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Food and the Marine

Miniaturization and Automation of Dutch mini Luke extraction of Pesticides in Fruits and Vegetables

Klaudia Dyrda

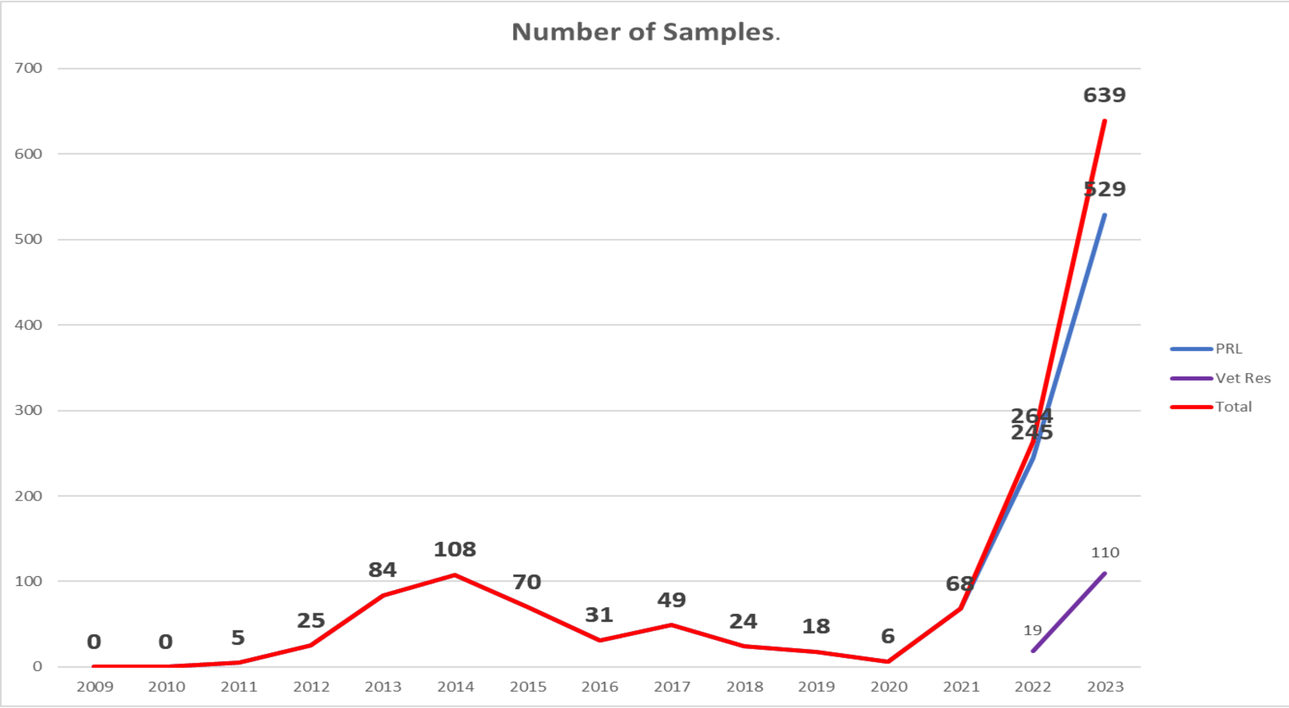
17th June 2025

The reasons for carrying out the analysis

- To provide a monitoring service in accordance with the harmonised EU monitoring programme.
- REGULATION (EC) No 396/2005 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
- on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC

Sampling and sample plans - 2024

Routine samples	F & V	TAT 4 weeks	817 samples
	FAO	TAT 4 weeks	290 samples
BCP Samples		TAT 48 hours	146 samples

