



Validated LC-MS Library and Strategy for Identifying Harmful Organics in Drinking Water

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- ORD- National Homeland Security Research Center
- OW- Water Security Division

Mention of Vendor Name Does Not Constitute Product Endorsement

Summary of Issue

- HPLC/MS/MS is a powerful technique to screen drinking water for a host of organic contaminants.
- To date, no widely applicable HPLC/MS/MS libraries exist; but gas chromatography mass spectral (GC/MS) libraries (Wiley, NIST ...) have been in widespread use.
- Our goal is to provide laboratories with an HPLC/MS/MS Library that would be a helpful new tool in monitoring the quality of drinking water for possible threat agents.
 - Current Library is comprised of 129 contaminants
 - Library will include 300+ contaminants



Rationale

What are we looking for and why??

- We are undertaking an initiative to develop a robust screening method for drinking water using LC/MS and LC/MS/MS technologies.
- This method will provide detection and identification of deleterious organic compounds not amenable to GC/MS.
- Organic analytes amenable to analysis via LC/MS and LC/MS/MS include:
 - Agrochemicals: pesticides and insecticides
 - Controlled substances (LSD, methamphetamine, ecstasy)
 - Pharmaceuticals

System to Tentatively Identify a Compound Using an LC/MS Library

- Standards were infused in order to create full scan (MS) and product ion scan library spectra (MS/MS).
- These spectra were designed to contain characteristic fragmentation while maintaining the integrity of the molecular ion at approximately 10% relative intensity to insure proper tentative identification.



Analytical System

- **HPLC**
 - Waters Alliance 2695 HPLC unit
- **Mass Spectrometers**
 - Micromass® ZQ™ Mass Spectrometer
 - Micromass® Quattro micro API mass spectrometer (capable of single and triple quadrupole usage)
- **Software**
 - MassLynx

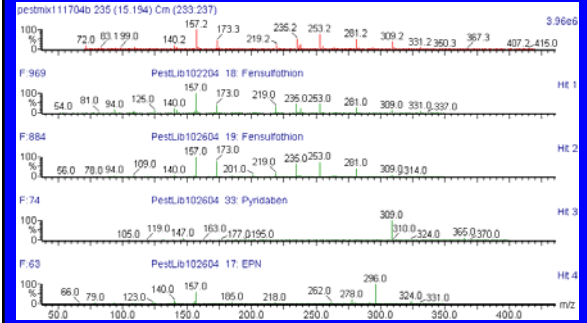
Experimental Conditions

- **Columns**
 - XBridge C₁₈ 2.1 X 150mm column, 3.5 µm particle diameter
- **Mobile Phase**
 - Acetonitrile/Water
- **Modifiers**
 - 5 mmolar NH₄HCO₃ adjusted to pH 10 with NH₄OH
- **Flow**
 - Gradient
 - Flow Rate: 0.300 mL/min
- **Injection**
 - Injection volume: Variable up to 100 µL

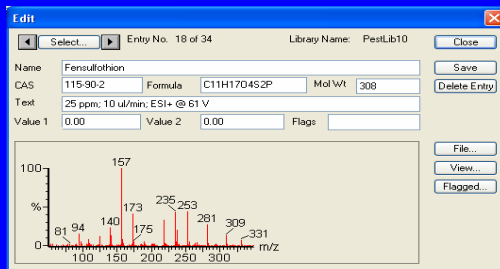
Unknown Identification Scenario

- Perform LC/MS on the water sample using full scan detection at a variety of cone voltages.
- This gives retention time data and full scan spectra which can be matched to MS libraries
- A TIC gives presumptive identification which can be further confirmed by generating it's product ion spectrum under set MS/MS conditions which can be matched to MS libraries.

Library Search of Spectrum 19.19 minutes @ 60 Volts



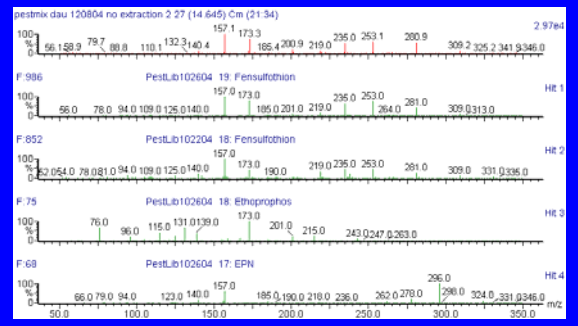
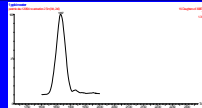
Fensulfothion Full Scan Library Spectrum



