

*Joint EURL pesticides meeting
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Almeria Spain*



LC-HRMS: Challenges for Routine Implementation

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www.fera.defra.gov.uk

Presentation Outline



- The drivers for qualitative screening methods
- Key requirements
- Challenges for routine implementation
- Emerging technologies



The need for HRMS screening



- Targeted pesticide analysis such as LC-MS/MS will answer the question;

*“Which pesticides from a **predefined list** are present in the sample at, or above, a specified concentration? “*

- Only detects pesticides in the ‘predefined list’
- Other residues present will not be detected
 - *essentially false negatives*

Stakeholders

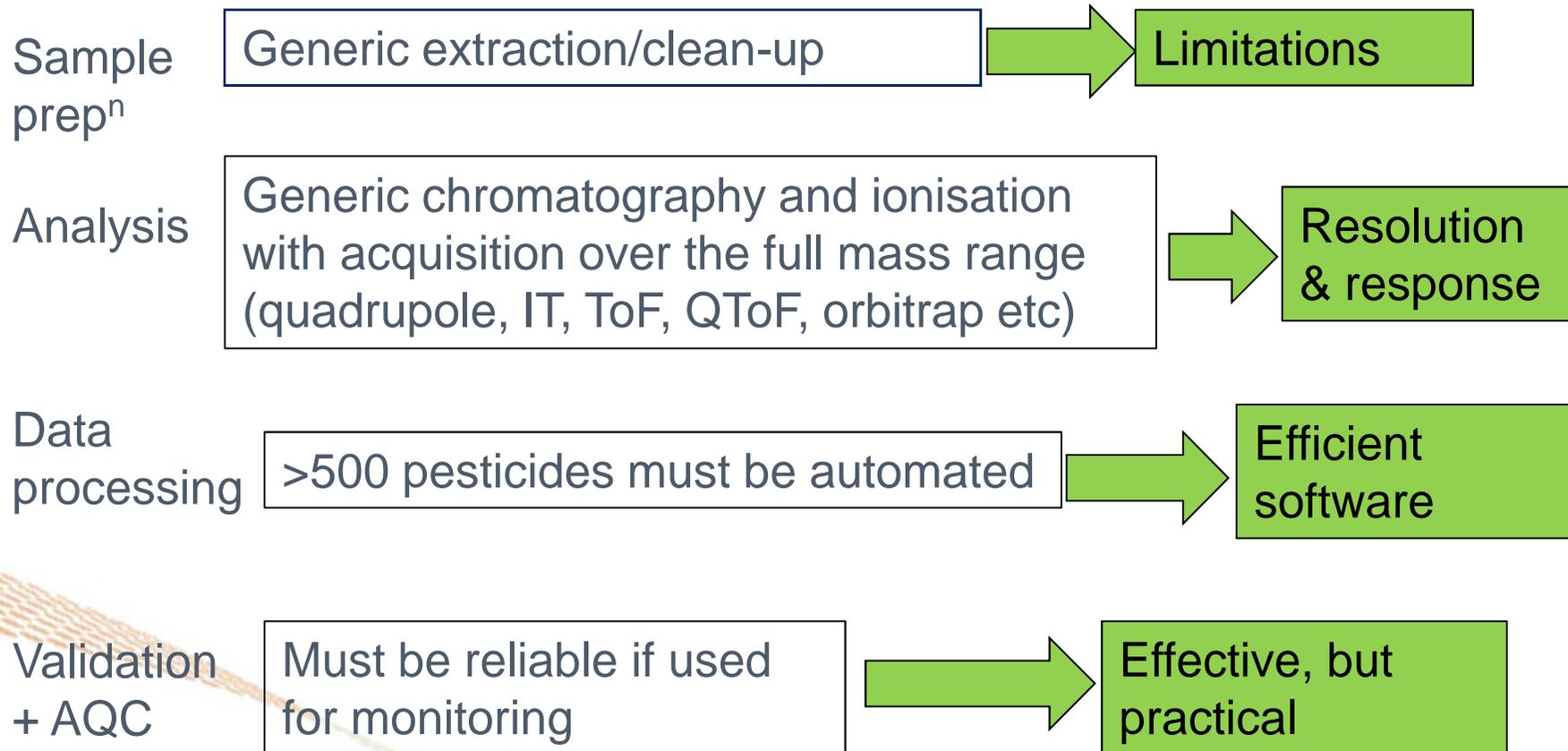


- **Consumers**
 - concerns regarding residues
- **Regulators**
 - residues do not affect consumer health
 - residues do not exceed MRL
 - seen to be proactive regarding unexpected residues
- **Laboratories**
 - increase scope of methods (more analytes)
 - increase speed of methods (more samples) retrospective
 - search capability



Can non-targeted analysis help meet these goals?

Key requirements of qualitative MS screening methods



View from other laboratories



- December 2012 - EURL training on screening
- Invited participants (good record in screening PT)
- Laboratories adopt different approaches to screening PTs
- Screening PT (relatively high concentrations)
- Common issues – resolving power, selectivity, sensitivity, data processing

LC Separation



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System	Acquity UPLC™ I-Class
Column	BEH C18 100 mm x 2.1 mm, 1.7 µm
Column temp.	45 °C
Flow	0.45 mL/min
Injection vol.	6 µL
Mobile phase	(A) 0.01M Amm. acetate aq. (B) 0.01M Amm. Acetate in MeOH
Gradient	17 min

Detection : Xevo G2-S QToF™

Ionization Mode	ESI + (1.0 kV)
Cone voltage	25V
Desolvation Temperature	550 °C
Reference Mass	Leucine enkephalin [M+H] ⁺ =556.2766
Acquisition Range	50-1200 m/z
Acquisition Rate	8 spectra/second
Mass resolution (FWHM)	19000 at <i>m/z</i> 142.0087 26500 at <i>m/z</i> 284.1417 30000 at <i>m/z</i> 413.1284 41000 at <i>m/z</i> 732.4695

