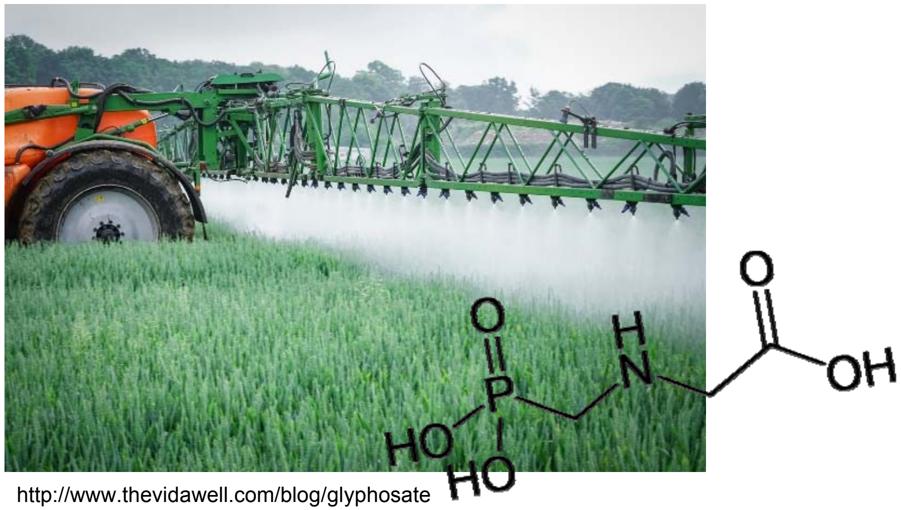
European Commission

EURL-SRM

EU Reference Laboratories for Residues of Pesticides
Single Residue Methods

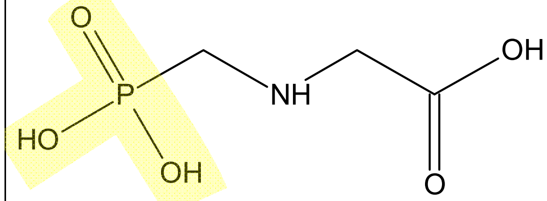
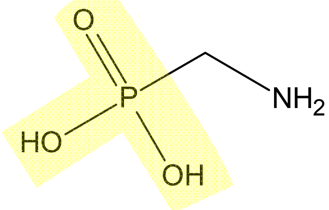
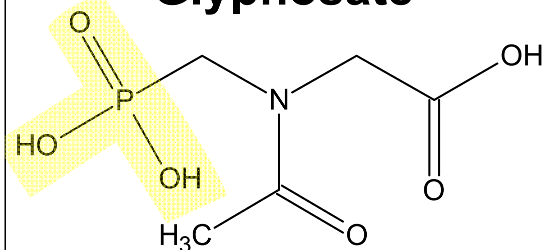
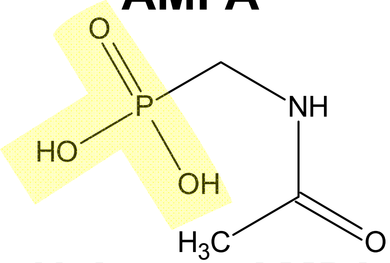
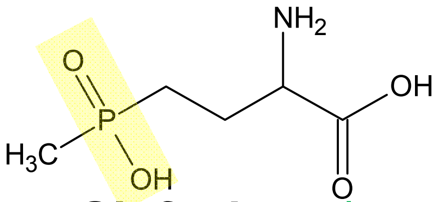
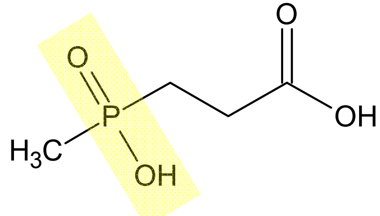
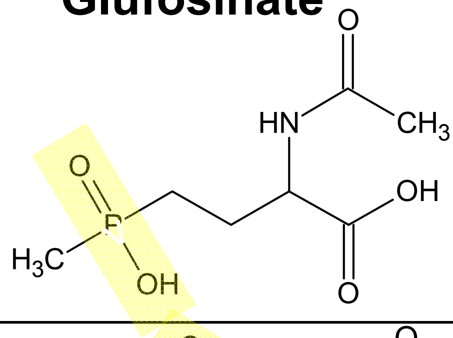
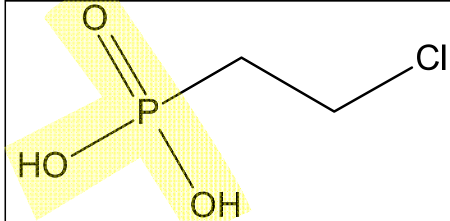
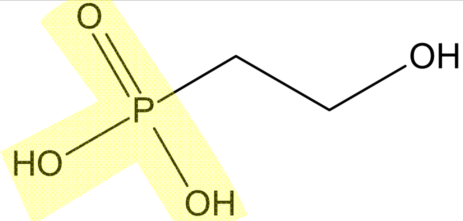
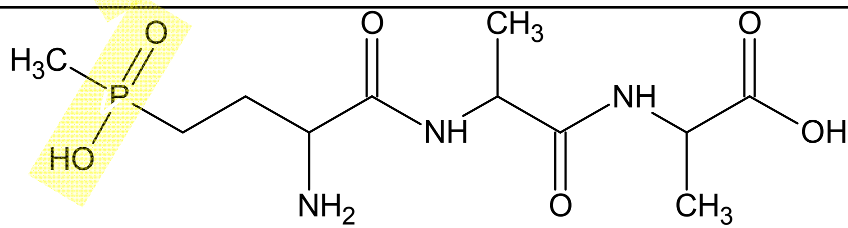
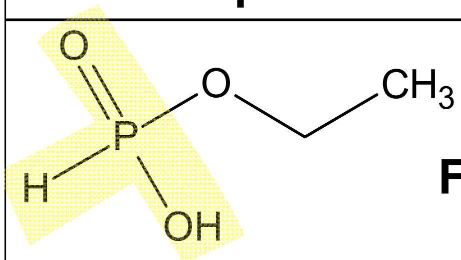
Selective Analysis of Glyphosate and other polar organo-phosphorous Compounds in Foods of plant Origin using on-line ligand-exchange SPE-HPLC-MS/MS



http://www.thevidawell.com/blog/glyphosate

Eric Eichhorn
Anne Benkenstein
Cristin Wildgrube
Ellen Scherbaum
Michelangelo Anastassiades

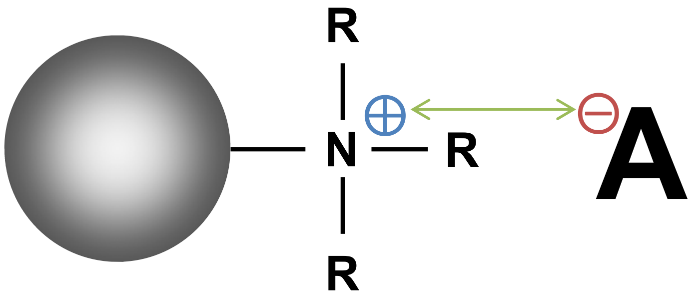
Joint EURL/NRLs Pesticide Residue Workshop 2018
27th-28th September 2018, Almeria, Spain

Phosphonic acid group containing	Phosphinic acid group containing
 **Glyphosate***  **AMPA***  **N-Acetylglyphosate***  **N-Acetyl-AMPA**	 **Glufosinate***  **MPPA***  **N-Acetylglufosinate***
 **Ethephon***  **HEPA**	 **Bialaphos**
 **Fosetyl***	* Included in the actual Residue Definition <u>or</u> currently discussed to be included

(Selected) Possible Mechanisms for an on-line Purification and Enrichment:

(Selected) Possible Mechanisms for an on-line Purification and Enrichment:

Anion Exchange



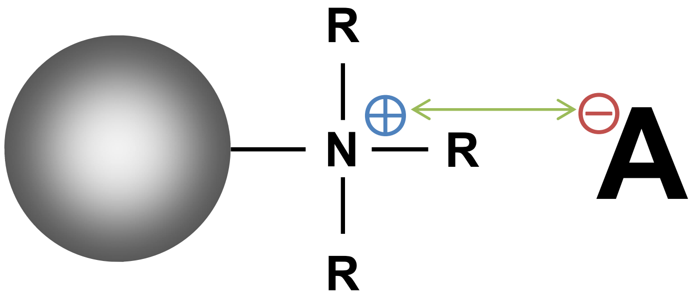
electrostatic interaction

A^- = Phosphate, Sulfate, Carbonic acids, Chloride, Nitrate,…

$$A^{3-} > A^{-2} > A^{-}$$

(Selected) Possible Mechanisms for an on-line Purification and Enrichment:

Anion Exchange



electrostatic interaction

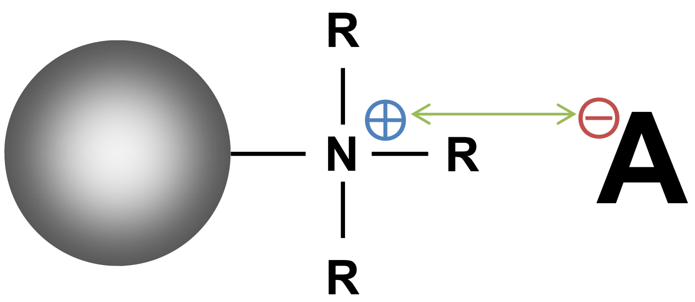
A^- = Phosphate, Sulfate, Carbonic acids, Chloride, Nitrate,…

$A^{3-} > A^{-2} > A$

Non-selective!

