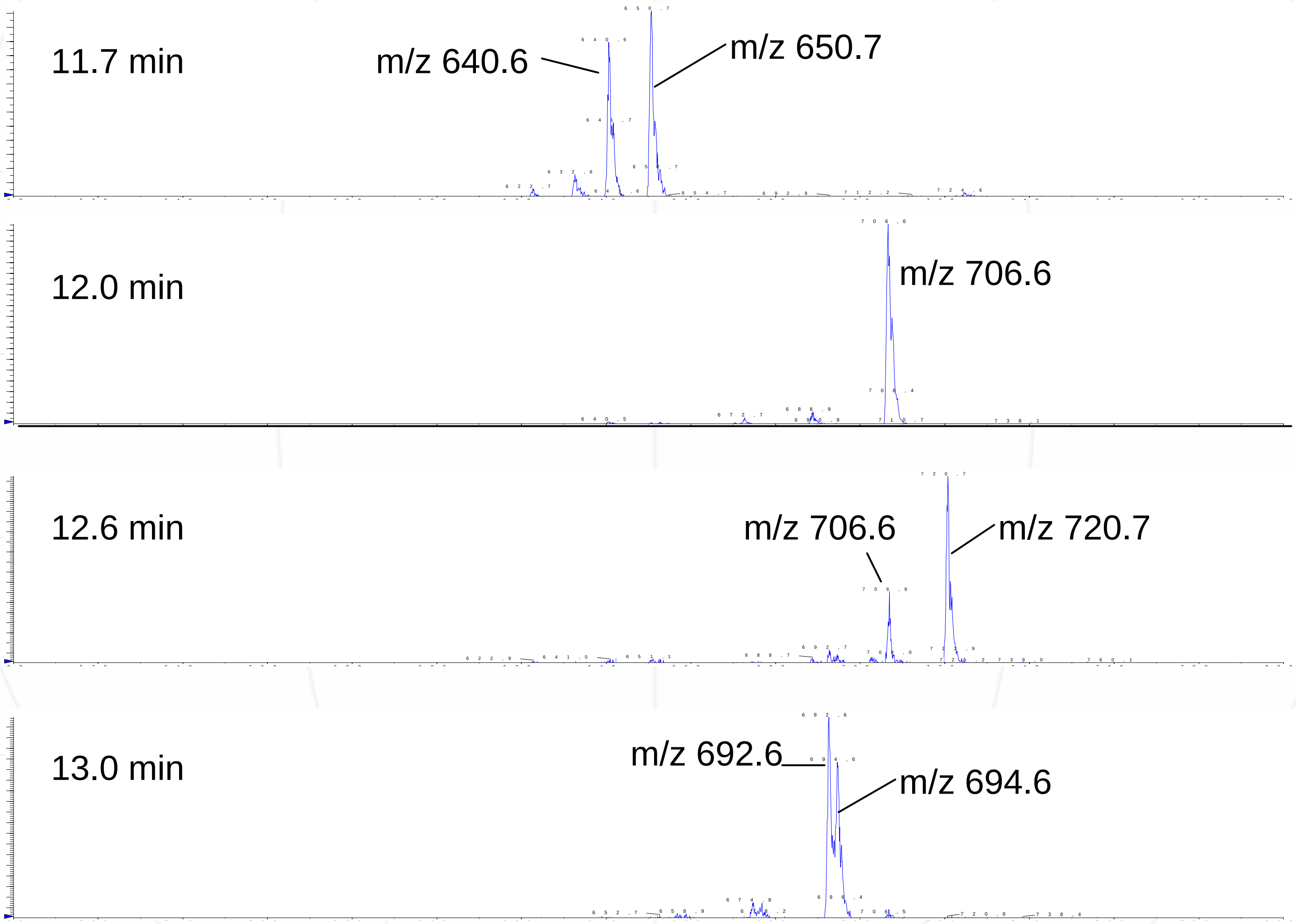
Using the characteristic fragment for the detection of unknown spirolides

