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Solving challenges associated with the UPLC-MS/MS analysis of polar analytes in foodstuffs

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Introduction

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- Goal of multi-residue analyses determine as many residues as possible in the smallest number of analyses
  - Generic extraction, no/limited cleanup, highly selective determination step (GC- and LC-MS/MS or HRMS)
  - A number of different very successful implementations
    - *e.g*. QuEChERS, SweEt, mini Luke…
- Some polar and ionic pesticides and metabolites are NOT "amenable" to common multi-residue methods
  - Need alternative conditions for extraction and LC retention/separation
- Historically treated as a series of selective single residue methods (SRM) adding significant costs so were often excluded from surveillance
- Now many included in surveillance programs as mandatory
  - *e.g.* Europe (national and EU coordinated), India (export certification)

Examples of polar and ionic pesticides

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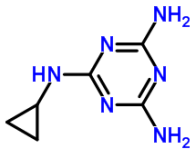
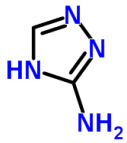
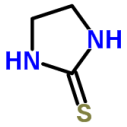
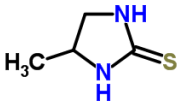
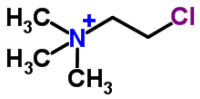
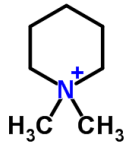
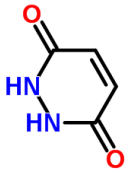
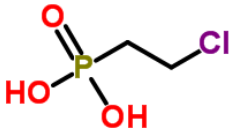
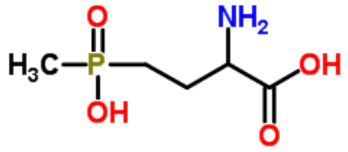
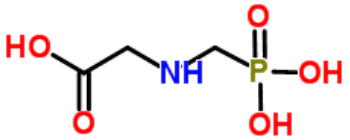
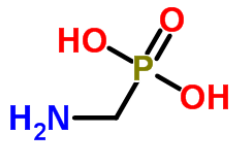
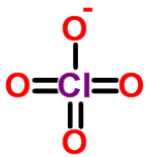
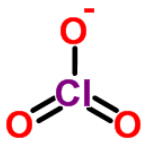
Anionic/acidic	Cationic/basic
Ethephon	Cyromazine
Glufosinate	Amitrole
Glyphosate	Ethylenethiourea
Phosphonic acid	Propylenethiourea
Fosetyl-Aluminium	Chlormequat
Maleic hydazide	Mepiquat
Perchlorate	Diquat
Chlorate	Paraquat
Plus metabolites	Plus metabolites

A selection of polar/ionic pesticides

- Cyromazine 
- Amitrole 
- Ethylenethiourea 
- Propylenethiourea 
- Chlormequat 
- Mepiquat 
- Maleic hydazide 

- Ethephon 
- Glufosinate 
- Glyphosate 
- Aminomethylphosphonic acid (AMPA) 
- Perchlorate 
- Chlorate 

Glyphosate and glufosinate

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- Extremely widely used herbicides
  - Glyphosate residues common in cereals, rice and pulses and associated finished products such as bread, breakfast cereals and infant foods
    - Detected in 44% of the oats analysed in EU coordinated survey in 2013
  - GM herbicide-resistant crops have a greater likelihood of residues due to repeated spraying of the plants with the herbicide
- Controversy and confusion over carcinogenic risk of glyphosate to humans from exposure through the diet
  - Glyphosate products have been banned in some countries
- Amount of monitoring has increased in Europe
  - Increase in laboratories returning results for glyphosate in EUPT
    - In 2009 (cereal) only 9/153
    - In 2015 (maize flour) 62/110 with 85% results acceptable
      - 54% QuPPe and 30% FMOC

Ethephon

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- Ethephon, 2-chloroethylphosphonic acid, is a plant growth regulator (PGR)
  - It is mainly used to enhance the ripening of fruits and to prevent lodging of cereal crops
- The increased application of PGRs has led to more concerns about their presence in different commodities
- Commonly detected during analysis of home-ground and imported fresh produce (*e.g.* grapes, peppers, tomato and pineapples) including > MRL

Fosetyl-aluminium and phosphonic acid

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- Phosphonic acid is the main metabolite resulting from the use of fosetyl
  - Hence its is included in the residue definition for fosetyl-Al
- The use of other pesticides also leads to the formation of phosphonic acid in treated crops
- Residues also arise from their use as fertilisers and as biostimulants
- The default MRL for fosetyl-Al in agricultural products where there is no approval was set to 2 mg/kg in January 2016
- MRLs on crops where there is approval are much higher
  - *e.g.* Citrus fruits 75 mg/kg fosetyl-Al (sum of fosetyl, phosphonic acid and their salts, expressed as fosetyl)

Chlorate and perchlorate

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- The issue of residues of perchlorate and chlorate on fresh produce within the EU is not related to their use as pesticides but is likely to be derived from contamination from fertilizer and disinfectants used for washing fresh crops
  - Although now banned, chlorate and perchlorate used to be approved as plant protection products so a default MRL of 0.01 mg/kg was applied
  - Lots of residues were reported > 0.01 mg/kg, which were impacting trade
  - Lack of consistent enforcement
  - Resulted in setting of higher levels for perchlorate just for intra-community trade
    - Not the same as maximum limits
  - Discussions ongoing at EU aimed at setting more realistic MRLs for chlorate
  - For baby food, a maximum residue limit of 0.01 mg/kg for ready-to-eat food products is still applicable, irrespective of the source of the residue.
  - Most contract laboratories often offer a service for chlorate and perchlorate using 0.01 mg/kg as a reporting limit
- Both compounds continue to be detected in fruit and vegetables

Residue definition impacts on analytical scope

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- For MRL compliance testing, metabolites are excluded if a minor part of the residue or if difficult or expensive to analyse
  - **For any risk assessment, metabolites and transformation products with properties similar to those of the parent substance, are included**
  - Specific MRLs are set in Regulation (EC) No 609/2013 for food intended for infants and young children, typically 0.01 mg/kg, which should include all metabolites
- MRLs are not routinely set for processed products, such as flour, bread, *etc.* as are normally set for raw agricultural commodities
- Check residue definitions and customers' requirements
  - The sum of glufosinate and its salts, 3-methyl-phosphinico-propionic acid (MPP) and N-acetyl-glufosinate (NAG), expressed as glufosinate equivalents

Maximum residue levels (MRLs) dictate required sensitivity

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- Check MRL for analyte/commodity combination
  - http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.selection&language=EN
- Where usage is approved, MRLs are often set relatively high
  - Glyphosate in barley: 20 mg/kg
  - Ethephon on blueberries: 20 mg/kg
  - Maleic hydrazide in onion: 15 mg/kg
  - Fosetyl-aluminium in blackberries: 100 mg/kg
- Where no MRLs have been set the default MRL "at or about the limit of determination" applies
  - These tend to be higher than the 0.01 mg/kg set for most pesticides
  - They have been updated and reduced over last few years (0.3-0.1 mg/kg)
- Exception is for food intended for infants and young children where the MRL for these compounds is 0.01 mg/kg throughout
- Some polar pesticides have temporary MRLs or national action limits

Challenges associated with the analysis of polar and ionic pesticides

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- Diverse range of analytes
- Inclusion of certain metabolites
- Wide range of MRLs but customer expectations are for < 0.01 mg/kg LOQs
- Not amenable to multi-residue methods
- One generic extraction or multiple optimal extractions?
- Cleanup improves performance but reduces scope
- Insufficient retention with reverse phase
- Stable isotope analogues for quantification
- Multiple chromatographic methods available
- Too many chromatographic methods without peer review
- Many compounds have low molecular weights and/or are labile

Chromatographic options

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- Reversed-phase (RP) LC
- Porous graphitised/graphitic carbon (PGC)
- Ion chromatography (IC)
- Hydrophilic interaction liquid chromatography (HILIC)
- Other including "Mixed Mode"

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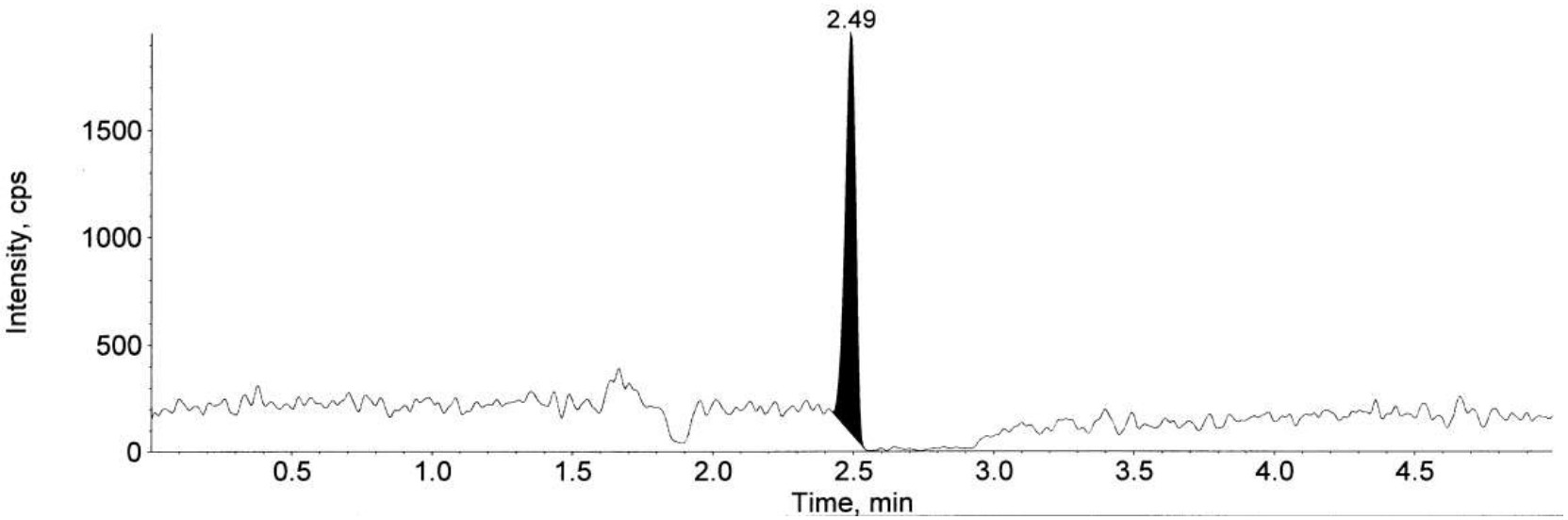
So where to start?

Let's take a look at the type of chromatography in use for most other applications

Reversed-phase LC (*e.g.* C18)



- Need retention greater than that equivalent to two column volumes
  - Void volume for 4.6 x 150 mm column is 1.74 ml
  - Flow rate is 0.75 ml/min so T_0 is 2.33 minutes
- Peak is un-retained along with lots of other co-extractives...
- Improve retention by:
  - Ion pair reagent
  - Derivatisation (*e.g.* FMOC)

BEH C18 with FMOC on Xevo TQ-XS (ESI pos)

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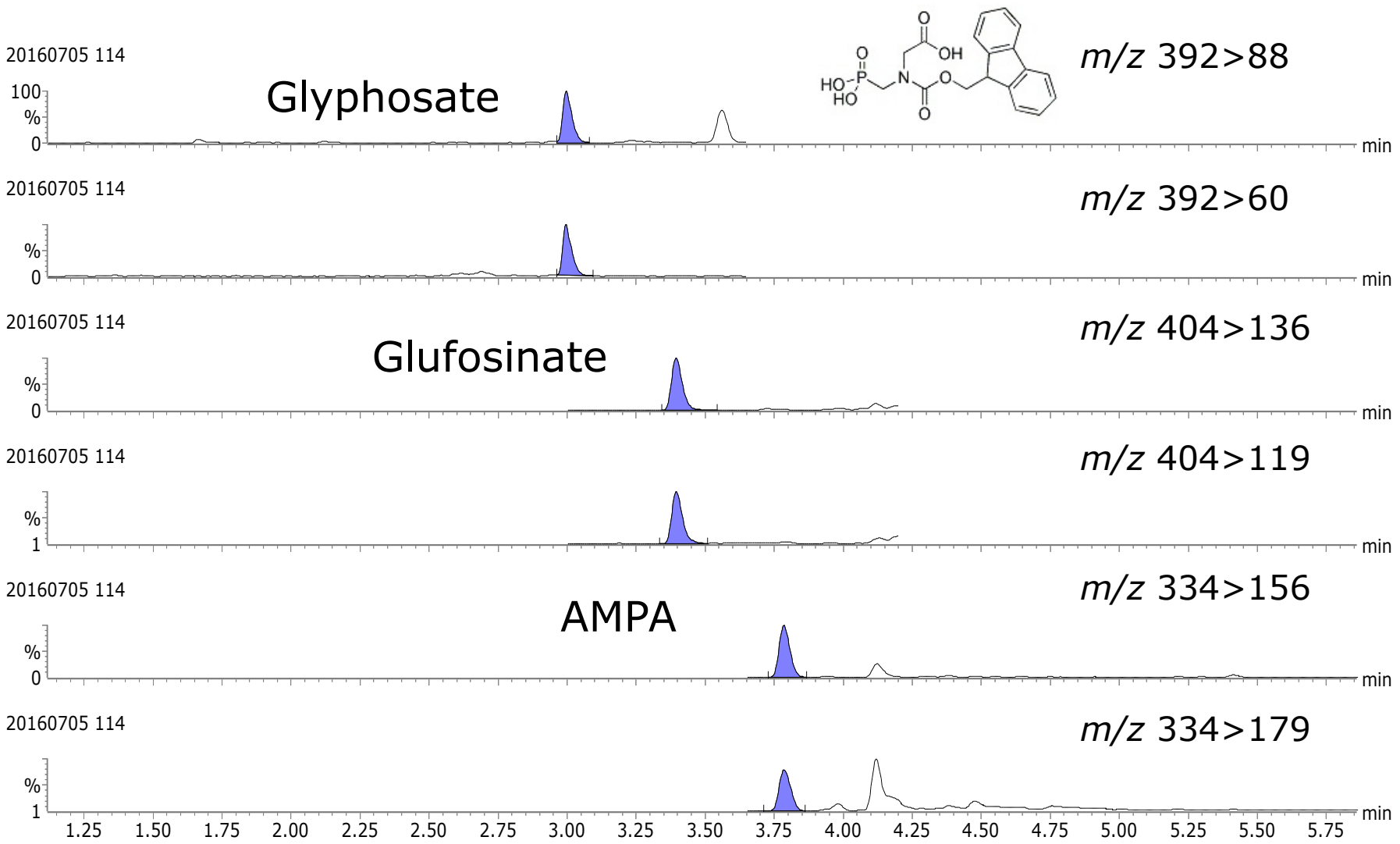

0.02 mg/kg in barley

 Extracts courtesy of Primoris, Belgium

Derivatisation using FMOC

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Advantages

- Allows for retention on C18 columns (reverse phase)
- Access to UPLC columns
- Molecular mass increased so more selective SRM transitions
- Ionises in positive ion mode
- Can be automated to reduce handling and increase efficiency
- Established approach (in water)
  - Ibanez *et al.* (2006)
- Examples of use for foodstuffs
  - Ehling and Reddy (2015)

Disadvantages

- Multiple steps need to be optimised, such as reagent concentration and reaction time
  - Oulkar *et al.* (2017)
- Lack of specificity as FMOC reacts with co-extractives
- Sample cleanup is typically required before and/or after derivatisation
- Scope is limited to analytes with primary and secondary amines
  - Excludes N-acetyl metabolites and many other polar pesticides

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So what do I do if I want to analyse more anionic compounds or required direct analysis of glyphosate?

What options have been published/promoted?

