A Suite of Sampling Suite for the In-Field, SPME Collection of Analytes from Air, Particulates, and Surfaces

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Rationale

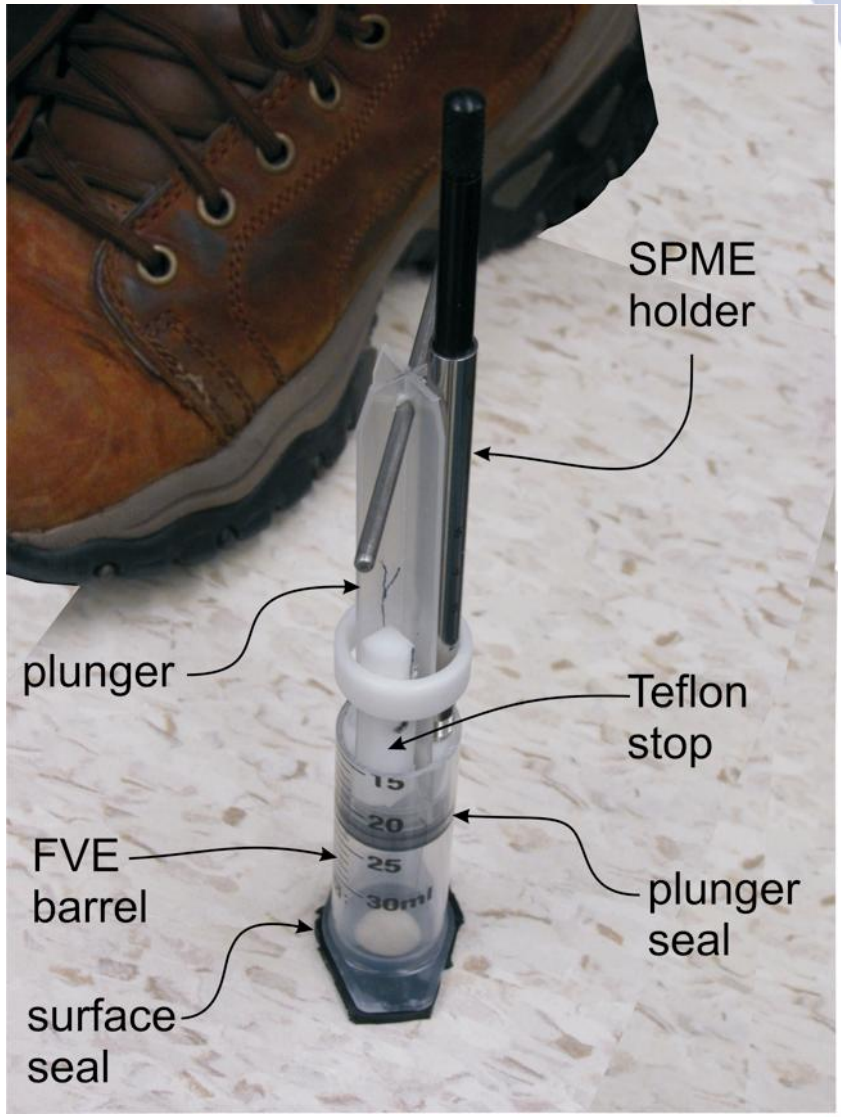
- **GUARDION™ / TRIDION™-9** field portable GC-TMS relies on Solid Phase Microextraction (**SPME)** for sampling and sample introduction
  - Low impact on vacuum pumps, low power chromatography.
- GC/MS applications are typically volatile or semi-volatile organic compounds in air, liquids, solids, surfaces and particulates.
- Important to co-develop sampling aids and strategies to support field analysis.

TRIDION™-9 Sampling Accessories

- Torion developing a suite of accessories to assist the user in acquire samples in the field w/o imposing significant additional fieldability issues (weight, utilities, chemicals, power, etc.)
  - Surfaces – **Field Vacuum Extractor (FVE)**
  - Biological particulates – **BioAgent Chemical System (BACS)**
  - Air sampling for SPME - **AIRION™**
  - Field calibration standard – **CALION™**
  - Sample preconcentration - **Needle Trap**

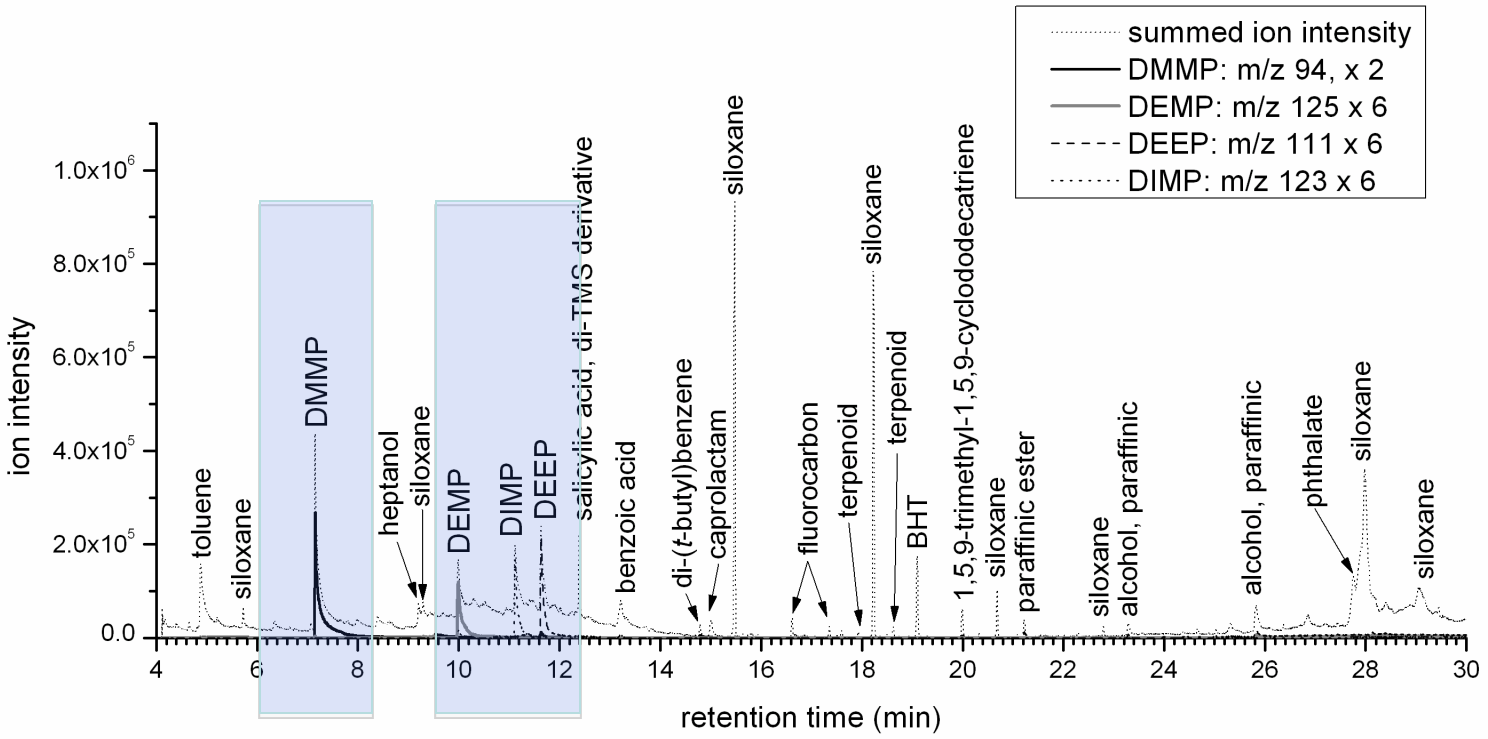
Field Vacuum Extractor (FVE)

- **Field Vacuum Extractor**
  - Developed at Idaho National Laboratory to improve recovery of surface analytes.
  - Uses moderate vacuum to increase sample availability.
  - Simple proof-of-concept using modified laboratory syringes.
  - Licensed from INL and commercialization underway at Torion.

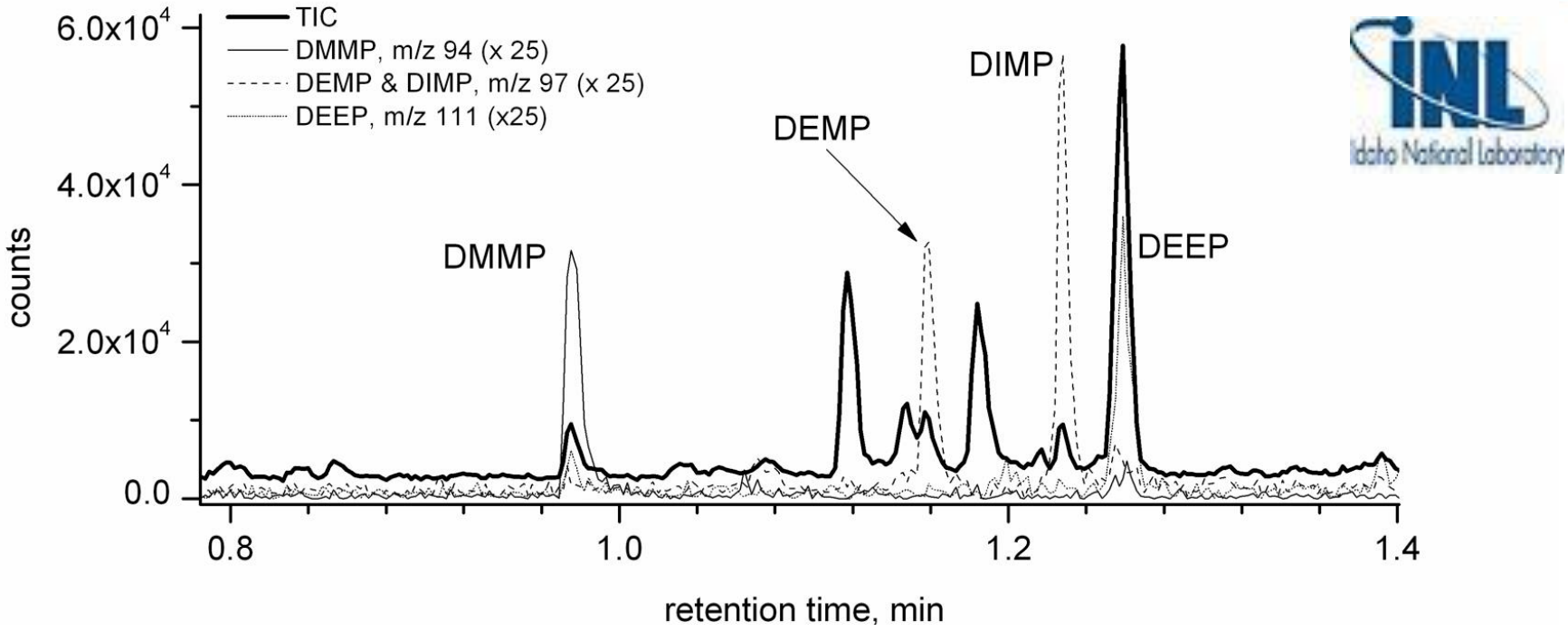


Chromatogram of a Spiked, Dirty Tile Surface

- Sample substrate contains significant signal from common industrial industrial compounds
- Target organophosphoryl compounds are easily observed using extracted ion chromatograms



Alkyl Phosphonates on Floor Tile



- *FVE headspace sampled over vinyl floor tile*
- *SPME fiber used for sampling the exposed tile to:*
  - *DMMP (70 ng) DEMP (33 ng) DIMP (38 ng) DEEP (46 ng)*
  - *Note: 30 minute sampling does not exhaust sample from surface (multiple analyses possible).*
  - ***Fast GC/TMS analysis allows multiple sampling sites to be screened***

