European Commission

EURL-SRM

EU Reference Laboratories for Residues of Pesticides

Single Residue Methods

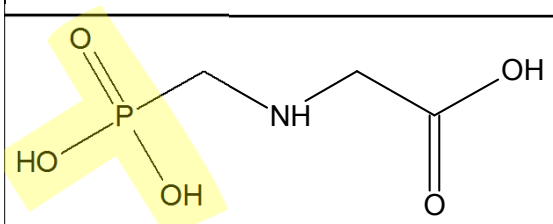
Selective Analysis of Glyphosate and other polar organo-phosphorous Compounds in Foods of plant Origin using on-line ligand-exchange SPE-HPLC-MS/MS

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

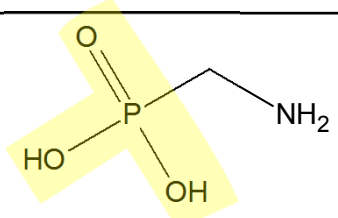
Eric Eichhorn
Anne Benkenstein
Cristin Wildgrube
Ellen Scherbaum
Michelangelo Anastassiades

12th European Pesticide Residue Workshop
22th-25th May 2018, Munich, Germany

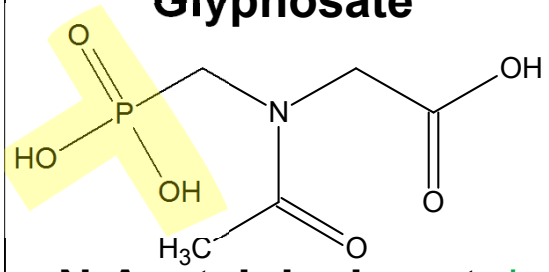
Phosphonic acid group containing



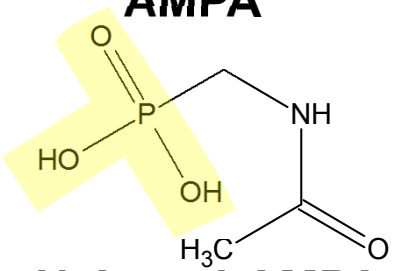
Glyphosate*



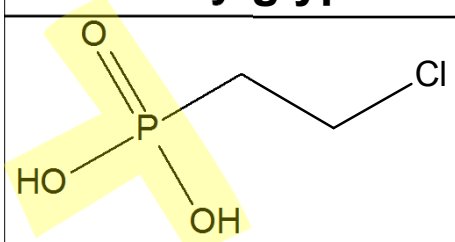
AMPA*



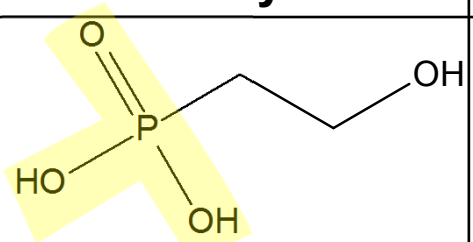
N-Acetylglyphosate*



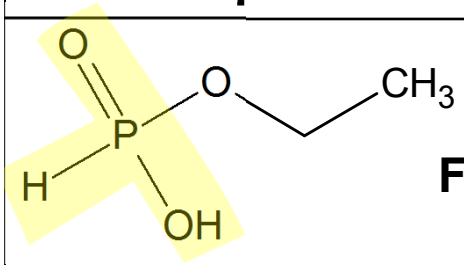
N-Acetyl-AMPA



Ethephon*

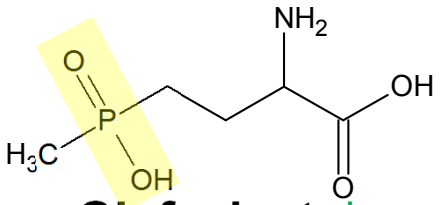


HEPA

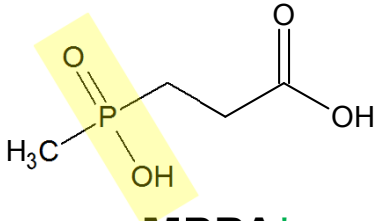


Fosetyl*

Phosphinic acid group containing



Glufosinate*



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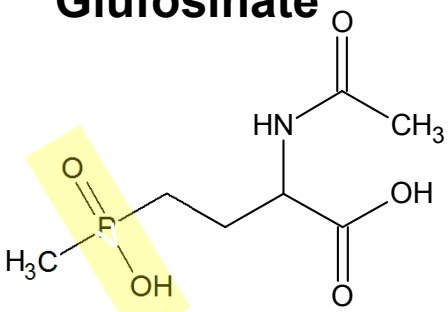
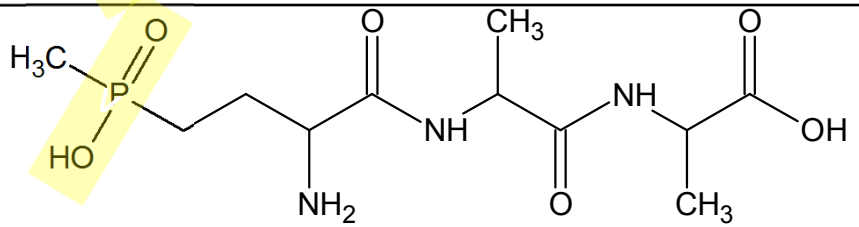


N-Acetylglufosinate*



Bialaphos

* Included in the actual Residue Definition
or currently discussed to be included

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

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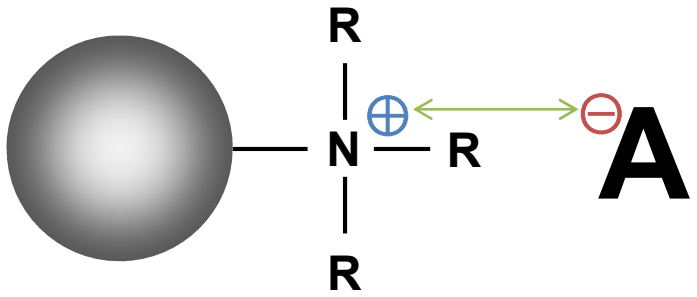
Anion Exchange



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

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

electrostatic interaction

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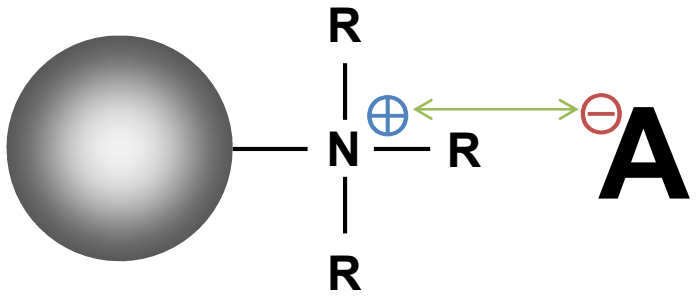

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Non-selective!

