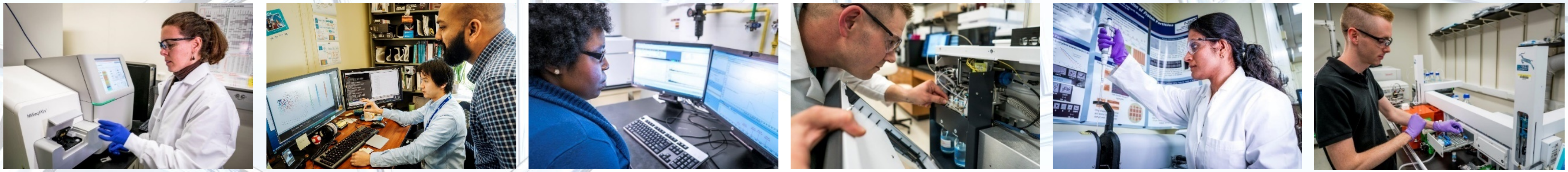
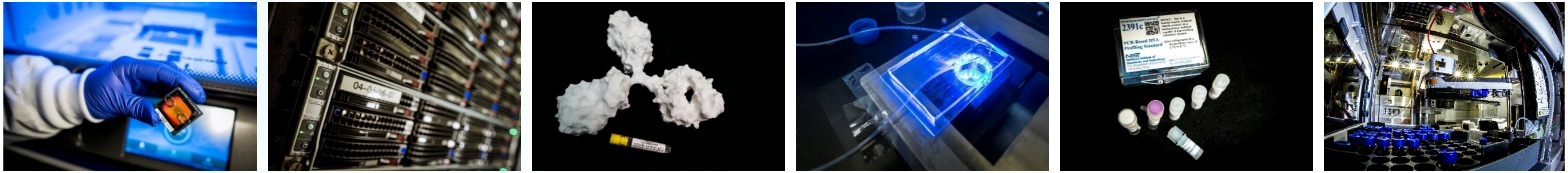


BIOMOLECULAR MEASUREMENT DIVISION

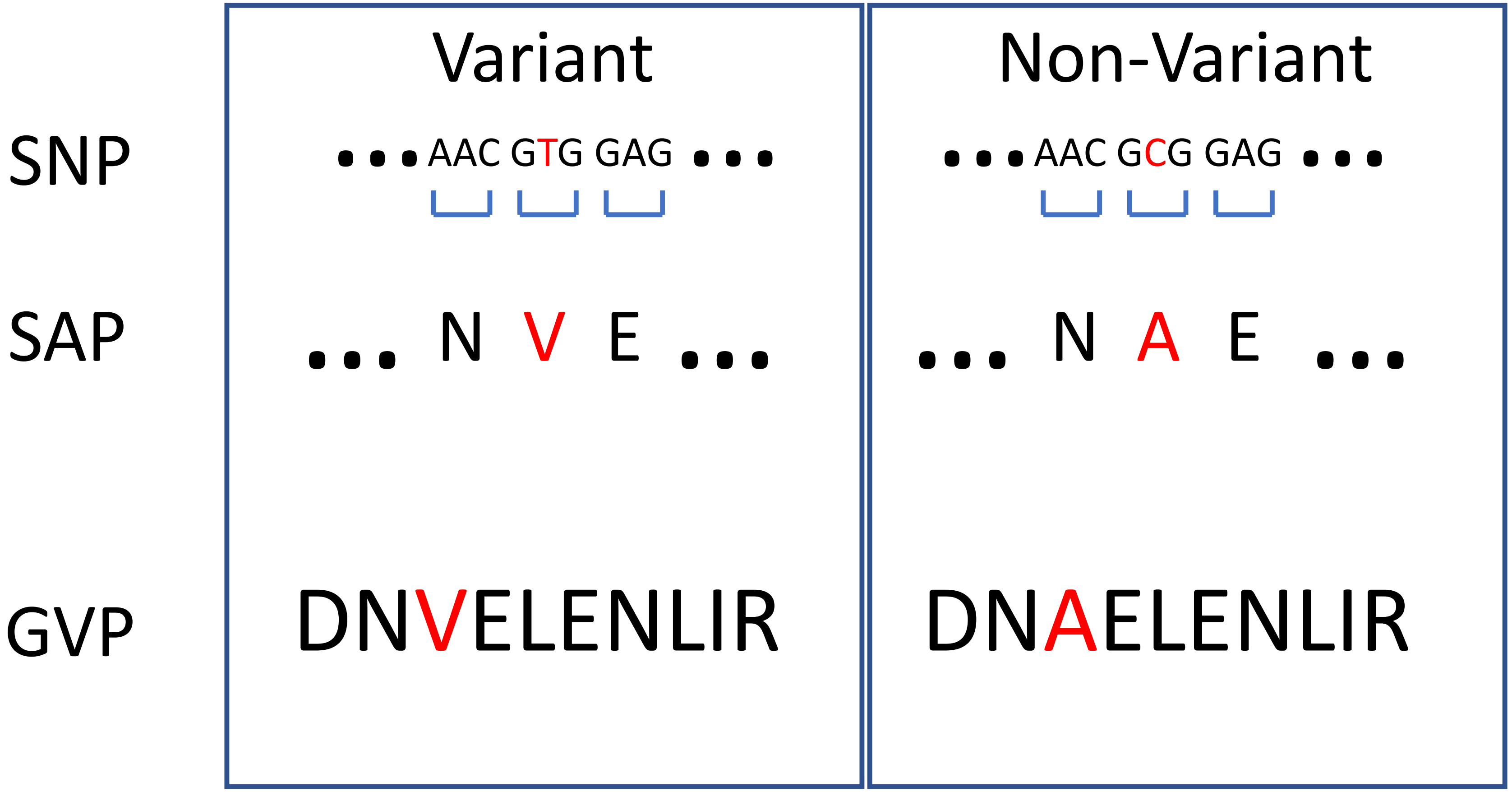
Human Identification from Hair Keratins

- Development of a more comprehensive and extensible hair peptide spectral library



MATERIAL MEASUREMENT LABORATORY

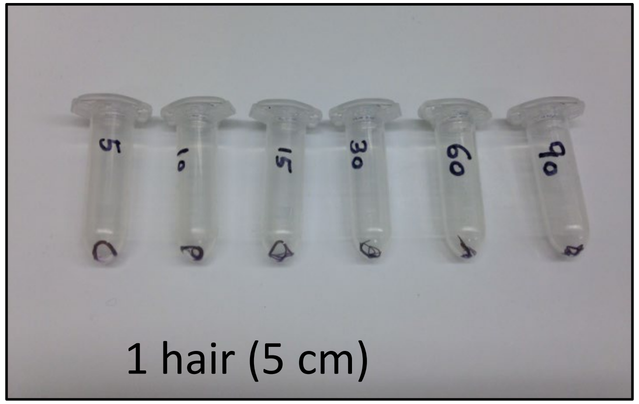
Motivation: Can GVPs Be Used as Alternative Evidence without SNPs?



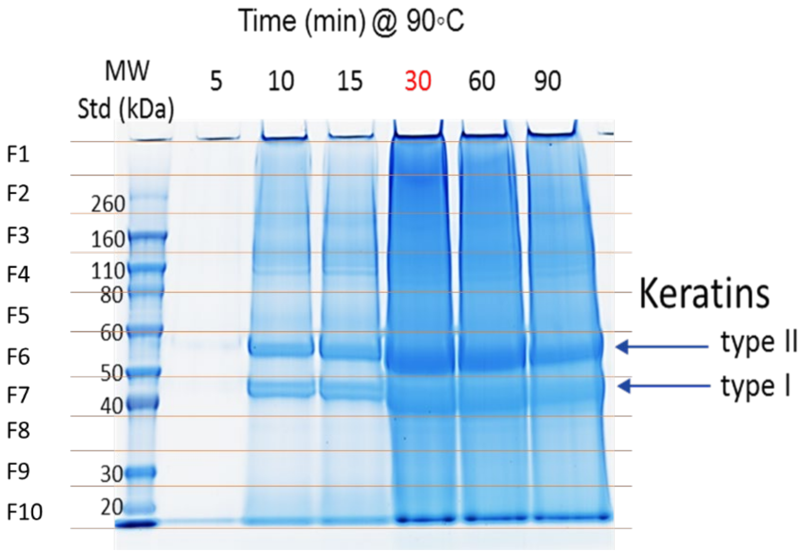
	Variant	Non-Variant
SNP	…AAC GTG GAG …	…AAC GCG GAG …
SAP	… N V E …	… N A E …
GVP	DNVELENLIR	DNAELENLIR

SNP: Single Nucleotide Polymorphism; SAP: Single Amino Acid Polymorphism; GVP: Genetically Variant Peptide

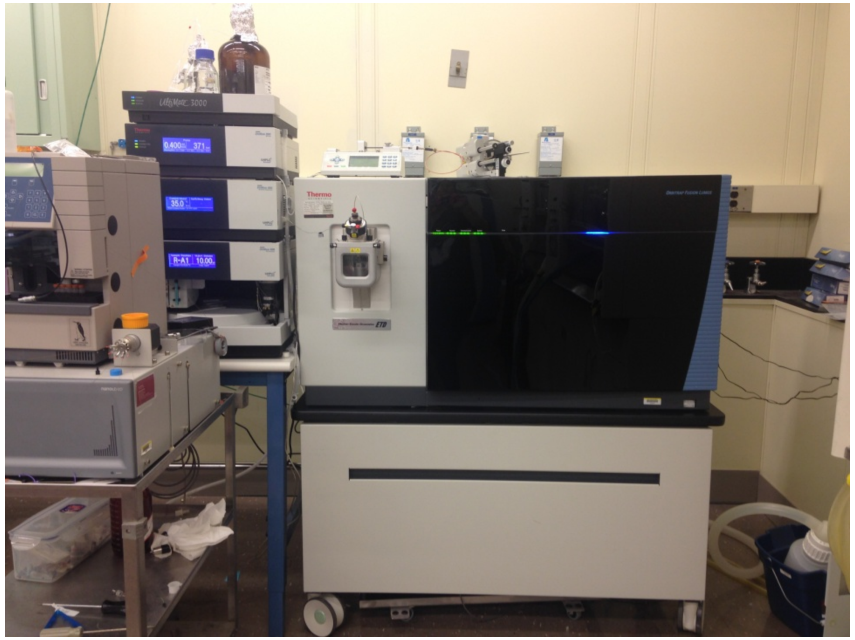
NIST Custom Workflow



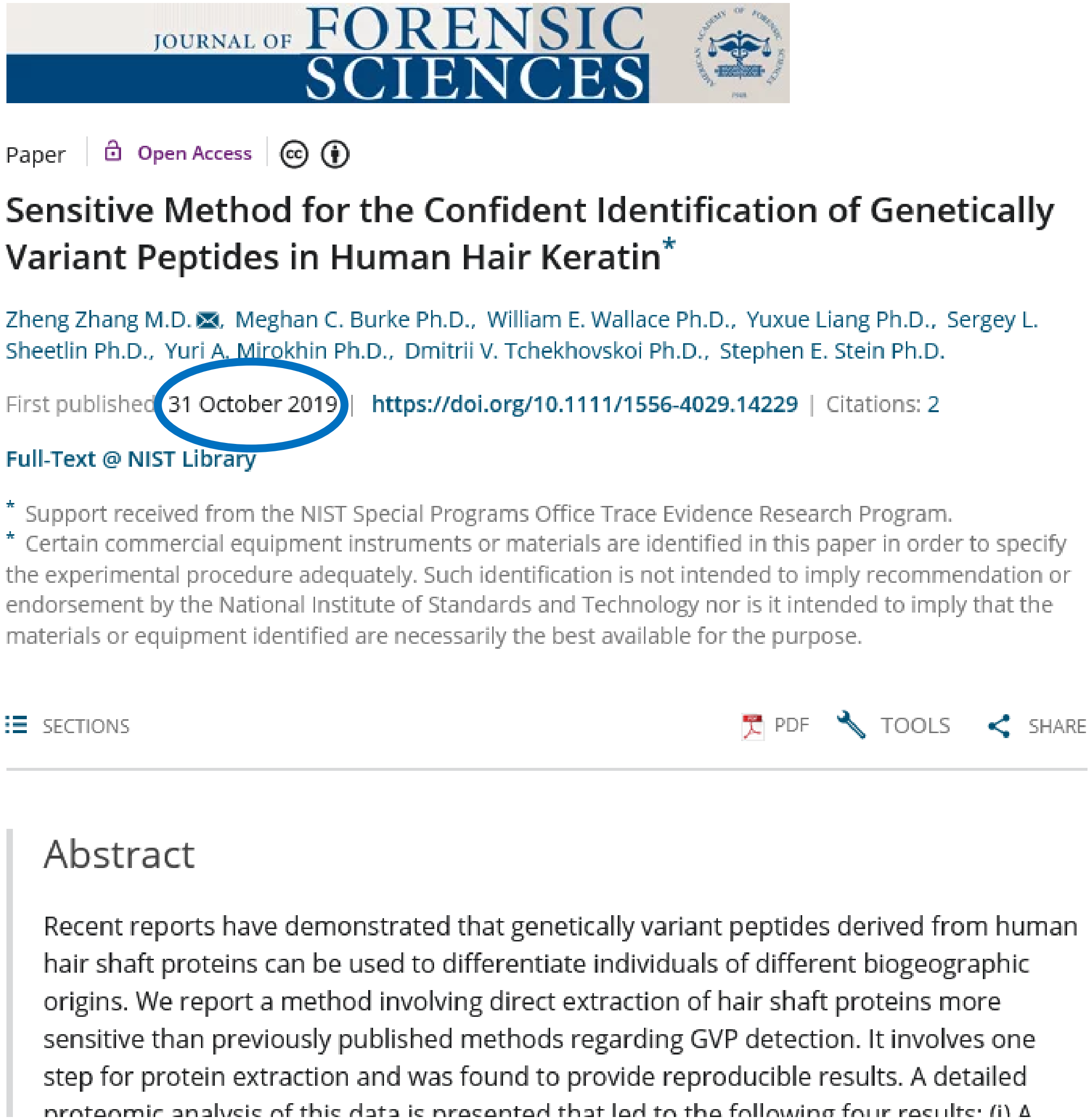
1 hair (5 cm)



SDS-PAGE



- **Manuscript published in the *Journal of Forensic Sciences***
- **Public release of human head hair keratin library on chemdata.nist.gov**


JOURNAL OF FORENSIC SCIENCES

Paper | Open Access

Sensitive Method for the Confident Identification of Genetically Variant Peptides in Human Hair Keratin*

Zheng Zhang M.D., Meghan C. Burke Ph.D., William E. Wallace Ph.D., Yuxue Liang Ph.D., Sergey L. Sheetlin Ph.D., Yuri A. Mirokhin Ph.D., Dmitrii V. Tchekhovskoi Ph.D., Stephen E. Stein Ph.D.

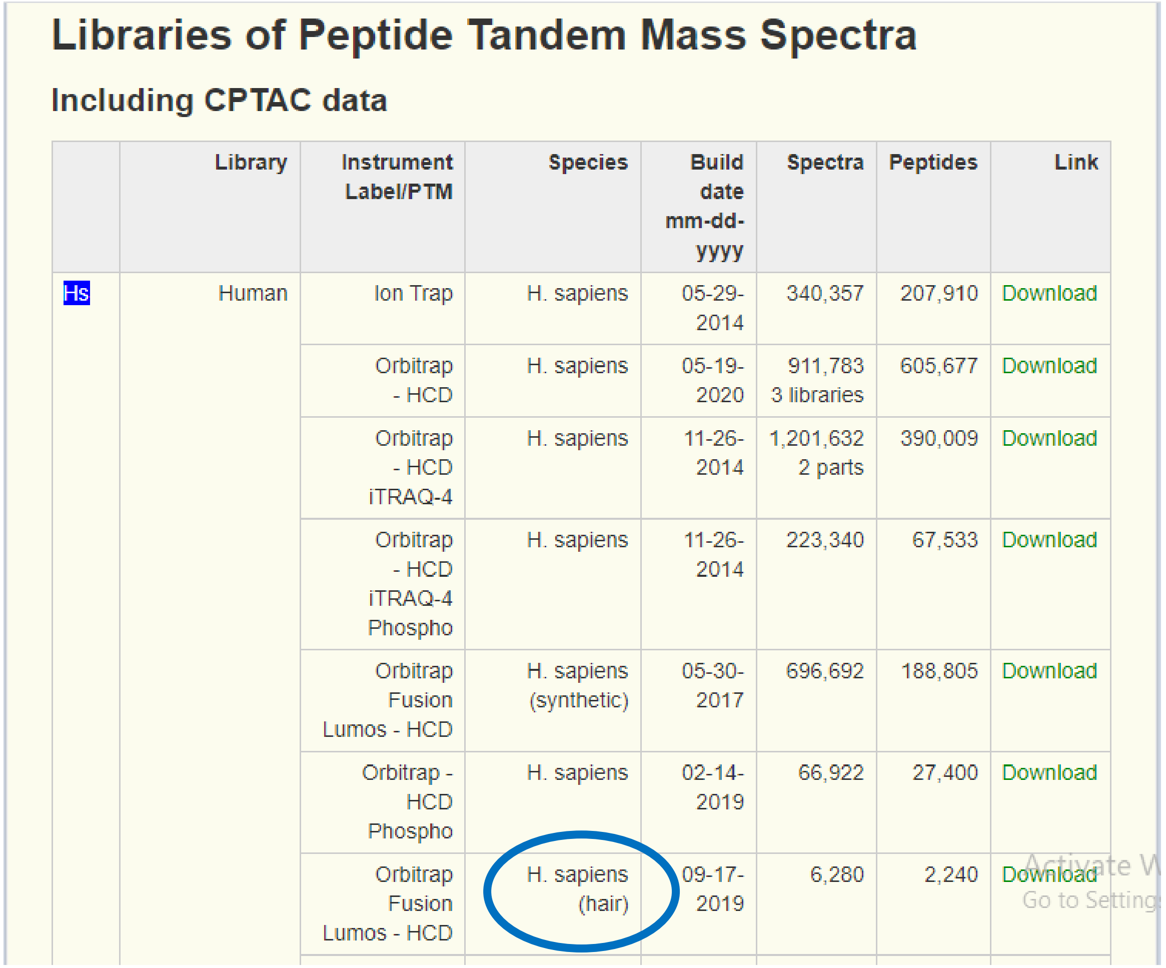
First published 31 October 2019 | https://doi.org/10.1111/1556-4029.14229 | Citations: 2

Full-Text @ NIST Library

* Support received from the NIST Special Programs Office Trace Evidence Research Program.
* Certain commercial equipment instruments or materials are identified in this paper in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

Abstract

Recent reports have demonstrated that genetically variant peptides derived from human hair shaft proteins can be used to differentiate individuals of different biogeographic origins. We report a method involving direct extraction of hair shaft proteins more sensitive than previously published methods regarding GVP detection. It involves one step for protein extraction and was found to provide reproducible results. A detailed proteomic analysis of this data is presented that led to the following four results: (i) A



Libraries of Peptide Tandem Mass Spectra

Including CPTAC data

	Library	Instrument Label/PTM	Species	Build date mm-dd-yyyy	Spectra	Peptides	Link
Hs	Human	Ion Trap	H. sapiens	05-29-2014	340,357	207,910	Download
		Orbitrap - HCD	H. sapiens	05-19-2020	911,783 3 libraries	605,677	Download
		Orbitrap - HCD iTRAQ-4	H. sapiens	11-26-2014	1,201,632 2 parts	390,009	Download
		Orbitrap - HCD iTRAQ-4 Phospho	H. sapiens	11-26-2014	223,340	67,533	Download
		Orbitrap Fusion Lumos - HCD	H. sapiens (synthetic)	05-30-2017	696,692	188,805	Download
		Orbitrap - HCD Phospho	H. sapiens	02-14-2019	66,922	27,400	Download
		Orbitrap Fusion Lumos - HCD	H. sapiens (hair)	09-17-2019	6,280	2,240	Download

Impact

Originating from Rich Press' Outreach Report –
A Solution to a Hairy Problem in Forensic Science

- Original reporting in *Science*, *Popular Mechanics, Forensic Magazine*
- *Science* article included an interview with NIST MSDC staff
- Blog post in *News in Proteomics Research*, the leading blog in proteomics
- Reprinted in *Evidence Technology Magazine*

Wiley Almetric Score for *JFS* paper

In the top 5% of all research outputs scored by Altmetric

Mentioned by

🟥 **8** news outlets
🟧 **2** blogs
🟦 **6** tweeters
🟦 **1** Redditor

Three Steps for Building a More Comprehensive Hair MS Library

1. Synthetic GVPs

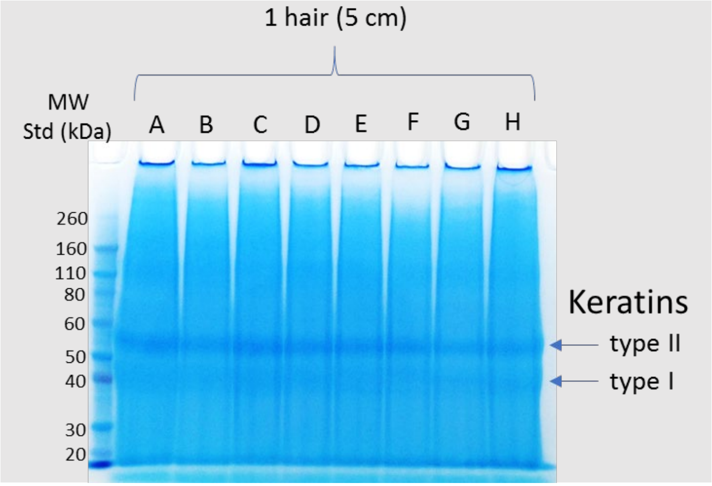
2. Minor Modifications of Hair Peptides
   - GVPs, post-translational modifications, chemical modifications

3. Main Human Library Expanded with Hair Peptides

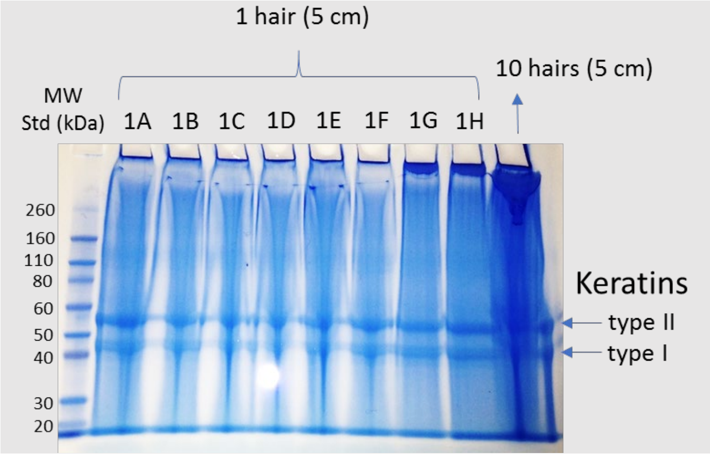
- Synthetic GVPs
  - Published GVPs
    - Type I hair cuticular keratins
    - Type II hair cuticular keratins
    - Other hair proteins
  - Total 164 “pairwise” sequences synthesized by New England Peptide
    - GVPs
    - Their regular non-variant sequences
    - Detailed information is available in excel sheets
  - Arrived as two batches
    - First 96 well vials
      - ☐ Quality checked
    - Second 68 well vials
      - ☐ Waiting to be checked

- Method Comparison: Minor Modifications of Hair Peptides
  - Direct Extraction (Direct) Method
  - Modified NaOH-based SDS Repeated Extraction (NaOH+SDS) Method

NIST Direct Extraction method



NaOH+SDS method



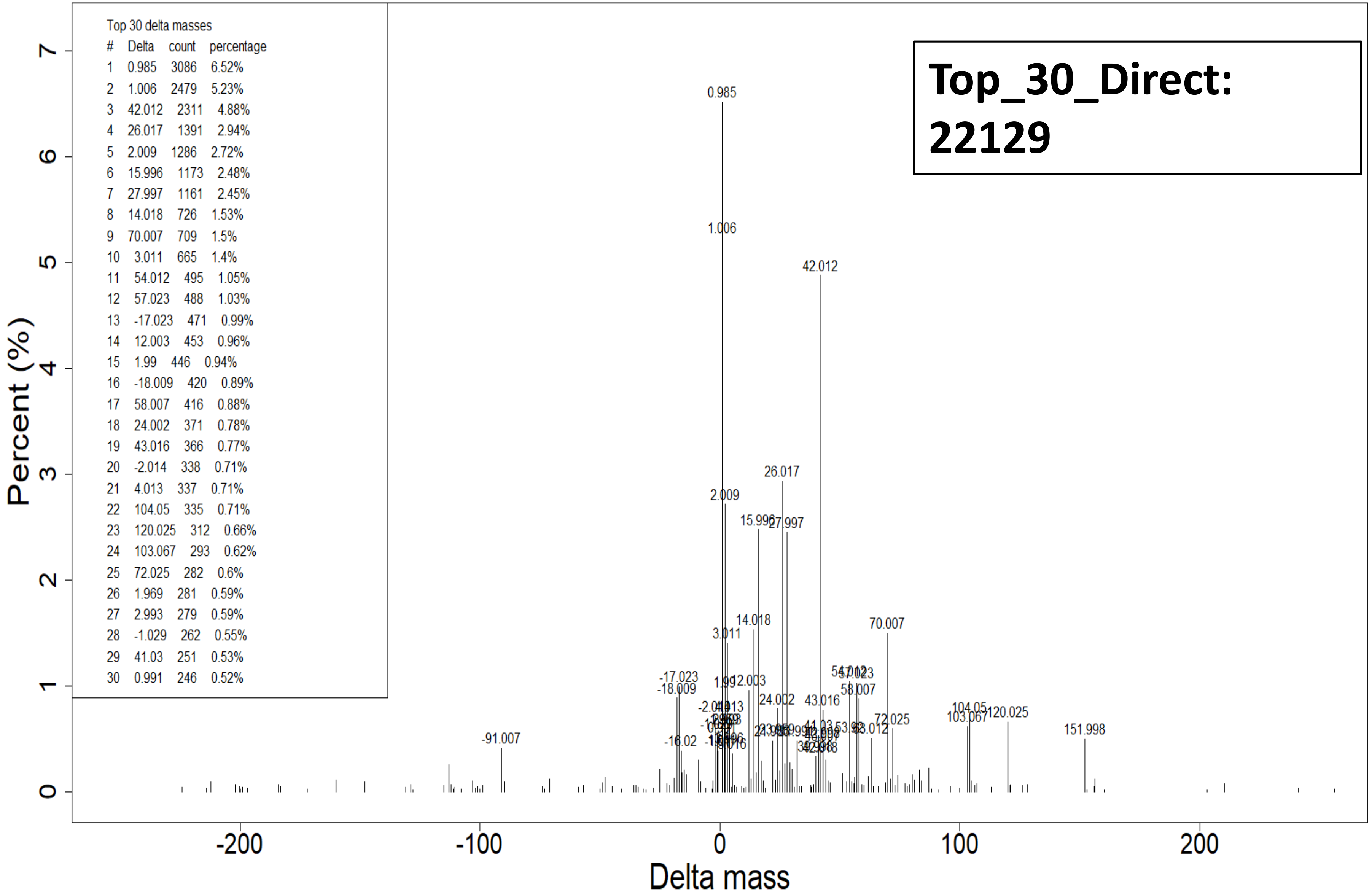
Total Cluster Number of DeltaMass Values in Two Methods

Method	# of DeltaMass	Counts
Direct	421	37126
NaOH+SDS	273	24220

← More Sensitive

DeltaMass is the difference in precursor mass between matched query and library spectra in hybrid search. It reflects a chemical or post-translational modification or an amino acid substitution in the query spectrum.

Histogram of Peptide Modifications

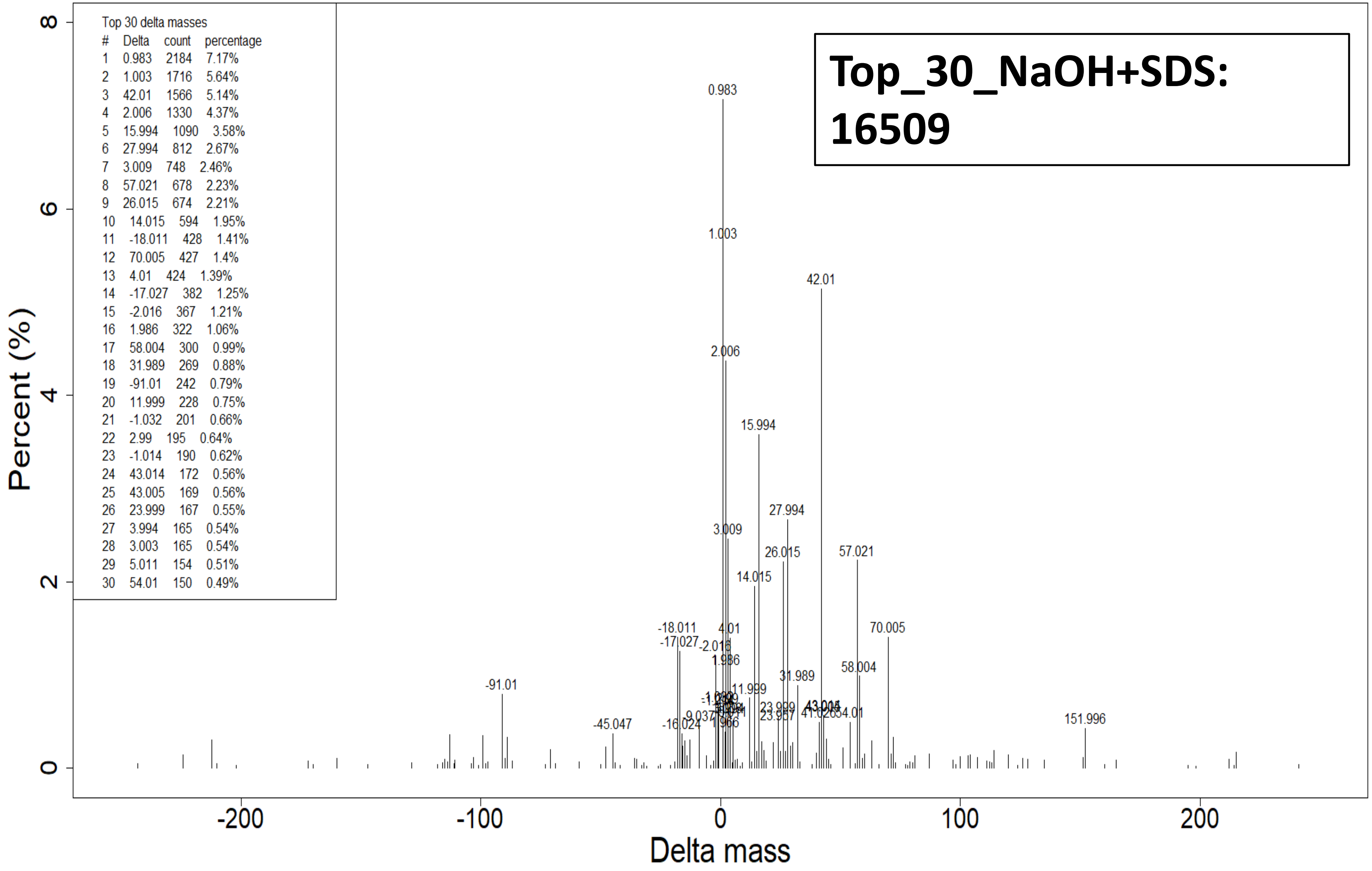


Top 30 delta masses

#	Delta	count	percentage
1	0.985	3086	6.52%
2	1.006	2479	5.23%
3	42.012	2311	4.88%
4	26.017	1391	2.94%
5	2.009	1286	2.72%
6	15.996	1173	2.48%
7	27.997	1161	2.45%
8	14.018	726	1.53%
9	70.007	709	1.5%
10	3.011	665	1.4%
11	54.012	495	1.05%
12	57.023	488	1.03%
13	-17.023	471	0.99%
14	12.003	453	0.96%
15	1.99	446	0.94%
16	-18.009	420	0.89%
17	58.007	416	0.88%
18	24.002	371	0.78%
19	43.016	366	0.77%
20	-2.014	338	0.71%
21	4.013	337	0.71%
22	104.05	335	0.71%
23	120.025	312	0.66%
24	103.067	293	0.62%
25	72.025	282	0.6%
26	1.969	281	0.59%
27	2.993	279	0.59%
28	-1.029	262	0.55%
29	41.03	251	0.53%
30	0.991	246	0.52%

Top_30_Direct:
22129

Histogram of Peptide Modifications



Top 30 delta masses

#	Delta	count	percentage
1	0.983	2184	7.17%
2	1.003	1716	5.64%
3	42.01	1566	5.14%
4	2.006	1330	4.37%
5	15.994	1090	3.58%
6	27.994	812	2.67%
7	3.009	748	2.46%
8	57.021	678	2.23%
9	26.015	674	2.21%
10	14.015	594	1.95%
11	-18.011	428	1.41%
12	70.005	427	1.4%
13	4.01	424	1.39%
14	-17.027	382	1.25%
15	-2.016	367	1.21%
16	1.986	322	1.06%
17	58.004	300	0.99%
18	31.989	269	0.88%
19	-91.01	242	0.79%
20	11.999	228	0.75%
21	-1.032	201	0.66%
22	2.99	195	0.64%
23	-1.014	190	0.62%
24	43.014	172	0.56%
25	43.005	169	0.56%
26	23.999	167	0.55%
27	3.994	165	0.54%
28	3.003	165	0.54%
29	5.011	154	0.51%
30	54.01	150	0.49%

Top_30_NaOH+SDS: 16509

1) Major modifications (from Top_30 list in above two histograms) are similar in the compared two methods

2) Minor modifications usually are more varied, as the analyses go deeper, with 10 fractions separately analyzed and the results combined – we want to see every modification of the hair, this depth of analysis has never been performed before

Example Minor Modifications that favor Direct Method*

Minor Mods	Direct				NaOH+SDS			
	Rank	DeltaMass	Count	%	Rank	DeltaMass	Count	%
Tris or other artifacts								
Tris adduct	22	104.05	335	0.71	88	104.046	42	0.14
Desulferization + CAM + DTT	23	120.025	312	0.66	86	120.024	44	0.14
Tris + Amidation	24	103.067	293	0.62	93	103.063	40	0.13
Cations								
Cation:Fe[II]	31	53.92	243	0.51	62	53.917	67	0.22
Sodiated	35	21.985	228	0.48	55	21.984	82	0.27
Cation:Zn[II] / Cation:Cu[I]	84	61.916	68	0.14	-	-	-	-
Unique GVP								
Gly->His	76	80.039	77	0.16	-	-	-	-

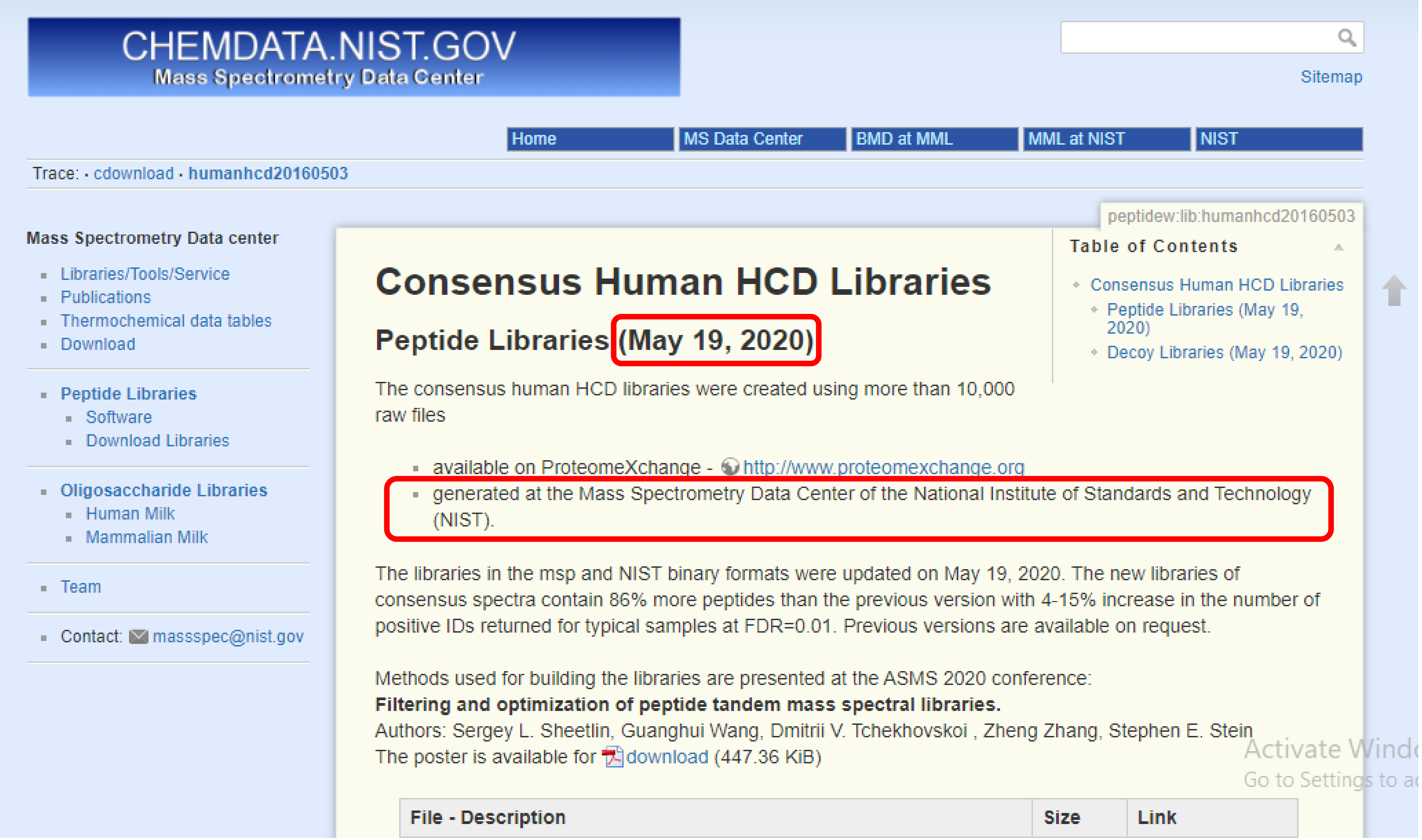
*note: Detailed analysis is available in excel sheets and separate ppxt files

Example Minor Modifications that favor NaOH+SDS Method*

Minor Mods	Direct				NaOH+SDS			
	Rank	DeltaMass	Count	%	Rank	DeltaMass	Count	%
Semi-tryptics or In-source?								
Loss of I/L	55	-113.083	122	0.26	39	-113.084	109	0.36
Loss of V	196	-99.066	29	0.06	41	-99.068	105	0.34
Loss of A	100	-71.035	55	0.12	66	-71.037	59	0.19
Ubiquitinylation								
"GG"	-	-	-	-	68	114.042	57	0.19
Unique artifact due to added NaOH								
Loss of CAM and loss of Sulfur	-	-	-	-	43	-88.993	101	0.33
Unique artifact due to added ß-ME								
+ Mercaptoethanol (ME)	-	-	-	-	81	60.002	46	0.15

*note: Detailed analysis is available in excel sheets and separate ppxt files

Main Library Recently Expanded with Additional Verified Hair Peptides



Consensus Human HCD Libraries

Peptide Libraries (May 19, 2020)

The consensus human HCD libraries were created using more than 10,000 raw files

- available on ProteomeXchange - http://www.proteomexchange.org
- generated at the Mass Spectrometry Data Center of the National Institute of Standards and Technology (NIST).

The libraries in the msp and NIST binary formats were updated on May 19, 2020. The new libraries of consensus spectra contain 86% more peptides than the previous version with 4-15% increase in the number of positive IDs returned for typical samples at FDR=0.01. Previous versions are available on request.

