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GCT-MS applied to the food and beverage analysis

Universidad de Murcia, 17 Junio 2008

Overview

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- Exact mass TOF
  - Selectivity / Mass accuracy
  - Dynamic Range Enhancement
- Screening in food: Analytical challenges
  - Targeted screening versus untargeted screening
  - Quantification of 'known' compounds
  - Deconvolution, identification
  - Detection of a non-targeted residue
- Flavour profiling: Beer analysis

GCT Premier - Features
Orthogonal Acceleration TOF/MS

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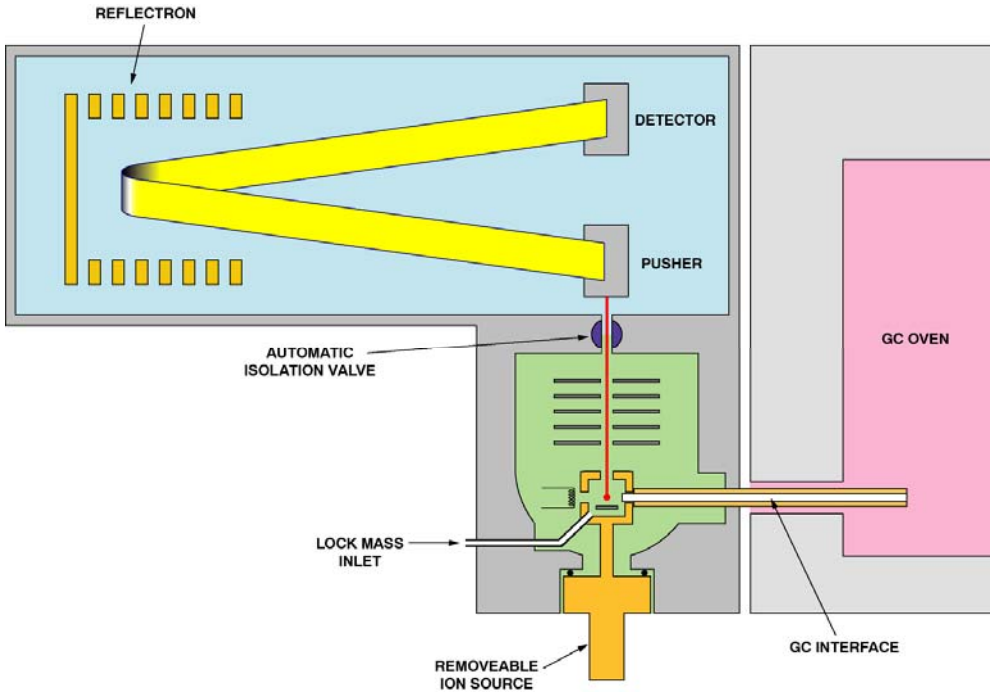
- Plug in EI/CI ion volumes for rapid change over
- Resolution >7000 (Full width half maximum)
- Exact mass less than 5ppm
- 4 orders linear dynamic range
- Spectral acquisition rate of 20 spectra per second



GCT Premier - Benefits
Orthogonal Acceleration TOF / MS

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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

Orthogonal Acceleration TOF
High resolution = Selectivity

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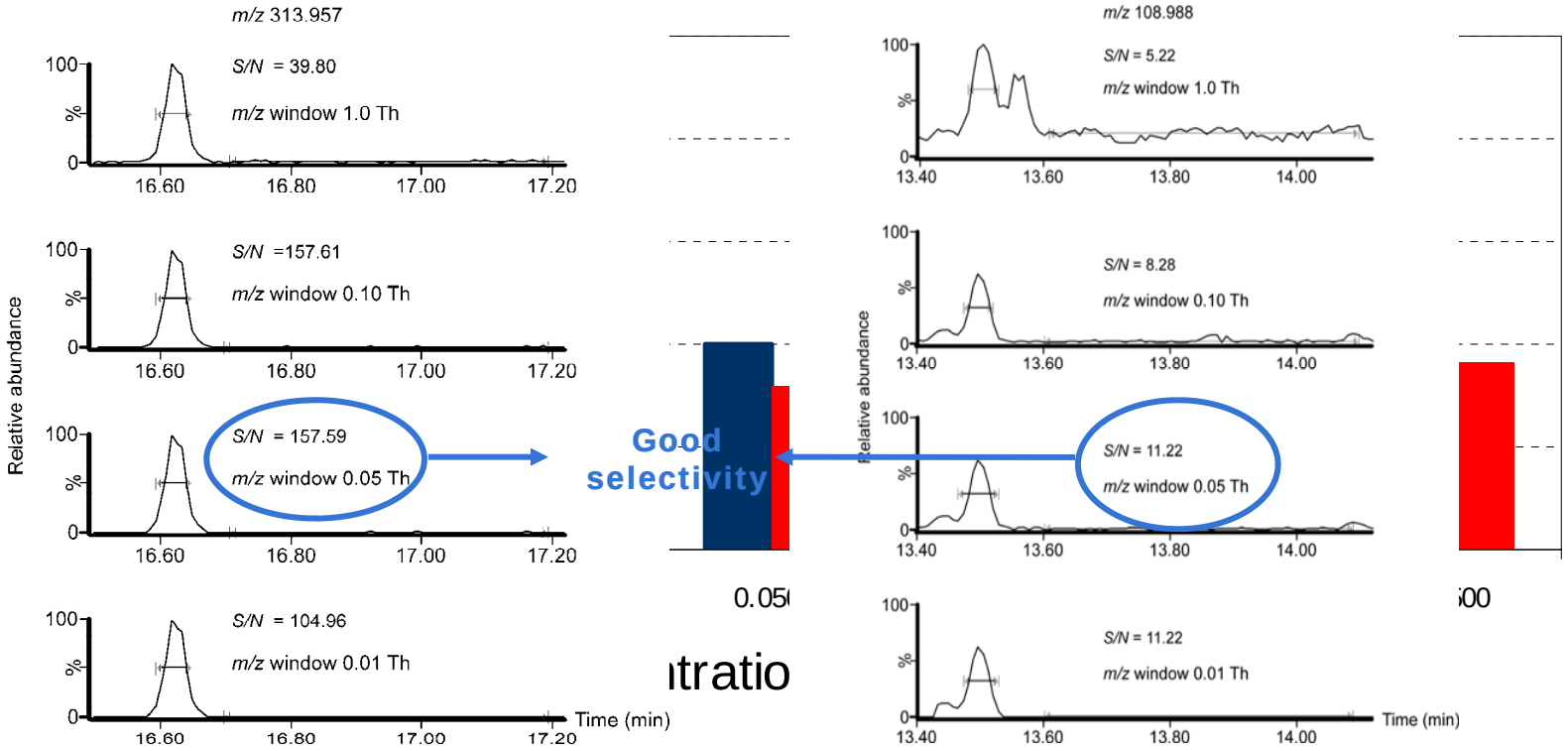
- Elevated mass accuracy allows for greater selectivity
  - Mass accuracy in fruit based baby food matrix matched standards
  - Chlorpyrifos (m/z 313.9574) and Fonofos (m/z 108.9877) in fruit based baby food
  - 0.05 Th is the routine mass window applied



Orthogonal Acceleration TOF DRE

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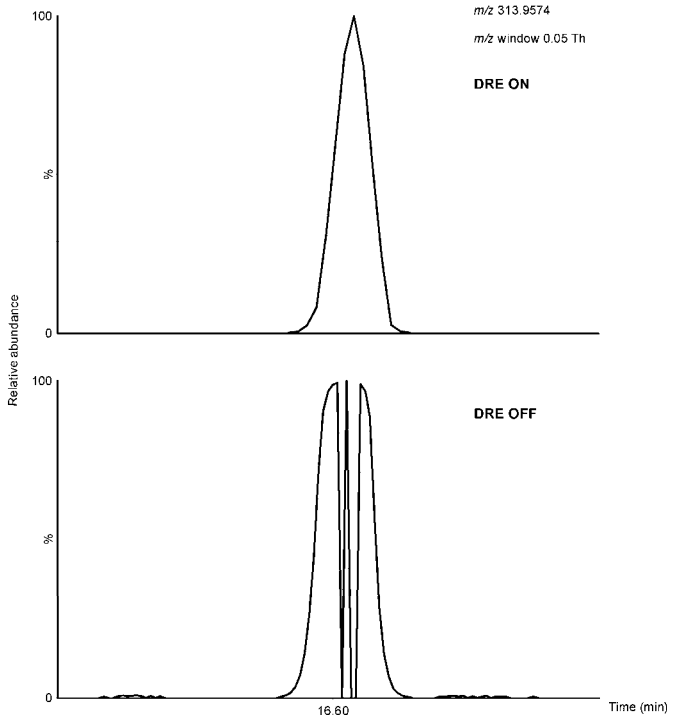
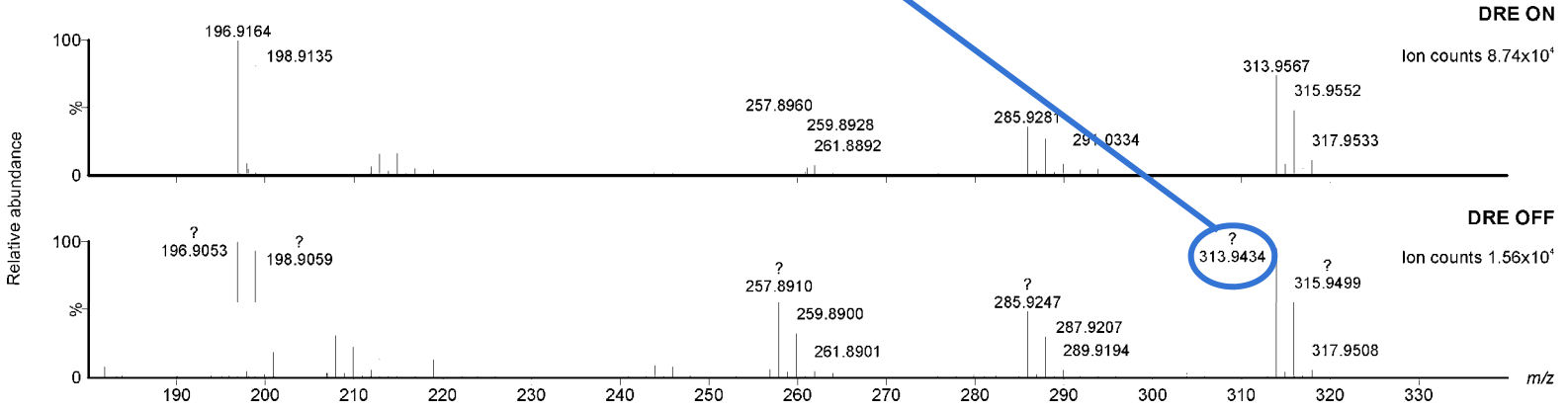
- DRE offers enhanced dynamic range
  - Chlorpyrifos in lettuce
  - Reduced saturation
  - With DRE off too many ions arrive at the detector simultaneously
    - Response falls outside mass window
- Enhanced mass accuracy
  - ? = Saturated peak, Mass error =14 mDa





