The development of small molecule profiling technology for the detection of complex food fraud

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Where is Keele University?

- Between Manchester and Birmingham
- Approximately 3 hour drive to London
- Keele was granted to the Knights Templar by King Henry II in 1168-69
- Owned by the Sneyd family from mid 15th Century until late 1940s
- Keele University (UCNS) opened in 1950

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Food fraud

- Deliberate

- Adulteration – adjusting the product
  - e.g. horse meat scandal

- Substitution – replacing a product with a less expensive one
  - e.g. refill whisky bottle

- Misrepresentation – labelling/advertising a product incorrectly
  - e.g. labelling cage eggs as organic

1. Fake versions of branded vodka, such as Smirnoff, containing methanol or anti-freeze – more than 35,000 counterfeit bottles were seized at Dover.
2. Offal taken from slaughterhouses to be used in pet food has been diverted for use in catering as big events.
3. Shellfish sold from locations known to be a health risk – such as sewage contamination.
4. Takeaways which have replaced lamb with cheaper off-cuts of meat – one in five were found to be at fault.
5. Pubs and restaurants found selling meat and produce labelled as organic and local which comes from cheaper sources.
6. Olive oil mislabelled as extra virgin or adulterated.
7. One in ten consignments of basmati rice bulked up with other rice.
8. One in three jars of manuka honey fail to meet the label claims covering quality or botanical origin.
9. Nineteen out of 78 packs of oregano were bulked up with cheap ingredients such as olive leaves.
10. Deadly diet pills are flooding back online.
11. Millions of cheap cage eggs have been mislabelled as organic or free range.

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FOOD CRIME

annual strategic assessment 2016

Food Standards Agency
food.gov.uk

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Food fraud

- Cost to the global economy estimated to be up to $40 billion per year
- Can have serious medical effects i.e. orange juice adulterated with grapefruit juice
- Focus of my research group is to investigate complex food frauds that are difficult to detect using current methods
- We use the biology of the foodstuff to tell us something about its history

Food fraud - metabonomics

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The comprehensive profiling of low molecular weight (typically <1000 Da) molecules in organic tissues and biofluids and the observation how this profile changes due to disease, intervention or environmental influences

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Food fraud - metabonomics

- Untargeted analysis
- Large data sets
- Generation of markers (RT-m/z pairs)
- Markers arranged in a table with the intensity of each recorded for each sample
- Multivariate statistical analysis is carried out to identify the key differences between the markers for each sample set

Metabonomic workflow

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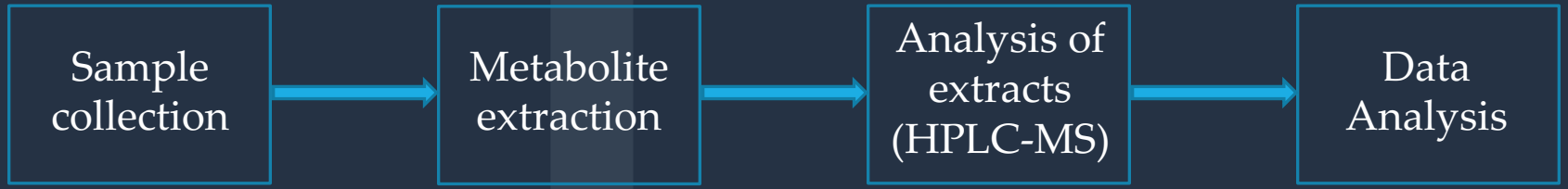
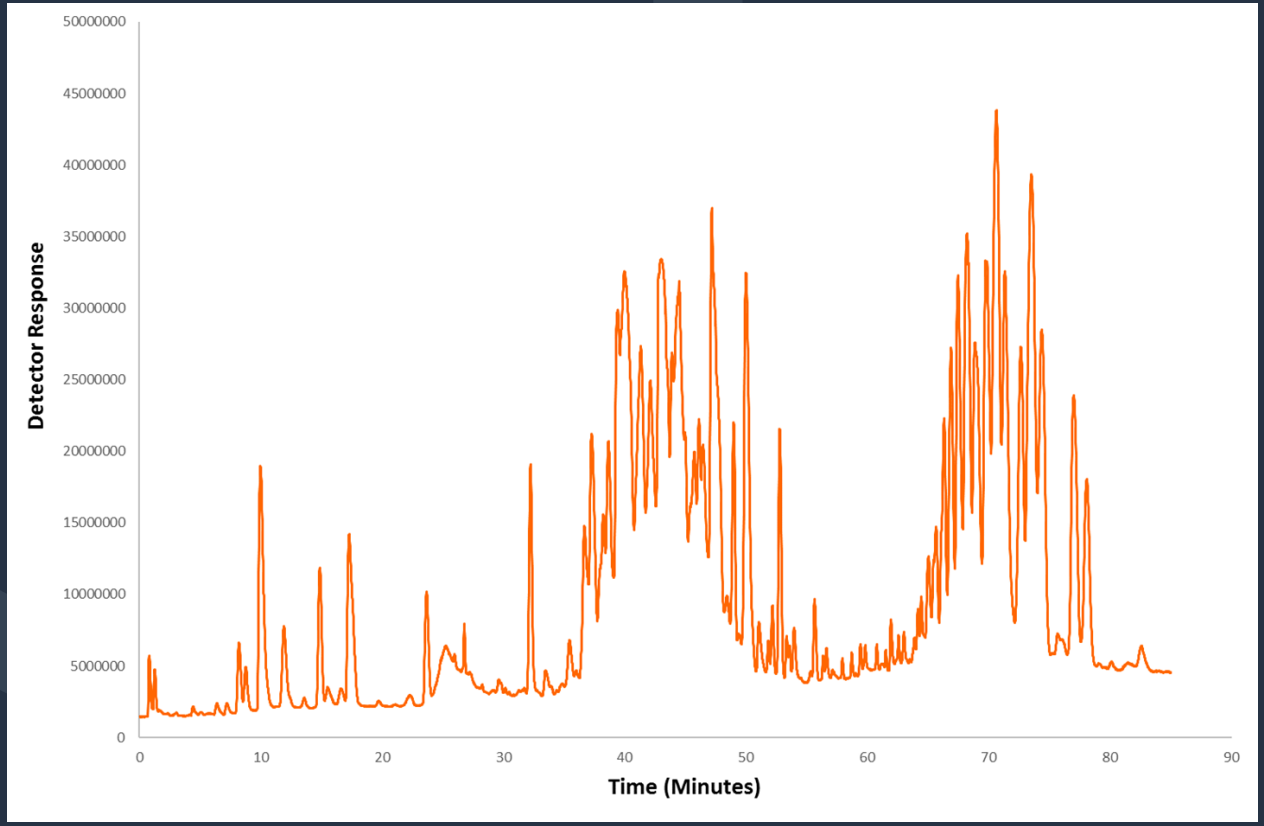


- Untargeted analysis
- Experiments need to be carefully designed so as to ensure useful results are obtained
- Polar and non-polar extractions carried out
- QC samples are vital
- Careful consideration of the data analysis methods used

Food fraud - metabonomics



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How do we ensure that the analysis and data is robust?