Methods used for building the libraries are presented at the ASMS 2020 conference:
Filtering and optimization of peptide tandem mass spectral libraries.
Authors: Sergey L. Sheetlin, Guanghui Wang, Dmitrii V. Tchekhovskoi , Zheng Zhang, Stephen E. Stein
The poster is available for download (447.36 KiB)

File - Description	Size	Link

The impact of COVID-19

- Due to lab shutdown in March, second batch of synthetic GVPs as well as other donor’s hair are waiting for measurement on orbitrap mass spectrometer
- Instrument currently occupied with SARS-CoV-2 spike protein measurements
- Z.Z. invited speaker at Green Mountain DNA Conference (postponed to July 2021 from July 2020)

Collaborations and Stakeholders

- Lawrence Livermore National Laboratory– Deon Anex
- FBI– Joseph Donfack
- IARPA Proteos– Kristen Jordan
  - Skin keratins

Advances in Dynamic Vapor Sampling Towards Reliable Field Deployment

Megan Harries, Cheryle Beuning, Adam Friss, Kavita Jeerage, Tara Lovestead, Jason Widegren

Fluid Characterization Group, NIST Boulder

Vapor sampling in the field

Cleaner: which is why we use it for fire debris analysis and many environmental analyses

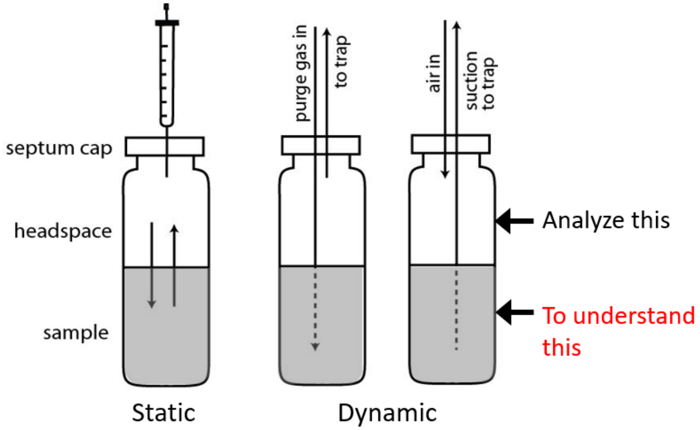
Safer: does not require direct contact or even close proximity to a sample, with the right sampling probe

Non-destructive: a big advantage in evidence analysis or when sample availability is limited

Low-volatility samples are difficult & require preconcentration

Indirect measurement: requires good fundamental understanding of sample properties

Requires sample stability: hours-days-weeks between collection and laboratory analysis




Environmental whole-air sampling


Cargo screening

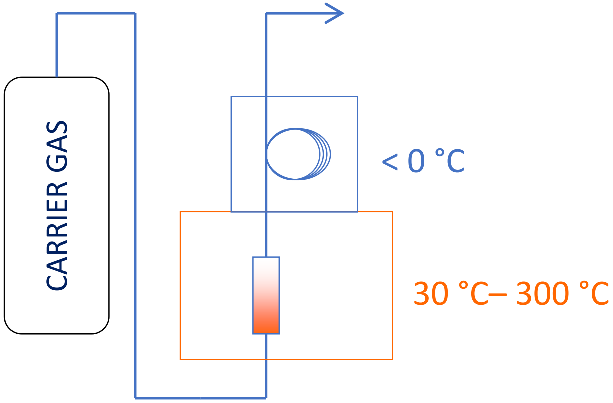

Personal exposure badges

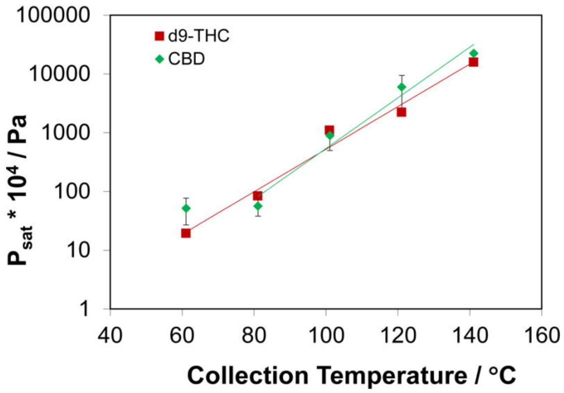
NIST’s dynamic vapor capture technology (since 2009)

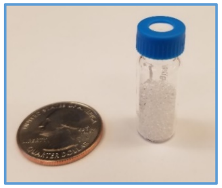
A headspace preconcentration method that uses an adsorbent capillary trap chilled to low temperatures to enhance efficiency + stabilize analytes

Advantages

- Cheap setup w/ many adsorbent phases readily available
- Small sample quantities (mg)
- Fast (minutes to days)
- Sample temps 30-300 °C
- Low-volatility compounds (TNT, plasticizers)
- Reactive compounds are preserved in cryostat
- Quantitative (vapor pressure!)

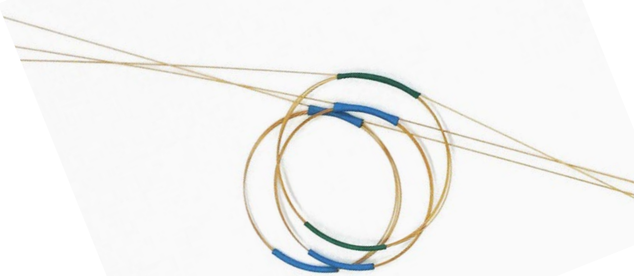






Bruno, T.J. J. Chromatogr. Sci., 47, 569-574, 2009.
Lovestead, T. M., Bruno, T. J., Detecting gravesoil with headspace analysis with adsorption on short porous layer open tubular (PLOT) columns. *Forens Sci Int* 2011, *204*, 156-161.
Nichols, J. E., Harries, M. E., et al., Analysis of arson fire debris by low temperature dynamic headspace adsorption porous layer open tubular columns. J Chromatogr A 2014, 1334, 126-138.
Lovestead, T. M., Bruno, T. J., Trace Headspace Sampling for Quantitative Analysis of Explosives with Cryoadsorption on Short Alumina Porous Layer Open Tubular Columns. Anal Chem 2010, 82, 5621-5627.
Lovestead, T. M., Bruno, T. J., Detection of poultry spoilage markers from headspace analysis with cryoadsorption on a short alumina PLOT column. Food Chem 2010, 121, 1274-1282.
Lovestead, T. M., Bruno, T. J., Determination of cannabinoid vapor pressures to aid in vapor phase detection of intoxication. Forensic Chemistry 2017, 5 (Supplement C), 79-85.

DVME (Dynamic Vapor Microextraction)

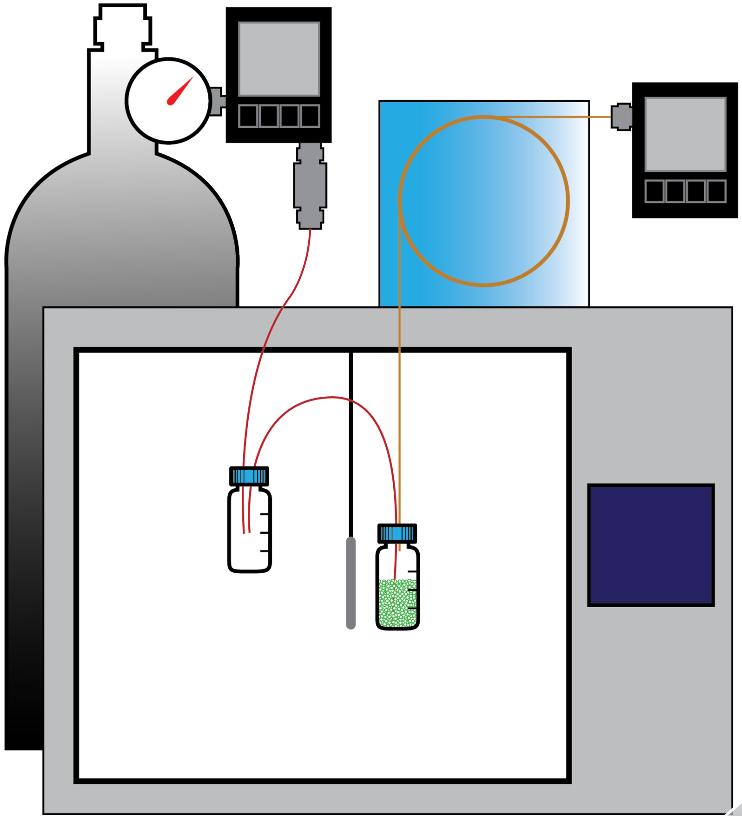


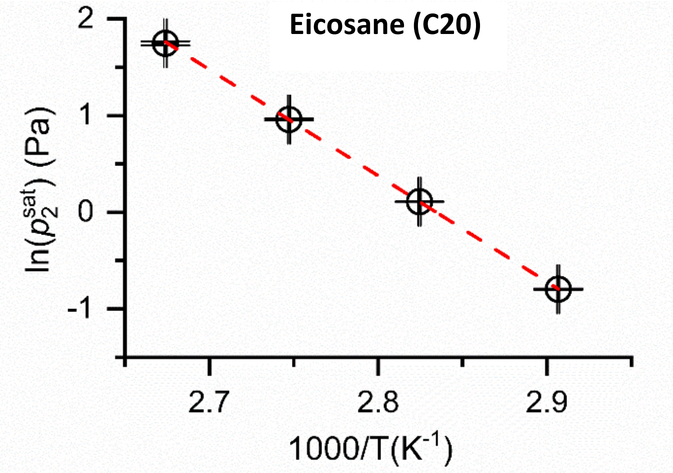
Key Innovations:

- Miniature vapor-equilibration vessel (the “saturator”) **minimizes temperature gradients**, internal volume, and equilibration time
- Capillary vapor trap minimizes internal volume
- Helium carrier gas minimizes non-ideal mixture behavior
- **Direct pressure measurement** inside the saturator enables us to account for overpressure caused by viscous flow

New state-of-the-art!

Fast (< 15 min), low uncertainty (2% at 1 Pa) measurement with only ~20 mg of sample!

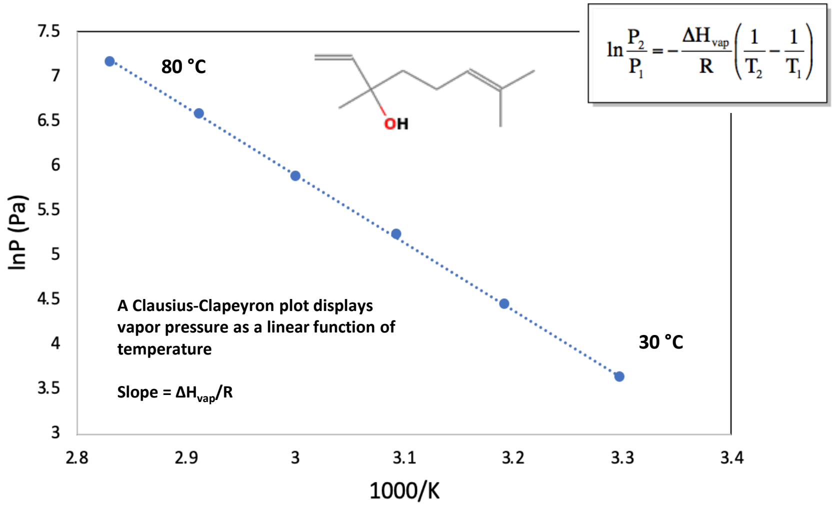




M. E. Harries, C. N. Beuning, B. L. Johnston, T. M. Lovestead, J. A. Widegren. Rapid Vapor Collection Method for Vapor Pressure Measurements of Low-Volatility Compounds. *Anal. Chem.* in review.

DVME for terpenoid vapor pressures (preliminary data)

- Terpenoids, cannabinoids, and metabolites are all potential target molecules in the development of a reliable field test for recent cannabis use
- We need data: vapor pressures, partition coefficients, VLE

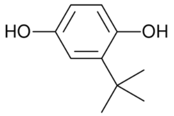


$$\ln\frac{P_2}{P_1} = -\frac{\Delta H_{vap}}{R}\left(\frac{1}{T_2}-\frac{1}{T_1}\right)$$

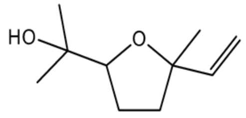
A Clausius-Clapeyron plot displays vapor pressure as a linear function of temperature

Slope = $\Delta H_{vap}/R$

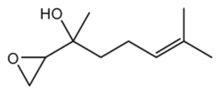
Antioxidant: 0.2% w/w tert-Butylhydroquinone



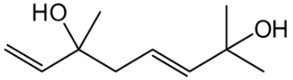
Linalool decomposes within hours at 30 ℃ and at 80 ℃



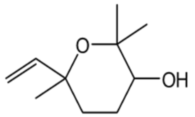
Linalool oxide (furanoid)



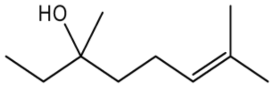
a-Methyl-a-[4-methyl-3-pentenyl]oxiranemethano



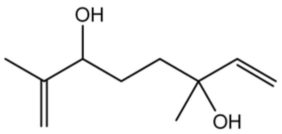
2,6-dimethyl-3,7-Octadiene-2,6-diol



2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-



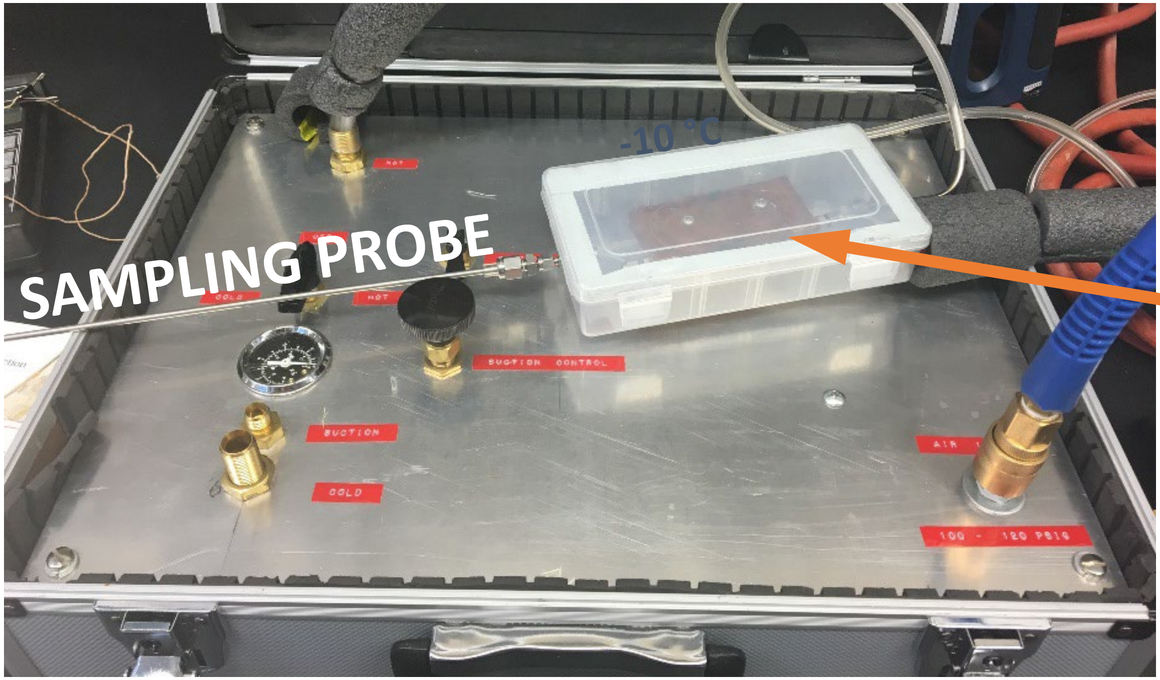
Dihydrolinalool

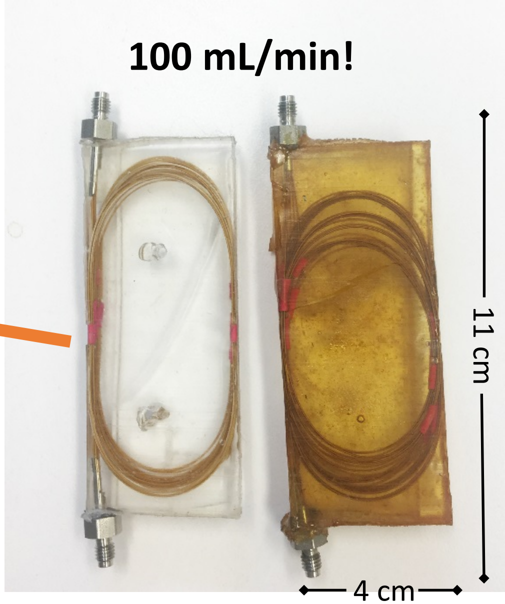


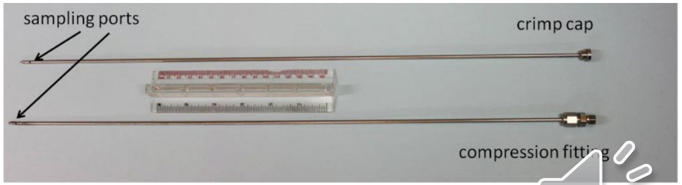
2,6-dimethyl-1,7-Octadiene-3,6-diol

Portable vapor capture device

- Robust, sturdy, hand-portable
- Powered by compressed air
- A probe to enable sampling from a distance
- Capable of rapid sampling times (higher flow rate)
- Field-based sample elution and capillary reactivation







Bruno, T.J., Field portable low temperature porous layer open tubular cryoadsorption headspace sampling and analysis part I: Instrumentation, Journal of Chromatography A 1429, 2016, Pages 65-71.
Harries, M.E., Bukovsky-Reyes, S., Bruno, T. J., Field portable low temperature porous layer open tubular cryoadsorption headspace sampling and analysis part II: Applications, Journal of Chromatography A, 1429, 72-78, 2016.

Bunker study

Bunker exterior

Bunker interior

Challenges:
- Large, open system
- Not well mixed – equilibration unlikely
- Mother nature

Sources of compressed air:
- Diesel compressors
- SCBA canister
- House air

Bunker study

Four experiments

Naphthalene	Pure compound	Check device functioning
Explosives-related	4-part mixture	Solvents and plasticizers Vapor pressures $5*10^{-4}$ – 0.084 kPa
Protein decomposition markers	8-part mixture	Sulfur compounds Diamines putrescine and cadaverine Vapor pressures 3 – 38 mm Hg
Gasoline	Highly complex	≈ ruptured fuel tank

Test compound volatility $5*\ 10^{-4}$ kPa to 5 kPa

Range of test conditions

$T_{ambient}$ = 3 to 37 °C

T_{bunker} = 2.2 to 43 °C

RH = 9 % to 92 %

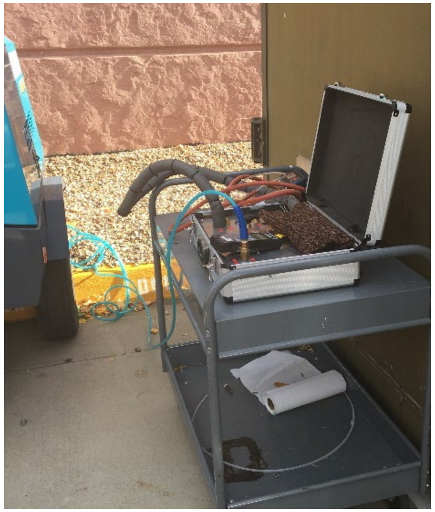
Sample times = 30 s – 20 min

Findings

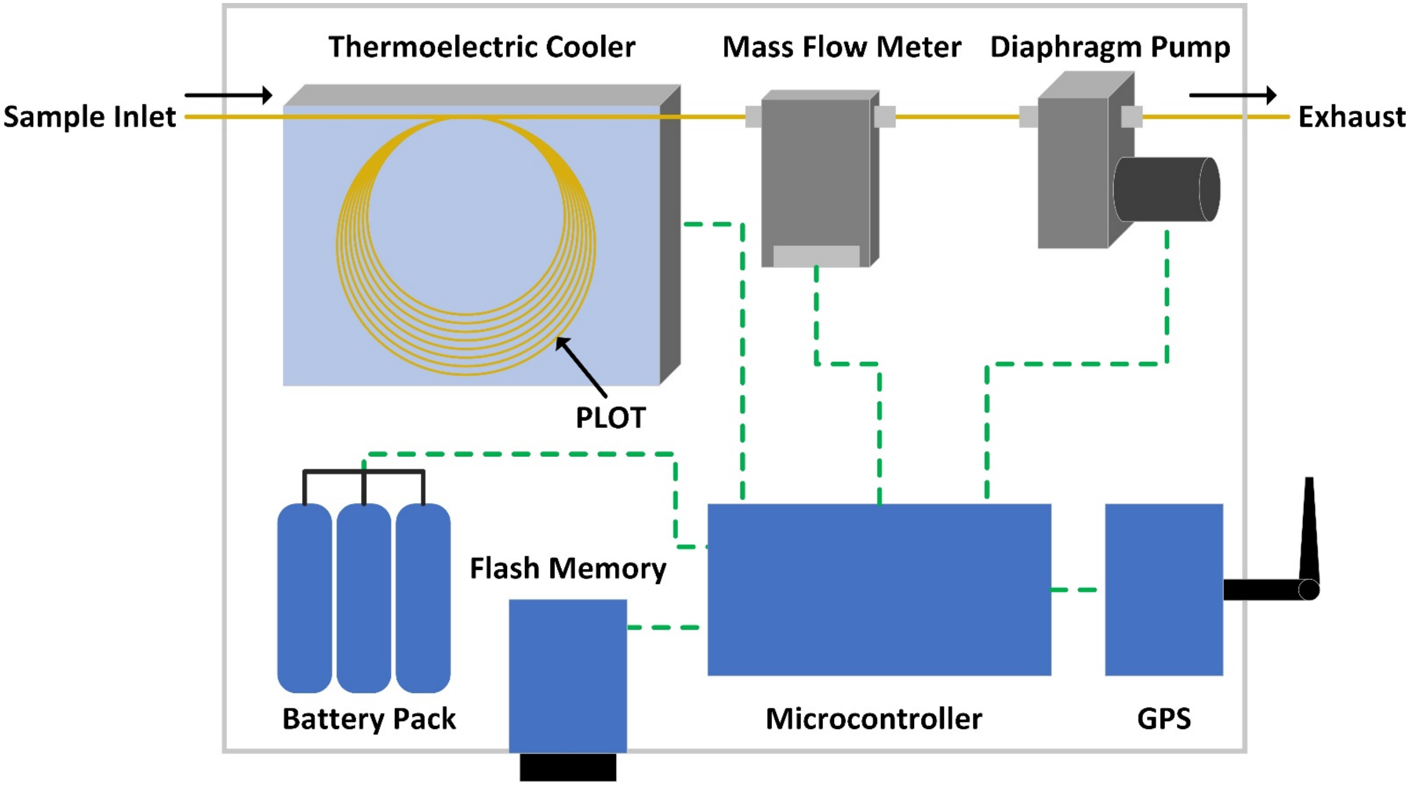
- Strong bunker background signal: degrading polyurethane foam insulation
- Higher temps eased detection
- Humidity > 90% increased sampling time; humid compressed air caused interruption to refrigeration
- Diethyl phthalate (lowest-volatility compound)
- Gasoline in 3 seconds
- **Costs of compressed air power outweigh the benefits**

Harries. M. and Bruno, T.J. Field demonstration of portable vapor sampling in a simulated cargo container. Forensic Chemistry 2019, 16, 100182.

Redesigned portable device



- Battery-operated design is lighter and more portable
- Better flow rate and temperature control
- Thermoelectric cooler capable of -20 °C eliminates heavy/loud vortex tube
- PWM-diaphragm pump provides a range of flow rates and eliminates the vacuum generator
- Advanced data logging capabilities
- Redesign capillary wafer materials for better heat transfer



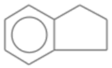
Stability of DVME samples over time

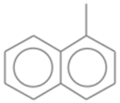
Effect of storage on collected vapor samples

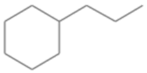
Methods

- Sample: 50% weathered gasoline – range of volatilities and carbon classes
- Storage: heat-sealed Kapak/Ampac evidence bags (polyester/PE) @ room temp
- Long-term study: 0, 6, 13, 20 weeks


toluene


indane

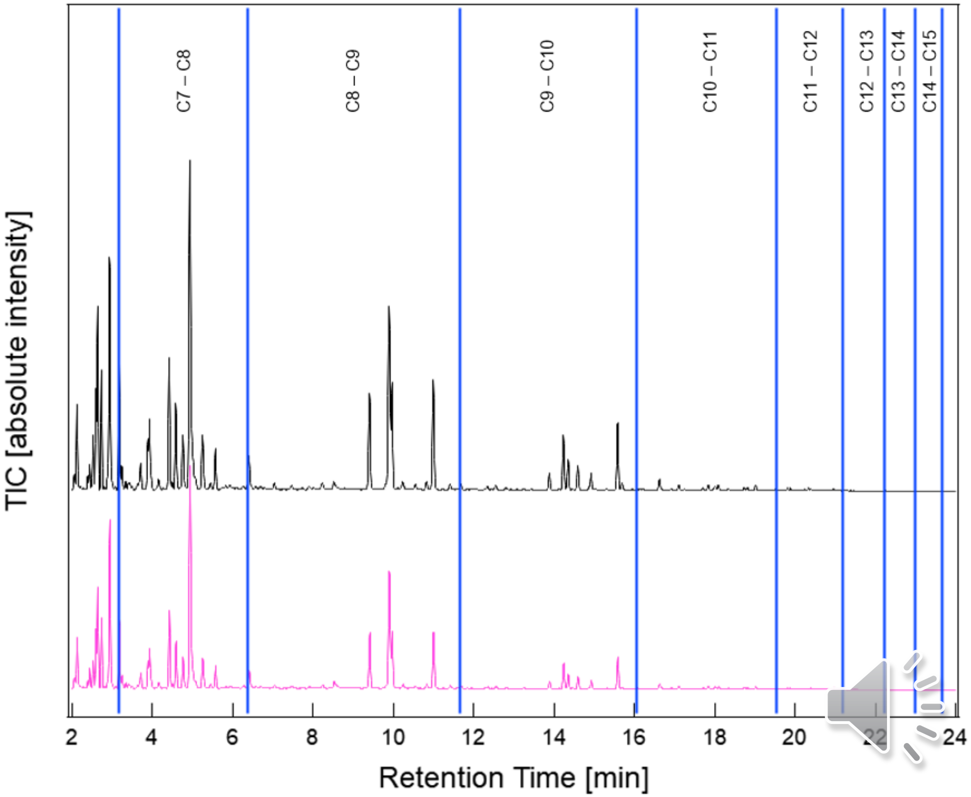

1-methylnaphthalene


propylcyclohexane


dodecane

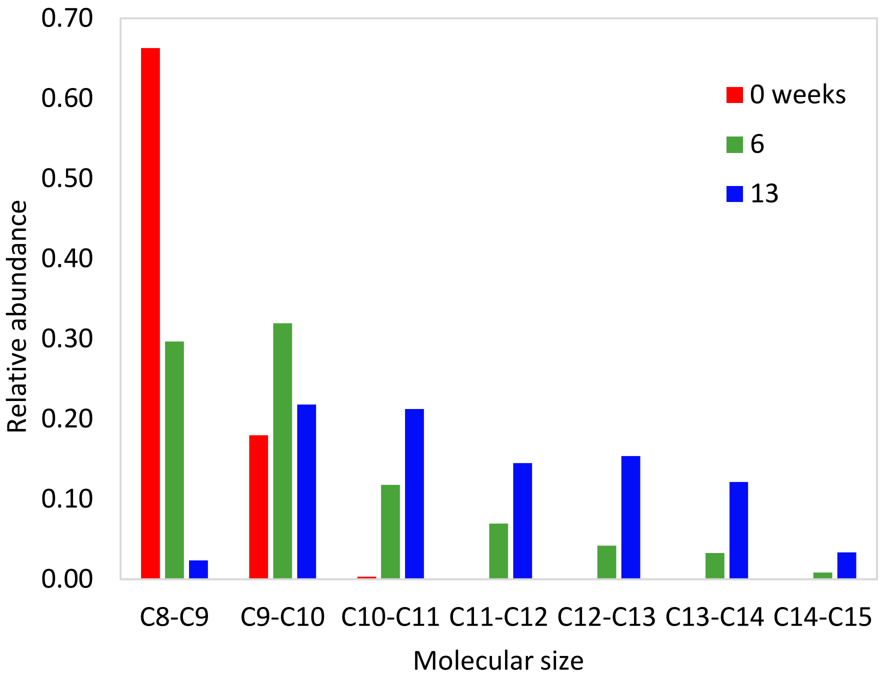
GC-MS Analysis

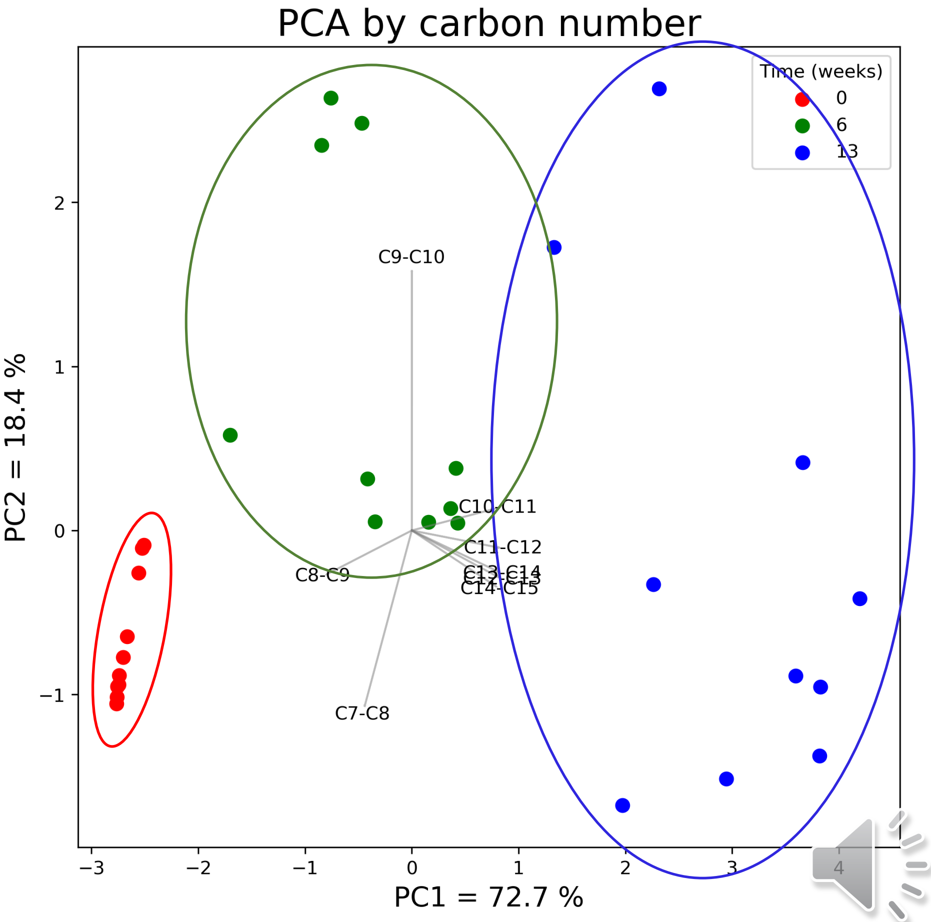
- Carbon number distribution
- Compare carbon class distribution (17 SIM ions)
- Principal Component Analysis (PCA)
- Total usable signal



Analysis by carbon number

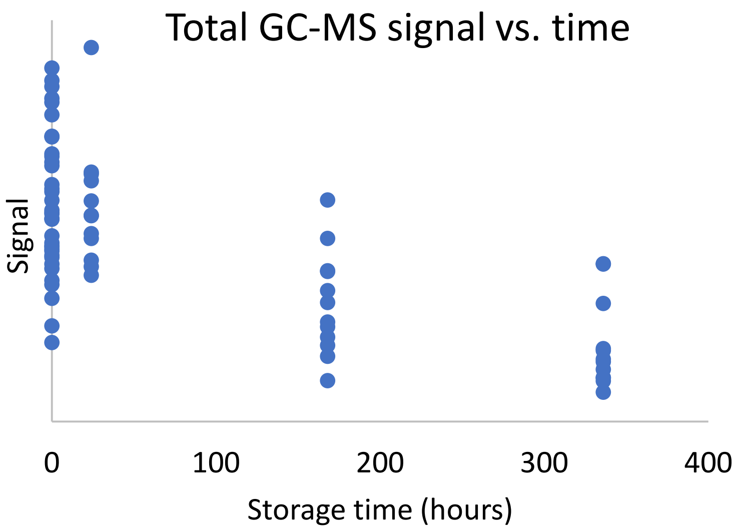
- Stability strongly affected by molecular size/volatility
- Clear influence of storage interval
- Dramatic changes in 6 weeks led to design of short-term study

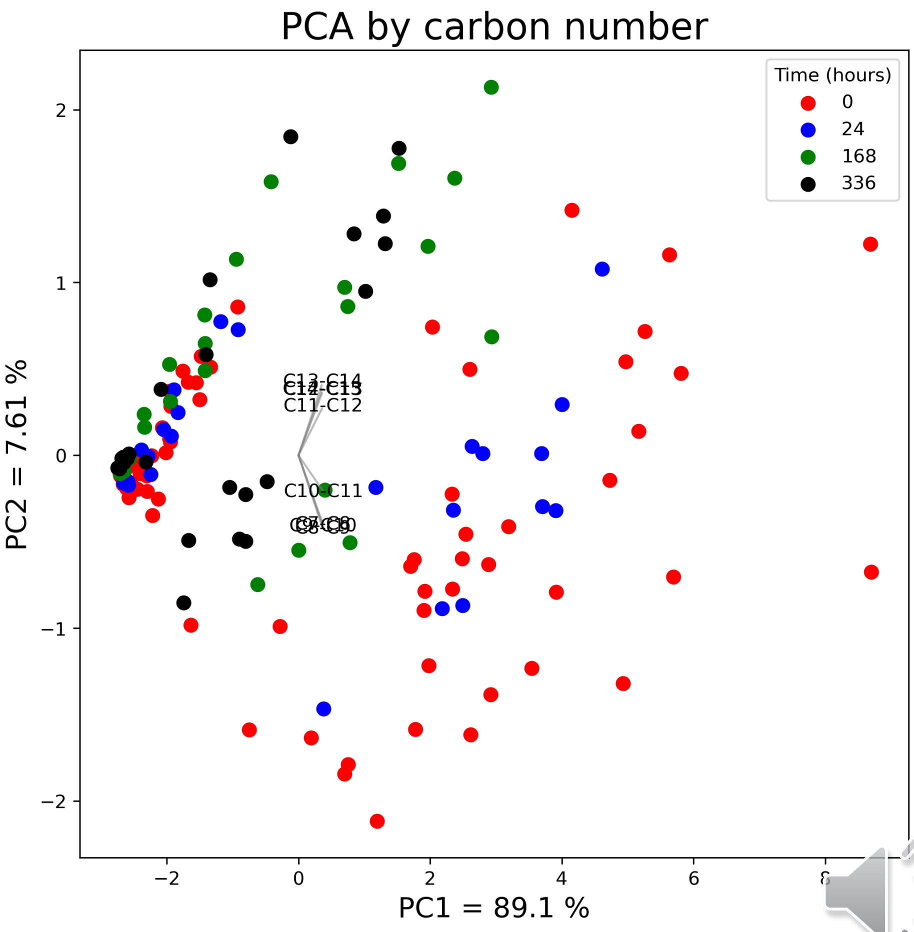




Analysis by carbon number: short term study

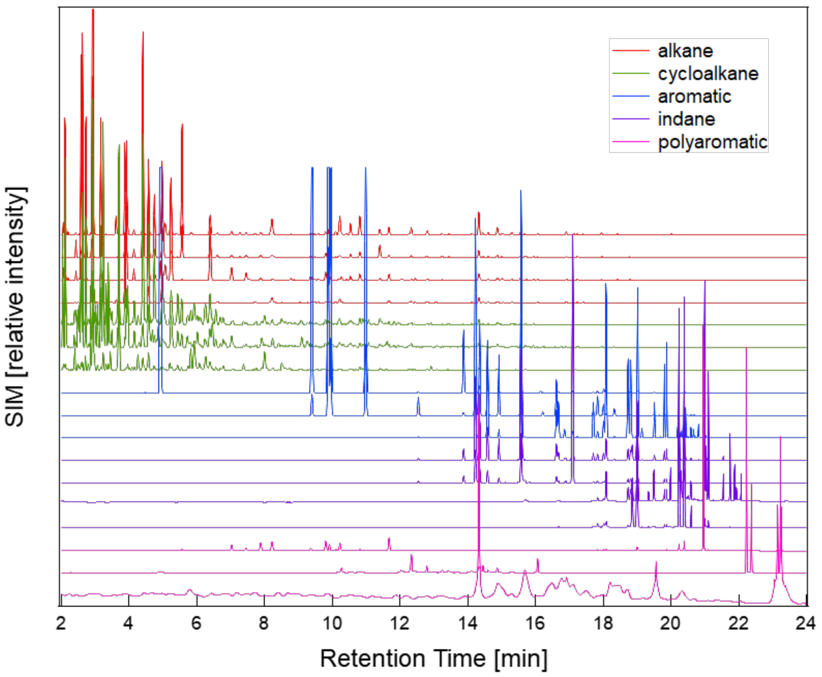
- Time points: 0, 24, 168, 336 hours
- High explanation of variance and high scatter
- Decreases in total GC-MS signal were observed with time
- TIC analysis

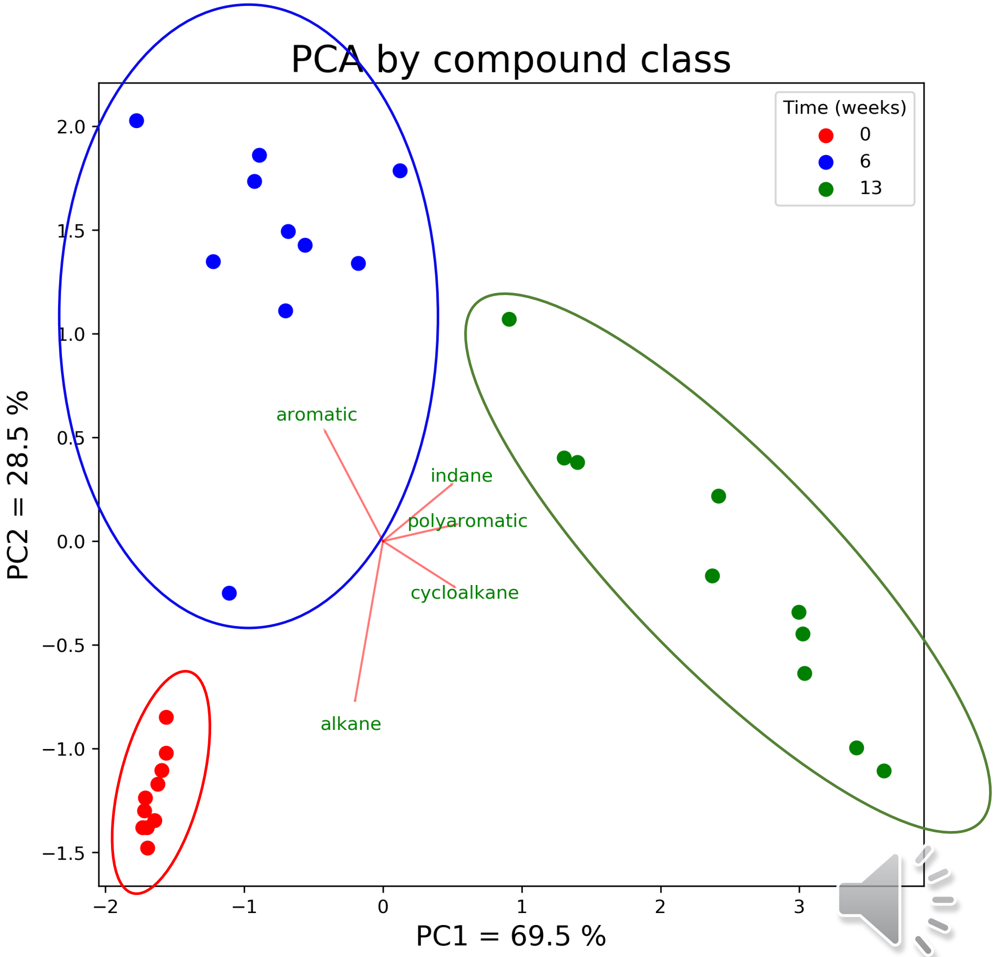




Analysis by hydrocarbon class

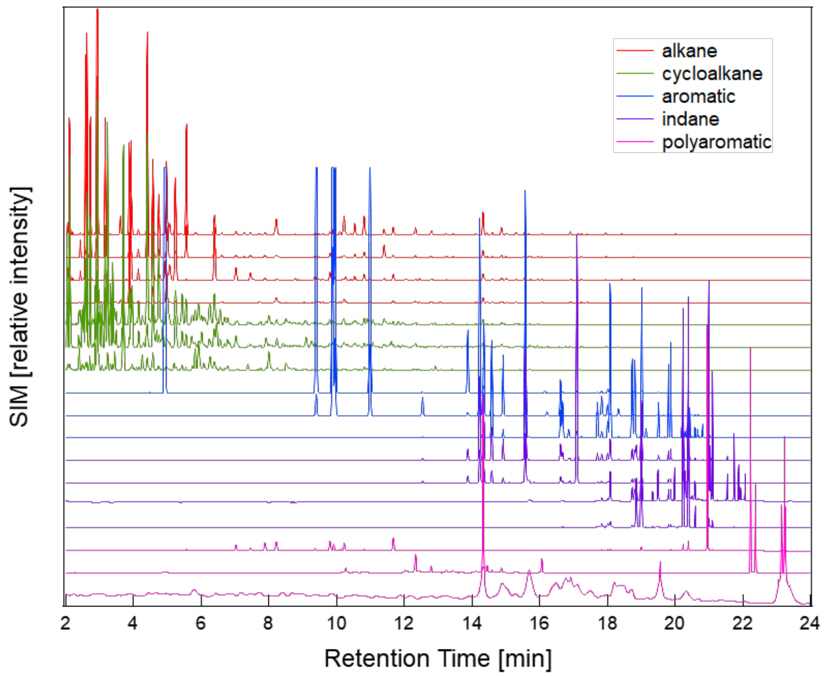
- SIM ions: 57, 71, 85, 99, 55, 69, 83, 91, 105, 119, 117, 118, 131, 132, 128, 142, 156 m/z
- Scatter within time points increases with storage time
- 13 wk shift toward polycyclics is consistent with carbon number analysis

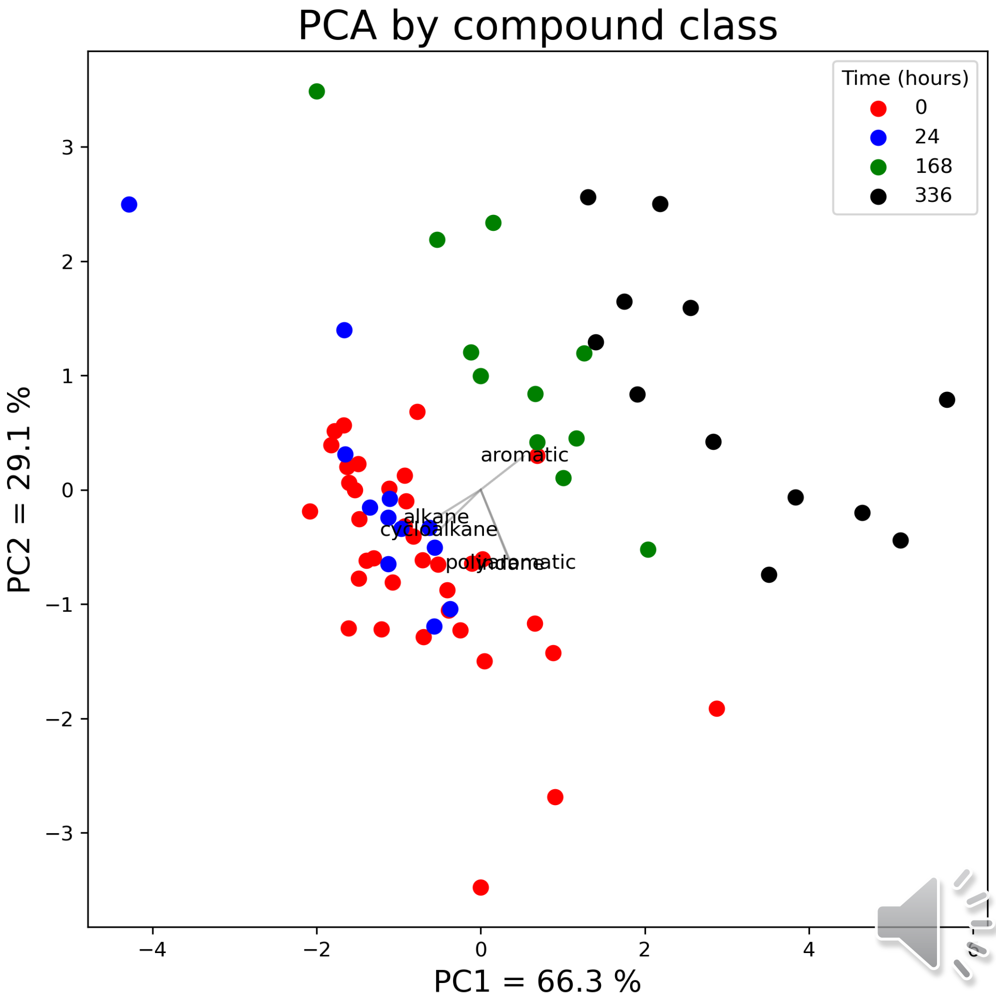




Analysis by hydrocarbon class: short term study

- Changes not observed in 24 hours; shifts emerge after 168 hours
- This analysis is more sensitive to changes than the carbon number analysis





Conclusions + future work

- Dynamic vapor microextraction (DVME) can measure VP of low-volatility, reactive species with unprecedented uncertainty < 2 %
- Future measurements of cannabis compounds will provide a foundation for developing cannabis breath collection devices
- Field deployment of NIST device, while successful, indicated areas for improvement incorporated into 2nd generation design

- PCA provides a method to reduce the dimensionality of chromatographic data by analysis of compound class (SIM) or carbon number (TIC)
- Samples were stable after 24 h of room temperature storage, but low MWs are lost after storage ≥ 7 days
- These methods can be applied when vapor samples are collected in a field setting and preserved until analysis.

