PROGRAM

All sessions will take place in the Belleair-Redington Room
Speakers – please arrive ½ hour before you session begins to connect laptop.

FRIDAY, JANUARY 28

6:00 - 8:00 pm	**Registration and Poster Set-Up** Posters may be set up on the boards in Indian Shores/Madeira, *See page 4 - 6 for poster board numbers.* Posters should be removed Monday after closing comments.
8:00 – 8:45- PM	**Special Presentation** with Introduction by Julia Dolan **Ken Alibek,** *Former First Deputy Chief, secret Soviet germ warfare program; George Mason University* 2001 Anthrax Attack: Was the Anthrax Weaponized?
8:45 – 9:45 PM	**Reception,** *Indian Shores/Madeira*

SATURDAY, JANUARY 29

8:30 – 8:40 am	**Opening Remarks/Welcome,** Pete Dreifuss, Brian Eckenrode and Julia Dolan
8:45 – 10:00 am	**Session I: Stable Isotopes in Forensics and Counterterrorism** James Ehleringer, Moderator
8:45 – 9:10 am	**Mary Russ**, *Oak Ridge Institute of Science and Education (ORISE) / FBI Academy* Isotope Ratio Contributions to Homeland Security Challenges: Technical Advances
9:20 – 9:50 am	**James R. Ehleringer**, *University of Utah* Isotope Ratio Contributions to Homeland Security Challenges: Case Studies
10:00 – 10:20 am	**Coffee Break**
10:20 am – 12:25 pm	**Session II: New Ionization and Instrumental Method** Bob Cotter, Moderator
10:20 – 10:50 am	**R. Graham Cooks,** *Purdue University* Miniature Mass Spectrometers and Other Developments for Explosive and CW Detection
10:50 - 11:20 am	**Robert B. Cody,** *JEOL USA, Inc.* and **James A. Laramee,** *EAI Corporation* DART: Direct Analysis in Real time for Drugs, Explosives, Accelerants, Chemical Agents and More…
11:30 - 11:55 am	**Alisha Mitchell-Roberts,** *University of Florida* The Role of FAIMS and MS/MS in Biological Agent Analysis by AP-MALDI
12:00 – 12:25 am	**Robert J. Cotter,** *Johns Hopkins University* Biodetection Instrumentation
12:30 – 2:00 pm	**Lunch for conferees.** Name badge is required. Sorry no guests. *Watercolour Grille/Veranda*

2:00 – 3:00 pm	**Special Presentation** with Introduction by Brian Eckenrode
2:10 – 3:00 pm	**Steven Burmeister,** *FBI Counter Terrorism Division* Terrorist Explosives Device Analytical Center (TEDAC), FBI
3:10 – 3:40 pm	**Coffee Break**
3:45 – 5:15 pm	**Session III: Analysis and Detection of Explosives** Pete Dreifuss, Moderator
3:45 – 4:10 pm	**John V. Goodpaster,** *Bureau of Alcohol, Tobacco, Firearms and Explosives* Novel Analyses of Black Powder Substitutes and Their Post-Blast Residues by Reverse Phase ESI LC/MS
4:15 – 4:40 pm	**Mike Sigman,** *National Center for Forensic Science, University of Central Florida* Characterization of Emulsions, Water Gels, Slurries, and Plastic Bonded Explosives
4:45 – 5:10 pm	**Aviv Amirav,** *Tel Aviv University* Improved Confidence Level in Sample Identification with the Supersonic GC-MS and LC-EI-MS
5:15 – 7:00 pm	**Dinner on your own**
7:15 – 8:00 pm	**Edward C. Bender,** *Forensic Science Laboratory, Natl. Laboratory Center, ATF* The Analysis of Explosive Post Blast Seats, Debris Fields and Device Components – The First Step
8:00 – 9:00 pm	**Reception,** *Indian Shores/Madeira Foyer*

SUNDAY, JANUARY 30

8:00 – 10:00 am	**Session IV: Drug Testing, Toxicology and Accelerant Applications** Julia Dolan, Moderator
8:00 – 8:25 am	**Larry D. Bowers,** *US Anti-Doping Agency* Application of Mass Spectrometry in Athletic Drug Testing: Steroids to Protein Hormones
8:30 – 8:55 am	**Julia Pearson,** *Virginia State Crime Laboratory* Case Studies in Homicidal Poisonings
9:00 – 9:25 am	**Glenn Frysinger,** *U. S. Coast Guard* Applications of Comprehensive GCxGC-MS to Fire Debris Analysis
9:30 – 9:55 am	**Jack Nowicki,** *Illinois State Police, Chicago* Analysis and Detection of Accelerants
10:00 – 10:20 am	**Coffee Break**

SUNDAY, continued

10:25 am – 12:30 pm	**Session V: Analysis and Detection of Biological Warfare Agents** Catherine C. Fenselau, Moderator
10:25 – 10:55 am	**Pete Snyder,** *US Army, Aberdeen Proving Ground* Field and Laboratory Analytical Analyses of Bioaerosols and Chemical Agents
11:00 – 11:25 am	**Steven Hofstadler,** *IBIS Therapeutics* TIGER – Detecting Newly Emerging Pathogens by MS
11:30 – 11:55 am	**Bruce Budowle,** *Federal Bureau of Investigation* MS in Microbial Forensics
12:00 – 12:25 pm	**Catherine Fenselau,** *University of Maryland* Microbial Forensics Using MS
12:30 am	**Lunch on your own; afternoon free**
7:00 – 8:00 pm	**Session VI: Poster Previews** (5 minutes – limit of 2 technical slides each**)** Pete Dreifuss, Moderator
8:00 – 10:00 pm	**Evening Poster Session and Reception,** *Indian Shores/Madeira* Please see list starting on page 4. Poster abstracts begin on page 7.

MONDAY, JANUARY 31

8:30am – 9:55 am	**Session VII: Analysis and Detection of Chemical Weapons and OPCW Monitoring** John G. Reynolds, Moderator
8:30 – 8:55 am	**Gary Mallard,** *National Institutes of Standards and Technology* OPCW Proficiency Issues and New Developments with AMDIS
9:00 – 9:25 am	**Marcus Wise,** *Oak Ridge National Laboratory* New Applications and Development in Block II Chemical and Bio-Detection
9:30 – 9:55 am	**John G. Reynolds,** *Lawrence Livermore National Labs* Overview of Chemical Weapons Convention and High Explosives Programs at LLNL
10:00 – 11:15 am	**Coffee Break and Poster Viewing**
11:15 am – 12:15 pm	**Session VIII: MS Methods for Biological Detection and Identification** Brian Eckenrode, Moderator
11:15 – 11:45 am	**Dirk van den Boom,** *Sequenome* High Performance Nucleic Acid Analysis With Array MALDI TOF
11:50 – 12:15 am	**Jacek P. Dworzanski,** *Geocenters, Inc.* Mass Spectrometry-Based Proteomics for Classification and Identification of Airborne Bacteria
12:20 – 1:50 pm	**Lunch for conferees.** Name badge is required. Sorry no guests. *Watercolour Grille/Veranda*

2:00 – 3:15 pm	**Session IX: Final Session – Selected Poster Oral Presentations**
2:00 – 2:15 pm	**Danielle N. Dickinson**, *Oak Ridge Institute for Science and Education and Federal Bureau of Investigation* Mass Spectrometry Investigations of *Bacillus anthracis-cerus-thuringiensis* Group Spores
2:20 – 2:35 pm	**Christopher J. Wolyniak**, *Division of Nutritional Sciences, Cornell University* Potential Application of Position Specific Isotope Analysis for Sourcing
2:40 – 2:55 pm	**Lateefah A. Stanford**, *Department of Chemistry and Biochemistry, Florida State University* Forensic Environmental Fingerprinting: Monitoring Biotic and Abiotic Compositional Changes in Heavy Crude Oil Determined by ESI FT-ICR Mass Spectrometry
3:00 – 3:15 pm	**Miriam Fico**, *Purdue University* Design Considerations, Simulation, and Fabrication of an Array of Micron-scaled Cylindrical Ion Trap Mass Analyzers
3:20 – 3:25 pm	**Closing Remarks** Pete Dreifuss, Julia Dolan and Brian Eckenrode

Thank you for attending the 17h Sanibel Conference on Mass Spectrometry.
Please take a moment to complete the survey on page 37.

Directory of Participants begins on page 39.

POSTERS

Please set up posters on Friday evening and remove Monday after the close of the program.

1. Corporate Poster: **Ionics Mass Spectrometry Group**

2. **Isotope Abundance Analysis Software for Improved Sample Identification with the Supersonic GC-MS and LC-EI-MS**; Aviv Amirav and Tal Alon; *School of Chemistry, Tel Aviv University*

3. **Purification and Characterization of the Plant Lectin Abrin from *Abrus precatorius,* Using Galactose-affinity Chromatography in Concert With LC-MS/MS**; Jason L. Dabbs, Neil J. Bonzagni and Robert L. Bull; *Biological Defense Research Directorate, Naval Medical Research Cente*

4. **Implications of Miniaturising Linear Ion Trap Arrays for Portable Detectors**; S.N. Cairns, D.P.A. Kilgour, M.D. Brookes, *Defence Science and Technology Laboratory (Dstl)*; J.F.J. Todd, *University of Kent*

5. **Growth Media Differentiation of *Bacillus subtilis* Spores by Elemental Characterization Using Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS);** [1]John Cliff, [1]Daniel Gaspar, [2]Stephen Golledge, [1]Kristin Jarman, [1]Nancy Valentine, [1]Karen Wahl, and [1]David Wunschel; *[1]Pacific Northwest National Laboratory*, *[2]University of Oregon*

6. **Analysis and Characterization of Tryptamine Analogues using Electrospray Ionization Mass Spectrometry (ESI-MS);** Sandra E. Rodriguez-Cruz, Ph.D. *Forensic Chemist, Drug Enforcement Administration Southwest Laboratory*

7. **Analysis of Chemical Warfare Agents in Contaminated Indoor Sample Media by High Resolution LC-ESI-MS/MS Analysis** ; Paul A. D'Agostino, Claude L. Chenier and James R. Hancock, *DRDC*

8. **Broad and Rapid Identification of Biological Threat Agents Using Atmospheric Pressure MALDI Tandem Mass Spectrometry;** Vladimir M. Doroshenko, Nelli I. Taranenko, Gavin E. Black, Vadym D. Berkout, Robert M. Serino, H. Sang Lee, *Science & Engineering Services, Inc.*

9. ***Clostridium botulinum*** **Neurotoxin Identification Using, Atmospheric Pressure MALDI Ion Trap Mass Spectrometry;** Nelli I. Taranenko, Gavin E. Black, Robert M. Serino and Vladimir M. Doroshenko*, Science & Engineering Services, Inc*

10. **Mass Spectrometry Investigations of *Bacillus anthracis-cerus-thuringiensis* Group Spores;** Danielle N. Dickinson[1,2], Brian A. Eckenrode[2], David H. Powell[3], Kasthuri Venkateswaran[4], Bruce Budoule[5]; *[1]Oak Ridge Institute for Science and Education*; *[2]Federal Bureau of Investigation, Counterterrorism and Forensic Science Research Unit*; *[3]Department of Chemistry, University of Florida*; *[4]Jet Propulsion Laboratory*, *California Institute of Technology*; *[5]Federal Bureau of Investigation, Chem-Bio Sciences Unit*

11. ***Coxiella burnetii*** **Strain Identification by Partial Least Squares-Discriminant Analysis of MALDI-TOF MS Biomarker Fingerprints'** Carrie L. Young[1,2], John R. Barr[1], Adrian R. Woolfitt[1], Hercules Moura[1], Ed I. Shaw[1], Herbert A. Thompson[1], Facundo M. Fernandez[2], *[1]Centers for Disease Control and Prevention, National Center for Environmental Health and National Center for Infectious Diseases*; *[2]School of Chemistry and Biochemistry, Georgia Institute of Technology*

