



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

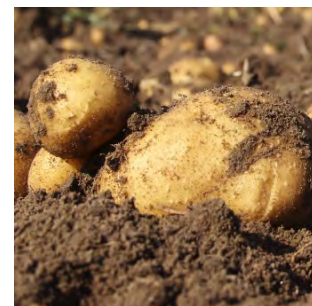
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SCIEX, Concord, Ontario (Canada)

RUO-MKT-11-3896-A For Research Use Only. Not for use in diagnostic procedures.



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



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 often not available in labs, e.g.:**

- *“Exotic” columns for LC- or GC-separation*
- *“Exotic” mobile phases (LC)*
- *“Exotic” Instrumental-configurations*

Objective of our Project

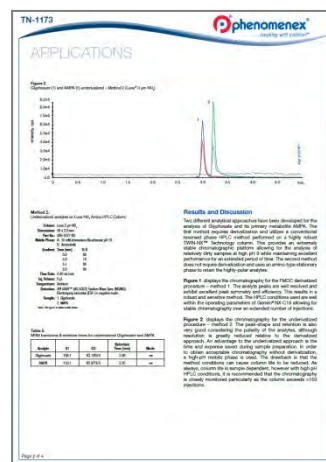
- Evaluation of published glyphosate methods
 - Routine quantitation of glyphosate, glufosinate and metabolites using LC-MS/MS without derivatization
 - Target LOQ of 100 ppb for food testing (after QuPPE extraction)
 - Target LOQ of 100 ppt for water testing (dilute and shoot)
 - High confidence in 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)

Authors: M. Anastassiades, G. Koutsouris, A. Benveniste, E. Sjöström, S. Zaccaroni
© M. A., G. K., A. B., E. S., S. Z.

Mass Changes from 10.1 to 10.5 are highlighted in yellow

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24	Method 12: Glyphosate & Co. Hypocrell"
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26	Method 14: Glyphosate & Co. Hypocrell"
27	Method 15: Glyphosate & Co. Hypocrell"
28	Method 16: Glyphosate & Co. Hypocrell"
29	Method 17: Glyphosate & Co. Hypocrell"
30	Method 18: Glyphosate & Co. Hypocrell"
31	Method 19: Glyphosate & Co. Hypocrell"
32	Method 20: Glyphosate & Co. Hypocrell"
33	Method 21: Glyphosate & Co. Hypocrell"
34	Method 22: Glyphosate & Co. Hypocrell"
35	Method 23: Glyphosate & Co. Hypocrell"
36	Method 24: Glyphosate & Co. Hypocrell"
37	Method 25: Glyphosate & Co. Hypocrell"
38	Method 26: Glyphosate & Co. Hypocrell"
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Journal of Regulatory Science
Regulatory Science

Direct Determination of Glyphosate, Glufosinate, and AMPA in Milk by Liquid Chromatography/Tandem Mass Spectrometry

Ning Chamkasem¹, Cynthia Morris¹, Tiffany Hansen¹

¹Food Safety Laboratory, U.S. Food and Drug Administration, 2015M Street, N.E., Atlanta, GA 30303, USA

Abstract

A simple high-throughput liquid chromatography/tandem mass spectrometry (LC-MS/MS) method was developed for the determination of glyphosate, glufosinate, and AMPA in milk. The method involves the use of a C18 column and a mobile phase consisting of water and acetic acid. The results show that the method is capable of detecting and quantifying these pesticides in milk at levels as low as 100 ppb.

1. Introduction

Glyphosate (N-(phosphonomethyl)glycine) and glufosinate (N-(2-(phosphorylmethyl)amino)ethanesulfonic acid) are two selective post-emergence herbicides used for the control of a broad spectrum of grasses and broad leaf weed species in agricultural and industrial fields. Glyphosate is the most widely used herbicide in the world, and it is also one of the most controversial. It has been classified as a probable human carcinogen by the International Agency for Research on Cancer (IARC) in 2015. Glufosinate is also a herbicide, but it is not classified as a carcinogen. AMPA is the active metabolite of glyphosate, and it is also a herbicide. It has been classified as a probable human carcinogen by the IARC in 2015. The European Union has banned the use of glyphosate in food crops, and it has also banned the use of glufosinate in food crops. The U.S. Food and Drug Administration (FDA) has also banned the use of glyphosate in food crops, and it has also banned the use of glufosinate in food crops. The purpose of this study was to develop a simple, sensitive, and specific method for the determination of glyphosate, glufosinate, and AMPA in milk. The method described in this application note involves the use of a C18 column and a mobile phase consisting of water and acetic acid. The results show that the method is capable of detecting and quantifying these pesticides in milk at levels as low as 100 ppb.

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

Phenomenex TN-1173

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

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 700°C
 - 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

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 3 μ m
- 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 μ L

- Method 2 (US-FDA)

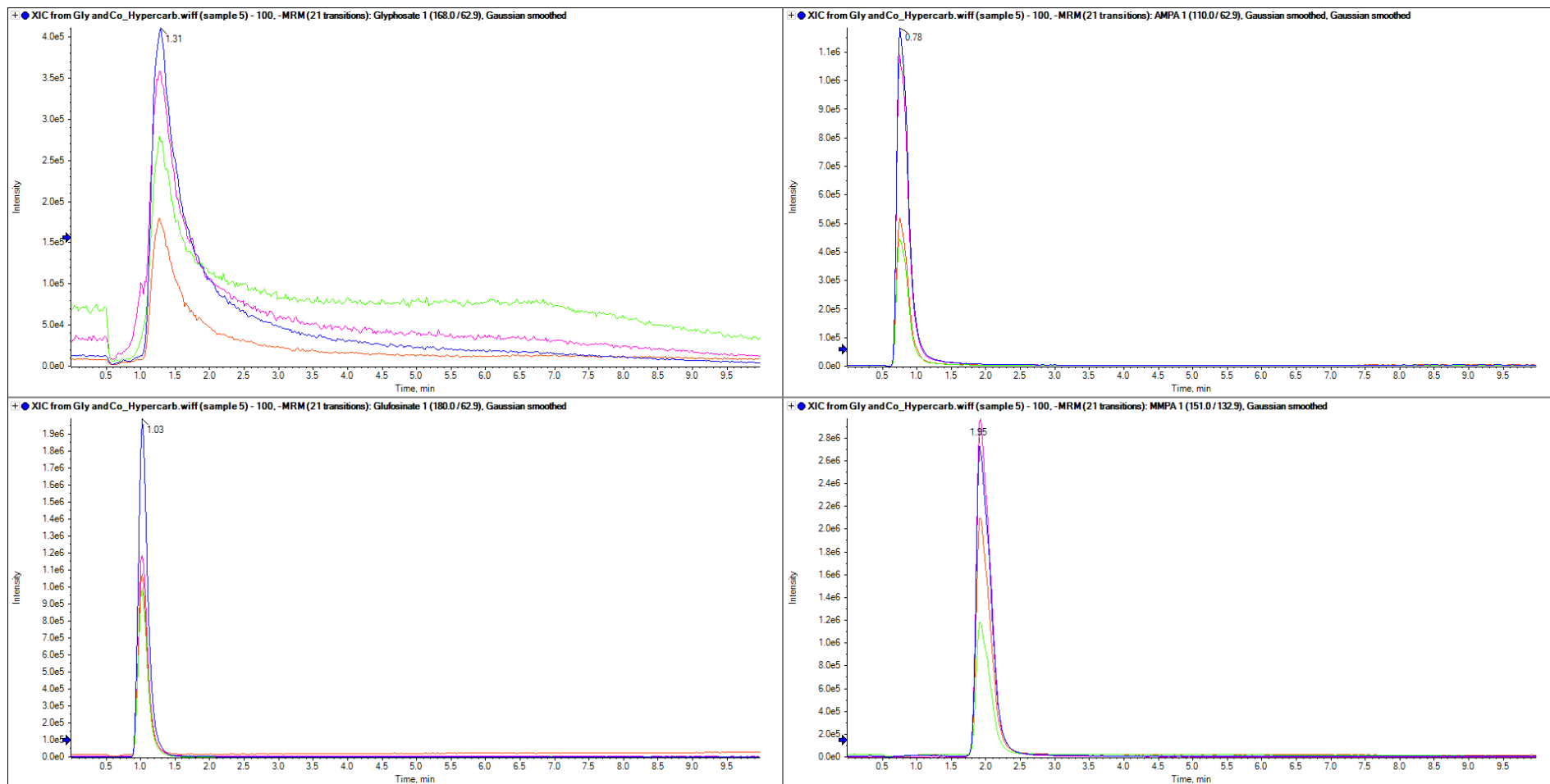
- Acclaim Trinity Q1 100 x 3 mm 3 μ m
- Gradient of water + 50 mM ammonium formate/formic acid (pH=2.9) and acetonitrile at flow rate 0.5 mL/min
- Injection of 10 - 50 μ L

- Method 3 (PHX)

- LUNA NH₂ 50 x 2 mm 3 μ m
- Gradient of water + 10 mM ammonium bicarbonate (pH=10) at flow rate 0.4 mL/min
- Injection of 10 - 50 μ L

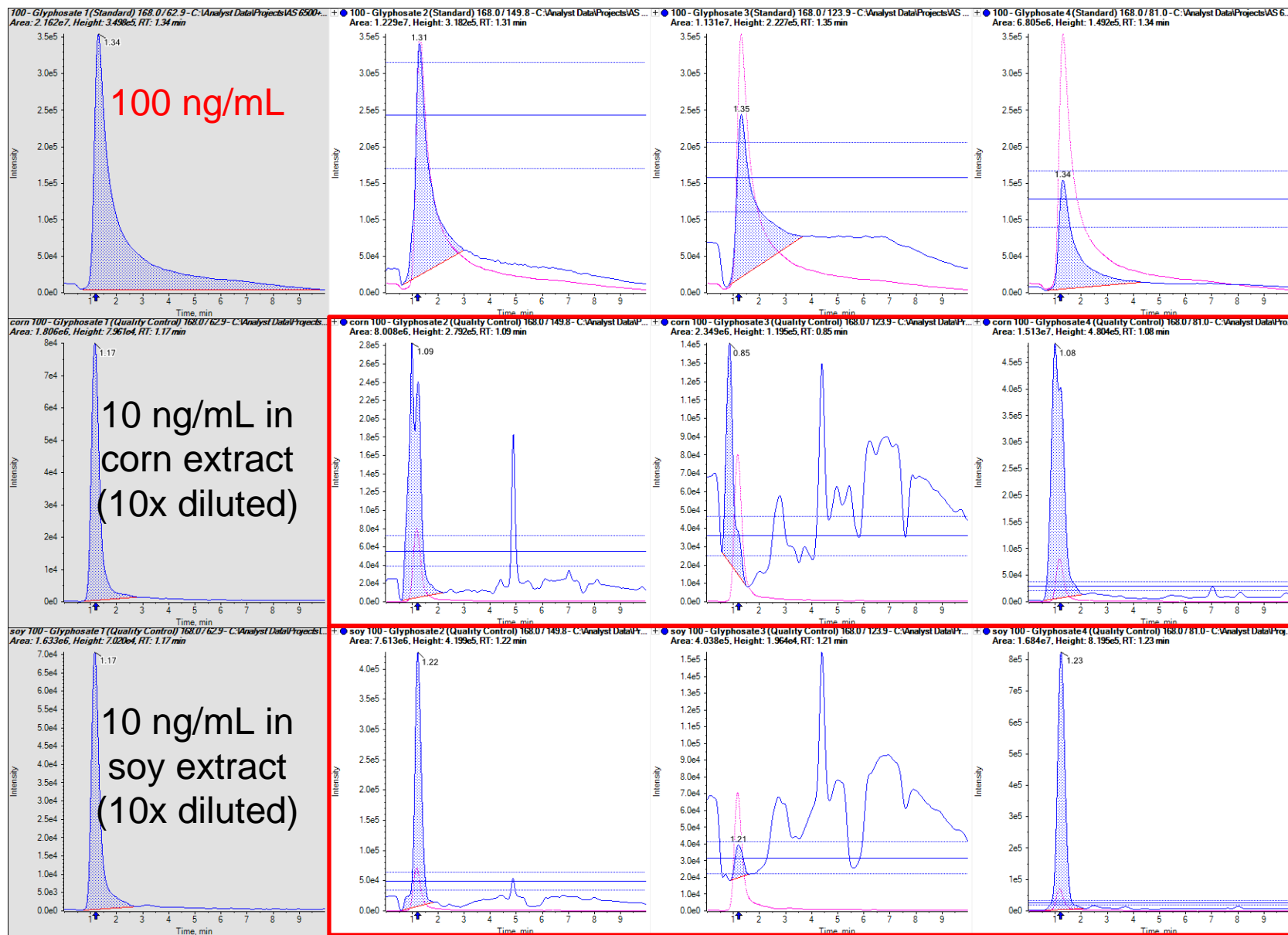
Method 1 (Hypercarb water/methanol + 1% acetic acid)

