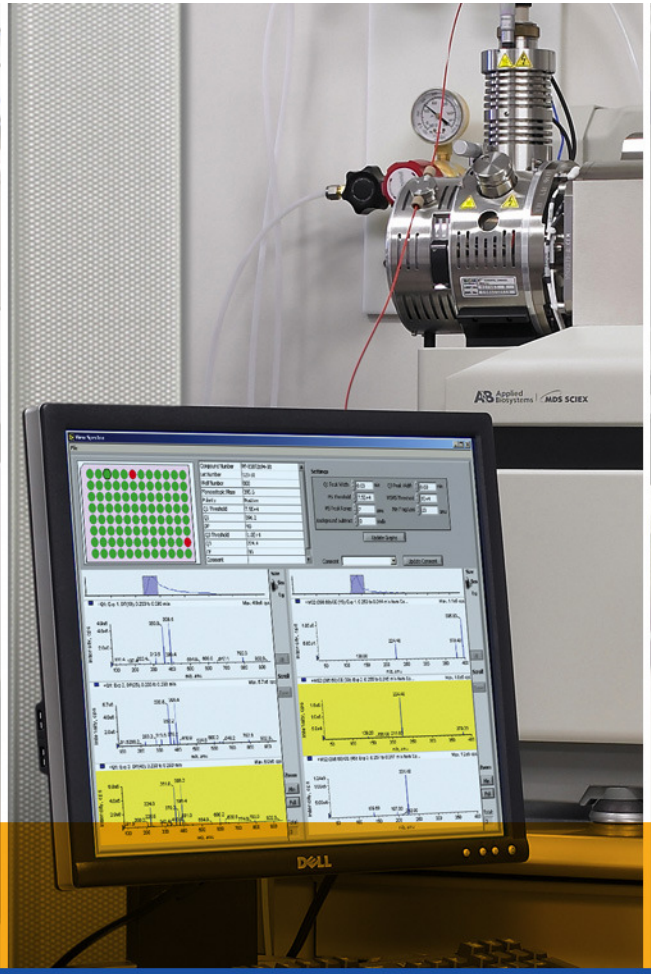
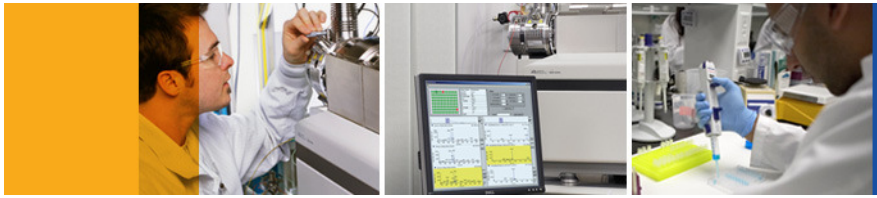


A Generic LC/MS/MS Method for the Analysis of Pesticides at 0.1µg/L in Water Samples by Direct Injection

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

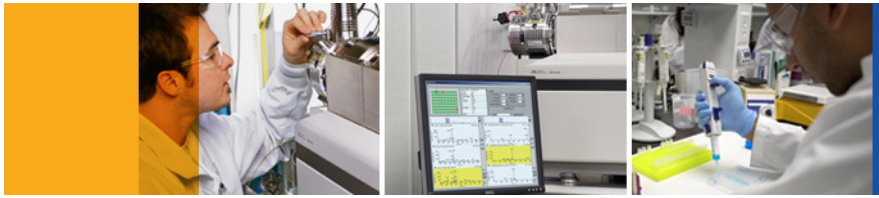
Senior Field Applications Specialist, Toronto, Canada

Applied Biosystems | MDS SCIEX



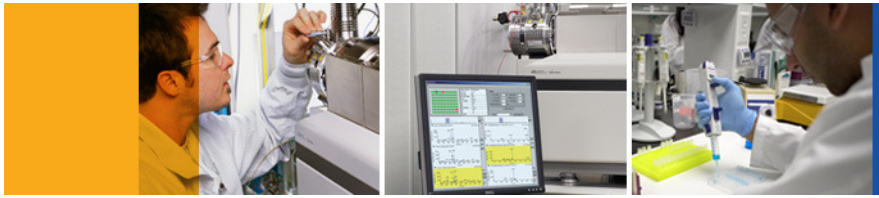
Outline

- 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.



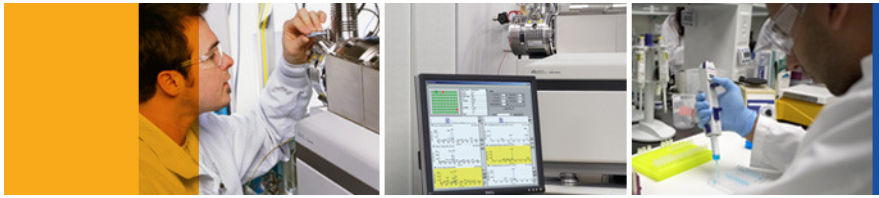
Experimental – Generic Pesticide Screening

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



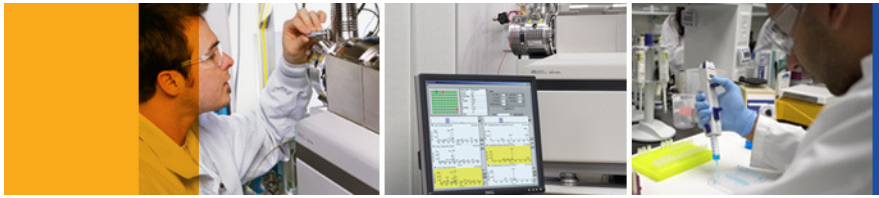
Experimental – Generic Pesticide Screening

- HPLC:
  - Phenomenex Synergi 4um Fusion-RP 80A (50x2mm)
  - Eluent A: H_2O + 5mM ammonium formate
  - Eluent B: CH_3OH + 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

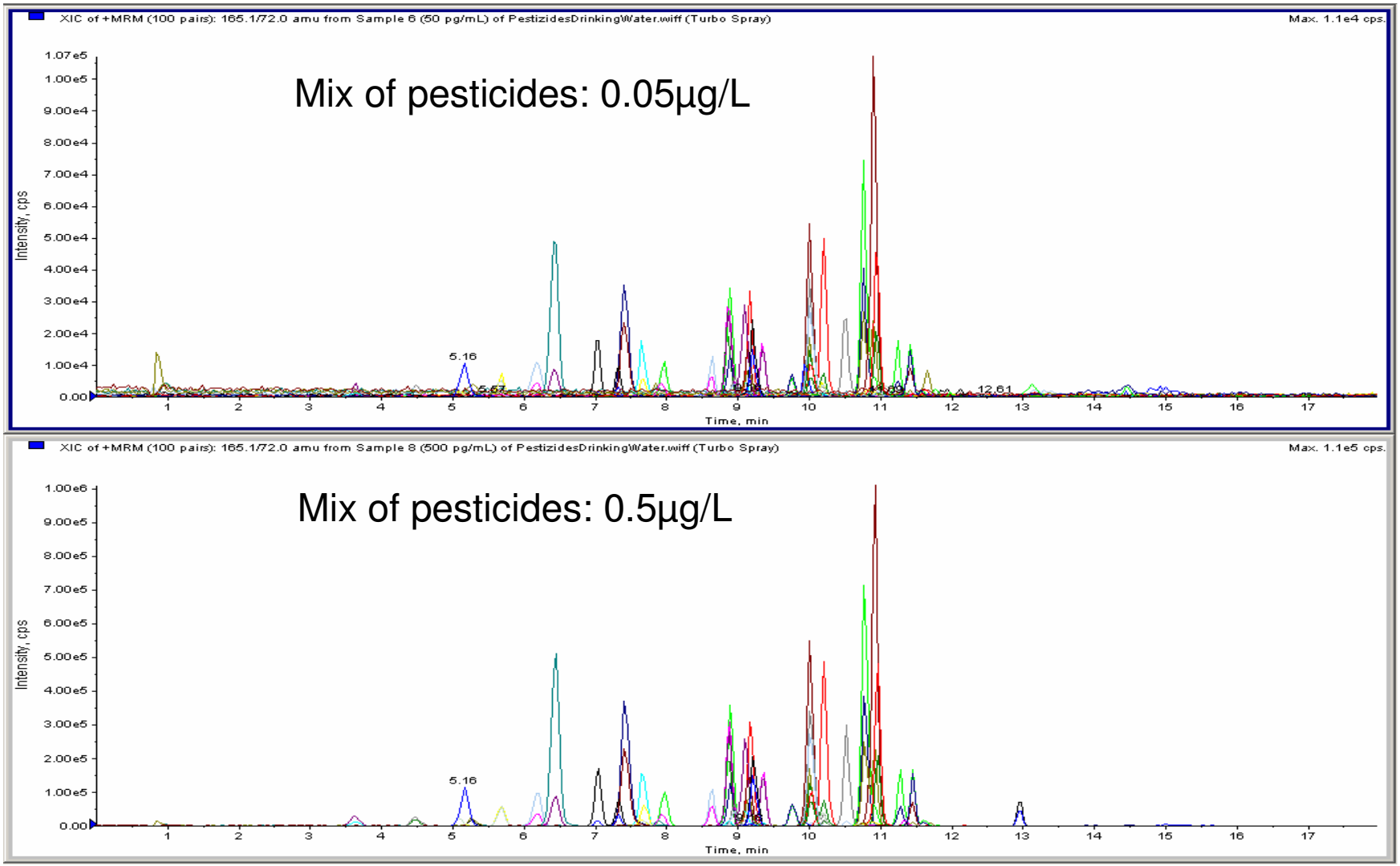


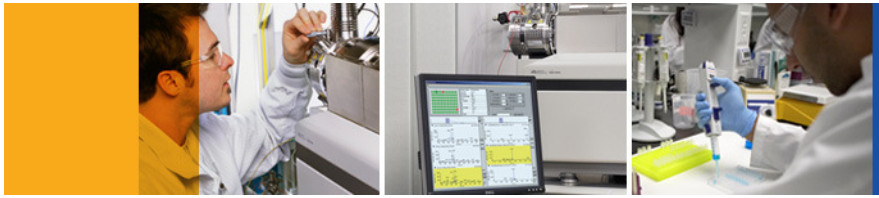
Experimental – Generic Pesticide Screening

- 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

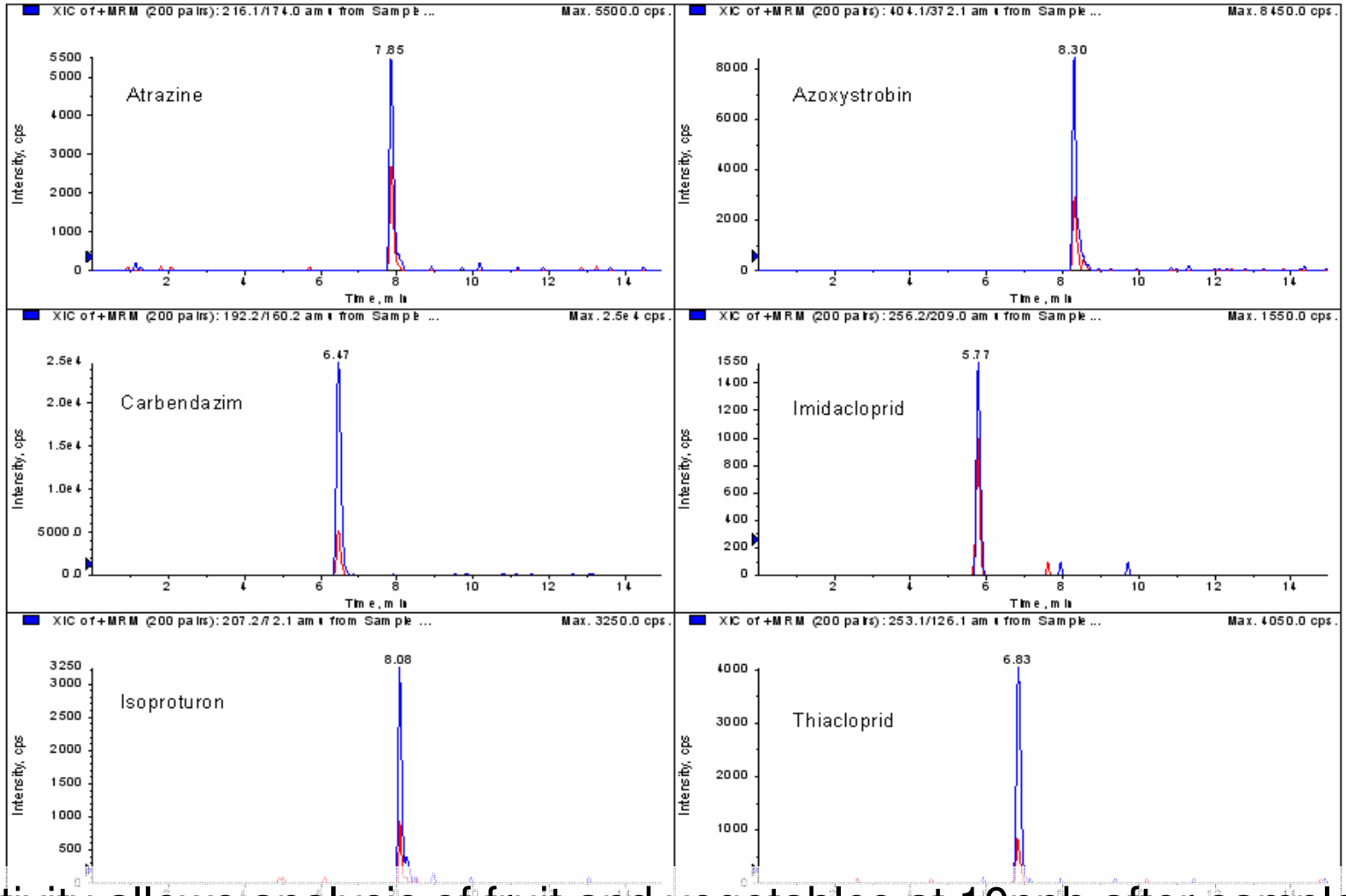


Separation and Detection of 50 Pesticides (100 MRM)