(Selected) Possible Mechanisms for an on-line Purification and Enrichment:

Anion Exchange



A^- = Phosphate, Sulfate, Carbonic acids, Chloride, Nitrate,…

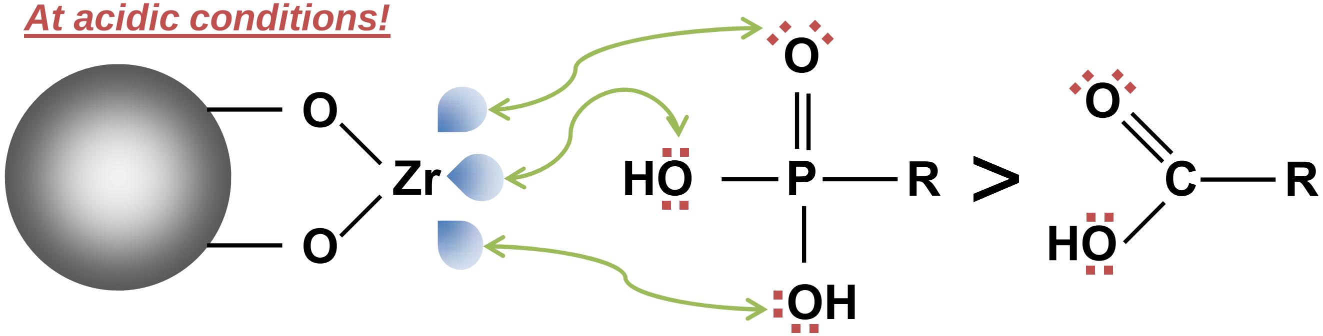
$A^{3-} > A^{-2} > A^-$

Non-selective!

electrostatic interaction

Ligand Exchange

At acidic conditions!



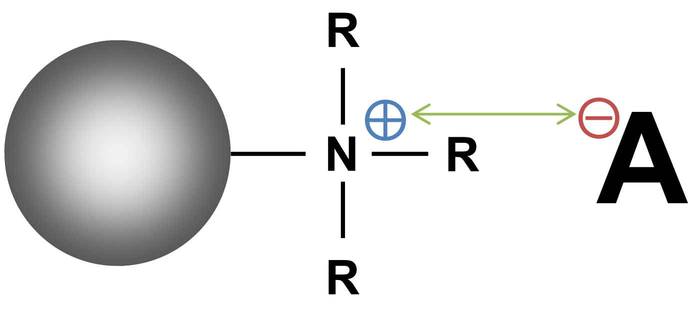
Lewis-Acid-Base-Interaction

Phosph(on)ate

Carbonic acid

(Selected) Possible Mechanisms for an on-line Purification and Enrichment:

Anion Exchange



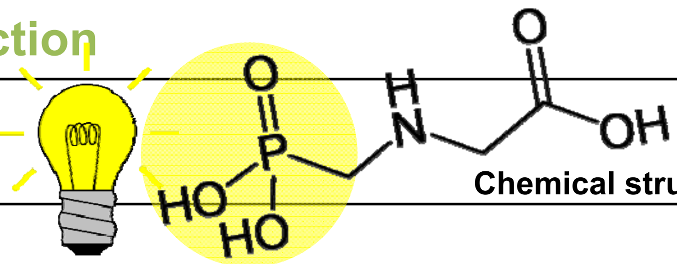
A^- = Phosphate, Sulfate, Carbonic acids, Chloride, Nitrate,…

$A^{3-} > A^{-2} > A^-$

Non-selective!

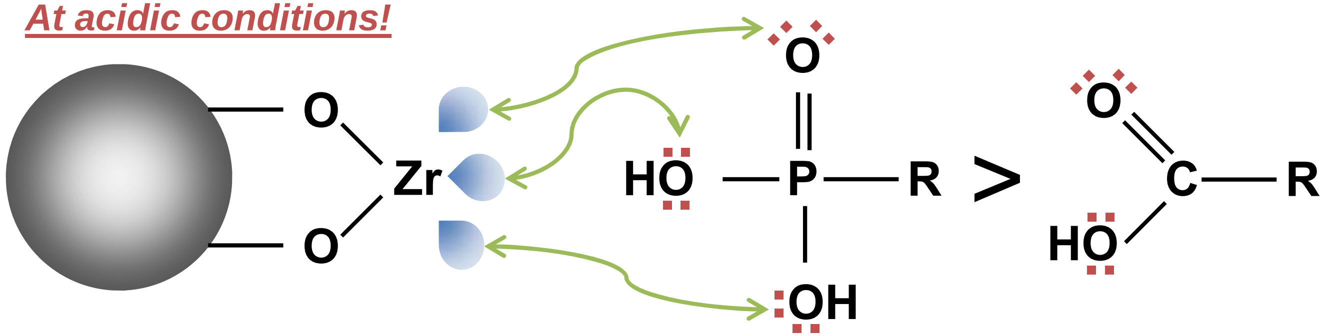
electrostatic interaction

Ligand Exchange



Chemical structure of Glyphosate

At acidic conditions!



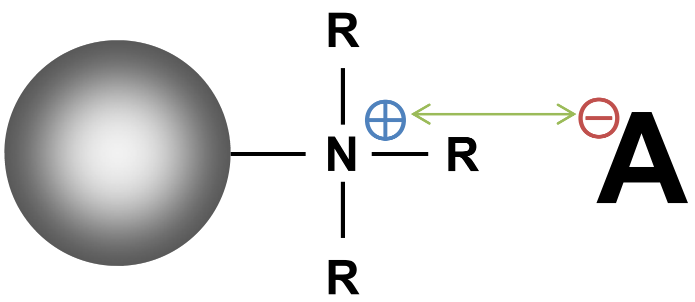
Phosph(on)ate

Carbonic acid

Lewis-Acid-Base-Interaction

(Selected) Possible Mechanisms for an on-line Purification and Enrichment:

Anion Exchange



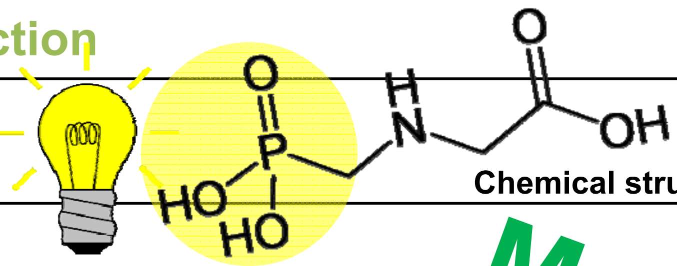
A^- = Phosphate, Sulfate, Carbonic acids, Chloride, Nitrate,…

$A^{3-} > A^{-2} > A^-$

Non-selective!

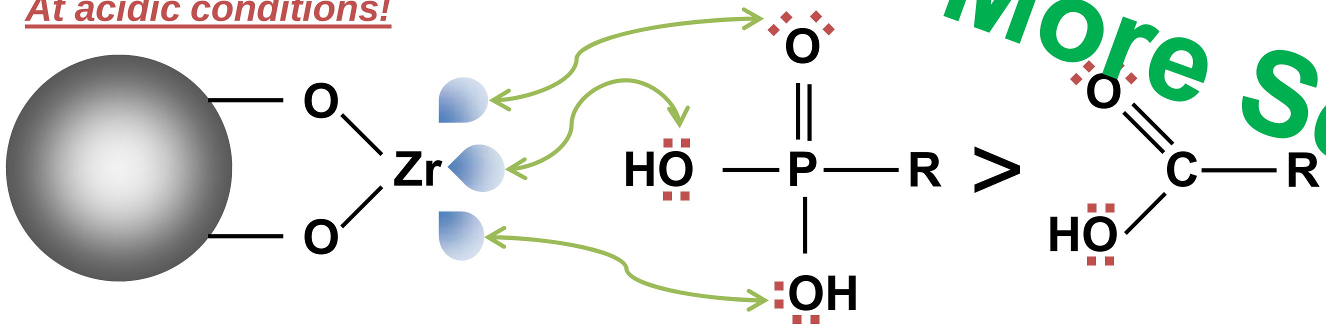
electrostatic interaction

Ligand Exchange



Chemical structure of Glyphosate

At acidic conditions!