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Screening in food: analytical challenges

Screening

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- Definition as "an examination, usually methodically, in order to make a separation into different groups"
- In chemistry we refer to a rapid check of a sample for a large number of analytes, most of which will result in a negative result.
- Identification and monitoring of residues in different commodities.
  - Food safety
    - Pesticides, veterinary drugs, banned additives…
  - Environmental
    - Drugs of abuse, endocrine disruptors…
  - Impurity control

Residues screening

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- **Screening**: to (rapid) detect the presence of a compound
  - Sample positive for an analyte? → **YES** or **NO**
  - Typically a second analysis is required to:
    - Correctly quantify or
    - Confirm the identity of the compounds detected
- **Confirmation**: to confirm the identity of the compound detected
- **Quantification**: to give information (accurate) on the analyte concentration in sample
- **Elucidation**: to discover (elucidate) the identity of a compound detected that is not a target analyte
  - Non-target analysis.

Analytical Challenges

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- ***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.

Analytical Challenges

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- ***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 (ie. Having broad scope)…
    - 'Food scares' and changes in legislation require updating of methods and re-analysis of samples

Pre-targeted screening

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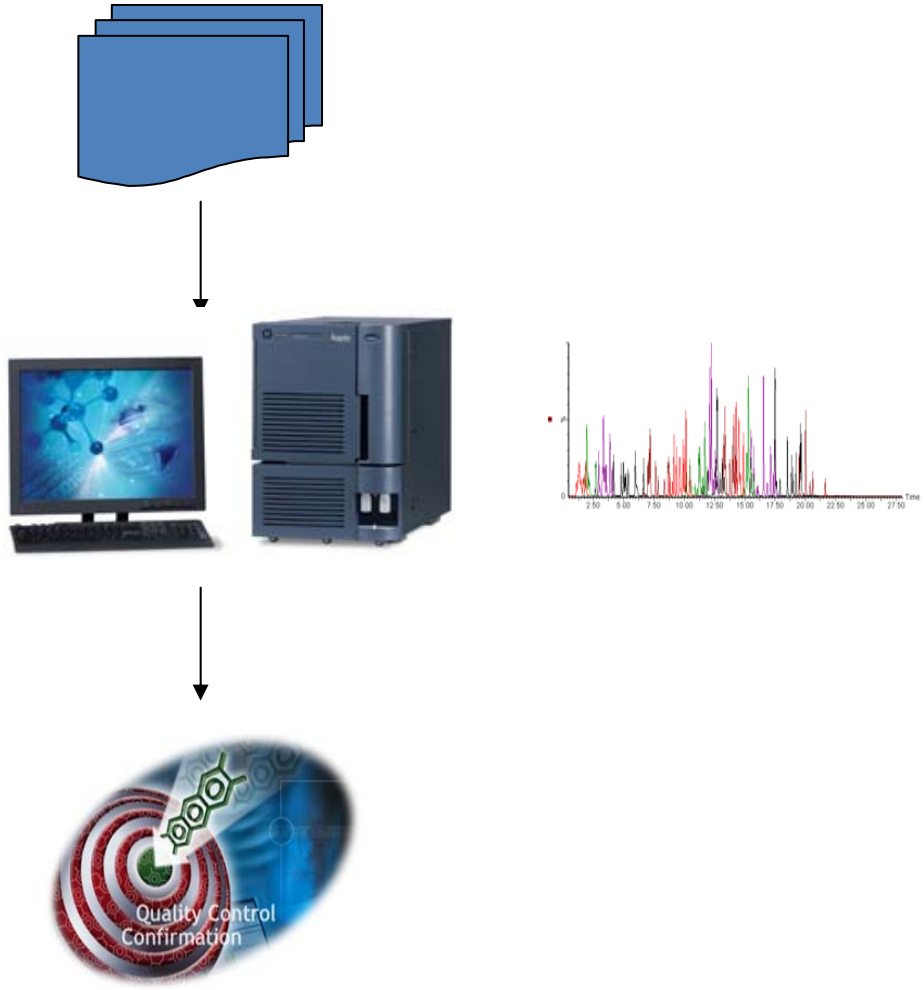
- First the analytes to be screened are selected
- Data acquisition
  - ACQUITY – TQD
  - ACQUITY – QP XE
  - GC-MS/MS
- Data processing
  - TargetLynx



Pre-target analysis tandem quadrupoles

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Strengths

- High sensitivity
  - MRM in triple quadrupoles
- High selectivity
  - Providing the MRM transitions are specific
- High dynamic range
  - Quantitation "gold standard"
- Legislation compliant
  - One trace for quantitation and one for confirmation

But…

- Sensitivity dependent on the number of analytes
  - Duty cycle vs. # transitions
- Not capable of reprocess historical data
  - New target needed, the analysis needs to be repeated.

Post-target screening

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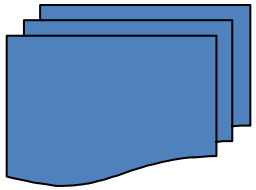
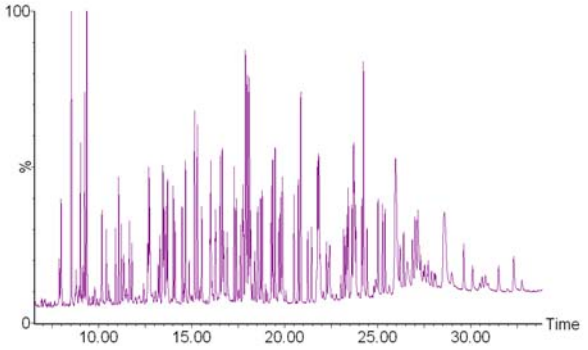
1. Data are acquired
   - LCT Premier
   - GCT Premier

2. Analytes to be screened are decided
   - templates

3. Data processing
   - TargetLynx





Post-target analysis TOF

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Strengths

- High sensitivity in full scan
  - Duty cycle not affected by # of analytes to screen
- High selectivity
  - Exact mass
  - Possibly retention time
- Dynamic range
  - DRE feature
- Capable of reprocessing historical data
  - Data are acquired, is just a matter of processing

But…

- Sensitivity less than most tandem quadrupoles in MRM mode
- Dynamic range less to the one of triple quadrupoles
- Legislation does not recognise exact mass as a proof of identity
  - Presumptive positives need to be submitted for confirmatory analysis be repeated.

Analytical Challenges - *New* approaches with ToF/MS

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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

System Solution

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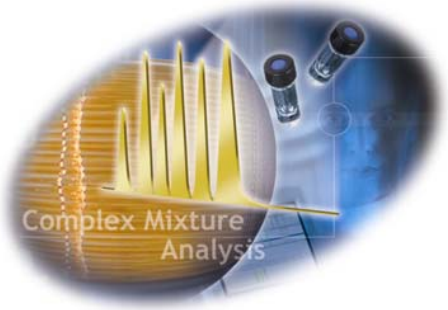
GCT Premier

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

Sample Preparation and GC-TOF parameters

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- Extraction of the baby food, lettuce and pear samples
  - Modified QuEChERS extraction

GCT Premier

- Electron Impact (EI+) with a mass range of *m/z* 50 → 500
- Acquisition Speed = 4 spectra / s
- Dynamic Range Enhancement (DRE) On
- 4GHz TDC
- Pusher interval 35µs
  - 28571 raw spectra s^{-1}
- RXi-5ms GC column
  - 5µL PTV injection
  - Simple oven temperature ramp

Total Ion Chromatogram (TIC)

