Fast Screening of Over 1000 Pesticides and Toxins Using Multiple Techniques at the US Food and Drug Administration

3rd Latin American Pesticide Residue Workshop

May 8-11, 2011

Chris A. Sack, David Eide, Michael Smoker, Jon Wong, Kai Zhang, Greg Mercer, Lora Lin, Sara Edison

Introduction

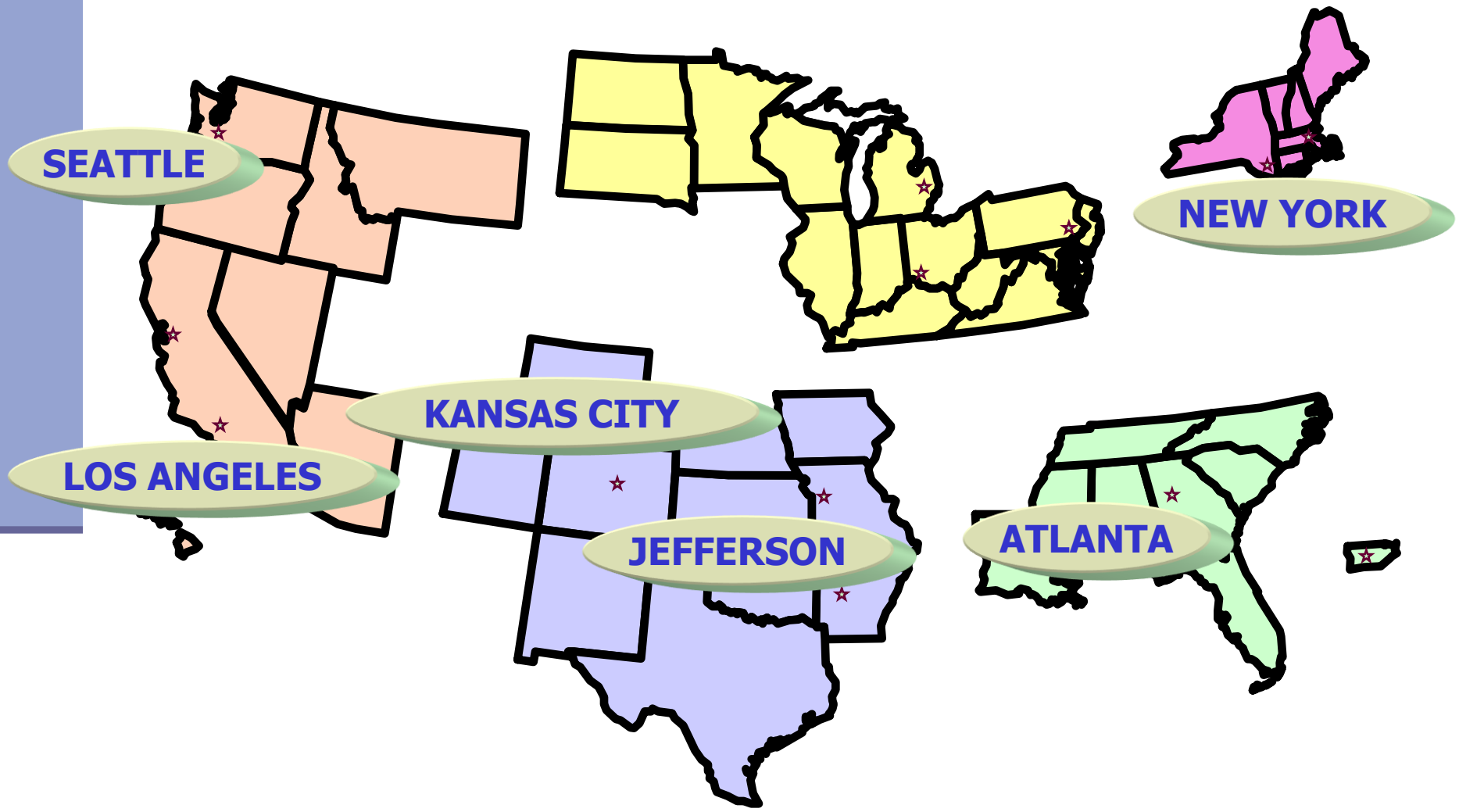
- Overview
- Residue methods
- Future
- Pesticide Residue Analysis Pilot Course

Overview - Challenges

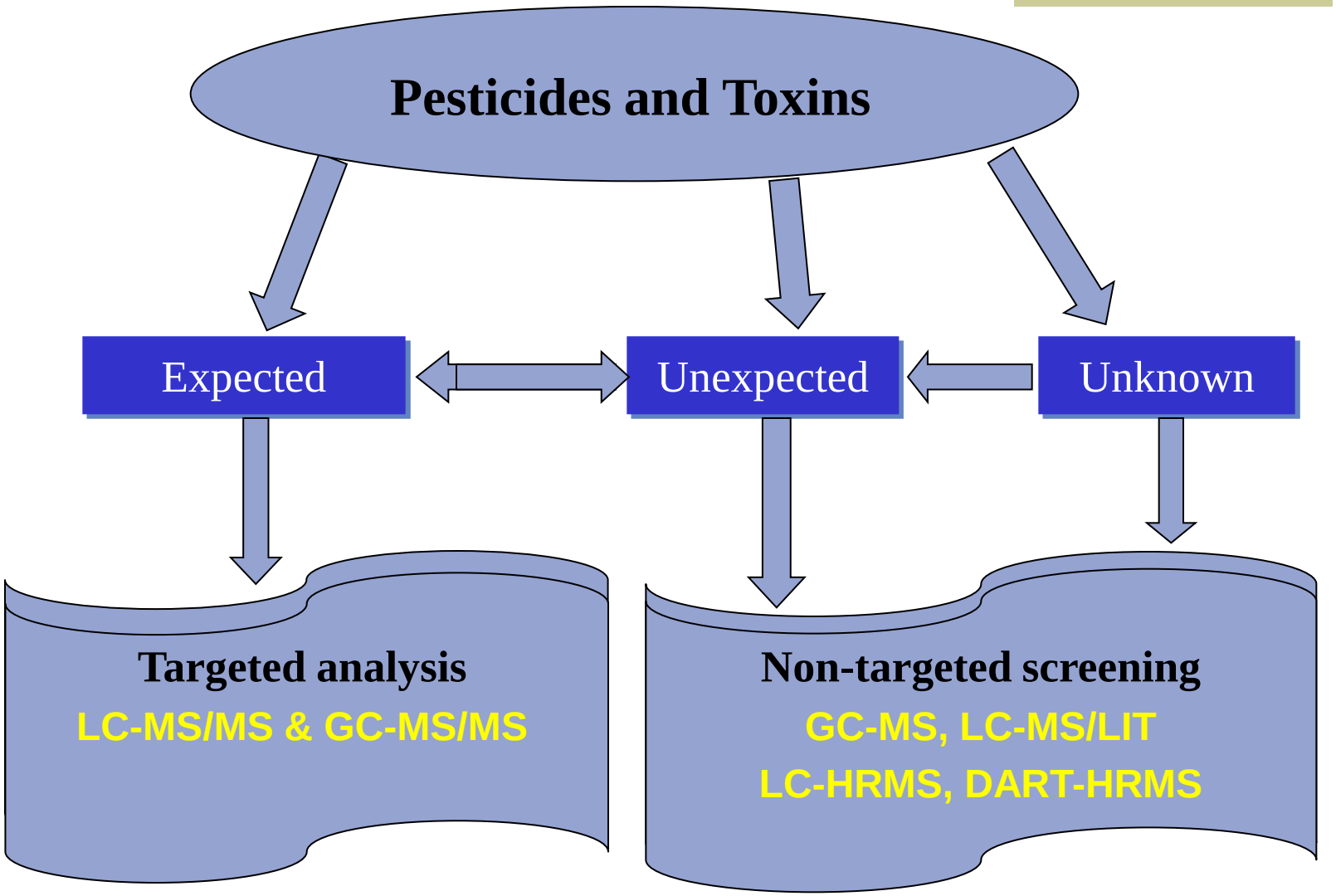
- Food and feed matrices
  - Imports: > 11 million per year
  - Domestic: ???
- Pesticides and other contaminants
  - 1000s that are known
  - Range: 10 ppb - ???
- Analyses
  - Up to 50 samples per day per lab
  - Timeframe: 1 day for imports

Overview

6 PESTICIDE LABORATORIES



Analytical Strategy



Analytical Strategy

- Targeted Quantitative Analysis
  - LC-MS/MS and GC-MS/MS
  - >350 Selected analytes
    - Historical findings
    - Anticipated findings
  - Calibration Standard Mixtures
    - Designed by FDA
    - Prepared by commercial vendors
    - Available for purchase

