From:	Sack, Chris A
To:	Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Thompson, Richard L.; Vonderbrink, John
Cc:	Noonan, Gregory; Viner, Marianna
Subject:	Minutes for PMC meeting Jan 13, 2017
Date:	Monday, January 23, 2017 10:30:00 AM
Attachments:	Glyphosate method 1-23-17.docx

PesTAG PMC Meeting Minutes

Date: January 13, 2017

Attendance: Greg Mercer (PNW), Eugene Chang, (PSW), Richard Thompson(ARL), Marianna Viner (NRL), Chris Sack, (CFSAN) Moh Islam, Connie Drake(ORA-ORS)

Agenda: Glyphosate method progress

- KAN (via email) has the reagents and supplies and plan to start next week
- PNW does not have LC supplies yet
- NRL is injecting standards using 2 mm column; they have ordered the 4.6 mm column
- SRL (via email) is waiting for LC reagents
- CFSAN (via email) has ordered supplies
- ARL is analyzing samples using 2 mm column, method working great
- LA is analyzing samples, conducting validation, and investigating N-acetylglyphosate

ARL adjusted the organic mobile phase from (b) (4) . Eugene agreed the (b) (4) works better. Richard will provide instructions for preparing the (b) (4) . Eugene will provide instructions to adjust the alternate preparation of the (b) (4) . Richard also adjusted the ionization temperature from (b) (4) °C to (b) (4) °C. Eugene mentioned the ionization temperature could be adjusted as low as (b) (4) °C depending upon the individual instrument.

Richard mentioned he is having difficulty with reproducibility of the standards on the LCMS system. Eugene said the N-acetyl-glyphosate standard he received from Toronto Research Chemicals was not pure. However, he collected the compound eluted from HPLC, then (b) (4) and found the compound itself was stable. Moh mentioned that Claude also complained about problems with the N-acetyl-glyphosate. Sack will check with Monsanto about availability of good N-acetyl-glyphosate reference material.

Richard will mail avocado next week. KAN will ship carrots next week also.

Everyone encouraged to keep us all in the loop re their progress/problems with implementing the method.

Chris is starting a method SOP (attached). Please review and return with comments and corrections. NOTE: Method is current as of 1/23/17. Standard preparation from Narong's LIB.

Happy New Year,

Chris

From:	Sack, Chris A
To:	Chamkasem, Narong; Chang, Eugene; Cooke, William; Islam, Mohammed R; Masse, Claude; Mercer, Gregory E; Noonan, Gregory; Thompson, Richard L.; Vonderbrink, John; Wong, Jon
Cc:	Drake, Connie P.
Subject:	Minutes for PesTAG PMC meeting January 26, 2017
Date:	Wednesday, February 08, 2017 8:16:00 AM
Attachments:	avocado interference and solution.pdf pH effect on chromatography.pdf Glyphosate method 1-26-17.docx

PesTAG PMC Meeting Minutes

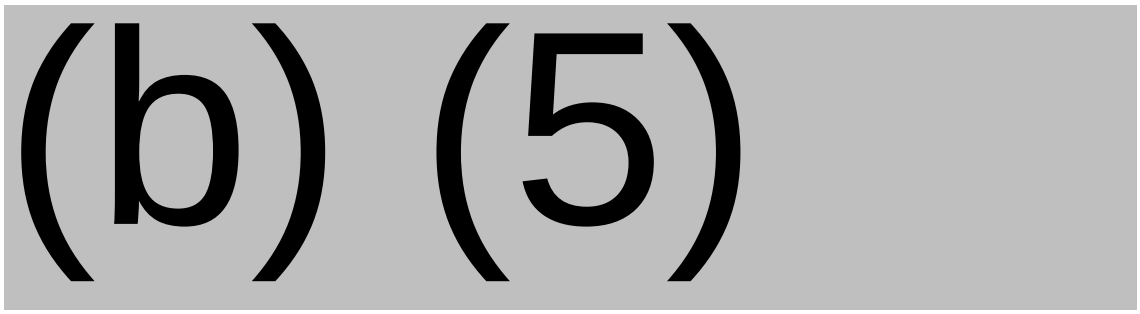
Date: January 26, 2017

Attendance: Greg Mercer and Bill Cooke (PNW), Eugene Chang, (PSW), Richard Thompson (ARL), Claude Masse (NRL), Narong Chamkasem (SRL), John Vonderbrink (KAN), Chris Sack, (CFSAN) Moh Islam (ORA-ORS)

Agenda: Glyphosate method progress

We need to begin the collaboration as soon as possible. (b) (5) (b) (5) We need minimum of 3 labs to begin. The collaboration matrices have all been shipped. Richard mentioned that the avocado might have a trace of glyphosate.

Eugene reported LA is almost finished with the validation. (b) (4) (b) (4) In the table below MDLs and LOQs were calculated (b) (4) Recovery, RSD and Linearity were calculated (b) (4) Note AMPA stats (b) (4) all others (b) (4) Eugene mentioned that some of the carrot data and all of the avocado data is missing because he had instrument problems; he will provide rest of data later. Excellent job Eugene!



What about N-acetylglyphosate? Sack contacted Monsanto and they said they get their N-acetylglyphosate standard from Toronto Research Company (TRC). They were willing to provide FDA with about 200 mg if we needed it. Bill Cooke procured an EPA standard and compared to the TRC. After correcting for declared purities the two standards exhibited the same response. EPA gets the standard from DuPont chemical. Has anyone conducted recovery of N-acetylglyphosate? Eugene did one recovery and got good results when correcting for the impurity of the TRC standard he used.

Richard had been working with the 2 mm column, however he just received the 4.6 mm column and

is trying it out. NOTE the flow rate for the 2 mm column is 0.3 ml/min vs the flow rate of 0.6 for the 4.6 mm column.

Eugene commented he encountered some matrix interferences for glufosinate in avocado. Specifically the (b) (4) was compromised – see attached chromatogram. He needed to use the (b) (4) for glufosinate in avocado.

Moh asked about the spiking protocol? Narong conducted stability studies and found that (b) (4) analytes were stable in various matrices. Eugene found the same.

Mercer recommended using (b) (5)

Each lab reported on their status:

(b) (5)

Prior to participating the collaboration each lab is requested to demonstrate both instrument and method proficiencies. Instrument proficiency consists of

(b) (4)

Method proficiency includes: (b) (4)

The latest version of the method is attached. Please use it when conducting analyses. (b) (5)

(b) (5) Send Sack the results of your instrument and method proficiencies. Simple data dumps in a spreadsheet is OK.

Everyone encouraged to inform the group about their progress/problems with implementing the method. We will meet again at the same time next week,

Chris

From:	Sack, Chris A
To:	Cassias, Irene; Chamkasem, Narong; Eide, David J; Islam, Mohammed R; Katsoudas, Eugenia; MacMahon, Shaun; Mercer, Gregory E; Noonan, Gregory; Sack, Chris A; Thompson, Richard L.; Wong, Jon; Chang, Eugene; Cooke, William; Masse, Claude; Parker, Christine; Vonderbrink, John; Wong, Jon
Subject:	Minutes for PesTAG PMC meeting Feb 28, 2017
Date:	Wednesday, March 15, 2017 6:15:27 AM
Attachments:	Collab-Glyphosate Final.xlsx Glyphosate method Collab Final.docx SEA Layout 2-17-17.PNG

PesTAG PMC Meeting Minutes

Date: February 28, 2017

Attendance: Greg Mercer and Bill Cooke (PNW), Eugene Chang, Shannon Lane (PSW), Richard Thompson (ARL), Claude Masse (NRL), Narong Chamkasem (SRL), John Vonderbrink (KAN), Chris Sack, Greg Noonan, Jon Wong (CFSAN), Moh Islam (ORA-ORS)

Agenda: Glyphosate method progress

All ORA labs have demonstrated proficiency with the LC-MS/MS determination. Only KAN and SRL have not reported on N-acetylglyphosate.

The LC-MS/MS MS/MS parameters changed slightly when Bill found a couple alternative transitions. For the method we agreed to use (b) (4)
(b) (4)
(b) (4)
(b) (4)
(b) (4)
(b) (4)

Eugene pointed out that (b) (4)
(b) (4)
(b) (4)
Sack updated (b) (4)

Four ORA pesticide labs have completed method proficiency. PSW had previously reported excellent recoveries for all three matrices during their single laboratory validation. NRL reported they are still getting low recoveries for the analysis of avocado.

ORA

* IS used for AMPA and N-acetyl

ORA

When integrating the chromatographic smoothing as needed to ensure proper integration. Richard mentioned (b) (5)

Each lab reported on their status:

(b) (5)

(b) (5)

I attached data layout that PNW provided earlier that meet my specifications in the protocol. I don’t need the chromatograms, just the data fields in Excel.

Good luck with the collaboration,

Chris

From:	Sack, Chris A
To:	Cassias, Irene; Chamkasem, Narong; Eide, David J; Islam, Mohammed R; Katsoudas, Eugenia; Liang, Charlotte; MacMahon, Shaun; Mercer, Gregory E; Noonan, Gregory; Sack, Chris A; Thompson, Richard L.; Wong, Jon
Cc:	Parker, Christine
Subject:	Minutes from PesTAG call March 15, 2017
Date:	Monday, March 27, 2017 11:31:00 AM
Attachments:	ORA-LAB_5_4_5 Section 6 Method Verification.docx

PesTAG Meeting Minutes

Date: March 15, 2017

Attendance: Greg Mercer (PNW), Irene Cassias (PSW), Richard Thompson (ARL), Jenny Katsoudas and Mike Iorsh (NRL), Narong Chamkasem (SRL), David Eide (KAN), Chris Sack, Greg Noonan, Jon Wong, Christine Parker (CFSAN), Moh Islam (ORA-ORS)

1. Glyphosate collaboration progress

The collaboration is progressing as planned. Sack submitted MLV forms with collaboration protocol to CMVS last week. The CRCG met yesterday to review. PSW, PNW and ARL have submitted collaboration data.

(b) (5)

(b) (5)

Greg noted that it takes a while to rinse the TBA from the LCMS system.

(b) (5)

Thanks Richard!

(b) (5)

2. Calculation of residue levels in processed products

(b) (5)

(b) (5)

The regulations about the calculation of residues in processed products, yielding those listed below.

1. PAM Vol. 1, Section 102: Preparation of Analytical Samples

ORA

residues

3. 21 CFR 170.19 - Pesticide chemicals in processed foods
4. 40 CFR 180.1(e) – Definitions and interpretations
5. 40 CFR 180.1(h)(10) – Definitions and interpretations

(b) (5)

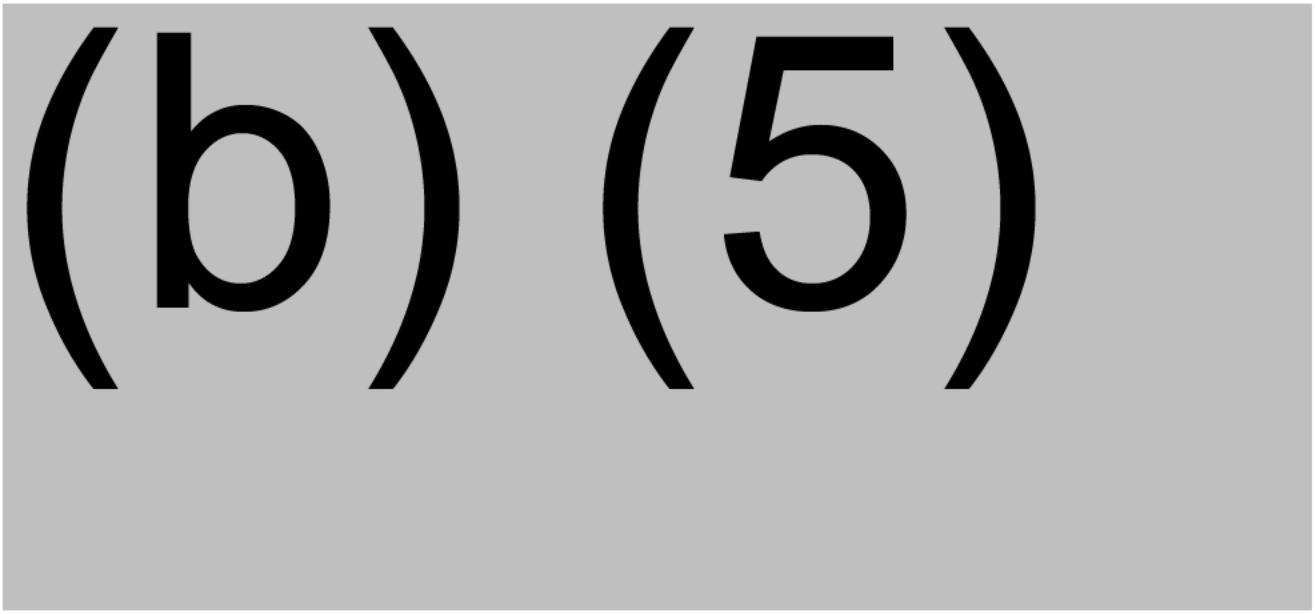
CFSAN consulted with EPA and agreed that the protocol in the PP Memo stands so **there is no change in the way we handle processed products.** However, in the event that a problem arises with a specific processed product found to contain a pesticide that exceeds the tolerance established for the corresponding raw agricultural product, the FDA would consider taking action.

3. **ORA-LAB.10**

Jon suggested (b) (5)

Thanks a lot Jon!

4. **Method verification and validation protocol for the pesticide program**



All agreed to look at the ORA-LAB.5.4.5 and provide suggestions for (b) (5)
(b) (5)

Really enjoy working with all of you,

Chris

From:	Sack, Chris A
To:	Cassias, Irene; Chamkasem, Narong; Eide, David J; Islam, Mohammed R; Katsoudas, Eugenia; Liang, Charlotte; MacMahon, Shaun; Mercer, Gregory E; Noonan, Gregory; Sack, Chris A; Thompson, Richard L.; Wong, Jon; Chang, Eugene; Cooke, William; Masse, Claude; Parker, Christine; Vonderbrink, John
Cc:	Humphries, Susan; Kontas, Cassandra; Kwan, Thao T.; Knox, Valerie; Noe, Danny A
Subject:	Minutes for PesTAG call April 19, 2017
Date:	Wednesday, April 26, 2017 12:16:39 PM
Attachments:	CMVS Review Glyphosate MLV Proposal - PesTAG Reply.docx Glyphosate method postCollab.docx MS Identification Criteria 4-19-17.xlsx Glyphosate MLV Rpt 4-24-17.docx

---

PesTAG Meeting Minutes

Date: April 19, 2017

Attendance: Greg Mercer, Bill Cooke (PNW), Irene Cassias, Eugene Chang (PSW), Richard Thompson, Steve Gonzalez, Russell Fairchild (ARL), Jenny Katsoudas, Claude Masse, Marianna Viner (NRL), David Eide, John Vonderbrink (KAN), Chris Sack, Greg Noonan, Jon Wong, Christine Parker (CFSAN), Moh Islam (ORA-ORS); and special guests from QMS: Susan Humphries, Cassandra Kontas (PNW), Thao Kwan, Brian Agan (PSW), Valerie Knox (ARL)

Agenda,

1. **Glyphosate collaboration and assignment implementation**
2. **ORA-LAB.10**
3. **Method verification of pesticide procedures**

1. Glyphosate collaboration progress

The glyphosate assignment implemented in April, 2016 was (b) (4) completed when it was temporarily suspended because the glyphosate laboratory (SRL) was reassigned to other programs. Attempts to unify and implement the SRL glyphosate methods in other ORA pesticide laboratories were unsuccessful. In the fall and winter of 2016 PSW developed an alternative LC-MS/MS determination that proved to be rugged and sensitive. Collaboration of the modified PSW glyphosate method was begun in March and continues. The original assignment for glyphosate analysis of milk, eggs, corn, and soy was modified and is awaiting the implementation of the glyphosate method at the three ORA labs assigned to analyze glyphosate for the assignment: PSW, PNW, and ARL.

The collaboration is progressing as planned. The CRCG and CMVS reviewed the MLV forms, including the proposed procedure and collaboration protocol, and submitted comments and questions to the PesTAG the first week of April. The PesTAG revised the method SOP (attached) (b) (5) and provided a response to the CMVS questions and comments (attached) on April 11, and is awaiting further correspondence.

Along with responses to the CRCG and CMVS comments and questions about the collaboration protocol, the PesTAG submitted a preliminary multi-laboratory validation (MLV) report (attached) based upon data from the single laboratory validation (SLV) submitted by PNW, and the collaboration data submitted by PSW, PNW, and ARL. The MLV report includes the method, protocols, and specifications for the successful completion of the collaboration. The data is summarized for all three participating laboratories in the main body of the report, and results from each laboratory are provided as attachments. Additionally, the results of the SLV conducted by PSW are provided as an attachment. The report clearly demonstrates that the method is fit for purpose for quantitative determination of glyphosate, glufosinate and N-acetylglyphosate residues for

the pesticide program.

Special Note re the MLV report. The visiting QSMs and QSSs reviewed the report attached with the draft minutes of this meeting and found a few errors that have been corrected in the attached version of the report. All corrections to the MLV are highlighted in the attached report. None of the corrections affects the validity of the report. The CMVS will be alerted to the changes in the MLV report.

The CRCG and CMVS declined to conduct a final review of the preliminary MLV report because the protocol indicates that 7-8 laboratories are participating. However, given the urgency of the need to begin the glyphosate assignment, they agreed to conduct a preliminary review to expedite the implementation of the method in the three laboratories that have completed the collaboration. Preliminary review and approval of the MLV protocol and report is sufficient for the labs that have completed the collaboration to implement the method. Upon completion of the preliminary review the CRCG and CMVS agreed to provide documentation that review of the preliminary MLV report indicates the method meets the requirements of a Level III multiple laboratory validation. Hope to get that by next week. Furthermore, the CMVS asserted their opinion that participation in the collaboration is a demonstration of method verification.

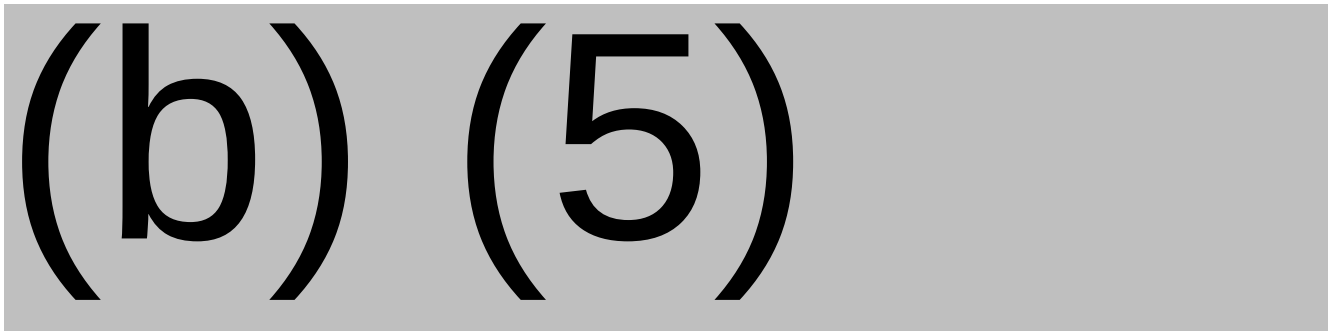
Richard prepared an SOP for the final glyphosate method that includes modifications (b) (5) to clarify some of the instructions. Can all the labs use Richard's SOP? Or is each laboratory required to rewrite the SOP to match their style and local protocols. All agreed the common SOP for all labs would be best and ORA-ORS supported this position.

What about using surrogates to monitor method recovery? Eugene suggested (b) (5) (b) (5) Most everyone agreed this was unnecessary and could create new issues if the surrogate doesn't mimic the analytes. Someone mentioned using another isotope for a surrogate, but the general consensus was not to pursue the use of surrogates.

What about standing method up, what does CFSAN require? At this point, assuming the CRCG and CMVS agree that the MLV report demonstrates that the method is fit for purpose, CFSAN has no additional requirements to implement the method for the glyphosate assignment. CFSAN does not want to analyze any other commodities until the assignment is completed.

Status for implementation of the method in the labs assigned to conduct glyphosate analysis:

(b) (5)



What about including FCC, ADRC?

2. **ORA-LAB.10**

All agreed to look at the ORA-LAB.5.4.5 and provide suggestions for (b) (5)
(b) (5)

Valerie Knox is on the committee to revise 5.4.5 and she would be glad to hear suggestion – Thanks Valerie!

Special thanks to QSMs and QSSs that joined us today. We are grateful for the opportunity of working with you to ensure the quality of the FDA pesticide program.

Really enjoy working with all of you,

Chris

From:	Sack, Chris A
To:	Cassias, Irene; Eide, David J; Islam, Mohammed R; Katsoudas, Eugenia; Liang, Charlotte; MacMahon, Shaun; Mercer, Gregory E; Noonan, Gregory; Sack, Chris A; Thompson, Richard L.; Wong, Jon
Subject:	Minutes for PesTAG meeting June 28, 2017
Date:	Wednesday, July 12, 2017 1:40:16 PM

Date: June 28, 2017

Attendance: Greg Mercer (PNL), Richard Thompson, Steve Gonzalez, (ARKL), Jenny Katsoudas, Angelo Damanti (NFFL), David Eide (KCL), Chris Sack, Charlotte Liang, Shaun MacMahon, Jon Wong (CFSAN), Moh Islam (ORA-ORS); and new QMS members: Cassandra Kontas (PNL), Thao Kwan, Brian Agan (PSFFL), Valerie Knox (ARKL), Gary Hinshaw (KCL), Sharna Pratt, and Ebony Laster (SFFL)

Agenda

1. PesTAG business (Sack)
    a. New co-chairs
    b. New members
2. ORA update (Moh)
3. Glyphosate update (Sack)
4. NACRW meeting (Sack)
5. Pesticide standard classification in LIMS (Eide)
6. Unfinished business (Sack)
    a. ORA-LAB.10
    b. ORA-LAB.5.4.5

1. PesTAG business

- The new co-chairs for the PesTAG are Greg Mercer and Moh Islam. Sack is grateful for the opportunity to serve as chair and looks forward to working with the new co-chairs. Congratulations and condolences to Greg and Moh and thanks to everyone for participation in the process.
- Three members from FDA's quality system: Cassandra Kontas (QSS at PNL), Thao Kwan (QSM at PSFFL), and Valerie Knox (QSM at ARKL) have volunteered to join the PesTAG to provide guidance re the current FDA quality system requirements and become familiar with the issues and challenges of the FDA pesticide program. Mercer, Moh, and Sack agree that the addition of more than one QSM to the PesTAG is unnecessary, and requested the QSMs to select a representative for the PesTAG, preferably someone familiar with pesticides or residue analysis.
- The original draft of the PSC (PesTAG) charter will need to be updated to add a (b) (5) to the composition of the PesTAG. The charter is located on the FDA pesticide SharePoint site http://sharepoint.fda.gov/orgs/ORA-RegulatoryOperations-ORS/FoodFeedSS/ORAPesticide/default.aspx. The charter is in the PSC Library). Sack will work with Moh to update the charter.

2. ORA update (Moh)

ORA

3. Glyphosate update (Sack)

(b) (5)

(b) (5)

4. **NACRW meeting (Sack)**
    - Because of limited attendance at the NACRW, the annual PesTAG meeting at the NACRW has been cancelled.

5. **Pesticide standard classification in LIMS (Eide)**
    - KCL submission of a Project Change Request for a new pesticide material Flurazole Technical was challenged because "Technical" is not in the naming convention for the pesticide standards. The Material Change Management Process experts requested that the PesTAG provide guidance.
    - Technical grade indicates the material is a mixture of components. PesTAG agrees we need the "technical" designation.

6. **Unfinished business (Sack)**

ORA

Closing

Jenny mentioned (b) (5)

Really enjoy working with all of you,

Chris

PesTAG Meeting Minutes

Date: July 23, 2017 at NACRW

Attendees: Angelo Damanti (NY), Eugene Chang (LA), Greg Mercer (Seattle), Jon Wong (CFSAN), Alex Krynitsky and Mike Farrow (ORS)

Attendees by Phone/WebEx: Chris Sack (CFSAN) and briefly by Mohammed Islam (ORS)

Original Agenda:

1. **Around the horn**
2. **Glyphosate update**
3. **Std Mixes**
4. **Charter Revision**
5. **Multi-Lab Collaboration of GC Procedures**
6. **ORA-LAB.10**
7. **Future Projects/Instrumentation**
8. **HRMS Update**
9. **Open discussion/Close out**

This PesTAG meeting was hampered by some technical difficulties in getting a good internet signal for the WebEx. This was overcome by using Mike Farrow's cell phone as a hotspot. Our time was also limited due to a schedule change to the FDA-State forum (it was moved up to 4 pm instead of 5 pm). Attendance was lower than expected too. Richard Thompson, Narong Chamkasem and David Eide had changes to their travel plans so they were not able to attend. Equipment status/needs, standard mixes and a few other agenda items were discussed amongst the five pesticide regulatory lab attendees for about an hour over lunch on Tuesday (7/25). These discussions are summarized below the PesTAG meeting minutes.

1. **Around the horn**

CFSAN: Jon and Jim Wittenberg did the MLV work for glyphosate and produced excellent results. They have not tried eggs or the MWCO filtration step yet.

2. Glyphosate

(b) (5)

3. General discussion of PLRs and analyst hours

(b) (5)

The attending PesTAG members were unanimous in feeling something needs to give and priority established. Most analysts feel the sample work should be the higher priority.

Another thought was to revisit the definition of an FTE and how we record time for samples, maintenance, PLRs, standards preparation, etc. Given the reorganization, maybe a separate group should be formed where all they do are PLRs? A group dedicated to PLRs would also provide more uniformity to FDA's review process. There are several ways this could be accomplished. (b) (5)

(b) (5)

4. **Concerns with QA**

(b) (5)

5. **Standard Mixes**

(b) (5)

(b) (5)

6. **ORA-LAB.010**

ORA

7. **Close out**

Due to time constraints, the remaining agenda items were not discussed. Next up was the FDA-State Forum. There was some minor grumbling from members regarding what FDA has gotten in return for buying MS equipment for the FERN state labs and how it ate into potential funding for the regulatory labs. One of the complaints FDA continues to hear is that it isn't doing enough for polar pesticides. (b) (5)

Summary of Reg Lab Attendee Meeting (Angelo, David, Eugene, Greg, and Richard)

Future equipment needs were discussed. All felt the next technology shift was going to be to HRMS. This opinion was supported by the large number of talks and posters on the topic over the last few years at NACRW. It has also been about five years since a new GCQQQ has been incorporated into the program. A Shimadzu GCQQQ was purchased for ARL a couple years ago but it has yet to be used to report results for a regulatory sample. This is a perfect example of why it is risky to just go with the low bid on this type of equipment. They simply don't get used and become a waste of our tax dollars.

We also discussed the status of equipment at each lab. NY is the only lab with just (b) (4) systems (b) (4) If we are going to expect the NY lab to run the glyphosate and/or AcH methods, they will need a (b) (4) system. Hopefully ORS can help NY see what is available in the field. Angelo mentioned the (b) (4) that was received by all other pesticide labs went to their mycotoxin group and they are no longer running samples.

This seemed like a logical place to start for them to obtain a (b) (4) We settled on the following equipment priorities and hoped ORS could help NY out.

Equipment priorities
1) (b) (4) system with method ready to roll out
2) (b) (4) or GC-HRMS (method ready for GC-HRMS?)
3) (b) (4) – backups will be needed – especially in NY

There were no volunteers to coordinate bulk purchase of the instrumentation as that has become a very tedious process.

Standard Mixes and ORA-LAB.010: Revisited what was discussed during Sunday's meeting but included KC and ARK.

(b) (5)

PesTAG Meeting Minutes

Date: Aug 23, 2017 Conference Call
Time: 11:00 AM EST

Attendance: PNL, PSFFL, KCL, ARKL, NFFL CFSAN, ORA-ORS,

Agenda Items:

1) **ORA-LAB.010**
2) **New Commercial Standard Mixes**
3) **Glyphosate Update and CMVS review of MLV**
4) **Charter – assign roles for revision**

Before the meeting started, Charlotte Liang introduced herself. She is working in Office of Food Safety in CFSAN with Chris Sack.

1) ORA-LAB.10

Additional ORA-LAB.010 discussions are highlighted below:

2) New Commercial Standard Mixes

3) Glyphosate Update and CMVS review of MLV

(b) (5)

4) Charter – assign roles for revision

(b) (5)

Memorandum

TO: Chris Sack, Chair, Pesticides Technical Advisory Group (TAG)

FROM: Shaun MacMahon, Chair, Chemistry Methods Validation Subcommittee (CMVS)

RE: MLV Proposal, “Determination of Glyphosate and Glufosinate Residues in Food”

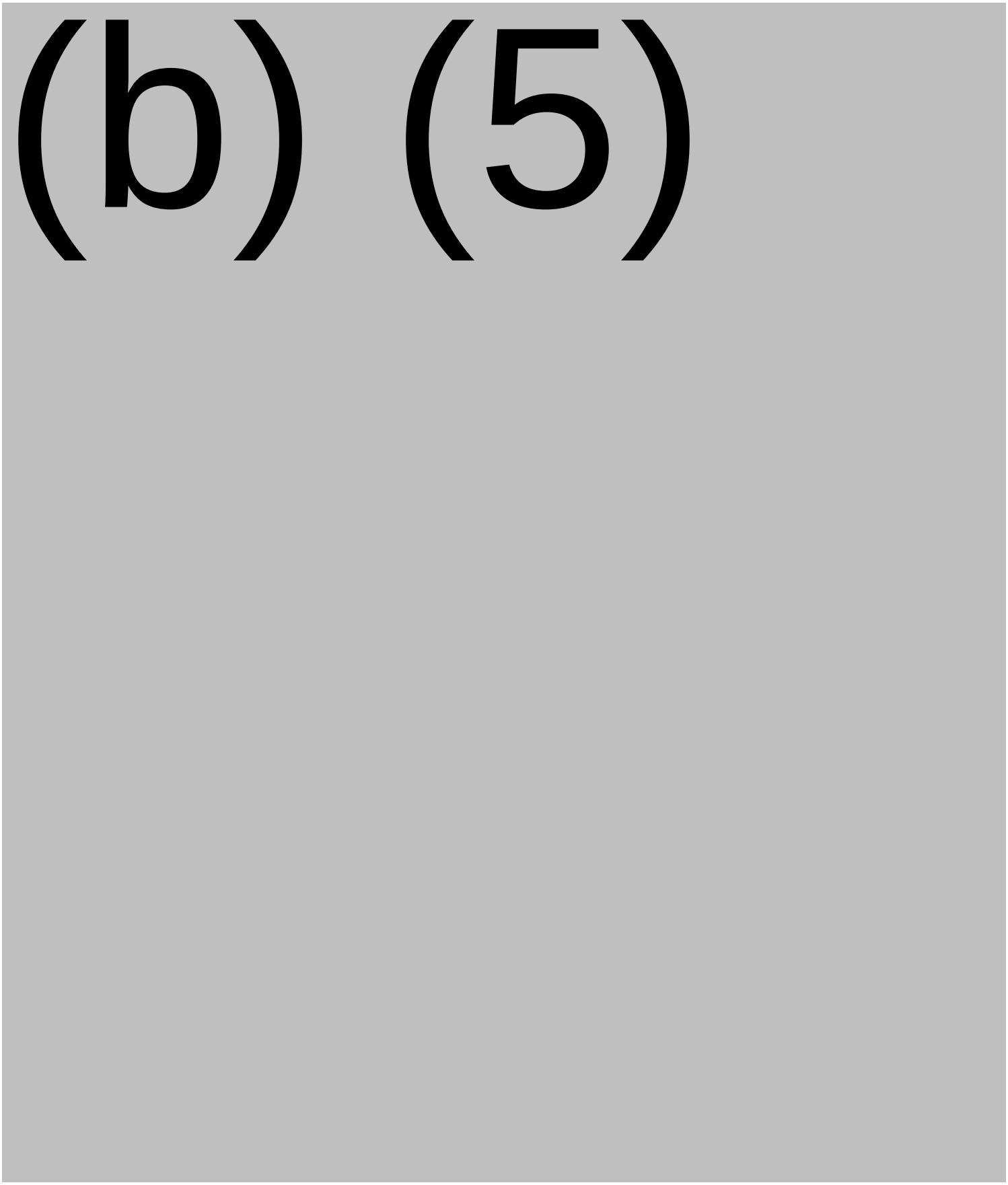
DATE: 3/27/2017

The CMVS has reviewed your submission of a multi-laboratory validation plan for the method, “Determination of Glyphosate and Glufosinate Residues in Food.” The enclosed report summarizes the findings of the subcommittee and includes a number of comments and suggestions which need to be addressed before the MLV proposal can be approved.

CMVS Report on MLV Proposal

Method Title: Determination of Glyphosate and Glufosinate Residues in Food

MLV POC: Chris Sack (CFSAN)



(b) (5)

4) Revisions for the Method SOP

ORA

From: Sack, Chris A
To: Hrdy, David
Subject: RE: Glyphosate
Date: Tuesday, January 03, 2017 4:14:00 PM
Attachments: LIB 4596 Glyphosate in Corn and Soybean Chamkasem 2015.pdf

---

Hi David,

We have heard the same thing and fielded the same request. First of all, we have found no violations for glyphosate in any official sample we have tested including corn, soybean, milk and egg. While the data to support this statement has not been made public, FDA has made this clear through our media outlet. If you like I can refer you to our lead media person.

Re the over the tolerance finding of glyphosate in corn, the author of the glyphosate method (Narong Chamkasem) did publish such a finding in one of his LIBs he wrote (attached). While developing his method he tested grain corn and found one sample to contain 6.5 ppm. The corn he tested was not an official sample, therefor no regulatory status can be assigned. The LIB has been made public through FOIA and it is the source of these requests. I am not sure if Narong published a journal article with this finding. I will ask him.

