
Waters™
Targeted and Non-Targeted Analytical Approaches for Authenticity Testing and Identification of Food Fraud
Waters
UNLOCKING THE POTENTIAL OF SCIENCE
PMN RAJESH
Waters

Scope of Food Testing

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The Good

Vitamins, sugars, amino acids, polyphenols….

What is in a label? Product claims? Buying choices?

Consistency, Quality

The Bad

Pesticides, natural toxins, antibiotics, allergens….

Contaminants and residues

The Fake

More than 10% of the food we consume could be adulterated

Costs the food industry $50 Billion/year

Food Quality

Food Safety

Food Fraud

Targeted vs Non-Targeted Analysis

When you know what you're looking for!

When you want to see it all!

Technologies for Food Authentication

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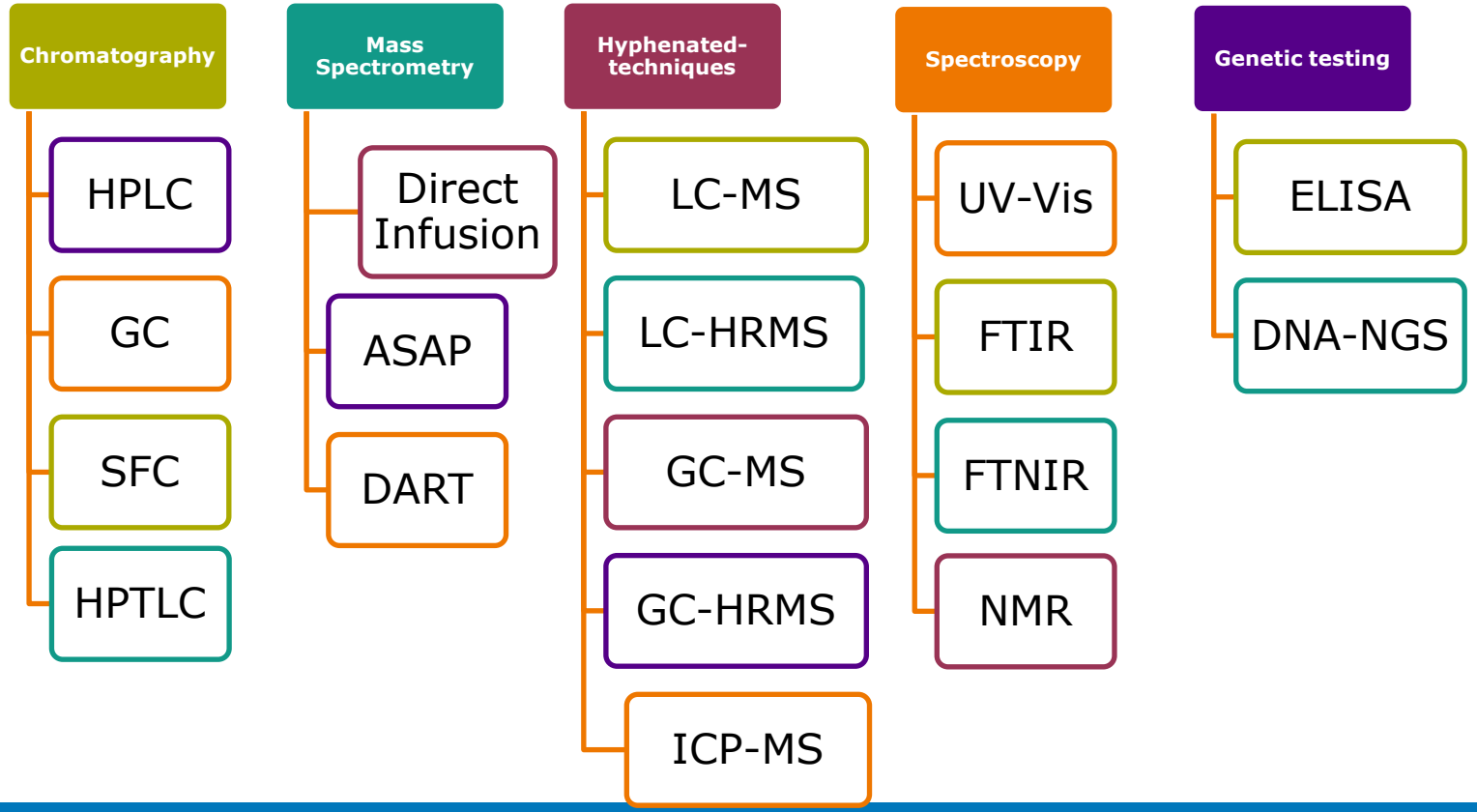

Several Considerations for Robust, Reliable and Fit for Purpose Methods

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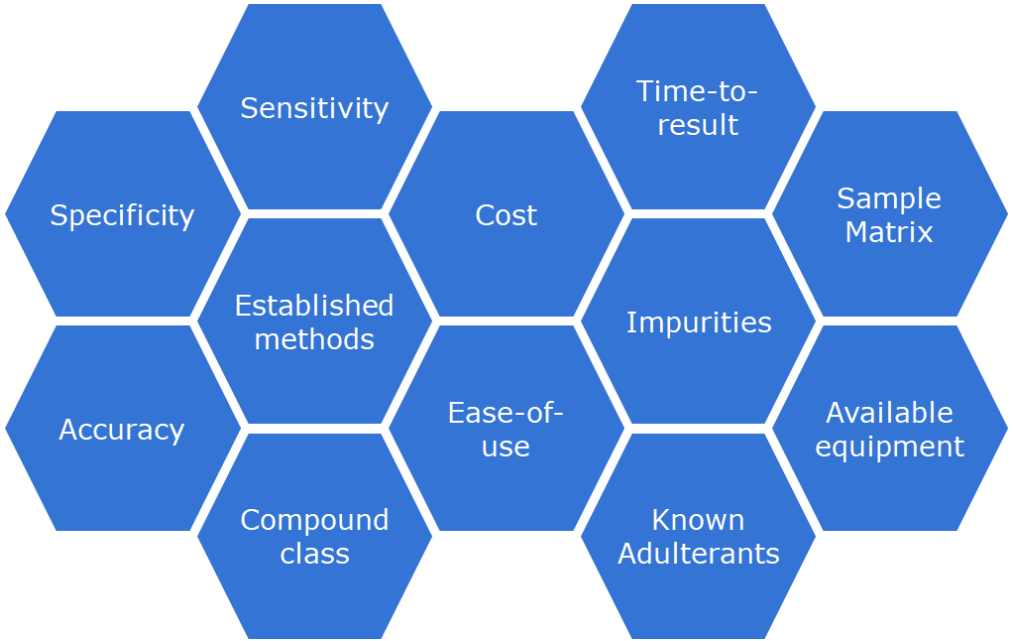

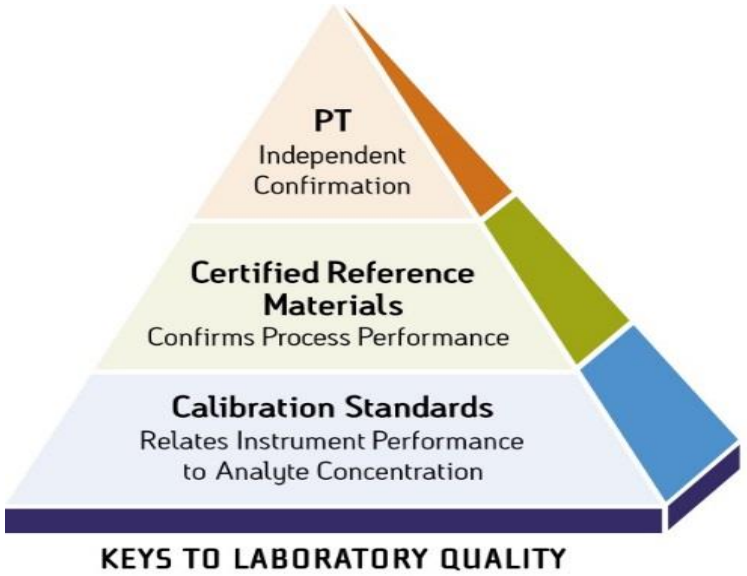

Chemometric Software - Progenesis QI

Find differences between samples, identify and quantify markers

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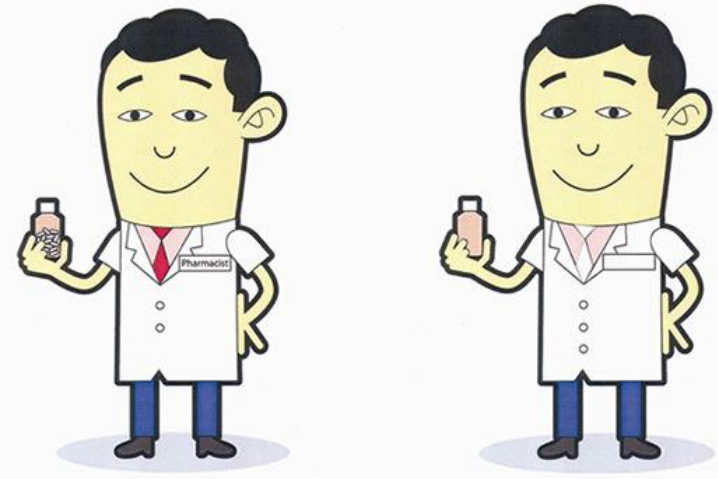
Detect → **Quantify** → **Compare** → **Identify**