- **Confidence in sample stability and property data are both important considerations in field sampling of vapors**

Thanks

Adam J. Broerman

Dr. Thomas J. Bruno

NIST Special Programs Office

National Institute of Justice

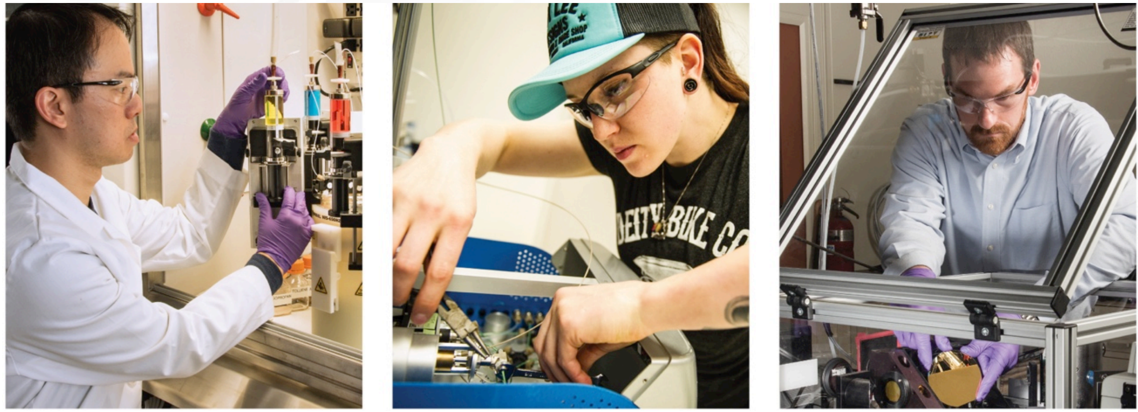
National Research Council Fellowship*

Contact

megan.harries@nist.gov

*Apply for an NRC postdoc:

https://sites.nationalacademies.org/PGA/RAP/index.htm



Bulk and Micro-Scale Trace Element Analysis of Glass SRMs Using Modern Nuclear Analytical Methods and LA-ICP-MS

Nicholas Sharp[1], Jamie L. Weaver[1], Maria Vega Martinez[1], Ruthmara Corzo[1], Rick Paul[1], Jose Almirall[2], and Eric Steel[1]

1 National Institute of Standards and Technology

2 Florida International University

NIST
National Institute of Standards and Technology
U.S. Department of Commerce

NIST FORENSIC SCIENCES

MATERIAL
MEASUREMENT
LABORATORY

Outline

1) Background
2) Project Goals
3) Creating a new Standard Reference Material
4) Laser Ablation Inductively-Coupled Plasma Mass Spectrometry Overview
5) Neutron Activation Analysis Overview
6) Preliminary comparison of LA-ICP-MS and NAA of SRMs 610, 614, and 1831

BACKGROUND

NIST is the USA’s metrology lab and is responsible for providing traceable Standard Reference Materials (SRMs) to the US community

Analysis of glass samples is done in labs all around the country

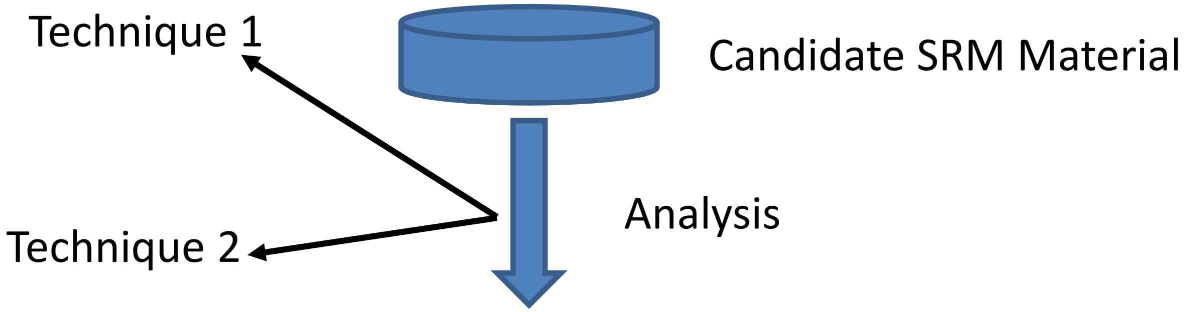
NIST Center for Neutron Research (NCNR)

AML Complex[1]

1: Clark Construction Group, LLC

PROJECT GOALS

- Analysis of glass fragments relies on the use of NIST-SRMs for **instrument calibration** (LA-ICP-MS)
  - *Many trace elements are measured which **have not** been assigned certified, reference and/or information values*
  - *No glass SRMs were designed for micro-scale analyses*
- This project aims to address this gap by:
  1. *<u>Quantifying</u> Trace Element Concentration on a bulk and microscopic levels*
  2. *Determining level of <u>heterogeneity</u> of the trace elements in the glasses on the bulk and microscopic levels*




National Institute of Standards & Technology

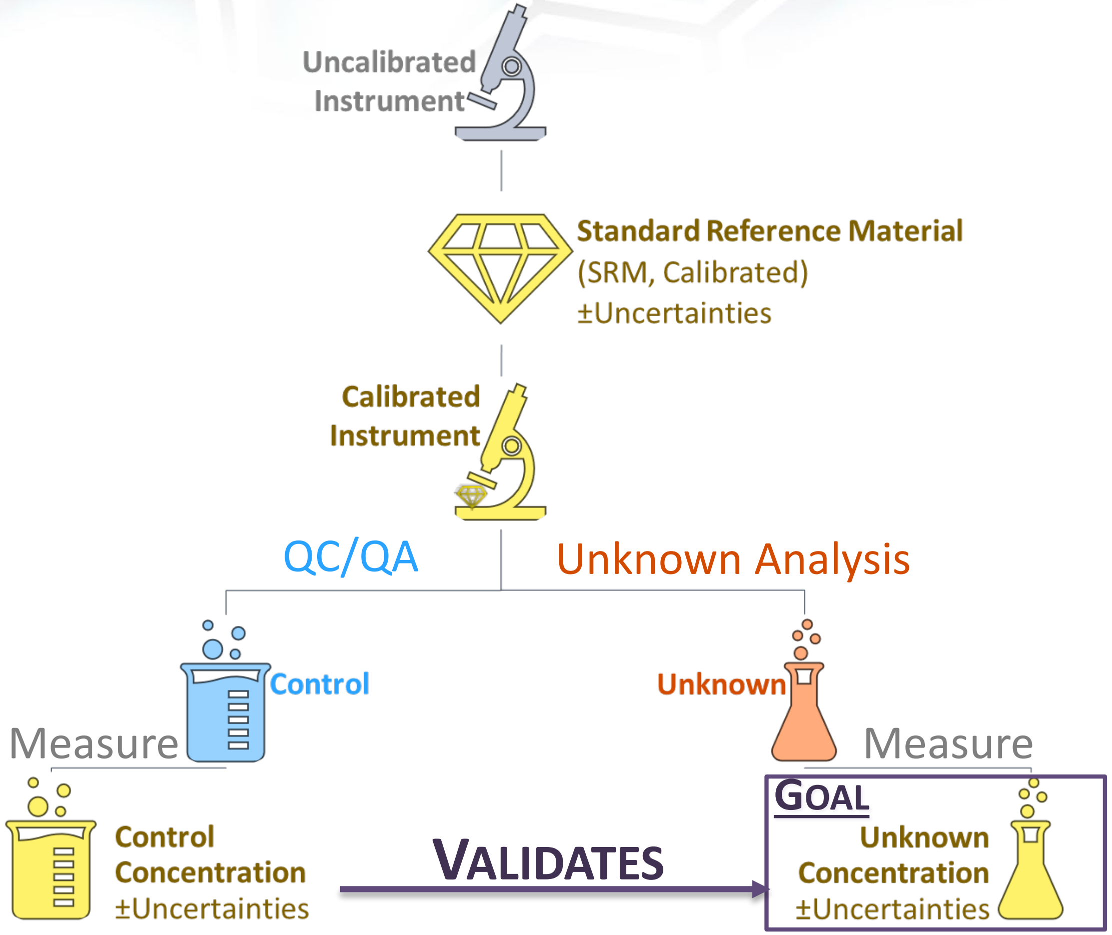
Certificate of Analysis

Standard Reference Material® 612

Trace Elements in Glass

WHY WORRY ABOUT STANDARDS?

- Standards define the minimal level of uncertainty a measurement can achieve
- Other SRMs can be used as quality control checks
    - Using an SRM outside of its CoA parameters, or for elements that are not certified, can give false positives or negatives
    - False QC checks can cost laboratories $$$ and hurt trustworthiness

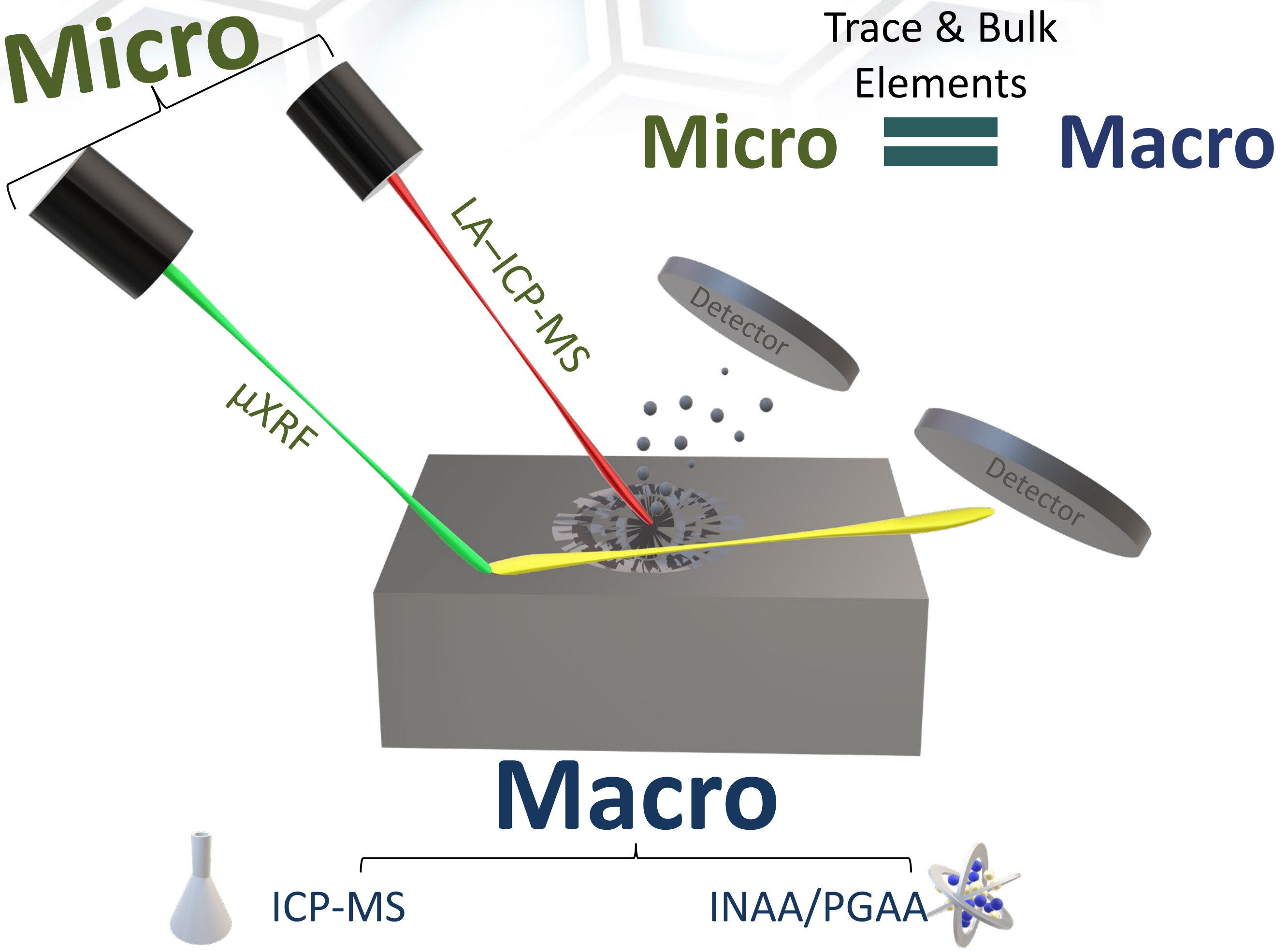


NEW FORENSIC GLASS SRM

Goal is to combine bulk and micro methods

Primary methods are required for element certification

Initial characterization with LA-ICP-MS for forensic purposes and NAA for bulk non-destructive analyses



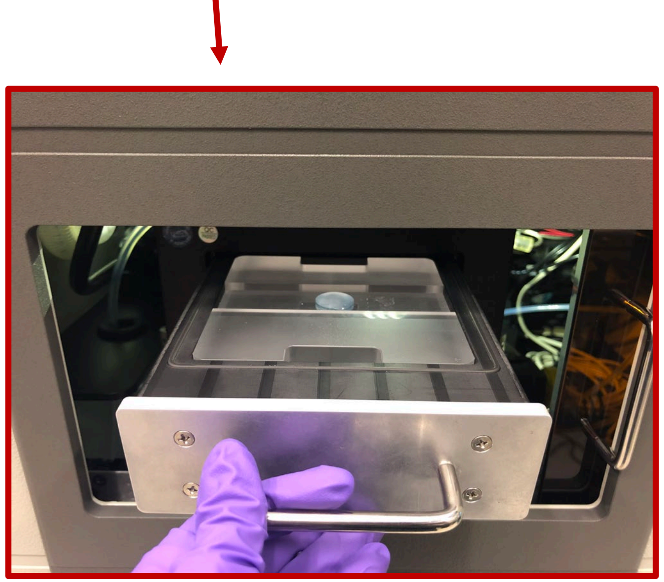
LA-ICP-MS

Laser

- Applied Spectra* J200 LA
- 213 nm Nd:YAG laser
- Analysis parameters: 100 % energy (2.73 mJ), 10 Hz, 100 µm spot size, 60 second dwell, 0.9 L/min carrier gas (Helium) flow rate

ICP-MS

- Thermo iCAP Q ICP-MS
- Single-point calibration using FGS2 as the calibration standard (with drift correction)
- Analysis parameters: 1500 W plasma power, 0.7 L/min nebulizer gas (Argon) flow rate, 10 to 15 ms dwell (depending on element)



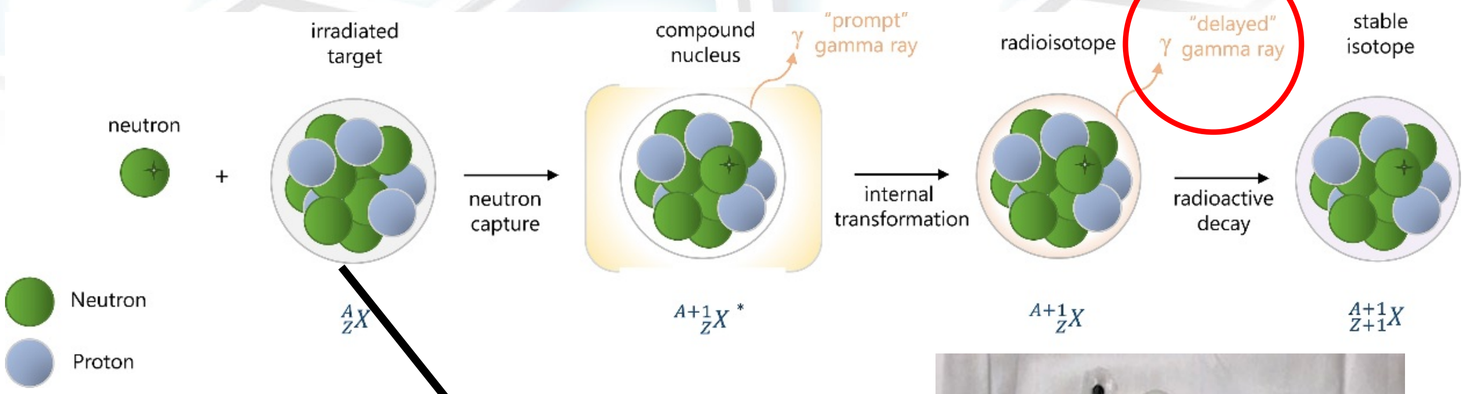
*Any mention of commercial products is for information only; it does not imply recommendation or endorsement by NIST.

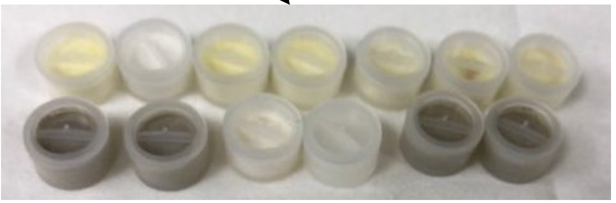
NAA OVERVIEW

A neutron is captured by the analyte nucleus

The analyte nucleus is converted into a radioactive isotope of the initial element

The gamma-rays emitted from the decay of the radioisotope are measured and the analyte is quantified



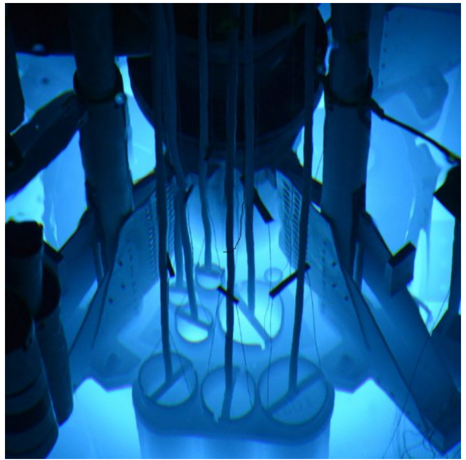


Rabbit

Also Rabbit

IRRADIATION PROCESS

Neutrons



MURR reactor core
copyright Steve Morris

Loaded Rabbit

PRELIMINARY DATA

- Excellent agreement in 610
- 614 has lower concentration of elements, beginning to see heterogeneity issues
- 1831 was not certified for anything outside of weight % elements

	610 (LA=38, NAA=4)		614 (LA=5, NAA=4)		1831 (LA=38, NAA=4)	
	Conc (mg/kg)	En score (LA vs NAA)	Conc (mg/kg)	En score (LA vs NAA)	Conc (mg/kg)	En score (LA vs NAA)
Rb	425.7	0.001	0.855	N/A	(6.11)	-1.40
La	(440)	0.008	0.83*	-6.58	(2.12)	-1.77
Ce	(453)	-0.001	(0.813)	-1.33	(4.54)	-1.47
Hf	(435)	0.002	(0.711)	-5.15	(1.1)	-0.56
Cr	415	0.001	(1.19)	1.09	(1.71)	N/A
Sb	415.3	0.000	1.06*	-1.76	(0.26)	N/A
Co	390*	0.011	0.73*	-1.56	(0.241)	N/A
Zn	433*	0.006	(2.79)	-0.85	(7.90)	N/A
W	(444)	0.004	(0.806)	N/A	N/A	N/A
Th	457.2	0.003	0.748	N/A	N/A	N/A

- *: Not certified in SRM CoA
- N/A: not analyzed
- (): “consensus” values from literature, GeoREM, and this research

En-score from ISO 13528

En > |1| is not a match according to ASTM 2927 (±4σ or ±4*3%)

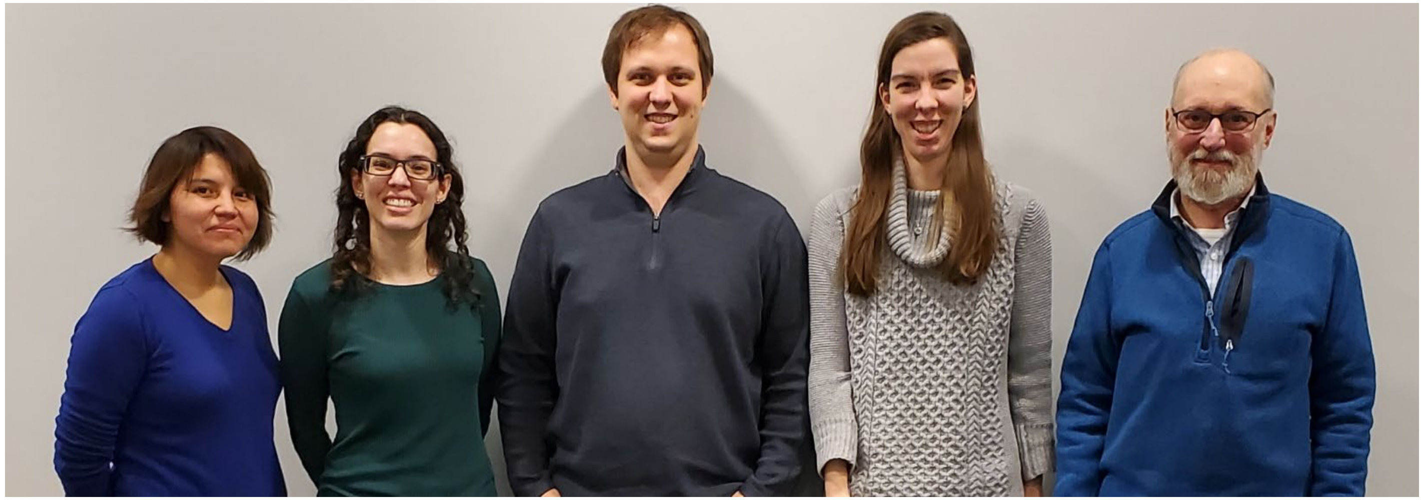
CONCLUSIONS

The forensic glass examination community is currently using a calibration standard (FGS 1 and 2) designed for microsampling for use in ASTM E2927-16e1 with demonstrated success and predictable bias and precision performance

The existing NIST 1831, 1830, 610-4 SRMs were not designed for micro-sampling and some elements of interest to forensic glass examiners are not certified and show heterogeneity issues

New calibration standards that are designed for micro sampling have been created and the process to certify them as Standard Reference Materials (SRMs) suitable for micro-analyses by techniques such as LA-ICP-MS is currently being explored

An interlaboratory series of exercises has been funded by NIJ and are being coordinated at FIU to evaluate the utility of the new standards for forensic glass analysis



GLASS FORENSICS TEAM

Left to right: Maria Vega, Ruthmara Corzo, Nick Sharp, Jamie Weaver, and Eric Steel

Not pictured: Pam Chu and Jose Almirall

Funding acknowledgments:

Funding for this project was provided through NIST Forensics (Special Programs Office).

Also through NIJ announcement # NIJ-2018-13600 - April 23rd, 2018

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An Interlaboratory Study Evaluating the Interpretation of Forensic Glass Evidence Using Refractive Index Measurements and Elemental Composition

- Ruthmara Corzo
- Materials Measurement Science Division

NIST
National Institute of Standards and Technology
U.S. Department of Commerce

MATERIAL
MEASUREMENT
LABORATORY

Glass as Forensic Evidence

- Ubiquity of glass makes it a commonly encountered type of forensic evidence in crime scenes (hit-and-runs, burglaries)
  - Soda-lime float glass (automotive and architectural) is the most common type of glass encountered in crime scenes

https://www.officeworks.com

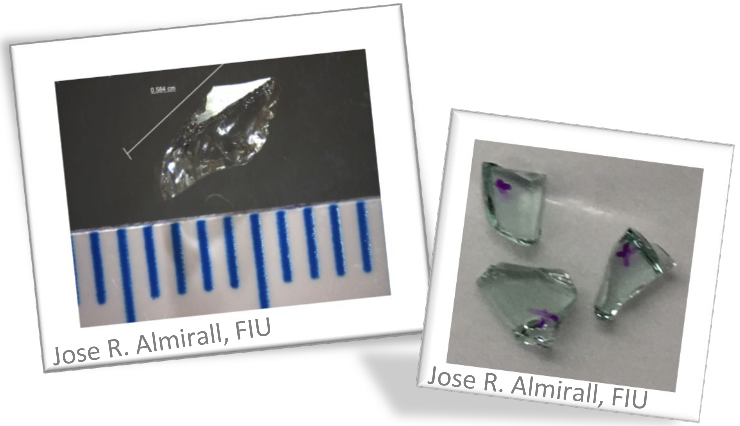
https://www.packagingstrategies.com

https://www.groupon.com/deals/low-cost-auto-glass-1-5

https://www.cmog.org/article/staying-line-coated-glass

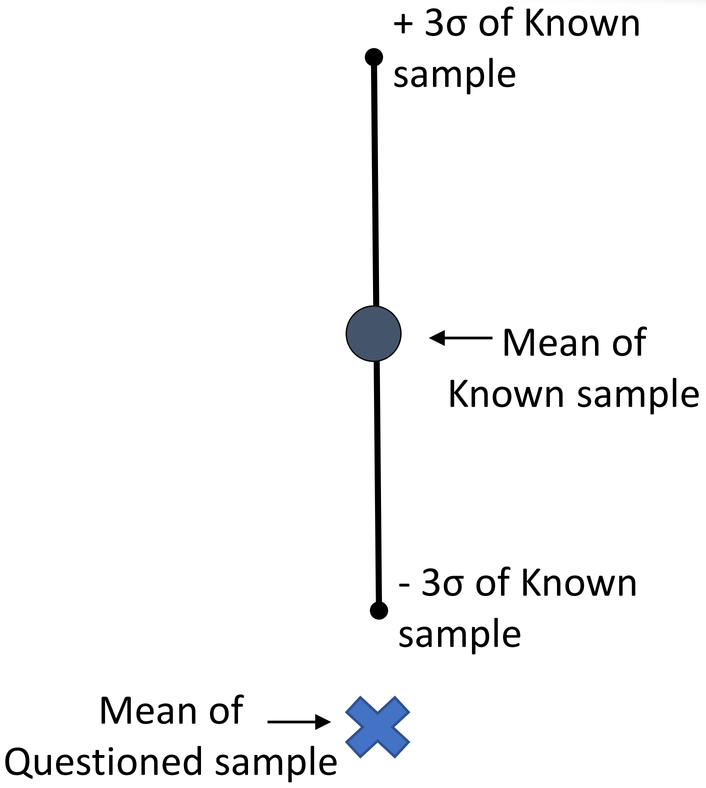
Casework Comparison of Glass Fragments

- Refractive index measurements and/or elemental analysis is conducted on Known sample and Questioned sample
    - Physicochemical properties of the K and Q are compared to determine if the Q sample could have originated from the K source
- Standard methods for techniques used in casework:
    - ASTM E2926: Micro X-ray Fluorescence Spectrometry (μXRF)
    - ASTM E2927: Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS)
    - ASTM 1967: Refractive Index
- No standard method for Laser Induced Breakdown Spectroscopy (LIBS)
- Hypothesis testing (match criterion) is used to compare the Known and Questioned samples



Jose R. Almirall, FIU

Jose R. Almirall, FIU



+ 3σ of Known sample

Mean of Known sample

- 3σ of Known sample

Mean of Questioned sample

Evidence Interpretation

- **Verbal Scale**
  - Includes: Physical Match, Association, Exclusion, and Inconclusive
  - Multiple levels for an Association
    - Level of Association depends on discrimination power of technique used and the presence of usual characteristics in glass specimens
- **Numerical Approaches (Require a Database)**
  - ***Random Match Probability (RMP)***: estimated by false inclusion rate
  - ***Frequency of Occurrence:*** Known sample is compared to every sample in database and the number of matches is reported
  - ***Likelihood Ratio:*** continuous approach that compares the probability of the evidence given two competing hypotheses (Association vs. Exclusion)

Jose R. Almirall, FIU

Jose R. Almirall, FIU

Glass Interpretation Working Group (GIWG)

- Interlaboratory study led by Dr. Almirall group at Florida International University (FIU)
- Three exercises designed as a mock forensic case
  - Blind samples from vehicle windshield
- Seventeen participating laboratories
  - µXRF: 7 participants
  - LIBS: 5 participants
  - Refractive Index: 11 participants
- Objectives:
  - Evaluate the significance of a match
  - Collect and evaluate existing glass databases for interpretative purposes
  - Assess statistical models for the numerical evaluation of glass evidence
  - Establish a common reporting language
  - Develop new float glass standards (Corning Inc.)

R. Corzo, et al. *Forensic Chemistry*, **2020** *submitted.* R. Corzo, et al. *Talanta*, **2018** (186) 655-651. T. Hoffman, et al. *Forensic Chemistry*, **2018** (11) 65-76.

Interlaboratory Exercise 1

Sample	Make	Model	Year	Remarks
$\mathbf{K1}_{\text{inner \& outer}}$	Mitsubishi	Galant	2009	Same make/model/year as Q1
$\mathbf{K2}_{\text{inner \& outer}}$	Subaru	Impreza	2008	Outer pane same as Q2
$\mathbf{Q1}_{\text{outer}}$	Mitsubishi	Galant	2009	Same make/model/year as K1
$\mathbf{Q2}_{\text{outer}}$	Subaru	Impreza	2008	Same as K2 outer

- Participants received **2 Known** samples and **2 Questioned** samples (3 fragments for each source)
    - K2/Q2_{outer} originated from the same source
    - K1/Q2 and K2/Q1 originated from different sources (easily distinguished by elemental composition)
    - K1/Q1 originated from similar sources (difficult to distinguish by elemental composition)

R. Corzo, et al. *Forensic Chemistry*, **2020** *submitted.*

Exercise 1 Results

Technique	False Exclusion (%)	False Inclusion (%)
µXRF (range overlap, ± 3s)	0	8.3 %
LIBS (varied match criteria)	0	16.7 %
Refractive Index (varied match criteria)	0	34.1 %

- For µXRF and LIBS, all false inclusions were comparisons between glass from similar vehicles (same make/year)
- Refractive index was unable to distinguish glass from vehicles with different make/year
- Refractive index provided complementarity to elemental techniques
    - Most labs found reproducible differences between glass from similar vehicles

R. Corzo, et al. *Forensic Chemistry*, **2020** *submitted.*

Exercise 1 Findings

- Reporting language varied between labs (need for standardization)
- Most labs simply stated whether K and Q were “Associated” or “Excluded”
  - “The Questioned sample originated from the Known source, or another source with the same characteristics as the Known source”
- Few labs provided a numerical interpretation and/or verbal scale
  - µXRF: verbal scale (one lab)
  - Refractive Index: frequency (one lab)
  - LIBS: none
- Refractive index can provide complementary information to elemental techniques

R. Corzo, et al. *Forensic Chemistry*, **2020** *submitted.*

Interlaboratory Exercises 2 and 3

Exercise 2

Sample	Make	Model	Year	Remarks
K1$_{\text{inner \& outer}}$	Honda	Civic	2006	K1 same as Q1
Q1$_{\text{inner \& outer}}$	Honda	Civic	2006	2 fragments same as K1 inner, 1 fragment same as K1 outer
Q2$_{\text{outer}}$	BMW	2 Series	2014	Different than K1

Exercise 3

Sample	Make	Model	Year	Remarks
K1$_{\text{inner \& outer}}$	Honda	Civic	2007	K1 outer same as Q1
Q1$_{\text{outer}}$	Honda	Civic	2007	Q1 same as K1 outer
Q2$_{\text{outer}}$	Mercedes	R-Class	2009	Different than K1

R. Corzo, et al. *Forensic Chemistry*, **2020** *submitted.*

Interlaboratory Exercises 2 and 3 Results

Exercise 2

Technique	False Exclusion (%)	False Inclusion (%)
µXRF (range overlap, ± 3s)	16.7 %	0
LIBS (varied match criteria)	0	0
Refractive Index (varied match criteria)	0	0

Exercise 3

Technique	False Exclusion (%)	False Inclusion (%)
µXRF (range overlap, ± 3s)	0	0
LIBS (varied match criteria)	0	0
Refractive Index (varied match criteria)	0	0

R. Corzo, et al. *Forensic Chemistry*, **2020** *submitted.*

Exercises 2 and 3 Findings

- More labs used verbal scales and/or numerical interpretation approaches
  - XRF: frequency (one lab), verbal scale (two labs)
  - LIBS: verbal scale (one lab)
  - Refractive Index: frequency (three labs), LR (one lab)
- For Exercise 2, 6/17 labs increased support for an association because of the presence of Q specimens that matched both K panes
- Potential for sharing XRF glass databases was evaluated (using element ratios)
  - High false exclusion rate between labs (100% false exclusion)
  - Normalizing to SRM 1831 improved results, but false exclusion rate was still unacceptable (77%)
- Poor precision observed for LIBS
  - 2 labs excluded element ratios with RSDs > 20 %

R. Corzo, et al. *Forensic Chemistry*, **2020** *submitted.*

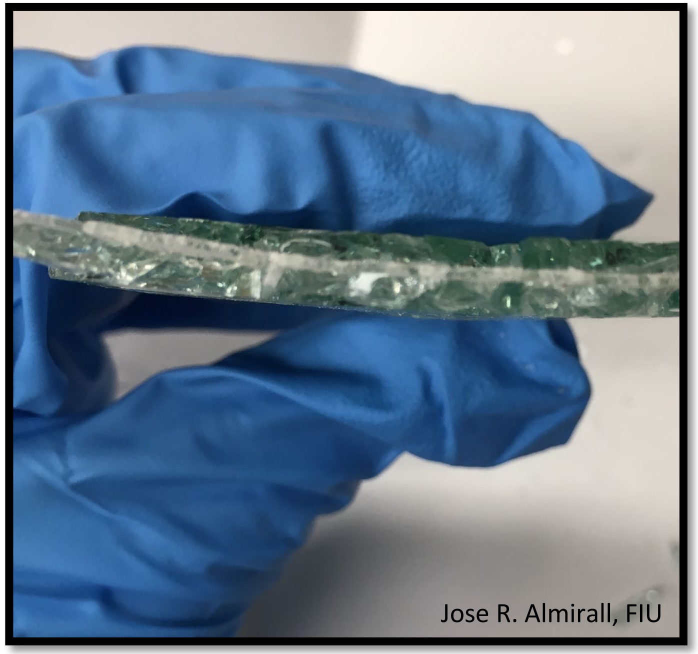
GIWG Overall Findings

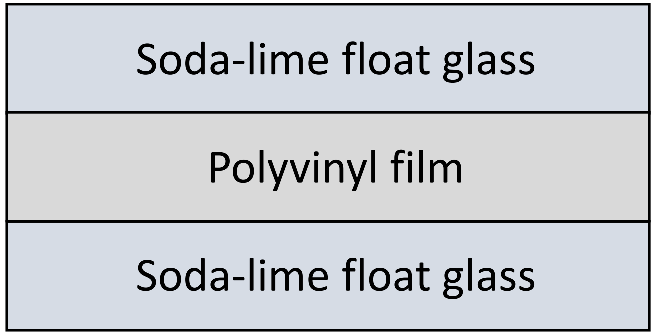
- > 92 % correct association for all techniques
- 82 %, 86 %, and 96 % discrimination for refractive index, LIBS, and µXRF, respectively
- Few labs use a verbal scale and/or numerical interpretation approaches
  - *“The Q originated from K source or another glass source with the same characteristics as the K”*
- **µXRF**
  - Development of shared database remains a challenge (database comprised of Known specimens may be feasible)
  - Quantitative database would be ideal (potential to combine XRF and LA-ICP-MS databases)
- **LIBS**
  - Poor precision needs to be addressed
  - Need for standardized methodology (different match criteria used between labs)
- **Refractive Index**
  - Available databases, but underutilized in forensic community (3/11 labs used a database to calculate frequency)
  - Can provide additional discrimination to elemental techniques
  - Different match criteria used between labs (updated ASTM method recommends range overlap for comparisons)

R. Corzo, et al. *Forensic Chemistry*, **2020** *submitted.* R. Corzo, et al. *Talanta*, **2018** (186) 655-651. T. Hoffman, et al. *Forensic* Chemistry, **2018** (11) 65-76.