Data Acquisition

- MS^E scan (low CE): 4 V
- MS^E scan (ramp CE): 10-45 V

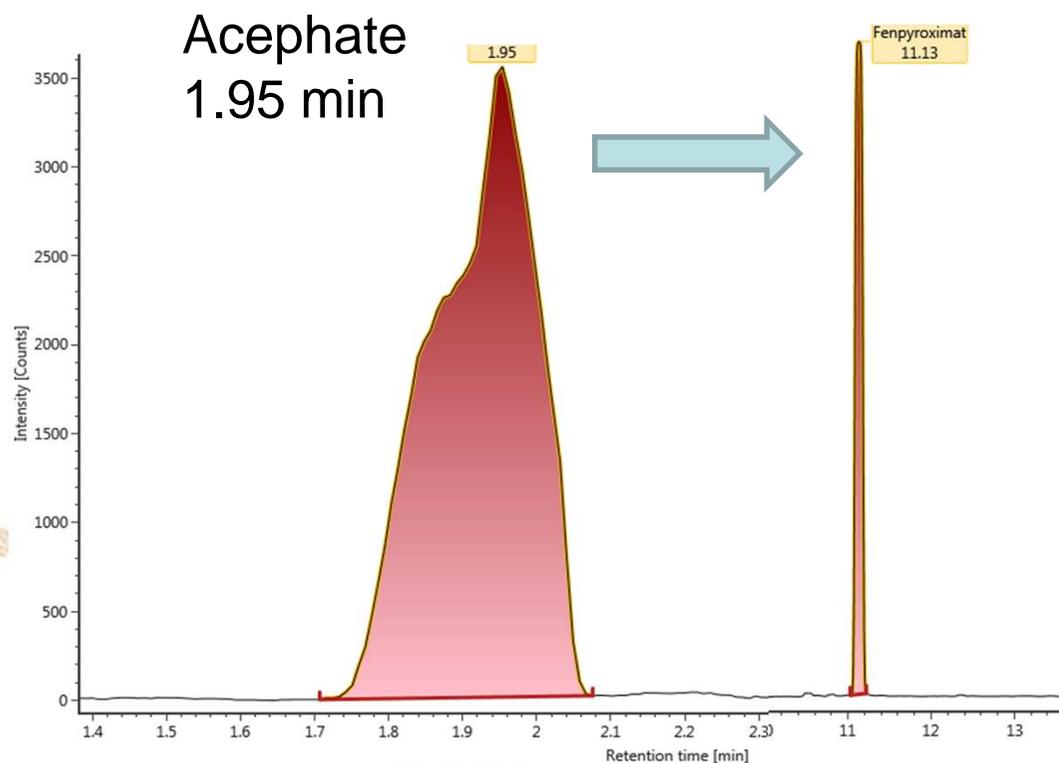
The chromatography compromise



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- 25:75% MeCN:water, 6 μ L injection volume

fenpyroximate
11.13 min



Component evaluation



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Waters UNIFI - ASMS Pest QuanQual: Analysis Center

My Work Welcome to UNIFI ASMS Pest QuanQual: An... ASMS Pest Master: Analy... Search folders...

Review Investigate Report

Review Results Limits Process Edit Tools File

Workflow Unknown in Red Pepper 2 Sample position: 1:40 Replicate: 1 Dicrotophos The sample set is not found, modifications will only be saved to the analysis

Workflow

- Summary
 - Batch Overview
 - Result Summary
- Review
 - IDs with no flags - summary
 - IDs with no flags - details
 - IDs with no flags - quan
 - IDs with flags - summary
 - IDs with flags - details
 - Excluded targets

Component name	Expected RT (min)	Observed RT (min)	Mass error (ppm)	Expected Fragments Count	Identified High Energy Fragments	Adducts	Isotop
1 Atrazine	7.53	7.46	-1.42	2		2 +H	
2 Azoxystrobin	8.47	8.44	-1.49	3		1 +H, +Na, +K	
3 Chlortoluron	7.26	7.23	0.61	0		0 +H, +Na, +K	
4 Dicrotophos	4.11	4.21	0.41	3		3 +H, +Na, +K	
5 Diuron	7.71	7.84	0.05	1		1 +H, +Na	
6 Fenpropimorph	11.52	11.63	1.09	1		1 +H	
7 Hexazinone	6.63	6.60	1.80	2		2 +H, +Na, +K	
8 Metolachlor	9.33	9.28	-2.16	2		2 +H, +Na, +K	

View: *F and E Qual View 1

Chromatograms

Item name: Unknown in Red Pepper 2
Channel name: Integrated : Smoothed : Mass Chrom...