4 differences

Waters “Omics” Research Platform Solutions with Progenesis QI Informatics

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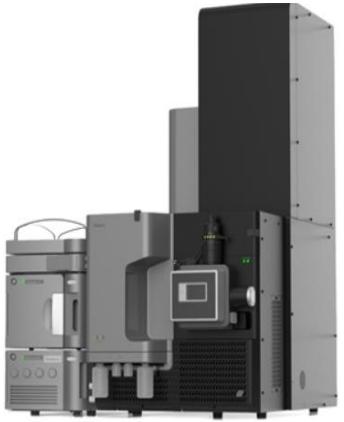
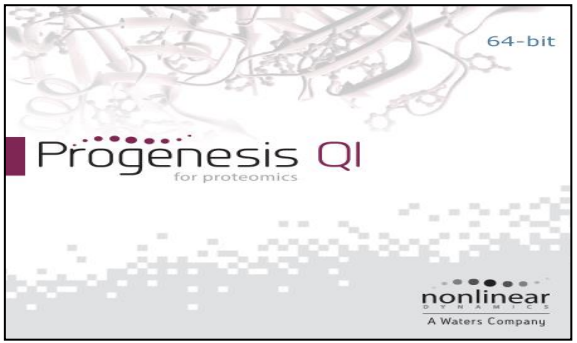


Lipidomics

Proteomics

Plant Metabolomics



Metabolomics

Authenticity

Plant/Food Profiling

Research to Routine

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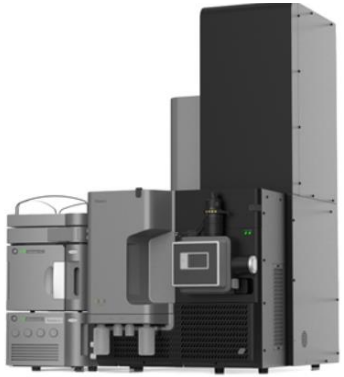
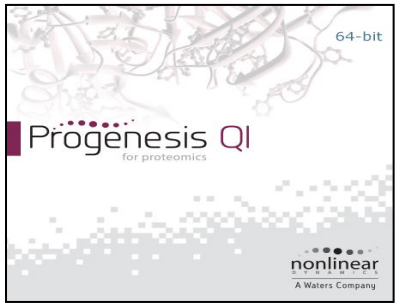
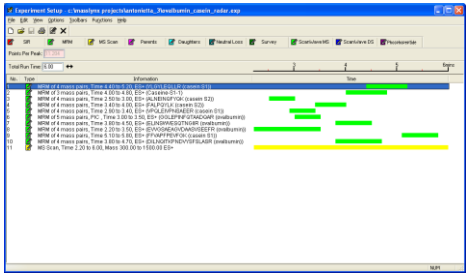
Xevo G3-S Q-ToF





