FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

IAEA
International Atomic Energy Agency

The impact of different parameters on LC-MS/MS and HRMS determination for toxins and pesticides

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Livsmedelsverket
National Food Agency

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Red Analítica de Latinoamérica y el Caribe

Outline

- SweEt

Pesticides in F&V, CER and AO

- Optimizing important parameters for LC-MS/MS
- Optimizing important parameters for LC-HRMS

Toxins in water

- Optimizing important parameters for LC-HRMS

514

Extraction

10 g sample
20 ml EtOAc+$NaHCO_3$
3 min Spex shaker
Na_2SO_4

Centrifugation

Filtration

GC-MS/MS

UHPLC-MS/MS + HRMS

359

Extraction

5 g sample
10 ml water
10 ml EtOAc (1% HAc)
3 min Spex shaker
Na_2SO_4

Centrifugation

Filtration

GC-MS/MS

UHPLC-MS/MS

119

Extraction

5 g sample
PSA + C18
10 ml EtOAc
3 min Spex shaker
Na_2SO_4

Centrifugation

Filtration

GC-MS/MS

UHPLC-MS/MS

78

Fat determination

Extraction

0.5 g fat
10 ml EtOAc:CH (1+1)
3 min Spex shaker
Na_2SO_4

Centrifugation

Filtration

GPC SX-3
(1+1 EtOAc/CH)

Evaporation

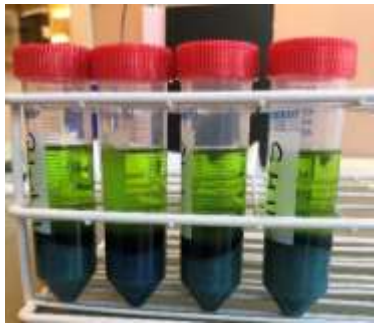
Reconstitution in EtOAc

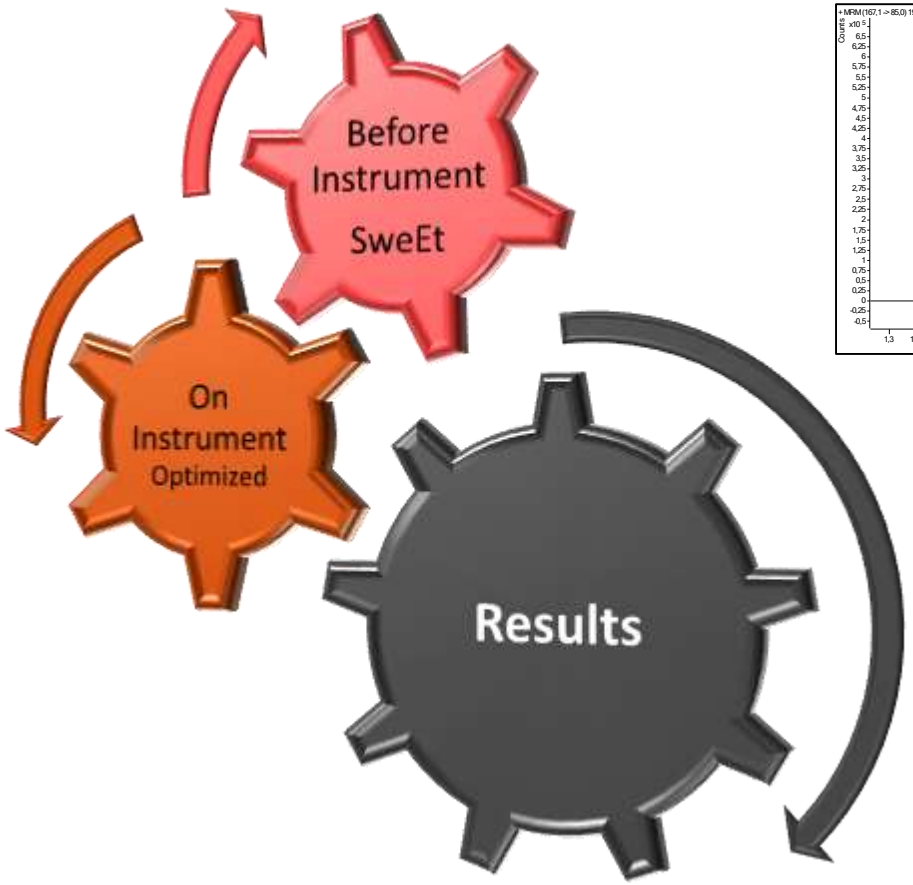
GC-MS/MS

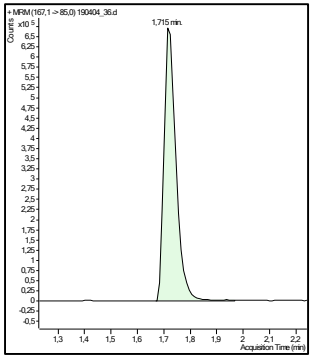
UHPLC-MS/MS

SweEt multiresidue method – a NMKL method and an ongoing CEN-method

Chain reaction







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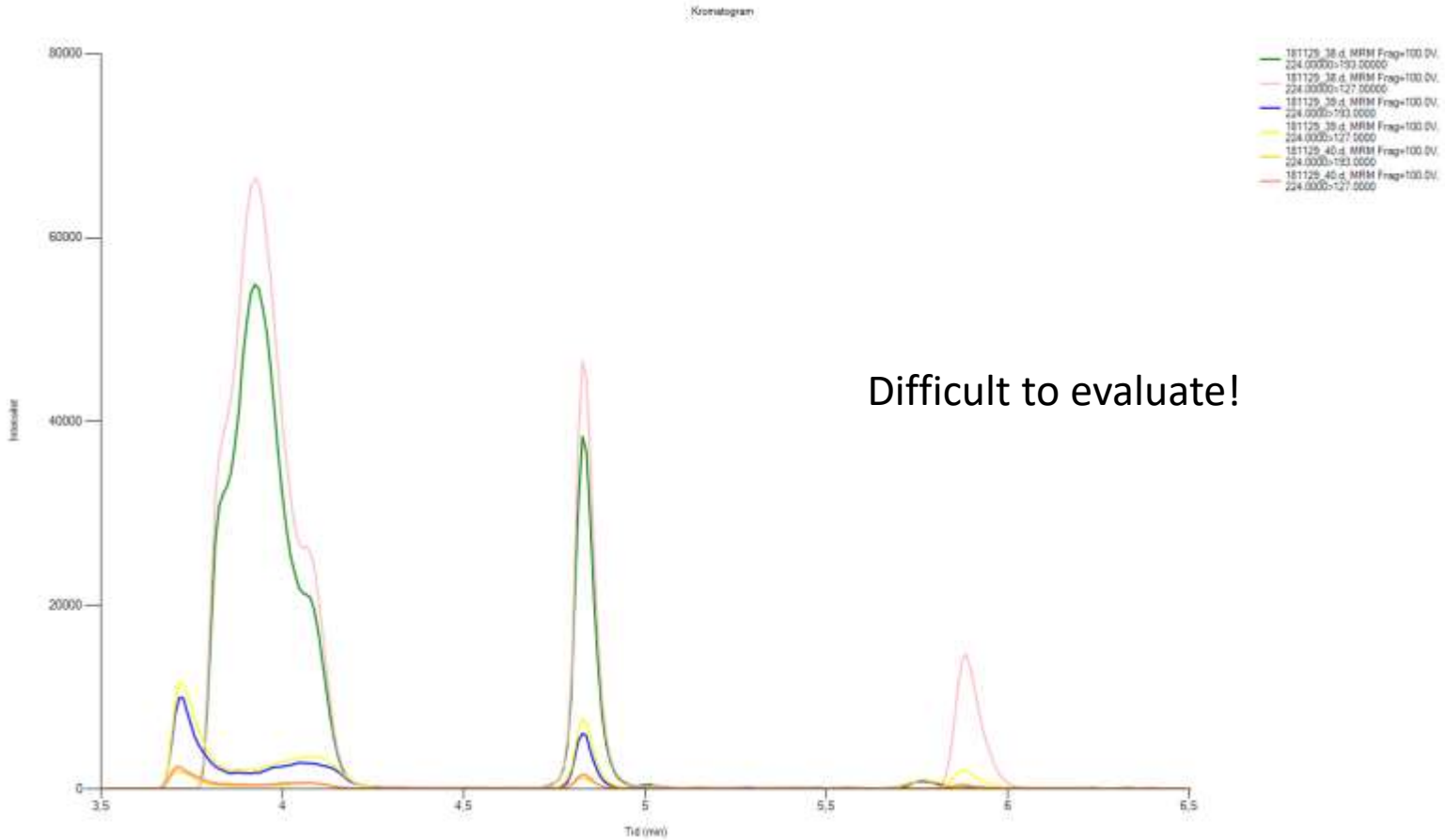
SweEt - pros and cons

+ Fast
+ Cost effective
+ Environmentally friendly
+ Extract can be analyzed directly on GC- and LC-MS/MS

- Analyses of EtAc (or ACN with QuEChERS) on LC-MS/MS where water buffer and methanol are used as mobile phase, solvent effects arise due to that the solubility between sample extract and mobile phase is limited.

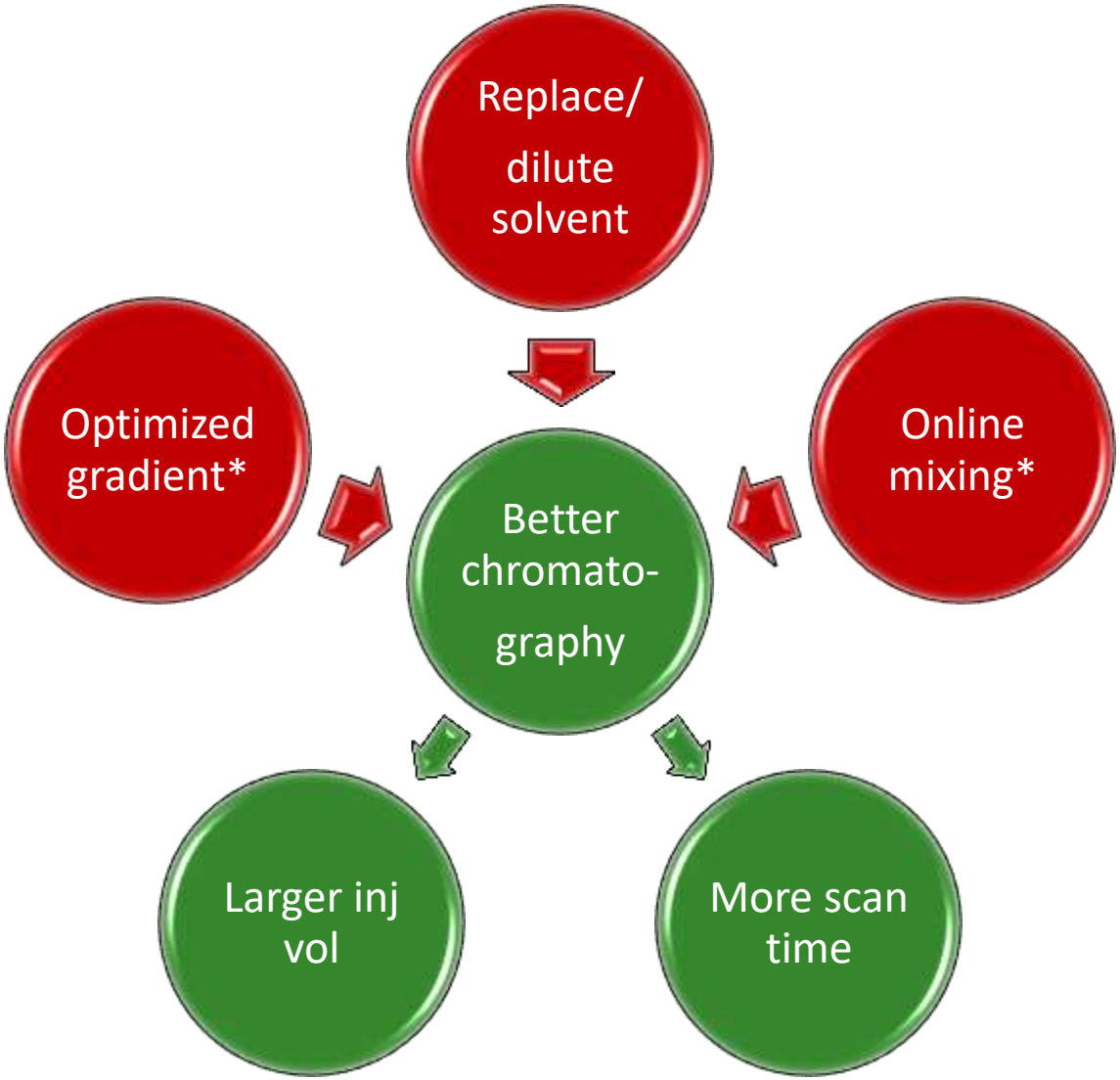
Solvent effects on LC-MS/MS Monocrotophos



Difficult to evaluate!

