LC-MS/MS Quantitation of Underivatized Glyphosate and Other Polar Pesticides

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SCIEX

Overview - Glyphosate Analysis

- Glyphosate, broad spectrum herbicide used
  - Against weeds and grasses
  - As desiccants to dry off crops before harvest
  - On transgenic crops resistant to glyphosate / glufosinate
  - Glyphosate ~ 60% of the worldwide herbicide sales
- Before LC-MS there was LC-FLD
  - Derivatization using FMOC and o-Phthalaldehyde
- Early LC-MS/MS methods
  - Flow injection analysis (FIA)
  - Ion chromatography (IC)
- More recent LC-MS/MS methods
  - HILIC
  - Ion exchange chromatography
  - Porous graphitic carbon (Hypercarb)
- Sample preparation
  - QuPPe (M. Anastassiades et al. EURL-SRM (2016) version 9)
  - Dilute and shoot to analyze liquid samples




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The Problem

SRM-Phobia...

SRMs often require...

- **tedious or unfamiliar sample preparation steps e.g.:**
  - *pH adjustments*
  - *Derivatization*
  - *Cleavage*
  - *Head-space sampling*
- **separation/detection techniques different from those typically used in MRMs and <u>often not available</u> in labs, e.g.:**
  - *"Exotic" columns for LC- or GC-separation*
  - *"Exotic" mobile phases (LC)*
  - *"Exotic" Instrumental-configurations*

* Michelangelo Anastassiades at FPRW 2010

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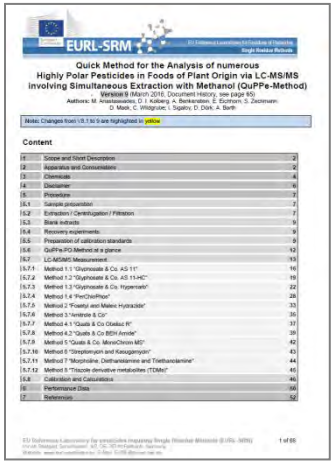
Objective of our Project

- Evaluation of published glyphosate methods
  - Routine quantitation of glyphosate, glufosinate and metabolites using LC-MS/MS without derivatization
  - Target LOQ of 100 ppb for food testing (after QuPPe extraction)
  - Target LOQ of 100 ppt for water testing (dilute and shoot)
  - High confidence in identification due to expected matrix interferences
    - MRM ratios, Full scan MS/MS, Differential Mobility Separation using SelexION® Technology, HR-MS/MS (pending)
  - Detection using the SCIEX QTRAP® 6500+ system

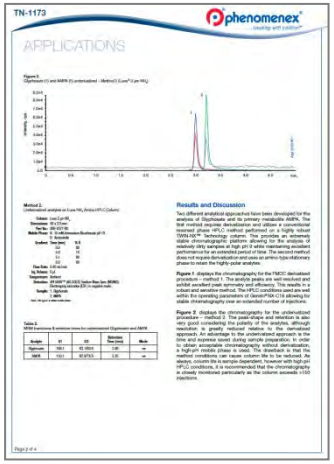

EURL-SRM

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 (March 2016, Document History, see page 65)

Content

M. Anastassiades et al.
QuPPe of EURL-SRM (2016)


TN-1173

phenomenex

APPLICATIONS

Results and Discussion

Phenomenex TN-1173


Journal of Regulatory Science

TDLORG

Regulatory Science

Direct Determination of Glyphosate, Glufosinate, and AMPA in milk by Liquid chromatography/tandem mass spectrometry

Narong Chamkasem[a,*], Cynthia Morris[a], Tiffany Harmon[a]

Abstract

1. Introduction

N. Chamkasem et al.
J. of Regulatory Science (2015)

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MS/MS Conditions

- MS/MS detection using QTRAP® 6500+ system
  - IonDrive™ Turbo V source with ESI probe (negative polarity)
  - IS -4500V, CUR 30 psi, Gas1 50 psi, Gas2 70 psi, CAD high, TEM 700ºC
  - Multiple Reaction Monitoring (Q3 low resolution)
  - Full scan MS/MS with CE -35 V and CES 15

Compound	Q1 (amu)	Q3 (amu)	DP (V)	CE (V)
Glyphosate	168	63	-30	-26
	168	150	-30	-14
	168	124	-30	-16
	168	81	-30	-20
AMPA	110	63	-15	-26
	110	79	-15	-36
	110	81	-15	-16
	110	80	-15	-24
Glufosinate	180	63	-50	-66
	180	95	-50	-24
	180	136	-50	-22
	180	85	-50	-24
MMPA	151	133	-10	-18
	151	63	-10	-44
	151	107	-10	-20
	151	78	-10	-28

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LC Conditions

- Method 1 (EURL-SRM)
  - Modified since the column was not available in the original dimension and particle size
  - Hypercarb 50 x 2.1 mm 3µm
  - Gradient of water/methanol (95/5) + 1% acetic acid and methanol + 1% acetic acid at flow rate 0.2 to 0.4 mL/min
  - Injection of 10 - 50 µL
- Method 2 (US-FDA)
  - Acclaim Trinity Q1 100 x 3 mm 3µm
  - Gradient of water + 50 mM ammonium formate/formic acid (pH=2.9) and acetonitrile at flow rate 0.5 mL/min
  - Injection of 10 - 50 µL
- Method 3 (PHX)
  - LUNA NH_2 50 x 2 mm 3µm
  - Gradient of water + 10 mM ammonium bicarbonate (pH=10) at flow rate 0.4 mL/min
  - Injection of 10 - 50 µL

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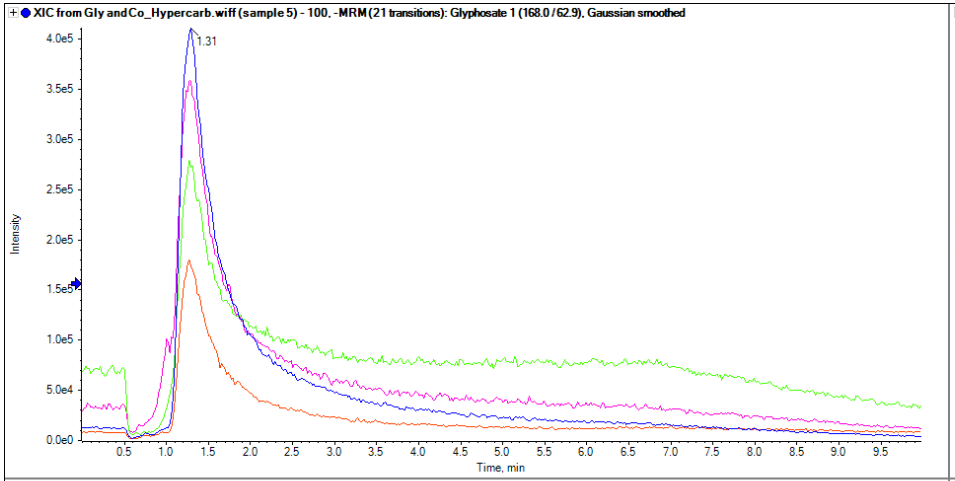
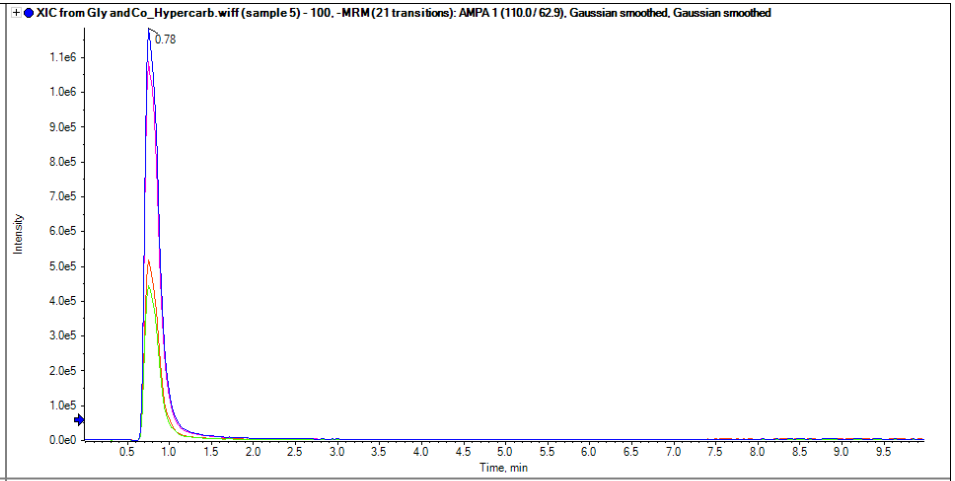
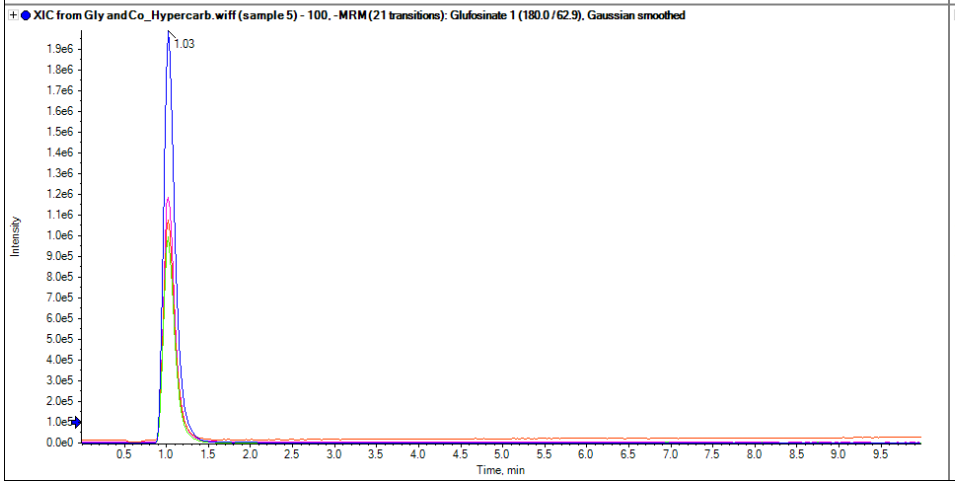
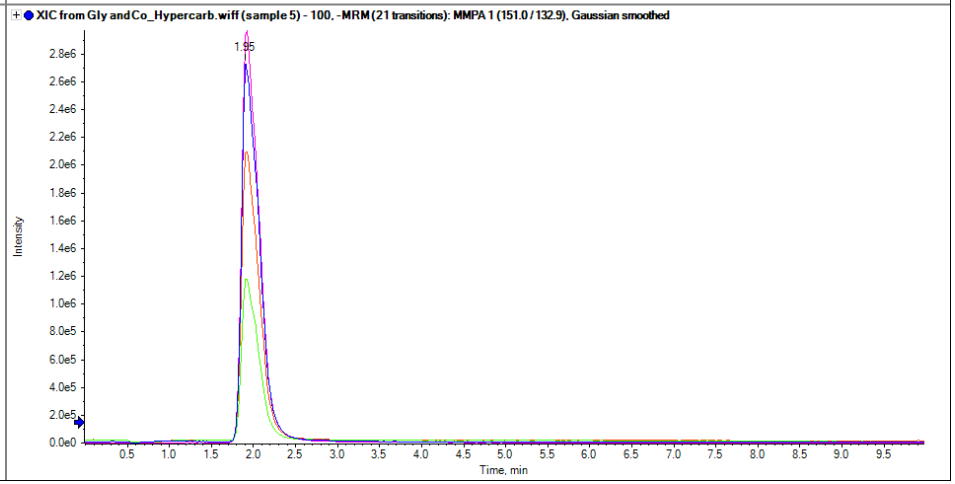
Method 1 (Hypercarb water/methanol + 1% acetic acid)

100 ng/mL Standard









Column was primed with 30 injections of QuPPe extracts of spinach

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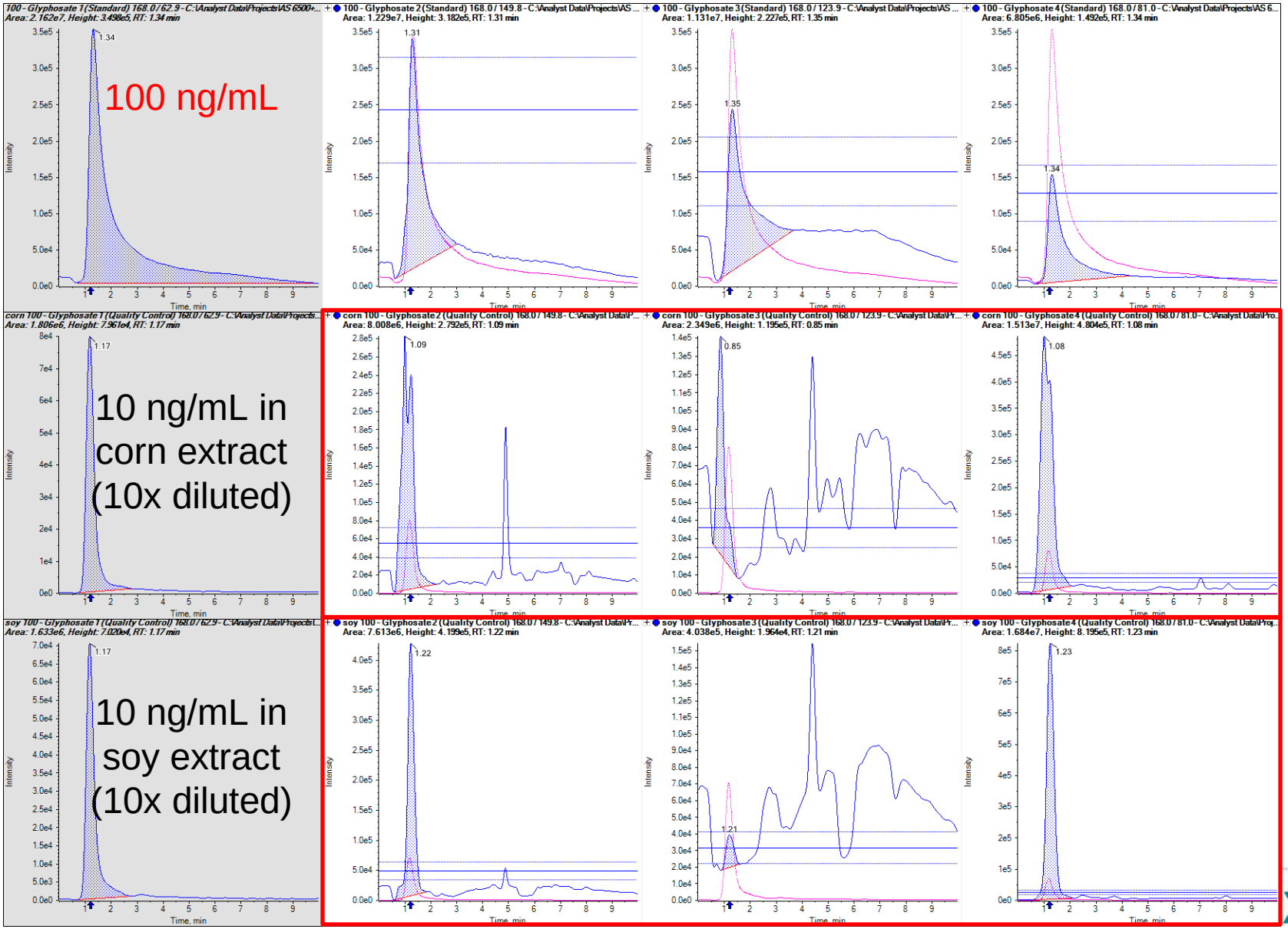
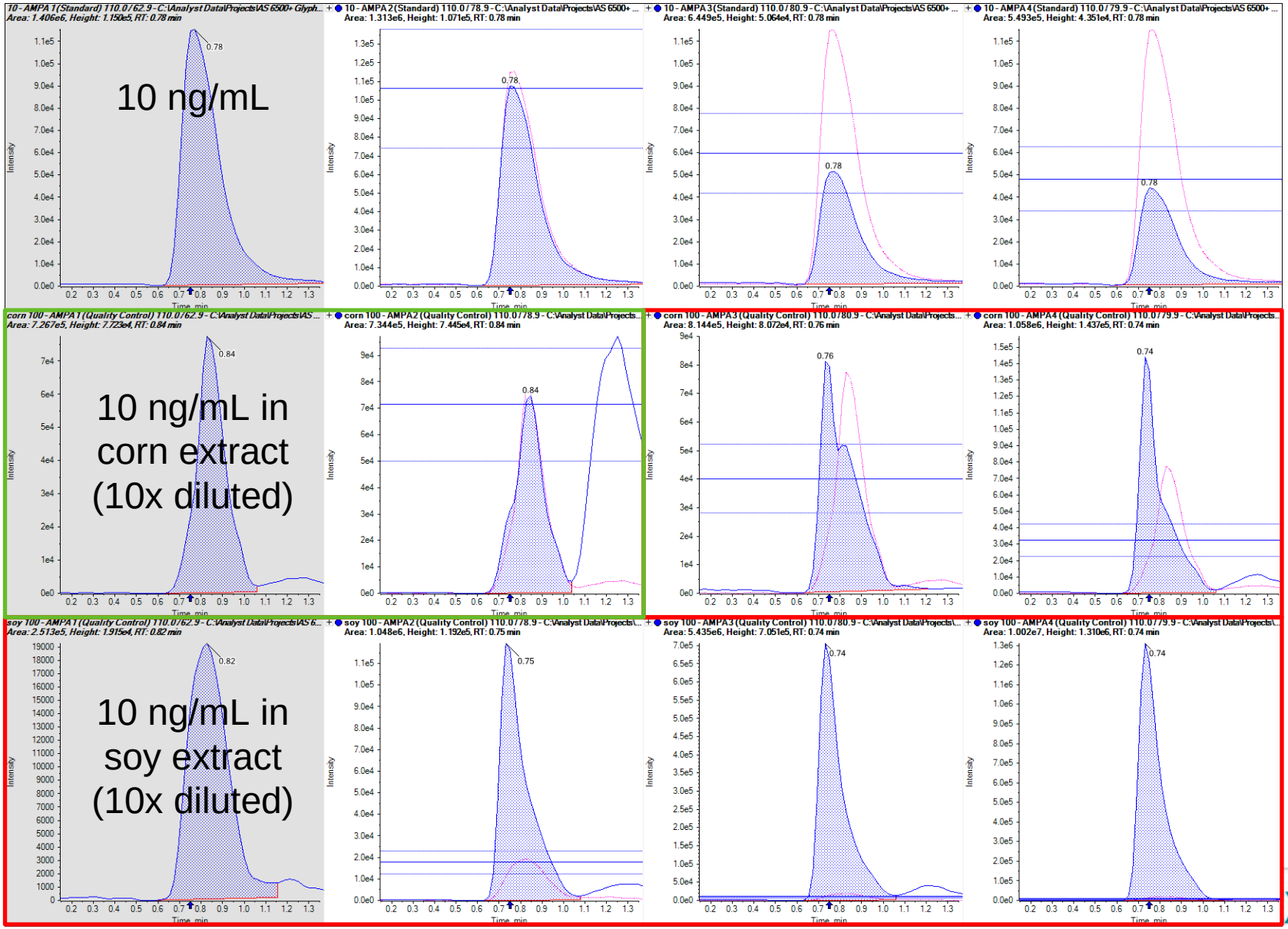
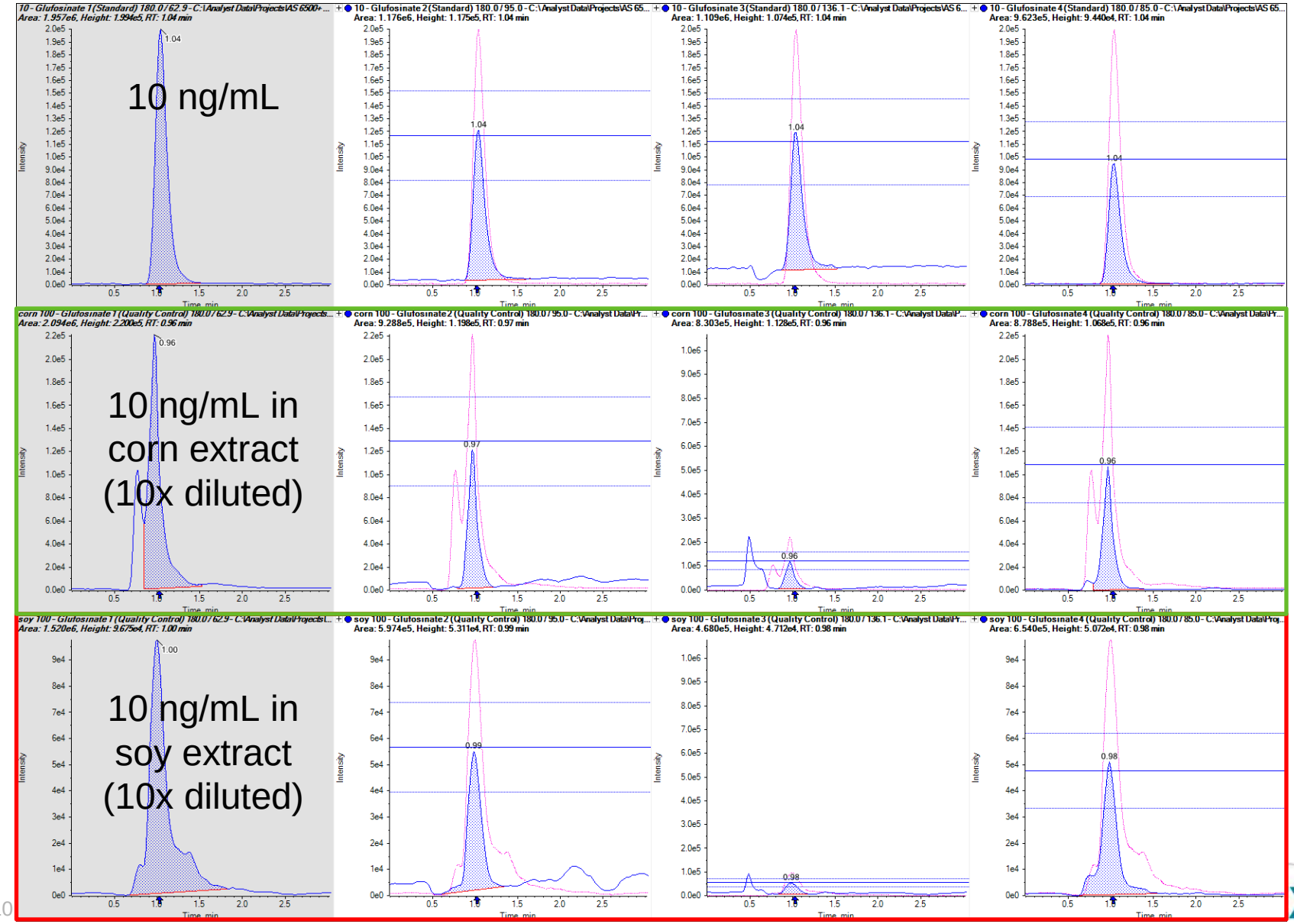
Method 1 Performance – Glyphosate