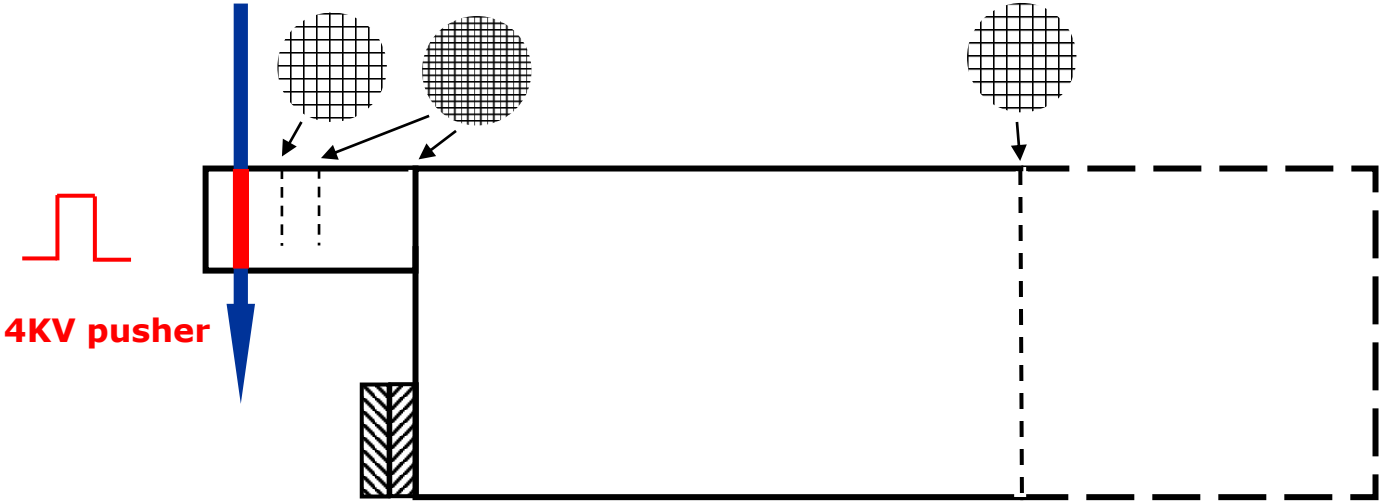
SYNAPT-XS HDMS

XEVO-TANDEM QUAD



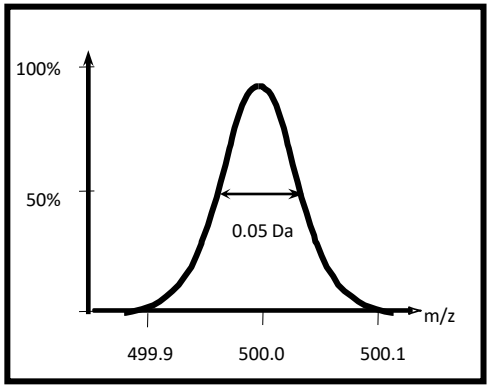
RADIAN™ ASAP

Q-TOF 1



Resolution = 40000

Spectral Resolution



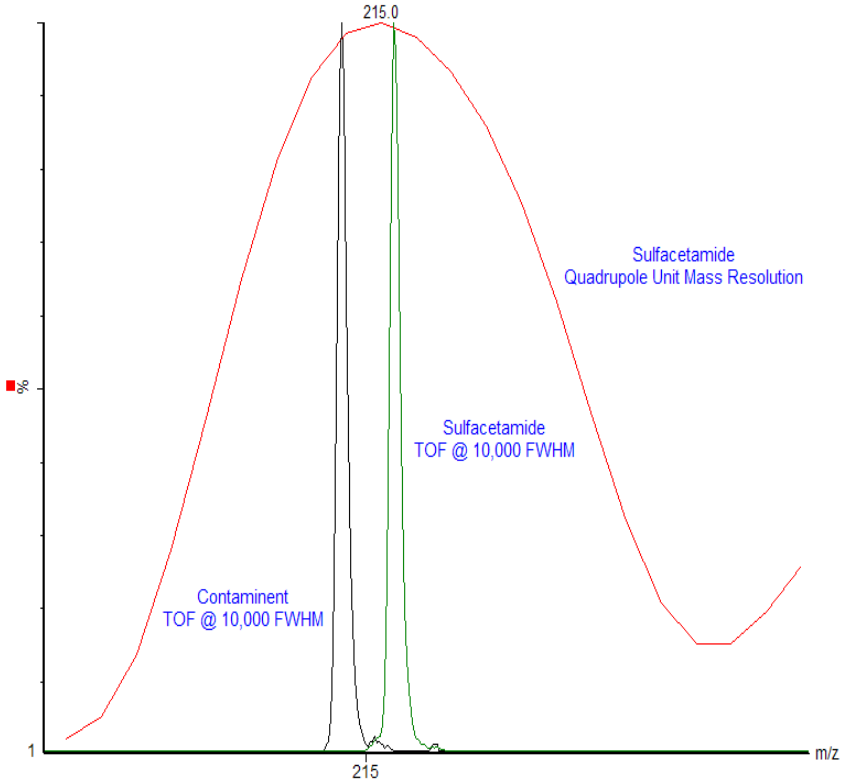
Mass = 500

Peak width (@ 50%) = 0.05Da

$$\text{Resolution (FWHM)} = \frac{500}{0.05} = 10000$$

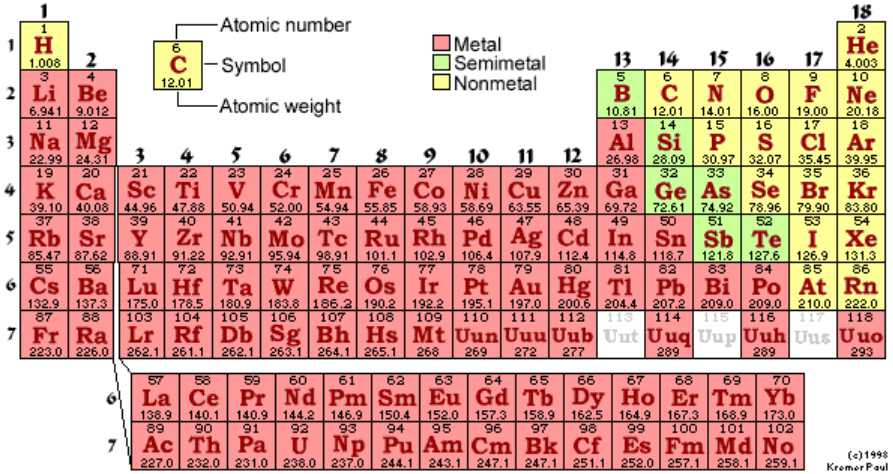
FWHM = Full Width Half Maximum

Resolution & Exact Mass



- Quadrupole resolution is not sufficient to differentiate these two compounds
- ToF data with a resolving power of >10,000, clearly shows two distinct peaks.
- These can be accurately mass measured to < 5ppm

Periodic Table



- Every element found in nature has a unique mass
- Elements are combined to produce compounds with distinct masses and physical properties
- Compounds can be detected by mass spectrometry and thus their masses measured
- If a compound mass can be measured with sufficient accuracy, a unique elemental composition can be inferred – the benefit of exact mass

Exact Mass and Elemental Composition

CO	=	27.9949
N_2	=	28.0061
C_2H_4	=	28.0313

- These elemental combinations have the same nominal mass but different exact mass
- A nominal mass measurement cannot distinguish these
- If any compounds differ in their elemental compositions by substitution of any of these elements, then the exact mass measurement will show this

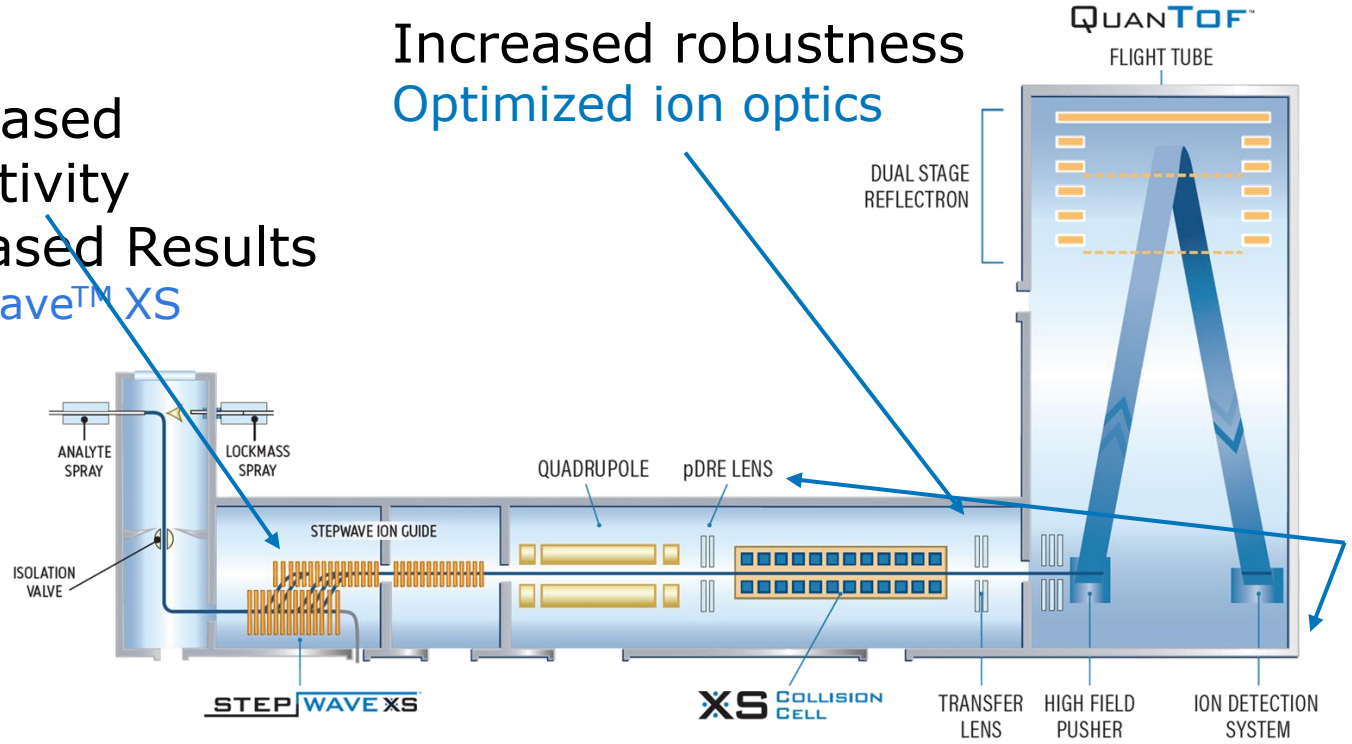
- Measurement of mass to 4 decimal places
- High confidence in confirming expected compounds
  - Distinguishes them from compounds of similar mass
- Confirmation of elemental composition
  - Identification of unknown compounds
  - Patent support and scientific journals

Overview of New Technology Xevo™ G3 Q Tof

Xevo™ G3 QTof

Increased sensitivity
Unbiased Results
StepWave™ XS

Increased robustness
Optimized ion optics



16,000 m/z Calibration
Native MS & New Modalities

Increased quan/qual dynamic range
Targeted and non-targeted

Overview

- Food authenticity and safety
  - Food supply network
  - Why adulterate?
- Honey a unique food commodity
  - Composition of honey
- Determination of botanical origin
  - Non-targeted profiling
  - Metabolomics study & results
- Introduction of REIMS
  - Rapid profiling technique for meat speciation
- Summary

Some history…

- **“Food fraud”** has been around for centuries with examples such as the adulteration of imported tea with iron filings in the 19th century
- 2008 “melamine scandal”
- 2013 “horse meat scandal”
- Tip of the iceberg!
- New vocabulary
  - Food Security
  - Food defence
  - Food fraud
  - Threat Assessment Critical Control Point (TACCP)

But why adulterate?