AOSH 2: Precursor m/z 164.1

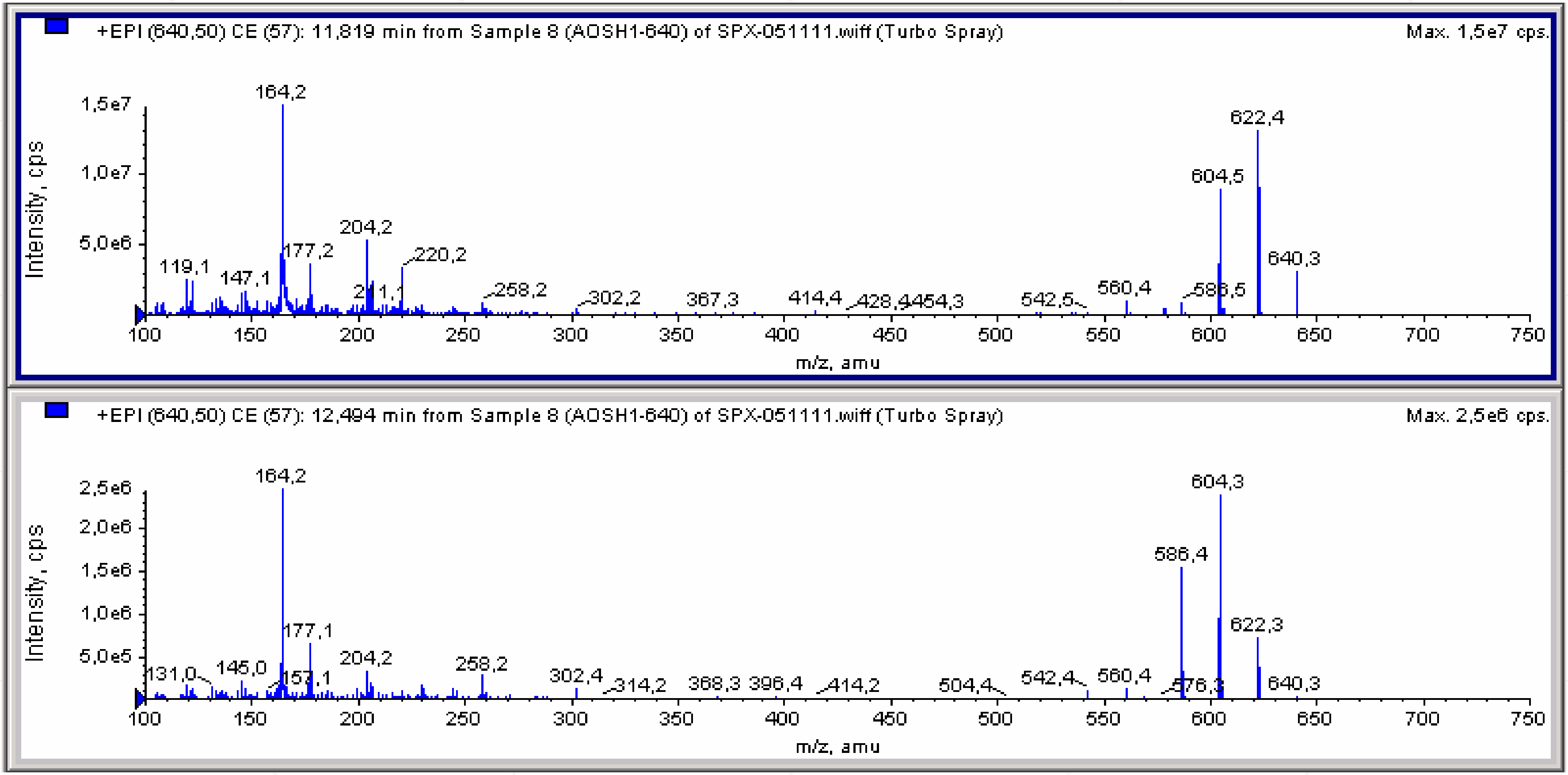


Spirolide masses

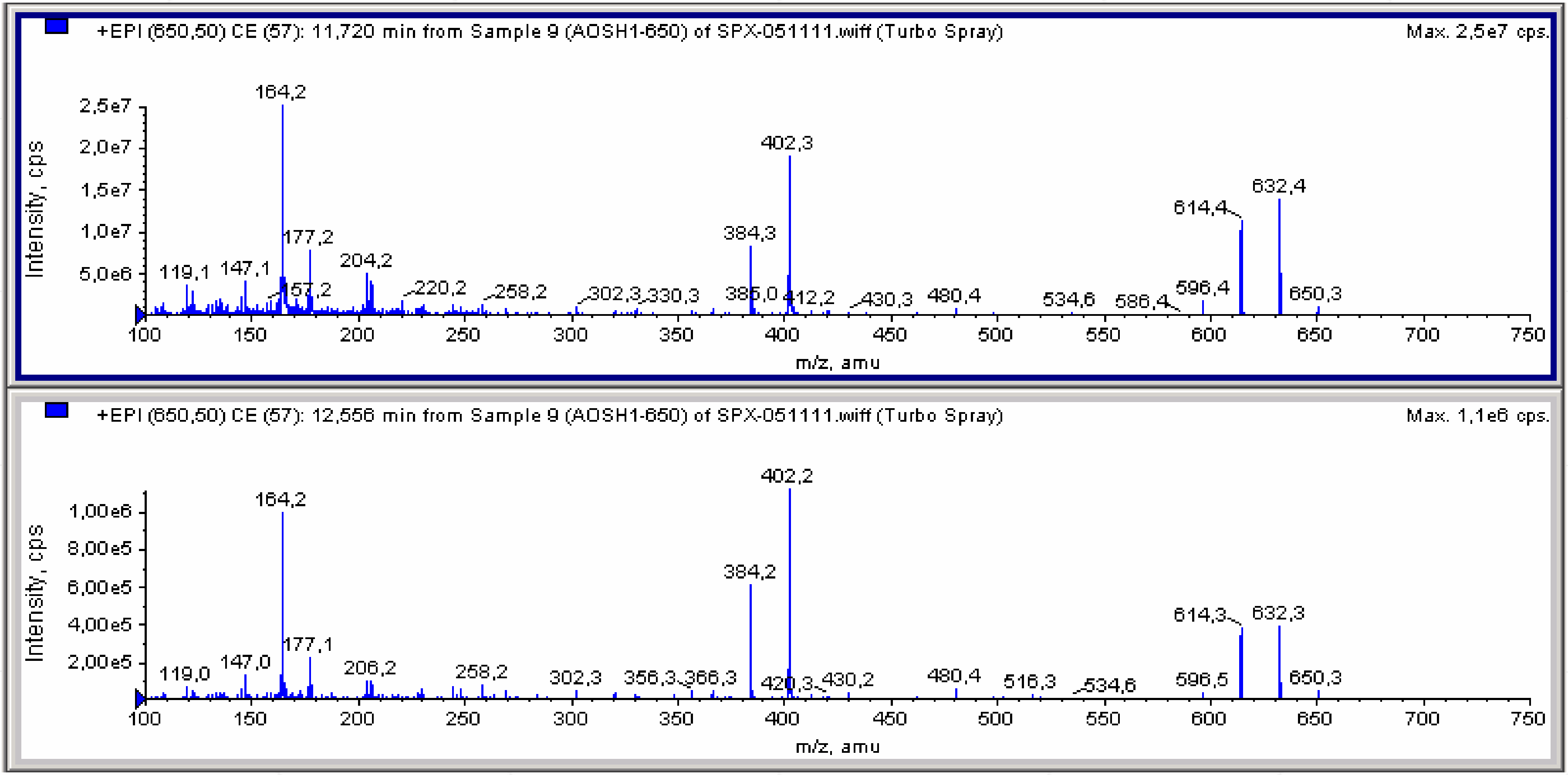
AOSH 2: Precursor m/z 164.1



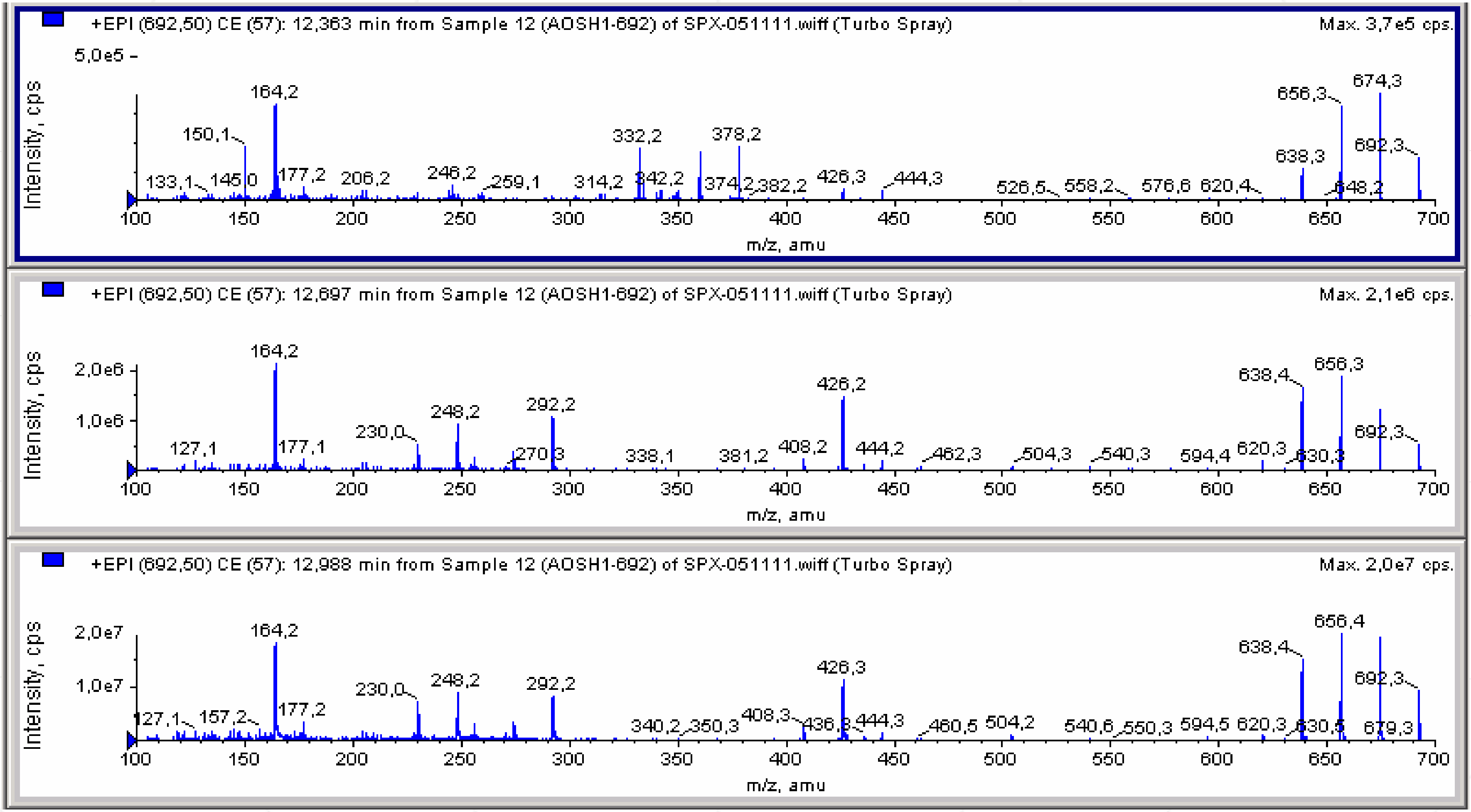
