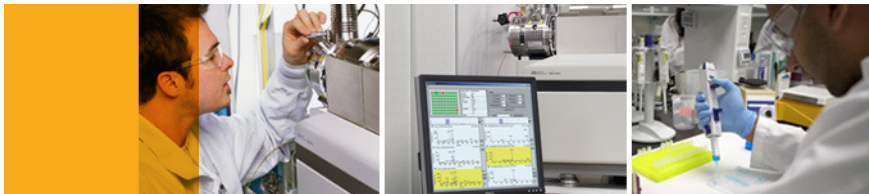


A Generic LC/MS/MS Method for the Analysis of Pesticides at 0.1 μ g/L in Water Samples by Direct Injection

Hesham Ghobarah, André Schreiber, Axel Besa, Jens Dahmann, Yuriko Ozeki, CJ Baker

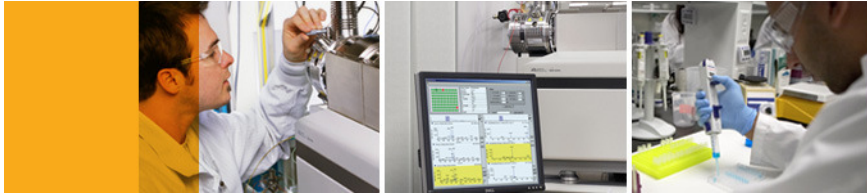
Senior Field Applications Specialist, Toronto, Canada





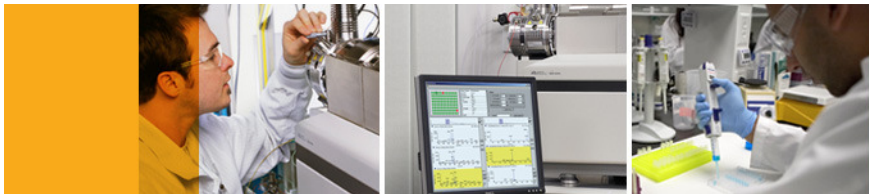
Outline

- Examination of generic direct injection methodology.
- Evaluation of sensitivity.
- Enabling technologies for assaying large numbers of analytes.
- Approaches to confirmation:
 - MRM Ratios
 - MS/MS Library Searching
- Software turn-key solutions for streamlining routine analysis and report generation.



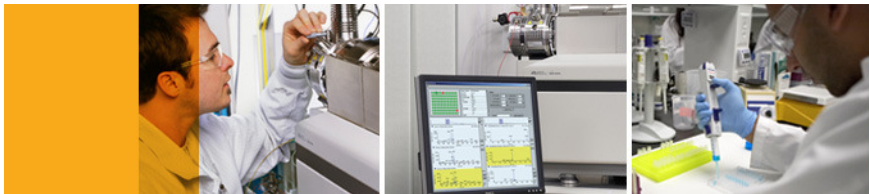
Experimental – Generic Pesticide Screening

- Sample Preparation
 - Surface water, tap water or bottled water.
 - Filtered through 0.2 μm syringe filter (Nalgene PTFE single use filter).
 - Direct injection.



Experimental – Generic Pesticide Screening

- HPLC:
 - Phenomenex Synergi 4um Fusion-RP 80A (50x2mm)
 - Eluent A: H₂O + 5mM ammonium formate
 - Eluent B: CH₃OH + 5mM ammonium formate
 - Gradient: 80/20 – 10/90 over 11 min
 - Hold time: 5 min. Re-equilibration 9 min
 - Flow rate: 200µL/min
 - Injection volume: 20µL

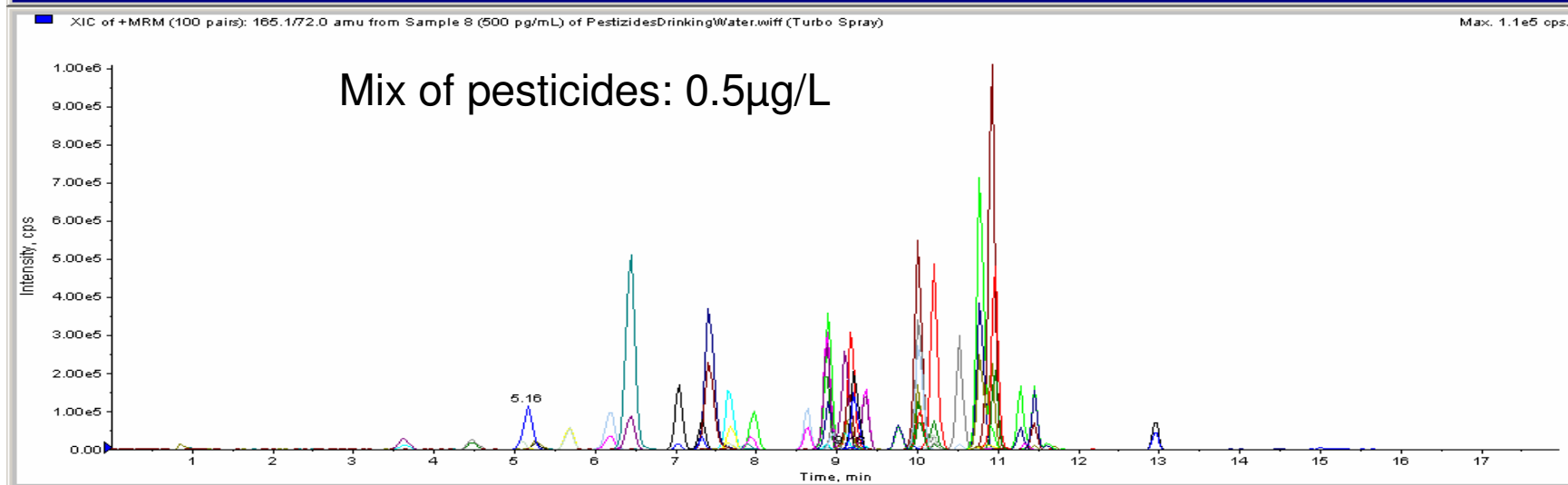
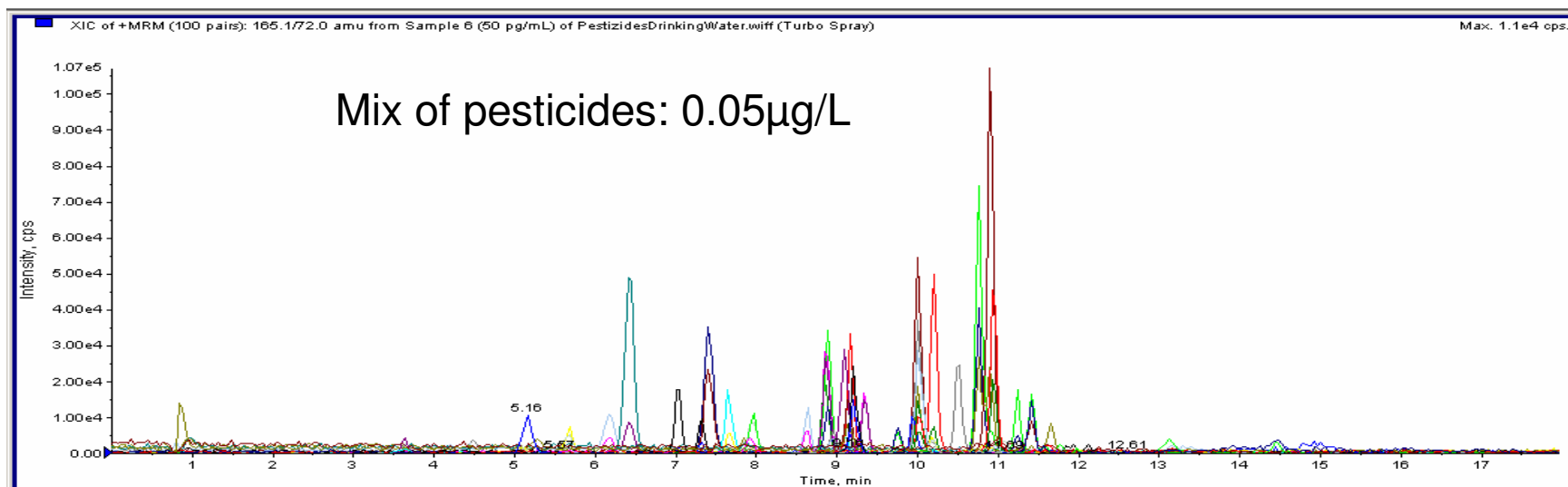


Experimental – Generic Pesticide Screening

- MS/MS:
 - 3200 Q TRAP[®] and API 5000[™] LC/MS/MS system
 - Turbo V[™] source with Electrospray probe
 - 2 MRM transitions per pesticide
 - Curtain gas: 20 psi
 - Nebulizer (Gas 1): 45 psi
 - Auxiliary (Gas 2): 65 psi
 - Temperature: 500 °C
 - IS Voltage: 5500V

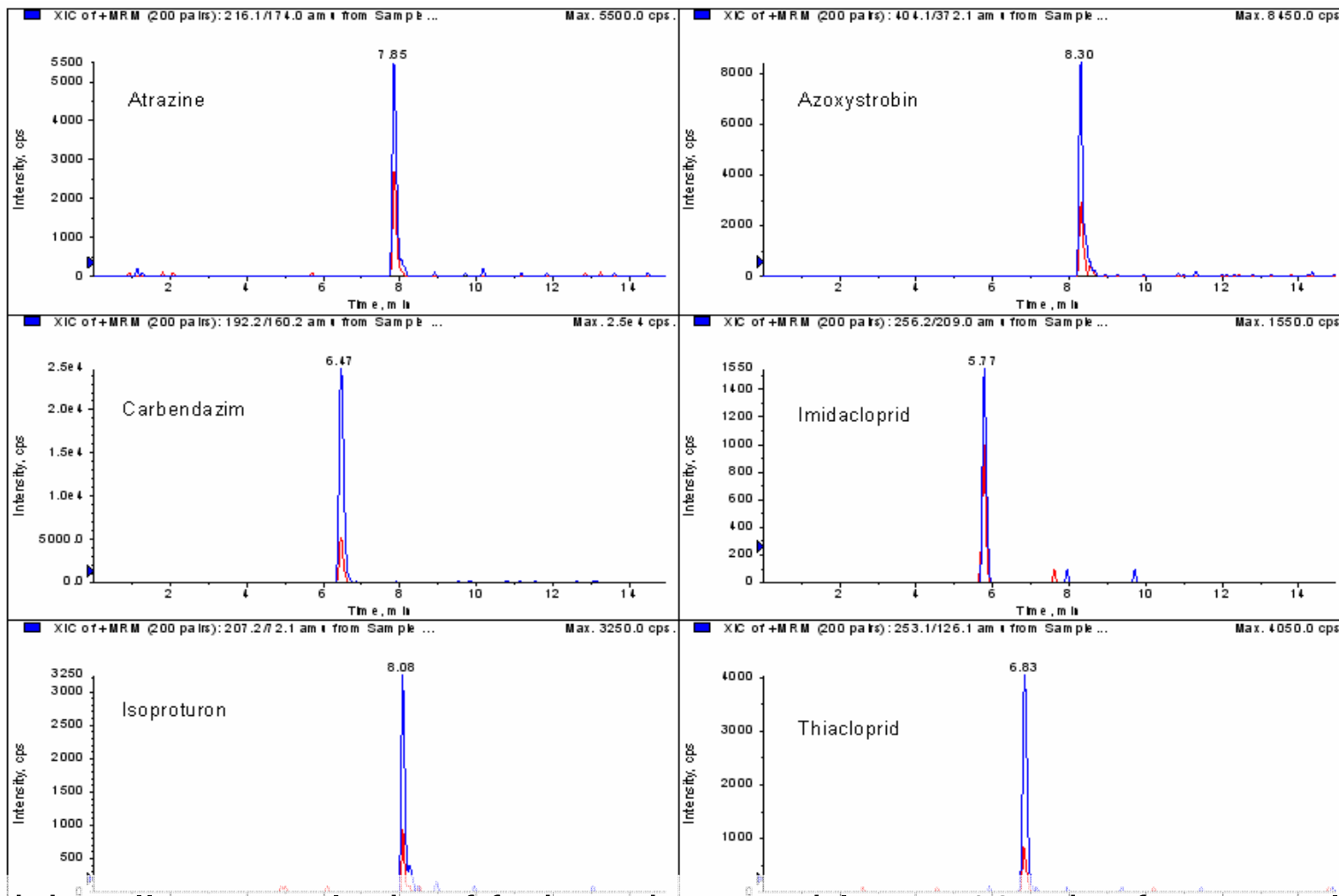


Separation and Detection of 50 Pesticides (100 MRM)