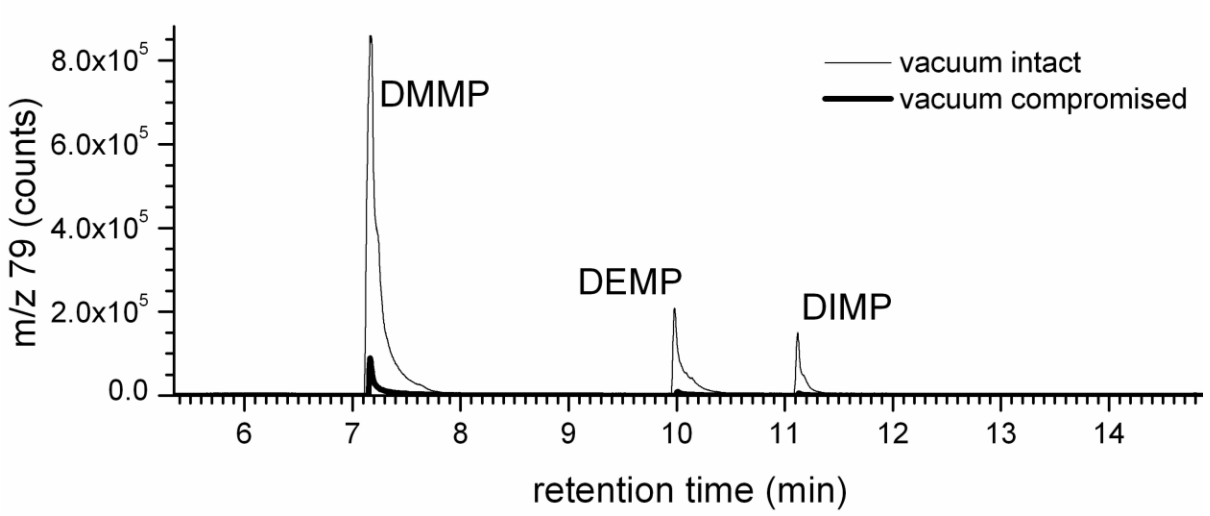
Effect of Vacuum

- Without the vacuum, the semivolatile compounds studied are not captured by the SPME samplers

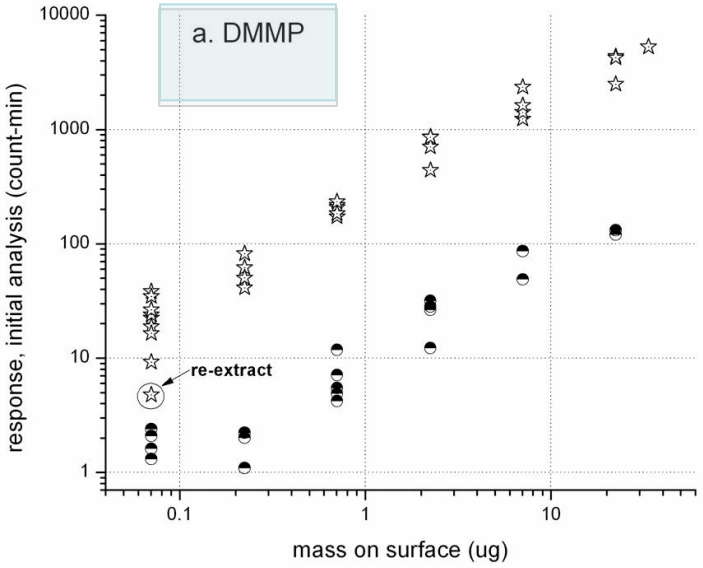
- Effect of vacuum (~60 torr)

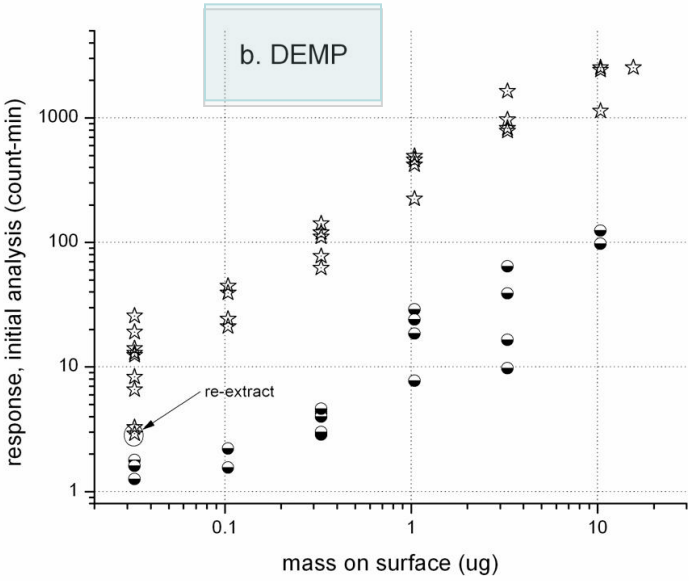
  illustrated by a comparison of analyses:

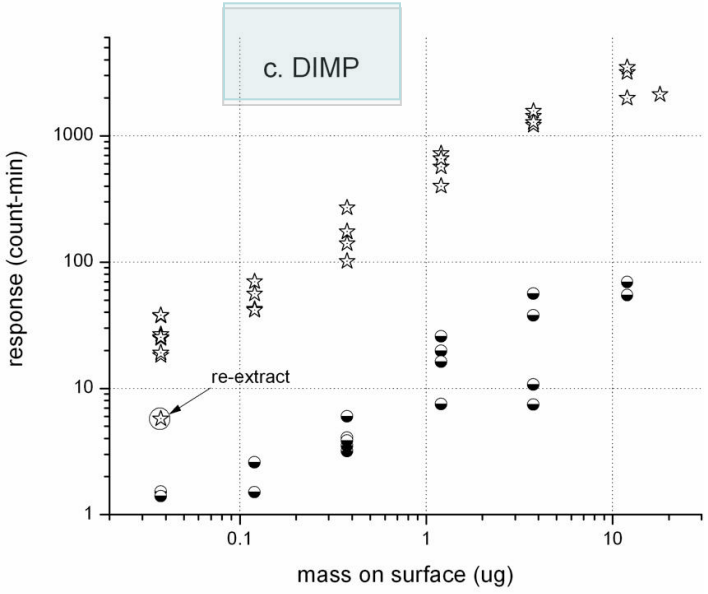
  - vacuum intact throughout analysis
  - vacuum compromised (seal failed during extraction)
  - Note, without <u>any</u> vacuum, no signal detected

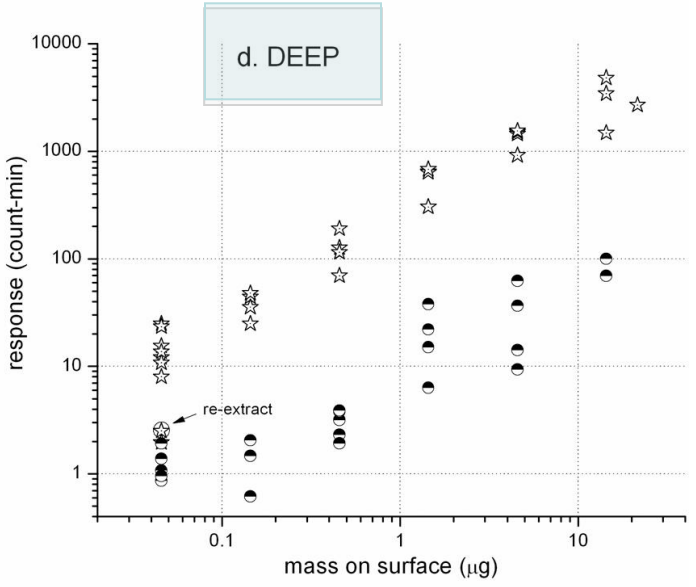


Alkyl-phosphonates Concentration Study







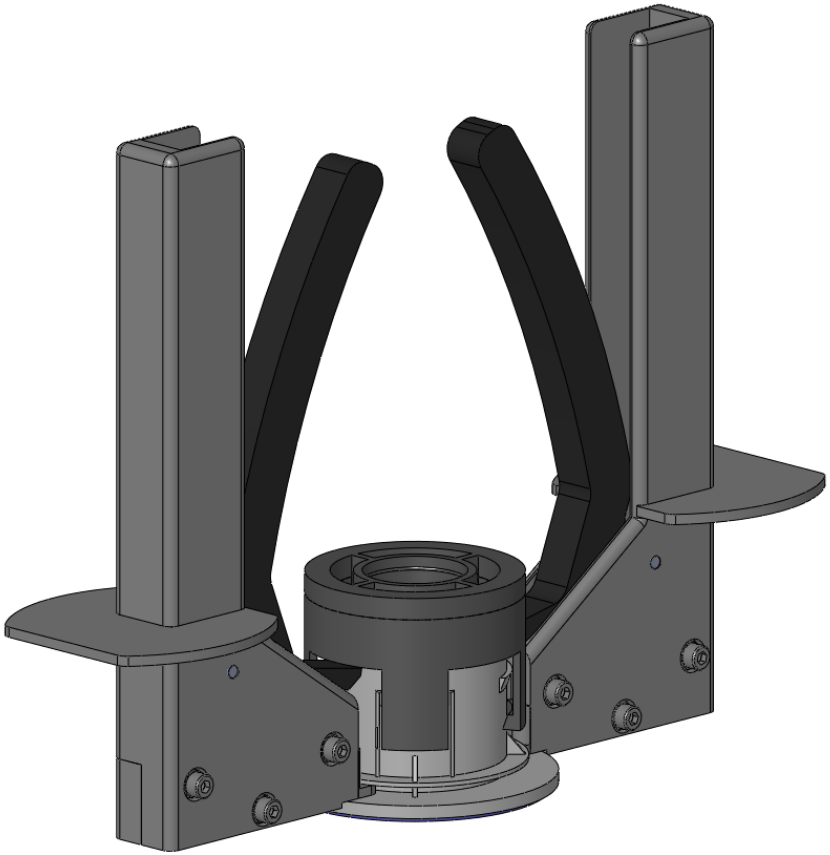


Anal. Chim. Acta, (submitted)

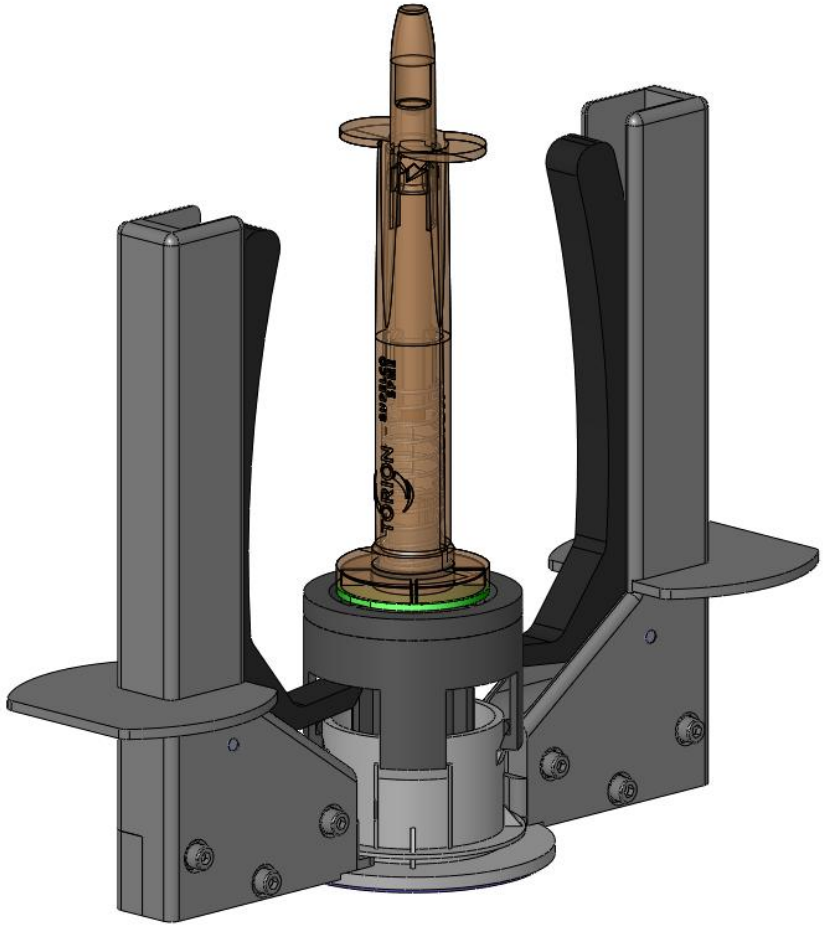
Prototype Operational Concept

- Multiple samplers supported – low cost, disposable chambers

No Vacuum Position



Vacuum and Sampling Position

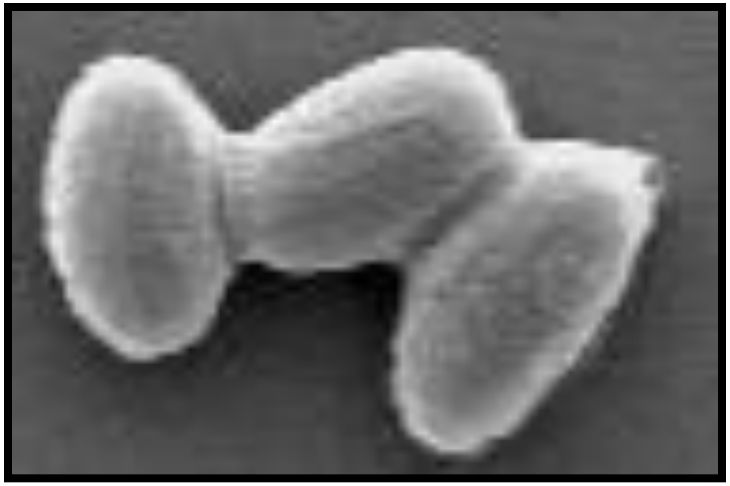


Biological Threat Detection

- **Bioagent Chemical System (BACS)**

- Portable GC-MS provides definitive detection of chemical threat agents.
- Biological threat agents such as *Bacillus anthracis* (BA) spores pose a similar threat and have been produced in weaponized form by various nations and groups
- Can field-portable GC-MS provide rapid, targeted detection of biological threats such as BA?