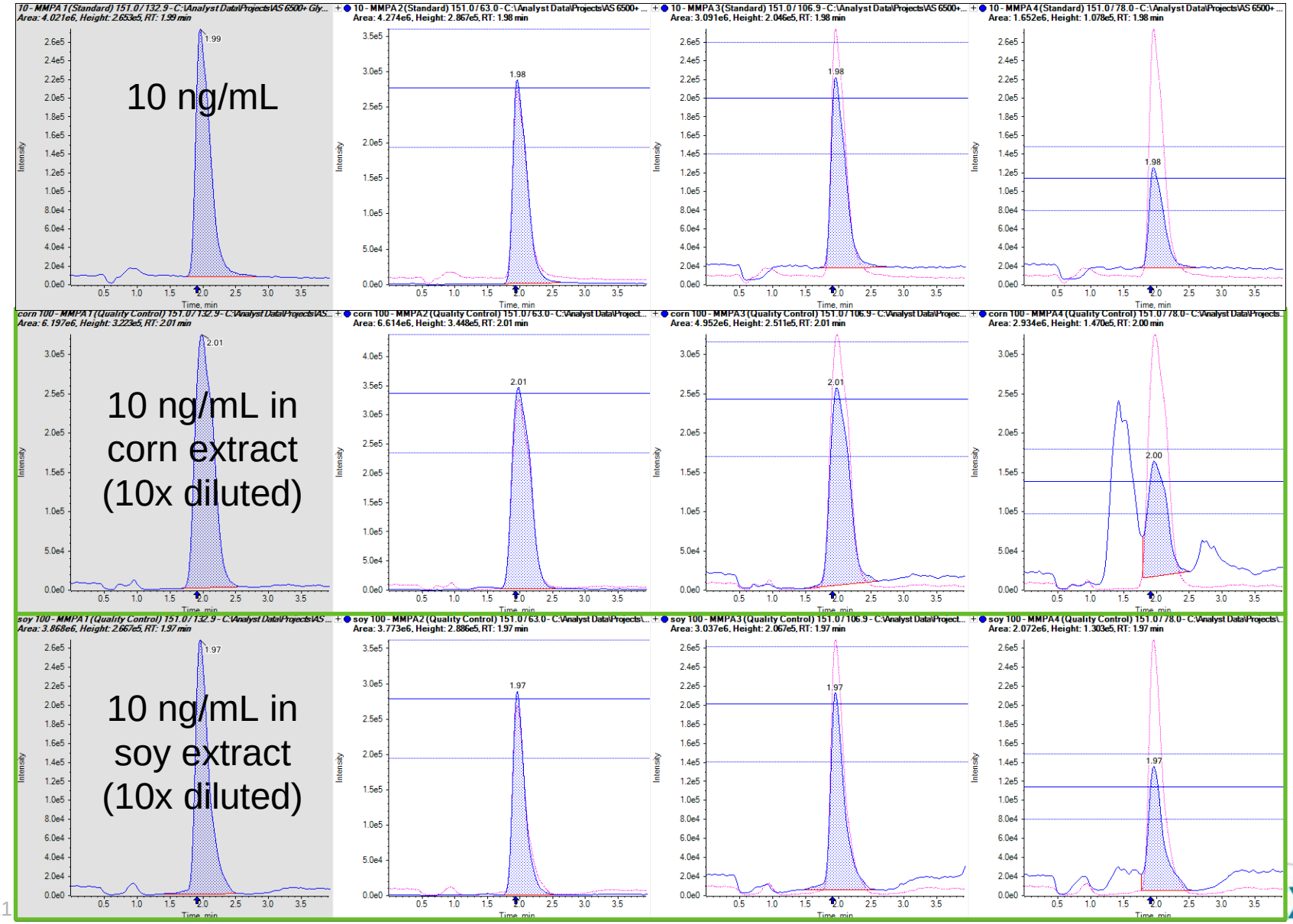
Method 1 Performance – AMPA



Method 1 Performance – Glufosinate

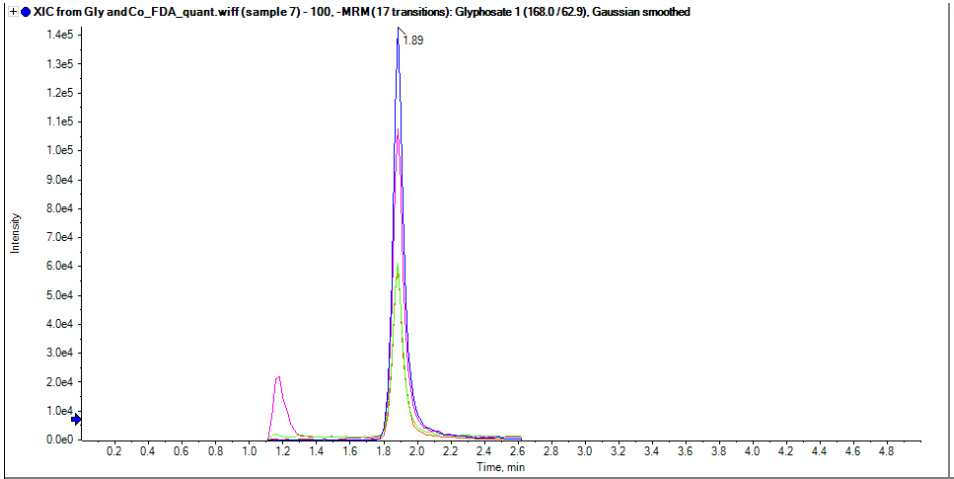


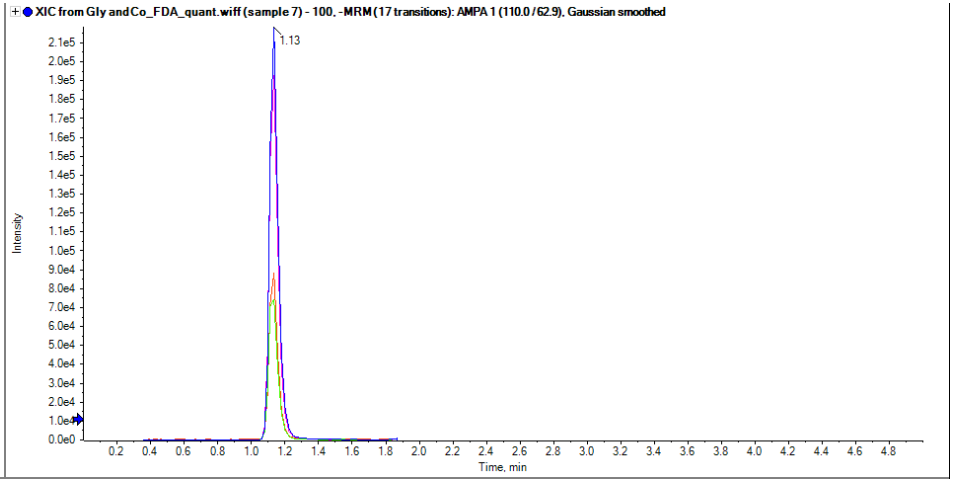
Method 1 Performance – MMPA

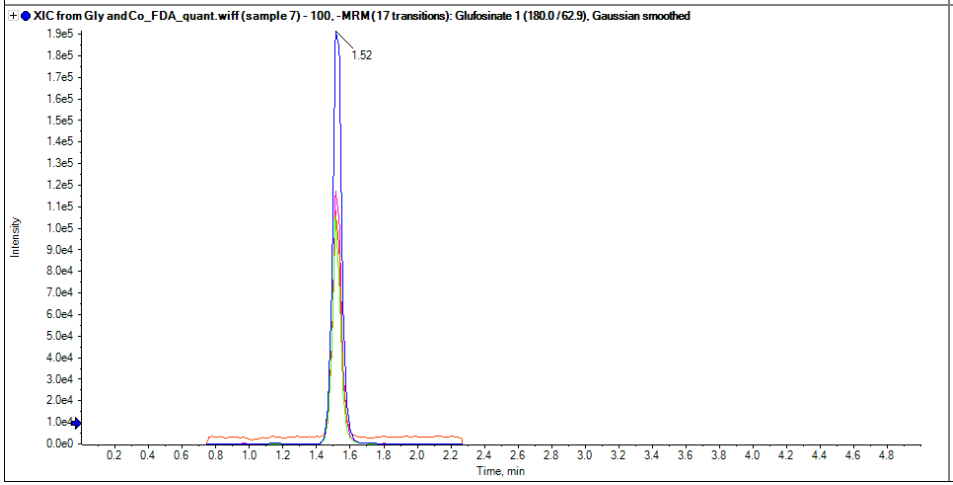


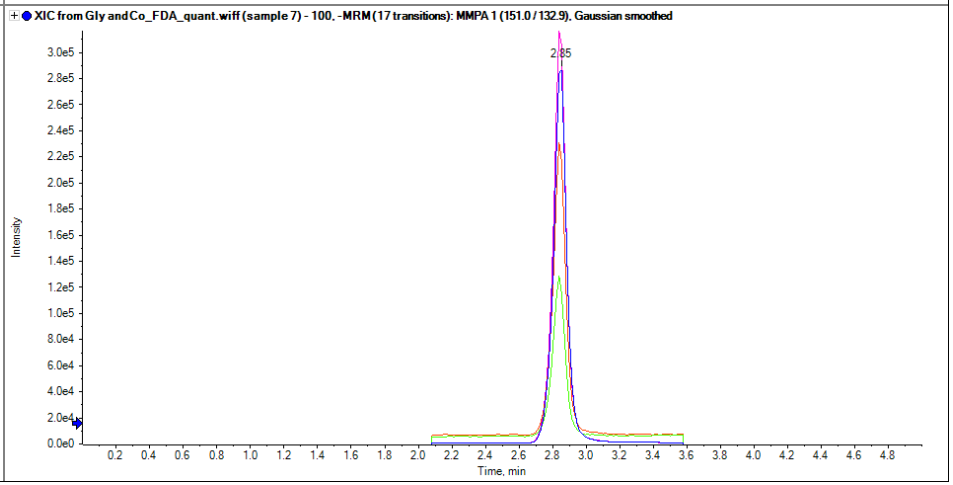
Method 2 (Trinity Q1 ammonium formate pH=2.9)

100 ng/mL Standard









Good separation of compounds to use the *Scheduled* MRM™ algorithm

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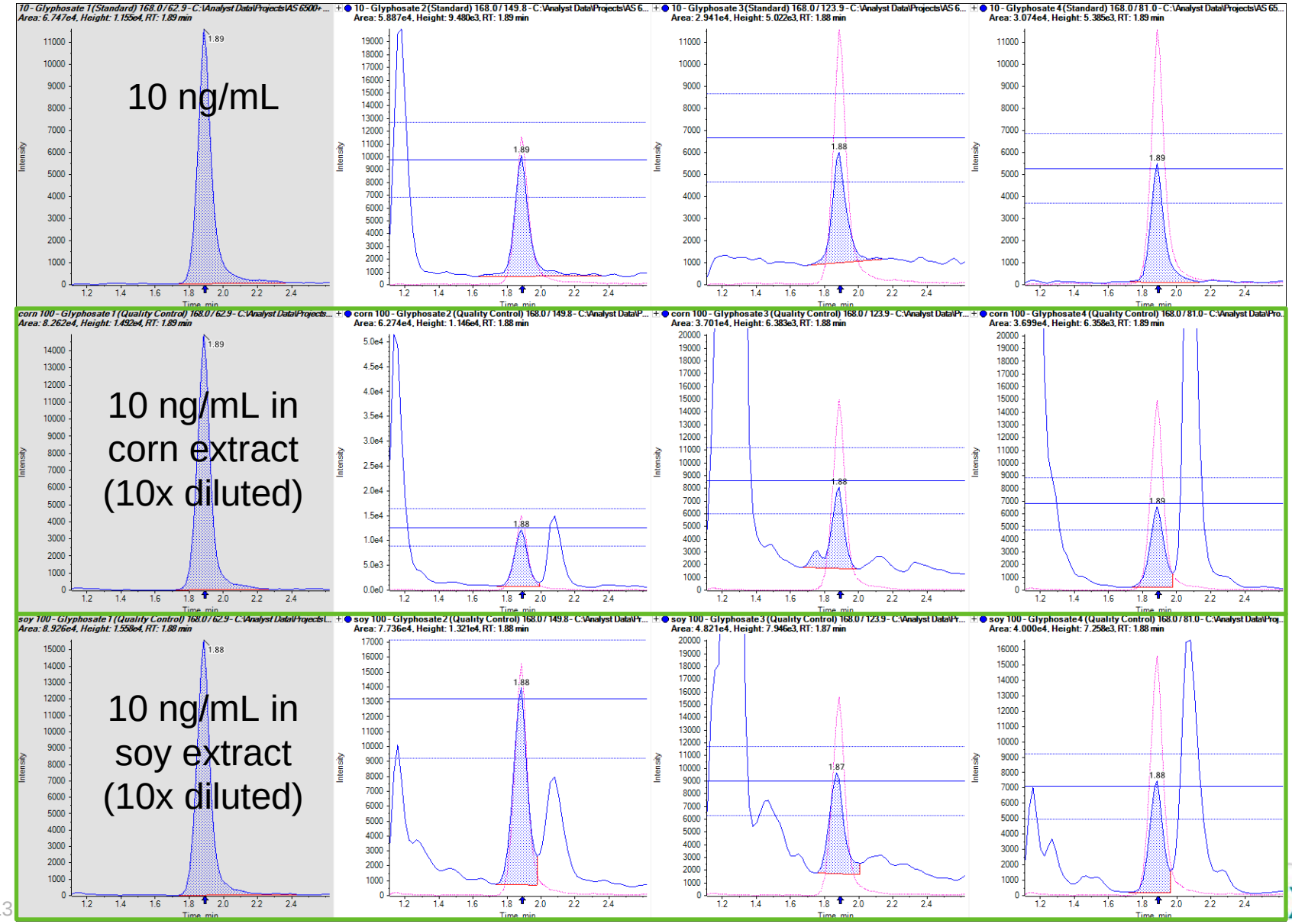
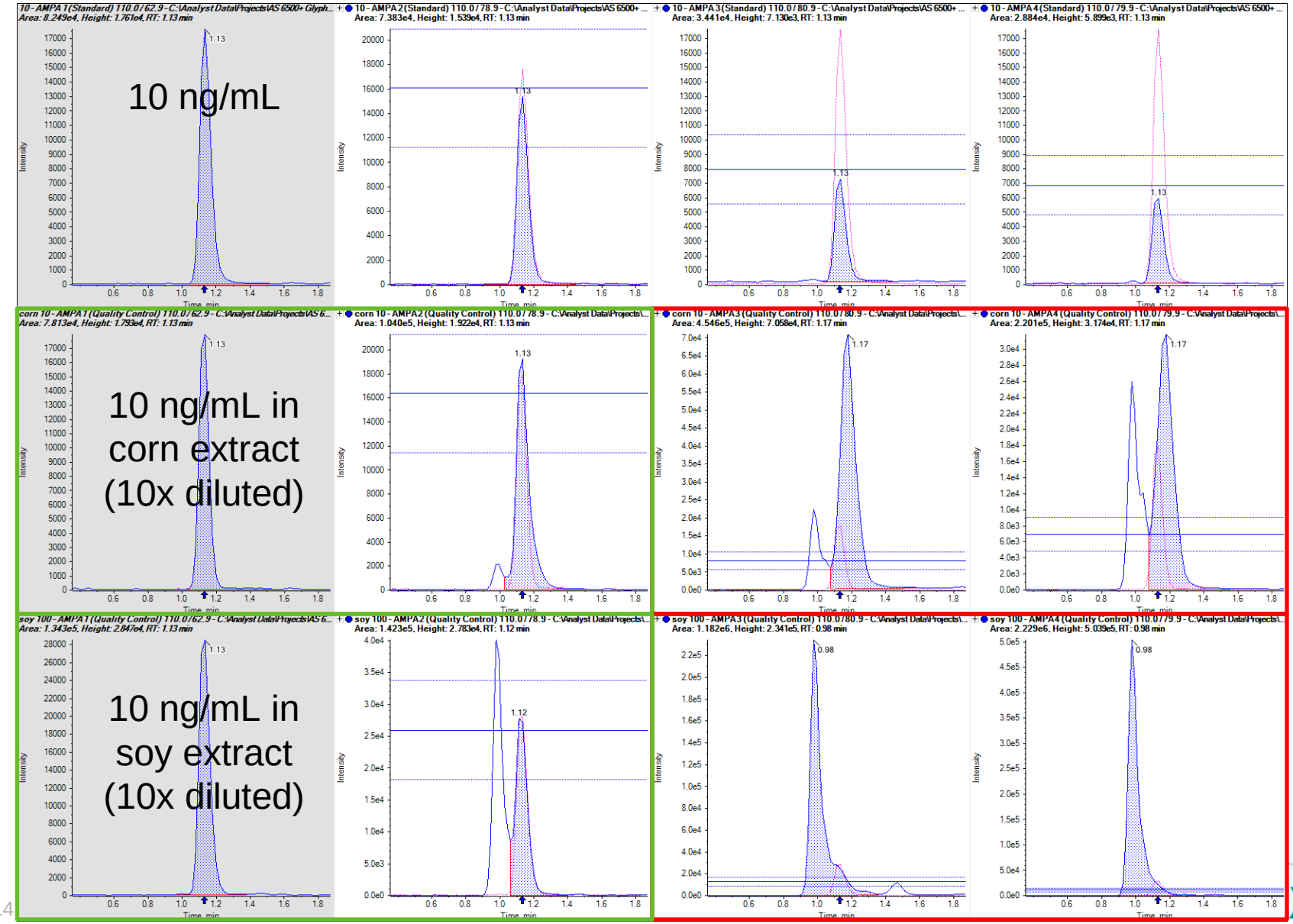
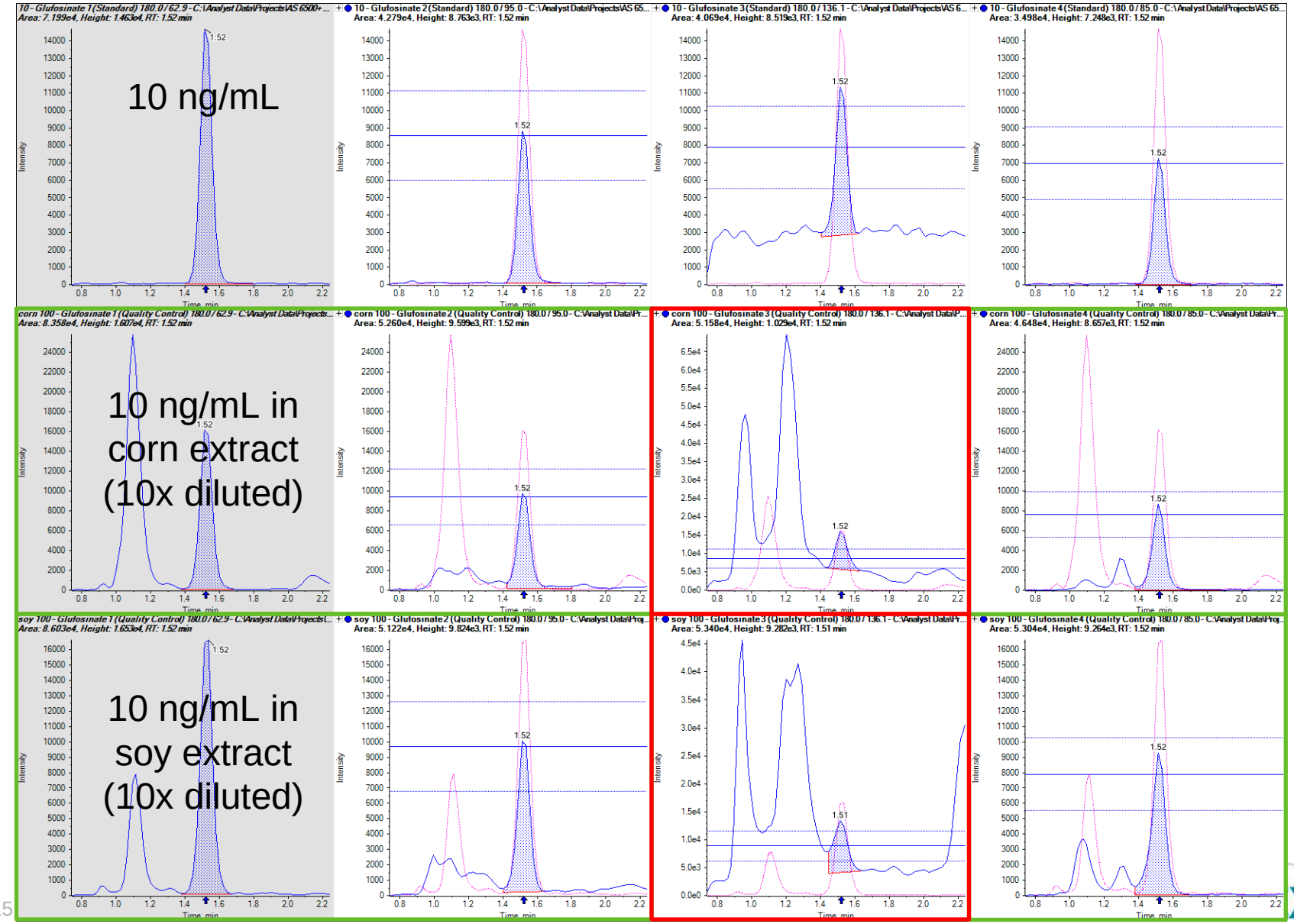
Method 2 Performance – Glyphosate