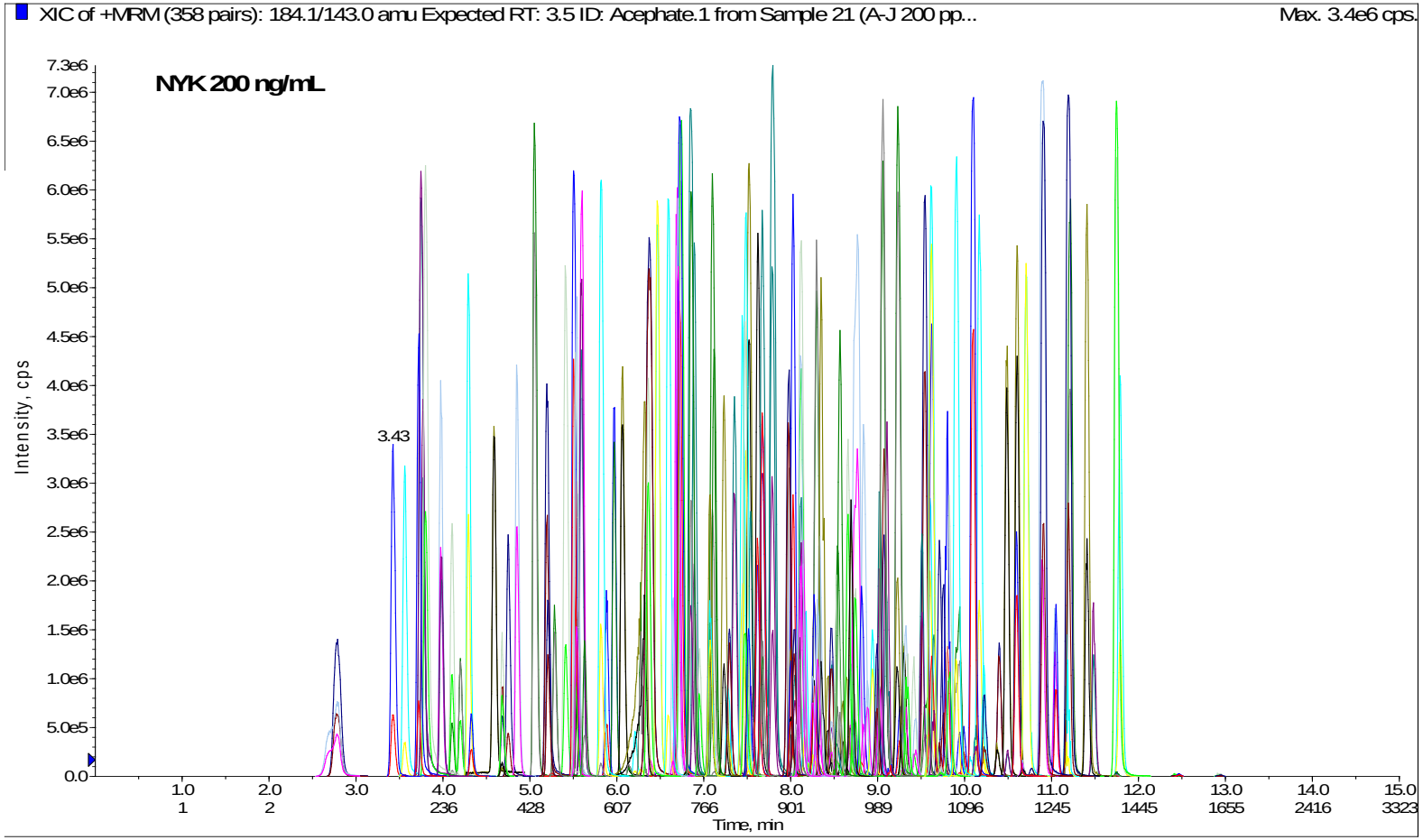
Analytical Strategy

- Qualitative Screening Analysis
  - No standards required
  - Spectral Library Techniques
    - GC-MS (fullscan mode)
    - LC-MS/LIT (IDA-EPI)
  - Exact Mass Techniques (Exactive Orbitrap)
    - LC-HRMS
    - DART-HRMS

LC-MS/MS Determination

- Scope: >240 pesticides in 15 minutes
- Chromatography: reverse phase
- Columns: C_{18}
  - 100 x 2.1 mm, ~3 µm
  - 50 cm x 4.6 mm, 1.9 µm
- Mass Spectrometer parameters
  - Ionization: Positive electrospray
  - Detection: scheduled MRM (two transitions)
- Detection Limit: < 10 ppb for most compounds

LC-MS/MS Determination

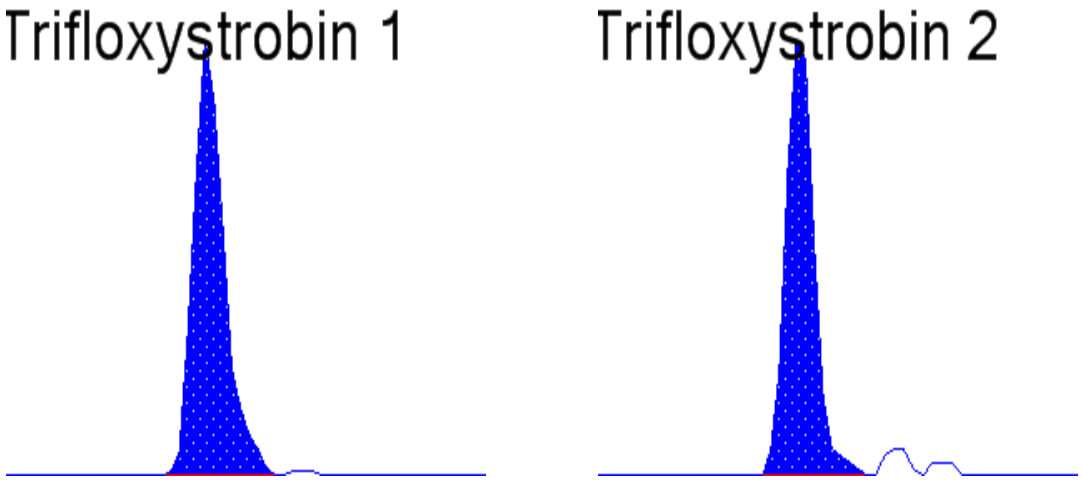


LC-MS/MS Determination

Residues in strawberry sample

Pesticide	PPB	Pesticide	PPB
Boscalid	128	Novaluron	9
Pyraclostrobin	54	Hexythiazox	5
Cyprodinil	47	Spiromesifen	2
Azoxystrobin	45	Propiconazole	1
Pyrimethanil	41	Acetamiprid	1
Chlorantranilaprole	23	Carbendazim	1
Fludioxinil	22	Methomyl	0.7
Fenhexamid	20	Thiophanate methyl	0.5
Bifenazate	9	Methoxyfenozide	0.3
Myclobutanil	9	Fenpyroximate	0.2

LC-MS/MS Determination



Trifloxystrobin @ 0.2 ppb in applesauce

GC-MS/MS Determination

- Scope: > 200 pesticides in 20 minutes
- GC
  - Retention time locking
  - Backflushing mid column
- MS/MS detection
  - MRM (2-3 transitions/compound)
- Detection Limit: 1 - 10 ppb for most analytes

