Joint EURL pesticides meeting
October 23rd-25th, 2013
Almeria Spain

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LC-HRMS: Challenges for Routine Implementation

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Presentation Outline

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

The need for HRMS screening

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- 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

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- 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

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Sample prep^{n}	Generic extraction/clean-up	Limitations
Analysis	Generic chromatography and ionisation with acquisition over the full mass range (quadrupole, IT, ToF, QToF, orbitrap etc)	Resolution & response
Data processing	>500 pesticides must be automated	Efficient software
Validation + AQC	Must be reliable if used for monitoring	Effective, but practical

View from other laboratories

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- 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

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

Data Acquistion

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

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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 *m/z* 142.0087 26500 at *m/z* 284.1417 30000 at *m/z* 413.1284 41000 at *m/z* 732.4695

The chromatography compromise

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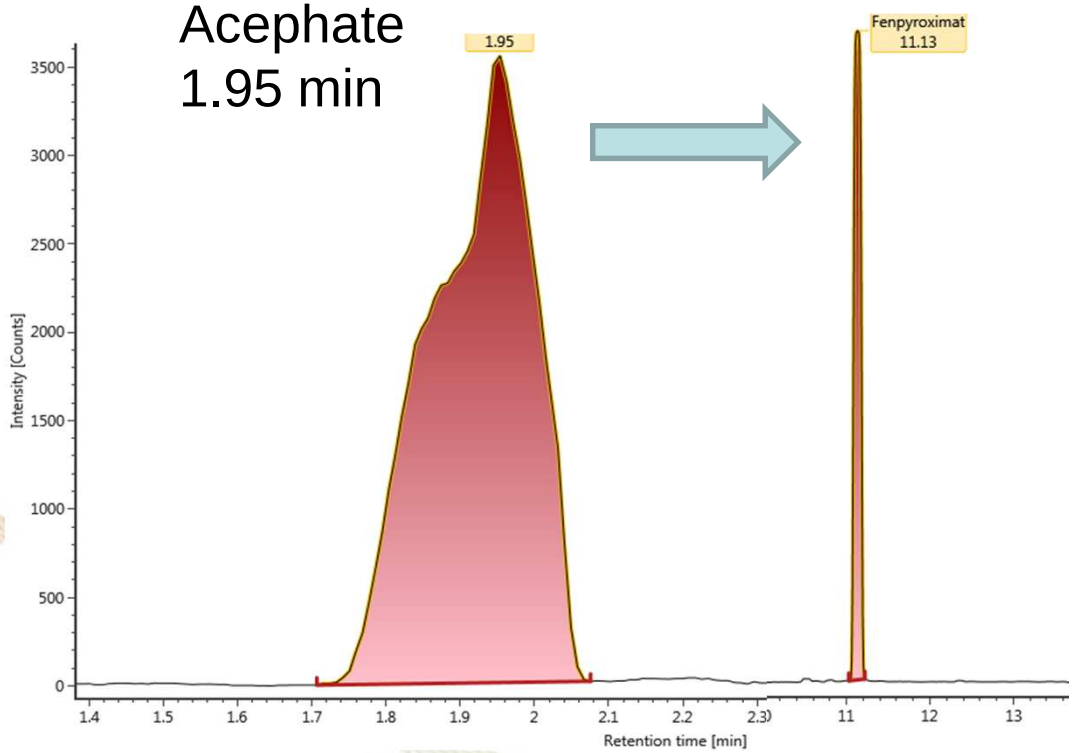
- 25:75% MeCN:water, 6 µL injection volume