Dicrotophos
4.21

Intensity [Counts]

Spectra

Item name: Unknown in Red Pepper 2
Description: Unknown in Red Pepper

Intensity [Counts]

230.03398
296.13665
112.07553
193.02560
297.13935
387.14193
467.16853
545.17181

Administrator, UNIFI [Administrator]

Database



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Maintenance up to date

In this example: Waters pesticide database

- 520 entries
- Available information:
 - ❖ Name
 - ❖ Chemical formula
 - ❖ Structure
 - ❖ Retention time
 - ❖ Accurate mass
 - ❖ Fragment ion(s)
 - ❖ Isotopic patterns
 - ❖ Isotope intensity

Component	Formula	Structure	RT	Frag 1	Frag 2	Frag 3	Frag 4	Classification
2,3,5-Trimethacarb	C11H15NO2	2,3,5-Trimethacarb.mol	7.76	137.0916				Insecticides
2,6-Dichlorbenzamid	C7H5Cl2NO	2,6-Dichlorbenzamid.mol	3.37	172.9511				Breakdown product
3,4,5-Trimethacarb	C11H15NO2	3,4,5-Trimethacarb.mol	7.8	137.0916				Insecticide
3-Hydroxycarbofuran	C4H7NO3	3-Hydroxycarbofuran.mol	4.72	163.0709	107.0447			Metabolite
Acephate	C12H15NO4	Acephate.mol	2.02	124.9776				Insecticide
Acetamiprid	C12H15NO3PS	Acetamiprid.mol	4.69	126.0061				Fungicide
Acibenzolar-S-methyl	C10H11ClN4	Acibenzolar-S-methyl.mol	8.15	136.0045				Insecticides
Akton	C8H6N2O5	Akton.mol	10.87	114.9569	147.0998	238.0949	132.0763	insecticides/nematicides
Alachlor	C12H14Cl3O3PS	Alachlor.mol	9.26	162.1233	91.0498			Insecticide/Acaricide
Alenycarb	C14H20ClNO2	Alenycarb.mol	9.67	238.0852	89.0375	69.0528		Metabolite
Aldicarb	C12H14Cl3O3PS	Aldicarb.mol	5.6	116.0484	148.0382			insecticides
Aldicarb sulfone	C12H14Cl3O3PS	Aldicarb sulfone.mol	2.84	86.0556				herbicides
Aldicarb sulfoxide	C14H20ClNO2	Aldicarb sulfoxide.mol	2.64	89.0375	135.076			Herbicide
Allethrin	C17H25N3O4S2	Allethrin.mol	10.79	121.0967	96.0512	68.0199		Insecticide
Amethrin	C7H14N2O4S	Amethrin.mol	5.76	98.092				Insecticide
Allidochlor	C7H14N2O3S	Allidochlor.mol	5.76	186.0763	152.1025	122.0556		plant growth regulators
Aminocarb	C19H26O3	Aminocarb.mol	8.39	261.0244				Fungicide
Amitraz	C8H12ClNO	Amitraz.mol	4.39	137.0791				Herbicide
Ancymidol	C19H26O3	Ancymidol.mol	6.25	132.0763				
Anilazine	C8H17N5S2	Anilazine.mol	11.42	239.1134				
	C15H16N2O2		6.75		170.0853	124.9776		
	C19H23N3		8.72					
	C15H16N2O2		9.78					
	C8H5Cl3N4							
	C13H19ClNO2PS2							

Qualitative screening: validation



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Document N° SANCO/12495/2011

Supersedes Document No. **SANCO/10684/2009**

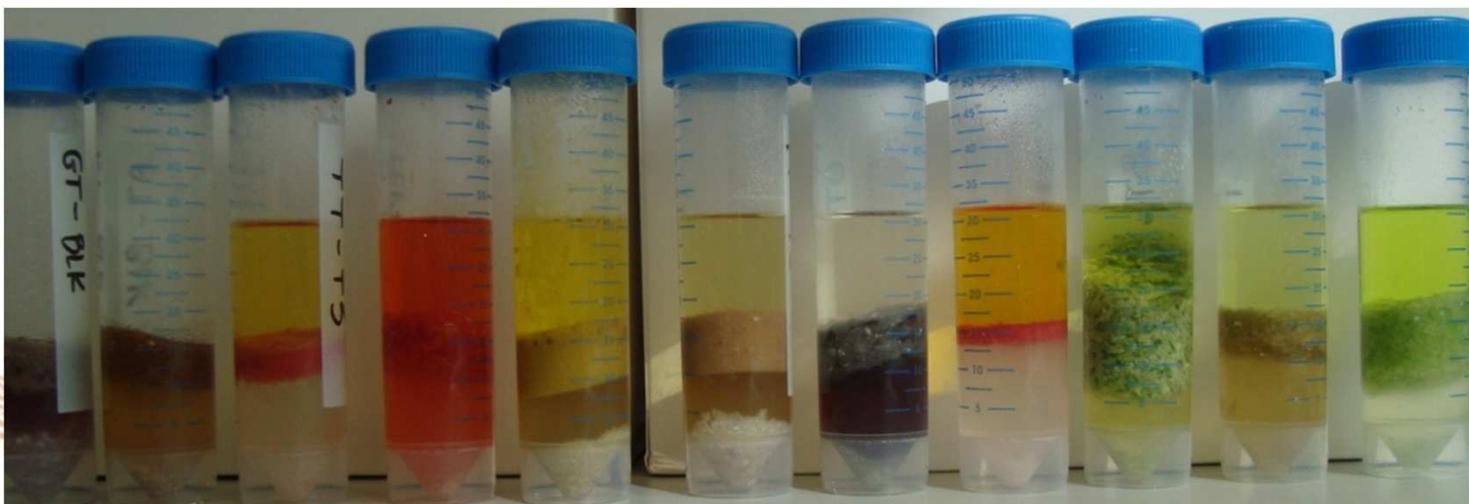
Implemented by 01/01/2012

- Based on detectability (< 5% false negatives)
- Analysis of at least 20 samples spiked at SDL
- Multiple matrices from commodity groups:
2 different samples for each matrix & representative of the scope of the laboratory
- Analysis of non-spiked samples to determine number of 'False detects'

Validation in practice



- 11 different matrices
- DisQuE™ QuEChERS (citrate buffered version)
- Samples spiked with pesticides at 0.01-0.05 mg/kg



