Bundesamt für Verbraucherschutz und Lebensmittelsicherheit

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jas joint analytical systems

Implementing a 2D-LC-Q-Tof screening method

Using the EPIC-System by JAS

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Starting point of investigations

- broaden analytical scope for Q-ToF screening method to include
  - „borderline“ SRM analytes (propamocarb, cyromazine, nicotine)
  - metabolites from complex residue definitions
- HPLC-Q-ToF method not valid using QuEChERS for 45 out of 78 analytes (at SDL 0.01 mg/kg) ☹
- causes:
  - analytes not detected because of ion suppression by Matrix

How to get rid of matrix?

→ by orthognonal LC separation using HILIC und RP

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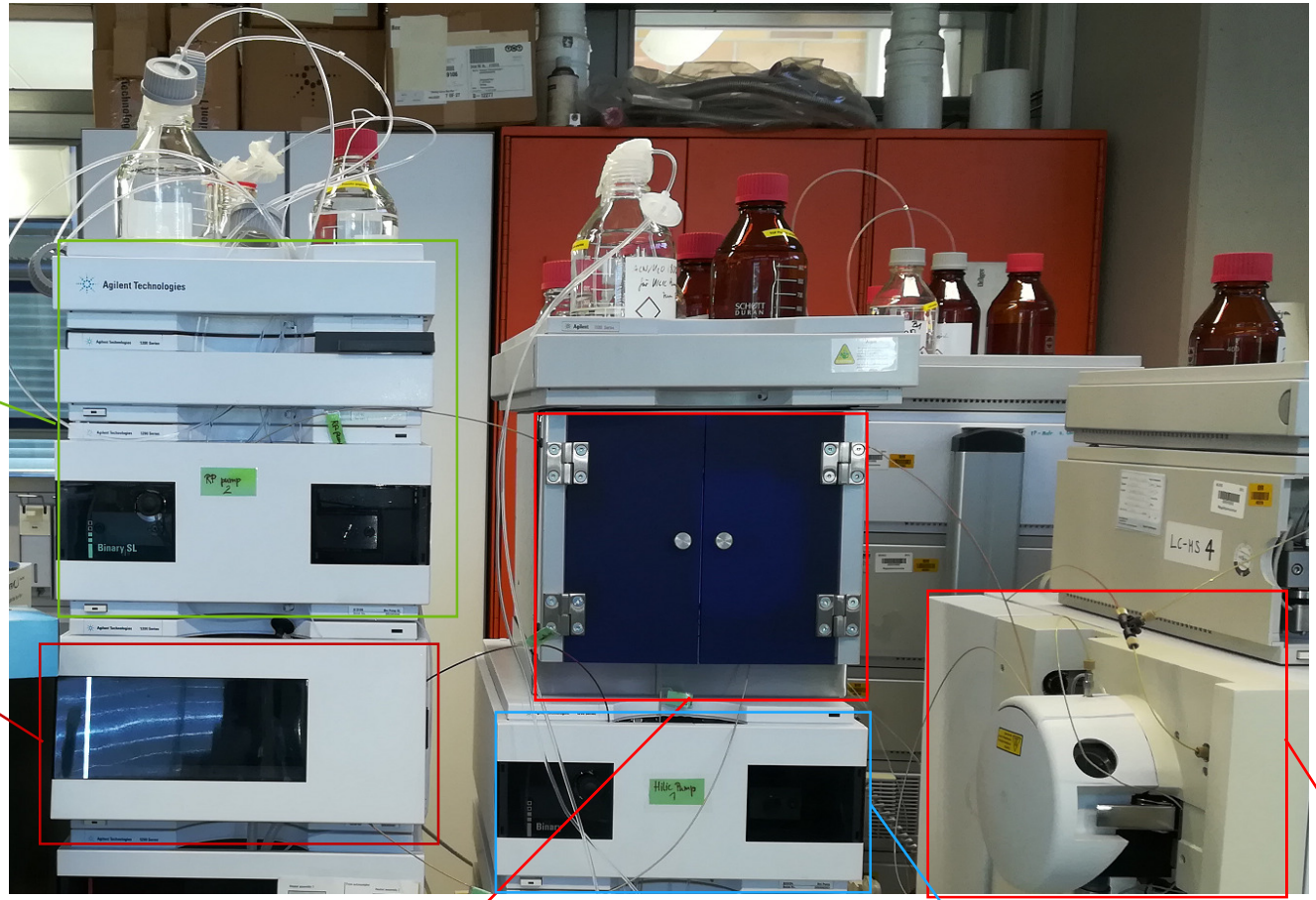
2D-LC consists of:



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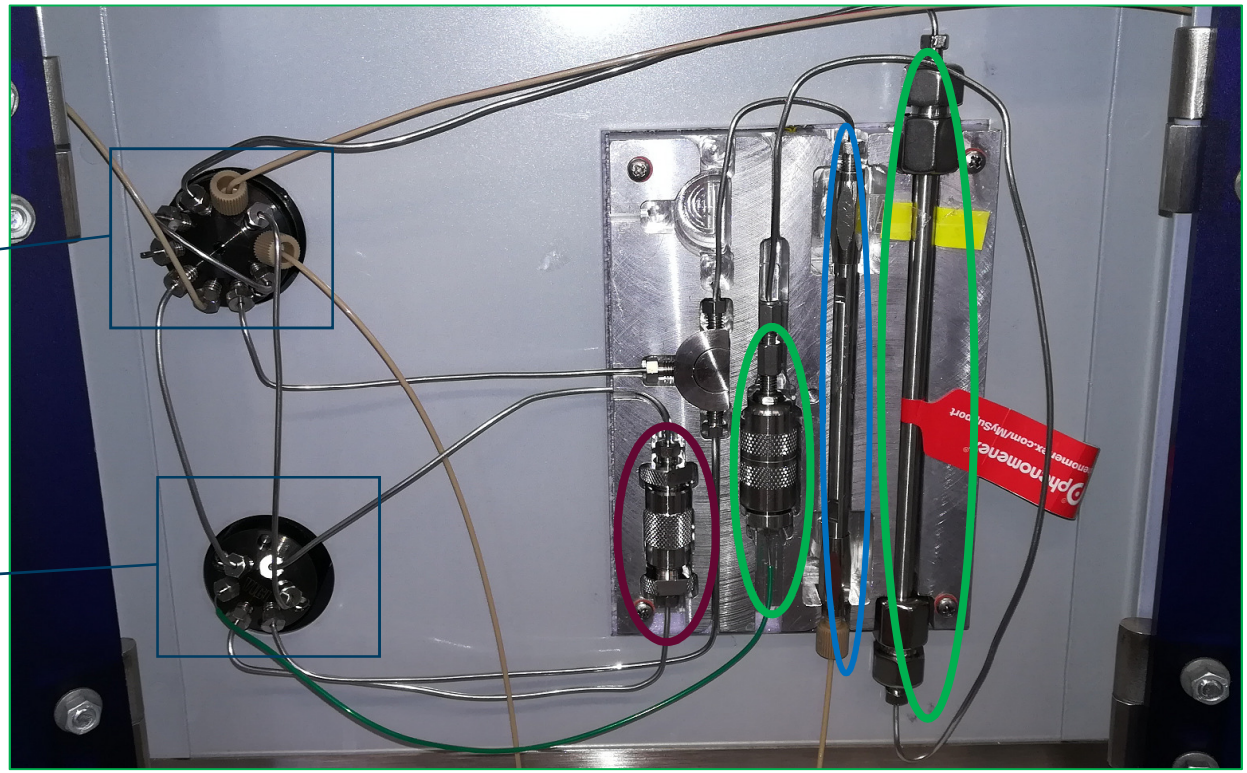
EPICS consists of:

Easy Pesticide Isolation and Concentration System (EPICS)



Valve 1 (10 port)

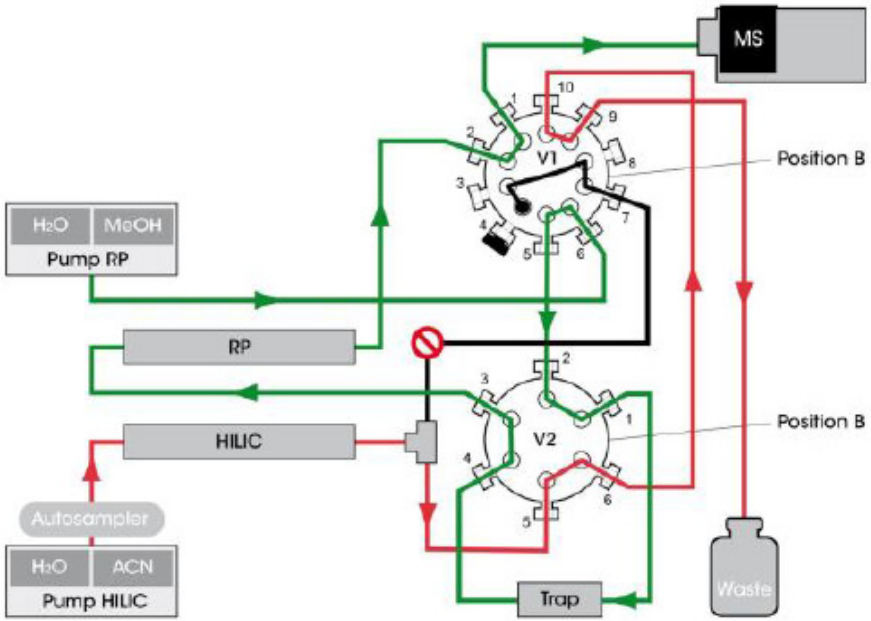
Valve 2 (6 port)

C8 Trap

HILIC

RP

How does it work?



Phase I

- **column equilibration**

Fig: Operating Manual EPICS Version 1.05 (Fa. JAS)

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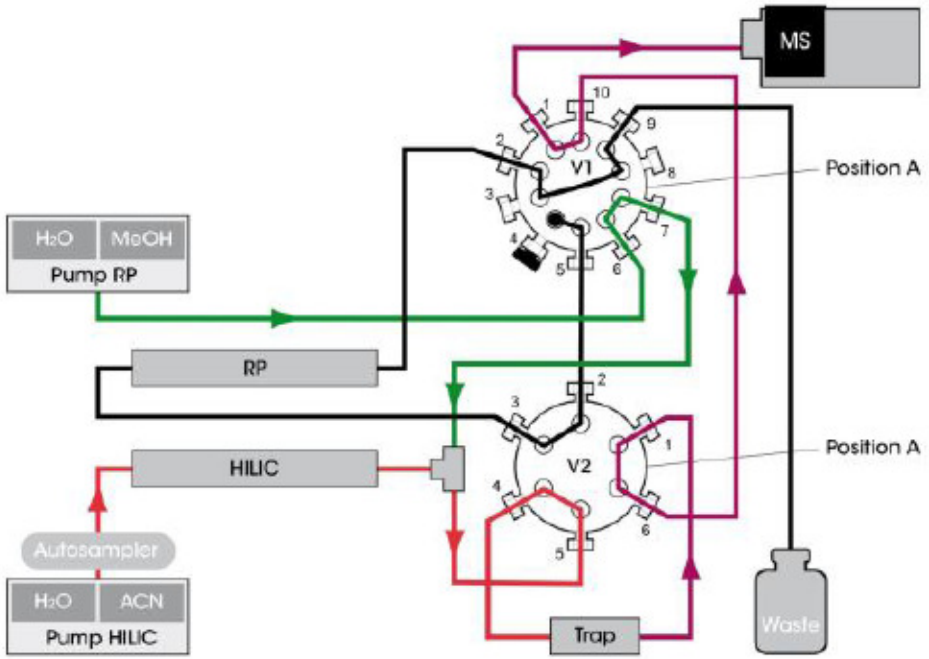
How does it work?