AOSH 2: m/z = 640.5



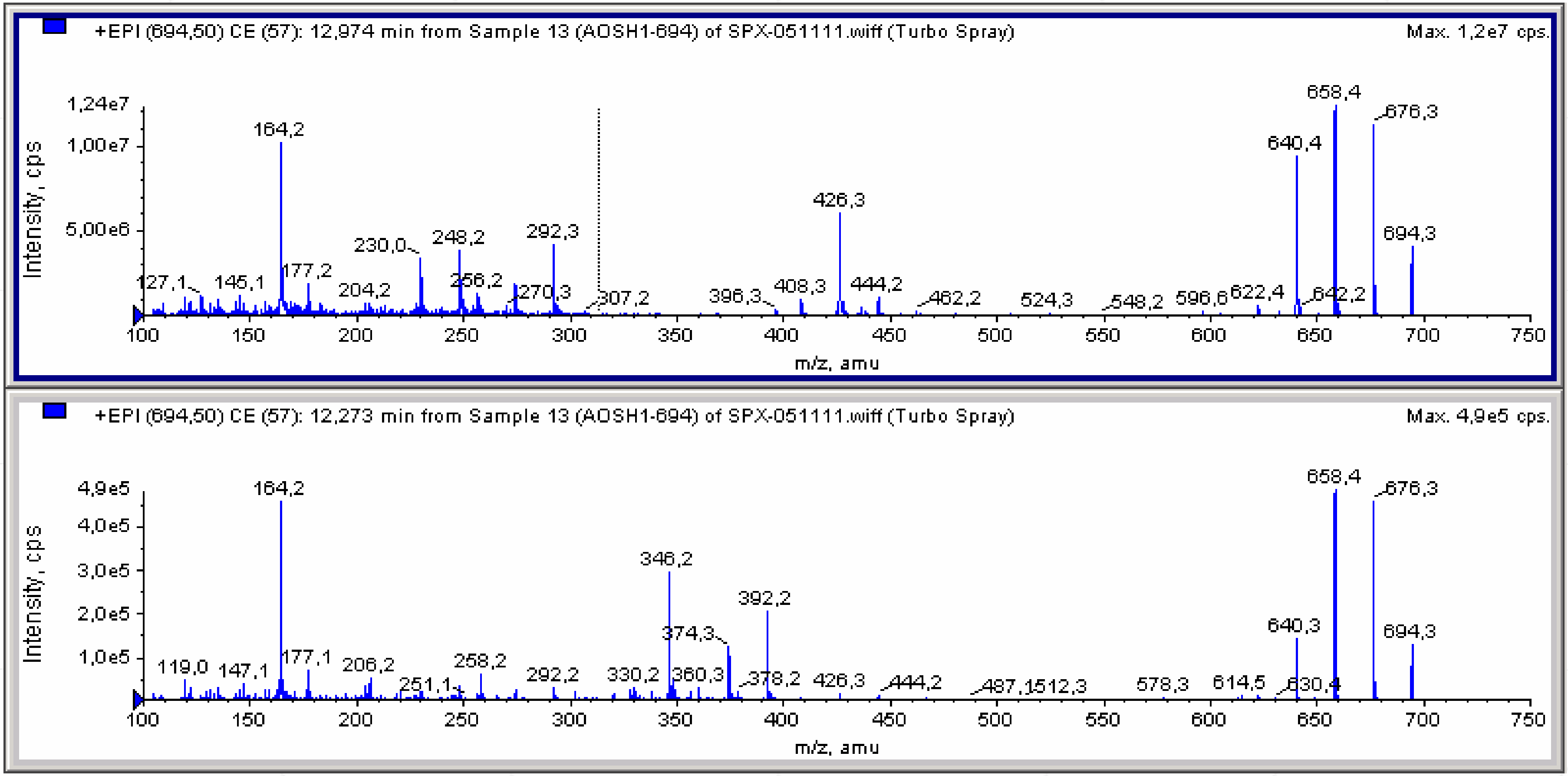
AOSH 2: m/z = 650.5



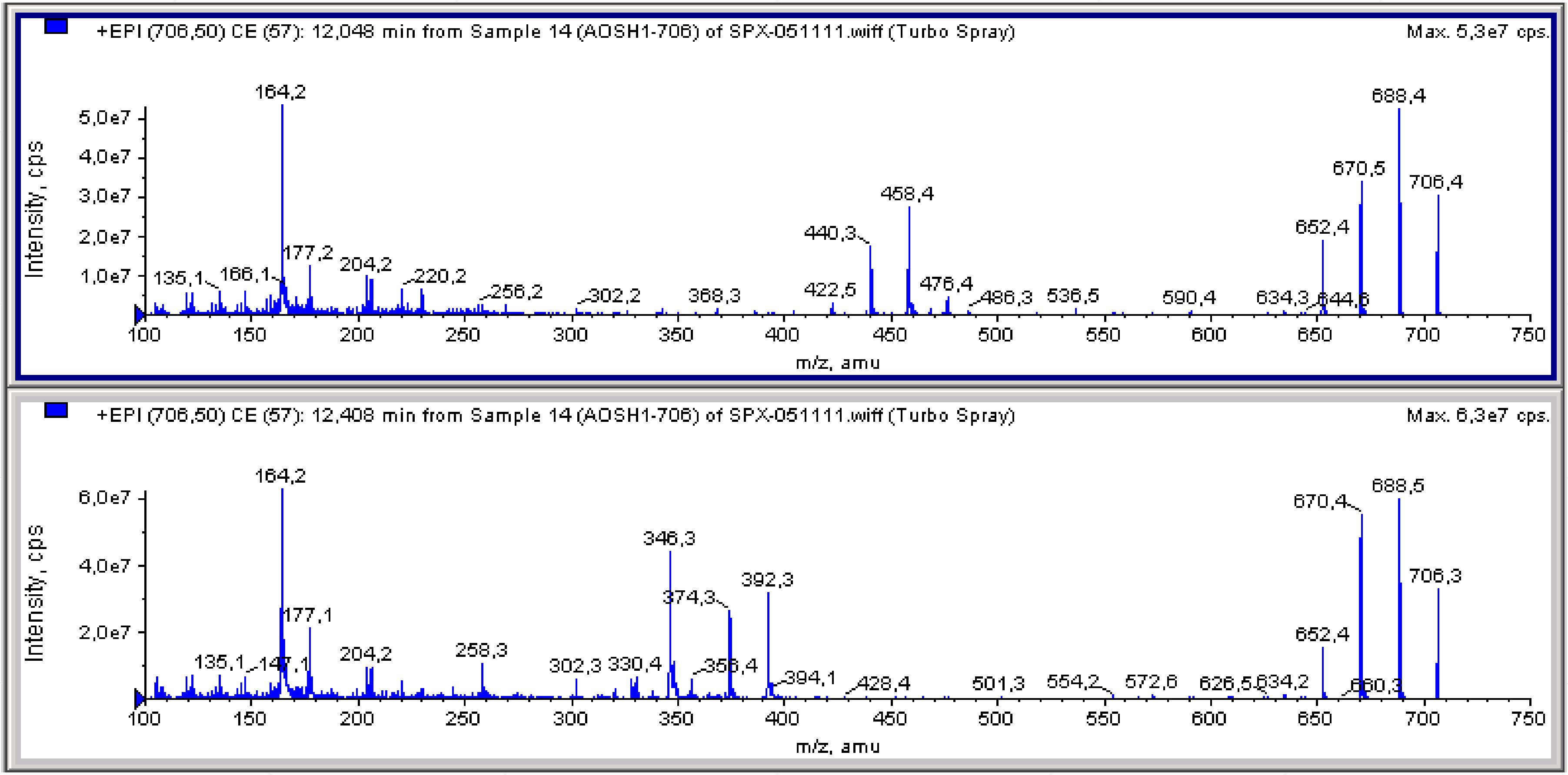
AOSH 2: m/z = 692.5



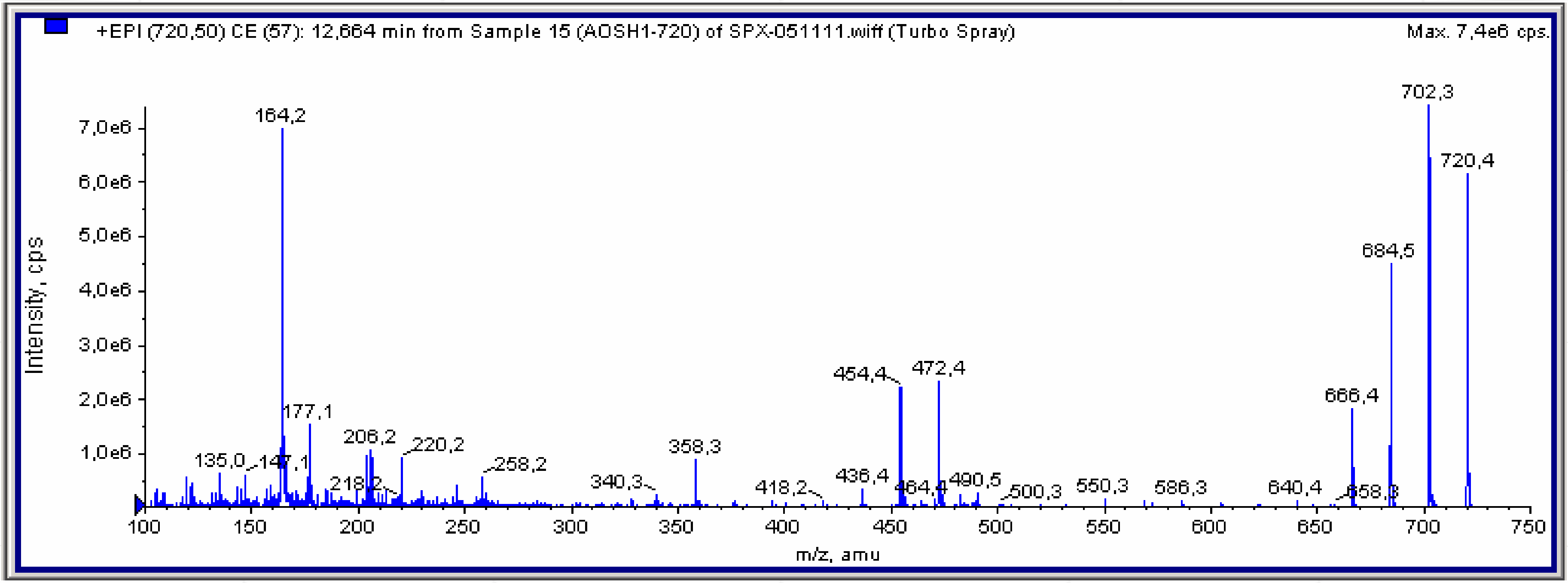