GUARDION® GC-TMS



Approaches to Bacterial Identification

- **Large molecule** (proteins, DNA, triglycerides)
  - Immunoassay (Antibody)
  - PCR (DNA)
  - Protein Identification (MALDI, Electrospray MS)
    - Except for PCR, not very fieldable, significant utility and reagent requirements, sample preparation not particularly suited for field analysis
- **Small Molecule** (cell wall components, carbohydrates, etc.)
  - MIDI (fatty acid analysis by Chromatography)
  - Direct MS (no chromatography)
    - DoD Block II Chemical and Biological Mass Spectrometer (ORNL)
    - Both techniques focus primarily on fatty acid profiles, very susceptible to media and growth conditions.
  - GC/MS has proven to be fieldable

- Most notable among pathogenic bacterial threats is ***Bacillus Anthracis***

Bacterial Species Relationships

All bacteria contain a range of **fatty acids**. Gram positive bacterial organisms (*Bacillus, Clostridium)* are spore-forming and typically contain **dipicolinic acid** in this stage.

***Bacillus cereus* group** includes 6 very closely related species: BC, BA, BT, *B. mycoides*, *B. pseudomycoides*, and *B. weihenstephanensis*.

Anthrose has been claimed to be highly specific to BA. Can differentiate from *Clostridium.* However, BC and BT spores can contain different levels of anthrose; e.g., BT (Al Hakam) can produce 42% of the BA anthrose level.[1]

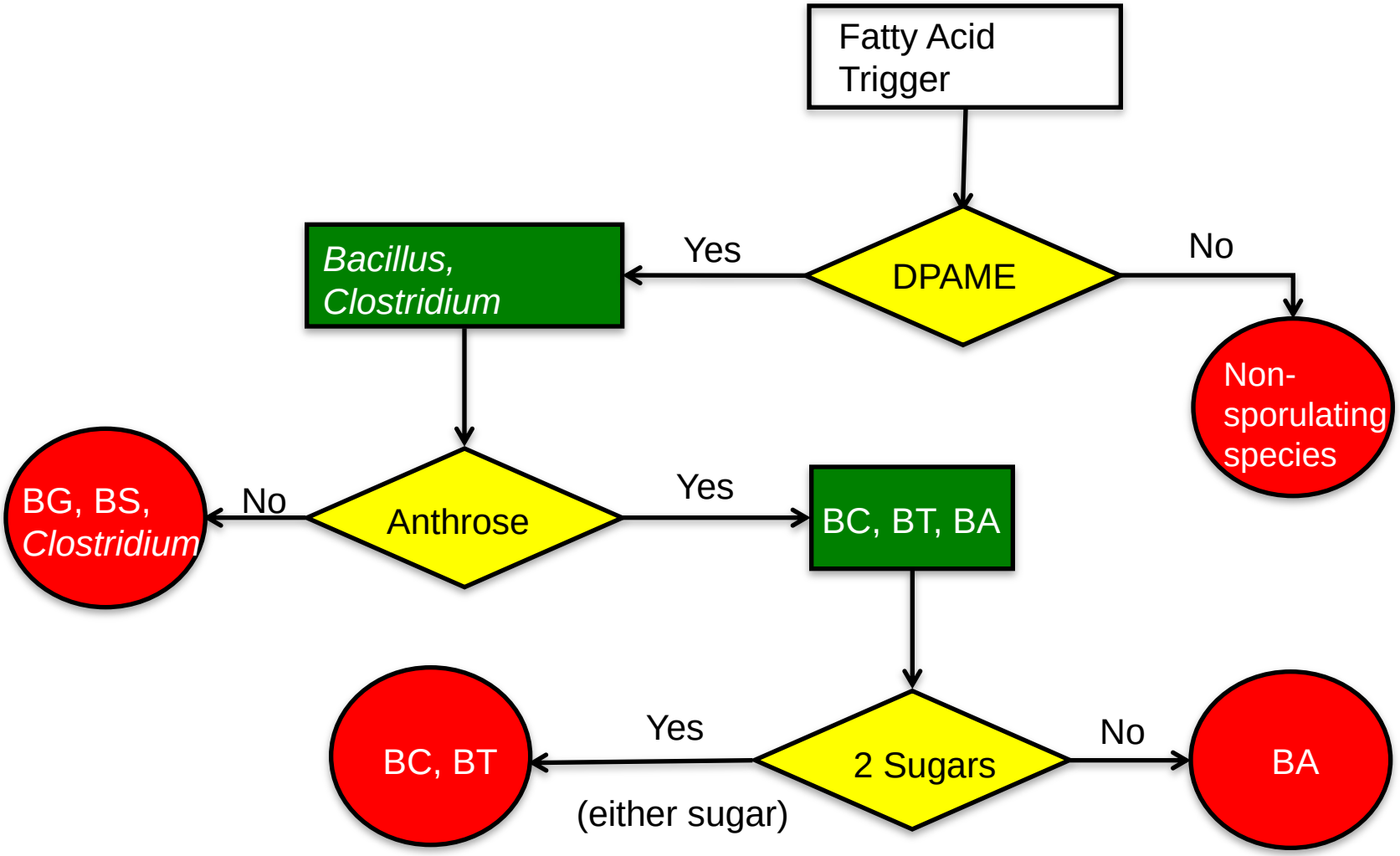
BC, BA, and BT are often considered to be members of a single species,[2-5] since they share a high degree of similarity as demonstrated by their 16S rRNA nucleotide sequences.[6]

BG is not closely related to BA, but has been used as a nonpathogenic surrogate for BA.

Milton Lee group (Brigham Young University) have determined that the presence or absence of **two additional sugars** can differentiate between BA, and BC/BT

[1]S. Dong et al. *J. Bacteriol.* **2008,** *190*, 2350.
[2]D. Daffonchio et al. *Appl. Environ. Microbiol.* **2000,** *66*, 5460.
[3]E. Helgason et al.*Appl. Environ. Microbiol.* **2000,** *66*, 2627.
[4]E. Helgason et al. *Clin. Microbiol.* **2000,** *38*, 1615.
[5]S. Valjevac et al. *Appl. Environ. Microbiol.* **2005,** *71*, 6613.
[6]C. Ash et al. *Int. J. Syst. Bacteriol.* **1991,** *41*, 343.

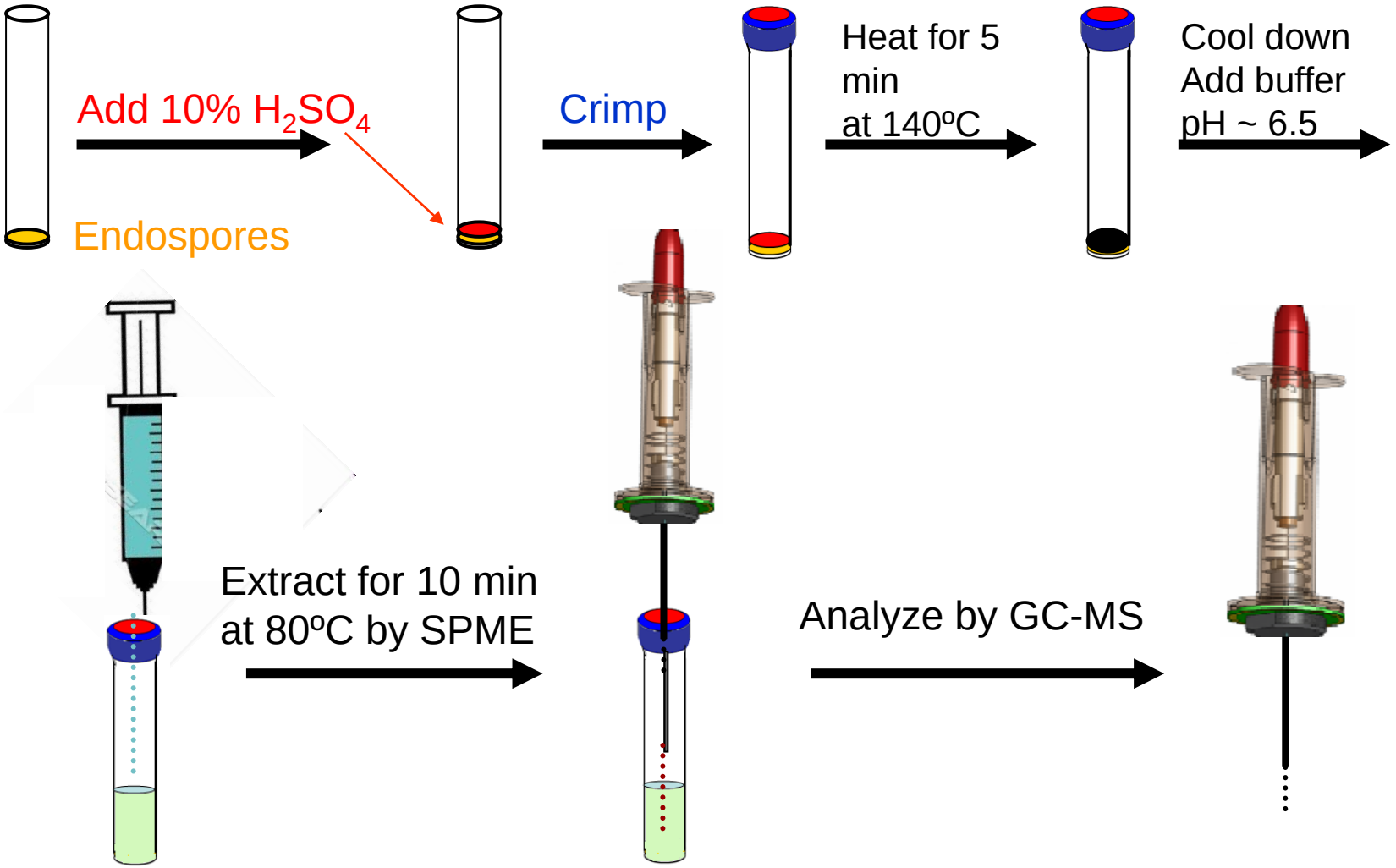
Identification Decision Tree



TORION

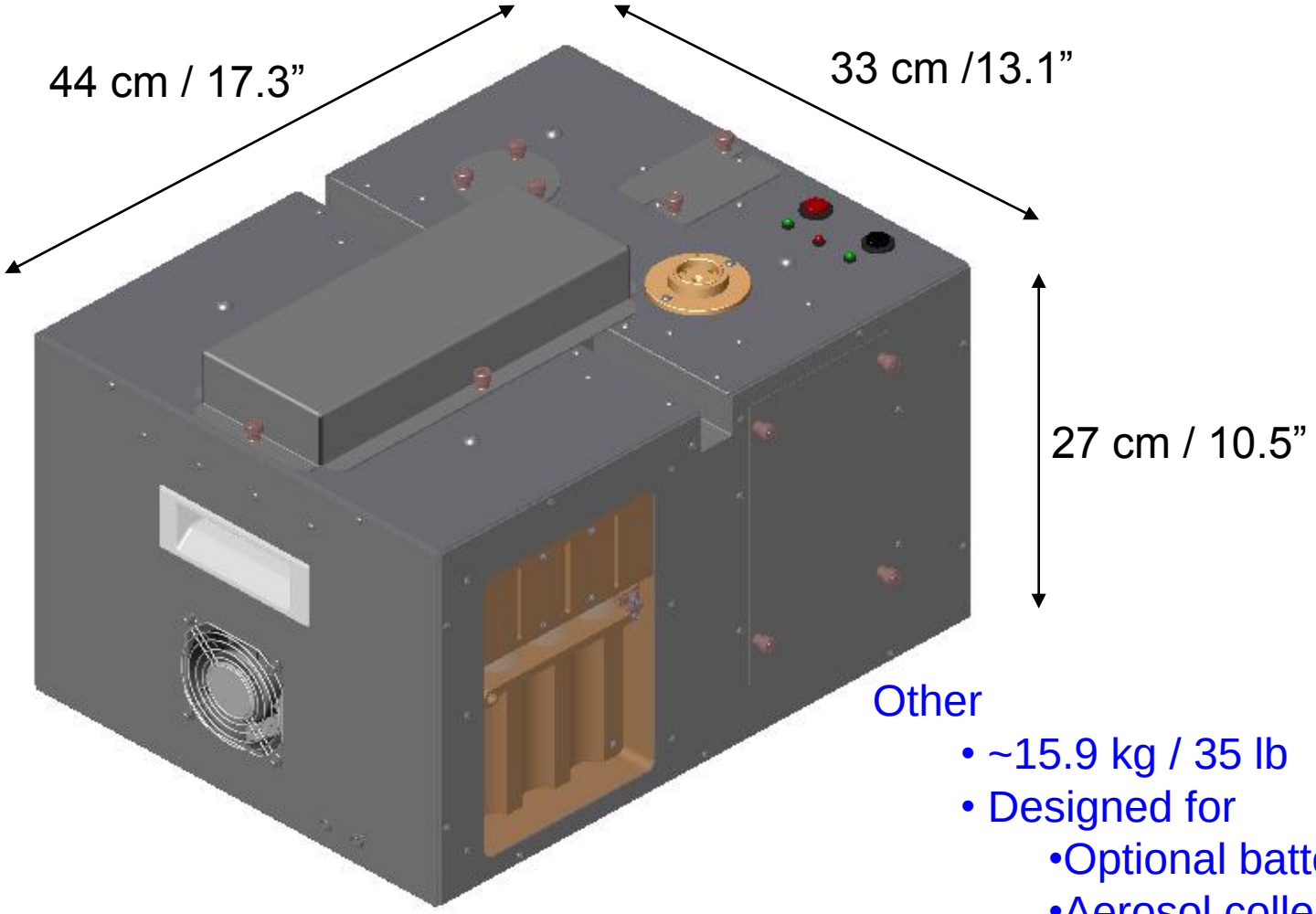
T.V. Truong et al., ***Anal. Methods***, 2010, 2, 638-644