Glass Collection Set for Quantitative XRF Database

- Windshield (laminated) glass from 99 vehicles (198 panes)
- OEM windshields collected from salvage yard
- Modern glass formulations
  - Vehicles were manufactured between 2013-2019
- Quantitative analysis using fundamental parameters method (standardless approach)
  - Use of standards can improve accuracy: three new float glass standards manufactured by Corning

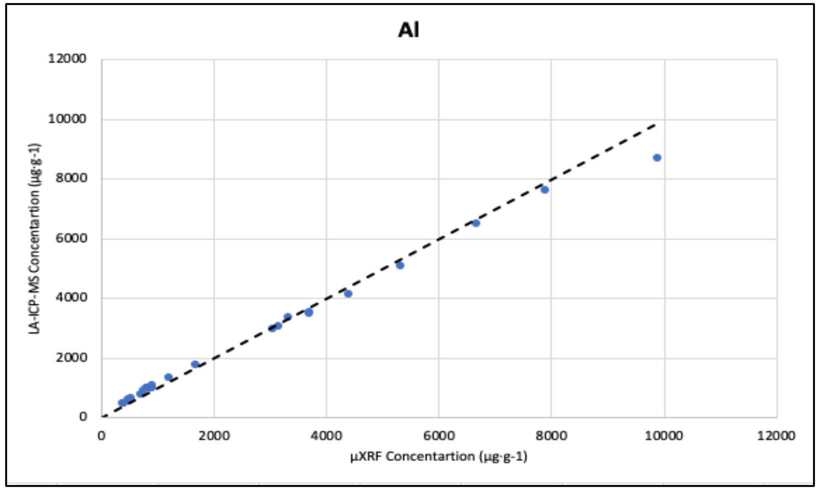

Jose R. Almirall, FIU


Soda-lime float glass

Polyvinyl film

Soda-lime float glass

LA-ICP-MS vs. µXRF for 25 Vehicle Glass Samples



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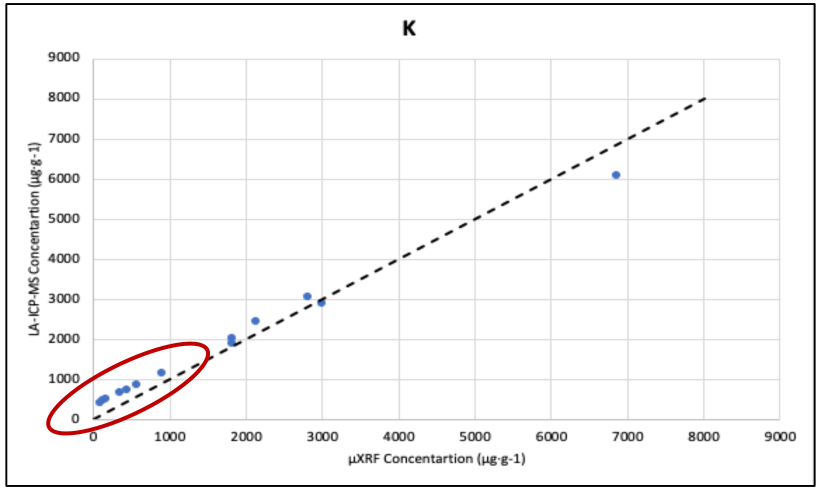
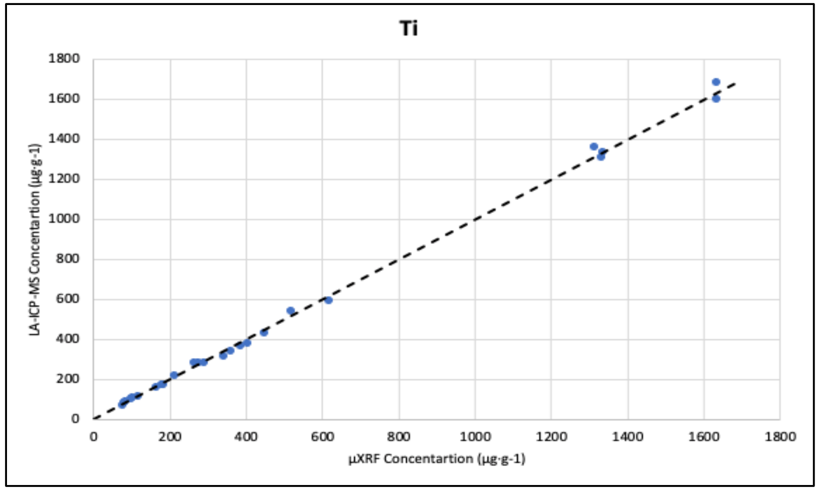


K



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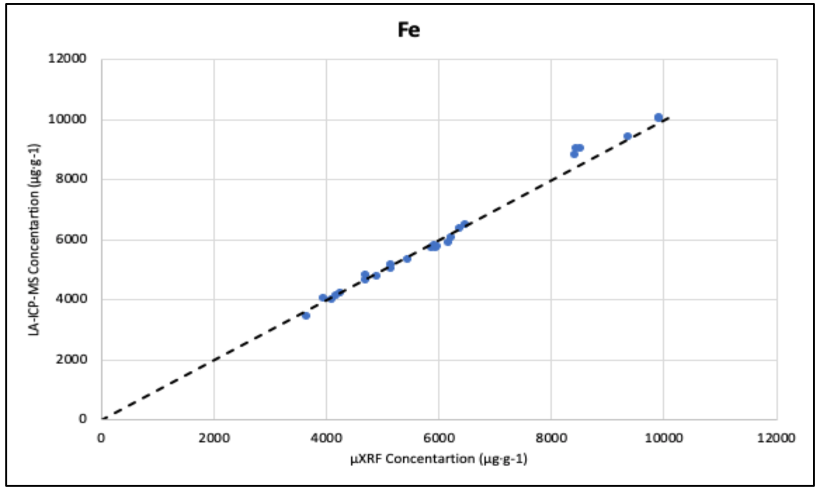

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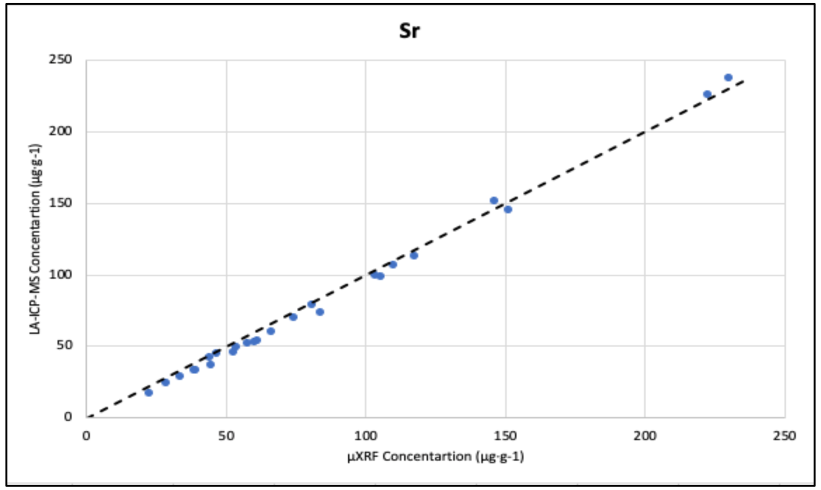

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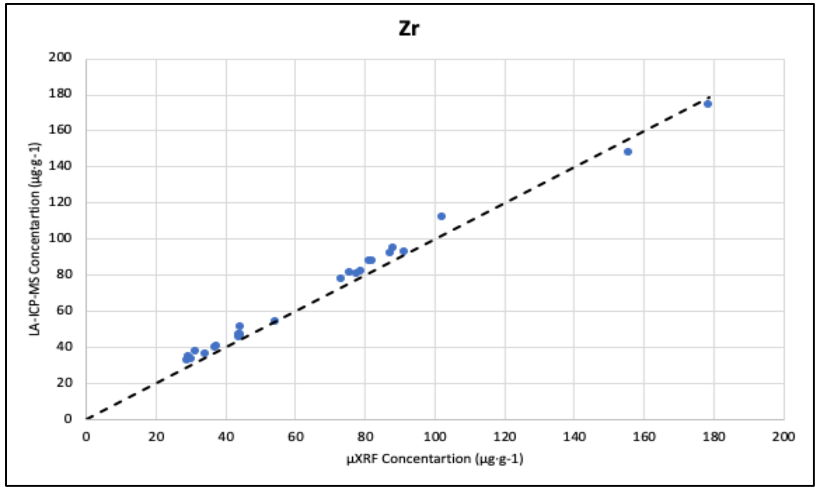


Zr

Conclusions

- Glass Interpretation Working Group (GIWG)
  - Interpretation of evidence varies widely across practitioners
  - Verbal scales and databases are underutilized
    - OSAC interpretation guide that aims to standardize a verbal scale used in trace evidence
- Development of Quantitative µXRF Glass Database
  - Potential to combine µXRF and LA-ICP-MS databases
    - Database needs to be validated to evaluate agreement between µXRF and LA-ICP-MS
  - Additional reference materials and/or other quantitative approaches (e.g., multivariate calibration) can potentially improve results
- **Overall Aims:**
  - **Reach a consensus for the interpretation of glass evidence**
  - **Encourage practitioners to use more objective methods to evidence interpretation**

Acknowledgements

- GIWG led by Dr. Jose Almirall at Florida International University (FIU)
- Funding to FIU:
  - NIJ: awards 2015-DN-BX-K049 and 2018-DU-BX-0194
  - NSF: award 1739805
- New float glass standards manufactured by Corning Inc.
- GIWG Participating Labs:
  - *Michigan State Police, Grand Rapids Forensic Laboratory*
  - *West Virginia University*
  - *Florida Department of Law Enforcement*
  - *Institute of Environmental Science and Research*
  - *Bundeskriminalamt*
  - *Centre of Forensic Sciences*
  - *Sacramento County DA’s Office, Laboratory of Forensic Services*
  - *Missouri State Highway Patrol Crime Lab*
  - *Kansas City Police Crime Laboratory*
  - *Microtrace LLC*
  - *Landeskriminalamt Sachsen*
  - *Health Sciences Authority, Singapore*
  - *Minnesota Bureau of Criminal Apprehension*
  - *Tennessee Bureau of Investigation*
  - *Oregon State Police Forensic Laboratory*
  - *Applied Spectra Incorporated*

A Standard Methodology for Analysis of Paint by FTIR

Presenter: Stephanie Watson
Team: William J. Hickling, Kimberly Kha, and LiPiin Sung
Infrastructure Materials Group, Engineering Laboratory

MML Sponsor: Marcela Najarro, MML Forensics Project

November 6, 2020

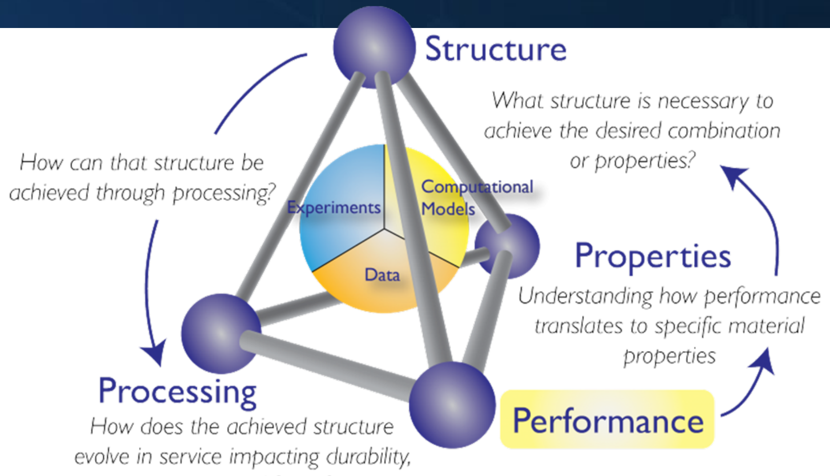
Outline

- Infrastructures Materials Group-Research Focus
- Trace Paint Project Background
- Automotive Coatings
  - Fourier-transform Infrared Spectroscopy (FTIR)
- Standard Set of Spectra for Common Binders
- Conclusions/Future Work

Infrastructure Materials Group –Aron Newman Leader

- **IMG Overview**: Improving infrastructure resilience for acute and chronic hazards requires understanding the characteristics of materials- concrete and polymers.

- Focus on high performance materials related to infrastructure (e.g. polymer insulation of wires, PVC pipes, sealants, fiber reinforced concrete foundations, and PV applications)
  - *Service Life Prediction*
  - *Materials Selection and Assessment*
  - *Metrology development to track degradation of materials and systems*



Structure

What structure is necessary to achieve the desired combination or properties?

Properties

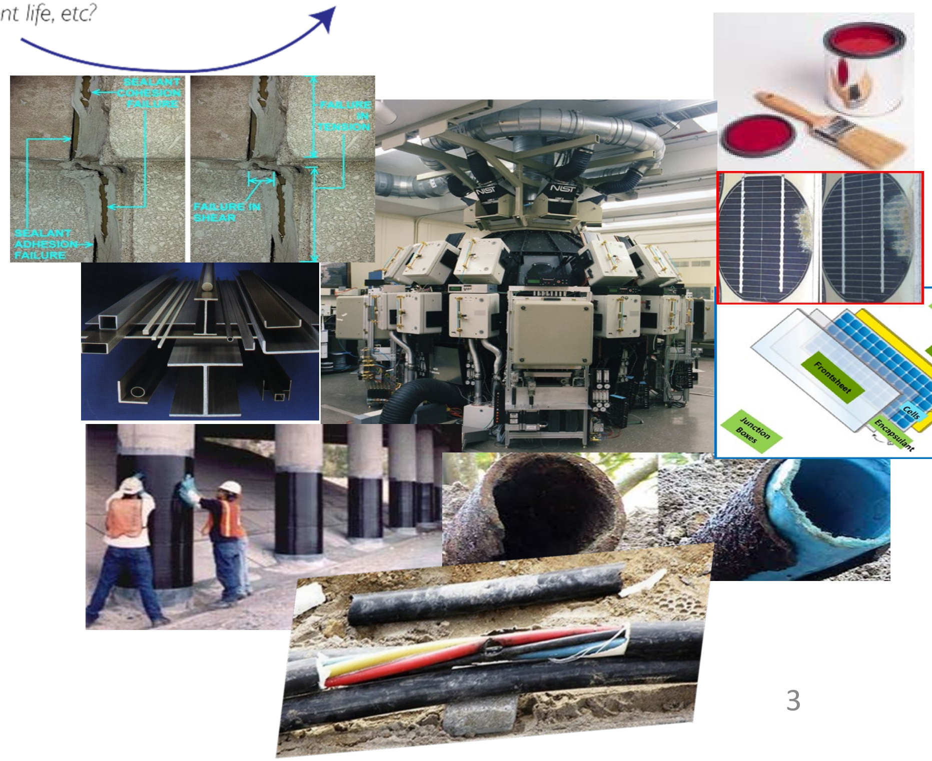
Understanding how performance translates to specific material properties

Performance

Processing

How does the achieved structure evolve in service impacting durability, component life, etc?

How can that structure be achieved through processing?



Trace Evidence-Paint Project Background

- Trace evidence is often the **forensic evidence of last resort** due to cost, time and the inability to predict the efficacy of the evidence
- Trace evidence can help explain crime scenes and identify criminals, but often the results yield a class of evidence
  - In paint evidence, the class may be manufacturer or year of vehicle, rarely an individual vehicle
- Analysis of trace evidence relies on experience of analyst and visual comparisons of materials

Can comparison become more quantitative and rigorous leading to stronger correlations?

Trace Evidence-Paint Project Goals

- Provide **approaches to quantitatively identify and compare** trace evidence materials
- Provide methods, reference materials, and data to **test and support** existing methods and technologies
- Develop rigorous approaches to microanalytical spectroscopies and microscopies to allow for **quantitative comparison** with **calculable uncertainties** on these comparisons
- Combine statistically complex multi-dimensional physical and chemical data into one overarching quantitative comparison with defined uncertainties
- **Engage with the forensics community** to identify research gaps, including review of literature, tour forensics laboratories (FBI-Wright and VA State-Weimer), and participate in appropriate standard committee (OSAC Materials (Trace) Subcommittee)

Trace Evidence-Paint Project Goals - FTIR

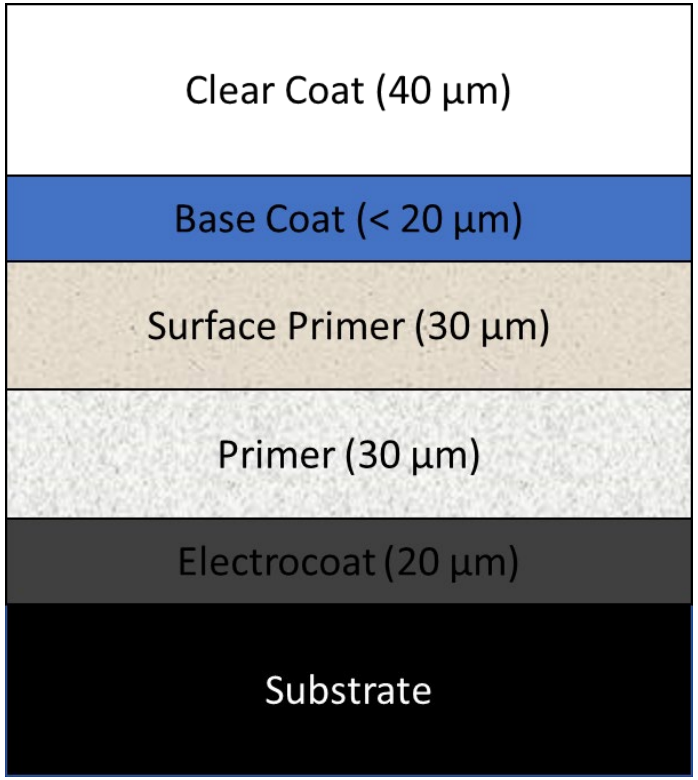
FTIR

Provide standardized guide for identification
    Improving methods
    Decreasing uncertainty
    Increase efficiency, accuracy
Ultimately, striving for individual identification

Automotive Coating Samples

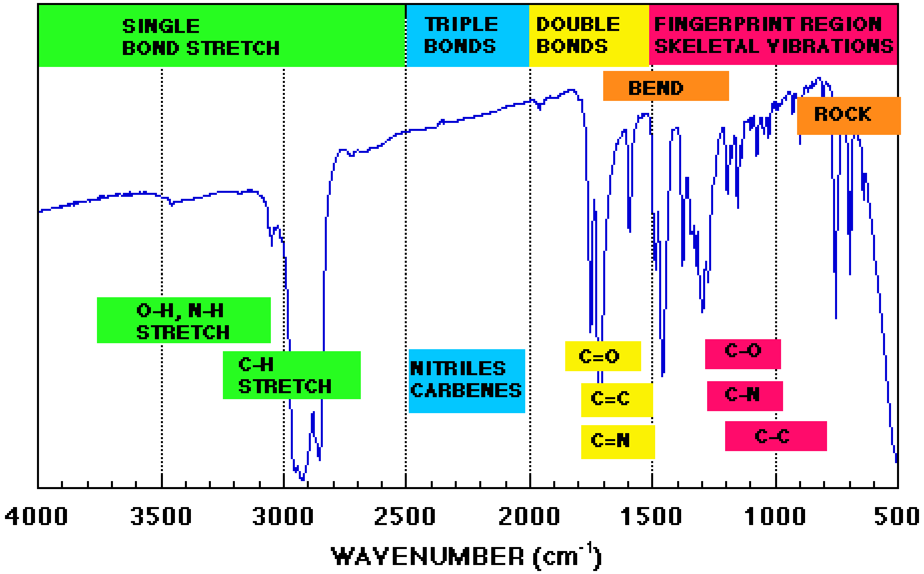
Composed of multiple layers of polymeric binders

- Clear coat – physical/chemical protection
- Base coat – appearance (color, etc.)
- Surface primer – surface uniformity, chip resistance
- Primer – chip resistance
- Electrocoat – corrosion protection for substrate
- Substrate – typically metal, sometimes plastic

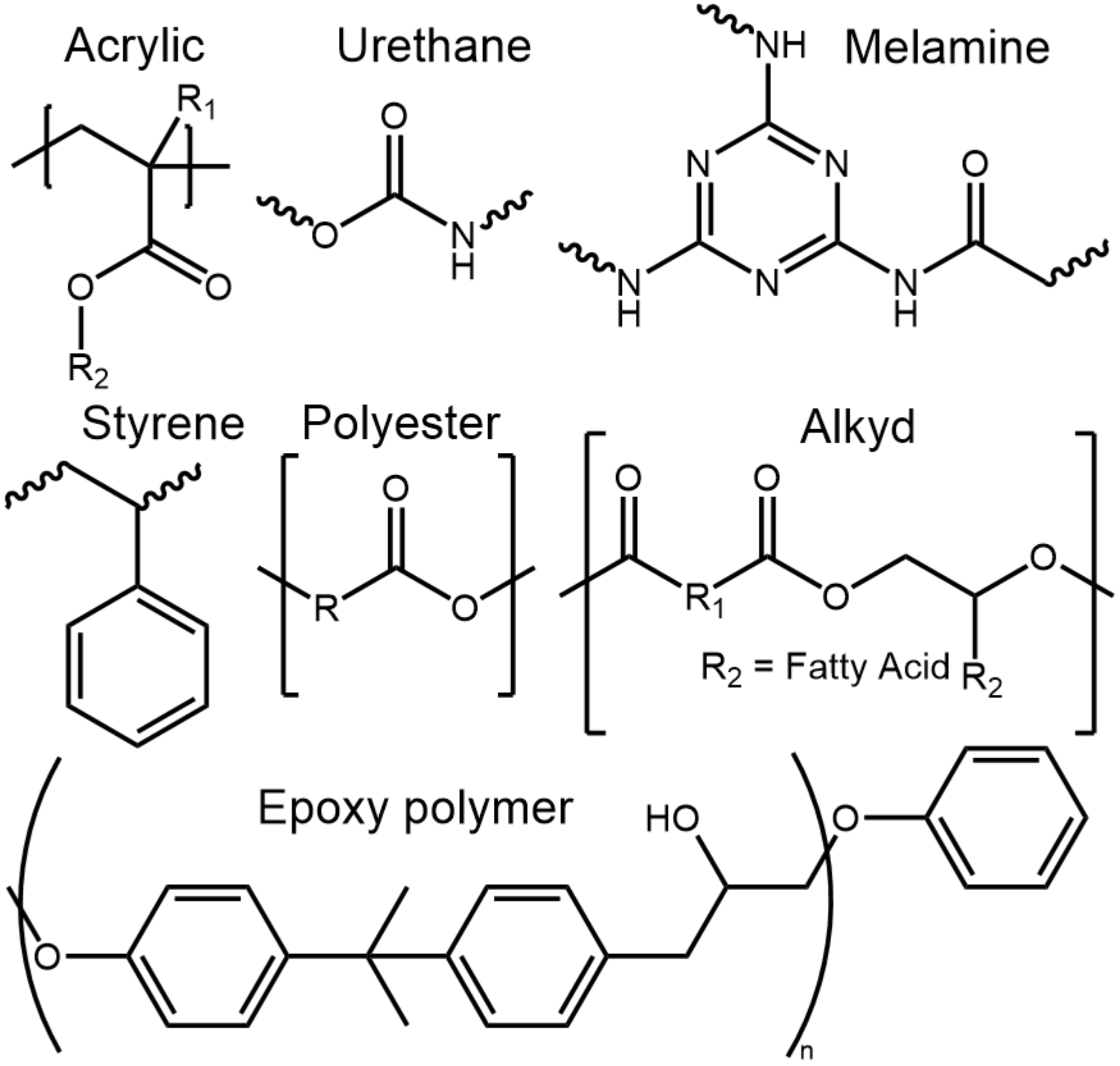


Fourier-transform Infrared Spectroscopy

- Relies on the interaction of molecules and atoms with infrared light
- Produces a characteristic spectrum dependent on the bonding environment
- Well-suited for analyzing binders due to changes, differences in bonding
    - C-C vs C=C
    - C-N vs C-O
    - C=N vs C=O vs C=C



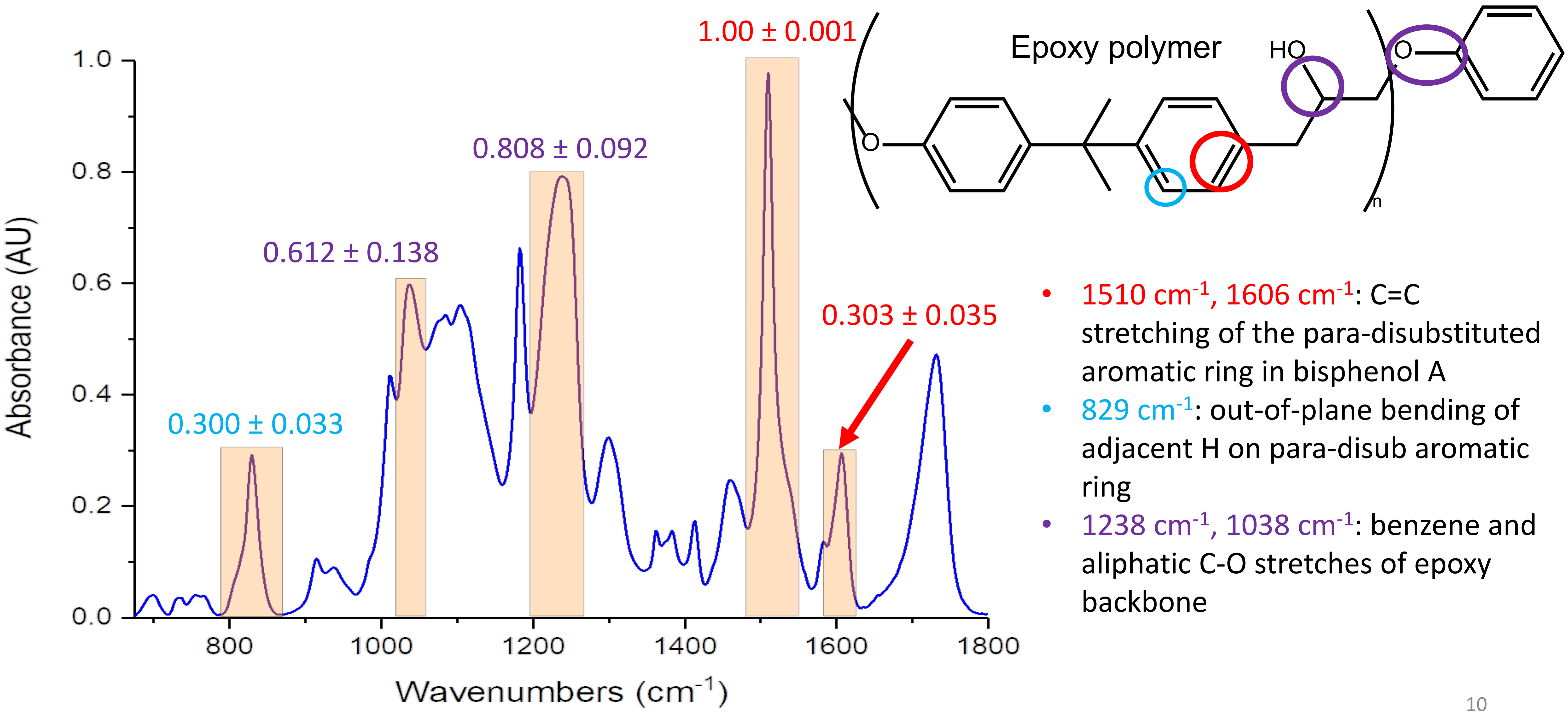
Automotive Coatings



Coatings layers are mostly composed of polymeric binders

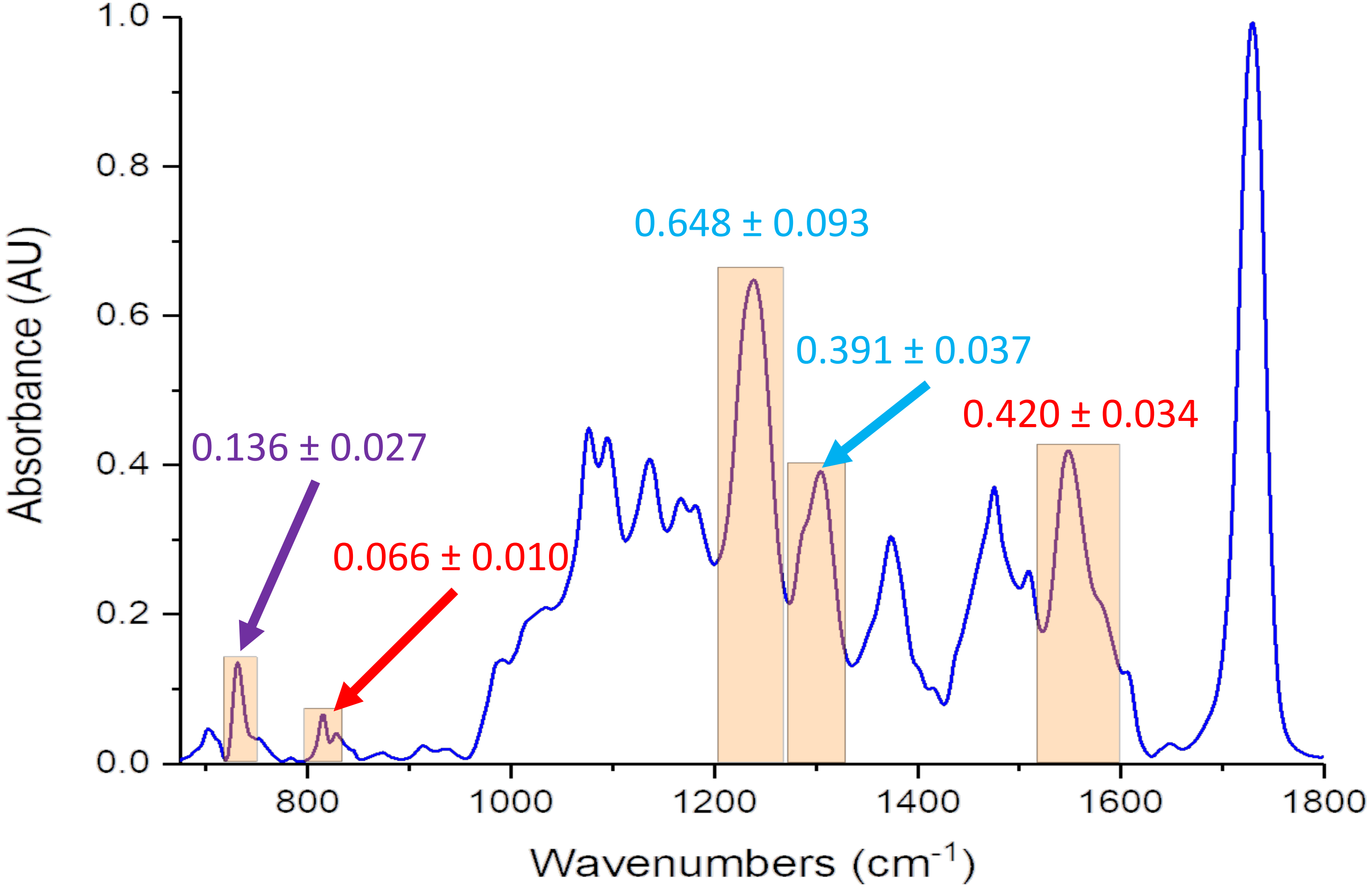
Binder Type Identified	Number of Spectra
Acrylic Melamine	33
Acrylic Urethane Melamine Styrene	13
Acrylic Styrene	6
Epoxy	45
Polyester Melamine	17
“Oxithane”-type	6

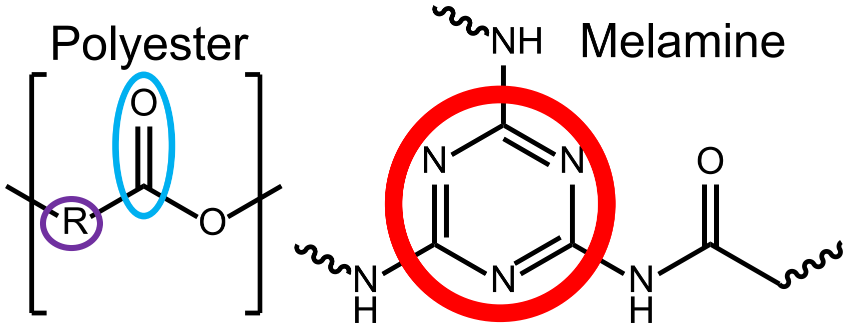
Epoxy Binders



- 1510 cm^{-1}, 1606 cm^{-1}: C=C stretching of the para-disubstituted aromatic ring in bisphenol A
- 829 cm^{-1}: out-of-plane bending of adjacent H on para-disub aromatic ring
- 1238 cm^{-1}, 1038 cm^{-1}: benzene and aliphatic C-O stretches of epoxy backbone

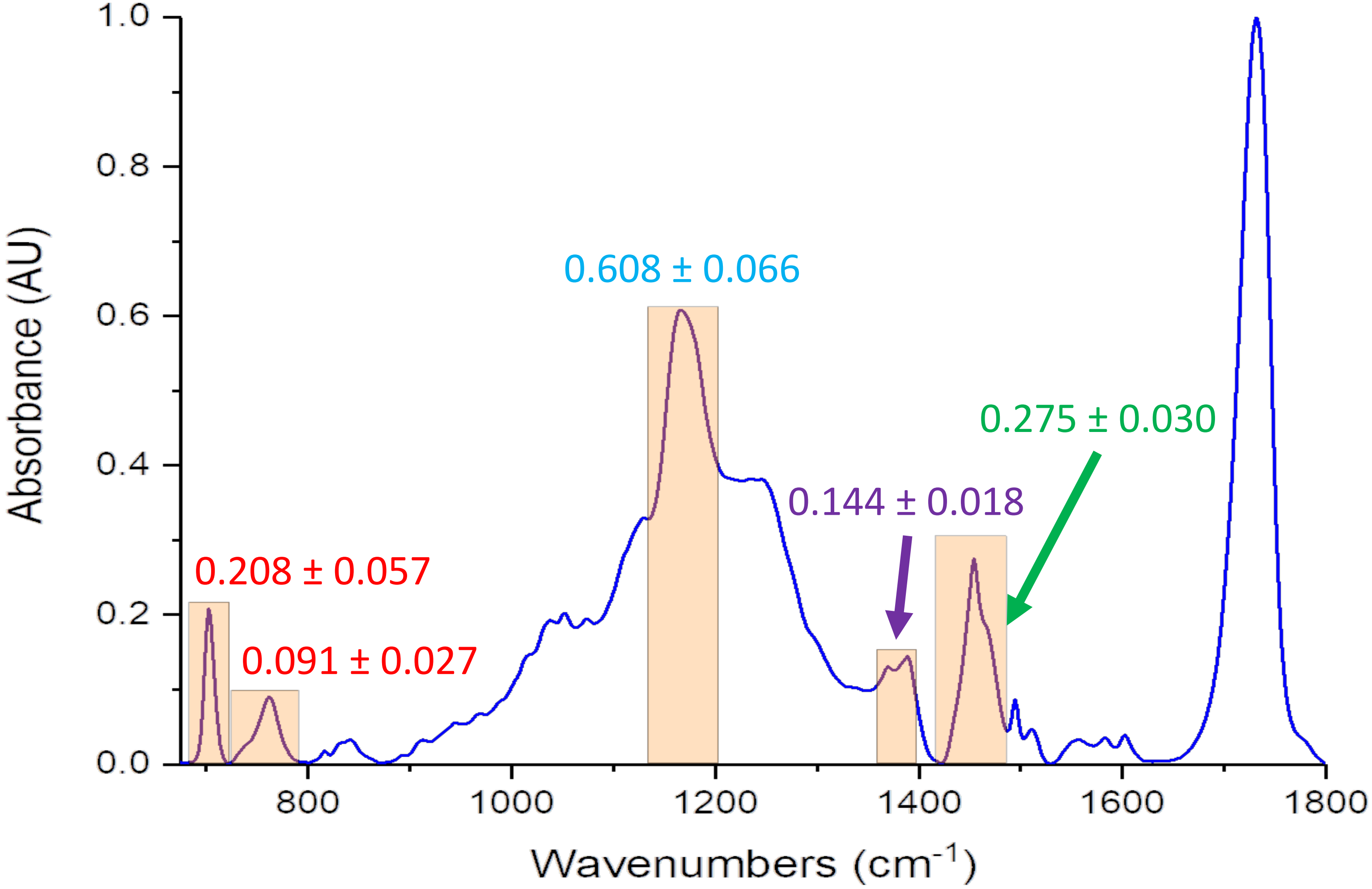
Polyester Melamine Binders

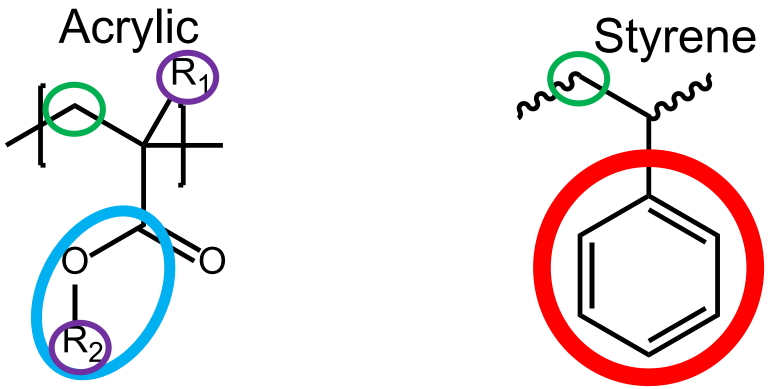




- 1549 cm^{-1}, 814 cm^{-1}: in-plane deformation of the triazine ring and out-of-plane triazine ring vibration, respectively
- 1238 cm^{-1}, 1304 cm^{-1}: C=O stretches of isophthalic acid, typical in Polyester Melamine binders
- 731 cm^{-1}: Hydrogens on aromatic ring of isophthalate

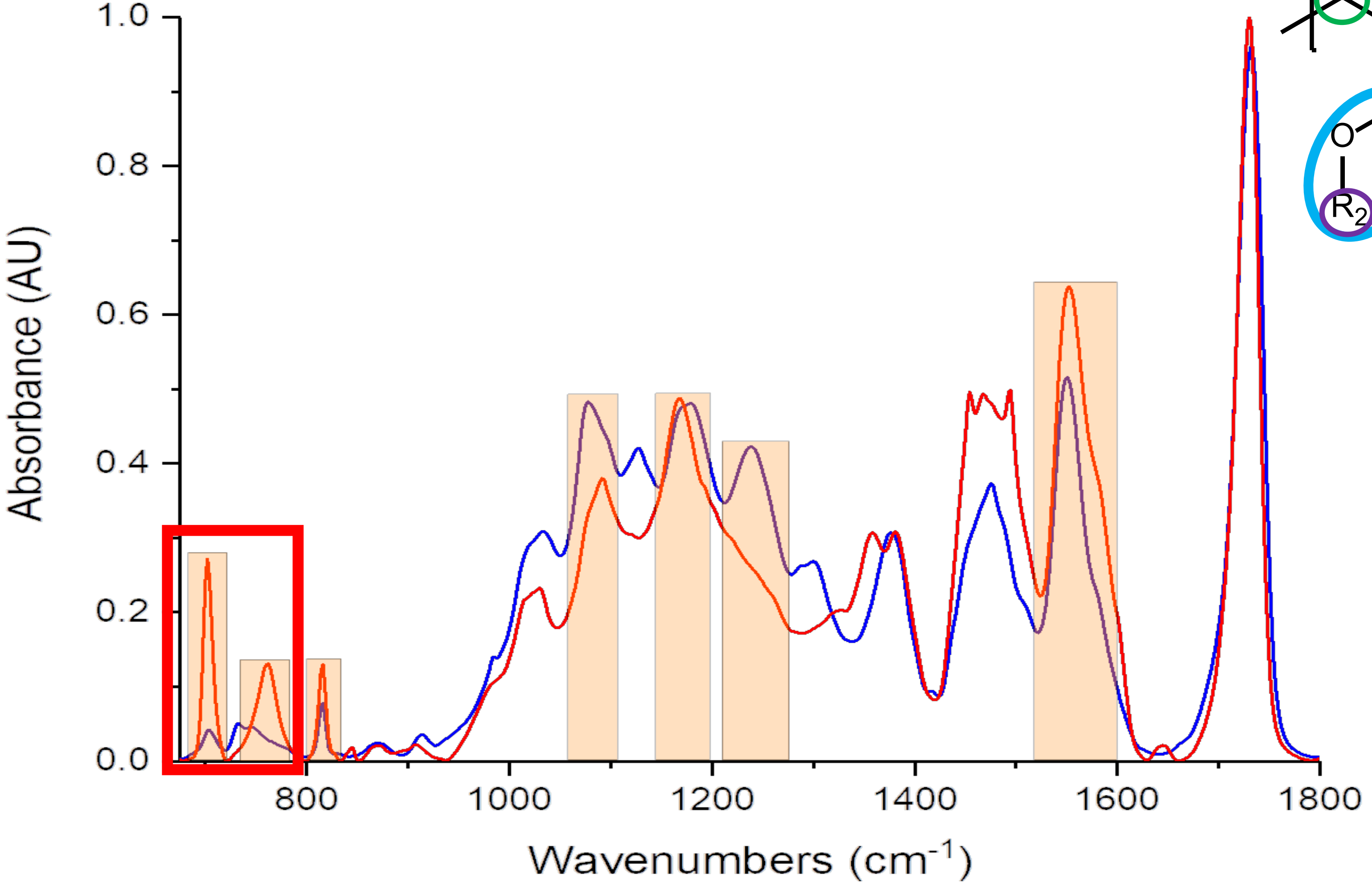
Acrylic Styrene Binders

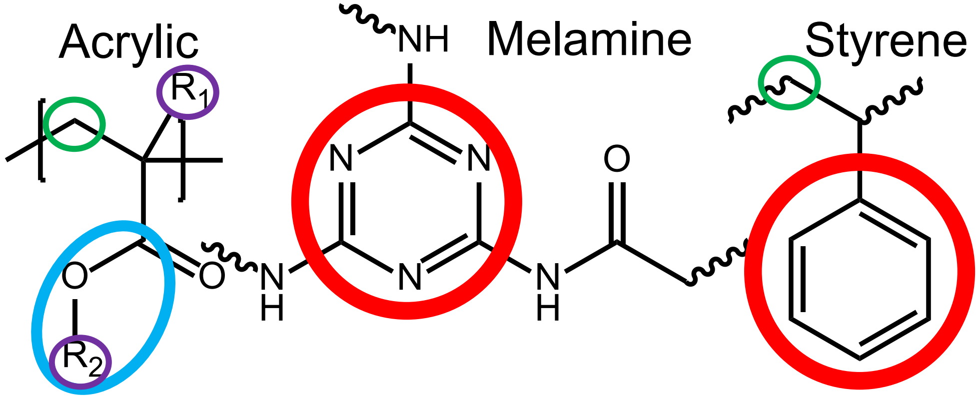




- 702 cm^{-1}, 762 cm^{-1}: monosubstituted ring out-of-plan bend and in-phase wag, respectively
- 1167 cm^{-1}: C-O-C stretching of aliphatic ester
- 1389 cm^{-1}: methyl ($-CH_3$) group bending
- 1454 cm^{-1}: methylene ($-CH_2-$) scissor bend