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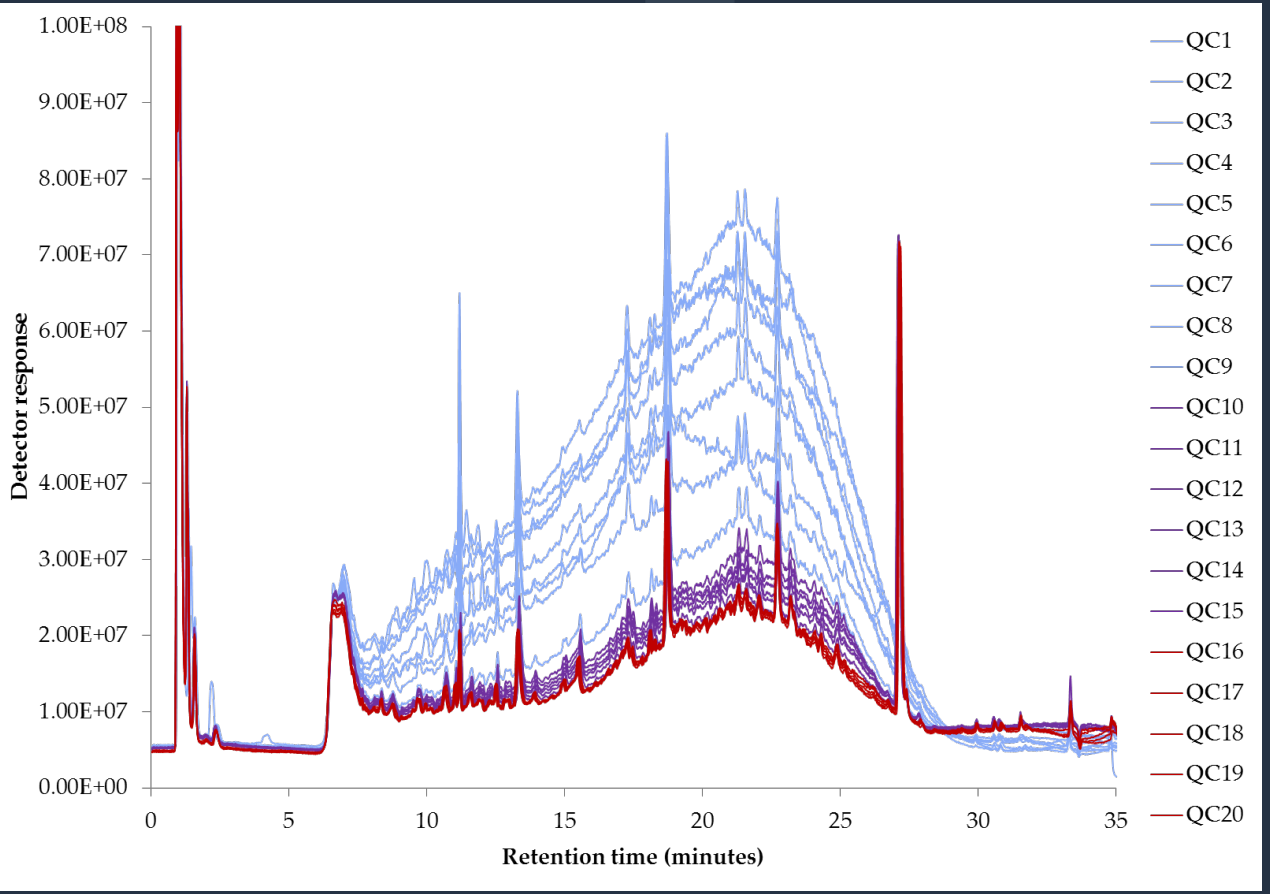
- Column conditioning
- Quality control samples
- Robust approach to statistics – marker generation
- Always refer back to the raw data – marker confirmation
- Use of analytical standards for marker identity confirmation

Column conditioning



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Current research

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- Dead on arrival v normally slaughtered poultry
- Effect of storage time on the profiles of poultry eggs

Dead on arrival study

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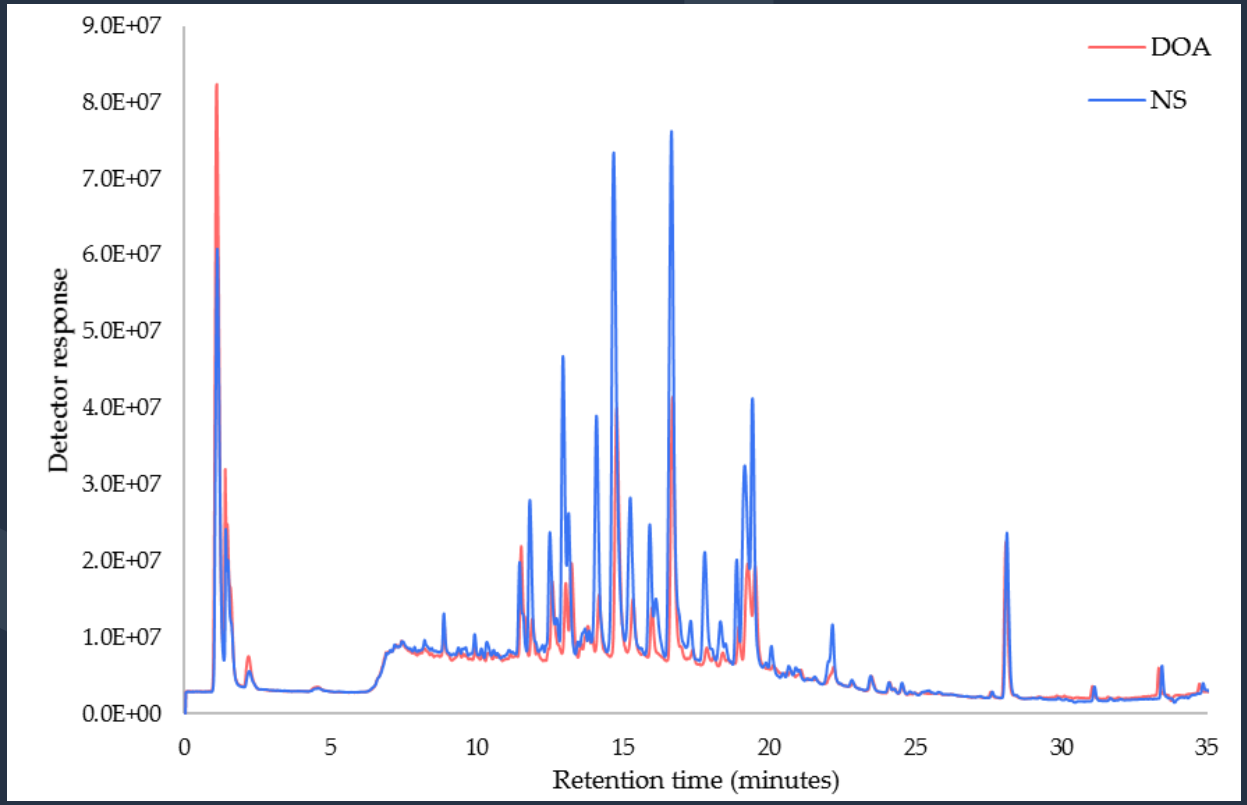
The Animal By-Products (Enforcement) Regulations 2013:

"Any animal found to be dead on arrival to the slaughterhouse must be removed and stained with a colouring agent in order to distinguish it as a product not fit for human consumption“

Dead on arrival study



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- 5 dead on arrival birds and 10 normally slaughtered (non-stunned) birds used
- These were the same breed and from the same flock
- Sent for slaughter on the same day

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Dead on arrival study - liver

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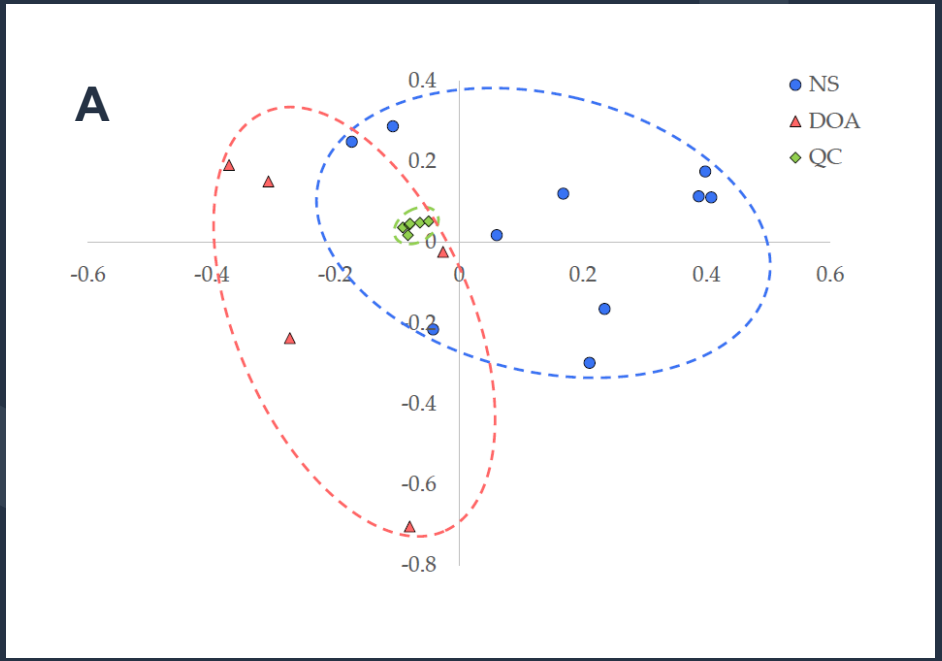
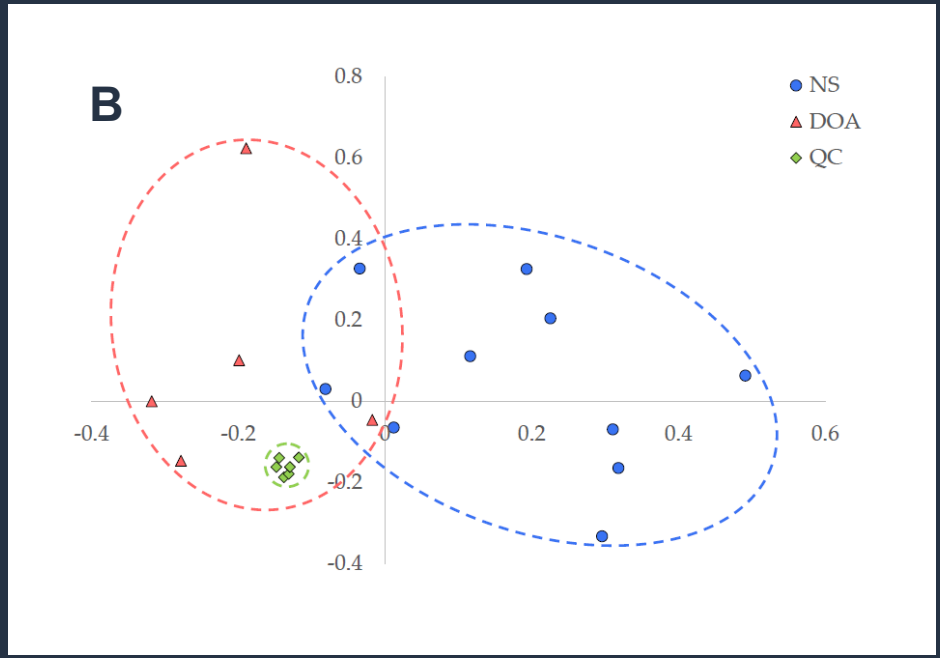
A – positive ionisation, B – negative ionisation





Dead on arrival study - muscle

A – positive ionisation, B – negative ionisation

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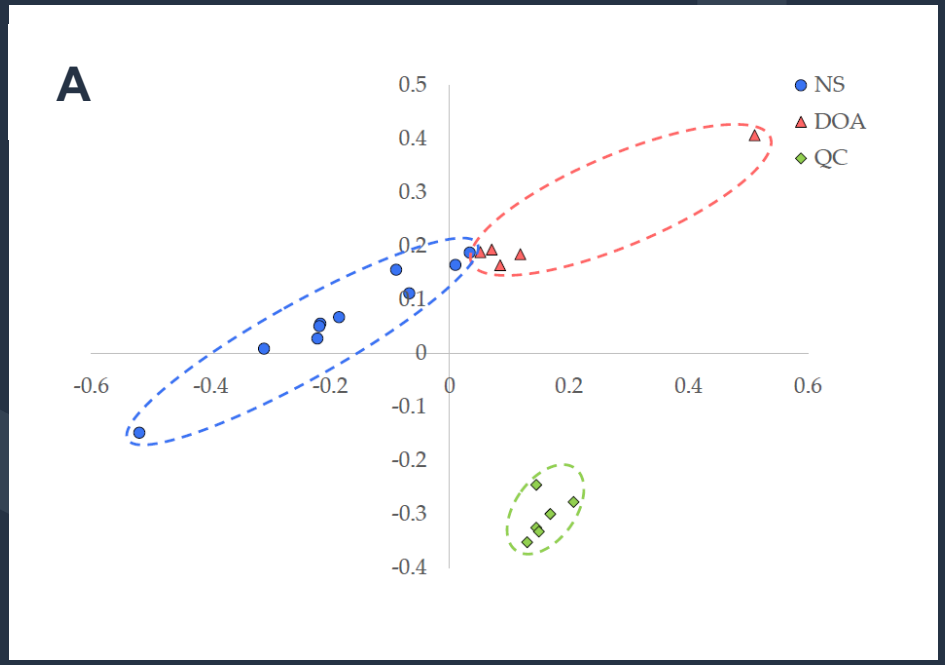