fenpyroximate
11.13 min

Acephate
1.95 min



Component evaluation

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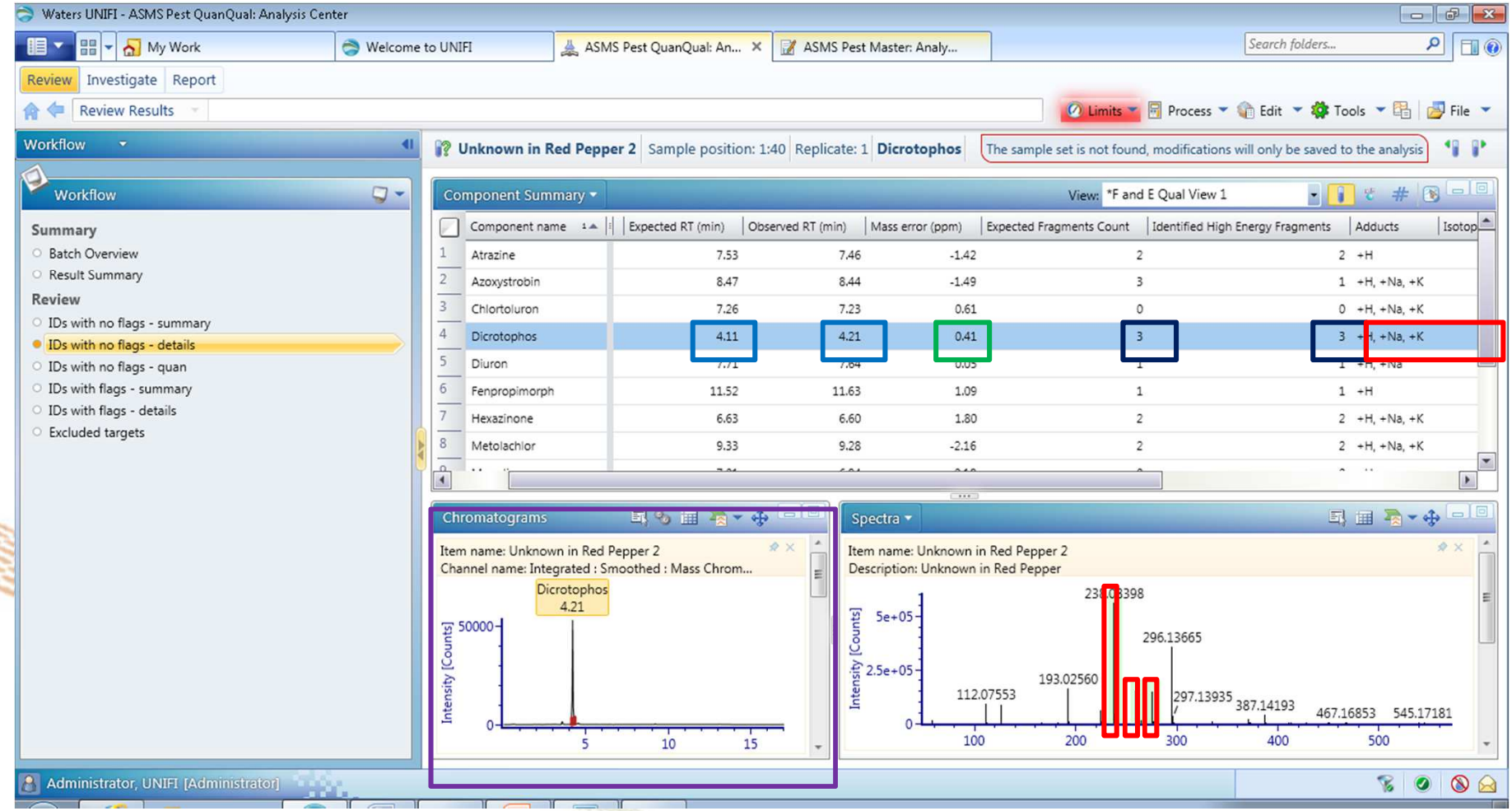