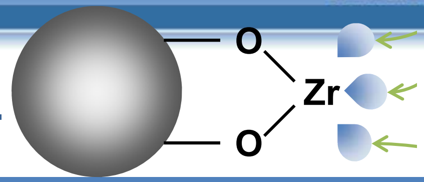
More Selective!

Lewis-Acid-Base-Interaction

Phosph(on)ate

Carbonic acid

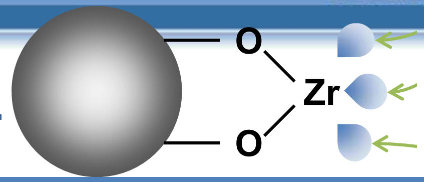
Selected substance affinities towards Zirconia [1]:



phosph(on)ates > tricarbonic acids > dicarbonic acids > carbonic acids >>> monovalent anions

[1] Blackwell and Carr, Development of an Eluotropic Series for the Chromatography of Lewis Bases on Zirconium Oxide, Anal. Chem., 1992, 64, 863-873

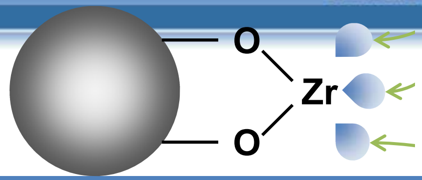
Selected substance affinities towards Zirconia [1]:



phosph(on)ates >	tricarbonic acids >	dicarbonic acids >	carbonic acids >>>	monovalent anions
e.g. phosphate, phospholipids	e. g. fruit acids		e.g. gallic acid and salicylic acid derivatives	e.g. chloride, nitrate

[1] Blackwell and Carr, Development of an Eluotropic Series for the Chromatography of Lewis Bases on Zirconium Oxide, Anal. Chem., 1992, 64, 863-873

Selected substance affinities towards Zirconia [1]:

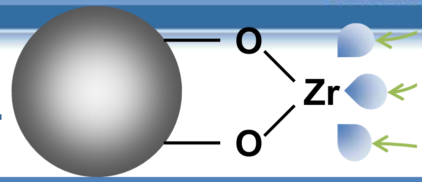


phosph(on)ates >	tricarbonic acids >	dicarbonic acids >	carbonic acids >>>	monovalent anions
e.g. phosphate, phospholipids	e. g. fruit acids		e.g. gallic acid and salicylic acid derivatives	e.g. chloride, nitrate

do not bind substantially

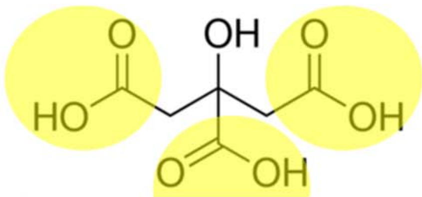
[1] Blackwell and Carr, Development of an Eluotropic Series for the Chromatography of Lewis Bases on Zirconium Oxide, Anal. Chem., 1992, 64, 863-873

Selected substance affinities towards Zirconia [1]:

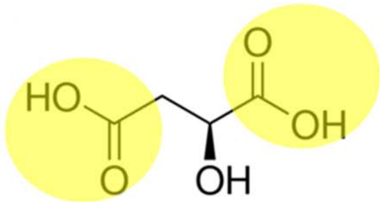


phosph(on)ates >	tricarbonic acids >	dicarbonic acids >	carbonic acids >>>	monovalent anions
e.g. phosphate, phospholipids	e. g. fruit acids		e.g. gallic acid and salicylic acid derivatives	e.g. chloride, nitrate

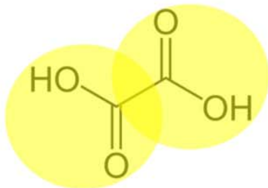
do not bind substantially



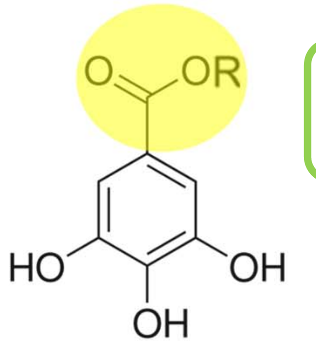
Citric acid
approx.47,000 ppm/kg lemon



Malic acid
approx.12,500 ppm/kg rhubarb



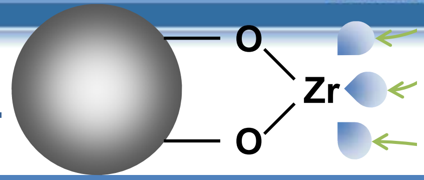
Oxalic acid
approx. 5,000 ppm/kg rhubarb



Gallic acid
approx. 10,000 ppm/kg Green Tea

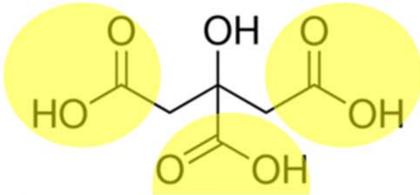
[1] Blackwell and Carr, Development of an Eluotropic Series for the Chromatography of Lewis Bases on Zirconium Oxide, Anal. Chem., 1992, 64, 863-873

Selected substance affinities towards Zirconia [1]:

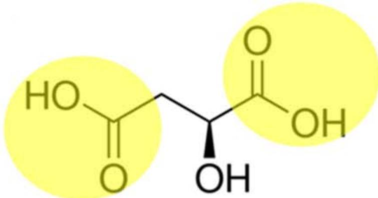


phosph(on)ates >	tricarbonic acids >	dicarbonic acids >	carbonic acids >>>	monovalent anions
e.g. phosphate, phospholipids	e. g. fruit acids		e.g. gallic acid and salicylic acid derivatives	e.g. chloride, nitrate

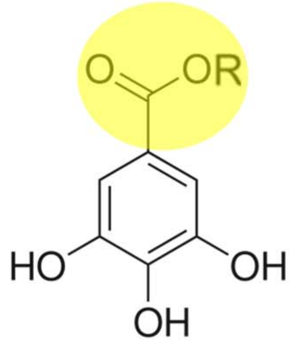
do not bind substantially



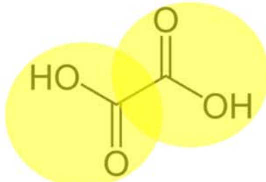
Citric acid
approx.47,000 ppm/kg lemon



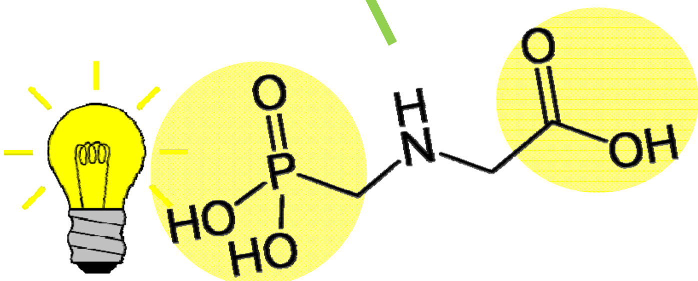
Malic acid
approx.12,500 ppm/kg rhubarb



Gallic acid
approx. 10,000 ppm/kg Green Tea



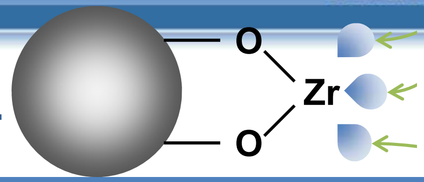
Oxalic acid
approx. 5,000 ppm/kg rhubarb



Chemical structure of Glyphosate

[1] Blackwell and Carr, Development of an Eluotropic Series for the Chromatography of Lewis Bases on Zirconium Oxide, Anal. Chem., 1992, 64, 863-873

Selected substance affinities towards Zirconia [1]:



phosph(on)ates > tricarbonic acids > dicarbonic acids > carbonic acids >>> monovalent anions