100 ng/mL Standard

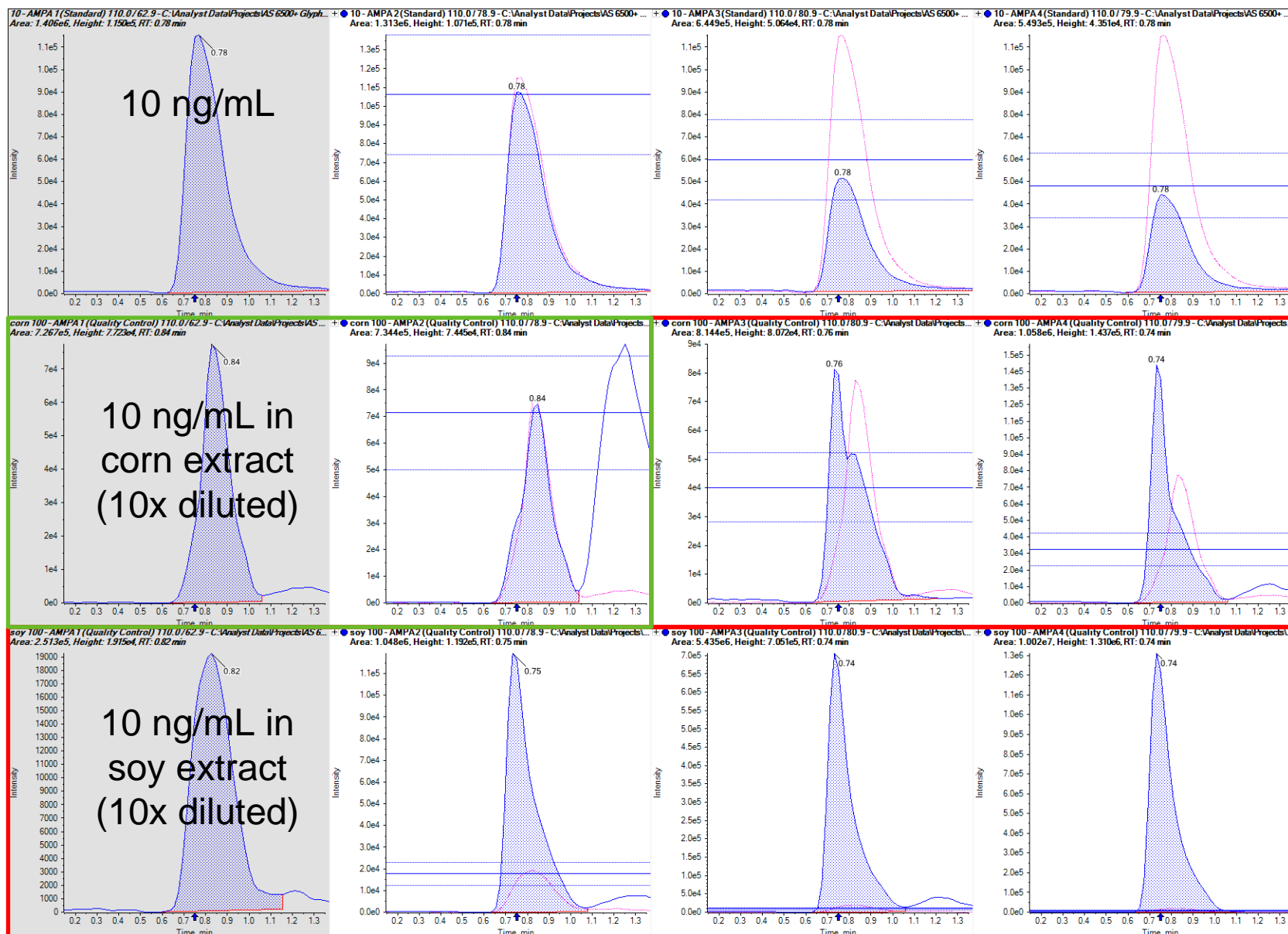


Column was primed with 30 injections of QuPPE extracts of spinach

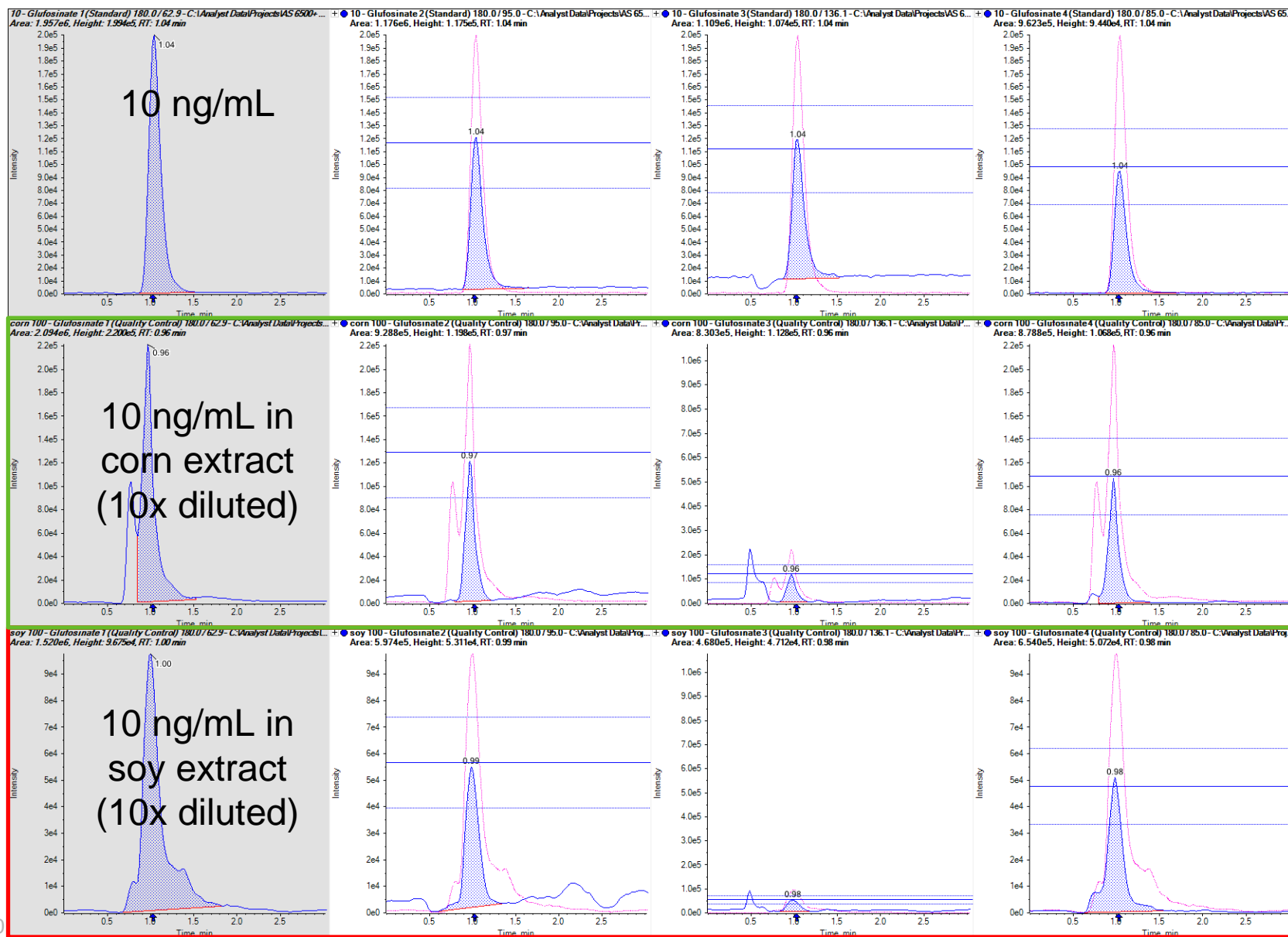
Method 1 Performance – Glyphosate



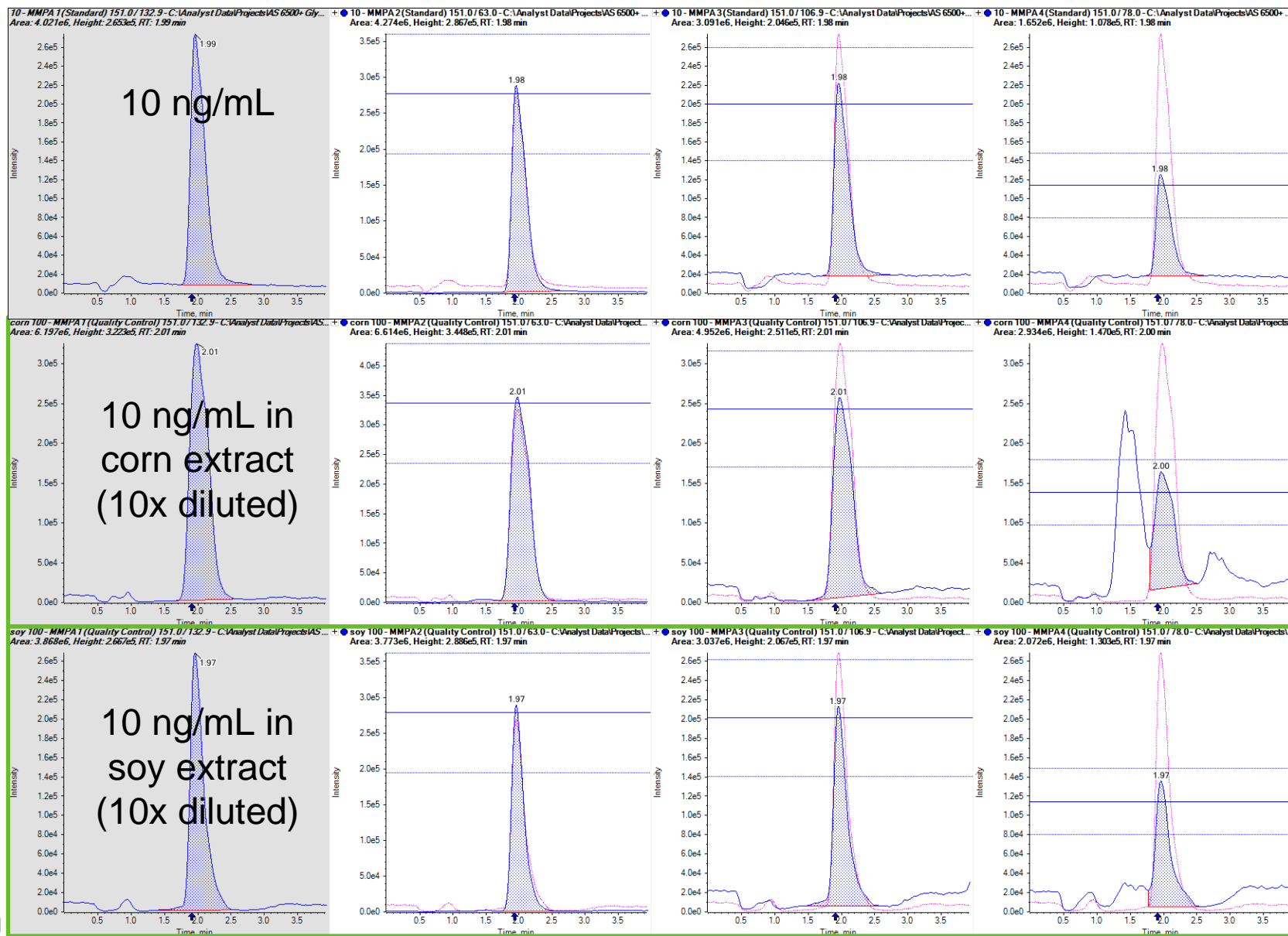
Method 1 Performance – AMPA



Method 1 Performance – Glufosinate



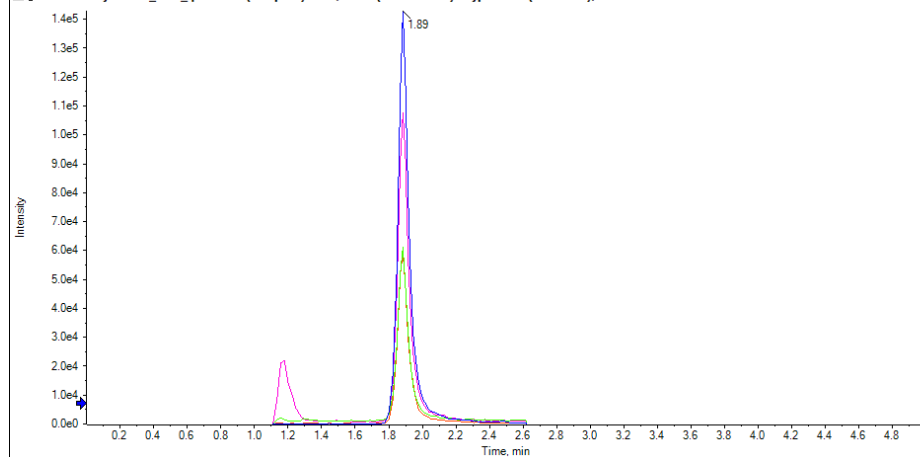
Method 1 Performance – MMPA



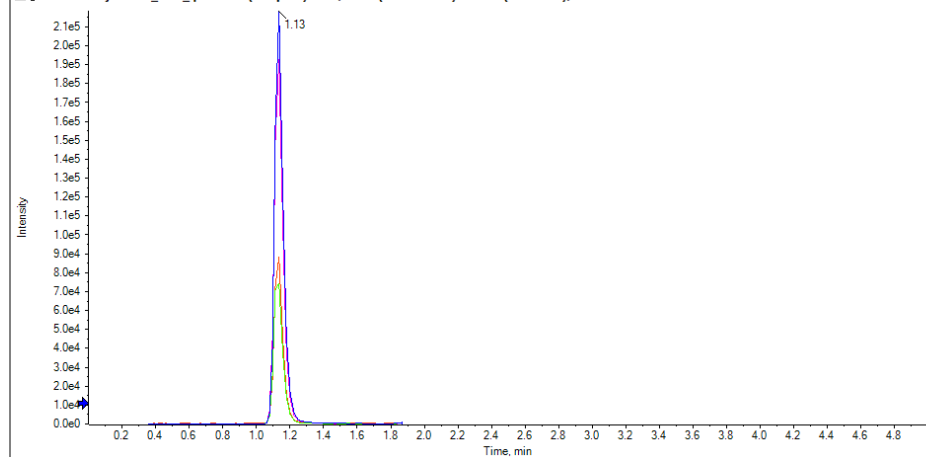
Method 2 (Trinity Q1 ammonium formate pH=2.9)

100 ng/mL Standard

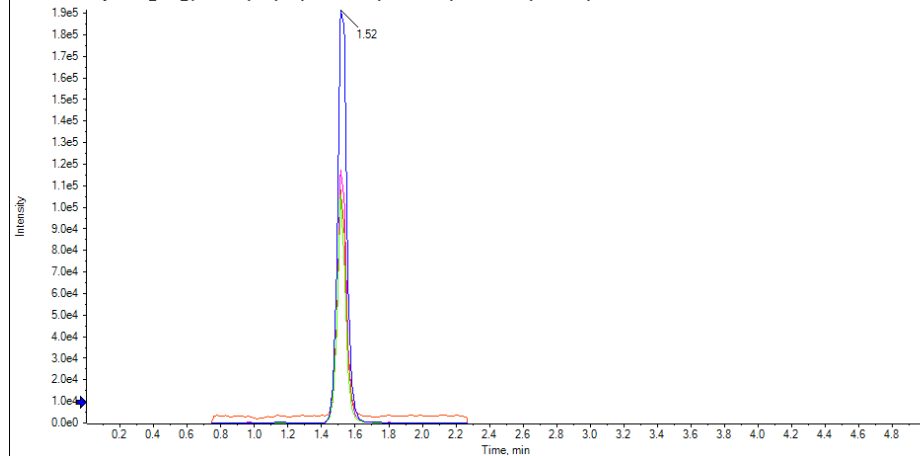
± ● XIC from Gly and Co_FDA_quant.wiff (sample 7) - 100, -MRM(17 transitions): Glyphosate 1 (168.0/62.9), Gaussian smoothed



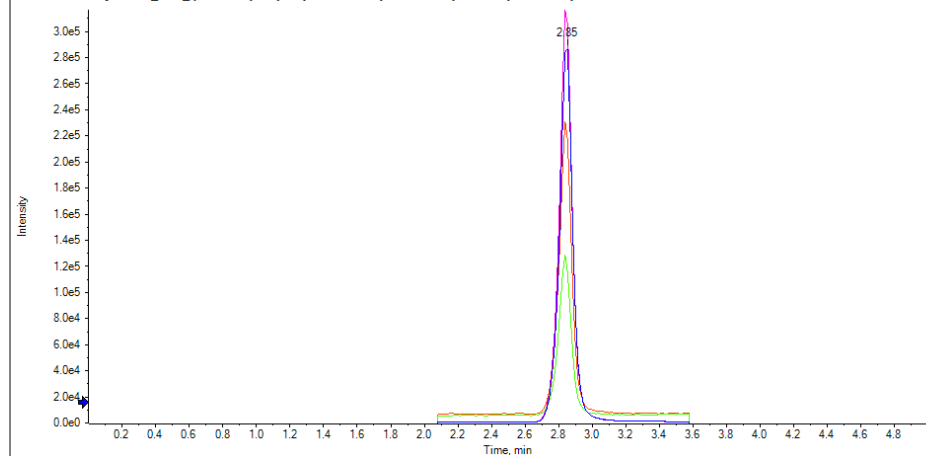
± ● XIC from Gly and Co_FDA_quant.wiff (sample 7) - 100, -MRM(17 transitions): AMPA 1 (110.0/62.9), Gaussian smoothed



± ● XIC from Gly and Co_FDA_quant.wiff (sample 7) - 100, -MRM(17 transitions): Glufosinate 1 (180.0/62.9), Gaussian smoothed

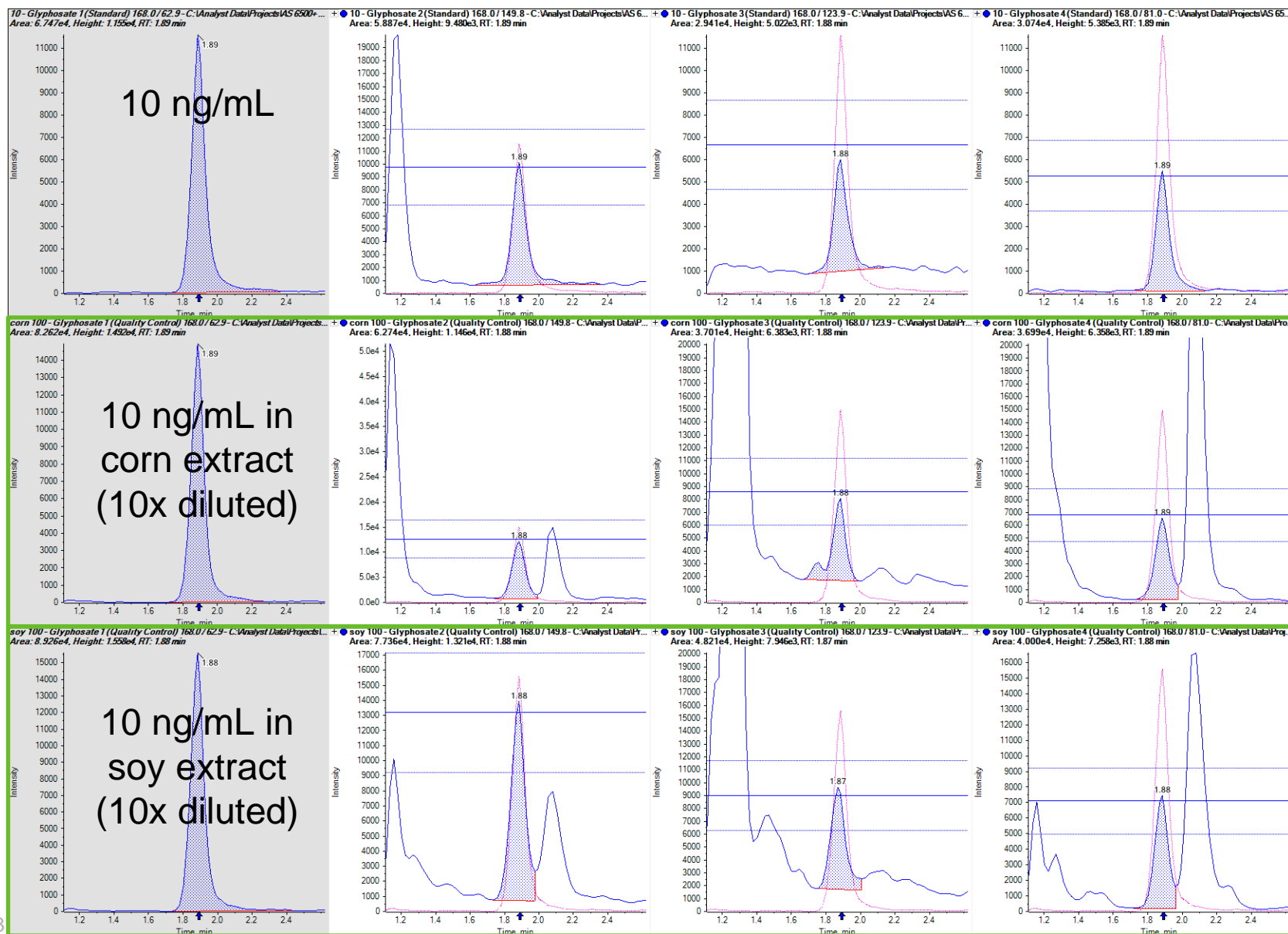


± ● XIC from Gly and Co_FDA_quant.wiff (sample 7) - 100, -MRM(17 transitions): MMPA 1 (151.0/132.9), Gaussian smoothed