12. **Design Considerations, Simulation, and Fabrication of an Array of Micron-scaled Cylindrical Ion Trap Mass Analyzers;** Miriam Fico, Christopher Mulligan, Andrew Guymon, Guangxiang Wu, R. Graham Cooks; *Purdue University*; Matthew G. Blain, Dolores Cruz, Daniel E. Austin, James G. Fleming; *Sandia National Laboratory*

13. **MALDI – TOF Mass Spectrometry as a Tool for the Rapid & Specific Identification of Pathogens Linked with Homeland Defence**; D. J. Dare[a], H. E. Sutton[a], C. J. Keys[b], H. N. Shah[b], T. McKenna[c], M. Luntc, J.C. Gebler[d, a]*Manchester Metropolitan University, Research Development Unit, [b]Health Protection Agency, Specialist and Reference Microbiology Division, [c]Waters Corporation, MS Technology Centre*, [d]Waters Life Sciences R&D

14. **On the Risk of False Positive Identification Using Multiple Ion Monitoring in Qualitative Mass spectrometry: Large-Scale Intercomparisons With a Comprehensive Mass Spectral Library**; David N. Heller[1], Stephen E. Stein[2], *[1] FDA Center for Veterinary Medicine, [2] National Institute of Standards and Technology*

15. **Enhanced Trace Explosive Detection Using High Power Strobe Desorption;** Hayden E. Hernandez, Kent D. Henry, and [*]John S. Lovell, *ADA Technologies Inc*

16. **The Mis-identification of Anatoxin-a Using Mass Spectrometry in the Forensic Investigation of Acute Neurotoxic Poisoning;** Kevin J. James, Janet Crowley, Brett Hamilton, Mary Lehane, Ambrose Furey, *PROTEOBIO, Mass Spectrometry Centre for Proteomics and Biotoxin Research, Department of Chemistry, Cork Institute of Technology*

17. **Analysis of Explosives and Chemical Warfare Agents Using Liquid Chromatography/Mass Spectrometry (LC/MS) Employing a Multi-modal Ionization Process**; Stuart A. Oehrle, *Waters Field Lab, Northern Kentucky University, Chemistry Department*

18. **Multiple Techniques for Simultaneous Quantitative and Qualitative Data Acquisition Using a Hybrid Quadrupole/Linear Ion Trap Mass Spectrometer**; Gary Impey and Nadia Pace, *MDS Sciex*

19. **Dye Analysis Using 9-aminoacridine Matrix for Negative Ion MALDI-TOF Mass Spectrometry.;** L.J. Soltzberg and Amanda Hagar, *Department of Chemistry, Simmons College*' Richard Newman, *Research Lab., Museum of Fine Arts, Boston, MA*

20. **Forensic Environmental Fingerprinting: Monitoring Biotic and Abiotic Compositional Changes in Heavy Crude Oil Determined by ESI FT-ICR Mass Spectrometry**; Lateefah A. Stanford[1], Sunghwan Kim[2], Ryan P. Rodgers[2], Alan G. Marshall[1,2]; *[1] Department of Chemistry and Biochemistry, Florida State University; [2] Ion Cyclotron Resonance Program, National High Magnetic Field Laboratory, Florida State University*

21. **Potential Application of Position Specific Isotope Analysis for Sourcing**; Christopher J. Wolyniak, Gavin L. Sacks, J. Thomas Brenna, *Division of Nutritional Sciences, Cornell University*

22. **A LC/MS Multi-Analyte Screening Method for Deleterious Organicsin Drinking Water;** Jim Krol; Joe Romano, *Waters Corporation*, and Lawrence Zintek, *EPA Region 5 Laboratory*

23. **Rapid Non-Enzymatic Protein Digestion Procedures for Proteomic-Based Microorganism Identification;** Nicolas Hauser, Mariah Lawrence, Katherine Smetana and Franco Basile*, *University of Wyoming, Department of Chemistry*

24. **Confirmation and Quantification of Protein Toxins in Complex Food Matrices;** John H. Callahan, Kevin J. Shefcheck, Tracie L. Williams and Steven M. Musser, *Instrumentation and Biophysics Branch, Center for Food Safety and Applied Nutrition, U.S. FDA*

25. **High-Throughput Field Detection of Toxic Chemicals Using Fast Gas Chromatography-Mass Spectrometry;** Frederick J. Cox Jr., Shannon B. Fox, and James C. Peterson, *Battelle Eastern Science and Technology Center*

26. **A MALDI Quadrupole Time-of-Flight Mass Spectrometric Approach to Bacterial Identification in Bioaerosols;** Alton J. Dugas Jr., Jae-Kuk Kim, Kermit K. Murray, *Louisiana State University, Baton Rouge, LA*

27. **Performance Advantages of Broadband-Modulated Time-of-Flight Mass Spectrometry**; Brian G. Frederick, *Stillwater Scientific Instruments*, *Laboratory for Surface Science and Technology, University of Maine*, Orono, ME

28. **Characterization of Chemical Warfare Agent Residues using EI/SI Ion Trap Mass Spectrometry;** G. L. Gresham, G. S. Groenewold, A. D. Appelhans, and J. E. Olson, *Idaho National Engineering and Environmental Laboratory*

29. **GC and Direct SPME Characterization Using a Portable Cylindrical Ion Trap (CIT) Mass Spectrometer;** Garth Patterson, J. Mitchell Wells, John Grossenbacher, Anthony Cochran, Brent Knecht, Brent Rardin, Mark Gregory, Jason Springston, Dennis J. Barket, Jr., *Griffin Analytical Technologies, Inc*.

30. **Trace Analysis of Peroxide Explosives by High Performance Liquid Chromatography - Atmospheric Pressure Chemical Ionization - Tandem Mass Spectrometry (HPLC-APCI-MS/MS) for Forensic Applications**; Xiaoma Xu, Ph.D.; Anick M. van de Craats, Ph.D.; Eric M. Kok, B.Sc.; and Peter C.A.M. de Bruyn, M.Sc., *Netherlands Forensic Institute (NFI)*

POSTER 2

Isotope Abundance Analysis Software for Improved Sample Identification with the Supersonic GC-MS and LC-EI-MS

Aviv Amirav and Tal Alon; School of Chemistry, Tel Aviv University, Tel Aviv 69978, Israel.,
Phone: 9723-6408253 Email: amirav@tau.ac.il

It is well known and established in the scientific literature that the relative abundance of the various isotopomers (molecular ion peaks with different isotopes) can provide accurate elemental and empirical formulas information, provided that the molecular ion is available and that the relative abundances of the various isotopomers can be accurately measured. While this isotope abundance analysis (IAA) method is well documented in the literature for many years, it is currently basically ignored and unused.

We have developed and are evaluating an isotope abundance analysis (IAA) software and method that automatically converts experimental mass spectral data into elemental formulas information and/or improve the confidence level in library identification. IAA seems as ideally applicable to GC-MS and LC-EI-MS with supersonic molecular beams since IAA requires having a trustworthy and high abundance true molecular ion, plus absence of self CI and vacuum background that are unique to the Supersonic GC-MS (and LC-EI-MS).

In addition to the provision of elemental formulas, the IAA software is also applicable to high mass fragments and provides both fragment and lost neutral elemental information for improved structural elucidation.

The IAA approach will be compared with accurate mass alternatives and several results will be shown. The IAA software and its several features will be demonstrated live at the poster place on a laptop

POSTER 3

Purification and Characterization of the Plant Lectin Abrin from *Abrus precatorius,* Using Galactose-affinity Chromatography in Concert with LC-MS/MS

Jason L. Dabbs, Neil J. Bonzagni and Robert L. Bull
Biological Defense Research Directorate, Naval Medical Research Center, 503 Robert Grant Ave., Silver Spring MD 20910

Abrin is a plant lectin from the Jequiriti bean *Abrus precatorius*, whose lethality as a naturally occurring protein toxin warrants its consideration as a potent biological warfare agent. Abrin, like the plant lectin Ricin, is a Ribosome-inactivating protein (RIP) capable of arresting protein synthesis through the depurination of the adenine residue of A4324 in 28S rRNA. While a number of immunochemical and molecular diagnostic assays exist for the detection of Ricin, there are no such detection strategies for Abrin.

Towards these ends, we have devised a definitive assay for Abrin, in which the crude extract of Jequiriti beans was purified via affinity chromatography on a galactosamine-agarose column, and subsequently analyzed by LC-MS/MS. Following purification on the galactose affinity column under a pH gradient elution, 435 □g/mL of purified Abrin was collected. The 60 KDa band corresponding to Abrin was then excised and subject to trypsin digestion prior to analysis via LC-MS/MS, using a quadrupole ion-trap mass spectrometer.

In this method, the tryptic digest was loaded to an in-line Picofrit column with an aqueous mobile phase containing 0.1% formic acid. Tryptic peptides were eluted under an acetonitrile gradient, and interrogated at various chromatographic retention times (r_t). In this process, survey scans were collected together with data dependent MS/MS scans for each of the ten most abundant chromatographic signatures. This process was repeated at regular time intervals across the chromatographic profile, thereby mining the MS data for less abundant species and generating their respecting MS/MS spectra.

Using this chromatographic protocol, we are able to resolve key components of the tryptic Abrin into individual peptides in the MS spectra, from which was gathered sequence specific MS/MS data. Taken together, these MS and MS/MS data were collected on multiple peptide fragments of various r_t, generating thousands of tandem mass spectra that were subsequently analyzed in a simultaneous fashion via Sequest Browser. Selection criteria and quality of data for a given peptide match observed in Sequest were based upon cross correlation values of 1.9, 2.7 and 3.5 for molecular ion charges of +1, +2 and +3, respectively. The Sequest results obtained from a 1 □g quantity of Abrin tryptic digest indicated that 44% of the Abrin sequence space was identified, after searching the NCBI nonredundant database. In this analysis, 11 peptides unique to Abrin were identified using the selection criteria mentioned above.

POSTER 4

Implications of Miniaturising Linear Ion Trap Arrays for Portable Detectors

S.N. Cairns, D.P.A. Kilgour, M.D. Brookes*; Defence Science and Technology Laboratory (Dstl), Fort Halstead, Sevenoaks, Kent, TN14 7BP, United Kingdom.
J.F.J. Todd, University of Kent, Canterbury, Kent, CT2 7NH, United Kingdom.
* Author for correspondence: mdbrookes@dstl.gov.uk

Much work has been published on the benefits of reducing the scale of quadrupole instruments in relation to power consumption, pressure tolerance and other critical parameters for robust fieldable systems. However, there is a cost associated with these benefits, namely a potential loss of performance below the useable threshold. This poster reports preliminary results arising from modelling and simulation studies examining the implications of size versus performance trade-offs for miniaturised Linear Ion Trap (LIT) arrays. The effects of miniaturisation have been modelled using both SIMION and CPO (Charged Particle Optics), at values of the quadrupole internal radius from $r_0 = 1$mm down to 200 µm.

Assuming that axial ejection would be required in a miniaturised LIT array, the efficiency of ion ejection (and hence detection) becomes an inverse function of rod length. However, reducing the rod length also reduces the space charge capacity which may also affect the detection sensitivity. As r_0 decreases, the pseudopotential well depth across the quadrupole decreases. When this becomes significantly lower than the fragmentation energy, collisionally induced fragmentation will cease to be practical. Therefore, the potential for MS/MS within quadrupoles below a certain size will also be adversely affected. While the decreasing potential well depth may be ameliorated by increasing the frequency, this will increase the power requirements of the instrument as a whole to the extent that the expected power saving anticipated by miniaturisation is not realised.

POSTER 5

Growth Media Differentiation of *Bacillus subtilis* Spores by elemental Characterization Using Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS)

[1]John Cliff, [1]Daniel Gaspar, [2]Stephen Golledge, [1]Kristin Jarman, [1]Nancy Valentine, [1]Karen Wahl, and [1]David Wunschel; [1]Pacific Northwest National Laboratory, [2]University of Oregon.