Thermal Chemolysis and Methylation (TCM) For SPME



T.V. Truong et al., *J. Chromatogr. A* 1216 (2009) 6852

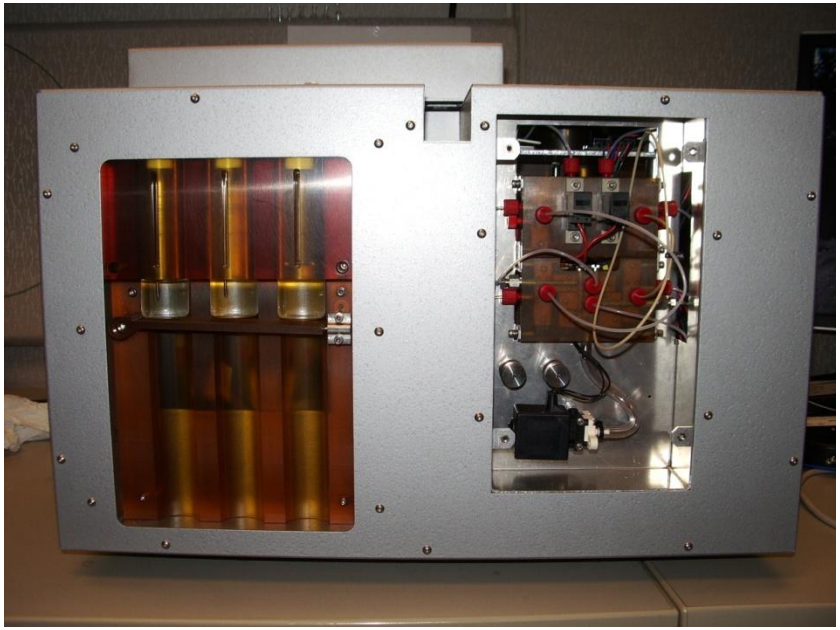
BACS Mechanical Design

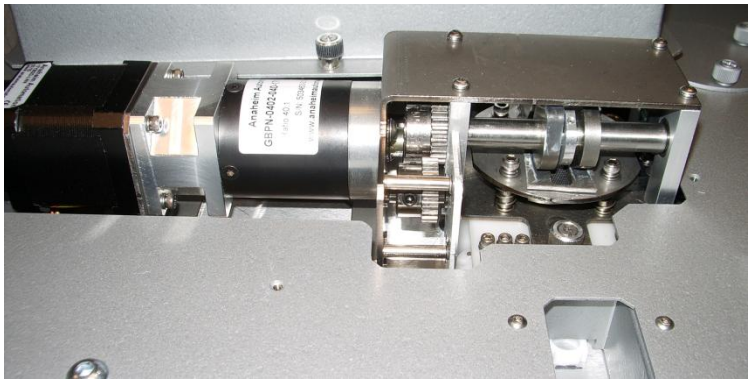


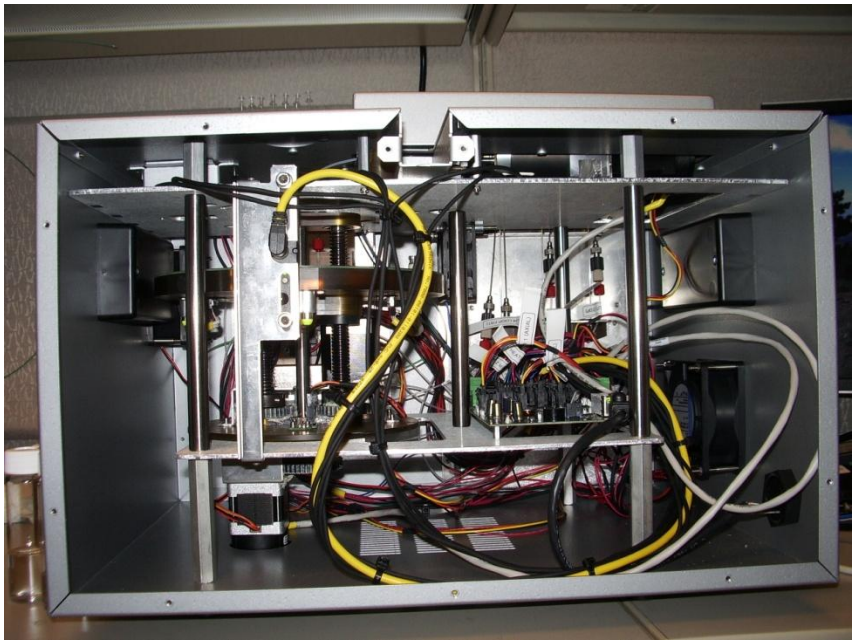
Other

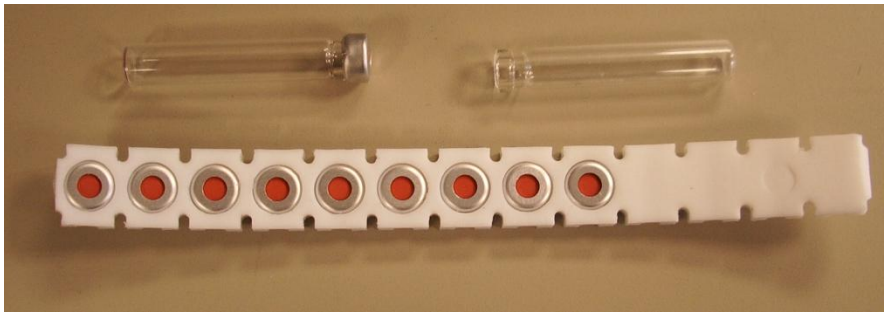
- ~15.9 kg / 35 lb
- Designed for
  - Optional battery operation
  - Aerosol collection (future)

