Is HILIC the best approach for highly polar anionic pesticides determination? The case of animal origin products and feed samples

Jonatan Dias, Wageningen Food Safety Research (WFSR), The Netherlands

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14th European Pesticide Residue Workshop

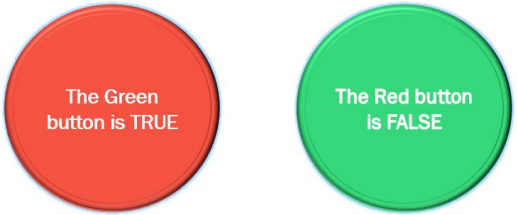
Outline

- The challenges of polar pesticides analysis
- To which extend different kind of matrices can make it even more difficult
- Sample preparation strategies
- Hydrophilic interaction liquid chromatography (HILIC)
- HILIC column options and comparison

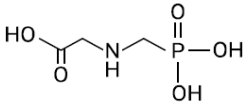
The challenging analysis of polar pesticides...

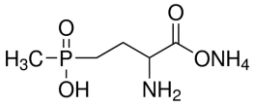
Glyphosate is the most widely used agrochemical in the world; the most hardly determined by analytical methods.

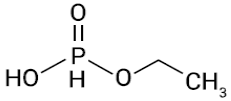
"Glyphosate paradox"

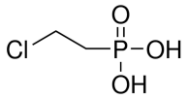


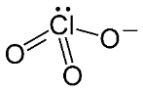
The challenging analysis of polar pesticides...

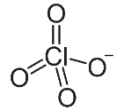

Glyphosate


Glufosinate

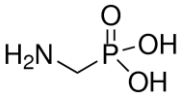

Fosetyl

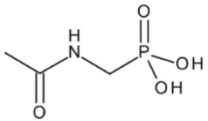

Ethephon

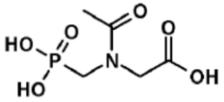

Chlorate

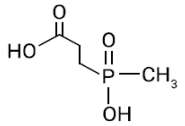

Perchlorate

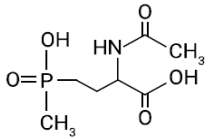
Br^-
Bromide

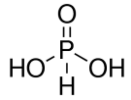

AMPA

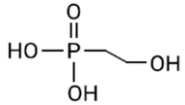

N-a-AMPA


N-a-Glyphosate


MPPA


N-a-Glufosinate


Phosphonic acid


HEPA

1- Highly polar
2- Low molecular mass
3- Chelating properties (phosphonate group)
4- Combination matrices/MRL/extraction techniques

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The challenging analysis of polar pesticides...

Sample prep.

- Poor extractability using typical organic solvents
- Analytes can bind to metal ions present in samples

Separation

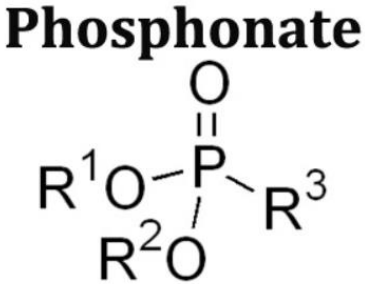
- Poor retention, bad separation, bad peak shapes
- Analytes can bind to metal ions in the system

Analysis

- Lack of selectivity (low molecular masses)
- Low sensitivity
- Matrix interferences

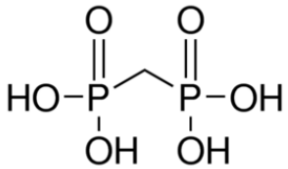
Overcoming the issues...

- Some of the polar pesticides can bind to metals during extraction and/or during analysis.
- Solution: passivation of the chromatographic system or additives in the eluents.



Passivation:

HNO_3 and EDTA



Medronic acid

Overcoming the issues...

- Derivatisation
  - FMOC (9-fluorenylmethyl chloroformate) is widely applied;
  - Improves chromatographic performance by chemical structure modification

  However...
  - Time consuming procedure;
  - Not all molecules are prone to derivatization (N-acetyl metabolites)

Direct analysis

* Easy sample preparation
* Wider scope (+ N-a compounds)
* Lower LOQs

- Especial separation columns
- Higher dilution factors (⇧ sensitivity)
- More sensitive detectors

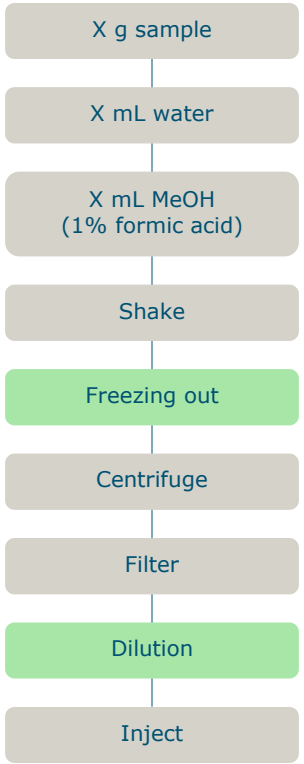
Sample preparation – F&V + beverages

- X g sample
- X mL water
- X mL MeOH (1% formic acid)
- Shake
- Centrifuge
- Filter
- Dilution
- Inject

- X g sample
- X mL MeOH (1% formic acid)
- Shake
- Centrifuge
- Filter
- Dilution
- Inject

Sample preparation – Feed

- Byproducts of cereals, pulses and oilseeds;
- Complexity + diversity + wide variety of constituents and additives (grains, milling products, added minerals, vitamins and fats)



Sample preparation – Animal origin

X g sample

X mL water

X mL MeOH
(1% formic acid)

Shake

Freezing out

Centrifuge

Extra clean-up*

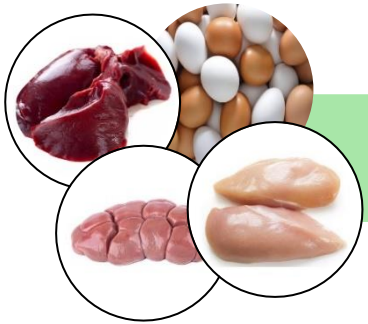
Filter

Dilution

Inject

Addition of EDTA

Melted sample +
warm water



*SPE clean-up
(Oasis MCX)

The challenging analysis of polar pesticides **in AO**

- Matrices included in the scope:
  - Kidney (bovine, swine), liver (bovine, swine and poultry), chicken eggs, chicken meat, swine fat and milk

Very complex matrices
- Proteins
- Fat
- Carbohydrates
- Minerals (Na, K, Ca, Mg, Fe, ...)
- Amino acids
- Fatty acids