- More difficult than expected

Data Processing Software



- Complex data sets
- Adducts, fragments and isotopes
- Automated peak detection, integration
- Balance between False detects and False negatives
- Ease and speed of review of data
- Automated reporting
- Storage and retrieval of data

UNIFI automated data processing

Parameters evaluation



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Balance between false negatives and False detects

	Orange 50 ppb		Grape 50 ppb	
	Detection rate (%)	False detects rate (%)	Detection rate (%)	False detects rate (%)
± 10 ppm, ± 0.5 min, > 100 counts	88	15	94	6
± 5 ppm, ± 0.5 min, > 100 counts	86	12	94	5
± 5 ppm, ± 0.2 min, > 100 counts	85	9	94	4
± 5 ppm, ± 0.2 min, > 100 counts, isotope m/z match (10 ppm)	79	6	93	4
± 5 ppm, ± 0.2 min, > 100 counts, fragment(s)	69	1	83	0,4
± 5 ppm, ± 0.2 min, > 100 counts, isotope m/z match (10 ppm), fragment(s)	65	0,8	83	0,4

Ease & speed of data review and report outputs

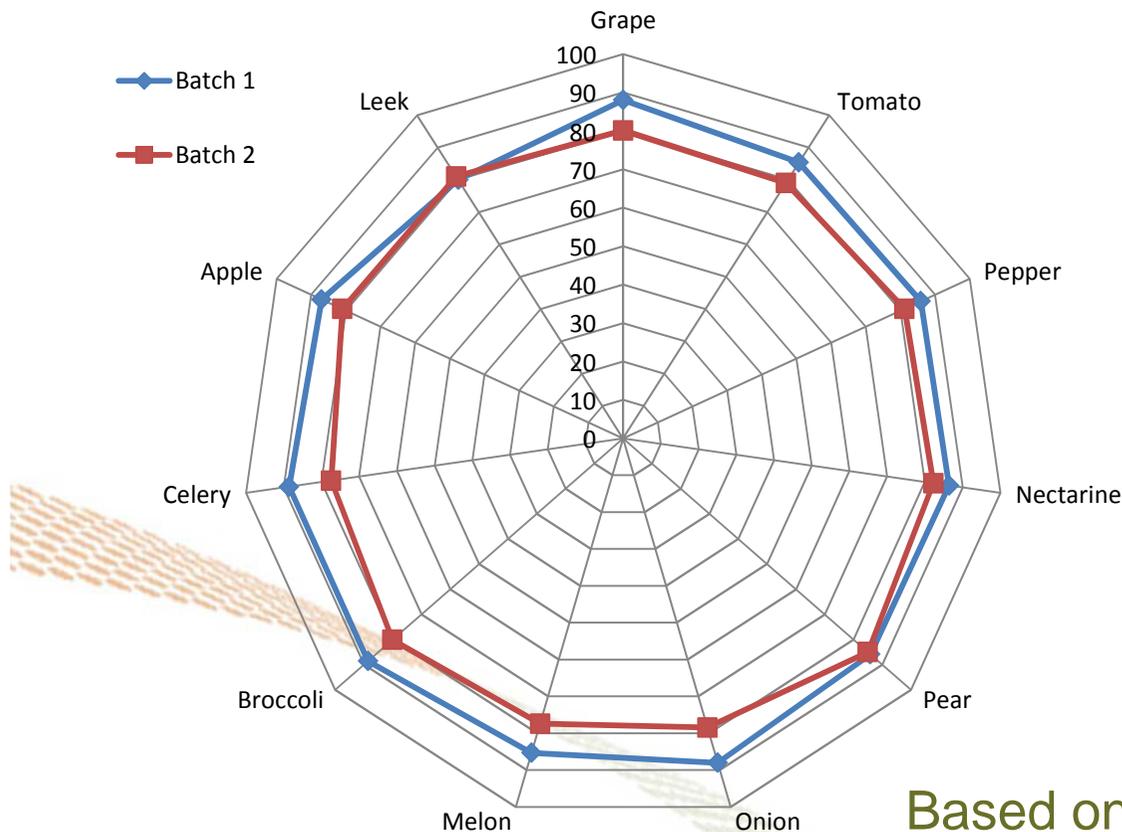


Automated detection (%)

Samples spiked with pesticides at 0.01 mg/kg



Settings: ± 10 ppm, ± 0.5 min, detector counts threshold 100



	Batch 1	Batch 2
Detection rate mean (%) \pm SD	86 \pm 2	80 \pm 2
False negatives rate mean (%) \pm SD	14 \pm 2	20 \pm 2

Based on 186 compounds included in the Waters database ESI + data

Screening Detection Limits



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- Two different samples for each matrix
- Analysis (ESI (+) mode) on 2 different days (>2 months apart)

Apple, grape, tomato, pepper, nectarine, pear, orange, melon, broccoli celery and leek	Screening Detection Limits	
	0.01 mg/kg	0.05 mg/kg
Number of compounds detected in $\geq 95\%$ of the samples	130	150
% of pesticides detected in $\geq 95\%$ of the samples	71	81

Based on 186 compounds included in the Waters database

Settings: ± 10 ppm, ± 0.5 min, detector counts threshold 100

Evaluation of data processing parameters (EUPT test materials)



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Mandarin FV-SM-03

Retention time window (\pm min)	Fragments observed?	Mass error (\pm ppm)	Number of targets	Number in DB	Total detects	Detects	% target detected (DB)	Total FD	%FD
0,5	No	10	27	22	67	18	82	49	9
0,5	No	5			50	18	82	31	6
0,5	No	3			43	18	82	23	4
0,5	No	1			18	9	41	9	2
0,2	No	5			40	18	82	22	4
0,5	Yes	5			20	18	82	2	0,4