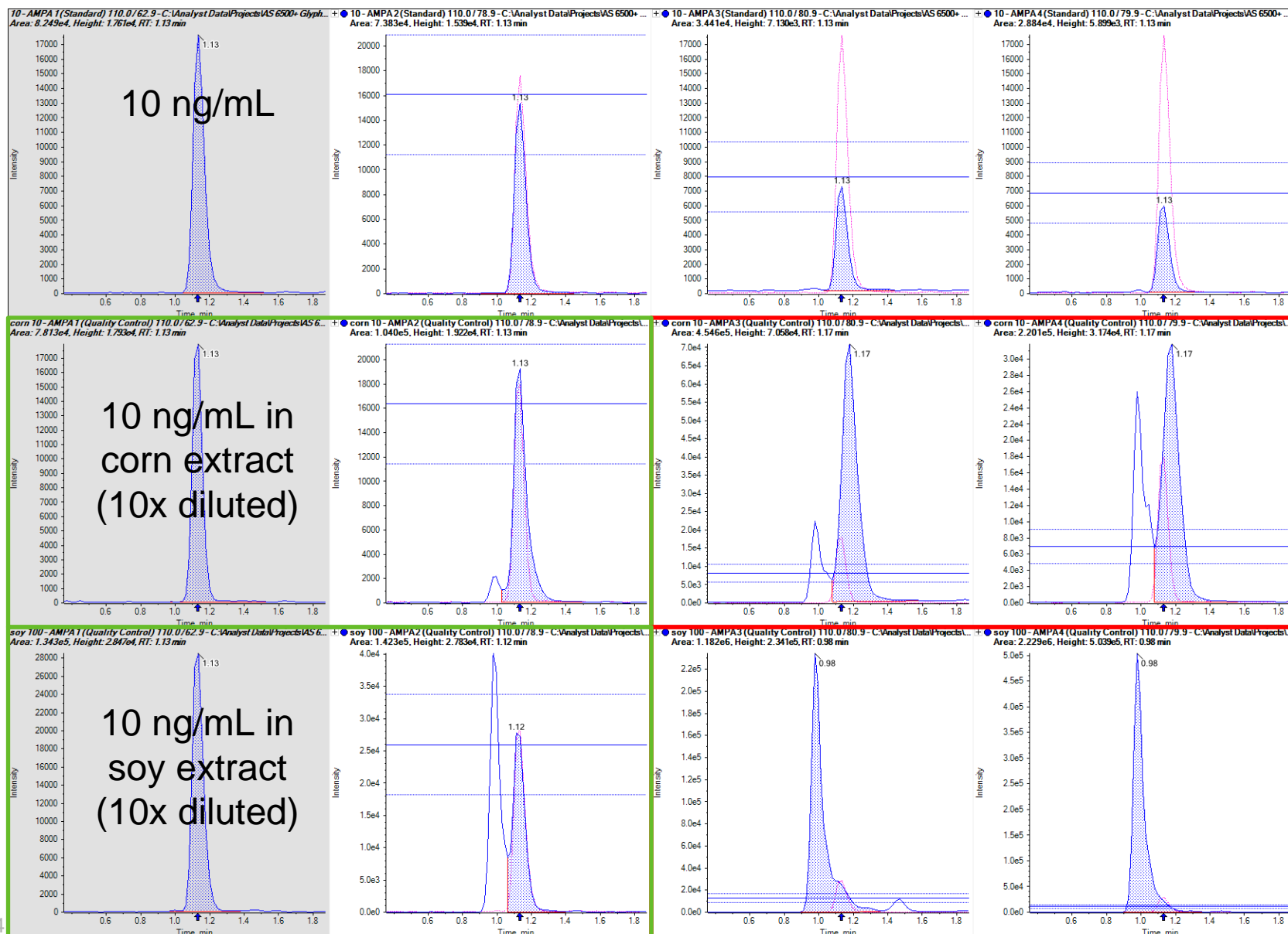


Good separation of compounds to use the *Scheduled* MRM™ algorithm

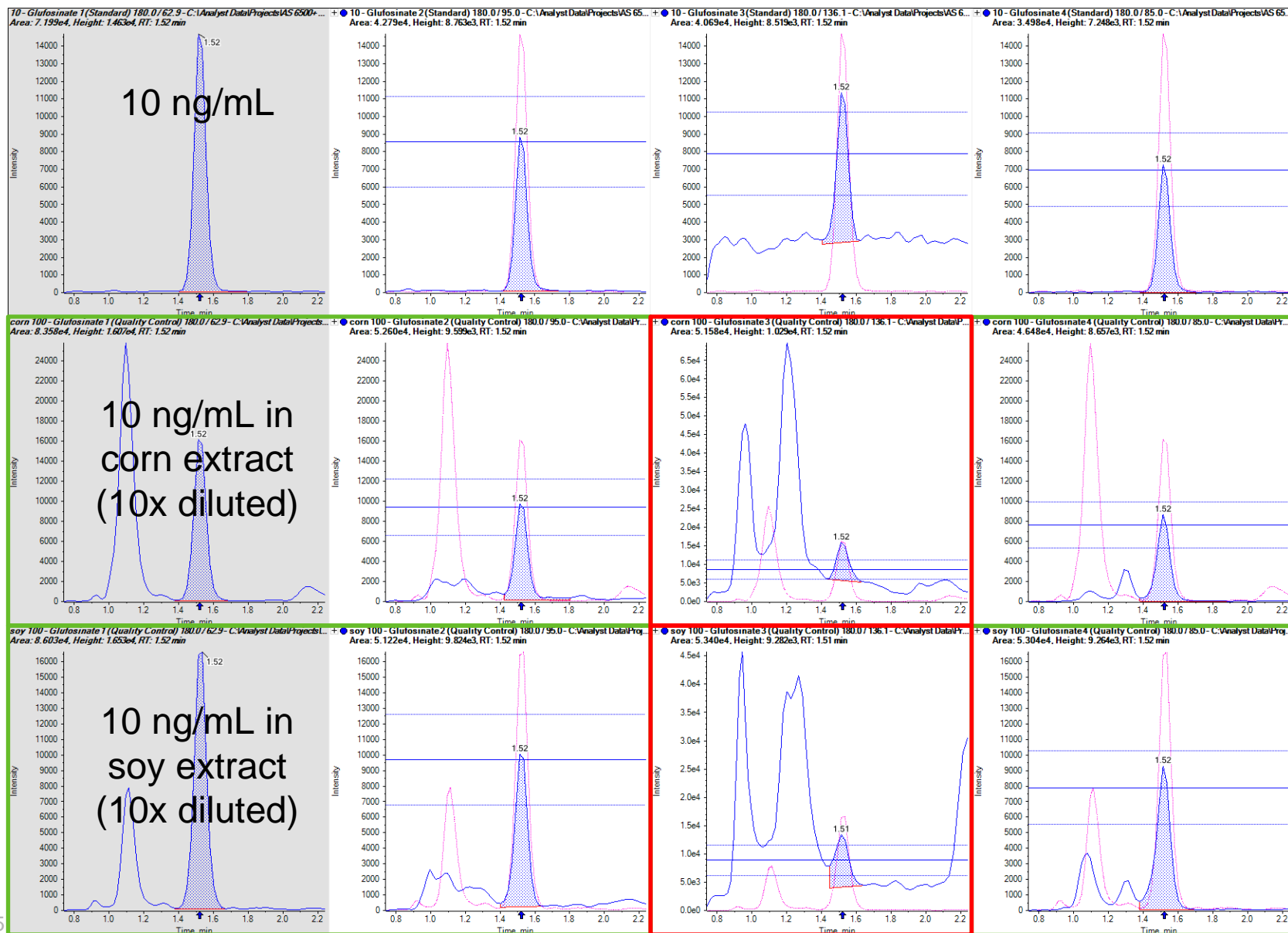
Method 2 Performance – Glyphosate



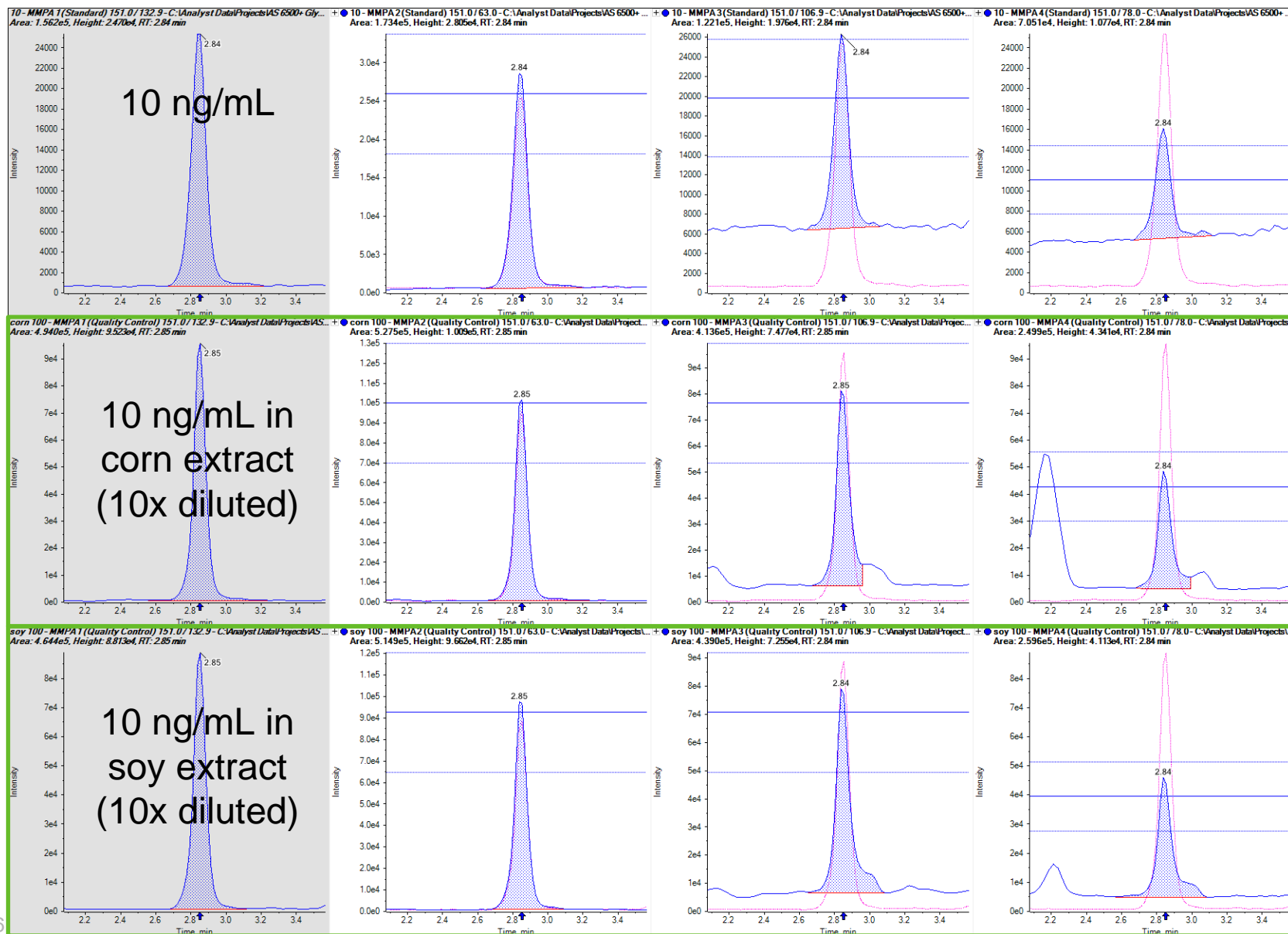
Method 2 Performance – AMPA



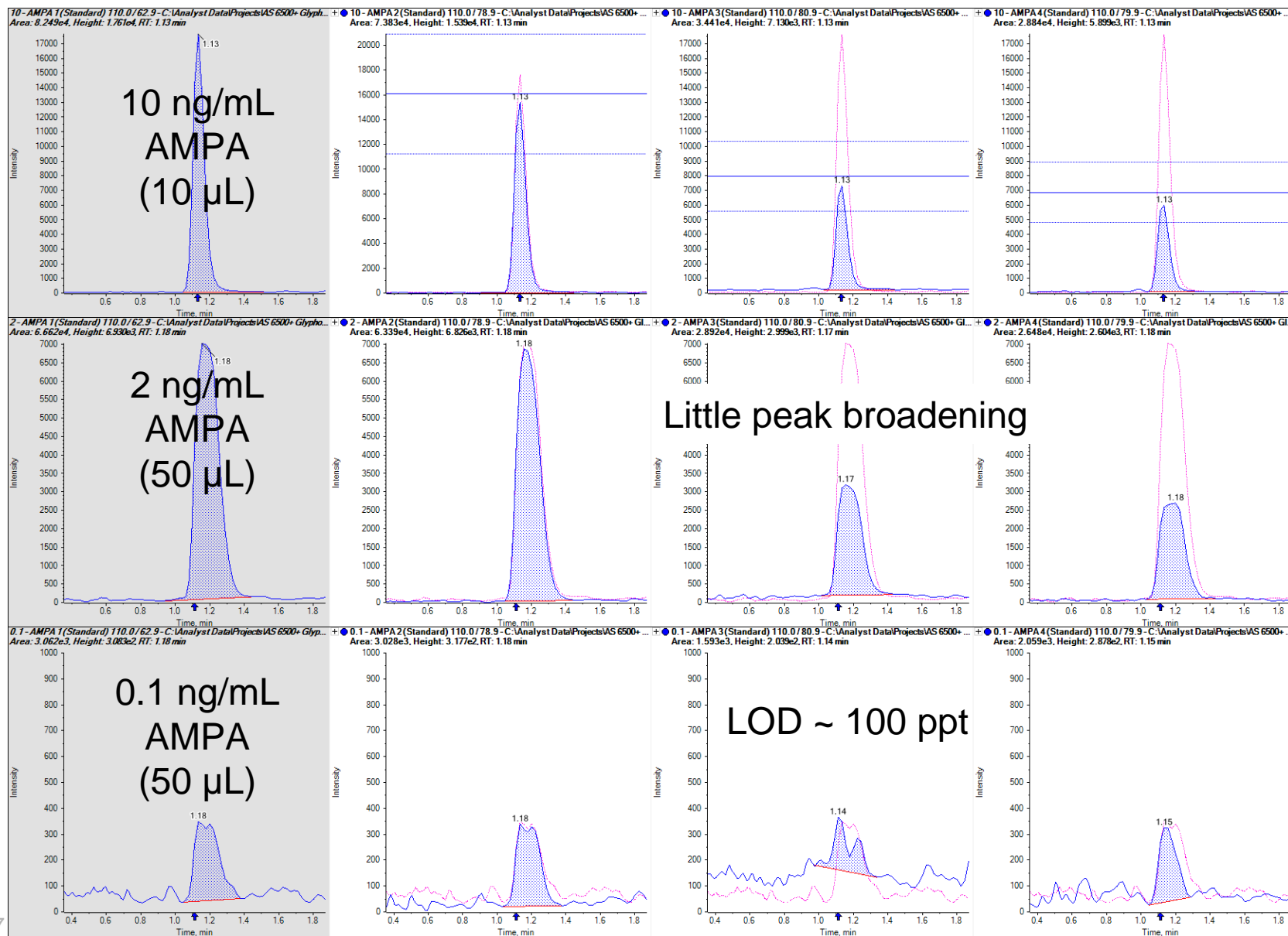
Method 2 Performance – Glufosinate



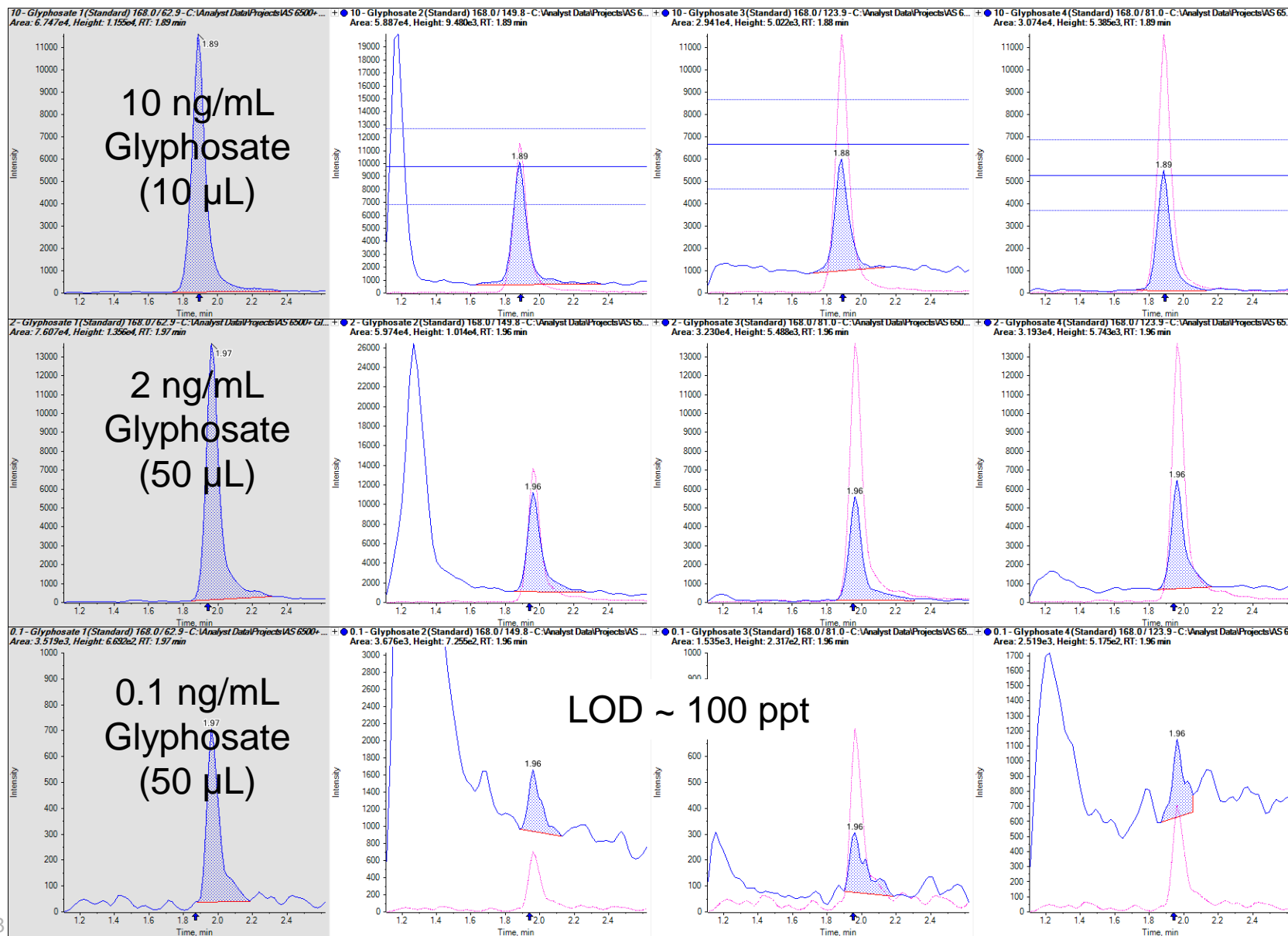
Method 2 Performance – MMPA



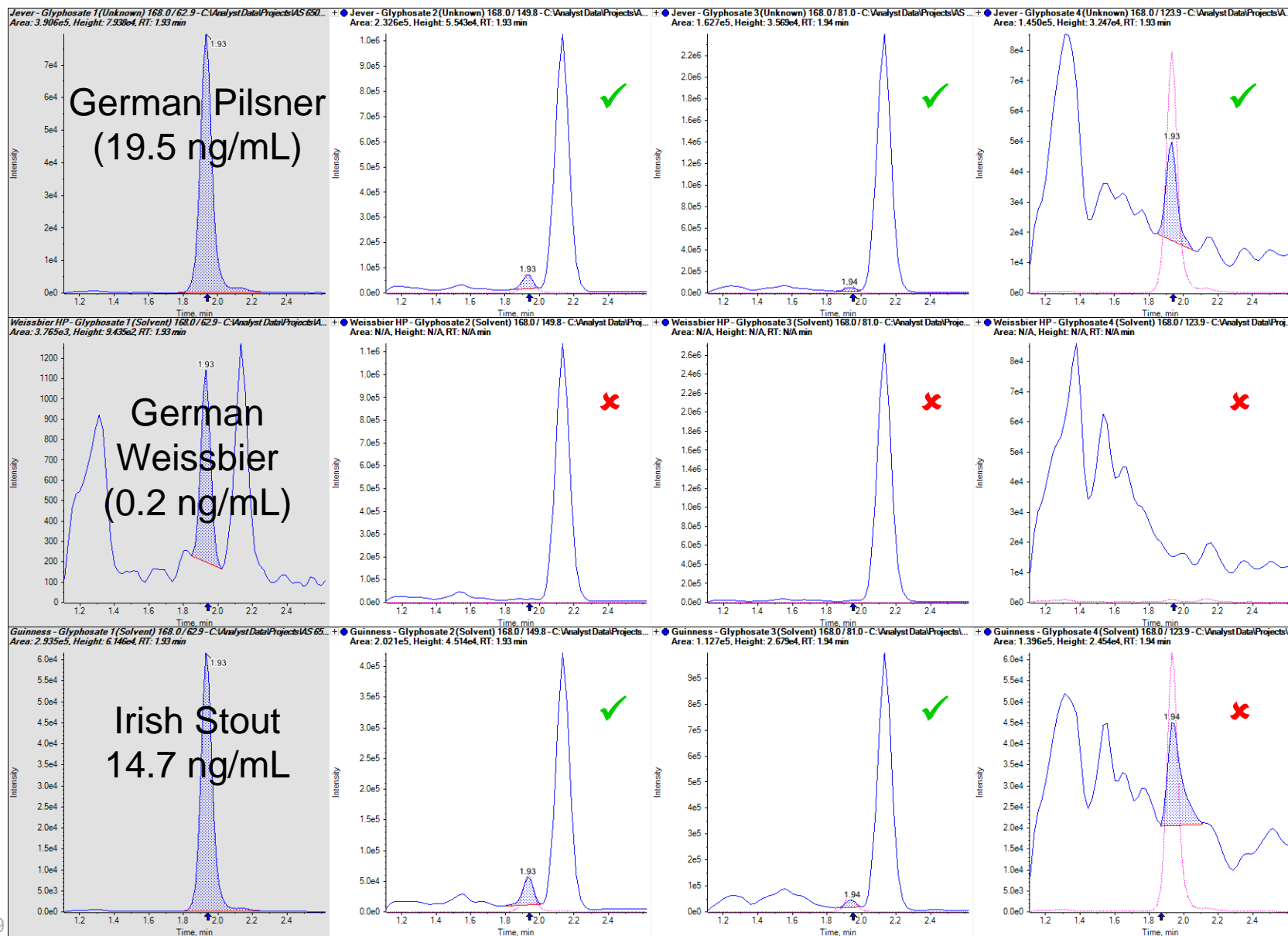
Method 2 Performance – Large Volume Injection (50 μ L)



Method 2 Performance – Large Volume Injection (50 μ L)