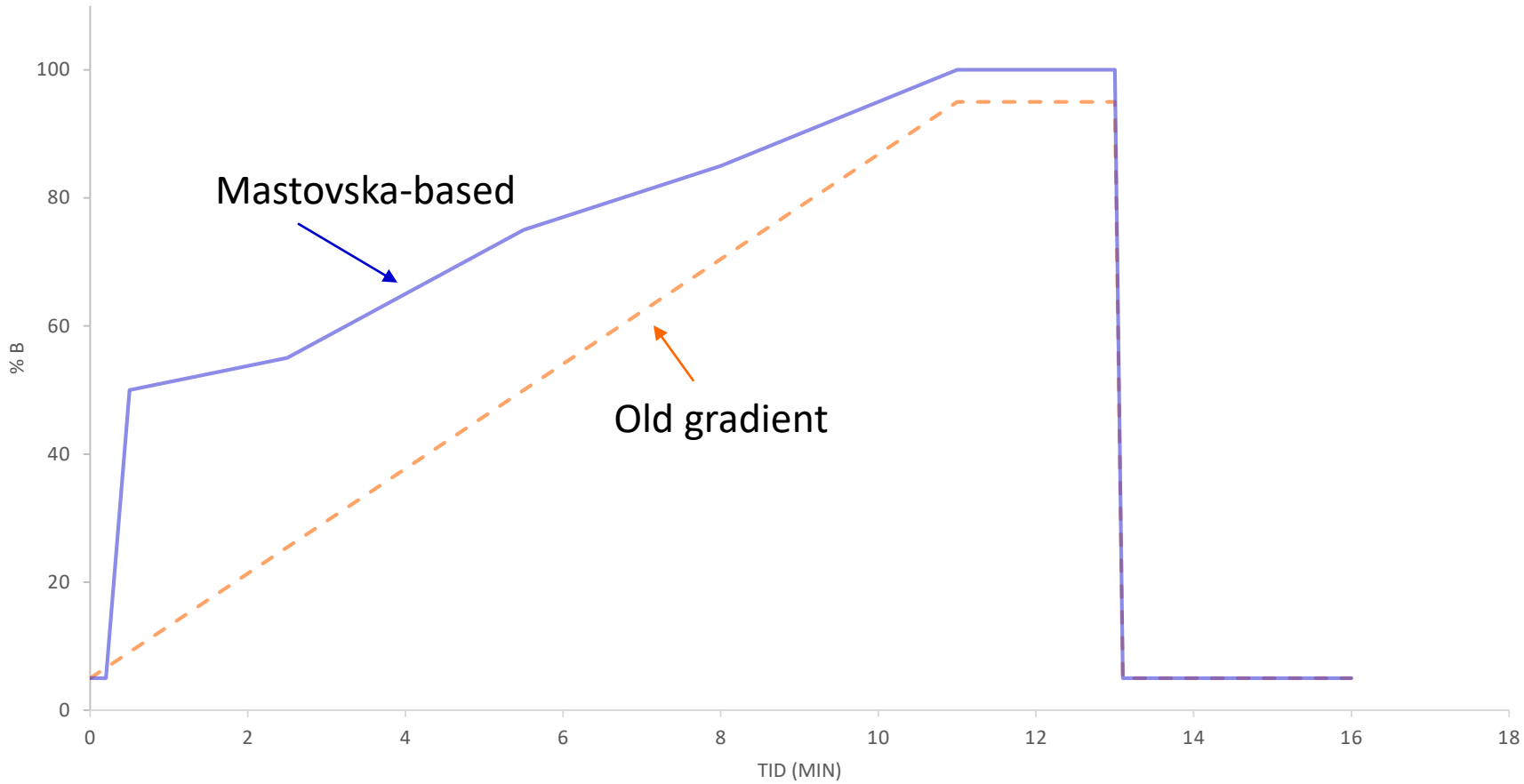
Livsmedelsverket

Solvent effects



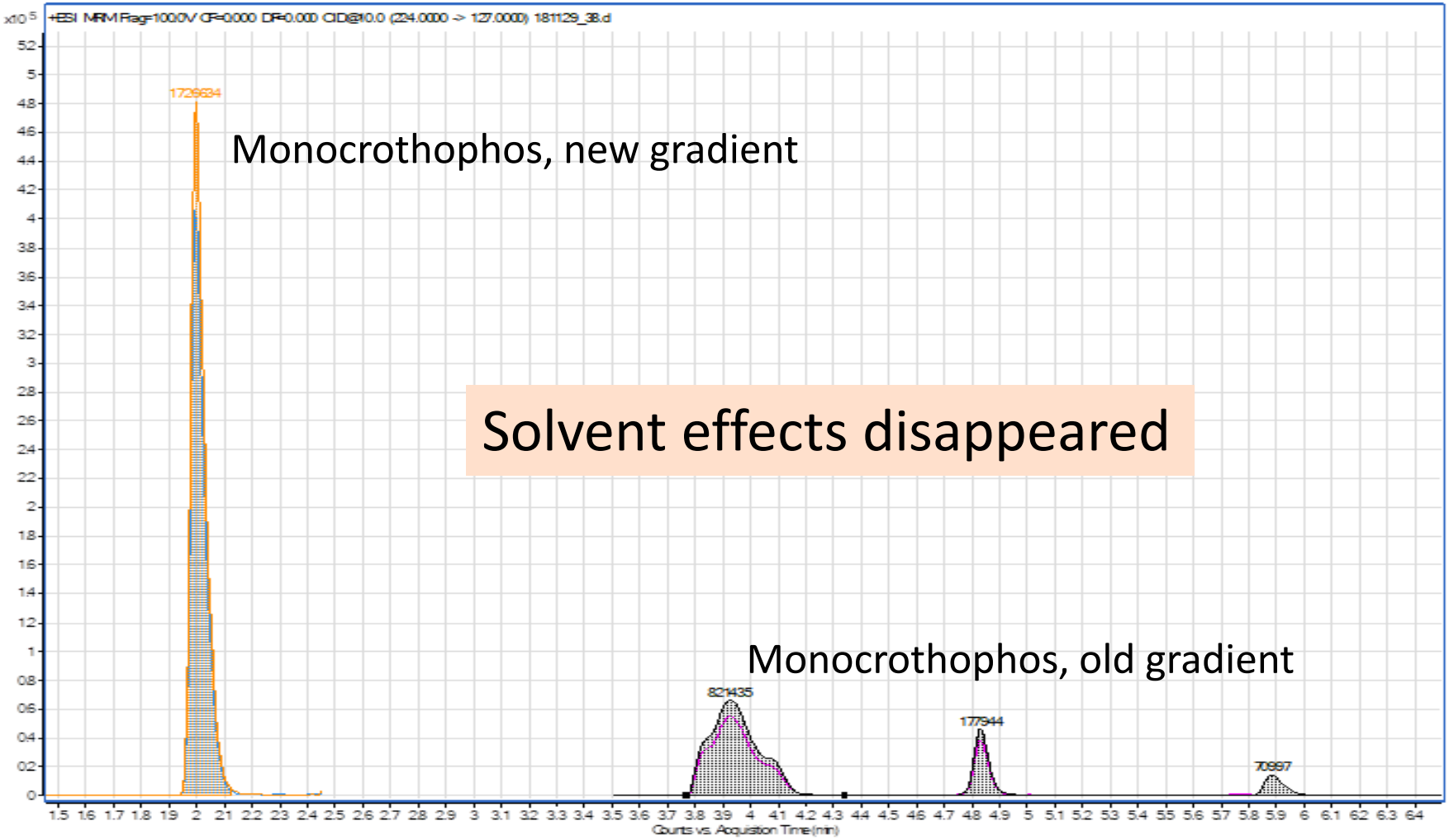
*EPRW 2016
Katerina Mastovska

Optimized gradient for LC-MS/MS



Livsmedelsverket

Results – gradient change

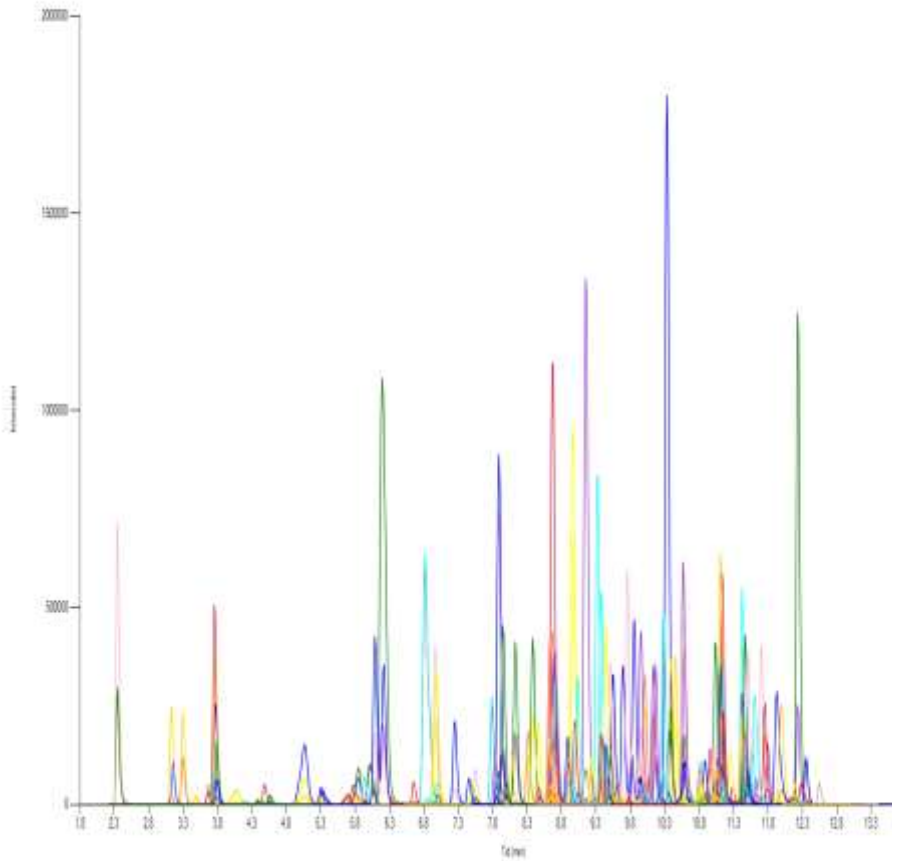


Livsmedelsverket

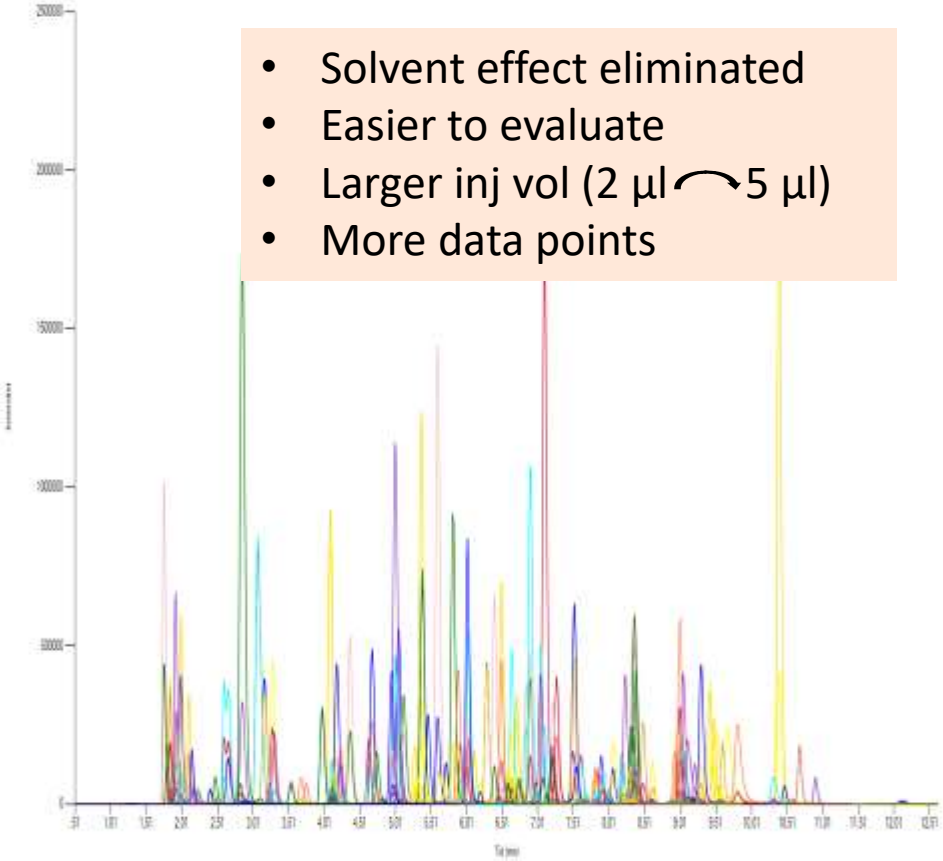
Eluting profile for LC-MS/MS

Mixture of 185 analytes in carrot

Old gradient



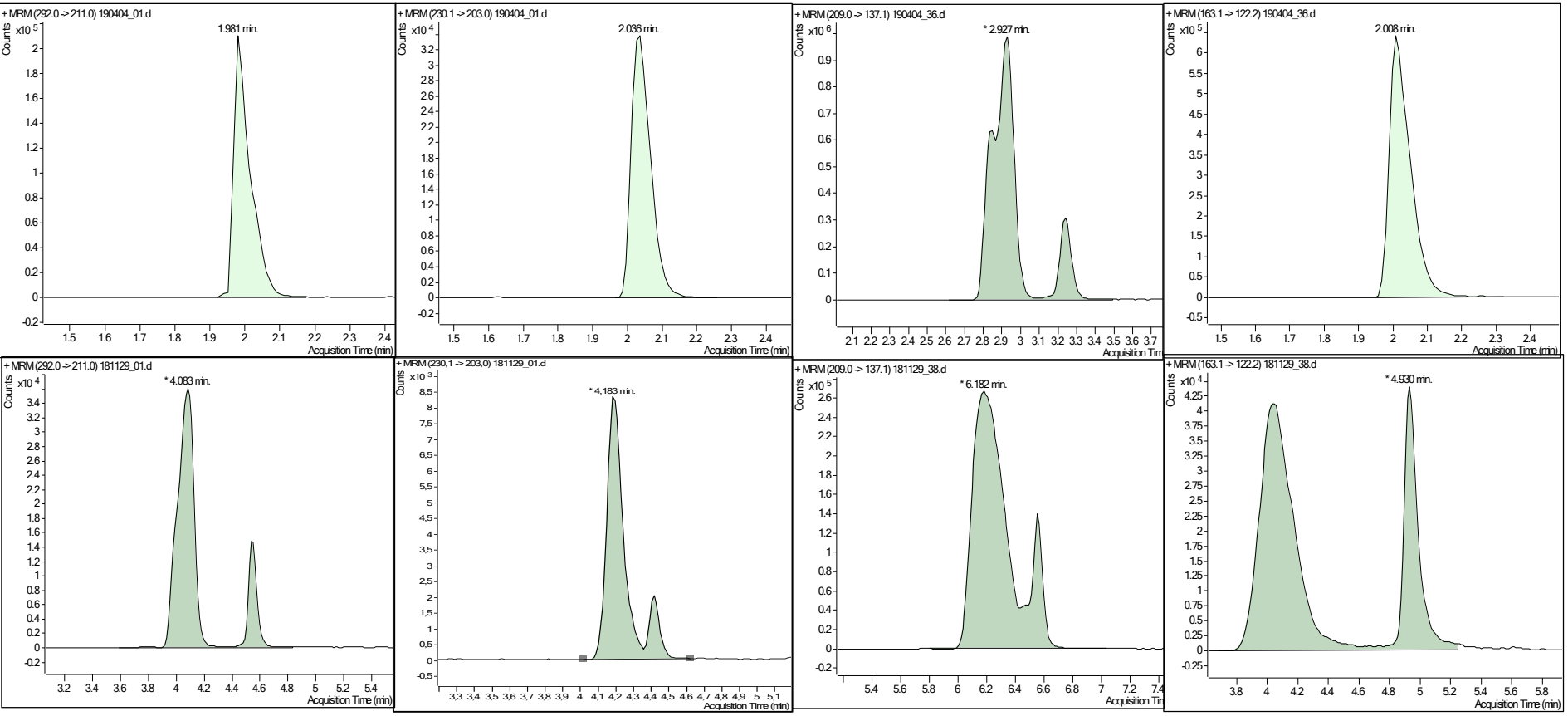
New gradient



- Solvent effect eliminated
- Easier to evaluate
- Larger inj vol (2 µl → 5 µl)
- More data points

New gradient vs old

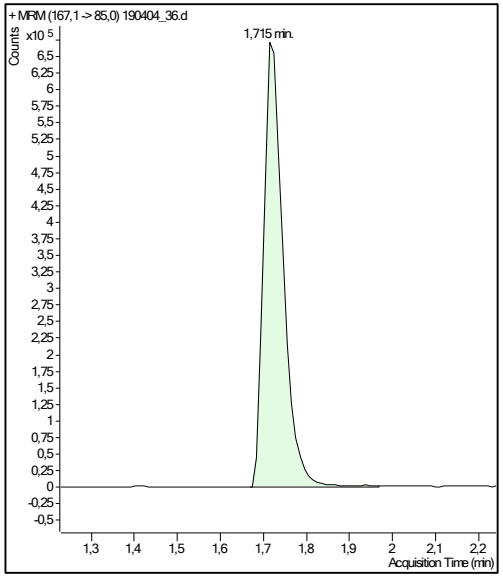
Thiamethoxam | Flonicamid | Aminocarb | DMPF



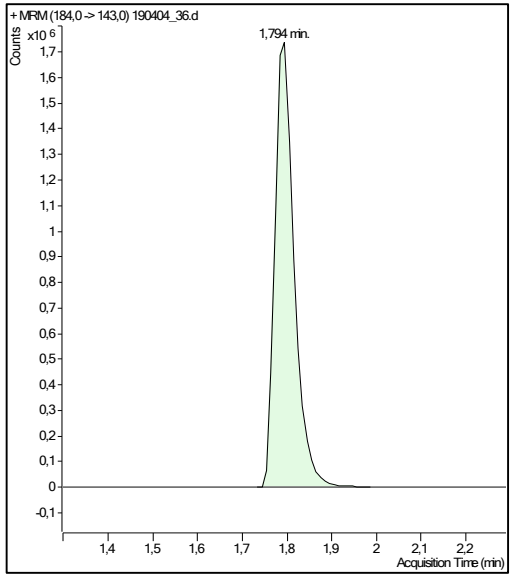