Can interfere during clean-up for some compounds

Compounds can be lost during extraction due to chelating properties

The challenging analysis of polar pesticides **in AO**

COMPOUNDS	Kidney		Liver		Chicken			Fat (swine)	Milk
	Bovine	Swine	Bovine	Swine	Poultry	Eggs	Muscle		
Ethephon	**0.4**	**0.4**	**0.4**	**0.4**	**0.08**	**0.05+**	**0.05+**	**0.05+**	**0.05+**
HEPA									
Fosetyl	**8**	**6**	**1.5**	**0.8**	**0.7**	**0.7**	**0.7**	**1.5**	**0.5**
Phosphonic Acid									
Glufosinate	**3**	**3**	**3**	**3**	**0.1**	**0.05**	**0.05**	**0.1**	**0.03+**
MPPA									
N-A-Glufosinate									
Glyphosate	**2**	**0.5**	**0.2**	**0.05+**	**0.05+**	**0.05+**	**0.05+**	**0.05+**	**0.05+**
AMPA									
N-A-AMPA									
N-A-Glyphosate									
Bromide					**0.05+**				
Chlorate	**0.05**	**0.05**	**0.05**	**0.05**	**0.05**	**0.05**	**0.05**	**0.1+**	**0.1**
Perchlorate*									

**No EU-MRL yet, indicative action limit*
+ Indicates lower limit of analytical determination

* Multiple possible combinations matrix/compound/MRL

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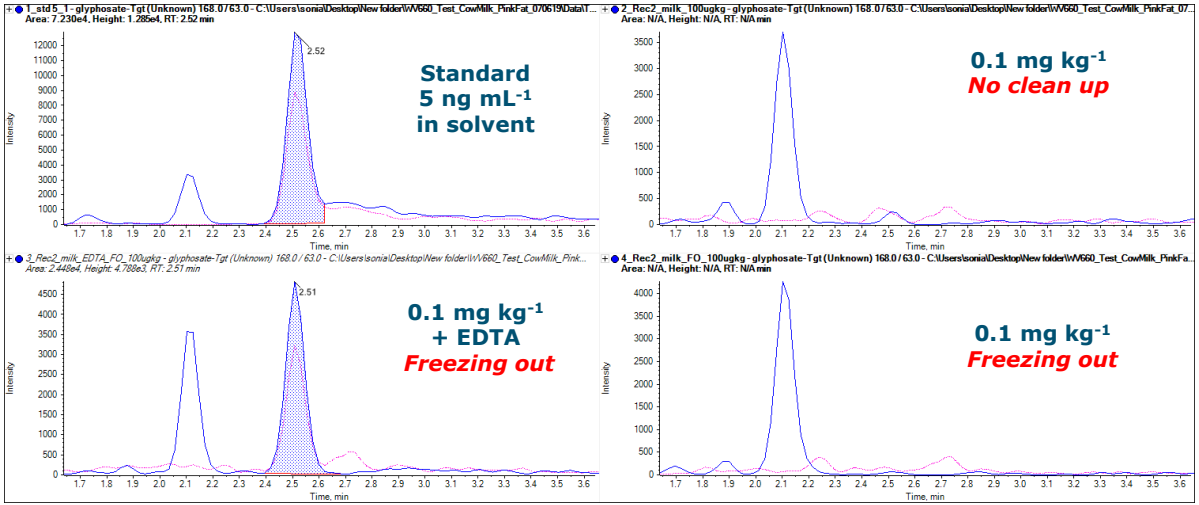
Sample preparation – Animal origin

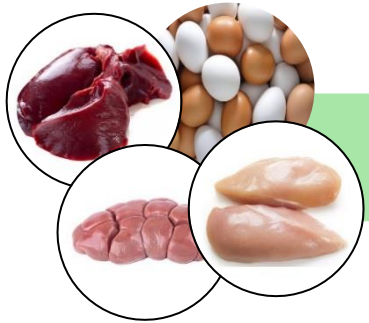
Addition of EDTA

Glyphosate in milk samples with and without EDTA

Compared to EDTA, glyphosate is considered a weak chelator!



Sample preparation – Animal origin

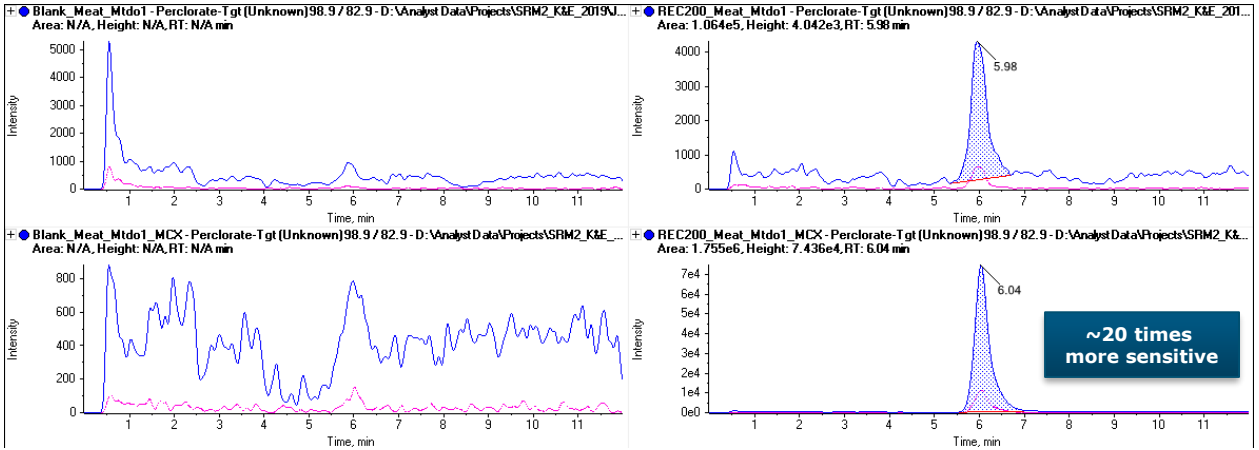


*SPE clean-up
(Oasis MCX)

Perchlorate in Chicken meat

Blank

**Sample
+ 0.2 mg kg^{-1}**



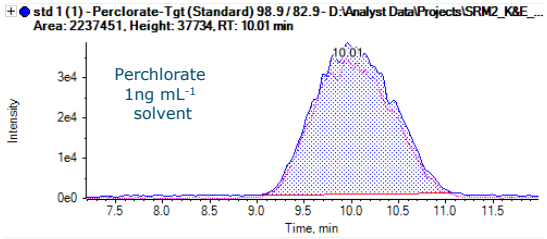
Sample dilx100
AcN/H_2O (60:40)
+ 0.2% TFA

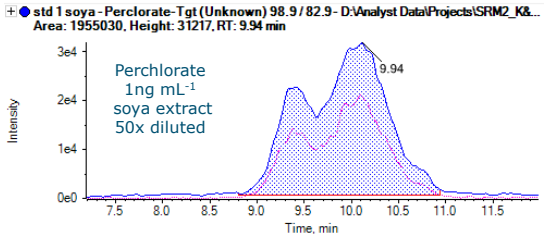
Sample dilx100
AcN/H_2O (60:40)
+ 0.2% TFA
Clean-up: MCX