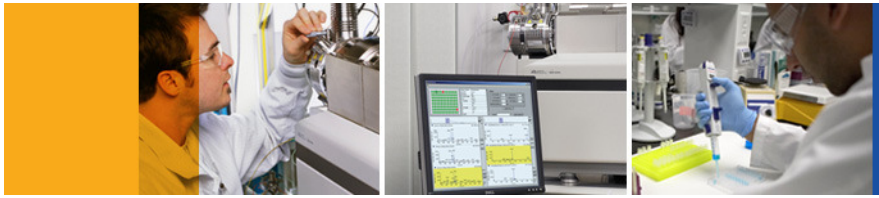




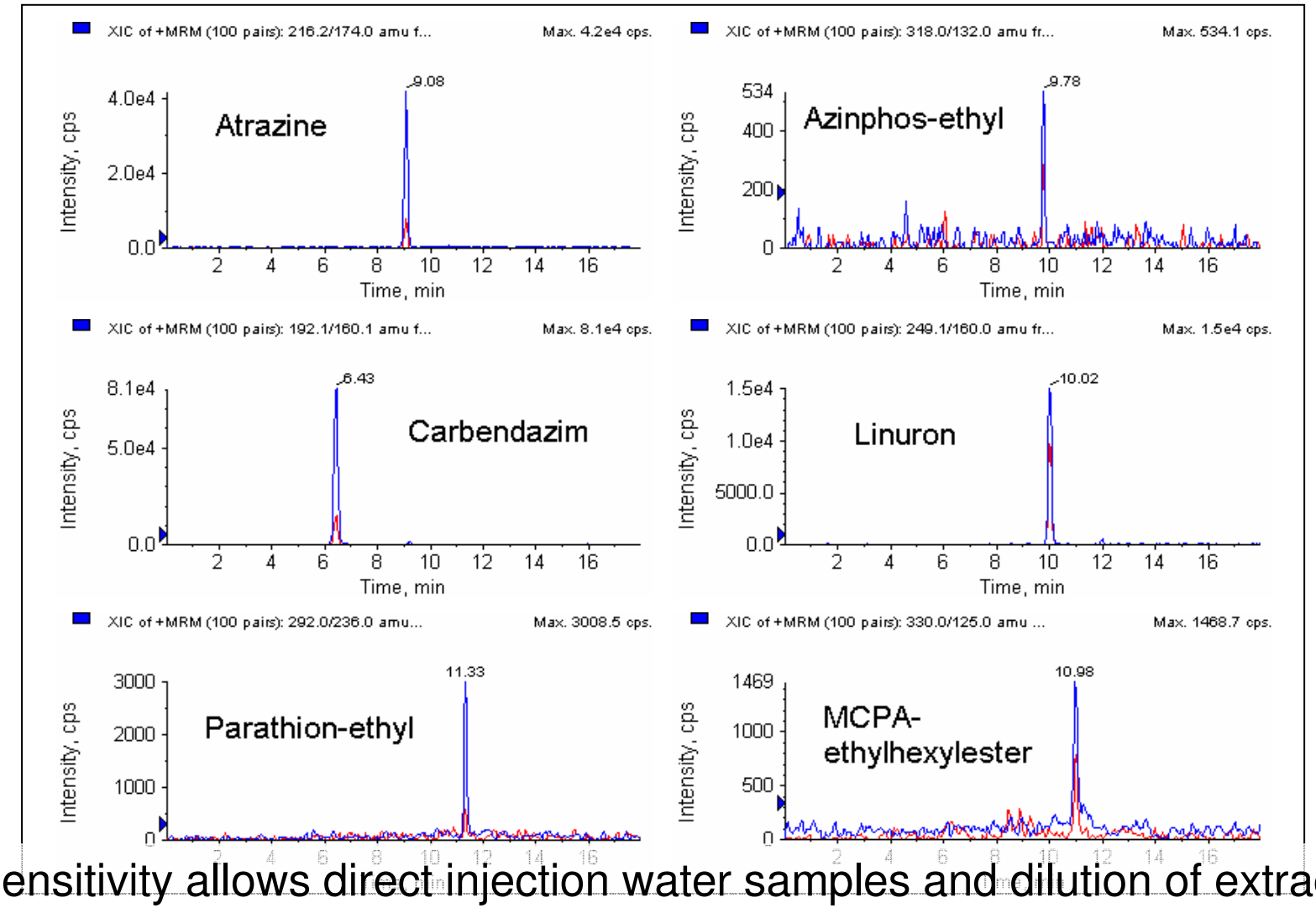
3200 Q TRAP®: 1µg/L of Selected Pesticides



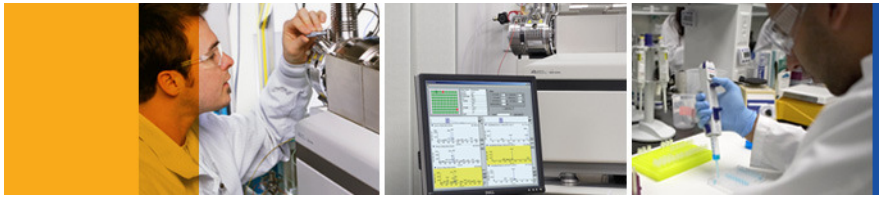
Sensitivity allows analysis of fruit and vegetables at 10ppb after sample prep



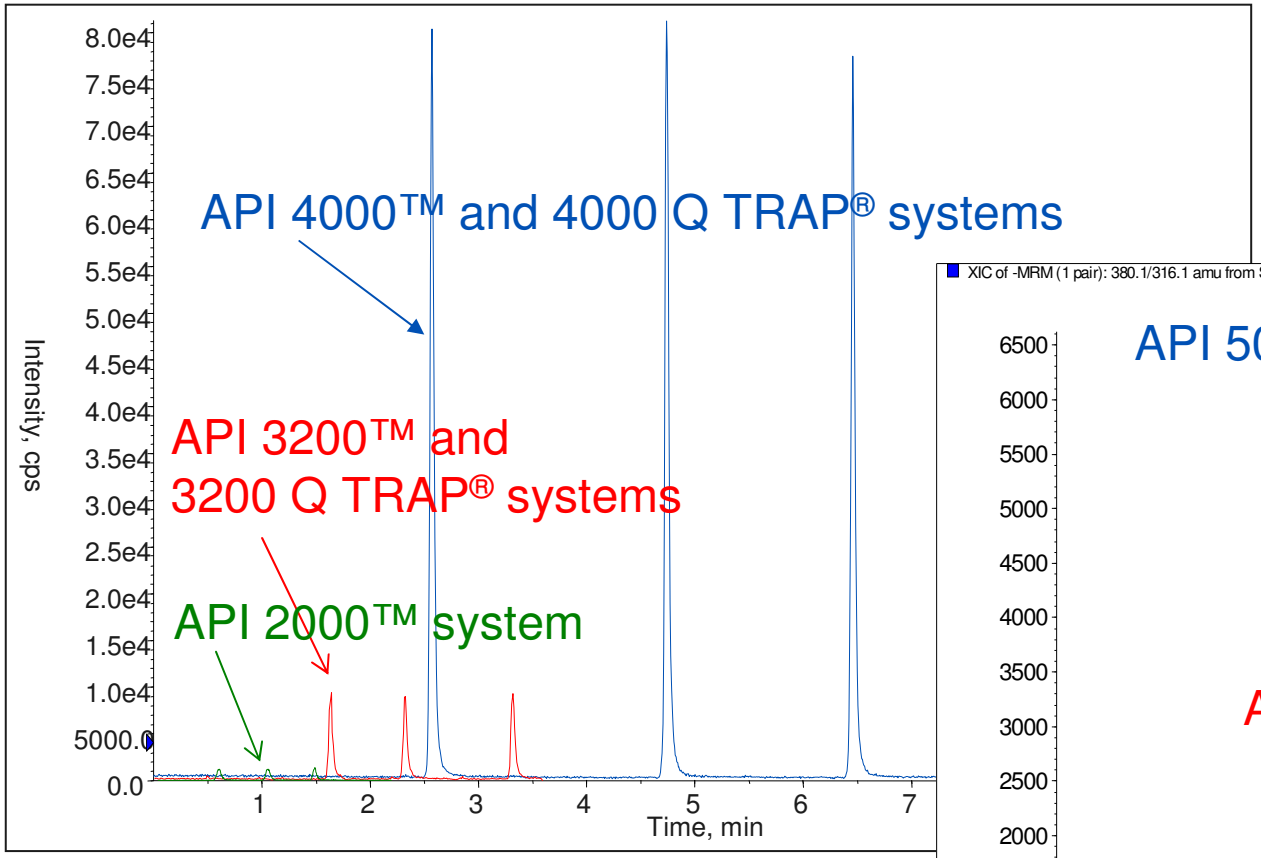
API 5000™: 0.1µg/L of Selected Pesticides

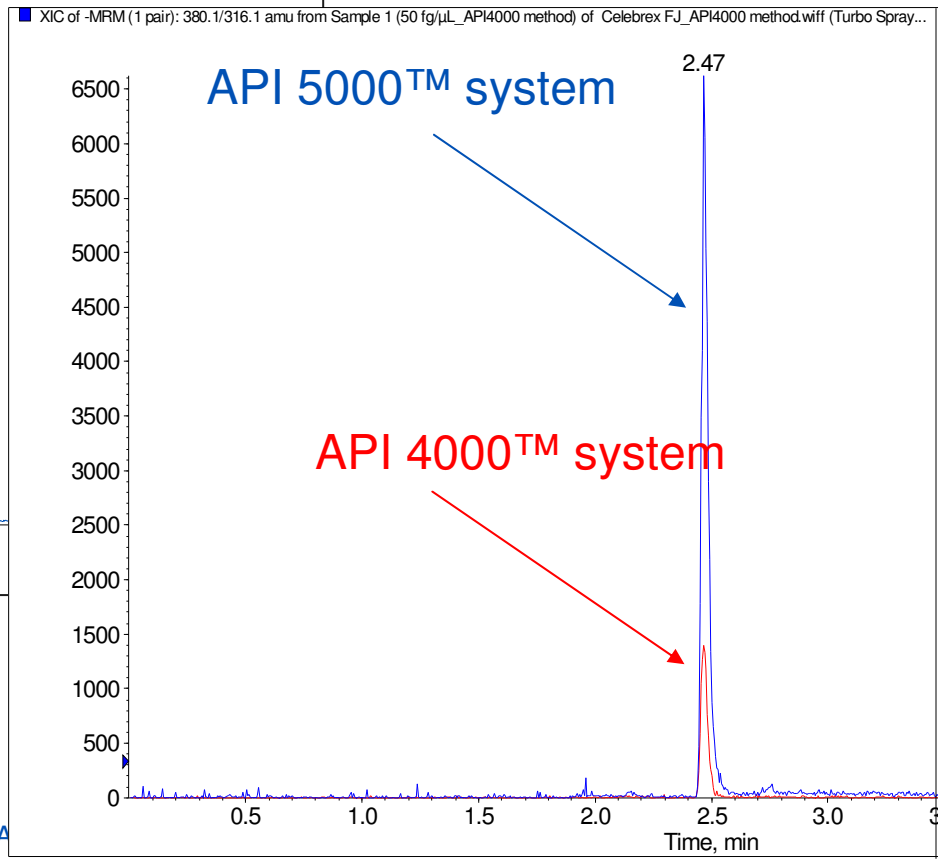


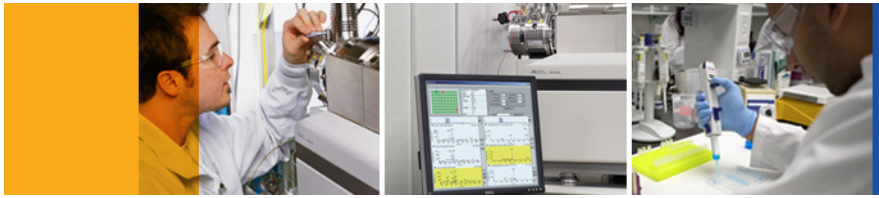
Sensitivity allows direct injection water samples and dilution of extracts