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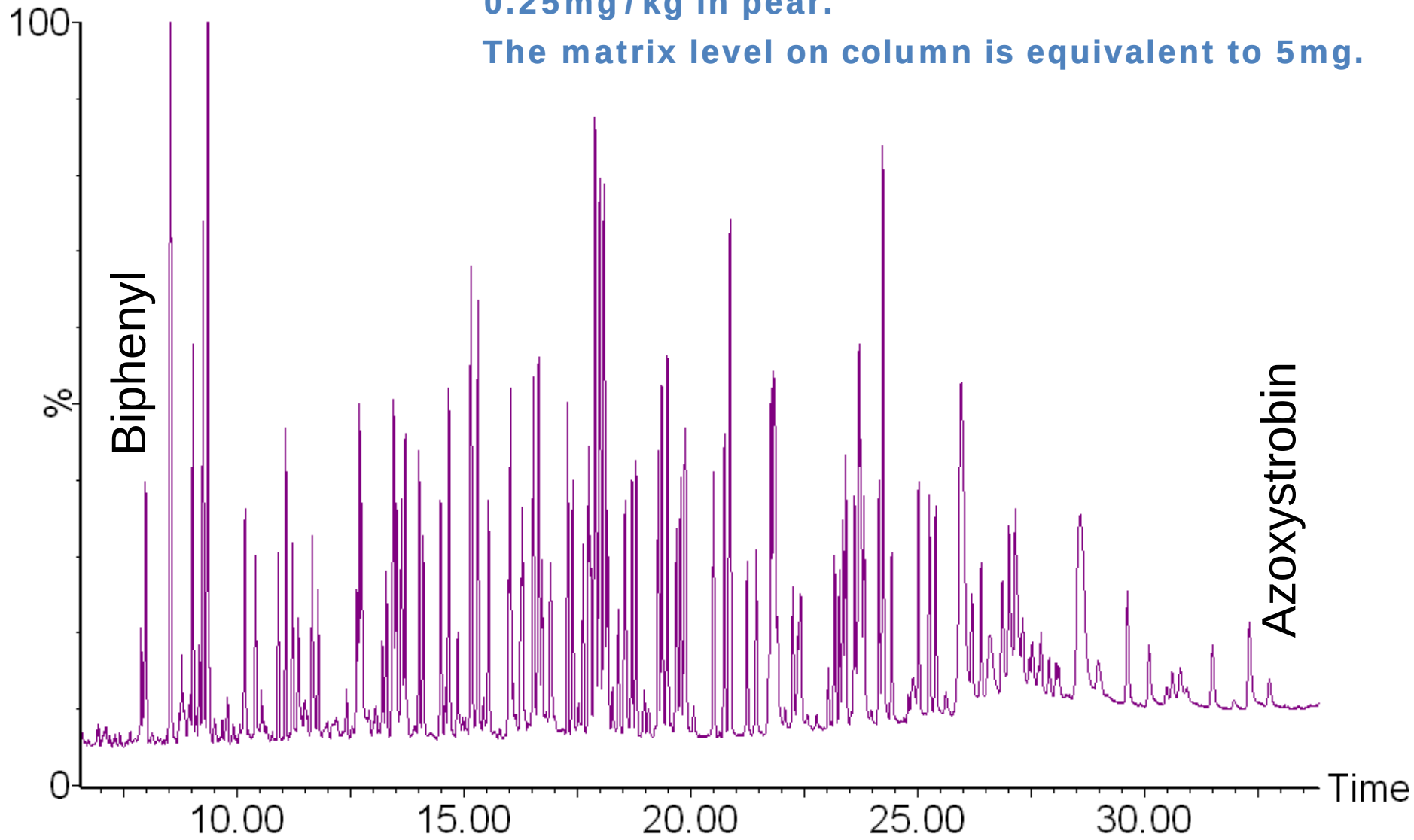
The residue concentrations are equivalent to 0.25mg/kg in pear.

The matrix level on column is equivalent to 5mg.



Targeted Screening GC-TOF/MS and TargetLynx

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- Compound list is known
- Detection is based on extracting exact mass chromatograms with 0.02 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
  - o If a peak is found within a defined time window the target compound is detected
  - o Ion ratios can be used to increase confidence in detection
  - o 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

Incurred Residue in Lettuce
Fenitrothion – 0.023mg/kg

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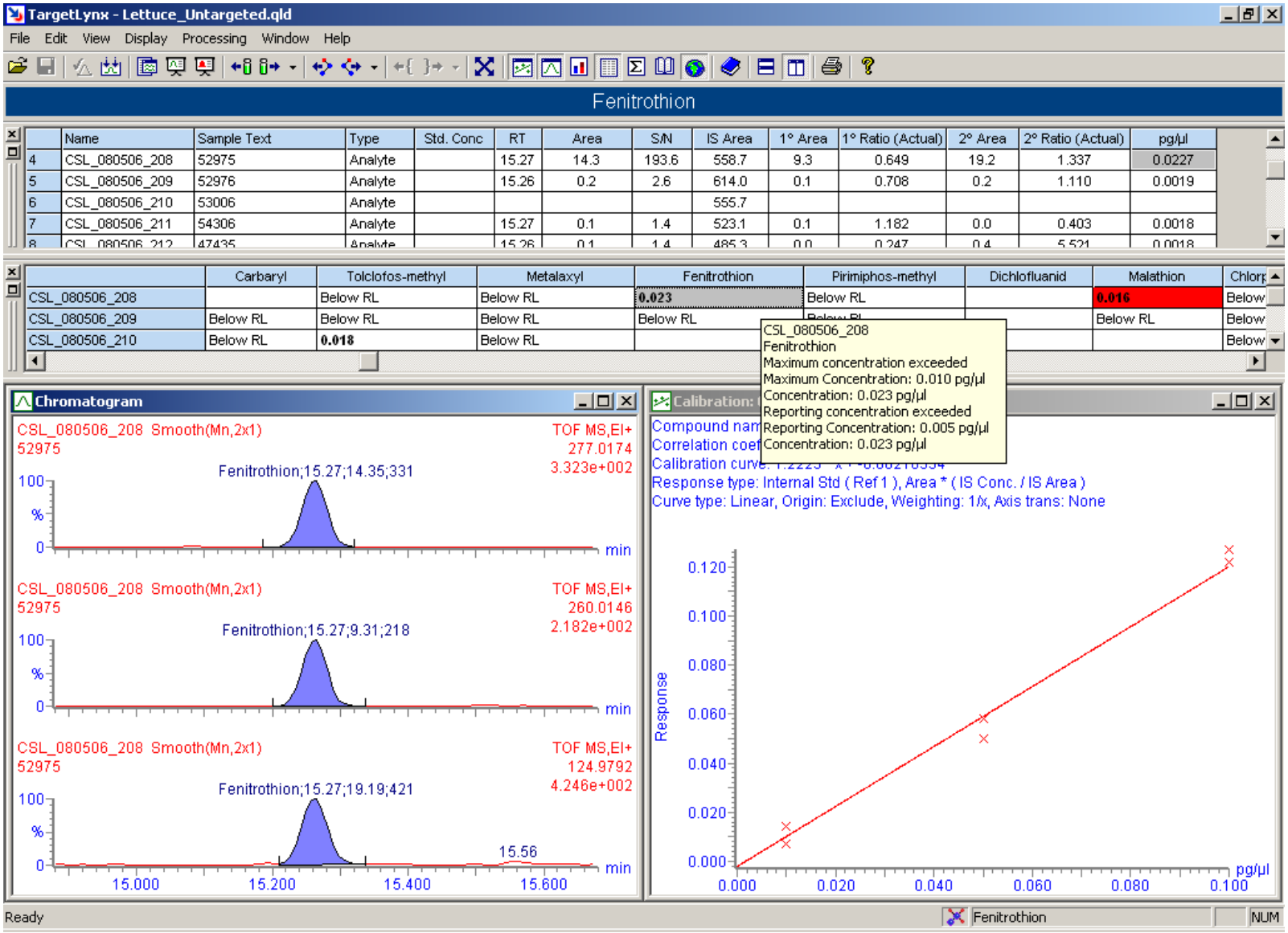

	Name	Sample Text	Type	Std. Conc	RT	Area	S/N	IS Area	1° Area	1° Ratio (Actual)	2° Area	2° Ratio (Actual)	pg/µl
4	CSL_080506_208	52975	Analyte		15.27	14.3	193.6	558.7	9.3	0.649	19.2	1.337	0.0227
5	CSL_080506_209	52976	Analyte		15.26	0.2	2.6	614.0	0.1	0.708	0.2	1.110	0.0019
6	CSL_080506_210	53006	Analyte					555.7					
7	CSL_080506_211	54306	Analyte		15.27	0.1	1.4	523.1	0.1	1.182	0.0	0.403	0.0018

	Carbaryl	Tolclofos-methyl	Metalaxyl	Fenitrothion	Pirimiphos-methyl	Dichlofluanid	Malathion	Chlorp
CSL_080506_208		Below RL	Below RL	**0.023**	Below RL		**0.016**	Below
CSL_080506_209	Below RL	Below RL	Below RL	Below RL			Below RL	Below
CSL_080506_210	Below RL	**0.018**	Below RL					Below

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Non-Targeted Screening GC-TOF/MS and ChromaLynx

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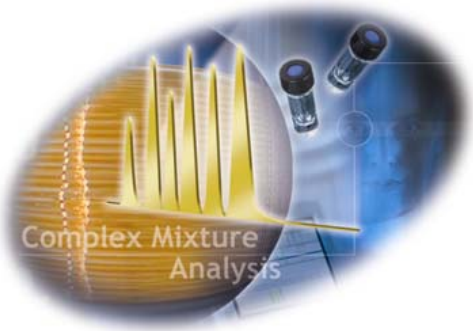
- Compound list is unknown
- ChromaLynx application manager
  - Automatic peak detection and deconvolution
  - Automatic production of "clean" component spectra
    - Standard EI spectra are generated by the GCT source
  - Automatic identification via library searching
    - Commercial or user generated 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