- **Mainly for financial gain**
  - Product extension
  - Improve perceived quality attributes
  - Brand/product substitution
  - Reduce manufacturing costs
- May also be **accidental** or **malicious**
  - Ignorance
  - Reputation damage
  - Terrorism

How can this happen?
(Global) food supply chain vulnerability

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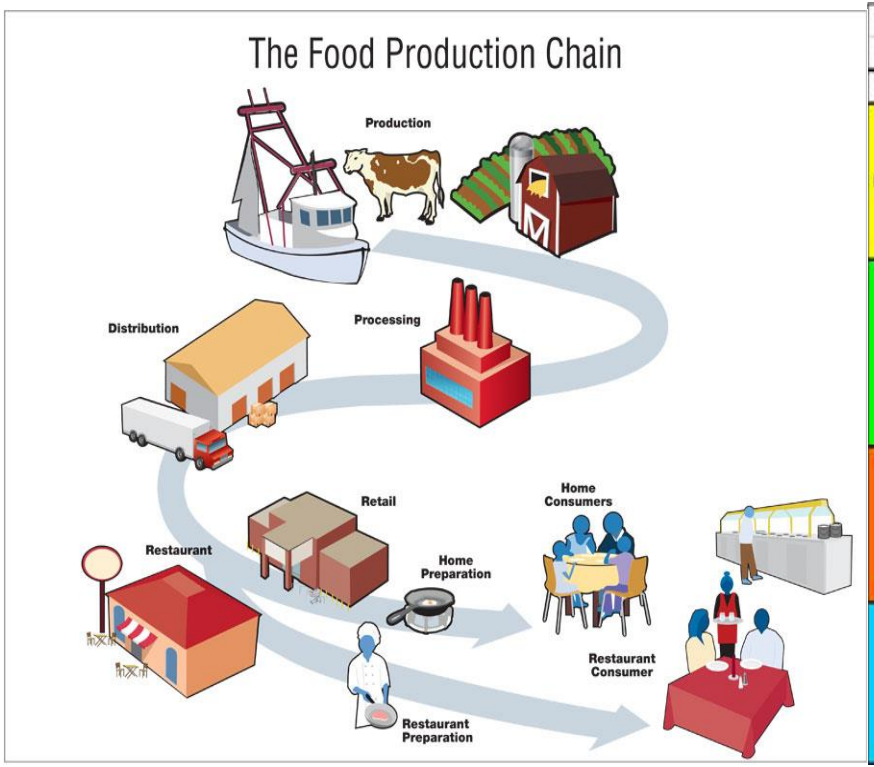

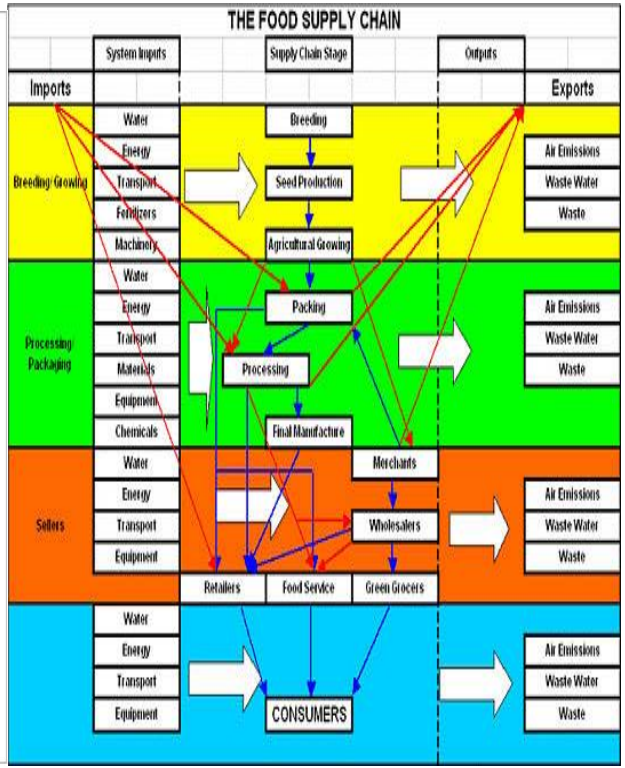

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Mars recalls chocolate in 55 countries

23 February 2016 Business

Chocolate maker Mars has ordered a recall of chocolate products in 55 countries.

In the UK the products affected include funsize Mars and Milky Way bars and boxes of Celebrations.

In the Netherlands Mars and Snickers bars were also affected.

It comes after a customer in Germany found bits of plastic in a Snickers bar in January. The plastic was traced back to the Mars factory in the southern town of Veghel in the Netherlands.

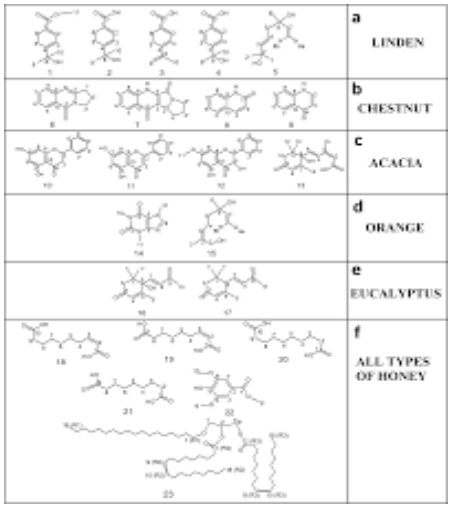
Determination of the botanical origin of honey

Honey – metabolomic profile

Every honey is an unique, complex matrix made up of plant secondary metabolites including:

- *flavonoids*
- *phenolics*
- *sugars*
- *natural compounds from bees*

- This “fingerprint” will differ depending on
  - *region*
  - *forage targets*
  - *biologi rt*



What is Manuka honey?

- Produced in New Zealand by bees that pollinate the native manuka bush
  - *Leptospermum spp* is a shrub native to New Zealand and southern Australia
- It has been reported to have **potent biocidal activity**
- Unique non-peroxide activity (NPA) – methylglyoxal (MG) from conversion of dihydroxyacetone *(++manuka flower nectar)*
- Suppliers have to demonstrate activity for labelling and to attract premium price
- Reputed health benefits; SIBO, acid reflux, acne, eczema, MRSA, burns, wounds, ulcers, sinusitus…

Manuka Honey – potential for economic adulteration

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Life & style › Food & drink

Manuka honey costing £55 a jar creates a buzz

Cornwall-made manuka honey has healing qualities, say creators

Steven Morris
guardian.co.uk, Monday 18 May 2009 14.00 BST
Article history

Mail Online

Home | News | Sport | TV&Showbiz | Femail | Health | Science&Tech | Money | Debate

Health Home | Health Directory | Health Boards | Diets

The treatment that's the bee's knees

By PETA BEE, Daily Mail

Last updated at 10:20 13 July 2006

Comments (7) | Add to My Stories

Medicine may be increasingly high-tech, but the latest wonder treatment which is being offered to patients is - honey.

Last week, it was announced that bandages soaked in manuka honey are to be given to mouth cancer patients at the Christie Hospital in Manchester to reduce their chances of contracting the MRSA superbug and to lessen wound inflammation following surgery.

This is just the latest study investigating this particular type of honey's healing powers.

It is used routinely at the Manchester Royal Infirmary for dressing wounds, and other research has found it can fight gum disease, ease digestive problems and soothe sore throats.

All honey contains hydrogen peroxide, a substance once used as a wound disinfectant in hospitals - it comes from an enzyme that bees add to nectar.

Honey analysis

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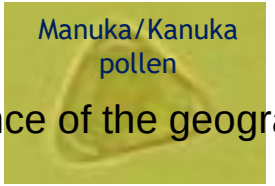


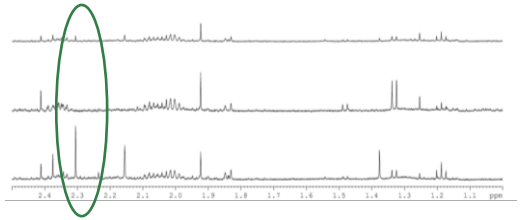
- **Melissopalynology (microscopy)**
  - By studying the pollen in a sample of honey, it is possible to gain evidence of the geographical location and genus of the plants that the honey bees visited
    - *Pollen analysis cannot distinguish between Manuka & Kanuka*

- **Nuclear magnetic resonance profiling**
  - Proton (^{1}H) NMR can detect any metabolites containing hydrogen
  - Signals can be assigned by comparison with libraries of reference compounds, or by 2D NMR

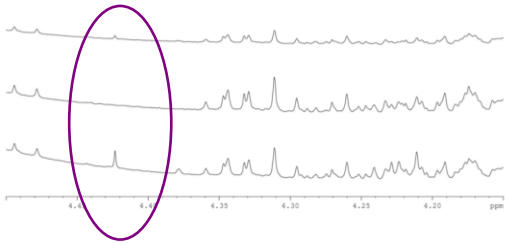
- **Non-targeted HRMS & chemometrics**
  - Identification chemical markers of botanical origin
  - Biochemical markers of Manuka honey have been identified
  - Donarski *et al*. (2010) Identification of botanical biomarkers found in Corsican honey. Food Chemistry 118, (4): 987-994
  - Z. Jandric´ *et al*. (2013) Discrimination of honey of different floral origins by a combination of various chemical parameters, Food Chemistry 89 (2015) 52-59

Profiling of honey - NMR

- Initial ^{1}H NMR results

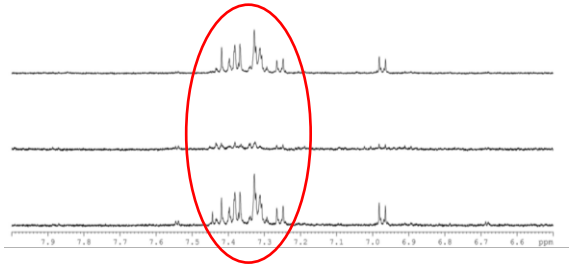


Active ingredient
(methylglyoxal)



Active ingredient precursor
(Dihydroxyacetone)

Upper trace: Non active manuka (kanuka?)
Centre trace: Non-manuka
Lower trace: Active (5+) Manuka



New potential biomarker?