Leek FV-SM-02

Retention time window (\pm min)	Fragments observed?	Mass error (\pm ppm)	Number of targets	Number in DB	Total detects	Detects	% target detected (DB)	Total FD	%FD
0,5	No	10	22	21	76	21	100	55	11
0,5	No	5			47	21	100	21	4
0,5	No	3			37	21	100	15	3
0,5	No	1			13	7	33	5	1
0,2	No	5			35	19	90	15	3
0,5	Yes	5			22	20	95	4	0,4

DB: database, FD: false detects

EUPT results automated data processing

Mass error range ± 5 ppm
 ± 0.5 min
 >100 counts
 isotope match (10 ppm)
 fragments (y/n)
 ESI +



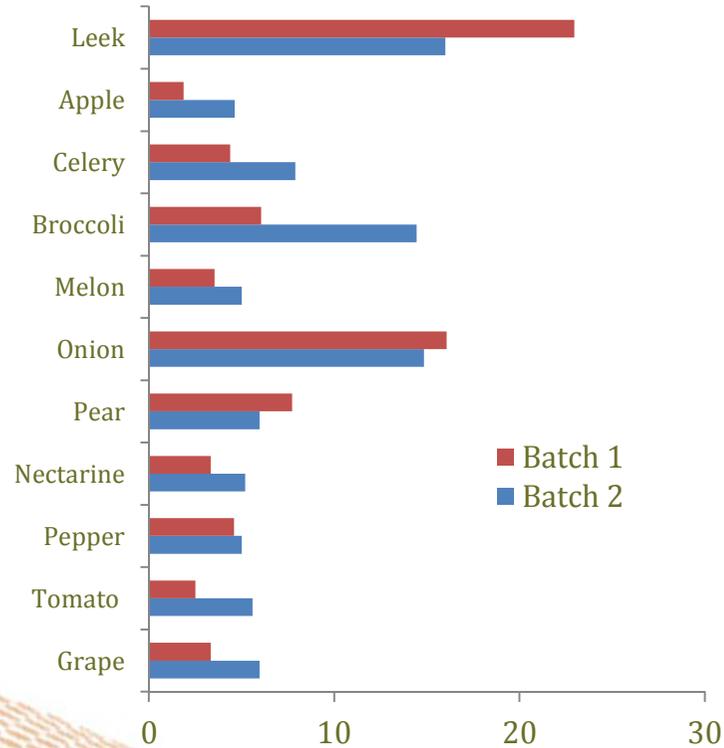
Sample ID	Leek SM-02	Mandarin SM-03	Pear SM-04
No. of targets	22	27	21
No. of targets in database	21	22	18
No. of target pesticides detected	20	18	15
No. of FDs	2	2	3
Max RT diff (min) across all pesticides	0.4	0.1	0.1
Mass error range (ppm)	0.1 – 2.9	0.0 – 2.6	0.0 – 2.6
Detection rate (%) (No. of pesticides in DB)	95	82	83



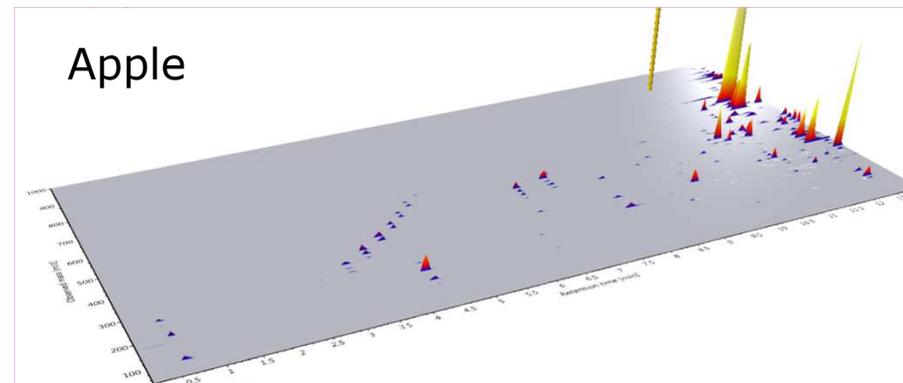
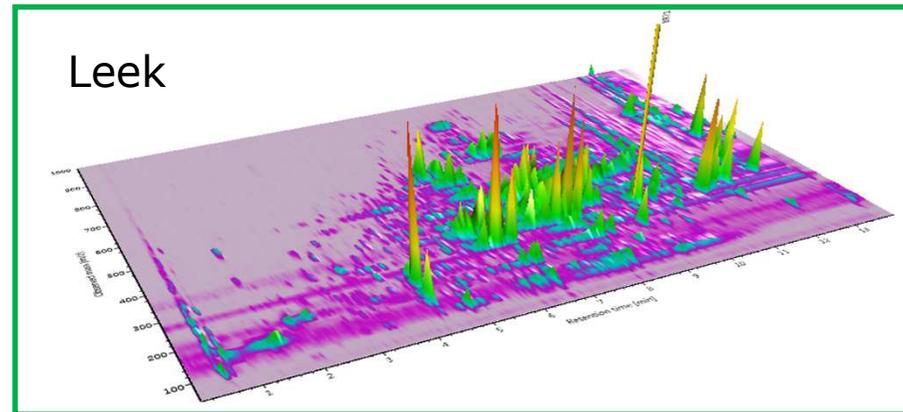
False detection rate (%)



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False detection rate calculated in blank samples against 479 compounds in batch 1 and versus 519 in batch 2



System Maintenance



- Batch to batch control of retention time (library maintenance)
- Cleanliness of system (adduct formation)
- Batch to batch control of sensitivity



