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Effective screening and quantitation strategies for trace analysis in food and environmental samples

Dr. Peter Hancock

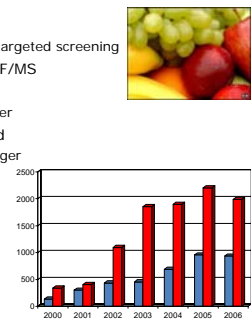
Florida Pesticide Residue Workshop 2007

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Overview

- Analytical challenges
 - Targeted screening versus untargeted screening
- Introduction to exact mass TOF/MS
- Targeted screening in food
 - TargetLynx application manager
- Non-targeted screening in food
 - ChromaLynx application manager



EU Alert and Information Notifications per Year

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EU Legislation

- Regulation 396/2005/EC amending 91/414/EC
 - Harmonised for all pesticide/product combinations
 - Range from "not detectable" to approximately 50mg/kg
 - "Not detectable" defaults to 0.01 mg/kg if no other level is assigned
- Recommendation 2006/26/EC of 18th January 2006
 - Coordinated monitoring programme to ensure compliance with maximum levels of pesticide residues in or on cereals and certain other products of plant origin for 2006-08.
 - Lists pesticides and products to be monitored
- http://ec.europa.eu/food/plant/protection/pesticides/index_en.htm

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Worldwide Legislation

- US
 - Food Quality Protection Act (FQPA) of 1996
 - <http://www.epa.gov/oppfod01/fqpa/backgrnd.htm>
 - 40 CFR 180
 - http://www.access.gpo.gov/nara/cfr/waisidx_04/40cfr180_04.html
 - USDA, Foreign Agricultural Service
 - <http://www.fas.usda.gov/hfp/mrl.htm>
- Japan
 - Positive List System for Agricultural Chemical Residues in Foods
 - Enforced from 29th May 2006
 - Uniform limit set to 0.01 mg/kg
 - http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/eng_h-page
 - <http://www.m5.ws001.squarestart.ne.jp/foundation/search.html>

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Analytical Challenges

- **Challenge #1:** Pesticide residue methods must cover a very wide range of analytes and matrices
- Numerous chemically and structurally diverse analytes exist
 - Approximately 1000 pesticides registered worldwide
- Wide variety of matrices (fruits, vegetables, herbs, spices) need to be monitored
- Over 17,000 EU community MRLs have been established for about 140 pesticides.

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Analytical Challenges

- **Challenge #2:** High demands are placed on analytical method performance to meet legislative requirements
- Methods must be...
 - **Sensitive**, low default reporting limit of 0.01mg/kg
 - **Selective**, reduce or eliminate matrix interferences
 - **Multiresidue**, multiple targets in a single run
 - **Rugged**, complex samples with reduced or no sample clean-up
 - But also **generic** (i.e. having broad scope)
 - 'Food scares' and changes in legislation require updating of methods and re-analysis of samples

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Analytical Challenges Waters
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- **Challenge #3: Delivering high performance, cost effective methods in the laboratory**
- Effective methods need to be targeted on multiple residues
- Generic extraction methods such as QuEChERS produce complex extracts with entrained matrix components
- Separation/detection strategies must be selective and sensitive (accepted standard is tandem quadrupole MS/MS)
- This is a targeted analysis – what about non-target species?
 - e.g. In (2004) UK lettuce contained residues of oxadixyl, chlorothalonil and vinclozolin, all fungicides not licensed for use
- Extending sensitive screening to really large numbers of compounds is difficult with tandem quad MS/MS as duty cycle of instruments is compromised

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Analytical Challenges
New approaches with ToF/MS Waters
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- Time-of-Flight MS (with both GC and LC inlets) is currently creating much interest for pesticide residue analysis.
- ToF/MS can support development of more generic screening methodologies
 - Can detect both target and non-target compounds using ion ratios and/or spectral matching to increase confidence
 - Very large numbers of residues may be determined in a single analysis
 - Presumptive positives can be submitted for confirmatory analysis by MS/MS if desired
 - Automated software processing
 - Ability to re-process historical data

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System Solution Waters
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GCT Premier



LCT Premier XE



ChromaLynx
Peak detection, deconvolution and library searching



TargetLynx
Advanced quantitation and automated QC

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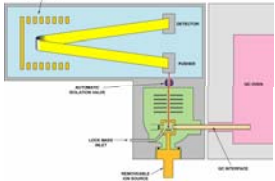
Application Example 1