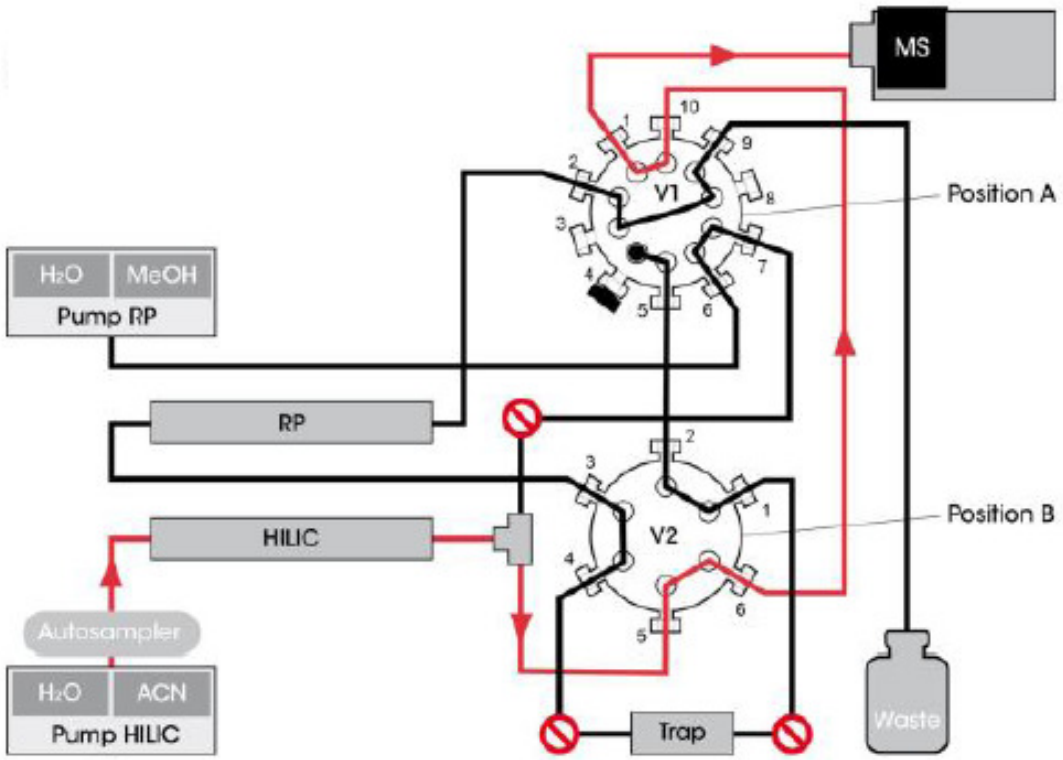


Phase II

- HILIC: nonpolar analytes elute first
- Trap: trapping (water addition from RP!)
- polar analytes and matrix retain strongly on HILIC

Fig: Operating Manual EPICS Version 1.05 (Fa. JAS)

How does it work?



Phase III

- Trap: no flow, analytes remain on trap column
- HILIC: polar analytes are measured directly

Fig: Operating Manual EPICS Version 1.05 (Fa. JAS)

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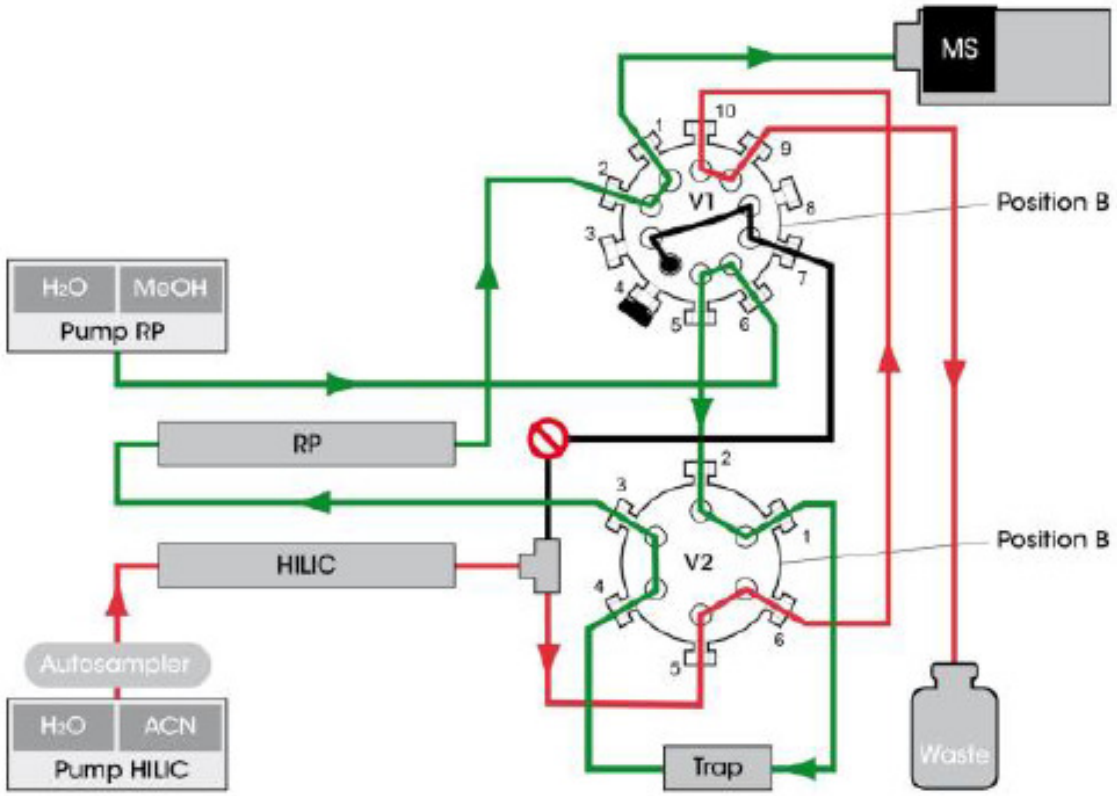
How does it work?