Deconvolution

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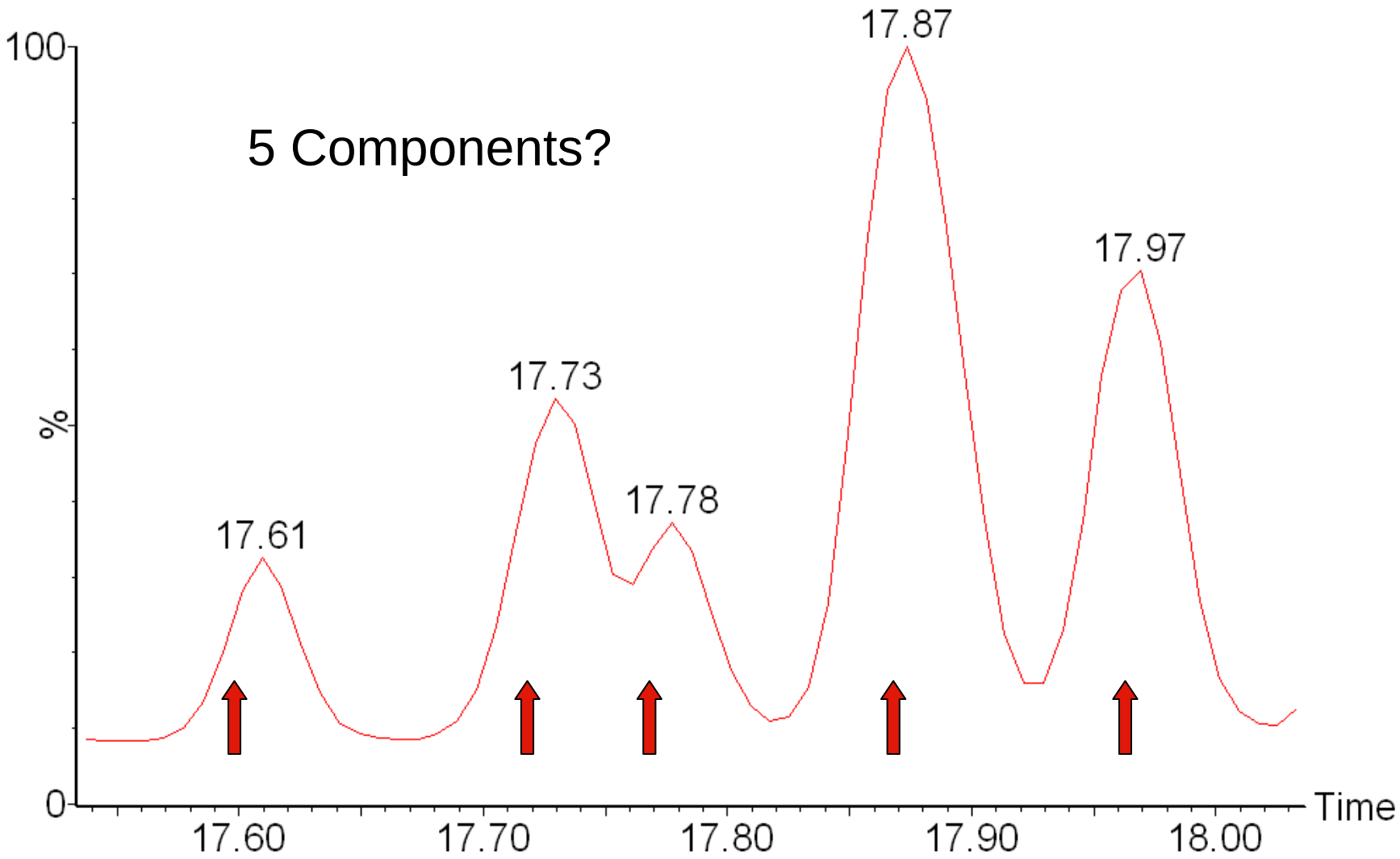

ChromaLynx Browser - Deconvolution

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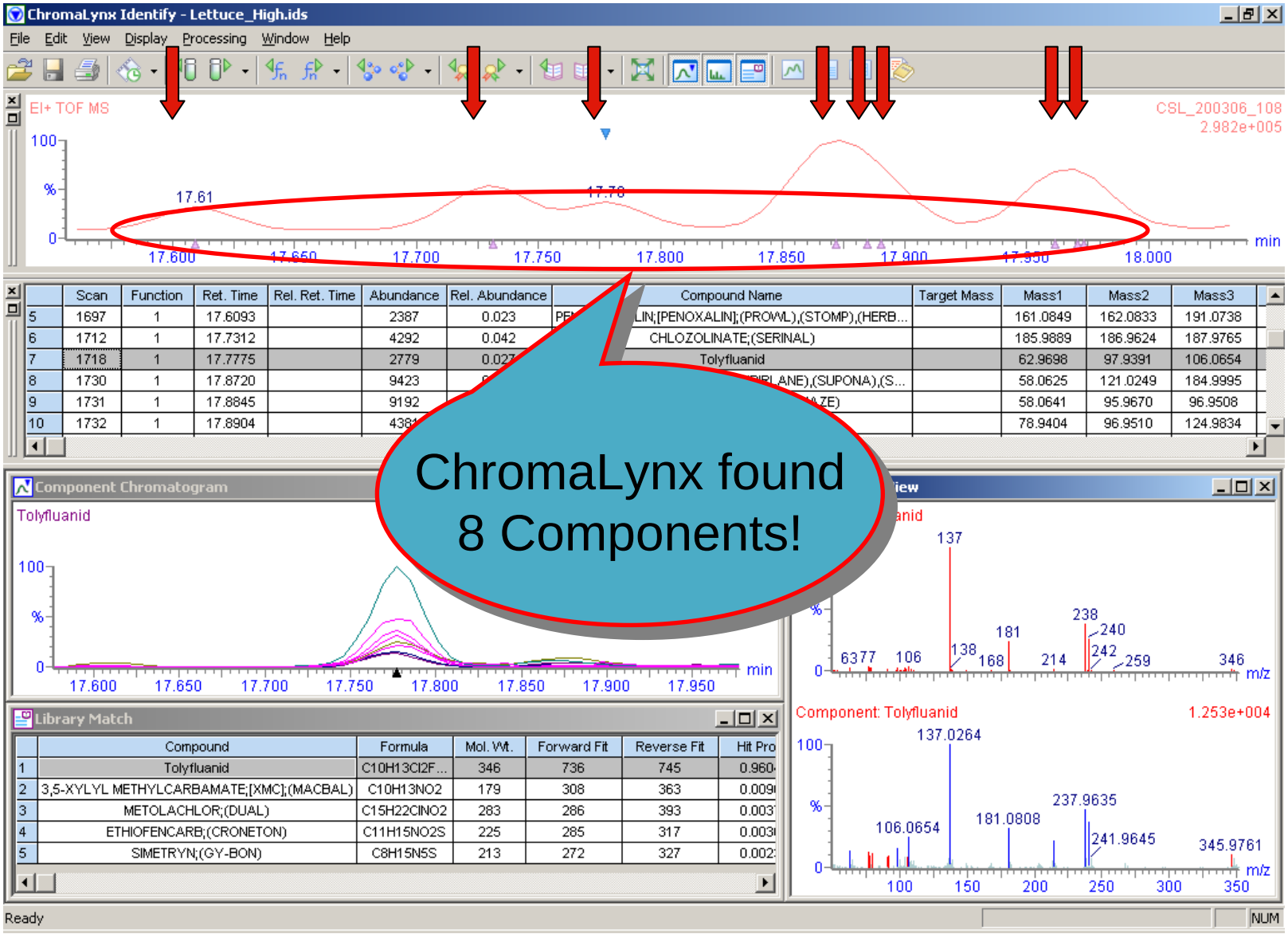