MRM Sensitivity of Different LC/MS/MS Systems







Pesticides in Drinking, Bottled and Pond Water

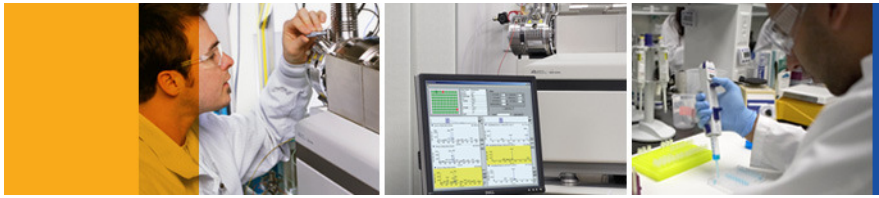
50 Pesticides detected using 2 MRM transitions

Area counts >3000

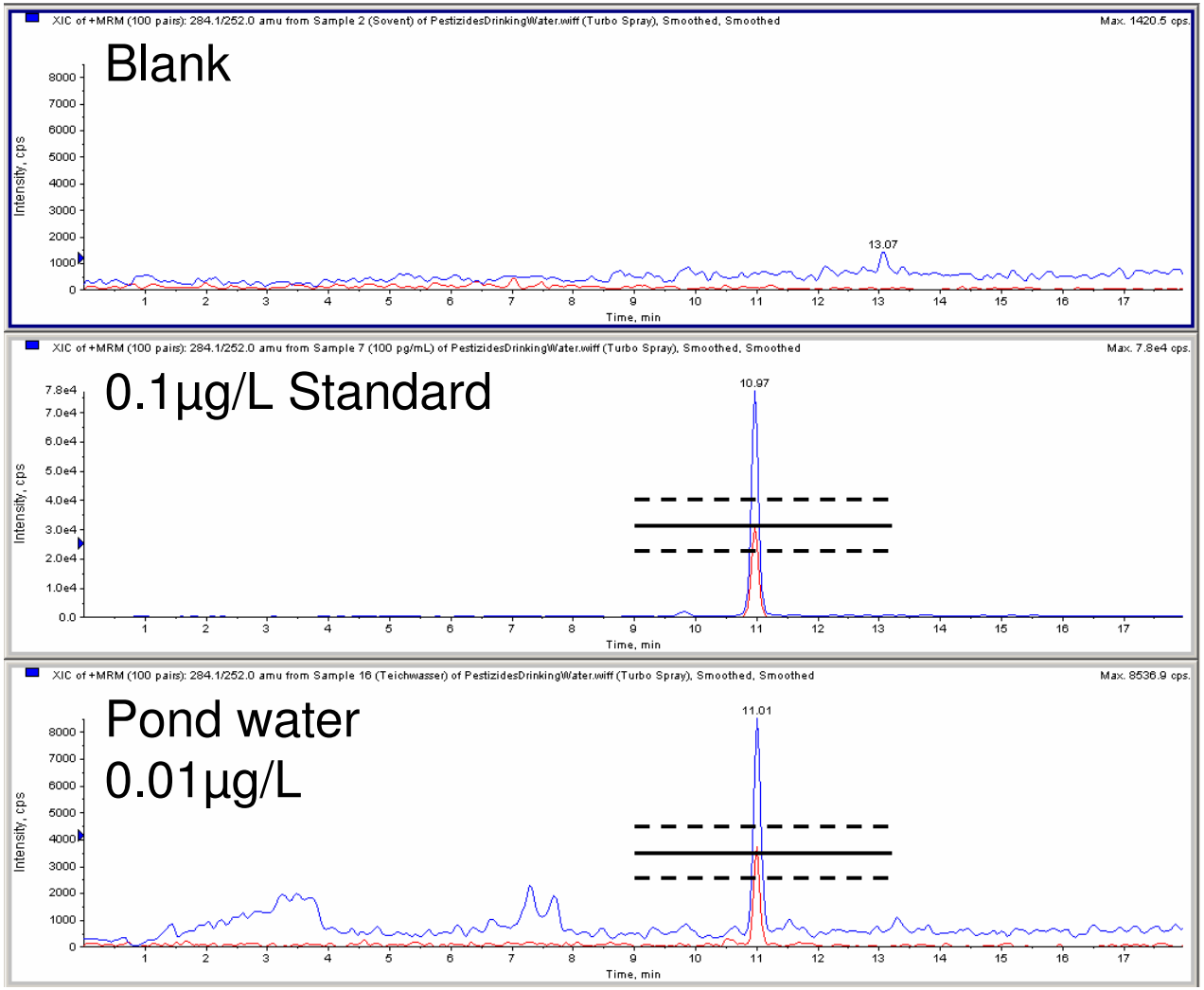
S/N >6

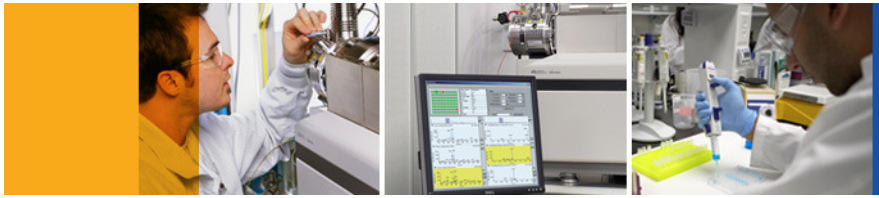
Full Layout
Query: SampleCheck, weg, SampleCheck, weg
Idle
Sort : Unsorted

	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	Metalaxyl / 220.0	2.15e+004	280.0/220.0 amu	0.008
11	Teichwasser	Unknown	Metalaxyl / 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

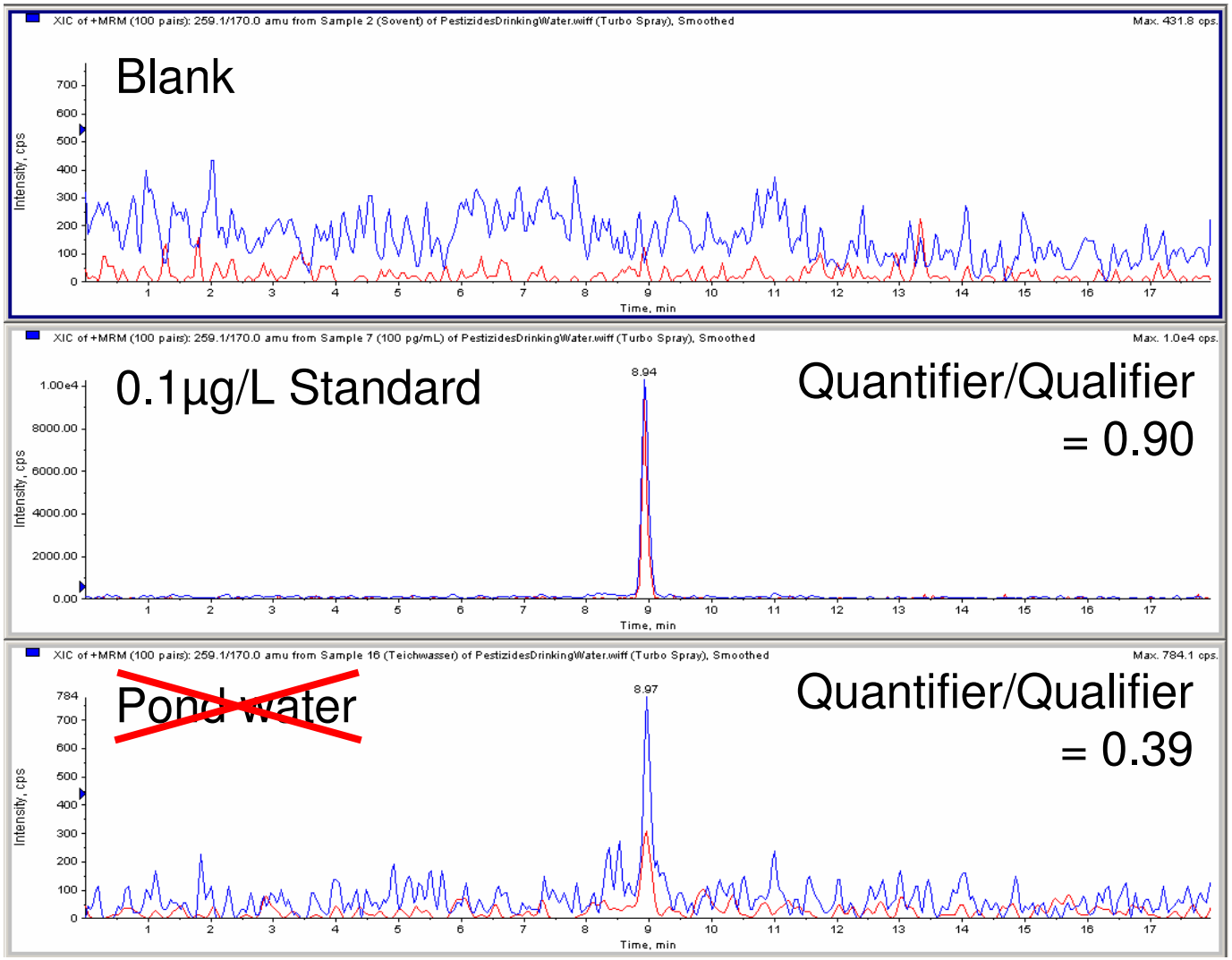


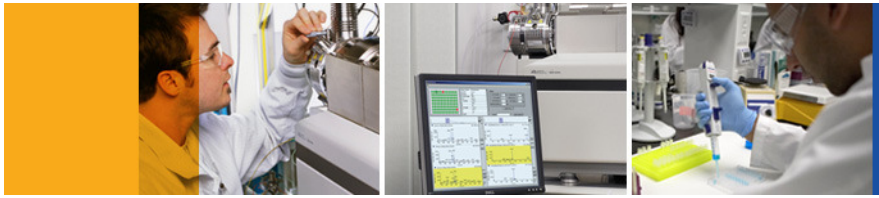
Positive: Metolachlor (API 5000™ system)





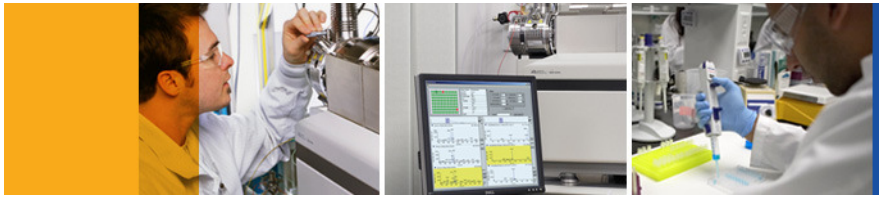
Negative: Metobromuron (API 5000™ system)





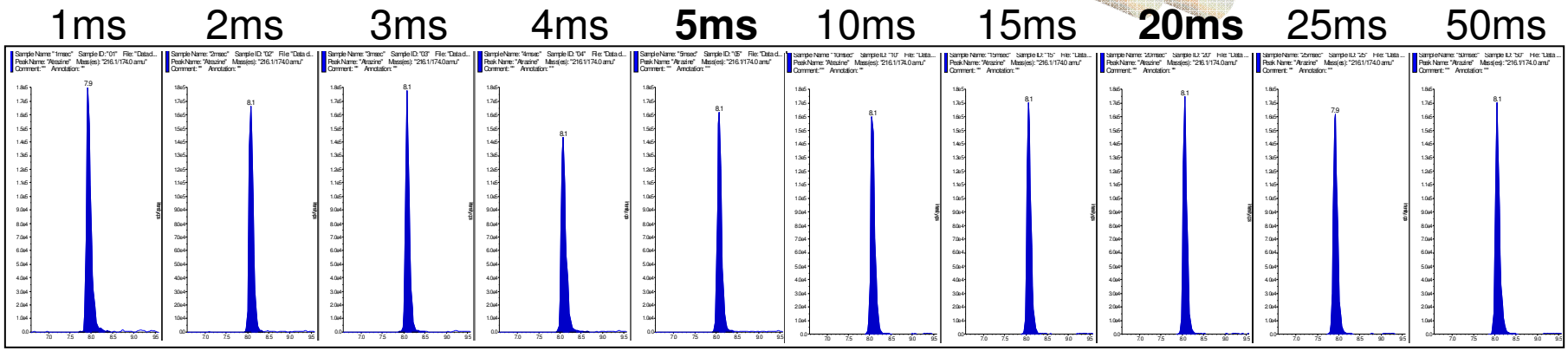
Enabling Technologies for Screening and Quantitation of Large Analytes Sets