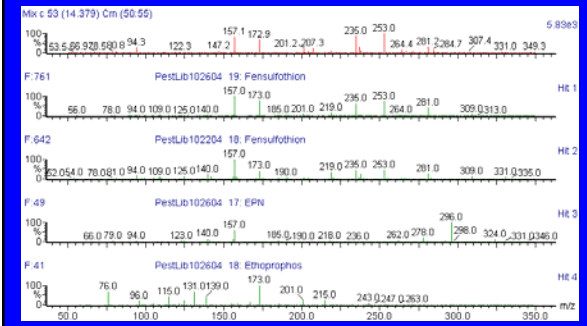
Further Tentatively Identified Compound Confirmation using LC/MS/MS

- MS/MS Method set to acquire a product ion spectrum of 309 around the retention time at 19.19 minutes at a cone voltage of 33 and a collision voltage of 19.
- The MS/MS library was acquired utilizing these same conditions.
- The spectrum generated is then compared to the MS and MS/MS libraries for added confirmation.
- Fensulfothion is tentatively identified at this point by matching of the Full scan spectra and daughter spectrum to our libraries.

Product Ion Spectrum of 1 ppb Fensulfothion in Water (no extraction) matched to both libraries



Fensulfothion at 1 ppb Product Ion Spectrum Matched to Both Libraries Analyzed at Waters Corporation, Milford, MA



Conclusions

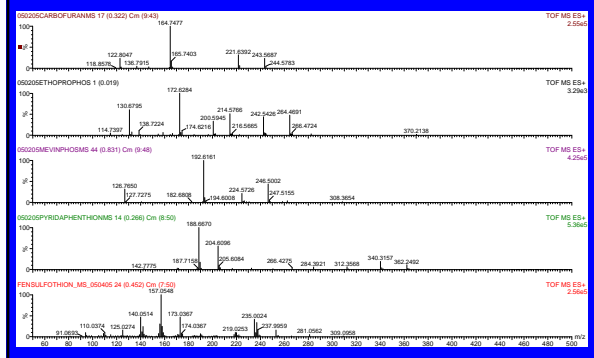
- Sensitivity is Compound Dependent
- All of the compounds are successfully searchable at 100 ppb full scan or product ion scan.
- Many are successfully searchable at 1 ppb product ion scan.

Applicability for Time of Flight Data

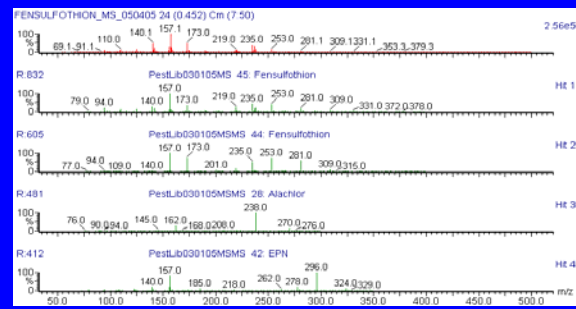
Marc Mills and Bryan Boulanger US EPA ORD-NRMRL Cincinnati, OH

- Quadrupole Time of Flight Mass Spectrometer (Q-ToF micro™)
- Exact mass MS measurement enables compounds to be more easily identified
- Great for “True Unknown” Analysis

TOF Spectra Data



MS TOF Match

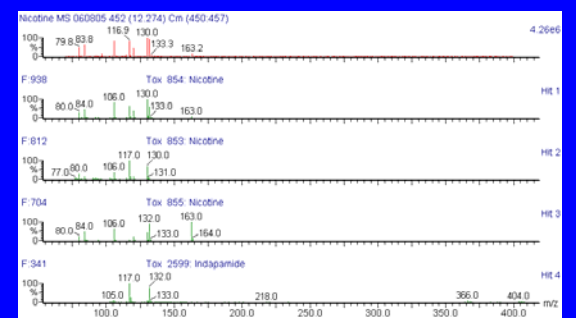


Calmette Hospital's Toxicological Library Lille, France



- Contains over 500 toxicological analytes at multiple voltages
- Total of 2692 Spectra!
- 12 Analytes on our list are in the toxicological library
- The Tox library was setup with the following generic conditions.
 - A, 5mM ammonium formate + 0.05% formic acid
 - B, Acetonitrile + 0.05% formic acid
 - Gradient, 0min 95% A, 16min 90% B, 20min 90% B
 - 2.1 x 100mm, 3.5um C18 MS XTerra @ 35 degrees C