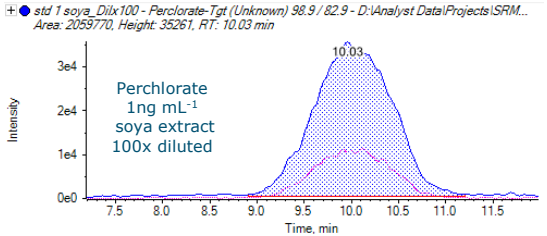
Sample preparation – Dilution of extracts

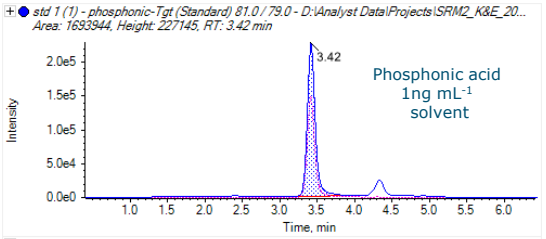
Effect of dilution factor on peak shape, response and retention time: Perchlorate and phosphonic acid in soya beans extract

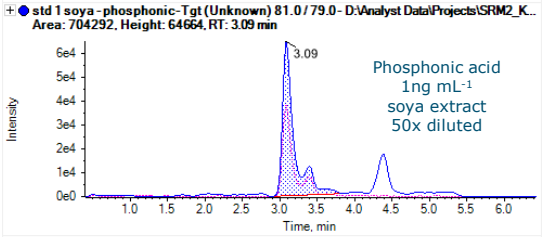
1. *Retention time stability*
2. *Better Peak shape*
3. *Higher Response*

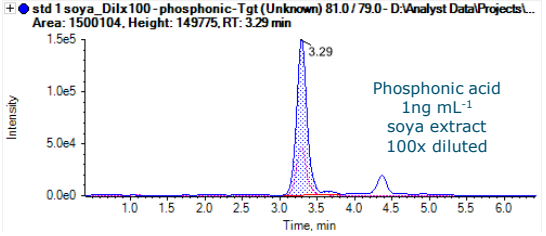












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Sample preparation – Dilution of extracts