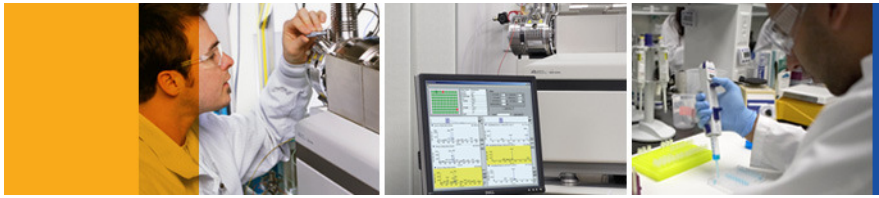
- LINAC® Collision Cell
- Scheduled MRM (sMRM)



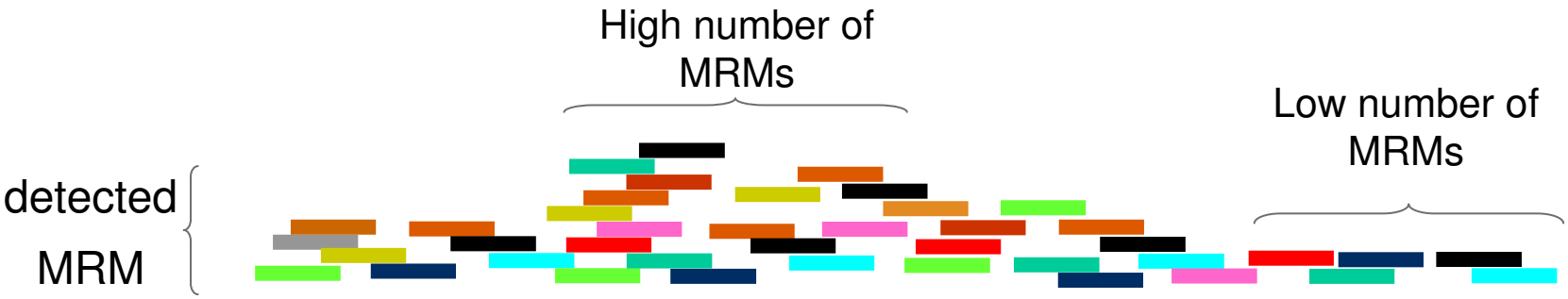
LINAC® Collision Cell – Fast MS/MS Experiments

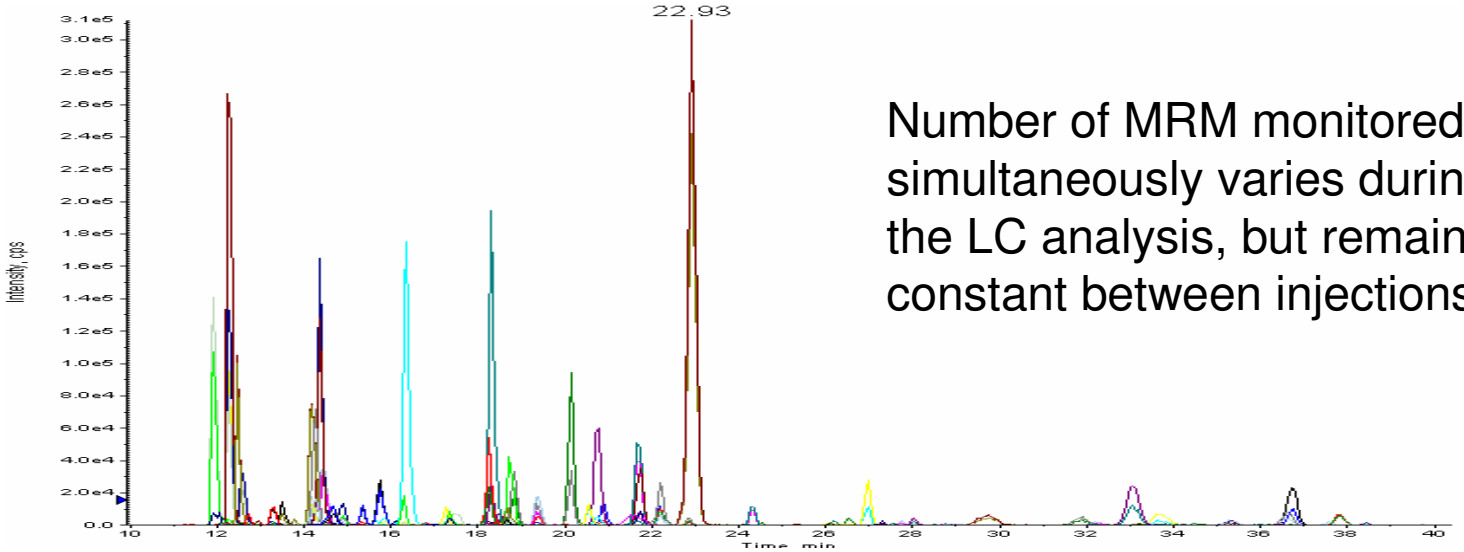
- **Lin**ear **Ac**celerator (LINAC®): Tilted quadrupole rods of the collision cell cause an electric field gradient which accelerates product ions after fragmentation.
- Faster MS/MS experiments without loss in sensitivity and without false positive results due to cross talk.
- Experiment: Atrazine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



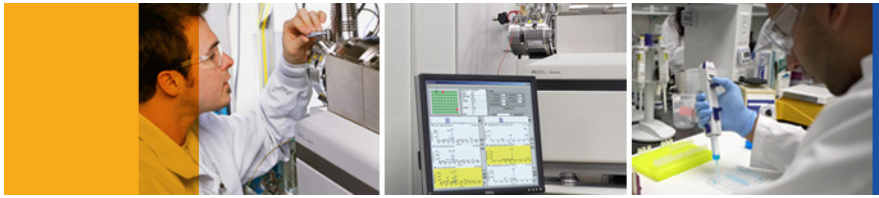


Scheduled MRM (sMRM)

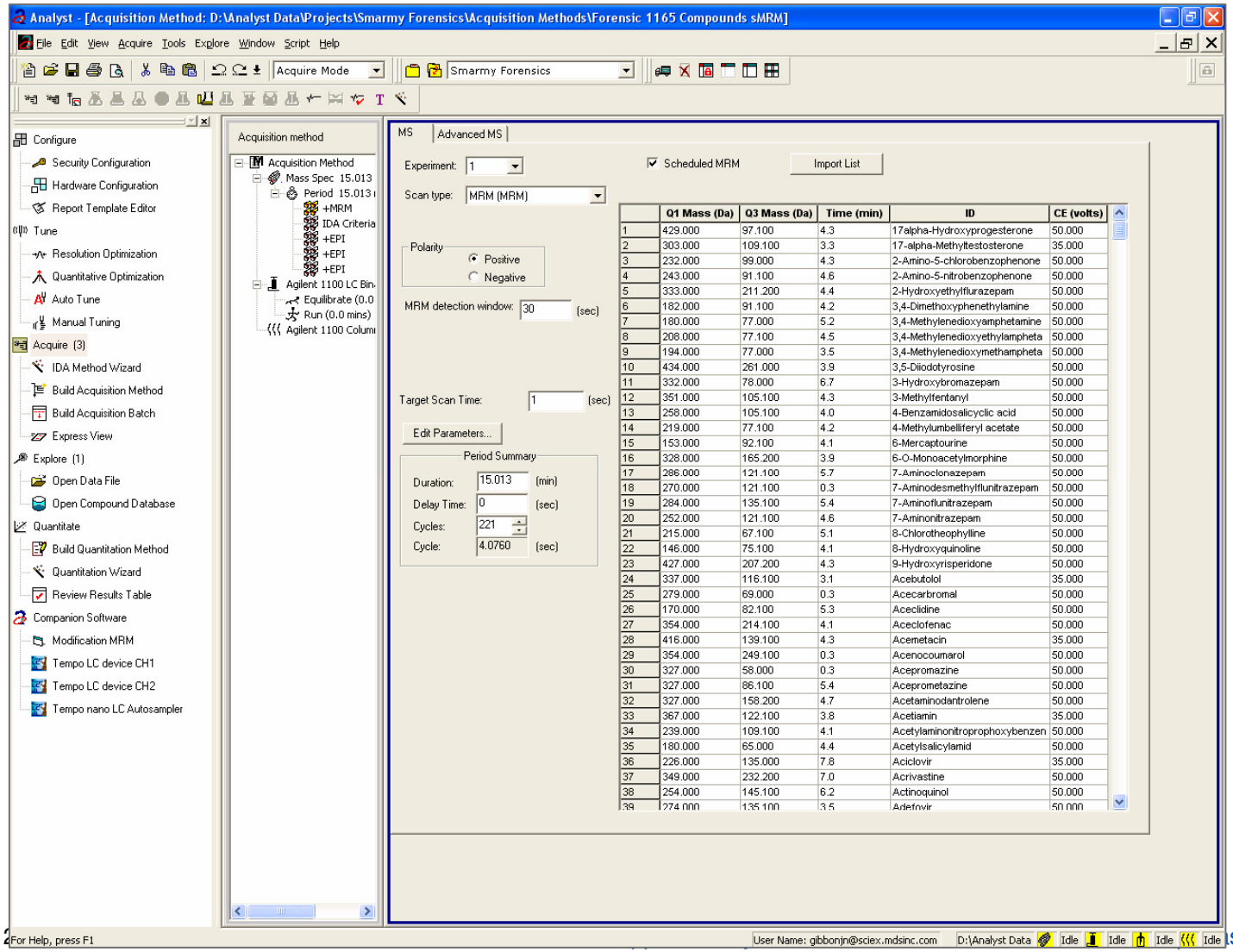


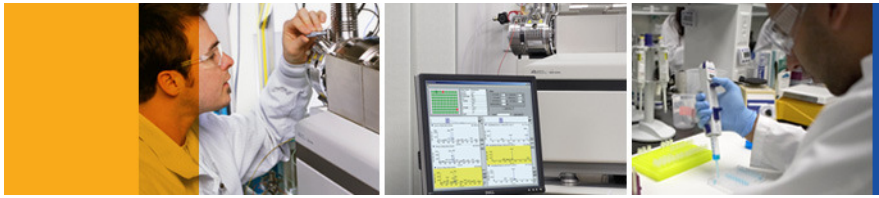


Number of MRM monitored simultaneously varies during the LC analysis, but remains constant between injections



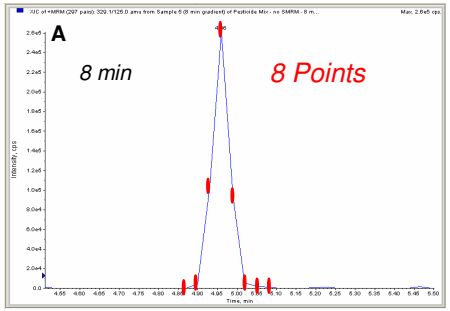
Acquisition Method with sMRM

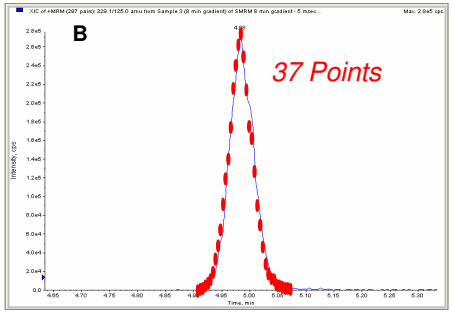


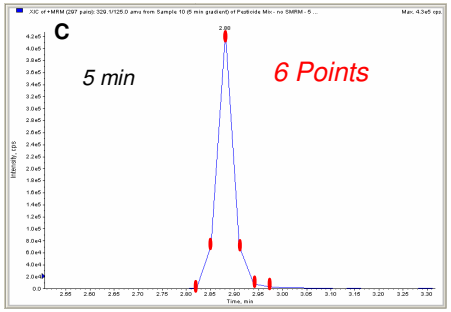


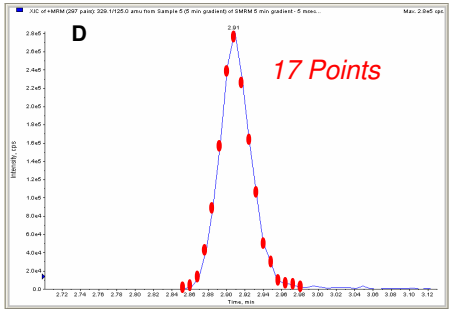
Advantages of sMRM

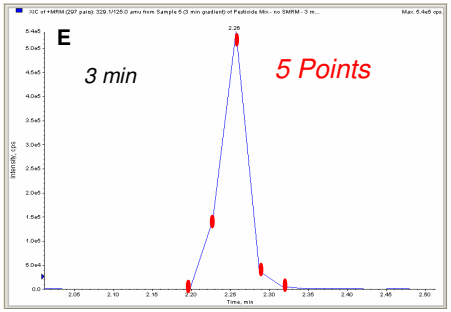
Fixed MRM sMRM

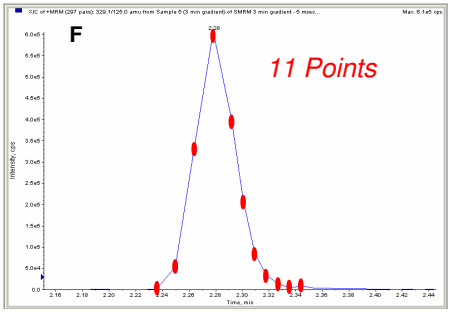




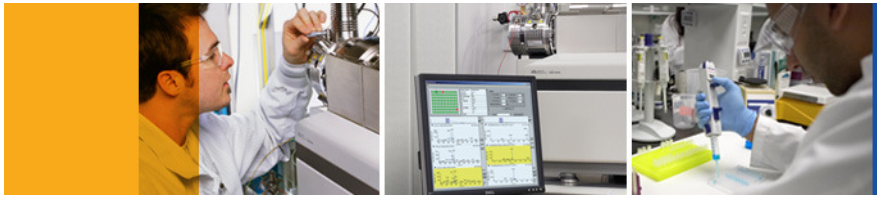








- More data points over HPLC peaks to improve data quality
- More MRM transitions per experiment to increase number of monitored compounds
- More time for confirmatory analysis
- High resolution HPLC with MS/MS detection



34 MRM Transitions in 4min with sMRM

XIC of +MRM (6 pairs): Period 4, 293.2/237.0 amu from Sample 8 (All Periods) of Periods RT Testing II.wif... Max. 4.1e5 cps.