BACS Prototype Unit

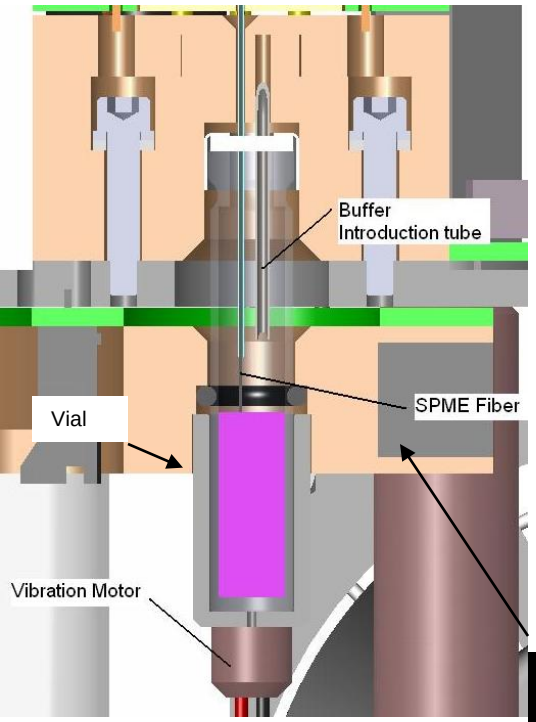


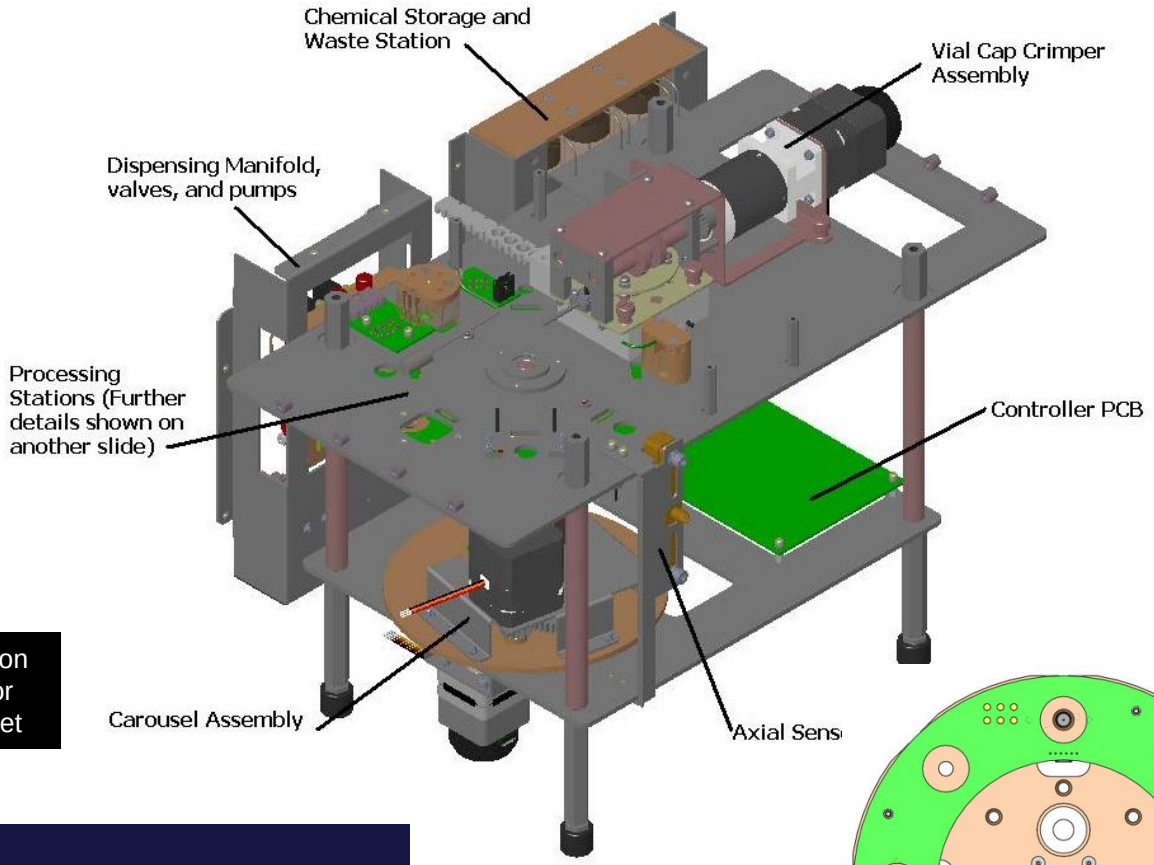


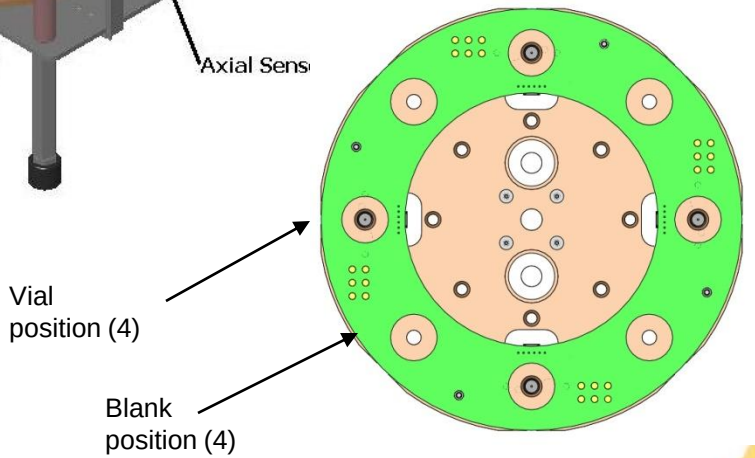




Inside View



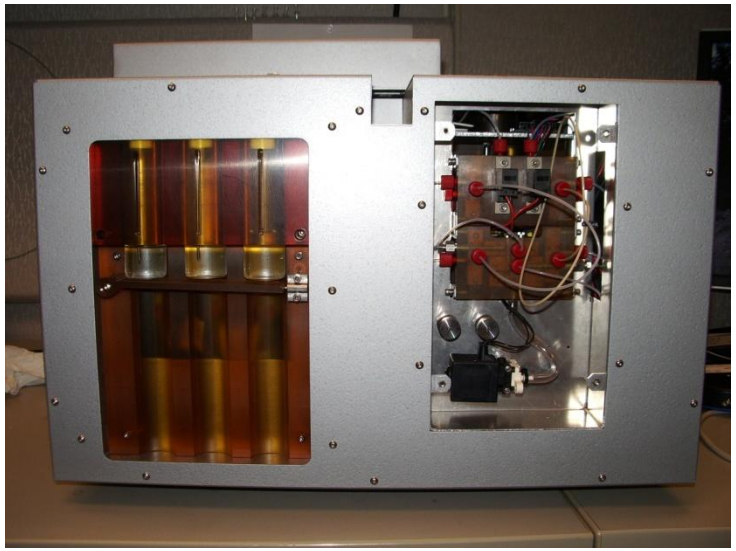




Stage 1 - Vial insertion verification
Stage 2 - Virtual Impactor Particle Collector (future)
Stage 3 - Sulfuric acid introduction
Stage 4 - Vial Crimping
Stage 5 - Heating of sulfuric acid/sample
Stage 6 - Buffer introduction and SPME insertion/sampling*
Stage 7 - Idle (possible future capabilities)
Stage 8 - Insertion and removal of vial*

***Currently manual operation**

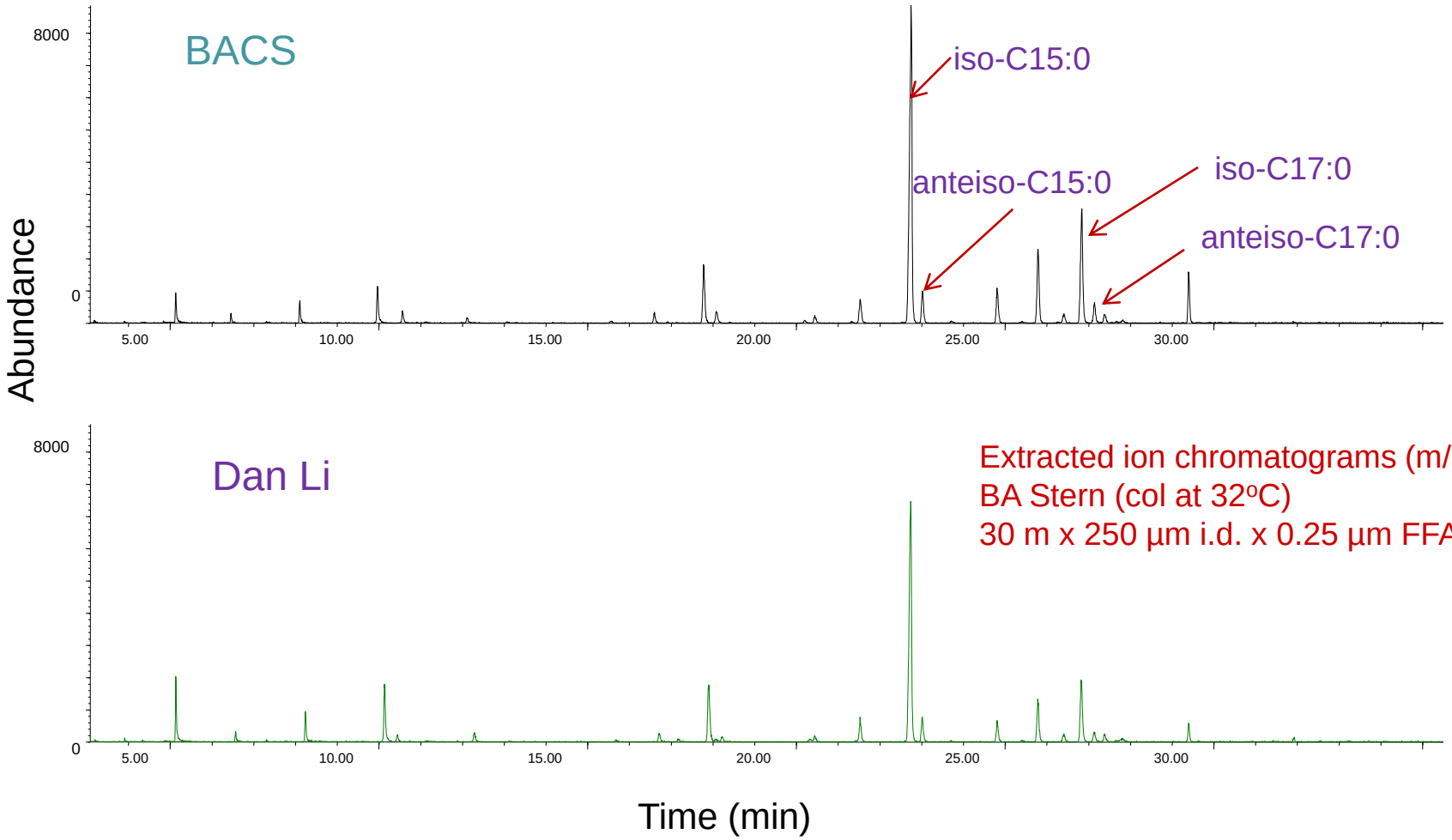
The Main Event: Dan Li vs. 'The Machine'



- Two current system tests (one ongoing)
  - Can the prototype reactor system replace the majority of the operator sample preparation steps?
  - Can the entire system accurately and robustly identify *B. anthracis* from non-virulent *Bacillus* and other gram-positive, gram-negative organisms in complex environmental backgrounds?

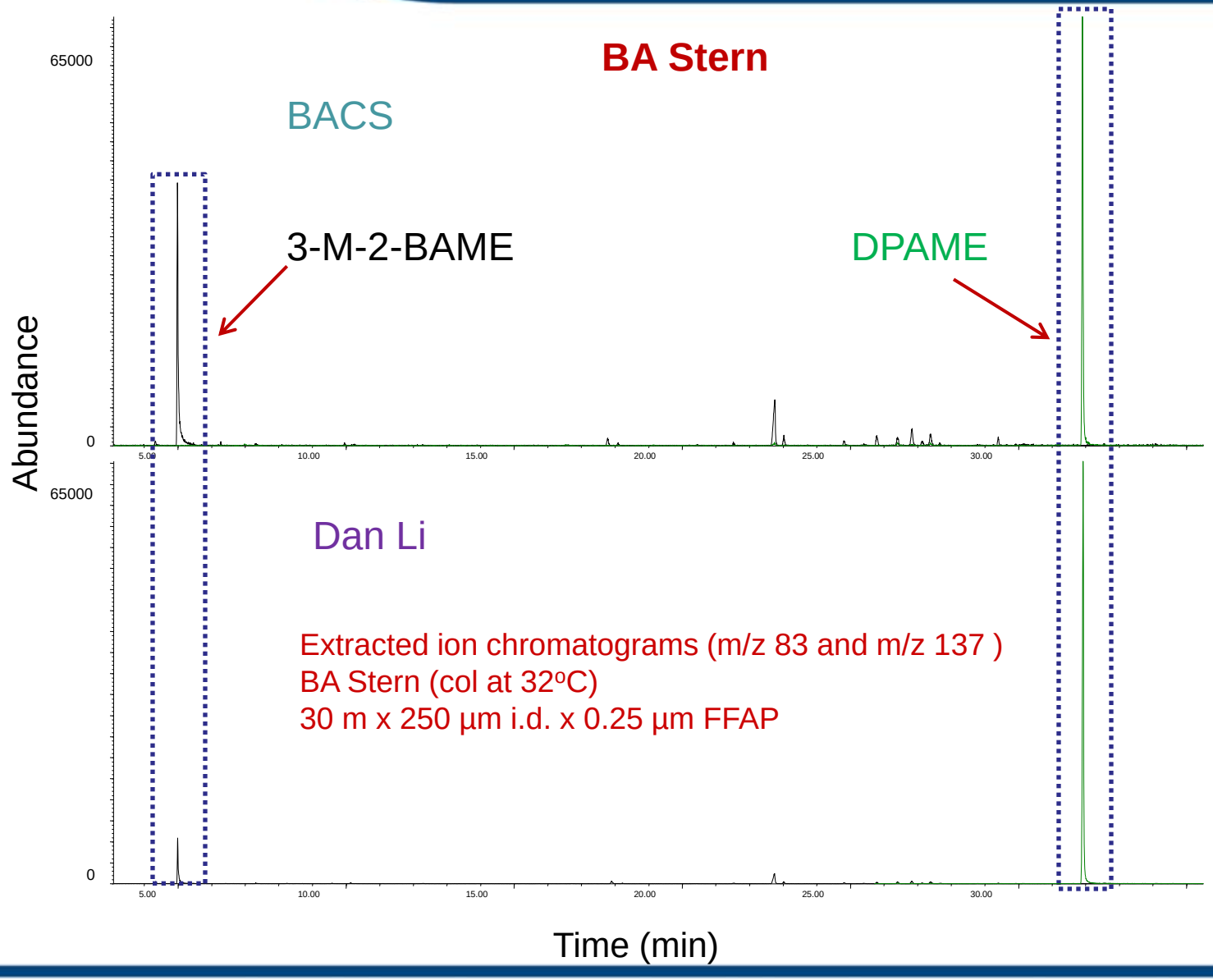
Fatty Acid Comparison

BA Stern FAME profile

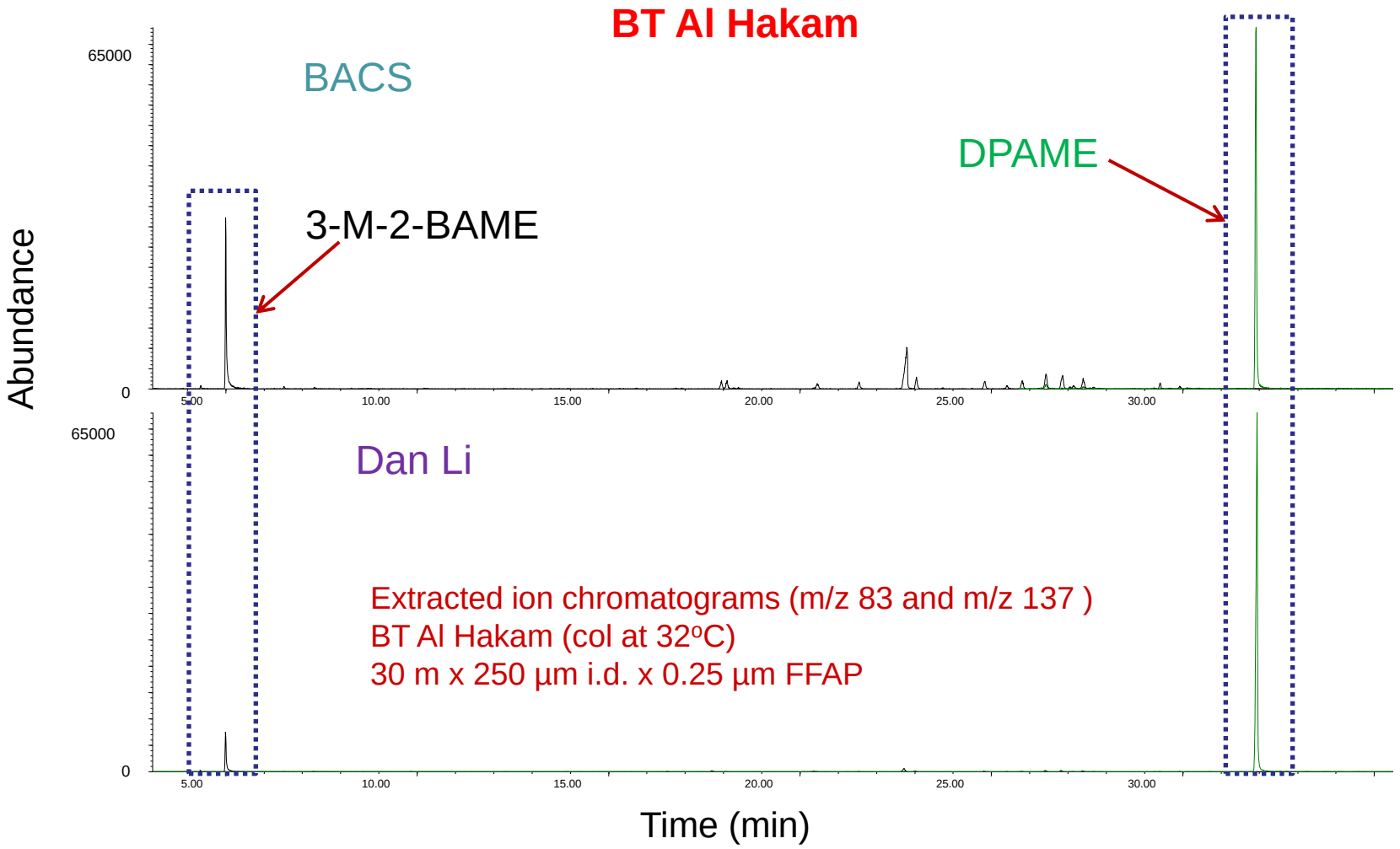


Extracted ion chromatograms (m/z 74)
BA Stern (col at 32°C)
30 m x 250 µm i.d. x 0.25 µm FFAP