Database

Maintenance up to date

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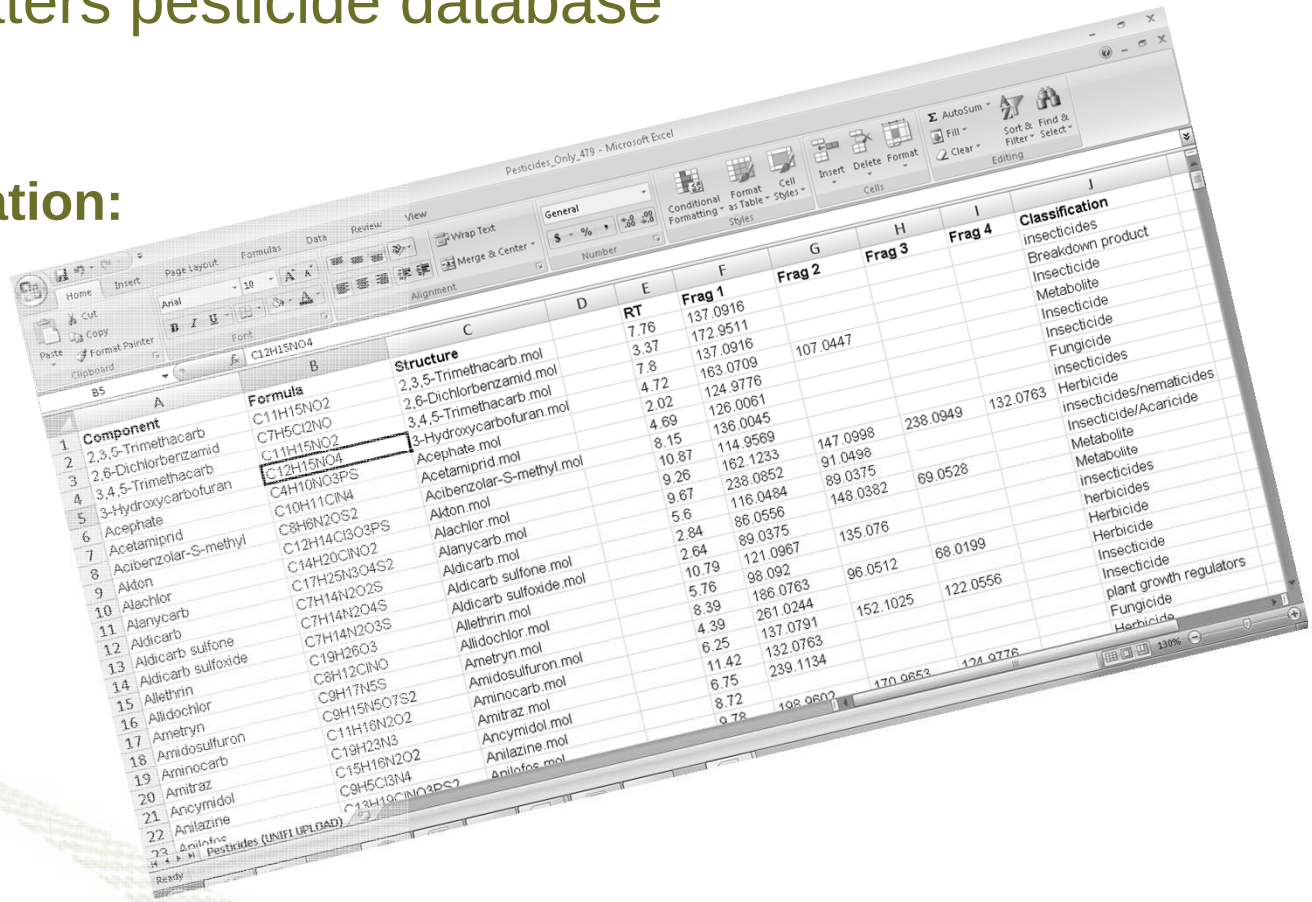
In this example: Waters pesticide database

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



Qualitative screening: validation

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Document N° SANCO/12495/2011

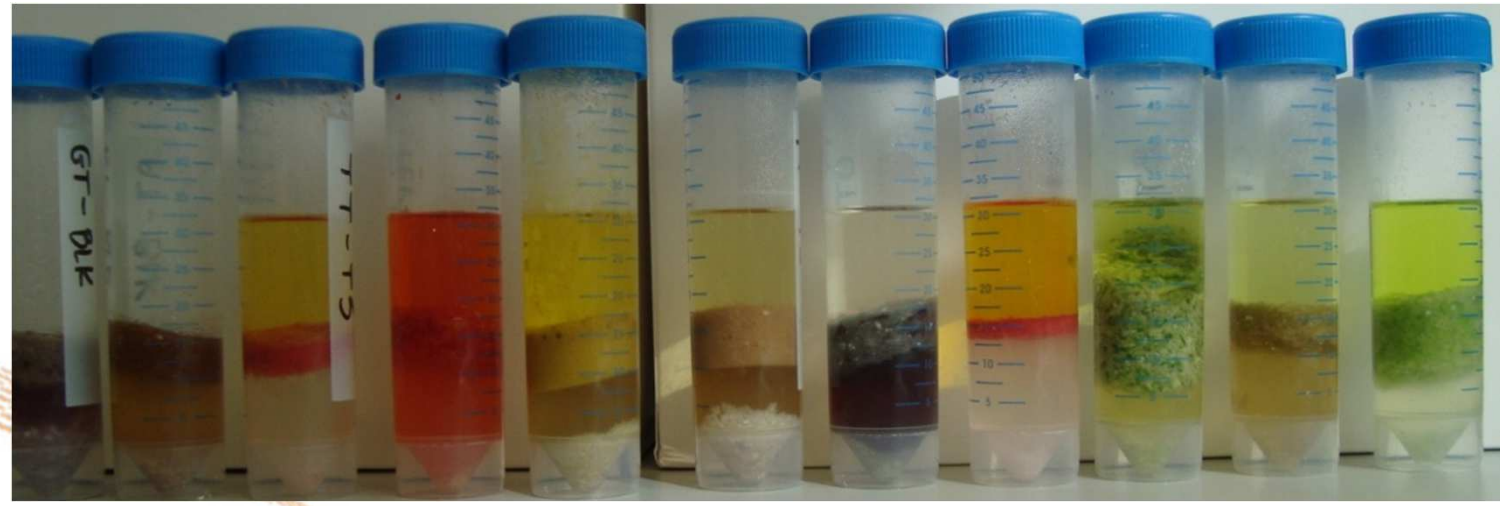
Supersedes Document No. **SANCO/10684/2009**