Time	Flow (mL/min)	A/B (%)
0.0	2.0	95/5
0.3	2.0	95/5
2.0	2.0	33/67
3.0	2.4	5/95
3.3	2.7	5/95
4.2	2.0	95/5
5.0	2.0	95/5

(Agilent 1.8µm column)

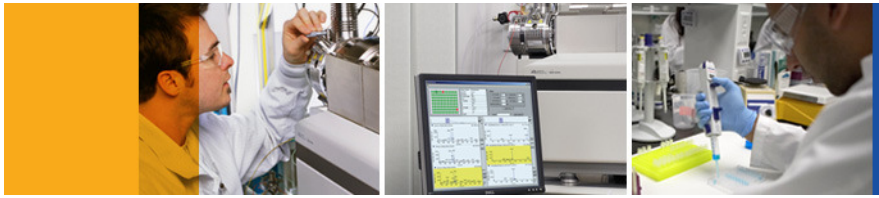
2.26

0.03min

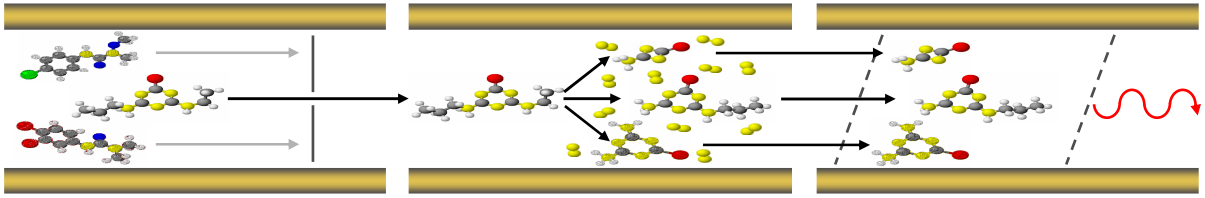
4.0e5 3.8e5 3.6e5 3.4e5 3.2e5 3.0e5 2.8e5 2.6e5 2.4e5 2.2e5 2.0e5 1.8e5 1.6e5 1.4e5 1.2e5 1.0e5 8.0e4 6.0e4 4.0e4 2.0e4 0.0

0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0

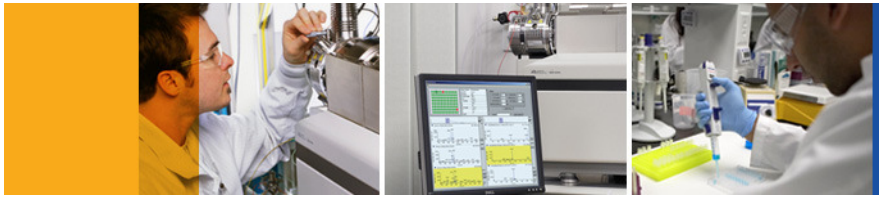
Time, min



Higher Degree of Confirmation using MS/MS Spectra



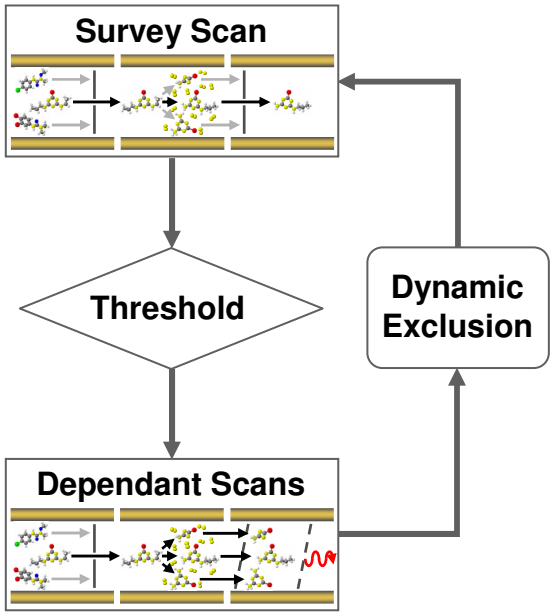
- Enhanced Product Ion (EPI) scan of Q TRAP® systems
  - → MS/MS Spectra contains information of all MRM transitions of the analyte.
  - → EPI has sensitivity level of MRM.
  - → EPI is less time consuming than acquisition of many confirmatory MRM transitions (100MRM with 5ms dwell + 5ms pause ~ 1s vs.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

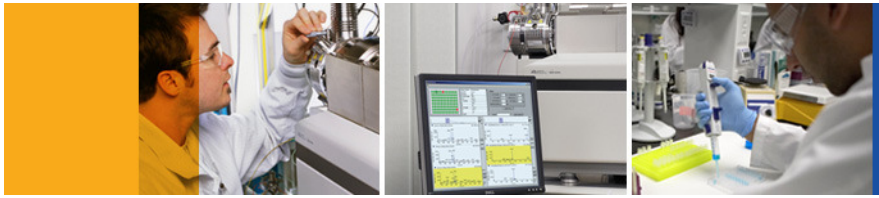


Information Dependent Acquisition (MRM to EPI)

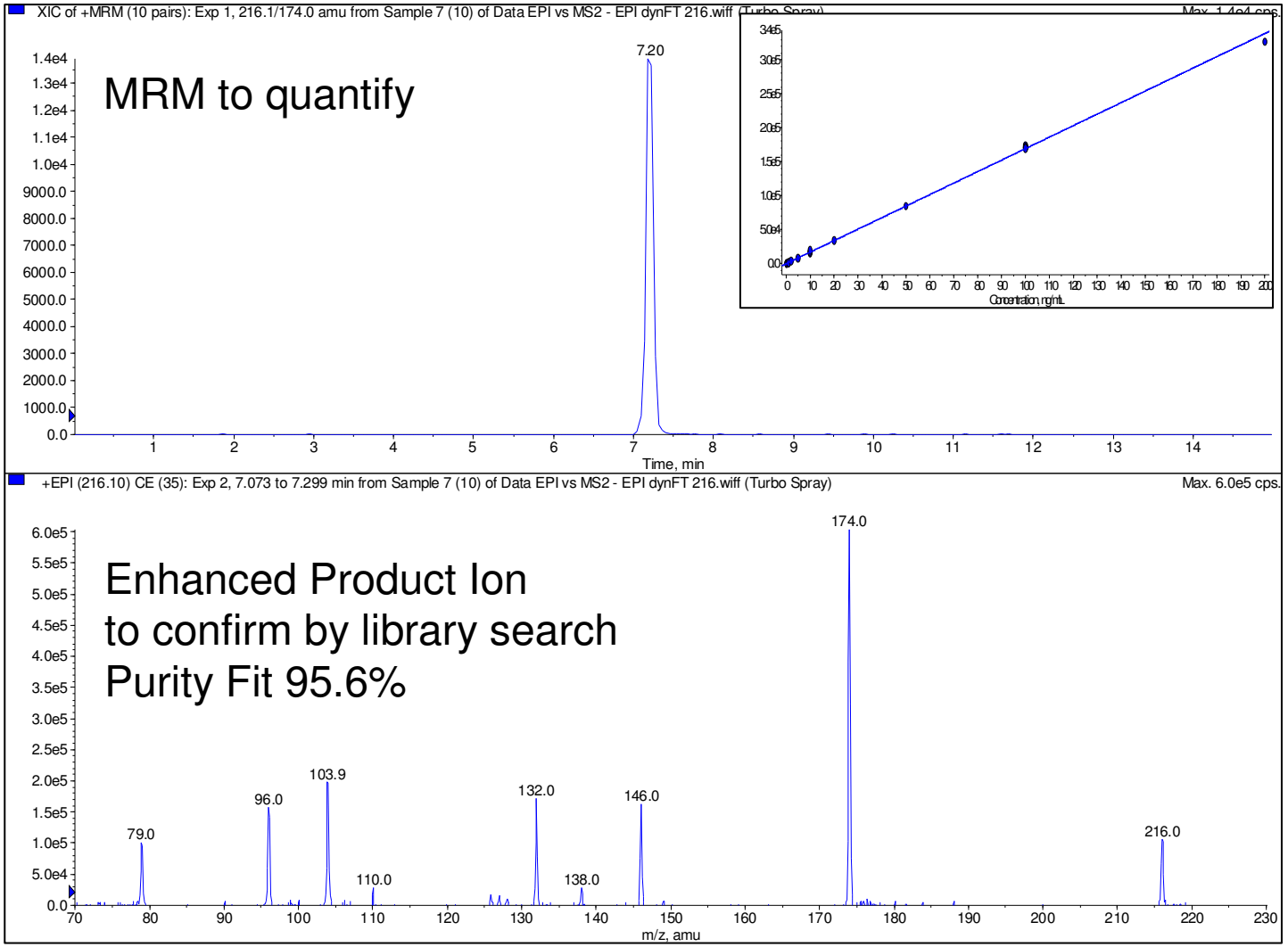
- Information Dependent Acquisition (IDA) of EPI spectra
  - MRM with high selectivity and sensitivity triggers automatically EPI scan

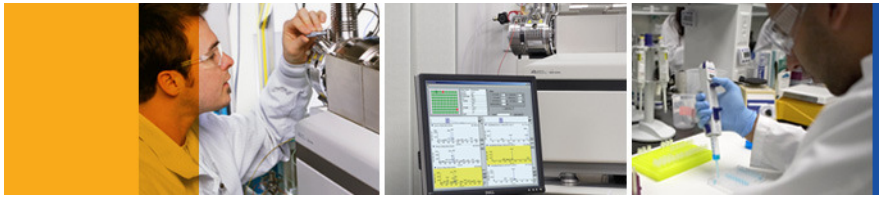
- Dynamic background subtraction to confirm co-eluting compounds