DPA and Anthrose Comparison

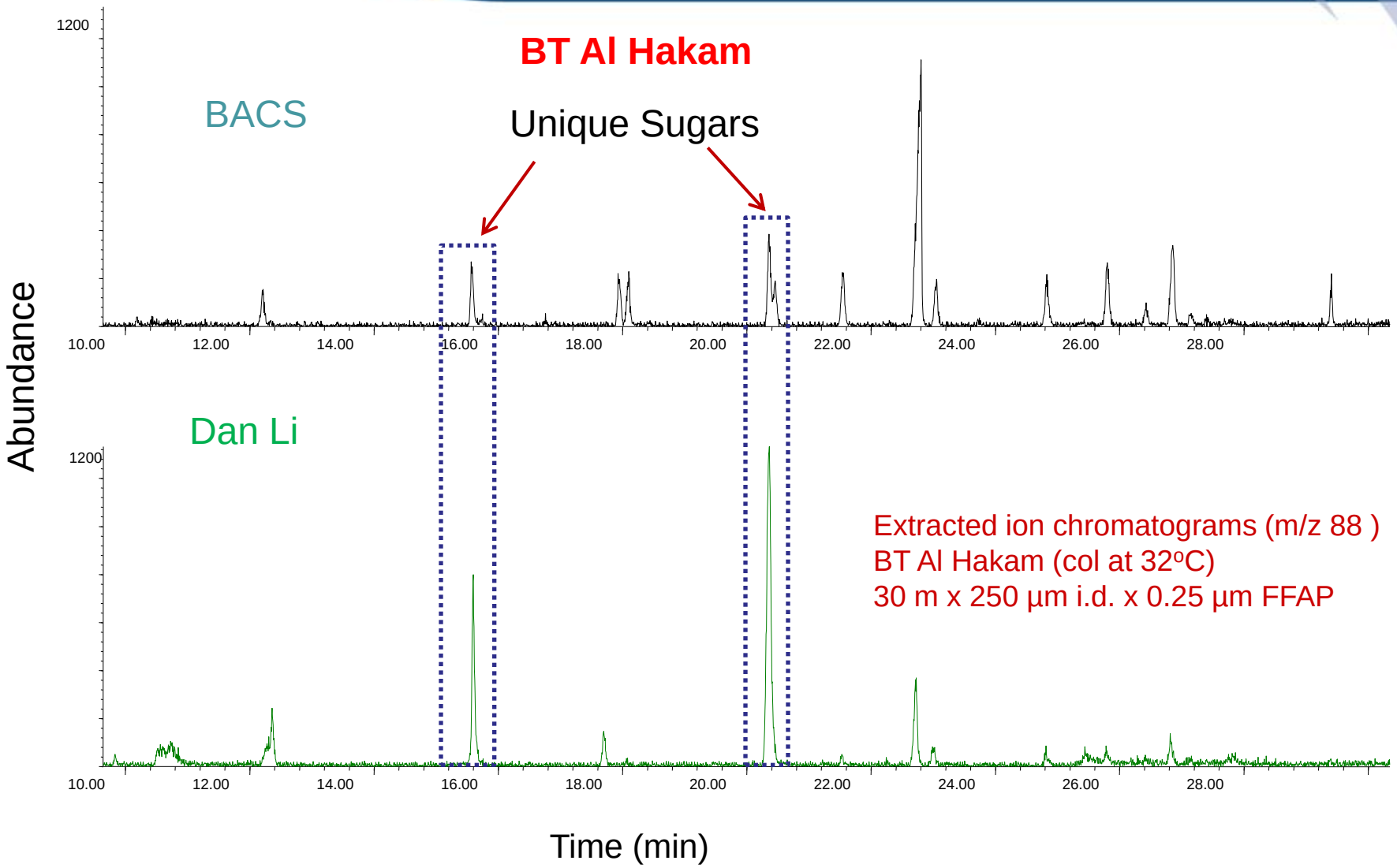


DPA and Anthrose Comparison

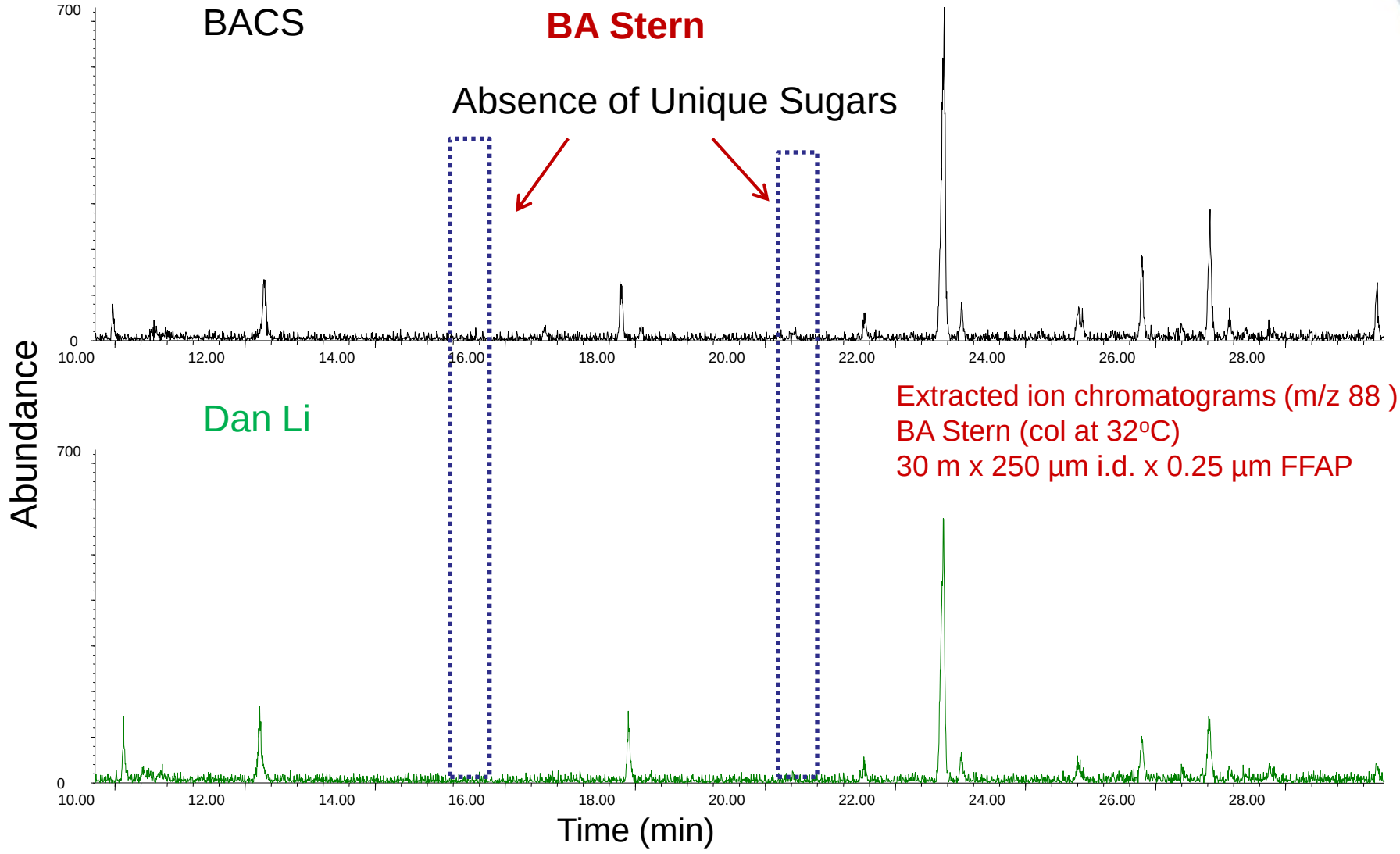


Extracted ion chromatograms (m/z 83 and m/z 137)
BT Al Hakam (col at 32°C)
30 m x 250 µm i.d. x 0.25 µm FFAP

Differentiating Sugars Comparison



Differentiating Sugars Comparison



AIRION-3 PDAS Specifications

- Palm portable size
  - 107 x 58 x 173 mm w x d x h
- Weight: 1.8 lbs (0.82 Kg)
- Rechargeable battery
  - 27 hrs at 1 L/min
- High impact steel fiber filled Lexan®
  - antistatic and RFI/EMI-shielded
- LCD display
  - 2 lines of 16 characters
- Flow rates: 5 mL/min to 5 L/min
- SPME Manifold: inert Teflon®
- Particulate/chemical in-line filter
- RS-232 COM port