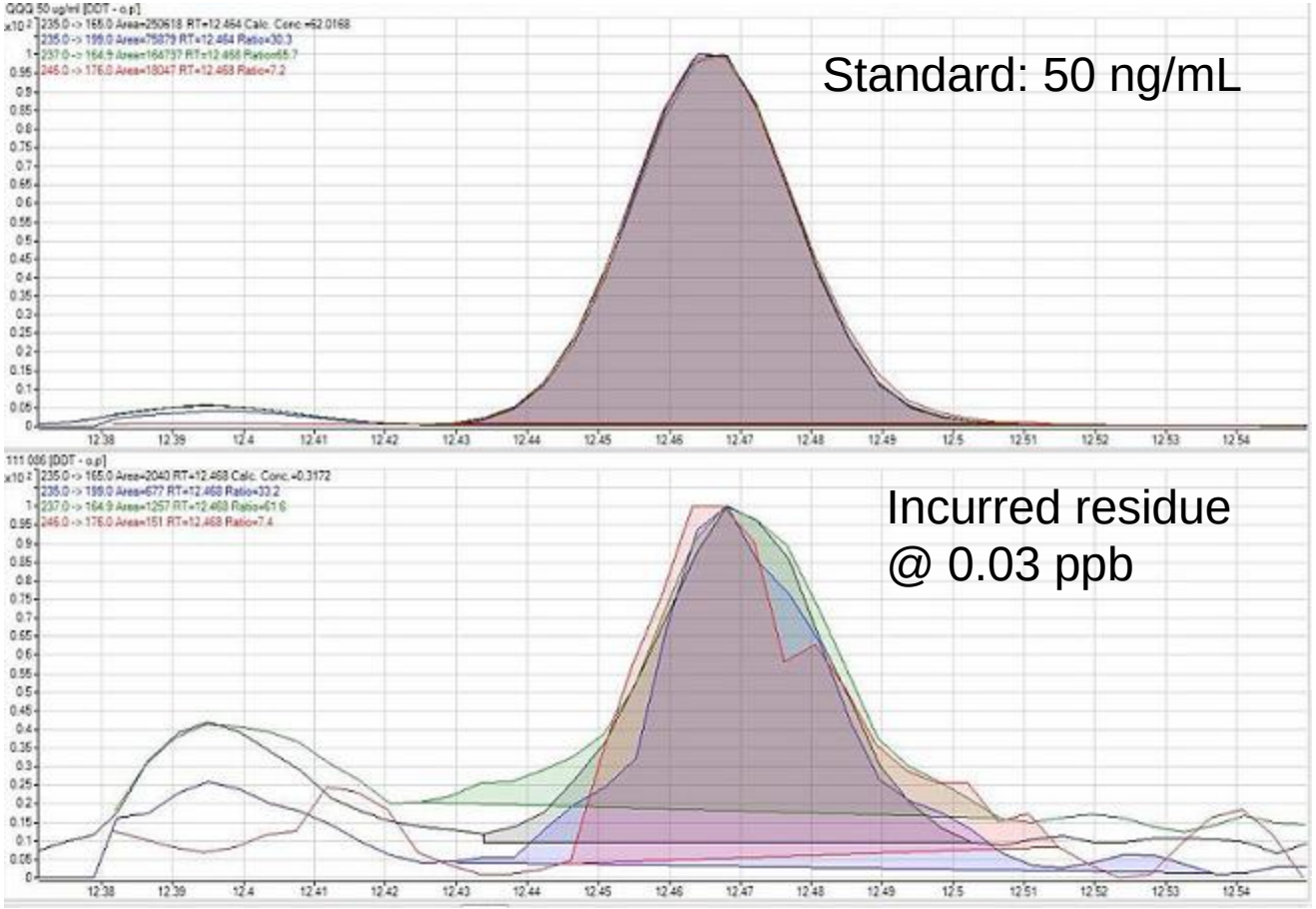
GC-MS/MS Determination

Residues in strawberry sample

Pesticide	PPB	Pesticide	PPB
THPI	237	Bifenthrin	7
Boscalid	128	Quinoxyfen	3
Pyraclostrobin	54	Spiromesifen	2
Cyprodinil	47	Propiconazole	1
Pyrimethanil	41	Fenpropathrin	0.7
Azoxystrobin	37	Biphenyl	0.3
Captan	27	Chlorpyrifos	0.1
Fludioxinil	22	Folpet	0.1
Fenhexamid	20	p,p’-DDE	0.1
Bifenazate	9	p,p’-DDT	0.04
Myclobutanil	9	o,p’-DDT	0.03
Malathion	8		

GC-MS/MS Determination

o,p'-DDT in strawberry

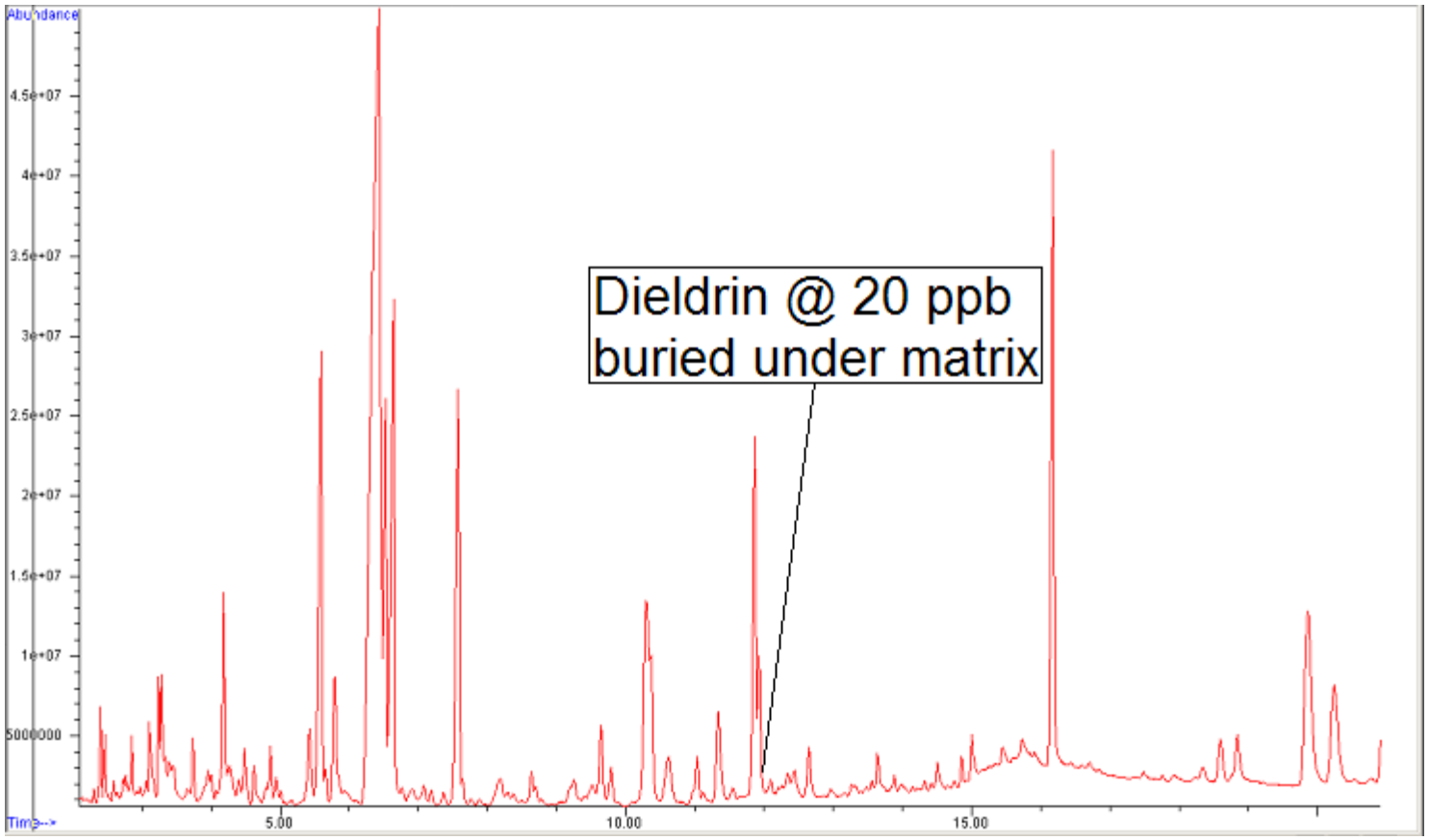


GC-MS Fullscan Screen

- Scope: > 900 compounds in 20 minutes
- Screen for library matches - no standards
- Agilent GC-MSD pesticide library
- Identification:
  - Spectral matching of AMDIS deconvoluted spectra
  - Retention time