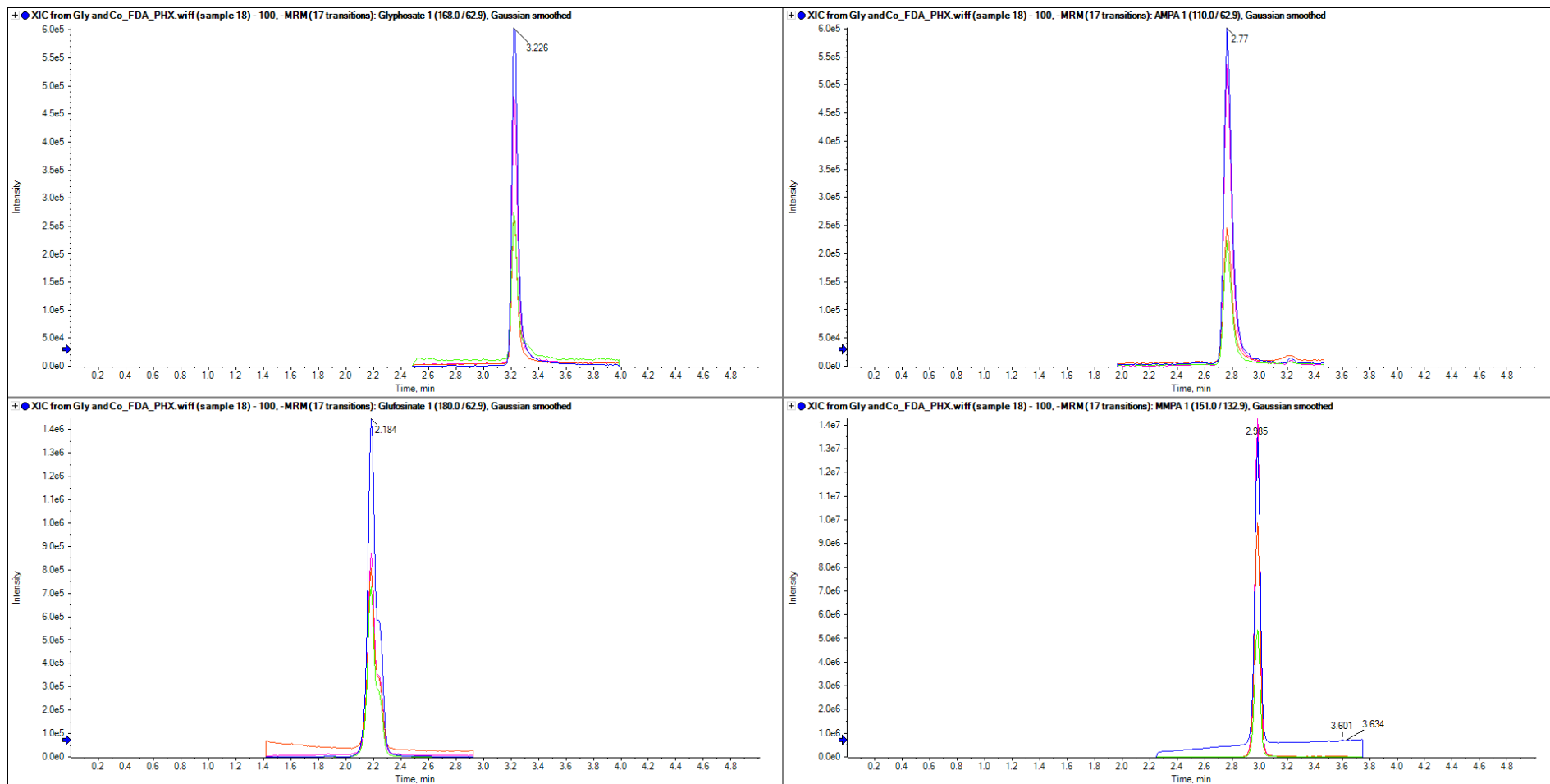


Method 2 Performance – Glyphosate in Beer (2x Dilution)



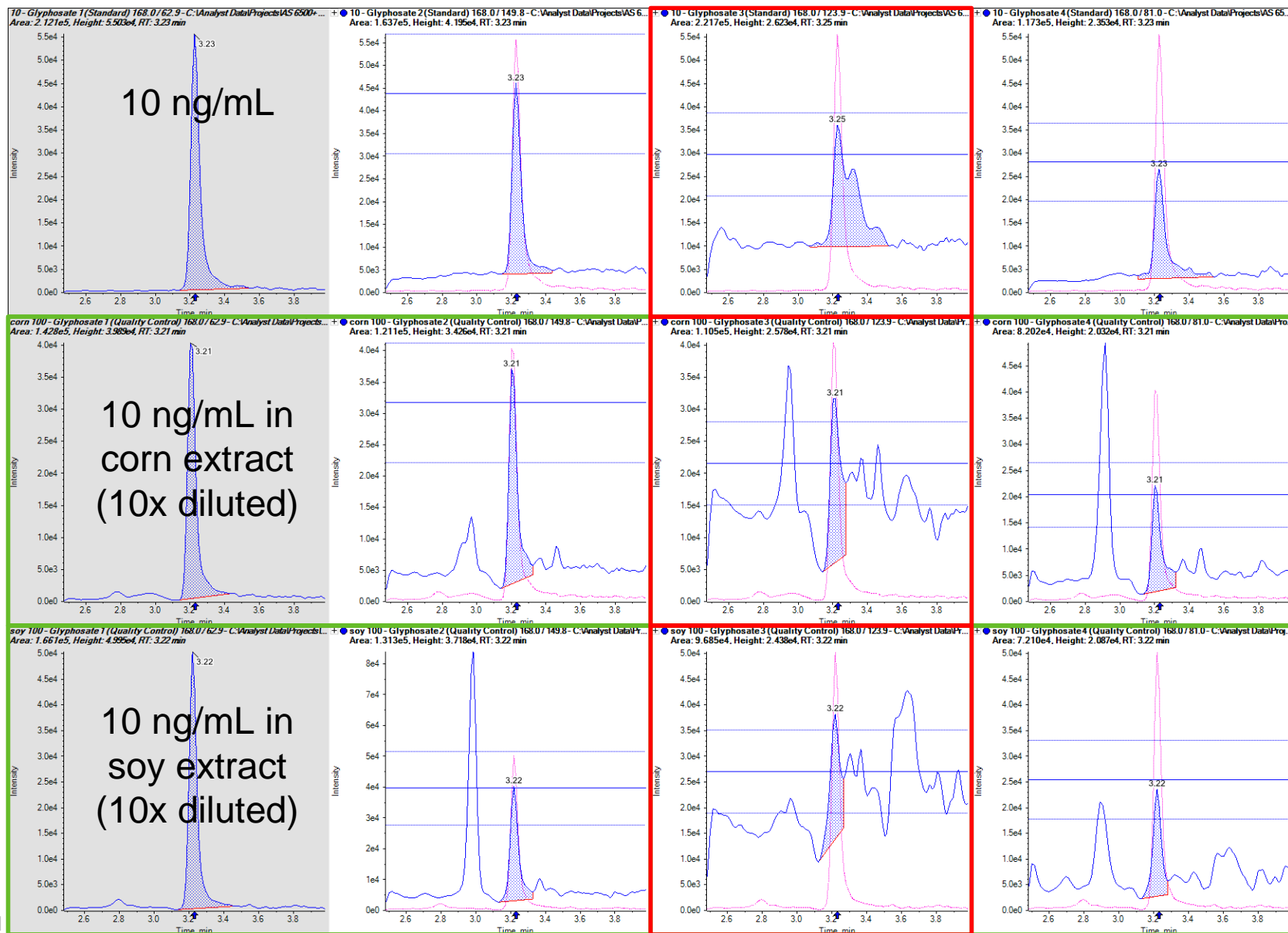
Method 3 (LUNA NH2 ammonium bicarbonate pH=10)