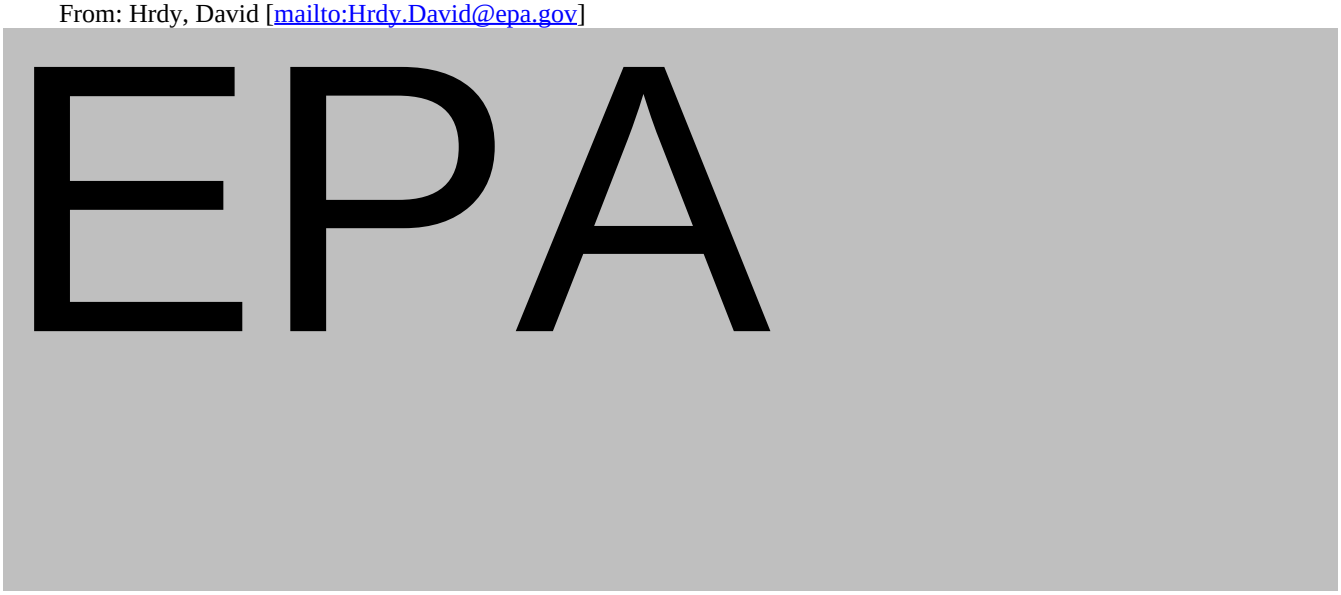
Does this help? Let me know if you would like to correspond with our media person. She is really good – all the info for FDA is funneling through her.

Stay warm,

Chris

Ph: 240-402-2464

From: Hrdy, David [mailto:Hrdy.David@epa.gov]




EPA

From: South, Paul
To: Sack, Chris A
Cc: Robin, Lauren P
Subject: FW: URGENT Media Inquiry - Glyphosate - CBS Evening News- 5:00 pm today deadline
Date: Friday, January 27, 2017 3:11:10 PM

Hi Chris,

Can you take a quick look at the response below.

Thanks, Paul

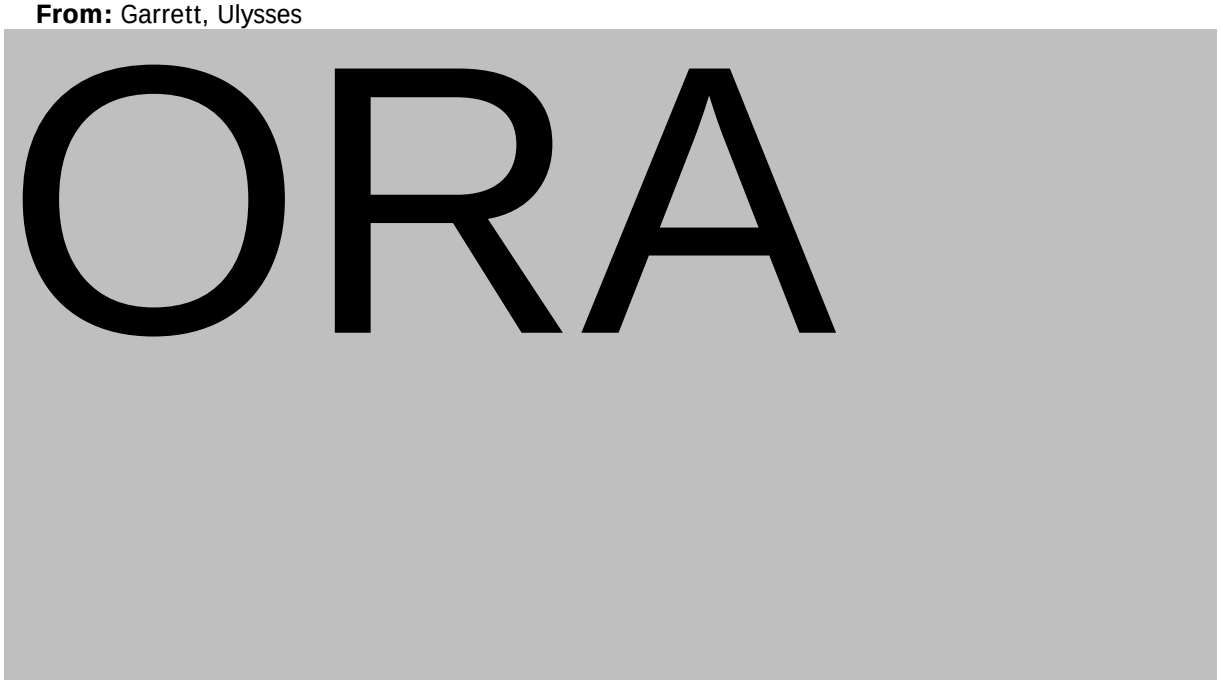
From: McSeveney, Megan

OC

From: Islam, Mohammed R

From: McLaughlin, Michael A

From: Garrett, Ulysses



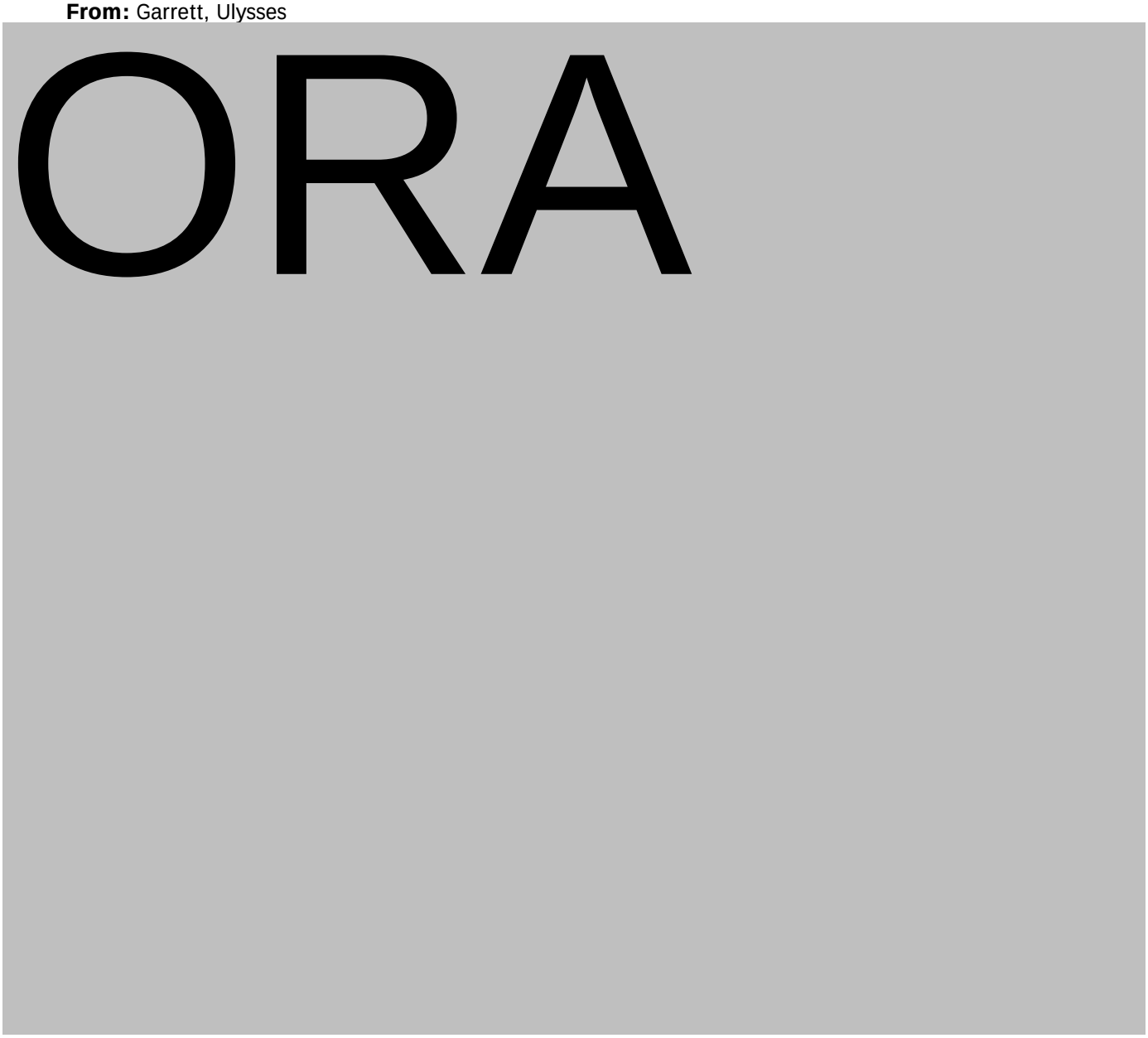
From: McLaughlin, Michael A

ORA

From: Garrett, Ulysses

ORA

From: Garrett, Ulysses




ORA

From:	Sack, Chris A
To:	Thompson, Richard L.; Chang, Eugene
Cc:	Mercer, Gregory E; Islam, Mohammed R; Cooke, William; Vonderbrink, John; Masse, Claude; Chamkasem, Narong
Subject:	RE: Glyphosate Method
Date:	Wednesday, January 04, 2017 2:06:00 PM

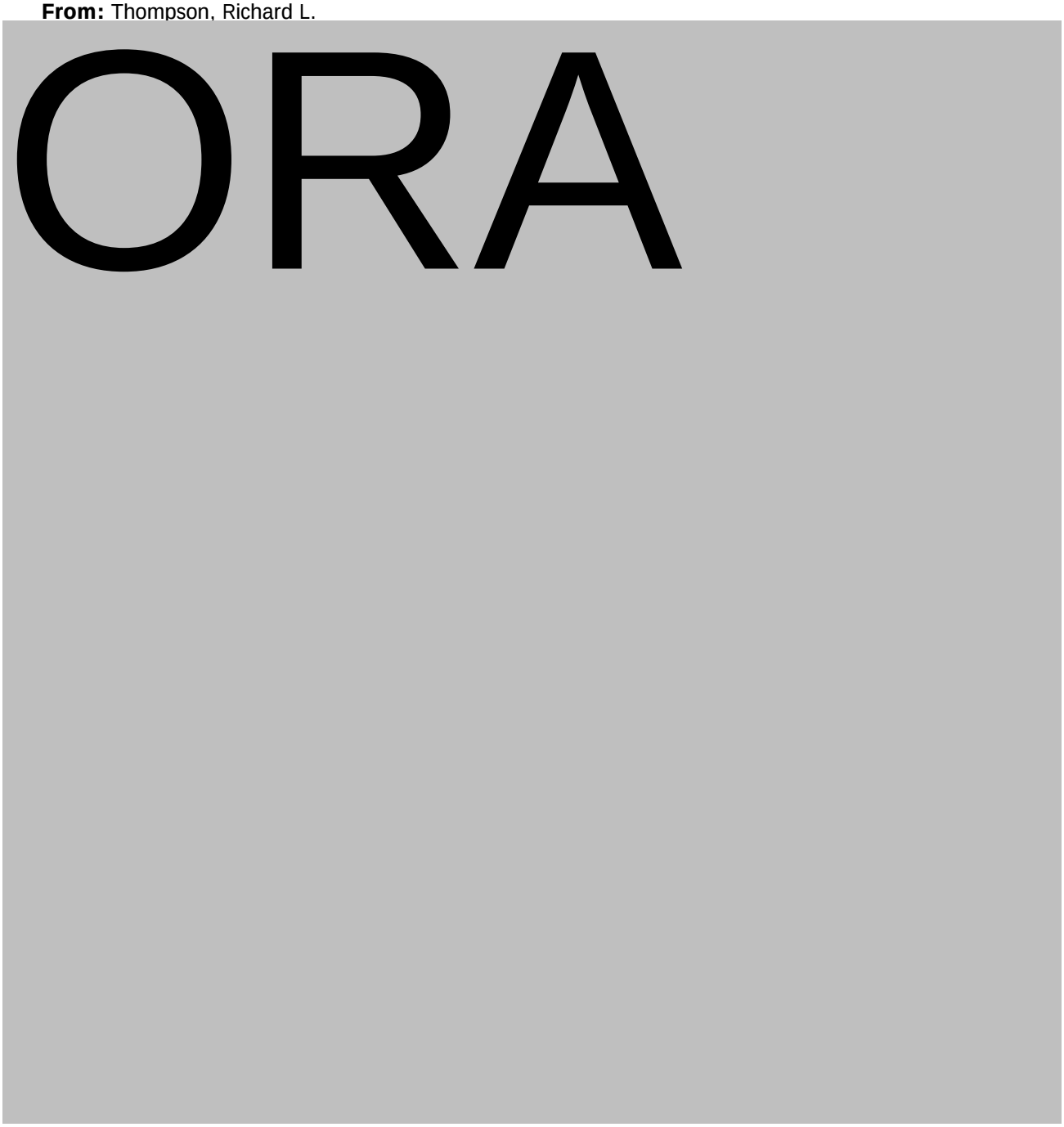
Thanks Richard. That is awesome!

Happy New Year to everyone,

Chris

Ph: 240-402-2464

From: Thompson, Richard L.



ORA

Glyphosate Method Collaboration Report

Introduction

A multi-laboratory validation (collaboration) was conducted of a method for the determination of residue levels of glyphosate, glufosinate, and two degradants of glyphosate N-acetylglyphosate and AMPA. Single laboratory validation of the method was conducted at PSW prior to the collaboration. Seven FDA pesticide laboratories plan to participate in the collaboration eventually. Data from three laboratories (ARL, PNW, and PSW) have been received at this time; this preliminary collaboration report summarizes data submitted from those three laboratories only. A final report encompassing all participating laboratories will be issued after all data from all laboratories has been submitted.

In addition to the collaboration summary of data from all three laboratories, an abbreviated report for the single laboratory validation and each collaborating laboratory are included as attachments to the collaboration report.

Conclusion

The collaboration data indicates the method is suitable for the purpose of quantitative determination for residues of glyphosate, glufosinate and N-acetylglyphosate and semi quantitative determination of AMPA residues in the three primary matrix types analyzed in the FDA pesticide program, i.e., high moisture, low moisture, and high fat items. The collaboration meets all the requirements of a level three multi-laboratory validation as per the "Guidelines for Validation of Chemical Methods for the FDA FVM Program, 2nd Edition.

Protocols and Procedure

Commodities were selected to represent the three major food commodity types analyzed in the FDA pesticide program, i.e. grain corn for dry products, carrots for high moisture products, and avocados for high lipid commodities. Composites of each of these three study matrices were prepared, composited, and distributed to the participating laboratories (PNW, PSW, KAN, ARL, SRL, NRL and CFSAN). Note: avocados were prepared without the outer peel. Each lab analyzed all matrices fortified with each analyte at the fortification levels in replicate as listed below:

i none: 2x
ii 0.050 ppm: 2x
iii 0.250 ppm: 2x
iv 0.500: 2x

Each lab was additionally sent two samples previously found to contain incurred glyphosate residues when analyzed at SRL using the method described in LIB 4596, i.e., ground grain corn in which 0.04 ppm was found and ground soy beans in which 4.5 ppm was found.

Glyphosate Method Collaboration Report

A detailed protocol is provided in attachment A and the method is provided in attachment B.

PSW conducted a single laboratory validation (SLV) of the procedure using the same procedure and collaboration protocol. The SLV results and protocols are reported in the C attachments.

Prior to conducting multiple laboratory method validation each participating laboratory was required to demonstrate proficiency with the procedure. Instrument proficiency was demonstrated conducting system suitability tests that included determination of accuracy, precision, linearity and LOQ by preparing and injecting standards. Results of the system suitability testing are reported with the attached individual laboratory reports (attachments C, F, G and H).

The concentrations and spike recoveries were calculated by single level calibration using average responses of matrix matched standards bracketing the samples and prepared at the same concentration as the spiked sample. For glyphosate and glufosinate residue levels were calculated using corresponding isotopic internal standards added to the extraction solvent prior to analysis. AMPA residues were calculated against the glyphosate isotopic internal standard. Residues of N-acetylglyphosate were calculated using external standard calibration.

The mean recoveries for all three spike levels (50, 250, and 500 ng/g) were calculated by matrix for each laboratory. The overall mean, RSD and method uncertainty (MU) of all three laboratories was calculated for each matrix. The linearity coefficient of determination (R^2) was calculated from the concentrations found at each level for each matrix and laboratory by squaring the Excel correlation function (Correl); the average R^2 of the three laboratories is reported in Table 1. Method specificity was evaluated by the analysis of control matrices. Acceptable validation specifications for the collaboration study are listed below.

Specificity: No residues found in blank control matrices

Recovery: 70-120 % RSD: 15% MU: 30% R^2: 0.990

Results and Discussion

The method collaboration results in this report were provided by three of the participating laboratories: ARL, PNW and PSW. Table 1 contains the summary statistical analysis of all collaboration analyses; results that did not meet specifications are highlighted in red font. Scatter plots of the recoveries are provided in attachment D. No residues were found in the control samples analyzed for each matrix. All results for glyphosate, glufosinate, and N-acetylglyphosate were within the validation specifications. The linearity of the AMPA results did not meet the specification of R2 = 0.99 in any of the three matrices studied, however all were above 0.95. One lab reported low recoveries (48.6 % and 61.3 %) of AMPA in avocado and carrot, respectively.

Glyphosate Method Collaboration Report

Table 1. Summary data includes the average spike recovery for each lab, overall average recovery, RSD, method uncertainty (MU) of the spike recoveries and the average coefficient of determination (R^2) of the spike concentrations.

Matrix	ARL	PNW	PSW	Mean	RSD	MU	R^2
Glyphosate							
Avocado	85.3	87.2	96.6	89.7	(b) (5)	(b) (5)	0.9990
Carrot	80.0	85.9	83.7	83.2	(b) (5)	(b) (5)	0.9995
Corn	91.4	95.1	101.8	96.1	(b) (5)	(b) (5)	0.9995
Glufosinate							
Avocado	82.9	87.0	94.4	88.1	(b) (5)	(b) (5)	0.9970
Carrot	81.0	90.4	84.6	85.3	(b) (5)	(b) (5)	0.9991
Corn	98.4	101.4	102.0	100.6	(b) (5)	(b) (5)	0.9994
N-acetylglyphosate							
Avocado	(b) (5)	90.3	106.3	(b) (5)	(b) (5)	(b) (5)	0.9941
Carrot	79.7	86.7	97.7	88.0	(b) (5)	(b) (5)	0.9965
Corn	93.1	94.4	117.9	101.8	(b) (5)	(b) (5)	0.9979
AMPA							
Avocado	48.6	87.3	85.9	74.0	(b) (5)	(b) (5)	0.9744
Carrot	61.3	83.4	90.9	78.5	(b) (5)	(b) (5)	0.9824
Corn	95.8	76.5	90.3	87.5	(b) (5)	(b) (5)	0.9624

The matrix effect for each analyte/matrix combination was evaluated by calculating residue concentrations using both matrix matched standards and standards prepared in solvent and comparing the slopes of the corresponding linearity charts. Results of the matrix study are tabulated in Table 2 and linearity charts for each analyte/matrix combination are provided in attachment E. Results indicate none of the matrices in the study had much effect on the determination of glyphosate, glufosinate and N-acetylglyphosate. However, all three matrices had a significant impact on residues of AMPA with matrix effects of 391 % in avocado, 327 % in carrot, and 455 % in corn. These results also reflect the advantage of using isotopically labelled internal standards.

Table 2. Matrix effects as percentages of slope ratios of residues calculated for the three spike levels using standards prepared in solvent vs matrix extracts

Compound	Avocado	Carrot	Corn
Glyphosate	91.1	102.2	100.7
Glufosinate	89.4	90.5	103.3
N-acetylglyphosate	108.1	103.1	101.3
AMPA	391	327	455

Each laboratory analyzed a corn sample and a soy sample previously analyzed and found to contain incurred residue of glyphosate. Results of the incurred residue analysis, tabulated in Table 3, are in excellent agreement.

Table 3. Incurred residues (ppb) in corn and soy samples.

Matrix	Original	ARL	PNW	PSW	Mean	RSD
Corn	40	36	35	46	39.3	(12.7)
Soy	4500	4290	4610	4620	4510	(3.4)

For the method collaboration study spike recoveries were calculated based upon a single level calibration at the same concentration as the spike level, i.e., the 50 ng/g spikes were calculated based upon calibration at 50 ng/g equivalence, or 10 ng/ml. Once implemented for routine analysis calibration will be conducted at a single level equivalent to 250 ng/g in the sample. In Table 4 the relative percent difference (RPD) of spike recoveries from the collaboration and the same spike recoveries calculated using a single level standard at concentration equivalent to 250 ng/g. Very low RPDs demonstrate the linearity of the method and accuracy of residue levels calculated from a single level calibration.

Table 4. Relative Percent Difference (RPD) of average recoveries for all levels and laboratories calculated based upon a single level calibration at 250 ng/g vs. calibration per each individual spike level.

Matrix	Single Level	Per Level	RPD	Single Level	Per Level	RPD
	Glyphosate			*Glufosinate*		
Avocado	90.1	89.7	0.4	87.6	88.1	0.6
Carrot	84.7	83.2	1.7	86.8	85.3	1.7
Corn	98.4	96.1	2.4	101.2	100.6	0.6
	N-acetylglyphosate			*AMPA*		
Avocado	87.6	96.9	10.1	65.9	74	11.5
Carrot	86.8	88	1.4	76.9	78.5	2.0
Corn	101.2	101.8	0.6	90.6	87.5	3.4

Glyphosate Method Collaboration Report

Attachments

A. Collaboration Protocol
B. Analytical Method
C. Single Laboratory Validation
    C_1 SLV Method Recovery Charts
    C_2 SLV Method Linearity Charts
D. Method Collaboration Recovery Charts
E. Method Collaboration Matrix Effects Charts
F. PSW Collaboration Data and System Suitability
    F_1 PSW Recovery Charts
    F_2 PSW Linearity Charts
G. PNW Collaboration Data and System Suitability
    G_1 PNW Recovery Charts
    G_2 PNW Linearity Charts
H. ARL Collaboration Data and System Suitability
    H_1 ARL Recovery Charts
    H_2 ARL Linearity Charts

Collaboration Protocol

Matrices: corn (dry), carrot (high moisture), avocado (high fat)

Analyses:

Recovery Study

Level	N*
Control	2
Spike 50	2
Spike 250	2
Spike 500	2

** replicates per matrix*

Incurred Residues

Matrix	Level
Corn	~40 ng/g
Soybean	~4.5 µg/g

Preparation of Standards: Prepare calibration/fortification standards in both solvent and in matrix extracts and listed below.

Calibration Standards in Solvent				Matrix Calibration Standards			
Std Conc (ng/ml)	**Spk Std[1] Conc (µg/ml)**	**Spk Std Volume Added (µl)**	**Dilution[2] Volume (ml)**	**Std Conc (ng/ml)**	**Spk Std[1] Conc (µg/ml)**	**Spk Std Volume Added (µl)**	**Dilution[3] Volume (ml)**
	corn (2 g sample)						
10	1	100	10	10	1	50	5
50	5	100	10	50	5	50	5
100	5	200	10	100	5	100	5
	carrot/avocado (5 g sample)						
10	5	50	25	10	1	100	10
50	5	250	25	50	5	100	10
100	50	50	25	100	50	20	10

[1] *Prepare mixed native standards as directed in method step C.4*

[2] *Dilute with 50 ng/ml IS fortified extraction solvent*

[3] *Dilute with control sample matrix*

Fortification Procedure:

Spike Level (ng/g)	Spk Std Conc (µg/ml)	Volume Added (µl)
corn (2 g/sample)		
50	1	100
250	5	100
500	5	200

Collaboration Protocol

carrot/avocado (5 g/sample)		
50	5	50
250	5	250
500	50	50

Extraction Cleanup for Avocado:

Follow method as written. Re the cleanup option for avocadoes; i.e. dichloromethane (DCM) vs petroleum ether (PE) three ORA labs agreed to use DCM and the remaining three ORA labs agreed to use PE. CFSAN can choose either.

DCM	PE
ARL	PNW
SRL	PSW
KAN	NRL

LCMS Transition Names:

AMPA[110-63] 1
AMPA[110-79] 2
AMPA[110-81] 3
Glu[180-63] 1
Glu[180-95] 2
Glu[180-85] 3
Glu[183-63] IS
Gly[168-63] 1
Gly[168-79] 2
Gly[168-150] 3
Gly[171-63] IS
N-acetyl[210-150] 1
N-acetyl[210-63] 2
N-acetyl[210-168] 3

LCMS Calibration: Calibrate using single level calibration for each spike level. Assign the internal standards as below.

Analyte	Internal Standard
Glyphosate:	Glyphosate-^{13}C
N-acetylglyphosate:	Glyphosate-^{13}C
AMPA:	Glyphosate-^{13}C
Glufosinate:	Glufosinate-D^{3}

Collaboration Protocol

Inj Sequence: Group by spike level. Assign Sample Name to Sample description and the Sample Types and Actual Concentrations listed in the table below.

Description	Sample Name	Sample Type	Actual Conc
50 ng/g spike level			
10 ng/ml calibration std in solvent	CalStd10	Standard	50
10 ng/ml calibration std in solvent	CalStd10	Standard	50
10 ng/ml corn matrix calibration std	MatStd10 Corn	QC	50
Corn control	Control Corn	Unknown	
Corn spike 50 #1	Spk50-1 Corn	QC	50
Corn spike 50 #2	Spk50-2 Corn	QC	50
Corn incurred residue	Corn Incur	Unknown	
10 ng/ml corn matrix calibration std	MatStd10 Corn	QC	50
10 ng/ml calibration std in solvent	CalStd10	Standard	50
10 ng/ml carrot matrix calibration std	MatStd10 Carrot	QC	50
Carrot control	Control Carrot	Unknown	
Carrot spike 50 #1	Spk50-1 Carrot	QC	50
Carrot spike 50 #2	Spk50-2 Carrot	QC	50
10 ng/ml carrot matrix calibration std	MatStd10 Carrot	QC	50
10 ng/ml calibration std in solvent	CalStd10	Standard	50
10 ng/ml avocado matrix calibration std	MatStd10 Avocado	QC	50
Avocado control	Control Avocado	Unknown	
Avocado spike 50 #1	Spk50-1 Avocado	QC	50
Avocado spike 50 #2	Spk50-2 Avocado	QC	50
10 ng/ml avocado matrix calibration std	MatStd10 Avocado	QC	50
10 ng/ml calibration std in solvent	CalStd10	Standard	50
250 ng/g spike level			
50 ng/ml calibration std in solvent	CalStd50	Standard	250
50 ng/ml calibration std in solvent	CalStd50	Standard	250
50 ng/ml corn matrix calibration std	MatStd50 Corn	QC	250
Corn spike 250 #1	Spk250-1 Corn	QC	250
Corn spike 250 #2	Spk250-2 Corn	QC	250
50 ng/ml corn matrix calibration std	MatStd50 Corn	QC	250
50 ng/ml calibration std in solvent	CalStd50	Standard	250
50 ng/ml carrot matrix calibration std	MatStd50 Carrot	QC	250
Carrot spike 250 #1	Spk250-1 Carrot	QC	250
Carrot spike 250 #2	Spk250-2 Carrot	QC	250
50 ng/ml carrot matrix calibration std	MatStd50 Carrot	QC	250
50 ng/ml calibration std in solvent	CalStd50	Standard	250
50 ng/ml avocado matrix calibration std	MatStd50 Avocado	QC	250
Avocado spike 250 #1	Spk250-1 Avocado	QC	250
Avocado spike 250 #2	Spk250-2 Avocado	QC	250
50 ng/ml avocado matrix calibration std	MatStd50 Avocado	QC	250
50 ng/ml calibration std in solvent	CalStd50	Standard	250

Collaboration Protocol

500 ng/g spike level

100 ng/ml calibration std in solvent	CalStd100	Standard	500
100 ng/ml calibration std in solvent	CalStd100	Standard	500
100 ng/ml corn matrix calibration std	MatStd100 Corn	QC	500
Corn spike 500 #1	Spk250-1 Corn	QC	500
Corn spike 500 #2	Spk250-2 Corn	QC	500
100 ng/ml corn matrix calibration std	MatStd100 Corn	QC	500
100 ng/ml calibration std in solvent	CalStd100	Standard	500
100 ng/ml carrot matrix calibration std	MatStd100 Carrot	QC	500
Carrot spike 500 #1	Spk250-1 Carrot	QC	500
Carrot spike 500 #2	Spk250-2 Carrot	QC	500
100 ng/ml carrot matrix calibration std	MatStd100 Carrot	QC	500
100 ng/ml calibration std in solvent	CalStd100	Standard	500
100 ng/ml avocado matrix calibration std	MatStd100 Avocado	QC	500
Avocado spike 500 #1	Spk250-1 Avocado	QC	500
Avocado spike 500 #2	Spk250-2 Avocado	QC	500
100 ng/ml avocado matrix calibration std	MatStd100 Avocado	QC	500
100 ng/ml calibration std in solvent	CalStd100	Standard	500
100 ng/ml soy matrix calibration std	MatStd100 Soy	QC	500
Soy control	Control Corn	Unknown	
Soy incurred residue	Soy Incur	Unknown	
Soy incurred residue Dil 1-10	Soy Incur (1-10)	Unknown	
100 ng/ml soy matrix calibration std	MatStd100 Soy	QC	500
100 ng/ml calibration std in solvent	CalStd100	Standard	500

Data: Provide the following data fields when reporting results

Index
Sample Name
Sample Type
Dilution Factor
Peak Name (Transition Name)
Peak Area
IS Peak Area
Retention Time (RT)
Actual Concentration (Spk level or Std conc)
Calculated concentration

Glyphosate Method Collaboration Report Attachment B

Analytical Method

A. Reagents and Supplies

1. Acetonitrile, HPLC grade
2. Petroleum ether
3. Methylene chloride
4. Water, HPLC grade
5. Formic acid, 98% solution
6. Acetic acid
7. Ammonium formate
8. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA)
9. Tetrabutylammonium hydroxide (TBAOH) titrant, 0.4 M in Water, HPLC Grade, ACROS Organics
10. Tetrabutylammonium acetate (TBuAA), Aldrich No. 335991-10G (optional)
11. Tetrabutylammonium acetate 1 M (TBuAA 1M), Aldrich No. 401803 – 50 ML (optional)
12. 50-mL plastic centrifuge tubes
13. Filter, 0.2 µm, 25 mm, nylon
14. Waters Oasis HLB SPE, 60 mg, 3cc, 30 µm
15. Extraction solvent (50 mM acetic acid/10 mM Na_2EDTA): mix 2.9 mL acetic acid and 3.7 g Na_2EDTA in 1000-mL of purified water.
16. 50 ng/ml IS fortified extraction solvent: dilute IS 20 µg/ml mixed isotope internal standard, prepared in step C.2.a, 1:400 using extraction solvent, prepared in step A.15, e.g. 2.5 ml (IS 20 µg/ml) to 1000 ml extraction solvent
17. Mobile phase A (4 mM tetrabutylammonium formate)
    a. Add 10.0 ml of 0.4 M TBAOH to ~900 mL HPLC water, and adjust the pH to 2.8±0.05 using formic acid (~ 3 ml). OR
    b. Add 1.20 g TBA acetate in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL). OR
    c. 4 ml 1M TBuAA in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL).

B. Standard Reference Materials

1. Glyphosate
2. Glufosinate
3. AMPA
4. N-acetyl-glyphosate, available from EPA and Toronto Research Chemicals (TRC No A178245)
5. Glyphosate-^{13}C
6. Glufosinate-D^3

C. Standard Solutions

1. General instructions
    a. Unless otherwise indicated prepare standards in DI water
    b. Store standard solutions in plastic containers because glass can leach standard reference material from solution. Use of glass volumetric flasks for standard preparation is OK if solution is removed from the glassware after preparation.
    c. Store standard solutions in a refrigerator. Do not store standards prepared with water or aqueous media in the freezer.
2. Stock standards 1 mg/ml

a. Includes all native and isotopic standards listed in Section B
b. Prepare individual stock standard for each compound

3. Isotopic working solutions
   a. IS 20 µg/ml mixed isotope internal standard
      i) Combine isotopes Glyphosate-^{13}C and Glufosinate-D^3 (step B.5 & 6)
      ii) Dilute 1 mg/ml stock isotope internal standards, prepared in step C.2, 1:50
4. Intermediate mixed standards
   a. 50 µg/ml mixed native standard
      i) Combine native 1 mg/ml stock standards, prepared in step C.2
      ii) Include glyphosate, glufosinate, AMPA, and N-acetyl-glyphosate (Step B.1-4)
      iii) Dilute 1:20
   b. 5.0 µg/ml mixed native standard
      i) Dilute 50 µg/ml mixed standard, prepared in step C.4.a, 1:10
   c. 1.0 µg/ml mixed native standard
      i) Dilute50 µg/ml mixed standard, prepared in step C.4.a, 1:50
5. LC-MS/MS calibration standard 50 ng/ml
   a. Dilute 5.0 µg/ml mixed native standard, prepared in step C.4.b, 1:100, using 50 ng/ml IS fortified extraction solvent (A.16)

D. **Equipment and Instrumentation**

1. Genogrinder
2. Centrifuge
3. Pipettes
4. LC-MS/MS
   a. Shimadzu HPLC system: two LC-20AD pumps, Sil-20AC autosampler, CTO-20AC column oven
      NOTE: Replace all metal LC tubing with PEEK tubing between the autosampler and injection valve because glyphosate can be retained on metal surfaces.
   b. AB model 5500, or 6500, Q-TRAP mass spectrometer
   c. HPLC columns: Phenomenex Luna C8(2), 100 Å, 5 µm, 150 x 4.6 mm, Phenomenex No. 00F-4249-E0; Or Phenomenex Luna C8, 100 Å, 5 µm, 150 x 2 mm, Phenomenex No. 00F-4040-B0
   d. HPLC guard column: Phenomenex guard column KrudKatcher P/N AFO-8497

      NOTE: Install peek tubing between the autosampler and column because metal can affect glyphosate and glufosinate chromatography

E. **Extraction Procedure**

1. 5 g sample + 25 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15
   For dry products containing less than 50 % moisture: 2 g sample plus 10 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15 for dry products
2. Add 10 ml PE, or $MeCl_2$, for matrices containing more than 3 % fat.
3. Shake @ 1000 shakes per min for 10 min
4. Centrifuge at ≥ 3000 rpm for 5 min NOTE: When using PE to remove lipid co-extractants high fat matrices, the PE will be the top layer. When using $MeCl_2$, the $MeCl_2$ will be the bottom layer in the centrifuge tube.