Use of GC-ToF/MS for screening of pesticides residue in fruits, vegetables and baby foods

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GCT Premier - Features
Orthogonal Acceleration TOF/MS Waters
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- Plug in EI/CI ion volumes for rapid change over
- Resolution >7000 (Full width half maximum)
- Exact mass less than 5ppm
- 4 orders linear dynamic range
- Spectral acquisition rate of 20 spectra per second



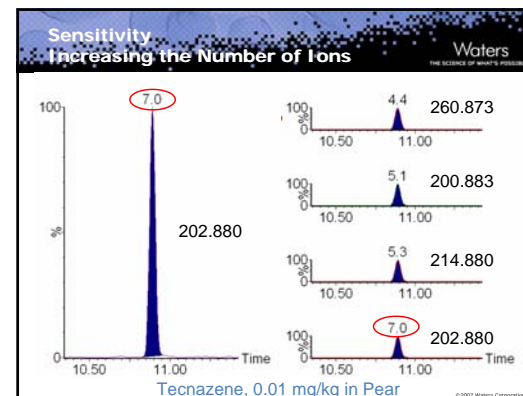
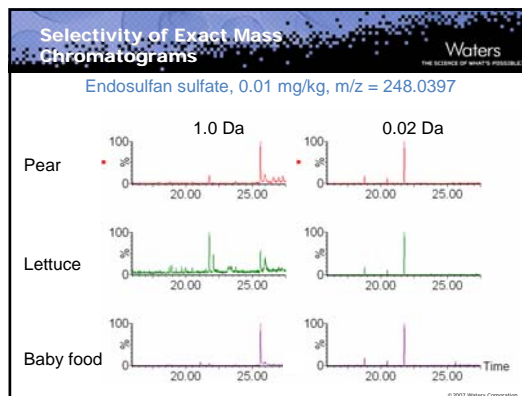
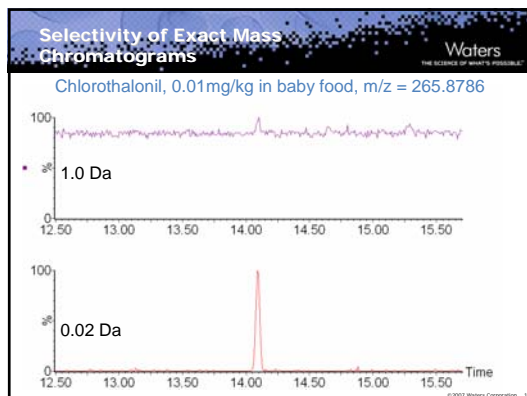
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GCT Premier - Benefits
Orthogonal Acceleration TOF/MS Waters
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- **Rapid full spectral acquisition rates**
 - Compatible with narrow GC chromatographic peaks
 - Produces Non-skewed spectra
 - Enables accurate chromatographic peak profiling and deconvolution
- **Non scanning instrument with a high duty cycle**
 - High full scan sensitivity
- **Produces EI library searchable spectra**
 - Allows compound identification using standard or user libraries
- **Spectra recorded with accurate mass**
 - Enables confirmation or elucidation of structure
- **Elevated Resolution**
 - Enhanced selectivity