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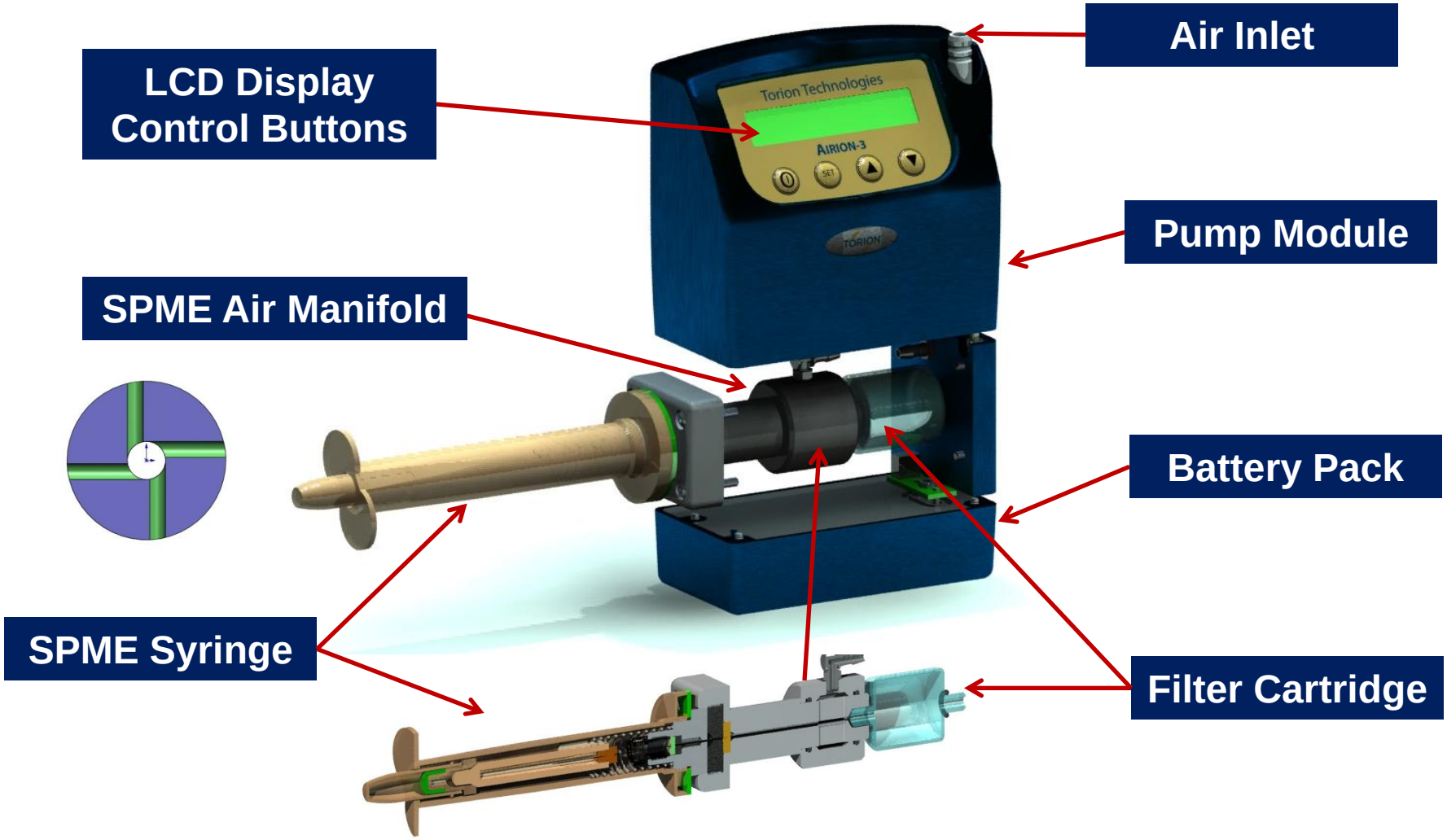
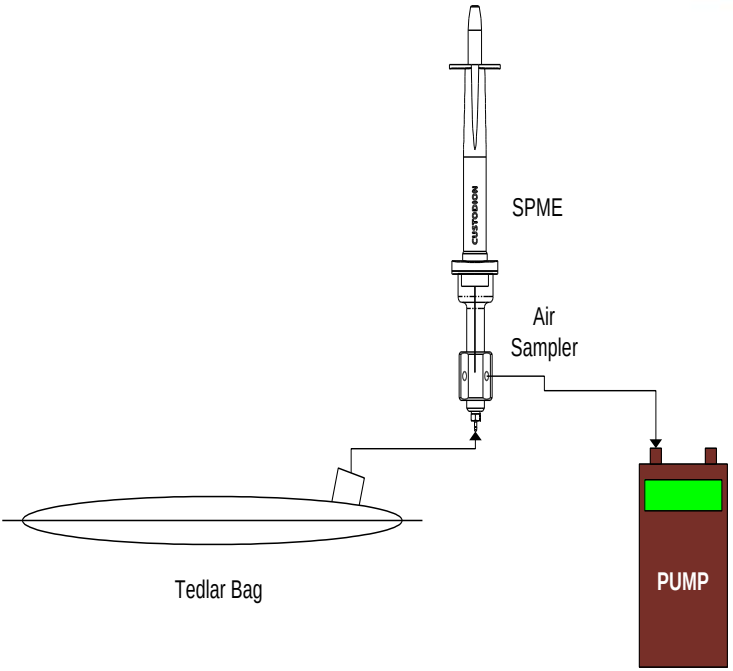
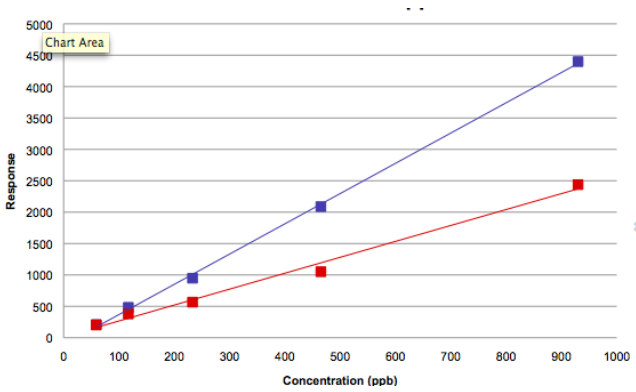
AIRION-3 PDAS Design Features

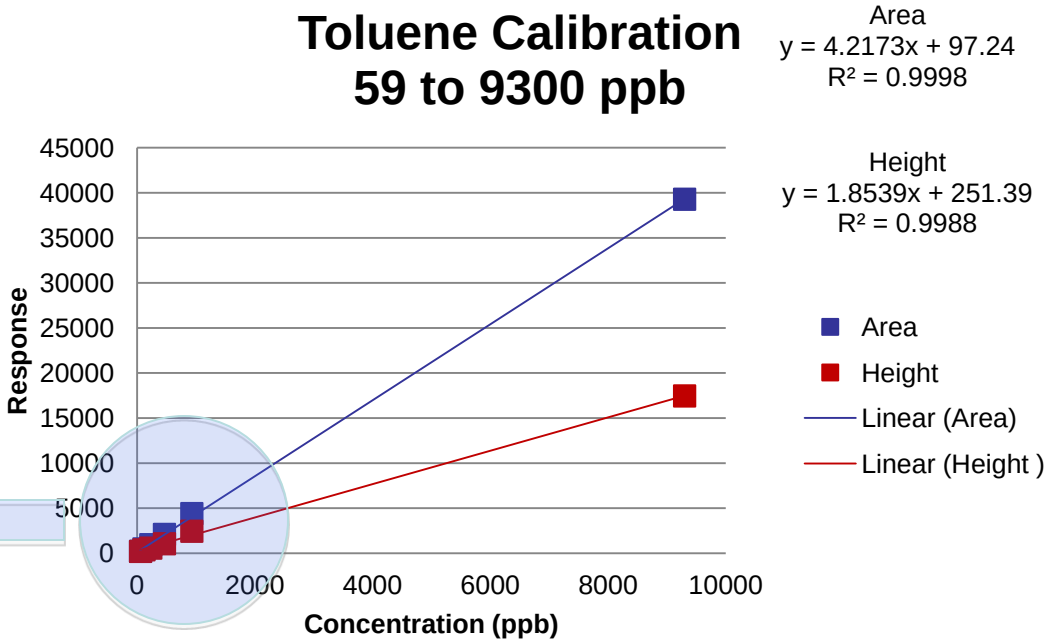


Tedlar Bag Air Vapor Sampling



- Tedlar Bag: 3 L
- Pump Flow Rate: 1 L/min
- SPME Phase: PDMS/DVB
- GC-TMS Analysis





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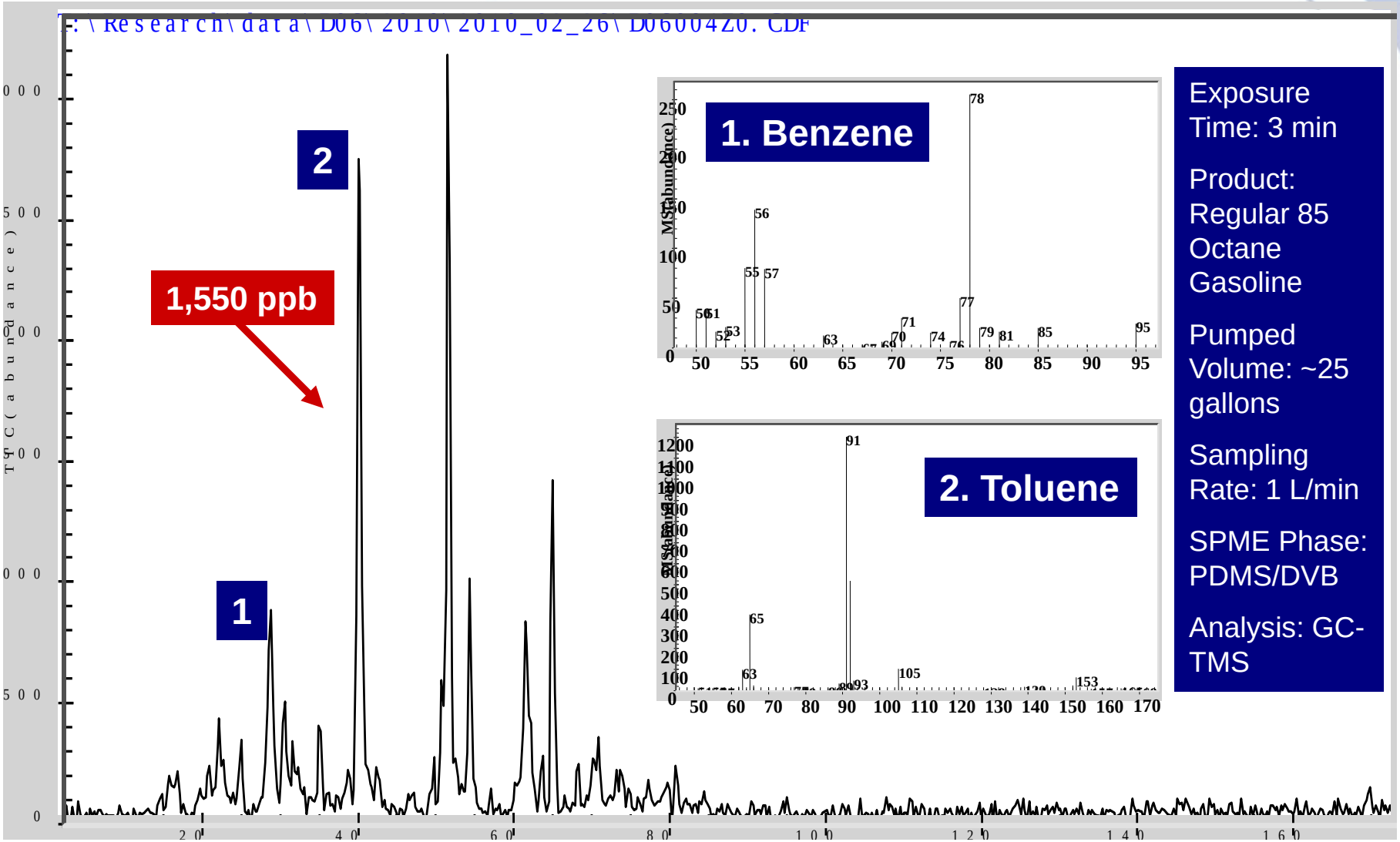
Gasoline Vapor Sampling

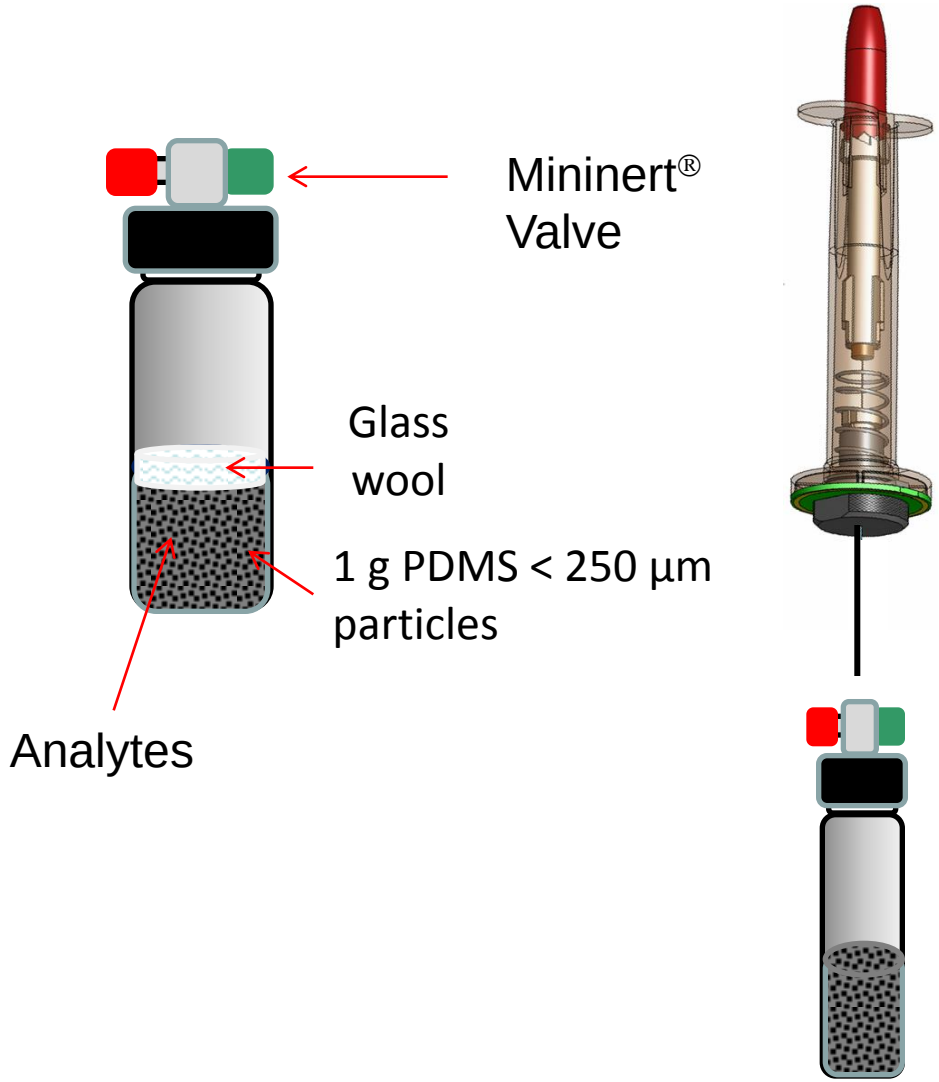


- Exposure Time: 3 min
- Product: Regular 85 Octane Gasoline
- Pumped Volume: ~25 gallons
- Sampling Rate: 1 L/min
- SPME Phase: PDMS/DVB
- Analysis: GC-TMS