AOSH 2: m/z = 694.5



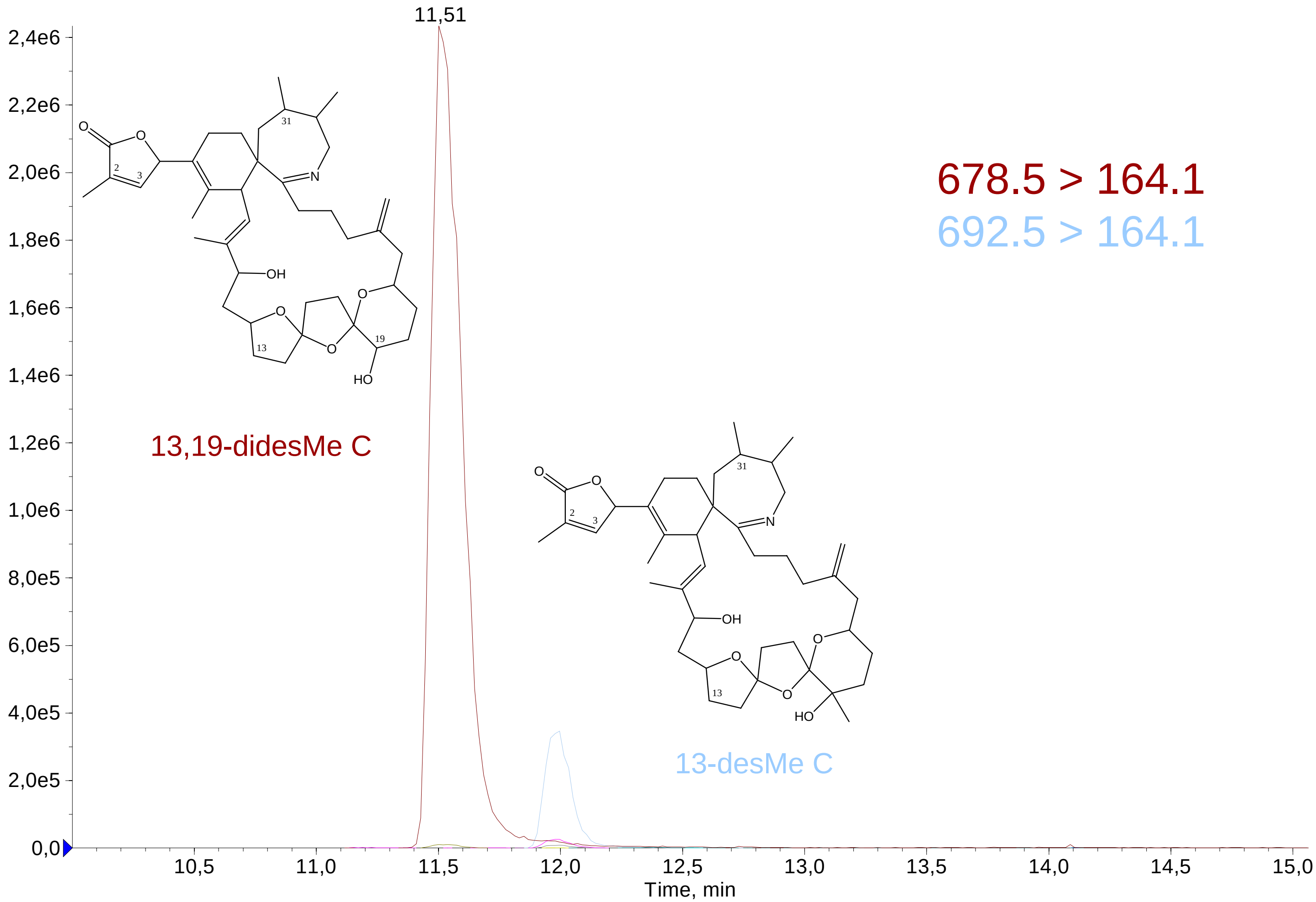
AOSH 2: m/z = 706.5



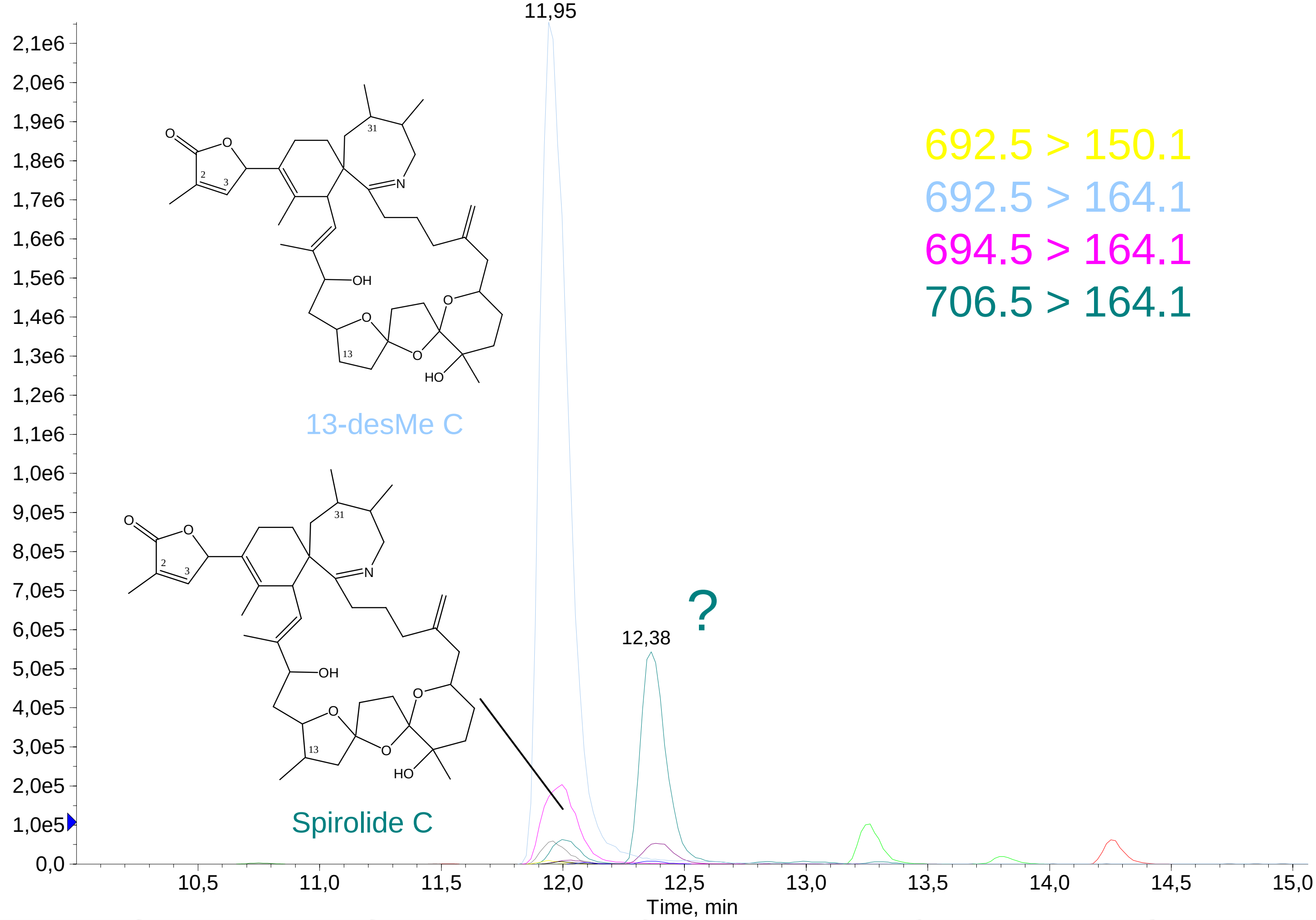
AOSH 2: m/z = 720.5