New improved gradient gives less peaks with solvent effects (due to direct injection with in ethyl acetate).

Examples of early eluting analytes < 2 minutes

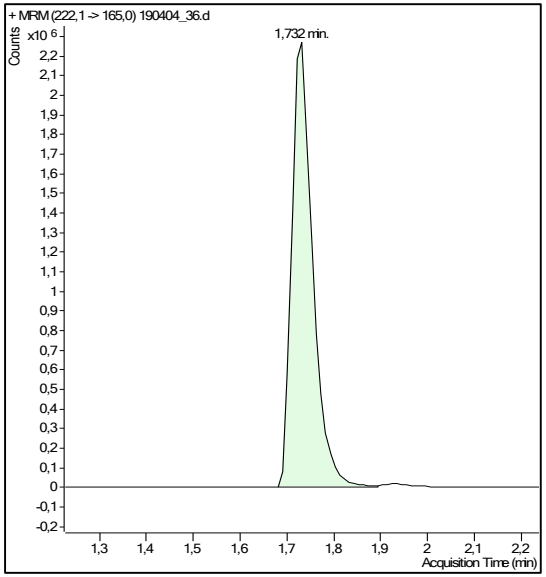
Cyromazine



Acephate



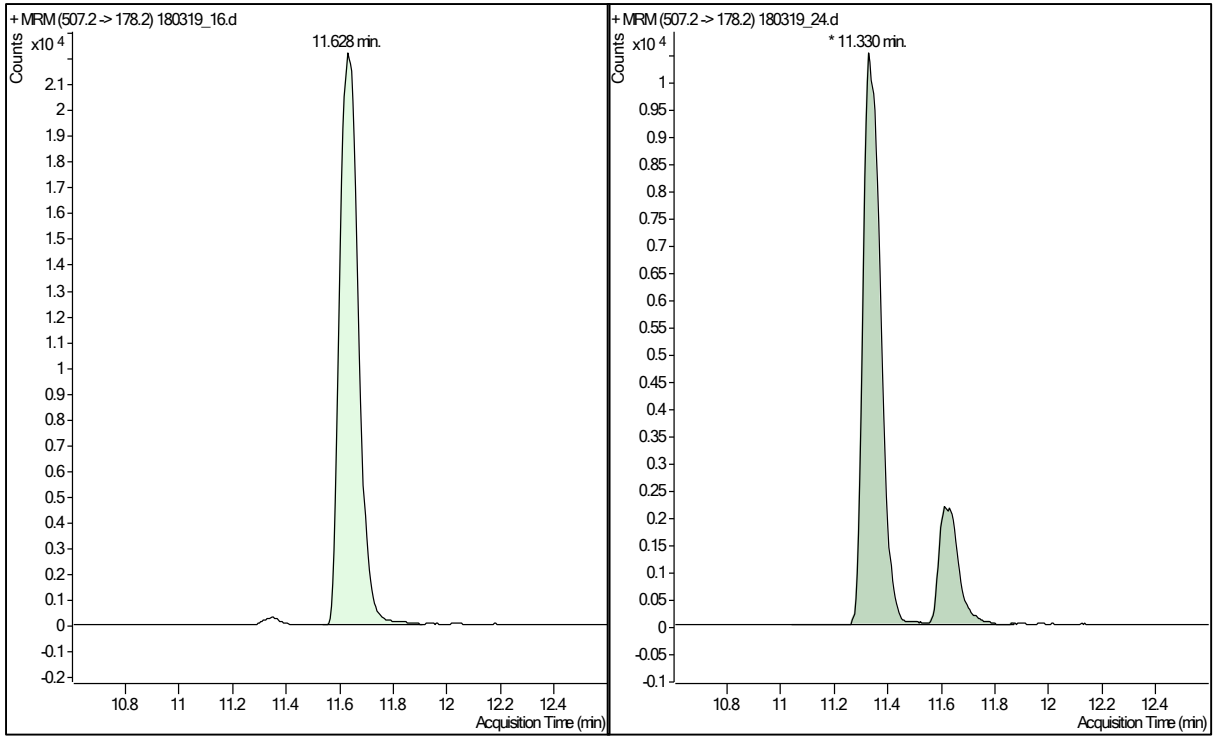
Formetanate



Livsmedelsverket

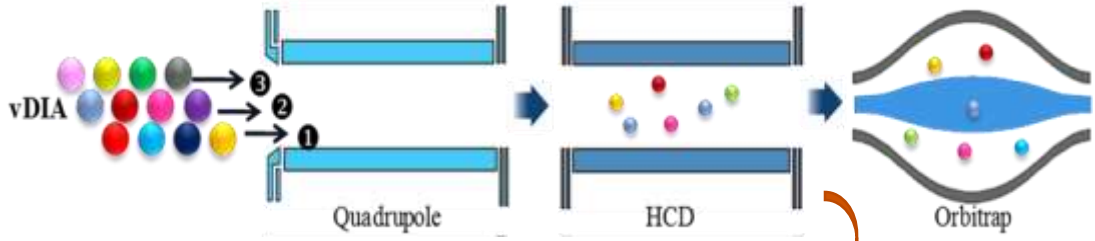
Isomers

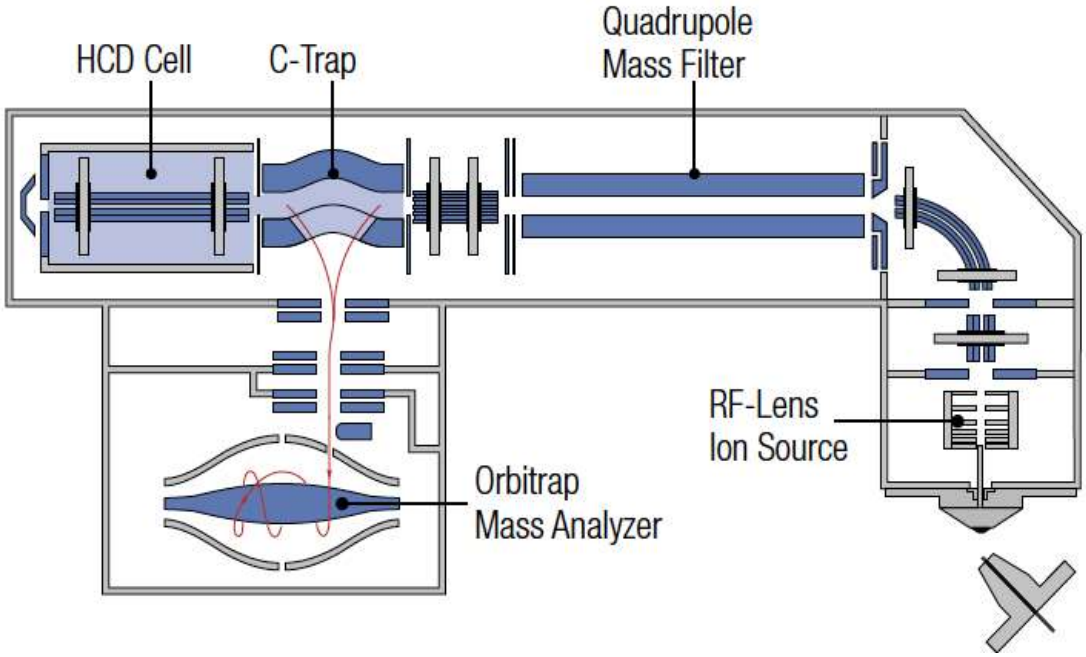
Metaflumizone; sum of E- and Z-isomers with different proportions depending on the commercial formulation.



Livsmedelsverket

Full scan combined with vDIA acquisition





Non-target acquisition

- No fragmentation (Full Scan)
- Fragmentation in HCD cell (vDIA)

vDIA is not available in the U.S.