Porous graphitised carbon (PGC)

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- Shown in QuPPe document for the simultaneous analysis of a large number of anionic pesticides (method 3.1)
- Particles are spherical and fully porous
  - Thermo Hypercarb available in 3 and 5 µm formats
- The surface of PGC is crystalline and highly reproducible with no micro pores
- At the molecular level, PGC is made up of sheets of hexagonally arranged carbon atoms
  - Mechanism is charge-induced interaction of polar analyte with polarisable surface of graphite
  - Polar compounds are well retained so mobile phases with a high proportion of organic solvent can be employed, which improves sensitivity

PGC using Thermo Hypercarb on Xevo TQ-XS (ESI neg)

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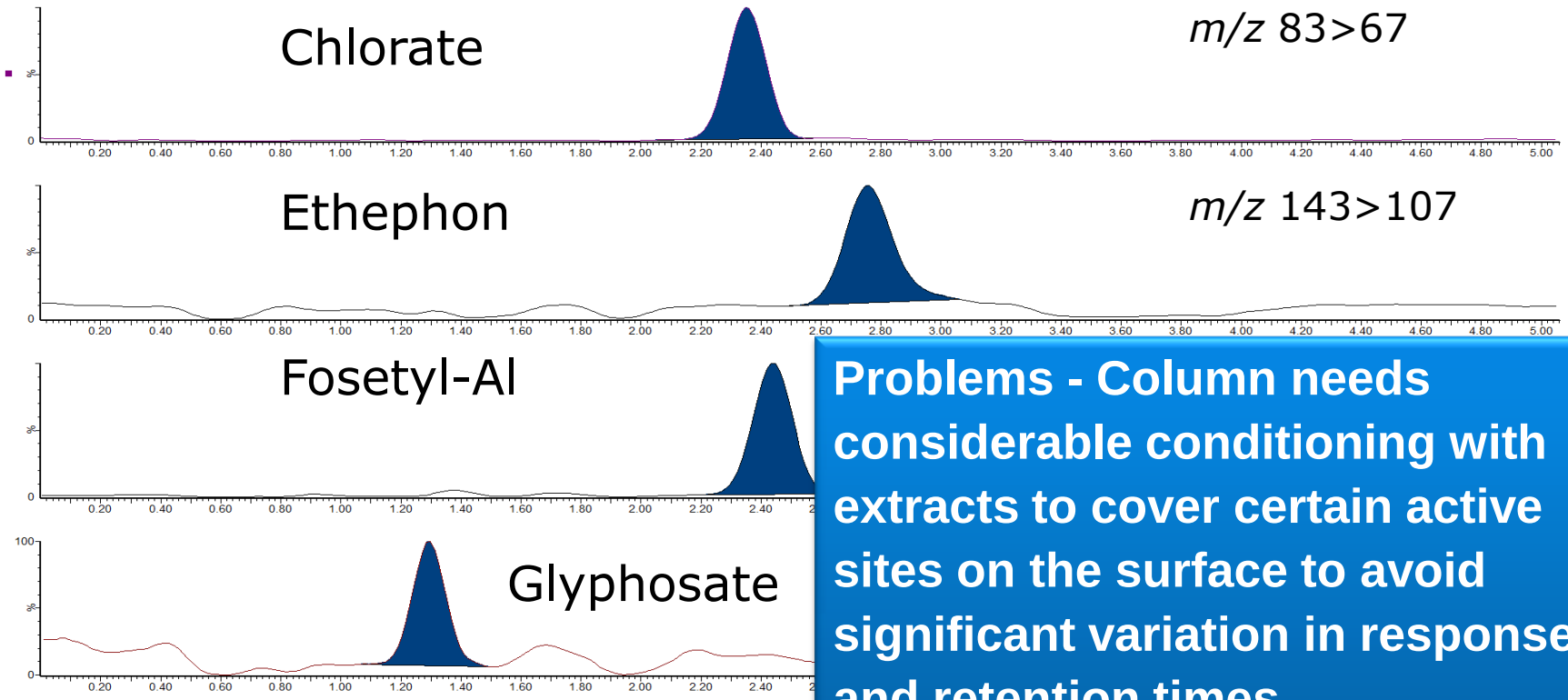
Extraction and LC-MS/MS - QuPPe method 1.3
1% acetic acid (*aq*)/methanol gradient



Problems - Column needs considerable conditioning with extracts to cover certain active sites on the surface to avoid significant variation in response and retention times

Spiked mango sample (0.01 mg/kg)

 Extracts courtesy of Nofalab, The Netherlands

Suppressed IC for anionic compounds

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- Columns are available for anion exchange using hydroxide or carbonate eluents
- MS is not compatible with high salt eluent, which is converted to water by either an electrolytic or chemical suppressor
- Analysis of a wide range of anionic pesticides in one run
  - Detection of 14 different anion/zwitterionic pesticides is possible using a specialist suppressed IC-MS/MS system

Problems - Needs specialist IC kit if KOH is used, conductivity detector to be placed in series to monitor suppressor failure, hold up observed on suppressors resulting in broad, asymmetrical peaks, poor efficiency of desolvation with high aqueous mobile phase and high risk of contamination of MS with salts over time

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What options are there for implementation of ion chromatography on standard HPLC/UPLC kit?

Mixed mode (WCX, WAX, RP) using Thermo Acclaim Trinity Q1 on Xevo TQ-XS (ESI neg)

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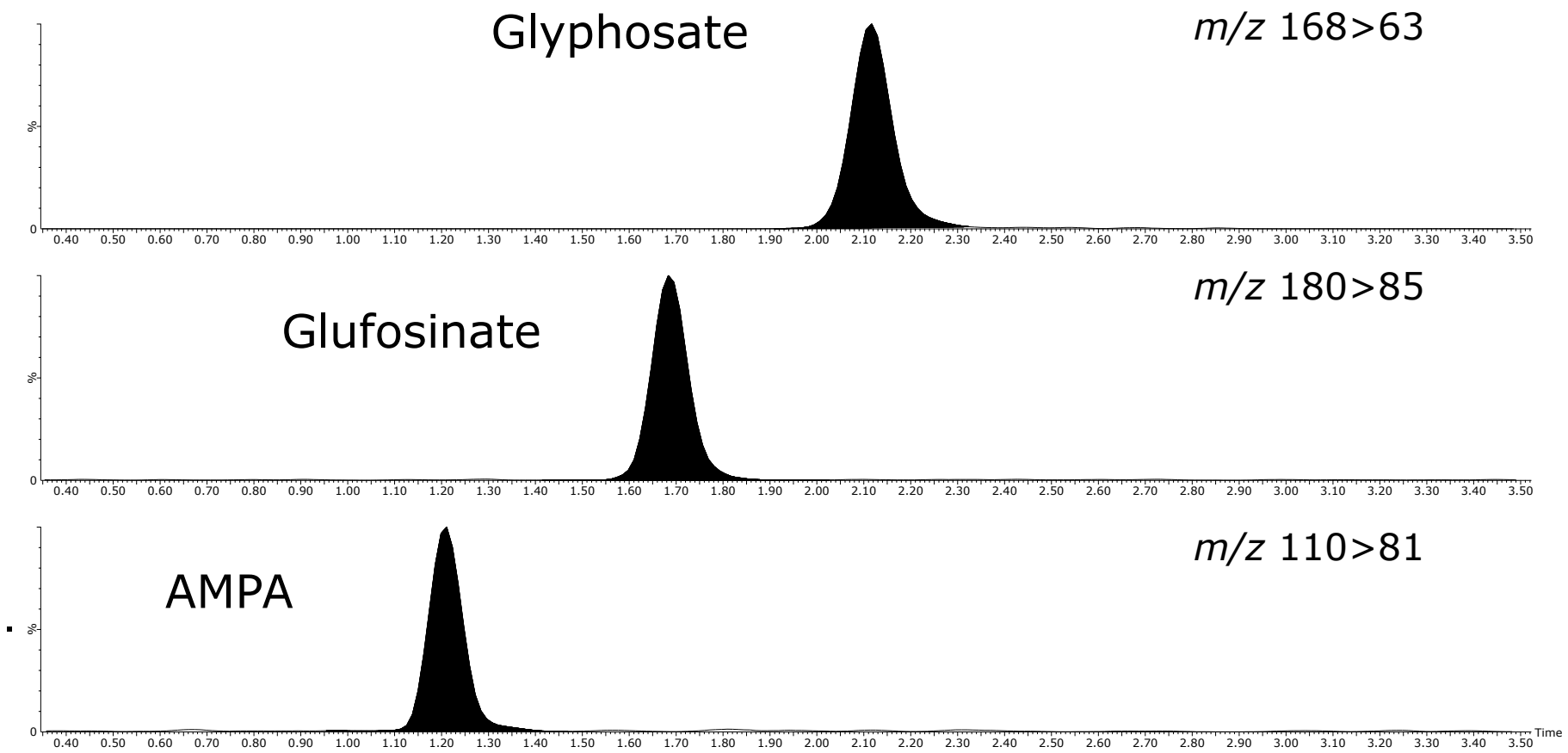
Extraction and LC-MS/MS method published by Chamkasem *et al.* (2015, 2016, 2017)

50 mM Ammonium formate @ pH 2.9/MeCN gradient



Matrix-matched standard in lentils (0.05 mg/kg)

AMPA peak shape after extraction by Quick Polar Pesticides Method (QuPPe)

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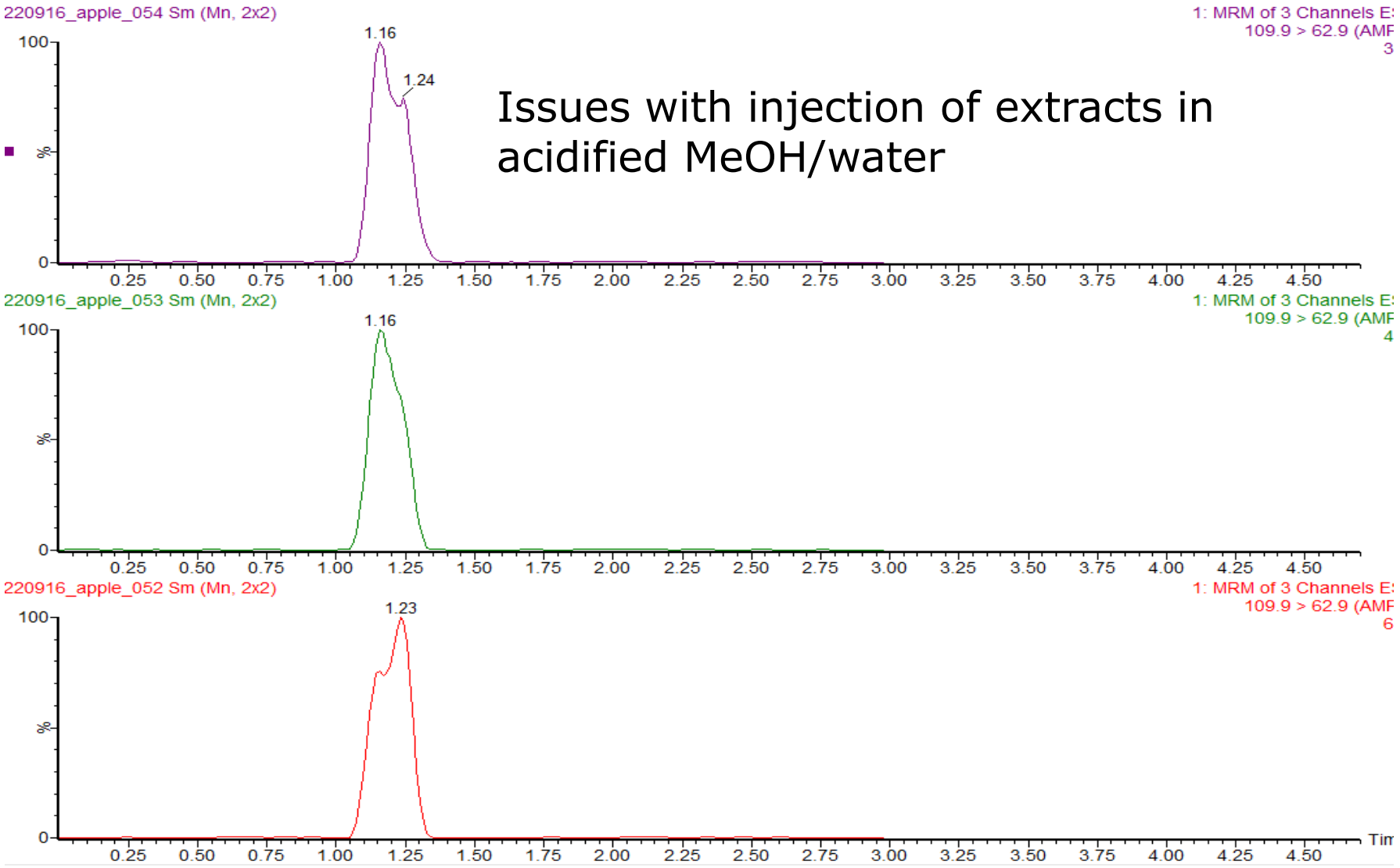

Issues with injection of extracts in acidified MeOH/water

Metrohm Metrosep A supp 5

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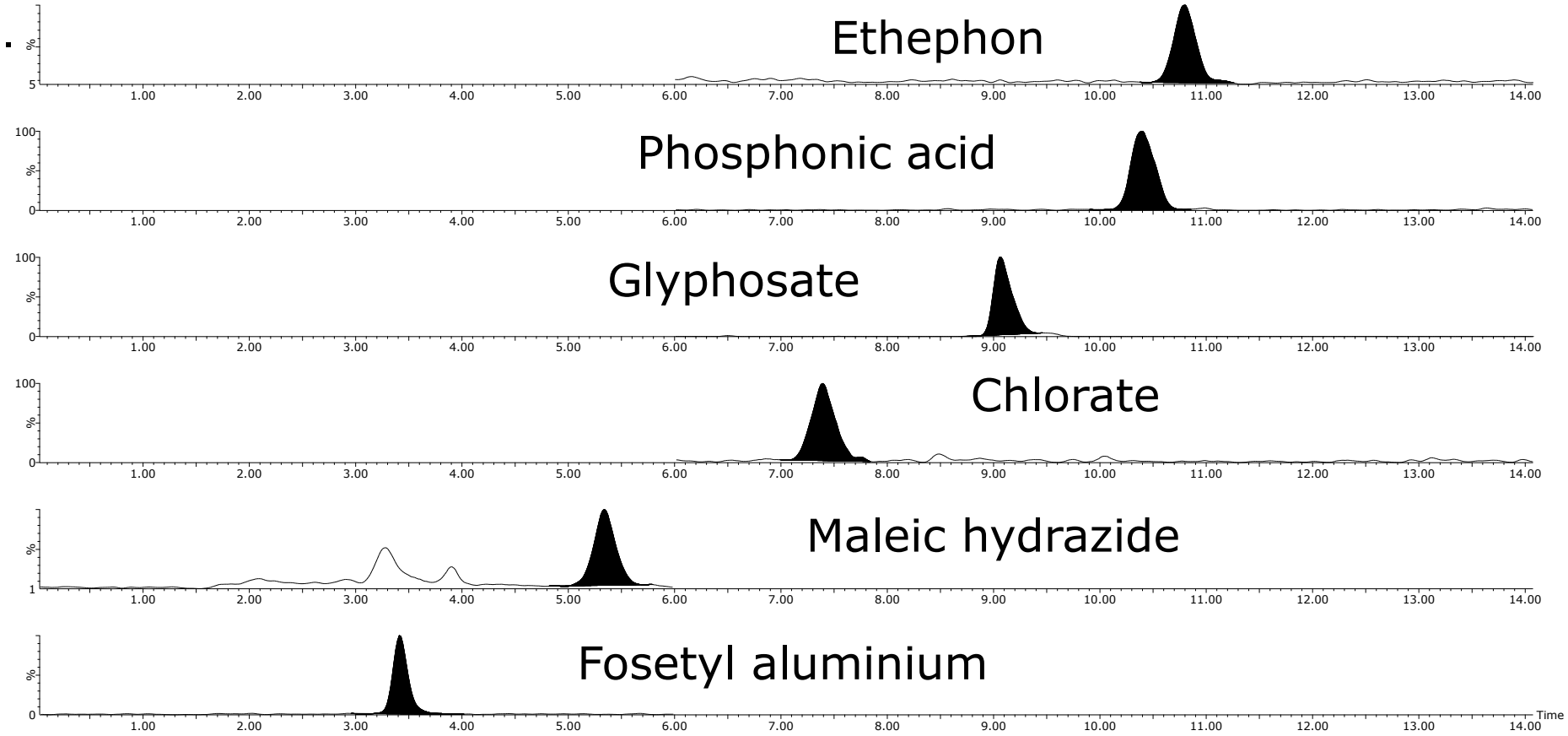
Quaternary ammonium as functional groups
50 mM Ammonium bicarbonate/water/MeCN gradient



Spiked mango sample (0.01 mg/kg)