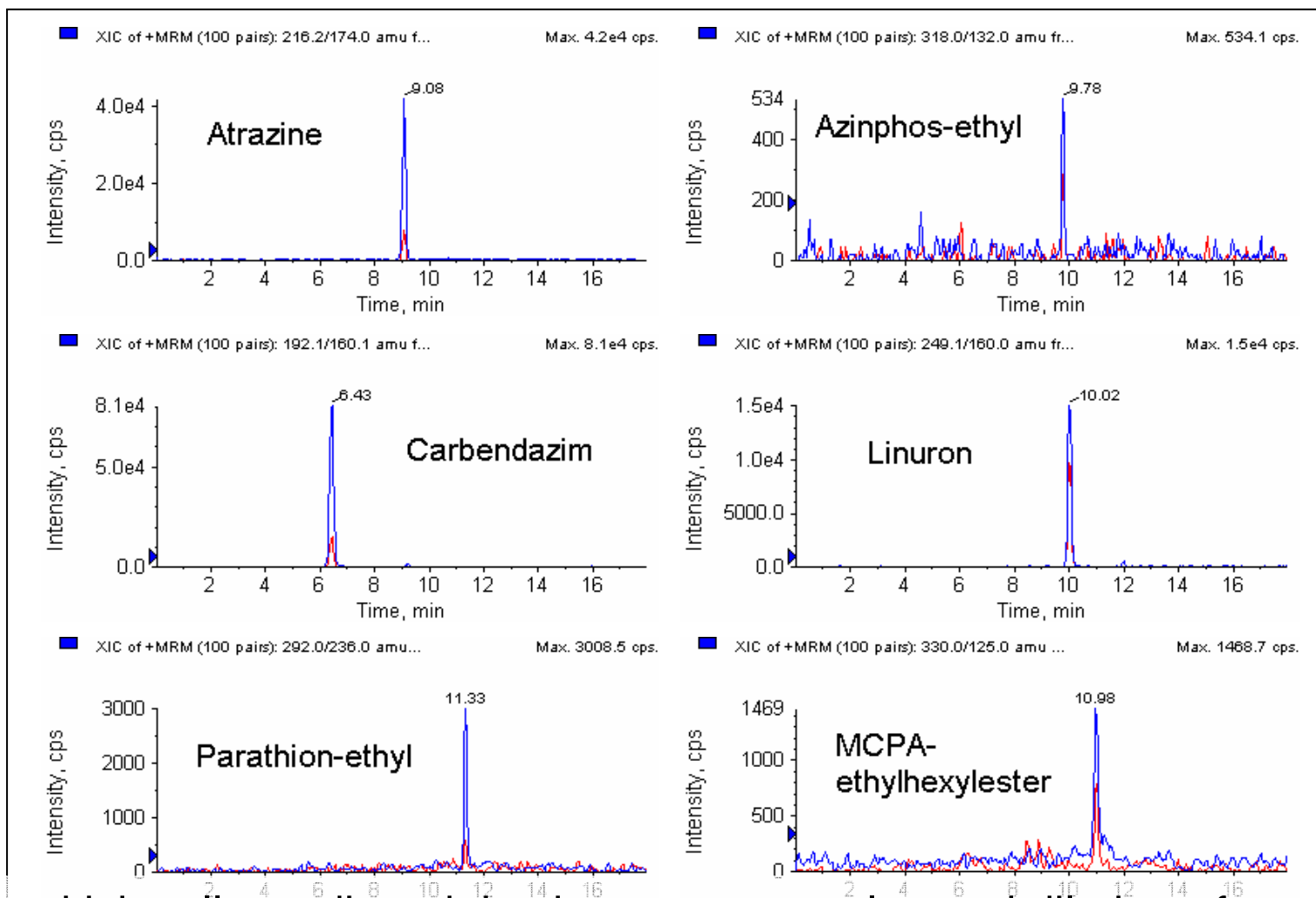
3200 Q TRAP[®]: 1 μg/L of Selected Pesticides



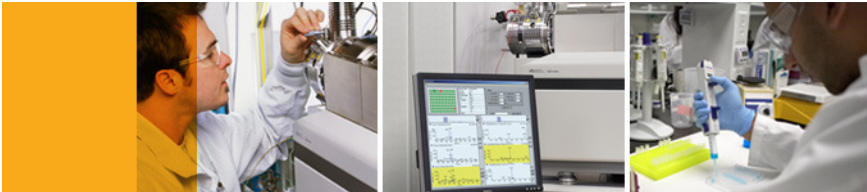
Sensitivity allows analysis of fruit and vegetables at 10ppb after sample prep



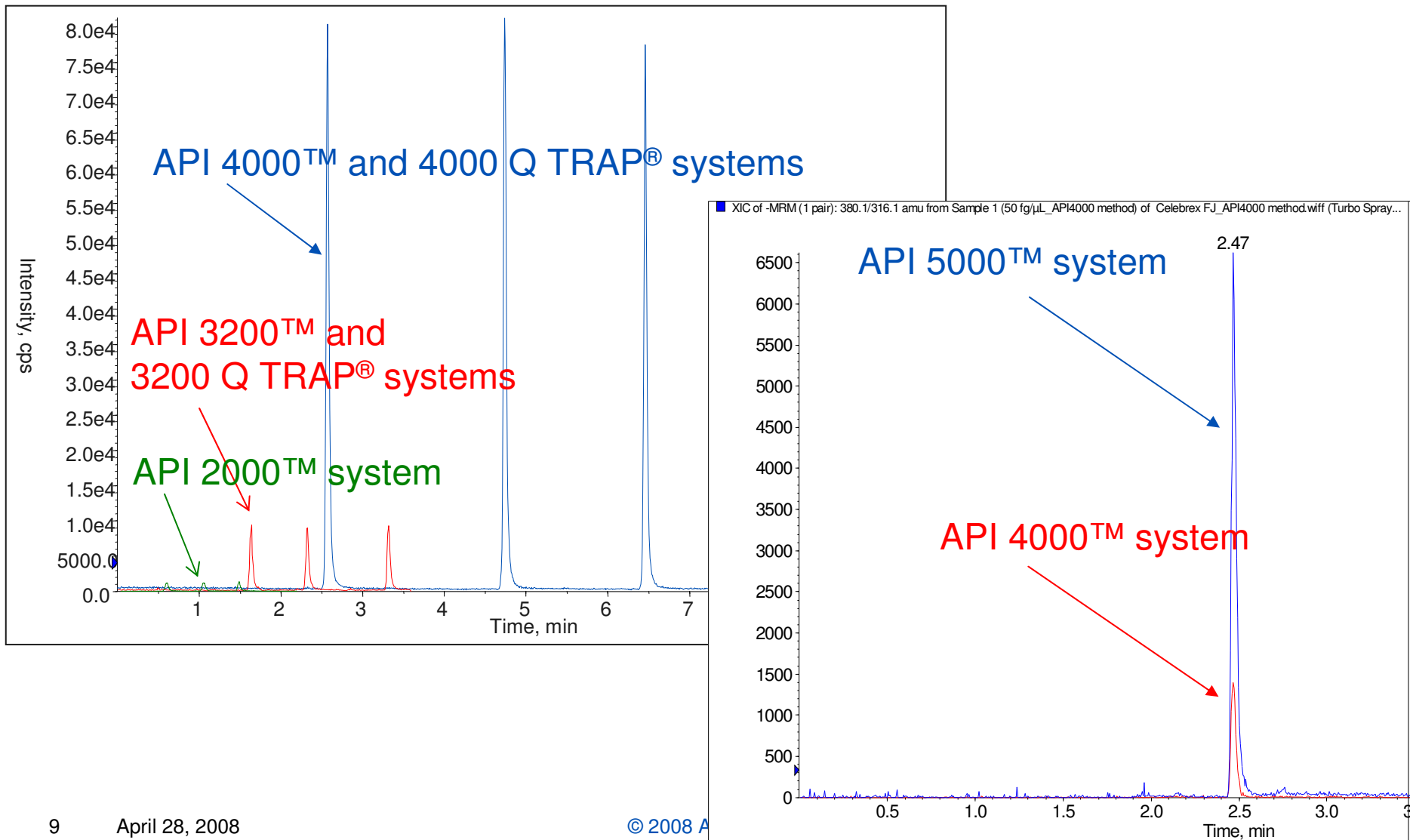
API 5000™: 0.1 µg/L of Selected Pesticides