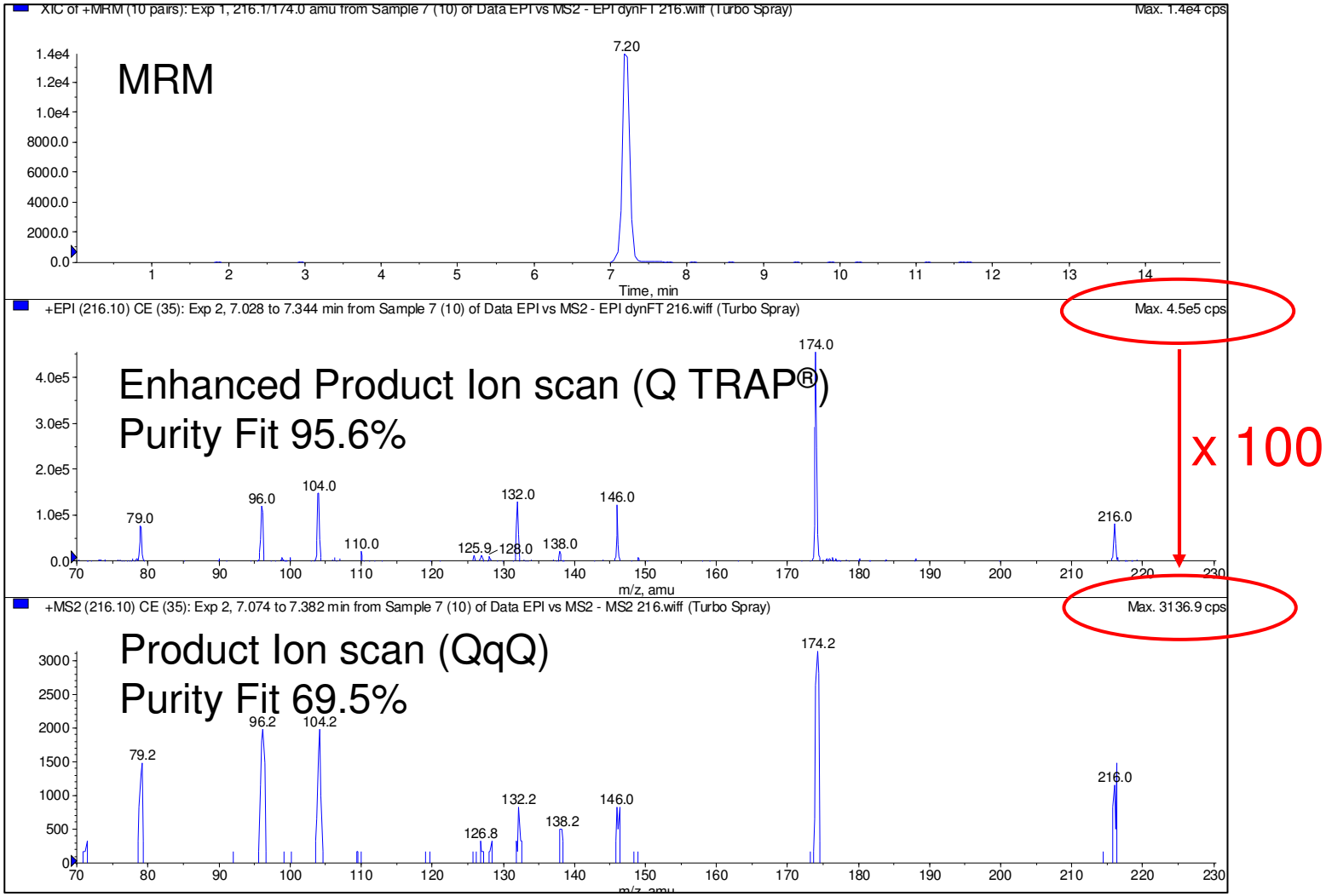


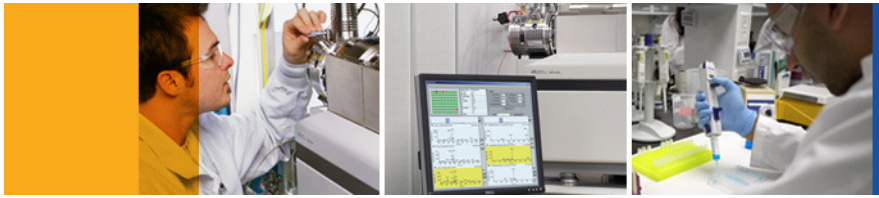
Quantitation and Confirmation



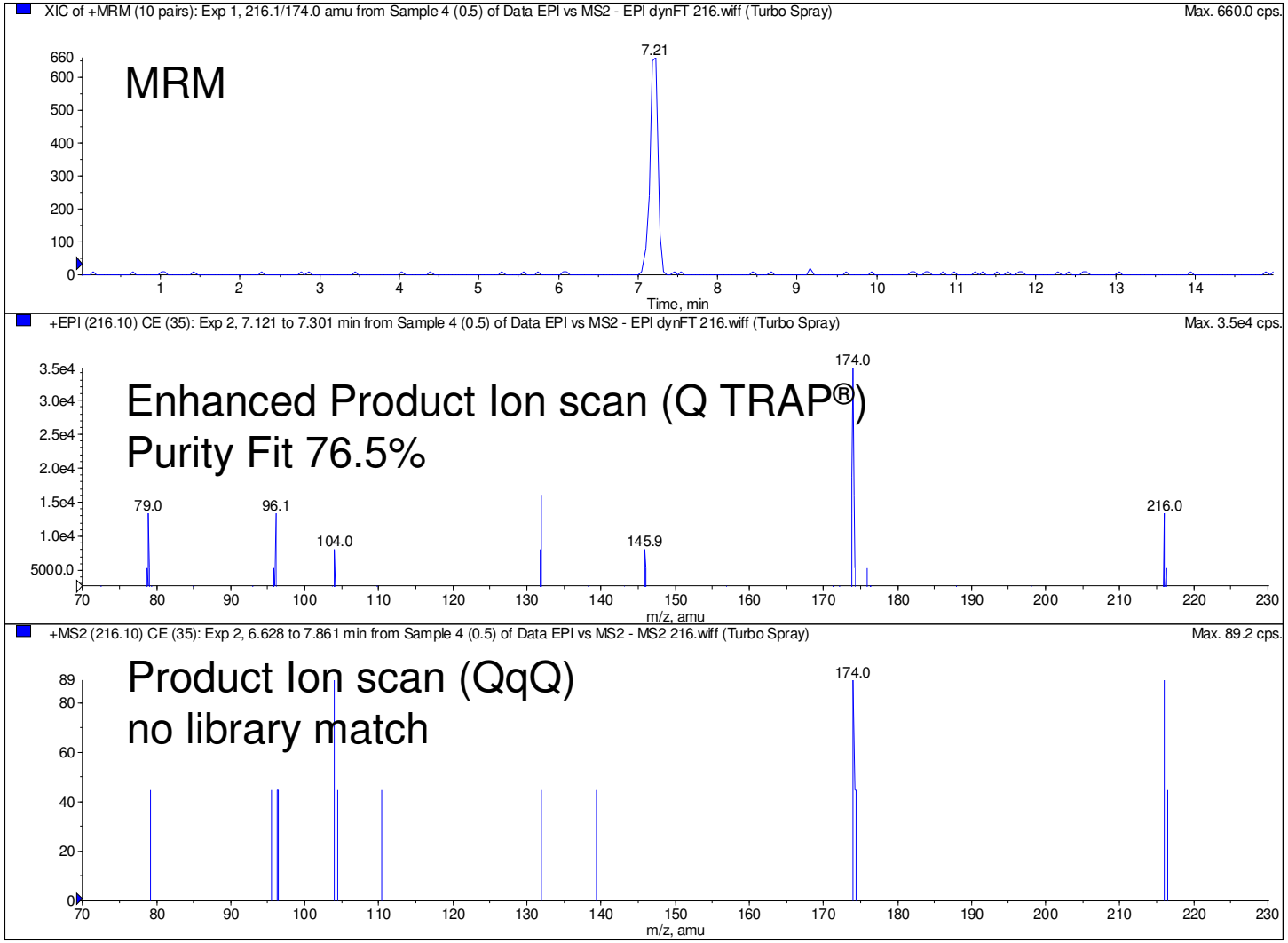


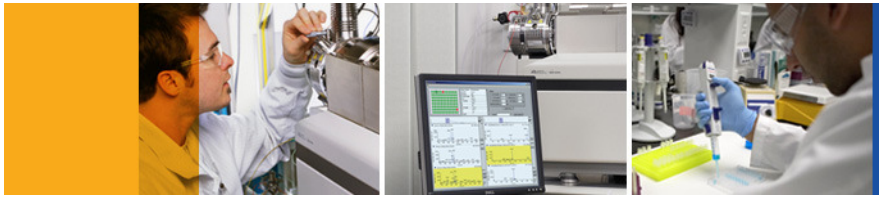
Confirmation by Q TRAP® or QqQ (10ng/mL Atrazine)



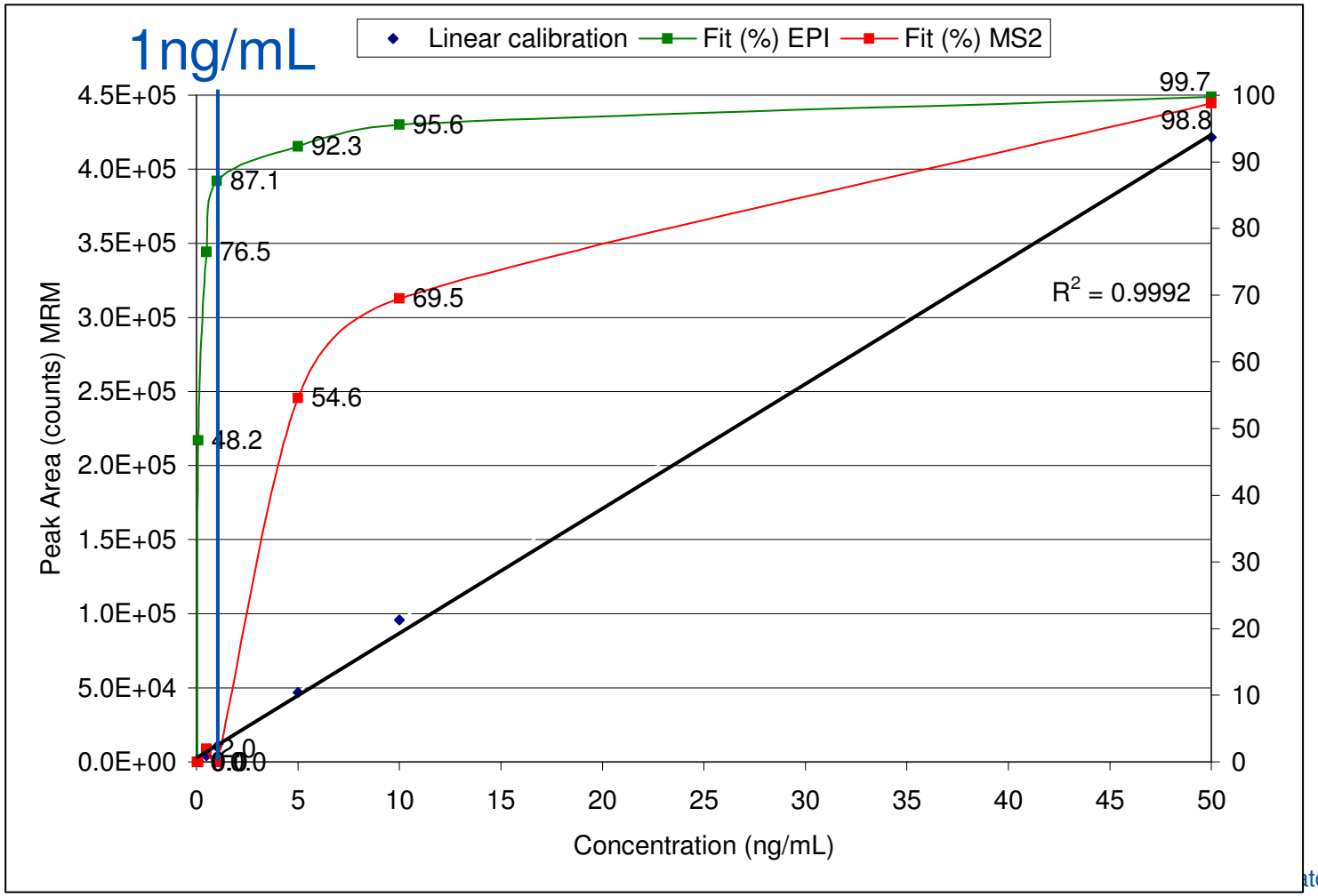


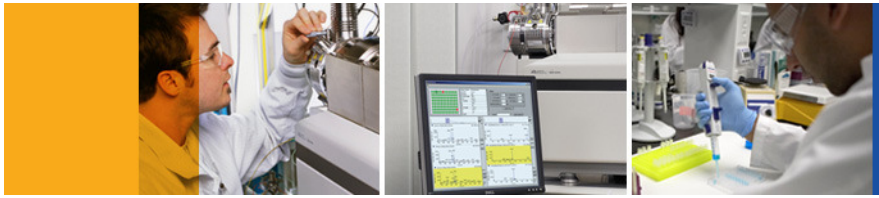
Confirmation by Q TRAP® or QqQ (0.5ng/mL Atrazine)



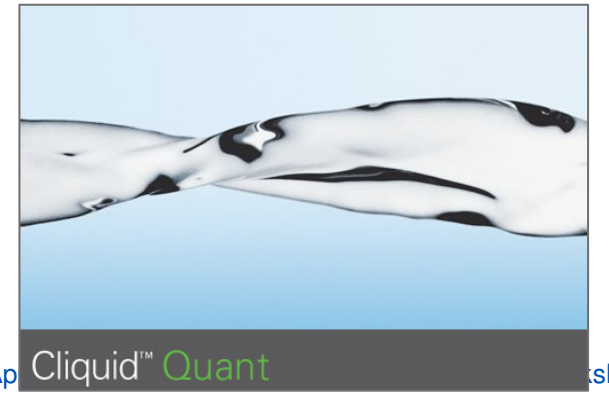


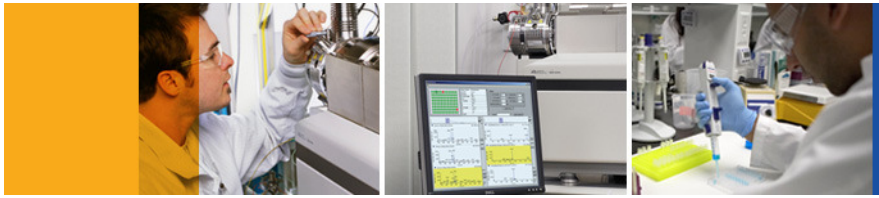
Library Search Results using QqQ or Q TRAP® in comparison to a calibration line in MRM