Analytical Method

5. Filter aqueous extract thru HLB SPE cartridge, limit filter volume to less than 2 mls.
6. Filter for injection (could be included with SPE step)
7. Sample concentration: 0.2 g/ml

F. **LC-MS/MS method**

LC Parameters		Gradient	
Column:	Phenomenex Luna C8(2), 150 x 4.6 mm, 5 µm OR Phenomenex Luna C8, 150 x 2 mm, 5 µm Guard Column: Phenomenex KrudKatcher	**Time**	**MPB**
MP A:	4 mM tetrabutlyammonium formate + 0.1 % formic acid in water (pH 2.8±0.05)	0.00	5
MP B:	MeCN	1.00	5
Flow:	0.45 mL/min (4.6 mm column)	5.00	90
	0.3 mL/min (2.0 mm column)	7.00	90
Inj Vol:	10 µL	8.00	5
Temp	40 °C	14.00	5
Divert Valve	Divert flow from mass spectrometer about 30 seconds before the first analyte and 60 seconds after the last analyte elutes		

MS/MS Parameters (5500 & 6500)

Q1	Q3	RT	Transition	DP*	EP	CE	CXP
110	63	1.3	AMPA 1	-40	-11	-30	-9
110	79	1.3	AMPA 2	-40	-11	-34	-9
110	81	1.3	AMPA 3	-40	-11	-34	-9
112	63	2.5	AMPA IS	-60	-11	-26	-9
180	63	3.0	Glufosinate 1	-60	-11	-66	-9
180	95	3.0	Glufosinate 2	-60	-11	-19	-5
180	85	3.0	Glufosinate 3	-60	-11	-25	-9
183	63	3.0	Glufosinate IS	-60	-11	-40	-9
168	63	4.4	Glyphosate 1	-30	-11	-28	-9
168	79	4.4	Glyphosate 2	-30	-11	-56	-9
168	150	4.4	Glyphosate 3	-30	-11	-16	-9
171	63	4.4	Glyphosate IS	-30	-11	-28	-9
210	150	5.3	N-acetyl glyphosate 1	-20 (-40)	-11	-20	-13
210	63	5.3	N-acetyl glyphosate 2	-20 (-40)	-11	-40	-13
210	168	5.3	N-acetyl glyphosate 3	-20 (-40)	-11	-18	-13

**DP: if more than one DP is provided the first is optimized for the 6500 and the DP in () is optimized for the 5500*

Analytical Method

MS Parameters

Ionization:	Ionspray in negative ionization mode		
CUR:	35	TEM:	450 °C (6500)
CAD:	medium		650 °C (5500)
IS:	-4000	Q1:	unit
GAS 1 & 2:	65	Q3:	unit

G. **Quantitation of Residues**

1. Calibrate instrument using single level calibration standard at 50 ng/ml
2. Calibrate using internal standard calibration for glyphosate, glufosinate and AMPA
   a. Assign internal standard calibration standards
      i) Glyphosate: Glyphosate-^{13}C
      ii) Glufosinate: Glufosinate-D_3
      iii) AMPA: Glyphosate-^{13}C
3. Calibrate using external calibration for N-acetylglyphosate
4. Reportable residues must meet the identification criteria provided in Appendix A “Identification of Residues” in ORA-LAB.10
5. Quantitate residues per instructions in Appendix B “Quantitation of Residues” in ORA-LAB.10. Give preference to quantitation using the primary MS/MS transition, e.g. “Glyphosate 1”, however, use of secondary transitions for quantitation may be advisable if/when matrix coextractants interfere with the primary transition response.

Single Laboratory Validation

The PSW laboratory conducted single laboratory validation (SLV) for the procedure "Analysis of Glyphosate in Food by HPLC-MS/MS" (Att. B). Standards were prepared as per glyphosate procedure (Att. B) at 1, 2, 10, 50, 100, 200, 250, 350, 400 and 500 ng/ml in extraction solvent fortified at 50 ng/ml with isotopic internal standards. The matrices studied were the collaboration samples of corn, carrot and avocado. Recovery studies were conducted using the calibration protocols and analysis sequences prescribed in the collaboration protocol (Att. A). Each matrix was analyzed as an unfortified control and fortified in duplicate at three different levels: 50, 250, and 500 ng/g; i.e. six analyses per matrix, 21 analyses altogether. For the MDL study each of the three matrices was fortified at 20 ng/ml and seven replicates were analyzed per the instructions of 40 CFR 136 Appendix B.

Prior to starting the collaboration, instrument system suitability (SS) was demonstrated. Standards were injected at concentrations of 10, 50, 100, 200, 350, and 500 ng/ml to determine accuracy and linearity. Five replicates of the 50 ng/ml standard were injected to determine precision. The instrument LOQ was determined as per ORA-LAB.10 by injecting a 2 ng/ml standard in solvent and determining the S/N of the quantifier and qualifier ions. The LOQ was calculated as the lowest level where the S/N of the quantifier ion ≥ 10 and the S/N of the qualifier ion ≥ 3. Results for the instrument system suitability study are listed in the table below.

SS Factor	Glyphosate		Glufosinate		AMPA		N-acetylglyphosate	
LOQ (ng/mL)	0.3		0.3		0.5		0.2	
Precision (RSD)	99.1	(1.4)	99.8	(2.3)	97.7	(2.1)	102.3	(1.2)
Accuracy (R^2)	100.4	(0.9997)	104.4	(0.9996)	96.1	(0.9998)	96.6	(0.9998)

For the recovery study the average recovery, RSD, method uncertainty (MU), and the coefficient of determination (R^2) for all levels was determined for each matrix and overall. MU at the 95 % confidence level was calculated as 2 * the RSD as prescribed in ORA-LAB.5.4.6. Linearity (R^2) was calculated by squaring the Excel correlation function (Correl) of the spike level and calculated concentrations of the spiked samples. The method LOQ was determined by multiplying the standard deviation of the concentrations of seven replicate 20 ng/ml spikes per matrix by 10. For the overall method LOQ the standard deviation was calculated by adding the variances and degrees of freedom of the individual matrix concentrations taking the square root. Specificity was determined by the analysis of the control samples. Acceptable method validation specifications for each method performance metric are listed below.

Recovery: 70-120 % RSD: 15% MU: 30%
R^2: 0.990 LOQ: ≤ 10 ng/g

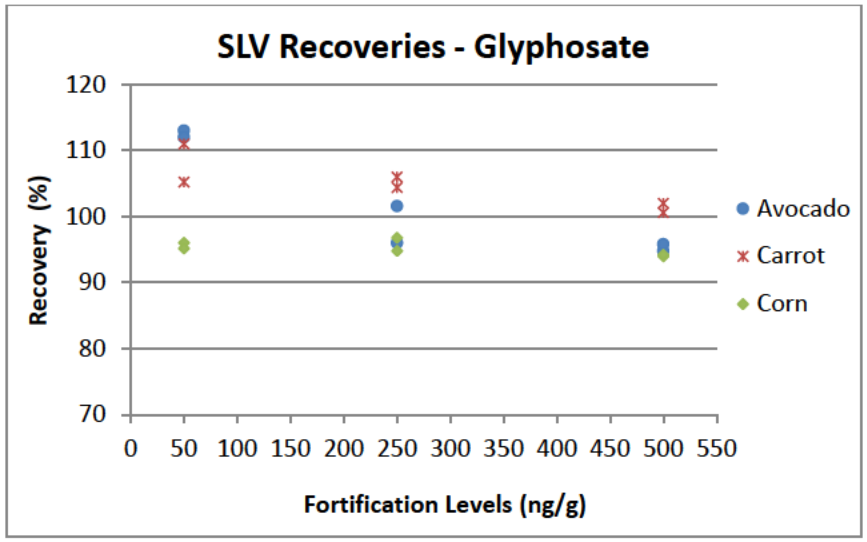
Results of the SLV are summarized in the Table C1 below; results that were not within validation specifications are indicated in red font. Scatter plots of recoveries and linearity charts for each analyte are provided in attachments C_1 and C_2. Results for both of the pesticides, glyphosate and glufosinate met all validation performance specifications and results for the glyphosate degradant N-acetylglyphosate met all specifications with the exception of the R^2 of

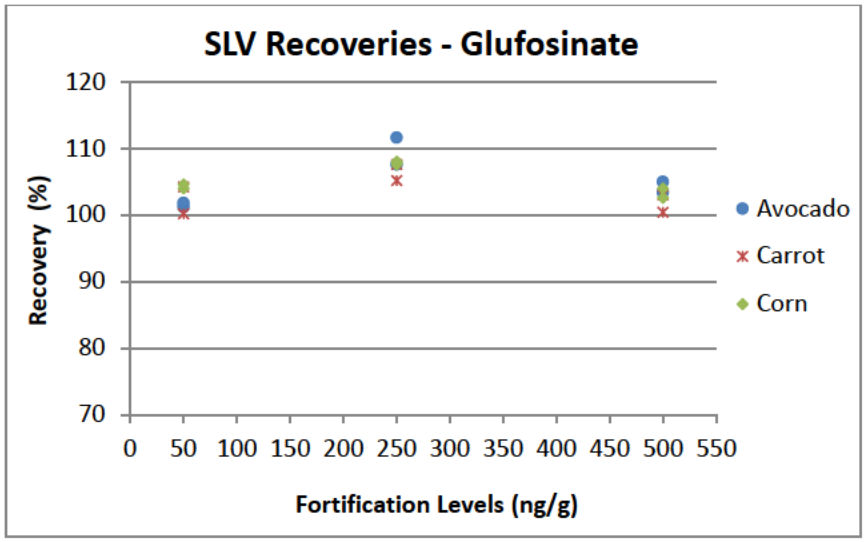
0.9871 for avocado recoveries was just below the specification of 0.99. Recoveries of the glyphosate degradant AMPA were very low, averaging 19.8 %, however it did meet most of the other specifications. AMPA will be considered qualitative and will not be reported for routine analyses

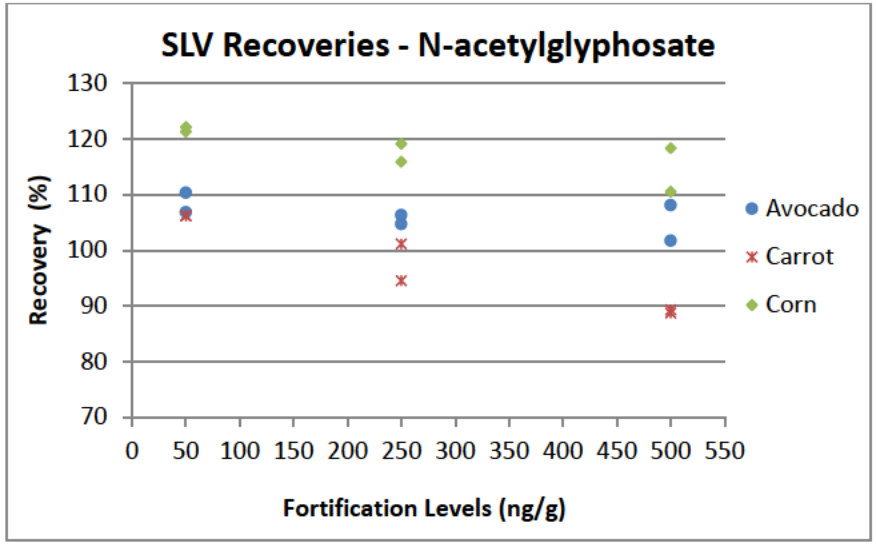
Table 1. Summary data includes the average, RSD, method uncertainty (MU) and coefficient of determination (R^2) from the recovery study and method limit of quantitation (LOQ) from the LOQ study.

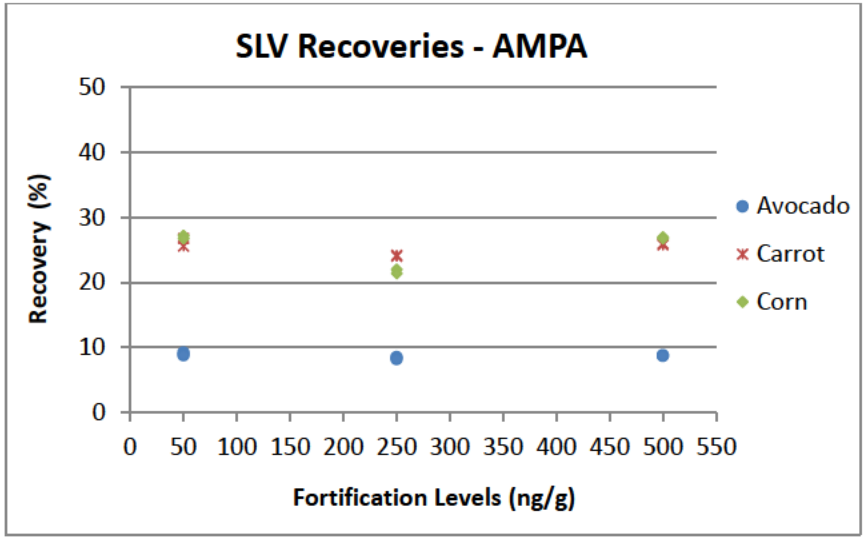
	Recoveries				Limits
Matrix	**Average**	**RSD**	**MU**	**R^2**	**LOQ**
Glyphosate					
Avocado	102.2	8.2	(b) (5)	0.9993	3.5
Carrot	104.9	3.5	(b) (5)	0.9994	7.5
Corn	95.2	1.1	(b) (5)	0.9998	5.2
Overall	100.7	6.5	(b) (5)	0.9957	5.7
Glufosinate					
Avocado	105.1	3.7	(b) (5)	0.9984	7.4
Carrot	103.4	2.8	(b) (5)	0.9986	8.8
Corn	105.1	2.1	(b) (5)	0.9991	10
Overall	104.6	2.9	(b) (5)	0.9984	8.8
N-acetylglyphosate					
Avocado	(b) (5)	(b) (5)	(b) (5)	(b) (5)	8.4
Carrot	(b) (5)	(b) (5)	(b) (5)	(b) (5)	4.4
Corn	(b) (5)	(b) (5)	(b) (5)	(b) (5)	7.6
Overall	(b) (5)	(b) (5)	(b) (5)	(b) (5)	7.0
AMPA					
Avocado	8.7	3.8	(b) (5)	0.9986	6.1
Carrot	25.4	4.3	(b) (5)	0.9978	9.9
Corn	25.2	10.8	(b) (5)	0.9853	3.9
Overall	19.8	41.5	(b) (5)	0.6745	7.1