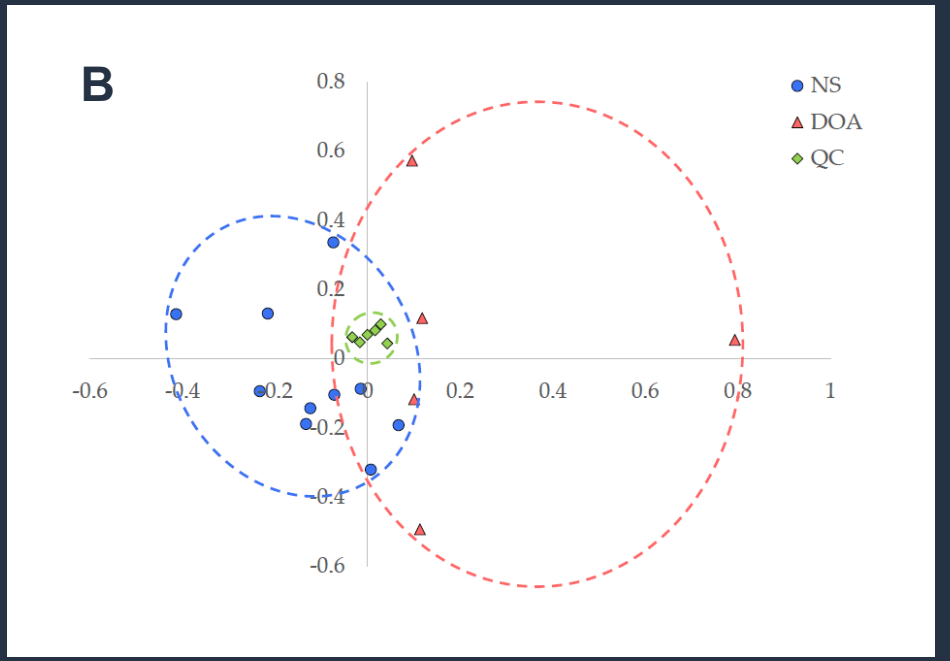

Dead on arrival study - heart

A – positive ionisation, B – negative ionisation

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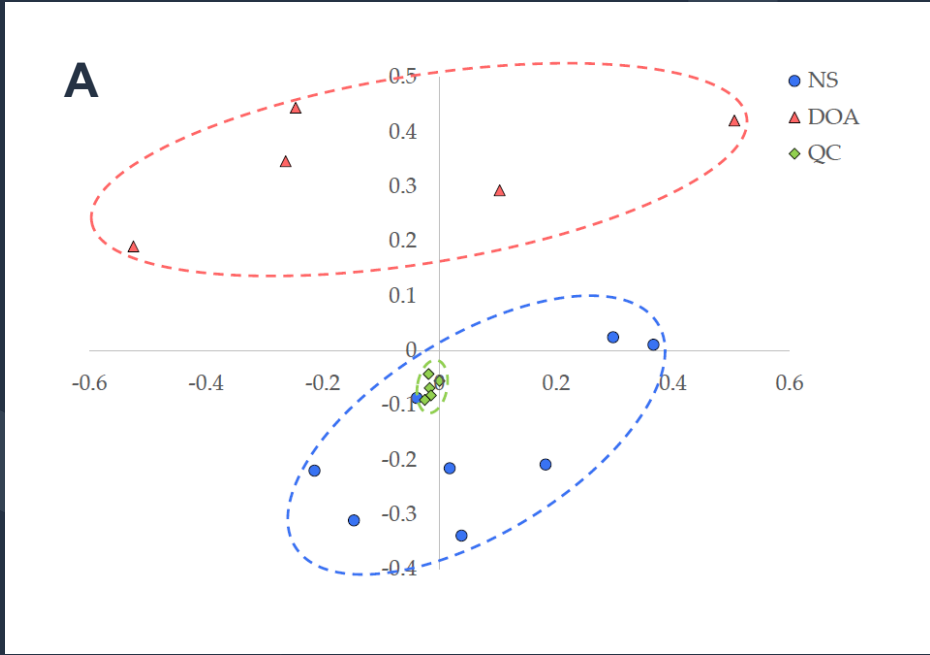

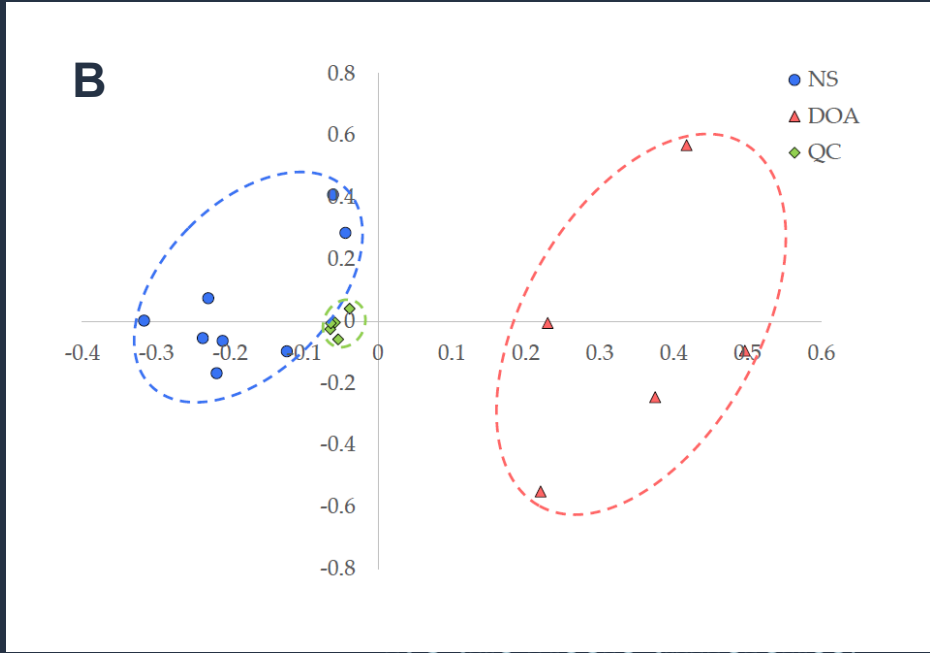

Dead on arrival study - sphingosine

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- Muscle extracts in positive ionisation mode
- Upregulated in normally slaughtered samples
- P-value = 0.009 (normalised data) and 0.015 (raw data)
- CV% = 6.61% (normalised data) and 7.08% (raw data)
- Tentative identification from METLIN database
- Confirmed identification from chemical standard