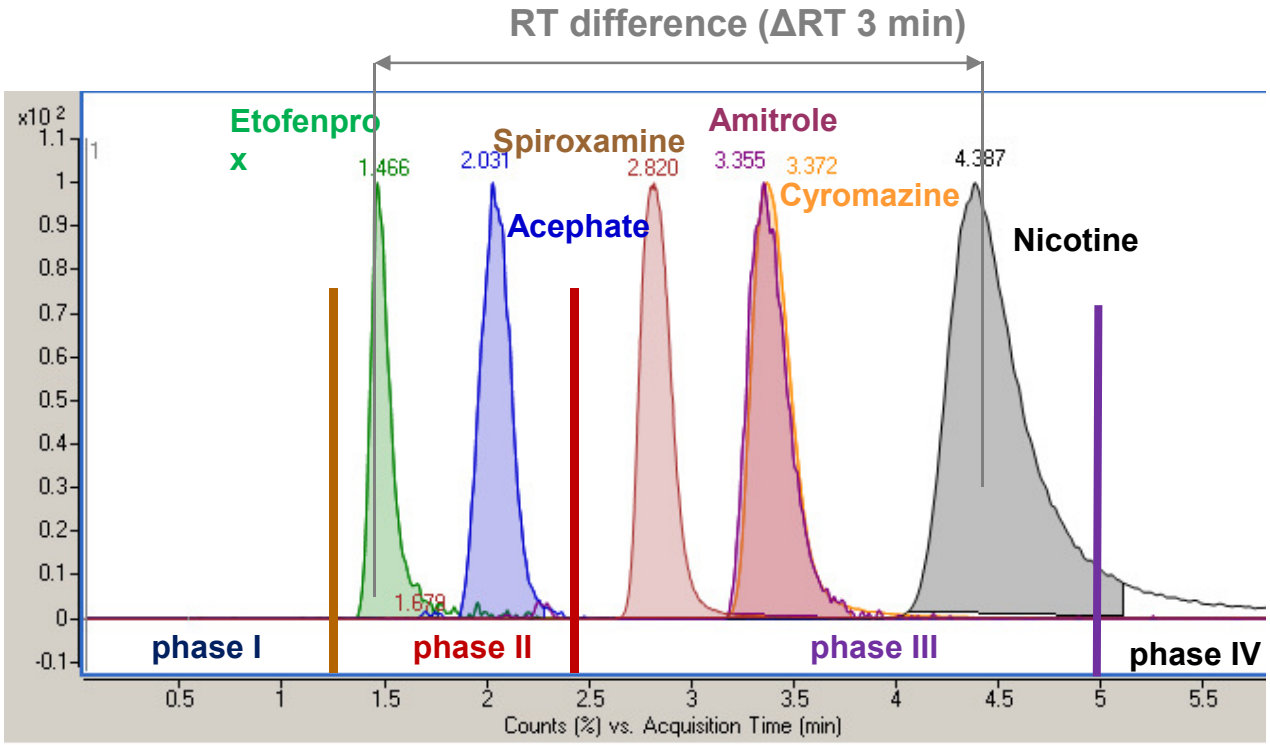


Phase IV

- HILIC: elution of matrix components to waste
- Trap: elution of trapped pesticides to RP
- RP: gradient for pesticide separation, detection via Q-ToF

Fig: Operating Manual EPICS Version 1.05 (Fa. JAS)

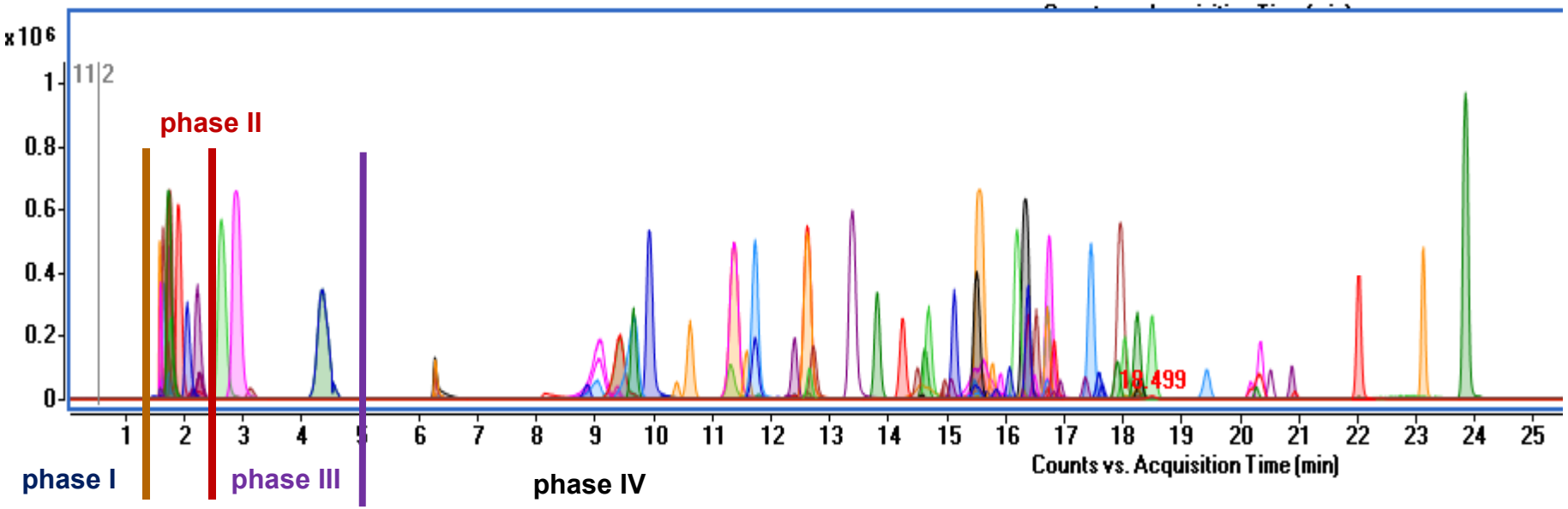
EPICS Phases



phase I: column equilibration
phase II: trapping
phase III: detection of polar analytes eluting from HILIC
phase IV: RP separation of trapped analytes