Extracted Mass Chromatograms 3 Components

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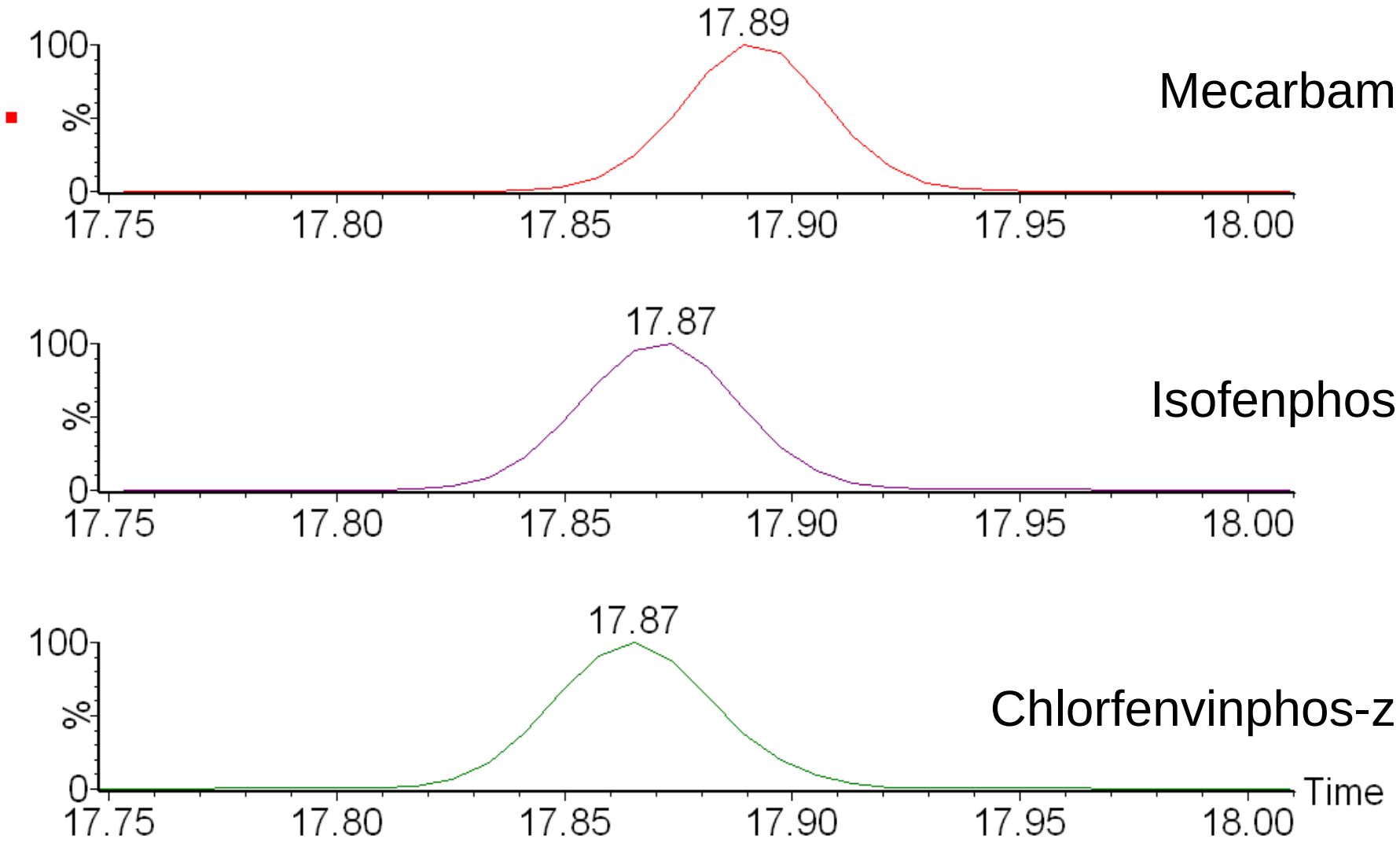

Pear Extract - ChromaLynx

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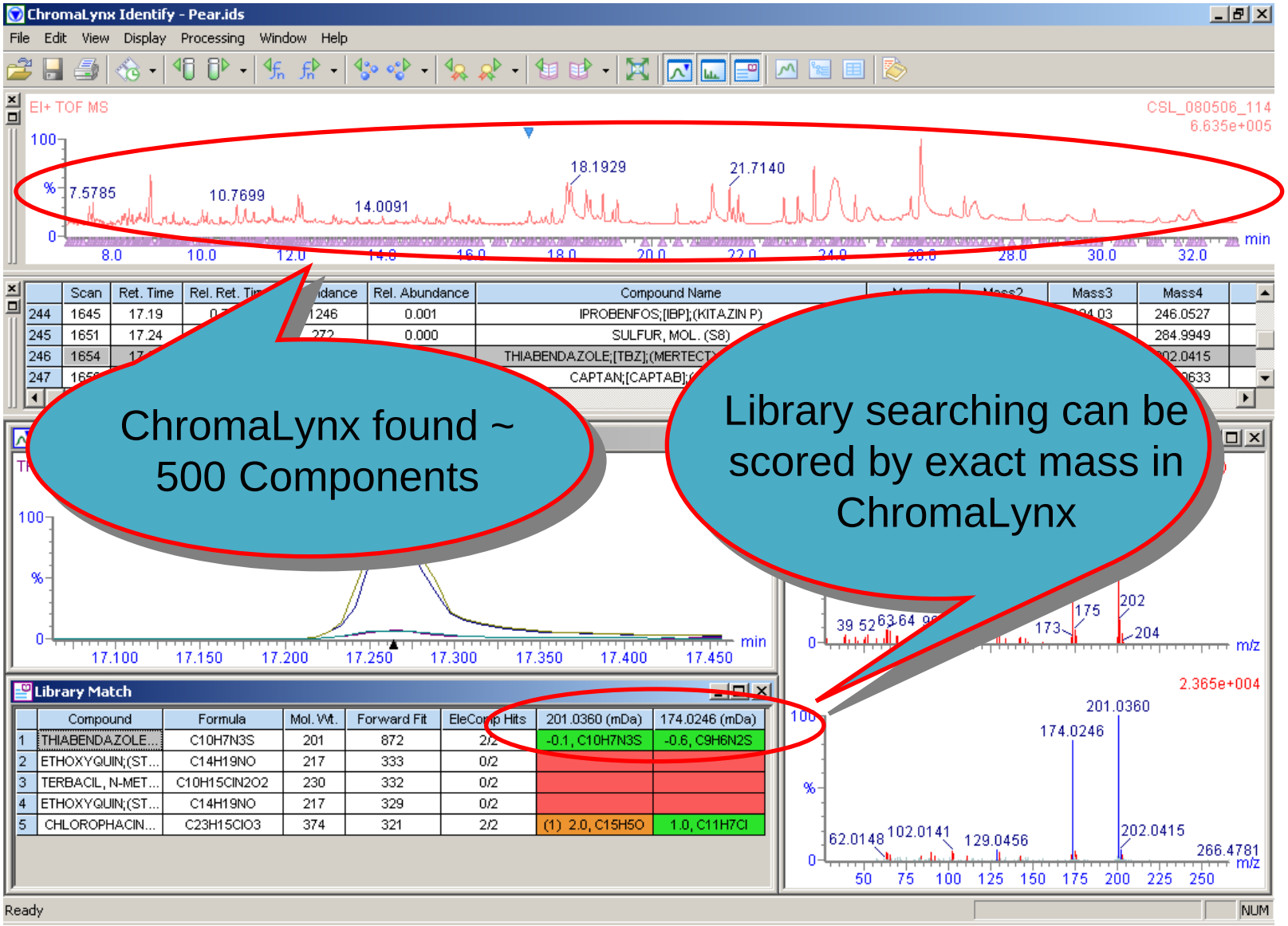

Example of Untargeted Screening DCPA in Lettuce

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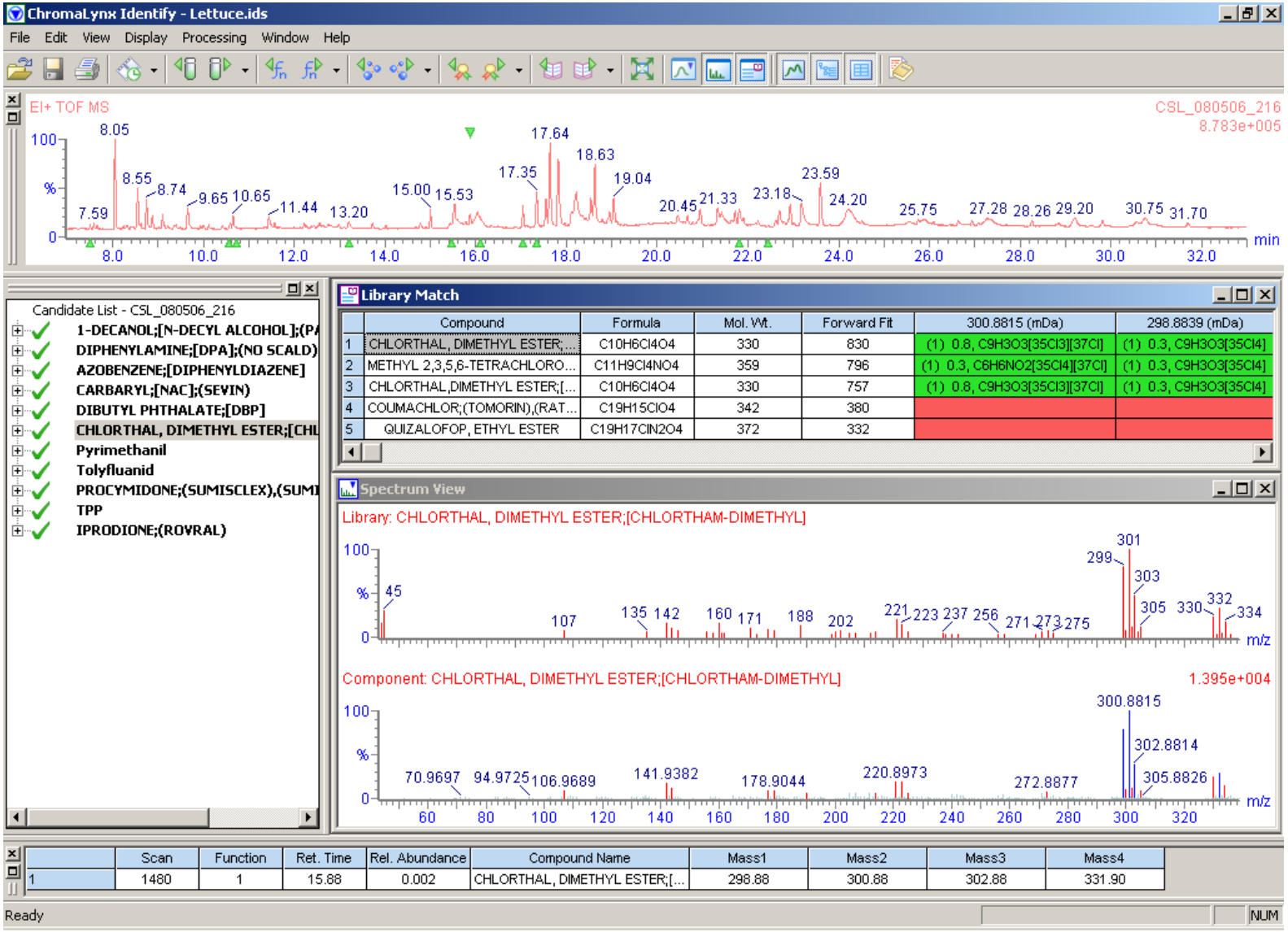