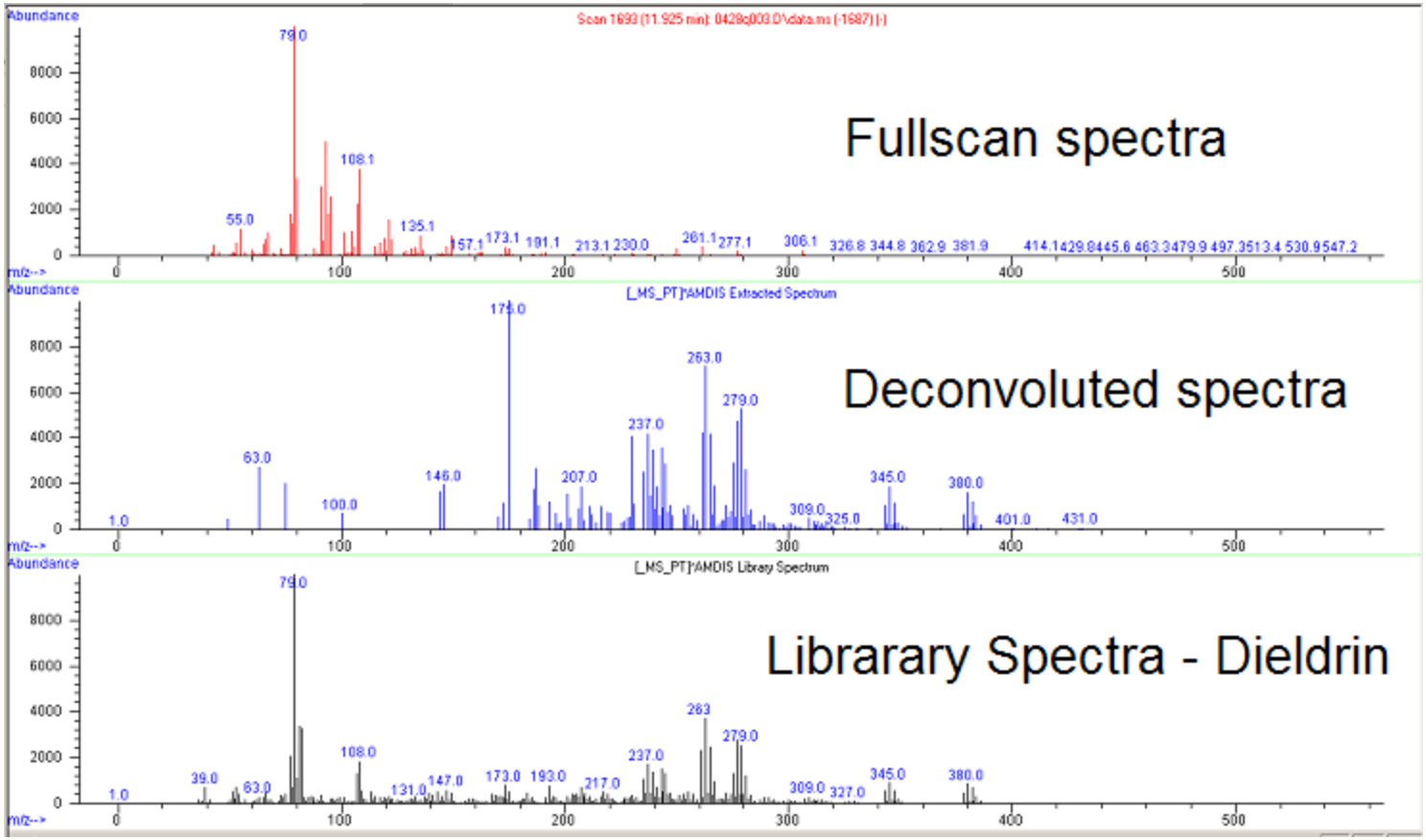
GC-MS Fullscan Screen

Celery fullscan TIC



GC-MS Fullscan Screen

Celery fullscan TIC



GC-MS Fullscan Screen

Celery deconvolution report

		AMDIS		NIST	
R.T.	Compound Name	Match	R.T. Diff	Reverse Match	Hit Num.
2.5859	Naphthalene-d8	99	-8.0	91	1
2.842	Carvone	75	-8.3	81	2
3.341	EPTC	94	-7.2	89	2
6.977	Anthracene	80	-5.4	88	5
7.9576	Diisobutyl phthalate	75	1.2	84	11
9.214	Di-n-butylphthalate	65	0.8	90	18
9.658	Parathion	82	2.5	76	2
11.925	**Dieldrin**	**79**	**1.5**	**61**	**2**
14.3189	Chrysene-d12	79	-12.1	91	1
14.8515	Bis(2-ethylhexyl)phthalate	96	3.3	85	7
15.7388	Permethrin I	60	6.5	63	2
15.833	Permethrin II	63	7.0	79	
16.518	Cypermethrin II	43	11.7	57	1

GC-MS Fullscan Screen

Residues discovered and transferred to the LC-MS/MS and GC-MS/MS targeted analyses:

- Fluopicolide
- Spiromesifen
- Spirodiclofen
- Flonicamide
- Chlorfenapyr
- Etoxazole
- Bifenazate
- Etoxazole
- Fenamidone
- Famoxadone
- Quinoxyfen
- Sudan I*

**Food color prohibited in US - not added to target analysis*

Looking to the Future

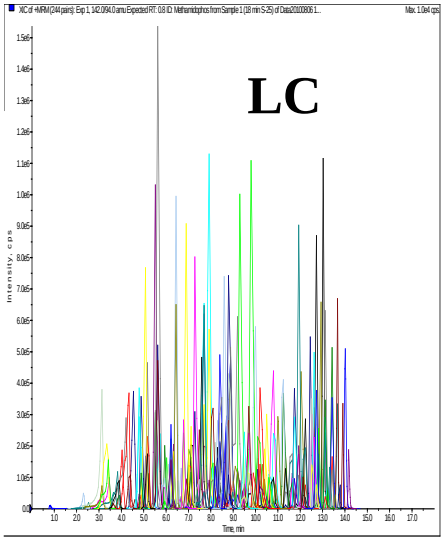
LC-MS/LIT Mass Spectral Libraries

- One instrument and Two Approaches
  - MRM by LC-MS/MS
  - MS library matching using LC-MS-Linear Ion Trap Hybrid Mass Spectrometry
- Targeted and library screening

Kai Zhang[1], Jon W. Wong[1], Paul Yang[2]
1. Food and Drug Administration, College Park, MD, U.S.A.
2. Ontario Ministry of the Environment, Ontario, Canada