 Extracts courtesy of Nofalab, The Netherlands

Shodex HILICpak VT-50 2D

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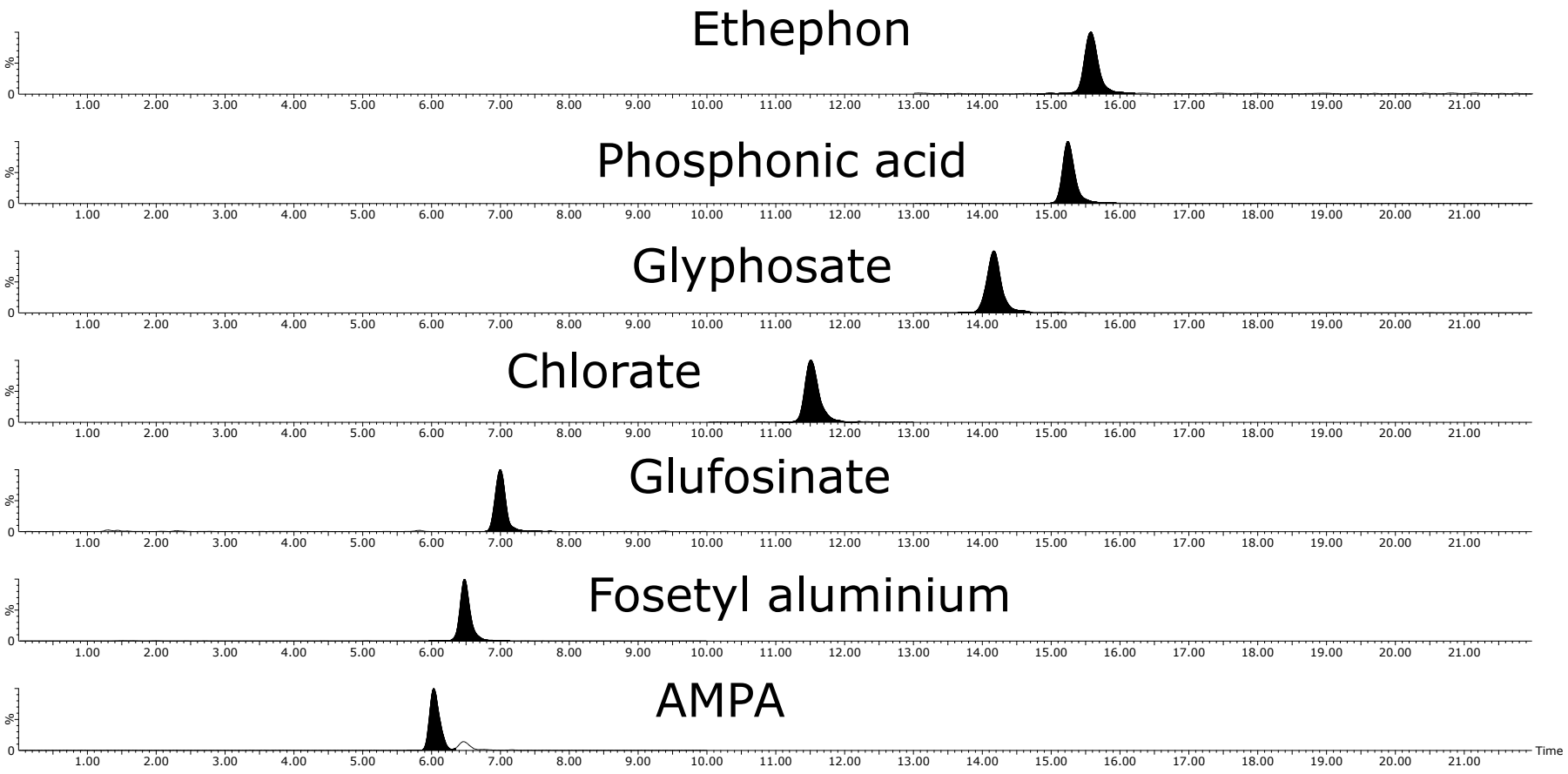
Quaternary ammonium as functional groups
50 mM Ammonium formate @ pH 2.9/MeCN gradient
Extracts from QuPPe method



Matrix-matched standard in beer (0.01 mg/kg)

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Promising but all had problems

Insufficient retention of AMPA on Acclaim Trinity Q1 leads to worse precision and ion suppression from matrix and unable to use methanol in the mobile phase

The flow rate available on Metrohm and Shodex columns was restricted by back pressure issues and columns not available in < 2 µm format

Conclusion – we needed to develop something in house…

A new Waters prototype column in a <2 µm format

Benefits of HPLC with high efficiency - speed

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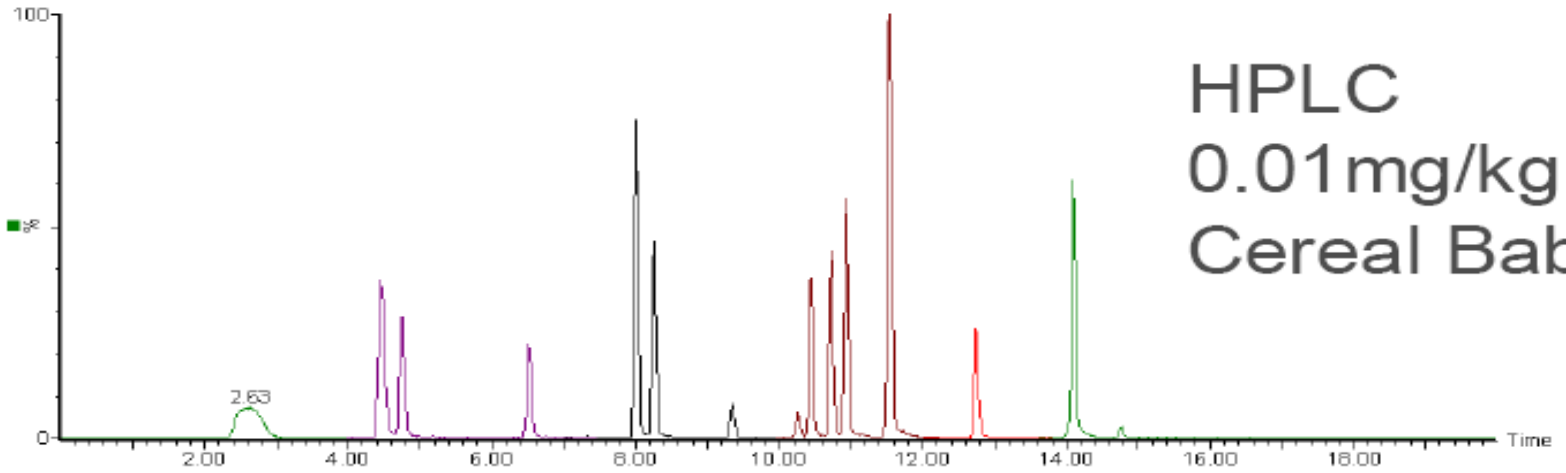
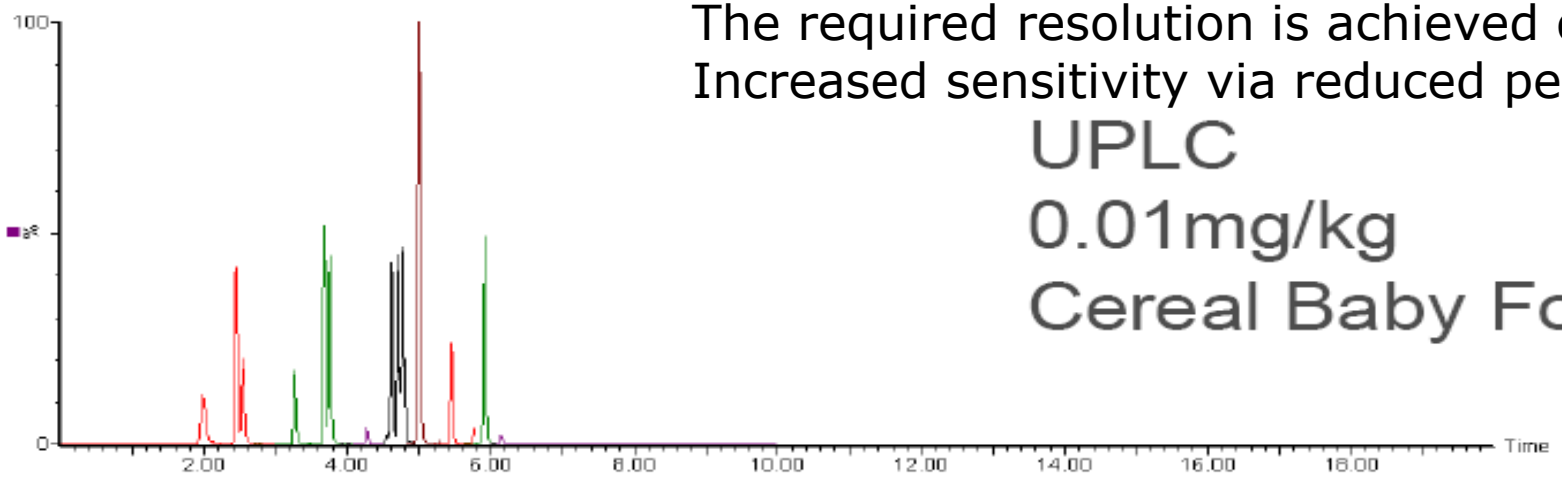


HPLC
0.01mg/kg
Cereal Baby Food



The required resolution is achieved quicker
Increased sensitivity via reduced peak widths

UPLC
0.01mg/kg
Cereal Baby Food

C.C. Leandro *et al*, J. Chromatogr. A., (2206) 1103, 94-101

Why can't we just increase the flow rate with a conventional column?

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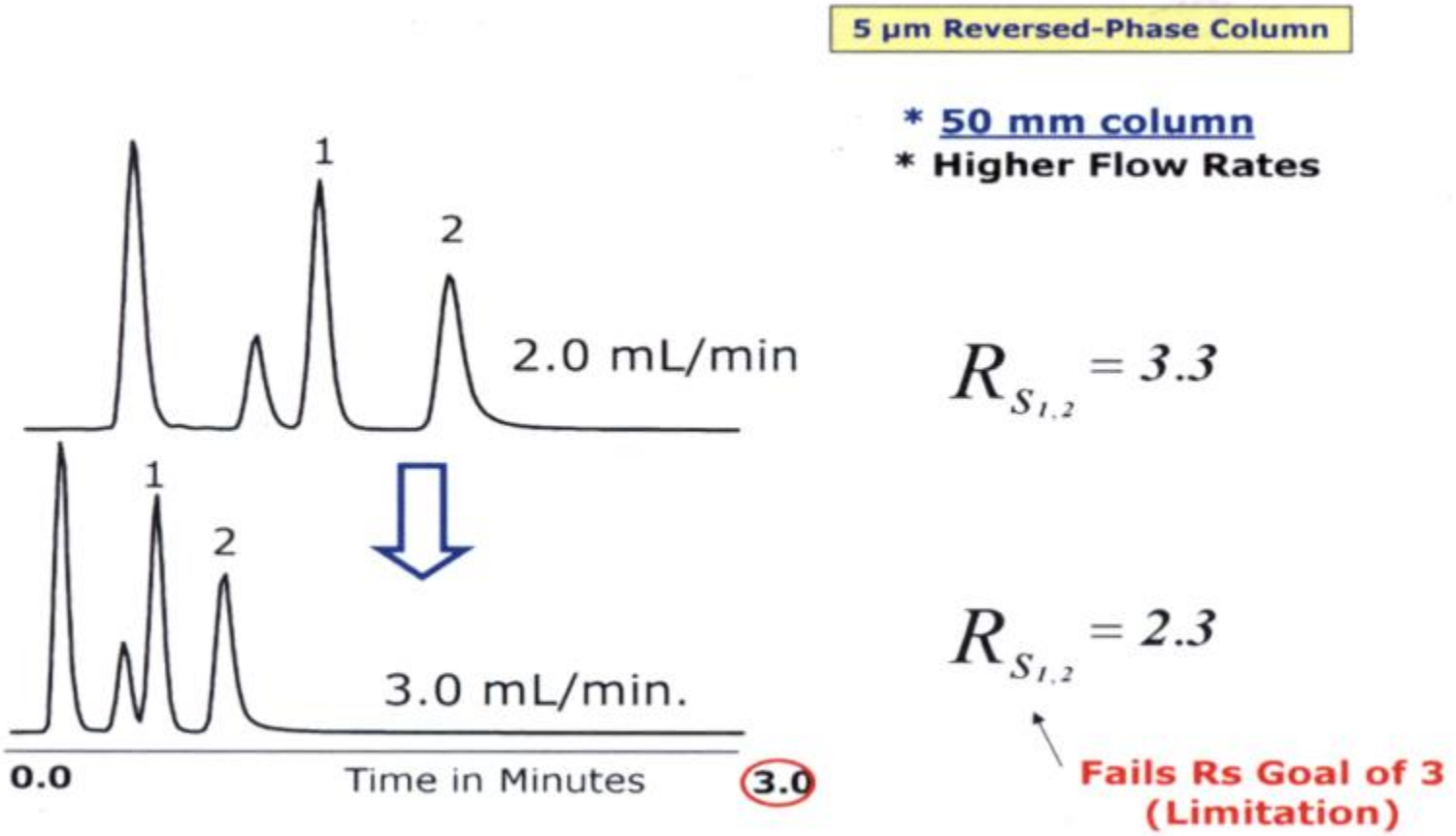

Insufficient time for complete partition between mobile and stationary phase

Van Deemter curves

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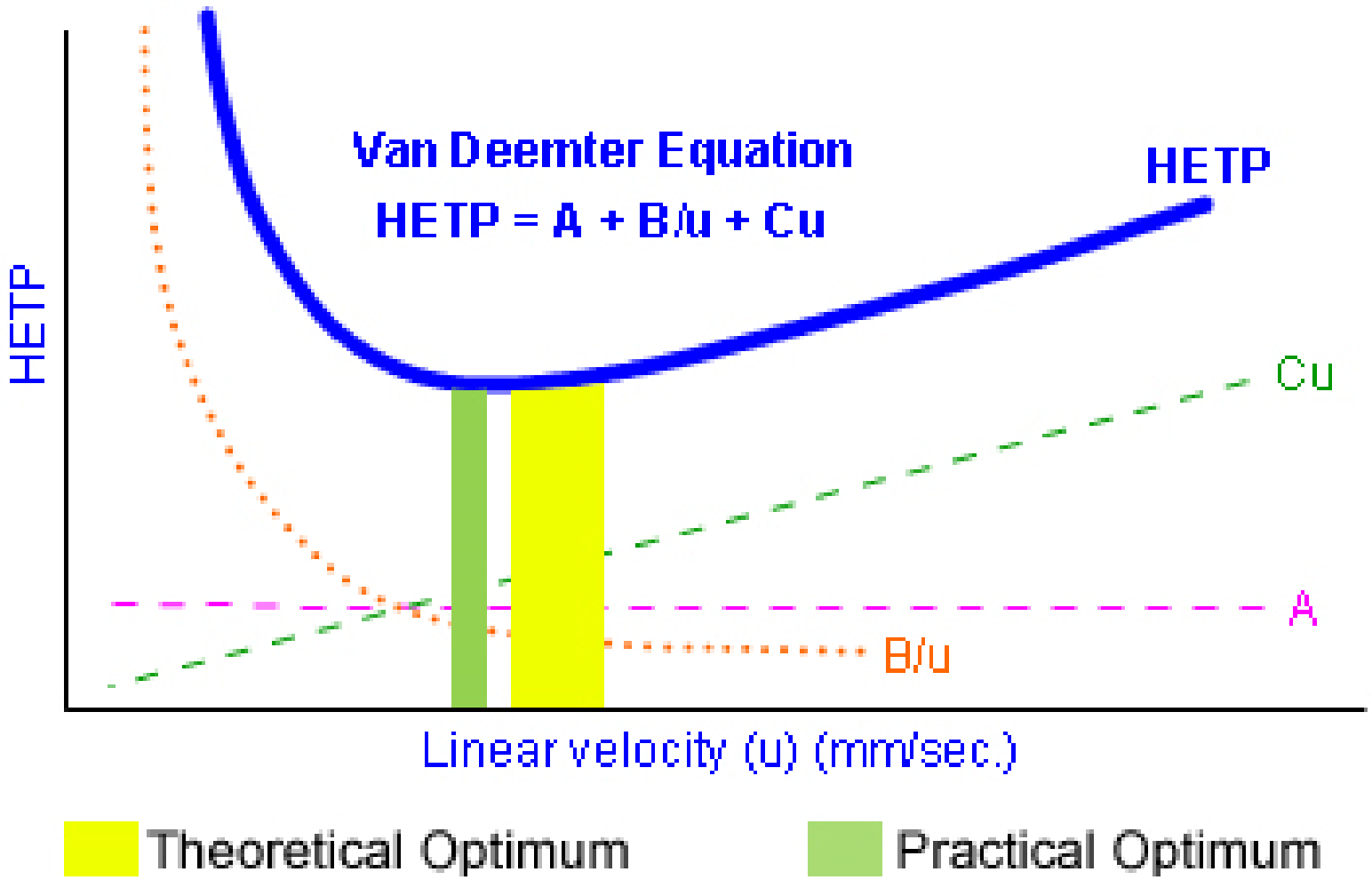
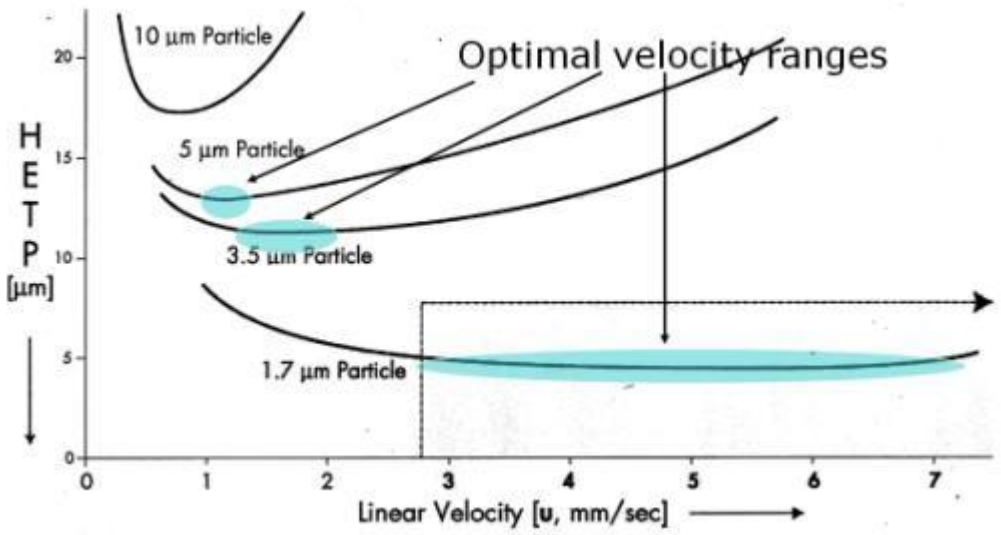
- Height equivalent of a theoretical plate (HETP, H) (y-axis) against the eluent linear velocity (m)
- Curve is a composite of curves made up from three individual effects which contribute to band broadening
  - Eddy Diffusion (A-Term)
  - Longitudinal Molecular Diffusion (B-Term)
  - Mass Transfer Effects (C-Term)