PERCHLORATE

Standard in solvent
5 ng mL^{-1}

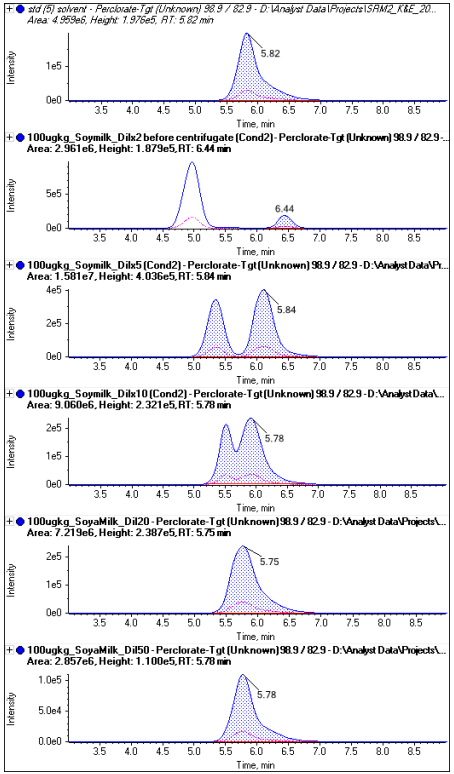
Sample extract
2 x dilution

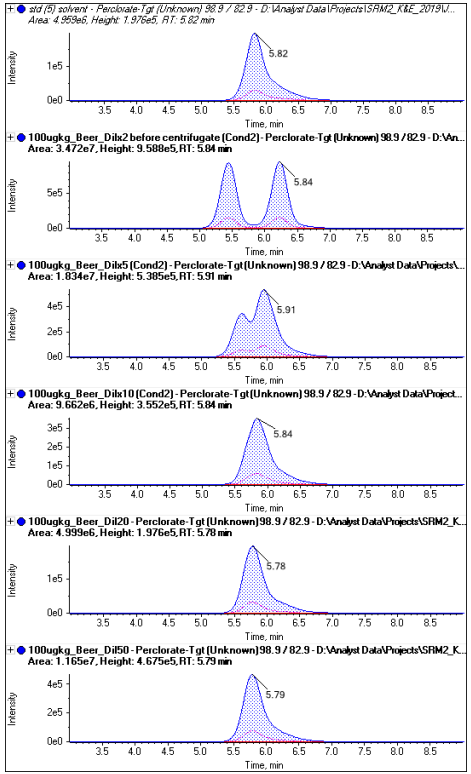
Sample extract
5 x dilution

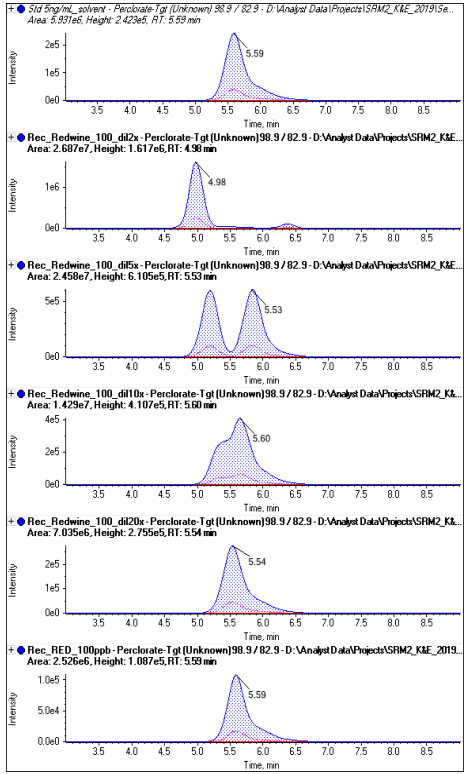
Sample extract
10 x dilution

Sample extract
20 x dilution

Sample extract
50 x dilution

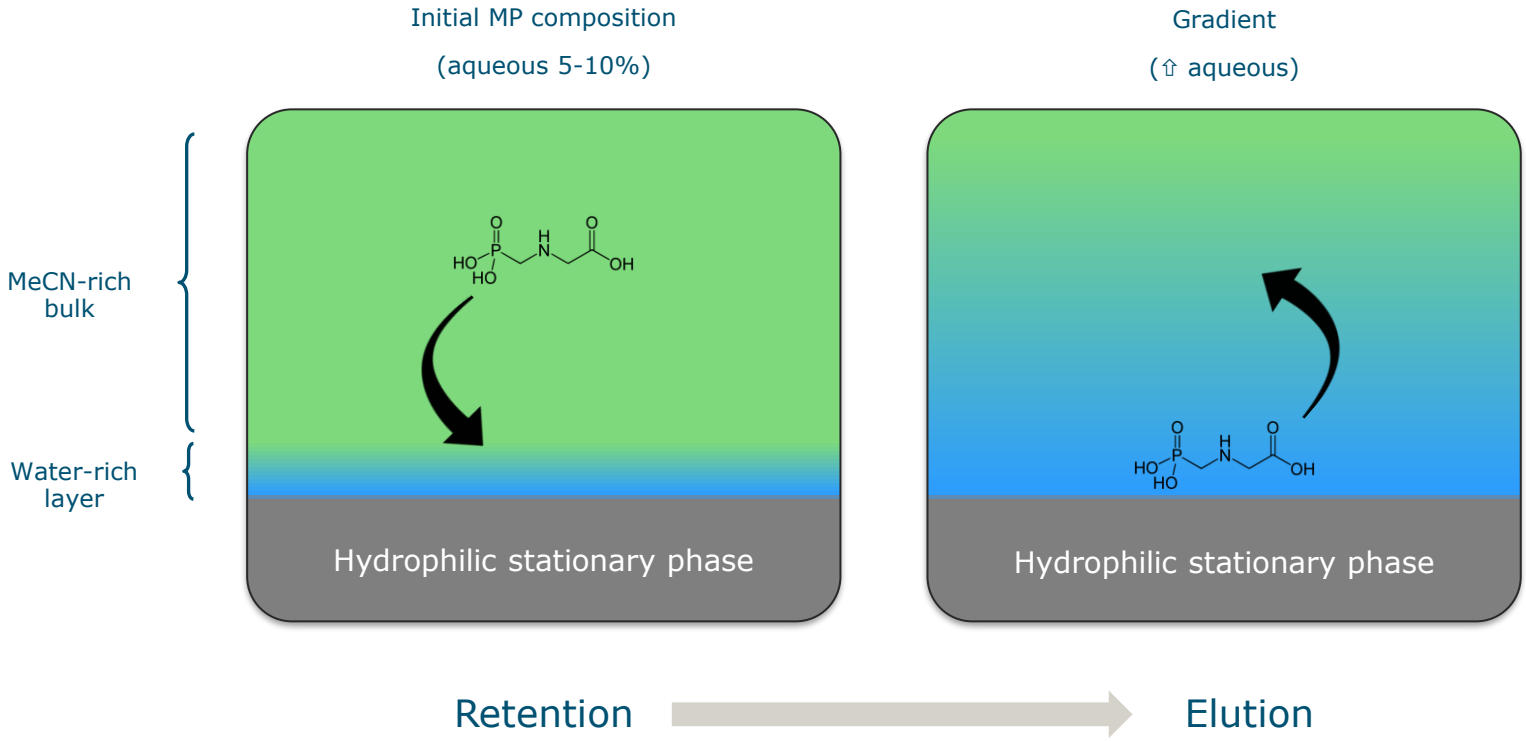






Soya milk **Beer** **Red wine**

Hydrophilic interaction liquid chromatography - HILIC



Hydrophilic interaction liquid chromatography - HILIC

- Sufficient retention of hydrophilic compounds;
- High proportion of organic solvent allows higher flow rates (⇩ viscosity, ⇩ back pressure);
- Efficient dessolvation: lower detection limits.

- Limited applicability;
- Slow columns equilibration*;
- Lack of robustness*;
- High organic solvent consumption.

HILIC columns options (already tested!)

- Obelisc-N (SIELC): Use of multiple separation mechanisms
- Poroshell 120 HILIC-Z (Agilent): Zwitterionic phase
- Anionic Polar Pesticides - APP (Waters): BEH (Ethylene Bridged Hybrid) particles with tri-functionally bonded diethylamine (DEA) ligants.
- Raptor Polar X (Restek): Multiple separation mechanisms (HILIC + ion-exchange)
- Poroshell 120 CS-C18 (Agilent): Charged surface C18

Feed matrices used for the evaluation!

Evaluation of different HILIC columns

Obelisc N

A: 1% formic acid in water
B: acetonitrile

Dilution solvent:
Acetonitrile/water (6:4)
+ 0.2% TFA

HILIC-Z

A: 1% formic acid in water
B: acetonitrile

Dilution solvent:
Acetonitrile/water (6:4)
+ 0.2% TFA

APP

A: 0.9% formic acid in water
B: 0.9% formic acid in acetonitrile

Dilution solvent:
MeOH 1% formic acid/water
(1:1)

Polar X

A: 0.5% formic acid in water
B: 0.5% formic acid in acetonitrile

Dilution solvent:
MeOH 1% formic acid/water
(1:1)

Poroshel 120 CS

A: 0.1% formic acid in water
B: 0.1% formic acid in methanol

Dilution solvent:
MeOH 1% formic acid/water
(1:1)