LC-HRMS – method development

- Injection solvent, comparison EtOAc and MeOH
- Optimizing MS1
- Optimizing MS2

SANTE

- >5 scans per peak
- Mass accuracy < 5 ppm
- Target ion + confirming ion/fragment
- Back-calculated concentration ≤±20 %
- Ion ratio ± 30 %
- Drift ± 30 %
- Retention time ±0.1 min

Challenging work

1. Standard mixture ES+ alpha 185 analytes
2. Standard mixture ES+ beta 169 analytes
3. Standard mixture ES- 33 analytes

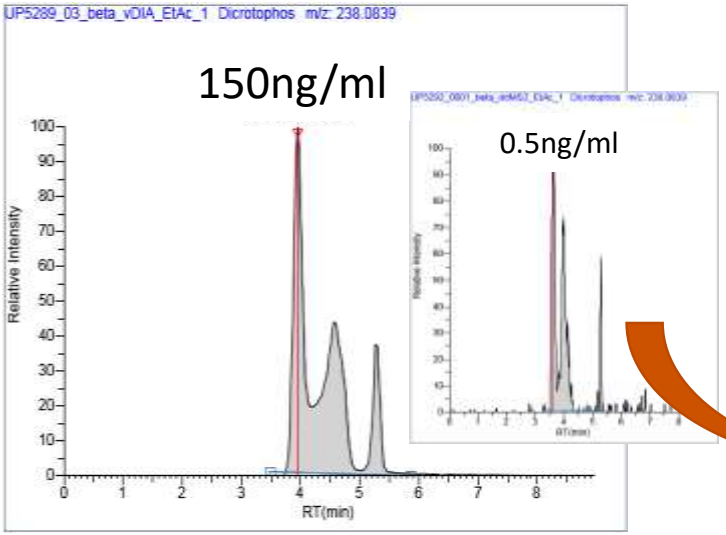
Livsmedelsverket

Properties

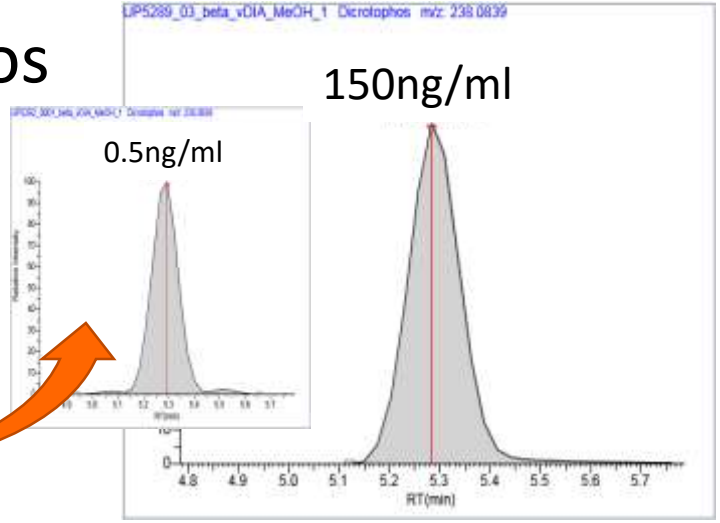
Mobile phase:
A: 5mM ammonium formiate and 0.1 % formic acid in water
B: 5 mM ammonium formiate and 0.1 % formic acid in methanol

Flow: 0.3 ml/min
Inj volume: 2 µl
Column: Accucore aQ column Thermo Scientific (100 mm x 2.1 mm ID)
Column temp: 30 °C

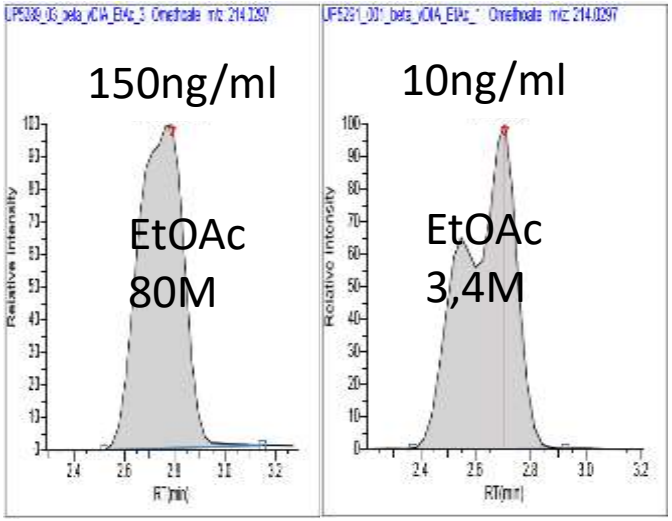
EtOAc vs MeOH in carrot



Dicrotophos



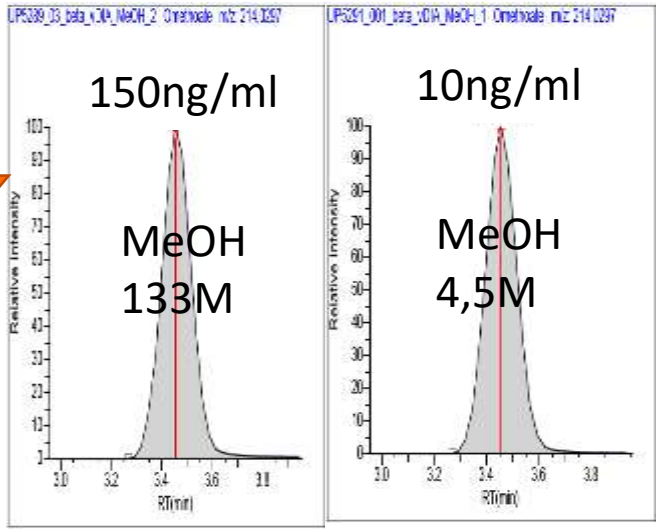
Nicer & easier peaks



Higher signal-intensity

Omethoate

M=Millions

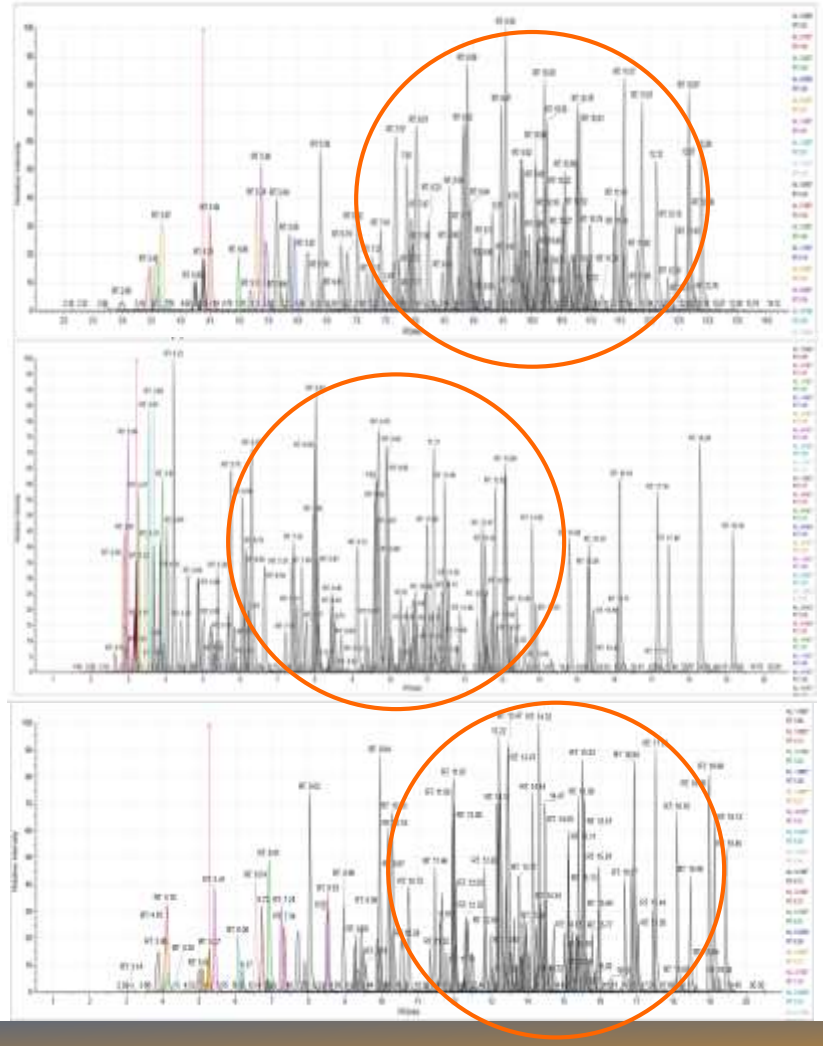


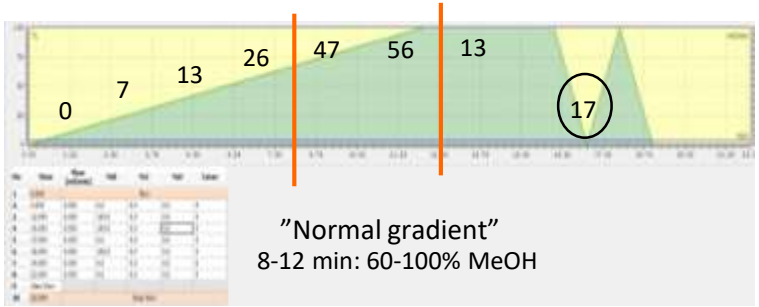
LC-MS Orbitrap
Optimizing MS1-parameters

- **Gradient**, a longer gradient with more spread out analytes results in less ion suppression , less matrix effects from other analytes, i.e. higher MS1 peaks and general higher sensitivity.
- **Flow**, a lower flow results in broader peaks with more data points per peak, i.e. better reproducability.
- **Injection volume**, higher sensitivity

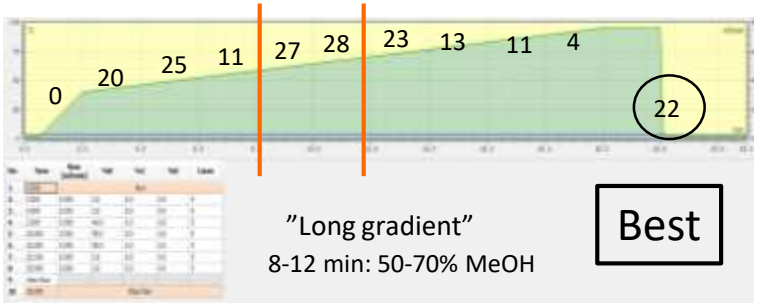
Livsmedelsverket

Standard solution with 162 analytes in different gradients



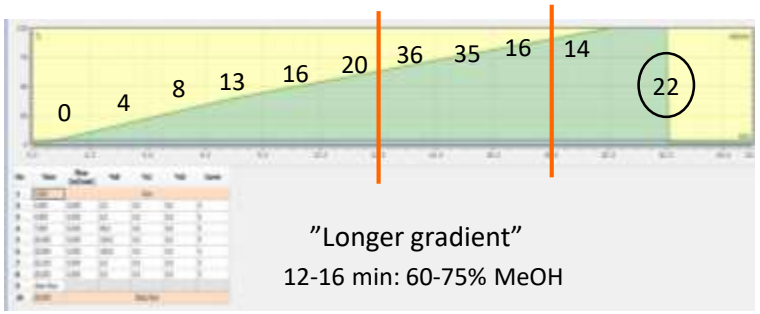


”Normal gradient”
8-12 min: 60-100% MeOH



”Long gradient”
8-12 min: 50-70% MeOH

Best



”Longer gradient”
12-16 min: 60-75% MeOH

MS1-parameter: scans

More scans per peak → more reproducable results

10 scans/peak= good but not necessary

5-7 scans/peak = good

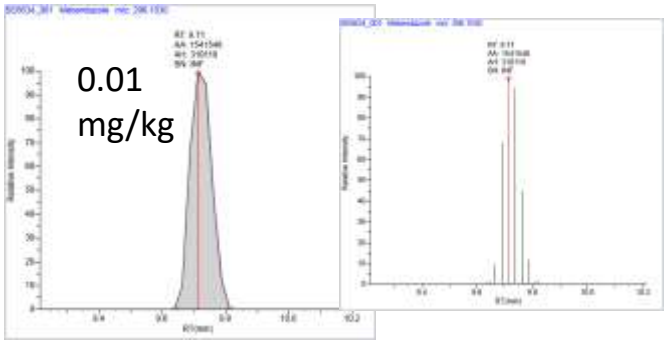
<5 scans/peak = tricky

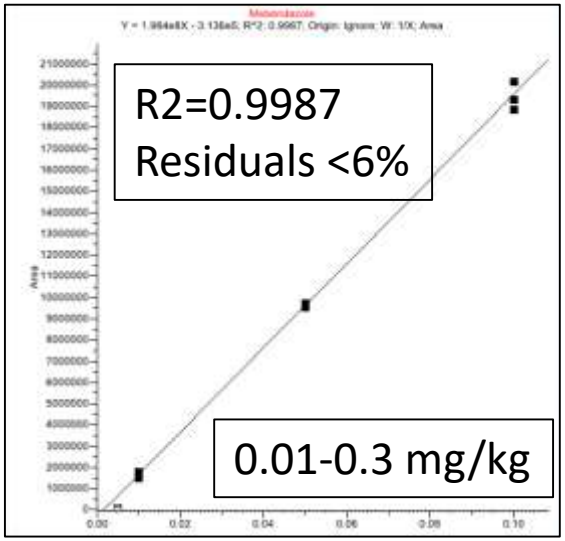
3-4 scans/peak = too low

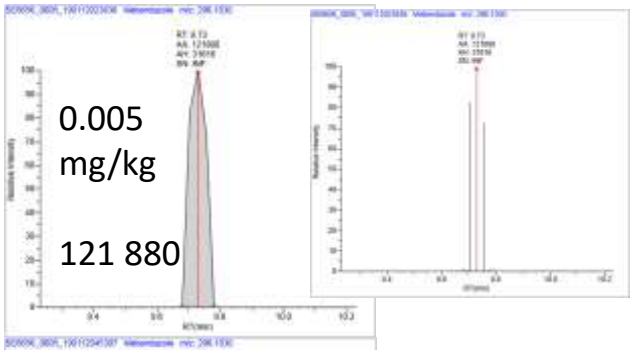
Livsmedelsverket

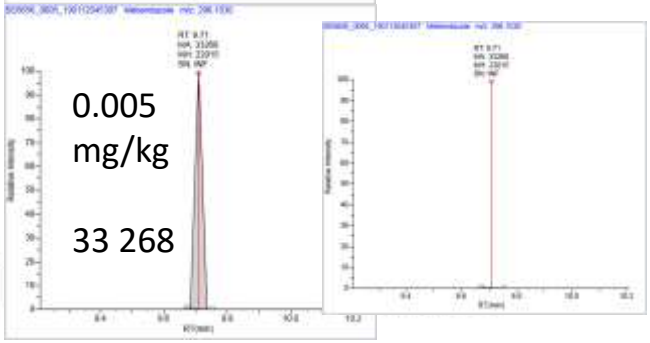
Mebendazole

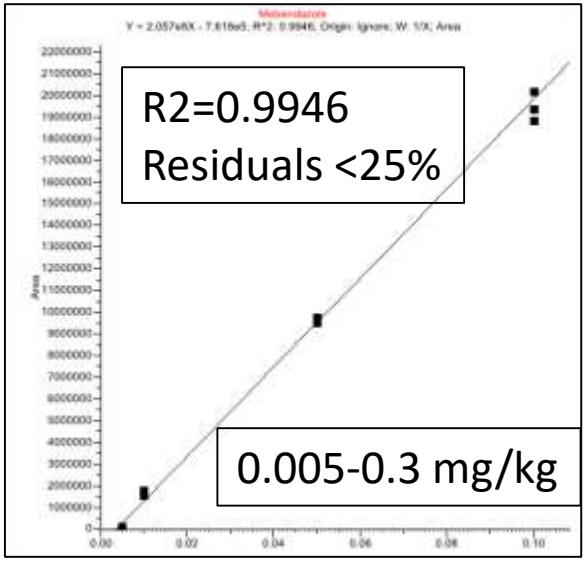
More scans per peak, more reproducable results