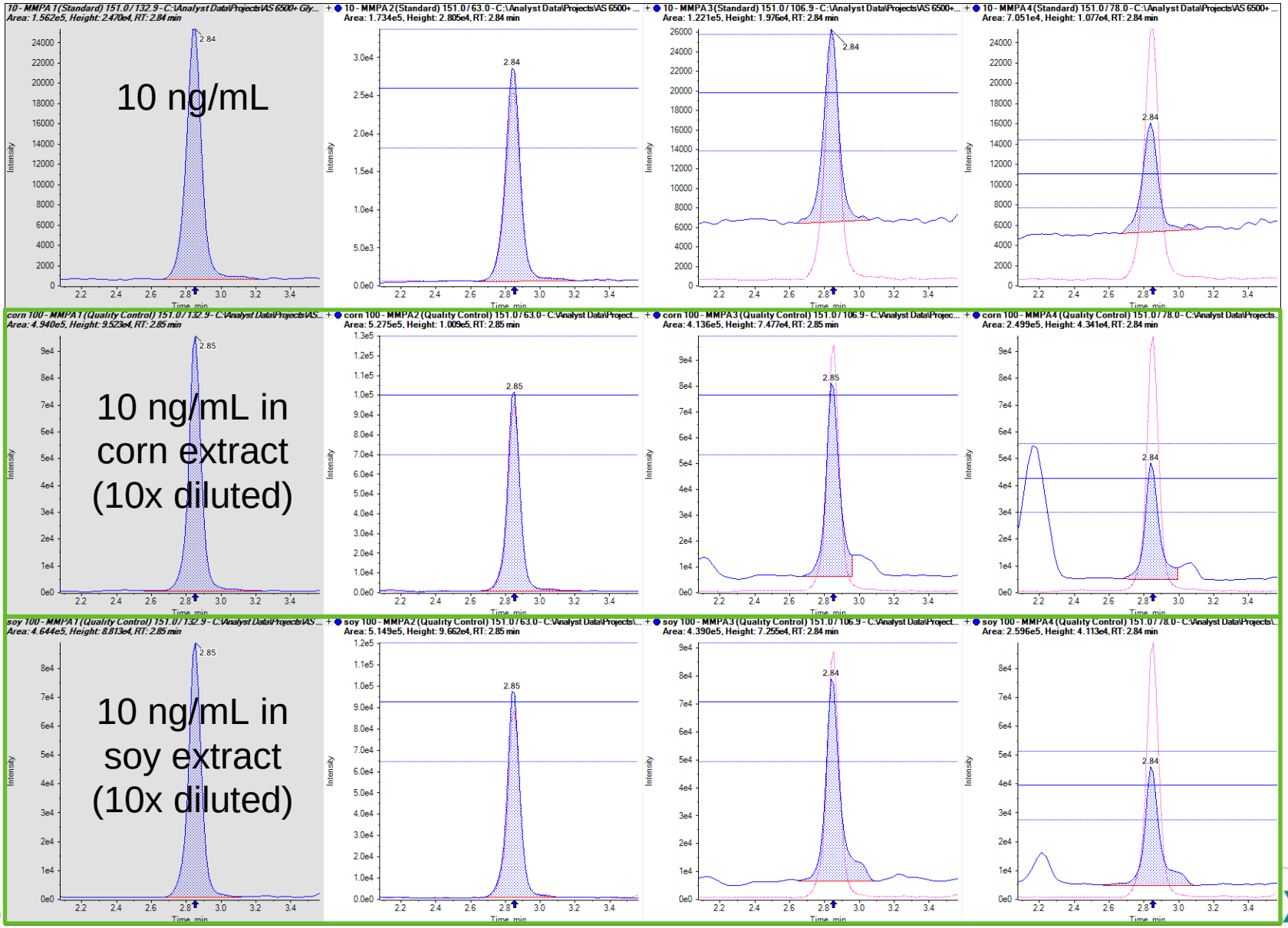
Method 2 Performance – AMPA



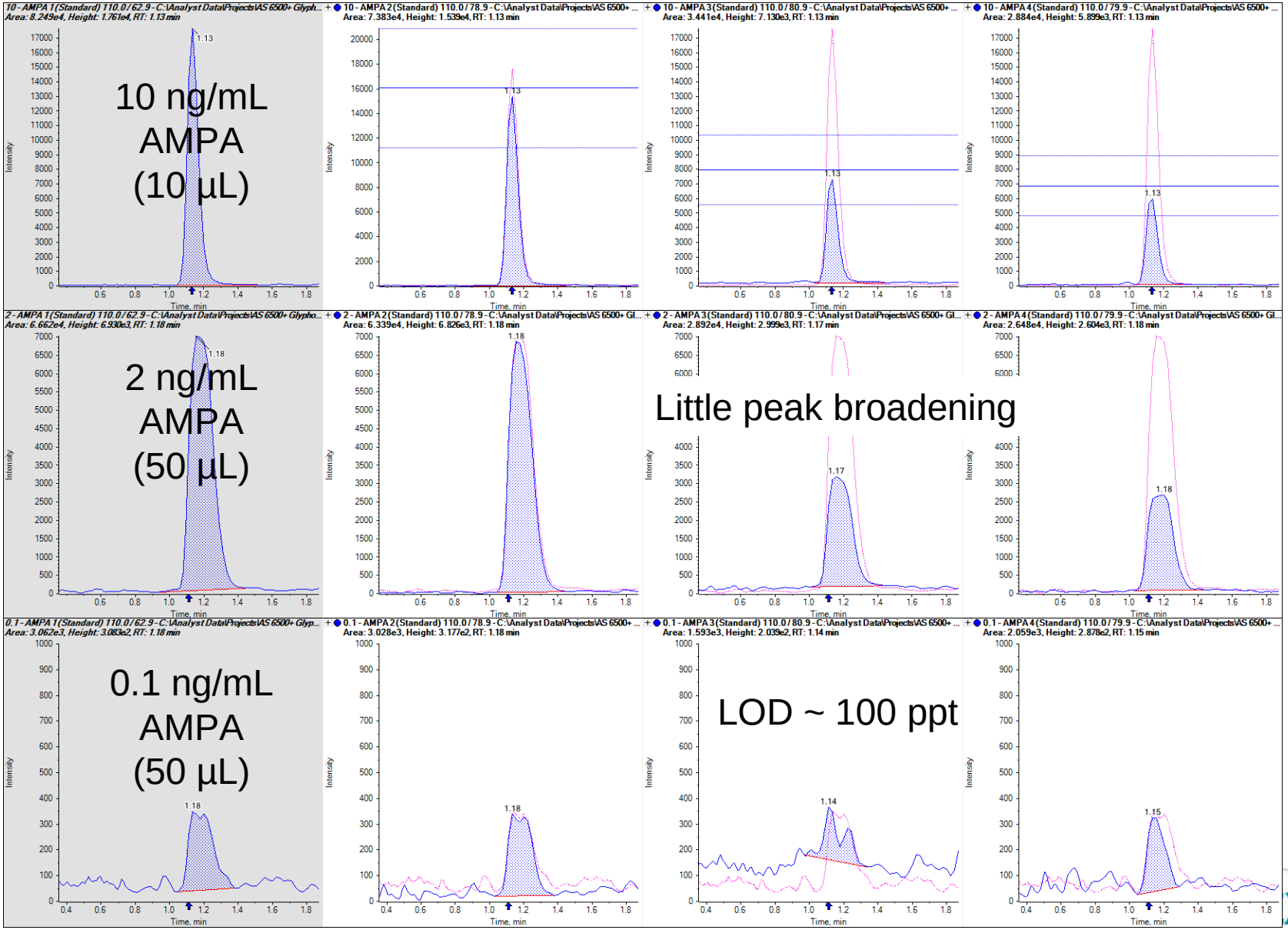
Method 2 Performance – Glufosinate



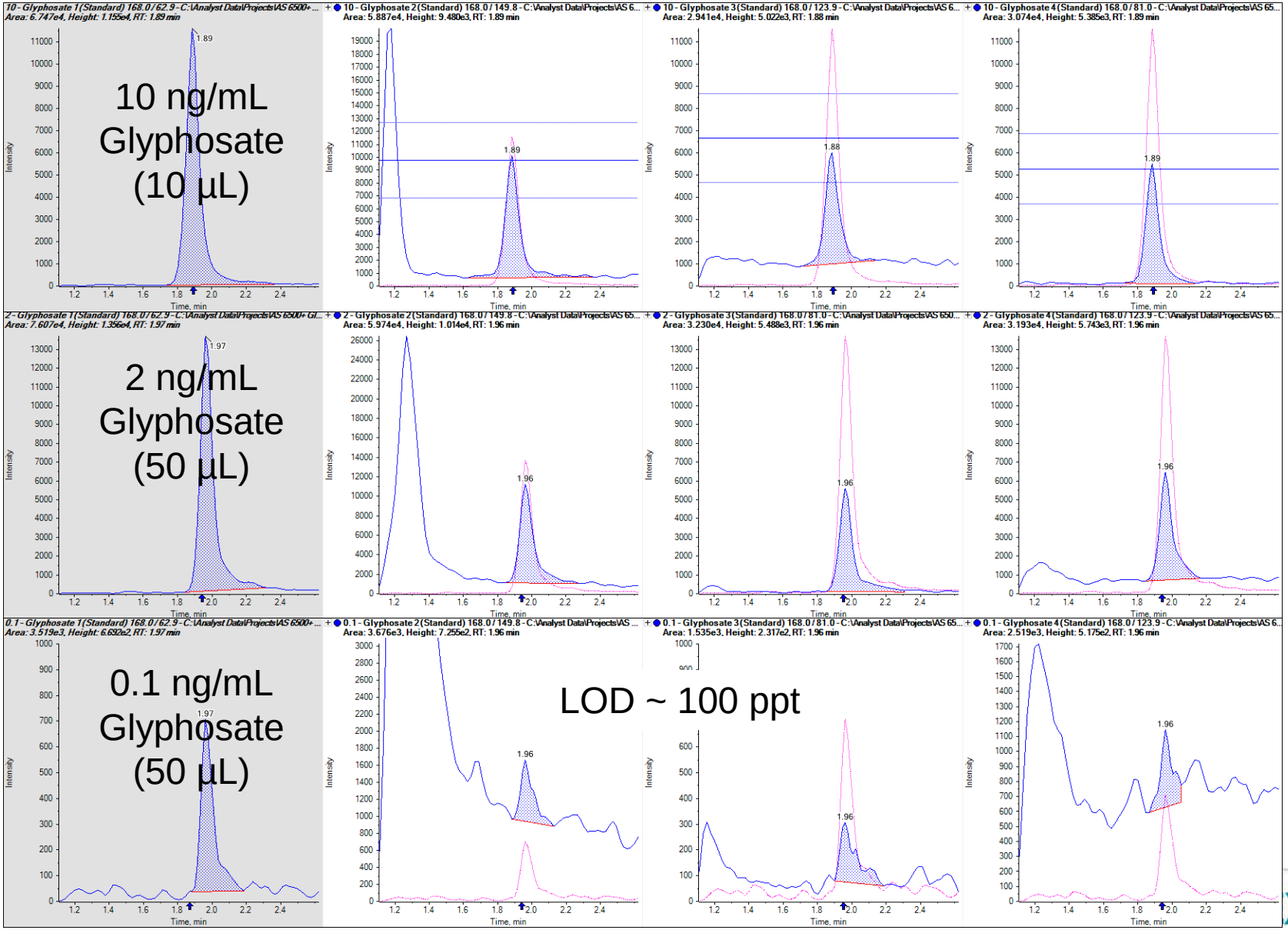
Method 2 Performance – MMPA



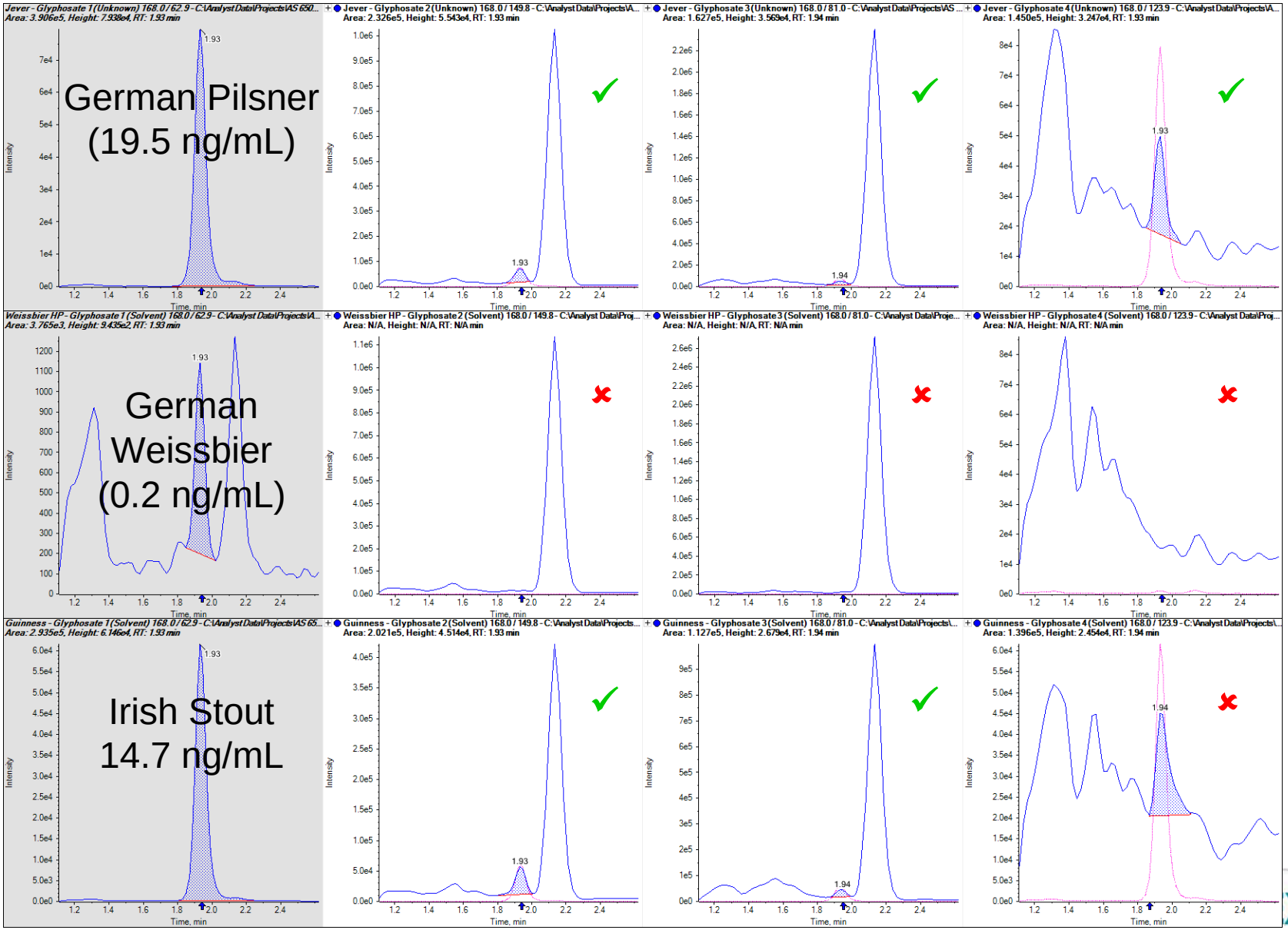
Method 2 Performance – Large Volume Injection (50 µL)



Method 2 Performance – Large Volume Injection (50 µL)

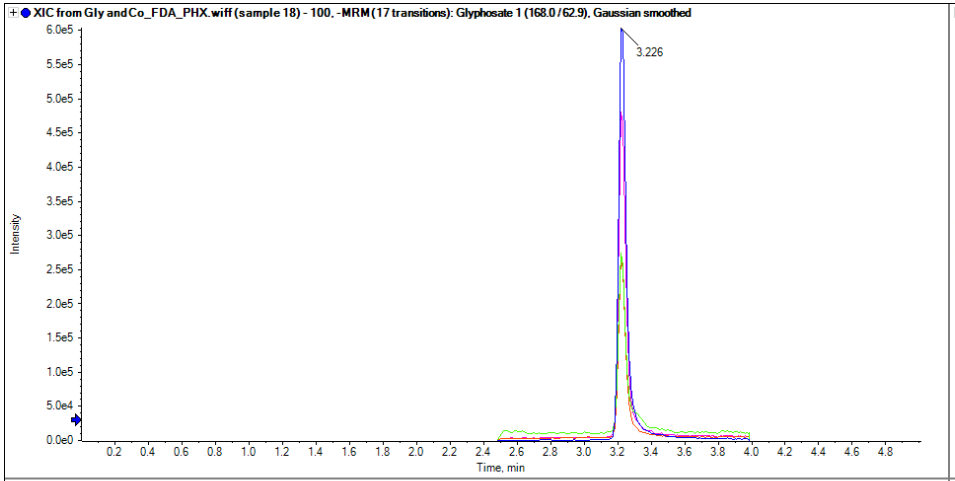


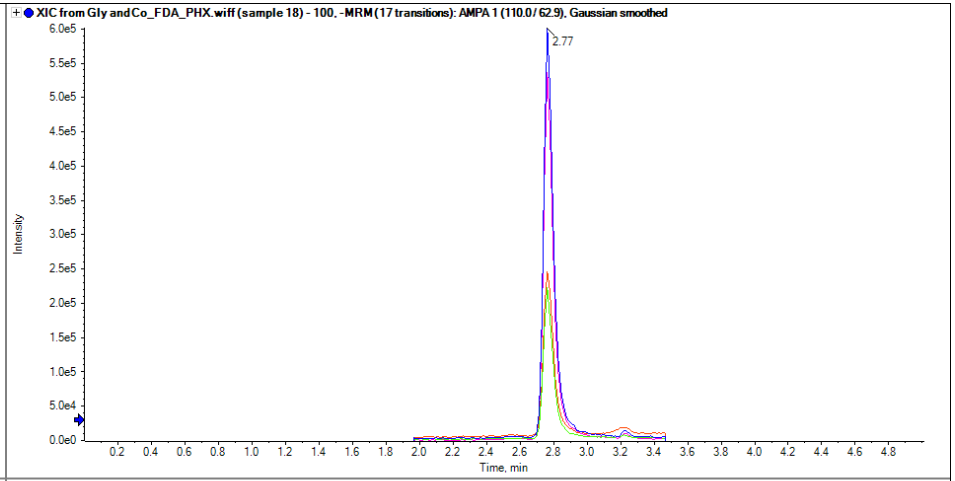
Method 2 Performance – Glyphosate in Beer (2x Dilution)

