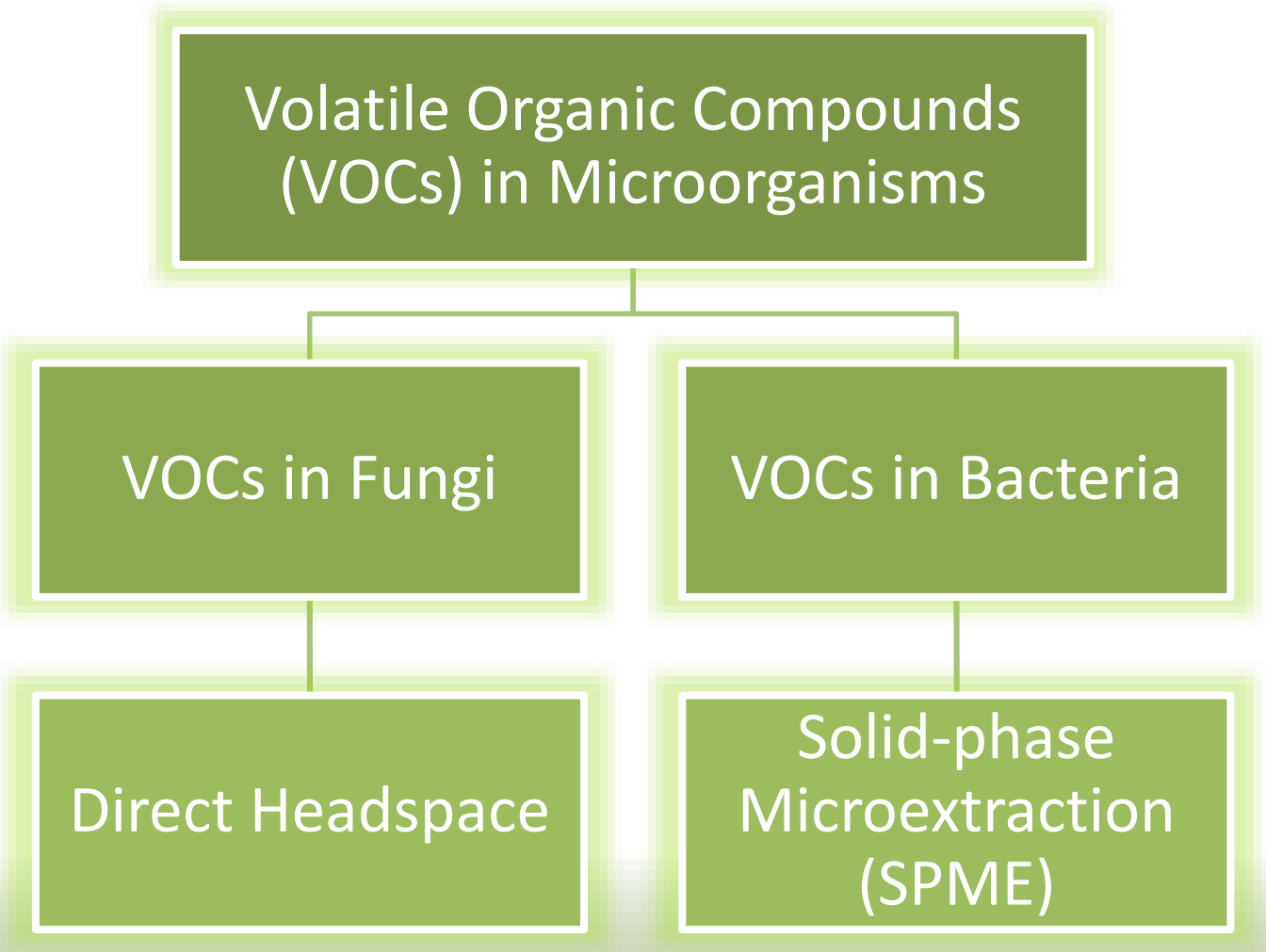
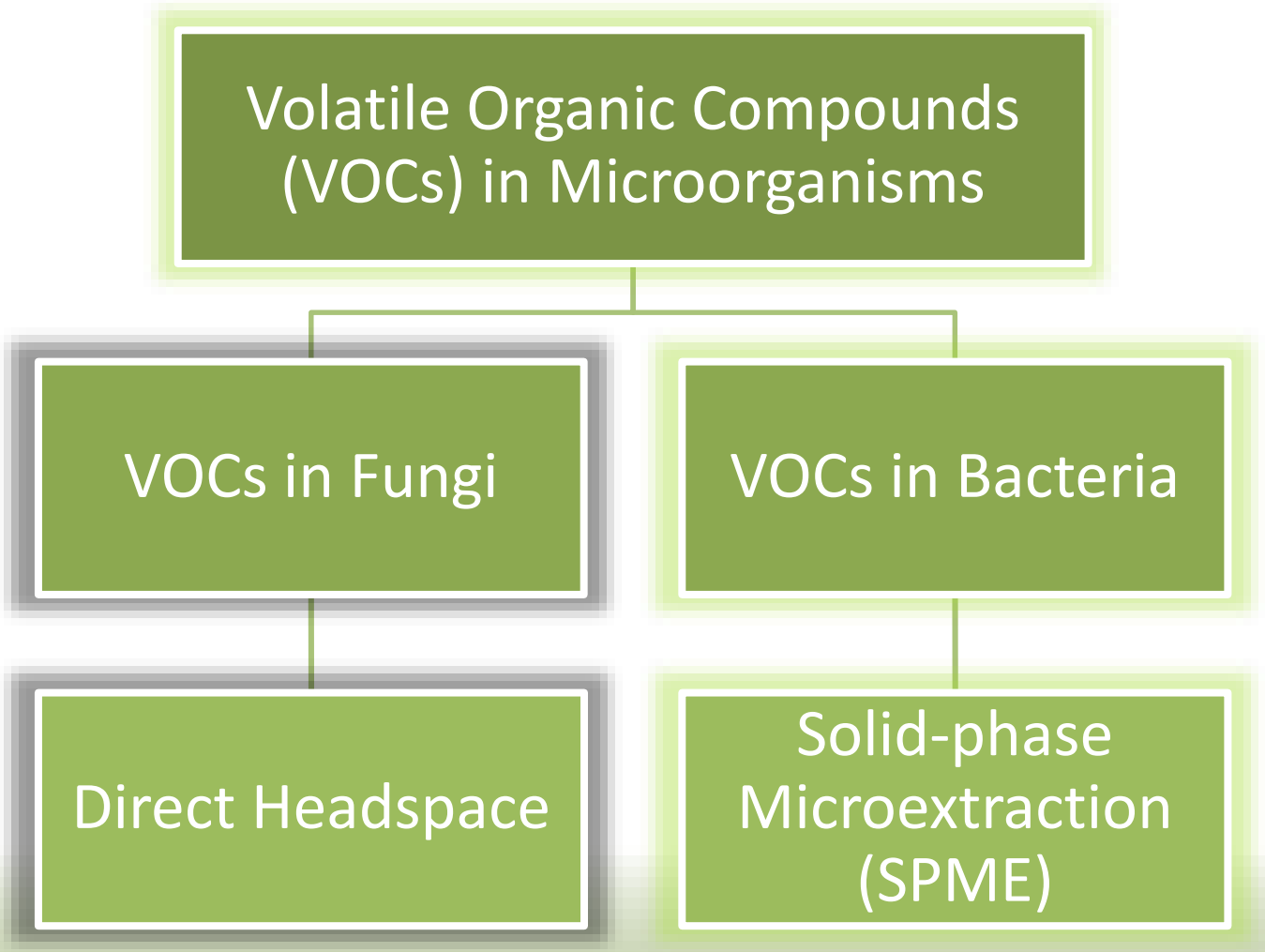
Microbe farts:
detecting volatile organic compounds from fungi and bacteria using GC/MS

William Trebelcock & James Erdmann

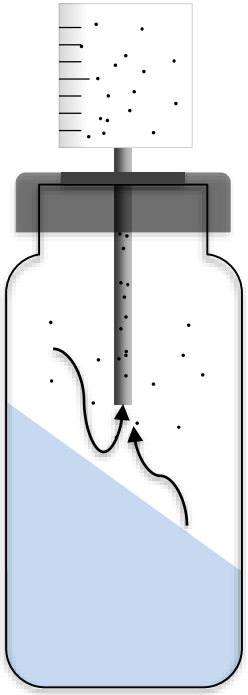
GC-MS Sampling Methods



GC-MS Sampling Methods

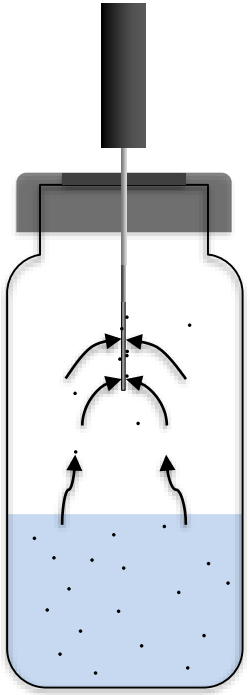


GC-MS Sampling Methods



Direct Headspace Sampling:

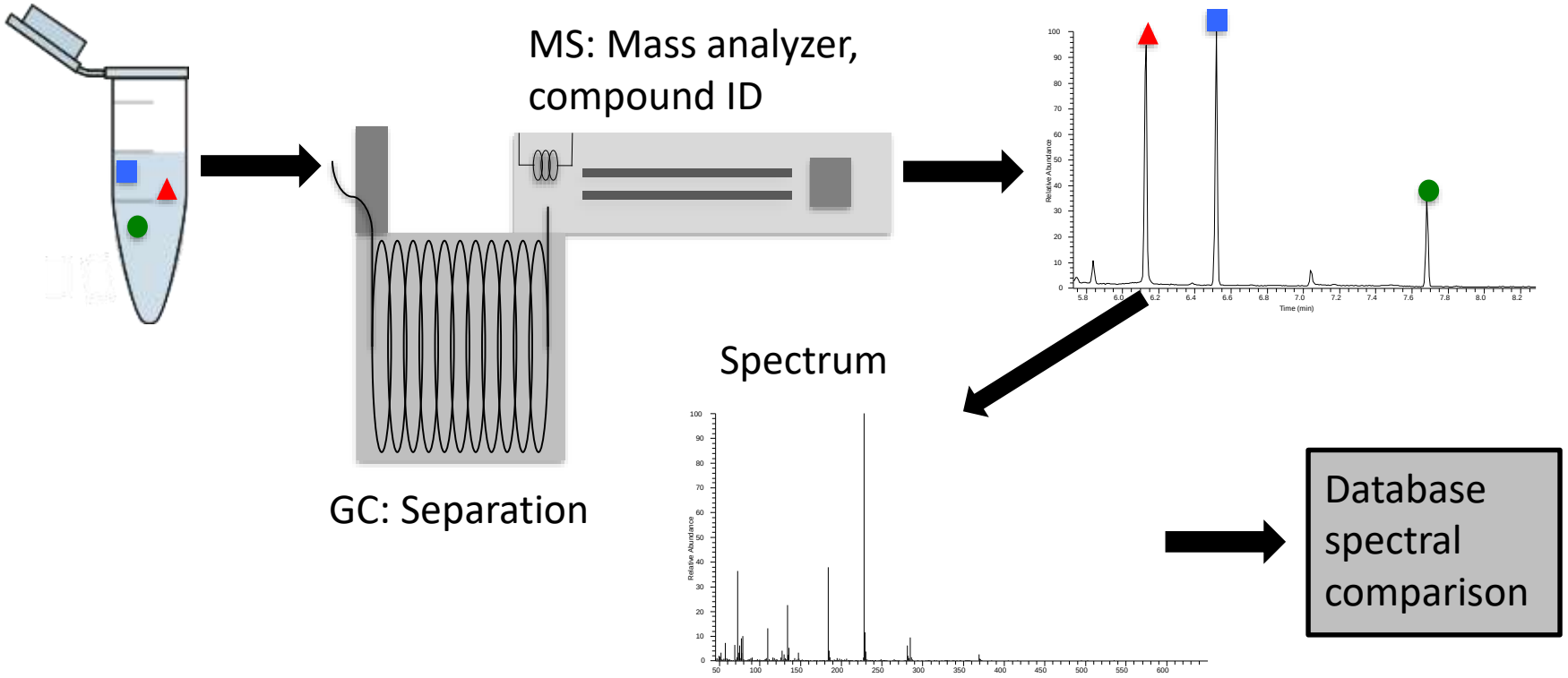
- **Quantification of VOCs using 1 mL of headspace gas.**