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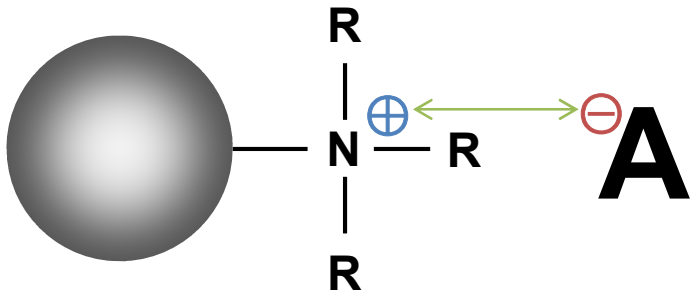

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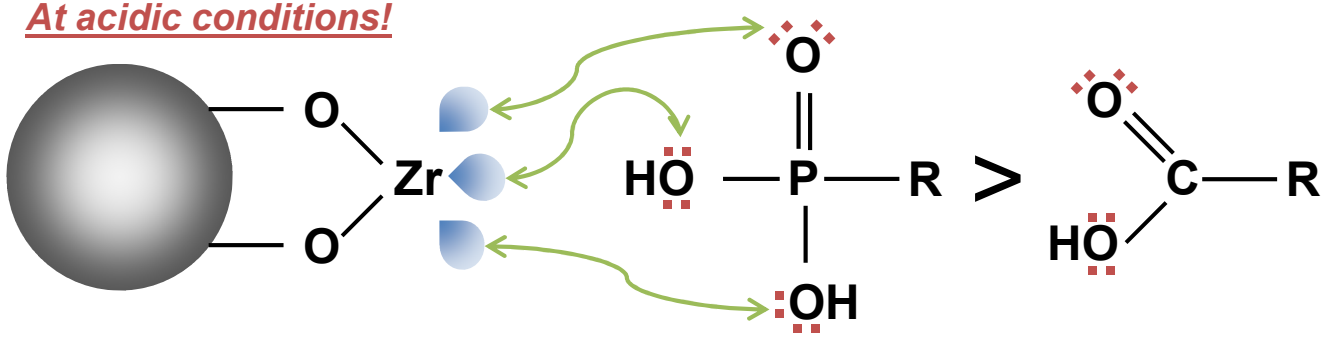
Non-selective!

electrostatic interaction

Ligand Exchange

At acidic conditions!



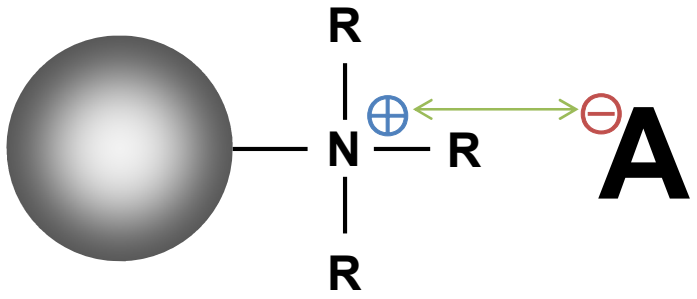
Lewis-Acid-Base-Interaction

Phosph(on)ate

Carbonic acid

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

Anion Exchange



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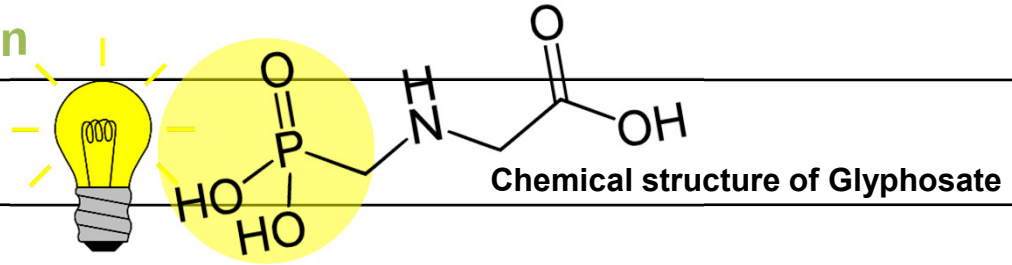
Non-selective!

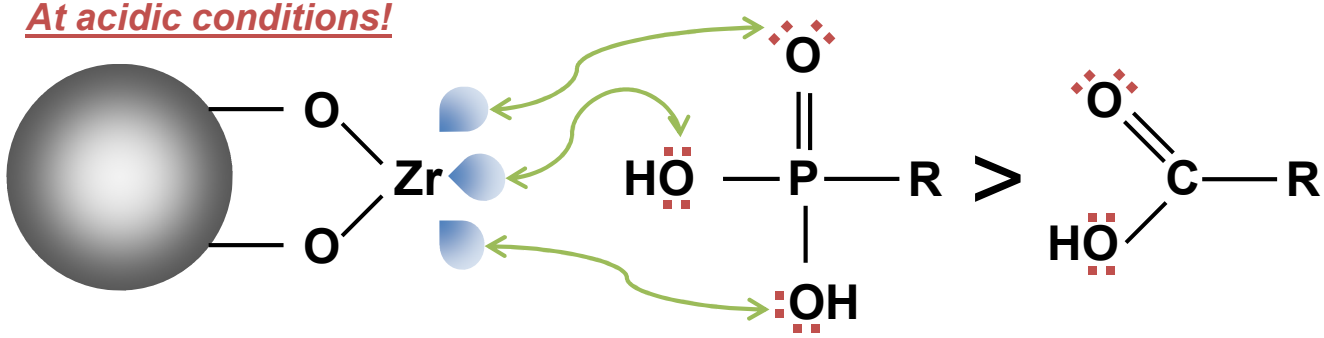
electrostatic interaction

Ligand Exchange



Chemical structure of Glyphosate

At acidic conditions!