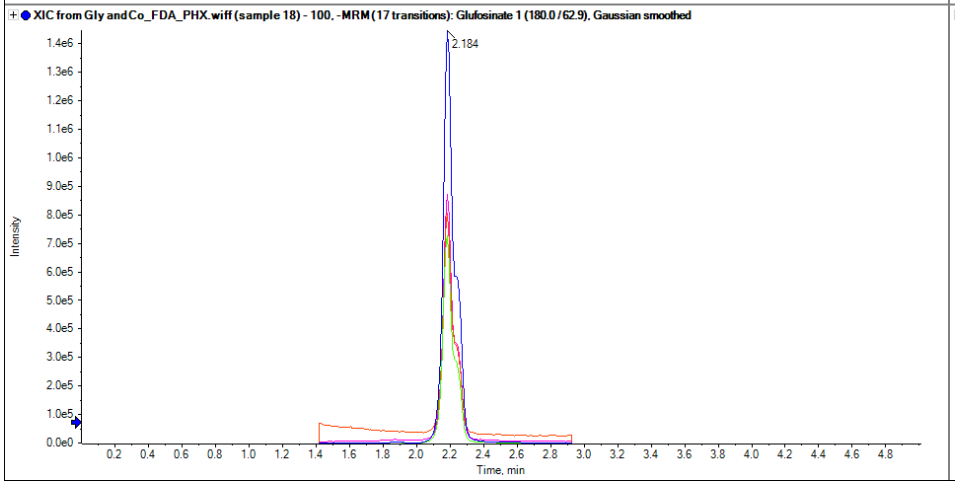


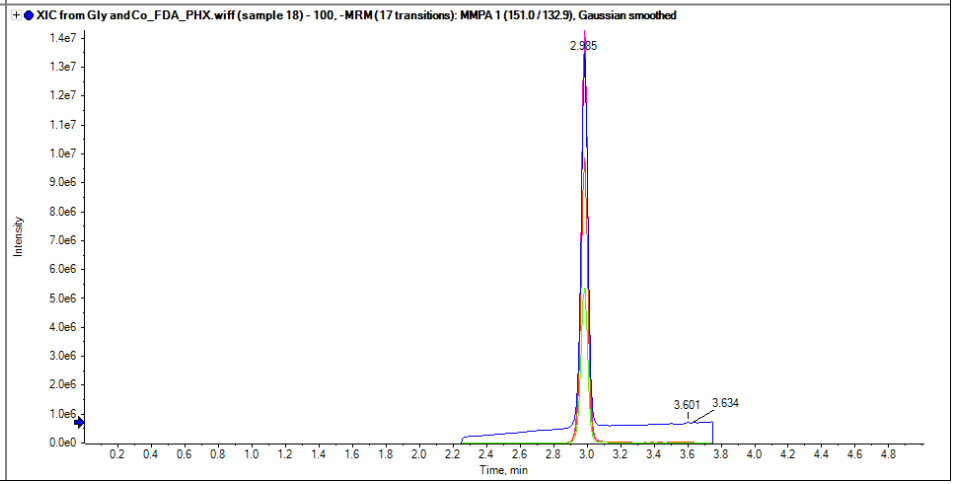
Method 3 (LUNA NH2 ammonium bicarbonate pH=10)

100 ng/mL Standard









Good separation of compounds to use the *Scheduled* MRM™ algorithm

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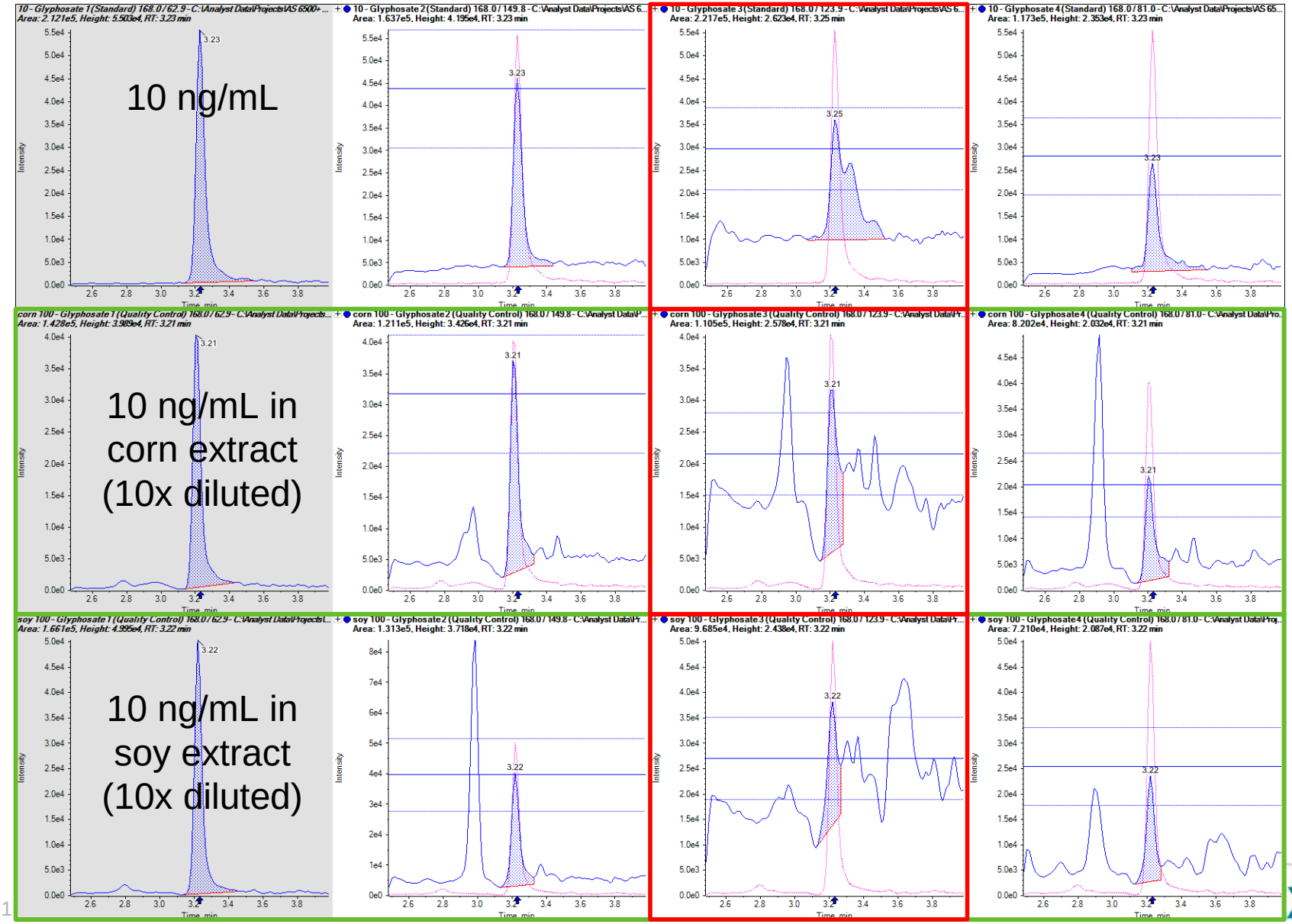
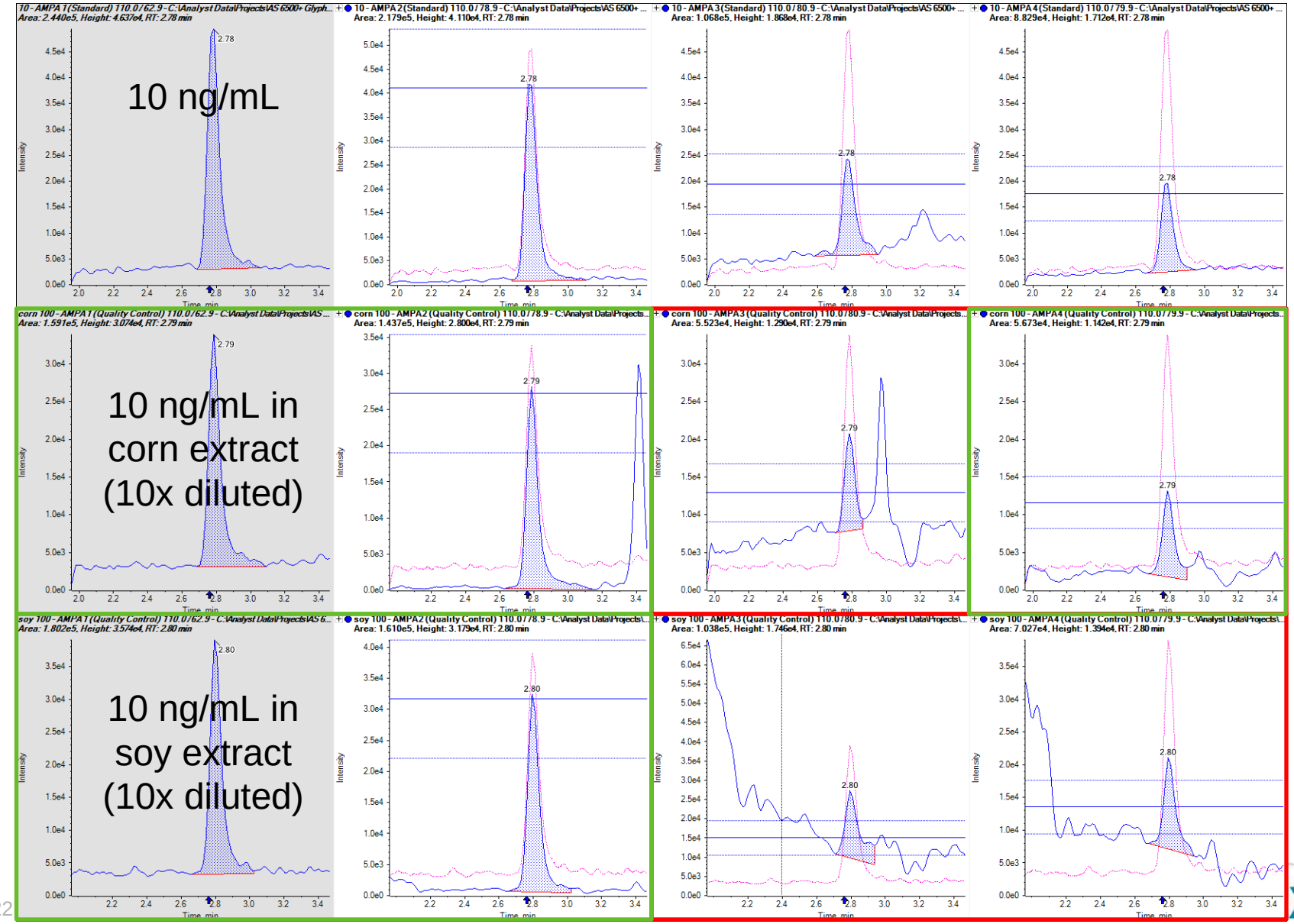
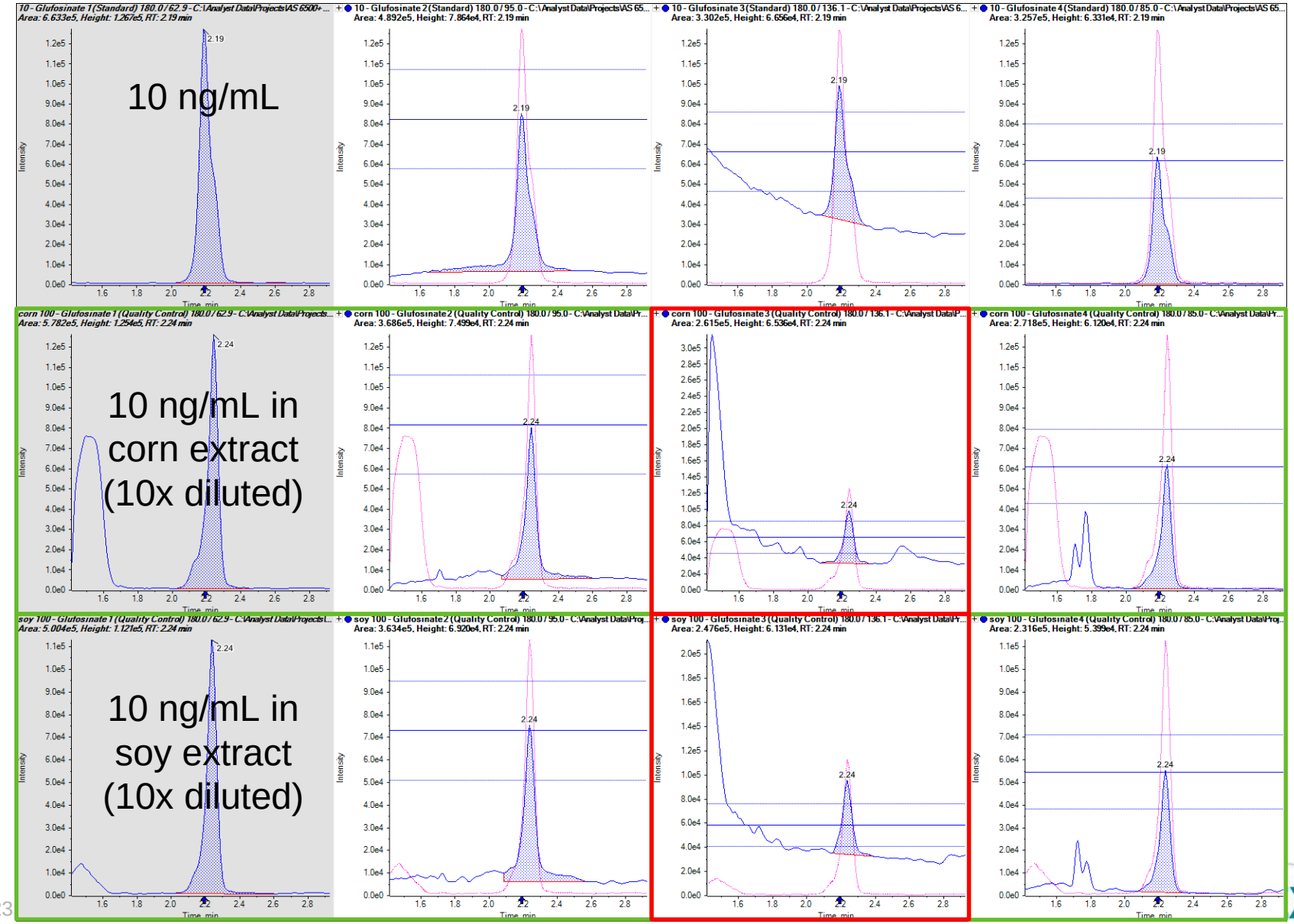
Method 3 Performance – Glyphosate



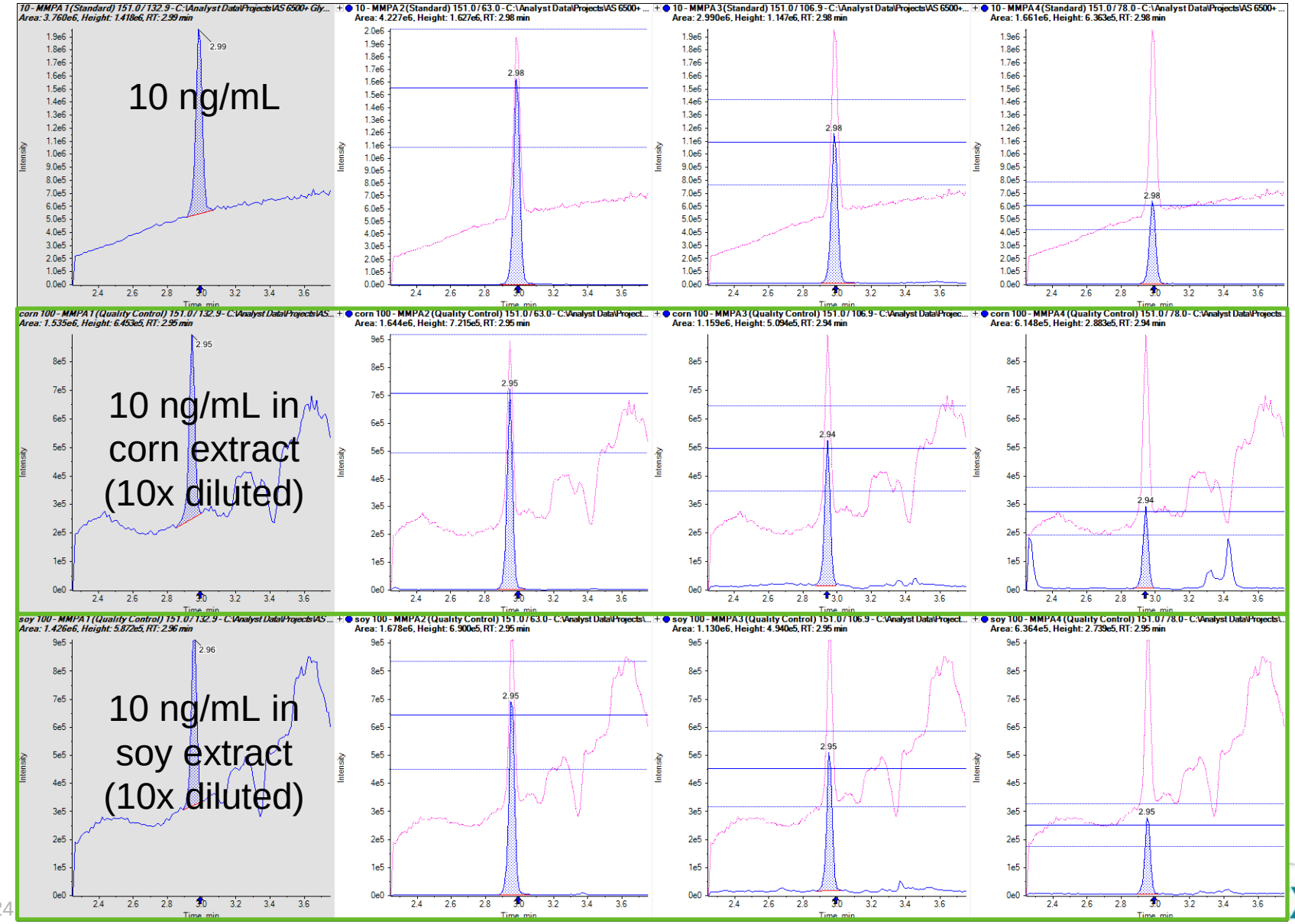
Method 3 Performance – AMPA



Method 3 Performance – Glufosinate



Method 3 Performance – MMPA



Method Comparison

	Method 1 (Hypercarb)	Method 2 (Trinity)	Method 3 (LUNA NH2)
LOD Glyphosate	2	**0.2**	0.2
LOD AMPA	0.2	**0.2**	1
LOD Glufosinate	0.5	**0.5**	0.5
LOD MMPA	0.5	**0.5**	5
%CV at 10 ng/mL Glyphosate	7.6%	**2.5%**	5.2%
%CV at 10 ng/mL AMPA	1.2%	**3.0%**	1.4%
%CV at 10 ng/mL Glufosinate	1.6%	**2.8%**	2.4%
%CV at 10 ng/mL MMPA	0.8%	**1.2%**	3.8%
#MRM at 10 ng/mL Glyphosate	2	**4**	3
#MRM at 10 ng/mL AMPA	4	**4**	4
#MRM at 10 ng/mL Glufosinate	4	**4**	4
#MRM at 10 ng/mL MMPA	4	**4**	4
LC peak shape	poor for glyphosate	**very good**	very good
RT stability	ok	**Very good**	long term (pH=10)?
Matrix interferences	many	**few**	few + high background
Potential for LVI (100 ppt in water)	not tested	**yes**	peak broadening
Ease to setup	most challenging	**yes**	yes