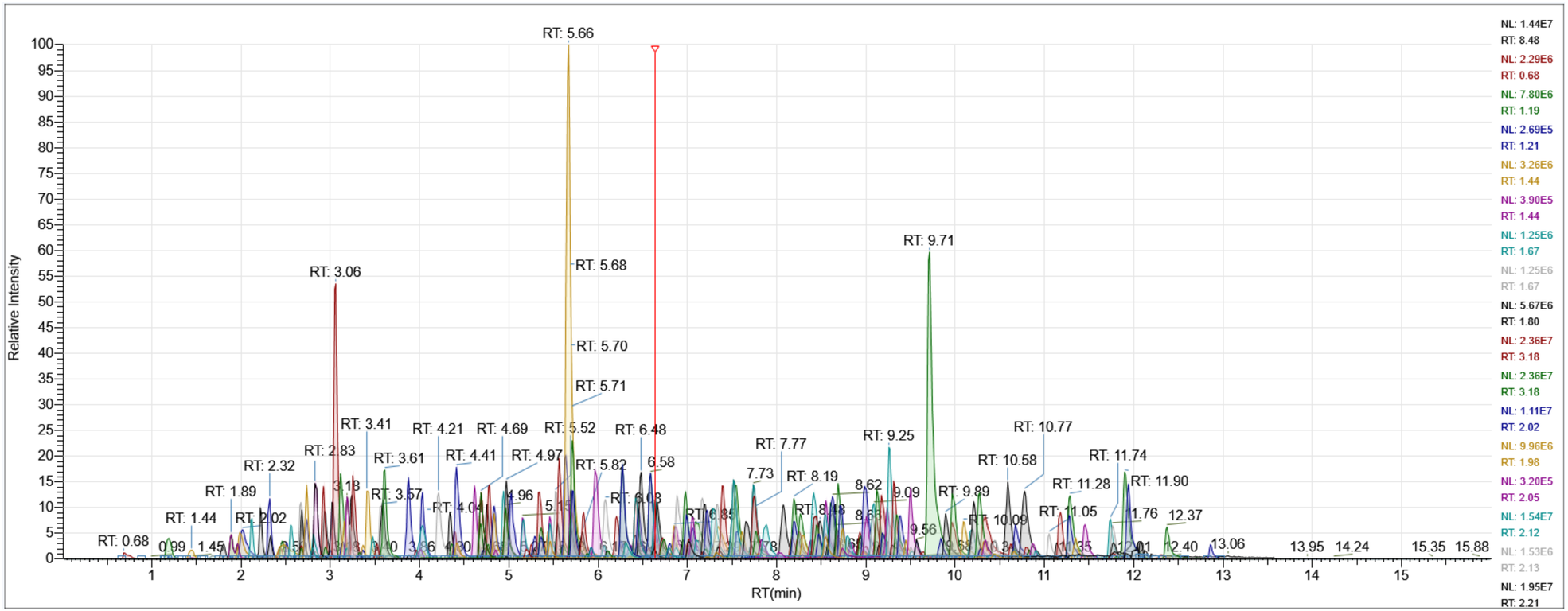


Scope

501 analytes

397 analytes accredited

79% of Fruit and vegetable accredited



Dutch mini-Luke Extraction

Advantages

- Well established
- Sensitive
- Low matrix effects
- Broad Spectrum Extraction
- Amenable with GC-MS and LC-MS

Disadvantages

- High solvent consumption
- Usage of chlorinated solvent
- Labour-intensive
- High glassware usage

Dutch mini-Luke Extraction

Samples are homogenised

15g aliquots

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Centrifuged

Evaporated down to low volume

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Diluted 1in20 in Methanol (LC analysis)

Dutch mini-Luke Extraction

Dutch mini-Luke Extraction

Samples are homogenised

15g aliquots

Extracted with

Acetone

Dichloromethane

Petroleum Ether

Sodium Sulphate is added to the extract to salt out the polar pesticides.

Centrifuged

Evaporated down to low volume

Re-constituted in Ethyl Acetate (GC analysis)

GC Calibration standard matrix matched

Diluted 1in20 in Methanol (LC analysis)

Dutch mini-Luke Extraction

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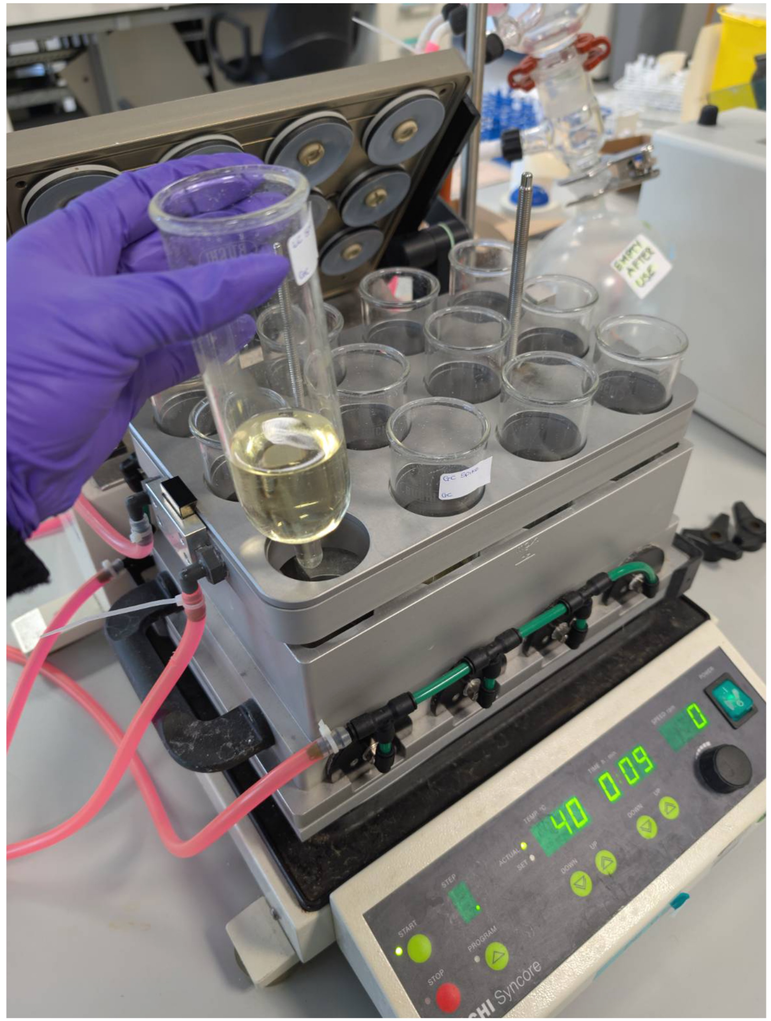