EPICS Phases

Complete 2D-LC separation chromatogram



phase I: column equilibration
phase II: trapping
phase III: detection of polar analytes eluting from HILIC
phase IV: RP separation of trapped analytes

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Implementing a 2D-LC-Q-Tof screening method

Optimisation of HILIC and RP separation

Extraction/Injection

	JAS	NRL
Extraction	ACN + water*	ACN + water + QuEChERS salts
Injection	ACN/water mixture**	ACN phase
Injection volume	5 µL	20 µl

***problems because phase separation for some matrices (kiwi, beet root etc.)**
****peak shape very broad for some analytes (cyromazine, propamocarb etc.)**

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2D-LC - first dimension

	JAS	NRL
flow	0.2 ml/min	0.3 ml/min
temperature	30 °C	40 °C
buffer		ESI+ 100 mM NH_4-formate (pH 3.2) ESI- 100 mM NH_4-acetate (pH 5.8)
HILIC eluent	A: 5 mM NH_4-formate in H_2O + 0.1 % formic acid B: 5 mM NH_4-formate in ACN/H_2O (90/10) + 0.1 % formic acid	A: H_2O/buffer (95/5) B: ACN/H_2O/buffer (95/5/5)

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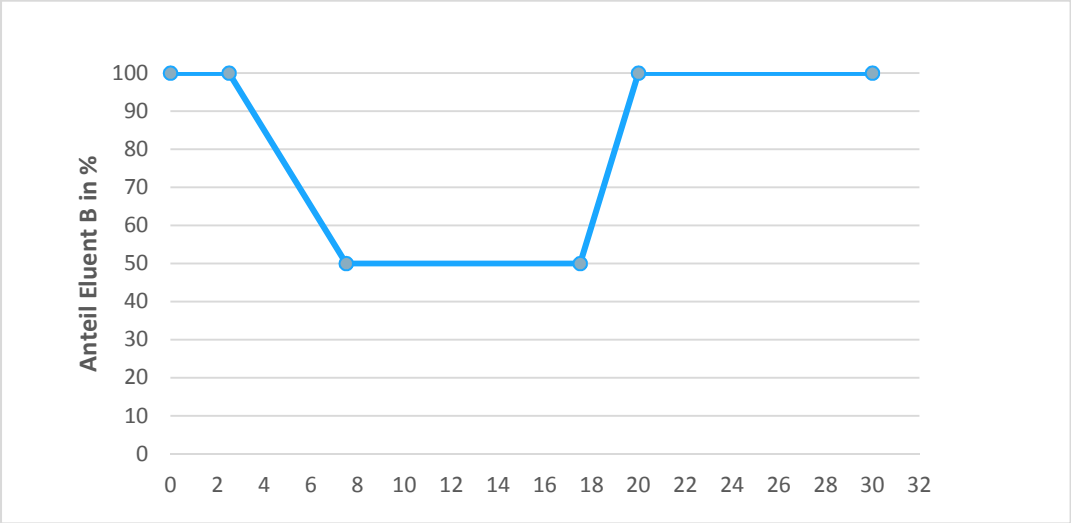
2D-LC - first dimension

	JAS	NRL
column	YMC-Pack Diol	YMC-Triart Diol
HILIC gradient % B		

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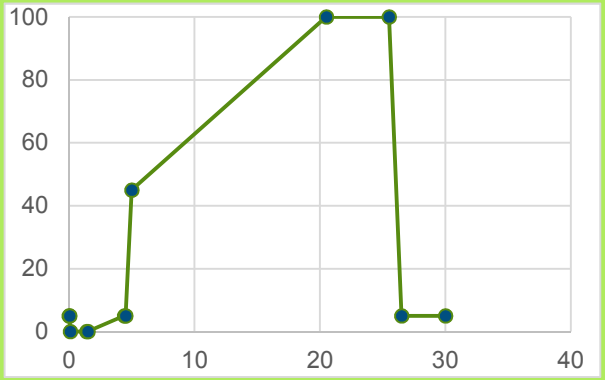
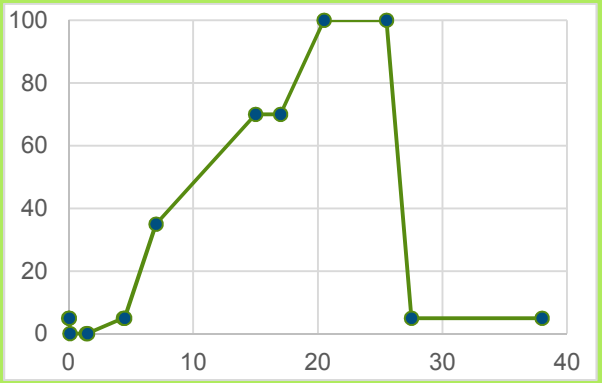
2D-LC - second dimension

	JAS	NRL
RP eluent	A: 5 mM NH_4-formate in H_2O + 0.1 % formic acid B: 5 mM NH_4-formate in MeOH + 0.1 % formic acid	A: H_2O/buffer (95/5) B: ACN/buffer (95/5)
RP column	Poroshell-C18 (2.1 mm × 100 mm; 2.7 µm; 120 Å)	Synergi Fusion-RP (2,0 mm × 150 mm; 4 µm; 80 Å)
RP gradient % B		