Evaluation of different HILIC columns

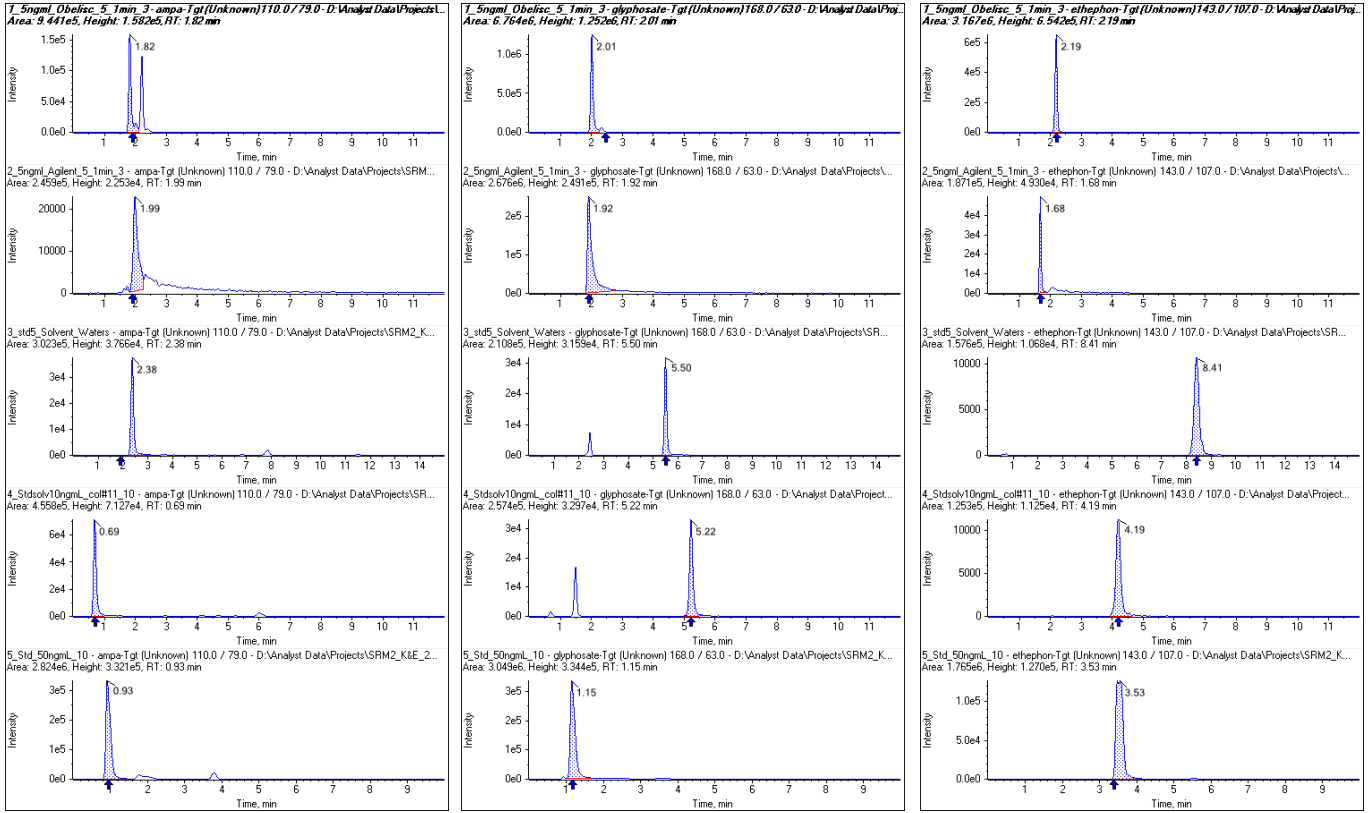
Obelisc N

HILIC-Z

APP

PolarX

CS-C18

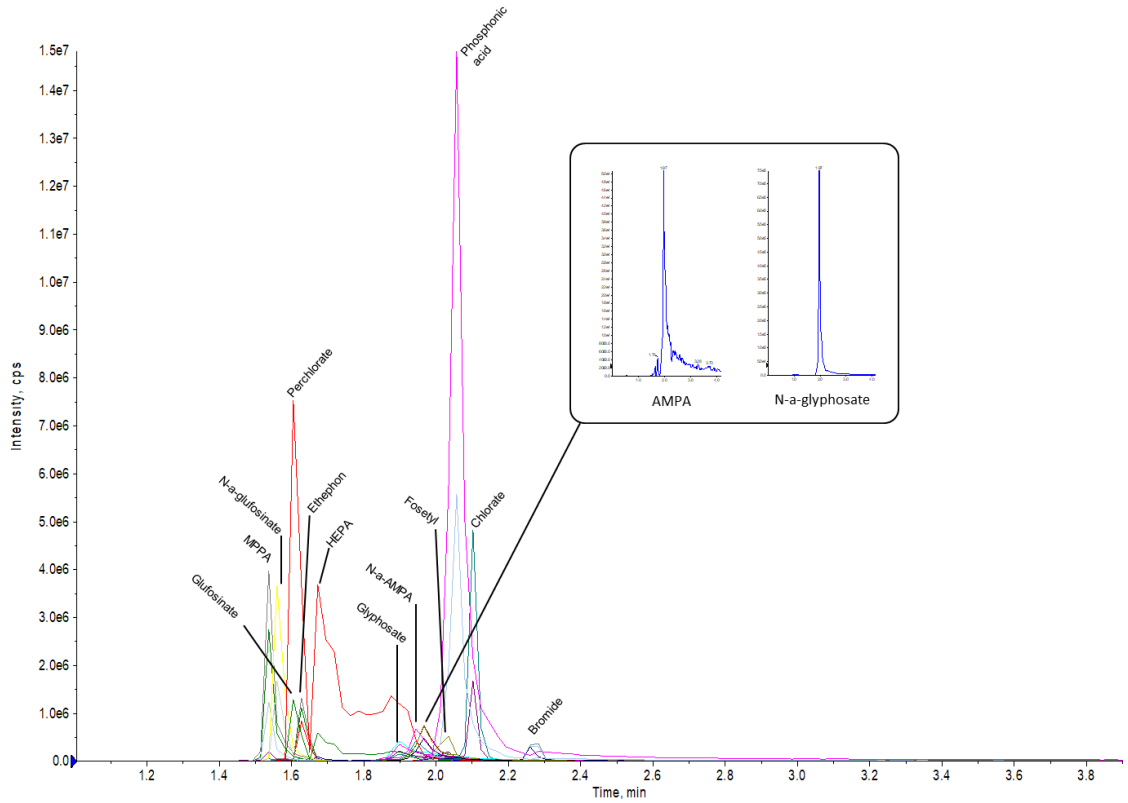


AMPA Glyphosate Ethephon

Evaluation of different HILIC columns

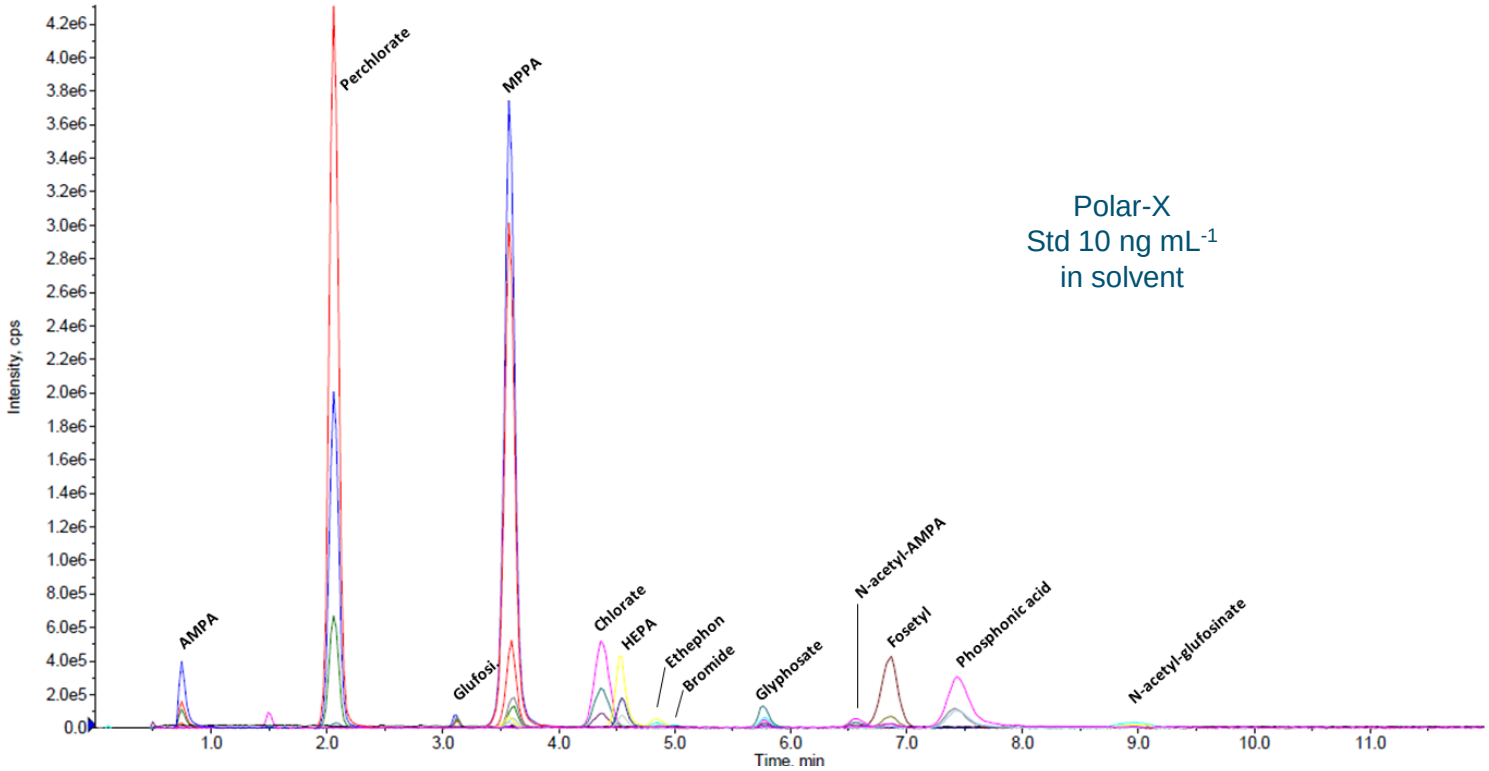
- Hilic-Z and Poroshell CS-18 were excluded from the evaluation due to multiple issues related to selectivity, peak splitting and lack of retention for most of the compounds.
- Validation performed for feed samples applying Obelisc N and APP for dry peas, soya meal and sunflower seeds meal