Sensitivity allows direct injection water samples and dilution of extracts



MRM Sensitivity of Different LC/MS/MS Systems





Pesticides in Drinking, Bottled and Pond Water

50 Pesticides detected using 2 MRM transitions

Area counts >3000

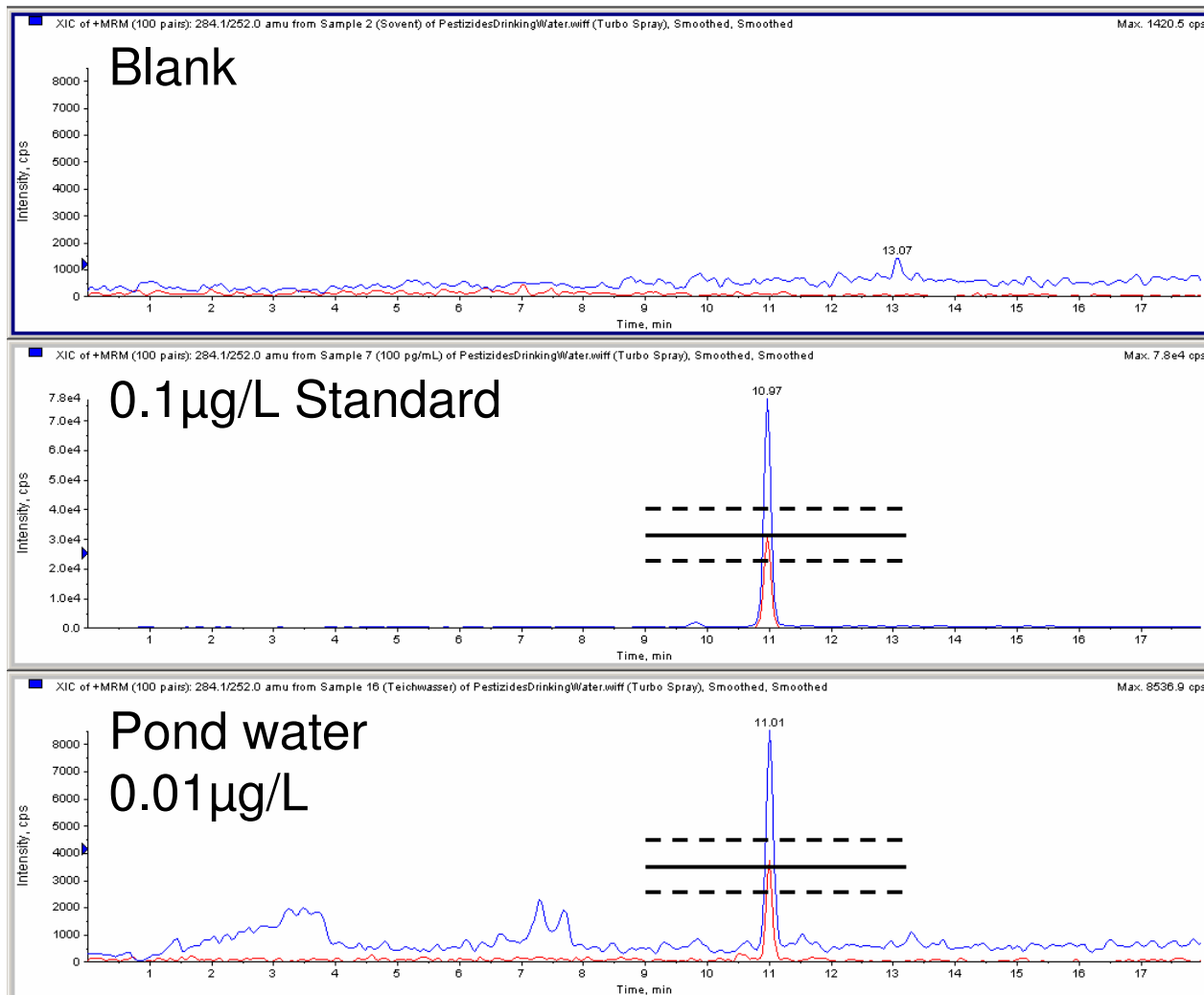
S/N >6

Full Layout
 Query: SampleCheck_weg, SampleCheck_weg
 Idle
 Sort: Unsorted

| | Sample Name | Sample Type | Analyte Peak Name | Analyte Peak Area (counts) | Analyte Mass Ranges (amu) | Calculated Concentration (ng/mL) |
|----|-------------------|-------------|---------------------------|----------------------------|---------------------------|----------------------------------|
| 1 | Leitungswasser DA | Unknown | Desethyl-atrazine / 104.0 | 5.20e+003 | 188.0/104.0 amu | 0.008 |
| 2 | Leitungswasser DA | Unknown | Hexaconazole / 159.0 | 6.07e+003 | 314.1/159.0 amu | 0.025 |
| 3 | Leitungswasser DA | Unknown | Flufenoxuron / 158.0 | 4.38e+003 | 489.1/158.0 amu | 0.005 |
| 4 | Leitungswasser DA | Unknown | Flufenoxuron / 141.1 | 3.02e+003 | 489.1/141.1 amu | 0.006 |
| 5 | Mineralwasser | Unknown | Desethyl-atrazine / 146.0 | 1.92e+004 | 188.0/146.0 amu | 0.008 |
| 6 | Teichwasser | Unknown | Fenuron / 72.0 | 1.78e+004 | 165.1/72.0 amu | 0.008 |
| 7 | Teichwasser | Unknown | Fenuron / 120.1 | 6.18e+003 | 165.1/120.1 amu | 0.073 |
| 8 | Teichwasser | Unknown | Cyanazine / 214.1 | 1.30e+004 | 241.1/214.1 amu | 0.014 |
| 9 | Teichwasser | Unknown | Metobromuron / 170.0 | 6.81e+003 | 259.1/170.0 amu | 0.008 |
| 10 | Teichwasser | Unknown | Metolachlor / 220.0 | 2.15e+004 | 280.0/220.0 amu | 0.008 |
| 11 | Teichwasser | Unknown | Metolachlor / 160.0 | 1.25e+004 | 280.0/160.0 amu | 0.006 |
| 12 | Teichwasser | Unknown | Metolachlor / 252.0 | 7.35e+004 | 284.1/252.0 amu | 0.011 |
| 13 | Teichwasser | Unknown | Metolachlor / 176.1 | 3.12e+004 | 284.1/176.1 amu | 0.012 |
| 14 | Teichwasser | Unknown | Parathion ethyl / 97.0 | 7.61e+003 | 292.0/97.0 amu | 0.216 |
| 15 | Teichwasser | Unknown | Hexaconazole / 159.0 | 6.71e+003 | 314.1/159.0 amu | 0.029 |

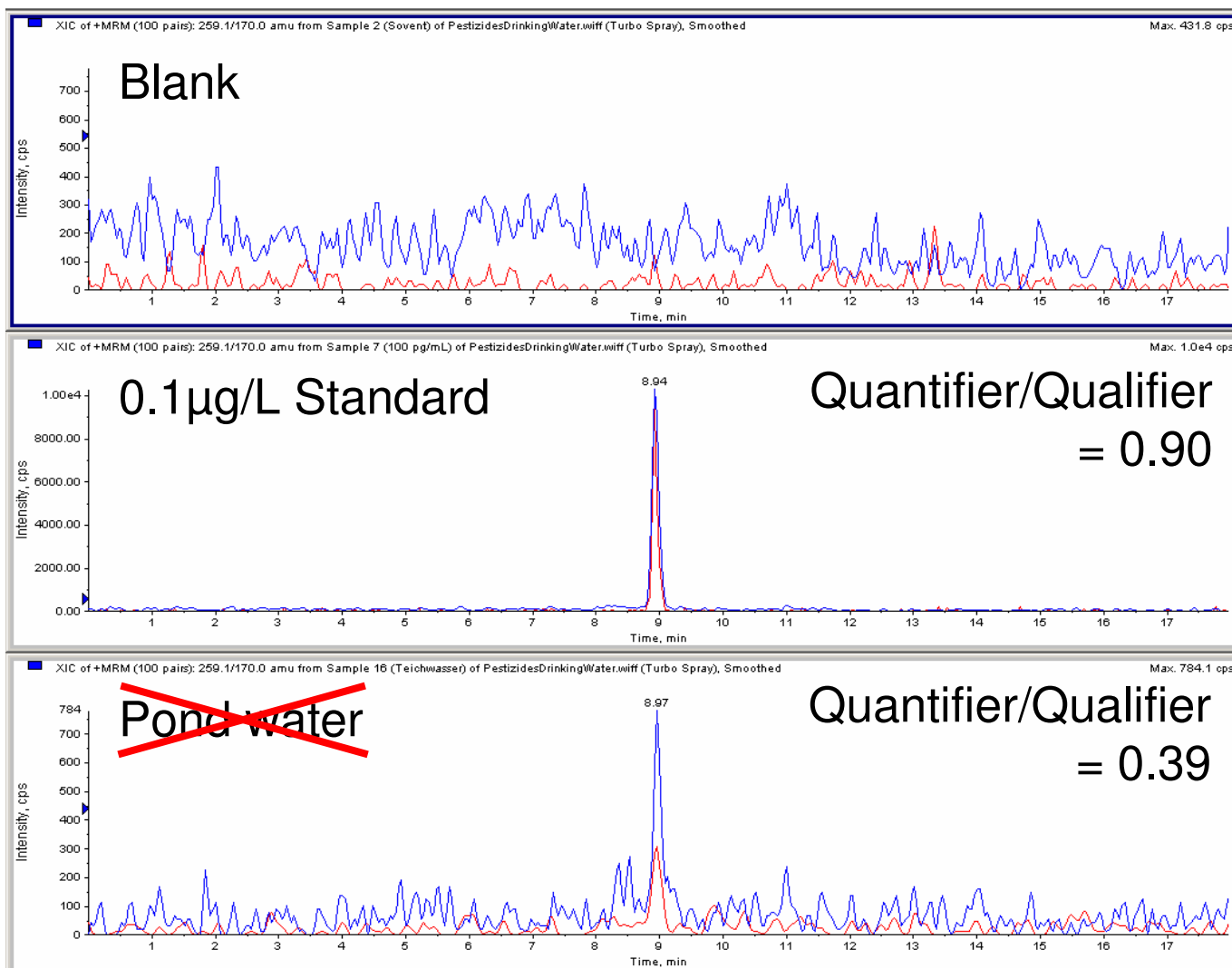


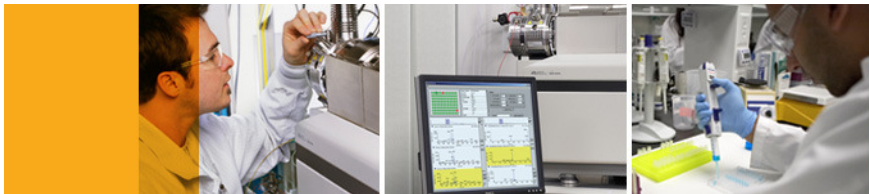
Positive: Metolachlor (API 5000™ system)





Negative: Metobromuron (API 5000™ system)





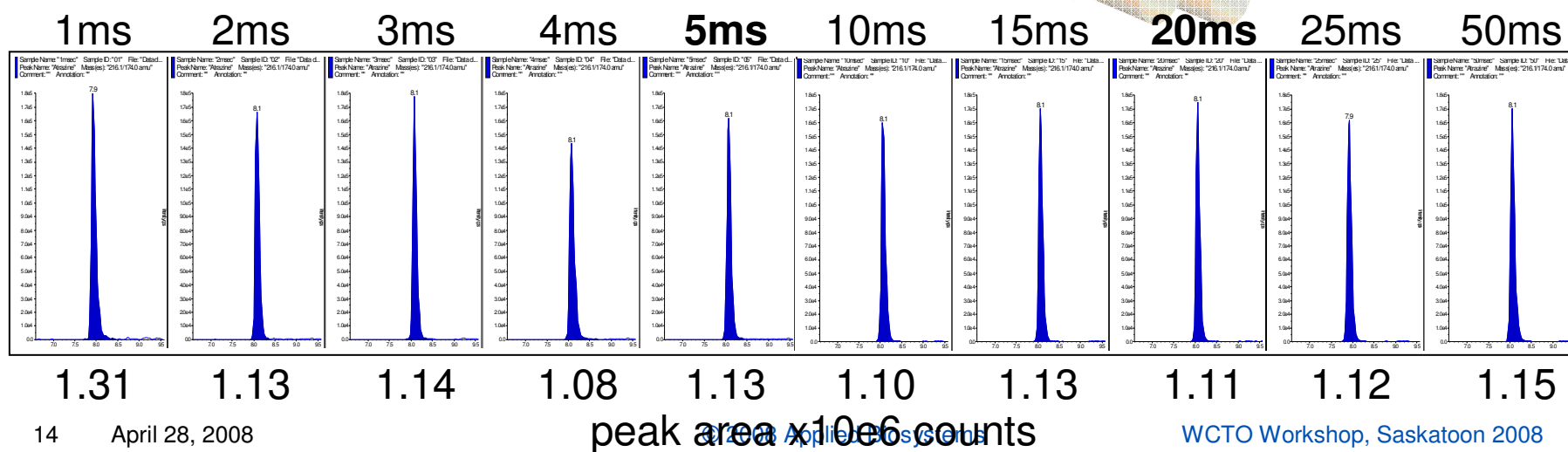
Enabling Technologies for Screening and Quantitation of Large Analytes Sets

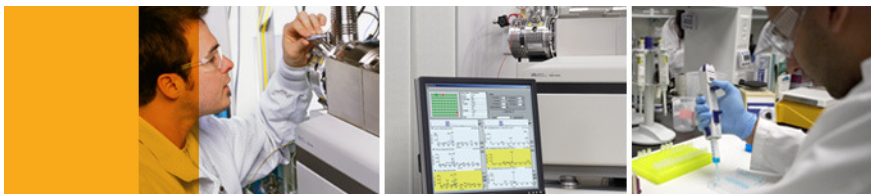
- LINAC[®] Collision Cell
- Scheduled MRM (sMRM)



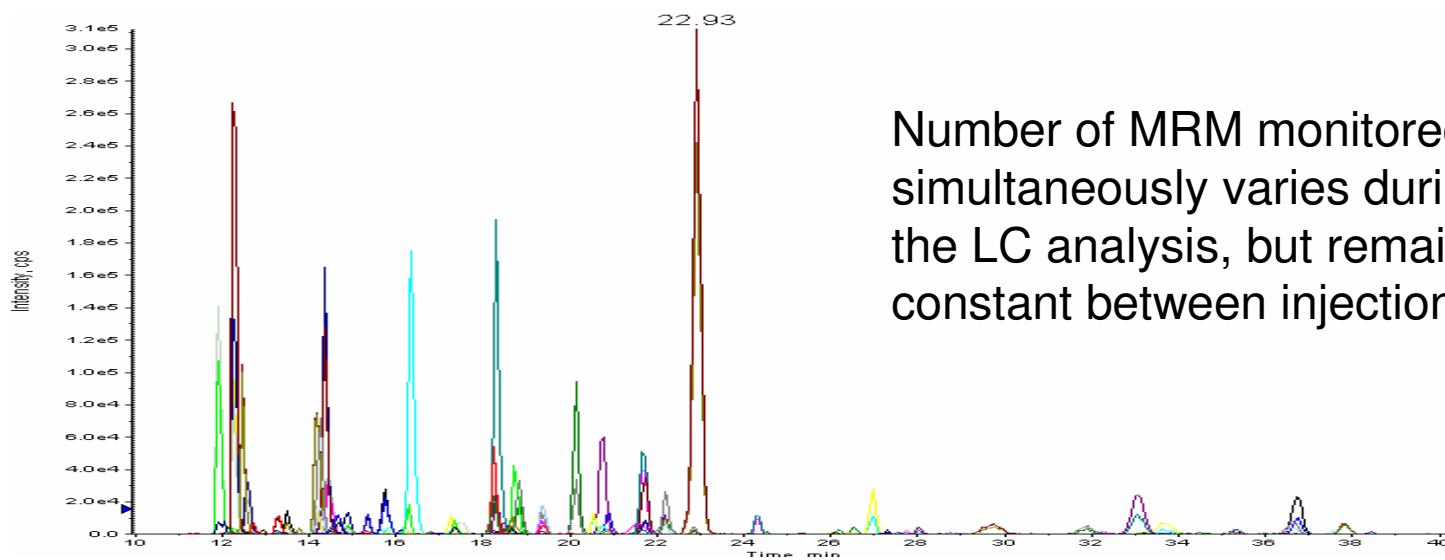
LINAC[®] Collision Cell – Fast MS/MS Experiments