100 ng/mL Standard

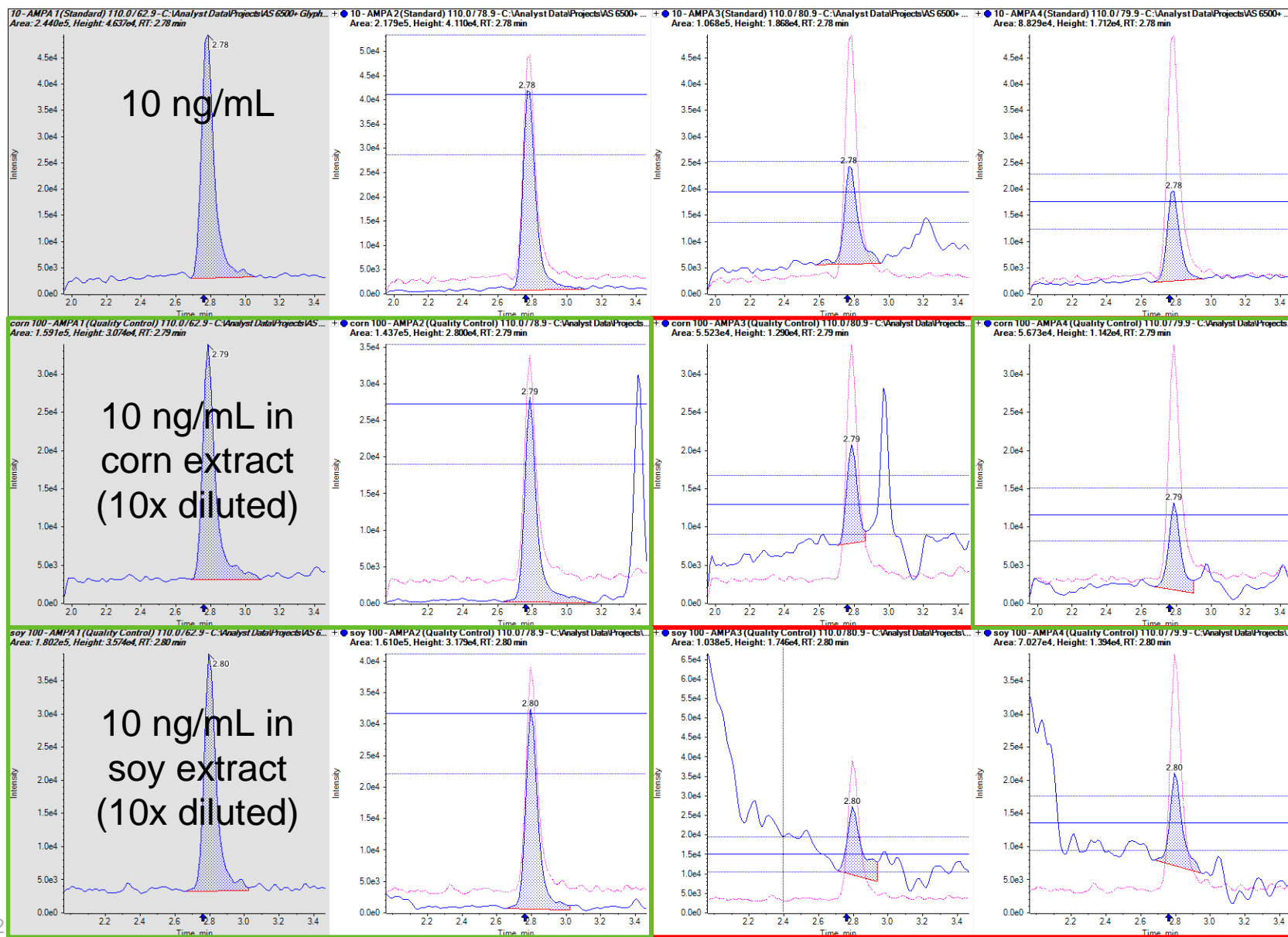


Good separation of compounds to use the *Scheduled* MRM™ algorithm

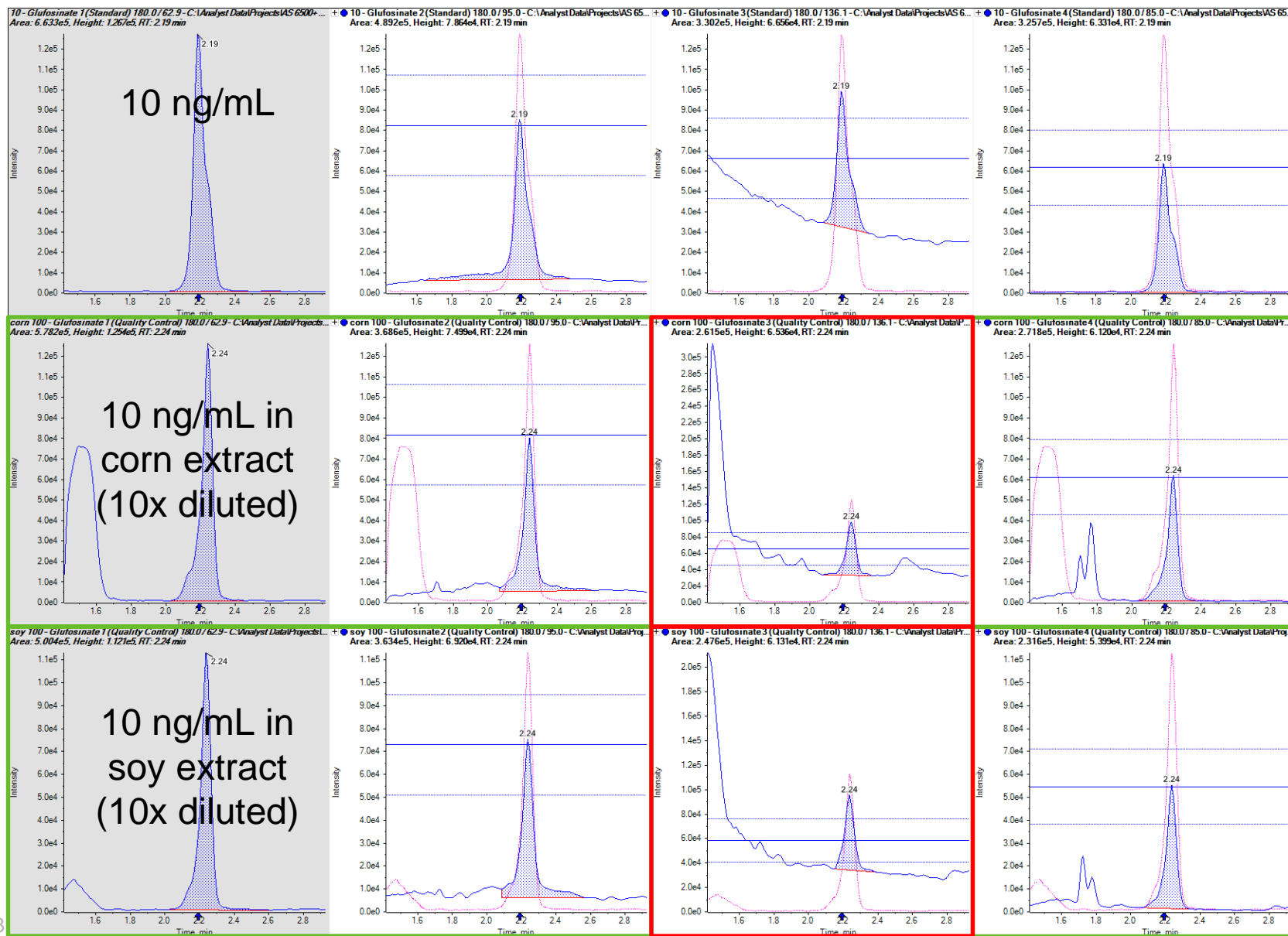
Method 3 Performance – Glyphosate



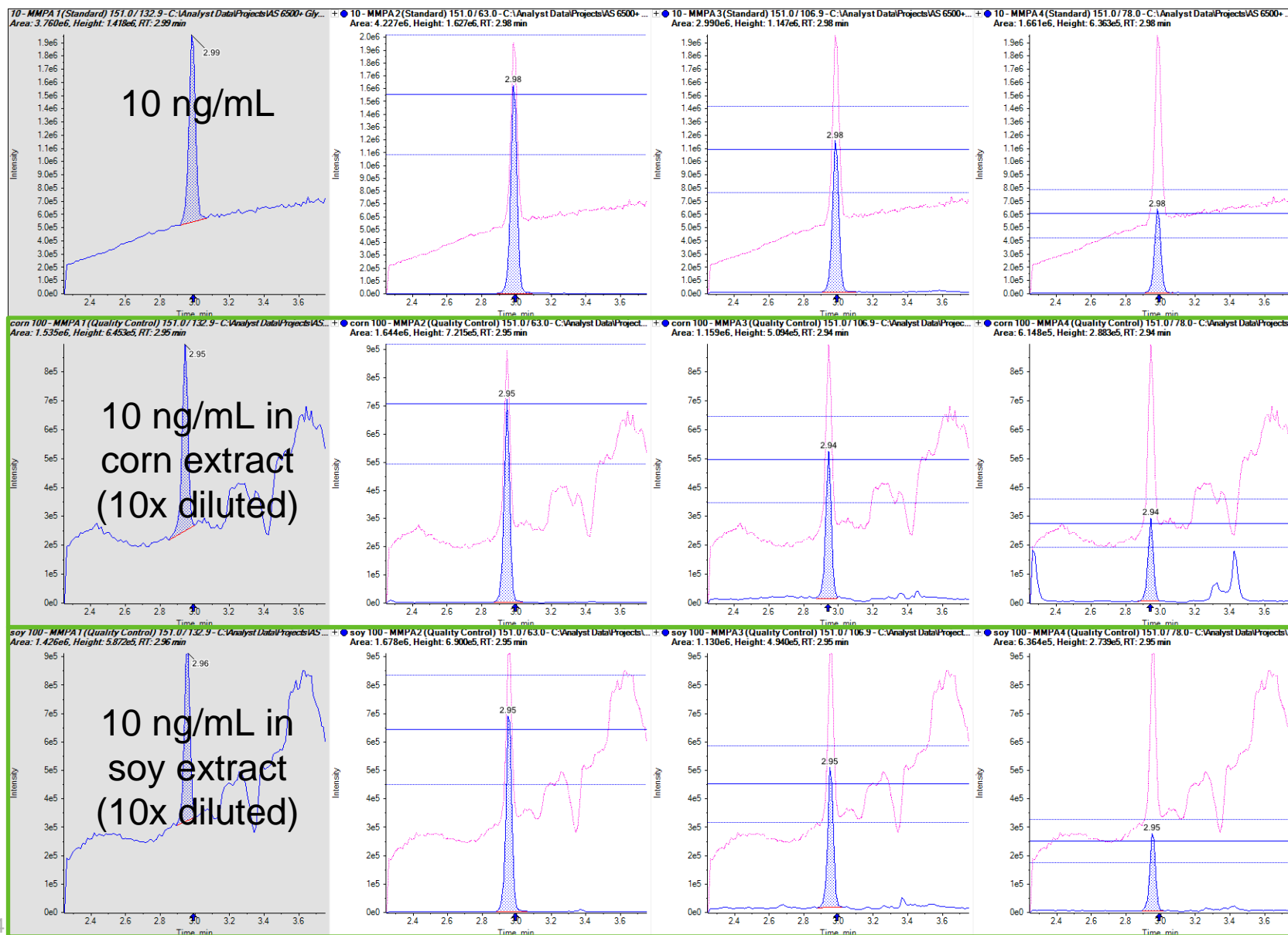
Method 3 Performance – AMPA



Method 3 Performance – Glufosinate



Method 3 Performance – MMPA



Method Comparison

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



Application Data

– Glyphosate, AMPA, Glufosinate and MMPA in Foods –



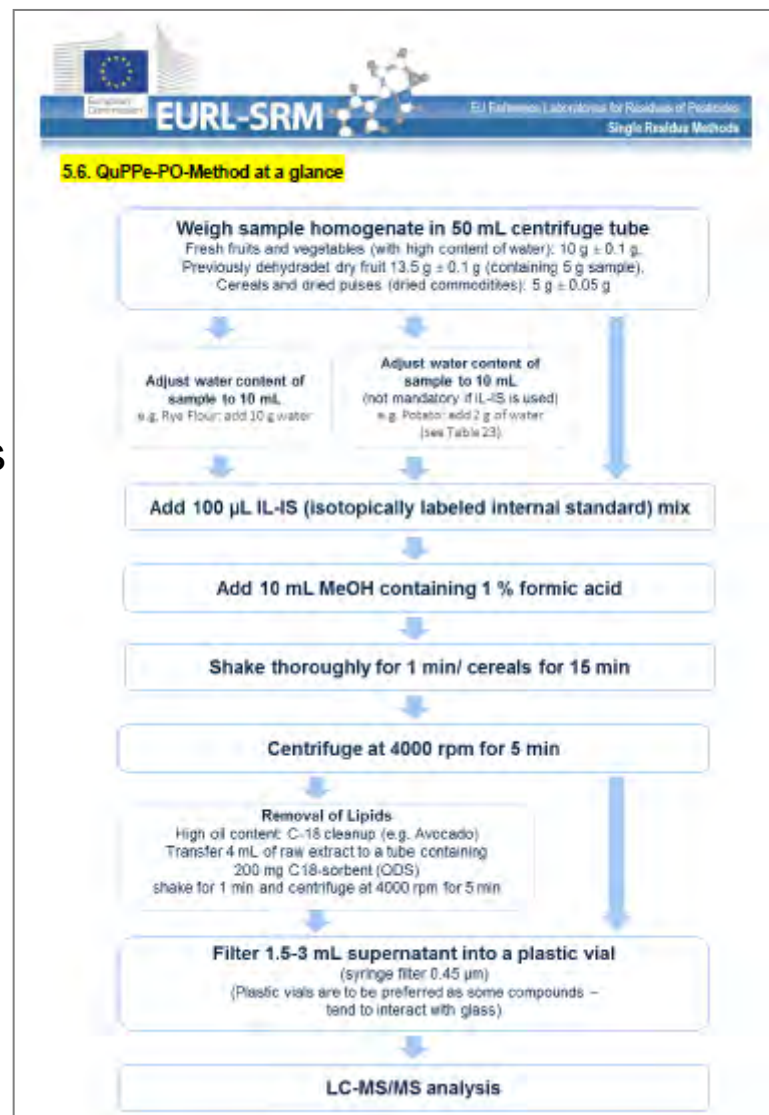
SCIEX ExionLC™ AC system



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



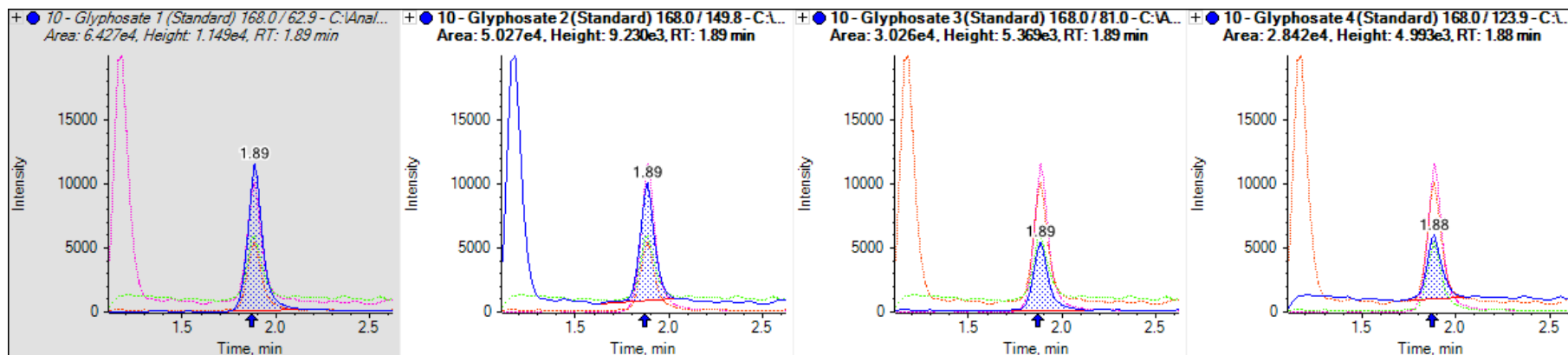
Experimental

- 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 700°C
 - Scheduled* MRM[™] algorithm (Q3 low resolution)
 - Full scan MS/MS with CE -35 V and CES 15

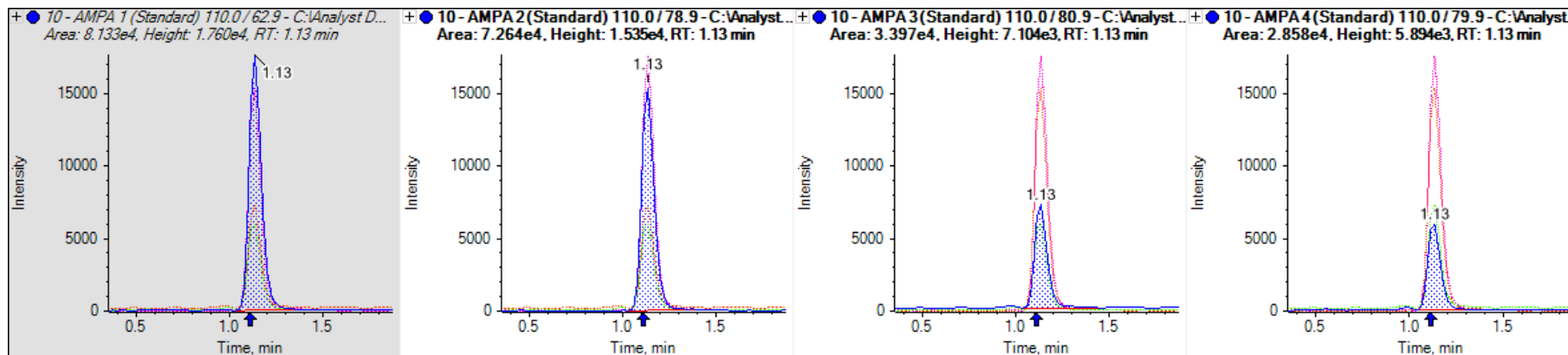
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	168	150	-30	-14
	168	124	-30	-16
	168	81	-30	-20
AMPA	110	63	-15	-26
	110	79	-15	-36
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	180	95	-50	-24
	180	136	-50	-22
	180	85	-50	-24
MMPA	151	133	-10	-18
	151	63	-10	-44
	151	107	-10	-20
	151	78	-10	-28

Glyphosate and AMPA at 10 ng/mL (10 μ L Injection)

Glyphosate %CV = 2.99% (n = 5)

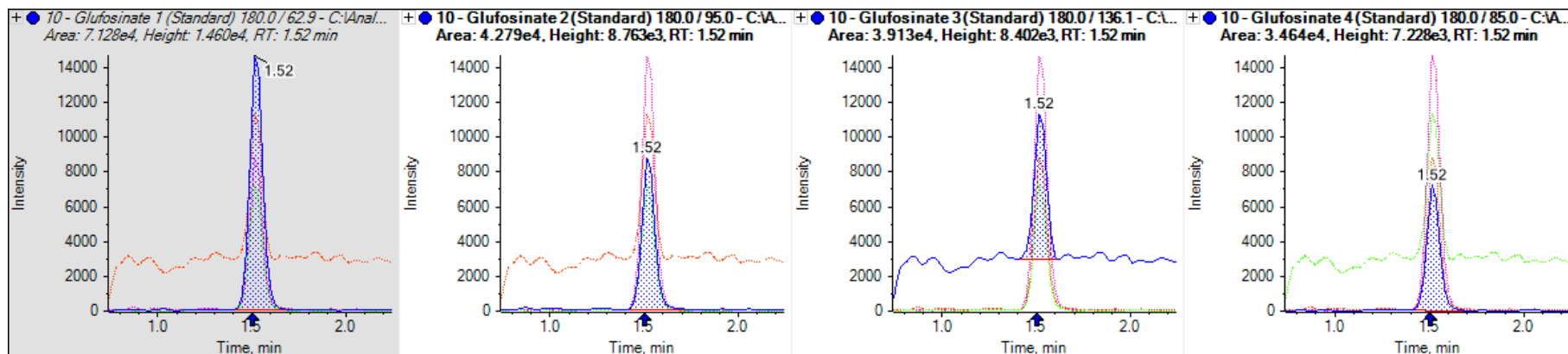


AMPA %CV = 2,85% (n = 5)

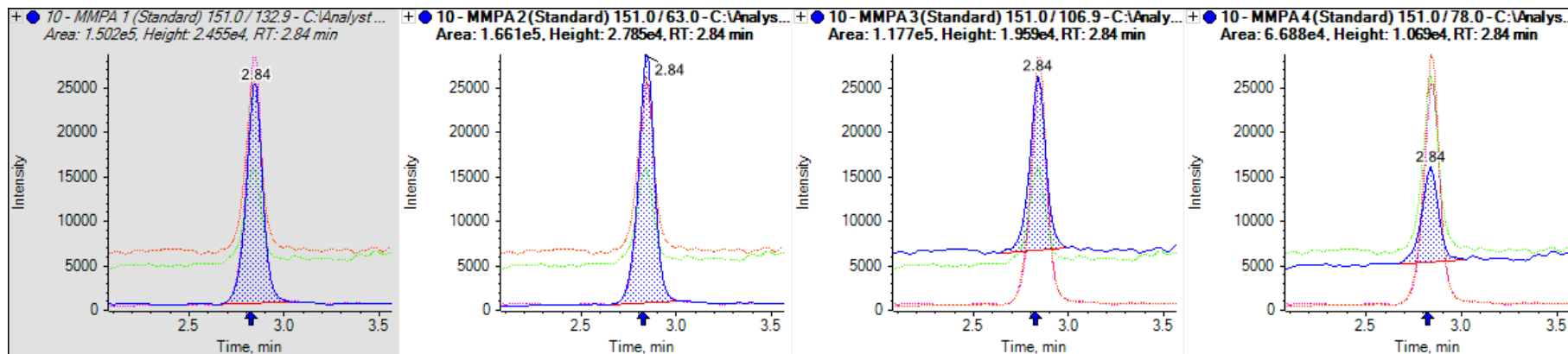


Glufosinate and MMPA at 10 ng/L (10 μ L Injection)

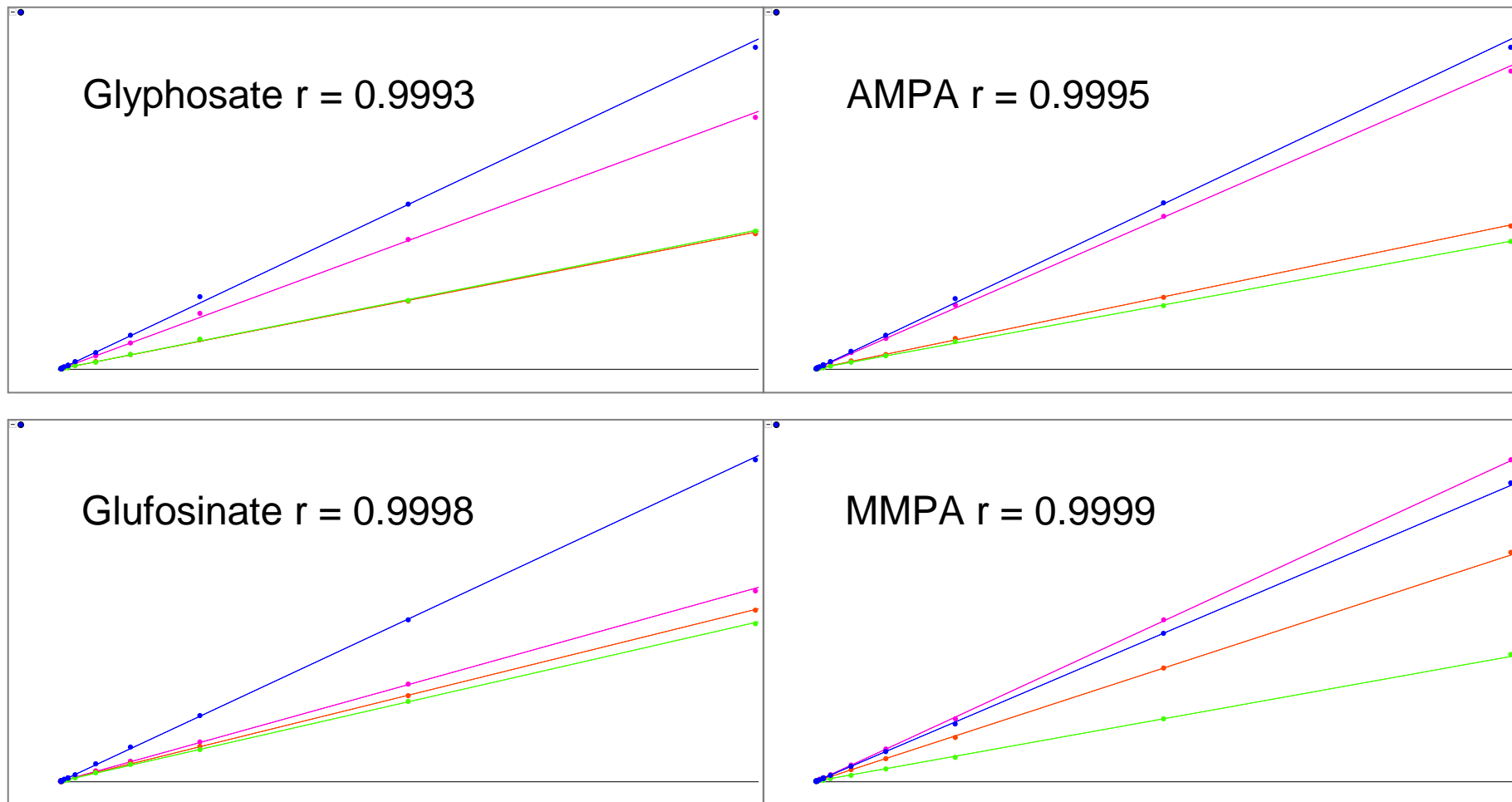
Glufosinate %CV = 3.26% (n = 5)



MMPA %CV = 2.39% (n = 5)