Van Deemter curve



Reducing particle size improves efficiency

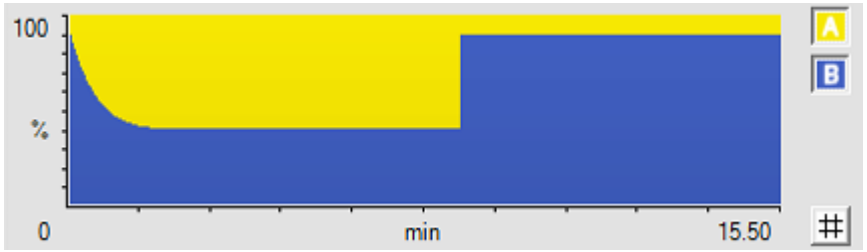
- Reduces analyte dispersion by reducing pore depth and distance between pores
- Reduces analyte dispersion by minimising Eddy diffusion
- Small particle size also facilitates use of higher flow rates without loss of efficiency

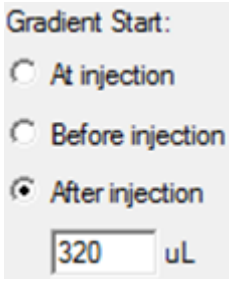


LC conditions

Column	Prototype (1.7 µm, 2.1 x 100 mm)
LC System	I Class FL (also have used FLN)
Solvent A	50 mM Ammonium Formate pH 2.9 (0.9% Formic Acid)
Solvent B	MeCN + 0.9% Formic Acid
Column Temp	50°C
Sample Temp	10°C
Injection Volume	10 µL
Flow rate	0.5 mL/min

Time (min)	%A	%B	Curve
0	10	90	-
4.50	60	40	2
8.50	60	40	6
15.50	10	90	1





Typical chromatography in solvent (25 ppb)

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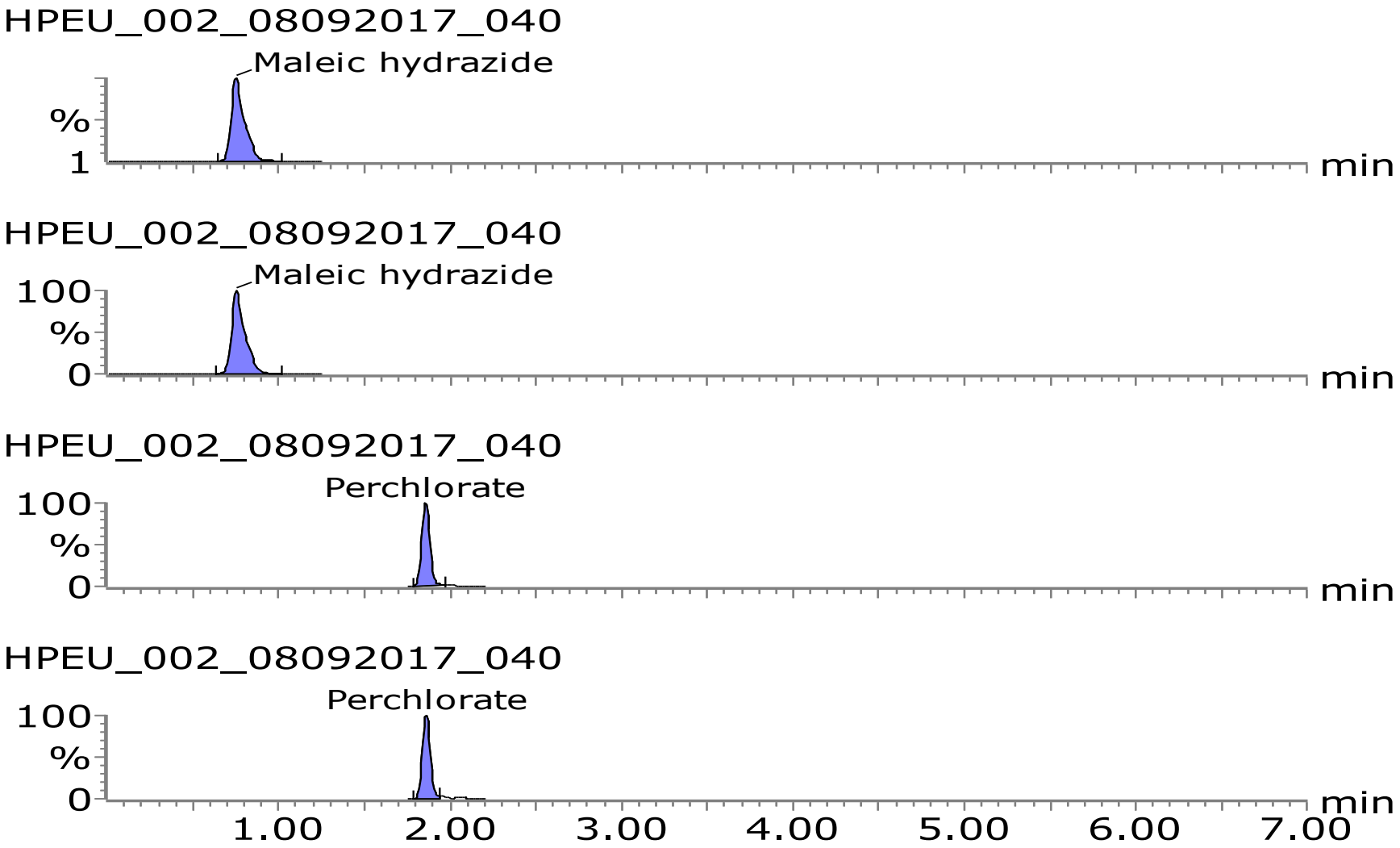

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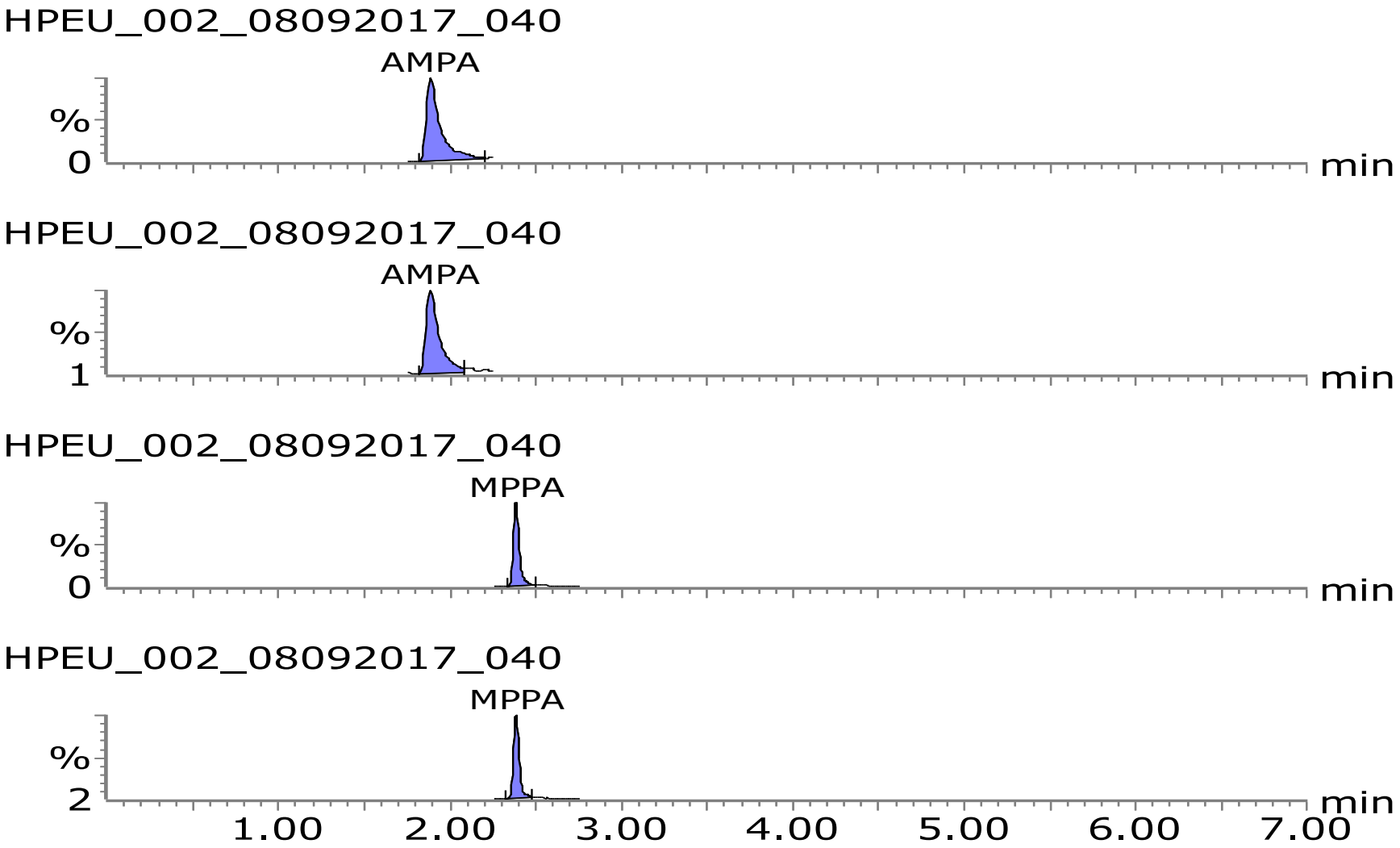

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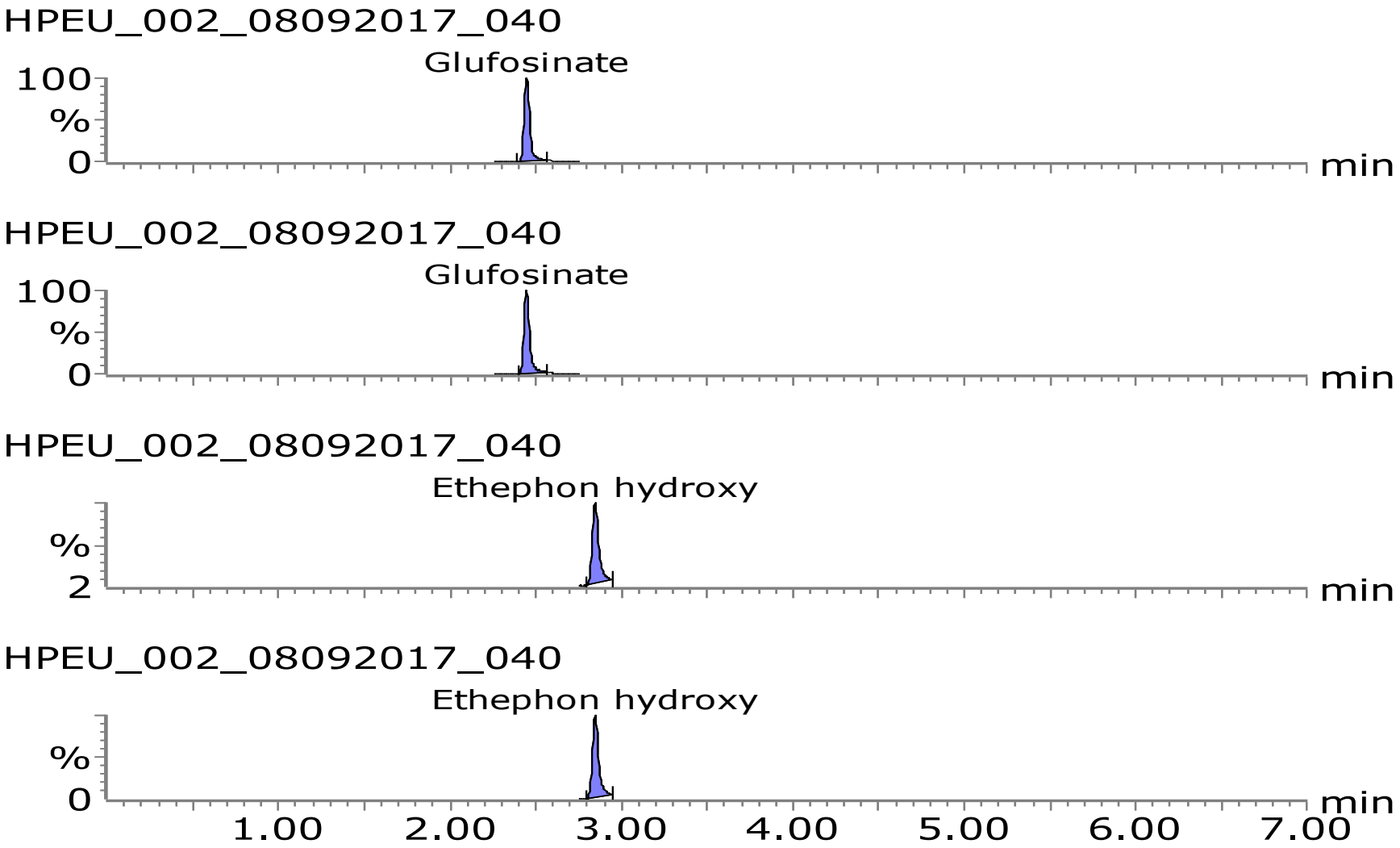

Typical chromatography in solvent (25 ppb)