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Application Example 2

Profiling of different beers

Importance for the Food Industry

Challenges for Analytical Chemists

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- Some of the challenges for scientists in the food industry
  - Identifying **process changes** that may lead to a change in quality
  - Detecting **adulteration** in any ingredient
  - Identifying the **geographical origin** of raw materials
- Challenges for scientists in regulatory agencies
  - Detecting economic fraud due to product substitution and adulteration, as well as health risks from possible contamination
- These QC issues are traditionally assessed by experts
  - Intensive training to be able to determine a product's quality

Taste and Smell

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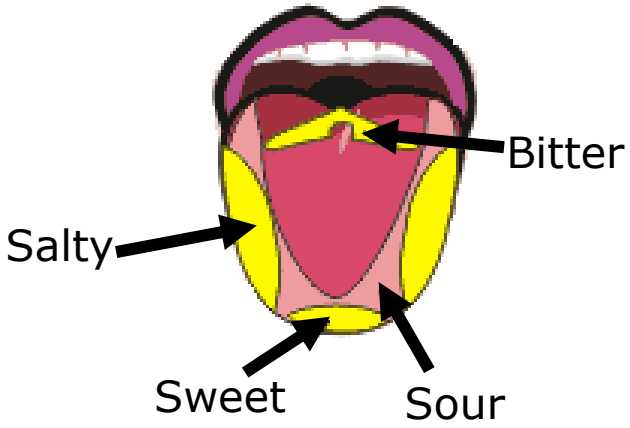
Taste

- Occurs on the tongue
- Detects 4 primary tastes
- Mouthfeel (e.g. viscosity, smoothness)
- Pungency



Smell

- Occurs in the nose
- Detects 32 primary tastes e.g. vanilla, smoky, cereals, fruity, sulphury, floral

Appearence

- Colour
- But sometimes altered by the addition of caramel

(Whisky) Flavour Chart

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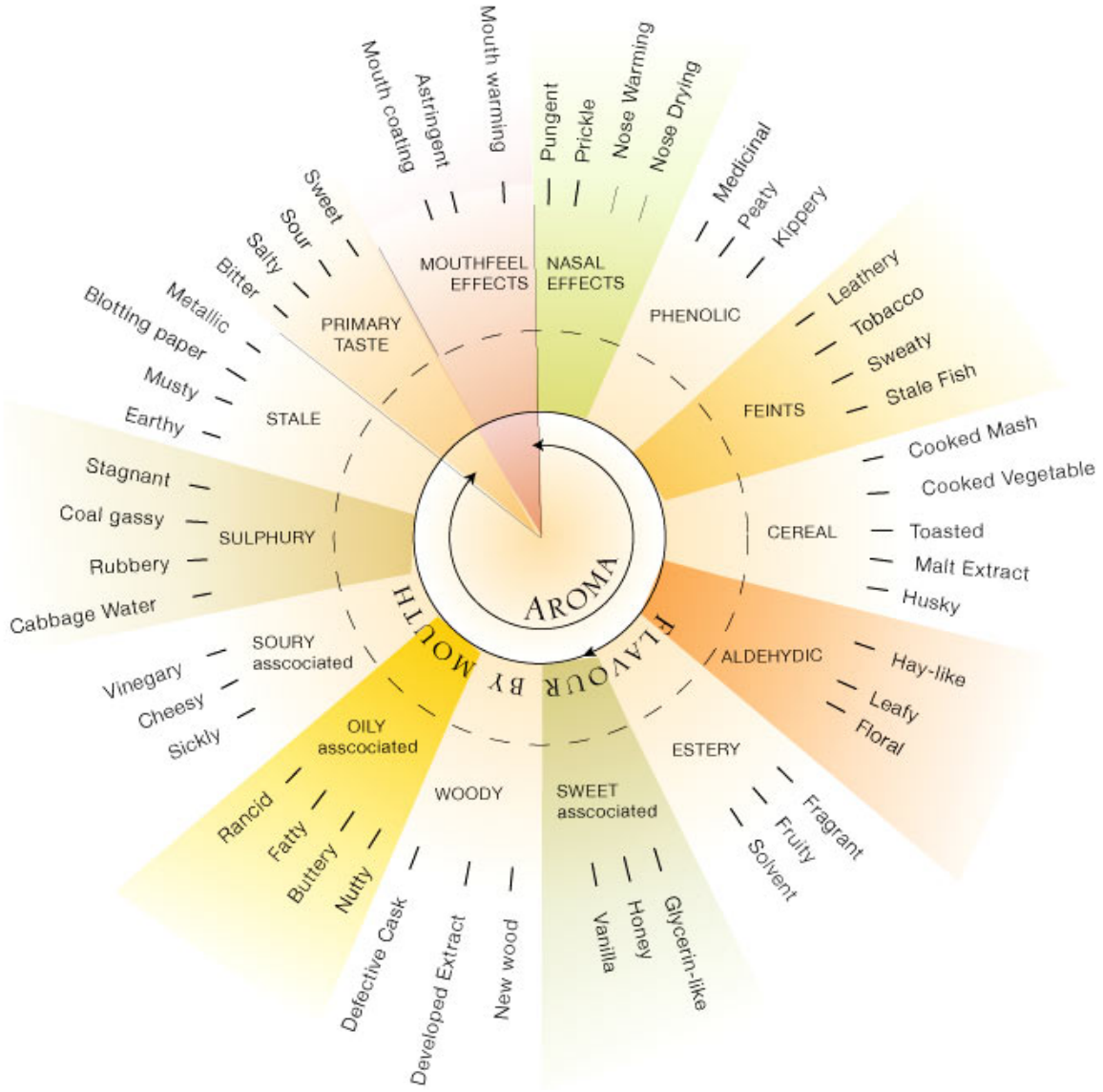

The Challenge…

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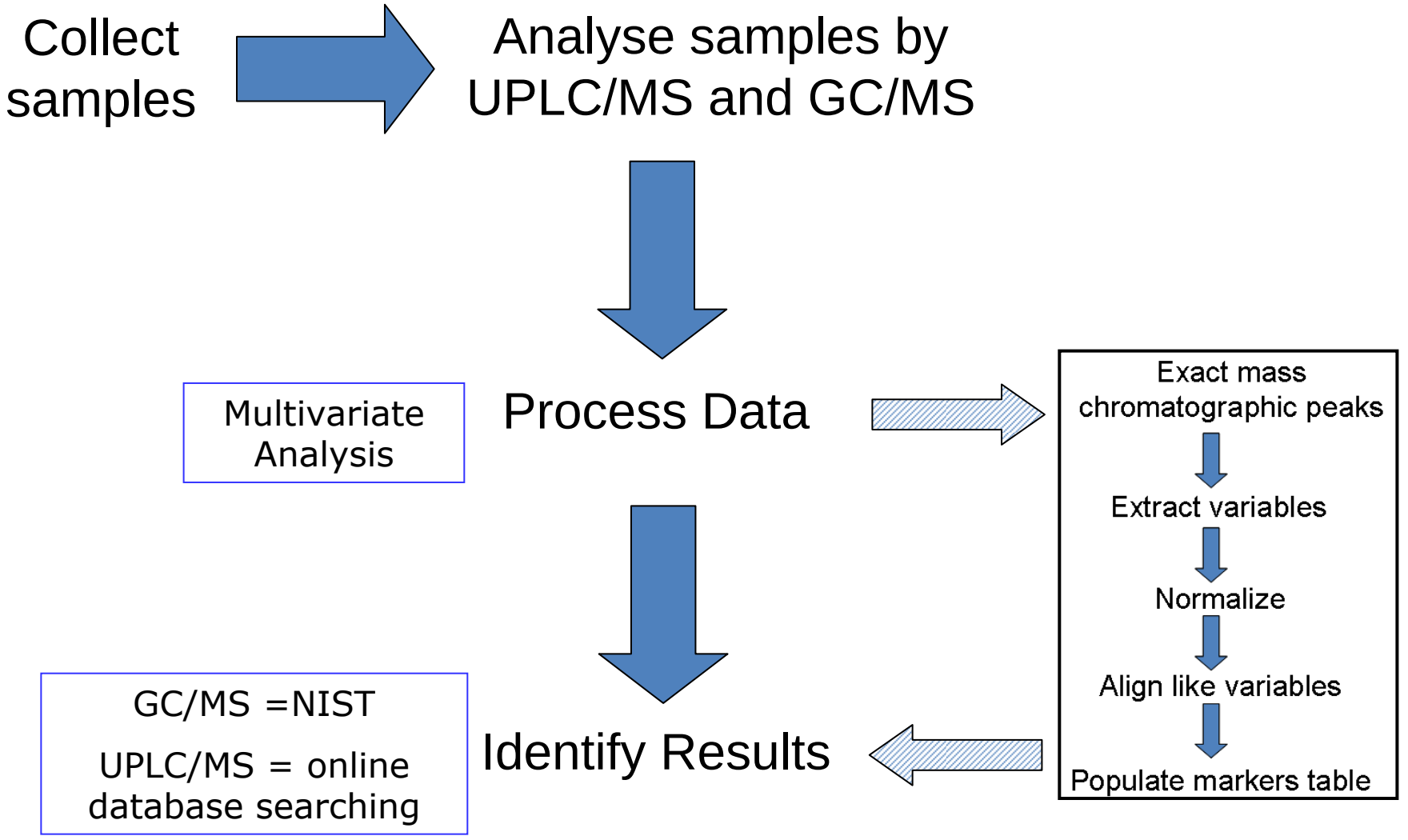
Work Flow