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





Cliquid™ Software – ‘Run Samples’ Wizard

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

Cliquid(TM)
Software for Routine Food Testing

Run Samples

Home | Help | Log Out

Step 1
Choose test

Step 2
Build
sample list

Step 3
Customize
report

Step 4
Submit
samples

Choose a test

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

Cancel | < Back

Instrument Panel

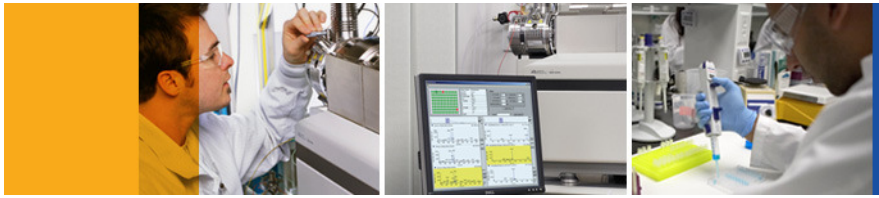
Stop | Live View
Standby | Event Log
Restart

Standby

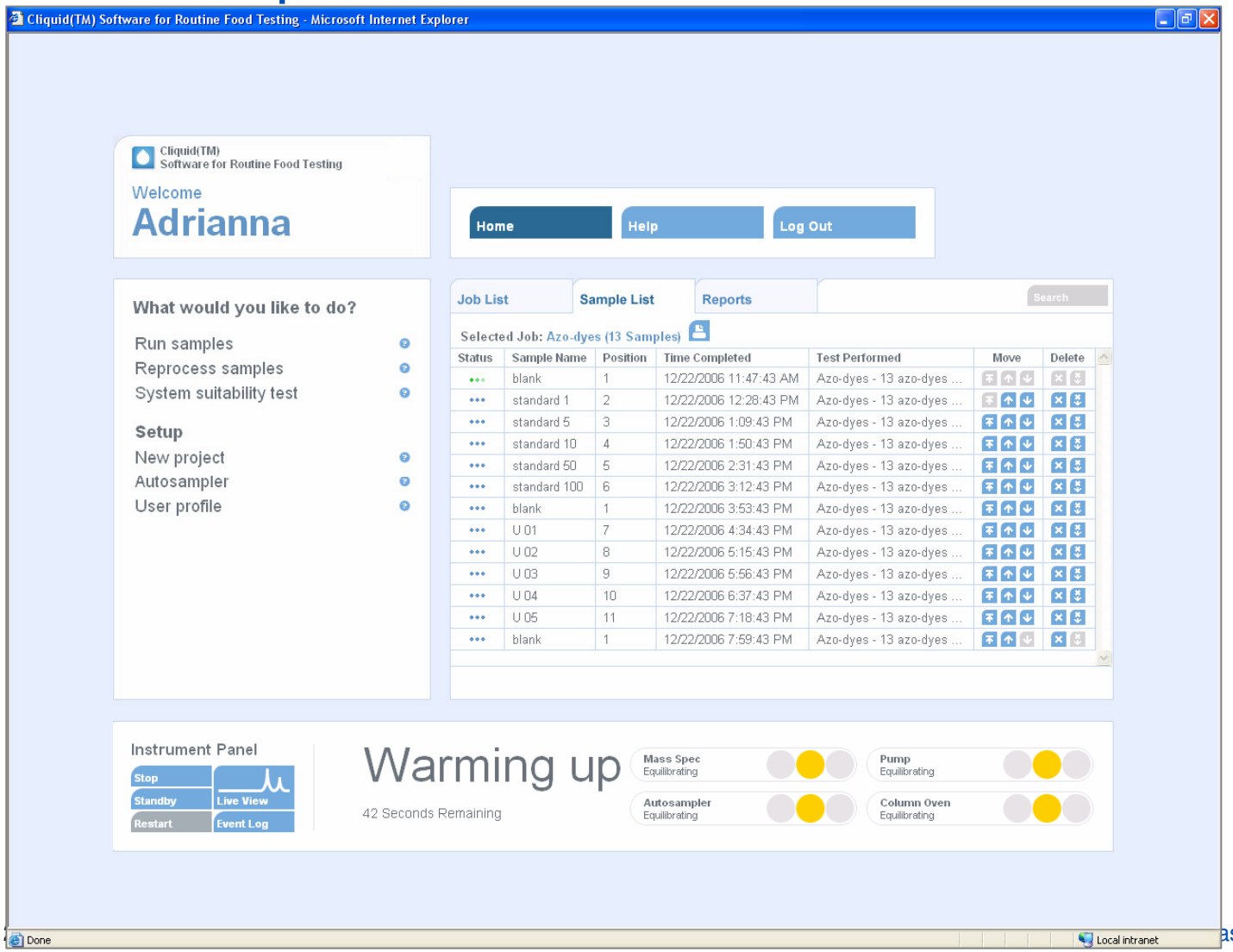
Mass Spec
Standby

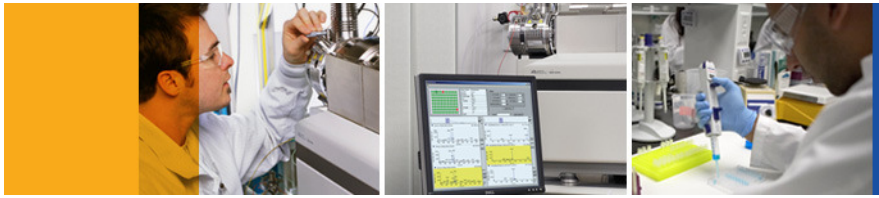
Autosampler
Standby

Done | Local intranet



Automatic Acquisition





Automatic Reporting

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

Cliquid™ Software for Food Testing

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

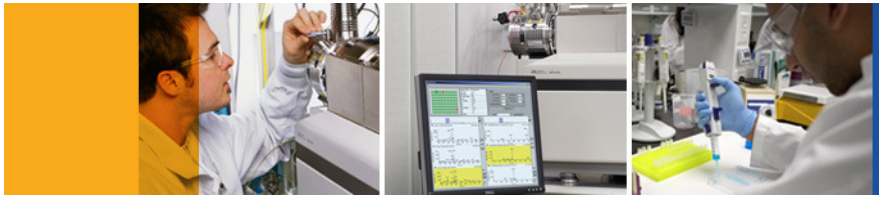
Stop | Standby | Restart | Live View | Event Log

Acquiring

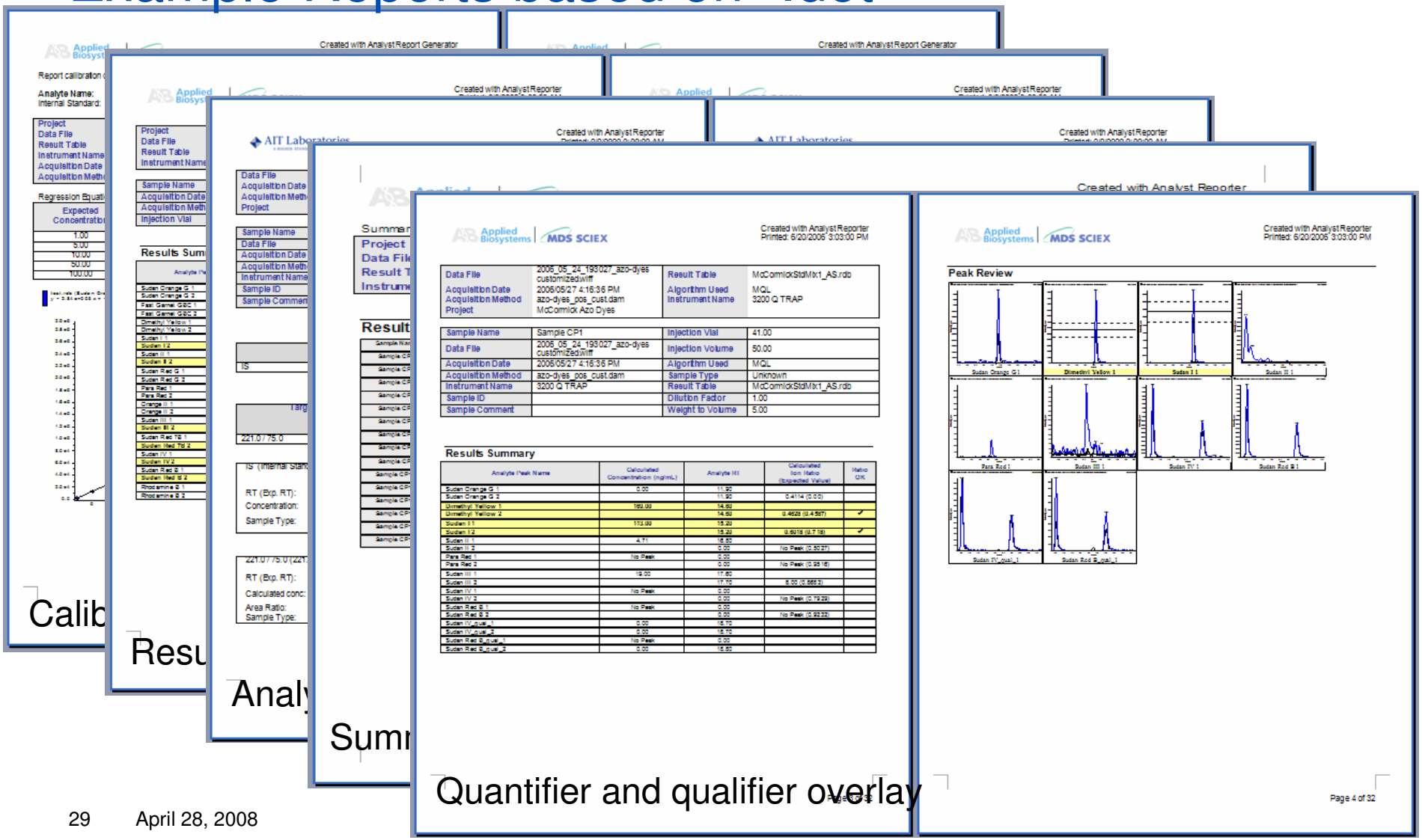
Current Sample: standard 1

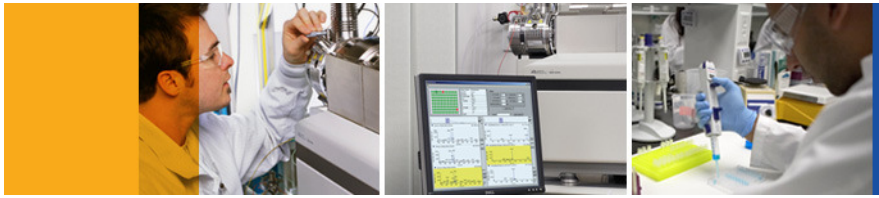
Mass Spec — Waiting
Pumps — Equilibrating
Autosampler — Waiting
Column Oven — Waiting