Single Laboratory Validation – Method Recovery Charts

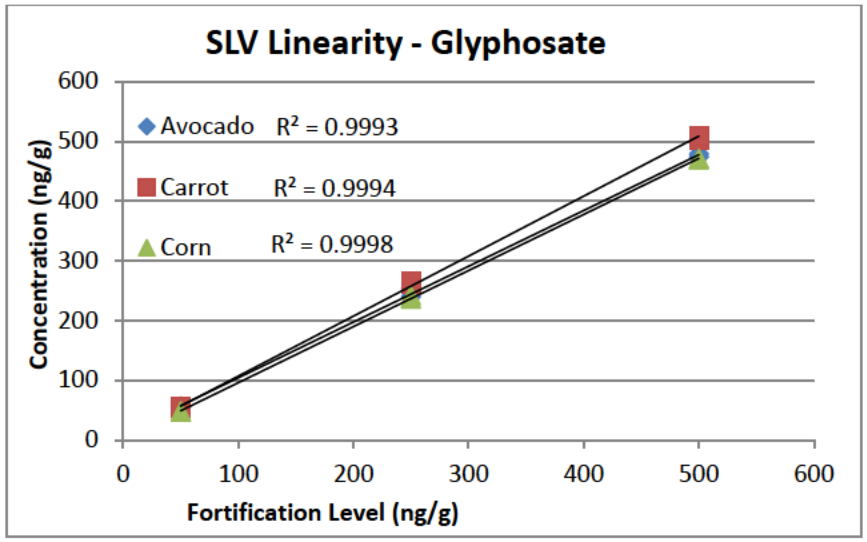


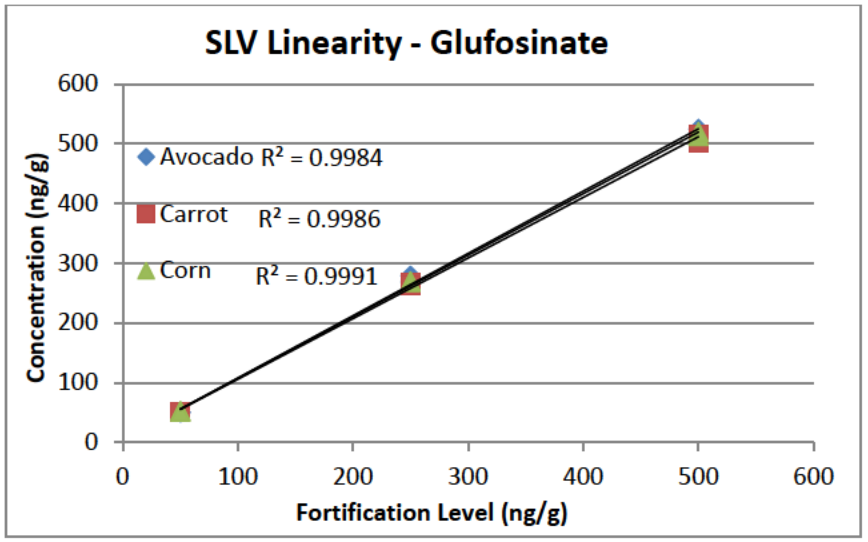


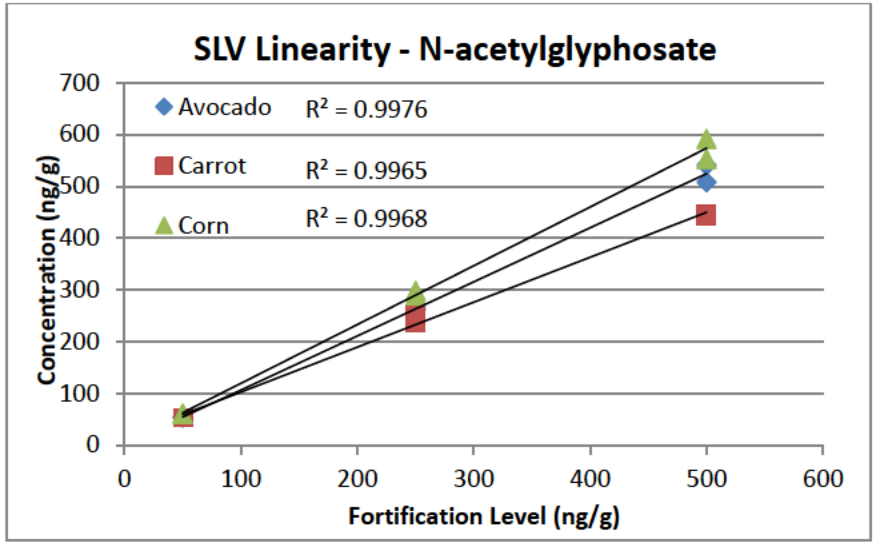


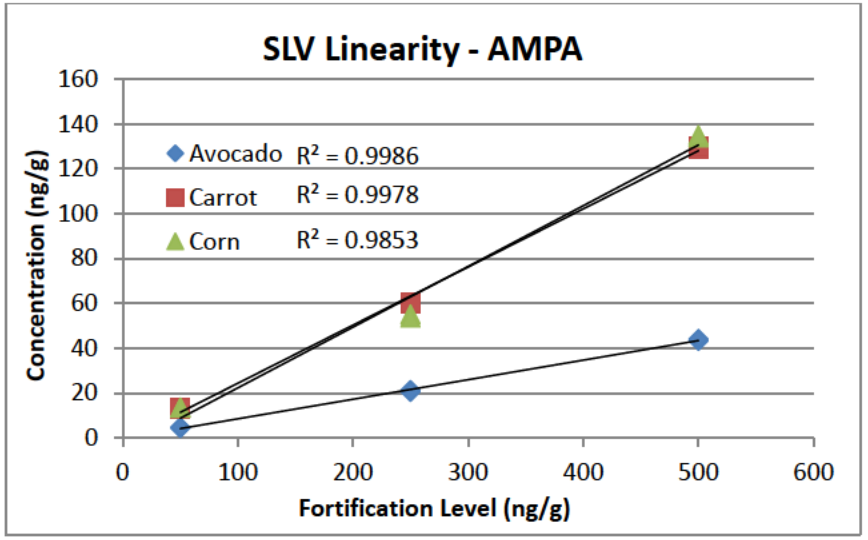


Single Laboratory Validation – Method Linearity Charts

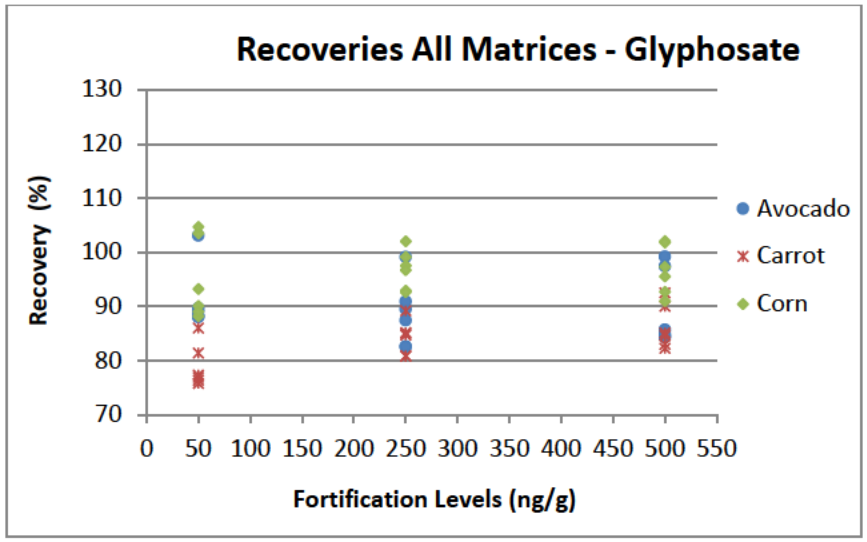


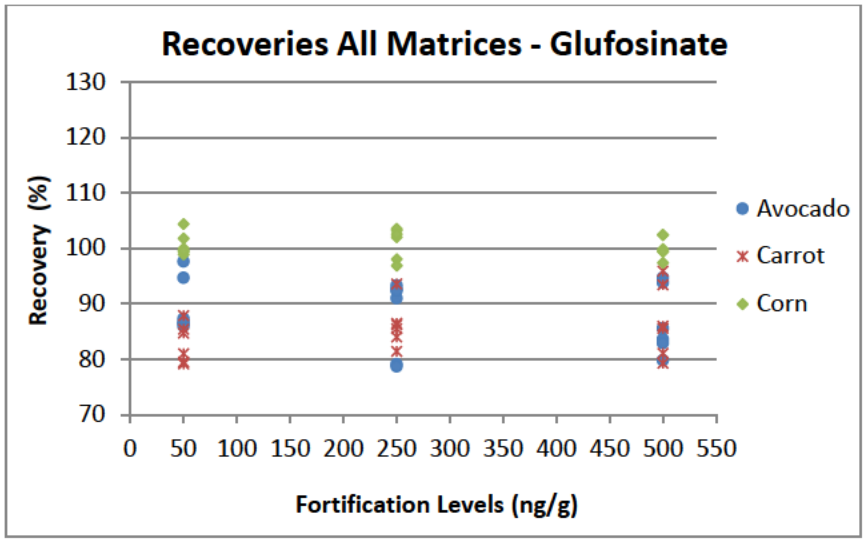


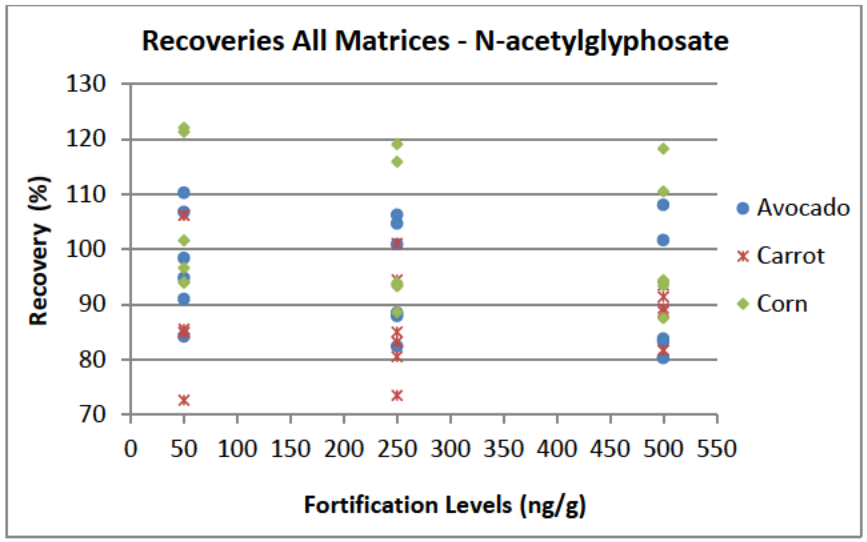


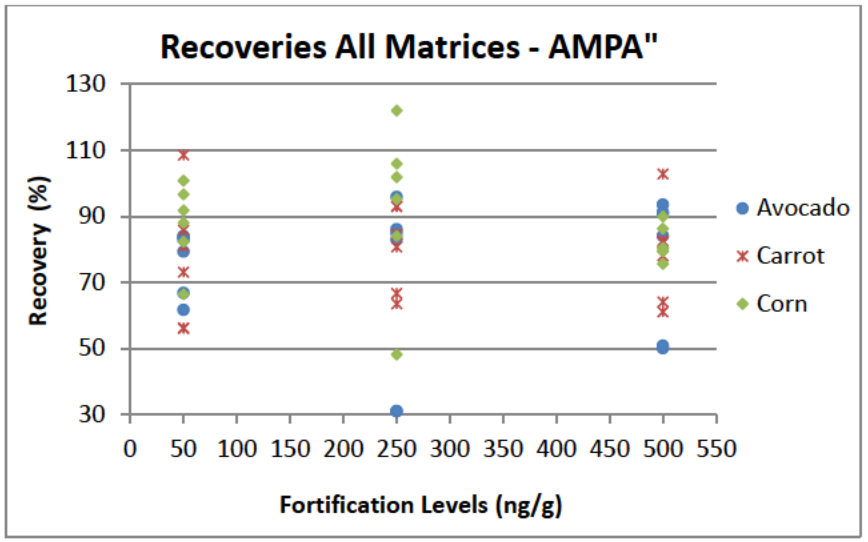


Method Collaboration Recovery Charts

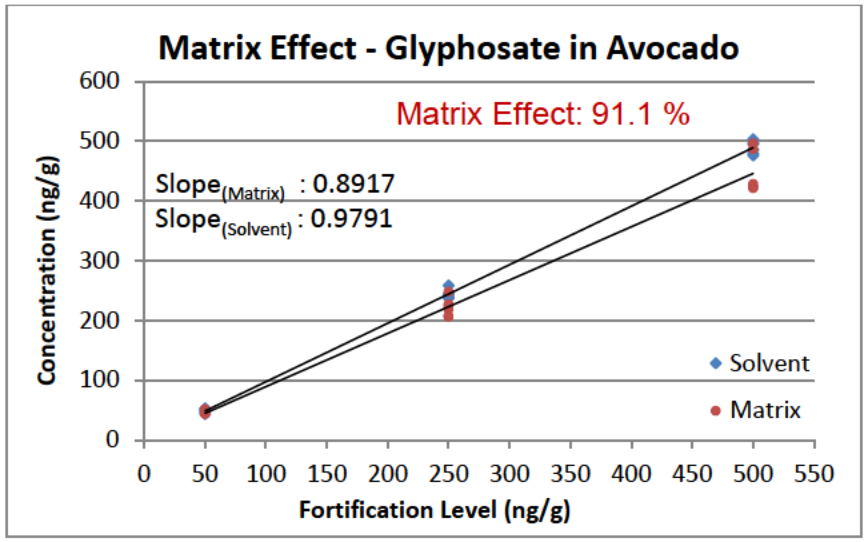


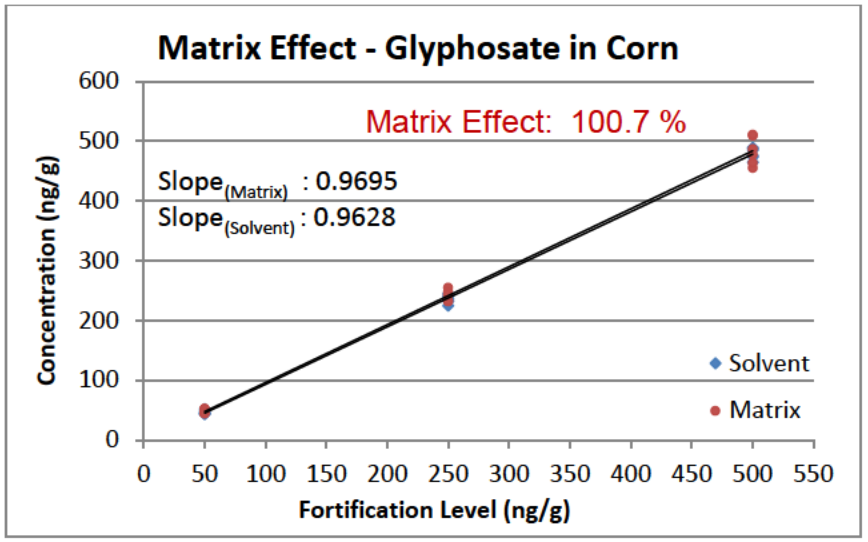


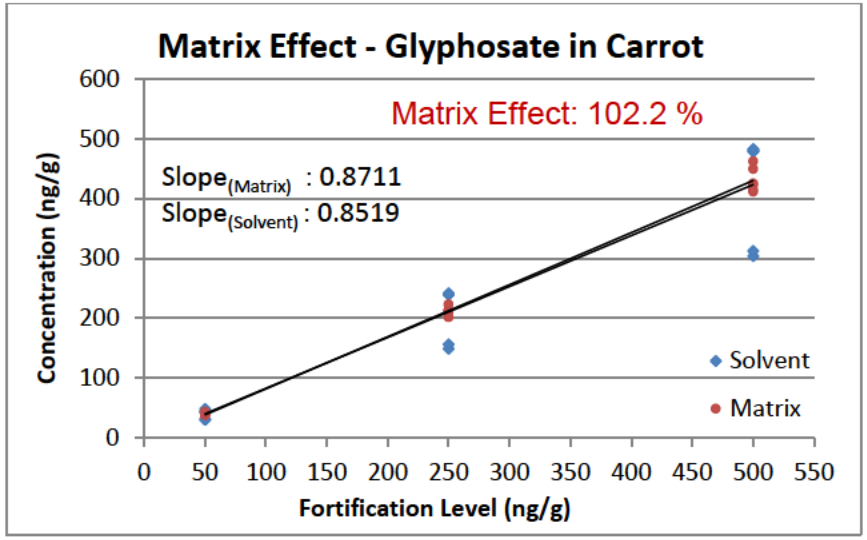


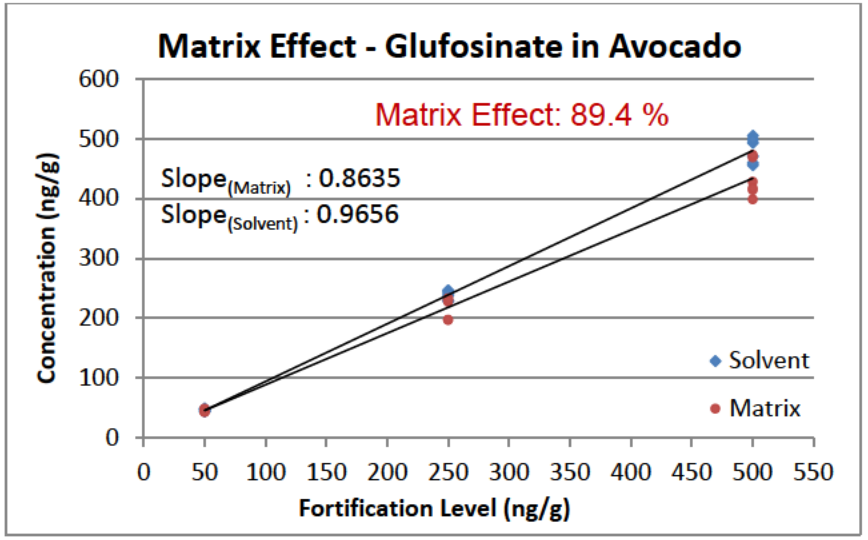


Method Collaboration Matrix Effects Charts

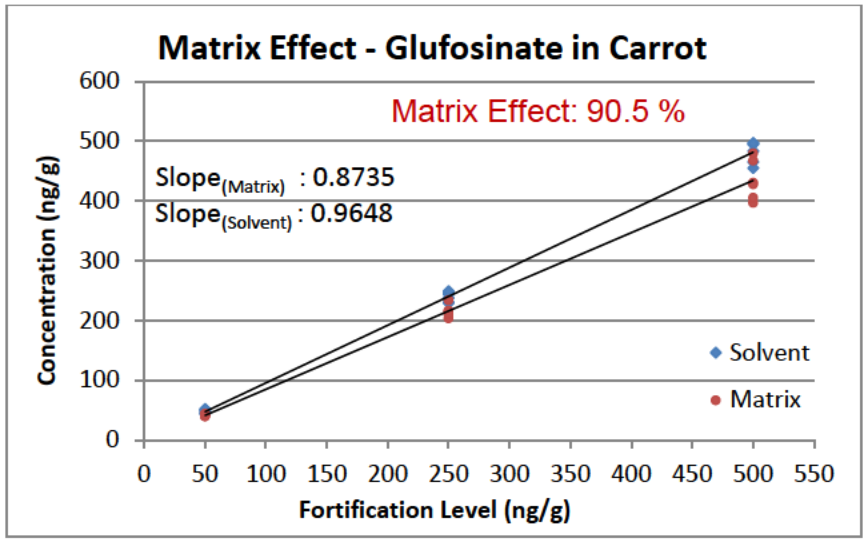


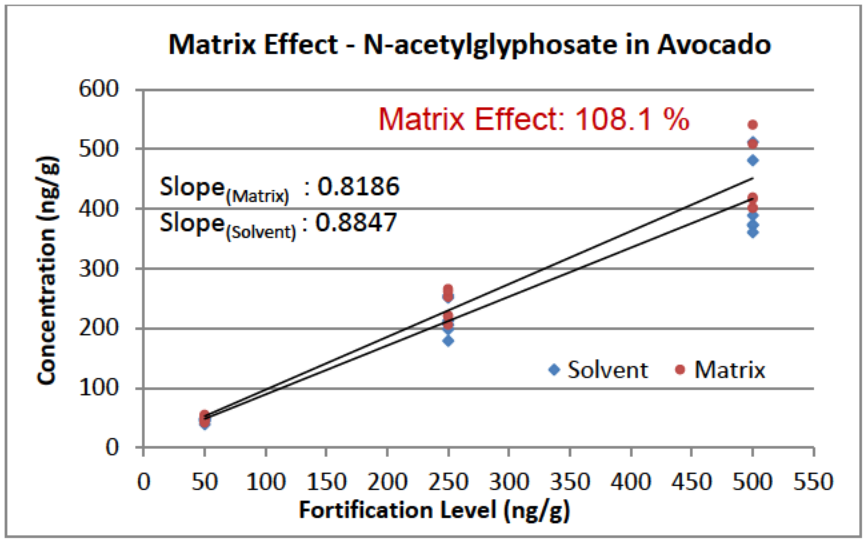


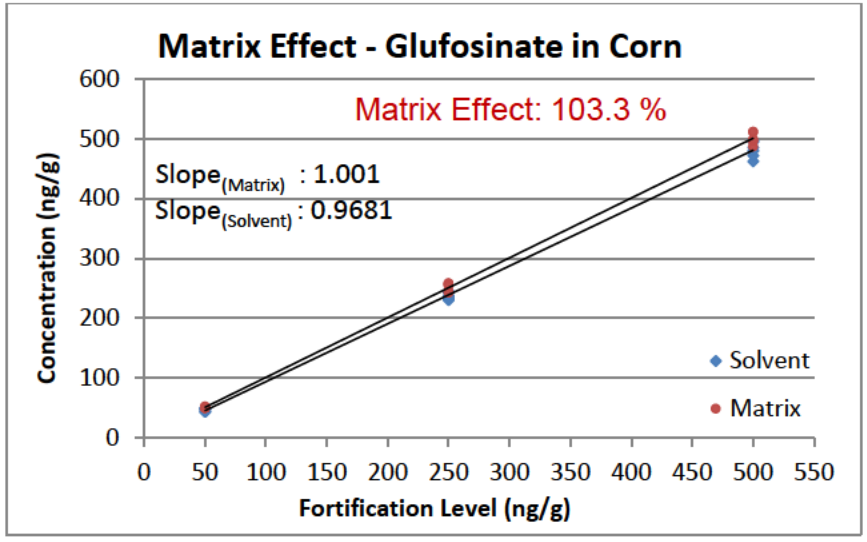


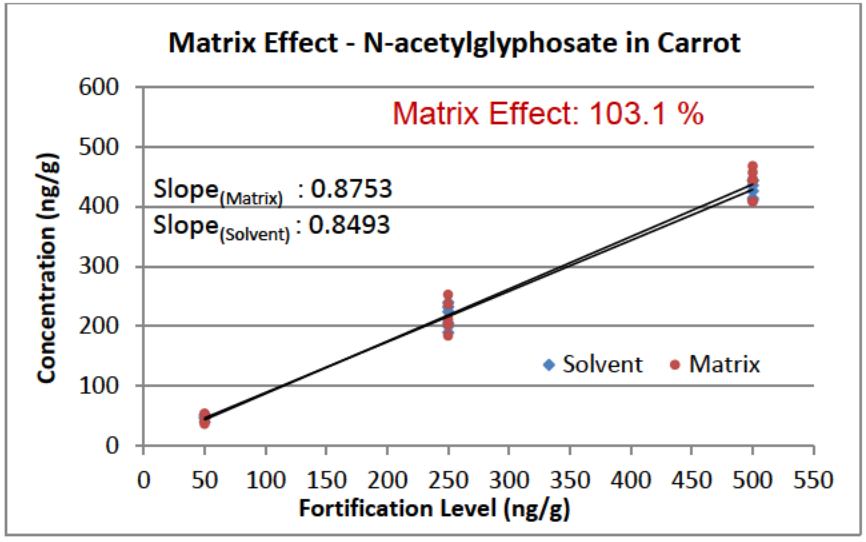


Method Collaboration Matrix Effects Charts

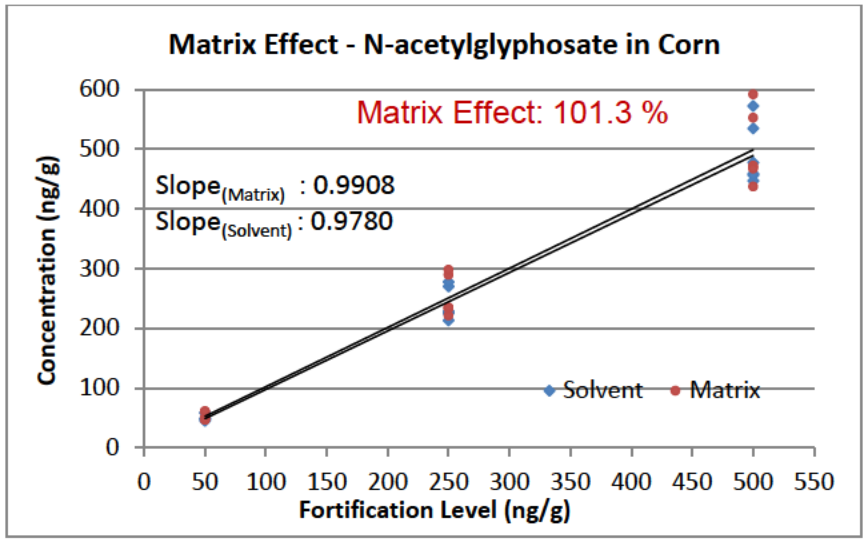


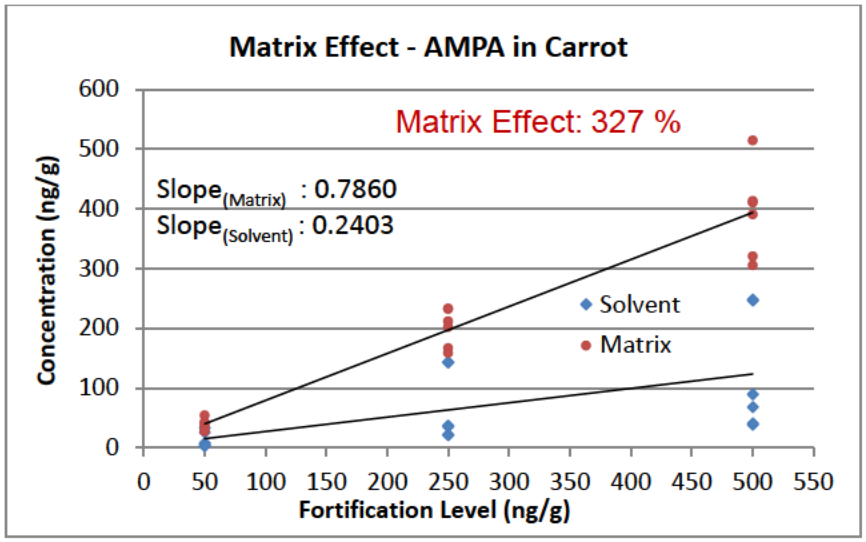


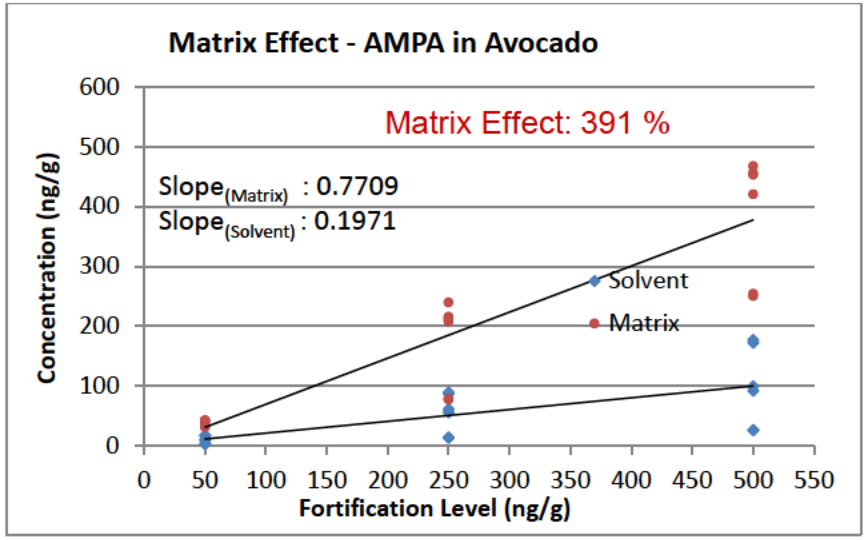


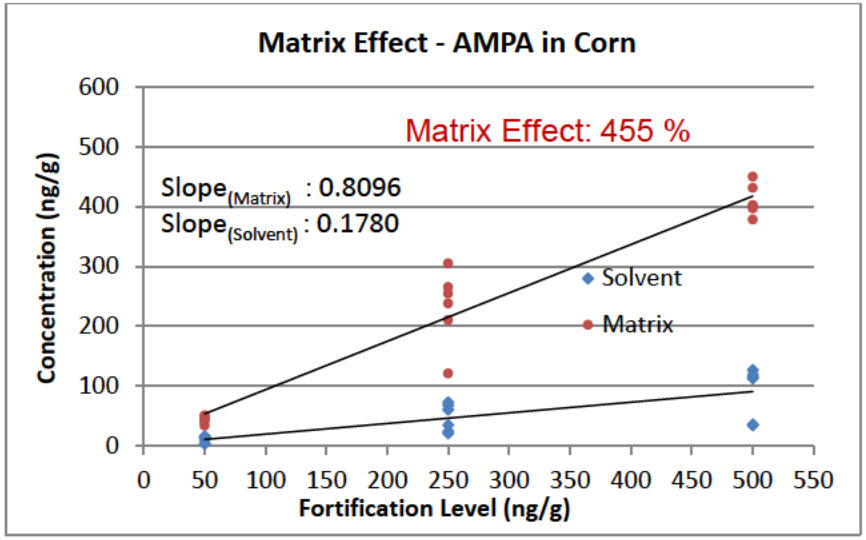


Method Collaboration Matrix Effects Charts









PSW Collaboration Data and System Suitability

All data and derived statistics in this attachment are from the method collaboration analyses conducted at PSW only. Results of the instrument system suitability and method collaboration verify that PSW is able to the method proficiently.

Instrument System Suitability

Prior to starting the collaboration instrument system suitability (SS) was demonstrated. Standards were injected at concentrations of 10, 50, 100, 200, 350, and 500 ng/ml to determine accuracy and linearity. Five replicates of the 50 ng/ml standard were injected to determine precision. The instrument LOQ was determined as per ORA-LAB.10 by injecting a 2 ng/ml standard and determining the S/N of the quantifier and qualifier ions. The LOQ was calculated as the lowest level where the S/N of the quantifier ion ≥ 10 and the S/N of the qualifier ion ≥ 3. Results for the instrument system suitability study are listed in the table below. Criteria for instrument system suitability are tabulated below.

LOQ (ng/ml)	Precision (RSD)	Accuracy (%)	Linearity (R^2)
≤ 2	≤ 10	90 - 110	0.995

Results for the instrument system suitability study, listed in the table below, are all within acceptable criteria.

SS Factor	Glyphosate		Glufosinate		AMPA		N-acetylglyphosate	
LOQ (ng/mL)	0.3		0.3		0.5		0.2	
Precision (RSD)	99.1	(1.4)	99.8	(2.3)	97.7	(2.1)	102.3	(1.2)
Accuracy (R^2)	100.4	(0.9997)	104.4	(0.9996)	96.1	(0.9998)	96.6	(0.9998)

Method Collaboration

The method and collaboration protocol are described in attachments A and B, respectively. The mean, RSD, method uncertainty (MU) of the recoveries for all three spike levels (50, 250, and 500 ng/g) were determined by matrix and overall. The linearity coefficient of determination (R^2) was calculated from the concentrations found at each level for each matrix by squaring the Excel correlation function (Correl). Statistics for all matrices were calculated from the whole set of data without correction for matrix bias. Acceptable method validation specifications for the collaboration study are listed below.

Recovery: 70-120 % RSD: 15% MU: 30% R^2: 0.990

Method collaboration results contributed by PSW are summarized in the Table F1 below; results that did not meet specifications are highlighted in red font. Scatter plots of the recoveries and linearity charts are provided in attachments F_1 and F_2, respectively. All results were within the validation specifications, with the exception of the R^2 for AMPA in corn of 0.9721 was just below the 0.99 specification.

PSW Collaboration Data and System Suitability

Table F1. Summary data includes the mean, RSD, method uncertainty (MU) of spike recoveries and coefficient of determination (R^2) of the three spike levels for each matrix.

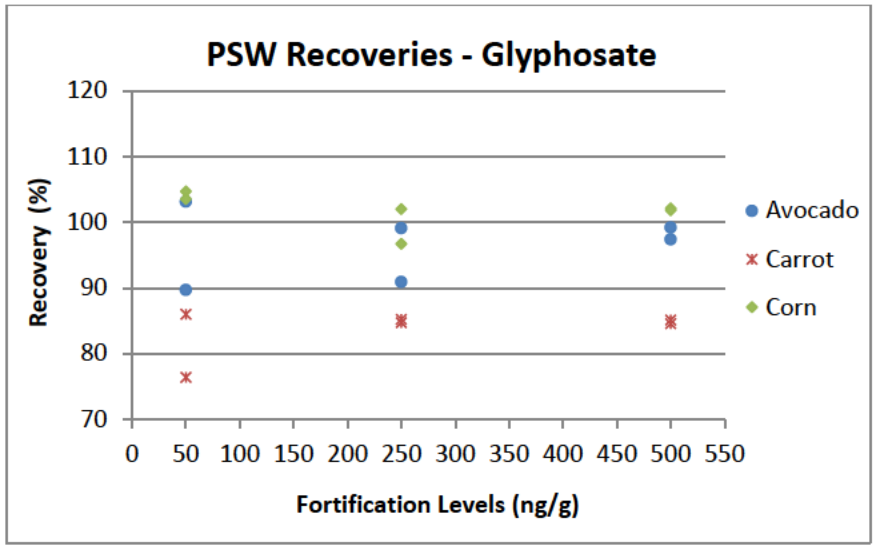
Matrix	N	Mean	RSD	MU	R^2
Glyphosate					
Avocado	6	96.6	5.4	(b) (5)	0.9982
Carrot	6	83.7	4.3	(b) (5)	0.9999
Corn	6	101.8	2.7	(b) (5)	0.9993
Glufosinate					
Avocado	6	94.4	1.8	(b) (5)	0.9998
Carrot	6	84.6	3.0	(b) (5)	0.9999
Corn	6	102.0	1.9	(b) (5)	0.9995
N-acetylglyphosate					
Avocado	6	(b) (5)	(b) (5)	(b) (5)	0.9976
Carrot	6	(b) (5)	(b) (5)	(b) (5)	0.9965
Corn	6	(b) (5)	(b) (5)	(b) (5)	0.9968
AMPA					
Avocado	6	85.9	6.3	(b) (5)	0.9971
Carrot	6	90.9	10.9	(b) (5)	0.9943
Corn	6	90.3	11.2	(b) (5)	0.9721

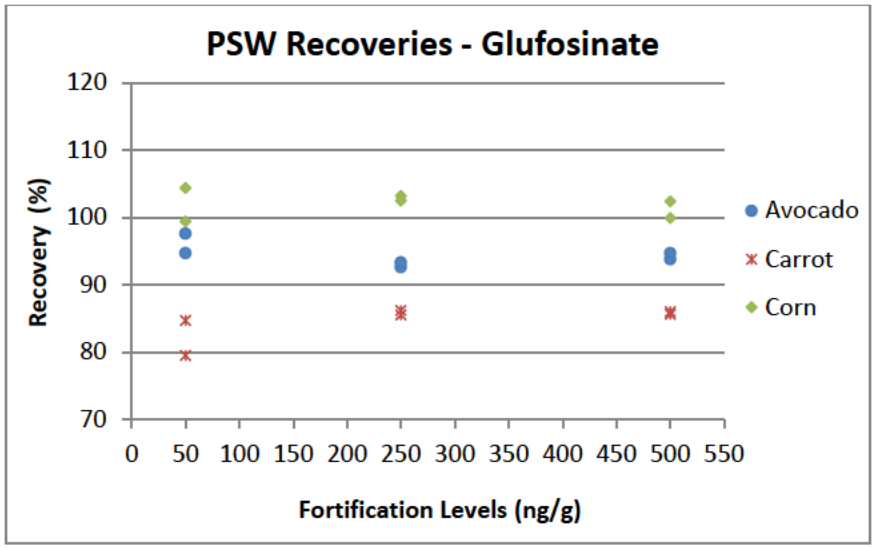
Analysis of Incurred Residues

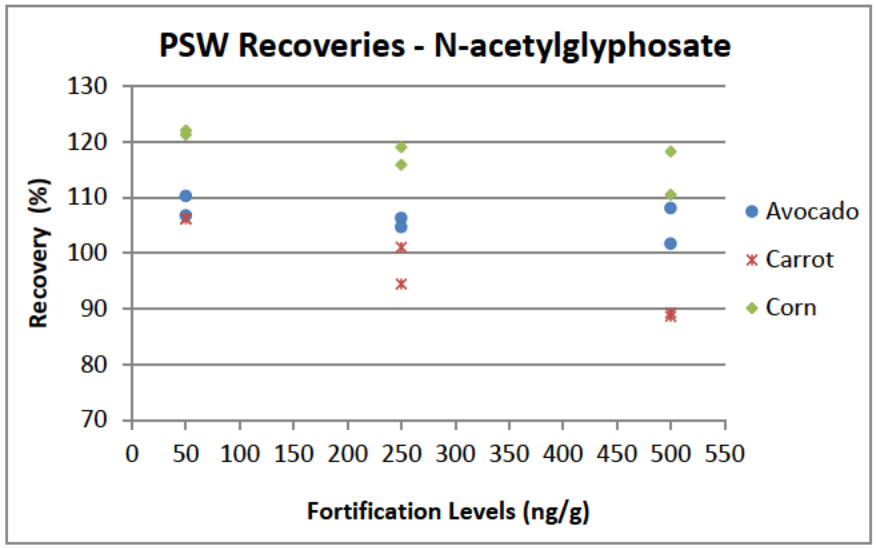
Results of the analysis of corn and soy containing incurred glyphosate residues are tabulated below. PSW findings are consistent with the range of residues levels reported from four different laboratories.

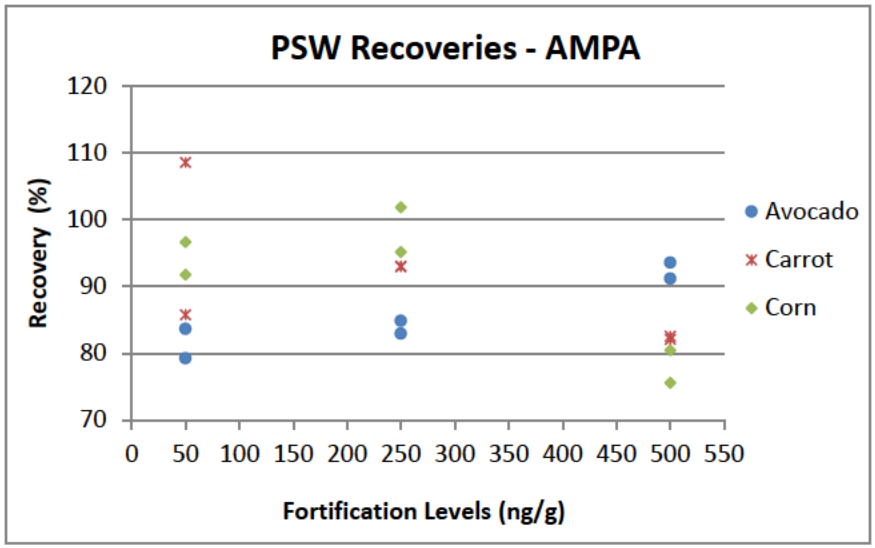
Matrix	Range	PSW
Corn	35-46	46
Soy	4290-4620	4620

PSW Collaboration Data and System Suitability – Recovery Charts

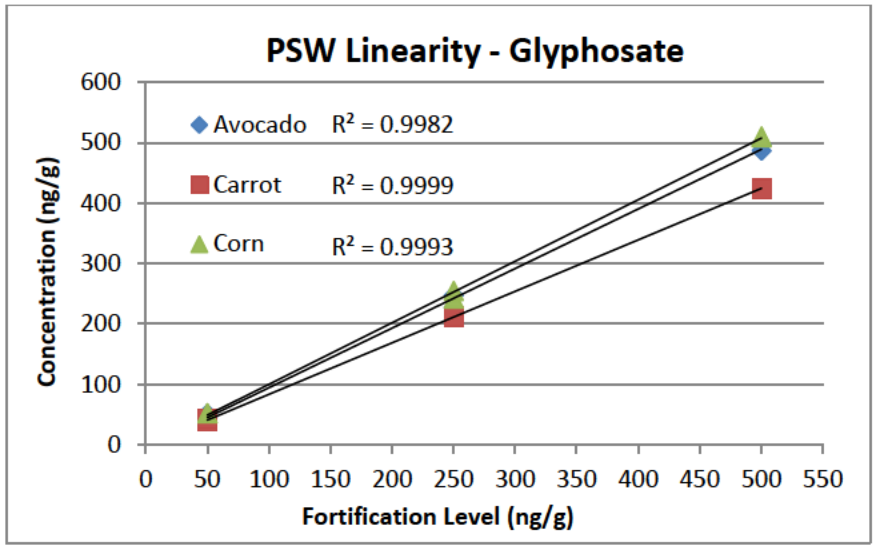


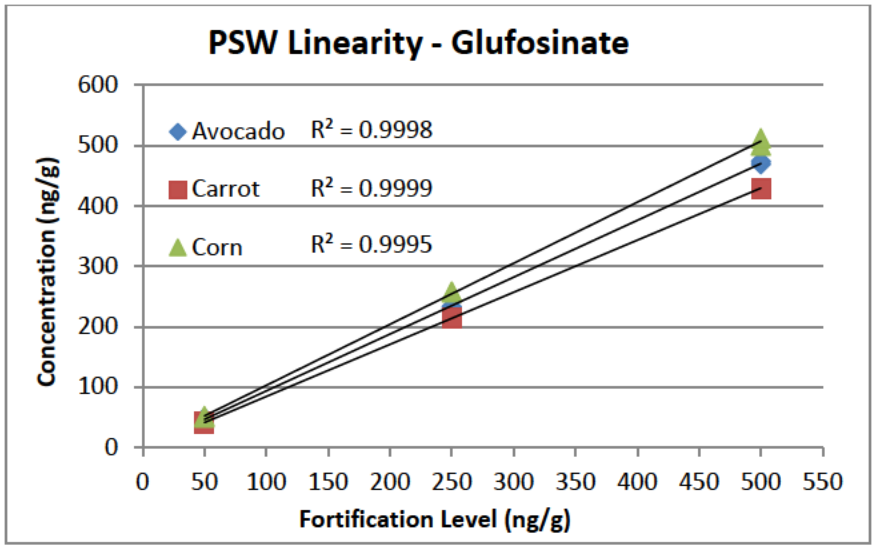


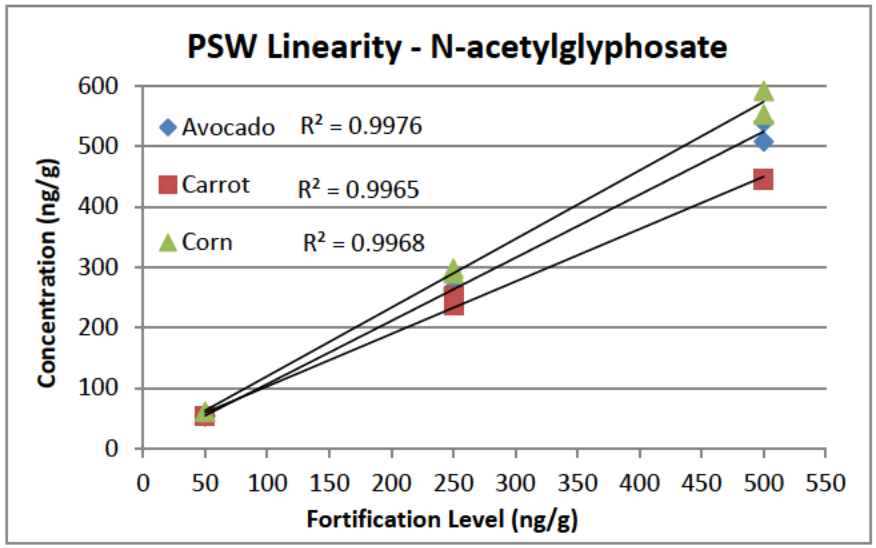


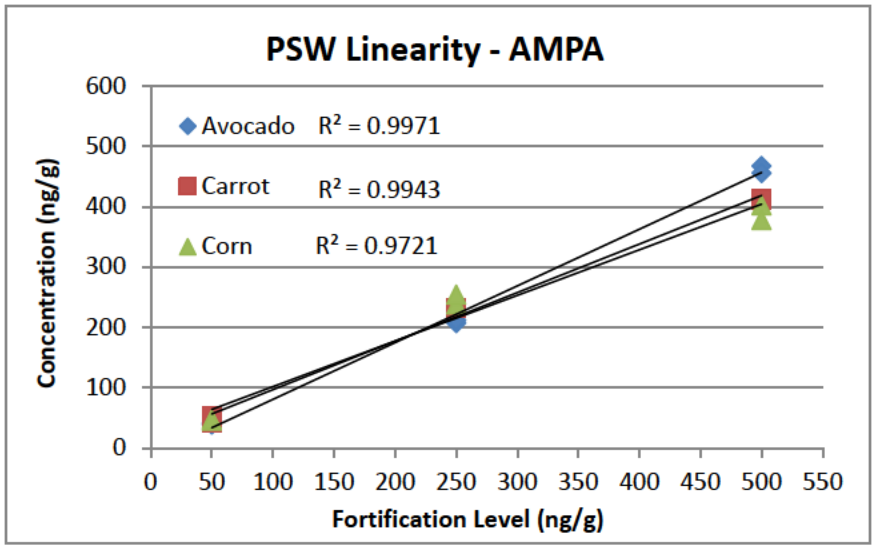


PSW Collaboration Data and System Suitability – Linearity Charts









PNW Collaboration Data and System Suitability

All data and derived statistics in this attachment are from the method collaboration analyses conducted at PNW only. Results of the instrument system suitability and method collaboration verify that PNW is able to the method proficiently.

Instrument System Suitability

Prior to starting the collaboration instrument system suitability (SS) was demonstrated. Standards were prepared and injected at concentrations of 1, 2, 5 10, 25, 50, 100, 200, 500, and 1000 ng/ml to determine accuracy and linearity; the standards at concentrations of 500 and 1000 ng/ml were not included in the accuracy and linearity calculations. Seven replicates of the 50 ng/ml standard were injected to determine precision. The instrument LOQ was determined as per ORA-LAB.10 by injecting standards at concentrations of 1, 2, 5, and 50 ng/ml and determining the S/N of the quantifier and qualifier ions. The LOQ was calculated as the lowest level where the S/N of the quantifier ion ≥ 10 and the S/N of the qualifier ion ≥ 3. Criteria for instrument system suitability are tabulated below.

LOQ (ng/ml)	Precision (RSD)	Accuracy (%)	Linearity (R^2)
≤ 2	≤ 10	90 - 110	0.995

Results for the instrument system suitability study, listed in the table below, are all within acceptable criteria with the exception of the LOQ for N-acetylglphosate at 6 ng/ml exceeded the maximum acceptable level of 2 ng/ml.

SS Factor	Glyphosate		Glufosinate		AMPA		N-acetylglyphosate	
LOQ (ng/mL)	0.4		1.4		2		6	
Precision (RSD)	98.4	(2.8)	96.2	(0.7)	96.4	(3.3)	97.2	(6.7)
Accuracy (R^2)	101	(0.9998)	99.4	(0.9999)	98.9	(0.9999)	101.1	(0.9998)

Method Collaboration

The method and collaboration protocol are described in attachments A and B, respectively. The mean, RSD, method uncertainty (MU) of the recoveries for all three spike levels (50, 250, and 500 ng/g) were determined by matrix and overall. The linearity coefficient of determination (R^2) was calculated from the concentrations found at each level for each matrix by squaring the Excel correlation function (Correl). Statistics for all matrices were calculated from the whole set of data without correction for matrix bias. Acceptable method validation specifications for the collaboration study are listed below.

Recovery: 70-120 % RSD: 15% MU: 30% R^2: 0.990

Method collaboration results contributed by PNW are summarized in the Table G1 below; results that did not meet specifications are highlighted in red font. Scatter plots of recoveries and

linearity charts for each analyte are provided in attachments G_1 and G_2. All results were within the validation specifications, with the exception of the R^2 of 0.9871 for N-acetylglyphosate, the R^2 of 0.9556 and 0.9571 for AMPA in carrot and corn, respectively, were just below the 0.99 specification. The precision and MU for AMPA in corn, 23.2 and 46.4 % also did not meet specifications of 15 and 30 %, respectively.

Table G1. Summary data includes the mean, RSD, method uncertainty (MU) of spike recoveries and coefficient of determination (R^2) of the three spike levels for each matrix.

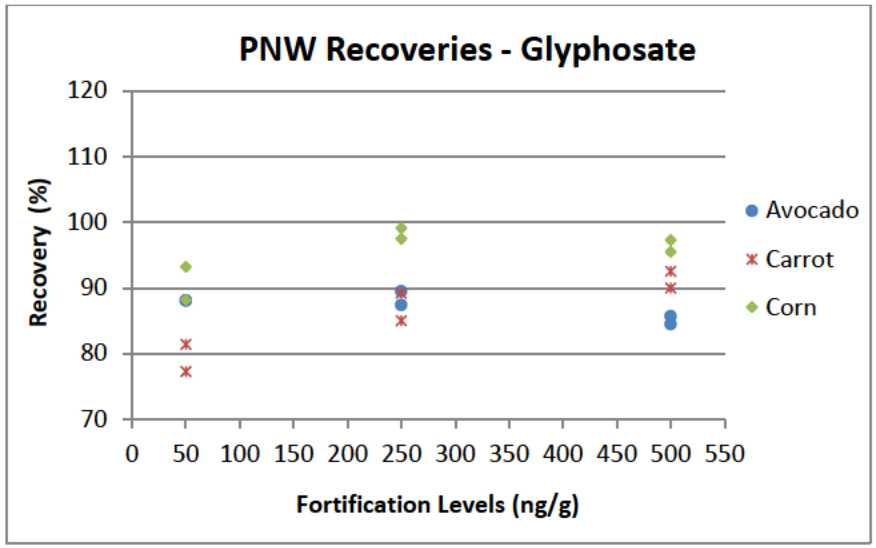
Matrix	N	Mean	RSD	MU	R^2
Glyphosate					
Avocado	6	87.2	2.1	(b) (5)	0.9992
Carrot	6	85.9	6.7	(b) (5)	0.9988
Corn	6	95.1	4.2	(b) (5)	0.9994
Glufosinate					
Avocado	6	87.0	5.1	(b) (5)	0.9925
Carrot	6	90.4	4.8	(b) (5)	0.9981
Corn	6	101.4	1.6	(b) (5)	0.9993
N-acetylglyphosate					
Avocado	6	90.3	9.0	(b) (5)	0.9871
Carrot	6	86.7	5.5	(b) (5)	0.9957
Corn	6	94.4	1.3	(b) (5)	1.0000
AMPA					
Avocado	6	87.3	5.7	(b) (5)	0.9938
Carrot	6	83.4	12.3	(b) (5)	0.9556
Corn	6	76.5	23.2	(b) (5)	0.9571

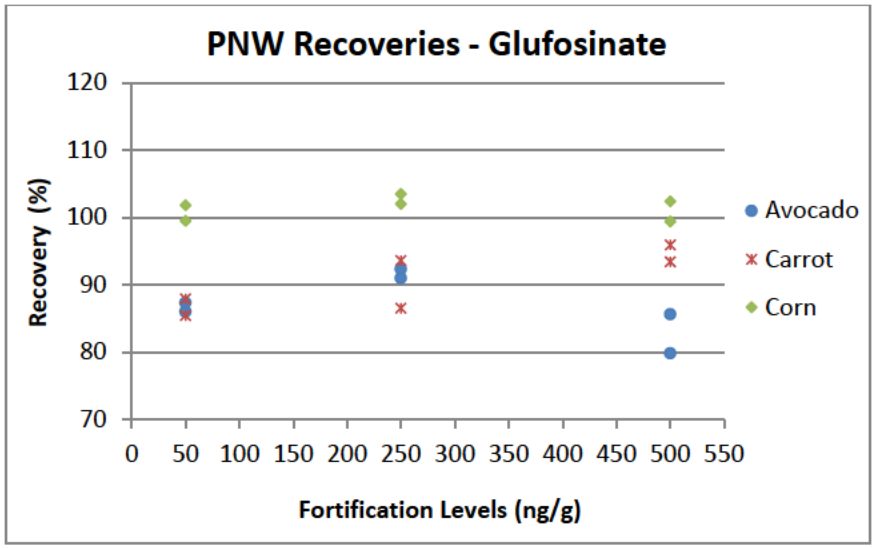
Analysis of Incurred Residues

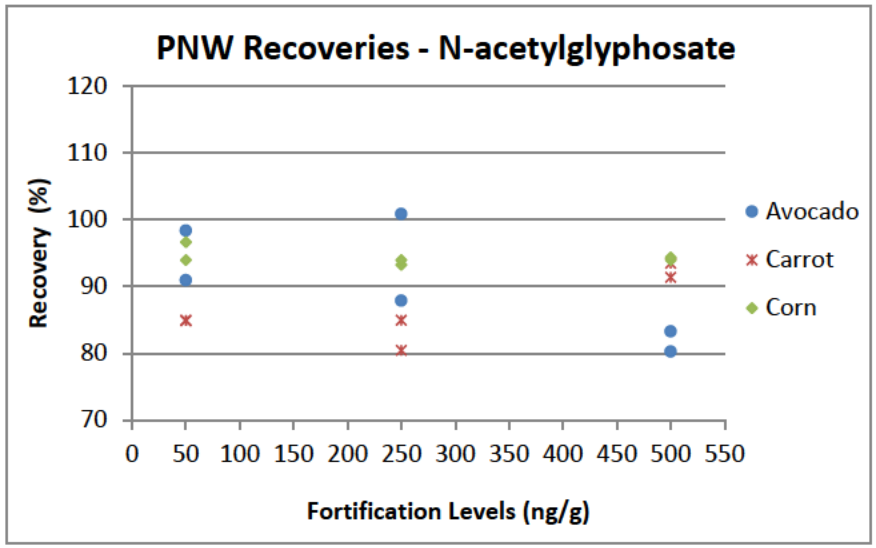
Results of the analysis of corn and soy containing incurred glyphosate residues are tabulated below. PNW findings are consistent with the range of residues levels reported from four different laboratories.