Dead on arrival study - sphingosine

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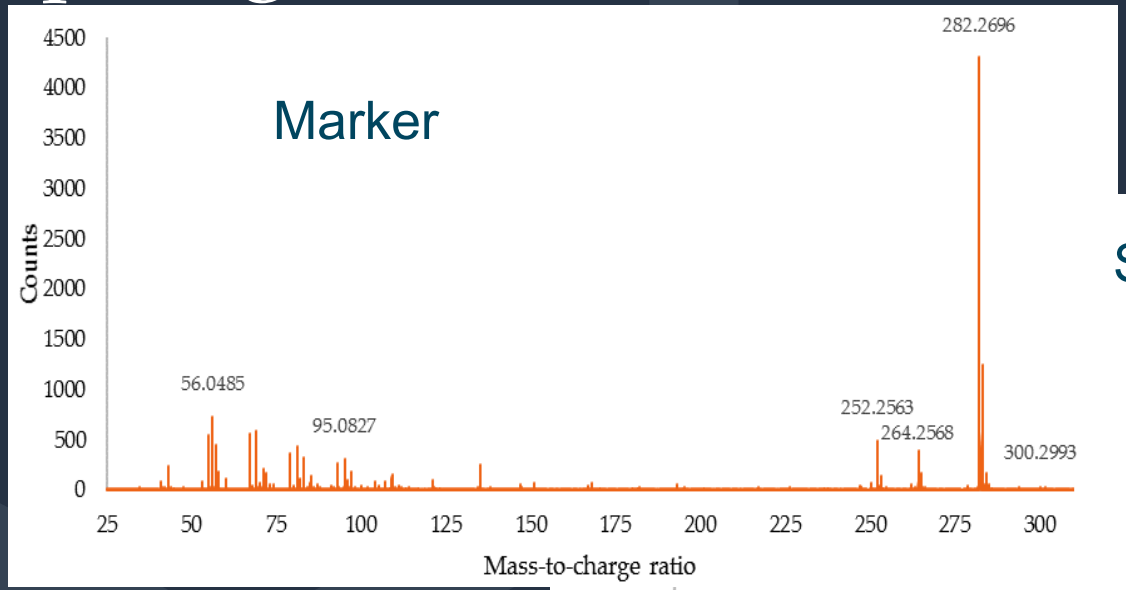

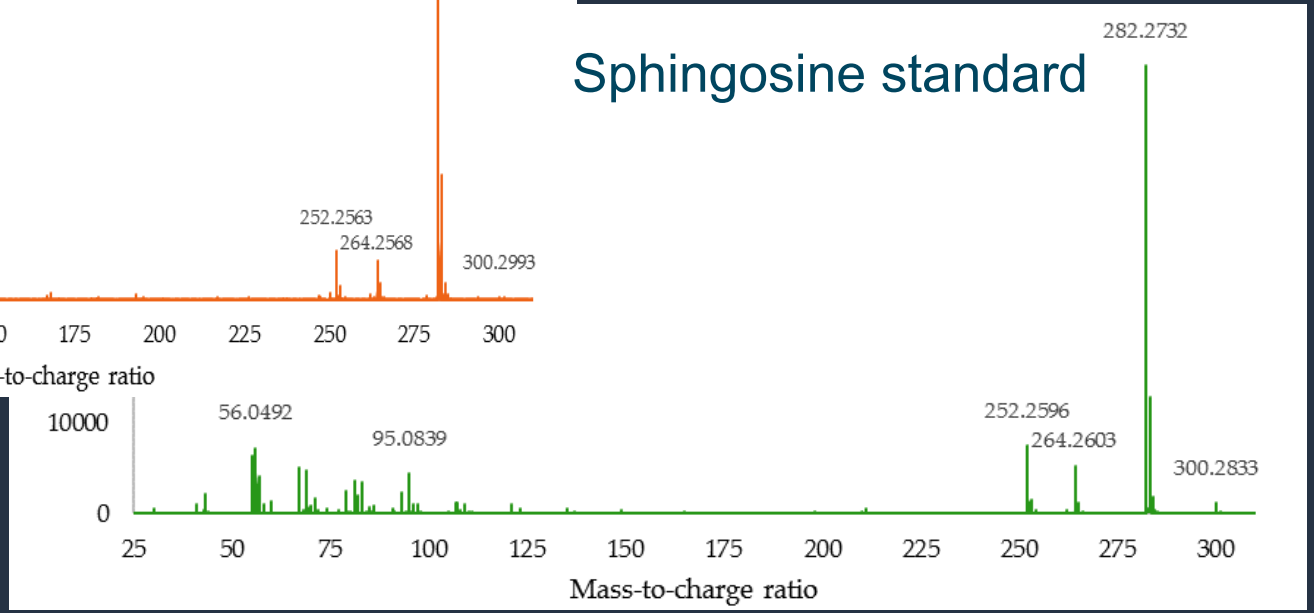

Dead on arrival study

analytical chemistry

Article

Cite This: Anal. Chem. XXXX, XXX, XXX-XXX

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Use of Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry and Metabonomic Profiling To Differentiate between Normally Slaughtered and Dead on Arrival Poultry Meat

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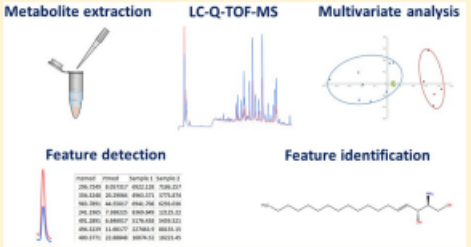
Supporting Information

ABSTRACT: Metabonomic profiling techniques, with established quality control methods, have been used to detect subtle metabolic differences in tissue that could aid in the discovery of fraud within the food industry. Liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS) was utilized to acquire metabolic profiles of muscle, heart, and liver tissue from normally slaughtered and dead on arrival chickens. A workflow including *XCMS Online* for data processing and robust confirmatory statistics was used in order to differentiate between the two sample types. It was found that normally slaughtered and dead on arrival chicken can be differentiated based on the metabolic profile and multivariate analysis. Markers were found to be significantly different between the two sample types in all samples. With the use of the METLIN database and MS/MS analysis of chemical standards, sphingosine was identified as a marker in the muscle tissue samples which may offer potential for the detection of



Anal. Chem., 2017, 89 (22), pp 12131–12136

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Egg storage study

- Eggs must reach the consumer within 21 days post lay
- Date of minimum durability is 28 days post lay

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John Morgan's son, Joseph Morgan, was fined by Llandrindod Wells magistrates for submitting the incorrect date of lay for the family's eggs.