Lewis-Acid-Base-Interaction

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Carbonic acid

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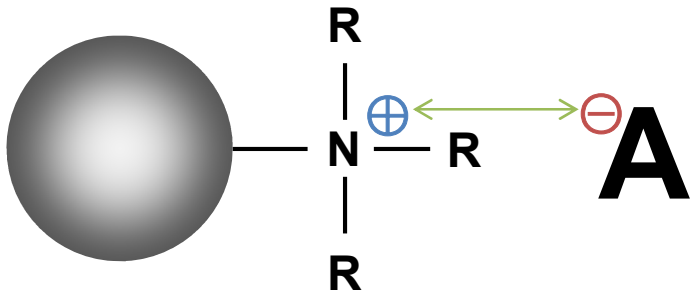
Anion Exchange



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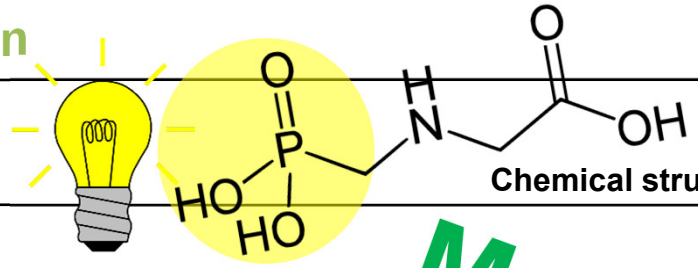
electrostatic interaction

Ligand Exchange



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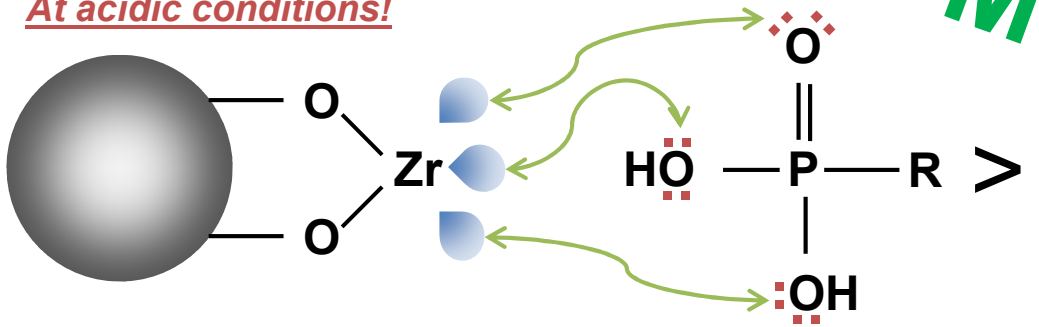


Phosph(on)ate

Carbonic acid

Lewis-Acid-Base-Interaction

More Selective!

Adsorption Behaviour on Zircona depending on pH:

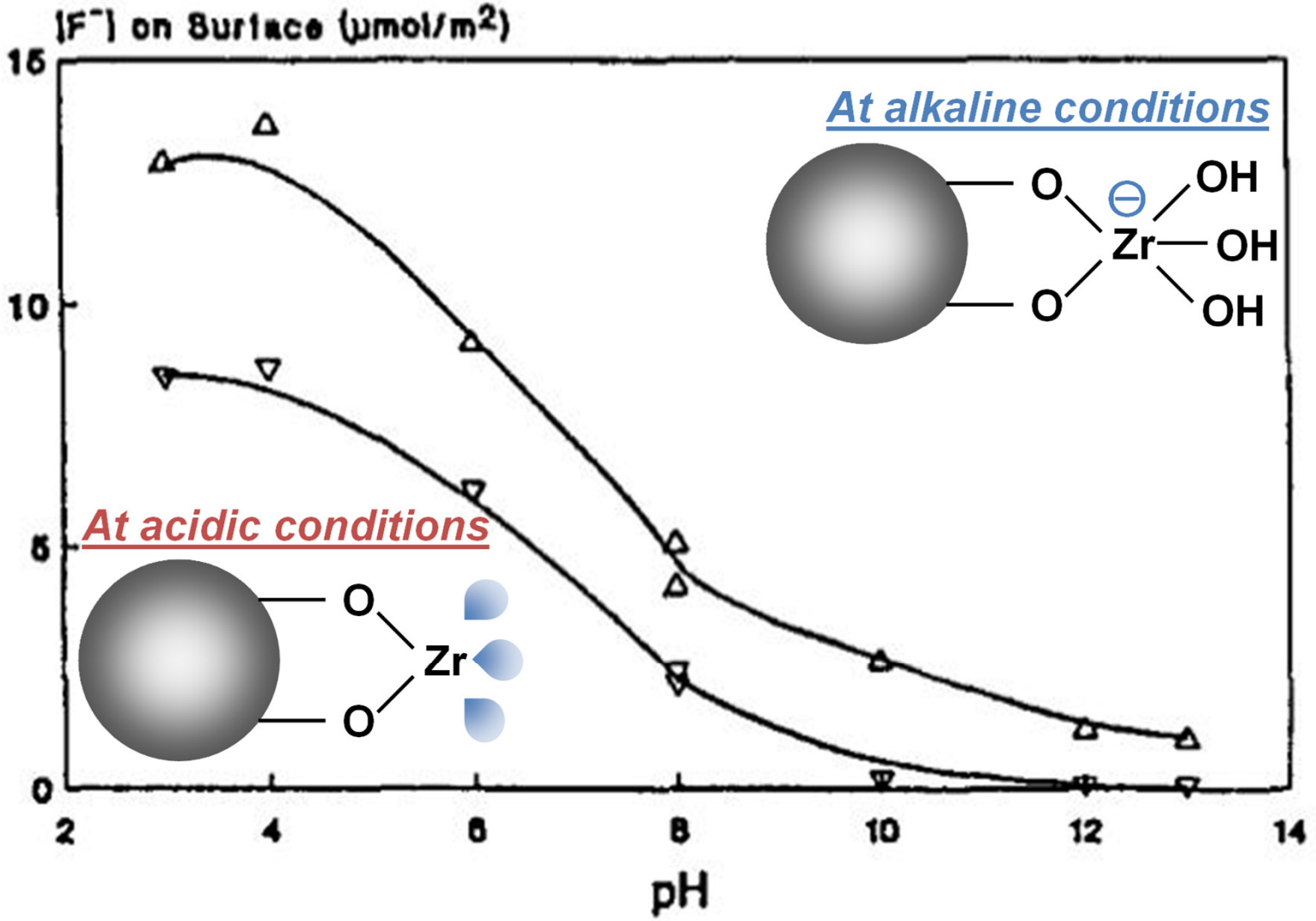
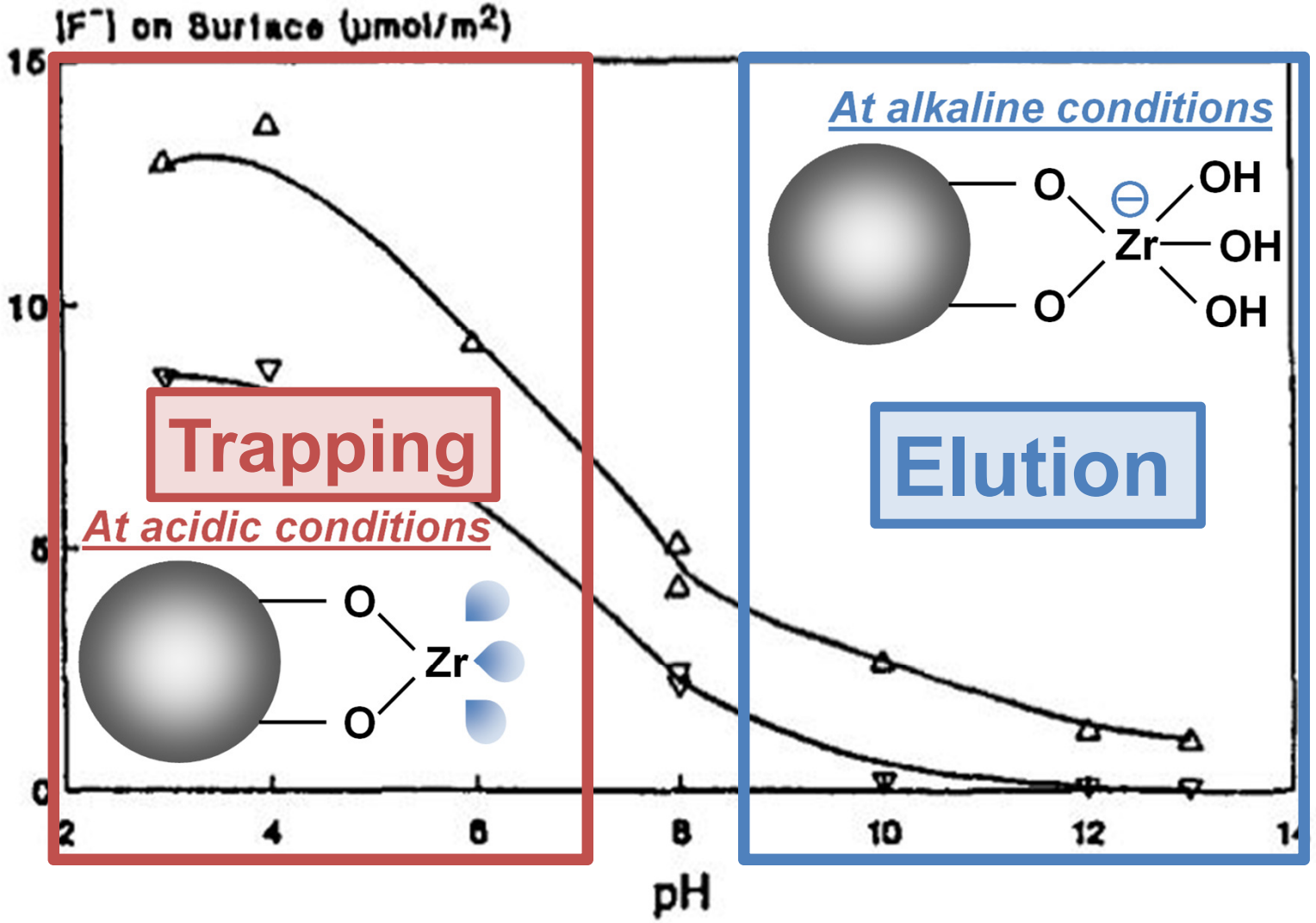


Fig. 42. Fluoride adsorption as a function of pH. Δ =

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:



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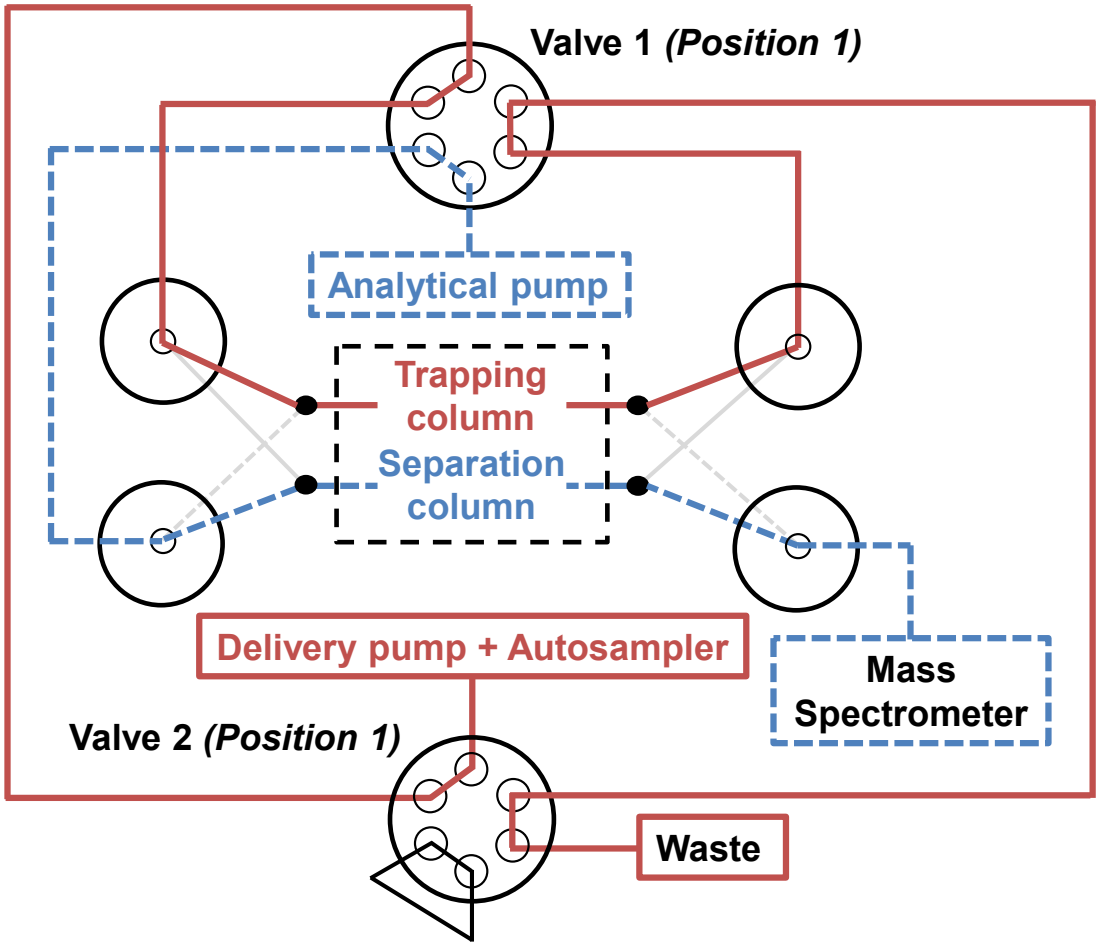
System Set-up and used Eluents