QuEChERS -ToFMS: batch to batch variability

samples spiked with 197 pesticides at 0.01 mg/kg

Batch N°	1	2	3	4	5
matrix	grape	grape	pear	pear	lettuce
% of pesticides detected	85	96	85	88	94
‘Blank samples’					
Total N° of peaks detected	26	33	37	27	57
N° of peaks (noise removed)	19	25	30	18	39

On-going Analytical Quality Control



- Detects at low concentration for compounds with high response
- Detector saturation at high analyte concentrations although in these cases quantification can be made using isotope ions
- Representative analytes
- Which standards should be included in the representative mix?
- Minimum requirements (MACCP)

MACCP



- Method Analysis Critical Control Points
- The requirement is to identify all critical points in the method (typically ~10) and link 1 analyte to each critical control point. A QC spike containing the selected analytes is included with each batch.
- When all 10 analytes are detected, it is assumed that all critical points are in control, the method performance is acceptable and the batch can be approved

Proposal by Hans Mol

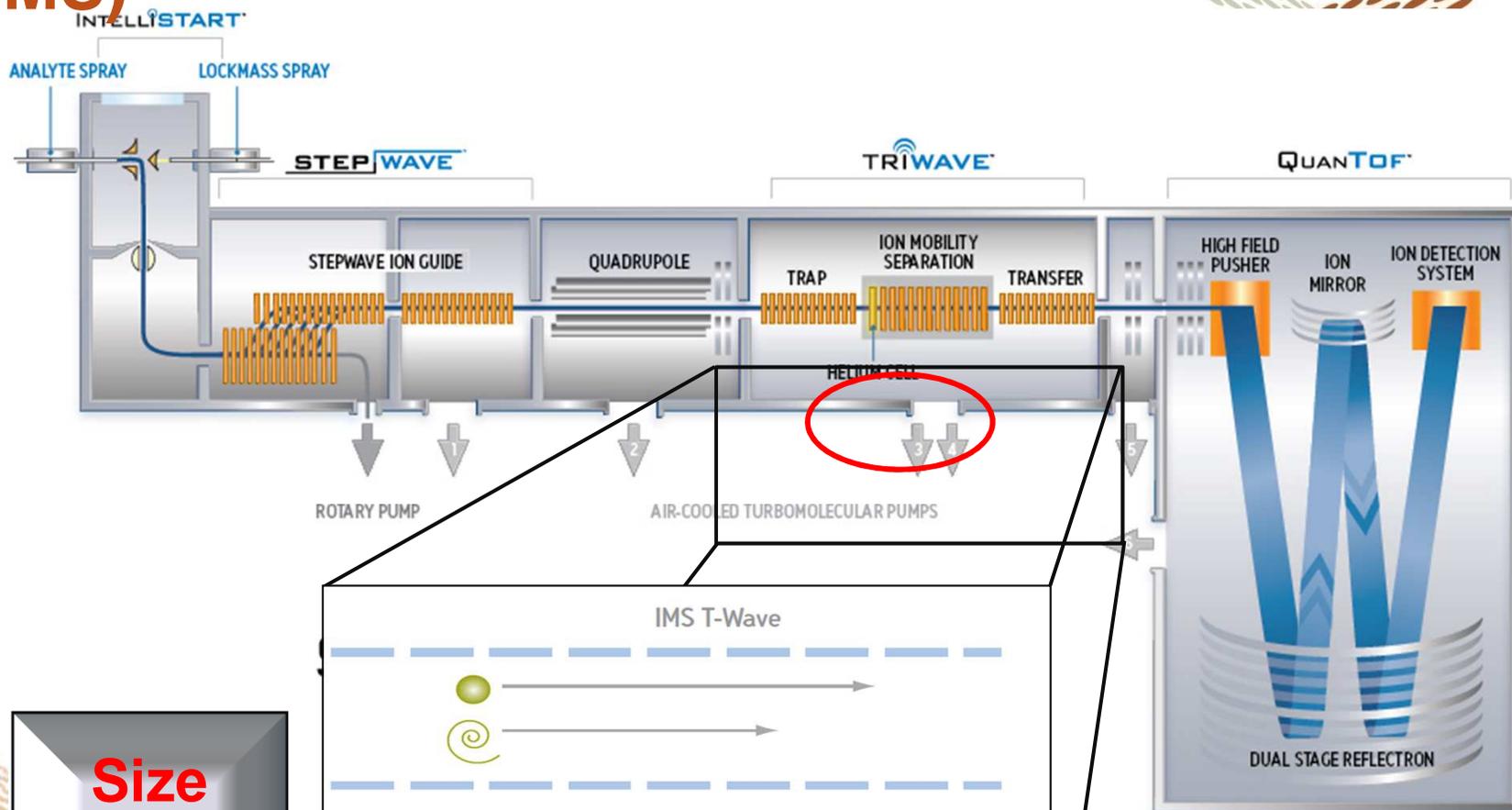
Use of Fragments



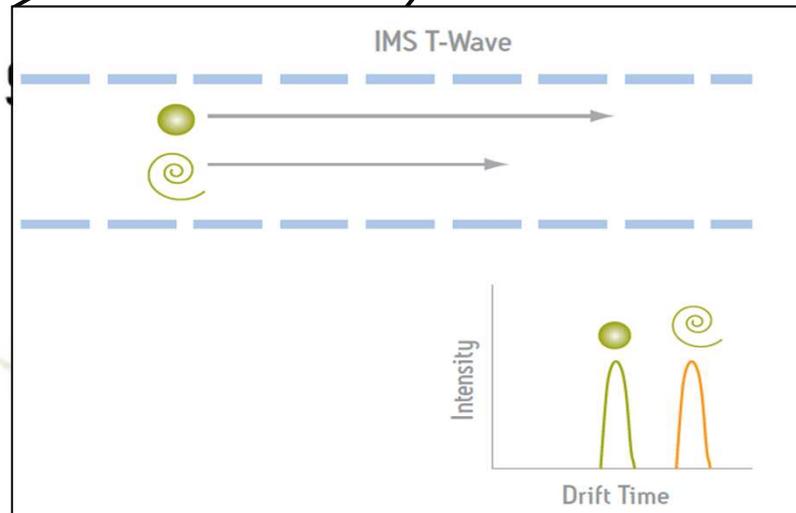
- Relative value of fragments without precursor ion selection
- *When analysing pesticides at 100 ppb with a high cone voltage, 57 of the 83 compounds only gave M+H (no fragments)*
- All ions or MS^e do not always produce detectable fragments