- **Linear Accelerator (LINAC[®])**: Tilted quadrupole rods of the collision cell cause an electric field gradient which accelerates product ions after fragmentation.
- Faster MS/MS experiments without loss in sensitivity and without false positive results due to cross talk.
- Experiment: Atrazine detected with different dwell times but constant total cycle time of 1.5s (20 MRM transitions with 5ms pause time and dummy MRM)





Scheduled MRM (sMRM)



Number of MRM monitored simultaneously varies during the LC analysis, but remains constant between injections



Acquisition Method with sMRM

Analyst - [Acquisition Method: D:\Analyst Data\Projects\Smarmy Forensics\Acquisition Methods\Forensic 1165 Compounds sMRM]

File Edit View Acquire Tools Explore Window Script Help

Acquire Mode Smarmy Forensics

Configure

- Security Configuration
- Hardware Configuration
- Report Template Editor

Tune

- Resolution Optimization
- Quantitative Optimization
- Auto Tune
- Manual Tuning

Acquire (3)

- IDA Method Wizard
- Build Acquisition Method
- Build Acquisition Batch
- Express View

Explore (1)

- Open Data File
- Open Compound Database

Quantitate

- Build Quantitation Method
- Quantitation Wizard
- Review Results Table

Companion Software

- Modification MRM
- Tempo LC device CH1
- Tempo LC device CH2
- Tempo nano LC Autosampler

Acquisition method

- Mass Spec 15.013
 - Period 15.013
 - +MRM
 - IDA Criteria
 - +EPI
 - +EPI
 - +EPI
 - Agilent 1100 LC Bin.
 - Agilent 1100 Column

MS Advanced MS

Experiment: 1 Scheduled MRM Import List

Scan type: MRM (MRM)

Polarity: Positive Negative

MRM detection window: 30 (sec)

Target Scan Time: 1 (sec)

Edit Parameters...

Period Summary

Duration: 15.013 (min)

Delay Time: 0 (sec)

Cycles: 221

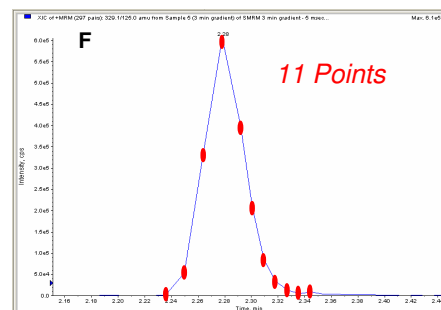
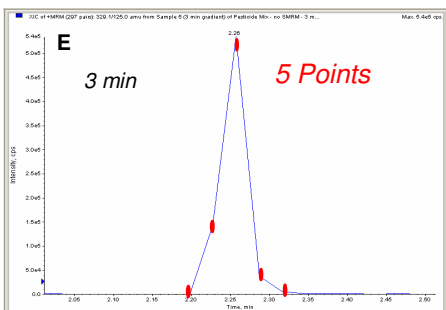
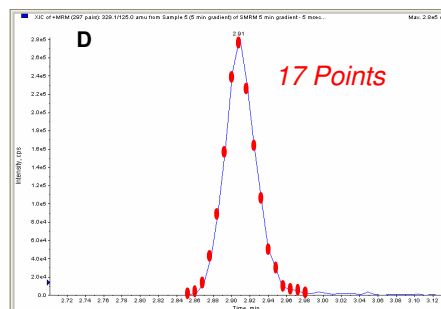
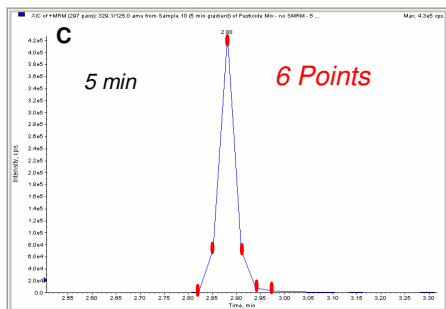
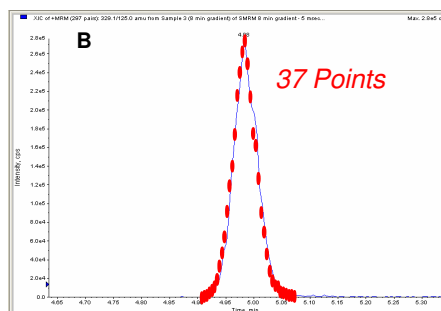
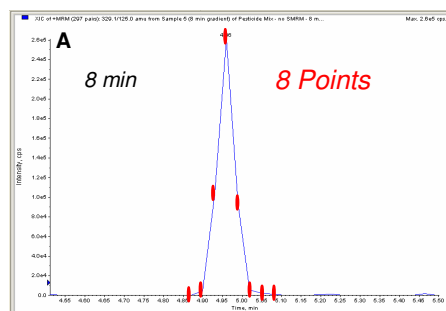
Cycle: 4.0760 (sec)

| | Q1 Mass (Da) | Q3 Mass (Da) | Time (min) | ID | CE (volts) |
|----|--------------|--------------|------------|--------------------------------|------------|
| 1 | 429.000 | 97.100 | 4.3 | 17-alpha-Hydroxyprogesterone | 50.000 |
| 2 | 303.000 | 109.100 | 3.3 | 17-alpha-Methyltestosterone | 35.000 |
| 3 | 232.000 | 99.000 | 4.3 | 2-Amino-5-chlorobenzophenone | 50.000 |
| 4 | 243.000 | 91.100 | 4.6 | 2-Amino-5-nitrobenzophenone | 50.000 |
| 5 | 333.000 | 211.200 | 4.4 | 2-Hydroxyethylflurazepam | 50.000 |
| 6 | 182.000 | 91.100 | 4.2 | 3,4-Dimethoxyphenethylamine | 50.000 |
| 7 | 180.000 | 77.000 | 5.2 | 3,4-Methylenedioxyamphetamine | 50.000 |
| 8 | 208.000 | 77.100 | 4.5 | 3,4-Methylenedioxyethylampheta | 50.000 |
| 9 | 194.000 | 77.000 | 3.5 | 3,4-Methylenedioxyampheta | 50.000 |
| 10 | 434.000 | 261.000 | 3.9 | 3,5-Diiodotyrosine | 50.000 |
| 11 | 332.000 | 78.000 | 6.7 | 3-Hydroxybromazepam | 50.000 |
| 12 | 351.000 | 105.100 | 4.3 | 3-Methylfentanyl | 50.000 |
| 13 | 258.000 | 105.100 | 4.0 | 4-Benzamidosalicylic acid | 50.000 |
| 14 | 219.000 | 77.100 | 4.2 | 4-Methylumbelliferyl acetate | 50.000 |
| 15 | 153.000 | 92.100 | 4.1 | 6-Mercaptourine | 50.000 |
| 16 | 328.000 | 165.200 | 3.9 | 6-O-Monoacetylmorphine | 50.000 |
| 17 | 286.000 | 121.100 | 5.7 | 7-Aminoclonazepam | 50.000 |
| 18 | 270.000 | 121.100 | 0.3 | 7-Aminodesmethylflunitrazepam | 50.000 |
| 19 | 284.000 | 135.100 | 5.4 | 7-Aminoflunitrazepam | 50.000 |
| 20 | 252.000 | 121.100 | 4.6 | 7-Aminonitrazepam | 50.000 |
| 21 | 215.000 | 67.100 | 5.1 | 8-Chlorotheophylline | 50.000 |
| 22 | 146.000 | 75.100 | 4.1 | 8-Hydroxyquinoline | 50.000 |
| 23 | 427.000 | 207.200 | 4.3 | 9-Hydroxyrisperidone | 50.000 |
| 24 | 337.000 | 116.100 | 3.1 | Acebutolol | 35.000 |
| 25 | 279.000 | 69.000 | 0.3 | Acecarbromal | 50.000 |
| 26 | 170.000 | 82.100 | 5.3 | Aceclidine | 50.000 |
| 27 | 354.000 | 214.100 | 4.1 | Aceclofenac | 50.000 |
| 28 | 416.000 | 139.100 | 4.3 | Acemetacin | 35.000 |
| 29 | 354.000 | 249.100 | 0.3 | Acenocoumarol | 50.000 |
| 30 | 327.000 | 58.000 | 0.3 | Acepromazine | 50.000 |
| 31 | 327.000 | 86.100 | 5.4 | Aceprometazine | 50.000 |
| 32 | 327.000 | 158.200 | 4.7 | Acetaminodantrolene | 50.000 |
| 33 | 367.000 | 122.100 | 3.8 | Acetiamin | 35.000 |
| 34 | 239.000 | 109.100 | 4.1 | Acetylaminoantiprophoxybenzen | 50.000 |
| 35 | 180.000 | 65.000 | 4.4 | Acetylsalicylamid | 50.000 |
| 36 | 226.000 | 135.000 | 7.8 | Aciclovir | 35.000 |
| 37 | 349.000 | 232.200 | 7.0 | Acrivastine | 50.000 |
| 38 | 254.000 | 145.100 | 6.2 | Actinoquinol | 50.000 |
| 39 | 274.000 | 135.100 | 3.5 | Adefnvir | 50.000 |

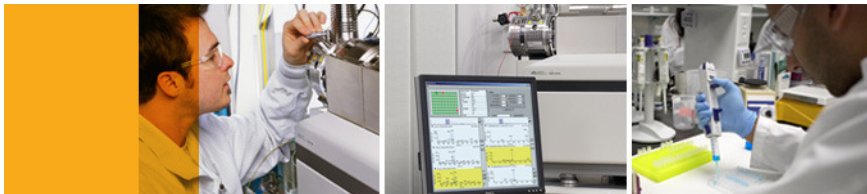
Advantages of sMRM

Fixed MRM

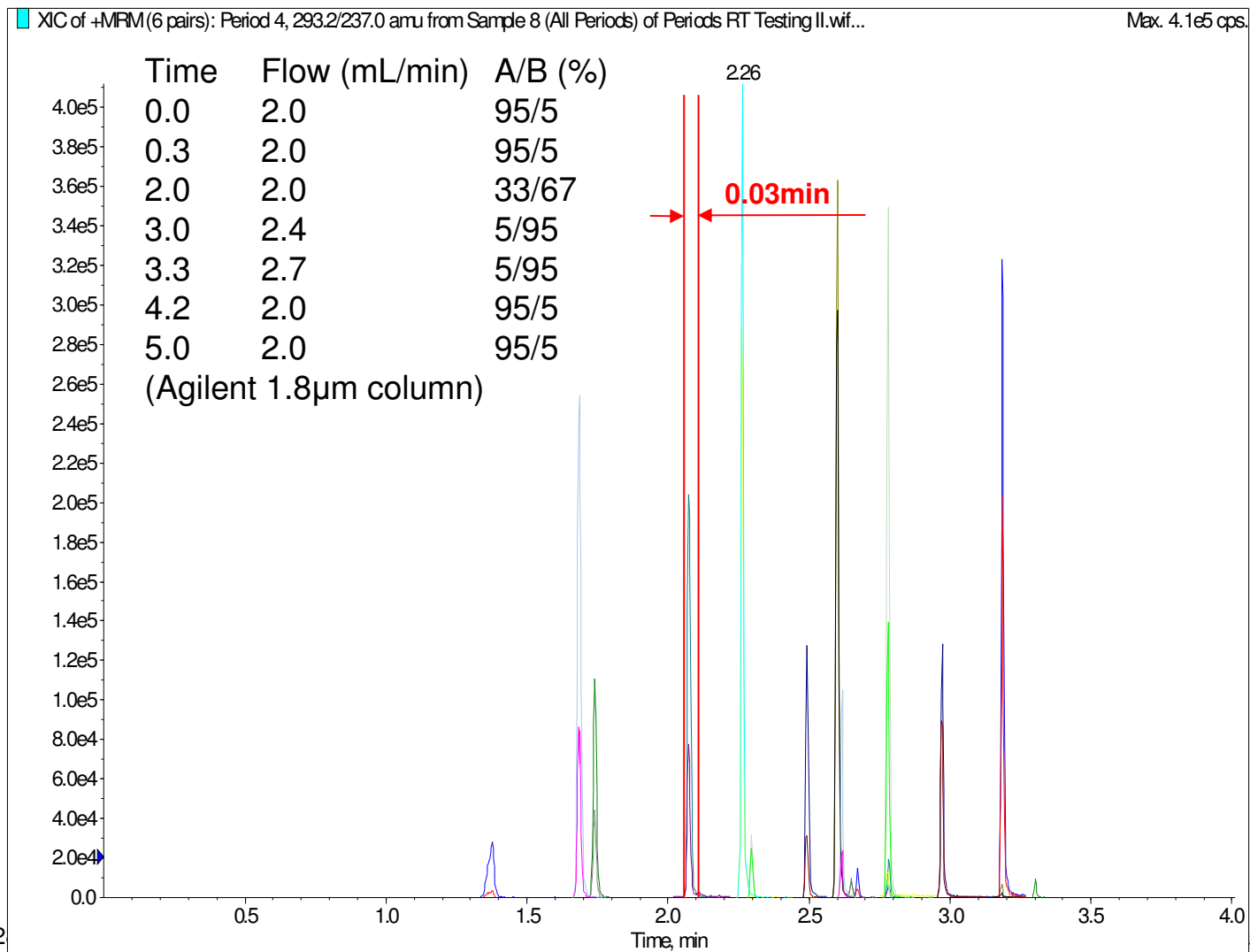
sMRM



- More data points over HPLC peaks to improve data quality
- More MRM transitions per experiment to increase number of monitored compounds
- More time for confirmatory analysis
- High resolution HPLC with MS/MS detection