Evaluation of different HILIC columns



HILIC-Z
Std 10 ng mL^{-1}
in solvent

Evaluation of different HILIC columns



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Evaluation of different HILIC columns

LOQs (mg kg^{-1}) obtained during validation study.

Compound	Obelisc N			APP		
	Sunflower seed cake	Peas	Soya cake	Sunflower seed cake	Peas	Soya cake
AMPA	0.05*	0.05*	0.05 (0.02*)	0.02	0.1	0.5
Bromide	n.a	n.a	0.05	n.d	n.d	n.d
Chlorate	0.02	0.02	0.02	n.d	n.d	n.d
Ethephon	0.02	0.02	0.02	0.02	0.02	0.02
Fosetyl	0.5	n.f.r	n.f.r	0.02	0.02	0.02
Glufosinate	0.02	0.02	0.02	0.02	0.02	0.02
Glyphosate	0.1 (0.02*)	0.5 (0.02*)	0.02	0.05	0.02	0.02
HEPA	0.1 (0.02*)	0.02	0.5	0.02	0.02	0.02
MPPA	0.1 (0.02*)	0.1	n.f.r	0.02	0.02	0.02
N-acetyl-AMPA	0.02	0.1	0.1	0.02	0.02	0.02
N-acetyl-Glufosinate	0.02	0.02	0.02	0.02	0.02	0.02
N-acetyl-Glyphosate	0.5	0.02	n.f.r	n.d	n.d	n.d
Perchlorate	0.02	0.02	0.05	n.d	n.d	n.d
Phosphonic acid	0.2	0.2	0.2	0.2	0.2	0.2

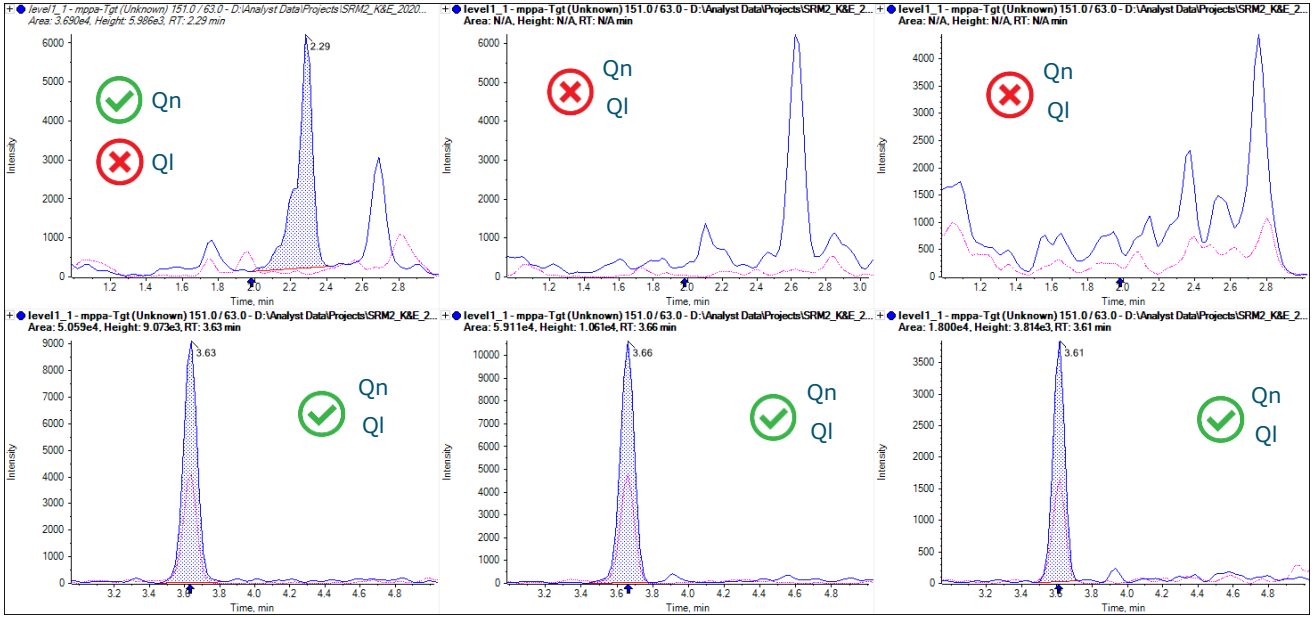
* Screening detection limit, only taking the quantifier into account
n.a.: not analysed due to high background levels in the blank
n.d.: not detectable in the same run
n.f.r.: not fulfilling requirements for a quantitative method