MS1-parameter: scans

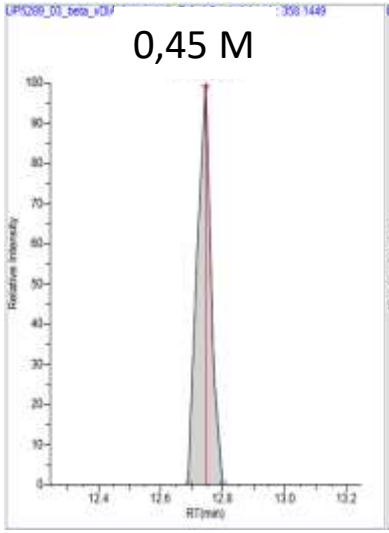
Cyhalofop-butyl

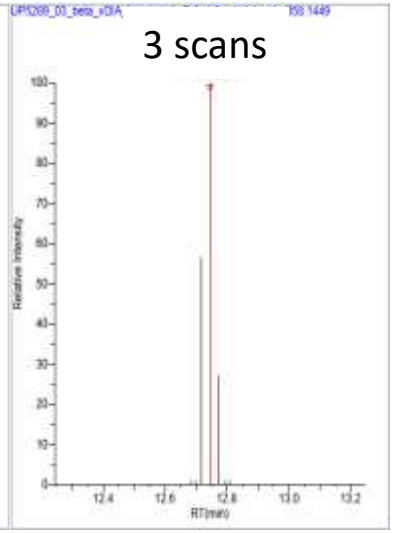
Normal gradient

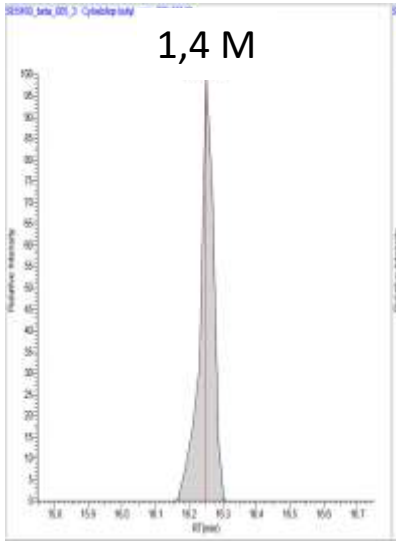
0.3ml/min
2 µl inj vol

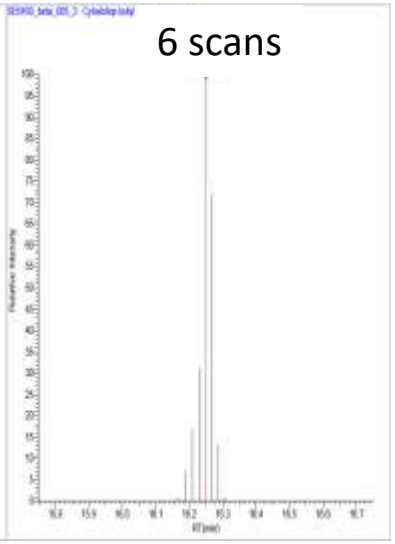
0.2ml/min
4 µl inj vol

Long gradient







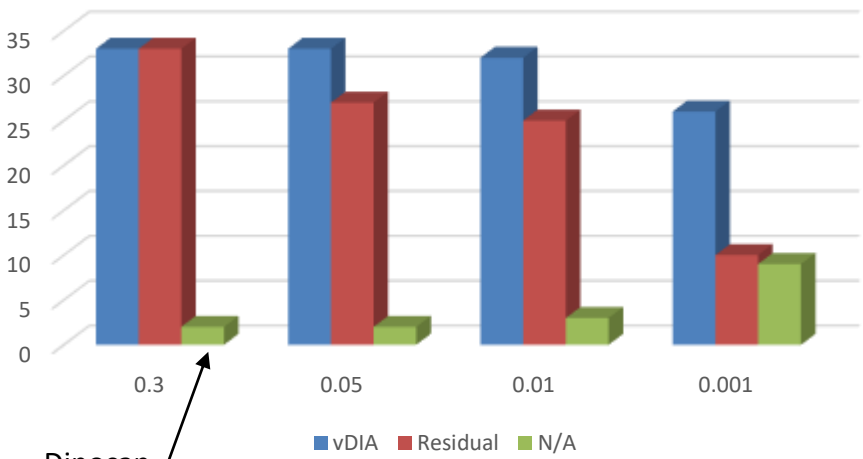


Livsmedelsverket

ESI neg

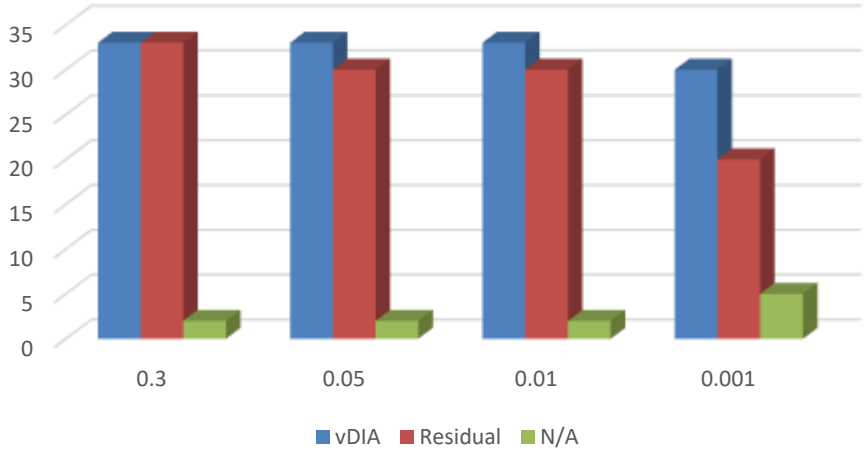
Long gradient, lower flow

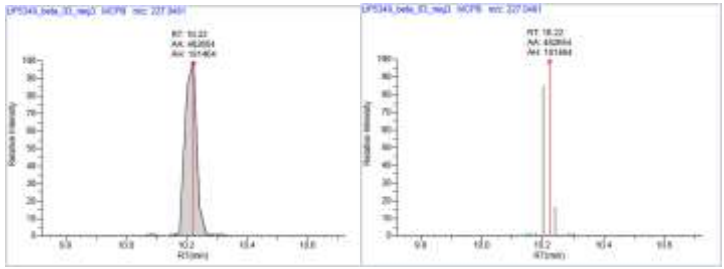
ESI-neg-old



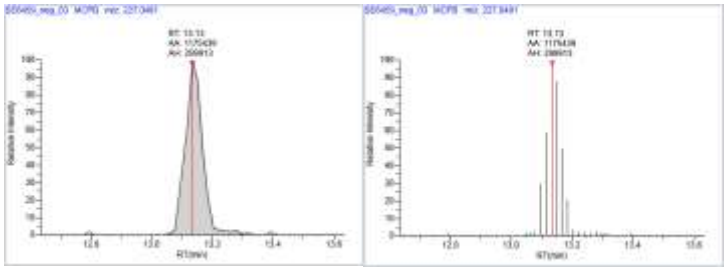
Dinocap
Mesotrione

ESI-neg-new

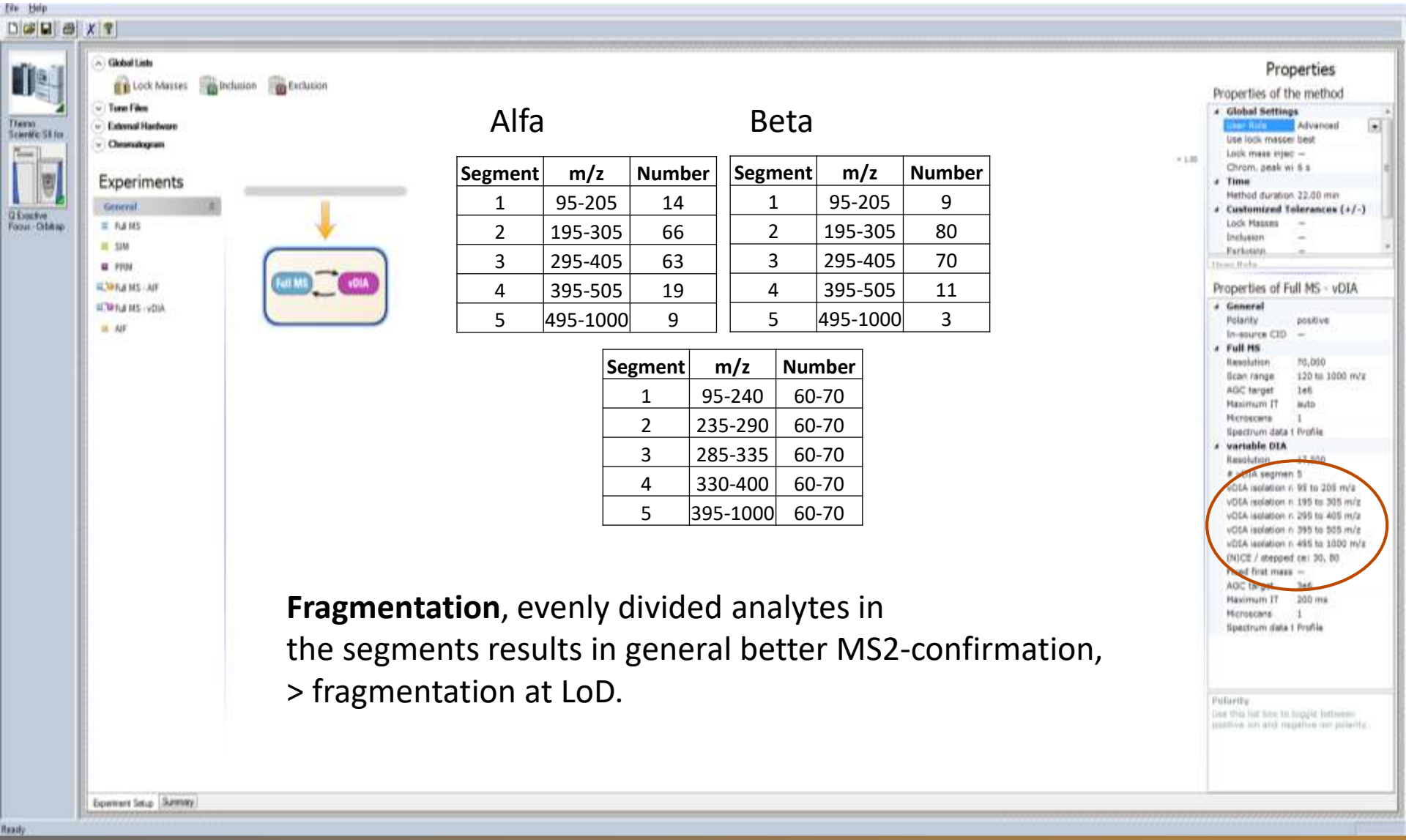




MCPB
150 ng/ml



Optimizing MS2-parameters



Alfa

Segment	m/z	Number
1	95-205	14
2	195-305	66
3	295-405	63
4	395-505	19
5	495-1000	9

Beta

Segment	m/z	Number
1	95-205	9
2	195-305	80
3	295-405	70
4	395-505	11
5	495-1000	3

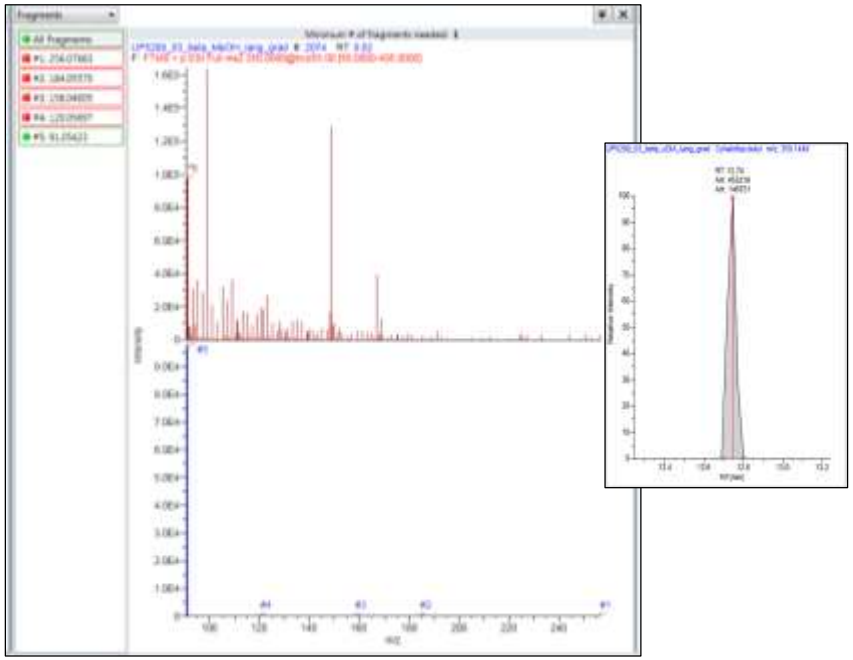
Segment	m/z	Number
1	95-240	60-70
2	235-290	60-70
3	285-335	60-70
4	330-400	60-70
5	395-1000	60-70