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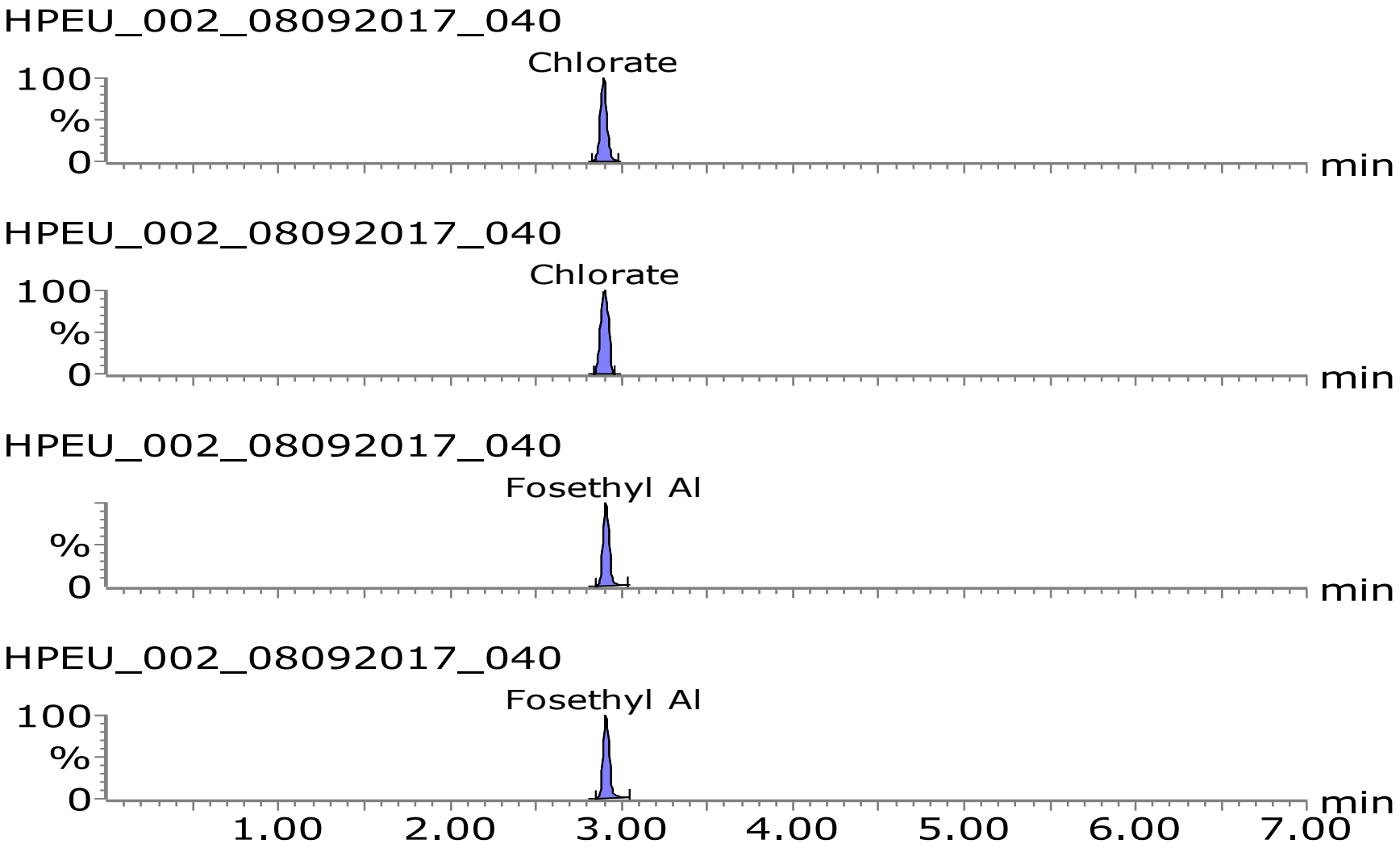
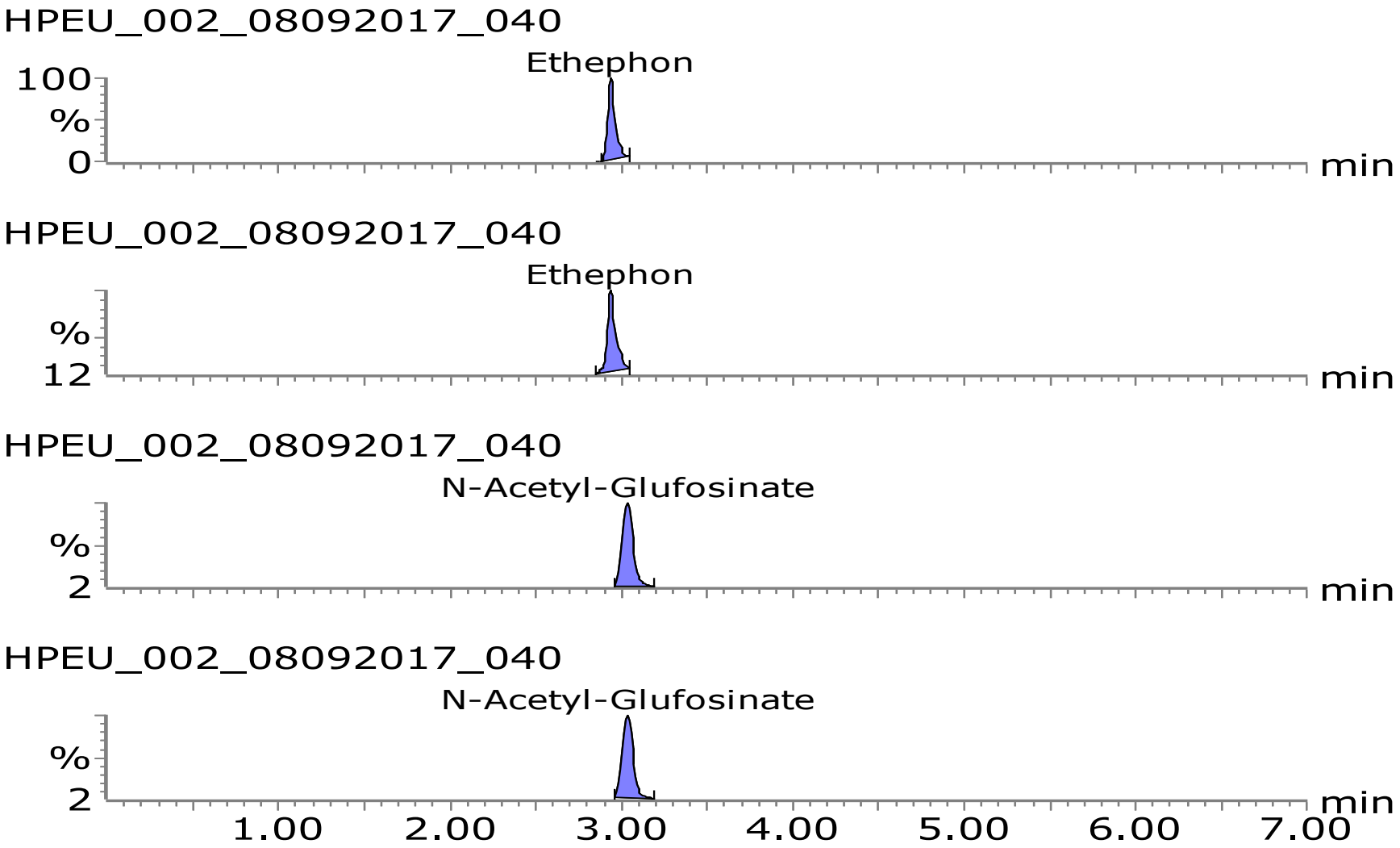


Typical chromatography in solvent (25 ppb)



Typical chromatography in solvent (25 ppb)

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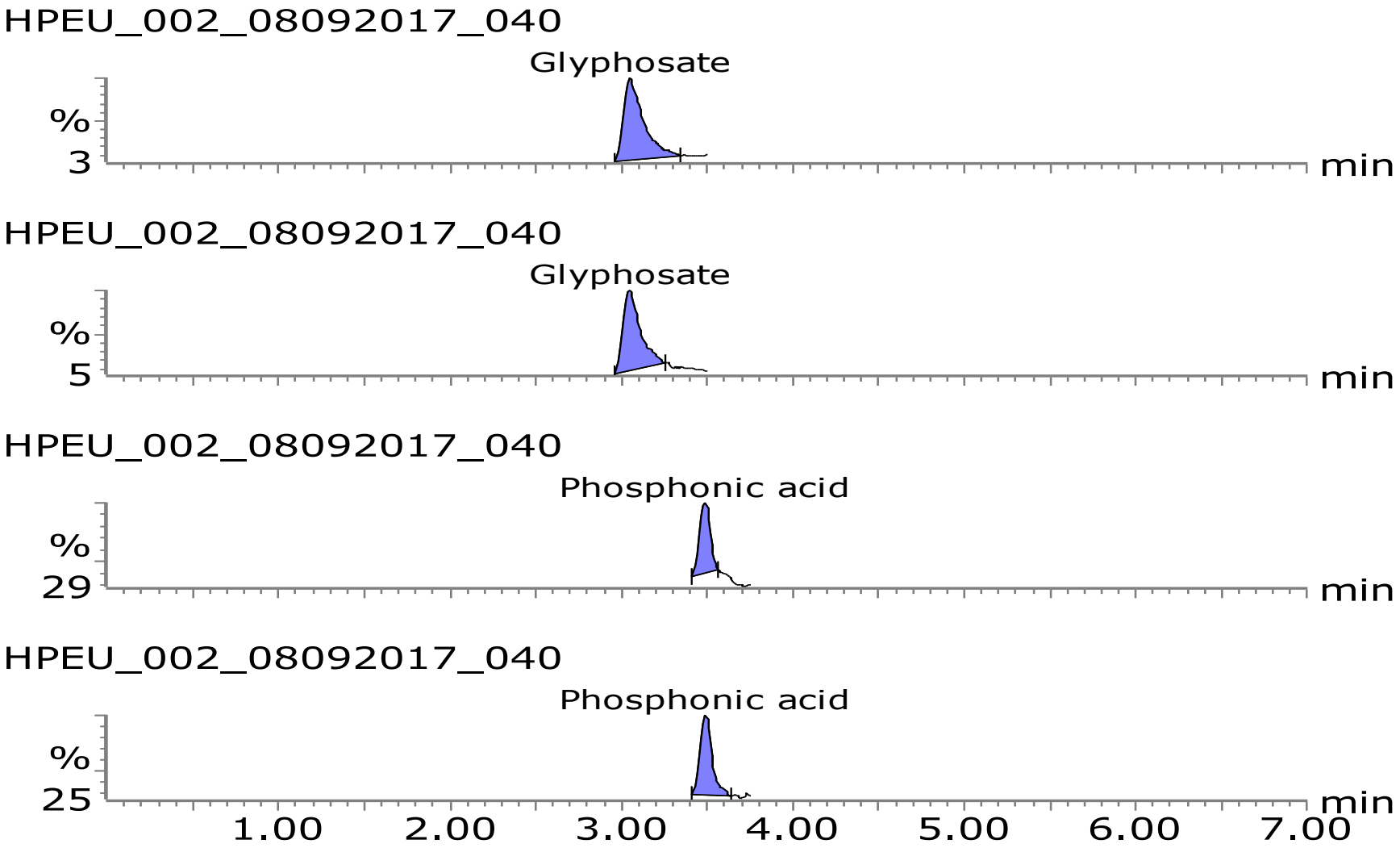
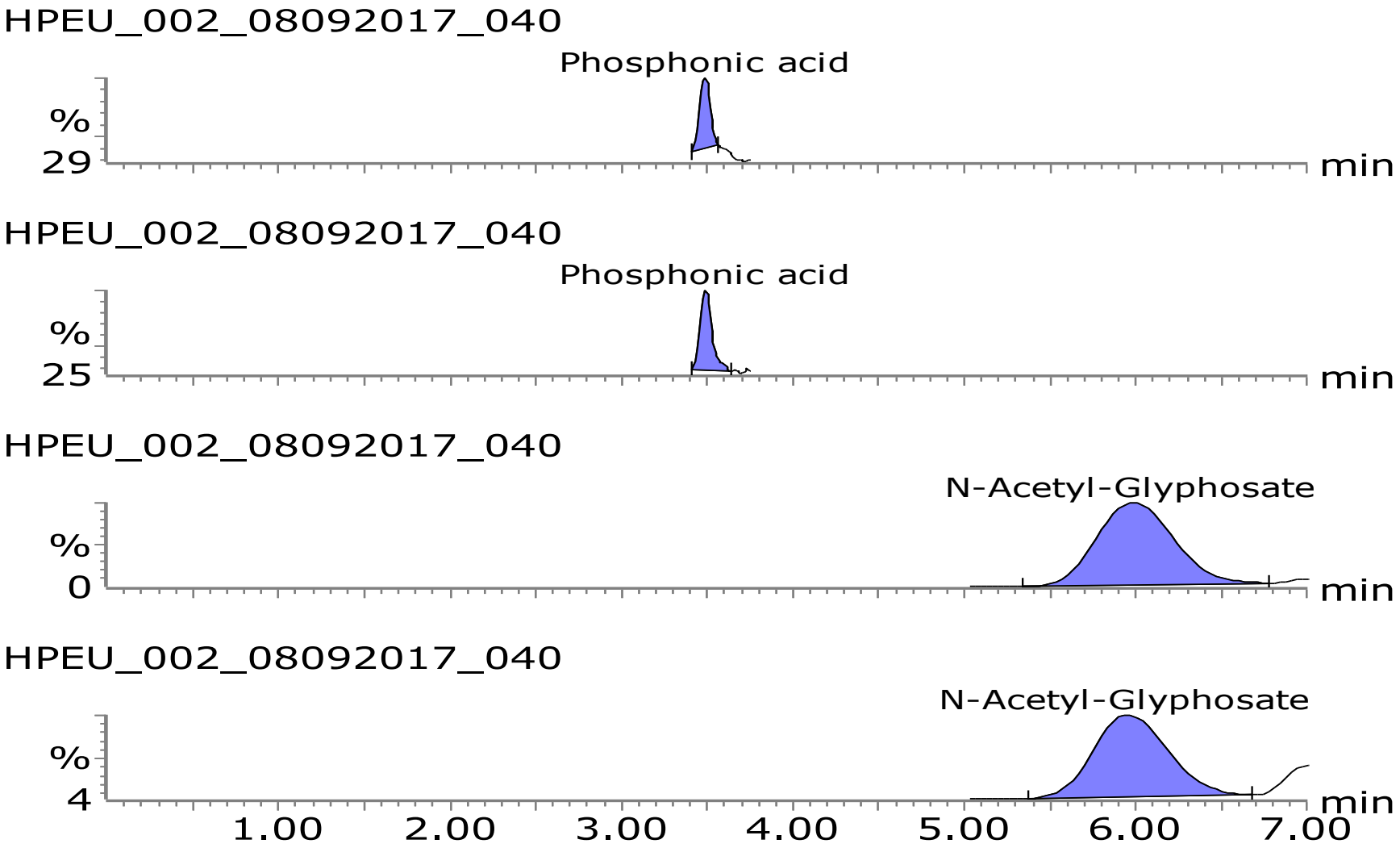


Typical chromatography in solvent (25 ppb)



Repeatability from injections of standards in solvent (n=6)

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	Conc level (ppb)	RSD (%)		Conc level (ppb)	RSD (%)
N-Acetyl-Glufosinate	5 25	2.1 2.3	Fosetyl Al	5 25	2.7 0.7
N-Acetyl-Glyphosate	5 25	2.4 0.9	AMPA	5 25	3.3 2.8
Glufosinate	5 25	3.3 1.2	Perchlorate	5 25	5.5 3.5
Glyphosate	5 25	2.6 1.8	Chlorate	5 25	11.9 3.0
MPPA	5 25	1.9 2.7	Ethephon Hydroxy	5 25	2.2 2.2
Ethephon	5 25	4.8 4.0	Phosphonic acid	5 25	9.4 4.7
Maleic Hydrazide	5 25	3.7 2.0			

Extraction method

QuPPe
5g of sample for onion & spinach / 2.5g for lentils
Blank or Spike @ 0.01 and 0.05 mg/kg (n=5)
Shake
Room temp for 2h
+ 5mL H_2O for lentils
+ 5 mL acidified MeOH (1% Formic Acid) for onions and spinach / + 2.5 mL for lentils
Vortex 2 min
For lentils: place in freezer for 60 min, vortex
Centrifuge, 5 min, 6000 rpm
Filter (0.25µm, PVDF, Spin Filters)
Place in TruView vial