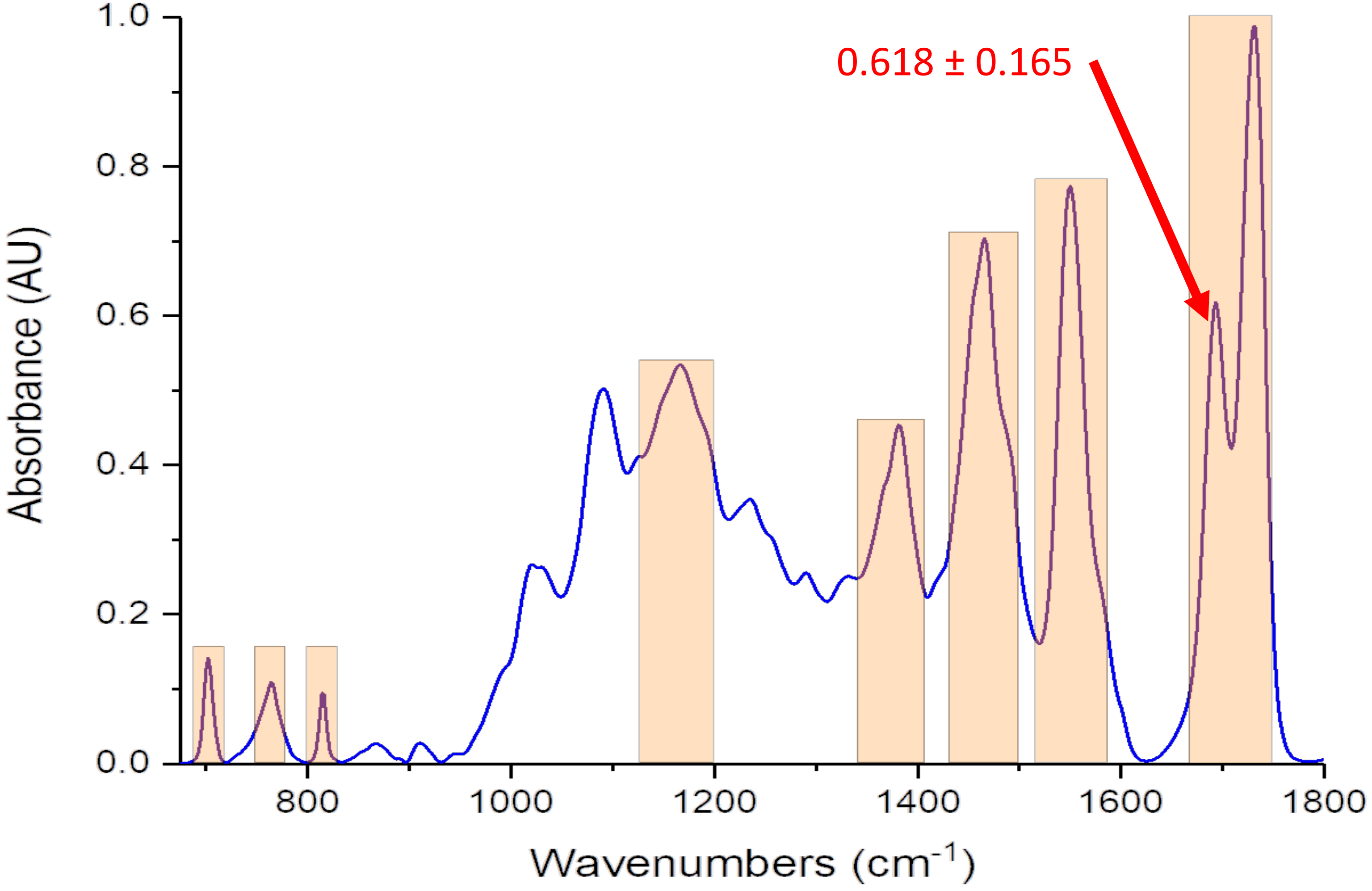
Acrylic Melamine (blue) and Acrylic Melamine Styrene (red) Binders



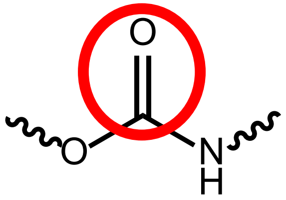


- Previously mentioned bands used to ID these binders appear
- Overlap demonstrates FTIR sensitivity to bonding environment
- Red spectrum shows bands for styrene, absent in blue spectrum

Acrylic Urethane Melamine Styrene Binders

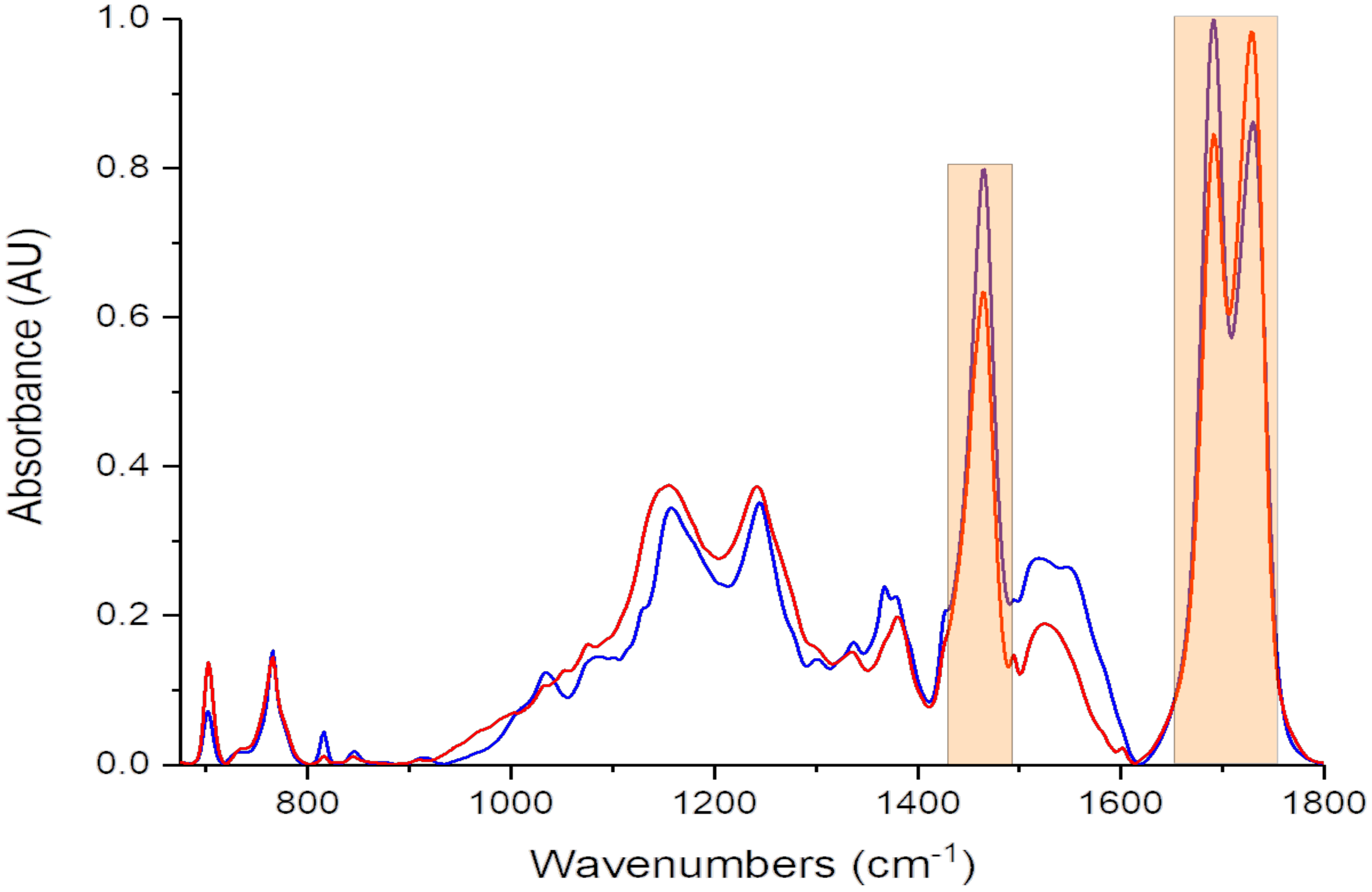


Urethane



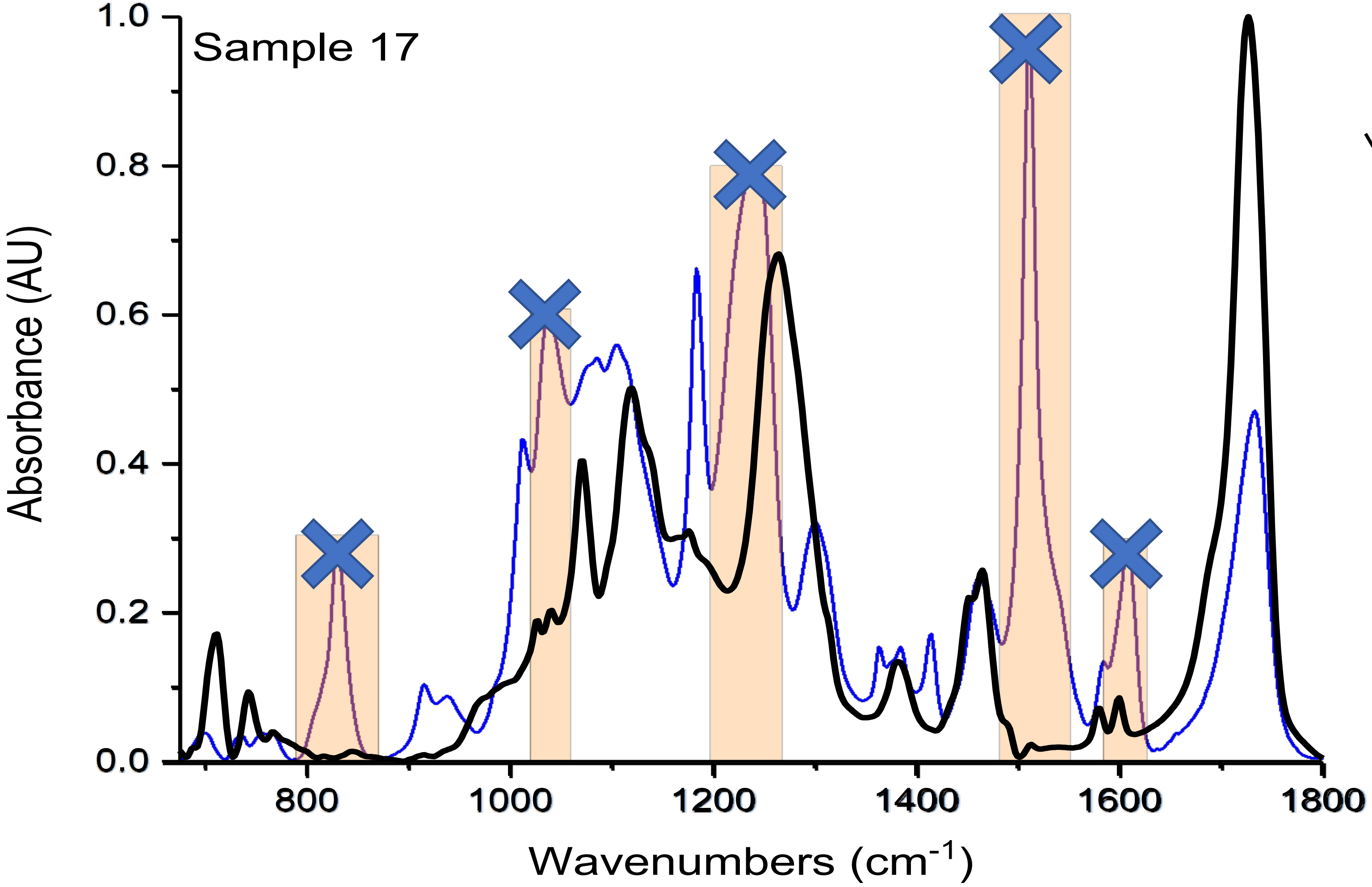
- 1693 cm^{-1}, 1732 cm^{-1}: splitting of carbonyl (C=O) stretch with addition of new carbamate moiety [(N-(C=O)-O vs (C-(C=O)-O)]

Urethane (red) vs Oxithane Modification (blue)

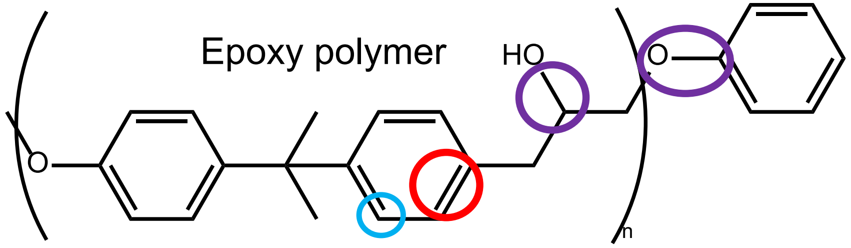


- Since 1987, distinguishable type of acrylic-urethane clear coat binders used compared to older generations
- **Intensity @ ~1690 cm^{-1} ≥ intensity @ ~1730 cm^{-1} ≈ intensity @ ~1470 cm^{-1}**

Example of Unknown—implement analysis



Scion (2016)



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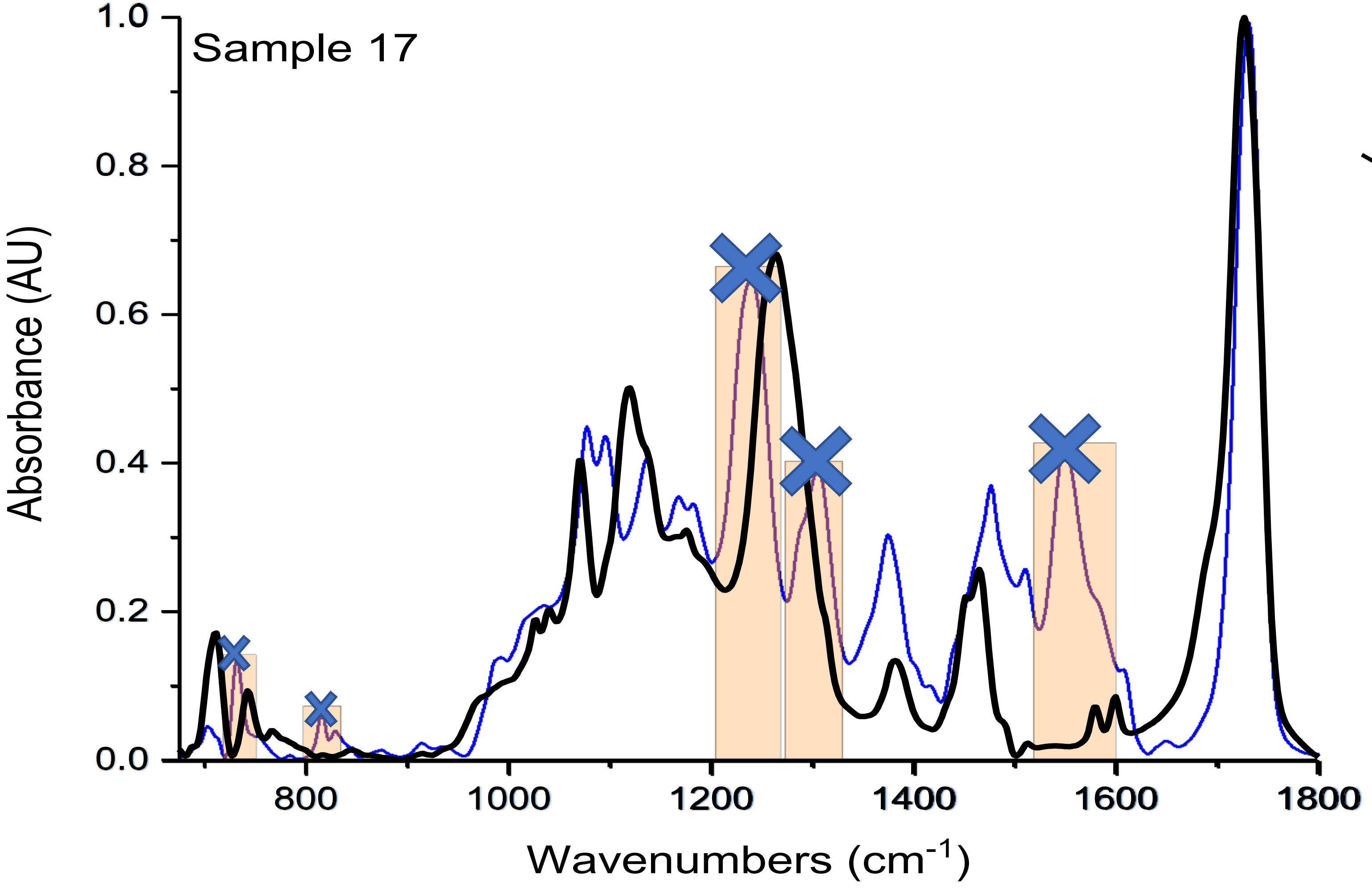

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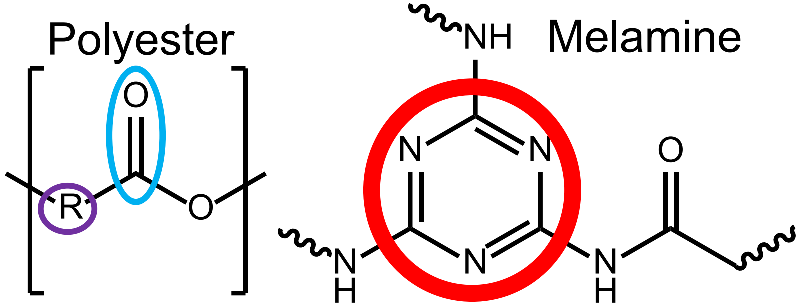

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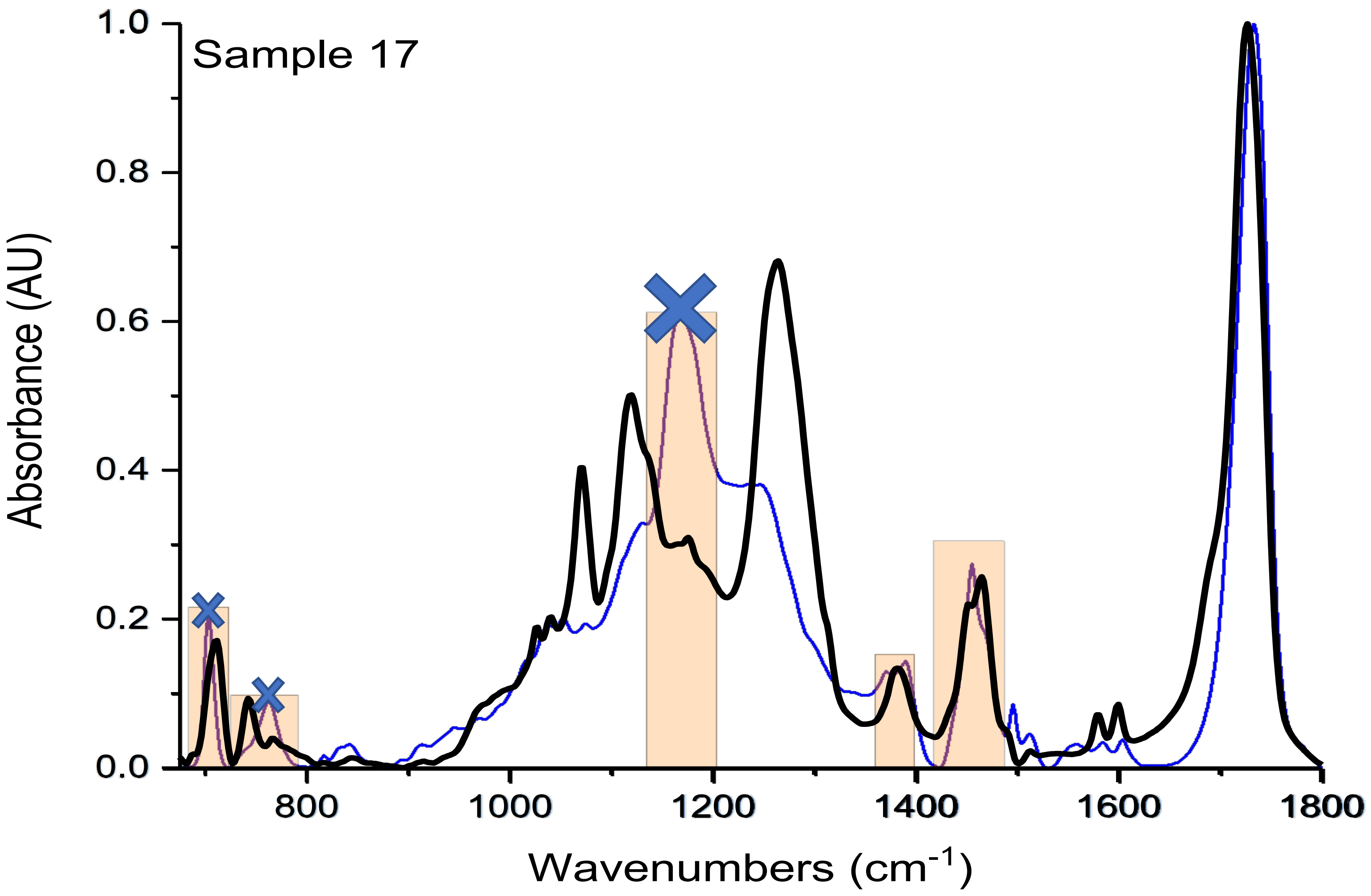

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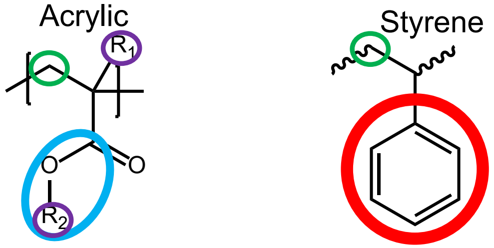

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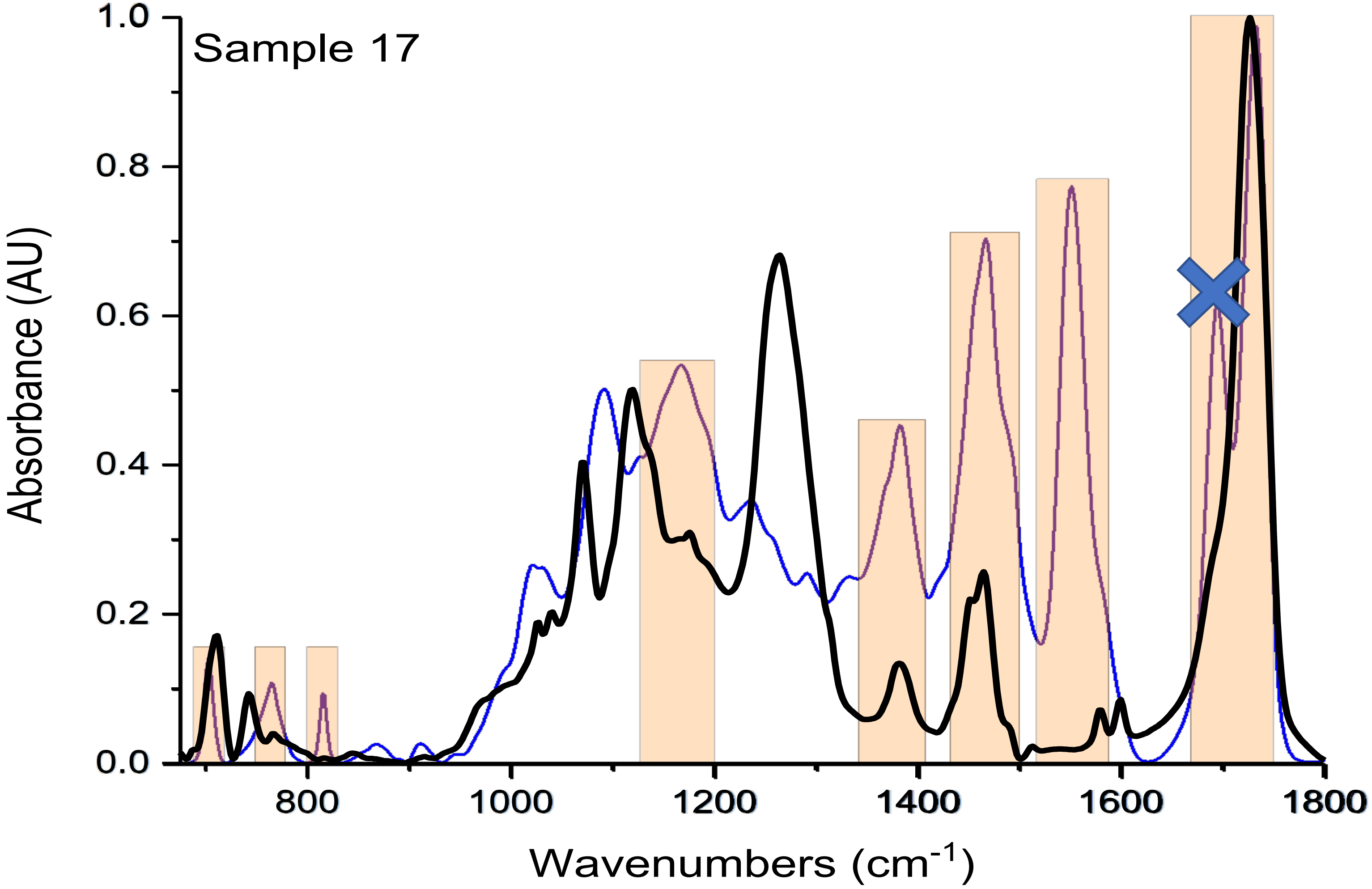
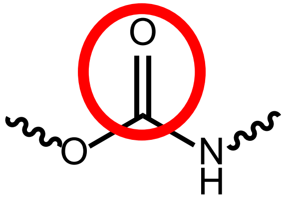
Example of Unknown—implement analysis



Scion (2016)

Urethane



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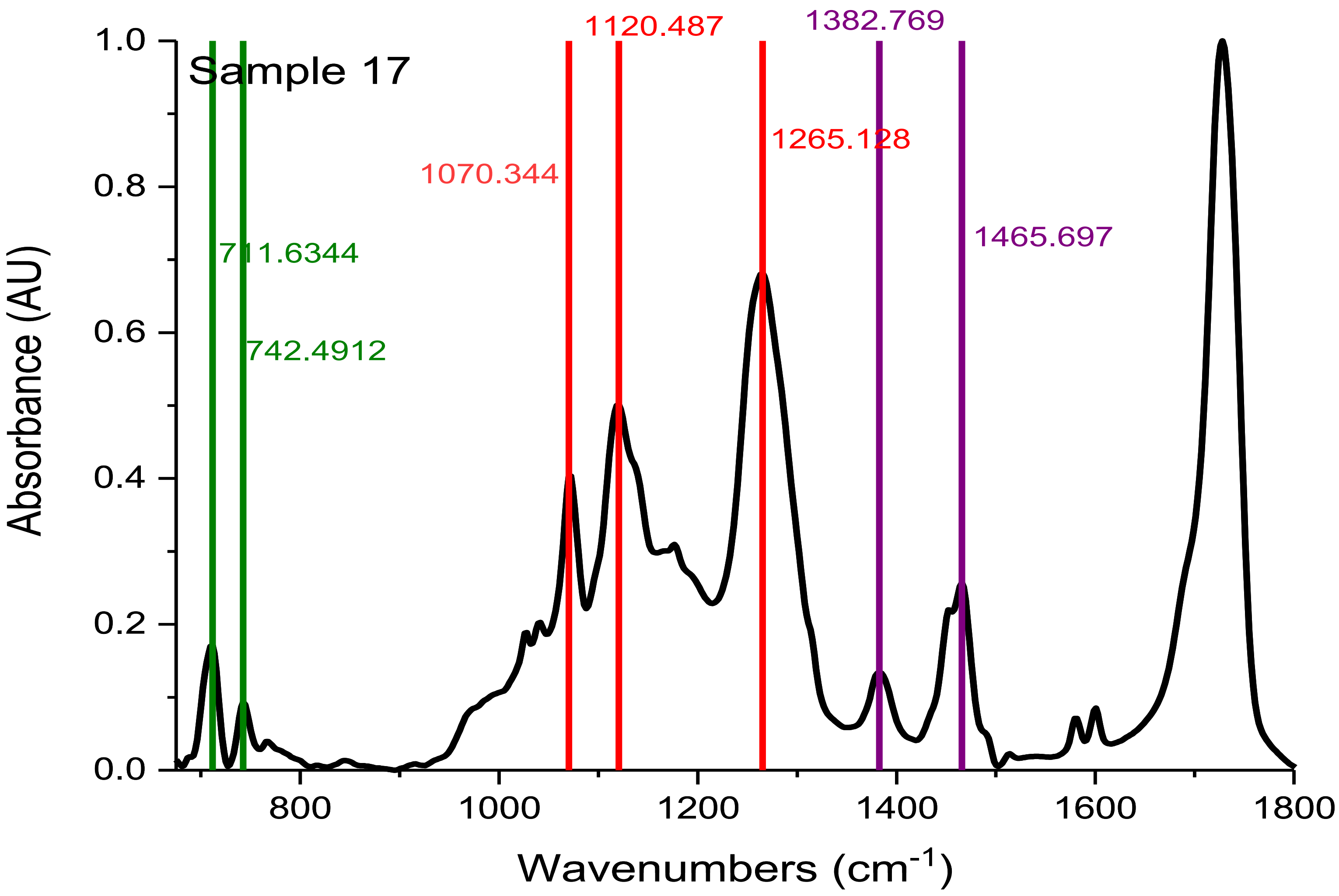
Example of Unknown—implement analysis



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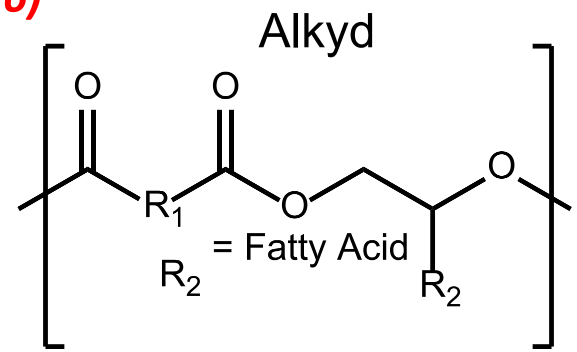


- 1070 cm^{-1}, 1120 cm^{-1}, 1265 cm^{-1}: Alkyd trio (ester C-O), 1265 dominates
- 1383 cm^{-1}, 1466 cm^{-1}: methyl ($-CH_3$) and methylene ($-CH_2-$) bending, respectively
- 712 cm^{-1}, 742 cm^{-1}: aromatic ring bending, out-of-plane bending of adjacent hydrogens, respectively
- Nothing @ 1550/815 cm^{-1} and 1510 cm^{-1}

Conclusions

FTIR is invaluable regarding the analysis and identification of automotive binder chemistries

- Identification relies on tandem absorption band presence for identification
  - Melamine: 815 cm^{-1} and 1550 cm^{-1}, **if only one present cannot conclusively say melamine is present in that sample**

Future Work

- Define oxithane modification
  - By understanding this modification, more analyses could be utilized
- Raman standard spectra?
  - Raman spectroscopy often used in conjunction with FTIR

Hybrid thermal desorption-ambient mass spectrometry developments for the trace detection of explosives

Thomas P. Forbes
Surface and Trace Chemical Analysis Group
Materials Measurement Science Division
Correspondence: thomas.forbes@nist.gov

Forensics@NIST 2020

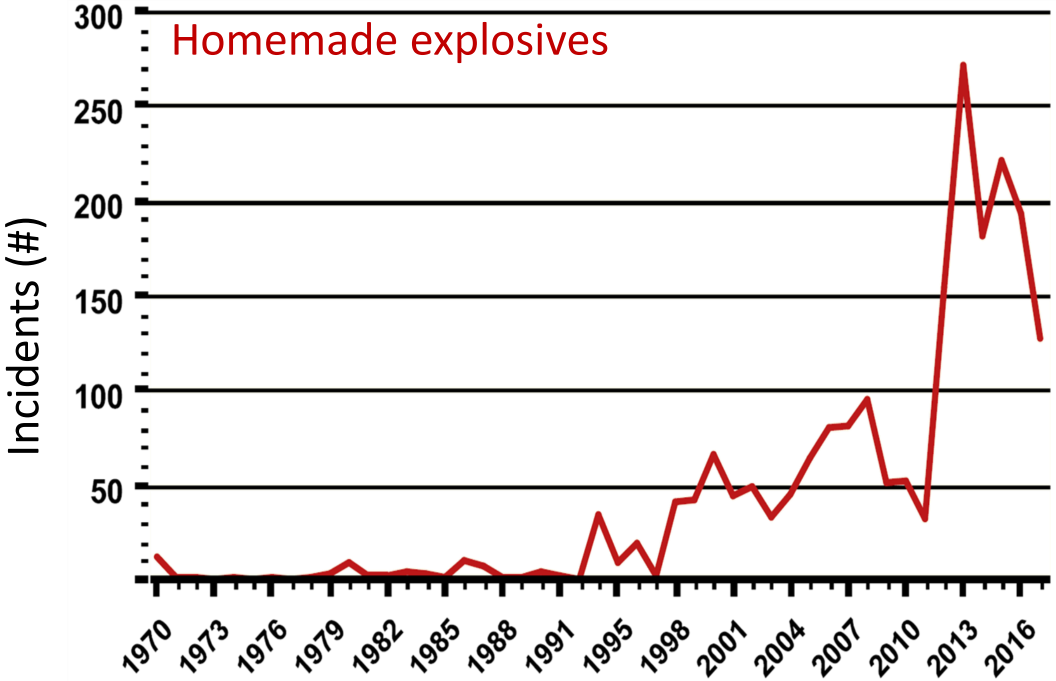
Trace Detection of Explosives

1) Checkpoint screening: defense, customs & border protection, transportation agencies

2) Forensic & criminal investigations

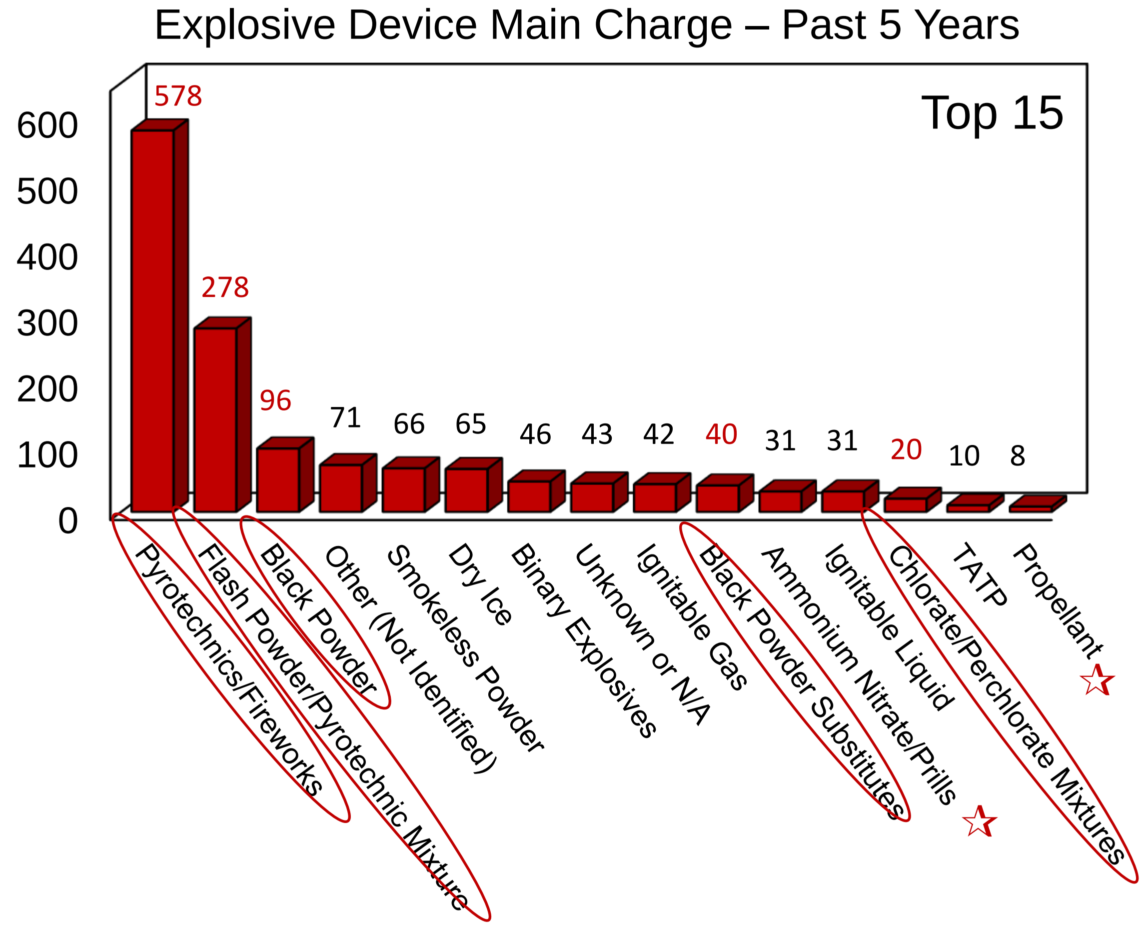
- Expanding list of explosive and related energetic materials
  - *2019 Annual List of Explosive Materials by the ATF included close to 240 compounds & mixtures*

- The open source Global Terrorism Database reveals a surge in attacks using homemade explosives (HMEs) or improvised explosive devices (IEDs) in recent years



* *National Consortium for the Study of Terrorism and Responses to Terrorism (START), Global Terrorism Database 2016; Available from: (https://www.start.umd.edu/gtd).*

Trace Detection of Explosives



The wide range of physicochemical properties presents difficulties for analytical techniques

Inorganic-based low explosives:

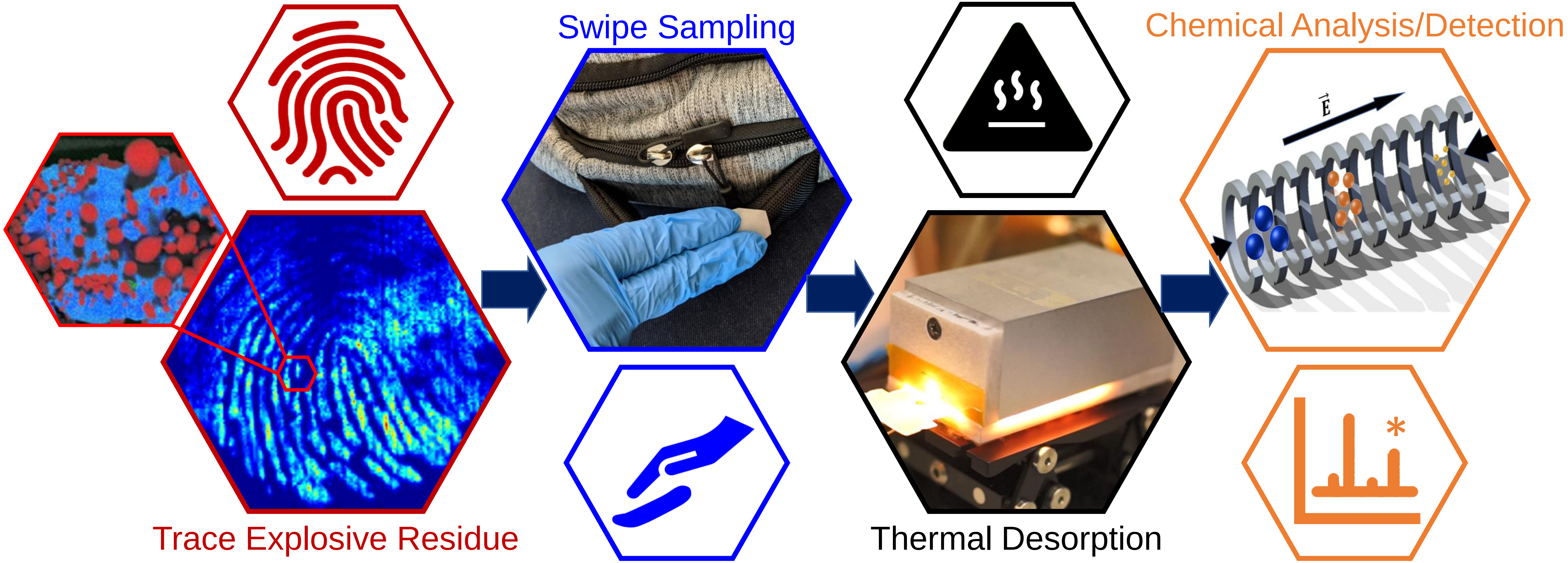
- Black & smokeless powders
- Flash powders
- Pyrotechnic mixtures
- Tertiary fuel-oxidizer mixtures

Remain among most abused IED charges in US

** from the 2017 U.S. Bomb Data Center (USBDC) Annual Explosives Incident Report (EIR),* www.atf.gov

Detection, Identification, & Differentiation

- Swipe sampling: used for both high-throughput screening to forensic analysis



Trace Explosive Residue

Swipe Sampling

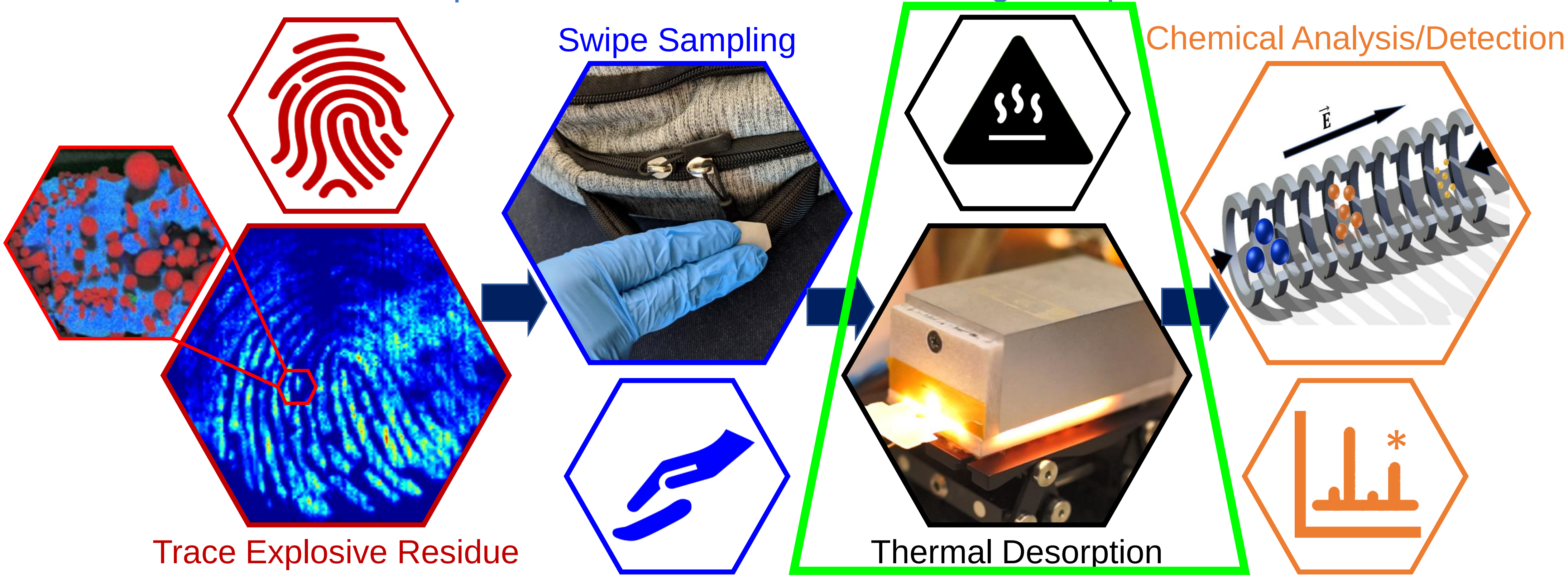
Thermal Desorption

Chemical Analysis/Detection

Forbes, T.P., Krauss, S.T., Gillen, G. (2020) *Trends in Analytical Chemistry,* **131**, 116023. (https://doi.org/10.1016/j.trac.2020.116023)