Simultaneous screening of origin, authenticity & activity

NMR spectra crowded with overlapping signals & complicated by spin-spin couplings

Metabolomics study: background

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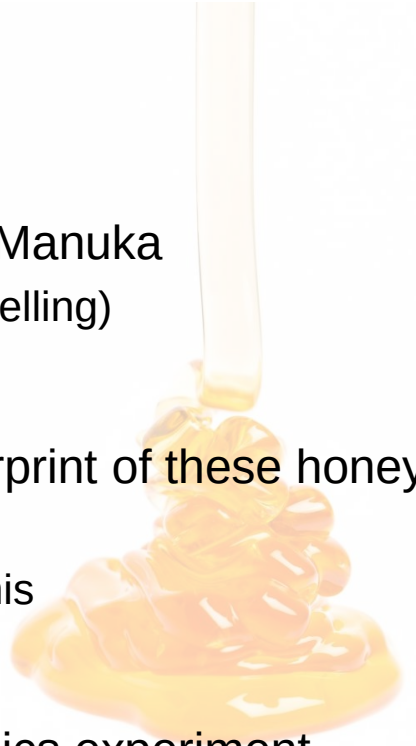
- Commercial honey is either
  - ***Polyfloral*** *– derived form many plant species*
  - ***Unifloral*** *– derived from one plant species*
- Premium price associated to some uniflorals, e.g. Manuka
  - Target for food fraud to occur (e.g. adulteration, mislabelling)
- Need to understand and profile the chemical fingerprint of these honeys to help fight against fraud
  - Different analytical techniques have been used to do this
- One approach: use of MS using a food metabolomics experiment



Honey samples provided

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Fera code	Main botanical origin	Country	Year sampled	
S15-051658	Heather	Norway	2014	
S15-051659	Heather	Norway	2014	
S15-051666	Heather	Norway	2014	
S15-051668	Heather	Norway	2014	
S15-051679	Heather	Denmark	2014e	
S15-051680	Heather	Denmark	2014e	
S15-051752	Heather	Lithuania	2011	
S15-051758	Heather	Lithuania	2011	
S15-051759	Heather	Lithuania	2014	
S15-051687	Buckwheat	Poland	2014	
S15-051688	Buckwheat	Poland	2014	
S15-051689	Buckwheat	Poland	2014	
S15-051747	Buckwheat	Lithuania	2011	
S15-051749	Buckwheat	Lithuania	2011	
S15-051686	Rape	Poland	2014	
S15-051754	Rape	Lithuania	2011	
S15-051760	Rape	Lithuania	2009	
S12-017129	Manuka	New Zealand	2012	
S12-045359	Manuka	New Zealand	2012	
S13-004671	Manuka	New Zealand	2013	
S15-000329	Manuka	New Zealand	2014	
S15-000328	Manuka	New Zealand	2014	
S15-000336	Manuka	New Zealand	2014	
S15-000333	Manuka	New Zealand	2014	
S15-000326	Manuka	New Zealand	2014	
			e = estimated	

Sample preparation

- Honey samples (0.5 g) were diluted with 10 mL 1% of formic acid in methanol/water (1/1, v:v), shaken, sonicated (20 min), and centrifuged using a high speed centrifuge
- Each sample analysed in triplicate (n=3)

LC Parameters
ACQUITY I-Class

Waters™

LC System	ACQUITY I-Class
Column:	ACQUITY BEH C18, 2.1. x 100mm, 1.7 um
Mobile phase A:	10 mM aqueous ammonium acetate
Mobile phase B:	Acetonitrile
Column temp:	45°C
Injection volume:	5 uL
Sample temp:	5°C

Time	Flow rate	%A	%B	Curve
0.00	0.5	99	1	6
0.75	0.5	99	1	6
2.00	0.5	95	5	6
3.00	0.5	95	5	6
6.50	0.5	45	55	6
8.50	0.5	10	90	6
9.00	0.5	10	90	6
9.10	0.5	99	1	6
12.00	0.5	99	1	6

Acquity I UPLC® CLASS

MS Parameters
SYNAPT G2-S*i* HDMS

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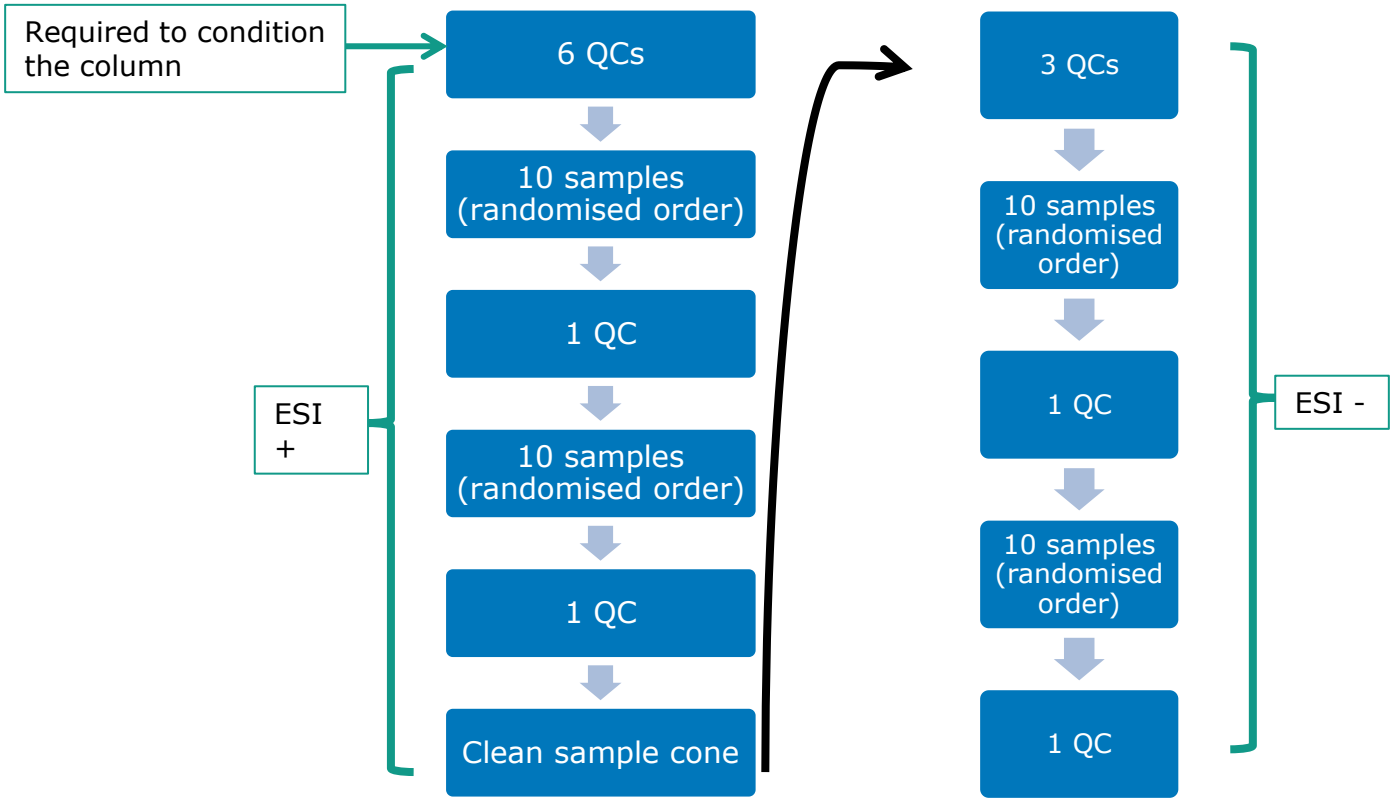
ESI Negative	
Polarity	ES-
Capillary (kV)	3.2
Source Temperature (°C)	130
Sampling Cone	30
Source Offset	80
Source Gas Flow (mL/min)	0
Desolvation Temperature (°C)	600
Cone Gas Flow (L/Hr)	50
Desolvation Gas Flow (L/Hr)	800
Nebuliser Gas Flow (Bar)	5
Acquisition mass range	
Start mass	50
End mass	1200
Experiment Reference Compound Name	Leu enk
Survey Scan Time	0.1
Survey Interscan Time	0
Survey Data Format	
Analyser Mode	Resolution
High energy ramp	
Transfer MS Collision Energy Low (eV)	15
Transfer MS Collision Energy High (eV)	55