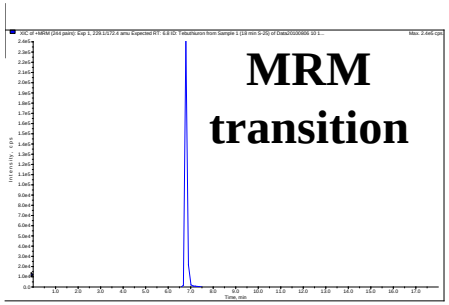
Looking to the Future

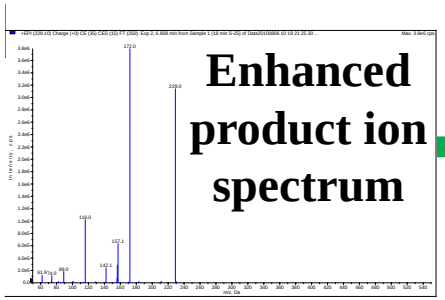
LC-MS/LIT Mass Spectral Libraries

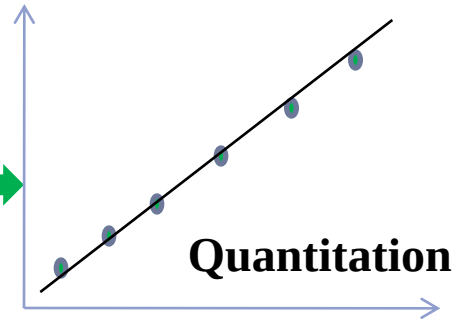


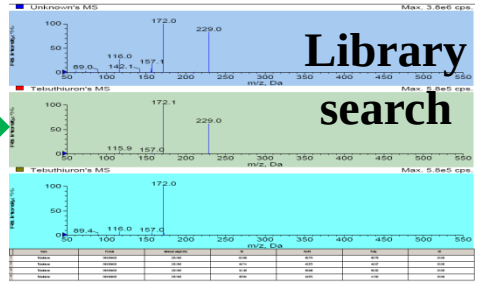
Survey scan

Intensity threshold $[M+H]^+$





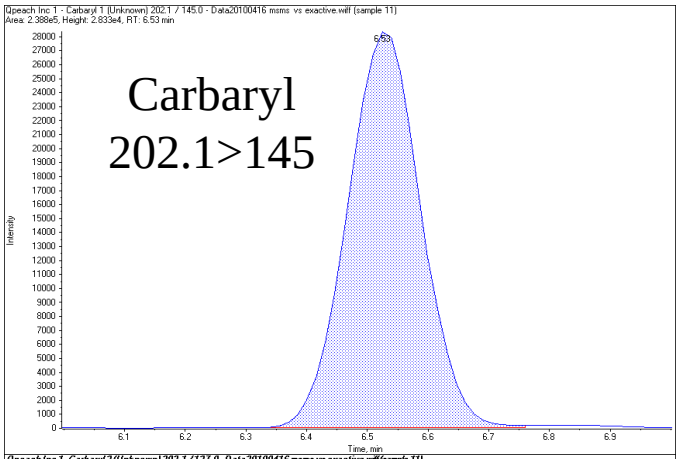


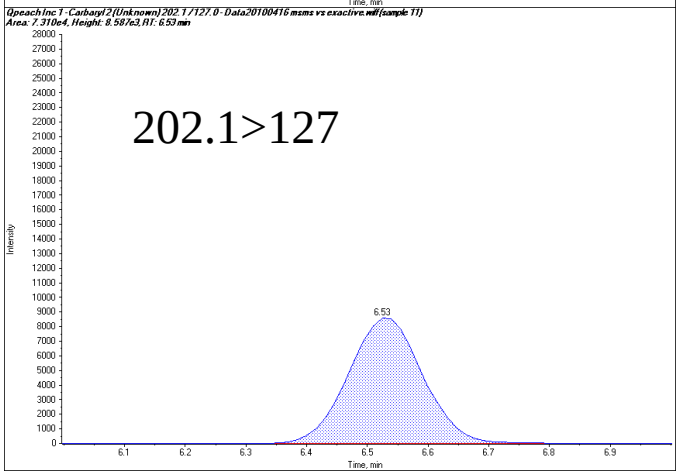


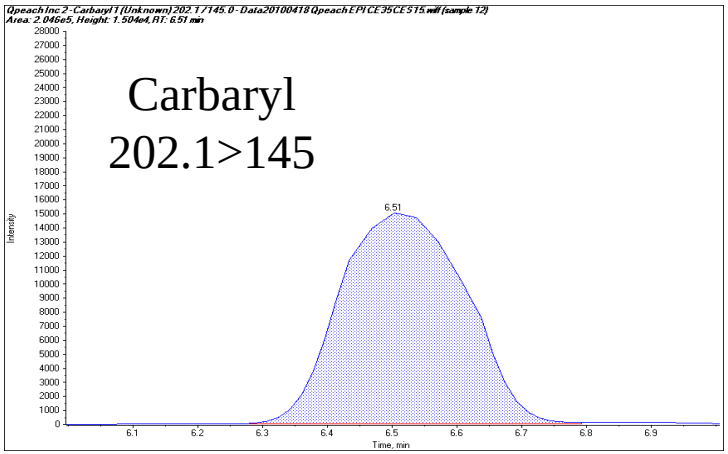
Targeted screening: information dependent acquisition-enhanced product ion (IDA-EPI) method

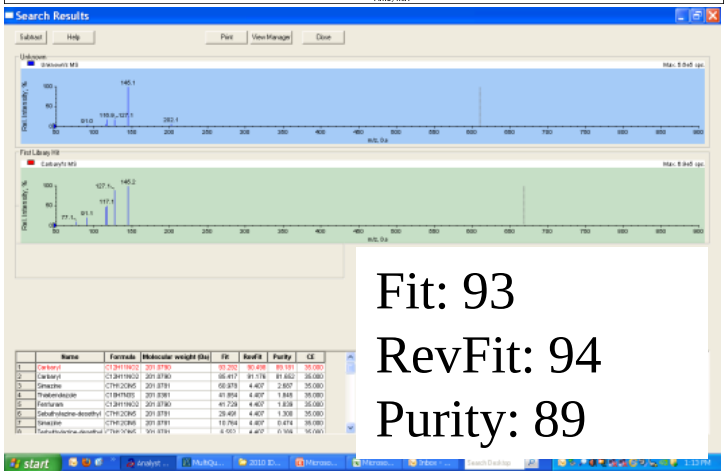
Looking to the Future

LC-MS/LIT Mass Spectral Libraries





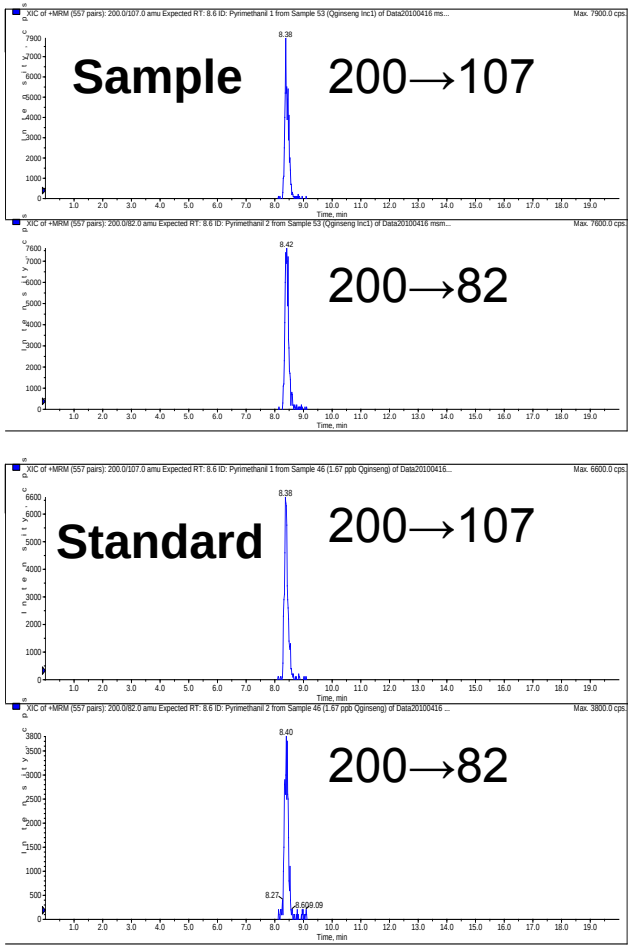




Carbaryl confirmed in peach sample

Looking to the Future

LC-MS/LIT Mass Spectral Libraries

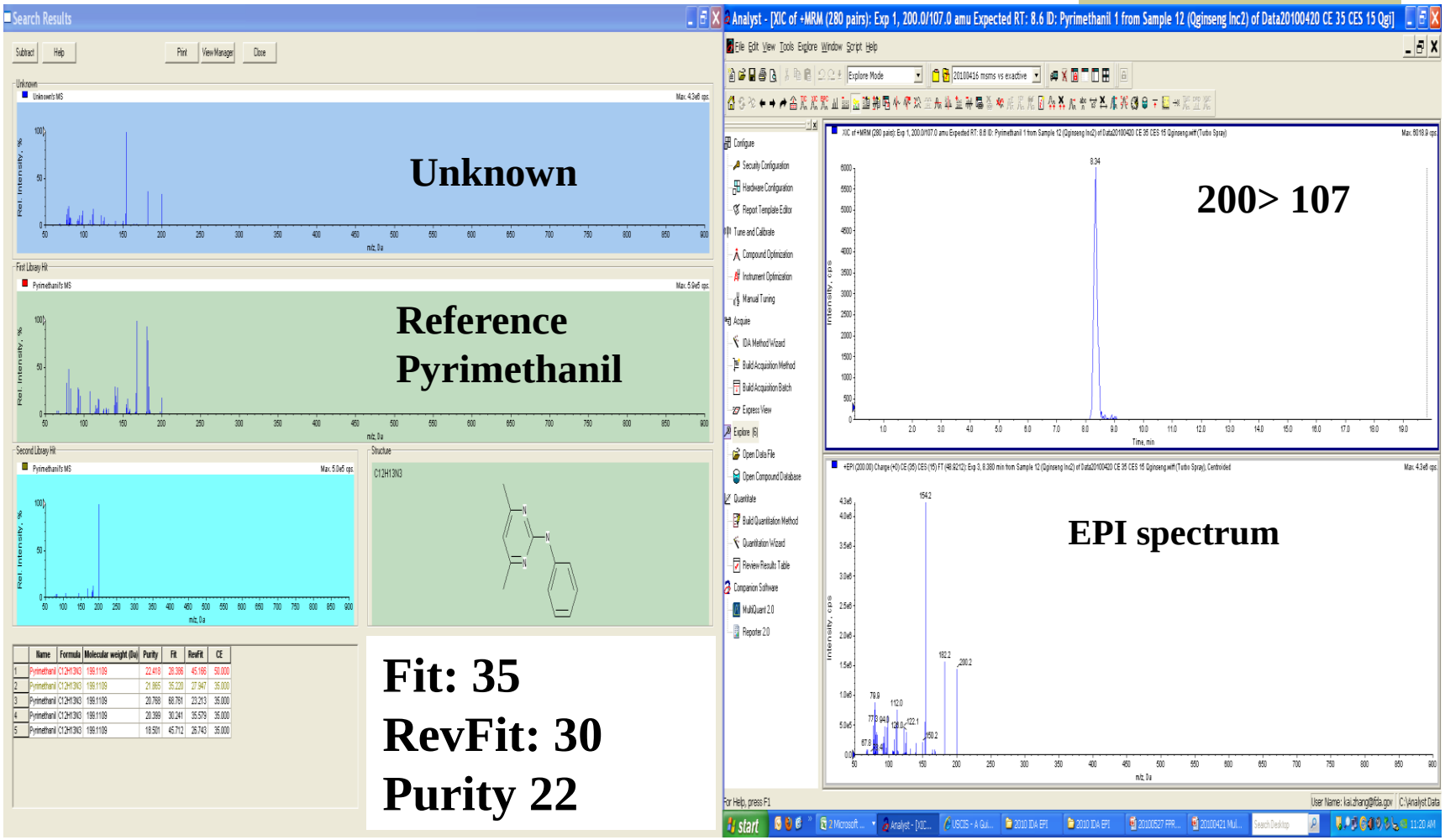


False Positive: Pyrimethanil?