*Information from Amadeo Fernandes Alba

SYNAPT G2-S High Definition MS (HDMS)



Size
Shape
Charge



Future developments : ion mobility

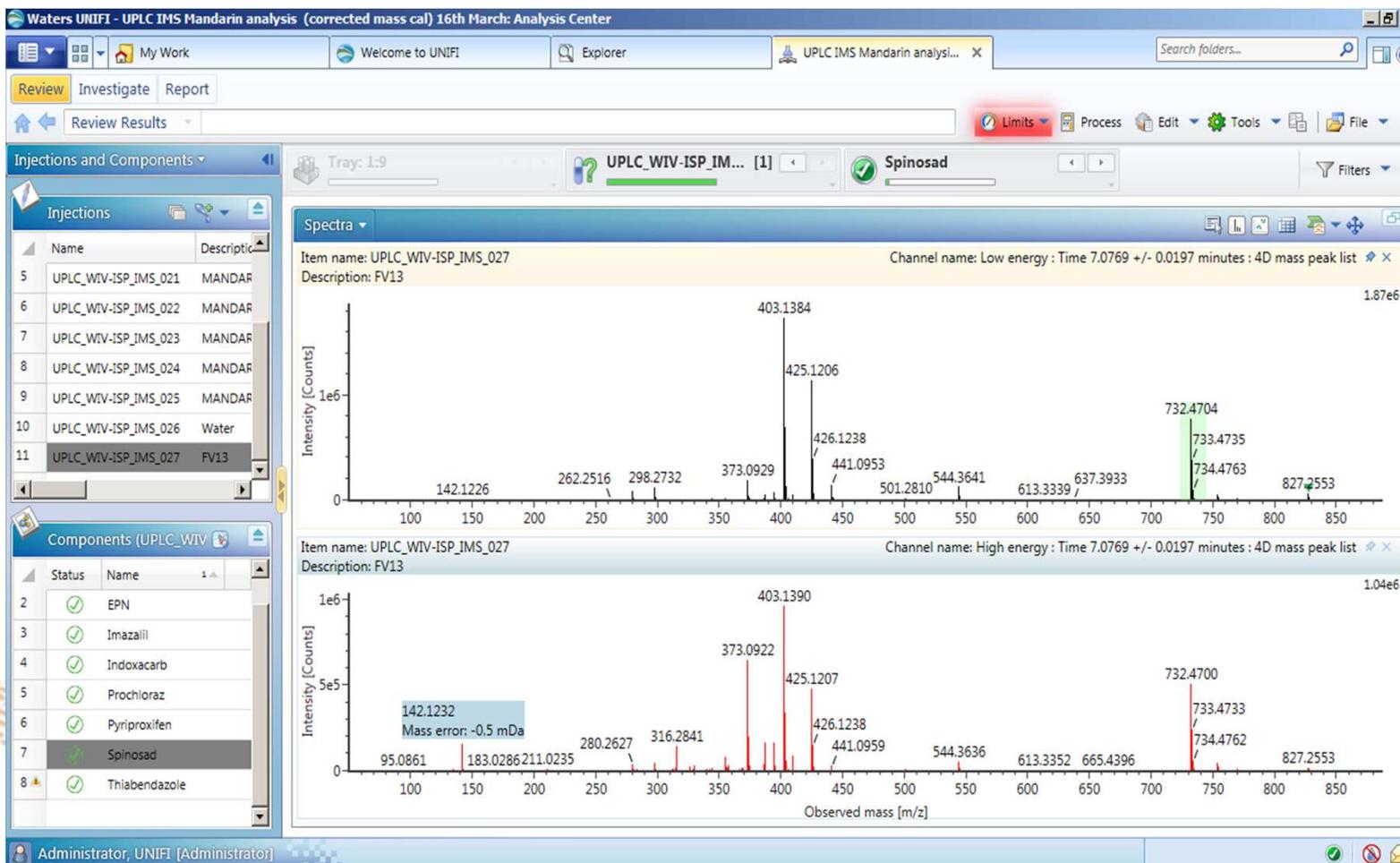


- Another dimension of separation (size and shape of the molecule)
- Drift time Independent of matrix
- Selectivity needs to be proven
- Can CCS value be used as an Identification point?