Selection of chromatograms at 10 ppb in spinach

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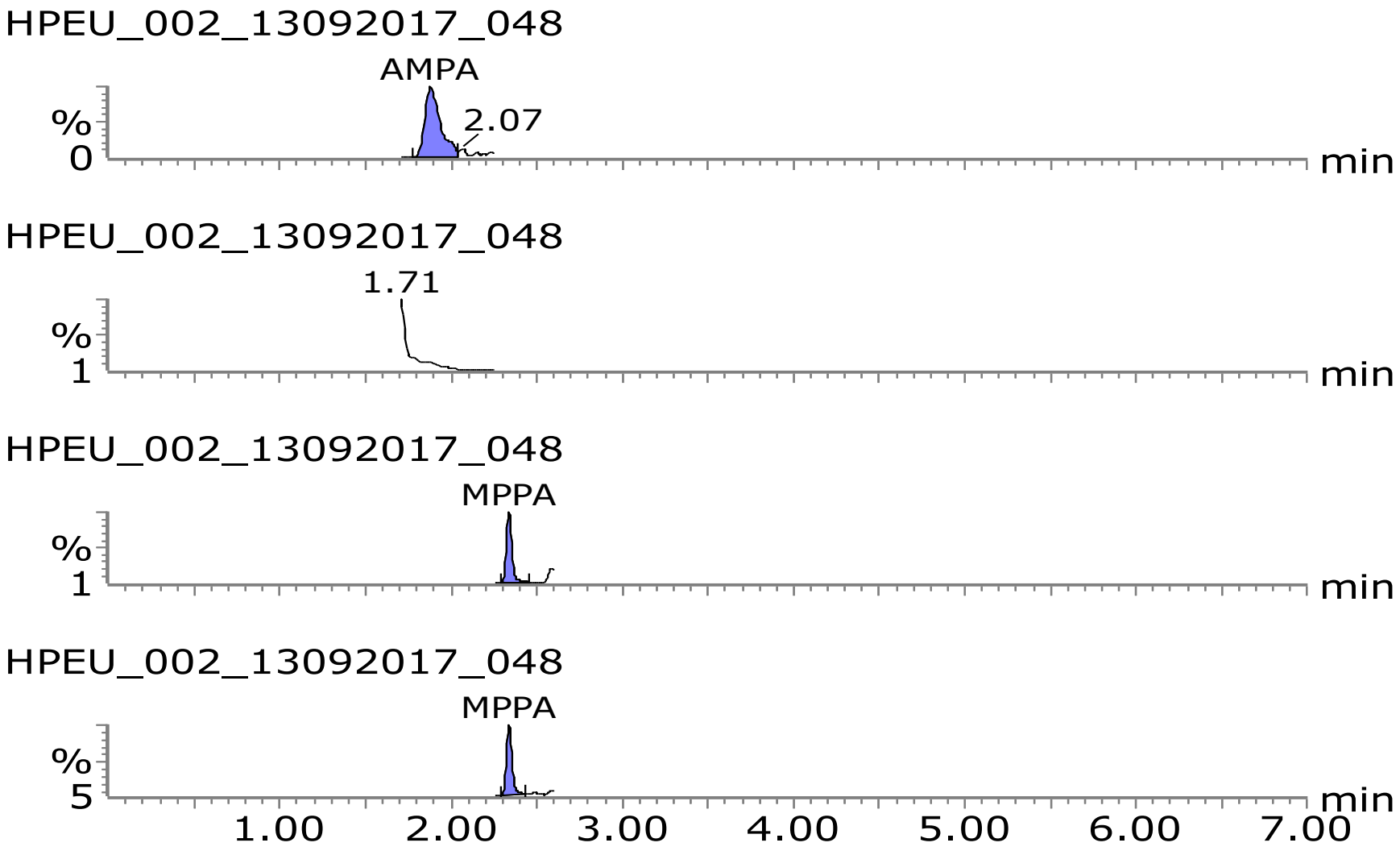
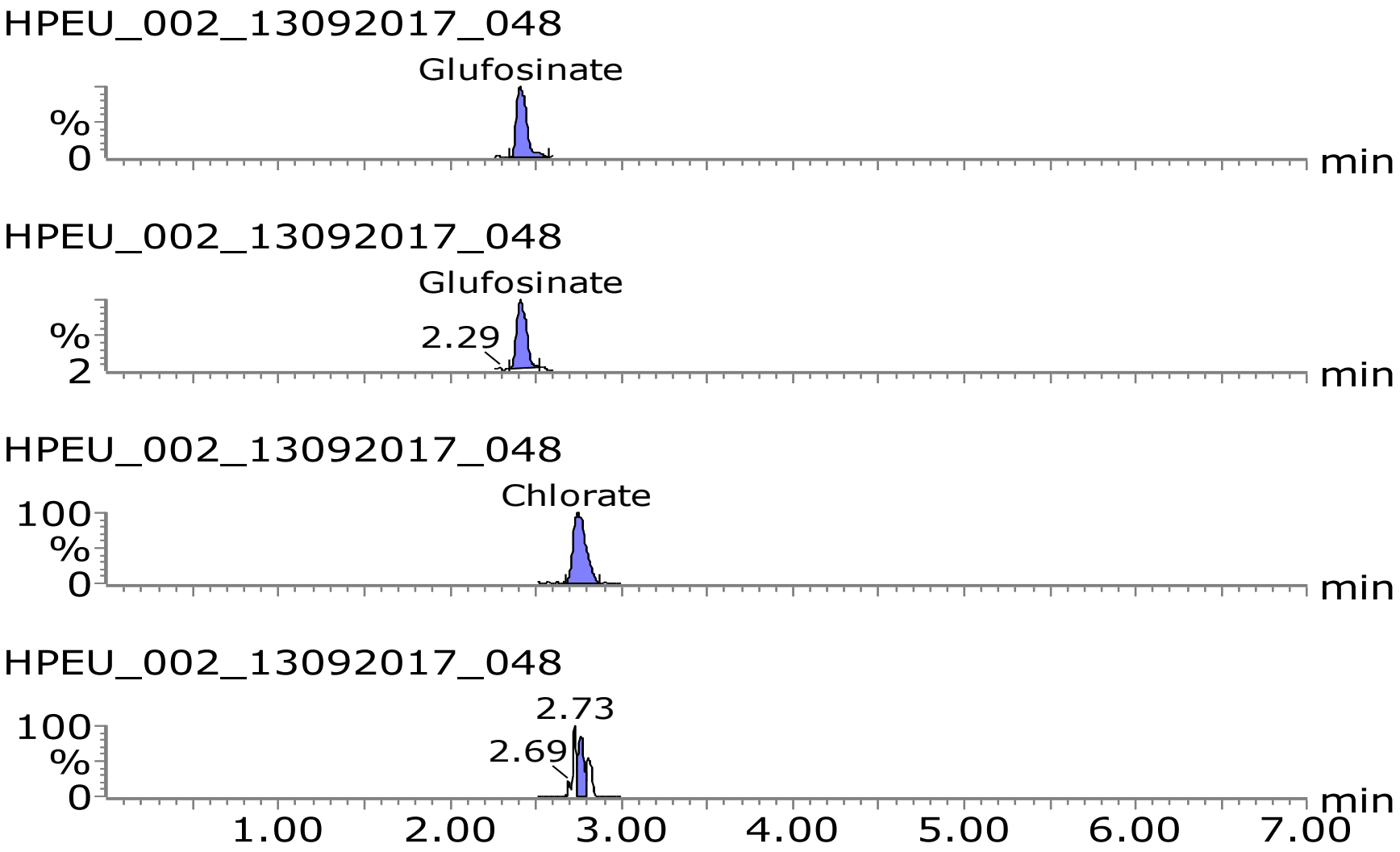


Selection of chromatograms at 10 ppb in spinach



Selection of chromatograms at 10 ppb in spinach

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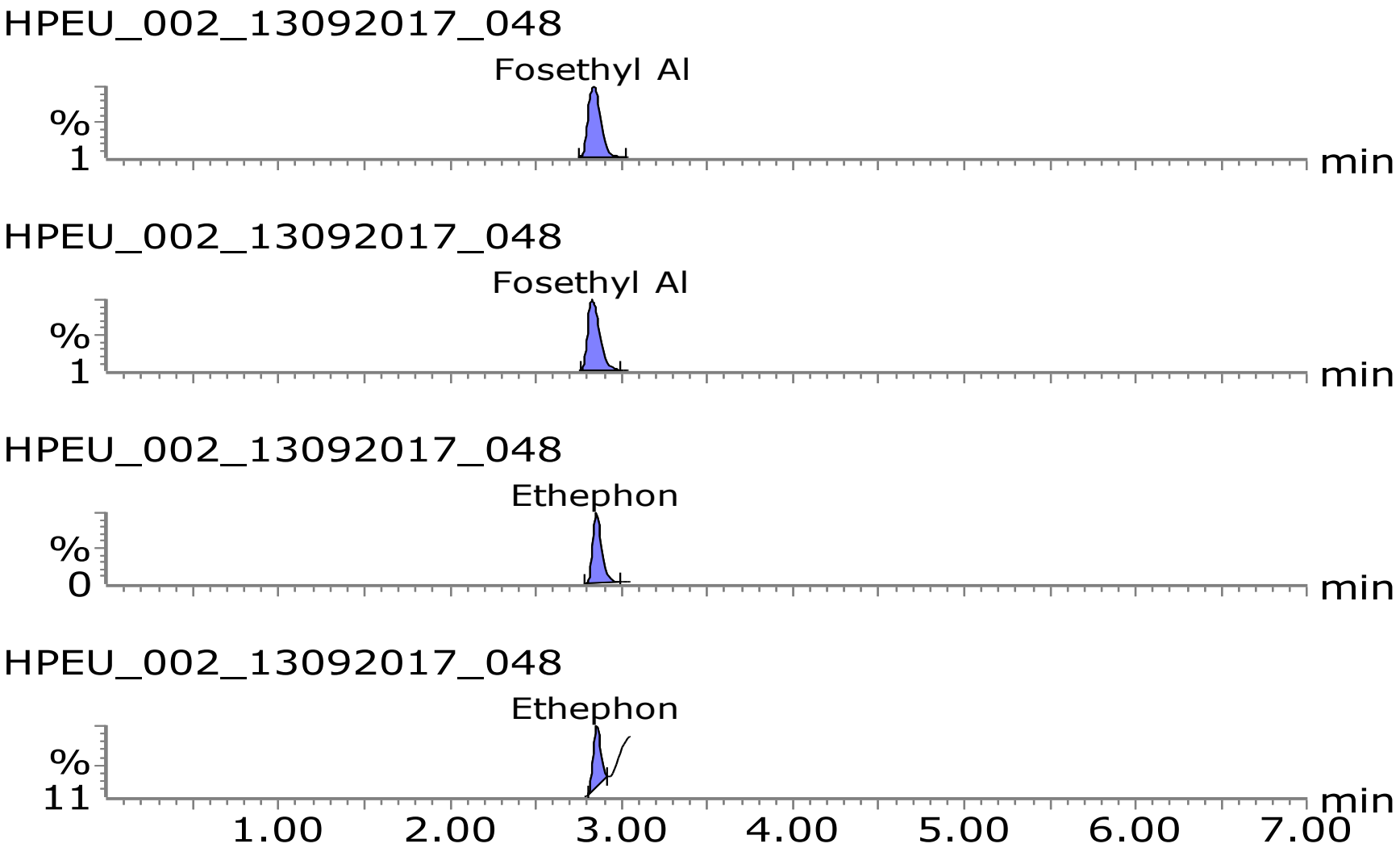

Selection of chromatograms at 10 ppb in spinach

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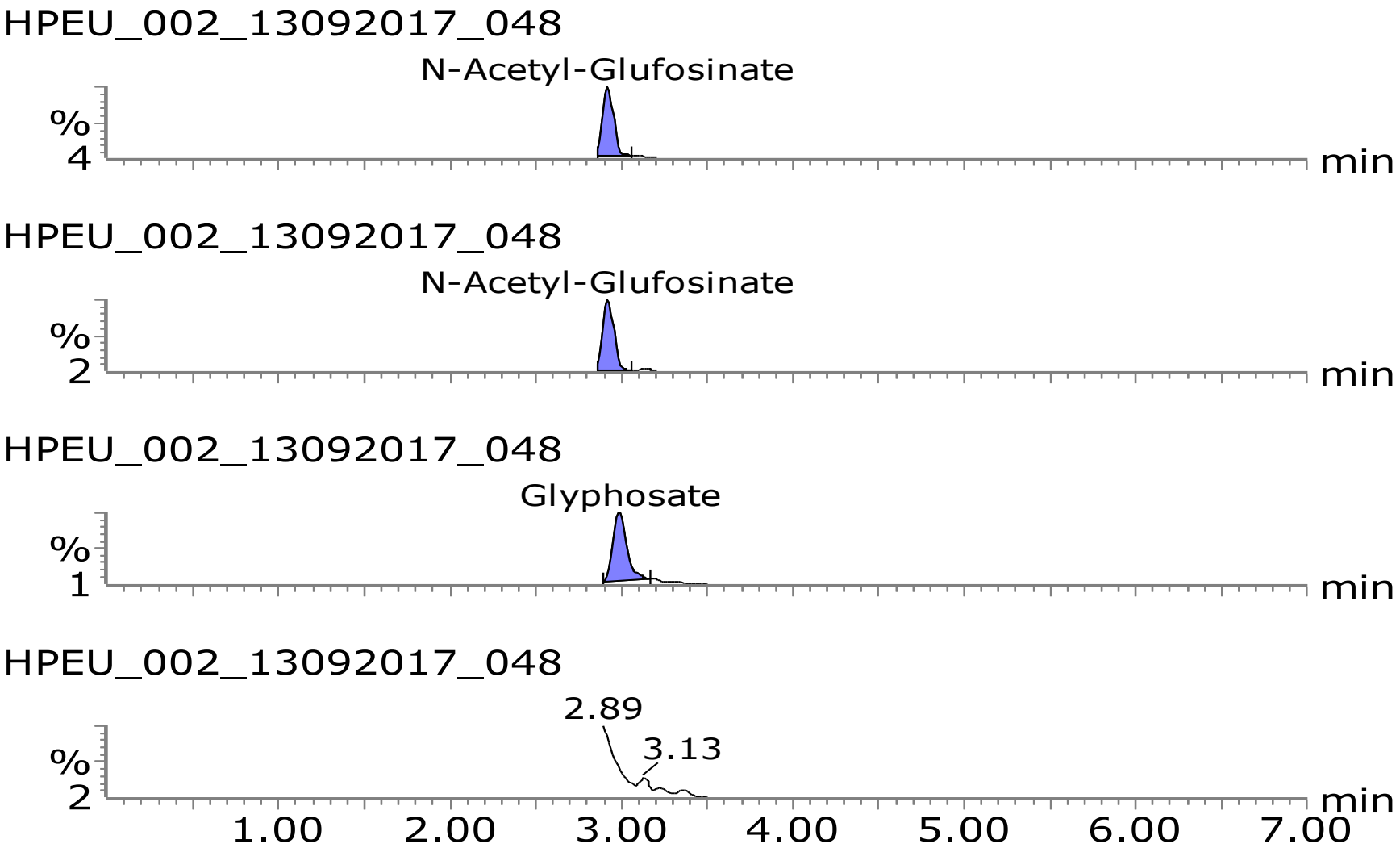
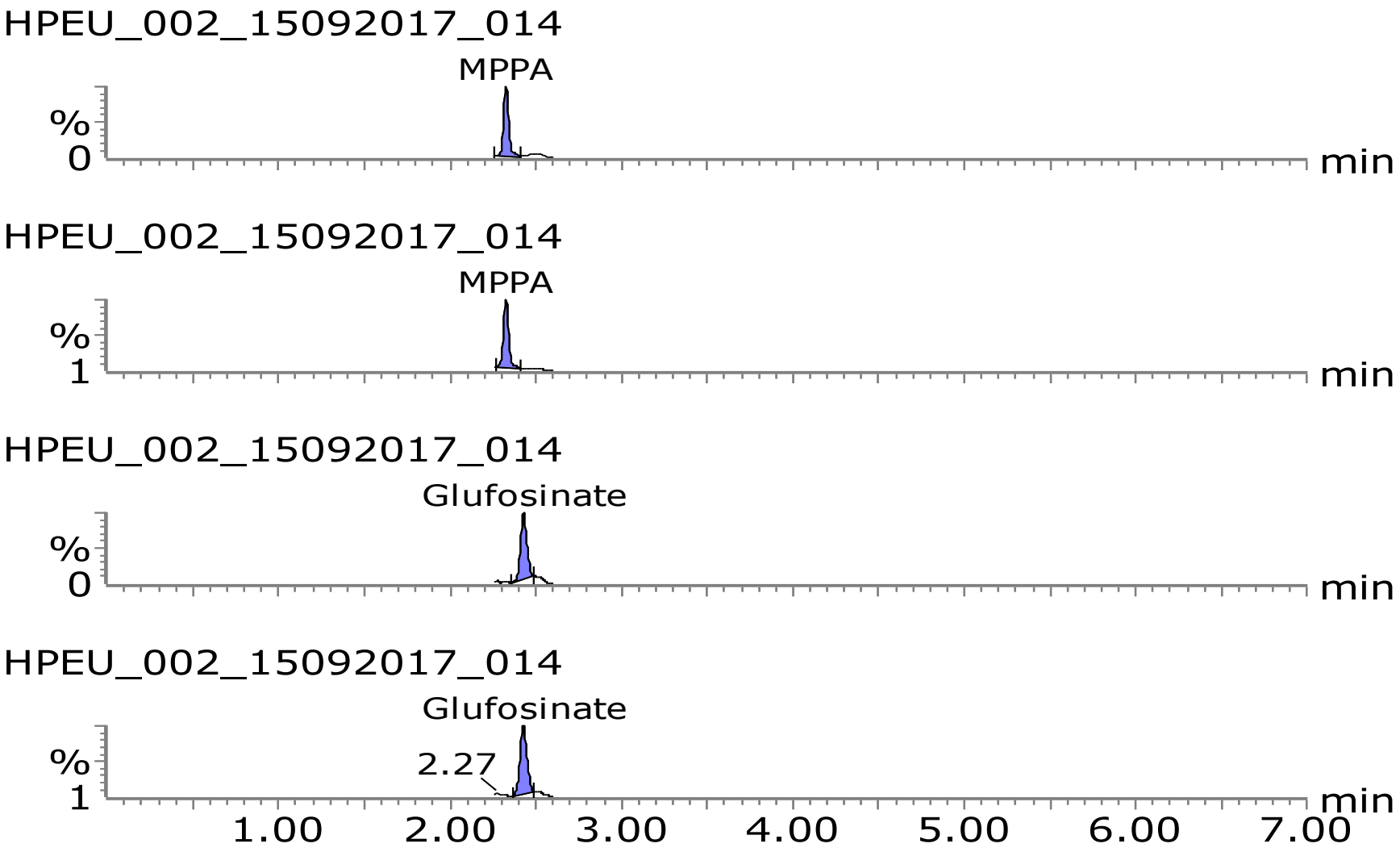