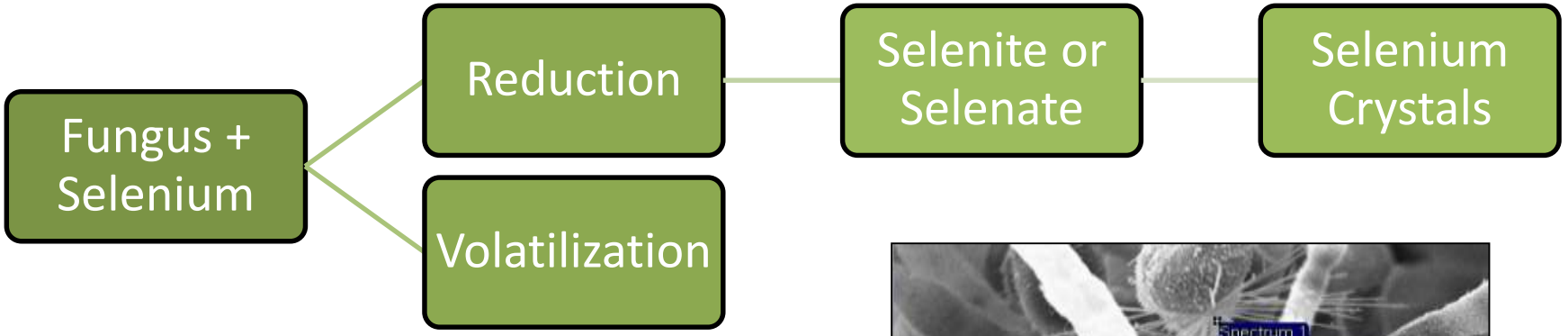
Solid Phase Microextraction (SPME):

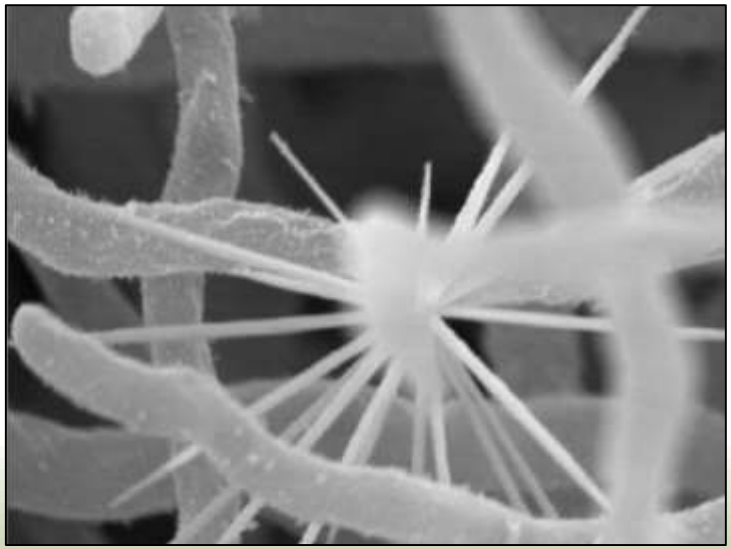
- **Concentration of VOCs on absorbent fiber.**

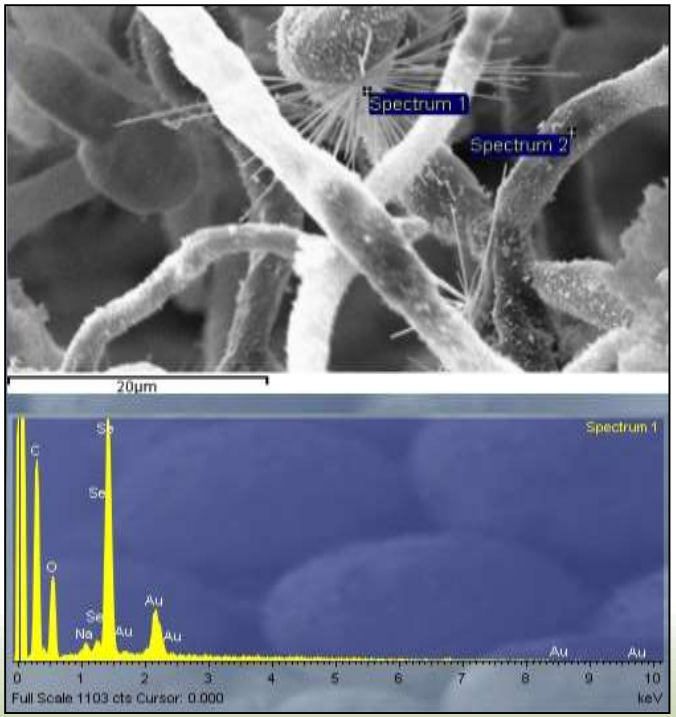
Why use GC-MS?


Chromatogram
MS: Mass analyzer, compound ID
Spectrum
GC: Separation
Database spectral comparison

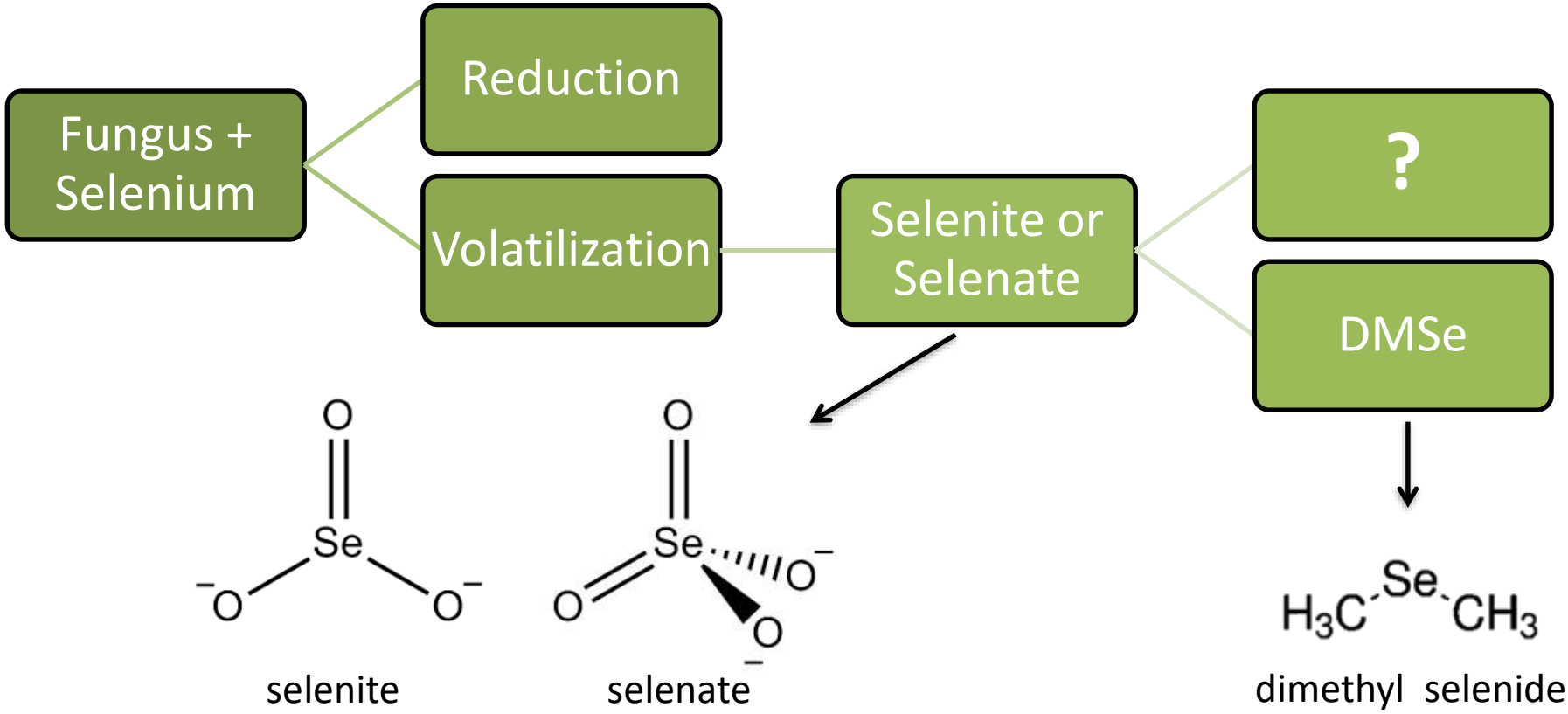
Selenium Tolerance - Reduction







Selenium Tolerance - Volatilization



Fungal Isolates

Fungal Isolate Name and Abbreviated ID	
Isolate	ID
Aspergillus leporis	AS117
Aspergillus leporis	AS2
Absidia spinosa	AB134
Alternaria seleniphilia	A1
Alternaria tenuissima	A2
Alternaria astragali	A3
Fusarium acuminatum	F30

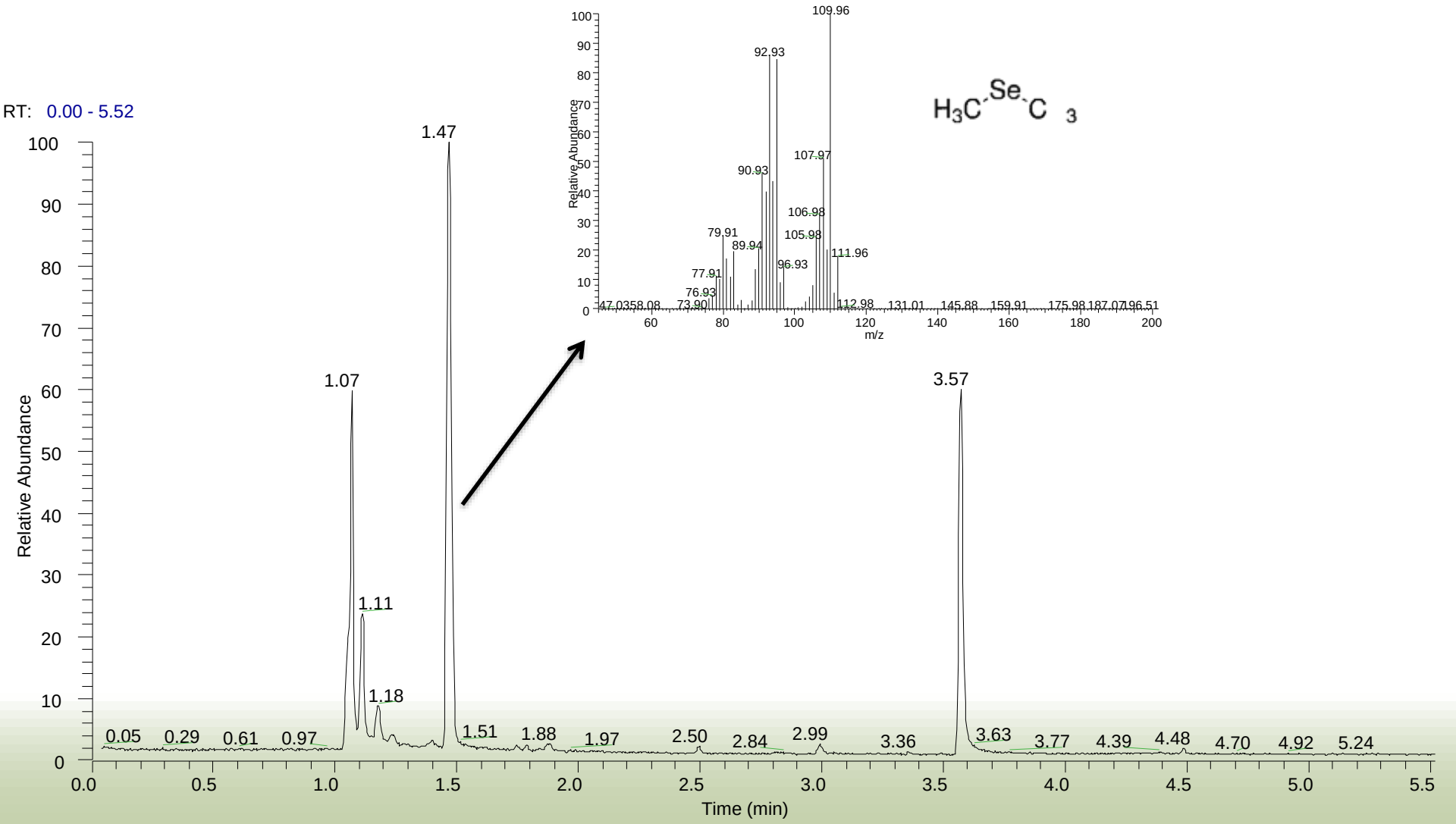
Fungal Treatments:

- Selenate, selenite, and control
- 30 ppm and 100 ppm concentrations
- 21 days growth in darkness at 22°C