We explored the use of time of flight secondary ion mass spectrometry (ToF-SIMS) to distinguish *Bacillus subtilis* spores based on the elemental signatures inherited from the media in which the spores were grown. Triplicate culture replicates grown in each of four different media were analyzed. Spore signatures comprised of 16 elemental intensities or normalized intensities (i.e. to Ca) gleaned from TOF-SIMS spectra were analyzed using analysis of variance (ANOVA) and principal components analysis (PCA). No single element clearly separated spores grown in the four media types using ANOVA (Tukey's HSD, □=0.01), however, visual inspection of PCA scores confirmed separation of the spores based on the media in which they were grown (Figure 1). Confusion matrices constructed using nearest neighbor classification on the PCA scores confirmed the predictive utility of the PCA in differentiating sporulation medium i.e., perfect classification was achieved when data was autoscaled prior to performing PCA. Loading vector magnitudes of raw and autoscaled data support the inference that elements with lower relative signal provided information valuable for separating spores grown in different media. These data confirm the utility of ToF-SIMS elemental analysis for media typing of *B. subtilis* spores. We are currently expanding these studies with greater replication and additional media and spore types to determine the relative role of ToF-SIMS elemental analysis in a microbial forensics toolbox.

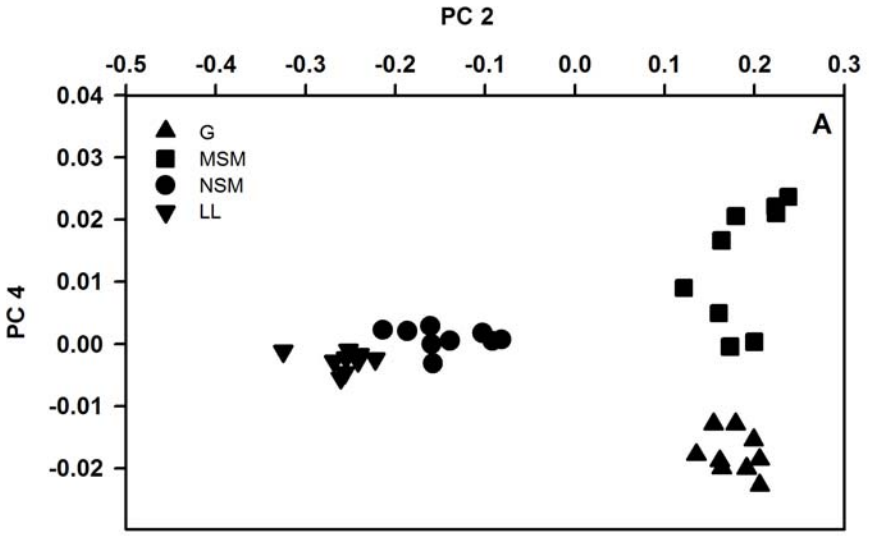


Figure 1. Scores plot for PCA of ToF-SIMS elemental signatures of *B. subtilis* spores. Each symbol represents an analytical replicate for each of three culture replicates from four different growth media.

Acknowledgements. This research was conducted under the Laboratory Directed Research and Development Program of the U.S. Department of Energy. The Pacific Northwest National Laboratory is operated by Battelle for the U.S. Department of Energy under contract DE-AC05-76RLO1830. Support from the NSF (DMR-0216639) for the ToF-SIMS instrumentation at the University of Oregon is gratefully acknowledged. A portion of the research described in this manuscript was performed at the W. R. Wiley Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by the U.S. Department of Energy's Office of Biological and Environmental Research and located at Pacific Northwest National Laboratory. PNNL is operated for the Department of Energy by Battelle.

POSTER 6

Analysis and Characterization of Tryptamine Analogues using Electrospray Ionization Mass Spectrometry (ESI-MS)

Sandra E. Rodriguez-Cruz, Ph.D. Forensic Chemist, Drug Enforcement Administration Southwest Laboratory, 2815 Scott Street, Vista, CA 92081

The characterization of new designer drugs requires analysis by multiple analytical techniques able to provide unequivocal identification of a novel structure. Techniques like infrared (IR) spectroscopy, mass spectrometry (MS) and nuclear magnetic resonance (NMR) are commonly used for this purpose, providing the basis for structural elucidation and subsequently allowing the production of spectral libraries. Commercial libraries currently available to forensic laboratories, although containing thousands of compounds, are unable to provide final identification of new, non-previously encountered compounds. In those situations, the combination of multiple and complementary analytical techniques is invaluable in correctly determining the final structure.

For decades, the use of electron ionization (EI) mass spectrometry as a detector for gas chromatography (GC) instruments has been a main step in the structure elucidation process used by analytical and synthetic chemists worldwide. Electron ionization spectra, normally collected under 70 eV of energy, are readily available from reference databases and instrument manufacturers. The role of mass spectrometry, however, has greatly expanded since the development of ionization techniques like electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), making possible the analysis of polar and thermally labile compounds. Through the ESI process, ions in solution are transported into the gas phase by a series of solvent evaporation and Coulomb explosion steps, preserving the original intact ions and introducing them into the vacuum-housed mass analyzer without significant fragmentation. As a result, this process produces singly and/or multiply charged ions which, after mass spectrometric analysis, provide direct molecular weight information.

The interface of an electrospray ionization source to an ion-trap mass spectrometer provides for not only molecular weight determinations, but also allows for tandem and MS^n fragmentation analysis of intact gas-phase ions via collision-induced dissociation experiments using a target gas. By performing these experiments under controlled conditions, structural elucidation can be accomplished and the spectra generated can be collected and stored as part of a laboratory-generated library.

This poster will present the analysis of 12 tryptamine analogues using ESI-MS. Figure 1 shows the core structure of a tryptamine-type molecule. Some of these compounds were recently encountered during an investigation targeting their sale via the internet. Initially, these compounds were characterized at the DEA Southwest Laboratory using GC, IR and NMR spectroscopy, and GC-MS techniques. Analysis by ESI-MS provides complementary information valuable for the full characterization of these compounds. For most of the analogues, the generation of pseudo-molecular ions via ESI provides molecular weight information not available via GC-MS analysis. MS^n fragmentation experiments complement the structural information obtained from GC-MS, and allows the characterization of thermally labile compounds.

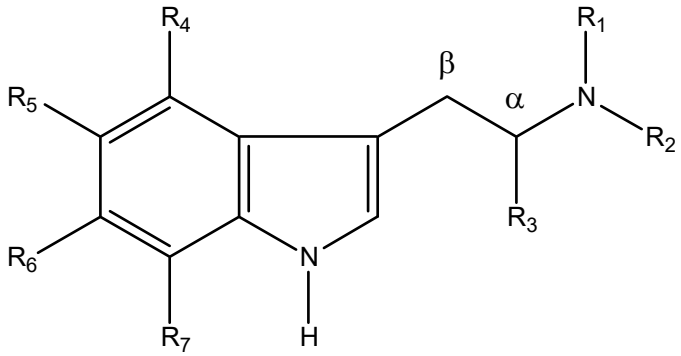


Figure 1: Core structure of a tryptamine-type compound.

POSTER 7

Analysis of Chemical Warfare Agents in Contaminated Indoor Sample Media by High Resolution LC-ESI-MS/MS Analysis

Paul A. D'Agostino, Claude L. Chenier and James R. Hancock; DRDC Suffield, P.O. Box 4000, Medicine Hat, Alberta, Canada, T1A 8K6

The Chemical Weapons Convention has reduced the likelihood of battlefield chemical weapons use, but there remains a serious concern that other parties may make use of these weapons against civilian or military targets. Analytical methods need to be developed to ensure that suspect samples collected under these circumstances can be analysed for the presence of chemical warfare agents in a timely manner. LC-ESI-MS and LC-ESI-MS/MS methods have been used for this purpose, with methods having been developed for the identification of chemical warfare agents, their hydrolysis products and related compounds in aqueous and other environmental samples.

The present study focused on the development of analytical methods for contaminated sample media that might be collected in an indoor environment, including flooring, wall surfaces, office fabrics, window coverings and paper products or packaging. Typical indoor media were spiked with either a complex munitions grade sample of tabun (GA) or with a standard containing three nerve agents, sarin (GB), cyclohexyl methylphosphonofluoridate (GF), soman (GD) and the nerve agent simulant, triethyl phosphate (TEP), to evaluate the potential of LC-ESI-MS and LC-ESI-MS/MS for forensic purposes.

Samples were spiked at the µg/g level, extracted with water using ultrasonic vibration, centrifuged to reduce the presence of fines and analysed by LC-ESI-MS and LC-ESI-MS/MS. The spiked chemical warfare agents were recovered in all cases from the media investigated and recovery efficiency was estimated. In some cases the aqueous extracts contained numerous co-extracted sample components that complicated LC-ESI-MS analysis and hampered identification. These interferences were minimized during LC-ES-MS/MS analysis, where each of the chemical warfare agents was identified on the basis of acquired product ion mass spectra. Data for all the spiked compounds in the prepared nerve agent standard and the munitions grade tabun were acquired with a resolution of 9000, which typically resulted in mass measurement errors of 0.001 Da or less. The developed methodology will be extended to include other forensic media that may be collected during an investigation, with application anticipated during future analyses where chemical warfare agent contamination is suspected.

POSTER 8

Broad and Rapid Identification of Biological Threat Agents Using Atmospheric Pressure MALDI Tandem Mass Spectrometry

Vladimir M. Doroshenko, Nelli I. Taranenko, Gavin E. Black, Vadym D. Berkout, Robert M. Serino, H. Sang Lee; Science & Engineering Services, Inc., 6992 Columbia Gateway Drive, Columbia, MD 21046

The purpose of this study is to develop a mass spectrometry (MS)-based technology to rapidly and unambiguously identify all known and unknown biological threat agents for using in homeland security applications, counterterrorism and forensic science. Here we report the use of atmospheric pressure (AP) matrix-assisted laser desorption/ionization (MALDI) tandem MS (or MS/MS) for identification of real threat bioagents and threat simulants. The use of an AP-MALDI ion source results in no time lost for breaking the vacuum of a mass spectrometer as well as in easy automation of the analysis process and low maintenance in field environment. The proposed method relies on bioinformatics approach for agent protein biomarker identification using publicly available proteome/genome databases and, thus, does not require any spectral library build-up prior its use.

The utilized protocol includes the use of: (a) fast (compared to chromatography and molecular affinity methods) *in situ* isolation of a small number of proteins (1 to 10, depending on the class of agent) using selective protein solubility method; (b) fast (within a few minutes) *in situ* protein proteolysis using trypsin immobilized on Agarose beads; (c) AP-MALDI ionization of the tryptic peptides without breaking the vacuum of a mass spectrometer; (d) tandem ion trap MS analysis to get peptide amino acid sequence information; and finally (e) searching publicly available proteome/genome databases for reliable identification of bioagents.

Among bioagents studied and positively tested are those from all bioagent classes, namely: spores (*Bacillus anthracis*), vegetative cells (*Yershinia pestis*), viruses (*Vaccinia*) and toxins (*Clostridium botulinum* neurotoxin A). The total analysis time including sample preparation is within 5-10 minutes. A reliable detection at 20 ng/sample level has been demonstrated for spore agents. In addition, the effect of cluttered environment on the utilized protocol is studied by adding industrial dust, natural bacilli, pollens, and trypsin inhibitors to the sample.

POSTER 9

Clostridium botulinum Neurotoxin Identification Using Atmospheric Pressure MALDI Ion Trap Mass Spectrometry

Nelli I. Taranenko, Gavin E. Black, Robert M. Serino and Vladimir M. Doroshenko; Science & Engineering Services, Inc., 6992 Columbia Gateway Drive, Columbia, MD 21046

Atmospheric pressure (AP) matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) has become a useful technique in a number of important applications, including protein identification, oligosaccharide analysis, phosphopeptide study, and bacterial sample analysis due to its tandem MS (or MS/MS) capabilities when interfaced with ion trap MS. Here we report a successful application of AP-MALDI-MS/MS for detection and identification of toxins, a major class of biological warfare agents which cannot be detected by polymerase chain reaction (PCR) technique commonly used for bacterial identification.