Selection of chromatograms at 10 ppb in lentils



Selection of chromatograms at 10 ppb in lentils

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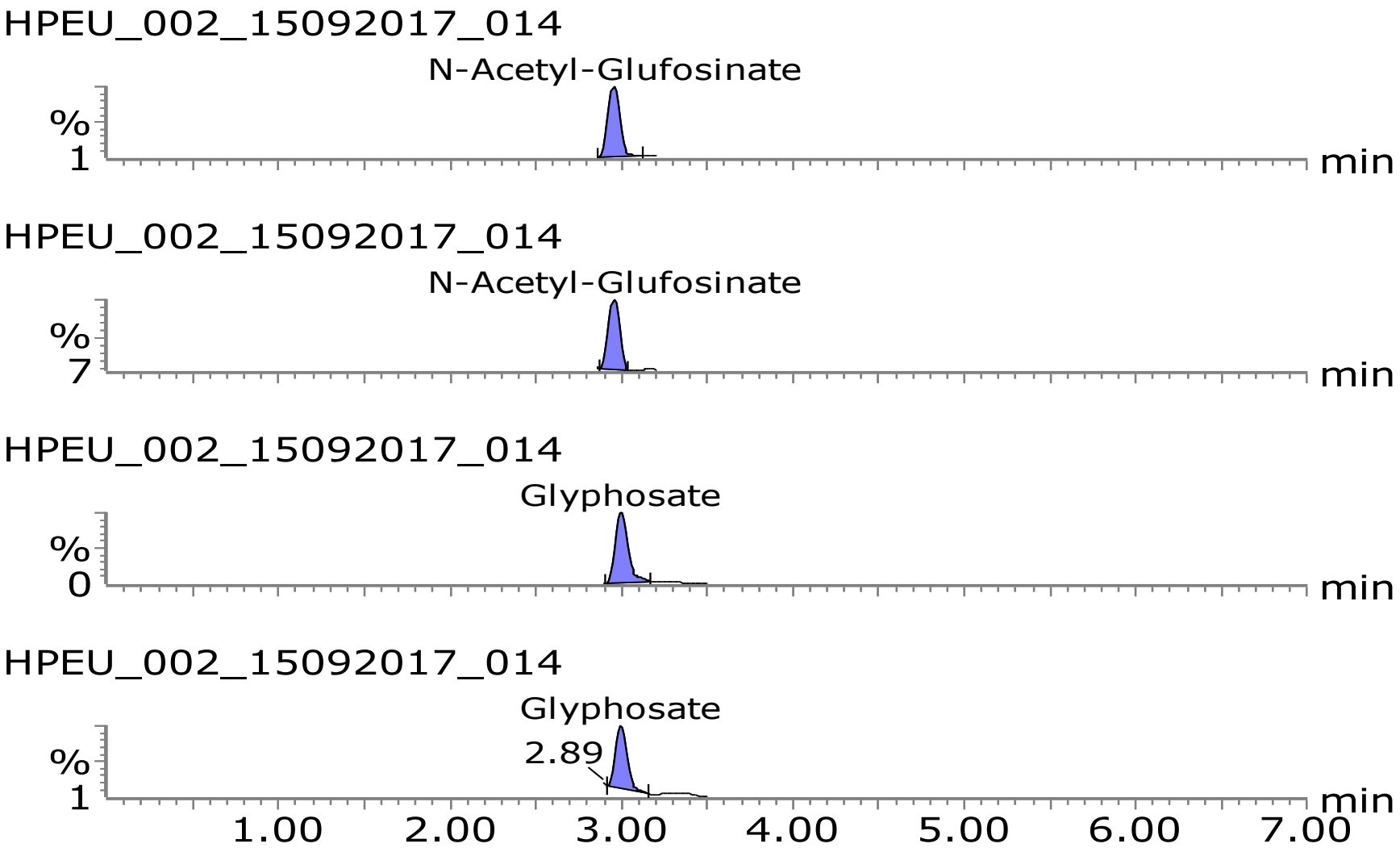
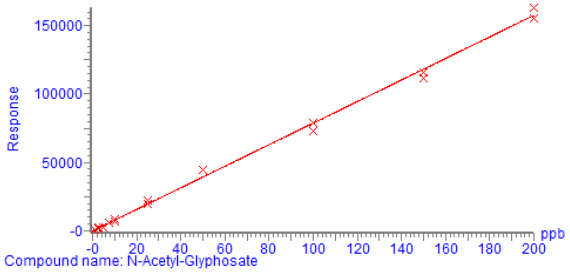


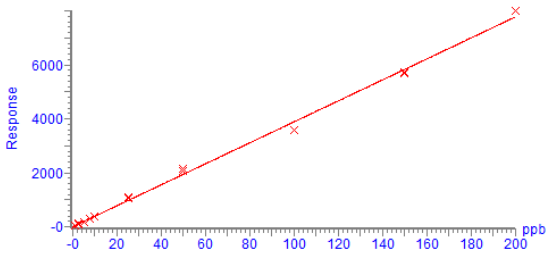
Calibration in spinach

- **Residuals < 20%, R^2 > 0.995**

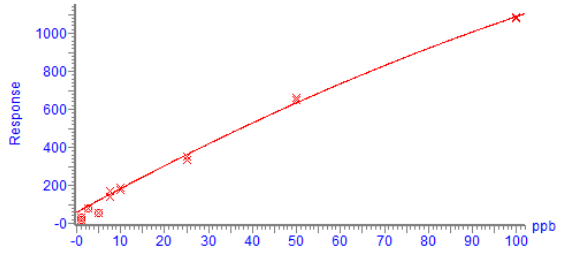
Compound name: N-Acetyl-Glufosinate
Correlation coefficient: r = 0.997737, r^2 = 0.995479
Calibration curve: 788.889 * x + -159.502
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



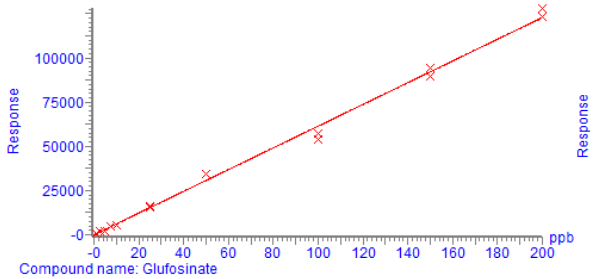
Compound name: Glyphosate
Correlation coefficient: r = 0.998237, r^2 = 0.996477
Calibration curve: 38.9395 * x + 0.278108
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



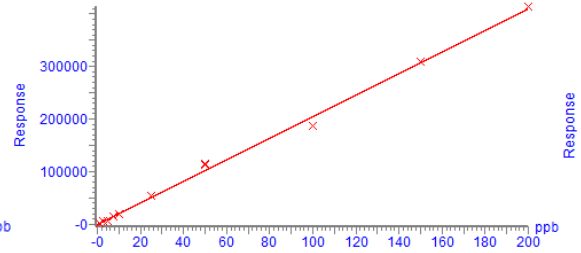
Compound name: Maleic hydrazide
Coefficient of Determination: R^2 = 0.995489
Calibration curve: -0.0243381 * x^2 + 12.735 * x + 58.2459
Response type: External Std, Area
Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x, Axis trans: None



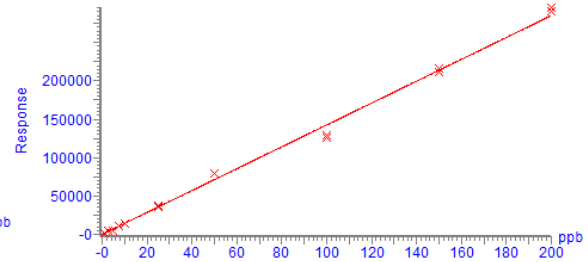
Compound name: N-Acetyl-Glyphosate
Correlation coefficient: r = 0.997325, r^2 = 0.994658
Calibration curve: 614.502 * x + 2.7827
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



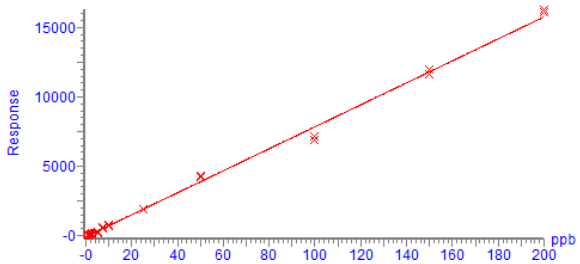
Compound name: MPPA
Correlation coefficient: r = 0.997948, r^2 = 0.995900
Calibration curve: 2051.01 * x + -716.426
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



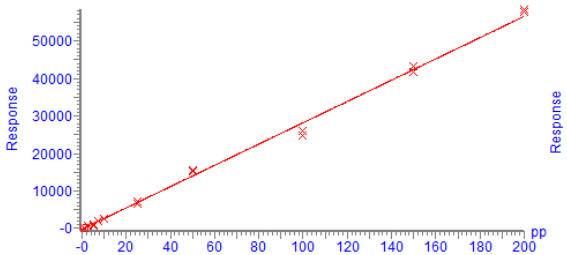
Compound name: Fosethyl Al
Correlation coefficient: r = 0.997503, r^2 = 0.995012
Calibration curve: 1425.29 * x + 64.453
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



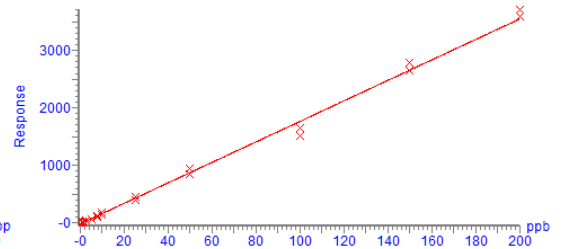
Compound name: Glufosinate
Correlation coefficient: r = 0.997631, r^2 = 0.995267
Calibration curve: 79.2335 * x + -75.6364
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Compound name: Ethephon
Correlation coefficient: r = 0.997604, r^2 = 0.995214
Calibration curve: 283.618 * x + -166.779
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



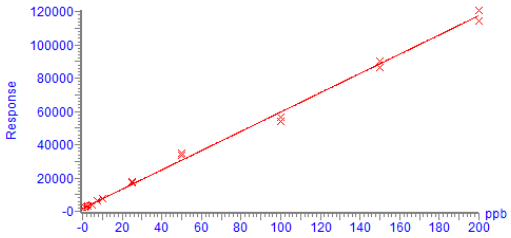
Compound name: AMPA
Correlation coefficient: r = 0.997517, r^2 = 0.995041
Calibration curve: 17.8285 * x + -16.6438
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



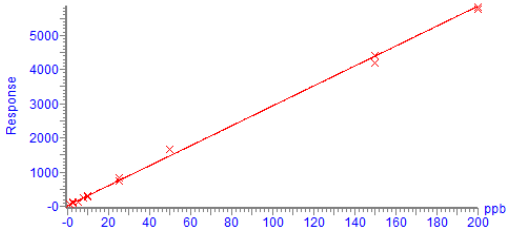
Calibration in spinach

- **Residuals < 20%, R² > 0.995**

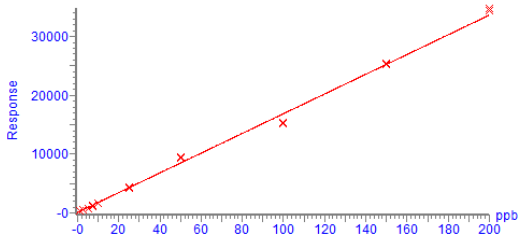
Compound name: Perchlorate
Correlation coefficient: r = 0.997430, r^2 = 0.994867
Calibration curve: 579.71 * x + 1720.79
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Compound name: Chlorate
Correlation coefficient: r = 0.997807, r^2 = 0.995620
Calibration curve: 29.1713 * x + 22.9957
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

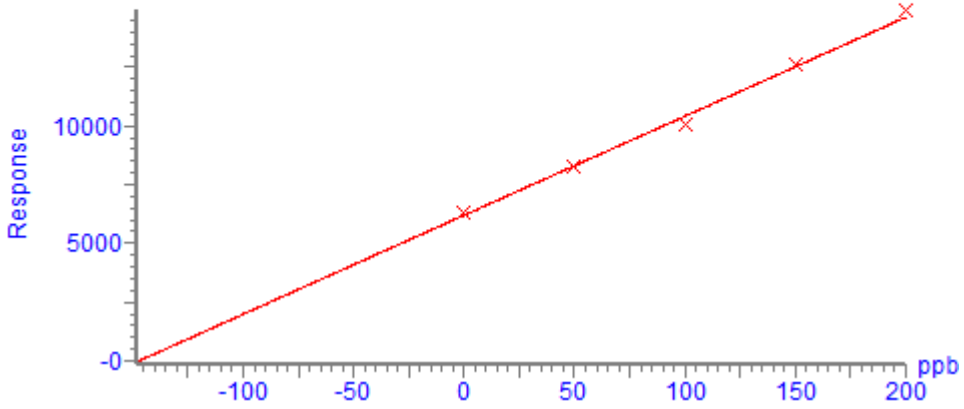


Compound name: Ethephon hydroxy
Correlation coefficient: r = 0.997581, r^2 = 0.995167
Calibration curve: 167.756 * x + 101.963
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



- **Standard addition**

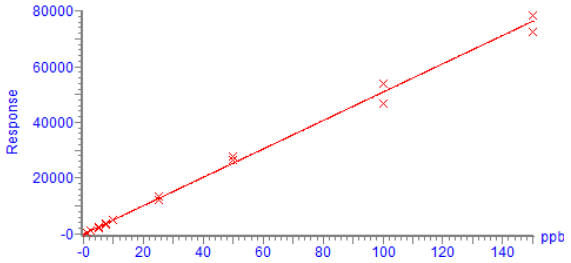
Compound name: Phosphonic acid
Correlation coefficient: r = 0.997988, r^2 = 0.995979
Calibration curve: 42.1393 * x + 6199.54
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None
Standard Addition Concentration : 147.12



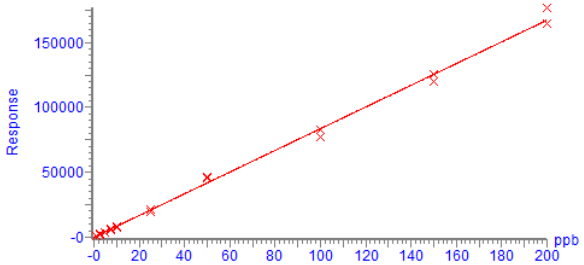
Calibration in lentils

- **Residuals < 20%, R² > 0.995**

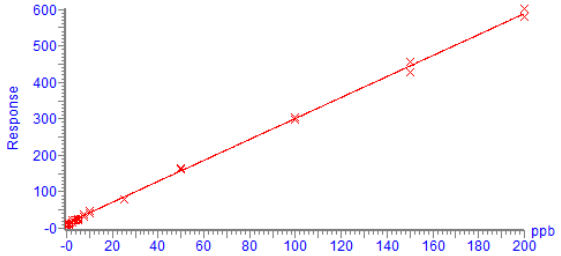
Compound name: N-Acetyl-Glufosinate
Correlation coefficient: r = 0.998105, r^2 = 0.996213
Calibration curve: 509.044 * x + -62.9974
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



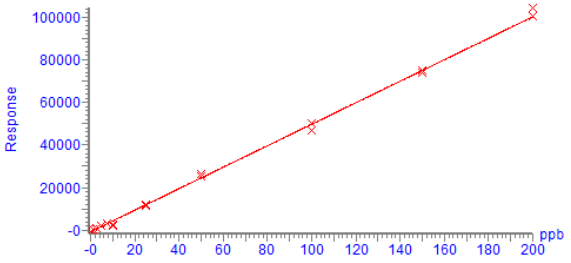
Compound name: MPPA
Correlation coefficient: r = 0.998448, r^2 = 0.996898
Calibration curve: 836.319 * x + 39.7887
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