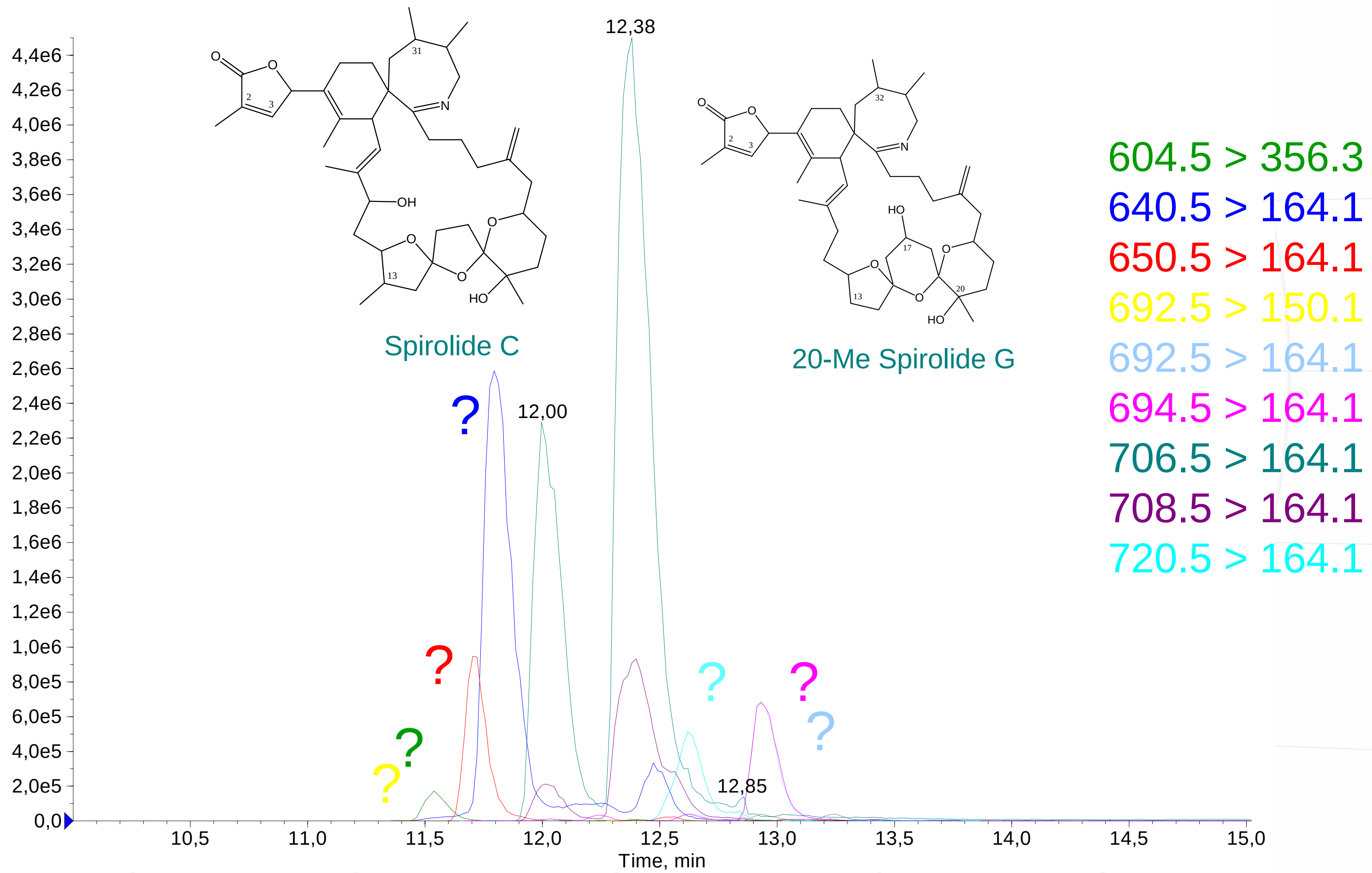
CCMP 1773, Denmark



AOSH 1, Canada

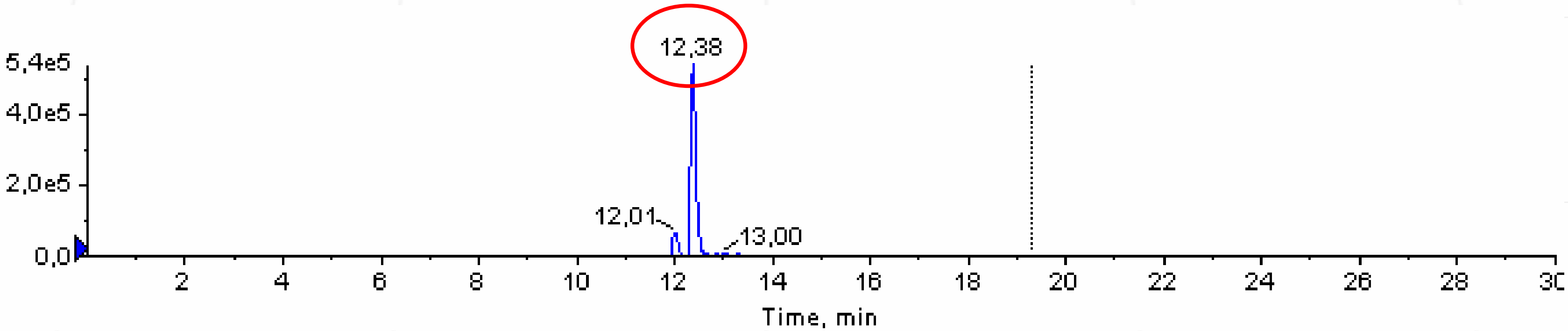


AOSH 2, Canada

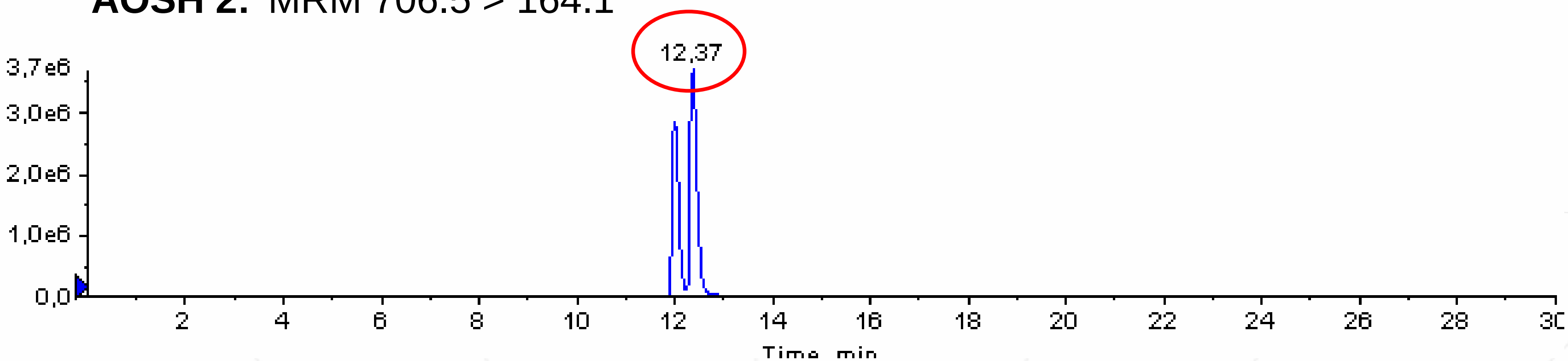


Identical retention time and mass transition,