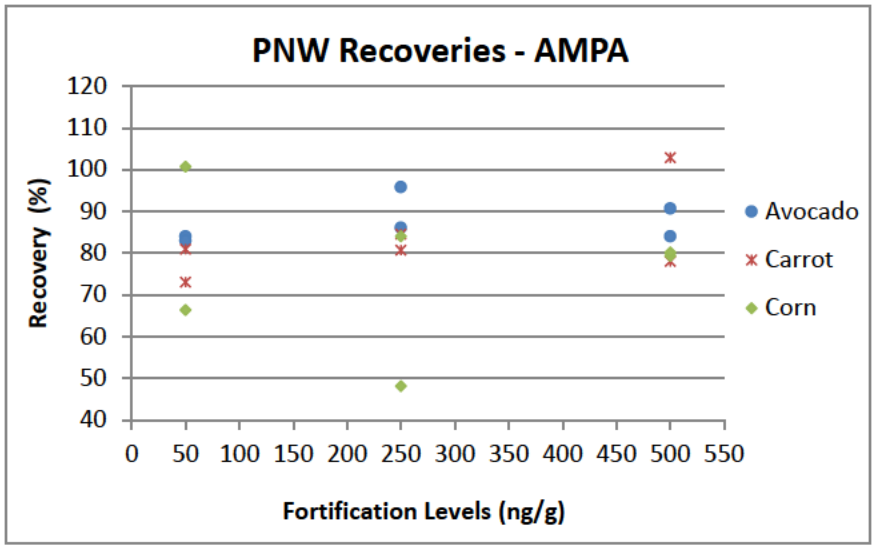
Matrix	Range	PNW
Corn	35-46	35
Soy	4290-4620	4610

PNW Collaboration Data and System Suitability – Recovery Charts

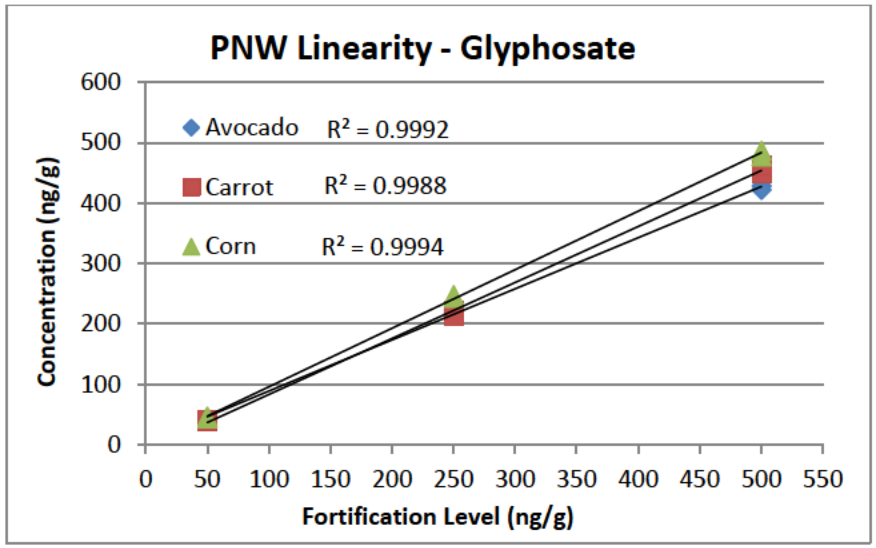


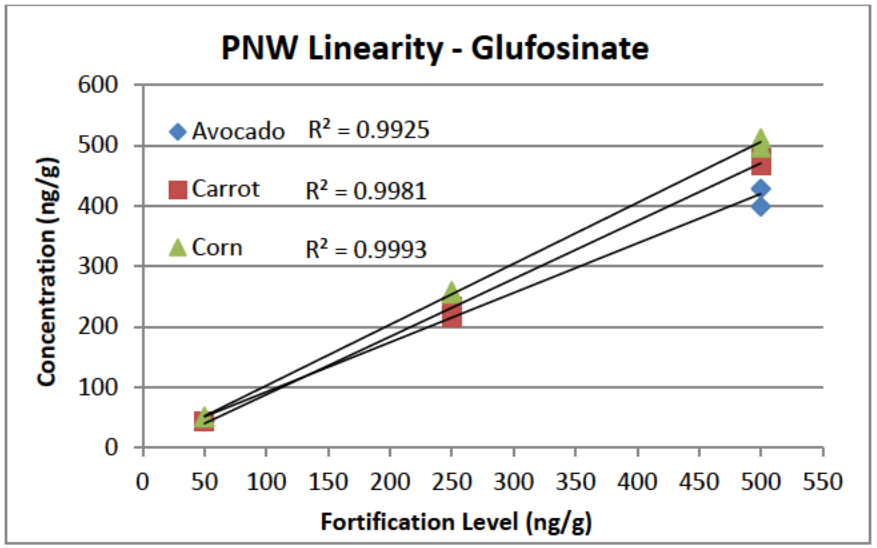


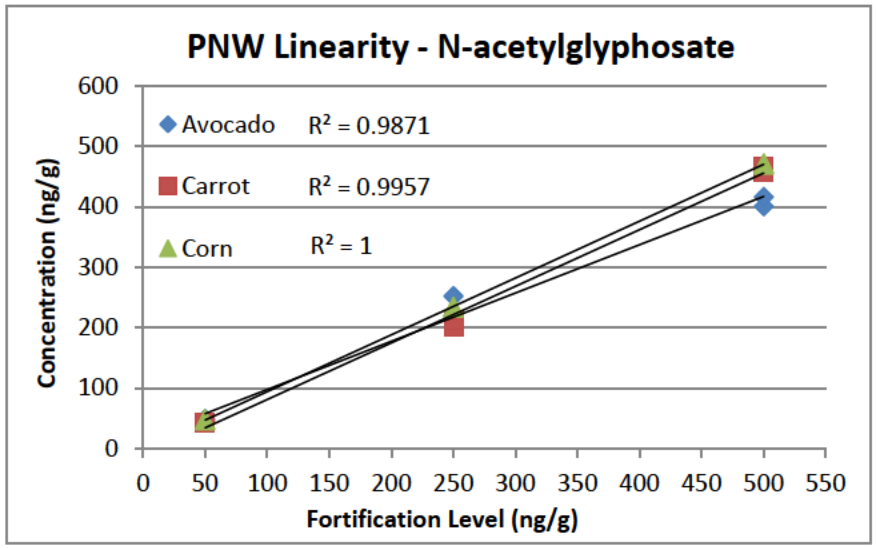


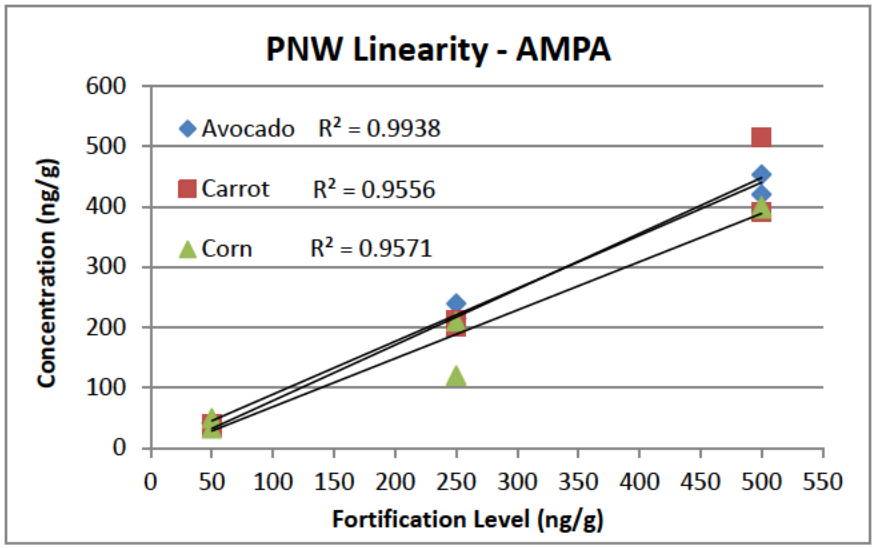


PNW Collaboration Data and System Suitability – Linearity Charts









ARL Collaboration Data and System Suitability

All data and derived statistics in this attachment are from the method collaboration analyses conducted at ARL only. Results of the instrument system suitability and method collaboration verify that ARL is able to the method proficiently.

Instrument System Suitability

Prior to starting the collaboration instrument system suitability (SS) was demonstrated. Standards were prepared and injected at concentrations of 1, 2, 5, 10, 25, 50, 100, 250, 500 and 1000 ng/ml to determine accuracy and linearity. Eight replicates of the 50 ng/ml standard were injected to determine precision. The instrument LOQ was determined as per ORA-LAB.10 by injecting standards at concentrations of 1, 2, 5, and 10 ng/ml and determining the S/N of the quantifier and qualifier ions. The LOQ was calculated as the lowest level where the S/N of the quantifier ion ≥ 10 and the S/N of the qualifier ion ≥ 3. Results for the instrument system suitability study are listed in the table below. Criteria for instrument system suitability are tabulated below.

LOQ (ng/ml)	Precision (RSD)	Accuracy (%)	Linearity (R^2)
≤ 2	≤ 10	90 - 110	0.995

Results for the instrument system suitability study, listed in the table below, are all within acceptable criteria.

SS Factor	Glyphosate		Glufosinate		AMPA		N-acetylglyphosate	
LOQ (ng/mL)	0.2		0.3		0.2		1.8	
Precision (RSD)	100.0	(1.0)	100.0	(1.0)	100.0	(1.8)	100.0	(1.7)
Accuracy (R^2)	102.8	(0.9998)	99.3	(0.9999)	106.7	(0.9996)	99.8	(0.9998)

Method Collaboration

The method and collaboration protocol are described in attachments A and B, respectively. Results from the analysis of spiked avocado, carrot, and corn matrices are summarized in Table E1. The mean, RSD, method uncertainty (MU) of the recoveries for all three spike levels (50, 250, and 500 ng/g) were determined by matrix and overall. The linearity coefficient of determination (R^2) was calculated from the concentrations found at each level for each matrix by squaring the Excel correlation function (Correl). Statistics for all matrices were calculated from the whole set of data without correction for matrix bias. Acceptable method validation specifications for the collaboration study are listed below.

Recovery: 70-120 % RSD: 15% MU: 30% R^2: 0.990

ARL Collaboration Data and System Suitability

Method collaboration results contributed by ARL are summarized in the Table H1 below; results that did not meet specifications are highlighted in red font. Scatter plots of individual recoveries and linearity charts for each matrix are provided in attachments H_1 and H_2, respectively. All results were within the validation specifications for glyphosate, glufosinate and the N-acetylglyphosate. Almost all results for AMPA failed validation specifications.

Table H1. Summary data includes the mean, RSD, method uncertainty (MU) of spike recoveries and coefficient of determination (R^2) of the three spike levels for each matrix.

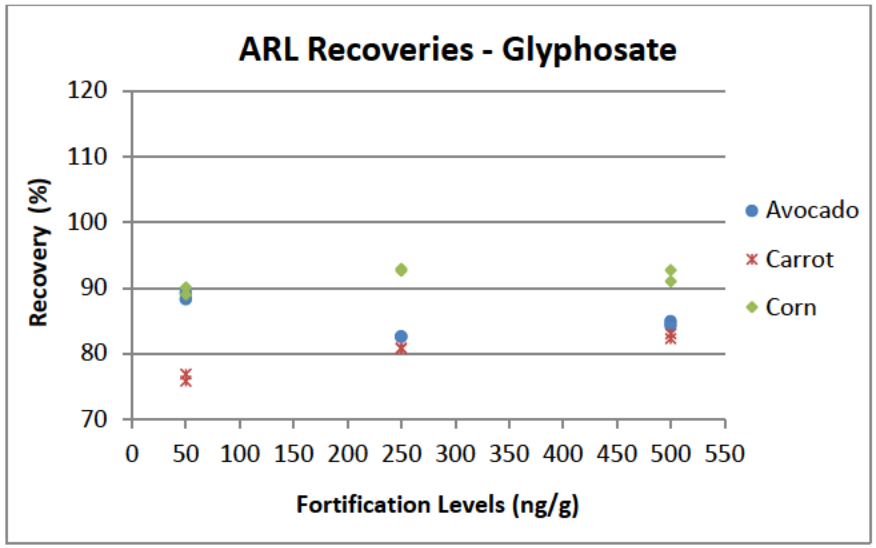
Matrix	N	Mean	RSD	MU	R^2
Glyphosate					
Avocado	6	85.3	3.3	(b) (5)	0.9996
Carrot	6	80.0	3.7	(b) (5)	0.9999
Corn	6	91.4	1.8	(b) (5)	0.9997
Glufosinate					
Avocado	6	82.9	4.2	(b) (5)	0.9987
Carrot	6	81.0	2.2	(b) (5)	0.9991
Corn	6	98.4	1.2	(b) (5)	0.9997
N-acetylglyphosate					
Avocado	6	85.7	6.1	(b) (5)	0.9975
Carrot	6	79.7	6.7	(b) (5)	0.9972
Corn	6	93.1	5.4	(b) (5)	0.9968
AMPA					
Avocado	6	48.6	30.9	(b) (5)	0.9324
Carrot	6	61.3	7.1	(b) (5)	0.9972
Corn	6	95.8	15.9	(b) (5)	0.9587

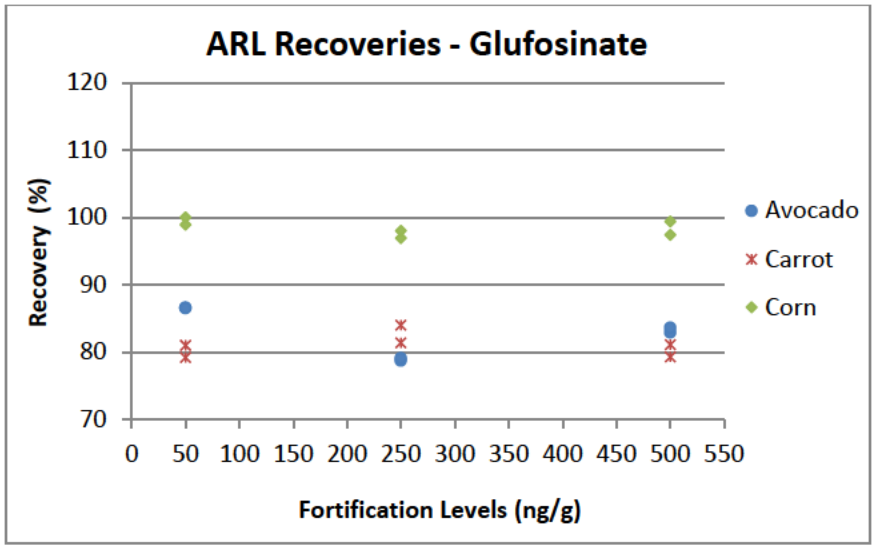
Analysis of Incurred Residues

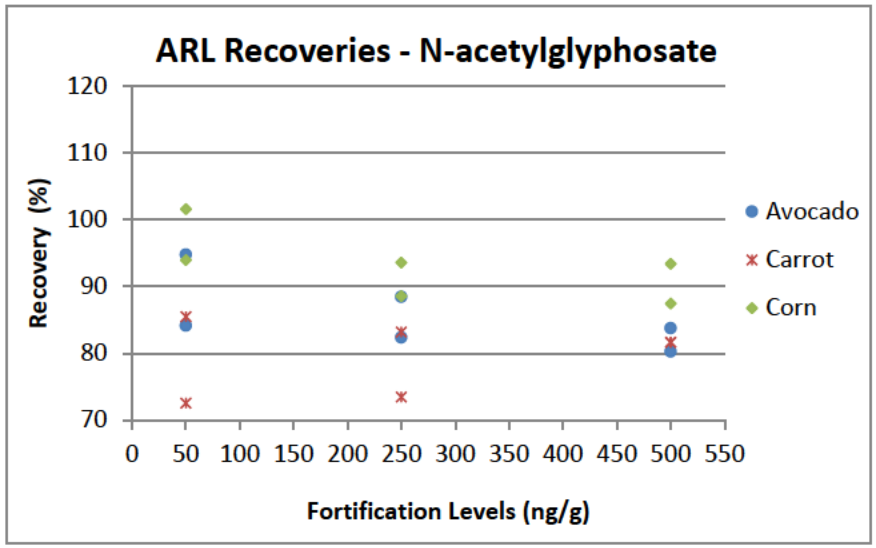
Results of the analysis of corn and soy containing incurred glyphosate residues are tabulated below. ARL findings are consistent with the range of residues levels reported from four different laboratories.

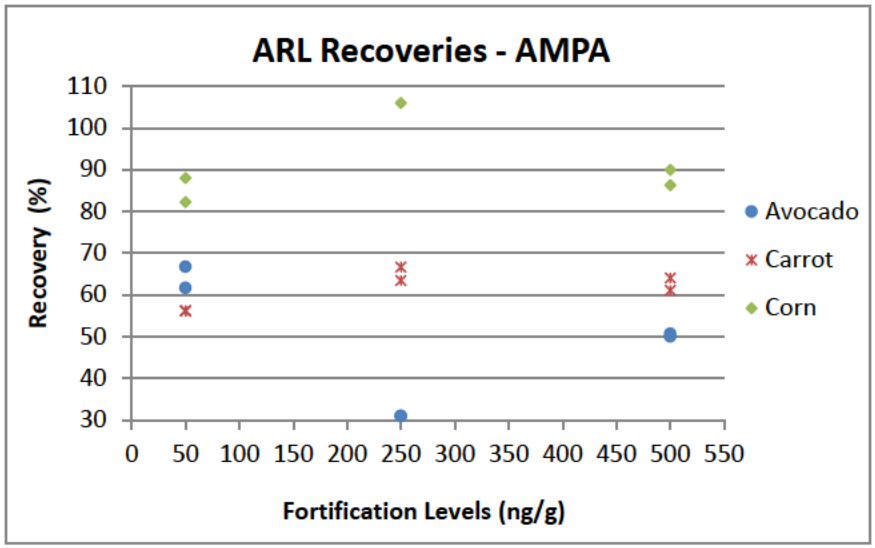
Matrix	Range	ARL
Corn	35-46	36
Soy	4290-4620	4290

ARL Collaboration Data and System Suitability – Recovery Charts

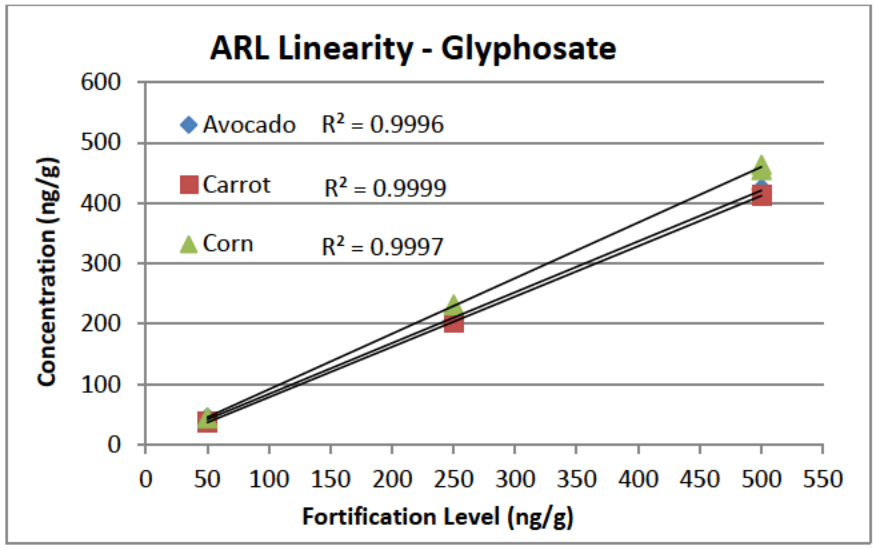


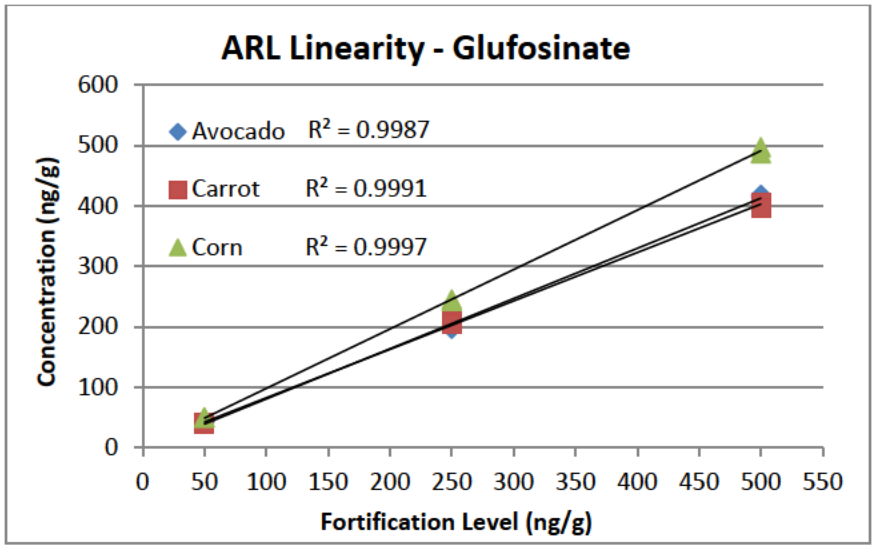


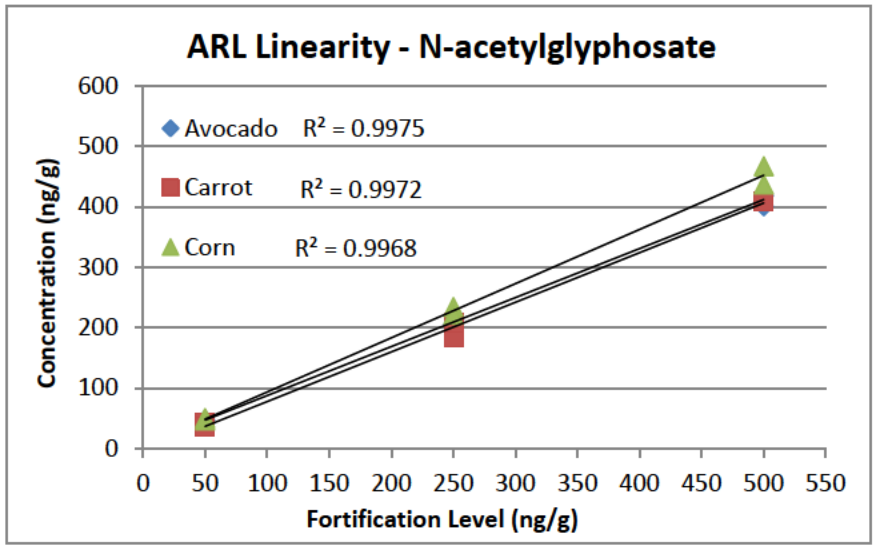


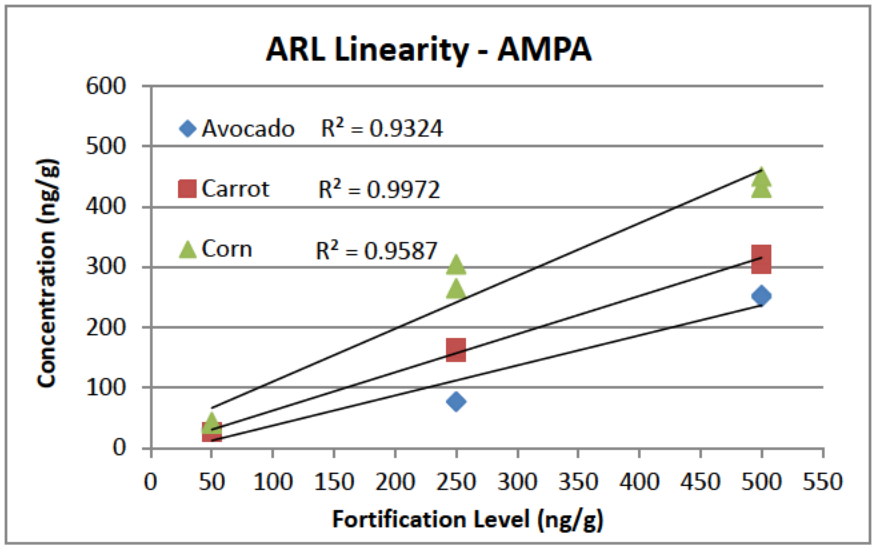


ARL Collaboration Data and System Suitability – Linearity Charts









Date: May 18, 2017

From: Consumer Safety Officer, Program Assignment and Monitoring Branch, Division of Field Programs and Guidance, Office of Compliance, CFSAN, HFS-615

Subject: AMENDED: Collection of Selected Domestic and Imported Foods for Herbicides Analysis

Priority: Routine

DFPG#: 16-08
FACTS Assignment #: 11618100
ORA Concurrence #:FF16020501

To: DIBs, DCBs and FPM: HAF 3E, HAF 6E, HAF 5E, HAF 3W, HAF 4W, HAF 6E, HAF 4E, HAF 2W, HAF 5W, HAF 1W, HAF 5E, HAF 1E, and HAF 6W
Lab Directors: KCL, PSFFL and PNL

Info: DDs: HAF 3E, HAF 6E, HAF 5E, HAF 3W, HAF 4W, HAF 6E, HAF 4E, HAF 2W, HAF 5W, HAF 1W, HAF 5E, HAF 1E, and HAF 6W

This assignment has been amended since its issuance on February 11, 2016 to include analyzing laboratory information and revised sample numbers to be collected in FY 17. The amended section has been highlighted in yellow in the word document.

Please distribute copies of this assignment to all appropriate district/regional personnel. If your district is not listed then consider this copy for informational purposes only.

NOT FOR PUBLIC DISTRIBUTION

1. General

- **Scope:**
  - Sample collection assignment to determine the presence and levels of glyphosate and acid herbicides in domestic cereal grains, corn, soybean, root/tuber vegetables, milk and eggs; and imported cereal grains, corn, soybean, and root/tuber vegetables.
  - Amount of samples collected: (b) (5)
- **Overall timeframe:** Sample collection start upon receipt of the assignment and (b) (5) (b) (5) Note: there are specific months for the collection of eggs and milk samples.
- **Analyte:** Glyphosate and Acid herbicides
- **Percentage domestic and import:** (b) (5)

- **Where to collect:** Domestic samples are to be collected at the warehouse and retail stores only. Import samples are to be collected at the port of entry.

2. Combined Interest for Investigators, Labs, and Compliance Officers

2.1 Objectives

- To determine the levels of selected herbicides (glyphosate and acid herbicides) in selected foods and generate data on the levels of herbicides in foods consumed by U.S. citizens.

- To take appropriate regulatory actions when violations of FD&C Act are found.

2.2 Background

Herbicides are widely used in the U.S. and around the world for weed control and as plant growth regulators for agricultural crops, lawns, and gardens. Herbicide active ingredients account for more than all the other types of pesticides combined, comprising over 60 % of the U.S. pesticide sales in 2007; fourteen of the top 25 most commonly used pesticides in the U.S. are herbicides. Included among them are glyphosate and the acid herbicides. Glyphosate is the most widely used pesticide in the world and the acid herbicides include five of the top 10 active ingredients used in the home and garden sector: mecoprop, dicamba, triclopyr, pelargonic acid, and 2,4-D (also one of the most commonly used pesticides in the world). Usage of 2,4-D is expected to triple in the coming year when crops genetically modified to resist it are introduced into the agrochemical market.

Most government reviews have concluded that glyphosate is relatively safe but controversy occurred recently when the International Agency for Research on Cancer (IARC) said glyphosate-containing formulations are probably carcinogenic to humans. The health effects of other herbicides include affecting the nervous system and hormone or endocrine system, and some are carcinogens.

FDA has never monitored glyphosate and the acid herbicides in its regulatory pesticide program. In its audit of the FDA's pesticide program, GAO noted that glyphosate and 2,4-D were among the most commonly used pesticides in the United States, but that FDA has rarely tested for these pesticides in its regulatory monitoring program or disclosed the fact that it does not test for these pesticides. In its response to the audit, FDA stated that it was considering whether glyphosate and 2,4-D would be added to its pesticide residue monitoring program.

2.3 Sampling Framework

Sampling Framework	
Product	cereal grains, corn, soybean, root/tuber vegetables, milk and eggs
Product Code	Barley 02A--02 Corn 02A--01 Oats 02A--03 Rice 02A--05/02A--06 Soybean 02A--10 Wheat 02A--09 Beet, garden 25J--08 Beet, sugar 25J--13 Carrot 25J--01 Potato 25J--06 Radish 25J--07 Sweet potato, yams 25J—12/25J--37 Turnip 25J--14 Peanuts 23A--07/23B--07 Egg 15A--01 (domestic samples only) Milk 09C--09 (domestic samples only)
Sample Size	No separate 702(b) portion is required. **Grains and Soybeans:** For each sample, collect 1 kg (2.2 lb.) grain sample representing the lot. **Potato, Beet, Carrot, Radish, Turnip and Sweet potato:** For each sample, collect 1 kg (2.2 lb.) or at least 10 units. **Peanuts:** For each sample, collect 0.5 kg (1.1 lb.) shelled/in shell peanuts. **Eggs:** For each sample, collect 12 (one dozen) whole chicken eggs from one lot. Domestic collection only. **Milk**: For each sample, collect 0.5 L of homogenized whole milk at retail from single lot. Domestic collection only.
Sample Type	Official
Sample Basis	Surveillance
FACTS Assignment Number	11618100
ORA Concurrence Number	ORA
DFPG Assignment #	16-08
Estimated cost per Sample	N/A

2.4 Resources and Reporting

Resources and Reporting	
Priority	Routine
Planning PAC	04F810 DSC Herbicide 03F810 DSC Emerging Issues 03F810 Entry Review 04F810 Import Field Examinations
Analytical planning PAC	04F810 DSA Herbicide
Reporting PAC	04004A
PAF	PES
Operation Code	31 DSC (Domestic Sample Collections) 41 DSA (Domestic Sample Analysis) 21 IFE (Import Field Examinations) 33 ISC (Import Sample Collections) 43 ISA (Import Sample Analysis)
Estimated accomplishment hours per– domestic sample collection **Estimated accomplishment hours per– import sample collection**	3.8 hours/operation 1.7 hours/operation
Estimated accomplishment hours per– domestic sample analysis **Estimated accomplishment hours per– import sample analysis** **Estimated accomplishment hours per– Field Examination**	5.9 hours/operation 5.4 hours/operation 4.4 hours/operation

2.5 State or External Involvement when applicable: N/A

2.6 Status Tracker

If needed, Contact Kaniz Shireen at kaniz.shireen@fda.hhs.gov.

3. Sampling Assignment and Information

3.1 Inspection Approach:

3.1.1 Import Entry Review

Review of the entry documents may be required prior to collection of a sample to determine if the product meets the criteria for collection.

Ensure proper PAC/PAF combinations are selected when creating work in OASIS. This may include updating product codes and rescreening lines. Note: If the proper PAC/PAF combinations are not available, please contact the DIO and Import Compliance Systems Branch (ICSB) contacts for this assignment.

- SAM/PES (PAC: 04004A)

Samples are to be collected from all countries of origin with the exception of U.S. Goods returned.

To the extent our current product coding system will allow, CFSAN has provided the most likely product code that fits each product. Some targeted products may not correlate with any of the available product codes. To ensure that these products are not overlooked for sampling, the districts must rely on the importer description, entry review documents or field examinations to determine if the correct product code was used.

3.1.2 Import Field Examinations

Field examination of the product labeling is necessary to determine if the product was properly declared. If examination of the product labeling indicates that the product does not meet the sampling criteria outlined in this assignment, the district may un-accomplish the sample and release the entry.

3.2 Sampling Approach

3.2.1 Import Sample Collections

- Collect samples per the normal sample collection procedure which can be found in the IOM, Chapter 6, Section 6.5, "Import Sample Collection".

Collect unprocessed single ingredient commodity only. Do not collect frozen products.

Refer to the Sampling Framework section above for sample size information.

Ship import samples to KAN-LAB only.

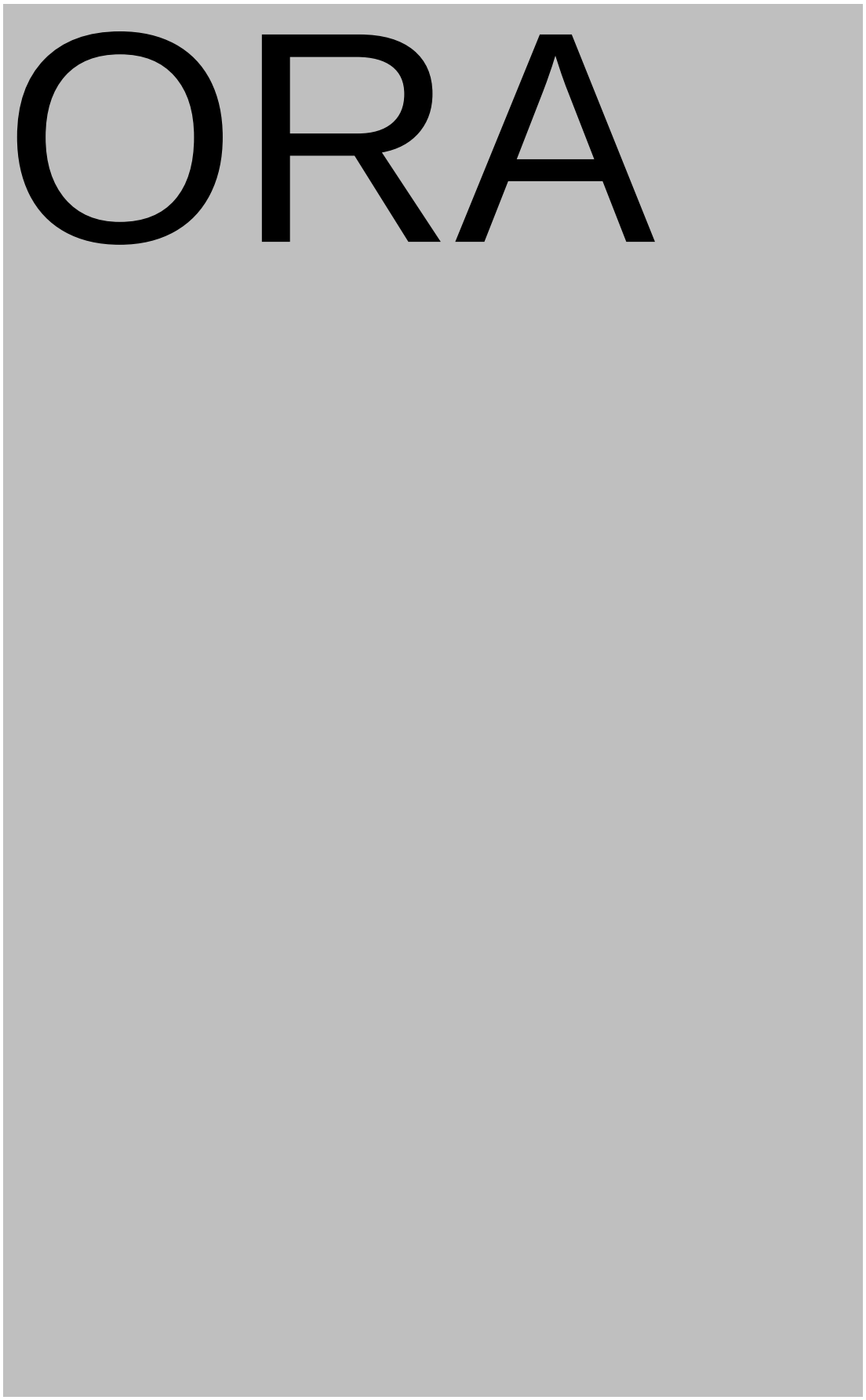
Collection Report Documentation

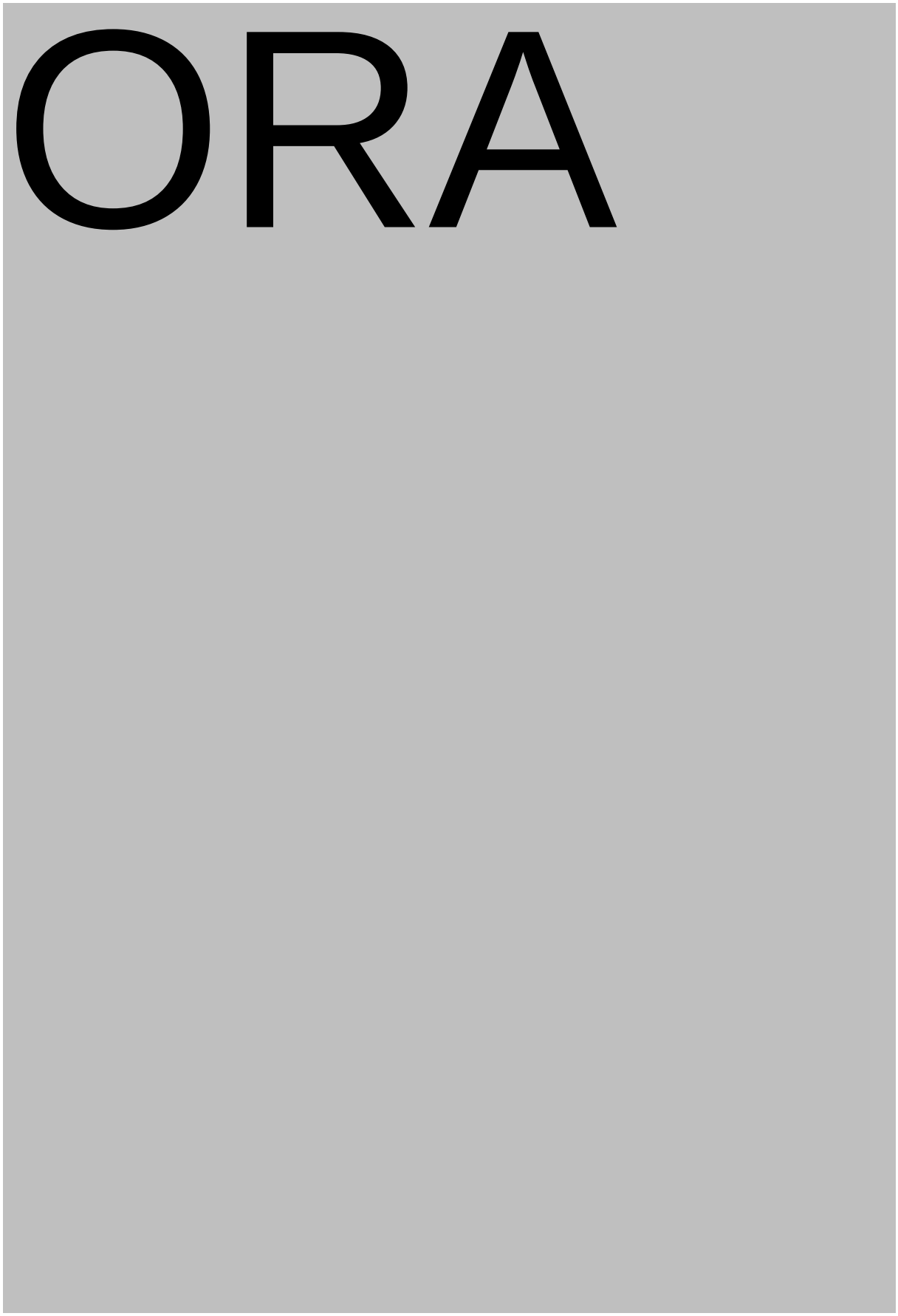
Please enter the following information in the REMARKS section of the collection report:

- "Analyze for Acid Herbicides per CFSAN/DFPG assignment #16-08."
- Contact information for district compliance so they can be contacted if the sample analysis is found to be CRO.