ESI Positive	
Polarity	ES+
Capillary (kV)	3.1
Source Temperature (°C)	130
Sampling Cone	35
Source Offset	35
Source Gas Flow (mL/min)	0
Desolvation Temperature (°C)	600
Cone Gas Flow (L/Hr)	50
Desolvation Gas Flow (L/Hr)	800
Nebuliser Gas Flow (Bar)	5
Acquisition mass range	
Start mass	50
End mass	1200
Experiment Reference Compound Name	Leu enk
Survey Scan Time	0.1
Survey Interscan Time	0
Survey Data Format	
Analyser Mode	Resolution
High energy ramp	
Transfer MS Collision Energy Low (eV)	15
Transfer MS Collision Energy High (eV)	55

- **ESI positive and negative modes were run**
- **Tof MS detector**
- **MS/MS approach using high and low collision energies– MS ^{E}**
- **Ion mobility pre-fragmentation = High Definition MS**

Metabolomics experimental workflow



QC samples: *Pool of all of the honeys that are being analysed*

Software solutions used

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Data analysis performed using:

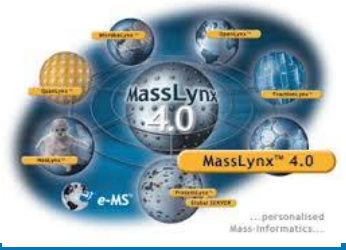
Progenesis QI

www.nonlinear.com

- Algorithm for data alignment, peak picking and peak deconvolution
- Plethora of other tools including peak ID, simple univariate and multivariate statistics

UMETRICS
AN MKS COMPANY

- EZinfo - option for more sophisticated multivariate stats, many “modelling” options as built into SIMCA



- To reverse search, i.e. check raw data (XIC’s)

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ESI Negative Results

Progenesis® QI

HDMS^E (ESI Negative)

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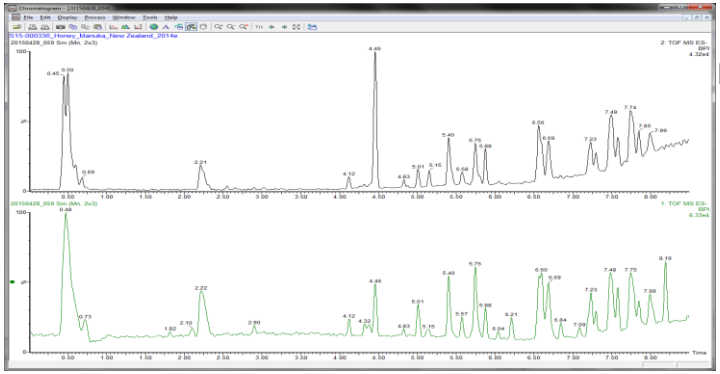
- **Manuka**

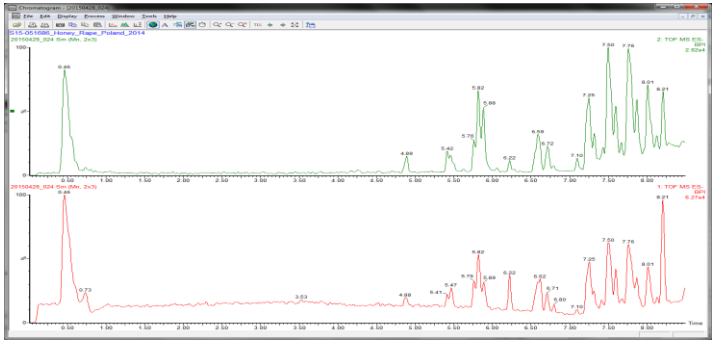


High energy CE ramp

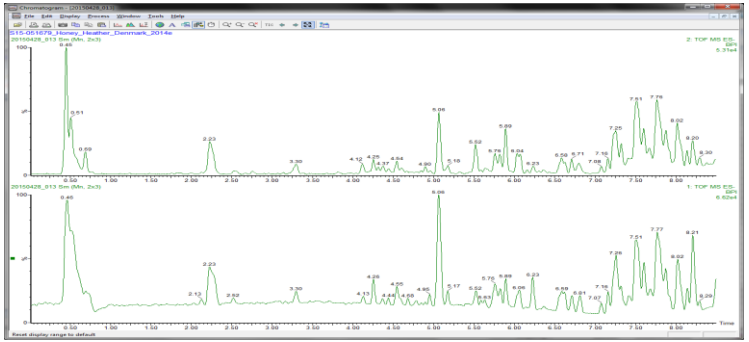
Low energy

- **Buckwheat**

- **Rape**



- **Heather**



HDMS^E (ESI Negative)
Pool Honey

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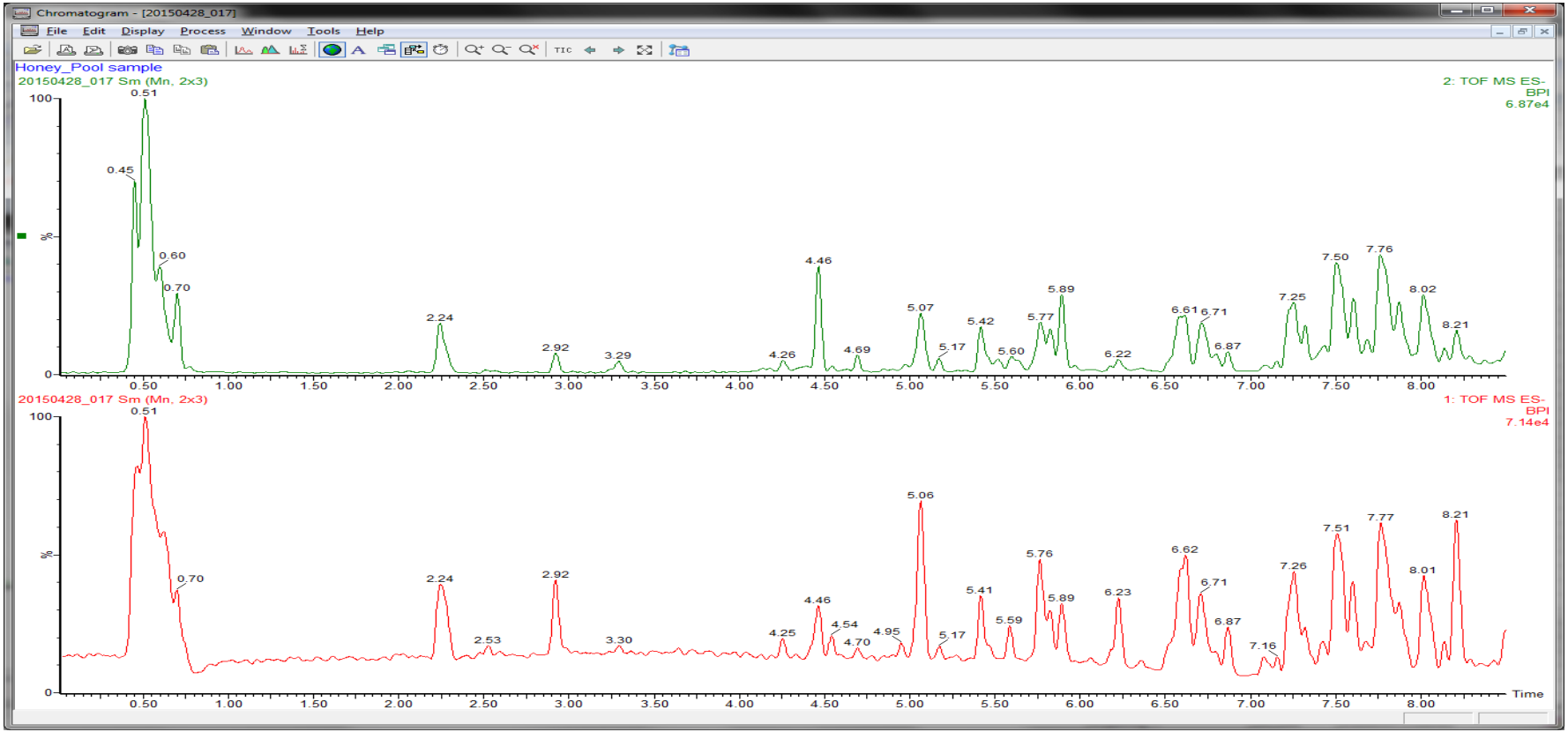