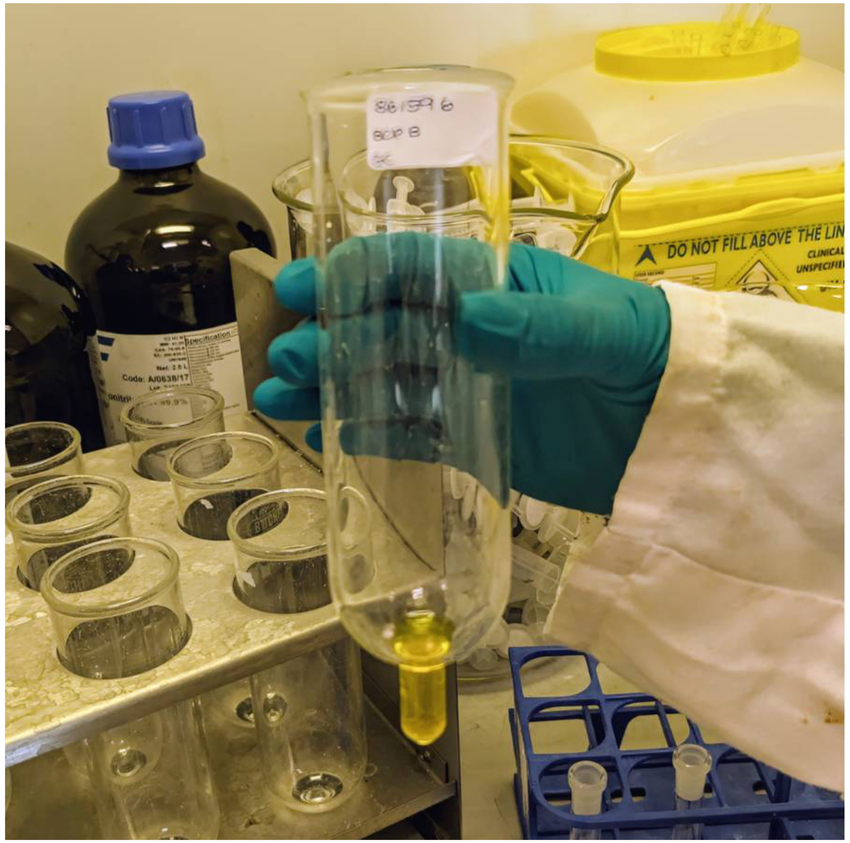

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Dutch mini-Luke Extraction

Samples are homogenised

15g aliquots

Centrifuged

Evaporated down to low volume

Diluted 1in20 in Methanol (LC analysis)

Solvent usage

Acetone	30ml + extra for cleaning
Dichloromethane	30ml
Petroleum Ether	30ml
Ethyl Acetate	25ml
Methanol	9.5ml
Total Solvent usage per sample	>124.5ml

2024 Samples

- 817 Fruit and Vegetables analysed
- Over 101 L of solvent consumed

- 5 water coolers

How do we validate methods?

ANALYTICAL QUALITY CONTROL AND METHOD VALIDATION PROCEDURES FOR PESTICIDE RESIDUES ANALYSIS IN FOOD AND FEED SANTE 11312/2021 v2

Supersedes Document No. **SANTE/11312/2021. Implemented by 01/01/2024**

Initial Full Validation

Tomato
Avocado
Potato

Validation needs to be performed

For all analytes within the scope of the method

For at least 1 commodity from each of the commodity groups

- *High water content – Apple, onion, broccoli*
- *High acid content and high water content – Lemon, strawberry, grape*
- *High sugar content and low water content – dried fruit, honey, fruit jam*
- *High oil content and very low water content – walnut, sunflower seed, peanut butter*
- *High oil content and intermediate water content – olives, avocado*
- *High starch and/or protein content and low water and fat content – lentils, barely, pasta*

Experimental set up

10ppb
20ppb
50ppb
100ppb

Sample set

- Reagent blank
- 1 blank sample
- 5 Spiked samples at target LOQ
- 5 Spiked samples at 2-10 x target LOQ

Instrumental sample sequence

- Conditioning blanks
- Calibration standards
- Reagent blank
- Sample blank
- 5 spiked samples at target LOQ
- 5 spiked samples at 2-10 x Target LOQ
- Calibration standards

Validation parameters and criteria

Parameter	What/How	Criterion
Sensitivity/Linearity	Linearity check from 5 levels	Deviation of back-calculated concentration from true concentration ≤+ 20%
Matrix effect	Difference of response from standard in matrix extract and standard in solvent	In case of more than 20% signal suppression or enhancement, matrix effects need to be addressed in Calibration
LOQ	Lowest spike level meeting the identification and method performance criteria for recovery and precision	≤ MRL
Specificity	Response in reagent blank and blank control samples	≤ 30% of Reporting Limit
Recovery	Average recovery for each spike level tested	70 – 120%
Precision (RSD_r)	Repeatability RSD_r for each spike level tested	≤ 20%
Precision (RSD_{wR})	Within-laboratory reproducibility, derived from on-going method validation / verification	≤ 20%
Robustness	Average recovery and RSD_{wR} derived from on-going method validation / verification	
Ion ratio	Check compliance with identification requirements for MS techniques	
Retention time		± 0.1 min

Identification requirements for different MS techniques

MS Detector/Characteristics		Acquisition	Requirements for identification	
Resolution	**Typical systems (Examples)**		**Minimum number of ions**	**Additionally**
Unit Mass resolution	Single MS Quadrupole, ion trap, TOF	Full scan, limited m/z range, SIM	3 ions	S/N ≥ 3 Analyte peaks from both product ions in the extracted ion chromatograms must fully overlap. Ion ratio from sample extracts should be within ±30% (relative) of average of calibration standards from same sequence
	MS/MS Triple Quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap	Selected or multiple reaction monitoring. Mass resolution for precursor-ion isolation equal to or better than unit mass resolution	2 products ions	
Accurate mass measurement	High Resolution MS: (Q-)TOF (Q-)Orbitrap	Full scan, limited m/z range, SIM fragmentation with or without precursor-ion selection, or combination thereof	2 ions with mass accuracy ≤ 5ppm Preferably include the molecular ion Include at least 1 fragment ion	S/N ≥ 3 Analyte peaks from precursor and/or products ion(s) in the extracted ion chromatograms must fully overlap.

Is Miniaturization possible?

Protocol

1. Aliquot 1g sample into 50ml tube
2. Spike
3. Add 2ml Acetone shake with ceramic bead
4. Add 2ml Petroleum ether, 2ml Dichloromethane and 2g of sodium sulphate
5. Shake using shaker
6. Centrifuge
7. Transfer 3ml into glass vial, evaporate to dryness using Turbovap
8. Reconstitute with 0.5ml of Ethyl Acetate
9. Filter the extract through a 0.2 µm filer – GC Fraction
10. Transfer 50µl of extract into 2ml vial and add 950µl Methanol – LC Fraction

Solvent usage

Acetone	2ml
Dichloromethane	2ml
Petroleum Ether	2ml
Ethyl Acetate	0.5ml
Methanol	0.95ml
Total Solvent usage per sample	7.45ml