Clostridium botulinum neurotoxin A (BoNT-A) used in this work has a 97 kDa chain of 832 amino acid residues and a 53 kDa chain of 448 amino acid residues linked by at least one disulfide bond. Chicken ovalbumin (MW 45 kDa) was used as a model toxin simulant to study the effect of various environmental factors on AP-MALDI analysis of toxins. The proteolysis was carried out by using trypsin immobilized on Agarose beads at 65^{o}C. The digest peptide mixture was purified by C18 ZipTips, analyzed by AP-MALDI-MS/MS, and the MS/MS data were searched against the proteome/genome database using Mascot search software. Using this approach a fast (within several minutes) and reliable detection/identification of BoNT-A has been demonstrated at 1 pmol/sample level. In addition, the effect of industrial and environmental dust purchased from NIST or collected by an aerosol concentrator on the above protocol has been studied. It has been shown that ZipTips effectively eliminate the effects of contaminants contained in the sample in the amount as large as 100 ug per sample.

POSTER 10

Mass Spectrometry Investigations of *Bacillus anthracis-cerus-thuringiensis* Group Spores

Danielle N. Dickinson[1,2], Brian A. Eckenrode[2], David H. Powell[3], Kasthuri Venkateswaran[4], Bruce Budoule[5; 1]Oak Ridge Institute for Science and Education, Oak Ridge, TN; [2]Federal Bureau of Investigation, Counterterrorism and Forensic Science Research Unit, Quantico, VA; [3]Department of Chemistry, University of Florida, Gainesville, FL; [4]Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA; [5]Federal Bureau of Investigation, Chem-Bio Sciences Unit, Quantico, VA

Great attention has been focused recently on the analysis of *B. anthracis* spores. *B. anthracis*, the causative agent of the disease anthrax, is closely related to a group of bacteria that includes *B. cereus* and *B. thuringiensis*. Collectively this group can be refered to as the BACT group spores. The precise discrimination and classification of this group of bacteria remains a topic of debate for most taxonomists. The ambiguities within the BACT group present a daunting challenge for species or strain level differentiation based solely on phenotypic characteristics and/or genetic analyses.

In the field of mass spectrometry there have been two widely accepted and investigated approaches for the analysis of microbial spores. One strategy, commonly referred to as whole cell protein profiling, involves the analysis of intact proteins extracted from whole spores followed by the use of statistical algorithms for spectral fingerprint identification. The second, more proteomic-based approach, focuses on the selective release of small acid soluble proteins (SASPs) followed by tryptic digestion and database searching for species level identification. Both of these approaches have proven useful for small subsets of *Bacillus* species which have included single strains of *B. cereus, B. anthracis, B. thuringiensis, B. globgii, and B. subtilis*. However differentiation between the *B. anthracis, B. cereus*, and *B. thuringiensis*, proved to be challenging even with the small subset of strains that were examined. To ascertain the effectiveness of mass spectrometry for the identification of closely related species and strains such as the BACT group, a more thorough study was undertaken.

To establish their phylogenetic affiliations, 26 BACT group strains were thoroughly characterized using genetic analysis including 16s rDNA, DNA hybridization, and gyrB sequencing. MALDI-TOFMS based protein profiling of these 26 spores was performed using both 30% formic acid and 10% TFA as sample pretreatments. The resulting protein profiles were analyzed using linear correlation and hierarchal cluster analysis. Protein profiles produced using the two solvent systems did vary and the discriminating power of the sample pretreatment will be addressed. Six of the strains tested did not fall into clusters using the linear correlation analysis. Further proteomic-based analysis of these six strains was explored. Treated and untreated spores were subjected to tryptic digestion and the resulting peptides were analyzed using both MALDI-TOFMS and the ESI on a LTQ mass spectrometer. MS/MS of the tryptic peptides was performed and peptide sequence information was collected and searched against protein databases via Sequest. The majority of positively identified proteins were SASPs and the discriminating capability of this family of proteins was evaluated using all of the BACT group spores in this study.

POSTER 11

Coxiella burnetii Strain Identification by Partial Least Squares-Discriminant Analysis of MALDI-TOF MS Biomarker Fingerprints

Carrie L. Young[1,2], John R. Barr[1], Adrian R. Woolfitt[1], Hercules Moura[1], Ed I. Shaw[1], Herbert A. Thompson[1], Facundo M. Fernandez[2]; [1]Centers for Disease Control and Prevention, National Center for Environmental Health and National Center for Infectious Diseases, Atlanta, Georgia 30341; [2]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332

Accurate and rapid bacterial identification is important in diagnosing disease and for public health. Recent studies have shown that matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a promising technique for the fast identification of whole microorganisms. Generally, two approaches have been adopted for microorganism identification. The most robust and time-intensive approach relies on the sequencing of protein biomarkers using MS-based proteomics techniques. A faster, complementary approach relies on pattern recognition of the protein biomarker fingerprint. The pattern recognition step (sometimes referred to as "database building") is usually done by classification methods such as artificial neural networks. In this work we investigate if a different pattern recognition method known as Partial Least Squares-Discriminant Analysis (PLS-DA) is useful in whole-bacteria forensic identification applications. An attractive feature of PLS-DA is that it reduces the number of original spectral variables used by attempting to identify characteristic latent variables which can be directly used to segregate the samples into classes. For this reason, PLS-DA has enjoyed considerable success in a variety of applications. PLS-DA is essentially the inverse-least-squares approach to Linear Discriminant Analysis (LDA) and produces essentially the same result but with the noise reduction and variable selection advantages of PLS. In the present study, PLS-DA was used to identify and characterize distinct strains of the whole organism C*oxiella burnetii* from MALDI-TOF MS spectra. *C. burnetii* is an intracellular bacterium that causes the human disease Q fever and is a category B bioterrorism agent. The aim was to detect unique biomarkers in the MALDI TOF MS spectrum of *C. burnetii* to generate a predictive model for differentiating strains of *C. burnetii*. The grouping for the different isolates is studied. Cluster analysis of these spectra showed that MALDI-TOF MS when coupled with chemometrics constitutes a powerful approach to discrimination and prediction of *C. burnetii* to the strain level.

POSTER 12

Design Considerations, Simulation, and Fabrication of an Array of Micron-scaled Cylindrical Ion Trap Mass Analyzers

Miriam Fico[1], Christopher Mulligan[1], Andrew Guymon[1], Guangxiang Wu[1], R. Graham Cooks[1]; [1]Purdue University, West Lafayette, IN; Matthew G. Blain[2], Dolores Cruz[2], Daniel E. Austin[2], James G. Fleming[2], [2]Sandia National Laboratories, Albuquerque, NM

Security and environmental concerns have necessitated the development of advanced portable sensors for chemical warfare agents and toxic industrial compounds. Mass spectrometers are important analytical tools in this regard due to their capability for in-situ measurements without a reduction in speed or sensitivity. Developing a fieldable mass spectrometer calls for careful redesign to optimize instrument size, as well as to meet power and vacuum requirements. Until recently, technology did not exist to miniaturize mass spectrometers to a hand-held scale. This project focuses on the design and implementation of a hand-held mass spectrometer utilizing micro-fabricated arrays of cylindrical ion trap (CIT) mass analyzers. Miniaturizing the mass analyzer to the micrometer level in principle allows operation at much higher pressures and lower voltages compared to conventional CIT-based instruments. Using micro-electro-mechanical systems (MEMS) fabrication methods to define trap electrodes in tungsten metal, arrays of 10^3 to 10^6 r_0 = 1.0 μm and r_0 = 2 μm CITs have been successfully constructed. Simulations have predicted favorable operation of these arrays at tens of volts and frequencies in the 100 to 1000 MHz range, at the limit of one ion per trap. The assembly of the hand-held device will be completed by utilizing small rf electronics, battery power, and a miniaturized turbomolecular vacuum pump.

Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy under contract DE-AC04-94AL85000.

POSTER 13

MALDI – TOF Mass Spectrometry as a Tool for the Rapid & Specific Identification of Pathogens Linked With Homeland Defence

D. J. Dare[a], H. E. Sutton[a], C. J. Keys[b], H. N. Shah[b], T. McKenna[c], M. Lunt[c] J.C. Gebler[d]; [a]Manchester Metropolitan University, Research Development Unit, Manchester UK; [b]Health Protection Agency, Specialist and Reference Microbiology Division, London UK; [c]Waters Corporation, MS Technology Centre, Manchester UK; [d]Waters Life Sciences R&D, Milford MA USA

Rapid identification of pathogens associated with homeland defence is currently required due to the potential release of biological agents. Of particular interest is *B. anthracis*, since this pathogen has been the subject of recent investigations. However the genus *Bacillus* is immensely complex and is presently undergoing major taxonomical revisions based upon 16S rDNA sequence analysis. For many isolates, identification to the sub-genus level is difficult due to the high degree of homogeneity within this region of the gene. This study examines the potential use of matrix-assisted laser desorption ionization time of flight (MALDI) mass spectrometry as an alternative rapid identification technique.

Intact *Bacillus* cells are overlaid with the MALDI matrix 5-chloro-2-mercaptobenzothiazole (CMBT) followed by analyzes with MALDI. This produces a unique mass spectral fingerprint pattern, which forms the basis of identification against a database containing representative spectra of the genus. In this study data ~50 NCTC strains were used to challenge a database of ~3500 spectral entries representing over 400 different bacterial species. Approximately 90 strains of *Bacilli* covering the species *B. badius, B. cereus, B. circulans, B. coagulans, B. firmus, B. lentus, B. licheniformis, B. megaterium, B. mycoides, B. pumulis, B. sphaericus and B. subtilis* were included. Accurate identification to the genus level was achieved for 92% of the isolates tested while 82% were also successfully speciated. This implies that identification of these problematic genuses can be achieved without the need for the more complex and time-consuming DNA extraction techniques using one or more genes. These results therefore demonstrate that MALDI mass spectrometry has the potential to rapidly identify pathogens linked with homeland defence.

The results of testing 66 of the 85 database strains of *Bacillus* by parallel data against a total database of 3424 spectral entries will be presented. *B. atrophaeus*, *B. badius*, *B. firmus*, *B lentus*, *B. mycoides* and *B. subtilis* were all identified correctly to species; all *B. cereus* strains tested were identified correctly to species when filtering for the correct culture conditions; the majority of *B. licheniformis* were identified correctly to species, the remaining 2 correctly identified to genus; 6 of the 7 *B. pumulis* samples tested were correctly identified. The results for *B. circulans*, *B. coagulans* and *B. megatarium* were generally correct to genus with the correct species appearing among the top 5 matches (for *B. coagulans* NCTC 11213 reclassification as *B. licheniformis* is suggested, noisy data was obtained for B*. megatarium* NCTC 5637, *Bacillus coagulans* NCTC 3993 and *Bacillus megaterium* NCTC 5637 demonstrated no top matches to the *Bacillus* genus); typical results for *B. cereus, B. pumulis & B. licheniformis, B subtilis* are give the majority of the top database matches as the same species. Thus, MALDI-TOF MS is an appropriate tool for the rapid & specific identification of pathogens linked with Homeland Defence

POSTER 14

On the Risk of False Positive Identification Using Multiple Ion Monitoring in Qualitative Mass Spectrometry: Large-scale Intercomparisons With a Comprehensive Mass Spectral Library.

David N. Heller[1], Stephen E. Stein[2]; [1] FDA Center for Veterinary Medicine, Laurel, MD' [2] National Institute of Standards and Technology, Gaithersburg, MD

Analysts involved in qualitative mass spectrometry have long debated the minimum data requirements for demonstrating that signals from an unknown sample are identical to those from a known compound. Often this process is carried out by comparing a few selected ions acquired by multiple ion monitoring (MIM), with due allowance for expected variability in response. In a few past experiments with electron-ionization mass spectrometry (EI-MS), the number of ions selected and the allowable variability in relative abundance were tested by comparing one spectrum against a library of mass spectra, where library spectra served to represent potential false positive signals in an analysis. Such experiments supported the selection of three or more diagnostic ions for maximum identification confidence. These peak constraints are known as confirmation criteria.

We have extended these experiments by carrying out large-scale intercomparisons between thousands of spectra and a library of one hundred thousand EI mass spectra. The results were analyzed to gain insights into the identification confidence associated with various numbers of selected ions. A new parameter was investigated for the first time, to take into account that a library spectrum with a different base peak than the search spectrum may still cause a false positive identification. The influence of peak correlation among the specific ions in all the library mass spectra was also studied.