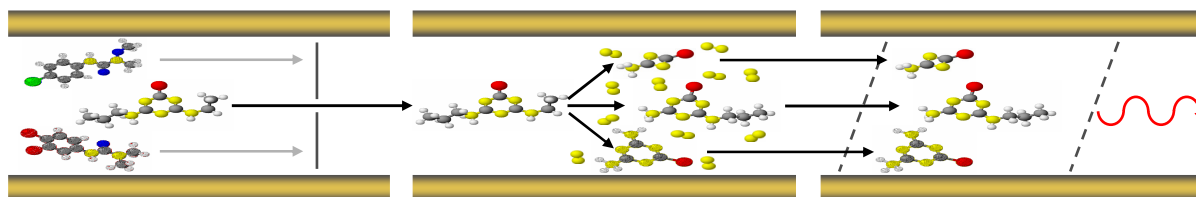


34 MRM Transitions in 4min with sMRM





Higher Degree of Confirmation using MS/MS Spectra

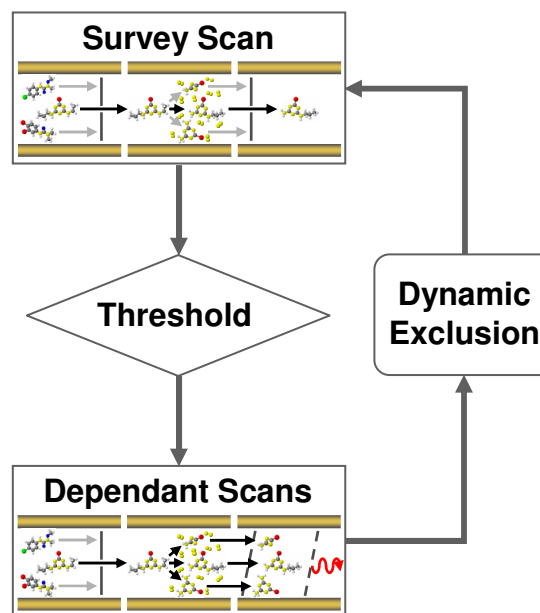


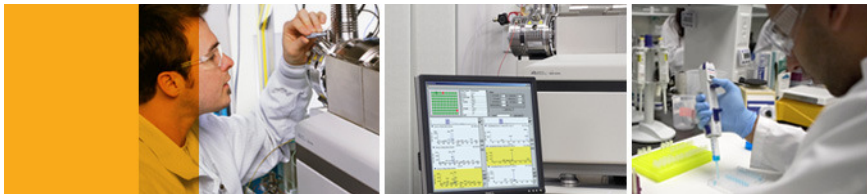
- Enhanced Product Ion (EPI) scan of Q TRAP[®] systems
 - → MS/MS Spectra contains information of all MRM transitions of the analyte.
 - → EPI has sensitivity level of MRM.
 - → EPI is less time consuming than acquisition of many confirmatory MRM transitions (100MRM with 5ms dwell + 5ms pause ~ 1s vs. 2 EPI over 500amu with 4000amu/s and 100ms fill time ~ 1s).
 - → Confirmation with library searching.



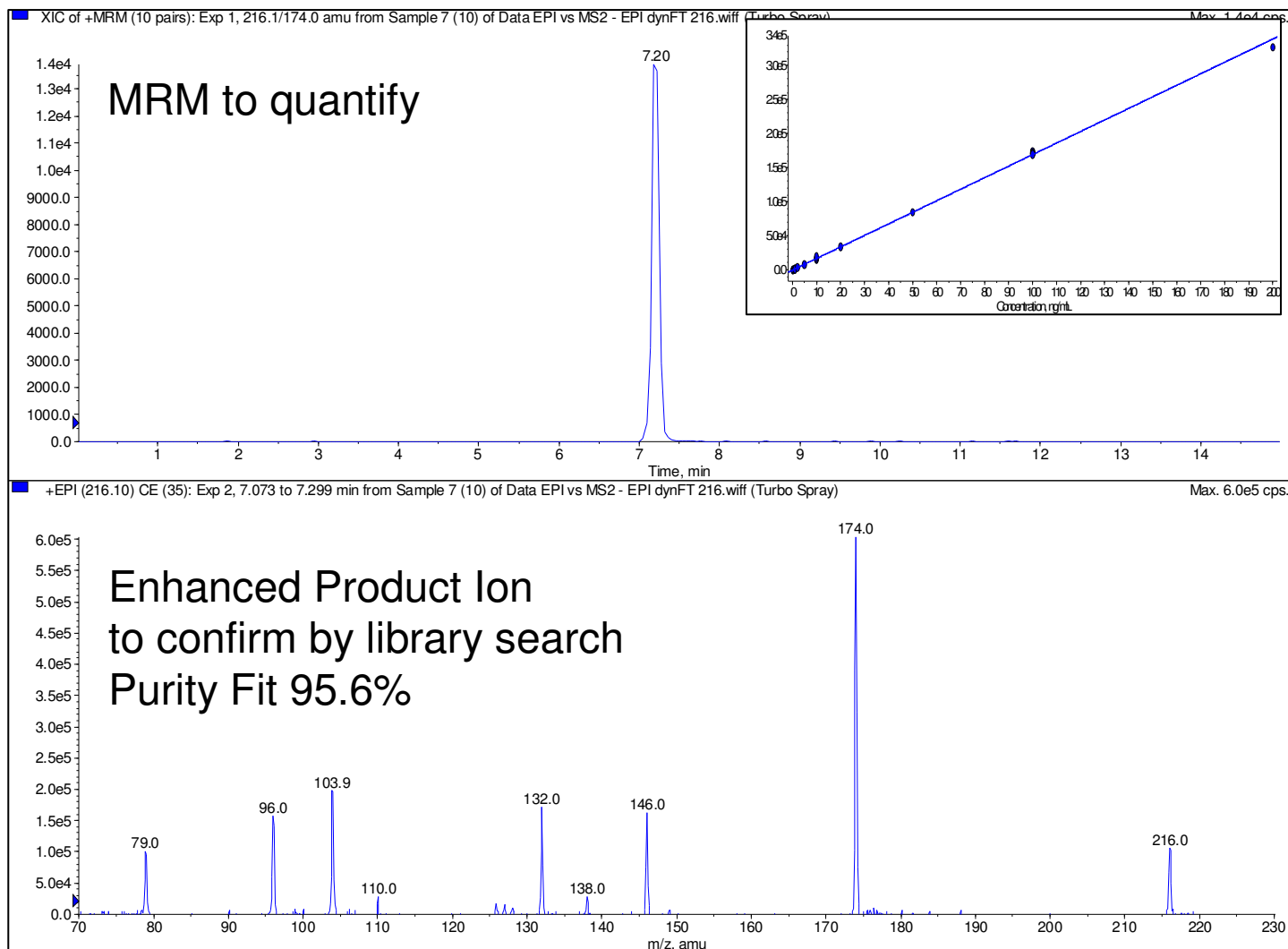
Information Dependent Acquisition (MRM to EPI)

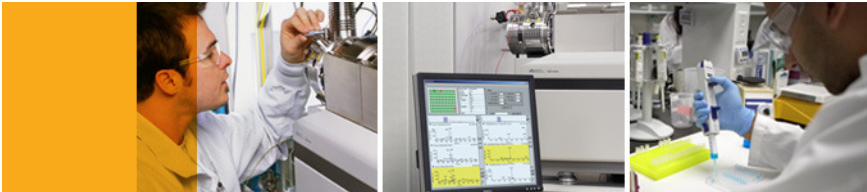
- Information Dependent Acquisition (IDA) of EPI spectra
 - MRM with high selectivity and sensitivity triggers automatically EPI scan
- Dynamic background subtraction to confirm co-eluting compounds