Statistical Analysis
Scores and Loadings Plot

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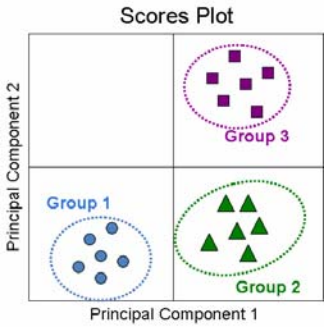
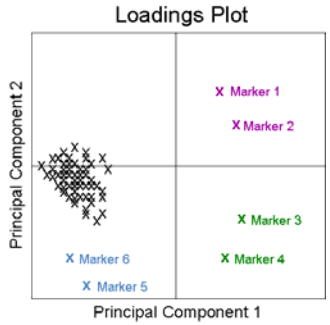
- PCA is an unsupervised technique that enables ellucidation of main sources of variability in datasets, detects clustering formation and enables identification of 'markers' (t_R, m/z pairs)
- Scores plot



Two principal components make up a plane. When points are projected onto the plane similarities between objects are described

- Loadings plot



Describes the variables relationships and can interpret the scores plot by telling which variables are responsible for similarities

Scores and Loadings plot

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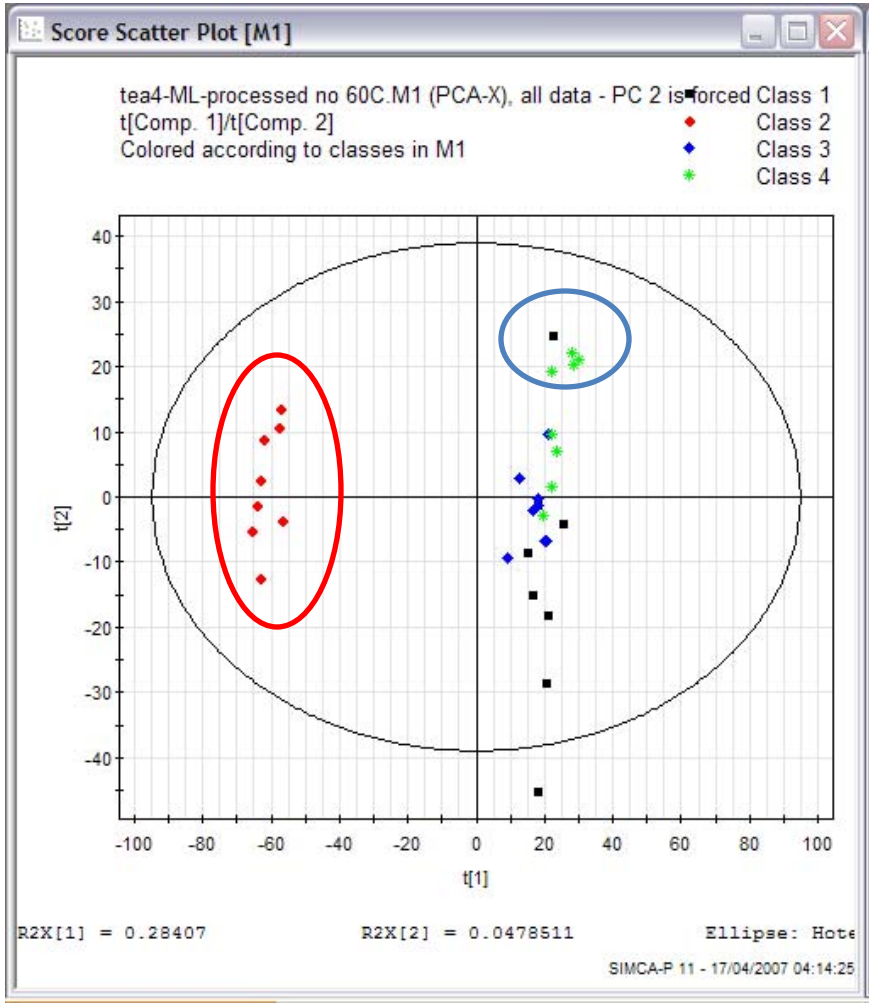

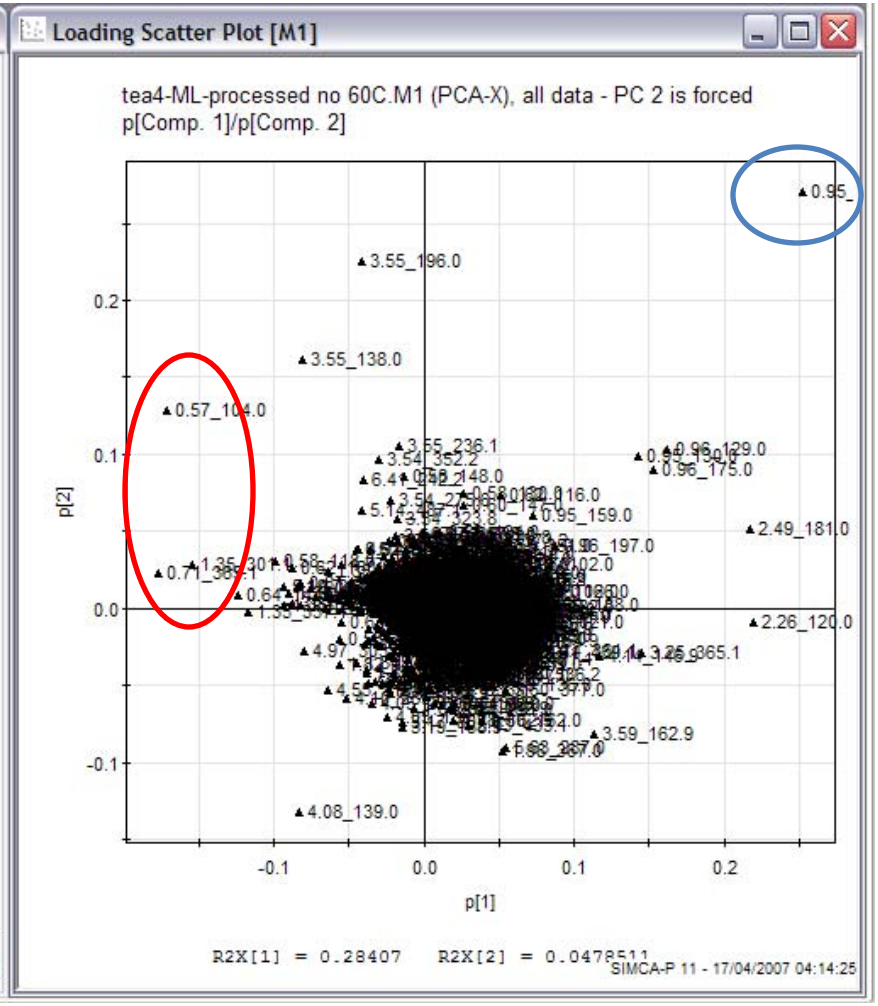

Data Acquisition

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GC/MS

LC/MS

Aims of the experiment

- Instrument: GCT
- Ten samples:
  - Nine commercially available beers
  - One home-made beer
- Mix of dark and light beers, from different geographical locations

Questions:

- Do any of the beers have markers that identify them from the other beers
- How similar are the brands to one another
- Is there anything else that we can learn about the beers