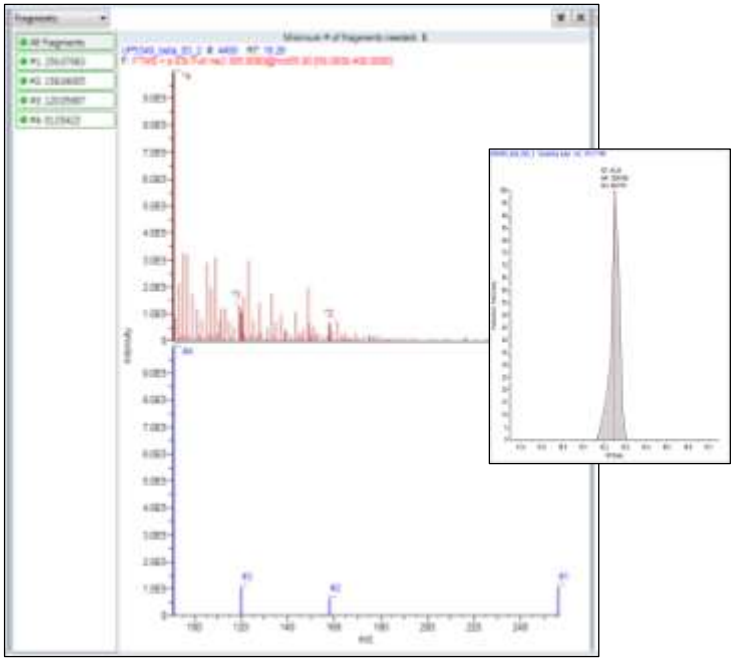
Fragmentation, evenly divided analytes in the segments results in general better MS2-confirmation, > fragmentation at LoD.

MS2-parameters: segment

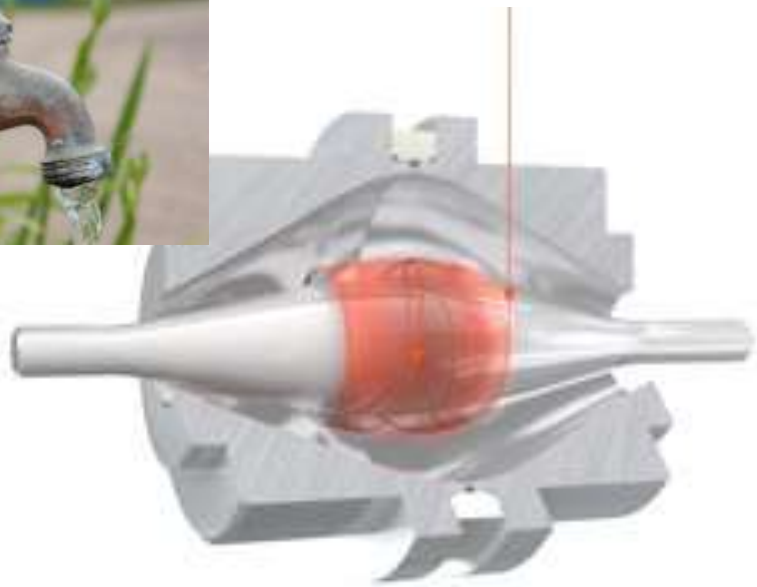
Cyhalofop-butyl 150 ng/ml

New segments, better fragmentation.





Method development of Saxitoxines on the Orbitrap



Livsmedelsverket

Aim of the project

1. To develop a targeted HRMS method on the Orbitrap and compare it to the running method on LC-MS/MS.

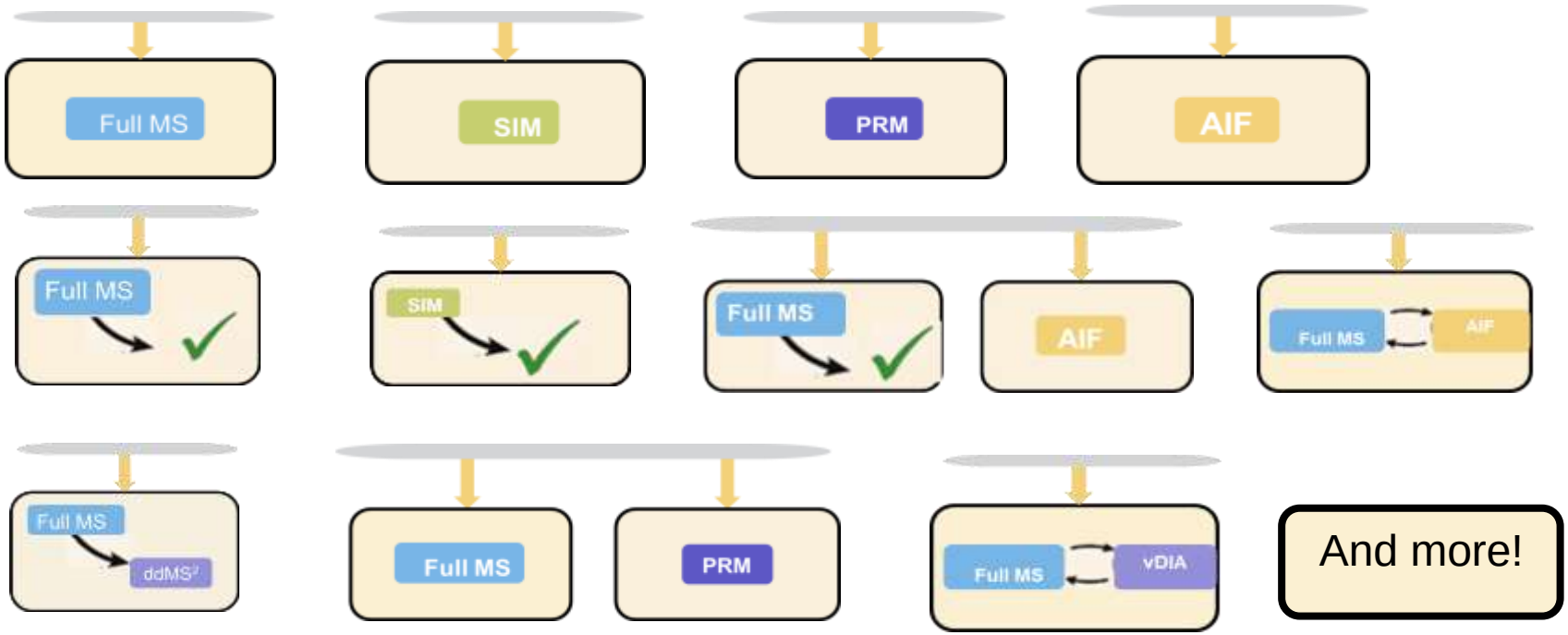
2. To develop a semi-targeted HRMS method on the Orbitrap to be able to screen for a larger scope of toxins

3. Lower the LOD, obsticles: no laboratory can perform analyses at 3 µg/l for Saxitoxins (action threshold). The sensitivity (LOD) needs to be lower, around 20 times.

LC-MS Orbitrap

ESI pos: Saxitoxin, dc-Saxitoxin, Neosaxitoxin and dc-Neosaxitoxin

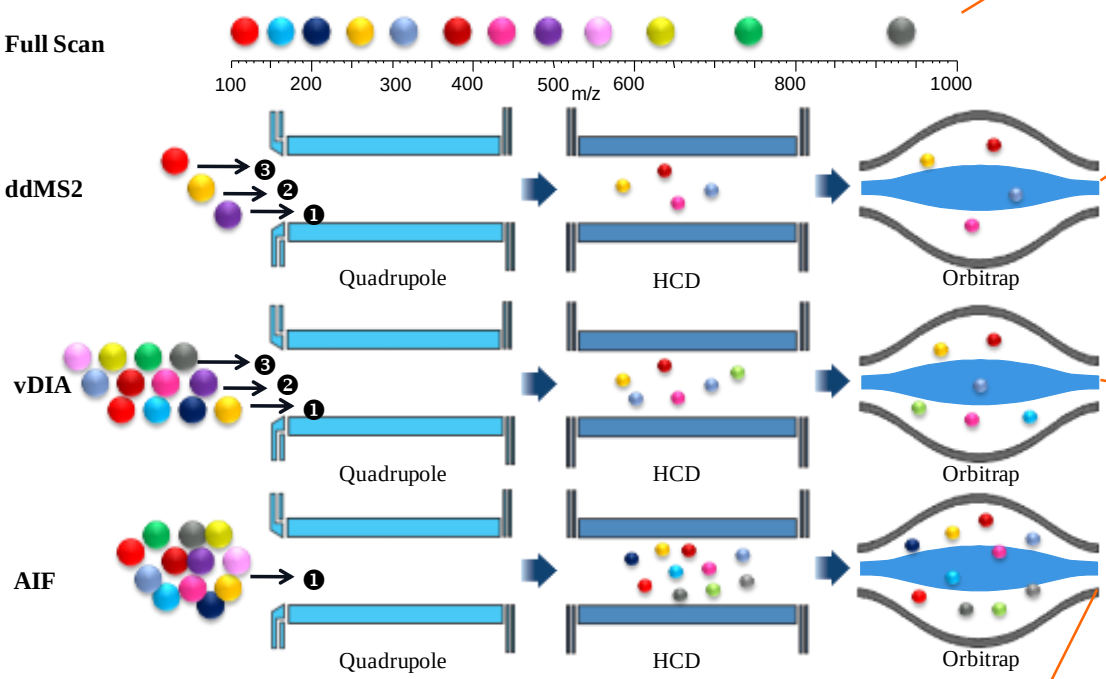
ESI neg: Gonyautoxin series (C1-C4), dcGTX1—4, GTX1—6



Livsmedelsverket

vDIA is not available in the U.S.

Scan modes

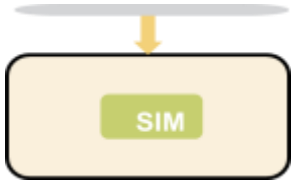


- All ions inside the scan range of the quadrupole are analyzed. Compare to SIM, Single Ion Monitoring.

- Selected parent ions from the preceding full scan are isolated and fragmented, all fragments are analyzed. **Inclusion list. Does not always trigger fragmentation.**

- Ions from selected mass ranges (typically 100 to 500 amu) are fragmented and all fragments are analyzed
- Mass ranges are lined up for fragmentation, the full scan is covered by MS^2 scans
- **Segments improves selectivity and sensitivity, otherwise similar to AIF. Non targeted acquisition - no inclusion list.**

- All ions present in full scan are fragmented all together and all fragments are analyzed. **All the ions all the time, no inclusion list. Regarded as difficult, non-selective and not sensitive.**



Single Ion Monitoring

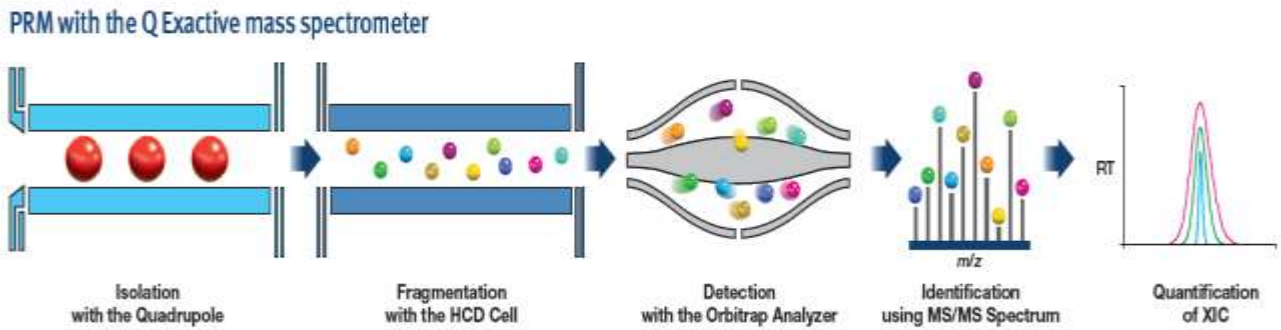
SIM isolates 1 only precursor which gives longer injection time and in turn better sensitivity. SIM is only working in MS1-level and does not fragment.

SIM can connect with ddMS2, and both modes uses the same inclusion list.

SIM is the most sensitive MS1-mode and suitable for complex matrices.