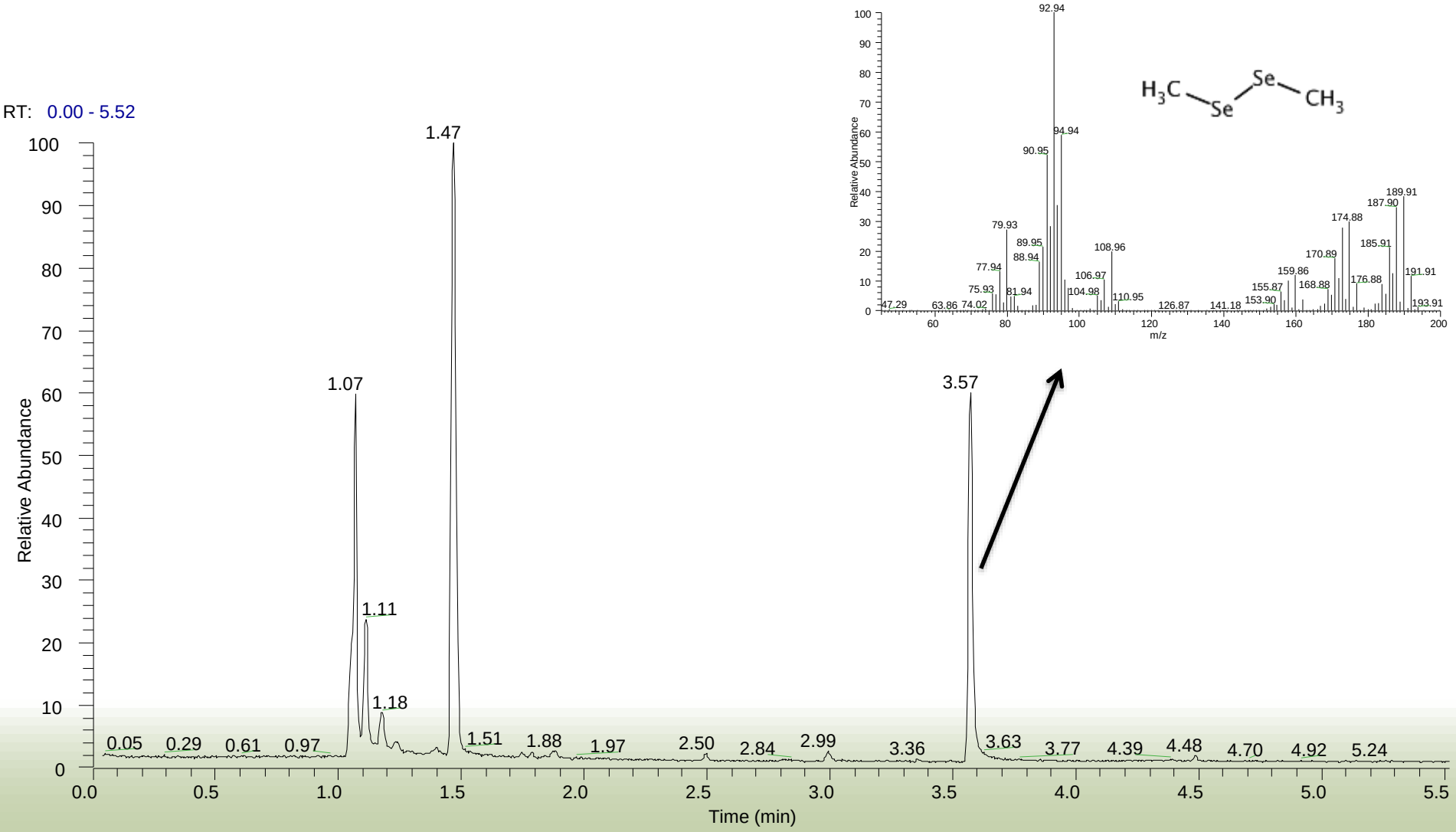
AS117 plate without selenium

AS2 headspace treatment vials

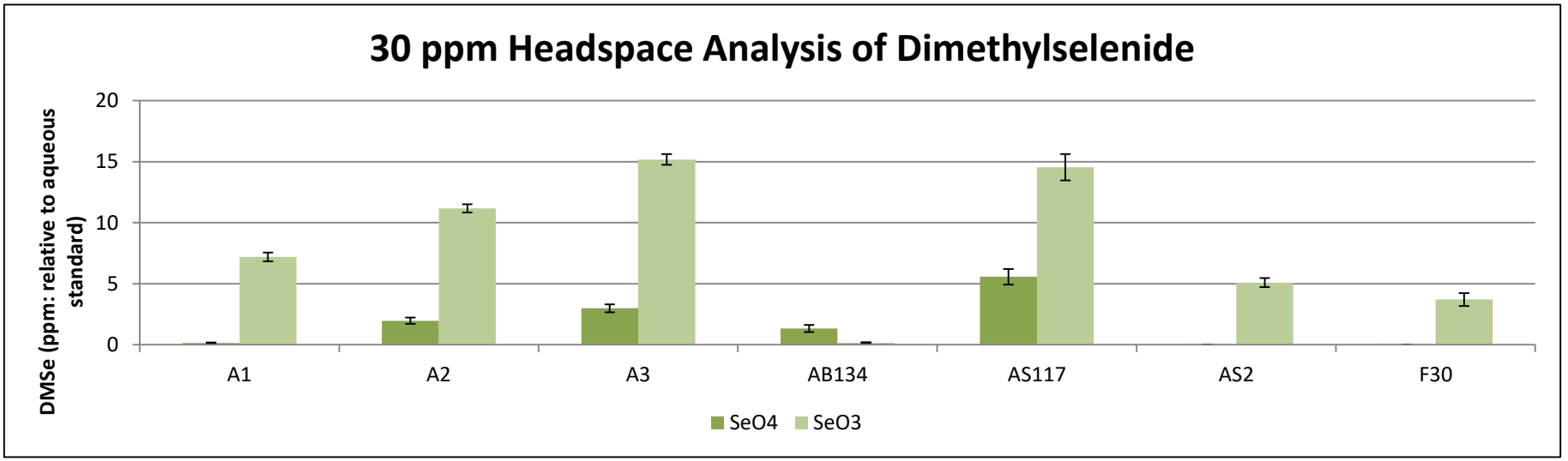
Dimethyl selenide Characterization

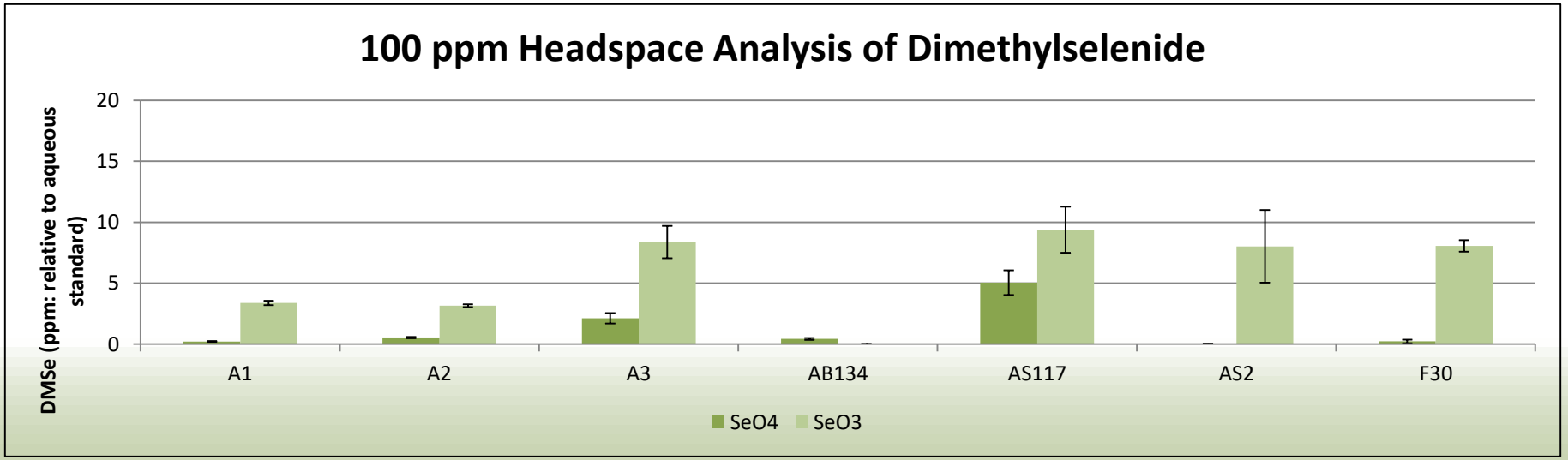


Dimethyl diselenide Characterization

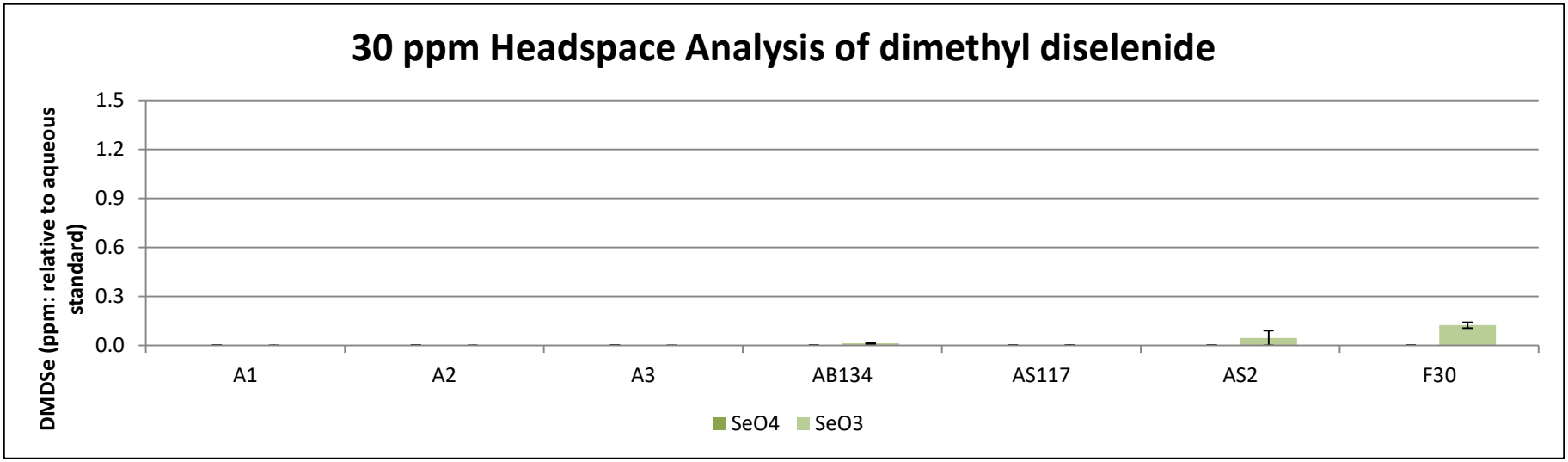


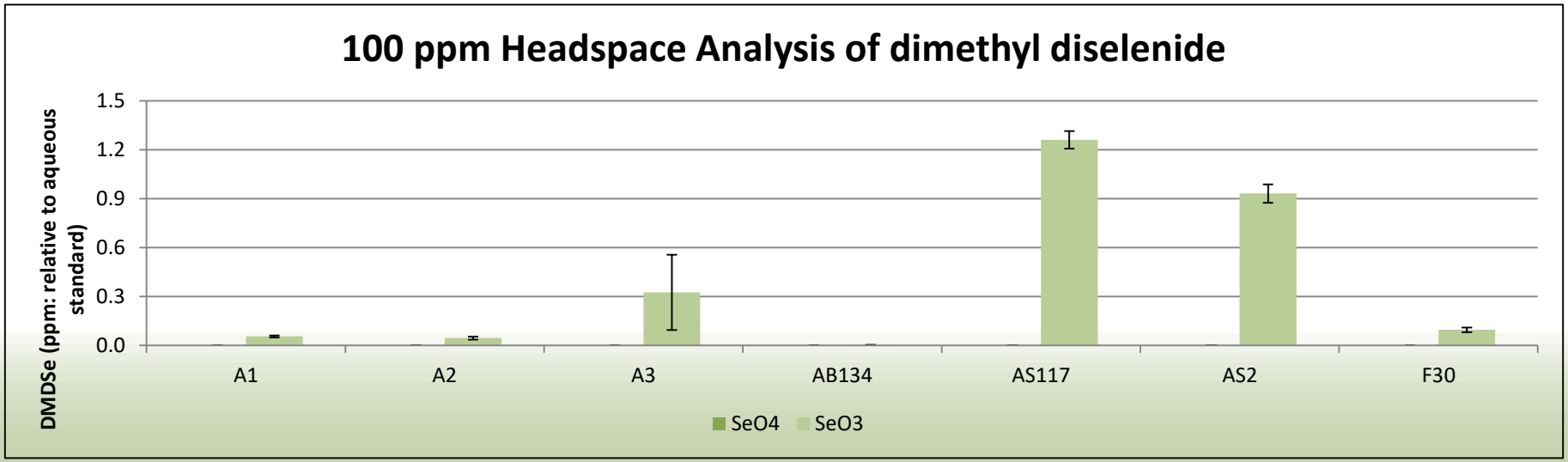
Dimethyl selenide Quantification



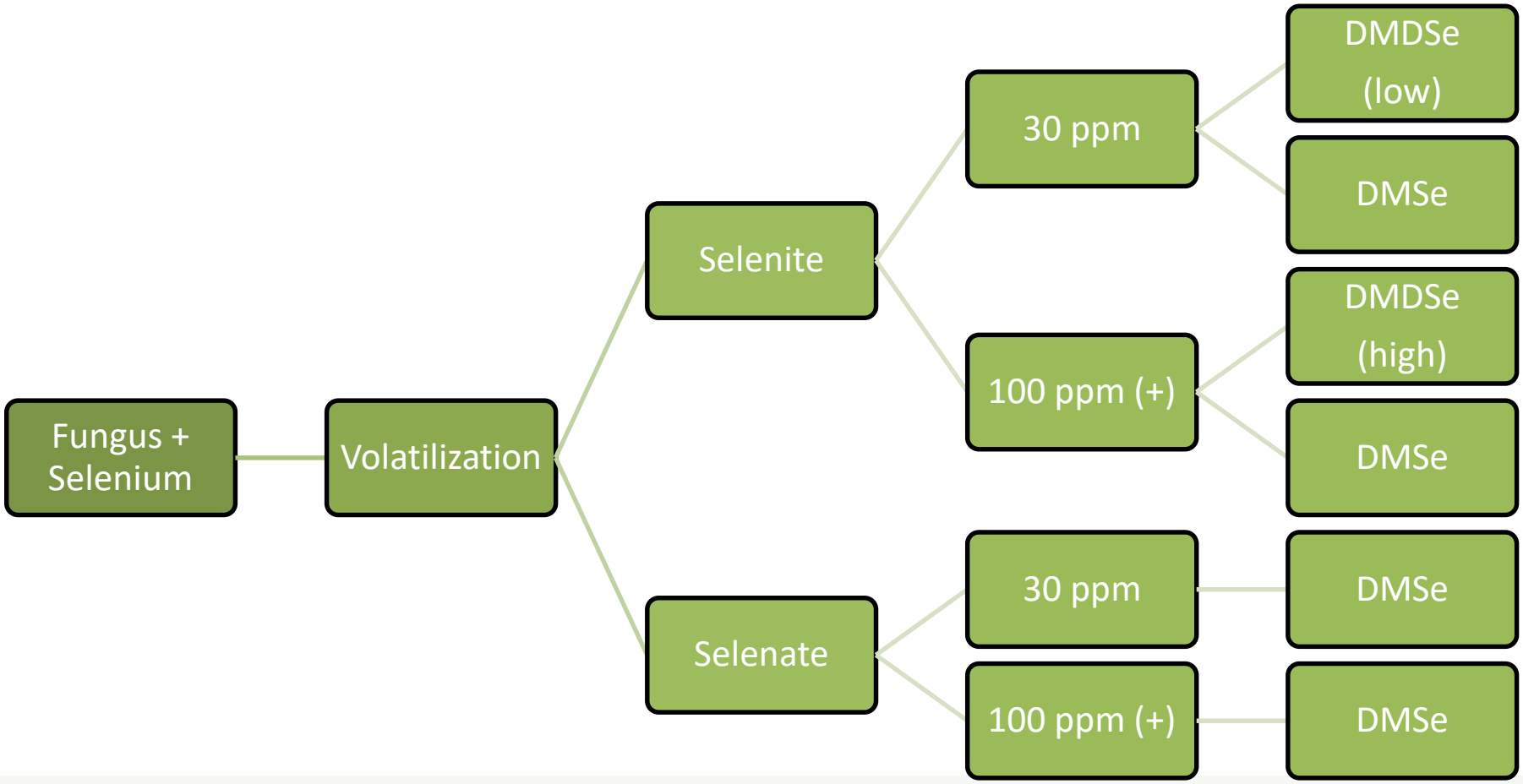