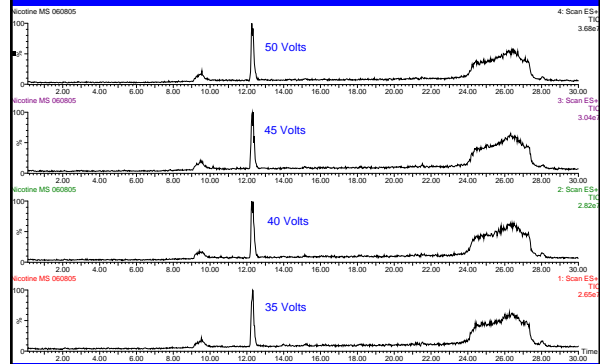
Nicotine Analyzed at CRL using Tox Library (BEFORE I KNEW OTHER LIBRARY EXISTED)



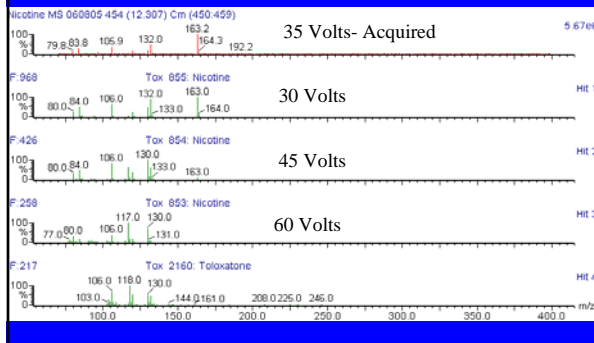
Nicotine in French Library

- Nicotine Entry at 6 voltages
 - 15, 30, 45, 60, 75, 90 volts
- Good for a single-quad screening method
- Results in multiple matches of the same compound at different voltages- additional confirmation!

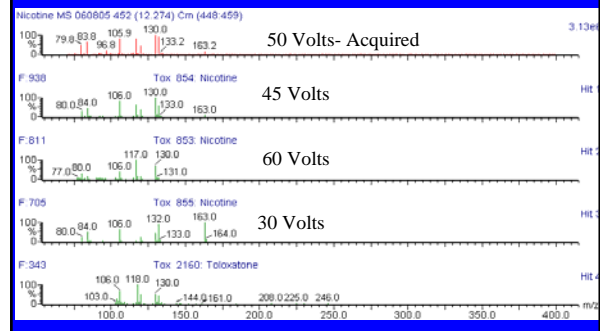
Nicotine Acquired at Multiple Voltages



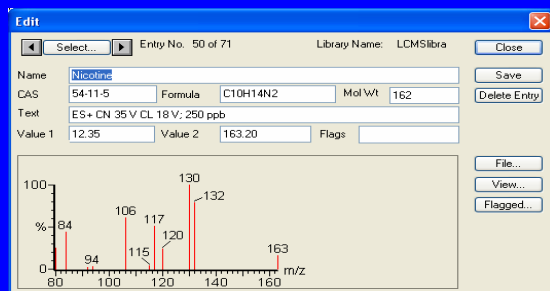
35 Volt Scan



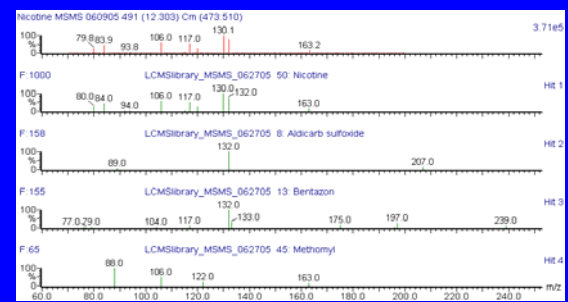
50 Volt Scan



MS/MS Library Confirmation



MS/MS Confirmation



12 out of 12 are the First Hit using the French Library!

- Do I need to say anything more?
- Another version of the EPA/Waters library is in process to include multiple voltages for added confirmation for those screening using a single quad MS
 - Data acquired, need to build the library
- MS/MS users will have additional confirmation
- Further develop the French Library for MS/MS confirmation?