Example: Analyze for Acid Herbicides per CFSAN/DFPG assignment #16-08. CO Jane Doe, (123)456-7890

3.2.2 **Where to Collect:** Domestic samples are to be collected at the warehouse and retail stores only. Import samples are to be collected at the port of entry.


ORA


ORA

3.2.2 How to Collect

Samples for this assignment are surveillance in nature. Districts are requested to collect domestic samples from warehouse and retail stores. Collect only samples of commodities intended for human food. Do not collect commodities for use in animal feed.

When samples are collected from the bulk containers the sampling operations must be carried out using techniques that ensure the sample is representative of the lot.

Collect unprocessed single ingredient commodity only. **Do not collect frozen products**. For more information on sample collection technique, please see IOM subchapter 4.3.

If possible, record grower information for domestic samples in the collection report.

As we are unsure if there would be any residue in the samples collected, please ensure you wear gloves while handling samples.

Grain, Root and Tuber Samples

Fresh produce samples decompose quickly in plastic bags under warm and hot ambient conditions. Do not use plastic bags for packaging fresh produce samples.

Egg Samples

Collect domestic egg samples at retail stores. Egg samples should be securely packaged in egg cartons. Seal each carton in a plastic bag to contain any leakage. Thoroughly cushion each bag with shredded paper, bubble pack or other suitable material for shipment. Ensure that each sample is properly refrigerated. Please refer to IOM 4.5.3 – Sample Handling for additional information.

Milk Samples

Collect domestic refrigerated, non-flavored whole milk samples at retail stores. Milk samples should be stored refrigerated until ready for shipment. Milk samples should be shipped refrigerated within 48 hours of collection. Do not freeze milk samples.

3.2.3 Special Instructions for Sampling

We recommend CSOs wear gloves while handling products.

3.2.4 Sample Shipment

(b) (5)

- Ship import samples, root and tuber samples, and barley, oat, wheat and rice samples to KAN-LAB.

See IOM 4.4.10.1.11 to flag split samples.

Ship samples so that they arrive during the week. **Do not** ship on Fridays. Follow procedures in the IOM section 4.5.5.5 for notifying the lab of sample shipment.

Milk and egg samples should be shipped refrigerated. Refer to IOM section 4.5.3.6 for instruction on shipping refrigerated samples.

(b) (5)

3.3 Special Instructions for Evidence development

N/A

3.3.1 Considerations

N/A

3.3.2 Additional Evidence

N/A

3.3.3 Additional Documentation

Document for interstate commerce

Record grower information for domestic samples.

3.3.4 When to Contact Other FDA Offices

N/A

3.3.5 Contact External to FDA

N/A

4. Lab

4.1 Method

(b) (5)

- LIB 4592 “Analysis of Acid Herbicides Using Modified QuEChERS with FastSwitching ESI+/ESI- LC-MS/MS Determination”.

All confirmation and quantitation should comply with specifications set forth in ORA-LAB.10. (Note: Laboratories may use abbreviated worksheets for NAI sample).

4.2 Analyte

This assignment is testing for glyphosate and Acid herbicides.

4.3 Special Instructions for Labs

(b) (5)

The laboratory shall maintain a portion of the composited sample as the 702(b) portion.

4.4 Communication and Reporting

4.4.1 Analyzing Laboratory:

- Communicate confirmed positive results to ORACFSANSurveillanceSamples@fda.hhs.gov and the home district with following information:
  - Firm name
  - FEI
  - Sample number
  - Analyte
  - Commodity
  - Collecting District

- Release analytical results if necessary (704(d) Letter) to the dealer listed in the Remarks field of the Collection Report in accordance to FMD-147

4.4.2 Collecting District:

- Notify the firm (Importer, broker, etc.) of analytical results.

5. Enforcement Information for Compliance Officers

5.1 Findings

5.1.1 Analytical Specific Results

Level below which no action needs to be taken: All residue findings below 0.01 ppm and/or below established tolerance levels for the pesticide/commodity combination.

Levels between which require Center review and concurrence: Levels found between 0.01 ppm and 0.05 ppm require Center review and concurrence.

Levels for which the district has Direct Reference Authority: When residues are found at or above the established tolerance level if one exist, or above 0.05 ppm for the pesticide/commodity combination for which EPA has not established a tolerance level.

(Note: If Finding is above the Tolerance (LC3), then Laboratories should be provided a full violative package).

5.1.2 Other Unique Assignment Factors

N/A

5.2 Possible Charges

5.2.1 Domestic

Charge Adulteration Section 402(a)(2)(B).

5.2.2 Imports

"The article is subject to refusal of admission pursuant to Section 801(a)(3) in that it appears to contain a pesticide chemical, namely _____, which is in violation of Section 402(a)(2)(B)."
OASIS charge code - PESTICIDE2

5.3 Actions

5.3.1 Initial Actions

Domestic Foods: District should consider either meeting with the grower/shipper to discuss corrective action, or issuing a warning letter when following-up on each sample classified as "Lab Class 3". The most effective way to remove adulterated food with pesticides from domestic channels has been through voluntary recalls. Where voluntary recall actions are not effective, consider seizure if there is a seizable size lot under embargo or voluntary hold.

Imported Foods: Recommendations for center review of a detained shipment for refusal and detention without physical examination involving pesticides residues should be forwarded to CFSAN's Division of Enforcement, Food Adulteration Assessment Branch, HFS-607 and DIO, HFC-172.

5.3.2 Firm Information

N/A

5.3.3 Compliance Activities

- FDA response to findings based on the considerations, charges, and initial actions identified: See above
- Who will drive the follow-up action: FDA
- Actions for Field: See Direct Reference Authority, refer to CPG 575.100
- Under what circumstances positive finding leads to no regulatory action by the FDA: N/A
- Additional regulatory activities for this particular assignment: N/A.

Finding	Considerations	Charges	Evidence to Support Charges	Potential actions	Link
		N/A			

5.4 Follow Up

5.4.1 Non-Regulatory Follow Up

Immediately notify the regional EPA office when investigation of a domestic food reveals possible misuse of pesticides. Information on regional EPA offices are available online. The link is http://www2.epa.gov/aboutepa/visiting-regional-office

5.4.2 Follow Up with Firm

Discuss with CFSAN compliance officer before following up with firm.

6. Contacts—*this information is intended for anyone executing portions of this assignment*

6.1 Frequently Asked Questions

N/A

6.2 Project Coordinator Contact Information

If your question cannot be found on the FAQ list or requires an immediate response, please direct any questions to the appropriate Project Coordinator for this assignment listed below.

Note that for regulatory purposes, Investigators should first reach out to Project Coordinators and Compliance Offices should reach out to the Enforcement Contacts.

Project Coordinator Name	Email Address	Phone	Project Coordinator Organization
Kaniz Shireen	kaniz.shireen@fda.hhs.gov	240-402-2775	**CFSAN OC/DFPG**
Chris Sack	Chris.sack@fda.hhs.gov	240-402-2464	**CFSAN OFS**
Rina Vora	Rina.Vora@fda.hhs.gov	562-256-9292	**ORA OHAFO**
Sam Rudnitsky	Samuel.Rudnitsky@fda.hhs.gov	562-256-9214	**ORA OHAFO**
Michael Pasternak	Michael.Pasternak@fda.hhs.gov	301-796-5932	**ORA OHAFO**
Mark Preciados	Mark.Preciados@fda.hhs.gov	301-348-1821	**ORA OHAFO**
Moh (Mohammed) R. Islam	Mohammed.Islam@fda.hhs.gov	240-402-0552	**ORA OHAFO**
Enforcement Contacts Name	**Email Address**	**Phone**	**Enforcement Contacts Organization**
Standra Purnell	Standra.Purnell@fda.hhs.gov	240-402-1613	**CFSAN DE**

7 Attachments

7.1 List of firms by district and instructions for substituting firms N/A

7.2 Seasonality information N/A

7.3 District and lab sampling schedule (See section 3 above)

7.4 Detailed lab methodologies (See section 4 above)

7.5 Phone and email addresses for contacts (only needed if assignment involves states)

FDA Glyphosate Method

A. Reagents and Supplies

1. Acetonitrile, HPLC grade
2. Petroleum ether
3. Methylene chloride
4. Water, HPLC grade
5. Formic acid, 98% solution
6. Acetic acid
7. Ammonium formate
8. Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA)
9. Tetrabutylammonium hydroxide (TBAOH) titrant, 0.4 M in Water, HPLC Grade, ACROS Organics
10. Tetrabutylammonium acetate (TBuAA), Aldrich No. 335991-10G (optional)
11. Tetrabutylammonium acetate 1 M (TBuAA 1M), Aldrich No. 401803 – 50 ML (optional)
12. 50-mL plastic centrifuge tubes
13. Filter, 2 µm, 25 mm,
14. Waters Oasis HLB SPE, 60 mg, 3cc, 30 µm
15. Extraction solvent (50 mM acetic acid/10 mM Na_2EDTA): mix 2.9 mL acetic acid and 3.7 g Na_2EDTA in 1000-mL of purified water.
16. 50 ng/ml IS fortified extraction solvent: dilute IS 20 µg/ml mixed isotope internal standard, prepared in step C.2.a, 1:400 using extraction solvent, prepared in step A.15, e.g. 2.5 ml (IS 20 µg/ml) to 1000 ml extraction solvent
17. Mobile phase A (4 mM tetrabutylammonium formate)
    a. Add 10.0 ml of 0.4 M TBAOH to ~900 mL HPLC water, and adjust the pH to 2.8±0.05 using formic acid (~ 3 ml). OR
    b. Add 1.20 g TBA acetate in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL). OR
    c. 4 ml 1M TBuAA in 1 L HPLC water; and adjust the pH to 2.8±0.05 using formic acid (~2 mL).

B. Standard Reference Materials

1. Glyphosate
2. Glufosinate
3. AMPA
4. N-acetyl-glyphosate, available from EPA and Toronto Research Chemicals (TRC No A178245)
5. Glyphosate-^{13}C
6. Glufosinate-D^3

C. Standard Solutions

1. General instructions
    a. Unless otherwise indicated prepare standards in DI water
    b. Store standard solutions in plastic containers because glass can leach standard reference material from solution. Use of glass volumetric flasks for standard preparation is OK if solution is removed from the glassware after preparation.
    c. Do not store standards prepared with water or aqueous media in the freezer.

2. Stock standards 1 mg/ml
   a. Includes all native and isotopic standards listed in Section B
   b. Prepare individual stock standard for each compound
3. Isotopic working solutions
   a. IS 20 µg/ml mixed isotope internal standard
      i) Combine isotopes Glyphosate-^{13}C and Glufosinate-D^3 (step B.5 & 6)
      ii) Dilute 1 mg/ml stock isotope internal standards, prepared in step C.2, 1:50
4. Intermediate mixed standards
   a. 50 µg/ml mixed native standard
      i) Combine native 1 mg/ml stock standards, prepared in step C.2
      ii) Include glyphosate, glufosinate, AMPA, and N-acetyl-glyphosate (Step B.1-4)
      iii) Dilute 1:20
   b. 5.0 µg/ml mixed native standard
      i) Dilute 50 µg/ml mixed standard, prepared in step C.4.a, 1:10
   c. 1.0 µg/ml mixed native standard
      i) Dilute50 µg/ml mixed standard, prepared in step C.4.a, 1:50
5. LC-MS/MS calibration standard 50 ng/ml
   a. Dilute 5.0 µg/ml mixed native standard, prepared in step C.4.b, 1:100, using 50 ng/ml IS fortified extraction solvent (A.16)

D. **Equipment and Instrumentation**
1. Genogrinder
2. Centrifuge
3. Pipettes
4. LC-MS/MS
   a. Shimadzu HPLC system: two LC-20AD pumps, Sil-20AC autosampler, CTO-20AC column oven
      NOTE: Replace all metal LC tubing with PEEK tubing between the autosampler and injection valve because glyphosate can be retained on metal surfaces.
   b. AB model 5500, or 6500, Q-TRAP mass spectrometer
   c. HPLC column: Phenomenex Luna C8(2), 100 Å, 5 µm, 150 x 4.6 mm, Phenomenex 00F-4249-E0
   d. HPLC guard column: Phenomenex guard column KrudKatcher P/N AFO-8497

      NOTE: Install peek tubing between the autosampler and column because metal can affect glyphosate and glufosinate chromatography

E. **Extraction Procedure**
1. 5 g sample + 25 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15
   For dry products containing less than 50 % moisture: 2 g sample plus 10 ml 50 ng/ml IS fortified extraction solvent prepared in step A.15 for dry products
2. Add 10 ml PE, or $MeCl_2$, as needed for fatty or dirty matrices
3. Shake @ 1000 for 10 min
4. Centrifuge at $\geq$ 3000 rpm for 5 min
5. Filter aqueous extract thru HLB SPE cartridge, limit filter volume to less than 2 mls.
6. Filter for injection (could be included with SPE step)

7. Sample concentration: 0.2 g/ml

F. **LC-MS/MS method**

LC Parameters		Gradient	
Column:	Phenomenex Luna C8(2), 150 x 4.6 mm, 5 µm OR Phenomenex Luna C8, 150 x 2 mm, 5 µm, with Phenomenex KrudKatcher guard column	**Time**	**MPB**
MP A:	4 mM tetrabutlyammonium formate + 0.1 % formic acid in water (pH 2.8±0.05)	0.00	5
MP B:	MeCN	1.00	5
Flow:	0.45 mL/min (4.6 mm column)	5.00	90
	0.3 mL/min (2.0 mm column)	7.00	90
Inj Vol:	10 µL	8.00	5
Temp	40 °C	14.00	5

MS/MS Parameters (5500 & 6500)

Q1	Q3	RT	Transition	DP*	EP	CE	CXP
110	63	1.3	AMPA 1	-40	-11	-30	-9
110	79	1.3	AMPA 2	-40	-11	-34	-9
110	81	1.3	AMPA 3	-40	-11	-34	-9
112	63	2.5	AMPA IS	-60	-11	-26	-9
180	63	3.0	Glufosinate 1	-60	-11	-66	-9
180	95	3.0	Glufosinate 2	-40	-11	-19	-5
180	85	3.0	Glufosinate 3	-60	-11	-25	-9
183	63	3.0	Glufosinate IS	-60	-11	-40	-9
168	63	4.4	Glyphosate 1	-30	-11	-28	-9
168	79	4.4	Glyphosate 2	-30	-11	-56	-9
168	150	4.4	Glyphosate 3	-30	-11	-16	-9
171	63	4.4	Glyphosate IS	-30	-11	-28	-9
210	150	5.3	N-acetyl glyphosate 1	-20 (-40)	-11	-20	-13
210	63	5.3	N-acetyl glyphosate 2	-20 (-40)	-11	-40	-13
210	168	5.3	N-acetyl glyphosate 3	-20 (-40)	-11	-18	-13

**DP: if more than one DP is provided the first is optimized for the 6500 and the DP in () is optimized for the 5500*

MS Parameters	
CUR	35

FDA Glyphosate Method

CAD	MEDIUM
IS	-4000
GAS 1	65
GAS 2	65
TEM	450 °C (6500) 650 °C (5500)
Q1	UNIT
Q3	UNIT

U. S. Food and Drug Administration

Laboratory Information Bulletin

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

Narong Chamkasem, *SRL, Atlanta, GA*
Cynthia Morris, *SRL, Atlanta, GA*
Tiffany Harmon, *SRL, Atlanta, GA*

ABSTRACT

A simple high-throughput liquid chromatography/tandem mass spectrometry (LC-MS/MS) method was developed for the determination of glyphosate, glufosinate and aminomethylphosphonic acid (AMPA) in soybean and corn using a reversed-phase with weak anion-exchange and cation-exchange mixed-mode Acclaim™ Trinity™ Q1 column. Two grams of sample were shaken with ten milliliters of water containing ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) and acetic acid for 10 min to precipitate protein. After centrifugation, the supernatant was passed thru an Oasis HLB SPE to retain suspended particulates and non-polar interferences. The sample was directly injected and analyzed in 6 min by LC-MS/MS with no sample concentration or derivatization steps. Two multiple reaction monitoring (MRM) channels were monitored in the method for each target compound to achieve true positive identification. Three internal standards corresponding to each analyte were used to counter matrix suppression effect. Linearity of the detector response with a minimum coefficient of determination (R^2) of more than 0.995 was demonstrated in the range of 10 to 1000 ng/mL for each analyte.

INTRODUCTION

Glyphosate (N-phosphonomethyl glycine) and glufosinate [ammonium(S)-2-amino-4-[hydroxyl (methyl) phosphinoyl] butyrate] are non-selective post emergence herbicides used for the control of a broad spectrum of grasses and broad-leaf weed species in agricultural and industrial fields. AMPA is the major metabolite of glyphosate and also classified as a toxicologically significant compound (1). According to recent reports, there has been a dramatic increase in the usage of

these herbicides which are of risk to both human health and the environment (2). Glyphosate and glufosinate have high efficacy, low toxicity and an affordable price, when compared with other pesticides. These factors lead to its wide utilization on several crops. Farmers also use glyphosate as a desiccant to rapidly kill above ground growth of crops such as wheat. This allows for rapid dry down for easy harvest. Due to the low toxicity of glyphosate, the maximum residues levels (MRLs) established around the world are generally greater than the limits for other pesticides. According to FDA (40CFR180.364 and 40CFR180.364), the tolerance of glyphosate for soybean and corn are 20 and 5 µg/g and the tolerance of glufosinate in soybean and corn are 2 and 0.2 µg/g (3). However, some crops such as wheat and oats do not have a tolerance for glyphosate. Therefore, any glyphosate detected above the limit of quantification would be violative. A quick, accurate, and sensitive method to determine these herbicides in food grains must be developed to support the regulatory actions.

Glyphosate, glufosinate, and AMPA are very polar compounds and insoluble in organic solvents. These properties make the use of classical organic solvent extraction very difficult. Alferness and Iwata used an aqueous extraction method to extract glyphosate and AMPA from soil, plant and animal matrices (4). This method required the use of lengthy cleanup procedures that involved both anion and cation exchange columns. Typical silica based reversed-phase C18 columns experience difficulty with the retention of such polar compounds, and may generate non-resolved co-eluting peaks, often with polar analytes eluting in the void volume. The lack of chromophophore or fluorophore also necessitates the use of derivatization techniques for the determination of these analyte residues by liquid chromatography and gas chromatography (5-7). Vreeken and co-workers developed an analytical method to analyze glyphosate, AMPA and glufosinate in water samples using a reversed phase liquid chromatography separation after pre-column derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) and detection by LC-MS/MS (8). Schreiber and Cabrices streamlined the derivatization by using a special autosampler for automation to determine these polar analytes in corn and soybean (9). The derivatization technique is not highly regarded by analysts as it requires the optimization of a number of parameters (temperature, reaction time, concentration and purity of the reagents, laboratory handling time). Anion exchange, Hydrophilic Interaction Liquid Chromatography (HILIC), and mixed-mode columns were used with LC-MS/MS to determined underivatized glyphosate and other polar pesticides in food matrixes with limited success (10,11,12).

This LIB describes a single laboratory validation of an LC-MS/MS method under a negative ion-spray ionization mode for the direct determination of glyphosate, glufosinate, and AMPA in soybean and corn. It also explains a quick and reliable extraction method that requires small sample size, non-toxic solvent, and an effective sample cleanup procedure to ensure a rugged, sensitive, and selective method.

MATERIAL AND METHODS

Chemicals and Materials

Pesticide standard (≥ 99% purity) were purchased from LGC Standards (Manchester, NH) consisting of glyphosate, AMPA, glufosinate, glyphosate $^{13}C2^{15}N$ (100 µg/mL), AMPA ^{13}C ^{15}N (100 µg/mL), and glufosinate D3. Methanol, acetonitrile, and water of HPLC grade were

obtained from Fisher Scientific (Pittsburgh, PA). Formic acid was obtained as 98% solution for mass spectrometry from Fluka (Buchs, Switzerland.). Acetic acid, Ammonium formate and Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) were purchased from Fisher Scientific (Pittsburgh, PA). Extracting solvent (50 mM acetic acid/10 mM Na_2EDTA) was prepared by mixing 572 µL of acetic acid and 0.74 g of Na_2EDTA in 200-mL of purified water. Oasis HLB (60 mg) solid phase extraction cartridge was obtained from Waters (Milford, MA). EDP 3 electronic pipettes at different capacities (0-10 µL, 10-100 µL, and 100-1000 µL) were purchased from Rainin Instrument LLC (Oakland, CA) and were used for standard fortification.

A solution of 500 mM ammonium formate/formic acid (pH 2.9) was prepared as follows: 15.76 g of ammonium formate were dissolve in approximately 300 mL of HPLC water and adjusted with 98% formic acid (approx. 28.3 mL) until the pH reached 2.9 (using pH meter), and the solution was diluted to 500 mL with water. The HPLC mobile phase was prepared by mixing 100 mL of the 500 mM buffer solution with 900 mL of purified water so the final concentration was 50 mM.

Standard Preparation

The stock solution of glyphosate, glufosinate, and AMPA at 50, 10, and 1 µg/mL were prepared by dissolving the stock standard in 1:1 water:methanol solution. The solutions were maintained at 4 °C in polypropylene tubes to avoid adsorption to glass. The internal standard (IS) solution of glyphosate $^{13}C2^{15}N$, AMPA $^{13}C^{15}N$, and glufosinate D3 at 2 and 10 µg/mL were prepared by dissolving the stock standard in 1:1 water:methanol solution. The calibration standards were prepared in the extracting solvent or blank matrix extract (after SPE cleanup) with IS solutions for the calibration curves as described in Table 1.

Sample Preparation and Extraction Procedure

Organic soybean and corn were obtained from a local market. The samples were ground with a food processor until they had powder-like texture. The samples were weighed at 2 g each in 50-mL centrifuge tubes (Fisher Scientific, Pittsburgh, PA) and fortified with native standard solutions at 0.1, 0.5 and 2 µg/g (7 replicates) using Table 2. The IS solution (100 µL) at the concentration of 10 µg/mL was added into the samples so the concentration was 0.5 µg /g for all samples. The samples were allowed to stand at room temperature for 1 hour and then stored in a freezer overnight to let the analytes to be absorbed by the sample. A set of five non-fortified samples without IS were also prepared and used for matrix matched standard. On the extraction day, the spiked samples were allowed to thaw to room temperature. The extracting solvent (10 mL) was added to each tube using an automatic pipette. The tubes were capped tightly and shaken for 10 min on a SPEX 2000 Geno grinder (SPEX Sample Prep LLC, Metuchen, NJ) at 1000 stroke/min then centrifuged at 3,000 x g for 5 min using a Q-Sep 3000 centrifuge (Restek, Bellefonte, PA). Three milliliters of the supernatant were passed through an Oasis HLB cartridge (60 mg), previously conditioned with 2 mL methanol and 2 mL of the extracting solvent, and the last milliliter of the extract was collected into an autosampler vial. A 10 µL volume of sample was injected into the LC-MS/MS system.

LC-MS/MS Analysis

LC-MS/MS analysis was performed by using a Shimadzu HPLC system. The instrument was equipped with two LC-20AD pumps, a Sil-20AC autosampler, and a CTO-20AC column oven (Shimadzu, Kyoto, Japan), coupled with a 5500 Q-TRAP mass spectrometer from AB SCIEX (Foster City, CA). The Analyst software (version 1.6) was used for instrument control and data acquisition. Nitrogen and air from TriGas Generator (Parker Hannifin Co., Haverhill, MA) were used for nebulizer and collision gas in LC-MS/MS. An Acclaim™ Trinity™ Q1 (3 µm, 100 x 3 mm) from Thermo Scientific (Sunnyvale, CA) and a C18 SecurityGuard guard column (4 x 3 mm) from Phenomenex (Torrance, CA) were used for HPLC separation at 35 °C with sample injection volume of 10 µL. The mobile phase is 50 mM ammonium formate (pH 2.9) at a flow rate of 0.5 mL/min for a total run time of 6 min. The MS determination was performed in negative electrospray mode with monitoring of the two most abundant MS/MS (precursor/product) ion transitions using a scheduled MRM program of 60 seconds for each analyte. Analyte-specific MS/MS conditions and LC retention times for the analytes are shown in Table 3. The MS source conditions were as follows: curtain gas (CUR) of 30 psi, ion spray voltage (ISV) of -4500 volts, collisionally activated dissociation gas (CAD) is high, nebulizer gas (GS1) of 60 psi, heater gas (GS2) of 60 psi, source temperature (TEM) of 350 ºC.

RESULTS AND DISCUSSION

Chromatography Optimization

Glyphosate, glufosinate, and AMPA possess negative charges in aqueous solution that make them difficult to be retained by a reversed-phase column. Several mixed phase mode columns containing reversed-phase, anion and cation exchange properties were evaluated for use in the study. They were a) Obelisc R (SIELC Technologies, Wheeling, IL), zwitterionic-type mixed mode, b) Scherzo SM-C18 (Imtakt USA, Philadelphia, PA), mixed beads of cation and anion exchange particles, and c) Nanopolymer Silica Hybrid , Acclaim™ (Thermo Scientific, Sunnyvale, CA). Among the Acclaim™ columns, three different columns were also evaluated. They are Acclaim™ Trinity™ P1 (strong cation, weak anion/reversed-phase), Acclaim™ Trinity P2 (weak cation, strong anion/HILIC), and Acclaim™ Trinity™ Q1 (week cation, weak anion/reversed-phase). Since these columns have both cation and anion exchange properties, they are the ideal columns for the analysis of both cationic charge pesticides (paraquat, diquat, mepiquat, chlormequat, amitrole, and daminozide) and anionic charge pesticides (glyphosate, AMPA, glufosinate, forsetyl alumina, ethephon, and maleic hydrazide). The idea is to use a single column to determine all these very polar pesticides with one LC-MS/MS instrument.

After a lengthy column evaluation period, it was found the columns with strong cation exchange functionality would strongly retain paraquat and diquat. Therefore, they were not considered as the column of choice. Different mobile phase parameters were evaluated which included pH (2.8 to 5), acetonitrile concentration (0 – 100%), and salt concentration (0 – 100 mM). The best column so far was the Acclaim™ Trinity™ Q1 which provided good peak shape and reasonable retention for all analytes. The most important parameter was the pH of the mobile phase. At low

pH (2.9), glyphosate eluted well while paraquat and diquat were strongly retained. At higher pH (3.5), glyphosate was a late eluter with a wide and tailing peak shape while paraquat and diquat had good peak shape. Therefore, two analyses on a single column should be done isocratically with two different mobile phases. Higher acetonitrile content in the mobile phase enhanced sensitivity and increased the retention time of the analytes. If too high, the acetonitrile content in the mobile phase resulted in very broad and late-eluting glyphosate peak at pH 2.9. High salt concentration shortened the retention time of the analytes and decreased analyte response due to ion-suppression. All of these three parameters must be chosen appropriately to achieve optimal separation and peak sensitivity for the target analytes.

It was found that the mobile phase containing 50 mM ammonium formate (pH 2.9) at a flow rate of 0.5 mL/min for the Acclaim™ Trinity™ Q1 (3 µm, 100 x 3 mm) produced the optimal conditions for peak shape, retention time, and sensitivity for these three analytes.

Optimization of Sample Extraction Procedure

For high protein sample such as soybean, protein precipitation is a common protocol for rapid sample clean-up and extraction (13). An organic solvent and acid have been used for effecting protein precipitation by exerting specific interactive effects on the protein structure. An organic solvent lowers the dielectric constant of the protein solution and also displaces the ordered water molecules around the hydrophobic regions on the protein surface, the former enhancing electrostatic attractions among charged protein molecules and the latter minimizing hydrophobic interactions among the proteins. Acidic reagents form insoluble salts with the positively charged amino groups of the proteins at pH values below their isoelectric points. EDTA was used to improve extracting efficiency of tetracycline in milk (14,15,16). Aqueous solution containing 50 mM acetic acid/10 mM Na_2EDTA was successfully used in extracting glyphosate, glufosinate, and AMPA in milk sample with recovery over 90% (17). Acetic acid lowered the pH of the sample to precipitate the protein and Na_2EDTA prevented chelation complex between polyvalent metal ions in the sample and the analytes.

Lecithin is a phospholipid found in soybeans that could be extracted along with the analytes in aqueous solution. They may accumulate at the head of the analytical column under high aqueous mobile phase condition and degrade column performance. Therefore, the Oasis HLB cartridge was added to the method to filter the aliquot and trap the phospholipid and other non-polar compounds in the final extract. Special cleanup cartridges specifically designed for phospholipids such as Captiva (Agilent Technology, Santa Clara, CA) and HybridSPE-plus (Supelco, Bellefonte, PA) were also evaluated with poor recovery because glyphosate and glufosinate have phosphate functional groups similar to those in phospholipids.

To evaluate the optimal extraction time, a soybean sample containing incurred residue of glyphosate (~10 µg/g) was put in five 50-mL plastic centrifuge tubes and 10 mL of the extracting solvent was added into each tube. The tubes were shaken on the SPEX 2000 Geno grinder at 1000 stroke/min at 2, 5, 10, 30, and 60 min, and then centrifuged at 3000 x g for 5 min using the Q-Sep 3000 centrifuge. The supernatant was passed thru an Oasis HLB cartridge (60 mg), previously conditioned with 2 mL methanol and 2 mL of the extracting solvent, and the last milliliter of the extract was collected into an autosampler vial. Ten microliters of the sample

extract were injected into the LC-MS/MS system. The results showed that there was no significant difference in glyphosate concentration in sample extract after the samples were shaken at 5, 10, 30, and 60 min. At 2 min of shaking, the concentration of glyphosate was approximately 70% of the sample shaken at 5 min. This suggested that five minute was long enough to extract glyphosate effectively. However, the ten minutes extraction time was chosen as the optimum extraction time for this method.

Evaluation of Matrix Effects

Matrix effect (%ME) in the sample extract was calculated as the slope of calibration curve of analyte in sample matrix divided by the slope of calibration curve of analyte in solvent and multiplied by 100 (Figure 1) . Therefore, a value of 100% means that no matrix effect is present. If the value is less than 100%, it means that there is matrix suppression. If the value is more than 100%, it means that there is matrix enhancement. Table 4 shows the %ME of all three analytes in both matrices. Glyphosate had minimum degree of suppression (95-101 %) in both matrices, while AMPA had severe suppression (17- 30%). Glufosinate has less % ME in soybean (74%) than in corn (92%). Based on this data, IS may not be needed for glyphosate and glufosinate analysis in soybean and corn (reduces the cost of analysis). However, it is necessary to use IS for AMPA analysis to correct for matrix suppression.

Method Validation

The calibration standard solutions at concentrations from 10 to 1000 ng/mL were prepared in both sample matrices (soybean and corn) and extracting solvent with the addition of IS (Table 1). These standard solutions were injected along with the fortified samples and sample blank as described in the Table 2. For comparison purposes, four different quantification methods were used to determine the accuracy and precision of the recovery results. They were a) standard in matrix with internal standard calibration method, b) standard in matrix with external calibration method, c) standard in solvent with internal standard calibration method, and d) standard in solvent with external standard calibration method (Table 5). The linearity was evaluated and they showed satisfactory linearity with coefficient of determination (R^2) of more the 0.995. The specificity of the method was evaluated by analyzing reagent blank, blank sample and blank sample spiked at the lowest fortification level (0.1µg/g). No relevant signal (above 30%) was observed at any of the transitions selected in the blank sample. A reagent blank was injected immediately after the 1000 ng/mL standard and no analyte signals were detected above 10% of the 10 ng/mL standard.

The method detection limit (MDL) for each compound was calculated according to FDA guidelines with 7 replicates of the lowest calibration standard (10 ng/mL). The MDL was calculated by multiplying standard deviation of 7 replicates with t value at a degree of freedom of 6 (3.14). By using matrix matched standard with IS, the MDL for glyphosate, glufosinate, and AMPA were 2.3, 2.3, and 4 ng/mL for soybean sample and 2.0, 4.8, and 5.5 ng/mL for corn sample, respectively. The method quantification limit (MQL) was three times the MDL which were 6.9, 6.9, and 11.9 ng/mL for soybean and 5.9, 14.4, and 16.5 ng/mL for corn, respectively.