Observed MSE spectra for spinosad in Mandarin extract



Environment
Agency



Authors:

Michael McCullagh¹, Severine Gosciny², Vincent Hanot², David Douce¹, Dominic Roberts¹, Sara Stead¹ and Ramesh Rao¹

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Mobility resolved MSE spectra for spinosad in Mandarin extract



The Food and Environment



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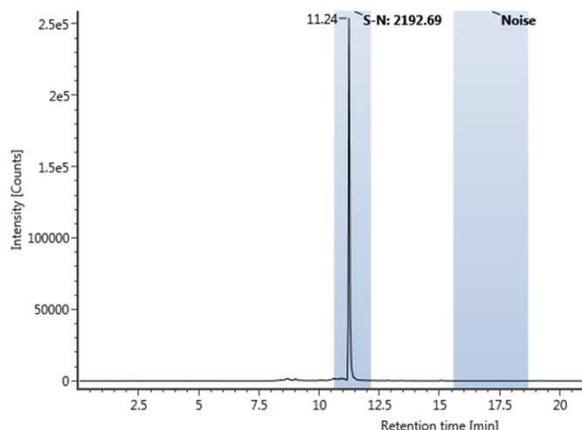
Future Development- Micro flow LC



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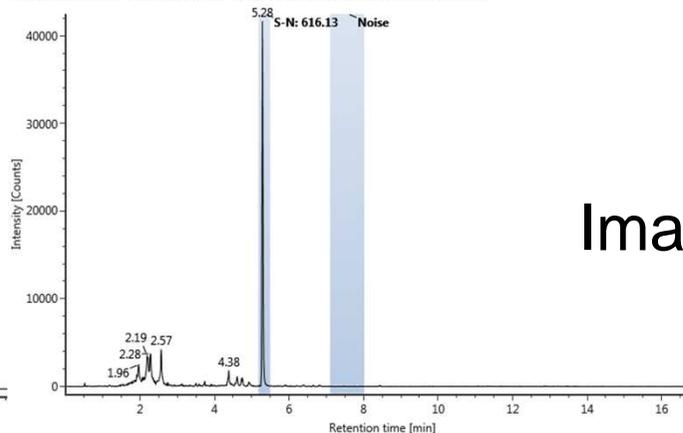
Prototype Microfluidic device

Item name: T150_PSS_ACN_290
Channel name: Mass Chromatogram (16.7 PPM) : +297.0561 : 1: TOF MSe (50-1200) 4eV ESI+



UPLC

Item name: UPLC_WIV-ISP_ACN_021
Channel name: Mass Chromatogram (16.7 PPM) : +297.0556 : 1: TOF MSe (50-1200) 4eV ESI+



Imazalil 10pg/ μ L

	S/N	RESPONSE	Amount on Column	Calculated Gain
Prototype Microfluidic Device	2163	2.5e ⁵	20pg	S/N 8.8 Response 15
UPLC	616	4.2e ⁴	50pg	

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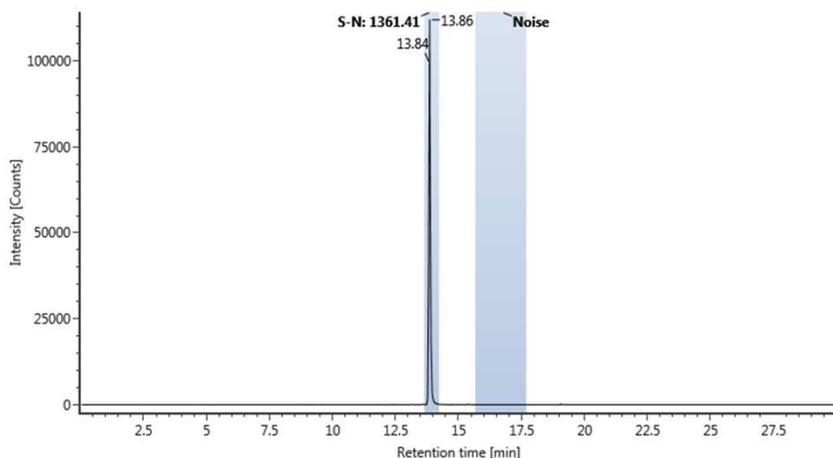
Micro fluidic Device/UPLC response



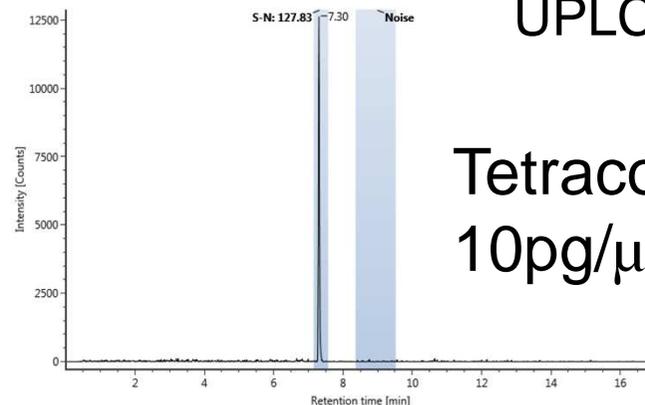
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Prototype Microfluidic device

Item name: T150_PSS_ACN_290
Channel name: Mass Chromatogram (16.7 PPM) :+372.0294 : 1: TOF MSe (50-1200) 4eV ESI+



Item name: UPLC_WIV-ISP_ACN_021
Channel name: Mass Chromatogram (16.7 PPM) :+372.0288 : 1: TOF MSe (50-1200) 4eV ESI+



UPLC

Tetraconazole
10pg/ μ L

	S/N	RESPONSE	Amount On Column	Calculated Gain
Prototype Microfluidic Device	1361	1.3e ⁵	20pg	S/N 27 Response 26.6
UPLC	128	12500	50pg	

Authors:

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Summary



- Substantial developments to hardware and software, but further improvements still required.
- In the interim period then it is likely that non-targeted screening approaches will be use in parallel with targeted methods.
- In the future, detection, identification, quantification and non targeted analysis will be combined into a single analysis.



Acknowledgements



Sara Stead, Mike McCullagh and Dominic Roberts:

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Culture and Sport for her postdoctoral contract

Further information on qualitative screening:
H G J Mol et al, Drug Testing and Analysis 2012, 4 (Suppl. 1), 10-16

Hans Mol , Non target is our Target, The Analytical Scientist #5,
May 2013.