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Application Data

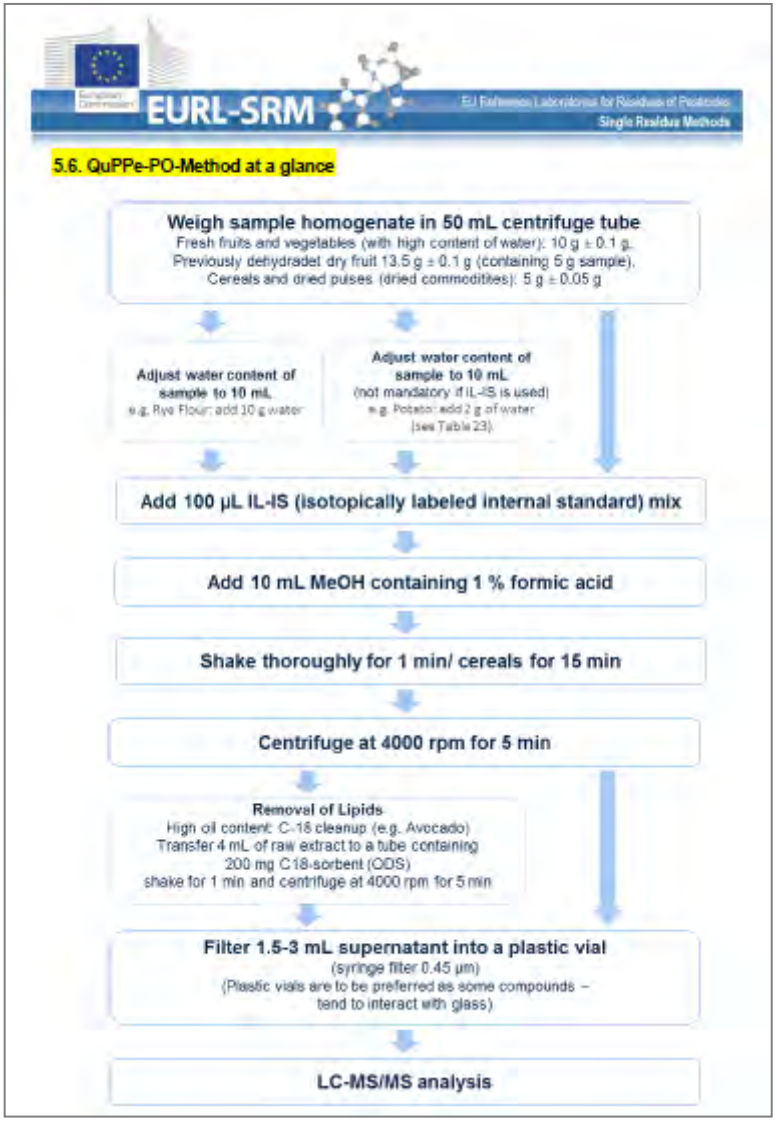
– Glyphosate, AMPA, Glufosinate and MMPA in Foods –

SCIEX ExionLC™ AC system

QTRAP® 6500+ system

Experimental

- QuPPe extraction
  - 10 g of sample
  - Adjust water content to 10 mL
  - (Add internal standard)
  - Add 10 mL methanol + 1% formic acid
  - Extraction + centrifugation
  - (Lipid removal for high oil content samples using C18 dSPE)
  - Dilution with water (10x)
- LC separation (ExionLC™ AC)
  - Acclaim Trinity Q1 100 x 3 mm 3µm
  - Gradient of water + 50 mM ammonium formate/formic acid (pH=2.9) and acetonitrile at flow rate 0.5 mL/min
  - 3 min 100% A and 3 min acetonitrile wash
  - Injection of 10 - 50 µL



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Experimental

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  - *Scheduled* MRM™ algorithm (Q3 low resolution)
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MMPA	151	133	-10	-18
	151	63	-10	-44
	151	107	-10	-20
	151	78	-10	-28

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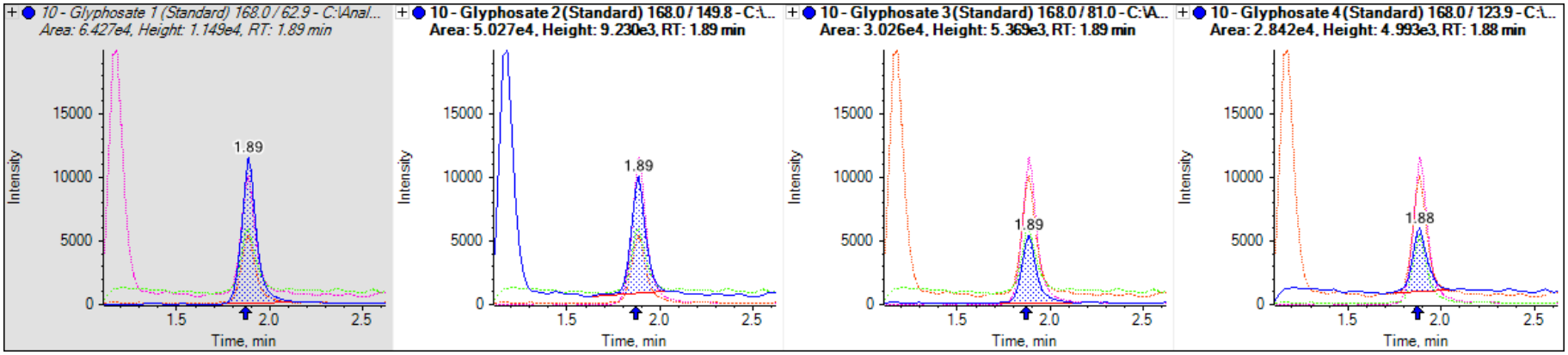
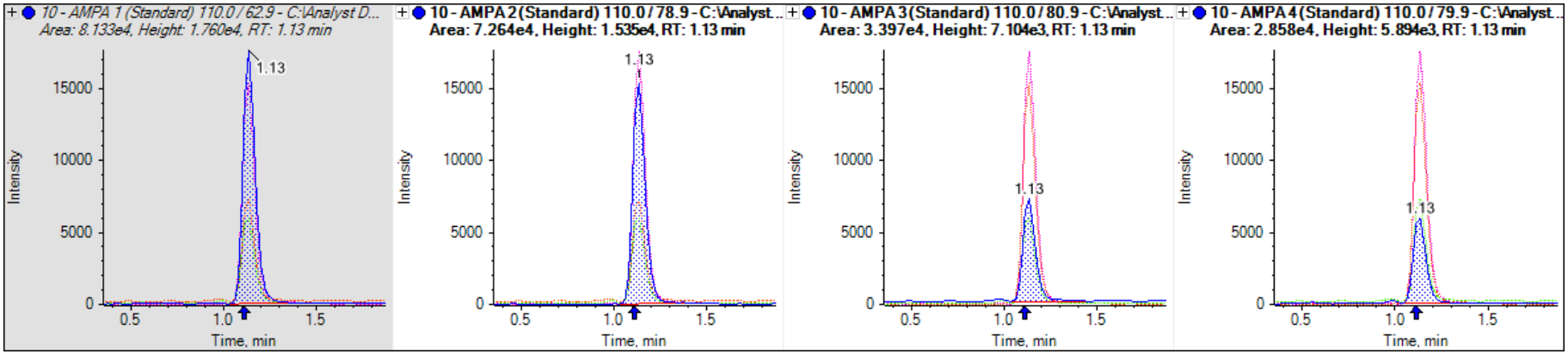
Glyphosate and AMPA at 10 ng/mL (10 µL Injection)

Glyphosate %CV = 2.99% (n = 5)



AMPA %CV = 2,85% (n = 5)



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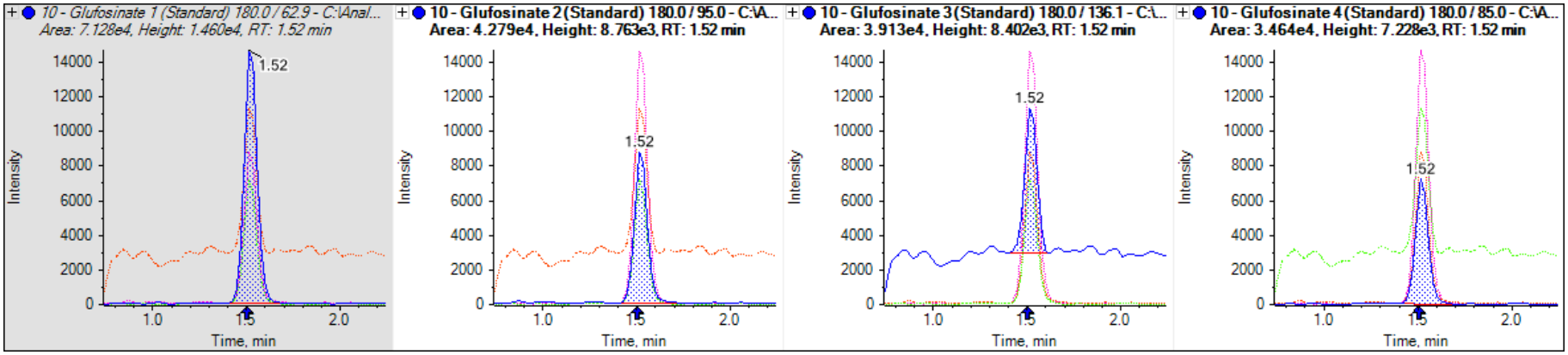
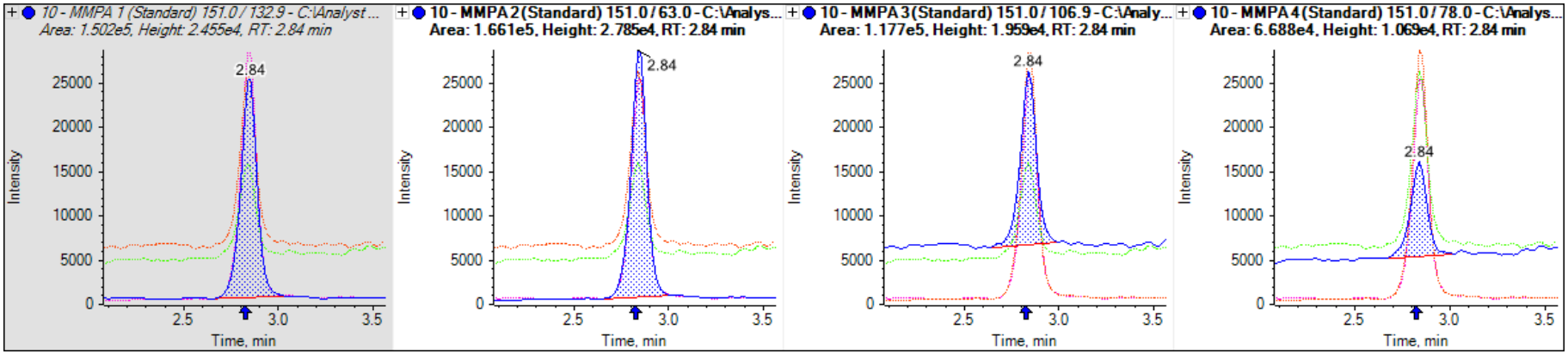
Glufosinate and MMPA at 10 ng/L (10 µL Injection)

Glufosinate %CV = 3.26% (n = 5)



MMPA %CV = 2.39% (n = 5)



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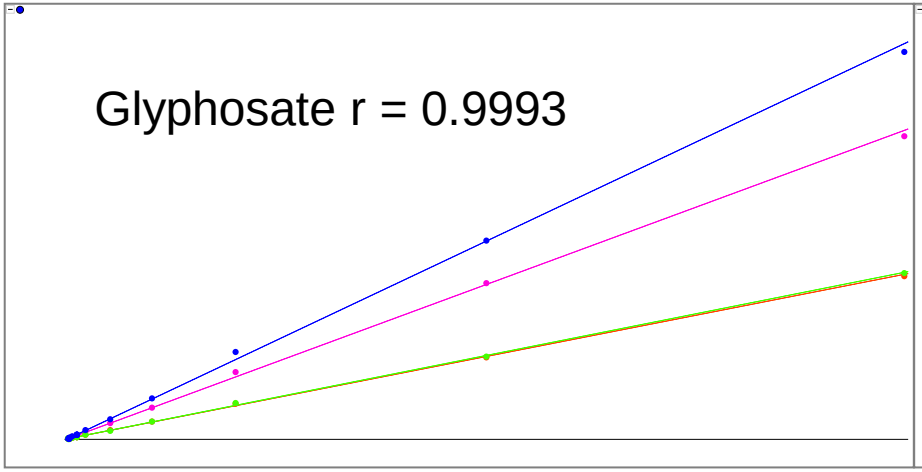
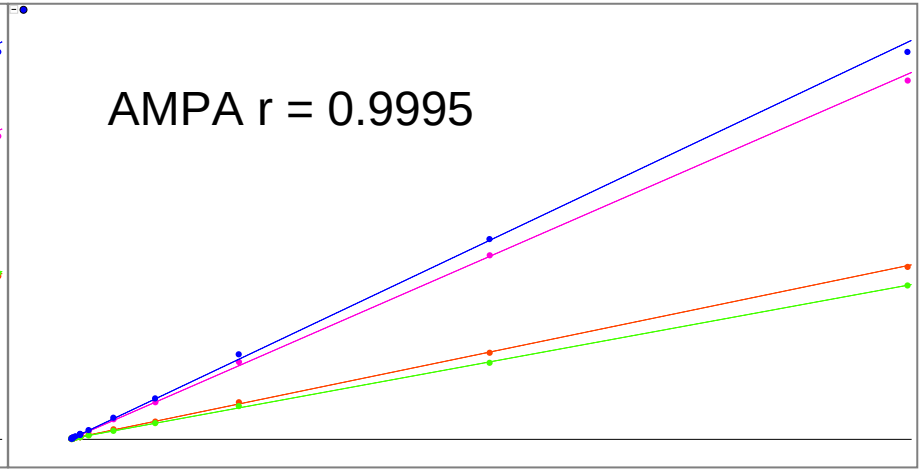
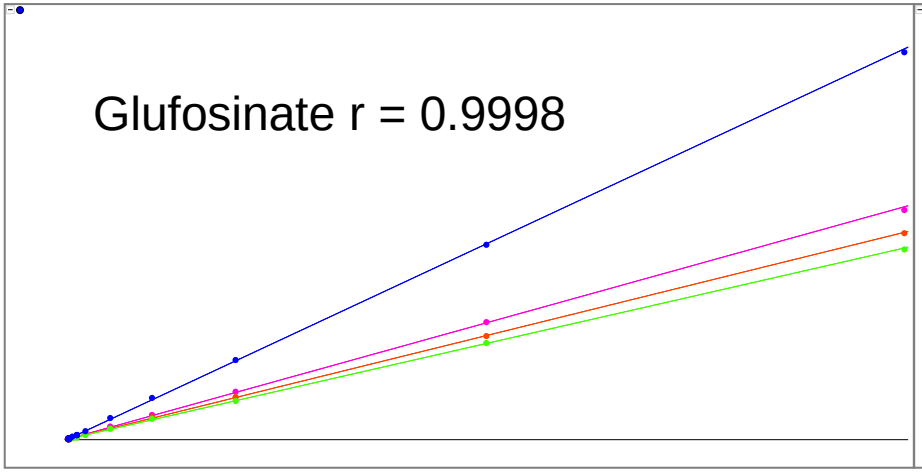
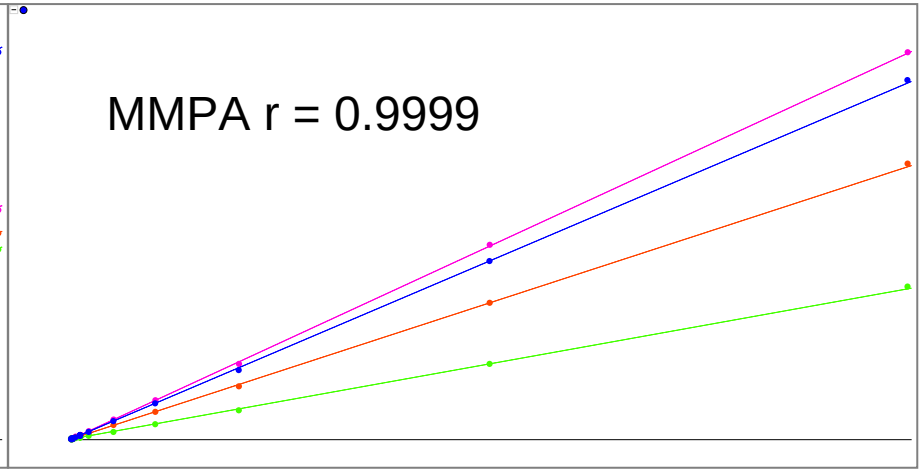
Linear Dynamic Range 1 to 1000 ng/mL