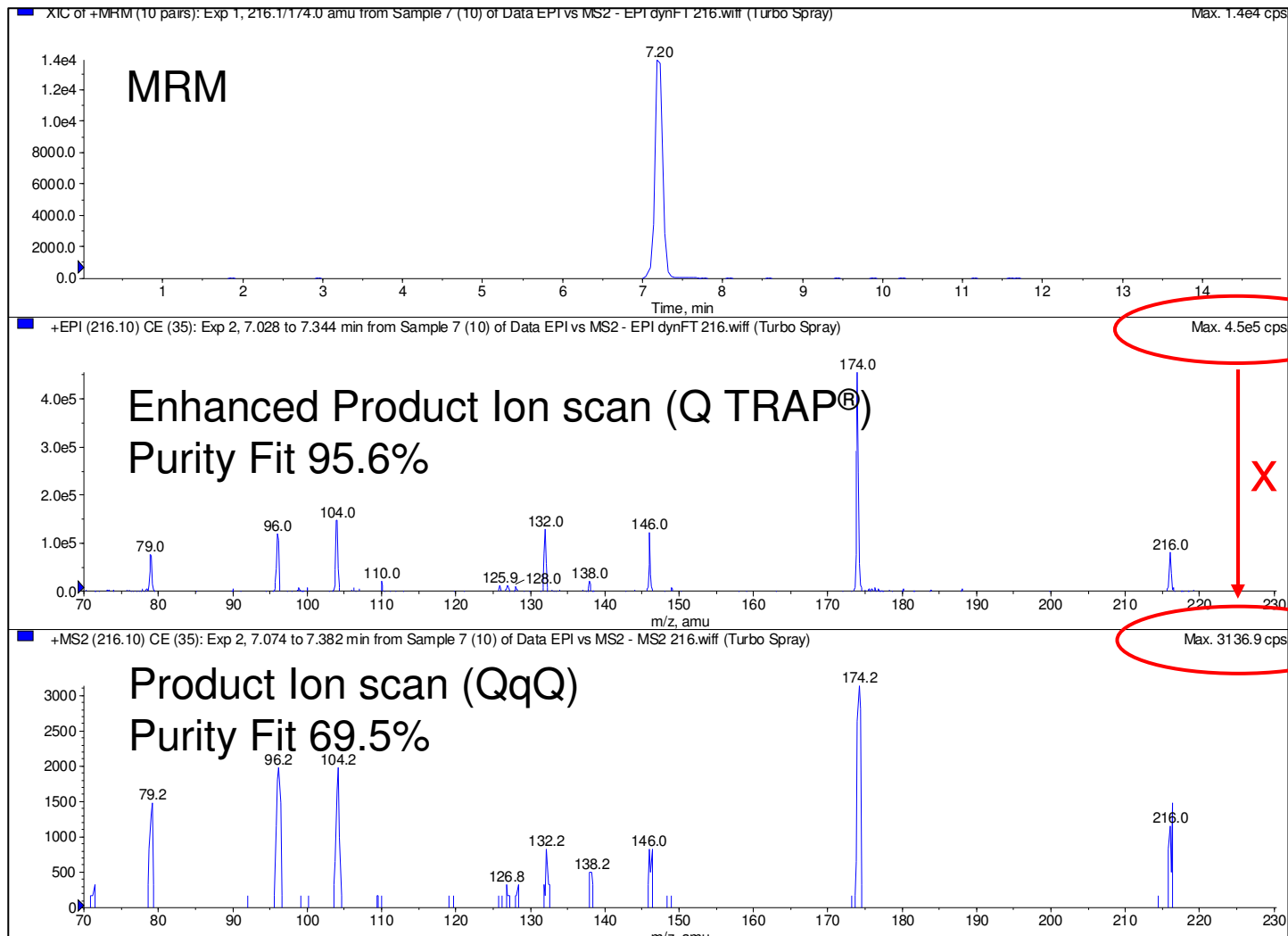


Quantitation and Confirmation



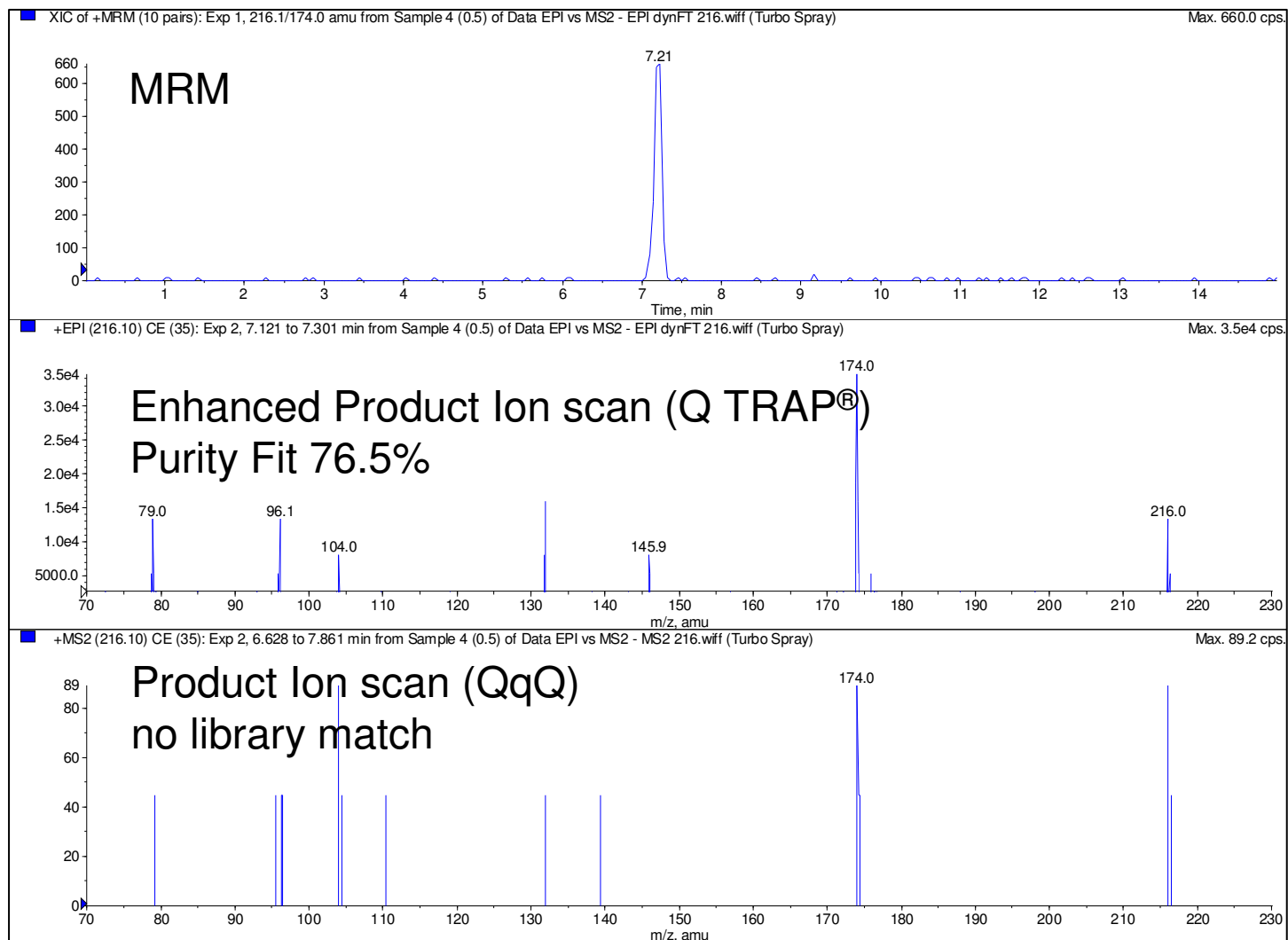


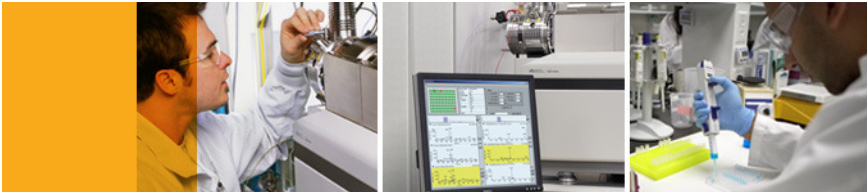
Confirmation by Q TRAP[®] or QqQ (10ng/mL Atrazine)



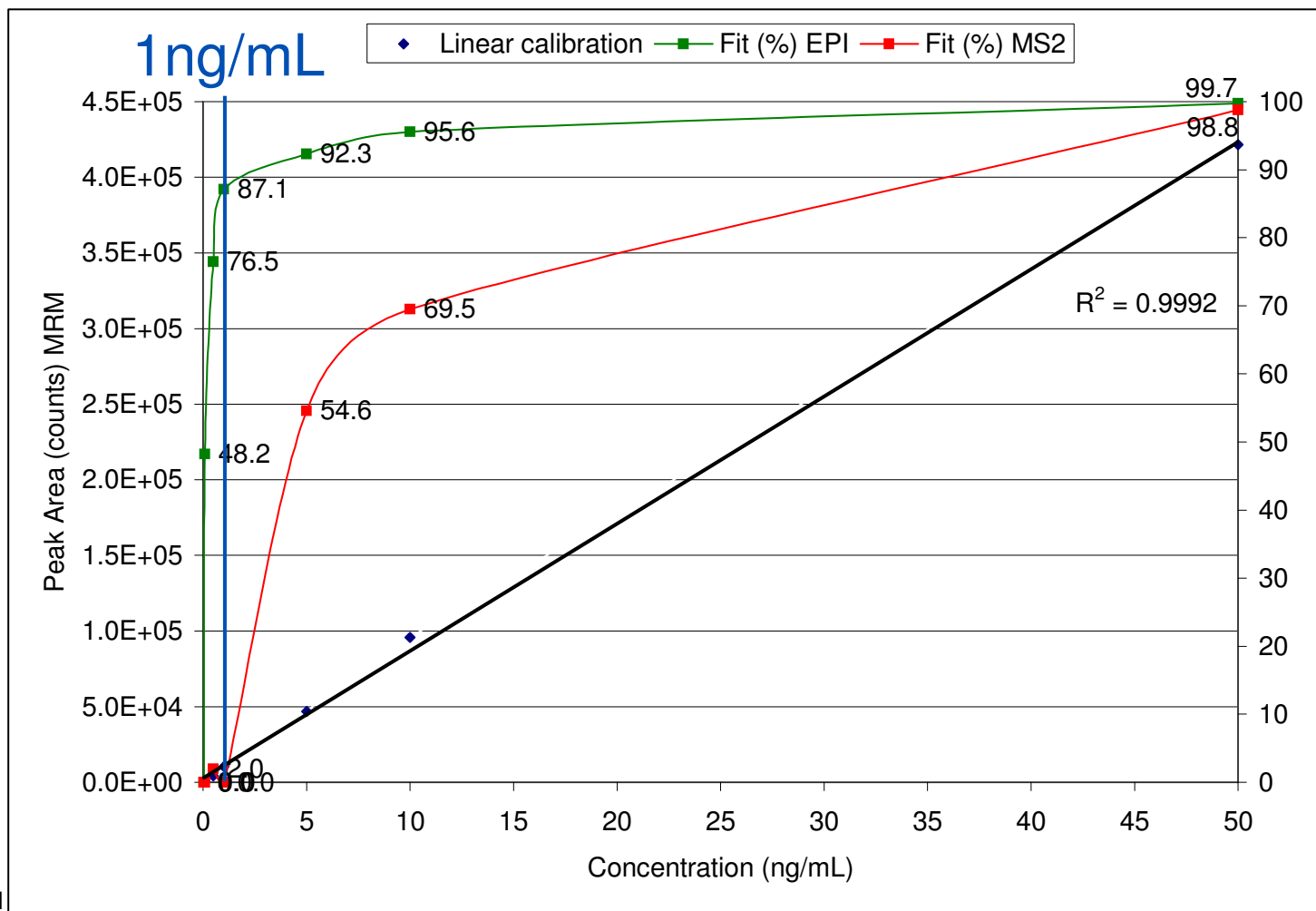


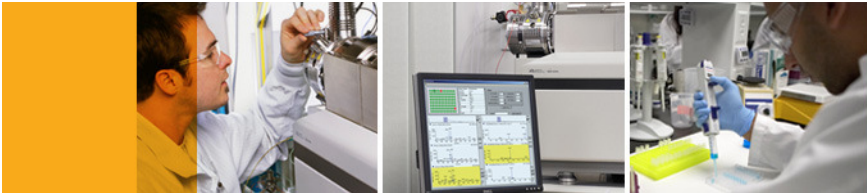
Confirmation by Q TRAP[®] or QqQ (0.5ng/mL Atrazine)





Library Search Results using QqQ or Q TRAP[®] in comparison to a calibration line in MRM





Ease-of-Use Software for LC/MS/MS Analysis



Cliquid™ Food & Beverage



Cliquid™ Quant



Cliiquid™ Software – ‘Run Samples’ Wizard

Cliiquid(TM) Software for Routine Food Testing - Microsoft Internet Explorer

Run Samples

Home Help Log Out

Step 1
Choose test →

Choose a test

- Pesticides - Acidic pesticides
- Pesticides - Carbamates
- Pesticides - Organophosphorus
- Pesticides - Phenyl ureas
- Pesticides - Triazines
- Nitrofurans metabolites
- Chloramphenicol
- Azo-dyes - 13 azo-dyes in positive polarity
- Azo-dyes - Orange II in negative polarity
- Malachite Green

Cancel < Back

Step 2
Build sample list

Step 3
Customize report

Step 4
Submit samples

Instrument Panel

Stop Live View

Standby Event Log

Restart

Standby

Mass Spec
Standby

Autosampler
Standby

Cliiquid™ Software for Routine Food Testing

Benzo[a]pyrene C.I. No. CAS: 22781-22-3 Benzo[a]pyrene C.I. No. CAS: 82580-54-1

Benzo[b]fluoranthene C.I. No. CAS: 17804-95-2 Benzo[k]fluoranthene C.I. No. CAS: 34911-10-2

Benzo[e]pyrene C.I. No. CAS: 34811-24-8 Benzo[a]anthracene C.I. No. CAS: 34811-23-7

Carbazol C.I. No. CAS: 69-35-2 Carbazolone C.I. No. CAS: 1953-66-2

Cliiquid™ Software for Routine Food Testing

HPLC [Go to top](#)

Column: Phenomenex Synergi Ac Fusion RP (150x4.6mm)

Mobile Phase: 0.1% HCl in water / 5% MeCN

Gradient:

| Step | Total Time | Flow rate | A (%) | B (%) |
|------|------------|-----------|-------|-------|
| 1 | 5.00 | 1.00 | 95 | 5 |
| 2 | 14.00 | 1.00 | 95 | 5 |
| 3 | 15.00 | 1.00 | 95 | 5 |

MS/MS Detection [Go to top](#)

API 2000™ and 2000 Q TRAP LC/MS/MS system

ScanMode: SIM

Positive polarity

CEP: 100V
CAD: 5
ESI: 5000V
TSM: 500°C
SIC: 0.1
PC: 0

Multiple Reaction Monitoring

| Compound | Q1 MS/MS (m/z) | Q3 MS/MS (m/z) | Decl. (m/z) | Parameter | Value |
|---------------------|----------------|----------------|-------------|-----------|-------|
| 3-Hydroxyanthracene | 238.1 | 183.1 | 25 | DP | 42 |
| | | | | CE | 35 |
| | | | | CF | 21 |
| | | | | CP | 42 |



Automatic Acquisition

Cliiquid(TM) Software for Routine Food Testing - Microsoft Internet Explorer

Welcome
Adrianna

Home Help Log Out

What would you like to do?