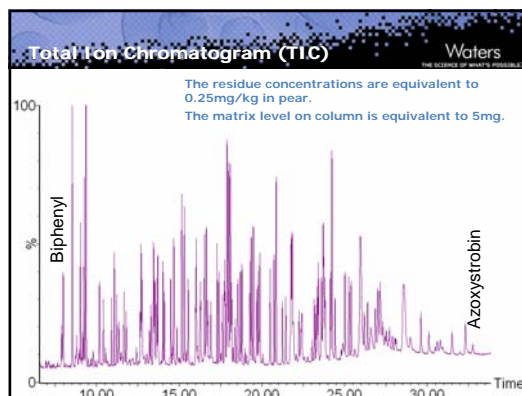


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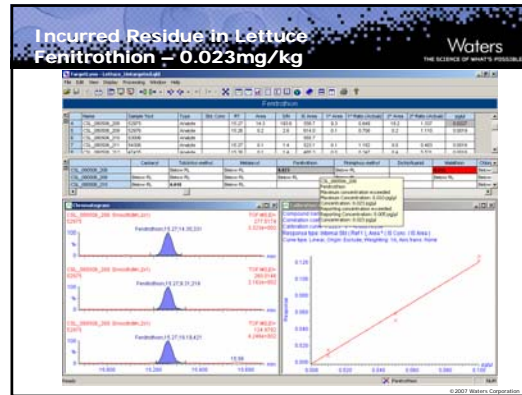
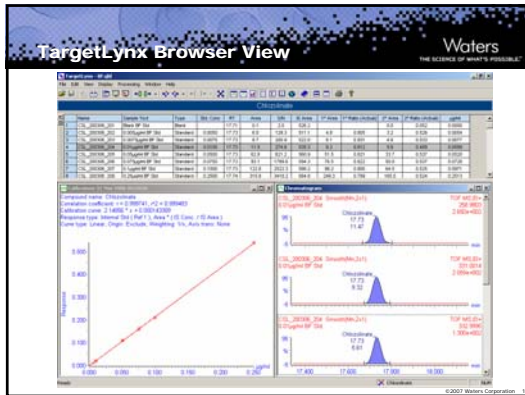
Experimental background Fruit based baby foods

- Over 100 residues were targeted using GC/ToF/MS
- Sample preparation was acidified QuEChERS, 1g/mL
- Recoveries at 0.01mg/kg
 - 81% of residues showed recoveries 70-110%
 - All RSDs for recovery were < 30%
 - Meeting requirements of 2006 EU AQC Guidance document
 - Some low recoveries were attributed to losses in dispersive SPE clean-up, 150mg MgSO₄ + 50mg PSA + 50mg carbon
 - e.g. quintozene, pyrimethanil, chlorothalonil
 - Generic, multi-residue methods often require acceptance of compromise
- GC analysis on a DB-5ms column using a cryo cooled PTV injection in solvent vent mode
 - Quantitation uses matrix matched standards



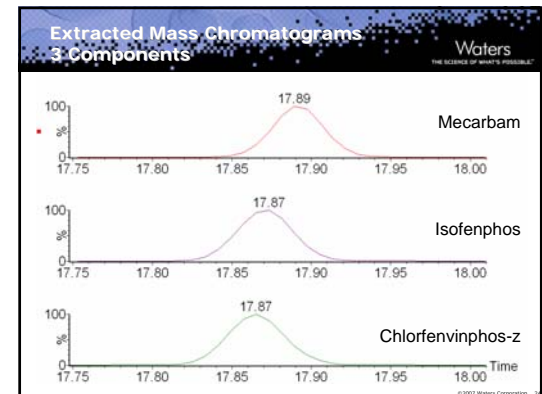
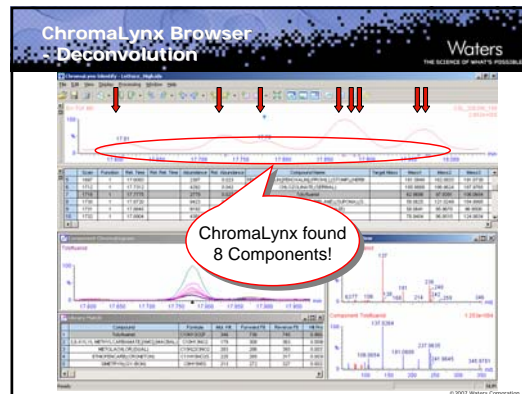
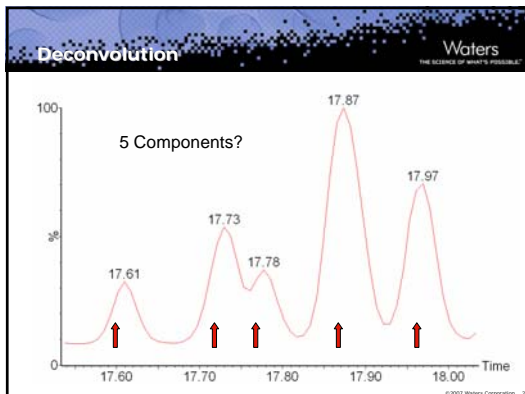
Targeted Screening GC-TOF/MS and TargetLynx