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

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- 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

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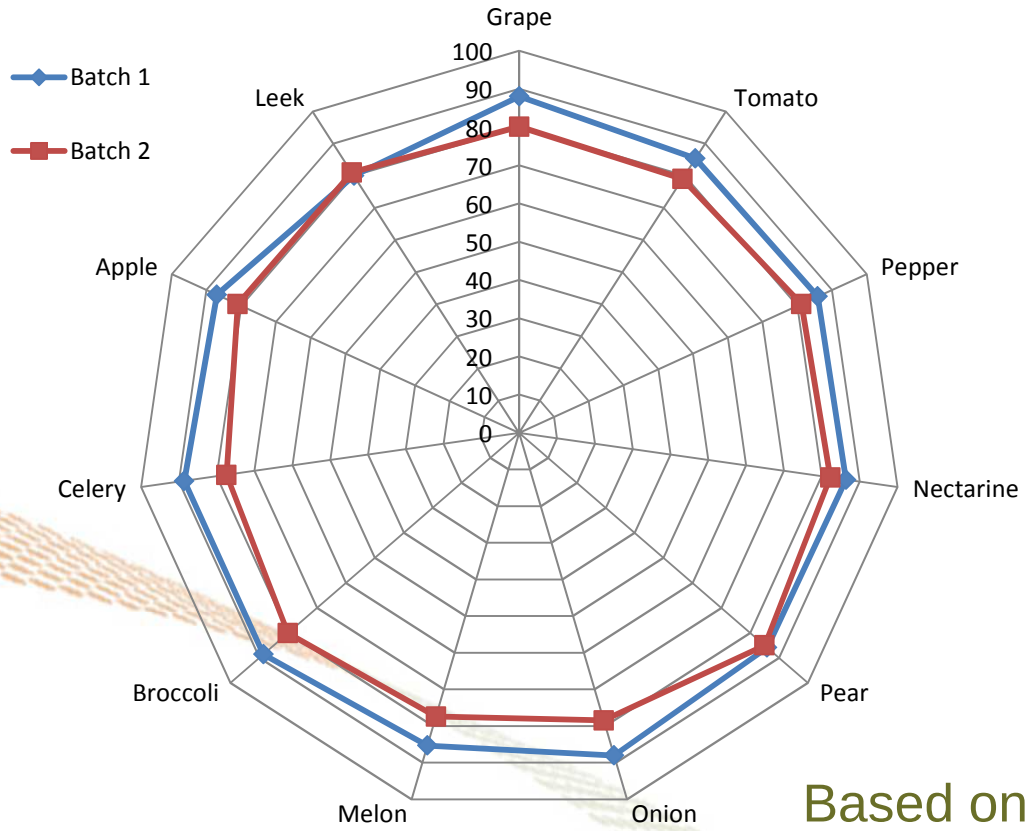
Settings: ± 10 ppm, ± 0.5 min, detector counts threshold 100



	Batch 1	Batch 2
Detection rate mean (%) ± SD	**86 ± 2**	**80 ± 2**
False negatives rate mean (%) ± SD	**14 ± 2**	**20 ± 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 ≥ 95% of the samples	130	150
% of pesticides detected in ≥ 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 (± min)	Fragments observed?	Mass error (±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 (± min)	Fragments observed?	Mass error (±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