He pleaded guilty to a single charge of failing to comply with the Eggs and Chicks (Wales) Regulations 2010, by not recording the laying date of eggs laid from September 1, 2013 to May 1, 2015.

fined…for submitting the incorrect date of lay

News story

London egg packer and wholesaler found guilty of egg marketing fraud

From: Animal and Plant Health Agency
First published: 11 February 2016

A London egg packer and wholesaler has beer fined for extending 'best before' dates on eggs and breaching compliance notices issued by APHA egg marketing inspectors.

fined for extending 'best before' dates on eggs

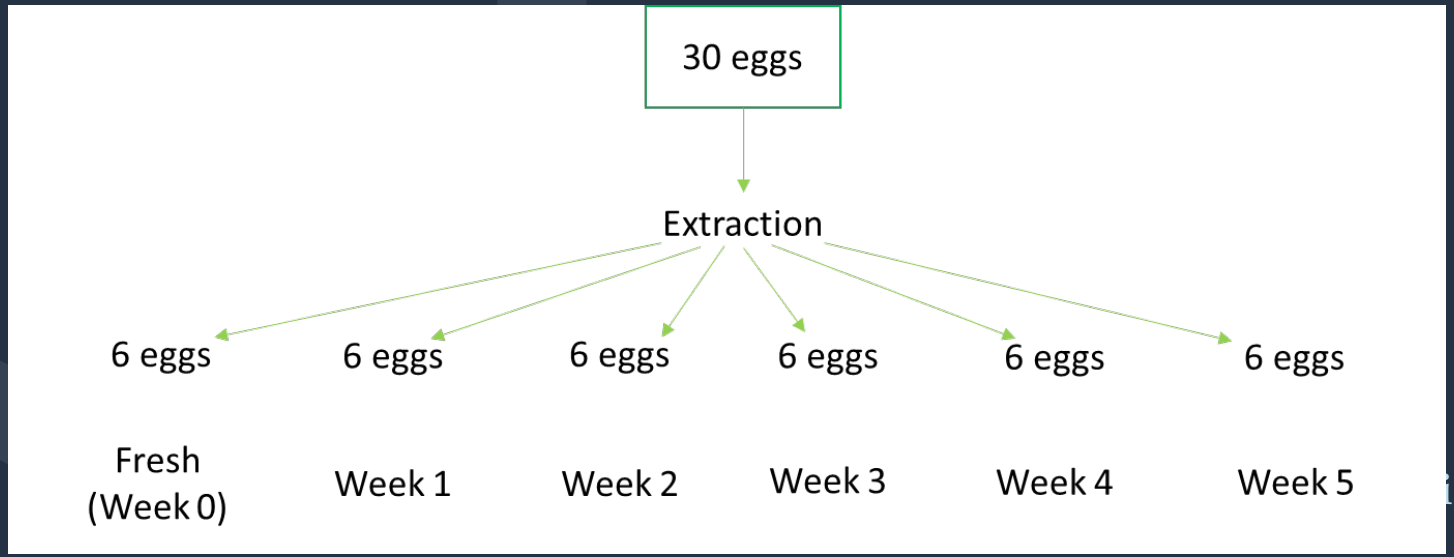
The court was told that Cavill's company, G&L Cavill and Son, had supplied eggs to a shop in Dryslwyn. A Defra egg inspector called at the shop and tests showed that some eggs packaged as free range were, in fact, cage eggs. The 'best before' dates on the eggs were also wrong.

Cavill, of Trimsaran Road, Llanelli, was fined £800 for each of the first two offences relating to the age of the eggs. She was fined £2,000 for the Food Safety Act offence, which related to the misdescription of the eggs. She was also ordered to pay full costs of £1,203.48.

fined £800 for each of the first two offences relating to the age of the eggs

Egg storage study

- 30 eggs collected from Hy-line brown hens
- 6 eggs were extracted on day of collection (0 weeks storage)
- Remaining 24 eggs stored at 23° C
- Organic liquid extraction of 6 eggs every week for 5 weeks



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ifference.