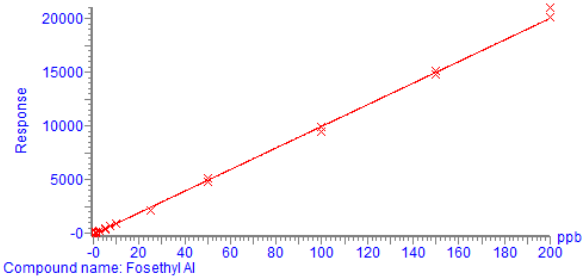
Compound name: AMPA
Correlation coefficient: r = 0.998976, r^2 = 0.997953
Calibration curve: 2.87346 * x + 13.9921
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



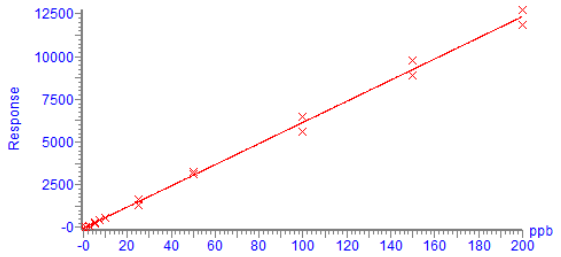
Compound name: N-Acetyl-Glyphosate
Correlation coefficient: r = 0.997375, r^2 = 0.994756
Calibration curve: 504.364 * x + -693.543
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



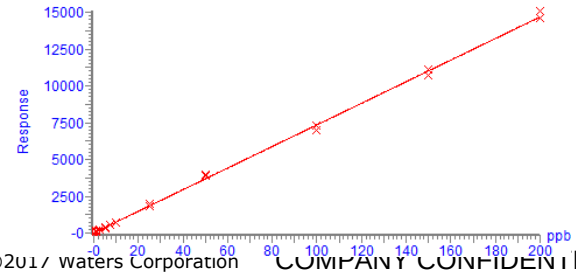
Compound name: Ethephon
Correlation coefficient: r = 0.999095, r^2 = 0.998191
Calibration curve: 100.588 * x + -107.117
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



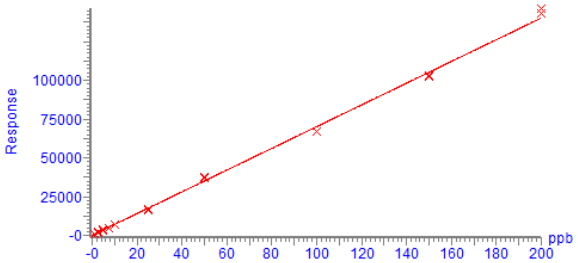
Compound name: Perchlorate
Correlation coefficient: r = 0.998349, r^2 = 0.996702
Calibration curve: 61.9144 * x + -33.9533
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



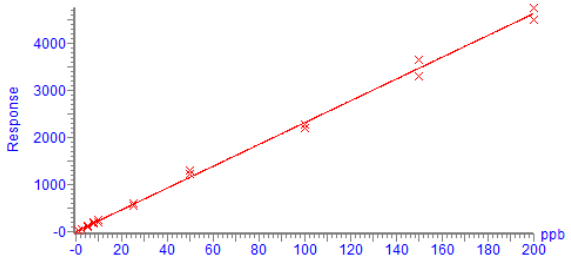
Compound name: Glufosinate
Correlation coefficient: r = 0.999268, r^2 = 0.998537
Calibration curve: 73.3767 * x + 13.2463
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Compound name: Fosethyl Al
Correlation coefficient: r = 0.999108, r^2 = 0.998216
Calibration curve: 700.869 * x + 31.9699
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



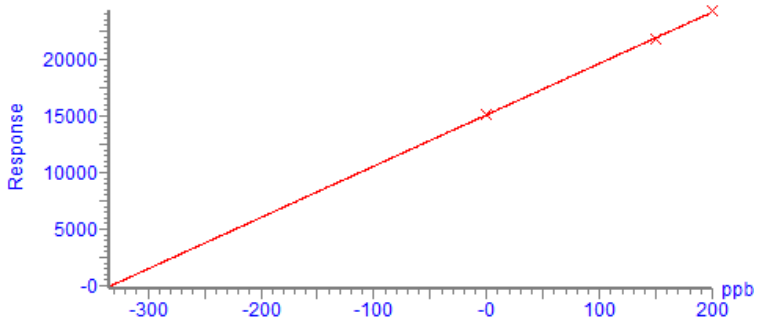
Compound name: Chlorate
Correlation coefficient: r = 0.998649, r^2 = 0.997300
Calibration curve: 23.1008 * x + 2.3057
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



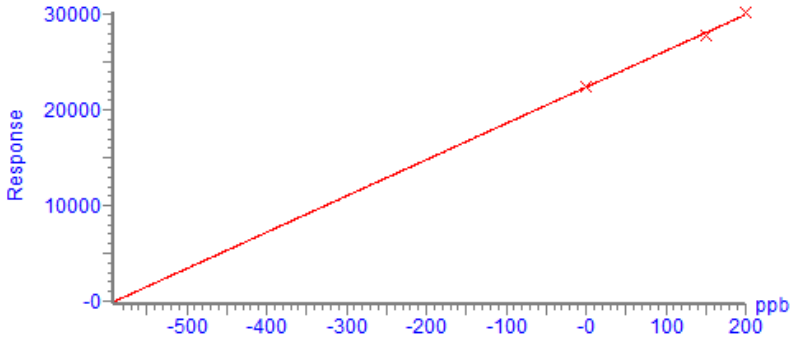
Calibration in lentils

- **Standard addition**

Compound name: Glyphosate
Correlation coefficient: r = 0.999693, r^2 = 0.999387
Calibration curve: 45.3795 * x + 15108.9
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None
Standard Addition Concentration : 332.945



Compound name: Phosphonic acid
Correlation coefficient: r = 0.998386, r^2 = 0.996775
Calibration curve: 37.9855 * x + 22407.3
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None
Standard Addition Concentration : 589.891



Maintaining peak shape by removing metals

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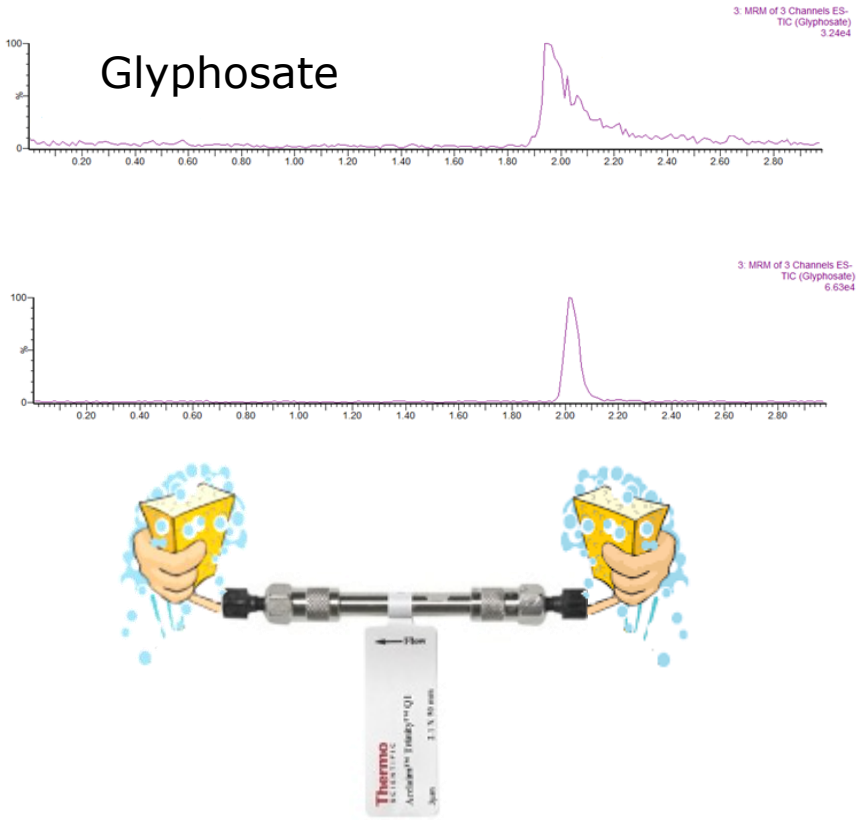
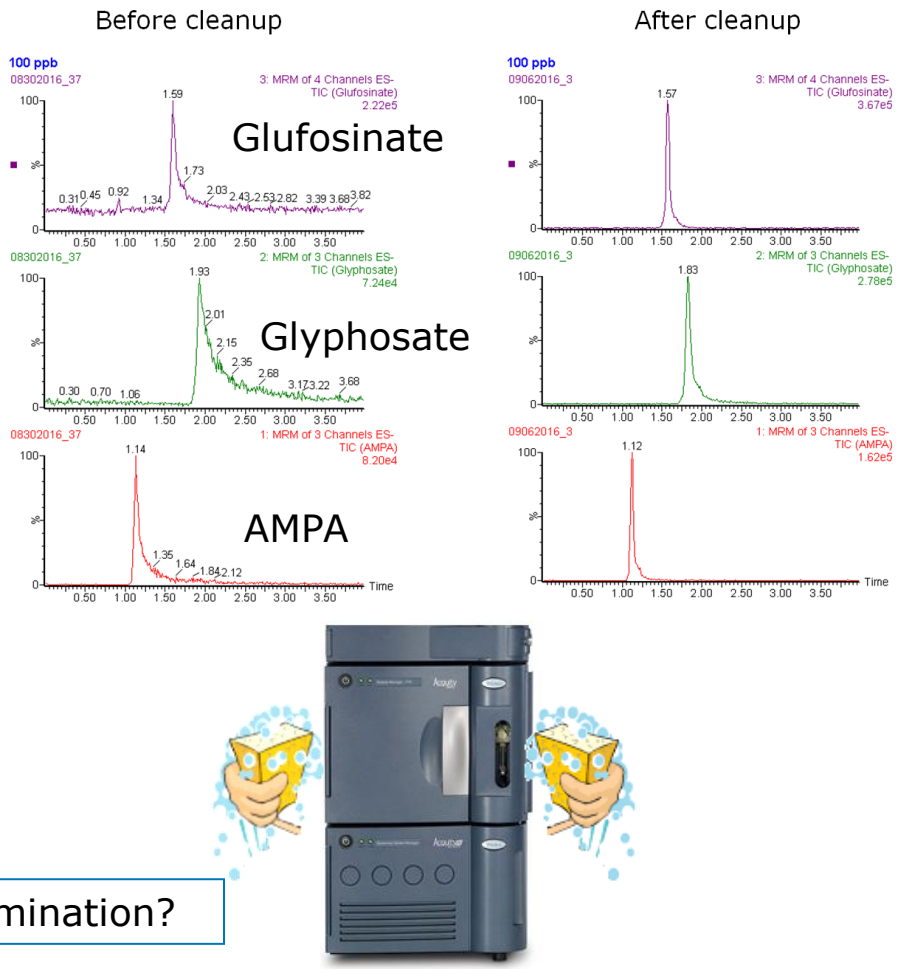
- Before and after flushing column



- Before and after flushing LC system



Peak tailing glyphosate ⟷ metal ion contamination?

Impact of using an UPLC system with an inert flow path: ACQUITY H-Class Bio

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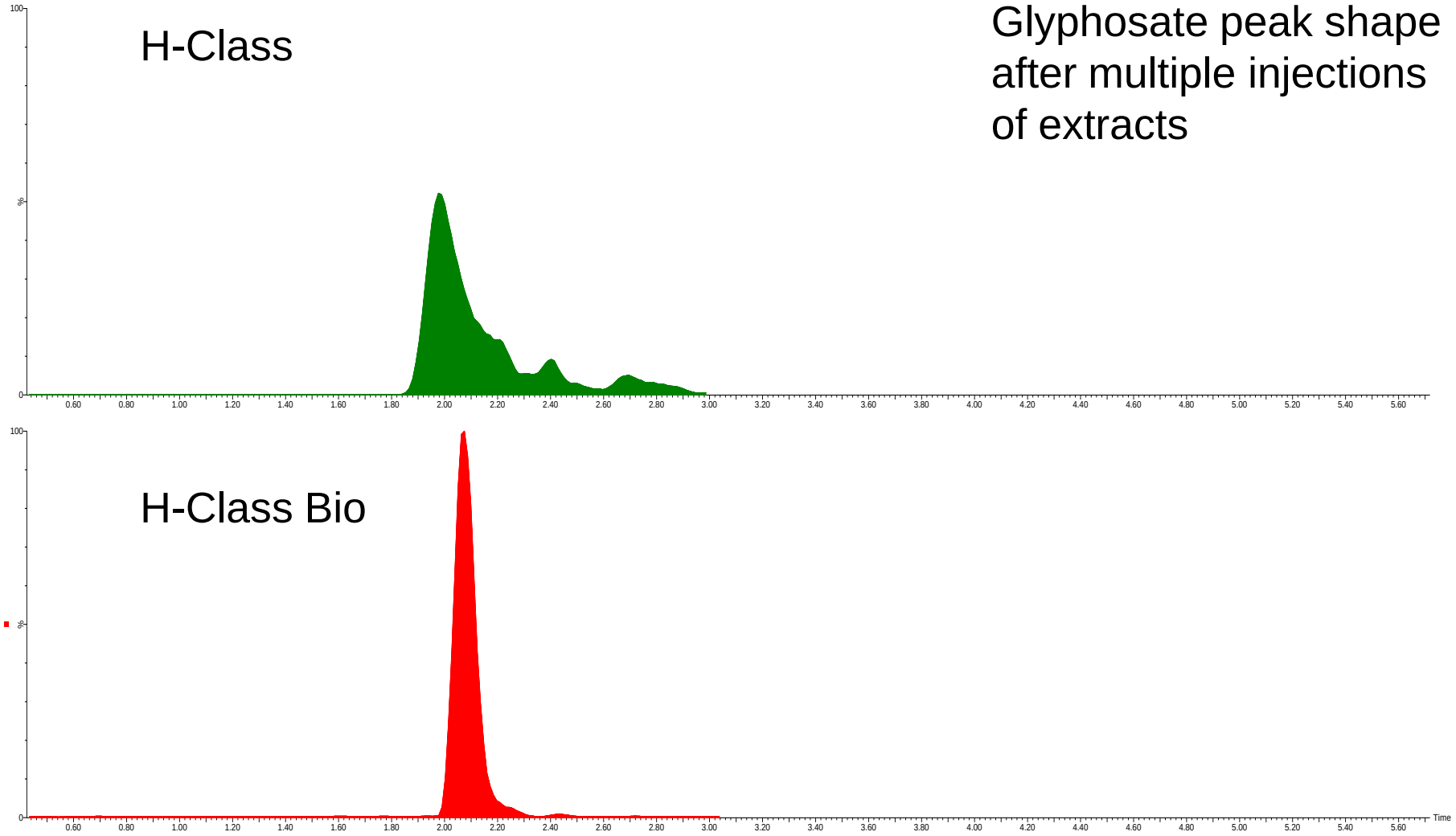

Glyphosate peak shape after multiple injections of extracts

Next Steps

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- Continue to evaluate the performance of the prototype column on different ACQUITY UPLC platforms
- Commercialise as a product
  - Batch testing
- Investigate cleanup options
- Validate UPLC-MS/MS performance (accuracy and repeatability) based upon spiking a range of representative commodities at concentrations relevant for checking MRL compliance
  - Xevo TQ-XS
  - Xevo TQ-S micro

Conclusions

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- The Xevo TQ-XS offers sufficient sensitivity for determination of a range of anionic, polar pesticides using two different approaches to sample extraction
- A number of existing chromatographic methods have been evaluated
  - **BEH C18 after FMOC**
    - Good sensitivity in ESI+ but method is complicated and scope limited
  - **Thermo Hypercarb**
    - Good analyte coverage but issues with ease of use and variability in retention and response
  - **Thermo Acclaim Trinity Q1**
    - Good for glyphosate (and some others) but we were unable to generate consistent peak shape for AMPA using QuPPe extracts

Conclusions

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- Other methods using ion chromatography/HILIC columns have been developed
  - **Metrohm Metrosep A Supp 5**
    - Good analyte coverage but flow rate restricted due to backpressure issues and does not fit in standard Waters column ovens
  - **Shodex HILICpak VT-50 2D**
    - Good analyte coverage, quantitative performance and repeatability but flow rate restricted due to backpressure issues and not suitable for perchlorate
      - Chlorate and perchlorate can be analysed in a separate method
  - **Waters prototype column**
    - Good analyte coverage, repeatability and chromatographic and quantitative performance

Acknowledgements

- Benjamin Wuyts
- Dimple Shah
- Euan Ross
- Eimear McCall
- Jacob Fairchild
- NofaLab Laboratories, Netherlands
- Primoris, Belgium
- GALAB Laboratories, Germany