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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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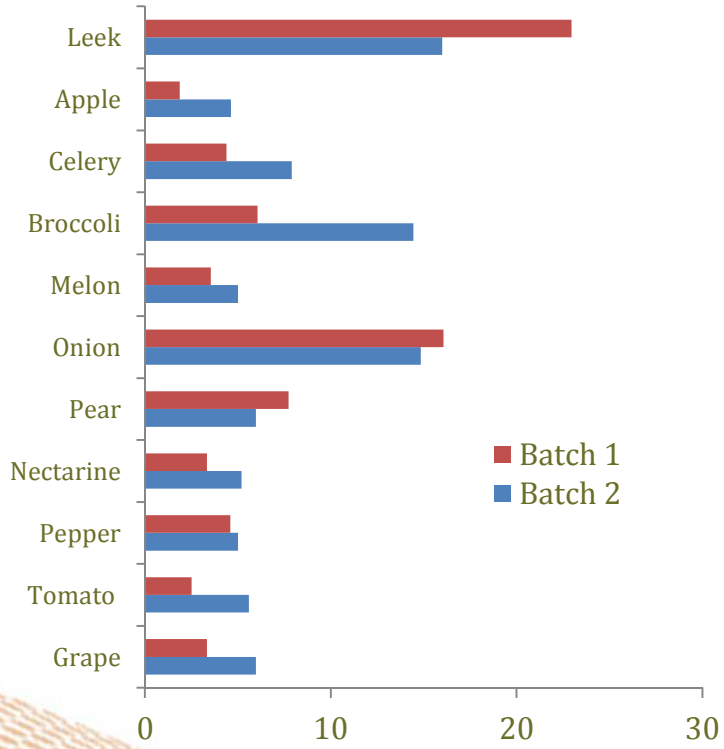
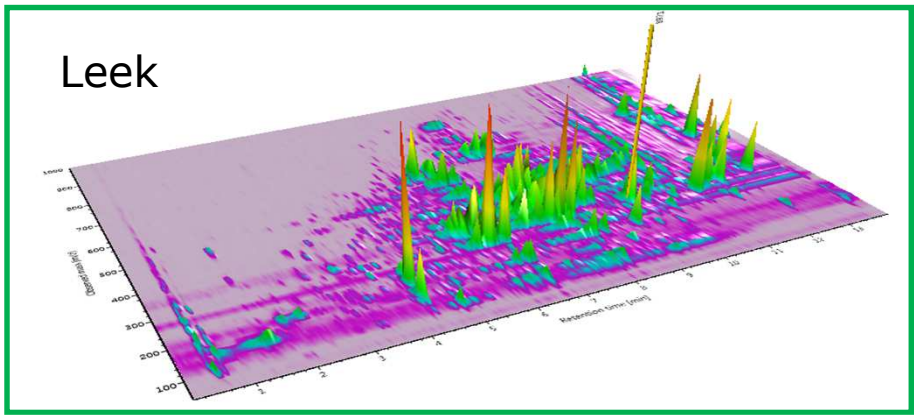
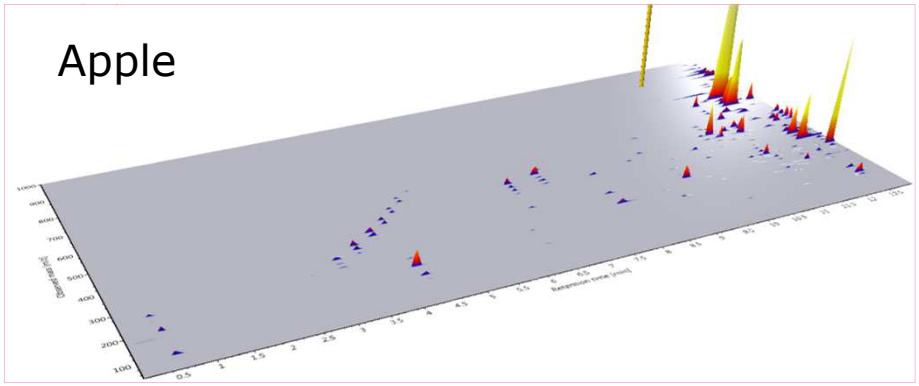


False detection rate calculated in blank samples against 479 compounds in batch 1 and versus 519 in batch 2





System Maintenance

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- 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

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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

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- 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

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- 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