Accuracy (recovery %) and precision (relative standard deviation or RSD %) were evaluated at the fortification levels of 0.1, 0.5, and 2 ng/g in seven replicates in both soybean and corn samples (Table 7 and 8) using all 4 calibration methods. For glyphosate and glufosinate, the average recovery using a) standard in matrix with internal standard calibration method, b) standard in matrix with external calibration method, and c) standard in solvent with internal standard calibration method was in the range of 92-104% with the RSD of less than 6 %. The calibration of standard in solvent without the IS had average recovery ranged from 96-98% with the RSD of less than 5% for glyphosate. However, it had average recovery range from 75-76 % with the RSD of less than 5%. This demonstrates that glyphosate can be effectively extracted from the sample and does not have significant matrix suppression. External standard calibration without the IS can used to accurately quantify glyphosate in these samples. On the other hand, IS should be used to accurately quantify glufosinate to compensate for the matrix suppression

The recovery of AMPA using calibration curve without IS in both matrices were very low due to matrix suppression as expected from the results in Table 4. The calibration curve from matrix match standard (without IS) improves the recovery of AMPA somewhat, but it is still less than 70%. AMPA was eluted near the solvent front where polar interferences in the matrix were present. The concentration of these interferences was not predictable depending upon the type of matrix. Therefore, the IS (AMPA ^{13}C ^{15}N) should be used to accurately quantify AMPA in these samples. The recovery of AMPA using IS in sample matrix and in solvent were in the range of 96-113% with the RSD of less than 12% in both matrices. Therefore, standard in solvent with IS may be used for the quantification of AMPA to save time and cost of analysis.

Chromatograms of glyphosate, glufosinate, and AMPA in soybean blank and soybean blank fortified at 0.1 µg/g are shown in Figures 3 and 4. Chromatograms of glyphosate, glufosinate, and AMPA in corn blank and corn blank fortified at 0.1 µg/g are shown in Figures 5 and 6. No significant inferences were observed the blank sample where the analytes were eluted. The Acclaim™ Trinity Q1 combined reverse-phase, weak anion, and weak cation exchange properties in one column. This column retained glyphosate, glufosinate, and AMPA by the ion-exchange mechanism similar to the previous work done by Hao et. al. on the Acclaim™ WAX-1 column (9). However, a lower concentration of salt in the mobile phase (50 mM ammonium formate) at a much lower pH, significantly improved peak shape and sensitivity with simple isocratic elution. The column was rugged and gave good peak shape and retention time reproducibility over 100 injections of sample matrix without the need for column reconditioning as previously recommended by Hao and coworkers.

A soybean sample and a corn sample collected from the market that contained incurred residue were analyzed by this method. The soybean sample contained 11 ppm of glyphosate and 4.9 ppm of AMPA (Figure 7). The corn sample contained 6.5 ppm of glyphosate and 0.065 ppm of AMPA (Figure 8). There was no glufosinate detected above 0.03 ppm in either sample.

CONCLUSION

This work describes a ten-minute extraction with aqueous solution of acetic acid and Na_2EDTA which allows a rapid and direct determination of glyphosate, glufosinate, and AMPA residue in soybean and corn samples. Acetic acid precipitates soluble protein (major interference) from the

sample extract while Na_2EDTA prevents the analytes from forming a chelation complex with polyvalent metal. Oasis HLB SPE is used to filter the sample extract and trap the phospholipids and other non-polar compounds. The SPE cleanup step is used to maintain HPLC column performance and minimize matrix concentration in the final extract. The mixed-mode Acclaim™ Trinity™ Q1 HPLC column allows the analytes to be retained on the column and separated from each other without a derivatization step. These analytes were commonly derivatized before HPLC analysis to improve their chromatographic retention in reversed-phase LC. Negative mode ion-spray with MS/MS measurement gives excellent sensitivity and selectivity that produce distinct chromatographic peaks with minimal interference. Severe matrix effect on AMPA was clearly observed because it co-eluted with other polar interferences near the solvent front. The use of isotope-labeled AMPA eliminates the matrix suppression problem and provides accurate quantification.

The proposed method is quick, rugged, selective, and sensitive enough to determine glyphosate, glufosinate and AMPA in soybean, corn and other food grains at or above the 50 ng/g level . It can be used as an alternate method to the traditional FMOC-bases methods which require tedious and time-consuming derivatization and concentration steps.

LITERATURE CITED

1) EUROPEAN COMMISSION HEALTH & CONSUMER PROTECTION DIRECTORATEGENERAL. Review report for the active substance glyphosate. http://ec.europa.eu/food/plant/protection/evaluation/existactive/list1_glyphosate_en.pdf (accessed Jan 27, 2015)

2) United States EPA 2007 Pesticide Market Estimates http://www.epa.gov/opp00001/pestsales/07pestsales/table_of_contents2007.htm (accessed Jan 1, 2015)

3) http://www.ecfr.gov/cgi-bin/text-idx?c=ecfr&sid=f41eea8cfec706a8f961b47685450107&tpl=/ecfrbrowse/Title40/40cfr180_main_02.tpl (accessed Jan 27, 2015)

4) Alferness, P.L.; Iwata Y. Determination of glyphosate and (aminomehtyl) phosphonic acid in soil, plant and animal matrices, and water by capillary gas chromatography with mass selective detection. *J. Agric. Food Chem.* **1994**, 42, 2751-2759

5) Qian, K.; Tang, T.; Shi, T.; Li, P.; Cao, P. Solid-phase extraction and residue determination of glyphosate in apple by ion-pairing reverse-phase chromatography with pre-column derivatization . *J. Sep Sci*. **2009,** 32(4), 2494-2300

6) Ibanez, M, P; Ozo, O.J.; Sancho, J.V.; Lopez, F.J.; Hernandez, F. Residue determination of glyphosate, glufosinate and aminomethylphosphonic acid in water and soil samples by liquid chromatography coupled to electrospray tandem mass spectrometry . *J. Chromatogr A*. **2005**, 1081 (2), 145-155.

7) http://www.crl-pesticides.eu/library/docs/srm/meth_QuPPe.pdf (accessed Jan 27, 2015)

8) Vreeken, R. J.; Speksnijder, P.; Bobeldijk-Pastorova, I. & Noij, Th. H. M. (1998). Selective analysis of the herbicides glyphosate and amonimethylphosphonic acid in water by on-line solid-phase extraction-high-performance liquid chromatography electrospray ionization mass spectrometry. **1988** *Journal of Chromatography A*, 794, 1-2, 187-199,

9) Schreiber, A.; Cabrices, O.G. Automated derivatization, SPE cleanup and LC-MS/MS determination of glyphosate and other polar pesticides : application note 8013813-01 http://www.newfoodmagazine.com/wp-content/uploads/Glyphosate_QTRAP-4500_Gerstel_AB-SCIEX_8013813-01.pdf

10) Hao, C.; Morse, D.; Morra, F.; Zhao, X.; Yang, P.; Nunn B. Direct aqueous determination of glyphosate and related compounds by liquid chromatography/tandem mass spectrometer using reversed-phase and weak anion-exchange mixed-mode column. *J. Chromatogr* A. **2011,** 1218 (33), 5638-5643.

11) http://www.crl-pesticides.eu/library/docs/srm/meth_QuPPe.pdf (accessed Jan 27, 2015)

12 Botero-Coy, A.M.; Ibanez, M.; Sancho, J.V.; Hernandez, F. Direct liquid chromatography–tandem mass spectrometry determination of underivatized glyphosate in rice, maize and soybean. *J. Chromatogr* A. **2013,** 1313 (1), 157-165

13) Helio A. Martins-Júnior, Daniel T. Lebre, Alexandre Y. Wang, Maria A. F. Pires and Oscar V. Bustillos (2011). Residue Analysis of Glyphosate and Aminomethylphosphonic Acid (AMPA) in Soybean Using Liquid Chromatography Coupled with Tandem Mass Spectrometry, Soybean - Biochemistry, Chemistry and Physiology, Prof. Tzi-Bun Ng (Ed.), ISBN: 978-953-307-219-7,

14) Neuvonen, P.J. Interactions with the absorption of tetracycline. *Drugs* **1976,** 11(1), 45-54

15) Bruno, F.; Curini, R.; Di Corcia, A.; Nazzari, M.; Pallagrosi, M. An original approach to determining traces of tetracycline antibiotics in milk and eggs by solid-phase extraction and liquid chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry* **2002,** 16(4), 1365-1376

16) Pena, A.; Lino, C. M.; Silveira, M. I. Determination of oxytetracycline, tetracycline and chlortetracycline in milk. *JAOAC Int*. **1999**, *82*, 55-60.

17) Chamkasem, N.; Harmon, T.; Morris, C. Direct determination of glyphosate, glufosinate, and AMPA in milk by liquid chromatography/tandem mass spectrometer. *Laboratory Information Bulletin* U.S. Food and Drug Administration, Office of Regulatory Affairs, Rockville, MD (pending).

Table 1. Preparation of Calibration Standard.

sample extract or extracting solvent (µL)	425	425	425	425	425	425	425
extracting solvent (µL)	45	37.5	25	0	37.5	25	0
pesticide mix 1 µg/mL (µL)	5	12.5	25	50	0	0	0
pesticide mix 10 µg/mL (µL)	0	0	0	0	12.5	25	50
IS 2 µg/mL (µL)	25	25	25	25	25	25	25
total volume (µL)	500	500	500	500	500	500	500
IS concentration (ng/mL)	**100**	**100**	**100**	**100**	**100**	**100**	**100**
final concentration (ng/mL)	**10**	**25**	**50**	**100**	**250**	**500**	**1000**

Table 2. Preparation of fortified samples (for each 2 g of sample and a final volume of 10 mL)

fortification level	standard mix	standard mix	IS mix 10 µg/mL	expected conc.
(µg/g)	10 ng/ µL (µL)	50 ng/ µL (µL)	(µL)	in the extract (ng/mL)
0	0	0	**100**	**0**
0.1	20	0	**100**	**20**
0.5	100	0	**100**	**100**
2.0		80	**100**	**400**

Table 3. Retention time and MRM conditions for LC-MS/MS analysis.

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	DP	CE	EP	CXP	Retention Time (min)
AMPA.1	110	63	-60	-24	-10	-10	1.1
AMPA.2	110	79	-60	-26	-10	-10	1.1
AMPA $^{13}C^{15}N$ (IS)	112	63	-60	-24	-10	-10	1.1
Glufosinate.1	180	95	-46	-23	-10	-10	1.65
Glufosinate.2	180	85	-46	-26	-10	-10	1.65
Glufosinate D3 (IS)	183	63	-46	-26	-10	-10	1.65
Glyphosate.1	168.2	63	-110	-30	-10	-10	2.05
Glyphosate.2	168.2	79	-110	-55	-10	-10	2.05
Glyphosate $^{13}C2^{15}N$ (IS)	171	63	-110	-30	-10	-10	2.05

Compound dependent parameters: DP = declustering potential, CE = collision energy, EP = entrance potential, CXP = collision cell exit potential

Table 4. Matrix effect evaluation soybean (using calibration curve with linear fit)

Soybean

	Slope of cal. curve in solvent	Slope of cal. curve matrix	Matrix effect (%ME)
glyphosate	772	731	95
glufosinate	755	562	74
AMPA	1499	258	17

Corn

	Slope of cal. curve in solvent	Slope of cal. curve matrix	Matrix effect (%ME)
glyphosate	812	823	101
glufosinate	779	718	92
AMPA	1516	455	30

Table 5. Linear regression of the calibration curve (1/x weighing) using four different methods (soybean).

Analyte	Calibration curve type	Slope	intercept	coefficient of determination (R2)
glyphosate	Matrix with IS	0.0156	0.0392	0.9995
	Matrix without IS	733	1720	0.9999
	Solvent with IS	0.0158	0.0371	0.9985
	Solvent without IS	765	1770	0.9998
glufosinate	Matrix with IS	0.0151	0.0189	0.9994
	Matrix without IS	559	1240	0.9998
	Solvent with IS	0.0158	0.00773	0.9994
	Solvent without IS	760	365	0.9996
AMPA	Matrix with IS	0.0436	2.72	0.9987
	Matrix without IS	261	12400	0.9991
	Solvent with IS	0.0413	2.72	0.9985
	Solvent without IS	1480	81000	0.9991

Table 6. Linear regression of the calibration curves (1/x weighing) using four different methods (corn).

Analyte	Calibration curve type	Slope	Intercept	Coefficient of determination (R^2)
Glyphosate	Matrix with IS	0.0156	0.0597	0.9985
	Matrix without IS	796	3750	0.9996
	Solvent with IS	0.0158	0.0378	0.9993
	Solvent without IS	791	1310	0.9993
Glufosinate	Matrix with IS	0.0153	0.0722	0.9979
	Matrix without IS	711	1800	0.987
	Solvent with IS	0.0157	0.0126	0.9999
	Solvent without IS	763	546	0.9994
AMPA	Matrix with IS	0.0439	2.92	0.9986
	Matrix without IS	474	48200	0.9989
	Solvent with IS	0.0423	2.77	0.9993
	Solvent without IS	1500	84500	0.9993

Table 7. Recovery (%) and RSD (%) data obtained in the validation experiments (soybean) (n = 7)

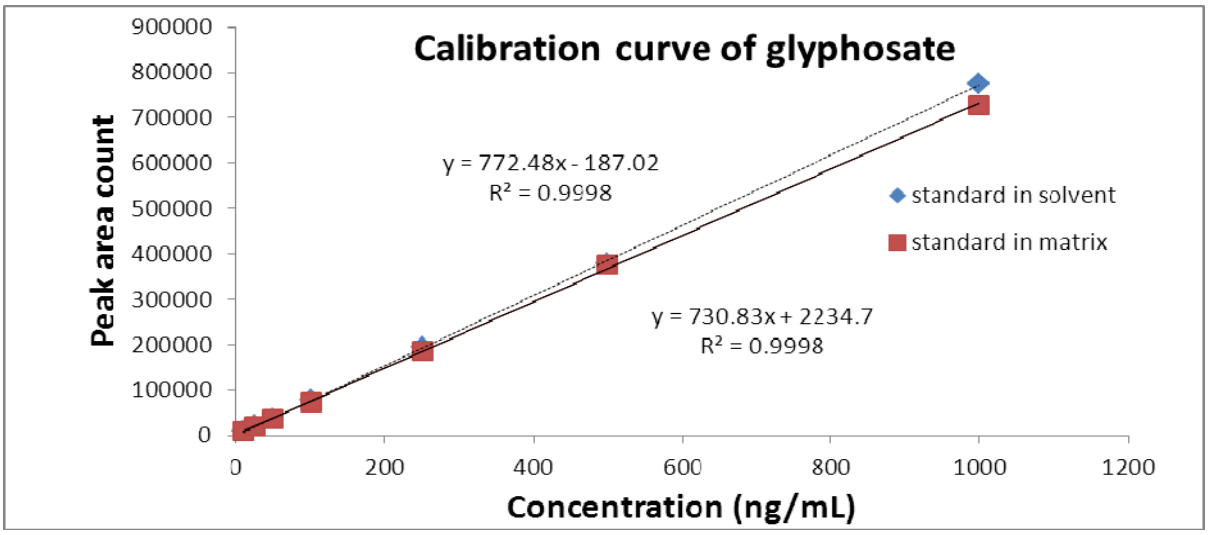
Analyte	Fortification level (µg/g)		Calibration method			
			Matrix with IS	Matrix no IS	Solvent with IS	Solvent no IS
Glyphosate	0.1	Recovery (%)	103	101	102	97
		RSD (%)	4.26	4.72	3.34	4.66
	0.5	Recovery (%)	102	100	101	96
		RSD (%)	3.98	2.96	3.51	3
	2	Recovery (%)	102	103	100	98
		RSD (%)	2.43	3.07	2.36	3
Glufosinate	0.1	Recovery (%)	102	95	101	76
		RSD (%)	4.28	5.2	4.13	4.86
	0.5	Recovery (%)	102	100	98	75
		RSD (%)	3.95	1.6	3.83	1.69
	2	Recovery (%)	98	104	94	76
		RSD (%)	2.99	3.85	3.07	3.75
AMPA	0.1	Recovery (%)	101	57	106	NA
		RSD (%)	6.3	28.83	4.53	NA
	0.5	Recovery (%)	108	78	107	NA
		RSD (%)	6.35	5.76	4.36	NA
	2	Recovery (%)	105	80	108	2
		RSD (%)	7.59	11.21	5.85	63.53

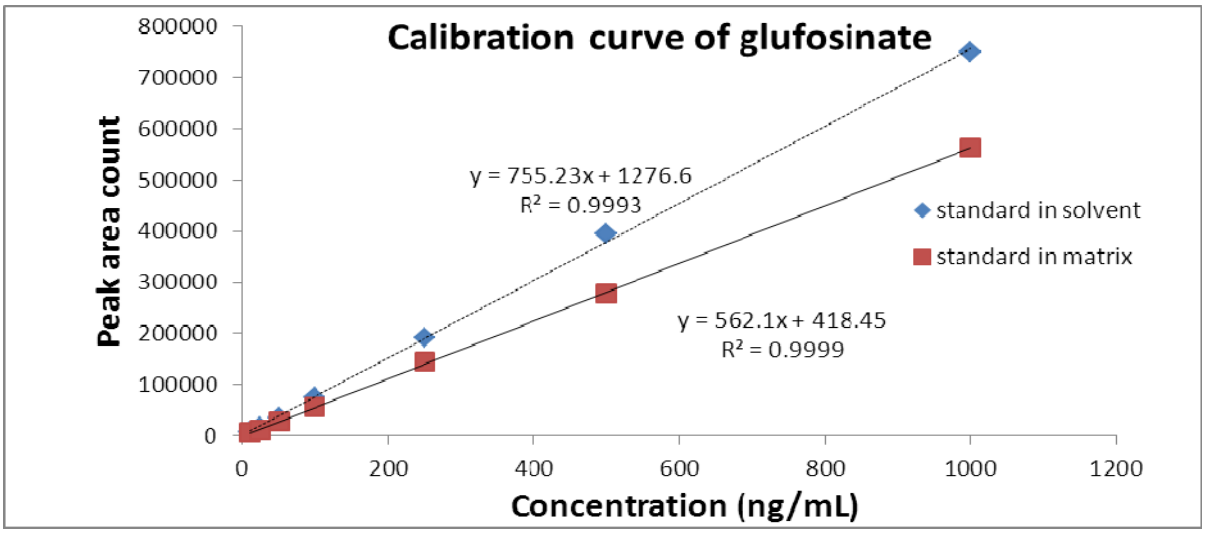
Table 8. Recovery (%) and RSD (%) data obtained in the validation experiments (n = 7).

(Corn)

Analyte	Fortification level (µg/g)		Calibration method			
			Matrix with IS	**Matrix no IS**	**Solvent with IS**	**Solvent no IS**
Glyphosate	0.1	Recovery (%)	100	89	104	105
		RSD (%)	4.78	6.3	3.59	5.4
	0.5	Recovery (%)	104	96	104	99.4
		RSD (%)	4.24	4.0	4.18	3.9
	2	Recovery (%)	107	97	106	98
		RSD (%)	3.79	2.7	3.77	2.8
Glufosinate	0.1	Recovery (%)	92	96	99	97
		RSD (%)	8.64	9.9	4.8	9.1
	0.5	Recovery (%)	103	99	104	94
		RSD (%)	3.98	3.7	3.7	3.6
	2	Recovery (%)	103	99	101	92
		RSD (%)	5.29	3.4	5.25	3.3
AMPA	0.1	Recovery (%)	96	NA	113	NA
		RSD (%)	11.96	NA	6.48	NA
	0.5	Recovery (%)	103	8.2	111	NA
		RSD (%)	8.26	48.6	7.8	NA
	2	Recovery (%)	105	52	110	10.4
		RSD (%)	6.89	5.8	6.95	9.28

Figure 1. Calibration curves of analytes in solvent and in blank soybean matrix





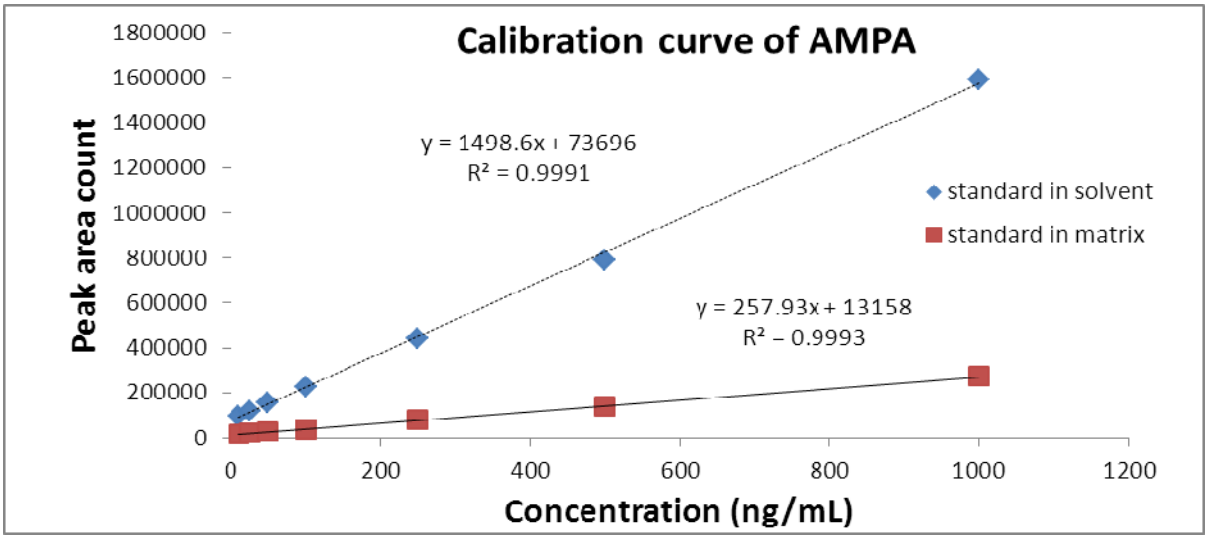
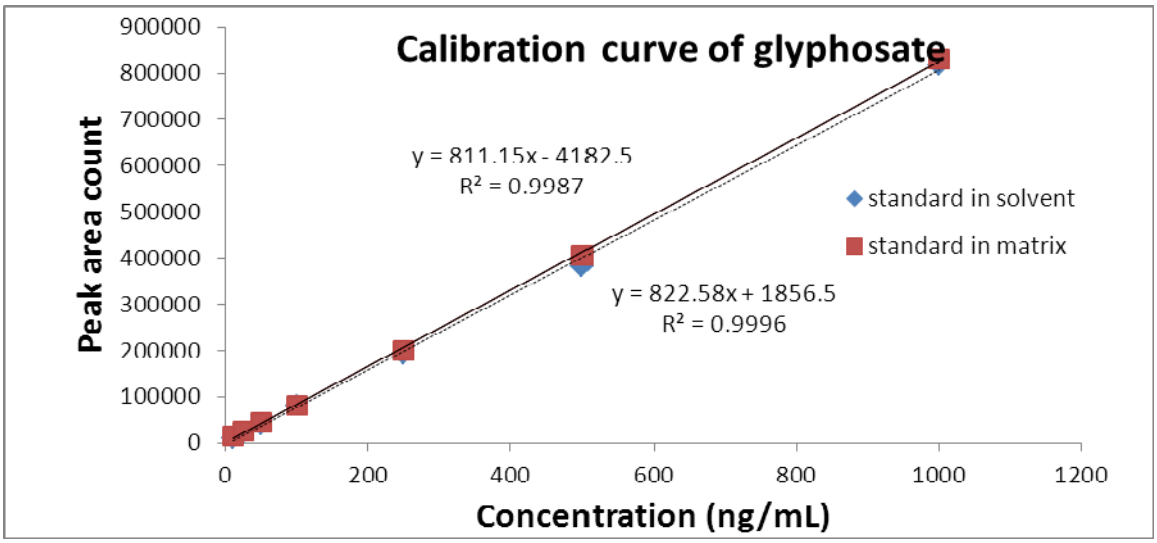
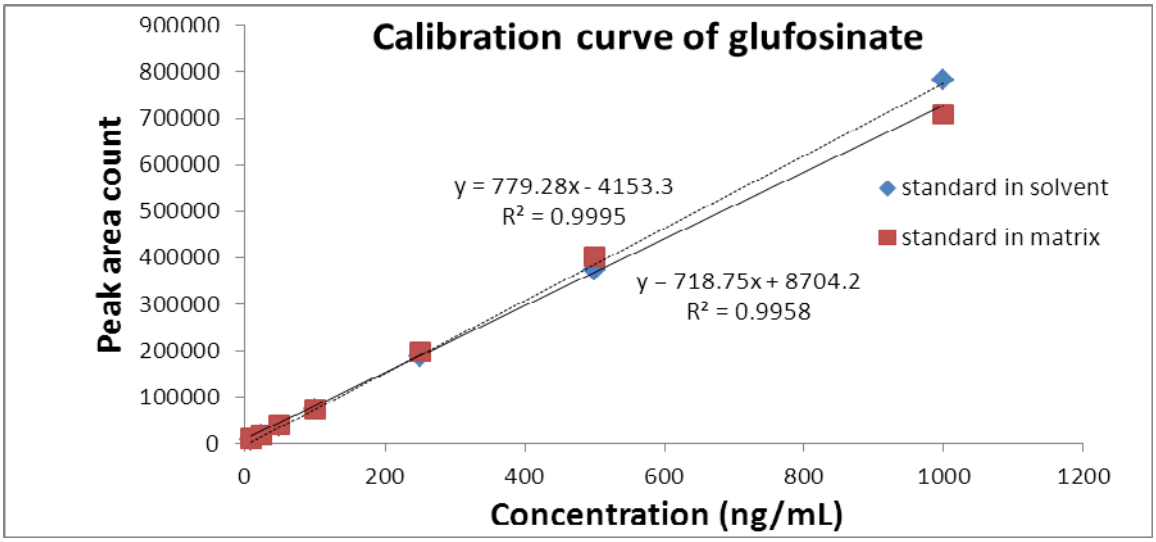


Figure 2. Calibration curves of analytes in solvent and in blank corn matrix





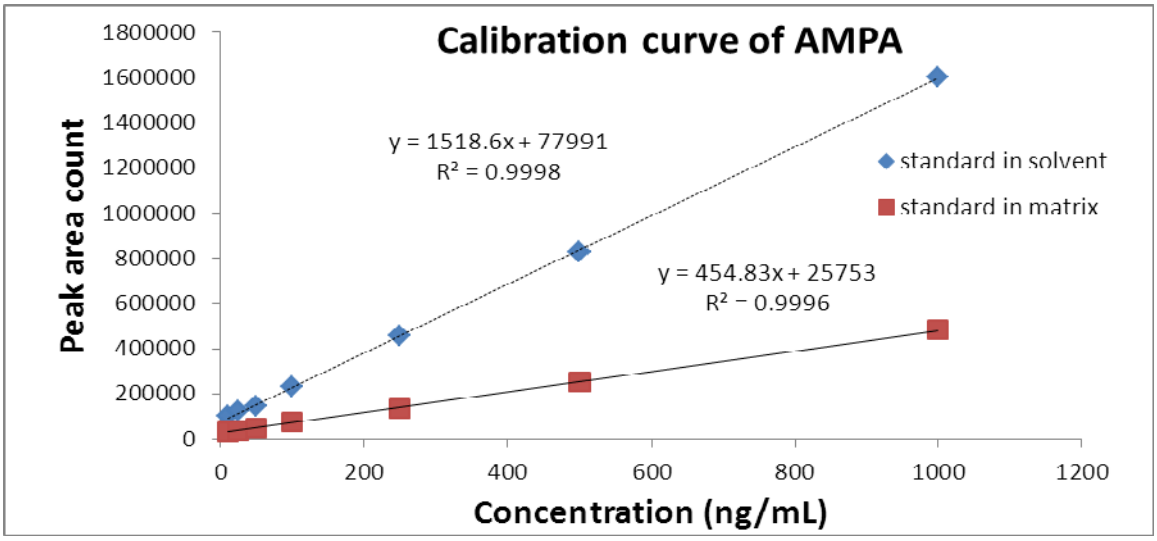
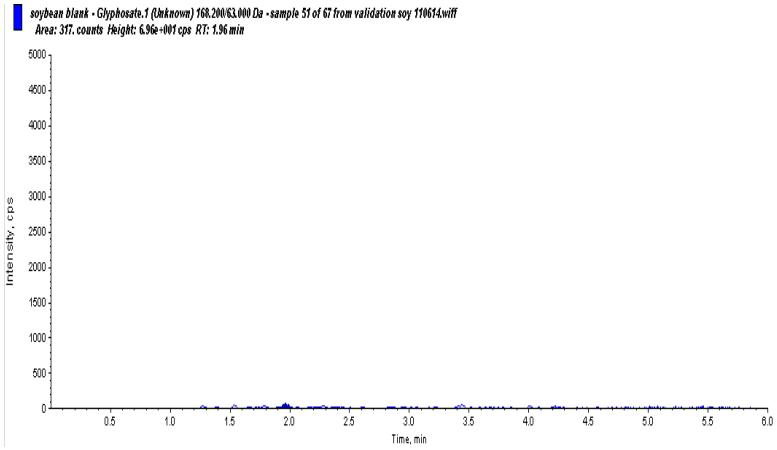
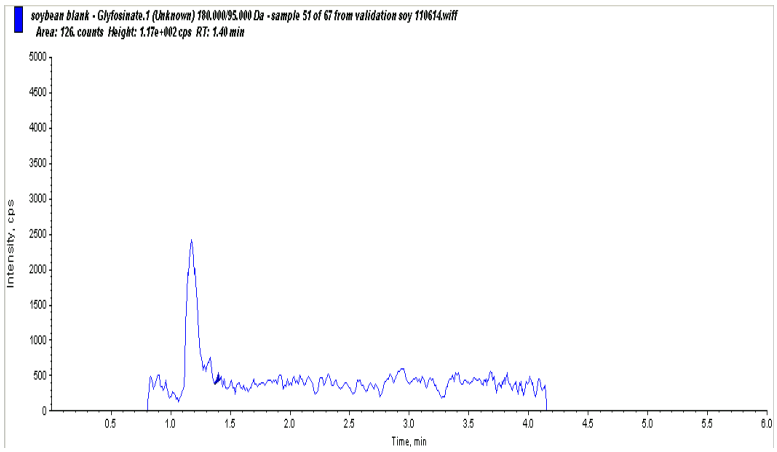


Figure 3 Chromatogram of soybean blank

Glyphosate channel



Glufosinate channel



AMPA channel

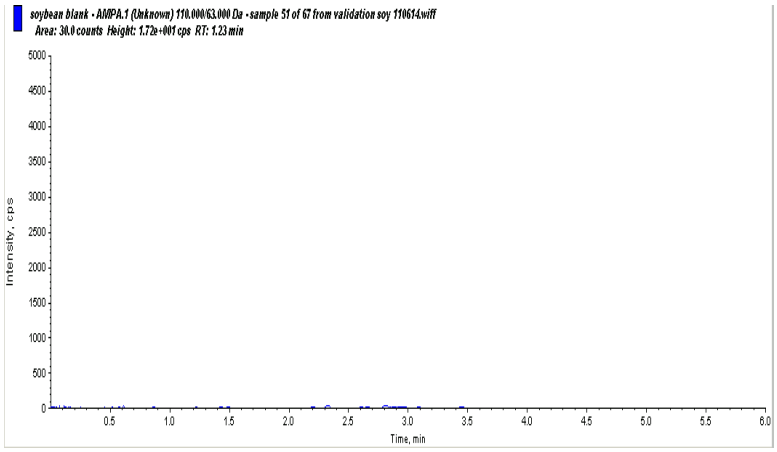
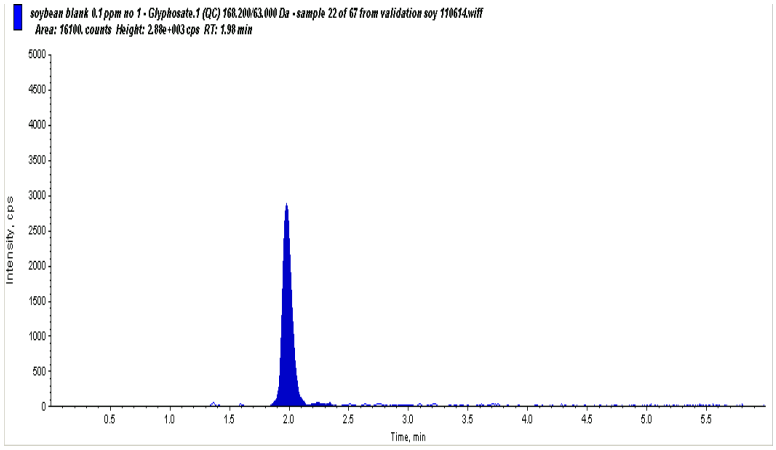
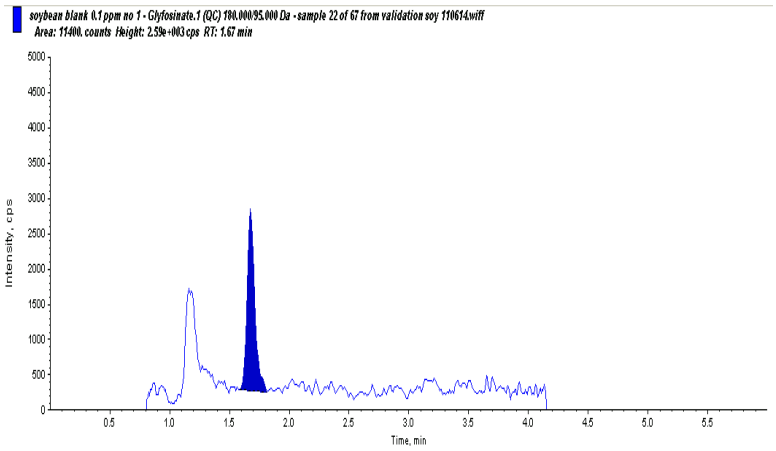


Figure 4 Chromatogram of soybean blank fortified at 0.1 ng/g of glyphosate, glufosinate and AMPA