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2D-LC vs QuEChERS

Comparison of validation results

Non-valid QuEChERS analytes

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Acetamiprid	✓	✓	✓	✓	✗	✗	✓	✓	✓	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
Aldicarb	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓	✗	✓	✓
Azoxystrobin	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✗	✗	✗	✓	✓	✓	✓
Bitertanol	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Boscalid	✓	✓	✓	✓	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Bromuconazole	✓	✓	✓	✓	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Buprofezin	✗	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Carbendazim	✓	✓	✓	✗	✗	✗	✗	✗	✓	✓	✓	✓	✗	✗	✗	✗	✗	✗	✗	✗
Carbofuran	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓
Carboxin	✗	✗	✗	✗	✗	✗	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Chlordimeform	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✗	✗	✗	✓	✗	✓	✓	✓	✓
Clethodim	✓	✓	✗	✗	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Coumaphos	✓	✓	✓	✓	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Cyprodinil	✓	✓	✓	✓	✗	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Cyromazine	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
Demeton-S-methyl	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
Demeton-S-methylsulfoxide	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
Diazinon	✓	✓	✓	✗	✗	✗	✗	✗	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Dimethoate	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
Dimethomorph	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗
Diniconazole	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✗	✗	✗	✓	✓	✓	✓	✓
Ethion	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
Ethoxyquin	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Fenamidone	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✗	✓	✓	✓	✓	✓	✓
Fenpiclonil	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
Flucythrinate	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
Furathiocarb	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✗	✓	✗
Hexaconazole	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓
Malathion	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Mecarbam	✗	✗	✗	✓	✓	✓	✗	✗	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Methidathion	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
Myclobutanil	✗	✗	✓	✓	✓	✓	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Nicotine	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
Paraoxon-methyl	✗	✗	✓	✓	✗	✗	✓	✓	✗	✓	✗	✗	✗	✗	✗	✗	✓	✗	✗	✓
Picoxystrobin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✗	✓	✓	✓	✓
Pirimicarb	✓	✓	✓	✓	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pirimiphos-methyl	✓	✓	✓	✓	✗	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓
Propiconazole	✓	✓	✓	✓	✗	✗	✓	✓	✓	✓	✗	✓	✗	✗	✗	✗	✓	✓	✓	✓
Pyraclostrobin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✗	✗	✗	✓	✓	✓	✓
Pyridaben	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓	✓	✓	✗	✓	✗	✓	✓	✓	✓
Quinoclamine	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗
Quizalofop-ethyl	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✗	✗	✗	✗	✗	✗	✓	✓	✓
Sulfotep	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓	✗	✓	✗	✗	✓	✓	✓	✓	✓
Tebufenpyrad	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Thiacloprid	✓	✓	✓	✓	✗	✗	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓

45 non valid with QuEChERS at SDL 0.01 mg/kg

Short Validation 2D-LC

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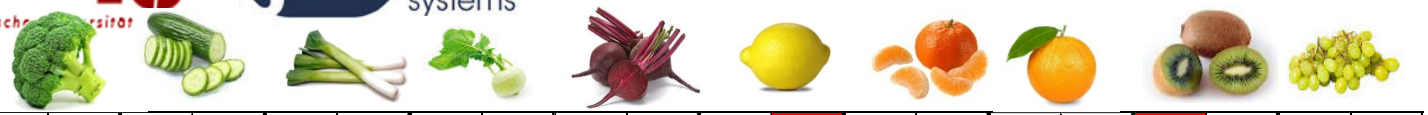

Acetamiprid	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	x	✓	✓	✓	✓	x	✓	✓	✓
Aldicarb	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Azoxystrobin	✓	✓	✓	✓	x	x	✓	✓	✓	✓	✓	✓	x	✓	x	x	✓	✓	✓	✓
Bitertanol	x	x	✓	✓	x	x	✓	✓	✓	✓	✓	✓	✓	✓	x	x	✓	✓	✓	✓
Boscalid	✓	✓	✓	✓	x	x	✓	✓	✓	✓	✓	✓	✓	✓	x	x	✓	✓	✓	✓
Bromuconazole	✓	✓	✓	✓	x	x	✓	✓	✓	✓	✓	✓	✓	✓	x	x	✓	✓	✓	✓
Buprofezin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Carbendazim	x	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	x	x	✓	x	✓	✓
Carbofuran	x	x	✓	✓	x	x	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Carboxin	✓	✓	✓	✓	x	x	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Chlordimeform	x	x	✓	✓	x	x	x	x	✓	✓	x	x	x	x	x	x	x	x	x	x
Clethodim	✓	✓	✓	✓	x	x	✓	✓	✓	✓	✓	✓	✓	x	x	x	✓	✓	✓	✓
Coumaphos	✓	✓	✓	✓	x	x	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Cyprodinil	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Cyromazine	x	x	✓	✓	x	x	✓	✓	✓	✓	✓	✓	x	x	x	x	✓	✓	x	x
Demeton-S-methyl	x	x	x	x	x	x	✓	✓	✓	✓	✓	✓	x	x	x	x	x	x	x	x
Demeton-S-methylsulfoxide	✓	✓	✓	✓	x		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Diazinon	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Dimethoate	✓	✓	✓	✓	x		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Dimethomorph	✓	✓	✓	✓	x		✓	✓	✓	✓	✓	✓	x	x	x	x	✓	✓	✓	✓
Diniconazole	✓	✓	✓	✓	x		✓	✓	✓	✓	✓	✓	x	x	✓	✓	✓	✓	✓	✓
Ethion	✓	✓	✓	✓	x		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ethoxyquin	x	x	x	x	x	x	✓	✓	✓	✓	✓	✓	✓	x	✓	✓	✓	✓	✓	✓
Fenamidone	✓	✓	✓	✓	x	x	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Fenpiclonil	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Flucythrinate	✓	✓	✓	✓	x	x	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Furathiocarb	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Hexaconazole	✓	✓	✓	✓	x	x	✓	✓	✓	✓	x	x	✓	✓	x	x	✓	✓	✓	✓
Malathion	✓	✓	✓	✓	x	x	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Mecarbam	✓	✓	✓	✓	x	x	✓	✓	✓	✓	✓	✓	✓	✓	x	✓	✓	✓	✓	✓
Methidathion	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Myclobutanil	x	x	✓	✓	x	x	x	x	✓	✓	✓	✓	✓	✓	✓	x	✓	✓	✓	✓
Nicotine	x	x	✓	✓	x	x	✓	✓	x	x	x	x	x	x	x	x	x	x	x	x
Paraoxon-methyl	x	x	✓	✓	x	x	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Picoxystrobin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pirimicarb	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pirimiphos-methyl	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Propiconazole	✓	✓	✓	✓	x	x	✓	✓	✓	✓	✓	✓	x	x	x	x	✓	✓	✓	✓
Pyraclostrobin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	x	x	x	x	✓	✓	x	✓
Pyridaben	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	x	x	✓	✓	✓	✓
Quinoclamine	x	x	x	x	x	x	x	x	x	x	x	x	x	✓	x	x	✓	✓	✓	✓
Quizalofop-ethyl	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	x	x	x	x	x	x	✓	✓
Sulfotep	✓	✓	✓	✓	x	x	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Tebufenpyrad	x	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Thiacloprid	✓	✓	✓	✓	x	x	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Detection rate could be improved with 2D-LC at SDL 0.01 mg/kg