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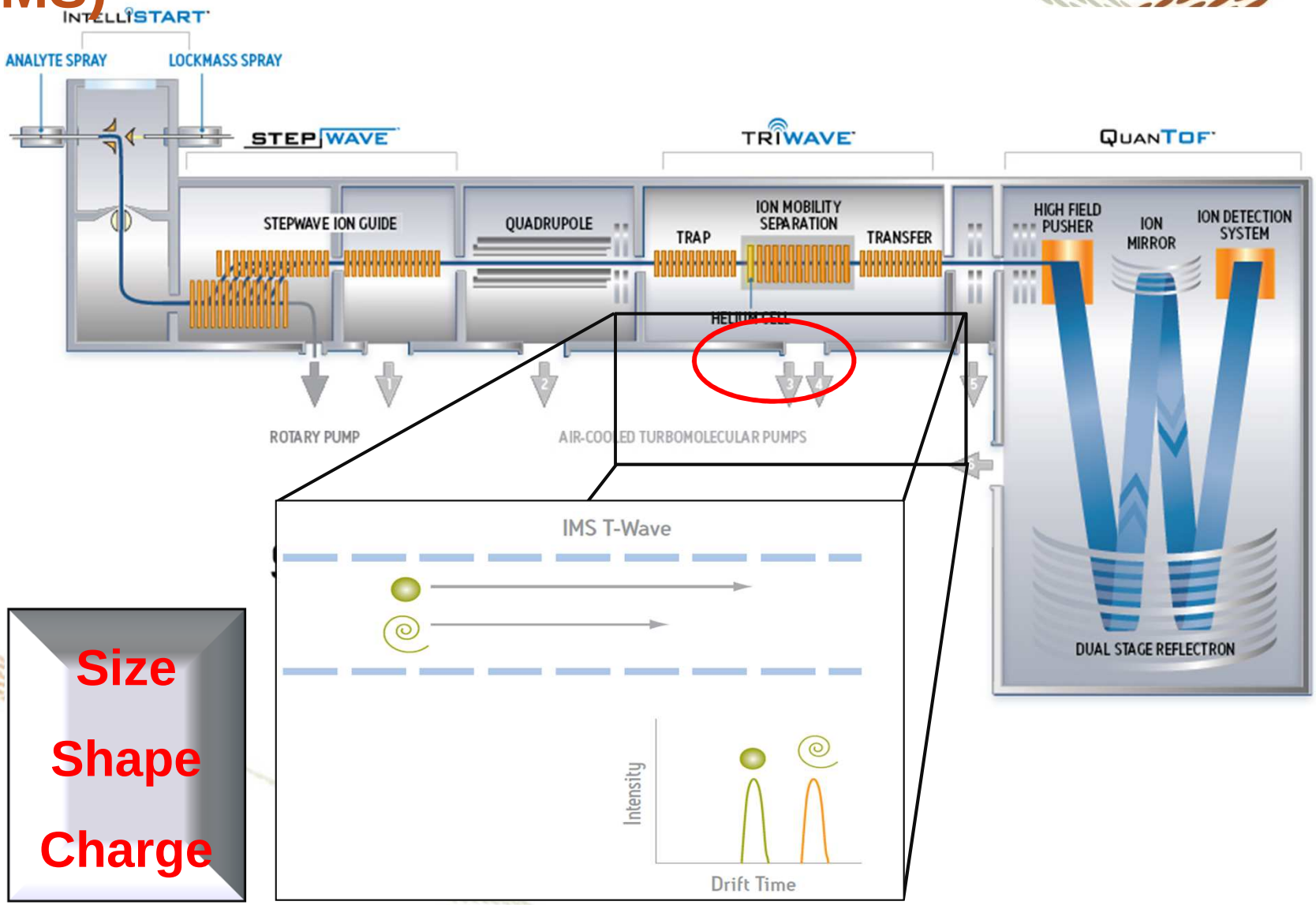
- Relative value of fragments without precursor ion selection
- *When analysing pesticides at 100 ppb with a high cone voltage, 57 of the 83 compounds only gave M+H (no fragments)*
- All ions or MS^e do not always produce detectable fragments

*Information from Amadeo Fernandes Alba

SYNAPT G2-S High Definition MS (HDMS)