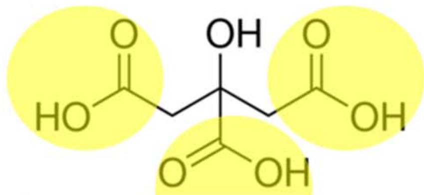
e.g. phosphate, phospholipids

e. g. fruit acids

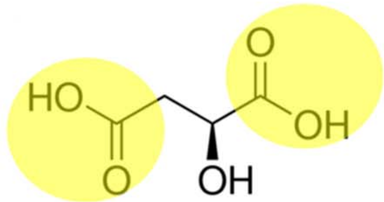
e.g. gallic acid and salicylic acid derivatives

e.g. chloride, nitrate

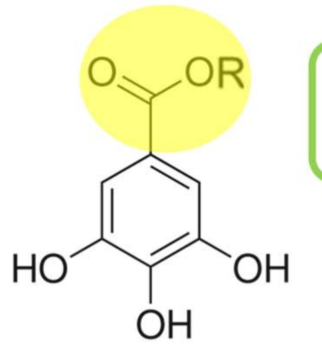
do not bind substantially



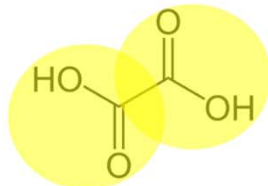
Citric acid
approx.47,000 ppm/kg lemon



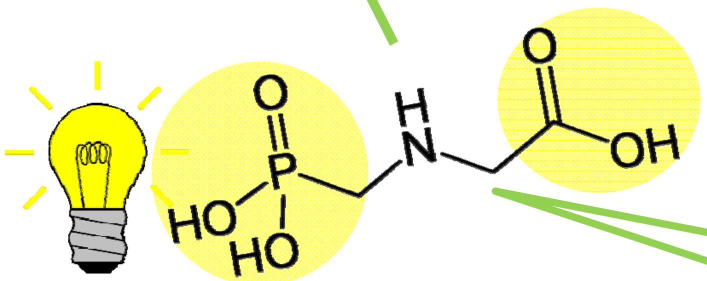
Malic acid
approx.12,500 ppm/kg rhubarb



Gallic acid
approx. 10,000 ppm/kg Green Tea



Oxalic acid
approx. 5,000 ppm/kg rhubarb



Chemical structure of Glyphosate

Satisfactory (absolute) recoveries even in presence of concurring matrix compounds

[1] Blackwell and Carr, Development of an Eluotropic Series for the Chromatography of Lewis Bases on Zirconium Oxide, Anal. Chem., 1992, 64, 863-873

Adsorption Behaviour on Zircona depending on pH [2]:

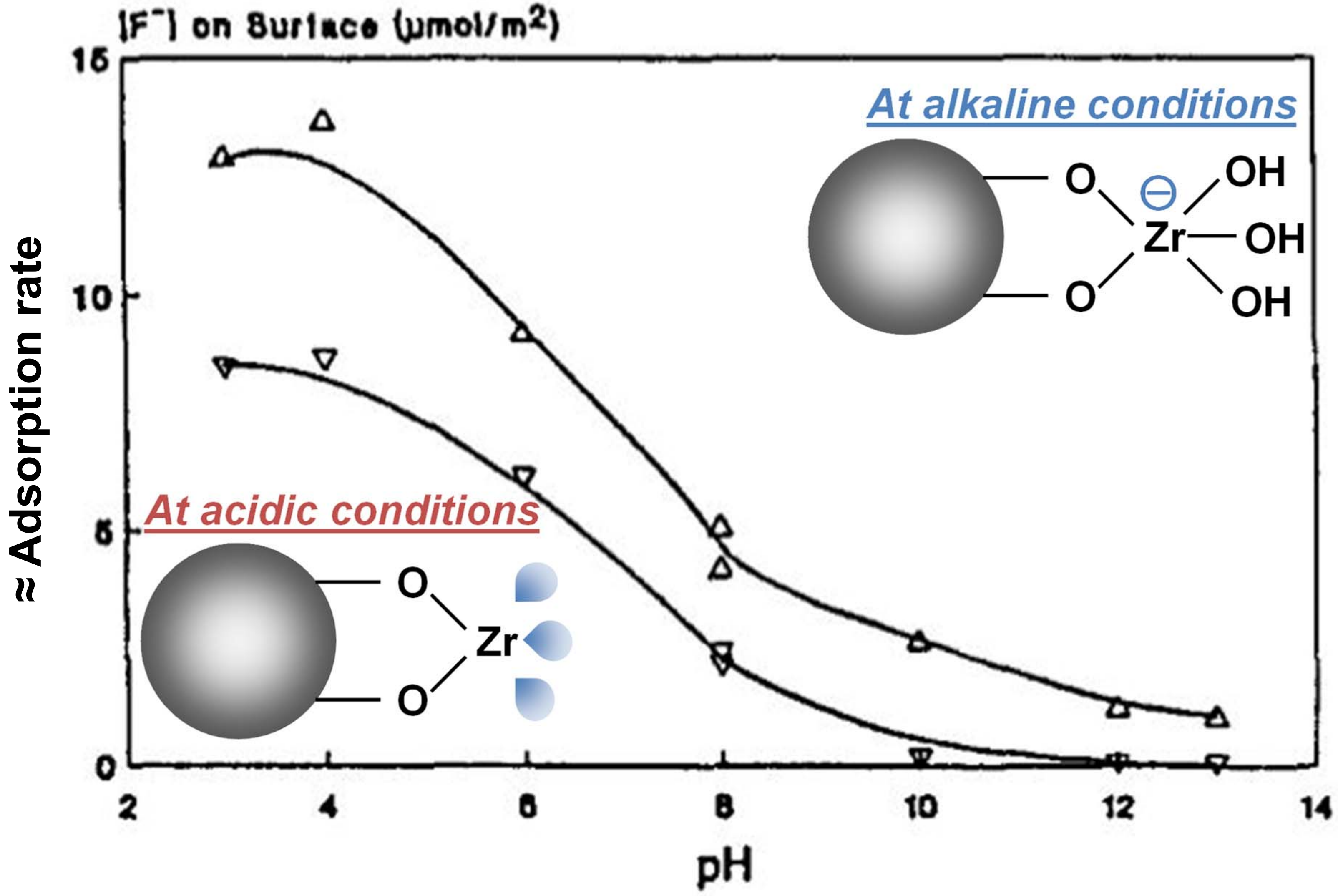


Fig. 42. Fluoride adsorption as a function of pH. Δ=

[2] Nawrocki,J., Rigney, M.P, McCormick,A., Carr,P.W.; Chemistry of zirconia and ist use in chromatography; Journal of Chromatography A, 657 (1993), 229-282

Adsorption Behaviour on Zircona depending on pH [2]:

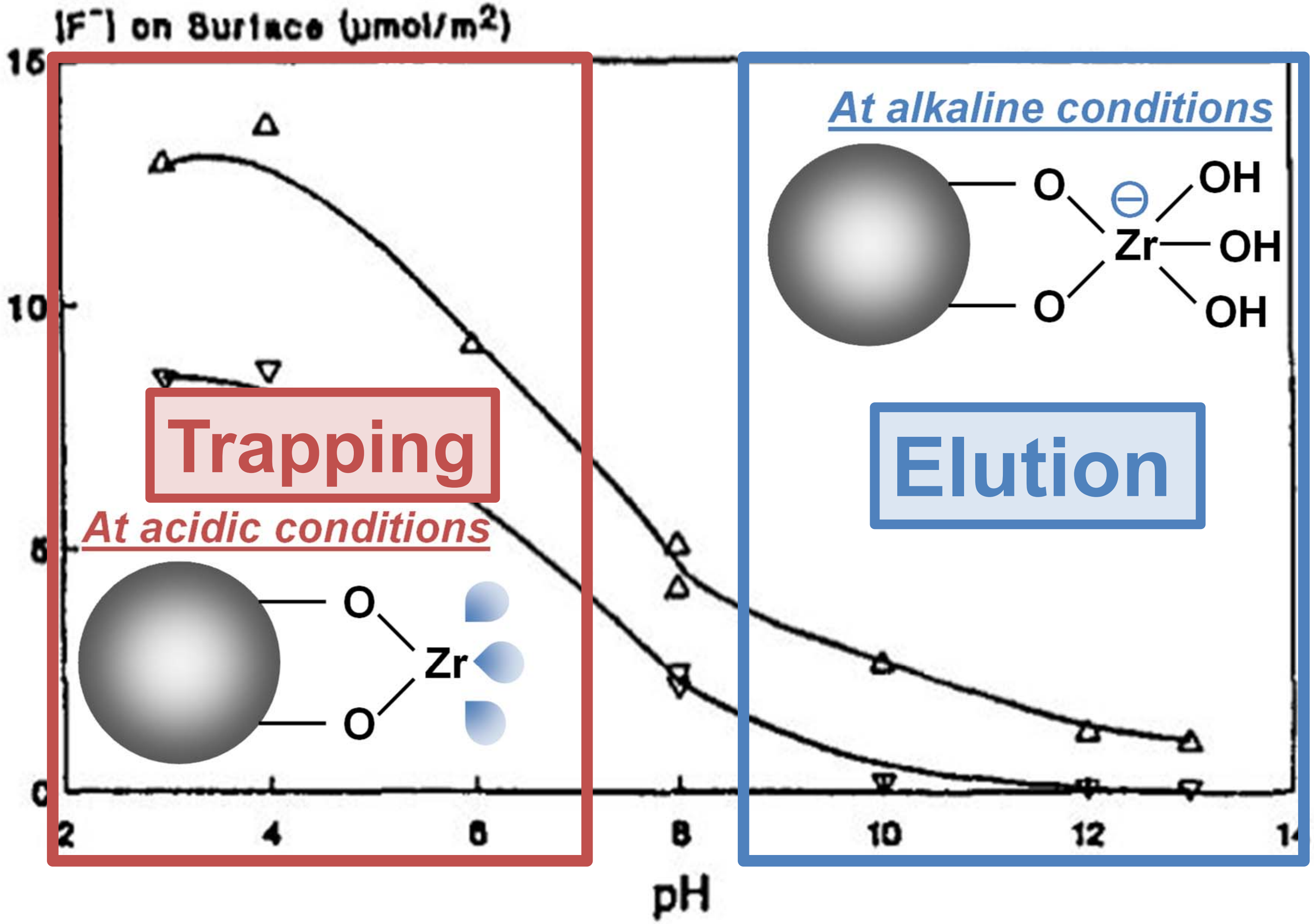
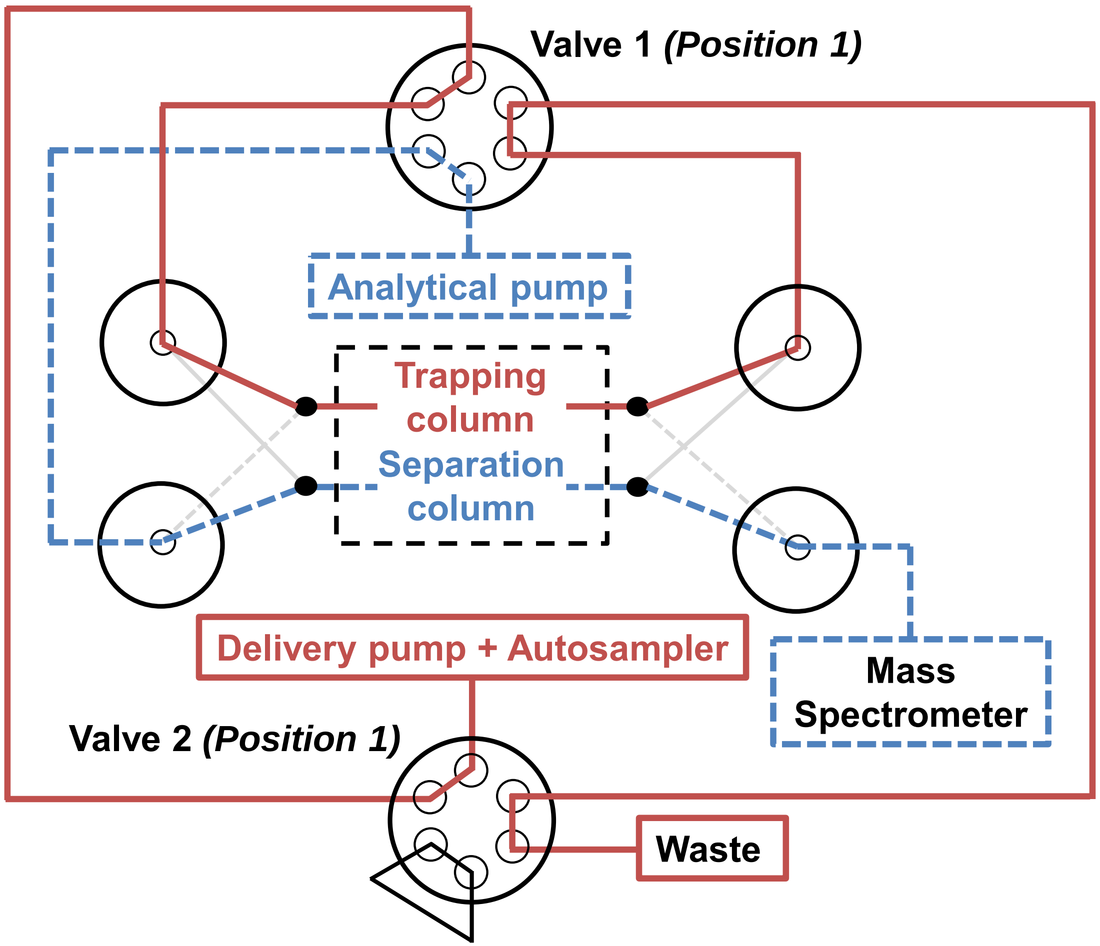


Fig. 42. Fluoride adsorption as a function of pH. Δ =

[2] Nawrocki,J., Rigney, M.P, McCormick,A., Carr,P.W.; Chemistry of zirconia and ist use in chromatography; Journal of Chromatography A, 657 (1993), 229-282

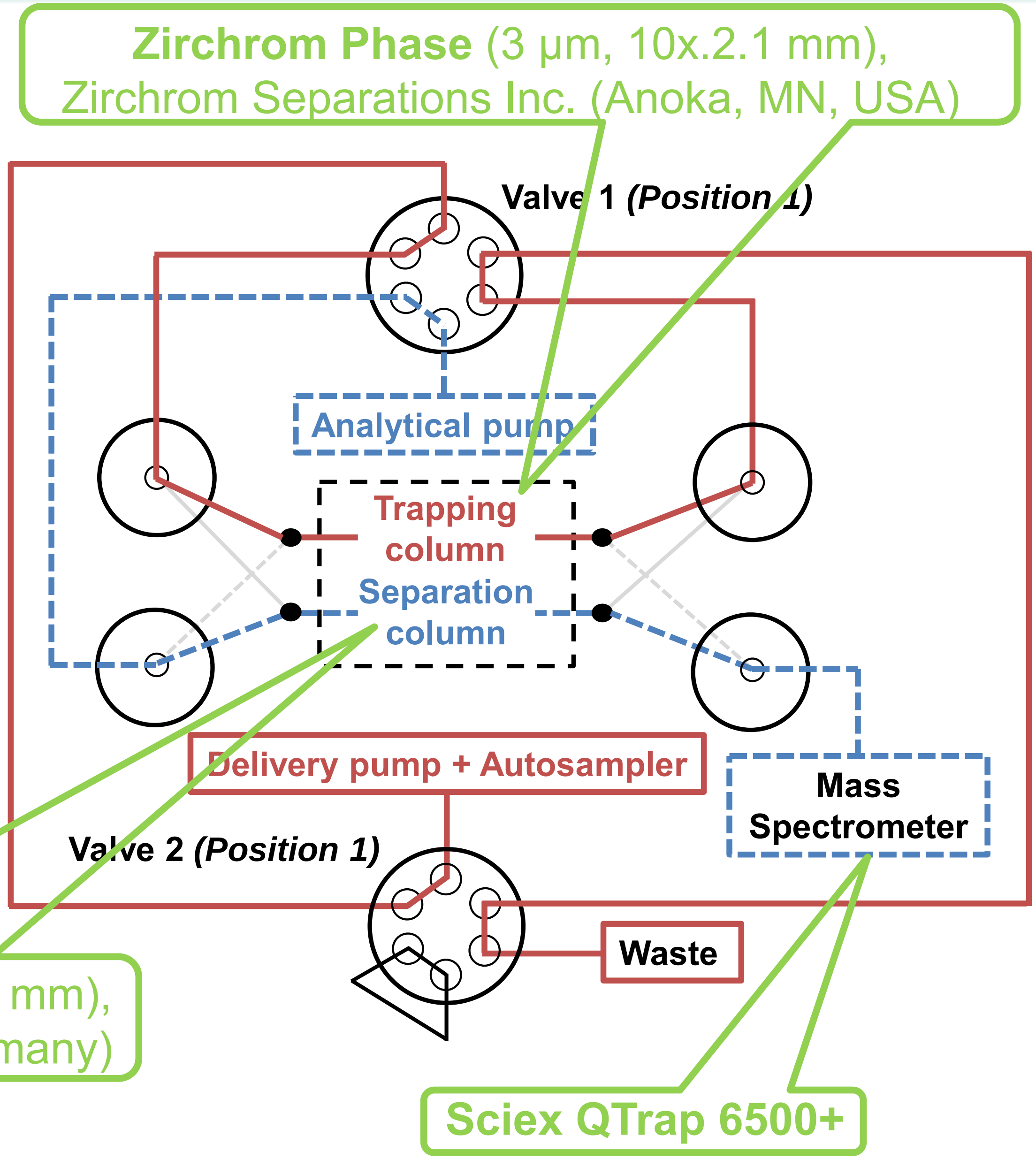
System Set-up and used Eluents

Step 1	***Sample Trapping***
Run time	**0.0-5.0 min**
Delivery Pump	50 mM Formic acid in Water/Methanol 1/1 (v/v)
Analytical Pump	A: 5 % Acetonitrile in Water B: 50 mM NH_4OH in 5 % Acetonitrile in Water
Valve 1	Position 1
Valve 2	Position 1