Untargeted Metabolomics

iClass
Xevo G3
Waters conect

LC-MS^E

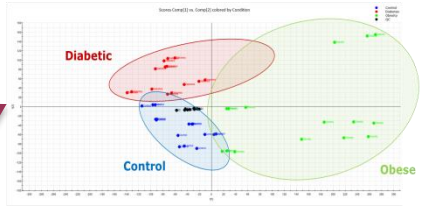
Fragment Ion Library Plugins
METLIN
NIST
Metabolic Profiling CCS

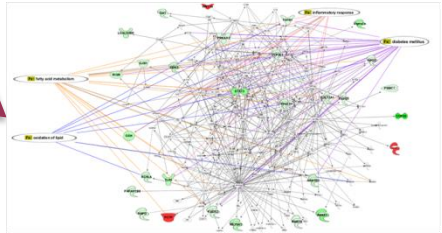
Progenesis QI

Progenesis Metascope (SDF)
HMDB
ChemSpider
Other

Theoretical Fragmentation

EZ Info

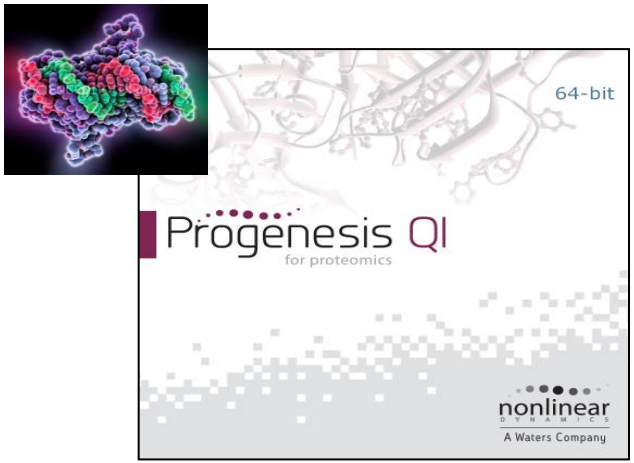




Pathway Analysis
IGA - IMPaLA

The Progenesis range

- For discovery ‘Omics analysis of LC-MS data



- QI: small molecules
- QI for proteomics: large molecules (proteins)
- **Q**uantify and **I**dentify