Future developments : ion mobility

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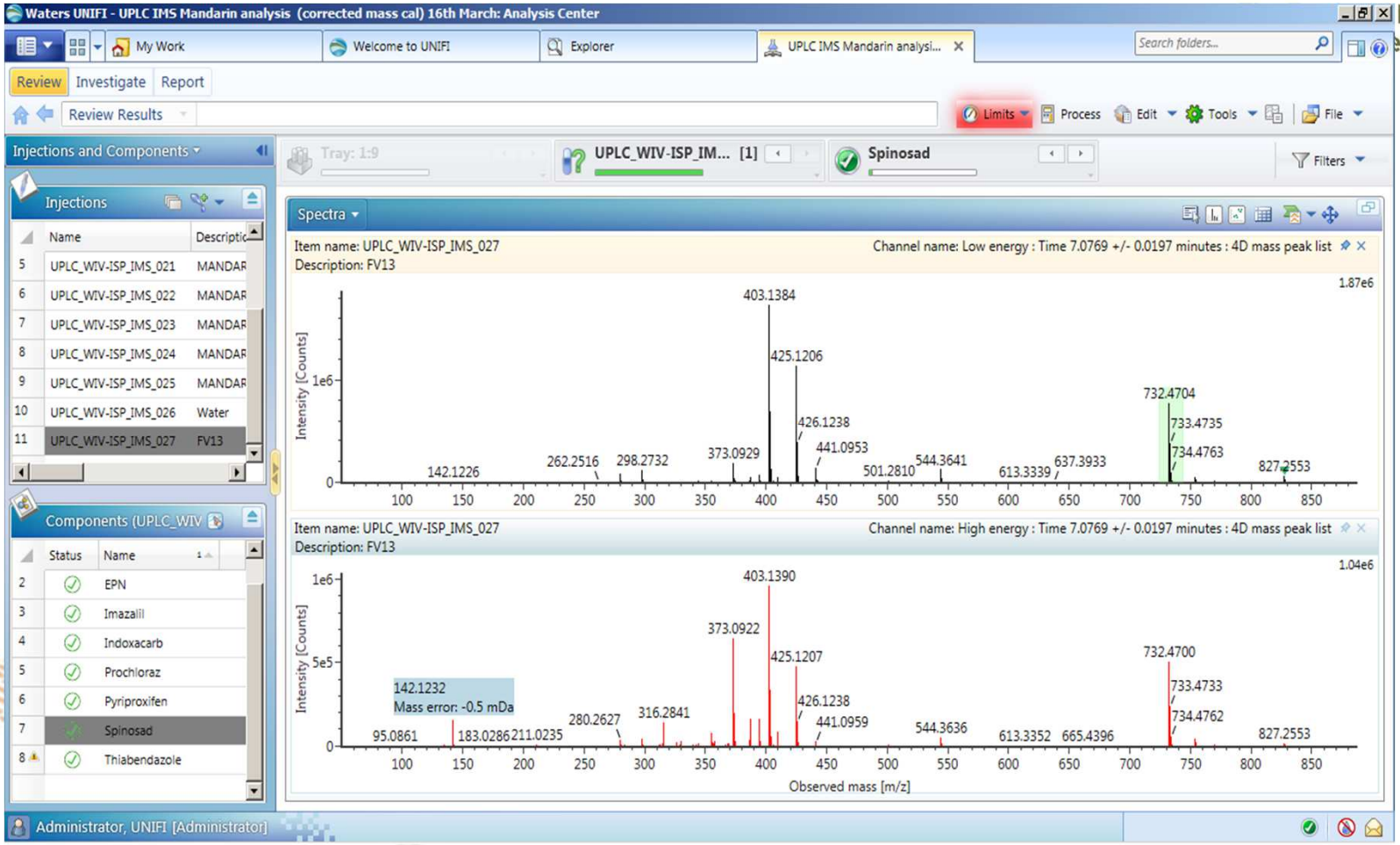
- Another dimension of separation (size and shape of the molecule)
- Drift time Independent of matrix
- Selectivity needs to be proven
- Can CCS value be used as an Identification point?