Step 1 **Run time**	***Sample Trapping*** **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



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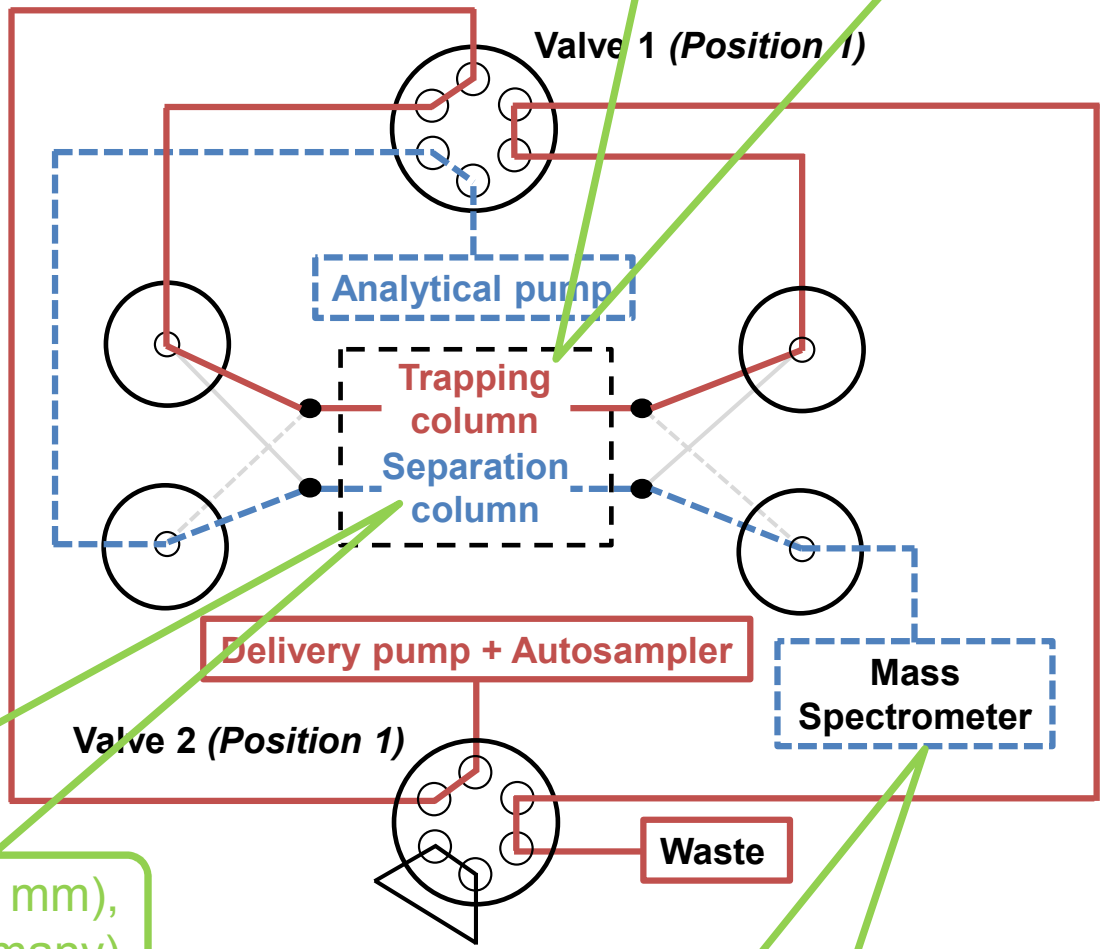
Valve 1	Position 1
Valve 2	Position 1

Zirchrom Phase (3 µm, 10x.2.1 mm), Zirchrom Separations Inc. (Anoka, MN, USA)