Detection, Identification, & Differentiation

- **Major hurdle - common inorganic oxidizers are nonvolatile, low vapor pressures**
  - Common temperatures for organic species are insufficient for thermal desorption
  - Constant elevated temperature – detrimental to labile organic species



Swipe Sampling

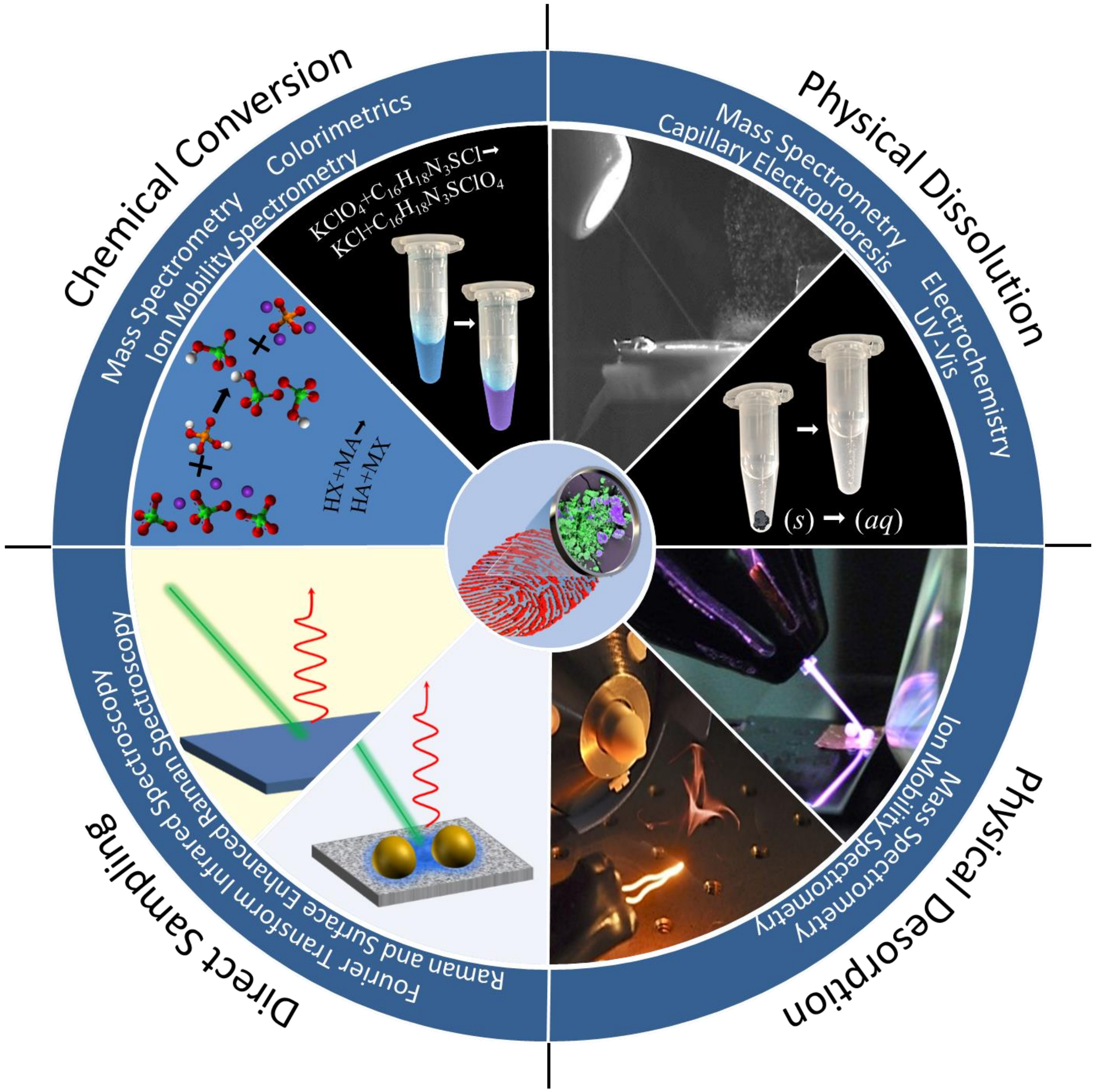
Chemical Analysis/Detection

Trace Explosive Residue

Thermal Desorption

Forbes, T.P., Krauss, S.T., Gillen, G. (2020) *Trends in Analytical Chemistry,* **131**, 116023. (https://doi.org/10.1016/j.trac.2020.116023)

Strategies for Analysis

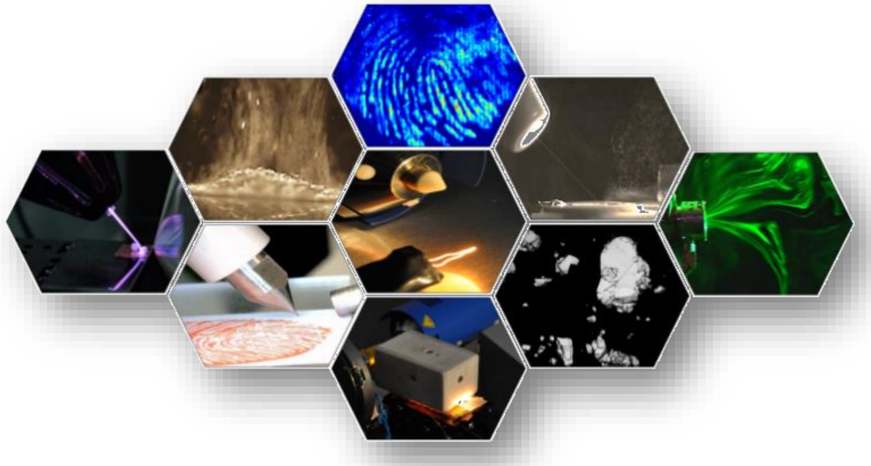


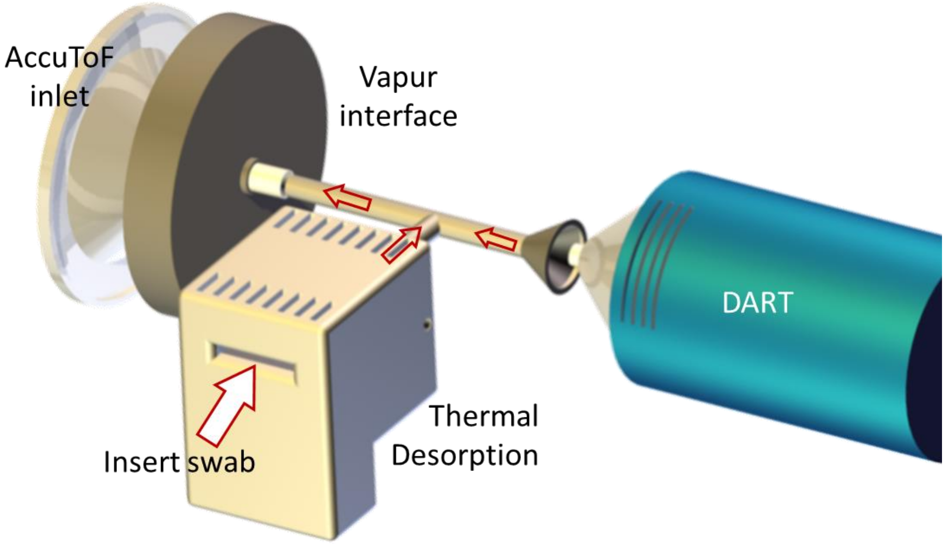
1. **Chemical Conversion**
   - Colorimetrics
   - Acidic reagent
2. **Physical Dissolution**
   - Capillary electrophoresis
   - Liquid extraction ambient MS
3. **Physical Desorption**
   - High temperature thermal desorption
   - High power plasma
4. **Direct Sampling**
   - Raman spectroscopy/SERS
   - FTIR spectroscopy

Forbes, T.P., Krauss, S.T., Gillen, G. (2020) *Trends in Analytical Chemistry,* **131**, 116023. (https://doi.org/10.1016/j.trac.2020.116023)

Hybrid Thermal Desorption-Ambient MS

- Ambient ionization MS of explosives
  - Class of techniques and platforms providing great utility for explosives detection[1]





- DART for wipe-based sample collections
  - Coupling with a resistance-based thermal desorber[2-3]
  - Demonstrated with drugs,[2-4] metabolites,[2] rodenticides,[5] and evidence packaging[6]

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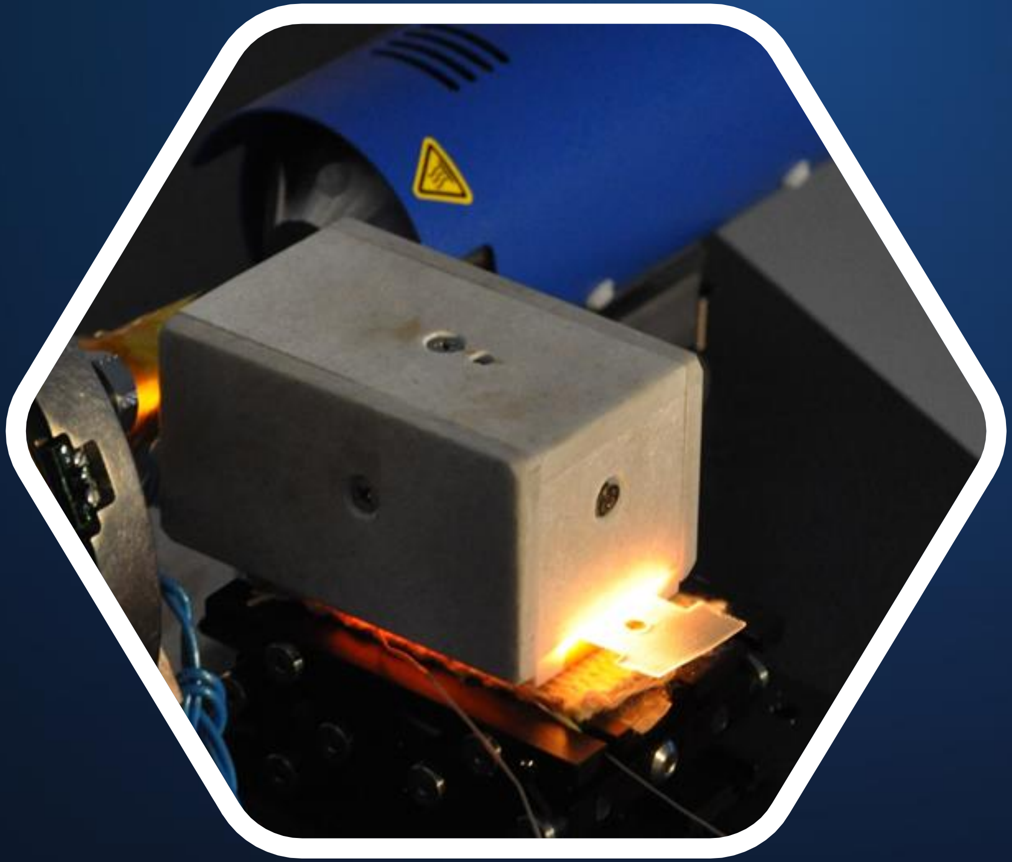
1. Forbes, T.P. and Sisco, E. (2018) *Analyst*, **143**(9), 1948-1969. (https://doi.org/10.1039/C7AN02066J)
2. Sisco, E., Forbes, T.P., Staymates, M., Gillen, G. (2016) *Analytical Methods,* **8**(35), 6494-6499. (https://doi.org/10.1039/C6AY01851C)
3. Sisco, E., Staymates, M., Forbes, T.P. (2020) *Analyst*, **145**(7), 2743-2750. (https://doi.org/10.1039/D0AN00031K)
4. Sisco, E., Verkouteren, J.R., Staymates, J., Lawrence, J. (2017) *Forensic Chemistry,* **4**, 108-115. (https://doi.org/10.1016/j.forc.2017.04.001)
5. Robinson, E.L. and Sisco, E. (2019) *Journal of Forensic Sciences*, **64**(4), 1026-1033. (https://doi.org/10.1111/1556-4029.13978)
6. Sisco, E., Robinson, E.L., Burns, A., Mead, R. (2019) *Forensic Science International,* **304,** 109939. (https://doi.org/10.1016/j.forsciint.2019.109939)

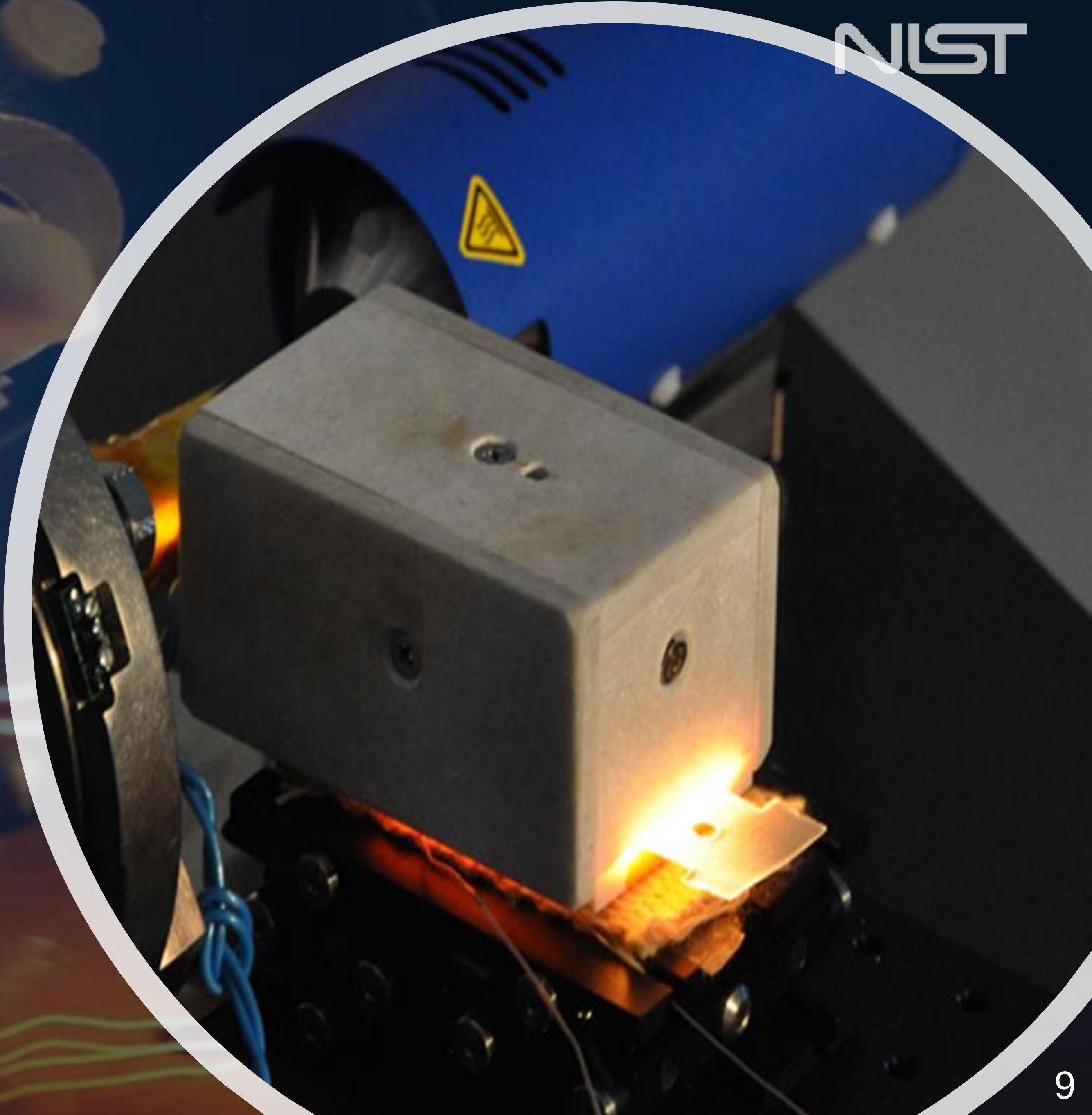
Hybrid Thermal Desorption-Ambient MS

Infrared Thermal Desorption (IRTD)

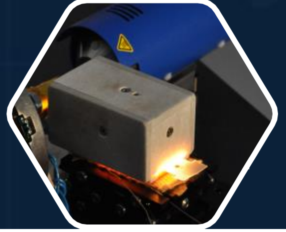


Joule Heating Thermal Desorption (JHTD)

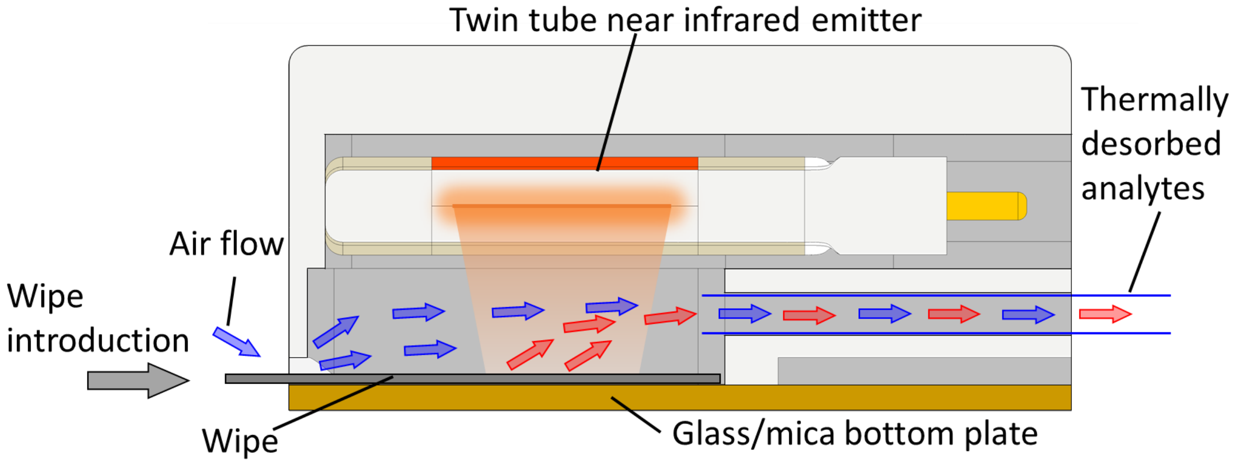
Infrared Thermal Desorption (IRTD)

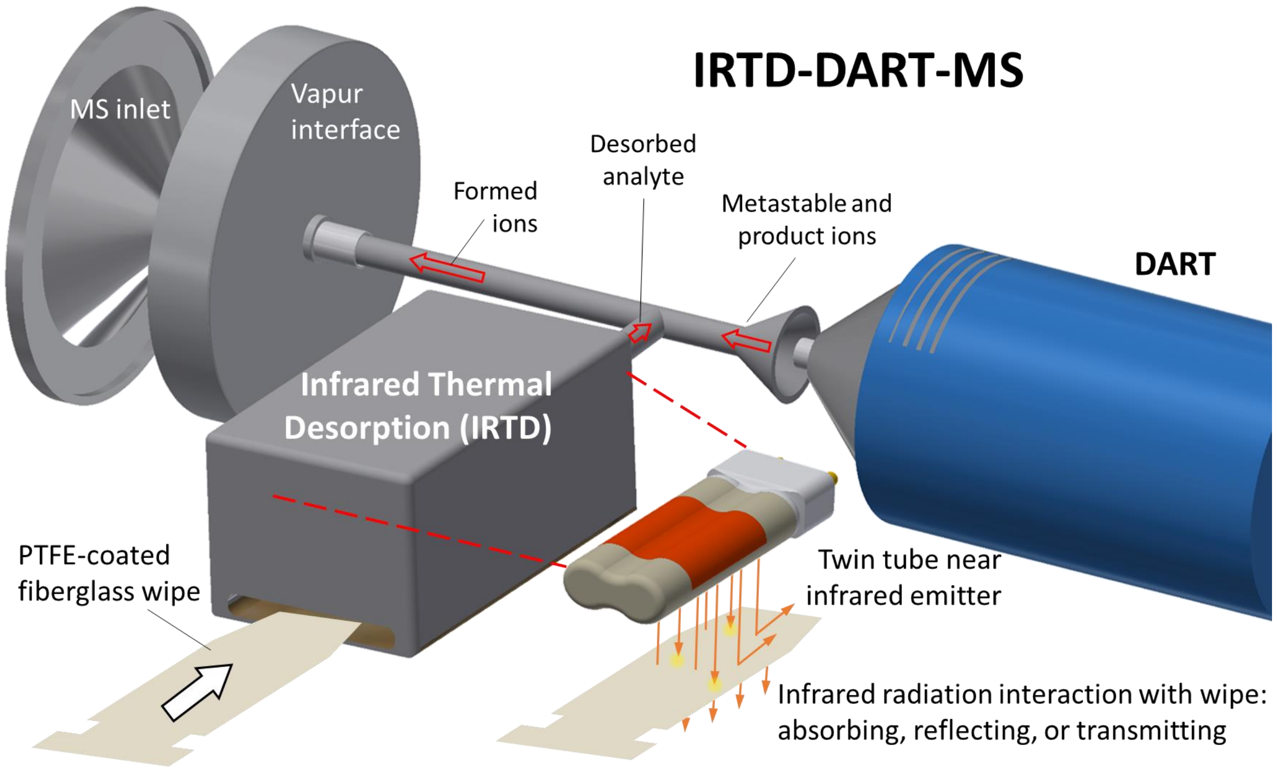


Infrared Thermal Desorption (IRTD)



- Filament-based near infrared emitter
  - Wipe-based sample introduction

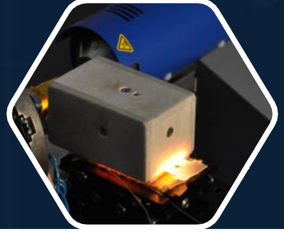




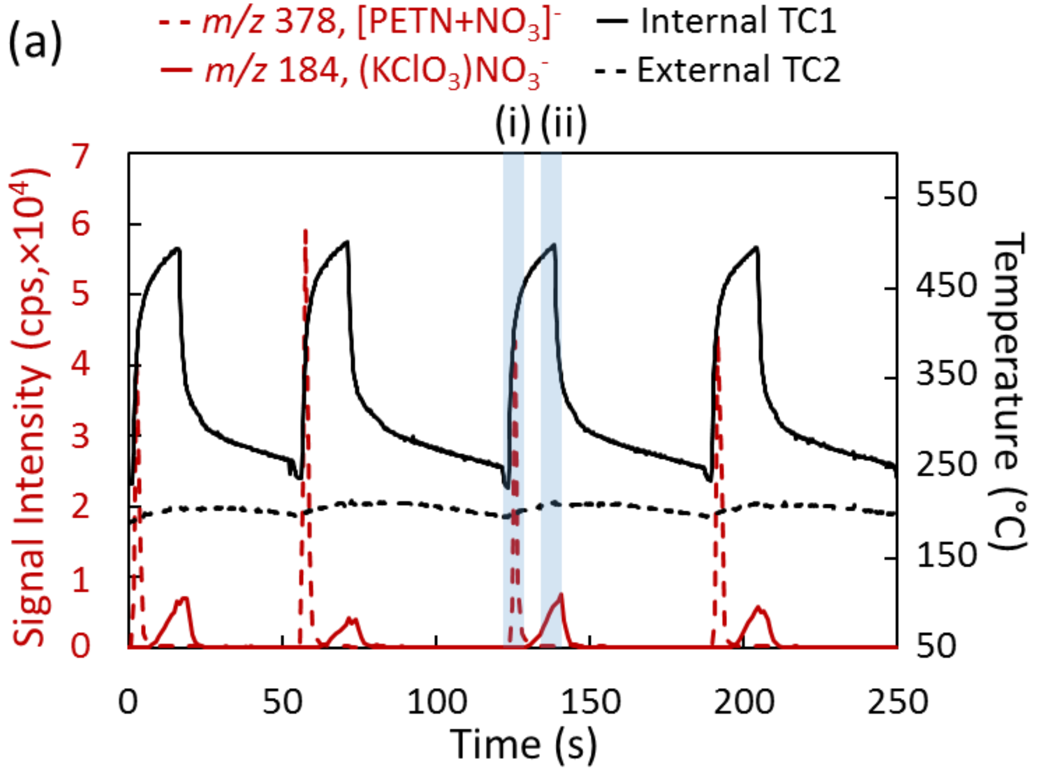
- Tunable broad spectrum
- Rapid response times (~1s)
- Temporally discrete emission
- Intrinsic temperature ramp
- Multi-mode heating

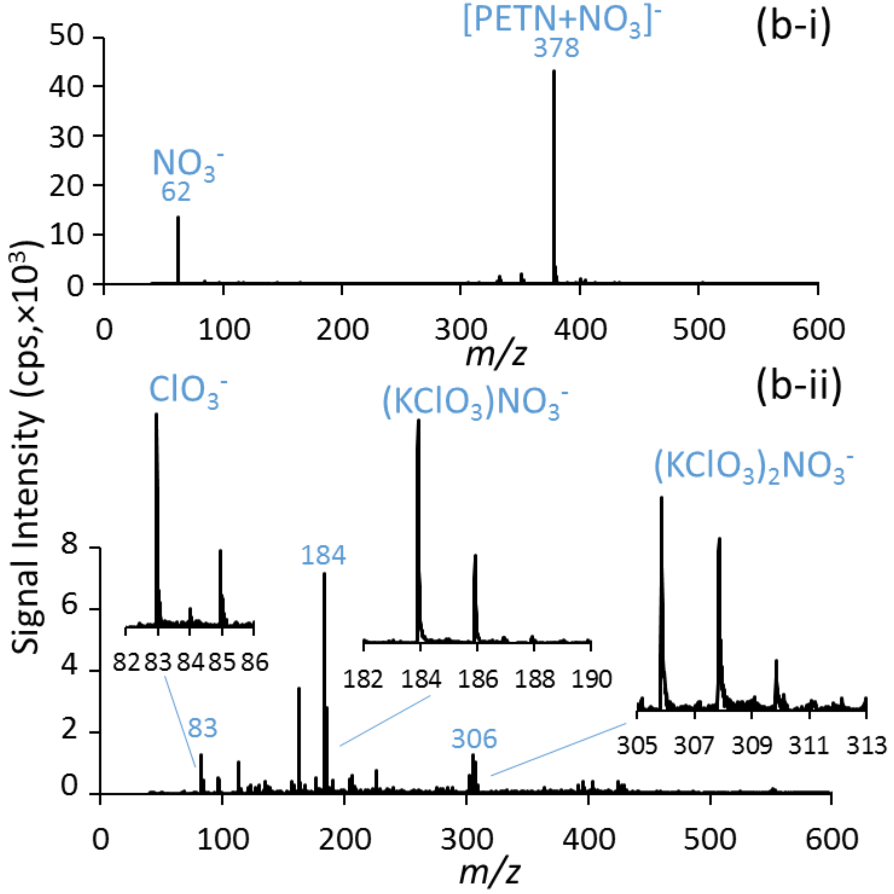
Forbes, T.P., Sisco, E., Staymates, M., (2018) *Analytical Chemistry,* **90**, 6419-6425. (https://doi.org/10.1021/acs.analchem.8b01037)
Forbes, T.P., Staymates, M., Sisco, E., (2017) *Analyst*, **142**(16), 3002-3010. (: https://doi.org/10.1039/C7AN00721C)
U.S. Provisional Patent Application # 62/958,873 “System and Method for Infrared Thermal Desorption.”

Infrared Thermal Desorption (IRTD)



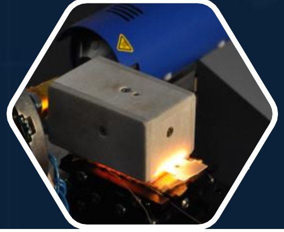
- Organic explosive / inorganic oxidizer mixtures
  - 10 ng PETN / 250 ng $KClO_3$
  - Replicate 15s / 100% infrared emission intervals





Forbes, T.P., Sisco, E., Staymates, M., (2018) *Analytical Chemistry,* **90**, 6419-6425. (https://doi.org/10.1021/acs.analchem.8b01037)

Infrared Thermal Desorption (IRTD)



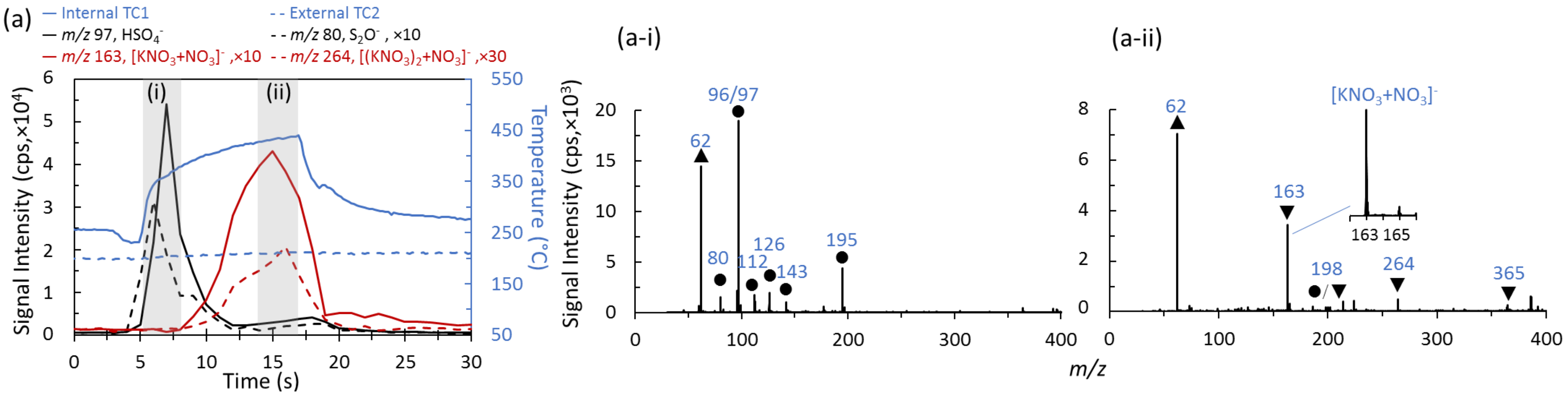
- Black powders and black powder substitutes

Elephant BP

Ions related to:

● Sulfur

▼ Potassium nitrate



Triple Seven BPS

Ions related to:

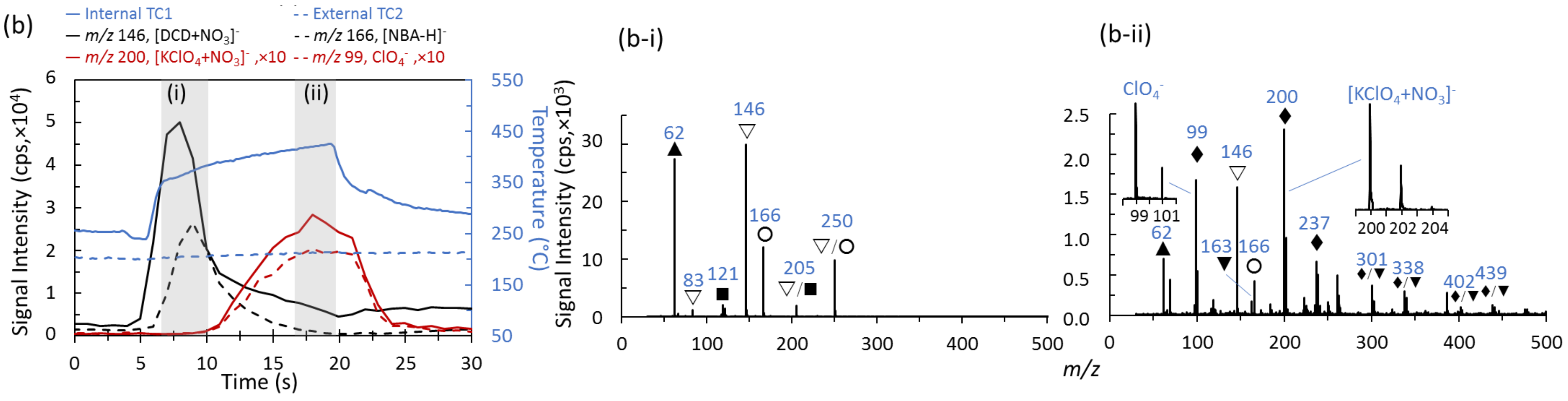
▼ Potassium nitrate

♦ Potassium perchlorate

▽ Dicyandiamide

○ 3-Nitrobenzoic acid

■ Sodium benzoate

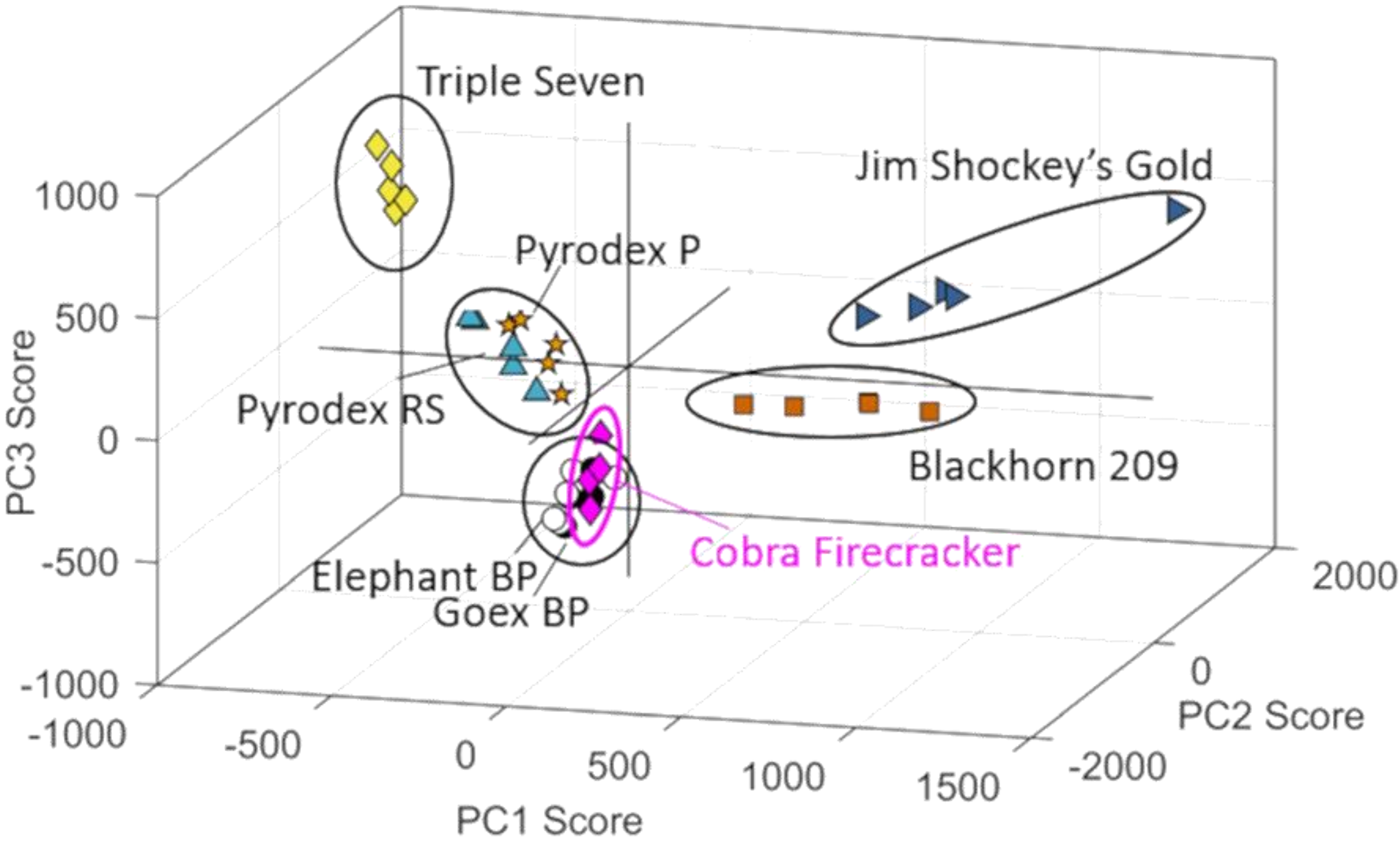


Forbes, T.P., and Verkouteren, J.R., (2019) *Analytical Chemistry*, **91**, 1089-1097. (https://doi.org/10.1021/acs.analchem.8b04624)

PCA of Black Powders & BPS

- Early in emission interval – moderate temperatures
  - Separation based on more volatile organic or inorganic fuels
- Late in emission interval – elevated temperatures
  - Separation based on nonvolatile oxidizers

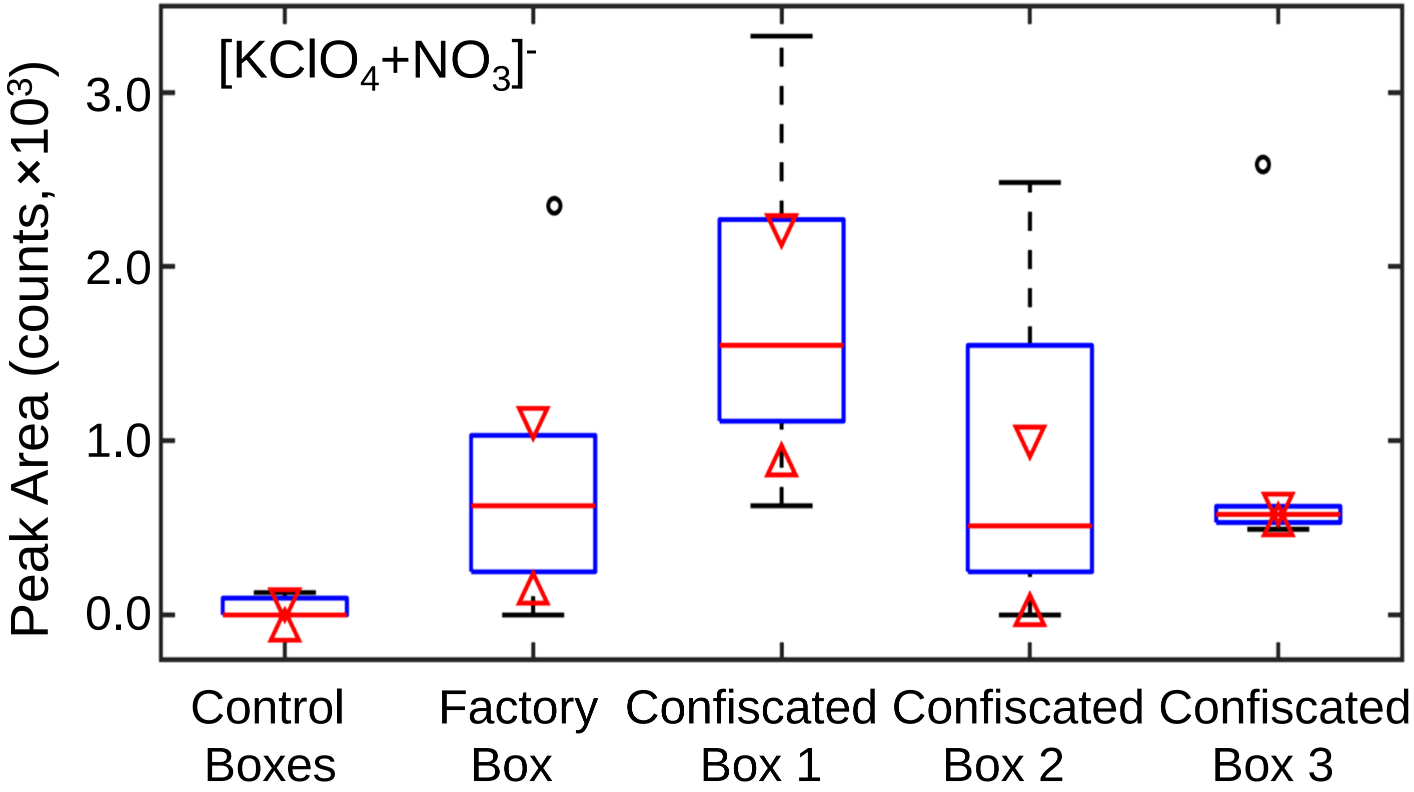
Propellants & Pyrotechnics



Forbes, T.P., and Verkouteren, J.R., (2019) *Analytical Chemistry,* **91**, 1089-1097. (https://doi.org/10.1021/acs.analchem.8b04624)

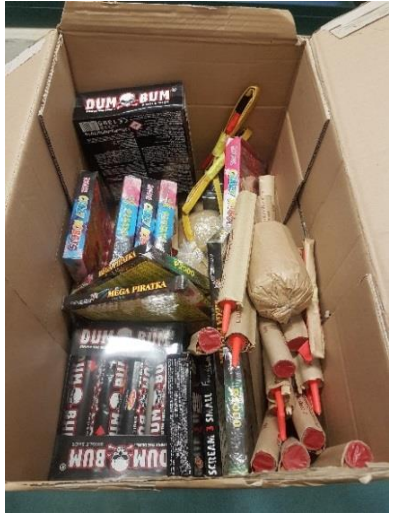
Illicit Pyrotechnics from Postal Packages

- Collaboration with Netherlands Forensic Institute
- Packages seized by Dutch police – wipe samples
  - Detection of flash powder oxidizer – Al / $KClO_4$