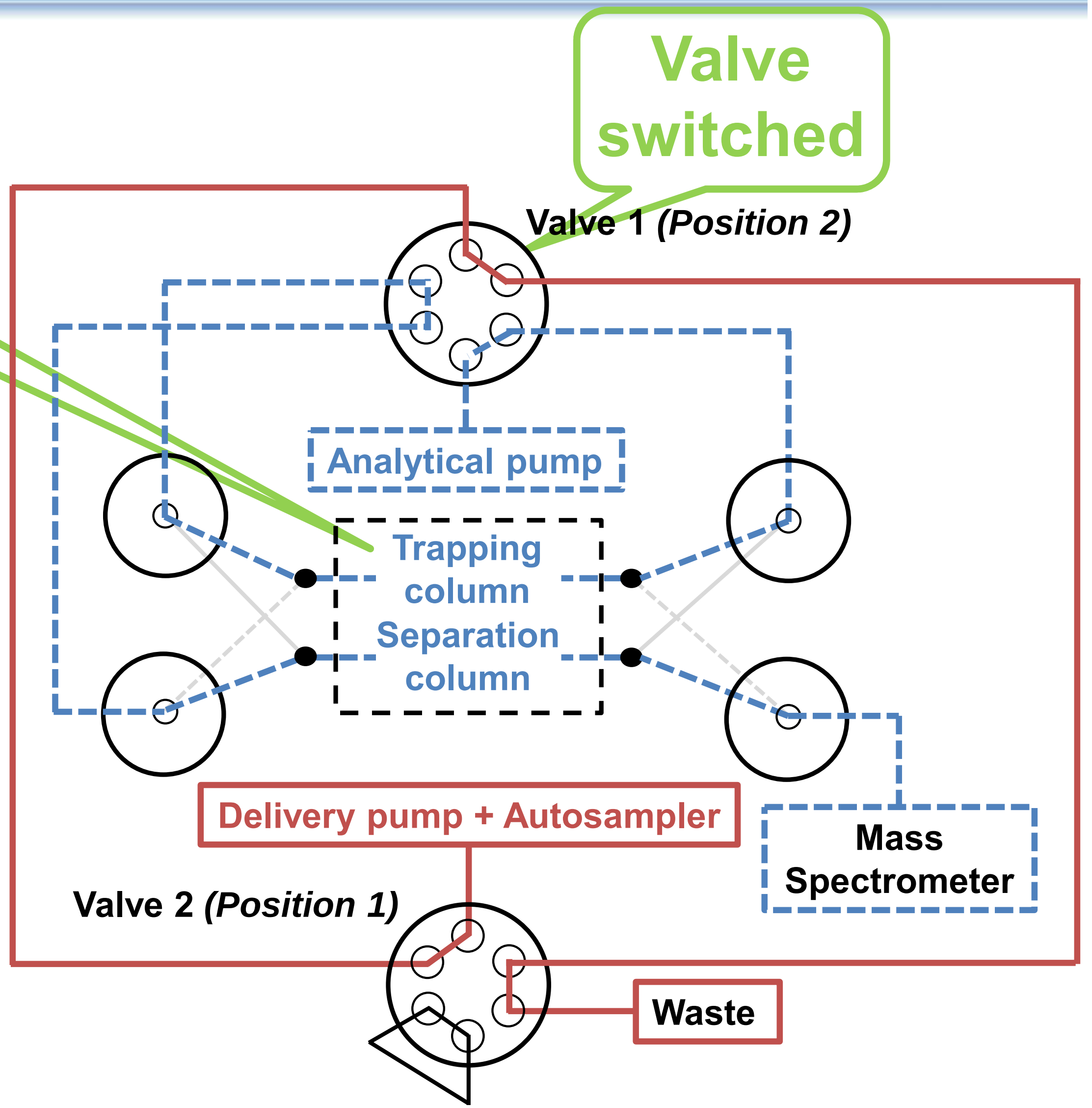
System Set-up and used Eluents

Step 1	***Sample Trapping***
Run time	**0.0-5.0 min**
Delivery Pump	50 mM Formic acid in Water/Methanol 1/1 (v/v)
Analytical Pump	A: 5 % Acetonitrile in Water B: 50 mM NH_4OH in 5 % Acetonitrile in Water
Valve 1	Position 1
Valve 2	Position 1



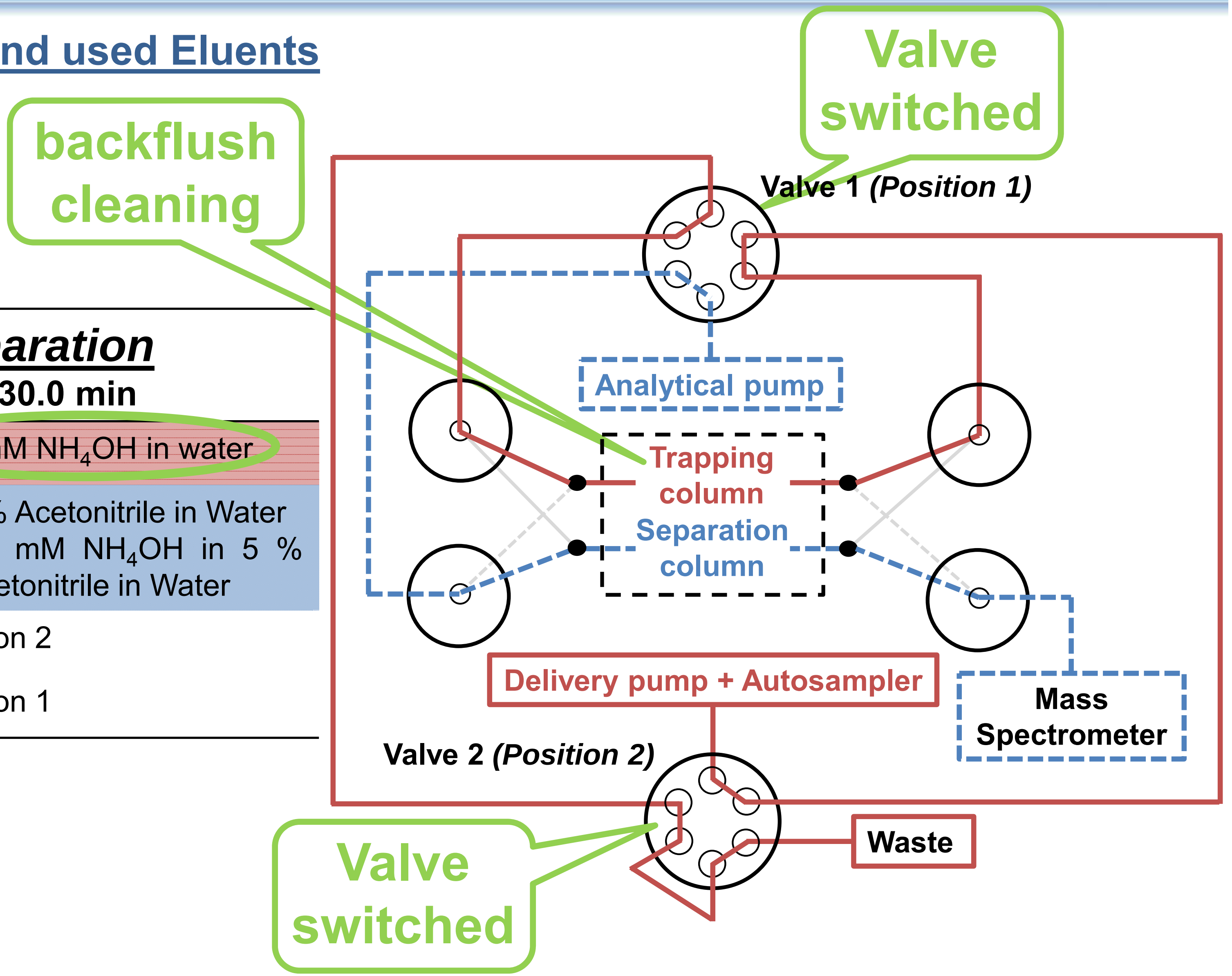
System Set-up and used Eluents

Step 2	***Elution***
Run time	**5.0-15.0 min**
Delivery Pump	100 mM NH_4OH in water
Analytical Pump	A: 5 % Acetonitrile in Water B: 50 mM NH_4OH in 5 % Acetonitrile in Water
Valve 1	Position 2
Valve 2	Position 1