Accuracies between 80 and 120%

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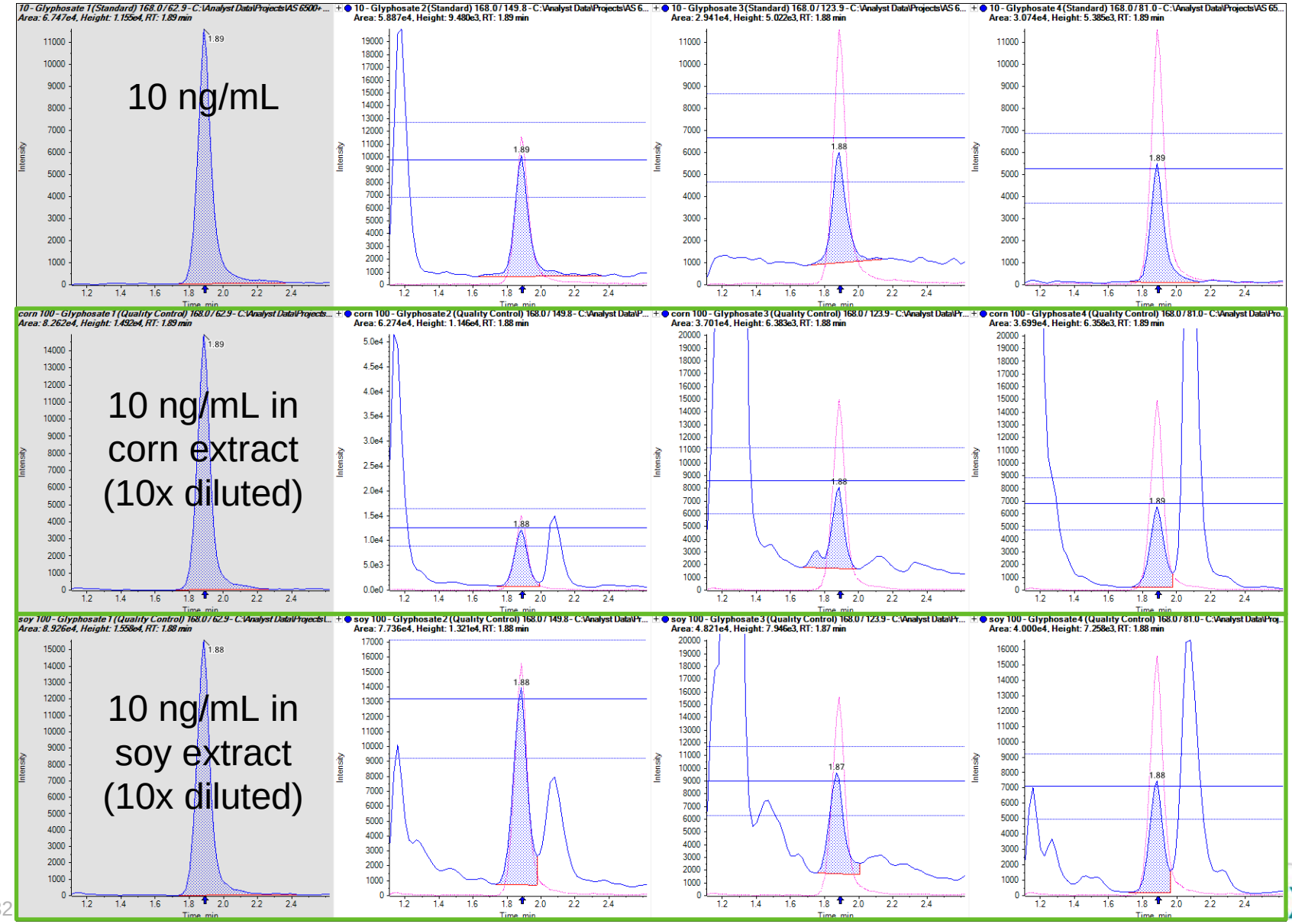
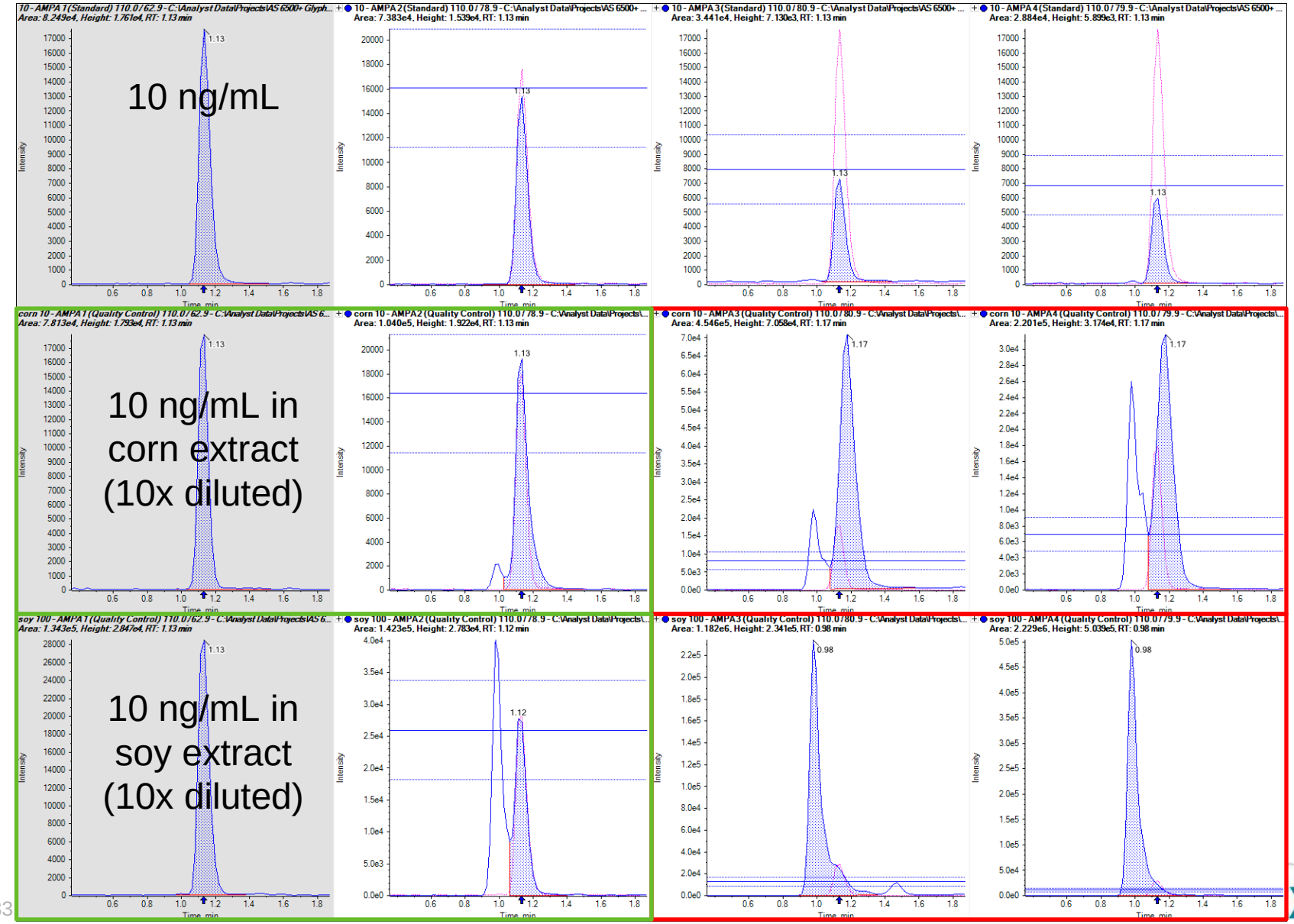
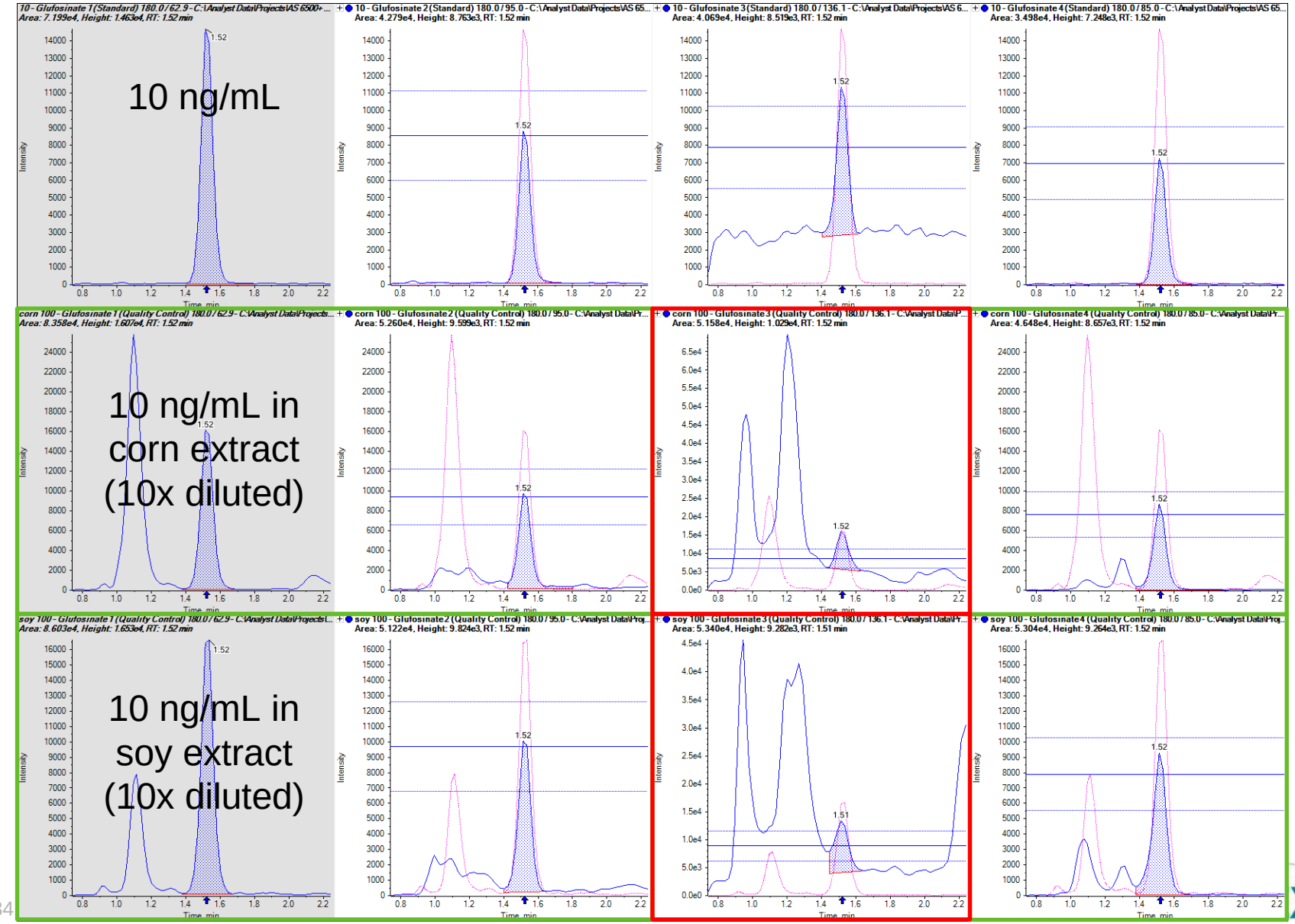
Glyphosate in Corn and Soy Extract



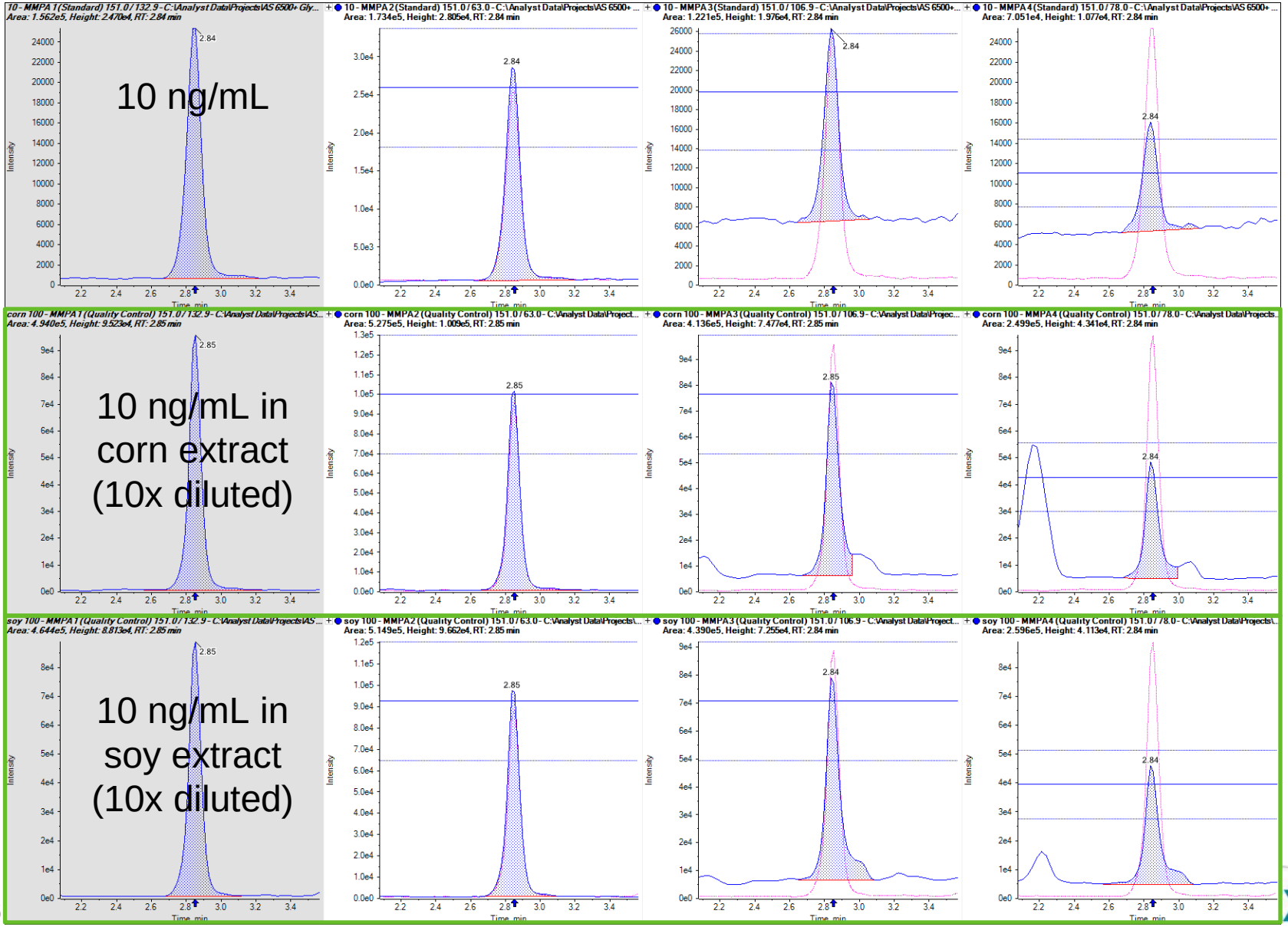
AMPA in Corn and Soy Extract



Glufosinate in Corn and Soy Extract



MMPA in Corn and Soy Extract



Application Data

– Increasing Selectivity using SelexION® Technology – (Differential Mobility Separation)

SCIEX ExionLC™ AC system

QTRAP® 6500+ system
with SelexION®+ DMS technology

Glyphosate with and without SelexION® DMS Technology

~7x Less Signal but Slightly Reduced Interferences and Background

10 ng/mL no DMS

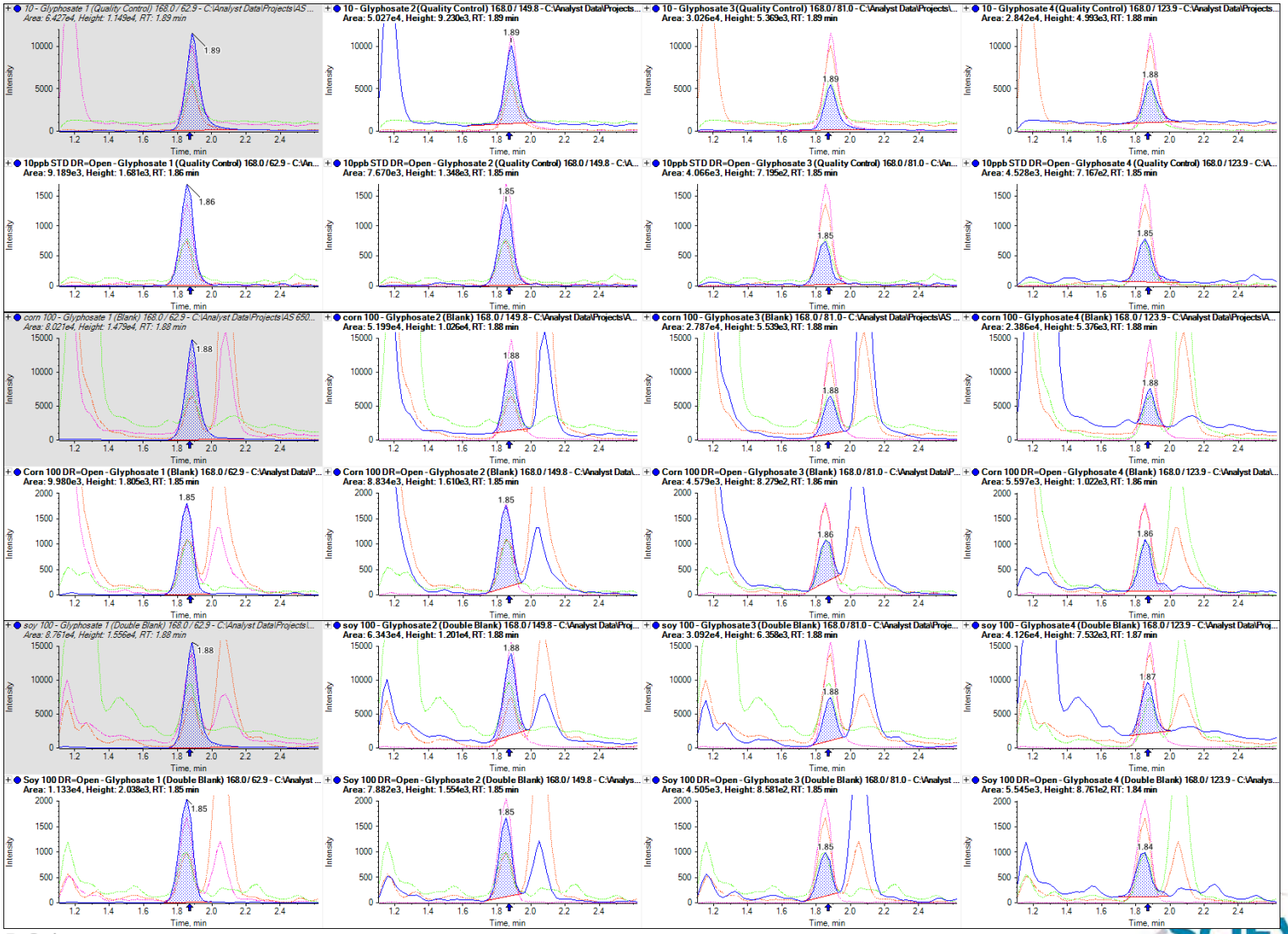
with DMS

Corn no DMS

with DMS

Soy no DMS

with DMS



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AMPA with and without SelexION® DMS Technology

~11x Less Signal but Greatly Reduced Interferences and Background

10 ng/mL no DMS

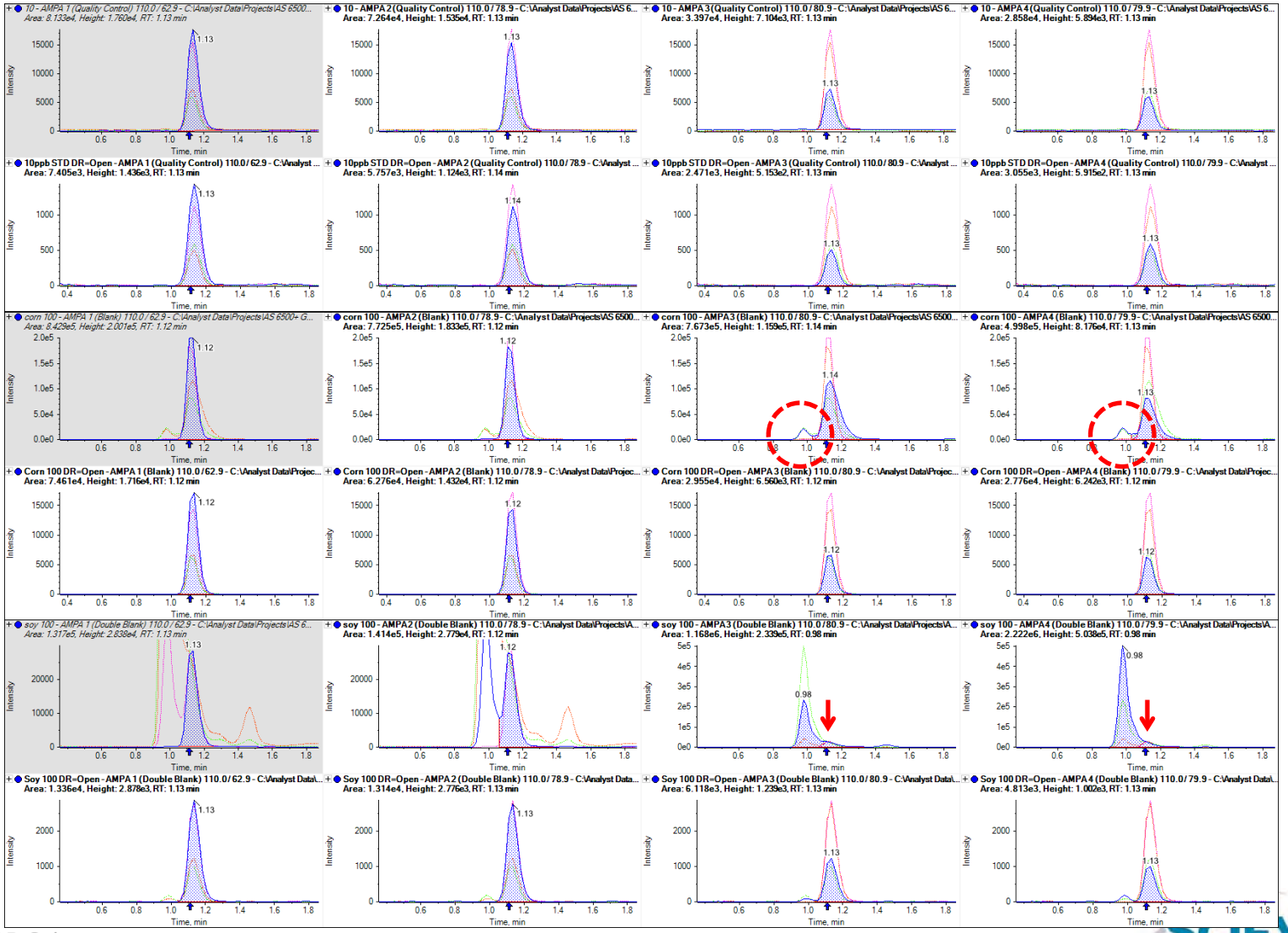
with DMS

Corn no DMS

with DMS

Soy no DMS

with DMS



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Glufosinate with and without SelexION® DMS Technology