AOSH 1: MRM 706.5 > 164.1

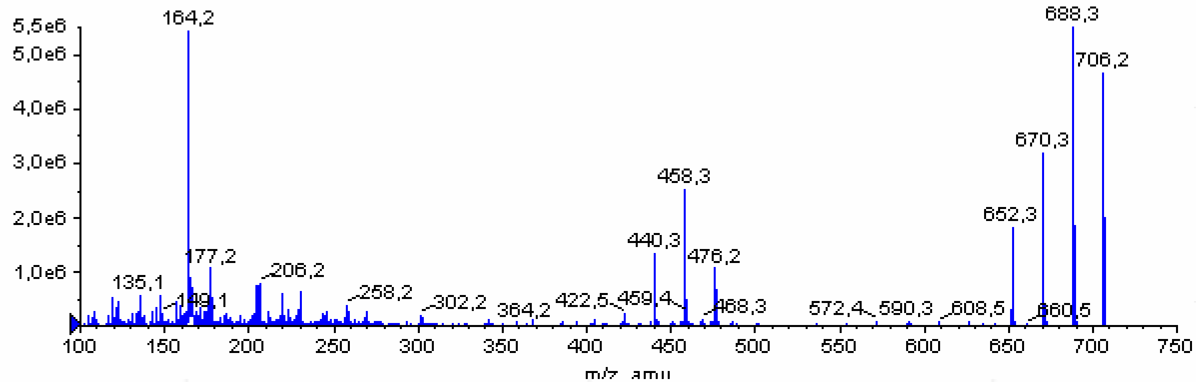


AOSH 2: MRM 706.5 > 164.1

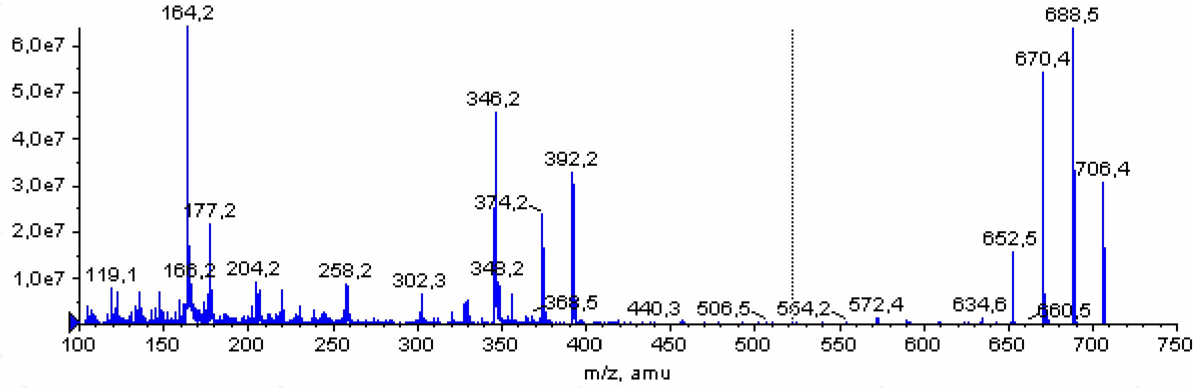


but different mass spectra