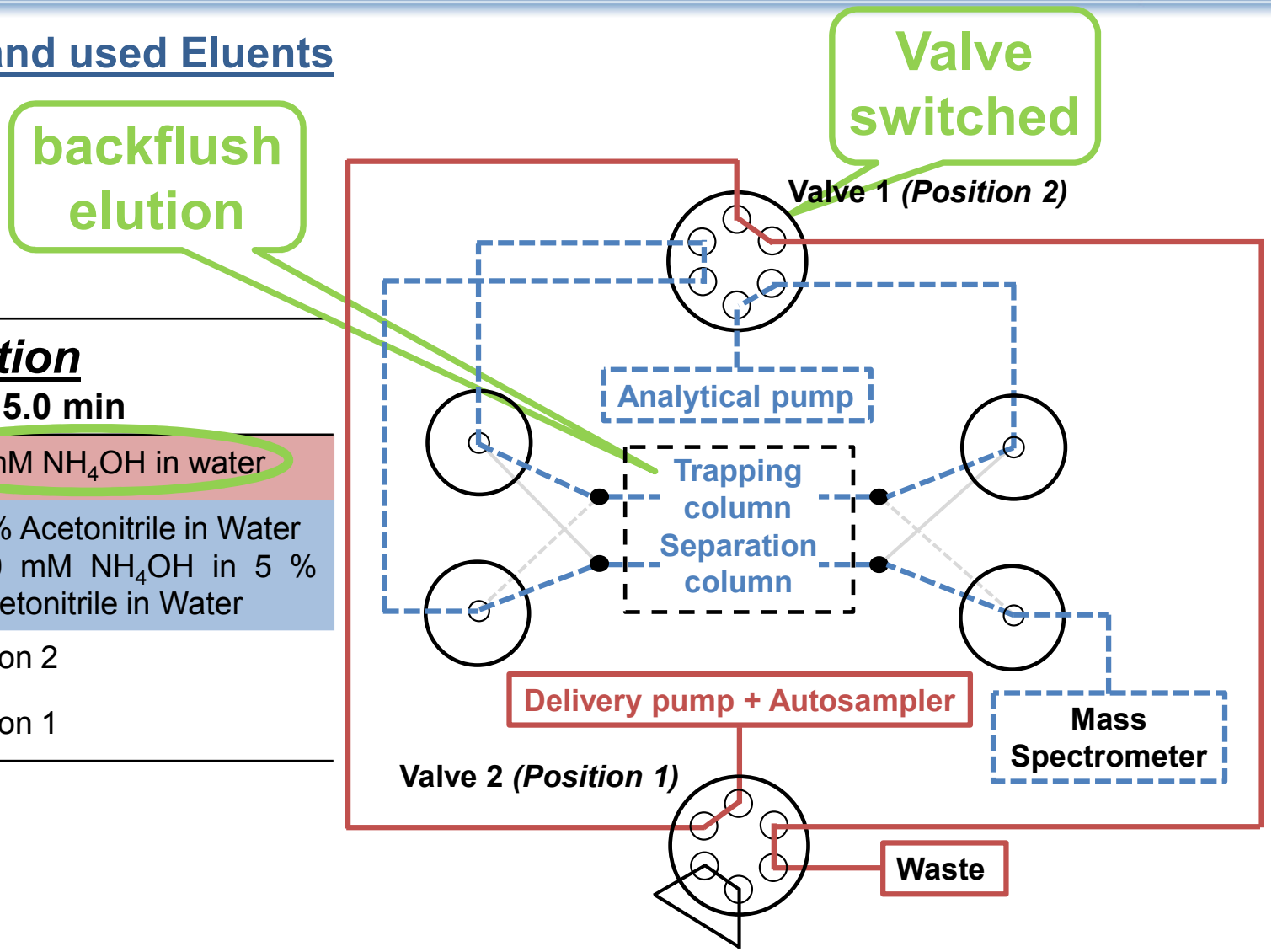


Asahipak NH2P-4D (5 µm, 4.6x150 mm), Showa Denko Europe (Munich, Germany)