Progenesis – Similar, easy to follow workflows

Waters™

Progenesis QI

Metabolomics/Lipidomics

Alignment

↓

Peak Detection

↓

Deconvolution

↓

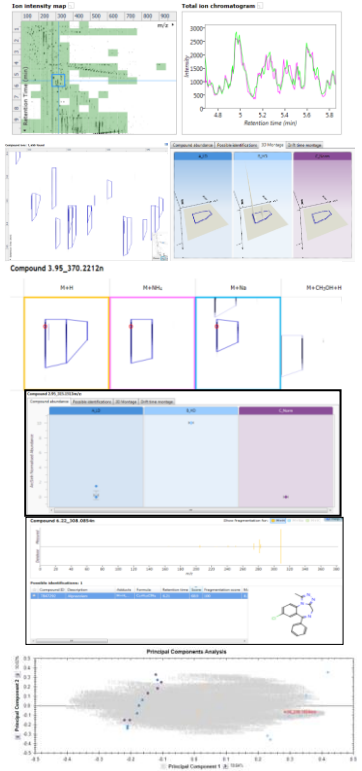
Compound Quantification

↓

Statistics

↓

Identification



Alignment

↓

Peak Detection

↓

Peptide Quantitation

↓

Identification

↓

Protein Quantitation

↓

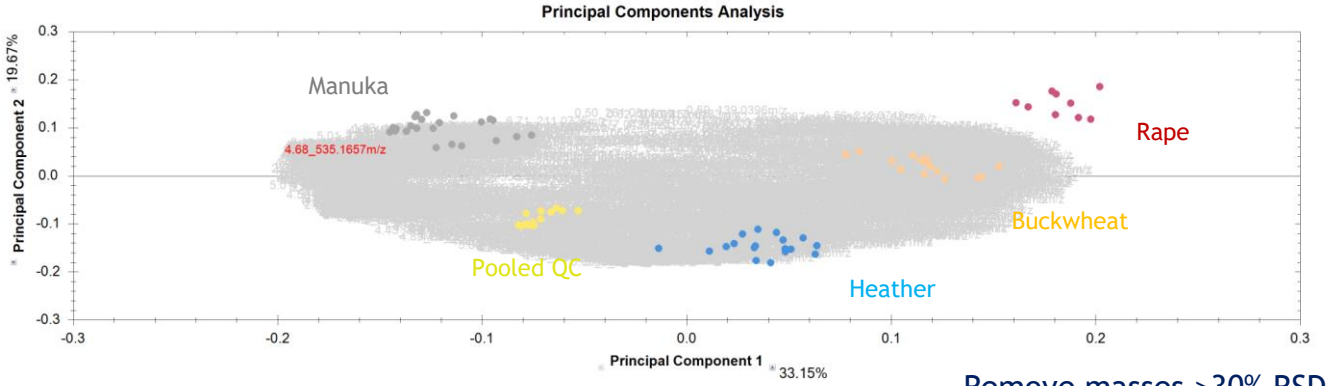
Statistics

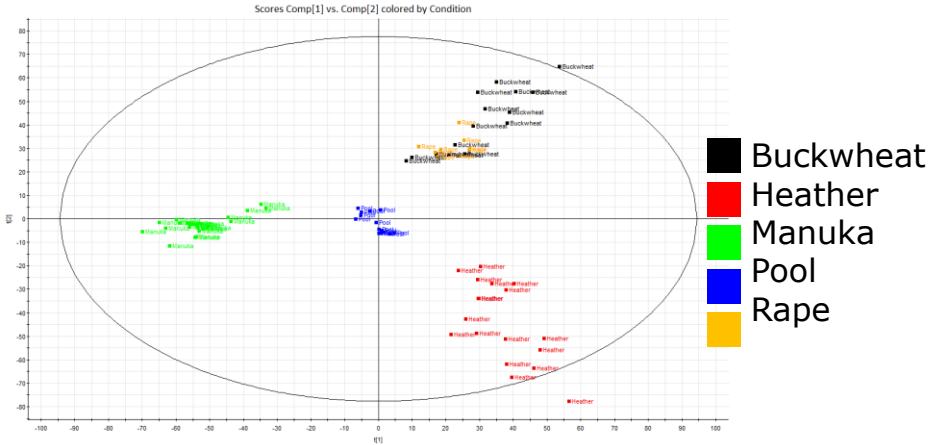
Progenesis QI for proteomics

Proteomics

Pathway Analysis

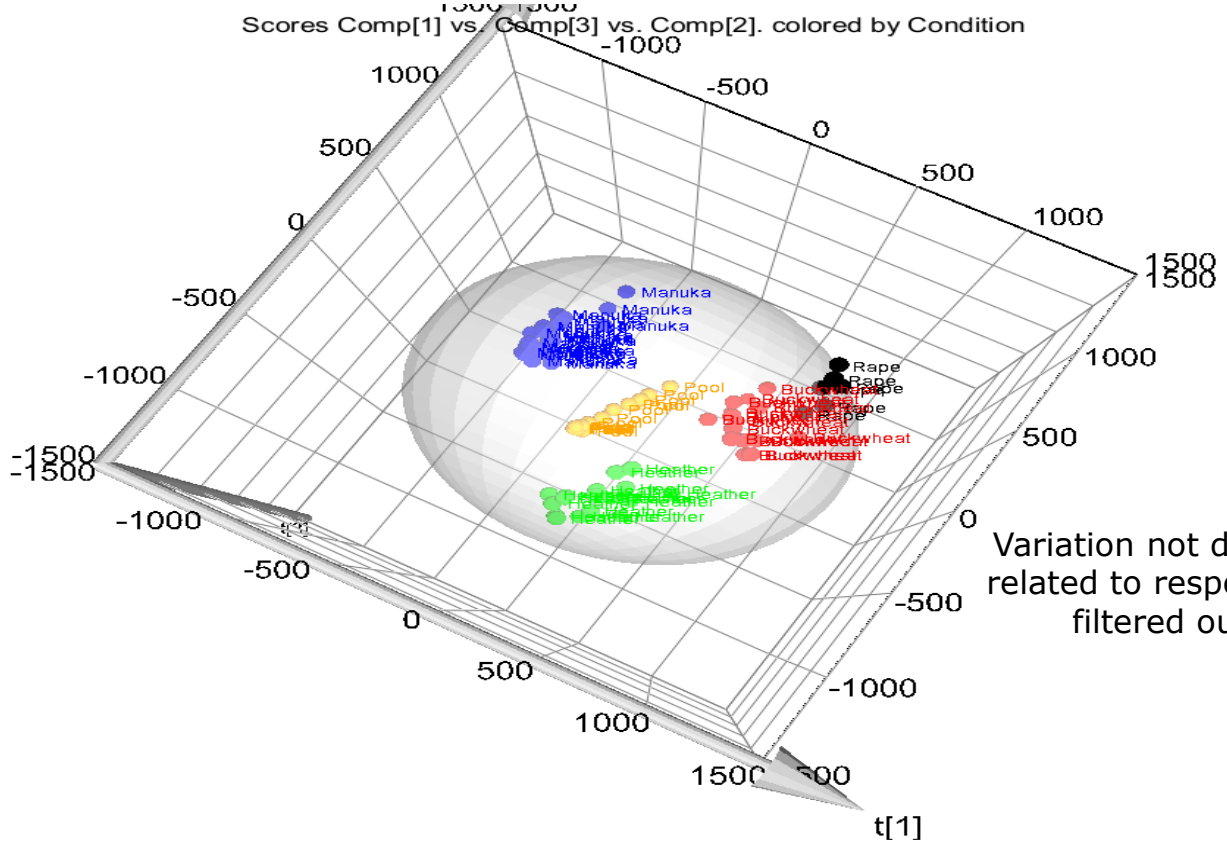
PCA Plots (unsupervised)





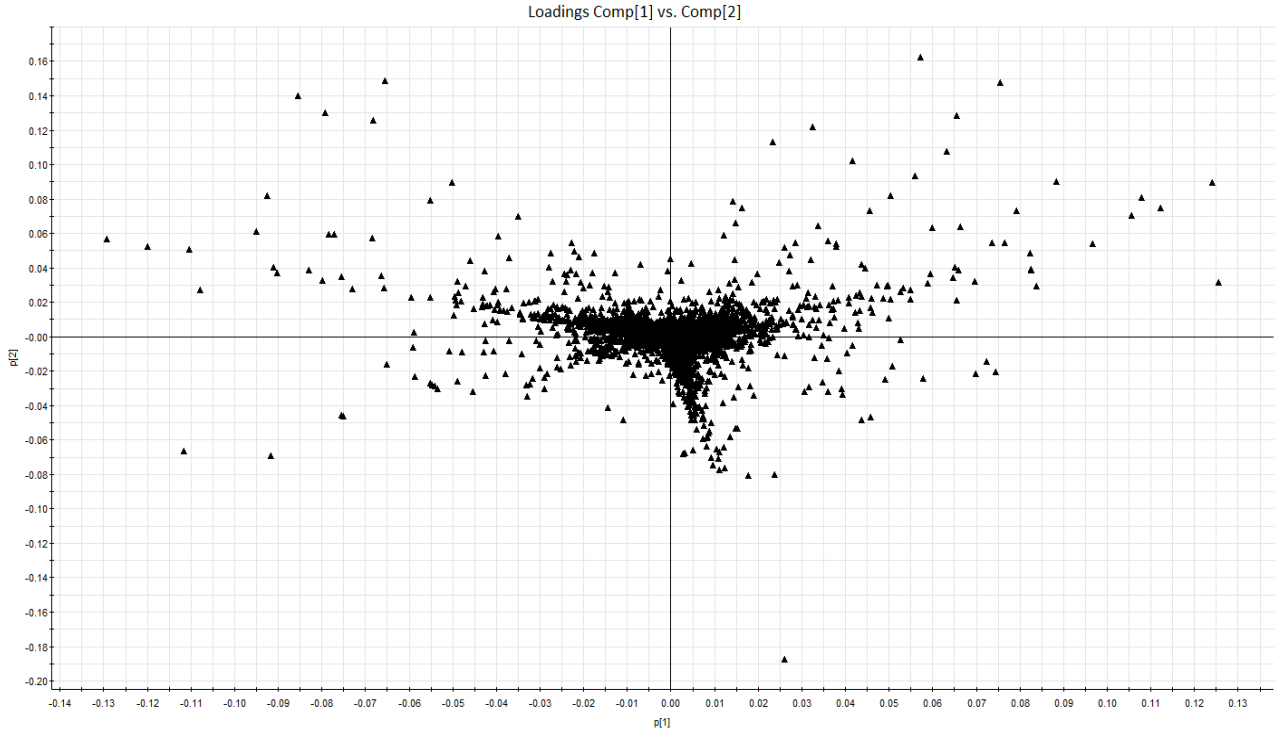
Remove masses >30% RSD between sample replicates

OPLS-DA scores plot (Pareto scaled)



Variation not directly related to response is filtered out

Pareto loadings plot

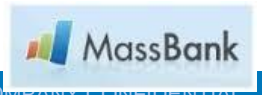


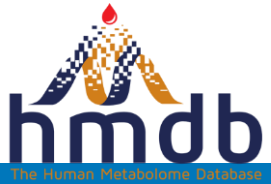
Searching for Manuka markers – other strategies: S-Plot

Searching for unique markers

Waters™

- Experimental design: comparison of different floral origins against all others
- Filtered masses on **P value <0.0001** and **mean abundance >5 fold**
- Applied HMDB database (contains accurate mass MS data) to remaining masses for first stage annotation (identification)
- Also used Metlin (https://metlin.scripps.edu/index.php) and MassBank databases (http://www.massbank.jp/) to annotate masses of interest.





Website-based Food Databases

Waters™

- Human Metabolome Database

- **FooDB** (http://www.foodb.ca/) is a **food component database**
  - Will eventually provide information on over **28,000** food components and food additives, including many of the constituents that give foods their flavour, colour, taste, texture, and aroma

- **Phenol Explorer** (http://phenol-explorer.eu/)
  - Contains values for **500 dietary polyphenols** and their known human metabolites in over 400 foods

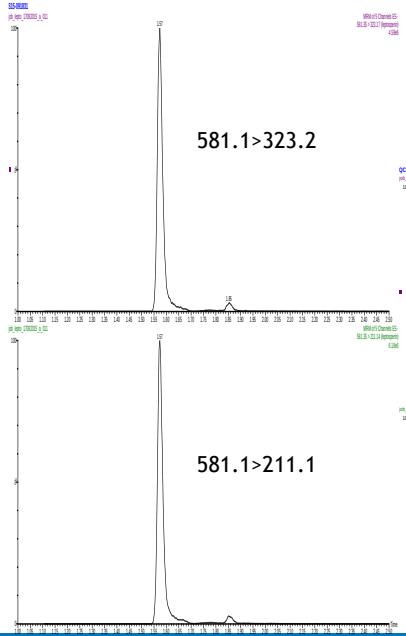
- ***Nutritional Epidemiology*** *(PhenoMeNep)*
  - *Upcoming database, called PhytoHUB, will contain a comprehensive inventory of dietary phytochemicals and their human metabolites, using structures obtained from both previous publications and in silico predictions*

Confirmation Leptosperin

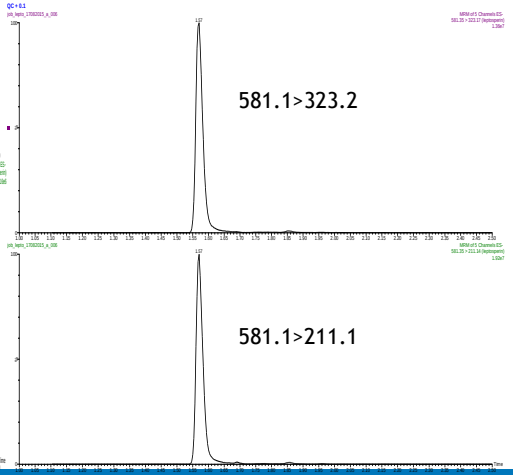
Waters™

- Using **targeted** metabolomics
  - UPLC-MS/MS system : **Waters Xevo TQ-S**

Analytical standard, RT: 1.6 mins, Ion ratio: 0.72



Manuka honey sample, RT: 1.6 mins, Ion ratio: 0.69



Non Manuka honey sample