Dimethyl diselenide Quantification



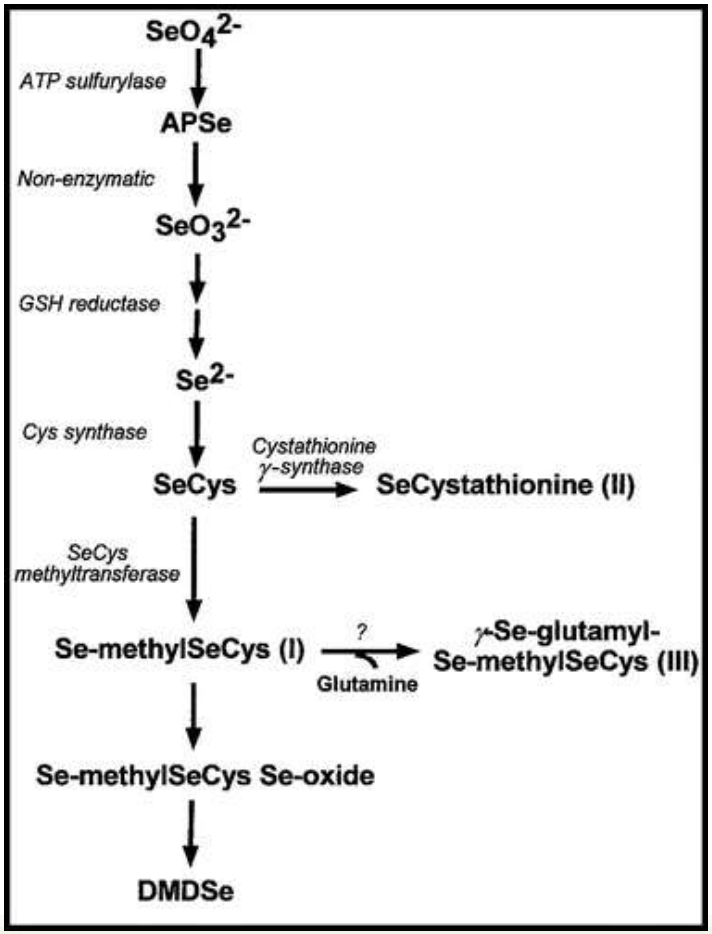


Selenium Tolerance - Volatilization



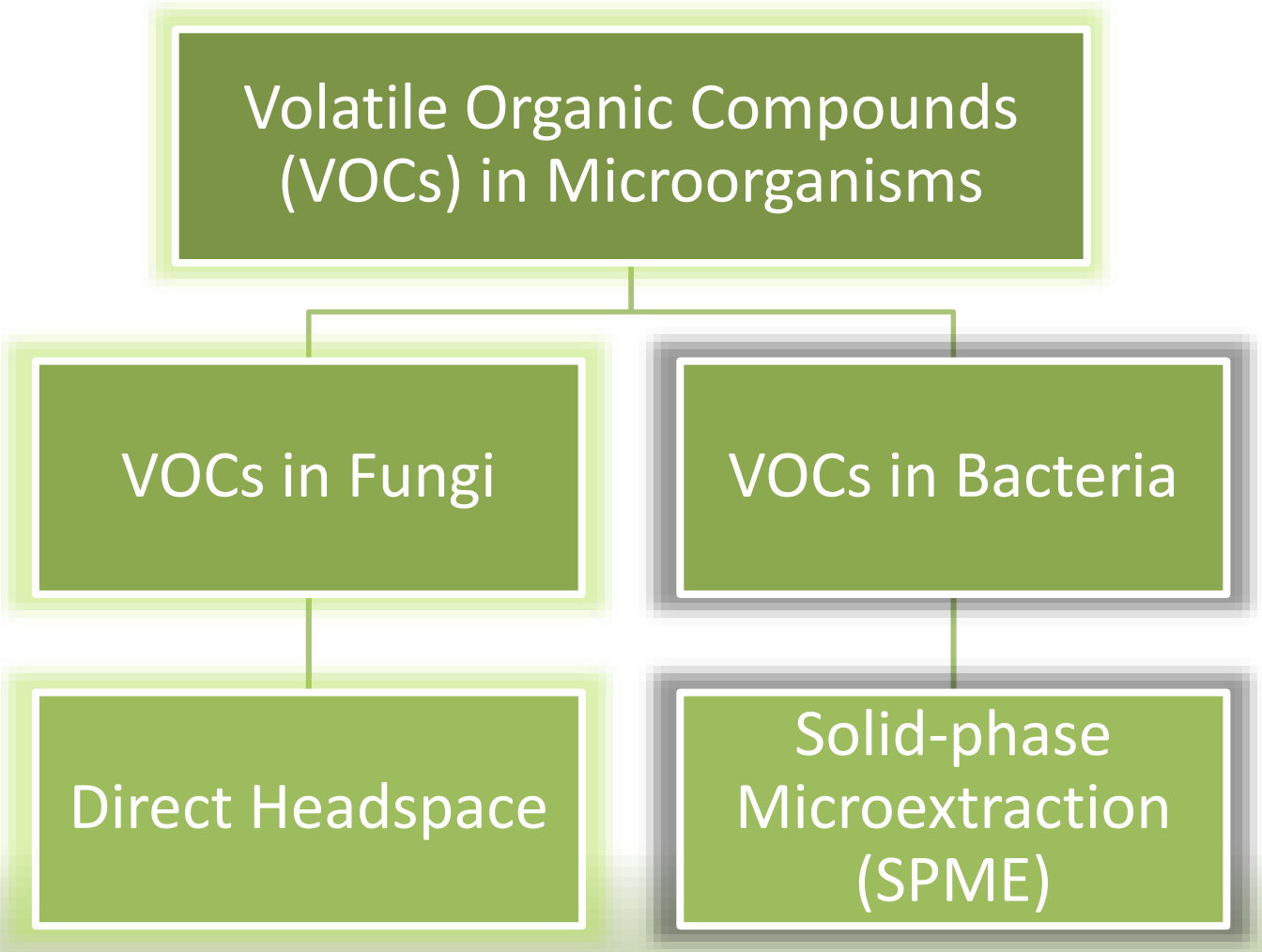
Conclusions

- Selenium metabolism different between plants and fungi?
- DMDSe analysis novel in fungi
- DMDSe production both concentration and species dependent
- Production varies between species



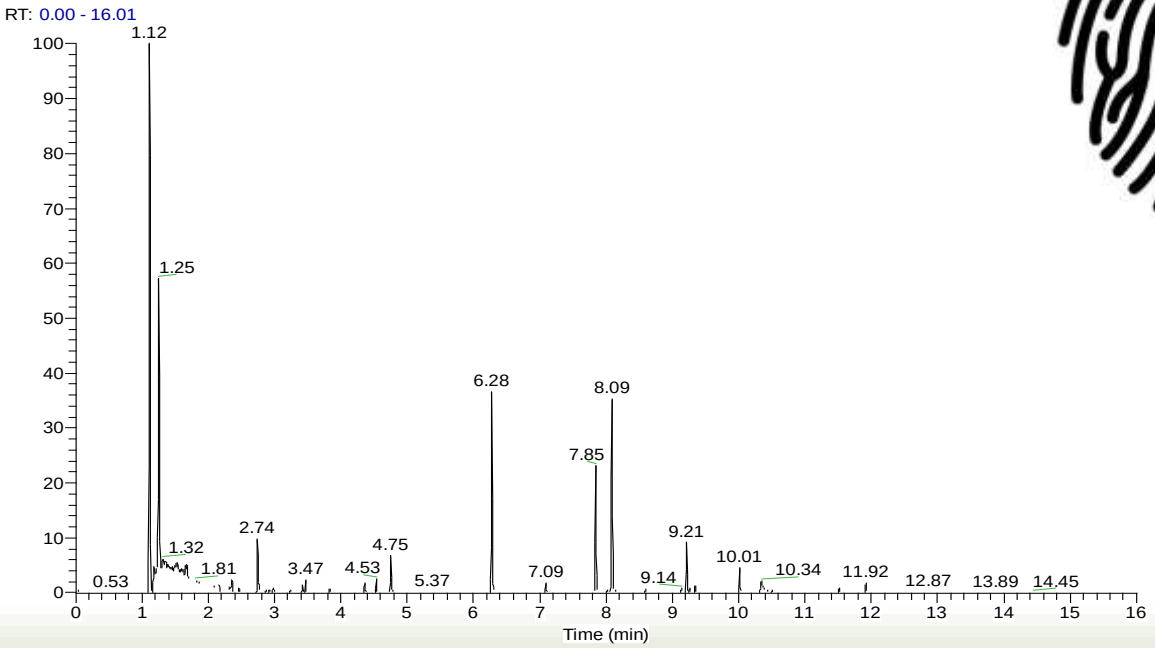
Terry et al. 2000. ARPPPMB 15:401-432.