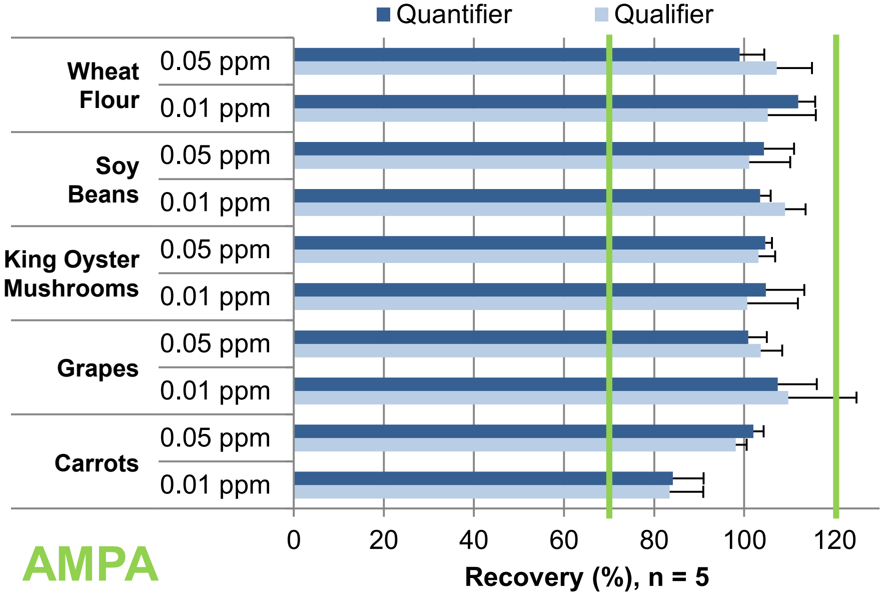


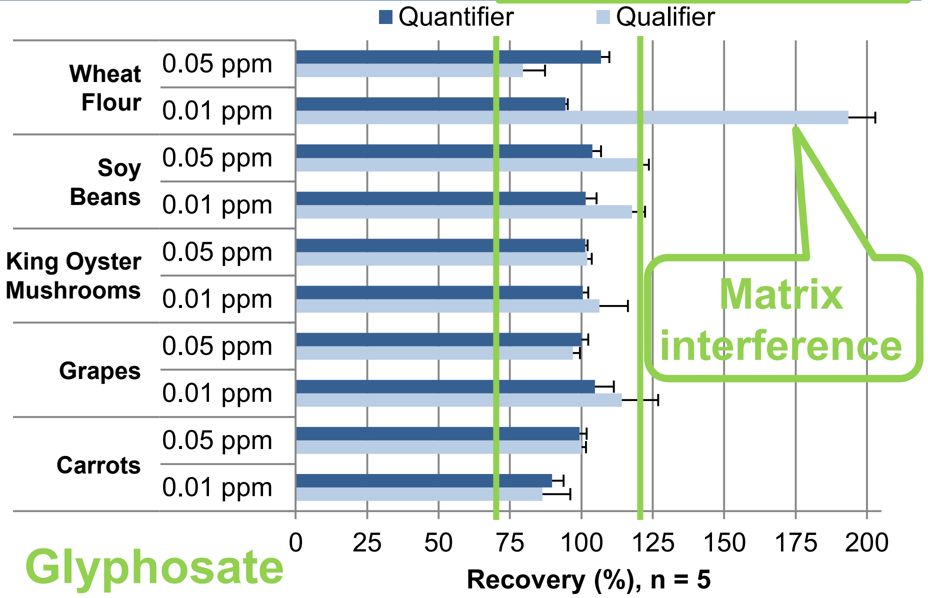
System Set-up and used Eluents

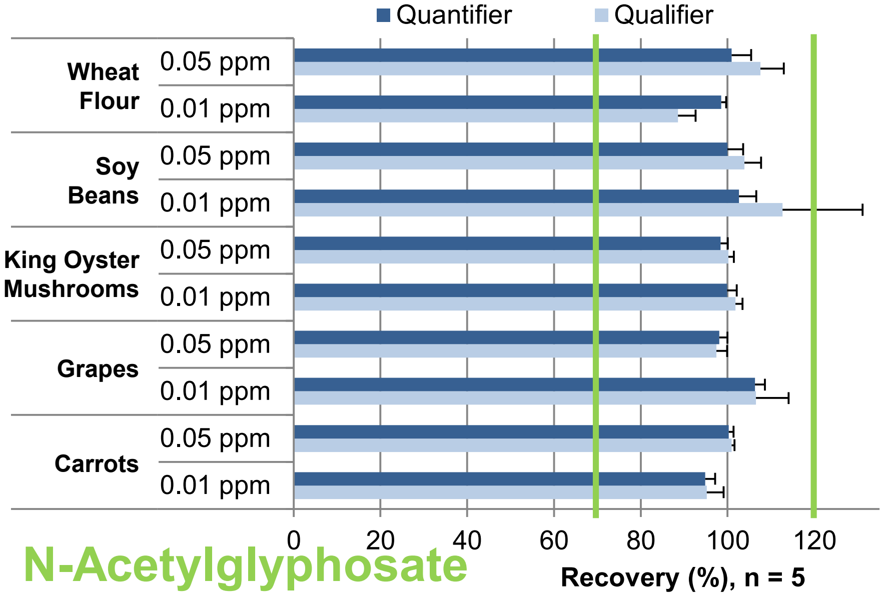
Step 3 **Run time**	***Separation*** **15.1-30.0 min**
Delivery Pump	100 mM NH_4OH in water
Analytical Pump	A: 5 % Acetonitrile in Water B: 50 mM NH_4OH in 5 % Acetonitrile in Water
Valve 1	Position 2
Valve 2	Position 1



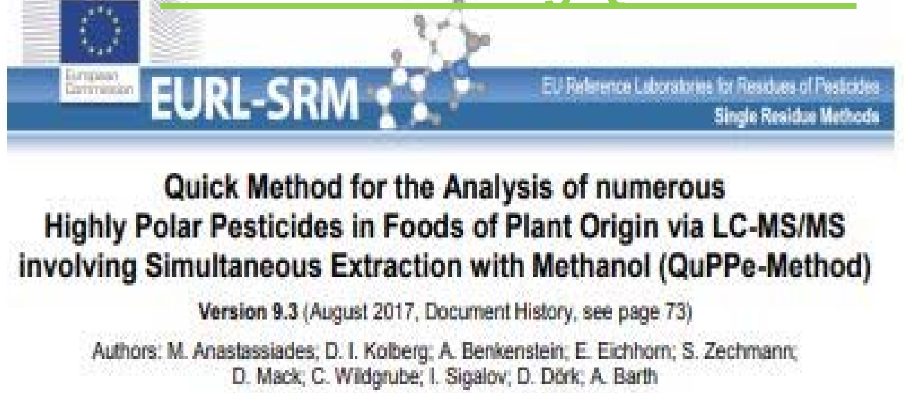
Validation according to SANTE/11945/2015 – Exemplary Data (Matrix Cal+ILIS):