Sciex QTrap 6500+

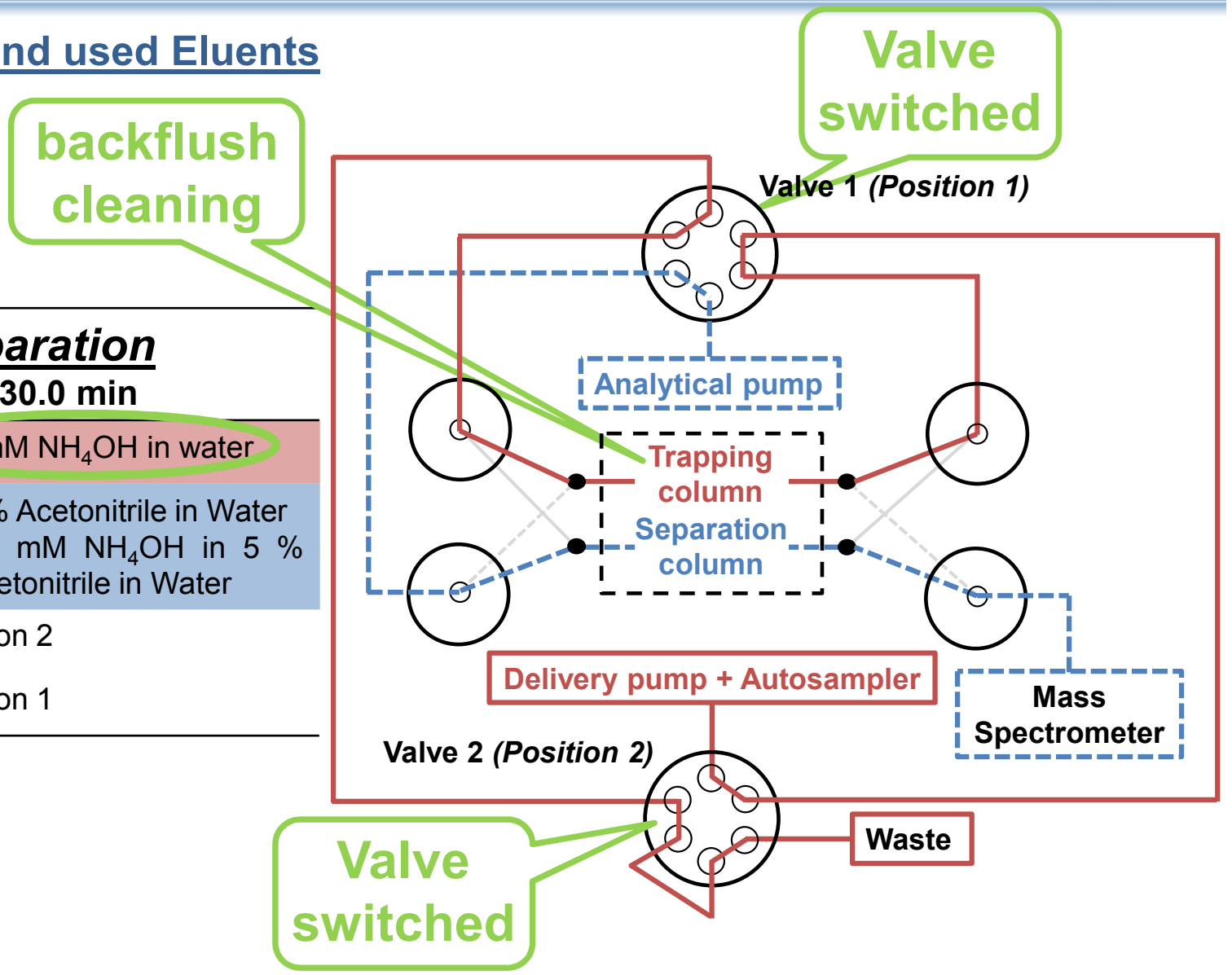
System Set-up and used Eluents

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

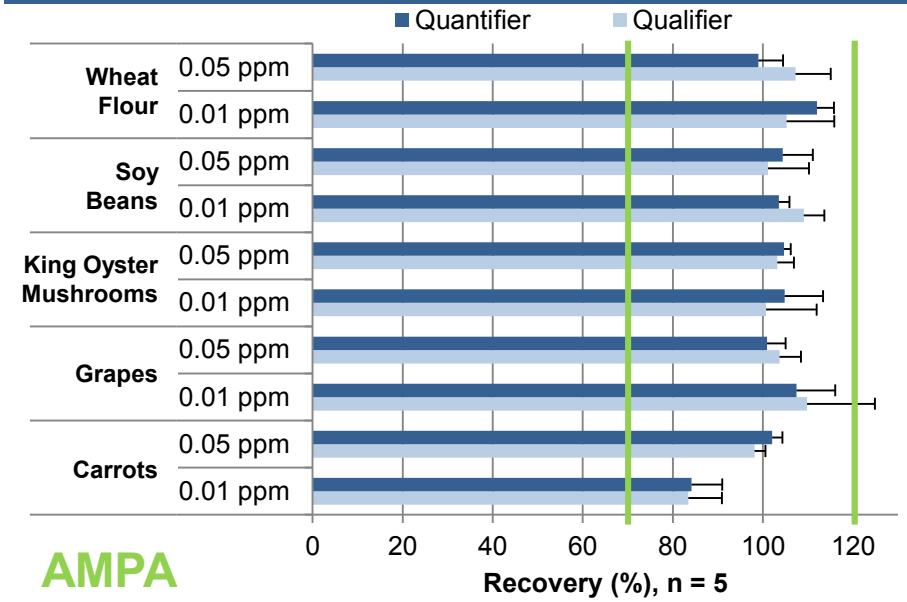


System Set-up and used Eluents

Step 3	***Separation***
Run time	**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):



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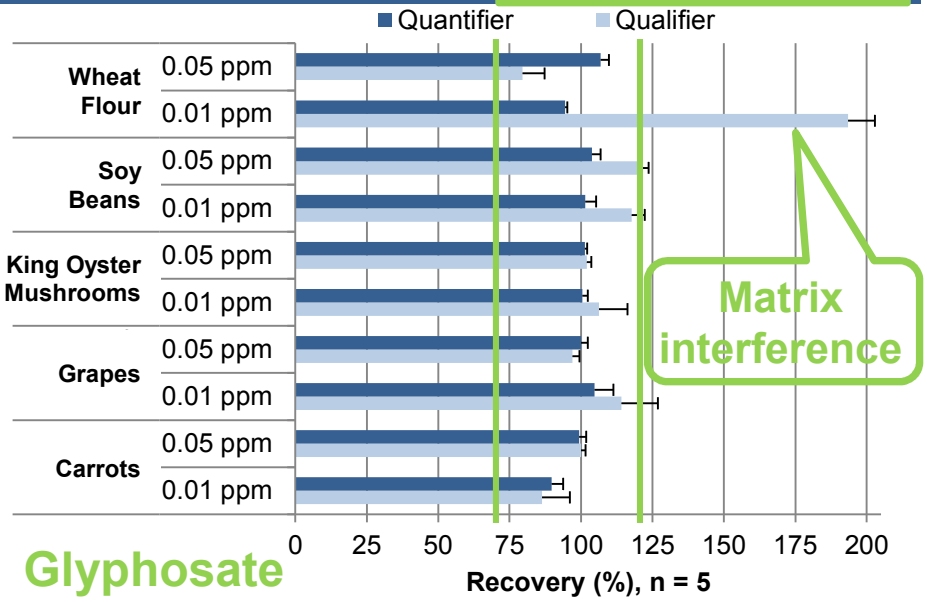
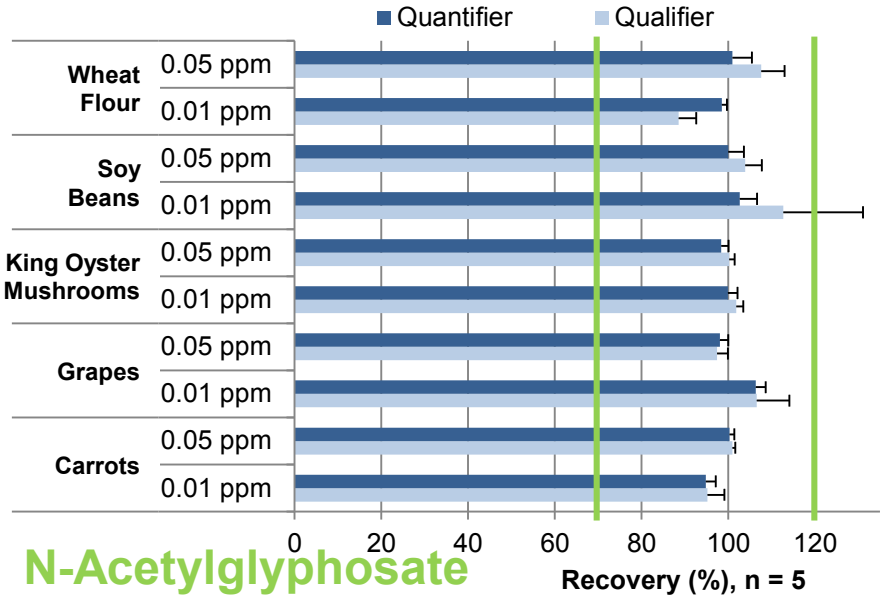
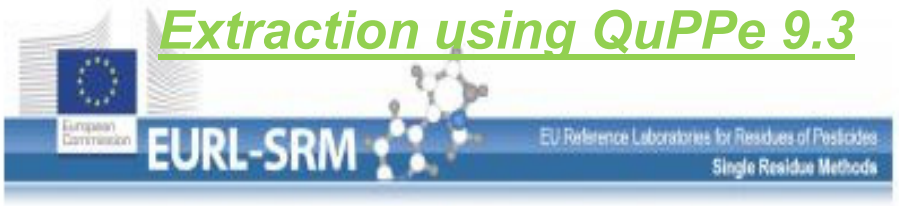