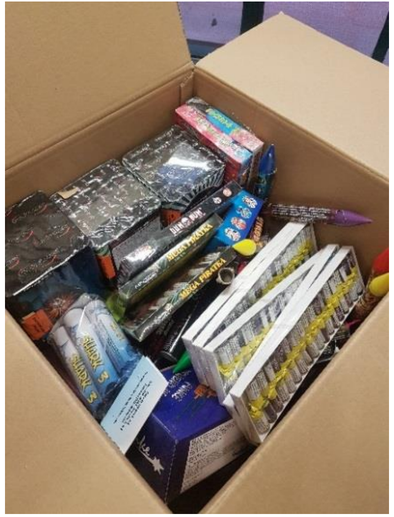


Factory Box

Conf. Box 1



Conf. Box 2



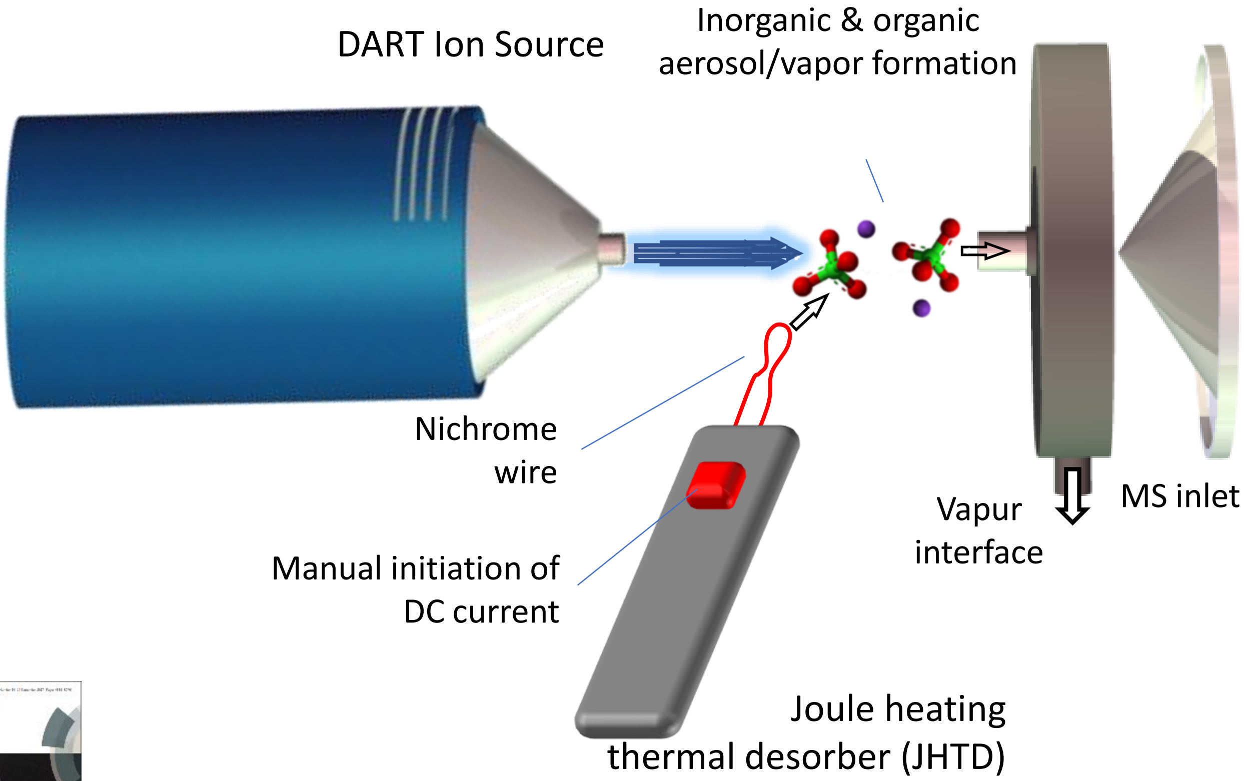
Conf. Box 3

Bezemer, K.D.B., Forbes, T.P., Hulsbergen, A.W.C., *et al.* (2020) *Forensic Science International*, **308**, 110160. (https://doi.org/10.1016/j.forsciint.2020.110160)

Joule Heating Thermal Desorption

Joule Heating Thermal Desorption (JHTD)

- Ohmic heating through nichrome wire
  - Discrete liquid and particle analysis



DART Ion Source

Inorganic & organic
aerosol/vapor formation

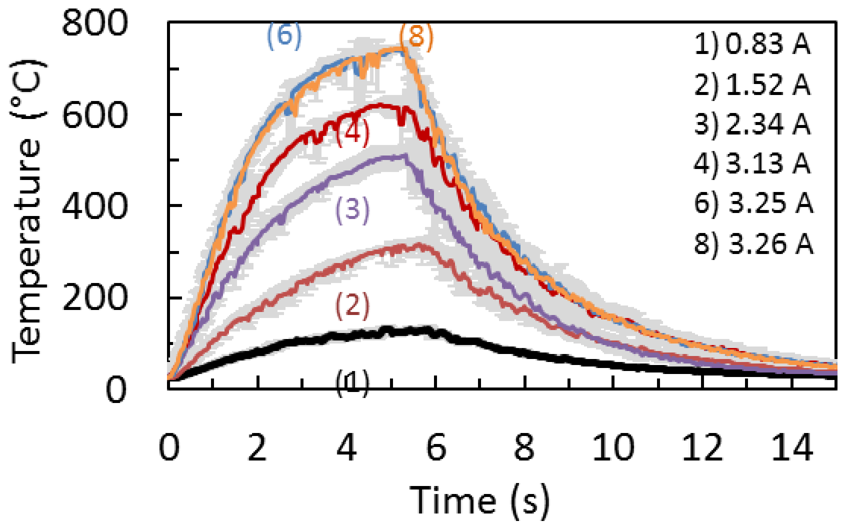
Nichrome
wire

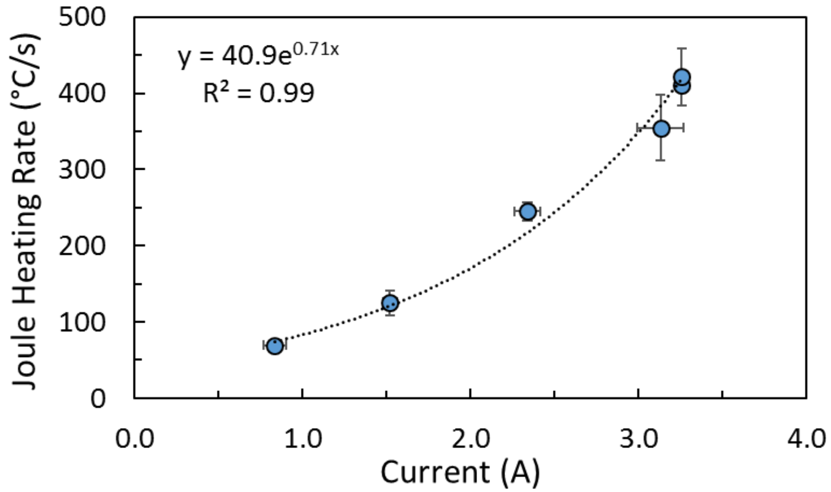
Manual initiation of
DC current

Vapur
interface

MS inlet

Joule heating
thermal desorber (JHTD)

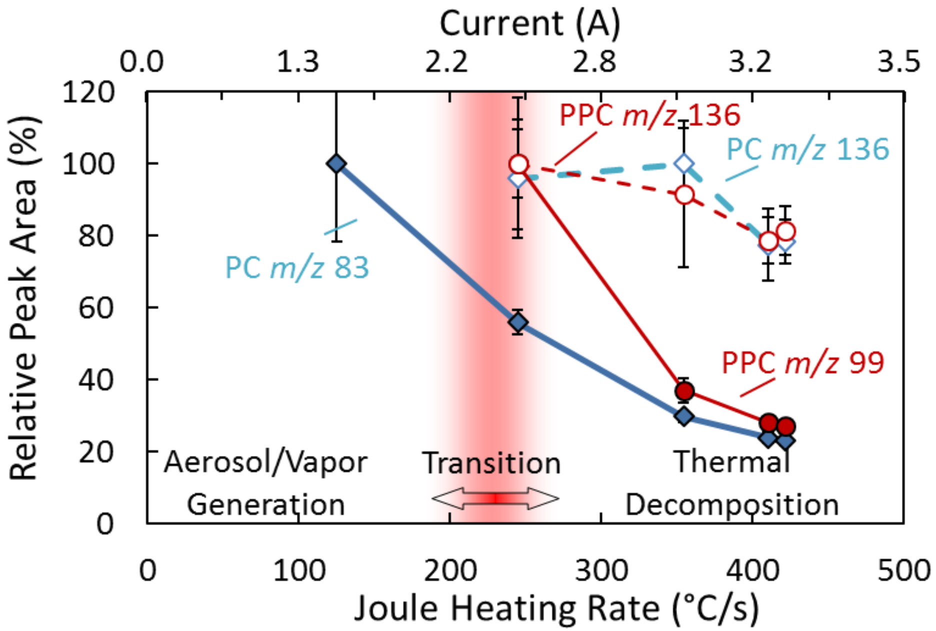


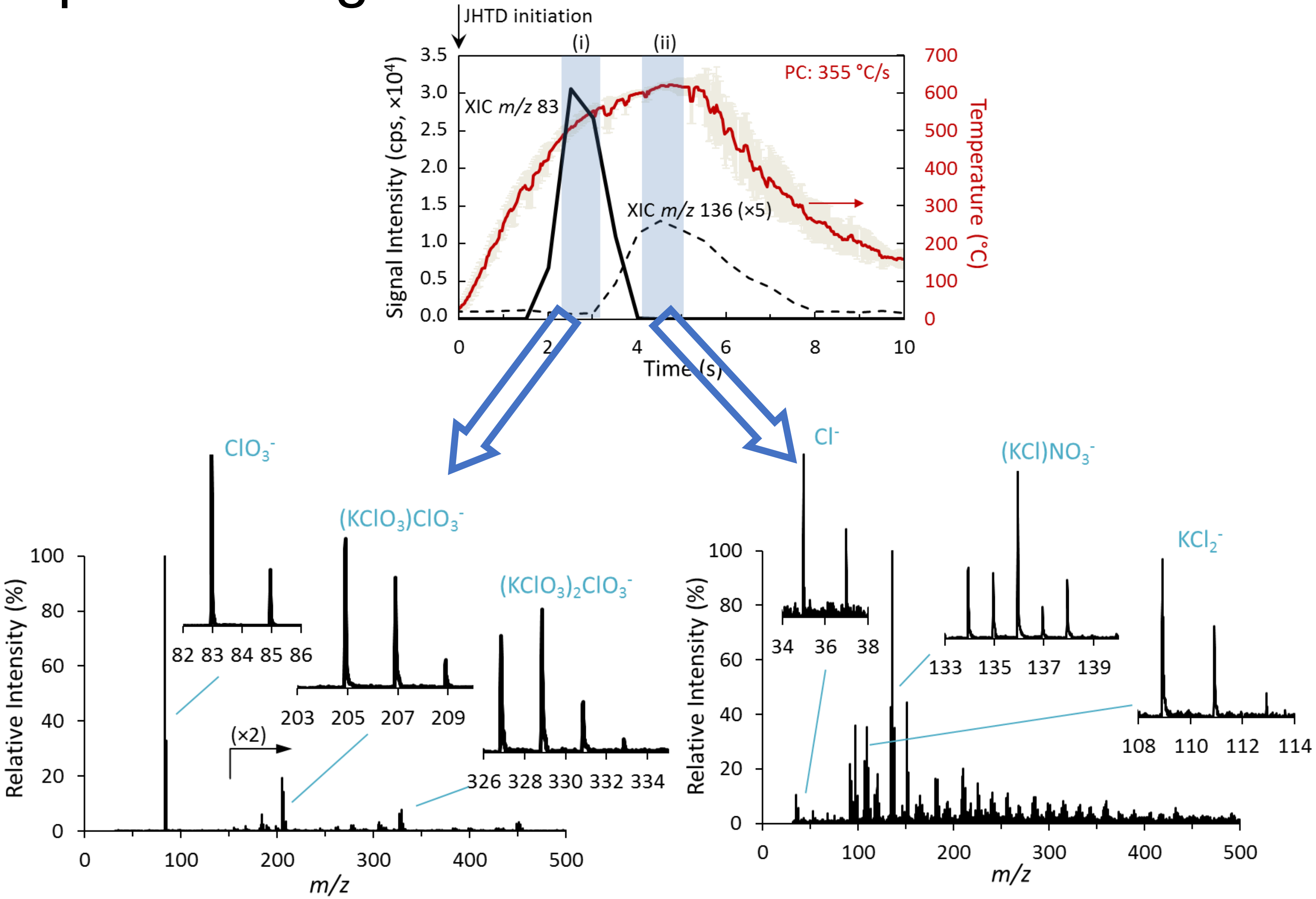


Forbes, T.P., Sisco, E., Staymates, M., and Gillen, G. (2017) *Analytical Methods,* **9**(34), 4988-4996. (https://doi.org/10.1039/C7AY00867H)

JHTD-DART-MS

- Elevated temperatures and rapid heating rates
- Regimes:
  - Aerosol/vapor generation
  - Thermal decomposition



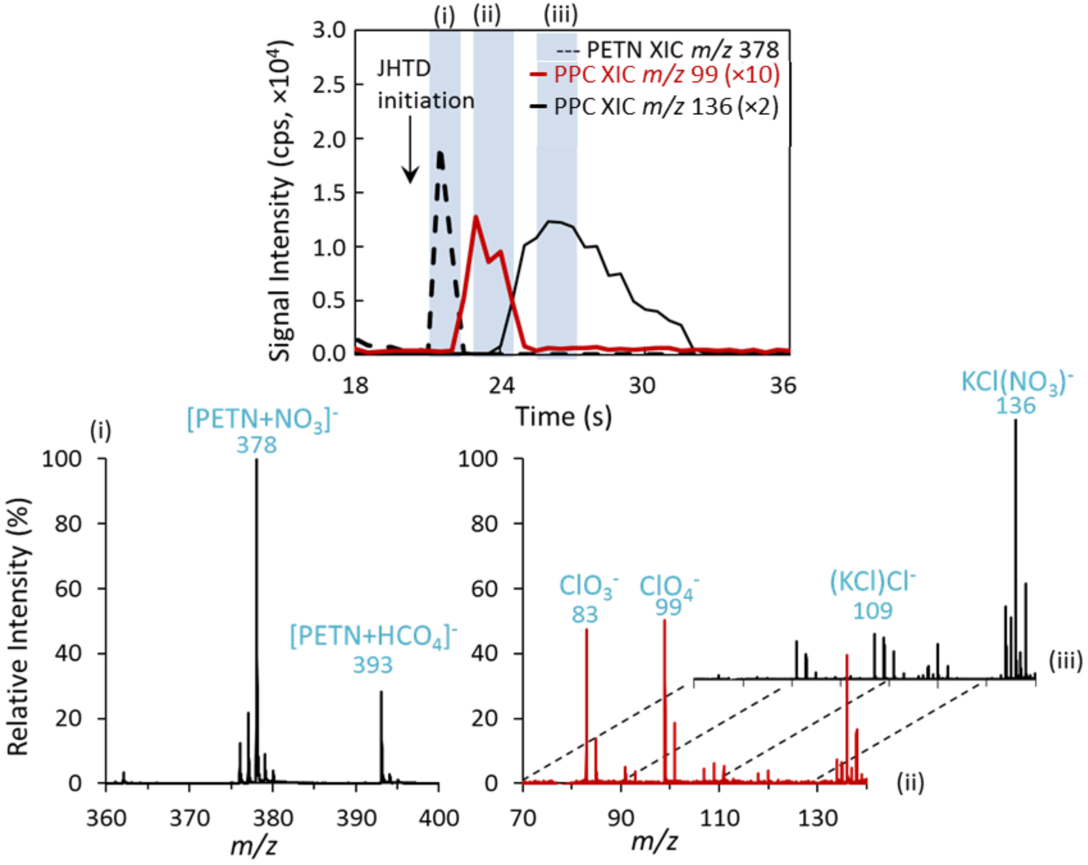


Forbes, T.P., Sisco, E., Staymates, M., and Gillen, G. (2017) *Analytical Methods,* **9**(34), 4988-4996. (https://doi.org/10.1039/C7AY00867H)

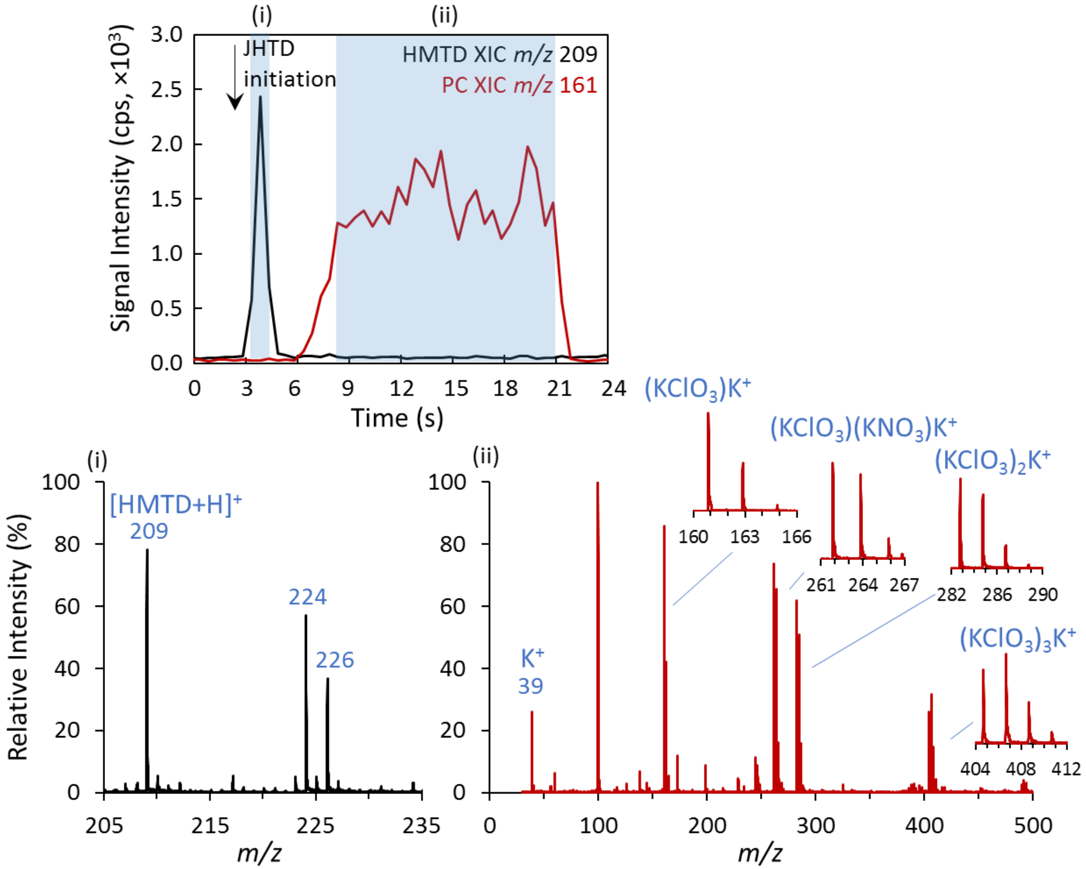
JHTD-DART-MS

- Detection of organic and inorganic mixtures
- Negative and positive mode operation

PETN-PPC Mixture



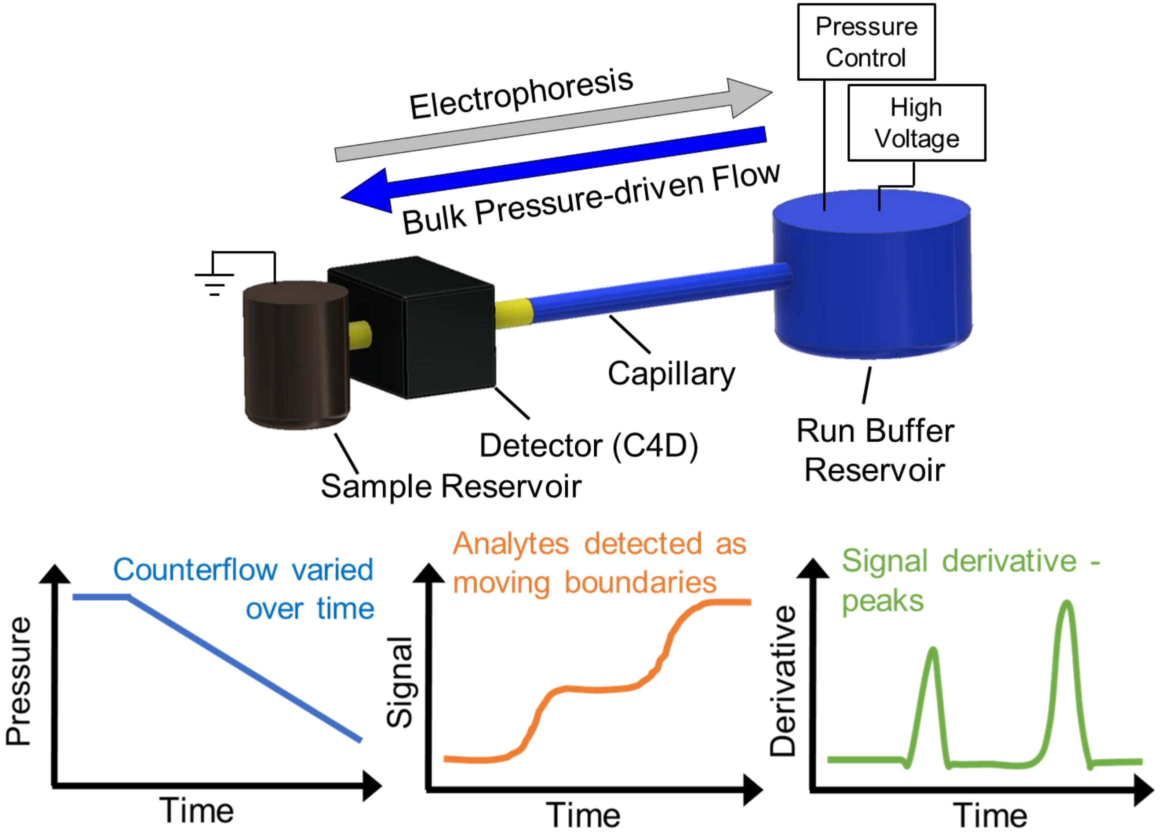
HMTD-PC Mixture

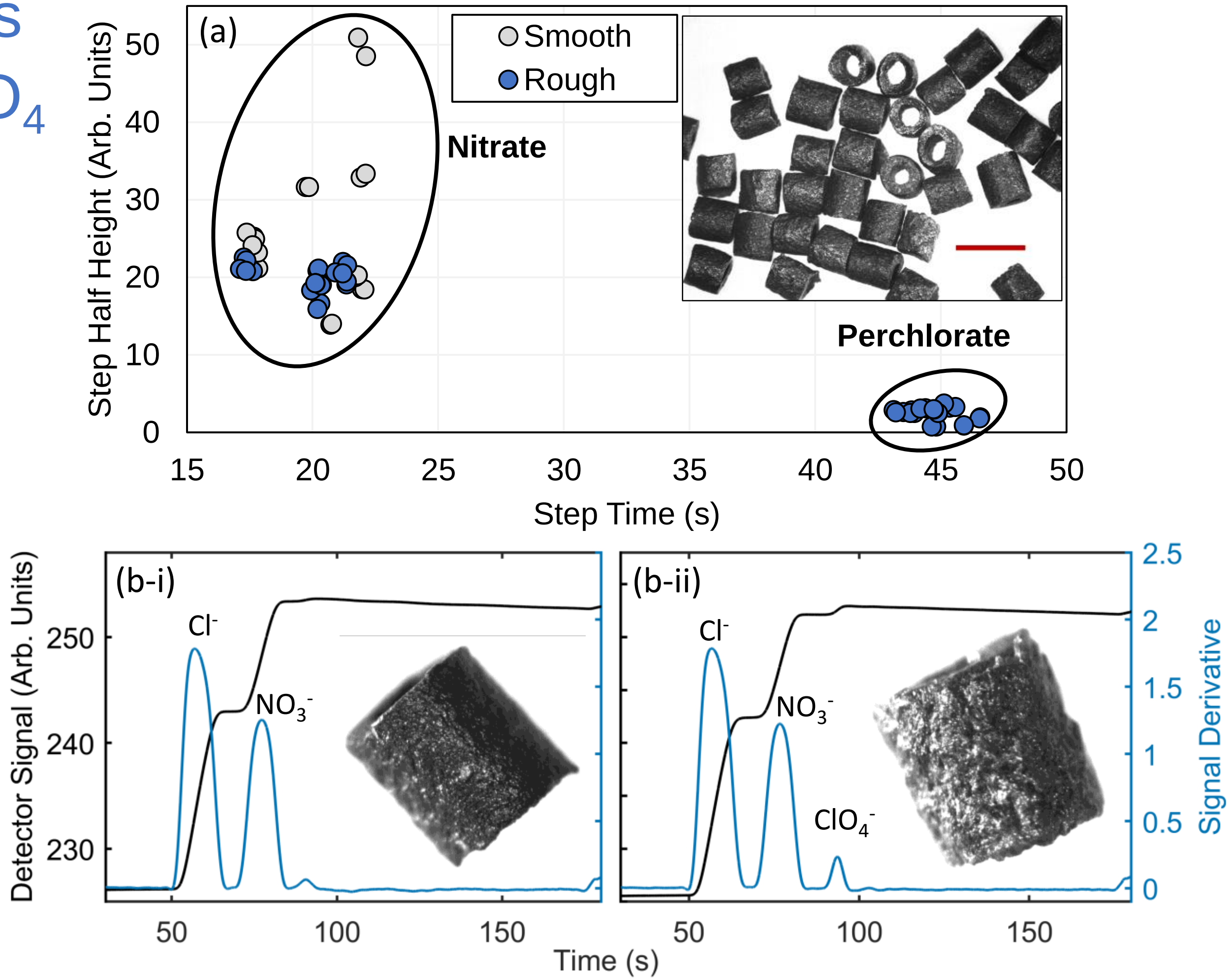


Forbes, T.P., Sisco, E., Staymates, M., and Gillen, G. (2017) *Analytical Methods*, **9**(34), 4988-4996. (https://doi.org/10.1039/C7AY00867H)

Single Particle Analysis – Blackhorn 209

- Gradient elution moving boundary electrophoresis (GEMBE)
  - Inorganic oxidizers – sample differences
  - Two subpopulations – NO_3 and NO_3/ClO_4

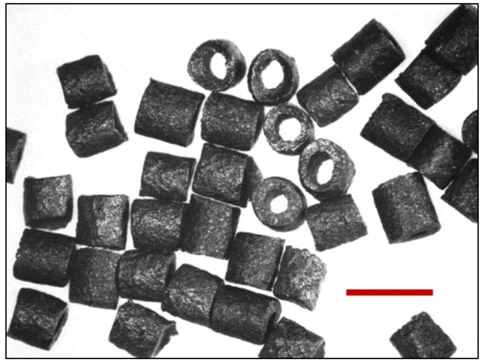


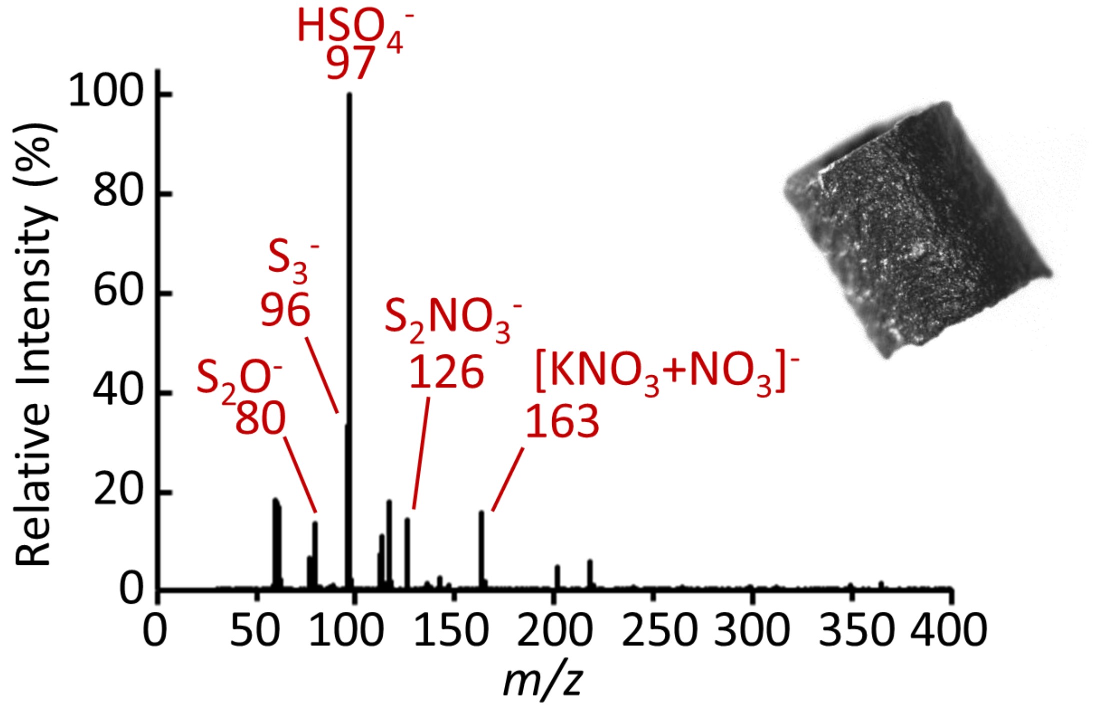


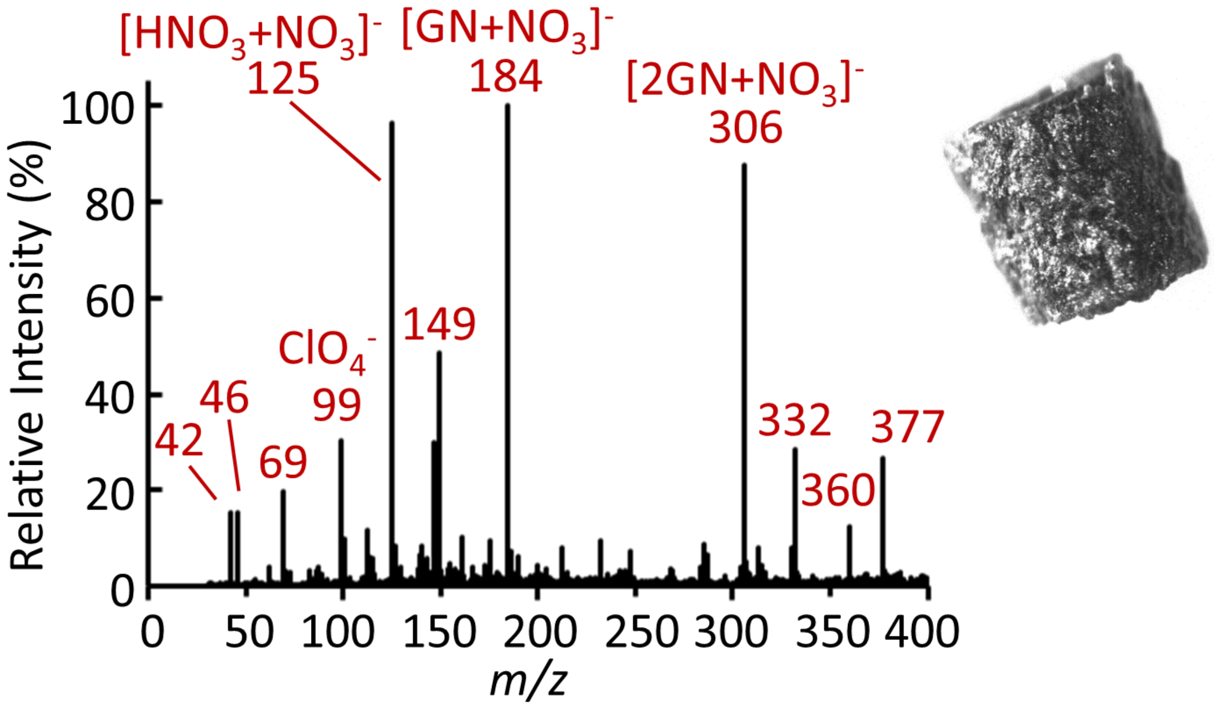
Krauss, S.T., Ross, D., Forbes, T.P. (2019) *Analytical Chemistry,* **91**, 13014-13021. (https://doi.org/10.1021/acs.analchem.9b03083)
Krauss, S.T., Forbes, T.P., Jobes, D., *Electrophoresis,* **Under Review**.

Single Particle Analysis – Blackhorn 209

- Two subpopulations observed:
  1. Black powder based – KNO_3/S/C
  2. Guanidine nitrate (GN) and $KClO_4$



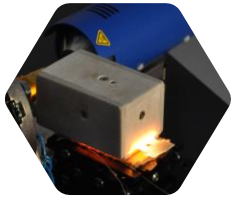




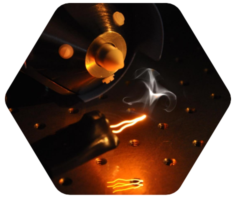
Krauss, S.T., Forbes, T.P., Jobes, D., *Electrophoresis,* **Under Review**.

Conclusions

- Hybrid thermal desorption–ambient MS enabled unique capabilities



  - IRTD-DART-MS: wipe-based samples for rapid screening



  - JHTD-DART-MS: discrete particle or liquid samples
    - Elevated temperatures – necessary for nonvolatile species
    - Rapid heating ramps – desorption at each compound’s optimal temperature
    - No degradation of organic or more volatile compounds
    - Heating profiles temporally separate mixtures
- Demonstrated for explosives
  - Homemade explosive fuel-oxidizer mixtures, propellants, pyrotechnics, powders
  - Nanogram sensitivities

Acknowledgements

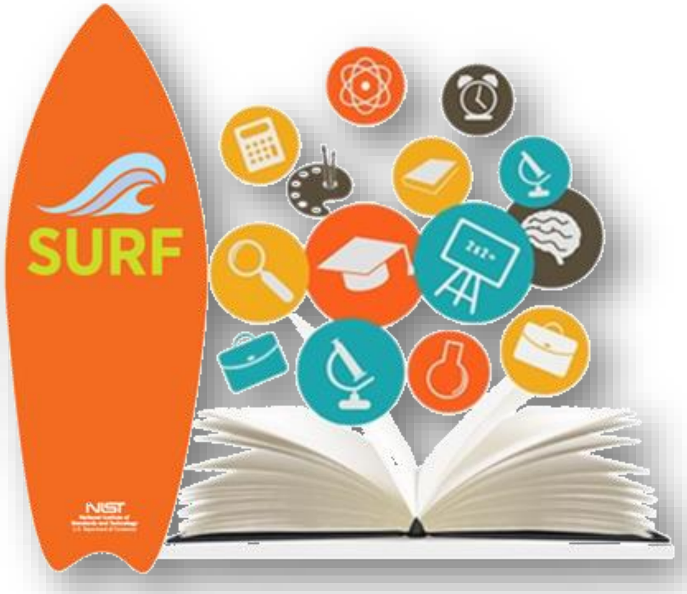
- Surface and Trace Chemical Analysis Group, NIST
  - Edward Sisco, Jennifer Verkouteren, Shannon Krauss, Matthew Staymates, Greg Gillen

- Funding
  - The U.S. Department of Homeland Security, Science and Technology Directorate sponsored a portion of the production of this material under Interagency Agreements IAA HSHQDC-12-X-00024 and HSHQPM-15-T-00050 with the National Institute of Standards and Technology.

Postdoctoral & Student Opportunities

- **NRC NIST Postdoctoral Research Associate Program**
  - http://www.nist.gov/iaao/postdoc.cfm
  - http://sites.nationalacademies.org/pga/rap/
    - Mass Spectrometry Metrology for Trace Detection and Chemical Imaging – RO# 50.64.31.B8187
    - Mass Spectrometry and Chemometrics for Forensic Science – RO# 50.64.31.C0217
    - Advanced Forensic Toxicology Measurements by Ambient Ionization MS– RO# 50.64.31.C0344
    - Sensor Technology, Measurement Science, and Standards – RO# 50.64.31.B8551
      - *U.S. citizens and held Ph.D. less than five years*
      - *Application deadlines of February 1 and August 1*

- **NIST Summer Undergraduate Research Fellowship (SURF)**
  - https://www.nist.gov/surf/surf-gaithersburg
    - *U.S. citizens or U.S. permanent residents*
    - *Must be enrolled as a full-time undergraduate at an accredited U.S. college/university*



Thank you

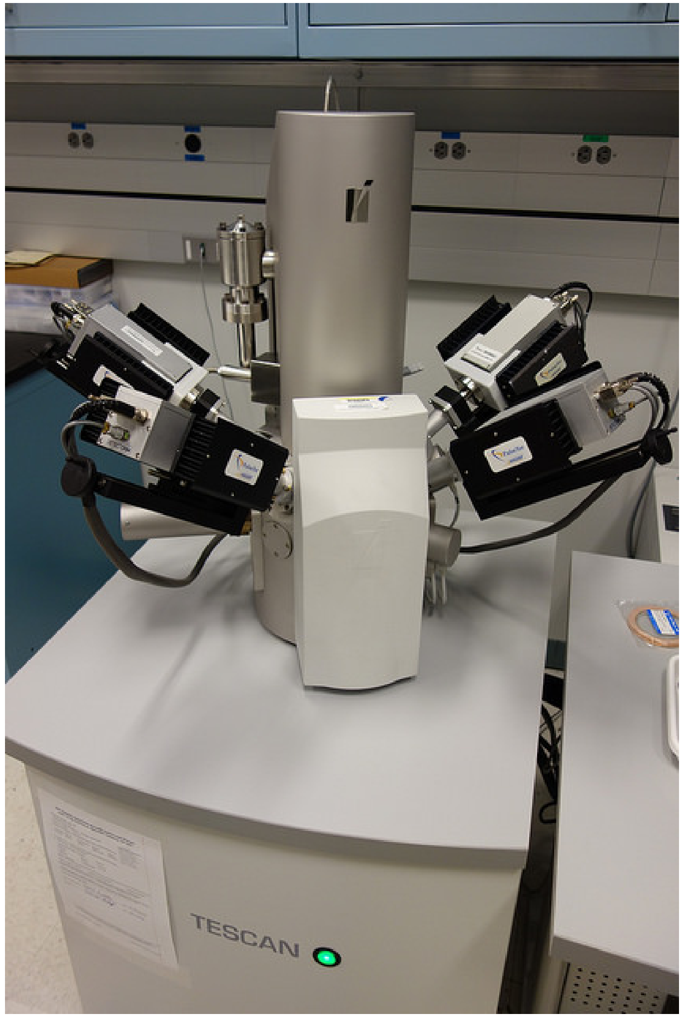
Correspondence: thomas.forbes@nist.gov

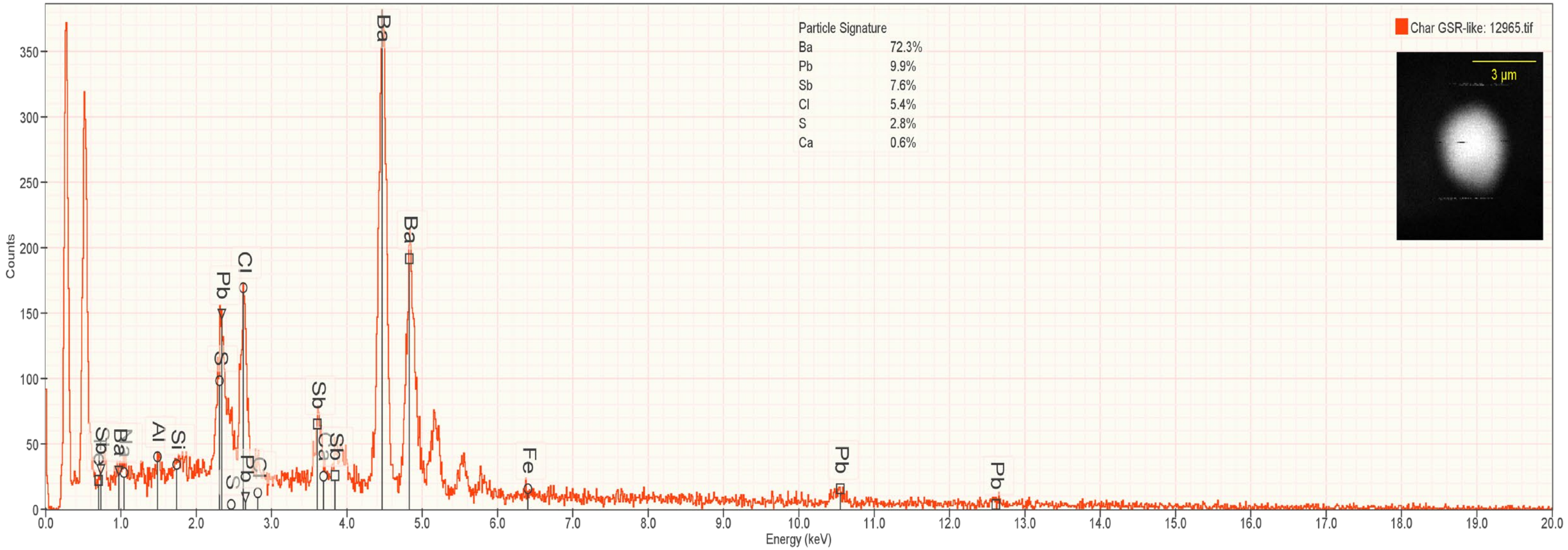
An objective, quantitative, statistically defensible scheme based on fitted intensity ratios for GSR analysis.