These analyses showed that false positive probability decreases roughly one order of magnitude for each additional ion selected. Our computations also showed that false positive identifications can result from both structurally-similar compounds and low-abundance peaks in unrelated compounds (if the method calls for detection at very low levels). The results establish that standardized confirmation criteria do not result in the same identification confidence when applied to compounds with different structures. In other words, standardized confirmation criteria cannot be configured to act with the same degree of confidence in all cases.

There are implications for residue chemists who would rely on standardized confirmation criteria to assess the validity of a given confirmatory method: (1) Standardized confirmation criteria should not be used in the absence of interference testing, rational selection of diagnostic ions, and an evaluation of the existence of similar compounds; (2) A MIM method's identification confidence improves in a roughly continuous manner as more ions are monitored; (3) full scan spectra still represent the best alternative, if instrument sensitivity is adequate. The use of large scale intercomparisons with a comprehensive library is the only way to provide direct evidence in support of these conclusions, which otherwise depend on the judgment and experience of individual analysts.

POSTER 15

Enhanced Trace Explosive Detection Using High Power Strobe Desorption

Hayden E. Hernandez, Kent D. Henry, and *John S. Lovell, ADA Technologies Inc.; 8100 Shaffer Parkway; Suite 130; Littleton, CO 80127 USA 303/792-5615

It is estimated that as many as 120 million land mines are distributed across the world. According to the International Committee of the Red Cross, 20-30,000 people are killed or maimed every year in mine related incidents. Antipersonnel mines may date from the Second World War, but the largest concentrations are in regions that have experienced recent warfare such as South and Central America, Africa, Asia and Europe. Table 1 summarizes the numbers of landmines in some of the most severely afflicted countries, but it is estimated that there are mines in over 65 countries.

Table 1 Principal concentrations of landmines.

Country	Total number of mines
Bosnia/Herzegovina	3-6,000,000
Croatia	3,000,000
Cambodia	6,000,000
Egypt	23,000,000
Iraq	10,000,000
Afghanistan	10,000,000
Angola	15,000,000
Vietnam	3,500,000
Iran	16,000,000
Rwanda	250,000
Eritrea	1,000,000
Mozambique	3,000,000
Somalia	1,000,000
Sudan	1,000,000
Ethiopia	500,000

ADA Technologies has developed an enhanced trace explosive detection system using high power stroboscopic light desorption. The technique uses the instantaneous (1/1000th sec.) power of a 2,400 joule strobe lamp to create a readily detectable plume of explosive vapor from traces of low-volatility explosives on surfaces.

To confirm the potential of this technique, a sample of sand was coated with 1 part per million (ppm) triethyl phosphate (b.p. 215°C) in a low boiling point solvent. The solvent was evaporated and 1% water was mixed into the sand. The sand was then placed in sealed metal box with a glass cover. A stream of nitrogen was passed through the box into a gas chromatograph fitted with an open 1/8" stainless steel column and a flame photometric detector (FPD) with a 526nm filter for phosphorus detection. The source of the strobe illumination was a Speedotron Blackline 2403 power pack and single strobe head that can be varied between 400 and 2,400 watt-seconds, all of the tests described were carried out at full power. An example result is shown in Figure 1.

Illustrated in Figure 2, an additional experiment was designed to measure the particle distribution of material released when the strobe was used on a pair of hands. Using a LASAIR particle monitor, the experiment showed that the strobe was very effective at desorbing small particulates from the hands which in turn would be amenable to further chemical analyses techniques.

The poster presentation will present the High Power Strobe Desorption apparatus designed for buried landmine detection and results obtained with an amplifying fluorescent polymer detection system and using mass spectrometry.

Figure 1: FPD response to pulse of triethyl phosphate vapor following strobe flash.

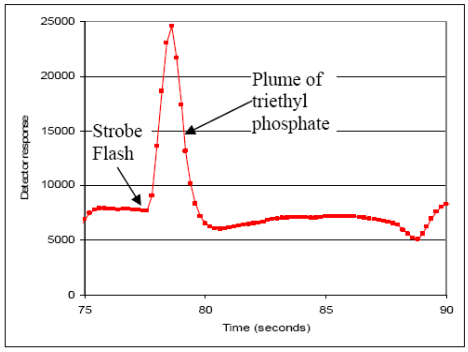
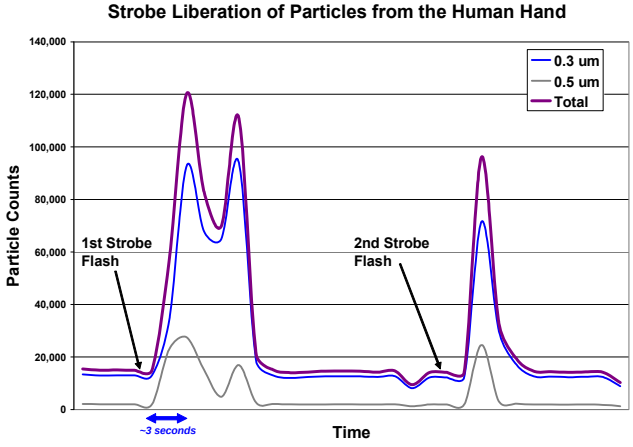


Figure 2: Particle Desorption from Strobing a pair of Hands



POSTER 16

The Mis-identification of Anatoxin-a Using Mass Spectrometry in the Forensic Investigation of Acute Neurotoxic Poisoning

Kevin J. James, Janet Crowley, Brett Hamilton, Mary Lehane, Ambrose Furey; PROTEOBIO, Mass Spectrometry Centre for Proteomics and Biotoxin Research, Department of Chemistry, Cork Institute of Technology, Bishopstown, Cork, Ireland. E-mail: kjames@cit.ie

Anatoxin-a (AN) is a potent neurotoxin, produced by a number of cyanobacterial species, and consumption of freshwater contaminated with this toxin has led to animal deaths throughout the world. The fatality of a young adult, that was ascribed by a US coroner to be due to AN poisoning, based on liquid chromatography – mass spectrometry (LC-MS) identification, has recently been shown to be incorrect. Forensic investigations of suspected AN poisonings are frequently hampered by difficulties in detecting this toxin in biological matrices due to its rapid decay. In addition, detection of AN using single quadrupole mass spectrometry (MS) is suspect due to the widespread occurrence of phenylalanine (Phe), since these compounds are isobaric and elute similarly in reversed phase LC. Approaches to prevent the misidentification of AN that have been explored in these studies include; a) fluorimetric LC following derivatisation using 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F), b) methylation using diazomethane prior to LC-MS determination, c) multiple tandem MS using a quadrupole ion-trap (LC-MS^3) and d) hybrid quadrupole time-of-flight (QqTOF) MS. Interference from Phe was not observed in any of procedures, a) – c), and the high mass accuracy obtained in method d), readily distinguished between AN (165.11536) and Phe (165.07898). LC-MS^n was also employed to study the fragmentation pathway of Phe and multi-stage spectra provided characteristic fragmentation information that clearly distinguished between AN and Phe. The difficulties associated with the over-reliance on low resolution MS without MS/MS data in forensic toxicology are discussed.

POSTER 17

Analysis of Explosives and Chemical Warfare Agents using Liquid Chromatography/Mass Spectrometry (LC/MS) employing a Multi-modal Ionization Process

Stuart A. Oehrle, Waters Field Lab, Northern Kentucky University, Chemistry Department, Nunn Drive, SC313, Highland Heights, KY 41076

With the added complexities of the world we live in, explosives analysis in counterterrorism efforts or crime scene investigation has grown. Previously, a large majority of explosives analysis were done in conjunction with military base closures. While many field techniques for identifying major explosive pollutants, such as TNT, exist and have been incorporated into standard methods, HPLC continues to be a main analytical tool for identification and verification of the presence of explosives. Many of these techniques use a dual column verification process whereby two separate analysis are done. Added to this array of explosives analysis is the need of the forensic chemist to detect and confirm the identity of explosives. One novel way to analyze and verify many of the explosives and degradation products is to use LC/MS. This hyphenated technique allows one to obtain both LC data (such as retention time) and mass spectral information. In addition, the incorporation of photodiode array (PDA) detection allows for UV spectra to be obtained and used for identification. Atmospheric pressure ionization (API) techniques were investigated with good results obtained for many of the target compounds. In addition a multi-mode ionization technique which incorporates simultaneous electrospray ionization (ESI) and atmospheric pressure chemical ionization (APcI) detection in a single run, was used for detection of various explosives and degradation products. In addition the use small particle size columns at accelerated flow rates enabled the analysis times to be substantially reduced. This technique was also used for the analysis of the phosphonic acid degradation products of chemical warfare agents.

POSTER 18

Multiple Techniques for Simultaneous Quantitative and Qualitative Data Acquisition Using a Hybrid Quadrupole/Linear Ion Trap Mass Spectrometer

Gary Impey and Nadia Pace, MDS Sciex, 71 Four Valley Drive, Concord, Ontario, Canada, L4K 4V8

Multiple reaction monitoring (MRM) is the standard technique for quantitative LC/MS/MS experiments. The LODs/LOQs, precision, and accuracy achieved with this technique are difficult to match. However, it is often desirable to obtain confirmatory or qualitative information in addition to quantitative data. This can help determine possible interferences or troubleshoot an analysis. The ability to acquire both types of data in a single experiment would save significant amounts of both time and money, with the advantages of obtaining additional information. A hybrid triple quadrupole/linear ion trap mass spectrometer has the capabilities to perform such an experiment in a variety of ways. These include, single MRM transitions per analyte, multiple MRM transitions per analyte (one for quantitation and the others for confirmation), MRM transitions used as survey scans to trigger the collection of MS/MS in an automated fashion, dedicated full scan MS/MS per analyte, or even the specificity achieved with MS^3.

As with any technique, the possibility of interferences can cause significant problems with reproducibility as well as inaccurate results, even in MS/MS mode. To improve specificity, several solutions have been proposed; 1) improve chromatographic separation, or 2) increase resolution to improve parent mass selection. Improving chromatographic separation generally leads to increases in analysis time, which is undesirable for large sample sets. Using higher resolution can improve parent mass selection, but a compromise between resolution and sensitivity must be realized. Taking advantage of improved specificity as opposed to resolution can be achieved using MS^3 in a quantitative application, without the need for lengthy chromatography.

Each one of these techniques was evaluated for a quantitative application. The quantitative data obtained from a typical MRM experiment was used as a benchmark in terms of LOD/LOQs, precision and accuracy. Statistical analysis was performed on all sets of data and the results compared.

POSTER 19

Dye Analysis Using 9-aminoacridine Matrix for Negative Ion MALDI-TOF Mass Spectrometry.

L.J. Soltzberg and Amanda Hagar, Department of Chemistry, Simmons College, Boston, MA 02115;
Richard Newman, Research Laboratory, Museum of Fine Arts, Boston, MA 02115

Identification of dye materials is important for forensic and art authentication applications. MALDI-TOF mass spectrometry, typically employed for the study of large molecules, is potentially an attractive tool for forensic analysis because of its high sensitivity and ease of sample preparation. With appropriate matrices and operating conditions, MALDI-TOF analysis can also be useful for small molecule analysis. We have successfully employed 9-aminoacridine (9AA)[1] for analyzing a variety of dye samples from the Schweppe library of dyes. We employ a simple overlay technique, in which a drop of dye solution is spotted on top of a film of 9AA on a stainless steel target. Negative ion reflectron mode yields clean spectra in which a molecular ion related species such as $[M-H]^-$, $[M-Na]^-$ or $[M-Cl]^-$ is usually the most prominent peak. We are easily able to detect nanomole quantities of the analyte.