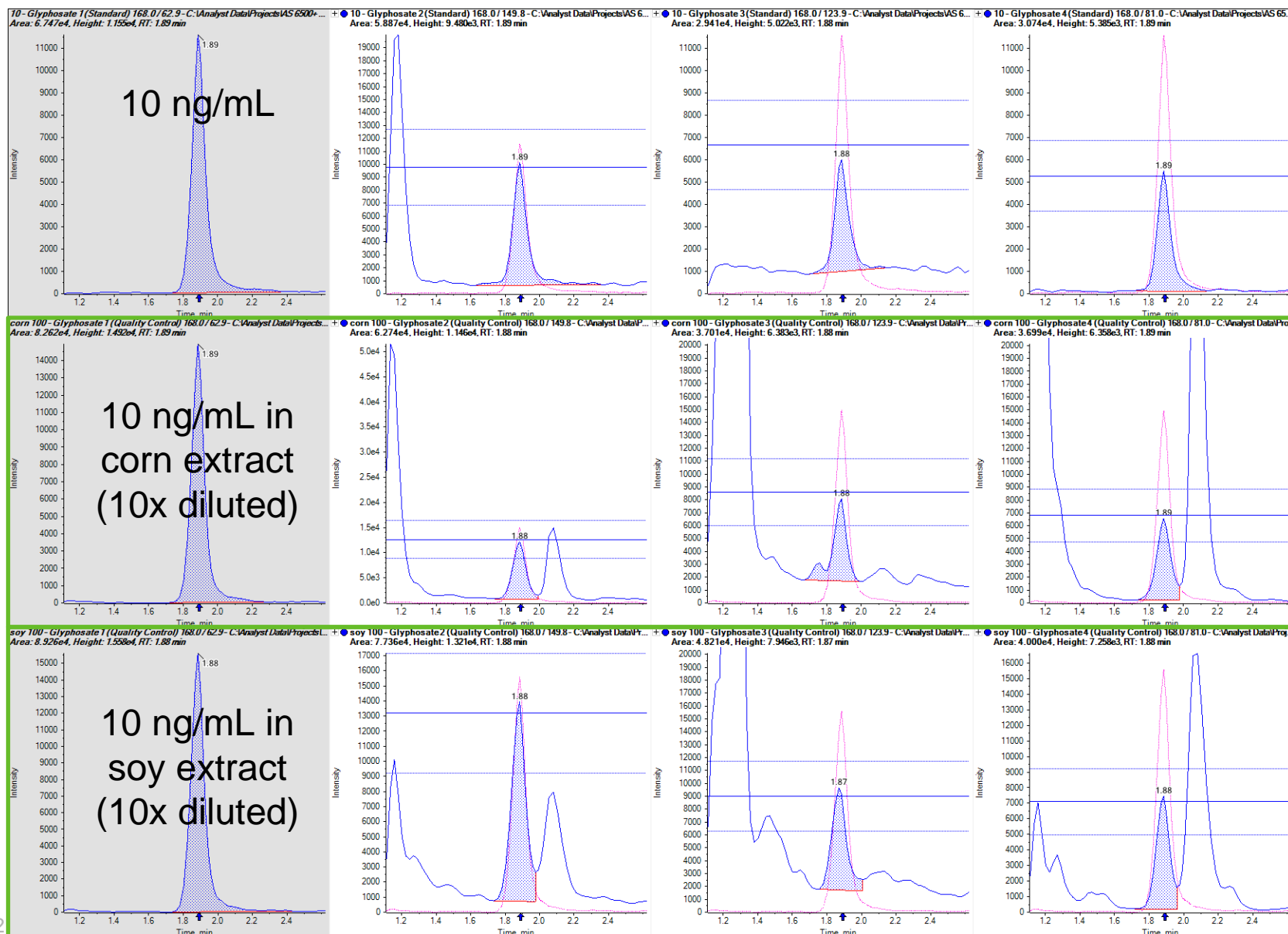


Linear Dynamic Range 1 to 1000 ng/mL

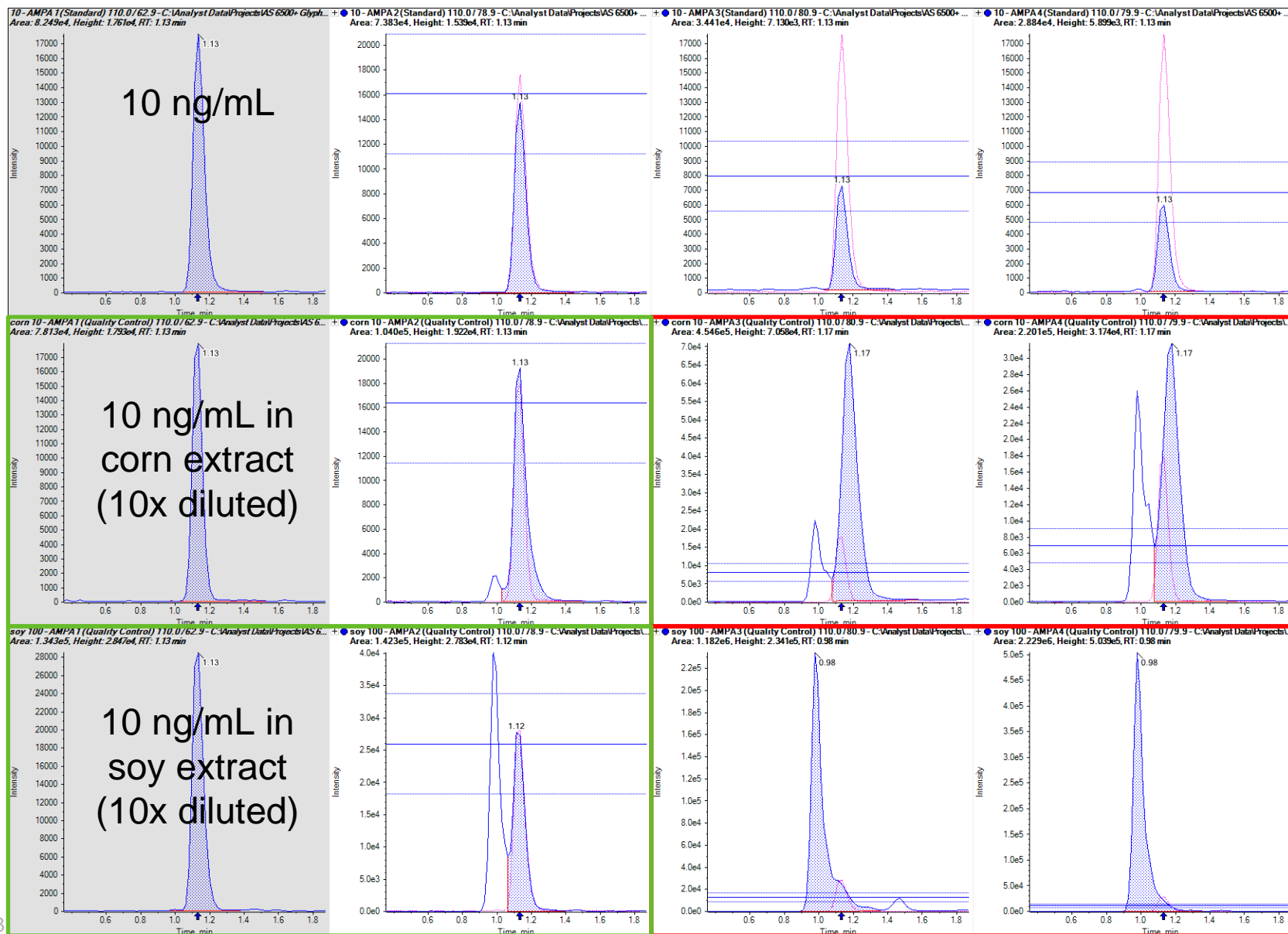


Accuracies between 80 and 120%

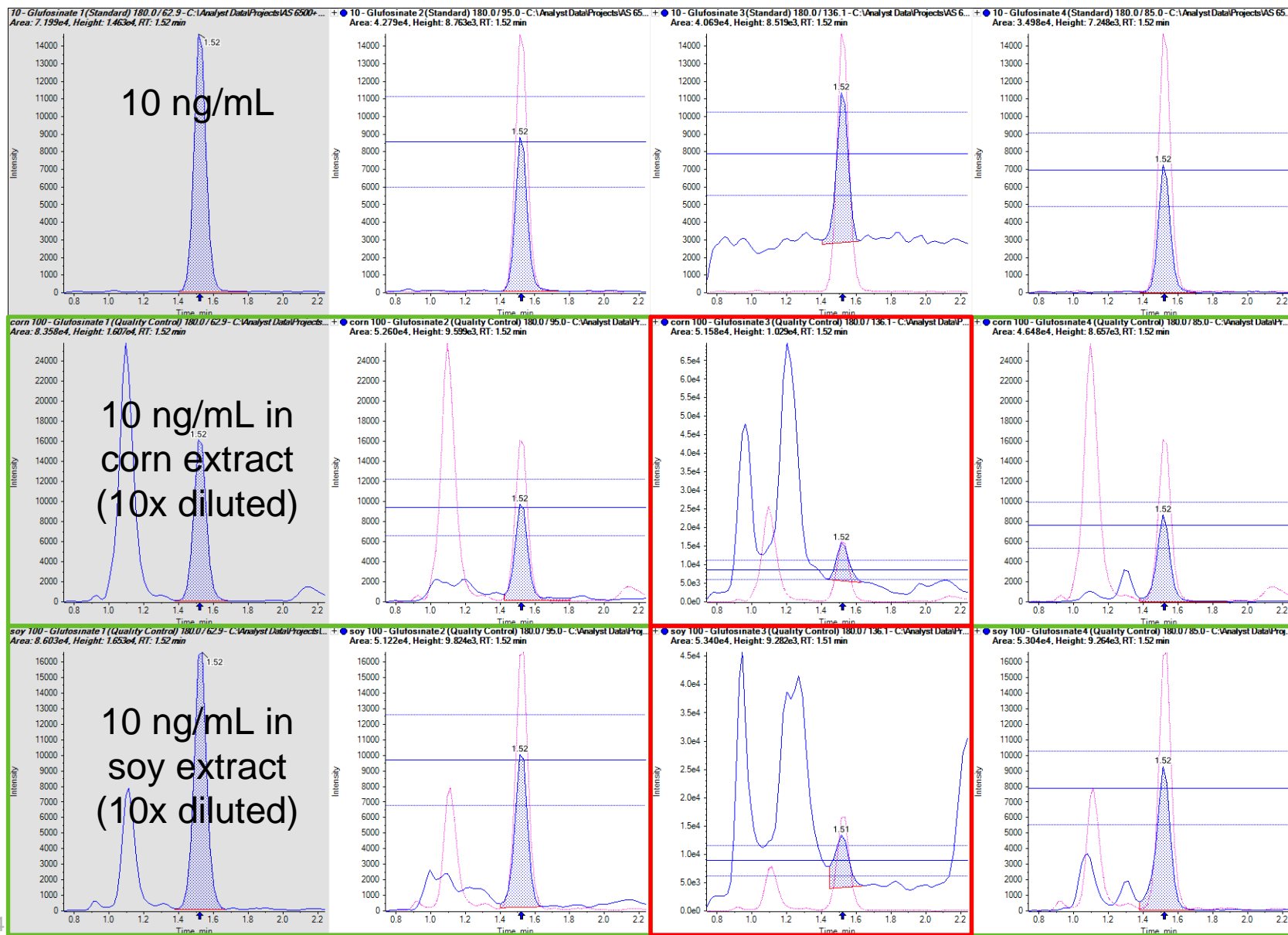
Glyphosate in Corn and Soy Extract



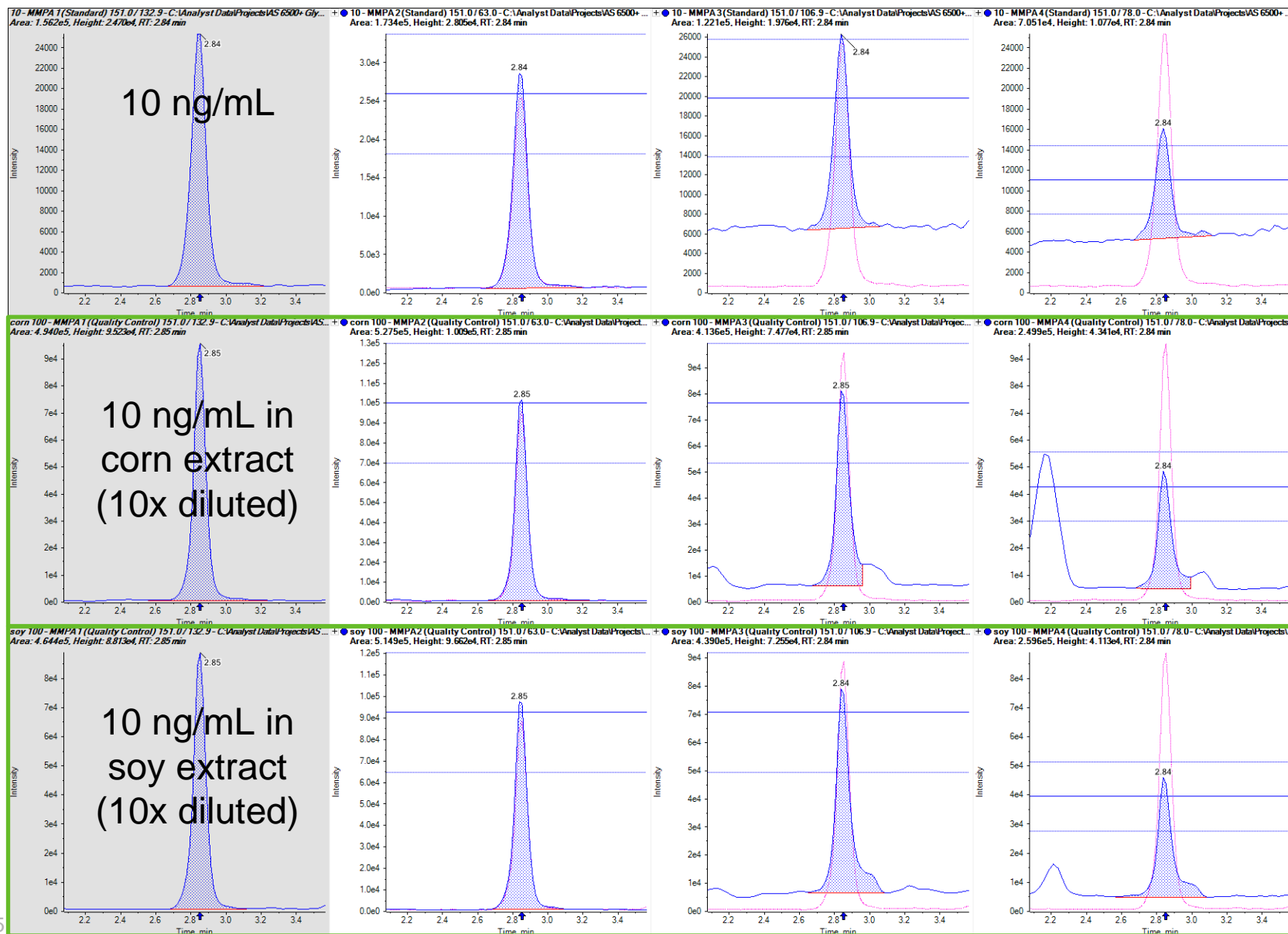
AMPA in Corn and Soy Extract



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

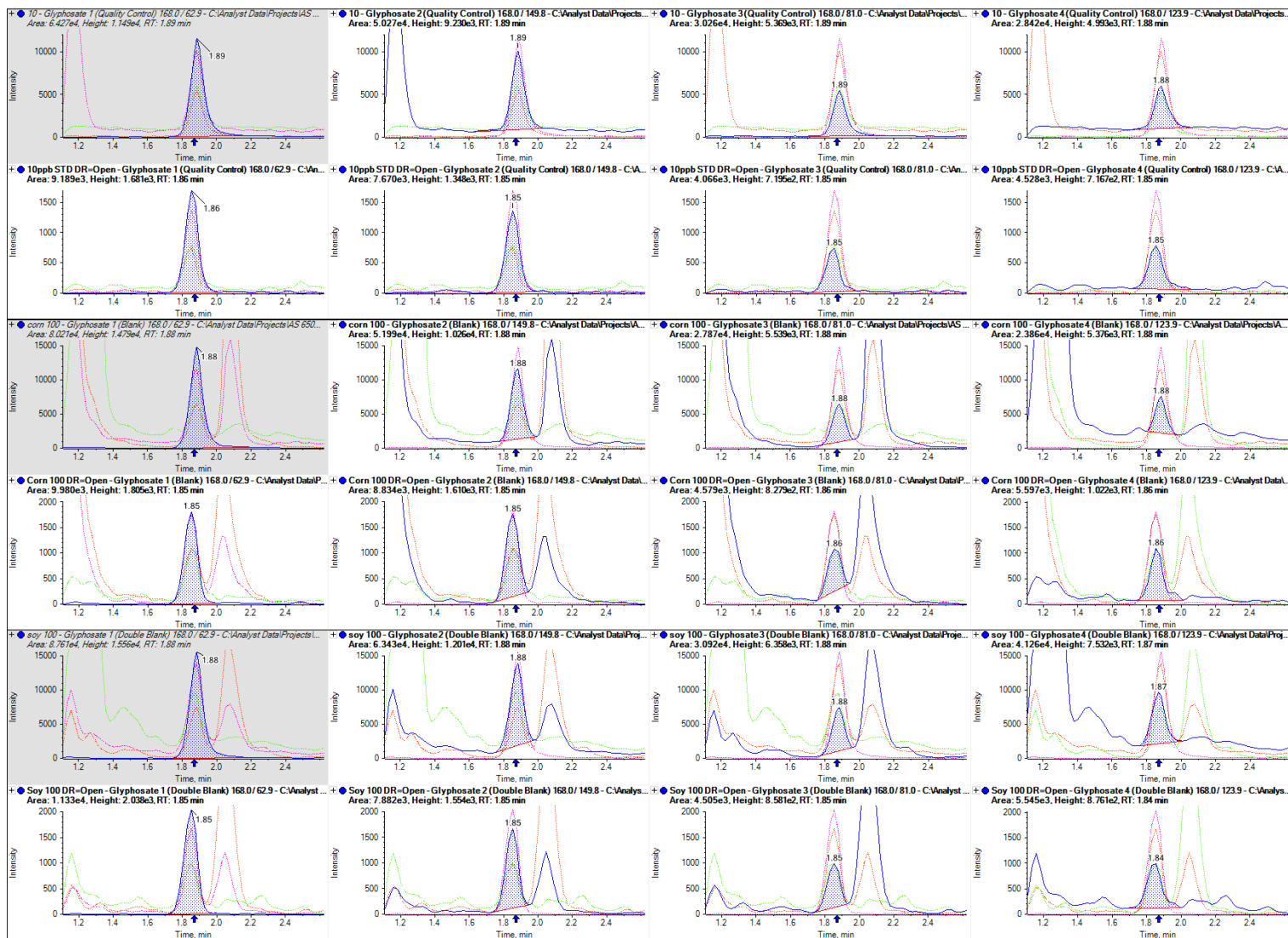
with DMS

Corn
no DMS

with DMS

Soy
no DMS

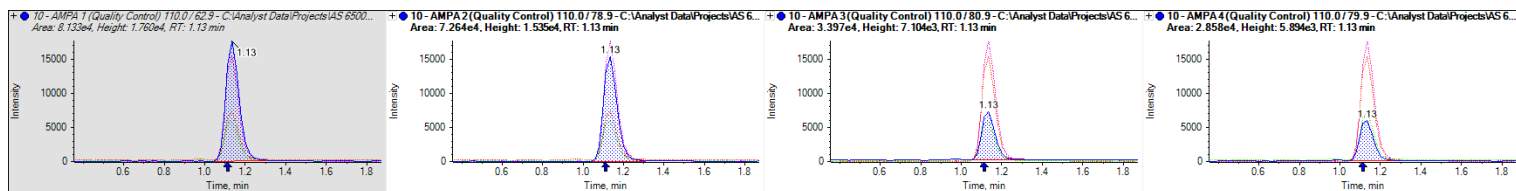
with DMS



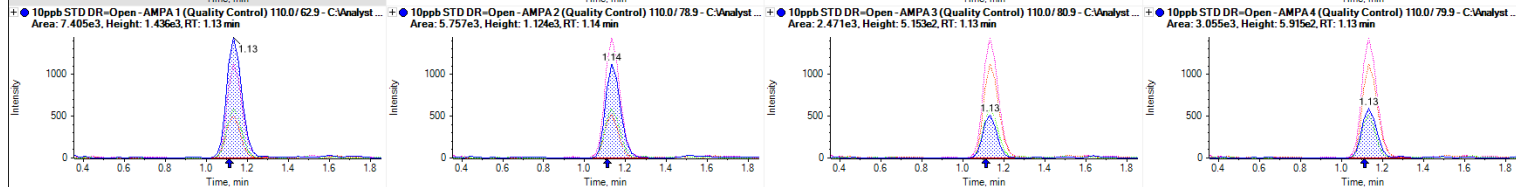
AMPA with and without SelexION® DMS Technology

~11x Less Signal but Greatly Reduced Interferences and Background

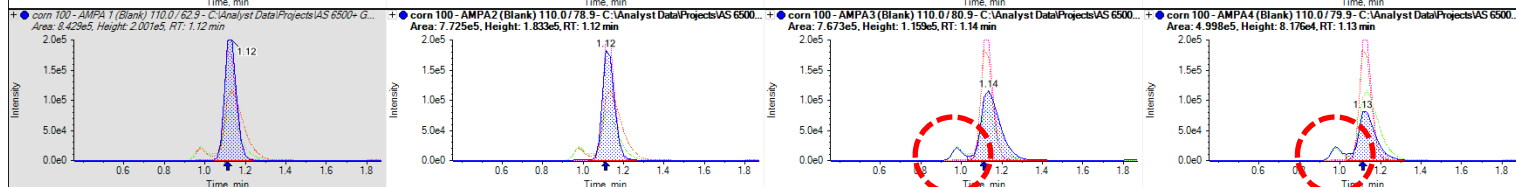
10 ng/mL
no DMS



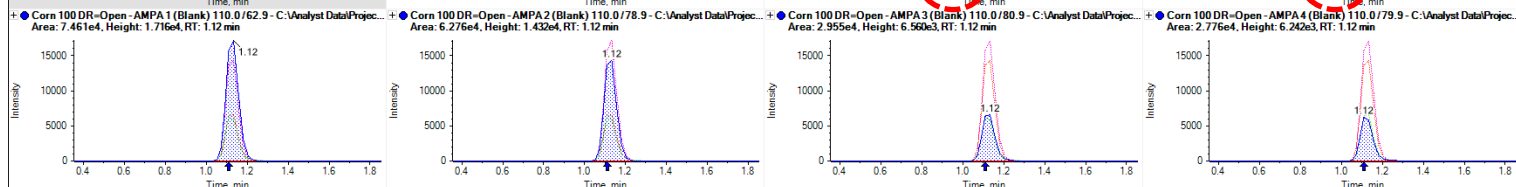
with DMS



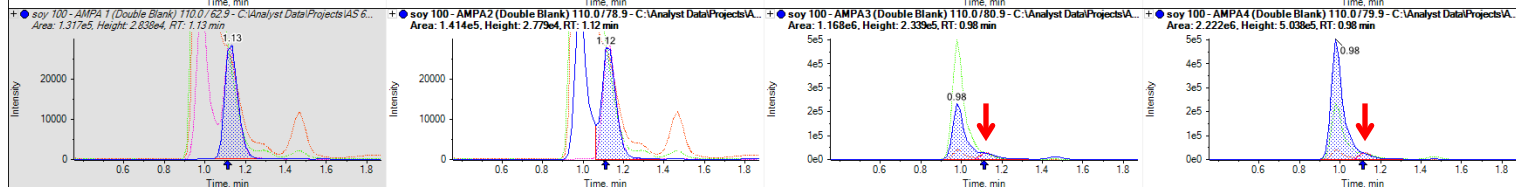
Corn
no DMS



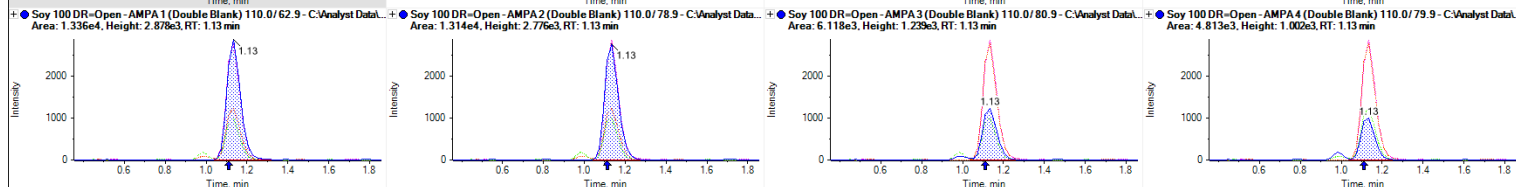
with DMS



Soy
no DMS



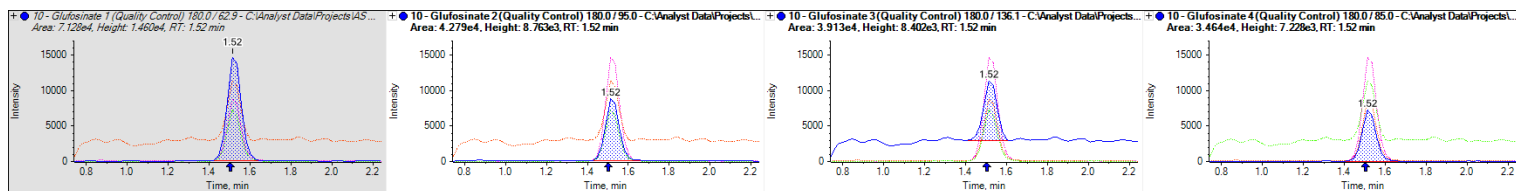
with DMS



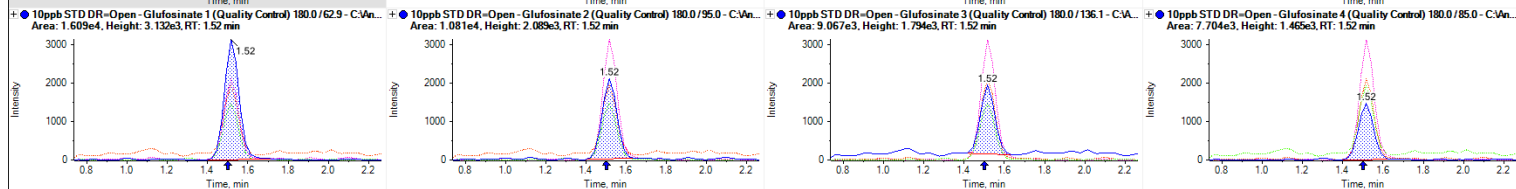
Glufosinate with and without SelexION® DMS Technology