Glyphosate



N-Acetylglyphosate

Extraction using QuPPe 9.3

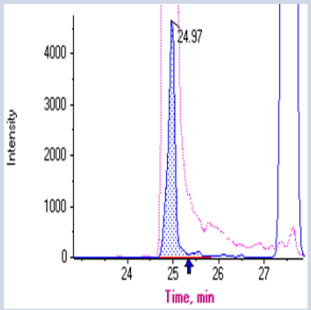
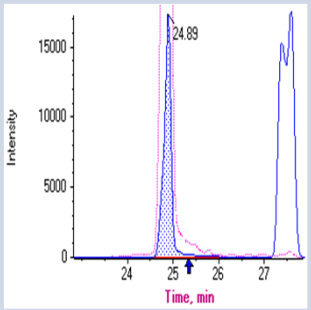
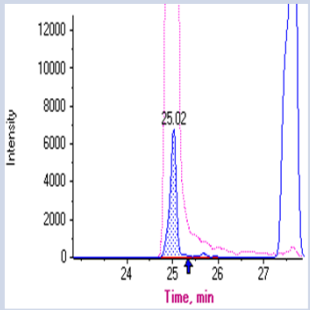
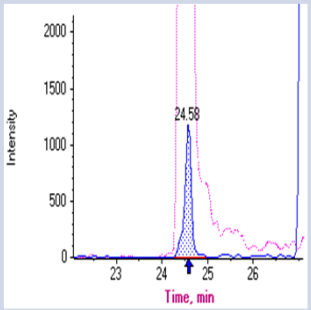
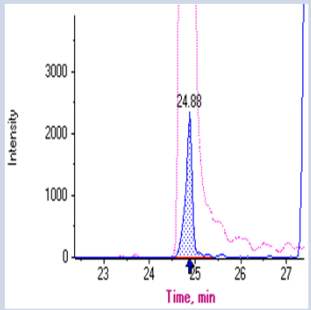
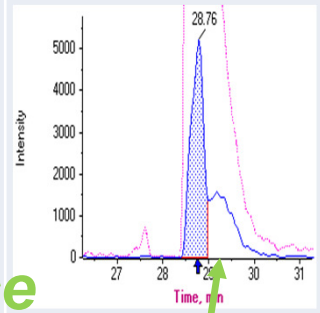
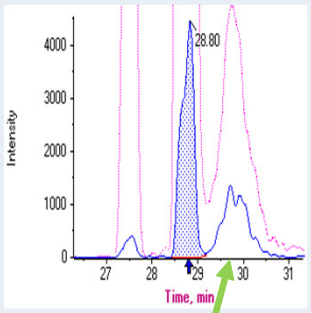
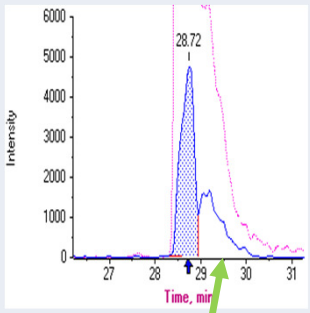
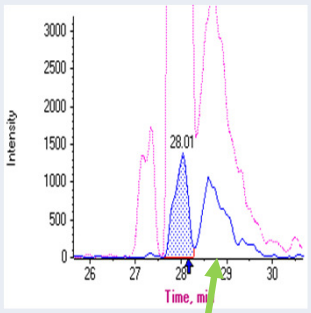
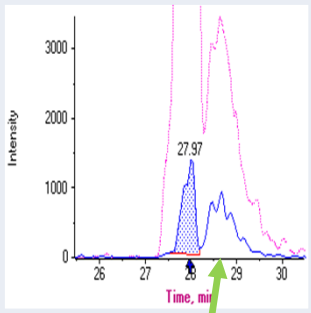
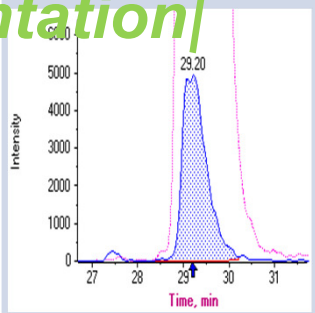
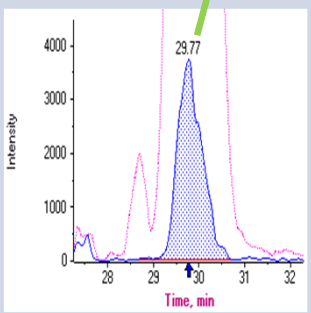
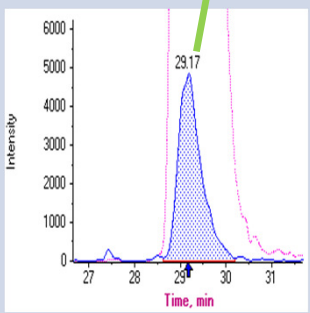
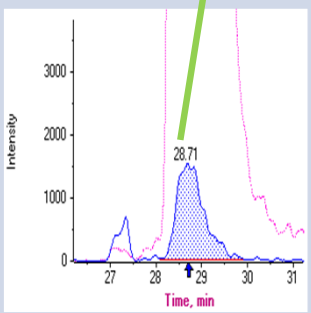
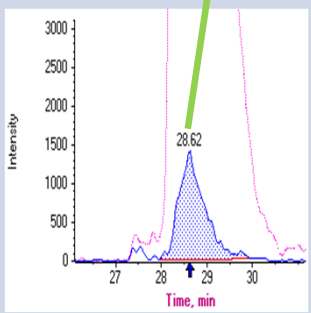


Quick Method for the Analysis of numerous Highly Polar Pesticides in Foods of Plant Origin via LC-MS/MS involving Simultaneous Extraction with Methanol (QuPPe-Method)

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

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