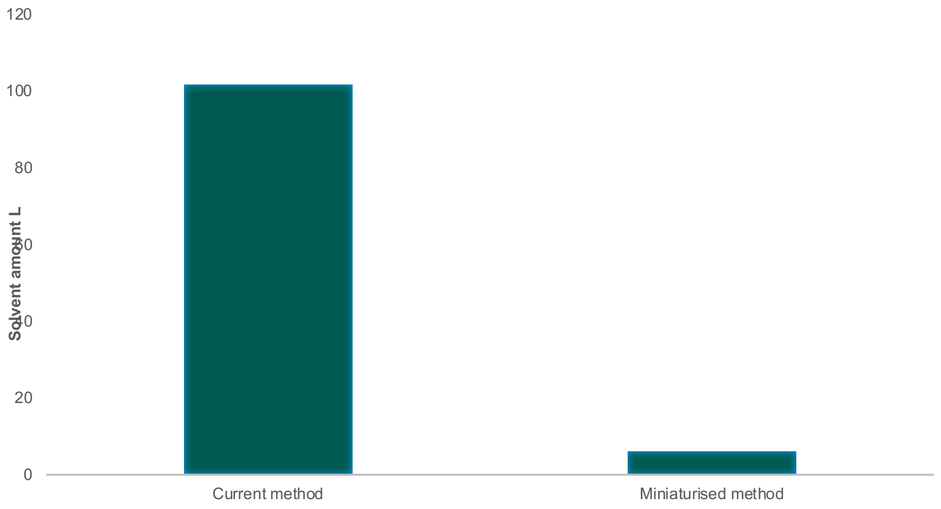
Solvent usage

Acetone	15ml + extra for cleaning
Dichloromethane	15ml
Petroleum Ether	15ml
Ethyl Acetate	25ml
Methanol	9.5ml
Total Solvent	>124.5ml

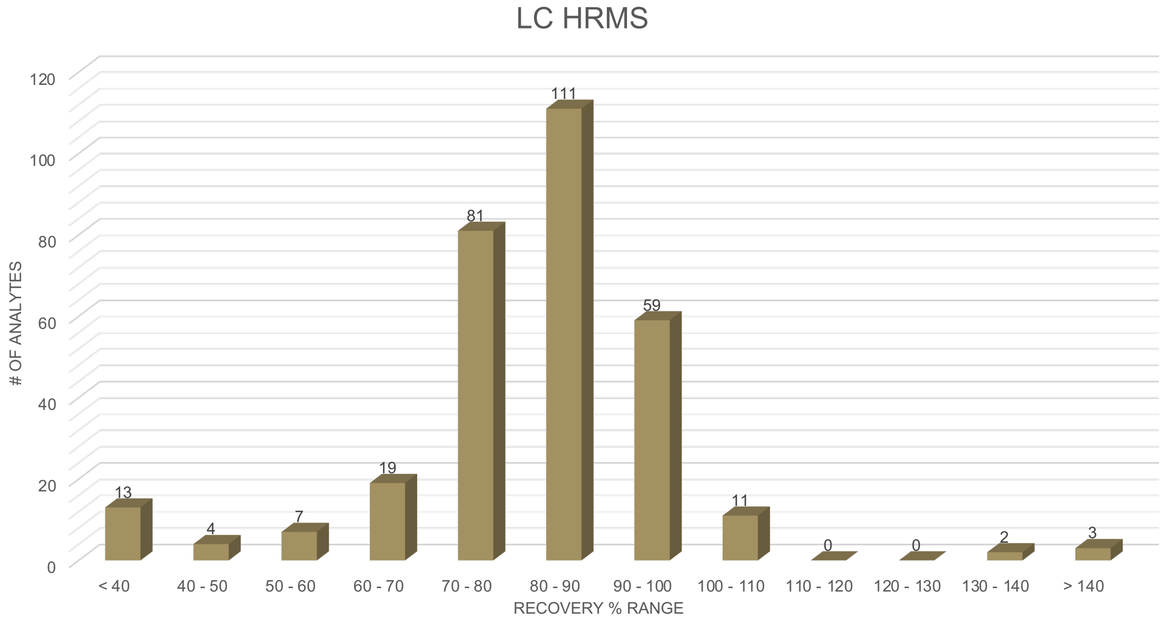
Acetone	2ml
Dichloromethane	2ml
Petroleum Ether	2ml
Ethyl Acetate	0.5ml
Methanol	0.95ml
Total Solvent	7.45ml

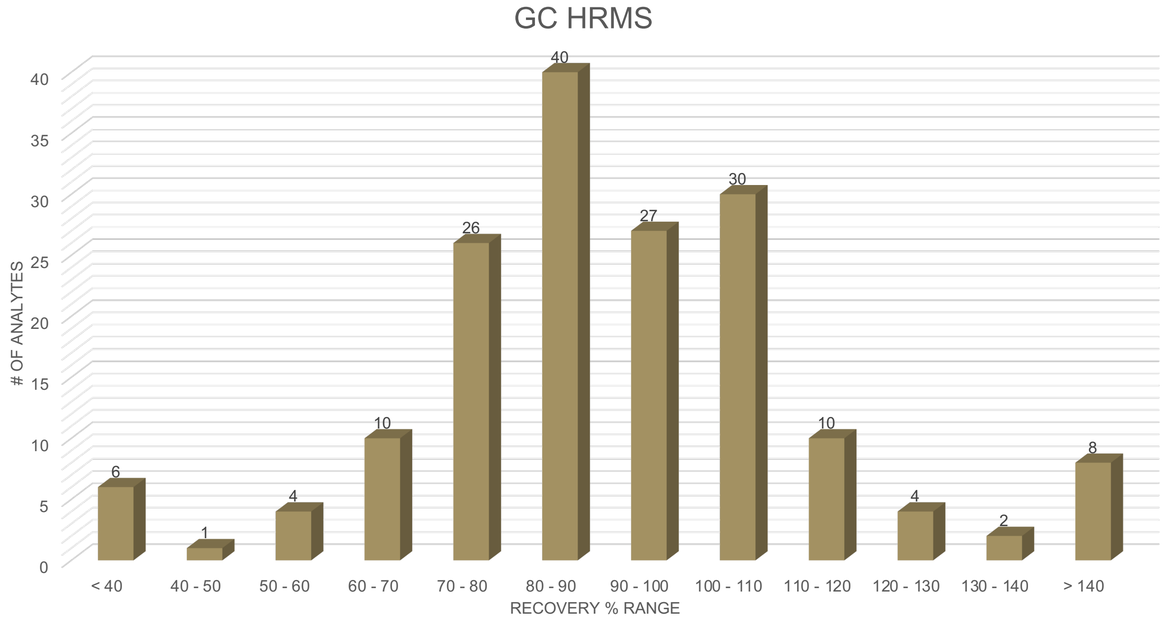
2024 Samples

- 817 Fruit and Vegetables analysed
- Over 101L of solvent consumed
- Miniaturised method only uses 6 L
- ~17 times less solvent would be consumed