Parallel Reaction Monitoring

- Similar to SIM but also MS2, fragments
- Similar to triple-quads
- Needs inclusion list
- Does not handle multi methods, co-eluting
- Good choice for very complex/dirty matrices



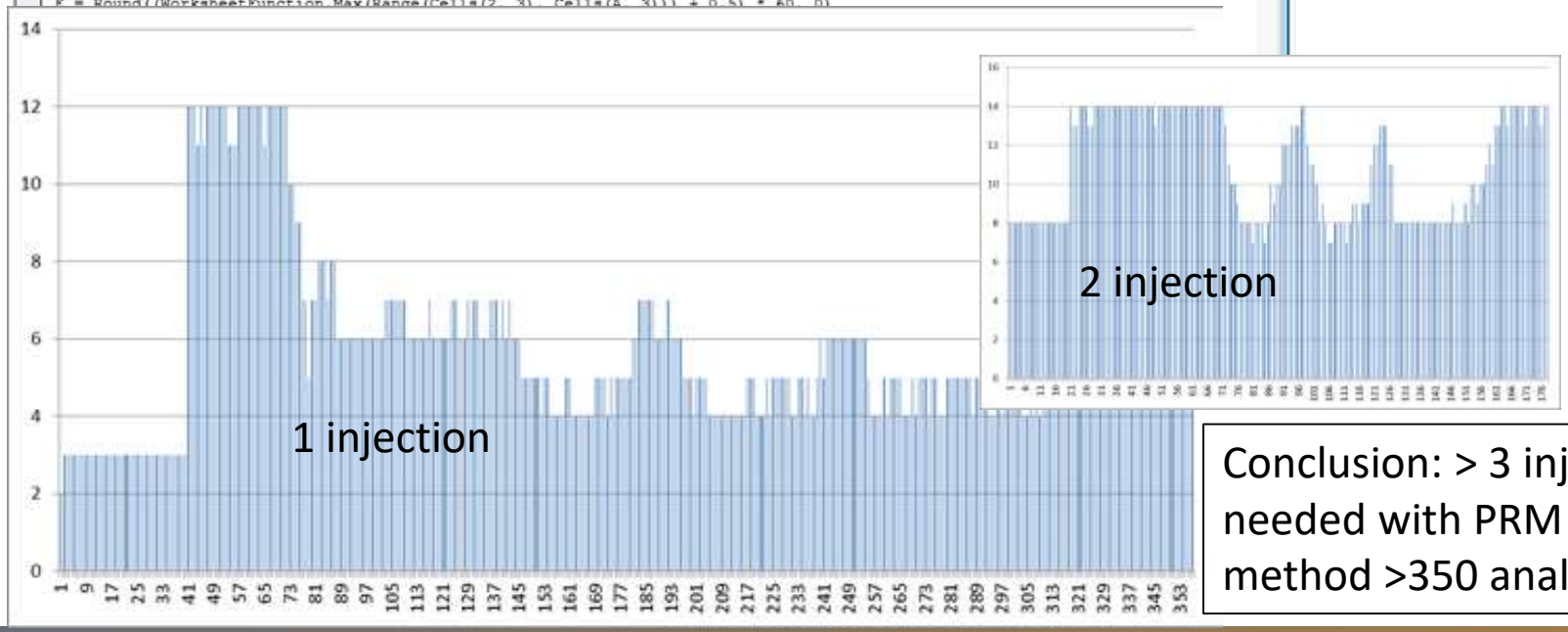
The feasibility of PRM for multiresidue methods

SIM-PRM feasibility on QE Focus MAKro.xlsm - Module2 (kod)

(Allmänt) | Scan_per_peak_calc_02

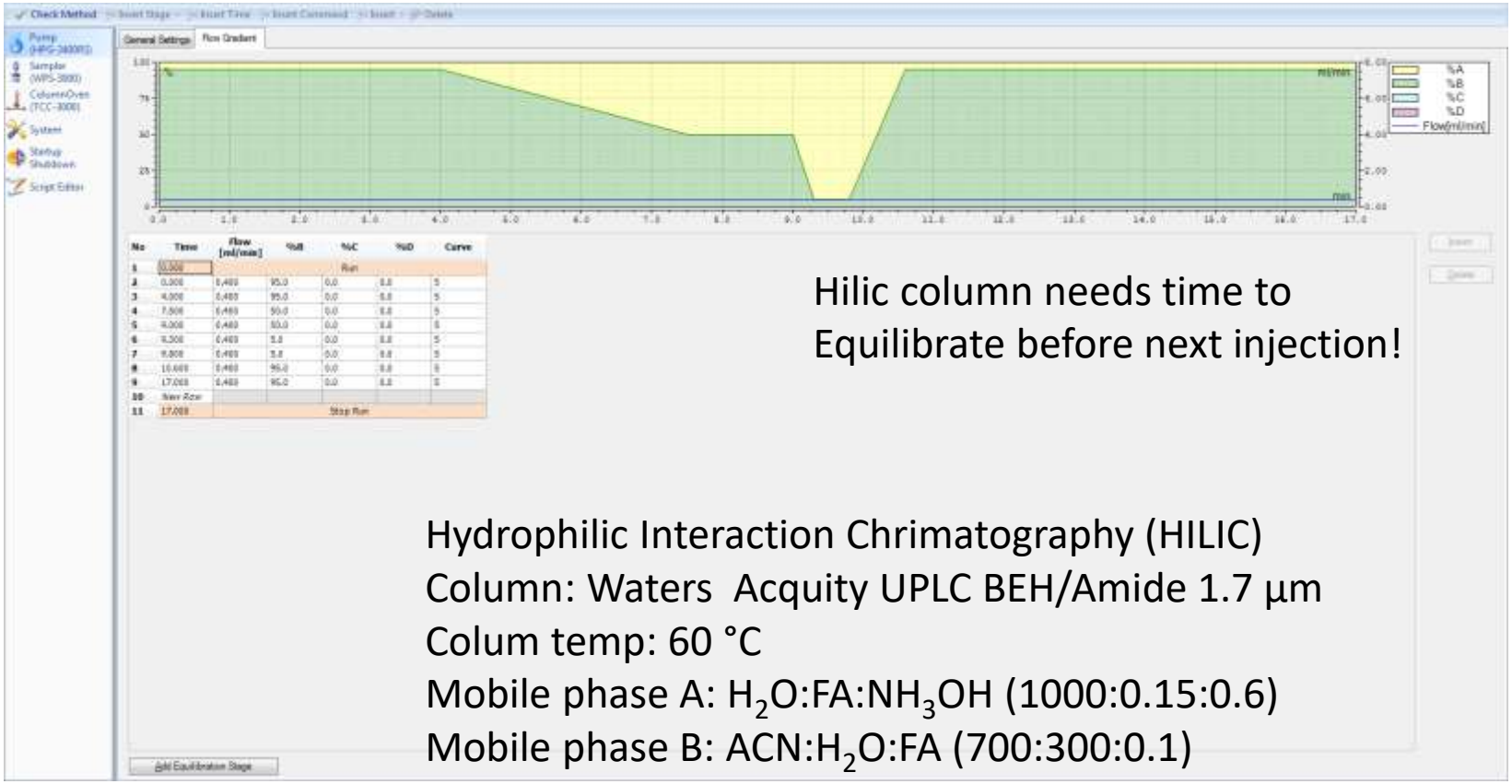
```
Sub Scan_per_peak_calc_02()
'peak width 12 seconds, resolution 17500 (11 sscans/seconds)

Dim A, B, C, D, E, F, G, H, I, J, K, L As Integer

A = ActiveSheet.UsedRange.Rows.Count

'peak width in seconds
B = Sheets("Parameters").Cells(1, 2)


'window in seconds before and after peak
C = Sheets("Parameters").Cells(2, 2)
J = Sheets("Parameters").Cells(3, 2)
G = 2
'start RT in seconds
D = Round((WorksheetFunction.Min(Range(Cells(2, 3), Cells(A, 3))) - 0.5) * 60, 0)
'end RT in seconds
E = Round((WorksheetFunction.Max(Range(Cells(2, 3), Cells(A, 3))) + 0.5) * 60, 0)
```

Calculate the expected number of scans under the LC peak under given parameters (peak width, scan speed, search window), the aim is to have 10+ scans under peak for every single compound.



Conclusion: > 3 injections is needed with PRM for pesticide method >350 analytes

Properties - HILIC



Hilic column needs time to Equilibrate before next injection!

Hydrophilic Interaction Chrimatography (HILIC)
Column: Waters Acquity UPLC BEH/Amide 1.7 µm
Colum temp: 60 °C
Mobile phase A: H_2O:FA:NH_3OH (1000:0.15:0.6)
Mobile phase B: ACN:H_2O:FA (700:300:0.1)

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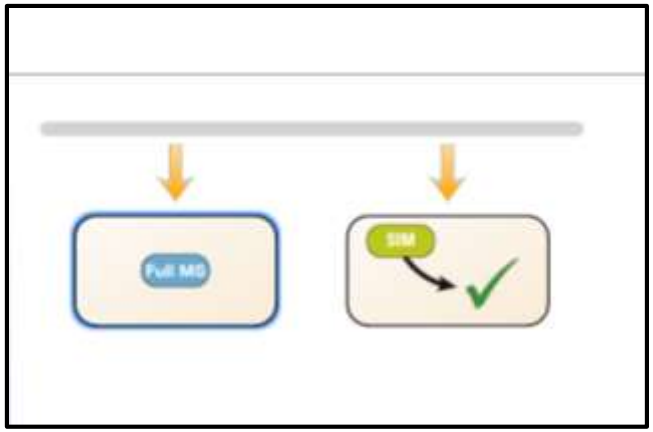
National Food Agency

Properties - HRMS

Properties of Full MS

General	
Polarity	positive
dd-MS²	—
In-source CID	—
Full MS	
Resolution	17,500
# Scan ranges	1
Scan range	100 to 500 m/z
AGC target	1e6
Maximum IT	20 ms
Microscans	1
Spectrum data t	Profile



Properties of SIM

General	
dd-MS²	Confirmation
In-source CID	—
SIM	
Resolution	70,000
Isolation windov	10.0 m/z
Isolation offset	—
AGC target	2e5
Maximum IT	300 ms
Loop count	1
MSX count	10
Microscans	1
Spectrum data t	Profile
dd-MS² Confirmation	
Resolution	17,500
Isolation windov	6.0 m/z
Isolation offset	—
(N)CE / stepped	nce: 10, 60
Fixed first mass	—
Default charge s	1
AGC target	1e5
Maximum IT	80 ms
Loop count	3
Minimum AGC t	8.00e3
Intensity thresh	1.0e5
Apex trigger	2 to 6 s
Dynamic exclus	auto
Spectrum data t	Profile

dd-MS²
Specify which mode of data dependent scans is performed.

MSX Count, multiplexing is a feature where several precursors are combined, → more duty cycles and more data points per analyte in SIM, only works in MS1.

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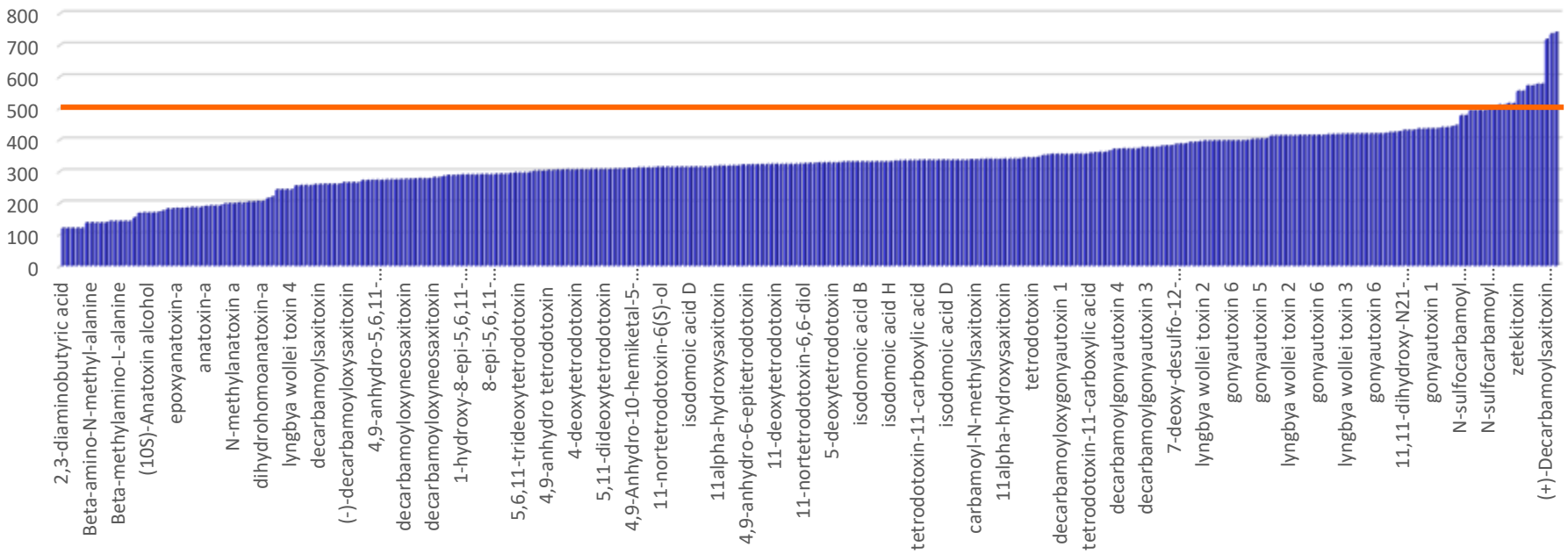
105 Saxitoxins in HRMS database

Most Saxitoxins m/z < 500



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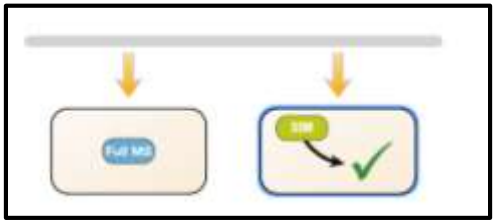
Combination of MS-modes