~4x Less Signal but Reduced Interferences and Background

10 ng/mL no DMS

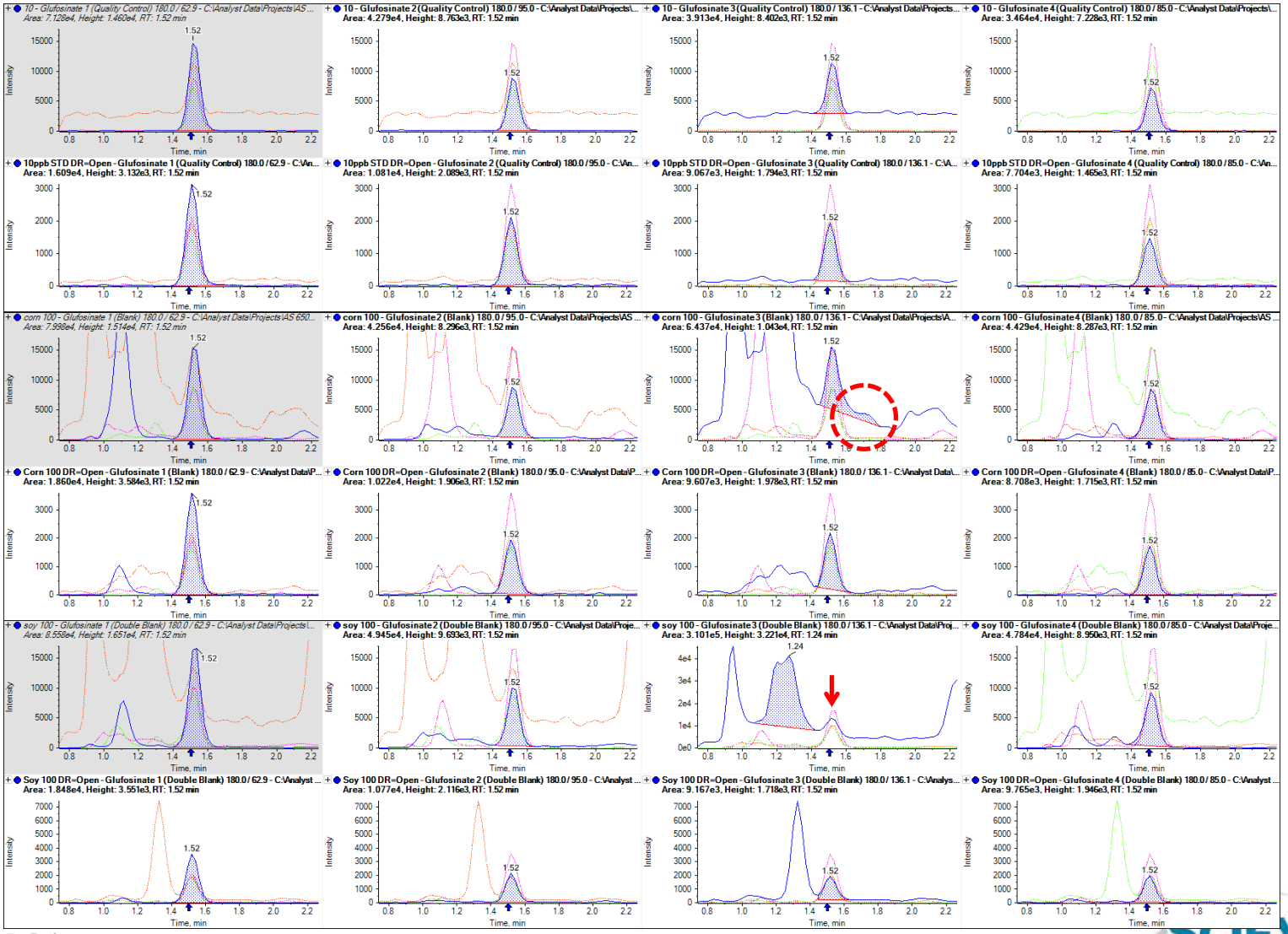
with DMS

Corn no DMS

with DMS

Soy no DMS

with DMS



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MMPA with and without SelexION® DMS Technology

~5x Less Signal but Reduced Interferences and Background

10 ng/mL
no DMS

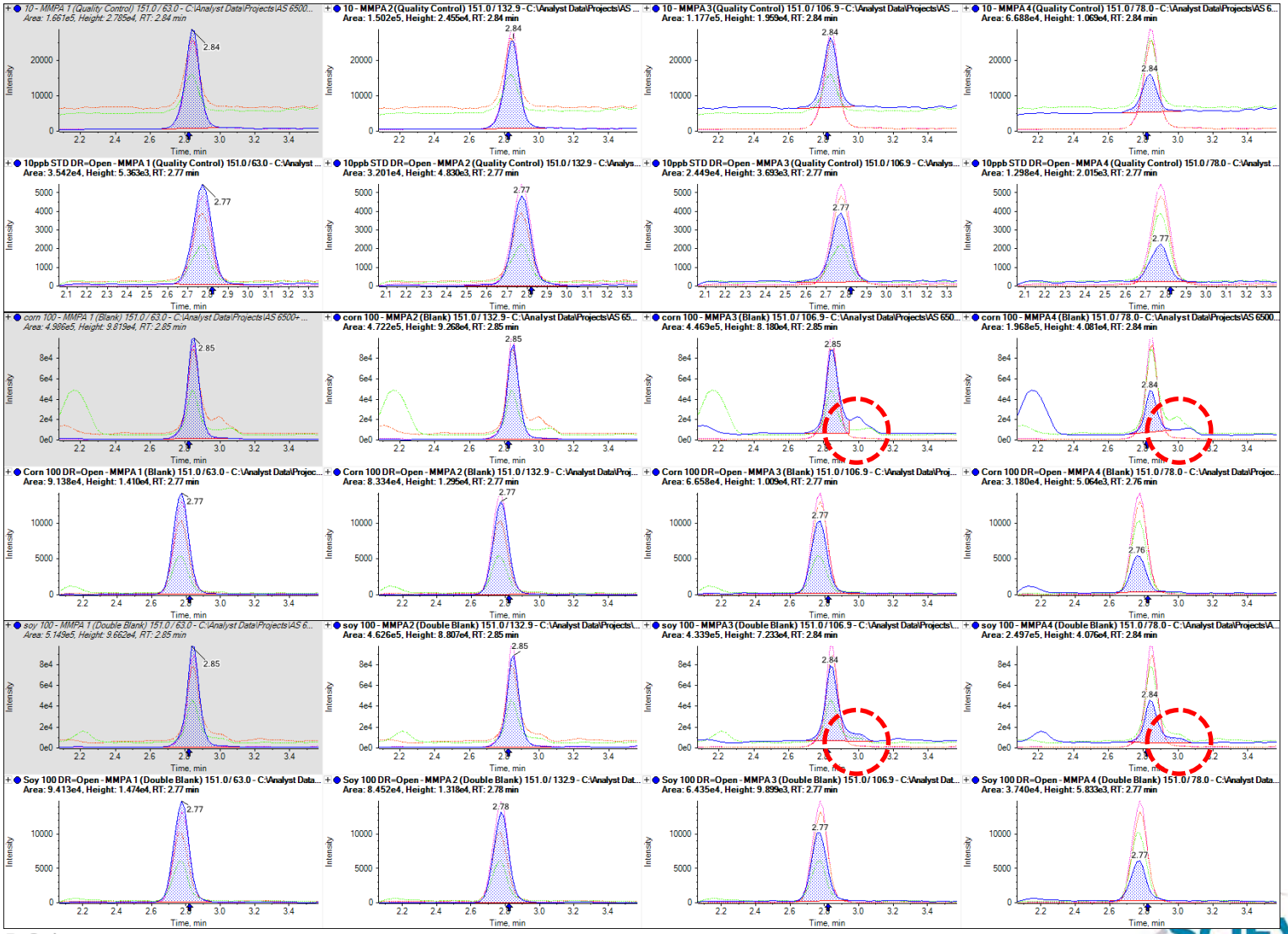
with DMS

Corn
no DMS

with DMS

Soy
no DMS

with DMS



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Application Data

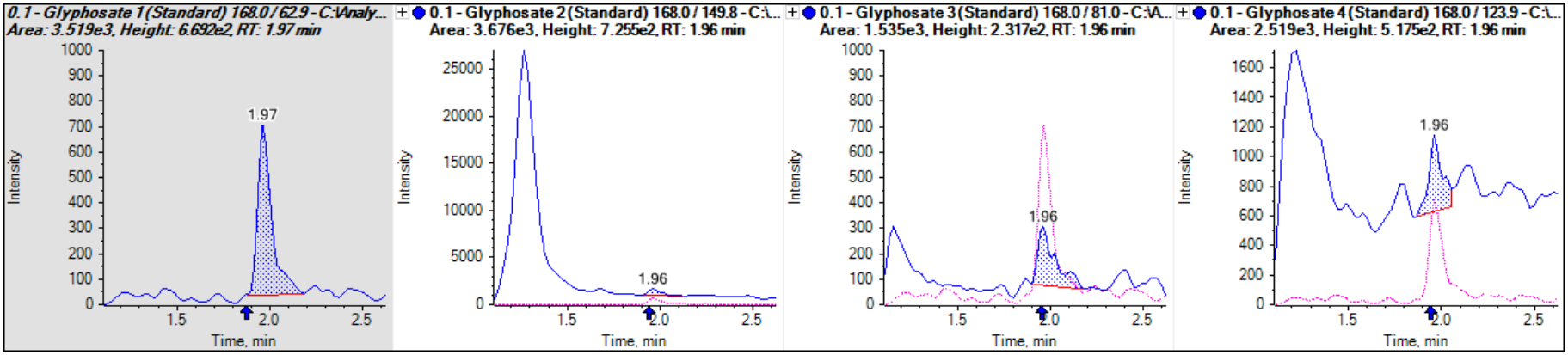
– Glyphosate, AMPA, Glufosinate and MMPA in Water and Beer –

SCIEX ExionLC™ AC system

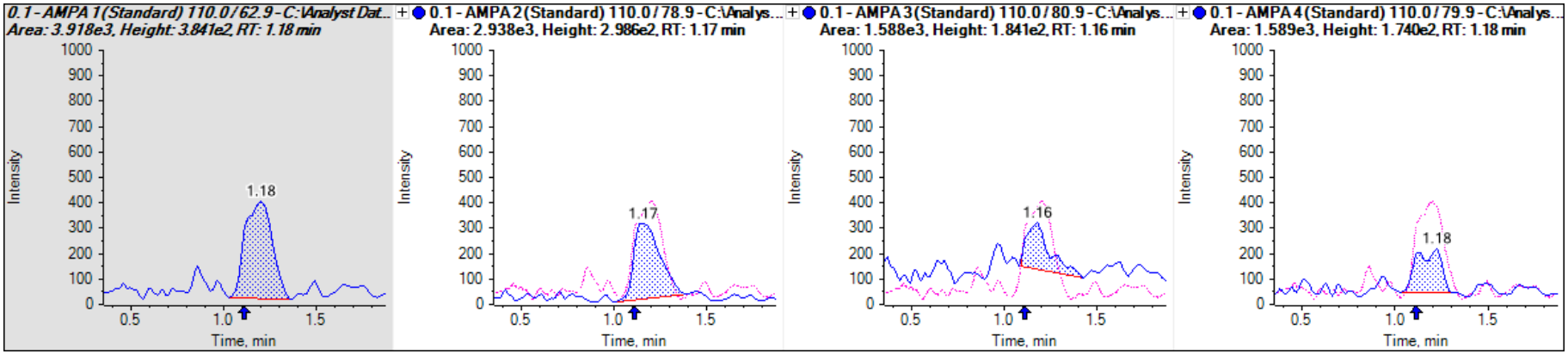
QTRAP® 6500+ system

Glyphosate and AMPA at 100 ng/L (50 µL Injection)

Glyphosate %CV = 3.32% (n = 5)



AMPA %CV = 11.4% (n = 5)



(Slight peak broadening observed for AMPA)

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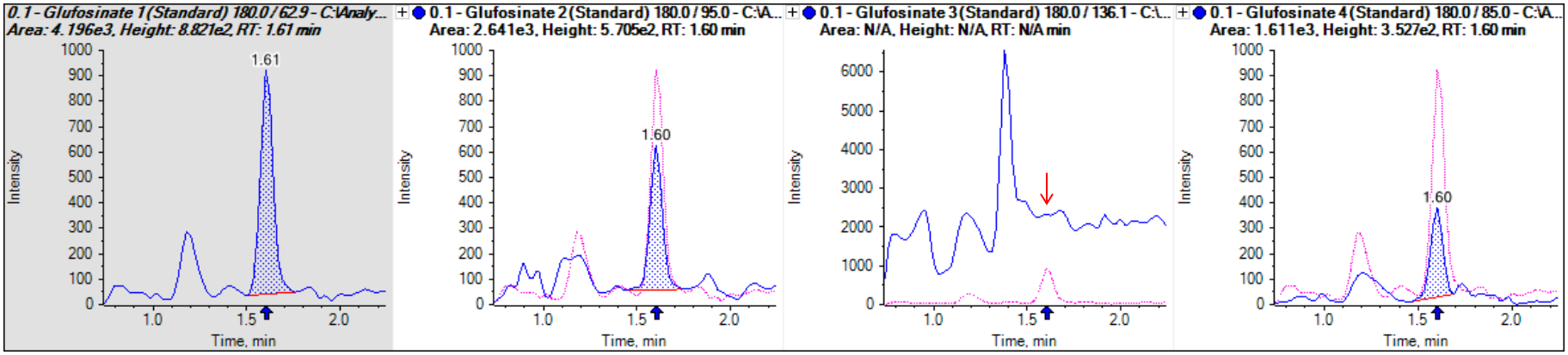
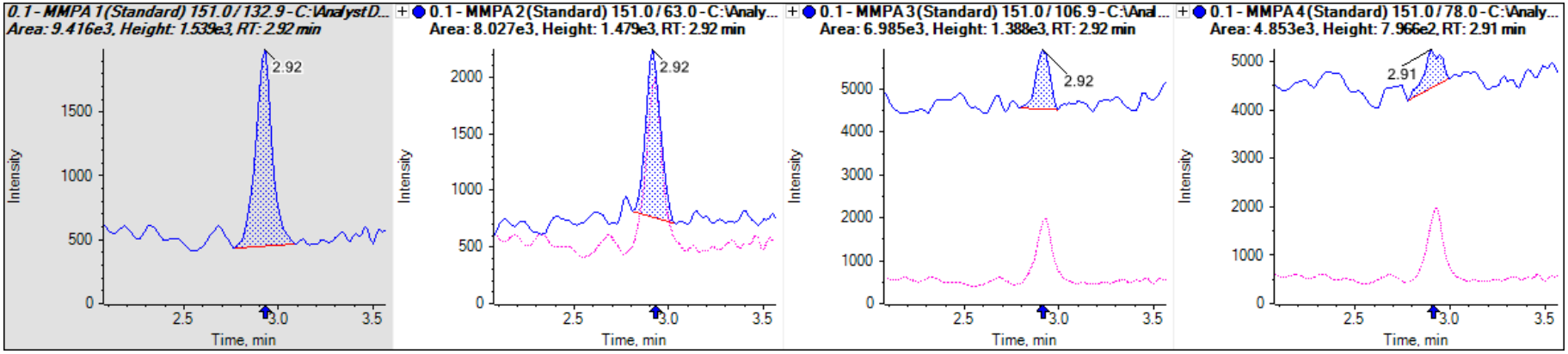
Glufosinate and MMPA at 100 ng/L (50 µL Injection)

Glufosinate %CV = 8.70% (n = 5)



MMPA %CV = 8.35% (n = 5)



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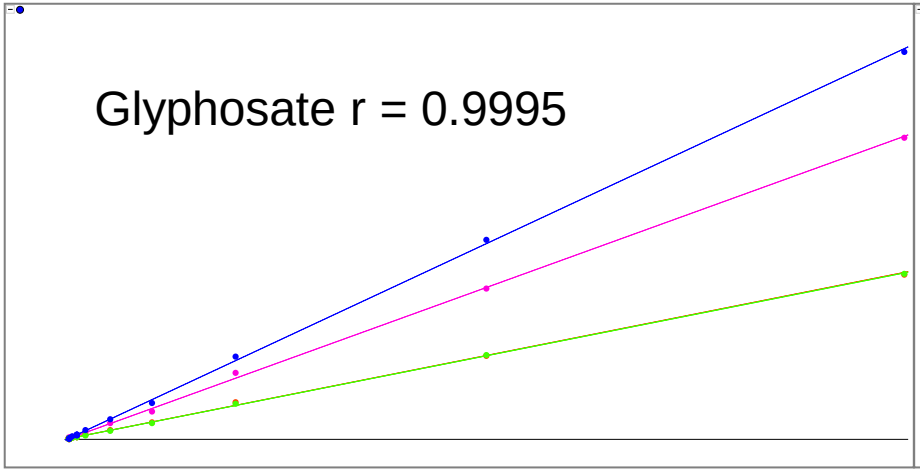
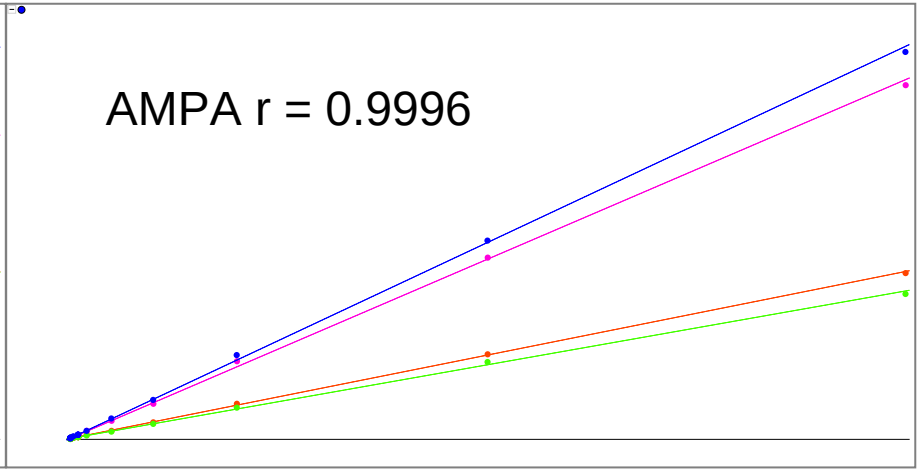
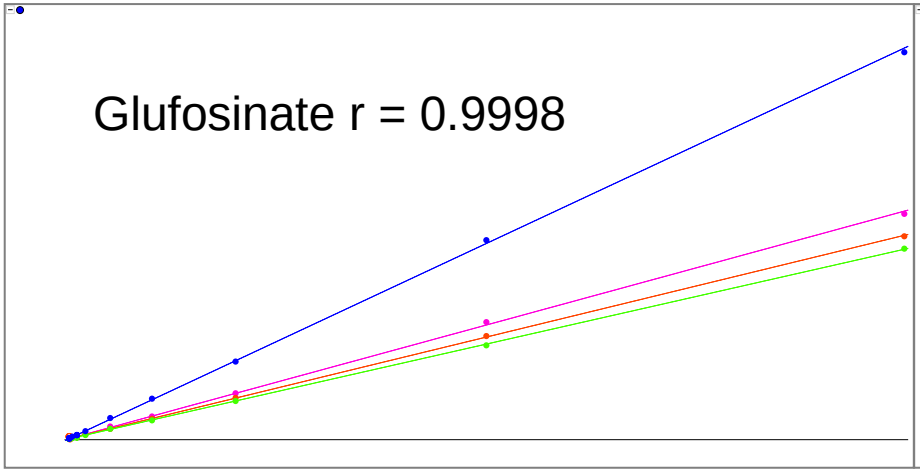
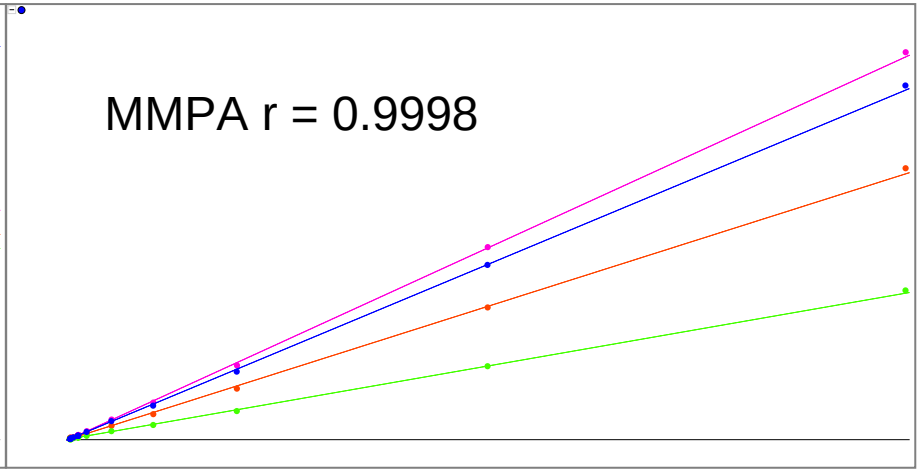
Linear Dynamic Range 0.1 to 100 ng/mL









Accuracies between 80 and 120%

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Glyphosate in Beer

German Beer Industry in Shock over Glyphosate Contamination

Posted on Feb 25 2016 - 3:54pm by Sustainable Pulse

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The Munich Environmental Institute (Umweltinstitut München) has released shocking results Thursday of laboratory testing it has completed on 14 of the most sold beers in Germany. The probable carcinogen and World's most used herbicide - glyphosate – was found in all of the 14 beers tested.