Results





310 analytes
262 analytes acceptable recovery

85% acceptable

168 analytes
133 analytes acceptable recovery

79 % acceptable

Can it be automated?

Collaboration with Da Vinci Laboratory Solutions UK and Ireland Ltd.

Colin Hastie – Application Chemist

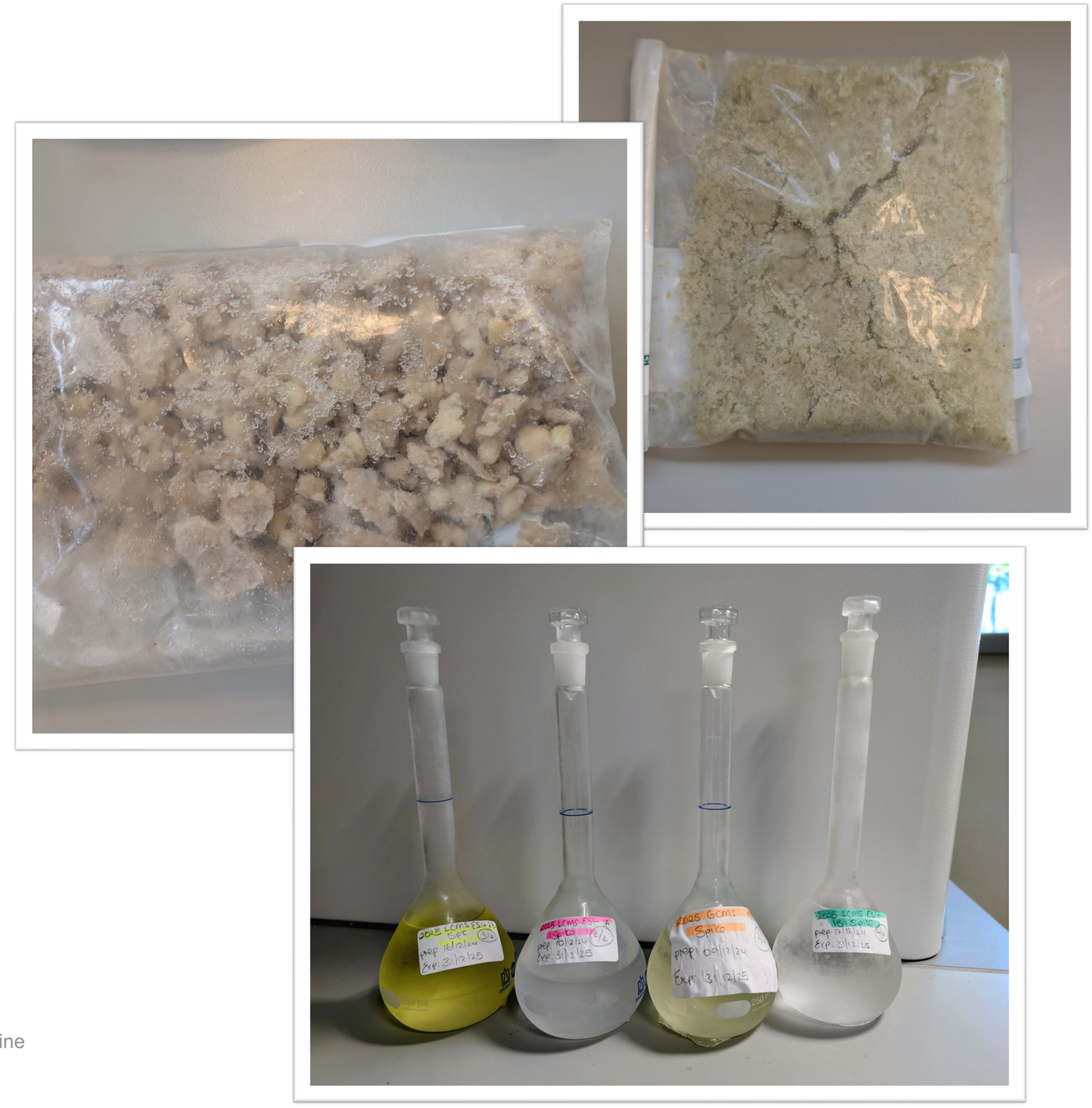
Use of Gerstel Dual head Robotic Pro Multipurpose sample (MPS)

MPS Dual Head configuration for sample preparation and sample introduction to an HPLC system.

Project Plan

Provide Colin with:

- Standards mixes
- Matrix – tomato, avocado & potato
- GC MS/MS methods



Protocol

Into a 10 mL glass vial 1 g of samples weigh out and 2 g of sodium sulphate, this is then be loaded on to the MPS which is programmed to carry out the following actions on the samples.

Sample is spiked at 4 levels with n=6 replicates at each level.