- RT match
- Two Pyrimethanil MRMs
  - 200 → 107
  - 200 → 82
- Ion Ratios (200→107/200→82)
  - Requirement: ± 20 %
  - Sample/Standard: ~ 60 %

Looking to the Future

LC-MS/LIT Mass Spectral Libraries



Looking to the Future

LC-MS/LIT Mass Spectral Libraries

- Currently building MS libraries for targeted compounds
- Plan to increase screening capability by adding other non-targeted compounds

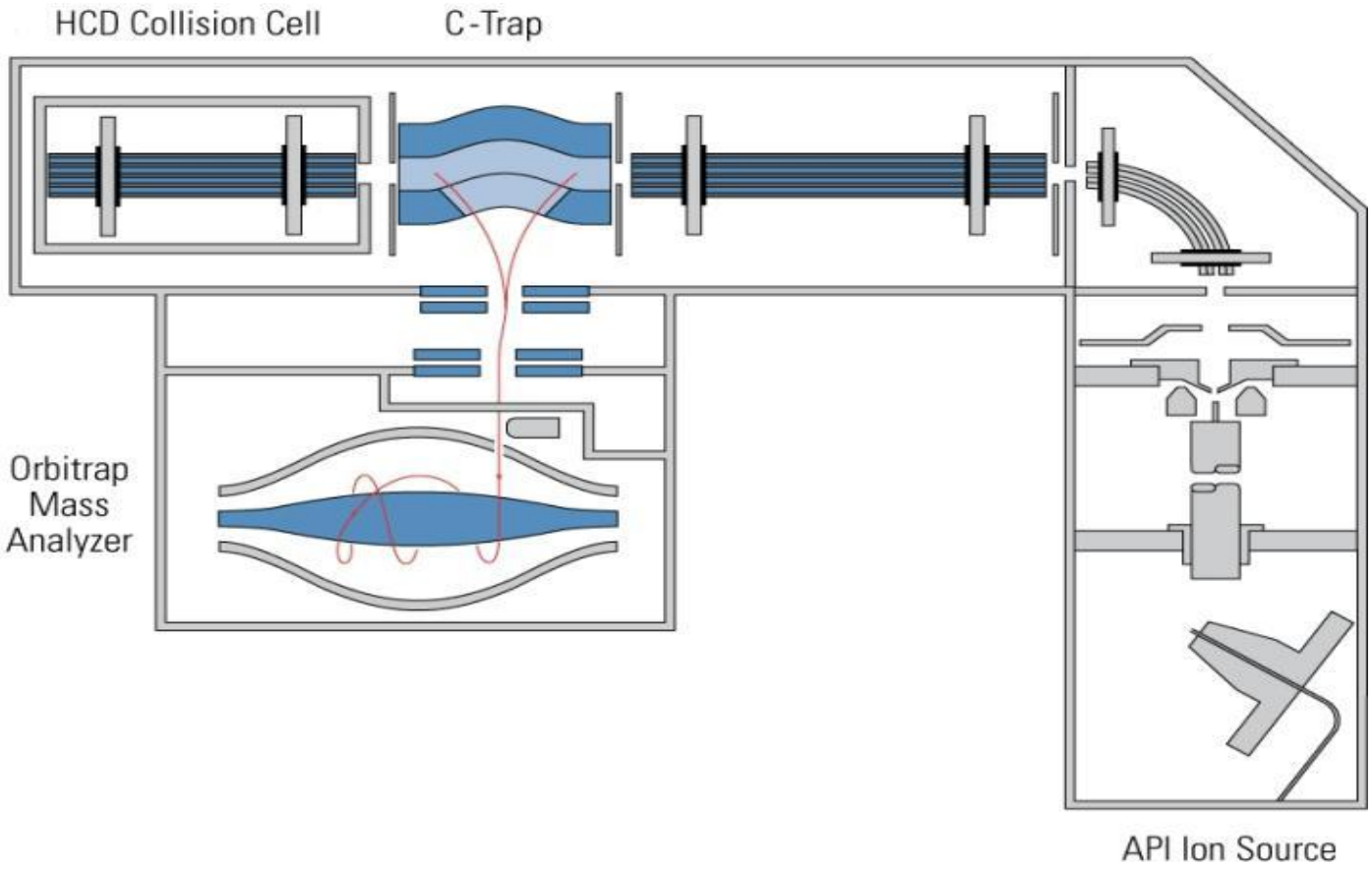
Looking to the Future

Exactive Orbitrap® High Resolution MS

- Resolution
  - 100,000 @ 1 scan per second
  - 10,000 at 10 scans per second
- Mass accuracy: <1 ppm
- Scan speed: → 10 per second
- Scan range: 50 – 4000 m/z
- Detection Limit: < 10 ng/ml (100 ppb) most compounds
- Sample introduction
  - LC (shown here)
  - DART

Looking to the Future

Exactive Orbitrap® High Resolution MS



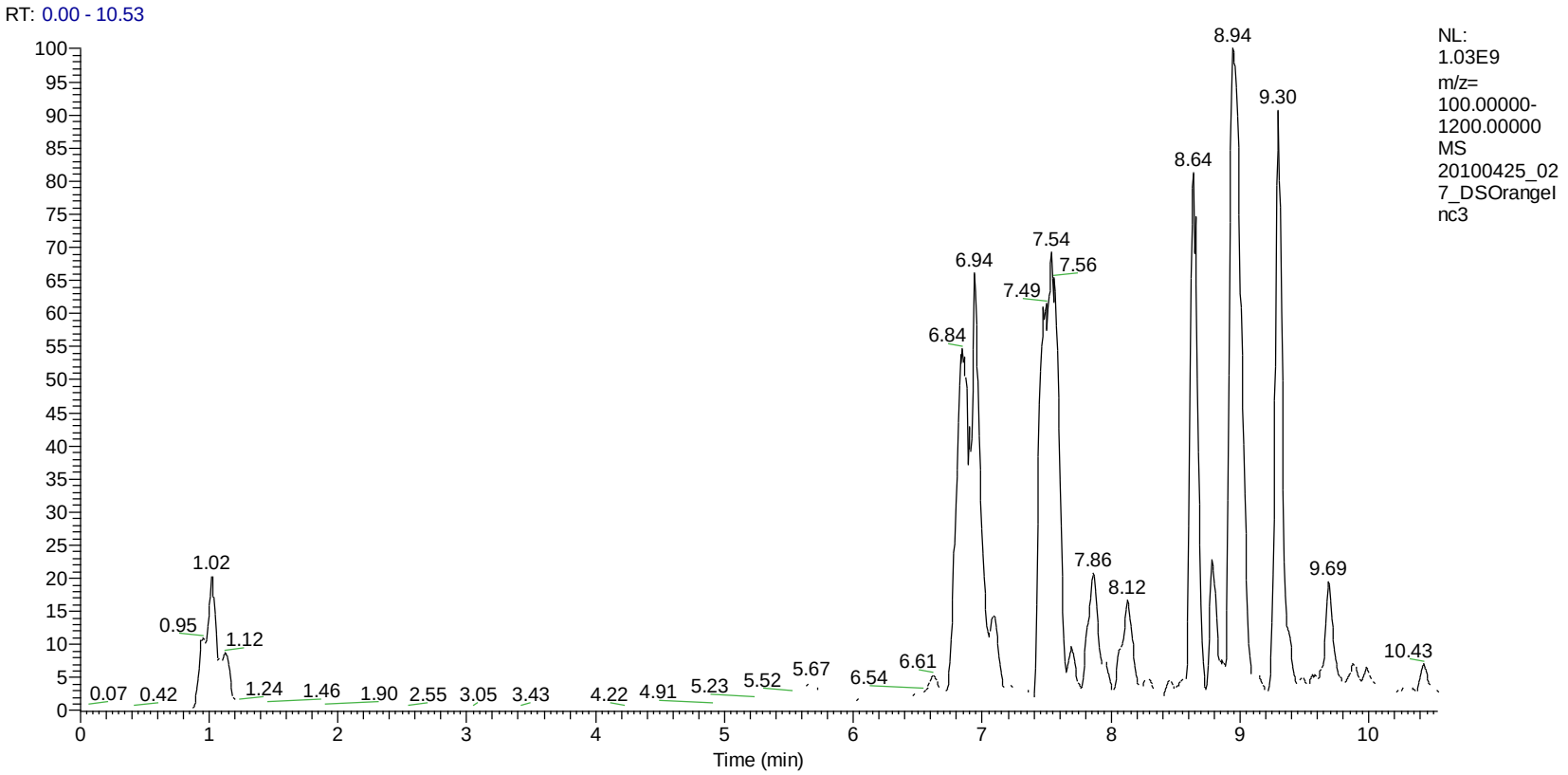
Looking to the Future

LC-HRMS Pesticide Screening Analysis

- Screen > 500 pesticides
- Sample introduction by reverse phase uHPLC
  - Column: C18, 100 x 2.1 mm, 1.9 µm particles
- Identification
  - Retention time
  - HRMS Resolution of 100,000
- Limit of Detection : < 10 ng/ml (100 ppb)
- Plan to expand to other chemical contaminants: mycotoxins, plant toxins, veterinary drugs, dyes, emerging organic pollutants, etc…