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2D-LC vs QuEChERS

Comparison EUPT-FV-SM09 Lemon

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2D-LC vs QuEChERS – EUPT-FV-SM09

2D-LC ESI +	QuEChERS ESI +	2D-LC ESI -	QuEChERS ESI -
Bromuconazole	Bromuconazole	Fipronil	Fipronil
Cyflufenamide		Flubendiamid	Flubendiamid
Fenpyrazamin	Fenpyrazamin	Isopyrazam	Isopyrazam
Isopyrazam	Isopyrazam	Novaluron	Novaluron
Novaluron	Novaluron	Penthiopyrad	Penthiopyrad
Penthiopyrad	Penthiopyrad		
Pyridalyl	Pyridalyl		
Spinetoram	Spinetoram		
Spirotetramat-enol	Spirotetramat-enol		
Tricyclazole			
Vailfenate	Vailfenate		

- Orthosulfamuron: FN not in Database and no analytical standard in 2017
- Tricyclazole: FN not reported because not verified with GC
- Spirotetramat-enol not identified by organizers (FP)

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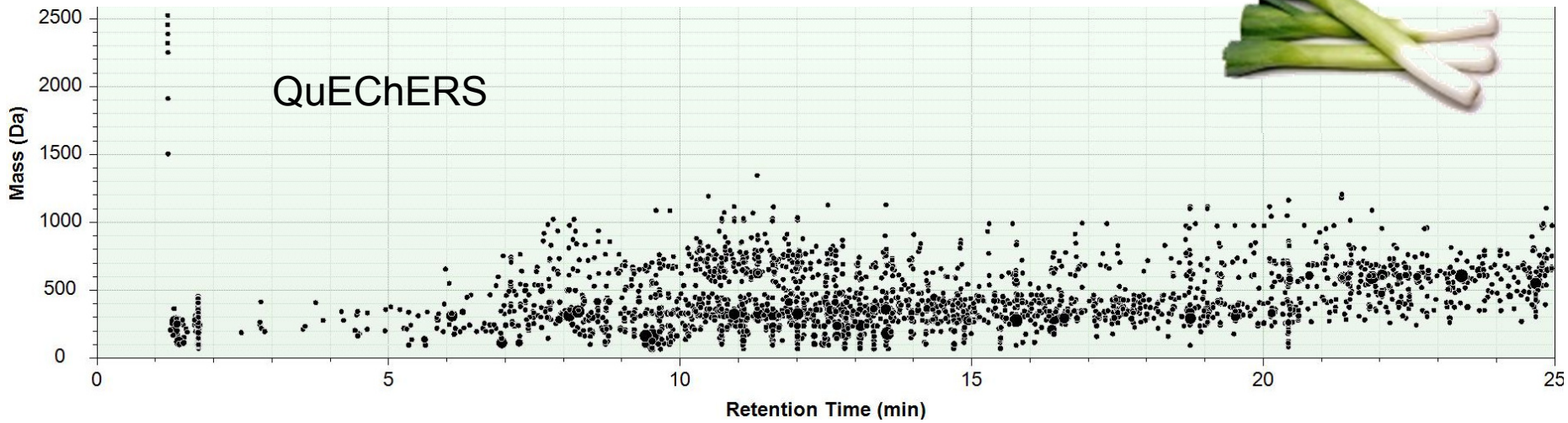
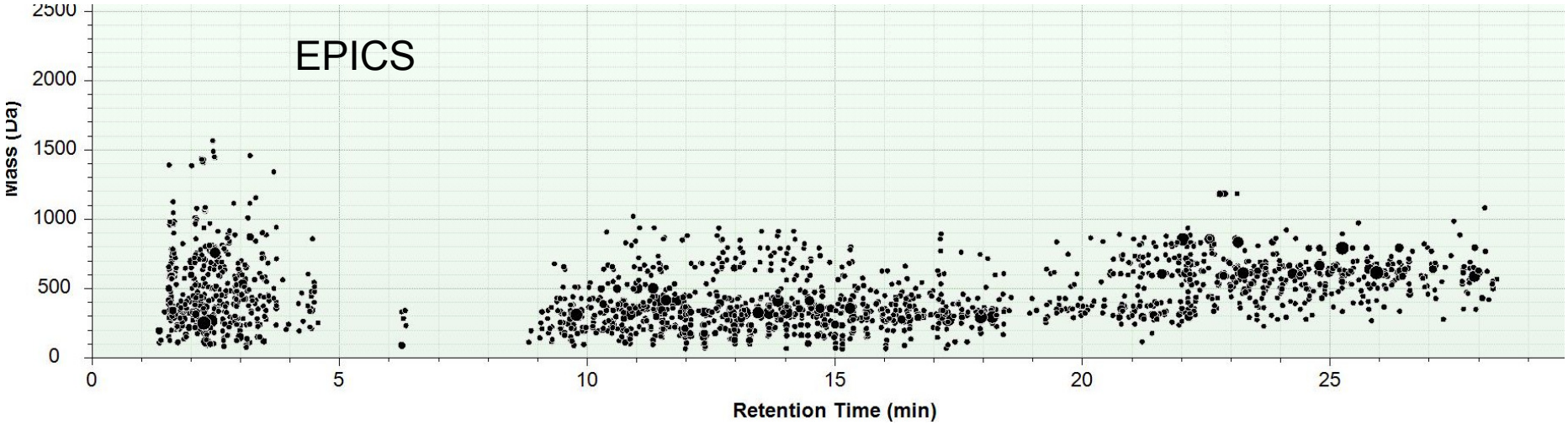
2D-LC vs QuEChERS