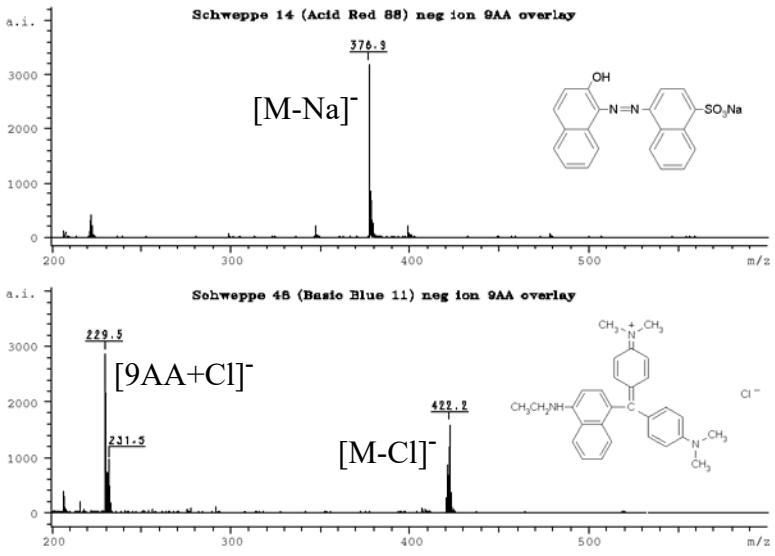


Figure 1. Negative ion MALDI-TOF mass spectra of Acid Red 88 and Basic Blue 11 from 9AA overlay samples.

[1] R.L. Vermillion-Salsbury and D.M. Hercules, *Rapid Communications in Mass Spectrometry* (2002), **16**, 1575-1581.

Research supported by the National Science Foundation (Grant number: NSF-CHE-0216268).

POSTER 20

Forensic Environmental Fingerprinting: Monitoring Biotic and Abiotic Compositional Changes in Heavy Crude Oil Determined by ESI FT-ICR Mass Spectrometry

Lateefah A. Stanford[1], Sunghwan Kim[2], Ryan P. Rodgers[2], Alan G. Marshall[1,2]; [1] Department of Chemistry and Biochemistry, Florida State University, Tallahassee, Florida 32306; [2] Ion Cyclotron Resonance Program, National High Magnetic Field Laboratory, Florida State University,1800 E Paul Dirac Drive, Tallahassee, Florida 32310-4005

The recent application of (ESI) Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) to petrochemical analyses assists in unraveling the complexity of crude oil and petroleum products, detecting >20,000 compositionally distinct polar acyclic, polycyclic, and polyaromatic polar –NSO compounds in a single sample, and generating detailed ”compositional fingerprints” without the need for pre-chromatographic separation. High mass accuracy and mass resolution forensic environmental fingerprinting allows for a higher level of confidence in the prosecution of negligent environmental crimes violating the Oil Pollution Act and the Clean Water Act. However, post oil spill environmental stressors such as bacterial and fungal remediation, solar UV photochemical transformations, and volatilization “smear” this fingerprint by generating new and removing previously identified components via dearomatization, denitrogenation, desulfurization, hydroxylation, and carboxylation, volatilization, further complicating source-to-sink forensic identification of crude oil spills. Environmental modifications that increase the polar nature of a crude oil are of concern since they may result in increased water-solubility and toxicological impact. ESI FT-ICR MS provides ultrahigh-resolution mass analysis of polar species in complex mixtures, such as crude oil, achieving high resolving power $m/\Delta m_{50\%} > 300{,}000$ and high mass accuracy (< 1 ppm). Therefore FT-ICR MS provides an effective method of monitoring (on a component by component basis) environmentally induced changes in complex organic materials. Accurate methods of isolation, identification, and extrapolated characterization of solubility trends for –NSO species within crude oil merit attention in order better predict environmental impact, as –NSO species are orders of magnitude more water soluble than their pure hydrocarbon counterparts. Compiled FT-ICR MS solubility predictors and compositional mass spectral libraries will provide a tool for linking environmentally transformed spilled oil and their generated water-solubles to the oil producer source.

Here we apply ESI negative-ion and positive-ion FT-ICR MS to three crude oils and their water-soluble acids and bases of differing continental origin for the purpose of developing environmental forensic compositional fingerprints for polar –NSO containing species to monitor water solubility changes as a result of abiotic and biotic stressors as well as linking changes within the spilled oils to their water-soluble samples. The analysis of a seven oil biodegradation sample ranging in quality from undegraded to severely biodegraded is included to emphasize changes in polar –NSO containing species as a function of increased biodegradation. Molecular formulas (elemental compositions) from each sample are assigned from accurate mass measurement combined with a Kendrick mass sorting procedure. Both class based, aromaticity, and carbon number variations in the samples are highlighted by a combination of three dimensional van Krevelen, Kendrick and aromaticity plots. This work was supported by the NSF National High Field FT-ICR Facility (CHE-99-09502), Florida State University, and the National High Magnetic Field Laboratory in Tallahassee, FL.

POSTER 21

Potential Application of Position Specific Isotope Analysis for Sourcing

Christopher J. Wolyniak, Gavin L. Sacks, J. Thomas Brenna; Division of Nutritional Sciences, Cornell University, Ithaca, NY

Bulk and compound specific isotope analysis (BSIA/CSIA) are established methods for sourcing. However, these methods are vulnerable to intentional forgery by careful mixing of adulterants to simulate authentic isotope ratios. Position specific isotope analysis (PSIA) adds a level of specificity that is expected to be very difficult to replicate. We are developing techniques to measure relative position specific isotope ratios of carbon (□□^{13}C) and □^{15}N at natural abundance levels. We present here our first results showing that amino acids can be sourced based on intramolecular isotope measurements.

PSIA of carbon and nitrogen were measured in several amino acids by strategies developed recently (Sacks & Brenna, 2003, 2004; Wolyniak et al., 2005). Samples were purchased from four commercial sources. Nitrogen analysis was done on Asn, Gln, Lys, and His, and carbon analysis was done on Leu, Met, Ala, and Phe.

Fig. 1a shows CSIA □^{15}N Asn measurements. The total range of □^{15}N for the four samples is 1‰ to 5‰. Fig. 1b is a plot of □^{15}N for the side-chain N vs. the peptide N. Error bars reflect total analysis error including preparation from the original sample. CSIA was unable to distinguish between Acros and Avocado Asn (fig. 1a), while intramolecular isotope signatures are unique and provide unequivocal identification of all sources (fig. 1b).

Fig. 2 shows results for PSIA of Ala. Here, δ^{13}C are referenced arbitrarily to values for the Fluka sample, and thus data are reported as $\Delta\delta^{13}$C. Amino acids from all sources had CSIA (total) δ^{13}C in the narrow range of 3‰ variation (at the right side of the figure 2), and thus can be distinguished with low certainty. In contrast, the PSIA results showed that the four sources separated into two groups, (Acros/Aldrich and Fluka/Sigma) based on variation at specific position of up to 50‰, observed for the C1 position.

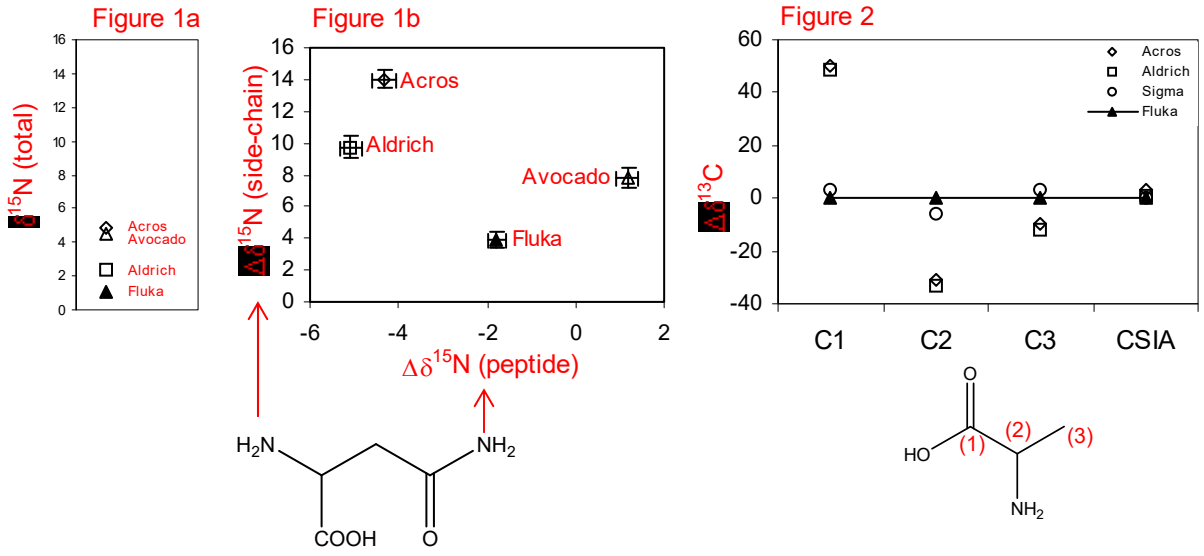


Figure 1: Nitrogen PSIA of Asparagine. a) Total δ^{15}N values. Errors (SDs) are about the size of the markers. b) PSIA isotopic fingerprint for each source.

Figure 2: Carbon PSIA of Alanine. Individual carbon positions are shown with compound specific (CSIA) data.

References:
Sacks GL, Brenna JT *Anal Chem* **2003**, 75, 5496-5503.
Sacks GL, Brenna JT *Anal Chem* **2004**, in press.
Wolyniak CJ, Sacks GL, Pan BS, Brenna JT, submitted.

POSTER 22

A LC/MS Multi-Analyte Screening Method for Deleterious Organics in Drinking Water

Jim Krol, Sr. Applications Chemist; Joe Romano, Environmental Marketing Manager, Waters Corporation, 34 Maple St, Milford, Massachusetts, 01757, Office 508/482-2131, Email Jim_Krol@Waters.com, Joseph_P_@Waters.com.; and Lawrence Zintek, Sr. Chemist, EPA Region 5 Laboratory, 536 Clark St, Chicago, IL, 60605 Office 312/886-2925

The determination of deleterious organics in drinking water is one of the particular areas of the Homeland Security Presidential Directive (HSPD-9) that will impact the EPA. It mandates that the EPA Office of Water expand monitoring and surveillance systems for recognizing a terrorist attack, or a significant change in water quality. This is a daunting task because of the breadth of organics, coupled with the numerous water sources required to be monitored.

The ability to perform a multi-analyte “screen” for numerous organics simultaneously would help maximize efforts to note the presence and significance of poisonous agents. This requires a broad analytical approach strategy utilizing the specificity of Liquid Chromatography / Mass Spectrometry (LC/MS and LC/MS/MS). Many of these organics are not amendable to Gas Chromatography / Mass Spectrometry) GC/MS. Universal detection with high sensitivity is the key.

For non-MS detection methods, analyte resolution is critical for identification and quantification. However, the capability of MS to detect a single m/z (molecular weight/charge) gives analyte detection specificity that does not require chromatographic resolution. Thus, a “universal” reversed phase gradient providing a degree of analyte separation coupled with the specificity of mass spectrometry allows for the “screening” for multi-analytes simultaneously.

This presentation will discuss the development of a single, multi-analyte screening strategy for several deleterious pesticides and herbicides in drinking water using HPLC/ Electrospray Mass Spectrometry. This work is being conducted in collaboration with USEPA Central Region Laboratory Region 5. Several analytical issues will be raised to stimulate audience discussion and to solicit input to evolve this LC/MS strategy into a validated screening method template.

POSTER 23

Rapid Non-Enzymatic Protein Digestion Procedures for Proteomic-Based Microorganism Identification

Nicolas Hauser, Mariah Lawrence, Katherine Smetana and Franco Basile*; University of Wyoming, Department of Chemistry, 1000 E. University Ave. (Dept. 3838), Laramie, Wyoming 82071

Specific microorganism identification can be achieved by applying Proteomic-based techniques based on peptide mass tags or micro-sequencing. This approach is very attractive since it has the potential to specifically identify *mixtures of microorganisms*. This approach requires *site-specific digestion* of microbial proteins for subsequent identification via mass-tags and database search, where microorganisms are identified by the source of the identified protein(s) in the database. However, in most proteomic studies digestions are performed with the enzyme trypsin which catalyzes the hydrolysis at the C-terminus of arginine (R) and lysine (K). Trypsin-catalyzed hydrolysis is conducted under a narrow range of pH and temperature, although, this reaction has been carried out to digest proteins of intact cells deposited on a MALDI probe at room temperature and in minutes. *However, when developing methods for field-detection, issues concerning reproducible enzyme activity, correct molar ratio between enzyme and substrate and enzyme shelf life could hinder this approach from being fully automated and dependable.*

Our long range goal is to develop rapid MS-based bio-detection techniques using non-enzymatic site-specific protein digestion and proteomic identification. In pursuit of this goal, *we are currently developing several approaches to non-enzymatic site-specific microorganism lysis and protein digestion*. One of these approaches is the specific cleavage at the aspartyl residue (mostly C-terminus) by hydrolysis at mild acid conditions (pH ~ 2) and microwave heating (108^{o}C) for 5 min.