- Run samples ➤
- Reprocess samples ➤
- System suitability test ➤
- Setup**
- New project ➤
- Autosampler ➤
- User profile ➤

Job List
Sample List
Reports
Search

Selected Job: Azo-dyes (13 Samples)

| Status | Sample Name | Position | Time Completed | Test Performed | Move | Delete |
|--------|--------------|----------|------------------------|----------------------------|--------|--------|
| ... | blank | 1 | 12/22/2006 11:47:43 AM | Azo-dyes - 13 azo-dyes ... | ⬆️⬆️⬆️ | ✕ |
| ... | standard 1 | 2 | 12/22/2006 12:28:43 PM | Azo-dyes - 13 azo-dyes ... | ⬆️⬆️⬆️ | ✕ |
| ... | standard 5 | 3 | 12/22/2006 1:09:43 PM | Azo-dyes - 13 azo-dyes ... | ⬆️⬆️⬆️ | ✕ |
| ... | standard 10 | 4 | 12/22/2006 1:50:43 PM | Azo-dyes - 13 azo-dyes ... | ⬆️⬆️⬆️ | ✕ |
| ... | standard 50 | 5 | 12/22/2006 2:31:43 PM | Azo-dyes - 13 azo-dyes ... | ⬆️⬆️⬆️ | ✕ |
| ... | standard 100 | 6 | 12/22/2006 3:12:43 PM | Azo-dyes - 13 azo-dyes ... | ⬆️⬆️⬆️ | ✕ |
| ... | blank | 1 | 12/22/2006 3:53:43 PM | Azo-dyes - 13 azo-dyes ... | ⬆️⬆️⬆️ | ✕ |
| ... | U 01 | 7 | 12/22/2006 4:34:43 PM | Azo-dyes - 13 azo-dyes ... | ⬆️⬆️⬆️ | ✕ |
| ... | U 02 | 8 | 12/22/2006 5:15:43 PM | Azo-dyes - 13 azo-dyes ... | ⬆️⬆️⬆️ | ✕ |
| ... | U 03 | 9 | 12/22/2006 5:56:43 PM | Azo-dyes - 13 azo-dyes ... | ⬆️⬆️⬆️ | ✕ |
| ... | U 04 | 10 | 12/22/2006 6:37:43 PM | Azo-dyes - 13 azo-dyes ... | ⬆️⬆️⬆️ | ✕ |
| ... | U 05 | 11 | 12/22/2006 7:18:43 PM | Azo-dyes - 13 azo-dyes ... | ⬆️⬆️⬆️ | ✕ |
| ... | blank | 1 | 12/22/2006 7:59:43 PM | Azo-dyes - 13 azo-dyes ... | ⬆️⬆️⬆️ | ✕ |

Instrument Panel

Stop

Standby

Restart

Live View

Event Log

Warming up

42 Seconds Remaining

Mass Spec
Equilibrating

Pump
Equilibrating

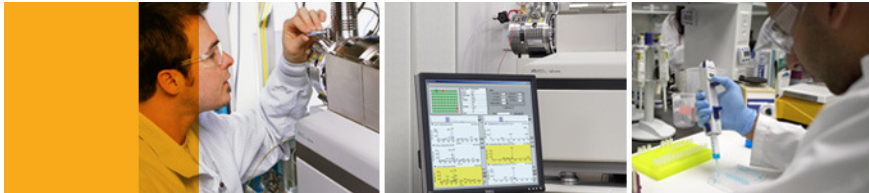
Autosampler
Equilibrating

Column Oven
Equilibrating

27

April

askatoon 2008



Automatic Reporting

Cliquid™ Software for Food Testing - Microsoft Internet Explorer provided by MDS Sciex

Welcome **Adrianna**

Home Help Log Out

What would you like to do?

- Run samples
- Reprocess samples
- System suitability test

Setup

- New project
- Autosampler
- User profile

Job List Sample List Reports Filter Job List

Selected Job: Azo-dyes_Foodlab 01234 (3 reports)

| Status | Report | Report Name | Report Style | Move | Delete |
|--------|--------|------------------------------|-----------------------------------|----------------|--------|
| ... | View | 2006_06_14_170621_Azo-dye... | Report calibration curve (1 pe... | ⬅️ ⬆️ ⬇️ ⬆️ ⬅️ | ✖️ ⬇️ |
| ... | View | 2006_06_14_170621_Azo-dye... | Report summary of unknown... | ⬅️ ⬆️ ⬇️ ⬆️ ⬅️ | ✖️ ⬇️ |
| ... | View | 2006_06_14_170621_Azo-dye... | Report unknown samples wit... | ⬅️ ⬆️ ⬇️ ⬆️ ⬅️ | ✖️ ⬇️ |

Instrument Panel

Stop Standby Restart Live View Event Log

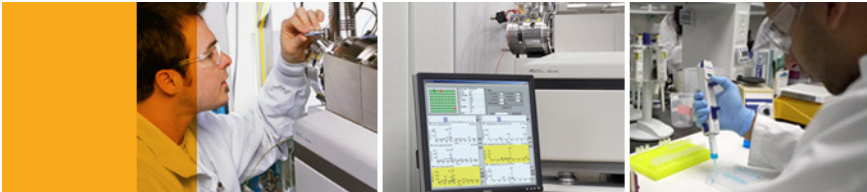
Acquiring

Current Sample: standard 1

Mass Spec Wating Pumps Equilibrating

Autosampler Wating Column Oven Wating

Done Local intranet Saskatoon 2008



Example Reports based on *.dot

Report calibration

Analyte Name:
Internal Standard:

Project Data File
Result Table
Instrument Name
Acquisition Date
Acquisition Method

Regression Equation:
Expected Concentration:

| |
|--------|
| 1.00 |
| 3.00 |
| 10.00 |
| 30.00 |
| 100.00 |

Calib

AIT Laboratories

Project Data File
Acquisition Date
Acquisition Method
Instrument Name

Sample Name
Data File
Acquisition Date
Acquisition Method
Injection Vial

Results Summary

| Sample Name | Sample CP1 |
|------------------|------------|
| Suden Orange G 1 | |
| Suden Orange G 2 | |
| Suden II 1 | |
| Suden II 2 | |
| Suden III 1 | |
| Suden III 2 | |
| Suden IV 1 | |
| Suden IV 2 | |
| Suden Red 1 | |
| Suden Red 2 | |
| Suden Red 3 | |
| Suden Red 4 | |
| Suden Red 5 | |
| Suden Red 6 | |
| Suden Red 7 | |
| Suden Red 8 | |
| Suden Red 9 | |
| Suden Red 10 | |
| Suden Red 11 | |
| Suden Red 12 | |
| Suden Red 13 | |
| Suden Red 14 | |
| Suden Red 15 | |
| Suden Red 16 | |
| Suden Red 17 | |
| Suden Red 18 | |
| Suden Red 19 | |
| Suden Red 20 | |
| Suden Red 21 | |
| Suden Red 22 | |
| Suden Red 23 | |
| Suden Red 24 | |
| Suden Red 25 | |
| Suden Red 26 | |
| Suden Red 27 | |
| Suden Red 28 | |
| Suden Red 29 | |
| Suden Red 30 | |
| Suden Red 31 | |
| Suden Red 32 | |
| Suden Red 33 | |
| Suden Red 34 | |
| Suden Red 35 | |
| Suden Red 36 | |
| Suden Red 37 | |
| Suden Red 38 | |
| Suden Red 39 | |
| Suden Red 40 | |
| Suden Red 41 | |
| Suden Red 42 | |
| Suden Red 43 | |
| Suden Red 44 | |
| Suden Red 45 | |
| Suden Red 46 | |
| Suden Red 47 | |
| Suden Red 48 | |
| Suden Red 49 | |
| Suden Red 50 | |

Resu

Summary

Project Data File
Result Table
Instrument Name

Sample Name
Data File
Acquisition Date
Acquisition Method
Instrument Name
Sample ID
Sample Comment

Result

| Sample Name | Sample CP1 |
|------------------|------------|
| Suden Orange G 1 | |
| Suden Orange G 2 | |
| Suden II 1 | |
| Suden II 2 | |
| Suden III 1 | |
| Suden III 2 | |
| Suden IV 1 | |
| Suden IV 2 | |
| Suden Red 1 | |
| Suden Red 2 | |
| Suden Red 3 | |
| Suden Red 4 | |
| Suden Red 5 | |
| Suden Red 6 | |
| Suden Red 7 | |
| Suden Red 8 | |
| Suden Red 9 | |
| Suden Red 10 | |
| Suden Red 11 | |
| Suden Red 12 | |
| Suden Red 13 | |
| Suden Red 14 | |
| Suden Red 15 | |
| Suden Red 16 | |
| Suden Red 17 | |
| Suden Red 18 | |
| Suden Red 19 | |
| Suden Red 20 | |
| Suden Red 21 | |
| Suden Red 22 | |
| Suden Red 23 | |
| Suden Red 24 | |
| Suden Red 25 | |
| Suden Red 26 | |
| Suden Red 27 | |
| Suden Red 28 | |
| Suden Red 29 | |
| Suden Red 30 | |
| Suden Red 31 | |
| Suden Red 32 | |
| Suden Red 33 | |
| Suden Red 34 | |
| Suden Red 35 | |
| Suden Red 36 | |
| Suden Red 37 | |
| Suden Red 38 | |
| Suden Red 39 | |
| Suden Red 40 | |
| Suden Red 41 | |
| Suden Red 42 | |
| Suden Red 43 | |
| Suden Red 44 | |
| Suden Red 45 | |
| Suden Red 46 | |
| Suden Red 47 | |
| Suden Red 48 | |
| Suden Red 49 | |
| Suden Red 50 | |

Anal

Applied Biosystems | MDS SCIEX

Created with Analyst Reporter
Printed: 6/20/2008 3:03:00 PM

| Data File | 2006_05_24_193027_azo-dyes customized.wiff | Result Table | McCormickStdMtx1_AS.rdb |
|--------------------|--|-----------------|-------------------------|
| Acquisition Date | 2006-05-27 4:16:36 PM | Algorithm Used | MQL |
| Acquisition Method | azo-dyes_206_cust.dam | Instrument Name | 3200 Q TRAP |
| Project | McCormick_Azo Dyes | | |

| Sample Name | Sample CP1 | Injection Vial | 41.00 |
|--------------------|--|------------------|-------------------------|
| Data File | 2006_05_24_193027_azo-dyes customized.wiff | Injection Volume | 50.00 |
| Acquisition Date | 2006-05-27 4:16:36 PM | Algorithm Used | MQL |
| Acquisition Method | azo-dyes_206_cust.dam | Sample Type | Unknown |
| Instrument Name | 3200 Q TRAP | Result Table | McCormickStdMtx1_AS.rdb |
| Sample ID | | Dilution Factor | 1.00 |
| Sample Comment | | Weight to Volume | 5.00 |

Quantifier and qualifier overlay

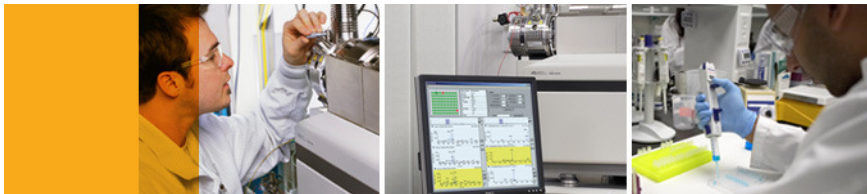
Applied Biosystems | MDS SCIEX

Created with Analyst Reporter
Printed: 6/20/2008 3:03:00 PM

Peak Review

29 April 28, 2008

Page 4 of 32



Quickly Build Methods with the MRM catalog

Cliiquid(TM) Software for Routine Quantitation - Microsoft Internet Explorer

Tests

Home Help Log Out

What would you like to do?