1. Spike with 1 mg/L Std mix (0, 10 ,50 or 100 µL as appropriate)
2. Add 2 mL of acetone to the sample
3. Move the sample vial to the quick mix and mix at 2000 rpm for 30 seconds
4. Add 2 mL of Petroleum ether to the sample
5. Add 2 mL of Dichloromethane to the sample
6. Mix the sample at 2000 rpm for 30 seconds
7. Move the sample to the centrifuge and centrifuge at 2000 G for 3 minutes.
8. Transfer 3 mL of sample extract to a 4 mL vial
9. Add 50 µL of nonane to the 4 mL vial as a keeper solvent
10. Evaporate the extract in the MVap
11. The vial is reconstituted in 0.45 mL of Ethyl acetate and is ready for GC analysis
12. Transfer 50 µL of sample extract to separate 2 mL vial
13. Add 950 µL of methanol to the new vial ready for LC-MS/MS analysis


MPS
SFS
GERSTEL

Experimental

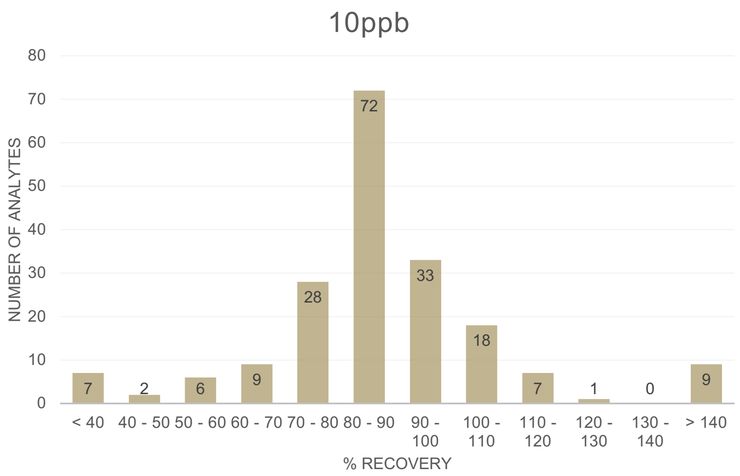
Validation Batch of 30 samples ~7hrs

Approx 15 min per sample

Routine Batches ~ 18 samples

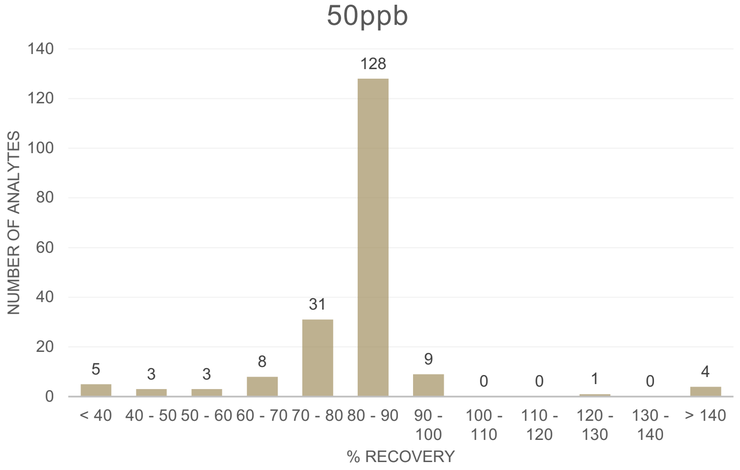
Approx 4.5hrs

Results – tomato GC



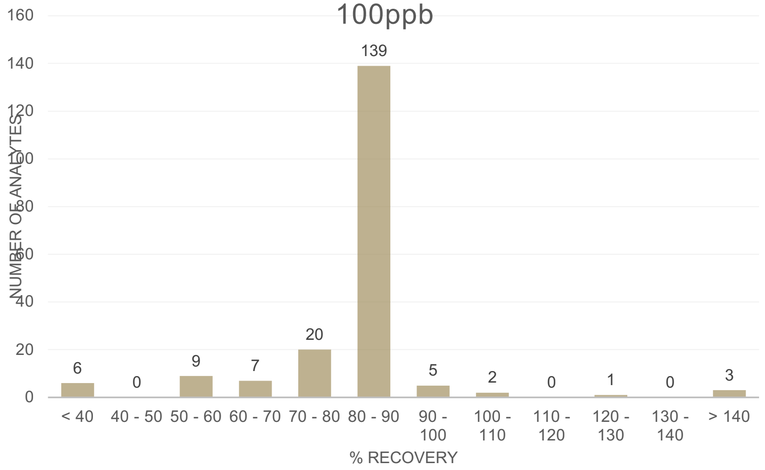
192 analytes
160 analytes acceptable recovery