Glyphosate channel



Glufosinate channel



AMPA channel

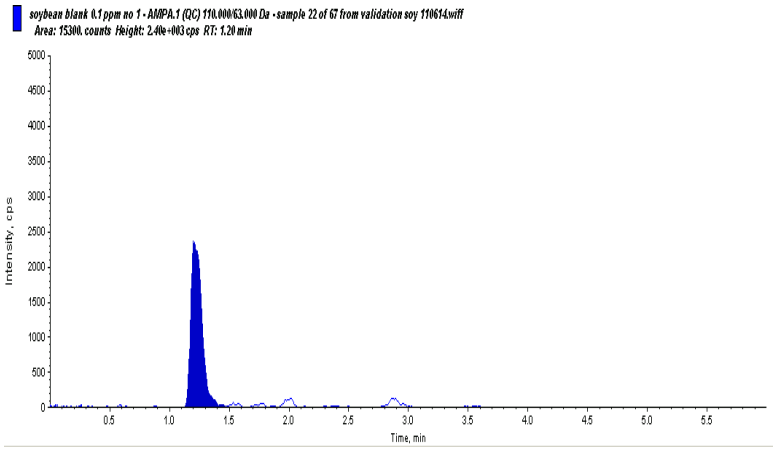
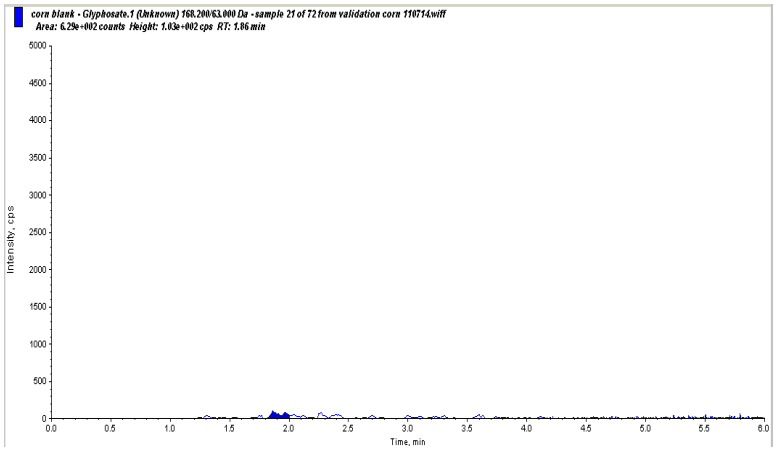
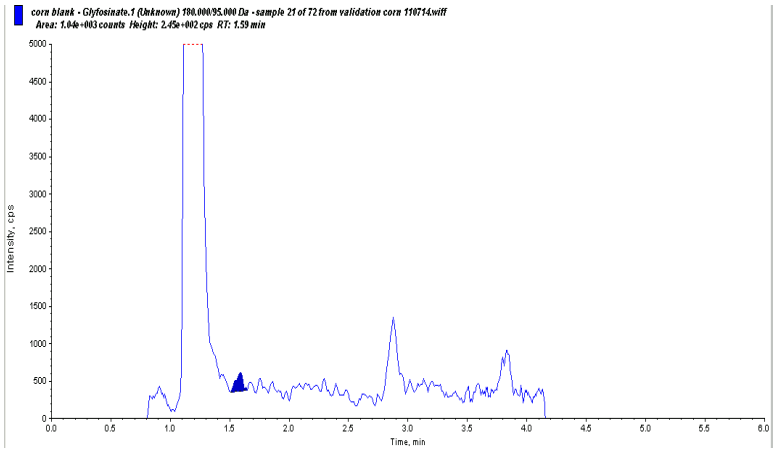


Figure 5 Chromatogram of corn blank

Glyphosate channel



Glufosinate channel



AMPA channel

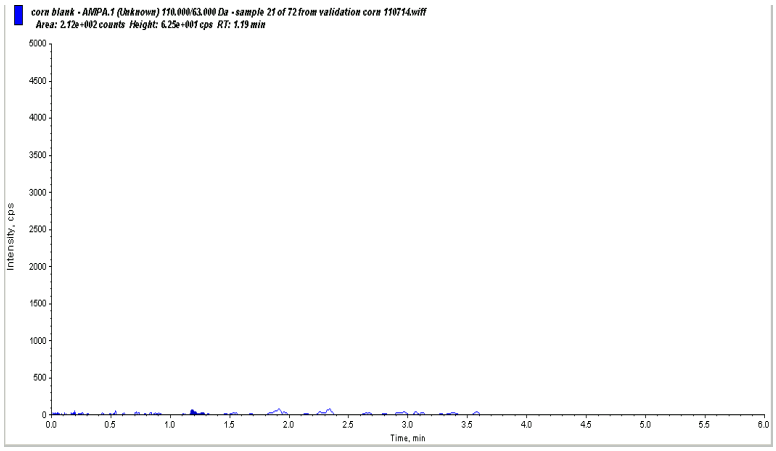
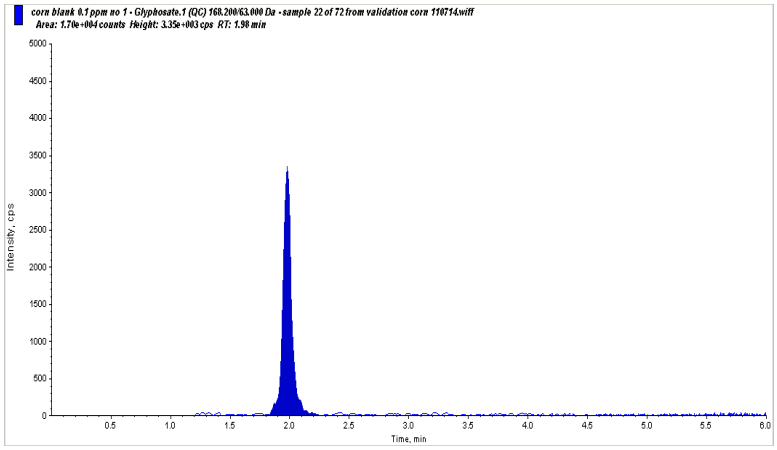
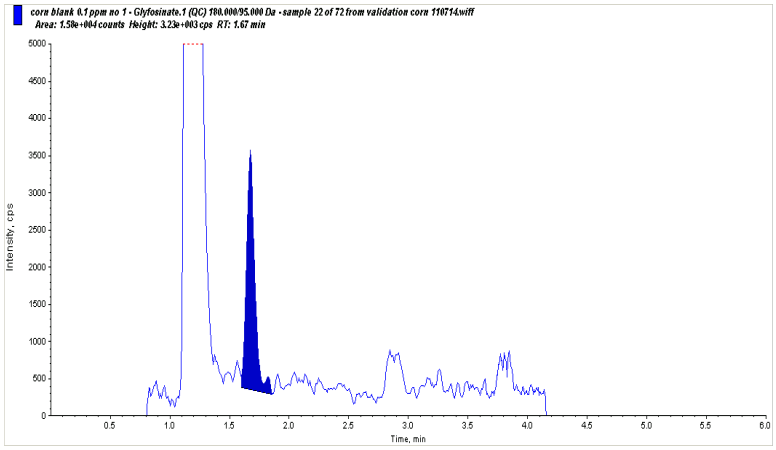


Figure 6 Chromatogram of corn blank fortified at 0.1 ng/g of glyphosate, glufosinate and AMPA

Glyphosate channel



Glufosinate channel



AMPA channel

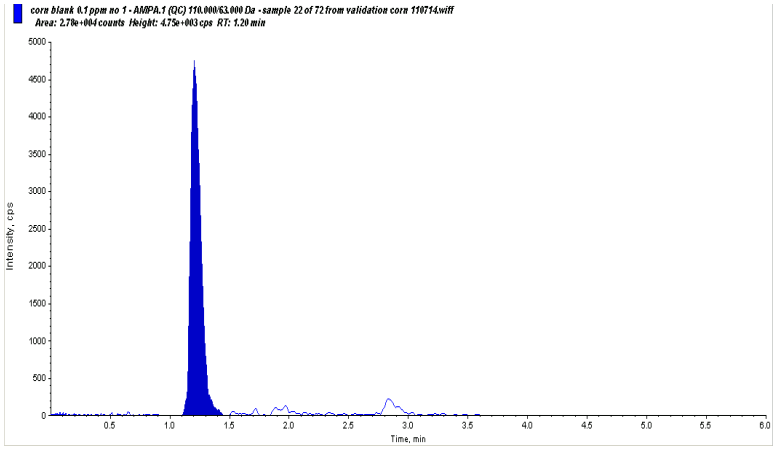
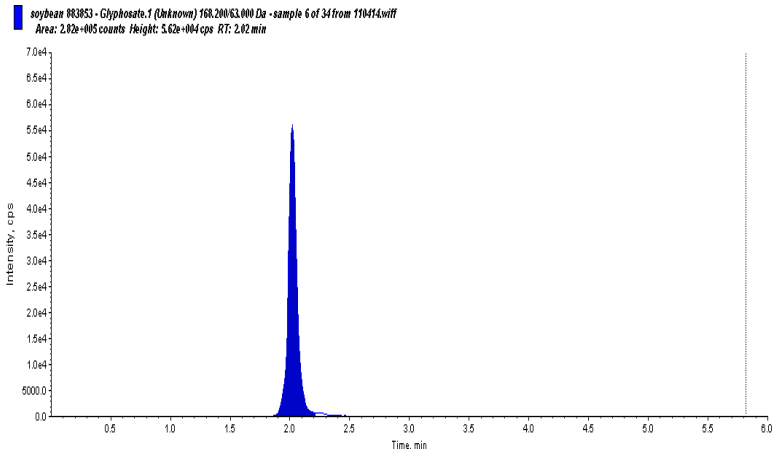
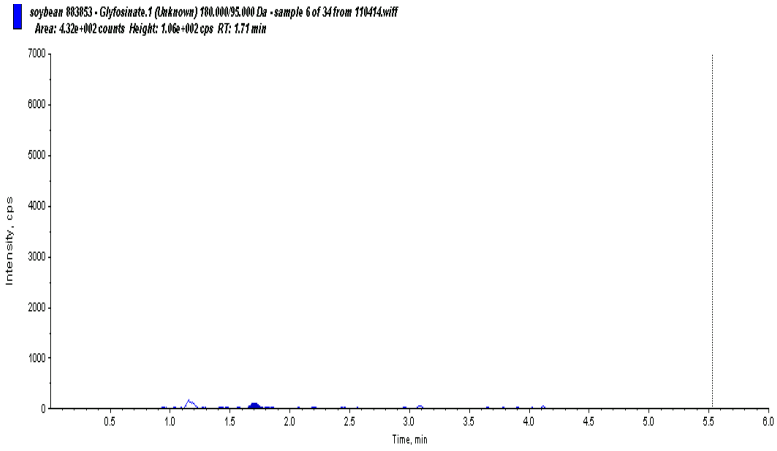


Figure 7 Chromatogram of soybean containing 11.0 ppm of glyphosate and 4.9 ppm of AMPA

Glyphosate channel



Glufosinate channel



AMPA channel

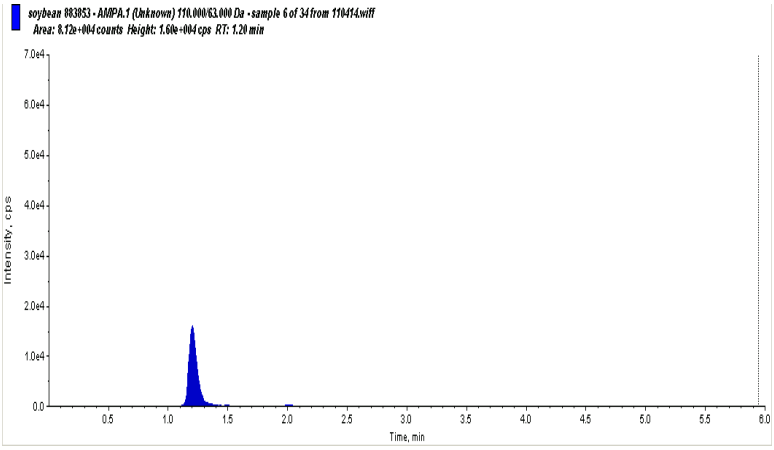
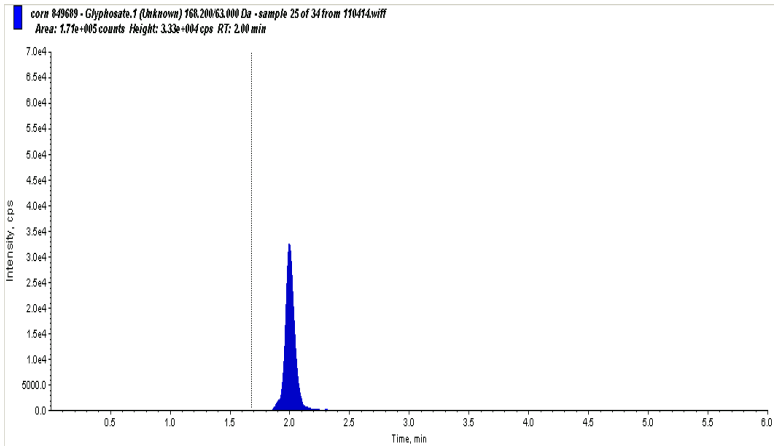
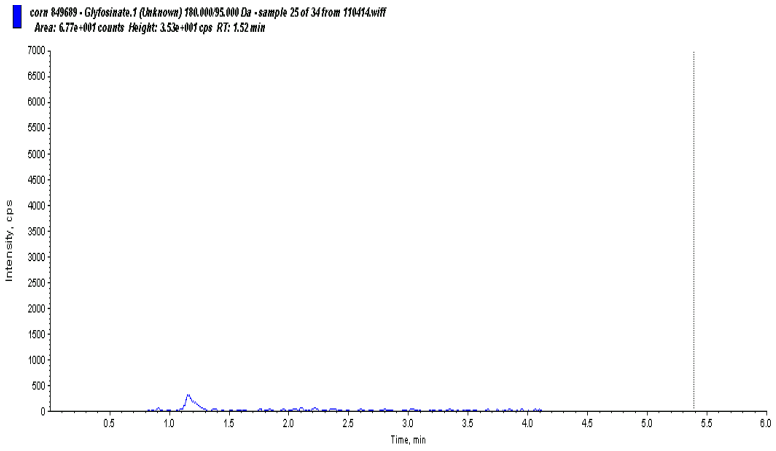


Figure 8 Chromatogram of corn containing 6.5 ppm of glyphosate and 0.065 ppm of AMPA

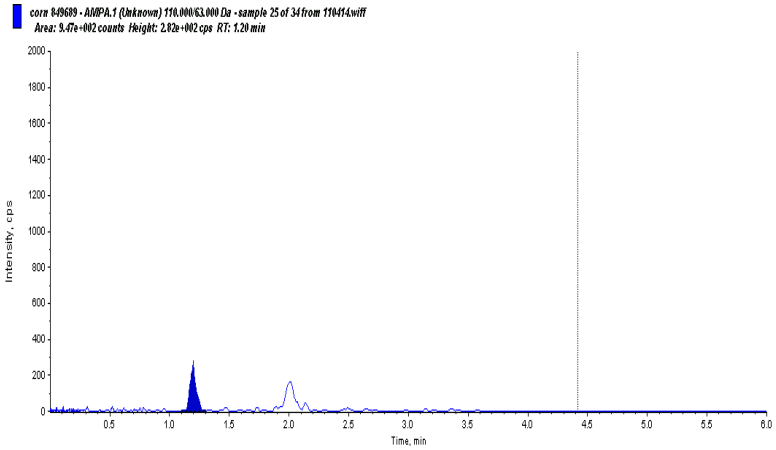
Glyphosate channel

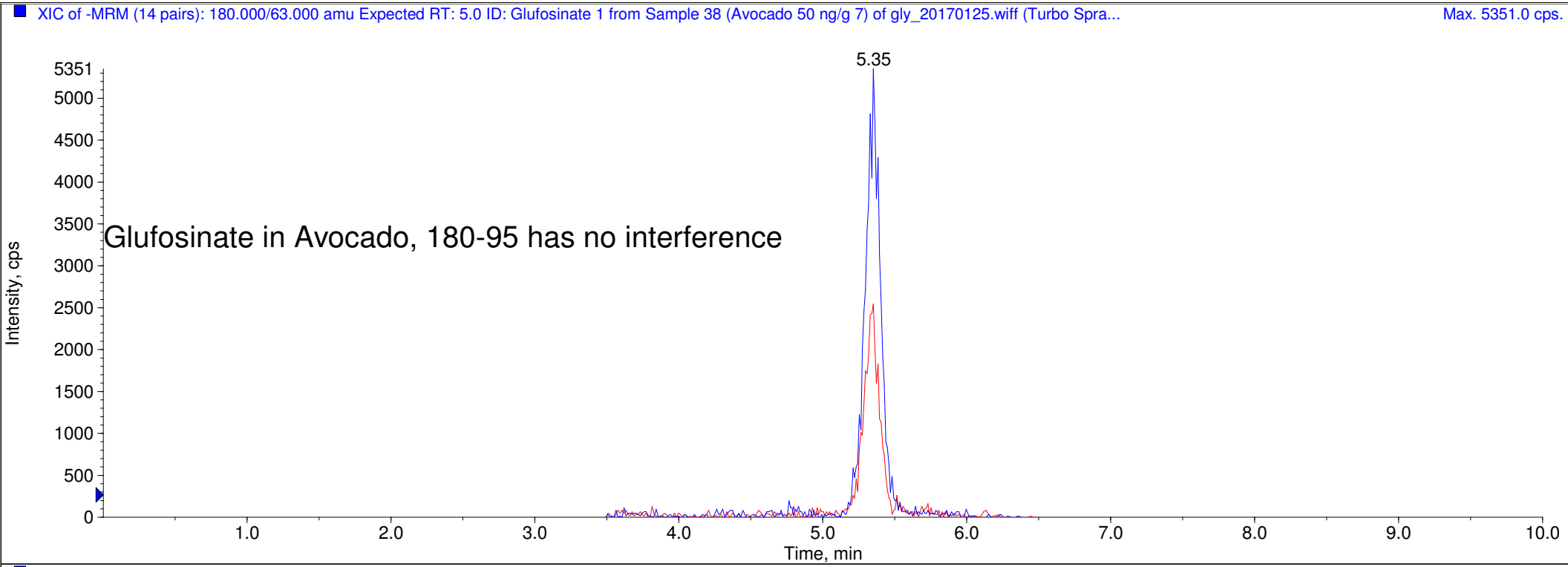


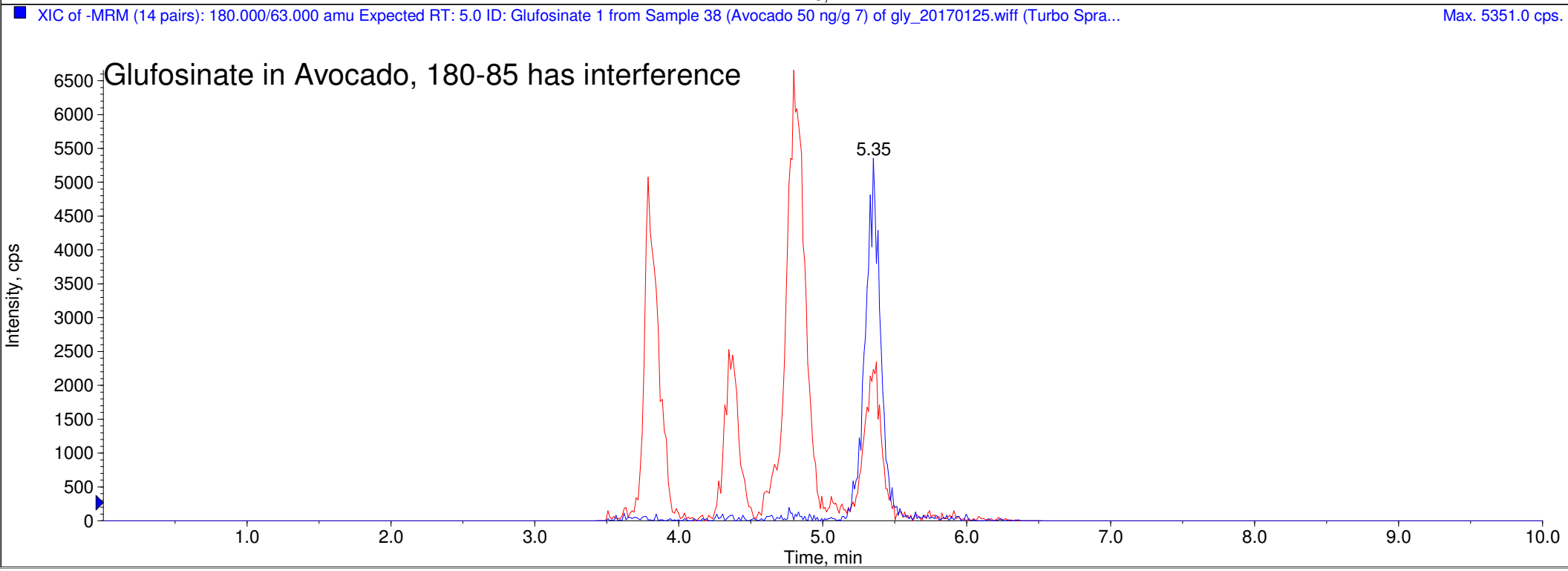
Glufosinate channel

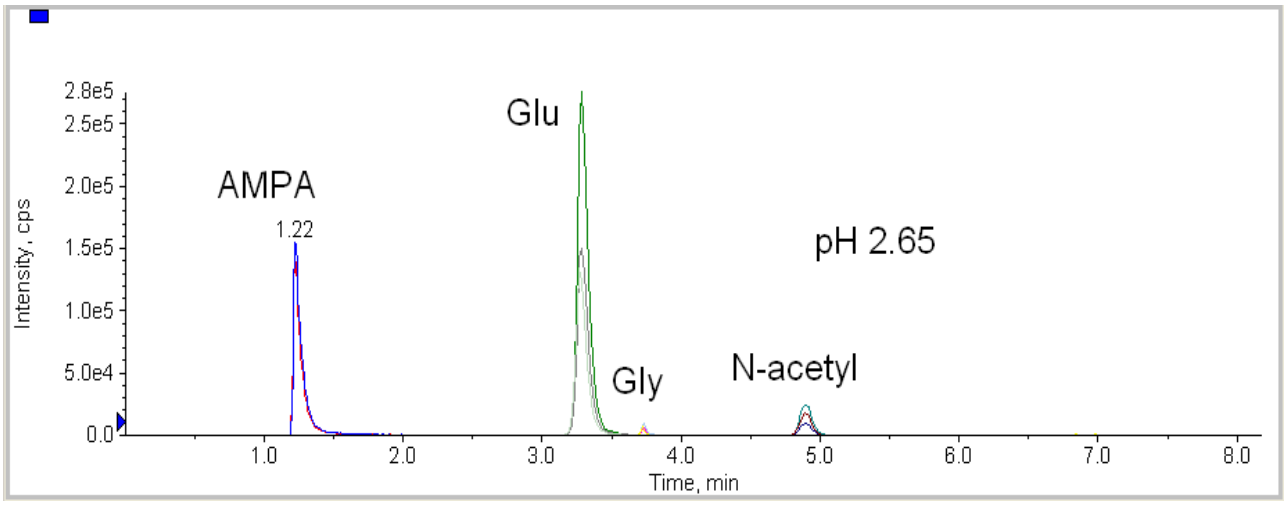


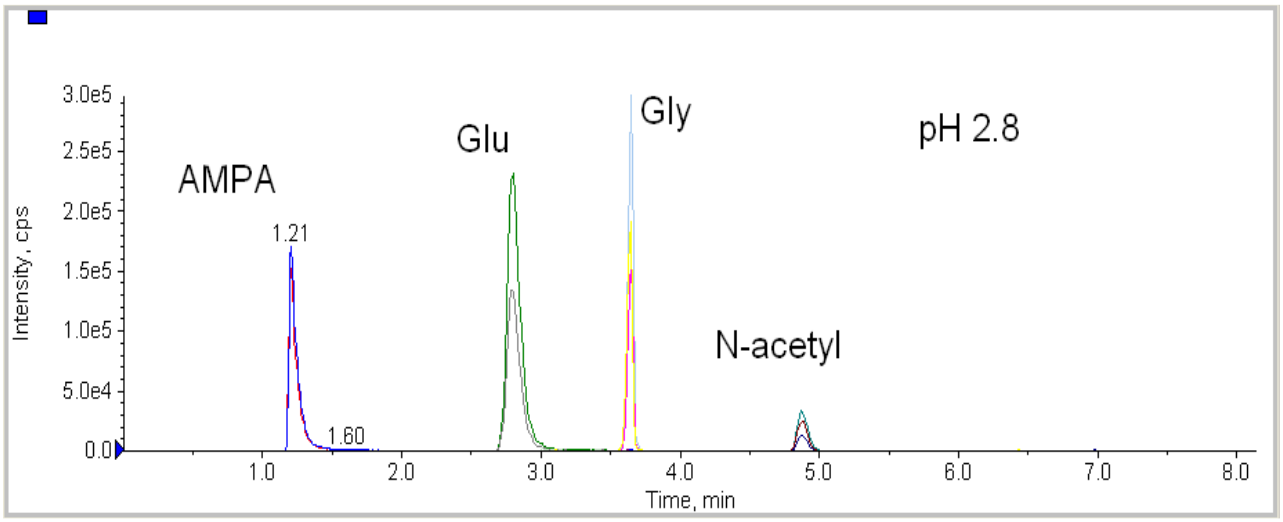
AMPA channel

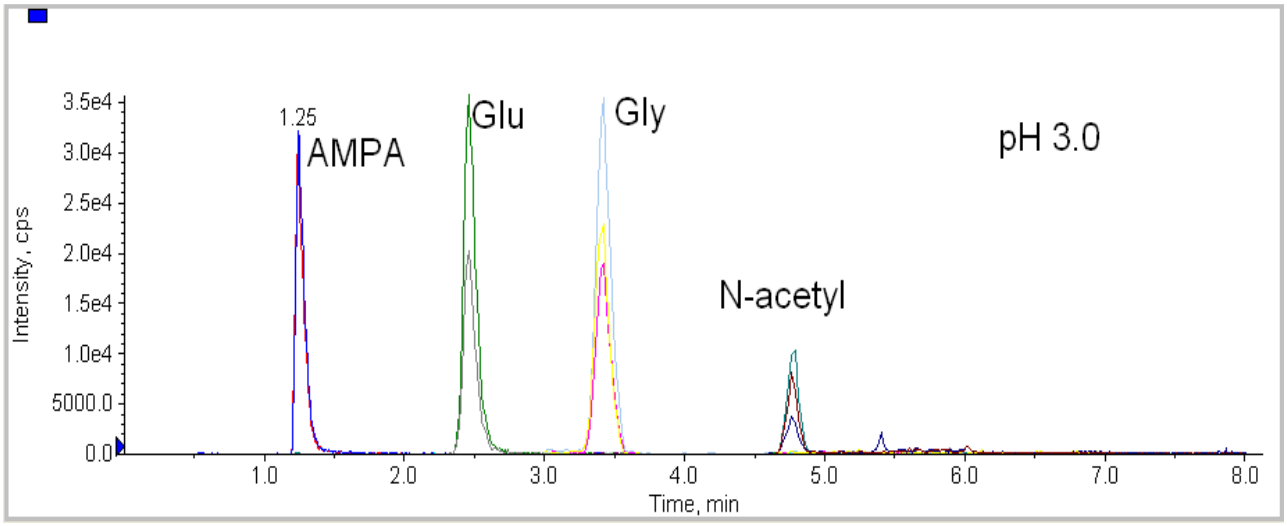



XIC of -MRM (14 pairs): 180.000/63.000 amu Expected RT: 5.0 ID: Glufosinate 1 from Sample 38 (Avocado 50 ng/g 7) of gly_20170125.wiff (Turbo Spra...
Max. 5351.0 cps.
Glufosinate in Avocado, 180-95 has no interference
5.35
Intensity, cps
Time, min


XIC of -MRM (14 pairs): 180.000/63.000 amu Expected RT: 5.0 ID: Glufosinate 1 from Sample 38 (Avocado 50 ng/g 7) of gly_20170125.wiff (Turbo Spra...
Max. 5351.0 cps.
Glufosinate in Avocado, 180-85 has interference
5.35
Intensity, cps
Time, min


Intensity, cps
2.8e5
2.5e5
2.0e5
1.5e5
1.0e5
5.0e4
0.0
AMPA
1.22
Glu
Gly
N-acetyl
pH 2.65
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
Time, min


Intensity, cps
3.0e5
2.5e5
2.0e5
1.5e5
1.0e5
5.0e4
0.0
AMPA
1.21
1.60
Glu
Gly
N-acetyl
pH 2.8
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
Time, min


Intensity, cps
3.5e4
3.0e4
2.5e4
2.0e4
1.5e4
1.0e4
5000.0
0.0
1.25
AMPA
Glu
Gly
N-acetyl
pH 3.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
Time, min

Matrices: Corn (dry)
Carrot (wet)
Avocado (fat)

Analyses: (per matrix)

Fortification Study

Level	N
Control	2
Spike 50	2
Spike 250	2
Spike 500	2

Incurred Residues

Matrix	Level
Corn	40 ng/g
Soybean	4.5 µg/g

Standards:

Calibration Standards in Solvent

Std Conc (ng/ml)	Spk Std[1] Conc (µg/ml)	Spk Std Volume Added (µl)	Dilution[2] Volume (ml)
corn (2 g sample)			
10	1	100	10
50	5	100	10
100	5	200	10
carrot/avocado (5 g sample)			
10	5	50	25
50	5	250	25
100	50	50	25

Matrix Calibration Standards

Std Conc (ng/ml)	Spk Std[1] Conc (µg/ml)	Spk Std Volume Added (µl)	Dilution[3] Volume (ml)
10	1	50	5
50	5	50	5
100	5	100	5
10	1	100	10
50	5	100	10
100	50	20	10

[1] *Prepare mixed native standards as directed in method step C.4*

[2] *Dilute with 50 ng/ml IS fortified extraction solvent*

[3] *Dilute with control sample matrix*

Fortification:

Spike Level (ng/g)	Spk Std[1] Conc (µg/ml)	Spk Std Volume Added (µl)
corn (2 g sample)		
50	1	100
250	5	100
500	5	200
carrot/avocado (5 g sample)		
50	5	50
250	5	250
500	50	50

Extraction Method: Follow method as written. Re the cleanup option for avocadoes; i.e. dichloromethane (DCM) vs petroleum ether (PE) three ORA labs agreed to use DCM and the remaining three ORA labs agreed to use PE. CFSAN can choose either.

DCM	PE
ARL	PNW
SRL	PSW
KAN	NRL

LCMS Transitions:
AMPA[110-63] 1
AMPA[110-79] 2
AMPA[110-81] 3
Glu[180-63] 1
Glu[180-95] 2
Glu[180-85] 3
Glu[183-63] IS
Gly[168-63] 1
Gly[168-79] 2
Gly[168-150] 3
Gly[171-63] IS
N-acetyl[210-150] 1
N-acetyl[210-63] 2
N-acetyl[210-168] 3

LCMS Calibration: Single level calibration for each spike level
Internal standard calibration

Analyte	Internal Standard
Glyphosate:	Glyphosate-^{13}C
N-acetyl gyphosate:	Glyphosate-^{13}C
AMPA:	Glyphosate-^{13}C
Glufosinate:	Glufosinate-D^{3}

Inj Sequence: Group by spike level

Description	Sample Name	Sample Type	Concentration
50 ng/g spike level			
10 ng/ml calibration std in solvent	CalStd10	Standard	50
10 ng/ml calibration std in solvent	CalStd10	Standard	50
10 ng/ml corn matrix calibration std	MatStd10 Corn	Quality Control	50
Corn control	Control Corn	Unknown	
Corn spike 50 #1	Spk50-1 Corn	Quality Control	50
Corn spike 50 #2	Spk50-2 Corn	Quality Control	50
Corn incurred residue	Corn Incur	Unknown	
10 ng/ml corn matrix calibration std	MatStd10 Corn	Quality Control	50
10 ng/ml calibration std in solvent	CalStd10	Standard	50
10 ng/ml carrot matrix calibration std	MatStd10 Carrot	Quality Control	50
Carrot control	Control Carrot	Unknown	
Carrot spike 50 #1	Spk50-1 Carrot	Quality Control	50
Carrot spike 50 #2	Spk50-2 Carrot	Quality Control	50
10 ng/ml carrot matrix calibration std	MatStd10 Carrot	Quality Control	50
10 ng/ml calibration std in solvent	CalStd10	Standard	50
10 ng/ml avocado matrix calibration std	MatStd10 Avocado	Quality Control	50
Avocado control	Control Avocado	Unknown	
Avocado spike 50 #1	Spk50-1 Avocado	Quality Control	50
Avocado spike 50 #2	Spk50-2 Avacado	Quality Control	50
10 ng/ml avocado matrix calibration std	MatStd10 Avocado	Quality Control	50
10 ng/ml calibration std in solvent	CalStd10	Standard	50
250 ng/g spike level			
50 ng/ml calibration std in solvent	CalStd50	Standard	250
50 ng/ml calibration std in solvent	CalStd50	Standard	250
50 ng/ml corn matrix calibration std	MatStd50 Corn	Quality Control	250
Corn spike 250 #1	Spk250-1 Corn	Quality Control	250
Corn spike 250 #2	Spk250-2 Corn	Quality Control	250
50 ng/ml corn matrix calibration std	MatStd50 Corn	Quality Control	250
50 ng/ml calibration std in solvent	CalStd50	Standard	250
50 ng/ml carrot matrix calibration std	MatStd50 Carrot	Quality Control	250
Carrot spike 250 #1	Spk250-1 Carrot	Quality Control	250
Carrot spike 250 #2	Spk250-2 Carrot	Quality Control	250
50 ng/ml carrot matrix calibration std	MatStd50 Carrot	Quality Control	250
50 ng/ml calibration std in solvent	CalStd50	Standard	250
50 ng/ml avocado matrix calibration std	MatStd50 Avocado	Quality Control	250
Avocado spike 250 #1	Spk250-1 Avocado	Quality Control	250
Avocado spike 250 #2	Spk250-2 Avacado	Quality Control	250
50 ng/ml avocado matrix calibration std	MatStd50 Avocado	Quality Control	250
50 ng/ml calibration std in solvent	CalStd50	Standard	250
500 ng/g spike level			
100 ng/ml calibration std in solvent	CalStd100	Standard	500
100 ng/ml calibration std in solvent	CalStd100	Standard	500
100 ng/ml corn matrix calibration std	MatStd100 Corn	Quality Control	500
Corn spike 500 #1	Spk250-1 Corn	Quality Control	500
Corn spike 500 #2	Spk250-2 Corn	Quality Control	500
100 ng/ml corn matrix calibration std	MatStd100 Corn	Quality Control	500
100 ng/ml calibration std in solvent	CalStd100	Standard	500
100 ng/ml carrot matrix calibration std	MatStd100 Carrot	Quality Control	500
Carrot spike 500 #1	Spk250-1 Carrot	Quality Control	500
Carrot spike 500 #2	Spk250-2 Carrot	Quality Control	500
100 ng/ml carrot matrix calibration std	MatStd100 Carrot	Quality Control	500
100 ng/ml calibration std in solvent	CalStd100	Standard	500
100 ng/ml avocado matrix calibration std	MatStd100 Avocado	Quality Control	500
Avocado spike 500 #1	Spk250-1 Avocado	Quality Control	500
Avocado spike 500 #2	Spk250-2 Avacado	Quality Control	500
100 ng/ml avocado matrix calibration std	MatStd100 Avocado	Quality Control	500
100 ng/ml calibration std in solvent	CalStd100	Standard	500
100 ng/ml soy matrix calibration std	MatStd100 Soy	Quality Control	500
Soy control	Control Corn	Unknown	
Soy incurred residue	Soy Incur	Unknown	
Soy incurred residue Dil 1-10	Soy Incur (1-10)	Unknown	

100 ng/ml soy matrix calibration std	MatStd100 Soy	Quality Control	500
100 ng/ml calibration std in solvent	CalStd100	Standard	500

Data Fields:
Index
Sample Name
Sample Type
Dilution Factor
Peak Name (Transition Name)
Peak Area
IS Peak Area
RT
Concentration (Spk level or Std conc)
Calc concentration