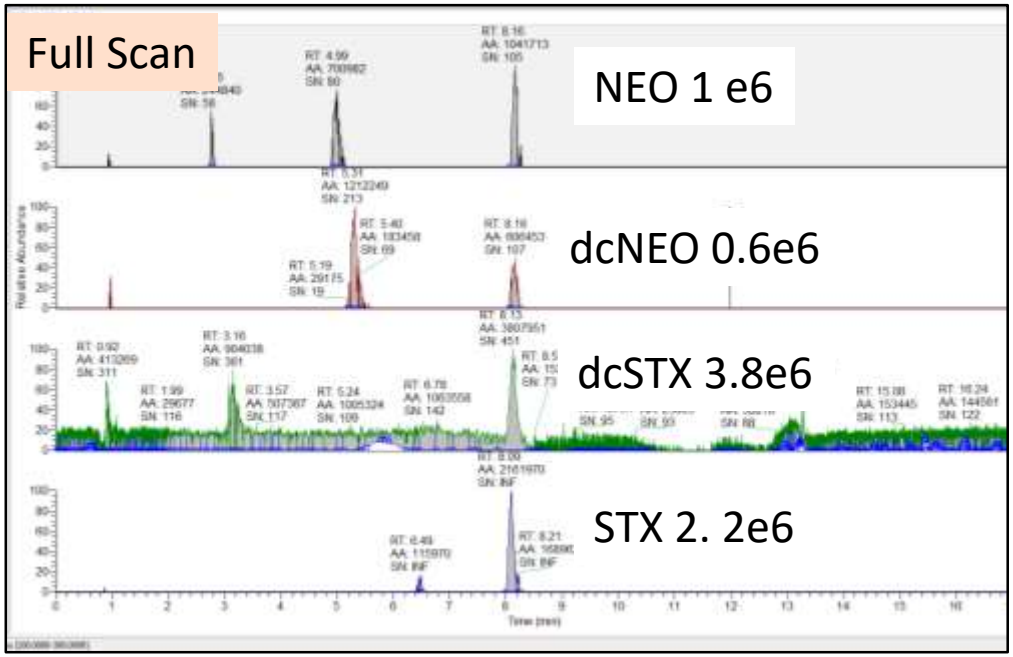


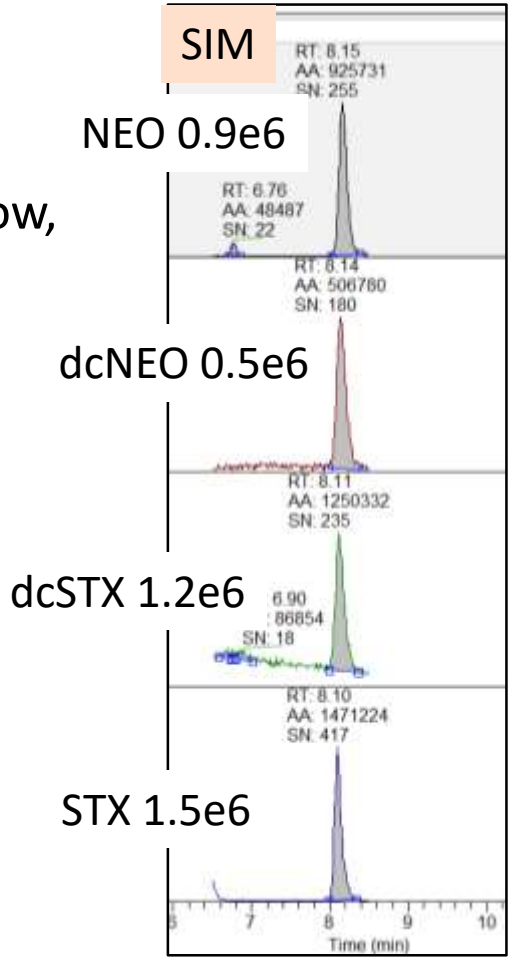
Full MS-SIMddMS2

Full MS shows what is happening in the analysis that is not possible with only SIM.

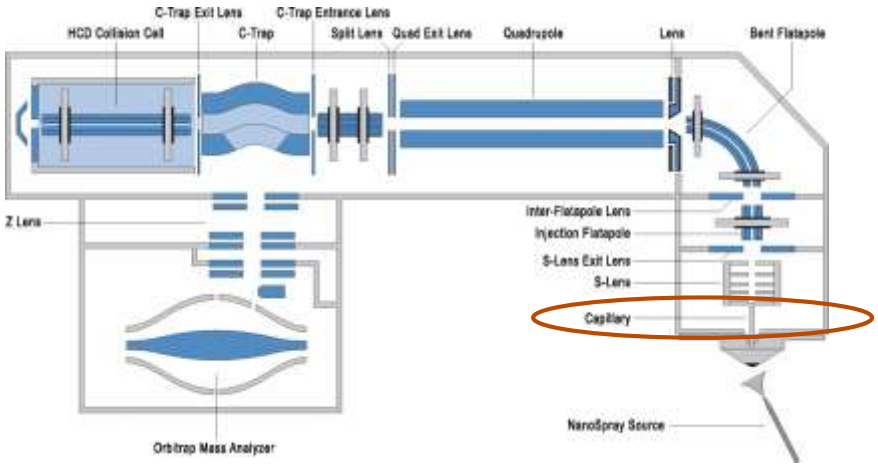
FS generates larger areas than SIM, higher AGC (Automatic Gain Control) target is possible.

But, SIM is more reproducible because of smaller mass window, less interfering ions.





Impact of capillary temp

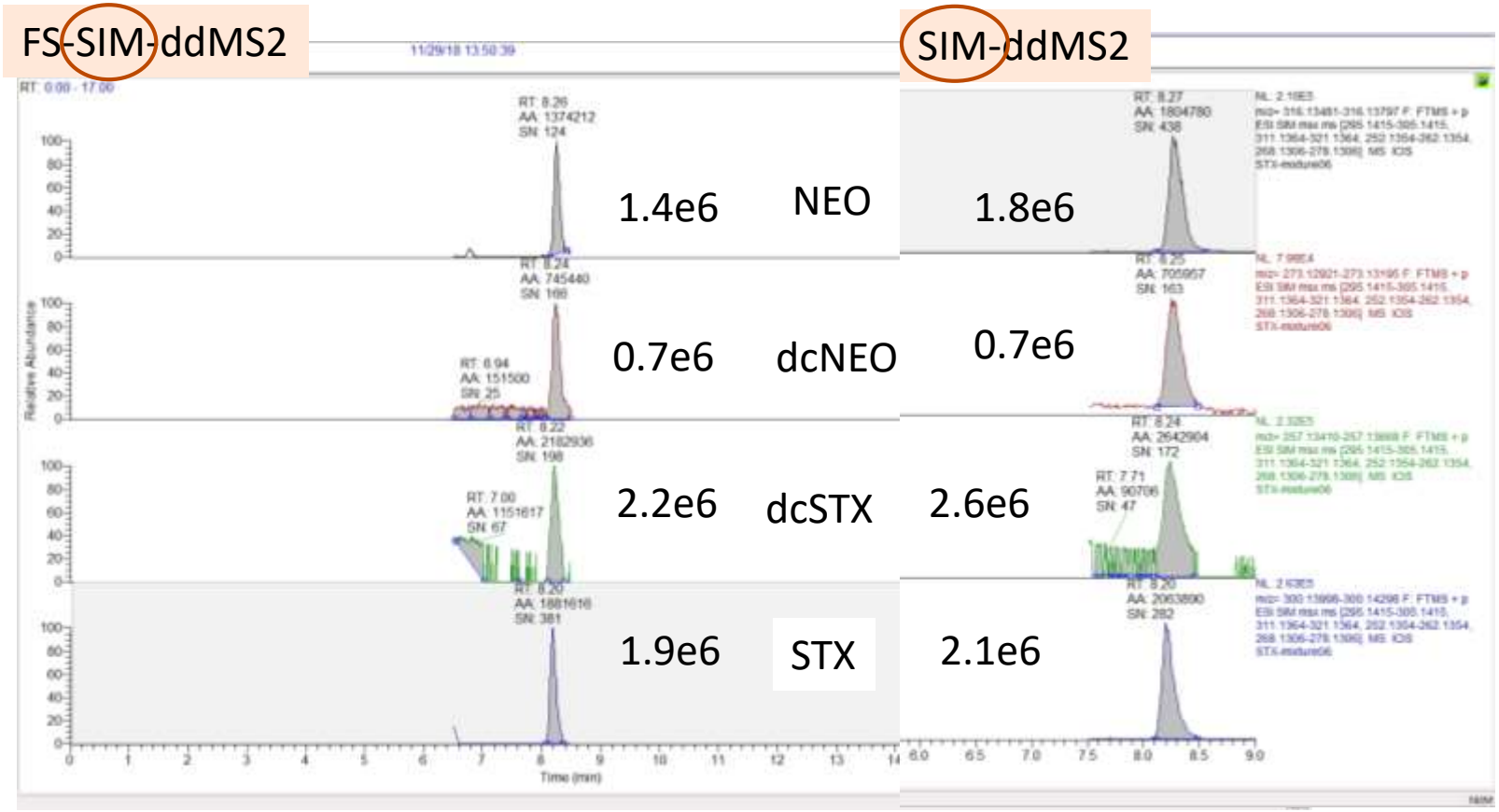


Temp Capillary	NEO e3	dcNEO e3	STX e3	dcSTX e3
200	294	178	288	284
300	1833	838	2422	2105

Significant higher signals with higher capillary temperature.

Livsmedelsverket

How much to you loose by adding FS to your experiment?

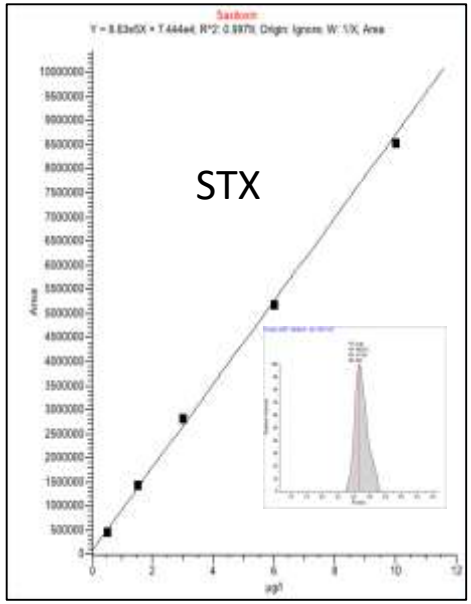


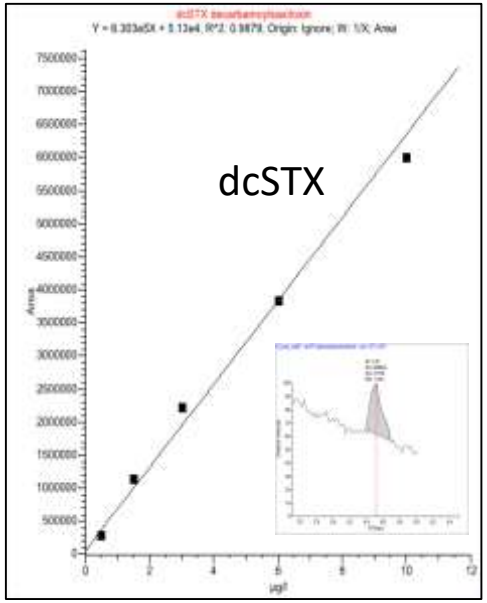
Livsmedelsverket

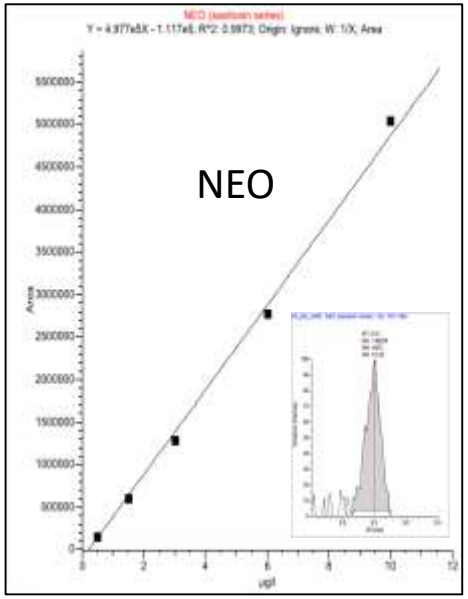
Minor negative impact of adding Full Scan to ddMS2.
Small decrease in peak area.

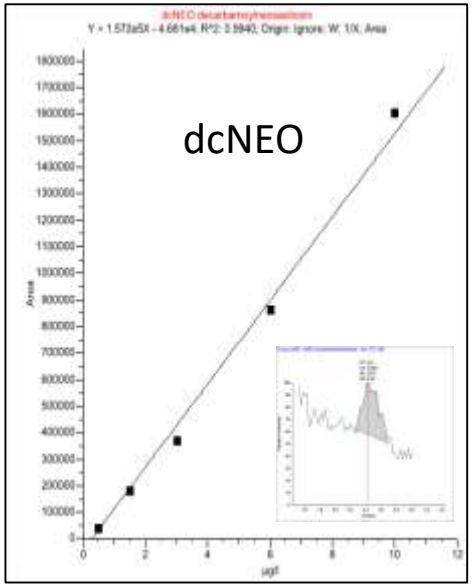
Calibration curve ESI POS 0.5-10 µg/l

Inj vol 50 µl









Final method FS-SIM-ddMS2: capillary temp 300 °C, S-lens 55 V, Loop Count 1, MSX 10, stepped collision energy (10.30 (N)CE), AGC 1e6 (FS), AGC 1e5 (SIM)

Final method is not only comparable to results on LC-MS/MS but also more sensitive, **AND** we are able to screen for other toxins in the same run!

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- Anas Kamleh, SE, Application Specialist NPI- Omics and LSMS

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National Food Agency