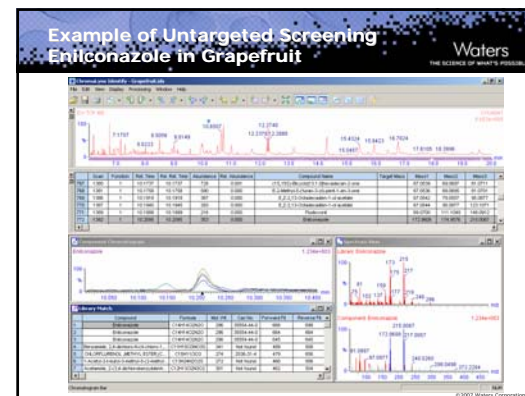
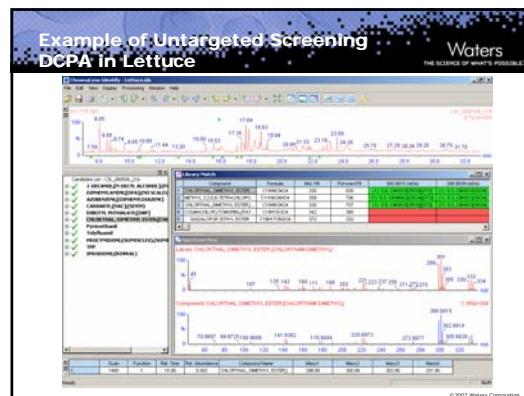
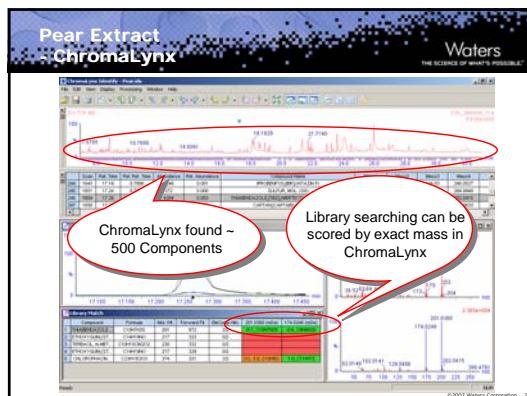
- Compound list is known
- Detection is based on extracting exact mass chromatograms with 0.02 or 0.05 Da windows
 - Any ion can be chosen for detection and/or quantification
 - Number of residues/ions can be increased without loss in sensitivity
 - Post-acquisition addition of target compounds is possible
- Screening is based on one or more exact mass chromatograms
 - If a peak is found within a defined time window the target compound is detected
 - Ion ratios can be used to increase confidence in detection
 - Quantitation against standards allows presumptive positives to be identified
- TargetLynx application manager
 - Highlights samples with concentrations above the reporting level
 - Highlights samples that do not pass quality control criteria



Non-Targeted Screening GC-TOF/MS and ChromaLynx

- Compound list is unknown
- ChromaLynx application manager
 - Automatic peak detection and deconvolution
 - Automatic production of "clean" component spectra
 - Standard EI spectra are generated by the GCT source
 - Automatic identification via library searching
 - Commercial or user generated libraries may be used
 - Exact mass scoring provides additional confirmation of identity
 - Exact mass spectra can facilitate structural elucidation if component is not in the library





Application Example 2

Use of UPLC-ToF/MS for screening of pesticides residue in fruits and vegetable based baby foods

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LCT Premier XE - Features Orthogonal Acceleration TOF/MS

- ESI and ESI (APCI and APPI options)
- Resolution >6,000 (FWHM) in V mode, >12,000 in W mode
- Exact mass less than 3ppm
- 4 orders linear dynamic range
- Spectral acquisition rate of 20 spectra per second

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LCT Premier XE - Benefits Orthogonal Acceleration TOF/MS

- General benefits of LCT ...
 - Rapid full spectrum acquisition, elevated resolution, mass accuracy, full scan sensitivity
- Major difference to GC/MS in the ionisation process
 - Produces ES spectra with few (if any) fragment ion
 - Benefit: Can often characterise molecular ion from ES spectra
 - Challenge: Lack fragments that could aid structural elucidation or spectral library matching
 - Users have to build their own libraries
 - Reproducibility, accuracy and validation considerations
 - Fragmentation can be induced by in-source CID
- Ideally suited to use with Waters Acquity UPLC systems