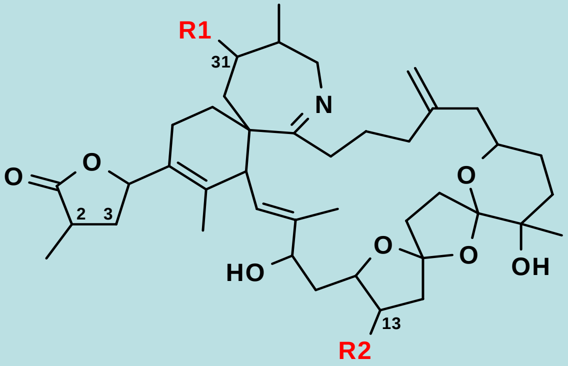
AOSH 1: EPI m/z 706.5; 12.37 min

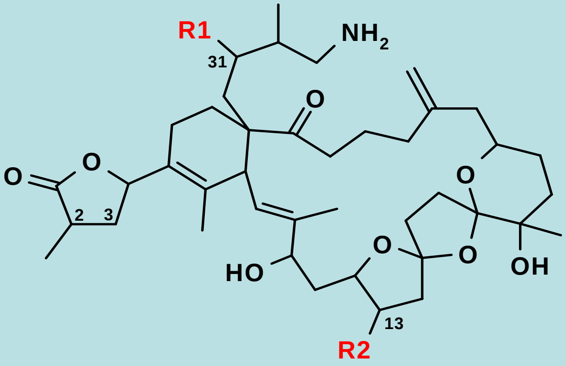


AOSH 2: EPI m/z 706.5; 12.37 min



Compound or isotopic peak?