Observed MSE spectra for spinosad in Mandarin extract



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[1]Waters, Floats Road, Manchester, United Kingdom
[2]Wetenschappelijk Instituut Volksgezondheid Institut Scientifique de Santé Publique, Rue Juliette Wytsmanstraat 14 | 1050 Brussels

Mobility resolved MSE spectra for spinosad in Mandarin extract

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Future Development- Micro flow LC

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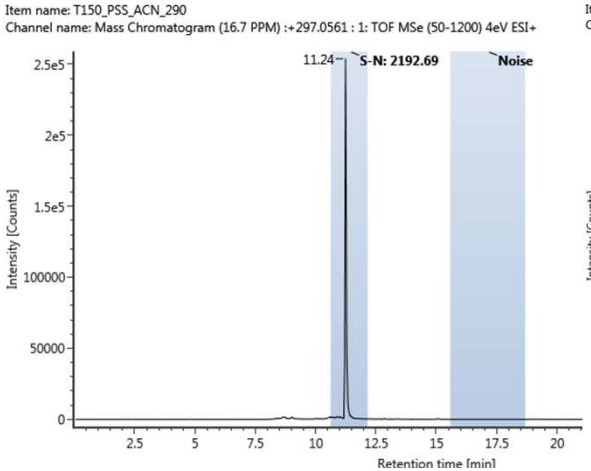
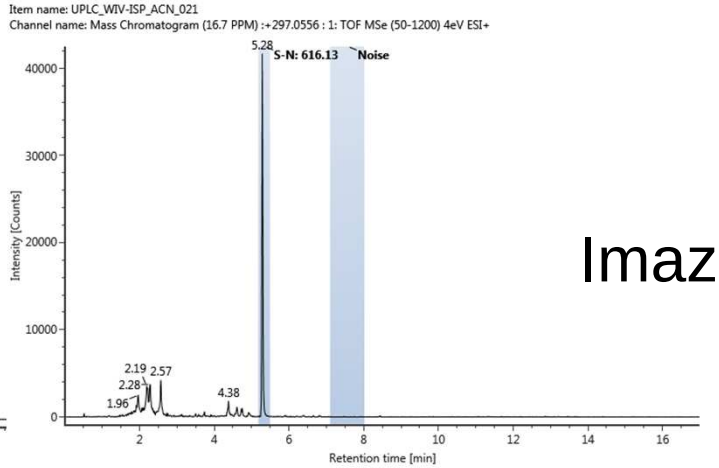
Prototype Microfluidic device



UPLC



Imazalil10pg/µL

	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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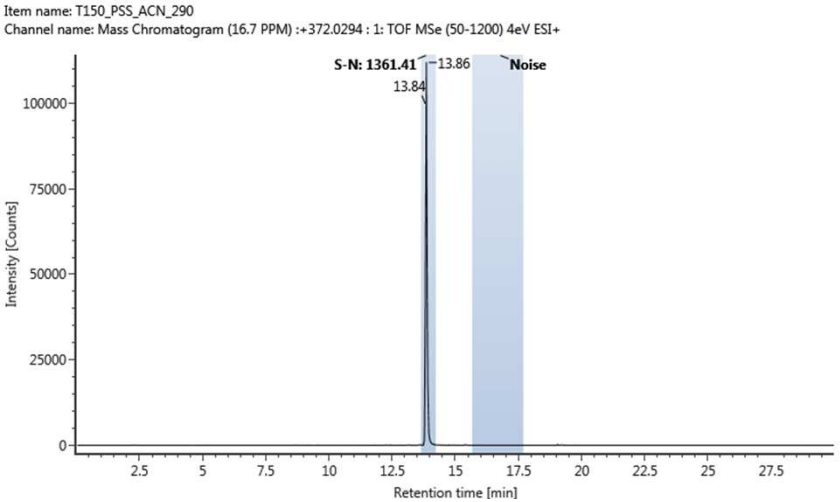
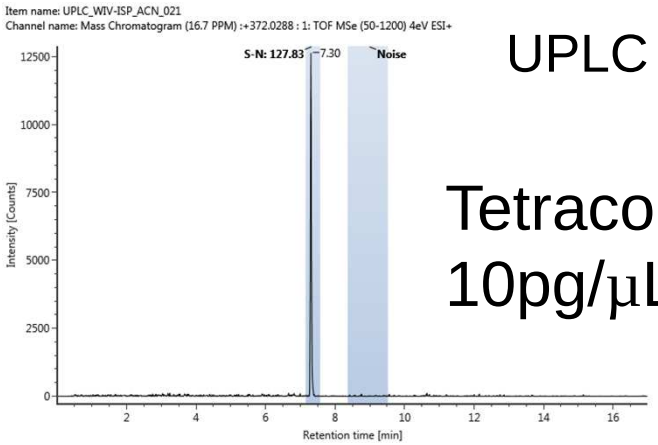
Prototype Microfluidic device





UPLC

Tetraconazole 10pg/µL

	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	

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Summary

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- 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.

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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.