Results will be shown from our investigations on model peptides, proteins and intact microorganisms.

Acknowledgement:
The authors are grateful to the Northern Rockies BRIN NIH NCRR Grant #RR-16474 for instrumentation and facility upgrade and the University of Wyoming Research Office for funding.

POSTER 24

Confirmation and Quantification of Protein Toxins in Complex Food Matrices

John H. Callahan, Kevin J. Shefcheck, Tracie L. Williams and Steven M. Musser
Instrumentation and Biophysics Branch, Center for Food Safety and Applied Nutrition, U.S. FDA, 5100 Branch Parkway, College Park, MD 20740

Introduction: The detection of deliberate contamination of food supplies by protein toxins depends on very sensitive immunoassay-based (ELISA) tests that can detect such toxins at sub-part-per-billion levels in food. However, such tests are sometimes subject to false positives due to cross reactivity, and definitive confirmation of the protein is required. Mass spectrometry-based proteomics tools can be used as a confirmatory method for the identification of toxins in food, although there are significant technical barriers due to the presence of high concentrations of biopolymers in the sample matrix. This work describes mass spectrometry approaches that can be used to detect and quantify protein toxins in the presence of complex food matrices at part-per-billion concentrations.

Methods: Staphylococcus enterotoxin B (SEB) was the model protein toxin used for this study. Powdered nonfat milk, 2 % low fat milk and apple juice were used as model food matrices. Toxin was spiked directly into the liquid samples. Milk samples were prepared by extraction of fats by hexane, followed by centrifugation to remove insoluble protein. Apple juice samples were not extracted prior to analysis. Various methods were then used to prepare the sample for analysis by mass spectrometry, including ultrafiltration, cation exchange, antibody-based extraction as well as conventional enzymatic digestion with trypsin. Both whole protein analysis and peptide analysis approaches were used to analyze the samples, using microspray/nanospray LC/MS and MS/MS on a Micromass QTOF Micro or Quattro Premier triple quad.

Results: The model problem undertaken in this study is to detect, identify and confirm the presence of toxin in liquid food matrices at sub-ppm levels. The first step was to survey the available methods to determine if they have the sensitivity to address the measurement requirement (detection of nanogram quantities). Whole protein microspray LC/MS can reproducibly measure SEB at the nanogram (per injection level) level. Digestion of the protein, followed by microspray LC/MS (and MS/MS) can also be used to detect SEB at the low nanogram level; lower detection levels can be achieved using nanospray LC/MS. However, measuring a protein toxin in a complex matrix is more problematic. Even a relatively simple, non-protein food matrix such as apple juice (which has a wide molecular weight range of polysaccharides) generates significant electrospray background and signal suppression. For apple juice, the best approach is a molecular weight shifting approach in which low molecular weight interferents are removed with 5 kD molecular weight cutoff filters. The sample (containing the retained 28 kD protein and high molecular weight biopolymers) is subsequently digested with trypsin and filtered through a 10K cutoff filter, thereby collecting the digest products without high molecular weight interferents. Internal standardization is a significant issue, and to minimize the cost, we utilize peptide standards with conservative amino acid replacements (e.g. valine for leucine) instead of stable isotope labeled standards. Microspray LC/MS on a QTOF can be used to quantify at the 50-100 ppb level (100 ng/ml in the original sample), as well as to definitively identify the protein. Use of SIM and MRM on a triple quad can further lower detection limits, and by increasing sample size, levels of SEB as low as 5 ppb can be confirmed in apple juice. The problem becomes more complex with a protein-containing matrix such as milk. Here, other sample preparation methods, including antibody extraction, have been employed to some benefit. Although the approaches described here are still an order of magnitude less sensitive than ELISA, adaptation of the methodology to nanoflow LC/MS could achieve this goal, since nanoflow methods are more sensitive and less subject to competitive suppression effects. Nevertheless, there are still significant issues of method robustness when nanoflow methods are employed with complex matrices. Multidimensional approaches will undoubtedly be necessary to optimize the nanoflow approach.

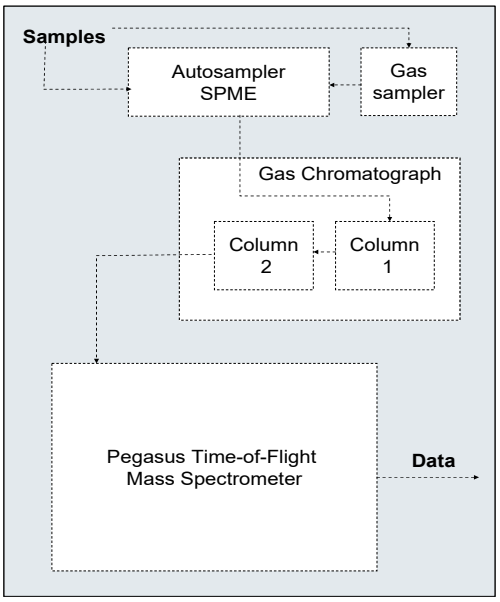
POSTER 25

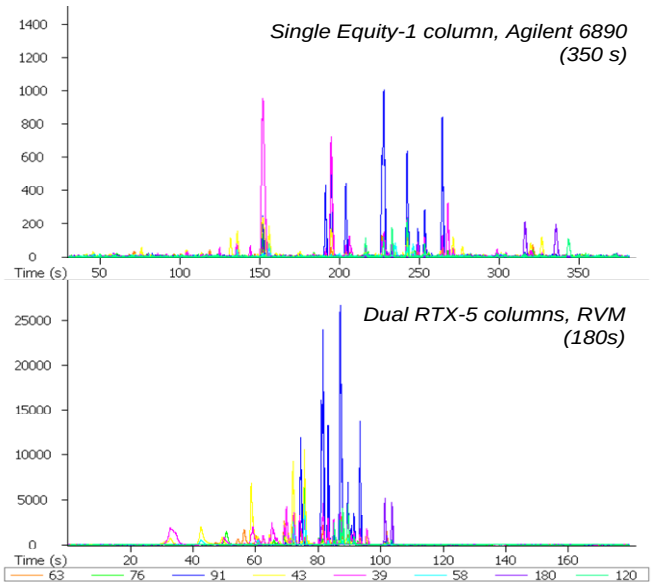
High-Throughput Field Detection of Toxic Chemicals Using Fast Gas Chromatography-Mass Spectrometry

Frederick J. Cox Jr., Shannon B. Fox, and James C. Peterson; Battelle Eastern Science and Technology Center, 1204 Technology Drive, Aberdeen, MD 21001

Fast gas chromatography and time-of-flight mass spectrometers with high spectrum acquisition rates make possible GC/MS analysis of a well-defined, prepared sample from injection to end of run in less than a few minutes. However, implementation of the technique by itself for rapid analysis of large numbers of samples is difficult, because the total time for turnaround, including time for sample preparation from diverse sample matrices or GC oven cool-down, and other considerations ancillary to the GC run itself, is much longer. Additionally, technologies available to increase throughput in fixed laboratories, such as cryogenic cooling, are not practical in a mobile environment. In this work, practical strategies and commercial technologies were integrated to overcome these limitations and reduce sample turnaround.

For example, rather than performing solvent extraction, samples are analyzed by headspace solid phase microextraction (SPME) with an autosampler (CTC CombiPAL), reducing waste, human intervention and time. This has the added benefit of reducing GC/MS contamination due to liquid injection, as only compounds adsorbed from headspace on the fiber are introduced into the GC. Although the Pegasus III mass spectrometer (LECO) was found sufficiently rugged, fast, and required little modification, the GC (Agilent 6890) normally used was modified by the addition of two low thermal mass columns (RVM Scientific) in series. The columns have extreme heating and cooling rates (~1200 °C/min) which in turn permit extremely short chromatographic runs. The two columns in series permit a wider volatility range of compounds to be resolved by alternately trapping, desorbing, and separating analytes. Below left, a schematic of integrated system is shown. To the right, an analysis of a78 component EPA Method 524.2 VOC mixture (Absolute Standards, Inc.), 10 ppm in air, is shown, comparing the dual column and traditional single column separations, cutting run time more than half.





POSTER 26

A MALDI Quadrupole Time-of-Flight Mass Spectrometric Approach to Bacterial Identification in Bioaerosols

Alton J. Dugas Jr., Jae-Kuk Kim, Kermit K. Murray; Louisiana State University, Baton Rouge, LA 70803

The growing global threat of biological warfare and terror has increased the demand for rapid detection and identification of these threats. A principle detection method is mass spectrometry, as this method is rapid, sensitive and selective. Development of new mass spectrometric techniques will be essential for maintaining homeland security. We are developing methods for the rapid detection of aerosol-borne biological agents based on matrix-assisted laser desorption ionization (MALDI) coupled to a hybrid quadrupole time-of-flight (TOF) mass spectrometer.

Recently, several research groups have demonstrated results showing that bacteria can be identified through their characteristic mass spectra using MALDI mass spectrometry.[1] These mass spectra are typically free of interferences from growth medium constituents or contaminants. One approach is based on proteomic bacterial identification by database comparison of identified peaks in the mass spectra.[2] The difficulty in this approach is that the database search may obtain multiple results for a given peak from the protein database. A second, more general, approach compares the spectra obtained to those found in a "fingerprint" library to identify bacteria.[3] The difficulty in this method is in reproducing mass spectra from sample to sample due to differences in the proteome over time.

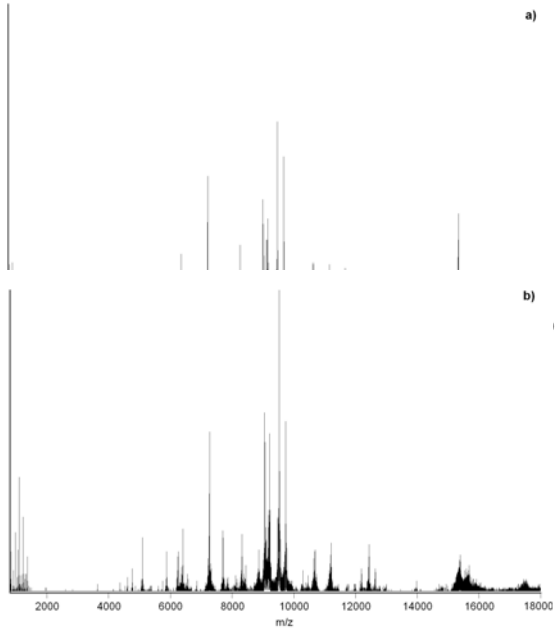


Figure 1. MALDI mass spectra of *E. coli* in a) SA matrix b) CHCA matrix.

Our lab has recently demonstrated a technique of collecting bioaerosol particles followed by MALDI TOF-MS.[4] This technique has been used to analyze bioaerosol particles deposited directly on MALDI targets with minimal post-deposit sample preparation. Our goal is to extend this collection technique for analysis on a hybrid quadrupole TOF mass spectrometer. To demonstrate the feasibility of this approach, we obtained mass spectra of lyophilized *E. coli* in both α-cyano-4-hydroxy-cinnamic acid (CHCA) and sinapinic acid (SA) matrix (Figure 1) using a dried droplet method.