German Beer – Glyphosate Testing Results:

Hasseröder Pils – 29,74 µg/l (ppb)
Jever Pils – 23,04 µg/l
Warsteiner Pils – 20,73 µg/l
Radeberger Pilsner – 12,01 µg/l
Veltins Pilsener – 5,78 µg/l
Oettinger Pils – 3,86 µg/l
König Pilsener – 3,35 µg/l
Krombacher Pils – 2,99 µg/l
Erdinger Weißbier – 2,92 µg/l
Paulaner Weißbier – 0,66 µg/l
Bitburger Pils – 0,55 µg/l
Beck's Pils – 0,50 µg/l
Franziskaner Weißbier – 0,49 µg/l
Augustiner Helles – 0,46 µg/l

- Beer: 0.5 to 30 µg/L
- Water: glyphosate < 100 ng/L
- Hops and yeast: “too small amount” added to beer to explain residues
- Grain: glyphosate used as desiccants to dry off crops before harvest, however, the direct use of glyphosate on barley turned into malt is banned in Germany

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Experimental

- Sample preparation
  - Degassing of beer
  - Dilution with water (2x) – LOQ ~ 0.2 µg/L
- LC separation
  - Acclaim Trinity Q1 100 x 3 mm 3µm
  - Gradient of water + 50 mM ammonium formate/formic acid (pH=2.9) and acetonitrile at flow rate 0.5 mL/min
  - 3 min 100% A and 3 min acetonitrile wash
  - Injection of 50 µL
- MS/MS detection using QTRAP® 6500+ system
  - IonDrive™ Turbo V source with ESI probe (negative polarity)
  - IS -4500V, CUR 30 psi, Gas1 50 psi, Gas2 70 psi, CAD high, TEM 700ºC
  - *Scheduled* MRM™ algorithm (Q3 low resolution)

Compound	Q1 (amu)	Q3 (amu)	DP (V)	CE (V)
Glyphosate	168	63	-30	-26
	168	150	-30	-14
	168	124	-30	-16
	168	81	-30	-20

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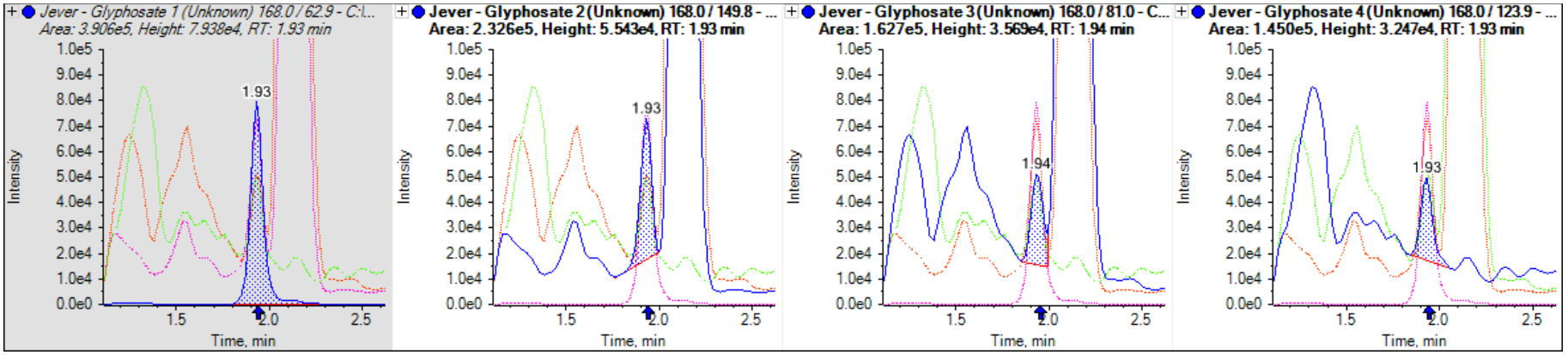
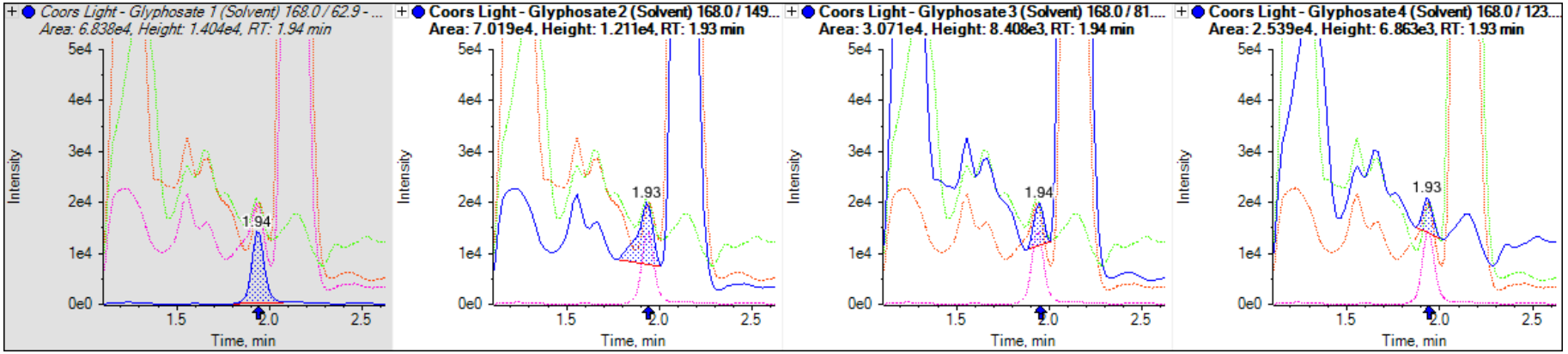
Examples of Beer Tested for Glyphosate (1)

German pilsner: glyphosate = 21.6 µg/L (identified using 4 MRM transitions ± 30%)



American Light beer: glyphosate = 3.8 µg/L (identified using 3 MRM transitions ± 30%)



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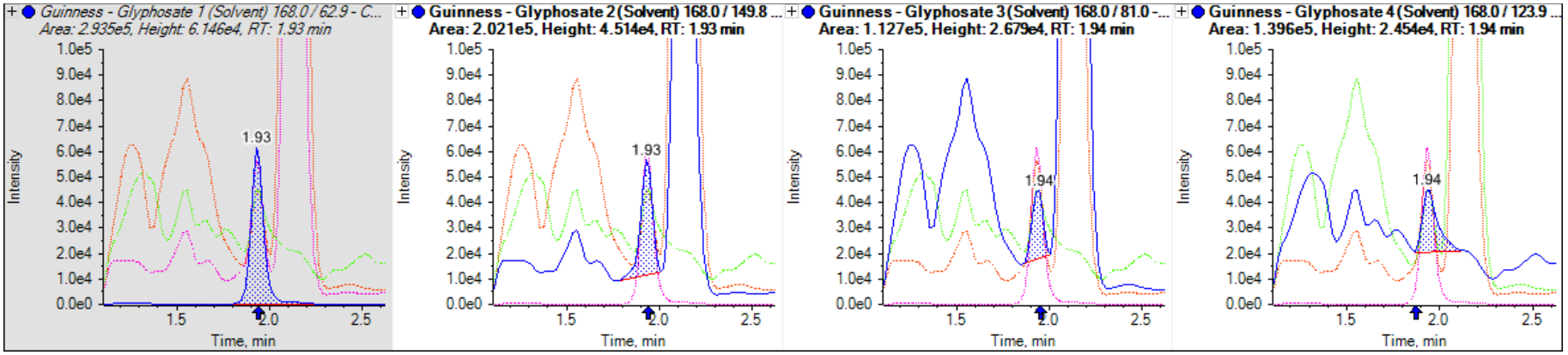
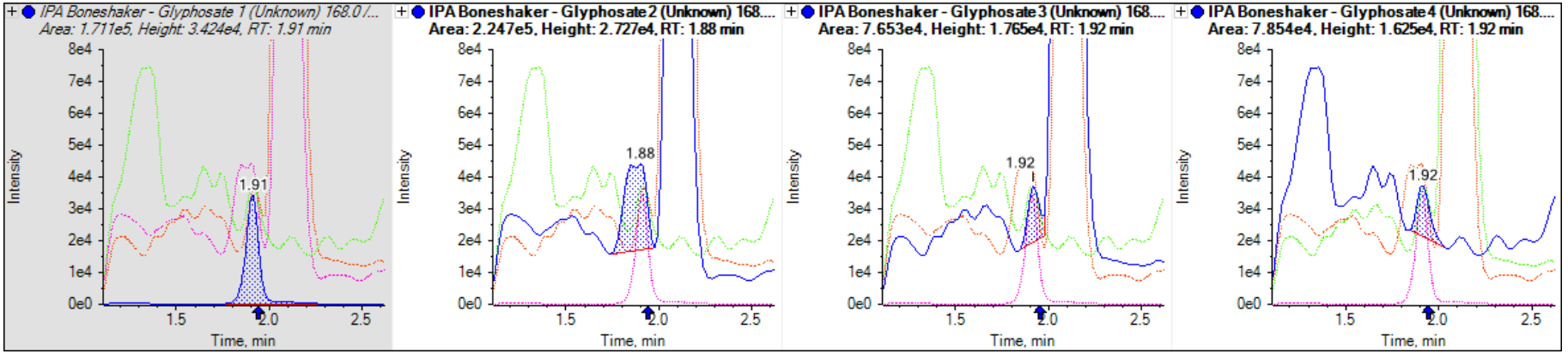
Examples of Beer Tested for Glyphosate (2)

Irish stout: glyphosate = 16.2 µg/L (identified using 4 MRM transitions ± 30%)



Canadian craft IPA: glyphosate = 9.5 µg/L (identified using 3 MRM transitions ± 30%)



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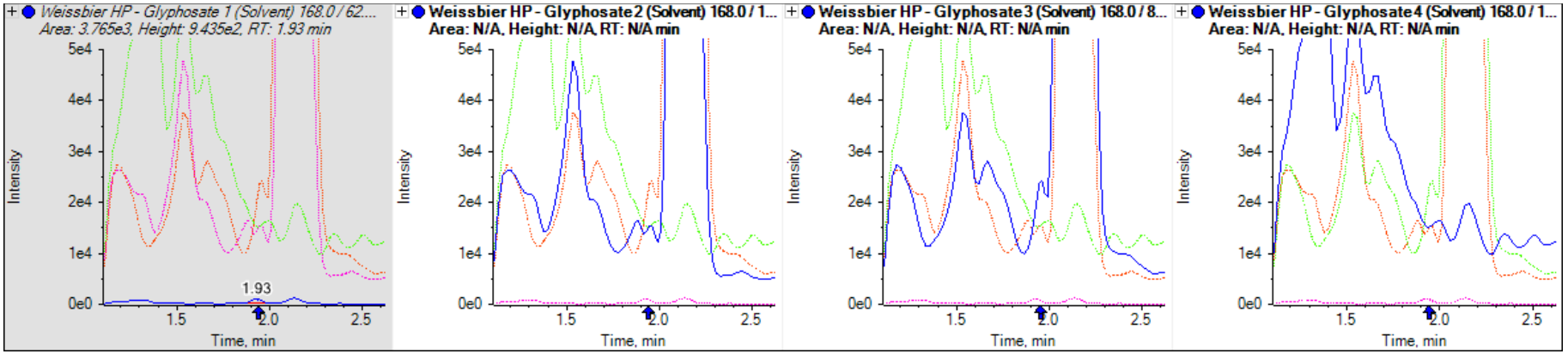
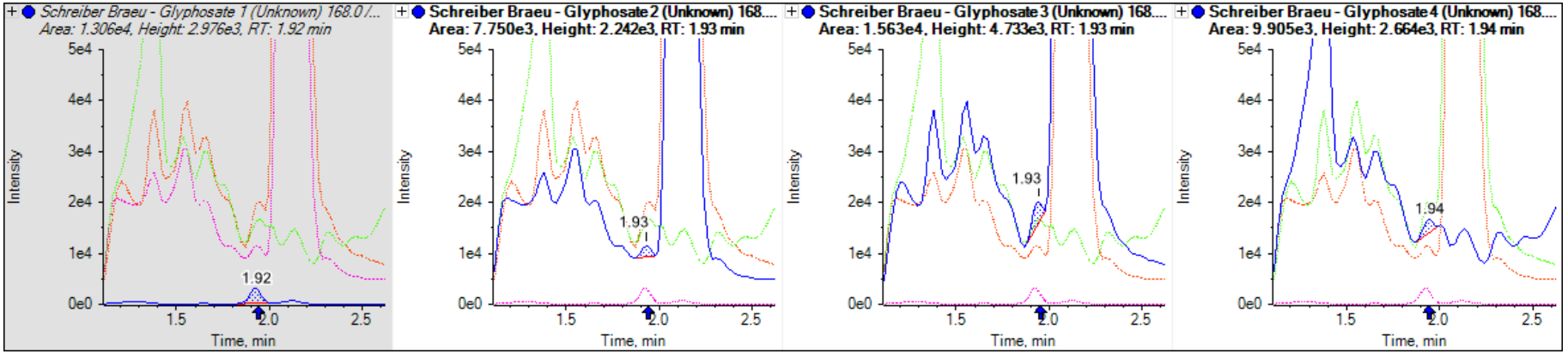
Examples of Beer Tested for Glyphosate (3)

German Weissbier (wheat): glyphosate = 0.2 µg/L (no secondary transitions for ID)



Homemade ale: glyphosate = 0.7 µg/L (identified using 2 MRM transitions ± 30%)



Homemade beer was brewed in Canada using barley malted in Germany.

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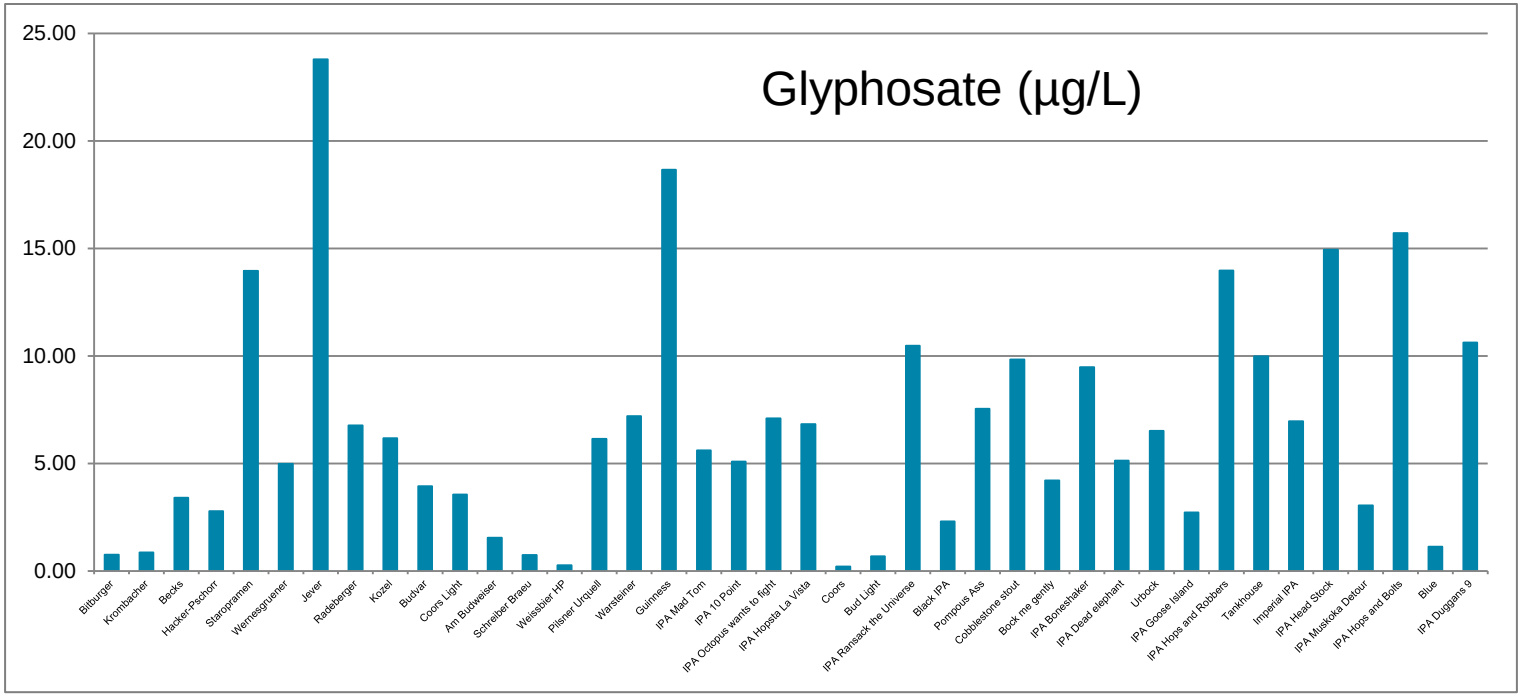
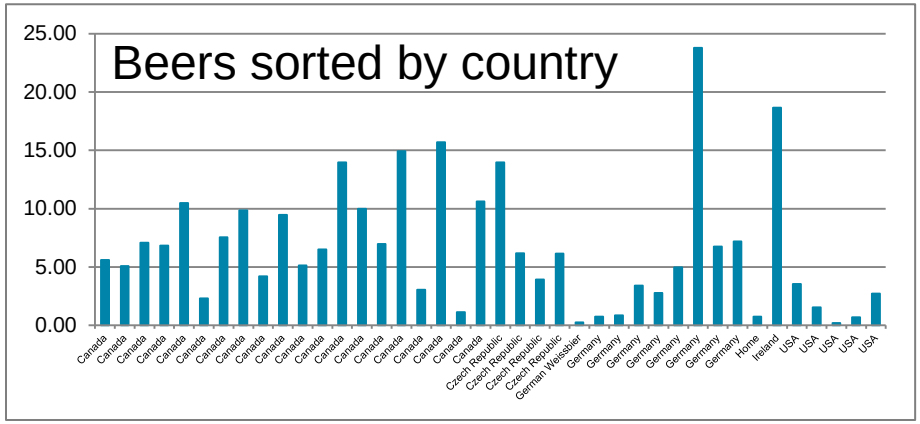
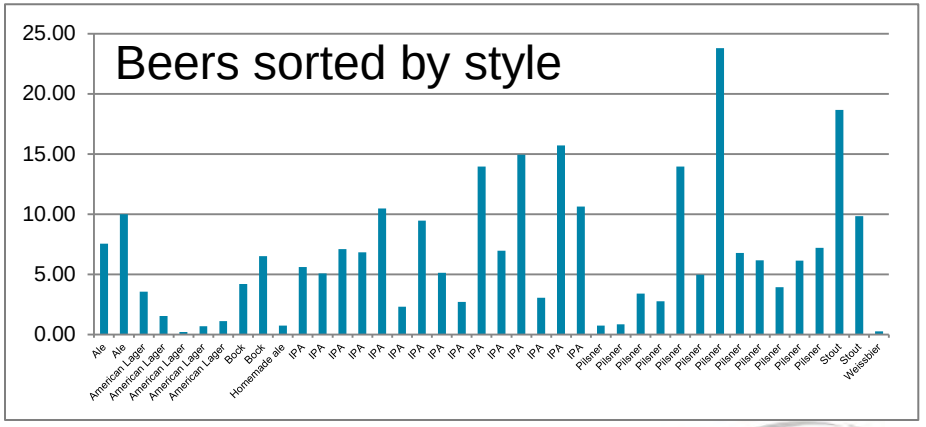
Glyphosate in Beers… no Correlation to Origin or Style







Insufficient number of samples for good conclusion.

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Summary

- LC-MS/MS method for the analysis of underivatized glyphosate and other polar pesticides was developed.
  - Sample preparation: QuPPe with 10x dilution with water
  - Direct injection of 2x diluted beer and undiluted water
  - LC: Trinity Q1 with water + 50 mM ammonium formate/formic acid (pH=2.9) and acetontrile (N. Chamkasem et al.)
  - MS/MS using QTRAP® 6500+ system with IonDrive™ Turbo V source with ESI probe in negative polarity
  - 4 MRM transitions for simultaneous identification and quantitation
- Method successfully applied to:
  - Extracts of corn and soy with an LOQ of 100 µg/kg (10 ng/mL in extract)
  - Interference can be reduced using SelexION® DMS technology
  - Water with an LOQ of 100 ng/L (potential improvements of sensitivity using post-column pH adjustments)
  - Beer with an LOQ of 0.2 µg/L

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