Evaluation of different HILIC columns

MPPA at 0.02 mg kg^{-1}

Obelisc N

APP



Sunflower seed meal

Peas

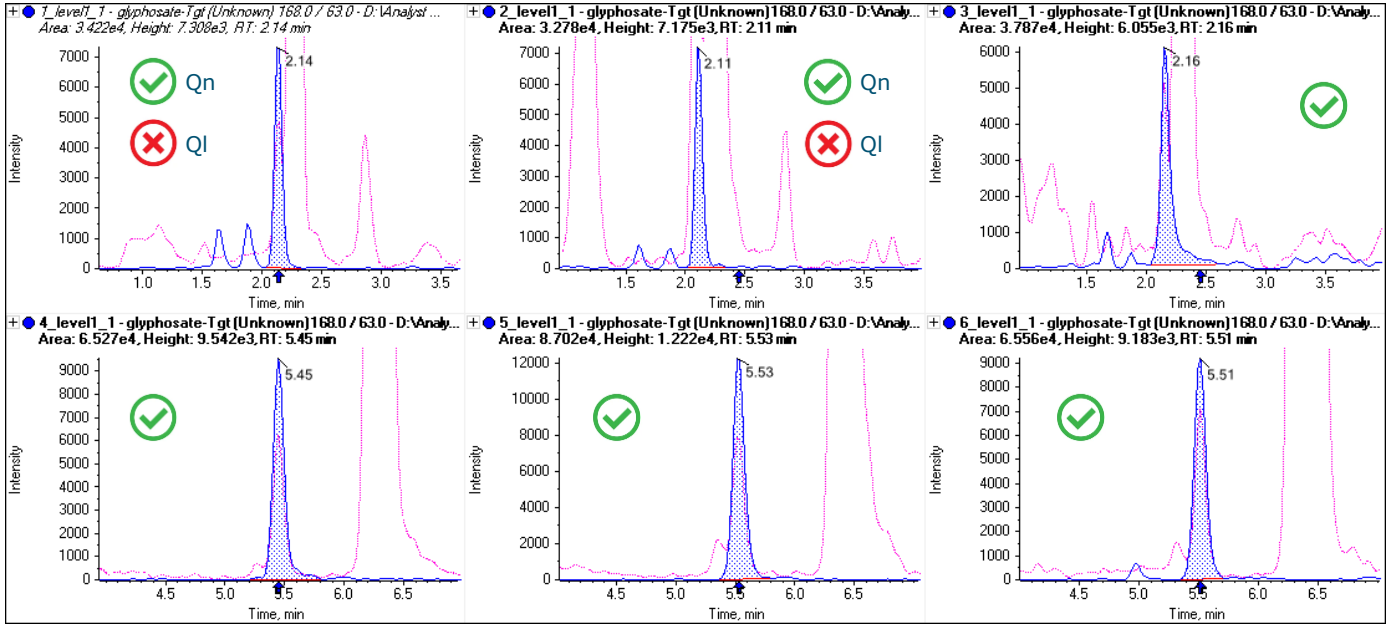
Soya meal

Evaluation of different HILIC columns

Glyphosate at 0.02 mg kg^{-1}

Obelisc N →

APP →



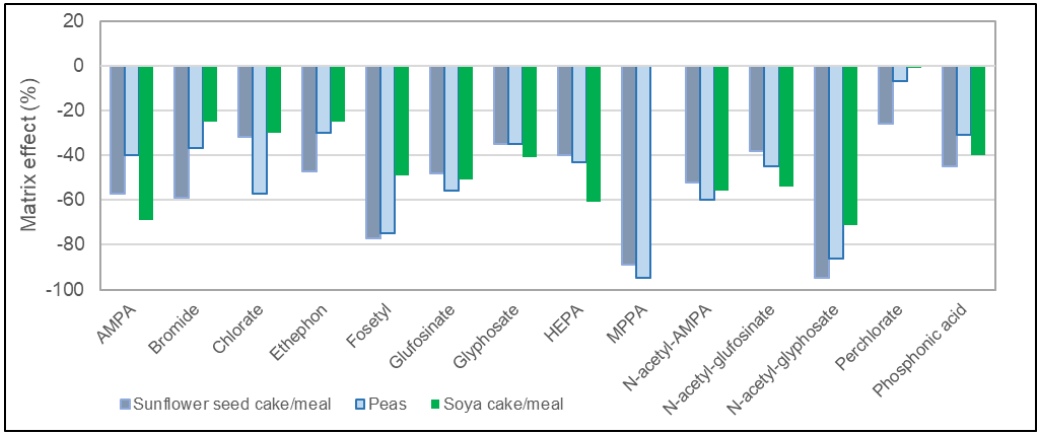
Sunflower seed meal

Peas

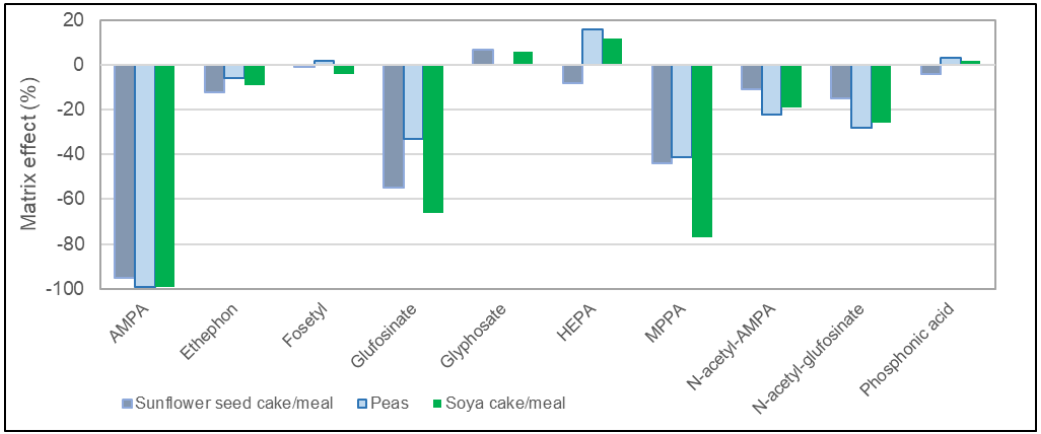
Soya meal

Evaluation of different HILIC columns

Obelisc N



APP



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Evaluation of different HILIC columns

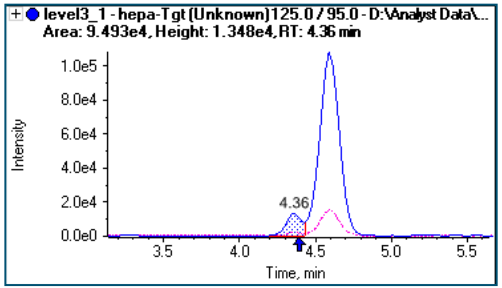
- APP column presented very good results when compared to Obelisc-N.
- This column was used to perform validations for dry garlic powder and dry parsley powder (the most challenging feed matrices).
- In addition, a new column available (Polar X) was also used for the same set of validations.