The next phase of work will be to perform MALDI MS/MS on bacteria samples of selected peaks from the initial survey scan. In the product ion scan, we will fragment the selected mass via collision-induced dissociation (CID) and send the fragmented products to the TOF region to obtain a spectrum of the fragments. Using the generated spectrum, we can perform both *de novo* sequencing and a database search or use them simultaneously to enhance identification by narrowing the database search criteria. By using MALDI MS/MS, we will be able to provide an improved capability to identify organisms and potentially distinguish between endogenous and engineered cells.

This research is supported by the National Institute of Health (Grant No. R01ES10497) and Louisiana State University.

References:

1. Lay, J. O. Jr. *Mass Spectrom. Rev. B* **2001**, 20(4), 172-194.
2. Demirev, P. A.; Ramirez, J.; Fenselau, C. *Anal. Chem.* **2001**, 73(23), 5725-5731.
3. Wahl, K. L.; Wunschel, S. C.; Jarman, K. H.; Valentine, N.B.; Petersen, C. E.; Kingsley, M. T.; Zartolas, K. A.; Saenz, A. J. *Anal. Chem.* **2002**, 74(24), 6191-6199.
4. Jackson, S. N.; Murray, K. *Anal. Chem.* **2003**, 74(18), 4841-4844.

POSTER 27

Performance Advantages of Broadband-Modulated Time-of-Flight Mass Spectrometry

Brian G. Frederick, Stillwater Scientific Instruments, Orono, ME 04473; Laboratory for Surface Science and Technology,University of Maine, Orono, ME 04469

Mass spectrometry is the method of choice for identification of low-level amounts of highly toxic substances, such as chemical warfare agents, pesticides, and toxic industrial chemicals. Stillwater's Broadband-Modulation Time-of-Flight technology offers the sensitivity of SIM mode while obtaining full scan spectra, necessary for irrefutable confirmation of the identity of hazardous volatile or simi-volatile substances. The mode of operation utilizes the detector more efficiently, increasing the dynamic range by a factor of 100 to 1000. For field applications, smaller size and power requirements are important. The design of the Stillwater prototype miniature mass spectrometer has advantages of reduced vacuum requirements, lower operating voltages, and simpler optics than orthogonal acceleration TOF instruments and is being further miniaturized.

Pseudo random binary sequence (PRBS) modulation, first demonstrated in neutron scattering and molecular beam scattering, achieves a throughput advantage by operating at a time-domain duty cycle of 50%, although in practice the behavior of modulators used to chop the beam has limited the performance of such instruments[1,2]. By characterizing the chopper, both theoretically and experimentally [3], this information can be utilized in a probability-based data recovery method[4,5] to achieve an order of magnitude improvement in resolution and a multiplex advantage, as well as the orders of magnitude improvements in dynamic range and throughput expected. Stillwater has built both mass spectrometers and high resolution electron energy analyzers utilizing the method. We will discuss the basis of the method[5] and present results from a prototype miniature mass spectrometer.

(1) Brock, A.; Rodriguez, N.; Zare, R. N. *Anal. Chem.* **1998**, *70*, 3735.
(2) Kimmel, J. R.; Fernandez, F. M.; Zare, R. N. *J Am Soc Mass Spectrom* **2003**, *14*, 278.
(3) Jackson, R. H.; LeGore, L. J.; Yang, Z.; Kleban, P.; Frederick, B. G. *Surf. Sci.* **2002**, *502-503*, 240.
(4) LeGore, L. J.; Jackson, R. H.; Yang, Z.; Kleban, P.; DeNoyer, L. K.; Frederick, B. G. *Surf. Sci.* **2002**, *502-503*, 232.
(5) LeGore, L. J.; III, R. H. J.; Y.Yang, Z.; DeNoyer, L. K.; Kleban, P. H.; Frederick, B. G. Spectroscopy Instrument Using Broadband Modulation and Statistical Estimation Techniques to Account for Component Artifacts; US Patent 6,782,342 B2, 2002.

POSTER 28

Characterization of Chemical Warfare Agent Residues using EI/SI Ion Trap Mass Spectrometry

G. L. Gresham, G. S. Groenewold, A. D. Appelhans, and J. E. Olson; Idaho National Engineering and Environmental Laboratory, Idaho Falls, Idaho

In the event of a release of a chemical warfare agent (CWA) many environmental and synthetic surfaces (soils, vegetation, concrete, asphalt, automobiles) will be contaminated requiring accurate and rapid analytical information to guide the response and attribution efforts. Since CWA are modestly sized organic compounds, they have a good affinity for environmental and synthetic surfaces, and consequently there is a strong possibility that CWA may act to permeate into synthetic materials, and strongly adsorb onto non-permeable surfaces. In either scenario, the CWA would be sequestered on or in the surface, where it would be protected against environmental hydrolysis or intentional decontamination. This persistence poses a significant toxicity risk via dermal contact. In addition to strong adsorptive nature of CWA, many will under go degradation, which can occur as a result of environmental exposure or decontamination efforts resulting in degradation products that may retain high toxicity. The occurrence of multiple degradation pathways also complicates the investigation of CWA behavior. The adsorptive nature of CWA can make them difficult to analyze and analytical methodologies can be complex and labor intensive. A novel analytical technique that addresses these difficulties is secondary ion mass spectrometry (SIMS) modified to perform electron ionization (EI) analyses (EI/SI-ITMS), which enables direct detection of trace levels of CWA, toxins, and toxic metals, directly on environmental and synthetic surfaces.

The EI/SI-ITMS is based on the ion trap-secondary ionization mass spectrometers (IT-SIMS) that have been effectively used to characterize strongly adsorbed surface residues derived from V, G, N, and H classes of chemical warfare nerve and blister agents. The IT-SIMS was capable of analyzing both intact CW agents, hydrolysis products, and products of alkylation and polymerization occurring in the environment. The addition of EI enabled analysis of more volatile compounds that would evaporate from the samples once they are placed in vacuum.

The applicability of EI/SI-ITMS was recently demonstrated with the investigation of samples removed from steel 1-ton containers of the organoarsenical 2-cholorovinylarsenic III chloride, which is known as Lewisite or L. The analysis of the blister agent L is a particularly vexing analytical challenge because the As-Cl moiety is highly reactive; as a result, it cannot be analyzed chromatographically without extensive derivatization. This approach is cumbersome, prone to false negatives and ambiguous compound identification. On the other hand, analysis of L-contaminated samples using the EI/SI-ITMS showed L, and the synthetic impurities, *bis*(2-chlorovinyl)arsenic III chloride (L-2), and *tris*-(2-chlorovinyl)arsine (L-3). The instrumentation was also used to identify VX hydrolysis pathways and kinetics by direct analysis of contaminated surfaces: VX rapidly degrades on the surfaces of concrete to form diisopropylaminoethanethiol (DESH) and a diisopropylvinylamine isomer at a rate that would be effective for detoxification of small concentrations. Conversely, VX was found to be detectable on silicate soil samples well over a year after application at the ppm level. Analysis of soils contaminated with nitrogen blister agents HN-2 [bis(2-chloroethyl)methylamine] and HN-3 [tris(2-chloroethyl) amine] were shown to undergo hydrolysis to form N-methyldiethanolamine (MDEA) and triethanolamine (TEA), respectively; these compounds can be readily detected as adsorbed species on soil particles. When organic alcohols were present, the HN compounds formed 2-alkoxyethylamine derivatives on the sample surfaces. This result showed that nitrogen blister agents will undergo condensation reactions with nucleophilic compounds to form compounds of unknown toxicity, which emphasizes the need for an analytical methodology capable of detecting a range of degradation and condensation products on environmental surfaces.

POSTER 29

GC and Direct SPME Characterization Using a Portable Cylindrical Ion Trap (CIT) Mass Spectrometer

Garth Patterson, J. Mitchell Wells, John Grossenbacher, Anthony Cochran, Brent Knecht, Brent Rardin, Mark Gregory, Jason Springston, Dennis J. Barket, Jr.; Griffin Analytical Technologies, Inc., West Lafayette, IN

Introduction

Field-deployable chemical analysis has become a necessity for a wide variety of applications. By performing analyses in the field rather than collecting samples to be studied in the lab at a later date, valuable time, resources, and chemical information content are retained. Interfacing a novel inlet technology and a low thermal mass GC with a fieldable mass spectrometer, in conjunction with sample extraction techniques, has yielded a powerful chemical analysis tool capable of chemical detection and identification of a variety of compounds of interest in forensic science and counterterrorism.

Methods

The portable GC-MS utilizes a low thermal mass GC (LTM-GC) and a mass spectrometer containing a cylindrical ion trap (CIT) mass analyzer. Samples were introduced to the GC inlet via liquid injection, and also with a solid phase microextraction (SPME) fiber. Data are also presented on the use of a direct insertion SPME inlet, where the SPME fiber is introduced directly to the mass spectrometer vacuum to provide rapid desorption and analysis of sampled compounds. Multidimensional mass analysis (MS^n) can be performed in conjunction with or instead of the separation technique to yield highly selective analyses.

Results

Data are presented from each inlet technique and include spectra collected from analyses using live chemical warfare agents and mixtures of agents. Limits of detection (LODs) for CW simulants and live agents on the order of tens of picograms were obtained. Use of the direct SPME technique allows for sample desorption and analysis in less than 15 seconds. Explosive materials have also been analyzed with the LTM-GC and the direct SPME interface; representative chromatograms and mass spectra will be presented. Multidimensional mass analyses will also be presented. These experiments demonstrate highly selective, very sensitive, and rapid analyses using a portable GC-MS system.

This material is based upon work supported by MARCORSYSCOM, United States Marines, under Contract No. M67854-04-C-3002.

POSTER 30

Trace Analysis of Peroxide Explosives by High Performance Liquid Chromatography - Atmospheric Pressure Chemical Ionization - Tandem Mass Spectrometry (HPLC-APCI-MS/MS) for Forensic Applications

Xiaoma Xu, Ph.D.; Anick M. van de Craats, Ph.D.; Eric M. Kok, B.Sc.; and Peter C.A.M. de Bruyn, M.Sc.; Netherlands Forensic Institute (NFI), P.O. Box 24044, 2490 AA The Hague, The Netherlands

In recent years, the analysis and detection of one class of explosives, namely peroxide explosives, has become particularly important in forensic investigations because of the emergence of terrorist threats and crimes in which these explosives were applied.

An HPLC-APCI-MS/MS method for the (trace) analysis of the most commonly encountered peroxide explosives, hexamethylenetriperoxidediamine (HMTD) and triacetonetriperoxide (TATP), has been developed. HMTD and TATP have been analyzed in the same run. (Pseudo-)molecular ions of these peroxides have been obtained as base peak under the same condition. A series of product ions was produced from these pseudo-molecular ions ($[HMTD-1]^+$ and $[TATP+NH_4]^+$) in the MS/MS analysis. We also pioneered in showing that a TATP molecular ion $[TATP+H]^+$ can be observed with HPLC-MS/MS. The limit of detection for HMTD and TATP was 0.26 and 3.3 ng, respectively, on column by HPLC-MS in the Full Scan mode and 0.08 and 0.8, respectively, by HPLC-APCI-MS/MS in Selected Reaction Monitoring mode. The method presented has been applied successfully for the identification of peroxides in the bulk solid state (powder sample), as well as in post-blast extract originating from a forensic cases. For the post-blast extracts, the use of tandem MS has been shown clearly to be of crucial importance for the identification and detection of the peroxide explosives.

SANIBEL CONFERENCE PARTICIPANT SURVEY
MS in Forensic Science and Counterterrorism

Please circle appropriate number below

	Poor	Fair	Good	Excellent
PROGRAM CONTENT				
Topics	1	2	3	4
Lectures	1	2	3	4
Discussion	1	2	3	4
MATERIALS (Program)				
Pertinent	1	2	3	4
Current	1	2	3	4
Quality	1	2	3	4
ORGANIZATION				
Value with respect to fee charged	1	2	3	4
Location & facilities	1	2	3	4
Refreshments	1	2	3	4

We may relocate the conference semi permanently to this location (from the Sundial). Do you favor this change? ☐ Yes ☐ No

COMMENTS.

Suggestions for future Conference topics

Please give to Cindi Lilly at registration before you depart or fax to ASMS, 505-989-1073

Thank you.