Scores Plot View: 10 Beers

3 Outliers

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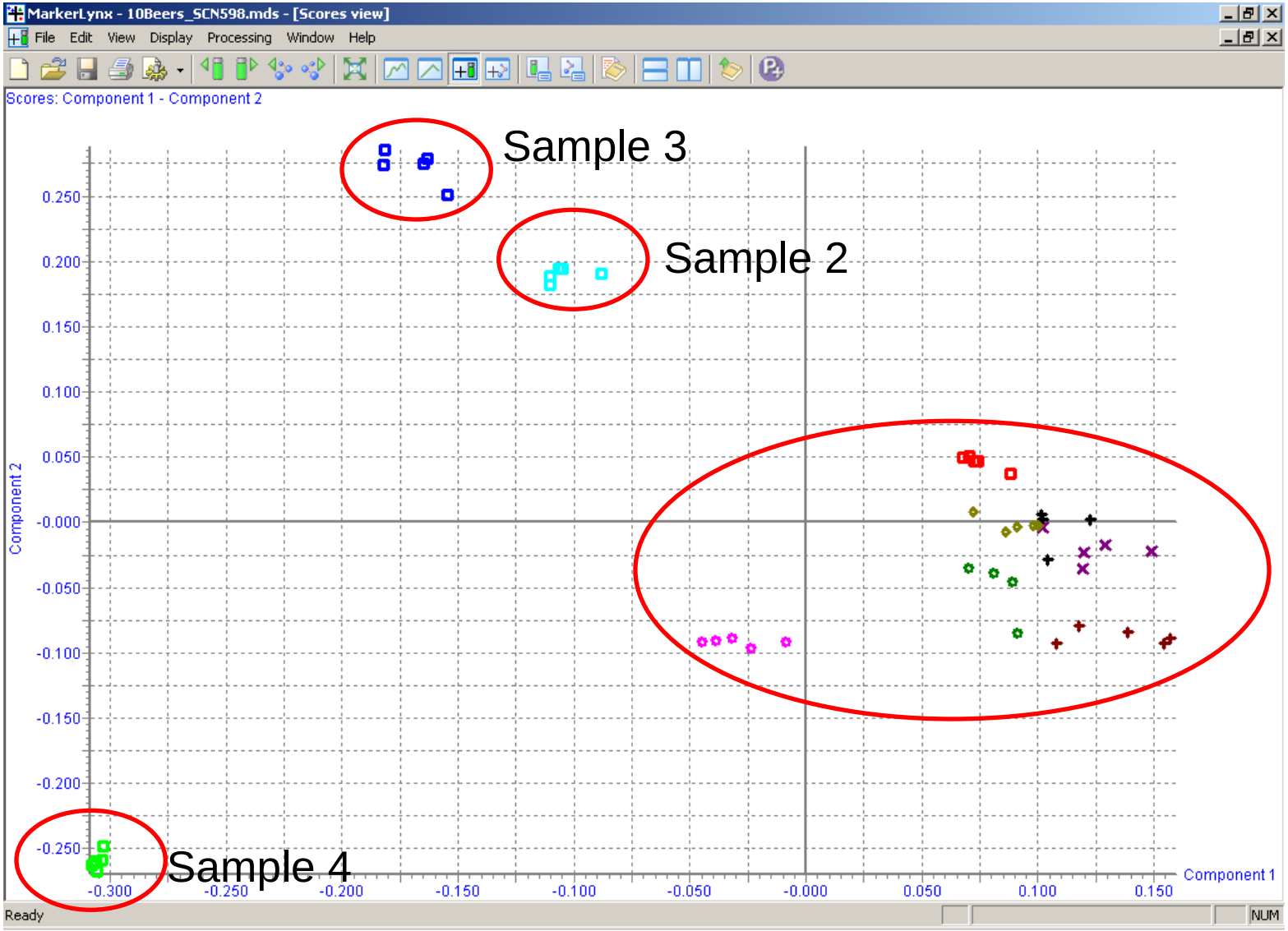

Library Search: 10 Beers

R_t = 14.53, m/z = 164.0717 ≡ Eugenol

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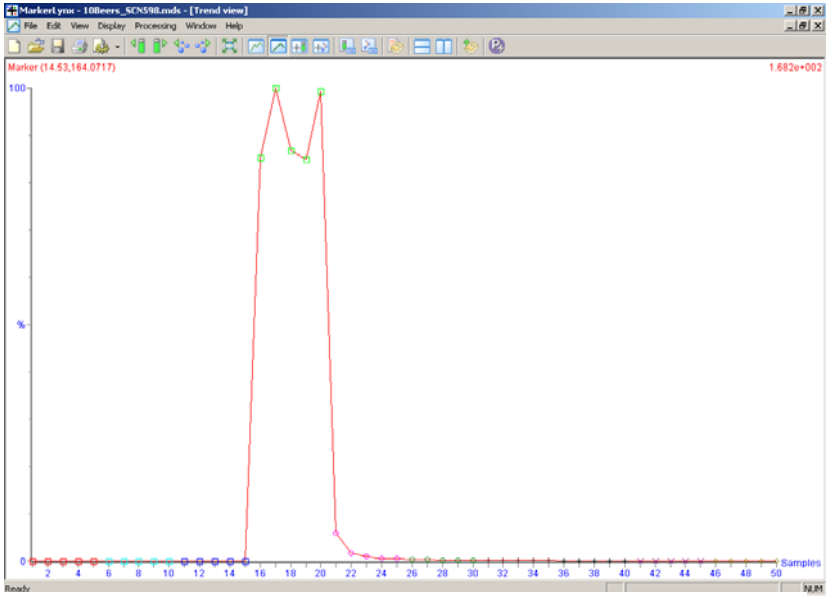

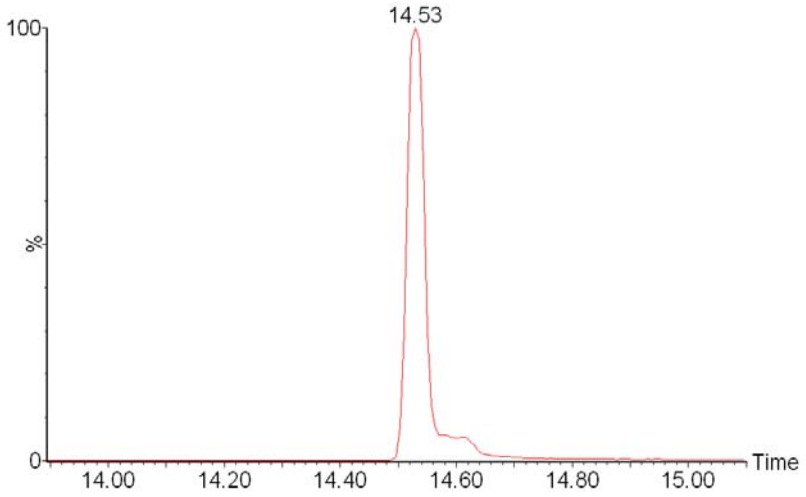

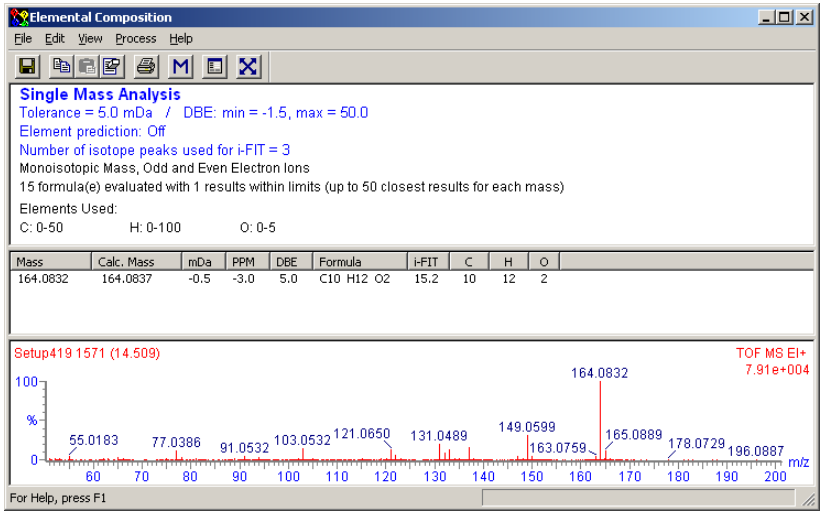

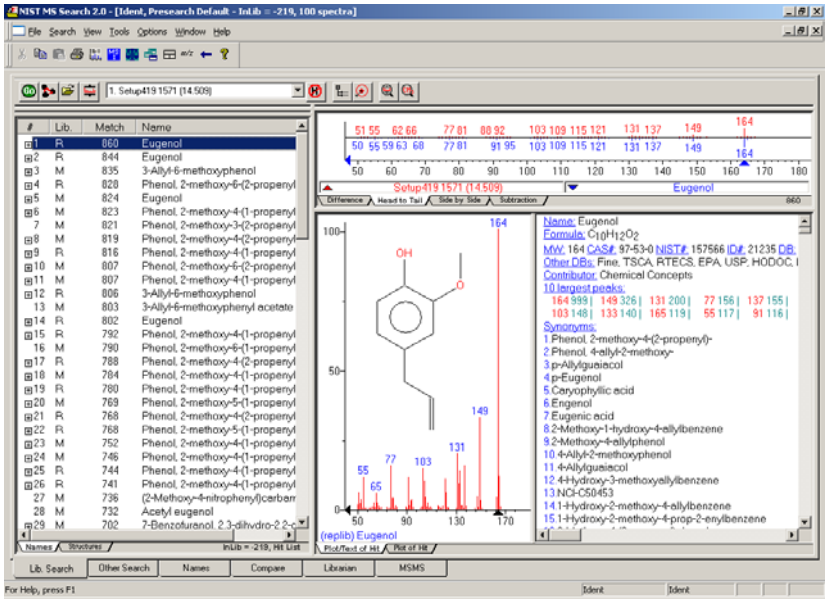

10 Beers
Marker Identification

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RT	*m/z*	Sample	Name	Formula
14.53	164.0717	4	Eugenol	$C_{10}H_{12}O_2$
10.64	93.0674	2, 3	3-Carene	$C_{10}H_{16}$
17.34	85.0231	3	Persicol	$C_{11}H_{20}O_2$
9.86	70.0744	3	Isoamyl butylate	$C_9H_{18}O_2$
16.03	85.0259	3	Decanolactone	$C_{10}H_{18}O_2$
12.25	93.0689	2, 3, 4	Terpinyl acetate	$C_{12}H_{20}O_2$
15.13	178.0976	4	Methyl eugenol	$C_{11}H_{14}O_2$
9.39	93.0691	4	Limonene	$C_{10}H_{16}$
8.71	71.0478	3	Butyl butylate	$C_8H_{16}O_2$
8.02	106.0404	3	Benzaldehyde	C_7H_6O

Decreasing significance

Conclusions

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- GCT is a powerful, versatile technology for a wide range of applications
- ToF are by principle an unbiased technology
  - Open data
  - Post-target analysis
  - Require POWERFUL SOFTWARE tools to mine the data
- GCT can be succesfully used for
  - Screening of compounds
  - Profiling of commodities - NIST libraries facilitate the identification of markers