83% acceptable



192 analytes
167 analytes acceptable recovery

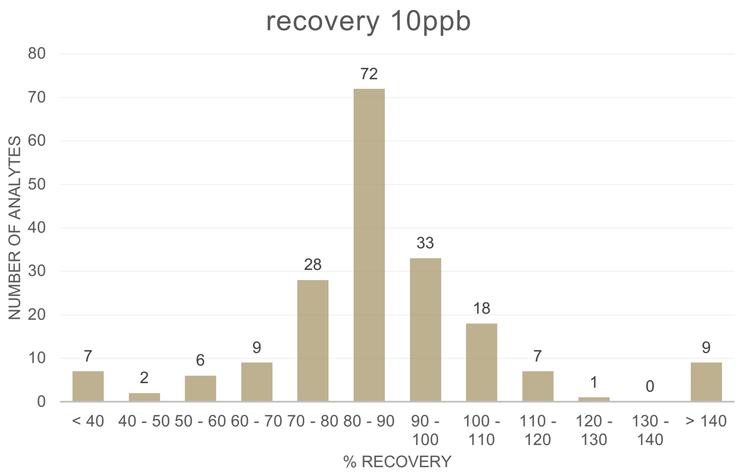
87% acceptable



192 analytes
167 analytes acceptable recovery

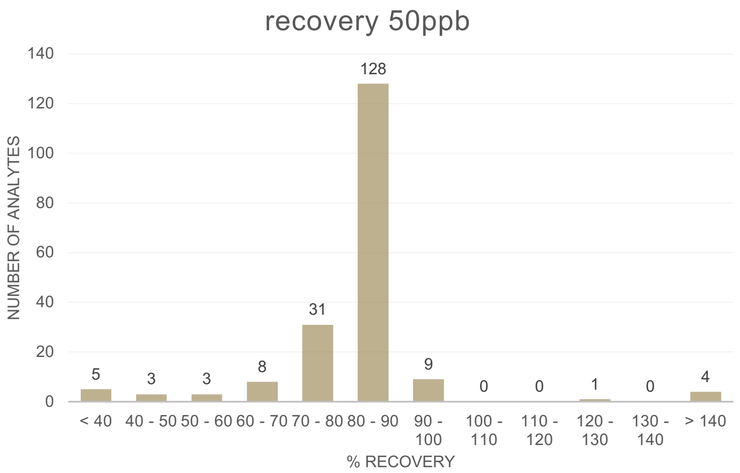
87% acceptable

Results – potato GC



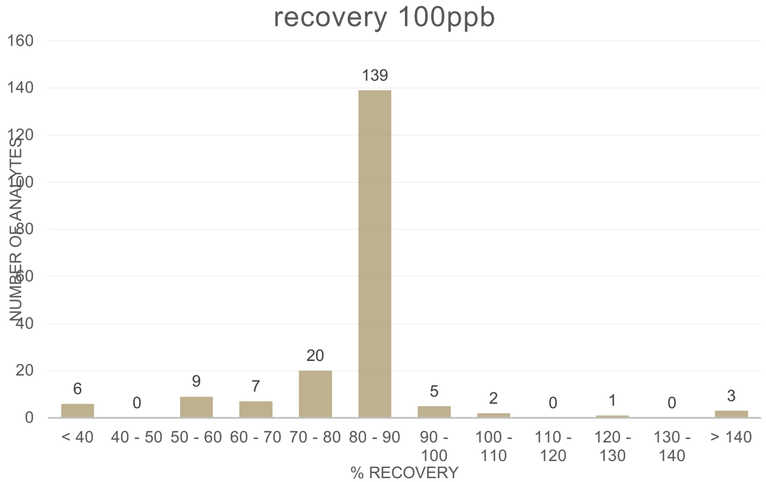
192 analytes
158 analytes acceptable recovery

82% acceptable



192 analytes
168 analytes acceptable recovery

88% acceptable



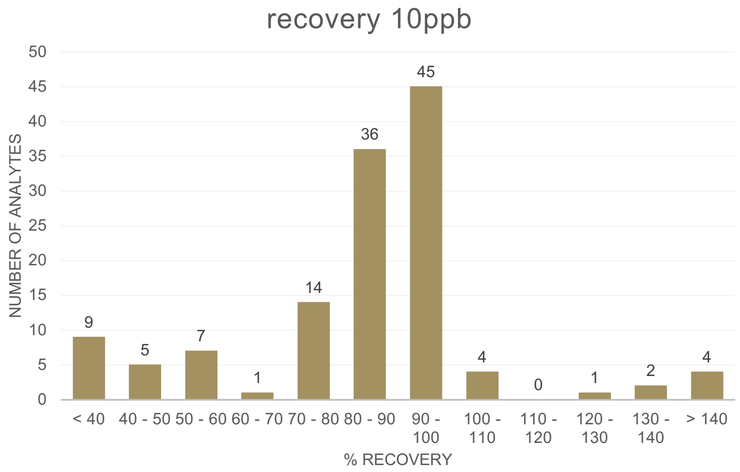
192 analytes
166 analytes acceptable recovery