Nicholas W. M. Ritchie
Microanalysis Group, Material Measurement Laboratory
National Institute of Standards and Technology

Forensics@NIST 2020

iGSR particles

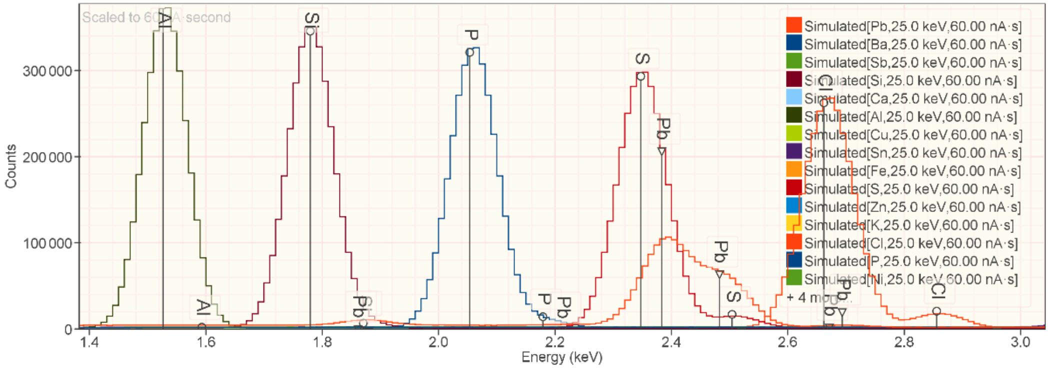




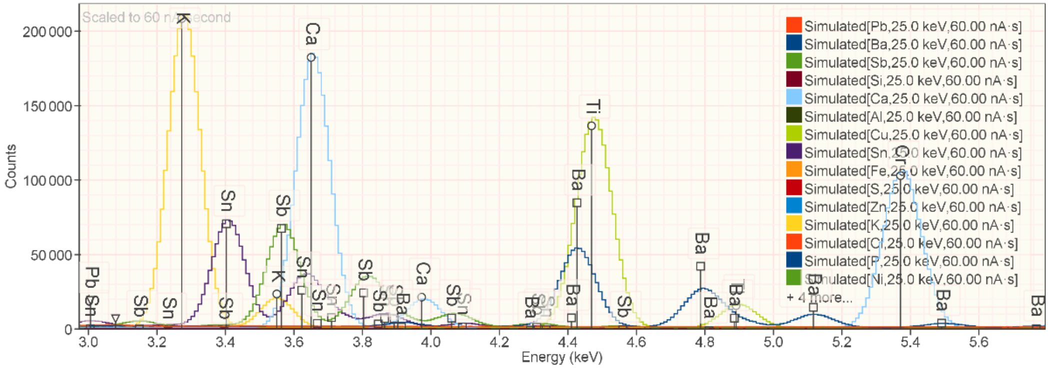
Particle Signature	
Ba	72.3%
Pb	9.9%
Sb	7.6%
Cl	5.4%
S	2.8%
Ca	0.6%

Characteristic Particle Types

Name	Required	Optional	Trace	Specials
Sinoxid	Pb, Ba, Sb	Si, Ca, Al, Cu, Sn	Fe, S, Zn, K, Cl, P, Ni	Co, Cr
SBP	Pb, Ba, Ca, Si, Sn	Cu, Fe, S, Zn, K, Cl		
Sintox – RUAG	Ca, Ti, Zn		Ca, S	
Sintox – MEN	Ca, Cu, Sn		K, S	



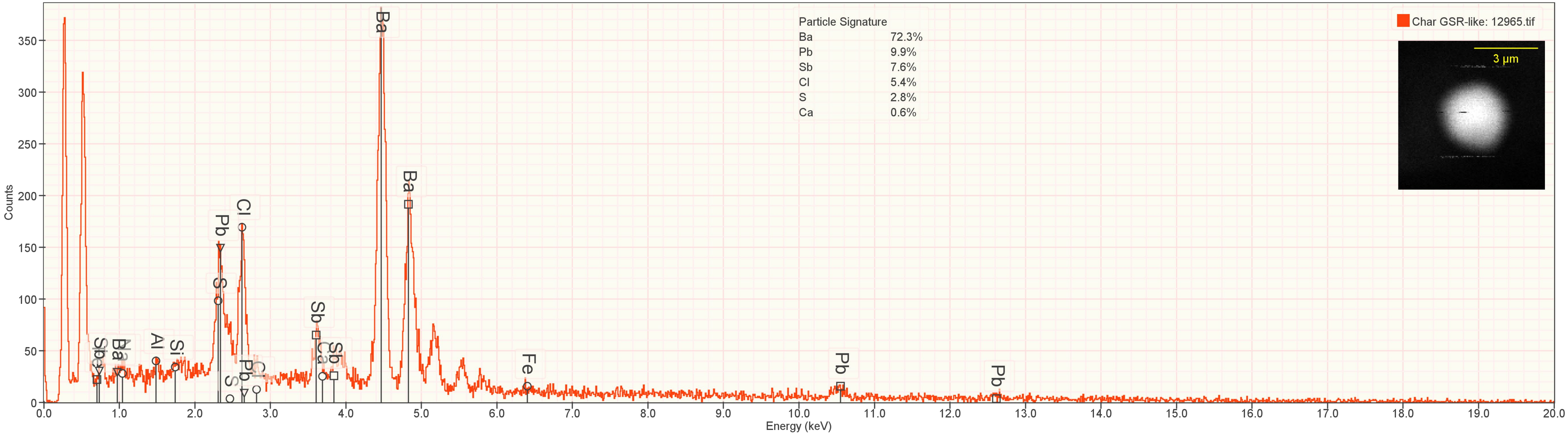
(S, Pb)



(Sn, K, Sb), (Sb, Ca), (Ba, Ti)

Source: SWGGSR – Guide for Primer Gunshot Residue Analysis by Scanning Electron Microscope / Energy Dispersive X-ray Spectrometer.

Manual Review



Particle Signature	
Ba	72.3%
Pb	9.9%
Sb	7.6%
Cl	5.4%
S	2.8%
Ca	0.6%

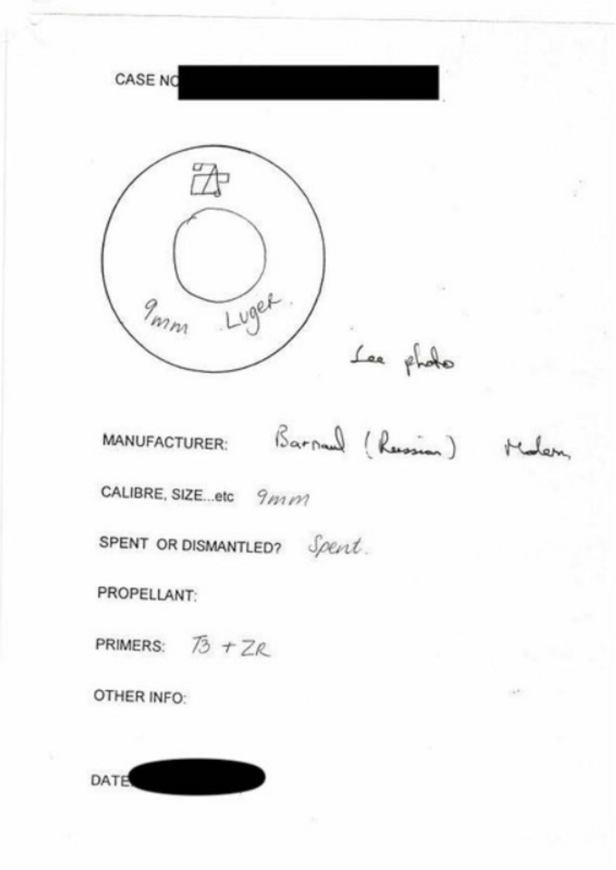
Does anybody know of a published article stating that pGSR particles from Barnaul ammunition contain Zr? Or, does anyone have well-documented spectra from Barnaul ammo that shows Zr in the spectra?

I know several of us have done some testing regarding this, but I'm tired of re-inventing the wheel every time I get a Barnaul ammo case and see Zr…

Thanks,

Rusty

Hey Rusty - this help?



CASE NO [redacted]

See photo

MANUFACTURER: Barnaul (Russian) Modern

CALIBRE, SIZE...etc 9mm

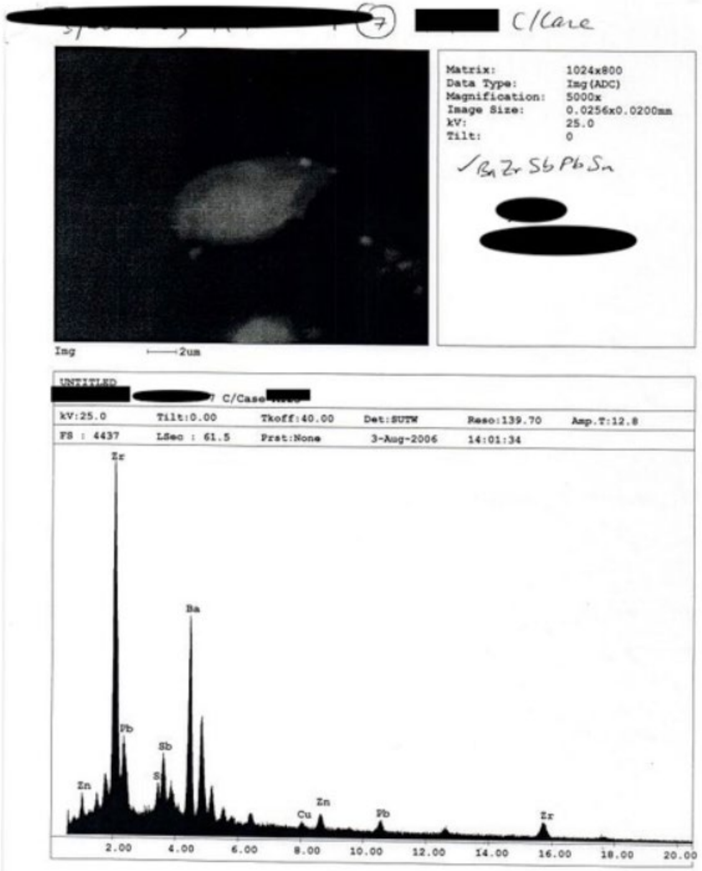
SPENT OR DISMANTLED? Spent.

PROPELLANT:

PRIMERS: B + ZR

OTHER INFO:

DATE [redacted]



[redacted] ⑦ [redacted] C/Case

Matrix: 1024x800
Data Type: Img (ADC)
Magnification: 5000x
Image Size: 0.0256x0.0200mm
kV: 25.0
Tilt: 0

✓Ba Zr Sb Pb Sn

[redacted]

Img 2um

UNTITLED
[redacted] 7 C/Case [redacted]

kV:25.0 Tilt:0.00 Tkoff:40.00 Det:SUTW Reso:139.70 Amp.T:12.8
FS : 4437 LSec : 61.5 Prst:None 3-Aug-2006 14:01:34

Angela Shaw BSc(Hons) MCSFS, MEWI
Director
www.forensicfirearmsconsultancy.com
Tel: +44 7919 392397
Secure email: angela.shaw@ffc.cjsm.net

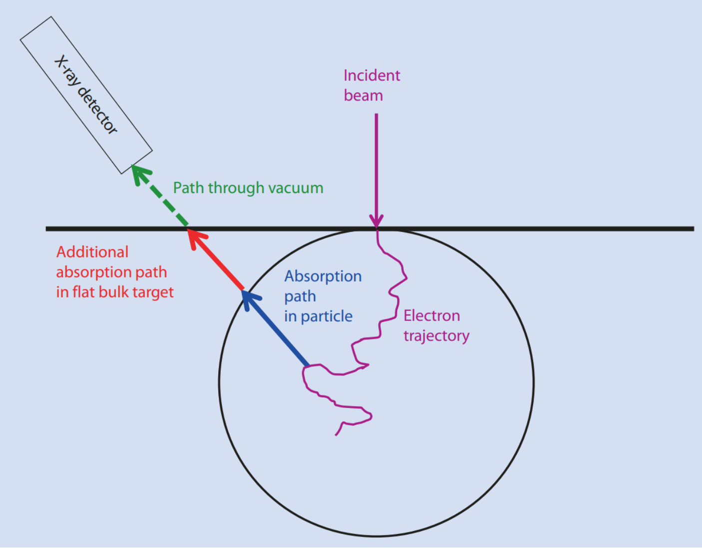
What can we learn from this example?

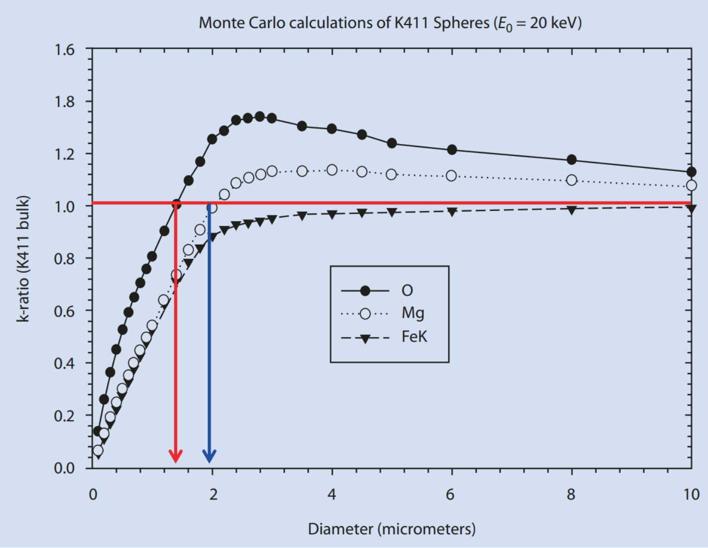
- The GSR community is tight-knit and helpful
- iGSR has distinctive characteristics that provide clues to assist with attribution
- Knowledge of these clues is available but not well documented or well shared
  - Local expert knowledge
- Even when documented, the information is largely qualitative

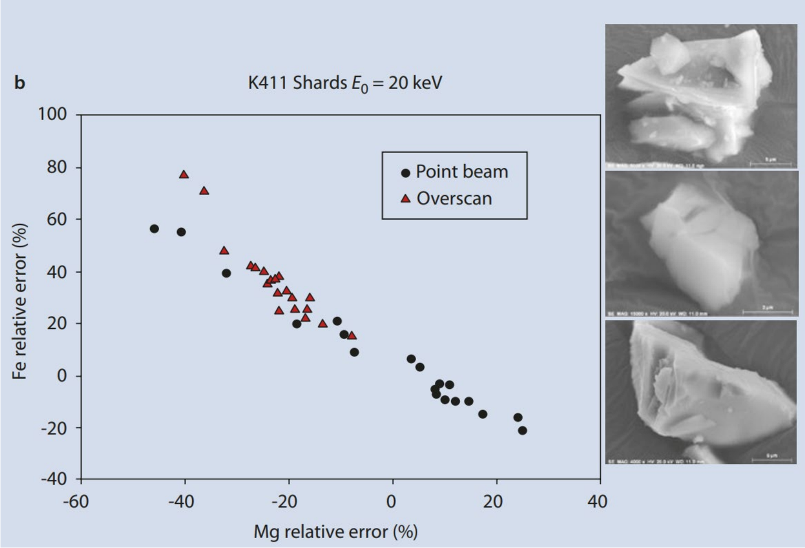
Why is this?

- Quantification of particles is difficult
    - Estimating the mass fraction of an element in a particle
- Fortunately, quantification isn’t necessary to determine the presence of an element
    - K-ratios are sufficient
- Linear regression using standard spectra is our friend
    - Linear regression provides uncertainty metrics
- Uncertainty metrics can be used to make defensible, quantitative statements about the presence or absence of an element
    - Even in the presence of interfering elements!

Bulk vs. Particles







$$C_{\text{unk}} = k_z \, C_{\text{std}} \frac{\text{ZAF}\left(C_{\text{std}};P\right)}{ZAF\left(C_{\text{unk}};P\right)}$$

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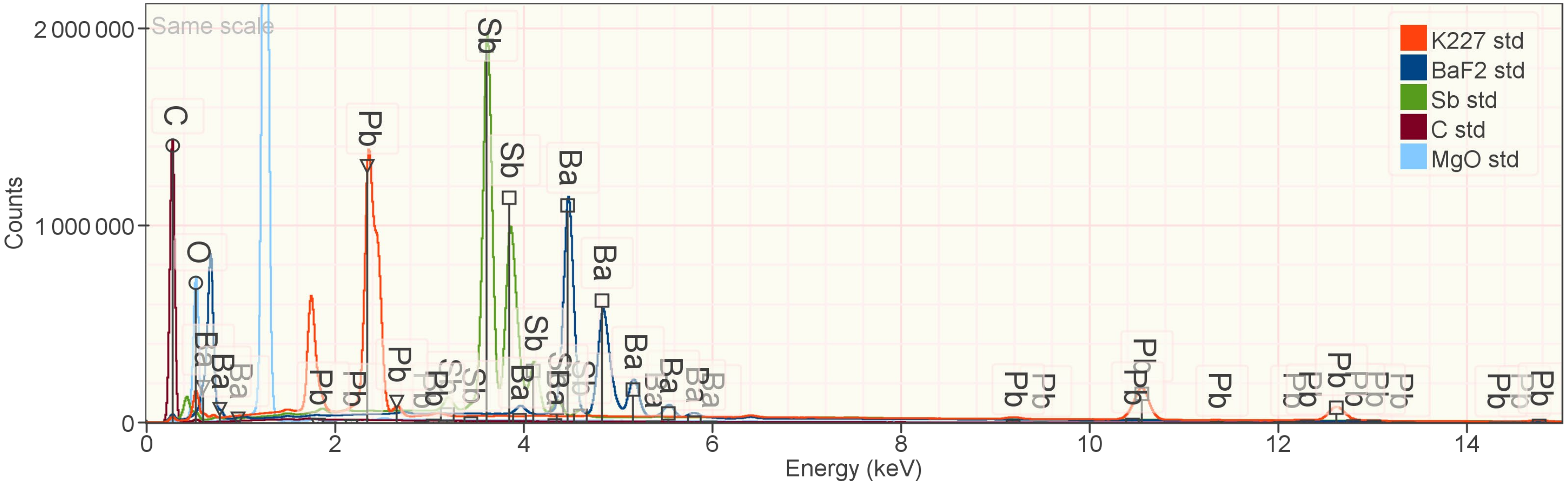
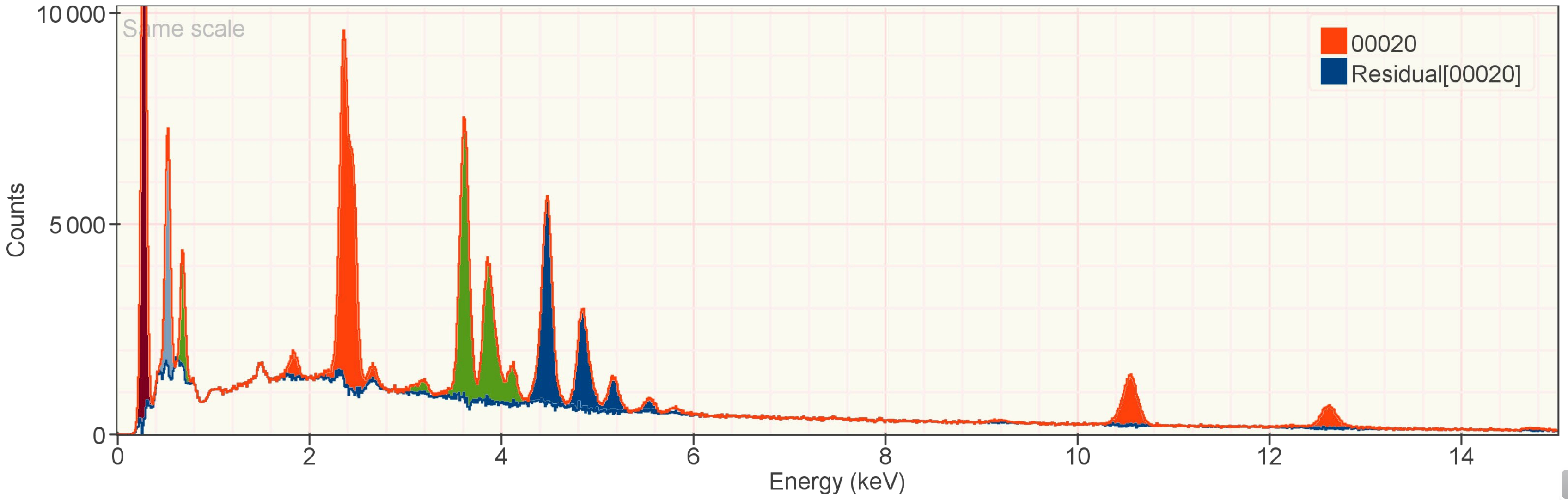
    - Even in the presence of interfering elements!

k-Ratios

C K-L2 = 0.22317 ± 0.00051
O All = 0.14212 ± 0.00086
F All = 0.13810 ± 0.00133
Sb L3-M5 = 0.06922 ± 0.00028
Ba L3-M5 = 0.08751 ± 0.00042
Ba L3-M1 = 0.08589 ± 0.01249
Pb L2-M4 = 0.14162 ± 0.00406
Pb L2-N4 = 0.12756 ± 0.02388
Pb L1-O3 = 0.22361 ± 0.2899
Pb L2-M1 = 0.21235 ± 0.14087
Pb L3-M1 = 0.10739 ± 0.02426
Pb Lα = 0.13907 ± 0.00229
Pb M3-O5 = 0.22722 ± 0.02723
Pb M5-N7 = 0.11897 ± 0.00061





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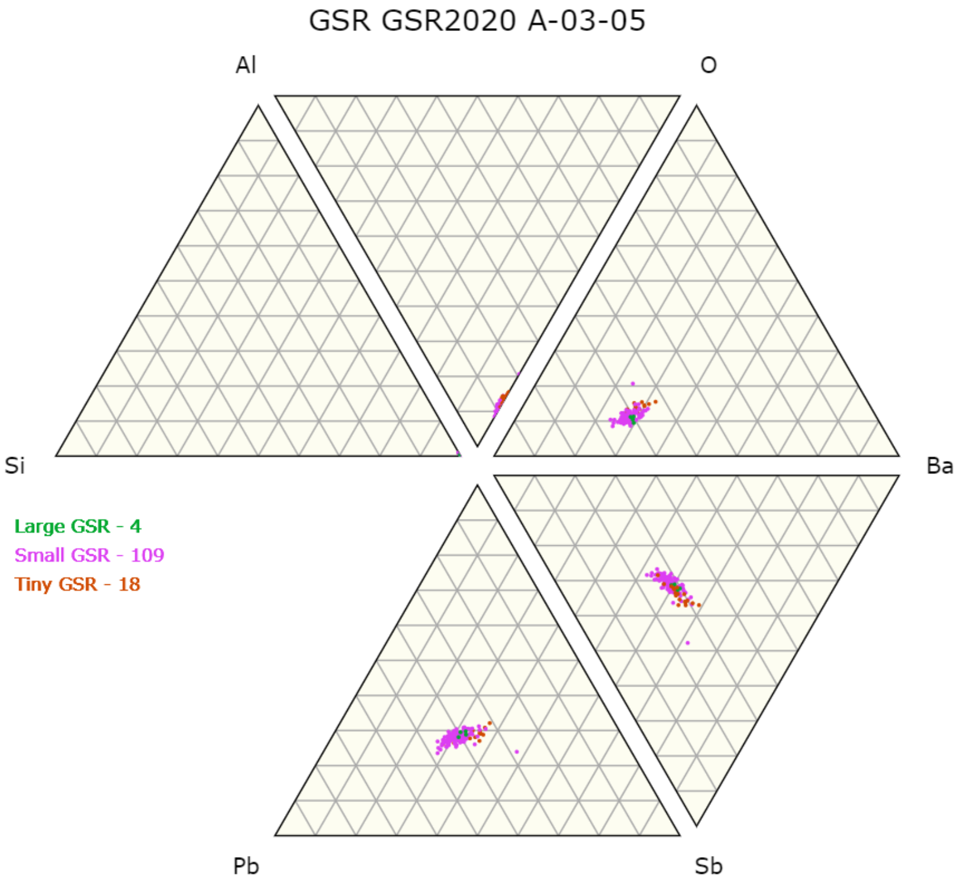
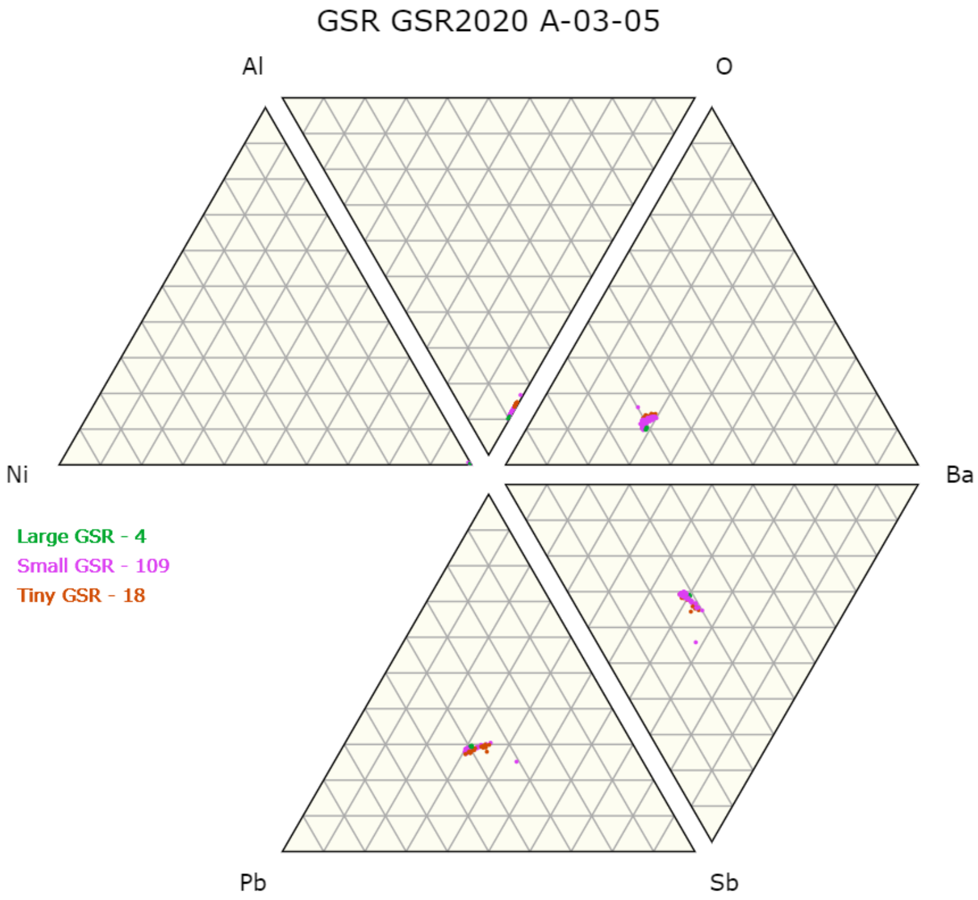
Automated



Live time = 0.4 s

Manual



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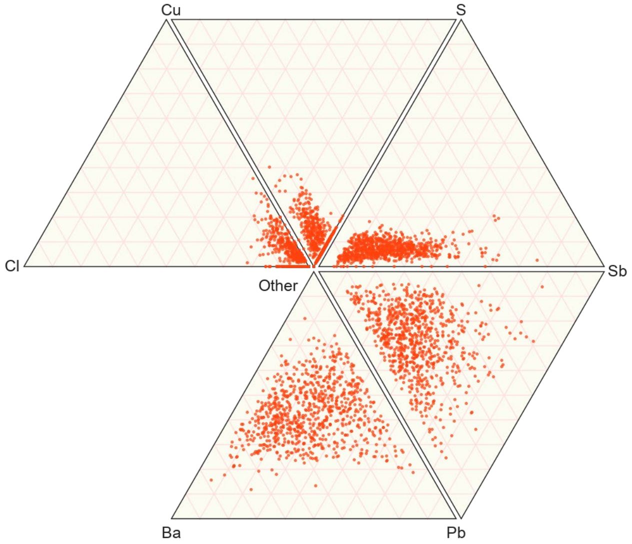
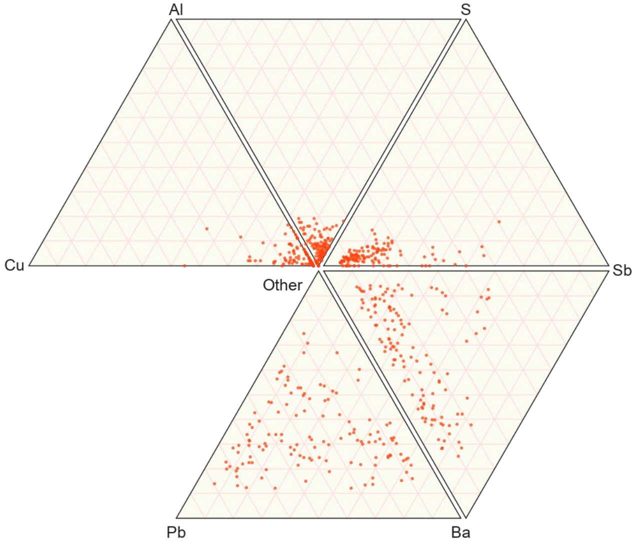


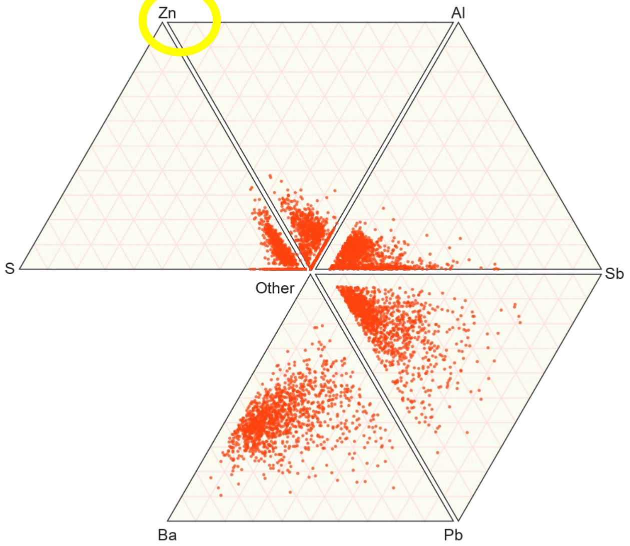
Char GSR-like

Shooter #1



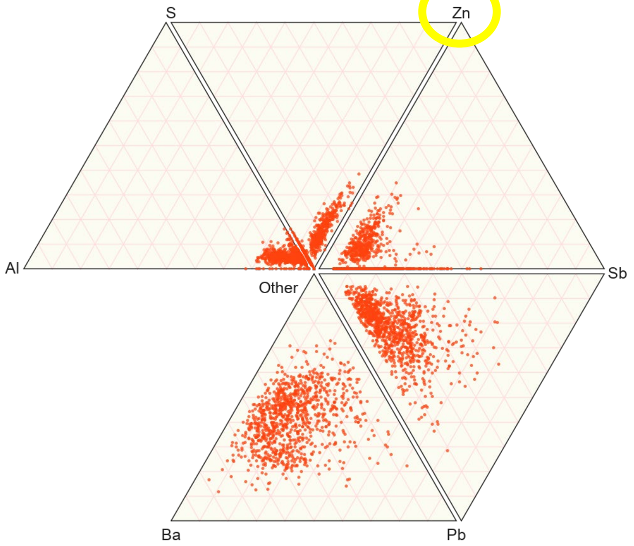
Char GSR-like

Shooter #2



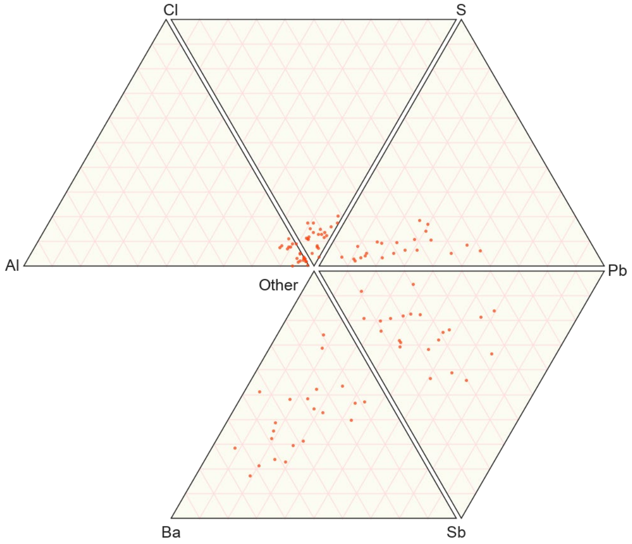
Char GSR-like

Shooter #3L



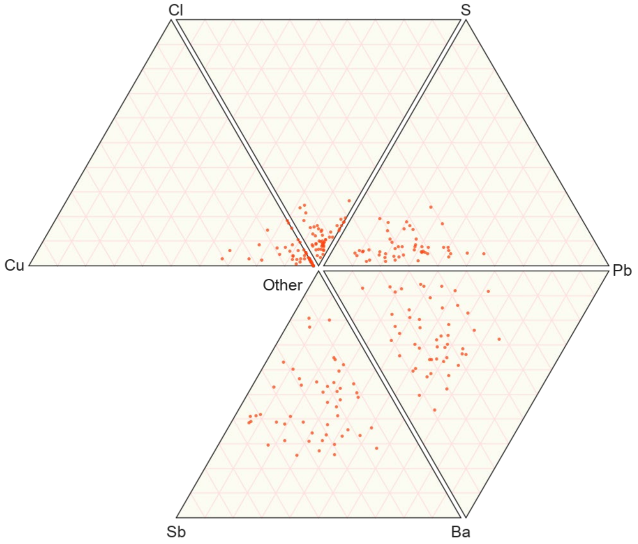
Char GSR-like

Shooter #3R



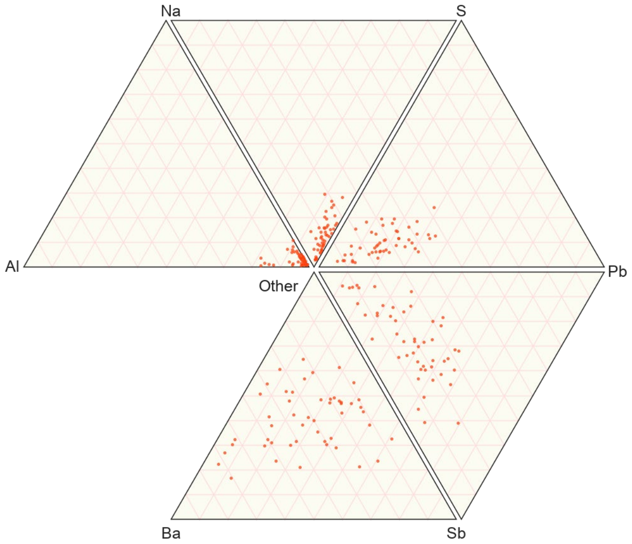
Char GSR-like

Shooter #4L



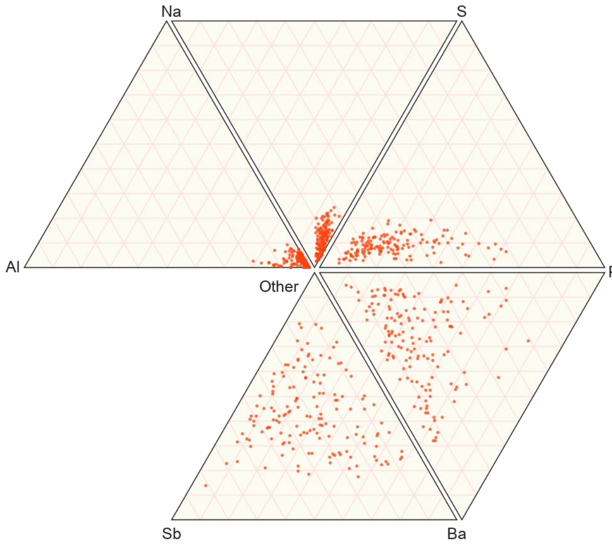
Char GSR-like

Shooter #4R



Char GSR-like

Shooter #5L



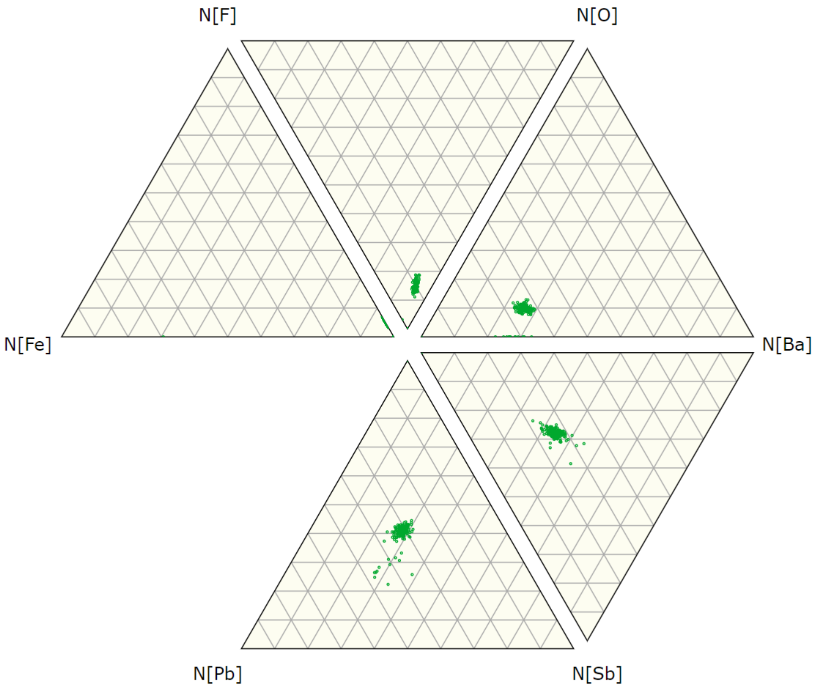
Char GSR-like

Shooter #5L

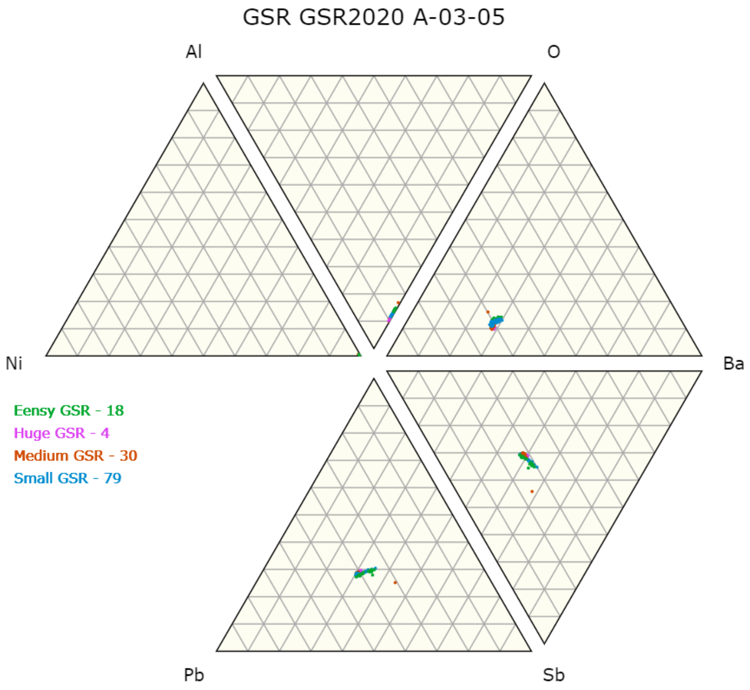
Challenges

- Community buy-in
  - Community is quite content with current way of doing things
- Vendor support
  - Must have support from the major GSR tool vendors
  - The vendors must produce similar answers
- Necessary Research
  - Who is going to perform the research needed to catalog and characterize the different types of GSR?

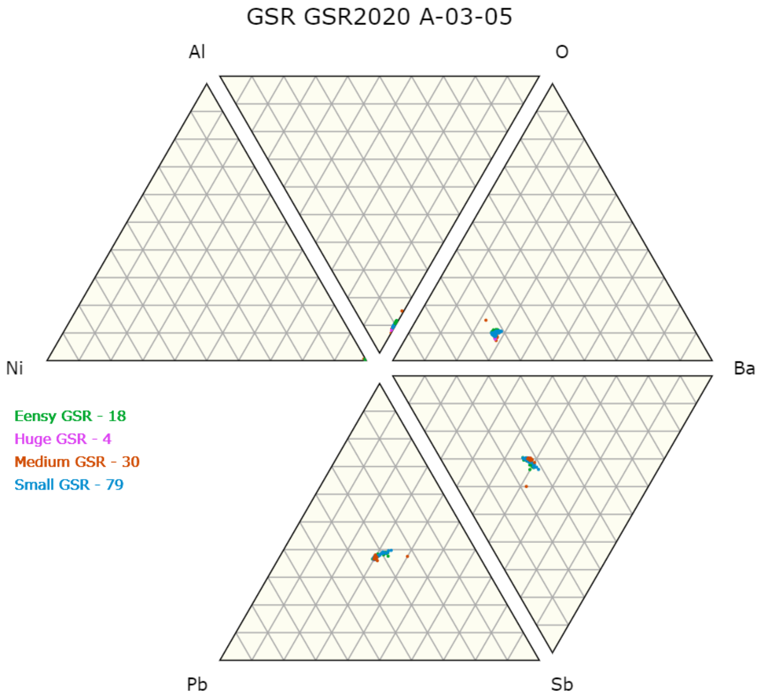
Reproducibility across vendors



Vendor A



NIST A



NIST B

Challenges

- Community buy-in
  - Community is quite content with current way of doing things
- Vendor support
  - Must have support from the major GSR tool vendors
  - The vendors must produce similar answers
- Necessary Research
  - Who is going to perform the research needed to catalog and characterize the different types of GSR?
    - Not a “do it and done”
    - Requires a long-term commitment with steady, reliable funding

Thank you!