GC-MS Sampling Methods

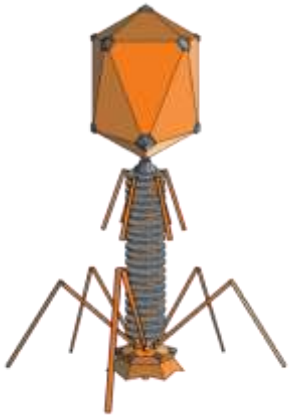


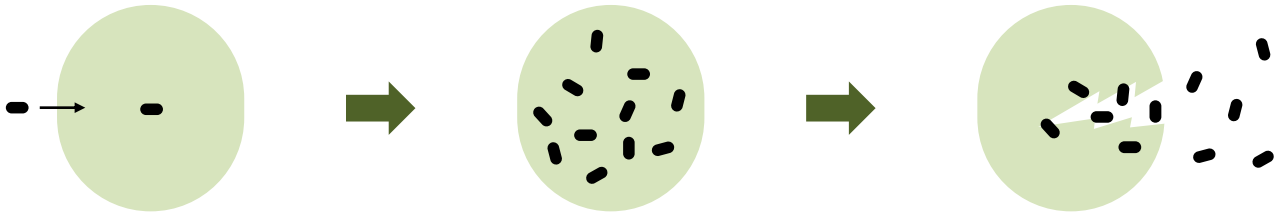
Bacterial VOCs

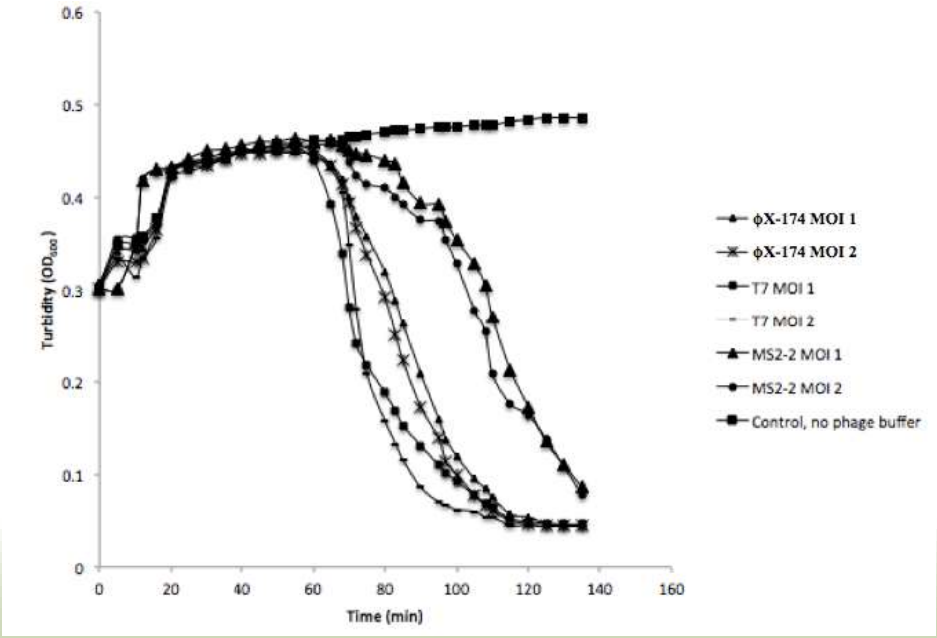
- Produced by all species
- Potential for rapid ID



Infection and Lysis







Brewster et al. 2012. JEMI 16:139-143.

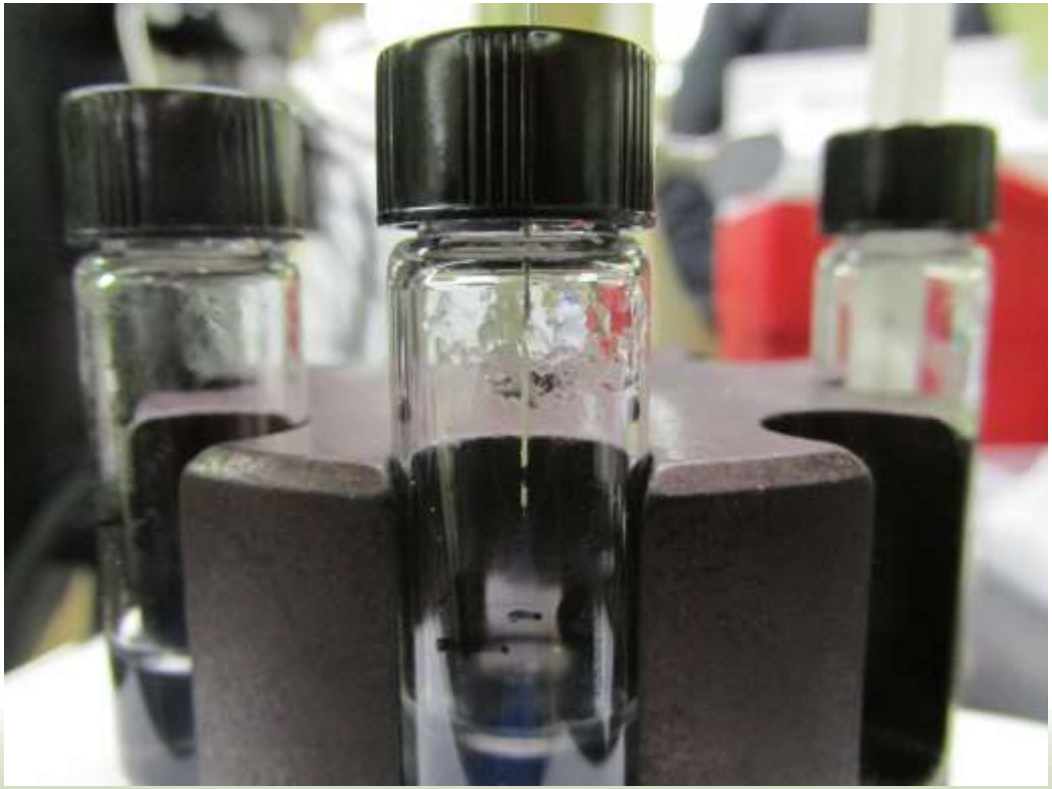
Method Development

- Escherichia coli K12
- MS2 bacteriophage

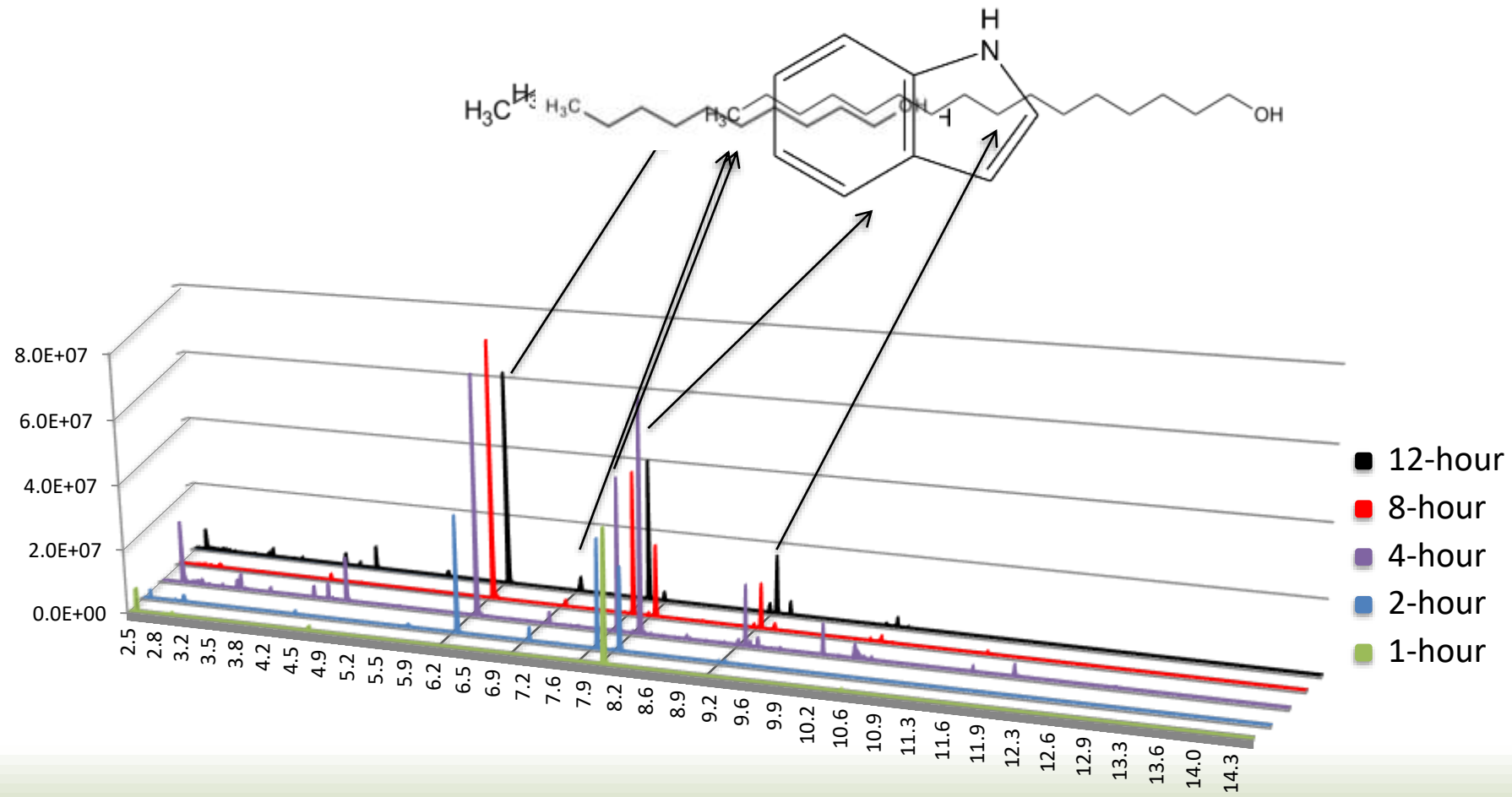
- Multiplicity of infection
- Bacterial concentration
- Sampling and infection time

- SPME/GC-MS settings

SPME



Time-step Analysis



Conclusions

- Large variation in VOCs across infection time
  - Most VOCs show up by 4 hr
- Other parameters optimized for SPME/GC-MS analysis

What does it all mean?

Fungus Project

- Successful quantitation of selenocompounds
  - Mycoremediation
- Novel identification of dimethyl diselenide production
  - Ecoevolution
  - Se biochemical pathways

Phage Project

- Preliminary data show good potential
- Use in bacterial VOC fingerprint development
- Analysis of foodborne pathogens and other health concerns

Acknowledgements

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- Dr. Ami Wangeline
- Sami Haller
- Josh Sharpe

Phage Project

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- Holden Bindl (Wyoming EPSCoR SRAP)