Egg storage study

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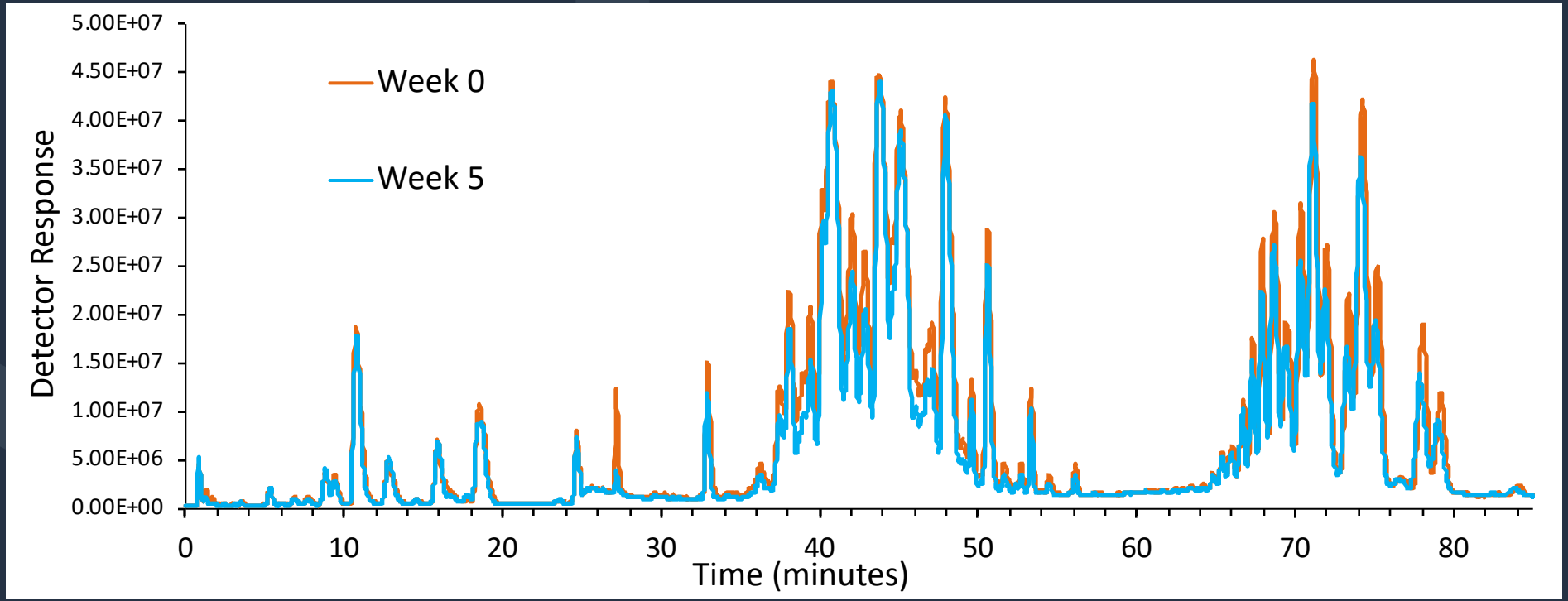
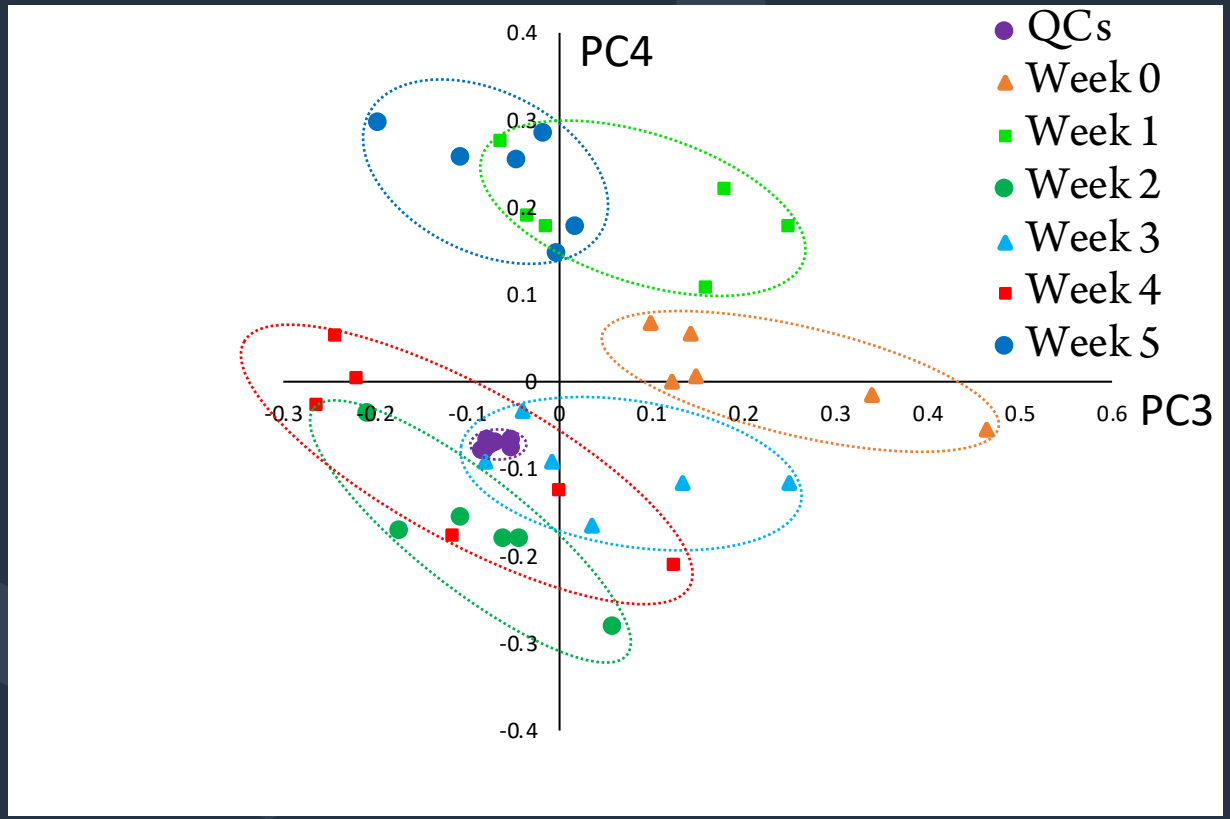


Egg storage study



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- QC samples tightly clustered
- Fresh eggs form own separate group
- Most difference between first few weeks

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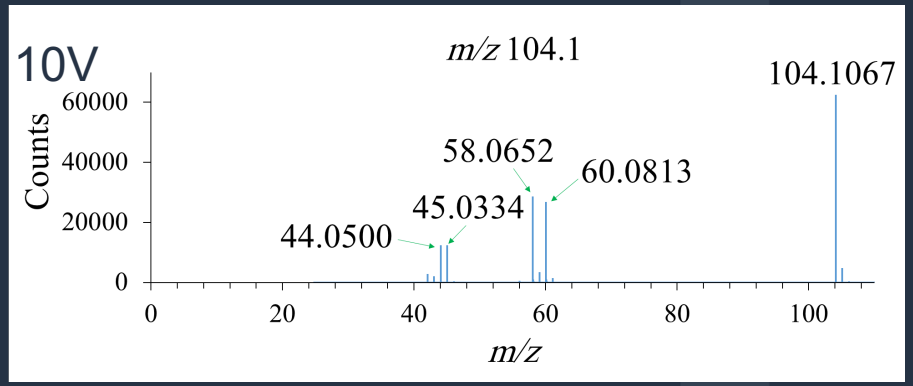
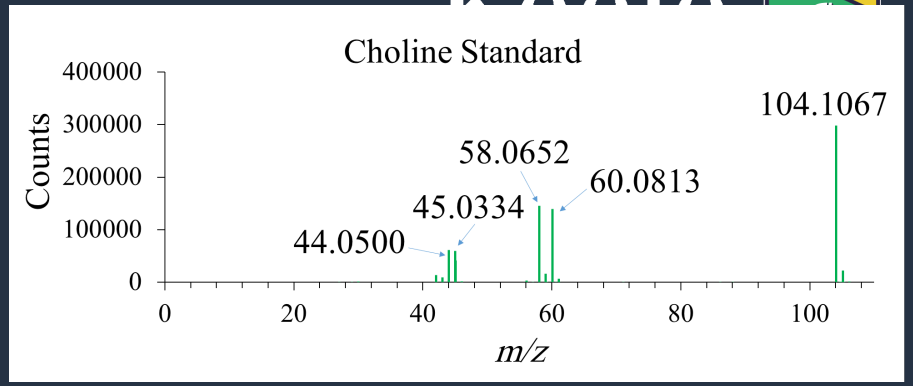
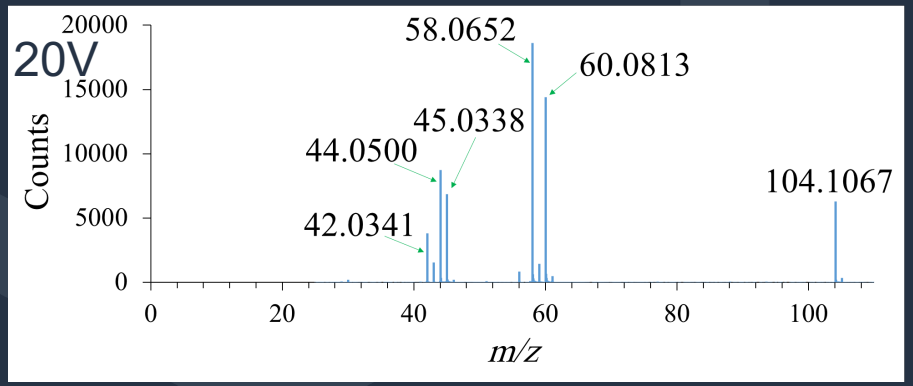
Egg storage study