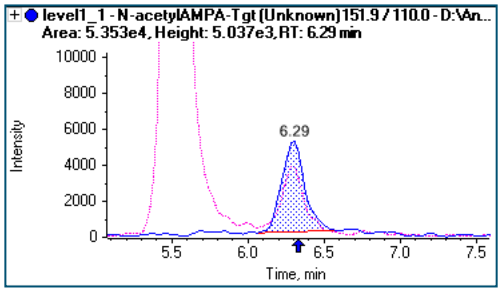
Evaluation of different HILIC columns

LOQs (mg kg^{-1}) obtained during validation study.

Compound	APP		Polar X	
	Garlic	Parsley	Garlic	Parsley
AMPA	0.1	0.02	0.02	nfr
Bromide	nd	nd	na	na
Chlorate	nd	nd	0.02	0.02
Ethephon	0.02	0.02	0.02	0.02
Fosetyl	0.02	0.02	0.02	0.02
Glufosinate	0.02	0.02	0.02	0.02
Glyphosate	0.02	0.02	0.02	0.02
HEPA	0.02	0.1	nfr	nfr
MPPA	0.02	0.02	0.02	0.02
N-acetyl-AMPA	0.02	0.02	nfr	nfr
N-acetyl-Glufosinate	0.02	0.02	0.02	0.02
N-acetyl-Glyphosate	nd	nd	nd	nd
Perchlorate	nd	nd	0.02	0.02
Phosphonic acid	0.2	0.2	0.2	0.2

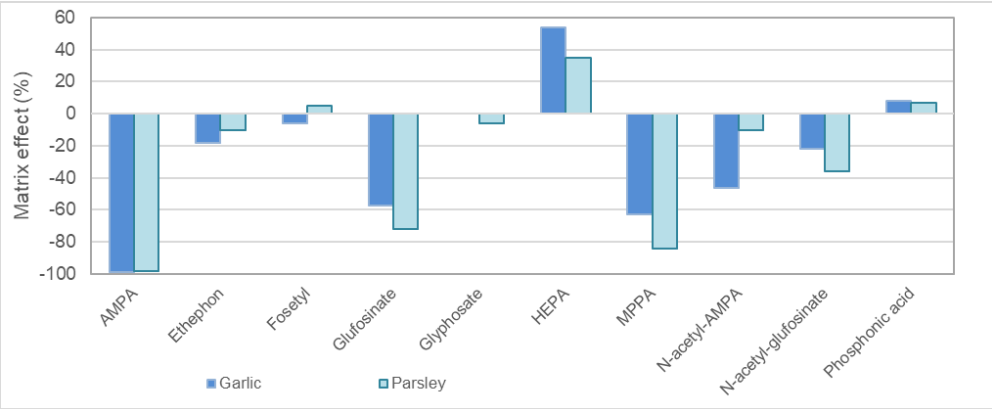
* Screening detection limit, only taking the quantifier into account
n.a.: not analysed due to high background levels in the blank
n.d.: not detectable in the same run
n.f.r.: not fulfilling requirements for a quantitative method



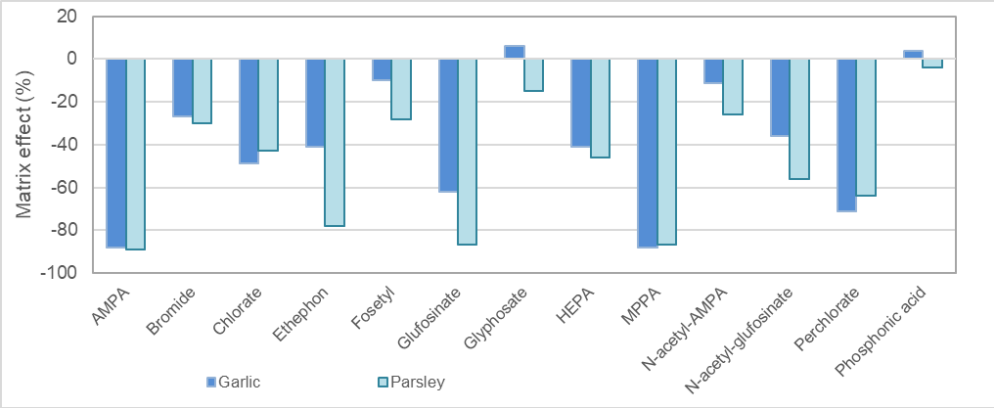


Evaluation of different HILIC columns

APP



Polar X



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Which column to use?

Compound	Performance (peak shape and detectability)				
	Obelisc N	APP	HILIC-Z	PolarX	Poroshell 120
AMPA	✓*	✓	✗+#	✓	✓
Bromide	✓	nd	✓	✓*	✓
Chlorate	✓	nd	✓	✓	✓
Ethephon	✓	✓	✓#	✓	✓
Fosetyl	✓	✓	✓#	✓	✓
Glufosinate	✓	✓	✓#	✓	✓
Glyphosate	✓*	✓	(✗)#	✓*	✗*
HEPA	✓*	✓	(✗)	✓	✗*
MPPA	✓	✓	✓	✓	✗*+
N-a-AMPA	✓	✓	✓	✓	✗*
N-a-Glufosinate	✓	✓	✓#	✓	✗*+
N-a-Glyphosate	✓	nd	(✗)	nd	✓
Perchlorate	✓	nd	✗+#	✓	✓
Phosphonic acid	✓	✓	✓	✓	✗*

nd.: not detected under these conditions
*Strong suppression in some matrices
+Peak splitting
#Lack of retention
()Tailing

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Which column to use?

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RESEARCH ARTICLE

JOURNAL OF SEPARATION SCIENCE

Influence of different hydrophilic interaction liquid chromatography stationary phases on method performance for the determination of highly polar anionic pesticides in complex feed matrices

Jonatan Dias | Sonia Herrera López | Hans Mol | André de Kok

The future...

- New columns being developed;
- New matrices to be included (honey);
- New challenges...

- The Netherlands, Portugal, Spain, Italy, France, Czech Republic, Slovenia, Switzerland, Croatia, Denmark and Argentina.
- Method development for food, feed and biological samples (urine and **feces**).

↓

Gly and AMPA + MeOH + HILIC (APP and PolaX)

To sum up...

- Why to choose HILIC? Allows direct analysis (no derivatisation needed)
- New HILIC materials are being developed specifically for highly polar pesticides analysis.
- The choice for one or other column will depend on the goal of the analysis (matrix, scope, LOQ).
- The use of ILIS is essential in order to correct for matrix effects and recovery losses.
- Large dilution factors will increase sensitivity and improve peak shapes and Rt stability. However, requires very sensitive MS systems.
- Polar pesticides analysis are challenging. However, new materials/technologies are always being created to overcome the difficulties.

Thank you for your attention!

Sonia Herrera

André de Kok

Hans Mol

Agnieszka Kiedrowska

Yuklai Liu

Franciska Kas

Joost Memelink

Gerco den Hertog

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