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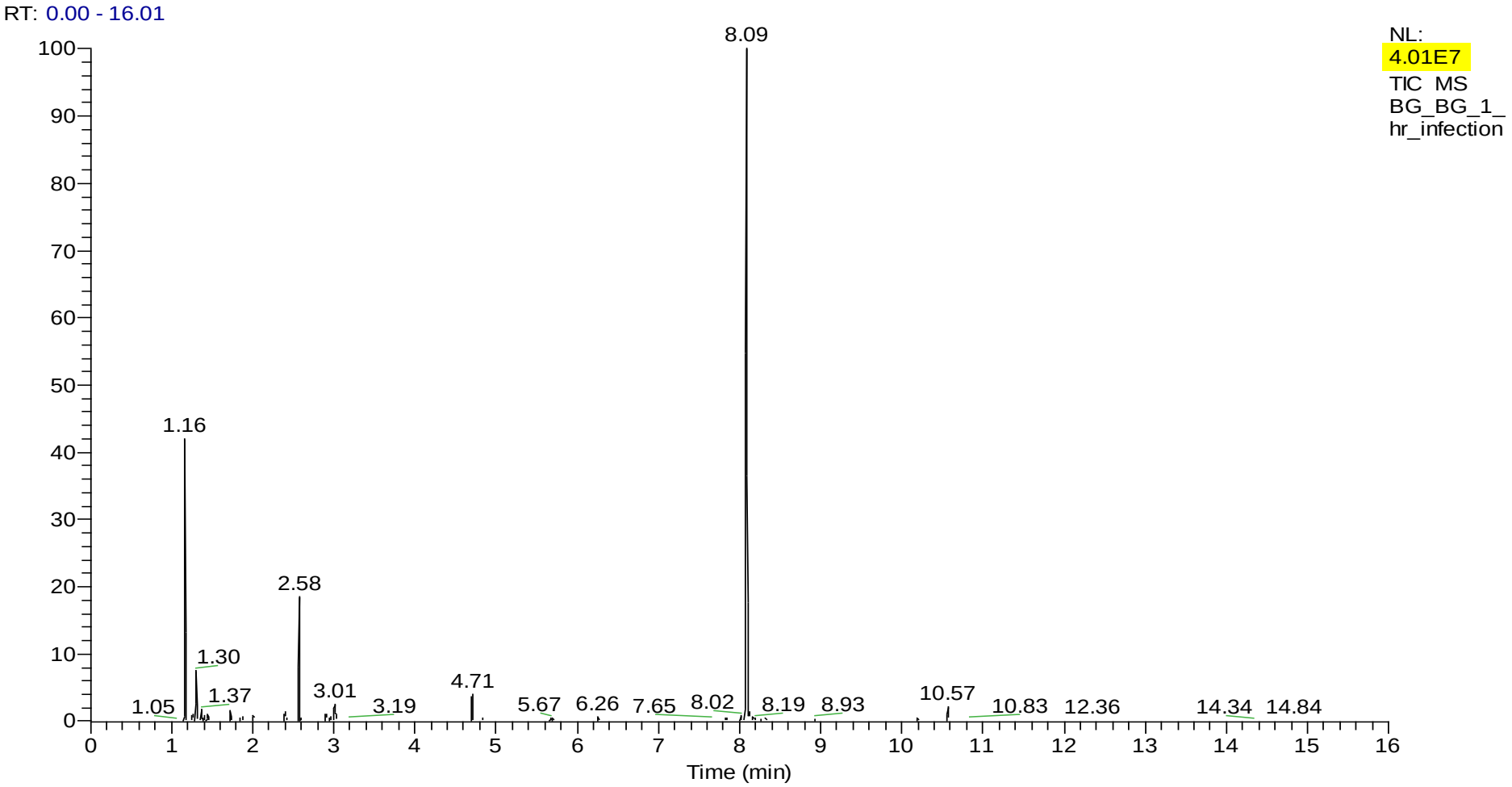
Thank you

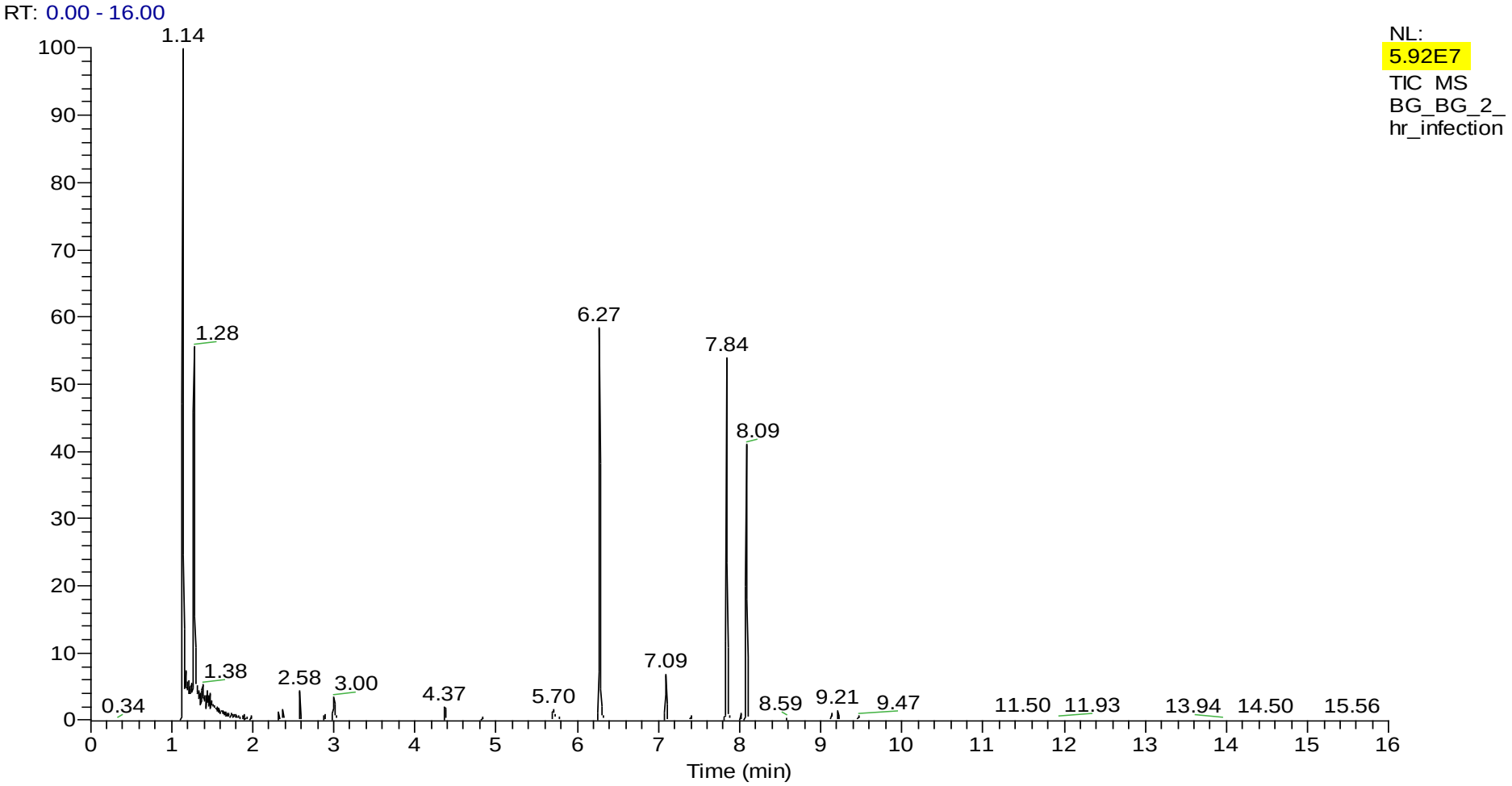
Questions?