Done | Local intranet

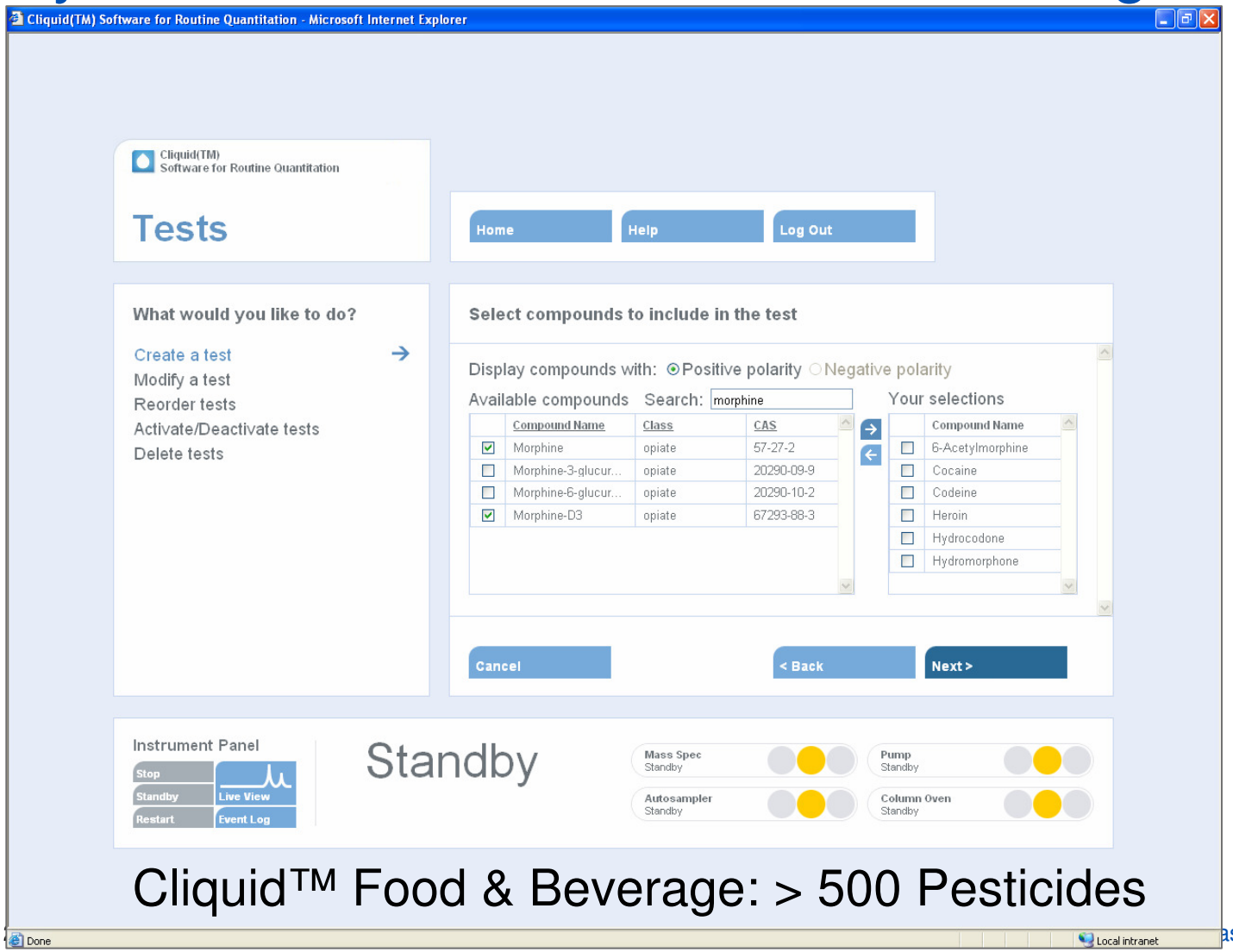


Example Reports based on *.dot

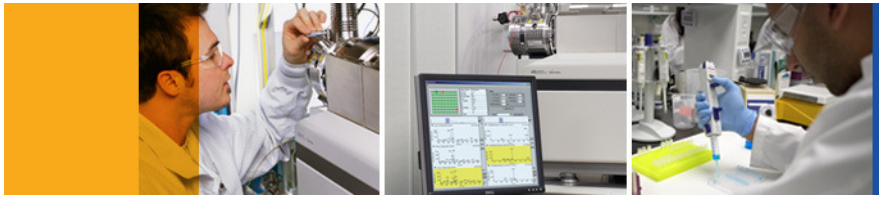




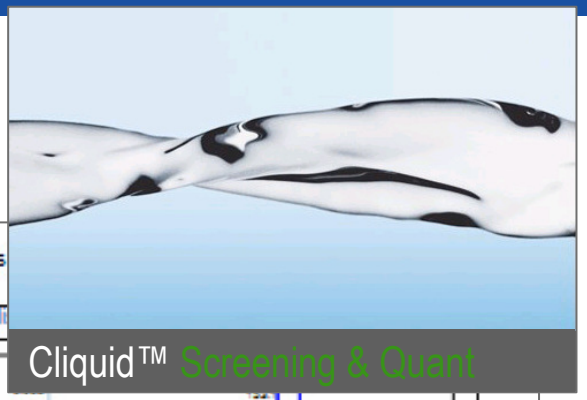
Quickly Build Methods with the MRM catalog



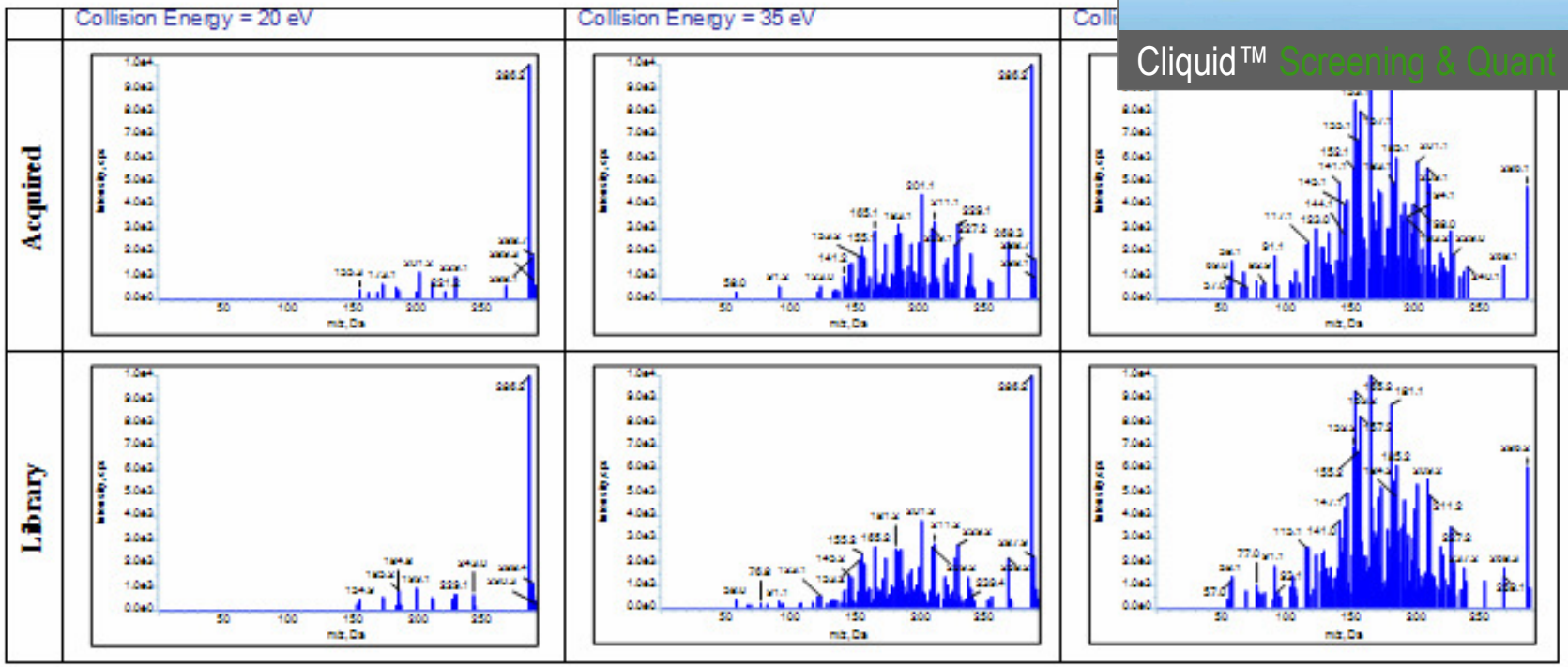
Cliquid™ Food & Beverage: > 500 Pesticides



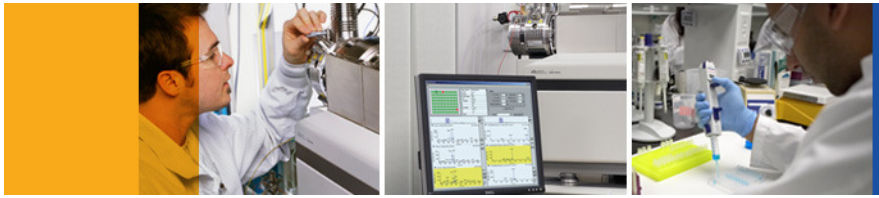
Automatic Library Searching



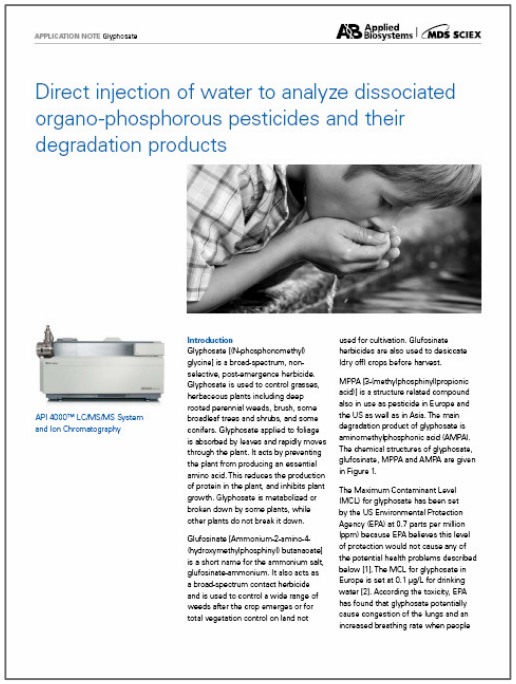
Peak 5 **Q1/Q3:** 286.1/201.1 amu **Retention Time:** 2.34 minutes **Area counts:** 127000 cps



	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

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

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

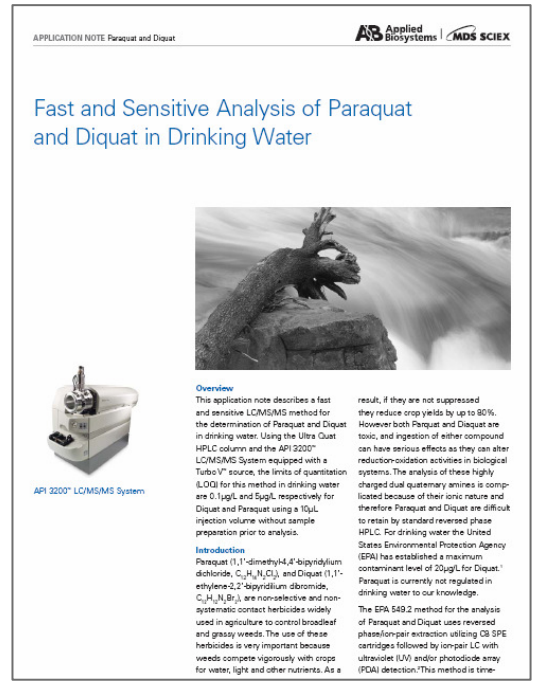
Introduction

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.

Glufosinate [Ammonium-2-amino-4-(hydroxymethylphosphinyl) butanoate] is a short name for the ammonium salt, glufosinate-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. Glufosinate herbicides are also used to desiccate (dry off) crops before harvest.

MPPA [3-(methylphosphinyl)propionic 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 the toxicity, EPA has found that glyphosate potentially cause congestion of the lungs and an increased breathing rate when people



APPLICATION NOTE Paraquat and Diquat

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 TurboV™ source, the limits of quantitation (LOQ) for this method in drinking water are 0.1µg/L and 5µg/L respectively for Diquat and Paraquat using a 10µL injection volume without sample preparation prior to analysis.

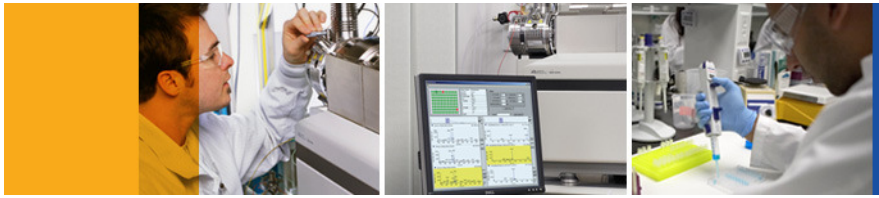
Introduction

Paraquat (1,1'-dimethyl-4,4'-bipyridylium dichloride, $C_{12}H_{14}N_2Cl_2$), and Diquat (1,1'-ethylene-2,2'-bipyridilium dibromide, $C_{12}H_{12}N_2Br_2$), are non-selective and non-systematic 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 Diaquat 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 20µg/L for Diquat.[1] 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.[1]This method is time-

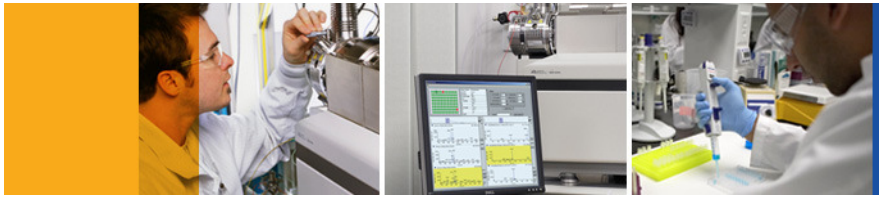
Direct injection of water samples to analyze:
Glyphosate, AMPA, Glyphosinate, MPPA
Dionex IonPac AG11 (50x2mm) column
Water, citric acid, triethylamine
API 4000™ LC/MS/MS system

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



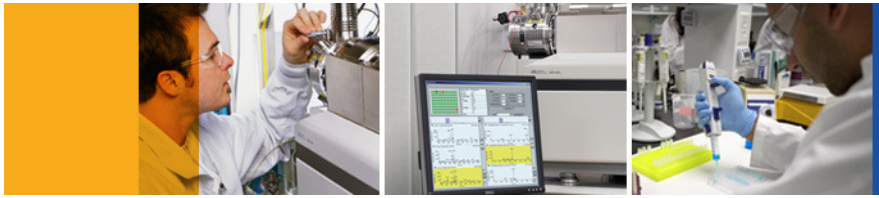
Conclusion

- 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)
- Cliquid™ 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



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Thanks for Listening!

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