- Create a test →
- Modify a test
- Reorder tests
- Activate/Deactivate tests
- Delete tests

Select compounds to include in the test

Display compounds with: Positive polarity Negative polarity

Available compounds Search: morphine

| | Compound Name | Class | CAS |
|-------------------------------------|----------------------|--------|------------|
| <input checked="" type="checkbox"/> | Morphine | opiate | 57-27-2 |
| <input type="checkbox"/> | Morphine-3-glucur... | opiate | 20290-09-9 |
| <input type="checkbox"/> | Morphine-6-glucur... | opiate | 20290-10-2 |
| <input checked="" type="checkbox"/> | Morphine-D3 | opiate | 67293-88-3 |

Your selections

- 6-Acetylmorphine
- Cocaine
- Codeine
- Heroin
- Hydrocodone
- Hydromorphone

Cancel < Back Next >

Instrument Panel

Stop Standby Restart Live View Event Log

Standby

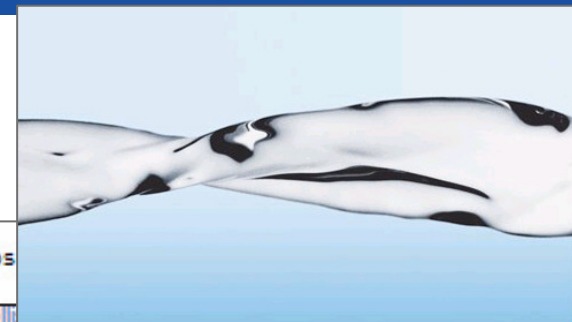
Mass Spec Standby Pump Standby Autosampler Standby Column Oven Standby

Cliiquid™ Food & Beverage: > 500 Pesticides

30 April Done Local intranet Saskatoon 2008

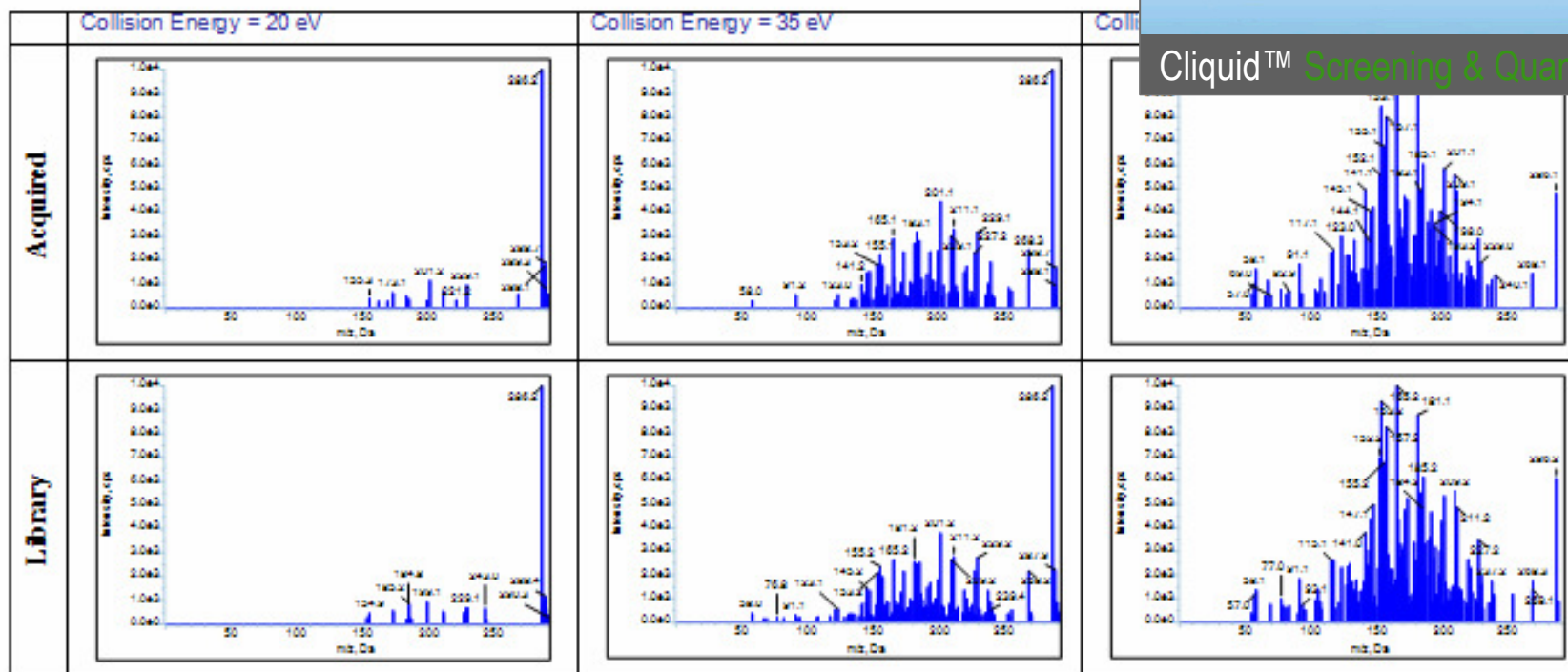


Automatic Library Searching



Cliquant™ Screening & Quant

Peak 5 Q1/Q3: 286.1/201.1 amu Retention Time: 2.34 minutes Area counts: 127000 cps



| | Compound Name | Purity (%) | Compound Name | Purity (%) | Compound Name | Purity (%) |
|---|-------------------|------------|---------------|------------|---------------|------------|
| 1 | Hydromorphone | 91.1 | Morphine | 85.8 | Morphine | 87.0 |
| 2 | Morphine | 90.6 | Hydromorphone | 63.1 | | |
| 3 | 7-Aminoclonazepam | 61.2 | | | | |





LC/MS/MS Analysis of Very Polar Pesticides

APPLICATION NOTE: glyphosate

Applied Biosystems | MDS SCIEX

Direct injection of water to analyze dissociated organo-phosphorous pesticides and their degradation products

API 4000™ LC/MS/MS System and Ion Chromatography

Introduction
Glyphosate (N-(phosphonomethyl)glycine) is a broad-spectrum, non-selective, post-emergence herbicide. Glyphosate is used to control grasses, herbaceous plants including deep rooted perennial weeds, brush, some broadleaf trees and shrubs, and some conifers. Glyphosate applied to foliage is absorbed by leaves and rapidly moves through the plant. It acts by preventing the plant from producing an essential amino acid. This reduces the production of protein in the plant, and inhibits plant growth. Glyphosate is metabolized or broken down by some plants, while other plants do not break it down. Glyphosinate (Ammonium-2-amino-4-hydroxymethylphosphonyl butanoate) is a short name for the ammonium salt, glyphosate-ammonium. It also acts as a broad-spectrum contact herbicide and is used to control a wide range of weeds after the crop emerges or for total vegetation control on land not used for cultivation. Glyphosinate herbicides are also used to desiccate (dry off) crops before harvest.



MPPA (S-(methylphosphinyloxypropionic acid)) is a structure related compound also in use as pesticide in Europe and the US as well as in Asia. The main degradation product of glyphosate is aminomethylphosphonic acid (AMPA). The chemical structures of glyphosate, glufosinate, MPPA and AMPA are given in Figure 1.

The Maximum Contaminant Level (MCL) for glyphosate has been set by the US Environmental Protection Agency (EPA) at 0.7 parts per million (ppm) because EPA believes this level of protection would not cause any of the potential health problems described below [1]. The MCL for glyphosate in Europe is set at 0.1 µg/L for drinking water [2]. According to the toxicity, EPA has found that glyphosate potentially causes congestion of the lungs and an increased breathing rate when people

APPLICATION NOTE: Paraquat and Diquat

Applied Biosystems | MDS SCIEX

Fast and Sensitive Analysis of Paraquat and Diquat in Drinking Water

API 3200™ LC/MS/MS System

Overview
This application note describes a fast and sensitive LC/MS/MS method for the determination of Paraquat and Diquat in drinking water. Using the Ultra Quat HPLC column and the API 3200™ LC/MS/MS System equipped with a Turbo V™ source, the limits of quantitation (LOQ) for this method in drinking water are 0.1 µg/L and 0.5 µg/L, respectively for Diquat and Paraquat using a 10 µL injection volume without sample preparation prior to analysis.

Introduction
Paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride, C₁₂H₁₁N₄Cl₂) and Diquat (1,1'-ethylene-2,2'-bipyridinium dibromide, C₁₂H₁₁N₄Br₂), are non-selective and non-systemic contact herbicides widely used in agriculture to control broadleaf and grassy weeds. The use of these herbicides is very important because weeds compete vigorously with crops for water, light and other nutrients. As a result, if they are not suppressed they reduce crop yields by up to 80%. However both Paraquat and Diquat are toxic, and ingestion of either compound can have serious effects as they can alter reduction-oxidation activities in biological systems. The analysis of these highly charged dual quaternary amines is complicated because of their ionic nature and therefore Paraquat and Diquat are difficult to retain by standard reversed phase HPLC. For drinking water the United States Environmental Protection Agency (EPA) has established a maximum contaminant level of 20 µg/L for Diquat. Paraquat is currently not regulated in drinking water to our knowledge. The EPA 549.2 method for the analysis of Paraquat and Diquat uses reversed phase/ion-pair extraction utilizing C8 SPE cartridges followed by ion-pair LC with ultraviolet (UV) and/or photodiode array (PDA) detection. This method is time-

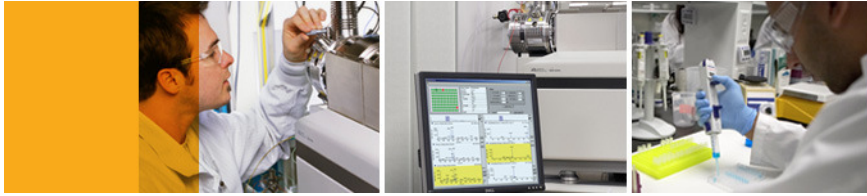
Direct injection of water samples to analyze:
 Glyphosate, AMPA, Glyphosinate, MPPA
 Dionex IonPac AG11 (50x2mm) column
 Water, citric acid, triethylamine
 API 4000™ LC/MS/MS system

Paraquat, Diquat
 Restek Ultra Quat 50x2.1mm
 Water/Acetonitril + HFBA
 API 3200™ LC/MS/MS system



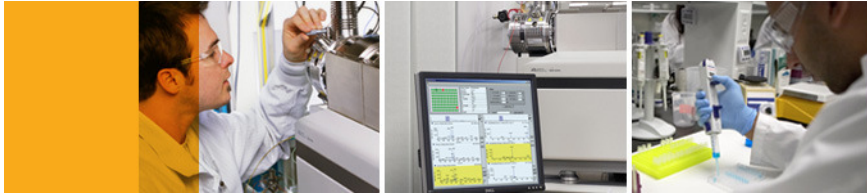
Conclusion

- LC/MS/MS: ideal technology for multi targeted screening of pesticides in food, water and environmental samples
 - 2 Multiple Reaction Monitoring transitions at correct retention time are typically used to quantify and confirm
 - API 3200™ / 3200 Q TRAP® LC/MS/MS systems give sufficient sensitivity to analyze food at 10ppb levels
 - Higher sensitivity (allows direct injections of water samples and dilution of extracts to reduce matrix effects)
- Cliquant™ Software makes LC/MS/MS easy to use (automatic report generation and MRM catalogue of > 500 pesticides)
- Scheduled MRM to detect more MRM transitions per time
- Enhanced Product Ion scan with higher degree of confirmation
- LC/MS/MS analysis of very polar compounds with special columns



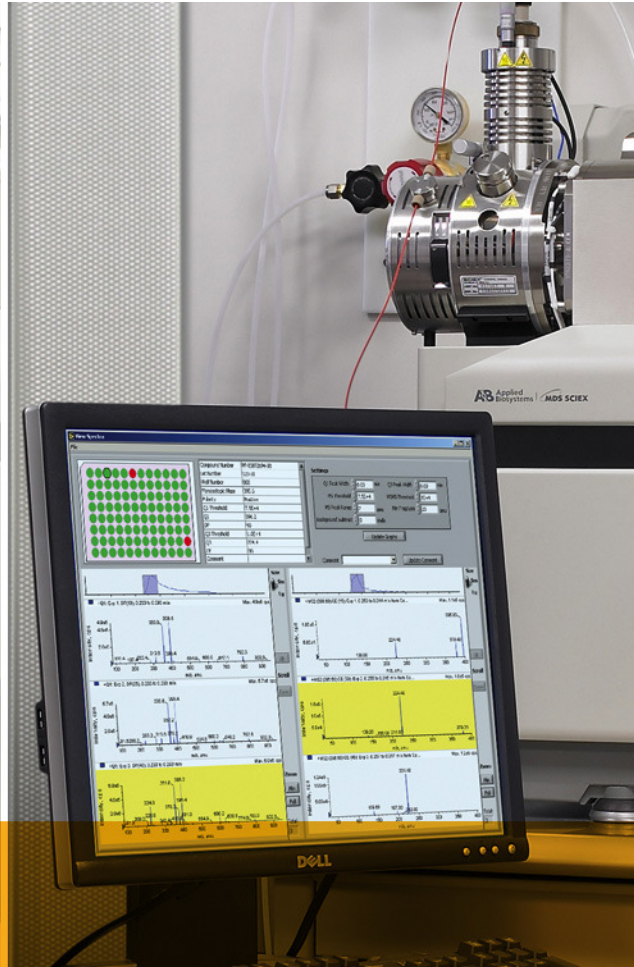
Acknowledgements

- Federal Institute for Risk Assessment – Berlin (Germany)
- Chinese Institute for Quarantine – Dalian (China)
- Kobe Quarantine Station – Kobe (Japan)
- Restek and Dionex for assistance in HPLC development
- Colleagues of Applied Biosystems / MDS Sciex



Legal acknowledgements

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Thanks for Listening!