Extraction using QuPPe 9.3

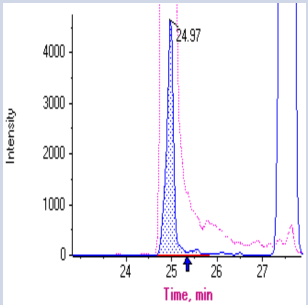
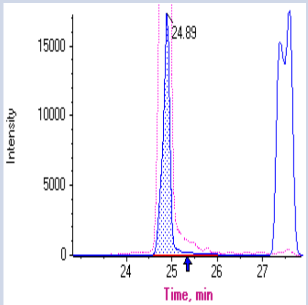
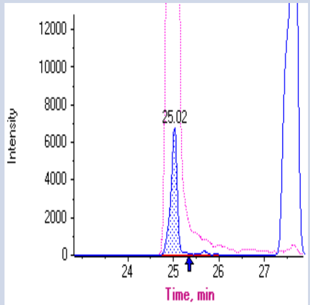
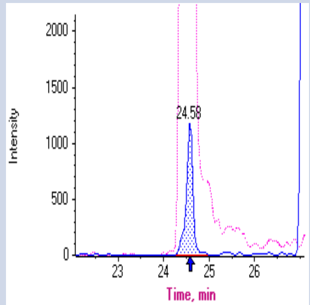
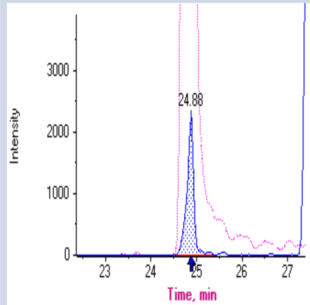
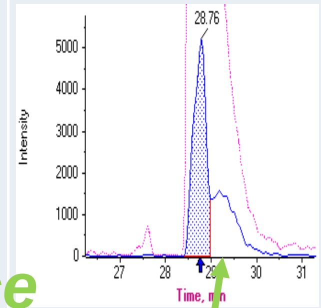
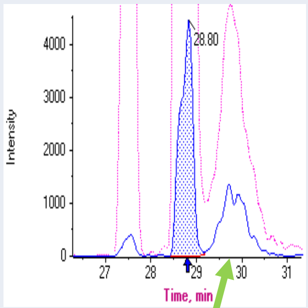
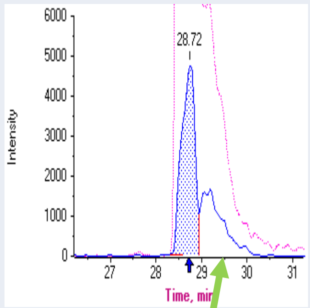
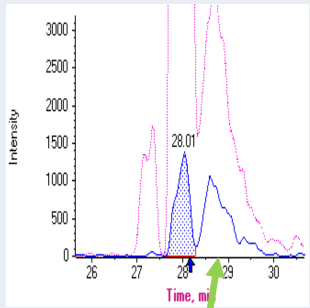
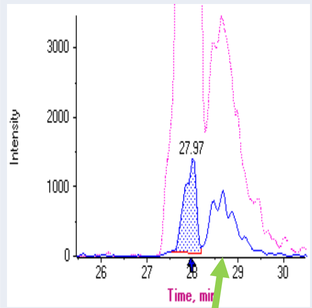
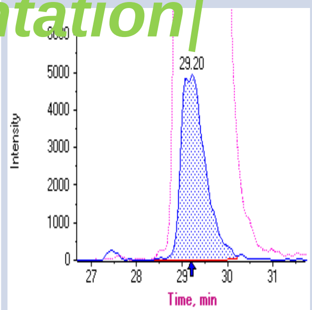
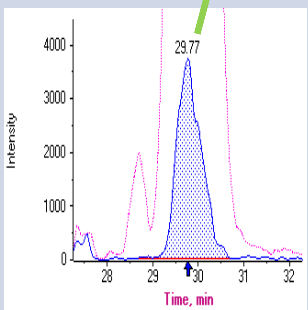
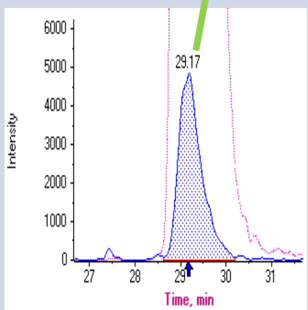
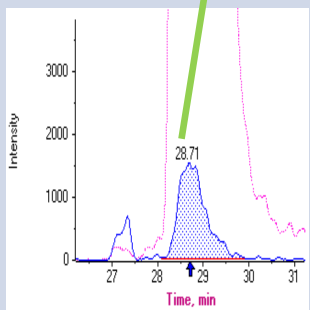
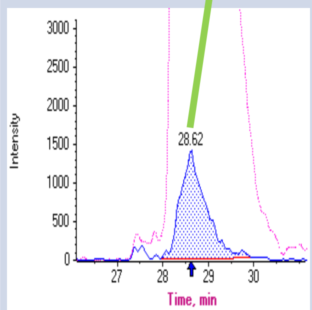

European Commission
EURL-SRM
EU Reference Laboratories for Residues of Pesticides
Single Residue Methods

Quick Method for the Analysis of numerous Highly Polar Pesticides in Foods of Plant Origin via LC-MS/MS involving Simultaneous Extraction with Methanol (QuPPe-Method)

Version 9.3 (August 2017, Document History, see page 73)

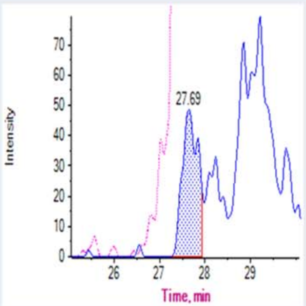
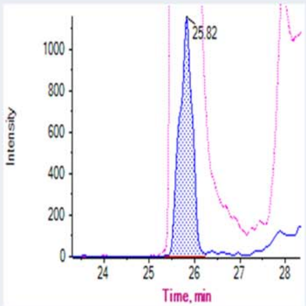
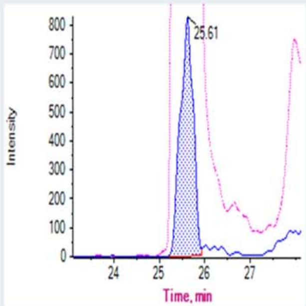
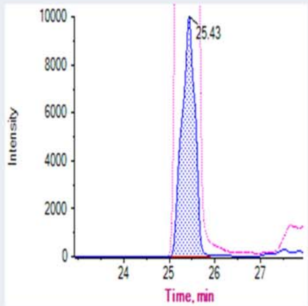
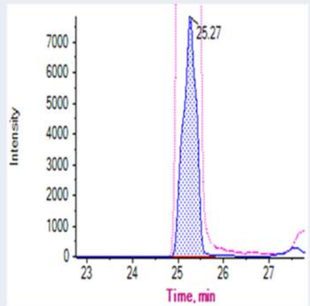
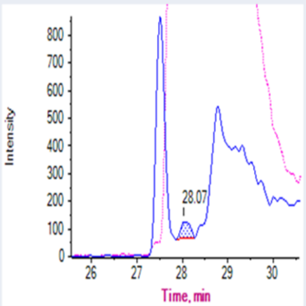
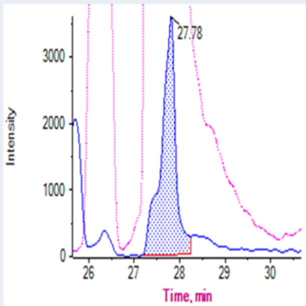
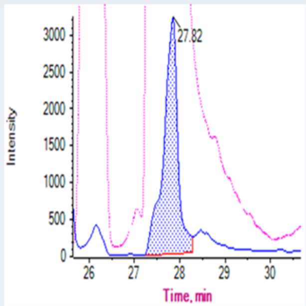
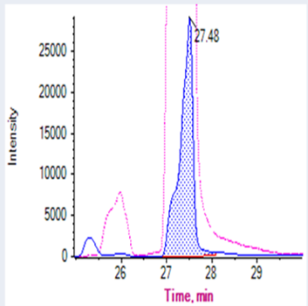
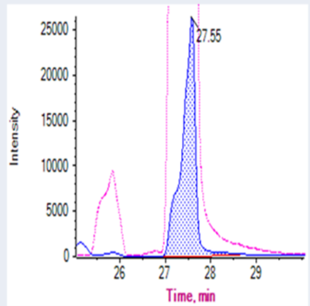
Authors: M. Anastassiades; D. I. Kolberg; A. Benkenstein; E. Eichhorn; S. Zechmann; D. Mack; C. Wildgrube; I. Sigalov; D. Dörk; A. Barth

Validation according to SANTE/11945/2015 – Exemplary Chromatograms:

Recovery 0.01 ppm	Carrots	King Oyster Mushrooms	Grapes	Soy Beans	Whole Wheat Flour
AMPA					
Glyphosate					
N-Acetyl-glyphosate					

In-source Fragmentation

Determination of Green Tea Samples:

All results n = 2	Blank	Level 10 ppb		Level 100 ppb	
		Matrix calibration	*Recovery (ILIS corrected)*	*Matrix calibration*	*Recovery (ILIS corrected)*
AMPA *m/z 110/63*					
	matrix effect* -34 %		***73 %***		***79 %***
Glyphosate *m/z 110/63*					
	matrix effect* +36 %		***89 %***		***95 %***

$$* \, matrix \; effect = 100\,\% \times \frac{area \; matrix \; calibration}{area \; solvent \; calibration} - 100\,\%$$

Thank you for your attention!

Questions to EURL-SRM@cvuas.bwl.de

Special thanks:

Pesticide Team of the CVUA Stuttgart

Ellen Scherbaum
Head of Department

Michelangelo Anastassiades
Head of EURL-SRM