~4x Less Signal but Reduced Interferences and Background

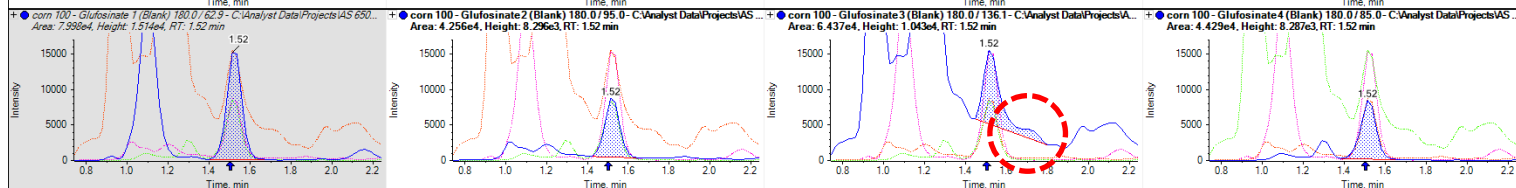
10 ng/mL
no DMS



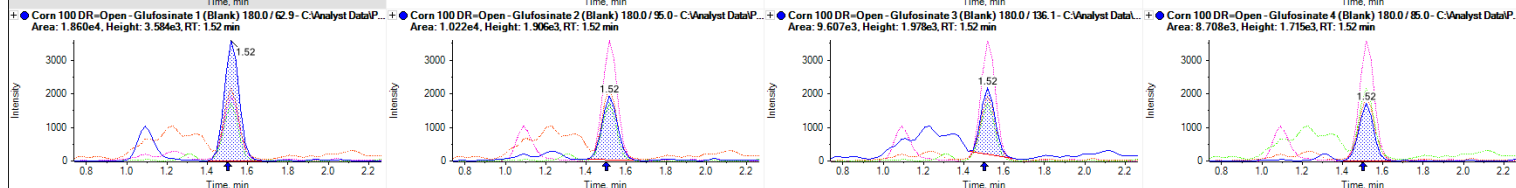
with DMS



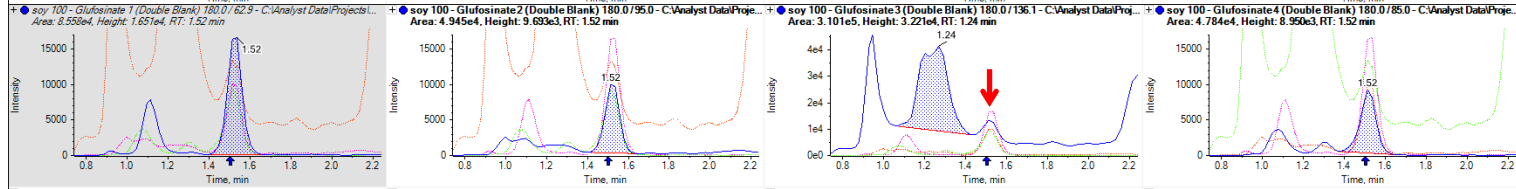
Corn
no DMS



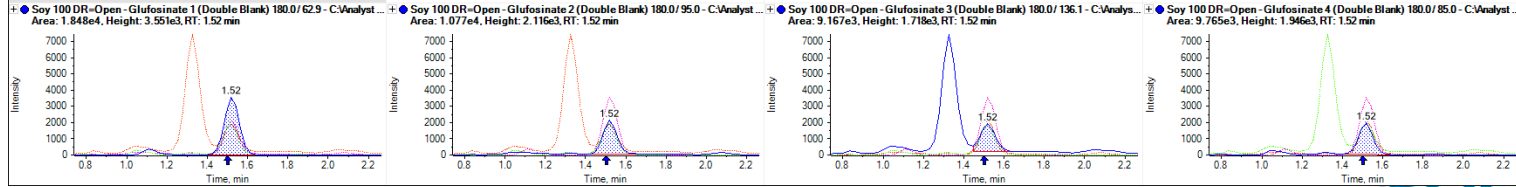
with DMS



Soy
no DMS



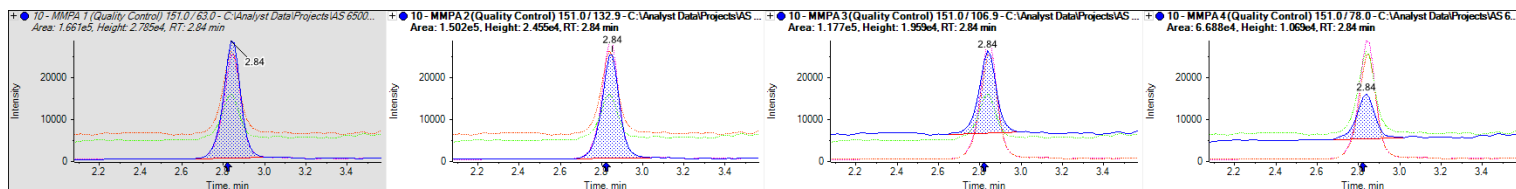
with DMS



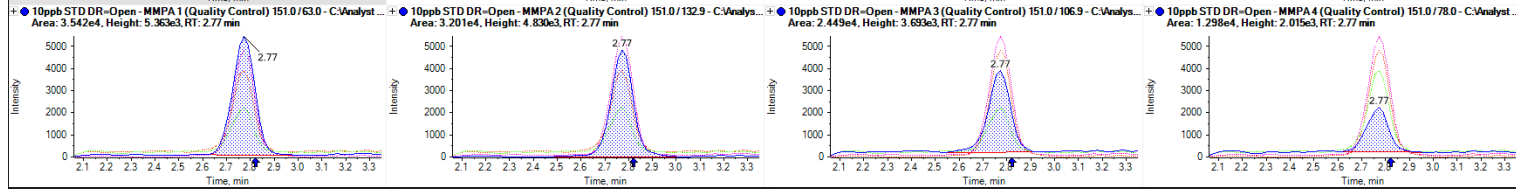
MMPA with and without SelexION® DMS Technology

~5x Less Signal but Reduced Interferences and Background

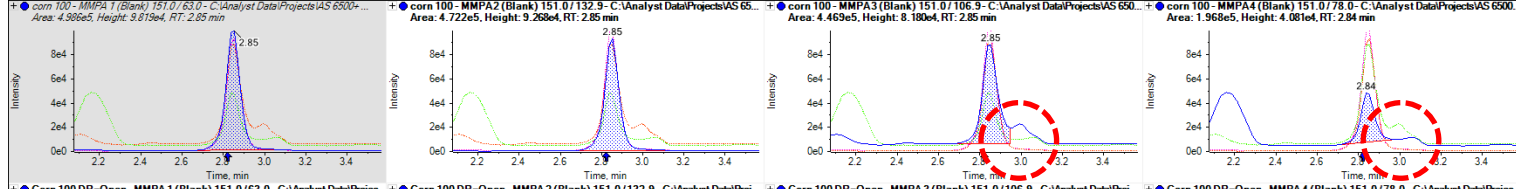
10 ng/mL
no DMS



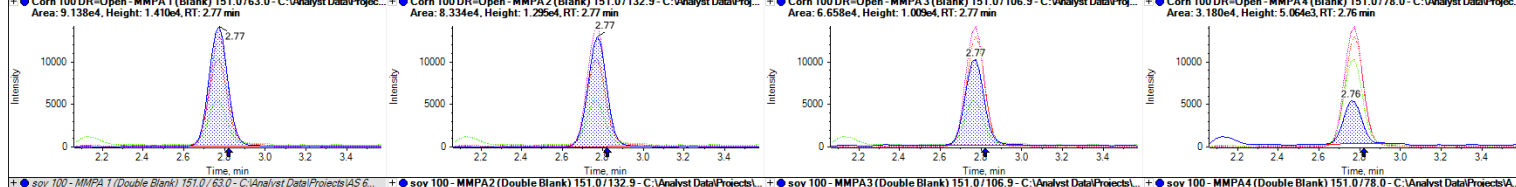
with DMS



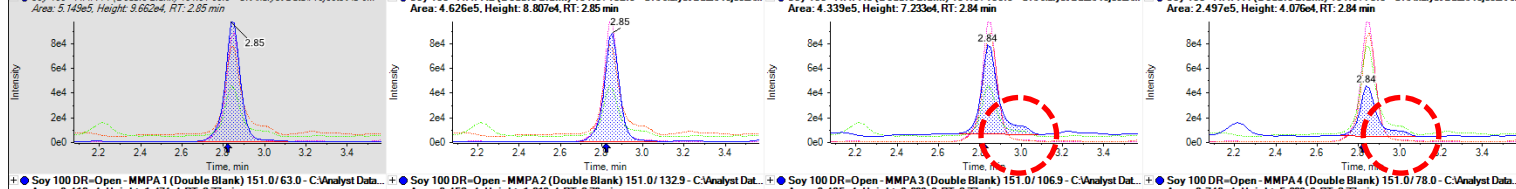
Corn
no DMS



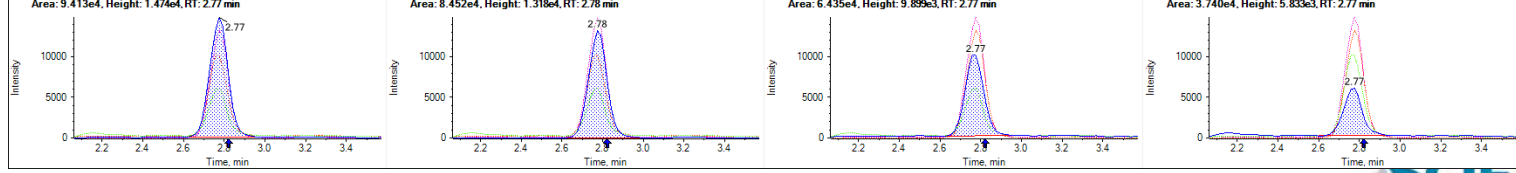
with DMS



Soy
no DMS



with DMS



Application Data

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



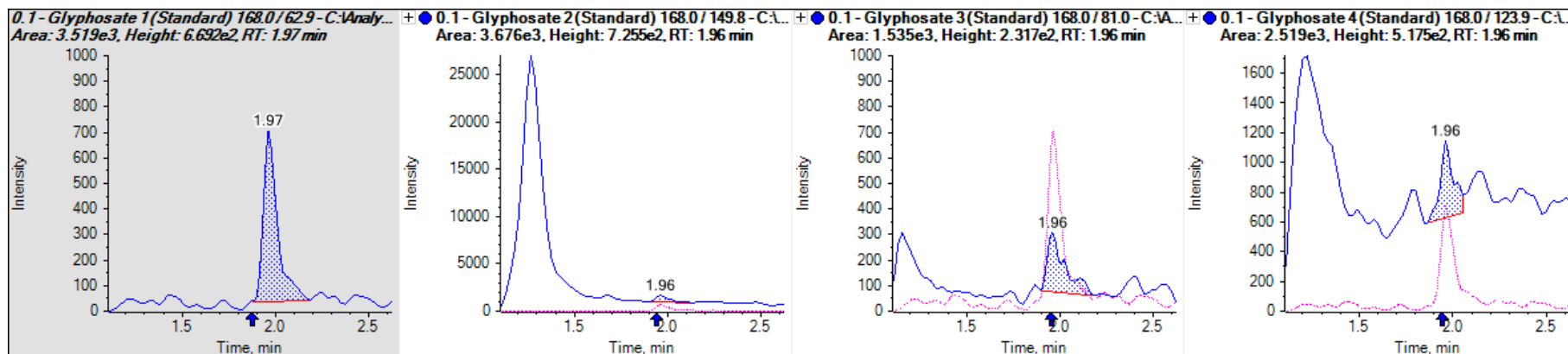
SCIEX ExionLC™ AC system



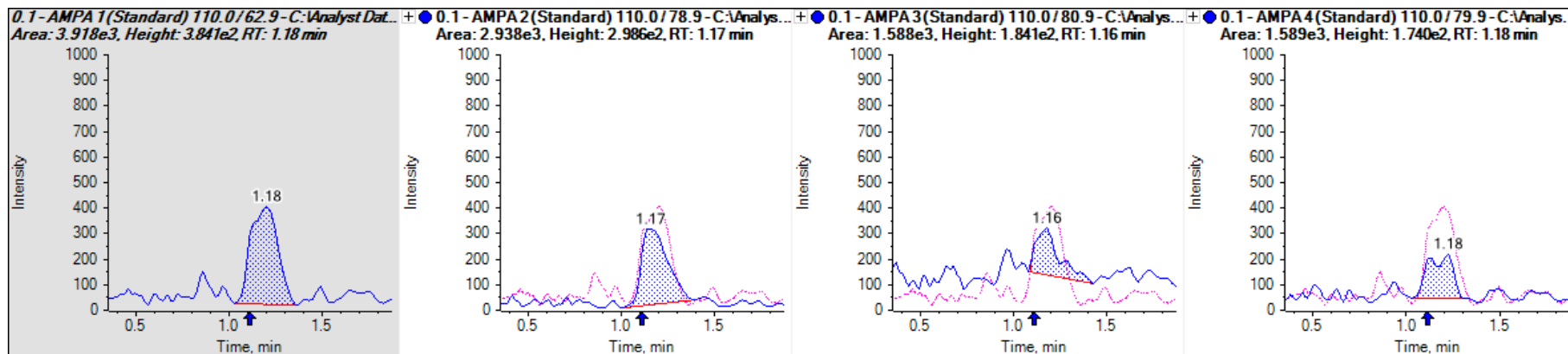
QTRAP® 6500+ system

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

Glyphosate %CV = 3.32% (n = 5)



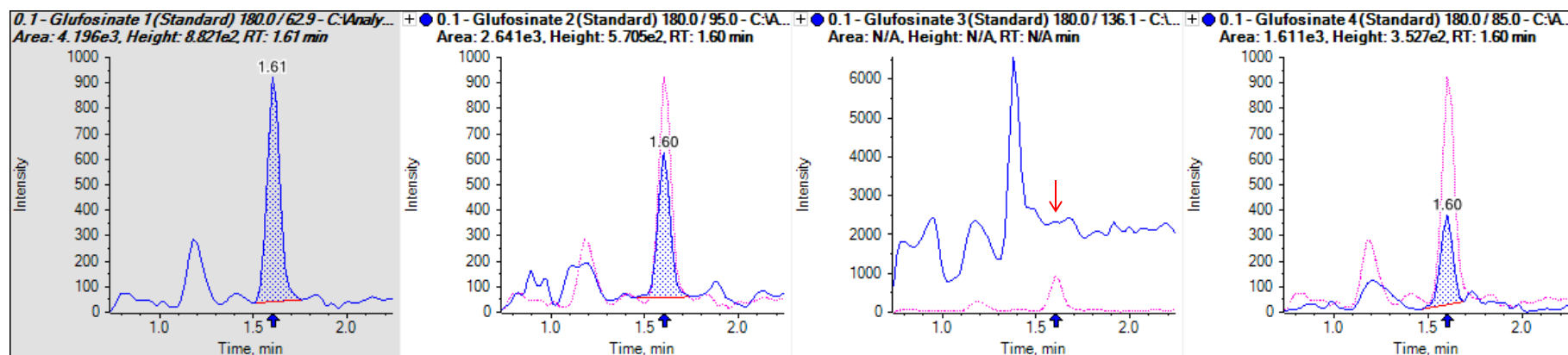
AMPA %CV = 11.4% (n = 5)



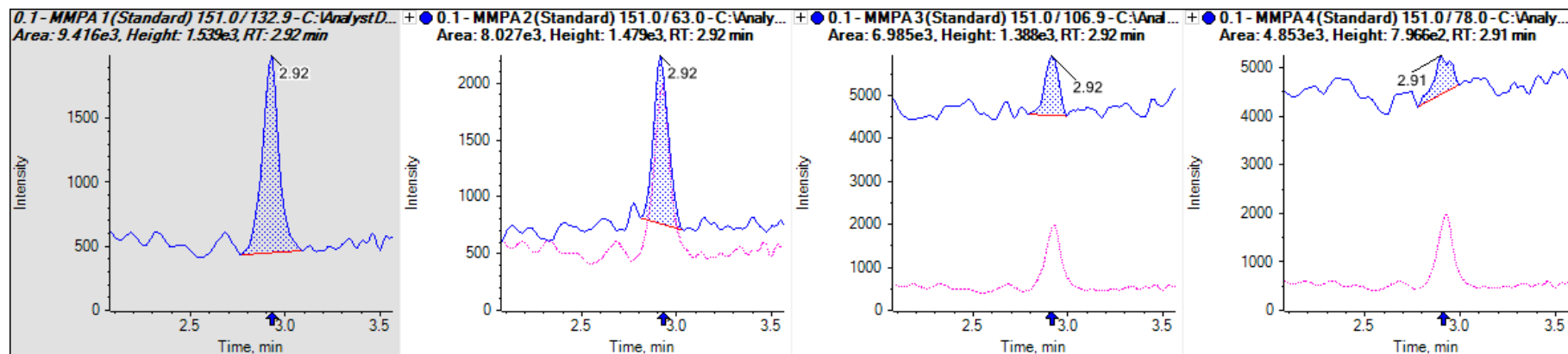
(Slight peak broadening observed for AMPA)