Evaluation of clean up in terms of matrix components

Using MassProfiler





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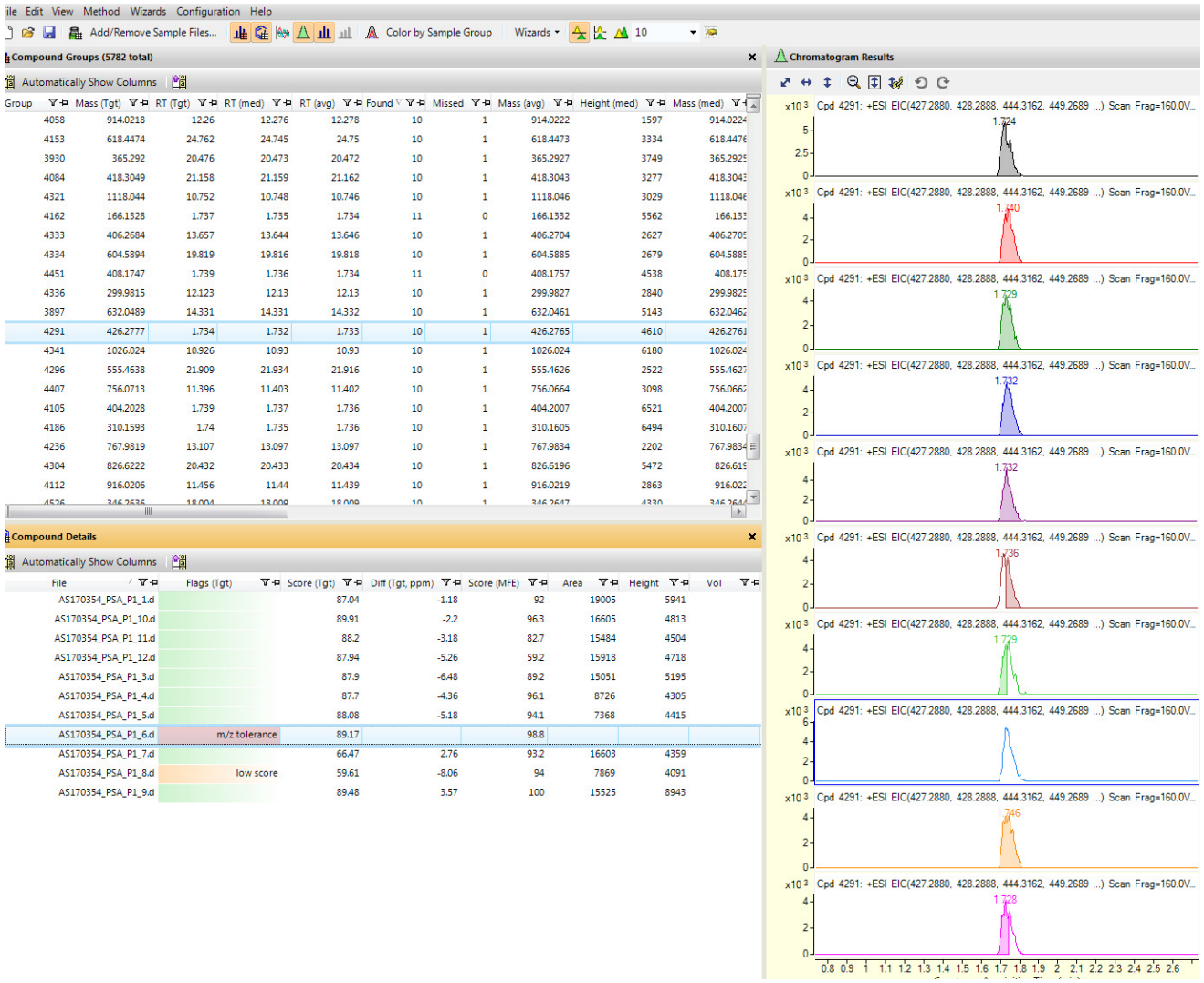
Compound Mass Profinder



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Summary and Outlook

- 2D-LC results in a better detection rate of analytes that could not be validated after QuEChERS clean-up
  - this could be due to changed elution profiles => no co-elution of analyte and matrix compounds
- higher injection volumes are possible with HILIC
- no analyte loss during sample preparation in 2D-LC

- further investigations to compare the two clean-up methods and to better understand matrix effects are in progress

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