LC/MS Library Validation Results

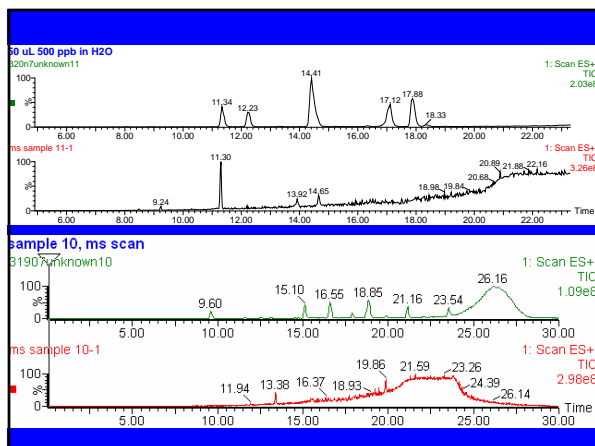
- LC/MS Library Protocol, methods, libraries and electronic files sent to participating labs last week of March 2007.
- Thirteen unknown mixes sent to eight labs
 - Six officially in the study
 - Two extra participants

Results

- Lab 1 – 91% Correct, 54/59
- Lab 2- 84% Correct, 58/69
- Lab 3- 62% Correct, 37/59
- Lab 4- Not in Study
- Lab 5- 86% Correct, 50/58
- Lab 6- 87% Correct, 62/71
- Lab 7- 83% Correct, 57/69
- Lab 8- Not in Study

Lab 3 Problems

- Did not analyze in negative mode
- High background noise
- Peaks missing due to interferences



Validation Results

- 107 Validated
- 16 Not Validated
 - Identified by Only One Lab
- 6 Not Validated
 - Not Identified by Any Lab

(RS)-methoprene	Clodinafop-propargyl	Imazethapyr	Propamocarb
2-Aminobenzimidazole	Clomazone	Imidacloprid	Propoxur
3-Hydroxy carbofuran	Colchicine	Isofenphos	Prosulfuron
Acetaminiprid	Cotinine	Isoxaflutole	Pyridaben
Acetochlor	Cyanazine	Kresoxim-methyl	Pyridaphenthion
Aconitine	Cyclanilide	LAMPA	Quinine
Alachlor	Cycloheximide	LSD	Sethoxydim
Aldicarb	Cyprodinil	Malathion	Simazine
Aldicarb sulfone	Cyromazine	Mesotrione	Simetryn
Aldicarb sulfoxide	Daminozide	Metalaxyl	Spiroxamine
Allethrin	Desethyl hydroxy atrazine	Methamidophos	Strychnine
Ametryn	Desisopropyl atrazine	Methiocarb	Tebuconazole
Amitraz	Diazinon	Methomyl	Tebufenpyrad

ANTU	Dicrotophos	Metolachlor	Temephos
Atrazine	Digitoxin	Metsulfuron-methyl	Terbumeton
Atropine	Diphacinone	Mevinphos	Terbutylazine
Azinphos-methyl	Diuron	Molinate	Thiabendazole
Azoxystrobin	EPTC	Monocrotophos	Thiamethoxam
Bromoxynil	Ethiofencarb	Napropamide	Thifensulfuron-methyl
Buprofezin	Ethoprophos	Naptalam	Thiram
Butylate	Fensulflothion	Nicotine	Tralkoxydim
Carbaryl	Formothion	Oxamyl	Triadimefon
Carbendazim	Heroin	Pirimicarb	Triasulfuron
Carbofuran	Hexazinone	Pirimiphos-methyl	Trinexapac-ethyl
Chlorimuron-ethyl	Imazalil	Prometon	Triticonazole
Chlorsulfuron	Imazamethabenz-methyl	Prometryn	Warfarin
Clethodim	Imazaquin	Propachlor	

Compounds Not Validated

Acibenzolar-s-methyl	Emetin, HCl	Alanine
Bentazon	Ethion	Fenitrothion
Chloramben	Fenthion	Phosalone
Chlorobenzilate	Naled	Resmethrin
Coumarin	Permethrin	Tri-allate
DDVP	Phorate	Trichlorfon
Digoxin	Phosmet	
Dodine	Trifloxystrobin	

Peer Review and Distribution

- Peer Review by ORD/NERL and NHSRC
- Library System Protocol Version 1.2 Available Free of Charge to Federal and State Labs.

Present Activity

- System Monitoring Compounds Evaluation
 - Warfarin (Phenyl-d5)
 - Simazine (Ring-13C3)
 - Carofuran (Ring-13C6)
- Standardized Analytical Methods (SAM) for use During Homeland Security Events Evaluation and Incorporation of Target Analytes
- Inclusion of additional compounds of interest
- Collaborating lab network expansion and training
- Automatic data processing- Chromalynx™
- Scientific Data Management System- NuGenesis

Conclusion

- This will allow us to Screen Water Samples for Deleterious Organics in Environmental Waters
 - Drinking Water
 - Well Water
 - Clean Surface Waters
- Limit of Detection is Compound Dependent
- Improvements and additions to the libraries are continually being made as more applied science is conducted.
- Applicable and Transferable for LC/MS, LC/MS/MS, UPLC/MS/MS and TOF MS/MS