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

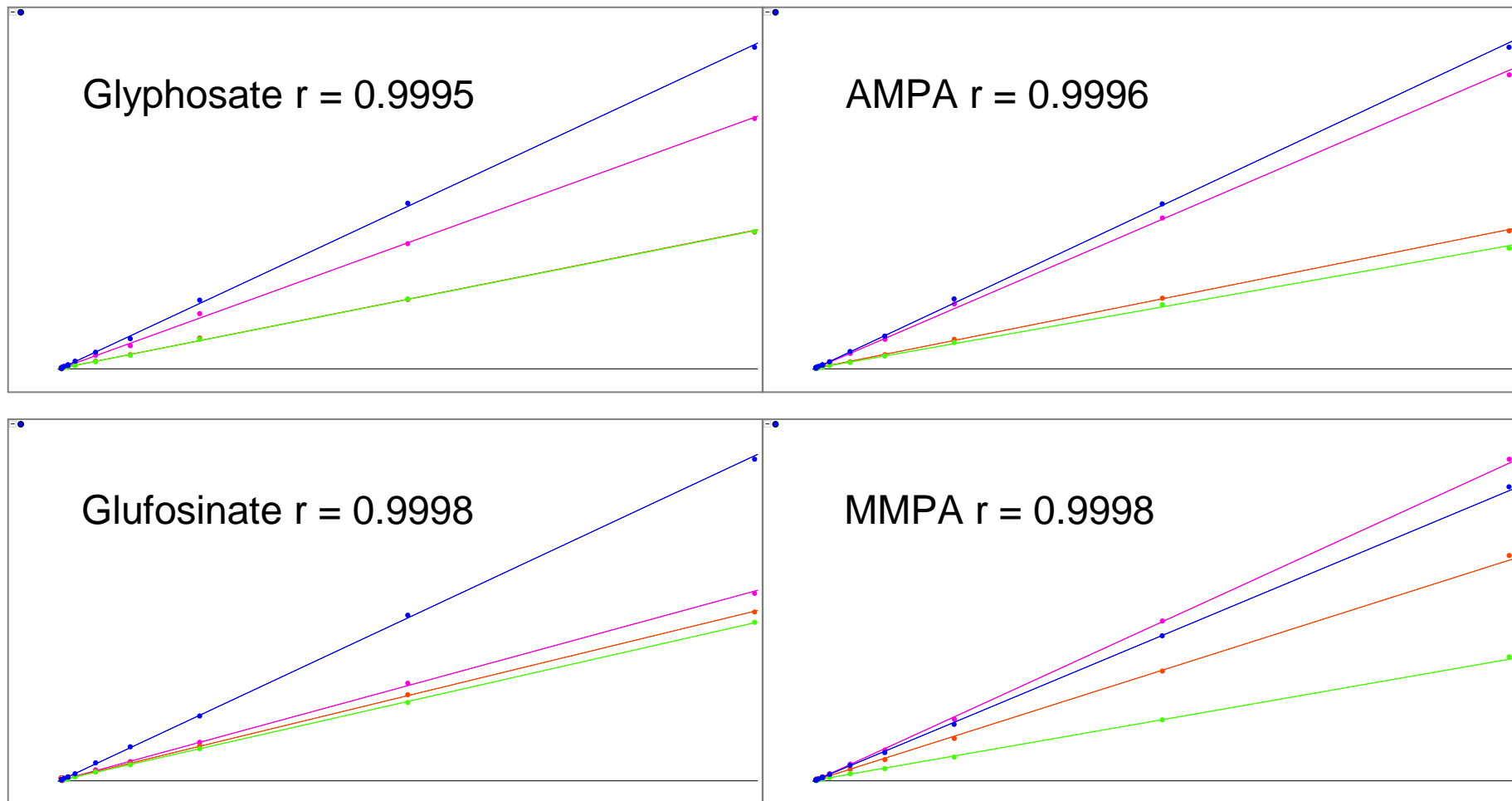
Glufosinate %CV = 8.70% (n = 5)



MMPA %CV = 8.35% (n = 5)



Linear Dynamic Range 0.1 to 100 ng/mL



Accuracies between 80 and 120%

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

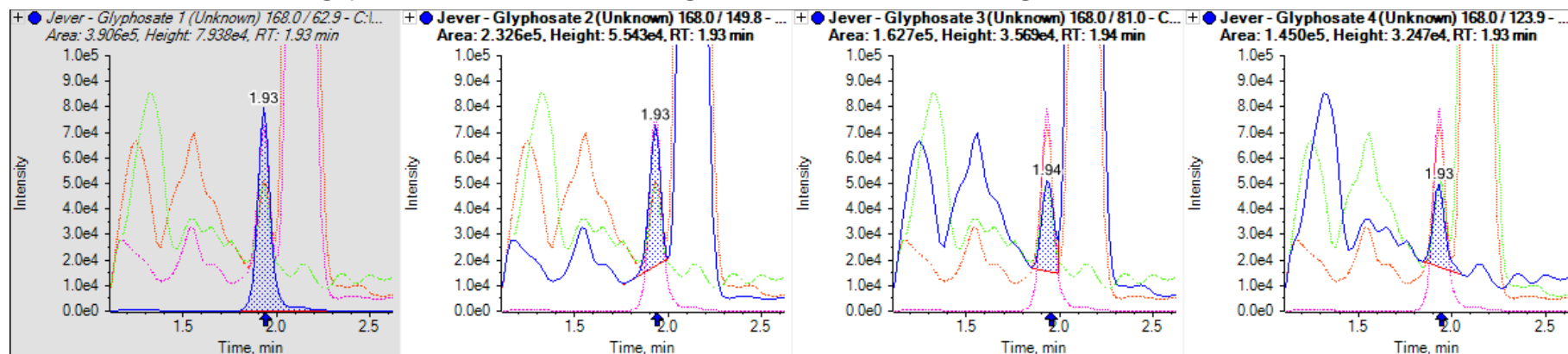
Experimental

- 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 700°C
 - *Scheduled* MRM[™] algorithm (Q3 low resolution)

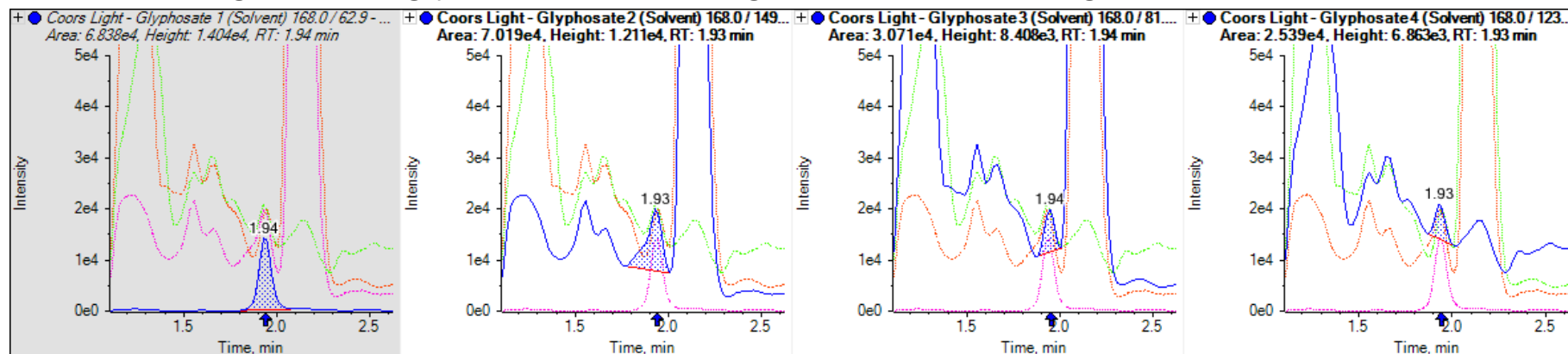
Compound	Q1 (amu)	Q3 (amu)	DP (V)	CE (V)
Glyphosate	168	63	-30	-26
	168	150	-30	-14
	168	124	-30	-16
	168	81	-30	-20

Examples of Beer Tested for Glyphosate (1)

German pilsner: glyphosate = 21.6 $\mu\text{g/L}$ (identified using 4 MRM transitions $\pm 30\%$)

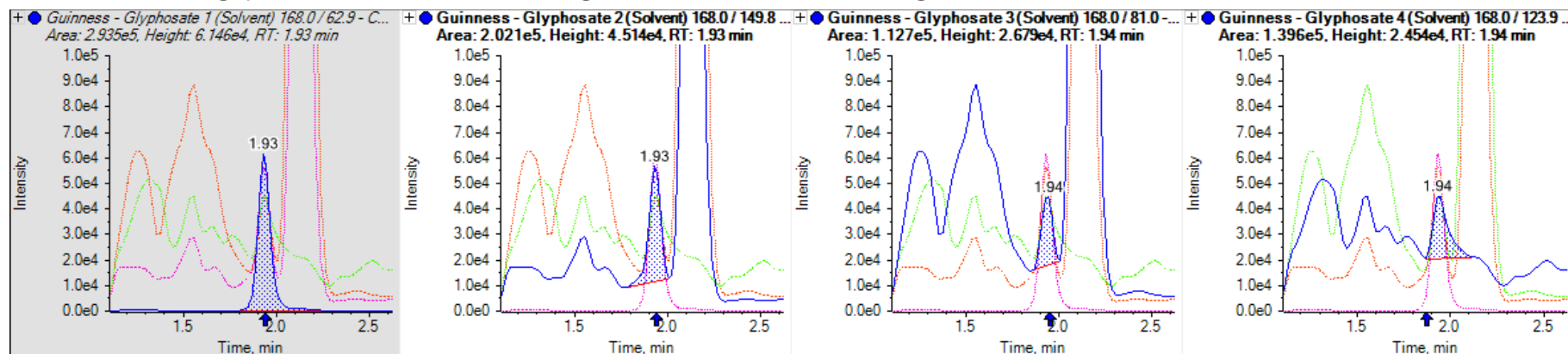


American Light beer: glyphosate = 3.8 $\mu\text{g/L}$ (identified using 3 MRM transitions $\pm 30\%$)

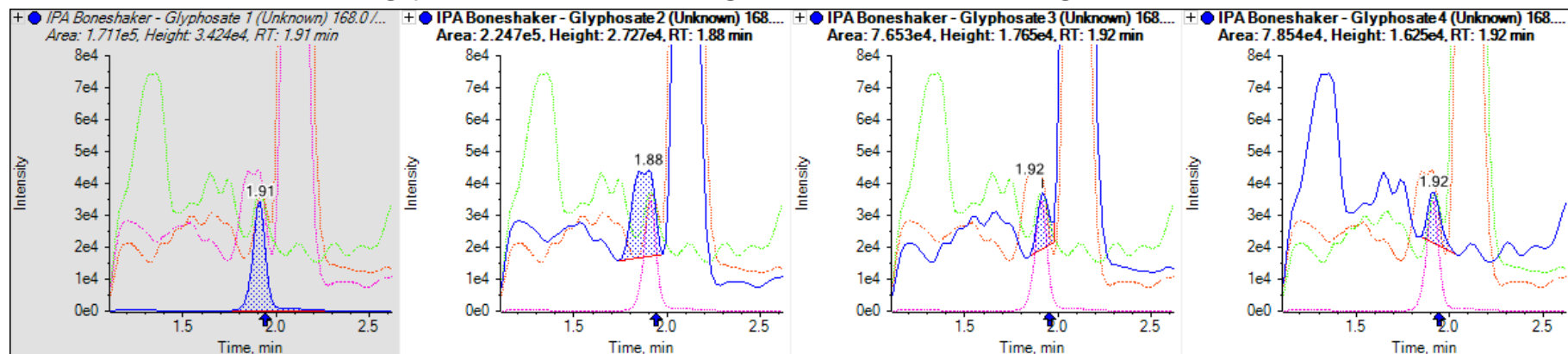


Examples of Beer Tested for Glyphosate (2)

Irish stout: glyphosate = 16.2 $\mu\text{g/L}$ (identified using 4 MRM transitions $\pm 30\%$)

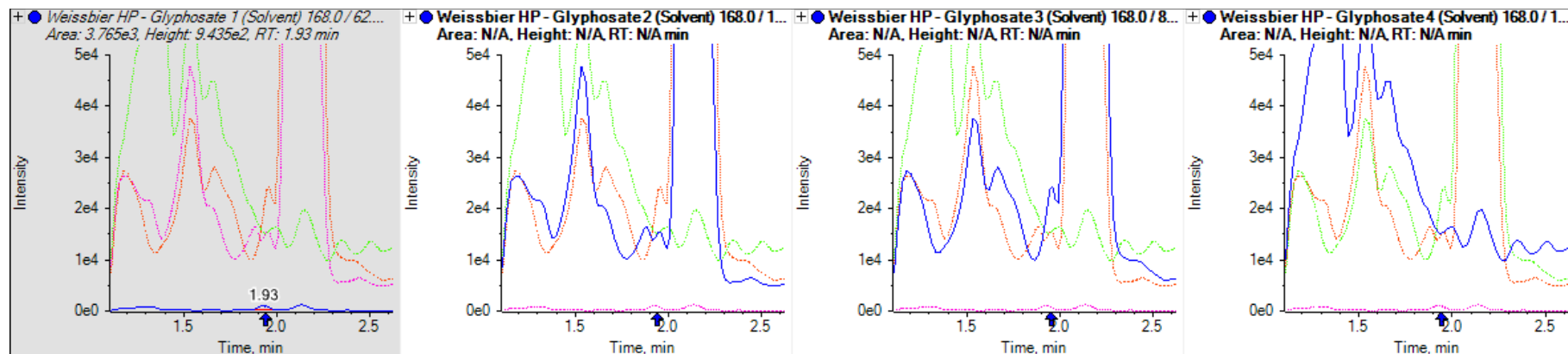


Canadian craft IPA: glyphosate = 9.5 $\mu\text{g/L}$ (identified using 3 MRM transitions $\pm 30\%$)

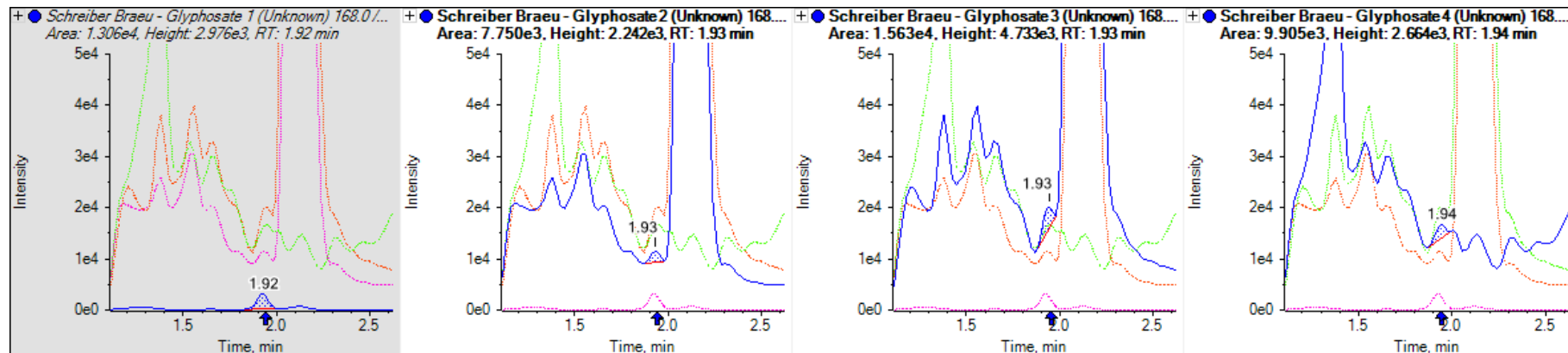


Examples of Beer Tested for Glyphosate (3)

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

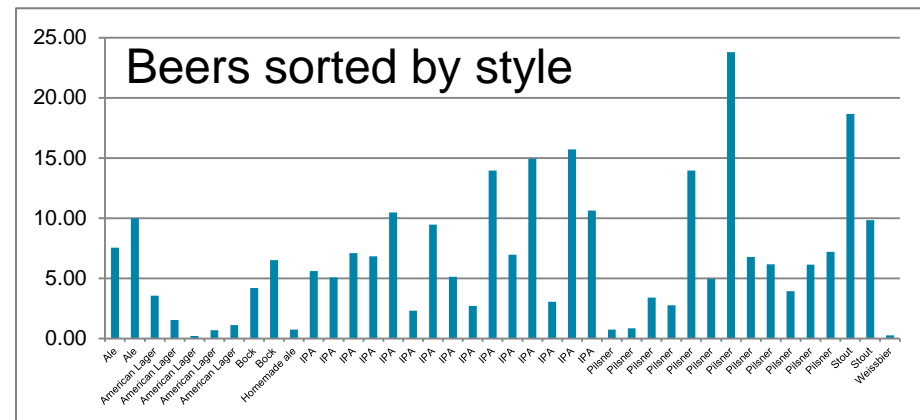
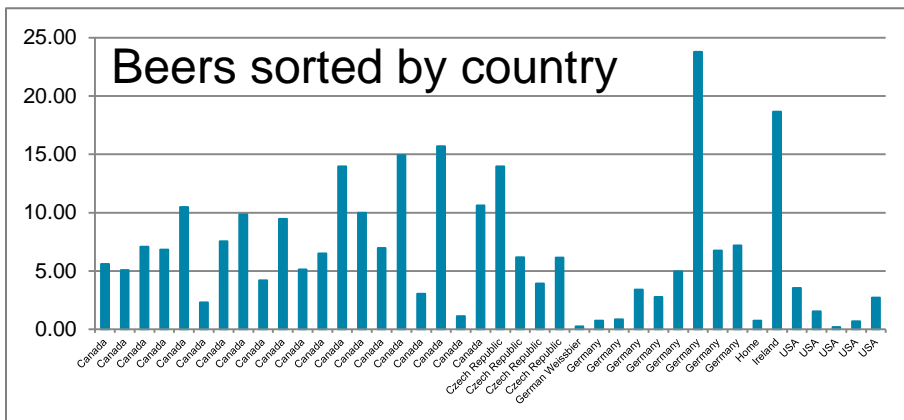
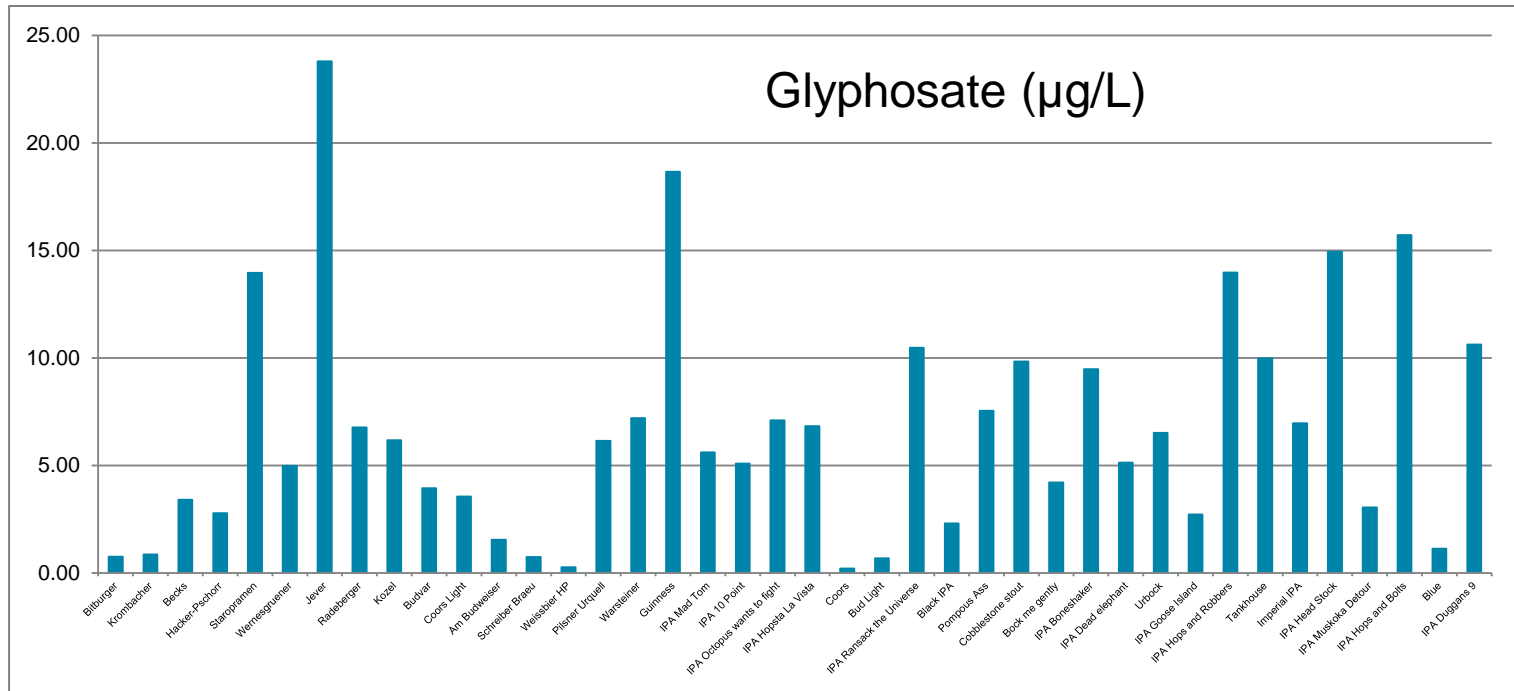


Homemade ale: glyphosate = 0.7 µg/L (identified using 2 MRM transitions ± 30%)



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

Glyphosate in Beers... no Correlation to Origin or Style



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 acetonitrile (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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