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D	CH_3	CH_3		707.5
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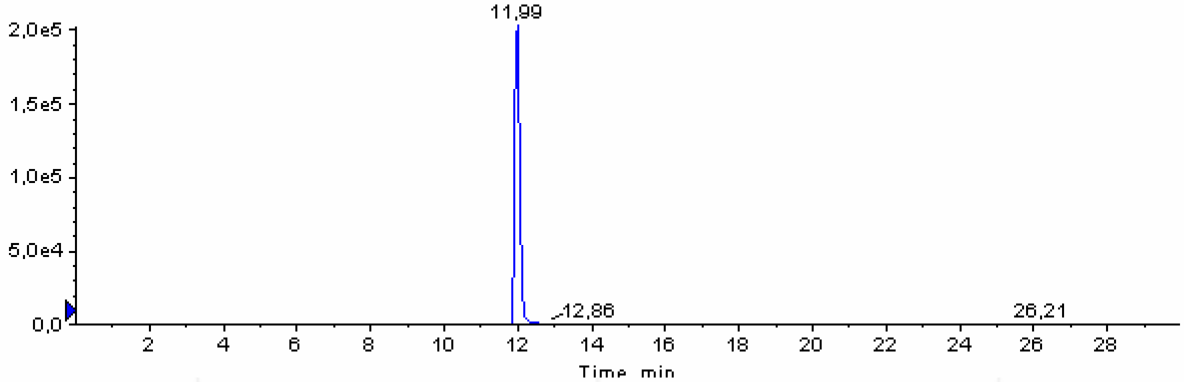
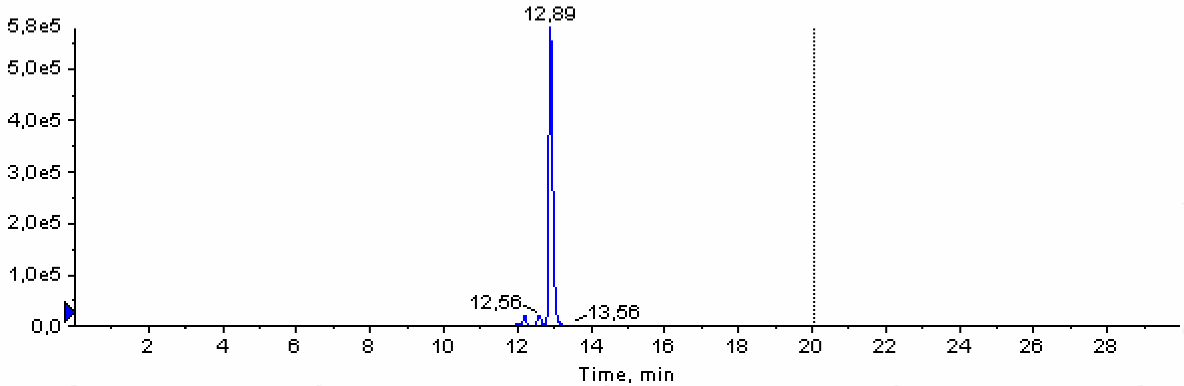
F	H	CH_3		711.5

Compound or isotopic peak?

AOSH 1: MRM 694.5 > 164.1



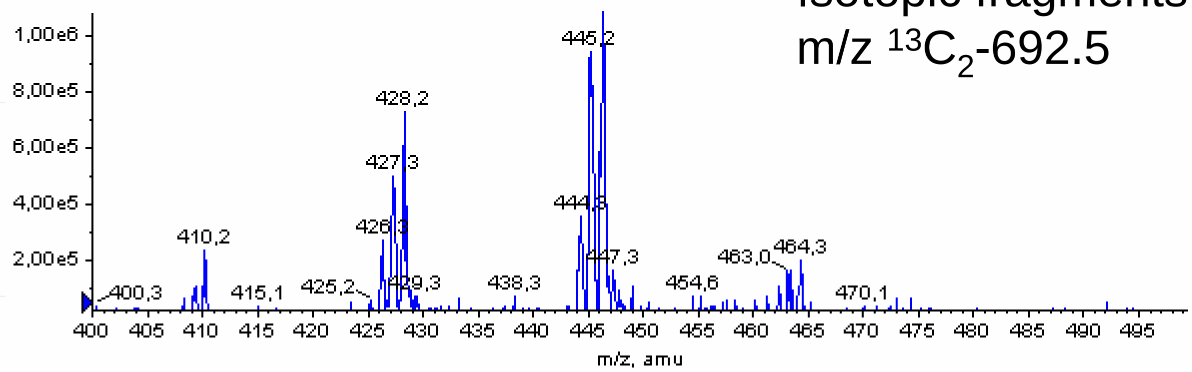
AOSH 2: MRM 694.5 > 164.1



Product ion spectra reveal isotopic pattern

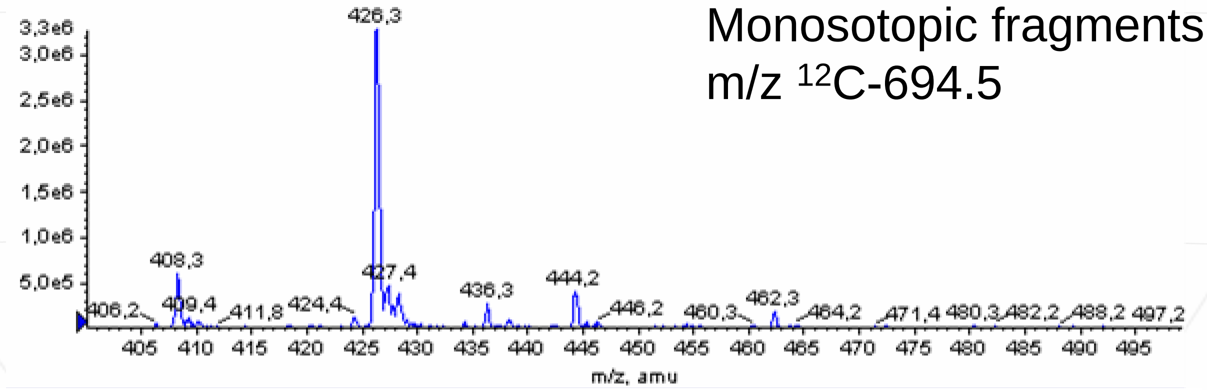
AOSH 1: EPI m/z 694.5; 12.0 min

Isotopic fragments of m/z $^{13}C_2$-692.5



AOSH 2: EPI m/z 694.5; 12.9 min

Monosotopic fragments of m/z ^{12}C-694.5



Conclusions:

Triple quadrupole tandem mass spectrometry is a powerful tool for the determination and quantitation of spirolides

Unknown toxic spirolides can be detected in the precursor ion mode of the characteristic cyclo imino fragments at m/z 150 and 164, respectively

Structural information can be obtained by product ion spectra of parent ions

Co-eluting compounds with identical mass transitions can be differentiated by their product ion spectra

Product ion spectra can be used to differentiate between isotope statellites and monoisotopic peaks

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Thank You!