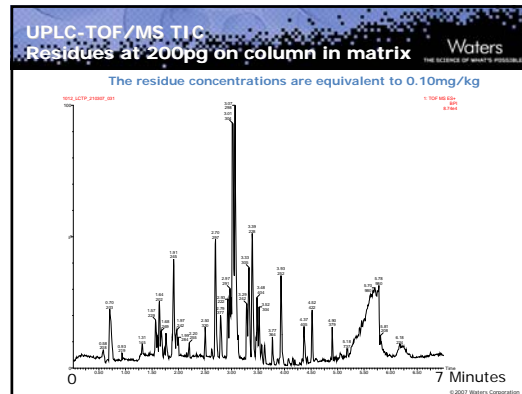
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Experimental background Fruit based baby foods

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- 45 residues were targeted using UPLC-LCT-PXE
- Sample preparation was using a QuEChERS based approach
- UPLC analysis on a 2.1 x 100 mm BEH C18, 1.7 μ m column, with a water/acetonitrile gradient over 7 minutes
- LCT-PXE was run in ES+ mode and experiments were run with both V and W mode
 - A targeted approach was adopted similar to that described for the GCT using extracted exact mass chromatograms for detection of residues.
 - A non-target approach can be applied using ChromaLynx
 - Must make use of user generated spectra
 - Can use isotope pattern fitting ("i-Fit") to increase confidence in the identity of detected components.

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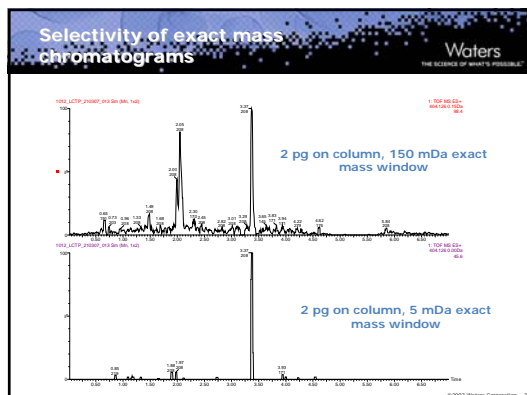


Targeted Approach TargetLynx

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- Components added manually specifying exact mass and expected retention time
 - Mass tolerance window 15 mDa
 - Retention time window 0.05 min
- Compounds were spiked at 0.25 mg/kg equivalent
- LoD and mass accuracy were estimated for both V and W mode
 - Sensitivity in V mode: ca. 3 times better than in W
 - Mass accuracy
 - Poorer in V mode and lower precision
 - 11 compounds present isobaric interferences with the matrix in V mode and not in W mode.
- All compounds were successfully detected:
 - No false positives
 - Typical LoDs were <0.01 mg/kg

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Non-Target Approach ChromaLynx and spectra

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- No commercial libraries exist so we have a choice
 - Generate *User library* (Have done this for veterinary drugs)
 - Generate *Theoretical library* from the isotope library generator
 - At present the library search is nominal mass based
- The approach is then similar to that described for the GCT
 - ChromaLynx is set to ...
 - Peak detect and deconvolute
 - Produce clean component spectra
 - Identify components by searching against the library
 - User library or theoretical library

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"Theoretical Libraries" and Isotope Ratios

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Theoretical isotope spectra

13C = 15%

Scanred Component

Lowest i-Fit score is correct elemental composition

Elecomp results ranked by i-FIT. Lowest value = best agreement to theoretical isotope ratio

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Summary

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**Summary
ToF/MS Screening**

- ToF/MS is an attractive method for multi-residue screening
 - "All of the data, all of the time"
 - Compounds can be targeted post-acquisition allowing historical review of data
 - Systems are less matrix tolerant than tandem quadrupole
 - May need to consider limited sample clean-up
- Targeted screening (TargetLynx approach)
 - Residues can be screened to low concentrations
 - e.g. <0.01 mg/kg in fruit, vegetables and baby foods with GCT
 - No false negatives were generated
 - Measuring multiple ions and ion ratios assists identification and limits false positives

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**Summary
ToF/MS Screening**



- Untargeted screening (ChromaLynx approach)
 - Can successfully detect and identify non-targeted residues
 - eg. DCPA in lettuce
 - Can use exact mass to confirm spectral matches and elucidate structures
 - Isotope pattern library generation and "i-Fit" scoring facilitate use with LCT Premier XE data
 - Cannot achieve same ultimate sensitivity as targeted method
 - Due to efficiency of deconvolution and spectral matching for very low level components in presence of major matrix peaks

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