SI

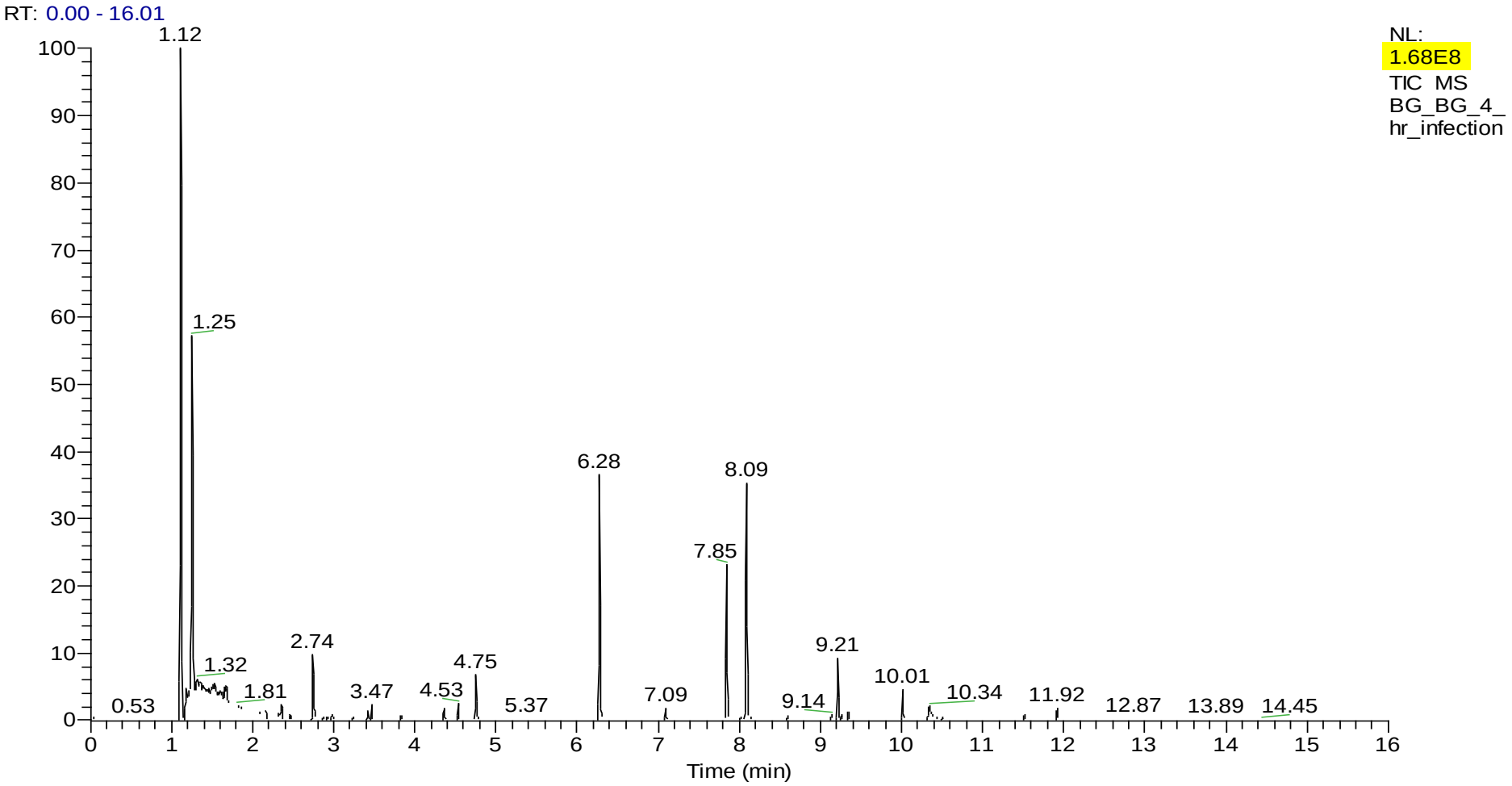
1-hour Infection



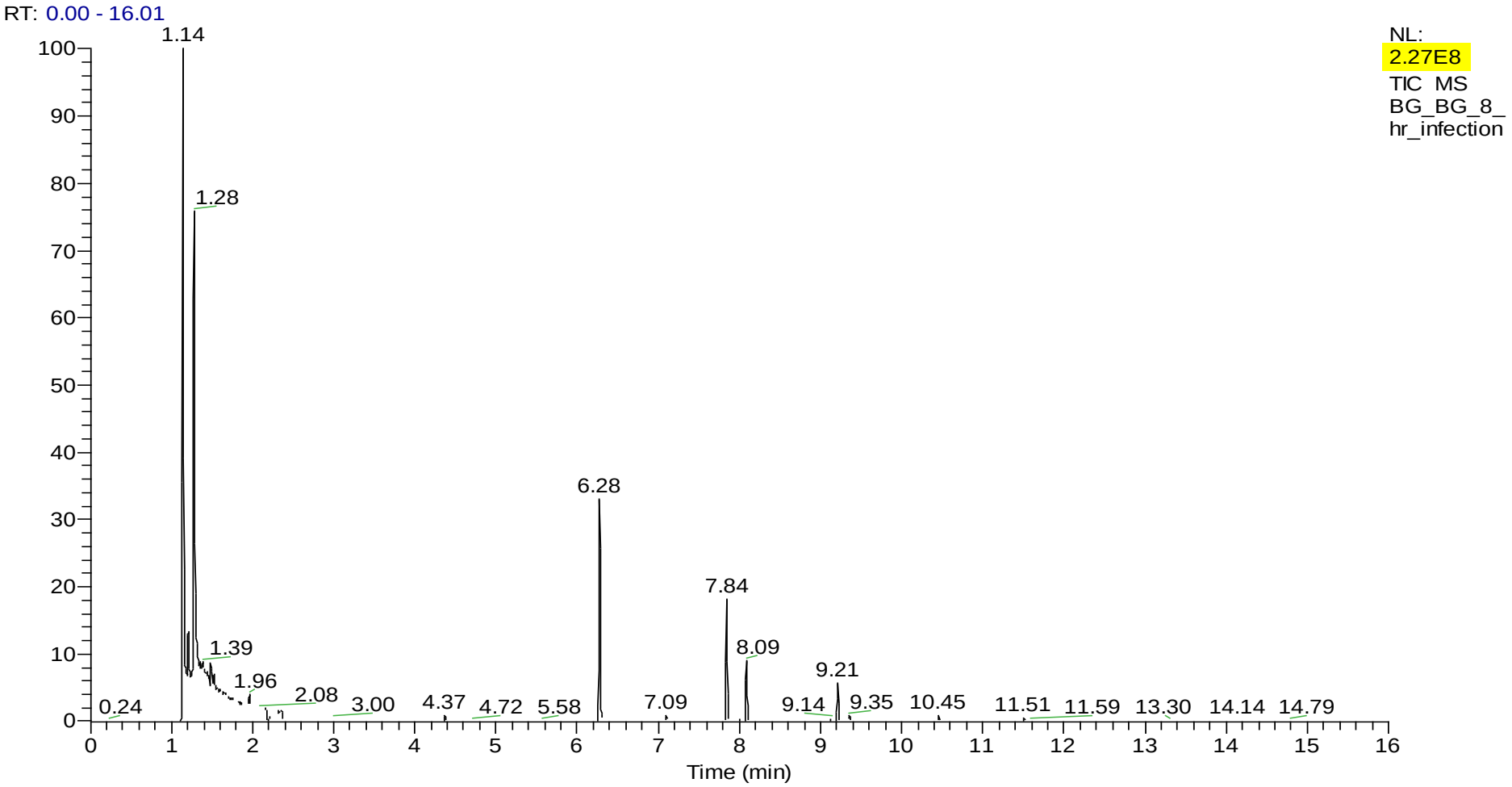
2-hour Infection



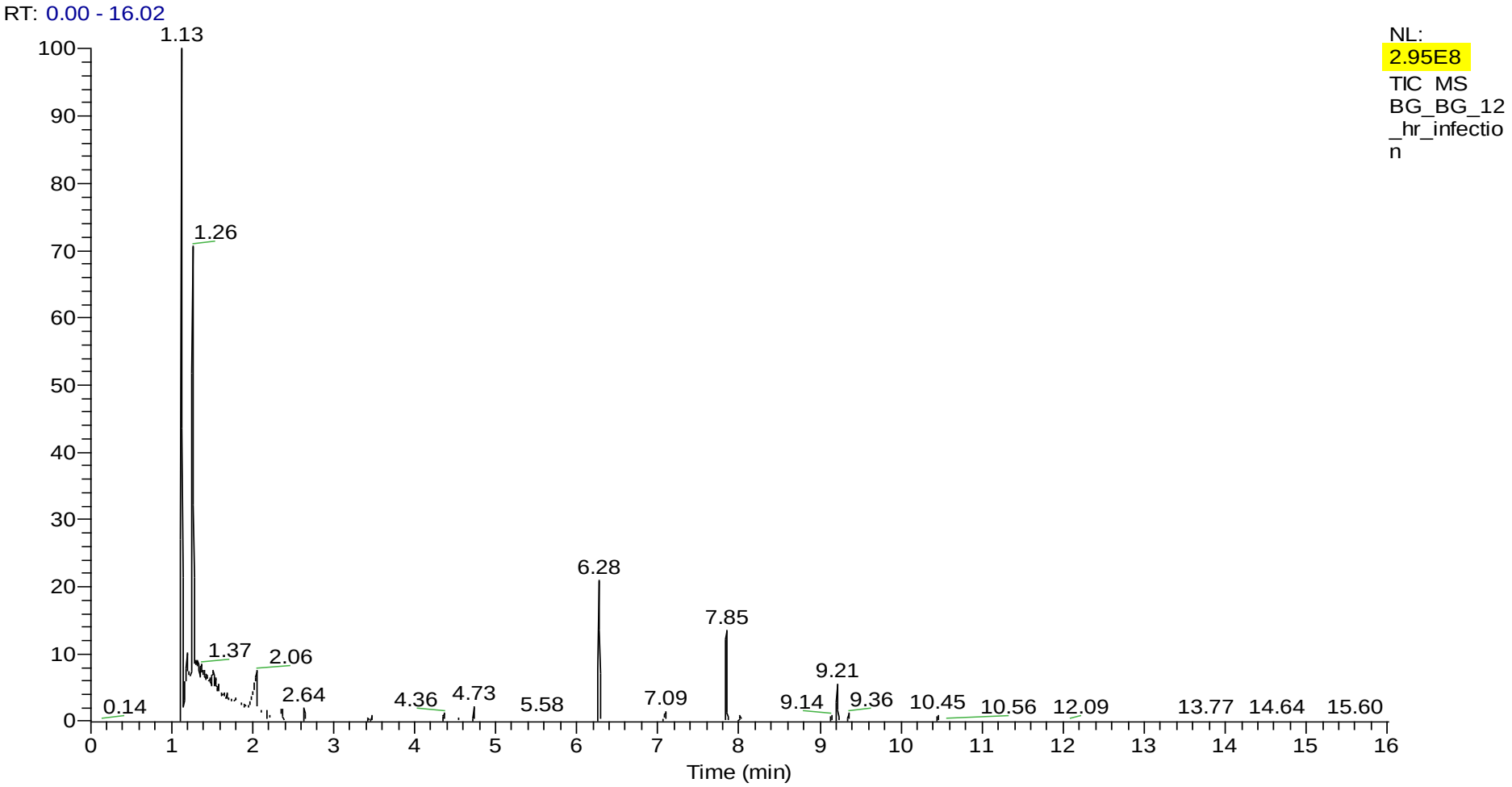
4-hour Infection

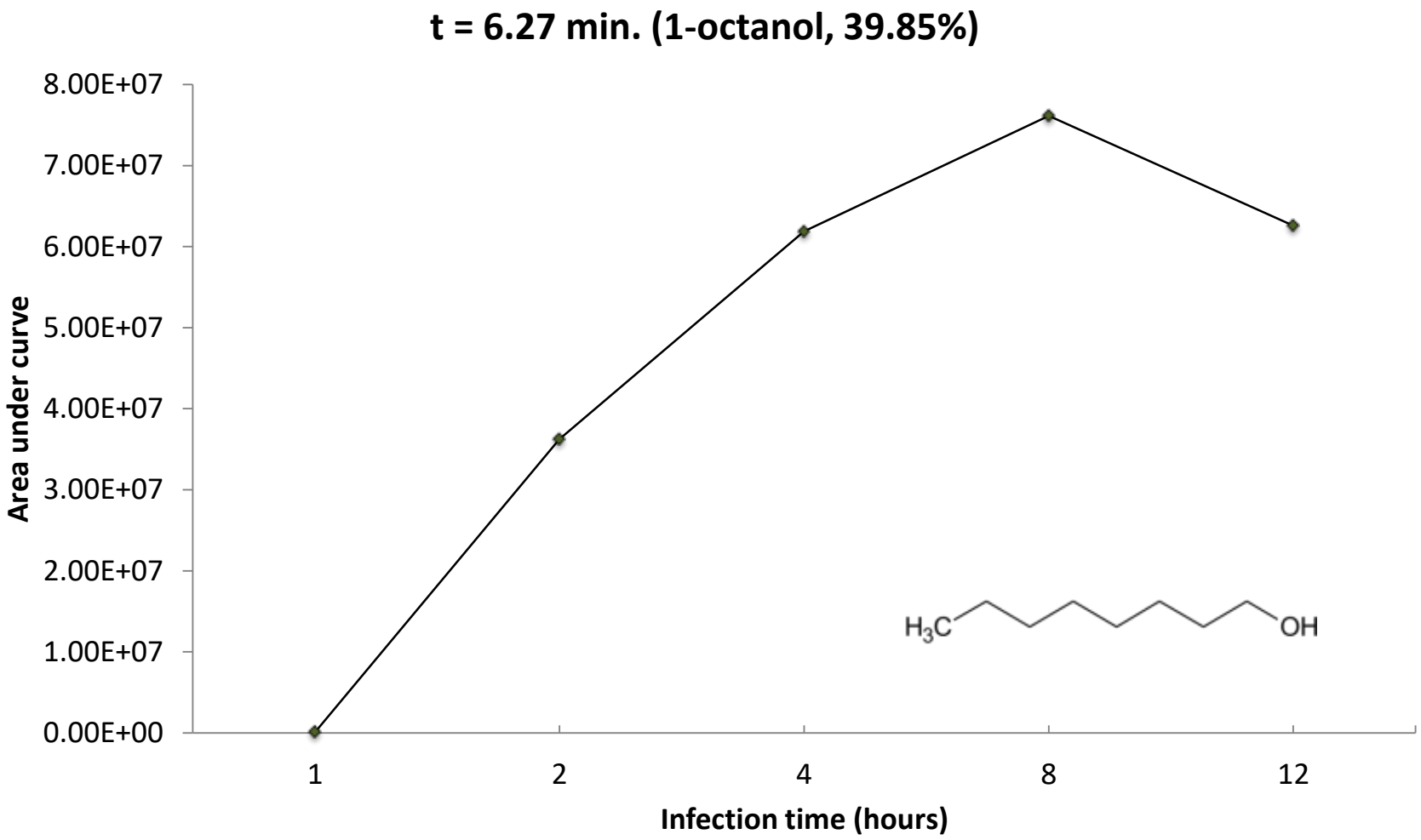


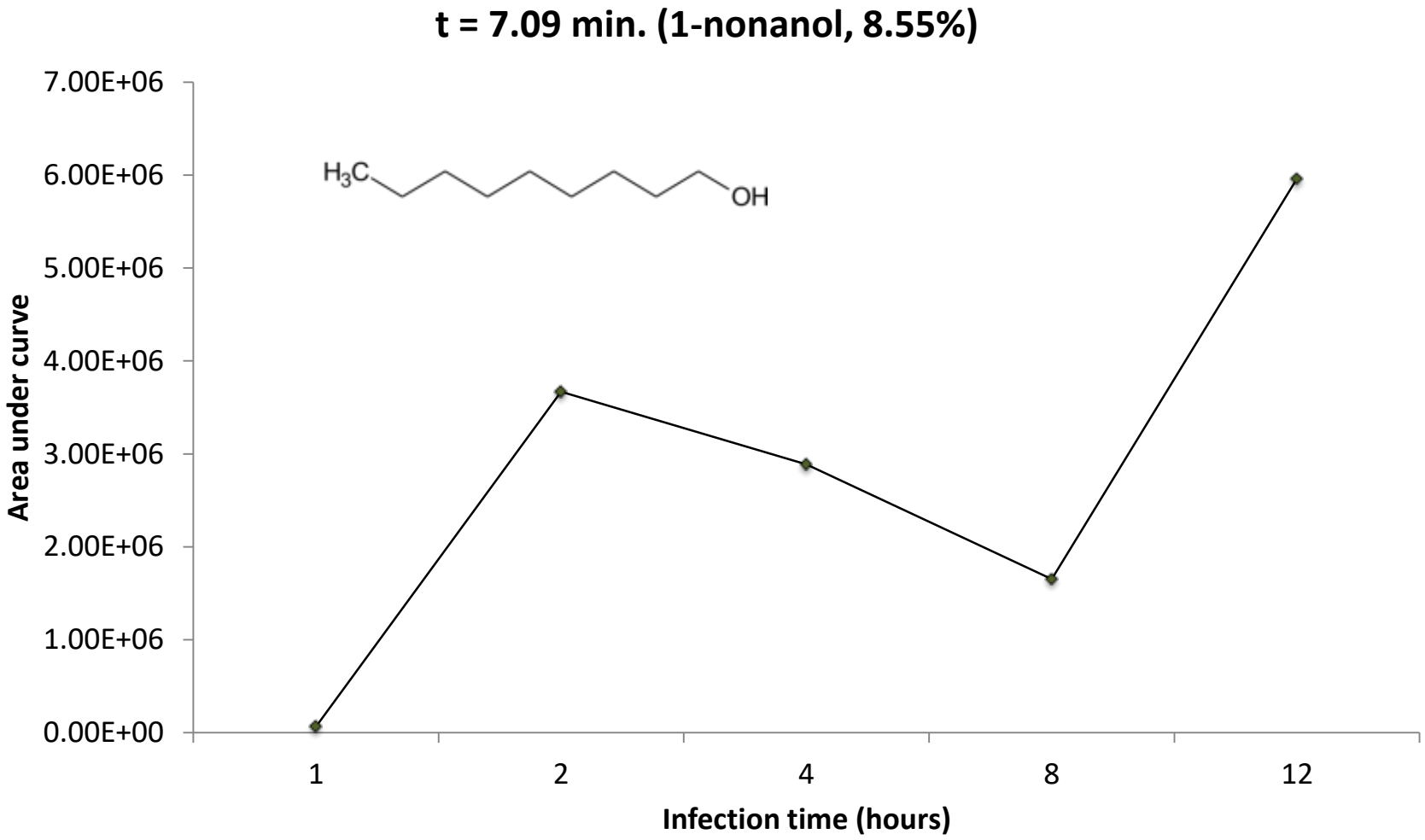
8-hour Infection

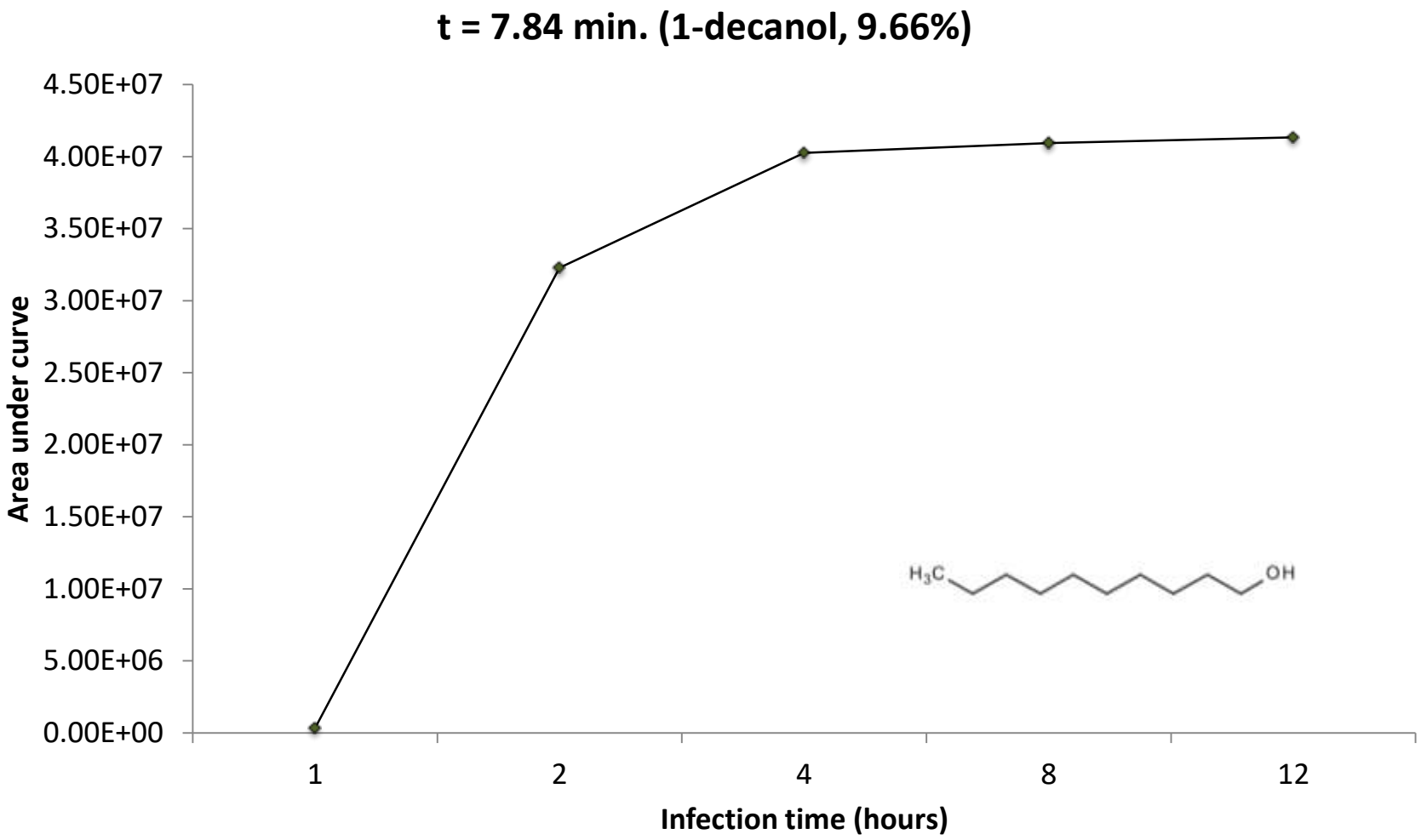


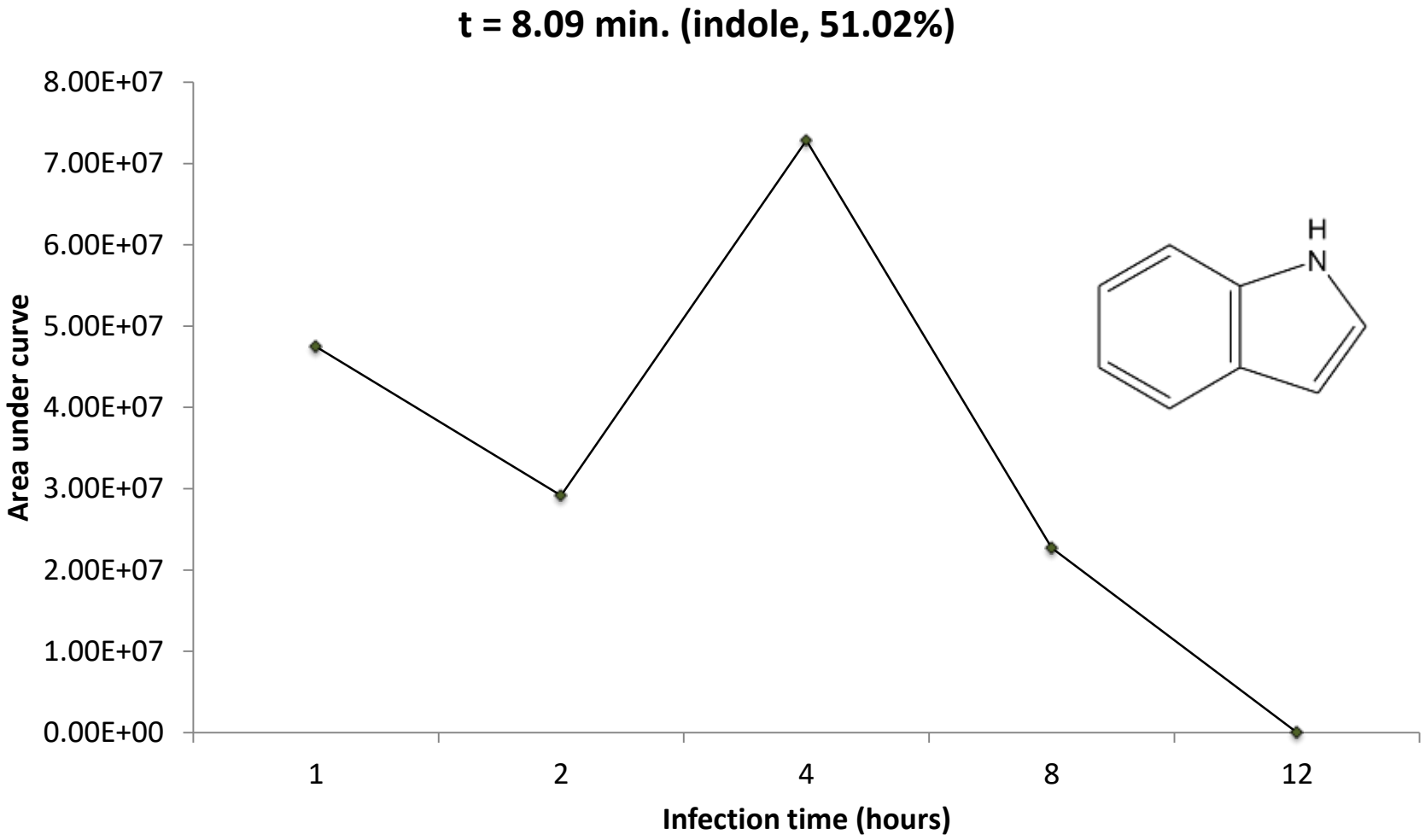
12-hour Infection

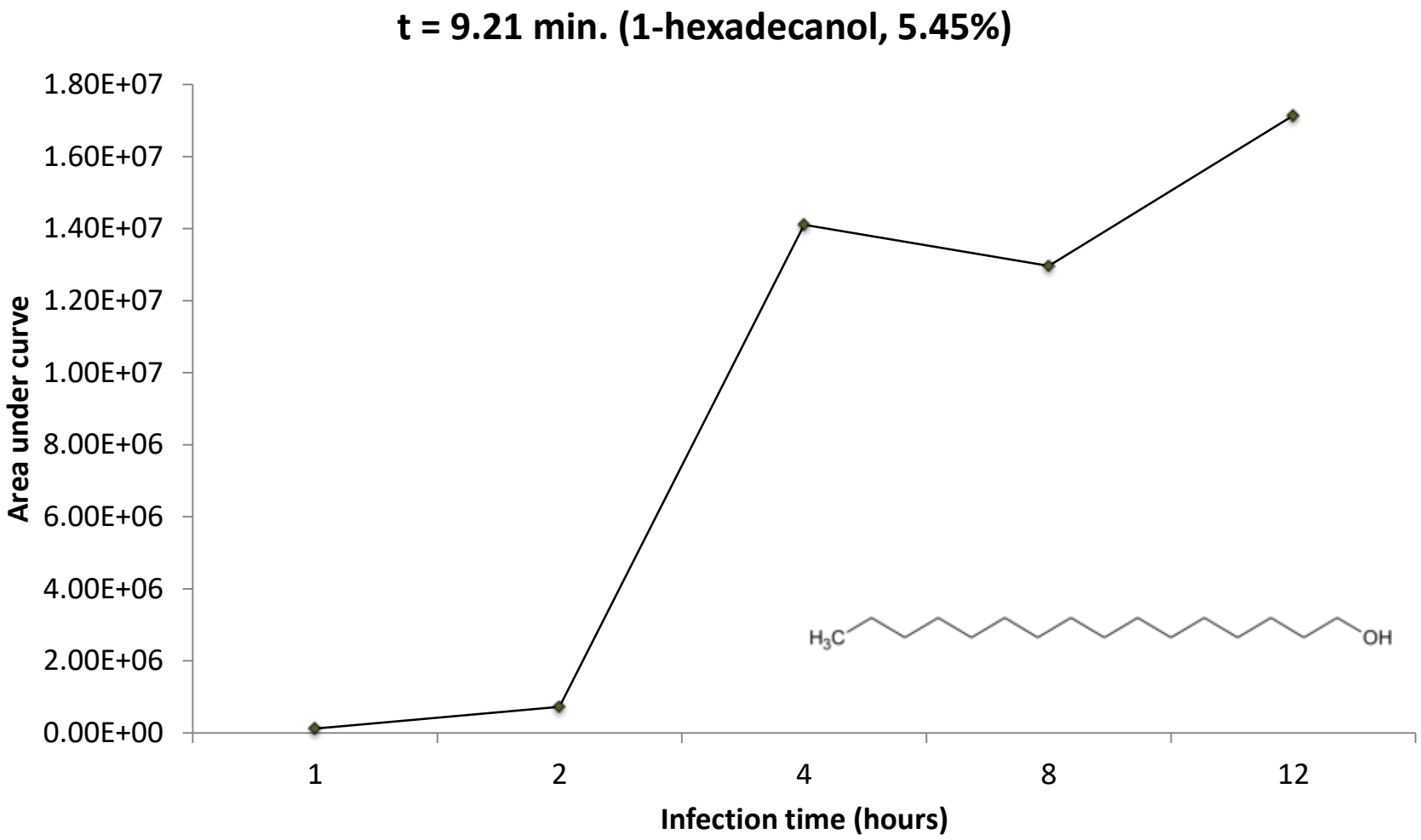



t = 6.27 min. (1-octanol, 39.85%)
8.00E+07
7.00E+07
6.00E+07
5.00E+07
4.00E+07
3.00E+07
2.00E+07
1.00E+07
0.00E+00
Area under curve
1
2
4
8
12
Infection time (hours)
H3C
OH


t = 7.09 min. (1-nonanol, 8.55%)
7.00E+06
6.00E+06
5.00E+06
4.00E+06
3.00E+06
2.00E+06
1.00E+06
0.00E+00
Area under curve
H3C
OH
1
2
4
8
12
Infection time (hours)


t = 7.84 min. (1-decanol, 9.66%)
4.50E+07
4.00E+07
3.50E+07
3.00E+07
2.50E+07
2.00E+07
1.50E+07
1.00E+07
5.00E+06
0.00E+00
Area under curve
1
2
4
8
12
Infection time (hours)
H3C
OH


t = 8.09 min. (indole, 51.02%)
8.00E+07
7.00E+07
6.00E+07
5.00E+07
4.00E+07
3.00E+07
2.00E+07
1.00E+07
0.00E+00
Area under curve
1
2
4
8
12
Infection time (hours)
H
N


t = 9.21 min. (1-hexadecanol, 5.45%)
1.80E+07
1.60E+07
1.40E+07
1.20E+07
1.00E+07
8.00E+06
6.00E+06
4.00E+06
2.00E+06
0.00E+00
Area under curve
1
2
4
8
12
Infection time (hours)
H3C
OH