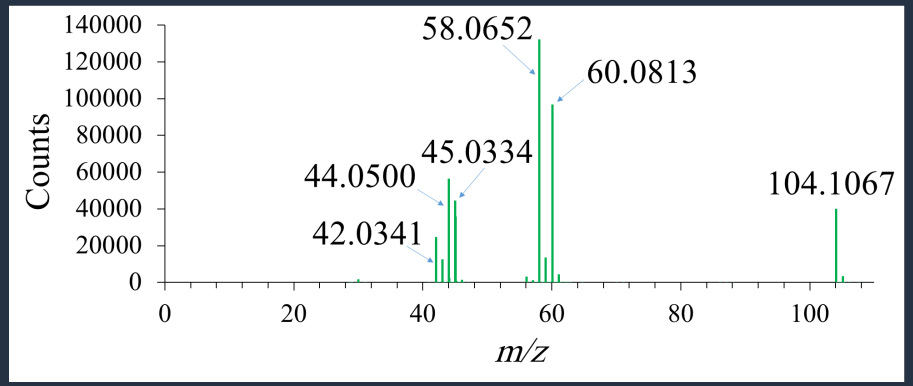
- METLIN – find metabolite matches for potential formulae
- METLIN – comparison of mass spectra between metabolite matches and potential biomarkers
- Compound 104.1 m/z predicted formula $C_5H_{13}NO$ with score of 98.85
- Was then putatively identified via METLIN as choline
- Identification of choline confirmed through use of a chemical standard

Egg storage study

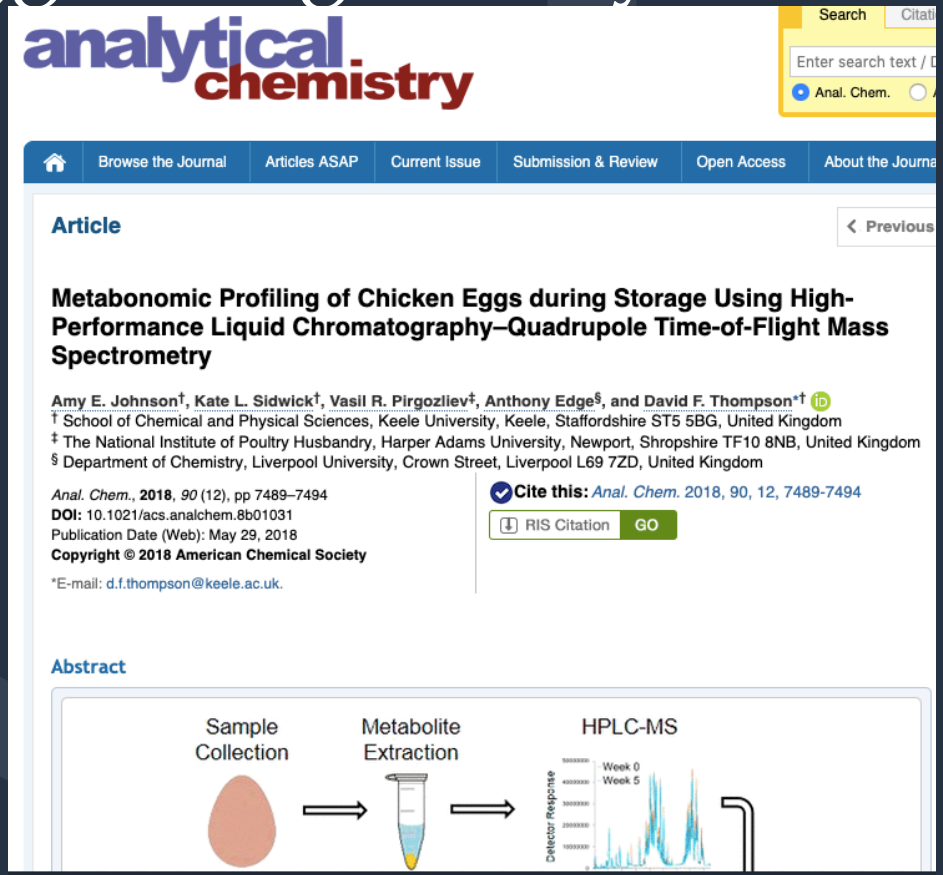








Egg storage study



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Article

Previous

Metabonomic Profiling of Chicken Eggs during Storage Using High-Performance Liquid Chromatography–Quadrupole Time-of-Flight Mass Spectrometry

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Anal. Chem., **2018**, *90* (12), pp 7489–7494

DOI: 10.1021/acs.analchem.8b01031

Publication Date (Web): May 29, 2018

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Cite this: *Anal. Chem.* 2018, 90, 12, 7489-7494

RIS Citation GO

Abstract

Sample Collection

Metabolite Extraction

HPLC-MS

Week 0

Week 5

Detector Response

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Anal Chem, 2018, 90 (12), pp 7489–7494

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Conclusions

- A metabonomic approach to food fraud has been used to investigate dead on arrival poultry and the age of an egg
- Both studies have shown that this approach can be used to identify molecular differences between the sample types
- Markers have been identified and confirmed by comparison with an analytical standard
- Many more markers were present but could not be identified
- Next step is to carry out targeted studies to confirm these results

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Acknowledgements

- Kate Sidwick (left) – Dead on arrival project
- Amy Johnson (right) – Egg age project
- Luisa Pereira – ThermoFisher
- Tony Edge – Agilent
- Vasil Pirgozliev – Harper Adams University

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BBSRC bioscience for the future

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Thank you for listening

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