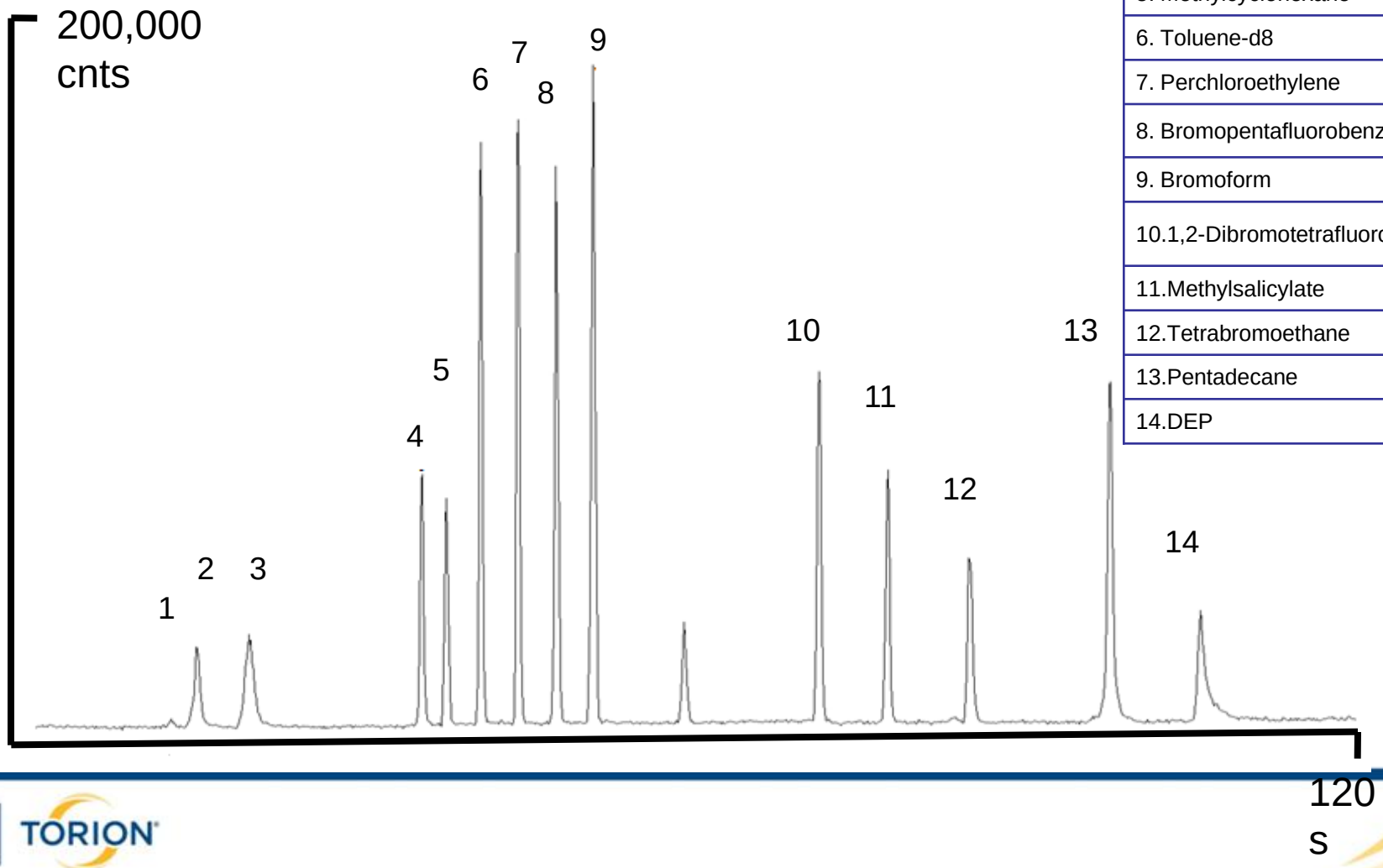
TORION

CALION Standards

- **CALION**
- Need for reliable field calibration standards
  - Robust to environment
  - Easily transportable
  - Long lasting
  - Easy to use
- Gas and Liquid standards have MANY pitfalls
- CALION employs standards encapsulated in ground PDMS particles (high surface area) in a sealed container.
  - Headspace concentration rapidly equilibrates (seconds to minute)
  - Highly repeatable headspace concentration (only temp dependence)
  - MANY (>100) samples per vial



TIC of Analytes in Calibration Vial



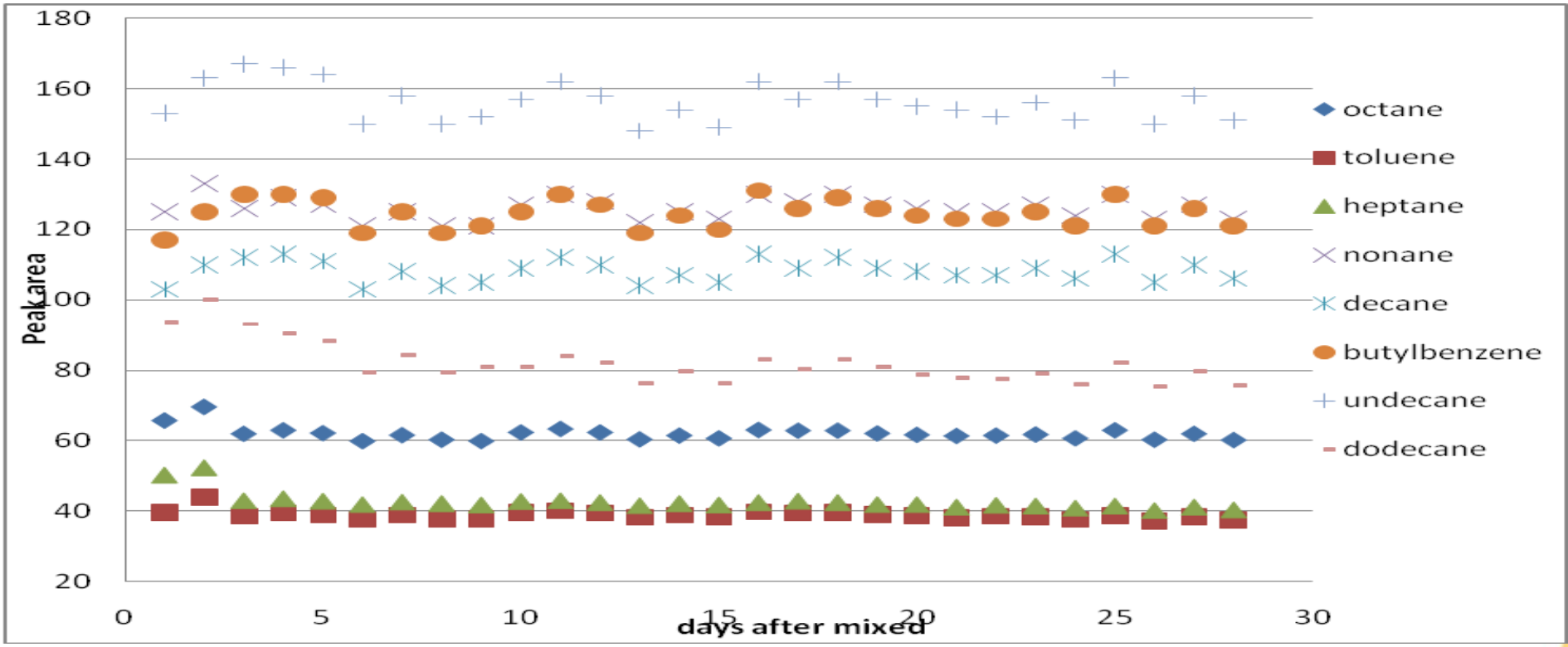
1. Acetone
2. Methylene chloride
3. Methyl-tert-butyl ether
4. Heptane
5. Methylcyclohexane
6. Toluene-d8
7. Perchloroethylene
8. Bromopentafluorobenzene
9. Bromoform
10.1,2-Dibromotetrafluorobenzene
11.Methylsalicylate
12.Tetrabromoethane
13.Pentadecane
14.DEP

Reproducibility for a single PDMS sample vial

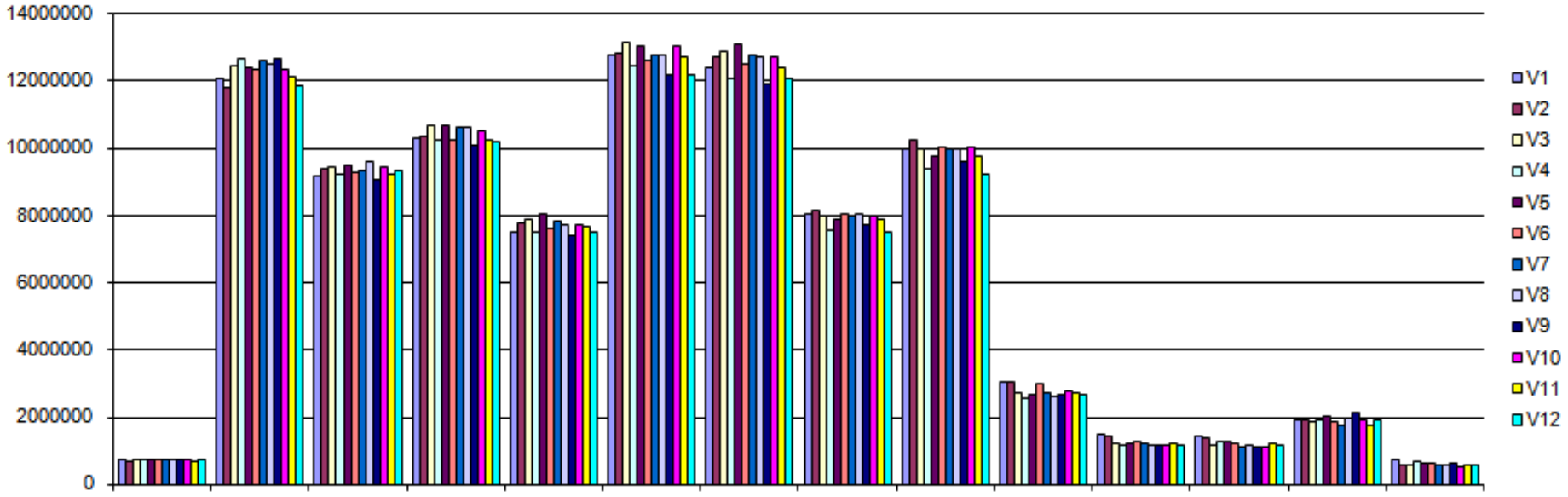
2.5 gram of PDMS, 2-dram vial was sampled more than 100 times.

All conditions were held constant.

RSD <10% in 27 days



Reproducibility for 12 PDMS calibration vials



Analyte	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Aver.	8.E+05	1.E+07	9.E+06	1.E+07	8.E+06	1.E+07	1.E+07	8.E+06	1.E+07	3.E+06	1.E+06	1.E+06	2.E+06	6.E+05
STD	3.E+04	3.E+05	1.E+05	2.E+05	2.E+05	3.E+05	4.E+05	2.E+05	3.E+05	2.E+05	1.E+05	1.E+05	1.E+05	5.E+04
RSD	3.36	2.36	1.59	2.08	2.50	2.44	2.86	2.61	2.98	5.61	8.72	8.16	5.24	8.50

Conclusions

- Automated and/or simple-to-use sampling accessories that interface directly to the field-portable TRIDION GC-TMS instrument allow non-expert field users to acquire and analyze samples with high data integrity.
- Wide range of capabilites (air/liquid preconcentration, bio-threats, surface analysis
- Sampling accessories carry the same fieldable features of the GC-TMS analysis instrument
  - Small, lightweight
  - Easy-to-use
  - Inexpensive
  - Rugged
  - Low or no power
  - Low or no additional utilities and reagents

Acknowledgments

- FVE:
  - Gary Groenewold (INL), Jill Scott (INL),
  - Chris Bailey, Jeff Jones, Ed Lee (Torion)
  - Funding: Battelle, DHS
- BACS:
  - Dan Li, Tai Truong, Richard Robison, Milton Lee (BYU)
  - Torion: Tony Rands
  - Funding SBIR Phase II Funding from DoD, US Army Dugway Proving Ground Contract No. W911S6-09-C-001
- AIRION, CALION:
  - Tony Rands, Nathan Porter, Chris Bailey, Jacob Later, Doug Later, Ed Lee (Torion)
  - Funding: Smiths Detection
- Needle Trap:
  - Tony Rands, Joe Oliphant, Nathan Porter, Tai Truong (Torion)
  - Capt. Simon Strating (USUHS), Phil Smith (OSHA)
  - Funding: Technical Support Working Group (TSWG)