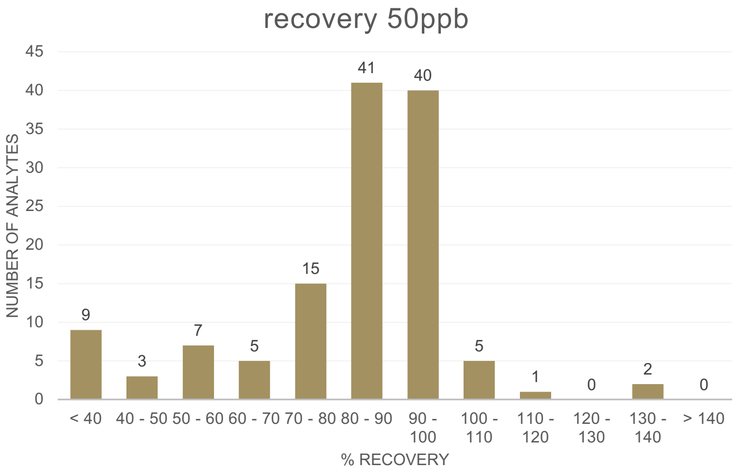
86% acceptable

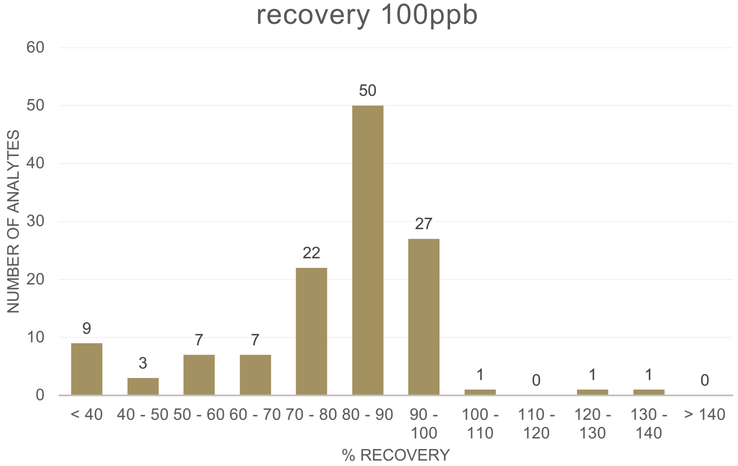
Problematic compounds

Not currently accredited	Tomato	Potato	Not on GC Scope
1,4-Dimethylnapthalene	1,4 dimethylnapthalene	1,4 dimethylnapthalene	2,4,6-Trichlorophenol
Anthraquinone	Binapacryl	Acephate	3,5-Dichloroaniline
Captofol	biphenyl	Aclonifen	3-chloroaniline
Captan	Biteranol-II	Aldrin	Methamidophos
Dicofol	Captan	Anthraquinone	Molinate
Dimoxystrobin	Carbofuran	Azaconazole	Simazine
Endosulfan-alpha	Chlorothalonil	Biteranol-II	Terbuthylazine
Folpet	Dichlobenil	Bromophos-ethyl	
Formothion	Dichlorvos	Chlorbufam	
Heptachlor endo-epoxide,trans	Etridazole	Cyanofenphos I	
Isofenphos-oxon	methacrifos	Diazinon	
Nitrofen	O-phenylphenol	Diphenylamine	
Oxadixyl	Phorate	Heptachlor exo epoxide	
Paraoxon methyl	Propham	hexachlorobenzene	
PCB28	Tecnazene	Iprovalicarb II	
PCB52	Triadimenol	Omethoate	
PCB101		Procymidone	
PCB118		Propachlor	
PCB138			
PCB153			
PCB180			
Pentachloroaniline			
Phorate			
Pirimicarb desmethyl			
Resmethrin			
Silthiofam			
Tefluthrin			

Results – Avocado LC







128 analytes
99 analytes acceptable recovery

77% acceptable

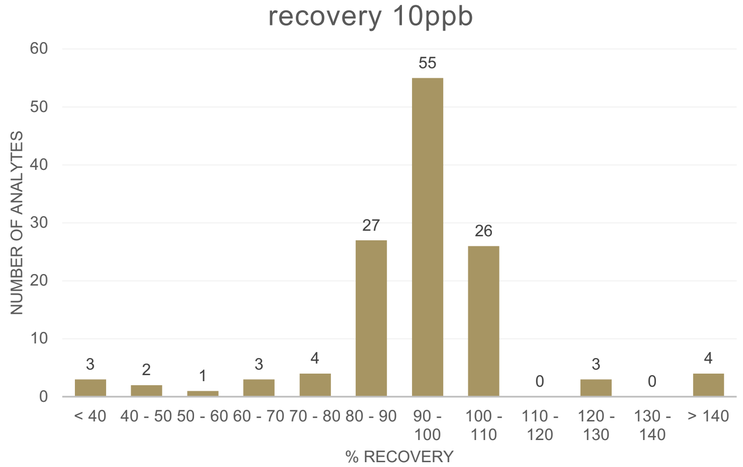
128 analytes
102 analytes acceptable recovery

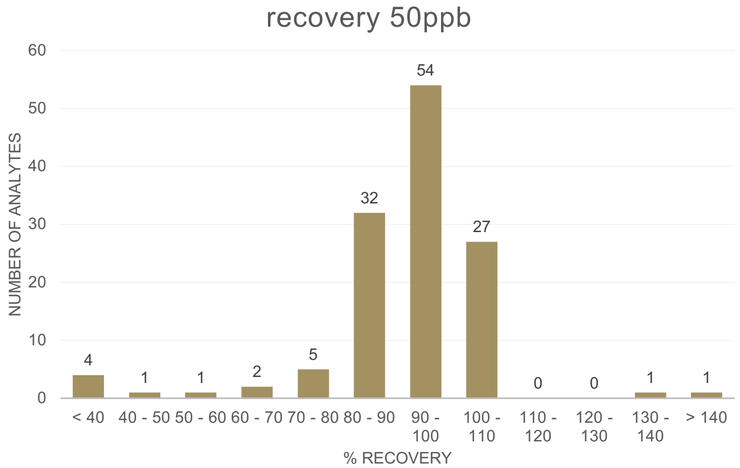
80% acceptable

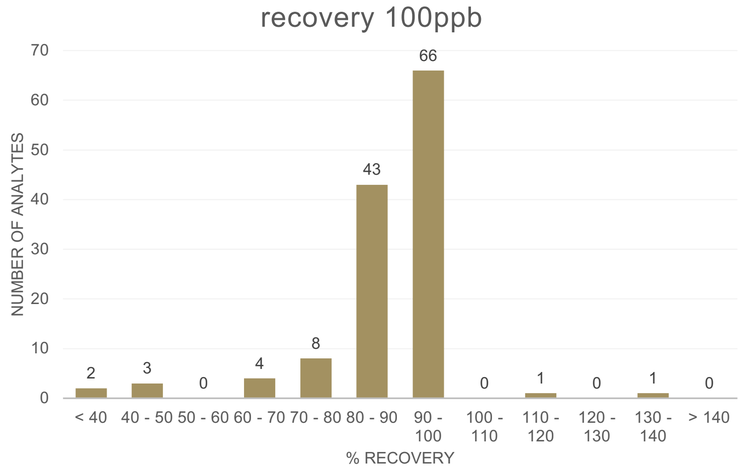
128 analytes
100 analytes acceptable recovery

78% acceptable

Results – Tomato LC







128 analytes
112 analytes acceptable recovery

88% acceptable

128 analytes
118 analytes acceptable recovery

92% acceptable

128 analytes
118 analytes acceptable recovery

92% acceptable

Success!

>79% of assessed analytes passed recovery criteria

Potential Issues

Homogenisation – current protocol proves difficult to obtain small enough particles to have representative sample

Next steps?

Next steps

1. Purchase Gerstel Dual head Robotic Robotic Pro Multipurpose sample (MPS)

2. Explore and revise the homogenisation protocols i.e. cryogenic milling.

3.Validation and Accreditation of Fruit and Vegetables method

4. Extend the protocol to honey and cereals?

Thank you for your attention!

Thank you

Da Vinci Laboratory Solutions UK and Ireland Ltd.
Colin Hastie
Jim Garvey
Sadbh Healy

Any questions?