Looking to the Future

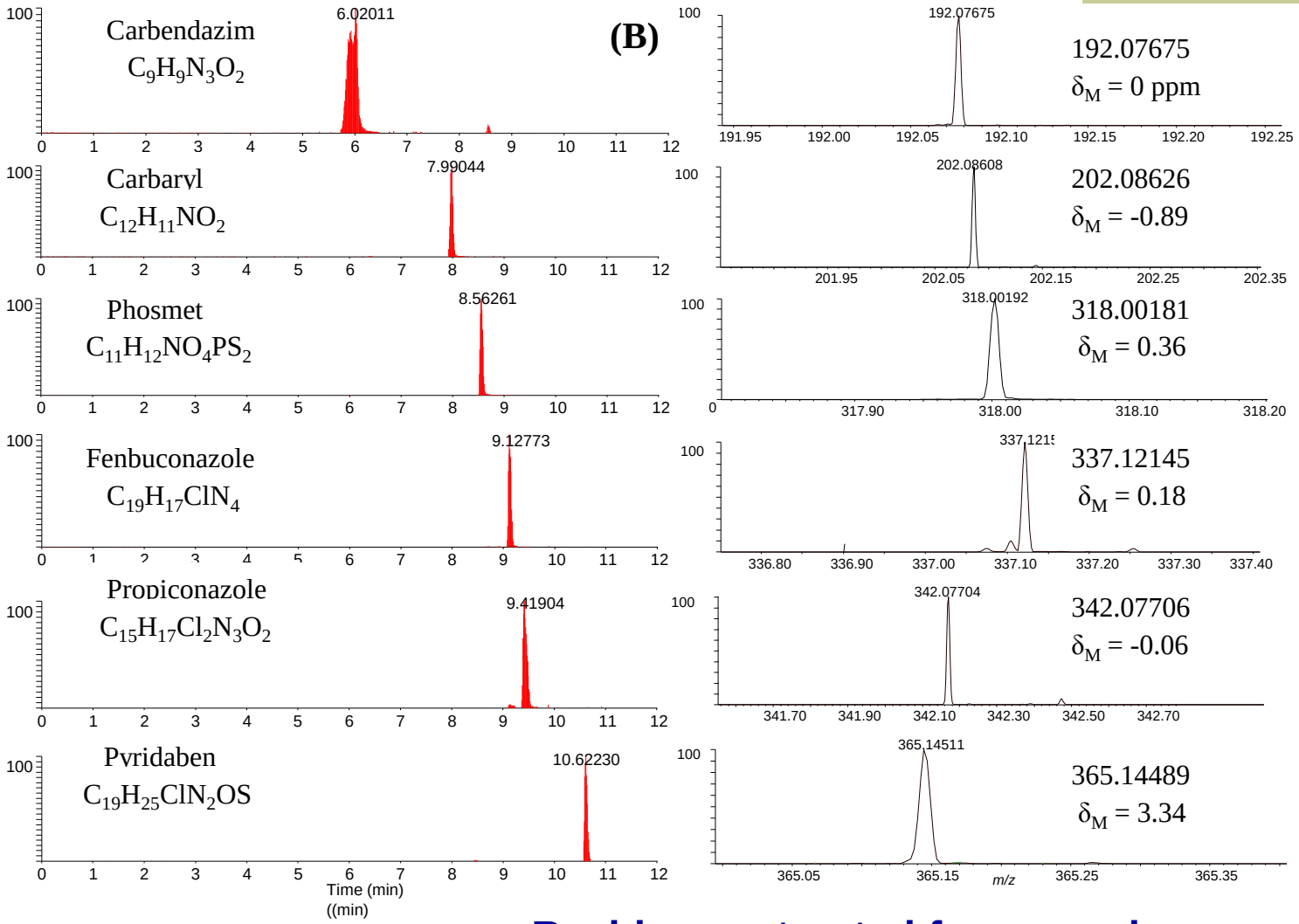
LC-HRMS Pesticide Screening Analysis



QuEChERS extract of a Peach Sample analyzed by uHPLC-single stage Orbitrap MS (full scan analysis)

Looking to the Future

LC-HRMS Pesticide Screening Analysis



Residues extracted from peaches

Looking to the Future

Screening using DART-HRMS

- Sample introduction by Direct Analysis in Real Time (DART)
- HRMS: Resolution = 100,000
- Scope: > 500 pesticides and toxins
- Analysis time: 7.5 minutes/sample

Looking to the Future

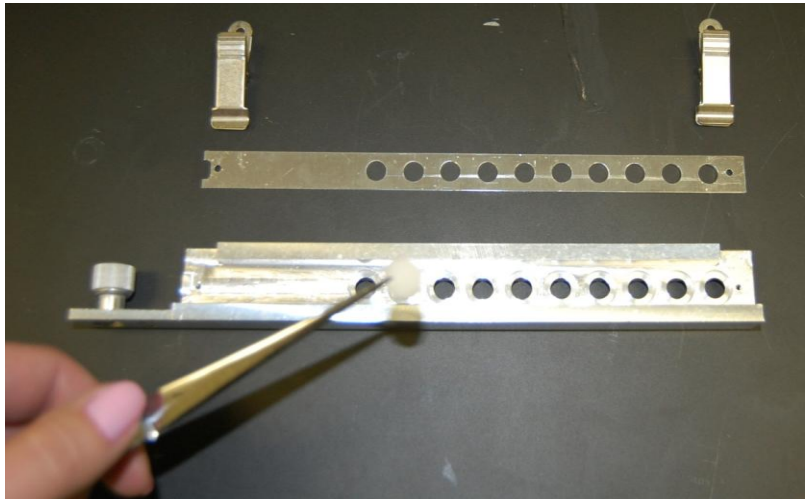
Screening using DART-HRMS

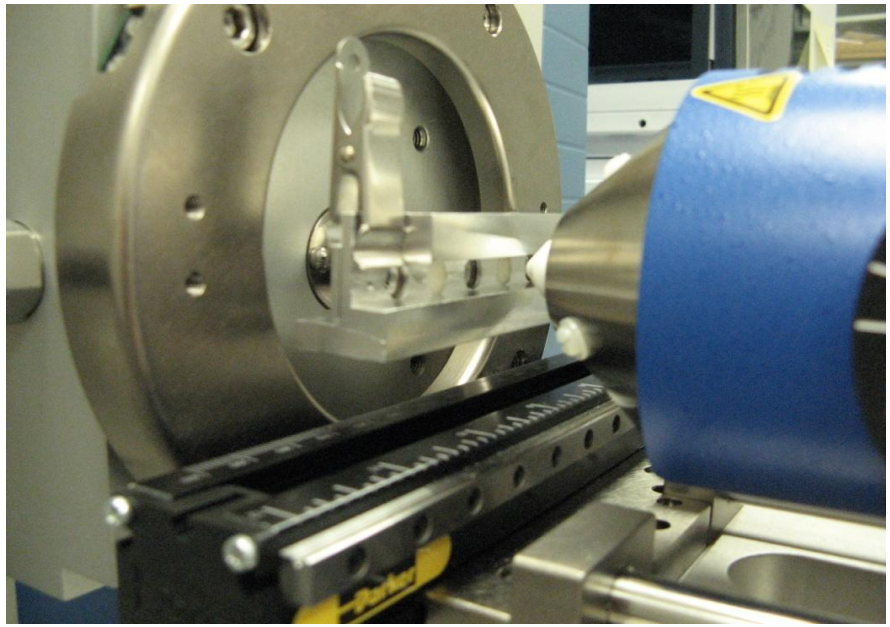
- Method:
  - Surface of raw agricultural commodities are moistened with solvent and swabbed with foam
    - **No sample preparation or extraction!**
  - Swabs are directly analyzed using a DART ionization source combined with high resolution mass spectrometry
    - **No chromatography!**

Looking to the Future

Screening using DART-HRMS

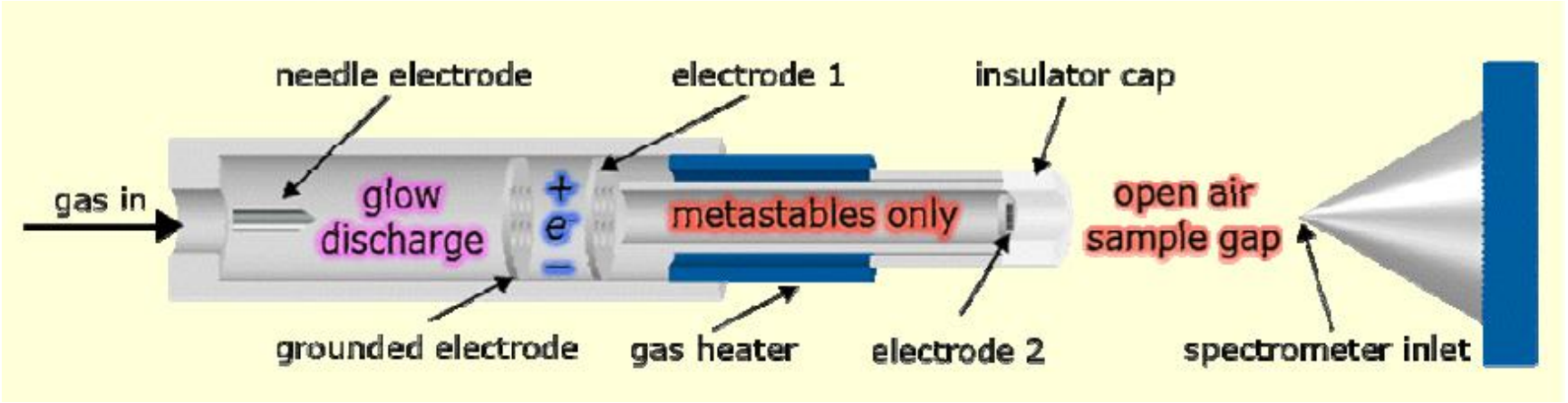
Custom foam rail autosampler





Looking to the Future

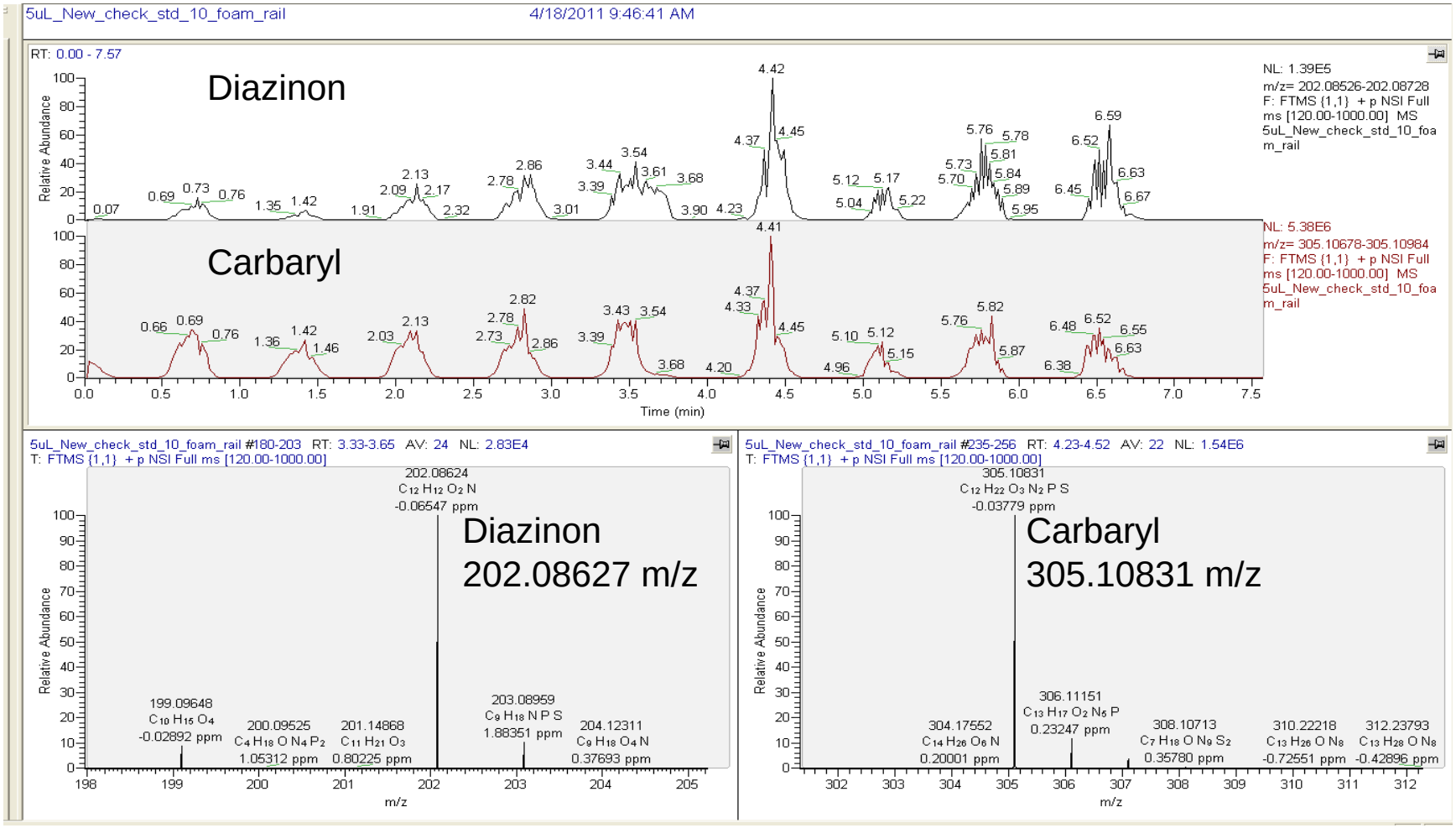
DART Ionization



$$He(2^3S) + nH_2O \rightarrow [(H_2O)_{n-1} + H]^+ + OH^- He(1^1S)$$
$$[(H_2O)_n + H]^+ + M \rightarrow [M + H]^+ + nH_2O$$

Looking to the Future

Screening using DART-HRMS



Looking to the Future

Screening using DART-HRMS

- Findings
  - Up to 10 pesticides per sample
  - Average 2-3 pesticides per sample
- Publications
  - Rapid Commun. Mass Spectrom. 2011, 25, 127–139 Sara. E. Edison[1], Lora A. Lin[1], Bryan M. Gamble[1], Jon Wong[2] and Kai Zhang[2]
    - *[1]USFDA, Forensic Chemistry Center, Cincinnati, OH, USA*
    - *[2]USFDA, Center for Food Safety and Applied Nutrition, College Park, MD, USA*
  - Practical considerations for rapid screening for pesticides using ambient pressure desorption ionization with high-resolution mass spectrometry, Sara E. Edison[1]*, Lora A. Lin[1], Lenin Parrales[2] (Submitted to Food Additives and Contaminants**)**
    - *[1]Food and Drug Administration, Forensic Chemistry Center, Cincinnati, OH*
    - *[2]Food and Drug Administration, San Francisco District Office, Alameda, CA*

Pesticide Residue Analysis Pilot Course

June 6-10, 2011

- Location: University of Maryland
  College Park, MD, USA
- By invitation only: Latin American countries
- Scope: Focus on fresh fruits and vegetables
- Hands-on laboratory training
- Instrumental Techniques: LC-MS/MS
- Certificate of Accomplishment of Pesticide Residue Analysis in Fresh Fruit and Produce

Pesticide Residue Analysis Pilot Course

Agenda

	Morning -Lecture	Afternoon -Laboratory
Monday	Pesticide compliance in LA Sample preparation	Laboratory SOPs Sample preparation
Tuesday	Principles of GC and LC Detection: Element and Mass LC-MS	Group A: LC-MS training Group B: Calibration and other sample preparation
Wed	Quantitative analysis Identification and confirmation QC/QA	Group A: Calibration and other sample preparation Group B: LC-MS training
Thursday	Method Development Validation of single lab method Regulations	Sample analysis Results interpretation
Friday	Review and evaluation	

Pesticide Residue Analysis Pilot Course

Course Instructors

Pesticide Residue Analysis Pilot Course

Registration

contact Dr. Janie Dubois at:

jifsantraining@umd.edu

Acknowledgements

- David Eide, Michael Smoker (USFDA-Kansas City)
- Jon Wong, Ronald Roy, Kai Zhang (USFDA-CFSAN)
- Greg Mercer, Barb Neuhaus (USFDA-Seattle)
- Luis Suguiyama, US Environmental Protection Agency
- US Department of Agriculture - Foreign Agriculture Service